

# **Fragment-Based Drug Discovery of Photopharmacological Agents**

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## **Declarations**

### **Statement of originality**

I, Kim Tai Tran, affirm that all content presented within this thesis reflects my independent efforts and was generated during my enrolment in the Doctor of Philosophy programme at Imperial College London. I attest that any data, concepts, or materials originating from the work of others or through collaborative efforts, whether in part or in whole, are fully acknowledged and appropriately credited to their original sources through citation.

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## Abstract

A fundamental challenge for current pharmacotherapy is the lack of drug selectivity that can lead to off-target toxicity and high attrition rates. Photopharmacology is an emerging field that attempts to address this critical issue by employing light-responsive molecules, potentially enabling more precise drug action. However, integrating efficient light-responsiveness with strong target engagement and drug-like features within a single molecule poses a daunting task, and current late-stage approaches that install photoswitchable motifs into established drugs often yield suboptimal outcomes. Here, we show that fragment-based drug discovery can serve as a viable alternative methodology for constructing photopharmacological agents. In a first study, a large-sized general fragment library was screened against the SARS-CoV-2 methyltransferase NSP16/10, identifying a biarylsulfonamide fragment hit **B1**. Ligand-based fragment optimisation with careful monitoring of target engagement, photochemical properties and lipophilicity ultimately resulted in the photoswitchable lead compound **B130**, which concurrently displayed a strong single-digit micromolar target affinity, more than an order of magnitude fold change in activity upon photoswitching and an improved lipophilic efficiency. In a second study, the ultra-small and universal fragment probe, 4-bromopyrazole, was employed as a starting point for developing photoswitchable inhibitors against the cancer target, cyclin-dependent kinase 2. Through rational structure-based and computer-aided drug design, the fragment probe was elaborated into a sub-micromolar potent and photoswitchable inhibitor **C20** at unprecedented speed. Collectively, these findings suggest that fragments can serve as powerful starting points for achieving multi-parameter optimisation of key photopharmacological properties and arriving at efficient endpoints, competitive with those of late-stage approaches. These are the first studies to demonstrate the utility of a fragment-based approach for constructing photoswitchable ligands, and we anticipate that the methodology will aid in addressing the core challenge of reconciling light-responsiveness with drug-like features—ultimately enabling the development of more clinically translatable photopharmacological agents.

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## Abbreviations

|                  |                                                |
|------------------|------------------------------------------------|
| APCI             | Atmospheric pressure chemical ionisation       |
| Ar               | Aryl                                           |
| ADP              | Adenosine diphosphate                          |
| ATP              | Adenosine triphosphate                         |
| Boc              | <i>tert</i> -Butyloxycarbonyl                  |
| CDK              | Cyclin-dependent kinase                        |
| DIAD             | Diisopropyl azodicarboxylate                   |
| DIPEA            | <i>N,N</i> -Diisopropylethylamine              |
| DMAP             | 4-Dimethylaminopyridine                        |
| DMF              | <i>N,N</i> -Dimethylformamide                  |
| DMF-DMA          | <i>N,N</i> -Dimethylformamide dimethyl acetal  |
| DMSO             | Dimethyl sulfoxide                             |
| DNA              | Deoxyribonucleic acid                          |
| DIBAL-H          | Diisobutylaluminium hydride                    |
| DSF              | Differential scanning fluorimetry              |
| DTT              | Dithiothreitol                                 |
| EC <sub>50</sub> | Half-maximal effective concentration           |
| EDC              | 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide  |
| EDTA             | Ethylenediaminetetraacetic acid                |
| ESI              | Electrospray ionisation                        |
| FBDD             | Fragment-based drug discovery                  |
| FA               | Formic acid                                    |
| FC               | Fold change                                    |
| FDA              | The United States Food and Drug Administration |
| FP               | Fluorescence polarisation                      |
| H bond           | Hydrogen bond                                  |
| HAC              | Heavy atom count                               |
| HBA              | H bond acceptor                                |
| HBD              | H bond donor                                   |
| HOBt             | 1-Hydroxybenzotriazole                         |
| HPLC             | High-performance liquid chromatography         |
| HRMS             | High-resolution mass spectrometry              |
| HTRF             | Homogeneous time resolved fluorescence         |
| HTS              | High-throughput screening                      |
| IC <sub>50</sub> | Half-maximal inhibitory concentration          |
| K <sub>D</sub>   | Dissociation constant                          |
| LE               | Ligand efficiency                              |
| LED              | Light-emitting diode                           |
| LC–MS            | Liquid chromatography-mass spectrometry        |
| LHAr             | Left-hand aryl ring                            |

|                     |                                                 |
|---------------------|-------------------------------------------------|
| LHS                 | Left-hand side                                  |
| LiHMDS              | Lithium bis(trimethylsilyl)amide                |
| LiPE                | Lipophilic efficiency                           |
| Ms                  | Methanesulfonyl (mesyl)                         |
| MTA                 | 5'-Methylthioadenosine                          |
| MTase               | Methyltransferase                               |
| MW                  | Molecular weight                                |
| NA                  | Not active                                      |
| ND                  | Not determined                                  |
| MOE                 | Molecular Operating Environment                 |
| NMR                 | Nuclear magnetic resonance                      |
| PDB                 | Protein Data Bank                               |
| PDT                 | Photodynamic therapy                            |
| PSD                 | Photostationary distribution                    |
| PS-PPh <sub>3</sub> | Polystyrene-bound triphenylphosphine            |
| PSS                 | Photostationary state                           |
| RHAr                | Right-hand aryl ring                            |
| RHS                 | Right-hand side                                 |
| RNA                 | Ribonucleic acid                                |
| Ro3                 | Rule of 3                                       |
| RPC                 | Reversed-phase chromatography                   |
| rt                  | Room temperature                                |
| SAD                 | Single-wavelength anomalous diffraction         |
| SAH                 | S-Adenosyl-L-methionine                         |
| SAM                 | S-Adenosyl-L-methionine                         |
| SAR                 | Structure-activity relationship                 |
| SARS-CoV-2          | Severe acute respiratory syndrome coronavirus 2 |
| SEM                 | Standard error of the mean                      |
| SFN                 | Sinefungin                                      |
| SGC                 | Structural Genomics Consortium                  |
| SGCC                | Silica gel column chromatography                |
| SPA                 | Scintillation proximity assay                   |
| SPR                 | Surface plasmon resonance                       |
| TFA                 | Trifluoroacetic acid                            |
| THF                 | Tetrahydrofuran                                 |
| TLC                 | Thin-layer chromatography                       |
| T <sub>m</sub>      | Melting temperature                             |
| TM                  | Target molecule                                 |
| TOF                 | Time-of-flight                                  |
| TR-FRET             | Time-resolved fluorescence energy transfer      |
| Ts                  | 4-Toluenesulfonyl (tosyl)                       |
| TSA                 | Thermal shift assay                             |

|        |                     |
|--------|---------------------|
| UV     | Ultraviolet         |
| UV–Vis | Ultraviolet-visible |

Standard chemical naming abbreviations and one- and three-letter amino acid codes have been employed throughout.

# 1. Introduction

## 1.1. The challenge of drug selectivity

Currently, 90% of drug candidates that enter clinical trials do not succeed—and in 30% of cases, this is due to adverse events causing intractable toxicity or excessive side-effects.<sup>1</sup> Accordingly, there has been a rising advocacy for implementing safety risk considerations in the early stages of drug discovery, where safety is to be regarded as another crucial property dimension during drug design and development.<sup>2</sup> A major factor contributing to toxicity stems from the agent engaging other unintended biological targets, which can be understood as a fundamental problem of selectivity (**Figure 1.1A**).<sup>3</sup> Poor target selectivity does not only hamper clinical development but also constitutes a significant issue for chemical probes employed for interrogating biological systems—where the use of non-selective probes has led to widespread erroneous conclusions in the scientific literature.<sup>4, 5</sup>

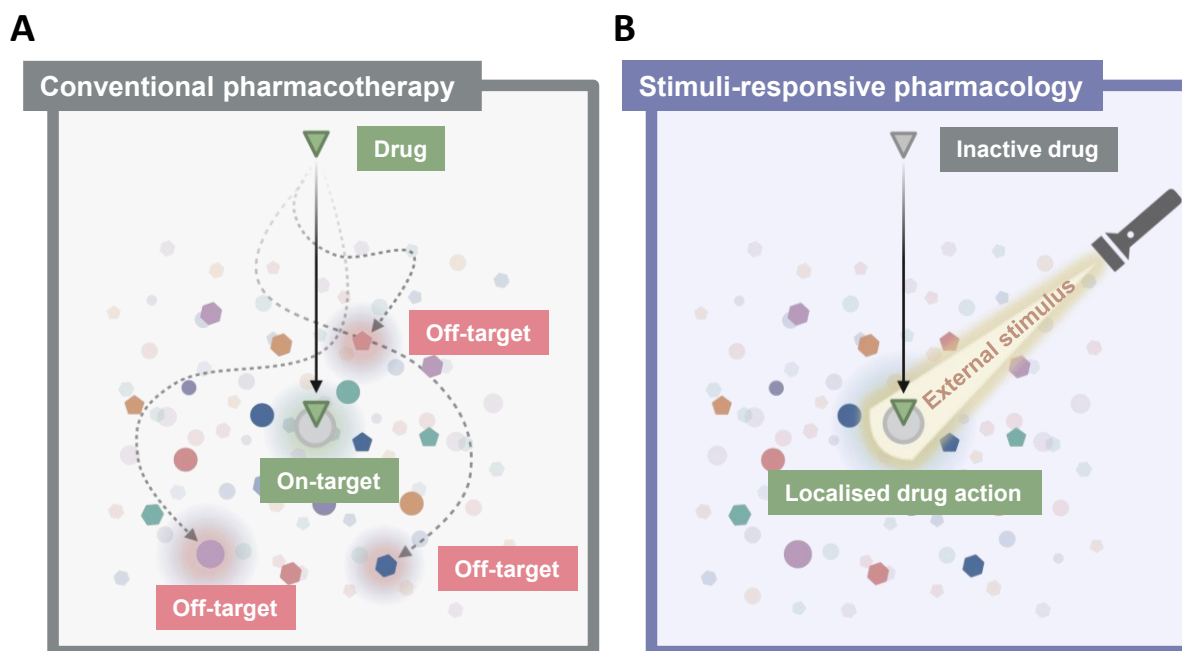


**Figure 1.1. The challenge of drug selectivity.** (A) Conceptual illustration of the selectivity issue of conventional drugs. (B) Conceptual illustration of stimuli-responsive drugs. Created with BioRender.com.

A number of strategies have been taken by researchers to overcome the challenge of selectivity. Rational structure-guided approaches in tandem with ligand-based considerations of e.g. “molecular obesity” have

become increasingly applied by researchers to design molecules that fit more uniquely to their intended biological target.<sup>6</sup> However, biological systems consist of a vast number of macromolecules that can often display high structural homology (the kinase family being a canonical example) and engaging a single target herein may not be feasible when relying solely on ligand design. As a response to the challenge of drug selectivity, medicinal chemists have explored moving beyond the rudimentary principle of ligand-target complexation towards higher-order functional systems. One such manifestation is stimuli-responsive drugs or drug-delivery systems, where drug action is put under the control of either an endogenous or external conditional trigger, e.g., pH, redox activity, enzymatic activity, temperature, ultrasound, or light (**Figure 1.1B**).<sup>7,8</sup> By coupling drug activity with such stimuli, higher selectivity may be achieved.

## 1.2. Light-controlled modalities

### 1.2.1. Light as a stimulus

Light has especially emerged as a powerful external stimulus for controlling drug action or delivery. Firstly, light can be regulated with extremely high spatio-temporal precision—for instance, using well-established microscopy and laser technologies, it is possible to achieve sub-cellular resolution with light.<sup>9</sup> Secondly, light does not interfere with the majority of biological tissues except for specialised cells carrying photo-receptors and is therefore a highly bioorthogonal signal.<sup>10</sup> The primary challenge of applying light for controlling drug action is its poor penetration depth—but by moving towards more red-shifted wavelengths, the amount of absorption and scattering by natural chromophores of the skin and other tissues can be significantly reduced.<sup>11</sup> In this regard, a wavelength range of 600–1200 nm is considered the therapeutically optimal light window.<sup>12</sup> Light is additionally safe across a range of wavelengths. UV-light is well-known to be able to cause mutagenic DNA lesions, and exposure to UV radiation is associated with skin carcinogenesis and immunosuppression.<sup>13-15</sup> However, low-dose 365 nm UV-light has been shown not to change the phenotype or proteome of various cell types and be safe for cell-based experiments.<sup>16</sup>

The use of light has made significant impact on medicine and biological research. For instance, the genetic incorporation of photoresponsive proteins into cells has allowed for unprecedented detection and manipulation of biological processes, which has revolutionised the neuroscientific field and also found application within cardiology and other medical sciences.<sup>17</sup>

### 1.2.2. Photodynamic therapy

Arguably, a more elegant and translational approach to using light entails the employment of photore-sponsive ligands, which do not require genetic engineering of target proteins. The earliest manifestation of coupling light to drug action arose with photodynamic therapy (PDT). This modality relies on the use of photosensitisers, which upon light irradiation react with molecular oxygen, resulting in the generation of highly reactive oxygen species that cause cell damage and death (**Figure 1.2A**).<sup>18</sup> PDT has found numerous clinical applications within cancer treatment and has more recently been adapted to non-oncological diseases as well (e.g., infections, cardiovascular conditions, and neurological disorders).<sup>18</sup> However, PDT has its limitations—beyond not being able to address the anoxic environments of solid tumours, PDT is fundamentally limited to a non-specific cell-killing mode-of-action, which may not always be the desirable ultimate outcome for a drug.<sup>18</sup>

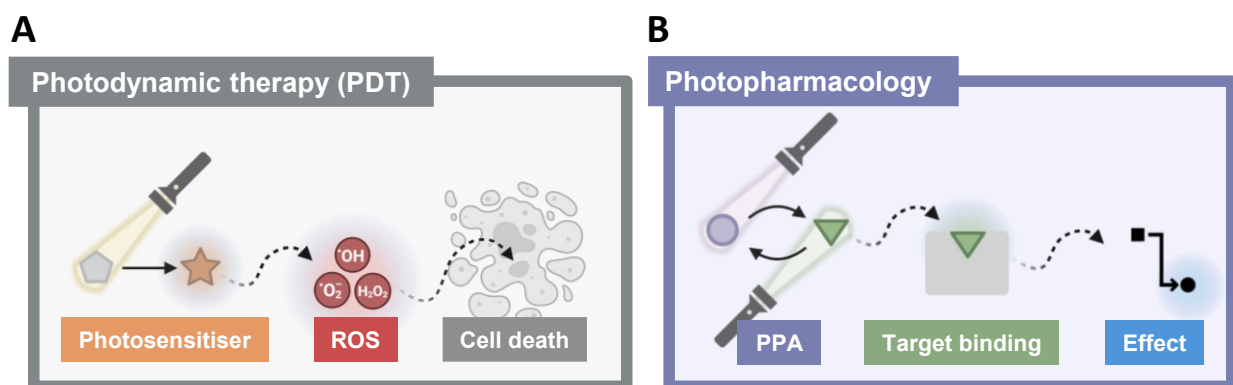


**Figure 1.2. Light-controlled modalities.** (A) Conceptual illustration of photodynamic therapy (PDT). ROS = Reactive oxygen species. (B) Conceptual illustration of photopharmacology using photoswitches. PPA = Photopharmacological agent. Created with BioRender.com.

### 1.2.3. Photopharmacology

Recently, a more targeted approach has emerged with the field of “photopharmacology”, which relies on the use of light to modulate the binding event of a ligand to its biological target(s) (**Figure 1.2B**). This has firstly materialised in the use of photolabile protective groups called photocages. These are used to mask and render the drug molecule inactive, whereas light irradiation causes specific uncaging and restoration of the drug activity.<sup>19</sup> It is important to highlight that photodecaging is an irreversible process that solely grants the capability to “turn on” the drug activity.

Another and perhaps conceptually more attractive approach relies on the use of photoswitches—molecules that can reversibly be activated and deactivated via light irradiation.<sup>19</sup> Reversibility allows—in principle—complete control over drug activity across both the dimensions of space and time. Within



chemical biology, the ability to modulate the very same system between an on and off state over a very short time course serves as a strong (within-subject) experimental design that is difficult to achieve with “static” ligands.<sup>20</sup> In downstream therapeutical applications, the possibility for deactivating the drug again means that potential adverse effects occurring from permanent on-target activity can be mitigated, including toxicity, drug addiction and resistance development.<sup>20</sup> Furthermore, the reversibility allows for appropriately probing and modulation of physiological events that operate at short millisecond timescales and where fast drug deactivation would be a desirable property.<sup>21</sup>

A plethora of photopharmacological campaigns have been published to date against a wide array of target classes (including the major categories of ion channels, transporters, GPCRs and kinases), where light-dependent control of biological function has been demonstrated in a range of systems—spanning from isolated biomacromolecules and cells to live organisms.<sup>22</sup> Although a majority of these studies remain at the proof-of-concept stage, there has recently been exciting translational development in the area of vision restoration, where a photopharmacological agent is currently in clinical trials for treatment of patients suffering from retinitis pigmentosa.<sup>23</sup> Together, photopharmacology constitutes as a new modality carrying enormous potential for transforming chemical biology as well as clinical therapy.

To fully realise the tremendous potential of photopharmacology, especially using reversible photoswitches, we must take a closer look at their structure and function.

### 1.3. Photoswitches

Photoswitches are molecules that can reversibly toggle (switch) between two (or more) structural states through light absorption, and the structural changes include isomerisation across double bonds or unimolecular ring-opening/closing reactions.<sup>19</sup> A multitude of photoswitchable motifs have been disclosed to date, including azobenzenes, stilbenes, diarylethenes, hemithioindigos, spiropyrans, fulgides and fulgimides. Amongst these, azobenzene has indisputably been subject to the most extensive studies and has found most applications.<sup>19</sup>

#### 1.3.1. Azobenzene

Azobenzene can reversibly and efficiently be switched between its thermodynamically stable *E*-isomeric form and its metastable (10–12 kcal/mol higher-energy) *Z*-isomeric state upon light irradiation with different wavelengths (**Figure 1.3A**).<sup>24</sup> The isomerisation yields both a large geometrical difference (resulting

in a 3.5 Å change in the end-to-end distance of the 4-position carbons) as well as causing a significant perturbation to the electronic system (with a 3 Debye change in the dipolar moment).<sup>25, 26</sup> These structural and electronic changes are exploited for driving the difference in target binding and biological activity between the *E*- and the *Z*-state.

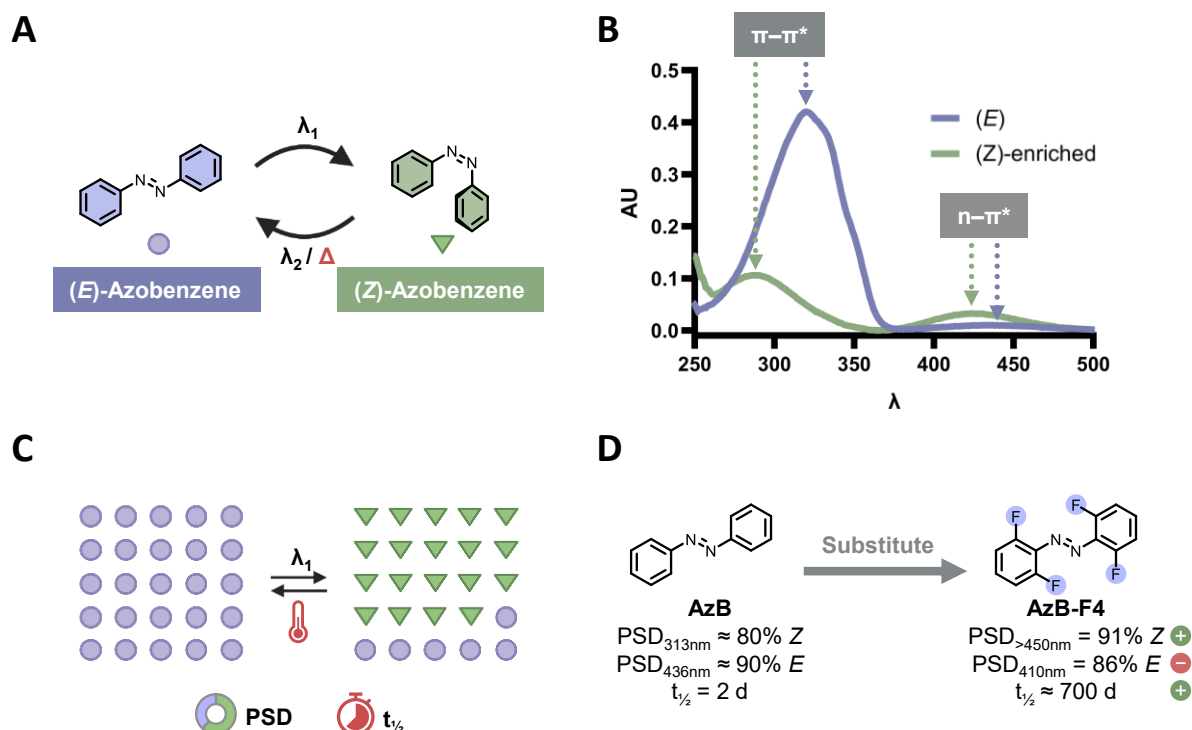


**Figure 1.3. Structure and property of azobenzene.** (A) Structure of *E*- and *Z*-azobenzene that can be interconverted using light with different wavelengths ( $\lambda_1/\lambda_2$ ) or through thermal relaxation ( $\Delta$ ). (B) UV-Vis absorbance spectrum of azobenzene upon photoswitching with the key absorbance bands highlighted. (C) Conceptual illustration of the photo-stationary distribution (PSD) and thermal *Z*-isomer half-life ( $t_{1/2}$ ). (D) Tetra-*ortho*-fluoro substitution of azobenzene (AzB) yields AzB-F4 with improved forward photoconversion and thermal *Z*-isomer stability.<sup>27</sup>

In the *E*-form, azobenzene is characterised by having an intense absorbance band at around 320 nm, corresponding to a symmetry-allowed  $\pi-\pi^*$  transition and the excitation from its ground electronic state ( $S_0$ ) to its second excited state ( $S_2$ ) (Figure 1.3B).<sup>28</sup> Additionally, a weak absorbance band around 450 nm is observed and arises from a weakly allowed  $n-\pi^*$  transition and the excitation from  $S_0$  to the first singlet excited state ( $S_1$ ). In the *Z*-form, a dramatic change in the absorbance profile is observed, with the  $\pi-\pi^*$  transition being markedly attenuated and blue-shifted, whereas the  $n-\pi^*$  band is significantly intensified (and slightly blue-shifted). Upon excitation to either the  $S_1$  or  $S_2$  excited states, the molecule can undergo non-radiative decay back to the ground state via a conical intersection, which promotes isomerisation.<sup>28</sup>

The separation between the absorbance bands of the *E*- and the *Z*-isomers is what allows for the predominant excitation of one isomer and thereby net accumulation of the other isomer. Upon continuous light irradiation, the system eventually reaches a photochemical equilibrium, where the ratio of *E*- and *Z*-isomers no longer changes—this is known as the photostationary state (PSS) (**Figure 1.3C**).<sup>28</sup> The distribution of *E*- and *Z*-isomers at PSS (the photostationary distribution, PSD) depends on the band separation between the isomers, the quantum yields associated with their interconversion and the choice of wavelength. For azobenzene, light irradiation at 313 nm yields a PSD  $\approx$  80% *Z*, whereas 436 nm light gives a PSD  $\approx$  90% *E*.<sup>30</sup>

*Z*-Azobenzene can in addition to undergoing photoisomerisation also isomerise back to the thermodynamically stable *E*-isomeric form via a thermal non-photochemical process, and this occurs with a half-life ( $t_{1/2}$ ) of approximately 2 days at room temperature (rt).<sup>28</sup>

These crucial properties of azobenzene—the completeness of photochemical switching (PSD) and thermal *Z*-isomer stability ( $t_{1/2}$ )—can be tuned via structural modifications. Prominently, the substitution with tetra-*ortho*-fluorines results in a selective hypsochromic shift (lower  $\lambda$ ) of the *Z*-isomer  $n\text{-}\pi^*$  transition (due to lowering of the  $n$ -orbital energy) and thus a significant separation between the bands of the two isomers (**Figure 1.3D**).<sup>27</sup> This confers substantial improvement to the *E*-to-*Z* photoconversion with a reported PSD of 91% *Z* at  $> 450$  nm.<sup>27</sup> The substitution with  $\pi$ -electron-donating substituents increases the electron density in the  $\pi^*$  orbital and reduces the bond order of the N=N double bond, whereas  $\pi$ -electron withdrawing substituents stabilises the transition state for the thermal isomerisation—in both cases, this leads to a decrease in the thermal isomerisation energy barrier and an increase in the back-conversion rate.<sup>31–33</sup> Crucially, the substitution with the  $\sigma$ -electron withdrawing tetra-*ortho*-fluorines causes a stabilisation of the *Z*-isomer ground state, resulting in an exceptional thermal half-life of 700 days at rt in the dark.<sup>27</sup>

### 1.3.2. Azoheteroarenes

Beyond substitutions, the replacement of one or more the benzene rings with heterocyclic motifs has been extensively explored and given rise to a diverse range of new photoswitches with remarkable properties.<sup>29</sup> 5- and 6-membered *N*-heterocyclic rings have especially been investigated and include pyridines,<sup>34</sup> pyrazoles,<sup>35–37</sup> imidazoles,<sup>38–40</sup> triazoles,<sup>41, 42</sup> and oxazoles amongst others.<sup>43</sup> In all cases, photochromism is preserved, but the introduction of one or more heteroatoms in the azo system can significantly perturb its electronics, its structural geometry, and photochemical properties.<sup>29</sup>

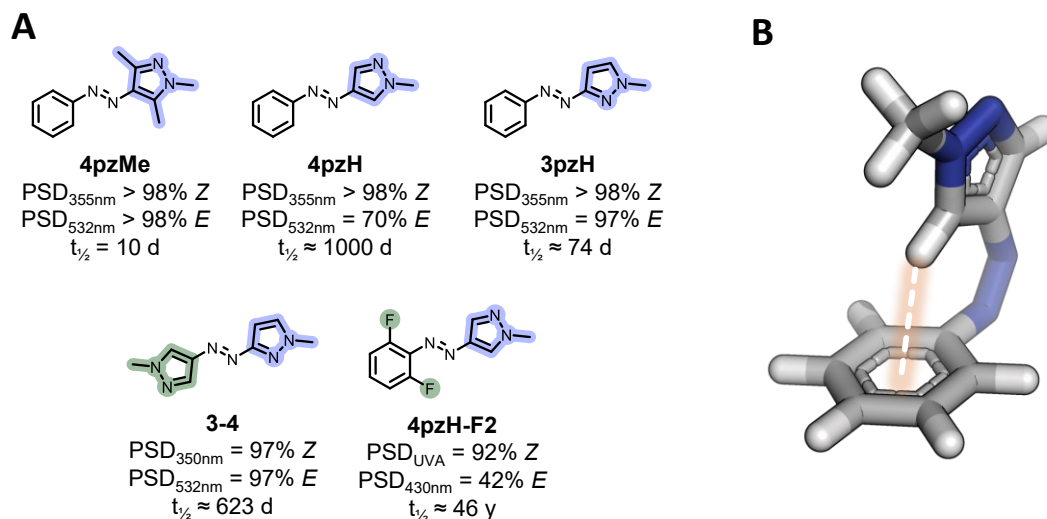


**Figure 1.4. Structure and properties of azopyrazoles.** (A) Representative examples of azopyrazoles: **4pzMe**,<sup>35</sup> **4pzH**,<sup>35</sup> **3pzH**,<sup>36</sup> **3-4**,<sup>37</sup> and **4pzH-F2**.<sup>44</sup> (B) Illustration of the T-shaped Z-isomer conformation accessed by certain 5-membered azoheteroarenes featuring an intramolecular CH– $\pi$  aromatic interaction (indicated with an orange stippled line).<sup>36</sup> The conformation was generated from UFF optimisation using Avogadro.<sup>45,46</sup> Created with PyMOL (version 2.5.4).<sup>47</sup>

Of these, the class of arylazopyrazoles have especially attracted attention and currently constitute one of the most studied and applied azoheteroaryl systems (**Figure 1.4A**).<sup>29</sup> In one aspect, some members of this family offer excellent band separation between their constituent *E*- and *Z*-isomers, resulting in quantitative bidirectional photoswitching—with **4pzMe** being a noticeable example (PSD > 98% in both directions).<sup>35</sup> In another aspect, arylazopyrazoles can also offer exceptionally long *Z*-isomer thermal stabilities, e.g., with the compound **4pzH** exhibiting a thermal half-life of 2.7 years.<sup>35</sup> The significant stability demonstrated by some arylazopyrazoles and other 5-membered azoheteroarenes has been attributed to their adoption of a T-shaped *Z*-isomer conformation, involving an intramolecular CH– $\pi$  aromatic interaction, which stabilises the *Z*-isomer ground state (**Figure 1.4B**).<sup>36</sup> This geometry cannot be accessed by azobenzene and its 6-membered ring derivatives, making it a unique structural property for 5-membered azoheteroarenes.<sup>28</sup> Further structural modifications to these early pioneering arylazopyrazole designs have furnished photoswitches which integrate both near-quantitative switchability as well as very long thermal half-lives (e.g., **3pzH** and **3-4**) or which show extremely high *Z*-isomer stabilities (e.g. **4pzH-F2** with a t<sub>1/2</sub>  $\approx$  46 years).<sup>36, 37, 44</sup> Together, azoheteroarenes present impressive photoswitching performance and a high degree of tunability in their properties, enabling tailoring for specific application endpoints. Accordingly, they have been increasingly explored as photoswitch motifs in photopharmacology.<sup>48</sup>

## 1.4. Development of photopharmacological agents

To develop an efficient photopharmacological agent, the above reviewed photochemical properties of the photoswitch needs to be integrated with all the properties related to their biological target engagement. This is not a trivial undertaking, and clearly, the success of such endeavour will rely significantly on the development and design approach taken. Consequently, there has been a growing interest in determining the most effective strategies for constructing these agents.<sup>49</sup>

### 1.4.1. Photopharmacological parameters

Prior to delving into these strategies, it is essential to first define the criteria by which we evaluate the effectiveness of photopharmacological agents.

Firstly, similarly to conventional (“static”) chemical probes and drug candidates, we still rely on the agent possessing a strong target engagement to elicit its biological activity in its activated/“on” state. We can quantify the target engagement either by the ligand-target dissociation constant ( $K_D$ ) or by the potency of the functional target modulation ( $IC_{50}/EC_{50}$ ) (**Figure 1.5A**). Using the criteria set by the Structural Genomics Consortium (SGC) for high-quality chemical probes, an *in vitro* target affinity/potency of < 100 nM and a cellular on-target activity at 1  $\mu$ M must be demonstrated.<sup>50</sup> The SGC further sets criteria for selectivity (> 30-fold selectivity over other related targets), but we may be more lenient with our photopharmacological agent, since its selectivity can here (at least in part) derive from its light-addressability.

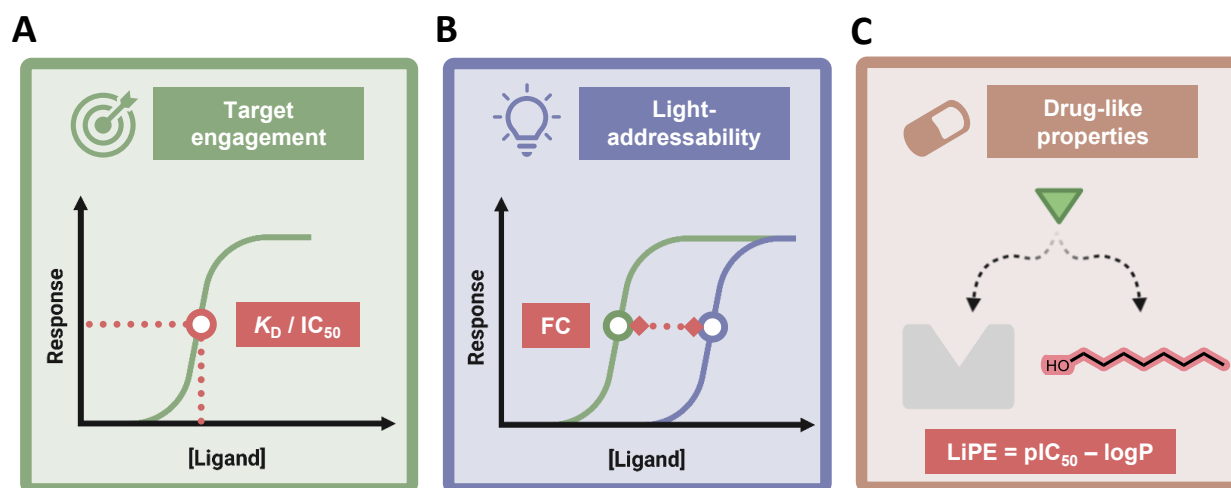


**Figure 1.5. The crucial properties of photopharmacological agents.** (A) Target engagement measured by the dissociation constant from the target ( $K_D$ ) or the half-maximum inhibition concentration ( $IC_{50}$ ). (B) Light addressability as measured by the fold change (FC) in activity upon photoswitching. (C) Drug-like physicochemical properties as measured by the lipophilicity efficiency (LiPE). Created with BioRender.com.

Secondly, it is imperative that the activity of the photopharmacological agents be highly controllable by light. Ideally, we aspire to a scenario where the agent exhibits high potency in one state (in alignment with the aforementioned criterion) and complete inactivity in the other state—thus achieving an “on/off”-like control over its biological activity. We can quantify this light-responsiveness as the fold change (FC) in activity observed upon photoswitching between the two isomeric states (**Figure 1.5B**). It is noteworthy that the apparent FC will be a composite of various factors, including the inherent activities of the *E*- and *Z*-isomers, the completeness of switching at the particular wavelength of light utilised, and the degree of thermal back-isomerisation that has occurred from the point of light irradiation to the point of measurement.

Thirdly, other drug-related properties need to be considered as well in order for the agent to be adaptable to biological (*in vivo*) settings, including its drug metabolism and pharmacokinetics (DMPK) profile.<sup>22,48</sup> One crucial parameter that has garnered increasing attention in drug discovery endeavours is the lipophilicity of the compound—this property does not only influence target affinity and selectivity but also significantly impacts solubility, permeability, and metabolic liability.<sup>51</sup> Recognising the strong correlation between lipophilicity and potency, the concept of lipophilicity efficiency (LiPE) has been introduced (**Figure 5C**).<sup>51</sup> This metric scales the compound potency to its lipophilicity ( $\text{LiPE} = -\log(K_D \text{ or } \text{IC}_{50}/\text{EC}_{50}) - \log P$ ) and is increasingly employed in drug discovery optimisation campaigns to constrain the molecular obesity of lead compounds.

#### 1.4.2. Canonical design of photopharmacological agents

With the aforementioned criteria in mind, let's now examine the strategies that researchers are employing to design and develop photopharmacological agents.

Currently, the canonical approach to discovering photoswitchable bioactive ligands relies on transforming a known drug molecule or advanced lead compound into a photoswitchable ligand in a late-stage fashion (**Figure 1.6A**).<sup>49</sup> Arguably, this has been most widely achieved through a ligand-based approach, where the parent ligand structure and knowledge on its structure-activity relationships (SAR) are employed as the sole basis for the structural transformation. The approach has first manifested in a structural modification coined “azologisation”.<sup>52</sup> Analogous to scaffold hopping, a substructure consisting of two ring-systems connected via a two-atom linker is identified in the drug molecule and replaced by an azo motif.<sup>52, 53</sup> Several such substructures have been shown to be replaceable (i.e., be isosteric) with azo(hetero)arenes, including stilbenes, diarylethanes, *N*-benzyl arylamines, benzyl aryl (thio)ethers, *N*-arylbenzamides, and biarylsulfonamides.<sup>49</sup> As an alternative structural modification, the drug molecule has

been extended with an azo motif (so called “azo extension”), with a preexisting (hetero)aryl ring becoming part of the new azo(hetero)aryl system or with the whole azo motif being appended onto the periphery of the parent ligand structure via a linker.<sup>49</sup>

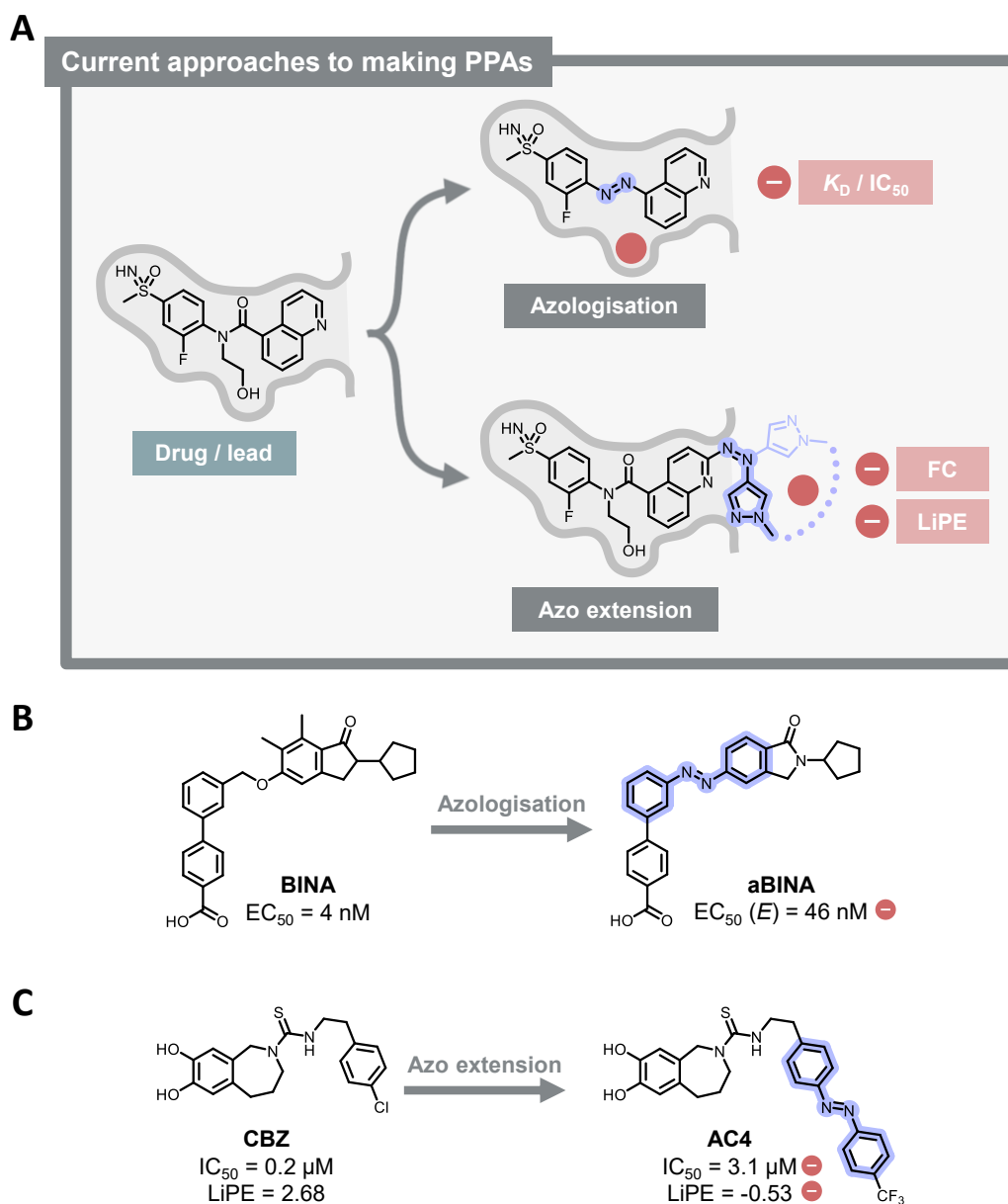


**Figure 1.6. Current approaches to making photopharmacological agents.** (A) Schematic illustration of late-stage azo transformation strategies for making photopharmacological agents (PPAs) and their potential limitations. (B) Example of an azologisation programme.<sup>54</sup> (C) Example of an azo extension programme.<sup>55</sup>

Employing these methodologies, numerous ligands exhibiting reversible light-responsive target activities have been disclosed to date.<sup>22, 49</sup> Nonetheless, these structural alterations to the parent ligand

are not without drawbacks, and many of the reported photopharmacological agents can arguably not be considered optimal endpoints. In a majority of cases, azologisation results in a disruption of the original ligand binding mode with a concomitant decrease in target affinity and potency.<sup>12, 49</sup> For instance, Donthamsetti *et al.* conducted azologisation on the well-known and potent metabotropic glutamate receptor 2 (mGluR2) agonist, **BINA** ( $EC_{50}$  = 4 nM), resulting in a photoswitchable ligand, **aBINA**, which displayed an  $EC_{50}$  of 46 nM in its active state—this corresponds to a decrease in potency by more than one order of magnitude (**Figure 1.6B**).<sup>54</sup> Conversely, azo extension on advanced lead molecules often yields excessively large and lipophilic molecules, raising concerns regarding their suitability for translational drug discovery. For instance, azo extension on the established vanilloid receptor 1 (TRPV1) antagonist **CBZ** ( $IC_{50}$  = 0.2  $\mu$ M,  $LiPE$  = 2.68) produced the photoswitchable ligand **AC4** demonstrating a  $LiPE$  = -0.53 ( $IC_{50}$  = 3.1  $\mu$ M) (**Figure 1.6C**).<sup>55</sup> The negative lipophilic efficiency indicates that the azo transformation constituted a highly unfavourable structural modification, disproportionately affecting the compound lipophilicity compared to its potency.<sup>51</sup>

More recently, researchers have progressed beyond merely relying on 2D structural similarity for azo transformations, transitioning towards more rational structure-guided approaches that take into account the 3D conformation and binding mode of the parent ligand.<sup>49</sup> However, one might still question whether the fundamental approach of utilising a pre-optimised advanced molecule as the foundation for photoswitchable ligand design constitutes the optimal strategy. As recognised by the photopharmacology community, this late-stage modification will almost inevitably compromise the pre-optimised parameters, including biological target engagement and physicochemical properties relevant to drug development.<sup>12,</sup>

49

Rather than considering the photoswitch motif as an add-on feature that must be integrated toward the end of the optimisation process for the photopharmacological agent, we pondered whether a better strategy would be to implement the photoswitch motif earlier on as a core part of the ligand structure.

### 1.4.3. Fragment-based drug discovery

Over the past decades, fragment-based drug discovery (FBDD) has matured into a well-established and powerful methodology for interrogating biological macromolecules and for developing drug-like chemical matter.<sup>56</sup> Indeed, FBDD has as of present furnished seven FDA-approved drugs and numerous clinical candidates, underscoring the significant utility of this approach.<sup>57</sup>



The fundamental principle behind FBDD is to screen smaller and structurally less complex molecules, called fragments (**Figure 1.7A**).<sup>58</sup> In an attempt to confine the property space of these molecules, fragments have classically been defined using the “rule of 3” (Ro3), stating that they should have a molecular weight (MW) < 300 Da, H bond donor (HBD) count ≤ 3, H bond acceptor (HBA) count ≤ 3, and cLogP ≤ 3.<sup>59</sup> However, it is important to note that these criteria are merely guidelines and have been rightfully challenged.<sup>60, 61</sup> Indeed, there has been a gradual movement towards screening even smaller molecules in recent years, with some companies applying a MW cutoff of 230 Da, for instance.<sup>56, 62</sup>

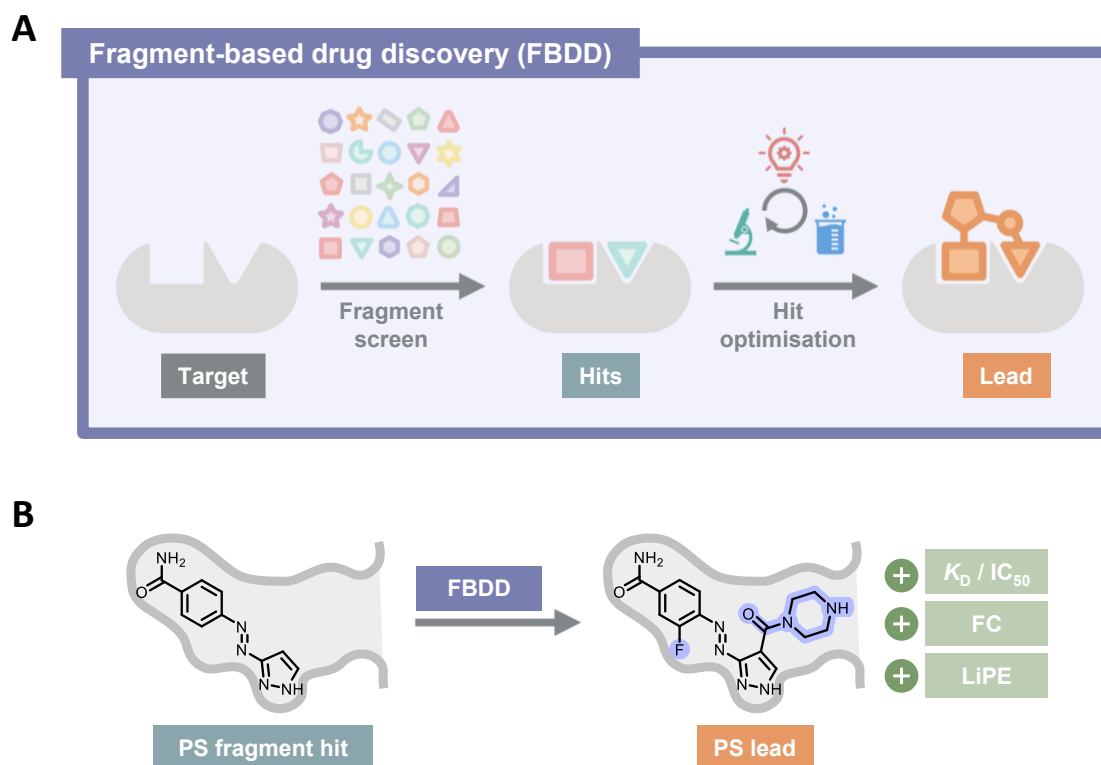


**Figure 1.7. A fragment-based approach.** (A) General workflow of FBDD. Created with BioRender.com. (B) Adaption of FBDD to the development of photoswitchable (PS) ligands.

The shift towards screening smaller molecules has several notable implications. Firstly, smaller molecules sample chemical space more efficiently, and the probability of finding hit molecules with the requisite features for probing the target interaction surface increases.<sup>60, 63</sup> Consequently, fragment screening generally yields higher hit rates and requires smaller-sized compound libraries compared with high-throughput screening (HTS) methods. Most successful FBDD campaigns have employed fragment libraries consisting of 1000–2000 members.<sup>64, 65</sup>

Secondly, given that fragments have fewer atoms at disposal to bind their target with, they usually display weak binding affinities with  $K_D$  values in the  $\mu\text{M}$ – $\text{mM}$  range.<sup>65</sup> However, fragment hits generally feature high-quality interactions with the target, since they must overcome roughly the same translational and rotational entropic barrier as larger-sized molecules.<sup>66</sup> The hits therefore often have high ligand efficiencies (LE, defined as the Gibbs free energy of binding divided by the heavy atom count), which is frequently employed as a metric to assess the size-scaled affinity or potency of fragments hits and of their analogues during optimisation.<sup>67</sup>

Thirdly, the fragment hits require extensive optimisation, which constitutes the most challenging aspect of the FBDD campaign—yet it is also at this stage where the power of leveraging fragments is fully manifested.<sup>56,58</sup> Fragment hit optimisation has been conducted via linking, merging, or growing strategies, and in a majority of instances, this process is guided heavily by a detailed structural understanding of the ligand-target binding mode.<sup>56</sup> Crucially, commencing the optimisation phase with a small and non-complex structural starting point offers more space and flexibility for enhancing and balancing the multiple drug-relevant properties.<sup>58</sup> Careful elaboration of fragment hits can therefore furnish smaller and more drug-like endpoints.<sup>56</sup>

We hypothesised that FBDD could serve as a novel and effective approach for developing photopharmacological agents (**Figure 1.7B**). By initiating the photopharmacology campaign with a small photoswitchable fragment starting point, the light-addressability can be optimised in tandem with target engagement and drug-like physicochemical properties en route to the lead compound. This approach can possibly enable more effective multi-parameter optimisation compared to the canonical late-stage azo transformation approach—thus potentially yielding more balanced and efficient photopharmacological agents.

## 1.5. Aims and objectives

### 1.5.1. Background

Photopharmacology using photoswitchable molecules has emerged as a promising light-responsive modality for potentially addressing the fundamental problem of selectivity faced by current drugs and chemical probes. While significant progress has been made in photopharmacological research, most studies remain at the proof-of-concept stage. One of the critical challenges is integrating light addressability and photophysical properties with the numerous properties relevant for drug development in a single

molecule. We believe solving this problem will be crucial for accelerating the practical use and translational development of photopharmacological agents. Currently, the canonical approach for constructing these agents involves late-stage azo transformation of established drugs or lead compounds. However, this approach often compromises pre-optimised parameters, leading to low target engagement or poor drug-likeness. FBDD offers an efficient and elegant alternative, emphasising design over brute-force screening. We hypothesise that this philosophy may be especially useful for the development of photopharmacological agents.

### **1.5.2. Aims**

The overarching aim of the research is to investigate the prospect of applying FBDD to develop photopharmacological agents. Based on these research findings, we seek to establish a generally applicable workflow tailored towards developing photoswitchable ligands against any biological target of interest.

### **1.5.3. Objectives**

To achieve these aims, we plan to adopt an empirical approach and conduct FBDD campaigns towards photoswitchable azo ligands against two–three different disease-relevant targets. The results from these campaigns will allow us to evaluate the merit and broader applicability of the fragment-based methodology.

To this end, the following specific objectives have been set for the research project:

- A. Determine and evaluate strategies for identifying photoswitchable azo fragment hits, including screening a library of photoswitchable fragments and screening general fragments followed by early-stage azo transformation.
- B. Establish assays for assessing the target binding affinity and/or functional activity of fragments and analogues, validating these using suitable control compounds.
- C. Optimise the fragment hits through structure-based, computer-aided, and ligand-based design strategies. Design and synthesise analogues and track their photochemical performance using UV–Vis spectroscopy, their absolute and differential biological activity using aforementioned assays and their drug-likeness using LiPE as a metric.
- D. To assess the endpoints from the campaigns, we apply chemical probe criteria but with some leeway and aim for an affinity/potency of  $K_D/IC_{50} \leq 1 \mu M$ . In terms of differential activity, we consider an  $FC \geq 10$  to be desired. When possible, we further aim to develop comparator compounds using late-stage azo transformation on established compounds to serve as a benchmark.

- E. Formulate the empirical findings from the FBDD campaigns into concrete guidelines for applying fragments to identify photoswitchable ligands.

This research has the potential to unveil a novel methodology for developing photopharmacological agents, offering the possibility of accessing more efficient and more drug-like chemical matter. Such advancements could broaden their applications in chemical biology and expedite their translation into clinically relevant entities.

## 2. Photoswitchable ligands from general fragments

### 2.1. Introduction

#### 2.1.1. A photoswitchable fragment library

Other members of our research group commenced the study by assembling a library of fragment-like azo photoswitches, as this emerged as the most obvious and direct route towards a photoswitchable fragment starting point. The compounds were acquired from our in-house collection of photoswitches and were therefore not designed de novo. Structurally, these compounds mostly consisted of well-studied azoheteroarene scaffolds optionally decorated with a limited number of functional groups (**Appendix Figure A1**). In the selection of library members, the Ro3 was employed for limiting the size and complexity of the molecules but with room for some divergence, including allowing for a larger number of HBA in accordance with the trend in FBDD literature (**Appendix Table A1**).<sup>65</sup> In total, the group was able to assemble a library of 35 compounds.

As a case study, our research group was interested in targeting fibrillarin—a methyltransferase enzyme which has been implicated in various cancers and emerged as a potentially promising therapeutic target against paramyxovirus infections.<sup>68, 69</sup> However, upon screening the photoswitchable fragment library against fibrillarin using a surface plasmon resonance (SPR) assay, our collaborators at CSIRO did not obtain any hits (data not shown).

#### 2.1.2. The challenges of obtaining a photoswitchable fragment hit

Upon obtaining the above negative results with the photoswitchable fragment library, we considered the possible factors. Firstly, the result may not be surprising given the relatively small library size compared with conventional fragment screening campaigns (in the order of thousand compounds).<sup>70</sup> This is a direct consequence of the logistic problem of accessing such a large-sized azo fragment library. To our awareness, fragment libraries enriched with azo frameworks are not commercially available through common chemical vendors. One could assemble a custom-build library through synthesis or commercial acquisition, but herein arises a second and more inherent challenge. It might be expected that a library of core azo photoswitch frameworks will not efficiently cover chemical space in terms of molecular shape diversity, as they will all (by definition) include two constituent ring systems connected via a two-atom linker (**Figure 2.1A**). Indeed, an analysis of 489 published co-crystal structures, revealed that such framework only constitutes 3% of fragment hits.<sup>71</sup> The low sampling of chemical space by these scaffolds will

inevitably yield a lower hit rate. To create more diversity, one could certainly functionalise the azo scaffolds with substituents and/or extend their carbon framework (**Figure 2.1B**). However, these decorations pose the risk that the molecular size and complexity will increase to the extent that the compounds will diverge from fragment-like property space.

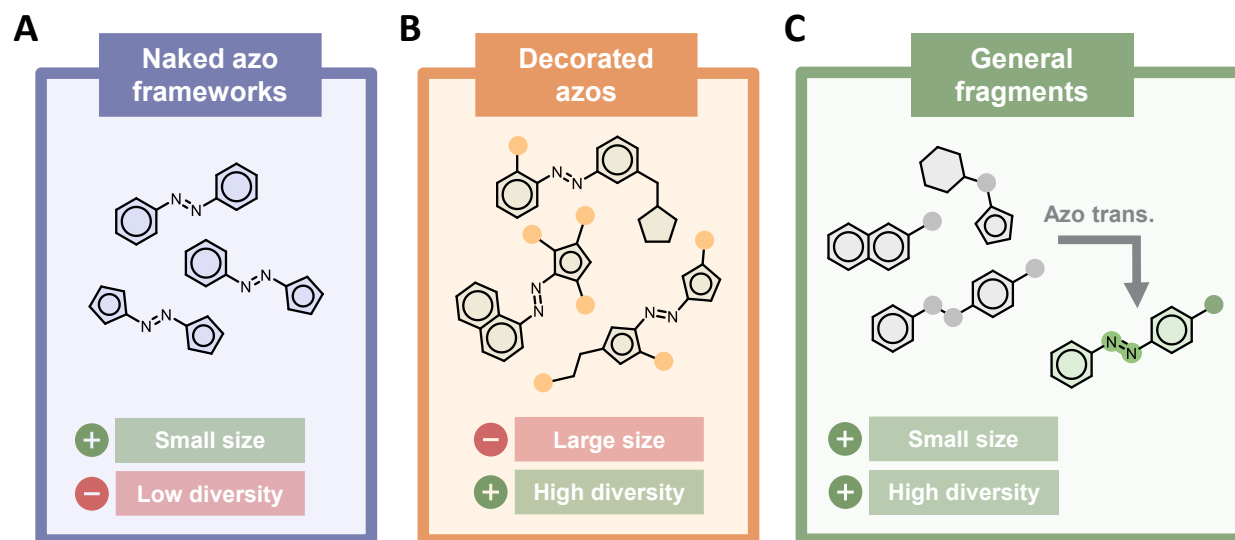


**Figure 2.1. The challenges of screening photoswitchable azo fragment libraries.** (A) A library of naked azo frameworks would possess low diversity. (B) A library of decorated azo scaffolds may suffer from too large compound size/complexity. (C) A general fragment library possesses low complexity and high diversity.

To circumvent these challenges, one solution lies in screening a general fragment library. These are accessible via common chemical vendors and will typically include compounds in the order of hundreds to thousands. They generally also integrate a lot of shape and functional group diversity whilst retaining a low molecular complexity (as they are not limited to two ring systems connected with a two-atom linker).<sup>65</sup> These libraries will not be enriched with azo scaffolds (and might not contain any azo compounds at all). However, through either azologisation or azo extension, one could transform the resulting fragment hit into a photoswitchable molecule at an early stage. This process may still harvest the flexibility and efficiency of a fragment-based approach, whilst circumventing the logistical and diversity issues of screening a photoswitchable fragment library (**Figure 2.1C**).

In this project, we explored a general fragment screening approach on the methyltransferase target SARS-CoV-2 NSP16/10.

### 2.1.3. SARS-CoV-2

In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged and rapidly spread across the world causing the coronavirus disease pandemic (COVID-19); as of February 2024, this has resulted in more than 700 million confirmed cases and 7 million associated deaths globally.<sup>72</sup> Although COVID-19 vaccines have caused significant reduction in morbidity and mortality, they are challenged by waning immunity and the emergence of resistant SARS-CoV-2 variants.<sup>73-75</sup> To address these issues, there is currently an urgent demand for the development of antiviral drugs to use as an orthogonal strategy for combatting COVID-19.<sup>76</sup> To date, two drugs have been FDA approved for emergency use against COVID-19: Paxlovid, which consists of the main protease ( $M^{Pro}$ ) inhibitor nirmatrelvir; and Lagevrio, consisting of the nucleoside analogue molnupiravir. Despite these measures, a panel of drugs with diverse modes of action is necessary to ensure the strongest armament against COVID-19 and related viruses, given their large mutation capacity, as well as to protect against future pandemics.<sup>77</sup>

### 2.1.4. NSP16/10

Several encoded viral proteins have been identified as potential targets for therapeutic intervention against SARS-CoV-2, including  $M^{Pro}$ , the spike (S) protein and the RNA-dependent RNA polymerase (RdRp).<sup>78</sup> The 2'-O-methyltransferase (MTase) NSP16 plays a critical part of the viral mRNA capping machinery that ensures RNA stability, enhanced translation and evasion of the host innate immune response.<sup>79</sup> Specifically it catalyses the final step of the capping pathway, in which viral mRNA in the cap0 (m7GpppA-RNA) state is converted to the cap1 (m7GpppAm-RNA) state via 2'-O-methylation (**Figure 2.2A**).<sup>80, 81</sup> For this purpose, NSP16 employs the co-factor S-adenosyl-L-methionine (SAM) as its methyl donor and further forms a heterodimeric complex with NSP10 to stabilise the binding to both SAM and the cap0 RNA substrate, thus enhancing its catalytic activity (**Figure 2.2B-D**).<sup>81, 82</sup> Crucially, studies on the related SARS-CoV-1 have shown that loss-of-function mutations of the NSP16 gene leads to significant viral attenuation both *in vitro* and *in vivo*.<sup>83</sup> Thus, NSP16 has been proposed as a promising target for the development of antiviral drugs against SARS-CoV-2.<sup>84, 85</sup> Importantly, analysis of > 10 million SARS-CoV-2 genome sequence samples showed no mutations in > 90% of NSP16 regions, and targeting NSP16 may therefore be associated with lower resistance development compared with the popular targets,  $M^{Pro}$  and S proteins, where the highest mutation frequency have been observed.<sup>86</sup> In addition, a recent study has identified a cryptic binding pocket in NSP16, conserved across multiple coronaviruses (including SARS-CoV1 and MERS) but not found in the human homologue CMTr1, which presents a potential opportunity for the development of safe antiviral treatments across multiple coronaviruses by targeting NSP16.<sup>87</sup>

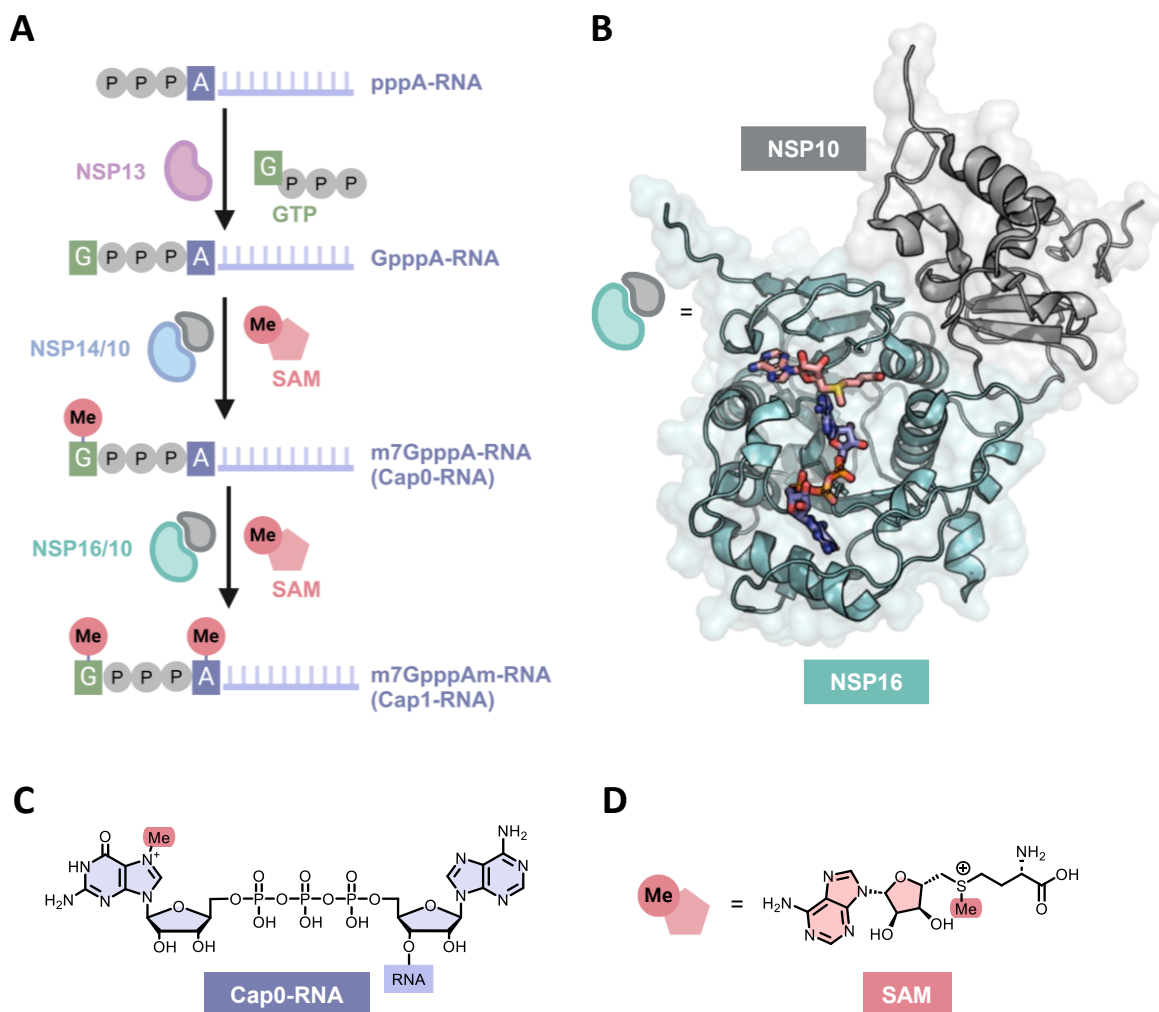


**Figure 2.2. The biology and structure of NSP16/10.** (A) The mRNA capping pathway employed by SARS-CoV-2. The final step is catalysed by NSP16/10. Created with BioRender.com. (B) Structure of NSP16/10 in complex with the co-factor SAM (pink sticks) and the cap0-RNA analogue **m7GpppA** (purple sticks) (PDB ID 6WKS).<sup>88</sup> Created with PyMOL (version 2.5.4).<sup>47</sup> (C) Structure of cap0-RNA, the substrate for NSP16/10. (D) Structure of SAM, the co-factor for NSP16/10.

Despite the therapeutic promises, NSP16/10 target validation using pharmacological inhibition has not yet been conducted, which is presumable due to the lack of suitable high quality and high potency chemical probes.

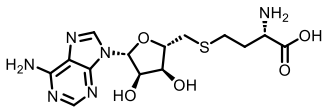
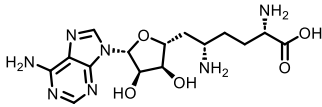
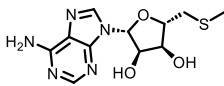
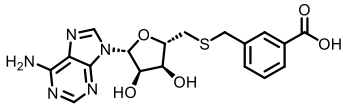
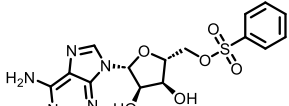
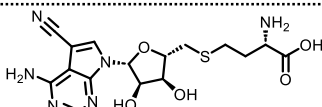
### 2.1.5. Reported NSP16/10 inhibitors

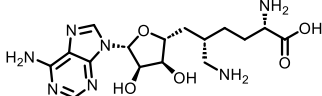
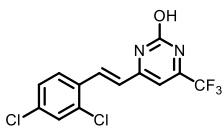
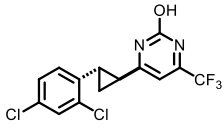
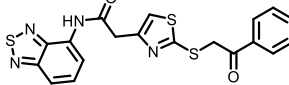
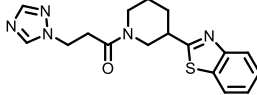
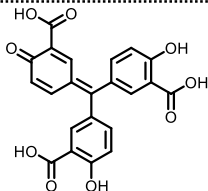
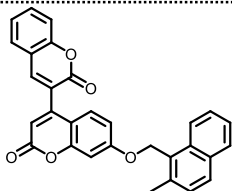
Currently, most reported inhibitors of NSP16/10 are SAM-analogues, which—although exhibiting moderate to good biochemical potencies—lack selectivity over non-viral human MTases and/or show no cellular activities due to poor permeabilities (**Table 2.1**).<sup>89</sup> These are direct consequences of the co-factor binding site being generally conserved across SAM-dependent MTases and its highly hydrophilic nature.<sup>90</sup>



Recently, non-SAM mimicking NSP16/10 inhibitors have also been disclosed, some of which do not engage the SAM binding site. Of note, Inniss *et al.* reported the covalent inhibitor **5a**, which was shown to bind a cryptic pocket adjacent to the SAM binding site in a fashion that disrupts and prevents co-factor binding.<sup>91</sup> However, both **5a** and the non-covalent analogue **5i** exhibited cytotoxicity against human cell lines, making them unsuitable as lead compounds for further development.<sup>91</sup> Bobrovs *et al.* conducted a high-throughput virtual screen of the ZINC15 library, which identified two hits, **ZINC38661771** and **ZINC23398144**, both shown to inhibit NSP16/10 in a biochemical assay.<sup>92</sup> However, both displayed rather weak potencies and low selectivity for NSP16/10 over the related NSP14/10 and the abundant human glycine *N*-methyltransferase (hGNMT).<sup>92</sup> Other compounds (Aurintricarboxylic acid and **ZINC2121012**) display promising biochemical potencies but present poor drug-like features (multiple charged motifs and high lipophilicity).<sup>80, 93</sup>

**Table 2.1. Structure and activity of reported NSP16/10 inhibitors. <sup>a</sup>**

| Cmpd ID                                | Structure                                                                           | Biological activity (assay)                                                                                                                                                                                                                                                                                                                                                  | PDB ID |
|----------------------------------------|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|
| <b>S-Adenosyl-L-homocysteine (SAH)</b> |   | $K_D$ (NSP16/10) = 1.2 $\mu$ M (SPR, in-house)<br>$IC_{50}$ (NSP16/10) = 0.28 $\mu$ M (HTRF assay) <sup>92</sup><br>$IC_{50}$ (NSP16/10) = 5.9 $\mu$ M (radiometric assay) <sup>94</sup><br>$K_D$ (NSP14/10) = 0.020 $\mu$ M (SPR, in-house)<br>$IC_{50}$ (NSP14/10) = 0.14 $\mu$ M (HTRF assay) <sup>92</sup><br>$IC_{50}$ (hGNMT) = 1.7 $\mu$ M (HTRF assay) <sup>92</sup> | 6WJT   |
| <b>Sinefungin (SFN)</b>                |  | $K_D$ (NSP16/10) = 5.5 $\mu$ M (SPR, in-house)<br>$IC_{50}$ (NSP16/10) = 0.86 $\mu$ M (HTRF assay) <sup>89</sup><br>$K_D$ (NSP14/10) = 1.7 $\mu$ M (SPR, in-house)<br>$IC_{50}$ (NSP14/10) = 0.45 $\mu$ M (HTRF assay) <sup>89</sup><br>$IC_{50}$ (hGNMT) = 316 $\mu$ M (HTRF assay) <sup>89</sup>                                                                           | 6WKQ   |
| <b>5'-Methylthioadenosine (MTA)</b>    |  | $K_D$ (NSP16/10) = 2.4 $\mu$ M (SPR, in-house)<br>$IC_{50}$ (NSP16/10) = 1.0 $\mu$ M (HTRF assay) <sup>92</sup><br>$IC_{50}$ (NSP14/10) = 1.4 $\mu$ M (HTRF assay) <sup>92</sup><br>$IC_{50}$ (hGNMT) = 1.8 $\mu$ M (HTRF assay) <sup>92</sup>                                                                                                                               | 8C5M   |
| <b>2a</b>                              |  | $IC_{50}$ (NSP16/10) = 4.0 nM (HTRF assay) <sup>89</sup><br>$IC_{50}$ (NSP14/10) = 8.0 nM (HTRF assay) <sup>89</sup><br>$IC_{50}$ (hGNMT) = 8.6 nM (HTRF assay) <sup>89</sup>                                                                                                                                                                                                | -      |
| <b>ZINC3861767</b>                     |  | $IC_{50}$ (NSP16/10) = 1.1 $\mu$ M (HTRF assay) <sup>92</sup><br>$IC_{50}$ (NSP14/10) = 1.5 $\mu$ M (HTRF assay) <sup>92</sup><br>$IC_{50}$ (hGNMT) = 2.8 $\mu$ M (HTRF assay) <sup>92</sup>                                                                                                                                                                                 | -      |
| <b>SS148</b>                           |  | $IC_{50}$ (NSP16/10) = 1.0–1.7 $\mu$ M (radiometric assay) <sup>93, 95</sup>                                                                                                                                                                                                                                                                                                 | 7R1T   |

|                                      |                                                                                     |                                                                                                                                                                                                      |               |
|--------------------------------------|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| <b>WZ16</b>                          |    | IC <sub>50</sub> (NSP16/10) = 2.5–4.6 μM (radiometric assay) <sup>93, 95</sup>                                                                                                                       | 7R1U          |
| <b>5a</b>                            |    | K <sub>inact</sub> /K <sub>i</sub> (NSP16/10) = 4.6 μM <sup>-1</sup> min <sup>-1</sup> (radiometric assay) <sup>91</sup><br>IC <sub>50</sub> (NSP16/10) = 9–100 μM (radiometric assay) <sup>91</sup> | 8F4S,<br>8F4Y |
| <b>5i</b>                            |    | IC <sub>50</sub> (NSP16/10) = 35–88 μM (radiometric assay) <sup>91</sup>                                                                                                                             | -             |
| <b>ZINC38661771</b>                  |    | IC <sub>50</sub> (NSP16/10) = 51 μM (HTRF assay) <sup>92</sup><br>IC <sub>50</sub> (NSP14/10) = 118 μM (HTRF assay) <sup>92</sup><br>IC <sub>50</sub> (hGNMT) = 58 μM (HTRF assay) <sup>92</sup>     | -             |
| <b>ZINC23398144</b>                  |    | IC <sub>50</sub> (NSP16/10) = 197 μM (HTRF assay) <sup>92</sup><br>IC <sub>50</sub> (NSP14/10) = 77 μM (HTRF assay) <sup>92</sup><br>IC <sub>50</sub> (hGNMT) = 315 μM (HTRF assay) <sup>92</sup>    | -             |
| <b>Aurintricarboxylic acid (ATA)</b> |   | IC <sub>50</sub> (NSP16/10) = 2.1 μM (radiometric assay) <sup>80</sup><br>IC <sub>50</sub> (NSP14/10) = 6.4 μM (radiometric assay) <sup>80</sup>                                                     | -             |
| <b>ZINC2121012</b>                   |  | K <sub>D</sub> (NSP16/10) = 23.1 μM (MST assay) <sup>93</sup>                                                                                                                                        | -             |

<sup>a</sup> Representative examples from each chemotype are presented. Compounds that have only been hypothesised to engage NSP16/10 based on molecular docking have not been included. SPR = surface plasmon resonance. HTRF = homogeneous time-resolved fluorescence. MST = microscale thermophoresis. In-house = unpublished data generated by collaborators at CSIRO.

## 2.2. The fragment hit

### 2.2.1. Screening of general fragment libraries

The commercial Maybridge Ro3-compliant fragment library of 1536 compounds as well as the internal CSIRO fragment library of 720 compounds were screened against NSP16/10 using a surface plasmon resonance (SPR) assay by our collaborators at CSIRO.<sup>96</sup> The 2256 compounds were screened at 100 μM, and primary hits giving top responses were subsequently assessed in multi-concentration format (top

concentration of 100  $\mu\text{M}$ , two-fold dilution, four points). From this screen, our collaborators obtained 40 fragment hits that showed detectable and dose-dependent binding responses (data not shown).

### 2.2.2. A biarylsulfonamide fragment hit

Compound **B1** emerged as the most promising hit (**Figure 2.3A**). Firstly, the data quality on its binding to NSP16/10 was the most robust, showing clear dose-responsiveness (data not shown). This amounted to a decent binding affinity of  $K_D = 350 \mu\text{M}$ , which given the heavy atom count (HAC) of 18 and lipophilicity ( $\text{clogP} = 2.07$ ) corresponded to an  $\text{LE} = 0.26 \text{ kcal/mol/HAC}$  and a  $\text{LiPE} = 1.39$ .

Structurally, **B1** is a biarylsulfonamide consisting of a left-hand unsubstituted benzene ring (LHAr) and a right-hand 4-amino substituted benzene ring (RHAr) spaced by an *N*-methylated sulfonamide linker. Synthetically, biarylsulfonamides can be readily accessed by reacting anilines with benzenesulfonyl halide building blocks—both widely commercially available (**Figure 2.3B**). To prevent chemoselectivity issues, the RHAr amino group would need to either be protected with a suitable protective group (e.g. a Boc-group) or be masked as a different functional group (e.g., a nitro group). The sulfonamide *N*-methyl group can also be installed by exploiting the acidity of the sulfonamide *NH* ( $\text{pK}_A \approx 8$ , predicted), which upon deprotonation will reveal a good nucleophile for *N*-alkylation reactions. Finally, the nucleophilicity of the deprotonated arylsulfonamides allows for a third disconnection strategy, where they can be utilised for *C-N* cross-couplings. Thus, **B1** appeared to be highly synthetically tractable with several robust synthetic pathways at disposal for accessing the fragment and its derivatives.

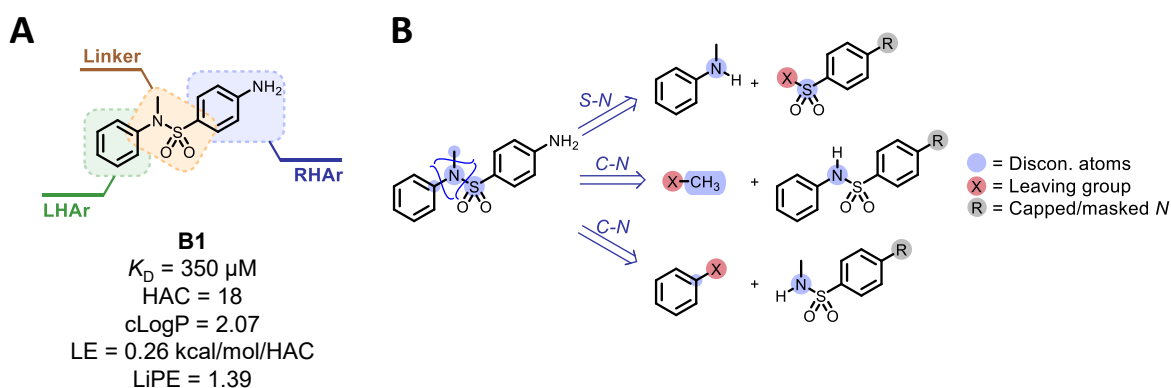
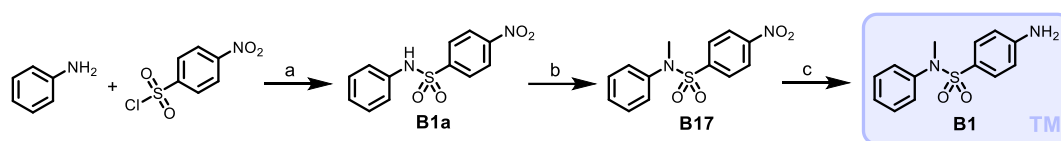


**Figure 2.3. The NSP16/10 fragment hit B1.** (A) Structure and activity of **B1**. LHAr = Left-hand aromatic ring. RHAr = Right-hand aromatic ring (B) Synthetic disconnection of **B1** into commercially available building blocks.

### 2.2.3. Synthesis and retest of B1

To confirm the activity of **B1**, the fragment hit was synthesised to allow for retesting and exclusion of any false positive activity arising from possible purity and/or identity problems of the originally screened library batch. The fragment hit was synthesised by first reacting aniline with 4-nitrobenzenesulfonyl chloride in the presence of pyridine, followed by *N*-methylation of the sulfonamide with methyl iodide and a final reduction of the nitro group by Pd/C-catalysed hydrogenation to reveal the amino group (**Scheme 2.1**). This gave the target molecule (TM) **B1** at an overall decent yield of 62% over three steps.

**Scheme 2.1. Synthesis of fragment hit B1.** <sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) Pyridine, DCM, reflux, 3 h, 65%. (b) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, rt, 5 h, 96%. (c) Pd/C, MeOH, H<sub>2</sub> (1 bar), rt, 18 h, quant. TM = Target molecule.

With the new batch of **B1** in hand, the fragment hit was retested in the SPR assay by our collaborators at CSIRO, employing **SFN** as the positive control (**Appendix Figure A2A–B**). Here, they could confirm target engagement for the fragment with a  $K_D \approx 200\text{--}300\ \mu\text{M}$  (**Appendix Figure A2C**).

## 2.3. Azologisation

### 2.3.1. Biarylsulfonamides as isosteres of Z-azobenzene

The fragment hit **B1** was further prioritised given the observation that biarylsulfonamides are known to preferentially adopt a staggered Gauche conformation, in which both the *N*-lone pair and the aryl *C* p-orbital bisect the sulfonyl *S–O* double bonds (**Figure 2.4A**).<sup>97</sup> This gives biarylsulfonamides a Z-like conformation, which shows high structural and electronic similarity with Z-azobenzenes based on modelling (**Figure 2.4B**).<sup>98</sup> The possibility of transforming the fragment hit into an azo photoswitch through replacement of the sulfonamide linker with a diazene was therefore recognised early on during hit prioritisation.

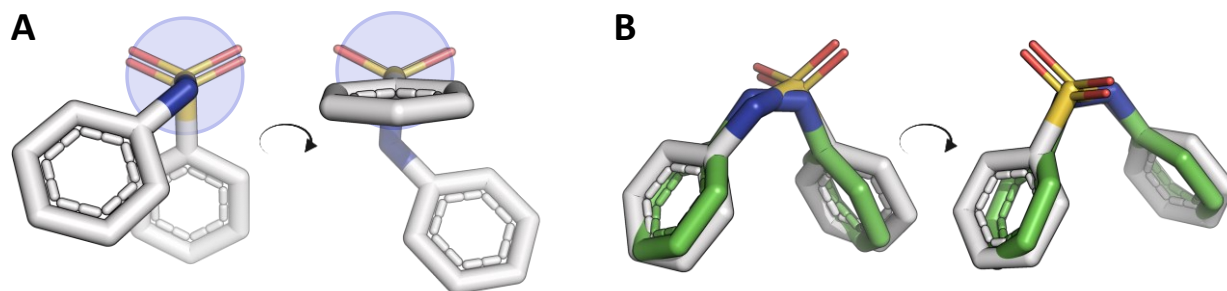


**Figure 2.4. The conformation of biarylsulfonamides.** (A) Illustration of the staggered conformation adopted by biarylsulfonamides. (B) The similarity of biarylsulfonamides with Z-azobenzene. The conformations were generated from MMFF94S optimisation using Avogadro.<sup>45, 46</sup> The conformations were aligned using LS-Align.<sup>99</sup> Created with PyMOL (version 2.5.4).<sup>47</sup>

### 2.3.2. Azologisation of B1

We proceeded to carry out this azologisation to obtain the analogue **B2**, and the sulfonamide linker was further exchanged with an amide to give analogue **B3** (**Figure 2.5**). In opposition to arylsulfonamides, arylamides are known to strongly favour a planar *E*-conformation,<sup>97</sup> so the latter transformation would be useful to probe the conformational preference of the fragment hit.

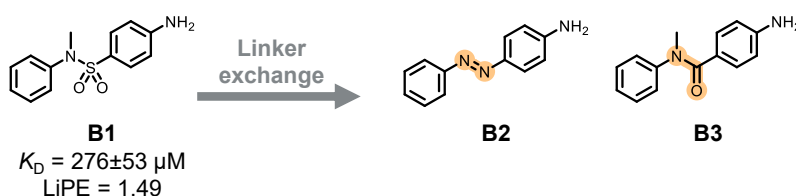
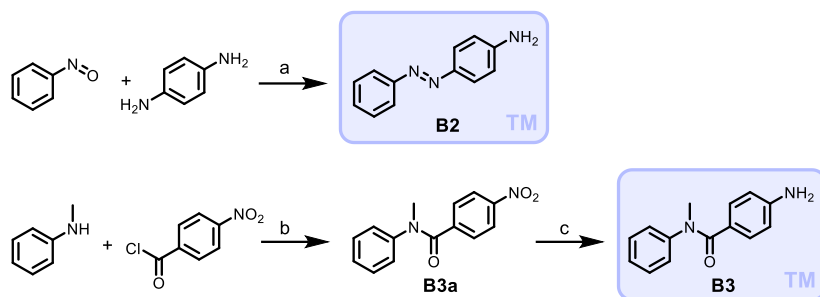


**Figure 2.5. Design of linker analogues of B1.** Structural changes from **B1** have been highlighted.

The azo analogue **B2** was readily synthesised in a single step via a previously reported acid-catalysed Bae-  
 yer-Mills reaction protocol (**Scheme 2.2**).<sup>100</sup> Here, 1,4-phenylenediamine was condensed with nitrosoben-  
 zene in acetic acid to afford the TM in 41% yield. The amide analogue **B3** was accessed by reacting *N*-  
 methylaniline with 4-nitrobenzoyl chloride followed by a nitro reduction via standard hydrogenation con-  
 ditions.

## Scheme 2.2. Synthesis of linker analogues **B2** and **B3**.<sup>a</sup>

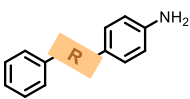


<sup>a</sup> Reagents and conditions: (a) AcOH, 40 °C, 16 h, 41%. (b) Et<sub>3</sub>N, DCM, 0 °C–rt, 44 h, 48%. (c) Pd/C, MeOH, H<sub>2</sub> (1 bar), rt, 6 h, 81%. TM = Target molecule.

Based on SPR, the azologue **B2** retained the binding affinity of **B1** with a calculated  $K_D = 301 \mu\text{M}$  (Table 2.2). However, no difference in its activity could be observed upon photoirradiation with 365 nm light. Photophysical characterisation of **B2** in the aqueous assay buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 10 w/v% glycerol, 0.05 v/v% Tween-20, 2% DMSO) revealed that this is likely due to an extremely low thermal half-life of the *Z*-isomer: no photoisomerisation could be observed by UV–Vis spectroscopy, suggesting that the photoswitch thermally relaxes back within the timescale of the measurement ( $\approx 5$  s) (Figure 2.6A). On the other hand, photoisomerisation could be detected when carrying out the experiment in 100% DMSO as the medium (Figure 2.6B). This is in agreement with the known poor stability of *NH*-substituted *Z*-azobenzenes in polar protic media, as they gain access to a hydrazone mechanism for the thermal back-isomerisation (Figure 2.6C).<sup>101</sup> Additionally, the electron-donating capacity of the amino group also reduces the bond order of the *N=N* double bond (c.f. section 1.3.1). As a result, only the *E*-isomer of **B2** has in practice been tested in the SPR assay, explaining the undifferentiated binding responses from the two experiments.

The retained activity of **B2** in its stable *E*-form might suggest that **B1** correspondingly binds in an extended *E*-like conformation (rather than the presumed staggered *Z*-like conformation). However, the corresponding amide analogue **B3** showed no activity. This suggests that the linker exchanges to either the diazene or the amide might not only have impacted the geometrical conformation of the fragment but also affected other aspects of the binding, e.g., the rotational/translational conformation and/or intermolecular interactions.

**Table 2.2. SPR results of linker analogues B2 and B3.** <sup>a</sup>



| Cmpd ID   | R                                                                                 | Dark $K_D$ ( $\mu\text{M}$ ) | Irr. $K_D$ ( $\mu\text{M}$ ) | LiPE |
|-----------|-----------------------------------------------------------------------------------|------------------------------|------------------------------|------|
| <b>B1</b> |  | $276 \pm 53$ <sup>b</sup>    | ND                           | 1.51 |
| <b>B2</b> |  | 301                          | 301                          | 0.17 |
| <b>B3</b> |  | NA                           | ND                           | ND   |

<sup>a</sup> Compounds were initially screened at 100  $\mu\text{M}$  and then retested at multiple concentrations (6.25–100  $\mu\text{M}$ , 5 points, 2-fold dilution). Photoswitchable compounds were tested both in the dark (Dark  $K_D$ ) and after photoirradiation (Irr.  $K_D$ ) with 365 nm light. NA = no significant response above noise. ND = not determined. Highlighted in grey are previously tested compounds. Structural changes from **B1** have been highlighted.

<sup>b</sup>  $K_D$  value reported as an average of two independent determinations  $\pm$  SEM.

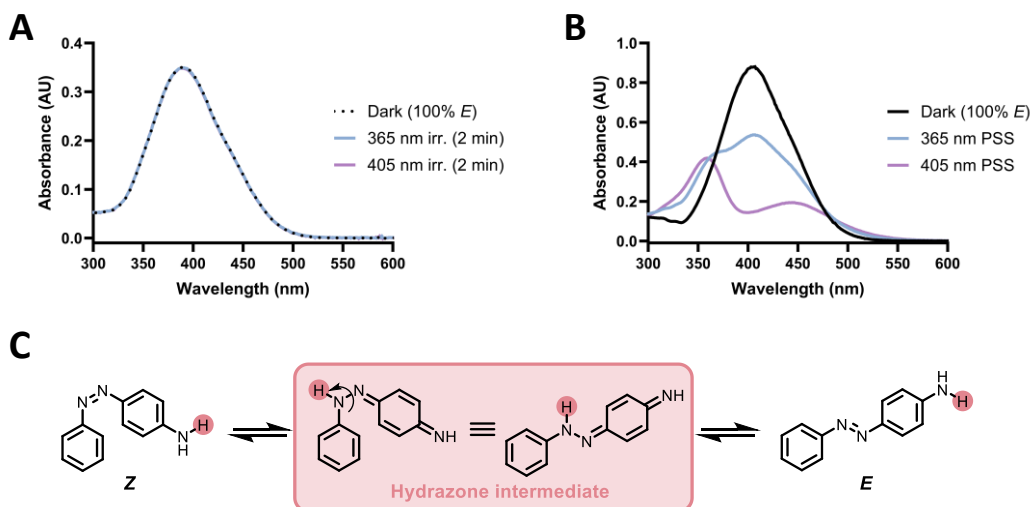


**Figure 2.6. Photochemical characterisation of B2.** Photoswitching was determined by UV–Vis spectroscopy at 20 °C and at 20  $\mu\text{M}$ . (A) Photoswitching in 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 10 w/v% glycerol, 0.05 v/v% Tween-20, 2% DMSO. (B) Photoswitching in 100% DMSO. (C) Proposed thermal back-isomerisation of **B2** via a hydrazone mechanism.

### 2.3.3. Capping modifications on B2

To proceed, we decided to make modifications on **B2** in an attempt to improve the thermal Z-isomer stability (**Figure 2.7**). We here focused on installing capping groups onto the amino N or replacing it with O-groups in order to either remove the acidic NH protons (and thereby disallowing back-isomerisation via the hydrazone mechanism) or to attenuate the donating capacity of the amino group.

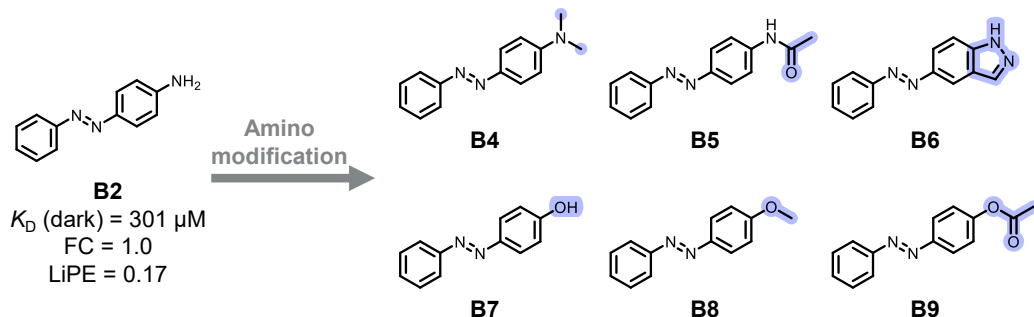
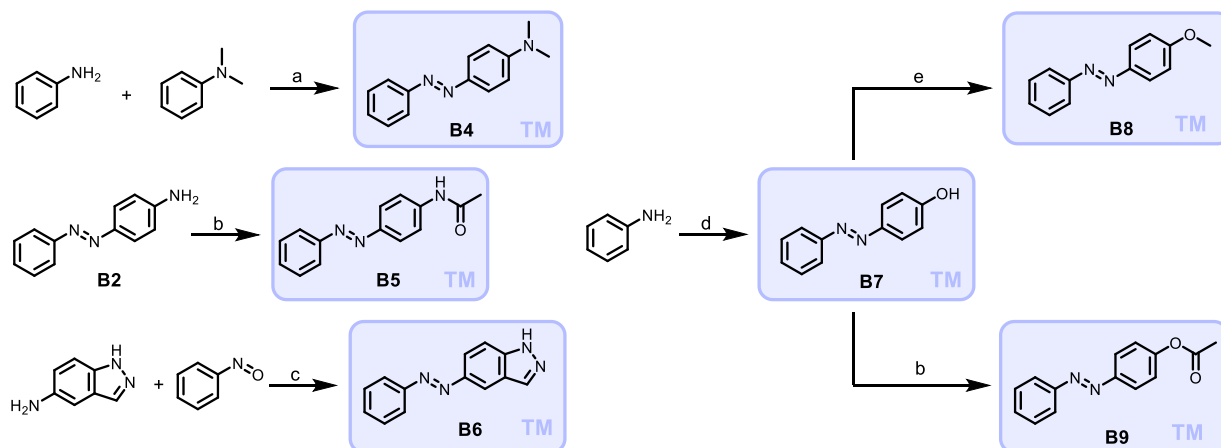


**Figure 2.7. Design of *N*-modified analogues of **B2**.** Structural changes from **B2** have been highlighted.

Analogues **B4** and **B7** were obtained via tandem diazotisation–azo coupling, analogue **B6** was obtained via a base-catalysed Baeyer-Mills reaction and analogues **B5**, **B8** and **B9** from conventional methylation or acetylation reactions (**Scheme 2.3**).

**Scheme 2.3. Synthesis of *N*-modified analogues **B4**–**9**.<sup>a</sup>**



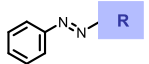
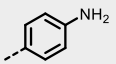
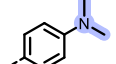
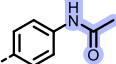
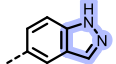
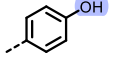
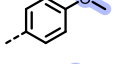
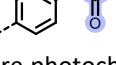
<sup>a</sup> Reagents and conditions: (a)  $\text{NaNO}_2$ , aq. HCl, 0 °C, 1 h; then *N,N*-dimethylaniline, NaOAc, THF, 0 °C–rt, 21 h, 24%. (b)  $\text{AcCl}$ ,  $\text{Et}_3\text{N}$ , THF, 0 °C–rt, 2–22 h, 73%–quant. (c) aq.  $\text{NaOH}$ , PhMe, 80 °C, 18 h, 5%. (d)  $\text{NaNO}_2$ , aq. HCl, 0 °C, 1 h; then aniline, 0 °C–rt, 15 h; then  $\text{NH}_4\text{OH}$ , 30%. (e)  $\text{MeI}$ ,  $\text{K}_2\text{CO}_3$ , DMF, 50 °C, 28 h, 82%. TM = Target molecule.

*N,N*-Dimethylating the amino group (**B4**) did not result in any sufficient stabilisation of the *Z*-isomer as apparent photoswitching could still not be observed (**Table 2.3**). On the other hand, acetylation (**B5**) and incorporating the amino group into an aromatic ring system (**B6**) both caused significant thermal stabilisation to the extent that photoswitching in the aqueous assay buffer could now be observed by UV–Vis spectroscopy and with only limited back-isomerisation occurring after 1 hour (**Appendix Figure A5A–B**). In both instances, the electron-pushing strength of the amino group is attenuated, suggesting that decreasing the basicity of the diazene *N* is more effective at increasing the *Z*-isomer thermal half-life (rather than reducing the acidity of the amino protons or removing them). Combined with the excellent



completeness of switching displayed by both photoswitches (PSD = 96%), it is therefore likely that a significant amount of the Z-isomer has been sampled in the SPR assay upon photoirradiation. Although the acetylated analogue **B5** retained some absolute affinity ( $K_D$  = 412  $\mu$ M), differential activity could still not be detected with our assay upon photoirradiation. This suggests that the two isomers inherently may not bind with significantly different affinities. For the *O*-analogues, capping similarly caused significant thermal stabilisation (< 1% back-conversion after 1 h). However, only the hydroxy analogue **B7** displayed binding, although differential activity could not be detected—again due to the low thermal half-life of the Z-isomer.

**Table 2.3. Photochemical properties and SPR results of *N*-modified analogues B4–9.** <sup>a</sup>

| Cmpd ID   |    |  |                             |                          |                          |       |
|-----------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-----------------------------|--------------------------|--------------------------|-------|
|           |                                                                                     | C <sub>1h</sub><br>(%)                                                            | PSD <sub>365nm</sub><br>(%) | Dark $K_D$<br>( $\mu$ M) | Irr. $K_D$<br>( $\mu$ M) | LiPE  |
| <b>B2</b> |    | 100                                                                               | ND                          | 301                      | 301                      | 0.17  |
| <b>B4</b> |   | 100                                                                               | ND                          | NA                       | NA                       | ND    |
| <b>B5</b> |  | 15                                                                                | 96                          | 412                      | 412                      | -0.16 |
| <b>B6</b> |  | <1                                                                                | 96                          | NA                       | NA                       | ND    |
| <b>B7</b> |  | 100                                                                               | ND                          | 288                      | 288                      | -0.42 |
| <b>B8</b> |  | <1                                                                                | 97                          | NA                       | NA                       | ND    |
| <b>B9</b> |  | <1                                                                                | 78                          | NA                       | NA                       | ND    |

<sup>a</sup> Compounds were photochemically characterised by UV–Vis spectroscopy in 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 10 w/v% glycerol, 0.05 v/v% Tween-20, 2% DMSO at 20  $\mu$ M, 20 °C. Compounds were initially screened by SPR at 100  $\mu$ M and then retested at multiple concentrations (6.25–100  $\mu$ M, 5 points, 2-fold dilution). The compounds were tested both in the dark (Dark  $K_D$ ) and after photoirradiation with 365 nm light (Irr.  $K_D$ ). C<sub>1h</sub> = percentage back-conversion after 1 h. NA = no significant response above noise. ND = not determined. Highlighted in grey are previously tested compounds. Structural changes from **B2** have been highlighted.

These results may suggest that the strategy of inducing a geometric change in the linker region of the fragment may not be ideal for achieving differential activity. However, this conclusion is based on analogues that are two transformations away from the original fragment hit **B1**. Furthermore, the

absolute fragment affinities are weak, and any small changes in affinity may simply be beyond the sensitivity of our SPR assay.

## 2.4. Scouting modifications

We proceeded to make conventional modifications on **B1** in order to 1) establish structure-activity relationships that might help elucidate how and where the fragment could be transformed into an azo photoswitch and 2) increase the absolute binding affinity of the fragment to allow for more granular detection of differential activities.

### 2.4.1. Deconstruction of B1

We first set out to systematically deconstruct the fragment to possibly remove any redundant or unproductive motifs and to isolate the key pharmacophore (**Figure 2.8**).

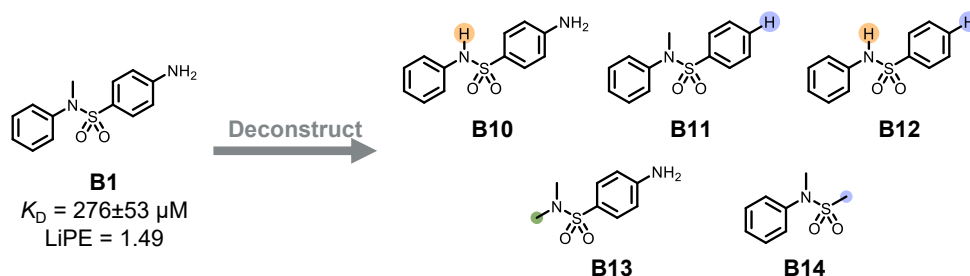
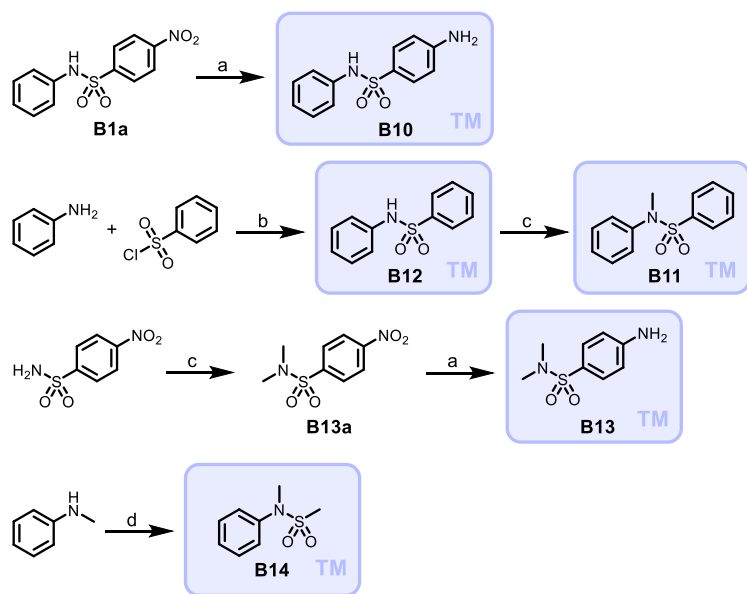


**Figure 2.8.** Design of deconstruction analogues of **B1**. Structural changes from **B1** have been highlighted.

The compounds were readily synthesised from previously employed transformations, i.e., nitro reduction, *N*-sulfonylation, and sulfonamide *N*-methylation (**Scheme 2.4**).

**Scheme 2.4. Synthesis of deconstruction analogues B10–14.** <sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) Pd/C, MeOH, H<sub>2</sub> (1 bar), rt, 20–22 h, quant. (b) Pyridine, DCM, reflux, 3 h, 52%. (c) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, rt, 5–6 h, 97%. (d) MsCl, pyridine, DCM, rt, 26 h, quant. TM = Target molecule.

**Table 2.4. SPR results of deconstruction analogues B10–14.** <sup>a</sup>

| Cmpd ID | R <sup>1</sup> | R <sup>2</sup> | R <sup>3</sup> | K <sub>D</sub> (μM)   | LiPE |
|---------|----------------|----------------|----------------|-----------------------|------|
| B1      | --Me           |                |                | 276 ± 53 <sup>b</sup> | 1.51 |
| B10     | --H            |                |                | NA                    | ND   |
| B11     | --Me           |                |                | 121                   | 1.12 |
| B12     | --H            |                |                | 309                   | 0.93 |
| B13     | --Me           |                | --H            | NA                    | ND   |
| B14     | --Me           | --Me           |                | NA                    | ND   |

<sup>a</sup> Compounds were initially screened at 100 μM and then retested at multiple concentrations (6.25–100 μM, 5 points, 2-fold dilution). NA = no significant response above noise. ND = not determined. Highlighted in grey are previously tested compounds. Structural changes from **B1** have been highlighted.

<sup>b</sup> K<sub>D</sub> value reported as an average of two independent determinations ± SEM.

SPR on the deconstruction analogues showed that the sulfonamide methyl group and both phenyl rings are required for activity given the inactivity of **B10**, **B13** and **B14** (Table 2.4). Curiously, the deaminated analogue **B11** displayed a small boost in affinity ( $K_D = 121 \mu\text{M}$ ), indicating that the amino group is not necessary for binding. However, it is worth noting that the analogue had a significantly reduced LiPE suggesting less specificity and poorer physicochemical properties.

### 2.4.2. Amino group modifications on B1

Given the above result, the amino group was next subjected to a range of modifications to further understand its role. These included capping the *N* with the same groups employed for **B2**, exchanging it with other functional moieties, spacing it from the aryl ring and changing its regioisomery (Figure 2.9). The resulting analogues were synthesised employing the same key *N*-sulfonylation strategy employed previously followed optionally by late-stage functionalisation (aniline *N*-alkylation and -acetylation to access **B15** and **B16**, respectively) or functional group conversion (nitrile reduction and hydration to yield **B22** and **B23**, respectively) (Scheme 2.5).

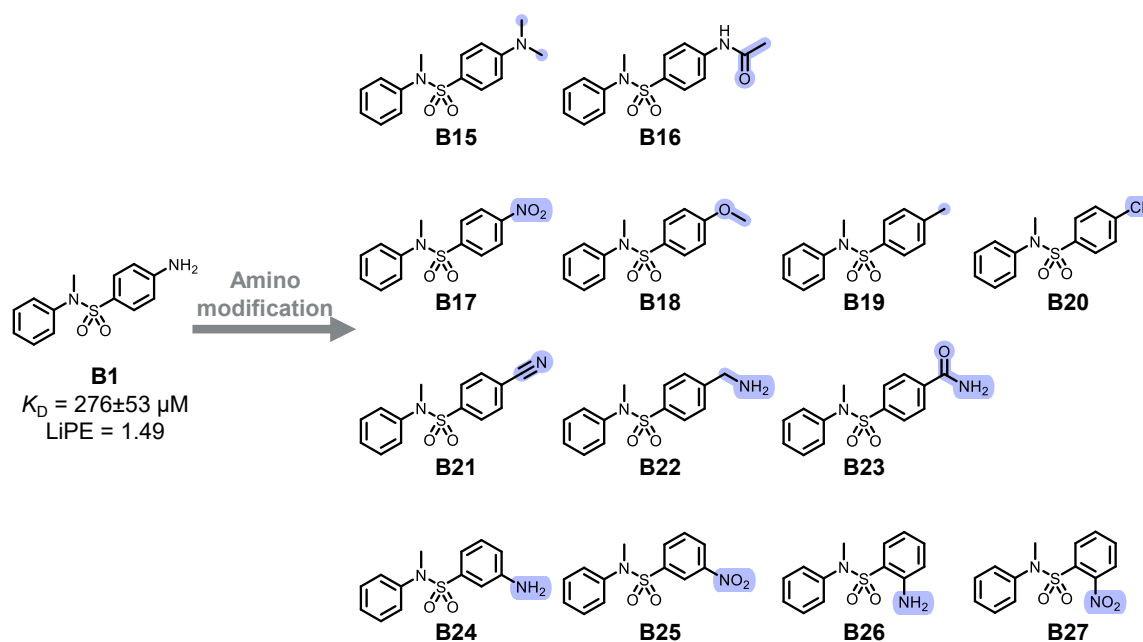
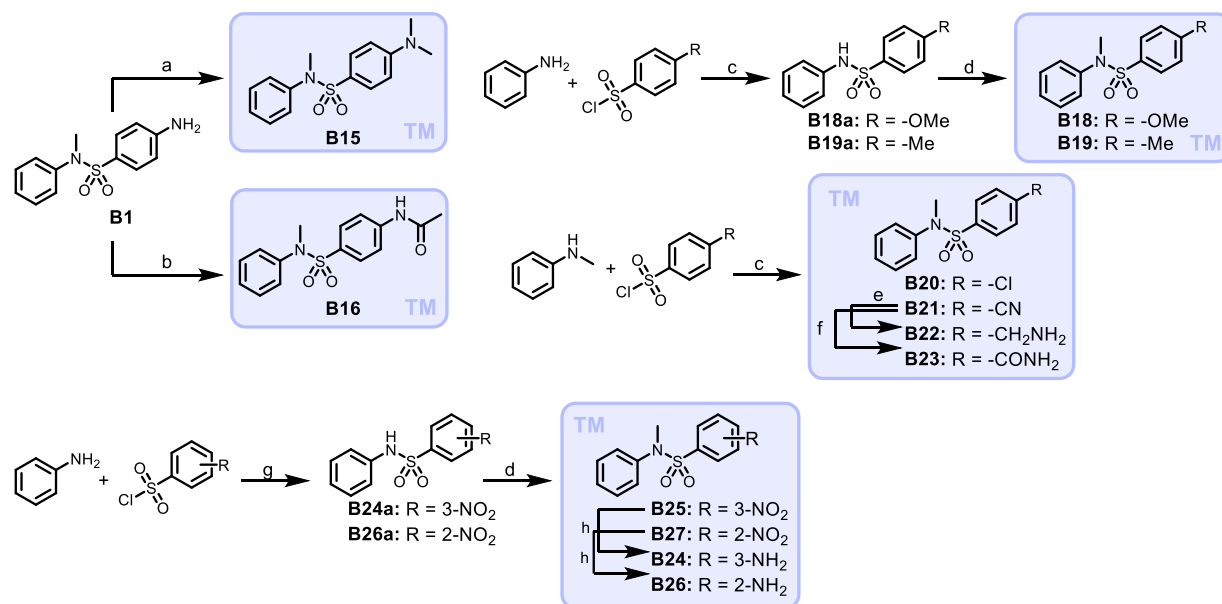


Figure 2.9. Design of *N*-modified analogues of **B1**. Structural changes from **B1** have been highlighted.

**Scheme 2.5. Synthesis of *N*-modified analogues B15–27. <sup>a</sup>**



<sup>a</sup> Reagents and conditions: (a) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, rt–95 °C, 21 h, 74%. (b) AcCl, Et<sub>3</sub>N, THF, 0 °C–rt, 4 h, 86%. (c) Pyridine, DCM, rt, 24–47 h, 82–99%. (d) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, rt, 16–18 h, 88%–quant. (e) Pd/C, conc. HCl, MeOH, H<sub>2</sub> (1 bar), rt, 32 h, 22%. (f) H<sub>2</sub>SO<sub>4</sub>, TFA, rt, 27 h, 78%. (g) Pyridine, DCM, reflux, 4.5–5.5 h, 42–55%. (h) Pd/C, MeOH, H<sub>2</sub> (1 bar), rt, 1.3–24 h, quant. TM = Target molecule.

**Table 2.5. SPR results of *N*-modified analogues B15–27. <sup>a</sup>**

| Cmpd ID    | R                   | SPR <i>K<sub>D</sub></i> (μM) | LiPE              |
|------------|---------------------|-------------------------------|-------------------|
| <b>B1</b>  | 4--NH <sub>2</sub>  | 276 ± 53 <sup>b</sup>         | 1.51              |
| <b>B11</b> | --H                 | 121                           | 1.12 <sup>a</sup> |
| <b>B15</b> | 4--NMe <sub>2</sub> | NA                            | ND                |
| <b>B16</b> | 4--NHAc             | NA                            | ND                |
| <b>B17</b> | 4--NO <sub>2</sub>  | NA                            | ND                |
| <b>B18</b> | 4--OMe              | NA                            | ND                |
| <b>B19</b> | 4--Me               | NA                            | ND                |
| <b>B20</b> | 4--Cl               | NA                            | ND                |
| <b>B21</b> | 4--CN               | NA                            | ND                |

|            |                                   |      |      |
|------------|-----------------------------------|------|------|
| <b>B22</b> | 4-CH <sub>2</sub> NH <sub>2</sub> | NA   | ND   |
| <b>B23</b> | 4-CONH <sub>2</sub>               | NA   | ND   |
| <b>B24</b> | 3-NH <sub>2</sub>                 | 58.4 | 2.16 |
| <b>B25</b> | 3-NO <sub>2</sub>                 | NA   | ND   |
| <b>B26</b> | 2-NH <sub>2</sub>                 | 108  | 1.16 |
| <b>B27</b> | 2-NO <sub>2</sub>                 | 145  | 1.48 |

<sup>a</sup> Compounds were initially screened at 100  $\mu$ M and then retested at multiple concentrations (6.25–100  $\mu$ M, 5 points, 2-fold dilution). NA = no significant response above noise. ND = not determined. Highlighted in grey are previously tested compounds. Structural changes from **B1** have been highlighted.

<sup>b</sup>  $K_D$  value reported as an average of two independent determinations  $\pm$  SEM.

Capping the amino group, replacing it with any other functional group or spacing it from the aryl ring with a one-carbon linker all resulted in complete loss of binding (**B15–23**), suggesting that the space probed by the amino group might be spatially and electronically confined (**Table 2.5**). Gratifyingly, a change of the regioisomery of the amino group from the 4-position to either the 3- or 2-position gave a significant boost in affinity. The 3-regioisomer **B24** emerged as the best with a 5-fold increased affinity compared with **B1** ( $K_D$  = 58.4  $\mu$ M, **Appendix Figure A2D**) as well as an increased LiPE.

#### 2.4.3. LHA<sub>r</sub> substitution on **B1**

In parallel to the above amino group modifications, we explored substitutions on the LHA<sub>r</sub> (**Figure 2.10**). We drew inspiration by the Topliss batchwise scheme to aromatic substitution, which infers the optimal substitution pattern on a benzene ring by analysing the relative biological activities of a small set of substituted analogues.<sup>102</sup> The 4-position was here selected for substitution with a range of functional groups, and the chloro substituent was further “walked” around the ring (**B28–34**). Scaffold hopping to a pyridine (**B35**) and ring fusion to an indazole (**B36**) were further attempted. Together, we envisioned that this would elucidate the electronic and steric demands of the LHA<sub>r</sub> ring.

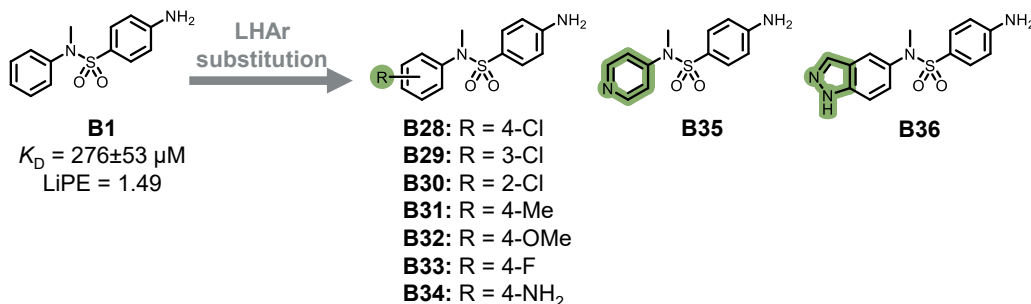
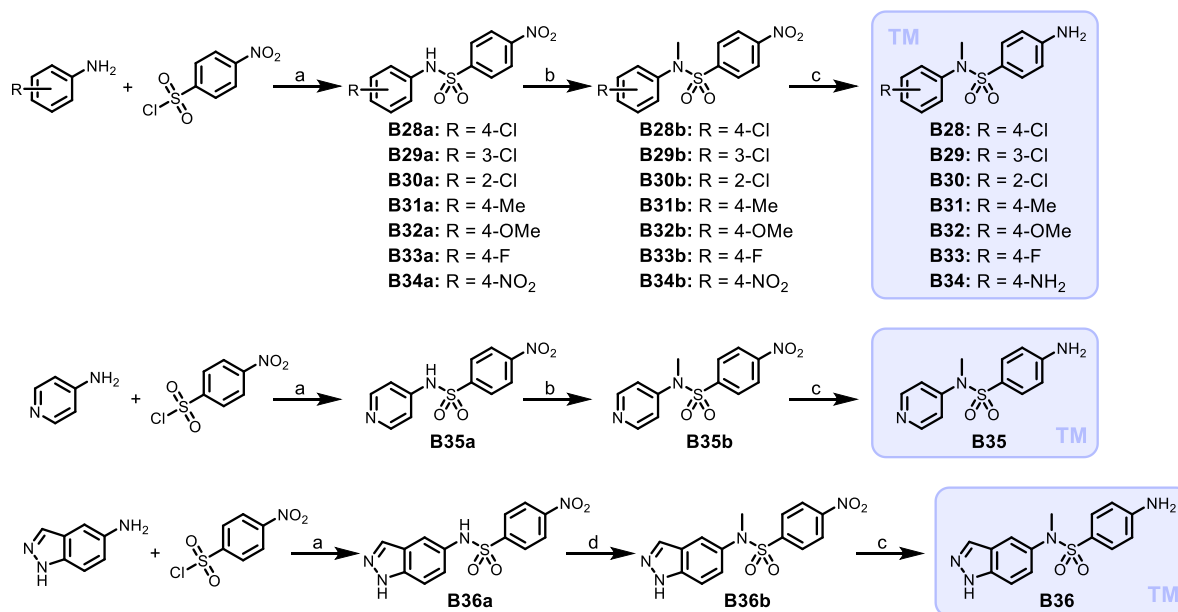


**Figure 2.10.** Design of LHAir substitution analogues of **B1**. Structural changes from **B1** have been highlighted.

The analogues were synthesised by following the common route of 1) *N*-sulfonylation, 2) sulfonamide *N*-methylation using methyl iodide, and 3) nitro reduction by hydrogenation (**Scheme 2.6**). However, for the indazole analogue **B36**, DMF-dimethyl acetal was used as the methylation agent instead of methyl iodide, as this has been reported to offer chemoselective methylation of *NH*-functionalities based on their acidity.

103

**Scheme 2.6.** Synthesis of LHAir substitution analogues **B28–36**.<sup>a</sup>

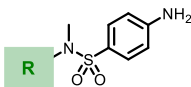


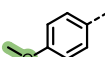
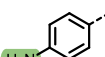
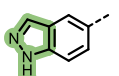
<sup>a</sup> Reagents and conditions: (a) Pyridine, DCM, rt–reflux, 3–46 h, 12–86%. (b) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, rt, 6–30 h. (c) Pd/C, MeOH, H<sub>2</sub> (1 bar), rt, 15–24 h, 41%–quant. (d) DMF-DMA, DMF, 60 °C, 18 h, 52%. TM = Target molecule.

The results of the SPR assay showed a preference for substitution on the 3- and 2-positions—with the 3-chloro analogue (**B29**) and 2-chloro analogue (**B30**) displaying slightly improved binding affinities ( $K_D = 211 \mu\text{M}$  and  $K_D = 153 \mu\text{M}$ ), whereas the 4-substituted analogues (**B28** and **B31–34**) showed weaker or no

activity at 100  $\mu\text{M}$ . More drastic changes to the LHA<sub>r</sub> such as scaffold hopping (**B35**) or ring fusion (**B36**) led to inactivity as well.

**Table 2.6. SPR results of LHA<sub>r</sub> substitution analogues B28–36.** <sup>a</sup>



| Cmpd ID | R                                                                                   | SPR $K_D$ ( $\mu\text{M}$ ) | LiPE |
|---------|-------------------------------------------------------------------------------------|-----------------------------|------|
| B1      |    | 276 $\pm$ 53 <sup>b</sup>   | 1.51 |
| B28     |    | 325                         | 0.54 |
| B29     |    | 211                         | 0.72 |
| B30     |    | 153                         | 1.69 |
| B31     |    | NA                          | ND   |
| B32     |   | NA                          | ND   |
| B33     |  | NA                          | ND   |
| B34     |  | 202                         | 2.85 |
| B35     |  | NA                          | ND   |
| B36     |  | NA                          | ND   |

<sup>a</sup> Compounds were initially screened at 100  $\mu\text{M}$  and then retested at multiple concentrations (6.25–100  $\mu\text{M}$ , 5 points, 2-fold dilution). NA = no significant response above noise. ND = not determined. Highlighted in grey are previously tested compounds. Structural changes from **B1** have been highlighted.

<sup>b</sup>  $K_D$  value reported as an average of two independent determinations  $\pm$  SEM.

## 2.5. Second generation modifications

The above scouting modifications led to identification of the significantly improved analogue **B24** ( $K_D$  = 58.4  $\mu\text{M}$ ). We proceeded to make additional modifications on this scaffold to further investigate the interaction space around the scaffold.



### 2.5.1. Linker modifications on B24

We initially focused our attention on the linker region (**Figure 2.11**). This included reattempting the azolisation (**B37**) as well as making the corresponding amide transformation (**B38**) as previously. An inverse linker analogue (**B39**) was further designed to probe whether the sulfonamide motif might be engaging in distance and orientation-sensitive interactions. Finally, growing from the sulfonamide *N* with a variety of functional groups (aliphatic, aromatic, and polar) was also sampled with a few analogues (**B40–43**) to assess whether the *N*-Me vector could be exploited to gain affinity.

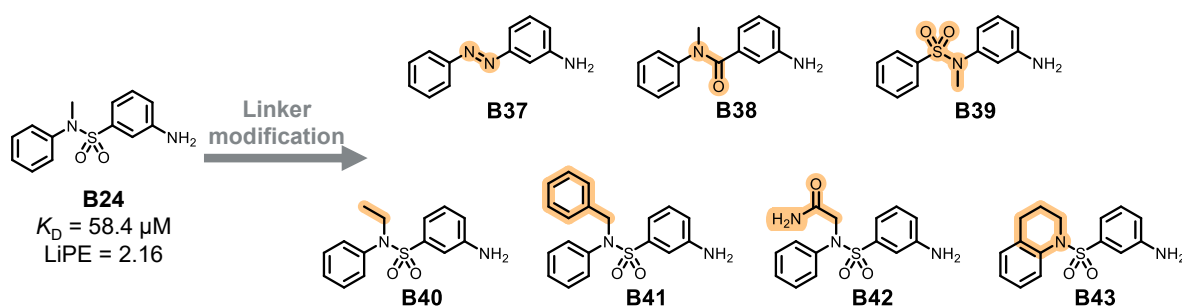
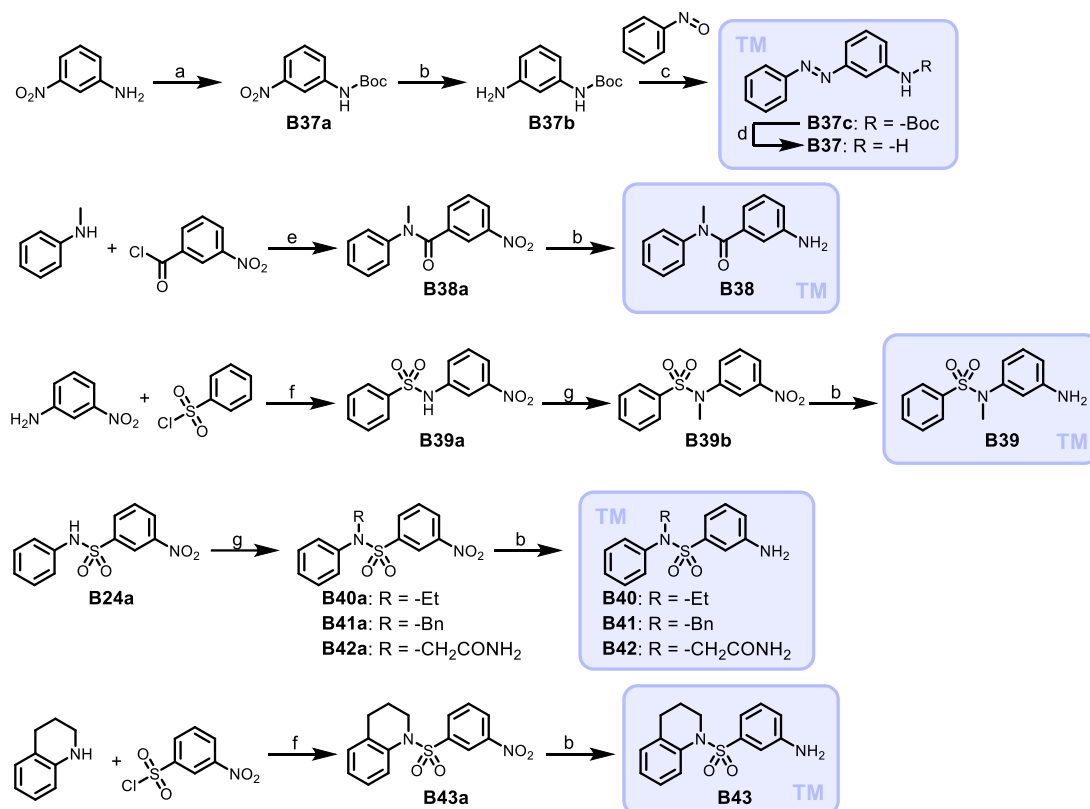


**Figure 2.11.** Design of linker modification analogues of **B24**. Structural changes from **B24** have been highlighted.

The TMs were generally synthesised using previously established protocols (**Scheme 2.7**). However, for compound **B37** a protective group route was opted for instead of the direct Baeyer-Mills reaction path employed for **B2** which relied on inherent amino group reactivity and statistics. However, this turned out not to be an efficient strategy, requiring many steps and afforded the product in a lower overall yield.

Changing the regioisomery of the 4-amino group of **B2** to the 3-position giving analogue **B37** resulted in significant thermal stabilisation of the *Z*-isomer with photoisomerisation now being detectable by UV–Vis spectroscopy and with no observed back-conversion occurring at 20 °C after 1 h (**Figure 2.12A–B**). This is presumably due to the amino group no longer being directly conjugated with the diazene bond. However, this also led to a hypsochromic shift of the *E*-isomer  $\pi$ – $\pi^*$  absorbance band, with a resulting poor photoconversion at 365 nm (64%). Still, a modest 2.8-fold change in binding affinity was observable by SPR upon photoirradiation (**Table 2.7**). However, the analogue suffered from a dramatic 8-fold loss in absolute activity compared with **B24** and a  $\text{LiPE} = 0.00$ . This azologue therefore did not emerge as a promising starting point for further optimisation.

**Scheme 2.7. Synthesis of linker modification analogues B37–43.** <sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) Boc<sub>2</sub>O, DIPEA, EtOH, rt, 51 h, 65%. (b) Pd/C, MeOH, (EtOAc), H<sub>2</sub> (1 bar), rt, 17–27 h, 75–99%. (c) AcOH, rt, 48 h, 38%. (d) HCl in 1,4-dioxane, rt, 22 h, 60%. (e) Et<sub>3</sub>N, DCM, 0 °C–rt, 20 h, 78%. (f) Pyridine, DCM, rt, 23–29 h, quant. (g) RX (X = Br, I), K<sub>2</sub>CO<sub>3</sub>, DMF, rt, 17–30 h, 92%–quant. TM = Target molecule.

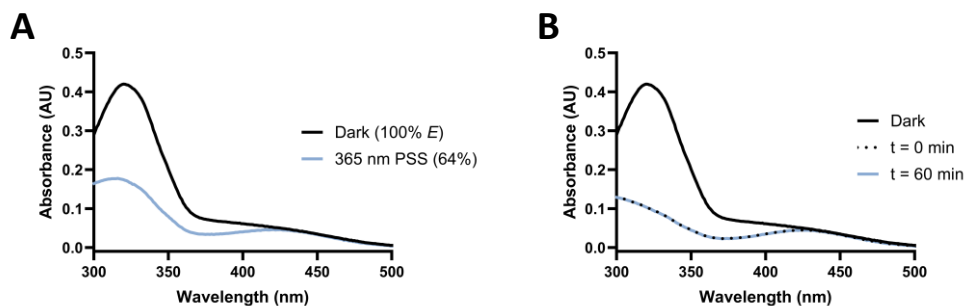
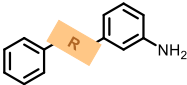
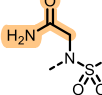
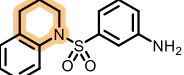


**Figure 2.12. Photochemical characterisation of B37.** Photoswitching and thermal kinetics were determined by UV–Vis spectroscopy at 20 °C and at 20 μM in 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 10 w/v% glycerol, 0.05 v/v% Tween-20, 2% DMSO. (A) PSD determination with 365 nm irradiation. (B) Thermal stability of the Z-isomer over 60 min.

Table 2.7. SPR results of linker modification analogues B37–B43. <sup>a</sup>



| Cmpd ID | R or structure                                                                      | Dark $K_D$<br>( $\mu\text{M}$ ) | Irr. $K_D$<br>( $\mu\text{M}$ ) | LiPE |
|---------|-------------------------------------------------------------------------------------|---------------------------------|---------------------------------|------|
| B24     |    | 58.4                            | ND                              | 2.16 |
| B37     |    | 444                             | 1270                            | 0.00 |
| B38     |    | NA                              | ND                              | ND   |
| B39     |    | 99 ± 46 <sup>b</sup>            | ND                              | 2.56 |
| B40     |    | 194                             | ND                              | 1.11 |
| B41     |    | NA                              | ND                              | ND   |
| B42     |   | 440                             | ND                              | 2.45 |
| B43     |  | 129                             | ND                              | 1.16 |

<sup>a</sup> Compounds were assayed at multiple concentrations (6.25–100  $\mu\text{M}$ , 5 points, 2-fold dilution). Photoswitchable compounds were tested both in the dark and after photoirradiation with 365 nm light. NA = no significant response above noise. ND = not determined. Highlighted in grey are previously tested compounds. Structural changes from **B24** have been highlighted.

<sup>b</sup>  $K_D$  value reported as an average of two independent determinations ± SEM.

The inactivity of the amide analogue **B38** again suggested a collapsed bioactive conformation of **B24** rather than a planar and extended conformation. Reversing the sulfonamide linker to give **B39** resulted in retained binding affinity ( $K_D \approx 100 \mu\text{M}$ ), which might indicate that the sulfonamide does not engage in specific interactions but only serves to stabilise the bioactive conformation. Of note, the inversion of the linker resulted in an increase in the compound polarity and thus in the LiPE.

Elaboration of the fragment from the *N*-Me vector to give the analogues **B40–43** all resulted in reduced binding, suggesting that this did not constitute a viable site for fragment growing.

### 2.5.2. Amino group modifications on B24

We next explored the amino group as a possible site for fragment elaboration (**Figure 2.13**). Here, we mainly sampled substitution with alkyl chains of various lengths and bulkiness (**B44–49**) but also made a modest effort to cap or exchange the amino group with other functional groups, including amides that would allow for high-throughput analogue synthesis (**B50–53**).

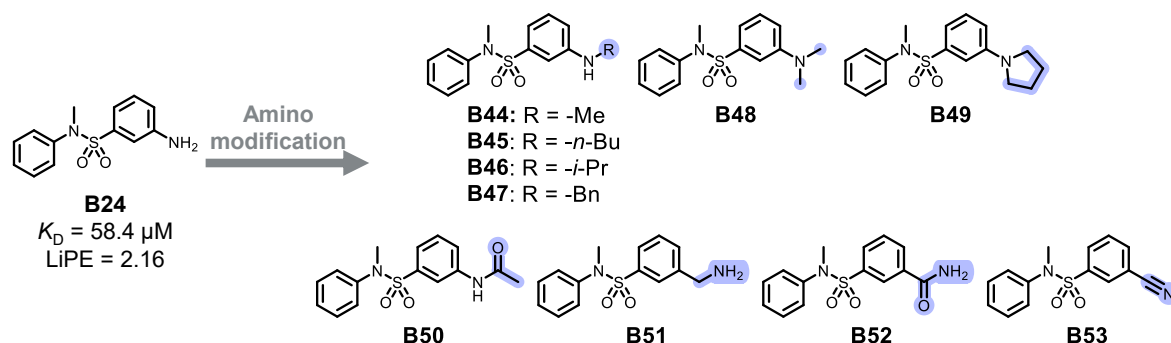
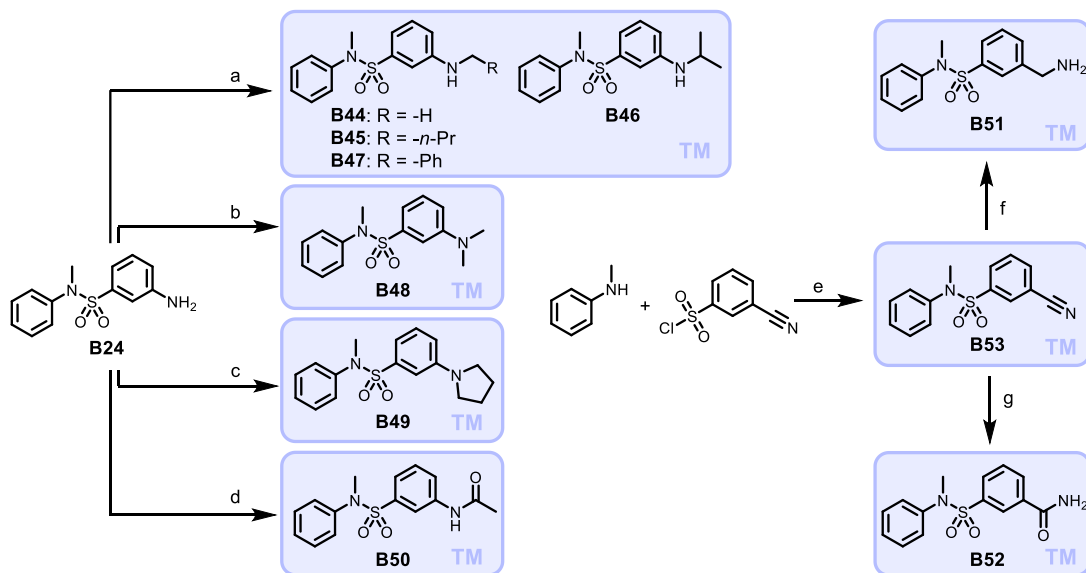


**Figure 2.13.** Design of *N*-modification analogues of **B24**. Structural changes from **B24** have been highlighted.

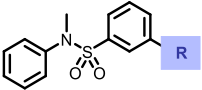
The *N*-substituted analogues were readily synthesised through late-stage derivatisation of **B24** either by reductive amination (**B44–47**), alkylation (**B48** and **B49**) or acylation (**B50**) (**Scheme 2.8**). Synthesis of the nitrile analogue **B53** also gave access to the aminomethyl analogue **B51** and the amide **B52** through late-stage reduction and hydration, respectively.

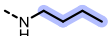
**Scheme 2.8.** Synthesis of *N*-modification analogues **B44–53**.<sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) RCHO or Me<sub>2</sub>CO, NaBH(OAc)<sub>3</sub>, AcOH, DCM, 0 °C–rt, 14 h–3 days, 42–86%. (b) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, 80 °C, 24 h, 77%. (c) 1,4-dibromobutane, KI, K<sub>2</sub>CO<sub>3</sub>, MeCN, 90 °C, 46 h, 65%. (d) AcCl, Et<sub>3</sub>N, THF, 0 °C–rt, 21 h, 55%. (e) Pyridine, DCM, rt, 24 h, 75%. (f) Pd/C, aq. HCl, MeOH, H<sub>2</sub> (1 bar), rt, 44 h, 18%. (g) H<sub>2</sub>SO<sub>4</sub>, TFA, rt, 21 h, 49%. TM = Target molecule.

**Table 2.8. SPR results of amino group modified analogues B44–53.** <sup>a</sup>



| Cmpd ID | R                                                                                   | SPR <i>K</i> <sub>D</sub> (μM) | LiPE |
|---------|-------------------------------------------------------------------------------------|--------------------------------|------|
| B24     |    | 58.4                           | 2.16 |
| B25     |    | NA                             | ND   |
| B44     |    | 3.96                           | 2.57 |
| B45     |    | 7.54                           | 0.70 |
| B46     |    | 84.0                           | 0.40 |
| B47     |    | 12.5                           | 0.62 |
| B48     |   | NA                             | ND   |
| B49     |  | NA                             | ND   |
| B50     |  | 82.0                           | 1.81 |
| B51     |  | 94.0                           | 2.28 |
| B52     |  | 86.0                           | 2.46 |
| B53     |  | 122                            | 1.68 |

<sup>a</sup> Compounds were assayed at multiple concentrations (6.25–100 μM, 5 points, 2-fold dilution; higher-affinity compounds retested at 0.313–20 μM or 0.625–40 μM, 7 points, 2-fold dilution). NA = no significant response above noise. ND = not determined. Highlighted in grey are previously tested compounds. Structural changes from **B24** have been highlighted.

The SPR results show that the amino group probes a large space that can be accommodated by even elaborate and sterically demanding motifs and yield affinity gains (**Table 2.8**). Locally around the *N*-atom there appears to be a preference for linear modifications, where the *n*-butyl analogue **B45** displayed a 7.7-fold boost in affinity relative to **B24** (*K*<sub>D</sub> = 7.54 μM), whereas the *i*-propyl analogue **B46** was only able to maintain binding (*K*<sub>D</sub> = 84.0 μM). However, distal from *N*-atom there is room for accommodating large moieties as demonstrated with the benzyl analogue **B47**, which had a 4.7-fold increased affinity (*K*<sub>D</sub> = 12.5 μM). Despite the fact that these large moieties were allowed and could yield affinity gains, the *N*-

monomethylated analogue **B44** emerged as the most outstanding with a 14.7-fold boost in activity, bringing the  $K_D$  down to the low single-digit micromolar range ( $K_D = 3.96 \mu\text{M}$ ). Of note, this also brought a significant increase in the LiPE (2.57). *N,N*-Dialkylation (**B48** and **B49**), on the other hand, yielded inactivity, suggesting that only one *NH* vector can be exploited for growing. Capping the *N* with an acetyl (**B50**) or spacing the *N* from the aryl ring with either a methylene (**B51**), a carbonyl (**B52**) or an *sp*-hybridised carbon (**B53**) were tolerable but not favourable for affinity.

### 2.5.3. LAr substitution on B24

We were mindful of the possibility that the regioisomeric change of the amino group from the 4-position (**B1**) to the 3-position (**B24**) may have changed the SAR around the rest of the molecule, so we proceeded to explore systematic substitution on the LAr of **B24** similarly to what was done previously on **B1** (Figure 2.14). Here, we sampled substitution with selected functional groups (chloride, methyl, methoxy, fluoride, trifluoromethyl and amino), and to cover more chemical space, we further examined regioisomerism for multiple of these substituents (**B54–65**). Beyond this, scaffold hopping was also explored to a modest extent (**B66** and **B67**).

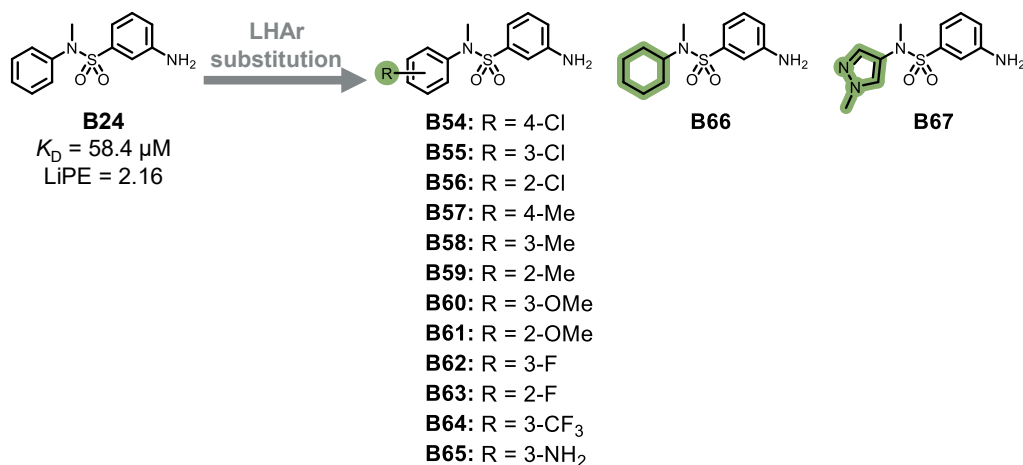
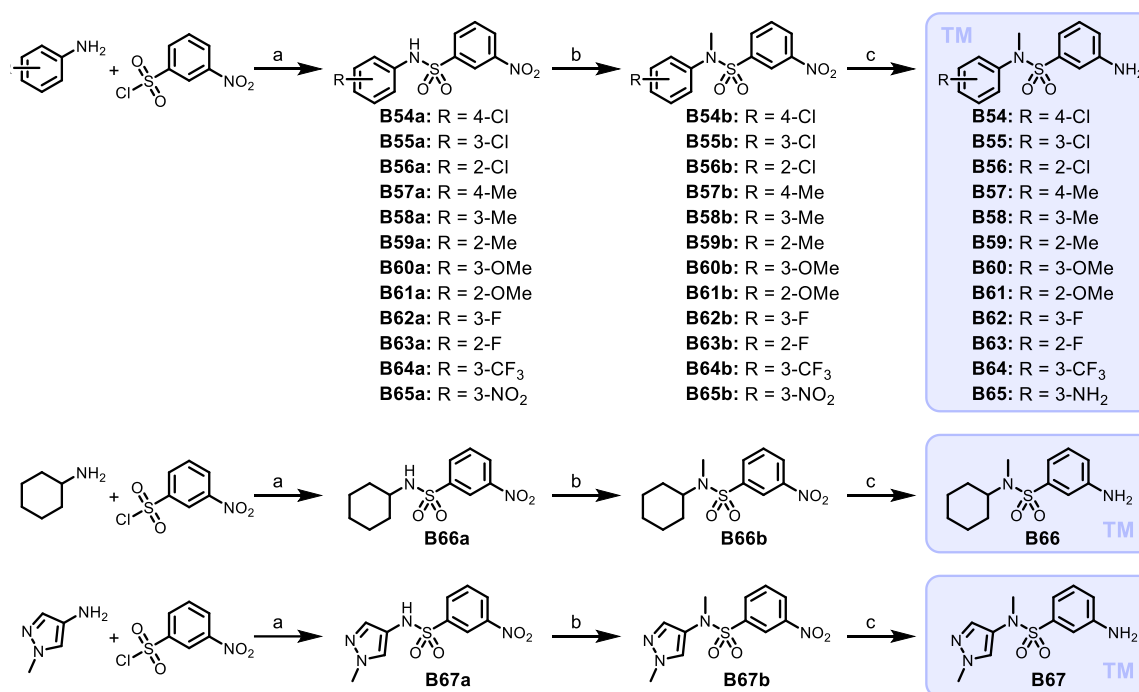


**Figure 2.14.** Design of LAr substituted analogues of **B24**. Structural changes from **B24** have been highlighted.

The analogues were rapidly synthesised from the previously established robust sequence of *N*-sulfonylation, sulfonamide *N*-methylation and nitro reduction by hydrogenation (Scheme 2.9).

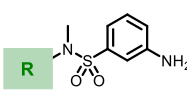
**Scheme 2.9. Synthesis of LHA<sub>r</sub> substituted analogues B54–67.<sup>a</sup>**

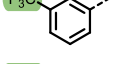
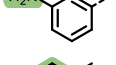


<sup>a</sup> Reagents and conditions: (a) Pyridine, DCM, rt, 17 h–3 days, 60–89%. (b) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, rt, 16 h–3 days, 80%–quant. (c) Pd/C, MeOH, (EtOAc), H<sub>2</sub> (1 bar), rt, 6–40 h, 19%–quant. TM = Target molecule.

From the significant sampling of various substitutions at various positions on the LHA<sub>r</sub> emerges firstly a clear regioisomeric preference—with substitution on the 3-position being consistently more favourable than the 2-position, which in turn was more favourable than substituting the 4-position (e.g. **B55** > **B56** > **B54**) (Table 2.9). Presumably, this preference arises from steric demands. As anticipated, this SAR is different from what was obtained for **B1**, where the 2-position was the most favoured from the “chloride walk” (however, for both **B1** and **B24**, the 4-position is the most disfavoured). Surprisingly, substitution on the 3-position with a chloride, methyl, methoxy and fluoride (**B55**, **B58**, **B60** and **B62**) all gave significant affinity boosts, whereas the trifluoromethyl (**B64**) and amino (**B65**) analogues only retained affinity. The equipotency of analogues carrying substituents of widely different nature (e.g., **B55**, **B60** and **B62** all having  $K_D \approx 10\text{--}20\ \mu\text{M}$ ) meant that the electronic and lipophilic preference of the aryl ring could not immediately be unravelled. Indeed, the relative affinities of the analogues could not be fitted into a Topliss scheme.<sup>102</sup> Crucially, the 3-methyl analogue **B58** stood out amongst the series displaying a  $K_D = 4.93\ \mu\text{M}$ , which corresponded to an 11.8-fold increase in affinity relative to **B24**. More drastic changes to the LHA<sub>r</sub> in the form of scaffold hopping into a saturated cyclohexane ring (**B66**) or a 5-membered heterocycle (**B67**) was not favourable for affinity.

Table 2.9. SPR results of LHA<sub>r</sub> modified analogues B54–67.<sup>a</sup>



| Cmpd ID | R                                                                                   | SPR $K_D$ ( $\mu$ M) | LiPE |
|---------|-------------------------------------------------------------------------------------|----------------------|------|
| B24     |    | 58.4                 | 2.16 |
| B54     |    | 167                  | 0.82 |
| B55     |    | 12.8                 | 1.94 |
| B56     |    | 29.9                 | 2.40 |
| B57     |    | 429                  | 0.80 |
| B58     |    | 4.93                 | 2.74 |
| B59     |    | 64.0                 | 1.97 |
| B60     |   | 16.8                 | 2.68 |
| B61     |  | 40.3                 | 2.37 |
| B62     |  | 11.8                 | 2.55 |
| B63     |  | 102                  | 2.02 |
| B64     |  | 96.0                 | 0.77 |
| B65     |  | 111                  | 3.11 |
| B66     |  | 165                  | 1.14 |
| B67     |  | NA                   | ND   |

<sup>a</sup> Compounds were assayed at multiple concentrations (6.25–100  $\mu$ M, 5 points, 2-fold dilution; higher-affinity compounds retested at 0.313–20  $\mu$ M or 0.625–40  $\mu$ M, 7 points, 2-fold dilution). NA = no significant response above noise. ND = not determined. Highlighted in grey are previously tested compounds. Structural changes from **B24** have been highlighted.

In summary, from the second-generation modifications of **B24** ( $K_D$  = 58.4  $\mu$ M, LiPE = 2.16), we identified several analogues displaying improved binding affinities and/or LiPE, including the inverse linker



analogue **B39** ( $K_D = 99 \mu\text{M}$ , LiPE = 2.56), the *N*-methylated analogue **B44** ( $K_D = 3.96 \mu\text{M}$ , LiPE = 2.57) and the LHA<sub>r</sub>-substituted analogues **B55** ( $K_D = 12.8 \mu\text{M}$ , LiPE = 1.94), **B58** ( $K_D = 4.93 \mu\text{M}$ , LiPE = 2.74), **B60** ( $K_D = 16.8 \mu\text{M}$ , LiPE = 2.68) and **B62** ( $K_D = 11.8 \mu\text{M}$ , LiPE = 2.55).

## 2.6. Identification of a potent non-photoswitchable lead

### 2.6.1. Combinatorial modification on B24

From having only made single-point mutations to the core fragment scaffold (**B1** or **B24**) thus far, we next turned to combining the modifications which previously had led to the largest improvement in affinity or LiPE: 1) inverting the linker (**B39**, LiPE = +0.40), 2) methylating the amino group (**B44**,  $K_D = \times 15$ ), and 3) substituting the LHA<sub>r</sub> 3-position (**B55**, **B58**, **B60** and **B63**,  $K_D = \times 3\text{--}12$ ). This led to a small series of combinatorial analogues (**Figure 2.15**).

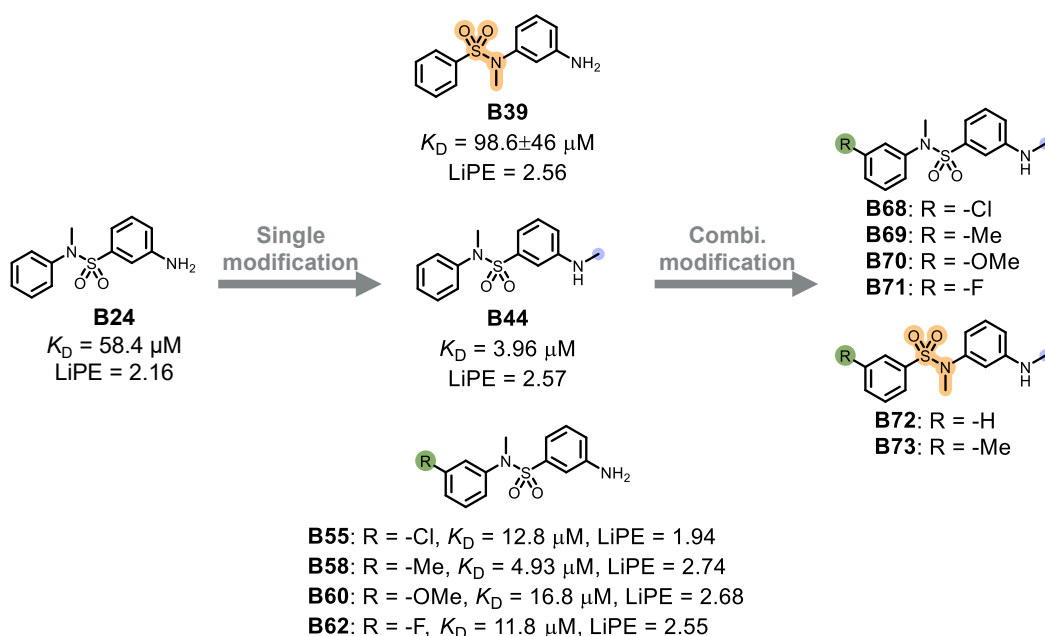
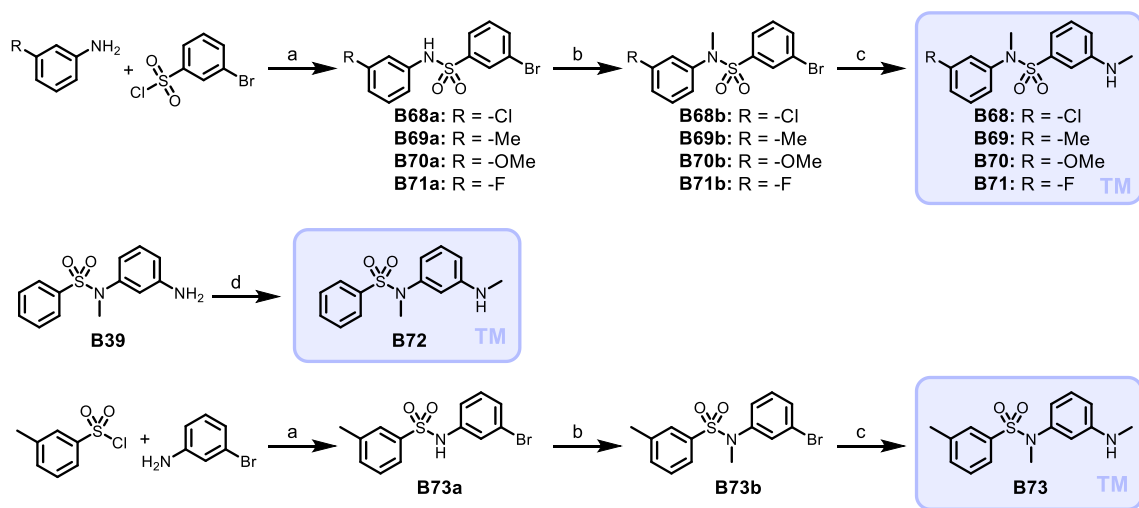


**Figure 2.15. Design of 3<sup>rd</sup> generation combinatorial modification analogues of B24.** Structural changes from **B24** have been highlighted.

To obtain these TMs, we considered a different approach than the late-stage reductive amination pathway that was followed in the synthesis of the **B44**. This had required a total of four steps, and the reductive amination reaction was operationally demanding (including time- and sequence-sensitive addition of reagents). We therefore opted for a late-stage aryl C-N bond formation instead, in which the methylamino

group was envisioned to be installed by cross-coupling late aryl halide intermediates with methylamine. A previously reported Ullmann-type reaction protocol using copper powder and carried out under aerobic atmosphere in a sealed pressure vial was here employed.<sup>104</sup> The reactions could be performed in a parallel fashion and offered the methylaminated products in decent to excellent yields. Furthermore, this strategy allowed for accessing **B68–71** and **B73** in a total of three steps (**Scheme 2.10**).

**Scheme 2.10. Synthesis of combinatorial modification analogues B68–73.** <sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) Pyridine, DCM, rt, 15–24 h, 87%–quant. (b) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, rt, 17–24 h, 70%–quant. (c) aq. MeNH<sub>2</sub>, Cu, air (sealed vial), 100 °C, 26 h–3 days, 33–92%. (d) CH<sub>2</sub>O, NaBH(OAc)<sub>3</sub>, AcOH, DCM, 0 °C–rt, 24 h, 24%. TM = Target molecule.

Interestingly, the combinatorial modifications did not yield additive effects for several of the analogues (**Table 2.10**). For **B70** and **B71**, where amino *N*-methylation had been combined with LHA<sub>R</sub> 3-methoxy and 3-fluoro substitution, respectively, reduced binding affinities were observed compared with both pairs of singly substituted analogues (i.e. these constituted destructive modifications). For compounds **B68**, **B72**, and **B73**, affinity had only been improved compared with one of the two modifications. These results suggest that the single modifications may alter the fragment binding mode (conformationally, rotationally, or translationally) so as to make additional modification unfavourable. Noticeably, the relative affinities of **B68–71** had also slightly changed compared with corresponding demethylated analogues **B55–62**. Only the combination of amino *N*-methylation with LHA<sub>R</sub> 3-methylation to give **B69** resulted in an additive improvement in binding affinity—the compound displayed a sub-micromolar binding affinity of *K<sub>D</sub>* = 0.73 μM, which corresponds to an approximate 6-fold increase in binding affinity relative to **B44** and **B58**

and an impressive 80-fold improvement compared to the non-substituted **B24**. This constituted the strongest binding compound made thus far.

**Table 2.10. SPR results of combinatorial modification analogues B68–73.** <sup>a</sup>

| Cmpd ID    | R <sup>1</sup> | R <sup>2</sup> | R <sup>3</sup> | SPR <i>K</i> <sub>D</sub> (μM) | LiPE |
|------------|----------------|----------------|----------------|--------------------------------|------|
| <b>B24</b> |                | --H            | --H            | 58.4                           | 2.16 |
| <b>B39</b> |                | --H            | --H            | 99 ± 46 <sup>b</sup>           | 2.56 |
| <b>B44</b> |                | --Me           | --H            | 3.96                           | 2.57 |
| <b>B55</b> |                | --H            | --Cl           | 12.8                           | 1.94 |
| <b>B58</b> |                | --H            | --Me           | 4.93                           | 2.74 |
| <b>B60</b> |                | --H            | --OMe          | 16.8                           | 2.68 |
| <b>B62</b> |                | --H            | --F            | 11.8                           | 2.55 |
| <b>B68</b> |                | --Me           | --Cl           | 2.76 ± 0.92 <sup>c</sup>       | 1.91 |
| <b>B69</b> |                | --Me           | --Me           | 0.73 ± 0.11 <sup>d</sup>       | 2.86 |
| <b>B70</b> |                | --Me           | --OMe          | 58 ± 12 <sup>b</sup>           | 1.40 |
| <b>B71</b> |                | --Me           | --F            | 38 ± 1 <sup>b</sup>            | 1.27 |
| <b>B72</b> |                | --Me           | --H            | 7.38                           | 2.84 |
| <b>B73</b> |                | --Me           | --Me           | 4.98 ± 0.26 <sup>c</sup>       | 2.51 |

<sup>a</sup> Compounds were assayed at multiple concentrations (1.56–100 μM, 7 points, 2-fold dilution; higher-affinity compounds retested at 0.313–20 μM or 0.625–40 μM, 7 points, 2-fold dilution). NA = no significant response above noise. ND = not determined. Highlighted in grey are previously tested compounds. Structural changes from **B24** have been highlighted.

<sup>b</sup> *K*<sub>D</sub> value reported as an average of two independent determinations ± SEM.

<sup>b</sup> *K*<sub>D</sub> value reported as an average of three independent determinations ± SEM.

<sup>b</sup> *K*<sub>D</sub> value reported as an average of four independent determinations ± SEM.

### 2.6.2. Modifications on B69

Further modifications on the newly identified lead compound **B69** were attempted (**Figure 2.16**). Given that the SAR from the preceding round of analogues had returned slightly non-linear results, we sought to also explore previously negative modifications, e.g., sulfonamide *N*-demethylation, linker cyclisation, aniline *N,N*-dialkylation and replacement of the amino group with different functionalities (**B74–80**). Scaffold hopping of the benzene rings to heterocycles was also attempted to address physicochemical properties and perhaps pick up new interactions (**B81**, **B86** and **B87**). Finally, we explored installation of synthetic handles to facilitate fragment growing (**B82–85**).

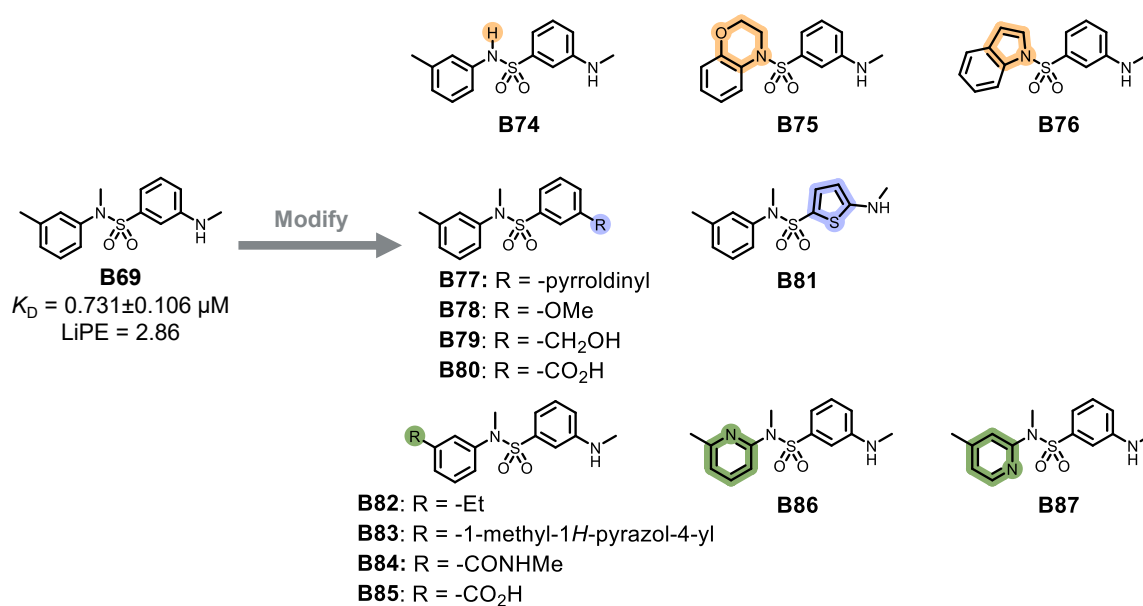
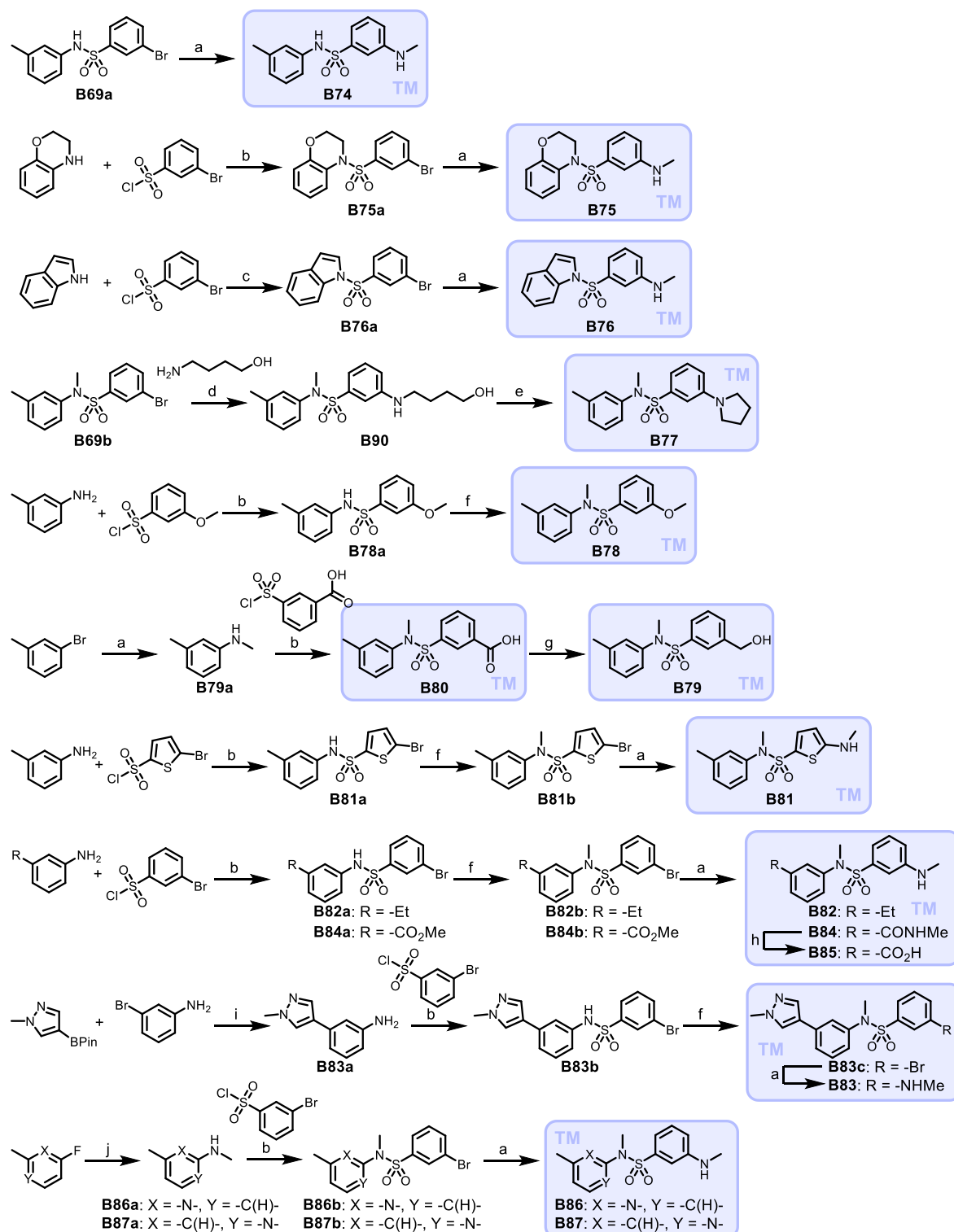


**Figure 2.16.** Design of modification analogues of **B69**. Structural changes from **B69** have been highlighted.

The analogues were generally synthesised employing previously established routes and protocols (**Scheme 2.11**). However, for the indole analogue **B76** a stronger base (NaH) was required to enable *N*-sulfonylation. The pyrrolidine analogue **B77** was serendipitously synthesised by firstly cross-coupling an amino alcohol chain onto the RHA<sub>r</sub> using a previously reported amino acid-promoted Ullmann-type reaction protocol.<sup>105</sup> This was followed by *O*-tosylation, which presumably resulted in pyrrolidine ring formation via an intramolecular S<sub>N</sub>2 displacement of the *O*-tosyl group by the aniline *N*. For **B83**, the elaborate pyrazolyl substituted aniline building block was synthesised using Suzuki-Miyaura cross-coupling conditions.<sup>106</sup>

**Scheme 2.11. Synthesis of modification analogues B74–B87.** <sup>a</sup>

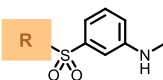
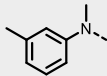
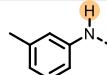
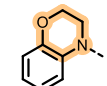
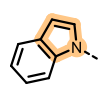
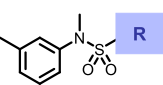
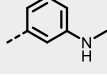
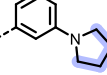
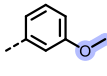
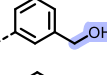
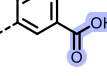
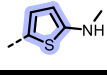


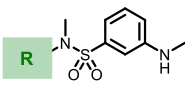
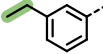
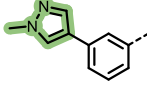
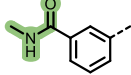
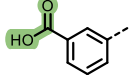
<sup>a</sup> Reagents and conditions: (a) aq. MeNH<sub>2</sub>, Cu, air (sealed vial), 100 °C, 19 h–3 days, 17–91%. (b) Pyridine, DCM, rt, 17–69 h, 52%–quant. (c) NaH, THF, 0 °C–rt, 23 h, 25%. (d) CuI, L-Proline, K<sub>3</sub>PO<sub>4</sub>, DMSO, 80 °C, 18 h, 82%. (e) TsCl, DMAP, Et<sub>3</sub>N, DCM, 0 °C–rt, 15 h, 68%. (f) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, rt, 17–30 h, 79%–quant. (g) BH<sub>3</sub>·THF, THF, 0 °C–rt, 7 h,

60%. (h) aq. NaOH, MeOH, reflux, 3 days, 11%. (i) Pd<sub>2</sub>dba<sub>3</sub>, PCy<sub>3</sub>, K<sub>3</sub>PO<sub>4</sub>, 1,4-dioxane, H<sub>2</sub>O, reflux, 17 h, quant. (j) aq. MeNH<sub>2</sub>, reflux, 24 h, quant. TM = Target molecule.

SPR assay on these analogues returned largely negative results with a majority of the compounds showing significantly reduced affinities (**Table 2.11**). The reduced activity of the negative modifications (e.g., the cyclisation analogues **B74–76**, the pyrrolidine analogue **B77** and the methoxy analogue **B78**) suggests there is some linearity in the SAR going from **B1** and **B24** to **B69**. Replacing the LHA<sub>r</sub> methyl group with functionalities that could facilitate synthetic elaboration (e.g., the pyrazolyl motif of **B83** and the amido group of **B84**) was not tolerable. Extension of the methyl group by one carbon (e.g., the ethyl analogue **B82**) was also unfavourable. This depicted a tight binding mode with limited space for growing on the LHA<sub>r</sub> side. Only scaffold hopping of the LHA<sub>r</sub> to the pyridine **B86** was tolerable with only a small drop in binding affinity but with an improvement in LiPE. Interestingly, the alternative pyridine regioisomer **B87** was inactive, suggesting perhaps the involvement of directional intermolecular interactions.

**Table 2.11.** SPR results of modification analogues **B74–87**. <sup>a</sup>

| Markush                                                                             | Cmpd ID    | R                                                                                   | SPR <i>K<sub>D</sub></i> (μM) | LiPE  |
|-------------------------------------------------------------------------------------|------------|-------------------------------------------------------------------------------------|-------------------------------|-------|
|  | <b>B69</b> |  | 0.73 ± 0.11 <sup>b</sup>      | 2.86  |
|                                                                                     | <b>B74</b> |  | 344 ± 54 <sup>c</sup>         | 0.52  |
|                                                                                     | <b>B75</b> |  | 390                           | 0.52  |
|                                                                                     | <b>B76</b> |  | 490                           | -0.36 |
|  | <b>B69</b> |  | 0.73 ± 0.11 <sup>b</sup>      | 2.86  |
|                                                                                     | <b>B77</b> |  | NA                            | ND    |
|                                                                                     | <b>B78</b> |  | 121 ± 33 <sup>c</sup>         | 0.49  |
|                                                                                     | <b>B79</b> |  | 24 ± 8 <sup>c</sup>           | 2.42  |
|                                                                                     | <b>B80</b> |  | NA                            | ND    |
|                                                                                     | <b>B81</b> |  | 14 ± 4 <sup>c</sup>           | 1.88  |

|                                                                                   |            |                                                                                   |                   |      |
|-----------------------------------------------------------------------------------|------------|-----------------------------------------------------------------------------------|-------------------|------|
|  | <b>B69</b> |  | $0.73 \pm 0.11^b$ | 2.86 |
|                                                                                   | <b>B82</b> |  | $3.6 \pm 1.7^c$   | 1.73 |
|                                                                                   | <b>B83</b> |  | $100 \pm 12^c$    | 1.12 |
|                                                                                   | <b>B84</b> |  | $130 \pm 2^c$     | 2.14 |
|                                                                                   | <b>B85</b> |  | NA                | ND   |
|                                                                                   | <b>B86</b> |  | 2.28              | 2.91 |
|                                                                                   | <b>B87</b> |  | NA                | ND   |

<sup>a</sup> Compounds were assayed at multiple concentrations (1.56–100  $\mu$ M, 7 points, 2-fold dilution; higher-affinity compounds retested from top concentration of 5–50  $\mu$ M, 11 points, 2-fold dilution). NA = no significant response above noise. ND = not determined. Highlighted in grey are previously tested compounds. Structural changes from **B69** have been highlighted.

<sup>b</sup>  $K_D$  value reported as an average of four independent determinations  $\pm$  SEM.

<sup>c</sup>  $K_D$  value reported as an average of two independent determinations  $\pm$  SEM.

From the 3<sup>rd</sup> generation combinatorial modifications, we arrived at the hitherto strongest binding fragment analogue **B69** displaying sub-micromolar binding affinity to NSP16/10 ( $K_D = 0.73 \mu$ M, LiPE = 2.86) and the heterocyclic variant **B86** appearing as a more drug-like candidate with a larger LiPE but still respectable single-digit micromolar affinity ( $K_D = 2.28 \mu$ M, LiPE = 2.91).

## 2.7. Binding mode investigation

From having significantly investigated ligand-based strategies to optimising fragment hit **B1** via multiple iterations of systematic structural modifications and biological assessment, we gratifyingly gained a >370-fold increase in binding affinity with compound **B69**. We next turned to a structure-based approach.

### 2.7.1. X-ray crystallography

To elucidate the binding mode of the compounds, our collaborators at CSIRO first attempted X-ray crystallography. They were successful at growing apo crystals of NSP16/10 and getting X-ray diffraction from

these; however, numerous attempts at obtaining a co-crystal structure of **B69** and its analogues were unsuccessful (data not shown).

### 2.7.2. Differential scanning fluorimetry

Thus far, our collaborators at CSIRO had only characterised the fragment hit and its analogues in a single binding assay, i.e., SPR. Although SPR constitutes a gold standard biophysical assay for fragment screening and characterisation of biomolecular interactions due to its high sensitivity and high-throughput nature,<sup>107</sup> the use of orthogonal secondary assays is strongly recommended for fragment screening.<sup>108</sup> This is firstly to exclude false positives and non-specific binders (which occur more frequently in fragment screening campaigns due to the employment of relatively high test concentrations); secondly, the use of an orthogonal assay based on a different measurement principle may also give additional information about the nature of the ligand binding mode.<sup>108</sup>

We here turned to a thermal shift assay (TSA), where ligand binding is detected by a change in the denaturation temperature of the protein upon heating ( $T_m$ )—this constitutes a rapid, inexpensive and high-throughput technique commonly employed in fragment screening.<sup>108</sup> In one version of the assay, called differential scanning fluorimetry (DSF), denaturation is detected via the use of a fluorescent dye (e.g., SYPRO Orange) which fluoresces preferentially when bound to the hydrophobic core of unfolded proteins whereupon it shows strong fluorescence, whilst its fluorescence is quenched in the polar environment of the bulk proteins (**Figure 2.17**).<sup>109</sup>

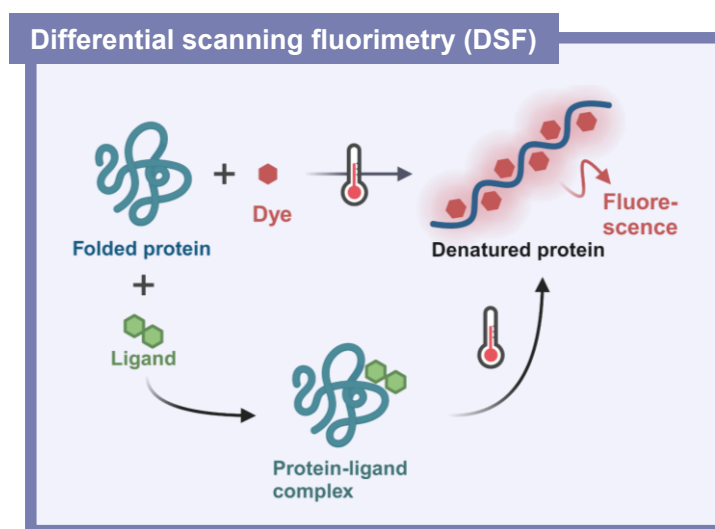


**Figure 2.17. Principle of differential scanning fluorimetry (DSF).** Denaturation of the protein upon increasing temperature is detected by the presence of a fluorescent dye, which binds to the hydrophobic clefts of unfolded proteins whereupon it shows strong fluorescence, whilst its fluorescence is quenched in the polar environment of the bulk



aqueous solution. The binding of a ligand causes stabilisation or destabilisation of the protein, which consequently increases or decreases the melting temperature, respectively. Created with BioRender.com.

An initial test was performed using DSF assay conditions for NSP16/10 previously established by our collaborators at CSIRO, testing the fragment hit **B1**, the advanced analogue **B69**, and **SFN** as a positive control—all at a single concentration of 100  $\mu\text{M}$  (**Figure 2.18A** and **Appendix Figure A3**). Concerningly, a significant change in the melting temperature for **SFN** was not observed, although the test concentration was in large excess over its measured binding affinity for NSP16/10 ( $K_D = 5.5 \mu\text{M}$ , in-house unpublished data). On the other hand, a significant but negative thermal shift for the fragment hit **B1** was seen ( $\Delta T_m = -0.53^\circ\text{C}$ ) and an even greater shift for **B69** ( $\Delta T_m = -1.48^\circ\text{C}$ ), in alignment with their relative affinities. Testing at multiple concentrations (100, 500, and 1000  $\mu\text{M}$ ) also established that the thermal shifts were dose-dependent, consistent with an occupancy-driven effect (**Figure 2.18B**).

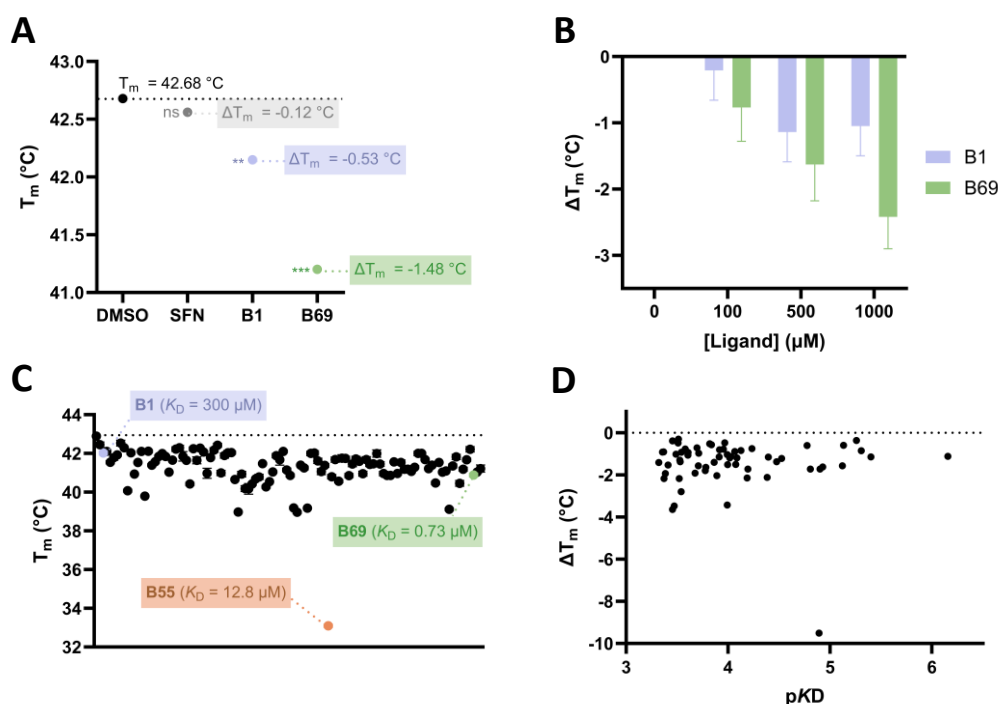


**Figure 2.18. DSF assay on NSP16/10.** The assay was performed on 50  $\mu\text{g}/\text{mL}$  (1.0  $\mu\text{M}$ ) NSP16/10 in 10 mM HEPES pH 7.4, 150 mM NaCl, 1% DMSO. SYPRO Orange was used as the detection dye. Error bars represent the standard deviation from technical triplicates. (A) Initial test on **SFN**, **B1** and **B69** at 100  $\mu\text{M}$ . Statistical significance relative to blank DMSO is indicated as ns = not significant ( $p > 0.05$ ), \*:  $p \leq 0.05$ , \*\*:  $p < 0.005$ , \*\*\*:  $p \leq 0.0005$  in a Student's two-tailed unpaired  $t$ -test. (B) Dose-response test on **B1** and **B69** at 100, 500, and 1000  $\mu\text{M}$ . (C) Larger screen on the library of **B1** analogues at 100  $\mu\text{M}$  with selected compounds and their associated SPR affinities highlighted. (D) A plot of the DSF determined  $\Delta T_m$  values from the larger screen against their SPR determined binding affinities.

We proceeded to screen the entire library of **B1** analogues together with the three initial test compounds (a total of 110 compounds) at 100  $\mu\text{M}$  (**Figure 2.18C**). Interestingly, although **B69** emerged as the strongest binder from the series based on SPR, we observed significantly greater thermal shifts for several other compounds. This included compound **B55**, which had an 18-fold weaker binding than **B69** by SPR but displayed the largest thermal shift of the series ( $\Delta T_m = -9.51\text{ }^\circ\text{C}$ ). Indeed, when plotting the thermal shifts against the binding affinities, we could not observe any apparent correlation (**Figure 2.18D**).

The observed destabilisation would perhaps explain why crystallisation of NSP16/10 with any of the compounds was unsuccessful. Ligand binding is usually expected to result in protein stabilisation and thereby a positive thermal shift, which will depend on the concentration and affinity of the ligand; however, it is well known that negative thermal shifts can also occur if the ligand binds preferentially to non-native or unfolded states of the protein.<sup>110</sup> Furthermore, the magnitude of the thermal shift will depend predominantly on the entropic component of ligand binding and can therefore correlate poorly with binding affinity (which is a summation of both entropic and enthalpic terms).<sup>111</sup> Still, the lack of an apparent thermal shift for the known inhibitor **SFN** calls into question the meaningfulness of these assay results. We speculated if this could be due to the presence of complexed endogenous SAM in the NSP16/10 batch that we tested, which would (at least to some extent) block SAM-competitive inhibitors such as **SFN** from binding. This could then perhaps suggest a different binding mode or mode of action for **B1** and its analogues compared with the SAM-mimicking inhibitor.

### 2.7.3. Competitive SPR studies

The DSF results prompted us to investigate whether the compounds might not be competitive with SAM. For this purpose, our collaborators at CSIRO reformed the SPR test of **B69** in the presence of excess SAM (100  $\mu\text{M}$ ) in the running buffer. For a SAM-competitive inhibitor, we would expect this to markedly decrease the measured binding affinity due to blockage of the SAM-binding site of the immobilised protein. For **B69**, no apparent change in the response could be observed by inspecting the resulting sensorgrams, and a binding affinity of  $K_D = 0.80\text{ }\mu\text{M}$  could be calculated from the dose-response curve, which is not significantly different from the previously determined  $K_D = 0.73 \pm 0.11\text{ }\mu\text{M}$  in the absence of SAM (**Figure 2.19A–B**). These results strongly suggest that **B69** is not SAM-competitive and therefore unlikely to bind in the SAM-binding site.

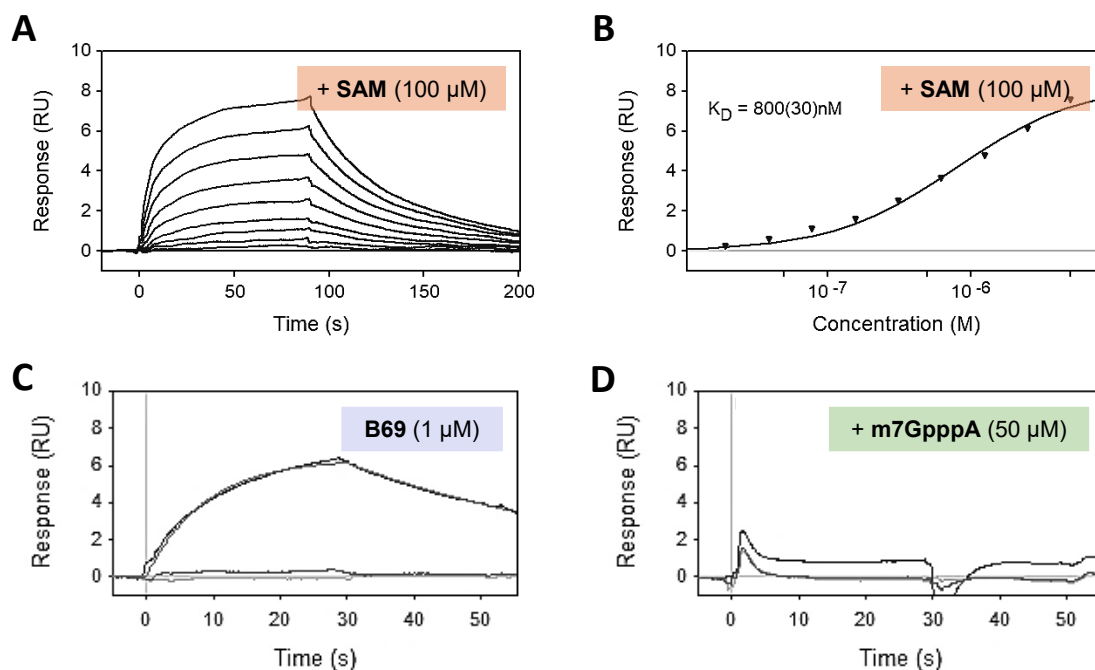


**Figure 2.19. Competitive SPR experiments on B69.** (A) Sensorgrams of a multi-concentration test of **B69** (9 points, 2-fold dilution from top concentration of 3  $\mu\text{M}$ ) in the presence of 100  $\mu\text{M}$  SAM in the running buffer. (B) Concentration-response curve from the multi-concentration test of **B69** in the presence of 100  $\mu\text{M}$  SAM gives a  $K_D = 0.80$   $\mu\text{M}$ . (C) Sensorgram of a single-dose test of **B69** at 1  $\mu\text{M}$ . (D) Sensorgram of a single dose test of **B69** at 1  $\mu\text{M}$  co-injected with 50  $\mu\text{M}$  **m7GpppA** after subtraction of the binding response of **m7GpppA**.

We next speculated if the compounds might bind to the RNA substrate binding site. To address this putative binding mode, our collaborators at CSIRO employed the cap0 RNA analogue **m7GpppA** which binds in the RNA pocket (PDB ID 6WQ3 and 6WKS) with a measured  $K_D = 2.4$   $\mu\text{M}$  (in-house unpublished data, which correlates well with a reported  $K_D = 6.6$   $\mu\text{M}$ ).<sup>84,112</sup> When testing **B69** at 1  $\mu\text{M}$  with 50  $\mu\text{M}$  co-injected **m7GpppA**, an almost complete abrogation of its binding response was observed (**Figure 2.19C–D**).

Although this remains indirect evidence, these competitive SPR assay results indicate that **B69** is competitive with **m7GpppA** and that the fragment analogue may be binding in the RNA substrate pocket.

#### 2.7.4. Molecular docking

With this binding pocket hypothesis in mind, we next turned to computational modelling, employing the published co-crystal structures of NSP16/10 in complex with **m7GpppA**. Previous crystallographic work on NSP16/10 in the presence and absence of **m7GpppA** has already established that the RNA binding pocket undergoes significant conformational changes upon binding of the cap0 RNA analogue especially around the loop regions flanked by Asp6824 and Leu6834 and between Ser6943 and Met6929.<sup>84</sup> These changes open up the pocket to allow for binding of the cap0 with formation of extensive H bond

interactions (**Figure 2.20A–B**). Based on this analysis, there appeared to be a large induced fit effect at play—the degree of which may depend on the structure of the ligand.

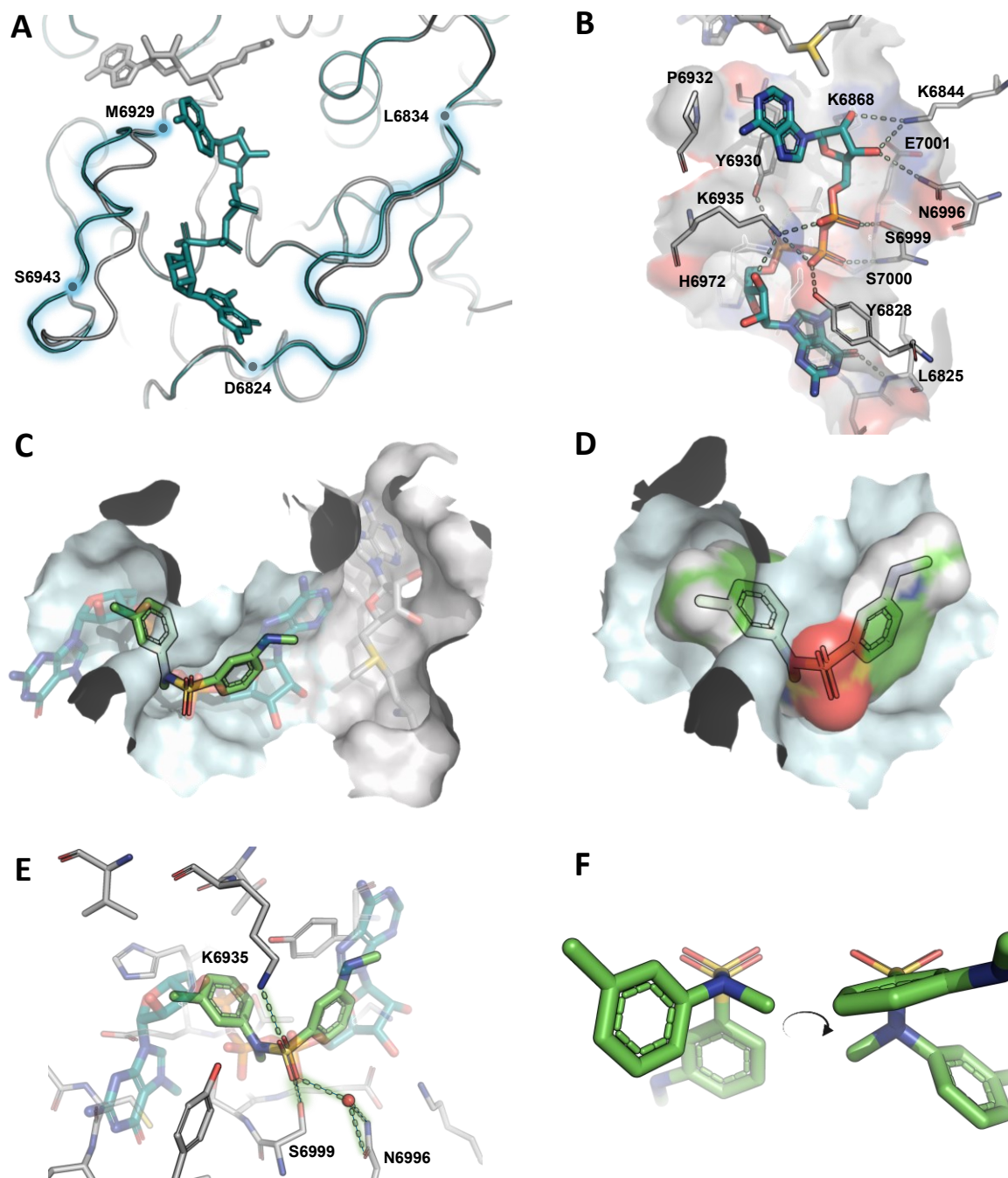


**Figure 2.20. Binding pocket analysis and molecular docking into NSP16/10.** Putative H bonds have been indicated as green stippled lines. Created with PyMOL (version 2.5.4).<sup>47</sup> (A) Co-crystal structure of NSP16/10 in complex with SAM (grey, PDB ID 6W4H) superposed with a co-crystal structure with SAM and m7GpppA (teal, PDB ID 6WKS).<sup>84, 88</sup> The loop regions that undergo significant translational and conformational change have been highlighted. (B) Co-crystal structure of NSP16/10 in complex with SAM (grey sticks) and m7GpppA (teal sticks) (PDB ID 6WKS). Nearby residues within 4 Å from m7GpppA are shown as sticks. (C) Induced fit docking pose of B69 (green sticks) into NSP16/10

(PDB ID 6WVN). (D) The solvent accessible surfaces of the pocket and the docking pose of **B69**. (E) The docking pose of **B69** (green sticks) superposed with the X-ray structure pose of **m7GpppA** (teal sticks, PDB ID 6WVN). (F) Conformational analysis of the docking pose of **B69**.

To predict the binding mode of **B69**, we employed Molecular Operating Environment (MOE) as the docking software.<sup>113</sup> In order to account for the expected large protein inducibility, we firstly performed the docking in an ensemble mode. Here, **B69** was docked into multiple published protein structures of NSP16/10 in different complexation states, i.e. 1) with SAM alone (PDB ID 6W4H),<sup>84</sup> 2) with SAM and **m7GpppA** (PDB ID 6WVN, 6WKS, 7JPE, and 7KOA),<sup>88, 112, 114</sup> and 3) with SAH and **m7GpppA** (PDB ID 6WQ3 and 6WRZ).<sup>84</sup> Furthermore, using the induced fit mode of MOE, we also sampled different conformational states of the nearby pocket residues during the docking process. The resulting docking results were assessed through visual inspection as well as from weighing their associated scores. From these computational experiments, we identified a binding pose of **B69** which appeared plausible for a number of reasons. Firstly, it is occupying the cap0 binding pocket in agreement with the **m7GpppA**-competition experiment, where it shows excellent complementarity with the pocket surface (**Figure 2.20C–D**). Additionally, the ligand effectively mimics the bent geometry of **m7GpppA** around the ribose and phosphate region. Crucially, the sulfonyl group of **B69** perfectly overlaps with the  $\gamma$ -phosphate of **m7GpppA** and is similarly strongly tethered in place through extensive H bonds or water-mediated H bond interactions with K6935, N6996, and S6999 (**Figure 2.20E**). The biarylsulfonamide also retains its preferred staggered conformation, where the *N*-lone pair and the aryl *C* p-orbital are bisecting the two *S*–*O* double bonds (**Figure 2.20F**). Finally, the binding mode conforms largely with some of the major SAR results, including the abrogated binding affinities of the azo analogue (**B37**) and the amide analogue (**B38**); these analogues would lose the extensive H bond interactions of the sulfonyl group and/or not be able to adopt the bent geometry required to complement the cap0 pocket surface. From these considerations, the identified docking pose emerged as a likely binding mode hypothesis.

#### 2.7.5. The bisubstrate inhibitor idea

Utilising the putative binding mode of **B69**, we next investigated carrying out more dramatic structure-guided transformations. We envisioned that one way of gaining a substantial amount of binding energy would be to probe the adjacent SAM binding pocket. We were especially intrigued by the possibility of linking **B69** to a SAM-like ligand to potentially obtain a bisubstrate inhibitor.

For this purpose, the *N*–Me vector emerged as the most suitable attachment point. As the linker, a non-functionalised alkyl chain appeared as the most obvious option to confer flexibility and simplicity of

design and synthesis. To commence this fragment linking study, a small set of scouting analogues with various linker lengths and end group functionalities were designed (**Figure 2.21**). These analogues were synthetically accessed via the previously employed amino acid-assisted Ullmann-type cross-coupling (**Scheme 2.12**).<sup>115</sup>

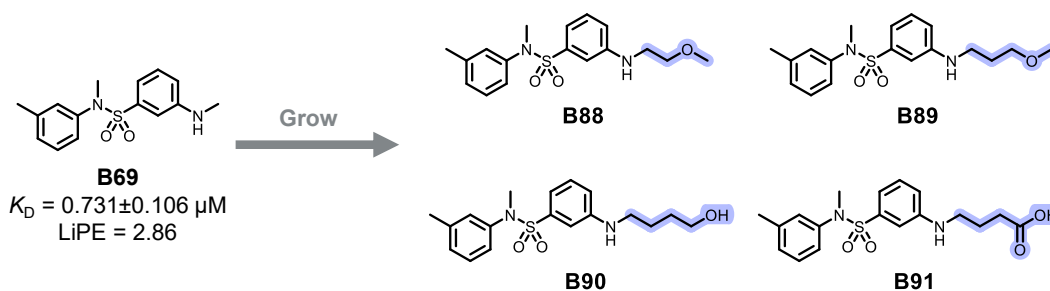
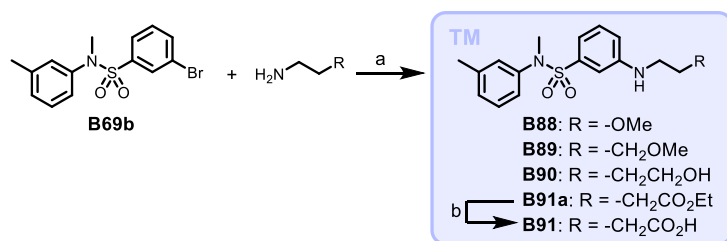


**Figure 2.21.** Design of linker probe analogues of **B69**. Structural changes from **B69** have been highlighted.

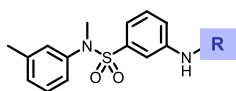
**Scheme 2.12.** Synthesis of linker probe analogues **B88–91**.<sup>a</sup>



<sup>a</sup> Reagents and conditions: (a)  $\text{CuI}$ , L-Proline,  $\text{K}_3\text{PO}_4$ , DMSO,  $80^\circ\text{C}$ , 18–22 h, 16–92%. (b) aq.  $\text{NaOH}$ , EtOH, rt, 3 h, 55%. TM = Target molecule.

Discouragingly, installing any of the linkers onto **B69** significantly reduced the SPR-determined affinity (**Table 2.12**). Given the low number of sampled linkers, it is difficult to derive any reliable SAR from the series; however, it appears that the longer linkers having polar cap groups are less favourable than the shorter, non-polar ones (i.e. **B69** > **B88** > **B89** > **B90** > **B91** in accordance with their linker lengths of 1, 4, and 5 atoms). According to the docking pose, the *N*-Me points to a large cavity that should be able to spatially accommodate all the linkers, i.e., we wouldn't expect any steric clash. Rather than unfavourable van der Waals contacts, the reduced affinities could perhaps instead be explained by a conformational penalty. This could either be of enthalpic nature, as the long linkers might result in a hydrophobically collapsed global energy conformation in solution, which needs to be disrupted in order for the ligand to bind. Alternatively (or additionally), an entropic effect may be at play, as the long alkyl chains can toggle between multiple conformational states in solvent, whereas binding may significantly freeze the linkers in place.

Table 2.12. SPR results of linker probe analogues B88–91. <sup>a</sup>



| Cmpd ID | R    | SPR $K_D$ ( $\mu$ M)         | LiPE  |
|---------|------|------------------------------|-------|
| B69     | --Me | $0.73 \pm 0.11$ <sup>b</sup> | 2.86  |
| B88     |      | $11 \pm 2$ <sup>c</sup>      | 1.64  |
| B89     |      | $115 \pm 34$ <sup>c</sup>    | 0.29  |
| B90     |      | $191 \pm 12$ <sup>c</sup>    | 0.79  |
| B91     |      | $420 \pm 14$ <sup>c</sup>    | -0.02 |

<sup>a</sup> Compounds were assayed at multiple concentrations (1.56–100  $\mu$ M, 7 points, 2-fold dilution; higher-affinity compounds retested from top concentration of 20–100  $\mu$ M, 7 points, 2-fold dilution). Highlighted in grey are previously tested compounds. Structural changes from **B69** have been highlighted.

<sup>b</sup>  $K_D$  value reported as an average of four independent determinations  $\pm$  SEM.

<sup>c</sup>  $K_D$  value reported as an average of two independent determinations  $\pm$  SEM.

Despite the negative results from these scouting analogues, we proceeded to attempt linkage. We reasoned that installing a SAM-ligand could increase the enthalpic gain of binding and compensate for the proposed conformational energy penalty. For the simplicity of the design and ease of synthesis, we opted for **MTA** as our SAM analogue, which still possesses good binding affinity ( $K_D$  = 2.4  $\mu$ M, in-house unpublished data). According to modelling, a 4-carbon linker in between the amino *N* of **B69** and the thioether *S* of **MTA** would be optimal for retaining the original putative binding mode of the fragment and the crystal structure pose of the SAM-analogue (**Figure 2.22A**). However, we also sampled a 3-carbon linker version, as the previous SAR suggested a slight favourability for shorter linkers over longer ones and to further account for conformational protein flexibility. For synthetic ease, we also explored exchanging the sulfur atom with an oxygen. This gave a total of three linked analogue designs (**Figure 2.22B**).

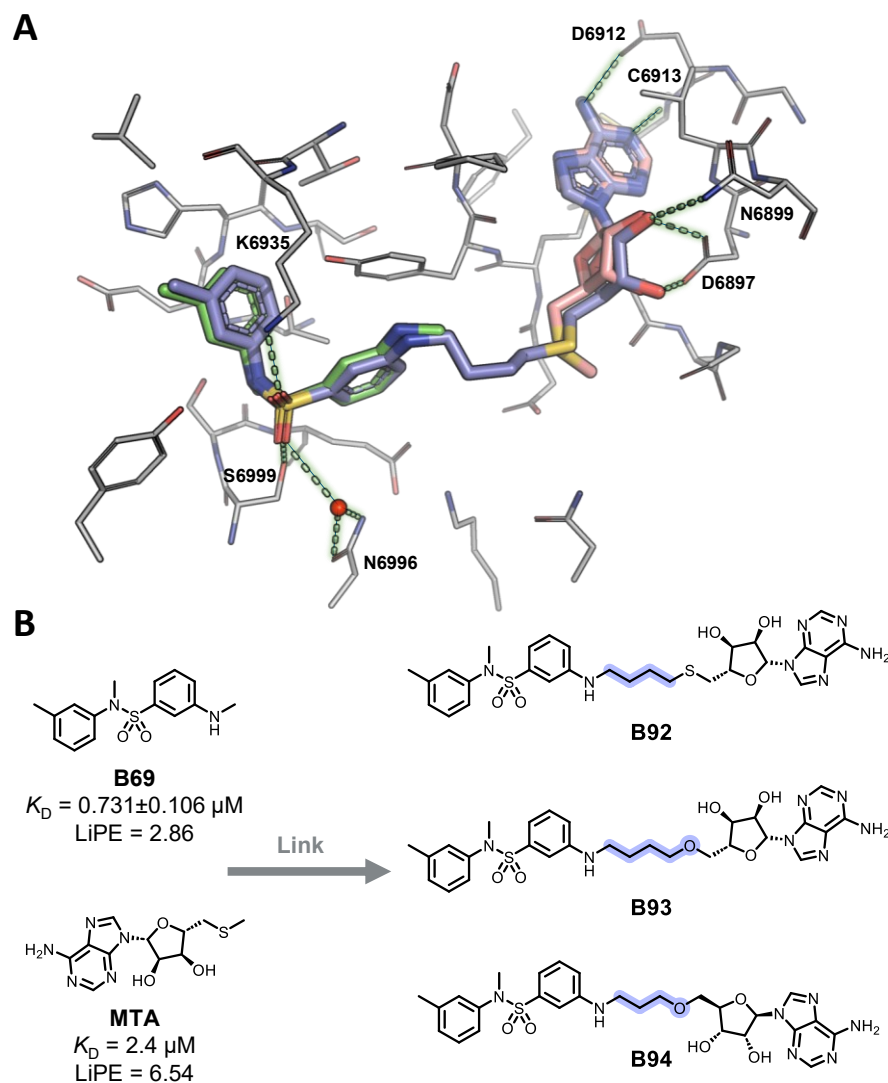
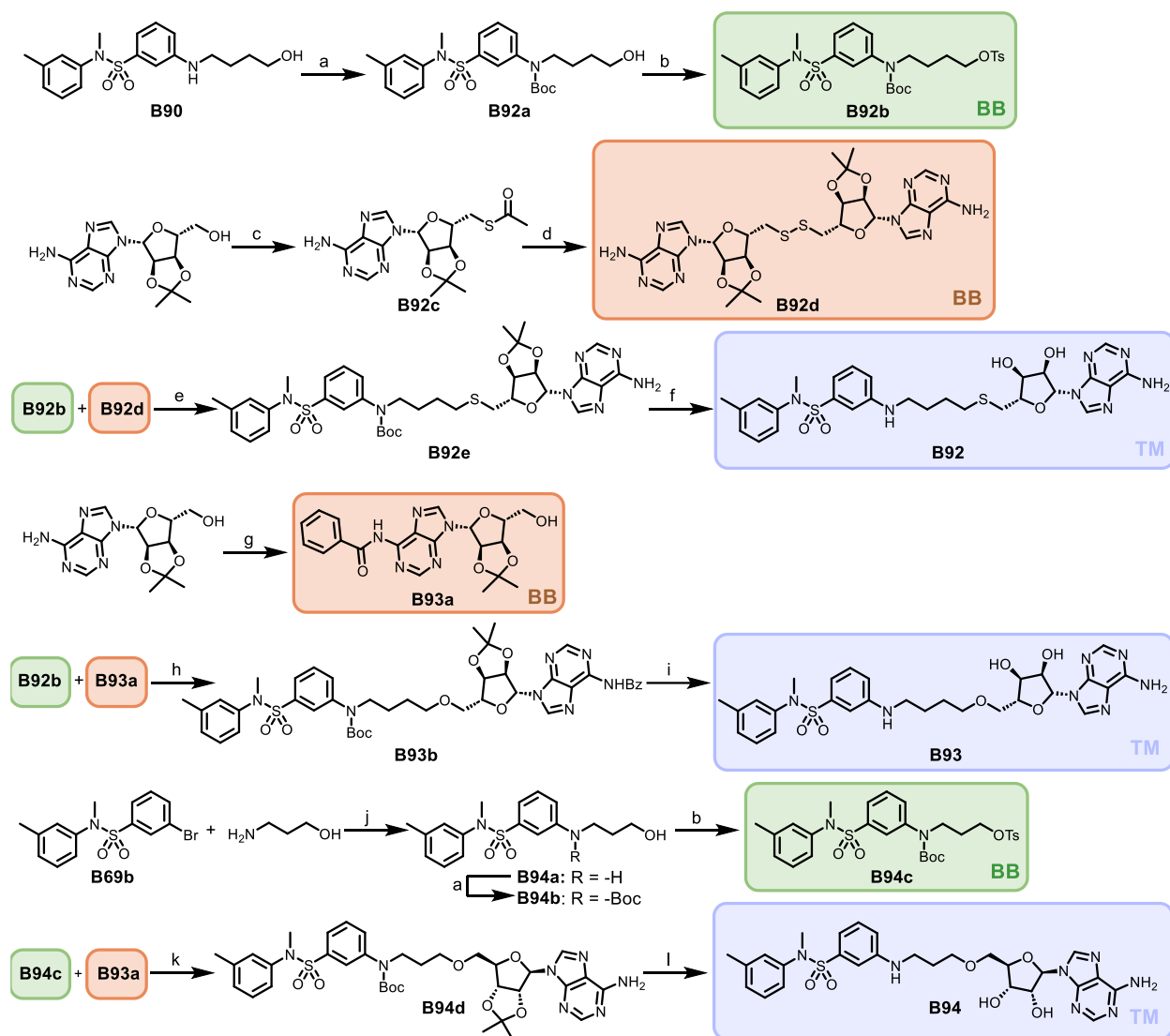


**Figure 2.22. Design of linking analogues of B69 and MTA.** (A) Docking pose of linked analogue **B92** (purple) superimposed with the docking pose of **B69** (green) and the X-ray crystal structure of **MTA** (PDB ID 8C5M). The docking poses were obtained from using the protein structure from PDB ID 6WVN as the receptor.<sup>84</sup> Created with PyMOL (version 2.5.4). (B) Schematic design of the linked analogue **B92–94**. Structural changes from **B69** and **MTA** have been highlighted.

The synthesis of these linked analogues relied on reacting two key intermediates in a late-stage (thio)ether bond formation: 1) the **B69**-derived fragment having the linker attached and functionalised with an electrophilic motif (referred to as the LHS intermediate) and 2) the **MTA** or adenosine-derived fragment protected with suitable protective groups to prevent chemoselectivity issues (referred to as the RHS intermediate) (**Scheme 2.13**). To access the LHS intermediate, the appropriate linker functionalised with a *O*-tosylate leaving group was installed onto the fragment scaffold, which was achieved by firstly cross-coupling the previously synthesised aryl bromide intermediate **B69b** with the appropriate alkanolamine (as done previously using Ullmann-type conditions to synthesise **B90**).



**Scheme 2.13. Synthesis of linked analogues B92–94.** <sup>a</sup>



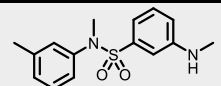
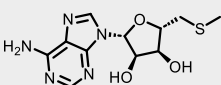
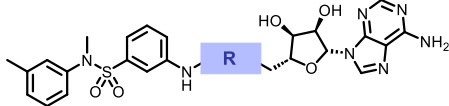
<sup>a</sup> Reagents and conditions: (a)  $\text{Boc}_2\text{O}$ ,  $80^\circ\text{C}$ , 25–50 h, 69–70%. (b)  $\text{TsCl}$ , DMAP,  $\text{Et}_3\text{N}$ , DCM,  $0^\circ\text{C}$ –rt, 16 h, 64–71%. (c) DIAD, PS- $\text{PPh}_3$ , THF,  $0^\circ\text{C}$ –rt, 4 h, 20%. (d)  $\text{NH}_4\text{OH}$ , MeOH, rt, 51 h, 46%. (e) NaOMe,  $\text{NaBH}_4$ , MeOH, rt, 16 h, 60%. (f) TFA, DCM, rt, 2 h; then  $\text{H}_2\text{O}$ , rt, 14 h, 36% (over 2 steps). (g) TMS-Cl, pyridine, rt, 30 min; then  $\text{BzCl}$ , rt, 4 h; then  $\text{NH}_4\text{OH}$ ,  $0^\circ\text{C}$ –rt, 30 min, 65% (over 3 steps). (h) NaH, DMF,  $0^\circ\text{C}$ –rt, 3 h, 26%. (i) TFA, DCM, rt, 4.5 h; then  $\text{H}_2\text{O}$ , rt, 41 h; then  $\text{NH}_4\text{OH}$ , MeOH, rt, 42 h, 15% (over 3 steps). (j)  $\text{CuI}$ , L-Proline,  $\text{K}_3\text{PO}_4$ , DMSO,  $80$ – $100^\circ\text{C}$ , 48 h, 30%. (k) KOH, DMSO, rt, 17 h; then aq. NaOH, 1,4-dioxane,  $50$ – $70^\circ\text{C}$ , 25 h, 49%. (l) TFA, DCM, rt, 3 h; then  $\text{H}_2\text{O}$ , rt, 2.5 h, 33% (over 2 steps). TM = Target molecule. BB = Building block.

To prevent cyclisation upon activation of the pendant hydroxy group, the aniline *N* was subsequently Boc-protected, which was chemoselectively achieved by heating the compound in neat Boc anhydride.<sup>116</sup> Finally, standard *O*-tosylation was performed by reacting the alcohols with tosyl chloride in the presence of catalytic amount of 4-dimethylaminopyridine (DMAP) to make the key LHS intermediates **B92b** and **B94c**. For the RHS intermediate required to make **B92**, 2,3-acetal-protected adenosine was firstly subjected to

Mitsunobu reaction conditions with thioacetic acid to install the sulfur atom (as a thioacetate). Upon hydrolysis of the thioester, the resulting thioadenosine intermediate **B92d** was found to exist solely in its oxidised disulfide form. In the central and convergent thioether bond step, sodium borohydride was added alongside a strong base (i.e., sodium methoxide) to reduce the disulfide and produce the sodium thiolate *in situ* which was captured by the alkyl tosylate **B92b**. Upon subsequent acetal- and Boc-deprotection, this yielded the TM **B92**. For the ethers, **B93** and **B94**, the protected adenosine building block **B93a** was directly reacted with either alkyl tosylate **B92b** or **B94c** using either sodium hydride or potassium hydroxide in DMSO as the base, which upon global deprotection yielded **B93** and **B94**, respectively.

Unfortunately, the SPR results from these linked analogues showed dramatically reduced binding affinities compared with both constituent fragments (**Table 2.13**). The best analogue was the 4-carbon thioether linker design **B92**, showing a  $K_D = 25.1 \mu\text{M}$ , which corresponds to a 34-fold and 10-fold weakened activity compared with **B69** and **MTA**, respectively. The analogue also displayed a lowered LiPE (1.82).

**Table 2.13.** SPR results of linked analogues **B92–94**. <sup>a</sup>

| Structure / Markush                                                                 | Cmpd ID    | R                                                                                   | SPR $K_D$ ( $\mu\text{M}$ ) | LiPE |
|-------------------------------------------------------------------------------------|------------|-------------------------------------------------------------------------------------|-----------------------------|------|
|   | <b>B69</b> | -                                                                                   | $0.73 \pm 0.11^b$           | 2.86 |
|  | <b>MTA</b> | -                                                                                   | 2.4                         | 6.54 |
|  | <b>B92</b> |  | 25.1                        | 1.82 |
|                                                                                     | <b>B93</b> |  | 265                         | 2.08 |
|                                                                                     | <b>B94</b> |  | 254                         | 2.05 |

<sup>a</sup> Compounds were assayed at multiple concentrations (1.56–100  $\mu\text{M}$ , 7 points, 2-fold dilution. Highlighted in grey are previously tested compounds. Structural changes from **B69** and **MTA** have been highlighted.

<sup>b</sup>  $K_D$  value reported as an average of four independent determinations  $\pm$  SEM.

These results may be a consequence of poor linker design. The flexibility of the linkers may come with a significant entropic penalty that cannot be compensated by the newly formed interactions. Alternatively (or additionally), the linkers may adopt a strained high-energy conformation in the intended binding mode or a collapsed conformation in solution. These entropic and conformational factors may not be accurately accounted for by standard molecular docking, and it is generally known that the accuracy of molecular

docking drops significantly with increasing number of rotatable bonds and molecular complexity. Alternatively (and perhaps more problematically), the binding mode hypothesis of **B69** may not be correct.

In summary, the structure-guided optimisation efforts did not yield any further improvement in the binding affinity of our lead compound **B69**. Given that NSP16/10 constitutes a heterodimeric complex that undergoes significant conformational changes upon ligand binding, experimental structural elucidation is probably required to reliably elucidate the binding mode of **B69** and thereby enable rational structure-based design.

## 2.8. Azo extension

From the extensive medicinal chemistry work on optimising **B1**, we firstly obtained a potent lead compound **B69** and further established a comprehensive structure-activity relationship map around the fragment scaffold. Armed with this knowledge, we revisited the original aim of transforming the fragment hit into a photoswitchable azo ligand. Our early azologisation efforts gave largely negative results, so we turned to azo extension as an alternative approach.

### 2.8.1. First-generation azobenzene-based extensions

In terms of selecting the scaffold for extension, we were conscious that substitution on the fragment had previously shown non-linear effects (i.e., substitution on the RHAr affects the optimal substitution on the LHAr) and that fragment elaboration may benefit affinity but be destructive for photochemical properties. As the fragment core, we therefore chose the simplified analogue **B24** ( $K_D = 58.4 \mu\text{M}$ ) rather than the decorated **B69**. We further chose to extend the original fragment hit **B1** ( $K_D = 276 \mu\text{M}$ ) and the reversed linker analogue **B39** ( $K_D = 99 \mu\text{M}$ ).

We initially attempted to extend the fragment scaffolds with an azobenzene motif. The amino group of each fragment core was here selected as the extension point, as they would serve as convenient synthetic handles to access the corresponding azo extension analogues. Furthermore, we were previously able to install a benzyl group onto the amino *N* of **B24**, which was tolerable and even resulted in a modest boost in binding affinity (**B47**,  $K_D = 12.5 \mu\text{M}$ ). In addition, our substitution studies on **B24** also uncovered a clear preference for growing from the 3-position of the LHAr, so we also chose this as a site for azo extension. A small series of four azobenzene-based extension analogues was designed in total (**Figure 2.23**).

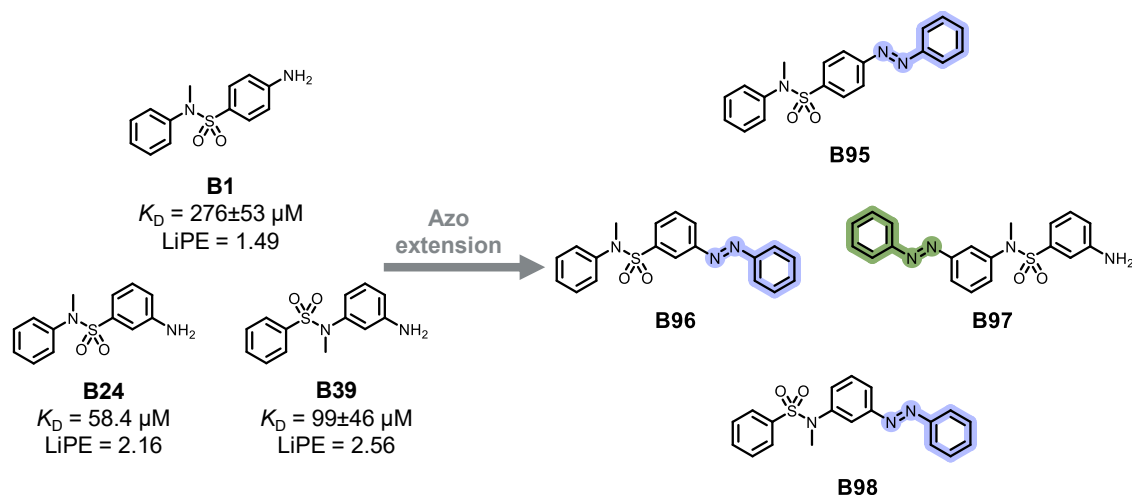
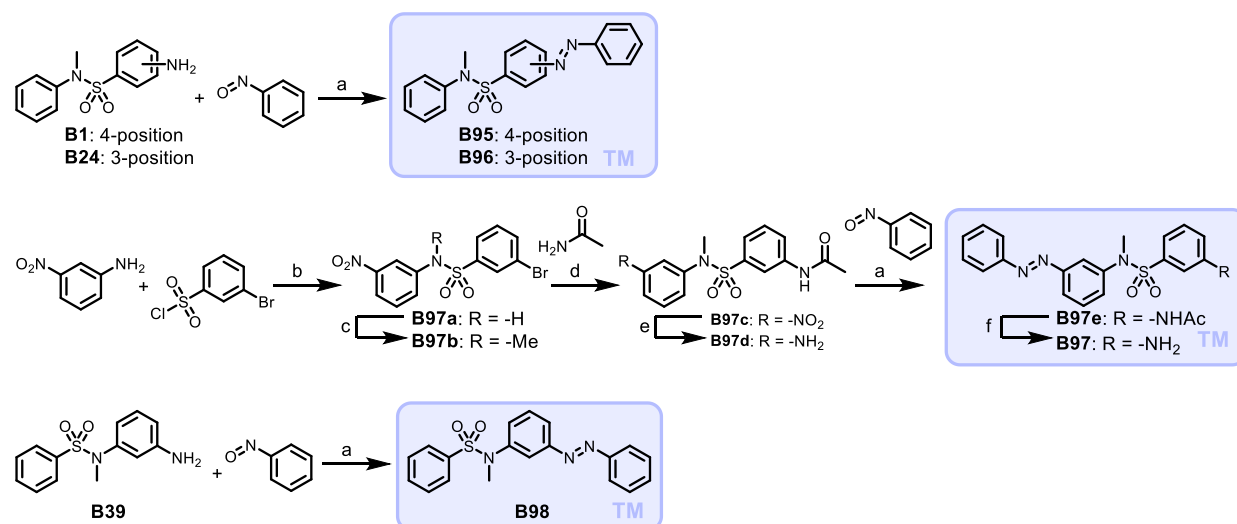


**Figure 2.23. Design of first-generation azobenzene-based extension analogues of B1/24/39.** Structural changes from **B1**, **B24** and **B39** have been highlighted.

The analogues **B95**, **B96**, and **B98** were synthesised from condensing **B1**, **B24**, and **B39** with nitrosobenzene using the previously employed acidic Baeyer-Mills reaction conditions (Scheme 2.14).<sup>100</sup> For the LHA<sub>r</sub> amino-functionalised analogue **B97**, protective chemistry was required. We here opted for an *N*-acetyl group as a protective group for the RHA<sub>r</sub>, which is orthogonal to the nitro group used to mask the LHA<sub>r</sub> amino group. Conventional *N*-sulfonylation of 3-nitroaniline and sulfonamide *N*-methylation afforded the biarylsulfonamide skeleton (**B97b**). This aryl bromide intermediate was then subjected to Buchwald-Hartwig cross-coupling conditions to install the RHA<sub>r</sub> acetamido group (giving **B97c**).<sup>117</sup> Hydrogenation selectively demasked the LHA<sub>r</sub> amino group (giving **B97d**), setting the stage for the Baeyer-Mills reaction with nitrosobenzene (affording **B97e**). A final deprotection via acid-catalysed amide hydrolysis furnished the TM **B97** in a total of six synthetic steps and in an overall yield of 26%.

In accordance with prior negative results from functionalising the RHA<sub>r</sub> 4-position of **B1**, the azo extension analogue **B95** showed inactivity in both the dark and photoswitched state (Table 2.14). In further accordance with the previous SAR, extension from the 3-position of either side of the fragments gave more positive results. Specifically, extension from the LHA<sub>r</sub> 3-position of **B24** (i.e., **B97**) and from the RHA<sub>r</sub> 3-position of **B39** (i.e., **B98**) both resulted in retained absolute affinity ( $K_D = 71.0$  and  $49.3 \mu\text{M}$ , respectively) and a modest 2–3-fold change in activity upon photoirradiation—with the photoswitched state yielding a stronger binding response in both cases. However, both **B97** and **B98** had negative LiPE values, suggesting that these were poor binders relative to their lipophilicities.

**Scheme 2.14. Synthesis of azobenzene-based extension analogues B95–98.** <sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) AcOH, 40 °C, 23 h–3 days, 64%–quant. (b) Pyridine, DCM, rt, 24 h, 90%. (c) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, rt, 18 h, 95%. (d) Pd(OAc)<sub>2</sub>, *t*BuBrettPhos, K<sub>3</sub>PO<sub>4</sub>, *t*-BuOH, H<sub>2</sub>O, 110 °C, 2.5 h, 69%. (e) Pd/C, MeOH, EtOAc, H<sub>2</sub> (1 bar), rt, 6 h, 92%. (f) SOCl<sub>2</sub>, MeOH, reflux, 16 h, 56%. TM = Target molecule.

**Table 2.14. Photochemical properties and SPR results of azobenzene-extended analogues B95–98.** <sup>a</sup>

| Markush | Cmpd ID | R                 | C <sub>1h</sub> (%) | PSD <sub>365nm</sub> (%) | Dark K <sub>D</sub> (μM) | Irr. K <sub>D</sub> (μM) | FC  | LiPE  |
|---------|---------|-------------------|---------------------|--------------------------|--------------------------|--------------------------|-----|-------|
|         | B1      | --NH <sub>2</sub> | -                   | -                        | 276 ± 53 <sup>b</sup>    | ND                       | -   | 1.51  |
|         | B95     |                   | <1                  | 72                       | NA                       | NA                       | ND  | ND    |
|         | B24     | --NH <sub>2</sub> | -                   | -                        | 58.4                     | ND                       | -   | 2.16  |
|         | B96     |                   | <1                  | 43                       | 15.6                     | 15.6                     | 1.0 | 0.15  |
|         | B24     | --H               | -                   | -                        | 58.4                     | ND                       | -   | 2.16  |
|         | B97     |                   | <1                  | 71                       | 196                      | 71.0                     | 2.8 | -0.07 |
|         | B39     | --NH <sub>2</sub> | -                   | -                        | 99 ± 46 <sup>b</sup>     | ND                       | -   | 2.56  |
|         | B98     |                   | <1                  | 72                       | 101                      | 49.3                     | 2.0 | -0.64 |

<sup>a</sup> Compounds were assayed at multiple concentrations (6.25–100 μM, 5 points, 2-fold dilution). Photoswitchable compounds were tested both in the dark (Dark K<sub>D</sub>) and after photoirradiation with 365 nm light (Irr. K<sub>D</sub>). NA = no significant response above noise. ND = not determined. Highlighted in grey are previously tested compounds. Structural changes from B1, B24, and B39 have been highlighted.

<sup>b</sup> K<sub>D</sub> value reported as an average of two independent determinations ± SEM.

On the other hand, extension from the RHA<sub>r</sub> 3-position of **B24** gave compound **B96** with a  $K_D = 15.6 \mu\text{M}$ , corresponding to a significant 3.7-fold increase in binding affinity relative to **B24** and is comparable to that of the *N*-benzylated analogue **B47**. Although the LiPE dropped significantly, it remained positive. Unfortunately, **B96** showed no significant difference in binding between the dark and photoirradiated state. This lack of differential activity could be attributed to two possible factors: 1) The *E*- and *Z*-isomers do not inherently have significantly different affinities and/or 2) photoirradiation does not result in a significant enrichment of the *Z*-isomer.

### 2.8.2. Substitution on B96

To address the first possibility that the intrinsic binding affinities of the *E*- and *Z*-isomer of **B96** are not significantly different, we set out to design and synthesise a set of substituted analogues in order to possibly pick up additional binding interactions and thereby increase the binding affinity for one of the isomers. We were here inspired by the positive results that were obtained previously from substituting the LHA<sub>r</sub> 3-position with a variety of functional groups and proceeded to reproduce these substitutions (**Figure 2.24**). A far from distal modifications, substitutions on the azobenzene scaffold itself was also attempted. The resulting analogues **B99–109** were all readily accessed by subjecting **B24** and its substituted analogues to Baeyer-Mills reaction conditions (**Scheme 2.15**).

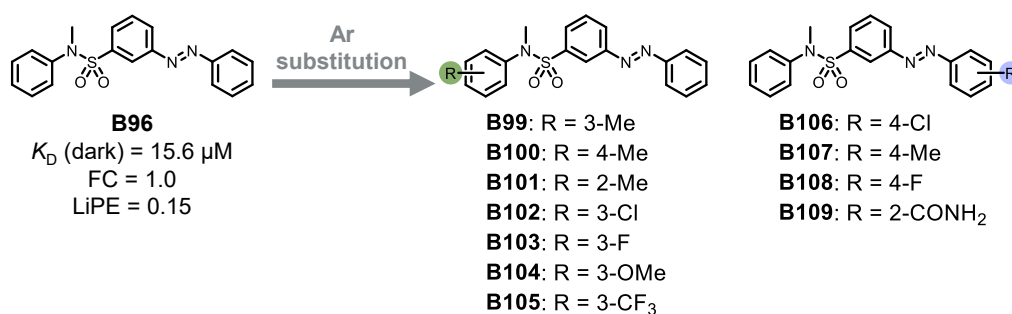
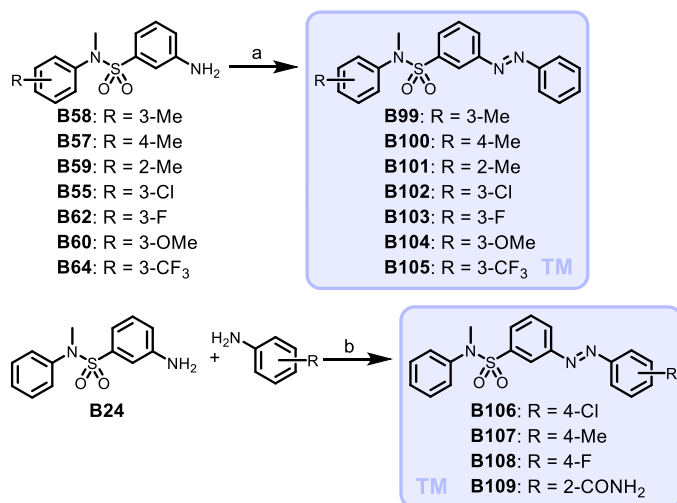


**Figure 2.24.** Design of substituted analogues of **B96**. Structural changes from **B96** have been highlighted.

Substitution of **B96** did result in an observed differential activity for some of the analogues with the RHA<sub>r</sub> 4-fluorosubstituted analogue **B108** emerging as the most promising with a 3.6-fold change in binding affinity (**Table 2.15**). However, the entire series suffered from a significant loss in absolute binding affinity with compound **B108** showing a 5.1-fold reduced binding relative to **B96**. The negative LiPE values also indicated that the compounds displayed low-quality binding.

**Scheme 2.15. Synthesis of substituted analogues B99–109.** <sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) AcOH, 40 °C, 22–33 h, 38%–quant. (b) Substituted aniline or **B24**, Oxone, DCM, H<sub>2</sub>O, rt, 3–16 h; then **B24** or substituted aniline, AcOH, 40–80 °C, 22 h–3 days, 14–42% (over 2 steps). TM = Target molecule.

**Table 2.15. Photochemical properties and SPR results of substituted analogues B99–109.** <sup>a</sup>

| Cmpd ID     | R <sup>1</sup>     | R <sup>2</sup>       | C <sub>1h</sub><br>(%) | PSD <sub>365nm</sub><br>(%) | Dark K <sub>D</sub><br>(μM) | Irr. K <sub>D</sub><br>(μM) | FC  | LiPE  |
|-------------|--------------------|----------------------|------------------------|-----------------------------|-----------------------------|-----------------------------|-----|-------|
| <b>B96</b>  | --H                | --H                  | <1                     | 43                          | 15.6                        | 15.6                        | 1.0 | 0.15  |
| <b>B99</b>  | 3--Me              | --H                  | <1                     | 57                          | 248                         | 500                         | 2.0 | -1.55 |
| <b>B100</b> | 4--Me              | --H                  | <1                     | 58                          | NA                          | NA                          | ND  | ND    |
| <b>B101</b> | 2--Me              | --H                  | <1                     | 54                          | 297                         | 281                         | 1.1 | -1.26 |
| <b>B102</b> | 3--Cl              | --H                  | <1                     | 53                          | 295                         | 248                         | 1.2 | -1.93 |
| <b>B103</b> | 3--F               | --H                  | <1                     | 54                          | 137                         | 256                         | 1.9 | -1.10 |
| <b>B104</b> | 3--OMe             | --H                  | <1                     | 50                          | 477                         | 458                         | 1.0 | -1.34 |
| <b>B105</b> | 3--CF <sub>3</sub> | --H                  | <1                     | 46                          | NA                          | NA                          | ND  | ND    |
| <b>B106</b> | --H                | 4--Cl                | <1                     | 76                          | NA                          | NA                          | ND  | ND    |
| <b>B107</b> | --H                | 4--Me                | <1                     | 82                          | NA                          | NA                          | ND  | ND    |
| <b>B108</b> | --H                | 4--F                 | <1                     | 63                          | 287                         | 79.8                        | 3.6 | -0.73 |
| <b>B109</b> | --H                | 2--CONH <sub>2</sub> | <1                     | 50                          | 232                         | 435                         | 1.9 | 0.00  |

<sup>a</sup> Compounds were assayed at multiple concentrations (0.625–40  $\mu\text{M}$ , 7 points, 2-fold dilution). Photoswitchable compounds were tested both in the dark and after photoirradiation with 365 nm light. NA = no significant response above noise. ND = not determined. Highlighted in grey are previously tested compounds. Structural changes from **B96** have been highlighted.

### 2.8.3. Scaffold hopping on B96

We next attempted to address the photochemical properties of **B96**. *Ex situ* photochemical analysis of the compound showed a very poor photoconversion with 365 nm UV-irradiation only resulting in a PSD of 43% Z-isomer (Table 2.14, Appendix Figure A5C–D). For this purpose, we explored scaffold hopping of the azobenzene core into heterocyclic variants and especially sampled the arylazopyrazoles, as these are known to possess superior photochemical properties including larger completeness of photoswitching.<sup>35, 36</sup> In total, a series of seven analogues were designed (Figure 2.25).

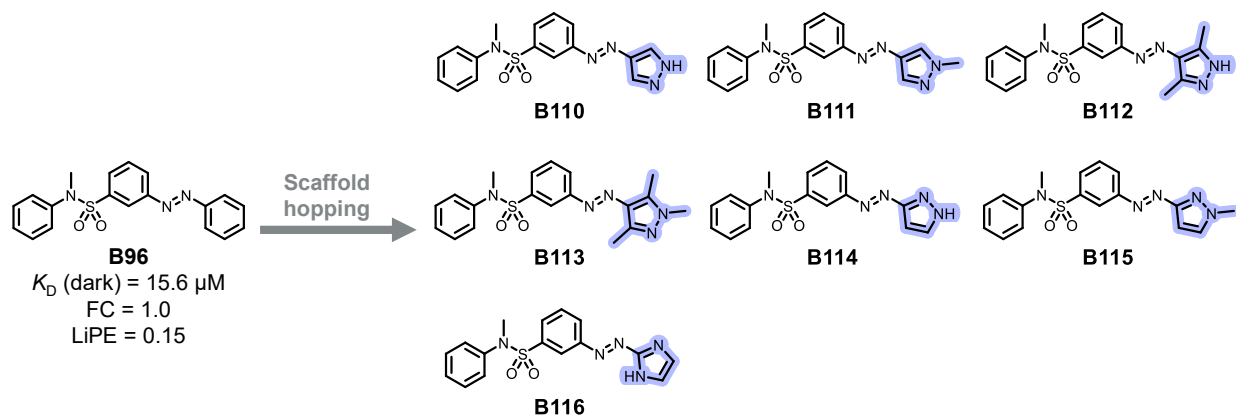


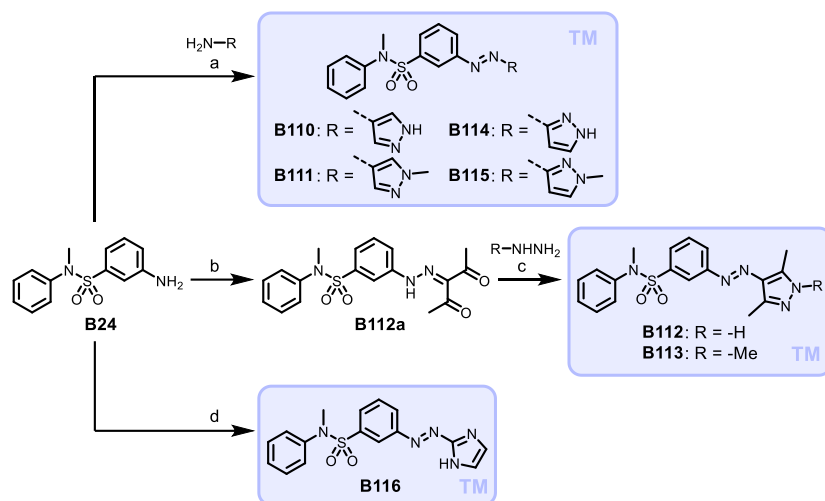
Figure 2.25. Design of scaffold hopping analogues of **B96**. Structural changes from **B96** have been highlighted.

The compounds were divergently synthesised through late-stage functionalisation of **B24** (Scheme 2.16). This included Baeyer-Mills reactions to access **B110**, **B111**, **B114**, and **B115** and tandem diazotisation-azo couplings to furnish **B112**, **B113** and **B116**.

From the library of heterocyclic analogues, we gratifyingly identified two analogues, **B114** and **B115**, which retained the binding affinity of **B96** but simultaneously displayed a significant fold change in activity upon photoirradiation (Table 2.16). Overall, compound **B115** emerged as the most promising and well-balanced analogue, showing a slightly improved binding affinity in its photoirradiated state ( $K_D$  = 11.8  $\mu\text{M}$ ), a 5.5-fold difference from the dark-adapted *E*-isomeric state, and a dramatically increased LiPE = 1.87.



**Scheme 2.16. Synthesis of scaffold hopping analogues B110–116.** <sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) **B24**, Oxone, DCM, H<sub>2</sub>O, rt, 16–17 h; then aminopyrazole, AcOH, rt–80 °C, 2 h–3 days, 10–32%. (b) NaNO<sub>2</sub>, aq. HCl, AcOH, 0 °C, 1 h; then Ac<sub>2</sub>CH<sub>2</sub>, NaOAc, EtOH, H<sub>2</sub>O, 0 °C–rt, 14 h, 87% (over 2 steps). (c) EtOH, reflux, 16 h, 80–91%. (d) NaNO<sub>2</sub>, aq. HCl, MeOH, H<sub>2</sub>O, 0 °C, 50 min; then imidazole, Na<sub>2</sub>CO<sub>3</sub>, H<sub>2</sub>O, 0 °C–rt, 22TM = Target molecule.

**Table 2.16. Photochemical properties and SPR results of scaffold hopping analogues B110–116.** <sup>a</sup>

| Cmpd ID     |  | C <sub>1h</sub><br>(%) | PSD <sub>365nm</sub><br>(%) | Dark K <sub>D</sub><br>(μM) | Irr. K <sub>D</sub><br>(μM) | FC  | LiPE  |
|-------------|--|------------------------|-----------------------------|-----------------------------|-----------------------------|-----|-------|
|             |  |                        |                             |                             |                             |     |       |
| <b>B96</b>  |  | <1                     | 43                          | 15.6                        | 15.6                        | 1.0 | 0.15  |
| <b>B110</b> |  | 57                     | 77                          | 338                         | 309                         | 1.1 | 0.02  |
| <b>B111</b> |  | <1                     | 83                          | 93.5                        | 41.4                        | 2.3 | 1.33  |
| <b>B112</b> |  | 100                    | ND                          | 350                         | 350                         | 1.0 | -0.58 |
| <b>B113</b> |  | <1                     | 100                         | NA                          | NA                          | ND  | ND    |
| <b>B114</b> |  | <1                     | 71                          | 150                         | 15.2                        | 9.9 | 1.32  |
| <b>B115</b> |  | <1                     | 84                          | 64.9                        | 11.8                        | 5.5 | 1.87  |
| <b>B116</b> |  | 100                    | ND                          | 102                         | 102                         | 1.0 | 1.07  |

<sup>a</sup> Compounds were assayed at multiple concentrations (0.625–40 μM, 7 points, 2-fold dilution). Photoswitchable compounds were tested both in the dark and after photoirradiation with 365 nm light. NA = no significant response

above noise. ND = not determined. Highlighted in grey are previously tested compounds. Structural changes from **B96** have been highlighted.

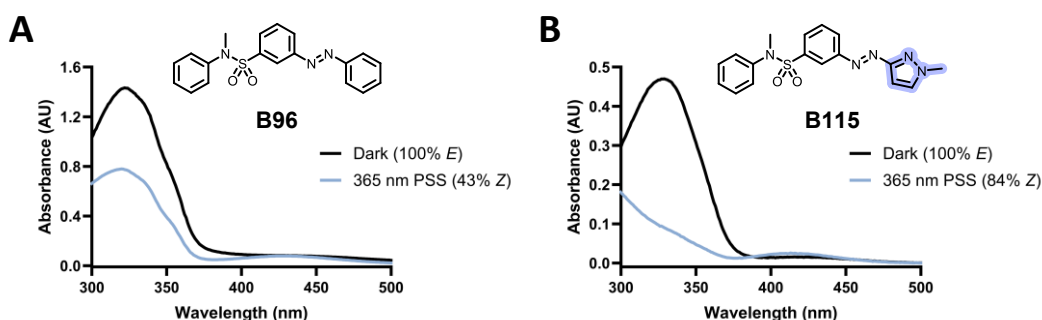


**Figure 2.26. Photochemical characterisation of B96 and B115.** Photoswitching was determined by UV–Vis spectroscopy at 20 °C and at 20  $\mu$ M in 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 10 w/v% glycerol, 0.05 v/v% Tween-20, 2% DMSO. The PSD was determined upon 365 nm irradiation. (A) Analysis of **B96**. (B) Analysis of **B115**.

We could partly attribute the larger FC to a substantial improvement in the photoconversion with the photoswitch exhibiting a PSD = 84% upon 365 nm light irradiation (corresponding to an almost 2-fold increase in PSD relative to **B96**), whilst retaining a high thermal stability (< 1% back-conversion after 1 h at 20 °C) (**Figure 2.26, Appendix Figure A5G–H**). Interestingly, analogue **B111** having a 4-regioisomery in the pyrazole ring (rather than the 3-regioisomery of **B115**) showed a 3.5-fold weaker binding, suggesting that the pyridine-like pyrazole *N* might be engaging in a specific distance- and orientation-dependent interaction (e.g., a H bond), which can further explain the observed difference in apparent *E*- and *Z*-binding.

#### 2.8.4. Azo walk

We proceeded to make a series of structural modifications to the newly identified **B115** scaffold in an attempt to further improve its properties.

An initial consideration was whether the optimal positioning of the photoswitch motif on the fragment scaffold had been identified. We had previously explored the regioisomery with the first-generation azo extension analogues, but only a limited number of positions had been sampled and solely with azobenzene as the extension motif. We therefore commenced the optimisation campaign on **B115** by systematically sampling all the aromatic positions on the fragment scaffold with the 3-azopyrazole motif. This is analogous to how one would perform a “methyl walk” in conventional medicinal chemistry, and we thus coined this an “azo walk”. We focused our attention on the 3-position of the LHAr, where the azobenzene analogue **B97** has previously shown good differential activity although possessing a poor absolute binding affinity and LiPE. Here, we additionally sampled different pyrazole connectivities and also explored the effect of methylating the RHAr amino group (which had previously given a dramatic affinity boost for the

non-photoswitchable series, cf. **B24** to **B44**). A series of seven compounds was designed in total (**Figure 2.27**).

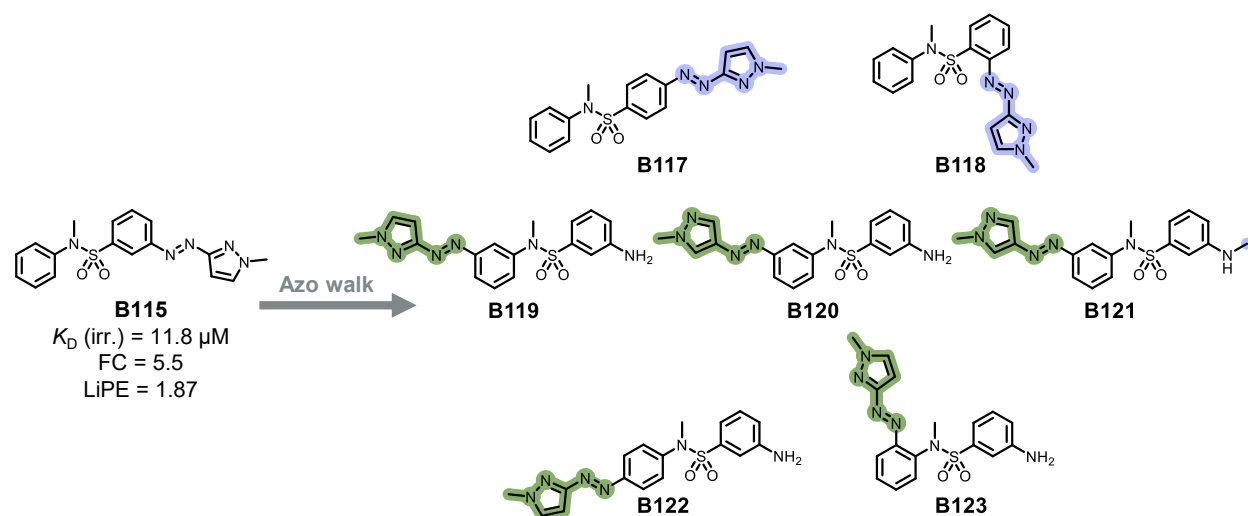


**Figure 2.27.** Design of azo walking analogues of **B115**. Structural changes from **B115** have been highlighted.

For the LAr azo-substituted analogues carrying an amino group on the RAr, synthesis of the structurally related azobenzene **B97** had previously been performed via an extensive six-step linear synthesis (**Figure 2.28A**). We envisioned an alternative synthetic route, in which the sulfonamide *N*-aryl *C* bond was disconnected first, corresponding to a late-stage cross-coupling in the forward sense (**Figure 2.28B**). Following this strategy, we arrived at a convergent route that would afford the TMs effectively in a total of three steps (**Figure 2.28C**).

The RAr azo-substituted analogues **B117** and **B118** were smoothly synthesised via late-stage Baeyer-Mills reactions on the corresponding anilines, **B1** and **B26** (**Scheme 2.17**). The previously synthesised intermediate **B97d** was also subjected to Baeyer-Mills reaction conditions, and following deacetylation, the analogue **B120** was obtained. To synthesise the LAr azo-substituted analogues, we employed the revised convergent synthetic pathway. For **B119**, **B122**, and **B123**, a previously reported amino acid-assisted Ullmann-type cross-coupling reaction was used for the convergent step.<sup>115</sup> This was followed by treatment with sodium sulfide to chemoselectively reduce the RAr nitro groups in the presence of the azo motifs and furnish the TMs.<sup>118</sup> For analogue **B121**, the *N*-methylated amino group was pre-installed onto the sulfonamide building block, and the central cross-coupling performed with the previously employed Buchwald-Hartwig conditions instead (which had only been reported for amide substrates but here shown to also be applicable to a sulfonamide nucleophile).<sup>117</sup>

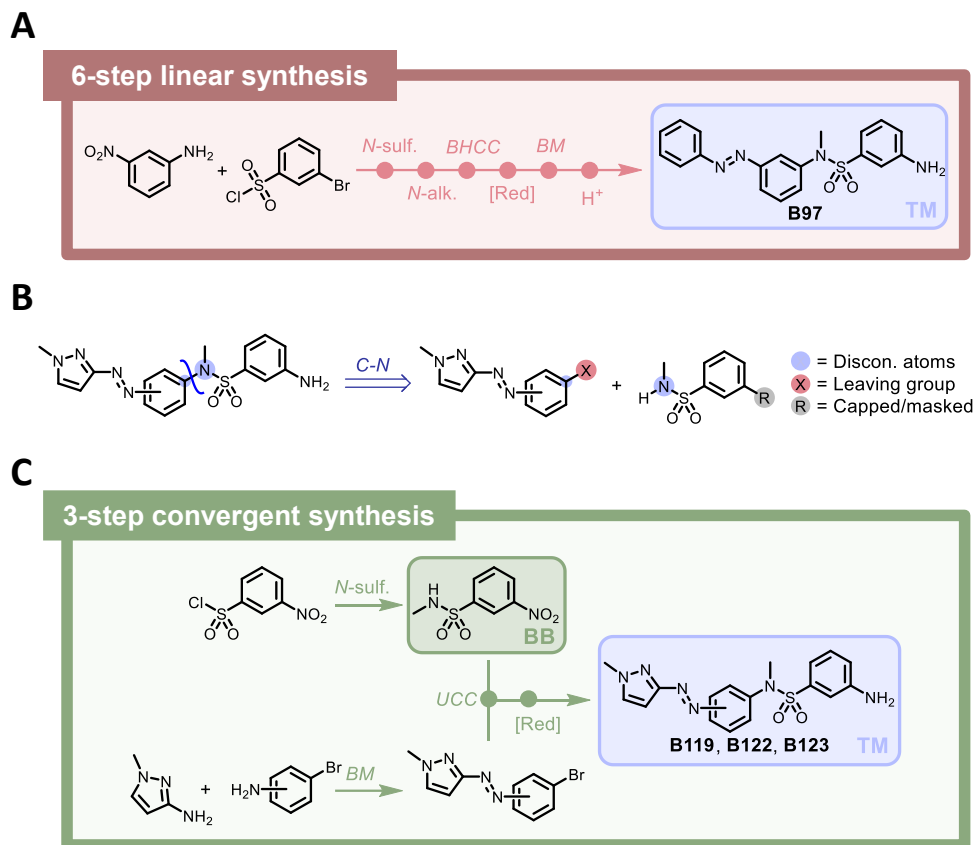
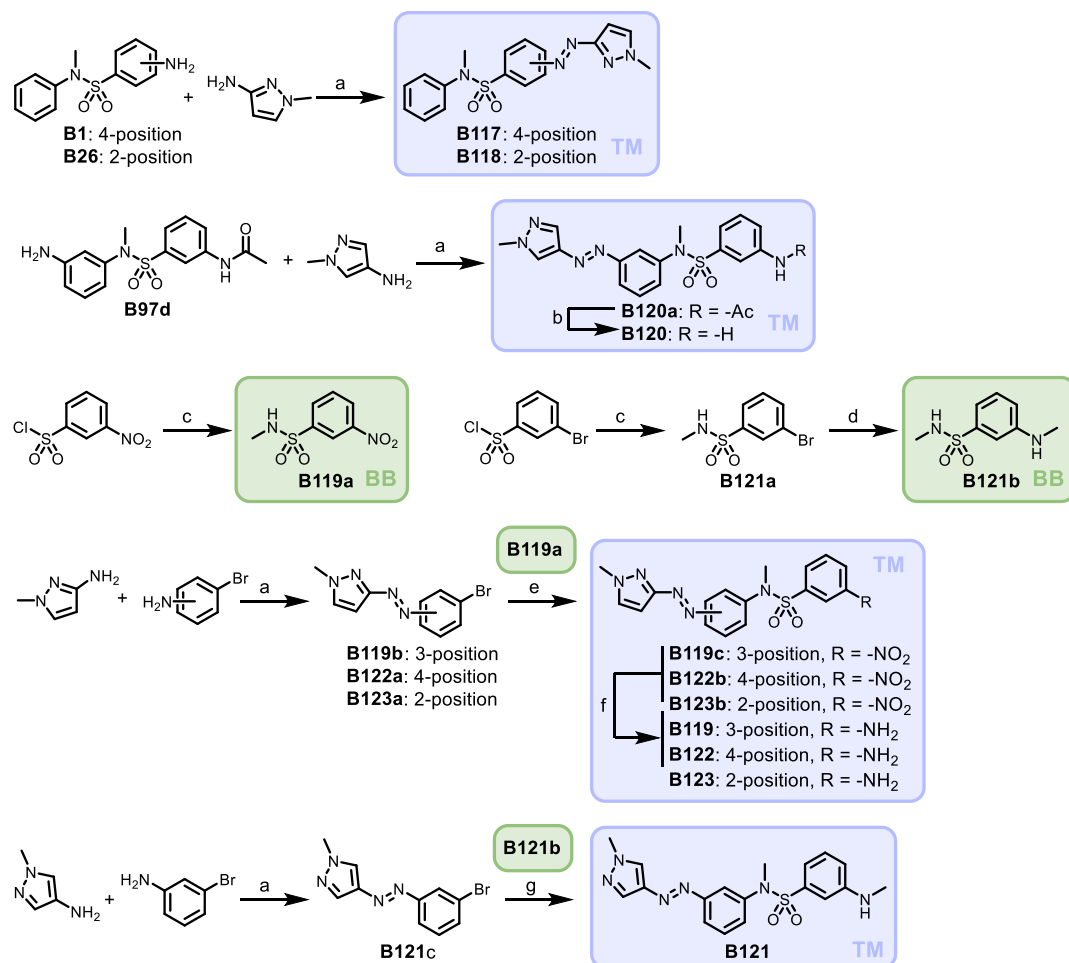


**Figure 2.28. Synthetic strategies towards LHAz azo walking analogues of B115.** *N*-sulf. = *N*-sulfonylation. *N*-alk. = *N*-alkylation. *BHCC* = Buchwald-Hartwig cross-coupling. *BM* = Baeyer-Mills reaction. *UCC* = Ullmann-type cross-coupling. (A) The original six-step linear synthetic route employed for synthesising **B97**. (B) Retrosynthetic analysis of the LHAz azo-substituted and RHAz amino-functionalised analogues. (C) An alternative three-step convergent synthesis pathway towards **B119**, **B122** and **B123**. TM = Target molecule.

**Scheme 2.17. Synthesis of azo walking analogues B117–123.** <sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) Oxone, DCM, H<sub>2</sub>O, rt–50 °C, 1.5 h–3 days; then aminopyrazole, AcOH, rt, 25–53 h, 20–78%. (b) SOCl<sub>2</sub>, MeOH, 65 °C, 1 h, 26%. (c) MeNH<sub>2</sub>·HCl, Et<sub>3</sub>N, DCM, rt, 2.5–8 h, 51–81%. (d) aq. MeNH<sub>2</sub>, Cu, air (sealed vial), 100 °C, 4.5 h, 78%. (e) CuI, *N,N*-dimethylglycine, K<sub>3</sub>PO<sub>4</sub>, DMF, 100 °C, 23 h–3 days, 19–64%. (f) Na<sub>2</sub>S, THF, H<sub>2</sub>O, reflux, 15 h–3 days, 26%–quant. (g) Pd(OAc)<sub>2</sub>, tBuBrettPhos, K<sub>3</sub>PO<sub>4</sub>, *t*-BuOH, H<sub>2</sub>O, 110 °C, 3.5 h, 67%. TM = Target molecule. BB = Building block.

Based on the series of azo walking analogues, **B115** remained the best overall in terms of absolute and differential binding affinity (**Table 2.17**). However, compound **B120** having a 4-azopyrazole motif substituted on the LHA<sub>r</sub> 3-position displayed equipotency with **B115** in its photoirradiated state ( $K_D = 10.8 \mu\text{M}$ ), had a significant change in binding from its dark state ( $FC = 2.6$ ) and showed the best lipophilicity efficiency of all photoswitchable analogues made thus far ( $LiPE = 2.42$ ). Interestingly, methylating the RHA<sub>r</sub> amino group to obtain **B121** resulted in a loss in binding affinity ( $K_D = 22.9 \mu\text{M}$ ) in opposition to the positive effect observed for the core fragment (cf. **B24** and **B44**). This suggested that extension with the azo motif had induced a different conformation for the ligand relative to the fragment core.

Table 2.17. Photochemical properties and SPR results of azo walking analogues B117–123.<sup>a</sup>

| Cmpd ID | R <sup>1</sup>     | R <sup>2</sup> | C <sub>1h</sub><br>(%) | PSD <sub>365nm</sub><br>(%) | Dark K <sub>D</sub><br>(μM) | Irr. K <sub>D</sub><br>(μM) | FC  | LiPE |
|---------|--------------------|----------------|------------------------|-----------------------------|-----------------------------|-----------------------------|-----|------|
| B115    |                    | --H            | <1                     | 84                          | 64.9                        | 11.8                        | 5.5 | 1.87 |
| B117    |                    | --H            | <1                     | 87                          | 195                         | 205                         | 1.1 | 0.65 |
| B118    |                    | --H            | <1                     | 89                          | 170                         | 254                         | 1.5 | 0.71 |
| B119    | 3--NH <sub>2</sub> |                | <1                     | 85                          | 78.0                        | 57.0                        | 1.4 | 1.70 |
| B120    | 3--NH <sub>2</sub> |                | <1                     | 84                          | 33.3                        | 10.8                        | 3.1 | 2.42 |
| B121    | 3--NHMe            |                | <1                     | 79                          | 58.8                        | 22.9                        | 2.6 | 1.33 |
| B122    | 3--NH <sub>2</sub> |                | <1                     | 100                         | 330                         | 320                         | 1.0 | 0.95 |
| B123    | 3--NH <sub>2</sub> |                | <1                     | 74                          | NA                          | NA                          | ND  | ND   |

<sup>a</sup> Compounds were assayed at multiple concentrations (1.56–100 μM, 7 points, 2-fold dilution; higher-affinity compounds retested from top concentration of 16–32 μM, 11 points, 2-fold dilution). Photoswitchable compounds were tested both in the dark (Dark K<sub>D</sub>) and after photoirradiation with 365 nm light (Irr. K<sub>D</sub>). NA = no significant response above noise. ND = not determined. Highlighted in grey are previously tested compounds. Structural changes from B115 have been highlighted.

We here speculated if azo substitution on the LHA<sub>R</sub> had resulted in B120 flipping its conformation so as to retain the azo motif in a similar region as B115. In that sense, B120 could be considered an analogue of B111 (and B115), in which the sulfonamide linker had been inversed and the LHA<sub>R</sub> substituted with an amino group—which would then explain why methylating the amino group did not produce the same affinity increase (Figure 2.29).

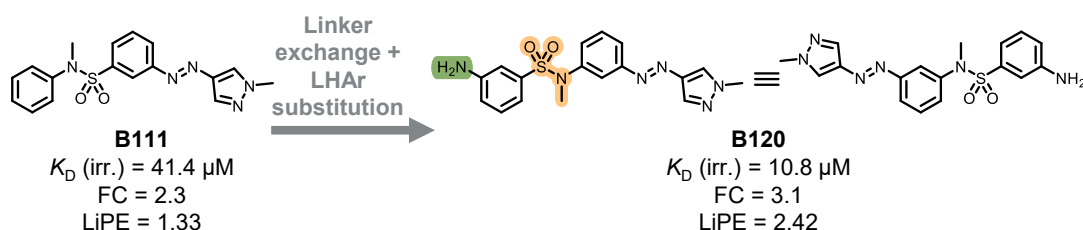


Figure 2.29. Structural relationship between B120 and B111. Structural changes from B111 have been highlighted.

### 2.8.5. LHA<sub>r</sub> substitution on B115

The above conjecture relies on the assumption that **B115** (and **B111**) adopts the same conformational orientation as the original core fragment **B24**. We investigated this hypothesis by next performing a series of structural modifications to the LHA<sub>r</sub>, which had previously resulted in positive effects (**B124** and **B126–128**) and to strengthen the study, we further supplemented the series with a single negative design (**B125**) (Figure 2.30).

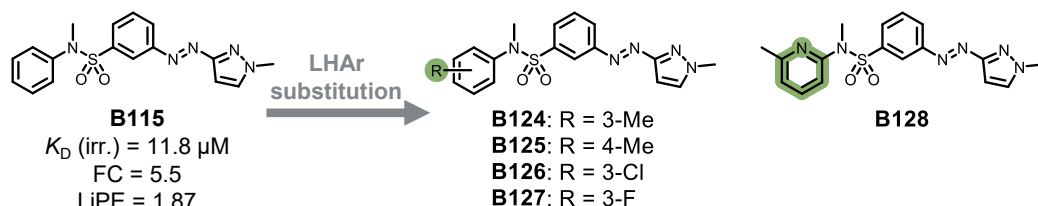
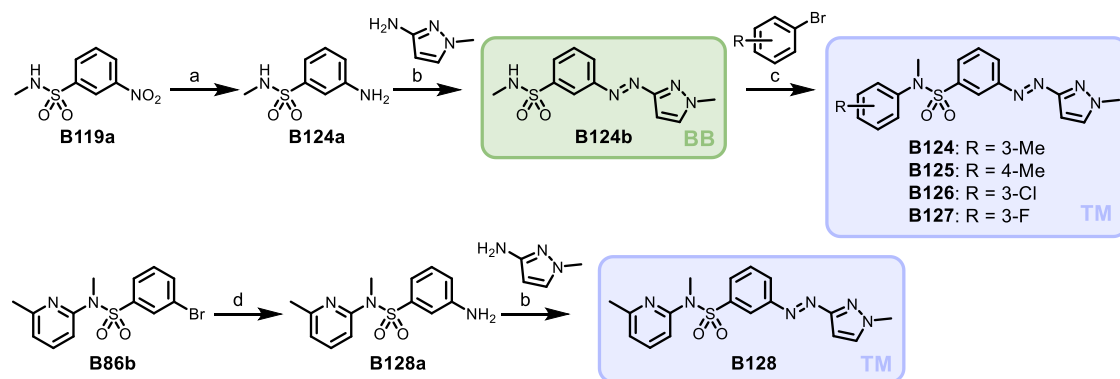


Figure 2.30. Design of LHA<sub>r</sub> substituted analogues of **B115**. Structural changes from **B115** have been highlighted.

To synthesise the LHA<sub>r</sub> substituted analogues **B124–127**, we again opted for a sulfonamide *N*-aryl halide cross-coupling strategy so as to introduce the divergent step late in the synthetic route and minimise the number of steps to a single for each analogue (Scheme 2.18). For this purpose, a common intermediate **B124b** was prepared by reducing the nitro group of the previously synthesised building block **B119a** and installing the azopyrazole motif with a tandem nitrosation–Baeyer–Mills reaction. For analogue **B128**, we alternatively utilised the previously synthesised aryl bromide intermediate **B86b** which was aminated and then also subjected to the two-step sequence of nitrosation and Baeyer–Mills coupling.

Scheme 2.18. Synthesis of LHA<sub>r</sub> substituted analogues **B124–128**. <sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) Pd/C, MeOH, H<sub>2</sub> (1 atm), rt, 15 h, 97%. (b) Oxone, DCM, H<sub>2</sub>O, rt, 2–5 h; then aminopyrazole, AcOH, rt, 17–44 h, 39–60%. (c) CuI, *N,N*-dimethylglycine, K<sub>3</sub>PO<sub>4</sub>, DMF, 100–140 °C, 16 h–3 days, 61–90%. (d) NH<sub>4</sub>OH, CuI, 130 °C, 20 h, 26%. TM = Target molecule. BB = Building block.

From the assessment of the compounds in our SPR assay, we saw an improved absolute binding affinity for all 3-substituted analogues **B124**, **B126** and **B127**, a reduced binding affinity for the 4-substituted analogue **B125** and maintained binding for the 2-pyridine analogue **B128**—in complete accordance with the previously established SAR on the **B24** fragment scaffold (**Table 2.18**). These results support the conjecture that **B115** and its analogues adopt a similar conformation to **B24**. Despite the affinity increases, these came at the cost of a lowered fold change in activity (although they all maintained the same completeness of switching and thermal Z-isomer stability as **B115**), i.e., the Z-isomers also benefitted from the structural modifications. This indicates that the LHS aryl side of the photoswitches keep in place whereas the RHS pyrazole side shifts its position upon photoswitching. Of the series of modifications, the 3-fluoro substitution (**B127**) stood out marginally, giving the strongest absolute binding affinity in the photoirradiated state ( $K_D = 1.52 \mu\text{M}$ ) and the largest lipophilic efficiency (LiPE = 2.45).

**Table 2.18. Photochemical properties and SPR results of LHA<sub>r</sub> substituted analogues B124–128.** <sup>a</sup>

| Cmpd ID     | R | C <sub>1h</sub><br>(%) | PSD <sub>365nm</sub><br>(%) | Dark $K_D$<br>( $\mu\text{M}$ ) | Irr. $K_D$<br>( $\mu\text{M}$ ) | FC  | LiPE |
|-------------|---|------------------------|-----------------------------|---------------------------------|---------------------------------|-----|------|
| <b>B115</b> |   | <1                     | 84                          | 64.9                            | 11.8                            | 5.5 | 1.87 |
| <b>B124</b> |   | <1                     | 85                          | 3.61                            | 2.40                            | 1.5 | 2.06 |
| <b>B125</b> |   | <1                     | 85                          | 194                             | 208                             | 1.1 | 0.16 |
| <b>B126</b> |   | <1                     | 85                          | 2.40                            | 1.90                            | 1.3 | 1.78 |
| <b>B127</b> |   | <1                     | 84                          | 1.66                            | 1.52                            | 1.1 | 2.45 |
| <b>B128</b> |   | <1                     | 84                          | 10.7                            | 13.3                            | 1.2 | 2.01 |

<sup>a</sup> Compounds were assayed at multiple concentrations (1.56–100  $\mu\text{M}$ , 7 points, 2-fold dilution; higher-affinity compounds retested from top concentration of 16–32  $\mu\text{M}$ , 11 points, 2-fold dilution). Photoswitchable compounds were tested both in the dark (Dark  $K_D$ ) and after photoirradiation with 365 nm light (Irr.  $K_D$ ). Highlighted in grey are previously tested compounds. Structural changes from **B115** have been highlighted.

#### 2.8.6. Combinatorial modification on B115

Based on the above campaigns of structural modifications on **B115**, we identified key analogues displaying favourable properties: 1) The pyrazole demethylated analogue **B114** displayed the largest difference in activity with an FC = 9.9; 2) the LHA<sub>r</sub> 3-fluorosubstituted analogue **B127** showed the strongest absolute



binding affinity with a  $K_D = 1.66 \mu\text{M}$  and LiPE = 2.45; 3) the inverse linker analogue **B120** showed improved absolute ( $K_D = 10.8 \mu\text{M}$ ) and differential (FC = 3.1) binding affinity as well as lipophilic efficiency (LiPE = 2.42) compared with **B111**. We next proceeded to combine these (mostly) single modifications in a systematic fashion to generate a library of combinatorial analogues (**Figure 2.31**).

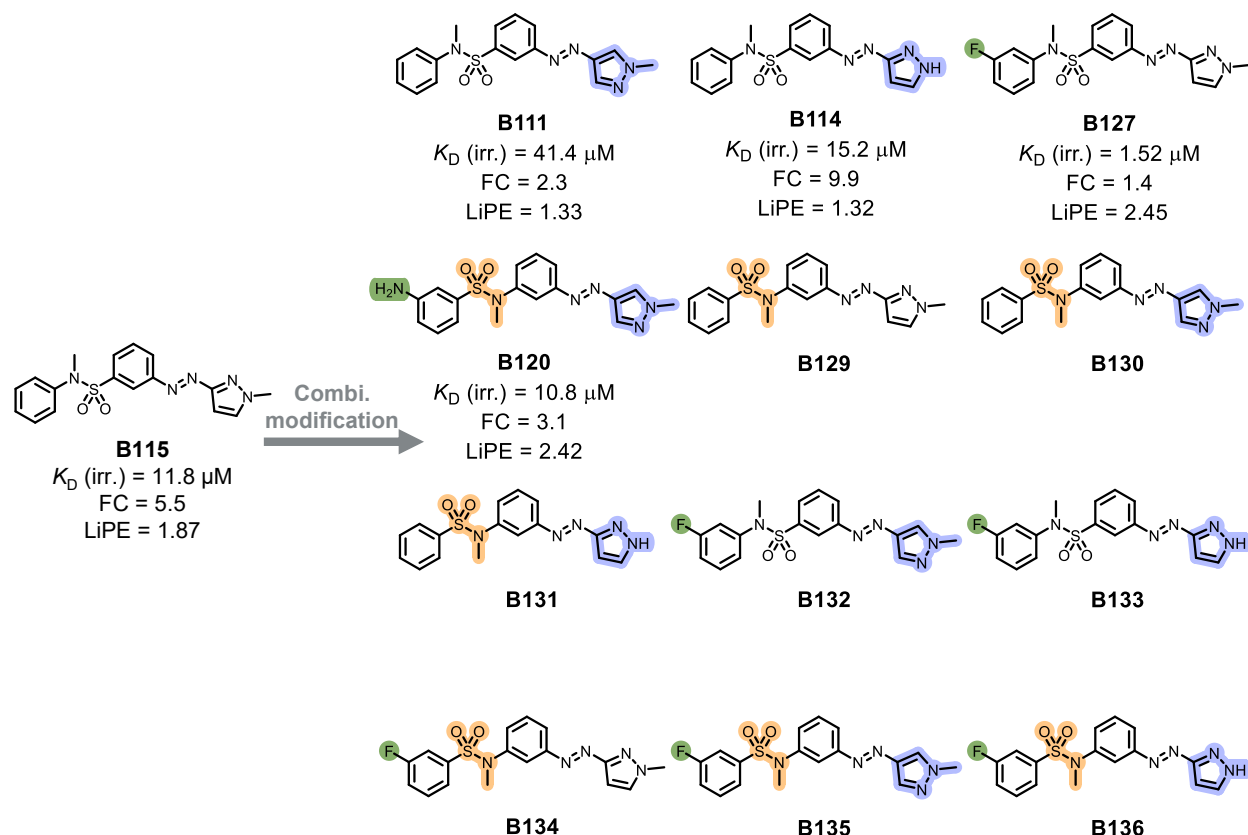
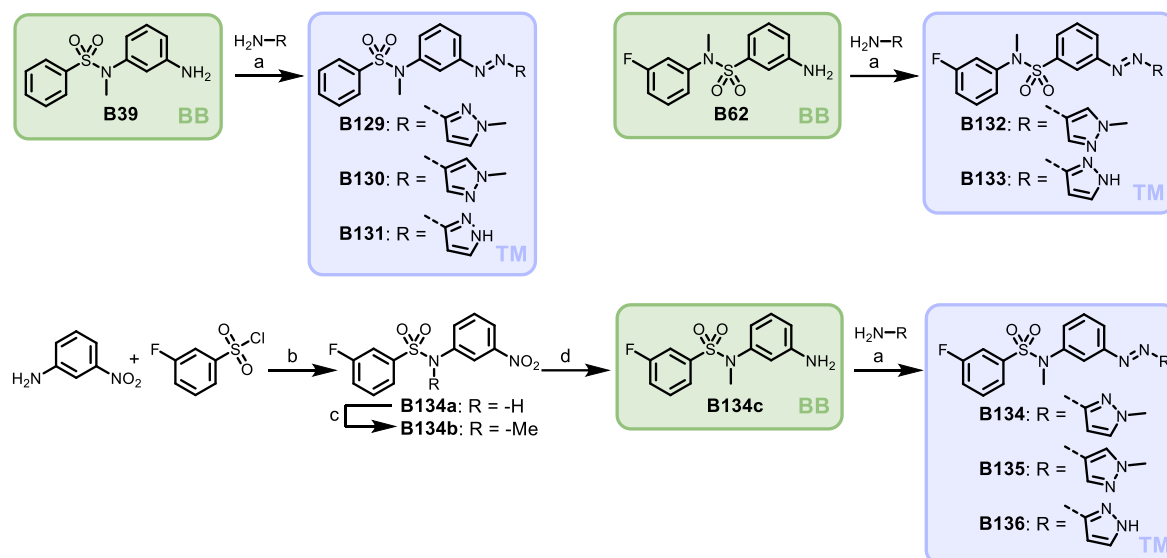


**Figure 2.31.** Design of combinatorial modification analogues of **B115**. Structural changes from **B115** have been highlighted.

These analogues were rapidly synthesised from late-stage tandem nitrosation–Baeyer–Mills reactions of common aniline building blocks (**B39**, **B62** and **B134c**) with the appropriate aminopyrazoles (**Scheme 2.19**).

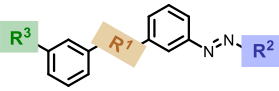
The SPR results on this library of combinatorial analogues showed that the activity effects of the structural modifications were highly non-linear. Whereas pyrazole demethylation of **B115** gave **B114** with an increased fold change in activity (FC = 9.9 versus 5.5) and retained absolute affinity ( $K_D = 11.8$  and 15.2  $\mu\text{M}$ ), the same transformation on, **B127**, **B129**, and **B134** (to give **B133**, **B131**, and **B136**, respectively) either did not improve the FC or improved it at the cost of a significant reduction in absolute binding affinity.

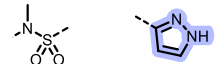
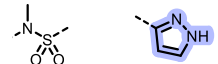
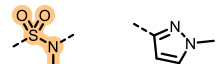
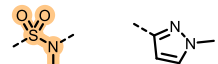
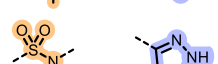
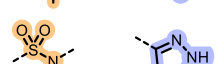
**Scheme 2.19. Synthesis of combinatorial modification analogues B129–136.** <sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) Oxone, DCM, H<sub>2</sub>O, rt–50 °C, 3–21 h; then aminopyrazole, AcOH, rt, 19 h–5 days, 9–63%. (b) Pyridine, DCM, rt, 43 h, 99%. (c) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, rt, 5 days, 96%. (d) Pd/C, MeOH, EtOAc, H<sub>2</sub> (1 atm), rt, 17 h, quant. TM = Target molecule. BB = Building block.

**Table 2.19. Photochemical properties and SPR results of combinatorial modification analogues B129–136.** <sup>a</sup>

| Cmpd ID |  |                                                                                     |                   | C <sub>1h</sub> (%) | PSD <sub>365nm</sub> (%) | Dark K <sub>D</sub> (μM) | Irr. K <sub>D</sub> (μM) | FC   | LiPE |
|---------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------|---------------------|--------------------------|--------------------------|--------------------------|------|------|
|         | R <sup>1</sup>                                                                      | R <sup>2</sup>                                                                      | R <sup>3</sup>    |                     |                          |                          |                          |      |      |
| B115    |  |  | --H               | <1                  | 84                       | 64.9                     | 11.8                     | 5.5  | 1.87 |
| B111    |  |  | --H               | <1                  | 83                       | 93.5                     | 41.4                     | 2.3  | 1.33 |
| B114    |  |  | --H               | <1                  | 71                       | 150                      | 15.2                     | 9.9  | 1.32 |
| B120    |  |  | --NH <sub>2</sub> | <1                  | 84                       | 33.3                     | 10.8                     | 3.1  | 2.42 |
| B127    |  |  | --F               | <1                  | 84                       | 1.66                     | 1.52                     | 1.1  | 2.45 |
| B129    |  |  | --H               | <1                  | 87                       | 65.7                     | 14.7                     | 4.5  | 1.56 |
| B130    |  |  | --H               | <1                  | 83                       | 69.0                     | 5.97                     | 11.6 | 1.95 |
| B131    |  |  | --H               | <1                  | 79                       | 74.0                     | 65.4                     | 1.1  | 0.61 |
| B132    |  |  | --F               | <1                  | 83                       | 124                      | 25.3                     | 4.9  | 1.23 |

|             |                                                                                   |                                                                                   |    |    |      |      |     |      |
|-------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|----|----|------|------|-----|------|
| <b>B133</b> |  |  | <1 | 70 | 10.7 | 8.40 | 1.3 | 1.27 |
| <b>B134</b> |  |  | <1 | 86 | 13.4 | 10.1 | 1.3 | 1.58 |
| <b>B135</b> |  |  | <1 | 83 | 236  | 76.0 | 3.1 | 0.71 |
| <b>B136</b> |  |  | <1 | 78 | 160  | 63.3 | 2.5 | 0.48 |

<sup>a</sup> Compounds were assayed at multiple concentrations (0.39–25  $\mu$ M, 7 points, 2-fold dilution; higher-affinity compounds retested from top concentration of 16  $\mu$ M, 11 points, 2-fold dilution). Photoswitchable compounds were tested both in the dark and after photoirradiation with 365 nm light. Previously tested compounds are highlighted in grey. Structural changes from **B115** have been highlighted.

It is noteworthy that these demethylated pyrazole photoswitches all displayed slightly lower completeness of switching (average PSD = 76%) relative to their methylated counterparts (average PSD = 86%), but this was also true for the **B115**–**B114** pair. LHA<sub>r</sub> 3-fluoro substitution on **B115** to furnish **B127** improved its absolute binding affinity significantly ( $K_D$  = 1.52  $\mu$ M versus 11.8  $\mu$ M, corresponding to almost 8 fold), but this effect did not translate well when substituting **B111**, **B114**, **B129**, **B130**, and **B131** (to obtain **B132**, **B133**, **B134**, **B135**, and **B136**, respectively)—maintained or reduced binding were mostly observed, and any improvements were only marginal (< 2-fold increase). Similarly, no apparent linear trends could be derived from changing the pyrazole connectivity or inverting the linker. However, the combination of inverting the sulfonamide linker and changing the pyrazole connectivity (from a 3- to a 4-regioisomer) gave the promising analogue **B130**. This compound maintained the favourable photochemical properties of **B115** (Appendix Figure A5M–N) and displayed a fairly weak binding in the dark state ( $K_D$  = 69.0  $\mu$ M) but a significantly improved affinity upon photoirradiation ( $K_D$  = 5.97  $\mu$ M), corresponding to a FC = 11.6 (Appendix Figure A2E–F). With a further increase in the lipophilic efficiency (LiPE = 1.95), compound **B130** displayed an enhancement of all three metrics, making it the hitherto best photoswitchable ligand.

In conclusion, the azo extension strategy on the original fragment hit **B1** ( $K_D$  = 276  $\mu$ M, LiPE = 1.51) yielded much more favourable results. An initial scouting with an azobenzene motif quickly identified key sites amenable to extension, and through a systematic series of scaffold hopping, regioisomer sampling, aryl substitution, and then combinatorial modification, this ultimately yielded a photoswitchable ligand **B130** featuring a single-digit micromolar binding affinity for NSP16/10, more than an order of magnitude difference in activity upon photoirradiation and an improved lipophilic efficiency compared with the fragment hit.

## 2.9. Functional activity

From our SPR assay, we had strong evidence that our compounds were binding to NSP16/10. Concurrent with the assessment for NSP16/10 binding, each compound had also been tested against the related NSP14/10 by our collaborators at CSIRO. From this counter screen, no significant binding response had been detected for **B1** or any of its analogues except for the linked analogues **B92–94** (data not shown). This provides confidence that the binding responses observed for the compounds against NSP16/10 were not artefactual (i.e., non-specific binding events). Binding was further partly supported by the DSF assay. However, it is well-established that protein binding does not necessarily yield inhibition. The SPR competition study suggested that our best binder **B69** was competitive with **m7GpppA**, which hints towards possible inhibition through substrate displacement. To firmly demonstrate inhibition, we next turned to functional assays.

### 2.9.1. Time-resolved fluorescence energy transfer MTase assay

As a functional assay, we were interested in the EPIgeneous time-resolved fluorescence energy transfer (TR-FRET) MTase assay kit which had previously been employed for assessing activity and inhibition of NSP16/10.<sup>89, 92</sup> This assay measures enzyme activity by quantifying the by-product of the enzymatic methyl transfer reaction, *S*-adenosylhomocysteine (SAH) (**Figure 2.32A**). The detection principle is based on competition with an immunoassay pair of d2 dye-coupled SAH (SAH-d2) and an antibody specific to SAH which has been labelled with a Lumi4-Tb cryptate (anti-SAH cryptate). Upon complexation, the d2 acceptor comes in close enough proximity with the Tb donor to give rise to a positive TR-FRET signal, whereas produced (unlabelled) SAH will displace SAH-d2 and reduce the signal. Enzyme activity will therefore be seen as a TR-FRET signal depression, whereas inhibition will rescue it.

To ensure that the assay detection principle was working, we firstly performed a titration experiment with SAH (in the absence of enzyme), from which an expected antagonistic sigmoidal dose-response curve was obtained (**Figure 2.32B**). We next turned to the enzymatic reaction, for which the enzyme (NSP16/10), the co-factor (SAM) and a capped RNA substrate are required. For the substrate, we first considered the cap0-analogue **m7GpppA**, which arguably constitutes the simplest potential substrate, containing the adenine A<sub>1</sub> to be *O*-methylated and which is readily commercially available. However, despite testing at high substrate concentrations (100 μM), high NSP16/10 concentrations (3 μM) and extended reaction times (17 h), we did not observe any significant SAH production using **m7GpppA** (**Table 2.20, entry 1–3**).

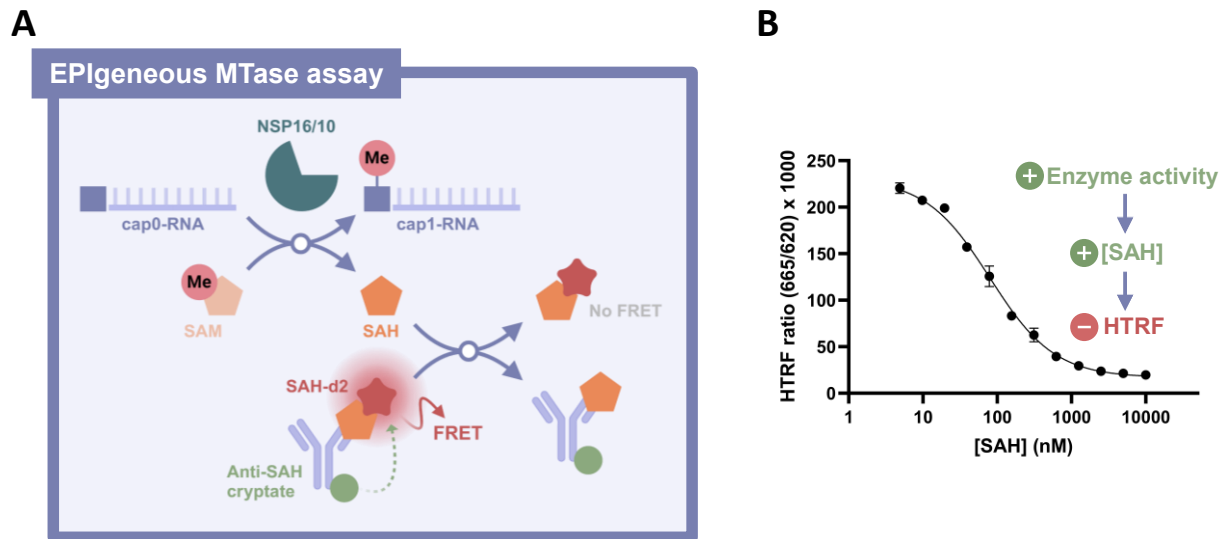


**Figure 2.32. The EPIgeneous TR-FRET MTase assay.** (A) Principle behind the assay. Created with BioRender.com. (B) A titration curve shows an inversely proportional sigmoidal relationship between the HTRF ratio and the SAH concentration and thus the enzyme activity. Error bars represent the standard deviation of technical duplicates.

**Table 2.20. Screened conditions for the MTase assay. <sup>a</sup>**

| Entry          | [NSP16/10]<br>(nM) | Substrate<br>(conc.)                           | [SAM]<br>( $\mu$ M) | Buffer                                                                              | Reaction<br>time (h) | Result |
|----------------|--------------------|------------------------------------------------|---------------------|-------------------------------------------------------------------------------------|----------------------|--------|
| 1              | 200                | <b>m7GpppA</b><br>(100 $\mu$ M)                | 10                  | 10 mM HEPES pH 7.5, 10 mM KCl, 2 mM MgCl <sub>2</sub> , 0.01% Tween-20, 1 mM DTT    | 1                    | NA     |
| 2              | 200                | <b>m7GpppA</b><br>(100 $\mu$ M)                | 10                  | 10 mM HEPES pH 7.5, 10 mM KCl, 2 mM MgCl <sub>2</sub> , 0.01% Tween-20, 1 mM DTT    | 17                   | NA     |
| 3              | 3000               | <b>m7GpppA</b><br>(100 $\mu$ M)                | 10                  | 40 mM Tris-HCl pH 8.0, 10 mM KCl, 2 mM MgCl <sub>2</sub> , 0.01% Tween-20, 1 mM DTT | 2                    | NA     |
| 4 <sup>b</sup> | 3000               | <b>Oligo RNA 0020</b><br>(25 $\mu$ M)          | 50                  | 40 mM Tris-HCl pH 8.0, 10 mM KCl, 2 mM MgCl <sub>2</sub> , 0.01% Tween-20, 1 mM DTT | 1.5                  | NA     |
| 5 <sup>b</sup> | 3000               | <b>Oligo RNA 0021</b><br>(25 $\mu$ M)          | 50                  | 40 mM Tris-HCl pH 8.0, 10 mM KCl, 2 mM MgCl <sub>2</sub> , 0.01% Tween-20, 1 mM DTT | 1.5                  | NA     |
| 6 <sup>c</sup> | 3000               | <b>m7GpppA-RNA<sub>34</sub></b><br>(5 $\mu$ M) | 10                  | 40 mM Tris-HCl pH 8.0, 10 mM KCl, 2 mM MgCl <sub>2</sub> , 0.01% Tween-20, 1 mM DTT | 2                    | NA     |

|   |      |                                                 |     |                                                                                           |   |        |
|---|------|-------------------------------------------------|-----|-------------------------------------------------------------------------------------------|---|--------|
| 7 | 3000 | <b>m7GpppA-RNA<sub>34</sub></b><br>(40 $\mu$ M) | 200 | 40 mM Tris-HCl pH 8.0, 10<br>mM KCl, 2 mM MnCl <sub>2</sub> , 0.01%<br>Tween-20, 1 mM DTT | 2 | Active |
|---|------|-------------------------------------------------|-----|-------------------------------------------------------------------------------------------|---|--------|

<sup>a</sup> NA = no significant ( $P > 0.05$  in unpaired t test) in reduction in HTRF compared with the no-substrate negative control. Active = Significant ( $P < 0.05$  in unpaired t test) reduction in HTRF compared with the no-substrate negative control.

<sup>b</sup> Conditions reported in Hsiao *et al.* 2022.<sup>119</sup> **Oligo RNA 0020**: 5'-ACAGAGA-3'. **Oligo RNA 0021**: 5'-UACAGAGAA-3'.

<sup>c</sup> Conditions similar to those reported in Benoni *et al.* 2021.<sup>120</sup> **m7GpppA-RNA<sub>34</sub>**: 5'-m7GpppAGGGAAGCGGG-CAUGCGGCCAGCCAUAGCCGAUCA-3'.

This correlates with previous reports that cap0 itself is not being accepted as a substrate by NSP16/10.<sup>120</sup> From X-ray crystallography experiments, it appears a pendant RNA chain is required to stabilise a conformation of the A<sub>1</sub> base in sufficient proximity with the SAM co-factor.<sup>112</sup>

Interestingly, recent studies have shown that NSP16/10 can use non-methylated RNA chains as substrates in addition to cap0-RNA.<sup>119, 120</sup> In particular, Hsiao *et al.* showed that the two short uncapped 7-meric and 9-meric oligoribonucleotides **oligo RNA 0020** and **oligo RNA 0021** could give rise to an enzymatic activity similar in magnitude to that of a longer 13-meric cap0-functionalised RNA chain in a bioluminescence assay.<sup>119</sup> However, despite employing reaction conditions very similar to those reported in Hsiao *et al.*, we were unable to detect any noticeable SAM-to-SAH conversion using these two uncapped oligoribonucleotides (**Table 2.20, entry 4–5**).

We finally turned to cap0-RNA substrates and selected the capped 36-meric oligoribonucleotide reported in Benoni *et al.* (**m7GpppA-RNA<sub>34</sub>**).<sup>120</sup> This required bespoke synthesis, which was achieved by *in vitro* RNA synthesis with co-transcriptional capping. As a negative control, we further synthesised the corresponding uncapped triphosphate RNA chain (**pppA-RNA<sub>34</sub>**). We could originally not observe any activity when testing the capped RNA substrate at 5  $\mu$ M (**Table 2.20, entry 6**). Only when increasing the concentration of the substrate to 40  $\mu$ M and of SAM to 200  $\mu$ M as well as making an adjustment to the assay buffer (replacing MgCl<sub>2</sub> with MnCl<sub>2</sub>),<sup>112</sup> did we finally observe significant enzyme activity (**Table 2.20, entry 7**). Applying the same conditions, no activity was detectable using either **pppA-RNA<sub>34</sub>** or **m7GpppA**, suggesting the lowered TR-FRET was substrate-specific (**Figure 2.33A**). A multi-concentration test in the presence and in the absence of NSP16/10 also established that the observed activity of **m7GpppA-RNA<sub>34</sub>** was dose- and enzyme-dependent (**Figure 2.33B**). However, extremely high substrate, SAM, and enzyme concentrations were required to achieve the HTRF change, which only corresponded to a SAM-to-SAH conversion of 0.3% (**Figure 2.33C**).

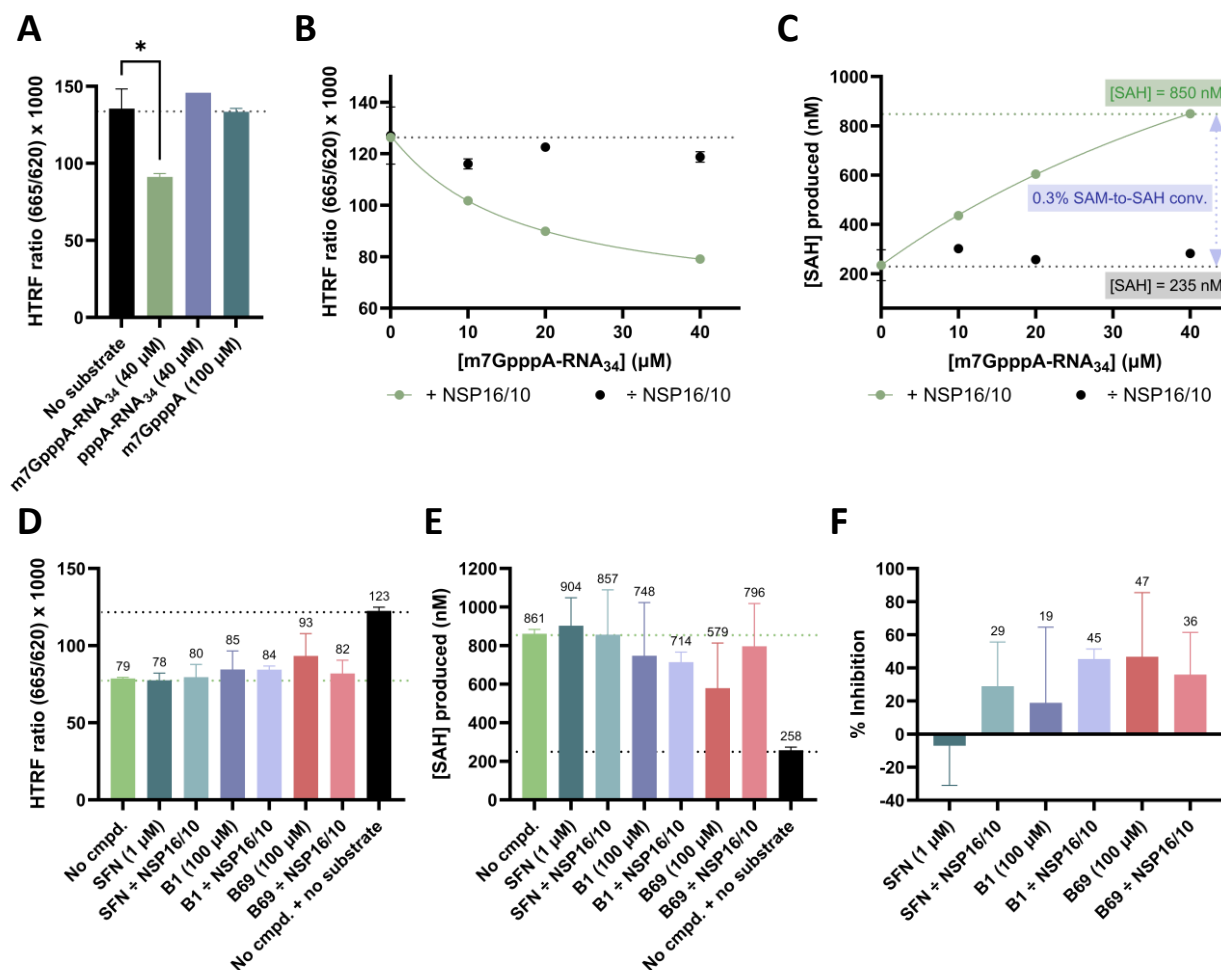


**Figure 2.33. MTase assay results using m7GpppA-RNA<sub>34</sub> as a substrate.** Error bars represent the standard deviation of technical duplicates. \* =  $P < 0.05$  in unpaired t test. (A) A single-dose test of m7GpppA-RNA<sub>34</sub>, alongside the uncapped RNA chain pppA-RNA<sub>34</sub> and the cap0-analogue m7GpppA. (B–C) Dose-response test of m7GpppA-RNA<sub>34</sub> with and without NSP16/10, expressed as HTRF and as [SAH] produced. (D–F) Inhibition test of SFN, B1, and B69 with and without NSP16/10.

Despite the narrow assay window, we proceeded to attempt inhibition test. SFN was chosen as a positive control, and as representative test compounds, B1 (the original fragment hit) and B69 (the strongest binding analogue) were selected. Testing the compounds at 100 μM (and SFN at 1 μM) gave largely inconclusive results due to poor data quality—given the large deviation between technical replicates, none of the compounds showed activities statistically different from the no-compound negative control (Figure 2.33D–F).

### 2.9.2. Scintillation proximity assay

Given that we could not obtain reliable data from the aforementioned MTase assay, we turned to an orthogonal and well-established scintillation proximity assay (SPA), which has previously been reported

in literature for measuring NSP16/10 activity.<sup>94</sup> This assay monitors the methyl transfer of tritium labelled SAM (<sup>3</sup>H-SAM) to a short biotinylated m7GpppA RNA substrate (5'-m7GpppACCCCC-7'), which is subsequently captured using streptavidin functionalised SPA plates. The radioactivity level (CPM) is then measured, and any changes used as a proxy for NSP16/10 activity.

Discouragingly, when our collaborators at University of Toronto screened the compounds up to a test concentration of 100  $\mu$ M, no inhibition could be detected. Further dose-response test from a top concentration of 500  $\mu$ M on selected high-affinity analogues (having SPR-determined affinities below 10  $\mu$ M) also did not give any reliable inhibition (**Appendix Figure A4**).

## 2.10. Summary

In summary, this study explored the prospect of applying a fragment-based approach to making photoswitchable ligands by screening a general (non-photoswitchable) library followed by early transformation of the resulting hit into an azo photoswitch. As a case study, we targeted the disease-relevant protein NSP16/10 from SARS-CoV-2. Using SPR, we screened general fragment libraries to identify the biarylsulfonamide hit **B1**. This was subjected to multiple iterations of design, synthesis, and test, whilst monitoring binding affinity ( $K_D$ ), fold change in activity (FC) and lipophilic efficiency (LiPE) (**Figure 2.34A**). We were unable to obtain any precise structural characterisation data on the fragment hit binding to the protein, so the optimisation study relied almost exclusively on ligand-based design and involved over 100 analogues. Of note, the initial azologisation of the hit to **B2** was unsuccessful, giving retained binding but no FC due to poor photochemical properties. Key to the success of the optimisation relied on a regioisomeric change of the RHA<sub>ar</sub> amino group to give the analogue **B24** with a 5-fold increased binding affinity. Further systematic substitution studies on this compound uncovered the SAR around the scaffold and resulted in the best overall ligand of the study, **B69**, displaying submicromolar binding affinity for NSP16/10. Guided by the structure-activity relationship data generated from this non-photoswitchable series, an azo extension strategy was adopted to ultimately give the best photoswitchable ligand **B130**, which showed a single-digit micromolar binding affinity and a more than 10-fold change in activity upon photoirradiation. Overall, this corresponded to a 370- and 46-fold improvement in binding affinity over **B1** for **B69** and **B130**, respectively, with concurrent increase in the LiPE (**Figure 2.34B**).



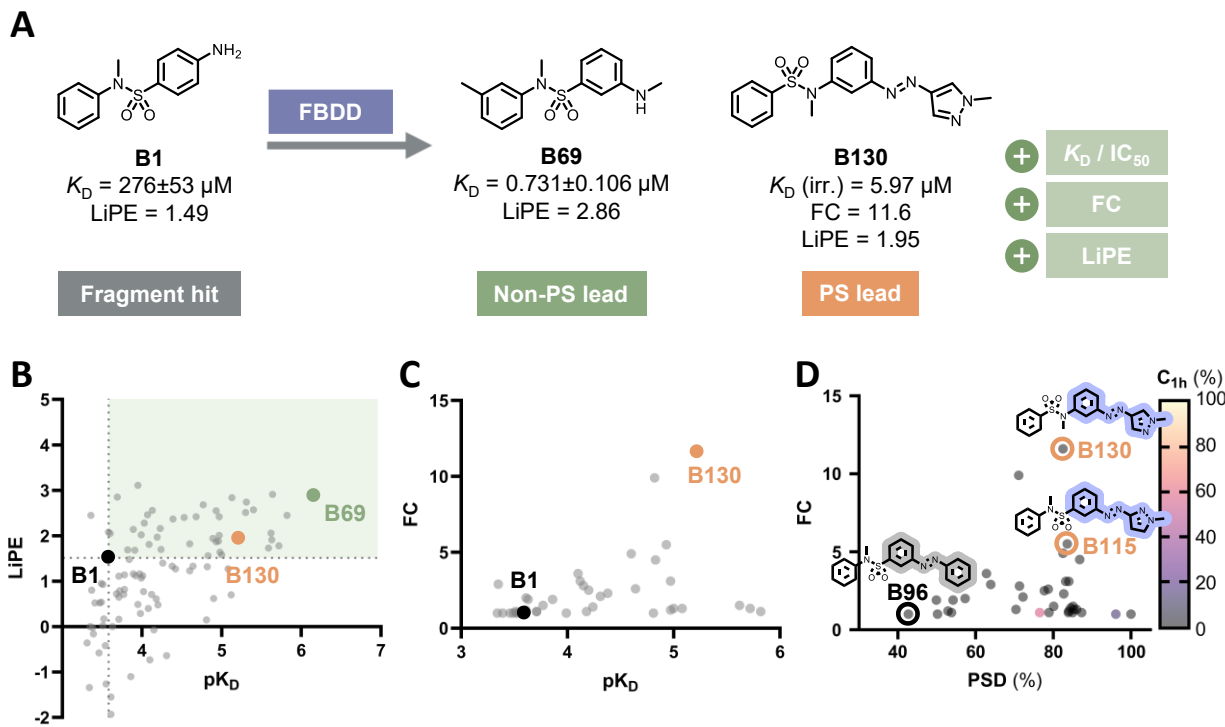


**Figure 2.34. Summary of the NSP16/10 FBDD campaign.** (A) Overview of key compounds from the optimisation study of the fragment hit **B1** into the lead compounds **B69** and **B130**. (B) A plot of lipophilic efficiency (LiPE) against SPR-determined binding affinity ( $pK_D$ ) for all compounds from the campaign showing activity. (C) A plot of the fold change in activity upon photoirradiation (FC) against  $pK_D$  for all photoswitchable compounds. (D) A plot of the FC against the photostationary distribution (PSD) and with the percentage thermal relaxation after 1 h ( $C_{1h}$ ) indicated by colour for all photoswitchable compounds.

Balancing the properties of the photoswitchable series was especially challenging, and many of the modifications yielded opposing effects on affinity and FC (**Figure 2.34C**). Key to the success of obtaining a significant increase in both  $K_D$  and FC involved a change from the initially explored azobenzene motif (e.g., **B96**) into the azopyrazoles (as represented by **B115** and **B130**)—presumably due to significantly increased photoconversions combined with potential new polar interactions endowed by the heteroatoms (**Figure 2.34D**).

Crucially, the structure of the best photoswitchable ligand, **B130**, cannot be easily derived from the best overall binder from the campaign, **B69**, exemplifying the limitations of the canonically employed late-stage azo-transformation approach. Rather, this photoswitchable lead molecule is the result of carefully balancing affinity, FC and LiPE through a bottom-up design- and synthesis-intensive approach. The ligands identified in this study still need to have their binding further characterised for their functional relevance as they do not appear to be inhibitory against NSP16/10 activity. Regardless, this study has

demonstrated that it is possible to employ a non-photoswitchable fragment hit as a starting point to develop efficient photoswitchable ligands.

## 3. Photoswitchable ligands from fragment probes

### 3.1. Introduction

#### 3.1.1. The idea of going smaller

The preceding strategy of screening a library of conventional fragments followed by early-stage optimisation of the fragment hit into an azo-based photoswitch gave modest success when applied to NSP16/10. However, the general applicability of such an approach has yet to be proven. One obvious concern is whether hits obtained from a general fragment library will always be amendable to azo transformation. There is a possibility that this may not be the case and that such transformation leads to unproductive binding and unfavourable properties.

To address this shortcoming, we considered if we could assemble a library of fragments which were poised to be transformed into azo-based ligands. Here, screening a fragment library comprising solely of azologable scaffolds (i.e., two ring systems connected via a two-atom linker) would likely provoke the same issues of low shape diversity as screening a photoswitchable fragment library, giving poor sampling of chemical space and result in a low hit rate.

This led us to contemplate if we could deconstruct the azo scaffolds into single-ring systems (**Figure 3.1A**). By reducing the size and complexity of the fragment further, a larger hit rate would be expected.<sup>63</sup> Extremely high hit rates (up to 60%) have been demonstrated when screening a library of ultra-small fragments called MiniFragments (MW < 100 Da, HAC of 5–7).<sup>121</sup> Firstly, this reduces the requirement to screen large numbers of fragments—indeed, the MiniFrag library comprises only 81 members.<sup>121</sup> Secondly, by starting with a smaller scaffold, rational extension with a suitable azo motif may allow the fragment to complement the protein interaction surface more snugly, giving both increased absolute and differential ligand binding affinity.

Of the single-ring systems, we took notice of 4-bromopyrazole (**Figure 3.1B**). This halogenated heterocycle has previously been coined a universal or privileged fragment probe, as it has been observed to bind specifically to multiple ligand-binding sites for multiple target proteins where it helps to identify hot spots (i.e. energetically favourable and frequently liganded pockets).<sup>122</sup> The inclusion of a halogen in the structure further facilitates solving co-crystal structures with the fragment through anomalous diffraction detection.<sup>122</sup>

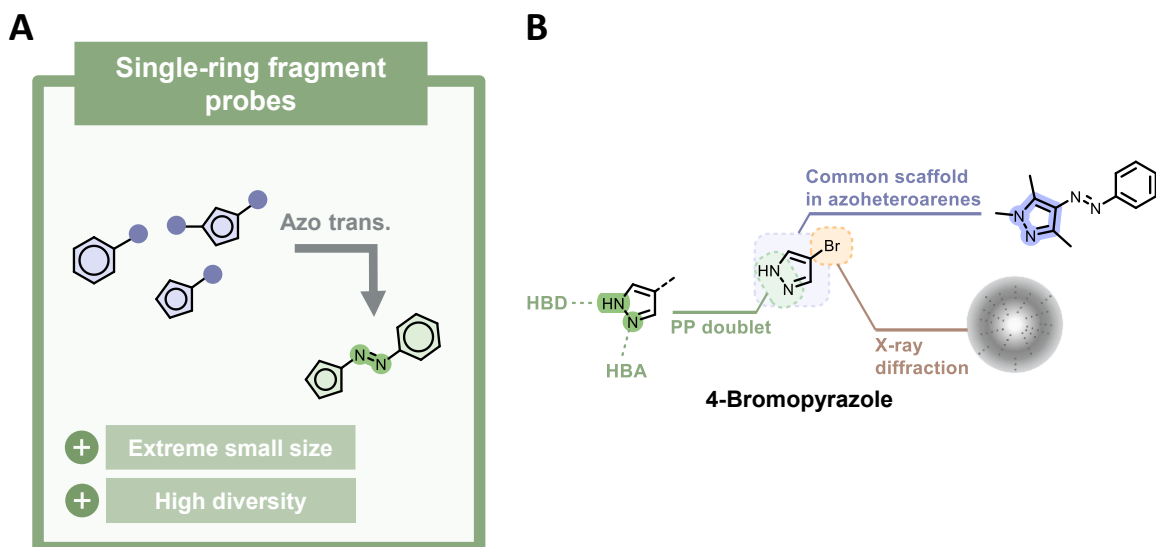


**Figure 3.1. Ultra-small fragment probes for photoswitch design.** (A) The screening of ultra-small substituted single-ring fragments requires a smaller library. Hits can be transformed into an azo hit via azo extension. (B) 4-Bromopyrazole is potentially a multi-functional fragment, which can probe the protein interaction landscape with its pharmacophore (PP) doublet, facilitate X-ray crystallography due to the inclusion of a halogen and serve as a basis for making azoheteroarene-based analogues.

The specific promiscuity of 4-bromopyrazole may be related to the inclusion of a pharmacophore doublet, i.e., two functional groups with H bonding capacity, confined in a minimally sized scaffold. More such halogenated fragments have been explored with the so-called FragLite library developed by Waring and co-workers.<sup>123</sup> This minimally sized 31-membered library has been demonstrated to identify and map druggable ligand binding sites in multiple protein targets.

Beyond its capacity to act as a fragment probe, we recognised that the pyrazole motif in 4-bromopyrazole is a well-represented scaffold in multiple next-generation azoheteroarene-based photoswitches and that it therefore may represent a fragment probe poised for azo photoswitch transformation (**Figure 1.4A**). From this observation arose the idea that one could crystallographically screen the target protein with 4-bromopyrazole, which would simultaneously sample the protein interaction surface for druggable binding sites as well as creating a basis for rapidly constructing azoheteroarene-based fragment analogues via azo extension.

### 3.1.2. Cyclin-dependent kinase 2

To test the hypothesis that 4-bromopyrazole could serve as an efficient fragment probe for making photoswitchable ligands, we were interested in targeting cyclin-dependent kinase 2 (CDK2) as a case study. This protein has previously been screened against with the FragLite library and been crystallised with 4-

bromopyrazole.<sup>123</sup> Given that CDK2 further represents a well-characterised enzymatic system that has been subjected to intense biological and drug discovery research for decades, we considered this target a robust model system to test our hypothesis.

CDK2 belongs to a family of serine-threonine protein kinases, which comprises a total of 20 members in humans (CDK1–20).<sup>124</sup> CDK2 is classically thought to play key regulatory roles in transcription initiation, centrosome duplication and DNA synthesis during the G1 and S-phases of the dividing cell (**Figure 3.2A**).<sup>125</sup> Due to its crucial responsibilities, CDK2 is in turn also strictly regulated—to gain catalytic activity, the enzyme needs to form a heterodimeric complex with cyclins (E1/E2 or A2) and further needs to be phosphorylated by the CAK complex and be dephosphorylated by CDC25A (**Figure 3.2B**).<sup>125</sup>

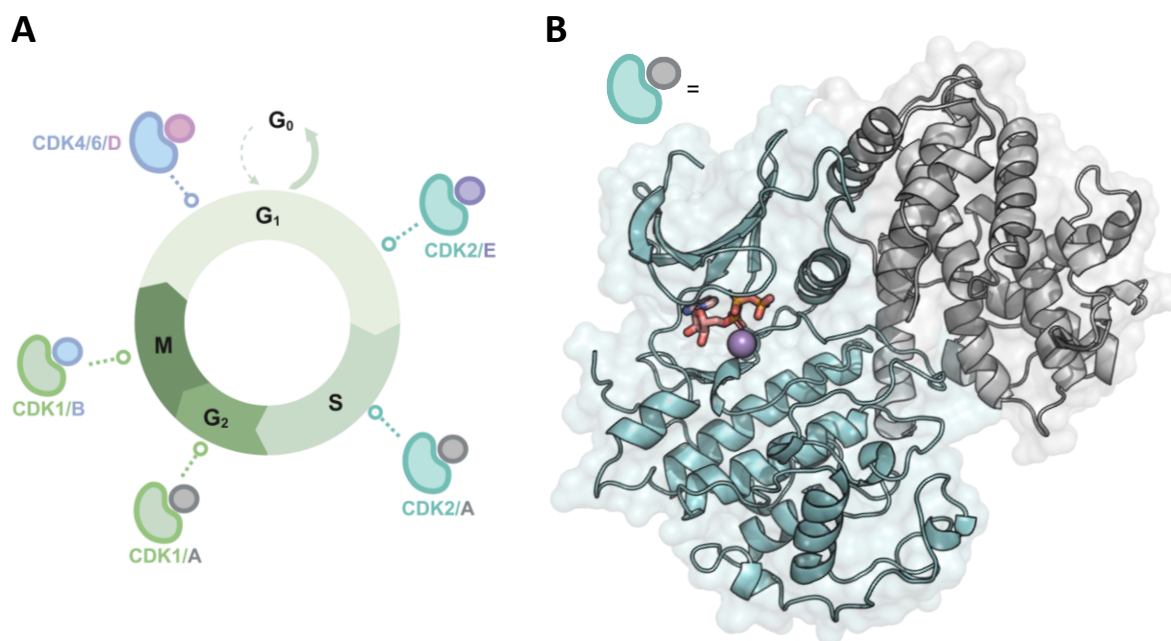


**Figure 3.2. The biology and structure of CDK2.** (A) An overview of the roles of various CDKs and their cognate cyclins in the cell cycle. Created with BioRender.com. (B) X-ray structure of CDK2 in complex with cyclin A and an ATP-derivative (ATP- $\gamma$ -S, shown as pink sticks) (PDB ID 1JST).<sup>126</sup> Created with PyMOL (version 2.5.4).<sup>47</sup>

Given its role in the cell cycle progression, CDK2 and other CDKs have been long-standing targets in cancer therapy. To this end, there have been conflicting data generated on the role of CDK2 in both normal as well as cancer cell development. Early studies using CDK2-targeting antibodies have shown that CDK2 is essential for S-phase initiation and thereby normal cell cycle progression.<sup>127</sup> However, animal model studies have shown that interphase CDKs including CDK2 is not vital for the division of most cell types but only required for specialised cells—indeed, genetic knock-out of CDK2 in mice results in no detectable defects.<sup>128</sup> Furthermore, a study based on genetic mutation as well as the use of antisense oligonucleotides and

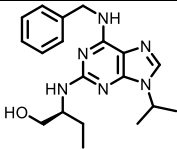
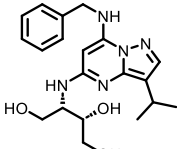
small interfering RNAs have shown that CDK2 is dispensable for osteosarcoma and cervical cancer proliferation—and concluded that CDK2 is a poor target for cancer therapy.<sup>129</sup> A more recent bump-and-hole chemical genetic study have, however, challenged this notion and demonstrated that CDK2 is crucial for both non-transformed cell growth as well as for the proliferation of various oncogene-transformed cancer cells.<sup>130</sup>

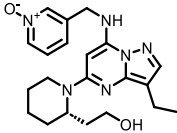
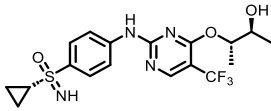
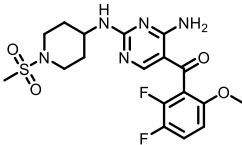
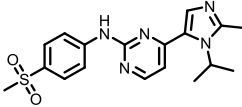
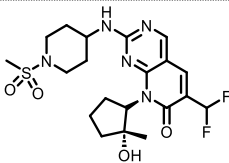
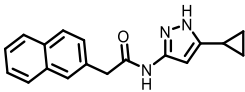
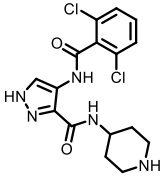
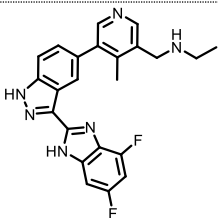
These inconsistent literature data may be due to the use of varying methods for modulating CDK2 activity, each having their set of limitations. Although mutation and RNA-silencing methods specifically target CDK2, they affect protein levels and will activate cellular compensatory mechanisms that muddies the phenotypic outcome. Thus, the current consensus is that CDK2 remains an attractive target for cancer therapy.<sup>125</sup>

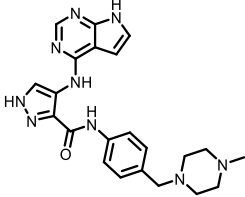
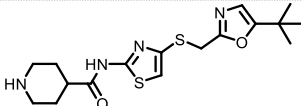
### 3.1.3. Reported CDK2 inhibitors

A multitude of small-molecule CDK2 inhibitors have been reported to date (**Table 3.1**). A variety of different chemotypes have here been explored, including nucleobase analogues (**Roscovitone**, **BS-194** and **Di-naciclib**), pyrimidines (**Roniciclib**, **R547**, **AZD5438**, and **PF-06873600**), pyrazoles (**PNU-292137**, **AT7519**, **AG-024322**, and **FN-1501**) and thiazoles (**SNS-032**). Most of these inhibitors possess excellent biochemical potencies in the low nanomolar range. However, none display appreciable selectivity for CDK2 over the other homologous CDKs. On the other hand, selective CDK4/6 inhibitors have been developed of which some have been FDA approved for the treatment of particular advanced forms of breast cancer.<sup>131</sup> This suggests that solving the problem of selectivity may be key for developing efficacious and safe therapeutic candidates targeting CDK2.

**Table 3.1. Structure and activity of reported CDK2 inhibitors.** <sup>a</sup>

| Cmpd ID                      | Structure                                                                           | Biological activity (target)                                                                                                                                                                                                                                                                                                                                                                                                                   | PDB ID |
|------------------------------|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|
| <b>Roscovitone (CYC-202)</b> |  | IC <sub>50</sub> = 0.7 μM ( <b>CDK2/A</b> ) <sup>132</sup><br>IC <sub>50</sub> = 0.7 μM ( <b>CDK2/E</b> ) <sup>132</sup><br>IC <sub>50</sub> > 100 μM ( <b>CDK4/D1</b> ) <sup>132</sup><br>IC <sub>50</sub> = 0.16 μM ( <b>CDK5/p35</b> ) <sup>132</sup><br>IC <sub>50</sub> > 100 μM ( <b>CDK6/D3</b> ) <sup>132</sup>                                                                                                                        | 2A4L   |
| <b>BS-194</b>                |  | IC <sub>50</sub> = 3 nM ( <b>CDK2/E</b> ) <sup>133</sup><br>IC <sub>50</sub> = 33 nM ( <b>CDK1/B1</b> ) <sup>133</sup><br>IC <sub>50</sub> = 20 μM ( <b>CDK4/D1</b> ) <sup>133</sup><br>IC <sub>50</sub> = 30 nM ( <b>CDK5/p35</b> ) <sup>133</sup><br>IC <sub>50</sub> = 35.5 μM ( <b>CDK6/D1</b> ) <sup>133</sup><br>IC <sub>50</sub> = 250 nM ( <b>CDK7/H</b> ) <sup>133</sup><br>IC <sub>50</sub> = 90 nM ( <b>CDK9/T</b> ) <sup>133</sup> | 3NS9   |

|                                |                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                             |      |
|--------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| <b>Dinaciclib (SCH 727965)</b> |    | $IC_{50} = 1 \text{ nM (CDK2)}$ <sup>134</sup><br>$IC_{50} = 3 \text{ nM (CDK1)}$ <sup>134</sup><br>$IC_{50} = 1 \text{ nM (CDK5)}$ <sup>134</sup><br>$IC_{50} = 4 \text{ nM (CDK9)}$ <sup>134</sup>                                                                                                                                                                                                                                                        | 5L2W |
| <b>Roniciclib (BAY1000394)</b> |    | $IC_{50} = 9 \text{ nM (CDK2/E)}$ <sup>135</sup><br>$IC_{50} = 7 \text{ nM (CDK1/B)}$ <sup>135</sup><br>$IC_{50} = 11 \text{ nM (CDK4/D1)}$ <sup>135</sup><br>$IC_{50} = 5 \text{ nM (CDK9/T1)}$ <sup>135</sup>                                                                                                                                                                                                                                             | 5IEV |
| <b>R547 (39)</b>               |    | $K_i = 3 \text{ nM (CDK2/E)}$ <sup>136</sup><br>$K_i = 2 \text{ nM (CDK1/B)}$ <sup>136</sup><br>$K_i = 1 \text{ nM (CDK4/D)}$ <sup>136</sup>                                                                                                                                                                                                                                                                                                                | 2FVD |
| <b>AZD5438</b>                 |    | $IC_{50} = 45 \text{ nM (CDK2/A)}$ <sup>137</sup><br>$IC_{50} = 6 \text{ nM (CDK2/E)}$ <sup>137</sup><br>$IC_{50} = 16 \text{ nM (CDK1/B1)}$ <sup>137</sup><br>$IC_{50} = 449 \text{ nM (CDK4/D1)}$ <sup>137</sup><br>$IC_{50} = 14 \text{ nM (CDK5/p25)}$ <sup>137</sup><br>$IC_{50} = 21 \text{ nM (CDK6/D3)}$ <sup>137</sup><br>$IC_{50} = 821 \text{ nM (CDK7/H)}$ <sup>137</sup><br>$IC_{50} = 20 \text{ nM (CDK9/T)}$ <sup>137</sup>                  | 6GUH |
| <b>PF-06873600 (22)</b>        |   | $K_i = 0.13 \text{ nM (CDK2/E1)}$ <sup>138</sup><br>$K_i = 4.5 \text{ nM (CDK1/A2)}$ <sup>138</sup><br>$K_i = 1.25 \text{ nM (CDK4/D1)}$ <sup>138</sup><br>$K_i = 0.11 \text{ nM (CDK6/D1)}$ <sup>138</sup><br>$K_i = 19.6 \text{ nM (CDK9/T1)}$ <sup>138</sup>                                                                                                                                                                                             | 7KJS |
| <b>PNU-292137 (41)</b>         |  | $IC_{50} = 37 \text{ nM (CDK2/A)}$ <sup>139</sup><br>$IC_{50} = 92 \text{ nM (CDK2/E)}$ <sup>139</sup><br>$IC_{50} = 270 \text{ nM (CDK1/B)}$ <sup>139</sup><br>$IC_{50} = >10 \text{ }\mu\text{M (CDK4/D1)}$ <sup>139</sup><br>$IC_{50} = 114 \text{ nM (CDK5/p35)}$ <sup>139</sup>                                                                                                                                                                        | 1VYW |
| <b>AT7519</b>                  |  | $IC_{50} = 47 \text{ nM (CDK2/A)}$ <sup>140</sup><br>$IC_{50} = 210 \text{ nM (CDK1/B)}$ <sup>141</sup><br>$IC_{50} = 360 \text{ nM (CDK3/E)}$ <sup>141</sup><br>$IC_{50} = 100 \text{ nM (CDK4/D1)}$ <sup>141</sup><br>$IC_{50} = 13 \text{ nM (CDK5/p35)}$ <sup>141</sup><br>$IC_{50} = 170 \text{ nM (CDK6/D3)}$ <sup>141</sup><br>$IC_{50} = 2.4 \text{ }\mu\text{M (CDK7/H/MAT1)}$ <sup>141</sup><br>$IC_{50} < 10 \text{ nM (CDK9/T)}$ <sup>141</sup> | 2VU3 |
| <b>AG-024322</b>               |  | $K_i = 3 \text{ nM (CDK2/A)}$ <sup>142</sup><br>$K_i = 2.3 \text{ nM (CDK1/B)}$ <sup>142</sup><br>$K_i = 2.9 \text{ nM (CDK4/D)}$ <sup>142</sup>                                                                                                                                                                                                                                                                                                            | -    |

|                             |                                                                                   |                                                                                                                                                                  |      |
|-----------------------------|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| <b>FN-1501 (50)</b>         |  | IC <sub>50</sub> = 2.47 nM (CDK2/A) <sup>143</sup><br>IC <sub>50</sub> = 0.85 nM (CDK4/D1) <sup>143</sup><br>IC <sub>50</sub> = 1.96 nM (CDK6/D1) <sup>143</sup> | -    |
| <b>SNS-032 (BMS-387032)</b> |  | IC <sub>50</sub> = 38 nM (CDK2/E) <sup>144</sup><br>IC <sub>50</sub> = 62 nM (CDK7/H) <sup>144</sup><br>IC <sub>50</sub> = 4 nM (CDK9/T) <sup>144</sup>          | 5D1J |

<sup>a</sup> Only representative examples from each compound series have been included.

Most reported CDK inhibitors (so-called type I and II inhibitors, including all entries in **Table 3.1**) exploit the ATP binding site, where they compete with the co-factor to achieve enzyme inhibition.<sup>145</sup> Specifically, most of the inhibitors occupy the adenine sub-pocket, where they form one or several H bonds to the “hinge region”—a key ATP-recognition segment of kinases (**Figure 3.3A–B**).<sup>145, 146</sup> However, the spatial structure of the ATP-binding pocket is highly conserved across the CDKs (and indeed amongst kinases), which explains the general poor selectivity displayed by these compounds.<sup>145</sup> Although allosteric CDK inhibitors (type III and IV inhibitors) that usually display much improved selectivity profiles have been reported, these are few in numbers and are challenging to discover on a rational basis.<sup>145</sup>

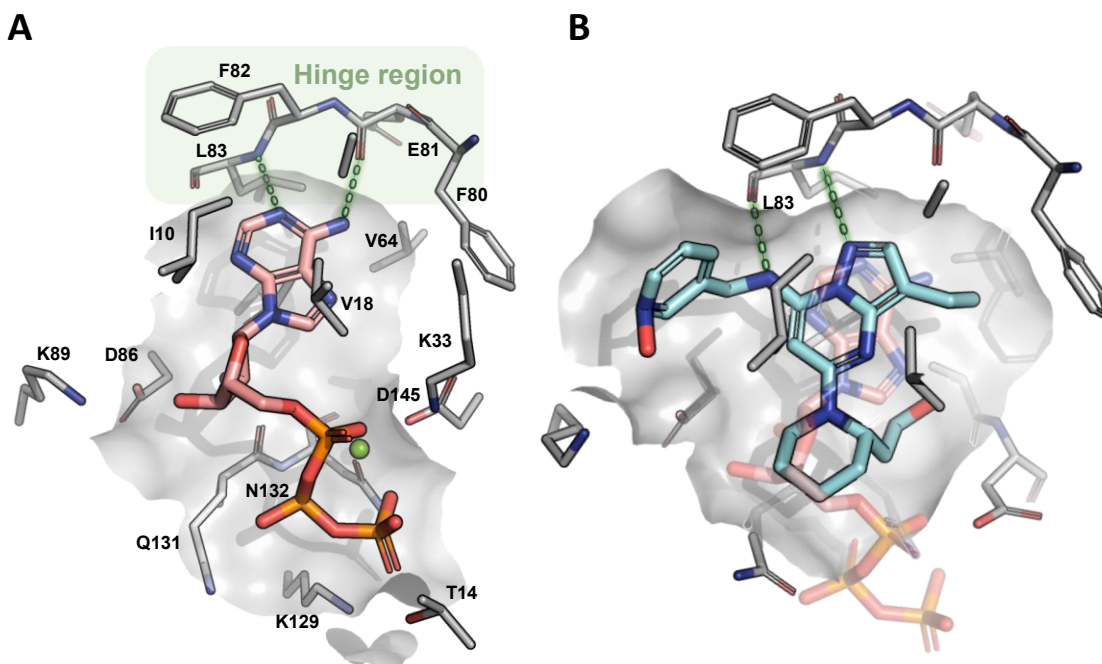


**Figure 3.3. The binding mode of CDK2 inhibitors.** (A) Binding mode ATP (pink sticks) to CDK2, highlighting the hinge region of the pocket (PDB ID 8FP5).<sup>147</sup> (B) Binding mode **Dinaciclib** (cyan sticks) as a canonical ATP-competitive inhibitor exploiting the Hinge region for binding affinity (PDB ID 5L2W).<sup>148</sup> Created with PyMOL (version 2.5.4).<sup>47</sup>



To this end, we may be able to exploit that different CDKs become active at different stages of the cell-cycle due to fluctuating cyclin concentrations and that they are therefore temporally resolved.<sup>149</sup> Furthermore, the localisation of the cyclins is subcellularly regulated and changes throughout the cell cycle, which result in the CDKs being active at different cellular sub-locations.<sup>150</sup> This creates an incentive for developing a photopharmacological agent, which—when combined with timed and ultra-precise photoirradiation—may allow for highly selective modulation of CDK2.

## 3.2. The fragment hit

### 3.2.1. Screening of the FragLite library

Waring and co-workers screened their 31-membered FragLite library of halogenated and pharmacophore-doublet containing fragments against CDK2 by X-ray crystallography from which they obtained nine hits that showed diffraction unambiguously assignable to ligand structure.<sup>123</sup> This corresponds to a large hit rate of 29%, as one could expect when screening such low-complexity compounds. The universal fragment 4-bromopyrazole (**C1**) was amongst the hits (**Figure 3.4A**).

### 3.2.2. Binding mode of **C1**

The co-crystal structure of **C1** in complex with CDK2 showed that the fragment is binding in the canonical hinge region of the ATP-binding site, overlapping with the adenine motif of ATP (**Figure 3.4B**). Here, the pyrazole is seen forming two H bond interactions with the backbone of Glu81 and Leu83 using its pharmacophore doublet, whereas the bromide is clamped in place by Val18 and Leu134 through hydrophobic interactions and possibly halogen bonding. The extensive intermolecular interactions made with a minimum of atoms supports the utility of 4-bromopyrazole as a universal fragment probe. Furthermore, one of the unsubstituted pyrazole positions is seen pointing towards the hydrophobic gatekeeper region constituting of Val64, Phe80, and Ala144 (the G vector), the other probing the more polar solvent-exposed region (the S vector) and the C-Br bond directed towards the ribose pocket (the B vector)—this would suggest plentiful of opportunities for fragment growing. Interestingly, an additional ligand occupancy was observed from the X-ray diffraction pattern, where the fragment flips such as to place the bromide substituent in the gatekeeper region instead (**Figure 3.4C**).

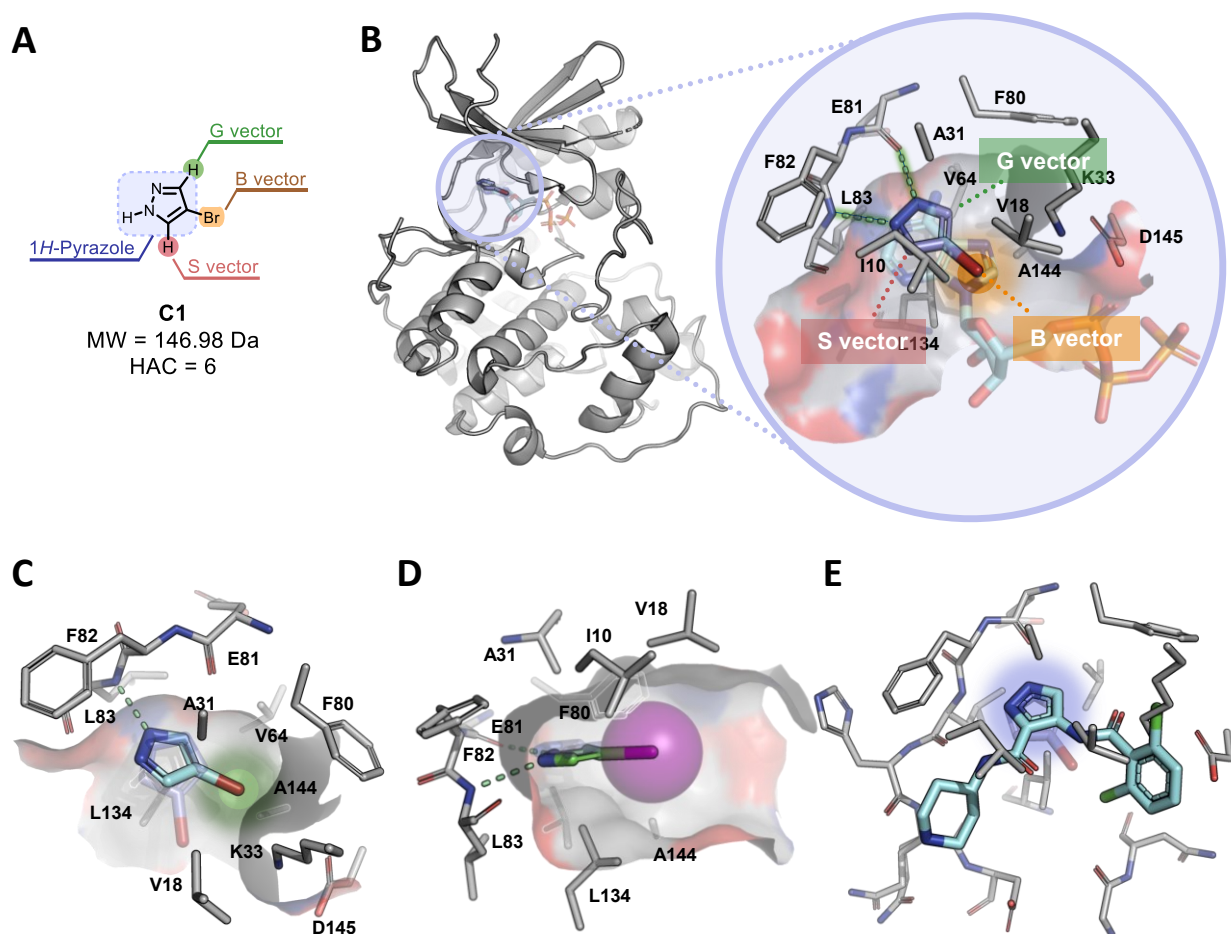


**Figure 3.4. The CDK2 fragment hit **C1**.** (A) Structure of **C1** and nomenclature of its growth vectors. (B) X-ray co-crystal structure of **C1** in complex with CDK2 (PDB ID 6Q3C).<sup>123</sup> **C1** shown in purple sticks. ATP (extracted from PDB ID 8FP5, aligned to the protein structure of 6Q3C) shown in cyan sticks.<sup>147</sup> (C) **C1** shows an additional binding mode in cyan sticks. (D) X-ray co-crystal structure of **C2** in complex with CDK2 (PDB ID 6Q3B).<sup>123</sup> **C2** shown in green sticks and the iodide additional shown as a sphere. (E) X-ray co-crystal structure of **AT7519** in complex with CDK2 (PDB ID 2VU3).<sup>140</sup> **AT7519** shown in cyan sticks. Created with PyMOL (version 2.5.4).<sup>47</sup>

In addition to **C1**, the 4-iodo analogue **C2** also emerged as a hit from the FragLite screening campaign. This showed the exact same binding mode as **C1**, retaining the dual H bonding with the hinge region (**Figure 3.4D**). The possibility of substituting the fragment with a large-sized iodide suggests that there is ample space for growing with bulky motifs from the B vector of the pyrazole ring.

We recognised that the pyrazole motif is represented in a number of reported CDK2 inhibitors (**Table 3.1**). Indeed, when superimposing the co-crystal structure of **AT7519** with **C1**, a well-preserved binding mode was observed (**Figure 3.4E**). Firstly, this underscores the favourability of the fragment binding mode, giving us confidence that the fragment can conserve its binding interactions even upon

extensive elaboration. Secondly, the B- and S-vectors of the pyrazole ring had been exploited to arrive at **AT7519**, which provided a guideline for subsequent fragment growing efforts.

### 3.2.3. Inhibitory activity of **C1** and **C2**

The affinity and the potency of the fragment probes **C1** and **C2** to CDK2 had not previously been determined. Here, we purchased the two fragments and firstly performed quality control by NMR spectroscopy to ensure their identity and purity. The compounds were tested for enzyme inhibition but showed no activity at 200  $\mu$ M in an ADP-Glo™ kinase assay (data not shown); this may however be expected given their small molecular sizes.

## 3.3. Azo transformation of fragment probe **C1**

### 3.3.1. Azo extension on **C1**

To commence the optimisation study, we immediately set out to perform azo extension on **C1**. As the growth moiety, we selected the phenyldiazenyl group due to synthetic ease and which would enable a plethora of subsequent functionalisation pathways (via aryl substitution). For the growth direction, we considered extension from all three vectors of the fragment. To assess the designs, we employed molecular docking using Molecular Operating Environment (MOE) and initially benchmarked the software by docking the fragment probe **C1**. Here, we were able to perfectly recapitulate the X-ray co-crystal structure pose, suggesting good fidelity (**Appendix Figure A6**).

Extension from the B vector involved replacing the bromide substituent with the phenyldiazenyl group, giving rise to the 4-phenylazopyrazole **C3** (**Figure 3.5A**). We assumed this substitution would be allowed given that the sterically demanding iodide group of **C2** can be accommodated and since the same pocket space is being probed by the isosteric benzamido group of **AT7519** (**Figure 3.4E**). Indeed, docking of **C3** into CDK2 showed that the constituent pyrazole ring could retain its binding interactions with the hinge region, whilst the phenyldiazenyl group probes the ribose sub-pocket, making favourable hydrophobic interactions with the side chains of Ile10, Val18 and Asn132 and possibly engaging in a  $\pi$ -cation interaction with Lys33 (**Figure 3.5B**). Interestingly, the Z-isomeric form of the compound could also recapitulate the fragment binding mode according to docking, but where the phenyldiazenyl group twists away to probe the more solvent exposed region of the ribose pocket (**Figure 3.5C**).

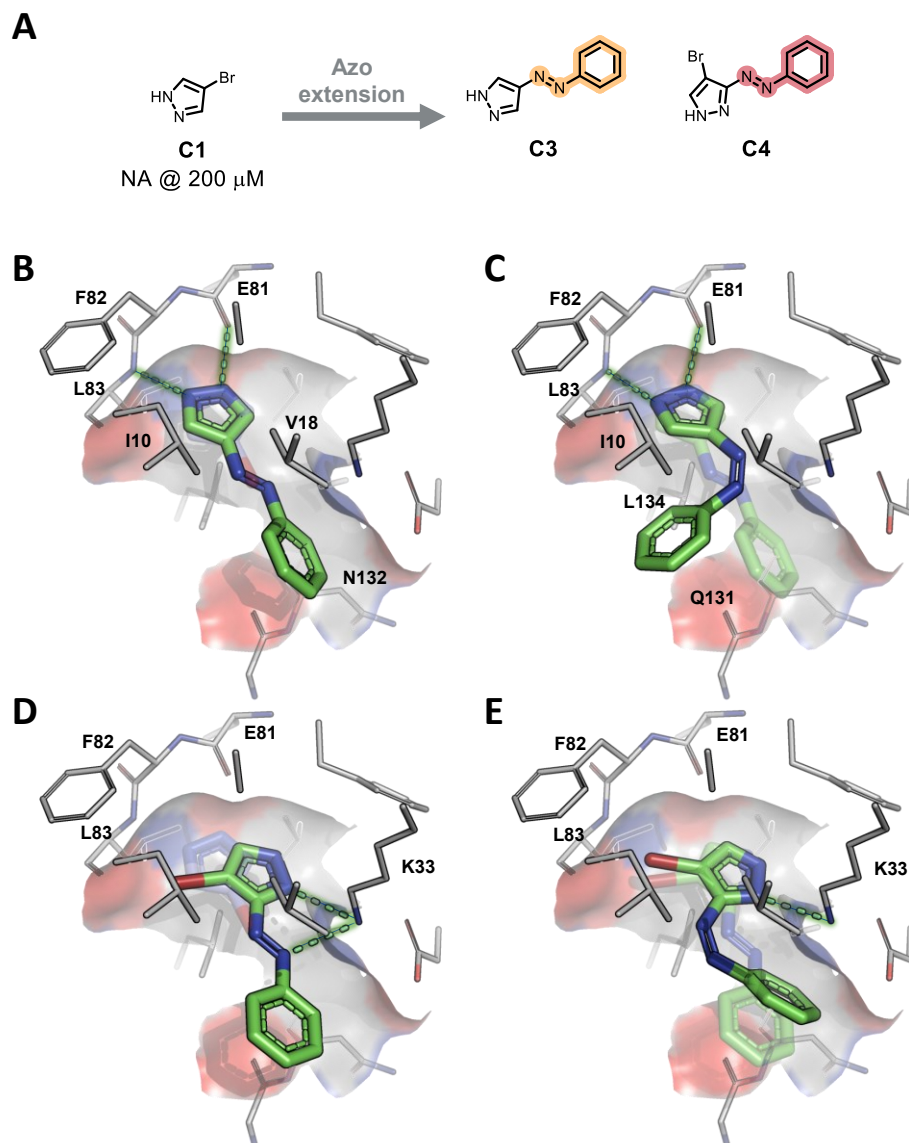


**Figure 3.5. Design of azo extension analogues of C1.** The analogues were docked in both the *E* and *Z*-isomeric form into CDK2 (protein receptor from PDB ID 6Q3C). X-ray structure of **C1** shown in cyan sticks and docking poses shown in green sticks. Created with PyMOL (version 2.5.4).<sup>47</sup> (A) Structure of **C3** and **C4**. (B) Docking pose of (*E*)-**C3**. (C) Docking pose of (*Z*)-**C3**. (D) Docking pose of (*E*)-**C4**. (E) Docking pose of (*Z*)-**C4**.

Still, given the more extensive contacts and a binding mode mimicking the high-affinity compound **AT7519**, we considered the *E*-isomeric form of **C3** to be more favourable.

Extension from either the G- or S-vector of the fragment can be treated to be chemically equivalent, due to the rapid tautomerisation that 3(5)-substituted pyrazoles are known to exhibit for a large set of substituents.<sup>151</sup> Extending from the 3(5)-position of **C1** thus yielded a single compound, the 3-phenylpyrazole **C4** (Figure 3.5A). Although the S vector appeared to be able to accommodate the phenyldi-azanyl group (e.g., given the structure of **AT7519** where a piperidin-4-ylcarbamoyl motif is seen placed in

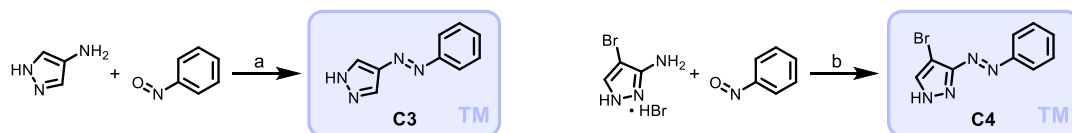
that region), docking of both the *E*- and *Z*-isomer of **C4** showed a flipped binding mode (**Figure 3.5D–E**). Here, the fragment has undergone translational and rotational change away from the hinge region to instead form a H bond with Lys33. Although these represent potentially interesting binding modes, we were concerned about their legitimacy.

From these computational investigations, the azo extension analogue **C3** emerged as the more promising design, having what we considered a more reliable binding mode. However, given the known inaccuracy and limitations associated with current molecular docking capabilities,<sup>152</sup> we proceeded to still pursue **C4** as well.

### 3.3.2. Synthesis of azo extension analogues **C3** and **C4**

The two analogues were readily synthesised by condensing the appropriate aminopyrazole building blocks with nitrosobenzene (**Scheme 3.1**). Performing the reaction with acetic acid which served both as a catalyst and solvent afforded **C3** in a decent 39% yield. The same acidic Baeyer-Mills reaction conditions were unsuccessful in giving **C4** (data not shown)—presumably due to usage of the aminopyrazole building block in the form of a hydrobromide salt, which renders the amino group too unreactive to act as a nucleophile. The reaction was instead performed under basic conditions with excess NaOH, which would desalt the aminopyrazole *in situ* and simultaneously catalyse the condensation reaction with nitrosobenzene.<sup>36</sup> Using these conditions, **C4** was obtained albeit in a low yield (18%).

**Scheme 3.1. Synthesis of azo extension analogues **C3** and **C4**.**<sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) AcOH, 40 °C, 23 h, 39%. (b) aq. NaOH, PhMe, 80 °C, 26 h, 18%. TM = Target molecule.

### 3.3.3. Photochemical characterisation of **C3** and **C4**

To gauge the light addressability of the synthesised azo extension analogues **C3** and **C4**, we next carried out photochemical characterisation on the compounds by UV–Vis spectrophotometry. We were especially interested in their photoswitching behaviour and thermal stability in an aqueous environment that would mimic biological systems and thus chose to perform the photochemical studies in an assay buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05 %[v/v] Tween-20/Surfactant P20) with 1% added DMSO as a co-solvent.

The 4-phenylazopyrazole **C3** showed only decent photoswitching completeness with 365 nm light (76%), whereas excellent photoconversion could be obtained using 340 nm (98%) (**Figure 3.6A**). Noticeably, the Z-isomeric form of the photoswitch possessed a relatively low thermal stability with a calculated half-life of 3.3 h at 20 °C (**Figure 3.6B**). This is in stark contrast to the thermal stability of the *N*-methylated analogue of **C3**, **4pzH**, which has a reported thermal half-life of  $\approx 1000$  days in DMSO at 25 °C.<sup>35</sup> The medium is known to be able to impart a dramatic effect on the thermal isomerisation rate of azo photoswitches,<sup>153</sup> and to exclude the effect of solvent (alongside other small differences in temperature and concentration) we performed photochemical analysis of **4pzH** with the same protocol (assay buffer with 1% DMSO at 20  $\mu$ M and 20 °C). However, we observed no detectable back-isomerisation for **4pzH** after 1 h (**Appendix Figure A7A–B**), implying an inherent difference in thermal stability between **C3** and the *N*-methylated analogue **4pzH**. This correlates with previous empirical studies on the effect of *N*-(de)methylation on the 4-phenylazopyrazole, although it is still not clear what is responsible for this phenomenon.<sup>154</sup>

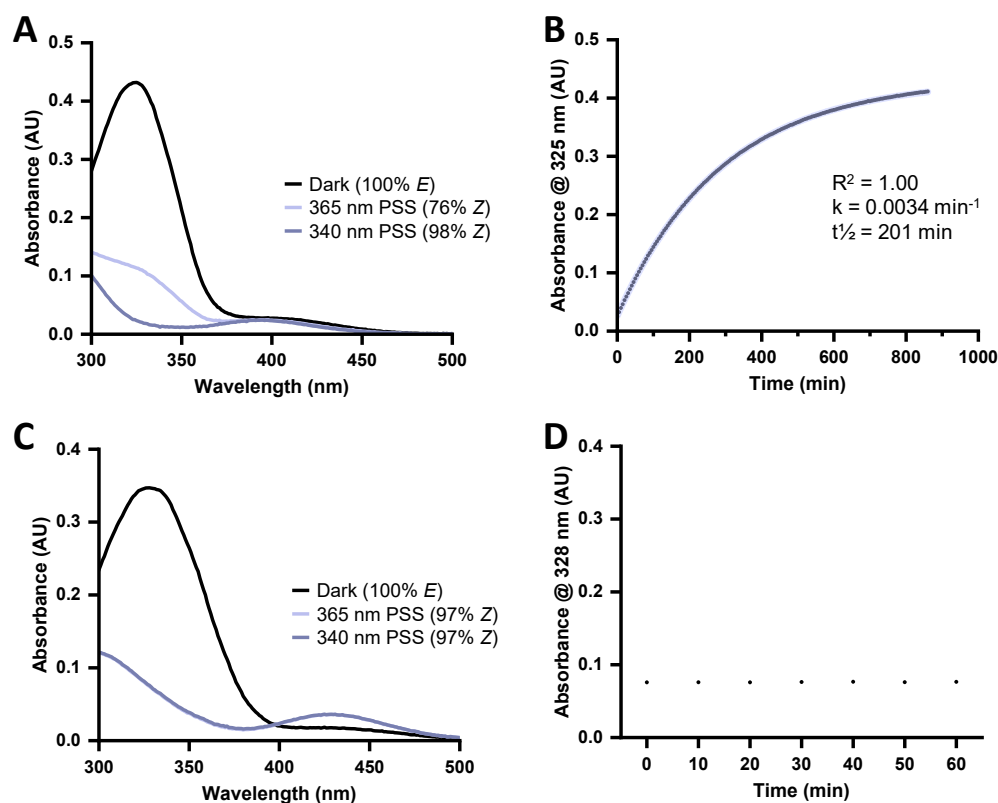


**Figure 3.6. Photochemical characterisation of **C3** and **C4**.** Photoswitching and thermal kinetics were determined by UV–Vis spectrophotometry at 20 °C and at 20  $\mu$ M in 10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05 %[v/v] Tween-20/Surfactant P20, 1% DMSO. (A) PSD determination for **C3**. (B) Thermal back-isomerisation of (**Z**)-**C3** fitted to a first-order kinetic model. (C) PSD determination for **C4**. (D) No significant thermal back-isomerisation was observed for (**Z**)-**C4** over 1 h.

Presumably due to a small bathochromic shift (higher  $\lambda$ ) of the *E*-isomer absorption band, the 4-bromo-3-phenylazopyrazole **C4** showed an equally excellent photoswitchability (PSD 97%) when photoirradiated with 340 and 365 nm light (**Figure 3.6C**). Interestingly, no thermal back-isomerisation could be observed to occur over a duration of 1 h for this compound, which suggested a thermal stability at least in the order of days (**Figure 3.6D**). This is in contrast to prior DFT analysis on 3-phenylazopyrazole, where fast isomerisation via a rotational pathway had been predicted.<sup>154</sup> This would allude to a stabilising effect caused by the bromide substituent, but further investigations are required to verify this conjecture.

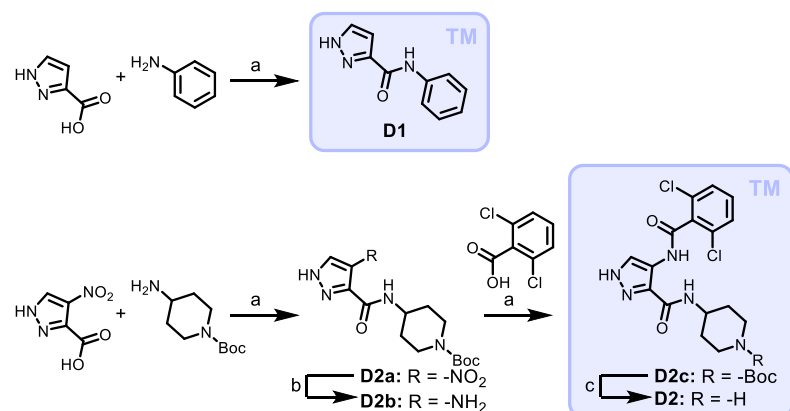
### 3.4. Biological characterisation

Having identified two possible azo extension analogues that could serve as the hit scaffolds for further optimisation, we first set out to establish assays for characterising their binding and functional activity.

#### 3.4.1. Control compounds

We first embarked on the acquisition of positive control compounds that would be useful for assay validation and serve as references to benchmark subsequent compound designs against. For this purpose, we selected the fragment-like compound **D1** ( $IC_{50}$  = 97  $\mu$ M), which due to its similar structure and size was expected to possess activity in the range of the initial fragment analogues.<sup>140</sup> In addition, we selected the advanced and potent CDK2 inhibitor **AT7519** (**D2**,  $IC_{50}$  = 47 nM) as a comparator for later and more elaborate analogues.<sup>140</sup> The compounds were readily synthesised using published procedures (**Scheme 3.2**).

**Scheme 3.2. Synthesis of control compounds D1 and D2.**<sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) EDC-HCl, HOBT, DMF, rt or 0 °C–rt, 18–30 h, 22%–quant. (b) Pd/C, MeOH, DMF, H<sub>2</sub> (1 atm), rt, 24 h, 96%. (c) TFA, DCM, rt, 17 h, 91%. TM = Target molecule.

### 3.4.2. Fluorescence polarisation assay

We were initially interested in establishing a fluorescence polarisation (FP) assay for detecting ligand binding to CDK2. The principle behind FP hinges on the phenomenon that when a fluorophore-labelled probe is excited with polarised light, the emitted light will be depolarised due to molecular tumbling during the lifetime of its excited state (**Figure 3.7A**).<sup>155</sup> However, upon binding to a high-molecular weight protein, the reorientation rate of the probe will slow down, and the emitted light will remain polarised to a larger degree.<sup>155</sup> Thus, FP can be applied to measure probe binding as well as ligand competition—as the degree of light polarisation will be proportional to the fraction of bound probe.

An FP assay for monitoring the binding of ligands to the ATP-binding site of protein kinases has previously been reported by Moreira *et al.*<sup>156</sup> This relies on the use of a fluorescent ATP derivative—the commercially available BODIPY<sup>TM</sup> ATP- $\gamma$ -S (**P1**, **Figure 3.7B**). We commenced the study of adapting the assay to CDK2 by first titrating free **P1** in an appropriate assay buffer in order to establish a suitable probe concentration (**Figure 3.7C**). We obtained an expected antagonistic sigmoidal curve, and upon inspecting its profile, a probe concentration of 5 nM was chosen—this resulted in significant depolarisation (i.e., low mP value) without causing full saturation of the signal. Next, we titrated in CDK2 where a protein concentration that yields approximately 75% repolarisation of the maximum signal is generally recommended to achieve a significant assay window.<sup>157</sup> Unexpectedly, an extremely high protein concentration (50  $\mu$ M) was required to gain a noteworthy repolarisation of  $\approx$  30% (**Figure 3.7D**). However, upon adapting the assay into a competition format, testing the cold tracer (i.e. unlabelled ATP), the two literature ligands **D1** and **D2** and our compound **C3**, we were unable to observe any significant depolarisation of the signal (**Figure 3.7E**). This would suggest that the small repolarisation obtained during protein titration was due to non-specific binding.

Upon inspecting the X-ray co-crystal structure of ATP binding to CDK2, we observed that the  $\gamma$ -phosphate may not constitute a favourable position to modify the endogenous ligand at, since this is relatively buried and engaged in extensive H bond interactions with protein residues (Lys129 and Thr14) and involved in chelating the Mg<sup>2+</sup> ion—a crucial co-factor for the proper catalytic functioning of kinases (**Figure 3.7F**).<sup>158</sup> Modification at the  $\gamma$ -position may therefore have perturbed the binding of ATP to CDK2 to a significant extent. We also considered modification at either the 2- or 3-positions of the ribose instead, as these hydroxy groups (although engaged in H bonds with Asp86) were much more solvent-exposed.



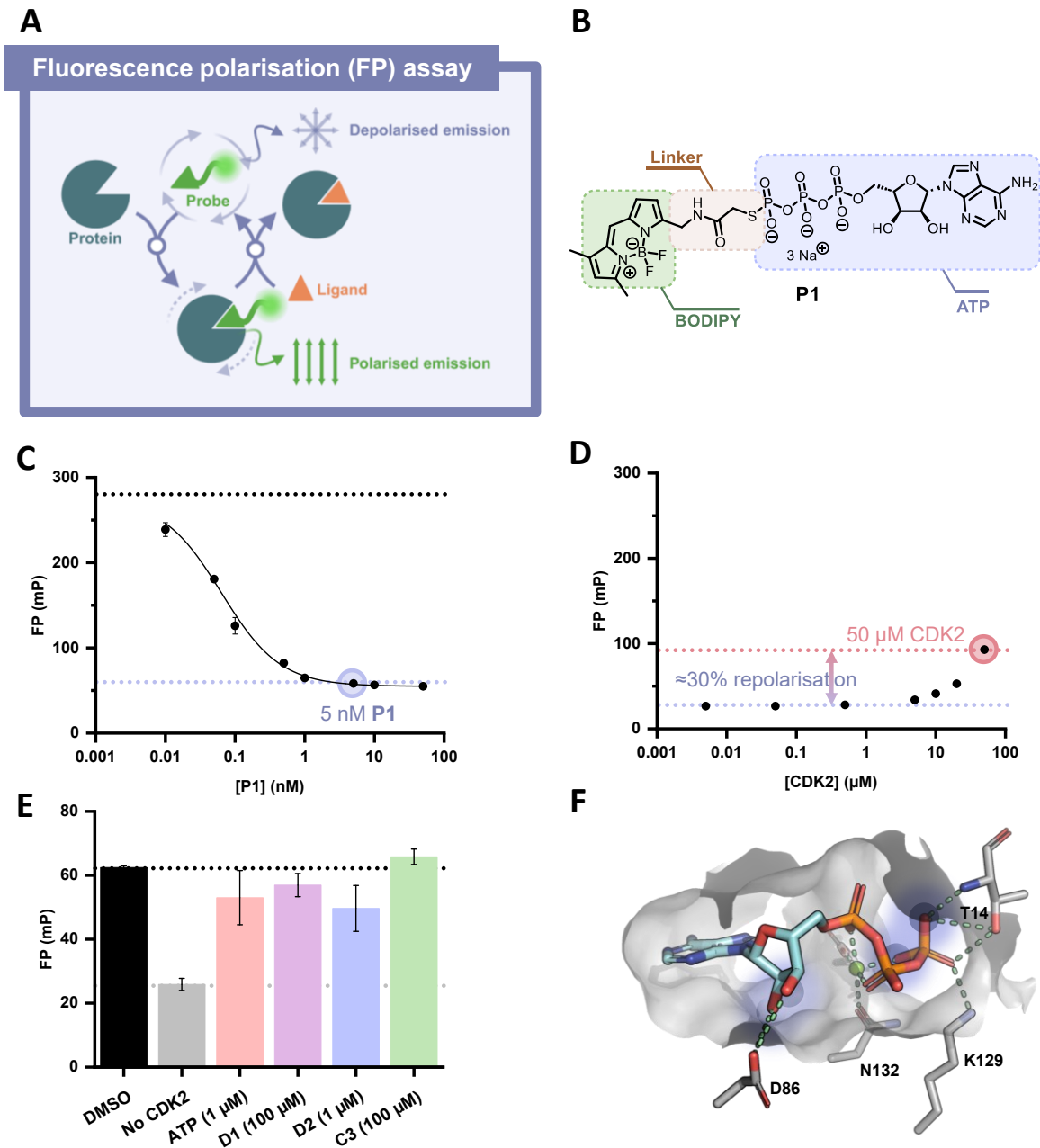


**Figure 3.7. FP assay on CDK2.** Error bars represent the standard deviation of technical triplicates. (A) The principle behind the FP assay. (B) Structure of the fluorescent ATP probe **P1**. (C) Titration of **P1** (0.01–50 nM) in assay buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.05% TWEEN20, 1% DMSO). (D) Titration of CDK2 (0.005–50 μM) in the presence of 5 nM **P1**. (E) Competition assay with ATP, **D1**, **D2** and **C3** tested at 1 or 100 μM against CDK2/**P1** (50 μM/5 nM). (F) X-ray co-crystal structure of CDK2 in complex with ATP (PDB ID 8FP5).<sup>147</sup> The possible attachment sites for the fluorescent tag on ATP has been highlighted in purple. Created with PyMOL (version 2.5.4).

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However, use of the alternative fluorescent tracer BODIPY<sup>TM</sup> ATP-2/3-O (**P2**) did not achieve a greater assay window, and the observed repolarisation was also similarly proven to be due to non-specific binding

(**Appendix Figure A8**). There may be several possible explanations for these poor assay results. Of note, monomeric (i.e., non-complexed) and non-phosphorylated CDK2 was used for these tests, and it is well-known that cyclin complexation results in a significant change in ligand-protein complex conformation and affinity.<sup>159</sup> However, it is known from crystallographic data that inactive CDK2 still has the capacity to bind ATP.<sup>160</sup> More significantly, when Moreira *et al.* applied **P1** against their model enzyme PKA, they also had to apply extremely high protein concentrations (> 200  $\mu$ M) and could still only achieve a narrow assay window ( $\approx$  20 mP).<sup>156</sup> This suggests these fluorescently tagged ATP-derivatives may inherently constitute poor kinase probes—perhaps because attachment of the fluorescent tag interferes significantly with their binding. Given these considerations, we ceased our attempts on establishing an FP assay for CDK2.

### 3.4.3. ADP-Glo™ kinase assay

To gauge the functional activity of the compounds, we next considered biochemical assays. We here opted for the ADP-Glo™ kinase assay which measures inhibition of kinase activity by tracking the formation of the by-product of the enzymatic reaction, ADP.<sup>161</sup> The generated ADP is detected with a system consisting of an ADP-to-ATP back-conversion step followed by a luciferase/luciferin reaction driven by the generated ATP, which produces luminescence. The luminescent readout will correlate positively with the ADP concentration and can thus be used as a proxy for kinase activity (**Figure 3.8A**).

We initiated the assay studies by first performing an ATP/ADP titration experiment, in which different ratios of ATP and ADP (but with a constant total concentration) were tested to simulate kinase reactions progressed to different extents. From this plot, we obtained an expected linearity with the luminescence signal, validating the basic assay principle (**Figure 3.8B**). Next, we introduced the enzymatic reaction components, where we employed functionally active CDK2 complexed with cyclin A2 (CDK2/A2) and histone H1 as the protein substrate. CDK2/A2 was titrated to establish a suitable working enzyme concentration, which would yield a sufficiently large assay window (**Figure 3.8C**). We here settled for a concentration of 2 ng/ $\mu$ L, which gave a signal-to-background (S/B) ratio above 10 and a Z prime (Z') value above 0.5, suggesting an excellent assay (**Figure 3.8D**).

Having established suitable assay conditions, we next turned to compound testing. The two positive controls **D1** and **D2** were here initially screened at a single concentration (200  $\mu$ M) against CDK2/A2 (**Figure 3.8E** and **Table 3.2**). Both compounds gave significant lowering of luminescence compared with the vehicle control, indicating inhibition. The fragment **D1** gave 44% inhibition at 200  $\mu$ M, which is in the same magnitude of the reported IC<sub>50</sub> of 97  $\mu$ M.<sup>140</sup>

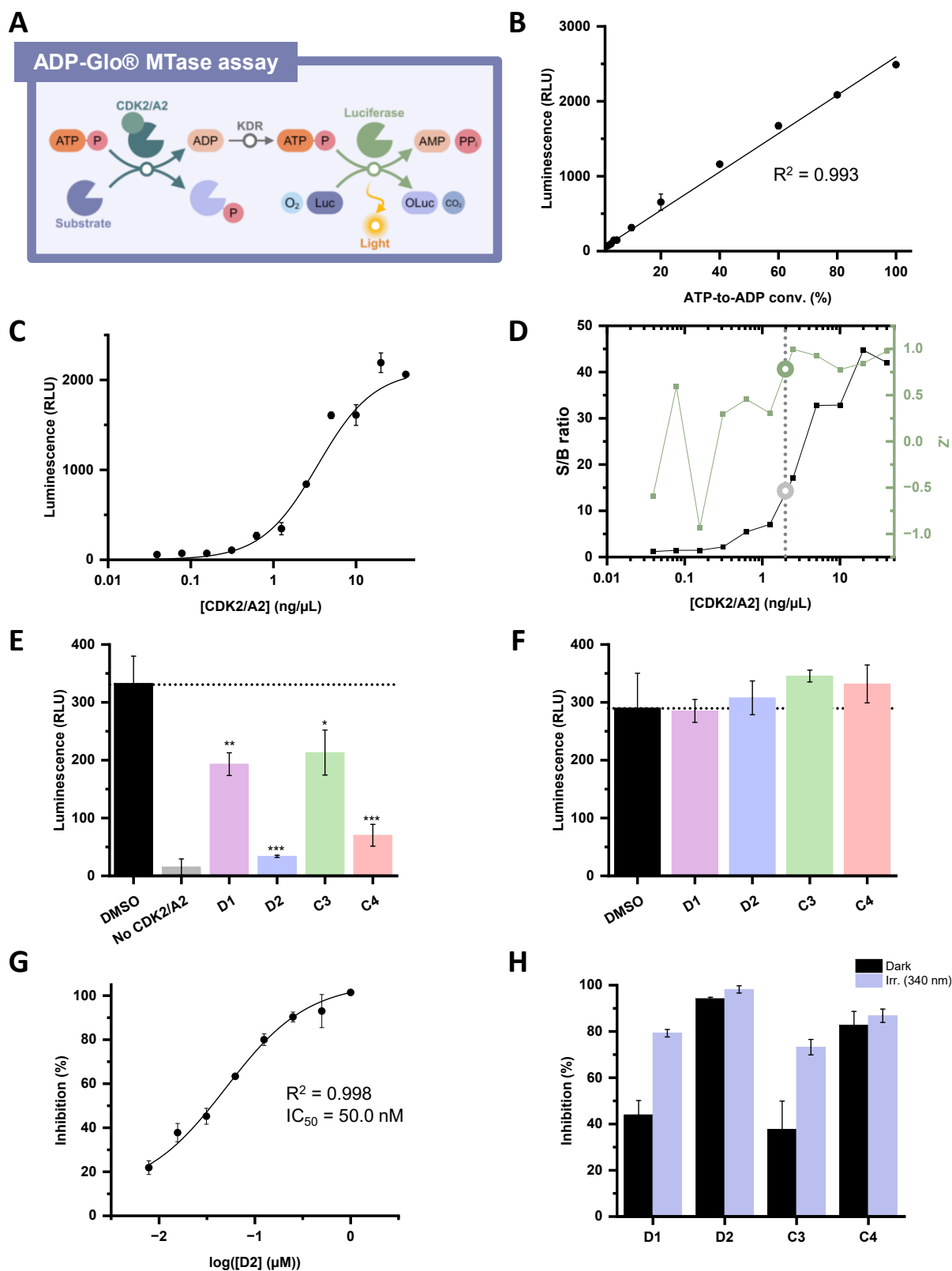
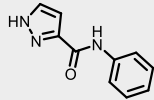
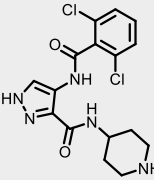
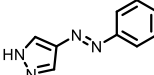
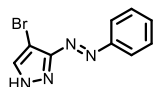


**Figure 3.8. ADP-Glo™ kinase assay on CDK2/A2.** Error bars represent the standard deviation of three technical replicates. \*:  $p \leq 0.05$ , \*\*:  $p < 0.005$ , \*\*\*:  $p \leq 0.0005$  in a Student's two-tailed unpaired  $t$ -test. (A) The principle behind the ADP-Glo™ kinase assay applied to CDK2/A2. KDR = Kinase detecting reagent. Luc = D-luciferin. OLuc =

oxyluciferin. Created with BioRender.com. (B) Titration of ATP/ADP (total concentration of 1  $\mu$ M) in a kinase reaction buffer (40 mM Tris pH 7.5, 50  $\mu$ M dithiothreitol (DTT), 20 mM  $MgCl_2$ , 1% DMSO) gives a linear correlation with the luminescence. (C) Titration of CDK2/A2 (0.04–40  $\mu$ M) in the presence of 1  $\mu$ M ATP and 0.2  $\mu$ g/ $\mu$ L histone H1 as the substrate. (D) A plot of the signal-to-background (S/B) ratio and the Z prime (Z') value as a function of [CDK2/A2]. (E) Test of positive controls (**D1** and **D2**) and the azo extension analogues (**C3** and **C4**) at 200  $\mu$ M with 2 ng/ $\mu$ L CDK2/A2, 0.2  $\mu$ g/ $\mu$ L H1 and 1  $\mu$ M ATP. (F) Counter screen of the compounds in the absence of CDK2/A2 but supplemented with 1  $\mu$ M ATP/ADP (83.8:16.2) to check for compound interference with the detecting steps. (G) Dose-response test of **D2** (0.01–1  $\mu$ M). (H) Test of the positive controls and the azo extension analogues in the dark and after photoirradiation with 340 nm light.

**Table 3.2. Photochemical properties and biochemical results of positive controls and C3–4.** <sup>a</sup>

| Cmpd ID   | Structure                                                                           | $t_{1/2}$<br>(h) | PSD <sub>340nm</sub><br>(%) | Dark activity                                     | Irr. activity         |
|-----------|-------------------------------------------------------------------------------------|------------------|-----------------------------|---------------------------------------------------|-----------------------|
| <b>D1</b> |    | -                | -                           | 44% inh.@200 $\mu$ M                              | 79% inh.@200 $\mu$ M  |
| <b>D2</b> |    | -                | -                           | IC <sub>50</sub> = 50 nM<br>100% inh.@200 $\mu$ M | 100% inh.@200 $\mu$ M |
| <b>C3</b> |  | 3.36             | 98                          | 38% inh.@200 $\mu$ M                              | 73% inh.@200 $\mu$ M  |
| <b>C4</b> |  | <1%<br>conv.@1h  | 97                          | 83% inh.@200 $\mu$ M                              | 87% inh.@200 $\mu$ M  |

<sup>a</sup> Compounds were assayed at a single dose (200  $\mu$ M) or in dose-response format (0.01–1  $\mu$ M, 8 points, 2-fold dilution) both in the dark and after photoirradiation with 340 nm light. Literature compounds are highlighted in grey.

The advanced lead compound **D2** on the other hand showed a complete reduction in luminescence, which was not statistically different from the no-enzyme control (indicating 100% inhibition)—in line with what would be expected for a compound with a reported IC<sub>50</sub> of 47 nM.<sup>140</sup> A counter screen was further conducted in which the compounds were tested in the absence of CDK2/A2 (but supplemented with ATP/ADP). Here, no lowered luminescence was observed, indicating no interference with the downstream detection steps and suggesting genuine enzyme-dependent inhibition (**Figure 3.6F**). As a final validation step, we also performed a dose-response test on **D2**, from which we determined an IC<sub>50</sub> of 50 nM—correlating well with the literature inhibitory constant (**Figure 3.8G**).

With a working assay in hand, we proceeded to test our azo extension compounds, **C3** and **C4**. We here saw significant enzyme-dependent inhibition at 200  $\mu$ M amounting to 38 and 83% for **C3** and **C4**, respectively (**Figure 3.8E–F** and **Table 3.2**). We further tested the compounds after photoirradiation at

340 nm and also included the non-photoswitchable compounds **D1** and **D2** as controls (**Figure 3.8H**). Interestingly, we saw an unexpected 35% difference in activity for **D1** between the dark and the photoirradiated state. We can firstly attribute this difference to the inherent inter-assay variability. Secondly, although we have attempted to minimise all other differences between the two assay formats (e.g., same incubation time), the photoirradiation may cause other non-photochemical events (e.g. slight heating of the assay plate) which may contribute to an apparent change in the activity of non-photoswitchable compounds. Further assay optimisation is required to disentangle and minimise these factors. For the photoswitchable compounds, **C4** showed a 36% difference in activity, but given the similarly sized variability seen for **D1**, we cannot confidently attribute this activity change to the effect of photoisomerisation.

In summary, we have successfully adapted the ADP-Glo™ kinase assay to testing inhibition of CDK2/A2 through the use of positive control compounds. Using the assay, we have detected significant inhibitory activities for the two azo extension fragment analogues **C3** and **C4** at 200 µM. This supports that the compounds may serve as useful scaffolds for building potent photoswitchable compounds.

### 3.5. An azologue comparator

In parallel to running our fragment-based campaign, we were interested in developing a photoswitchable CDK2 inhibitor using the conventional late-stage transformation approach, which would serve as a useful comparator to the chemical matter generated from the fragment-based approach.

#### 3.5.1. Design of an azologue of **D2**

From structural inspection of the collection of known CDK2 inhibitors, compound **D2** emerged as the most suitable ligand for azo transformation. Specifically, the structure appeared to be amendable to azologisation—by replacing the central amide linker connecting the pyrazole ring and the 2,6-dichlorobenzene motif, one would arrive at the phenylazopyrazole-based photoswitch **D3** (**Figure 3.9A**). Considering the binding mode of **D2** as visible from its X-ray co-crystal structure, the carbonyl O of the amide linker is engaged in a water-mediated H bond with the backbone of Asp145 (**Figure 3.9B**). However, the azo N can also act as a H bond acceptor and may be able to recapitulate this interaction. More crucially, the amide N appears to be forming an intramolecular H bond with the other distal carbonyl group, thus potentially pre-configuring and stabilising the ligand in its bioactive conformation.<sup>140</sup> This intramolecular H bond would be abolished upon azologisation, but the importance of this interaction has not been investigated by the authors.

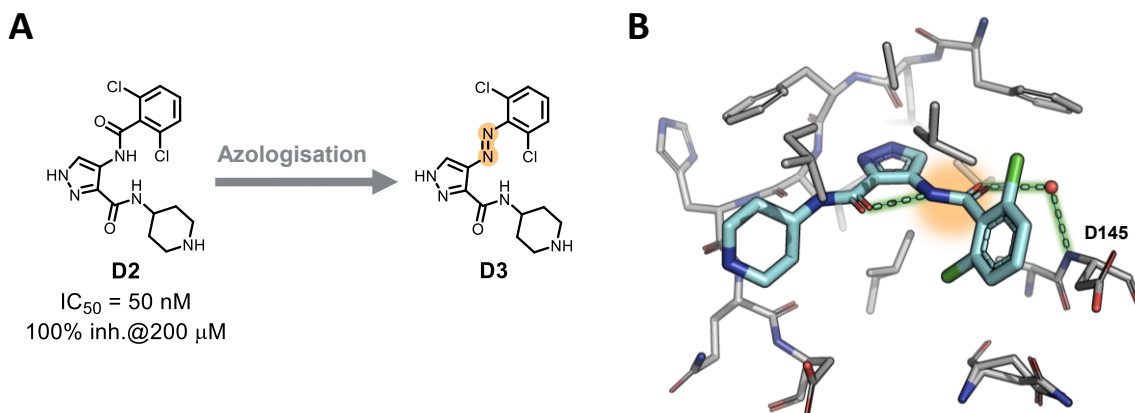


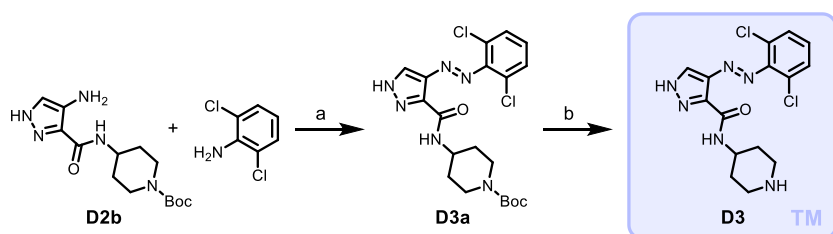
**Figure 3.9. Azologisation of D2.** (A) Structure of the azologue **D3**. The structural change from **D2** has been highlighted. (B) Binding mode of **D2** (PDB ID 2VU3).<sup>140</sup> The amide linker has been highlighted (orange) as well as key H bonds (green stippled lines). Created with PyMOL (version 2.5.4).<sup>47</sup>

Thus, we still considered the azologisation of **D2** to be a sensible transformation that would be representative of the current widely adopted late-stage and ligand-based azo transformation approach.

### 3.5.2. Synthesis of the azologue **D3**

The compound **D3** was synthesised by adapting the synthetic route for the parent compound **D2** (Scheme 3.3). Specifically, the previously synthesised aminopyrazole intermediate **D2b** was employed in a Baeyer-Mills reaction with 2,6-dichloronitrosobenzene (made from the corresponding aniline) to afford the azopyrazole **D3a**, which upon Boc-deprotection of the pendant piperidine *N* afforded the target molecule **D3**.

#### Scheme 3.3. Synthesis of azologue **D3**.<sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) Oxone, DCM, H<sub>2</sub>O, rt–50 °C, 27 h; then **D2b**, AcOH, rt, 45 h, 22%. (b) TFA, DCM, 0 °C–rt, 20 h, 38%. TM = Target molecule.

### 3.5.3. Photochemical analysis of **D3**

With the azologue in hand, we proceeded to perform photochemical analysis in an aqueous assay buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05 %[v/v] Tween-20/Surfactant P20, 1% DMSO).

Concerningly, the compound showed poor completeness of switching ( $\text{PSD} < 80\%$ ) with both wavelengths of light that were sampled (340 and 365 nm) (**Figure 3.10A**). A negative impact of di-*ortho*-chlorination on photoconversion has previously been observed for the parent photoswitch **4pzH**, which was attributed to a greater overlap in the  $\pi$ - $\pi^*$  bands between the *E*- and the *Z*-isomers.<sup>44</sup> Conversely, the photoswitch showed an excellent thermal *Z*-isomer stability, with no significant back-conversion observed after 24 h at 20 °C (**Figure 3.10B**).

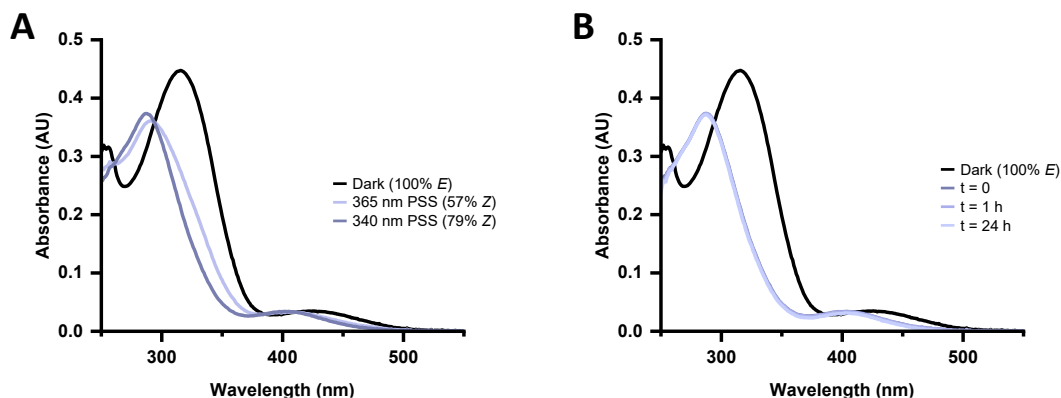


**Figure 3.10. Photochemical characterisation of D3.** Photoswitching and thermal kinetics were determined by UV–Vis spectrophotometry at 20 °C and at 20  $\mu\text{M}$  in 10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05 %[v/v] Tween-20/Surfactant P20, 1% DMSO. A) PSD determination for **D3**. (B) Thermal back-isomerisation of (*Z*)-**D3** after 1 and 24 h.

#### 3.5.4. Inhibitory activity of D3

The azologue was finally tested for its inhibition of CDK2/A2 in the ADP-Glo™ kinase assay (**Figure 3.11**). In the dark state, **D3** displayed an  $\text{IC}_{50}$  of 27  $\mu\text{M}$ , corresponding to a massive 540-fold decrease in inhibitory potency from the parent compound **D2**. The significant drop in absolute potency suggests a crucial importance of the amide linker. Upon photoirradiation with 340 nm light (which furnished the greatest photoconversion in the photochemical analysis), only a slight decrease in inhibition was observed ( $\text{IC}_{50}$  = 40  $\mu\text{M}$ ), corresponding to a 1.5-fold change in activity. The marginal differential activity may in part be explained by the poor photoconversion exhibited by the photoswitch and may also indicate that the azologisation process has disrupted the original binding mode to a significant extent (where the conformational placement of the 2,6-dichlorobenzene ring no longer makes a significant impact on binding).

In summary, we have successfully made and characterised an azologue of the literature inhibitor **D2**. As an endpoint, this compound **D3** does not exhibit great photopharmacological properties (low target engagement and low FC), but it serves as a good representative product of the current late-stage ligand-based azo transformation approach for discovering photopharmacological agents.

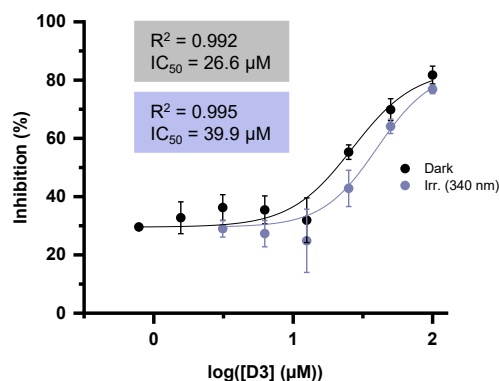


**Figure 3.11. Activity of D3 in the ADP-Glo™ kinase assay.** The compound was tested in the dark and after photoirradiation with 340 nm light. Error bars represent the standard deviation of three technical replicates.

### 3.6. Elaboration of azo fragments C3 and C4

Having identified two promising photoswitchable fragments **C3** and **C4** showing biochemical potency in our kinase assay, we next sought to further elaborate on their structure and identify more potent and light-addressable inhibitors. Although **C4** showed an apparent stronger inhibition at 200 μM (83% versus 38%), **C3** remained the more promising starting point given its more robust binding mode model, and we therefore focused most of our attention on this fragment scaffold.

#### 3.6.1. Growing of C3 via the G vector

In order to pick up more binding affinity, we were first interested in elaborating off the G vector of **C3**, which probes a hydrophobic sub-pocket constituting of Val64, Phe80 and Ala144. From the alternative X-ray structure pose of the parent fragment probe **C1**, we know that this region can accommodate a large and lipophilic bromide substituent. In addition, several known CDK2 inhibitors were observed to exploit the sub-pocket based on X-ray structure analysis. To complement the lipophilic nature of the sub-pocket we decided to sample a series of alkyl substituents and designed a total of four analogues (**Figure 3.12A**). Docking analysis of the methyl-substituted analogue **C5** suggests that the fragment can maintain its crucial binding mode of interacting with the hinge region both in its *E*- and *Z*-isomeric state, whilst the alkyl substituent protrudes into the hydrophobic sub-pocket as desired (**Figure 3.12B–C**).



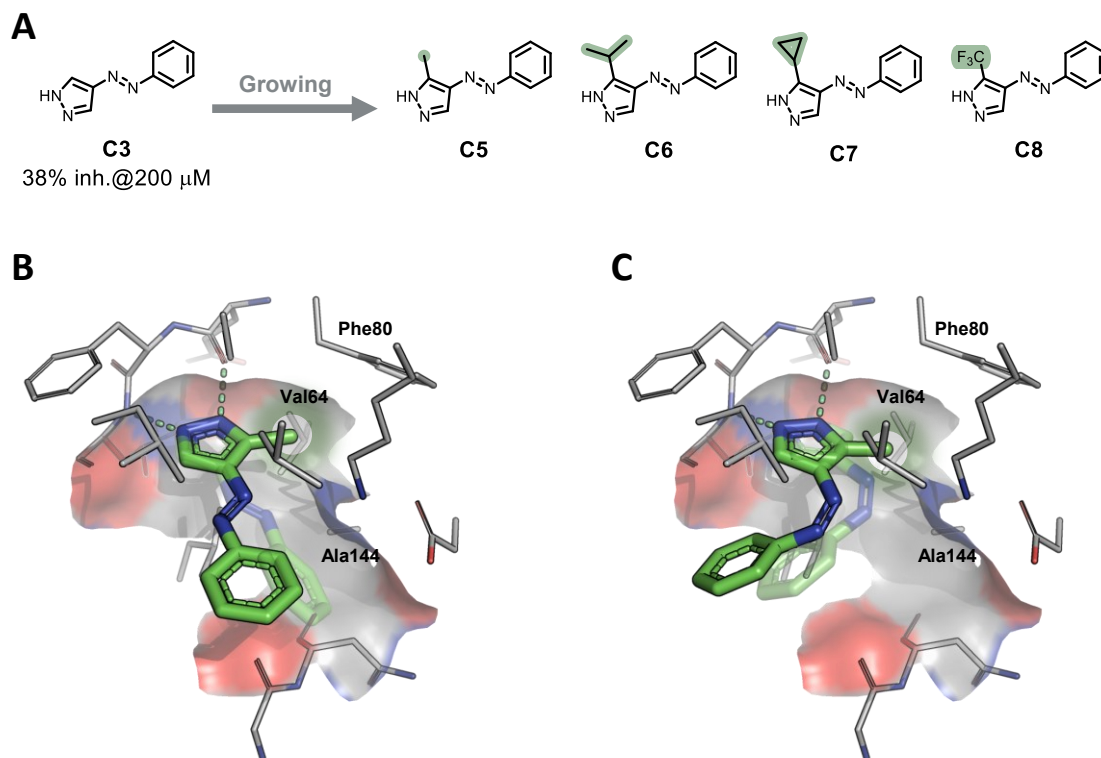
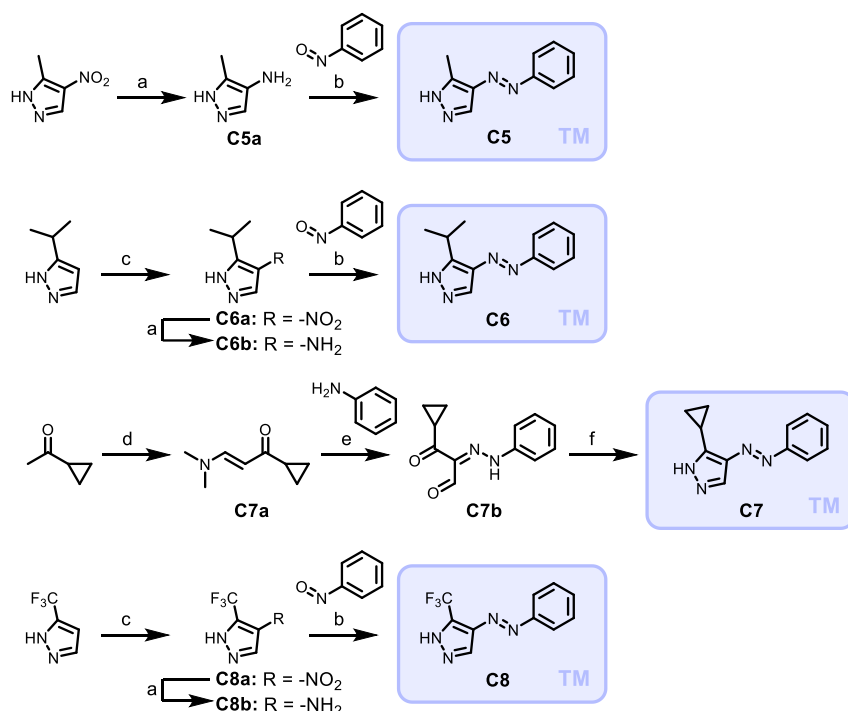


**Figure 3.12. Design of G-vector growing analogues of C3.** (A) Structure of **C5–8**. The structural changes from **C3** have been highlighted. (B) Docking of (*E*)-**C5** into CDK2. (C) Docking of (*Z*)-**C5** into CDK2. For both figures (B) and (C): The alkyl substituent has been highlighted. The protein receptor from the co-crystal structure of **C1** was employed (PDB ID 6Q3C).<sup>123</sup> The docking poses of **C3** are shown as transparent sticks. Created with PyMOL (version 2.5.4).<sup>47</sup>

The compounds **C5**, **C6** and **C8** were synthesised via an acidic Baeyer-Mills reaction, condensing nitrobenzene with the appropriately substituted aminopyrazole building blocks—these were in turn obtained from the corresponding nitropyrazoles, which were either commercially available or accessed through standard nitration conditions (**Scheme 3.4**). For analogue **C7**, the cyclopropyl-substituted pyrazole was not readily accessible and was therefore obtained via an azo coupling strategy followed by de novo ring synthesis.

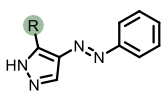
The obtained compounds were firstly assessed for their photochemical properties (**Table 3.3**). No significant perturbation of the high photoconversion efficiency at 340 nm (PSD > 90%) was observed except for the cyclopropyl analogue **C7**, where a slight decrease in PSD was noted (88% *Z*-isomer, **Appendix Figure A7A–J**). Noticeably, compounds **C5–7** exhibited a marked drop in the thermal *Z*-isomer stability to the order of minutes compared with the thermal half-life of 3.4 hours for **C3**. This correlates with literature studies on azoheteroaromatic systems, where the introduction of *ortho*-methyl groups was empirically found to result in shorter *Z*-isomer half-lives.<sup>36</sup>

**Scheme 3.4. Synthesis of growing analogues C5–8.** <sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) Pd/C, MeOH, (EtOAc), rt, 4–16 h, 95%–quant. (b) AcOH, rt–40 °C, 18 h–3 days, 79–86%. (c) HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, 0–115 °C, 3–4 h, 99%–quant. (d) DMF-DMA, 100 °C, 3 days, 21%. (e) NaNO<sub>2</sub>, aq. HCl, 0 °C, 30 min; then **C7a**, NaOAc, H<sub>2</sub>O, 0 °C, 1 h, 59%. (f) H<sub>2</sub>NNH<sub>2</sub>·H<sub>2</sub>O, EtOH, reflux, 16 h, 32%. TM = Target molecule.

**Table 3.3. Photochemical properties and biochemical results of growing analogues C5–8.** <sup>a</sup>

| Cmpd ID   | R <sup>1</sup>                                                                        | t <sub>1/2</sub><br>(min) | PSD <sub>340nm</sub><br>(%) |  |                           |
|-----------|---------------------------------------------------------------------------------------|---------------------------|-----------------------------|-------------------------------------------------------------------------------------|---------------------------|
|           |                                                                                       |                           |                             | Dark activity                                                                       | Irr. activity             |
| <b>C3</b> | --H                                                                                   | 201                       | 98                          | 38% inh.@200 μM                                                                     | 73% inh.@200 μM           |
| <b>C5</b> | --Me                                                                                  | 5.03                      | 95                          | 87% inh.@200 μM                                                                     | ND                        |
| <b>C6</b> | --<img alt="Chemical structure of a vinyl group." data-bbox="265 720 295 750"/>       | 3.28                      | 94                          | 100% inh.@200 μM<br>IC <sub>50</sub> = 2.4 μM                                       | IC <sub>50</sub> = 3.2 μM |
| <b>C7</b> | --<img alt="Chemical structure of a cyclopropyl group." data-bbox="265 760 295 780"/> | 1.38                      | 88                          | 100% inh.@200 μM                                                                    | 100% inh.@200 μM          |
| <b>C8</b> | --CF <sub>3</sub>                                                                     | <1%<br>conv.@1 h          | 97                          | 61% inh.@200 μM                                                                     | 72% inh.@200 μM           |

<sup>a</sup> Compounds were assayed at a single dose (200 μM) and optionally in dose-response format (0.78–100 μM, 8 points, 2-fold dilution) both in the dark and after photoirradiation with 340 nm light. Previously tested compounds are highlighted in grey. The structural changes from **C3** have been highlighted.

Interestingly, the trifluoromethylated analogue **C8** did not suffer from this loss in thermal Z-isomer stability and in fact displayed an even longer thermal half-life than the non-substituted **C3** (< 1% back-conversion after 1 h). This again aligns well with previous experimental data which demonstrated a stabilising effect of the trifluoromethyl group on Z-azopyrazoles.<sup>162</sup>

We proceeded to test the compounds in the ADP-Glo™ assay (**Table 3.3**). Encouragingly, a significant boost in the inhibitory potency was observed for all alkyl-substitutions (> 50% inhibition at 200 µM) relative to **C3**. Furthermore, the analogues carrying the bulkier isopropyl and cyclopropyl motifs (**C6** and **C7**) maxed out the assay window, showing 100% inhibition at the test concentration. A dose-response test was performed on **C6** (which outperformed **C7** in terms of photochemical properties), from which a single-digit IC<sub>50</sub> of 2.4 µM was determined (**Appendix Figure A9A**). This corresponds to a significant >60-fold boost in potency from **C3** (assuming an IC<sub>50</sub> > 200 µM). The positive results indicate that the alkyl substituents successfully engaged the hydrophobic sub-pocket. Given the poor thermal half-lives of **C5–7**, we did not expect to observe a significant apparent change in activity upon photoswitching. Indeed, photoirradiation of **C6** resulted in a similar IC<sub>50</sub> to that displayed in its dark state (3.2 µM, **Appendix Figure A9A**). Compound **C8** (the only analogue with high Z-isomer stability) showed no significant change in potency upon photoswitching either. However, this aligns well with the docking-guided hypothesis that the alkyl groups would not significantly change the differential E/Z-binding.

In conclusion, we were successful in picking up more absolute binding affinity by probing the hydrophobic sub-pocket via the G-vector.

### 3.6.2. Growing of C3 via the S vector

We next turned our attention to the S vector. As a first step, we were interested in probing the steric demands of this region with a small substituent and selected a methyl group for this purpose. The monomethylated analogue had already been made and tested (**C5**), and this methyl group is expected to preferentially occupy the G-vector. The dimethylated analogue **C9** was therefore designed to be able forcibly probe the S vector (**Figure 3.13A**). Further to the methyl group, we were intrigued by the idea of forging a new H bond with Leu83—a common interaction motif seen for several literature inhibitors. Inspired by these structures, we decided to sample a series of more elaborate carboxamide- and amino-substituted analogues carrying H bond donors (**Figure 3.13B–C**).

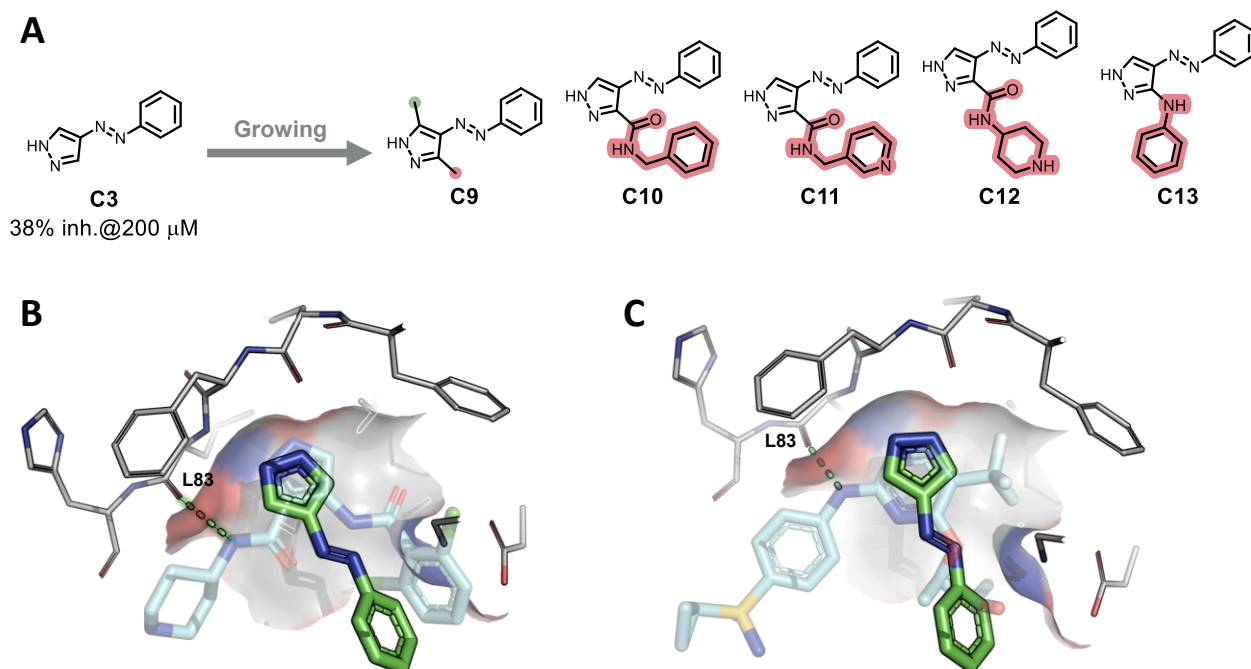
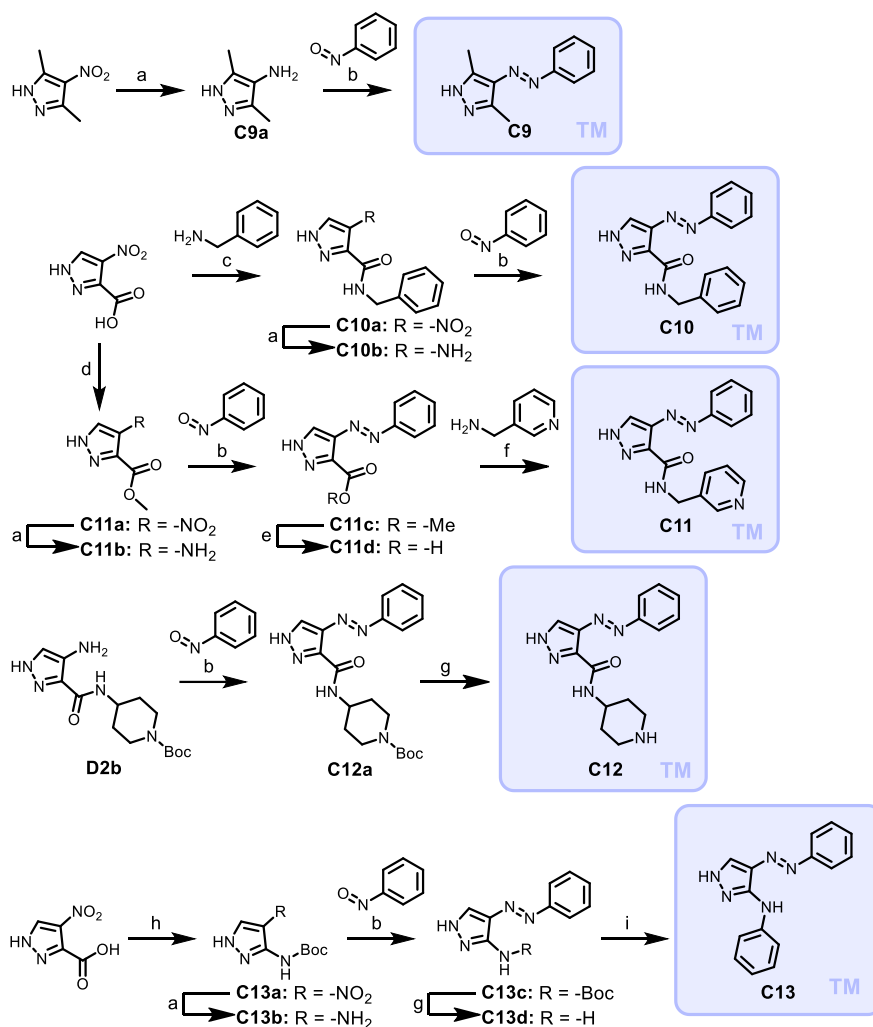


**Figure 3.13. Design of S-vector growing analogues of C3.** (A) Structure of **C9–13**. The structural changes from **C3** have been highlighted. (B) Comparison of the docking pose of (*E*)-**C3** (green sticks) with the X-ray structure of **AT7519** (cyan sticks, PDB ID 2VU3).<sup>140</sup> (C) Comparison of the docking pose of (*E*)-**C3** (green sticks) with the X-ray structure of **Roniciclib** (cyan sticks, PDB ID 5IEV).<sup>163</sup> Created with PyMOL (version 2.5.4).<sup>47</sup>

The dimethylated analogue **C9** was readily synthesised using the same approach as for the mono-alkylated analogues, starting from the corresponding dimethylated aminopyrazole building block (**Scheme 3.5**). The carboxamide substituted analogues **C10** and **C12** were accessed by performing an early-stage amide coupling on a nitropyrazolecarboxylic acid building block followed by the same two-step transformation sequence of nitro reduction–Baeyer–Mills reaction. Alternatively, an early-stage Baeyer–Mills reaction/late-stage amide coupling involving a key carboxy-substituted intermediate **C11d** was also explored to allow for late-stage amide coupling and access to new amide derivatives in a single step. Finally, the synthesis of the phenylamino analogue **C13** required more bespoke chemistry. Starting from the nitropyrazolecarboxylic acid building block, a tandem acyl azide formation–Curtius rearrangement (using diphenylphosphoryl azide) followed by trapping of the intermediate isocyanate with *tert*-butanol was performed in one pot to give the Boc-protected aminopyrazole **C13a**. A sequence of nitro-reduction, Baeyer–Mills reaction and Boc-deprotection was then carried out to arrive at the key amino-substituted phenylazopyrazole **C13d**. This was then subjected to a final C–N cross-coupling using a reported nickel(II)-catalysed Cham–Lam reaction to furnish the target molecule **C13**.<sup>164</sup>

**Scheme 3.5. Synthesis of growing analogues C9–13.** <sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) Pd/C, EtOAc and/or MeOH, H<sub>2</sub> (1 atm), rt, 3–16 h, 36%–quant. (b) AcOH, rt–40 °C, 21 h–3 days, 28–85%. (c) EDC-HCl, HOBT, DMF, 0 °C–rt, 22–24 h, 42–93%. (d) AcCl, MeOH, 0 °C–rt, 3 days, 88%. (e) aq. NaOH, MeOH, rt, 39 h, 86%. (f) TFA, DCM, 0 °C–rt, 9–15 h, 38%–quant. (g) DPPA, Et<sub>3</sub>N, *t*-BuOH, 1,4-dioxane, reflux, 24 h, 25%. (h) Ni(OAc)<sub>2</sub>·H<sub>2</sub>O, DBU, DMSO, air, 50–100 °C, 49 h, 16%. TM = Target molecule.

Expectedly, the dimethylated analogue **C9** presented an even lower thermal *Z*-isomer stability than the monomethylated counterpart **C5**—in fact, the relaxation rate was so fast that forward photoswitching could not be detected with our UV–Vis spectrophotometer (Table 3.4, Appendix Figure A7K). This aligns again with literature, where the inclusion of two *ortho*-methyl groups in azopyrazoles has been found to force the *Z*-isomers into a twisted conformation (rather than the stable T-shaped conformation), which is associated with shorter *Z*-isomer thermal half-lives.<sup>36</sup> In contrast, the carboxamide-substituted analogues **C10–12** all displayed extended thermal half-lives with only 1–2% back-conversion detected after 1 h (Appendix Figure A7M).

**Table 3.4. Photochemical properties and biochemical results of growing analogues C9–13.** <sup>a</sup>

| Cmpd ID    | R <sup>1</sup> | R <sup>2</sup> | t <sub>½</sub> (min) | PSD <sub>340nm</sub> (%) | Dark activity                                              | Irr. activity                                              |
|------------|----------------|----------------|----------------------|--------------------------|------------------------------------------------------------|------------------------------------------------------------|
| <b>C3</b>  | --H            | --H            | 201                  | 98                       | 38% inh.@200 μM                                            | 73% inh.@200 μM                                            |
| <b>C5</b>  | --Me           | --H            | 5.03                 | 95                       | 87% inh.@200 μM                                            | ND                                                         |
| <b>C9</b>  | --Me           | --Me           | ND                   | ND                       | 55% inh.@200 μM                                            | ND                                                         |
| <b>C10</b> | --H            |                | 1% conv.<br>@1 h     | 96                       | 87% inh.@200 μM <sup>b</sup><br>IC <sub>50</sub> = 11.1 μM | 97% inh.@200 μM <sup>b</sup><br>IC <sub>50</sub> = 24.9 μM |
| <b>C11</b> | --H            |                | 1% conv.<br>@1 h     | 96                       | NA @200 μM <sup>b</sup>                                    | ND                                                         |
| <b>C12</b> | --H            |                | 2% conv.<br>@1 h     | 97                       | 74% inh.@200 μM<br>IC <sub>50</sub> = 19.4 μM              | 89% inh.@200 μM<br>IC <sub>50</sub> = 12.4 μM              |
| <b>C13</b> | --H            |                | ND                   | ND                       | 92% inh.@200 μM <sup>b</sup>                               | 94% inh.@200 μM <sup>b</sup>                               |

<sup>a</sup> Compounds were assayed at a single dose (200 μM) and optionally in dose-response format (0.78–100 μM, 8 points, 2-fold dilution) both in the dark and after photoirradiation with 340 nm light. Previously tested compounds are highlighted in grey. The structural changes from **C3** have been highlighted.

<sup>b</sup> Compounds showed visible solubility issues.

This may be related to the electron donating capacity of the pyrrole-like pyrazole *N*, where the *N* lone pair can be pushed into the azo double-bond through resonance and result in a decreased bond order—thereby facilitating back-conversion.<sup>36</sup> This polarisation may be reduced by the electron withdrawing effect of the carboxamide substituents.

Finally, the phenylamino substituted analogue **C13** showed no apparent photoswitchability, presumably due to the extremely fast *Z*-isomer back-conversion kinetics (**Appendix Figure A7L**). This can be explained by a further polarisation of the azo double bond through  $\pi$ -electron pushing and/or be a consequence of a tautomeric shift of the NH proton onto the azo double bond to form a hydrazone species.

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In the kinase assay, the dimethylated analogue **C9** displayed a slight weaker activity than the corresponding monomethylated compound **C5**, suggesting that the methyl group may not be favourably placed in the S vector (**Table 3.4**). Conversely, the two carboxamide substituted analogues **C10** and **C12** both displayed significantly increased potencies (87% and 74% inhibition at 200 μM, respectively) relative

to **C3**. Their dose-responsive potencies were therefore quantified, which revealed IC<sub>50</sub> values of 11.1 and 19.4  $\mu$ M for **C10** and **C12** respectively, corresponding to a > 10-fold potency boost (**Appendix Figure A9B–C**). Upon photoswitching, no significant change in the potencies were however observed. Interestingly, exchanging the new pendant benzene ring of **C10** with a 3-pyridine to give **C11** resulted in a complete loss in activity (in both the dark and photoirradiated state). The sensitivity of the benzene ring to such small structural perturbation suggests an activity cliff, which would be interesting to understand and probe further. Lastly, the phenylamino-substituted compound **C13** displayed the strongest inhibition of the series (92%) but expectedly showed no change in activity upon photoswitching given its fast kinetics.

In conclusion, we managed to achieve a further potency boost by utilising the S vector of the pyrazole ring and installing carboxamide substituents. Still, no significant fold change in activity could be attained by any of the compounds from the series.

### 3.6.3. Growing of **C3** via the phenyl ring

To address the absence of differential activity, we next turned our attention to the phenyl ring of **C3**. Based on the binding mode hypothesis, this ring engages the ribose sub-pocket mostly via weak van der Waals interactions—and we thus propose that the majority of the binding energy is derived from the H bond interactions to the hinge region (*vide supra*). This may explain why upon photoswitching to the Z-isomer no significant difference in affinity/potency is seen. We hypothesised that the strategic installation of a functional group with H bonding capacity on the phenyl ring could help tether the ligand more strongly in place in one isomeric state and thus give rise to differential activity.

Here, we sampled a small series of three phenyl-substituted ligands which were designed to engage Lys33 and/or Asp145 in their *E*-isomeric state (**Figure 3.14A**). For instance, the 3-hydroxymethyl analogue **C14** was predicted to form a new H bond with Asp145 in its *E*-isomer, but not form any new polar interactions with protein residues in its Z-isomeric state (**Figure 3.14B–C**).

To access these phenyl-substituted compounds, 4-aminopyrazole was condensed with the appropriate ester-substituted nitrosobenzenes (made by oxidising the corresponding anilines) in an acidic Bae-  
yer-Mills reaction (**Scheme 3.6**). The ester intermediates were then either chemoselectively reduced using DIBAL-H to give the hydroxymethyl analogues **C14** and **C15** or hydrolysed and aminated to provide the carboxamide **C16**.

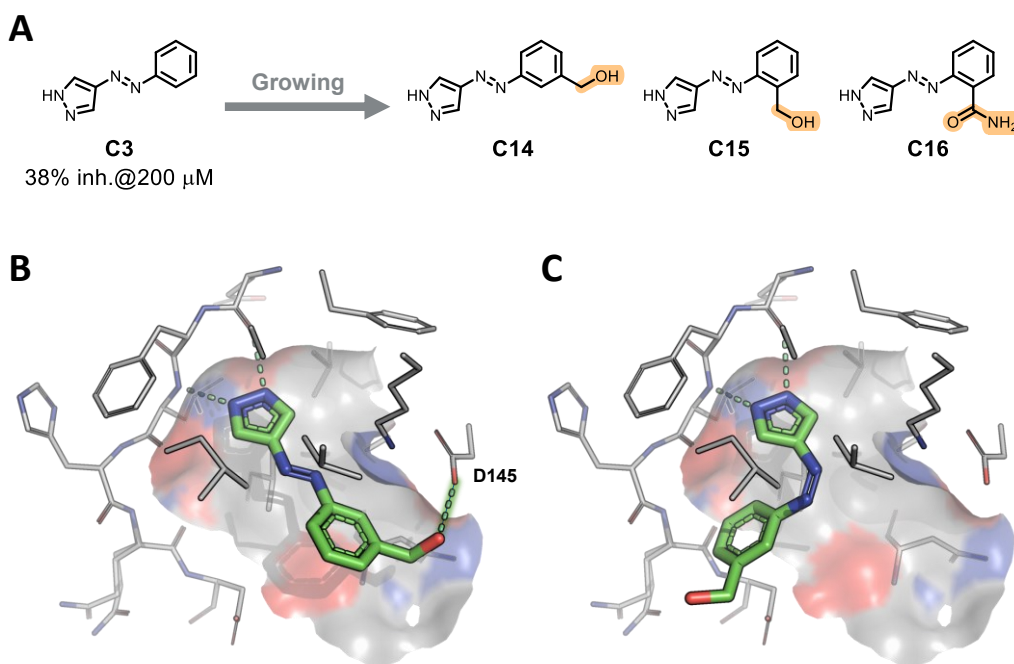
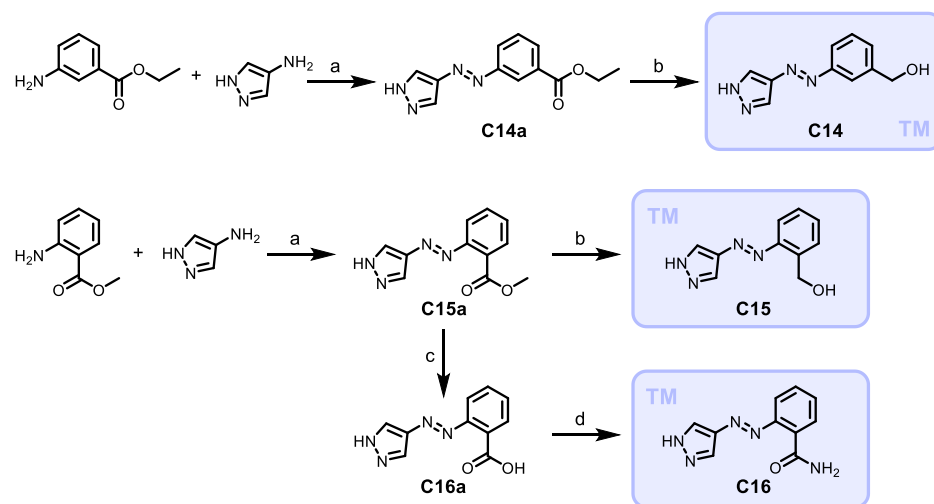


**Figure 3.14. Design of phenyl-substituted analogues of C3.** (A) Structure of **C14–16**. The structural changes from **C3** have been highlighted. (B) Docking pose of (*E*)-**C14** (green sticks) with the new H bond to D145 highlighted. (C) Docking pose of (*Z*)-**C14** (green sticks). For both figure (B) and (C): The protein receptor from the co-crystal structure of **C1** was employed (PDB ID 6Q3C).<sup>123</sup> Created with PyMOL (version 2.5.4).<sup>47</sup>

**Scheme 3.6. Synthesis of growing analogues C14–16.**<sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) Oxone, DCM, H<sub>2</sub>O, rt, 45 min–14 h; then 4-aminopyrazole, AcOH, DCM, rt, 30 h–3 days, 47–60%. (b) DIBAL-H in PhMe, THF, 0 °C–rt, 17–20 h, 10–53%. (c) aq. NaOH, MeOH, rt, 7 days, 92%. (d) EDC·HCl, HOBT, DIPEA, DMF, 0 °C, 30 min; then NH<sub>4</sub>Cl, rt, 24 h, 17 %. TM = Target molecule.



Photochemical analysis on the three compounds revealed no changes to the excellent near quantitative photoconversion exhibited by **C3** (PSD 97–98%) (**Table 3.5** and **Appendix Figure A70**). In terms of thermal Z-isomer stability, a similar thermal half-life could also be calculated for compounds **C14** and **C16** ( $t_{1/2} \approx 3.3$  h). Intriguingly however, **C15** displayed a significantly prolonged thermal stability with a calculated  $t_{1/2} = 19$  h—corresponding to a 6-fold improvement over the Z-isomer stability of **C3** (**Appendix Figure A7P**). It is unclear how the hydroxymethyl group exerts its stabilising effect on the Z-isomer, but we propose that it might be due to additional dispersive interactions affected by the methylene group on the Z-isomer ground state.

**Table 3.5. Photochemical properties and biochemical results of growing analogues C14–16.** <sup>a</sup>

| Cmpd ID    | R   | $t_{1/2}$<br>(min) | PSD <sub>340nm</sub><br>(%) | Dark activity        | Irr. activity        |
|------------|-----|--------------------|-----------------------------|----------------------|----------------------|
| <b>C3</b>  | --H | 201                | 98                          | 38% inh.@200 $\mu$ M | 73% inh.@200 $\mu$ M |
| <b>C14</b> | 3-- | 202                | 97                          | 45% inh.@200 $\mu$ M | 66% inh.@200 $\mu$ M |
| <b>C15</b> | 2-- | 1139               | 98                          | 53% inh.@200 $\mu$ M | 47% inh.@200 $\mu$ M |
| <b>C16</b> | 2-- | 195                | 97                          | 84% inh.@200 $\mu$ M | 78% inh.@200 $\mu$ M |

<sup>a</sup> Compounds were assayed at a single dose (200  $\mu$ M) both in the dark and after photoirradiation with 340 nm light. Previously tested compounds are highlighted in grey. The structural changes from **C3** have been highlighted.

In the kinase assay, a modest improvement in the dark state activity could be observed for **C15** and **C16**, displaying >50% inhibition at 200  $\mu$ M (**Table 3.5**). This would suggest that new polar interactions have been established by the groups in these analogues. Unfortunately, the improvements did not translate into any significant increase in the differential activity.

In conclusion, we identified substituents on the phenyl ring, which yielded slight potency boosts on **C3**. Importantly, the 2-hydroxymethyl group additionally caused a significant increase in the thermal Z-isomer stability.

### 3.6.4. Modifications on C4

In parallel to our efforts on modifying and improving the activity and properties of **C3**, we also made a modest effort to develop some structure-activity relationships on fragment **C4**. We were especially

interested in modifying the bromide-position of the fragment and first considered if the bromide was favourable for binding. We therefore designed the dehalogenated fragment **C17** (Figure 3.15A). Interestingly, docking predicted that this compound could recapitulate the favourable binding mode of the original fragment **C1** forming H bond interactions with the hinge region in both its *E*- and *Z*-isomeric form (Figure 3.15B–C). Given this binding mode, we considered if the bromide substituent could be replaced by more elaborate motifs, which could be accommodated in the *E*-isomeric form, but which could perhaps result in an unfavourable internal steric clash in the *Z*-isomeric state if the compound were to maintain the hinge binding mode. Here, we envisioned installing an aryl ring connected via an appropriate spacer, as this would then further provide a synthetic handle for subsequent functionalisation. We sampled a phenyl ring spaced by either an amino group (**C18**) or an amide (**C19**).

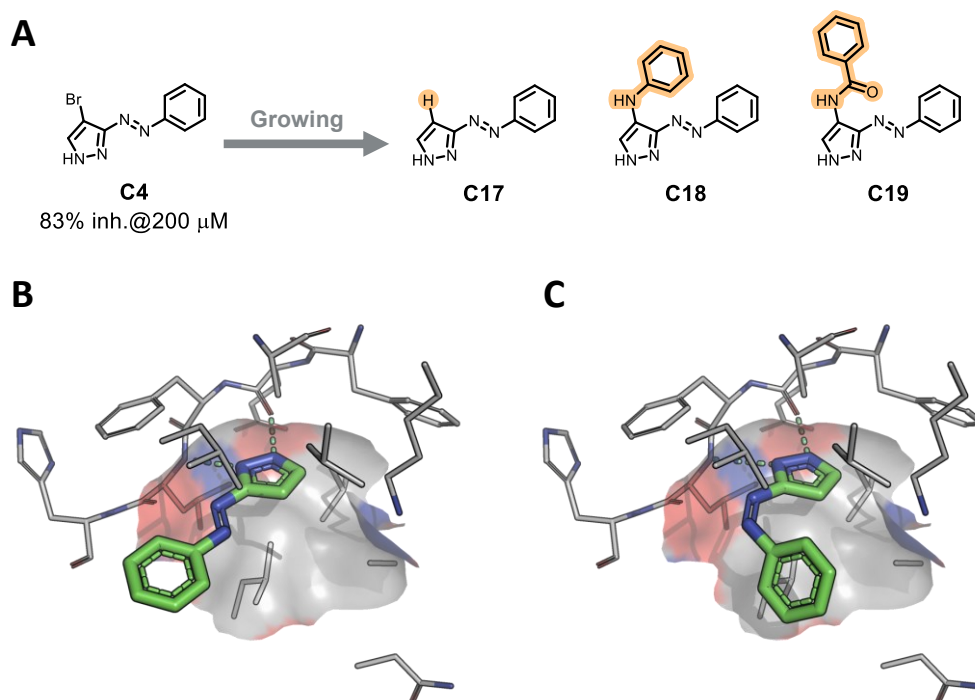
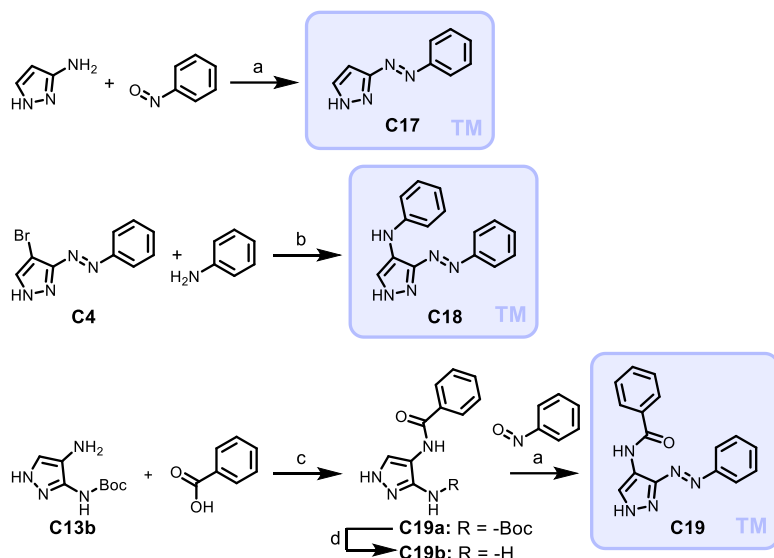


**Figure 3.15. Design of modification analogues of C4.** (A) Structure of **C17–19**. The structural changes from **C4** have been highlighted. (B) Docking pose of (*E*)-**C17** (green sticks). (C) Docking pose of (*Z*)-**C17** (green sticks). The protein receptor from the co-crystal structure of **C1** was employed (PDB ID 6Q3C).<sup>123</sup> Created with PyMOL (version 2.5.4).<sup>47</sup>

Compound **C17** was synthesised in a single step by condensing 3-aminopyrazole with nitrosobenzene (Scheme 3.7). For compound **C18**, a previously reported Buchwald-Hartwig cross-coupling on protocol compatible with unprotected pyrazole substrates was employed on the bromo-substituted phenylazopyrazole **C4** to afford the aminated product although in a low yield.

**Scheme 3.7. Synthesis of modification analogues C17–19.** <sup>a</sup>



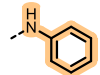
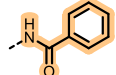
<sup>a</sup> Reagents and conditions: (a) AcOH, rt–50 °C, 41 h–3 days, 36–42%. (b) tBuBrettPhos Pd G3, tBuBrettPhos, LiHMDS, THF, 50 °C, 27 h, 25%. (c) EDC·HCl, HOBT, DMF, rt, 23 h, 80%. (d) TFA, DCM, 0 °C–rt, 5.5 h, 18%. TM = Target molecule.

Finally, compound **C19** was accessed by exploiting the previously synthesised diamino intermediate **C13b**, and performing an amide coupling, *N*-Boc-deprotection and a final Baeyer-Mills reaction efficiently yielded the target molecule.

Deletion of the bromide substituent (**C17**) resulted in no apparent changes to the photoconversion and the thermal stability (Table 3.6, Appendix Figure A7Q–R). This establishes that the bromide was not responsible for the unexpected high thermal stability observed for **C4** (*vide supra*) but that is rather an inherent property of the phenylazopyrazole scaffold. Despite the tolerance for the halide, introduction of a phenylamino substituent on the 4-position (**C18**) resulted in a complete loss of thermal *Z*-isomer stability, again, presumably due to either electron-pushing onto the azo double bond or tautomerisation-mediated back-conversion (Appendix Figure A7S). The introduction of a carbonyl group in the spacer (**C19**) rescued the thermal stability to some degree (with a respectable  $t_{1/2}$  = 59 min) but conversely yielded a very poor photoconversion (PSD 60%) (Appendix Figure A7T–U).

Compound **C17** showed no CDK2/A2 inhibition at 200 μM in the dark state, whereas only a weak activity could be detected upon photoirradiation ( $IC_{50}$  > 200 μM). The bromide substituent therefore appears to be crucial to the activity of **C4**. For compounds **C18** and **C19**, we curiously observed no activity in the dark state but apparent potent inhibition upon photoirradiation (≈ 90% inhibition at 200 μM), despite the poor photochemical properties of the compounds.

**Table 3.6. Photochemical properties and biochemical results of growing analogues C17–19.** <sup>a</sup>

| Cmpd ID    |  | $t_{1/2}$<br>(min) | PSD <sub>340nm</sub><br>(%) | Dark activity                                                  | Irr. activity                                                        |
|------------|-----------------------------------------------------------------------------------|--------------------|-----------------------------|----------------------------------------------------------------|----------------------------------------------------------------------|
|            |                                                                                   |                    |                             |                                                                |                                                                      |
| <b>C4</b>  | --Br                                                                              | <1% conv.@1h       | 97                          | 83% inh.@200 $\mu$ M                                           | 87% inh.@200 $\mu$ M                                                 |
| <b>C17</b> | --H                                                                               | <1% conv.@1h       | 96                          | NA @200 $\mu$ M                                                | 40% inh.@200 $\mu$ M                                                 |
| <b>C18</b> |  | ND                 | ND                          | NA @200 $\mu$ M                                                | 87% inh.@200 $\mu$ M <sup>b</sup>                                    |
| <b>C19</b> |  | 56.1               | 60                          | NA @200 $\mu$ M <sup>b</sup><br>IC <sub>50</sub> = 128 $\mu$ M | 90% inh.@200 $\mu$ M <sup>b</sup><br>IC <sub>50</sub> = 86.4 $\mu$ M |

<sup>a</sup> Compounds were assayed at a single dose (200  $\mu$ M) and optionally in dose-response format (0.78–100  $\mu$ M, 8 points, 2-fold dilution) both in the dark and after photoirradiation with 340 nm light. Previously tested compounds are highlighted in grey. The structural changes from **C4** have been highlighted.

<sup>b</sup> Compounds showed visual solubility issues.

However, for both compounds we observed significant solubility issues, which may have given rise to substantial heterogeneity between the assay repeats. Indeed, when tested in a dose-response format starting from a lower top concentration (100  $\mu$ M), we did not observe a significant activity difference between the dark and photoirradiated states for **C19** (FC < 2) and with a low absolute activity (IC<sub>50</sub>  $\approx$  100  $\mu$ M) (**Appendix Figure A9D**). The results of preliminarily modifying **C4** were therefore largely negative.

### 3.6.5. Combinatorial modification on C3

Upon making single-point modifications on the identified photoswitchable fragment **C3** and **C4**, cyclopropyl-substitution off the G-vector emerged as the best structural change in terms of absolute potency, with compound **C6** displaying low single-digit micromolar potency. However, this modification led to a 60-fold decrease in the Z-isomer thermal half-life to the order of minutes, explaining the low light-addressability of **C6** in the kinase assay. On the other hand, substitution of the phenyl ring at the 2-position with a simple hydroxymethyl group had yielded a significant 6-fold increase in the thermal stability to 19 h and a small boost in the potency as well. As a next step, we were intrigued by the idea of combining these two modifications to assess if we could harvest the high potency of **C6** and high thermal stability of **C15** in a single molecule (**Figure 3.16**).

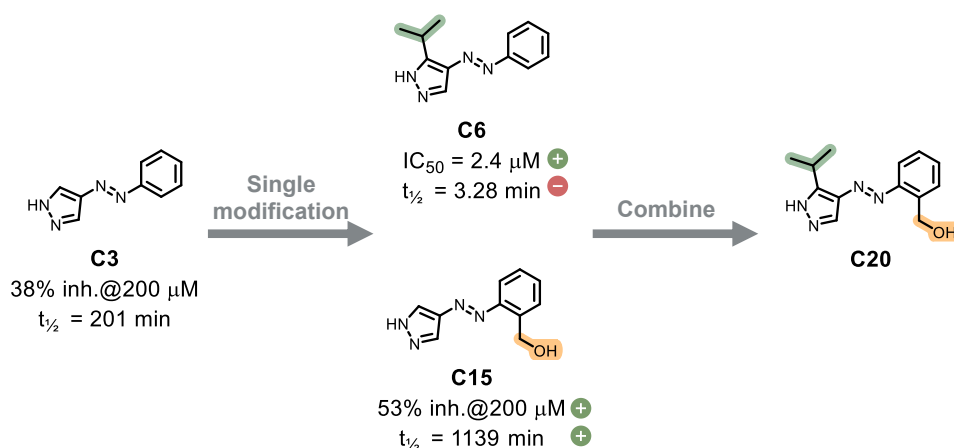
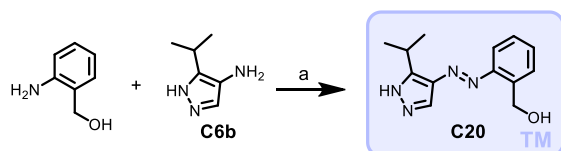


**Figure 3.16. Combinatorial modification on C3.** The structural changes from **C3** have been highlighted.

The hydroxymethyl-substitution had previously been installed on **C14** and **C15** via the aniline building block masked in the form of an ester (**Scheme 3.6**). This strategy had been taken because the direct two-step nitrosation–Baeyer-Mills reaction process on the hydromethylated anilines had earlier given extensive side reactions (data not shown)—presumably, because the hydroxy group can facilitate self-condensation during the nitrosation step. Here, we found that shortening the reaction time of the nitrosation step to 10 min allowed for a much cleaner subsequent Baeyer-Mills reaction, and compound **C20** could therefore be successfully obtained in a single step (**Scheme 3.8**).

**Scheme 3.8. Synthesis of combinatorial modification analogue C20.** <sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) Oxone, DCM, H<sub>2</sub>O, rt, 10 min; then **C6b**, AcOH, DCM, rt, 26 h, 51%. TM = Target molecule.

Photochemical analysis on the synthesised **C20** revealed no perturbation to the photoswitching completeness of **C3**, **C6** and **C15** (340 nm PSD of 97% *Z*) (**Figure 3.17A**). On the other hand, kinetic assessment showed a thermal half-life of 19 min, which interestingly corresponded to 6-fold increase in the thermal stability of **C6**—the same fold improvement observed when the hydroxymethyl group was introduced to **C3** (**Figure 3.17B**). This suggests that the stabilising effect of the group is highly transferrable.

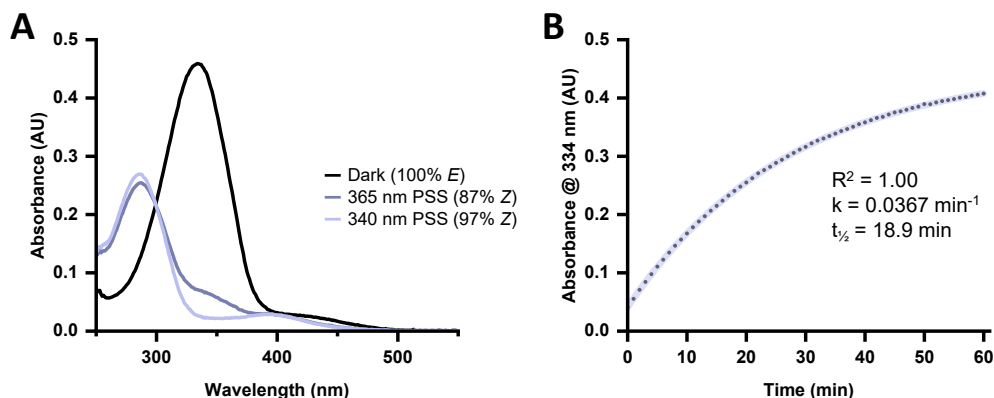


**Figure 3.17. Photochemical characterisation of C20.** Photoswitching and thermal kinetics were determined by UV–Vis spectrophotometry at 20 °C and at 20  $\mu\text{M}$  in 10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05 %[v/v] Tween-20/Surfactant P20, 1% DMSO. (A) PSD determination for **C20**. (B) Thermal back-isomerisation of **(Z)-C20** over 1 h.

When testing the compound in the kinase assay, we gratifyingly observed a further improvement to the potency of **C6**, with compound **C20** demonstrating apparent sub-micromolar  $\text{IC}_{50} = 0.56 \mu\text{M}$  in the dark state (**Figure 3.18**). Although more datapoints at lower compound concentrations are required to get accurate  $\text{IC}_{50}$  determination, it is apparent that the compound displays higher potency than **C6**. Furthermore, although the increased thermal stability did not translate into a substantial differential activity (with the photoirradiated state giving an  $\text{IC}_{50} = 0.94 \mu\text{M}$ ), the FC had still improved compared with **C6** (FC 1.7 versus 1.3).

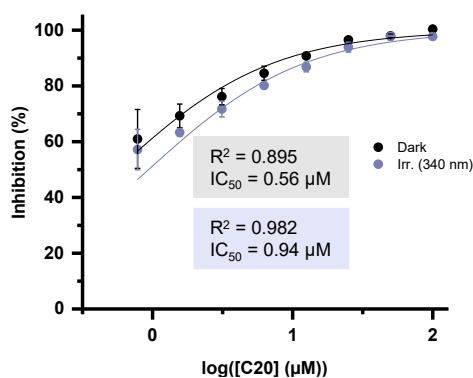


**Figure 3.18. Activity of C20 in the ADP-Glo™ kinase assay.** The compound was tested in the dark and after photoirradiation with 340 nm light. Error bars represent the standard deviation of three technical replicates.

### 3.7. Summary

In this study, we explored the prospect of utilising ultra-small fragment probes as starting points for making photoswitchable ligands. In particular, we hypothesised that the universal fragment, 4-bromopyrazole, in tandem with its known capability of mapping out ligand binding sites, could be used as a scaffold for rapidly building azoheteroarene-based photoswitchable ligands. As a case study, we targeted a cancer-related kinase, CDK2, which had previously been co-crystallised with 4-bromopyrazole (**C1**). To serve as a benchmark for our fragment-based campaign, we first performed late-stage azo transformation on a reported and highly potent CDK2 inhibitor **AT7519** (**D2**,  $IC_{50}$  = 50 nM), which emerged as the most suitable candidate for azologisation due to the inclusion of a core isosteric *N*-pyrazolylbenzamide scaffold. However, the resulting azologue **D3**, suffered from a 540-fold decrease in potency ( $IC_{50}$  = 27  $\mu$ M) and only presented a small light-dependent differential activity ( $FC$  = 1.5) (**Figure 3.19A**). With this reference point, we initiated a FBDD-campaign starting from **C1** (**Figure 3.19B**). Multi-directional azo extension on the fragment probe furnished two core photoswitchable fragments **C3** and **C4**, which both displayed detectable inhibition in the ADP-Glo™ kinase assay. Through rational structure-based and computer-aided design and by carefully monitoring both target engagement and photochemical properties, we identified the photoswitchable lead compound **C20**, which displayed an impressive sub-micromolar potency in the kinase assay ( $IC_{50}$  = 0.56  $\mu$ M). Compared with known literature inhibitors, this activity is still modest, and in terms of light-addressability, the compound could not achieve a substantial fold change in activity upon photoswitching ( $FC$  = 1.7). Despite this, our fragment-derived lead compound still outperformed the azologue **D3** across all measured parameters, including absolute and differential activity and drug-likeness as measured by the lipophilic efficiency metric ( $LiPE$  = 3.30 and 1.43 for **C20** and **D3**, respectively). Noticeably, **C20** is a surprisingly small molecule (MW = 244 Da, HAC = 18), and the size-scaled compound activity even surpasses that of the known inhibitor **AT7519** as measured by the ligand efficiency metric ( $LE$  = 0.47 and 0.40 for **C20** and **D2**, respectively). Crucially, this campaign has involved less than 20 compounds thus far and is still on-going. We expect that further elaboration on **C20** will improve its absolute potency as well as addressing the limited light-dependent differential activity.

Our preliminary results showcase the utility of 4-bromopyrazole as a fragment probe for making photoswitchable ligands.

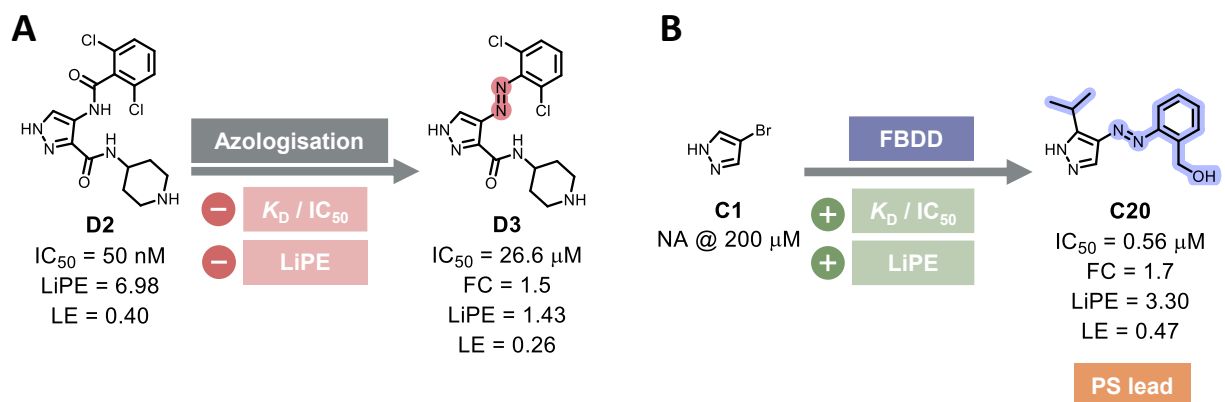


**Figure 3.19. Summary of the CDK2-targeting photopharmacology campaigns.** (A) Azologisation of the advanced lead molecule **D2** (**AT7519**), giving **D3**. The structural change from **D2** has been highlighted. (B) FBDD on the fragment probe **C1** (4-bromopyrazole), giving the photoswitchable lead molecule **C20**. The structural changes from **C1** have been highlighted.



## 4. Summary and outlook

### 4.1. Conclusion

Through the integration of light-responsiveness, photopharmacological agents offer the potential to refine drug action, enabling more precise interrogation and manipulation of biological systems than current drugs and chemical probes. However, the development of these agents is hampered by the challenge of reconciling light addressability with drug properties—the current late-stage azo transformation approach often yielding sub-optimal endpoints. We proposed that fragment-based drug discovery (FBDD) could offer a viable alternative approach to constructing photoswitchable bioactive ligands. By shifting the focus from treating light addressability as an add-on feature to considering it an intrinsic aspect of lead optimisation, FBDD might achieve more balanced and efficient photopharmacological agents.

To address this hypothesis, we embarked on multiple FBDD campaigns, applying three different hit identification approaches to three different disease-relevant targets. In all cases, target engagement (target-based affinity or potency), light addressability (the fold-change in activity upon photoswitching) and drug-likeness (LiPE) were carefully monitored.

We first attempted screening an in-house assembled 35-membered library of fragment-sized photoswitches against the methyltransferase fibrillarin, but this resulted in no identified hits. Beyond attributing the negative outcome to the small library size, we were concerned about the inherent low diversity of any such azo fragment library given the overall common structural topology that the fragments would share.

As a consequence, we next turned to screening a general and larger-sized 2256-membered fragment library against the methyltransferase target, NSP16/10. A biarylsulfonamide fragment hit **B1** having a  $K_D \approx 200\text{--}300\text{ }\mu\text{M}$  was identified and subjected to extensive medicinal chemistry optimisation focusing on installing an azo motif into the structure at an early stage. In the absence of precise structural data, systematic ligand-based design was applied, which ultimately yielded the photoswitchable lead compound **B130**. This compound displays a  $K_D = 6.0\text{ }\mu\text{M}$ , a  $FC = 12$  and an improved LiPE compared with **B1**. Although we could not meet our set criterion for target engagement ( $K_D/IC_{50} \leq 1\text{ }\mu\text{M}$ ), the compound still achieved a significant optimisation across all three property dimensions compared with the hit and displays a competitive profile compared with current literature standards in the photopharmacology field.

Finally, we explored the option of screening ultra-small monocyclic fragments as a basis for identifying photoswitchable ligands. We here adapted the universal fragment, 4-bromopyrazole (**C1**), which had previously been crystallised in complex with the kinase, CDK2. Structure-guided and computer-aided elaboration of **C1** very quickly yielded more potent and photoswitchable analogues, including compound **C20**, which demonstrated sub-micromolar potency ( $IC_{50} = 0.56 \mu M$ ). Although **C20** displayed a limited change in activity upon photoswitching ( $FC = 1.7$ ), it still outperformed the azologisation comparator **D3** across all measured parameters.

Several significant points emerge from these studies. Firstly, the NSP16/10 campaign was slow-paced and required the synthesis and test of a substantial number of compounds (>100) before the lead compound could be identified. This may at first be taken as an inherent consequence of the design-intensive fragment-approach. However, the CDK2 project demonstrated a significant multi-parameter enhancement at an unprecedented pace, achieved with a minimum number of compounds (< 20). The latter demonstrates that even when adding an additional property dimension, fragment optimisation can proceed rapidly and can produce better molecules than late-stage azo transformation. The difference in pace between the two campaigns may be a consequence of the two different hit identification approaches applied and would suggest that ultra-small fragments like 4-bromopyrazole may be better starting points for photoswitch design compared with more conventional fragments. However, there are several confounding factors in this equation. Crucially, the CDK2 project benefitted significantly from access to X-ray crystal structures (of the hit and of reference compounds) to guide design, whereas any precise structural data of relevant chemical matter was not available for NSP16/10. The results may therefore just substantiate the known fact that FBDD generally relies heavily on structural information to be efficient.<sup>56</sup> It can be argued that such structural knowledge may be especially important when adding the new property of light-addressability on top of the other drug-related properties. Secondly, there is obviously also a difference in the target: Whereas CDK2 has been heavily studied in the past, NSP16/10 still remains a quite novel target with fewer high-quality ligands.

In addition to the pace of optimisation, we observed a recurring tendency for structural modifications to induce opposing effects on photochemical properties and on target affinity/potency, similar to the findings of the late-stage azo transformation approach. However, fragments provide ample flexibility to rectify a decrease in one parameter and for sampling modifications that can yield multi-parameter enhancement. A notable example is the substitution of the original azobenzene scaffold of **B96** with azoheteroarene motifs, which yielded the analogue **B115** showing concurrent improvements in affinity, fold

change in activity and in LiPE. This further underscores that azoheteroarenes may be especially suitable for photopharmacological applications, possibly owing to their ability to achieve excellent photoconversions and thermal stabilities while simultaneously enabling directional polar interactions via their heteroatoms.<sup>48</sup> Another key example is how the hydroxymethyl group was found to give both potency enhancement and thermal Z-isomer stabilisation of **C3**, which was exploited to furnish the multi-parameter optimised lead compound **C20**.

Lastly, none of the photoswitchable lead compounds produced from the two campaigns can be readily accessed from structural transformations of either known or concurrently developed non-photoswitchable compounds. This indicates a potential capacity for FBDD to tap into a unique chemical space perhaps tailored for photopharmacological applications, which may not be easily accessed through late-stage methodologies.

In light of these findings, there is a compelling case for further exploring the use of FBDD in the development of photopharmacological agents.

## 4.2. Future work

To establish FBDD as a robust new methodology for developing photopharmacological agents and set forth concrete guidelines for its application, further work is required.

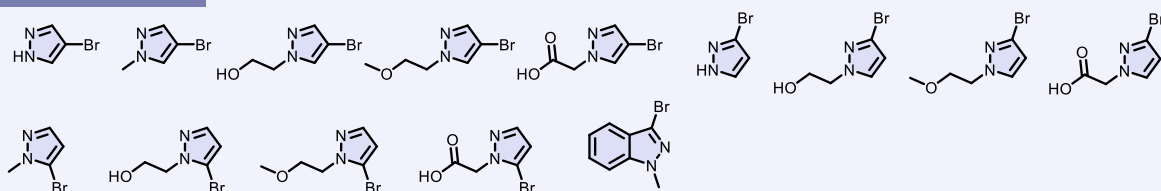
Firstly, the general applicability of the three different fragment-based approaches needs to be determined in order to attain a better understanding of their strengths and limitations. Regarding the photoswitchable fragment screening strategy, which was unsuccessful against fibrillarin, our collaborators at University of Leeds were able to obtain a single hit when screening against another protein target, METTL3-14. Another member of our research group is currently working on advancing the hit into more potent and light-addressable compounds. This indicates that our prior notion about the limited applicability of fragment-sized azo photoswitch libraries may be erroneous, and that screening such libraries may still be viable—especially if larger-sized compound collections can be accessed. In our research group, we are currently working on generating a large library of functionalised azo photoswitches via high-throughput synthesis, which could be employed for screening purposes.

Concerning the general fragment screening approach, it would be interesting to apply the strategy against a different protein target, especially where crystal structure data are more readily accessible, as this could substantially expedite the hit optimisation process. In particular, it would be compelling to apply the strategy against CDK2, allowing for a target-independent and more direct comparison with the photoswitchable probe strategy.

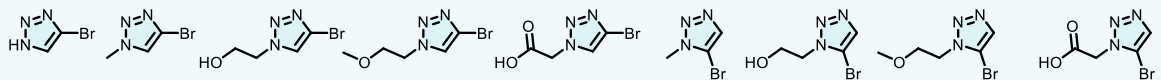
The latter approach emerged as the most promising based on the speed of hit optimisation. Nevertheless, it would likewise be crucial to evaluate the strategy against other targets, including non-enzymatic target classes such as receptors, ion channels and protein-protein interactions. In addition, it would be interesting to explore the practical utility of more halogenated minimal-sized heterocyclic fragment probes similar to 4-bromopyrazole. Building on the work of Waring and co-workers on the FragLites library, we have here designed a library 39 fragment probes (including 4-bromopyrazole) for this purpose (**Figure 4.1**).<sup>123</sup> These are all substituted with a bromide to facilitate crystallographic screening and optionally carry additional substituents to create pharmacophore doublets equivalent to the FragLites library. Uniquely, all the compounds include heterocyclic motifs which have previously been incorporated in efficient photoswitchable molecules—and we therefore hypothesise that the fragment probes will be poised for subsequent azo transformation. Consequently, we have coined these molecules “AzoFragS”. We are currently working on acquiring the molecules to test their utility as fragment probes for photopharmacological campaigns.

The power of a fragment-based approach lies in its potential for offering more efficient multi-parameter optimisation. In the present studies, we focused only on three parameters, i.e. target engagement, fold change in activity upon photoswitching and lipophilicity. However, future fragment-based studies could expand their scope to encompass additional crucial properties important for the development of photopharmacological agents into clinically relevant entities—aspects that were beyond the scope of the current studies. One significant challenge pertains to the wavelength of light required for photoswitching. Specifically, the UV light (340/365 nm) required to photoswitch compounds of the current studies is suboptimal for biocompatibility and tissue penetration. Another shortcoming of azo-based photoswitches involves their metabolic liability towards biological reductants such as glutathione and azoreductases.<sup>22</sup> While prior literature has tackled each of these challenges individually through structural modifications, the central dilemma persists: how to address them collectively within a single molecule. We are of the opinion that a fragment-based approach offers particular promise in solving this central problem.

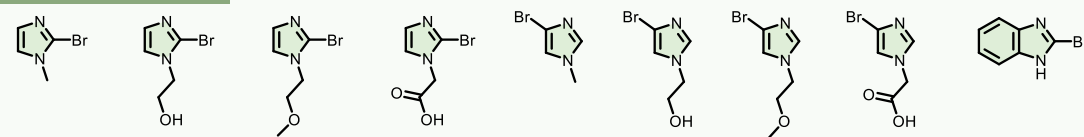
### Pyrazole-based



### Triazole-based



### Imidazole-based



### Pyridine-based

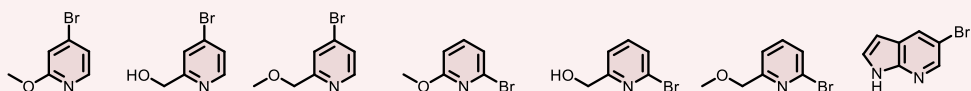


**Figure 4.1. AzoFrag.** The library consists of 39 halogenated and heterocyclic fragment probes that may be poised for development into photoswitchable ligands. The fragments have been divided into groups based on their heterocyclic core.

## 5. Experimental

### 5.1. Chemistry

#### 5.1.1. Materials and instrumentation

All reagents and solvents for chemical synthesis were obtained from commercial suppliers and used without prior purification. Thin-layer chromatography (TLC) analysis was performed on TLC silica gel 60 F<sub>254</sub> aluminium plates (Merck), and visualisation was achieved with UV light ( $\lambda = 254$  nm) and/or with appropriate TLC stains. Normal-phase silica gel column chromatography (SGCC) was carried out using prepacked silica cartridges (Biotage® Sfär or Teledyne ISCO RediSep®), and reversed-phase chromatography (RPC) purification was performed using C18 cartridges (Biotage® Sfär or Teledyne ISCO RediSep®)—both on a Biotage® Isolera™ automated purification system with eluent systems as specified in individual cases. <sup>1</sup>H, <sup>13</sup>C, and <sup>19</sup>F NMR spectra were acquired using a Bruker Avance III 400 spectrometer at 400 MHz, 101 MHz, and 377 MHz, respectively, and analysed at 300 K. Samples were dissolved in either DMSO-*d*<sub>6</sub> (99.9% D, Merck), CDCl<sub>3</sub> (99.8% D, Merck) or MeOD (99% D, Merck). Chemical shifts ( $\delta$ ) are given in ppm and referenced to residual non-deuterated solvent peaks of either DMSO-*d*<sub>6</sub> (<sup>1</sup>H: 2.500 ppm, <sup>13</sup>C: 39.520 ppm), CDCl<sub>3</sub> (<sup>1</sup>H: 7.260 ppm, <sup>13</sup>C: 77.160 ppm) or MeOD (<sup>1</sup>H: 3.310 and 4.870 ppm, <sup>13</sup>C: 49.000 ppm). Coupling constants (*J*) are reported in Hz. The following abbreviations for signal multiplicities were used throughout: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; p, pentet; m, multiplet. High-resolution mass spectrometry (HRMS) spectra were acquired either with atmospheric pressure chemical ionisation (APCI) or electrospray ionisation (ESI). For APCI, the ion source was connected to a Thermo Fisher Q-Exactive Orbitrap mass spectrometer using capillary temperature of 320 °C and discharge voltage set between 3.5 and 4.0 kV. The mass spectrometer operated in positive and negative ionisation. For ESI, analysis was performed using a Waters LCT Premier TOF mass spectrometer. The mass spectrometer operated in positive and negative ionisation with full MS scan. The mass range was from *m/z* 100 to 2000 with the following parameters: the source capillary was 2500 V, sample cone 35 V, desolvation temperature 350 °C and source temperature 120 °C. The TOF was calibrated with sulfadimethyloxidine [M]<sup>+</sup> with *m/z* value of 311.0814 and leucine enkephalin [M]<sup>+</sup>, *m/z* value of 556.2771. Liquid chromatography mass spectrometry (LC–MS) analysis was performed using either 1) a Waters LCT Premier TOF mass spectrometer with Waters ACQUITY UPLC system using a C18 column (1.7  $\mu$ m, 2.1 mm x 50 mm) and running with a linear gradient of mobile phase A containing H<sub>2</sub>O with 0.1% formic acid and B containing MeCN with 0.1% formic acid over 3 min or 2) an Agilent 1260 Infinity II Hybrid SFC/UHPLC-6250 MS system using a Raptor C18 column

running with a linear gradient of mobile phase A containing water and B containing MeCN with 20 mM aq. NH<sub>4</sub>OAc over 5 min.

### 5.1.2. General synthesis

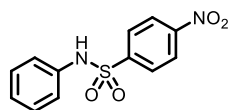
All reactions were performed under a nitrogen (N<sub>2</sub>) or an argon (Ar) atmosphere unless otherwise stated. Room temperature (rt) refers to temperatures of 20–25 °C. All final compounds that were tested biologically and photochemically had at least 95% purity based on <sup>1</sup>H NMR and HPLC (λ = 254 nm).

### 5.1.3. Series A compounds

The series A compounds (**A1–35**) were acquired from other members of the Fuchter research group.

### 5.1.4. Series B compounds

#### 4-Nitro-*N*-phenylbenzenesulfonamide (**B1a**)



**Synthetic procedure:** To a solution of aniline (0.931 g, 10.0 mmol, 1.00 equiv) and pyridine (0.809 mL, 10.0 mmol, 1.00 equiv) in DCM (10.0 mL) was added 4-nitrobenzenesulfonyl chloride (2.22 g, 10.0 mmol, 1.00 equiv), and the mixture was stirred at reflux for 3 h. Upon reaction completion as determined by TLC, the mixture was cooled to rt and added 1 M HCl (10 mL). The aq. layer was extracted with DCM (3 x 10 mL), and the combined organic layers were dried over MgSO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (1.81 g, 6.52 mmol, 65%). This is a known compound. Experimental data correlate with literature.<sup>187</sup>

**Physical state:** Beige solid.

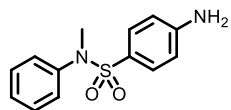
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.61 (s, 1H), 8.41–8.33 (m, 2H), 8.04–7.95 (m, 2H), 7.25 (tt, *J* = 7.7, 2.1 Hz, 2H), 7.14–7.02 (m, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.3, 145.3, 137.3, 129.8, 128.7, 125.3, 125.1, 121.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>12</sub>H<sub>9</sub>N<sub>2</sub>O<sub>4</sub>S [M-H]<sup>−</sup> 277.0283, found 277.0290.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.50.

#### 4-Amino-*N*-methyl-*N*-phenylbenzenesulfonamide (**B1**)



**Synthetic procedure:** A solution of **B17** (0.877 g, 3.00 mmol, 1.00 equiv) in MeOH (15.0 mL) was degassed with N<sub>2</sub> for 10 minutes. 10% Pd/C stabilised with 50% water (0.175 g, 10 wt%) was then added against a positive pressure of N<sub>2</sub>, and the mixture further degassed with N<sub>2</sub> for 5 minutes. The mixture was then degassed with H<sub>2</sub> for 5 minutes and stirred under H<sub>2</sub> (1 atm) at rt for 18 h. Upon reaction completion as determined by TLC, the mixture was degassed with N<sub>2</sub> for 10 minutes, filtered through a bed of celite and the filtrate concentrated to dryness *in vacuo* to provide the title compound (0.805 g, 3.00 mmol, quant.). This is a known compound. Experimental data correlate with literature.<sup>188</sup>

**Physical state:** Beige solid.

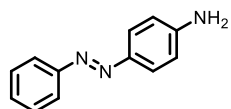
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.32 (t, *J* = 7.6 Hz, 2H), 7.24 (t, *J* = 6.9 Hz, 1H), 7.09 (dd, *J* = 8.8, 2.6 Hz, 4H), 6.55 (d, *J* = 8.5 Hz, 2H), 6.07 (s, 2H), 3.04 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 153.2, 141.9, 129.4, 128.6, 126.7, 126.0, 120.5, 112.5, 37.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>15</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 263.0854, found 263.0860.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.17.

#### (*E*)-4-(Phenyldiazenyl)aniline (**B2**)



**Synthetic procedure:** A previously reported Baeyer-Mills reaction protocol was followed with minor modifications.<sup>100</sup> To a solution of *p*-phenylenediamine (0.108 g, 1.00 mmol, 1.00 equiv) in glacial AcOH (7.0 mL) was degassed with N<sub>2</sub> for 10 minutes. Nitrosobenzene (0.107 g, 1.00 mmol, 1.00 equiv) was then added against a positive pressure of N<sub>2</sub>, and the mixture was stirred under N<sub>2</sub> at 40 °C for 16 h. Upon reaction completion as determined by TLC, the mixture was diluted with water (30 mL) and 1 M NaOH (30 mL) and extracted with EtOAc (3 x 50 mL). The combined organic phases were washed with sat. brine (20 mL), dried over MgSO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.082 g, 0.414 mmol, 41%). This is a known compound.<sup>189</sup>

**Physical state:** Orange solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.78–7.72 (m, 2H), 7.70–7.64 (m, 2H), 7.51 (dd, *J* = 8.4, 6.9 Hz, 2H), 7.44–7.38 (m, 1H), 6.71–6.63 (m, 2H), 6.12 (s, 2H).

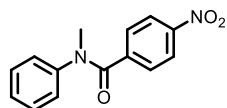
**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.9, 152.5, 142.8, 129.4, 129.2, 125.2, 121.7, 113.4.



**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{12}H_{12}N_3$   $[M+H]^+$  198.1031, found 198.1040.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.48.

#### ***N*-Methyl-4-nitro-*N*-phenylbenzamide (B3a)**



**Synthetic procedure:** A previously reported aniline acylation protocol was followed with minor modifications.<sup>165</sup> A solution of *N*-methylaniline (1.19 mL, 11.0 mmol, 1.10 equiv) and  $Et_3N$  (1.74 mL, 12.5 mmol, 1.25 equiv) in anhydrous DCM (8.0 mL) was cooled to 0 °C, and 4-nitrobenzoyl chloride (1.86 g, 10.0 mmol, 1.00 equiv) was added portion wise. The mixture was stirred at 0 °C and allowed to slowly reach rt over 44 h. Upon reaction completion as determined by TLC, the mixture was diluted with DCM (10 mL) and washed with 1 M HCl (2 x 10 mL) and sat. brine (10 mL), dried over  $MgSO_4$ , and concentrated to dryness *in vacuo* to provide the title compound (1.22 g, 4.75 mmol, 48%). This is a known compound.<sup>190</sup>

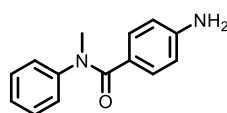
**Physical state:** White solid.

**$^1H$  NMR** (400 MHz,  $DMSO-d_6$ ):  $\delta$  8.06 (d,  $J$  = 8.3 Hz, 2H), 7.52 (d,  $J$  = 8.2 Hz, 2H), 7.30–7.21 (m, 4H), 7.20–7.13 (m, 1H), 3.39 (s, 3H).

**$^{13}C\{^1H\}$  NMR** (101 MHz,  $DMSO-d_6$ ):  $\delta$  167.7, 147.4, 143.6, 142.8, 129.3, 129.3, 127.3, 127.1, 123.0, 37.7.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.49.

#### **4-Amino-*N*-methyl-*N*-phenylbenzamide (B3)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B3a** (1.20 g, 4.68 mmol, 1.00 equiv), 10% Pd/C (0.120 g, 10 wt%) and MeOH (20.0 mL). Stirring at rt for 6 h and purification by SGCC (hex:EtOAc 0–100%) provided the title compound (0.860 g, 3.80 mmol, 81%). This is a known compound.<sup>191</sup>

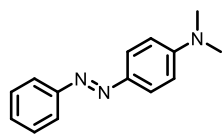
**Physical state:** White solid.

**$^1H$  NMR** (400 MHz,  $DMSO-d_6$ ):  $\delta$  7.31–7.23 (m, 2H), 7.14 (ddt,  $J$  = 8.0, 6.8, 1.2 Hz, 1H), 7.11–7.05 (m, 2H), 6.99–6.89 (m, 2H), 6.34–6.24 (m, 2H), 5.45 (s, 2H), 3.31 (s, 3H).

**$^{13}C\{^1H\}$  NMR** (101 MHz,  $DMSO-d_6$ ):  $\delta$  169.7, 150.3, 145.9, 130.6, 129.0, 126.7, 125.7, 122.1, 112.1, 38.2.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{14}H_{15}N_2O$   $[M+H]^+$  227.1184, found 227.1176.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.18.

**(E)-N,N-Dimethyl-4-(phenyldiazenyl)aniline (B4)**

**Synthetic procedure:** To a solution of aniline (0.137 mL, 1.50 mmol, 1.00 equiv) in THF (2.5 mL) and water (2.5 mL) was added 3 M aq. HCl (1.00 mL, 3.00 mmol, 2.00 equiv), and the mixture was stirred at 0 °C for 10 min. A solution of NaNO<sub>2</sub> (0.124 g, 1.80 mmol, 1.20 equiv) in water (1 mL) was then added dropwise, and the mixture was stirred at 0 °C for 40 min. NaOAc (0.246 g, 3.00 mmol, 2.00 equiv) was then added, and the mixture was stirred at 0 °C for 5 min. Finally, a solution of *N,N*-Dimethylaniline (0.190 mL, 1.50 mmol, 1.00 equiv) in THF (1.0 mL) was added dropwise, and the mixture was stirred at 0 °C and allowed to slowly reach rt over 21 h. Upon reaction completion as determined by TLC, the mixture was concentrated *in vacuo*, and the precipitate isolated by suction filtration, washed with water, and dried *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.081 g, 0.360 mmol, 24%). This is a known compound.<sup>189</sup>

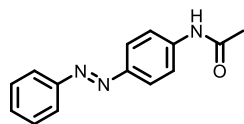
**Physical state:** Orange solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.83–7.75 (m, 4H), 7.56–7.49 (m, 2H), 7.46–7.40 (m, 1H), 6.87–6.80 (m, 2H), 3.06 (s, 6H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.5, 152.4, 142.5, 129.6, 129.2, 124.7, 121.8, 111.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>16</sub>N<sub>3</sub> [M+H]<sup>+</sup> 226.1344, found 226.1344.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.63.

**(E)-N-(4-(Phenyldiazenyl)phenyl)acetamide (B5)**

**Synthetic procedure:** A solution of **B2** (0.090 g, 0.456 mmol, 1.00 equiv) and Et<sub>3</sub>N (0.191 mL, 1.37 mmol, 3.00 equiv) in anhydrous THF (6.0 mL) was cooled to 0 °C and AcCl (0.097 mL, 1.37 mmol, 3.00 equiv) was added dropwise. The mixture was stirred at rt for 22 h. Upon reaction completion as determined by TLC, the mixture was added water (20 mL) and extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with 1 M HCl (20 mL) and sat. brine (20 mL), dried over MgSO<sub>4</sub> and concentrated to dryness *in vacuo* to provide the title compound (0.112 g, 0.456 mmol, quant.). This is a known compound.<sup>192</sup>

**Physical state:** Orange solid.

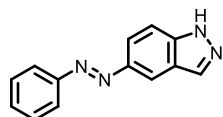
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.31 (s, 1H), 7.90–7.87 (m, 2H), 7.87–7.83 (m, 2H), 7.83–7.79 (m, 2H), 7.61–7.56 (m, 2H), 7.55–7.50 (m, 1H), 2.10 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 168.8, 152.0, 147.4, 142.5, 131.0, 129.4, 123.7, 122.3, 119.1, 24.2.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>14</sub>N<sub>3</sub>O [M+H]<sup>+</sup> 240.1137, found 240.1139.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.24.

#### (*E*)-5-(Phenyldiazenyl)-1*H*-indazole (B6)



**Synthetic procedure:** A previously reported Baeyer-Mills reaction protocol was followed with minor modifications.<sup>36</sup> A solution of 1*H*-indazol-5-amine in 40% aq. NaOH (1.00 mL, 14.7 mmol, 14.7 equiv) and toluene (1.0 mL) was degassed with N<sub>2</sub> for 10 min. Then nitrosobenzene (0.107 g, 1.00 mmol, 1.00 equiv) was added against a positive pressure of N<sub>2</sub>, and the mixture was stirred under N<sub>2</sub>, at 80 °C for 18 h. Upon reaction completion as determined by TLC, the mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic phases were washed with sat. brine (10 mL), dried over MgSO<sub>4</sub>, and concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–60%) provided the title compound (0.011 g, 0.047 mmol, 5%). This is a novel compound.

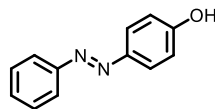
**Physical state:** Yellow-green solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.42 (dd, *J* = 1.9, 0.7 Hz, 1H), 8.33–8.30 (m, 1H), 8.15 (dd, *J* = 9.0, 1.7 Hz, 1H), 7.96–7.91 (m, 2H), 7.65 (d, *J* = 9.0 Hz, 1H), 7.57–7.51 (m, 2H), 7.51–7.45 (m, 1H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>11</sub>N<sub>4</sub> [M+H]<sup>+</sup> 223.0984, found 223.0990.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.52.

#### (*E*)-4-(Phenyldiazenyl)phenol (B7)



**Synthetic procedure:** Conc. HCl (6.5 mL, 78.0 mmol, 3.63 equiv) was cooled to 0 °C, and aniline (0.978 mL, 10.7 mmol, 1.00 equiv) and a solution of NaNO<sub>2</sub> (1.48 g, 21.5 mmol, 1.00 equiv) in water (5 mL) were added dropwise. The mixture was stirred at 0 °C for 1 h. A solution of aniline (0.978 mL, 10.7 mmol, 1.00 equiv) in 1 M aq. HCl (22 mL, 22.0 mmol, 1.00 equiv) was cooled to 0 °C and the diazonium solution prepared above was added dropwise. The mixture was stirred at 0 °C and allowed to slowly reach rt over 15

h. The mixture was then carefully poured into 1 M  $\text{NH}_4\text{OH}$  (30 mL), the pH adjusted to 8 with 30%  $\text{NH}_4\text{OH}$  and the mixture extracted with EtOAc (3 x 30 mL). The combined organic phases were washed with sat.  $\text{NaHCO}_3$  (30 mL) and sat. brine (30 mL), dried over  $\text{MgSO}_4$ , and concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (1.26 g, 6.36 mmol, 30%). This is a known compound. Experimental data correlate with literature.<sup>193</sup>

**Physical state:** Orange solid.

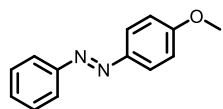
**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.95–7.85 (m, 4H), 7.56–7.47 (m, 2H), 7.47–7.40 (m, 1H), 7.01–6.93 (m, 2H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{CDCl}_3$ ):  $\delta$  158.6, 152.0, 146.7, 130.4, 128.9, 125.2, 122.4, 115.8.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{12}\text{H}_{11}\text{N}_2\text{O}$   $[\text{M}+\text{H}]^+$  199.0871, found 199.0860.

**$R_f$**  (silica gel, *n*-hexane:EtOAc 2:1): 0.69.

#### (*E*)-1-(4-Methoxyphenyl)-2-phenyldiazene (**B8**)



**Synthetic procedure:** To a solution of **B7** (0.197 g, 1.00 mmol, 1.00 equiv) and  $\text{K}_2\text{CO}_3$  (0.276 g, 2.00 mmol, 2.00 equiv) in anhydrous DMF (2.0 mL) was added MeI (0.125 mL, 2.00 mmol, 2.00 equiv), and the mixture was stirred at 50 °C for 28 h. Upon reaction completion as determined by TLC, the mixture was cooled to rt, added water (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with water (2 x 20 mL) and sat. brine (2 x 20 mL), dried over  $\text{MgSO}_4$  and concentrated to dryness *in vacuo* to provide the title compound (0.175 g, 0.823 mmol, 82%). This is a known compound.<sup>194</sup>

**Physical state:** Orange solid.

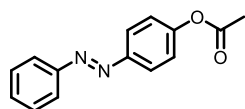
**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  7.93–7.88 (m, 2H), 7.86–7.81 (m, 2H), 7.61–7.55 (m, 2H), 7.55–7.49 (m, 1H), 7.16–7.11 (m, 2H), 3.87 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  162.0, 152.0, 146.1, 130.8, 129.4, 124.6, 122.2, 114.6, 55.7.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{13}\text{H}_{13}\text{N}_2\text{O}$   $[\text{M}+\text{H}]^+$  213.1019, found 213.1011.

**$R_f$**  (silica gel, *n*-hexane:EtOAc 1:1): 0.67.

#### (*E*)-4-(Phenyldiazenyl)phenyl acetate (**B9**)



**Synthetic procedure:** The same procedure employed for **B5** was followed starting from **B7** (0.197 g, 1.00 mmol, 1.00 equiv), AcCl (0.213 mL, 3.00 mmol, 3.00 equiv),  $\text{Et}_3\text{N}$  (0.418 mL, 3.00 mmol, 3.00 equiv) and

anhydrous THF (12.0 mL). Stirring at 0 °C–rt for 2 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.174 g, 0.727 mmol, 73%). This is a known compound.<sup>195</sup>

**Physical state:** Orange solid.

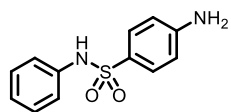
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.98–7.93 (m, 2H), 7.92–7.87 (m, 2H), 7.65–7.54 (m, 3H), 7.40–7.34 (m, 2H), 2.32 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 169.0, 152.8, 151.9, 149.5, 131.6, 129.5, 123.8, 123.0, 122.6, 20.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>13</sub>N<sub>2</sub>O<sub>2</sub> [M+H]<sup>+</sup> 241.0977, found 241.0979.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.69.

#### 4-Amino-*N*-phenylbenzenesulfonamide (B10)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B1a** (0.278 g, 1.00 mmol, 1.00 equiv), 10% Pd/C stabilised with 50% water (0.056 g, 10 wt%) and MeOH (5.0 mL). Stirring at rt for 22 h provided the title compound (0.278 g, 1.00 mmol, quant.). This is a known compound.<sup>188</sup>

**Physical state:** White solid.

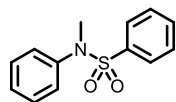
**<sup>1</sup>H NMR** (400 MHz, MeOD): δ 7.40–7.35 (m, 2H), 7.17–7.11 (m, 2H), 7.04–6.95 (m, 3H), 6.57–6.52 (m, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, MeOD): δ 153.2, 138.6, 129.3, 129.1, 125.9, 124.5, 121.3, 113.4.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>12</sub>H<sub>13</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 249.0698, found 249.0708.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.23.

#### *N*-Methyl-*N*-phenylbenzenesulfonamide (B11)



**Synthetic procedure:** To a solution of **B12** (0.467 g, 2.00 mmol, 1.00 equiv) and K<sub>2</sub>CO<sub>3</sub> (1.66 g, 12.0 mmol, 6.00 equiv) in anhydrous DMF (8.0 mL) was added MeI (0.187 mL, 3.00 mmol, 1.50 equiv), and the mixture was stirred at rt for 5 h. Upon reaction completion as determined by TLC, the mixture was added water (30 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with water (2 x 20 mL) and sat. brine (20 mL), dried over MgSO<sub>4</sub> and concentrated to dryness *in vacuo* to provide the title compound (0.481 g, 1.95 mmol, 97%). This is a known compound. Experimental data correlate with literature.<sup>196</sup>

**Physical state:** Off-white solid.

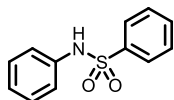
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.60–7.53 (m, 3H), 7.48–7.42 (m, 2H), 7.33–7.23 (m, 3H), 7.11–7.07 (m, 2H), 3.18 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 141.6, 136.6, 132.9, 129.0, 128.9, 128.0, 127.5, 126.8, 38.3.

**HRMS** (ESI-TOF): m/z calc. for C<sub>13</sub>H<sub>14</sub>NO<sub>2</sub>S [M+H]<sup>+</sup> 248.0745, found 248.0754.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.43.

### ***N*-Phenylbenzenesulfonamide (B12)**



**Synthetic procedure:** The same procedure employed for **B1a** was followed starting from aniline (0.931 g, 10.0 mmol, 1.00 equiv), benzenesulfonyl chloride (1.77 g, 10.0 mmol, 1.00 equiv), pyridine (0.809 mL, 10.0 mmol, 1.00 equiv) and DCM (10.0 mL). Stirring at reflux for 3 h and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (1.20 g, 5.15 mmol, 52%). This is a known compound. Experimental data correlate with literature.<sup>197</sup>

**Physical state:** White solid.

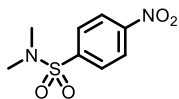
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.84–7.79 (m, 2H), 7.59–7.53 (m, 1H), 7.49–7.43 (m, 2H), 7.26 (ddt, *J* = 8.9, 7.7, 1.8 Hz, 2H), 7.17–7.08 (m, 3H), 6.88 (s, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 139.4, 136.8, 133.5, 129.8, 129.5, 127.7, 126.0, 122.3.

**HRMS** (ESI-TOF): m/z calc. for C<sub>12</sub>H<sub>10</sub>NO<sub>2</sub>S [M-H]<sup>−</sup> 232.0432, found 232.0440.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.41.

### ***N,N*-Dimethyl-4-nitrobenzenesulfonamide (B13a)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from 4-nitrobenzenesulfonamide (1.01 g, 5.00 mmol, 1.00 equiv), MeI (2.13 mL, 15.0 mmol, 3.00 equiv), K<sub>2</sub>CO<sub>3</sub> (4.15 g, 30.0 mmol, 6.00 equiv) and anhydrous DMF (20.0 mL). Stirring at rt for 6 h provided the title compound (1.11 g, 4.83 mmol, 97%). This is a known compound.<sup>198</sup>

**Physical state:** Off-white solid.

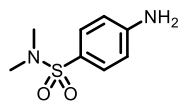
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.48–8.41 (m, 2H), 8.05–7.98 (m, 2H), 2.67 (s, 6H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.0, 140.5, 129.1, 124.7, 37.4.

**HRMS** (ESI-TOF): m/z calc. for C<sub>8</sub>H<sub>9</sub>N<sub>2</sub>O<sub>4</sub>S [M-H]<sup>−</sup> 229.0283, found 229.0294.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.55.

#### 4-Amino-*N,N*-dimethylbenzenesulfonamide (B13)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B13a** (0.460 g, 2.00 mmol, 1.00 equiv), 10% Pd/C (0.046 g, 10 wt%) and MeOH (10.0 mL). Stirring at rt for 20 h provided the title compound (0.399 g, 1.99 mmol, quant.). This is a known compound.<sup>199</sup>

**Physical state:** Off-white solid.

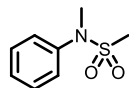
$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  7.38–7.33 (m, 2H), 6.69–6.59 (m, 2H), 6.05 (s, 2H), 2.50 (s, 6H).

$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):  $\delta$  153.1, 129.6, 119.1, 112.7, 37.7.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_8\text{H}_{13}\text{N}_2\text{O}_2\text{S}$   $[\text{M}+\text{H}]^+$  201.0698, found 201.0704.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.34.

#### *N*-Methyl-*N*-phenylmethanesulfonamide (B14)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from *N*-methylaniline (0.120 mL, 1.10 mmol, 1.10 equiv), methanesulfonyl chloride (0.077 mL, 1.00 mmol, 1.00 equiv), pyridine (0.243 mL, 3.00 mmol, 3.00 equiv) and DCM (4.0 mL). Stirring at rt for 26 h provided the title compound (0.287 g, 1.00 mmol, quant.). This is a known compound.<sup>200</sup>

**Physical state:** Yellow solid.

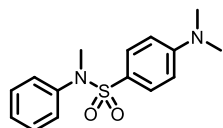
$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  7.41 (d,  $J$  = 4.3 Hz, 4H), 7.34–7.28 (m, 1H), 3.23 (s, 3H), 2.93 (s, 3H).

$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):  $\delta$  141.7, 129.1, 127.0, 126.3, 37.8, 35.0.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_8\text{H}_{12}\text{NO}_2\text{S}$   $[\text{M}+\text{H}]^+$  186.0589, found 186.0594.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.22.

#### 4-(Dimethylamino)-*N*-methyl-*N*-phenylbenzenesulfonamide (B15)



**Synthetic procedure:** The same procedure employed for **B8** was followed starting from **B1** (0.262 g, 1.00 mmol, 1.00 equiv), MeI (0.125 mL, 2.00 mmol, 2.00 equiv), K<sub>2</sub>CO<sub>3</sub> (0.276 g, 2.00 mmol, 2.00 equiv) and anhydrous DMF (2.0 mL). Stirring at rt for 17 h and then at 95 °C for 4 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.215 g, 0.739 mmol, 74%). This is a known compound.<sup>201</sup>

**Physical state:** White solid.

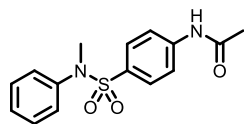
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.39–7.32 (m, 2H), 7.32–7.26 (m, 2H), 7.26–7.20 (m, 1H), 7.16–7.09 (m, 2H), 6.64–6.56 (m, 2H), 3.13 (s, 3H), 3.03 (s, 6H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 152.8, 142.3, 129.8, 128.8, 127.0, 126.8, 122.2, 110.8, 40.3, 38.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>19</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 291.1167, found 291.1174.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.47.

#### ***N*-(4-(*N*-Methyl-*N*-phenylsulfamoyl)phenyl)acetamide (B16)**



**Synthetic procedure:** The same procedure employed for **B5** was followed starting from **B1** (0.262 g, 1.00 mmol, 1.00 equiv), AcCl (0.213 mL, 3.00 mmol, 3.00 equiv), Et<sub>3</sub>N (0.418 mL, 3.00 mmol, 3.00 equiv) and anhydrous THF (12.0 mL). Stirring at 0 °C–rt for 4 h and purification by SGCC (*n*-hexane:EtOAc 50–100%) provided the title compound (0.261 g, 0.859 mmol, 86%). This is a known compound.<sup>202</sup>

**Physical state:** Colourless oil.

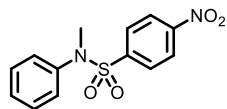
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.72 (s, 1H), 7.63–7.56 (m, 2H), 7.49–7.42 (m, 2H), 7.32–7.22 (m, 3H), 7.11–7.04 (m, 2H), 3.16 (s, 3H), 2.20 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 168.9, 142.3, 141.5, 131.2, 129.2, 129.1, 127.6, 126.8, 119.1, 38.3, 24.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>17</sub>N<sub>2</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 305.0960, found 305.0964.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.15.

#### ***N*-Methyl-4-nitro-*N*-phenylbenzenesulfonamide (B17)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B1a** (1.11 g, 4.00 mmol, 1.00 equiv), MeI (0.374 mL, 6.00 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (3.32 g, 24.0 mmol, 6.00 equiv) and



anhydrous DMF (16.0 mL). Stirring at rt for 5 h provided the title compound (1.12 g, 3.83 mmol, 96%). This is a known compound. Experimental data correlate with literature.<sup>188</sup>

**Physical state:** Beige solid.

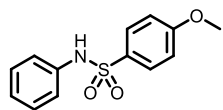
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.32–8.27 (m, 2H), 7.75–7.69 (m, 2H), 7.37–7.30 (m, 3H), 7.11–7.03 (m, 2H), 3.23 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 150.3, 142.4, 140.7, 129.4, 129.1, 128.1, 126.7, 124.1, 38.5.

**HRMS** (ESI-TOF): m/z calc. for C<sub>13</sub>H<sub>13</sub>N<sub>2</sub>O<sub>4</sub>S [M+H]<sup>+</sup> 293.0596, found 293.0593.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.42.

#### 4-Methoxy-*N*-phenylbenzenesulfonamide (B18a)



**Synthetic procedure:** A previously reported *N*-sulfonylation protocol was followed with minor modifications.<sup>166</sup> To a solution of aniline (1.00 mL, 11.0 mmol, 1.10 equiv) and pyridine (2.43 mL, 30.0 mmol, 3.00 equiv) in DCM (25.0 mL) was dropwise added a solution of 4-methoxybenzenesulfonyl chloride (2.07 g, 10.0 mmol, 1.00 equiv) in DCM (25 mL), and the mixture was stirred at rt for 47 h. Upon reaction completion as determined by TLC, the mixture was poured into water (30 mL), and the aq. phase extracted with DCM (2 x 30 mL). The combined organic layers were washed with 1 M HCl (2 x 30 mL), sat. NaHCO<sub>3</sub> (30 mL) and sat. brine (30 mL), dried over MgSO<sub>4</sub> and concentrated to dryness *in vacuo* to provide the title compound (2.15 g, 8.16 mmol, 82%). This is a known compound.<sup>203</sup>

**Physical state:** White solid.

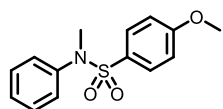
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.15 (s, 1H), 7.71–7.65 (m, 2H), 7.24–7.18 (m, 2H), 7.10–7.06 (m, 2H), 7.06–7.03 (m, 2H), 7.02–6.97 (m, 1H), 3.78 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 162.4, 137.9, 131.1, 129.1, 128.9, 123.9, 119.8, 114.3, 55.6.

**HRMS** (ESI-TOF): m/z calc. for C<sub>13</sub>H<sub>14</sub>NO<sub>3</sub>S [M+H]<sup>+</sup> 264.0694, found 264.0692.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.26.

#### 4-Methoxy-*N*-methyl-*N*-phenylbenzenesulfonamide (B18)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B18a** (1.05 g, 4.00 mmol, 1.00 equiv), MeI (0.374 mL, 6.00 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (3.32 g, 24.0 mmol, 6.00 equiv) and

anhydrous DMF (16.0 mL). Stirring at rt for 16 h provided the title compound (1.04 g, 3.76 mmol, 94%). This is a known compound. Experimental data correlate with literature.<sup>204</sup>

**Physical state:** Beige solid.

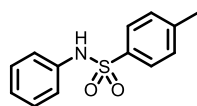
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.52–7.46 (m, 2H), 7.36–7.24 (m, 3H), 7.15–7.10 (m, 2H), 6.96–6.90 (m, 2H), 3.88 (s, 3H), 3.18 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 162.8, 141.5, 129.8, 128.6, 127.9, 127.0, 126.5, 113.7, 55.4, 37.9.

**HRMS** (ESI-TOF): m/z calc. for C<sub>14</sub>H<sub>16</sub>NO<sub>3</sub>S [M+H]<sup>+</sup> 278.0851, found 278.0843.

**R<sub>f</sub>**(silica gel, *n*-hexane:EtOAc 2:1): 0.49.

#### 4-Methyl-*N*-phenylbenzenesulfonamide (B19a)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from aniline (1.00 mL, 11.0 mmol, 1.10 equiv), 4-toluenesulfonyl chloride (1.91 g, 10.0 mmol, 1.00 equiv), pyridine (2.43 mL, 30.0 mmol, 3.00 equiv) and DCM (50.0 mL). Stirring at rt for 45 h provided the title compound (2.30 g, 9.29 mmol, 93%). This is a known compound. Experimental data correlate with literature.<sup>205</sup>

**Physical state:** Beige solid.

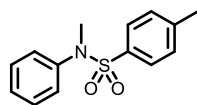
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.22 (s, 1H), 7.68–7.59 (m, 2H), 7.36–7.29 (m, 2H), 7.24–7.18 (m, 2H), 7.10–7.06 (m, 2H), 7.03–6.97 (m, 1H), 2.32 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 143.2, 137.8, 136.7, 129.7, 129.1, 126.7, 123.9, 119.9, 20.9.

**HRMS** (ESI-TOF): m/z calc. for C<sub>13</sub>H<sub>14</sub>NO<sub>2</sub>S [M+H]<sup>+</sup> 248.0745, found 248.0733.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.36.

#### *N*,4-Dimethyl-*N*-phenylbenzenesulfonamide (B19)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B19a** (0.989 g, 4.00 mmol, 1.00 equiv), MeI (0.374 mL, 6.00 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (3.32 g, 24.0 mmol, 6.00 equiv) and anhydrous DMF (16.0 mL). Stirring at rt for 16 h provided the title compound (1.05 g, 4.00 mmol, quant.). This is a known compound. Experimental data correlate with literature.<sup>206</sup>

**Physical state:** Beige solid.

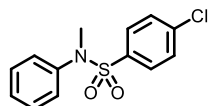
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.45–7.40 (m, 2H), 7.34–7.21 (m, 5H), 7.13–7.07 (m, 2H), 3.17 (s, 3H), 2.42 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 143.7, 141.7, 133.6, 129.4, 128.9, 128.0, 127.4, 126.7, 38.2, 21.7.

**HRMS** (ESI-TOF): m/z calc. for C<sub>14</sub>H<sub>16</sub>NO<sub>2</sub>S [M+H]<sup>+</sup> 262.0902, found 262.0907.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.53.

#### 4-Chloro-*N*-methyl-*N*-phenylbenzenesulfonamide (B20)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from *N*-methylaniline (0.596 mL, 5.50 mmol, 1.10 equiv), 4-chlorobenzenesulfonyl chloride (1.06 g, 5.00 mmol, 1.00 equiv), pyridine (1.21 mL, 15.0 mmol, 3.00 equiv) and DCM (20.0 mL). Stirring at rt for 24 h provided the title compound (1.40 g, 4.97 mmol, 99%). This is a known compound.<sup>207</sup>

**Physical state:** Yellow solid.

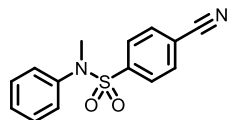
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.70–7.62 (m, 2H), 7.54–7.45 (m, 2H), 7.39–7.33 (m, 2H), 7.33–7.27 (m, 1H), 7.15–7.07 (m, 2H), 3.14 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 140.8, 138.2, 134.7, 129.4, 129.3, 129.0, 127.5, 126.4, 126.3, 37.9.

**HRMS** (ESI-TOF): m/z calc. for C<sub>13</sub>H<sub>13</sub>NO<sub>2</sub>SCl [M+H]<sup>+</sup> 282.0356, found 282.0346.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.71.

#### 4-Cyano-*N*-methyl-*N*-phenylbenzenesulfonamide (B21)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from *N*-methylaniline (1.19 mL, 11.0 mmol, 1.10 equiv), 4-cyanobenzenesulfonyl chloride (2.02 g, 10.0 mmol, 1.00 equiv), pyridine (2.43 mL, 30.0 mmol, 3.00 equiv) and DCM (40.0 mL). Stirring at rt for 24 h provided the title compound (2.51 g, 9.20 mmol, 92%). This is a known compound.<sup>208</sup>

**Physical state:** Yellow solid.

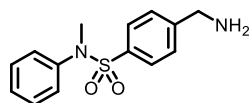
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.12–8.03 (m, 2H), 7.71–7.62 (m, 2H), 7.41–7.27 (m, 3H), 7.18–7.07 (m, 2H), 3.17 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 140.5, 140.0, 133.4, 129.1, 128.1, 127.7, 126.4, 117.6, 115.7, 38.1.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{14}H_{13}N_2O_2S$   $[M+H]^+$  273.0698, found 273.0691.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.62.

#### 4-(Aminomethyl)-*N*-methyl-*N*-phenylbenzenesulfonamide (**B22**)



**Synthetic procedure:** A previously reported nitrile reduction was followed with minor modifications.<sup>167</sup> A solution of **B21** (0.272 g, 1.00 mmol, 1.00 equiv) in conc. HCl (0.048 mL, 0.40 mmol, 0.40 equiv) and MeOH (10.0 mL) was degassed with  $N_2$  for 10 min. 10% Pd/C (0.027 g, 10 wt%) was then added against a positive pressure of  $N_2$ , and the mixture further degassed with  $N_2$  for 5 minutes. The mixture was then degassed with  $H_2$  for 5 minutes and stirred under  $H_2$  (1 atm) at rt for 32 h. Upon reaction completion as determined by TLC, the mixture was degassed with  $N_2$  for 10 minutes, filtered through a bed of celite and the bed washed with EtOAc (5 x 10 mL). The combined filtrates were extracted with 1 M HCl (3 x 15 mL), and the combined aq. phases basified to pH 12 with 10% NaOH and extracted with EtOAc (3 x 20 mL). The combined organic phases were dried over  $MgSO_4$  and concentrated to dryness *in vacuo*. Purification by SGCC (DCM:MeOH 0–15%) provided the title compound (0.061 g, 0.219 mmol, 22%). This is a known compound (CAS no.: 747358-59-4).

**Physical state:** White solid.

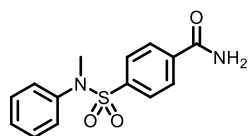
$^1H$  NMR (400 MHz,  $DMSO-d_6$ ):  $\delta$  7.54–7.50 (m, 2H), 7.45–7.40 (m, 2H), 7.37–7.31 (m, 2H), 7.31–7.24 (m, 1H), 7.12–7.07 (m, 2H), 3.79 (s, 2H), 3.12 (s, 3H), 1.95 (s, 2H).

$^{13}C\{^1H\}$  NMR (101 MHz,  $DMSO-d_6$ ):  $\delta$  150.1, 141.2, 133.7, 128.9, 127.5, 127.3, 127.1, 126.1, 45.1, 37.8.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{16}H_{20}N_3O_2S$   $[M+H]^+$  318.1276, found 318.1286.

$R_f$  (silica gel, DCM:MeOH 9:1): 0.20.

#### 4-(*N*-Methyl-*N*-phenylsulfamoyl)benzamide (**B23**)



**Synthetic procedure:** A previously reported acid-catalysed nitrile hydration protocol was followed with minor modifications.<sup>168</sup> To a solution of **B21** (0.272 g, 1.00 mmol, 1.00 equiv) in TFA (1.12 mL) was dropwise added conc.  $H_2SO_4$  (0.28 mL, 5.25 mmol, 5.25 equiv). The mixture was stirred at rt for 27 h. Upon reaction completion as determined by TLC, the mixture was poured into ice-cold water (60 mL) and

extracted with EtOAc (3 x 20 mL). The combined organic phases were dried over MgSO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (hex:EtOAc 0–100%) provided the title compound (0.225 g, 0.775 mmol, 78%). This is a known compound (CAS no.: 915905-61-2).

**Physical state:** Off-white solid.

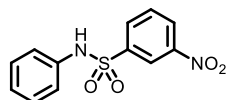
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.20 (s, 1H), 8.04–7.98 (m, 2H), 7.65 (s, 1H), 7.61–7.53 (m, 2H), 7.38–7.32 (m, 2H), 7.32–7.27 (m, 1H), 7.13–7.08 (m, 2H), 3.16 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 166.5, 140.9, 138.3, 138.2, 129.0, 128.3, 127.4, 126.3, 38.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>15</sub>N<sub>2</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 291.0803, found 291.0811.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:2): 0.25.

### 3-Nitro-*N*-phenylbenzenesulfonamide (B24a)



**Synthetic procedure:** The same procedure employed for **B1a** was followed starting from aniline (0.931 g, 10.0 mmol, 1.00 equiv), 3-nitrobenzenesulfonyl chloride (2.22 g, 10.0 mmol, 1.00 equiv), pyridine (0.809 mL, 10.0 mmol, 1.00 equiv) and DCM (10.0 mL). Stirring at reflux for 4.5 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (1.52 g, 5.46 mmol, 55%). This is a known compound.

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**Physical state:** White solid.

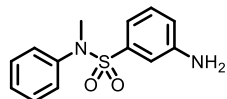
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.56 (s, 1H), 8.48 (t, *J* = 1.9 Hz, 1H), 8.44 (ddd, *J* = 8.2, 2.3, 1.0 Hz, 1H), 8.12 (ddd, *J* = 7.8, 1.8, 1.0 Hz, 1H), 7.85 (t, *J* = 8.0 Hz, 1H), 7.28–7.22 (m, 2H), 7.13–7.03 (m, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 147.9, 140.9, 136.8, 132.6, 131.4, 129.4, 127.6, 124.9, 121.4, 120.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>12</sub>H<sub>9</sub>N<sub>2</sub>O<sub>4</sub>S [M-H]<sup>−</sup> 277.0283, found 277.0290.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.30.

### 3-Amino-*N*-methyl-*N*-phenylbenzenesulfonamide (B24)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B25** (0.877 g, 3.00 mmol, 1.00 equiv), 10% Pd/C stabilised with 50% water (0.088 g, 5 wt%) and MeOH (15.0 mL). Stirring at rt for 80 min provided the title compound (0.786 g, 3.00 mmol, quant.). This is a known compound.<sup>210</sup>

**Physical state:** Light yellow solid.

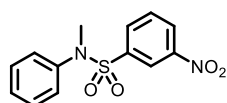
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.37–7.31 (m, 2H), 7.29–7.23 (m, 1H), 7.15 (t, *J* = 7.8 Hz, 1H), 7.13–7.09 (m, 2H), 6.78 (ddd, *J* = 8.1, 2.3, 1.0 Hz, 1H), 6.75 (t, *J* = 2.0 Hz, 1H), 6.55 (ddd, *J* = 7.7, 1.9, 1.0 Hz, 1H), 5.59 (s, 2H), 3.12 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.4, 141.4, 136.6, 129.4, 128.8, 127.0, 126.1, 117.9, 114.0, 111.7, 37.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>15</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 263.0854, found 263.0843.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.37.

### ***N*-Methyl-3-nitro-*N*-phenylbenzenesulfonamide (B25)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B24a** (1.11 g, 4.00 mmol, 1.00 equiv), MeI (0.374 mL, 6.00 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (3.32 g, 24.0 mmol, 6.00 equiv) and anhydrous DMF (16.0 mL). Stirring at rt for 17 h provided the title compound (1.03 g, 3.53 mmol, 88%). This is a known compound.<sup>210</sup>

**Physical state:** White solid.

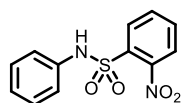
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.59–8.50 (m, 1H), 8.15–8.09 (m, 1H), 7.93–7.84 (m, 2H), 7.41–7.29 (m, 3H), 7.19–7.09 (m, 2H), 3.19 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 147.8, 140.5, 137.3, 133.3, 131.4, 129.2, 127.9, 127.8, 126.6, 122.0, 38.16.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>13</sub>N<sub>2</sub>O<sub>4</sub>S [M+H]<sup>+</sup> 293.0596, found 293.0609.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.38.

### **2-Nitro-*N*-phenylbenzenesulfonamide (B26a)**



**Synthetic procedure:** The same procedure employed for **B1a** was followed starting from aniline (0.93 g, 10.0 mmol, 1.00 equiv), 2-nitrobenzenesulfonyl chloride (2.22 g, 10.0 mmol, 1.00 equiv), pyridine (0.809 mL, 10.0 mmol, 1.00 equiv) and DCM (10.0 mL). Stirring at reflux for 5.5 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (1.17 g, 4.20 mmol, 42%). This is a known compound.

211

**Physical state:** Beige solid.

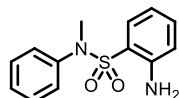
**<sup>1</sup>H NMR** (400 MHz, MeOD):  $\delta$  7.82 (d,  $J$  = 7.9 Hz, 1H), 7.78 (d,  $J$  = 8.0 Hz, 1H), 7.69 (td,  $J$  = 7.9, 1.0 Hz, 1H), 7.59 (t,  $J$  = 7.7 Hz, 1H), 7.20 (dd,  $J$  = 8.6, 7.0 Hz, 2H), 7.17–7.13 (m, 2H), 7.06 (t,  $J$  = 7.2 Hz, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, MeOD):  $\delta$  148.9, 136.9, 134.4, 132.4, 132.2, 131.4, 129.4, 125.7, 125.0, 122.4.

**HRMS** (ESI-TOF):  $m/z$  calc. for C<sub>12</sub>H<sub>9</sub>N<sub>2</sub>O<sub>4</sub>S [M-H]<sup>-</sup> 277.0283, found 277.0290.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.29.

### 2-Amino-*N*-methyl-*N*-phenylbenzenesulfonamide (B26)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B27** (0.877 g, 3.00 mmol, 1.00 equiv), 10% Pd/C stabilised with 50% water (0.088 g, 5 wt%) and MeOH (15.0 mL). Stirring at rt for 24 h provided the title compound (0.784 g, 2.99 mmol, quant.). This is a known compound.<sup>212</sup>

**Physical state:** Colourless oil.

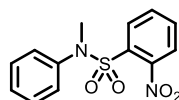
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.37 (dd,  $J$  = 8.3, 1.6 Hz, 1H), 7.31–7.21 (m, 4H), 7.20–7.17 (m, 2H), 6.68–6.62 (m, 2H), 4.64 (s, 2H), 3.21 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>):  $\delta$  146.2, 141.7, 134.4, 130.7, 129.0, 127.6, 127.2, 118.4, 117.6, 117.0, 38.4.

**HRMS** (ESI-TOF):  $m/z$  calc. for C<sub>13</sub>H<sub>15</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 263.0854, found 263.0846.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.56.

### *N*-Methyl-2-nitro-*N*-phenylbenzenesulfonamide (B27)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B26a** (1.11 g, 4.00 mmol, 1.00 equiv), MeI (0.374 mL, 6.00 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (3.32 g, 24.0 mmol, 6.00 equiv) and anhydrous DMF (16.0 mL). Stirring at rt for 18 h provided the title compound (1.06 g, 3.61 mmol, 90%). This is a known compound.<sup>213</sup>

**Physical state:** Beige solid.

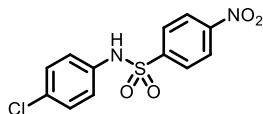
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.66 (ddd,  $J$  = 7.9, 5.7, 3.1 Hz, 1H), 7.59 (dt,  $J$  = 7.9, 0.7 Hz, 1H), 7.55–7.45 (m, 2H), 7.38–7.28 (m, 3H), 7.25–7.20 (m, 2H), 3.39 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>):  $\delta$  148.5, 140.5, 133.8, 131.8, 131.2, 131.1, 129.5, 128.2, 127.7, 123.9, 39.6.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{13}H_{13}N_2O_4S$   $[M+H]^+$  293.0596, found 293.0604.

$R_f$  (silica gel, *n*-hexane:EtOAc 2:1): 0.29.

#### ***N*-(4-Chlorophenyl)-4-nitrobenzenesulfonamide (B28a)**



**Synthetic procedure:** The same procedure employed for **B1a** was followed starting from 4-chloroaniline (1.28 g, 10.0 mmol, 1.00 equiv), 4-nitrobenzenesulfonyl chloride (2.22 g, 10.0 mmol, 1.00 equiv), pyridine (0.809 mL, 10.0 mmol, 1.00 equiv) and DCM (10.0 mL). Stirring at reflux for 3 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.736 g, 2.35 mmol, 24%). This is a known compound. Experimental data correlate with literature.<sup>214</sup>

**Physical state:** Beige solid.

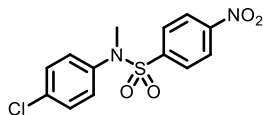
**$^1H$  NMR** (400 MHz,  $CDCl_3$ ):  $\delta$  8.34–8.28 (m, 2H), 7.95–7.89 (m, 2H), 7.29–7.23 (m, 2H), 7.06–6.99 (m, 2H), 6.63 (s, 1H).

**$^{13}C\{^1H\}$  NMR** (101 MHz,  $CDCl_3$ ):  $\delta$  145.9, 144.5, 142.0, 133.9, 130.0, 128.7, 124.6, 124.1.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{12}H_8N_2O_4S$   $[M-H]^-$  310.9893, found 310.9902.

$R_f$  (silica gel, *n*-hexane:EtOAc 2:1): 0.36.

#### ***N*-(4-Chlorophenyl)-*N*-methyl-4-nitrobenzenesulfonamide (B28b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B28a** (0.313 g, 1.00 mmol, 1.00 equiv), MeI (0.093 mL, 1.50 mmol, 1.50 equiv),  $K_2CO_3$  (0.829 g, 6.00 mmol, 6.00 equiv) and anhydrous DMF (4.0 mL). Stirring at rt for 13 h provided the title compound (0.286 g, 0.877 mmol, 88%). This is a known compound.<sup>188</sup>

**Physical state:** Beige solid.

**$^1H$  NMR** (400 MHz,  $CDCl_3$ ):  $\delta$  8.36–8.27 (m, 2H), 7.76–7.69 (m, 2H), 7.35–7.28 (m, 2H), 7.08–6.98 (m, 2H), 3.21 (s, 3H).

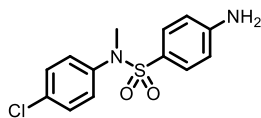
**$^{13}C\{^1H\}$  NMR** (101 MHz,  $CDCl_3$ ):  $\delta$  150.4, 142.1, 139.2, 134.0, 129.6, 129.1, 127.9, 124.3, 38.4.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{13}H_{12}N_2O_4S$   $[M+H]^+$  327.0206, found 327.0212.

$R_f$  (silica gel, *n*-hexane:EtOAc 2:1): 0.44.



#### 4-Amino-*N*-(4-chlorophenyl)-*N*-methylbenzenesulfonamide (B28)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B28b** (0.163 g, 0.500 mmol, 1.00 equiv), 10% Pd/C stabilised with 50% water (0.016 g, 5 wt%) and MeOH (5.0 mL). Stirring at rt for 24 h and purification by SGCC (*n*-hexane:EtOAc 0–80%) provided the title compound (0.098 g, 0.331 mmol, 66%). This is a known compound. Experimental data correlate with literature.<sup>188</sup>

**Physical state:** Off-white solid.

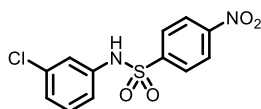
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.42–7.35 (m, 2H), 7.15–7.08 (m, 4H), 6.59–6.51 (m, 2H), 6.10 (s, 2H), 3.02 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 153.4, 140.8, 129.5, 128.6, 127.6, 112.6, 112.5, 112.5, 37.4.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>SCl [M+H]<sup>+</sup> 297.0465, found 297.0463.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.30.

#### *N*-(3-Chlorophenyl)-4-nitrobenzenesulfonamide (B29a)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 3-chloroaniline (0.395 mL, 5.50 mmol, 1.10 equiv), 4-nitrobenzenesulfonyl chloride (1.11 g, 5.00 mmol, 1.00 equiv), pyridine (1.21 mL, 15.0 mmol, 3.00 equiv) and DCM (20.0 mL). Stirring at rt for 18 h provided the title compound (1.27 g, 4.05 mmol, 81%). This is a known compound. Experimental data correlate with literature.

<sup>188</sup>

**Physical state:** Beige solid.

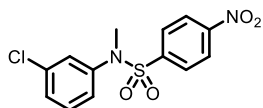
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.92 (s, 1H), 8.43–8.34 (m, 2H), 8.07–7.98 (m, 2H), 7.33–7.25 (m, 1H), 7.16–7.11 (m, 2H), 7.08 (ddd, *J* = 8.1, 2.0, 1.1 Hz, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.0, 144.4, 138.5, 133.6, 131.2, 128.3, 124.9, 124.5, 119.7, 118.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>12</sub>H<sub>8</sub>N<sub>2</sub>O<sub>4</sub>SCl [M-H]<sup>−</sup> 310.9893, found 310.9904.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.38.

#### ***N*-(3-Chlorophenyl)-*N*-methyl-4-nitrobenzenesulfonamide (B29b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B29a** (0.938 g, 3.00 mmol, 1.00 equiv), MeI (0.280 mL, 4.50 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (2.49 g, 18.0 mmol, 6.00 equiv) and anhydrous DMF (12.0 mL). Stirring at rt for 22 h provided the title compound (0.928 g, 2.84 mmol, 95%).

This is a known compound.<sup>215</sup>

**Physical state:** Beige solid.

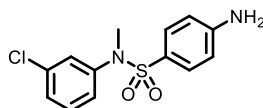
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.44–8.37 (m, 2H), 7.85–7.76 (m, 2H), 7.45–7.35 (m, 2H), 7.31–7.27 (m, 1H), 7.12 (dt, *J* = 6.5, 2.2 Hz, 1H), 3.20 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.2, 141.8, 141.1, 133.2, 130.7, 129.1, 127.7, 126.5, 125.0, 124.7, 37.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>SCl [M+H]<sup>+</sup> 327.0206, found 327.0206.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.92.

#### ***4*-Amino-*N*-(3-chlorophenyl)-*N*-methylbenzenesulfonamide (B29)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B29b** (0.327 g, 1.00 mmol, 1.00 equiv), 10% Pd/C (0.033 g, 10 wt%) and MeOH (5.0 mL). Stirring at rt for 17 h provided the title compound (0.315 g, 1.00 mmol, quant.). This is a known compound (CAS no.: 1226136-25-9).

**Physical state:** Off-white solid.

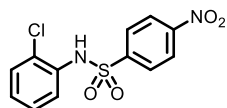
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.38–7.29 (m, 2H), 7.18–7.16 (m, 1H), 7.14–7.07 (m, 3H), 6.60–6.52 (m, 2H), 6.12 (s, 2H), 3.04 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 153.43, 143.28, 132.72, 130.19, 129.47, 126.51, 125.67, 124.30, 120.05, 112.54, 37.28.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>SCl [M+H]<sup>+</sup> 297.0465, found 297.0470.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.31.

### ***N*-(2-Chlorophenyl)-4-nitrobenzenesulfonamide (B30a)**



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 2-chloroaniline (0.579 mL, 5.50 mmol, 1.10 equiv), 4-nitrobenzenesulfonyl chloride (1.11 g, 5.00 mmol, 1.00 equiv), pyridine (1.21 mL, 15.0 mmol, 3.00 equiv) and DCM (20.0 mL). Stirring at rt for 46 h provided the title compound (1.34 g, 4.28 mmol, 86%). This is a known compound. Experimental data correlate with literature.

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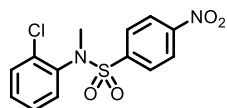
**Physical state:** Orange solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.47 (s, 1H), 8.42–8.36 (m, 2H), 7.98–7.92 (m, 2H), 7.45–7.41 (m, 1H), 7.34–7.29 (m, 1H), 7.29–7.23 (m, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.8, 145.9, 132.8, 130.1, 129.8, 128.5, 128.4, 128.3, 128.0, 124.6, 116.8, 115.4.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.40.

### ***N*-(2-Chlorophenyl)-*N*-methyl-4-nitrobenzenesulfonamide (B30b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B30a** (1.30 g, 4.16 mmol, 1.00 equiv), MeI (0.388 mL, 6.23 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (3.45 g, 24.9 mmol, 6.00 equiv) and anhydrous DMF (16.0 mL). Stirring at rt for 6 h provided the title compound (1.42 g, 4.16 mmol, quant.). This is a known compound.<sup>215</sup>

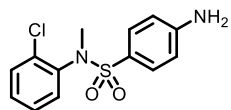
**Physical state:** Orange solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.48–8.42 (m, 2H), 8.02–7.97 (m, 2H), 7.59 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.46–7.39 (m, 1H), 7.35 (td, *J* = 7.7, 1.6 Hz, 1H), 7.12 (dd, *J* = 7.9, 1.7 Hz, 1H), 3.19 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 162.30, 150.06, 143.52, 137.50, 133.51, 130.63, 130.46, 128.99, 128.95, 128.35, 124.78, 38.34.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.65.

#### 4-Amino-*N*-(2-chlorophenyl)-*N*-methylbenzenesulfonamide (B30)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B30b** (1.42 g, 4.16 mmol, 1.00 equiv), 10% Pd/C (0.142 g, 10 wt%) and MeOH (20.0 mL). Stirring at rt for 22 h and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.862 g, 2.90 mmol, 70%). This is a known compound (CAS no.: 1226356-42-8).

**Physical state:** Beige solid.

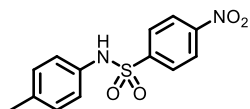
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.58–7.51 (m, 1H), 7.40–7.27 (m, 4H), 7.06–6.99 (m, 1H), 6.67–6.59 (m, 2H), 6.13 (s, 2H), 3.00 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 153.3, 139.0, 133.8, 130.4, 129.6, 129.6, 127.9, 122.2, 112.7, 38.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>SCl [M+H]<sup>+</sup> 297.0465, found 297.0470.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.35.

#### 4-Nitro-*N*-(*p*-tolyl)benzenesulfonamide (B31a)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 4-methylaniline (0.589, 5.50 mmol, 1.10 equiv), 4-nitrobenzenesulfonyl chloride (1.11 g, 5.00 mmol, 1.00 equiv), pyridine (1.21 mL, 15.0 mmol, 3.00 equiv) and DCM (20.0 mL). Stirring at rt for 18 h provided the title compound (1.25 g, 4.27 mmol, 85%). This is a known compound. Experimental data correlate with literature.<sup>216</sup>

**Physical state:** Orange solid.

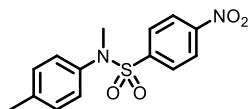
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.44 (s, 1H), 8.41–8.31 (m, 2H), 7.99–7.89 (m, 2H), 7.09–7.03 (m, 2H), 7.02–6.91 (m, 2H), 2.18 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.8, 144.9, 134.2, 129.8, 128.3, 124.6, 121.3, 20.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>13</sub>NO<sub>2</sub>SCl [M-H]<sup>−</sup> 291.0440, found 291.0443.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.66.

#### *N*-Methyl-4-nitro-*N*-(*p*-tolyl)benzenesulfonamide (B31b)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B31a** (0.877 g, 3.00 mmol, 1.00 equiv), MeI (0.280 mL, 4.50 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (2.49 g, 18.0 mmol, 6.00 equiv) and anhydrous DMF (12.0 mL). Stirring at rt for 23 h provided the title compound (0.831 g, 2.71 mmol, 90%). This is a known compound. Experimental data correlate with literature.<sup>188</sup>

**Physical state:** Orange solid.

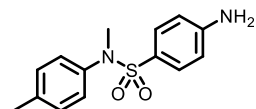
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.43–8.36 (m, 2H), 7.82–7.72 (m, 2H), 7.19–7.12 (m, 2H), 7.02–6.96 (m, 2H), 3.16 (s, 3H), 2.29 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.0, 141.5, 137.9, 137.3, 129.6, 129.1, 126.4, 124.5, 38.3, 20.6.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>15</sub>N<sub>2</sub>O<sub>4</sub>S [M+H]<sup>+</sup> 307.0753, found 307.0739.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.65.

#### 4-Amino-*N*-methyl-*N*-(*p*-tolyl)benzenesulfonamide (**B31**)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B31b** (0.306 g, 1.00 mmol, 1.00 equiv), 10% Pd/C (0.031 g, 10 wt%) and MeOH (5.0 mL). Stirring at rt for 24 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.167 g, 0.60 mmol, 60%). This is a known compound. Experimental data correlate with literature.<sup>188</sup>

**Physical state:** White solid.

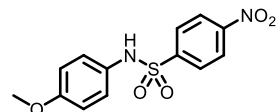
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.15–7.05 (m, 4H), 6.99–6.91 (m, 2H), 6.58–6.49 (m, 2H), 6.05 (s, 2H), 3.00 (s, 3H), 2.27 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 153.1, 139.3, 136.1, 129.5, 129.1, 126.0, 120.6, 112.5, 37.7, 20.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>17</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 277.1011, found 277.1021.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.27.

#### *N*-(4-Methoxyphenyl)-4-nitrobenzenesulfonamide (**B32a**)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 4-methoxyaniline (0.677 g, 5.50 mmol, 1.10 equiv), 4-nitrobenzenesulfonyl chloride (1.11 g, 5.00 mmol, 1.00 equiv), pyridine (1.21 mL, 15.0 mmol, 3.00 equiv) and DCM (20.0 mL). Stirring at rt for 22 h provided the title

compound (0.839 g, 2.72 mmol, 54%). This is a known compound. Experimental data correlate with literature.<sup>217</sup>

**Physical state:** Light pink solid.

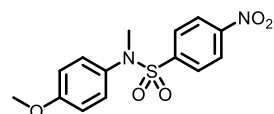
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.24 (s, 1H), 8.44–8.31 (m, 2H), 7.97–7.84 (m, 2H), 7.02–6.92 (m, 2H), 6.88–6.77 (m, 2H), 3.67 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 157.0, 149.7, 144.9, 129.2, 128.3, 124.6, 124.1, 114.5, 55.2.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>11</sub>N<sub>2</sub>O<sub>5</sub>S [M-H]<sup>-</sup> 307.0389, found 307.0396.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.23.

#### ***N*-(4-Methoxyphenyl)-*N*-methyl-4-nitrobenzenesulfonamide (B32b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B32a** (0.617 g, 2.00 mmol, 1.00 equiv), MeI (0.187 mL, 3.00 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (1.66 g, 12.0 mmol, 6.00 equiv) and anhydrous DMF (8.0 mL). Stirring at rt for 16 h provided the title compound (0.434 g, 1.35 mmol, 67%). This is a known compound.<sup>188</sup>

**Physical state:** Yellow solid.

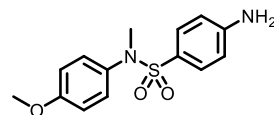
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.44–8.36 (m, 2H), 7.81–7.73 (m, 2H), 7.05–6.98 (m, 2H), 6.92–6.86 (m, 2H), 3.74 (s, 3H), 3.15 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 158.5, 150.0, 141.5, 133.0, 129.1, 128.1, 124.5, 114.3, 55.3, 38.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>15</sub>N<sub>2</sub>O<sub>5</sub>S [M+H]<sup>+</sup> 323.0702, found 323.0694.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.45.

#### **4-Amino-*N*-(4-methoxyphenyl)-*N*-methylbenzenesulfonamide (B32)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B32b** (0.322 g, 1.00 mmol, 1.00 equiv), 10% Pd/C (0.032 g, 10 wt%) and MeOH (5.0 mL). Stirring at rt for 23 h provided the title compound (0.295 g, 1.00 mmol, quant.). This is a known compound.<sup>188</sup>

**Physical state:** Off-white solid.

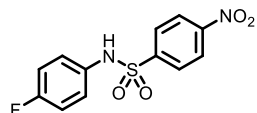
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.13–7.06 (m, 2H), 6.99–6.93 (m, 2H), 6.89–6.82 (m, 2H), 6.59–6.52 (m, 2H), 6.05 (s, 2H), 3.73 (s, 3H), 2.99 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 158.3, 153.6, 135.0, 130.0, 128.1, 121.0, 114.3, 112.9, 55.7, 38.4.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>17</sub>N<sub>2</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 293.0960, found 293.0974.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.33.

#### ***N*-(4-Fluorophenyl)-4-nitrobenzenesulfonamide (B33a)**



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 4-fluoroaniline (0.521 mL, 5.50 mmol, 1.10 equiv), 4-nitrobenzenesulfonyl chloride (1.11 g, 5.00 mmol, 1.00 equiv), pyridine (1.21 mL, 15.0 mmol, 3.00 equiv) and DCM (20.0 mL). Stirring at rt for 22 h provided the title compound (0.884 g, 2.99 mmol, 60%). This is a known compound. Experimental data correlate with literature.

188

**Physical state:** Orange solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.56 (s, 1H), 8.42–8.29 (m, 2H), 7.99–7.87 (m, 2H), 7.18–7.02 (m, 4H).

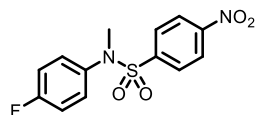
**<sup>19</sup>F NMR** (377 MHz, DMSO-*d*<sub>6</sub>): δ -117.43.

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 160.7, 158.3, 149.9, 144.6, 133.0, 128.3, 124.7, 123.6, 123.6, 116.2, 116.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>12</sub>H<sub>8</sub>N<sub>2</sub>O<sub>4</sub>SF [M-H]<sup>-</sup> 295.0189, found 295.0177.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.59.

#### ***N*-(4-Fluorophenyl)-*N*-methyl-4-nitrobenzenesulfonamide (B33b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B33a** (0.593 g, 2.00 mmol, 1.00 equiv), MeI (0.187 mL, 3.00 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (1.66 g, 12.0 mmol, 6.00 equiv) and anhydrous DMF (8.0 mL). Stirring at rt for 30 h provided the title compound (0.626 g, 2.00 mmol, quant.). This is a known compound. Experimental data correlate with literature.<sup>188</sup>

**Physical state:** Beige solid.

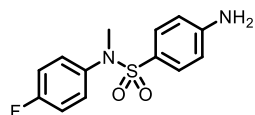
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.46–8.36 (m, 2H), 7.82–7.68 (m, 2H), 7.25–7.10 (m, 4H), 3.17 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  162.3, 159.8, 150.1, 141.1, 136.7, 136.7, 129.6, 129.1, 129.1, 128.9, 128.9, 126.4, 124.6, 116.1, 115.9, 38.3.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}_4\text{SF}$   $[\text{M}+\text{H}]^+$  311.0502, found 311.0510.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 1:1): 0.53.

#### 4-Amino-*N*-(4-fluorophenyl)-*N*-methylbenzenesulfonamide (**B33**)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B33b** (0.310 g, 1.00 mmol, 1.00 equiv), 10% Pd/C (0.031 g, 10 wt%) and MeOH (5.0 mL). Stirring at rt for 24 h provided the title compound (0.290 g, 1.00 mmol, quant.). This is a known compound. Experimental data correlate with literature.<sup>188</sup>

**Physical state:** White solid.

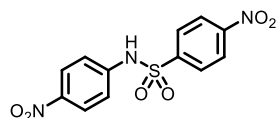
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  7.19–7.07 (m, 6H), 6.59–6.52 (m, 2H), 6.09 (s, 2H), 3.01 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  161.7, 159.3, 153.3, 138.1, 138.1, 129.5, 128.3, 128.2, 120.1, 115.5, 115.3, 112.5, 37.7.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}_2\text{SF}$   $[\text{M}+\text{H}]^+$  281.0760, found 281.0770.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 1:1): 0.25.

#### 4-Nitro-*N*-(4-nitrophenyl)benzenesulfonamide (**B34a**)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 4-nitroaniline (0.760 mL, 5.50 mmol, 1.10 equiv), 4-nitrobenzenesulfonyl chloride (1.11 g, 5.00 mmol, 1.00 equiv), pyridine (1.21 mL, 15.0 mmol, 3.00 equiv) and DCM (20.0 mL). Stirring at rt for 27 h provided the title compound (1.26 g, 3.88 mmol, 78%). This is a known compound. Experimental data correlate with literature.

205

**Physical state:** Orange solid.

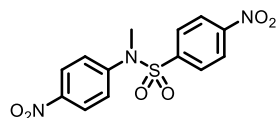
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  11.58 (s, 1H), 8.43–8.37 (m, 2H), 8.19–8.14 (m, 2H), 8.14–8.08 (m, 2H), 7.38–7.28 (m, 2H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  150.2, 144.2, 143.4, 143.1, 128.4, 125.5, 125.0, 118.6.



$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.43.

#### ***N*-Methyl-4-nitro-*N*-(4-nitrophenyl)benzenesulfonamide (B34b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B34a** (1.25 g, 3.87 mmol, 1.00 equiv), MeI (0.361 mL, 5.80 mmol, 1.50 equiv),  $K_2CO_3$  (3.21 g, 23.2 mmol, 6.00 equiv) and anhydrous DMF (10.0 mL). Stirring at rt for 23 h provided the title compound (0.963 g, 2.85 mmol, 74%). This is a known compound.<sup>218</sup>

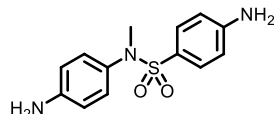
**Physical state:** Yellow solid.

**$^1H$  NMR** (400 MHz,  $DMSO-d_6$ ):  $\delta$  8.42–8.35 (m, 2H), 8.27–8.19 (m, 2H), 7.86–7.78 (m, 2H), 7.52–7.45 (m, 2H), 3.27 (s, 3H).

**$^{13}C\{^1H\}$  NMR** (101 MHz,  $DMSO-d_6$ ):  $\delta$  150.3, 146.2, 145.6, 140.8, 129.1, 126.4, 124.8, 124.5, 37.7.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.50.

#### **4-Amino-*N*-(4-aminophenyl)-*N*-methylbenzenesulfonamide (B34)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B34b** (0.337 g, 1.00 mmol, 1.00 equiv), 10% Pd/C (0.034 g, 10 wt%) and MeOH (5.0 mL). Stirring at rt for 15 h and purification by SGCC (hex:EtOAc 0–20% then 20–100%) provided the title compound (0.133 g, 0.481 mmol, 48%). This is a known compound. (CAS no.: 57771-76-3)

**Physical state:** White solid.

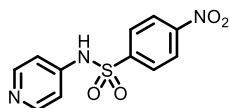
**$^1H$  NMR** (400 MHz,  $DMSO-d_6$ ):  $\delta$  7.15–7.05 (m, 2H), 6.69–6.61 (m, 2H), 6.60–6.52 (m, 2H), 6.46–6.39 (m, 2H), 6.01 (s, 2H), 5.14 (s, 2H), 2.93 (s, 3H).

**$^{13}C\{^1H\}$  NMR** (101 MHz,  $DMSO-d_6$ ):  $\delta$  152.9, 147.8, 129.9, 129.5, 127.4, 121.1, 113.4, 112.4, 38.2.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{13}H_{15}N_3O_2S$   $[M+H]^+$  278.0963, found 278.0972.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:2): 0.23.

#### 4-Nitro-*N*-(pyridin-4-yl)benzenesulfonamide (B35a)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 4-amino-pyridine (0.471 g, 5.00 mmol, 1.10 equiv), 4-nitrobenzenesulfonyl chloride (1.11 g, 5.00 mmol, 1.00 equiv), pyridine (1.21 mL, 15.0 mmol, 3.00 equiv) and DCM (20.0 mL). Stirring at rt for 21 h and purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.163 g, 0.583 mmol, 12%). This is a known compound. Experimental data correlate with literature.<sup>219</sup>

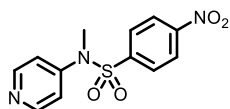
**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.32 (dq, *J* = 8.7, 2.2 Hz, 2H), 8.10–8.01 (m, 4H), 7.05–6.97 (m, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 148.9, 139.5, 127.6, 126.9, 124.3, 123.4, 114.9.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.22.

#### *N*-Methyl-4-nitro-*N*-(pyridin-4-yl)benzenesulfonamide (B35b)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B35a** (0.160 g, 0.573 mmol, 1.00 equiv), MeI (0.054 mL, 0.859 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.475 g, 3.44 mmol, 6.00 equiv) and anhydrous DMF (2.0 mL). Stirring at rt for 17 h provided the title compound (0.053 g, 0.179 mmol, 31%). This is a known compound.<sup>220</sup>

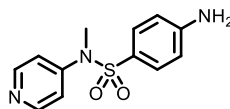
**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.32–8.25 (m, 2H), 8.21–8.15 (m, 2H), 7.60 (d, *J* = 7.1 Hz, 2H), 7.31 (d, *J* = 7.0 Hz, 2H), 3.90 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 31.0. Most carbon signals were missing due to dilute sample.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.33.

#### 4-Amino-*N*-methyl-*N*-(pyridin-4-yl)benzenesulfonamide (B35)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B35b** (0.053 g, 0.181 mmol, 1.00 equiv), 10% Pd/C (0.005 g, 10 wt%) and MeOH (2.0 mL). Stirring at rt for 20 h provided the title compound (0.019 g, 0.074 mmol, 41%). This is a known compound.<sup>220</sup>

**Physical state:** Grey solid.

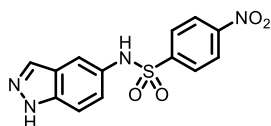
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.24 (d, *J* = 6.8 Hz, 2H), 7.56–7.49 (m, 2H), 7.18 (d, *J* = 6.8 Hz, 2H), 6.61–6.55 (m, 2H), 3.91 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 153.0, 144.0, 128.7, 125.2, 113.4, 112.7, 44.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>12</sub>H<sub>14</sub>N<sub>3</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 264.0807, found 264.0798.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.09.

#### ***N*-(1*H*-Indazol-5-yl)-4-nitrobenzenesulfonamide (**B36a**)**



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 1*H*-indazol-5-amine (0.666 g, 5.00 mmol, 1.00 equiv), 4-nitrobenzenesulfonyl chloride (1.11 g, 5.00 mmol, 1.00 equiv), pyridine (1.21 mL, 15.0 mmol, 3.00 equiv) and DCM (20.0 mL). Stirring at rt for 21 h and purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.535 g, 1.68 mmol, 34%). This is a known compound. Experimental data correlate with literature.<sup>219</sup>

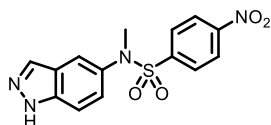
**Physical state:** Brown solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.07 (s, 1H), 10.54–10.31 (m, 1H), 8.37–8.32 (m, 2H), 8.00 (dd, *J* = 1.6, 1.0 Hz, 1H), 7.94–7.88 (m, 2H), 7.46–7.41 (m, 2H), 7.06 (dd, *J* = 9.0, 1.9 Hz, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.7, 144.8, 137.9, 133.6, 129.3, 128.32, 128.27, 124.5, 122.5, 113.8, 110.9.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.38.

#### ***N*-(1*H*-Indazol-5-yl)-*N*-methyl-4-nitrobenzenesulfonamide (**B36b**)**



**Synthetic procedure:** A previously reported chemoselective sulfonamide *N*-methylation protocol was followed with minor modifications.<sup>103</sup> To a solution of **B36a** (0.159 g, 0.500 mmol, 1.00 equiv) in anhydrous DMF (2.0 mL) was added DMF-DMA (0.332 mL, 2.50 mmol, 5.00 equiv). The mixture was stirred at 60 °C

for 18 h. Upon reaction completion as determined by TLC, the mixture was concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.087 g, 0.261 mmol, 52%). This is a novel compound.

**Physical state:** Off-white solid.

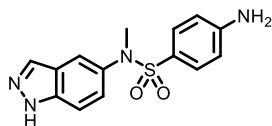
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.21 (s, 1H), 8.42–8.35 (m, 2H), 8.06 (t, *J* = 1.3 Hz, 1H), 7.81–7.74 (m, 2H), 7.53–7.47 (m, 2H), 7.06 (dd, *J* = 8.9, 2.0 Hz, 1H), 3.24 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.0, 141.6, 138.8, 134.0, 133.1, 129.2, 125.4, 124.5, 122.7, 119.1, 110.6, 38.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>13</sub>N<sub>4</sub>O<sub>4</sub>S [M+H]<sup>+</sup> 333.0658, found 333.0659.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.22.

#### 4-Amino-*N*-(1*H*-indazol-5-yl)-*N*-methylbenzenesulfonamide (B36)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B36b** (0.071 g, 0.214 mmol, 1.00 equiv), 10% Pd/C (0.007 g, 10 wt%) and MeOH (5.0 mL). Stirring at rt for 16 h and purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.046 g, 0.153 mmol, 72%). This is a novel compound.

**Physical state:** White solid.

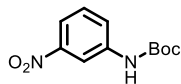
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.13 (s, 1H), 8.04 (s, 1H), 7.46 (dt, *J* = 8.9, 0.9 Hz, 1H), 7.39 (dd, *J* = 2.0, 0.7 Hz, 1H), 7.12–7.05 (m, 3H), 6.57–6.50 (m, 2H), 6.05 (s, 2H), 3.08 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 153.1, 138.5, 134.7, 133.9, 129.6, 125.7, 122.6, 120.6, 118.1, 112.5, 110.0, 38.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>15</sub>N<sub>4</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 303.0916, found 303.0909.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:2): 0.20.

#### *tert*-Butyl (3-nitrophenyl)carbamate (B37a)



**Synthetic procedure:** A previously reported *N*-Boc-protection protocol was followed with minor modifications.<sup>169</sup> To a solution of 3-nitroaniline (1.38 g, 10.0 mmol, 1.00 equiv) in abs. EtOH (30.0 mL) was added Boc<sub>2</sub>O (6.55 g, 30.0 mmol, 3.00 equiv). The mixture was stirred at rt for 33 h and then added DIPEA (1.74

mL, 10.0 mmol, 1.00 equiv) and additional  $\text{Boc}_2\text{O}$  (2.18 g, 10.0 mmol, 1.00 equiv). Upon reaction completion as determined by TLC, the mixture was concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (1.56 g, 6.53 mmol, 65%). This is a known compound.

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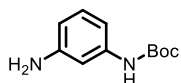
**Physical state:** White solid.

**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  9.92 (s, 1H), 8.48 (t,  $J = 2.2$  Hz, 1H), 7.81 (dd,  $J = 8.1, 2.3$  Hz, 1H), 7.76 (dd,  $J = 8.1, 2.1$  Hz, 1H), 7.54 (t,  $J = 8.2$  Hz, 1H), 1.49 (s, 9H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  152.7, 148.1, 140.9, 130.0, 124.1, 116.6, 111.9, 80.0, 28.0.

**$R_f$**  (silica gel, *n*-hexane:EtOAc 1:1): 0.70.

#### ***tert*-Butyl (3-aminophenyl)carbamate (B37b)**



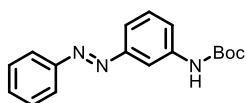
**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B37a** (1.55 g, 6.51 mmol, 1.00 equiv), 10% Pd/C (0.155 g, 10 wt%) and MeOH (30.0 mL). Stirring at rt for 24 h provided the title compound (1.35 g, 6.46 mmol, 99%). This is a known compound.<sup>222</sup>

**Physical state:** Grey solid.

**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  9.00 (s, 1H), 6.82 (dd,  $J = 14.6, 6.4$  Hz, 2H), 6.52 (d,  $J = 8.0$  Hz, 1H), 6.15 (dd,  $J = 8.0, 2.0$  Hz, 1H), 4.97 (s, 2H), 1.45 (s, 9H).

**$R_f$**  (silica gel, *n*-hexane:EtOAc 1:1): 0.43.

#### ***tert*-Butyl (*E*)-(3-(phenyldiazenyl)phenyl)carbamate (B37c)**



**Synthetic procedure:** To a solution of **B37b** (0.208 g, 1.00 mmol, 1.00 equiv) in glacial AcOH (10.0 mL) was added nitrosobenzene (0.107 g, 1.00 mmol, 1.00 equiv). The mixture was stirred at rt for 48 h. Upon reaction completion as determined by TLC, the mixture was concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–20%) provided the title compound (0.114 g, 0.382 mmol, 38%). This is a known compound.<sup>223</sup>

**Physical state:** Orange solid.

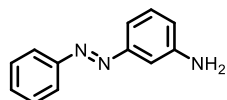
**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  9.66 (s, 1H), 8.12 (t,  $J = 2.0$  Hz, 1H), 7.91–7.86 (m, 2H), 7.63–7.54 (m, 4H), 7.52 (dt,  $J = 7.9, 1.6$  Hz, 1H), 7.47 (t,  $J = 7.8$  Hz, 1H), 1.50 (s, 9H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  152.8, 152.4, 151.9, 140.7, 131.5, 129.6, 129.5, 122.5, 121.0, 117.1, 111.2, 79.4, 28.1.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{17}\text{H}_{20}\text{N}_3\text{O}_2$   $[\text{M}+\text{H}]^+$  298.1556, found 298.1546.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 4:1): 0.48.

### (*E*)-3-(Phenyldiazenyl)aniline (**B37**)



**Synthetic procedure:** To **B37c** (0.089 g, 0.300 mmol, 1.00 equiv) was added 4 M HCl in 1,4-dioxane (0.75 mL, 3.00 mmol, 10.0 equiv). The mixture was stirred at rt for 22 h. Upon reaction completion as determined by TLC, water (10 mL) was added, and the mixture basified to pH 10 with 1 M NaOH. The mixture was extracted with EtOAc (3 x 10 mL), and the combined organic layers were dried over  $\text{Na}_2\text{SO}_4$  and concentrated to dryness *in vacuo*. Purification by SGCC ( $n$ -hexane:EtOAc 0–40%) provided the title compound (0.036 g, 0.180 mmol, 60%). This is a known compound.<sup>223</sup>

**Physical state:** Orange solid.

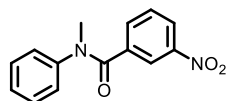
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  7.87–7.80 (m, 2H), 7.62–7.49 (m, 3H), 7.23 (t,  $J$  = 7.8 Hz, 1H), 7.13–7.06 (m, 2H), 6.76 (ddd,  $J$  = 7.9, 2.3, 1.0 Hz, 1H), 5.44 (s, 2H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  153.0, 152.0, 149.7, 131.1, 129.6, 129.4, 122.3, 117.3, 112.5, 105.3.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{12}\text{H}_{12}\text{N}_3$   $[\text{M}+\text{H}]^+$  198.1031, found 198.1034.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 1:1): 0.58.

### *N*-Methyl-3-nitro-*N*-phenylbenzamide (**B38a**)



**Synthetic procedure:** The same procedure employed for **B3a** was followed starting from 3-nitrobenzoyl chloride (0.371 g, 2.00 mmol, 1.00 equiv), *N*-methylaniline (0.238 mL, 2.20 mmol, 1.10 equiv),  $\text{Et}_3\text{N}$  (0.348 mL, 2.50 mmol, 1.25 equiv) and anhydrous DCM (2.0 mL). Stirring at 0 °C–rt for 20 h provided the title compound (0.398 g, 1.55 mmol, 78%). This is a known compound.<sup>224</sup>

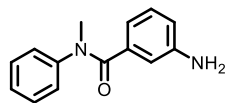
**Physical state:** Beige solid.

**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  8.12 (dd,  $J$  = 8.2, 2.3 Hz, 1H), 8.08 (s, 1H), 7.66 (d,  $J$  = 7.6 Hz, 1H), 7.50 (t,  $J$  = 7.9 Hz, 1H), 7.31–7.23 (m, 4H), 7.21–7.14 (m, 1H), 3.41 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  167.2, 147.0, 143.8, 137.9, 134.5, 129.5, 129.3, 127.3, 127.0, 124.1, 123.0.

**$R_f$**  (silica gel, *n*-hexane:EtOAc 1:1): 0.49.

### 3-Amino-*N*-methyl-*N*-phenylbenzamide (**B38**)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B38a** (0.398 g, 1.55 mmol, 1.00 equiv), 10% Pd/C (0.040 g, 10 wt%) and MeOH (10.0 mL). Stirring at rt for 17 h and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.264 g, 1.17 mmol, 75%). This is a known compound (CAS no.: 14309-78-5).

**Physical state:** White solid.

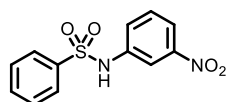
**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  7.26 (t,  $J$  = 7.7 Hz, 2H), 7.17–7.10 (m, 3H), 6.78 (t,  $J$  = 7.8 Hz, 1H), 6.57 (t,  $J$  = 1.9 Hz, 1H), 6.43 (ddd,  $J$  = 8.0, 2.4, 1.0 Hz, 1H), 6.25 (dt,  $J$  = 7.6, 1.3 Hz, 1H), 5.10 (s, 2H), 3.32 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  170.3, 148.3, 144.8, 136.9, 129.0, 128.0, 126.7, 126.1, 115.6, 114.8, 113.6, 37.9.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{14}\text{H}_{15}\text{N}_2\text{O}$   $[\text{M}+\text{H}]^+$  227.1184, found 227.1192.

**$R_f$**  (silica gel, *n*-hexane:EtOAc 1:1): 0.11.

### *N*-(3-Nitrophenyl)benzenesulfonamide (**B39a**)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 3-nitroaniline (0.304 g, 2.20 mmol, 1.10 equiv), benzenesulfonyl chloride (0.255 mL, 2.00 mmol, 1.00 equiv), pyridine (0.485 mL, 6.00 mmol, 3.00 equiv) and DCM (8.0 mL). Stirring at rt for 23 h provided the title compound (0.569 g, 2.00 mmol, quant.). This is a known compound. Experimental data correlate with literature.<sup>225</sup>

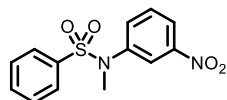
**Physical state:** Off-white solid.

**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  10.95 (s, 1H), 7.94 (t,  $J$  = 2.3 Hz, 1H), 7.87 (dt,  $J$  = 7.1, 2.3 Hz, 1H), 7.81 (dd,  $J$  = 7.2, 1.9 Hz, 2H), 7.67–7.61 (m, 1H), 7.61–7.50 (m, 4H).

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{12}\text{H}_9\text{N}_2\text{O}_4\text{S}$   $[\text{M}-\text{H}]^-$  277.0289, found 277.0289.

**$R_f$**  (silica gel, *n*-hexane:EtOAc 1:1): 0.57.

### ***N*-Methyl-*N*-(3-nitrophenyl)benzenesulfonamide (B39b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B39a** (0.557 g, 2.00 mmol, 1.00 equiv), MeI (0.187 mL, 3.00 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (1.66 g, 12.0 mmol, 6.00 equiv) and anhydrous DMF (6.0 mL). Stirring at rt for 24 h provided the title compound (0.552 g, 1.89 mmol, 94%). This is a known compound (CAS no.: 313670-14-3).

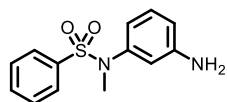
**Physical state:** Light yellow solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.13 (ddd, *J* = 8.2, 2.2, 1.1 Hz, 1H), 7.84 (t, *J* = 2.2 Hz, 1H), 7.68–7.59 (m, 2H), 7.57–7.44 (m, 5H), 3.23 (s, 3H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>13</sub>N<sub>2</sub>O<sub>4</sub>S [M+H]<sup>+</sup> 293.0591, found 293.0591.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.59.

### ***N*-(3-Aminophenyl)-*N*-methylbenzenesulfonamide (B39)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B39b** (0.540 g, 1.85 mmol, 1.00 equiv), 10% Pd/C (0.054 g, 10 wt%) and MeOH (10.0 mL). Stirring at rt for 26 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.441 g, 1.68 mmol, 91%). This is a known compound (CAS no.: 1258322-88-1).

**Physical state:** Off-white solid.

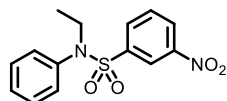
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.73–7.64 (m, 1H), 7.61–7.51 (m, 4H), 6.91 (t, *J* = 8.0 Hz, 1H), 6.44 (ddd, *J* = 8.1, 2.2, 0.9 Hz, 1H), 6.35 (t, *J* = 2.1 Hz, 1H), 6.10 (ddd, *J* = 7.9, 2.2, 0.9 Hz, 1H), 5.19 (s, 2H), 3.05 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.3, 141.8, 136.4, 133.1, 129.1, 129.0, 127.4, 112.8, 112.7, 111.8, 38.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>15</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 263.0849, found 263.0859.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.31.

### ***N*-Ethyl-3-nitro-*N*-phenylbenzenesulfonamide (B40a)**





**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B24a** (0.278 g, 1.00 mmol, 1.00 equiv), iodoethane (0.121 mL, 1.50 mmol, 1.50 equiv),  $K_2CO_3$  (0.829 g, 6.00 mmol, 6.00 equiv) and anhydrous DMF (3.0 mL). Stirring at rt for 18 h provided the title compound (0.282 g, 0.921 mmol, 92%). This is a known compound.<sup>226</sup>

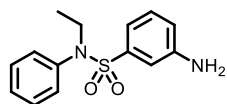
**Physical state:** Beige solid.

**$^1H$  NMR** (400 MHz,  $DMSO-d_6$ ):  $\delta$  8.54 (ddt,  $J$  = 8.2, 2.2, 1.1 Hz, 1H), 8.18 (d,  $J$  = 2.1 Hz, 1H), 7.98 (dq,  $J$  = 7.9, 1.3 Hz, 1H), 7.89 (t,  $J$  = 8.0 Hz, 1H), 7.42–7.35 (m, 3H), 7.14–7.06 (m, 2H), 3.64 (q,  $J$  = 7.1 Hz, 2H), 0.98 (t,  $J$  = 7.1 Hz, 3H).

**$^{13}C\{^1H\}$  NMR** (101 MHz,  $DMSO-d_6$ ):  $\delta$  147.9, 139.1, 137.8, 133.2, 131.4, 129.3, 128.7, 128.4, 127.7, 121.8, 45.4, 13.8.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 2:1): 0.45.

### 3-Amino-*N*-ethyl-*N*-phenylbenzenesulfonamide (**B40**)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B40a** (0.282 g, 0.921 mmol, 1.00 equiv), 10% Pd/C (0.028 g, 10 wt%), MeOH (5.0 mL) and EtOAc (5.0 mL). Stirring at rt for 18 h and purification by SGCC ( $n$ -hexane:EtOAc 0–50%) provided the title compound (0.193 g, 0.699 mmol, 76%). This is a known compound (CAS no.: 847171-53-3).

**Physical state:** White solid.

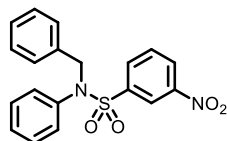
**$^1H$  NMR** (400 MHz,  $DMSO-d_6$ ):  $\delta$  7.40–7.26 (m, 3H), 7.17 (t,  $J$  = 7.8 Hz, 1H), 7.11–7.01 (m, 2H), 6.83–6.72 (m, 2H), 6.63 (dt,  $J$  = 7.8, 1.2 Hz, 1H), 5.58 (s, 2H), 3.56 (q,  $J$  = 7.1 Hz, 2H), 0.95 (t,  $J$  = 7.1 Hz, 3H).

**$^{13}C\{^1H\}$  NMR** (101 MHz,  $DMSO-d_6$ ):  $\delta$  149.4, 138.7, 138.4, 129.5, 128.9, 128.5, 127.7, 117.7, 113.8, 111.6, 45.0, 13.9.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{14}H_{17}N_2O_2S$   $[M+H]^+$  277.1011, found 277.1012.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 1:1): 0.41.

### *N*-Benzyl-3-nitro-*N*-phenylbenzenesulfonamide (**B41a**)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B24a** (0.278 g, 1.00 mmol, 1.00 equiv), BnBr (0.178 mL, 1.50 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.829 g, 6.00 mmol, 6.00 equiv) and anhydrous DMF (3.0 mL). Stirring at rt for 30 h provided the title compound (0.348 g, 0.944 mmol, 94%). This is a known compound (CAS no.: 324065-80-7).

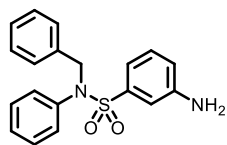
**Physical state:** Orange solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.54 (t, *J* = 2.0 Hz, 1H), 8.47 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.95 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.71 (t, *J* = 8.0 Hz, 1H), 7.31–7.24 (m, 8H), 7.02–6.96 (m, 2H), 4.82 (s, 2H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>19</sub>H<sub>17</sub>N<sub>2</sub>O<sub>4</sub>S [M+H]<sup>+</sup> 369.0904, found 369.0910.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.62

### 3-Amino-*N*-benzyl-*N*-phenylbenzenesulfonamide (**B41**)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B41a** (0.340 g, 0.923 mmol, 1.00 equiv), 10% Pd/C (0.034 g, 10 wt%) and MeOH (5.0 mL). Stirring at rt for 27 h and purification by SGCC (*n*-hexane:EtOAc 0–60%) provided the title compound (0.271 g, 0.800 mmol, 87%). This is a novel compound.

**Physical state:** White solid.

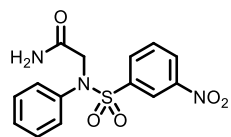
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.28–7.14 (m, 9H), 7.10–7.03 (m, 2H), 6.87 (t, *J* = 2.1 Hz, 1H), 6.85–6.79 (m, 1H), 6.72 (dt, *J* = 7.6, 1.2 Hz, 1H), 5.62 (s, 2H), 4.76 (s, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.5, 138.8, 138.4, 136.4, 129.7, 128.7, 128.4, 128.3, 128.0, 127.5, 127.4, 117.9, 113.9, 111.6, 53.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>19</sub>H<sub>19</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 339.1162, found 339.1166.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.44.

### 2-((3-Nitro-*N*-phenylphenyl)sulfonamido)acetamide (**B42a**)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B24a** (0.139 g, 0.500 mmol, 1.00 equiv), 2-bromoacetamide (0.103 g, 0.750 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.415 g, 3.00

mmol, 6.00 equiv) and anhydrous DMF (1.5 mL). Stirring at rt for 17 h provided the title compound (0.172 g, 0.500 mmol, quant.). This is a known compound (CAS no.: 1266448-69-4).

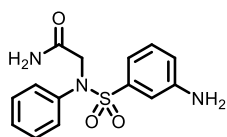
**Physical state:** Beige solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.51 (ddd, *J* = 8.3, 2.4, 1.1 Hz, 1H), 8.31 (t, *J* = 2.0 Hz, 1H), 7.99 (dt, *J* = 7.9, 1.3 Hz, 1H), 7.86 (t, *J* = 8.0 Hz, 1H), 7.47 (s, 1H), 7.41–7.28 (m, 3H), 7.27–7.13 (m, 3H), 4.27 (s, 2H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>14</sub>N<sub>3</sub>O<sub>5</sub>S [M+H]<sup>+</sup> 336.0649, found 336.0650.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.08.

## 2-((3-Amino-*N*-phenylphenyl)sulfonamido)acetamide (**B42**)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B42a** (0.172 g, 0.500 mmol, 1.00 equiv), 10% Pd/C (0.017 g, 10 wt%), MeOH (3.0 mL) and EtOAc (3.0 mL). Stirring at rt for 21 h and purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.109 g, 0.356 mmol, 71%). This is a novel compound.

**Physical state:** White solid.

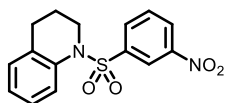
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.38 (s, 1H), 7.30 (ddd, *J* = 13.4, 7.9, 6.1 Hz, 3H), 7.21–7.11 (m, 4H), 6.81–6.74 (m, 2H), 6.65 (dt, *J* = 7.9, 1.2 Hz, 1H), 5.58 (s, 2H), 4.12 (s, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 168.8, 149.4, 139.8, 138.2, 129.5, 128.7, 127.7, 127.4, 117.9, 113.9, 111.6, 53.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>16</sub>N<sub>3</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 306.0912, found 306.0920.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.29.

## 1-((3-Nitrophenyl)sulfonyl)-1,2,3,4-tetrahydroquinoline (**B43a**)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 1,2,3,4-tetrahydroquinoline (0.138 mL, 1.10 mmol, 1.10 equiv), 3-nitrobenzenesulfonyl chloride (0.222 g, 1.00 mmol, 1.00 equiv), pyridine (0.243 mL, 3.00 mmol, 3.00 equiv) and DCM (4.0 mL). Stirring at rt for 29 h provided the title compound (0.247 g, 0.776 mmol, 78%). This is a known compound.<sup>227</sup>

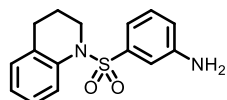
**Physical state:** Red solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.47 (t, *J* = 2.0 Hz, 1H), 8.37 (ddd, *J* = 8.2, 2.2, 1.0 Hz, 1H), 7.85 (dt, *J* = 7.9, 1.3 Hz, 1H), 7.78 (dd, *J* = 8.3, 1.1 Hz, 1H), 7.61 (t, *J* = 8.0 Hz, 1H), 7.27–7.20 (m, 1H), 7.12 (td, *J* = 7.5, 1.2 Hz, 1H), 7.02 (dd, *J* = 7.6, 1.5 Hz, 1H), 3.91–3.83 (m, 2H), 2.44 (t, *J* = 6.7 Hz, 2H), 1.76–1.65 (m, 2H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>15</sub>N<sub>2</sub>O<sub>4</sub>S [M+H]<sup>+</sup> 319.0747, found 319.0750.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.55.

### 3-((3,4-Dihydroquinolin-1(2H)-yl)sulfonyl)aniline (**B43**)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B43a** (0.240 g, 0.754 mmol, 1.00 equiv), 10% Pd/C (0.024 g, 10 wt%) and MeOH (5.0 mL). Stirring at rt for 27 h and purification by SGCC (*n*-hexane:EtOAc 0–60%) provided the title compound (0.163 g, 0.567 mmol, 75%). This is a known compound.<sup>228</sup>

**Physical state:** White solid.

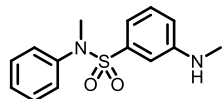
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.56 (d, *J* = 8.2 Hz, 1H), 7.18–7.01 (m, 4H), 6.85 (t, *J* = 2.0 Hz, 1H), 6.74 (dd, *J* = 8.1, 2.2 Hz, 1H), 6.62 (d, *J* = 7.6 Hz, 1H), 5.59 (s, 2H), 3.76–3.67 (m, 2H), 2.50 (d, *J* = 2.6 Hz, 2H), 1.62 (p, *J* = 6.4 Hz, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.5, 139.6, 136.6, 130.2, 129.7, 129.3, 126.1, 124.5, 123.5, 117.9, 113.3, 111.0, 46.1, 26.1, 21.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>17</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 289.1005, found 289.1006.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.38.

### *N*-Methyl-3-(methylamino)-*N*-phenylbenzenesulfonamide (**B44**)



**Synthetic procedure:** A previously reported reductive amination protocol was followed with minor modifications.<sup>170</sup> To a solution of **B24** (0.262 g, 1.00 mmol, 1.00 equiv) and 37% formaldehyde in water (0.082 mL, 1.10 mmol, 1.10 equiv) in DCM (5.0 mL) was added glacial AcOH (0.063 mL, 1.10 mmol, 1.10 equiv). The mixture was stirred at rt for 5 min. The mixture was then cooled to 0 °C and added NaBH(OAc)<sub>3</sub> (0.318 g, 1.50 mmol, 1.50 equiv) in portions. The mixture was stirred at 0 °C for 30 min and then at rt for 14 h. Upon reaction completion as determined by TLC, the mixture was added sat. NaHCO<sub>3</sub> (20 mL) and extracted with EtOAc (3 x 15 mL). The combined organic phases were washed with sat. NaHCO<sub>3</sub> (15 mL) and

sat. brine (15 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–60%) provided the title compound (0.115 g, 0.417 mmol, 42%). This is a known compound (CAS no.: 2396339-29-8).

**Physical state:** White solid.

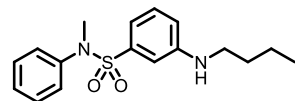
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.34 (dd, *J* = 8.5, 6.8 Hz, 2H), 7.30–7.21 (m, 2H), 7.12 (dd, *J* = 7.7, 1.7 Hz, 2H), 6.77 (dd, *J* = 8.2, 2.3 Hz, 1H), 6.65–6.59 (m, 1H), 6.53 (t, *J* = 2.1 Hz, 1H), 6.19 (q, *J* = 5.0 Hz, 1H), 3.11 (s, 3H), 2.58 (d, *J* = 5.0 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.0, 141.4, 136.5, 129.4, 128.7, 127.0, 126.1, 115.8, 113.8, 109.3, 37.8, 29.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>SNa [M+Na]<sup>+</sup> 299.0825, found 299.0836.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.46.

### 3-(Butylamino)-*N*-methyl-*N*-phenylbenzenesulfonamide (B45)



**Synthetic procedure:** The same procedure employed for **B44** was followed starting from **B24** (0.262 g, 1.00 mmol, 1.00 equiv), butyraldehyde (0.099 mL, 1.10 mmol, 1.10 equiv), glacial AcOH (0.063 mL, 1.10 mmol, 1.10 equiv), DCM (5.0 mL) and NaBH(OAc)<sub>3</sub> (0.318 g, 1.50 mmol, 1.50 equiv). Stirring at 0 °C–rt for 3 days and purification by SGCC (*n*-hexane:EtOAc 0–40%) provided the title compound (0.240 g, 0.752 mmol, 75%). This is a novel compound.

**Physical state:** White solid.

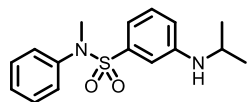
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.33 (dd, *J* = 8.4, 6.6 Hz, 2H), 7.30–7.24 (m, 1H), 7.21 (t, *J* = 7.9 Hz, 1H), 7.12 (dd, *J* = 7.6, 1.8 Hz, 2H), 6.79 (dd, *J* = 8.2, 2.4 Hz, 1H), 6.65–6.58 (m, 1H), 6.55 (d, *J* = 2.1 Hz, 1H), 6.13 (t, *J* = 5.4 Hz, 1H), 3.10 (s, 3H), 2.87 (q, *J* = 6.5 Hz, 2H), 1.44 (p, *J* = 7.0 Hz, 2H), 1.32 (h, *J* = 7.1 Hz, 2H), 0.88 (t, *J* = 7.2 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.3, 141.5, 136.5, 129.4, 128.7, 126.9, 126.0, 116.2, 113.6, 109.4, 42.2, 37.8, 30.4, 19.7, 13.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>23</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 319.1480, found 319.1480.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.61.

### 3-(Isopropylamino)-*N*-methyl-*N*-phenylbenzenesulfonamide (B46)



**Synthetic procedure:** The same procedure employed for **B44** was followed starting from **B24** (0.262 g, 1.00 mmol, 1.00 equiv), acetone (0.081 mL, 1.10 mmol, 1.10 equiv), glacial AcOH (0.063 mL, 1.10 mmol, 1.10 equiv), DCM (5.0 mL) and NaBH(OAc)<sub>3</sub> (0.318 g, 1.50 mmol, 1.50 equiv). Stirring at 0 °C–rt for 26 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.161 g, 0.527 mmol, 53%). This is a novel compound.

**Physical state:** Colourless oil.

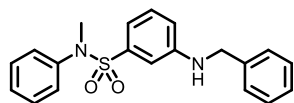
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.33 (dd, *J* = 8.3, 6.6 Hz, 2H), 7.29–7.24 (m, 1H), 7.21 (t, *J* = 7.9 Hz, 1H), 7.15–7.08 (m, 2H), 6.79 (ddd, *J* = 8.3, 2.4, 1.0 Hz, 1H), 6.62–6.54 (m, 2H), 5.99 (d, *J* = 7.7 Hz, 1H), 3.45–3.26 (m, 1H), 3.11 (s, 3H), 1.05 (d, *J* = 6.3 Hz, 6H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 148.4, 141.4, 136.6, 129.5, 128.8, 126.9, 126.0, 116.6, 113.5, 109.9, 43.0, 37.8, 22.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>16</sub>H<sub>21</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 305.1324, found 305.1326.

*R<sub>f</sub>* (silica gel, *n*-hexane:EtOAc 1:1): 0.60.

### 3-(Benzylamino)-*N*-methyl-*N*-phenylbenzenesulfonamide (B47)



**Synthetic procedure:** The same procedure employed for **B44** was followed starting from **B24** (0.262 g, 1.00 mmol, 1.00 equiv), benzaldehyde (0.112 mL, 1.10 mmol, 1.10 equiv), glacial AcOH (0.063 mL, 1.10 mmol, 1.10 equiv), DCM (5.0 mL) and NaBH(OAc)<sub>3</sub> (0.318 g, 1.50 mmol, 1.50 equiv). Stirring at 0 °C–rt for 14 h and purification by SGCC (*n*-hexane:EtOAc 0–40%) provided the title compound (0.302 g, 0.857 mmol, 86%). This is a novel compound.

**Physical state:** Off-white solid.

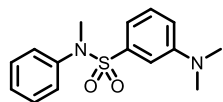
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.37–7.14 (m, 9H), 7.07–6.99 (m, 2H), 6.83 (q, *J* = 4.2 Hz, 2H), 6.65 (d, *J* = 2.1 Hz, 1H), 6.58 (d, *J* = 7.6 Hz, 1H), 4.22 (d, *J* = 6.0 Hz, 2H), 3.00 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 148.9, 141.4, 139.3, 136.6, 129.4, 128.8, 128.4, 127.1, 127.0, 126.8, 126.0, 116.6, 114.2, 110.1, 46.1, 37.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>20</sub>H<sub>21</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 353.1318, found 353.0335.

*R<sub>f</sub>* (silica gel, *n*-hexane:EtOAc 1:1): 0.62.

### 3-(Dimethylamino)-*N*-methyl-*N*-phenylbenzenesulfonamide (B48)



**Synthetic procedure:** The same protocol employed for **B8** was followed starting from **B24** (0.131 g, 0.500 mmol, 1.00 equiv), MeI (0.062 mL, 1.00 mmol, 2.00 equiv), K<sub>2</sub>CO<sub>3</sub> (0.276 g, 2.00 mmol, 4.00 equiv) and anhydrous DMF (1.0 mL). Stirring at 80 °C for 24 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.111 g, 0.383 mmol, 77%). This is a known compound (CAS no.: 86317-05-7).

**Physical state:** Light yellow oil

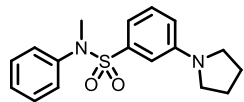
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.32–7.21 (m, 4H), 7.16–7.11 (m, 2H), 6.89 (ddd, *J* = 11.5, 7.9, 2.1 Hz, 2H), 6.70 (dd, *J* = 2.7, 1.7 Hz, 1H), 3.17 (s, 3H), 2.87 (s, 6H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 150.1, 142.0, 137.0, 129.4, 128.9, 127.3, 126.8, 116.5, 115.7, 111.3, 40.6, 38.2.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>19</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 291.1162, found 291.1171.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.50.

### *N*-Methyl-*N*-phenyl-3-(pyrrolidin-1-yl)benzenesulfonamide (B49)



**Synthetic procedure:** A previously reported *de novo* pyrrolidine ring formation was followed with minor modifications.<sup>171</sup> A mixture of **B24** (0.131 g, 0.500 mmol, 1.00 equiv), 1,4-dibromobutane (0.060 mL, 0.500 mmol, 1.00 equiv), KI (0.183 g, 1.10 mmol, 2.20 equiv) and K<sub>2</sub>CO<sub>3</sub> (0.152 g, 1.10 mmol, 2.20 equiv) in anhydrous MeCN (1.0 mL) was stirred in a pressure vial at 90 °C for 46 h. Upon reaction completion as determined by TLC, the mixture was cooled to rt, filtered through a bed of celite and the bed washed with EtOAc (3 x 10 mL). The combined filtrates were concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–40%) provided the title compound (0.104 g, 0.327 mmol, 65%). This is a novel compound.

**Physical state:** White solid.

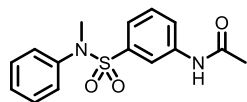
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.39–7.21 (m, 4H), 7.13 (d, *J* = 8.1 Hz, 2H), 6.79 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.68 (d, *J* = 7.6 Hz, 1H), 6.41 (t, *J* = 2.1 Hz, 1H), 3.12 (d, *J* = 7.1 Hz, 7H), 1.98–1.86 (m, 4H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 147.4, 141.5, 136.4, 129.5, 128.7, 127.0, 126.0, 115.9, 113.4, 109.2, 47.2, 37.7, 24.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>21</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 317.1318, found 317.1320.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.63.

### ***N*-(3-(*N*-Methyl-*N*-phenylsulfamoyl)phenyl)acetamide (B50)**



**Synthetic procedure:** The same procedure employed for **B5** was followed starting from **B24** (0.262 g, 1.00 mmol, 1.00 equiv), AcCl (0.213 mL, 3.00 mmol, 3.00 equiv), Et<sub>3</sub>N (0.418 mL, 3.00 mmol, 3.00 equiv) and anhydrous THF (12.0 mL). Stirring at 0 °C–rt for 21 h and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.169 g, 0.554 mmol, 55%). This is a known compound (CAS no.: 852665-21-5).

**Physical state:** Off-white solid.

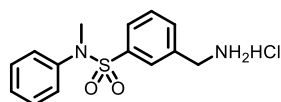
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.27 (s, 1H), 7.93–7.83 (m, 2H), 7.47 (t, *J* = 8.0 Hz, 1H), 7.38–7.31 (m, 2H), 7.31–7.25 (m, 1H), 7.15–7.05 (m, 3H), 3.14 (d, *J* = 1.1 Hz, 3H), 2.05 (d, *J* = 1.1 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 168.8, 141.1, 139.9, 136.6, 129.7, 128.9, 127.3, 126.2, 122.9, 121.6, 117.3, 38.0, 24.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>17</sub>N<sub>2</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 305.0960, found 305.0964.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.13.

### **3-(Aminomethyl)-*N*-methyl-*N*-phenylbenzenesulfonamide hydrochloride (B51)**



**Synthetic procedure:** The same protocol employed for **B22** was followed starting from **B53** (0.272 g, 1.00 mmol, 1.00 equiv), 10% Pd/C (0.027 g, 10 wt%), conc. HCl (0.048 mL, 0.400 mmol, 0.400 equiv) and MeOH (10.0 mL). After under H<sub>2</sub> at rt for 44 h and purification by SGCC (DCM:MeOH 0–50%), the product was redissolved in 2 M HCl in ether (2.00 mL, 2.00 mmol, 2.00 equiv) and stirred at rt for 5 min. The mixture was concentrated to dryness in vacuo to provide the title compound (0.055 g, 0.176 mmol, 18%). This is a known compound (CAS no.: 953753-31-6).

**Physical state:** White solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.52–8.36 (m, 3H), 7.87 (d, *J* = 1.7 Hz, 1H), 7.82 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.39–7.25 (m, 4H), 7.14–7.07 (m, 2H), 4.11 (s, 2H), 3.19 (s, 3H).

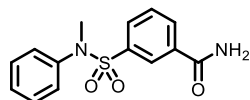
**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 141.0, 136.6, 135.9, 133.9, 129.4, 129.0, 127.8, 127.3, 127.3, 126.4, 41.7, 38.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>17</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 277.1016, found 277.1003.

**R<sub>f</sub>** (silica gel, DCM:MeOH 2:1): 0.33.



### 3-(*N*-Methyl-*N*-phenylsulfamoyl)benzamide (B52)



**Synthetic procedure:** The same protocol employed for **B23** was followed starting from **B53** (0.272 g, 1.00 mmol, 1.00 equiv), conc. H<sub>2</sub>SO<sub>4</sub> (0.28 mL, 5.25 mmol, 5.25 equiv) and TFA (1.12 mL). Stirring at rt for 21 h and purification by SGCC (hex:EtOAc 0–100%) provided the title compound (0.143 g, 0.493 mmol, 49%). This is a known compound (CAS no.: 1309138-86-0).

**Physical state:** Beige solid.

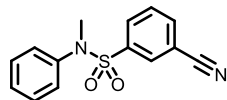
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.25 (s, 1H), 8.17 (dt, *J* = 7.8, 1.4 Hz, 1H), 8.06 (t, *J* = 1.8 Hz, 1H), 7.65 (t, *J* = 7.8 Hz, 1H), 7.62 (s, 1H), 7.56 (dt, *J* = 8.1, 1.3 Hz, 1H), 7.39–7.24 (m, 3H), 7.17–7.05 (m, 2H), 3.16 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 166.2, 140.9, 136.4, 135.1, 131.9, 129.9, 129.4, 129.0, 127.4, 126.5, 126.3, 38.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>15</sub>N<sub>2</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 291.0803, found 291.0813.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.08.

### 3-Cyano-*N*-methyl-*N*-phenylbenzenesulfonamide (B53)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from *N*-methylaniline (0.477 mL, 4.40 mmol, 1.10 equiv), 3-cyanobenzenesulfonyl chloride (0.806 g, 4.00 mmol, 1.00 equiv), pyridine (0.971 mL, 12.0 mmol, 3.00 equiv) and DCM (16.0 mL). Stirring at rt for 24 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.822 g, 3.02 mmol, 75%). This is a known compound.<sup>229</sup>

**Physical state:** White solid.

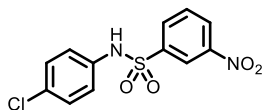
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.19 (dt, *J* = 7.5, 1.6 Hz, 1H), 7.98 (t, *J* = 1.7 Hz, 1H), 7.82–7.69 (m, 2H), 7.42–7.28 (m, 3H), 7.17–7.08 (m, 2H), 3.19 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 140.6, 137.2, 136.9, 131.8, 130.9, 130.7, 129.1, 127.7, 126.5, 117.5, 112.6, 38.2.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>SNa [M+Na]<sup>+</sup> 295.0512, found 295.0516.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.22.

### ***N*-(4-Chlorophenyl)-3-nitrobenzenesulfonamide (B54a)**



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 4-chloroaniline (0.281 g, 2.20 mmol, 1.10 equiv), 3-nitrobenzenesulfonyl chloride (0.443 g, 2.00 mmol, 1.00 equiv), pyridine (0.485 mL, 6.00 mmol, 3.00 equiv) and DCM (8.0 mL). Stirring at rt for 17 h provided the title compound (0.461 g, 1.47 mmol, 74%). This is a known compound.<sup>209</sup>

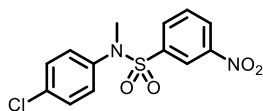
**Physical state:** Light pink solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.72 (s, 1H), 8.49 (t, *J* = 2.0 Hz, 1H), 8.46 (dd, *J* = 8.1, 2.3 Hz, 1H), 8.11 (dt, *J* = 7.9, 1.3 Hz, 1H), 7.86 (t, *J* = 8.0 Hz, 1H), 7.37–7.25 (m, 2H), 7.19–7.06 (m, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 147.9, 140.6, 135.8, 132.6, 131.5, 129.4, 129.1, 127.8, 122.5, 121.4.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.34.

### ***N*-(4-Chlorophenyl)-*N*-methyl-3-nitrobenzenesulfonamide (B54b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B54a** (0.450 g, 1.44 mmol, 1.00 equiv), MeI (0.134 mL, 2.16 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (1.19 g, 8.63 mmol, 6.00 equiv) and anhydrous DMF (5.0 mL). Stirring at rt for 24 h provided the title compound (0.422 g, 1.29 mmol, 90%). This is a known compound. Experimental data correlate with literature.<sup>188</sup>

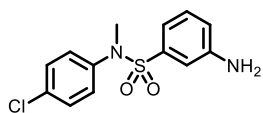
**Physical state:** Beige solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.50–8.42 (m, 2H), 7.80 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.69 (td, *J* = 7.7, 1.1 Hz, 1H), 7.34–7.28 (m, 2H), 7.07–7.00 (m, 2H), 3.22 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 148.3, 139.3, 138.7, 134.0, 133.36, 130.43, 129.6, 128.0, 127.6, 122.9, 38.5.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.47.

### **3-Amino-*N*-(4-chlorophenyl)-*N*-methylbenzenesulfonamide (B54)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B54b** (0.410 g, 1.26 mmol, 1.00 equiv), 10% Pd/C (0.041 g, 10 wt%), MeOH (5.0 mL) and EtOAc (5.0 mL). Stirring at rt for 21 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.317 g, 1.07 mmol, 85%). This is a known compound. Experimental data correlate with literature.<sup>188</sup>

**Physical state:** White solid.

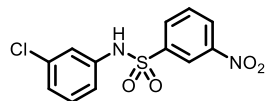
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.44–7.37 (m, 2H), 7.21–7.10 (m, 3H), 6.79 (ddd, *J* = 8.1, 2.3, 0.9 Hz, 1H), 6.72 (t, *J* = 2.1 Hz, 1H), 6.56 (ddd, *J* = 7.7, 1.9, 1.0 Hz, 1H), 5.62 (s, 2H), 3.09 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.5, 140.3, 136.1, 131.3, 129.6, 128.8, 127.7, 118.1, 114.0, 111.6, 37.7.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>SCl [M+H]<sup>+</sup> 297.0459, found 297.0467.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.32.

#### ***N*-(3-Chlorophenyl)-3-nitrobenzenesulfonamide (B55a)**



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 3-chloroaniline (0.158 mL, 2.20 mmol, 1.10 equiv), 3-nitrobenzenesulfonyl chloride (0.443 g, 2.00 mmol, 1.00 equiv), pyridine (0.485 mL, 6.00 mmol, 3.00 equiv) and DCM (8.0 mL). Stirring at rt for 17 h provided the title compound (0.447 g, 1.43 mmol, 72%). This is a known compound. Experimental data correlate with literature.

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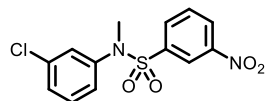
**Physical state:** Light pink solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.86 (s, 1H), 8.50 (t, *J* = 2.1 Hz, 1H), 8.46 (dd, *J* = 8.2, 2.3 Hz, 1H), 8.24–8.06 (m, 1H), 7.88 (t, *J* = 8.0 Hz, 1H), 7.37–7.24 (m, 1H), 7.21–7.12 (m, 2H), 7.09 (dd, *J* = 8.2, 2.1 Hz, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 148.0, 140.5, 138.5, 133.6, 132.6, 131.6, 131.2, 127.9, 124.6, 121.4, 119.9, 118.8.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.36.

#### ***N*-(3-Chlorophenyl)-*N*-methyl-3-nitrobenzenesulfonamide (B55b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B55a** (0.437 g, 1.40 mmol, 1.00 equiv), MeI (0.130 mL, 2.10 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (1.16 g, 8.38 mmol, 6.00 equiv) and

anhydrous DMF (4.0 mL). Stirring at rt for 24 h provided the title compound (0.430 g, 1.32 mmol, 94%). This is a known compound (CAS no.: 2217015-57-9).

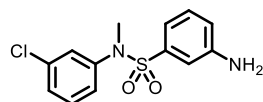
**Physical state:** Yellow-brown solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.62 (dd, *J* = 6.8, 1.3 Hz, 2H), 7.98 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.89–7.82 (m, 1H), 7.48–7.39 (m, 2H), 7.26 (t, *J* = 2.0 Hz, 1H), 7.18 (dt, *J* = 7.2, 2.0 Hz, 1H), 3.38 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 148.3, 142.0, 138.7, 134.9, 133.3, 130.5, 130.3, 128.4, 127.6, 126.9, 124.8, 122.9, 38.5.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.32.

### 3-Amino-*N*-(3-chlorophenyl)-*N*-methylbenzenesulfonamide (B55)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B55b** (0.420 g, 1.29 mmol, 1.00 equiv), 10% Pd/C (0.042 g, 10 wt%), MeOH (5.0 mL) and EtOAc (5.0 mL). Stirring at rt for 21 h and purification by SGCC (*n*-hexane:EtOAc 0–70%, then DCM:MeOH 0–10%) provided the title compound (0.071 g, 0.239 mmol, 19%). This is a known compound (CAS no.: 1226357-17-0).

**Physical state:** White solid.

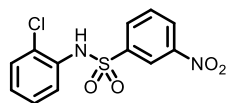
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.70 (d, *J* = 2.0 Hz, 1H), 8.58 (d, *J* = 2.0 Hz, 1H), 7.41–7.28 (m, 3H), 7.20 (q, *J* = 1.6 Hz, 1H), 7.13–7.04 (m, 2H), 6.99 (d, *J* = 2.2 Hz, 1H), 6.87–6.79 (m, 1H), 3.13 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.7, 142.6, 136.1, 132.9, 130.4, 129.4, 127.0, 125.9, 124.4, 117.6, 117.1, 110.7, 37.7.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>SCl [M+H]<sup>+</sup> 297.0459, found 297.0460.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.35.

### *N*-(2-Chlorophenyl)-3-nitrobenzenesulfonamide (B56a)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 2-chloroaniline (0.231 mL, 2.20 mmol, 1.10 equiv), 3-nitrobenzenesulfonyl chloride (0.443 g, 2.00 mmol, 1.00 equiv), pyridine (0.485 mL, 6.00 mmol, 3.00 equiv) and DCM (8.0 mL). Stirring at rt for 17 h provided the title compound (0.372 g, 1.19 mmol, 59%). This is a known compound.<sup>226</sup>

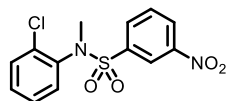
**Physical state:** Beige solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.46 (s, 1H), 8.50 (ddd, *J* = 8.2, 2.3, 1.0 Hz, 1H), 8.45 (t, *J* = 2.0 Hz, 1H), 8.09 (dt, *J* = 8.0, 1.3 Hz, 1H), 7.87 (t, *J* = 8.0 Hz, 1H), 7.42 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.34–7.29 (m, 1H), 7.26 (ddd, *J* = 8.8, 3.8, 2.1 Hz, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 147.8, 142.0, 132.7, 132.6, 131.4, 130.1, 129.7, 128.48, 128.45, 128.0, 127.6, 121.5.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.44.

#### ***N*-(2-Chlorophenyl)-*N*-methyl-3-nitrobenzenesulfonamide (B56b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B56a** (0.360 g, 1.15 mmol, 1.00 equiv), MeI (0.107 mL, 1.73 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.955 g, 6.91 mmol, 6.00 equiv) and anhydrous DMF (4.0 mL). Stirring at rt for 3 days provided the title compound (0.353 g, 1.08 mmol, 94%). This is a known compound (CAS no.: 353235-99-1).

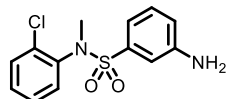
**Physical state:** Beige solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.58 (ddd, *J* = 8.2, 2.3, 1.0 Hz, 1H), 8.34 (t, *J* = 2.0 Hz, 1H), 8.16 (ddd, *J* = 7.9, 1.9, 1.0 Hz, 1H), 7.95 (t, *J* = 8.0 Hz, 1H), 7.59 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.43 (td, *J* = 7.7, 1.6 Hz, 1H), 7.35 (td, *J* = 7.7, 1.5 Hz, 1H), 7.15 (dd, *J* = 7.9, 1.6 Hz, 1H), 3.19 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 148.0, 139.5, 137.5, 133.5, 133.3, 131.6, 130.6, 130.5, 130.4, 128.3, 127.9, 122.0, 38.3.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.26.

#### **3-Amino-*N*-(2-chlorophenyl)-*N*-methylbenzenesulfonamide (B56)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B56b** (0.340 g, 1.04 mmol, 1.00 equiv), 10% Pd/C (0.034 g, 10 wt%) and MeOH (5.0 mL). Stirring at rt for 19 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.254 g, 0.855 mmol, 82%). This is a known compound (CAS no.: 353236-00-7).

**Physical state:** White solid.

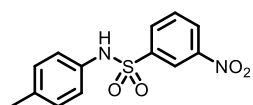
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.56 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.38 (td, *J* = 7.7, 1.8 Hz, 1H), 7.32 (td, *J* = 7.6, 1.7 Hz, 1H), 7.24 (t, *J* = 7.9 Hz, 1H), 7.03 (dd, *J* = 7.8, 1.7 Hz, 1H), 6.94 (t, *J* = 2.1 Hz, 1H), 6.86–6.81 (m, 1H), 6.81–6.77 (m, 1H), 5.65 (s, 2H), 3.08 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.6, 149.5, 138.5, 138.5, 133.8, 130.4, 129.9, 129.7, 128.0, 117.9, 114.0, 111.7, 38.2.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>SCl [M+H]<sup>+</sup> 297.0459, found 297.0464.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.25.

### 3-Nitro-*N*-(*p*-tolyl)benzenesulfonamide (B57a)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from *p*-toluidine (0.236 g, 2.20 mmol, 1.10 equiv), 3-nitrobenzenesulfonyl chloride (0.443 g, 2.00 mmol, 1.00 equiv), pyridine (0.485 mL, 6.00 mmol, 3.00 equiv) and DCM (10.0 mL). Stirring at rt for 22 h provided the title compound (0.434 g, 1.49 mmol, 74%). This is a known compound.<sup>230</sup>

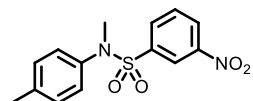
**Physical state:** Beige solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.39 (s, 1H), 8.47 (t, *J* = 2.0 Hz, 1H), 8.43 (ddd, *J* = 8.2, 2.4, 1.0 Hz, 1H), 8.08 (dt, *J* = 8.0, 1.3 Hz, 1H), 7.84 (t, *J* = 8.0 Hz, 1H), 7.05 (d, *J* = 8.2 Hz, 2H), 6.97 (d, *J* = 8.4 Hz, 2H), 2.18 (s, 3H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>11</sub>N<sub>2</sub>O<sub>4</sub>S [M-H]<sup>-</sup> 291.0440, found 291.0437.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.43.

### *N*-Methyl-3-nitro-*N*-(*p*-tolyl)benzenesulfonamide (B57b)



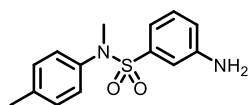
**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B57a** (0.434 g, 1.49 mmol, 1.00 equiv), MeI (0.139 mL, 2.23 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (1.23 g, 8.92 mmol, 6.00 equiv) and anhydrous DMF (5.0 mL). Stirring at rt for 16 h provided the title compound (0.432 g, 1.41 mmol, 95%). This is a known compound (CAS no.: 220400-34-0).

**Physical state:** Beige solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.43 (pd, *J* = 2.3, 1.1 Hz, 2H), 7.83 (dt, *J* = 7.9, 1.3 Hz, 1H), 7.70–7.62 (m, 1H), 7.12 (d, *J* = 8.1 Hz, 2H), 6.98–6.90 (m, 2H), 3.21 (s, 3H), 2.34 (s, 3H).

$R_f$  (silica gel, *n*-hexane:EtOAc 2:1): 0.34.

### 3-Amino-*N*-methyl-*N*-(*p*-tolyl)benzenesulfonamide (B57)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B57b** (0.430 g, 1.40 mmol, 1.00 equiv), 10% Pd/C (0.043 g, 10 wt%), MeOH (5.0 mL) and EtOAc (5.0 mL). Stirring at rt for 22 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.203 g, 0.735 mmol, 52%). This is a known compound (CAS no.: 1226253-74-2).

**Physical state:** Beige solid.

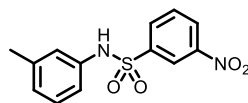
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.15 (dd, *J* = 15.4, 7.8 Hz, 3H), 6.97 (d, *J* = 8.2 Hz, 2H), 6.81–6.74 (m, 2H), 6.56 (dd, *J* = 7.7, 1.6 Hz, 1H), 5.59 (s, 2H), 3.08 (s, 3H), 2.27 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.3, 138.8, 136.7, 136.4, 129.4, 129.3, 126.1, 117.8, 114.0, 111.8, 38.1, 20.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>16</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 318.1276, found 318.1277.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.33.

### 3-Nitro-*N*-(*m*-tolyl)benzenesulfonamide (B58a)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from *m*-toluidine (0.236 mL, 2.20 mmol, 1.10 equiv), 3-nitrobenzenesulfonyl chloride (0.443 g, 2.00 mmol, 2.00 equiv), pyridine (0.485 mL, 6.00 mmol, 3.00 equiv) and DCM (8.0 mL). Stirring at rt for 23 h provided the title compound (0.436 g, 1.49 mmol, 75%). This is a known compound (CAS no.: 16936-93-9).

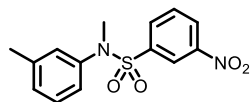
**Physical state:** Red solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.50 (s, 1H), 8.49 (t, *J* = 2.0 Hz, 1H), 8.47–8.41 (m, 1H), 8.12 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.85 (t, *J* = 8.0 Hz, 1H), 7.12 (t, *J* = 7.7 Hz, 1H), 6.96–6.86 (m, 3H), 2.19 (s, 3H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>11</sub>N<sub>2</sub>O<sub>4</sub>S [M-H]<sup>−</sup> 291.0445, found 291.0451.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.60.

### ***N*-Methyl-3-nitro-*N*-(*m*-tolyl)benzenesulfonamide (B58b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B58a** (0.420 g, 1.44 mmol, 1.00 equiv), MeI (0.134 mL, 2.16 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (1.19 g, 8.62 mmol, 6.00 equiv) and anhydrous DMF (5.0 mL). Stirring at rt for 24 h provided the title compound (0.404 g, 1.32 mmol, 92%). This is a known compound (CAS no.: 2216509-81-6).

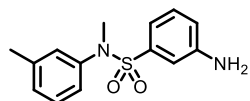
**Physical state:** Brown oil.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.43 (ddt, *J* = 4.6, 3.4, 2.1 Hz, 2H), 7.84 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.71–7.62 (m, 1H), 7.19 (t, *J* = 7.7 Hz, 1H), 7.12 (d, *J* = 7.6 Hz, 1H), 6.95 (d, *J* = 2.0 Hz, 1H), 6.81 (dt, *J* = 7.9, 1.6 Hz, 1H), 3.22 (s, 3H), 2.32 (s, 3H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>15</sub>N<sub>2</sub>O<sub>4</sub>S [M+H]<sup>+</sup> 307.0474, found 307.0749.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.45.

### ***3*-Amino-*N*-methyl-*N*-(*m*-tolyl)benzenesulfonamide (B58)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B58b** (0.390 g, 1.27 mmol, 1.00 equiv), 10% Pd/C (0.039 g, 10 wt%), MeOH (10.0 mL) and EtOAc (10.0 mL). Stirring at rt for 23 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.280 g, 1.01 mmol, 80%). This is a known compound (CAS no.: 1225581-97-4).

**Physical state:** Beige solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.18 (dt, *J* = 18.3, 7.8 Hz, 2H), 7.11–7.05 (m, 1H), 6.94 (d, *J* = 1.8 Hz, 1H), 6.87 (dd, *J* = 8.0, 2.3 Hz, 1H), 6.81–6.75 (m, 2H), 6.57 (ddd, *J* = 7.7, 1.8, 1.0 Hz, 1H), 5.59 (s, 2H), 3.09 (s, 3H), 2.26 (s, 3H).

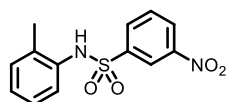
**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.4, 141.4, 138.1, 136.8, 129.4, 128.5, 127.7, 126.9, 123.0, 117.8, 114.0, 111.8, 38.0, 20.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>17</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 277.1005, found 277.1008.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.40.



### 3-Nitro-*N*-(*o*-tolyl)benzenesulfonamide (B59a)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from *o*-toluidine (0.118 g, 1.10 mmol, 1.10 equiv), 3-nitrobenzenesulfonyl chloride (0.222 g, 1.00 mmol, 1.00 equiv), pyridine (0.293 mL, 3.00 mmol, 3.00 equiv) and DCM (4.0 mL). Stirring at rt for 20 h provided the title compound (0.228 g, 0.779 mmol, 78%). This is a known compound. Experimental data correlate with literature.<sup>231</sup>

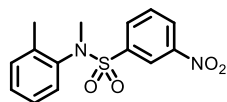
**Physical state:** Orange solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 9.96 (s, 1H), 8.49 (ddd, *J* = 8.2, 2.3, 1.1 Hz, 1H), 8.40 (t, *J* = 2.0 Hz, 1H), 8.04 (dt, *J* = 8.0, 1.3 Hz, 1H), 7.87 (t, *J* = 8.0 Hz, 1H), 7.24–7.02 (m, 4H), 6.89 (dd, *J* = 7.6, 1.6 Hz, 1H), 2.03 (s, 3H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>11</sub>N<sub>2</sub>O<sub>4</sub>S [M-H]<sup>-</sup> 291.0445, found 291.0445.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.56.

### *N*-Methyl-3-nitro-*N*-(*o*-tolyl)benzenesulfonamide (B59b)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B59a** (0.220 g, 0.753 mmol, 1.00 equiv), MeI (0.070 mL, 1.13 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.624 g, 4.52 mmol, 6.00 equiv) and anhydrous DMF (3.0 mL). Stirring at rt for 24 h provided the title compound (0.184 g, 0.600 mmol, 80%). This is a known compound (CAS no.: 2216509-50-9).

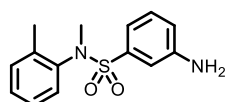
**Physical state:** Orange solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.58 (t, *J* = 2.0 Hz, 1H), 8.47 (ddd, *J* = 8.2, 2.3, 1.1 Hz, 1H), 8.01 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.74 (t, *J* = 8.0 Hz, 1H), 7.36–7.30 (m, 1H), 7.25 (td, *J* = 7.5, 1.2 Hz, 1H), 7.07 (td, *J* = 7.6, 1.6 Hz, 1H), 6.55 (dd, *J* = 7.9, 1.2 Hz, 1H), 3.21 (s, 3H), 2.40 (s, 3H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>SNa [M+Na]<sup>+</sup> 329.0566, found 329.0570.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.69.

### 3-Amino-*N*-methyl-*N*-(*o*-tolyl)benzenesulfonamide (B59)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B59b** (0.180 g, 0.589 mmol, 1.00 equiv), 10% Pd/C (0.018 g, 10 wt%), MeOH (3.0 mL) and EtOAc (3.0 mL). Stirring at rt for 40 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.114 g, 0.412 mmol, 70%). This is a known compound (CAS no.: 1226282-63-8).

**Physical state:** Off-white solid.

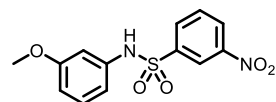
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.30 (d, *J* = 7.5 Hz, 1H), 7.23 (t, *J* = 7.7 Hz, 2H), 7.11 (t, *J* = 7.6 Hz, 1H), 6.88 (d, *J* = 2.1 Hz, 1H), 6.83 (dd, *J* = 8.1, 2.2 Hz, 1H), 6.78–6.70 (m, 1H), 6.67 (d, *J* = 7.8 Hz, 1H), 5.62 (s, 2H), 3.05 (s, 3H), 2.29 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.5, 140.3, 138.2, 138.1, 131.0, 129.6, 128.1, 127.0, 126.6, 117.8, 114.1, 111.9, 38.8, 17.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>17</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 277.1005, found 277.1006.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.15.

#### ***N*-(3-Methoxyphenyl)-3-nitrobenzenesulfonamide (B60a)**



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from *m*-anisidine (0.124 mL, 1.10 mmol, 1.10 equiv), 3-nitrobenzenesulfonyl chloride (0.222 g, 1.00 mmol, 1.00 equiv), pyridine (0.243 mL, 3.00 mmol, 3.00 equiv) and DCM (4.0 mL). Stirring at rt for 18 h provided the title compound (0.238 g, 0.773 mmol, 77%). This is a known compound (CAS no.: 16936-98-4).

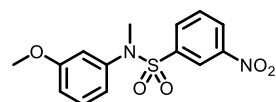
**Physical state:** Red solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.57 (s, 1H), 8.50 (t, *J* = 2.0 Hz, 1H), 8.45 (ddd, *J* = 8.2, 2.3, 1.0 Hz, 1H), 8.15 (dt, *J* = 7.9, 1.3 Hz, 1H), 7.86 (t, *J* = 8.0 Hz, 1H), 7.19–7.10 (m, 1H), 6.72–6.60 (m, 3H), 3.66 (s, 3H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>12</sub>N<sub>2</sub>O<sub>5</sub>Na [M+Na]<sup>+</sup> 331.0359, found 331.0362.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.22.

#### ***N*-(3-Methoxyphenyl)-*N*-methyl-3-nitrobenzenesulfonamide (B60b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B60a** (0.230 g, 0.746 mmol, 1.00 equiv), MeI (0.070 mL, 1.12 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.619 g, 4.48 mmol, 6.00 equiv)

and anhydrous DMF (3.0 mL). Stirring at rt for 19 h provided the title compound (0.234 g, 0.727 mmol, 97%). This is a known compound (CAS no.: 2217015-89-7).

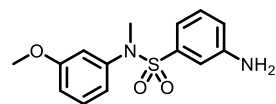
**Physical state:** Golden brown oil.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.43 (dd, *J* = 7.3, 1.2 Hz, 2H), 7.85 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.67 (td, *J* = 7.6, 1.3 Hz, 1H), 7.21 (t, *J* = 8.1 Hz, 1H), 6.85 (ddd, *J* = 8.4, 2.5, 0.9 Hz, 1H), 6.71 (t, *J* = 2.3 Hz, 1H), 6.59 (ddd, *J* = 8.0, 2.0, 0.9 Hz, 1H), 3.78 (s, 3H), 3.22 (s, 3H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>15</sub>N<sub>2</sub>O<sub>5</sub>S [M+H]<sup>+</sup> 323.0696, found 323.0700.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.54.

### 3-Amino-*N*-(3-methoxyphenyl)-*N*-methylbenzenesulfonamide (B60)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B60b** (0.234 g, 0.727 mmol, 1.00 equiv), 10% Pd/C (0.023 g, 10 wt%) and MeOH (7.0 mL). Stirring at rt for 6 h and purification by SGCC (*n*-hexane:EtOAc 0–60%) provided the title compound (0.164 g, 0.562 mmol, 77%). This is a known compound (CAS no.: 1226355-90-3).

**Physical state:** Yellow oil.

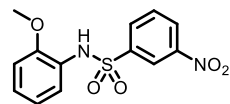
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.24 (t, *J* = 8.1 Hz, 1H), 7.17 (td, *J* = 7.6, 1.0 Hz, 1H), 6.85 (ddd, *J* = 8.3, 2.5, 0.9 Hz, 1H), 6.79 (dq, *J* = 9.5, 1.4 Hz, 2H), 6.68 (ddd, *J* = 8.0, 2.1, 0.9 Hz, 1H), 6.64 (t, *J* = 2.3 Hz, 1H), 6.62–6.55 (m, 1H), 5.60 (s, 2H), 3.69 (s, 3H), 3.11 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 159.3, 149.4, 142.6, 136.7, 129.4, 118.1, 117.9, 114.0, 112.6, 112.0, 111.7, 55.1, 38.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>17</sub>N<sub>2</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 293.0954, found 293.0942.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.47.

### *N*-(2-Methoxyphenyl)-3-nitrobenzenesulfonamide (B61a)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from *o*-anisidine (0.124 mL, 1.10 mmol, 1.10 equiv), 3-nitrobenzenesulfonyl chloride (0.222 g, 1.00 mmol, 1.00 equiv), pyridine (0.243 mL, 3.00 mmol, 3.00 equiv) and DCM (4.0 mL). Stirring at rt for 3 days provided the title compound (0.240 g, 0.780 mmol, 78%). This is a known compound (CAS no.: 169945-38-4).

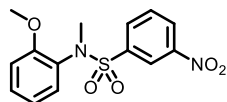
**Physical state:** Beige solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 9.98 (s, 1H), 8.50–8.40 (m, 2H), 8.06 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.83 (t, *J* = 7.9 Hz, 1H), 7.23 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.17 (td, *J* = 7.8, 1.7 Hz, 1H), 6.95–6.84 (m, 2H), 3.40 (s, 3H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>12</sub>N<sub>2</sub>O<sub>5</sub>Na [M+Na]<sup>+</sup> 331.0359, found 331.0358.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.41.

### ***N*-(2-Methoxyphenyl)-*N*-methyl-3-nitrobenzenesulfonamide (B61b)**



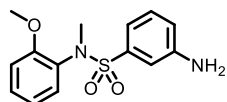
**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B61a** (0.239 g, 0.775 mmol, 1.00 equiv), MeI (0.072 mL, 1.16 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.643 g, 4.65 mmol, 6.00 equiv) and anhydrous DMF (3.0 mL). Stirring at rt for 43 h provided the title compound (0.226 g, 0.701 mmol, 90%). This is a known compound (CAS no.: 2216513-62-9).

**Physical state:** Golden-brown oil.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.53 (ddd, *J* = 8.2, 2.3, 1.0 Hz, 1H), 8.26 (t, *J* = 2.0 Hz, 1H), 8.09 (dt, *J* = 7.9, 1.3 Hz, 1H), 7.92 (q, *J* = 8.0 Hz, 1H), 7.41–7.31 (m, 1H), 7.24 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.04–6.93 (m, 2H), 3.35 (s, 3H), 3.16 (s, 3H).

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.29.

### **3-Amino-*N*-(2-methoxyphenyl)-*N*-methylbenzenesulfonamide (B61)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B61b** (0.220 g, 0.683 mmol, 1.00 equiv), 10% Pd/C (0.022 g, 10 wt%) and MeOH (7.0 mL). Stirring at rt for 22 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.174 g, 0.594 mmol, 87%). This is a known compound (CAS no.: 1225725-45-0).

**Physical state:** Off-white solid.

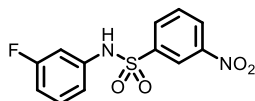
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.33–7.27 (m, 1H), 7.18 (t, *J* = 7.9 Hz, 1H), 7.09 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.01 (dd, *J* = 8.3, 1.3 Hz, 1H), 6.90 (td, *J* = 7.6, 1.3 Hz, 1H), 6.85 (t, *J* = 2.0 Hz, 1H), 6.78 (ddd, *J* = 8.1, 2.3, 1.0 Hz, 1H), 6.73 (ddd, *J* = 7.7, 1.8, 1.0 Hz, 1H), 5.55 (s, 2H), 3.49 (s, 3H), 3.07 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 156.4, 149.3, 139.5, 130.5, 129.5, 129.2, 129.1, 120.3, 117.4, 113.9, 112.6, 111.7, 55.3, 37.8.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{14}H_{17}N_2O_3S$   $[M+H]^+$  293.0960, found 293.0960.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.23.

#### ***N*-(3-Fluorophenyl)-3-nitrobenzenesulfonamide (B62a)**



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 3-fluoroaniline (0.106 mL, 1.10 mmol, 1.10 equiv), 3-nitrobenzenesulfonyl chloride (0.222 g, 1.00 mmol, 1.00 equiv), pyridine (0.243 mL, 3.00 mmol, 3.00 equiv) and DCM (4.0 mL). Stirring at rt for 31 h provided the title compound (0.264 g, 0.891 mmol, 89%). This is a known compound (CAS no.: 333440-85-0).

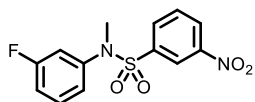
**Physical state:** Red-orange oil.

**$^1H$  NMR** (400 MHz,  $CDCl_3$ ):  $\delta$  8.66 (t,  $J$  = 2.0 Hz, 1H), 8.42 (ddd,  $J$  = 8.2, 2.2, 1.1 Hz, 1H), 8.14–8.06 (m, 1H), 7.69 (t,  $J$  = 8.0 Hz, 1H), 7.24 (td,  $J$  = 8.1, 6.2 Hz, 1H), 6.99–6.80 (m, 3H).

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{12}H_8N_2O_4SF$   $[M-H]^-$  295.0195, found 295.0195.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.47.

#### ***N*-(3-Fluorophenyl)-*N*-methyl-3-nitrobenzenesulfonamide (B62b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B62a** (0.260 g, 0.878 mmol, 1.00 equiv), MeI (0.082 mL, 1.32 mmol, 1.50 equiv),  $K_2CO_3$  (0.728 g, 5.27 mmol, 6.00 equiv) and anhydrous DMF (3.0 mL). Stirring at rt for 16 h provided the title compound (0.246 g, 0.608 mmol, 90%). This is a known compound (CAS no.: 2216507-86-5).

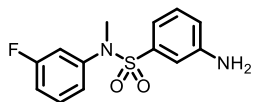
**Physical state:** Golden-brown oil.

**$^1H$  NMR** (400 MHz,  $CDCl_3$ ):  $\delta$  8.45 (dt,  $J$  = 7.3, 1.8 Hz, 2H), 7.83 (dt,  $J$  = 8.0, 1.3 Hz, 1H), 7.72–7.64 (m, 1H), 7.35–7.26 (m, 2H), 7.03 (tdd,  $J$  = 8.3, 2.4, 1.0 Hz, 1H), 6.94–6.84 (m, 2H), 3.23 (s, 3H).

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{13}H_{10}N_2O_4SF$   $[M-H]^-$  309.0351, found 309.0352.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.63.

#### **3-Amino-*N*-(3-fluorophenyl)-*N*-methylbenzenesulfonamide (B62)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B62b** (0.240 g, 0.773 mmol, 1.00 equiv), 10% Pd/C (0.024 g, 10 wt%), MeOH (4.0 mL) and EtOAc (4.0 mL). Stirring at rt for 17 h and purification by SGCC (*n*-hexane:EtOAc 0–60%) provided the title compound (0.201 g, 0.717 mmol, 93%). This is a known compound (CAS no.: 1226330-67-1).

**Physical state:** Yellow oil.

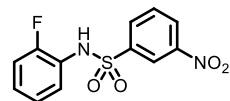
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.42–7.33 (m, 1H), 7.20–7.08 (m, 2H), 7.04–6.96 (m, 2H), 6.79 (ddd, *J* = 8.1, 2.3, 1.0 Hz, 1H), 6.75 (t, *J* = 2.0 Hz, 1H), 6.57 (ddd, *J* = 7.7, 1.9, 1.0 Hz, 1H), 5.61 (s, 2H), 3.13 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 162.9, 160.5, 149.5, 143.1, 143.0, 136.3, 130.2, 130.1, 129.5, 121.6, 121.6, 118.1, 113.9, 113.7, 113.5, 112.9, 112.7, 111.6, 37.6.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>SF [M+H]<sup>+</sup> 281.0755, found 281.0765.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.38.

#### ***N*-(2-Fluorophenyl)-3-nitrobenzenesulfonamide (B63a)**



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 2-fluoroaniline (0.106 mL, 1.10 mmol, 1.10 equiv), 3-nitrobenzenesulfonyl chloride (0.222 g, 1.00 mmol, 1.00 equiv), pyridine (0.243 mL, 3.00 mmol, 3.00 equiv) and DCM (4.0 mL). Stirring at rt for 27 h provided the title compound (0.203 g, 0.687 mmol, 69%). This is a known compound.<sup>232</sup>

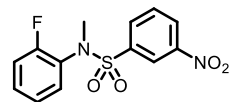
**Physical state:** Light orange solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.54 (s, 1H), 8.53–8.41 (m, 2H), 8.10 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.87 (t, *J* = 8.0 Hz, 1H), 7.25 (dtd, *J* = 7.5, 4.7, 1.9 Hz, 2H), 7.21–7.11 (m, 2H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>12</sub>H<sub>8</sub>N<sub>2</sub>O<sub>4</sub>SF [M-H]<sup>−</sup> 295.0194, found 295.0195.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.55.

#### ***N*-(2-Fluorophenyl)-*N*-methyl-3-nitrobenzenesulfonamide (B63b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B63a** (0.200 g, 0.675 mmol, 1.00 equiv), MeI (0.063 mL, 1.01 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.560 g, 4.05 mmol, 6.00 equiv) and anhydrous DMF (3.0 mL). Stirring at rt for 16 h provided the title compound (0.189 g, 0.608 mmol, 90%). This is a known compound (CAS no.: 2216238-68-3).

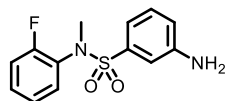
**Physical state:** Beige solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.53 (t, *J* = 2.0 Hz, 1H), 8.45 (ddd, *J* = 8.2, 2.3, 1.1 Hz, 1H), 7.99 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.70 (t, *J* = 8.0 Hz, 1H), 7.41 (td, *J* = 7.8, 1.7 Hz, 1H), 7.35 (tdd, *J* = 7.4, 4.9, 1.8 Hz, 1H), 7.19 (td, *J* = 7.7, 1.4 Hz, 1H), 7.02 (ddd, *J* = 10.9, 8.3, 1.4 Hz, 1H), 3.28 (d, *J* = 1.3 Hz, 3H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>10</sub>N<sub>2</sub>O<sub>4</sub>SF [M-H]<sup>-</sup> 309.0351, found 309.0354.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.87.

### 3-Amino-*N*-(2-fluorophenyl)-*N*-methylbenzenesulfonamide (B63)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B63b** (0.180 g, 0.580 mmol, 1.00 equiv), 10% Pd/C (0.018 g, 10 wt%), MeOH (3.0 mL) and EtOAc (3.0 mL). Stirring at rt for 17 h and purification by SGCC (*n*-hexane:EtOAc 0–60%) provided the title compound (0.150 g, 0.535 mmol, 92%). This is a known compound (CAS no.: 1226330-08-0).

**Physical state:** White solid.

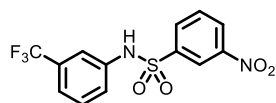
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.38 (dddd, *J* = 8.5, 7.1, 5.0, 1.9 Hz, 1H), 7.26 (ddd, *J* = 10.8, 8.3, 1.4 Hz, 1H), 7.23–7.15 (m, 2H), 7.13 (td, *J* = 7.8, 1.9 Hz, 1H), 6.85 (t, *J* = 2.0 Hz, 1H), 6.82 (ddd, *J* = 8.1, 2.4, 1.0 Hz, 1H), 6.73–6.67 (m, 1H), 5.62 (s, 2H), 3.10 (d, *J* = 1.0 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 160.5, 158.0, 150.0, 138.1, 130.49, 130.45, 130.4, 130.1, 129.1, 129.0, 125.2, 125.1, 118.5, 117.2, 117.0, 114.4, 112.1, 38.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>SF [M+H]<sup>+</sup> 281.0755, found 281.0765.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.39.

### 3-Nitro-*N*-(3-(trifluoromethyl)phenyl)benzenesulfonamide (B64a)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 3-(trifluoromethyl)aniline (0.687 mL, 5.50 mmol, 1.10 equiv), 3-nitrobenzenesulfonyl chloride (1.11 g, 5.00 mmol, 1.00 equiv), pyridine (1.21 mL, 15.0 mmol, 3.00 equiv) and DCM (25.0 mL). Stirring at rt for 18 h provided the title compound (1.13 g, 3.27 mmol, 65%). This is a known compound. Experimental data correlate with literature.<sup>233</sup>

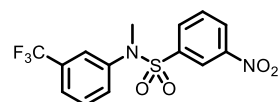
**Physical state:** Light orange solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.01 (s, 1H), 8.51–8.44 (m, 2H), 8.16 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.88 (t, *J* = 8.0 Hz, 1H), 7.52 (t, *J* = 8.2 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.42–7.36 (m, 2H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>8</sub>N<sub>2</sub>O<sub>4</sub>SF<sub>3</sub> [M+H]<sup>+</sup> 345.0157, found 345.0161.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.25.

### ***N*-Methyl-3-nitro-*N*-(3-(trifluoromethyl)phenyl)benzenesulfonamide (B64b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B64a** (1.13 g, 3.27 mmol, 1.00 equiv), MeI (0.306 mL, 4.91 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (2.71 g, 19.6 mmol, 6.00 equiv) and anhydrous DMF (15.0 mL). Stirring at rt for 22 h provided the title compound (1.19 g, 3.27 mmol, quant.). This is a known compound (CAS no.: 2216237-98-6).

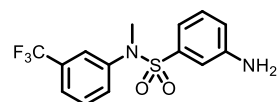
**Physical state:** Light orange solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.46 (ddd, *J* = 8.1, 2.3, 1.2 Hz, 1H), 8.42 (t, *J* = 2.0 Hz, 1H), 7.79 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.69 (t, *J* = 7.9 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.50 (t, *J* = 7.9 Hz, 1H), 7.38 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.27 (d, *J* = 2.1 Hz, 1H), 3.26 (s, 3H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>O<sub>6</sub>SF<sub>3</sub> [M-H]<sup>-</sup> 405.0368, found 405.0375.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.32.

### **3-Amino-*N*-methyl-*N*-(3-(trifluoromethyl)phenyl)benzenesulfonamide (B64)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B64b** (1.19 g, 3.27 mmol, 1.00 equiv), 10% Pd/C (0.119 g, 10 wt%), MeOH (15.0 mL) and EtOAc (15.0 mL). Stirring at rt for 22 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.543 g, 0.735 mmol, 50%). This is a novel compound.

**Physical state:** Beige solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.65 (d, *J* = 7.8 Hz, 1H), 7.59 (t, *J* = 8.2 Hz, 1H), 7.46–7.39 (m, 2H), 7.16 (t, *J* = 7.9 Hz, 1H), 6.80 (dd, *J* = 8.1, 2.3 Hz, 1H), 6.73 (t, *J* = 2.1 Hz, 1H), 6.57–6.49 (m, 1H), 5.63 (s, 2H), 3.16 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.6, 149.5, 142.1, 136.0, 130.1, 129.9, 129.7, 129.6, 129.6, 129.3, 129.0, 125.1, 123.6, 123.5, 122.7, 122.7, 122.6, 122.4, 118.2, 113.9, 111.6, 37.6.

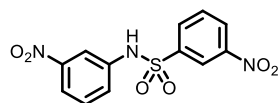


**$^{19}\text{F}$  NMR** (377 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  -61.18.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_2\text{F}_3\text{S}$   $[\text{M}+\text{H}]^+$  331.0728, found 331.0722.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 1:1): 0.40.

### 3-Nitro-*N*-(3-nitrophenyl)benzenesulfonamide (B65a)



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 3-nitroaniline (0.304 g, 2.20 mmol, 1.10 equiv), 3-nitrobenzenesulfonyl chloride (0.443 g, 2.00 mmol, 1.00 equiv), pyridine (0.485 mL, 6.00 mmol, 3.00 equiv) and DCM (8.0 mL). Stirring at rt for 18 h provided the title compound (0.486 g, 1.51 mmol, 75%). This is a known compound (CAS no.: 50891-99-1).

**Physical state:** Orange solid.

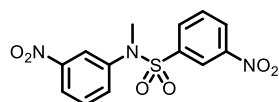
**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  11.22 (s, 1H), 8.53 (t,  $J$  = 2.1 Hz, 1H), 8.47 (dd,  $J$  = 8.1, 2.2 Hz, 1H), 8.23–8.14 (m, 1H), 7.97–7.90 (m, 2H), 7.87 (t,  $J$  = 8.1 Hz, 1H), 7.60–7.51 (m, 2H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  148.3, 148.0, 140.3, 138.2, 132.6, 131.7, 131.1, 128.1, 126.1, 121.5, 119.3, 114.3.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{12}\text{H}_8\text{N}_3\text{O}_6\text{S}$   $[\text{M}+\text{H}]^+$  322.0134, found 322.0142.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 2:1): 0.24

### *N*-Methyl-3-nitro-*N*-(3-nitrophenyl)benzenesulfonamide (B65b)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B65a** (0.480 g, 1.49 mmol, 1.00 equiv), MeI (0.139 mL, 2.23 mmol, 1.50 equiv),  $\text{K}_2\text{CO}_3$  (1.23 g, 8.91 mmol, 6.00 equiv) and anhydrous DMF (7.5 mL). Stirring at rt for 3 days provided the title compound (0.519 g, 1.49 mmol, quant.). This is a known compound (CAS no.: 2704754-64-1).

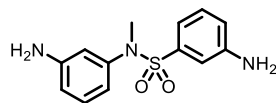
**Physical state:** Golden-brown oil.

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.48 (ddd,  $J$  = 8.1, 2.3, 1.1 Hz, 1H), 8.44 (t,  $J$  = 2.0 Hz, 1H), 8.19 (ddd,  $J$  = 7.9, 2.2, 1.4 Hz, 1H), 7.88 (t,  $J$  = 2.2 Hz, 1H), 7.83 (dt,  $J$  = 7.9, 1.4 Hz, 1H), 7.72 (t,  $J$  = 8.0 Hz, 1H), 7.65–7.60 (m, 1H), 7.58 (t,  $J$  = 8.0 Hz, 1H), 3.30 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{CDCl}_3$ ):  $\delta$  148.6, 148.4, 142.0, 138.3, 133.1, 133.0, 130.8, 130.3, 128.0, 122.9, 122.8, 120.7, 38.3.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.60.

### 3-Amino-*N*-(3-aminophenyl)-*N*-methylbenzenesulfonamide (B65)



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B65b** (0.510 g, 1.49 mmol, 1.00 equiv), 10% Pd/C (0.051 g, 10 wt%), MeOH (7.5 mL) and EtOAc (7.5 mL). Stirring at rt for 21 h and purification by SGCC (*n*-hexane:EtOAc 0–80%) provided the title compound (0.403 g, 1.45 mmol, 98%). This is a novel compound.

**Physical state:** Colourless oil.

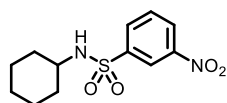
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.15 (t, *J* = 7.9 Hz, 1H), 6.91 (t, *J* = 8.0 Hz, 1H), 6.81 (t, *J* = 2.1 Hz, 1H), 6.80–6.73 (m, 1H), 6.61 (dt, *J* = 7.8, 1.2 Hz, 1H), 6.47–6.39 (m, 1H), 6.38 (t, *J* = 2.1 Hz, 1H), 6.22–6.11 (m, 1H), 5.57 (s, 2H), 5.17 (s, 2H), 3.04 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.3, 149.1, 142.1, 137.1, 129.3, 128.9, 117.7, 114.1, 112.9, 112.6, 111.9, 111.8, 38.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>16</sub>N<sub>3</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 278.0963, found 278.0956.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.13.

### *N*-Cyclon-hexaneyl-3-nitrobenzenesulfonamide (B66a)



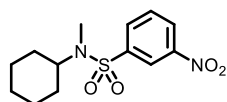
**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from cyclohexylamine (0.252 mL, 2.20 mmol, 1.10 equiv), 3-nitrobenzenesulfonyl chloride (0.443 g, 2.00 mmol, 1.00 equiv), pyridine (0.485 mL, 6.00 mmol, 3.00 equiv) and DCM (8.0 mL). Stirring at rt for 23 h provided the title compound (0.341 g, 1.20 mmol, 60%). This is a known compound.<sup>234</sup>

**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.55 (t, *J* = 2.0 Hz, 1H), 8.46 (ddd, *J* = 8.3, 2.3, 1.0 Hz, 1H), 8.23 (dt, *J* = 7.7, 1.3 Hz, 1H), 8.03 (d, *J* = 7.3 Hz, 1H), 7.89 (t, *J* = 8.0 Hz, 1H), 3.00 (s, 1H), 1.62–1.49 (m, 4H), 1.43 (d, *J* = 12.2 Hz, 1H), 1.20–0.94 (m, 5H).

$R_f$  (silica gel, *n*-hexane:EtOAc 2:1): 0.35.

### ***N*-Cyclohexyl-*N*-methyl-3-nitrobenzenesulfonamide (B66b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B66a** (0.325 g, 1.14 mmol, 1.00 equiv), MeI (0.142 mL, 2.29 mmol, 2.00 equiv), K<sub>2</sub>CO<sub>3</sub> (0.632 g, 4.57 mmol, 4.00 equiv) and anhydrous DMF (3.0 mL). Stirring at rt for 3 days provided the title compound (0.326 g, 1.04 mmol, 91%). This is a known compound (CAS no.: 885373-98-8).

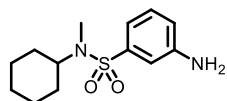
**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.49 (ddd, *J* = 8.2, 2.3, 1.0 Hz, 1H), 8.42 (t, *J* = 2.0 Hz, 1H), 8.26 (dt, *J* = 8.0, 1.3 Hz, 1H), 7.91 (t, *J* = 8.0 Hz, 1H), 3.70 (tt, *J* = 11.3, 4.2 Hz, 1H), 2.73 (s, 3H), 1.70–1.60 (m, 2H), 1.51 (d, *J* = 13.5 Hz, 1H), 1.44–1.17 (m, 6H), 0.98 (qt, *J* = 12.6, 3.6 Hz, 1H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>19</sub>N<sub>2</sub>O<sub>4</sub>S [M+H]<sup>+</sup> 299.1060, found 299.1065.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.50.

### ***3*-Amino-*N*-cyclohexyl-*N*-methylbenzenesulfonamide (B66)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B66b** (0.320 g, 1.02 mmol, 1.00 equiv), 10% Pd/C (0.032 g, 10 wt%), MeOH (5.0 mL) and EtOAc (5.0 mL). Stirring at rt for 22 h provided the title compound (0.234 g, 0.872 mmol, 85%). This is a known compound.<sup>235</sup>

**Physical state:** Off-white solid.

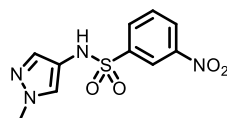
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.19 (t, *J* = 7.9 Hz, 1H), 6.96 (t, *J* = 2.0 Hz, 1H), 6.88–6.78 (m, 1H), 6.75 (dd, *J* = 8.1, 2.2 Hz, 1H), 5.58 (s, 2H), 3.56 (ddd, *J* = 15.4, 9.1, 6.7 Hz, 1H), 2.64 (s, 3H), 1.66 (d, *J* = 12.5 Hz, 2H), 1.51 (dt, *J* = 12.9, 3.2 Hz, 1H), 1.38–0.87 (m, 8H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.5, 140.1, 129.6, 117.3, 113.1, 111.0, 56.3, 29.4, 28.6, 25.3, 24.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>21</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 269.1318, found 269.1326.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.39.

### ***N*-(1-Methyl-1*H*-pyrazol-4-yl)-3-nitrobenzenesulfonamide (B67a)**



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from 1-methyl-1*H*-pyrazol-4-amine (0.194 g, 2.00 mmol, 2.00 equiv), 3-nitrobenzenesulfonyl chloride (0.443 g, 2.00 mmol, 1.00 equiv), pyridine (0.485 mL, 6.00 mmol, 3.00 equiv) and DCM (8.0 mL). Stirring at rt for 44 h provided the title compound (0.402 g, 1.423 mmol, 71%). This is a novel compound.

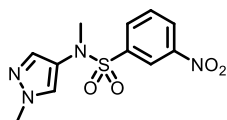
**Physical state:** Brown solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.00 (s, 1H), 8.51–8.42 (m, 2H), 8.07 (dt, *J* = 7.9, 1.3 Hz, 1H), 7.86 (t, *J* = 7.9 Hz, 1H), 7.51 (s, 1H), 7.08 (s, 1H), 3.70 (s, 3H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>10</sub>H<sub>11</sub>N<sub>4</sub>O<sub>4</sub>S [M+H]<sup>+</sup> 283.0496, found 283.0497.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.13.

#### ***N*-Methyl-*N*-(1-methyl-1*H*-pyrazol-4-yl)-3-nitrobenzenesulfonamide (**B67b**)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B67a** (0.390 g, 1.38 mmol, 1.00 equiv), MeI (0.172 mL, 2.76 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.764 g, 5.53 mmol, 4.00 equiv) and anhydrous DMF (3.0 mL). Stirring at rt for 42 h provided the title compound (0.397 g, 1.34 mmol, 97%). This is a novel compound.

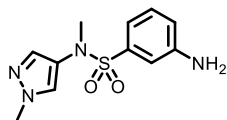
**Physical state:** Brown solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.54 (ddd, *J* = 8.2, 2.3, 1.1 Hz, 1H), 8.20 (t, *J* = 2.0 Hz, 1H), 7.97 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.90 (t, *J* = 8.0 Hz, 1H), 7.70 (s, 1H), 7.30 (s, 1H), 3.76 (s, 3H), 3.08 (s, 3H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>11</sub>H<sub>12</sub>N<sub>4</sub>O<sub>4</sub>S [M+H]<sup>+</sup> 297.0652, found 297.0665.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.61.

#### **3-Amino-*N*-methyl-*N*-(1-methyl-1*H*-pyrazol-4-yl)benzenesulfonamide (**B67**)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B67b** (0.390 g, 1.32 mmol, 1.00 equiv), 10% Pd/C (0.039 g, 10 wt%), MeOH (5.0 mL) and EtOAc (5.0 mL). Stirring at rt for 19 h provided the title compound (0.328 g, 1.23 mmol, 93%). This is a novel compound.

**Physical state:** Brown solid.

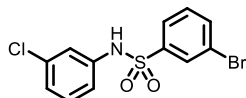
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.61 (s, 1H), 7.25 (d, *J* = 0.9 Hz, 1H), 7.16 (dd, *J* = 8.8, 7.6 Hz, 1H), 6.84–6.73 (m, 2H), 6.69–6.58 (m, 1H), 5.61 (s, 2H), 3.75 (s, 3H), 3.00 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.4, 136.1, 133.2, 129.4, 125.1, 124.3, 117.9, 113.9, 111.6, 38.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>11</sub>H<sub>15</sub>N<sub>4</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 267.0910, found 267.0922.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.42.

### 3-Bromo-*N*-(3-chlorophenyl)benzenesulfonamide (B68a)



**Synthetic procedure:** To a solution of 3-chloroaniline (0.053 mL, 0.500 mmol, 1.00 equiv) and pyridine (0.121 mL, 1.50 mmol, 3.00 equiv) in DCM (2.0 mL) was added 3-bromobenzenesulfonyl chloride (0.128 g, 0.500 mmol, 1.00 equiv) in portions. The mixture was stirred at rt for 18 h. Upon reaction completion as determined by TLC, the mixture was diluted with DCM (10 mL) and washed with 1 M HCl (2 x 5 mL), sat. NaHCO<sub>3</sub> (5 mL) and sat. brine (5 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo* to provide the title compound (0.188 g, 0.500 mmol, quant.). This is a known compound (CAS no.: 876576-28-2).

**Physical state:** Beige solid.

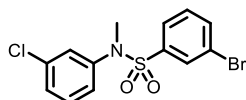
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.68 (s, 1H), 7.90 (d, *J* = 1.9 Hz, 1H), 7.89–7.84 (m, 1H), 7.75 (dt, *J* = 8.0, 1.3 Hz, 1H), 7.54 (t, *J* = 7.9 Hz, 1H), 7.30 (t, *J* = 8.0 Hz, 1H), 7.15–7.10 (m, 2H), 7.09–7.04 (m, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 141.0, 138.8, 136.2, 133.5, 131.8, 131.1, 129.0, 125.7, 124.3, 122.2, 119.5, 118.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>12</sub>H<sub>8</sub>BrClNO<sub>2</sub>S [M-H]<sup>−</sup> 345.9133, found 345.9131.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.63.

### 3-Bromo-*N*-(3-chlorophenyl)-*N*-methylbenzenesulfonamide (B68b)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B68a** (0.170 g, 0.500 mmol, 1.00 equiv), MeI (0.047 mL, 0.750 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.415 g, 3.00 mmol, 6.00 equiv) and anhydrous DMF (2.5 mL). Stirring at rt for 17 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.170 g, 0.472 mmol, 94%). This is a novel compound.

**Physical state:** Colourless oil.

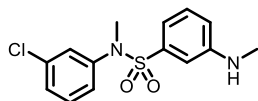
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.95 (ddd, *J* = 7.9, 2.0, 1.2 Hz, 1H), 7.60–7.55 (m, 2H), 7.51 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.44–7.37 (m, 2H), 7.25–7.21 (m, 1H), 7.19–7.09 (m, 1H), 3.16 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 142.1, 137.4, 136.4, 133.1, 131.7, 130.6, 129.6, 127.5, 126.5, 126.2, 125.0, 122.3, 37.8.

**HRMS** (APCI): *m/z* calc. for C<sub>13</sub>H<sub>12</sub>BrClNO<sub>2</sub>S [M+H]<sup>+</sup> 361.9435, found 361.9433.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.45.

### ***N*-(3-Chlorophenyl)-*N*-methyl-3-(methyamino)benzenesulfonamide (B68)**



**Synthetic procedure:** A previously reported Ullmann-type cross-coupling protocol was followed with minor modifications.<sup>172</sup> A pressure vial was charged with **B68b** (0.100 g, 0.277 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (1.0 mL, 11.6 mmol, 41.7 equiv) and powdered Cu (0.9 mg, 0.014 mmol, 0.050 equiv). The mixture was stirred under air at 100 °C for 3 days. Upon reaction completion as determined by TLC, the mixture was added water (10 mL) and EtOAc (10 mL), and the aq. phase extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness in vacuo. Purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.029 g, 0.092 mmol, 33%). This is a novel compound.

**Physical state:** White solid.

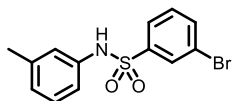
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.40–7.33 (m, 2H), 7.26 (t, *J* = 7.9 Hz, 1H), 7.20 (t, *J* = 1.7 Hz, 1H), 7.12 (dt, *J* = 7.1, 2.1 Hz, 1H), 6.79 (ddd, *J* = 8.3, 2.4, 0.9 Hz, 1H), 6.64 (ddd, *J* = 7.7, 1.8, 0.9 Hz, 1H), 6.53 (t, *J* = 2.1 Hz, 1H), 6.23 (q, *J* = 5.0 Hz, 1H), 3.11 (s, 3H), 2.60 (d, *J* = 4.9 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.1, 142.8, 136.1, 132.8, 130.3, 129.6, 126.9, 125.8, 124.5, 116.1, 113.7, 109.2, 37.6, 29.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub>SCI [M+MeCN+H]<sup>+</sup> 352.0887, found 352.0893.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.57.

### **3-Bromo-*N*-(*m*-tolyl)benzenesulfonamide (B69a)**



**Synthetic procedure:** The same procedure employed for **B18a** was followed starting from *m*-toluidine (0.118 mL, 1.10 mmol, 1.10 equiv), 3-bromobenzenesulfonyl chloride (0.256 g, 1.00 mmol, 1.00 equiv),

pyridine (0.243 mL, 3.00 mmol, 3.00 equiv) and DCM (4.0 mL). Stirring at rt for 24 h provided the title compound (0.353 g, 1.00 mmol, quant.). This is a known compound (CAS no.: 906270-79-9).

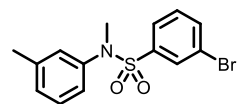
**Physical state:** Orange oil.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.31 (s, 1H), 7.87 (t, *J* = 1.8 Hz, 1H), 7.83 (ddd, *J* = 8.0, 2.1, 1.0 Hz, 1H), 7.76–7.69 (m, 1H), 7.51 (t, *J* = 7.9 Hz, 1H), 7.12 (t, *J* = 7.7 Hz, 1H), 6.92–6.84 (m, 3H), 2.20 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 141.5, 138.6, 137.2, 135.7, 131.5, 129.1, 129.0, 125.7, 125.3, 122.0, 121.0, 117.5, 21.0.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.38.

### 3-Bromo-*N*-methyl-*N*-(*m*-tolyl)benzenesulfonamide (B69b)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B69a** (0.350 g, 1.00 mmol, 1.00 equiv), MeI (0.093 mL, 1.50 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.829 g, 6.00 mmol, 6.00 equiv) and anhydrous DMF (5.0 mL). Stirring at rt for 24 h provided the title compound (0.311 g, 0.913 mmol, 91%). This is a known compound (CAS no.: 1456104-17-8).

**Physical state:** Brown oil.

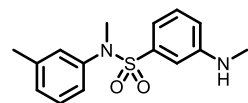
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.93 (dt, *J* = 7.6, 1.8 Hz, 1H), 7.60–7.49 (m, 3H), 7.24 (t, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 7.6 Hz, 1H), 6.93 (t, *J* = 1.9 Hz, 1H), 6.90 (s, 0H), 3.13 (s, 3H), 2.26 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 140.7, 138.5, 137.8, 136.1, 131.5, 129.7, 128.8, 128.3, 127.1, 126.5, 123.3, 122.1, 38.1, 20.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>15</sub>NO<sub>2</sub>SBr [M+H]<sup>+</sup> 340.0007, found 340.0022.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.45.

### *N*-Methyl-3-(methylamino)-*N*-(*m*-tolyl)benzenesulfonamide (B69)



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from **B69b** (0.310 g, 0.913 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (0.395 mL, 4.57 mmol, 5.00 equiv) and powdered Cu (3.0 mg, 0.046 mmol, 0.050 equiv). Stirring at 100 °C for 3 days and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.090 g, 0.312 mmol, 34%). This is a novel compound.

**Physical state:** Light brown oil.

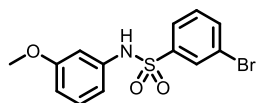
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.23 (dt, *J* = 13.2, 7.8 Hz, 2H), 7.09 (d, *J* = 7.6 Hz, 1H), 6.95 (s, 1H), 6.88 (d, *J* = 8.1 Hz, 1H), 6.81–6.75 (m, 1H), 6.66–6.60 (m, 1H), 6.56 (d, *J* = 2.0 Hz, 1H), 6.19 (q, *J* = 5.0 Hz, 1H), 3.08 (s, 3H), 2.60 (d, *J* = 4.8 Hz, 3H), 2.26 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.0, 141.4, 138.1, 136.6, 129.4, 128.5, 127.7, 126.9, 123.1, 115.8, 113.9, 109.4, 37.9, 29.3, 20.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>19</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 291.1167, found 291.1165.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.47.

### 3-Bromo-*N*-(3-methoxyphenyl)benzenesulfonamide (B70a)



**Synthetic procedure:** The same procedure employed for **B68a** was followed starting from *m*-anisidine (0.056 mL, 0.500 mmol, 1.00 equiv), 3-bromobenzenesulfonyl chloride (0.128 g, 0.500 mmol, 1.00 equiv), pyridine (0.121 mL, 1.50 mmol, 3.00 equiv) and DCM (2.0 mL). Stirring at rt for 18 h provided the title compound (0.177 g, 0.500 mmol, quant.). This is a known compound (CAS no.: 849511-37-1).

**Physical state:** Orange oil.

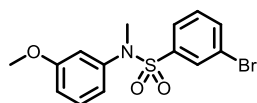
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.39 (s, 1H), 7.89 (t, *J* = 1.9 Hz, 1H), 7.84 (ddd, *J* = 8.0, 2.0, 1.0 Hz, 1H), 7.74 (ddd, *J* = 7.9, 1.8, 1.0 Hz, 1H), 7.52 (t, *J* = 7.9 Hz, 1H), 7.15 (td, *J* = 7.8, 1.2 Hz, 1H), 6.73–6.58 (m, 3H), 3.67 (s, 3H), 2.08 (s, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 159.7, 141.4, 138.4, 135.9, 131.6, 130.2, 129.0, 125.8, 122.1, 112.3, 109.6, 106.1, 55.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>11</sub>BrNO<sub>3</sub>S [M-H]<sup>−</sup> 341.9628, found 341.9628.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.56.

### 3-Bromo-*N*-(3-methoxyphenyl)-*N*-methylbenzenesulfonamide (B70b)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B70a** (0.170 g, 0.500 mmol, 1.00 equiv), MeI (0.047 mL, 0.750 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.415 g, 3.00 mmol, 6.00 equiv) and anhydrous DMF (2.5 mL). Stirring at rt for 17 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.125 g, 0.351 mmol, 70%). This is a known compound.<sup>236</sup>

**Physical state:** Colourless oil.



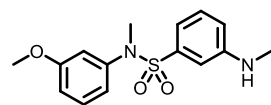
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.93 (dt, *J* = 7.2, 2.0 Hz, 1H), 7.61–7.50 (m, 3H), 7.27 (t, *J* = 8.1 Hz, 1H), 6.91 (ddd, *J* = 8.4, 2.5, 0.9 Hz, 1H), 6.72–6.62 (m, 2H), 3.70 (s, 3H), 3.14 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 159.4, 141.9, 137.8, 136.2, 131.5, 129.7, 129.7, 126.5, 122.1, 118.4, 113.2, 112.3, 55.3, 38.1.

**HRMS** (APCI): *m/z* calc. for C<sub>14</sub>H<sub>15</sub>BrNO<sub>3</sub>S [M+H]<sup>+</sup> 357.9930, found 357.9930.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.41.

### ***N*-(3-Methoxyphenyl)-*N*-methyl-3-(methylamino)benzenesulfonamide (B70)**



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from **B70b** (0.100 g, 0.281 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (1.0 mL, 11.6 mmol, 41.3 equiv) and powdered Cu (0.9 mg, 0.014 mmol, 0.050 equiv). Stirring at 100 °C for 3 days and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.029 g, 0.095 mmol, 34%). This is a novel compound.

**Physical state:** Colourless oil.

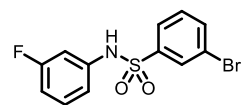
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.24 (td, *J* = 8.0, 3.9 Hz, 2H), 6.86 (ddd, *J* = 8.4, 2.5, 0.9 Hz, 1H), 6.78 (ddd, *J* = 8.3, 2.4, 0.9 Hz, 1H), 6.72–6.62 (m, 3H), 6.58 (t, *J* = 2.1 Hz, 1H), 6.20 (q, *J* = 5.0 Hz, 1H), 3.69 (s, 3H), 3.10 (s, 3H), 2.60 (d, *J* = 4.9 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 159.3, 150.0, 142.6, 136.6, 129.49, 129.45, 118.1, 115.8, 113.8, 112.5, 112.1, 109.4, 55.2, 37.9, 29.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>19</sub>N<sub>2</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 307.1116, found 307.1121.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.51.

### **3-Bromo-*N*-(3-fluorophenyl)benzenesulfonamide (B71a)**



**Synthetic procedure:** The same procedure employed for **B68a** was followed starting from 3-fluoroaniline (0.048 mL, 0.500 mmol, 1.00 equiv), 3-bromobenzenesulfonyl chloride (0.128 g, 0.500 mmol, 1.00 equiv), pyridine (0.121 mL, 1.50 mmol, 3.00 equiv) and DCM (2.0 mL). Stirring at rt for 18 h provided the title compound (0.144 g, 0.436 mmol, 87%). This is a known compound.<sup>237</sup>

**Physical state:** Beige solid.

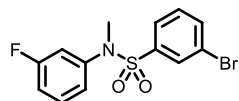
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.69 (s, 1H), 7.91 (t, *J* = 1.9 Hz, 1H), 7.86 (ddd, *J* = 8.1, 2.1, 1.0 Hz, 1H), 7.77 (dt, *J* = 7.9, 1.3 Hz, 1H), 7.53 (t, *J* = 7.9 Hz, 1H), 7.30 (ddd, *J* = 8.9, 7.8, 6.3 Hz, 1H), 6.96–6.84 (m, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 163.5, 161.0, 141.1, 139.2, 136.1, 131.7, 131.2 (d, *J* = 9.6 Hz), 129.0, 125.8, 122.2, 115.8, 111.2, 111.0, 106.9, 106.6.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>12</sub>H<sub>8</sub>BrFNO<sub>2</sub>S [M-H]<sup>−</sup> 329.9428, found 329.9428.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.59.

### 3-Bromo-*N*-(3-fluorophenyl)-*N*-methylbenzenesulfonamide (B71b)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B71a** (0.160 g, 0.500 mmol, 1.00 equiv), MeI (0.047 mL, 0.750 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.415 g, 3.00 mmol, 6.00 equiv) and anhydrous DMF (2.5 mL). Stirring at rt for 17 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.125 g, 0.363 mmol, 83%). This is a known compound (CAS no.: 1460276-67-8).

**Physical state:** Colourless oil.

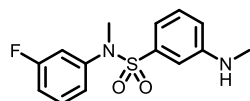
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.94 (ddd, *J* = 7.9, 2.0, 1.2 Hz, 1H), 7.59 (t, *J* = 1.8 Hz, 1H), 7.56 (t, *J* = 7.9 Hz, 1H), 7.51 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.41 (td, *J* = 8.2, 6.7 Hz, 1H), 7.19 (tdd, *J* = 8.4, 2.6, 0.9 Hz, 1H), 7.08–7.00 (m, 2H), 3.16 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 137.4, 136.4, 131.6, 130.6, 130.5, 129.6, 126.5, 122.2, 114.5, 114.3, 113.5, 113.2, 37.8.

**HRMS** (APCI): *m/z* calc. for C<sub>13</sub>H<sub>12</sub>BrFNO<sub>2</sub>S [M+H]<sup>+</sup> 345.9730, found 345.9731.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.43.

### *N*-(3-Fluorophenyl)-*N*-methyl-3-(methylamino)benzenesulfonamide (B71)



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from **B71b** (0.100 g, 0.291 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (1.0 mL, 11.6 mmol, 39.9 equiv) and powdered Cu (0.9 mg, 0.015 mmol, 0.050 equiv). Stirring at 100 °C for 3 days and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.042 g, 0.143 mmol, 49%). This is a novel compound.

**Physical state:** Colourless oil.

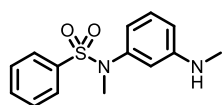
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.42–7.34 (m, 1H), 7.25 (t, *J* = 7.9 Hz, 1H), 7.16–7.09 (m, 1H), 7.04–6.98 (m, 2H), 6.78 (ddd, *J* = 8.3, 2.4, 0.9 Hz, 1H), 6.64 (ddd, *J* = 7.6, 1.8, 0.9 Hz, 1H), 6.54 (t, *J* = 2.1 Hz, 1H), 6.22 (q, *J* = 5.0 Hz, 1H), 3.12 (s, 3H), 2.59 (d, *J* = 4.9 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 162.9, 160.5, 150.1, 143.1, 143.0, 136.2, 130.3, 130.2, 129.6, 121.71, 121.68, 116.0, 113.8, 113.7, 113.6, 113.0, 112.7, 109.2, 37.6, 29.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>SF [M+H]<sup>+</sup> 295.0917, found 295.0905.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.57.

### ***N*-Methyl-*N*-(3-(methylamino)phenyl)benzenesulfonamide (B72)**



**Synthetic procedure:** The same procedure employed for **B44** was followed starting from **B39** (0.052 g, 0.200 mmol, 1.00 equiv), 37% formaldehyde in water (0.016 mL, 0.220 mmol, 1.10 equiv), NaBH(OAc)<sub>3</sub> (0.064 g, 0.300 mmol, 1.50 equiv), glacial AcOH (0.013 mL, 0.220 mmol, 1.10 equiv) and DCM (1.0 mL). Stirring at 0 °C–rt for 24 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.013 g, 0.048 mmol, 24%). This is a novel compound.

**Physical state:** Colourless oil.

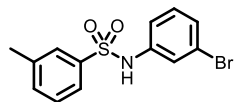
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.69 (ddt, *J* = 8.7, 6.5, 2.1 Hz, 1H), 7.63–7.52 (m, 4H), 6.99 (t, *J* = 8.0 Hz, 1H), 6.43 (dd, *J* = 8.1, 2.3 Hz, 1H), 6.22 (t, *J* = 2.2 Hz, 1H), 6.16 (dd, *J* = 7.8, 2.0 Hz, 1H), 5.78 (q, *J* = 5.0 Hz, 1H), 3.08 (s, 3H), 2.56 (d, *J* = 5.0 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.3, 141.9, 136.4, 133.1, 129.1, 127.4, 112.9, 110.6, 109.6, 38.2, 29.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>17</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 277.1011, found 277.1022.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.22.

### ***N*-(3-Bromophenyl)-3-methylbenzenesulfonamide (B73a)**



**Synthetic procedure:** The same procedure employed for **B68a** was followed starting from 3-bromoaniline (0.054 mL, 0.500 mmol, 1.00 equiv), *m*-toluenesulfonyl chloride (0.072 mL, 0.500 mmol, 1.00 equiv), pyridine (0.121 mL, 1.50 mmol, 3.00 equiv) and DCM (2.0 mL). Stirring at rt for 15 h provided the title compound (0.209 g, 0.500 mmol, quant.). This is a known compound (CAS no.: 568570-28-5).

**Physical state:** Beige solid.

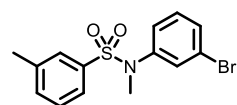
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.54 (s, 1H), 7.60 (s, 1H), 7.59–7.54 (m, 1H), 7.47–7.43 (m, 2H), 7.26–7.16 (m, 3H), 7.14–7.08 (m, 1H), 2.35 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 139.5, 139.2, 139.1, 133.9, 131.2, 129.3, 126.8, 126.6, 123.8, 121.9, 121.8, 118.3, 20.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>11</sub>BrNO<sub>2</sub>S [M-H]<sup>−</sup> 323.9699, found 323.9701.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.57.

#### ***N*-(3-Bromophenyl)-*N*,3-dimethylbenzenesulfonamide (B73b)**



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B73a** (0.190 g, 0.500 mmol, 1.00 equiv), MeI (0.047 mL, 0.750 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.415 g, 3.00 mmol, 6.00 equiv) and anhydrous DMF (2.5 mL). Stirring at rt for 17 h provided the title compound (0.186 g, 0.500 mmol, quant.). This is a novel compound.

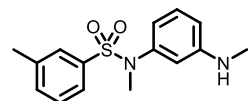
**Physical state:** Brown oil.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.50 (dq, *J* = 13.9, 7.6 Hz, 3H), 7.37–7.25 (m, 4H), 7.13 (ddd, *J* = 8.2, 2.2, 0.9 Hz, 1H), 3.12 (s, 3H), 2.36 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 142.6, 139.1, 135.5, 134.2, 130.7, 130.1, 129.2, 128.8, 127.6, 125.1, 124.6, 121.2, 37.7, 20.8.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.65.

#### ***N*,3-Dimethyl-*N*-(3-(methylamino)phenyl)benzenesulfonamide (B73)**



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from **B73b** (0.170 g, 0.500 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (2.0 mL, 23.1 mmol, 46.2 equiv) and powdered Cu (1.6 mg, 0.025 mmol, 0.050 equiv). Stirring at 100 °C for 26 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.133 g, 0.458 mmol, 92%). This is a novel compound.

**Physical state:** White solid.

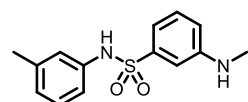
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.50 (d, *J* = 7.6 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 1H), 7.38 (d, *J* = 1.8 Hz, 1H), 7.33 (dt, *J* = 7.7, 1.8 Hz, 1H), 7.00 (t, *J* = 8.0 Hz, 1H), 6.43 (dd, *J* = 8.1, 2.3 Hz, 1H), 6.23 (t, *J* = 2.2 Hz, 1H), 6.20–6.13 (m, 1H), 5.78 (q, *J* = 5.1 Hz, 1H), 3.07 (s, 3H), 2.57 (d, *J* = 5.0 Hz, 3H), 2.36 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.3, 142.0, 138.8, 136.4, 133.7, 129.0, 128.9, 127.7, 124.7, 112.9, 110.6, 109.7, 38.2, 29.5, 20.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>19</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 291.1167, found 291.1158.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.54.

### 3-(Methylamino)-*N*-(*m*-tolyl)benzenesulfonamide (B74)



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from **B69a** (0.163 g, 0.500 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (2.0 mL, 23.1 mmol, 46.2 equiv) and powdered Cu (1.6 mg, 0.025 mmol, 0.050 equiv). Stirring at 100 °C for 23 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.112 g, 0.405 mmol, 81%). This is a novel compound.

**Physical state:** Off-white solid.

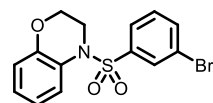
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.07 (s, 1H), 7.19 (t, *J* = 8.0 Hz, 1H), 7.09 (t, *J* = 7.7 Hz, 1H), 6.94–6.86 (m, 4H), 6.84–6.79 (m, 1H), 6.70–6.65 (m, 1H), 6.17 (q, *J* = 5.0 Hz, 1H), 2.63 (d, *J* = 4.9 Hz, 3H), 2.19 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.1, 140.3, 138.3, 138.0, 129.5, 128.9, 124.4, 120.2, 116.7, 115.5, 113.1, 108.7, 29.4, 21.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>17</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 277.1011, found 277.1008.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.19.

### 4-((3-Bromophenyl)sulfonyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (B75a)



**Synthetic procedure:** The same procedure employed for **B68a** was followed starting from 3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (0.135 g, 1.00 mmol, 1.00 equiv), 3-bromobenzenesulfonyl chloride (0.256 g, 1.00 mmol, 1.00 equiv), pyridine (0.243 mL, 3.00 mmol, 3.00 equiv) and DCM (4.0 mL). Stirring at rt for 17 h provided the title compound (0.358 g, 1.00 mmol, quant.). This is a known compound (CAS no.: 1090552-16-1).

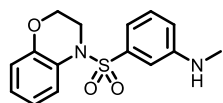
**Physical state:** Red oil.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.84–7.77 (m, 2H), 7.69 (ddd, *J* = 8.0, 2.0, 1.1 Hz, 1H), 7.52 (ddd, *J* = 7.9, 1.8, 1.0 Hz, 1H), 7.31 (t, *J* = 7.9 Hz, 1H), 7.09 (ddd, *J* = 8.2, 7.3, 1.6 Hz, 1H), 6.95 (ddd, *J* = 8.6, 7.3, 1.5 Hz, 1H), 6.82 (dd, *J* = 8.2, 1.5 Hz, 1H), 3.90 (dd, *J* = 5.3, 3.8 Hz, 2H), 3.80 (dd, *J* = 5.3, 3.9 Hz, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 147.3, 141.0, 136.8, 131.3, 130.5, 127.2, 126.1, 125.0, 123.8, 121.6, 118.1, 63.3, 44.9.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.63.

### 3-((2,3-Dihydro-4*H*-benzo[*b*][1,4]oxazin-4-yl)sulfonyl)-*N*-methylaniline (B75)



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from **B75a** (0.350 g, 1.00 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (3.0 mL, 34.7 mmol, 34.7 equiv) and powdered Cu (3.0 mg, 0.050 mmol, 0.050 equiv). Stirring at 100 °C for 53 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.126 g, 0.415 mmol, 42%). This is a novel compound.

**Physical state:** Light yellow solid.

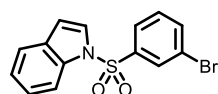
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.67 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.21 (t, *J* = 7.9 Hz, 1H), 7.06 (ddd, *J* = 8.5, 7.3, 1.6 Hz, 1H), 6.91 (ddd, *J* = 8.6, 7.3, 1.6 Hz, 1H), 6.80 (dd, *J* = 8.2, 1.5 Hz, 1H), 6.77–6.67 (m, 2H), 6.56 (t, *J* = 2.1 Hz, 1H), 6.21 (q, *J* = 4.9 Hz, 1H), 3.79 (dd, *J* = 5.3, 3.9 Hz, 2H), 3.66 (dd, *J* = 5.3, 3.9 Hz, 2H), 2.51 (d, *J* = 4.9 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.4, 146.5, 138.4, 129.9, 126.1, 124.3, 123.9, 120.5, 117.4, 116.6, 113.2, 108.3, 62.1, 43.9, 29.2.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>17</sub>N<sub>2</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 305.0960, found 305.0960.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.50.

### 1-((3-Bromophenyl)sulfonyl)-1*H*-indole (B76a)



**Synthetic procedure:** A solution of indole (0.117 g, 1.00 mmol, 1.00 equiv) in anhydrous THF (5.0 mL) was cooled to 0 °C and added 60% NaH in mineral oil (0.052 g, 1.30 mmol, 1.30 equiv). The mixture was stirred at 0 °C for 15 min. A solution of 3-bromobenzenesulfonyl chloride (0.256 g, 1.00 mmol, 1.00 equiv) in anhydrous THF (5.0 mL) was then added dropwise. The mixture was stirred at rt for 23 h. Upon reaction completion as determined by TLC, the mixture was cooled to 0 °C, carefully added H<sub>2</sub>O (10 mL) and

extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:DCM 0–40%) provided the title compound (0.083 g, 0.247 mmol, 25%). This is a known compound (CAS no.: 1809957-22-9).

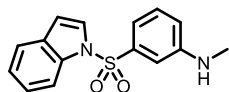
**Physical state:** Off-white solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.05 (t, *J* = 1.9 Hz, 1H), 8.01 (d, *J* = 8.3 Hz, 1H), 7.82 (dt, *J* = 7.9, 1.3 Hz, 1H), 7.68 (dt, *J* = 8.1, 1.4 Hz, 1H), 7.61–7.54 (m, 2H), 7.35 (dt, *J* = 16.1, 7.7 Hz, 2H), 7.31–7.25 (m, 2H), 6.73 (d, *J* = 3.7 Hz, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 140.0, 137.0, 134.9, 130.94, 130.88, 129.8, 126.3, 125.4, 125.1, 123.8, 123.3, 121.7, 113.6, 110.0.

**R<sub>f</sub>** (silica gel, *n*-hexane:DCM 2:1): 0.33.

### 3-((1*H*-Indol-1-yl)sulfonyl)-*N*-methylaniline (B76)



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from **B76a** (0.076 g, 0.226 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (1.00 mL, 11.6 mmol, 51.3 equiv) and powdered Cu (0.7 mg, 0.011 mmol, 0.050 equiv). Stirring at 100 °C for 3 days and purification by SGCC (*n*-hexane:EtOAc 0–40%) provided the title compound (0.059 g, 0.205 mmol, 91%). This is a known compound (CAS no.: 926664-19-9).

**Physical state:** Off-white solid.

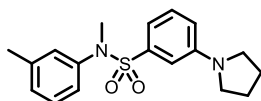
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.90 (d, *J* = 8.3 Hz, 1H), 7.76 (d, *J* = 3.7 Hz, 1H), 7.61 (d, *J* = 7.8 Hz, 1H), 7.33 (q, *J* = 6.7 Hz, 1H), 7.24 (td, *J* = 7.7, 4.9 Hz, 2H), 7.05–6.99 (m, 1H), 6.98 (d, *J* = 2.1 Hz, 1H), 6.83 (d, *J* = 3.7 Hz, 1H), 6.74 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.33 (q, *J* = 5.1 Hz, 1H), 2.63 (d, *J* = 4.9 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.4, 137.8, 134.2, 130.4, 130.1, 127.0, 124.5, 123.3, 121.6, 116.8, 113.1, 112.7, 109.1, 108.1, 29.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>15</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 287.0854, found 287.0854.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.40.

### *N*-Methyl-3-(pyrrolidin-1-yl)-*N*-(*m*-tolyl)benzenesulfonamide (B77)



**Synthetic procedure:** A previously reported *O*-tosylation protocol was followed with minor modifications.

<sup>173</sup> A solution of **B90** (0.174 g, 0.500 mmol, 1.00 equiv) in anhydrous DCM (2.0 mL) was cooled to 0 °C and added anhydrous Et<sub>3</sub>N (0.153 mL, 1.10 mmol, 2.20 equiv), DMAP (0.006 g, 0.050 mmol, 0.100 equiv) and TsCl (0.114 g, 0.600 mmol, 1.20 equiv). The mixture was stirred at 0 °C and slowly allowed to reach rt over 15 h. Upon reaction completion as determined by TLC, the mixture was diluted with DCM (10 mL) and washed with sat. NaHCO<sub>3</sub> (10 mL) and sat. brine (10 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.112 g, 0.339 mmol, 68%). This is a novel compound.

**Physical state:** Colourless oil.

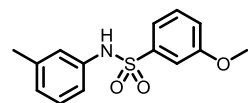
<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.32 (t, *J* = 8.0 Hz, 1H), 7.22 (t, *J* = 7.8 Hz, 1H), 7.10 (d, *J* = 7.5 Hz, 1H), 6.93 (d, *J* = 1.9 Hz, 1H), 6.90 (dd, *J* = 7.9, 2.2 Hz, 1H), 6.80 (dd, *J* = 8.4, 2.5 Hz, 1H), 6.69 (dd, *J* = 7.5, 1.6 Hz, 1H), 6.42 (t, *J* = 2.0 Hz, 1H), 3.14 (q, *J* = 5.0 Hz, 4H), 3.08 (s, 3H), 2.25 (s, 3H), 1.99–1.88 (m, 4H).

<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, DMSO-*d*<sub>6</sub>): δ 147.4, 141.5, 138.1, 136.5, 129.5, 128.5, 127.7, 126.8, 123.2, 115.9, 113.5, 109.3, 47.2, 37.8, 24.9, 20.9.

HRMS (ESI-TOF): *m/z* calc. for C<sub>18</sub>H<sub>23</sub>N<sub>2</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 331.1480, found 331.1487.

R<sub>f</sub> (silica gel, DCM:MeOH 9:1): 0.88.

### 3-Methoxy-*N*-(*m*-tolyl)benzenesulfonamide (**B78a**)



**Synthetic procedure:** The same procedure employed for **B68a** was followed starting from *m*-toluidine (0.054 mL, 0.500 mmol, 1.00 equiv), 3-methoxybenzenesulfonyl chloride (0.071 mL, 0.500 mmol, 1.00 equiv), pyridine (0.121 mL, 1.50 mmol, 3.00 equiv) and DCM (2.0 mL). Stirring at rt for 32 h provided the title compound (0.176 g, 0.500 mmol, quant.). This is a known compound. <sup>238</sup>

**Physical state:** Orange solid.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.19 (s, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.31 (ddd, *J* = 7.7, 1.7, 1.0 Hz, 1H), 7.24 (dd, *J* = 2.6, 1.7 Hz, 1H), 7.16 (ddd, *J* = 8.3, 2.6, 0.9 Hz, 1H), 7.10 (t, *J* = 7.7 Hz, 1H), 6.93–6.86 (m, 2H), 6.86–6.80 (m, 1H), 3.76 (s, 3H), 2.19 (s, 3H).

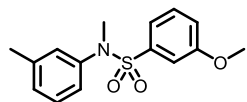
<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, DMSO-*d*<sub>6</sub>): δ 159.3, 140.8, 138.5, 137.6, 130.5, 129.0, 124.9, 120.6, 118.7, 118.6, 117.1, 111.7, 55.6, 21.0.

HRMS (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>14</sub>NO<sub>3</sub>S [M-H]<sup>-</sup> 276.0694, found 276.0700.

R<sub>f</sub> (silica gel, *n*-hexane:EtOAc 1:1): 0.68.



### 3-Methoxy-*N*-methyl-*N*-(*m*-tolyl)benzenesulfonamide (B78)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B78a** (0.140 g, 0.500 mmol, 1.00 equiv), MeI (0.047 mL, 0.750 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.415 g, 3.00 mmol, 6.00 equiv) and anhydrous DMF (2.5 mL). Stirring at rt for 30 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.152 g, 0.500 mmol, quant.). This is a novel compound.

**Physical state:** Colourless oil.

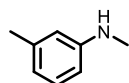
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.50 (t, *J* = 8.0 Hz, 1H), 7.27 (ddd, *J* = 8.3, 2.6, 0.9 Hz, 1H), 7.22 (t, *J* = 7.8 Hz, 1H), 7.10 (dddd, *J* = 10.4, 7.7, 1.7, 0.9 Hz, 2H), 6.94 (d, *J* = 2.0 Hz, 1H), 6.90–6.84 (m, 2H), 3.73 (s, 3H), 3.11 (s, 3H), 2.26 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 159.2, 141.1, 138.3, 137.1, 130.4, 128.6, 128.0, 126.9, 123.1, 119.6, 119.3, 112.1, 55.5, 37.9, 20.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>18</sub>NO<sub>3</sub>S [M+H]<sup>+</sup> 292.1007, found 292.0996.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.67.

### *N*,3-Dimethylaniline (B79a)



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from 3-bromotoluene (1.21 g, 10.0 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (10.0 mL, 116 mmol, 11.6 equiv) and powdered Cu (0.032 g, 0.500 mmol, 0.050 equiv). Stirring at 100 °C for 21 h and purification by SGCC (*n*-hexane:EtOAc 0–40%) provided the title compound (0.215 g, 1.78 mmol, 18%). This is a known compound. Experimental data correlate with literature.<sup>239</sup>

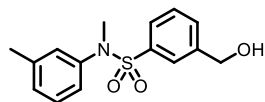
**Physical state:** Colourless oil.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.09 (t, *J* = 7.9 Hz, 1H), 6.55 (d, *J* = 7.4 Hz, 1H), 6.44 (d, *J* = 6.7 Hz, 2H), 3.64 (s, 1H), 2.83 (s, 3H), 2.30 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 149.5, 139.1, 129.2, 118.3, 113.3, 109.8, 30.9, 21.8.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.63.

### 3-(Hydroxymethyl)-*N*-methyl-*N*-(*m*-tolyl)benzenesulfonamide (B79)



**Synthetic procedure:** A previously reported carboxylic acid reduction protocol was followed with minor modifications.<sup>174</sup> A solution of **B80** (0.094 g, 0.308 mmol, 1.00 equiv) in anhydrous THF (1.2 mL) was cooled to 0 °C and dropwise added 1 M BH<sub>3</sub>·THF in THF (1.50 mL, 1.50 mmol, 1.50 equiv). The mixture was stirred at rt for 7 h. Upon reaction completion as determined by TLC, the mixture was cooled to 0 °C, dropwise added conc. HCl (0.5 mL) and stirred at rt for 15 min. The mixture was basified with 30% NH<sub>4</sub>OH to pH 12 and extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.054 g, 0.185 mmol, 60%). This is a known compound (CAS no.: 1466876-24-3).

**Physical state:** Colourless oil.

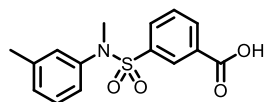
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.61 (d, *J* = 7.6 Hz, 1H), 7.56–7.46 (m, 2H), 7.37–7.31 (m, 1H), 7.21 (t, *J* = 7.8 Hz, 1H), 7.10 (d, *J* = 7.6 Hz, 1H), 6.91 (d, *J* = 2.0 Hz, 1H), 6.84 (dd, *J* = 8.0, 2.2 Hz, 1H), 5.41 (t, *J* = 5.7 Hz, 1H), 4.55 (d, *J* = 5.6 Hz, 2H), 3.10 (s, 3H), 2.25 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 144.0, 141.1, 138.3, 136.1, 130.9, 128.9, 128.7, 128.0, 127.0, 125.7, 125.0, 123.2, 62.0, 38.0, 20.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>18</sub>NO<sub>3</sub>S [M+H]<sup>+</sup> 292.1007, found 292.1001.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.53.

### 3-(*N*-Methyl-*N*-(*m*-tolyl)sulfamoyl)benzoic acid (B80)



**Synthetic procedure:** The same procedure employed for **B68a** was followed starting from **B79a** (0.061 g, 0.500 mmol, 1.00 equiv), 3-(chlorosulfonyl)benzoic acid (0.110 g, 0.500 mmol, 1.00 equiv), pyridine (0.121 mL, 1.50 mmol, 3.00 equiv) and DCM (2.0 mL). Stirring at rt for 22 h, washing with 1 M HCl (10 mL) for the workup and purification by SGCC (DCM:MeOH 0–15%) provided the title compound (0.114 g, 0.375 mmol, 75%). This is a known compound (CAS no.: 1225979-29-2).

**Physical state:** Off-white solid.

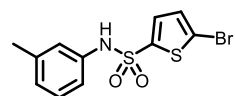
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.52 (s, 1H), 8.26–8.18 (m, 1H), 7.96 (q, *J* = 1.2 Hz, 1H), 7.78–7.68 (m, 2H), 7.22 (t, *J* = 7.8 Hz, 1H), 7.12 (d, *J* = 7.5 Hz, 1H), 6.92 (d, *J* = 2.0 Hz, 1H), 6.86 (dd, *J* = 7.8, 2.2 Hz, 1H), 3.11 (s, 3H), 2.25 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  165.9, 140.8, 138.5, 136.4, 133.7, 131.7, 131.4, 129.9, 128.8, 128.2, 128.0, 127.1, 123.3, 38.0, 20.8.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{15}\text{H}_{16}\text{NO}_4\text{S}$   $[\text{M}+\text{H}]^+$  306.0800, found 306.0799.

**$R_f$**  (silica gel, DCM:MeOH 9:1): 0.29.

#### 5-Bromo-*N*-(*m*-tolyl)thiophene-2-sulfonamide (**B81a**)



**Synthetic procedure:** The same procedure employed for **B68a** was followed starting from *m*-toluidine (0.054 mL, 0.500 mmol, 1.00 equiv), 5-bromothiophene-2-sulfonyl chloride (0.131 g, 0.500 mmol, 1.00 equiv), pyridine (0.121 mL, 1.50 mmol, 3.00 equiv) and DCM (2.0 mL). Stirring at rt for 29 h provided the title compound (0.148 g, 0.446 mmol, 89%). This is a known compound (CAS no.: 663166-74-3).

**Physical state:** Red oil.

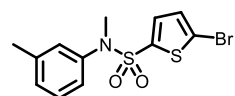
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  7.37 (d,  $J$  = 4.0 Hz, 1H), 7.28 (d,  $J$  = 4.0 Hz, 1H), 7.17 (td,  $J$  = 7.4, 1.4 Hz, 1H), 6.97–6.89 (m, 3H), 2.23 (s, 3H). (400 MHz, DMSO- $d_6$ ):  $\delta$  7.37 (d,  $J$  = 4.0 Hz, 1H), 7.28 (d,  $J$  = 4.0 Hz, 1H), 7.17 (td,  $J$  = 7.4, 1.4 Hz, 1H), 6.97–6.89 (m, 3H), 2.23 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  141.1, 138.7, 137.1, 133.0, 131.3, 129.1, 125.4, 121.0, 118.8, 117.5, 21.1.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{11}\text{H}_9\text{BrNO}_2\text{S}_2$   $[\text{M}-\text{H}]^-$  329.9264, found 329.9267.

**$R_f$**  (silica gel, *n*-hexane:EtOAc 2:1): 0.48.

#### 5-Bromo-*N*-methyl-*N*-(*m*-tolyl)thiophene-2-sulfonamide (**B81b**)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B81a** (0.148 g, 0.446 mmol, 1.00 equiv), MeI (0.042 mL, 0.669 mmol, 1.50 equiv),  $\text{K}_2\text{CO}_3$  (0.370 g, 2.68 mmol, 6.00 equiv) and anhydrous DMF (2.5 mL). Stirring at rt for 24 h provided the title compound (0.128 g, 0.371 mmol, 83%). This is a known compound (CAS no.: 1458443-41-8).

**Physical state:** Brown oil.

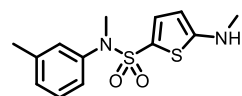
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  7.42 (d,  $J$  = 4.0 Hz, 1H), 7.33 (d,  $J$  = 4.0 Hz, 1H), 7.26 (t,  $J$  = 7.8 Hz, 1H), 7.15 (d,  $J$  = 7.6 Hz, 1H), 7.02 (d,  $J$  = 2.0 Hz, 1H), 6.95 (dd,  $J$  = 8.0, 2.2 Hz, 1H), 3.17 (s, 3H), 2.28 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  140.5, 138.5, 136.8, 133.8, 131.7, 128.9, 128.5, 127.2, 123.2, 119.6, 38.1, 20.8.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{12}\text{H}_{13}\text{NO}_2\text{S}_2\text{Br}$   $[\text{M}+\text{H}]^+$  345.9571, found 345.9562.

$R_f$  (silica gel,  $n$ -hexane:EtOAc 1:1): 0.67.

### ***N*-Methyl-5-(methylamino)-*N*-(*m*-tolyl)thiophene-2-sulfonamide (B81)**



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from **B81b** (0.128 g, 0.371 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (2.0 mL, 23.1 mmol, 62.3 equiv) and powdered Cu (1.2 mg, 0.019 mmol, 0.050 equiv). Stirring at 100 °C for 19 h and purification by SGCC ( $n$ -hexane:EtOAc 0–50%) provided the title compound (0.064 g, 0.216 mmol, 58%). This is a novel compound.

**Physical state:** Purple solid.

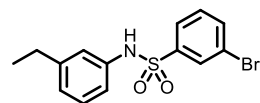
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  7.26–7.19 (m, 2H), 7.08 (ddt,  $J$  = 7.6, 1.8, 0.9 Hz, 1H), 7.03 (d,  $J$  = 4.2 Hz, 1H), 7.00 (d,  $J$  = 2.0 Hz, 1H), 6.98–6.90 (m, 1H), 5.86 (dd,  $J$  = 4.2, 1.2 Hz, 1H), 3.08 (s, 3H), 2.72 (d,  $J$  = 4.7 Hz, 3H), 2.28 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  164.1, 141.6, 138.0, 135.3, 128.5, 127.6, 127.0, 123.0, 112.6, 100.4, 37.7, 32.4, 20.9.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{13}\text{H}_{17}\text{N}_2\text{O}_2\text{S}_2$   $[\text{M}+\text{H}]^+$  297.0731, found 297.0741.

$R_f$  (silica gel,  $n$ -hexane:EtOAc 1:1): 0.45.

### **3-Bromo-*N*-(3-ethylphenyl)benzenesulfonamide B82a)**



**Synthetic procedure:** The same procedure employed for **B68a** was followed starting from 3-ethylaniline (0.062 mL, 0.500 mmol, 1.00 equiv), 3-bromobenzenesulfonyl chloride (0.128 g, 0.500 mmol, 1.00 equiv), pyridine (0.121 mL, 1.50 mmol, 3.00 equiv) and DCM (2.0 mL). Stirring at rt for 29 h provided the title compound (0.165 g, 0.484 mmol, 97%). This is a known compound (CAS no.: 1179666-53-5).

**Physical state:** Orange solid.

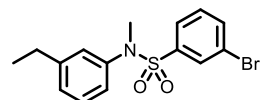
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  10.29 (s, 1H), 7.87–7.79 (m, 2H), 7.72 (dt,  $J$  = 8.1, 1.3 Hz, 1H), 7.51 (t,  $J$  = 7.9 Hz, 1H), 7.15 (t,  $J$  = 7.9 Hz, 1H), 6.94–6.85 (m, 3H), 2.53–2.46 (m, 2H), 1.07 (t,  $J$  = 7.6 Hz, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  144.9, 141.4, 137.2, 135.7, 131.5, 129.2, 129.1, 125.7, 124.2, 122.0, 119.9, 118.0, 28.0, 15.4.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{14}\text{H}_{13}\text{NO}_2\text{SBr}$   $[\text{M}-\text{H}]^-$  337.9856, found 337.9860.

$R_f$  (silica gel,  $n$ -hexane:EtOAc 2:1): 0.50

### 3-Bromo-*N*-(3-ethylphenyl)-*N*-methylbenzenesulfonamide (B82b)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B82a** (0.165 g, 0.484 mmol, 1.00 equiv), MeI (0.045 mL, 0.726 mmol, 1.50 equiv),  $\text{K}_2\text{CO}_3$  (0.401 g, 2.90 mmol, 6.00 equiv) and anhydrous DMF (2.5 mL). Stirring at rt for 24 h provided the title compound (0.139 g, 0.391 mmol, 81%). This is a novel compound.

**Physical state:** Brown oil.

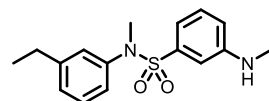
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  7.93 (dt,  $J$  = 7.4, 2.0 Hz, 1H), 7.61–7.47 (m, 3H), 7.27 (t,  $J$  = 7.8 Hz, 1H), 7.17 (dt,  $J$  = 7.8, 1.3 Hz, 1H), 6.95 (ddd,  $J$  = 7.9, 2.4, 1.2 Hz, 1H), 6.87 (t,  $J$  = 2.0 Hz, 1H), 3.14 (s, 3H), 2.59–2.51 (m, 2H), 1.10 (t,  $J$  = 7.6 Hz, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  144.8, 140.7, 137.8, 136.1, 131.5, 129.7, 128.9, 127.2, 126.5, 125.5, 124.0, 122.1, 38.1, 27.9, 15.5.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{15}\text{H}_{17}\text{NO}_2\text{SBr}$   $[\text{M}+\text{H}]^+$  354.0163, found 354.0165.

$R_f$  (silica gel,  $n$ -hexane:EtOAc 1:1): 0.71.

### *N*-(3-Ethylphenyl)-*N*-methyl-(methylamino)benzenesulfonamide (B82)



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from **B82b** (0.138 g, 0.391 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (2.0 mL, 23.1 mmol, 59.1 equiv) and powdered Cu (1.2 mg, 0.020 mmol, 0.050 equiv). Stirring at 100 °C for 19 h and purification by SGCC ( $n$ -hexane:EtOAc 0–50%) provided the title compound (0.027 g, 0.088 mmol, 23%). This is a novel compound.

**Physical state:** White solid.

**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  7.24 (t,  $J$  = 7.8 Hz, 2H), 7.12 (dt,  $J$  = 7.8, 1.3 Hz, 1H), 6.93 (ddd,  $J$  = 7.9, 2.3, 1.1 Hz, 1H), 6.89 (d,  $J$  = 1.9 Hz, 1H), 6.78 (ddd,  $J$  = 8.3, 2.4, 0.9 Hz, 1H), 6.64 (ddd,  $J$  = 7.6, 1.8, 0.9 Hz, 1H),

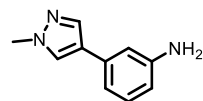
6.53 (t,  $J$  = 2.1 Hz, 1H), 6.18 (q,  $J$  = 5.0 Hz, 1H), 3.09 (s, 3H), 2.58 (d,  $J$  = 4.9 Hz, 3H), 2.57–2.51 (m, 2H), 1.10 (t,  $J$  = 7.6 Hz, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  150.0, 144.4, 141.5, 136.5, 129.4, 128.6, 126.6, 125.5, 123.6, 115.8, 113.9, 109.4, 38.0, 29.3, 27.9, 15.4.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{16}\text{H}_{21}\text{N}_2\text{O}_2\text{S}$   $[\text{M}+\text{H}]^+$  305.1324, found 305.1317.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 1:1): 0.48.

### 3-(1-Methyl-1H-pyrazol-4-yl)aniline (B83a)



**Synthetic procedure:** A previously reported Suzuki-Miyaura cross-coupling protocol was followed with minor modifications.<sup>106</sup> A pressure vial was charged with 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (0.114 g, 0.550 mmol, 1.10 equiv), 3-bromoaniline (0.054 mL, 0.500 mmol, 1.00 equiv),  $\text{Pd}_2\text{dba}_3$  (0.005 g, 0.005 mmol, 0.010 equiv),  $\text{PCy}_3$  (0.004 g, 0.013 mmol, 0.025 equiv) and  $\text{K}_3\text{PO}_4$  (0.180 g, 0.850 mmol, 1.70 equiv) and subjected to 3 x vacuum-Ar cycles. Degassed 1,4-dioxane (2.0 mL) and  $\text{H}_2\text{O}$  (0.8 mL) were then added by syringe. The mixture was stirred at reflux for 17 h. Upon reaction completion as determined by TLC, the mixture was added  $\text{H}_2\text{O}$  (5 mL) and extracted with DCM (2 x 5 mL). The combined organic phases were dried over  $\text{Na}_2\text{SO}_4$  and concentrated to dryness *in vacuo*. Purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.095 g, 0.500 mmol, quant.). This is a known compound.<sup>240</sup>

**Physical state:** Off-white solid.

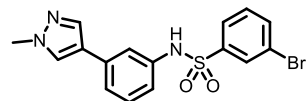
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  7.94 (d,  $J$  = 0.8 Hz, 1H), 7.67 (d,  $J$  = 0.8 Hz, 1H), 6.98 (t,  $J$  = 7.7 Hz, 1H), 6.71 (t,  $J$  = 1.9 Hz, 1H), 6.68 (dt,  $J$  = 7.6, 1.3 Hz, 1H), 6.40 (ddd,  $J$  = 8.1, 2.3, 1.1 Hz, 1H), 5.02 (s, 2H), 3.84 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  149.0, 135.7, 133.0, 129.3, 127.4, 122.6, 113.0, 112.0, 110.4.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{10}\text{H}_{12}\text{N}_3$   $[\text{M}+\text{H}]^+$  174.1031, found 174.1029.

**$R_f$**  (silica gel, DCM:MeOH 9:1): 0.53.

### 3-Bromo-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)benzenesulfonamide (B83b)



**Synthetic procedure:** The same procedure employed for **B68a** was followed starting from **B83a** (0.087 g, 0.500 mmol, 1.00 equiv), 3-bromobenzenesulfonyl chloride (0.128 g, 0.500 mmol, 1.00 equiv), pyridine

(0.121 mL, 1.50 mmol, 3.00 equiv) and DCM (2.0 mL). Stirring at rt for 69 h and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.133 g, 0.340 mmol, 68%). This is a novel compound.

**Physical state:** White solid.

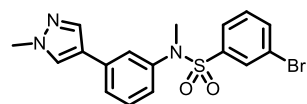
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.40 (s, 1H), 8.04 (s, 1H), 7.91 (t, *J* = 1.8 Hz, 1H), 7.83 (ddd, *J* = 8.0, 2.0, 1.0 Hz, 1H), 7.79–7.73 (m, 1H), 7.71 (d, *J* = 0.9 Hz, 1H), 7.51 (t, *J* = 7.9 Hz, 1H), 7.29–7.18 (m, 3H), 6.88 (dt, *J* = 7.6, 1.8 Hz, 1H), 3.85 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 141.4, 137.7, 135.8, 133.6, 131.6, 129.8, 129.1, 127.9, 125.8, 122.1, 121.4, 121.2, 118.0, 116.8, 38.7.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>16</sub>H<sub>13</sub>BrN<sub>3</sub>O<sub>2</sub>S [M-H]<sup>−</sup> 389.9917, found 389.9924.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.48.

### 3-Bromo-*N*-methyl-*N*-(3-(1-methyl-1*H*-pyrazol-4-yl)phenyl)benzenesulfonamide (B83c)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting **B83b** (0.133 g, 0.340 mmol, 1.00 equiv), MeI (0.032 mL, 0.510 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.282 g, 2.04 mmol, 6.00 equiv) and anhydrous DMF (2.0 mL). Stirring at rt for 17 h provided the title compound (0.109 g, 0.268 mmol, 79%). This is a novel compound.

**Physical state:** Yellow oil.

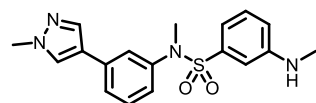
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.12 (s, 1H), 7.97–7.90 (m, 1H), 7.80 (d, *J* = 0.8 Hz, 1H), 7.63–7.46 (m, 4H), 7.36–7.28 (m, 2H), 6.90 (ddd, *J* = 8.0, 2.2, 1.0 Hz, 1H), 3.85 (s, 3H), 3.18 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 141.4, 137.9, 136.1, 133.6, 131.5, 129.7, 129.4, 128.1, 126.5, 124.1, 123.7, 122.9, 122.2, 121.0, 38.7, 38.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub>SBr [M+H]<sup>+</sup> 406.0225, found 406.0228.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.14.

### *N*-Methyl-*N*-(3-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-3-(methylamino)benzenesulfonamide (B83)



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from **B83c** (0.109 g, 0.268 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (1.0 mL, 11.6 mmol, 43.3 equiv) and powdered Cu (0.9 mg,

0.013 mmol, 0.050 equiv). Stirring at 100 °C for 22 h and purification by SGCC (EtOAc 100%) provided the title compound (0.025 g, 0.070 mmol, 26%). This is a novel compound.

**Physical state:** White solid.

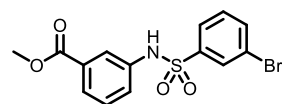
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.09 (s, 1H), 7.77 (d, *J* = 0.8 Hz, 1H), 7.46 (dt, *J* = 8.0, 1.2 Hz, 1H), 7.30 (t, *J* = 7.9 Hz, 1H), 7.28–7.22 (m, 2H), 6.91 (ddd, *J* = 8.0, 2.3, 1.1 Hz, 1H), 6.79 (dd, *J* = 8.2, 2.4 Hz, 1H), 6.67 (dt, *J* = 7.9, 1.0 Hz, 1H), 6.59 (t, *J* = 2.1 Hz, 1H), 6.19 (q, *J* = 4.9 Hz, 1H), 3.85 (s, 3H), 3.13 (s, 3H), 2.58 (d, *J* = 4.9 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.1, 142.1, 136.7, 136.1, 133.3, 129.5, 129.2, 128.0, 123.6, 123.4, 122.8, 121.2, 115.8, 113.9, 109.5, 38.7, 37.9, 29.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>18</sub>H<sub>21</sub>N<sub>4</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 357.1385, found 357.1391.

**R<sub>f</sub>** (silica gel, EtOAc): 0.28.

#### Methyl 3-((3-bromophenyl)sulfonamido)benzoate (B84a)



**Synthetic procedure:** The same procedure employed for **B68a** was followed starting from methyl 3-aminobenzoate (0.151 g, 1.00 mmol, 1.00 equiv), 3-bromobenzenesulfonyl chloride (0.256 g, 1.00 mmol, 1.00 equiv), pyridine (0.243 mL, 3.00 mmol, 3.00 equiv) and DCM (4.0 mL). Stirring at rt for 28 h provided the title compound (0.300 g, 0.811 mmol, 81%). This is a known compound.<sup>241</sup>

**Physical state:** Beige solid.

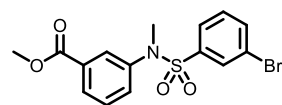
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.66 (s, 1H), 7.88 (d, *J* = 1.9 Hz, 1H), 7.84 (dd, *J* = 7.9, 2.0 Hz, 1H), 7.75–7.71 (m, 1H), 7.69 (d, *J* = 1.9 Hz, 1H), 7.65 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.52 (t, *J* = 8.0 Hz, 1H), 7.43 (t, *J* = 7.8 Hz, 1H), 7.37 (dt, *J* = 8.2, 1.6 Hz, 1H), 3.82 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 165.6, 141.1, 137.7, 136.1, 131.7, 130.6, 130.0, 129.0, 125.7, 125.2, 124.8, 122.2, 120.6, 52.4.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>11</sub>BrNO<sub>4</sub>S [M-H]<sup>-</sup> 367.9598, found 367.9599.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.47.

#### Methyl 3-((3-bromo-*N*-methylphenyl)sulfonamido)benzoate (B84b)





**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B84a** (0.300 g, 0.810 mmol, 1.00 equiv), MeI (0.076 mL, 1.22 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (0.672 g, 4.86 mmol, 6.00 equiv) and anhydrous DMF (4.0 mL). Stirring at rt for 22 h provided the title compound (0.303 g, 0.788 mmol, 97%). This is a novel compound.

**Physical state:** Beige solid.

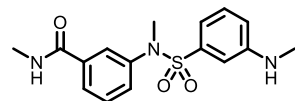
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.97–7.93 (m, 1H), 7.91 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.67 (t, *J* = 1.9 Hz, 1H), 7.60–7.47 (m, 4H), 7.41 (ddd, *J* = 8.1, 2.4, 1.1 Hz, 1H), 3.85 (s, 3H), 3.19 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 165.4, 141.1, 137.4, 136.4, 131.7, 130.9, 130.4, 129.72, 129.65, 128.1, 126.9, 126.5, 122.3, 52.4, 37.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>13</sub>BrNO<sub>4</sub>S [M-H]<sup>-</sup> 381.9754, found 381.9757.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.62.

#### ***N*-Methyl-3-((*N*-methyl-3-(methylamino)phenyl)sulfonamido)benzamide (**B84**)**



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from **B84b** (0.300 g, 0.781 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (1.0 mL, 11.6 mmol, 14.9 equiv) and powdered Cu (2.5 mg, 0.039 mmol, 0.050 equiv). Stirring at 100 °C for 21 h and purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.141 g, 0.423 mmol, 54%). This is a novel compound.

**Physical state:** White solid.

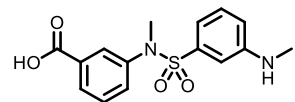
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.47 (q, *J* = 4.5 Hz, 1H), 7.74 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.63 (t, *J* = 2.0 Hz, 1H), 7.42 (t, *J* = 7.9 Hz, 1H), 7.30–7.18 (m, 2H), 6.82–6.74 (m, 1H), 6.61 (dt, *J* = 7.9, 1.0 Hz, 1H), 6.53 (t, *J* = 2.1 Hz, 1H), 6.20 (q, *J* = 4.9 Hz, 1H), 3.13 (s, 3H), 2.76 (d, *J* = 4.5 Hz, 3H), 2.58 (d, *J* = 4.9 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 165.7, 150.1, 141.6, 136.3, 135.0, 129.5, 128.7, 128.5, 125.5, 124.8, 116.0, 113.8, 109.2, 37.7, 29.3, 26.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>16</sub>H<sub>20</sub>N<sub>3</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 334.1225 found 334.1238.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.52.

#### **3-((*N*-Methyl-3-(methylamino)phenyl)sulfonamido)benzoic acid (**B85**)**



**Synthetic procedure:** To a solution of **B84** (0.100 g, 0.299 mmol, 1.00 equiv) in MeOH (3.0 mL) was added 2 M aq. NaOH (0.6 mL, 1.20 mmol, 4.00 equiv). The mixture was stirred at reflux for 43 h and then added powdered NaOH (0.092 g, 2.40 mmol, 8.00 equiv) and stirred at reflux for additional 24 h. Upon reaction completion as determined by TLC, the mixture was diluted with water (10 mL), acidified to pH 4 with 1 M HCl and extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. The crude was redissolved in 1 M NaOH (5 mL) and washed with EtOAc (3 x 5 mL). The aq. layer was then acidified to pH 4 with 1 M HCl and extracted with EtOAc (3 x 5 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo* to provide the title compound (0.011 g, 0.033 mmol, 11%). This is a novel compound.

**Physical state:** White solid.

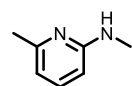
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.16 (s, 1H), 7.84 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.67 (t, *J* = 1.9 Hz, 1H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.37 (ddd, *J* = 8.1, 2.3, 1.2 Hz, 1H), 7.24 (t, *J* = 7.9 Hz, 1H), 6.78 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.62 (dt, *J* = 8.0, 1.0 Hz, 1H), 6.51 (t, *J* = 2.1 Hz, 1H), 6.21 (d, *J* = 5.5 Hz, 1H), 2.58 (d, *J* = 3.8 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 166.7, 150.1, 141.7, 136.2, 130.2, 129.6, 129.1, 127.7, 126.6, 116.0, 113.8, 109.2, 37.7, 29.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>17</sub>N<sub>2</sub>O<sub>4</sub>S [M+H]<sup>+</sup> 321.0909, found 321.0902.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.33.

### ***N*,6-Dimethylpyridin-2-amine (B86a)**



**Synthetic procedure:** A solution of 2-fluoro-6-methylpyridine (0.206 mL, 2.00 mmol, 1.00 equiv) in 40% MeNH<sub>2</sub> in H<sub>2</sub>O (8.0 mL, 92.4 mmol, 46.2 equiv) was stirred at reflux for 25 h. Upon reaction completion as determined by TLC, the mixture was cooled to rt and extracted with DCM (3 x 5 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.297 g, 2.00 mmol, quant.). This is a known compound.<sup>242</sup>

**Physical state:** Colourless oil.

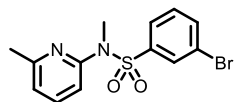
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.35 (dd, *J* = 8.3, 7.3 Hz, 1H), 6.45 (d, *J* = 7.3 Hz, 1H), 6.19 (d, *J* = 8.3 Hz, 1H), 4.54 (s, 1H), 2.88 (d, *J* = 5.3 Hz, 3H), 2.37 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 159.9, 157.4, 138.4, 112.6, 102.5, 29.8, 24.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>7</sub>H<sub>11</sub>N<sub>2</sub> [M+H]<sup>+</sup> 123.0922, found 123.0917.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.42.

### 3-Bromo-*N*-methyl-*N*-(6-methylpyridin-2-yl)benzenesulfonamide (B86b)



**Synthetic procedure:** The same procedure employed for **B68a** was followed starting from **B86a** (0.240 g, 2.00 mmol, 1.00 equiv), 3-bromobenzenesulfonyl chloride (0.511 g, 2.00 mmol, 1.00 equiv), pyridine (0.485 mL, 6.00 mmol, 3.00 equiv) and DCM (8.0 mL). Stirring at rt for 43 h and washing with sat. NaHCO<sub>3</sub> (2 x 10 mL) and sat. brine (10 mL) for the workup provided the title compound (0.380 g, 1.11 mmol, 56%).

This is a novel compound.

**Physical state:** Colourless oil.

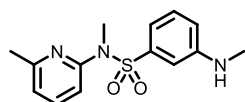
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.80 (t, *J* = 1.8 Hz, 1H), 7.67 (ddd, *J* = 8.0, 2.0, 1.0 Hz, 1H), 7.63–7.53 (m, 2H), 7.39–7.27 (m, 2H), 6.99 (d, *J* = 7.5 Hz, 1H), 3.27 (s, 3H), 2.39 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 157.6, 152.9, 139.7, 138.1, 135.9, 131.0, 130.3, 126.4, 122.8, 121.0, 117.3, 36.0, 24.2.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>SBr [M+H]<sup>+</sup> 340.9959, found 340.9950.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.53.

### *N*-Methyl-3-(methylanino)-*N*-(6-methylpyridin-2-yl)benzenesulfonamide (B86)



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from **B86b** (0.370 g, 1.08 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (3.0 mL, 34.7 mmol, 32.1 equiv) and powdered Cu (3.0 mg, 0.054 mmol, 0.050 equiv). Stirring at 100 °C for 24 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.053 g, 0.183 mmol, 17%). This is a novel compound.

**Physical state:** Colourless oil.

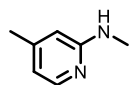
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.70 (t, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.22 (t, *J* = 7.9 Hz, 1H), 7.07 (d, *J* = 7.5 Hz, 1H), 6.80–6.70 (m, 2H), 6.67 (t, *J* = 2.1 Hz, 1H), 6.20 (q, *J* = 5.0 Hz, 1H), 3.20 (s, 3H), 2.60 (d, *J* = 4.9 Hz, 3H), 2.33 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 156.6, 152.4, 150.1, 138.2, 137.8, 129.5, 120.2, 116.0, 115.8, 113.5, 109.0, 35.2, 29.3, 23.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>18</sub>N<sub>3</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 292.1120, found 292.1119.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.41.

### ***N*,4-Dimethylpyridin-2-amine (B87a)**



**Synthetic procedure:** The same procedure employed for **B86a** was followed starting from 2-fluoro-4-methylpyridine (0.206 mL, 2.00 mmol, 1.00 equiv) and 40% MeNH<sub>2</sub> in H<sub>2</sub>O (8.0 mL, 92.4 mmol, 46.2 equiv). Stirring at reflux for 20 h and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.257 g, 2.00 mmol, quant). This is a known compound.<sup>243</sup>

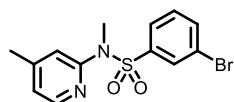
**Physical state:** Light yellow oil.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.95 (d, *J* = 5.2 Hz, 1H), 6.42 (dd, *J* = 5.2, 1.4 Hz, 1H), 6.20 (s, 1H), 4.45 (s, 1H), 2.90 (d, *J* = 5.2 Hz, 3H), 2.24 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 160.0, 148.6, 148.0, 114.5, 106.5, 29.3, 21.4.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.16.

### **3-Bromo-*N*-methyl-*N*-(4-methylpyridin-2-yl)benzenesulfonamide (B87b)**



**Synthetic procedure:** The same procedure employed for **B68a** was followed starting from **B87a** (0.250 g, 2.00 mmol, 1.00 equiv), 3-bromobenzenesulfonyl chloride (0.511 g, 2.00 mmol, 1.00 equiv), pyridine (0.485 mL, 6.00 mmol, 3.00 equiv) and DCM (8.0 mL). Stirring at rt for 45 h, washing with sat. NaHCO<sub>3</sub> (2 x 10 mL) and sat. brine (10 mL) for the workup and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.358 g, 1.05 mmol, 52%). This is a novel compound.

**Physical state:** White solid.

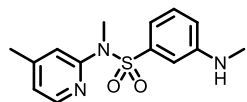
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.17 (d, *J* = 5.0 Hz, 1H), 7.90 (ddd, *J* = 7.9, 2.0, 1.1 Hz, 1H), 7.69 (t, *J* = 1.8 Hz, 1H), 7.61 (ddd, *J* = 7.9, 1.8, 1.1 Hz, 1H), 7.53 (t, *J* = 7.9 Hz, 1H), 7.36 (dt, *J* = 1.5, 0.8 Hz, 1H), 7.17–7.10 (m, 1H), 3.19 (s, 3H), 2.37 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 153.0, 149.4, 147.8, 138.9, 136.2, 131.5, 129.6, 126.4, 123.0, 122.1, 120.7, 35.9, 20.6.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>SBr [M+H]<sup>+</sup> 340.9959, found 340.9956.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.66.

### ***N*-Methyl-3-(methylamino)-*N*-(4-methylpyridin-2-yl)benzenesulfonamide (B87)**



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from **B87b** (0.350 g, 1.03 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (3.0 mL, 34.7 mmol, 33.7 equiv) and powdered Cu (3.0 mg, 0.051 mmol, 0.050 equiv). Stirring at 100 °C for 22 h and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.104 g, 0.357 mmol, 35%). This is a novel compound.

**Physical state:** White solid.

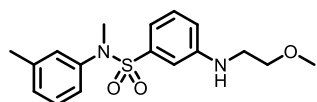
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.16 (d, *J* = 5.0 Hz, 1H), 7.40 (s, 1H), 7.21 (t, *J* = 7.9 Hz, 1H), 7.08 (d, *J* = 5.0 Hz, 1H), 6.74 (dd, *J* = 8.2, 2.3 Hz, 1H), 6.68 (d, *J* = 7.7 Hz, 1H), 6.63 (s, 0H), 6.19 (q, *J* = 5.1 Hz, 1H), 3.16 (s, 3H), 2.59 (d, *J* = 4.8 Hz, 3H), 2.35 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 153.3, 150.1, 148.7, 147.5, 137.3, 129.6, 122.3, 120.1, 115.9, 113.5, 109.0, 35.4, 29.3, 20.6.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>14</sub>H<sub>18</sub>N<sub>3</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 292.1120, found 292.1110.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.39.

### **3-((2-Methoxyethyl)amino)-*N*-methyl-*N*-(*m*-tolyl)benzenesulfonamide (B88)**



**Synthetic procedure:** A previously reported Ullmann-type cross-coupling protocol was followed with minor modifications.<sup>105</sup> A pressure vial was charged with **B69b** (0.102 g, 0.300 mmol, 1.00 equiv), 2-methoxyethan-1-amine (0.039 mL, 0.450 mmol, 1.50 equiv), CuI (0.006 g, 0.030 mmol, 0.100 equiv), L-Proline (0.007 g, 0.060 mmol, 0.200 equiv) and K<sub>3</sub>PO<sub>4</sub> (0.127 g, 0.600 mmol, 2.00 equiv) and purged with Ar. Degassed and anhydrous DMSO was then added by syringe (1.0 mL). The mixture was stirred at 80 °C for 22 h. Upon reaction completion as determined by TLC, the mixture was added H<sub>2</sub>O (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with sat. brine (10 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.072 g, 0.214 mmol, 71%). This is a novel compound.

**Physical state:** Colourless oil.

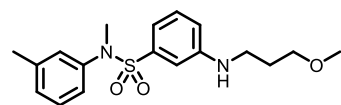
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.22 (td, *J* = 7.8, 6.2 Hz, 2H), 7.09 (d, *J* = 7.5 Hz, 1H), 6.94 (d, *J* = 2.0 Hz, 1H), 6.87 (ddd, *J* = 13.9, 7.8, 2.3 Hz, 2H), 6.68–6.60 (m, 2H), 6.21 (t, *J* = 5.6 Hz, 1H), 3.41 (t, *J* = 5.6 Hz, 2H), 3.25 (s, 3H), 3.10 (d, *J* = 9.6 Hz, 5H), 2.26 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  149.1, 141.4, 138.1, 136.7, 129.4, 128.5, 127.7, 126.8, 123.0, 116.2, 114.0, 109.7, 70.3, 58.0, 42.2, 37.9, 20.9.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{15}\text{H}_{16}\text{NO}_4\text{S}$   $[\text{M}+\text{H}]^+$  306.0800, found 306.0799.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 1:1): 0.68.

### 3-((3-Methoxypropyl)amino)-*N*-methyl-*N*-(*m*-tolyl)benzenesulfonamide (B89)



**Synthetic procedure:** The same procedure employed for **B88** was followed starting from **B69b** (0.102 g, 0.300 mmol, 1.00 equiv), 3-methoxypropan-1-amine (0.046 mL, 0.450 mmol, 1.50 equiv), CuI (0.006 g, 0.030 mmol, 0.100 equiv), L-Proline (0.007 g, 0.060 mmol, 0.200 equiv),  $\text{K}_3\text{PO}_4$  (0.127 g, 0.600 mmol, 2.00 equiv) and anhydrous DMSO (1.0 mL). Stirring at 80 °C for 18 h and purification by SGCC ( $n$ -hexane:EtOAc 0–60%) provided the title compound (0.096 g, 0.275 mmol, 92%). This is a novel compound.

**Physical state:** Colourless oil.

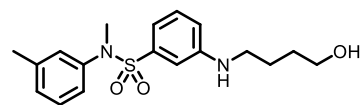
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  7.22 (q,  $J$  = 8.2 Hz, 2H), 7.09 (d,  $J$  = 7.5 Hz, 1H), 6.93 (d,  $J$  = 1.9 Hz, 1H), 6.88 (dd,  $J$  = 8.2, 2.2 Hz, 1H), 6.80 (dd,  $J$  = 8.3, 2.3 Hz, 1H), 6.67–6.60 (m, 1H), 6.57 (t,  $J$  = 2.1 Hz, 1H), 6.16 (t,  $J$  = 5.5 Hz, 1H), 3.36 (t,  $J$  = 6.2 Hz, 2H), 3.22 (s, 3H), 3.08 (s, 3H), 2.96 (q,  $J$  = 6.5 Hz, 2H), 2.25 (s, 3H), 1.68 (p,  $J$  = 6.6 Hz, 2H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  149.2, 141.4, 138.1, 136.6, 129.4, 128.5, 127.6, 126.8, 123.1, 116.1, 113.9, 109.6, 69.5, 57.9, 37.9, 28.4, 20.9.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{18}\text{H}_{25}\text{N}_2\text{O}_3\text{S}$   $[\text{M}+\text{H}]^+$  349.1586, found 349.1584.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 1:1): 0.57.

### 3-((4-Hydroxybutyl)amino)-*N*-methyl-*N*-(*m*-tolyl)benzenesulfonamide (B90)



**Synthetic procedure:** The same procedure employed for **B88** was followed starting from **B69b** (0.680 g, 2.00 mmol, 1.00 equiv), 4-aminobutan-1-ol (0.277 mL, 3.00 mmol, 1.50 equiv), CuI (0.038 g, 0.200 mmol, 0.100 equiv), L-Proline (0.046 g, 0.400 mmol, 0.200 equiv),  $\text{K}_3\text{PO}_4$  (0.849 g, 4.00 mmol, 2.00 equiv) and anhydrous DMSO (4.0 mL). Stirring at 80 °C for 18 h and purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.710 g, 2.04 mmol, 82%). This is a novel compound.

**Physical state:** Colourless oil.

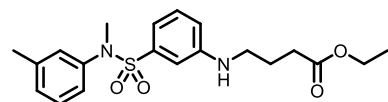
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.21 (td, *J* = 7.8, 4.7 Hz, 2H), 7.09 (d, *J* = 7.5 Hz, 1H), 6.94 (d, *J* = 2.0 Hz, 1H), 6.88 (dd, *J* = 7.8, 2.2 Hz, 1H), 6.80 (dd, *J* = 8.2, 2.4 Hz, 1H), 6.65–6.60 (m, 1H), 6.58 (t, *J* = 2.1 Hz, 1H), 6.15 (t, *J* = 5.4 Hz, 1H), 4.42 (t, *J* = 5.0 Hz, 1H), 3.40 (q, *J* = 5.7 Hz, 2H), 3.08 (s, 3H), 2.90 (q, *J* = 6.2 Hz, 2H), 2.25 (s, 3H), 1.57–1.41 (m, 4H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.2, 141.4, 138.1, 136.6, 129.4, 128.5, 127.7, 126.8, 123.1, 116.1, 113.7, 109.6, 60.5, 42.5, 37.9, 30.1, 25.0, 20.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>18</sub>H<sub>25</sub>N<sub>2</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 349.1586, found 349.1580.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.73.

#### Ethyl 4-((3-(*N*-methyl-*N*-(*m*-tolyl)sulfamoyl)phenyl)amino)butanoate (**B91a**)



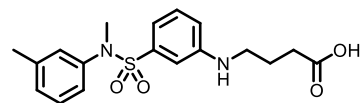
**Synthetic procedure:** The same procedure employed for **B88** was followed starting from **B69b** (0.102 g, 0.300 mmol, 1.00 equiv), ethyl 4-aminobutanoate hydrochloride (0.075 g, 0.450 mmol, 1.50 equiv), CuI (0.006 g, 0.030 mmol, 0.100 equiv), L-Proline (0.007 g, 0.060 mmol, 0.200 equiv), K<sub>3</sub>PO<sub>4</sub> (0.127 g, 0.600 mmol, 2.00 equiv) and anhydrous DMSO (1.0 mL). Stirring at 80 °C for 18 h and purification by SGCC (*n*-hexane:EtOAc 0–60%) provided the title compound (0.019 g, 0.049 mmol, 16%). This is a novel compound.

**Physical state:** Colourless oil.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.22 (dt, *J* = 10.7, 7.9 Hz, 2H), 7.08 (d, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 2.4 Hz, 1H), 6.91–6.85 (m, 1H), 6.80 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.63 (d, *J* = 7.6 Hz, 1H), 6.59 (t, *J* = 2.1 Hz, 1H), 6.21 (t, *J* = 5.5 Hz, 1H), 4.05 (q, *J* = 7.1 Hz, 2H), 3.08 (s, 3H), 2.94 (q, *J* = 6.6 Hz, 2H), 2.36 (t, *J* = 7.4 Hz, 2H), 2.25 (s, 3H), 1.71 (p, *J* = 7.2 Hz, 2H), 1.17 (t, *J* = 7.1 Hz, 3H).

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.67.

#### 4-((3-(*N*-Methyl-*N*-(*m*-tolyl)sulfamoyl)phenyl)amino)butanoic acid (**B91**)



**Synthetic procedure:** To a solution of **B91a** (0.019 g, 0.049 mmol, 1.00 equiv) in abs. EtOH (1.0 mL) was added 1 M aq. NaOH (0.200 mL, 0.196 mmol, 4.00 equiv). The mixture was stirred at rt for 3 h. Upon reaction completion as determined by TLC, the mixture was diluted with water (10 mL), adjusted to pH 4 with 1 M HCl and extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. This is a novel compound.

**Physical state:** Beige solid.

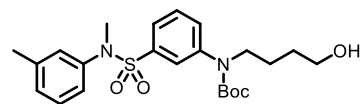
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 12.16 (s, 1H), 7.21 (q, *J* = 8.1 Hz, 2H), 7.08 (d, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 2.4 Hz, 1H), 6.88 (dd, *J* = 8.0, 2.2 Hz, 1H), 6.81 (dd, *J* = 8.2, 2.3 Hz, 1H), 6.63 (d, *J* = 7.7 Hz, 1H), 6.59 (t, *J* = 2.0 Hz, 1H), 6.21 (s, 1H), 3.08 (s, 3H), 2.97–2.88 (m, 2H), 2.27 (d, *J* = 10.9 Hz, 5H), 1.69 (p, *J* = 7.2 Hz, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 174.4, 149.1, 141.4, 138.1, 136.7, 129.5, 128.5, 127.7, 126.8, 123.0, 116.1, 113.9, 109.7, 41.9, 37.9, 31.2, 23.7, 20.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>18</sub>H<sub>23</sub>N<sub>2</sub>O<sub>4</sub>S [M+H]<sup>+</sup> 363.1379, found 363.1379.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.07.

***tert*-Butyl (4-hydroxybutyl)(3-(*N*-methyl-*N*-(*m*-tolyl)sulfamoyl)phenyl)carbamate (B92a)**



**Synthetic procedure:** A previously reported *N*-Boc protection protocol was followed with minor modifications.<sup>116</sup> A pressure vial was charged with **B90** (0.540 g, 1.55 mmol, 1.00 equiv) and Boc<sub>2</sub>O (0.338 g, 1.55 mmol, 1.00 equiv). The mixture was stirred under a constant jet of N<sub>2</sub> at 80 °C for 50 h. Upon reaction completion as determined by TLC, the mixture was directly purified by SGCC (*n*-hexane:EtOAc 0–100%) to provide the title compound (0.481 g, 1.07 mmol, 69%). This is a novel compound.

**Physical state:** Colourless oil.

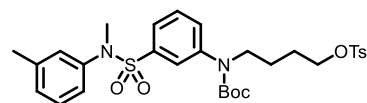
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.62–7.53 (m, 2H), 7.38 (ddd, *J* = 5.0, 3.3, 1.8 Hz, 1H), 7.25 (q, *J* = 1.4 Hz, 1H), 7.21 (t, *J* = 7.8 Hz, 1H), 7.11 (d, *J* = 7.6 Hz, 1H), 6.92 (d, *J* = 1.9 Hz, 1H), 6.85 (dd, *J* = 8.1, 2.2 Hz, 1H), 4.41 (t, *J* = 5.0 Hz, 1H), 3.55 (t, *J* = 6.8 Hz, 2H), 3.39–3.32 (m, 2H), 3.12 (s, 3H), 2.25 (s, 3H), 1.35 (s, 13H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 153.3, 142.6, 141.0, 138.4, 136.3, 131.1, 129.7, 128.7, 128.0, 126.9, 125.4, 124.5, 123.1, 80.0, 48.8, 38.0, 29.5, 27.8, 24.6.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>23</sub>H<sub>33</sub>N<sub>2</sub>O<sub>5</sub>S [M+H]<sup>+</sup> 449.2110, found 449.2102.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.46.

**4-((*tert*-Butoxycarbonyl)(3-(*N*-methyl-*N*-(*m*-tolyl)sulfamoyl)phenyl)amino)butyl 4-methylbenzenesulfonate (B92b)**



**Synthetic procedure:** The same procedure employed for **B77** was followed here starting from **B92a** (0.200 g, 0.446 mmol, 1.00 equiv), TsCl (0.102 g, 0.535 mmol, 1.20 equiv), DMAP (0.005 g, 0.045 mmol, 0.100



equiv), anhydrous Et<sub>3</sub>N (0.137 mL, 0.980 mmol, 2.20 equiv) and anhydrous DCM (2.0 mL). Stirring at 0 °C–rt for 16 h and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.192 g, 0.318 mmol, 71%). This is a novel compound.

**Physical state:** Colourless oil.

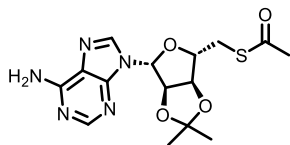
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.79–7.71 (m, 2H), 7.60–7.51 (m, 2H), 7.46 (d, *J* = 8.1 Hz, 2H), 7.39 (dt, *J* = 7.4, 1.6 Hz, 1H), 7.21 (d, *J* = 2.0 Hz, 1H), 7.18 (t, *J* = 7.8 Hz, 1H), 7.08 (d, *J* = 7.7 Hz, 1H), 6.92 (d, *J* = 2.1 Hz, 1H), 6.86–6.79 (m, 1H), 4.07–4.01 (m, 2H), 3.49 (t, *J* = 7.1 Hz, 2H), 3.10 (s, 3H), 2.40 (s, 3H), 2.23 (s, 3H), 1.99 (s, 2H), 1.56–1.44 (m, 2H), 1.33 (s, 11H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 136.3, 130.2, 128.6, 128.0, 127.5, 126.9, 125.4, 124.6, 123.0, 80.2, 70.5, 38.0, 27.8, 25.5, 20.8. Missing several peaks due to dilute sample.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>30</sub>H<sub>39</sub>N<sub>2</sub>O<sub>7</sub>S<sub>2</sub> [M+H]<sup>+</sup> 603.2199, found 603.2190.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.55.

***S*-(((3*aS*,4*S*,6*R*,6*aR*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl) ethanethioate (B92c)**



**Synthetic procedure:** A previously reported Mitsunobu reaction protocol was followed with minor modifications.<sup>175</sup> A solution of 1.4–2 mmol/g polymer-bound PPh<sub>3</sub> (1.29 g, 2.20 mmol, 2.20 equiv) in anhydrous THF (3.0 mL) was cooled to 0 °C, and DIAD (0.433 mL, 2.20 mmol, 2.20 equiv) was added dropwise over 5 min. The mixture was stirred at 0 °C for 30 min. 2',3'-*O*-Isopropylideneadenosine (0.307 g, 1.00 mmol, 1.00 equiv) was then added and the mixture stirred at 0 °C for 30 min. A solution of thioacetic acid (0.157 mL, 2.20 mmol, 2.20 equiv) in anhydrous THF (1.0 mL) was then added dropwise. The mixture was stirred at 0 °C for 4 h. Upon reaction completion as determined by TLC, the mixture was filtered, and the filtrate concentrated to dryness *in vacuo*. Purification by SGCC (DCM:MeOH 0–7%) provided the title compound (0.073 g, 0.200 mmol, 20%). This is a known compound.<sup>244</sup>

**Physical state:** White solid.

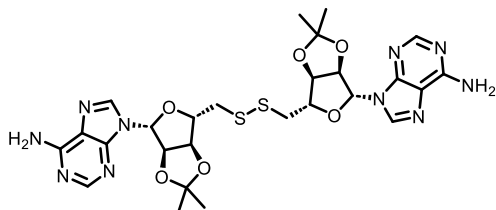
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.33 (d, *J* = 3.1 Hz, 1H), 8.17 (d, *J* = 2.8 Hz, 1H), 7.38 (d, *J* = 6.9 Hz, 3H), 6.23 (d, *J* = 2.4 Hz, 0H), 6.16 (d, *J* = 2.4 Hz, 1H), 5.49 (ddd, *J* = 6.3, 3.8, 2.4 Hz, 1H), 4.95 (dd, *J* = 6.3, 3.0 Hz, 1H), 4.15 (td, *J* = 7.0, 3.1 Hz, 1H), 3.21 (dd, *J* = 13.6, 7.2 Hz, 1H), 3.10 (dd, *J* = 13.6, 6.9 Hz, 1H), 2.33 (s, 3H), 1.51 (s, 3H), 1.31 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 194.6, 156.2, 152.8, 140.0, 119.1, 113.5, 89.0, 84.7, 83.2, 83.1, 54.9, 30.9, 30.5, 26.9, 25.2.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>S [M+H]<sup>+</sup> 366.1236, found 366.1229.

**R<sub>f</sub>** (silica gel, DCM:MeOH 95:5): 0.31.

**9,9'-((3*aR*,3*a'R*,4*R*,4'*R*,6*S*,6*aS*,6'*S*,6*a'S*)-(Disulfanediy)bis(methylene))bis(2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-6,4-diyl))bis(9*H*-purin-6-amine) (B92d)**



**Synthetic procedure:** A solution of **B92c** (0.560 g, 1.53 mmol, 1.00 equiv) in 2 M NH<sub>3</sub> in MeOH (7.70 mL, 15.3 mmol, 10.0 equiv) and 30% NH<sub>4</sub>OH (7.7 mL, 59.3 mmol, 38.8 equiv) was stirred rt for 51 h. Upon reaction completion as determined by TLC, the mixture was concentrated to dryness *in vacuo*. Purification by SGCC (DCM:MeOH 0–30%) provided the title compound (0.228 g, 0.705 mmol, 46%). This is a known compound.<sup>245</sup>

**Physical state:** Beige solid.

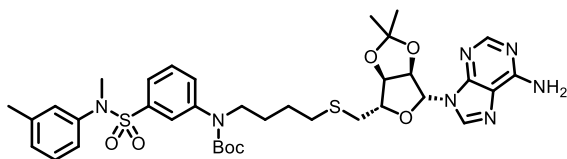
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.30 (s, 2H), 8.16 (s, 2H), 7.36 (s, 4H), 6.17 (d, *J* = 2.3 Hz, 2H), 5.50 (dd, *J* = 6.3, 2.3 Hz, 2H), 5.01 (dd, *J* = 6.3, 2.7 Hz, 2H), 4.33 (td, *J* = 7.1, 2.6 Hz, 2H), 3.16 (d, *J* = 5.3 Hz, 4H), 3.09–2.91 (m, 4H), 1.52 (s, 6H), 1.32 (s, 6H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 156.2, 152.3, 148.7, 140.1, 119.2, 113.3, 89.4, 84.7, 83.2, 48.6, 26.9, 25.1, 22.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>26</sub>H<sub>33</sub>N<sub>10</sub>O<sub>6</sub>S<sub>2</sub> [M+H]<sup>+</sup> 645.2026, found 645.2036.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.36.

***tert*-Butyl (4-(((3*aS*,4*S*,6*R*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)thio)butyl)(3-(*N*-methyl-*N*-(*m*-tolyl)sulfamoyl)phenyl)carbamate (B92e)**



**Synthetic procedure:** A previously reported thiol alkylation protocol was followed with minor modifications.<sup>176</sup> To a solution of **B92d** (0.107, 0.332 mmol, 1.00 equiv) in anhydrous MeOH (10.0 mL) was added

NaOMe (0.036 g, 0.664 mmol, 2.00 equiv), **B92b** (0.200 g, 0.332 mmol, 1.00 equiv) and then NaBH<sub>4</sub> (0.025 g, 0.664 mmol, 2.00 equiv). The mixture was stirred at rt for 16 h. Upon reaction completion as determined LC–MS, the mixture was carefully quenched with H<sub>2</sub>O (1.0 mL) and then concentrated to dryness *in vacuo*. The residue was partitioned between EtOAc (10 mL) and sat. NaHCO<sub>3</sub> (10 mL), and the aq. phase extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.150 g, 0.199 mmol, 60%). This is a novel compound.

**Physical state:** Colourless oil.

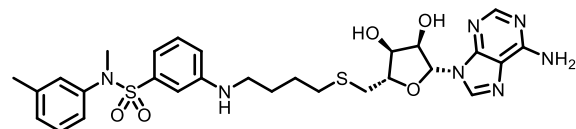
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.32 (s, 1H), 8.15 (s, 1H), 7.61–7.51 (m, 2H), 7.42–7.32 (m, 3H), 7.23–7.22 (m, 1H), 7.22–7.15 (m, 1H), 7.08 (d, *J* = 7.6 Hz, 1H), 6.91 (d, *J* = 2.0 Hz, 1H), 6.87–6.80 (m, 1H), 6.16 (d, *J* = 2.3 Hz, 1H), 5.51 (dd, *J* = 6.3, 2.4 Hz, 1H), 4.99 (dd, *J* = 6.3, 2.8 Hz, 1H), 4.22 (td, *J* = 7.3, 2.7 Hz, 1H), 3.49 (d, *J* = 6.8 Hz, 2H), 3.10 (s, 3H), 2.81–2.63 (m, 2H), 2.47–2.40 (m, 2H), 2.23 (s, 3H), 1.52 (s, 3H), 1.40–1.29 (m, 15H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 156.2, 152.7, 142.5, 138.4, 136.3, 131.1, 129.7, 128.7, 128.0, 123.0, 113.2, 89.3, 86.0, 83.4, 83.1, 80.1, 54.9, 38.0, 33.4, 30.9, 27.8, 26.9, 26.1, 25.1, 20.8.

**LC–MS** (APCI): *m/z* calc. for C<sub>36</sub>H<sub>48</sub>N<sub>7</sub>O<sub>7</sub>S<sub>2</sub> [M+H]<sup>+</sup> 754.3057, found 754.3.

**R<sub>f</sub>** (silica gel, DCM:MeOH 95:5): 0.39.

**3-((4-((((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)thio)butyl)amino)-*N*-methyl-*N*-(*m*-tolyl)benzenesulfonamide (**B92**)**



**Synthetic procedure:** To a solution of **B92e** (0.145 g, 0.192 mmol, 1.00 equiv) in DCM (10 mL) was added TFA (10 mL), and the mixture was stirred at rt for 1 h. Upon reaction completion (removal of the Boc-group) as determined by LC–MS, H<sub>2</sub>O was added (3.0 mL), and the mixture stirred at rt for 14 h. Upon reaction completion as determined by LC–MS, the mixture was concentrated to dryness *in vacuo*. Purification by RPC (H<sub>2</sub>O + 0.1% FA : MeCN + 0.1% FA 5–100%) provided the title compound (0.042 g, 0.069 mmol, 36% over two steps). This is a novel compound.

**Physical state:** White solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.36 (s, 1H), 8.15 (s, 1H), 7.32 (s, 2H), 7.27–7.15 (m, 3H), 7.08 (t, *J* = 8.6 Hz, 2H), 6.93 (q, *J* = 2.3 Hz, 2H), 6.87 (dd, *J* = 7.7, 2.4 Hz, 2H), 6.79 (ddd, *J* = 10.6, 8.0, 2.4 Hz, 2H), 6.62 (dd, *J* = 7.7, 1.8 Hz, 1H), 6.57 (dt, *J* = 6.9, 2.0 Hz, 2H), 6.14 (s, 1H), 5.89 (d, *J* = 5.7 Hz, 1H), 5.51 (d, *J* = 6.0 Hz,

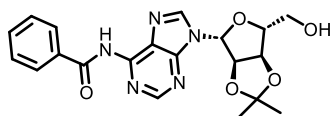
1H), 5.32 (d,  $J = 4.9$  Hz, 1H), 4.77 (q,  $J = 5.4$  Hz, 1H), 4.19–4.11 (m, 1H), 4.01 (td,  $J = 6.4, 3.6$  Hz, 1H), 3.07 (s, 3H), 2.86 (dtd,  $J = 28.9, 13.8, 6.4$  Hz, 4H), 2.24 (s, 3H), 1.66–1.39 (m, 6H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  156.0, 152.6, 149.5, 149.1, 141.4, 139.9, 138.1, 136.6, 129.4, 128.5, 127.7, 126.8, 123.0, 119.2, 113.8, 109.7, 87.4, 84.1, 73.7, 72.6, 42.0, 37.9, 33.9, 31.6, 27.4, 26.7, 20.9.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{28}\text{H}_{36}\text{N}_7\text{O}_5\text{S}_2$   $[\text{M}+\text{H}]^+$  614.2219, found 614.2213.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.28.

***N*-(9-((3*aR*,4*R*,6*R*,6*aR*)-6-(Hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purin-6-yl)benzamide (B93a)**



**Synthetic procedure:** A previously reported selective adenosine *N*-benzoylation protocol was followed with minor modifications.<sup>177</sup> To a solution of 2',3'-*O*-isopropylideneadenosine (1.54 g, 5.00 mmol, 1.00 equiv) in anhydrous pyridine (25.0 mL) was added TMS-Cl (3.17 mL, 25.0 mmol, 5.00 equiv), and the mixture was stirred at rt for 30 min. BzCl (0.755 mL, 6.50 mmol, 1.30 equiv) was then added, and the mixture was stirred at rt for 4 h. The mixture was cooled to 0 °C, diluted with H<sub>2</sub>O (5.0 mL), stirred at 0 °C for 10 min and then added 30% NH<sub>4</sub>OH (10.0 mL). The mixture was stirred at rt for 30 min. Upon reaction completion as determined by TLC, the mixture was concentrated to dryness *in vacuo*. Purification by SGCC (DCM:MeOH 0–10%) provided the title compound (1.34 g, 3.26 mmol, 65% over three steps). This is a known compound.<sup>246</sup>

**Physical state:** White solid.

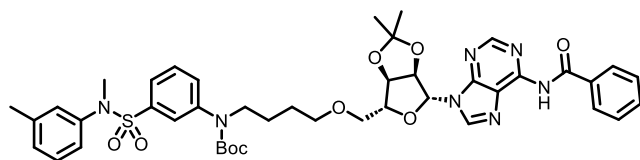
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  11.24 (s, 1H), 8.77 (s, 1H), 8.68 (s, 1H), 8.08–8.01 (m, 2H), 7.69–7.61 (m, 1H), 7.55 (dd,  $J = 8.3, 7.1$  Hz, 2H), 6.28 (d,  $J = 2.7$  Hz, 1H), 5.44 (dd,  $J = 6.2, 2.8$  Hz, 1H), 5.15 (t,  $J = 5.3$  Hz, 1H), 5.00 (dd,  $J = 6.1, 2.5$  Hz, 1H), 4.27 (td,  $J = 4.9, 2.4$  Hz, 1H), 3.56 (h,  $J = 6.4$  Hz, 2H), 1.57 (s, 3H), 1.35 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  151.7, 150.5, 143.1, 132.5, 131.2, 128.5, 128.2, 127.5, 113.1, 89.8, 86.9, 83.5, 81.4, 61.5, 54.9, 27.1, 25.2.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{20}\text{H}_{22}\text{N}_5\text{O}_5$   $[\text{M}+\text{H}]^+$  412.1621, found 412.1624.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.53.

**tert-Butyl (4-(((3a*R*,4*R*,6*R*,6a*R*)-6-(6-benzamido-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methoxy)butyl)(3-(*N*-methyl-*N*-(*m*-tolyl)sulfamoyl)phenyl)carbamate (B93b)**



**Synthetic procedure:** A previously reported adenosine *O*-alkylation protocol was followed with minor modifications.<sup>178</sup> A solution of **B93a** (0.137 g, 0.332 mmol, 1.00 equiv) in anhydrous DMF (2.5 mL) was cooled to 0 °C and added 60% NaH in mineral oil (0.024 g, 0.598 mmol, 1.80 equiv). The mixture was stirred at 0 °C and allowed to reach rt over 1 h. The mixture was cooled to 0 °C, and a solution of **B92b** (0.200 g, 0.332 mmol, 1.00 equiv) in anhydrous DMF (2.5 mL) was added. The mixture was stirred at 0 °C and allowed to reach rt over 3 h. Upon reaction completion as determined by TLC, the mixture was added MeOH (2 mL), EtOAc (10 mL) and H<sub>2</sub>O (10 mL) in that sequence. The aq. layer was extracted with EtOAc (4 x 10 mL), and the combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (DCM:MeOH 0–7%) provided the title compound (0.074 g, 0.087 mmol, 26%). This is a novel compound.

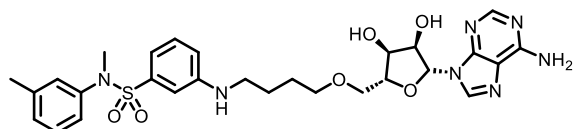
**Physical state:** White solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.22 (s, 1H), 8.75 (s, 1H), 8.57 (s, 1H), 8.07–8.00 (m, 2H), 7.89–7.84 (m, 0H), 7.69–7.60 (m, 1H), 7.60–7.50 (m, 4H), 7.36 (dt, *J* = 6.0, 2.4 Hz, 1H), 7.23–7.13 (m, 2H), 7.07 (d, *J* = 7.6 Hz, 1H), 6.90 (s, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 6.28 (d, *J* = 2.3 Hz, 1H), 5.45 (dd, *J* = 6.2, 2.4 Hz, 1H), 4.97 (dd, *J* = 6.1, 2.6 Hz, 1H), 4.37 (q, *J* = 4.6 Hz, 1H), 3.61–3.44 (m, 5H), 3.29 (s, 0H), 3.09 (s, 3H), 2.21 (s, 3H), 1.55 (s, 3H), 1.34 (d, *J* = 5.3 Hz, 7H), 1.31 (s, 9H).

**LC–MS** (APCI): *m/z* calc. for C<sub>43</sub>H<sub>51</sub>N<sub>7</sub>O<sub>9</sub>S [M+H]<sup>+</sup> 842.3547, found 842.3.

**R<sub>f</sub>** (silica gel, DCM:MeOH 95:5): 0.52.

**3-(((4-(((2*R*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)butyl)amino)-*N*-methyl-*N*-(*m*-tolyl)benzenesulfonamide (B93)**



**Synthetic procedure:** To a solution of **B93b** (0.062 g, 0.074 mmol, 1.00 equiv) in DCM (2.5 mL) was added TFA (2.5 mL), and the mixture was stirred at rt for 4.5 h. Upon reaction completion (removal of the Boc-group) as determined by LC–MS, H<sub>2</sub>O was added (0.75 mL), and the mixture stirred at rt for 41 h. Upon

reaction completion (removal of the acetal group) as determined by LC–MS, the mixture was concentrated to dryness *in vacuo*. The residue was redissolved in MeOH (2.5 mL), added 30% NH<sub>4</sub>OH (1.5 mL, 11.6 mmol, 156 equiv) and stirred at rt for 26 h. Then additional 30% NH<sub>4</sub>OH (1.5 mL, 11.6 mmol, 156 equiv) and MeOH (1.0 mL) were added, and the mixture stirred at rt for an additional 16 h. Upon reaction completion as determined by LC–MS, the mixture was concentrated to dryness *in vacuo*. Purification by RPC (H<sub>2</sub>O + 0.1% FA : MeCN + 0.1% FA 5–100%) provided the title compound (0.007 g, 0.011 mmol, 15% over three steps). This is a novel compound.

**Physical state:** White solid.

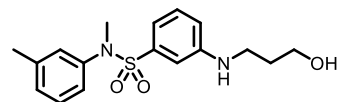
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.34–8.26 (m, 1H), 8.17–8.11 (m, 1H), 7.29 (s, 2H), 7.20 (q, *J* = 7.5 Hz, 2H), 7.07 (d, *J* = 7.5 Hz, 1H), 6.93 (s, 1H), 6.87 (d, *J* = 7.9 Hz, 1H), 6.79 (d, *J* = 8.1 Hz, 1H), 6.62 (d, *J* = 7.6 Hz, 1H), 6.57 (s, 1H), 6.15 (t, *J* = 5.6 Hz, 1H), 5.88 (dd, *J* = 13.0, 4.3 Hz, 1H), 5.52 (d, *J* = 5.8 Hz, 1H), 5.26 (d, *J* = 5.3 Hz, 1H), 4.56 (q, *J* = 5.4 Hz, 1H), 4.28–4.13 (m, 1H), 4.02 (d, *J* = 4.6 Hz, 1H), 3.66 (dd, *J* = 10.9, 3.7 Hz, 1H), 3.56 (dd, *J* = 10.7, 5.2 Hz, 1H), 3.44 (t, *J* = 6.3 Hz, 2H), 3.07 (d, *J* = 5.1 Hz, 3H), 2.91 (q, *J* = 6.3 Hz, 2H), 2.23 (d, *J* = 6.2 Hz, 3H), 1.54 (dd, *J* = 22.6, 7.9 Hz, 5H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 156.1, 152.7, 149.4, 149.2, 141.4, 139.2, 138.1, 136.6, 129.4, 128.5, 127.6, 126.8, 123.0, 119.0, 116.0, 113.7, 109.7, 87.5, 83.0, 73.4, 70.43, 70.38, 42.3, 37.9, 26.8, 25.0, 20.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>28</sub>H<sub>36</sub>N<sub>7</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 598.2448, found 598.2435.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.24.

### 3-((3-Hydroxypropyl)amino)-*N*-methyl-*N*-(*m*-tolyl)benzenesulfonamide (B94a)



**Synthetic procedure:** The same procedure employed for **B88** was followed starting from **B69b** (0.680 g, 2.00 mmol, 1.00 equiv), 3-aminopropan-1-ol (0.229 mL, 3.00 mmol, 1.50 equiv), CuI (0.038 g, 0.200 mmol, 0.100 equiv), L-Proline (0.046 g, 0.400 mmol, 0.200 equiv), K<sub>3</sub>PO<sub>4</sub> (0.849 g, 4.00 mmol, 2.00 equiv) and anhydrous DMSO (4.0 mL). Stirring at 80–100 °C for 48 h and purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.203 g, 0.606 mmol, 30%). This is a novel compound.

**Physical state:** Colourless oil.

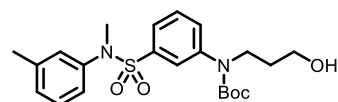
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.22 (q, *J* = 7.4 Hz, 2H), 7.09 (d, *J* = 7.6 Hz, 1H), 6.94 (s, 1H), 6.89 (d, *J* = 8.1 Hz, 1H), 6.84–6.77 (m, 1H), 6.65–6.58 (m, 2H), 6.13 (t, *J* = 5.4 Hz, 1H), 4.48 (td, *J* = 5.1, 1.2 Hz, 1H), 3.46 (q, *J* = 6.0 Hz, 2H), 3.08 (d, *J* = 1.2 Hz, 3H), 2.97 (q, *J* = 6.4 Hz, 2H), 2.26 (s, 3H), 1.62 (p, *J* = 6.6 Hz, 2H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  149.3, 141.4, 138.1, 136.7, 129.4, 128.5, 127.7, 126.8, 123.1, 116.1, 113.8, 109.6, 58.5, 37.9, 31.6, 20.9.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{17}\text{H}_{23}\text{N}_2\text{O}_3\text{S}$   $[\text{M}+\text{H}]^+$  335.1429, found 335.1417.

$R_f$  (silica gel,  $\text{DCM}:\text{MeOH}$  9:1): 0.53.

***tert*-Butyl (3-hydroxypropyl)(3-(*N*-methyl-*N*-(*m*-tolyl)sulfamoyl)phenyl)carbamate (B94b)**



**Synthetic procedure:** The same procedure employed for **B92a** was followed starting from **B94a** (0.200 g, 0.598 mmol, 1.00 equiv) and  $\text{Boc}_2\text{O}$  (0.131 g, 0.598 mmol, 1.00 equiv). Stirring at 80 °C for 25 h and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.181 g, 0.417 mmol, 70%). This is a novel compound.

**Physical state:** Colourless oil.

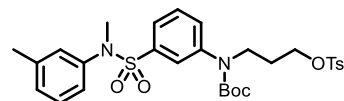
**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  7.58 (d,  $J$  = 7.2 Hz, 2H), 7.37 (dd,  $J$  = 6.9, 2.3 Hz, 1H), 7.28 (s, 1H), 7.21 (t,  $J$  = 7.8 Hz, 1H), 7.11 (d,  $J$  = 7.6 Hz, 1H), 6.93 (s, 1H), 6.85 (d,  $J$  = 8.0 Hz, 1H), 4.46 (t,  $J$  = 5.0 Hz, 1H), 3.61 (t,  $J$  = 7.3 Hz, 2H), 3.36 (d,  $J$  = 5.9 Hz, 2H), 3.12 (s, 3H), 2.25 (s, 3H), 1.54 (p,  $J$  = 6.4 Hz, 2H), 1.35 (s, 9H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  153.3, 142.8, 141.0, 138.4, 136.3, 131.0, 129.7, 128.7, 128.0, 126.8, 125.4, 124.4, 123.1, 80.0, 58.2, 46.6, 38.0, 31.3, 27.8, 20.8.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{22}\text{H}_{31}\text{N}_2\text{O}_5\text{S}$   $[\text{M}+\text{H}]^+$  435.1954, found 435.1949.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.26.

**3-((*tert*-Butoxycarbonyl)(3-(*N*-methyl-*N*-(*m*-tolyl)sulfamoyl)phenyl)amino)propyl 4-methylbenzenesulfonate (B94c)**



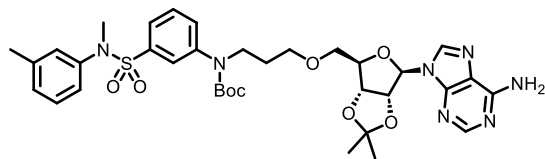
**Synthetic procedure:** The same procedure employed for **B77** was followed starting from **B94b** (0.180 g, 0.414 mmol, 1.00 equiv), TsCl (0.095 g, 0.497 mmol, 1.20 equiv), DMAP (0.005 g, 0.041 mmol, 0.100 equiv), anhydrous  $\text{Et}_3\text{N}$  (0.127 mL, 0.911 mmol, 2.20 equiv) and anhydrous DCM (2.0 mL). Stirring at 0 °C–rt for 16 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.155 g, 0.263 mmol, 64%). This is a novel compound.

**Physical state:** Colourless oil.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{29}\text{H}_{37}\text{N}_2\text{O}_7\text{S}_2$   $[\text{M}+\text{H}]^+$  589.2042, found 589.2056.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.60.

**tert-Butyl 3-(((3*aR*,4*R*,6*R*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methoxy)propyl)(3-(*N*-methyl-*N*-(*m*-tolyl)sulfamoyl)phenyl)carbamate (B94d)**



**Synthetic procedure:** A previously reported adenosine *O*-alkylation protocol was followed with minor modifications.<sup>179</sup> To a solution of **B94c** (0.150 g, 0.255 mmol, 1.00 equiv) and **B93a** (0.126 g, 0.306 mmol, 1.20 equiv) in anhydrous DMSO was added powdered KOH (0.026 g, 0.459 mmol, 1.80 equiv). The mixture was stirred at rt for 17 h. Upon reaction completion as determined by LC–MS, cold H<sub>2</sub>O (10 mL) was added and the mixture extracted with EtOAc (5 x 10 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. The crude alkylation product was redissolved in 1,4-dioxane (1.0 mL) and added 3 M aq. NaOH (1.0 mL, 3.00 mmol, 11.8 equiv). The mixture was stirred at 50 °C for 19 h. Then additional NaOH (0.120 g, 3.00 mmol, 11.8 equiv) was added and the mixture stirred at 70 °C for an additional 6 h. Upon reaction completion as determined by LC–MS, the mixture was diluted with H<sub>2</sub>O (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.090 g, 0.124 mmol, 49%). This is a novel compound.

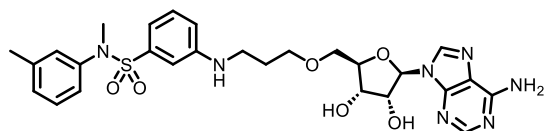
**Physical state:** Colourless oil.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.67 (s, 0H), 8.26 (s, 1H), 8.14 (s, 1H), 7.57–7.52 (m, 2H), 7.33 (s, 2H), 7.23 (s, 1H), 7.17 (t, *J* = 7.8 Hz, 1H), 7.07 (d, *J* = 7.6 Hz, 1H), 6.91 (d, *J* = 2.1 Hz, 1H), 6.82 (d, *J* = 7.9 Hz, 1H), 6.13 (d, *J* = 2.5 Hz, 1H), 5.40 (dd, *J* = 6.2, 2.5 Hz, 1H), 4.94 (dt, *J* = 5.5, 2.7 Hz, 1H), 4.28 (dd, *J* = 5.2, 2.7 Hz, 1H), 3.60–3.37 (m, 6H), 3.10 (s, 3H), 2.22 (s, 4H), 1.53 (s, 4H), 1.32 (s, 14H).

**LC–MS** (APCI): *m/z* calc. for C<sub>35</sub>H<sub>46</sub>N<sub>7</sub>O<sub>8</sub>S [M+H]<sup>+</sup> 724.3129, found 724.3.

$R_f$  (silica gel, DCM:MeOH 95:5): 0.38.

**3-(((3-(((2*R*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)propyl)amino)-*N*-methyl-*N*-(*m*-tolyl)benzenesulfonamide (B94)**





**Synthetic procedure:** The same procedure employed for **B92** was followed starting from **B94d** (0.085 g, 0.117 mmol, 1.00 equiv), TFA (5.0 mL) and DCM (5.0 mL). Stirring at rt for 3 h provided the intermediate Boc-protected intermediate, which was used in the subsequent step with H<sub>2</sub>O (1.5 mL). Stirring at rt for 2.5 h and purification by RPC (H<sub>2</sub>O + 0.1% FA : MeCN + 0.1% FA 5–100%) provided the title compound (0.023 g, 0.039 mmol, 33% over two steps). This is a novel compound.

**Physical state:** Off-white solid.

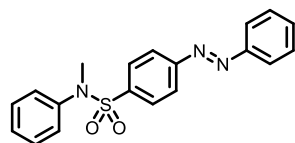
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.32 (s, 1H), 8.15 (s, 1H), 7.30 (s, 2H), 7.20 (q, *J* = 7.6 Hz, 2H), 7.06 (d, *J* = 7.5 Hz, 1H), 6.92 (d, *J* = 1.9 Hz, 1H), 6.87 (dd, *J* = 7.8, 2.2 Hz, 1H), 6.77 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.65–6.60 (m, 1H), 6.58 (t, *J* = 2.1 Hz, 1H), 6.16 (t, *J* = 5.4 Hz, 1H), 5.91 (d, *J* = 5.0 Hz, 1H), 5.53 (d, *J* = 5.9 Hz, 1H), 5.27 (d, *J* = 5.4 Hz, 1H), 4.58 (q, *J* = 5.3 Hz, 1H), 4.19 (q, *J* = 5.0 Hz, 1H), 4.03 (q, *J* = 4.5 Hz, 1H), 3.66 (dd, *J* = 10.8, 3.8 Hz, 1H), 3.57 (dd, *J* = 10.8, 5.2 Hz, 1H), 3.53–3.45 (m, 2H), 3.07 (s, 3H), 2.97 (q, *J* = 6.5 Hz, 2H), 2.23 (s, 3H), 1.71 (p, *J* = 6.6 Hz, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 156.1, 152.7, 149.4, 149.1, 141.4, 139.3, 138.1, 136.6, 129.5, 128.5, 127.7, 126.8, 123.0, 119.1, 116.0, 113.9, 109.8, 87.5, 83.0, 73.4, 70.6, 70.4, 68.4, 37.9, 28.5, 20.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>27</sub>H<sub>34</sub>N<sub>7</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 584.2291, found 584.2299.

*R<sub>f</sub>* (silica gel, DCM:MeOH 9:1): 0.24.

#### (*E*)-*N*-Methyl-*N*-phenyl-4-(phenyldiazenyl)benzenesulfonamide (**B95**)



**Synthetic procedure:** The same procedure employed for **B37c** was followed starting from **B1** (0.181 g, 0.690 mmol, 1.00 equiv), nitrosobenzene (0.074 g, 0.690 mmol, 1.00 equiv) and glacial AcOH (7.0 mL). Stirring at 40 °C for 42 h and purification by SGCC (*n*-hexane:EtOAc 0–40%) provided the title compound (0.156 g, 0.444 mmol, 64%). This is a known compound (CAS no.: 102840-95-9).

**Physical state:** Orange solid.

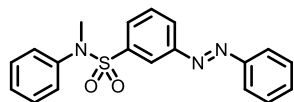
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.02 (d, *J* = 8.2 Hz, 2H), 7.94 (dd, *J* = 6.7, 3.0 Hz, 2H), 7.71 (d, *J* = 8.2 Hz, 2H), 7.66–7.61 (m, 3H), 7.34 (dq, *J* = 14.1, 7.3 Hz, 3H), 7.15 (d, *J* = 7.7 Hz, 2H), 3.20 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 154.1, 151.9, 140.9, 137.7, 132.6, 129.7, 129.0, 128.9, 127.5, 126.4, 123.1, 123.0, 38.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>19</sub>H<sub>18</sub>N<sub>3</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 352.1120, found 352.1125.

*R<sub>f</sub>* (silica gel, *n*-hexane:EtOAc 1:1): 0.74.

### (*E*)-*N*-Methyl-*N*-phenyl-3-(phenyldiazenyl)benzenesulfonamide (B96)



**Synthetic procedure:** The same procedure employed for **B37c** was followed starting from **B24** (0.262 g, 1.00 mmol, 1.00 equiv), nitrosobenzene (0.107 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at 40 °C for 28 h and purification by SGCC (*n*-hexane:EtOAc 0–30%) provided the title compound (0.278 g, 0.790 mmol, 79%). This is a novel compound.

**Physical state:** Orange solid.

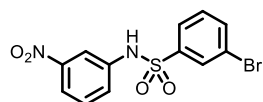
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.25–8.19 (m, 1H), 7.91 (dd, *J* = 6.7, 3.0 Hz, 2H), 7.86 (d, *J* = 2.0 Hz, 1H), 7.83 (t, *J* = 7.9 Hz, 1H), 7.67 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.64–7.59 (m, 3H), 7.40–7.27 (m, 3H), 7.16 (dd, *J* = 7.5, 1.9 Hz, 2H), 3.19 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 151.6, 140.9, 137.3, 132.4, 130.8, 129.7, 129.6, 129.0, 128.0, 127.5, 126.4, 122.9, 120.0, 38.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>19</sub>H<sub>18</sub>N<sub>3</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 352.1120, found 352.1140.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.62.

### 3-Bromo-*N*-(3-nitrophenyl)benzenesulfonamide (B97a)



**Synthetic procedure:** To a solution of 3-nitroaniline (0.691 g, 5.00 mmol, 1.00 equiv) and pyridine (1.213 mL, 15.0 mmol, 3.00 equiv) in DCM (20.0 mL) was added 3-bromobenzensulfonyl chloride (1.28 g, 5.00 mmol, 1.00 equiv) in portions. The mixture was stirred at rt for 24 h. Upon reaction completion as determined by TLC, the mixture was washed with 1 M HCl (2 x 15 mL), sat. NaHCO<sub>3</sub> (15 mL) and sat. brine (15 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo* to provide the title compound (1.61 g, 4.51 mmol, 90%). This is a known compound (CAS no.: 848906-31-0).

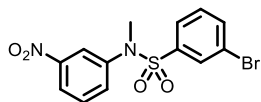
**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.04 (s, 1H), 7.98–7.91 (m, 3H), 7.88 (dd, *J* = 7.8, 2.0 Hz, 1H), 7.80 (dt, *J* = 8.0, 1.2 Hz, 1H), 7.62–7.51 (m, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 148.2, 140.8, 138.6, 136.3, 131.9, 131.0, 129.0, 125.8, 122.3, 119.0, 113.9.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.21.

### 3-Bromo-*N*-methyl-*N*-(3-nitrophenyl)benzenesulfonamide (**B97b**)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B97a** (1.60 g, 4.48 mmol, 1.00 equiv), MeI (0.418 mL, 6.72 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (3.72 g, 26.9 mmol, 6.00 equiv) and anhydrous DMF (20.0 mL). Stirring at rt for 18 h provided the title compound (1.58 g, 4.27 mmol, 95%). This is a novel compound.

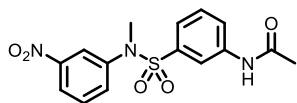
**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.19 (dt, *J* = 7.8, 1.8 Hz, 1H), 7.96 (dt, *J* = 9.8, 1.9 Hz, 2H), 7.71–7.62 (m, 3H), 7.56 (t, *J* = 7.8 Hz, 1H), 7.51 (dt, *J* = 8.0, 1.5 Hz, 1H), 3.23 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 147.9, 141.8, 137.2, 136.6, 132.5, 131.8, 130.5, 129.7, 126.6, 122.4, 122.2, 120.9, 37.7.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.33.

### *N*-(3-(*N*-Methyl-*N*-(3-nitrophenyl)sulfamoyl)phenyl)acetamide (**B97c**)



**Synthetic procedure:** A previously reported Buchwald-Hartwig cross-coupling protocol was followed with minor modifications.<sup>117</sup> A pressure vial was charged with Pd(OAc)<sub>2</sub> (0.002 g, 0.010 mmol, 0.010 equiv) and tBuBrettPhos (0.011 g, 0.022 mmol, 0.022 equiv) and subjected to 3 x vacuum-Ar cycles. Degassed *t*-BuOH (2.0 mL) and water (0.72 μL, 0.040 mol, 0.040 equiv) were then added by syringe. The mixture was stirred at 110 °C for 1.5 min. A separate pressure vial was charged with **B97b** (0.371 g, 1.00 mmol, 1.00 equiv), acetamide (0.071 g, 1.20 mmol, 1.20 equiv) and K<sub>3</sub>PO<sub>4</sub> (0.297 g, 1.40 mmol, 1.40 equiv) and subjected to 3 x vacuum-Ar cycles. The pre-activated catalyst solution prepared above was then added by syringe. The mixture was stirred at 110 °C for 2.5 h. Upon reaction completion as determined by TLC, the mixture was diluted with EtOAc (10 mL) and washed with water (10 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–80%) provided the title compound (0.242 g, 0.693 mmol, 69%). This is a novel compound.

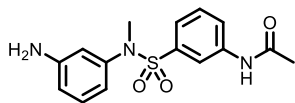
**Physical state:** Brown oil.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.27 (s, 1H), 8.17 (ddd, *J* = 7.9, 2.3, 1.3 Hz, 1H), 7.94 (t, *J* = 2.1 Hz, 1H), 7.87 (t, *J* = 1.9 Hz, 1H), 7.84 (ddd, *J* = 8.1, 2.3, 1.1 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.61 (dt, *J* = 8.2, 1.6 Hz, 1H), 7.50 (t, *J* = 8.0 Hz, 1H), 7.16 (ddd, *J* = 7.8, 1.9, 1.0 Hz, 1H), 3.19 (s, 3H), 2.03 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 168.9, 147.8, 142.1, 140.1, 135.7, 132.3, 130.4, 129.9, 123.3, 121.9, 121.6, 120.6, 117.2, 37.6, 24.0.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.09.

***N*-(3-(*N*-(3-Aminophenyl)-*N*-methylsulfamoyl)phenyl)acetamide (B97d)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B97c** (0.240 g, 0.687 mmol, 1.00 equiv), 10% Pd/C (0.024 g, 10 wt%), MeOH (5.0 mL) and EtOAc (5.0 mL). Stirring at rt for 6 h provided the title compound (0.203 g, 0.635 mmol, 92%). This is a novel compound.

**Physical state:** White solid.

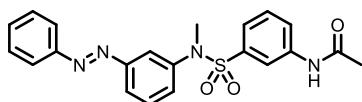
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.27 (s, 1H), 7.92 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.47 (t, *J* = 8.0 Hz, 1H), 7.15 (d, *J* = 7.9 Hz, 1H), 6.91 (t, *J* = 7.9 Hz, 1H), 6.44 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.36 (d, *J* = 2.4 Hz, 1H), 6.13 (d, *J* = 7.9 Hz, 1H), 5.19 (s, 2H), 3.07 (s, 3H), 2.05 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 168.8, 149.3, 141.8, 139.8, 137.0, 129.6, 129.0, 122.8, 121.6, 117.3, 112.9, 112.8, 111.9, 38.2, 24.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>18</sub>N<sub>3</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 320.1069, found 320.1086.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.10.

***(E)*-N-(3-(*N*-Methyl-*N*-(3-(phenyldiazenyl)phenyl)sulfamoyl)phenyl)acetamide (B97e)**



**Synthetic procedure:** The same procedure employed for **B37c** was followed starting from **B97d** (0.160 g, 0.500 mmol, 1.00 equiv), nitrosobenzene (0.054 g, 0.500 mmol, 1.00 equiv) and glacial AcOH (5.0 mL). Stirring at 40 °C for 3 days and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.175 g, 0.429 mmol, 86%). This is a novel compound.

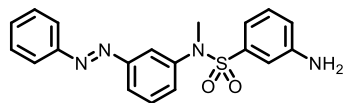
**Physical state:** Orange solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.27 (s, 1H), 7.93 (t, *J* = 1.9 Hz, 1H), 7.88 (dt, *J* = 7.1, 2.0 Hz, 3H), 7.85–7.81 (m, 1H), 7.64–7.54 (m, 5H), 7.50 (t, *J* = 8.0 Hz, 1H), 7.31 (ddd, *J* = 8.0, 2.2, 1.0 Hz, 1H), 7.18 (dt, *J* = 8.1, 1.2 Hz, 1H), 3.22 (s, 3H), 2.01 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 168.8, 152.2, 151.8, 142.2, 140.1, 136.3, 131.9, 130.0, 129.8, 129.5, 128.7, 123.1, 122.7, 121.6, 119.8, 117.3, 37.9, 24.0.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.58.

**(*E*)-3-Amino-*N*-methyl-*N*-(3-(phenyldiazenyl)phenyl)benzenesulfonamide (B97)**



**Synthetic procedure:** A previously reported chemoselective *N*-deacylation protocol was followed with minor modifications.<sup>180</sup> To a solution of **B97e** (0.175 g, 0.429 mmol, 1.00 equiv) in MeOH (1.0 mL) was added SOCl<sub>2</sub> (0.025 mL, 0.343 mmol, 0.800 equiv). The mixture was stirred at reflux for 16 h. Upon reaction completion as determined by TLC, the mixture was added 1 M NaOH (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.088 g, 0.240 mmol, 56%). This is a novel compound.

**Physical state:** Orange solid.

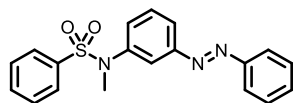
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.94–7.85 (m, 2H), 7.84–7.77 (m, 1H), 7.66 (d, *J* = 2.2 Hz, 1H), 7.64–7.52 (m, 4H), 7.36–7.28 (m, 1H), 7.17 (t, *J* = 7.7 Hz, 1H), 6.80 (d, *J* = 7.8 Hz, 2H), 6.60 (dd, *J* = 7.9, 1.6 Hz, 1H), 5.61 (d, *J* = 6.8 Hz, 2H), 3.20 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.1, 151.8, 149.5, 142.5, 136.3, 131.9, 129.8, 129.6, 129.5, 128.5, 122.7, 121.1, 119.9, 118.1, 114.0, 111.7, 37.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>19</sub>H<sub>19</sub>N<sub>4</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 367.1229, found 367.1239.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.44.

**(*E*)-*N*-Methyl-*N*-(3-(phenyldiazenyl)phenyl)benzenesulfonamide (B98)**



**Synthetic procedure:** The same procedure employed for **B37c** was followed starting from **B39** (0.026 g, 0.100 mmol, 1.00 equiv), nitrosobenzene (0.011 g, 0.100 mmol, 1.00 equiv) and glacial AcOH (1.0 mL). Stirring at 40 °C for 23 h and purification by SGCC (*n*-hexane:EtOAc 3:1) provided the title compound (0.038 g, 0.100 mmol, quant.). This is a novel compound.

**Physical state:** Orange oil.

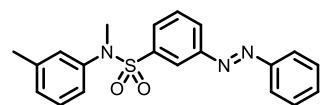
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.88 (dd, *J* = 7.6, 2.1 Hz, 2H), 7.82 (ddd, *J* = 8.0, 1.9, 1.0 Hz, 1H), 7.75–7.68 (m, 1H), 7.65–7.54 (m, 9H), 7.30 (ddd, *J* = 8.0, 2.3, 1.0 Hz, 1H), 3.22 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  152.1, 151.7, 142.2, 135.8, 133.5, 131.9, 129.9, 129.5, 129.4, 128.6, 127.4, 122.7, 121.4, 119.9, 37.8.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{19}\text{H}_{18}\text{N}_3\text{O}_2\text{S}$   $[\text{M}+\text{H}]^+$  352.1120, found 352.1109.

**$R_f$**  (silica gel, *n*-hexane:EtOAc 3:1): 0.36.

**(*E*)-*N*-Methyl-3-(phenyldiazenyl)-*N*-(*m*-tolyl)benzenesulfonamide (B99)**



**Synthetic procedure:** The same procedure employed for **B37c** was followed starting from **B58** (0.028 g, 0.100 mmol, 1.00 equiv), nitrosobenzene (0.011 g, 0.100 mmol, 1.00 equiv) and glacial AcOH (1.0 mL). Stirring at 40 °C for 23 h and purification by SGCC (*n*-hexane:EtOAc 3:1) provided the title compound (0.030 g, 0.082 mmol, 82%). This is a novel compound.

**Physical state:** Orange oil.

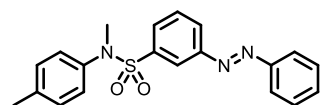
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  8.23 (dt,  $J$  = 8.0, 1.3 Hz, 1H), 7.94–7.89 (m, 2H), 7.88–7.80 (m, 2H), 7.69 (dt,  $J$  = 7.9, 1.4 Hz, 1H), 7.63 (dd,  $J$  = 5.0, 1.9 Hz, 3H), 7.23 (t,  $J$  = 7.8 Hz, 1H), 7.13 (d,  $J$  = 7.6 Hz, 1H), 6.96 (d,  $J$  = 1.8 Hz, 1H), 6.92 (dd,  $J$  = 7.9, 2.1 Hz, 1H), 3.16 (s, 3H), 2.24 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  151.6, 140.8, 138.4, 137.4, 132.3, 130.7, 129.7, 129.6, 128.8, 128.2, 128.0, 127.1, 123.4, 122.9, 119.9, 38.1, 20.8.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{20}\text{H}_{20}\text{N}_3\text{O}_2\text{S}$   $[\text{M}+\text{H}]^+$  366.1276, found 366.1261.

**$R_f$**  (silica gel, *n*-hexane:EtOAc 3:1): 0.39.

**(*E*)-*N*-Methyl-3-(phenyldiazenyl)-*N*-(*p*-tolyl)benzenesulfonamide (B100)**



**Synthetic procedure:** The same procedure employed for **B37c** was followed starting from **B57** (0.138 g, 0.500 mmol, 1.00 equiv), nitrosobenzene (0.054 g, 0.500 mmol, 1.00 equiv) and glacial AcOH (5.0 mL). Stirring at 40 °C for 24 h and purification by SGCC (*n*-hexane:EtOAc 0–30%) provided the title compound (0.113 g, 0.308 mmol, 62%). This is a novel compound.

**Physical state:** Orange solid.

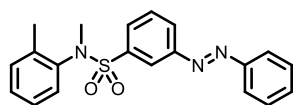
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  8.25–8.18 (m, 1H), 7.96–7.86 (m, 3H), 7.82 (t,  $J$  = 7.9 Hz, 1H), 7.72–7.57 (m, 4H), 7.15 (d,  $J$  = 8.0 Hz, 2H), 7.05–6.97 (m, 2H), 3.16 (s, 3H), 2.27 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  151.6, 138.3, 137.4, 137.0, 132.4, 130.8, 129.7, 129.6, 129.5, 127.9, 126.3, 122.9, 120.0, 38.2, 20.5.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_2\text{S}$   $[\text{M}+\text{H}]^+$  366.1276, found 366.1294.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 2:1): 0.55.

**(*E*)-*N*-Methyl-3-(phenyldiazenyl)-*N*-(*o*-tolyl)benzenesulfonamide (B101)**



**Synthetic procedure:** The same procedure employed for **B37c** was followed starting from **B59** (0.055 g, 0.200 mmol, 1.00 equiv), nitrosobenzene (0.021 g, 0.200 mmol, 1.00 equiv) and glacial AcOH (2.0 mL). Stirring at 40 °C for 22 h and purification by SGCC ( $n$ -hexane:EtOAc 3:1) provided the title compound (0.043 g, 0.117 mmol, 58%). This is a novel compound.

**Physical state:** Orange solid.

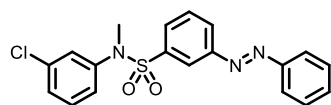
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  8.27 (dt,  $J$  = 7.5, 1.8 Hz, 1H), 8.03 (t,  $J$  = 1.8 Hz, 1H), 7.97–7.92 (m, 2H), 7.92–7.82 (m, 2H), 7.63 (dd,  $J$  = 5.0, 1.9 Hz, 3H), 7.34 (dd,  $J$  = 7.7, 1.6 Hz, 1H), 7.25 (td,  $J$  = 7.5, 1.3 Hz, 1H), 7.10 (td,  $J$  = 7.7, 1.6 Hz, 1H), 6.67 (dd,  $J$  = 7.9, 1.3 Hz, 1H), 3.14 (s, 3H), 2.33 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  151.8, 151.7, 139.8, 139.0, 138.1, 132.3, 131.2, 130.9, 129.8, 129.6, 128.4, 127.9, 127.0, 126.7, 122.9, 120.0, 38.8, 17.9.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{20}\text{H}_{20}\text{N}_3\text{O}_2\text{S}$   $[\text{M}+\text{H}]^+$  366.1276, found 366.1284.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 3:1): 0.40.

**(*E*)-*N*-(3-Chlorophenyl)-*N*-methyl-3-(phenyldiazenyl)benzenesulfonamide (B102)**



**Synthetic procedure:** The same procedure employed for **B37c** was followed starting from **B55** (0.148 g, 0.500 mmol, 1.00 equiv), nitrosobenzene (0.054 g, 0.500 mmol, 1.00 equiv) and glacial AcOH (5.0 mL). Stirring at 40 °C for 24 h and purification by SGCC ( $n$ -hexane:EtOAc 0–30%) provided the title compound (0.074 g, 0.191 mmol, 38%). This is a novel compound.

**Physical state:** Orange oil.

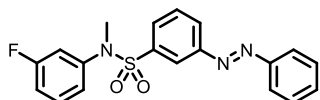
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  8.29–8.19 (m, 1H), 7.92 (dd,  $J$  = 6.8, 3.0 Hz, 2H), 7.88–7.80 (m, 2H), 7.69 (d,  $J$  = 7.9 Hz, 1H), 7.63 (p,  $J$  = 3.8 Hz, 3H), 7.40 (d,  $J$  = 4.8 Hz, 2H), 7.26 (d,  $J$  = 2.3 Hz, 1H), 7.16 (ddd,  $J$  = 7.9, 4.6, 2.7 Hz, 1H), 3.19 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  165.7, 159.0, 141.5, 139.4, 134.7, 132.1, 131.9, 131.2, 131.2, 130.4, 129.7, 127.6, 127.1, 126.0, 123.7, 121.8, 118.6, 111.7, 55.4.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{19}\text{H}_{17}\text{N}_3\text{O}_2\text{S}\text{Cl}$   $[\text{M}+\text{H}]^+$  386.0730, found 386.0718.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 4:1): 0.55.

**(*E*)-*N*-(3-Fluorophenyl)-*N*-methyl-3-(phenyldiazenyl)benzenesulfonamide (B103)**



**Synthetic procedure:** The same procedure employed for **B37c** was followed starting from **B62** (0.028 g, 0.100 mmol, 1.00 equiv), nitrosobenzene (0.011 g, 0.100 mmol, 1.00 equiv) and glacial AcOH (1.0 mL). Stirring at 40 °C for 23 h and purification by SGCC ( $n$ -hexane:EtOAc 3:1) provided the title compound (0.038 g, 0.100 mmol, quant.). This is a novel compound.

**Physical state:** Orange solid.

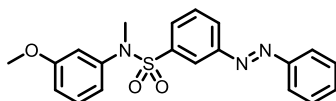
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  8.24 (dt,  $J$  = 8.1, 1.3 Hz, 1H), 7.94–7.89 (m, 2H), 7.87 (t,  $J$  = 1.9 Hz, 1H), 7.84 (t,  $J$  = 7.9 Hz, 1H), 7.68 (dt,  $J$  = 8.0, 1.3 Hz, 1H), 7.66–7.60 (m, 3H), 7.41 (td,  $J$  = 8.3, 6.8 Hz, 1H), 7.22–7.14 (m, 1H), 7.07 (td,  $J$  = 9.8, 2.2 Hz, 2H), 3.20 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  163.0, 160.6, 151.7, 151.6, 142.5, 142.4, 137.0, 132.4, 130.9, 130.5, 130.4, 129.6, 129.6, 128.2, 122.9, 122.2, 122.2, 119.8, 114.4, 114.2, 113.4, 113.2, 37.8.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{19}\text{H}_{14}\text{N}_3\text{O}_2\text{SF}$   $[\text{M}+\text{H}]^+$  370.0826, found 370.0833.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 3:1): 0.36.

**(*E*)-*N*-(3-Methoxyphenyl)-*N*-methyl-3-(phenyldiazenyl)benzenesulfonamide (B104)**



**Synthetic procedure:** The same procedure employed for **B37c** was followed starting from **B60** (0.029 g, 0.100 mmol, 1.00 equiv), nitrosobenzene (0.011 g, 0.100 mmol, 1.00 equiv) and glacial AcOH (1.0 mL). Stirring at 40 °C for 23 h and purification by SGCC ( $n$ -hexane:EtOAc 3:1) provided the title compound (0.027 g, 0.072 mmol, 72%). This is a novel compound.

**Physical state:** Orange oil.

**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  8.24 (dt,  $J$  = 8.1, 1.3 Hz, 1H), 7.92 (dd,  $J$  = 6.7, 3.0 Hz, 2H), 7.89 (d,  $J$  = 1.9 Hz, 1H), 7.84 (t,  $J$  = 7.9 Hz, 1H), 7.71 (dt,  $J$  = 7.9, 1.3 Hz, 1H), 7.63 (p,  $J$  = 3.7 Hz, 3H), 7.26 (t,  $J$  = 8.1 Hz, 1H), 6.90 (dd,  $J$  = 8.3, 2.5 Hz, 1H), 6.72 (dd,  $J$  = 7.9, 2.0 Hz, 1H), 6.69 (t,  $J$  = 2.3 Hz, 1H), 3.67 (s, 3H), 3.18 (s, 3H).

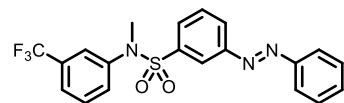


**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  159.4, 151.6, 142.0, 137.4, 132.4, 130.8, 129.7, 129.6, 128.1, 122.9, 119.9, 118.4, 113.1, 112.3, 55.2, 38.1.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{20}\text{H}_{20}\text{N}_3\text{O}_3\text{S}$   $[\text{M}+\text{H}]^+$  382.1225, found 382.1217.

**$R_f$**  (silica gel, *n*-hexane:EtOAc 3:1): 0.35.

**(*E*)-*N*-Methyl-3-(phenyldiazenyl)-*N*-(3-(trifluoromethyl)phenyl)benzenesulfonamide (B105)**



**Synthetic procedure:** The same procedure employed for **B37c** was followed starting from **B64** (0.165 g, 0.500 mmol, 1.00 equiv), nitrosobenzene (0.054 g, 0.500 mmol, 1.00 equiv) and glacial AcOH (5.0 mL). Stirring at 40 °C for 33 h and purification by SGCC (*n*-hexane:EtOAc 0–20%) provided the title compound (0.115 g, 0.275 mmol, 55%). This is a novel compound.

**Physical state:** Orange oil.

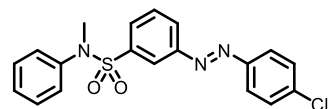
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  8.25 (d,  $J$  = 7.9 Hz, 1H), 7.89 (dd,  $J$  = 6.8, 3.0 Hz, 2H), 7.84 (t,  $J$  = 7.9 Hz, 1H), 7.80 (d,  $J$  = 2.1 Hz, 1H), 7.72–7.66 (m, 2H), 7.62 (p,  $J$  = 4.6 Hz, 4H), 7.51 (d,  $J$  = 8.3 Hz, 1H), 7.46 (s, 1H), 3.23 (s, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  151.7, 151.6, 141.6, 136.7, 132.4, 130.9, 130.4, 130.3, 129.9, 129.7, 129.6, 128.5, 124.1, 124.1, 122.9, 122.9, 122.8, 122.2, 119.7, 37.7.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{20}\text{H}_{17}\text{N}_3\text{O}_2\text{SF}_3$   $[\text{M}+\text{H}]^+$  420.0994, found 420.0985.

**$R_f$**  (silica gel, *n*-hexane:EtOAc 2:1): 0.54.

**(*E*)-3-((4-Chlorophenyl)diazenyl)-*N*-methyl-*N*-phenylbenzenesulfonamide (B106)**



**Synthetic procedure:** A solution of 4-chloroaniline (0.128 g, 1.00 mmol, 1.00 equiv) in DCM (10.0 mL) and water (10.0 mL) was degassed with Ar for 10 min. Oxone (1.23 g, 2.00 mmol, 2.00 equiv) was then added against a positive pressure of Ar. The mixture was stirred vigorously under Ar at rt for 3 h. Upon reaction completion as determined by TLC, the phases were separated, and the aq. phase extracted with DCM (2 x 10 mL). The combined organic phases were washed with sat. brine (20 mL), dried over  $\text{Na}_2\text{SO}_4$  and concentrated to dryness *in vacuo*. The crude nitroso intermediate was redissolved in glacial AcOH (10.0 mL) and degassed with  $\text{N}_2$  for 10 min. Then **B24** (0.262 g, 1.00 mmol, 1.00 equiv) was added. The mixture was stirred at 40 °C for 22 h. Upon reaction completion as determined by TLC, the mixture was concentrated

to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–20%) provided the title compound (0.141 g, 0.366 mmol, 37% over two steps). This is a novel compound.

**Physical state:** Orange solid.

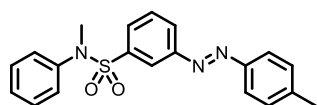
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.23 (d, *J* = 7.9 Hz, 1H), 8.02–7.78 (m, 4H), 7.69 (d, *J* = 8.4 Hz, 3H), 7.34 (dt, *J* = 13.2, 7.1 Hz, 3H), 7.15 (d, *J* = 7.6 Hz, 2H), 3.19 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 151.5, 140.9, 137.4, 130.9, 129.8, 129.0, 128.0, 127.5, 126.4, 124.6, 120.1, 38.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>19</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub>SCl [M+H]<sup>+</sup> 386.0730, found 386.0740.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.78.

#### (*E*)-*N*-Methyl-*N*-phenyl-3-(*p*-tolyldiazenyl)benzenesulfonamide (B107)



**Synthetic procedure:** The same procedure employed for **B106** was followed starting from *p*-toluidine (0.054 g, 0.500 mmol, 1.00 equiv), Oxone (0.615 g, 1.00 mmol, 2.00 equiv), DCM (5.0 mL) and water (5.0 mL). Stirring at rt for 7 h provided the crude nitroso intermediate, which was used directly in the next step with **B24** (0.131 g, 0.500 mmol, 1.00 equiv) and glacial AcOH (5.0 mL). Stirring at 40 °C for 40 h and purification by SGCC (*n*-hexane:EtOAc 0–25%) provided the title compound (0.041 g, 0.112 mmol, 22% over two steps). This is a novel compound.

**Physical state:** Orange solid.

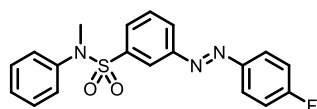
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.19 (dt, *J* = 8.2, 1.3 Hz, 1H), 7.86–7.77 (m, 4H), 7.64 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.42 (d, *J* = 8.1 Hz, 2H), 7.35 (dd, *J* = 8.4, 6.3 Hz, 2H), 7.33–7.27 (m, 1H), 7.18–7.13 (m, 2H), 3.19 (s, 3H), 2.42 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 151.7, 149.8, 142.8, 140.9, 137.3, 130.7, 130.1, 129.4, 129.0, 127.8, 127.5, 126.3, 122.9, 119.9, 38.0, 21.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>20</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 366.1276, found 366.1275.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 3:1): 0.46.

#### (*E*)-3-((4-Fluorophenyl)diazenyl)-*N*-methyl-*N*-phenylbenzenesulfonamide (B108)



**Synthetic procedure:** The same procedure employed for **B106** was followed starting from 4-fluoroaniline (0.056 g, 0.500 mmol, 1.00 equiv), Oxone (0.615 g, 1.00 mmol, 2.00 equiv), DCM (5.0 mL) and water (5.0 mL). Stirring at rt for 23 h provided the crude nitroso intermediate, which was used directly in the next step with **B24** (0.131 g, 0.500 mmol, 1.00 equiv) and glacial AcOH (5.0 mL). Stirring at 40 °C for 3 days and purification by SGCC (*n*-hexane:EtOAc 0–20%) provided the title compound (0.078 g, 0.211 mmol, 42% over two steps). This is a novel compound.

**Physical state:** Orange solid.

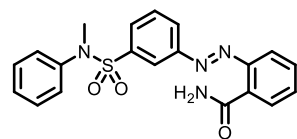
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.21 (ddd, *J* = 7.9, 2.0, 1.1 Hz, 1H), 8.03–7.94 (m, 2H), 7.86 (t, *J* = 1.9 Hz, 1H), 7.82 (t, *J* = 7.9 Hz, 1H), 7.66 (dt, *J* = 7.9, 1.3 Hz, 1H), 7.51–7.42 (m, 2H), 7.36 (dd, *J* = 8.4, 6.2 Hz, 2H), 7.33–7.27 (m, 1H), 7.18–7.08 (m, 2H), 3.19 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 165.5, 163.0, 151.5, 148.4, 148.4, 140.9, 137.4, 130.8, 129.7, 129.0, 127.9, 127.5, 126.3, 125.4, 125.3, 120.0, 116.7, 116.5, 38.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>19</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub>SF [M+H]<sup>+</sup> 370.1026, found 370.1029.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.76.

**(*E*)-2-((3-(*N*-Methyl-*N*-phenylsulfamoyl)phenyl)diazenyl)benzamide (**B109**)**



**Synthetic procedure:** The same procedure employed for **B106** was followed starting from **B24** (0.262 g, 1.00 mmol, 1.00 equiv), Oxone (1.23 g, 2.00 mmol, 2.00 equiv), DCM (10.0 mL) and water (10.0 mL). Stirring at rt for 16 h provided the crude nitroso intermediate, which was used directly in the next step with 2-aminobenzamide (0.136 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at 40 °C for 23 h and at 80 °C for 7 h and purification by SGCC (DCM:MeOH 0–50%) provided the title compound (0.056 g, 0.142 mmol, 14% over two steps). This is a novel compound.

**Physical state:** Orange solid.

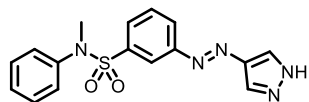
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.15 (ddd, *J* = 7.9, 2.0, 1.1 Hz, 1H), 7.95 (t, *J* = 1.9 Hz, 1H), 7.91 (s, 1H), 7.81 (t, *J* = 7.9 Hz, 1H), 7.71–7.57 (m, 6H), 7.36 (dd, *J* = 8.3, 6.6 Hz, 2H), 7.33–7.27 (m, 1H), 7.18–7.13 (m, 2H), 3.21 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 168.6, 151.9, 148.5, 140.8, 137.6, 136.9, 131.7, 130.7, 130.3, 129.8, 129.1, 128.8, 127.5, 127.1, 126.3, 121.1, 116.1, 38.2.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>20</sub>H<sub>19</sub>N<sub>4</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 395.1178, found 395.1175.

**R<sub>f</sub>** (silica gel, DCM:EtOAc 1:1): 0.42.

**(E)-3-((1H-Pyrazol-4-yl)diazenyl)-N-methyl-N-phenylbenzenesulfonamide (B110)**



**Synthetic procedure:** The same procedure employed for **B106** was followed starting from **B24** (0.262 g, 1.00 mmol, 1.00 equiv), Oxone (1.23 g, 2.00 mmol, 2.00 equiv), DCM (10.0 mL) and water (10.0 mL). Stirring at rt for 17 h provided the crude nitroso intermediate, which was used directly in the next step with 1H-pyrazol-4-amine (0.091 g, 1.10 mmol, 1.10 equiv) and glacial AcOH (10.0 mL). Stirring at 40 °C for 3 days and purification by SGCC (DCM:MeOH 0–5%) provided the title compound (0.080 g, 0.234 mmol, 23% over two steps). This is a novel compound.

**Physical state:** Yellow solid.

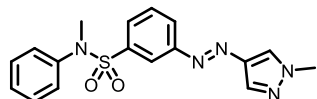
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.59 (s, 1H), 8.66 (s, 1H), 8.07 (dt, *J* = 8.2, 1.3 Hz, 1H), 8.03 (s, 1H), 7.79–7.72 (m, 2H), 7.55 (dt, *J* = 7.9, 1.3 Hz, 1H), 7.39–7.26 (m, 3H), 7.17–7.09 (m, 2H), 3.18 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.2, 140.9, 137.2, 131.3, 130.6, 129.0, 128.8, 128.5, 127.5, 126.3, 119.1, 119.1, 38.1, 38.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>16</sub>H<sub>16</sub>N<sub>5</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 342.1025, found 342.1035.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.58.

**(E)-N-Methyl-3-((1-methyl-1H-pyrazol-4-yl)diazenyl)-N-phenylbenzenesulfonamide (B111)**



**Synthetic procedure:** The same procedure employed for **B106** was followed starting from **B24** (0.262 g, 1.00 mmol, 1.00 equiv), Oxone (0.738 g, 1.20 mmol, 1.20 equiv), DCM (10.0 mL) and water (10.0 mL). Stirring at rt for 16 h provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1H-pyrazol-4-amine (0.097 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at 40 °C for 2 h and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.113 g, 0.317 mmol, 32% over two steps). This is a novel compound.

**Physical state:** Yellow solid.

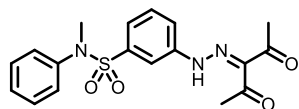
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.57 (s, 1H), 8.08–8.03 (m, 1H), 8.02 (s, 1H), 7.78–7.71 (m, 2H), 7.56 (dt, *J* = 8.0, 1.3 Hz, 1H), 7.38–7.26 (m, 3H), 7.17–7.10 (m, 2H), 3.93 (s, 3H), 3.17 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.2, 140.90, 140.87, 137.2, 132.5, 130.6, 129.3, 129.0, 128.5, 127.4, 127.2, 126.3, 119.2, 39.2, 38.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>19</sub>H<sub>21</sub>N<sub>6</sub>O<sub>2</sub>S [M+MeCN+H]<sup>+</sup> 397.1447, found 397.1441.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.20.

### 3-(2-(2,4-Dioxopentan-3-ylidene)hydrazineyl)-*N*-methyl-*N*-phenylbenzenesulfonamide (B112a)



**Synthetic procedure:** A previously reported tandem diazotisation–azo coupling protocol was followed with minor modifications.<sup>35</sup> A solution of **B24** (0.262 g, 1.00 mmol, 1.00 equiv) in conc. HCl (0.25 mL, 3.00 mmol, 3.00 equiv) and glacial AcOH (2.0 mL) was cooled to 0 °C and dropwise added a cooled solution of NaNO<sub>2</sub> (0.083 g, 1.20 mmol, 1.20 equiv) in water (1.0 mL). The mixture was stirred at 0 °C for 1 h. A solution of acetylacetone (0.133 mL, 1.30 mmol, 1.30 equiv) and NaOAc (0.246 g, 3.00 mmol, 3.00 equiv) in abs. EtOH (1.0 mL) and water (0.5 mL) was cooled to 0 °C and dropwise added the diazonium solution prepared above. The mixture was stirred at 0 °C and slowly allowed to reach rt over 14 h. Upon reaction completion as determined by TLC, the precipitate was collected by suction filtration, washed with water, water:EtOH 1:1 and *n*-hexane and dried *in vacuo* to provide the title compound (0.324 g, 0.867 mmol, 87% over two steps). This is a novel compound.

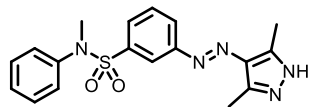
**Physical state:** Yellow solid.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.55 (s, 1H), 7.87 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.70 (t, *J* = 2.0 Hz, 1H), 7.59 (t, *J* = 8.0 Hz, 1H), 7.34 (td, *J* = 7.2, 1.6 Hz, 2H), 7.27 (ddt, *J* = 15.2, 7.8, 1.3 Hz, 2H), 7.13 (dd, *J* = 7.6, 1.8 Hz, 2H), 3.17 (s, 3H), 2.48–2.19 (m, 6H).

<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, DMSO-*d*<sub>6</sub>): δ 197.1, 196.3, 142.9, 141.0, 137.2, 134.7, 130.4, 128.9, 127.3, 126.2, 123.2, 120.6, 114.6, 38.0, 31.2, 26.2.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.50.

### (*E*)-3-((3,5-Dimethyl-1*H*-pyrazol-4-yl)diazenyl)-*N*-methyl-*N*-phenylbenzenesulfonamide (B112)



**Synthetic procedure:** A previously reported *de novo* pyrazole ring formation protocol was followed with minor modifications.<sup>35</sup> To a solution of **B112a** (0.149 g, 0.400 mmol, 1.00 equiv) in abs. EtOH (2.0 mL) was added hydrazine hydrate (0.045 mL, 0.800 mmol, 2.00 equiv). The mixture was stirred at reflux for 16 h. Upon reaction completion as determined by TLC, the mixture was concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.118 g, 0.320 mmol, 80%). This is a novel compound.

**Physical state:** Yellow solid.

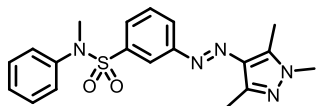
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 12.99 (s, 1H), 8.02 (dt, *J* = 8.2, 1.2 Hz, 1H), 7.72 (t, *J* = 7.8 Hz, 1H), 7.64 (t, *J* = 1.9 Hz, 1H), 7.54 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.35 (dd, *J* = 8.4, 6.3 Hz, 2H), 7.32–7.27 (m, 1H), 7.17–7.11 (m, 2H), 3.16 (s, 3H), 2.41 (s, 6H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.9, 141.0, 137.0, 134.3, 130.4, 128.9, 127.7, 127.4, 126.5, 126.3, 119.2, 59.8, 37.9, 14.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>18</sub>H<sub>20</sub>N<sub>5</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 370.1338, found 370.1324.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.13.

**(*E*)-*N*-Methyl-*N*-phenyl-3-((1,3,5-trimethyl-1*H*-pyrazol-4-yl)diazenyl)benzenesulfonamide (B113)**



**Synthetic procedure:** The same procedure employed for **B112** was followed starting from **B112a** (0.149 g, 0.400 mmol, 1.00 equiv), methylhydrazine (0.021 mL, 0.400 mmol, 1.00 equiv) and abs. EtOH (2.0 mL). Stirring at reflux for 16 h and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.140 g, 0.365 mmol, 91%). This is a novel compound.

**Physical state:** Yellow solid.

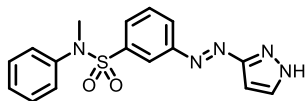
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.01 (dt, *J* = 8.2, 1.3 Hz, 1H), 7.71 (t, *J* = 7.9 Hz, 1H), 7.63 (t, *J* = 1.9 Hz, 1H), 7.54 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.34 (dd, *J* = 8.3, 6.3 Hz, 2H), 7.32–7.26 (m, 1H), 7.17–7.11 (m, 2H), 3.73 (s, 3H), 3.15 (s, 3H), 2.50 (s, 3H), 2.33 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.9, 141.0, 140.8, 140.7, 137.0, 134.5, 130.4, 128.9, 127.7, 127.4, 126.5, 126.2, 119.1, 38.0, 36.1, 13.8, 9.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>19</sub>H<sub>22</sub>N<sub>5</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 384.1494, found 384.1485.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.16.

**(*E*)-3-((1*H*-Pyrazol-3-yl)diazenyl)-*N*-methyl-*N*-phenylbenzenesulfonamide (B114)**



**Synthetic procedure:** The same procedure employed for **B106** was followed starting from **B24** (0.262 g, 1.00 mmol, 1.00 equiv), Oxone (1.23 g, 2.00 mmol, 2.00 equiv), DCM (10.0 mL) and water (10.0 mL). Stirring at rt for 16 h provided the crude nitroso intermediate, which was used directly in the next step with 1*H*-pyrazol-3-amine (0.160 g, 2.00 mmol, 2.00 equiv) and glacial AcOH (10.0 mL). Stirring at rt for 48 h and

purification by SGCC (DCM:MeOH 0–10% followed by *n*-hexane:EtOAc 0–100%) provided the title compound (0.033 g, 0.098 mmol, 10% over two steps). This is a novel compound.

**Physical state:** Yellow solid.

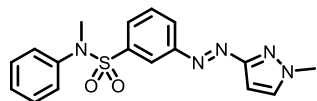
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.66 (s, 1H), 8.17 (dt, *J* = 8.2, 1.3 Hz, 1H), 7.90 (d, *J* = 2.5 Hz, 1H), 7.85 (d, *J* = 2.0 Hz, 1H), 7.80 (t, *J* = 7.9 Hz, 1H), 7.62 (dt, *J* = 8.2, 1.3 Hz, 1H), 7.35 (dd, *J* = 8.4, 6.4 Hz, 2H), 7.33–7.25 (m, 1H), 7.14 (dd, *J* = 7.5, 1.8 Hz, 2H), 6.61 (d, *J* = 2.5 Hz, 1H), 3.19 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.0, 140.9, 137.3, 130.8, 129.4, 129.0, 127.7, 127.5, 126.4, 119.8, 38.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>16</sub>H<sub>16</sub>N<sub>5</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 342.1025, found 342.1037.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:2): 0.40.

#### (*E*)-*N*-Methyl-3-((1-methyl-1*H*-pyrazol-3-yl)diazenyl)-*N*-phenylbenzenesulfonamide (B115)



**Synthetic procedure:** The same procedure employed for **B106** was followed starting from **B24** (0.262 g, 1.00 mmol, 1.00 equiv), Oxone (1.23 g, 2.00 mmol, 2.00 equiv), DCM (10.0 mL) and water (10.0 mL). Stirring at rt for 16 h provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1*H*-pyrazol-3-amine (0.097 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at 40 °C for 23 h and at 80 °C for 7 h and purification by SGCC (*n*-hexane:EtOAc 50–100%) provided the title compound (0.061 g, 0.172 mmol, 17% over two steps). This is a novel compound.

**Physical state:** Yellow solid.

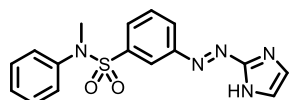
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.18–8.12 (m, 1H), 7.87 (d, *J* = 2.5 Hz, 1H), 7.84 (t, *J* = 1.9 Hz, 1H), 7.79 (t, *J* = 7.9 Hz, 1H), 7.62 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.35 (dd, *J* = 8.4, 6.3 Hz, 2H), 7.32–7.26 (m, 1H), 7.17–7.12 (m, 2H), 6.59 (d, *J* = 2.4 Hz, 1H), 3.99 (s, 3H), 3.19 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.3, 149.1, 142.1, 137.1, 129.3, 128.9, 117.7, 114.1, 112.9, 112.6, 111.9, 111.8, 38.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>18</sub>N<sub>5</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 356.1181, found 356.1194.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:3): 0.42.

#### (*E*)-3-((1*H*-Imidazol-2-yl)diazenyl)-*N*-methyl-*N*-phenylbenzenesulfonamide (B116)



**Synthetic procedure:** To 0.5 M aq. HCl (1.8 mL, 0.90 mmol, 0.90 equiv) cooled to 0 °C was dropwise added a cooled solution of NaNO<sub>2</sub> (0.090 g, 1.30 mmol, 1.30 equiv) in water (2.0 mL). This was then added dropwise to a cooled solution of **B24** (0.262 g, 1.00 mmol, 1.00 equiv) in 0.5 M aq. HCl (3.6 mL, 1.80 mmol, 1.80 equiv) and MeOH (3.0 mL). The mixture was stirred at 0 °C for 50 min. A solution of imidazole (0.068 g, 1.00 mmol, 1.00 equiv) and Na<sub>2</sub>CO<sub>3</sub> (0.238 g, 2.25 mmol, 2.25 equiv) in water (4.0 mL) was cooled to 0 °C, and the diazonium intermediate solution prepared above was added dropwise. The mixture was stirred at 0 °C and allowed to slowly reach rt over 22 h. Upon reaction completion as determined by TLC, the precipitate was collected by suction filtration, washed with cold water and *n*-hexane, and dried *in vacuo*. Purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.063 g, 0.185 mmol, 19% over two steps). This is a novel compound.

**Physical state:** Orange solid.

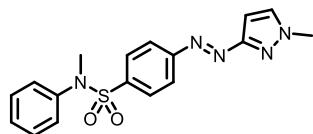
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.19 (s, 1H), 8.19–8.12 (m, 1H), 7.87 (t, *J* = 1.9 Hz, 1H), 7.81 (t, *J* = 7.9 Hz, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.48 (s, 1H), 7.34 (tt, *J* = 14.1, 6.4 Hz, 4H), 7.15 (dd, *J* = 7.2, 1.9 Hz, 2H), 3.20 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 154.4, 151.8, 140.9, 137.4, 132.6, 130.9, 129.5, 129.0, 127.5, 127.2, 126.4, 121.0, 120.2, 38.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>16</sub>H<sub>16</sub>N<sub>5</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 342.1025, found 342.1020.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.49.

**(*E*)-*N*-Methyl-4-((1-methyl-1*H*-pyrazol-3-yl)diazenyl)-*N*-phenylbenzenesulfonamide (**B117**)**



**Synthetic procedure:** A solution of **B1** (0.131 g, 0.500 mmol, 1.00 equiv) in DCM (5.0 mL) and H<sub>2</sub>O (5.0 mL) was degassed with Ar for 10 min. Oxone (0.615 g, 1.00 mmol, 2.00 equiv) was then added against a positive pressure of Ar, and the mixture was stirred under Ar at rt for 24 h. Upon reaction completion as determined by TLC, the layers were allowed to separate, and the organic layer was syringed over into an Ar-purged flask containing Na<sub>2</sub>SO<sub>4</sub>. A solution of 1-methyl-1*H*-pyrazol-3-amine (0.049 g, 0.500 mmol, 1.00 equiv) in glacial AcOH (5.0 mL) was degassed with Ar for 10 min. The dried solution of the nitroso intermediate prepared above was then added dropwise by syringe. Additional DCM (5.0 mL) was added by syringe into the reaction flask from the first step, and the mixture stirred for a few minutes to extract more of the nitroso intermediate. This organic layer was then syringed over into the same Na<sub>2</sub>SO<sub>4</sub>-containing flask to dry it before being syringed over into the reaction flask of the second step. The mixture



was stirred under Ar at rt for 53 h. Upon reaction completion, the mixture was concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.036 g, 0.102 mmol, 20% over two steps). This is a novel compound.

**Physical state:** Yellow solid.

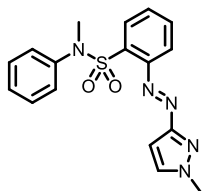
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.99–7.92 (m, 2H), 7.88 (d, *J* = 2.5 Hz, 1H), 7.72–7.64 (m, 2H), 7.36 (dd, *J* = 8.3, 6.3 Hz, 2H), 7.33–7.27 (m, 1H), 7.18–7.10 (m, 2H), 6.60 (d, *J* = 2.4 Hz, 1H), 4.00 (s, 3H), 3.19 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 163.0, 154.4, 140.9, 137.4, 133.6, 129.0, 128.9, 127.5, 126.4, 122.8, 95.0, 38.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>18</sub>N<sub>5</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 356.1181, found 356.1182.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.27.

**(*E*)-*N*-Methyl-2-((1-methyl-1*H*-pyrazol-3-yl)diazenyl)-*N*-phenylbenzenesulfonamide (B118)**



**Synthetic procedure:** The same procedure employed for **B117** was followed starting from **B26** (0.440 g, 1.68 mmol, 1.00 equiv), Oxone (2.06 g, 3.36 mmol, 2.00 equiv), DCM (15.0 mL) and H<sub>2</sub>O (15.0 mL). Stirring at rt for 19 h and then at 50 °C for 53 h provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1*H*-pyrazol-3-amine (0.163 g, 1.68 mmol, 1.00 equiv) and glacial AcOH (15.0 mL). Stirring at rt for 25 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.307 g, 0.863 mmol, 51% over two steps). This is a novel compound.

**Physical state:** Orange solid.

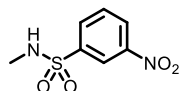
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.83 (d, *J* = 2.5 Hz, 1H), 7.82–7.74 (m, 2H), 7.65–7.56 (m, 2H), 7.27 (dd, *J* = 8.4, 7.0 Hz, 2H), 7.22–7.16 (m, 1H), 7.16–7.11 (m, 2H), 6.24 (d, *J* = 2.5 Hz, 1H), 3.99 (s, 3H), 3.31 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 163.1, 149.4, 141.0, 135.2, 134.4, 133.3, 130.8, 130.6, 129.0, 126.8, 126.0, 117.0, 95.6, 38.6.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>18</sub>N<sub>5</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 356.1181, found 356.1183.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.26.

### ***N*-Methyl-3-nitrobenzenesulfonamide (B119a)**



**Synthetic procedure:** To a solution of 3-nitrobenzenesulfonyl chloride (2.22 g, 10.0 mmol, 1.00 equiv) in DCM (50.0 mL) was added anhydrous Et<sub>3</sub>N (4.18 mL, 30.0 mmol, 3.00 equiv) and then MeNH<sub>2</sub>·HCl (0.898 g, 13.3 mmol, 1.33 equiv). The mixture was stirred at rt for 8 h. Upon reaction completion as determined by TLC, the mixture was washed with 1 M HCl (50 mL) and sat. brine (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo* to provide the title compound (1.11 g, 5.11 mmol, 51%). This is a known compound. Experimental data correlate with literature.<sup>247</sup>

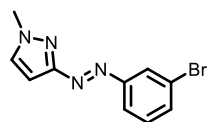
**Physical state:** Off-white solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.71 (t, *J* = 2.0 Hz, 1H), 8.45 (ddd, *J* = 8.3, 2.2, 1.0 Hz, 1H), 8.21 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.77 (t, *J* = 8.0 Hz, 1H), 4.71 (d, *J* = 6.8 Hz, 1H), 2.74 (d, *J* = 4.9 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 148.5, 141.5, 132.9, 130.7, 127.4, 122.6, 29.5.

**R<sub>f</sub>** (silica gel, DCM:MeOH 95:5): 0.30.

### ***(E)*-3-((3-Bromophenyl)diazenyl)-1-methyl-1*H*-pyrazole (B119b)**



**Synthetic procedure:** The same procedure employed for **B117** was followed starting from 3-bromoaniline (0.544 mL, 5.00 mmol, 1.00 equiv), Oxone (6.15 g, 10.0 mmol, 2.00 equiv), DCM (50.0 mL) and water (50.0 mL). Stirring at rt for 6 h provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1*H*-pyrazol-3-amine (0.486 g, 5.00 mmol, 1.00 equiv) and glacial AcOH (50.0 mL). Stirring at rt for 44 h and purification by SGCC (DCM:MeOH 0–10%) provided the title compound (1.03 g, 3.88 mmol, 78% over two steps). This is a novel compound.

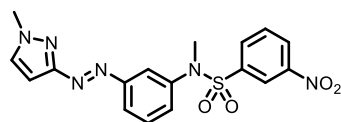
**Physical state:** Orange solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.10 (t, *J* = 1.9 Hz, 1H), 7.89 (ddd, *J* = 7.9, 1.9, 1.0 Hz, 1H), 7.57 (ddd, *J* = 7.9, 2.0, 1.0 Hz, 1H), 7.43–7.33 (m, 2H), 6.64 (d, *J* = 2.4 Hz, 1H), 4.03 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 163.5, 153.8, 133.8, 132.1, 130.5, 125.1, 123.2, 122.9, 96.6, 39.9.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.51.

**(E)-N-Methyl-N-(3-((1-methyl-1H-pyrazol-3-yl)diazenyl)phenyl)-3-nitrobenzenesulfonamide (B119c)**



**Synthetic procedure:** A previously reported Ullmann-type cross-coupling protocol was followed with minor modifications.<sup>115</sup> A pressure vial was charged with **B119a** (0.133 g, 0.500 mmol, 1.00 equiv), **B119b** (0.108 g, 0.500 mmol, 1.00 equiv), CuI (0.019 g, 0.100 mmol, 0.200 equiv), *N,N*-dimethylglycine (0.010 g, 0.100 mmol, 0.200 equiv) and K<sub>3</sub>PO<sub>4</sub> (0.265 g, 1.25 mmol, 2.50 equiv) and then purged with Ar. Degassed and anhydrous DMF (1.0 mL) was then added by syringe. The mixture was stirred at 100 °C for 3 days. Upon reaction completion as determined by TLC, the mixture was diluted with EtOAc (10 mL), filtered through a bed of celite and the bed washed with EtOAc (3 x 10 mL). The combined filtrates were concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.127 g, 0.318 mmol, 64%). This is a novel compound.

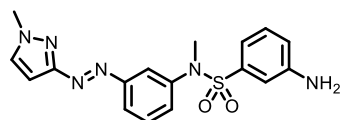
**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.47 (t, *J* = 2.0 Hz, 1H), 8.42 (ddd, *J* = 8.2, 2.3, 1.1 Hz, 1H), 7.91 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.80 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.64 (t, *J* = 8.0 Hz, 1H), 7.56–7.48 (m, 2H), 7.45 (ddd, *J* = 7.8, 2.3, 1.2 Hz, 1H), 7.40 (d, *J* = 2.5 Hz, 1H), 6.61 (d, *J* = 2.4 Hz, 1H), 4.02 (s, 3H), 3.29 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 163.8, 153.6, 148.6, 142.0, 139.0, 133.7, 132.5, 130.8, 130.30, 130.25, 127.9, 124.0, 123.3, 118.7, 97.0, 40.2, 38.8.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.27.

**(E)-3-Amino-N-methyl-N-(3-((1-methyl-1H-pyrazol-3-yl)diazenyl)phenyl)benzenesulfonamide (B119)**



**Synthetic procedure:** A previously reported chemoselective nitro reduction protocol was followed with minor modifications.<sup>118</sup> A pressure vial was charged with a solution of **B119c** (0.120 g, 0.300 mmol, 1.00 equiv) in THF (15.0 mL) and H<sub>2</sub>O (5.0 mL) and then added Na<sub>2</sub>S (0.351 g, 4.50 mmol, 15.0 equiv). The mixture was stirred at reflux 40 h. Upon reaction completion as determined by TLC, the mixture was added H<sub>2</sub>O (5 mL) and extracted with EtOAc (3 x 10 mL). The combined organic phases were washed with sat. NaHCO<sub>3</sub> (15 mL) and sat. brine (15 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (DCM:MeOH 0–10%, followed by *n*-hexane:EtOAc 0–50%) provided the title compound (0.067 g, 0.180 mmol, 60%). This is a novel compound.

**Physical state:** Yellow solid.

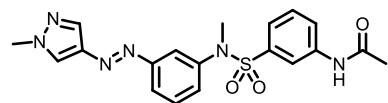
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.84 (d, *J* = 2.4 Hz, 1H), 7.74 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.59 (d, *J* = 2.1 Hz, 1H), 7.54 (t, *J* = 8.0 Hz, 1H), 7.33–7.26 (m, 1H), 7.16 (t, *J* = 7.8 Hz, 1H), 6.83–6.74 (m, 2H), 6.61–6.56 (m, 1H), 6.55 (d, *J* = 2.5 Hz, 1H), 5.61 (s, 2H), 3.98 (s, 3H), 3.19 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 162.8, 152.3, 149.5, 142.5, 136.2, 133.3, 129.7, 129.6, 128.1, 120.6, 119.5, 118.1, 113.9, 111.6, 94.7, 39.4, 37.7.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>19</sub>N<sub>6</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 371.1290, found 371.1289.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.57.

**(*E*)-*N*-(3-(*N*-Methyl-*N*-(3-((1-methyl-1*H*-pyrazol-4-yl)diazenyl)phenyl)sulfamoyl)phenyl)acetamide (B120a)**



**Synthetic procedure:** The same procedure employed for **B106** was followed starting from **B97d** (0.174 g, 0.544 mmol, 1.00 equiv), Oxone (0.669 g, 1.09 mmol, 2.00 equiv), DCM (5.0 mL) and water (5.0 mL). Stirring at rt for 1.5 h provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1*H*-pyrazol-4-amine (0.053 g, 0.544 mmol, 1.00 equiv) and glacial AcOH (5.0 mL). Stirring at 40 °C for 44 h and purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.119 g, 0.289 mmol, 53% over two steps). This is a novel compound.

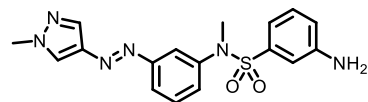
**Physical state:** Orange oil.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.27 (s, 1H), 8.51 (s, 1H), 7.97 (s, 1H), 7.91 (d, *J* = 2.0 Hz, 1H), 7.90–7.85 (m, 1H), 7.66 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.53–7.46 (m, 3H), 7.23–7.17 (m, 1H), 7.14 (dd, *J* = 7.9, 1.7 Hz, 1H), 3.92 (s, 3H), 3.20 (s, 3H), 2.02 (s, 3H).

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>19</sub>H<sub>21</sub>N<sub>6</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 413.1396, found 413.1389.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.44.

**(*E*)-3-Amino-*N*-methyl-*N*-(3-((1-methyl-1*H*-pyrazol-4-yl)diazenyl)phenyl)benzenesulfonamide (B120)**



**Synthetic procedure:** The same procedure employed for **B97** was followed starting from **B120a** (0.110 g, 0.267 mmol, 1.00 equiv), SOCl<sub>2</sub> (0.016 mL, 0.213 mmol, 0.800 equiv) and MeOH (1.0 mL). Stirring at 65 °C

for 1 h and purification by SGCC (DCM:MeOH 0–7%) followed by RPC (H<sub>2</sub>O:MeCN 0–100%) provided the title compound (0.026 g, 0.069 mmol, 26%). This is a novel compound.

**Physical state:** Yellow oil.

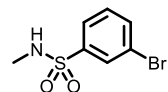
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.52 (s, 1H), 7.98 (s, 1H), 7.65 (ddd, *J* = 7.9, 1.9, 1.0 Hz, 1H), 7.53–7.47 (m, 2H), 7.22 (ddd, *J* = 8.0, 2.2, 1.0 Hz, 1H), 7.16 (t, *J* = 7.8 Hz, 1H), 6.81–6.75 (m, 2H), 6.58 (dt, *J* = 7.9, 1.3 Hz, 1H), 5.60 (d, *J* = 6.8 Hz, 2H), 3.92 (s, 3H), 3.18 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.5, 149.5, 142.4, 140.9, 136.4, 132.3, 129.6, 129.5, 128.8, 127.2, 120.3, 119.1, 118.0, 113.9, 111.6, 59.8, 39.2, 37.8, 20.8, 14.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>19</sub>N<sub>6</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 371.1290, found 371.1287.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.44.

### 3-Bromo-*N*-methylbenzenesulfonamide (B121a)



**Synthetic procedure:** The same procedure employed for **B119a** was followed starting from 3-bromobenzenesulfonyl chloride (0.256 g, 1.00 mmol, 1.00 equiv), MeNH<sub>2</sub>·HCl (0.090 g, 1.33 mmol, 1.33 equiv), anhydrous Et<sub>3</sub>N (0.418 mL, 3.00 mmol, 3.00 equiv) and DCM (5.0 mL). Stirring at rt for 2.5 h provided the title compound (0.203 g, 0.811 mmol, 81%). This is a known compound.<sup>248</sup>

**Physical state:** White solid.

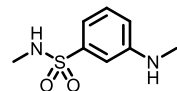
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.91 (t, *J* = 1.8 Hz, 1H), 7.88 (ddd, *J* = 7.9, 2.0, 1.0 Hz, 1H), 7.78 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.65–7.61 (m, 1H), 7.58 (t, *J* = 7.9 Hz, 1H), 2.43 (d, *J* = 5.0 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 141.3, 135.3, 131.6, 129.0, 125.8, 122.2, 28.7.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>7</sub>H<sub>9</sub>NO<sub>2</sub>SBr [M+H]<sup>+</sup> 249.9532, found 249.9531.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.52.

### *N*-Methyl-3-(methylamino)benzenesulfonamide (B121b)



**Synthetic procedure:** The same procedure employed for **B68** was followed starting from **B121a** (0.200 g, 0.811 mmol, 1.00 equiv), 40% MeNH<sub>2</sub> in water (3.0 mL, 34.7 mmol, 42.7 equiv) and powdered Cu (2.6 mg, 0.041 mmol, 0.050 equiv). Stirring at 100 °C for 4.5 h and purification by SGCC (*n*-hexane:EtOAc 0–100%)

provided the title compound (0.127 g, 0.633 mmol, 78%). This is a known compound. Experimental data correlate with literature.<sup>249</sup>

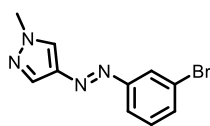
**Physical state:** White solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.31–7.22 (m, 2H), 6.93–6.86 (m, 2H), 6.74 (ddd, *J* = 8.2, 2.3, 1.1 Hz, 1H), 6.17 (q, *J* = 5.0 Hz, 1H), 2.69 (d, *J* = 5.0 Hz, 3H), 2.38 (d, *J* = 4.9 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 150.2, 139.7, 129.5, 115.0, 113.1, 108.9, 29.5, 28.7.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.32.

#### (*E*)-4-((3-Bromophenyl)diazenyl)-1-methyl-1*H*-pyrazole (**B121c**)



**Synthetic procedure:** The same procedure employed for **B106** was followed starting from 3-bromoaniline (0.172 g, 1.00 mmol, 1.00 equiv), Oxone (0.738 g, 1.20 mmol, 1.20 equiv), DCM (10.0 mL) and water (10.0 mL). Stirring at rt for 5 h provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1*H*-pyrazol-4-amine (0.097 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at rt for 22 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.161 g, 0.608 mmol, 61% over two steps). This is a novel compound.

**Physical state:** Yellow solid.

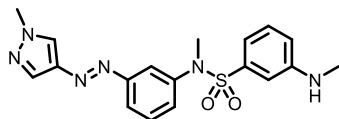
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.01 (s, 1H), 7.98 (s, 1H), 7.93 (t, *J* = 1.9 Hz, 1H), 7.74 (ddd, *J* = 8.0, 1.9, 1.0 Hz, 1H), 7.53 (ddd, *J* = 7.9, 2.0, 1.0 Hz, 1H), 7.36 (t, *J* = 7.9 Hz, 1H), 3.99 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 154.0, 141.8, 133.6, 133.0, 130.5, 127.5, 124.2, 123.2, 122.5, 39.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>10</sub>H<sub>10</sub>N<sub>4</sub>Br [M+H]<sup>+</sup> 265.0089, found 265.0076.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.56.

#### (*E*)-*N*-Methyl-*N*-(3-((1-methyl-1*H*-pyrazol-4-yl)diazenyl)phenyl)-3-(methylamino)benzenesulfonamide (**B121**)



**Synthetic procedure:** The same procedure employed for **B97c** was followed starting from **B121c** (0.100 g, 0.377 mmol, 1.00 equiv), **B121b** (0.091 g, 0.453 mmol, 1.20 equiv), Pd(OAc)<sub>2</sub> (0.85 mg, 0.004 mmol, 0.010 equiv), tBuBrettPhos (4.0 mg, 0.008 mmol, 0.022 equiv), K<sub>3</sub>PO<sub>4</sub> (0.112 g, 0.528 mmol, 1.40 equiv), *t*-BuOH

(1.0 mL) and H<sub>2</sub>O (0.27  $\mu$ L, 0.015 mmol, 0.040 equiv). Stirring at 110 °C for 3.5 h and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.077 g, 0.201 mmol, 67%). This is a novel compound.

**Physical state:** Yellow solid.

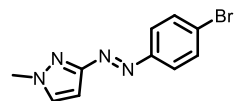
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  8.51 (s, 1H), 7.98 (s, 1H), 7.66 (ddd, *J* = 7.9, 1.9, 1.0 Hz, 1H), 7.53–7.46 (m, 2H), 7.28–7.21 (m, 2H), 6.78 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.68–6.63 (m, 1H), 6.56 (t, *J* = 2.0 Hz, 1H), 6.20 (q, *J* = 5.0 Hz, 1H), 3.92 (s, 3H), 3.17 (s, 3H), 2.57 (d, *J* = 4.8 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  152.5, 150.1, 142.4, 140.9, 136.3, 132.3, 129.62, 129.57, 128.8, 127.3, 120.4, 119.0, 116.0, 113.8, 109.2, 39.2, 37.7, 29.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>18</sub>H<sub>21</sub>N<sub>6</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 385.1447, found 385.1431.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.13.

#### (*E*)-3-((4-Bromophenyl)diazenyl)-1-methyl-1*H*-pyrazole (B122a)



**Synthetic procedure:** The same procedure employed for **B117** was followed starting from 4-bromoaniline (0.860 g, 5.00 mmol, 1.00 equiv), Oxone (6.15 g, 10.0 mmol, 2.00 equiv), DCM (25.0 mL) and water (25.0 mL). Stirring at rt for 3 h provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1*H*-pyrazol-3-amine (0.486 g, 5.00 mmol, 1.00 equiv) and glacial AcOH (50.0 mL). Stirring at rt for 38 h and purification by SGCC (by *n*-hexane:EtOAc 0–60%) provided the title compound (0.631 g, 2.38 mmol, 48% over two steps). This is a novel compound.

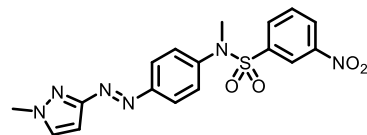
**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.86–7.78 (m, 2H), 7.68–7.59 (m, 2H), 7.40 (d, *J* = 2.4 Hz, 1H), 6.64 (d, *J* = 2.4 Hz, 1H), 4.03 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>):  $\delta$  164.0, 151.9, 132.7, 132.4, 125.9, 124.9, 96.8, 40.2.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.41.

#### (*E*)-*N*-Methyl-*N*-(4-((1-methyl-1*H*-pyrazol-3-yl)diazenyl)phenyl)-3-nitrobenzenesulfonamide (B122b)



**Synthetic procedure:** The same procedure employed for **B127** was followed starting from **B122a** (0.265 g, 1.00 mmol, 1.00 equiv), **B119a** (0.216 g, 1.00 mmol, 1.00 equiv), CuI (0.038 g, 0.200 mmol, 0.200 equiv), *N,N*-dimethylglycine (0.021 g, 0.200 mmol, 0.200 equiv), K<sub>3</sub>PO<sub>4</sub> (0.531 g, 2.50 mmol, 2.50 equiv) and anhydrous DMF (2.0 mL). Stirring at 100 °C for 23 h and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.247 g, 0.617 mmol, 62%). This is a novel compound.

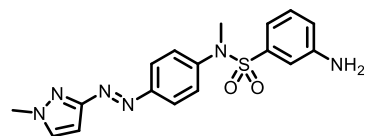
**Physical state:** Orange solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.51 (t, *J* = 1.9 Hz, 1H), 8.44 (ddd, *J* = 8.2, 2.3, 1.1 Hz, 1H), 7.93–7.86 (m, 2H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 2.4 Hz, 1H), 7.29–7.20 (m, 3H), 6.64 (t, *J* = 2.0 Hz, 1H), 4.04 (s, 3H), 3.29 (d, *J* = 1.5 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 163.7, 151.6, 148.3, 142.8, 138.7, 133.4, 132.1, 130.4, 127.6, 126.9, 123.8, 122.9, 96.5, 39.9, 38.4.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.19.

**(*E*)-3-Amino-*N*-methyl-*N*-(4-((1-methyl-1*H*-pyrazol-3-yl)diazenyl)phenyl)benzenesulfonamide (**B122**)**



**Synthetic procedure:** The same procedure employed for **B119** was followed starting from **B122b** (0.230 g, 0.574 mmol, 1.00 equiv), Na<sub>2</sub>S (0.448 g, 5.740 mmol, 10.0 equiv), THF (6.0 mL) and H<sub>2</sub>O (2.0 mL). Stirring at reflux for 3 days and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.144 g, 0.387 mmol, 67%). This is a novel compound.

**Physical state:** Orange solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.84 (d, *J* = 2.5 Hz, 1H), 7.83–7.76 (m, 2H), 7.39–7.32 (m, 2H), 7.17 (t, *J* = 7.9 Hz, 1H), 6.79 (dd, *J* = 8.0, 2.3 Hz, 1H), 6.75 (t, *J* = 2.1 Hz, 1H), 6.64–6.57 (m, 1H), 6.54 (d, *J* = 2.5 Hz, 1H), 5.62 (s, 2H), 3.97 (s, 3H), 3.18 (s, 3H).

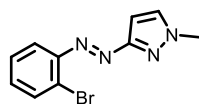
**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 163.0, 150.1, 149.5, 143.7, 136.3, 133.2, 129.6, 126.2, 122.6, 118.1, 113.9, 111.5, 94.6, 39.4, 37.6.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>19</sub>N<sub>6</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 371.1290, found 371.1292.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.19.



**(E)-3-((2-Bromophenyl)diazenyl)-1-methyl-1H-pyrazole (B123a)**



**Synthetic procedure:** The same procedure employed for **B117** was followed starting from 2-bromoaniline (0.226 mL, 2.00 mmol, 1.00 equiv), Oxone (2.46 g, 4.00 mmol, 2.00 equiv), DCM (20.0 mL) and water (20.0 mL). Stirring at rt for 7 h provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1H-pyrazol-3-amine (0.173 mL, 2.00 mmol, 1.00 equiv) and glacial AcOH (20.0 mL). Stirring at rt for 24 h and purification by SGCC (by *n*-hexane:EtOAc 0–50%) provided the title compound (0.394 g, 1.49 mmol, 74% over two steps). This is a novel compound.

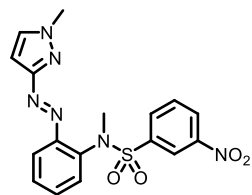
**Physical state:** Orange oil.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.78 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.73 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.40 (d, *J* = 2.6 Hz, 1H), 7.36 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.30 (td, *J* = 7.6, 1.7 Hz, 1H), 6.68 (d, *J* = 2.4 Hz, 1H), 4.04 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 164.1, 149.8, 133.8, 132.10, 132.05, 128.1, 125.9, 118.0, 96.0, 39.9.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.28.

**(E)-N-Methyl-N-(2-((1-methyl-1H-pyrazol-3-yl)diazenyl)phenyl)-3-nitrobenzenesulfonamide (B123b)**



**Synthetic procedure:** The same procedure employed for **B119c** was followed starting from **B123a** (0.133 g, 0.500 mmol, 1.00 equiv), **B119a** (0.108 g, 0.500 mmol, 1.00 equiv), CuI (0.019 g, 0.100 mmol, 0.200 equiv), *N,N*-dimethylglycine (0.010 g, 0.100 mmol, 0.200 equiv), K<sub>3</sub>PO<sub>4</sub> (0.265 g, 1.25 mmol, 2.50 equiv) and anhydrous DMF (1.0 mL). Stirring at 100 °C for 3 days and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.037 g, 0.093 mmol, 19%). This is a novel compound.

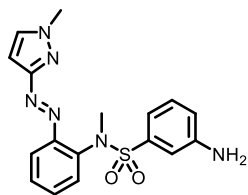
**Physical state:** Brown solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.54 (d, *J* = 2.1 Hz, 1H), 8.04–7.97 (m, 1H), 7.68 (d, *J* = 7.9 Hz, 1H), 7.63 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.58–7.52 (m, 2H), 7.44 (t, *J* = 7.7 Hz, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.21 (d, *J* = 2.4 Hz, 1H), 5.84 (d, *J* = 2.4 Hz, 1H), 3.99 (s, 3H), 3.52 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 163.8, 149.6, 148.4, 140.6, 138.7, 133.5, 133.2, 132.5, 132.2, 130.24, 130.17, 127.2, 122.8, 117.4, 94.9, 40.9, 40.2.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.29.

**(E)-3-Amino-N-methyl-N-(2-((1-methyl-1H-pyrazol-3-yl)diazenyl)phenyl)benzenesulfonamide (B123)**



**Synthetic procedure:** The same procedure employed for **B119** was followed starting from **B123b** (0.030 g, 0.075 mmol, 1.00 equiv), Na<sub>2</sub>S (0.058 g, 0.750 mmol, 10.0 equiv), THF (3.0 mL) and H<sub>2</sub>O (1.0 mL). Stirring at reflux for 15 h and purification by SGCC (*n*-hexane:EtOAc 0–100% followed by DCM:MeOH 0–5%) provided the title compound (0.031 g, 0.075 mmol, quant.). This is a novel compound.

**Physical state:** Yellow solid.

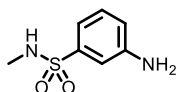
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.74 (d, *J* = 2.4 Hz, 1H), 7.61–7.52 (m, 2H), 7.48 (td, *J* = 7.6, 1.6 Hz, 1H), 7.41 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.05 (t, *J* = 7.9 Hz, 1H), 6.85 (t, *J* = 2.1 Hz, 1H), 6.69 (dd, *J* = 7.8, 1.8 Hz, 1H), 6.64 (dd, *J* = 8.0, 2.2 Hz, 1H), 6.11 (d, *J* = 2.4 Hz, 1H), 5.49 (s, 2H), 3.95 (s, 3H), 3.23 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 163.4, 151.2, 149.4, 149.2, 139.5, 138.6, 132.8, 131.4, 131.0, 129.3, 128.9, 117.6, 116.0, 113.9, 111.7, 94.6.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>19</sub>N<sub>6</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 371.1290, found 371.1287.

**R<sub>f</sub>** (silica gel, DCM:MeOH 95:5): 0.34.

**3-Amino-N-methylbenzenesulfonamide (B124a)**



**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B119a** (1.10 g, 5.09 mmol, 1.00 equiv), 10% Pd/C (0.110 g, 10 wt%) and MeOH (25 mL). Stirring at rt for 15 h provided the title compound (0.918 g, 4.93 mmol, 97%). This is a known compound.<sup>247</sup>

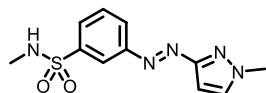
**Physical state:** Brown oil.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.28 (t, *J* = 7.0 Hz, 1H), 7.20 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.15 (t, *J* = 2.1 Hz, 1H), 6.84 (dd, *J* = 7.9, 2.4 Hz, 1H), 4.44 (d, *J* = 7.1 Hz, 1H), 3.94 (s, 2H), 2.65 (d, *J* = 5.4 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 147.4, 139.6, 130.3, 119.0, 116.9, 113.1, 29.6.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.11.

**(E)-N-Methyl-3-((1-methyl-1H-pyrazol-3-yl)diazenyl)benzenesulfonamide (B124b)**



**Synthetic procedure:** The same procedure employed for **B117** was followed starting from **B124a** (0.900 g, 4.83 mmol, 1.00 equiv), Oxone (3.94 g, 9.67 mmol, 2.00 equiv), DCM (50.0 mL) and H<sub>2</sub>O (50.0 mL). Stirring at rt for 5 h provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1H-pyrazol-3-amine (0.469 g, 4.83 mmol, 1.00 equiv) and glacial AcOH (50.0 mL). Stirring at rt for 44 h and recrystallisation from hot EtOH provided the title compound (0.530 g, 1.90 mmol, 39%). This is a novel compound.

**Physical state:** Yellow-brown solid.

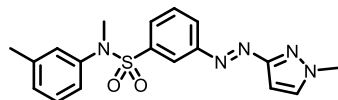
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.16 (t, *J* = 1.9 Hz, 1H), 8.12 (ddd, *J* = 7.9, 2.0, 1.2 Hz, 1H), 7.95–7.90 (m, 1H), 7.88 (d, *J* = 2.5 Hz, 1H), 7.83 (t, *J* = 7.8 Hz, 1H), 7.66 (q, *J* = 5.0 Hz, 1H), 6.61 (d, *J* = 2.4 Hz, 1H), 4.00 (s, 3H), 2.45 (d, *J* = 4.9 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 162.8, 152.2, 140.7, 133.5, 130.8, 128.8, 127.1, 118.9, 95.0, 28.7.

**LC–MS** (APCI): *m/z* calc. for C<sub>11</sub>H<sub>14</sub>N<sub>5</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 280.0868, found 280.1.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.39.

**(E)-N-Methyl-3-((1-methyl-1H-pyrazol-3-yl)diazenyl)-N-(*m*-tolyl)benzenesulfonamide (B124)**



**Synthetic procedure:** The same procedure employed for **B119c** was followed starting from **B117** (0.140 g, 0.500 mmol, 1.00 equiv), 3-bromotoluene (0.855 g, 5.00 mmol, 10.0 equiv), CuI (0.019 g, 0.100 mmol, 0.200 equiv), *N,N*-dimethylglycine (0.010 g, 0.100 mmol, 0.200 equiv), K<sub>3</sub>PO<sub>4</sub> (0.265 g, 1.25 mmol, 2.50 equiv) and anhydrous DMF (1.0 mL). Stirring at 100 °C for 20 h and then at 140 °C for 52 h and purification by SGCC (*n*-hexane:EtOAc 0–80%) provided the title compound (0.166 g, 0.450 mmol, 90%). This is a novel compound.

**Physical state:** Orange solid.

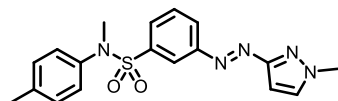
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.19–8.13 (m, 1H), 7.90–7.83 (m, 2H), 7.80 (t, *J* = 7.9 Hz, 1H), 7.67–7.60 (m, 1H), 7.22 (t, *J* = 7.7 Hz, 1H), 7.11 (d, *J* = 7.7 Hz, 1H), 6.96 (s, 1H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.59 (dd, *J* = 2.3, 1.4 Hz, 1H), 3.99 (s, 3H), 3.16 (d, *J* = 1.3 Hz, 3H), 2.24 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 162.7, 151.9, 140.8, 138.5, 137.4, 133.5, 130.7, 129.5, 128.8, 128.2, 127.6, 127.1, 123.3, 119.7, 95.0, 38.1, 20.8.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{18}H_{20}N_5O_2S$   $[M+H]^+$  370.1338, found 370.1338.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:2): 0.38.

**(*E*)-*N*-Methyl-3-((1-methyl-1*H*-pyrazol-3-yl)diazenyl)-*N*-(*p*-tolyl)benzenesulfonamide (B125)**



**Synthetic procedure:** The same procedure employed for **B119c** was followed starting from **B117** (0.140 g, 0.500 mmol, 1.00 equiv), 4-bromotoluene (0.855 g, 5.00 mmol, 10.0 equiv), CuI (0.019 g, 0.100 mmol, 0.200 equiv), *N,N*-dimethylglycine (0.010 g, 0.100 mmol, 0.200 equiv),  $K_3PO_4$  (0.265 g, 1.25 mmol, 2.50 equiv) and anhydrous DMF (1.0 mL). Stirring at 100 °C for 21 h and at 140 °C for 51 h and purification by SGCC (*n*-hexane:EtOAc 0–80%) provided the title compound (0.126 g, 0.342 mmol, 68%). This is a novel compound.

**Physical state:** Orange solid.

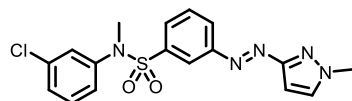
**$^1H$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  8.15 (d,  $J$  = 7.9 Hz, 1H), 7.90–7.83 (m, 2H), 7.79 (t,  $J$  = 7.9 Hz, 1H), 7.61 (d,  $J$  = 7.8 Hz, 1H), 7.14 (d,  $J$  = 8.0 Hz, 2H), 7.00 (d,  $J$  = 8.0 Hz, 2H), 6.59 (d,  $J$  = 2.4 Hz, 1H), 3.99 (s, 3H), 3.16 (s, 3H), 2.27 (s, 3H).

**$^{13}C\{^1H\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  162.7, 151.9, 138.3, 137.4, 137.0, 133.5, 130.8, 129.50, 129.46, 127.5, 126.3, 119.9, 95.1, 38.2, 20.5.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{18}H_{20}N_5O_2S$   $[M+H]^+$  370.1338, found 370.1327.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:2): 0.38.

**(*E*)-*N*-(3-Chlorophenyl)-*N*-methyl-3-((1-methyl-1*H*-pyrazol-3-yl)diazenyl)benzenesulfonamide (B126)**



**Synthetic procedure:** The same procedure employed for **B119c** was followed starting from **B117** (0.070 g, 0.250 mmol, 1.00 equiv), 1-bromo-3-chlorobenzene (0.479 g, 2.50 mmol, 10.0 equiv), CuI (0.010 g, 0.050 mmol, 0.200 equiv), *N,N*-dimethylglycine (0.005 g, 0.050 mmol, 0.200 equiv),  $K_3PO_4$  (0.133 g, 0.625 mmol, 2.50 equiv) and anhydrous DMF (1.0 mL). Stirring at 100 °C for 16 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.060 g, 0.155 mmol, 62%). This is a novel compound.

**Physical state:** Orange solid.

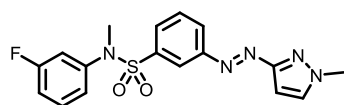
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.48 (ddd, *J* = 8.0, 2.0, 1.0 Hz, 1H), 7.22–7.15 (m, 2H), 7.11 (t, *J* = 7.9 Hz, 1H), 6.96–6.89 (m, 1H), 6.74–6.64 (m, 2H), 6.59–6.53 (m, 1H), 6.50–6.40 (m, 1H), 5.90 (d, *J* = 2.5 Hz, 1H), 3.30 (s, 3H), 2.50 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 162.7, 152.0, 142.2, 136.9, 133.5, 133.1, 130.9, 130.6, 129.4, 127.8, 127.5, 126.3, 124.9, 119.8, 95.1, 37.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>17</sub>N<sub>5</sub>O<sub>2</sub>SCl [M+H]<sup>+</sup> 390.0791, found 390.0804.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.28.

### (*E*)-*N*-(3-Fluorophenyl)-*N*-methyl-3-((1-methyl-1*H*-pyrazol-3-yl)diazenyl)benzenesulfonamide (B127)



**Synthetic procedure:** The same procedure employed for **B119c** was followed starting from **B117** (0.024 g, 0.086 mmol, 1.00 equiv), 1-bromo-3-fluorobenzene (0.150 g, 0.859 mmol, 10.0 equiv), CuI (0.003 g, 0.017 mmol, 0.200 equiv), *N,N*-dimethylglycine (0.002 g, 0.017 mmol, 0.200 equiv), K<sub>3</sub>PO<sub>4</sub> (0.046 g, 0.215 mmol, 2.50 equiv) and anhydrous DMF (1.0 mL). Stirring at 100 °C for 48 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.020 g, 0.052 mmol, 61%). This is a novel compound.

**Physical state:** Orange solid.

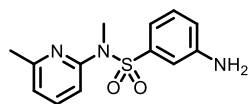
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.17 (ddd, *J* = 7.9, 2.0, 1.1 Hz, 1H), 7.90–7.86 (m, 2H), 7.80 (t, *J* = 7.9 Hz, 1H), 7.62 (dt, *J* = 8.0, 1.3 Hz, 1H), 7.40 (td, *J* = 8.2, 6.7 Hz, 1H), 7.21–7.11 (m, 1H), 7.11–7.00 (m, 2H), 6.60 (d, *J* = 2.4 Hz, 1H), 3.99 (s, 3H), 3.20 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 163.0, 162.7, 160.6, 152.0, 142.5, 142.4, 137.0, 133.5, 130.9, 130.5, 130.4, 129.4, 127.8, 122.21, 122.18, 119.8, 114.4, 114.2, 113.5, 113.2, 95.1, 37.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>17</sub>N<sub>5</sub>O<sub>2</sub>SF [M+H]<sup>+</sup> 374.1087, found 374.1077.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.27.

### 3-Amino-*N*-methyl-*N*-(6-methylpyridin-2-yl)benzenesulfonamide (B128a)



**Synthetic procedure:** A previously reported Ullmann-type cross-coupling protocol was followed with minor modifications.<sup>181</sup> A pressure vial was charged with a solution of **B86b** (0.171 g, 0.500 mmol, 1.00 equiv) in 35% NH<sub>4</sub>OH (2.00 mL, 17.6 mmol, 35.1 equiv), and the solution was degassed with Ar for 10 min. CuI (0.010 g, 0.050 mmol, 0.100 equiv) was then added against a positive pressure of Ar. The mixture was

stirred at 130 °C for 20 h. Upon reaction completion as determined by TLC, the mixture was diluted with H<sub>2</sub>O (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.036 g, 0.130 mmol, 26%). This is a novel compound.

**Physical state:** White solid.

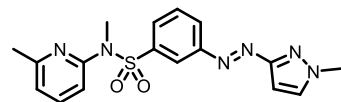
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.68 (t, *J* = 7.8 Hz, 1H), 7.32 (d, *J* = 8.2 Hz, 1H), 7.14 (t, *J* = 7.9 Hz, 1H), 7.05 (d, *J* = 7.4 Hz, 1H), 6.85 (t, *J* = 2.0 Hz, 1H), 6.76 (ddd, *J* = 8.1, 2.3, 0.9 Hz, 1H), 6.67 (ddd, *J* = 7.7, 1.9, 0.9 Hz, 1H), 5.60 (s, 2H), 3.22 (s, 3H), 2.33 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 156.6, 152.3, 149.5, 138.1, 137.9, 129.5, 120.0, 118.0, 115.4, 113.7, 111.4, 35.2, 23.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>16</sub>N<sub>3</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 278.0963, found 278.0954.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.51.

**(*E*)-*N*-Methyl-3-((1-methyl-1*H*-pyrazol-3-yl)diazenyl)-*N*-(6-methylpyridin-2-yl)benzenesulfonamide (B128)**



**Synthetic procedure:** The same procedure employed for **B117** was followed starting from **B128a** (0.360 g, 1.29 mmol, 1.00 equiv), Oxone (1.58 g, 2.57 mmol, 2.00 equiv), DCM (13.0 mL) and water (13.0 mL). Stirring at rt for 2 h provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1*H*-pyrazol-3-amine (0.125 g, 1.29 mmol, 1.00 equiv) and glacial AcOH (13.0 mL). Stirring at rt for 17 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.286 g, 0.772 mmol, 60% over two steps). This is a novel compound.

**Physical state:** Yellow solid.

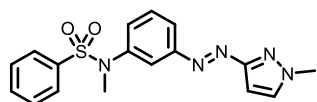
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.14 (dt, *J* = 6.9, 2.0 Hz, 1H), 8.03 (d, *J* = 1.9 Hz, 1H), 7.87 (d, *J* = 2.5 Hz, 1H), 7.84–7.78 (m, 2H), 7.75 (t, *J* = 7.9 Hz, 1H), 7.30 (d, *J* = 8.1 Hz, 1H), 7.10 (d, *J* = 7.5 Hz, 1H), 6.60 (d, *J* = 2.4 Hz, 1H), 3.99 (s, 3H), 3.26 (s, 3H), 2.27 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 162.7, 156.9, 152.4, 151.9, 139.0, 138.7, 133.5, 130.6, 129.5, 127.9, 120.9, 119.8, 116.1, 95.0, 35.8, 23.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>19</sub>N<sub>6</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 371.1290, found 371.1292.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.22.

**(*E*)-*N*-Methyl-*N*-(3-((1-methyl-1*H*-pyrazol-3-yl)diazenyl)phenyl)benzenesulfonamide (B129)**



**Synthetic procedure:** The same procedure employed for **B117** was followed starting from **B39** (0.262 g, 1.00 mmol, 1.00 equiv), Oxone (1.23 g, 2.00 mmol, 2.00 equiv), DCM (10.0 mL) and water (10.0 mL). Stirring at rt for 21 h provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1*H*-pyrazol-3-amine (0.097 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at rt for 19 h and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.223 g, 0.627 mmol, 63% over two steps). This is a novel compound.

**Physical state:** Orange solid.

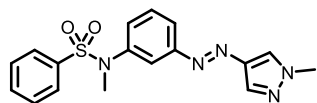
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.84 (d, *J* = 2.4 Hz, 1H), 7.78–7.73 (m, 1H), 7.73–7.67 (m, 1H), 7.63–7.50 (m, 6H), 7.29 (ddd, *J* = 8.0, 2.2, 1.0 Hz, 1H), 6.54 (d, *J* = 2.4 Hz, 1H), 3.97 (s, 3H), 3.21 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 162.8, 152.4, 142.1, 135.6, 133.5, 133.3, 129.9, 129.3, 128.3, 127.4, 121.1, 119.4, 94.7, 39.4, 37.7.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>18</sub>N<sub>5</sub>O<sub>2</sub>S [M+H]<sup>+</sup> 356.1181, found 356.1176.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.31.

**(*E*)-*N*-Methyl-*N*-(3-((1-methyl-1*H*-pyrazol-4-yl)diazenyl)phenyl)benzenesulfonamide (B130)**



**Synthetic procedure:** The same procedure employed for **B117** was followed starting from **B39** (0.262 g, 1.00 mmol, 1.00 equiv), Oxone (1.23 g, 2.00 mmol, 2.00 equiv), DCM (10.0 mL) and water (10.0 mL). Stirring at rt for 7 h provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1*H*-pyrazol-4-amine (0.097 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at rt for 3 days and purification by SGCC (DCM:MeOH 0–5% followed by *n*-hexane:EtOAc 0–70%) provided the title compound (0.131 g, 0.367 mmol, 37% over two steps). This is a novel compound.

**Physical state:** Orange oil.

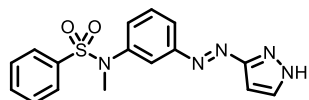
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.51 (s, 1H), 7.97 (s, 1H), 7.75–7.68 (m, 1H), 7.66 (ddd, *J* = 7.9, 1.9, 1.0 Hz, 1H), 7.61–7.52 (m, 4H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.46 (t, *J* = 2.1 Hz, 1H), 7.20 (ddd, *J* = 8.0, 2.3, 1.1 Hz, 1H), 3.92 (s, 3H), 3.20 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.6, 142.1, 140.9, 135.7, 133.5, 132.3, 129.8, 129.3, 128.9, 127.41, 127.36, 120.6, 119.1, 39.2, 37.8.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{17}H_{18}N_5O_2S$   $[M+H]^+$  356.1181, found 356.1177.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.25.

**(*E*)-*N*-(3-((1*H*-Pyrazol-3-yl)diazenyl)phenyl)-*N*-methylbenzenesulfonamide (B131)**



**Synthetic procedure:** The same procedure employed for **B117** was followed starting from **B39** (0.262 g, 1.00 mmol, 1.00 equiv), Oxone (1.23 g, 2.00 mmol, 2.00 equiv), DCM (10.0 mL) and water (10.0 mL). Stirring at rt for 18 h provided the crude nitroso intermediate, which was used directly in the next step with 1*H*-pyrazol-3-amine (0.083 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at rt for 4 days and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.116 g, 0.341 mmol, 34% over two steps). This is a novel compound.

**Physical state:** Orange solid.

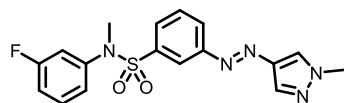
**$^1H$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  13.55 (s, 1H), 7.88 (s, 1H), 7.77 (ddd,  $J$  = 8.0, 1.9, 1.0 Hz, 1H), 7.74–7.67 (m, 1H), 7.62–7.52 (m, 6H), 7.28 (ddd,  $J$  = 8.1, 2.3, 1.0 Hz, 1H), 6.55 (s, 1H), 3.22 (s, 3H).

**$^{13}C\{^1H\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  163.7, 152.4, 142.1, 135.7, 133.5, 130.8, 129.9, 129.4, 128.3, 127.4, 121.1, 119.4, 93.6, 37.7.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{16}H_{16}N_5O_2S$   $[M+H]^+$  342.1025, found 342.1035.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.20.

**(*E*)-*N*-(3-Fluorophenyl)-*N*-methyl-3-((1-methyl-1*H*-pyrazol-4-yl)diazenyl)benzenesulfonamide (B132)**



**Synthetic procedure:** The same procedure employed for **B117** was followed starting from **B62** (0.280 g, 1.00 mmol, 1.00 equiv), Oxone (1.23 g, 2.00 mmol, 2.00 equiv), DCM (10.0 mL) and water (10.0 mL). Stirring at rt for 2.5 h and at 50 °C for 30 min provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1*H*-pyrazol-4-amine (0.097 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at rt for 5 days and purification by SGCC (*n*-hexane:EtOAc 0–80%) followed by RPC ( $H_2O$  + 0.1% FA : MeCN + 0.1% FA 5–95%) provided the title compound (0.045 g, 0.120 mmol, 12% over two steps). This is a novel compound.

**Physical state:** Orange oily solid.



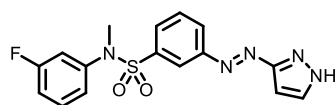
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.57 (s, 1H), 8.07 (dd, *J* = 8.0, 1.7 Hz, 1H), 8.02 (s, 1H), 7.80–7.72 (m, 2H), 7.57 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.45–7.35 (m, 1H), 7.17 (td, *J* = 8.5, 2.5 Hz, 1H), 7.10–7.00 (m, 2H), 3.93 (s, 3H), 3.18 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 163.0, 160.6, 152.2, 142.6, 142.5, 140.9, 136.8, 132.5, 130.7, 130.5, 130.4, 129.4, 128.5, 127.5, 122.2, 122.1, 119.1, 114.3, 114.1, 113.4, 113.2, 39.3, 37.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>17</sub>N<sub>5</sub>O<sub>2</sub>FS [M+H]<sup>+</sup> 374.1087, found 374.1094.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.24.

### (*E*)-3-((1*H*-Pyrazol-3-yl)diazenyl)-*N*-(3-fluorophenyl)-*N*-methylbenzenesulfonamide (B133)



**Synthetic procedure:** The same procedure employed for **B117** was followed starting from **B62** (0.280 g, 1.00 mmol, 1.00 equiv), Oxone (1.23 g, 2.00 mmol, 2.00 equiv), DCM (10.0 mL) and water (10.0 mL). Stirring at rt for 18 h provided the crude nitroso intermediate, which was used directly in the next step with 1*H*-pyrazol-3-amine (0.083 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at rt for 4 days and purification by SGCC (*n*-hexane:EtOAc 0–100%) followed by RPC (H<sub>2</sub>O + 0.1% FA : MeCN + 0.1% FA 5–95%) provided the title compound (0.031 g, 0.087 mmol, 9% over two steps). This is a novel compound.

**Physical state:** Yellow solid.

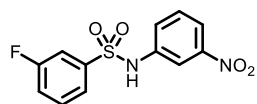
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.72 (s, 1H), 8.18 (dd, *J* = 7.7, 1.9 Hz, 1H), 7.93–7.85 (m, 2H), 7.81 (t, *J* = 7.9 Hz, 1H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.40 (q, *J* = 7.8 Hz, 1H), 7.16 (td, *J* = 8.5, 2.6 Hz, 1H), 7.11–7.01 (m, 2H), 6.61 (d, *J* = 2.6 Hz, 1H), 3.20 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 163.0, 160.6, 152.0, 142.5, 142.4, 137.0, 130.9, 130.5, 130.4, 129.4, 127.9, 122.19, 122.16, 119.7, 114.4, 114.2, 113.5, 113.3, 37.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>16</sub>H<sub>15</sub>N<sub>5</sub>O<sub>2</sub>SF [M+H]<sup>+</sup> 360.0930, found 360.0935.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.25.

### 3-Fluoro-*N*-(3-nitrophenyl)benzenesulfonamide (B134a)



**Synthetic procedure:** The same procedure employed for **B68a** was followed starting from 3-nitroaniline (1.38 g, 10.0 mmol, 1.00 equiv), 3-fluorobenzenesulfonyl chloride (1.33 mL, 10.0 mmol, 1.00 equiv),

pyridine (2.43 mL, 30.0 mmol, 3.00 equiv) and DCM (40.0 mL). Stirring at rt for 43 h provided the title compound (2.92 g, 9.86 mmol, 99%). This is a known compound.<sup>250</sup>

**Physical state:** Orange solid.

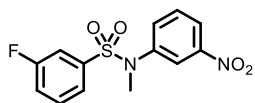
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.99 (dt, *J* = 7.8, 1.7 Hz, 1H), 7.95 (t, *J* = 2.1 Hz, 1H), 7.68–7.61 (m, 1H), 7.56 (dt, *J* = 8.0, 2.2 Hz, 1H), 7.48 (dt, *J* = 12.5, 7.7 Hz, 3H), 7.29 (td, *J* = 8.3, 2.5 Hz, 1H).

**<sup>19</sup>F NMR** (377 MHz, CDCl<sub>3</sub>): δ -108.38.

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 164.2, 161.7, 149.3, 140.82, 140.75, 137.9, 131.8, 131.7, 130.9, 127.0, 123.52, 123.49, 121.6, 121.4, 120.7, 116.0, 115.2, 115.0.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 2:1): 0.26.

### 3-Fluoro-*N*-methyl-*N*-(3-nitrophenyl)benzenesulfonamide (B134b)



**Synthetic procedure:** The same procedure employed for **B11** was followed starting from **B134a** (2.90 g, 9.79 mmol, 1.00 equiv), MeI (0.609 mL, 9.79 mmol, 1.50 equiv), K<sub>2</sub>CO<sub>3</sub> (8.12 g, 58.7 mmol, 6.00 equiv) and anhydrous DMF (20.0 mL). Stirring at rt for 5 days provided the title compound (2.92 g, 9.40 mmol, 96%). This is a novel compound.

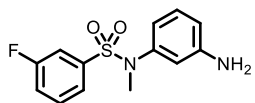
**Physical state:** Orange oil.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.16 (dt, *J* = 8.1, 1.6 Hz, 1H), 7.88 (t, *J* = 2.2 Hz, 1H), 7.63 (dt, *J* = 8.4, 1.4 Hz, 1H), 7.55 (t, *J* = 8.1 Hz, 1H), 7.48 (td, *J* = 8.0, 5.2 Hz, 1H), 7.37–7.27 (m, 3H), 3.25 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 163.7, 161.2, 142.5, 133.1, 131.2, 131.1, 130.0, 123.61, 123.58, 122.4, 121.0, 120.8, 120.6, 115.3, 115.1, 38.1.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.30.

### *N*-(3-Aminophenyl)-3-fluoro-*N*-methylbenzenesulfonamide (B134c)



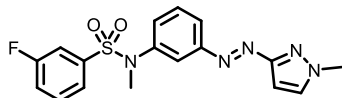
**Synthetic procedure:** The same procedure employed for **B1** was followed starting from **B134b** (2.90 g, 9.35 mmol, 1.00 equiv), 10% Pd/C (0.150 g, 5 wt%), MeOH (25.0 mL) and EtOAc (25.0 mL). Stirring at rt for 21 h provided the title compound (2.94 g, 9.35 mmol, quant.). This is a known compound (CAS no.: 1305719-38-3).

**Physical state:** Yellow solid.

**LC-MS** (ES-API):  $m/z$  calc. for  $C_{13}H_{14}FN_2O_2S$   $[M+H]^+$  281.0760, found  $m/z$  281.0  $[M+H]^+$ .

$R_f$  (silica gel, *n*-hexane:EtOAc 2:1): 0.34.

**(*E*)-3-Fluoro-*N*-methyl-*N*-(3-((1-methyl-1*H*-pyrazol-3-yl)diazenyl)phenyl)benzenesulfonamide (B134)**



**Synthetic procedure:** The same procedure employed for **B117** was followed starting from **B134c** (0.280 g, 1.00 mmol, 1.00 equiv), Oxone (1.23 g, 2.00 mmol, 2.00 equiv), DCM (10.0 mL) and water (10.0 mL). Stirring at rt for 4 h provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1*H*-pyrazol-3-amine (0.097 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at rt for 3 days and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.070 g, 0.189 mmol, 19% over two steps). This is a novel compound.

**Physical state:** Orange oily solid.

**$^1H$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  7.85 (d,  $J$  = 2.4 Hz, 1H), 7.81–7.74 (m, 1H), 7.68–7.53 (m, 4H), 7.43–7.34 (m, 2H), 7.34–7.26 (m, 1H), 6.54 (d,  $J$  = 2.5 Hz, 1H), 3.98 (s, 3H), 3.25 (s, 3H).

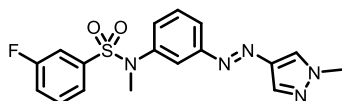
**$^{19}F$  NMR** (377 MHz, DMSO- $d_6$ ):  $\delta$  -110.03.

**$^{13}C\{^1H\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  162.9, 162.8, 160.5, 152.5, 141.8, 137.7, 137.6, 133.3, 131.83, 131.75, 130.0, 128.4, 123.8, 123.7, 121.3, 120.8, 120.6, 119.6, 114.6, 114.3, 94.8, 39.4, 37.9.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{17}H_{17}N_5O_2FS$   $[M+H]^+$  374.1087, found 374.1092.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.23.

**(*E*)-3-Fluoro-*N*-methyl-*N*-(3-((1-methyl-1*H*-pyrazol-4-yl)diazenyl)phenyl)benzenesulfonamide (B135)**



**Synthetic procedure:** The same procedure employed for **B117** was followed starting from **B134c** (0.280 g, 1.00 mmol, 1.00 equiv), Oxone (1.23 g, 2.00 mmol, 2.00 equiv), DCM (10.0 mL) and water (10.0 mL). Stirring at rt for 4 h provided the crude nitroso intermediate, which was used directly in the next step with 1-methyl-1*H*-pyrazol-4-amine (0.097 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at rt for 3 days and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.115 g, 0.307 mmol, 31% over two steps). This is a novel compound.

**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.51 (s, 1H), 7.97 (s, 1H), 7.72–7.55 (m, 3H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.48 (t, *J* = 2.1 Hz, 1H), 7.37 (ddd, *J* = 7.6, 3.6, 1.6 Hz, 2H), 7.22 (ddd, *J* = 7.9, 2.2, 1.0 Hz, 1H), 3.92 (s, 3H), 3.23 (s, 3H).

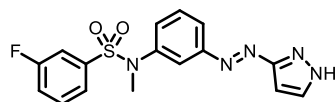
**<sup>19</sup>F NMR** (377 MHz, DMSO-*d*<sub>6</sub>): δ -110.08.

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 162.9, 160.5, 152.7, 141.8, 140.9, 137.8, 137.7, 132.3, 131.8, 131.7, 129.9, 128.9, 127.5, 123.8, 123.7, 121.0, 120.8, 120.6, 119.3, 114.6, 114.4, 39.2, 38.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>17</sub>N<sub>5</sub>O<sub>2</sub>FS [M+H]<sup>+</sup> 374.1087, found 374.1095.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.21.

#### (*E*)-*N*-(3-((1*H*-Pyrazol-3-yl)diazenyl)phenyl)-3-fluoro-*N*-methylbenzenesulfonamide (**B136**)



**Synthetic procedure:** The same procedure employed for **B117** was followed starting from **B134c** (0.280 g, 1.00 mmol, 1.00 equiv), Oxone (1.23 g, 2.00 mmol, 2.00 equiv), DCM (10.0 mL) and water (10.0 mL). Stirring at rt for 22 h provided the crude nitroso intermediate, which was used directly in the next step with 1*H*-pyrazol-3-amine (0.324 g, 2.00 mmol, 2.00 equiv) and glacial AcOH (10.0 mL). Stirring at rt for 24 h and purification by SGCC (DCM:MeOH 0–5%) provided the title compound (0.062 g, 0.171 mmol, 17% over two steps). This is a novel compound.

**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.55 (s, 1H), 7.92–7.86 (m, 1H), 7.85–7.76 (m, 1H), 7.70–7.52 (m, 4H), 7.38 (td, *J* = 7.8, 1.7 Hz, 2H), 7.33–7.27 (m, 1H), 6.55 (t, *J* = 2.2 Hz, 1H), 3.25 (s, 3H).

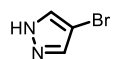
**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 163.7, 162.9, 160.4, 152.5, 141.8, 137.7, 131.8, 131.7, 130.8, 130.0, 128.4, 123.8, 123.7, 121.3, 120.8, 120.6, 119.7, 114.6, 114.3, 93.6, 37.9.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>16</sub>H<sub>15</sub>N<sub>5</sub>O<sub>2</sub>SF [M+H]<sup>+</sup> 360.0930, found 360.0928.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.48.

#### 5.1.5. Series C compounds

##### 4-Bromo-1*H*-pyrazole (**C1**)



**Synthetic procedure:** NA. This compound was procured (CAS no.: 2075-45-8).

**Physical state:** White solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.19 (s, 1H), 7.96 (s, 1H), 7.56 (s, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 139.1, 128.9, 91.9.

#### 4-Iodo-1*H*-pyrazole (C2)



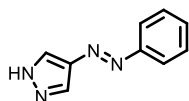
**Synthetic procedure:** NA. This compound was procured (CAS no.: 3469-69-0).

**Physical state:** Off-white solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.18 (s, 1H), 7.85 (s, 1H), 7.62 (s, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 144.1, 133.6, 56.8.

#### (*E*)-4-(Phenyldiazenyl)-1*H*-pyrazole (C3)



**Synthetic procedure:** A previously reported Baeyer-Mills reaction protocol was followed with minor modifications.<sup>100</sup> To 1*H*-pyrazol-4-amine (0.183 g, 2.20 mmol, 1.10 equiv) and nitrosobenzene (0.214 g, 2.00 mmol, 1.00 equiv) were added degassed glacial AcOH (20 mL). The mixture was stirred at 40 °C for 23 h. Upon reaction completion as determined by TLC, the mixture was concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.136 g, 0.788 mmol, 39%). This is a known compound.<sup>251</sup>

**Physical state:** Yellow solid.

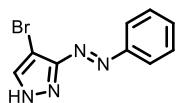
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.51–13.46 (m, 1H), 8.55 (s, 1H), 7.98 (d, *J* = 34.3 Hz, 1H), 7.79–7.70 (m, 2H), 7.54 (dd, *J* = 8.4, 6.6 Hz, 2H), 7.50–7.43 (m, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.4, 141.0, 130.3, 129.3, 121.8.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>9</sub>H<sub>9</sub>N<sub>4</sub> [M+H]<sup>+</sup> 173.0827, found 173.0822.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.20.

#### (*E*)-4-Bromo-3-(phenyldiazenyl)-1*H*-pyrazole (C4)



**Synthetic procedure:** A previously reported Baeyer-Mills reaction protocol was followed with minor modifications.<sup>36</sup> A solution of 4-bromo-1*H*-pyrazol-3-amine hydrobromide (0.534 g, 2.20 mmol, 1.10 equiv) in

40% aq. NaOH (4.00 mL, 48.0 mmol, 21.8 equiv) and toluene (4.0 mL) was degassed with N<sub>2</sub> for 10 min. Then nitrosobenzene (0.214 g, 2.00 mmol, 1.00 equiv) was added against a positive pressure of N<sub>2</sub>, and the mixture was stirred under N<sub>2</sub>, at 80 °C for 26 h. Upon reaction completion as determined by TLC, the mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic phases were washed with sat. brine (10 mL), dried over MgSO<sub>4</sub>, and concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–100%, followed by DCM:MeOH 0–10%) provided the title compound (0.092 g, 0.366 mmol, 18%). This is a novel compound.

**Physical state:** Orange solid.

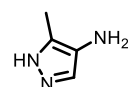
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.93 (s, 1H), 8.09 (s, 1H), 7.91–7.80 (m, 2H), 7.67–7.54 (m, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*D*<sub>6</sub>): δ 152.2, 131.9, 129.6, 122.4.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>9</sub>H<sub>8</sub>N<sub>4</sub>Br [M+H]<sup>+</sup> 250.9932, found 250.9929.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.48.

### 5-Methyl-1*H*-pyrazol-4-amine (C5a)



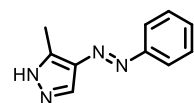
**Synthetic procedure:** A solution of 5-methyl-4-nitro-1*H*-pyrazole (0.254 g, 2.00 mmol, 1.00 equiv) in MeOH (10.0 mL) was degassed with N<sub>2</sub> for 10 minutes. 10% Pd/C (0.025 g, 10 wt%) was then added against a positive pressure of N<sub>2</sub>, and the mixture further degassed with N<sub>2</sub> for 5 minutes. The mixture was then degassed with H<sub>2</sub> for 5 minutes and stirred under H<sub>2</sub> (1 atm) at rt for 16 h. Upon reaction completion as determined by TLC, the mixture was degassed with N<sub>2</sub> for 10 minutes, filtered through a bed of celite and the filtrate concentrated to dryness *in vacuo* to provide the title compound (0.211 g, 2.00 mmol, quant.). This is a known compound.<sup>252</sup>

**Physical state:** Orange-brown solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.64 (s, 1H), 6.91 (s, 1H), 3.49 (s, 2H), 2.02 (s, 3H).

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.00.

### (*E*)-5-Methyl-4-(phenyldiazenyl)-1*H*-pyrazole (C5)



**Synthetic procedure:** The same procedure employed for **C3** was followed starting from **C5a** (0.210 g, 2.00 mmol, 1.00 equiv), nitrosobenzene (0.214 g, 2.00 mmol, 1.00 equiv) and glacial AcOH (20.0 mL). Stirring

at 40 °C for 22 h and purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.322 g, 1.73 mmol, 86%). This is a novel compound.

**Physical state:** Yellow-green solid.

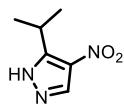
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.19 (s, 1H), 8.02–7.81 (m, 1H), 7.78–7.71 (m, 2H), 7.57–7.49 (m, 2H), 7.48–7.41 (m, 1H), 2.54 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.6 137.6, 129.9, 129.3, 121.6.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>10</sub>H<sub>11</sub>N<sub>4</sub> [M+H]<sup>+</sup> 187.0984, found 187.0975.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.56.

### 5-Isopropyl-4-nitro-1*H*-pyrazole (C6a)



**Synthetic procedure:** A previously reported pyrazole nitration was followed with minor modifications.<sup>182</sup>

To a solution of 5-isopropyl-1*H*-pyrazole (0.500 g, 4.54 mmol, 1.00 equiv) in H<sub>2</sub>SO<sub>4</sub> (2.0 mL) cooled to 0 °C was dropwise added 65% HNO<sub>3</sub> (0.730 mL, 11.4 mmol, 2.50 equiv), and the mixture was stirred at 0 °C for 10 min. The mixture was then stirred at 115 °C for 4 h. Upon reaction completion as determined by TLC, the mixture was cooled to rt, poured into ice-water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic phases were washed with sat. NaHCO<sub>3</sub> (10 mL) and sat. brine (10 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to dryness *in vacuo* to provide the title compound (0.700 g, 4.51 mmol, 99%). This is a known compound (CAS no.: 51355-77-2).

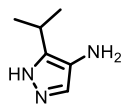
**Physical state:** Beige solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.20 (s, 1H), 3.78 (hept, *J* = 7.0 Hz, 1H), 1.39 (d, *J* = 7.0 Hz, 6H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 136.4, 25.9, 20.9.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.52.

### 5-Isopropyl-1*H*-pyrazol-4-amine (C6b)



**Synthetic procedure:** The same procedure employed for **C5a** was followed starting from **C6a** (0.695 g, 4.48 mmol, 1.00 equiv), 10% Pd/C (0.070 g, 10 wt%) and MeOH (20.0 mL). Stirring under H<sub>2</sub> at rt for 7 h provided the title compound (0.591 g, 4.48 mmol, quant.). This is a known compound (CAS no.: 1249021-45-1).

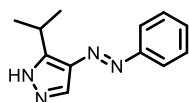
**Physical state:** Beige solid.

**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.15 (s, 1H), 3.00 (hept,  $J$  = 7.1 Hz, 1H), 1.30 (dd,  $J$  = 7.0, 1.1 Hz, 6H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{CDCl}_3$ ):  $\delta$  124.2, 60.5, 25.0, 21.7.

**$R_f$**  (silica gel, DCM:MeOH 9:1): 0.28.

**(*E*)-5-Isopropyl-4-(phenyldiazenyl)-1*H*-pyrazole (C6)**



**Synthetic procedure:** The same procedure employed for **C3** was followed starting from **C6b** (0.250 g, 2.00 mmol, 1.00 equiv), nitrosobenzene (0.214 g, 2.00 mmol, 1.00 equiv) and glacial AcOH (20.0 mL). Stirring at rt for 3 days and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.355 g, 1.66 mmol, 83%). This is a novel compound.

**Physical state:** Yellow solid.

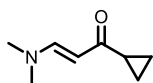
**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  13.17 (d,  $J$  = 42.7 Hz, 1H), 7.78 (s, 1H), 7.76–7.69 (m, 2H), 7.53 (dd,  $J$  = 8.4, 6.7 Hz, 2H), 7.47–7.40 (m, 1H), 3.67–3.37 (m, 1H), 1.39 (d,  $J$  = 7.1 Hz, 6H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  152.7, 129.9, 129.3, 128.9, 121.6, 24.8, 22.2.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{12}\text{H}_{15}\text{N}_4$   $[\text{M}+\text{H}]^+$  215.1297, found 215.1294.

**$R_f$**  (silica gel, *n*-hexane:EtOAc 1:1): 0.46.

**(*E*)-1-Cyclopropyl-3-(dimethylamino)prop-2-en-1-one (C7a)**



**Synthetic procedure:** A previously reported aldol condensation protocol was followed with minor modifications.<sup>183</sup> A neat mixture of 1-cyclopropylethan-1-one (0.991 mL, 10.0 mmol, 1.00 equiv) and *N,N*-dimethylformamide dimethyl acetal (1.33 mL, 10.0 mmol, 1.00 equiv) was stirred at 100 °C for 3 days. Upon reaction completion as determined by TLC, the mixture was concentrated to dryness *in vacuo*. Purification by SGCC (DCM:MeOH 0–15%) provided the title compound (0.291 g, 2.09 mmol, 21%). This is a known compound. Experimental data correlate with literature.<sup>253</sup>

**Physical state:** Yellow oil.

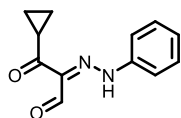
**$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.54 (d,  $J$  = 12.6 Hz, 1H), 5.18 (d,  $J$  = 12.6 Hz, 1H), 2.90 (d,  $J$  = 30.2 Hz, 6H), 1.77 (tt,  $J$  = 7.9, 4.6 Hz, 1H), 1.03–0.92 (m, 2H), 0.73 (dt,  $J$  = 7.9, 3.3 Hz, 2H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{CDCl}_3$ ):  $\delta$  197.4, 162.6, 151.8, 95.9, 36.6, 31.5, 20.0, 9.3.



$R_f$  (silica gel, DCM:MeOH 9:1): 0.42.

**(E)-3-Cyclopropyl-3-oxo-2-(2-phenylhydrazineylidene)propanal (C7b)**



**Synthetic procedure:** To a solution of aniline (0.149 mL, 1.63 mmol, 1.00 equiv) in 1 M aq. HCl (5.00 mL, 5.00 mmol, 3.07 equiv) cooled to 0 °C was dropwise added a solution of NaNO<sub>2</sub> (0.169 g, 2.45 mmol, 1.50 equiv) in H<sub>2</sub>O (1.0 mL), and the mixture was stirred at 0 °C for 30 min. To a solution of **C7a** (0.233 g, 1.67 mmol, 1.03 equiv) and NaOAc (0.201 g, 2.45 mmol, 1.50 equiv) in H<sub>2</sub>O (2.0 mL) cooled to 0 °C was dropwise added the diazonium intermediate prepared above. The pH was adjusted to 7.0 with NaOAc, and the mixture stirred at 0 °C for 1 h. Upon reaction completion as determined by TLC, the precipitate was isolated by suction filtration, washed with H<sub>2</sub>O, and dried *in vacuo* to provide the title compound (0.209 g, 0.967 mmol, 59%). This is a novel compound.

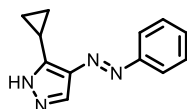
**Physical state:** Orange solid.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 14.71–14.00 (m, 1H), 10.20–9.28 (m, 1H), 7.67 (t, *J* = 11.0 Hz, 2H), 7.46 (t, *J* = 7.8 Hz, 2H), 7.25 (t, *J* = 7.4 Hz, 1H), 3.32–2.91 (m, 1H), 1.16–0.90 (m, 4H).

<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, DMSO-*d*<sub>6</sub>): δ 197.4, 190.2, 187.3, 141.4, 133.0, 132.3, 129.6, 128.9, 126.2, 126.1, 120.1, 116.9, 116.8, 18.6, 14.2, 12.7, 10.6.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.74.

**(E)-5-Cyclopropyl-4-(phenyldiazenyl)-1H-pyrazole (C7)**



**Synthetic procedure:** To a solution of **C7b** (0.200 g, 0.925 mmol, 1.00 equiv) in abs. EtOH (5.0 mL) was added hydrazine hydrate (0.052 mL, 0.925 mmol, 1.00 equiv). The mixture was stirred at reflux for 16 h. Upon reaction completion as determined by TLC, the mixture was concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–80%) provided the title compound (0.063 g, 0.297 mmol, 32%). This is a novel compound.

**Physical state:** Yellow solid.

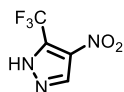
<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.07 (d, *J* = 16.3 Hz, 1H), 7.77 (d, *J* = 1.8 Hz, 1H), 7.76–7.69 (m, 2H), 7.52 (t, *J* = 7.7 Hz, 2H), 7.44 (q, *J* = 6.6 Hz, 1H), 2.43 (tt, *J* = 7.9, 5.7 Hz, 1H), 1.22–0.93 (m, 4H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  152.7, 151.1, 146.8, 138.0, 137.6, 129.9, 129.7, 129.3, 128.9, 121.6, 121.4, 9.2, 8.7, 8.1, 6.6.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{12}\text{H}_{13}\text{N}_4$   $[\text{M}+\text{H}]^+$  213.1130, found 213.1125.

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 1:1): 0.37.

#### 4-Nitro-5-(trifluoromethyl)-1H-pyrazole (C8a)



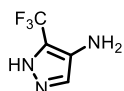
**Synthetic procedure:** The same procedure employed for **C6a** was employed starting from 5-(trifluoromethyl)-1H-pyrazole (0.680 g, 5.00 mmol, 1.00 equiv), 65%  $\text{HNO}_3$  (0.866 mL, 12.5 mmol, 2.50 equiv) and  $\text{H}_2\text{SO}_4$  (2.2 mL). Stirring at 0–115 °C for 3 h provided the title compound (1.13 g, 5.00 mmol, quant.). This is a known compound. Experimental data correlate with literature.<sup>254</sup>

**Physical state:** Colourless oil.

**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  14.73 (s, 1H), 9.17 (s, 1H).

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 1:1): 0.39.

#### 5-(Trifluoromethyl)-1H-pyrazol-4-amine (C8b)



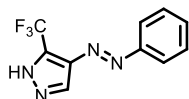
**Synthetic procedure:** The same procedure employed for **C5a** was followed starting from **C8a** (1.10 g, 5.00 mmol, 1.00 equiv), 10% Pd/C (0.11 g, 10 wt%), MeOH (25.0 mL) and EtOAc (25.0 mL). Stirring under  $\text{H}_2$  at rt for 4 h provided the title compound (0.717 g, 4.74 mmol, 95%). This is a known compound.<sup>255</sup>

**Physical state:** Brown solid.

**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  12.82 (s, 1H), 7.20 (s, 1H), 4.16 (s, 2H).

**$R_f$**  (silica gel,  $n$ -hexane:EtOAc 1:1): 0.18.

#### (E)-4-(Phenyldiazenyl)-5-(trifluoromethyl)-1H-pyrazole (C8)



**Synthetic procedure:** The same procedure employed for **C3** was followed starting from **C8b** (0.151 g, 1.00 mmol, 1.00 equiv), nitrosobenzene (0.107 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring

at rt for 18 h and purification by SGCC (*n*-hexane:EtOAc 0–50%) provided the title compound (0.190 g, 0.789 mmol, 79%). This is a novel compound.

**Physical state:** Yellow solid.

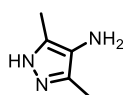
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 14.22 (s, 1H), 8.53 (s, 1H), 7.82–7.73 (m, 2H), 7.63–7.50 (m, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.1, 137.1, 131.4, 129.5, 122.9, 122.7, 122.1, 120.2.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>10</sub>H<sub>8</sub>N<sub>4</sub>F<sub>3</sub> [M+H]<sup>+</sup> 241.0701, found 241.0704.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.51.

### 3,5-Dimethyl-1*H*-pyrazol-4-amine (C9a)



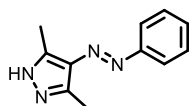
**Synthetic procedure:** The same procedure employed for **C5a** was followed starting from 3,5-dimethyl-4-nitro-1*H*-pyrazole (0.282 g, 2.00 mmol, 1.00 equiv), 10% Pd/C (0.028 g, 10 wt%) and EtOAc (10.0 mL). Stirring under H<sub>2</sub> at rt for 6.5 h provided the title compound (0.230 g, 2.00 mmol, quant.). This is a known compound. Experimental data correlate with literature.<sup>256</sup>

**Physical state:** Beige solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.40 (s, 1H), 3.22 (s, 2H), 1.99 (s, 6H).

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.88.

### (*E*)-3,5-Dimethyl-4-(phenyldiazenyl)-1*H*-pyrazole (C9)



**Synthetic procedure:** The same procedure employed for **C3** was followed starting from **C9a** (0.230 g, 1.00 mmol, 1.00 equiv), nitrosobenzene (0.214 g, 2.00 mmol, 1.00 equiv) and glacial AcOH (20.0 mL). Stirring at 40 °C for 22 h and purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.340 g, 1.70 mmol, 85%). This is a known compound.<sup>257</sup>

**Physical state:** Yellow solid.

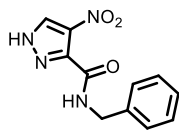
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 12.85 (s, 1H), 7.75–7.67 (m, 2H), 7.51 (dd, *J* = 8.4, 6.9 Hz, 2H), 7.46–7.37 (m, 1H), 2.45 (s, 6H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 153.0, 134.1, 129.4, 129.2, 121.4.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>11</sub>H<sub>13</sub>N<sub>4</sub> [M+H]<sup>+</sup> 201.1140, found 201.1141.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.62.

#### ***N*-Benzyl-4-nitro-1*H*-pyrazole-3-carboxamide (C10a)**



**Synthetic procedure:** To a solution of 4-nitro-1*H*-pyrazole-3-carboxylic acid (0.314 g, 2.00 mmol, 1.00 equiv), benzylamine (0.218 mL, 2.00 mmol, 1.00 equiv) and HOBt (0.337 g, 2.20 mmol, 1.10 equiv) in anhydrous DMF (10.0 mL) was added EDC·HCl (0.422 g, 2.20 mmol, 1.10 equiv). The mixture was stirred at rt for 22 h. Upon reaction completion as determined by TLC, the mixture was added H<sub>2</sub>O (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic phases were washed with sat. brine (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to dryness *in vacuo*. The residue was added H<sub>2</sub>O (10 mL) to form a precipitate, which was isolated by suction filtration, washed with H<sub>2</sub>O (3 x 5 mL) and dried *in vacuo* to provide the title compound (0.460 g, 1.87 mmol, 93%). This is a known compound.<sup>258</sup>

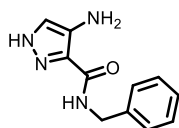
**Physical state:** Off-white solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 14.18 (s, 1H), 9.19 (s, 1H), 8.82 (s, 1H), 7.37–7.31 (m, 4H), 7.26 (tt, *J* = 5.7, 3.0 Hz, 1H), 4.48 (d, *J* = 6.0 Hz, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 138.7, 132.3, 128.3, 127.8, 127.3, 127.0, 124.5, 119.2, 109.6, 42.4.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): ND. The compound is streaking a lot.

#### **4-Amino-*N*-benzyl-1*H*-pyrazole-3-carboxamide (C10b)**



**Synthetic procedure:** The same procedure employed for **C5a** was followed starting from **C10a** (0.450 g, 1.83 mmol, 1.00 equiv), 10% Pd/C (0.045 g, 10 wt%) and MeOH (10.0 mL). Stirring under H<sub>2</sub> at rt for 3 h and purification by SGCC (DCM:MeOH 0–15%) provided the title compound (0.142 g, 0.655 mmol, 36%). This is a known compound.<sup>258</sup>

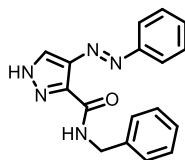
**Physical state:** Grey solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 12.58 (s, 1H), 8.41 (s, 1H), 7.30 (d, *J* = 4.3 Hz, 5H), 7.22 (q, *J* = 4.4 Hz, 1H), 7.11 (s, 1H), 5.11 (s, 2H), 4.39 (d, *J* = 6.4 Hz, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 164.1, 140.2, 132.9, 131.8, 128.2, 127.3, 127.1, 126.6, 124.4, 119.1, 115.0, 109.7, 41.5.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.29.

**(E)-N-Benzyl-4-(phenyldiazenyl)-1H-pyrazole-3-carboxamide (C10)**



**Synthetic procedure:** The same procedure employed for **C3** was followed starting from **C10b** (0.100 g, 0.462 mmol, 1.00 equiv), nitrosobenzene (0.050 g, 0.462 mmol, 1.00 equiv) and glacial AcOH (5.0 mL). Stirring at rt for 3 days and purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.040 g, 0.131 mmol, 28%). This is a novel compound.

**Physical state:** Orange solid.

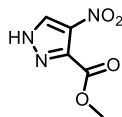
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 12.76 (s, 1H), 9.29 (t, *J* = 5.2 Hz, 1H), 8.24 (s, 1H), 7.48–7.31 (m, 10H), 4.74 (d, *J* = 5.1 Hz, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 152.4, 137.8, 137.7, 131.8, 129.7, 129.5, 128.9, 128.4, 122.8, 44.7.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>17</sub>H<sub>16</sub>N<sub>5</sub>O [M+H]<sup>+</sup> 306.1355, found 306.1355.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.40.

**Methyl 4-nitro-1H-pyrazole-3-carboxylate (C11a)**



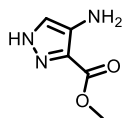
**Synthetic procedure:** To MeOH (30.0 mL) cooled to 0 °C was dropwise added AcCl (3.91 mL, 55.0 mmol, 11.0 equiv), and the mixture was stirred at rt for 10 min. 4-Nitro-1H-pyrazole-3-carboxylic acid (0.785 g, 5.00 mmol, 1.00 equiv) was then added and the mixture stirred at rt for 3 days. Upon reaction completion as determined by TLC, the mixture was added sat. NaHCO<sub>3</sub> (15 mL), concentrated *in vacuo* and extracted with EtOAc (3 x 15 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo* to provide the title compound (0.756 g, 4.42 mmol, 88%). This is a known compound. Experimental data correlate with literature.<sup>259</sup>

**Physical state:** Off-white solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 14.44 (s, 1H), 8.94 (s, 1H), 3.88 (s, 3H).

**R<sub>f</sub>** (silica gel, DCM:MeOH 4:1): 0.72.

### Methyl 4-amino-1H-pyrazole-3-carboxylate (C11b)



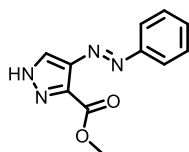
**Synthetic procedure:** The same procedure employed for **C5a** was followed starting from **C11a** (0.750 g, 4.38 mmol, 1.00 equiv), 10% Pd/C (0.075 g, 10 wt%) and MeOH (20.0 mL). Stirring under H<sub>2</sub> at rt for 16 h provided the title compound (0.502 g, 3.56 mmol, 81%). This is a known compound. Experimental data correlate with literature.<sup>260</sup>

**Physical state:** Beige solid.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 12.85–12.80 (m, 1H), 7.08 (s, 1H), 4.91–4.84 (m, 2H), 3.77 (s, 3H).

R<sub>f</sub> (silica gel, *n*-hexane:EtOAc 1:1): 0.19.

### Methyl (*E*)-4-(phenyldiazenyl)-1H-pyrazole-3-carboxylate (C11c)



**Synthetic procedure:** The same procedure employed for **C3** was followed starting from **C11b** (0.500 g, 3.54 mmol, 1.00 equiv), nitrosobenzene (0.379 g, 3.54 mmol, 1.00 equiv) and glacial AcOH (30.0 mL). Stirring at rt for 48 h and purification by SGCC (DCM:MeOH 0–15%) provided the title compound (0.643 g, 2.79 mmol, 79%). This is a known compound.<sup>261</sup>

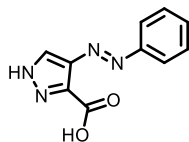
**Physical state:** Orange solid.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 14.04 (s, 1H), 8.26 (s, 1H), 7.85–7.78 (m, 2H), 7.62–7.49 (m, 3H), 3.90 (s, 3H).

<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.3, 139.7, 131.2, 129.4, 122.2, 52.1.

R<sub>f</sub> (silica gel, DCM:MeOH 9:1): 0.41.

### (*E*)-4-(Phenyldiazenyl)-1H-pyrazole-3-carboxylic acid (C11d)



**Synthetic procedure:** To a solution of **C11c** (0.500 g, 2.17 mmol, 1.00 equiv) in MeOH (20.0 mL) was added 1 M aq. NaOH (8.70 mL, 8.69 mmol, 4.00 equiv). The mixture was stirred at rt for 39 h. Upon reaction completion, the mixture was concentrated to dryness *in vacuo*. The residue was retaken in H<sub>2</sub>O (10 mL),

the pH adjusted to 3 and the mixture extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo* to provide the title compound (0.405 g, 1.87 mmol, 86%). This is a novel compound.

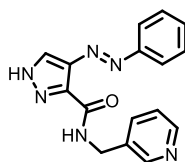
**Physical state:** Orange solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.83 (s, 1H), 8.09 (s, 1H), 7.83–7.75 (m, 2H), 7.62–7.48 (m, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.2, 139.5, 131.1, 129.5, 122.2.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.06.

**(*E*)-4-(Phenyldiazenyl)-*N*-(pyridin-3-ylmethyl)-1*H*-pyrazole-3-carboxamide (C11)**



**Synthetic procedure:** To a solution of **C11d** (0.108 g, 0.500 mmol, 1.00 equiv) in anhydrous DMF (10.0 mL) cooled to 0 °C was added EDC·HCl (0.115 g, 0.600 mmol, 1.20 equiv) and HOBT (0.092 g, 0.600 mmol, 1.20 equiv), and the mixture was stirred at 0 °C for 10 min. 3-Picolylamine (0.056 mL, 0.550 mmol, 1.10 equiv) was then added, and the mixture was stirred at rt for 24 h. Upon reaction completion as determined by TLC, the mixture was concentrated to dryness *in vacuo*. The crude was purified by SGCC (DCM:MeOH 0–20%), and the residue was added H<sub>2</sub>O (10 mL) to form a precipitate, which was isolated by suction filtration, washed with H<sub>2</sub>O (3 x 5 mL) and dried *in vacuo* to provide the title compound (0.064 g, 0.209 mmol, 42%). This is a novel compound.

**Physical state:** Yellow solid.

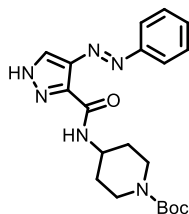
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 14.08 (d, *J* = 164.3 Hz, 1H), 8.92 (s, 1H), 8.63 (d, *J* = 2.2 Hz, 1H), 8.50 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.02 (s, 0H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.76–7.68 (m, 2H), 7.52 (dd, *J* = 5.2, 1.9 Hz, 3H), 7.39 (dd, *J* = 7.9, 4.8 Hz, 1H), 4.61 (s, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.1, 149.1, 148.4, 135.4, 131.2, 129.4, 123.6, 122.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>16</sub>H<sub>15</sub>N<sub>6</sub>O [M+H]<sup>+</sup> 307.1307, found 307.1314.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.32.

***tert*-Butyl (*E*)-4-(4-(phenyldiazenyl)-1*H*-pyrazole-3-carboxamido)piperidine-1-carboxylate (**C12a**)**



**Synthetic procedure:** The same procedure employed for **C3** was followed starting from **D2b** (0.155 g, 0.500 mmol, 1.00 equiv), nitrosobenzene (0.054 g, 0.500 mmol, 1.00 equiv) and glacial AcOH (5.0 mL). Stirring at rt for 3 days and purification by SGCC (DCM:MeOH 0–20%) provided the title compound (0.168 g, 0.420 mmol, 84%). This is a novel compound.

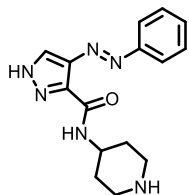
**Physical state:** Orange-brown solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 14.25 (s, 1H), 8.36 (d, *J* = 7.7 Hz, 1H), 8.04 (s, 1H), 7.78 (d, *J* = 7.4 Hz, 2H), 7.65–7.50 (m, 3H), 4.10–3.96 (m, 0H), 3.88 (d, *J* = 13.3 Hz, 2H), 2.94 (s, 2H), 1.89 (d, *J* = 9.7 Hz, 2H), 1.40 (s, 11H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 153.9, 152.2, 129.5, 122.1, 78.7, 54.9, 46.2, 31.1, 28.1.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.45.

**(*E*)-4-(Phenyldiazenyl)-*N*-(piperidin-4-yl)-1*H*-pyrazole-3-carboxamide (**C12**)**



**Synthetic procedure:** To a solution of **C12a** (0.160 g, 0.402 mmol, 1.00 equiv) in DCM (4.0 mL) cooled to 0 °C was dropwise added TFA (4.0 mL). The mixture was stirred and slowly allowed to reach rt over 9 h. Upon reaction completion as determined by LC–MS, the mixture was concentrated *in vacuo*. The residue was retaken in H<sub>2</sub>O (10 mL), basified with 1 M NaOH to pH 12 and extracted with EtOAc (3 x 15 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. The residue was redissolved in EtOAc (5 mL) and washed with 1 M NaOH (5 mL), and the aq. phase back-extracted with EtOAc (3 x 5 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo* to provide the title compound (0.045 g, 0.151 mmol, 38%). This is a novel compound.

**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.39 (d, *J* = 7.8 Hz, 1H), 8.09 (s, 1H), 7.83–7.74 (m, 2H), 7.65–7.47 (m, 3H), 3.97–3.84 (m, 1H), 2.95 (dt, *J* = 13.0, 3.8 Hz, 2H), 2.63–2.51 (m, 2H), 1.92–1.81 (m, 2H), 1.49–1.34 (m, 2H).

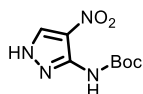


**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  157.8, 152.2, 137.4, 137.3, 131.2, 129.6, 127.6, 122.1, 46.8, 44.7, 32.7.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $\text{C}_{15}\text{H}_{19}\text{N}_6\text{O}$   $[\text{M}+\text{H}]^+$  299.1620, found 299.1621.

$R_f$  (silica gel,  $\text{DCM}:\text{MeOH}$  7:3): 0.12.

***tert*-Butyl (4-nitro-1*H*-pyrazol-3-yl)carbamate (C13a)**



**Synthetic procedure:** A previously reported Curtius rearrangement protocol was followed with minor modifications.<sup>184</sup> To a solution of 4-nitro-1*H*-pyrazole-3-carboxylic acid (0.785 g, 5.00 mmol, 1.00 equiv) and  $\text{Et}_3\text{N}$  (2.09 mL, 15.0 mmol, 3.00 equiv) in anhydrous *t*-BuOH (10.0 mL) and anhydrous 1,4-dioxane (20.0 mL) was added diphenyl phosphoryl azide (1.72 mL, 8.00 mmol, 1.60 equiv). The mixture was stirred at reflux for 24 h. Upon reaction completion as determined by TLC, the mixture was diluted with  $\text{H}_2\text{O}$  (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over  $\text{Na}_2\text{SO}_4$  and concentrated to dryness *in vacuo*. Purification by SGCC ( $\text{DCM}:\text{MeOH}$  0–5%) provided the title compound (0.284 g, 1.24 mmol, 25%). This is a known compound (CAS no.: 2101822-96-0).

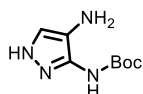
**Physical state:** White solid.

**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  13.60 (s, 1H), 9.41 (s, 1H), 8.50 (s, 1H), 1.44 (s, 9H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  130.4, 130.2, 126.1, 120.0, 120.0, 27.9, 13.6.

$R_f$  (silica gel,  $\text{DCM}:\text{MeOH}$  9:1): 0.78.

***tert*-Butyl (4-amino-1*H*-pyrazol-3-yl)carbamate (C13b)**



**Synthetic procedure:** The same procedure employed for **C5a** was followed starting from **C13a** (0.273 g, 1.20 mmol, 1.00 equiv), 10% Pd/C (0.027 g, 10 wt%), MeOH (10.0 mL) and EtOAc (10 mL). Stirring under  $\text{H}_2$  at rt for 16 h provided the title compound (0.235 g, 1.19 mmol, 99%). This is a known compound.<sup>262</sup>

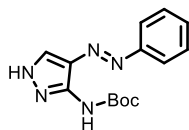
**Physical state:** Pink solid.

**$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  11.67 (s, 1H), 8.81 (s, 1H), 7.00 (s, 1H), 3.49 (s, 2H), 1.43 (s, 9H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  153.6, 122.4, 78.6, 40.0, 28.1.

$R_f$  (silica gel,  $\text{DCM}:\text{MeOH}$  9:1): 0.35.

***tert*-Butyl (*E*)-(4-(phenyldiazenyl)-1*H*-pyrazol-3-yl)carbamate (C13c)**



**Synthetic procedure:** The same procedure employed for **C3** was followed starting from **C13b** (0.230 g, 1.16 mmol, 1.00 equiv), nitrosobenzene (0.124 g, 1.16 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at rt for 21 h and purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.225 g, 0.783 mmol, 68%). This is a novel compound.

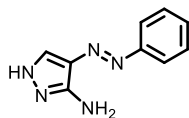
**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 11.41 (s, 1H), 9.60 (s, 1H), 8.10 (s, 1H), 7.78–7.72 (m, 2H), 7.48 (td, *J* = 7.4, 1.4 Hz, 2H), 7.44–7.36 (m, 1H), 1.57 (s, 9H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 153.1, 152.6, 140.0, 129.9, 129.3, 125.1, 121.8, 83.5, 28.3.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.73.

**(*E*)-4-(Phenyldiazenyl)-1*H*-pyrazol-3-amine (C13d)**



**Synthetic procedure:** The same procedure employed for **C12** was followed starting from **C13c** (0.225 g, 0.783 mmol, 1.00 equiv), TFA (8.0 mL) and DCM (8.0 mL). Stirring at 0 °C–rt for 15 h provided the title compound (0.153 g, 0.783 mmol, quant.). This is a novel compound.

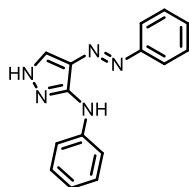
**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 12.70–11.67 (m, 1H), 7.73–7.67 (m, 2H), 7.46 (q, *J* = 8.1 Hz, 2H), 7.33 (dt, *J* = 14.3, 7.2 Hz, 1H), 6.91 (s, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.9, 129.1, 129.0, 128.6, 126.9, 121.0, 120.9.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.40.

**(*E*)-*N*-Phenyl-4-(phenyldiazenyl)-1*H*-pyrazol-3-amine (C13)**



**Synthetic procedure:** A previously reported Cham-Lam cross-coupling protocol was followed with minor modifications.<sup>164</sup> A solution of **C13d** (0.050 g, 0.267 mmol, 1.00 equiv), phenylboronic acid (0.065 g, 0.534 mmol, 2.00 equiv), Ni(OAc)<sub>2</sub>·4H<sub>2</sub>O (0.013 g, 0.053 mmol, 0.20 equiv) and DBU (0.080 mL, 0.534 mmol, 2.00 equiv) in anhydrous DMSO (1.0 mL) was stirred under air at 50 °C for 25 h and then at 100 °C for 24 h. Upon reaction completion as determined by TLC, the mixture was diluted with H<sub>2</sub>O (15 mL) and extracted with EtOAc (3 x 10 mL). The combined organic phases were washed with sat. brine (2 x 20 mL), dried over Na<sub>2</sub>SO<sub>4</sub> (1.0 mL) and concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–40%) provided the title compound (0.012 g, 0.044 mmol, 16%). This is a novel compound.

**Physical state:** Yellow solid.

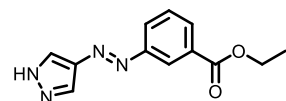
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 12.96 (s, 1H), 9.49 (s, 1H), 8.58 (s, 1H), 7.90–7.82 (m, 2H), 7.71 (d, *J* = 8.0 Hz, 2H), 7.54 (t, *J* = 7.7 Hz, 2H), 7.47–7.41 (m, 1H), 7.32–7.27 (m, 2H), 6.90 (t, *J* = 7.3 Hz, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.1, 141.1, 131.0, 129.4, 129.3, 128.9, 127.3, 126.9, 121.5, 120.3, 117.1.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>14</sub>N<sub>5</sub> [M+H]<sup>+</sup> 264.1249, found 264.1248.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.53.

#### Ethyl (*E*)-3-((1*H*-pyrazol-4-yl)diazenyl)benzoate (**C14a**)



**Synthetic procedure:** A solution of ethyl 3-aminobenzoate (0.330 g, 2.00 mmol, 1.00 equiv) in DCM (20.0 mL) and H<sub>2</sub>O (20.0 mL) was degassed with Ar for 10 min. Oxone (2.46 g, 4.00 mmol, 2.00 equiv) was then added against a positive pressure of Ar, and the mixture was stirred under Ar at rt for 45 min. Upon reaction completion as determined by TLC, the layers were allowed to separate, and the organic layer was syringed over into an Ar-purged flask containing Na<sub>2</sub>SO<sub>4</sub>. A solution of 1-methyl-1*H*-pyrazol-4-amine (0.183 g, 2.20 mmol, 1.10 equiv) in glacial AcOH (20.0 mL) was degassed with Ar for 10 min. The dried solution of the nitroso intermediate prepared above was then added dropwise by syringe. Additional DCM (10.0 mL) was added by syringe into the reaction flask from the first step, and the mixture stirred for a few minutes to extract more of the nitroso intermediate. This organic layer was then syringed over into the same Na<sub>2</sub>SO<sub>4</sub>-containing flask to dry it before being syringed over into the reaction flask of the second step. The mixture was stirred under Ar at rt for 3 days. Upon reaction completion, the mixture was concentrated to dryness *in vacuo*. Purification by SGCC (*n*-hexane:EtOAc 0–100%) provided the title compound (0.228 g, 0.935 mmol, 47% over two steps). This is a novel compound.

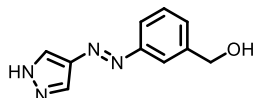
**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.55 (s, 1H), 8.63 (s, 1H), 8.26 (t, *J* = 1.8 Hz, 1H), 8.08–7.99 (m, 2H), 7.70 (t, *J* = 7.8 Hz, 1H), 4.36 (q, *J* = 7.1 Hz, 2H), 1.34 (t, *J* = 7.1 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 165.3, 152.4, 141.0, 131.1, 130.5, 130.0, 127.0, 121.3, 61.1, 14.2.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.35.

#### (*E*)-(3-((1*H*-Pyrazol-4-yl)diazenyl)phenyl)methanol (**C14**)



**Synthetic procedure:** A previously reported ester reduction protocol was followed with minor modifications.<sup>185</sup> To a solution of **C14a** (0.220 g, 0.901 mmol, 1.00 equiv) in anhydrous THF (20.0 mL) cooled to 0 °C was dropwise added 1 M DIBAL-H in PhMe (2.70 mL, 2.70 mmol, 3.00 equiv). The mixture was stirred and allowed to slowly reach rt over 17 h. Upon reaction completion as determined by TLC, the mixture was cooled back to 0 °C and dropwise added sat. aq. NH<sub>4</sub>Cl (2 mL) and then 10% Rochelle salt (20 mL) and EtOAc (20 mL). The resulting mixture was stirred for 1 h. The layers were separated, and the aq. layer extracted with EtOAc (2 x 20 mL). The combined organic phases were washed with sat. brine (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub> (1.0 mL) and concentrated to dryness *in vacuo*. Purification by SGCC (DCM:MeOH 0–100%) provided the title compound (0.096 g, 0.476 mmol, 53%). This is a novel compound.

**Physical state:** Yellow solid.

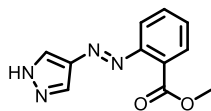
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.47 (s, 1H), 8.69–7.84 (brs, 1H), 7.72 (d, *J* = 1.9 Hz, 1H), 7.63 (dt, *J* = 7.9, 1.6 Hz, 1H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.41 (dt, *J* = 7.8, 1.4 Hz, 1H), 5.34 (t, *J* = 5.8 Hz, 1H), 4.59 (d, *J* = 4.0 Hz, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 152.4, 144.0, 141.0, 129.0, 128.2, 121.0, 118.8, 62.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>10</sub>H<sub>11</sub>N<sub>4</sub>O [M+H]<sup>+</sup> 203.0933, found 203.0934.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.45.

#### Methyl (*E*)-2-((1*H*-pyrazol-4-yl)diazenyl)benzoate (**C15a**)



**Synthetic procedure:** The same procedure employed for **C14a** was followed starting from methyl 2-aminobenzoate (0.302 g, 2.00 mmol, 1.00 equiv), Oxone (2.46 g, 4.00 mmol, 2.00 equiv), DCM (20.0 mL) and H<sub>2</sub>O (20.0 mL). Stirring at rt for 14 h provided the crude nitroso intermediate, which was used directly in

the next step with 1*H*-pyrazol-4-amine (0.183 g, 2.20 mmol, 1.10 equiv) and glacial AcOH (20.0 mL). Stirring at rt for 30 h and purification by SGCC (DCM:MeOH 0–20%) provided the title compound (0.277 g, 1.20 mmol, 60% over two steps). This is a novel compound.

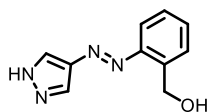
**Physical state:** Yellow-brown solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.54 (s, 1H), 8.58 (s, 1H), 7.96 (s, 1H), 7.70 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.66 (ddd, *J* = 8.7, 7.3, 1.5 Hz, 1H), 7.58 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.53 (td, *J* = 7.4, 1.3 Hz, 1H), 3.80 (s, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 167.9, 151.0, 141.0, 131.8, 129.4, 129.0, 128.0, 119.5, 52.2.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.19.

#### (*E*)-2-((1*H*-Pyrazol-4-yl)diazenyl)phenyl)methanol (**C15**)



**Synthetic procedure:** The same procedure employed for **C14** was followed starting from **C15a** (0.115 g, 0.500 mmol, 1.00 equiv), 1 M DIBAL-H in PhMe (1.50 mL, 1.50 mmol, 3.00 equiv) and anhydrous THF (10.0 mL). Stirring at 0 °C–rt for 20 h and purification by SGCC (DCM:MeOH 0–15%) provided the title compound (0.016 g, 0.051 mmol, 10%). This is a novel compound.

**Physical state:** Yellow solid.

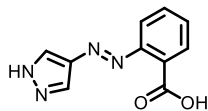
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.45 (s, 1H), 8.28 (s, 2H), 7.69–7.63 (m, 1H), 7.46 (td, *J* = 7.1, 1.2 Hz, 2H), 7.34 (td, *J* = 7.4, 1.5 Hz, 1H), 5.21 (s, 1H), 4.98 (s, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 148.7, 141.5, 140.1, 130.1, 127.7, 127.2, 114.5, 58.5.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>10</sub>H<sub>11</sub>N<sub>4</sub>O [M+H]<sup>+</sup> 203.0933, found 203.0920.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.35.

#### (*E*)-2-((1*H*-Pyrazol-4-yl)diazenyl)benzoic acid (**C16a**)



**Synthetic procedure:** The same procedure employed for **C11d** was followed starting from **C15a** (0.149 g, 0.647 mmol, 1.00 equiv), 1 M aq. NaOH (2.59 mL, 2.59 mmol, 4.00 equiv) and MeOH (6.0 mL). Stirring at rt for 7 days provided the title compound (0.128 g, 0.593 mmol, 92%). This is a novel compound.

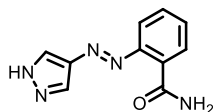
**Physical state:** Orange solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.40 (brs, 2H), 8.23 (s, 2H), 7.73 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.62 (td, *J* = 7.6, 1.5 Hz, 1H), 7.55–7.45 (m, 2H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  168.5, 151.2, 141.2, 131.7, 129.7, 129.4, 129.2, 118.2.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.27.

**(E)-2-((1H-Pyrazol-4-yl)diazenyl)benzamide (C16)**



**Synthetic procedure:** A solution of **C16a** (0.120 g, 0.555 mmol, 1.00 equiv) in anhydrous DMF (3.0 mL) cooled to 0 °C was added EDC·HCl (0.117 g, 0.611 mmol, 1.10 equiv), HOBT (0.102 g, 0.666 mmol, 1.20 equiv) and DIPEA (0.290 mL, 1.67 mmol, 3.00 equiv), and the mixture was stirred at 0 °C for 30 min. NH<sub>4</sub>Cl (0.059 g, 1.11 mmol, 2.00 equiv) was then added, and the mixture was stirred at rt for 24 h. Upon reaction completion as determined by TLC, the mixture was added H<sub>2</sub>O (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by SGCC (DCM:MeOH 0–20%) followed by RPC (H<sub>2</sub>O + 0.1% FA : MeCN + 0.1% FA 5–100%) provided the title compound (0.020 g, 0.094 mmol, 17%). This is a novel compound.

**Physical state:** Yellow solid.

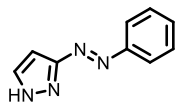
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  13.55 (s, 1H), 8.29 (s, 2H), 7.94 (s, 1H), 7.79 (dd,  $J$  = 6.5, 1.7 Hz, 1H), 7.61 (s, 1H), 7.58–7.47 (m, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  168.1, 149.4, 141.3, 133.7, 130.7, 129.9, 129.4, 115.8.

**HRMS** (ESI-TOF):  $m/z$  calc. for C<sub>10</sub>H<sub>10</sub>N<sub>5</sub>O [M+H]<sup>+</sup> 216.0885, found 216.0876.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.24.

**(E)-3-(Phenyldiazenyl)-1H-pyrazole (C17)**



**Synthetic procedure:** The same procedure employed for **C3** was followed starting from 1H-pyrazol-3-amine (0.183 g, 2.20 mmol, 1.10 equiv), nitrosobenzene (0.214 g, 2.00 mmol, 1.00 equiv) and glacial AcOH (20.0 mL). Stirring at rt for 3 days and purification by SGCC (*n*-hexane:EtOAc 0–80%) provided the title compound (0.138 g, 0.802 mmol, 36%). This is a known compound.<sup>263</sup>

**Physical state:** Yellow solid.

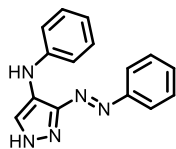
**$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  13.51 (s, 1H), 7.85 (ddd,  $J$  = 8.1, 3.8, 1.7 Hz, 3H), 7.63–7.50 (m, 3H), 6.60–6.55 (m, 1H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR** (101 MHz, DMSO- $d_6$ ):  $\delta$  152.2, 131.3, 130.7, 129.5, 122.3, 93.5.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_9H_9N_4$   $[M+H]^+$  173.0827, found 173.0820.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.39.

**(*E*)-*N*-Phenyl-3-(phenyldiazenyl)-1*H*-pyrazol-4-amine (C18)**



**Synthetic procedure:** A previously reported Buchwald-Hartwig cross-coupling protocol was followed with minor modifications.<sup>186</sup> A pressure vial was charged with **C4** (0.025 g, 0.100 mmol, 1.00 equiv), tBuBrettPhos Pd G3 (0.85 mg, 0.001 mmol, 0.010 equiv) and tBuBrettPhos (0.48 mg, 0.001 mmol, 0.010 equiv), evacuated and back-filled with Ar 3 x. Aniline (0.011 mL, 0.120 mmol, 1.20 equiv), anhydrous and degassed THF (1.0 mL) and 1 M LiHMDS in THF (0.220 mL 0.220 mmol, 2.20 equiv) were then added. The mixture was stirred under Ar at 50 °C for 27 h. Upon reaction completion as determined by LC–MS, the mixture was cooled to rt, carefully added 1 M HCl (4 mL) and then diluted with EtOAc (10 mL) and sat. NaHCO<sub>3</sub> (10 mL). The aq. phase was further extracted with EtOAc (3 x 10 mL), and the combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness *in vacuo*. Purification by RPC (H<sub>2</sub>O + 0.1% FA : MeCN + 0.1% FA 5–95%) provided the title compound (0.007 g, 0.025 mmol, 25%). This is a novel compound.

**Physical state:** Yellow solid.

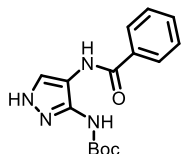
**<sup>1</sup>H NMR** (500 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  13.48 (s, 1H), 8.88 (s, 1H), 7.97 (s, 1H), 7.93–7.88 (m, 2H), 7.61–7.53 (m, 2H), 7.52–7.45 (m, 1H), 7.31–7.23 (m, 2H), 7.20–7.13 (m, 2H), 6.85 (tt,  $J$  = 7.3, 1.1 Hz, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (126 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  152.3, 142.9, 130.3, 129.4, 129.3, 121.9, 119.7, 115.6.

**HRMS** (ESI-TOF):  $m/z$  calc. for  $C_{15}H_{14}N_5$   $[M+H]^+$  264.1249, found 264.1239.

$R_f$  (silica gel, *n*-hexane:EtOAc 1:1): 0.45.

***tert*-Butyl (4-benzamido-1*H*-pyrazol-3-yl)carbamate (C19a)**



**Synthetic procedure:** To a solution of benzoic acid (0.106 g, 0.866 mmol, 1.10 equiv), EDC·HCl (0.181 g, 0.944 mmol, 1.20 equiv) and HOBt (0.145 g, 0.944 mmol, 1.20 equiv) in anhydrous DMF (15.0 mL) was added **C13b** (0.156 g, 0.787 mmol, 1.00 equiv). The mixture was stirred at rt for 23 h. Upon reaction

completion as determined by TLC, the mixture was concentrated *in vacuo*, added H<sub>2</sub>O (15 mL) and extracted with EtOAc (4 x 10 mL). The combined organic phases were washed with sat. brine (3 x 15 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to dryness *in vacuo*. Purification by SGCC (DCM:MeOH 0–15%) provided the title compound (0.191 g, 0.631 mmol, 80%). This is a novel compound.

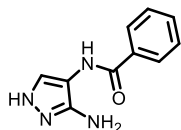
**Physical state:** Brown oil.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.64 (s, 3H), 12.38 (s, 1H), 9.85 (s, 1H), 9.44 (s, 1H), 7.72 (d, *J* = 8.3 Hz, 2H), 7.59–7.53 (m, 3H), 7.41 (t, *J* = 7.6 Hz, 3H), 1.46 (s, 9H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 134.1, 131.6, 128.6, 127.8, 127.4, 127.0, 124.5, 119.2, 109.6, 79.7, 28.0.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.70.

#### ***N*-(3-Amino-1*H*-pyrazol-4-yl)benzamide (C19b)**



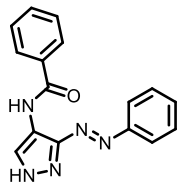
**Synthetic procedure:** The same procedure employed for **C12** was followed starting from **C19a** (0.185 g, 0.612 mmol, 1.00 equiv), TFA (3.0 mL) and DCM (3.0 mL). Stirring at 0 °C–rt for 5.5 h and purification by SGCC (DCM:MeOH 0–15%) provided the title compound (0.022 g, 0.108 mmol, 18%). This is a known compound.<sup>264</sup>

**Physical state:** Brown solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.42 (s, 1H), 9.60 (s, 1H), 7.96–7.89 (m, 2H), 7.72 (s, 1H), 7.58–7.53 (m, 1H), 7.51 (dd, *J* = 8.2, 6.3 Hz, 2H), 4.67 (s, 2H).

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.30.

#### **(*E*)-*N*-(3-(Phenyldiazenyl)-1*H*-pyrazol-4-yl)benzamide (C19)**



**Synthetic procedure:** The same procedure employed for **C3** was followed starting from **C19b** (0.020 g, 0.099 mmol, 1.00 equiv), nitrosobenzene (0.011 g, 0.099 mmol, 1.00 equiv) and glacial AcOH (1.0 mL). Stirring at rt for 25 h and at 50 °C for 16 h and purification by SGCC (DCM:MeOH 0–15%) provided the title compound (0.012 g, 0.041 mmol, 42%). This is a novel compound.



**Physical state:** Yellow solid.

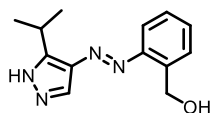
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.68 (s, 1H), 10.62 (s, 1H), 8.41 (s, 1H), 7.98–7.88 (m, 4H), 7.69–7.54 (m, 6H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 164.1, 151.8, 133.4, 132.1, 131.4, 129.6, 129.0, 127.0, 122.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>16</sub>H<sub>14</sub>N<sub>5</sub>O [M+H]<sup>+</sup> 292.1198, found 292.1188.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.72.

#### (*E*)-(2-((5-isopropyl-1*H*-pyrazol-4-yl)diazenyl)phenyl)methanol (**C20**)



**Synthetic procedure:** The same procedure employed for **C14a** was followed starting from (2-aminophenyl)methanol (0.123 g, 1.00 mmol, 1.00 equiv), Oxone (1.23 g, 2.00 mmol, 2.00 equiv), DCM (10.0 mL) and H<sub>2</sub>O (10.0 mL). Stirring at rt for 10 min provided the crude nitroso intermediate, which was used directly in the next step with 5-isopropyl-1*H*-pyrazol-4-amine (0.125 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at rt for 26 h and purification by SGCC (DCM:MeOH 0–10%) provided the title compound (0.125 g, 0.513 mmol, 51% over two steps). This is a novel compound.

**Physical state:** Yellow-orange solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 13.18 (s, 1H), 7.81 (s, 1H), 7.65 (d, *J* = 7.6 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.44 (t, *J* = 7.4 Hz, 1H), 7.33 (t, *J* = 7.6 Hz, 1H), 5.20 (t, *J* = 5.7 Hz, 1H), 4.97 (d, *J* = 5.0 Hz, 2H), 3.54 (s, 1H), 1.37 (d, *J* = 7.6 Hz, 6H).

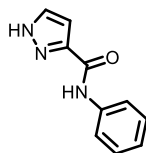
**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 149.0, 139.8, 137.0, 129.7, 127.5, 127.2, 114.6, 58.7, 24.9, 22.0.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>13</sub>H<sub>17</sub>N<sub>4</sub>O [M+H]<sup>+</sup> 245.1402, found 245.1407.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.35.

### 5.1.6. Synthesis and characterisation of series D compounds

#### *N*-Phenyl-1*H*-pyrazole-3-carboxamide (**D1**)



**Synthetic procedure:** The same procedure employed for **C19a** was followed starting from 1*H*-pyrazole-3-carboxylic acid (0.112 g, 1.00 mmol, 1.00 equiv), aniline (0.100 mL, 1.10 mmol, 1.10 equiv), EDC·HCl

(0.230 g, 1.20 mmol, 1.20 equiv) and HOBt (0.184 g, 1.20 mmol, 1.20 equiv) and anhydrous DMF (2.0 mL). Stirring at rt for 30 h and purification by SGCC (*n*-hexane:EtOAc 0–70%) provided the title compound (0.117 g, 0.624 mmol, 62%). This is a known compound.<sup>140</sup>

**Physical state:** Beige solid.

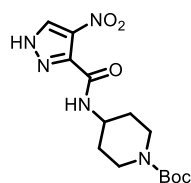
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 10.69 (s, 1H), 8.76 (s, 1H), 7.72–7.66 (m, 2H), 7.63 (d, *J* = 2.4 Hz, 1H), 7.41–7.32 (m, 2H), 7.19–7.09 (m, 1H), 6.97 (d, *J* = 2.5 Hz, 1H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, CDCl<sub>3</sub>): δ 138.2, 129.5, 124.7, 120.3, 107.0, 77.7.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>10</sub>H<sub>10</sub>N<sub>3</sub>O [M+H]<sup>+</sup> 188.0824, found 188.0815.

**R<sub>f</sub>** (silica gel, *n*-hexane:EtOAc 1:1): 0.38.

***tert*-Butyl 4-(4-nitro-1*H*-pyrazole-3-carboxamido)piperidine-1-carboxylate (D2a)**



**Synthetic procedure:** The same procedure employed for **C11** was followed starting from 4-nitro-1*H*-pyrazole-3-carboxylic acid (0.785 g, 5.00 mmol, 1.00 equiv), *tert*-butyl 4-aminopiperidine-1-carboxylate (1.10 g, 5.50 mmol, 1.10 equiv), EDC·HCl (1.15 g, 6.00 mmol, 1.20 equiv), HOBt (0.919 g, 6.00 mmol, 1.20 equiv) and anhydrous DMF (10.0 mL). Stirring at 0 °C–rt for 24 h provided the title compound (1.70 g, 5.00 mmol, quant.). This is a known compound. Experimental data correlate with literature.<sup>140</sup>

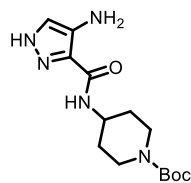
**Physical state:** Beige solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.75 (s, 1H), 8.64 (d, *J* = 7.8 Hz, 1H), 3.94 (pd, *J* = 6.6, 4.0 Hz, 1H), 3.85 (d, *J* = 13.3 Hz, 2H), 2.87 (d, *J* = 10.9 Hz, 1H), 1.85–1.74 (m, 2H), 1.39 (s, 12H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 154.2, 132.5, 79.0, 46.4, 31.1, 28.3.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.65.

***tert*-Butyl 4-(4-amino-1*H*-pyrazole-3-carboxamido)piperidine-1-carboxylate (D2b)**



**Synthetic procedure:** The same procedure employed for **C5a** was followed starting from **D2a** (1.70 g, 5.00 mmol, 1.00 equiv), 10% Pd/C (0.017 g, 10 wt%), MeOH (15.0 mL) and DMF (15 mL). Stirring under H<sub>2</sub>

at rt for 24 h provided the title compound (1.48 g, 4.80 mmol, 96%). This is a known compound. Experimental data correlate with literature.<sup>140</sup>

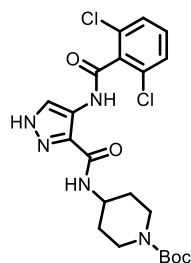
**Physical state:** Brown oil.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.70 (s, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 6.26 (s, 1H), 3.74 (s, 2H), 3.08 (d, *J* = 13.0 Hz, 4H), 1.66 (p, *J* = 1.9 Hz, 1H), 0.90–0.81 (m, 2H), 0.56 (s, 13H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 163.3, 153.9, 132.9, 131.8, 129.7, 114.9, 78.6, 45.3, 42.51–41.9 (m), 31.4, 28.1.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.43.

***tert*-Butyl 4-(4-(2,6-dichlorobenzamido)-1*H*-pyrazole-3-carboxamido)piperidine-1-carboxylate (D2c)**



**Synthetic procedure:** The same procedure employed for **C11** was followed starting from 2,6-dichlorobenzoic acid (0.109 g, 0.573 mmol, 1.10 equiv), **D2b** (0.161 g, 0.521 mmol, 1.00 equiv), EDC·HCl (0.119 g, 0.625 mmol, 1.20 equiv), HOBT (0.919 g, 6.00 mmol, 1.20 equiv) and anhydrous DMF (10.0 mL). Stirring at 0 °C–rt for 18 h and purification by SGCC (*n*-hexane:EtOAc 0–60% followed by DCM:MeOH 0–15%) provided the title compound (0.055 g, 0.114 mmol, 22%). This is a known compound. Experimental data correlate with literature.<sup>140</sup>

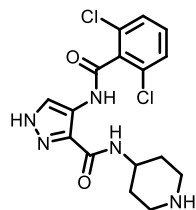
**Physical state:** White solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 10.57 (s, 1H), 9.87 (s, 1H), 8.51 (s, 1H), 7.38–7.26 (m, 3H), 6.81 (d, *J* = 8.1 Hz, 1H), 4.04 (ddd, *J* = 11.3, 7.4, 3.8 Hz, 1H), 2.88 (m, 4H), 2.02–1.93 (m, 2H), 1.46 (s, 9H).

**HRMS** (ESI-FT): *m/z* calc. for C<sub>21</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>5</sub>O<sub>4</sub> [M-H]<sup>−</sup> 480.1211, found 480.1211.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.42.

**4-(2,6-Dichlorobenzamido)-*N*-(piperidin-4-yl)-1*H*-pyrazole-3-carboxamide (D2)**



**Synthetic procedure:** The same procedure employed for **C12** was followed starting from **D2c** (0.050 g, 0.104 mmol, 1.00 equiv), TFA (1.0 mL) and DCM (1.0 mL). Stirring at rt for 17 h provided the title compound (0.036 g, 0.095 mmol, 91%). This is a known compound. Experimental data correlate with literature.<sup>140</sup>

**Physical state:** White solid.

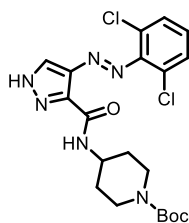
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.19 (s, 1H), 8.38–8.19 (m, 2H), 7.62–7.57 (m, 2H), 7.52 (dd, *J* = 9.3, 6.6 Hz, 1H), 3.76 (m, 1H), 2.92 (d, *J* = 12.1 Hz, 2H), 2.49–2.39 (m, 2H), 1.69–1.60 (m, 2H), 1.46 (qd, *J* = 12.0, 4.1 Hz, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 162.2, 160.4, 135.4, 132.6, 131.9, 131.3, 128.5, 121.5, 121.3, 46.6, 45.2, 32.6.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>16</sub>H<sub>18</sub>N<sub>5</sub>O<sub>2</sub>Cl<sub>2</sub> [M+H]<sup>+</sup> 382.0838, found 382.0848.

**R<sub>f</sub>** (silica gel, DCM:MeOH 7:3): 0.45.

***tert*-Butyl (*E*)-4-(4-((2,6-dichlorophenyl)diazenyl)-1*H*-pyrazole-3-carboxamido)piperidine-1-carboxylate (**D3a**)**



**Synthetic procedure:** The same procedure employed for **C14a** was followed starting from 2,6-dichloroaniline (0.162 g, 1.00 mmol, 1.00 equiv), Oxone (3.69 g, 6.00 mmol, 6.00 equiv), DCM (10.0 mL) and H<sub>2</sub>O (10.0 mL). Stirring at rt for 24 h and at 50 °C for 3 h provided the crude nitroso intermediate, which was used directly in the next step with **D2b** (0.309 g, 1.00 mmol, 1.00 equiv) and glacial AcOH (10.0 mL). Stirring at rt for 45 h and purification by SGCC (DCM:MeOH 0–15%) provided the title compound (0.104 g, 0.222 mmol, 22% over two steps). This is a novel compound.

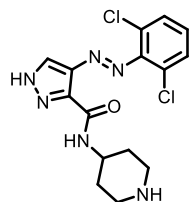
**Physical state:** Brown crystalline solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.15 (s, 1H), 7.65 (d, *J* = 8.1 Hz, 2H), 7.43 (t, *J* = 8.1 Hz, 1H), 4.00 (d, *J* = 7.0 Hz, 1H), 3.79 (d, *J* = 13.3 Hz, 2H), 2.85–2.79 (m, 2H), 1.85–1.77 (m, 2H), 1.38 (s, 12H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 153.9, 129.8, 126.3, 78.7, 46.1, 31.1, 28.1, 21.1.

**R<sub>f</sub>** (silica gel, DCM:MeOH 9:1): 0.38.

**(E)-4-((2,6-Dichlorophenyl)diazenyl)-N-(piperidin-4-yl)-1H-pyrazole-3-carboxamide (D3)**



**Synthetic procedure:** The same procedure employed for **C12** was followed starting from **D3a** (0.130 g, 0.278 mmol, 1.00 equiv), TFA (3.0 mL) and DCM (3.0 mL). Stirring at 0 °C–rt for 20 h and purification by SGCC (DCM:MeOH 0–100%) followed by RPC (H<sub>2</sub>O + 0.1% FA : MeCN + 0.1% FA 5–70%) provided the title compound (0.039 g, 0.105 mmol, 38%). This is a novel compound.

**Physical state:** Yellow solid.

**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.14 (s, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 7.65 (d, *J* = 8.2 Hz, 2H), 7.61 (d, *J* = 8.1 Hz, 0H), 7.42 (t, *J* = 8.1 Hz, 1H), 3.96–3.83 (m, 1H), 2.90 (dt, *J* = 12.7, 3.7 Hz, 2H), 2.56 (dd, *J* = 11.7, 2.6 Hz, 2H), 1.83–1.73 (m, 2H), 1.31 (qd, *J* = 11.4, 4.0 Hz, 2H).

**<sup>13</sup>C{<sup>1</sup>H} NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 157.7, 146.7, 138.9, 137.4, 129.9, 129.8, 127.2, 126.4, 46.6, 44.5, 32.3.

**HRMS** (ESI-TOF): *m/z* calc. for C<sub>15</sub>H<sub>17</sub>N<sub>6</sub>OCl<sub>2</sub> [M+H]<sup>+</sup> 367.0841, found 367.0848.

**R<sub>f</sub>** (silica gel, DCM:MeOH 4:1): 0.41.

## 5.2. Photochemical analysis

### 5.2.1. General

Photochemical analysis was performed by UV–Vis spectrophotometry. UV–Vis absorbance spectra were recorded using an Agilent Cary 60 UV–Vis spectrophotometer equipped with a thermostat and temperature probe. All compound samples were carefully protected from ambient light exposure during sample preparation and analysis. The samples were measured at 20 °C in a two-window 1 cm x 1 cm quartz cuvette in concentrations and media as specified in individual cases. All sample spectra were subtracted with a blank baseline obtained from pre-recording the absorbance of the medium minus the sample. Photoirradiation of UV–Vis samples were performed using custom-build setups from SAHLMANN photochemical solution. In brief, this consisted of 3 x 55–800 mW LEDs modules (incl. 340 and 365 nm) mounted on a passive heatsink and connected to a power and time controller.

### 5.2.2. Determination of photostationary state distribution

The photostationary state distribution (PSD) at a particular wavelength was determined by initially acquiring a UV–Vis absorbance spectrum of the photoswitch in its dark-adapted state—through chemical characterisation of the dry bulk sample by  $^1\text{H}$  NMR and HPLC (and by carefully protecting the samples from ambient light exposure), this UV–Vis spectrum was assumed to represent 100% *E*-isomer. The same sample was then photoirradiated through a blank side of the cuvette using LEDs with a particular wavelength positioned right up against the sample. Irradiation was performed in a closed box at 100% power and at 30 seconds intervals. After each interval, the sample was placed back into UV–Vis spectrophotometer and a spectrum acquired within an estimated timeframe of  $\approx 5$  s. Photoirradiation and subsequent spectral acquisition was continued until no further apparent changes could be observed on the absorbance spectrum (particular at the  $\pi$ – $\pi^*$  transition), at which point the photostationary state (PSS) was assumed to have been reached. For each compound, photoirradiation with at least two different wavelengths of light was performed. To quantify the PSD based on the acquired UV–Vis spectra, the method reported by Ernst Fischer was employed.<sup>265</sup> This method considers perturbation of a photoreversible system (between an *E* and *Z*-isomer) with two different wavelengths 1 and 2 and has two requirements/assumptions: 1) the photoisomerisation product upon irradiation is sufficiently stable thermally and chemically so that acquired UV–Vis spectra upon photoirradiation will represent the true PSS (and not represent a significant amount of the thermodynamically stable isomer formed from rapid thermal back-isomerisation or any photoinduced degradation products), and 2) the ratio of the quantum yields for the two inverse photoisomerisation reactions remains constant across the two wavelengths, which is generally true especially within one absorbance band.<sup>266</sup> Given these assumptions, the PSD can be calculated with the following equation:

$$\alpha_2 = \frac{\left(\frac{\Delta_1}{A_1} - \frac{\Delta_2}{A_2}\right)}{1 + \frac{\Delta_1}{A_1} - n\left(1 + \frac{\Delta_2}{A_2}\right)}$$
$$n = \frac{\Delta_{1,\max}}{\Delta_{2,\max}}$$
$$\alpha_1 = n \cdot \alpha_2$$

Where  $\alpha_1$  and  $\alpha_2$  are the extent of photoconversion at wavelength 1 and 2 (where wavelength 2 gives higher PSD),  $\Delta_1$  and  $\Delta_2$  represent the change in absorbance for the *E*-isomer at wavelength 1 and 2 after irradiation with wavelength 1 and 2,  $A_1$  and  $A_2$  represent the absorbance of the *E*-isomer at wavelength 1 and 2, and  $\Delta_{1,\max}$  and  $\Delta_{2,\max}$  represent the change in absorbance for the *E*-isomer at the maximum absorbance (where the maximum change in absorbance occurs) after irradiation with wavelength 1 and 2. As a

validation, the quantification method was benchmarked against NMR-determined PSD values for two photoswitchable compounds **A** and **B**, from which a good correlation was obtained (**Table 5.1**).

**Table 5.1. Comparison of the Fischer method for PSD determination with NMR.** <sup>a</sup>

| Cmpd | Wavelength (nm) | PSD <sub>Fischer</sub> (%) | PSD <sub>NMR</sub> (%) |
|------|-----------------|----------------------------|------------------------|
| A    | 365             | 97                         | 98                     |
| A    | 495             | 89                         | 88                     |
| B    | 365             | 97                         | 98                     |
| B    | 495             | 87                         | 87                     |

<sup>a</sup> PSD were determined at 20 °C in CH<sub>3</sub>CN and CD<sub>3</sub>CN at 25 μM and 20 mM by UV–Vis spectroscopy and NMR, respectively.

### 5.2.3. Determination of thermal back-conversion

The thermal stability of the *Z*-isomers was assessed by UV–Vis spectrophotometry at 20 °C. The sample was initially photoirradiated with a wavelength of light giving the best photoconversion to the *Z*-isomer. For relatively short-lived photoswitches, spectral requisition was performed at regular intervals until at least 50% back-conversion had occurred (i.e., for at least one thermal half-life). The absorbance at  $\lambda_{\max}$  for the *E*-isomer was then plotted against time, and the curve fitted to a first-order kinetic model, from which the rate constant (*k*) and the thermal half-life (*t*<sub>1/2</sub>) could be determined. For longer-lived photoswitches, spectral requisition was continued for a maximum of 1 h, and the percentage back-conversion after 1 h (*C*<sub>1h</sub>) calculated by constructing a linear plot between the absorbance for the *E*-isomer  $\lambda_{\max}$  and the PSD determined using the Fischer method (three points corresponding to the dark state, and the two PSS compositions from irradiation with wavelength 1 and 2). Interpolation from this linear plot at *t* = 0 and *t* = 1 h was then performed, and the two values were subtracted to give the estimated percentage back-conversion of the *Z*-isomer after 1 h.

## 5.3. Biological characterisation of NSP16/10 activity

### 5.3.1. Protein expression and purification

Expression and purification of NSP16/10 was carried out by Dr Alex Caputo from CSIRO. The complex was produced by co-expressing NSP16 (*N*-terminal His6-tagged with TEV cleavage site) and NSP10 (*N*-terminal FLAG tag) in BL21(DE3) competent *E. coli* cells using two separate pET vectors (pET43 and pET28) under different antibiotic selection. The cells were subsequently mechanically lysed, and the clarified lysate

applied to a TALON Cobalt affinity resin, washed, and eluted in a buffer supplemented with glycerol and  $\beta$ -mercaptoethanol. The eluted peak was cleaved by incubation with TEV protease (1 w/w%) overnight at 4 °C. The TEV, digested affinity tag and undigested complex were captured onto an IMAC resin, and the flowthrough concentrated and loaded onto a Superdex™ 200 column in HBS + 1 mM TCEP. The fractions containing the complex were pooled and concentrated to > 5 mg/mL.

### 5.3.2. Surface plasmon resonance

All surface plasmon resonance work on NSP16/10 was performed by Dr Matthew L. Dennis and Meghan Hattarki from CSIRO. The assay was carried out using a Biacore™ S200 instrument with series S sensor chip CM5 (Cytiva). Preconditioning was performed according to manufacturer's instructions (HCl/NaOH/SDS). Protein immobilisation was performed by first immobilising streptavidin at 25 °C with the following sequence of flow-through: NHS/EDC (600 s), streptavidin (100  $\mu$ g, pH 4.5, 420 s), and ethanolamine (420 s). This was followed by capture of minimally biotinylated NSP16/10 at 12 °C with 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 10 %[w/v] glycerol, 0.05 %[v/v] Tween-20 as the running buffer giving capture levels of 6.7–8.6 kRU. The assay was performed at 12 °C with 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 10 %[w/v] glycerol, 0.05 %[v/v] Tween-20, 2 %[v/v] DMSO as the running buffer and with a flowrate of 60  $\mu$ L/min, contact time of 30 s and dissociation time of 15 s. Compounds were initially screened at a single concentration of 100  $\mu$ M before testing in dose-response format with appropriate concentration ranges. **SFN** was tested as a positive control at the beginning and end of each experiment. For photoswitchable compounds, the 100  $\mu$ M compound stock solutions were irradiated with a Spectroline CM UV-viewing cabinet CM-10 at 365 nm for 30 min before plating and testing. An elapsed time period of 2 h is estimated from the point of irradiation till compound testing. Data processing was done in Scrubber (v2.0) where Fc1 was selected as the reference and blank RB injection as the double reference. Sensorgrams were scaled by MW and fitted to the  $R_{\text{max}}$  of **SFN** replicates. The “bound” for the steady-state was taken at 20–25 s.

### 5.3.3. Differential scanning fluorimetry

Differential scanning fluorimetry was performed on NSP16/10 on a C1000 Thermal Cycler with a CFX96 Real-Time PCR system (Bio-Rad) in 96-well skirted PCR plates (Thermo Scientific) according to a previously reported protocol.<sup>267</sup> SYPRO® Orange was used as the fluorescent dye (final volume of 0.3  $\mu$ L per well) and first pre-mixed with the NSP16/10 (final concentration of 50  $\mu$ g/mL = 1.01  $\mu$ M) in the assay buffer (10 mM HEPES pH 7.4, 150 mM NaCl) before mixing with either compound solution (final concentration of



100–1000  $\mu\text{M}$ ) or vehicle as a negative control (DMSO) to a final DMSO concentration of 1% (v/v) and final sample volume of 20  $\mu\text{L}$ . The assay was performed in triplicate. After dispensing the test solutions into the 96-well plate, the plate was sealed, spun down (10,000 rpm, 1 min), and then measured with a temperature increment of 0.5  $^{\circ}\text{C}/30\text{ s}$  from 20  $^{\circ}\text{C}$  up to 100  $^{\circ}\text{C}$ . Fluorescence was measured using the FRET scanning mode (reading at  $\lambda = 570\text{ nm}$ ). Data analysis including determination of melt temperatures ( $T_m$ ) was performed using the software, Meltdown.<sup>268</sup>

#### 5.3.4. In vitro capped oligonucleotide synthesis

Synthesis of the capped 36-meric oligonucleotide **m7GpppA-RNA<sub>34</sub>** (5'-m7GpppAGGGAAGCGGG-CAUGCGGCCAGCCAUAGCCGAUCA-3') was performed by co-transcriptional capping using the HiScribe® T7 Quick High Yield RNA Synthesis Kit (NEB). The DNA template was prepared by annealing the custom-made sense strand (5'-CAGTAATACGACTCACTATTAGGGAAGCGGGCATGCGGCCAGCCATAGCCGATCA-3', 100  $\mu\text{M}$ , 200  $\mu\text{L}$ ) and antisense strand (5'-TGATCGGCTATGGCTGGCCGCATGCCCCGCTTCCTAATAGTGAGTCG-TATTACTG-3', 100  $\mu\text{M}$ , 200  $\mu\text{L}$ ): The two substrates were mixed in a microtube and incubated at 95  $^{\circ}\text{C}$  for 5 min and then allowed to slowly cool to rt over 1 h to give the DNA template stock (50  $\mu\text{M}$ ). Co-transcription was performed in a microtube according to manufacturer's instructions using the DNA template (final amount of 25  $\mu\text{g}$ ), **m7GpppA** (final concentration of 40 mM), NTP buffer mix (final concentration of 10 mM of each NTP) and T7 RNA Polymerase Mix (50  $\mu\text{L}$ ) in water with total reaction volume of 500  $\mu\text{L}$ . The mixture was incubated in a dry-air incubator at 37  $^{\circ}\text{C}$  overnight (16 h) before being treated with DNase I (50  $\mu\text{L}$ ) and incubated at 37  $^{\circ}\text{C}$  for additional 15 min. The transcript was purified using the Monarch® RNA Cleanup Kit (NEB) according to manufacturer's instructions and the final concentration quantified using a NanoDrop spectrophotometer using  $1\text{ A}_{260} = 40\text{ }\mu\text{g/mL}$ .

#### 5.3.5. TR-FRET Methyltransferase assay

Methyltransferase activity was determined using the EPIgeneous Methyltransferase Assay Kit (Cisbio) according to manufacturer's instructions. The assay was performed in 384-well white, low-volume round-bottom microplates (Corning) with a total reaction volume of 8–10  $\mu\text{L}$  and with technical duplicates. Various reagent concentrations, buffers and reaction times were initially sampled, but the conditions that gave measurable enzyme activity are described here (**Table 20, entry 7**). The assay buffer consisted of 40 mM Tris-HCl pH 8.0, 10 mM KCl, 2 mM  $\text{MnCl}_2$ , 0.01% Tween-20, and 1 mM DTT. For compound testing, the working compound solutions (5X) were dispensed into the assay wells first (final concentration of 1 or 100  $\mu\text{M}$ , 2% DMSO, 2  $\mu\text{L}$ ). The assay plate was subsequently carefully protected from ambient light

using aluminium foil. The **m7GpppA-RNA<sub>34</sub>** substrate solution (5X, final concentration of 40  $\mu$ M, 2  $\mu$ L), SAM solution (5X, 200  $\mu$ M, 2  $\mu$ L) and the NSP16/10 solution (2.5X, final concentration of 3  $\mu$ M, 4  $\mu$ L) were then dispensed. A negative control having no compound (compensated with DMSO, a compound interference control having no protein (compensated with SAH), a positive/100% inhibition control having no substrate and buffer controls (having none of the enzyme reaction components) were also included. After dispensing, the plate was sealed, spun down (1000 rpm, 1 min) and then incubated at 37 °C for 2 h. For detection, detection buffer one (2  $\mu$ L) was dispensed into each well and the plate incubated at rt for 10 min. Then, detection buffer 2 (8  $\mu$ L) was added to all buffer control wells, while SAH d2 reagent (4  $\mu$ L) and SAH Tb cryptate antibody (4  $\mu$ L) were dispensed into all other wells. The plate was resealed, spun down (1000 rpm, 1 min) and incubated at rt for 1 h. HTRF measurement was performed on at CLARIOstar Plate Reader (BMG Labtech) detecting fluorescence emission wavelengths at 620 and 665 nm with an integration start time (delay) of 60  $\mu$ s and integration time of 400  $\mu$ s. All fluorescence readouts were blank corrected by individually subtracting the measurements at 620 and 665 nm from the buffer measurements. The HTRF values were then calculated as

$$HTRF = \frac{F_{665nm}}{F_{620nm}} \times 1000$$

(where  $F_{665nm}$  and  $F_{620nm}$  are the fluorescence intensities). For compound testing, these HTRF values were converted to [SAH] using a SAM-to-SA-H titration curve, and the percentage inhibition calculated as

$$\%Inh. = \left( 1 - \left( \frac{[SAH]_{compd} - [SAH]_{min}}{[SAH]_{max} - [SAH]_{min}} \right) \right) \times 100\%$$

where  $[SAH]_{compd}$ ,  $[SAH]_{min}$  and  $[SAH]_{max}$  are the SAH concentrations calculated for the compound, for the minimum signal (negative/no compound control) and for the maximum signal (positive/100% inhibition control).

### 5.3.6. Scintillation proximity assay

The scintillation proximity assay was performed by Dr Alice Li from Dr Masoud Vedadi's research group at University of Toronto in accordance with the previous literature protocol.<sup>94</sup> The compounds were first screened in singlet at 10, 50, and 100  $\mu$ M against 125 nM NSP16/10 (1:8 complex ratio) and employing 2  $\mu$ M <sup>3</sup>H-SAM and 0.8  $\mu$ M 5'-m7GpppACCCCC-7'. The enzyme reaction was performed at 23 °C for 30 min using 50 mM Tris-HCl pH 7.5, 0.01% Triton X-100, 1.5 mM MgCl<sub>2</sub>, 5 mM DTT and 5% DMSO as the assay buffer. 12 compounds were subsequently tested in a dose-response format (1.95–500  $\mu$ M) in duplicate.

## 5.4. Biological characterisation of CDK2 activity

### 5.4.1. Protein expression and purification

Monomeric CDK2 was expressed and purified by a member of Dr David Mann's research group at Imperial College London. In short, expression was performed using a pRSET-A plasmid containing a His-tagged CDK2 encoding sequence, and transformation was done in BL21(DE3) competent *E. coli* cells (NEB) by electroporation. Cells were subsequently lysed, and soluble material purified through a Ni-NTA HP column (Cytiva) followed by size-exclusion chromatography.

Full-length CDK2/cyclinA2 employed for the ADP-Glo™ assay was procured (Promega).

### 5.4.2. Fluorescence polarisation assay

A fluorescence polarisation (FP) assay on monomeric and His-tagged CDK2 (provided by Dr David Mann, Imperial College London) was attempted using BODIPY™ ATP-γ-S and BODIPY™ ATP-2/3-O (ThermoFischer) as fluorescent binding probes. The CDK2 protein stock was first buffer exchanged into the assay buffer consisting of 20 mM HEPES pH 7.4, 150 mM NaCl, 1 mM MgCl<sub>2</sub>, and 0.05% TWEEN20 and concentrated to 50 μM (quantified using NanoDrop with  $\epsilon = 37 \text{ M}^{-1} \text{ cm}^{-1}$ , MW = 37 kDa). The assay was performed in 384-well black flat bottom microplates (Corning) with a total assay volume of 20 μL and with technical triplicates. For compound testing, the compound solution (10X) was dispensed into the plate (final concentration of 1 or 100 μM, 1% DMSO, 7 μL). The assay plate was subsequently carefully protected from ambient light using aluminium foil. The probe solution (2X, final concentration of 1 nM, 10 μL) and the CDK2 solution (2.5X, final concentration of 20 μM, 8 μL) were then dispensed. A negative control having no compound (compensated with DMSO), a compound interference control having no protein, a positive/100% inhibition control having only the probe and a buffer control were also included. After dispensing, the plate was sealed, spun down (1000 rpm, 1 min) and then incubated at rt for 30 min. Fluorescence intensities were measured on a CLARIOstar Plate Reader (BMG Labtech) with an excitation wavelength of 482 nm and emission detection wavelength of 530 nm. FP values were calculated from the fluorescence intensities parallel ( $F_{||}$ ) and perpendicular ( $F_{\perp}$ ) to the excitation light plane by the equation:

$$FP = \frac{F_{||} - F_{\perp}}{F_{||} + F_{\perp}}$$

### 5.4.3. Bioluminescence kinase assay

Measurement of CDK2/A2 activity was performed using the ADP-Glo™ Kinase Assay (Promega) according to manufacturer's instructions. The assay was performed in white 384-well low-volume flat-bottom microplates (Greiner Bio-One) with a total reaction volume of 5 µL and with technical triplicates. The assay buffer consisted of 40 mM Tris pH 7.5, 50 µM DTT, and 20 mM MgCl<sub>2</sub>. Histone H1 (Promega) was here used as the enzyme substrate and was first mixed with ATP to make a master mix (2.5X H1 and 5X ATP). Compound working solutions were prepared in a dilution plate (5X, 5% DMSO) before being transferred to the assay plate (final DMSO concentration of 1%, 1 µL). The assay plate was subsequently carefully protected from ambient light using aluminium foil. CDK2/A2 was then added (5X, final concentration of 2 ng/µL, 1 µL), and the plate was sealed and spun down (1000 rpm, 1 min). If testing in the dark state, the plate was subsequently incubated in the dark at rt for 30 min. If testing in the photoirradiated state, the plate was irradiated with 340 nm LEDs (SAHLMANN photochemical solution) for 3 x 10 min (with 10 s breaks to avoid overheating the LEDs). The H1/ATP master mix was then dispensed (final concentrations of 0.2 µg/µL H1 and 1 µM ATP, 3 µL). A negative control having no compound (compensated with DMSO), a positive/100% inhibition control having no compound and CDK2/A2 and a buffer control were also included. After dispensing, the plate was sealed, spun down (1000 rpm, 1 min) and incubated at 30 °C for 30 min. For the detection, ADP-Glo reagent (5 µL) was added to all wells and the plate resealed, spun down (1000 rpm, 1 min) and mixed on a plate shaker for 2 min before being incubated at rt for 40 min. Then, kinase detecting reagent (10 µL, pre-made from mixing kinase detection buffer with kinase detection substrate) was added to all wells and the plate resealed, spun down (1000 rpm, 1 min) and incubated at rt for 30 min. The luminescence signals were measured on a CLARIOstar Plate Reader (BMG Labtech) at an emission detection wavelength of 555 nm. All luminescence readouts (RLUs) were blank corrected by subtracting the signal from the buffer control and were then used to calculate the percentage inhibition using the equation:

$$\%inh. = \left( \frac{RLU_{max} - RLU_{cmpd}}{RLU_{max} - RLU_{min}} \right) \times 100\%$$

where  $RLU_{cmpd}$ ,  $RLU_{max}$  and  $RLU_{min}$  are the luminescence for the compound, for the minimum signal (the negative/no compound control) and the maximum signal (the positive/no compound and CDK2/A2 control). Dose-response curves plotted on Origin 2024 (version 10.1) and fitted using the sigmoidal DoseResp function, from which IC<sub>50</sub> values (concentration for obtaining half-saturation of the curve) could be calculated.

## 5.5. Computational modelling

### 5.5.1. General

X-ray crystal structures employed for visualisation and for modelling were downloaded from the Protein Data Bank (PDB).<sup>269</sup> Visualisation and picture generation of X-ray crystal structures and docking poses (including alignment of structures) were performed using PyMOL (version 2.5.4).<sup>47</sup>

### 5.5.2. Conformational analysis

Global energy conformational analysis of compounds were performed in Avogadro (version 1.2).<sup>45, 46</sup> Structures were imported as SMILES strings and optimised with the Auto Optimization Tool using either MMFF94s or UFF as the force field and steepest descent as the energy minimisation algorithm. Optimisation was run until convergence was reached (dE = 0).

### 5.5.3. Molecular docking

Molecular docking studies were performed in Molecular Operating Environment (MOE) (version 2.4.0) using the Amber10:EHT force field.<sup>270</sup> The X-ray structure of the protein was imported and prepared for modelling using the QuickPrep module with default settings: The sequence was preserved, hydrogens added using Protonate3D (allowing Asn, Gln and His residues to flip), water molecules farther than 4.5 Å away from the co-crystallised ligand were removed, the receptor tethered in place using default settings, atoms farther than 8 Å away from the ligand fixed, hydrogens close to the binding site allowed to flip, and conformational refinement done using a root mean square gradient of 0.1 kcal/mol/Å<sup>2</sup>. Ligand structures were prepared for molecular docking in the Database Wash tool with default settings, but the protonation state was set to enumerate at pH 7 to a maximum of 10 protomers, and 3D ligand structures were rebuilt. The prepared ligands (all generated protomers) were docked into the prepared protein structure using the Dock module. The docking grid was generated based on the site of the co-crystallised ligand and with either retainment or removal of binding pocket waters on a case-by-case basis. Ligand conformations were sampled using the bond rotation method and placed into the docking grid using the triangle matcher method. Ranking was performed with the London dG scoring function, and the top 30 poses were carried through to final refinement in the rigid receptor or induced fit mode on a case-by-case basis. The top five poses were rescored using the GBVI/WSA scoring function. The top five highest-scoring docking poses were visually inspected, and the most sensible pose (based on interactions, pocket complementarity and alignment with known ligands) were reported.

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## 7. Appendix

### 7.1. Chapter 2 supporting information

#### 7.1.1. A photoswitchable fragment library

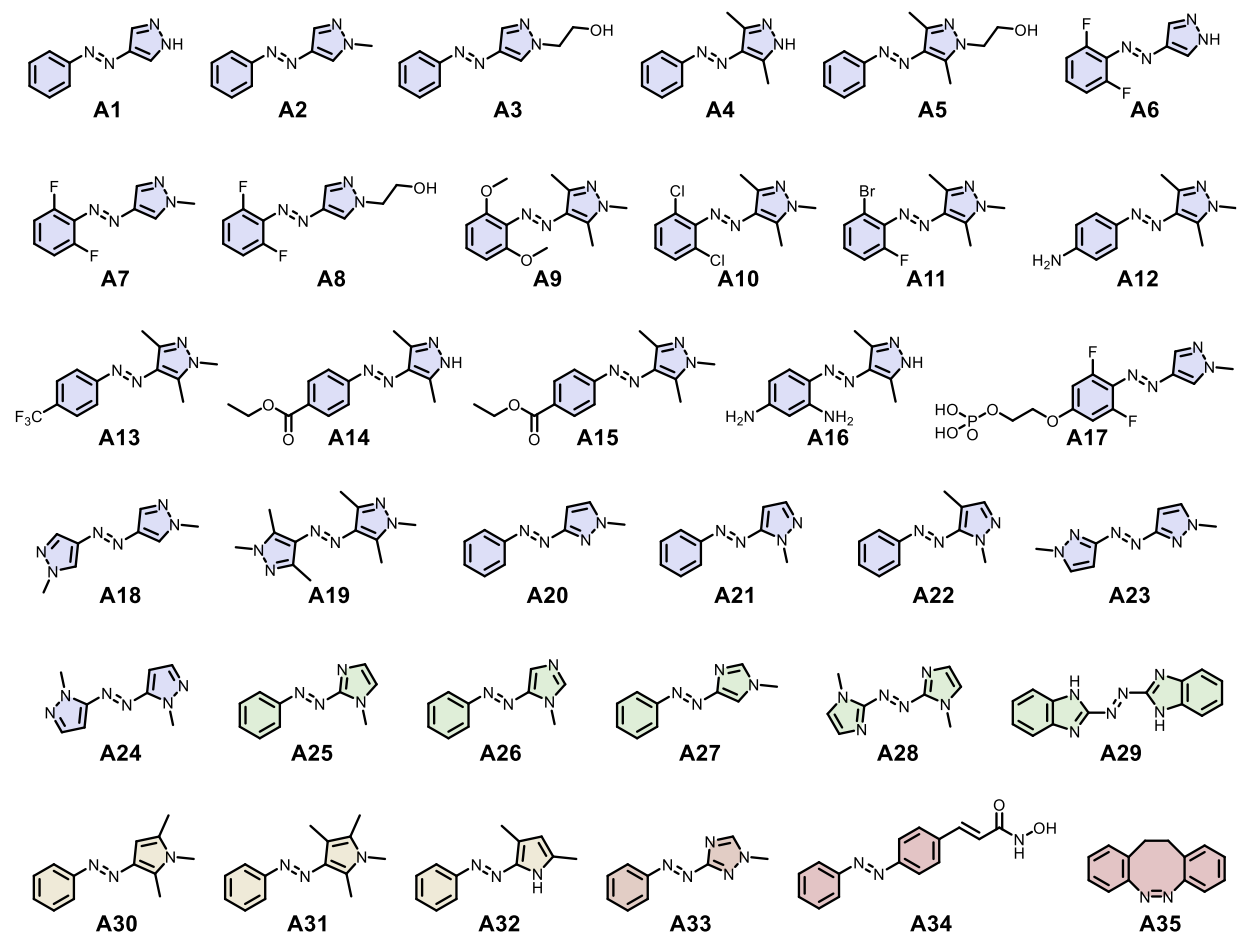


**Figure A1. The 35-membered azo fragment library.** The structures are colour coded according to their core azo motif.

**Table A1. Properties of the photoswitchable fragment library. <sup>a</sup>**

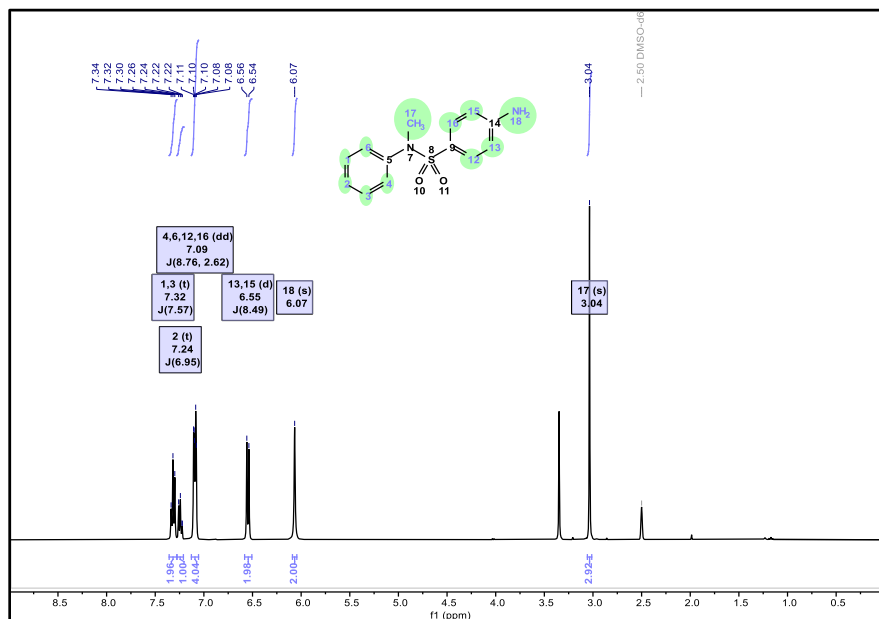
|                            | Average | Max   | Min   |
|----------------------------|---------|-------|-------|
| <b>MW (Da)</b>             | 226.8   | 362.2 | 172.2 |
| <b>HAC</b>                 | 17      | 24    | 13    |
| <b>HBD</b>                 | 0.5     | 3     | 0     |
| <b>HBA</b>                 | 4.9     | 8     | 2     |
| <b>ClogP</b>               | 2.51    | 4.60  | 0.10  |
| <b>RBC</b>                 | 2.6     | 7     | 0     |
| <b>PSA (Å<sup>2</sup>)</b> | 53.0    | 116   | 24.7  |

<sup>a</sup> MW = molecular weight; HAC = heavy atom count; HBD = hydrogen-bond donor count; HBA = hydrogen-bond acceptor count; ClogP = calculated *n*-octanol/water partition coefficient (ChemDraw version 22.2); RBC = rotatable bond count; PSA = topological polar surface area (ChemDraw version 22.2).

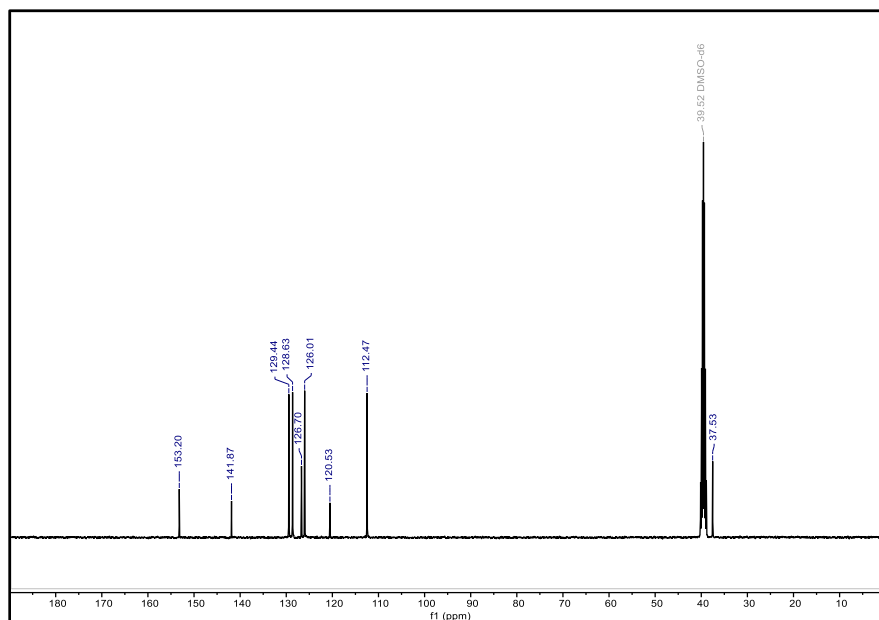
### 7.1.2. Chemical characterisation of key series B compounds

#### 4-Amino-*N*-methyl-*N*-phenylbenzenesulfonamide (B1)

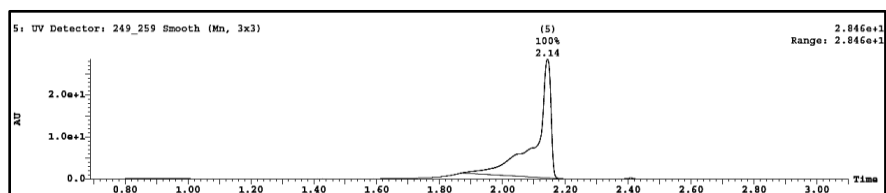
$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):



$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):

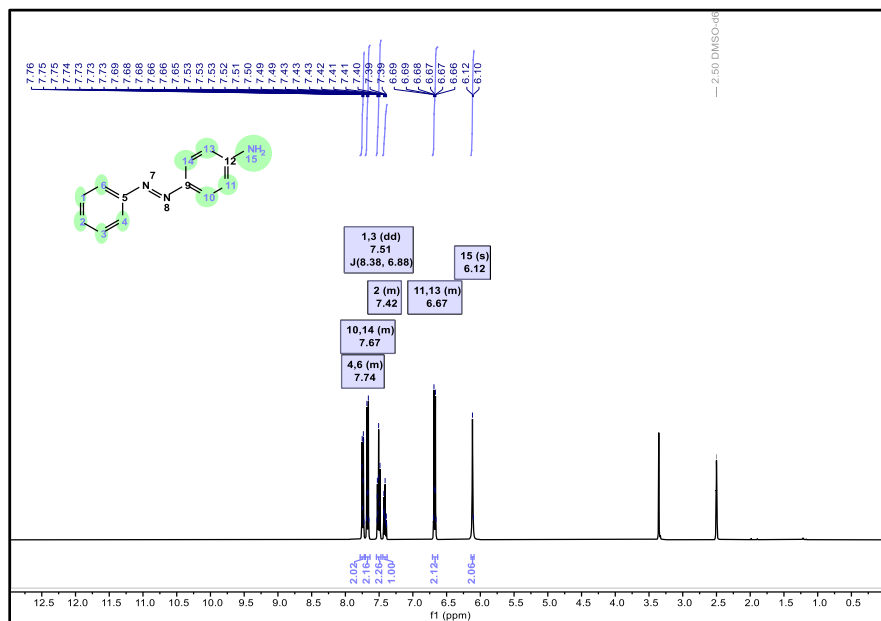


HPLC (UV 249+259 nm):

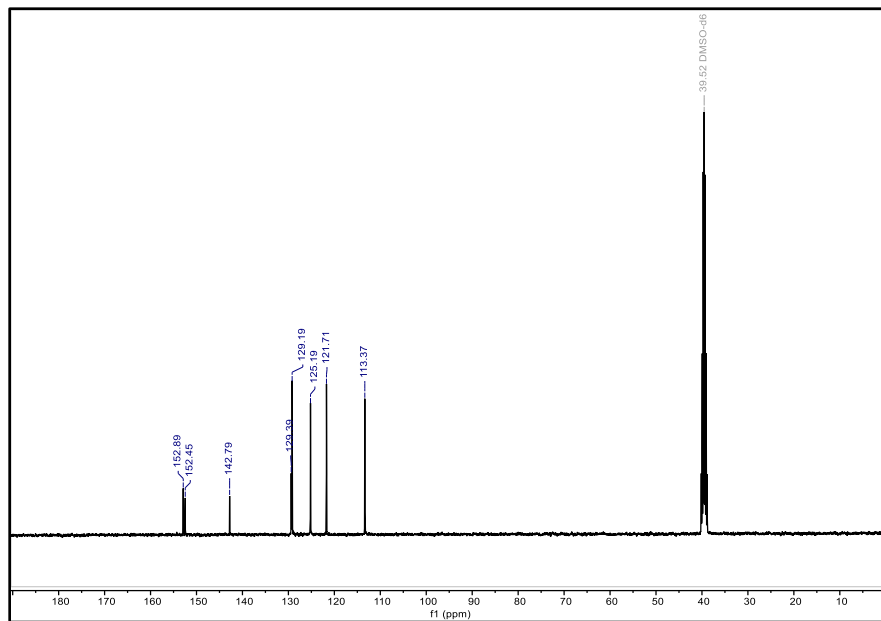


### (E)-4-(Phenyldiazenyl)aniline (B2)

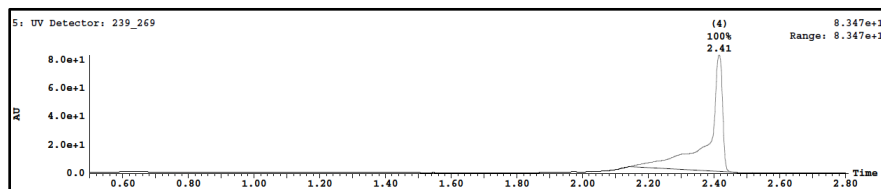
$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):



$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):

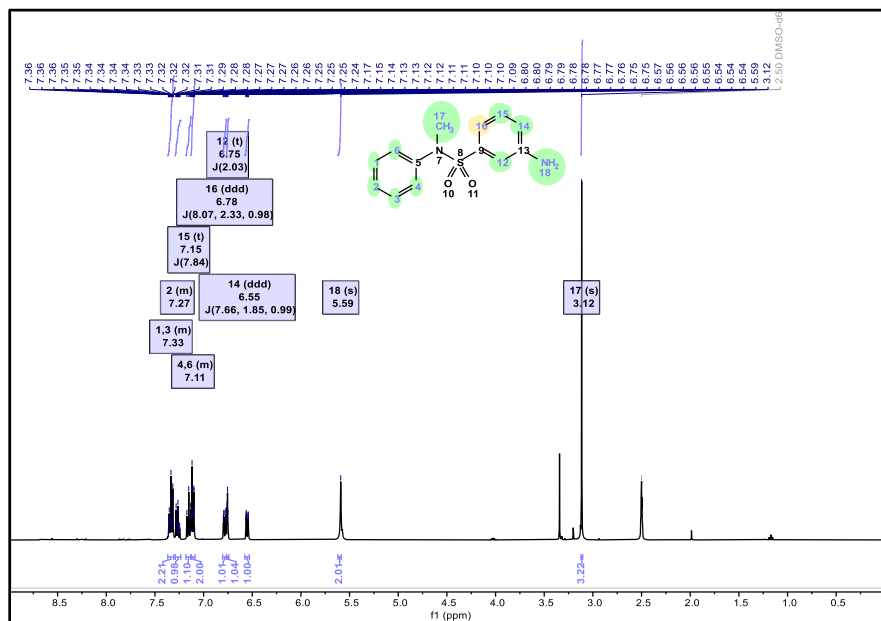


HPLC (UV 239+269 nm):

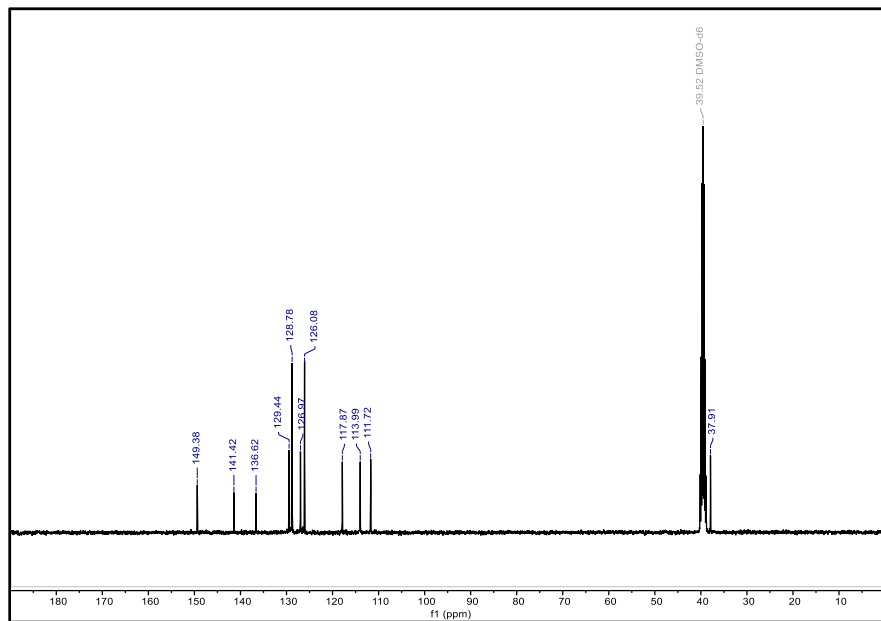


### 3-Amino-*N*-methyl-*N*-phenylbenzenesulfonamide (B24)

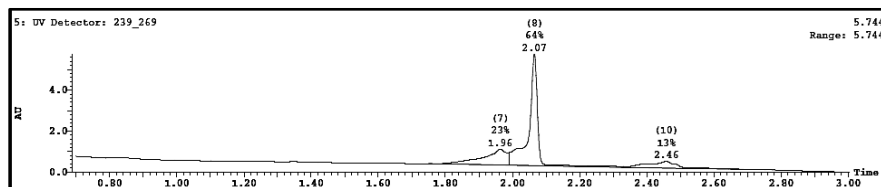
$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):



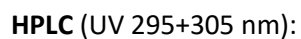
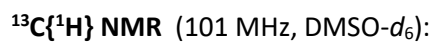
$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):



HPLC (UV 239+269 nm):

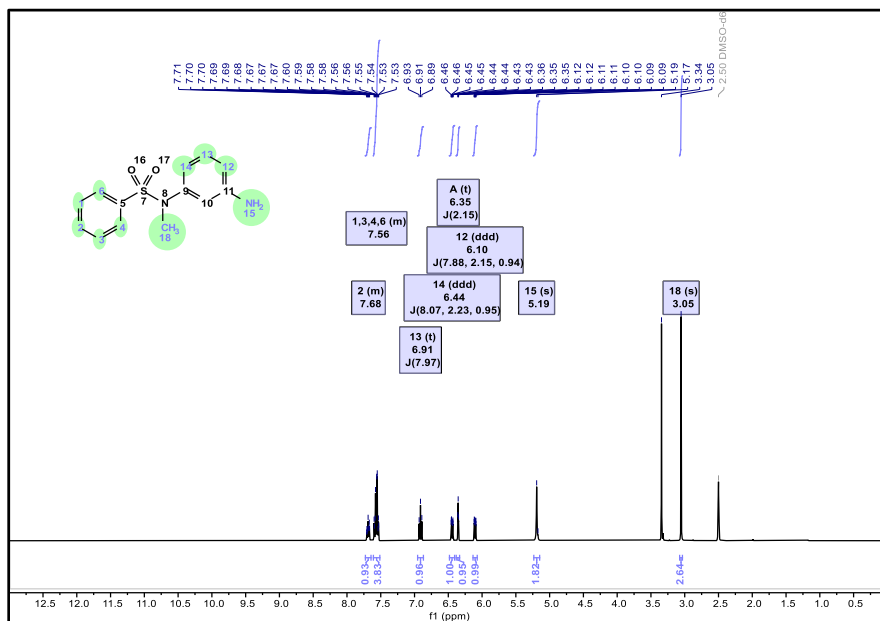


**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>):

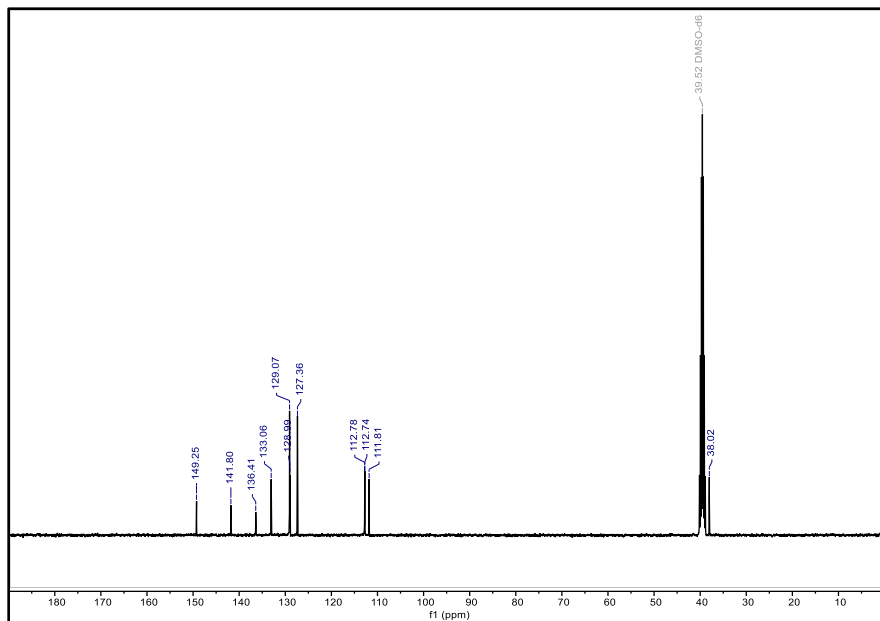


# ***N*-(3-Aminophenyl)-*N*-methylbenzenesulfonamide (B39)**

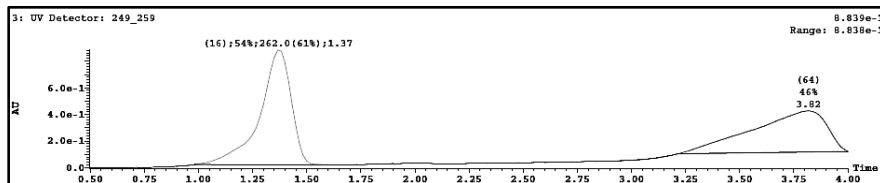
<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):



<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, DMSO-*d*<sub>6</sub>):

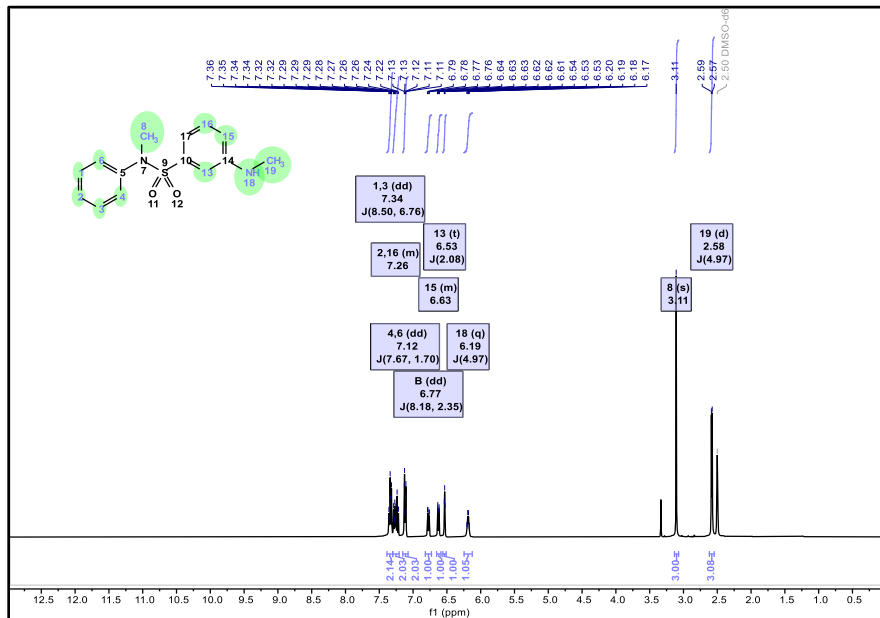


HPLC (UV 249+259 nm):

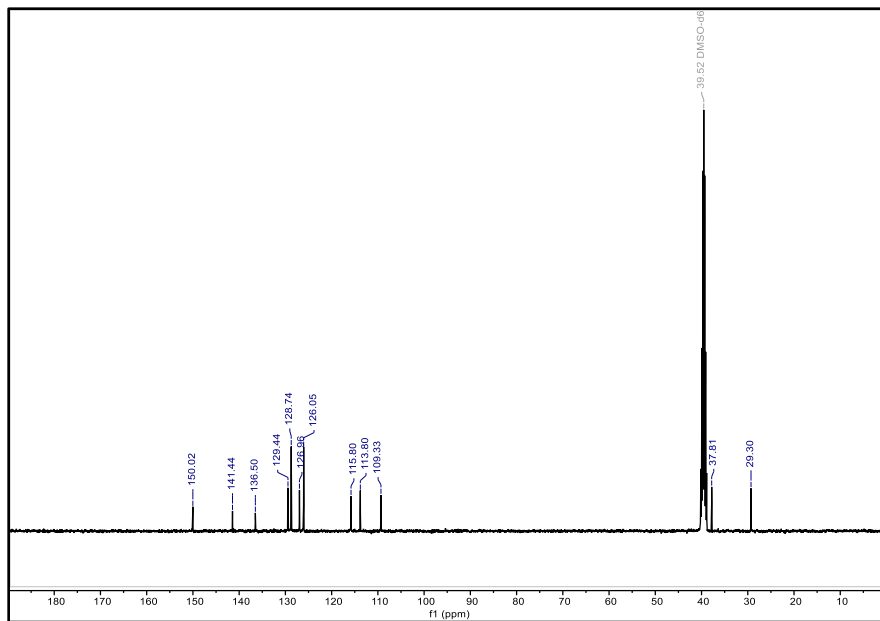


# **N-Methyl-3-(methylamino)-N-phenylbenzenesulfonamide (B44)**

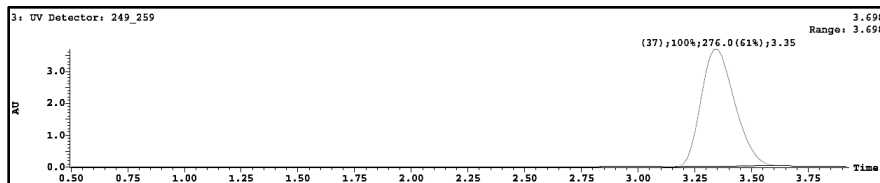
<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):



<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, DMSO-*d*<sub>6</sub>):



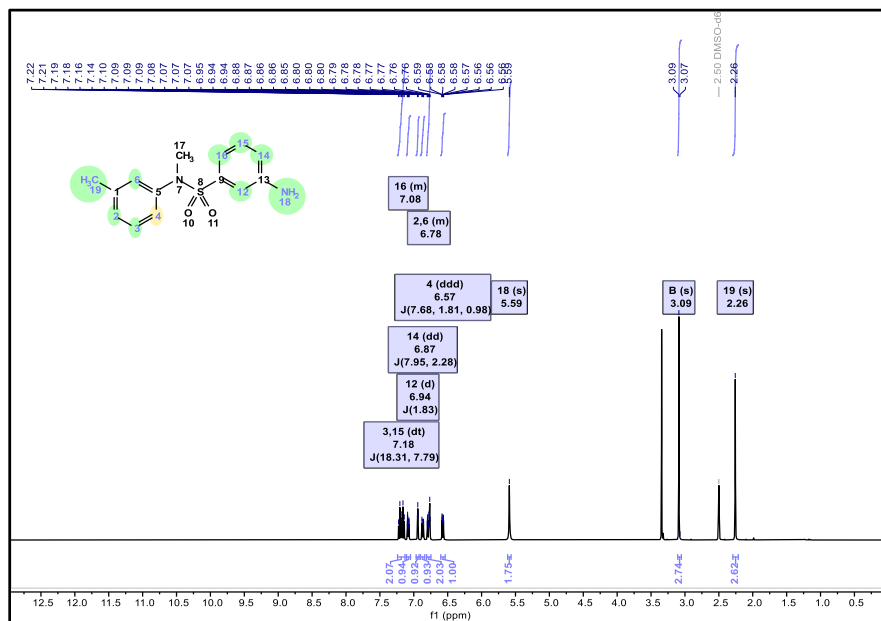
HPLC (UV 249+259 nm):



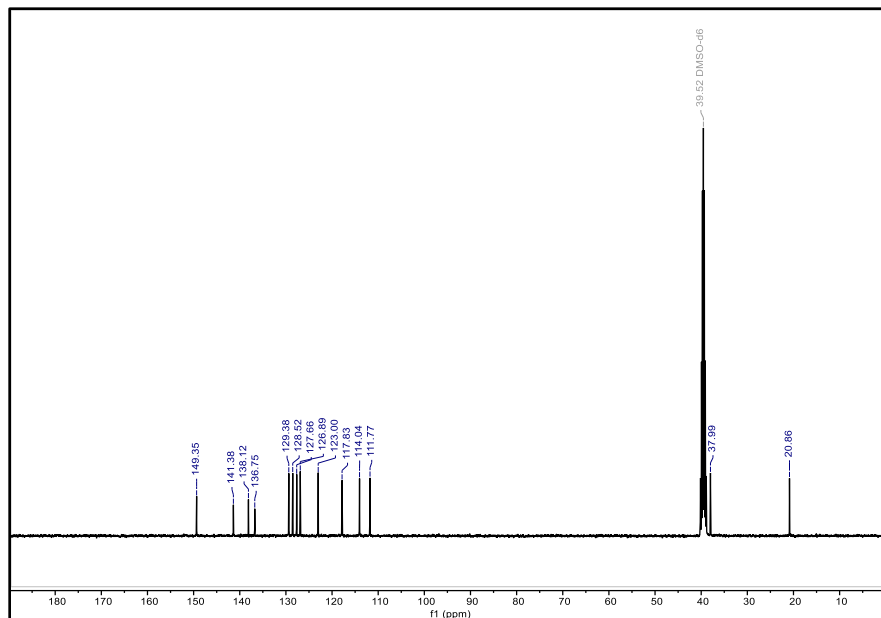


### 3-Amino-*N*-methyl-*N*-(*m*-tolyl)benzenesulfonamide (B58)

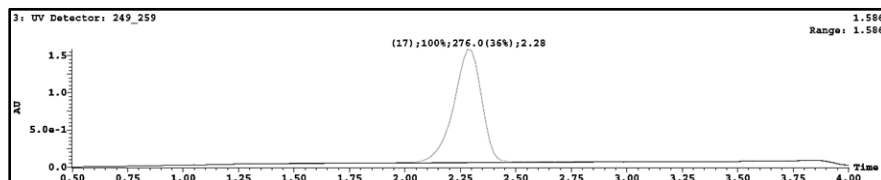
$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):



$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):

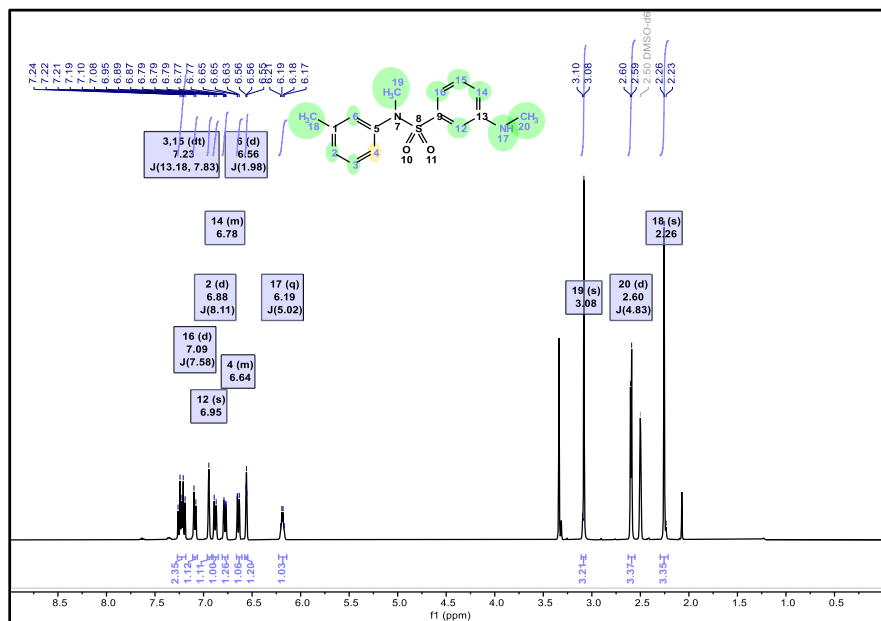


HPLC (UV 249+259 nm):

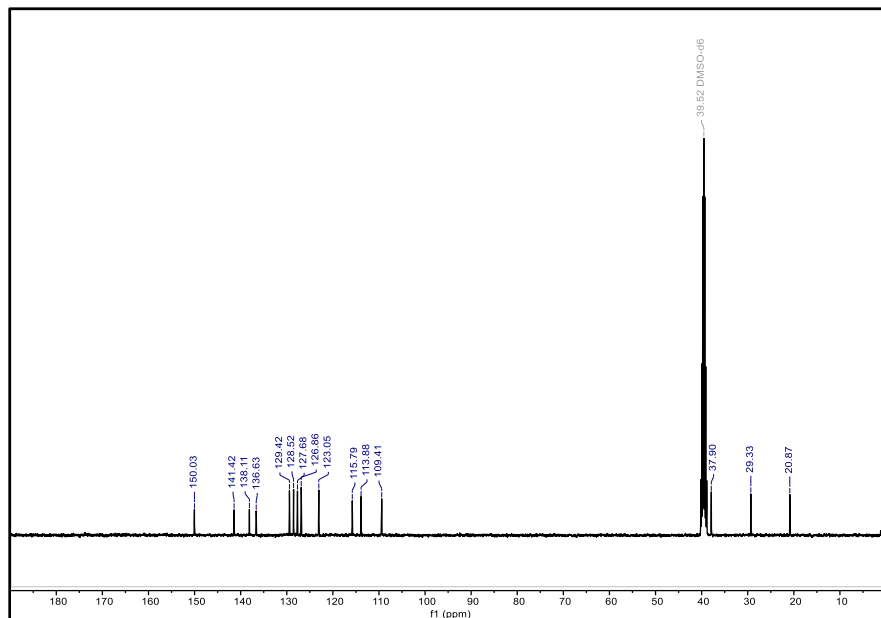


# ***N*-Methyl-3-(methylamino)-*N*-(*m*-tolyl)benzenesulfonamide (B69)**

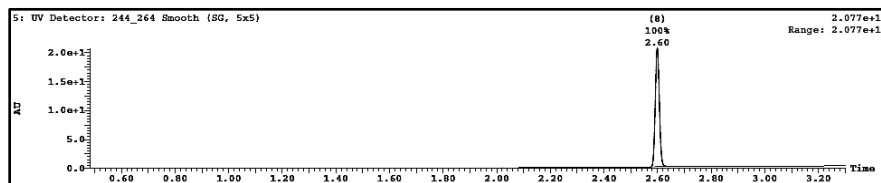
<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):



<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, DMSO-*d*<sub>6</sub>):

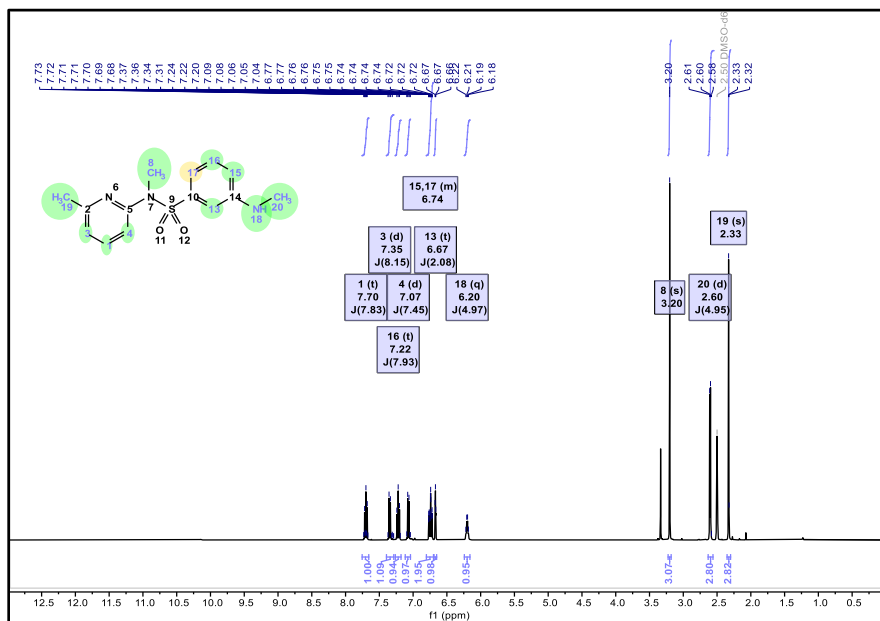


HPLC (UV 244+264 nm):

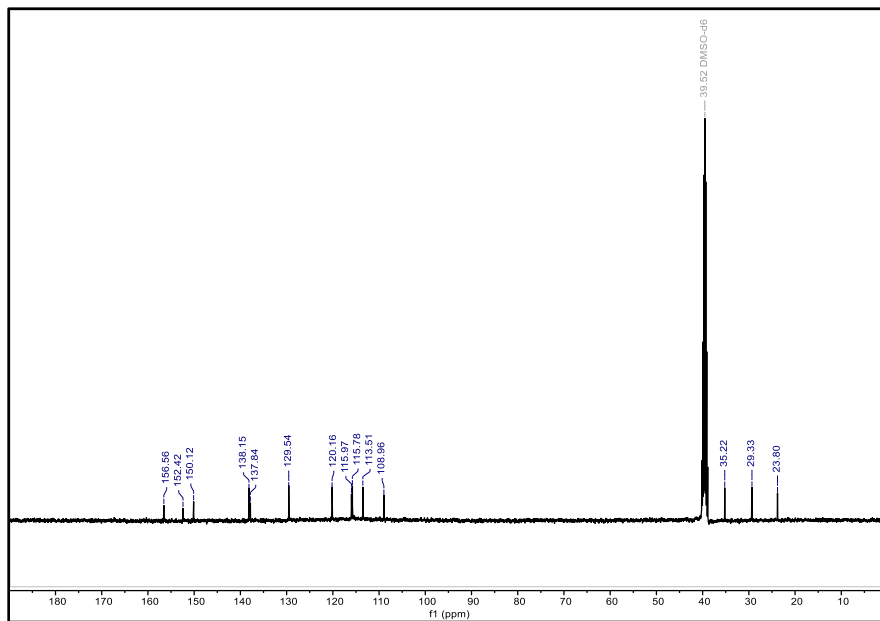


***N*-Methyl-3-(methylamino)-*N*-(6-methylpyridin-2-yl)benzenesulfonamide (B86)**

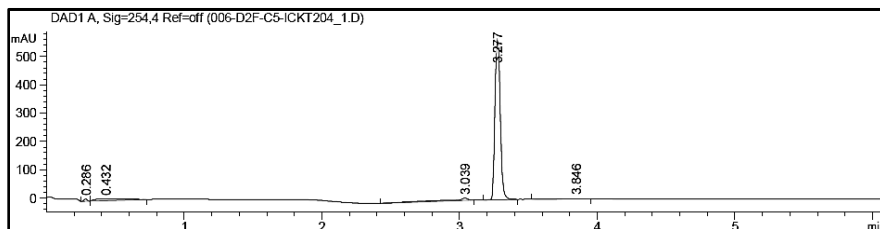
$^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ ):



$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz,  $\text{DMSO-}d_6$ ):

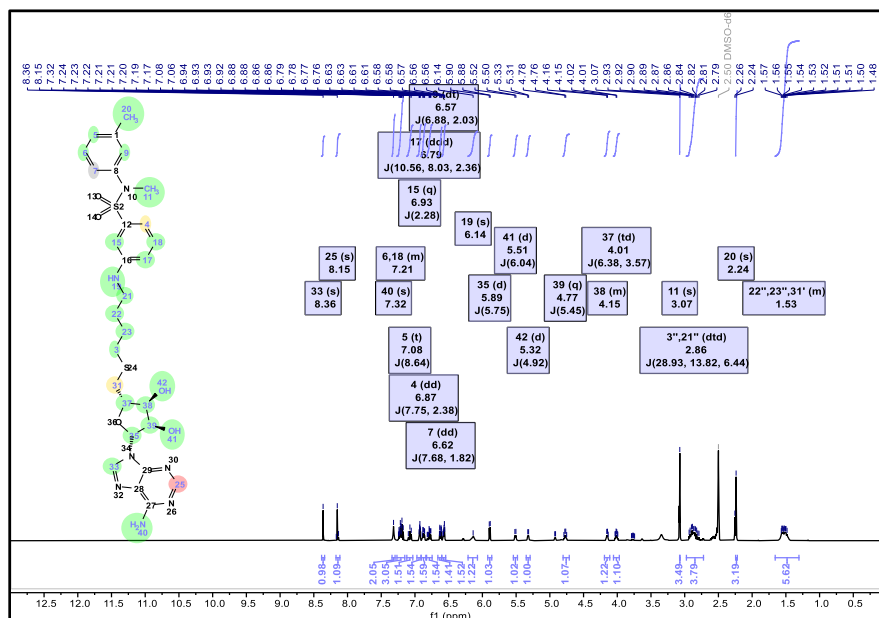


HPLC (UV 254 nm):

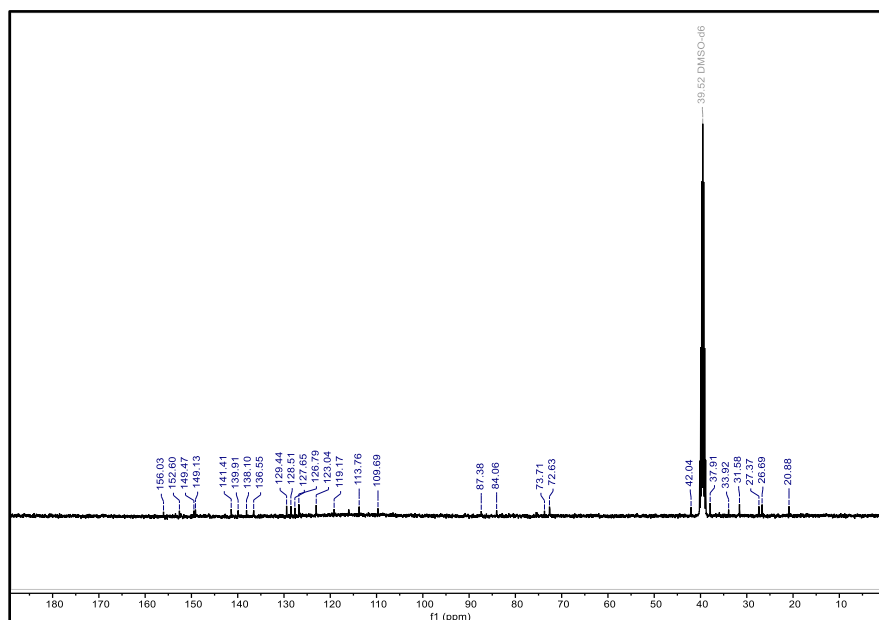


**3-((4-((((2*S*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)thio)butyl)amino)-*N*-methyl-*N*-(*m*-tolyl)benzenesulfonamide (B92)**

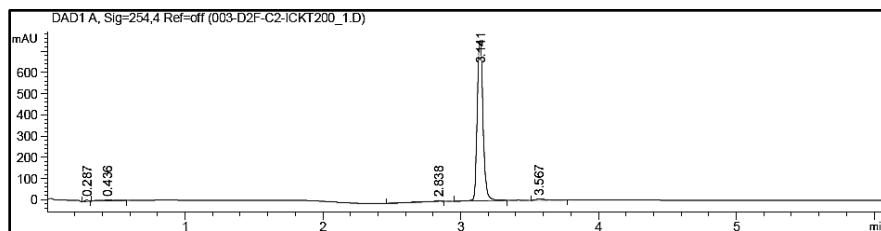
<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):



<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, DMSO-*d*<sub>6</sub>):

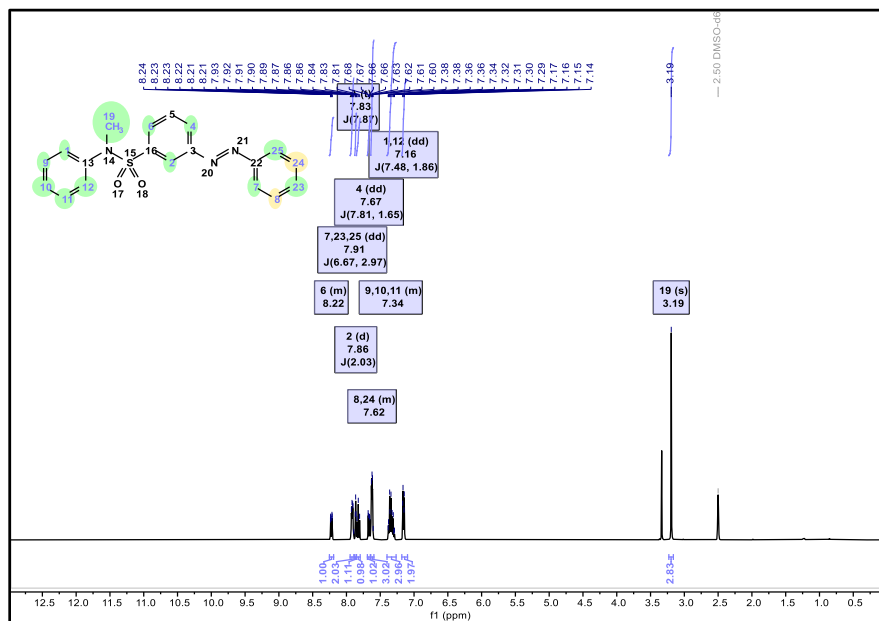


**HPLC (UV 254 nm):**

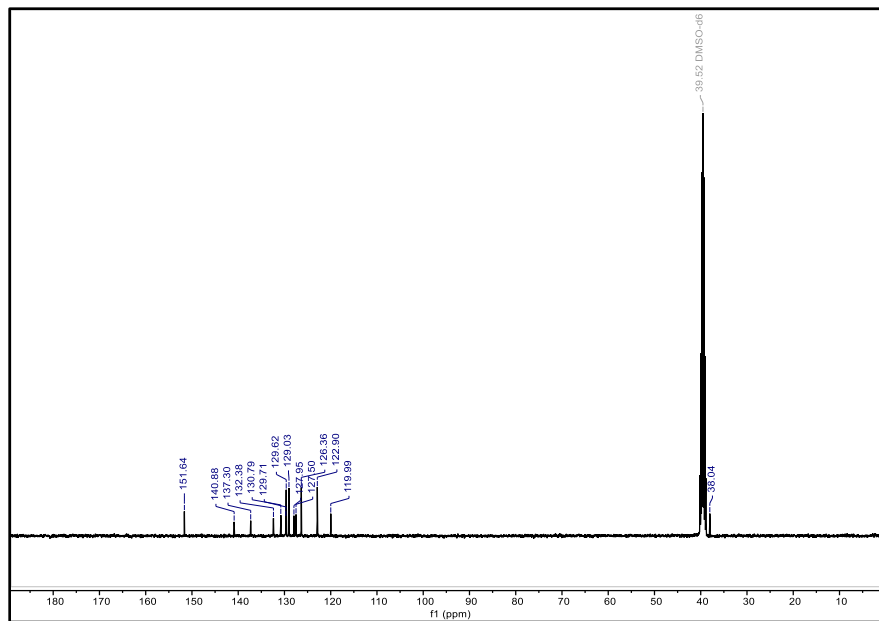


**(E)-N-Methyl-N-phenyl-3-(phenyldiazenyl)benzenesulfonamide (B96)**

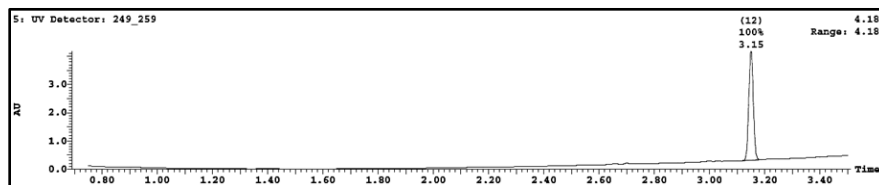
$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):



$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):

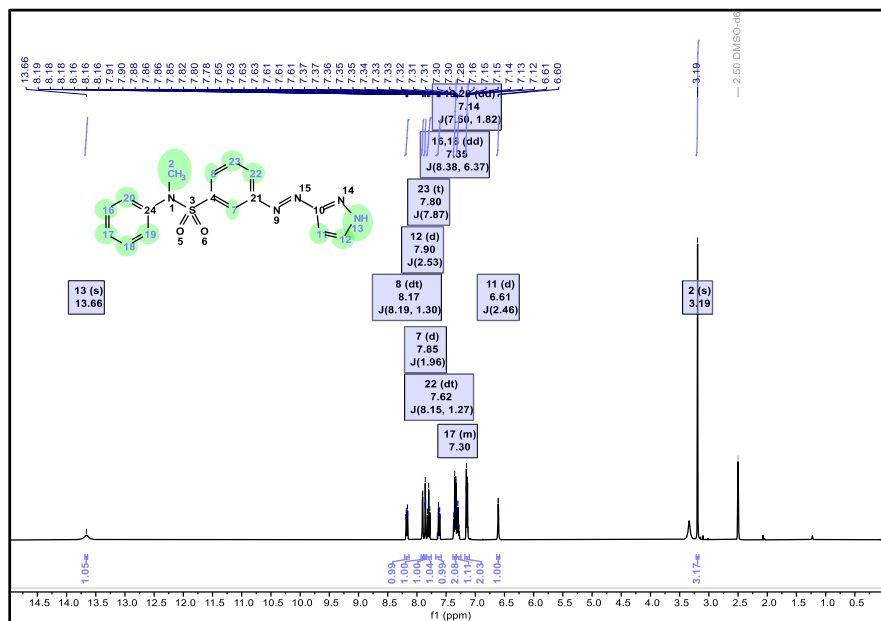


HPLC (UV 249+259 nm):

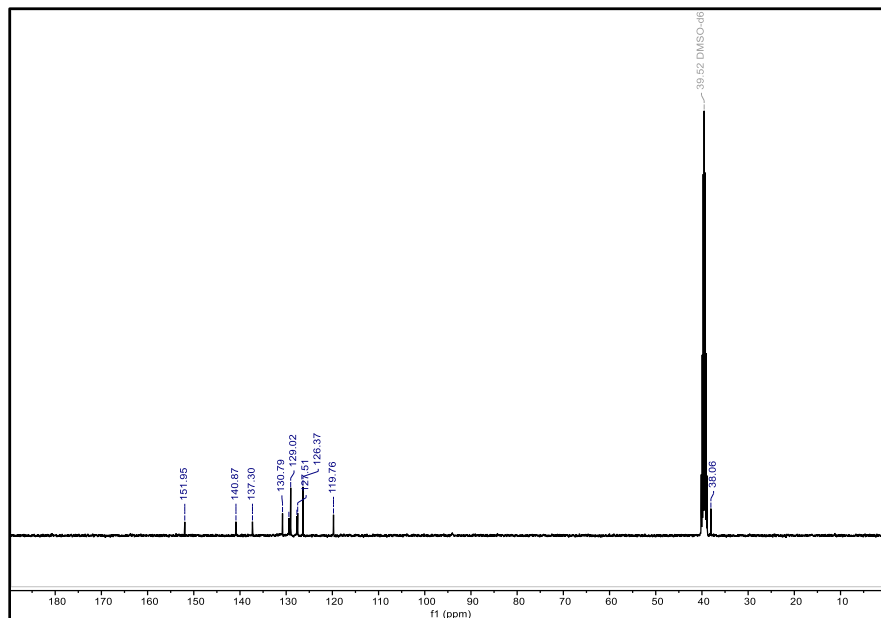


**(E)-3-((1*H*-Pyrazol-3-yl)diazenyl)-*N*-methyl-*N*-phenylbenzenesulfonamide (B114)**

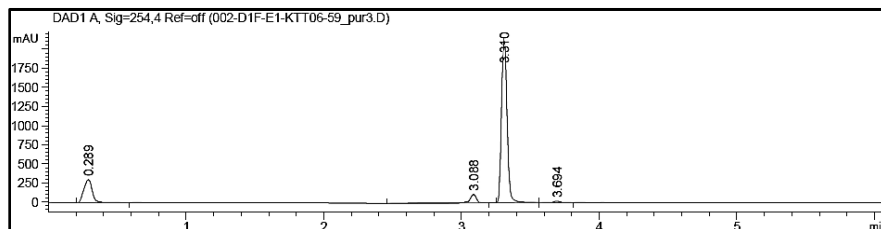
<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):



<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, DMSO-*d*<sub>6</sub>):

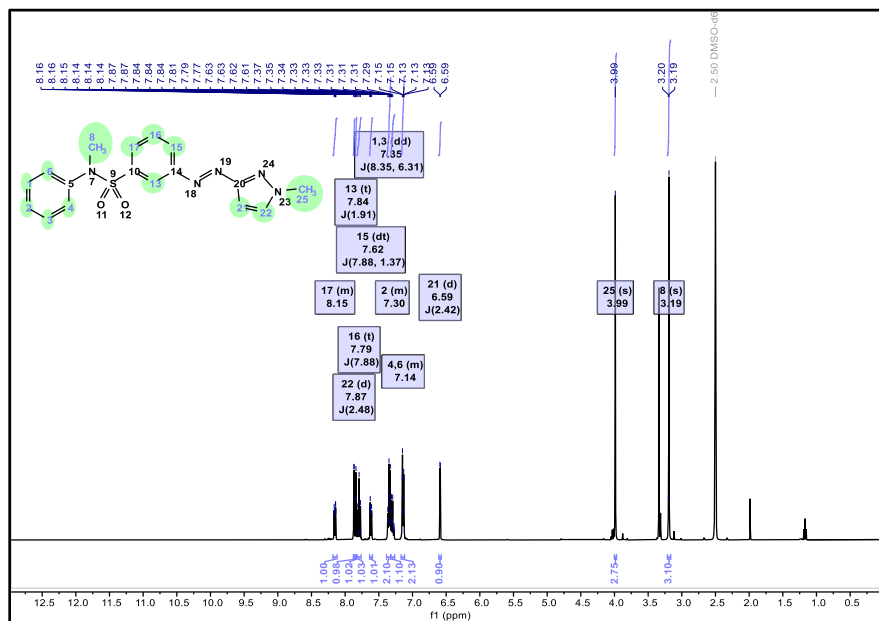


HPLC (UV 254 nm):

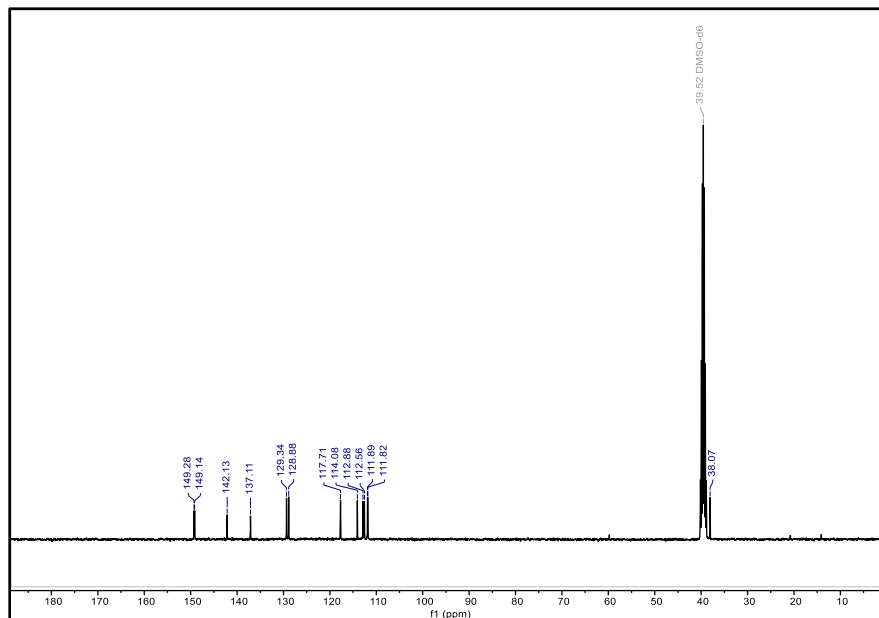


**(*E*)-*N*-Methyl-3-((1-methyl-1*H*-pyrazol-3-yl)diazenyl)-*N*-phenylbenzenesulfonamide (B115)**

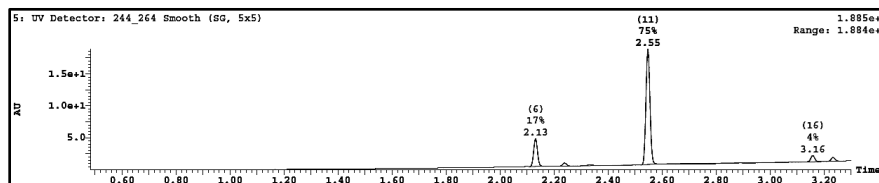
**$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):**



**$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):**



**HPLC (UV 254 nm):**

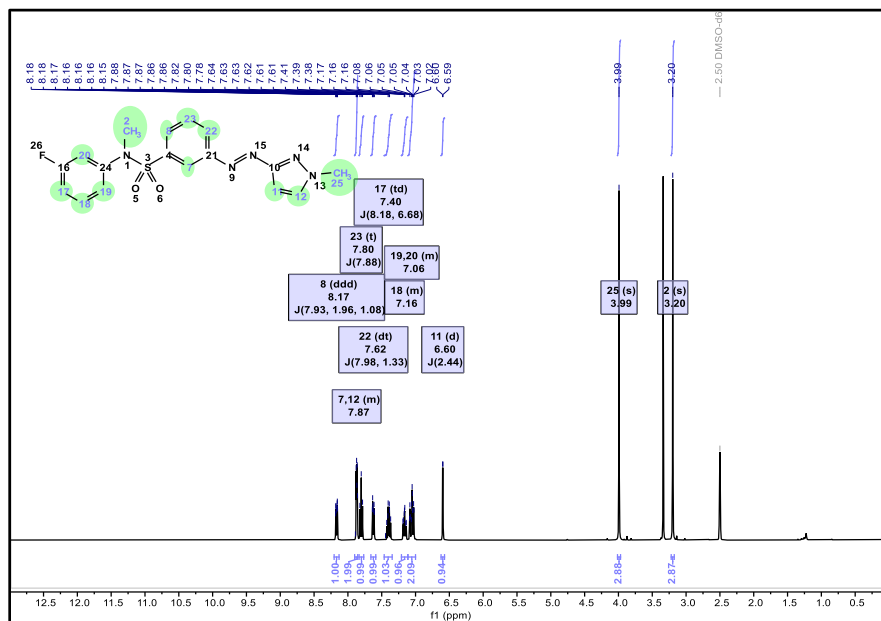




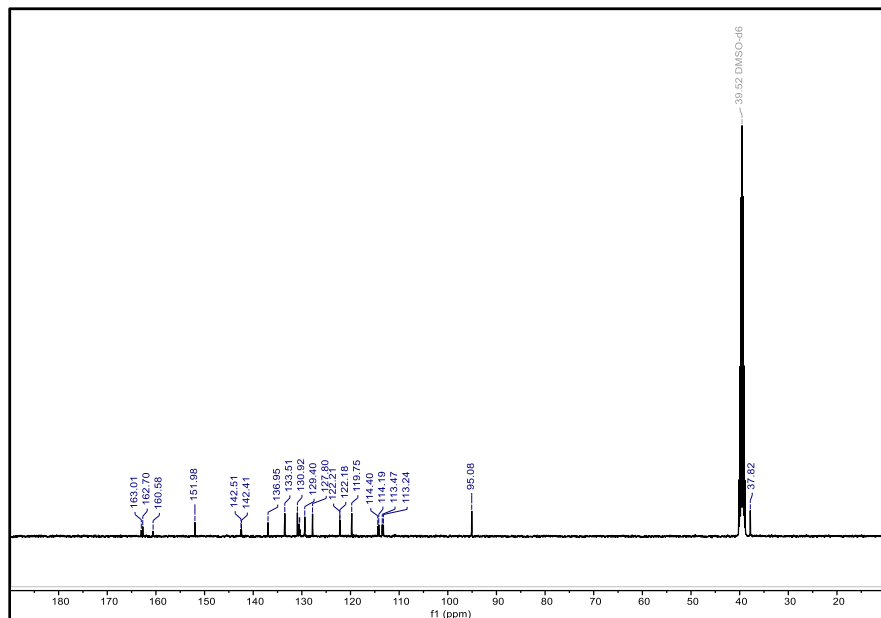
(The peak at 2.13 min arises from the Z-isomer, not detectable by NMR analysis and therefore attributed to ambient light photoswitching during sample preparation or analysis)

**(*E*)-*N*-(3-Fluorophenyl)-*N*-methyl-3-((1-methyl-1*H*-pyrazol-3-yl)diazenyl)benzenesulfonamide (B127)**

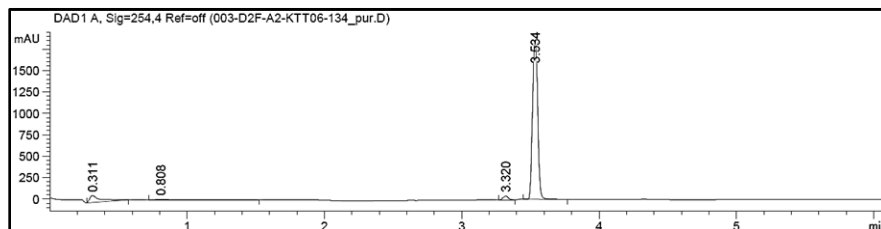
<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):



<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, DMSO-*d*<sub>6</sub>):

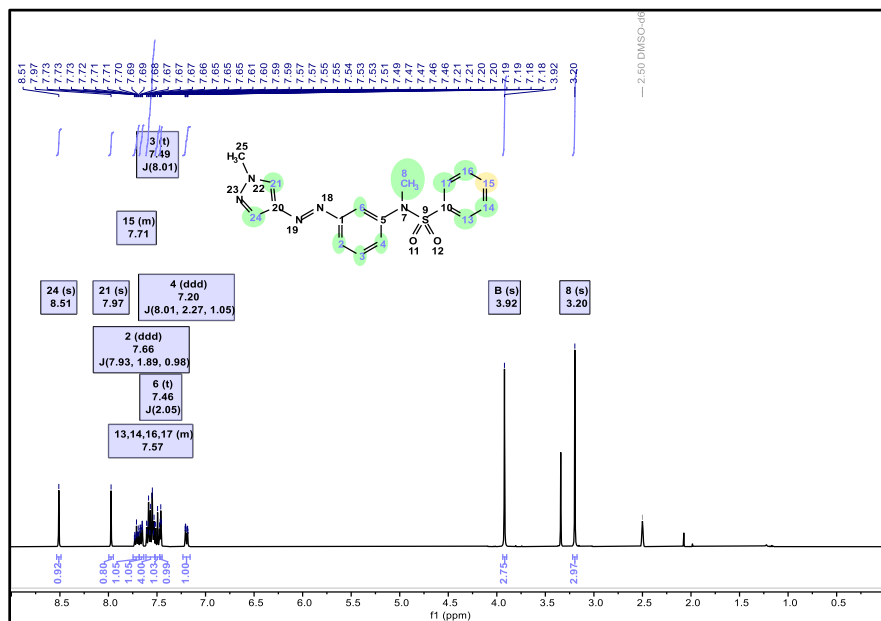


HPLC (UV 254 nm):

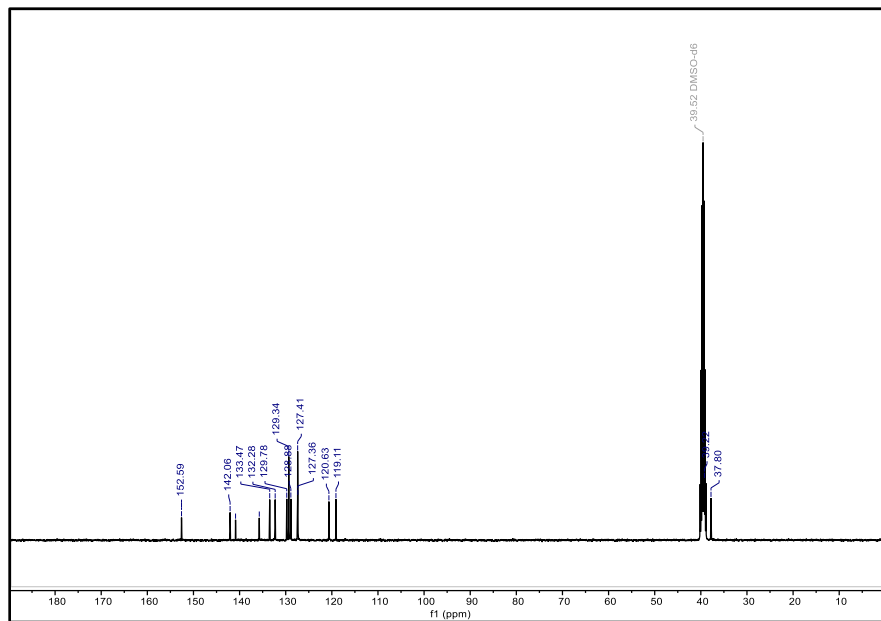


**(*E*)-*N*-methyl-*N*-(3-((1-methyl-1*H*-pyrazol-4-yl)diazenyl)phenyl)benzenesulfonamide (B130)**

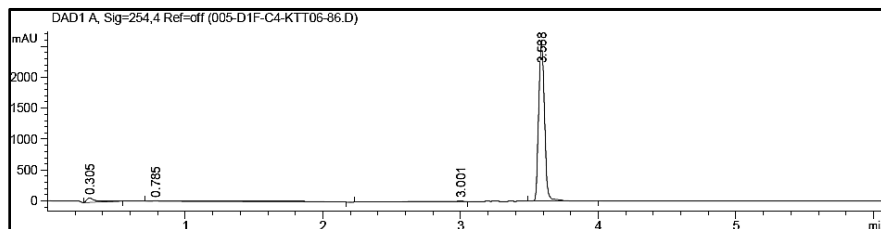
$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):



$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):



HPLC (UV 254 nm):



### 7.1.3. Biological assays on NSP16/10

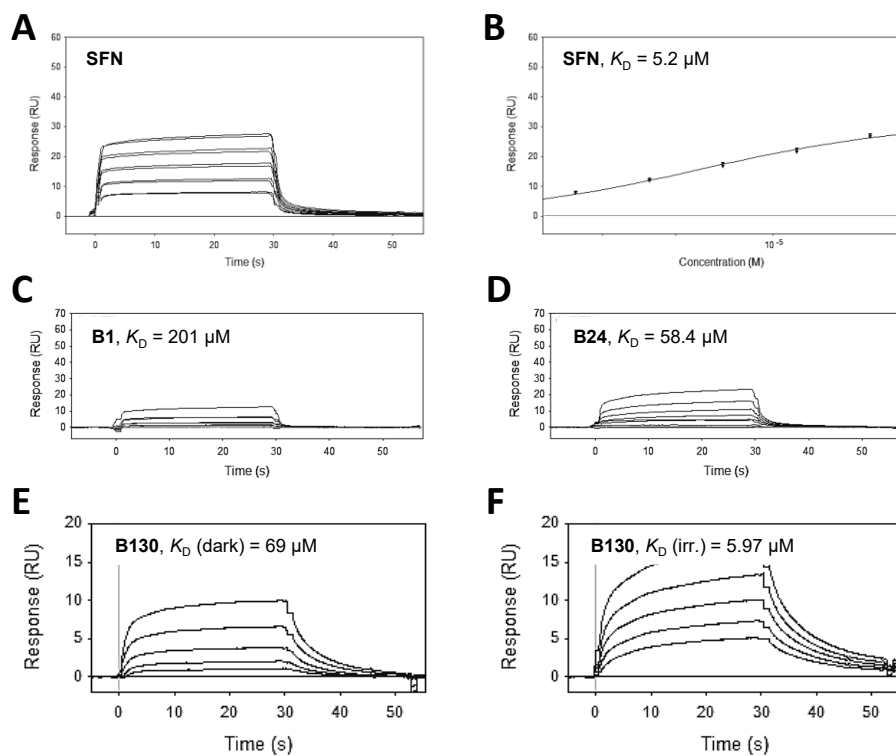


**Figure A2. SPR sensorgrams of selected compounds against NSP16/10.** (A) Sensorgrams of the positive control **SFN**. (B) Dose-response curve for **SFN**. (C) Sensorgrams for the fragment hit **B1** upon retest. (D) Sensorgrams for the key analogue **B24**. (E–F) Sensorgrams for the photoswitchable lead **B130** tested in the dark state and photoirradiated state.

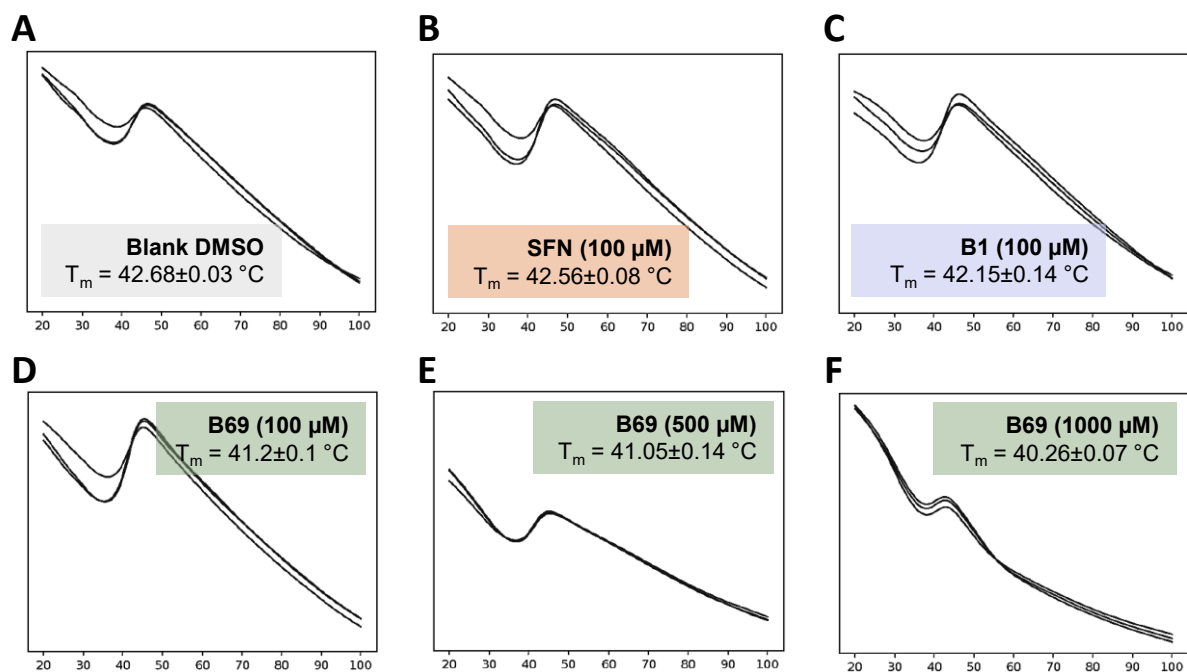


**Figure A3.** DSF assay melting curves of selected compounds against NSP16/10. Curves and average melting temperatures ( $\pm$  the standard deviation) from technical triplicates are shown. (A) Blank DMSO. (B) Test of **SFN** as positive control. (C) Test of **B1** at 100  $\mu\text{M}$ . (D) Test of **B69** at 100  $\mu\text{M}$ . (E) Test of **B69** at 500  $\mu\text{M}$ . (F) Test of **B69** at 1000  $\mu\text{M}$ .

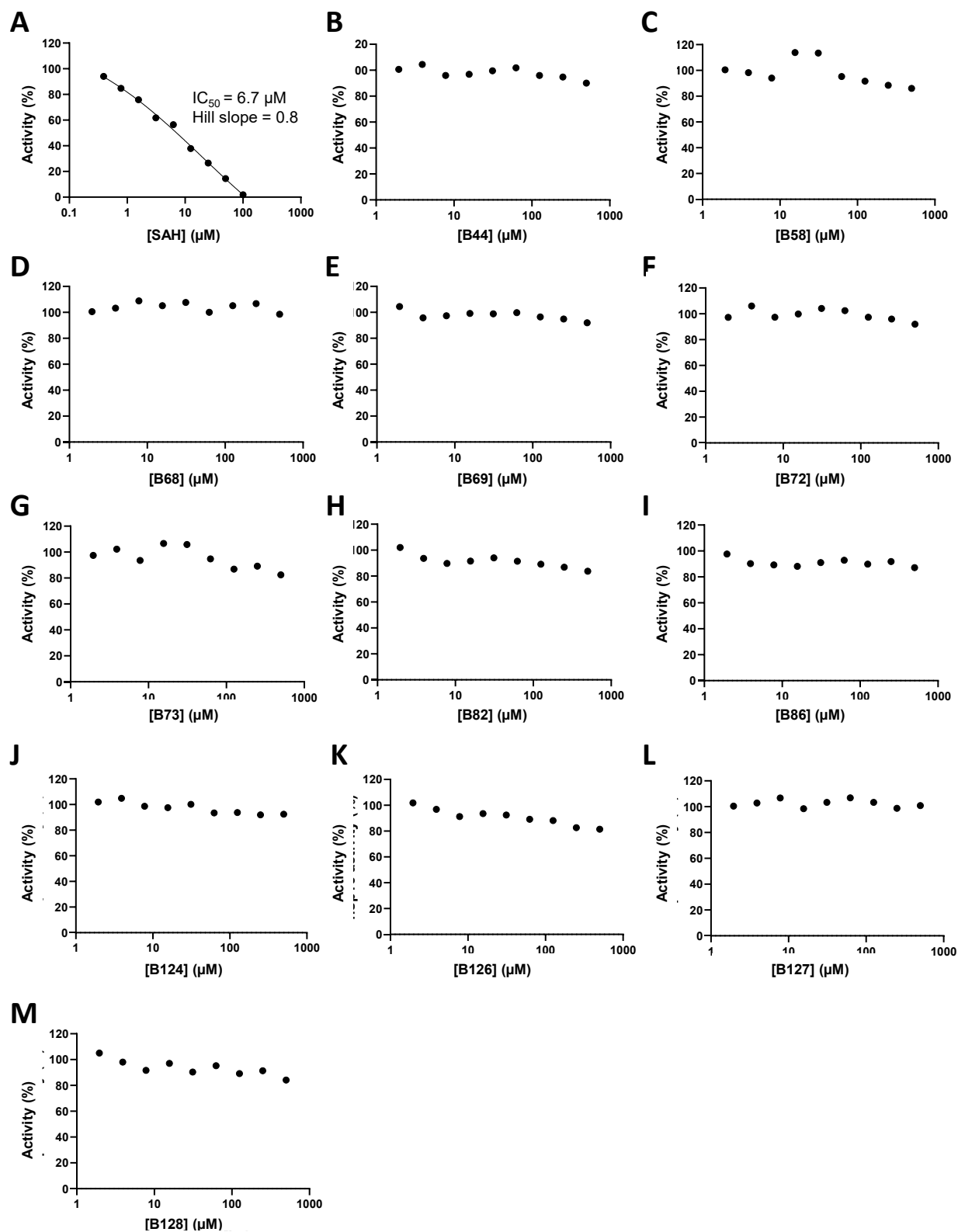
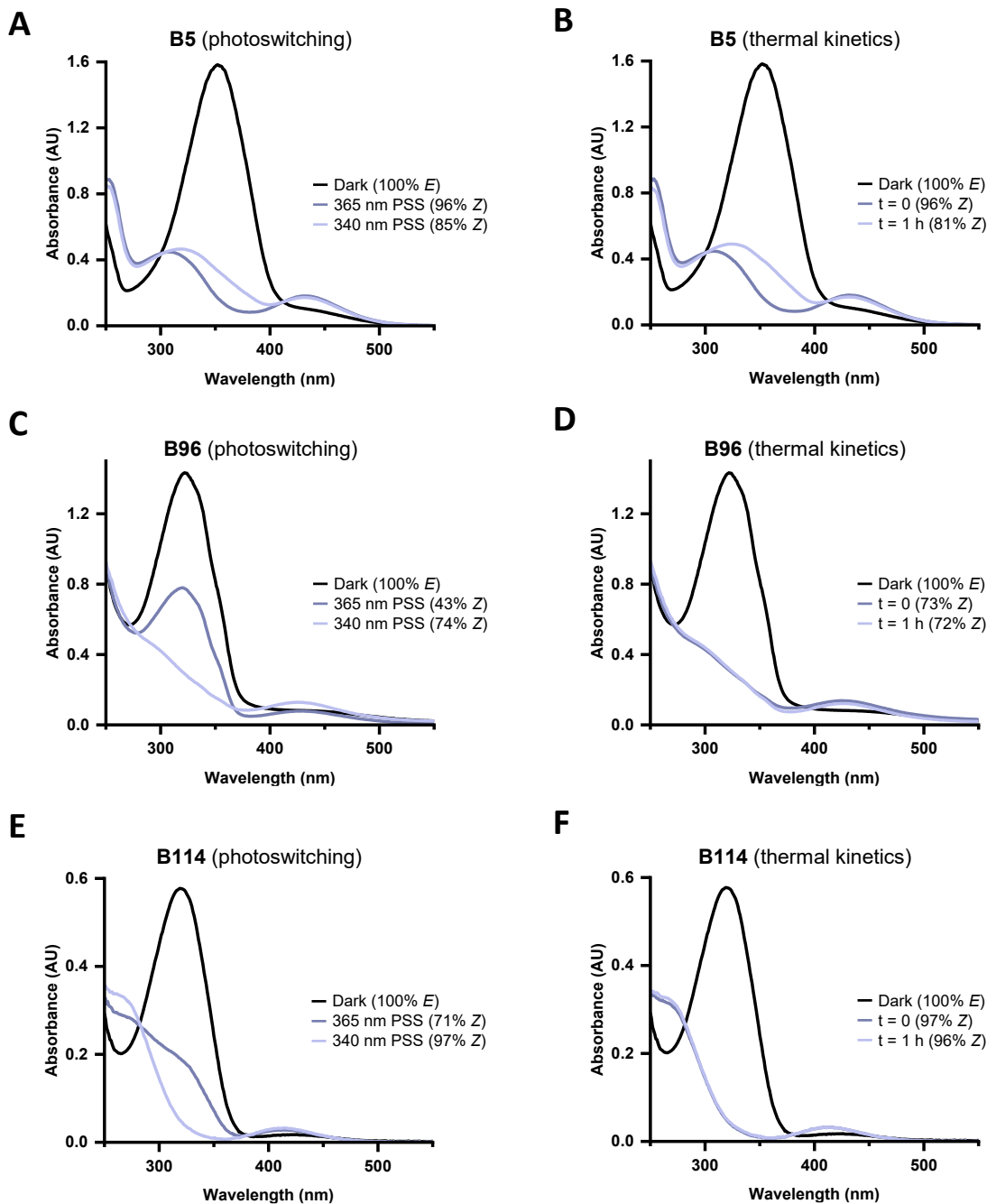


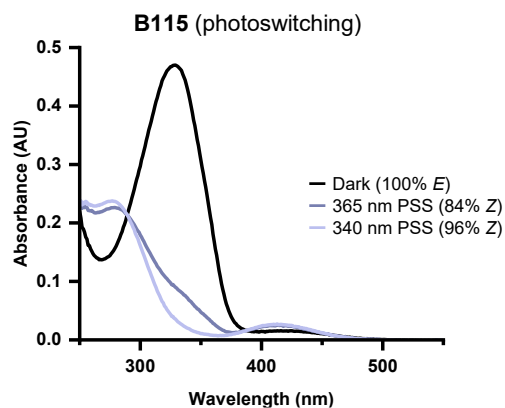
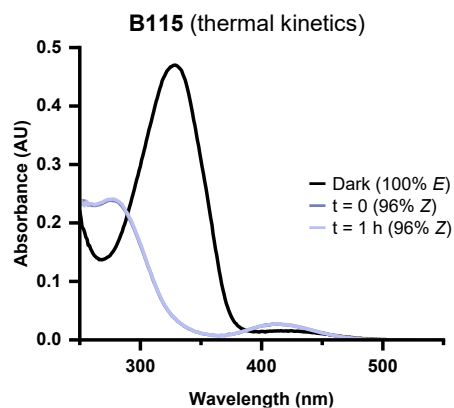
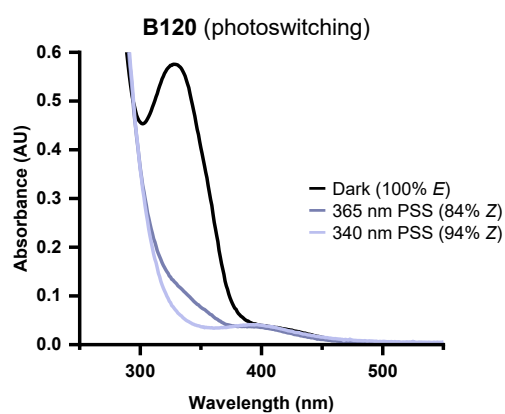
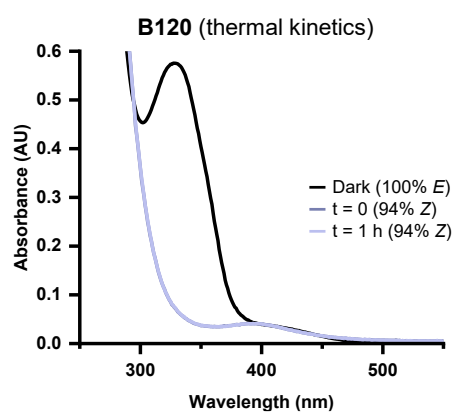
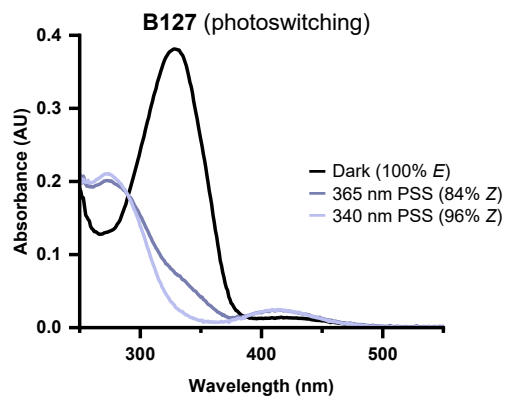
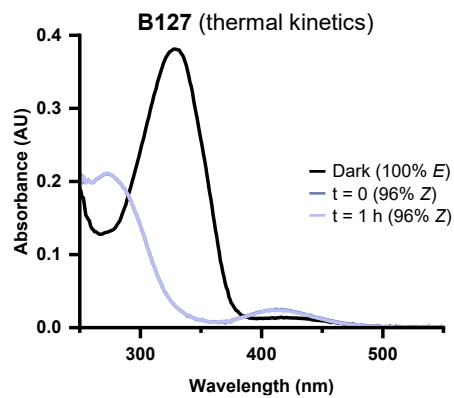
**Figure A4.** SPA assay dose-response curves of selected compounds against NSP16/10. The compounds were tested at 1.95–500  $\mu M$  with technical duplicates, but only one repeat has been shown for each compound. (A) The positive control SAH ( $K_D = 1.2 \mu M$ ). (B) **B44** ( $K_D = 3.96 \mu M$ ). (C) **B58** ( $K_D = 4.93 \mu M$ ). (D) **B68** ( $K_D = 2.76 \mu M$ ). (E) **B69** ( $K_D = 0.73$

$\mu\text{M}$ ). (F) **B72** ( $K_D = 7.38 \mu\text{M}$ ). (G) **B73** ( $K_D = 4.98 \mu\text{M}$ ). (H) **B82** ( $K_D = 3.56 \mu\text{M}$ ). (I) **B86** ( $K_D = 2.28 \mu\text{M}$ ). (J) **B124** ( $K_D$  (dark) =  $3.61 \mu\text{M}$ ). (K) **B126** ( $K_D$  (dark) =  $1.66 \mu\text{M}$ ). (L) **B127** ( $K_D$  (dark) =  $2.40 \mu\text{M}$ ). (M) **B128** ( $K_D$  (dark) =  $10.7 \mu\text{M}$ ).

#### 7.1.4. Photochemical characterisation of key series B compounds



(figure continued on next page)

**G****H****I****J****K****L**

(figure continued on next page)



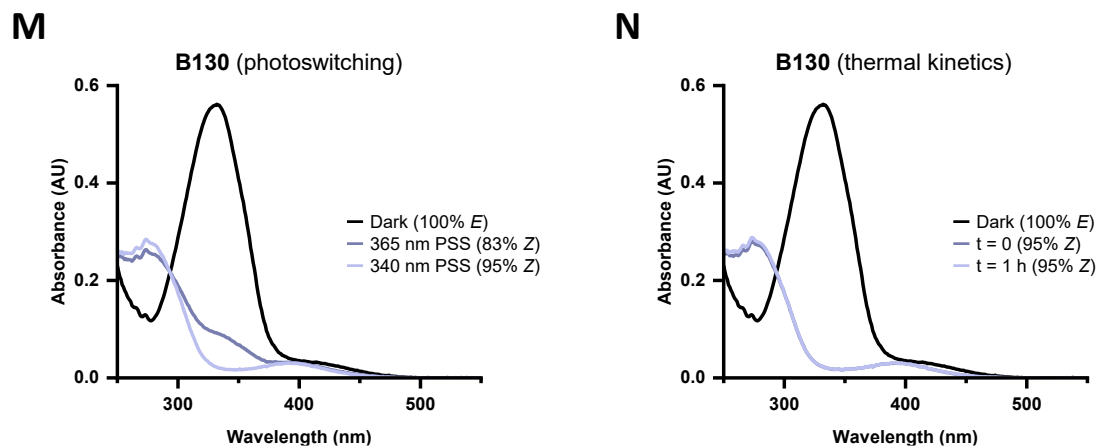


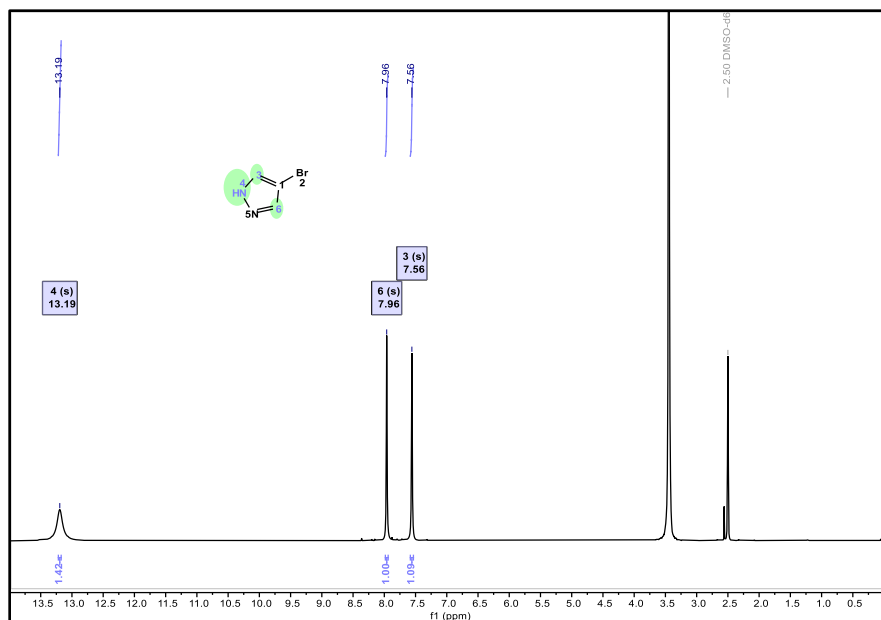
**Figure A5. Photochemical characterisation of selected series B compounds.** Photoswitching and thermal kinetics were determined by UV-Vis spectroscopy at 20 °C and at 20  $\mu$ M in 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 10 w/v% glycerol, 0.05 v/v% Tween-20, 2% DMSO.

## 7.2. Chapter 3 supporting information

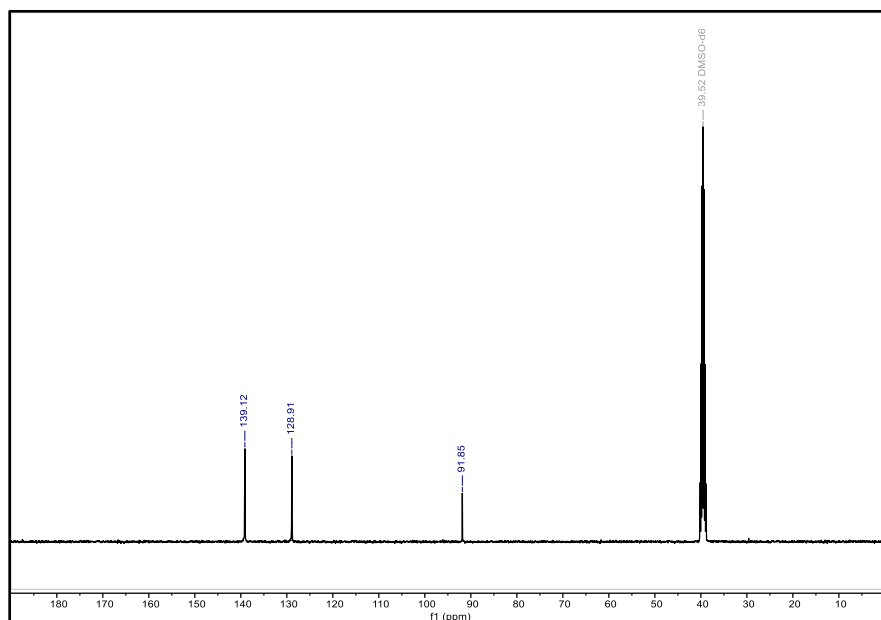
### 7.2.1. Chemical characterisation of key series C and D compounds

#### 4-Bromo-1*H*-pyrazole (C1)

$^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ ):

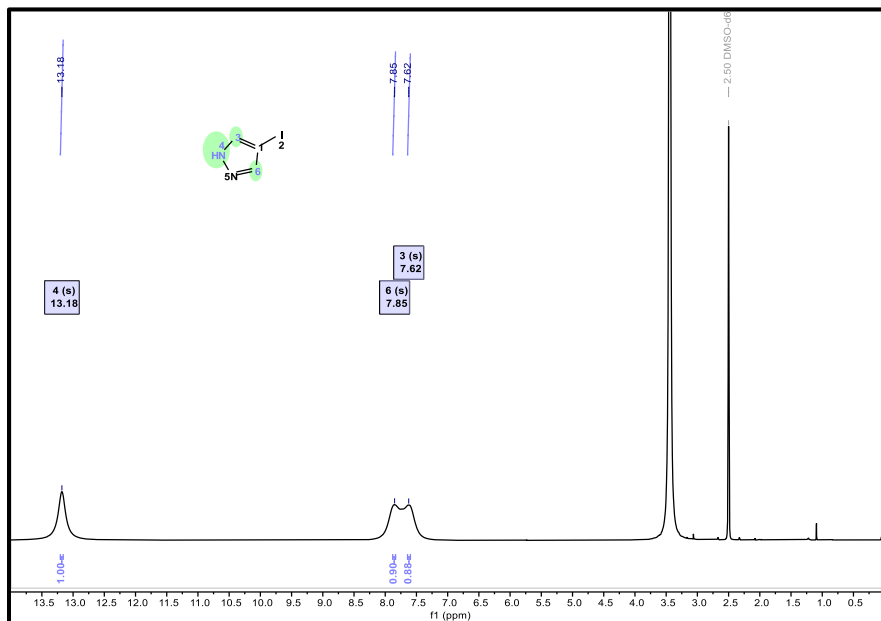


$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz,  $\text{DMSO-}d_6$ ):

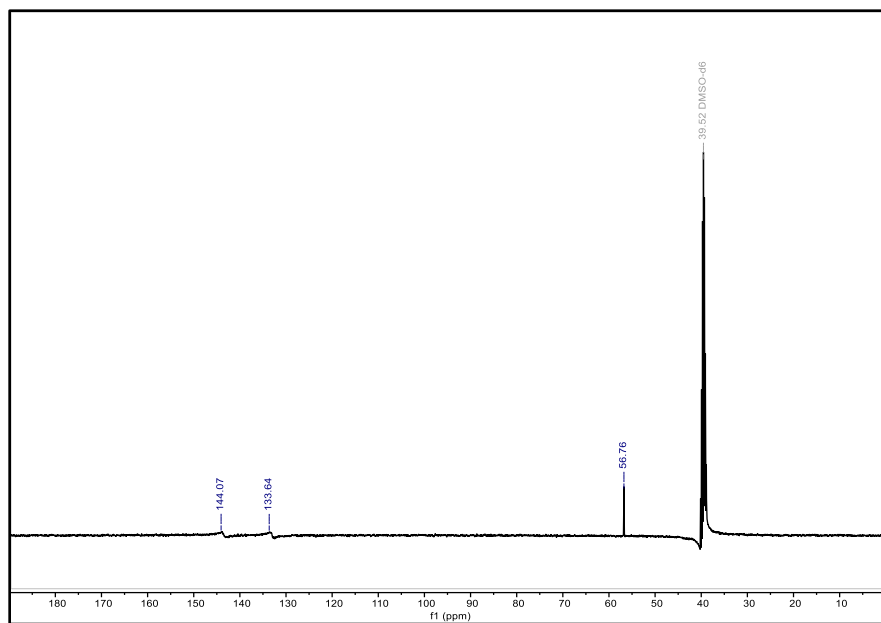


### 4-Iodo-1*H*-pyrazole (C2)

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):

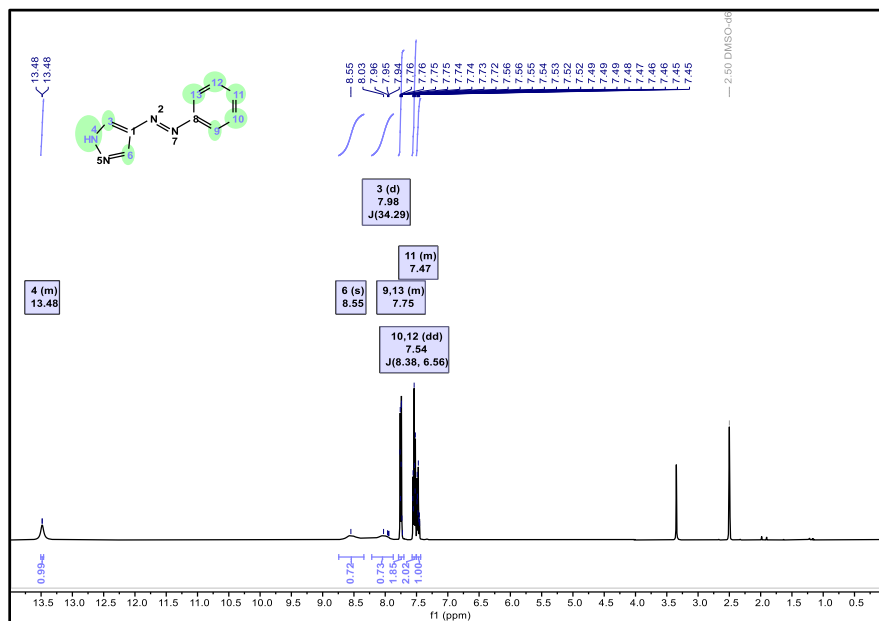


$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):

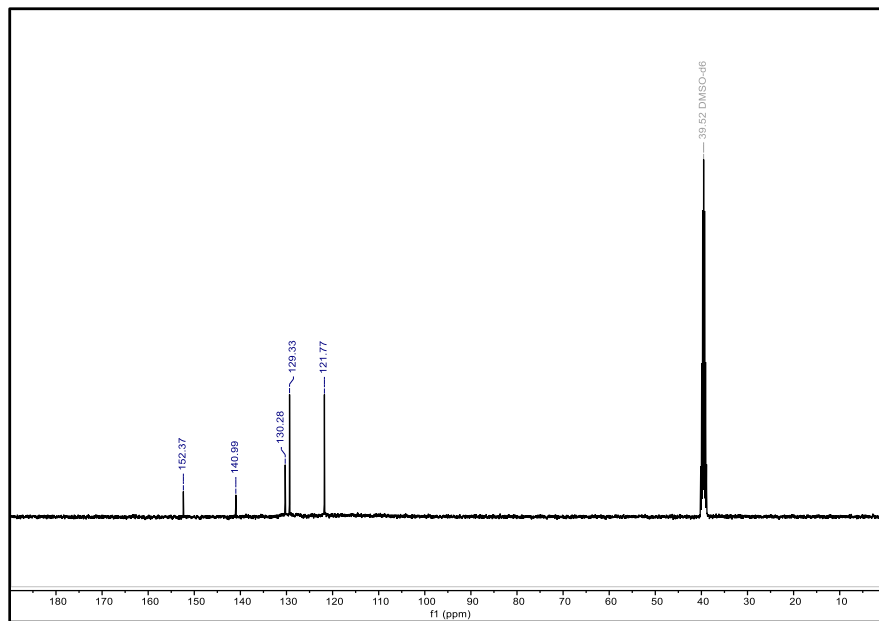


### (E)-4-(Phenyldiazenyl)-1H-pyrazole (C3)

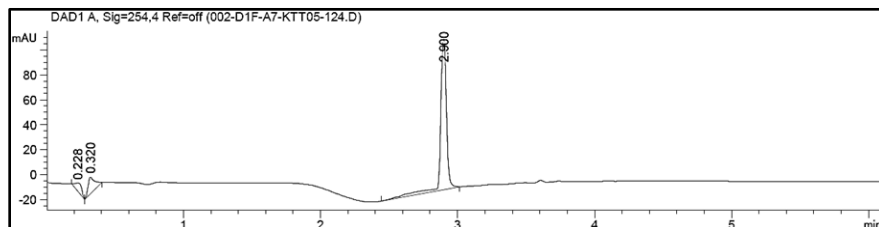
$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):



$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):

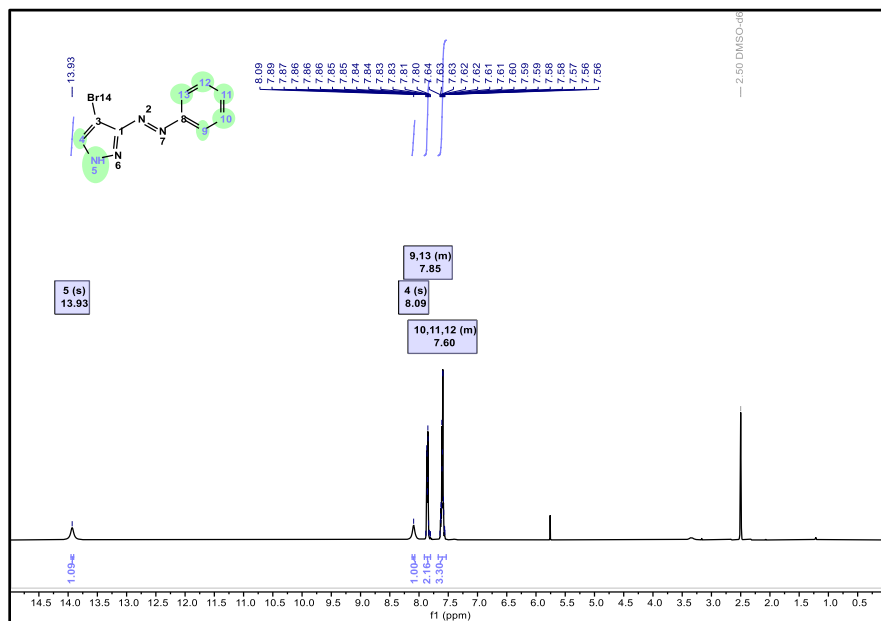


HPLC (UV 254 nm):

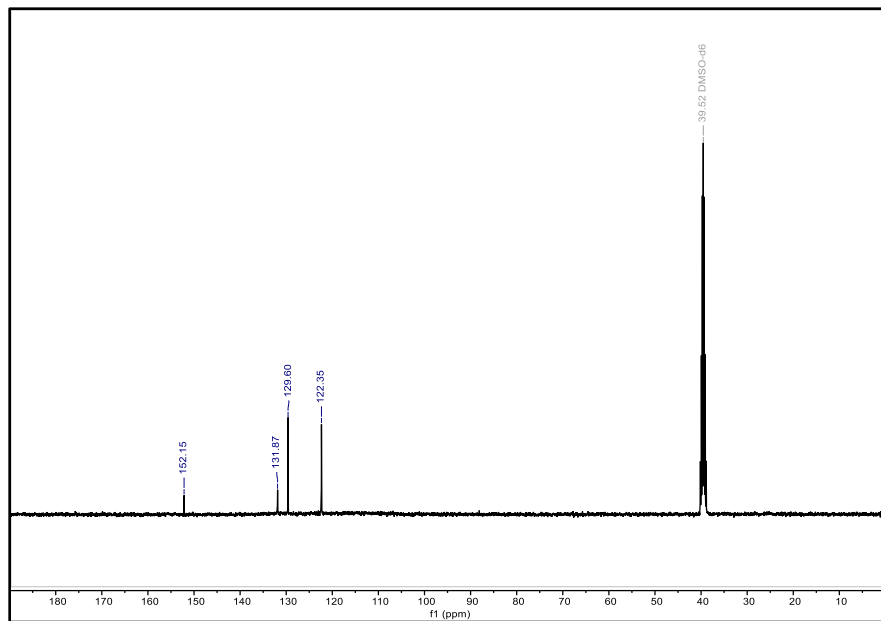


**(E)-4-Bromo-3-(phenyldiazenyl)-1H-pyrazole (C4)**

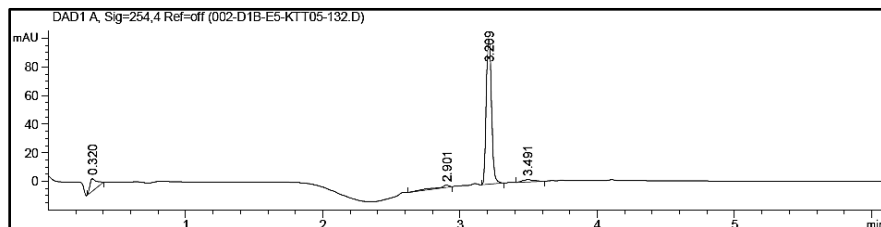
**$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):**



**$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):**

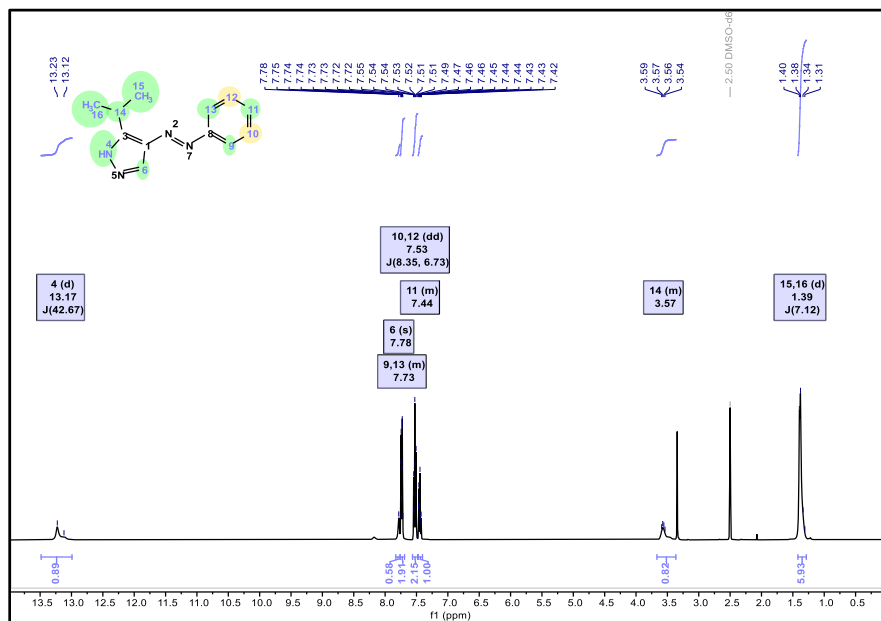


**HPLC (UV 254 nm):**

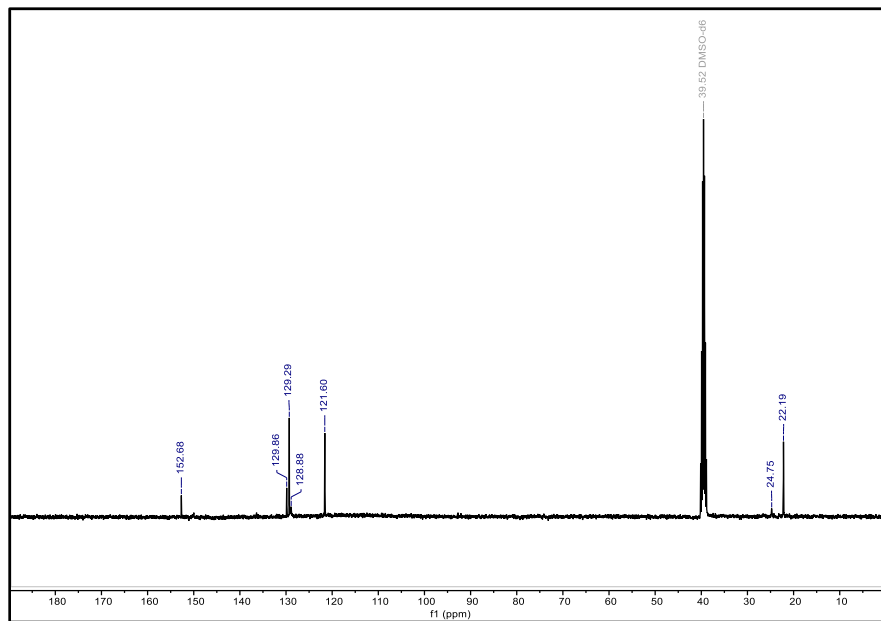


**(E)-5-Isopropyl-4-(phenyldiazenyl)-1H-pyrazole (C6)**

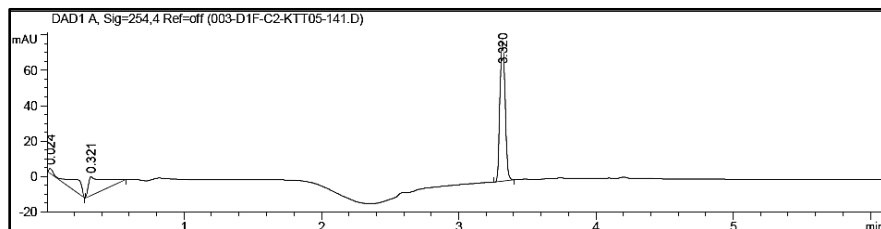
**$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):**



**$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):**

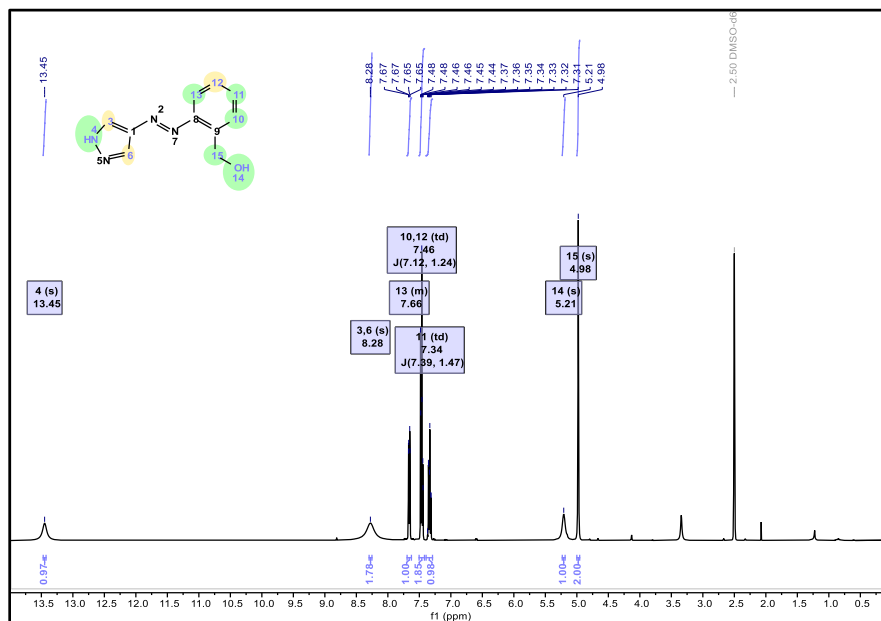


**HPLC (UV 254 nm):**

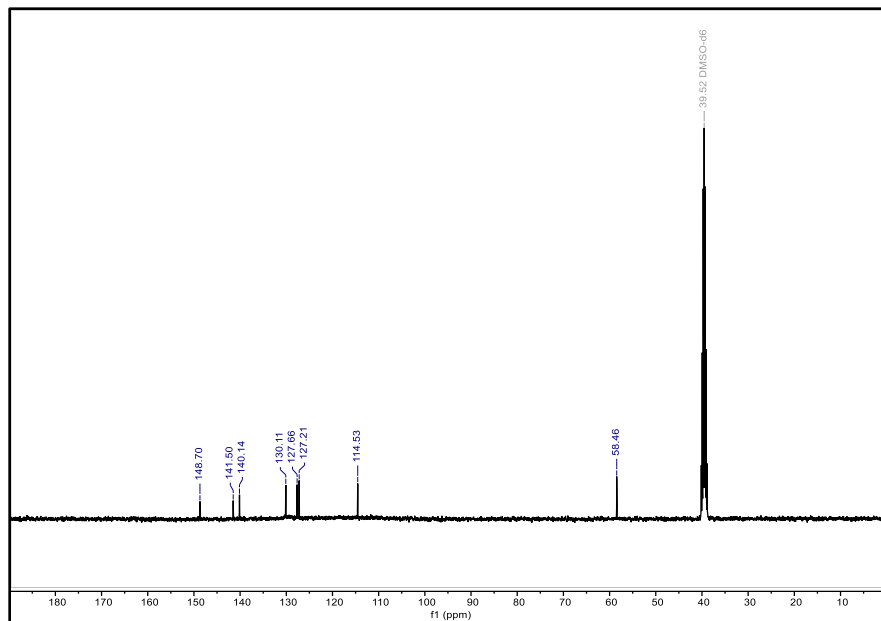


**(E)-2-((1H-Pyrazol-4-yl)diazenyl)phenyl)methanol (C15)**

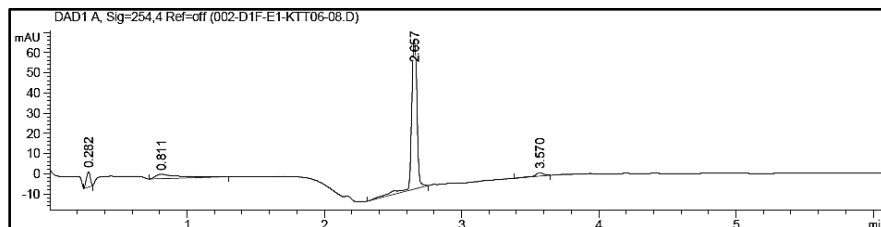
**$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):**



**$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):**

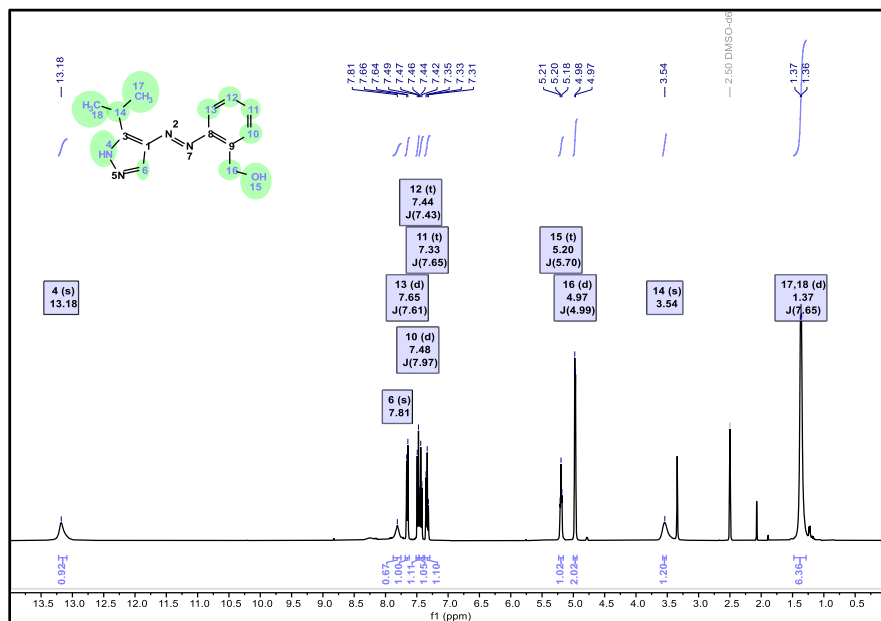


**HPLC (UV 254 nm):**

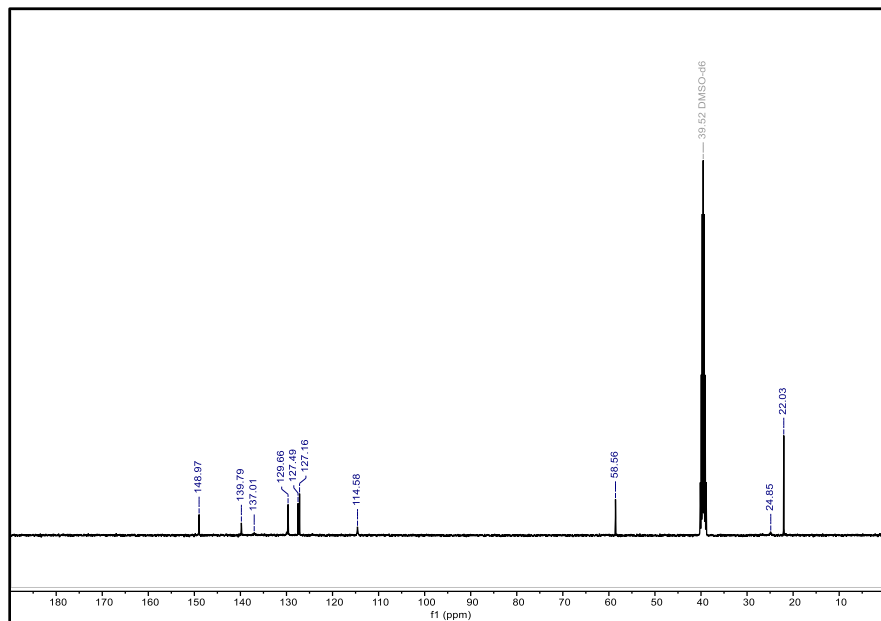


**(E)-2-((5-Isopropyl-1H-pyrazol-4-yl)diazenyl)phenyl)methanol (C20)**

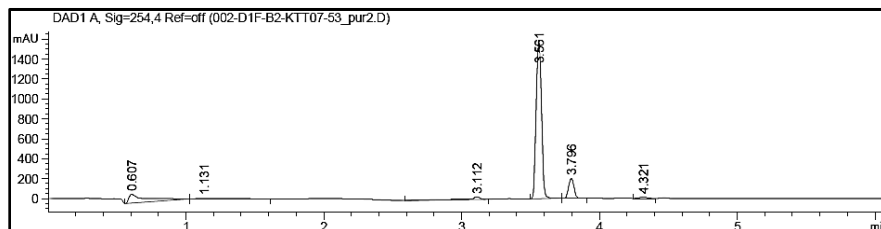
**$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):**



**$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):**



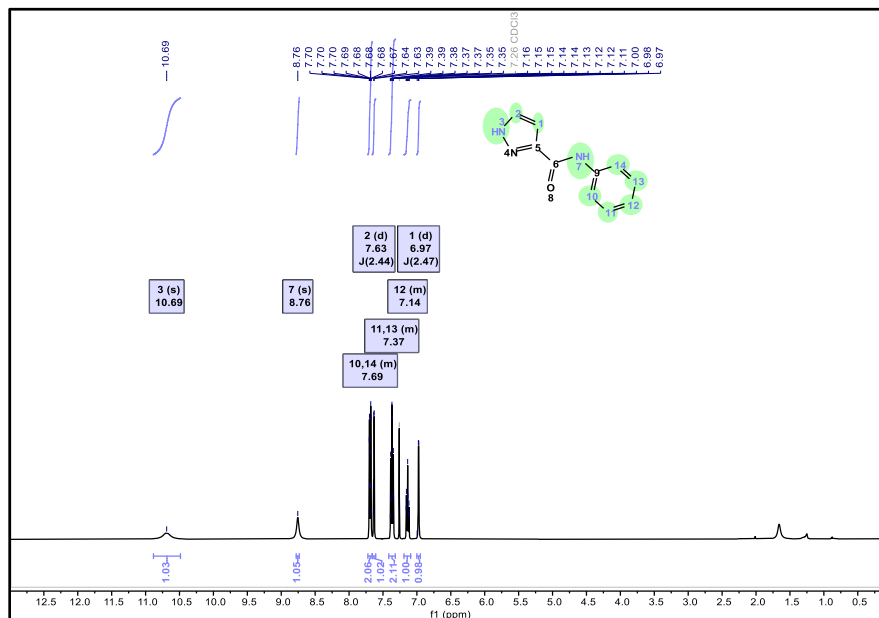
**HPLC (UV 254 nm):**



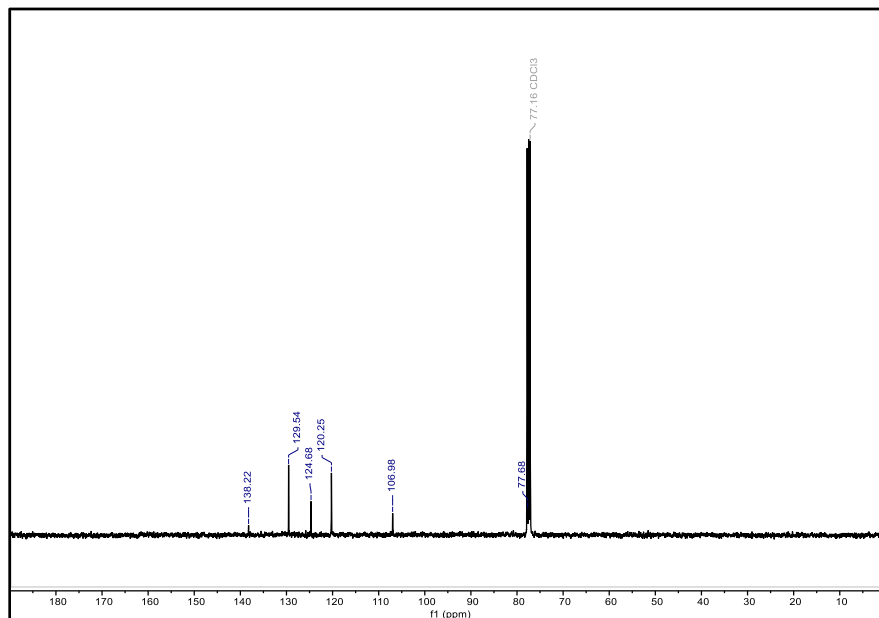


## ***N*-Phenyl-1*H*-pyrazole-3-carboxamide (D1)**

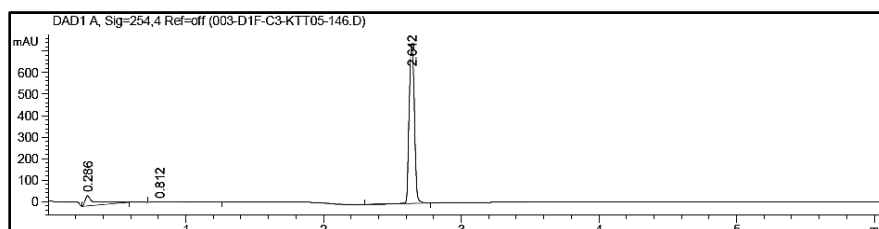
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):



<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, CDCl<sub>3</sub>):

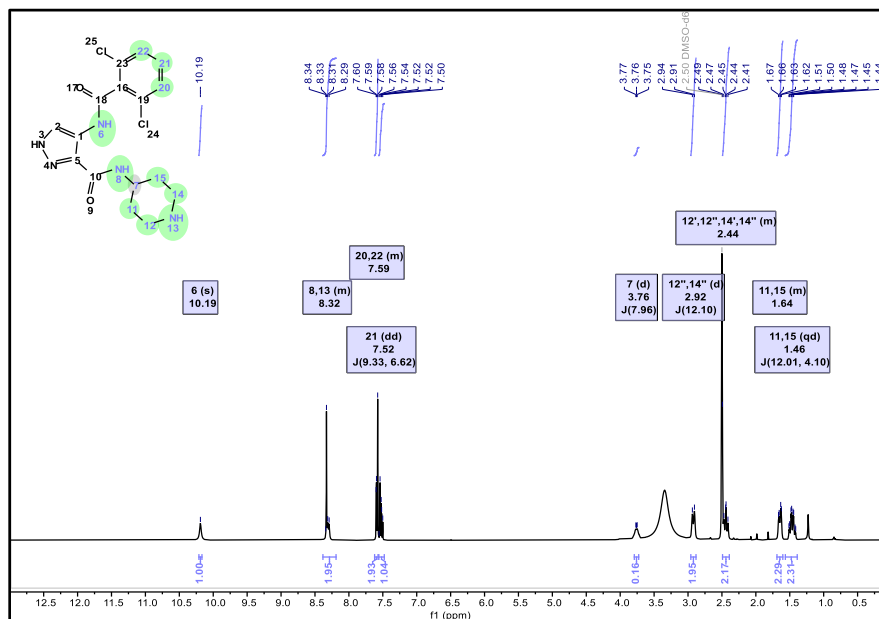


HPLC (UV 254 nm):

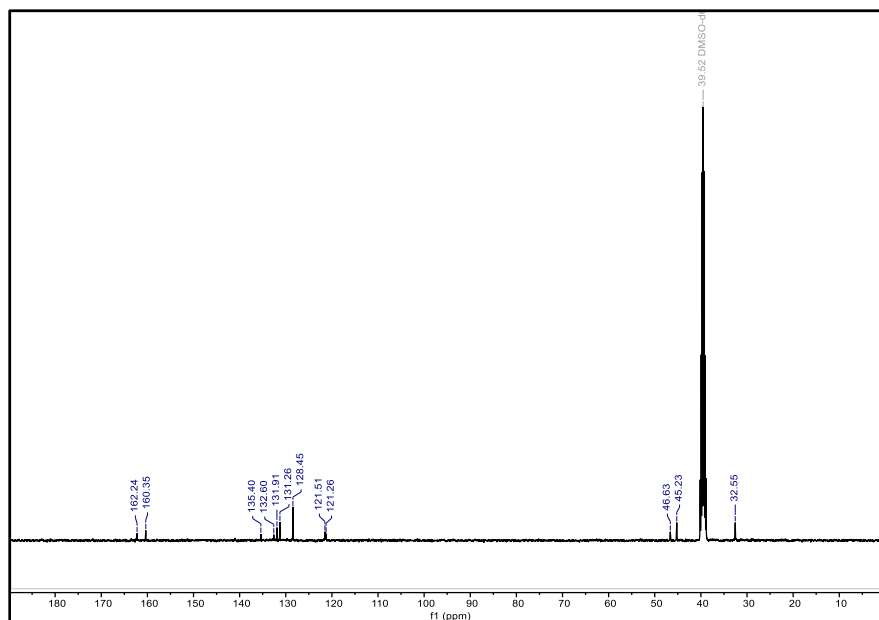


#### 4-(2,6-Dichlorobenzamido)-*N*-(piperidin-4-yl)-1*H*-pyrazole-3-carboxamide (D2)

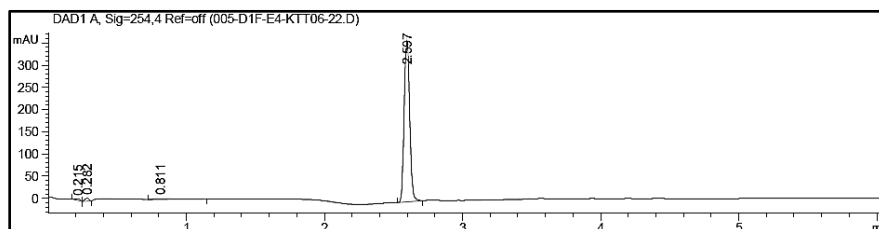
$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):



$^{13}\text{C}\{^1\text{H}\}$  NMR (101 MHz, DMSO- $d_6$ ):



HPLC (UV 254 nm):





### 7.2.2. Docking against CDK2

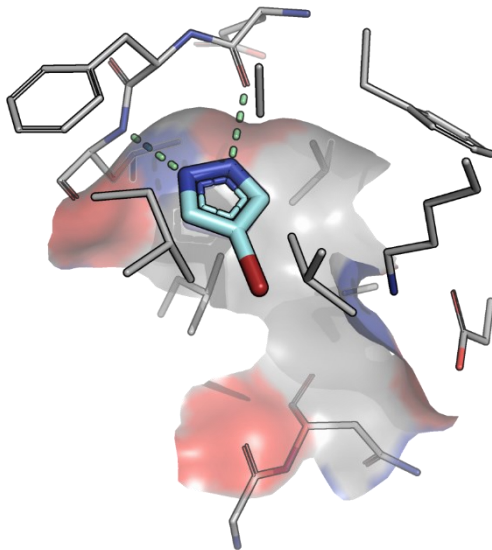
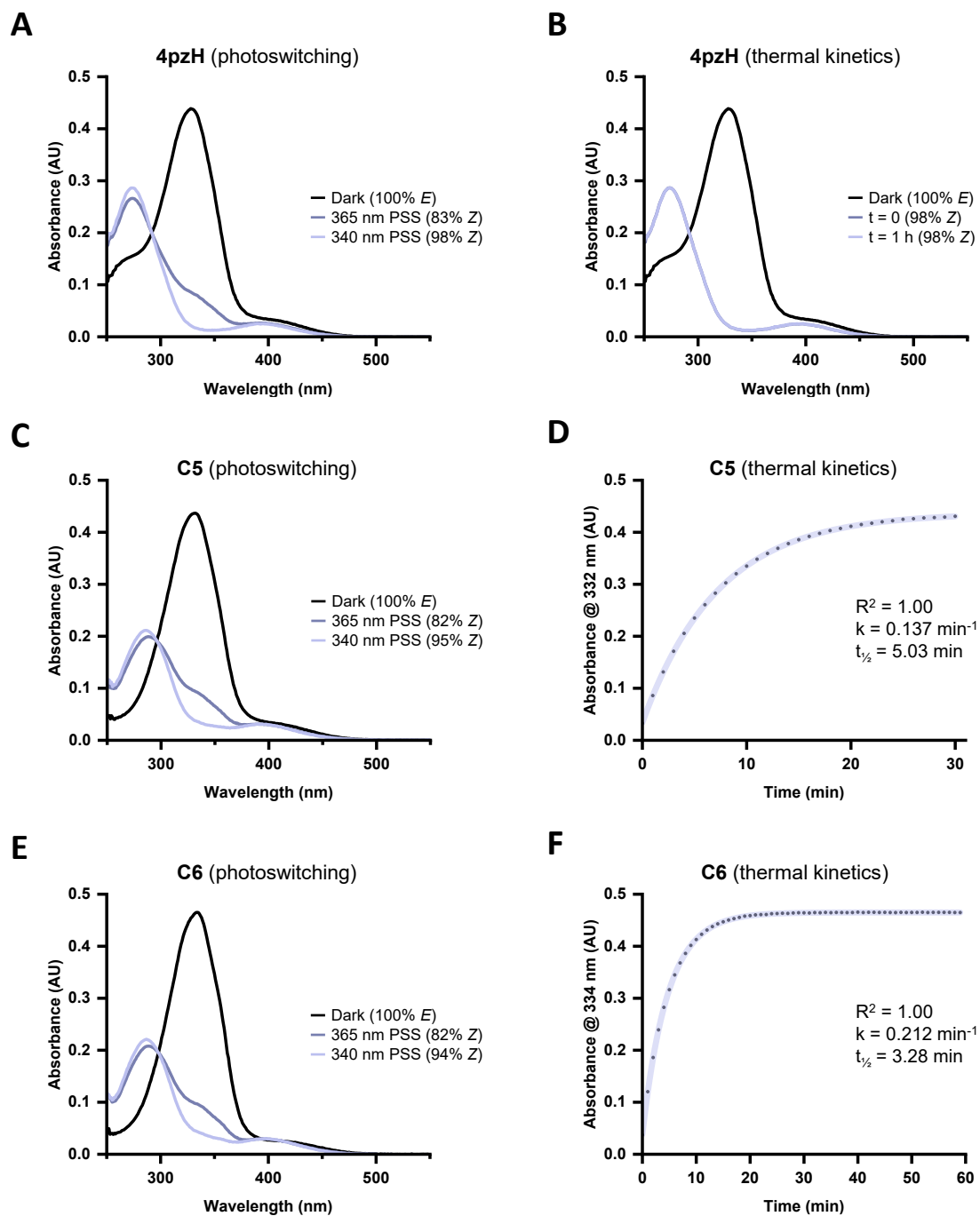
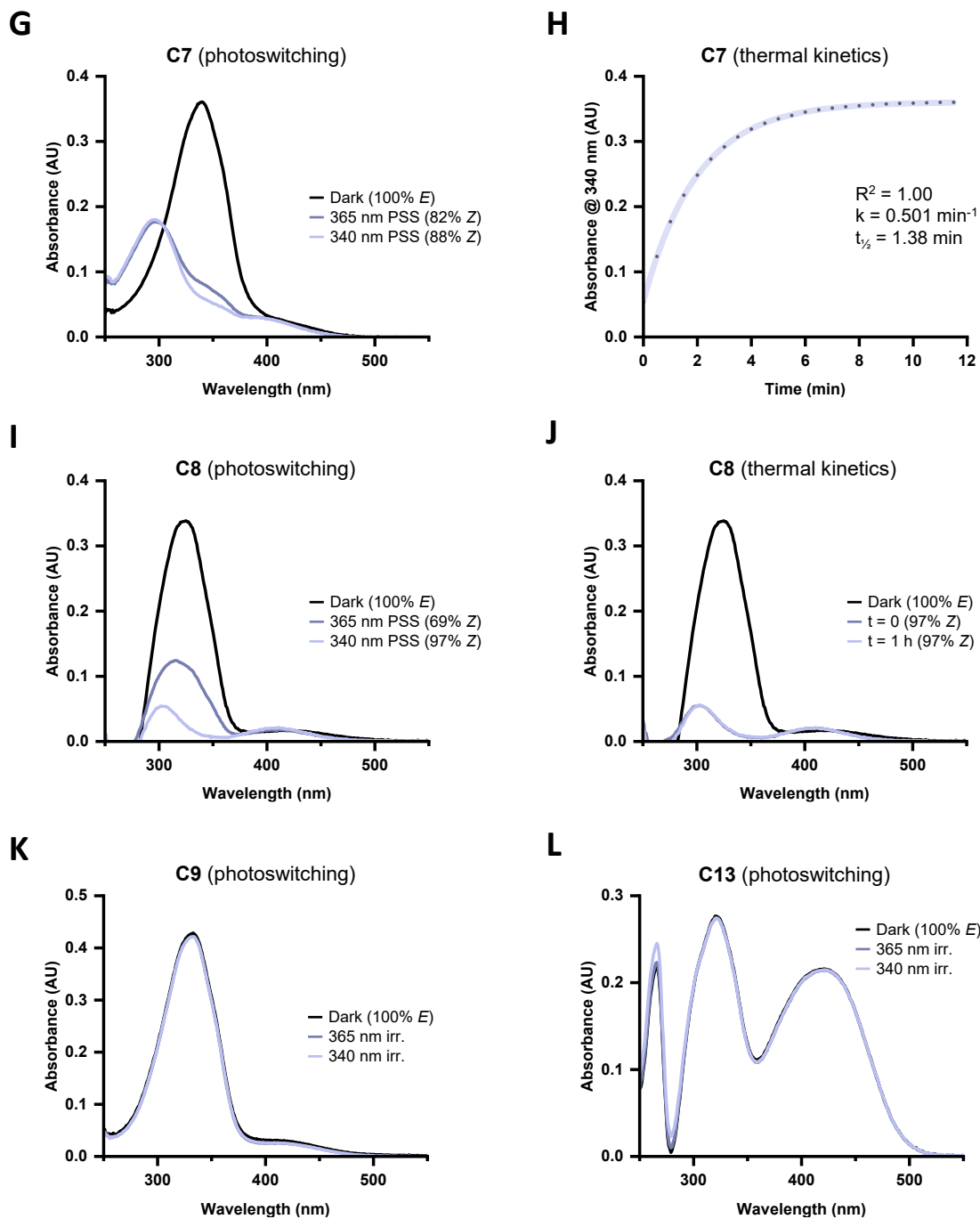


**Figure A6. Control docking study against CDK2.** Benchmarking of MOE against CDK2 by docking **C1** into CDK2 (protein receptor from PDB ID 6Q3C).<sup>123</sup> X-ray pose shown in purple sticks and docking pose shown in cyan sticks (an almost perfect recapitulation is observed).

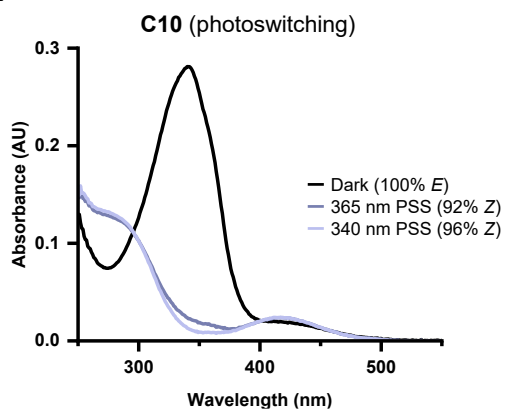
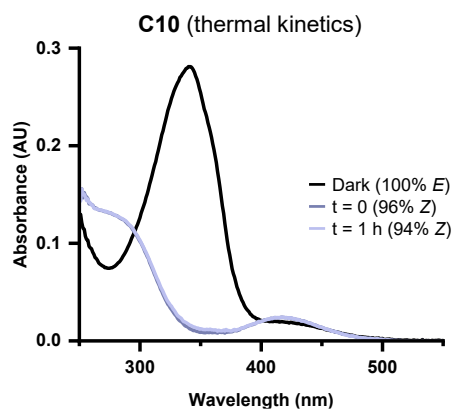
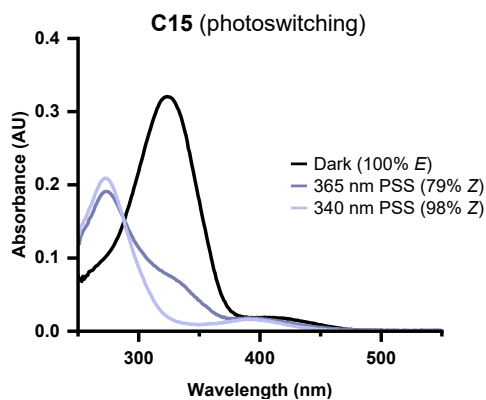
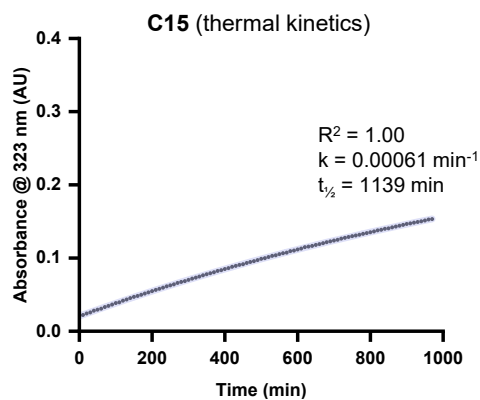
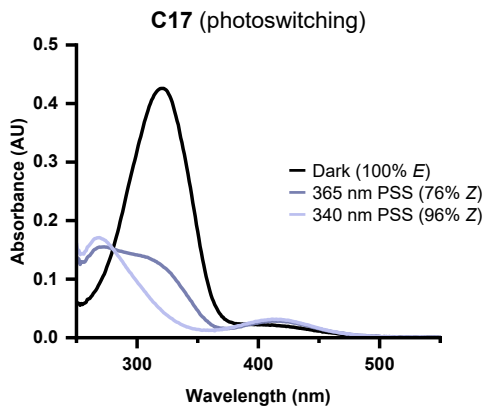
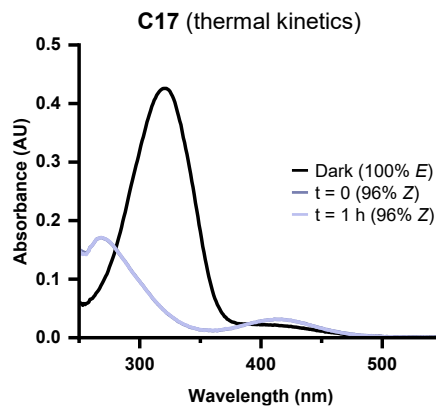
### 7.2.3. Photochemical characterisation of key series C compounds



(figure continued on next page)



(figure continued on next page)

**M****N****O****P****Q****R**

(figure continued on next page)

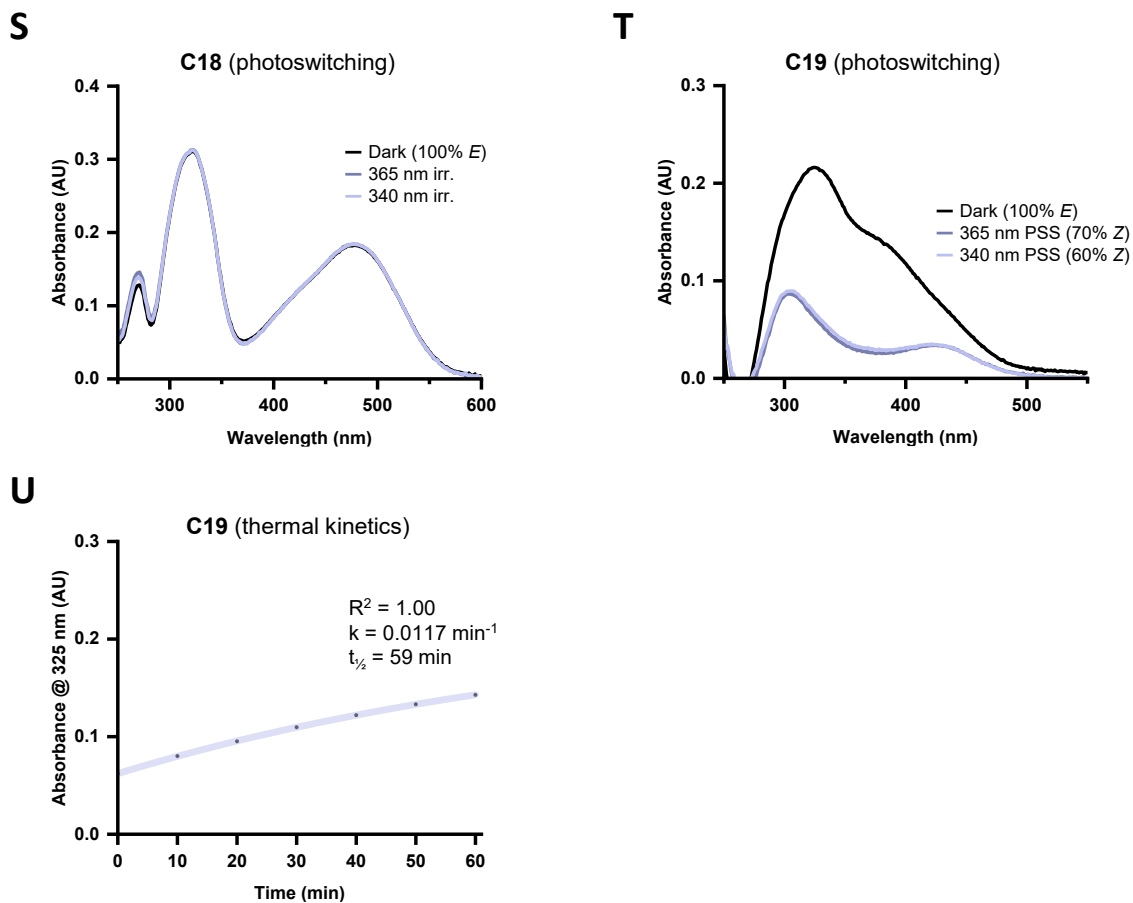


**Figure A7. Photochemical characterisation data of selected series C compounds.** Photoswitching and thermal kinetics were determined by UV–Vis spectroscopy at 20 °C and at 20  $\mu\text{M}$  in 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 10 w/v% glycerol, 0.05 v/v% Tween-20, 1% DMSO.



#### 7.2.4. Biological assays on CDK2

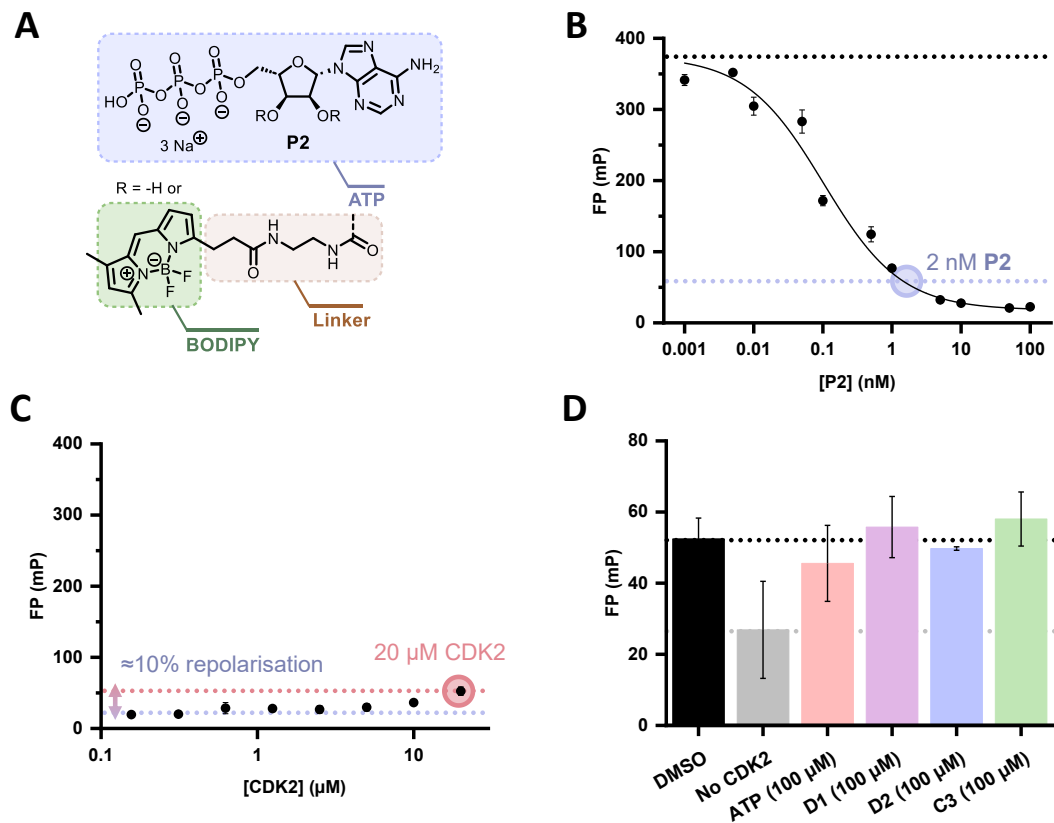


**Figure A8. FP assay on CDK2 using the alternative probe P2.** Error bars represent the standard deviation of technical triplicates. (A) Structure of **P2**. The BODIPY-tag is either attached to the 2- or 3-position of the ribose unit. (B) Titration of **P2** (0.001–100 nM) in assay buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.05% TWEEN20, 1% DMSO). (C) Titration of CDK2 (0.16–20 μM) in the presence of 2 nM **P2**. (D) Competition assay with ATP, **D1**, **D2** and **C3** tested at 100 μM against CDK2/**P2** (20 μM/2 nM).

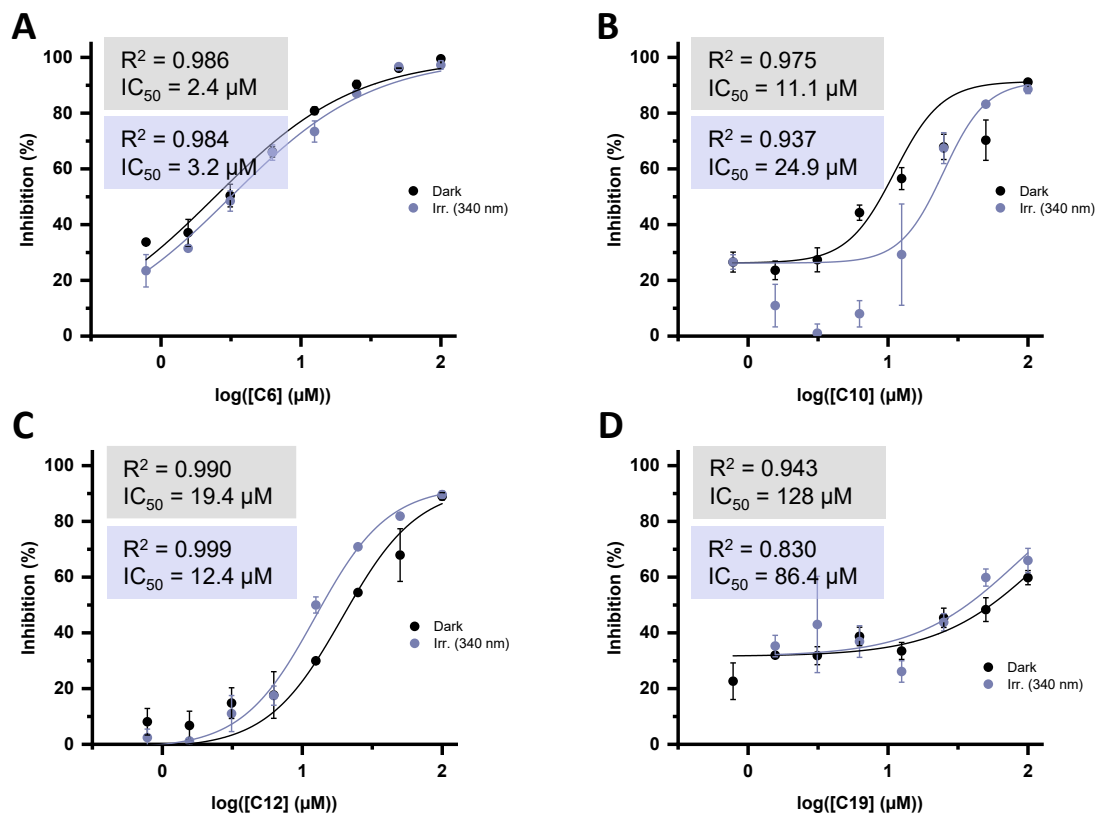


**Figure A9.** ADP-Glo™ kinase assay dose-response curves of selected compounds against CDK2/A2. The compounds were tested independently in the dark and after photoirradiation with 340 nm light. Error bars represent the standard deviation of technical triplicates. (A) Test of **C6**. (B) Test of **C10**. (C) Test of **C12**. (D) Test of **C19**.