Multiple-parameter optimisation in Drug Discovery : example of the 5-HT1B GPCR

Authors: Robert C. Glen, David R. Spring, Warren R.J.D Galloway, Gemma Liwiki.

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Abstract: Early phase drug discovery is a multi-parameter optimisation process. Finding drugable targets, discovering starting points for lead optimisation and creating novel structures with new biological properties within these constraints is challenging. As an example of a drug optimisation strategy, recent work on 5-HT1B antagonists will be described. This is put in the context of the drugability of the target, the desired physicochemical properties of the desired molecules and approaches to compound design to create high affinity, selective molecules that are optimised to have low Central Nervous System (CNS) penetration.

Introduction

The design of chemical structures for medicinal applications, particularly small molecules that are intended to be orally bioavailable, selective, have low toxicity and are efficacious is a multivariate problem. In order to optimise these properties within the constraints of the available chemistry, and also to include knowledge of what makes compounds drug-like, the medicinal chemist has to optimise the problem in multivariate physico-chemical and biological space to find an optimal solution. There are a multitude of software algorithms that can assist in this process. When combined with in-vitro assays and in-vivo assays, these can assist in the optimisation of putative drug candidates hence complementing the intellectual input of the medicinal chemist.

The process from identifying a disease target to a marketed small molecule product is complex and expensive. Drugs fail due to many factors including a lack of efficacy in the clinic, poor pharmacokinetics and toxicity. Using computational approaches early in the process can increase the chances of success. It should be noted that decisions on which small molecule candidates to develop are typically taken at an early stage in the development process; the lead becomes more and more difficult to change as investment increases, therefore to avoid costly expense, it is best to invest in early stage assessment using computational, in-vitro and in-vivo assessment, including human tissues, if available.

Before embarking on a drug discovery project, experience shows that careful consideration of the viability of the target is of the utmost importance – is it drugable? Currently most drugs typically fail due to a lack of efficacy in the clinic. This initial step is probably the most important in drug discovery – ideally, the target should be demonstrated to be of relevance in human clinical studies. Unfortunately, it is still the case that a major reason for failure of drug discovery projects which are based on non-phenotypic endpoints (e.g. molecular biology hypotheses which identify probable novel targets) or in-vivo animal models (which show species differences) are later shown to be irrelevant to the human disease process. In addition, we should consider:

- Is there a medical need
  - Patient population, current therapies ineffective, toxicity, cost
- Is an effective therapy already availability
  - First to market is always best
  - Is the current therapy out of patent, and therefore inexpensive

- Can you be first to market?
  - Drug discovery is very competitive (how many statins are there? I've lost count)
- Is it commercially viable
  - Investment requires returns!

Of course, as a valid academic investigation, we may simply be very interested in the chemistry and biology of the target or system, which may lead to new and exciting therapeutic opportunities that are discovered by the use of novel pharmacological probes of disease processes.

The basic scientific problem of course is optimising the effect of the putative drug (be it e.g. binding affinity, allosteric effects, interference with specific biochemical pathways etc.) and obtaining the desired efficacy at a functional level at the desired target in-vivo, followed by (or best practice is in parallel with) consideration of selectivity and ADME (Absorption, Distribution, Metabolism and Excretion). Toxicity must be considered early on (as this is another major point of failure in clinical studies), generally from consideration of structural alerts or undesirable physico-chemical properties coupled with targeted in-vitro testing (e.g. Ames and hERG assays to test for mutagenicity and cardiac toxicity). Getting a starting point for synthesis, particularly of course for very new targets, is often challenging, but if a reasonable starting point is available (e.g. from a natural hormone, screening or e.g. off target effects of existing drugs) then the process of structure optimisation can begin immediately if suitable validated assays are available.

Example Project: 5HT-1B

As an example, we are currently working to optimise a series of 5-HT1B antagonists for the treatment of Pulmonary Arterial Hypertension (PAH). 5-HT1B is a G-protein Coupled Receptor (GPCR) of family A, which binds the natural hormone 5-hydroxytryptamine (5-HT). PAH is characterized by vasoconstriction and vascular remodelling of pulmonary arteries and this leads to a progressive increase in pulmonary arterial pressure and right ventricular failure, leading to death, sometimes at an early age. Numerous studies have demonstrated that 5-HT is involved with the disease process of PAH. 5-HT (also called serotonin) is a potent vasoconstrictor in pulmonary vessels (including the lung) and also promotes proliferation of smooth muscle cells and hypertrophy. Patients with PAH have increased plasma levels of 5-HT, resulting from reduced 5-HT re-uptake (hence the interest in the Serotonin Transporter as a drug target for PAH). Importantly, the appetite-supressing drugs fenfluramine and aminorex - which enhance 5-HT release by platelets while inhibiting 5-HT uptake are seen to contribute to pulmonary hypertension. Mechanistically, 5-HT released by platelet accumulation in blood vessels of the lung and the coronary arteries induces vasoconstriction and proliferation, contributing to symptoms. The receptor target we are interested in, 5-HT1B is expressed in the lung and the coronary arteries and induces vasoconstriction in these arteries. This evidence suggests this target would be relevant for PAH and that a silent, selective antagonist for 5-HT1B would be an excellent probe of the therapeutic utility of this approach.[1] This project is a continuation of our long-standing interest in serotonin (5-hydroxytriptamine) and its effects in health and disease.

This is a drugable target:

- Molecules can be designed to bind to the receptor with the desired properties of affinity, potency and ADME. There are good starting points for synthesis and medicinal chemistry.
- 5-HT1B agonists are the top selling prescription medicines for migraine. Sumatriptan and zolmitriptan have each accumulated sales of > $6 Billion each

- Other drugs target the 5-HT family include e.g. mirtazapine (*Zispin,* Organon) antidepressant, buspirone (*Buspar*, BMS) anxiety, pizotifen (*Sanomigran*, Novartis) cluster headaches etc., locaserin (Arena), agomelatine (Phase III, Novartis) depression.

As a first step, we have defined the desirable characteristics of a successful compound for pre-clinical studies. This is important to measure progress and should be the initial step in all drug discovery projects. Here is a brief summary of a desirable 5-HT1B antagonist profile as an example:

- Orally bioavailable 5-HT1B neutral antagonist (main target) with:
- affinity better than 20nM at 5-HT1B receptors and >20-fold selectivity (particularly 5-HT1A) and against a panel of relevant receptors, and (80) screened at 5μM (selectivity)
- Solubility >30μg/ml, logD <1.0, Polar Surface Area (PSA) > 90.0 (to reduce CNS (Central Nervous System) penetration)
- pharmacokinetic half life >6 hours and a pharmacodynamic half-life compatible with daily or twice daily dosing (pharmcokinetics, PK)
- Oral bioavailability of >30%, clearance <35ml/min/kg with a reduced susceptibility to metabolism, an acceptable volume of distribution and inactive human metabolites. (bioavailability, metabolism)
- Plasma protein binding should be <99% and there should be little or no inhibition or induction of cytochrome p450's with no mutagenic or teratogenic indications (safety pharmacology).
- Toxicology should show no adverse cardiovascular effects (the important ion channel for cardiac arrhythmia hERG is assayed) and there should be no CNS side-effects at a multiple of at least 20 times the anticipated human dose (off-target CNS receptors and transporters such as DAT, SERT etc. are assayed). No DEREK toxicology alerts (these are warnings on possible toxicity using the DEREK software available from Lhasa Ltd).[2]
- Ames test negative (mutagenicity).
- There is no evidence of mechanism based toxicity (for the target).

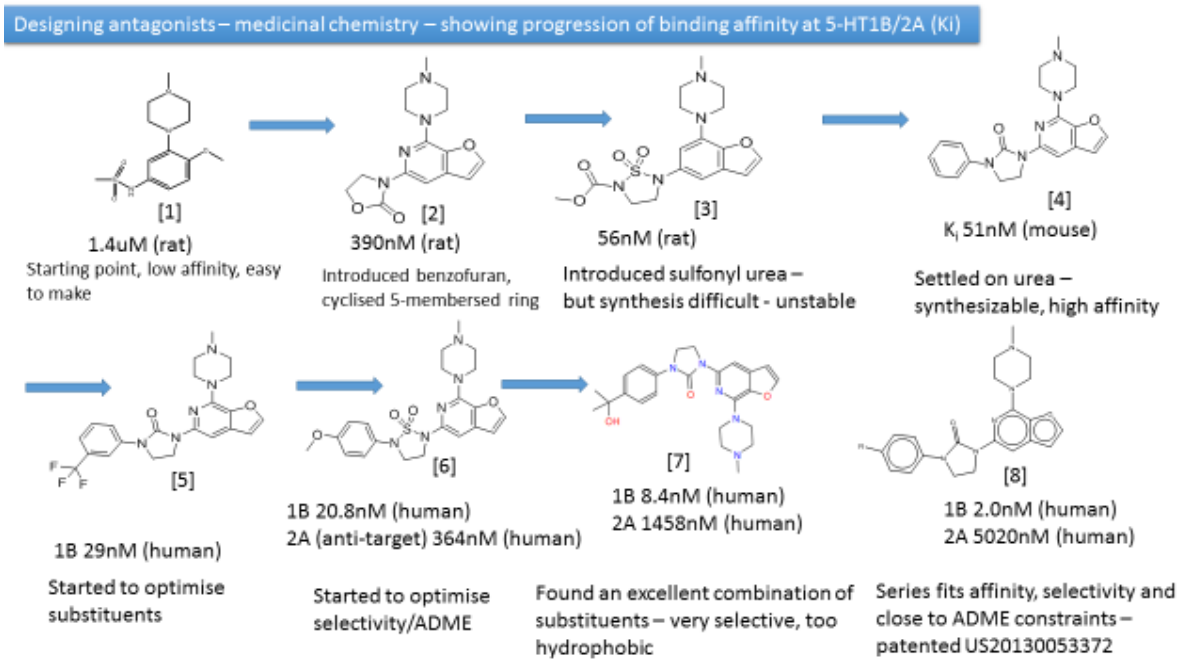
In-vitro and in-vivo assays are chosen to evaluate these parameters (e.g. in-vitro tests include Ames, hERG, bacterial toxicity, phospholipidosis etc. and are available from CRO's. Relevant in-vivo models of PAH are also available such as the monocrotiline rat, the Sugen rat and the hypobaric mouse[3]).

Of course, additional constraints are added as development of the program ensues e.g. the requirement to show tissue remodelling in a disease progression model of PAH was seen as beneficial and incorporated into the in-vitro screening using a cell proliferation assay. A full ADME and toxicity package (in two species) of the final lead compounds would generally be required before first exposure in man.

Of interest is that we defined 5-HT2A, a closely related 5-HT receptor, as an "anti-target" – one which would be associated with additional potentially undesirable effects (effects on blood clotting, platelet aggregation, CNS effects), and which due to its similarities to 5-HT1B is also commonly seen to have affinity for 5-HT1B ligands. Also, to validate the "5-HT1B" hypothesis that this receptor is a key component of PAH, we needed to discover highly selective ligands to probe this specific mechanism of the disease process.

The progression from "hit" to lead in this project is shown below. As the series is optimised, using both pharmacophore and site-directed constraints from homology models and X-ray crystallography, the progression from a low affinity (μM) hit to a high affinity (nM) selective series can be seen in Figure 1.

[Insert Figure 1 here]

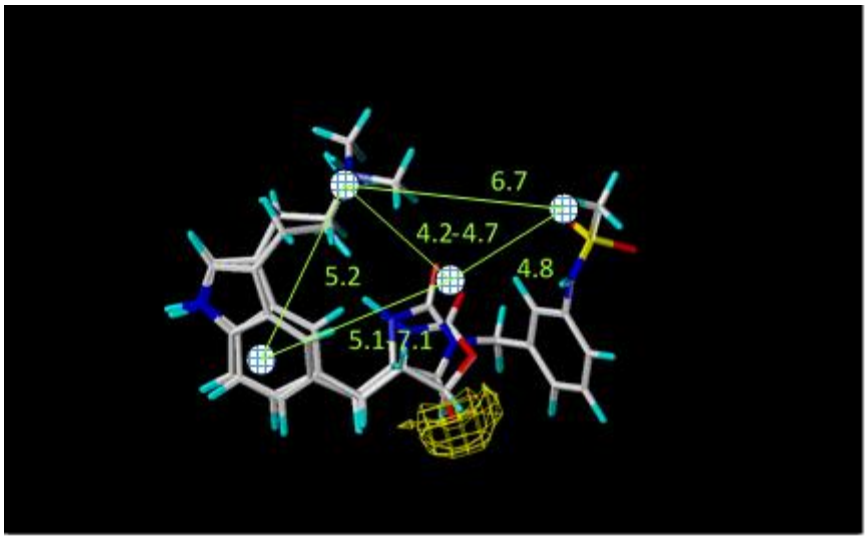


I will focus on a few aspects of this work: using the X-ray structures to optimise affinity and efficacy, and also the required ADME constraints, particularly in attempting to reduce CNS effects (the molecule should be optimised to exhibit peripheral cardiovascular effects while showing no or limited CNS effects).

Affinity and Efficacy

Previous work on 5-HT binding compounds has utilised a pharmacophore model developed for the optimisation of agonists, shown below in Figure 2 (distances shown in Angstroms)[4]. We have strong evidence from SAR (Structure Activity Relationships) that antagonists of similar structure bind in the same region and take advantage of the same interactions with the protein, therefore this pharmacophore model can be extended to include antagonists.

[Insert Figure 2 here]



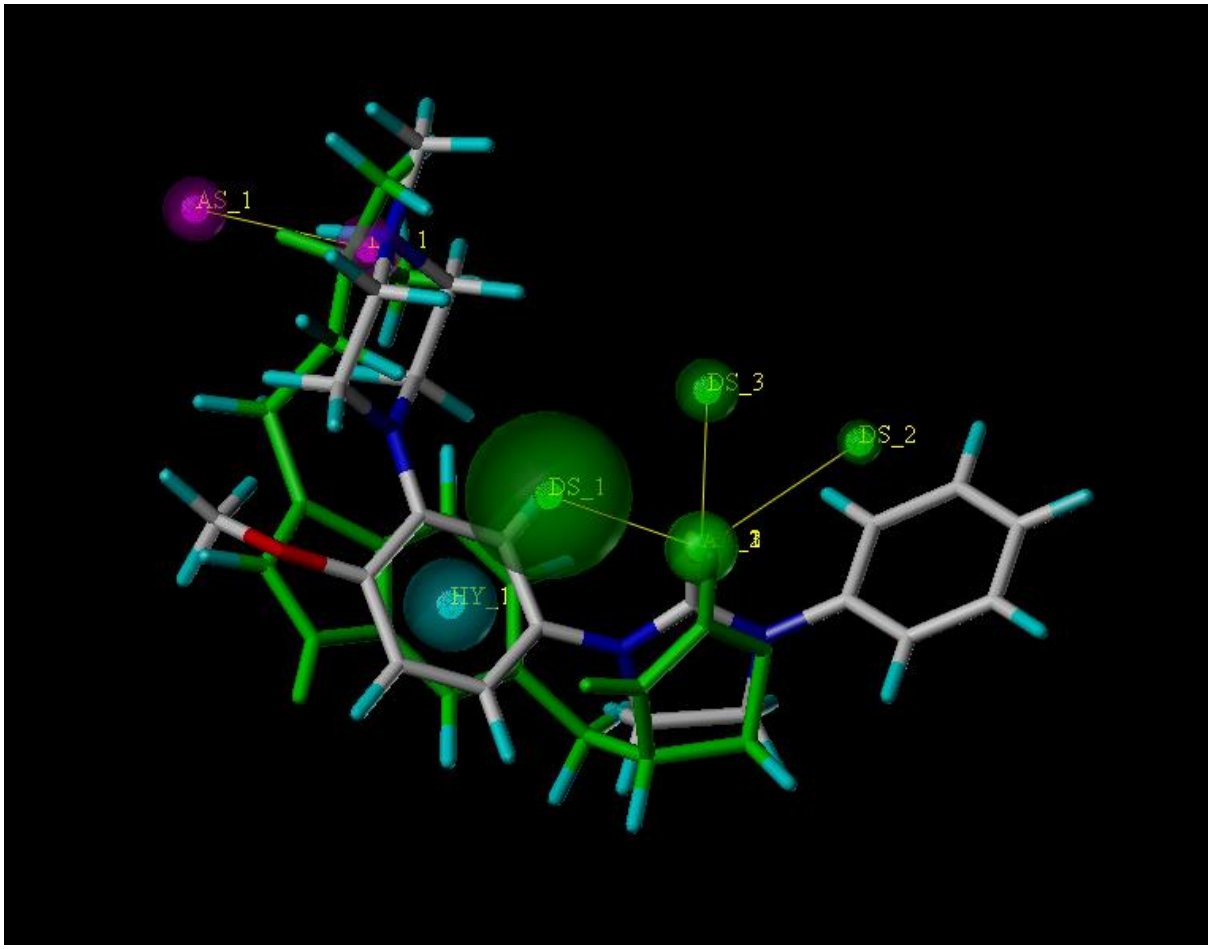
The big advantage of pharmacophore models is that the medicinal chemist can quickly and easily visualise the fit of new molecule ideas to the geometric constraints. Indeed, this model has been further developed, based on SAR to include extension of the original model as additional functional groups were added. This model was subsequently developed to discover 5-HT antagonists[5].

Computational modelling was used at each stage. Some of the screening approaches used were:

- Pharmacophore constraints were used as the initial design criteria.
- The novel molecules were docked into the binding site of the receptor using GOLD[6,7] and the goodness of fit determined from visual analysis and the docking score.
- clogD, MR, solubility, polar surface area (PSA) were computed.
- Compounds were evaluated for CNS penetration based on computational models (see below for an example model) using computed properties.
- Toxicity was evaluated using DEREK[2].
- Possible side products of metabolism were evaluated (MetaPrint2D[8]).
- Transporter interactions were evaluated using a read-across approach (MetraBase)[9].
- ADME/toxicity constraints were included (FAF-Drugs2)[10].

The antagonist model reduces to a series of simple rules that can be incorporated into compound design: we observed that ligands based on the 5-HT structure but without the complete indole ring were often partial agonists or antagonists. Adding a 2-substituent to the indole ring, with adjustment of the chain length of the ethylamine (to maintain the appropriate pharmacophore distances and hence affinity) often resulted in antagonists. Replacing the 3-substituted indole with a 4-substituted indole-piperazine could result in antagonists. Confirmation of this approach was the ability to reproducibly design 5-HT1B antagonists. A simple example molecule from this series is shown in Figure 3, the atom-coloured compound is an antagonist with a binding affinity of 106nM at 5-HT1B (human) and is compared to the 5-HT1B partial agonist zolmitriptan (binding affinity 19.9nM). Both molecules are assumed to occupy a similar binding site in the receptor – but one is a partial agonist and the other an antagonist. Indeed, receptor docking of the ligands into 5-HT1B shows very similar results to the previously determined pharmacophore overlay. The overlay was generated using GASP[11]. The indole is replaced by a methoxy-phenyl substructure, with the piperazine protonated nitrogen overlaying the protonated amine of the dimethylethylamine sidechain of zolmitriptan, while the hydrogen-bond acceptor (ketone) overlays with a similar acceptor in zolmitriptan. Note the pyrroline of the indole is replaced by a methoxy group, which, as described above, would be expected to give an antagonist. Indeed, substitution at the 2-position of the indole ring appeared to result in antagonists by (from steric-clashes with the receptor) moving the indole ring from the 'agonist site' (see the paper, *2,5-substituted tryptamine derivatives as vascular 5HT1B/1D receptor antagonists*[5]).

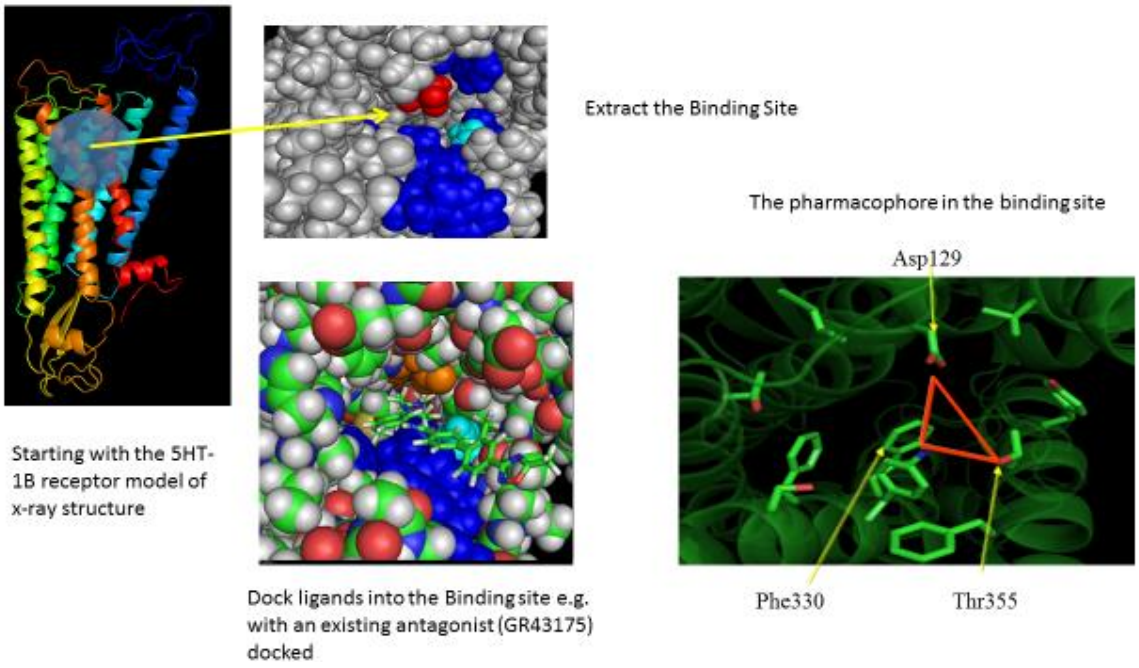
[Insert Figure 3 here]



From docking experiments, we hypothesised that the phenyl ring is involved with π-π stacking interactions with a complimentary phenyl ring in the receptor (see Figure 4 below, of GR43175, a non-selective 5HT1-B antagonist, docked into the receptor model).

Starting from homology models, but subsequently utilising the published X-ray structures of 5-HT1B (which became available in 2013, 4IAQ and 4IAR from the protein databank, PDB)[12], the binding site is identified as being adjacent to the important residue Asp129 (from mutagenesis studies thought to bind to the protonated amine of 5-HT) and inspection of the binding site topology, remarkably, shows the presence of the expected complimentary pharmacophore (Asp129, Thr355, Phe330), deduced from ligand-based studies. An example of the 5-HT1B binding antagonist GR43175[13], is shown docked into the binding-site cavity (using GOLD) in Figure 4.

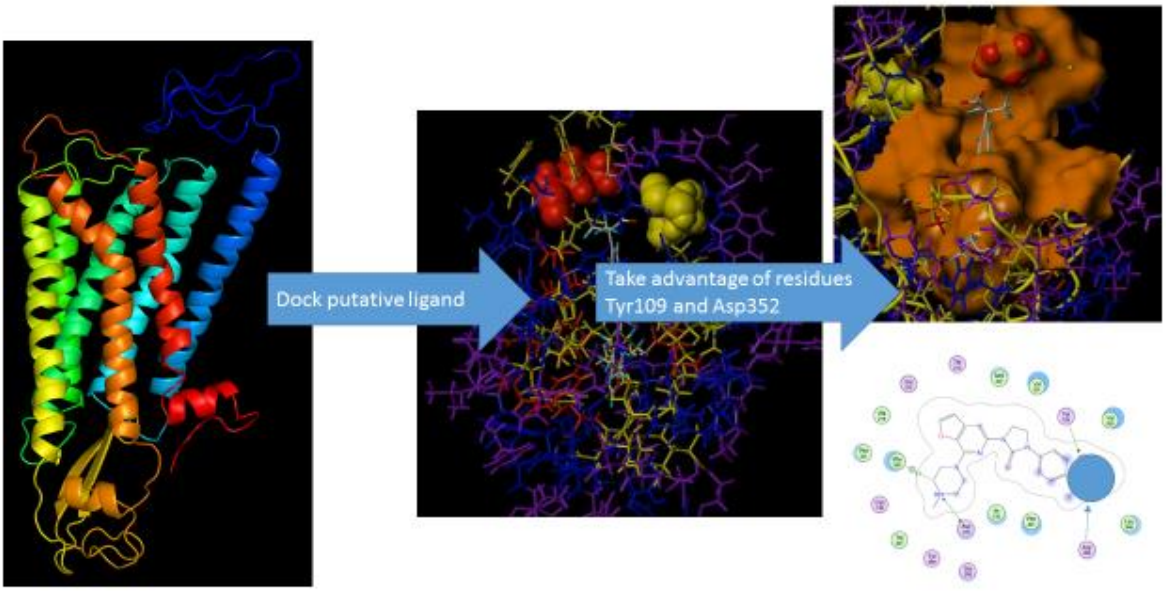
[Insert Figure 4 here]



To focus on aspects of the usefulness of the protein structure in optimising affinity and selectivity, the step-change in binding affinity and selectivity between structures [4] to [7] was driven by the realisation that there were two available hydrogen-bonding sidechains in the 5-HT1B transmembrane sequence (Tyr109 and Asp352) that were possible beneficial interactions if paired with the 'right' substituents (combining a hydrophobic and a hydrogen-bonding region). This resulted in affinity increasing from 51nM to 2nM, but more importantly, selectivity against 5-HT2A increasing from 17-fold to 174-fold while decreasing lipophilicity and increasing Polar Surface Area (making CNS penetration less likely). In going from 5-HT1B to 5-HT2A, residues Tyr109 and Asp352 are changed to isoleucine and asparagine (sequence alignment from EMBOSS Needle from EBI[14]).

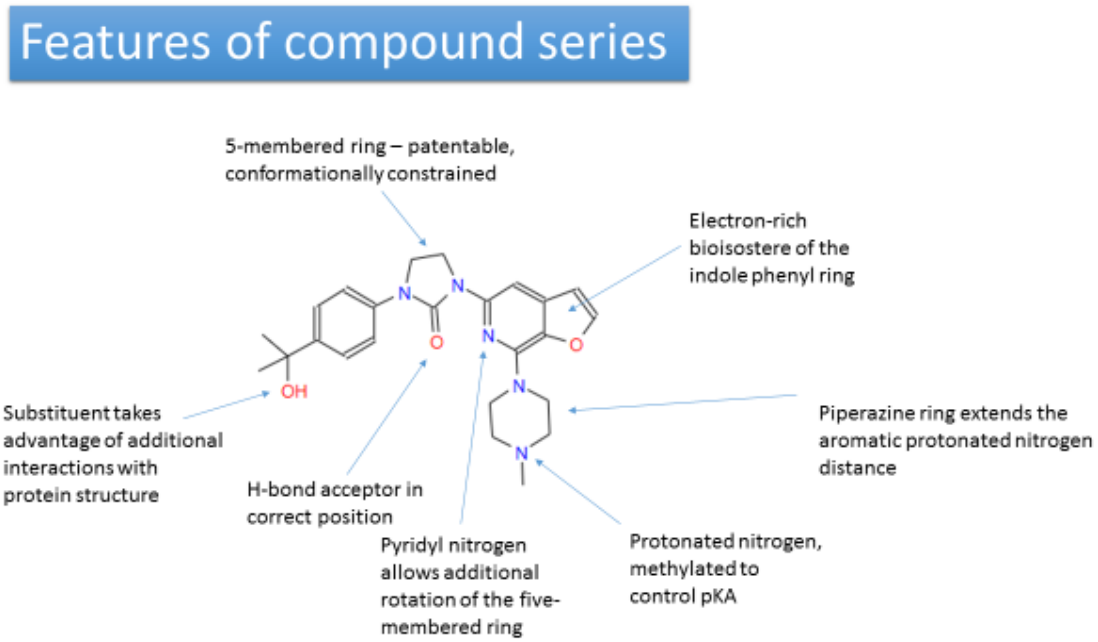
This changes the binding environment and allows optimisation of excellent selectivity for 5-HT1B over 5-HT2A to be obtained. This is shown in Figure 5.

[Insert Figure 5 here]



This trend has continued as the series continues to be optimised. The features of the compound series are shown below in Figure 6.

[Insert Figure 6 here]



CNS Penetration

Since the objective of this optimisation strategy is, among other factors, to optimise the compounds for peripheral cardiovascular effects and lower CNS penetration (or at least to have a higher probability of showing no CNS effects), a simple model developed by Clark et al.[15] using ClogP and polar surface area (PSA) can be useful here as an example to demonstrate the probability of CNS penetration. This model was developed from a set of measured compounds showing a range of CNS penetration.

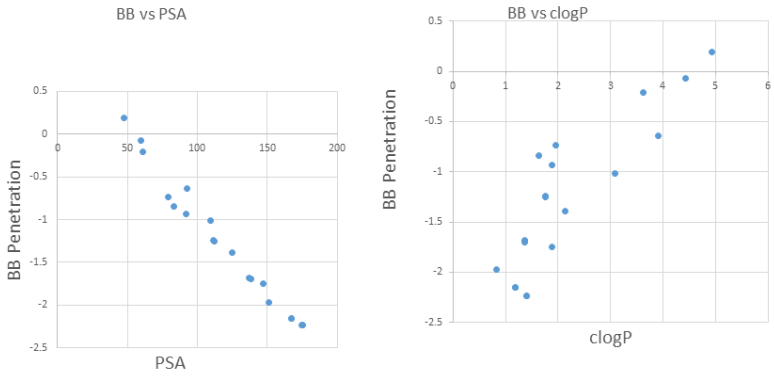
One of the published models is shown below.

log BB = -0.0148(0.001)PSA + 0.152(0.036)ClogP + 0.139(0.073)

From the equation, it is obvious that increased polar surface area (PSA) and a more hydrophilic clogP would be predicted to reduce BB (Blood-Brain penetration). A number of similar models could of course be chosen (and more sophisticated models[16], using a combination of the physico-chemical properties of the molecules (including the ionisation of the amine and other groups (pKa, logD) are of course more appropriate here).

We can therefore compute an estimate of BB, and incorporate this into our compound design. An example of modified compounds from this series and their BB penetration is shown below in Figure 7. Several of the compounds are predicted to have very low predicted BB.

[Insert Figure 7 here]



Additionally, we can also investigate whether transporters were likely to actively move compounds across the blood brain barrier and into/out-of the brain. A simple “read-across” (looking for similar structures) can be used to look for similar structures that are transported. Although no very similar compounds were found (in a databse of compounds and transporters, MetraBase[9]) with a Tanimoto >0.4, the closest compound was a substrate for MDR1, which in the capillary endothelial cells of the blood–brain barrier pumps from the brain into the capillaries (so this is promising). The amount of available experimental data on transporters is still a limiting factor in producing reliable models of compound transport.

Of course, in this short article, this is just a snapshot of some of the constraints applied. Importantly, it can be mentioned that in an experimantal panel of 60 diverse receptors, for the lead molecules there was only one significant off-target effect (screened at 10uM looking for >90% inhibition) which is currently being removed by additional compound design. Also, some analogs (having a protonated-amine combined with a phenyl ring, which can be associated with hERG binding) showed hERG toxicity, but again by design these effects have been removed. Drug discovery is often a cyclic-process of design/screening and re-design to remove unwanted effects and optimise the desired

profile. Importantly, in-vivo the compounds have shown significant desirable effects in treating symptoms of PAH (which will be published elsewhere). Development of this series continues.

Summary

The discovery of new medicines is a complex multivariate problem, and spans chemistry and biology. Deciding on which targets to invest time and effort in requires substantial evidence that the targets indeed modify disease. Having found a suitable target, a lead series and a relevant pharmacological assay is required before structural optimisation can begin. Described here is a current example of this process using the GPCR target 5-HT1B. Using a combination of pharmacophore and structural constraits we have shown how novel antagonists at the 5-HT1B receptor can be discovered. The introduction of additional constraints, such as blood brain penetration, is shown using a simple model utilising PSA and clogP, which is easy to incorporate into compound design. The resulting molecules were patented (US2013053372 (A1)), and have been investigated in in-vivo models of PAH, with positive results, which will be published elsewhere.

References

1. M.R. MacLean, Y. Dempsie, *Adv. Exp. Med. Biol.* **2010**, 661, 309-322.
2. C.A. Marchant, K.A. Briggs, A. Long, T*oxicol. Mech. Method.* **2008**, 18 (2-3), 177-187.
3. K.L. Colvin, M.E. Yaeger, *J. Pulm. Respir. Med.* **2014**, 4(4): 198.
4. R. C. Glen, G. R. Martin, A. P. Hill, R. M. Hyde, P. M. Woollard, J. A. Salmon, J. Buckingham, A. D. Robertson, *J. Med. Chem.* **1995**, 38(18), 3566-3580.
5. G. P. Moloney, A. D. Robertson, G. R. Martin, S. MacLennan, N. Mathews, S. Dodsworth, P. Y. Sang, C. Knight, R. Glen, *J. Med. Chem.* **1997**, 40(15), 2347-2362.
6. G. Jones, P. Willett, R. C. Glen*, J. Mol. Biol.* **1995**, 245(1), 43-53.
7. G. Jones, P. Willett, R. C. Glen, A. R. Leach, R. Taylor, *J. Mol. Biol.* **1997**, 267(3), 727-748
8. S. Boyer, C. H. Arnby, L. Carlsson, J. Smith, V. Stein and R. C. Glen, *J. Chem. Inf. Model.* **2007**, 47(2), 583-590.
9. L. Mak, D. Marcus, A. Howlett, G. Yarova, G. Duchateau, W. Klaffke, A. Bender, R. C. Glen, *J. Chem Inf.* **2015**, 7,31.
10. D. Lagorce, O. Sperandio, H. Galons, M. A. Miteva, B. O. Villoutreix, *BMC Bioinformatics,* **2008**, Sep 24, 9:396.
11. G. Jones, P. Willett, R. C. Glen, *J. Comput. Aid. Mol. Des.* **1995**, 9(6), 532-549.
12. C. Wang et al. *Science,* **2013**, 340, 610-614.
13. P. P. A. Humphrey, W. Feniuk, M. J. Perren, H. E. Connor, A. W. Oxford, I. H. Coates, D. Butina, Gr43175, *Brit. J. Pharmacol.* **1988**, 94(4), 1123-1132.
14. *EMBOSS: the European Molecular Biology Open Software Suite.* (2000 June) Trends in genetics : TIG 16 (6) :276-7.
15. D. E. Clark, *J. Pharm. Sci.* **1999**, 88(8), 815-821.
16. Blood-Brain Barrier in Drug Discovery: Optimizing Brain Exposure of CNS Drugs and Minimizing Brain Side Effects for Peripheral Drugs, **2015**. Editor(s): Li Di, Edward H. Kerns. John Wiley & Sons, Inc. Print ISBN: 9781118788356.

Figure Legends

Figure 1. The lead optimisation process for novel 5-HT1B antagonists. This shows the initial simple starting 'hit' and the development of the series as additional substituents are added to improve the affinity and other target properties such as selectivity. This Figure should be read in conjunction with Figure 6, which may explain in more detail the reasoning behind the compound development.

Figure 2. A pharmacophore model for ligand binding to the 5-HT1B receptor. This simple model is a powerful constraint to use in the design of novel analogues, which ideally would satisfy the geometrically defined interactions with the receptor.

Figure 3. Zolmitriptan is overlaid with a novel 5-HT1B antagonist showing the close proximity of the compatible pharmacophore components. Despite one molecule being a partial agonist, and the other an antagonist, they occupy similar positions from the docking study.

Figure 4. The 5-HT1B X-ray structure (4IAQ), with the binding site for 5-HT highlighted. The binding site is shown in more detail, with the exemplar antagonist compound GR43175 docked. The pharmacophore (from the protein side) which is deduced to interact with GR43175[13] (and zolmitriptan) is shown.

Figure 5. Optimisation of a substituent for affinity and selectivity using the homology models and X-ray crystal structures of 5-HT1B. In particular, this image shows the additional hydrogen-bonding interactions obtained by substitution of the phenyl ring with appropriate fragments. This translates into both higher affinity and selectivity.

Figure 6. Features of the novel 5-HT1B selective series of compounds. This shows the reasoning behind the adoption of the structural components of the compounds.

Figure 7. BB penetration predicted for a series of compounds based on the present 5-HT1B antagonist series. Lowering clogP and increasing PSA lowers BB penetration in this model. This is intended to show the trend in compound design, as not all the compound structures are shown here (they will be published elsewhere).