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Reactivities of *N*-Nitrosamines against Common Reagents and Reaction Conditions

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ABSTRACT: The knowledge of the reactivity of *N*-nitrosamines (NSAs) with common organic reagents in synthesis is essential in determining their presence in pharmaceutical products, if formed and retained during synthesis. In this study, we carried out a comprehensive survey of the Reaxys database for all reactions in which the NSA functional group is consumed. Very different reactivities for different classes of NSAs, e.g., *N*,*N*-dialkylnitrosamines and *N*,*N*-diphenylnitrosamine, were identified, suggesting substrates which should be included in any future reactivity screening. A classification of NSAs based on their reactivities, and corresponding reagents and transformations, was drawn up based on the data. Furthermore, the survey identified missing areas in the reported reactivities of NSAs with different reagents. This led to an experimental reactivity screening of 8 commercial NSAs with common synthetic reagents in the Mirabilis tool for purge assessment. The results showed $Na_2S_2O_4$ in 1 M aqueous NaOH at 50 °C to be highly effective at destroying NSAs without damaging other organic compounds.

KEYWORDS: *N-nitrosamines, reactivity, cheminformatics, impurity*

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■ INTRODUCTION

The presence of *N*-nitrosamines (NSAs) in pharmaceutical products has gained center stage from a regulatory perspective with the discovery of *N*-nitrosodimethylamine (NDMA) in valsartan and in other classes of drugs such as ranitidine.[1] Updated guidance from health authorities required NSA risk assessments for all synthetic drug products on the market and, if required, to carry out confirmatory testing and make changes to the product manufacture or control strategy.[2,3] The requirement also applies to biological products and medicines and marketing applications for new products. Thus, it is important for the chemistry community to develop complete understanding of how NSAs can be generated during active pharmaceutical ingredient (API) synthesis[4−6] and how NSAs may be entirely removed during synthetic processes without affecting the final products. These are essential to help the pharmaceutical industry develop robust risk assessment processes and control strategies to ensure the safety of patients.

In this context, the reactivity of NSAs with common chemical reagents in synthetic reactions is part of the foundation of risk assessment and control strategies. Despite their long history,[7] few quantitative measurements of reactivity, i.e., rate constants, are known and reactions of NSAs are few in the literature. Recent reviews by Swager and Borths showed that NSAs can undergo a small number of reactions under harsh conditions, e.g., strong acids, lithium bases, Grignard reagents, hydrogenation with Raney-Ni or 10% Pd/C, and metal reductants (Scheme 1).[8,9] Milder reagents and conditions, such as thiourea dioxide (TDO)/NaOH/50 °C or $Na_2S_2O_4$/NaOH,[10,11] are fewer in number with a limited reported substrate scope. This scarcity of data is partially due to the hazards associated with NSAs, which necessitates stringent safety protocols and dissuades extensive studies by synthetic chemists.

In this article, we report our complete survey of reported reactions of NSAs using a cheminformatics and data science-based approach. The data highlighted the gaps in our knowledge of the reactions of NSAs and biased data toward dialkyl NSAs due to their role in food and drug contamination. This led to an experimental campaign to collect reactivity data of a set of 8 different NSAs with focus on dialkyl NSAs with common reagents and workup/purification conditions in organic synthesis, where both reactions and nonreactions are important in evaluating purge factors of NSAs in chemical syntheses.[12] These highly valuable reaction data will inform medicinal and process chemists on the risks and mitigation strategies in drug design and development.

■ RESULTS AND DISCUSSION

Reactions of NSAs in the Literature. *Data Collection and Curation.* A search of all reactions in Reaxys database containing NMDA as a substructure of the starting material resulted in 15,163 reactions, which are exported in XML format (Figure 1a). For each reaction, SMILES strings for reactants and products, and reaction conditions, which included solvents and catalysts, were extracted with a Python

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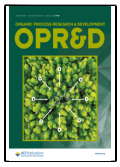

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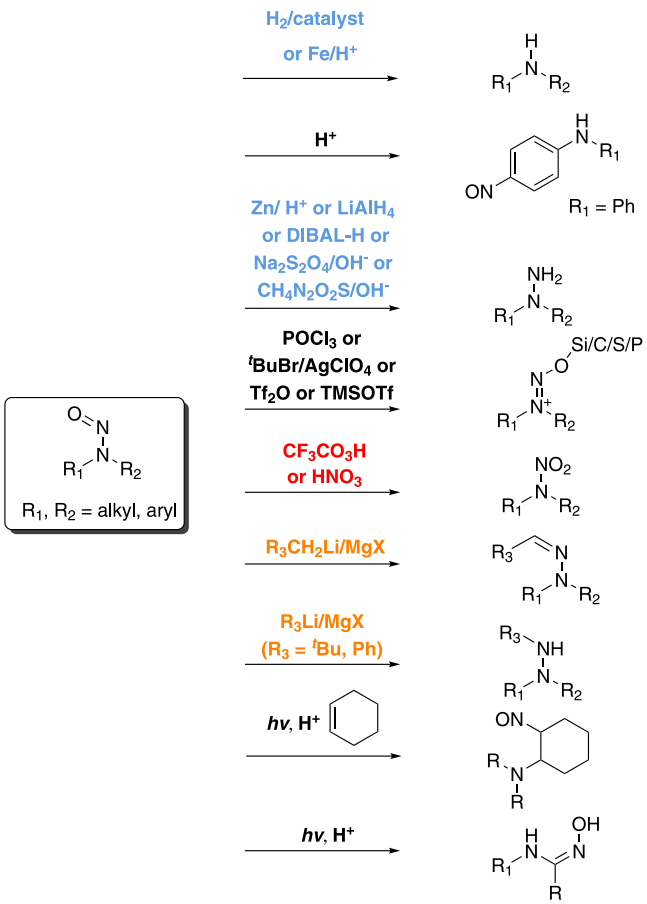
Scheme 1. Classes of Reactions of Nitrosamines Summarized in Previous Reviews (Red for Oxidative Conditions, Blue for Reductive Conditions, and Orange for Organometallic Reagents)[8,9]



script. The Python package ***rdkit*** was used to convert MDL Molfiles in the exported XML file to SMILES strings. This resulted in 11,413 reactions with extractable information. After this, multistep reactions were removed, leaving 11,087 single-step reactions. In order to perform functional group analysis and reaction classification at the later stages, the Reaction Decoder Tool (RDT)[13] was employed to perform atom−atom mapping (AAM) based on the computed reaction SMILES strings, giving 11,069 reactions. AAM is essential for reaction center generation,[14−16] which will underpin automated processing of the collected reactions. The RDT was reported to achieve 76% accuracy in mapping reactions in a curated data set containing a mixture of balanced and unbalanced reactions.[17] Against the unbalanced reactions from Reaxys, a 99.8% conversion rate was obtained due to the higher data quality of these curated reactions. AAM was followed by application of ReactionCode (version 1.2.2) software to encode the reactions into ReactionCodes. ReactionCodes are a layered string-based language which defines a reaction center as the atoms and bonds which change in a reaction, and the reaction can be portrayed at various depths.[18] The depth was evaluated (Figure 1b) and a depth 1 core was found to be sufficient to capture the changes to the NSA functional group. A total of 5486 reactions successfully underwent these transformations. Reactions which were not successfully transformed were often not balanced, taking into account the recorded reagents, or included side products in the reaction SMILES strings, which also led to balancing problems. An analysis of these discarded reactions showed similar reagents to those carried forward (Supporting Information, Section S1.3), suggesting that few transformations of NSAs were lost at this stage. The only significant class of the reaction which was removed at this stage are oxidation reactions (e.g., with H_2O_2 90%), which have been well-covered in previous reviews.[8,9] The correctly assigned reaction centers were analyzed, and reactions in which the *N*-nitroso substructure was not consumed were removed, leaving 2327 reactions (Figure 1c). Duplicated reactions, based on starting materials, products and reagent/conditions, were removed, leaving a data set of 1080 unique reactions of NSAs. Finally, manual classification of these reactions was performed, and 890 reactions were found to have adequate information to determine the reaction class. These reactions form the ***NSA_data set***.

Analysis of Literature Reported Reactions of NSAs. An analysis of the functional groups of the NSA starting materials is summarized in Figure 2a,b. While the regulatory important dialkyl-NSAs are significantly represented (116 reactions), there are many other classes of NSAs in the ***NSA_data set***, with alkyl,phenyl-NSAs being the most abundant (phenyl stands for any aryl groups). Thus, the data set represents a wide range of NSAs and their reactivity. Figure 2d shows the grouped transformations and their reactions with the NSAs in ***NSA_data set***. Many of these classes of NSAs have electron-withdrawing functional groups next to the N−N═O group, which enables class-specific reactions such as formation of diazonium salts with bases (32 reactions with alkyl,amide-NSAs, 16 reactions with alkyl,carbamate-NSAs, 24 reactions with alkyl,guanidine-NSAs, 39 reactions with alkyl,sulfone-NSAs and 62 reactions with alkyl,urea-NSAs (Supporting Information, Section S2.3, Figures S14, S17, S18, S20, and S21). While these are not strictly NSAs and have a different potential carcinogenic mechanism,[19] they are referred to as NSAs in the article for simplicity. Most of the reported reactions of *N*,*N*-diphenylnitrosamine are Fischer−Hepp reactions (migration of the nitroso group to the phenyl ring) under strongly acidic conditions. In addition, there are a large number of literature precedents that employ reducing conditions including dissolving metal and hydride reagents. These classes of reagents are linked with reduction to amines and hydrazines and diazonium formation (most commonly done with alkyl,urea-NSAs)[20−22] (Figure 2d). Although the documented reagents are reasonably diverse, the transformations that they achieve are limited. In-depth analysis of the formation of diazonium salts from NSAs under basic conditions showed that this transformation does not always require an electron-withdrawing group next to the −N−N═O functional group, e.g., Reaxys reaction ID = 240,906 (see Supporting Information, Section S2.4 and Scheme S4). This methodical and complete survey of the reported reactions of NSAs also identified a small number of reactions which were not covered in previous reviews by Swager and Borths, which focused on NSAs with alkyl and aryl substituents and did not include electrophilic NSAs:[8,9] reactions with transition-metal catalyst, arenes, N-nucleophiles, and stabilized carbanions. Examples of these reactions are listed in Scheme 2.

Reclassification of NSAs Based on their Reactivity in the Literature. Based on the reactivity described in Figure 2, the classes of NSAs were rearranged to reflect their similar reactivity with common reagents in the ***NSA_data set***. The new classification and process are described in Figure 3. This

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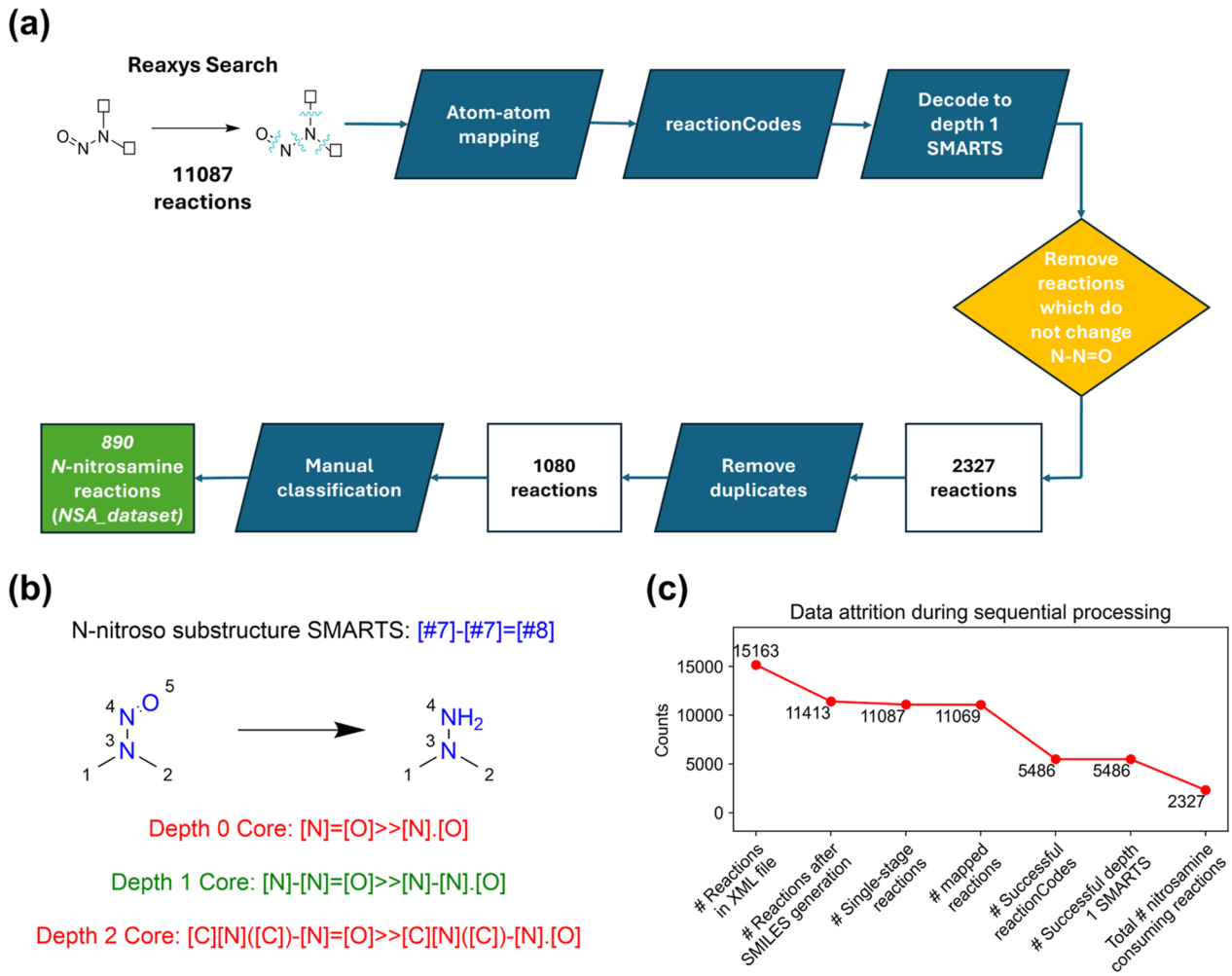

Figure 1. (a) Workflow for the collection and curation of the NSA reaction data set from Reaxys; (b) ReactionCode and SMARTS string at different depth cores for identifying reactions in which the NSA functional group is consumed; and (c) graph to show the data attrition during data collection.

was achieved by combining the nitrosamines into 8 classes (**C1−8**, Figure 4), reagents/conditions into 21 classes, and transformations into 10 classes. The streamlining of classification resulted in a loss of 251 reactions which belong to classes with fewer examples (<10 nitrosamines or <3 transformations) and a small number of reactions being included in more than 1 class of the substrate. On the other hand, it provides a straightforward identification of the most common reactions for each type of NSA through the use of Figure 4 at the cost of a small number of much less common transformations. The reaction classes which were reported by Swager and Borths are highlighted in red numbers[8,9] and accounted for 19 entries out of 43 in Figure 4.

Importantly, the analysis of the reactions in ***NSA_data set*** showed that reported reactions in the literature do not distribute evenly among different classes of NSA, reagents, and transformations, and there are many gaps in-between, which one cannot confidently conclude whether they are due to lack of activity or lack of prior investigation (Figure 2a). The reported acidic and basic conditions are also typically harsh. Examples include (i) strong acids at high concentrations required for Fischer−Hepp reactions[23] and (ii) strong bases for diazonium formation with NSAs which do not have an adjacent urea/carbamate functional group,[24] H_2/Raney-Ni, Zn/HCl, Zn/AcOH, and $LiAlH_4$ for reduction of NSAs to hydrazines and amines.[25−28] The most promising, and potentially general reaction, for destroying trace contamination of NSAs in APIs are reductions with inorganic S-based reductants, e.g., $Na_2S_2O_4$ and TDO (acute toxicity by inhalation).[10,11] Nevertheless, the reported substrate scopes with these reagents were somewhat limited: 6 NSAs with $Na_2S_2O_4$ (dibenzyl-NSA and 5 aromatic-NSAs) and 23 NSAs with TDO (dibenzyl-NSA and 22 NSAs containing an aryl-NSA substructure). Finally, they did not include any alkyl,alkyl-NSA, which is the most important class of NSA from a regulatory point of view. Thus, there is a clear need for a systematic investigation on reactivity of a wide range of NSAs with common reagents in organic synthesis and workup/purification.

Reactivity of NSAs with Common Reagents in Organic Reactions and Workup. Eight commercial NSAs were sourced from common chemical suppliers for this study (Figure 5a). These are mainly the alkyl,alkyl-NSAs which are most important from a regulatory standpoint, with one alkyl,carbamate-NSA (**N6**) and diphenyl-NSA (**N4**). Three of these NSAs have hydroxyl groups at the β- or δ-positions. These substrates represent a different distribution of NSAs compared to that of the ***NSA_data set***, with a better

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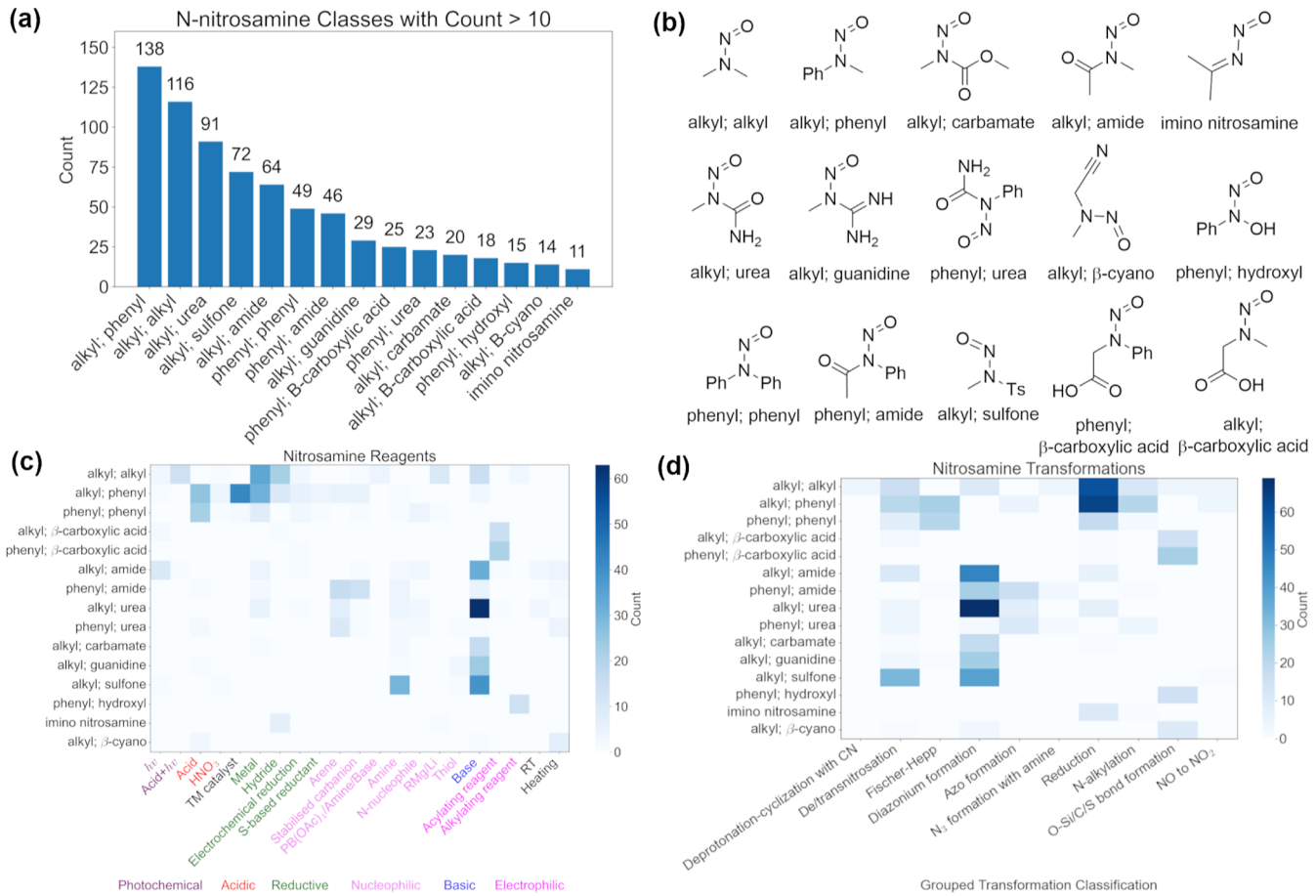

Figure 2. Literature reaction data analysis: (a) distribution of different classes of NSAs as starting materials in ***NSA_data set***, showing classes with 10 or more compounds; (b) representative structures of different classes of NSAs; (c) reaction counts (>3) between different reagents and classes of NSAs; and (d) transformation counts (>3) for different classes of NSAs.

representation of the electron-rich NSAs. Electron-rich NSAs are associated with carcinogenicity in the literature,[29] although hydroxyl groups at the *β*-position are considered to reduce carcinogenic potency.[19] A set of reaction conditions was initially selected based on those employed in the Mirabilis tool to estimate purge factor for contaminants in final APIs (Table 1).[12] These common conditions were carefully examined and removed based on 4 criteria: (i) reaction conditions which are already well-represented in the ***NSA_data set*** (>5 reactions) or prior reviews,[8,9] i.e., Grignard reagents, $LiAlH_4$, NaOEt/EtOH, *n*-BuLi, and H_2/Raney-Ni; (ii) reaction conditions for which rate constants have been reported, i.e., H_2/10% Pd/C and H_2/5% Pd/C; (iii) reaction conditions which are too harsh and indiscriminate for late-stage synthesis where many sensitive functional groups are present, i.e., $NaClO_4$, BH_3, and O_3; and (iv) reaction conditions that are not commonly used in the later stage of API syntheses, e.g., TEMPO, which has only 8 examples in *Org. Process Res. Dev.* between 2010 and 2020 based on Reaxys. Given the potential chemoselectivity of sulfur-based reductants, $Na_2S_2O_4$ and Na_2SO_3 were included, with and without NaOH and heating, in the reactivity screening in our study (Figure 5b).

Initial reactivity screening was performed in flow, using a Vapourtec flow reactor with static mixers, in order to minimize and contain the inventory of hazardous NSAs. However, most of the reaction conditions are biphasic with a long reaction time, and continuous and efficient mixing is required to ensure fast and reproducible reactions. Thus, subsequent reactions, which are summarized in Figure 5b, were performed in the batch mode. The reaction mixtures were quenched (see Supporting Information, Section S3.3) and analyzed with HPLC, GC−MS/MS, and LC−MS. The reaction conversions were calculated based on the consumption of the NSAs. The solvents were selected based on common solvents for the desired reactions, their miscibility, and solubility of the NSAs in them.

In some cases, conversion was reported in Figure 5b and no product was found by either GC−MS/MS or LC−MS/MS (Figure 5c). For example, in row 7 (2 M HCl/EtOH, 50 °C, 16 h) and 14 [$Na_2S_2O_4$ (1 M NaOH/EtOH), 50 °C, 16 h], the products of **N1**, **N2**, and **N8** were not observed. Where the products of higher molecular weight NSAs were successfully captured and assigned based on GC−MS/MS and LC−MS data, the unobserved products of the lower molecular weight NSAs were accordingly assumed. In some cases, particularly with **N1**, significant conversion was achieved under reaction conditions 7, 9, 10, 14, and 17, but no product was observed. This is attributed to the limitation of our analysis regime to capture and characterize volatile products and the loss of some products due to aqueous workup (described in Supporting Information, Section S3.2.3). Substrate **N6** displayed activity under all reaction conditions, except H_2O_2, but also no detected product. Our literature analysis above suggested that it is a highly activated NSA toward formation of the reactive

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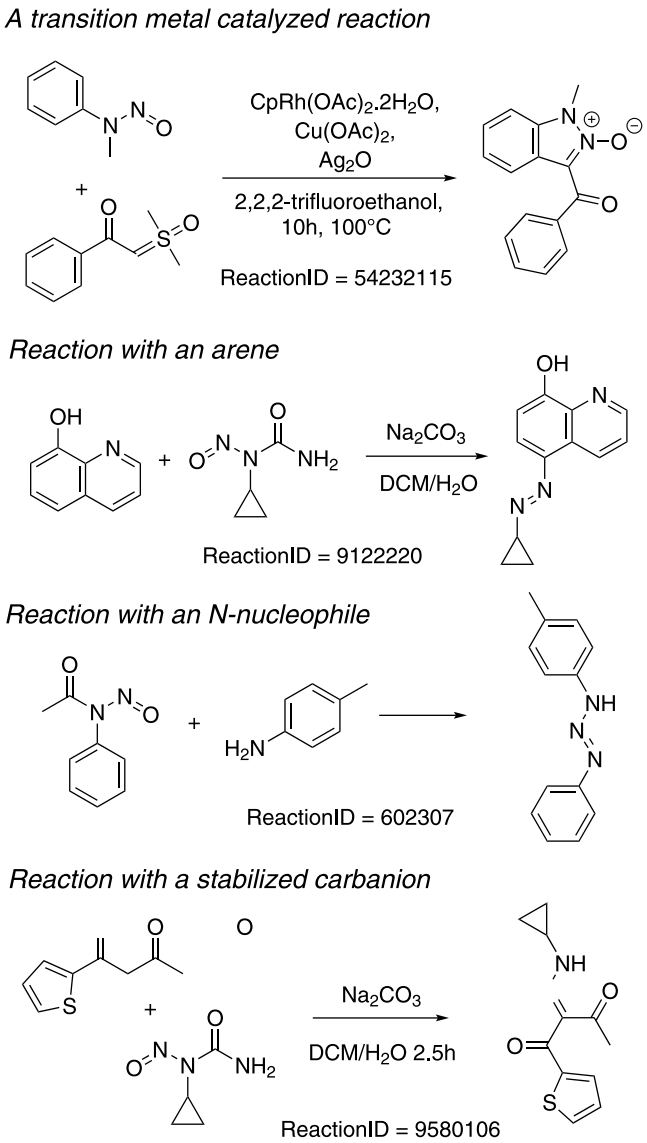
Scheme 2. Examples of Reactions of Other Classes of NSAs Not Covered by Swager and Borths and their Reaxys Reaction ID Numbers



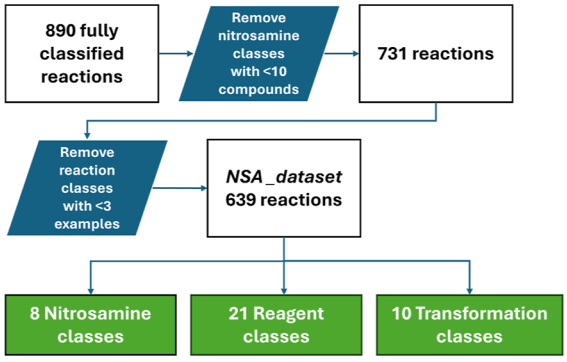


Figure 3. Workflow for reclassification and streamlining of the data set.

methyldiazonium cation, leading to products with low molecular weights and significant aqueous solubility.

Treatment of the NSAs with aqueous 2 M HCl resulted in no reaction except with **N4** and **N6**. **N6** was completely converted to the amine product, while the conversion was only moderate (37%) with **N4** after 24 h at room temperature. Gentle heating at 50 °C improved the conversion to 86%, giving the expected mixture of amine and transnitrosation products (Figure 5c). Williams and co-workers suggested that a strong nucleophile would improve the reactivity of nitrosamine under acidic conditions.[33] Thus, the solvent was changed from water to EtOH to reduce the level of solvation of the chloride anion and to improve its nuleophilicity. This led to an increase of conversion with **N4** to 100% at room temperature after 24 h. In addition, **N5** also gave 56% conversion to the amine product. Increasing the temperature to 50 °C led to reactions with all 8 NSAs, with higher reactivity observed for **N4**, **N5**, **N6**, and **N8** (Figure 4b, row 7). The more acidic reagent 33% HBr in AcOH did not show improved reactivity compared to HCl in EtOH with **N4**, **N5,** and **N8** at room temperature, and little product was observed with the rest of the NSAs (row 3).

Oxidative conditions were heavily under-represented in the ***NSA_data set***, with only 12 reported reactions using reagents such as H_2O_2 with Ac_2O, organic peroxide, and $NaClO_4$. Thus, two simple oxidants were included in the reactivity screening: aqueous 2 M H_2O_2 and AcOOH in EtOH. The latter is much more reactive with organic compounds,[38] with a mechanism based on transfer of an oxygen atom instead of a hydroxyl radical or a peroxide anion.[39] While little reactivity was observed with aqueous 2 M H_2O_2 at room temperature with all NSAs (Figure 5b, row 1). AcOOH showed near complete conversion with **N4** and **N6** after 24 h, and some conversion with other NSAs (Figure 5b, row 2). Substrates **N7** and **N8**, which contains oxidizable primary alcohol functional groups showed no reaction with aqueous 2 M H_2O_2 and low conversions with AcOOH, 14% and 12%, respectively. These results are consistent with the lack of report of noncatalytic oxidation of alcohols with H_2O_2 or AcOOH under ambient conditions in the literature. Heating was not applied given the potential hazards of oxidants at a high temperature. With **N4** as the substrate, AcOOH gave the expected *N*-nitroamine (O, Figure 5c) product, but only amine was observed with H_2O_2. This is attributed to the reagent solution being slightly acidic and therefore invoking denitrosation. **N6** gave the amine product with both peroxide reagents, highlighting its poor stability in acidic conditions (Figure 5c).

Strong reductants such as $LiAlH_4$ and Zn/HCl are well documented as effective reagents for reducing the NSA group to hydrazines and amines.[8,9] However, these are not mild reagents and may not be compatible with a wide range of functional groups at a late stage of synthesis. Thus, we included reaction screening for **N1−8** with $NaBH_4$ and DIBAL-H in their usual solvents. $NaBH_4$ showed no reactivity with most NSAs, low conversion with **N4,** and complete reduction of **N6** (Figure 5b, row 8). $NaBH_4$ is a mild reductant and normally does not reduce a carbamate functional group. The electron-withdrawing group −N═O manifests increased reactivity of the carbamate group, consistent with the observed disappearance of the C═O stretching IR signal when **N6** was subjected to $NaBH_4$ (see Supporting Information, Section S8). Hence, no product was observed for **N6** by GC−MS/MS with $NaBH_4$ and the stronger reductant DIBAL-H. DIBAL-H was effective to react with all NSAs, giving high conversions after 30 min at room temperature (Figure 5b, rows 9 and 10), providing the corresponding hydrazine and amine in most cases. The lack of detected product for **N1**, despite evidence of consumption by

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Transformation	Reagent	Nitrosamine class							
		C1	C2	C3	C4	C5	C6	C7	C8
De/transnitrosation	Acid	*3*	*3*						
	Acid + *hv*						*13*		
	Amine			34					
	N -nucleophile		*4*						
	Stabilised carbanion	4							
	TM catalyst	9							
	Thiol			5					
	hv			11					
Deprotonation/cyclization with CN	Base						*4*		
Diazonium formation	Amine			5					
	Base			179			10		
	Heating			5					
	N -nucleophile			4					
	Stabilised carbanion			16					
	RT			6					
Fischer-Hepp reaction	Acid	*23*	*19*						
N -alkylation	Heating			5					
	RMg/Li						*10*		
	TM catalyst	20							
N_3 formation	Amine			5					
	$Pb(OAc)_4$ /Amine/base						4		
Oxidation NO to NO_2	HNO_3	4							
O-Si/C/S bond formation	Acylating reagent				36				
	Alkylating reagent								14
	Heating					7			
Reduction	Electrochemical reduction	*6*	*3*						
	Hydride	*10*					*22*	7	
	Metal	*31*	*8*	10			*34*		
	S -based reductant	*4*	*1*						
	TM catalyst	*13*	*2*						
Diazo formation	Arene	5		33					

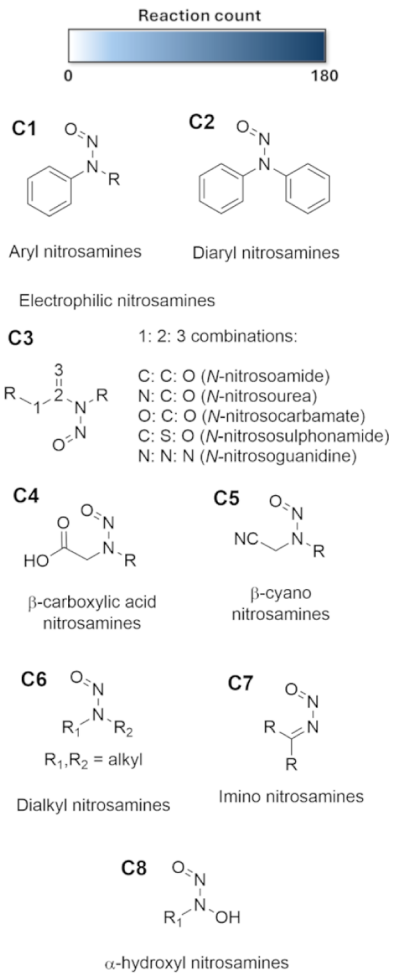

Figure 4. Classification table for major reactions of NSAs from Reaxys. The numbers represent the reaction counts in each case, with ***red***, italic numbers representing reactions which were listed in previous reviews.[8,9] Reaction counts lower than 3 were included for class **C2** due to the lower total count for this class, these highlight the similar reactivity between **C1** and **C2**.

HPLC, was attributed to the volatility of diethylamine and aqueous solubility of other products used during the workup. Interestingly, reduction of **N8** with DIBAL-H resulted in an unexpected product, which was tentatively assigned as **D** based on LC−MS and GC−MS data (Supporting Information, Section S9.5).

The sulfur-based reductants were included in addition to the original Mirabilis conditions (Table 1), due to their mild conditions and lack of activity with the most common organic functional groups. The aqueous $Na_2S_2O_4$ reagent is of particular interest, due to its tunable redox potential, depending on the pH of the solution (eq 1).[40−42] The standard redox potential E^0 can vary from 0.07 to −1.12 V at pH = 0 to 14.

$$S_2O_4^{2-} + 2H_2O \rightarrow 2HSO_3^- + 2H^+ + 2e^- \quad (1)$$

Consequently, the NSAs were treated with $Na_2S_2O_4$ under both neutral and basic conditions (NaOH 0.1, 1 M, and 20%). The results are summarized in Figure 5b, row 11−17. Changing from $Na_2S_2O_4$ to aqueous Na_2SO_3 (Figure 5b, row 18) led to no conversion with all NSAs but **N4** (22% after 24 h) and **N6** (100% conversion). The corresponding amines and hydrazines were observed as expected products (Figure 5c). To rule out the reactions mediated by basic conditions alone, all 8 NSAs were treated with aqueous 2 M NaOH and 21% NaOEt/EtOH (Figure 5b, rows 19 and 20). These resulted in no significant reaction with any NSAs other than **N6**. Compound **N6** contains an electron-withdrawing group next to the −N−N═O functional group, which activates it to produce diazonium salts under basic conditions.[43] These control experiments confirm the treatment of NSAs with $Na_2S_2O_4$ in aqueous NaOH 1 M at 50 °C as an effective method for removing NSA contamination. The only reported reactions of $Na_2S_2O_4$ with organic compounds without a metal catalyst are reduction of an aromatic nitro or nitroso group to an amine,[44,45] conversion of alkyl halides to sodium sulfonates,[46] and reductive cleavage of N−S bonds,[47] suggesting excellent functional group tolerance for this treatment. Base-sensitive functional groups, i.e., incompatible with NaOH, such as aldehydes and ketones, are also uncommon in APIs. It is worth noting that the products of the reactions between NSAs and $Na_2S_2O_4$ are amines and hydrazines, which have their own hazards and toxicity issues, albeit of lesser concerns than those of NSAs.

Finally, a general trend in reactivity was observed across the 8 NSAs. Electron-rich NSAs, such as **N1−3** (dialkyl-NSAs) and **N7** (with a hydroxyl group at the δ position), displayed the lowest reactivity, while **N4** (*N*,*N*-diphenylnitrosamine) and **N6** (electron-poor NSA with a −COOMe group on the *N* −1 position) are the most reactive. This is important when it

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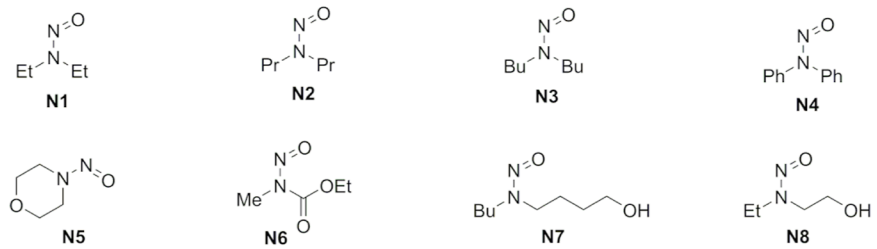
(a) Commercial NSAs in this study



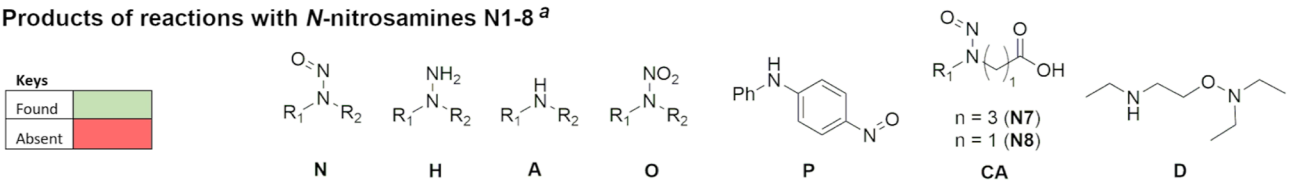
(b) Conversions of N1-8 against common reagents in organic syntheses

Conversion (%) 0 100

No.	Class	Reagents	Solvent	Temp. (°C)	Reaction time (h)	N1	N2	N3	N4	N5	N6	N7	N8
1	Oxidant	H_2O_2 2M	H_2O/EtOH	22	24	2	-3	4	17	0	2	2	5
2	Oxidant	CH_3CO_3H	EtOH	22	24	8	8	7	95	14	101	14	12
3	Acid	HBr	AcOH/EtOH	22	24	6	7	13	42	25	85	2	26
4	Acid	HCl 2M	H_2O/EtOH	22	24	2	-5	6	37	-1	101	-12	6
5	Acid	HCl 2M	H_2O/EtOH	50	16	1	-4	13	86	5	101	-14	6
6	Acid	HCl 2M	EtOH	22	24	-2	-2	3	100	56	101	-6	15
7	Acid	HCl 2M	EtOH	50	16	71	54	56	99	98	101	52	100
8	Reductant	$NaBH_4$ 16 eq	EtOH	22	24	3	0	3	23	-2	101	5	0
9	Reductant	DiBAL-H 5 eq.	THF/Hexane	22	0.5	72	66	61	76	101	100	51	69
10	Reductant	DiBAL-H 5 eq.	THF/Hexane	22	1	85	86	75	85	101	100	90	101
11	Reductant	$Na_2S_2O_4$	H_2O/EtOH	22	24	9	3	9	87	25	63	-5	5
12	Reductant	$Na_2S_2O_4$ + NaOH 0.1M	H_2O/EtOH	22	24	-4	2	9	87	32	100	-9	2
13	Reductant	$Na_2S_2O_4$ + NaOH 0.1M	H_2O/EtOH	50	16	18	7	11	99	67	101	6	40
14	Reductant	$Na_2S_2O_4$ + NaOH 1M	H_2O/EtOH	50	16	78	57	56	99	99	101	87	99
15	Reductant	$Na_2S_2O_4$ + NaOH 1M	H_2O/EtOH	50	3	18	1	11	99	75	101	14	32
16	Reductant	$Na_2S_2O_4$ + NaOH 1M	H_2O/EtOH	50	1	18	2	12	97	44	101	-4	17
17	Reductant	$Na_2S_2O_4$ + 20% NaOH	H_2O/EtOH	50	16	106	104	104	95	96	100	107	101
18	Reductant	Na_2SO_3	H_2O/EtOH	22	24	4	-2	6	22	-2	102	-6	3
19	Reductant	Na_2SO_3 + 1M NaOH	H_2O/EtOH	50	16	2	1	7	75	1	101	-3	-1
20	Base	NaOH 2M	H_2O/EtOH	22	24	0	-5	3	1	0	101	6	6
21	Base	NaOEt 21% w/v	EtOH	22	24	-7	1	6	-3	3	101	2	7

Reaction conversions were determined by HPLC

(c) Products of reactions with *N*-nitrosamines N1-8 [a]



No.	Reagent	Temp. (°C)	N1				N2				N3				N4					N5			N6		N7				N8				
			N	H	A	O	N	H	A	O	N	H	A	O	N	H	A	P	O	N	H	A	N	A	N	H	A	CA	N	H	A	CA	D
1	H_2O_2 2M	22															b							c									
2	CH_3CO_3H/EtOH	22																						c									
3	HBr/AcOH	22																															
5	HCl 2M	50																															
7	HCl/EtOH 2M	50																				b											
8	$NaBH_4$/EtOH	22																															
10	DiBAL-H 5 eq.	22																															
11	$Na_2S_2O_4$	22																															
14	$Na_2S_2O_4$ + NaOH 1M	50																															
19	Na_2SO_3 + 1M NaOH	50																															
21	NaOEt/EtOH 21% w/v	22																															

[a]Characterised by LC-MS or GC-MS/MS; [b]Characterised by ^{1}H NMR; [c]carbamate product;

Figure 5. (a) Commercial NSAs employed in this study; (b) reaction screening results with common reagents in organic syntheses; and (c) products from reaction screening with common reagents in organic syntheses.

comes to extrapolating the reactivity of electron-rich NSAs based on the known reactivity of safer NSAs such as **N4**. The presence of an oxygen atom at the β-position to the −N−N═O group improves the reactivity of the NSA, as demonstrated with **N8** vs **N1**−**3**, and with **N5** which was the third most reactive NSA, after **N4** and **N6**. Computational investigation of the influence of this oxygen atom at the β-position is ongoing and will be reported in due course.

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Table 1. Reaction Conditions Based on the Mirabilis Tool for Reactivity Screening with Commercial NSAs

no.	reagents	solvent	reactivity screen	reason for removal	no reaction
1	BnSH		no	acute toxicity (inhalation)	
2	$BnNH_2$	isooctane	no	literature data[a]	
3	EtMgBr	diethyl ether	no	literature data[31]	
4	DBU	DCM/THF	no	overlap with NaOEt/EtOH	
5	NaOH (10%)	H_2O	yes		
6	$NaHCO_3$ sat.	H_2O	yes		yes
7	NaOEt (21%)	EtOH	yes		
8	*n*BuLi	hexane/THF	no	literature data[32]	
9	HCl 2 M		yes	literature data, limited scope[33]	
10	H_2SO_4 95%	none	no	harsh conditions	
11	H_2SO_4 2 M	H_2O	yes	literature data[33]	yes[b]
12	HBr 33%	AcOH	yes		
13	HCl	EtOH	yes		
14	$NaBH_4$	EtOH	yes		
15	$LiAlH_4$	THF	no	literature data[34]	
16	DIBAL-H	THF	yes		
17	BH_3		no	harsh conditions	
18	H_2, 10% Pd/C	EtOH, iPrOH	no	literature data[35,36]	
19	H_2, 5% Pd/C	EtOH, iPrOH	no	literature data[9]	
20	H_2, Raney Ni		no	literature data[25]	
21	$NaClO_4$		no	harsh conditions	
22	H_2O_2 30%	H_2O	yes		
23	AcOOH 40%	H_2O	yes		
24	$KMnO_4$	DCM	no	literature data[37]	
25	IBX/DMP	DCE/MeCN	yes		yes[b]
26	TEMPO	MeCN	no	less common conditions	
27	*m*CPBA	DCE/MeCN	yes		yes[b]
28	O_3	THF	no	harsh conditions	

[a]Literature reactions with BnNHR resulting in transnitrosation. [b]Reaction performed in the continuous mode using Vapourtec R-series with internal static mixers.

CONCLUSIONS

We have carried out a comprehensive analysis of literature reaction data of NSAs using cheminformatics tools. The main reactivity trends were highlighted, and the NSAs were classified based on the analysis, with common transformations and reagents for each NSA class summarized in Figure 4. Importantly, the data show that *N*,*N*-dialkyl nitrosamines are the least reactive and often only react with highly reactive reagents or under harsh reaction conditions. Based on these insights, reactivities of a diverse range of eight NSAs with common organic reagents and workup conditions were measured. These are important in assessing the residual amount of possible NSAs in medicinal products. The results showed $Na_2S_2O_4$ in aqueous NaOH 1 M at 50 °C to be highly effective at reducing NSAs to the parent amine with a limited alteration of other functional groups.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.4c00217.

Reaction data of NSAs, experimental procedures, and characterization data of reactions and products (PDF)

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Notes

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