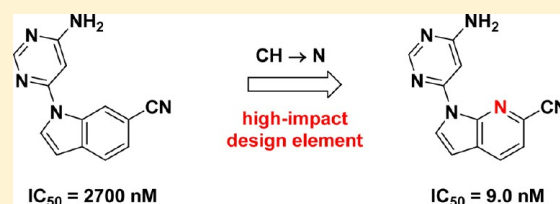


The Necessary Nitrogen Atom: A Versatile High-Impact Design Element for Multiparameter Optimization

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ABSTRACT: There is a continued desire in biomedical research to reduce the number and duration of design cycles required to optimize lead compounds into high-quality chemical probes or safe and efficacious drug candidates. The insightful application of impactful molecular design elements is one approach toward achieving this goal. The replacement of a CH group with a N atom in aromatic and heteroaromatic ring systems can have many important effects on molecular and physicochemical properties and intra- and intermolecular interactions that can translate to improved pharmacological profiles. In this Perspective, the “necessary nitrogen atom” is shown to be a versatile high-impact design element for multiparameter optimization, wherein ≥ 10 -, 100-, or 1000-fold improvement in a variety of key pharmacological parameters can be realized.



INTRODUCTION

Over 80 years since the advent of modern drug discovery and 20 years after the publication of Lipinski's rules,^{1,2} biomedical researchers still struggle to minimize the number and duration of design cycles required to optimize lead compounds into high-quality chemical probes or safe and efficacious drug candidates (Figure 1).^{3,4} Although computational and medicinal chemists generally strive for impactful molecular design elements during multiparameter optimization,⁵ this effort is challenged not only by synthetic tractability but also by the limits of their ability to predict how molecules will interact with complex biological systems.⁶ Contemporary computational modeling and data analysis tools greatly facilitate the molecular design process;⁷ however, the difficulty of multiparameter optimization in the context of the uncertainty that is inherent in biomedical research data continues to complicate the identification of compounds with desirable pharmacological profiles.⁸ For example, in the absence of statistical data to the contrary, 3-fold variability in measured values for bioassays is a generally accepted rule-of-thumb ($SD = 0.50 \log \text{ unit}$). Segall and co-workers have pointed out that because each of the uncertainties in a series of bioactivity profiling filters combine, the probability of even an ideal compound passing a sequence of five filters each having an accuracy of 80% is only 33%.⁸ To more efficiently prosecute multiparameter optimization efforts, continued improvement in the insightful application of high-impact design elements at critical stages of the multiparameter optimization process is needed, in which ≥ 10 -fold improvement ($\geq 1.0 \log \text{ unit}$) in key pharmacological parameters is achieved.

One well-known, high-impact design element is commonly referred to as “the magic methyl effect,” in which replacement of a H atom with a Me group results in profound potency improvement (>100 -fold).⁹ Several years ago, Jorgensen and co-workers determined that the most dramatic effects on

potency arise when the newly installed Me group not only fills a hydrophobic pocket but also enforces a favorable ligand conformation for binding to its receptor.¹⁰ This study also found from a survey of literature data that the likelihood of increasing or decreasing potency with this structure modification is approximately equal, and the probabilities of achieving potency gains greater than 10- or 100-fold are 8.0% and 0.40%, respectively.¹⁰ Another high-impact design element could be termed “the necessary nitrogen atom,” wherein substitution of a CH group with a N atom in aromatic and heteroaromatic ring systems gives rise to substantial improvement in a key pharmacological parameter (≥ 10 -fold). A dramatic example of this phenomenon is shown in Figure 2. While searching for potent inhibitors of Cdc7 kinase, Allen and co-workers discovered that replacing the C7 CH group of **1** with a N atom to give **2** resulted in a 300-fold improvement in biochemical potency (Cdc7 $IC_{50} = 2700$ and 9.0 nM for **1** and **2**, respectively).¹¹ This potency improvement was attributed to the different conformational preferences of **1** and **2** as shown by the torsional energy diagrams depicted in Figure 2. Notably, indole **1** prefers a biaryl dihedral angle $>150^\circ$, while aza-indole **2** favors a biaryl dihedral angle of 0° . These different conformational preferences are likely due to a steric clash of H7 and H6' in the 0° rotamer of **1** and lone pair repulsion between N7 and N2' in the 180° rotamer of **2**.¹¹ Although challenging to achieve, dramatic pharmacological improvements such as those discussed above can serve to greatly advance multiparameter optimization efforts.

Traditionally regarded as a classical bioisosteric replacement, the substitution of a CH group with a N atom in aromatic and heteroaromatic ring systems can have many important effects on molecular and physicochemical properties of relevance to

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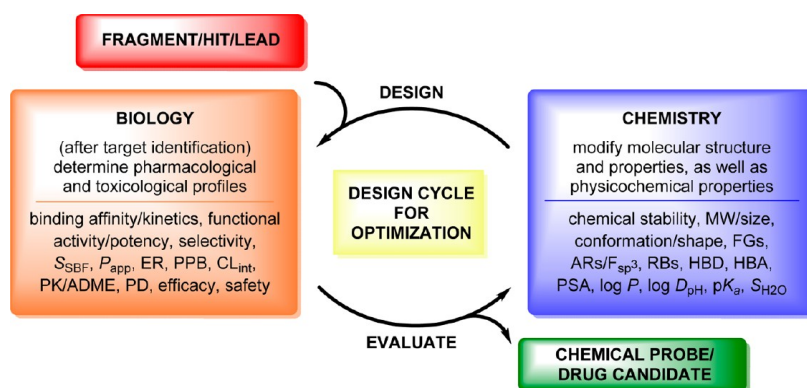


Figure 1. Minimizing the number and duration of design cycles during multiparameter optimization is a continued challenge for biomedical research.

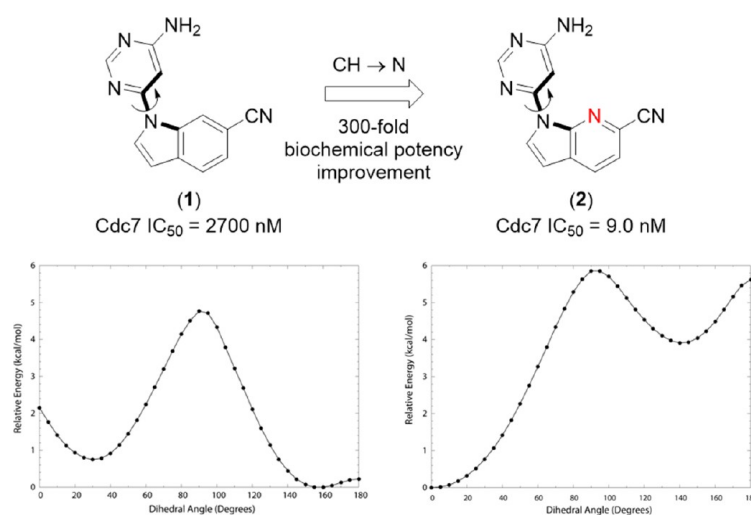
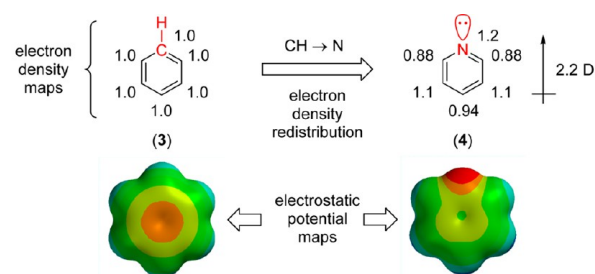


Figure 2. A dramatic example of a necessary nitrogen atom on potency improvement, likely due to different conformational preferences. The torsional energy diagrams were generated using quantum mechanical calculations at the B3LYP/6-31G* level of theory.¹¹

multiparameter optimization.¹² For example, whereas benzene (3) is a resonance-stabilized aromatic system ($E_{\text{res}} = 36$ kcal/mol) with an even distribution of electron density, pyridine (4) is a resonance-stabilized aromatic system ($E_{\text{res}} = 32$ kcal/mol) with variable electron density, a permanent dipole moment ($\mu = 2.2$ D), and a polar N atom bearing an unshared pair of electrons coplanar to the ring (Figure 3).¹³ While going from 3 to 4 imparts only a slight increase in molecular weight ($\Delta\text{MW} = +1$ Da) and decrease in van der Waals radius at the point of substitution ($r_{\text{W}} = 1.77$ and 1.55 Å for the CH group and the N atom, respectively),¹⁴ it introduces a strong hydrogen bond acceptor ($\text{p}K_{\text{BHX}} = 1.86$ for 4)¹⁵ with physiologically relevant basicity ($\text{p}K_{\text{a}} = 5.20$ for the conjugate acid of 4),¹⁶ and leads to an increase in polar surface area ($\text{tPSA} = 12.9$ Å² for 4),¹⁷ a decrease in lipophilicity ($\Delta\text{CLogP} = -1.50$),¹⁸ and improved aqueous solubility ($S_{\text{H}_2\text{O}} = 1.8$ g/L for 3 and complete miscibility for 4).¹⁹ The redistribution of electron density from the center of more π -electron rich 3 ($\text{IP} = 9.23$ eV)²⁰ to the N atom of more π -electron deficient 4 ($\text{IP} = 9.34$ eV)²⁰ can be visualized by the electrostatic potential maps in Figure 3,²¹ a result of the lower electronegativity of a C atom ($\chi = 2.55$) versus a N atom ($\chi = 3.04$) and its unshared electron pair. The N atom also leads to a reduced polarizability and spatial extent of the π -electron cloud for 4 versus 3 and serves to favor and

strengthen the optimal parallel-displaced π -stacking configuration for 4 as compared to 3 ($\Delta E = -2.71$ and -3.23 kcal/mol for the 3–3 and 3–4 complexes, respectively; $R = 3.5$ Å and $H = 1.6$ Å for both the 3–3 and 3–4 complexes).²² Additionally, the calculated binding energies of the Na^+ cation with 3 and 4 reveal a greatly diminished cation– π interaction for 4 as compared to 3 ($\text{BE} = -27.1$ and -20.0 kcal/mol, for 3 and 4, respectively) and, in fact, binding of the N atom of 4 to the Na^+ cation represents the true binding energy minimum ($\text{BE} = -33.4$ kcal/mol).²³ The polarity of the N atom in 4 also manifests itself in other ways: the CH groups flanking it are better weak hydrogen bond donors than the CH groups of 3 ($\text{IE} = -1.47$ and -2.23 kcal/mol for 3 and 4, respectively)²⁴ and the H atom at the para position of 4 is a more effective π -hydrogen bond donor than the H atoms of 3.²² Finally, benzylic carbanions appended to C2 or C4 of 4 experience greater charge demand into the ring than those attached to 3 ($\text{CD} = 0.29$ and 0.41 for 3 and 4, respectively).²⁵ The substitution of a CH group with a N atom can have similar effects on the molecular and physicochemical properties of other aromatic and heteroaromatic ring systems, many of which can be fine-tuned by the choice of location of the N atom.^{12–27}

As mentioned above, the changes in molecular and physicochemical properties incurred upon substitution of a



Property or Interaction	3	4
resonance stabilization energy (E_{res})	36 kcal/mol	32 kcal/mol
molecular weight (MW)	78 Da	79 Da
polar surface area (tPSA)	—	12.9 Å ²
lipophilicity (CLogP)	2.142	0.645
basicity (pK_a)	—	5.20
HBA strength (pK_{BHX})	—	1.86
C/N electronegativity (χ)	2.55	3.04
dipole moment (μ)	—	2.2 D
CH/N van der Waals radius (r_w)	1.77 Å	1.55 Å
aqueous solubility ($S_{\text{H}_2\text{O}}$)	1.8 g/L	miscible
ionization potential (IP)	9.23 eV	9.34 eV
3-3/3-4 π -stacking energy (ΔE)	-2.71 kcal/mol	-3.23 kcal/mol
Na ⁺ / π -system binding energy (BE)	-27.1 kcal/mol	-20.0 kcal/mol
H2 HBD interaction energy (IE)	-1.47 kcal/mol	-2.23 kcal/mol
C2/C4 carbanion charge demand (CD)	0.29	0.41

Figure 3. Substitution of a CH group with a N atom in aromatic and heteroaromatic ring systems is a small structure modification with potentially large effects on pharmacological profiles due to changes in molecular and physicochemical properties and intra- and intermolecular interactions.

CH group with a N atom in aromatic and heteroaromatic ring systems can impact a number of intra- and intermolecular orbital, steric, electrostatic, and hydrophobic interactions²⁸ such as lone pair, dipole–dipole, hydrogen bonding, metal coordination, van der Waals, σ -hole, $\sigma^*_{\text{S-X}}$, and π -system interactions, which in turn can translate to modified pharmacological profiles. To increase the likelihood of effecting dramatic improvement in pharmacological parameters such as binding affinity, functional activity, and selectivity for the desired therapeutic target, the molecular partners (in ligand,

receptor, or water) with which the original CH group, the newly installed N atom, and their associated rings may interact should be carefully considered when practicable. With greater knowledge of the structure, function, and pharmacophores of P-glycoprotein (Pgp),²⁹ cytochrome P450s (CYPs),³⁰ pregnane X receptor (PXR),³¹ and human ether- α -go-go-related gene product (hERG),³² such considerations are also increasingly possible for these common antitargets that can have important effects on pharmacological profiles. Though the above considerations can be particularly important for conformational control and molecular recognition, opportunities for serendipitous improvements should also be exploited when appropriate. For example, in the absence of knowing the preferred binding pose of the ligand or having a predictive structural model of the receptor or ligand–receptor complex, an alternative strategic approach to determine optimal placement of N atoms in the ligand is the systematic N atom scan (N-scan).³³

A matched molecular pair analysis (MMPA) of internal data at Abbott several years ago by Hajduk and Sauer found that as with most substituent replacements, there is an approximately equal probability of increasing or decreasing potency by exchanging CH groups and N atoms.³⁴ Notably, they found that in contrast to the majority of the other 26 most-common structure modifications in the database, the exchange of CH groups and N atoms resulted in a significantly broader potency distribution ($\text{SD} > 0.75$ log unit).³⁴ This analysis also determined that in general the probability of realizing a 10-fold increase in potency with most substituent replacements is 8.5%, and the likelihood of achieving a >100-fold gain is less than 1.0%.³⁴ Interestingly, these findings are very similar to those discussed above for the magic methyl effect.¹⁰ A more recent MMPA of public domain data by Bajorath and co-workers found that there are several structure modifications, most of them simple substituent replacements, which may lead to more consistent potency increases.³⁵ A previous MMPA by Bajorath et al. identified the exchange of CH groups and N atoms as one of many structure modifications that can induce activity cliffs.³⁶ A separate study by this same group found that for the phenyl ring to 2-pyridyl, 3-pyridyl, or 4-pyridyl ring transformations, the distribution of potency differences depended on the location of the N atom, with the 2-pyridyl ring transformation exhibiting a greater percentage of records with potency differences larger than 1 order of magnitude.³⁷

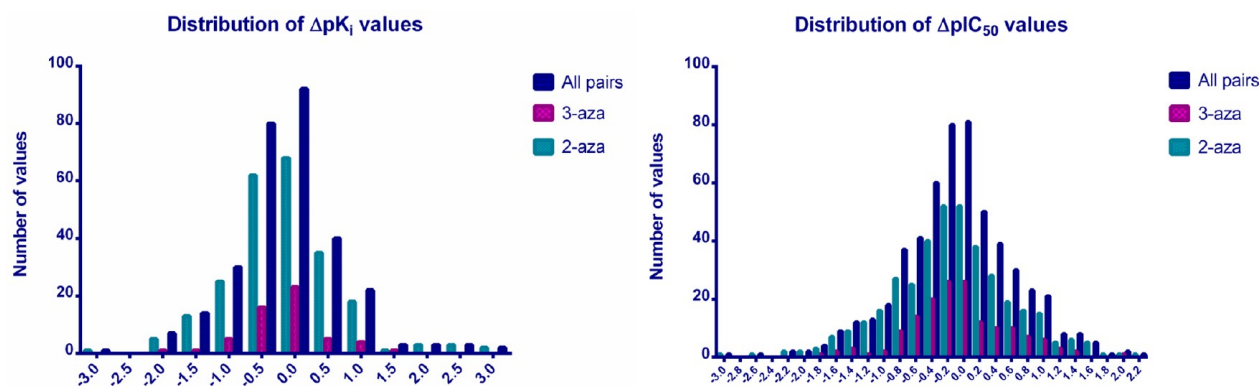


Figure 4. MMPA histograms for the exchange of a CH group and a N atom in aromatic and heteroaromatic ring systems. The ΔpK_i values are on the left, and the ΔpIC_{50} values are on the right. A ΔpK_i or ΔpIC_{50} value >0 reflects more potent activity for the N analogue versus the CH analogue.

With respect to microsomal stability, cellular permeability, and Pgp-mediated efflux, MMPAs of internal high-throughput in vitro assay data at Pfizer by Keefer, Chang et al. revealed that trends in pharmacological profile differences can depend on both the number and location of N atoms in the scaffold.³⁸ For example, a 1-phenyl ring to 3-pyridyl ring transformation was shown to result in greater efflux propensity; however, whereas a 3-pyridyl ring to 4-pyridazyl ring transformation was also found to give rise to greater efflux propensity, a 3-pyridyl ring to 2-pyrazyl ring transformation was found to impart lower efflux propensity.³⁸ Kramer and co-workers recently suggested that caution must be exercised when making predictions from insignificant effects identified in MMPAs of public domain data but indicated that predictions from profound effects can be made more reliably.³⁹ Another caution when leveraging statistical analyses of historical data sets to determine the probability that a structure modification will lead to a pharmacological improvement in a new situation is that in the historical data sets it is very difficult to differentiate instances of rational (i.e., hypothesis-driven) versus random (e.g., library synthesis) structure modifications and the specific context of their application, each of which may give rise to a profound difference for the chance of success.

Using previously generated binding affinity (pK_i) and functional activity (pIC_{50}) data sets from matched molecular pairs previously extracted from a recent version of the ChEMBL database,⁴⁰ an MMPA was conducted by filtering the two data sets to identify those matched molecular pairs that differed only by the exchange of a single CH group and a single N atom in aromatic and heteroaromatic ring systems (Figure 4). The filtered pK_i and pIC_{50} data sets contained 297 and 549 matched molecular pairs, respectively. The distributions of ΔpK_i and ΔpIC_{50} values were found to be normal and centered near zero (ΔpK_i mean value = -0.15 , SD = 0.81 ; ΔpIC_{50} mean value = -0.11 , SD = 0.72), where a zero value represents identical activity for the CH and N analogues, a positive value reflects more potent activity for the N analogue, and a negative value denotes less potent activity for the N analogue. The small negative mean values for both the ΔpK_i and ΔpIC_{50} distributions indicate that there is a general tendency for the N analogue to display slightly less potent activity than the CH analogue. Interestingly, the ΔpK_i distribution is somewhat asymmetrical with a greater number of negative values on the left side of the histogram, whereas the ΔpIC_{50} distribution is more symmetrical. These distributions indicate that for most matched molecular pairs the effect of exchanging CH groups and N atoms in aromatic and heteroaromatic ring systems on binding affinity and functional activity is modest but that the effect can be dramatic in some instances. This analysis determined that in general the probability of realizing a 10-fold increase in binding affinity (ΔpK_i) is 6.4% and the likelihood of achieving a >100-fold gain is less than 2.0%. Similarly, the probability of realizing a 10-fold increase in functional activity (ΔpIC_{50}) was found to be 6.6% and the likelihood of achieving a >100-fold gain was shown to be 0.40%. Encouragingly, this analysis is consistent with the previously reported MMPA of internal data at Abbott by Hajduk and Sauer.³⁴ Similar to the phenyl ring to pyridyl ring MMPA by Bajorath and co-workers,³⁷ the location of the N atom relative to the closest ring system substituent or fusion location did seem to affect the distributions of ΔpK_i and ΔpIC_{50} values in this study, with the 2-aza-analogues having broader distributions. For the CH group to 2-aza-analogue

MMPAs, the distributions of ΔpK_i and ΔpIC_{50} values were found to be normal and centered near zero (ΔpK_i mean value = -0.15 , SD = 0.84 ; ΔpIC_{50} mean value = -0.13 , SD = 0.76). For the CH group to 3-aza-analogue MMPAs, the distributions of ΔpK_i and ΔpIC_{50} values were also found to be normal and centered near zero (ΔpK_i mean value = -0.15 , SD = 0.60 ; ΔpIC_{50} mean value = -0.060 , SD = 0.69). For the CH group to 4-aza-analogue MMPAs, there were too few matched molecular pairs to calculate reliable mean and standard deviations for the distributions of ΔpK_i and ΔpIC_{50} values.

As discussed above, the reverse substitution, of a N atom with a CH group, can also lead to dramatic improvements in binding affinity, functional activity, or selectivity through beneficial effects on various intra- or intermolecular interactions, especially steric and hydrophobic interactions. This structure modification, however, is less likely to be broadly useful for multiparameter optimization. It has been shown previously that lipophilicity needs to be carefully controlled during multiparameter optimization, as high-throughput screening (HTS) hits and early stage leads often suffer poor lead- or drug-like properties and molecular weight tends to increase during these efforts.^{41,42} Importantly, however, this reverse exchange should be considered for compounds at stages of the optimization process in which the replacement of a N atom with a CH group in aromatic and heteroaromatic ring systems is not anticipated to have significantly deleterious effects on key pharmacological parameters or physicochemical properties. A similar caution for the substitution of a CH group with a N atom is that the benefit incurred for one pharmacological parameter can be offset by a detrimental effect on another, such as a reduction in potency or selectivity or the introduction of cytochrome P450 inhibition or aldehyde oxidase metabolism.^{43,44} As identifying where N atom substitution will be beneficial for the parameter(s) of interest while being benign for others is paramount, it is critically important to judiciously select the appropriate CH group to replace with a N atom to achieve the desired balance of pharmacological parameters that leads to the optimal pharmacological profile.

For this Perspective, a variety of examples from the literature published over the last 10 years has been selected to illustrate the range of pharmacological improvements that can be achieved by the replacement of a CH group with a N atom, wherein ≥ 10 -fold improvement in at least one key pharmacological parameter is realized. It is important to note that the examples discussed in this Perspective are not intended to suggest what pharmacological improvements will be realized upon the substitution of a CH group with a N atom, but rather to show the dramatic improvements that can be achieved under certain circumstances. The broad utility of this structure modification is suggested by a recent analysis of U.S. FDA-approved pharmaceuticals, which revealed that 59% of structurally unique, small-molecule drugs contain at least one nitrogen heterocycle with an average of 3.1 N atoms per drug.⁴⁵ Because of their ubiquitous presence in drug-like molecules⁴⁵ and to avoid confounding the interpretation of pharmacological profile differences resulting from the introduction of more basic N atoms,⁴⁶ only trivalent CH groups ($-\text{CH}=\text{}$) and trivalent N atoms ($-\text{N}=\text{}$) in aromatic and heteroaromatic ring systems are considered. The examples reviewed in this Perspective will demonstrate that although the substitution of a CH group with a N atom is one of the smallest structure modifications that can be made, it offers (if done thoughtfully) an excellent opportunity to dramatically impact a wide variety of

pharmacological parameters during early-, mid-, or late-stages of multiparameter optimization.

DISCUSSION

In Vitro Binding Affinity. Unlike the replacement of a H atom with a Me group, there are inherent hydrophobic and desolvation penalties incurred upon the substitution of a CH group with a N atom (Figure 5). For example, comparing the

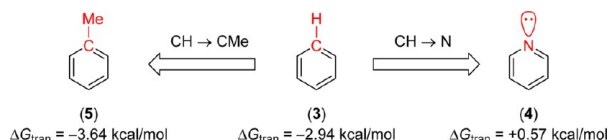


Figure 5. For improved binding affinity upon the substitution of a CH group with a N atom, there are inherent hydrophobic and desolvation penalties that must be overcome (ΔG_{tran} , difference in the free energies of solvation in hexadecane and in water).

solvation of benzene (3) and toluene (5) in water and hexadecane (a model of the lipid-like interior of a protein), the more lipophilic 5 ($\Delta \text{CLogP} = +0.5$)¹⁸ having a greater solvent-accessible surface area ($\Delta \text{SASA} = +32 \text{ \AA}^2$) exhibits a more favorable free energy of transfer ($\Delta G_{\text{tran}} = -2.94$ and -3.64 kcal/mol for 3 and 5, respectively).^{10,47} In actuality, the transfer of a ligand bearing a Me group from water to a hydrophobic pocket in a receptor is more complicated, as it relies on size and shape complementarity and may also result in the Me group displacing receptor-bound water molecules.^{10,48} Comparing the solvation of benzene (3) and pyridine (4) in these same two media, the less lipophilic 4 ($\Delta \text{CLogP} = -1.5$)¹⁸ that also possesses a strong HBA and a smaller solvent-accessible surface area ($\Delta \text{SASA} = -7.0 \text{ \AA}^2$) displays an unfavorable free energy of transfer ($\Delta G_{\text{tran}} = -2.94$ and $+0.57$ kcal/mol for 3 and 4, respectively). The difference in the free energy of transfer of 3 and 4 ($\Delta \Delta G_{\text{tran}} = +3.51$ kcal/mol) is quite significant when compared to the difference in the free energy of binding required for a 10-fold improvement in ligand–receptor affinity ($\Delta \Delta G = -1.36$ kcal/mol). In reality, replacing a CH group with a N atom in a larger and more complex drug-like molecule will generally impact a smaller proportion of its overall molecular and physicochemical properties than doing so in the simple case of benzene (3). For the substitution of a CH group with a N atom, improvements in binding affinity will typically hinge upon positive effects on ligand conformation or favorable electrostatic interactions, especially hydrogen bonding with specific donor residues in the receptor or receptor-bound water molecules that need to be satisfied. The case studies below illustrate the dramatic impact that the substitution of a CH group with a N atom can have on binding affinity.

An extraordinary improvement in binding affinity upon the substitution of a CH group with a N atom was reported by de Esch and co-workers in 2012 as part of an effort to obtain potent ligands of the 5-HT₃A receptor (5-HT₃AR), a pentameric ligand-gated ion channel for which no X-ray crystal structure existed at that time (Figure 6).^{49,50} A cell-based fragment screening effort discovered a set of quinolines and quinazolines as promising hits. Subsequent structure–activity relationship (SAR) studies, including partial N-scans, which were based on a competitive binding assay using the 5-HT₃-specific radioligand [³H]-granisetron and membrane preparations from HEK293 cells stably expressing 5-HT₃AR, identified

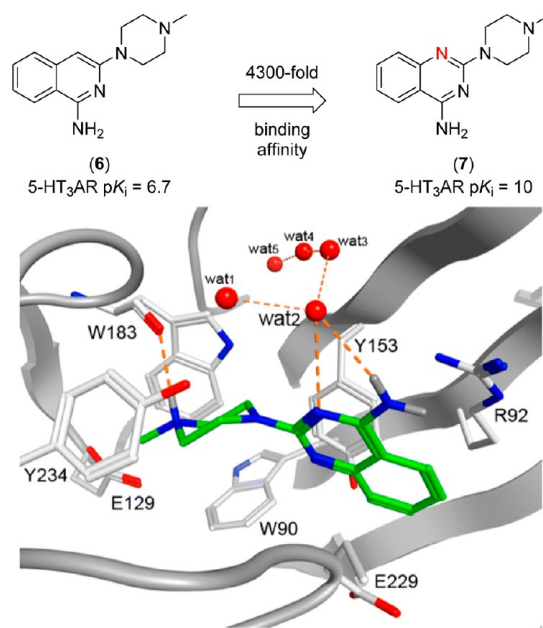


Figure 6.

isoquinoline 6 as a moderate-affinity ligand (5-HT₃AR $pK_i = 6.7$). Remarkably, quinazoline analogue 7 was found to display a 4300-fold improvement in binding affinity (5-HT₃AR $pK_i = 10$) despite the newly installed N atom not being predicted to engage in any favorable electrostatic interactions for the ligand docking pose in their acetylcholine binding protein (AChBP)-based homology model of the 5-HT₃AR binding site.

Another remarkable improvement in binding affinity was disclosed by Müller et al. in 2009, stemming from their studies to identify chemical probes of adenine receptors (AdeRs), which at that time were recently discovered purinergic G-protein-coupled receptors (GPCRs; Figure 7).⁵¹ Systematic

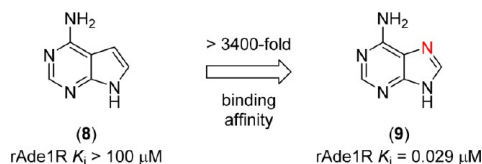


Figure 7.

SAR studies of a set of deazapurines using a competitive binding assay employing [³H]-adenine and rat cortical membrane preparations revealed that whereas both 1- and 3-deazaadenine show weak binding affinity (rAde1R $K_i = 11$ and $6.8 \mu\text{M}$, respectively), neither 7- deazaadenine (8) nor 9-deazaadenine exhibit measurable binding affinity (rAde1R $K_i > 100 \mu\text{M}$ for both compounds). This lack of binding affinity was in sharp contrast to that measured for adenine (9), which was found to bind with >3400 -fold better affinity than 8 (rAde1R $K_i = 0.029 \mu\text{M}$). The authors noted that similar observations had been made previously for 7-deazaadenosine at adenosine receptors (AdoRs).⁵² Likewise, an investigation of S-adenosyl-L-homocysteine hydrolase inhibitors reported in 2015 found that the 7-deazaadenine derivative of neplanocin A displays markedly reduced potency (>210 -fold).⁵³

Zhang and collaborators revealed in 2013 their investigations into novel naltrexone-based antagonists of the μ -opioid receptor (μ -OR), a class A GPCR (Figure 8).⁵⁴ Docking

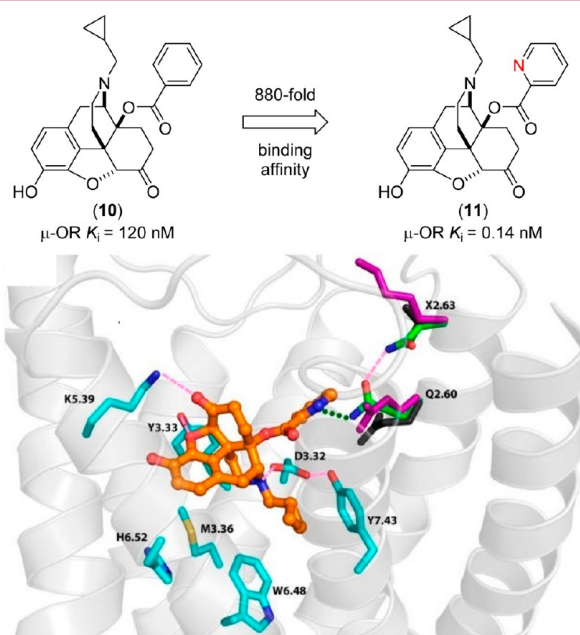


Figure 8.

studies with naltrexone using a bovine rhodopsin-based μ -OR homology model suggested that aromatic or heteroaromatic derivatives at the C14-hydroxyl group might increase binding affinity and selectivity through hydrogen bonding or π -stacking interactions with residues around extracellular loop III and the upper-level region of transmembrane helices VI and VII. A competitive binding assay using [^{3} H]-naltrexone and membrane preparations from CHO cells stably expressing murine μ -OR identified C14-benzoate analogue **10** as a moderate-affinity ligand (μ -OR K_i = 120 nM), albeit with a significant loss of binding affinity as compared to its parent naltrexone (μ -OR K_i = 0.26 nM). A N-scan around the C14-benzoate ring of **10** demonstrated that 2-aza-analogue **11** exhibits 880-fold better binding affinity than **10** (μ -OR K_i = 0.14 nM), as well as 11- and 40-fold better binding affinity than its 3-aza- and 4-aza-analogues (μ -OR K_i = 1.6 and 5.6 nM, respectively). 2-Aza-analogue **11** also displays the best selectivity against κ -OR and δ -OR (κ -OR K_i = 26 nM and δ -OR K_i = 120 nM, respectively). None of the compounds was found to show detectable agonist activity in a functional assay using [^{35} S]-GTP γ S and the cell membrane preparations described above. The favorable binding profile of **11** was explained by μ -, κ -, and δ -OR docking studies showing the formation of a propitious Asn^{2.63}-facilitated intermolecular hydrogen bonding network between **11** (orange) and Gln^{2.60} in the transmembrane helix II of μ -OR (green), which is not possible in κ -OR (black) and δ -OR (purple).

Pennington, Bartberger, and co-workers reported in 2015 their efforts to find structurally distinct GKRP-selective disrupters of glucokinase–glucokinase regulatory protein (GK–GKRP) binding, a protein–protein interaction that serves to sequester inactive GK in the nucleus of hepatocytes at low concentrations of glucose (Figure 9).⁵⁵ Early stage

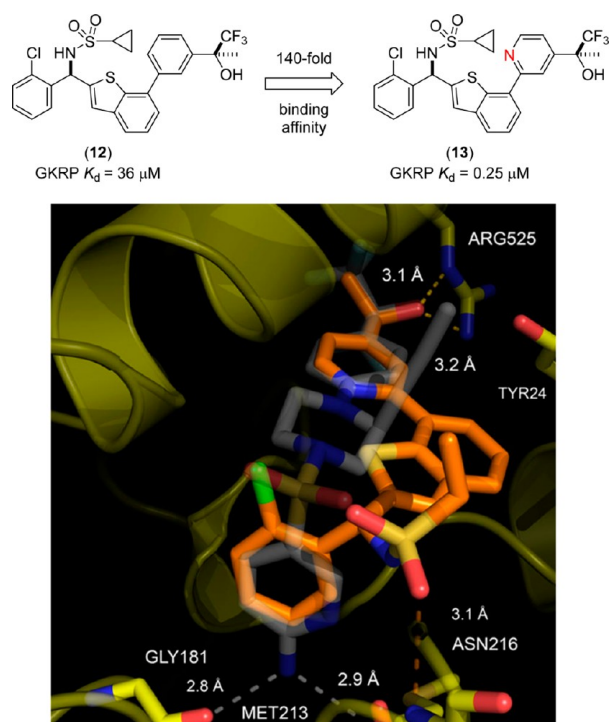


Figure 9.

optimization studies indicated that a benzodioxepinylsulfonamide group present in the HTS hit was metabolically labile and required truncation to a cyclopropylsulfonamide group. To recover dramatically reduced potency, it was proposed to append a suitably functionalized aryl moiety to C7 of a benzothioephene ring system in another part of the molecule to engage Arg525, a residue that resides in a sterically constrained region of the GKRP binding site. Density functional theory calculations and natural bond orbital analysis indicated that whereas a phenyl ring at C7 would prefer an unfavorable biaryl dihedral angle $>50^\circ$, a 2-pyridyl ring at this position would prefer a favorable dihedral angle of 0° due to the formation a $N \rightarrow S$ ($n_N \rightarrow \sigma^*_{S-X}$) interaction. Surface plasmon resonance experiments using a human GKRP construct found that whereas 7-phenylbenzothioephene **12** displays weak binding affinity (GKR P K_d = 36 μ M), 7-pyridylbenzothioephene **13** exhibits 140-fold improved binding affinity (GKR P K_d = 0.25 μ M). An overlay of the X-ray crystal structures of the *gem*-dimethyl carbinol analogue of **13** (orange) and a previously disclosed piperazine-based disrupter (transparent white) bound to hGKR P (yellow) is included in Figure 9 and shows the desired *syn*-locked orientation of the biaryl system.

Lawhorn et al. disclosed in 2015 their work to identify type I inhibitors as chemical probes of troponin I-interacting kinase (TNNI3K), a member of the tyrosine-like kinase family for which no substrates had been identified (Figure 10).⁵⁶ Initial SAR studies included probing the effects of replacing N atoms with CH groups in the purine ring system of their early lead compound. In a fluorescence polarization competitive binding assay using the fluorescent ligand 5-({[2-({[3-({[4-({5-hydroxy-2-methylphenyl)amino]-2-pyrimidinyl)amino]phenyl]-carbonyl)amino]-ethyl]amino}carbonyl)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid and a full-length human TNNI3K construct, pyrrolopyridine **14** was found to bind

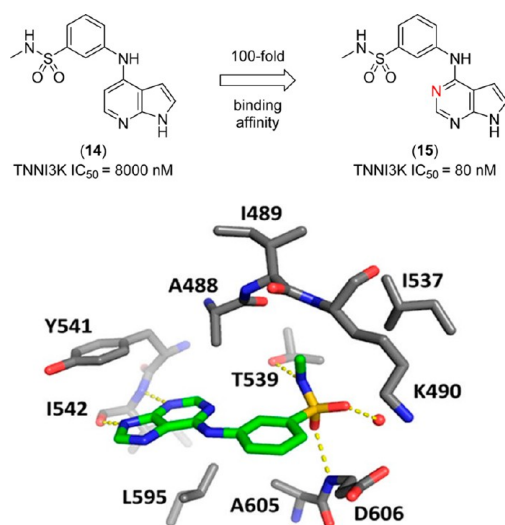


Figure 10.

with 100-fold less affinity than pyrrolopyrimidine **15** ($\text{TNNI3K IC}_{50} = 8000$ and 80 nM for **14** and **15**, respectively). The dramatic difference in binding affinity between **14** and **15** was proposed to be due to a steric clash of H1 and H2' in the coplanar aryl orientation of **14** that is required for effective binding, as illustrated in the X-ray crystal structure of their early lead compound bound to the ATP binding site of TNNI3K ($\text{TNNI3K IC}_{50} = 500 \text{ nM}$).

In Vitro Functional Activity. As discussed above, differences in ligand binding affinity can generally be explained by understanding differences in size, shape, and electrostatic complementarity of a low-energy ligand conformation with the receptor binding site, hydrogen bonding or displacement of receptor-bound water molecules, and hydrophobic and desolvation effects.²⁸ Although achieving a desired functional activity does not require knowledge of how a ligand binds to its receptor, rationalizing differences in functional activity can be complex and may require not only knowing how (and how selectively) a ligand binds to various conformations of its receptor, but also knowing the conformation and dynamics of the ligand–receptor complex, interactions or modifications of the complex with cofactors or other proteins, downstream signal transduction or feedback processes, as well as cellular permeability and efflux profiles for intracellular targets, among other possibilities. The substitution of a CH group with a N atom can lead to improvements in functional activity through separate or concerted effects on any number of the above phenomena. The case studies below illustrate the profound impact that the substitution of a CH group with a N atom can have on biochemical potency, cellular potency, target selectivity, and functional activity switches.

Biochemical Potency. Verhoest and collaborators revealed in 2009 their investigations into selective inhibitors of phosphodiesterase 10A (PDE10A), a phosphodiesterase gene family member that regulates both 3',5'-cyclic adenosine monophosphate (cAMP) and 3',5'-cyclic guanosine monophosphate (cGMP) in the medium spiny neurons of the striatum (Figure 11).⁵⁷ An HTS effort followed by further structure-guided screening of analogues of the HTS hit identified a novel class of pyrazole-based inhibitors. Remarkably, 4-phenyl analogue **16** was found to exhibit 610-fold lower

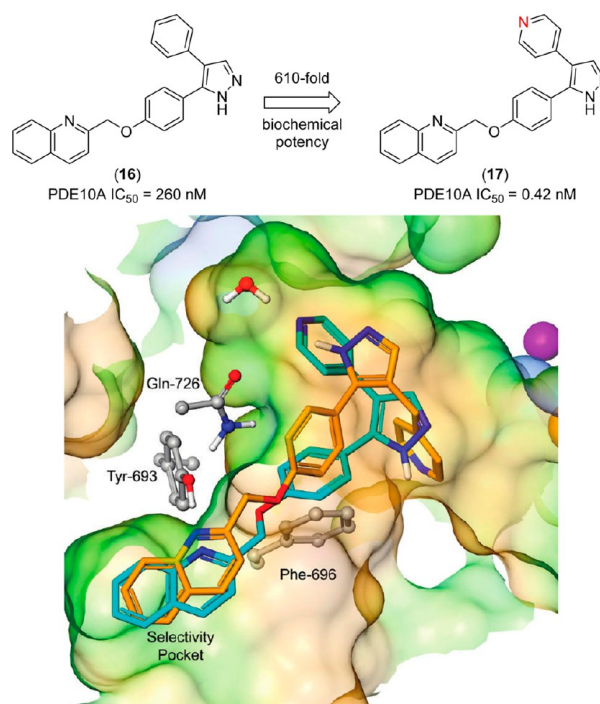


Figure 11.

potency than 4-pyridyl analogue **17** ($\text{PDE10A IC}_{50} = 260$ and 0.42 nM for **16** and **17**, respectively). This dramatic difference in potency was attributed to the pyridyl N atom of **17** forming a hydrogen bond with a water molecule that is bound to the backbone of the enzyme, as can be seen in the overlay of the X-ray crystal structures of the HTS hit (yellow) and **17** (blue) bound to the PDE10A catalytic site. Interestingly, the pyrimidinyl and pyridazinyl analogues of **17** also display markedly reduced potency ($\text{PDE10A IC}_{50} = 27$ and 12 nM , respectively), which was rationalized by their weaker HBA strengths versus **17** as determined by quantum mechanical calculations. Inhibitor **17** also shows >1000-fold selectivity over the other PDEs.

Vanotti and co-workers reported in 2008 their efforts to obtain the first inhibitors of cell division cycle 7 (Cdc7) kinase, which is involved in the phosphorylation and activation of the minichromosome maintenance complex (Figure 12).⁵⁸ A screening effort led to the identification of 4-pyridyl-substituted pyrrolopyridinone **19** ($\text{Cdc7 IC}_{50} = 0.010 \mu\text{M}$), which was found to be >500-fold more potent than its phenyl-substituted analogue **18** ($\text{Cdc7 IC}_{50} > 5.0 \mu\text{M}$). A three-dimensional homology model of Cdc7 based upon its similarity to other kinases and including water molecules was constructed and used to determine the binding mode of **19**. Using this homology model, the more favored docking arrangement of **19** had the pyridine N atom interacting with the hinge region of Cdc7 and accounted for the SAR trends, including the lack of measurable potency for **18** and the 3-pyridyl analogue of **18** ($\text{Cdc7 IC}_{50} > 5.0 \mu\text{M}$).

Pierre et al. disclosed in 2011 their studies to identify selective, ATP-competitive inhibitors of protein kinase CK2, a highly conserved protein kinase that is a heterotetrameric complex composed of different combinations of two catalytic isoforms and two regulatory subunits (Figure 13).⁵⁹ By comparison of the chemical structures of known CK2 inhibitors

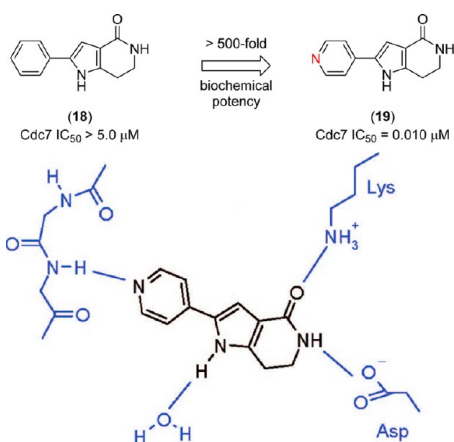


Figure 12.

to internal compounds, a promising lead series was identified. Focused SAR efforts guided by a published X-ray crystal structure of an architecturally related inhibitor bound to CK2 led to the discovery of substituted 4-aminothieno[3,2-*c*]-quinoline-7-carboxylate scaffolds with promising activity. Surprisingly, bioisosteric replacement of the fused thiophene

ring with a fused phenyl ring to give **20** resulted in a dramatic reduction in potency ($\text{CK2 IC}_{50} = 0.092$ and $>1.0 \mu\text{M}$, respectively). A N-scan on the newly installed fused phenyl ring of **20** revealed aza-analogue **21** with >170-fold improved potency versus **20** ($\text{CK2 IC}_{50} = 0.0060 \mu\text{M}$). Notably, the other three aza-analogues of **21** exhibit profoundly reduced potency ($\text{CK2 IC}_{50} > 0.50 \mu\text{M}$). This dramatic difference in potency was attributed to the newly installed N atom of **21** forming a hydrogen bond with Val116 in the hinge region of the enzyme, as can be seen in the optimized binding model of the 3-chloroaniline analogue of **21** (green) in the ATP binding pocket of CK2.

Ellis and collaborators revealed in 2015 their investigations into selective inhibitors of spleen tyrosine kinase (Syk), an enzyme that plays a key role in the immune response through its involvement in Fc and B cell receptor signaling (Figure 14).⁶⁰ A survey of reported Syk inhibitors, including an analysis of published X-ray crystal structures of inhibitors bound to Syk, followed by a focused SAR effort inspired by a known series of 3,5-diaminoheteroarylcarboxamides led to the identification of a promising series of 3,5-diaminopicolinamides represented by **22** ($\text{Syk IC}_{50} = 8.8 \text{ nM}$). Notably, aza-analogue **23** was found to exhibit 150-fold better potency than **22** ($\text{Syk IC}_{50} = 0.060 \text{ nM}$). This dramatic difference in potency was ascribed to an

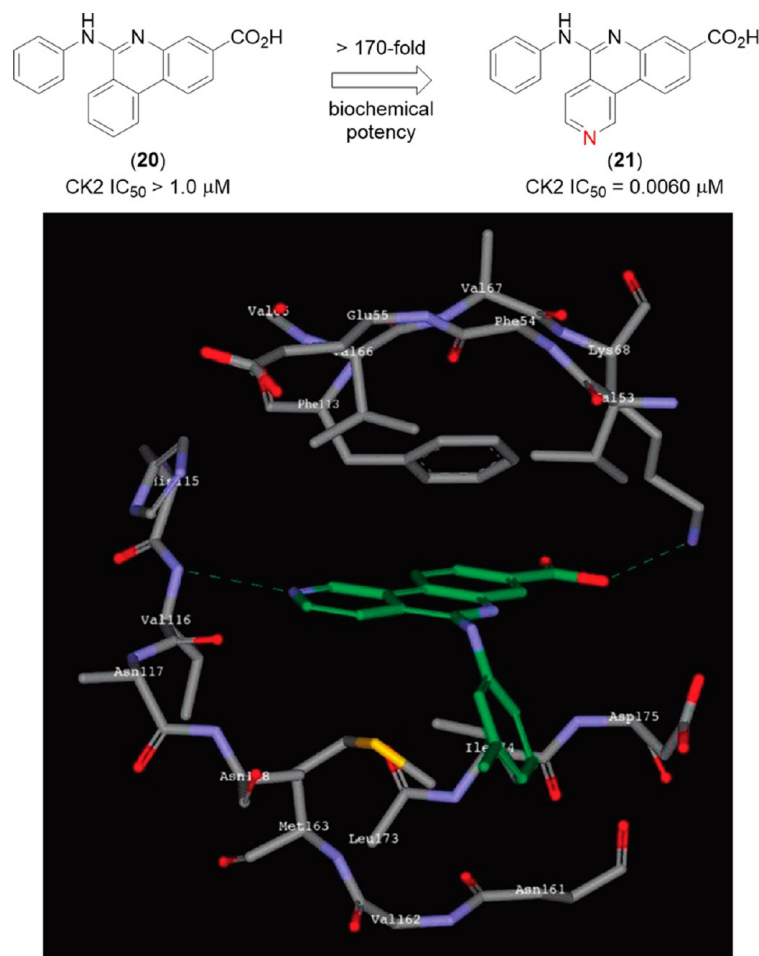


Figure 13.

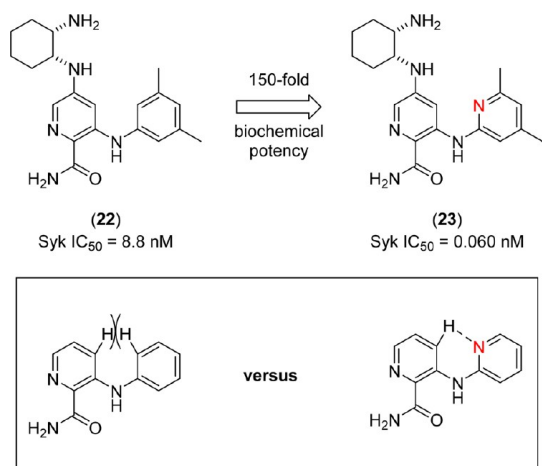


Figure 14.

unfavorable steric clash of H4 and H2' in the coplanar aryl orientation of **22** required for effective binding to Syk, which is replaced by a favorable intramolecular hydrogen bond between H4 and N2 in **23**. Interestingly, the aromatic N atom of the picolinamide core is also necessary for potency (Syk IC₅₀ = 2.8 nM for the des-aza-analogue of **23**), which was proposed to arise by the N atom helping to enforce a favorable conformation of the carboxamide moiety as well as decreasing its desolvation penalty.

D'Angelo and co-workers reported in 2011 their efforts to find dual inhibitors of phosphoinositide 3-kinase α (PI3K α), a lipid kinase involved in the regulation of cell proliferation, survival, and translation, and the mammalian target of rapamycin (mTOR), a member of the phosphatidylinositol 3-kinase-related kinase family that is also involved in the regulation of these cellular processes (Figure 15).⁶¹ An HTS effort followed by extensive SAR studies guided by an X-ray crystal structure of the screening hit bound to PI3K γ , the structure of which is likely closely related to that of PI3K α ,⁶¹ led to the evaluation of phenyl-linked sulfonamide **24** (PI3K α K_i = 48 nM; mTOR IC₅₀ = 1500 nM). A partial N-scan on the phenyl ring of **24** identified pyridyl-linked sulfonamide **25**

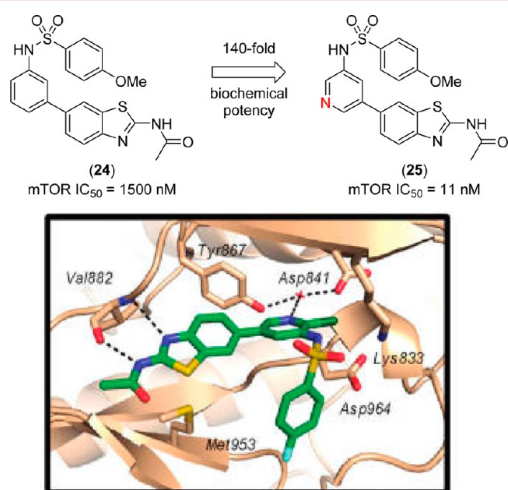


Figure 15.

displaying 30- and 140-fold improved potency toward PI3K α and mTOR, respectively (PI3K α K_i = 1.6 nM; mTOR IC₅₀ = 11 nM). An X-ray cocrystal structure of PI3K γ and a close analogue of **25** bearing a Cl atom at C2 of the pyridine ring and having a F atom in place of the MeO group on the sulfonamide moiety reveals the pyridyl N atom engaging in a hydrogen bond with an ordered water molecule located between Tyr867 and Asp 841 in the affinity pocket of the enzyme.

Cellular Potency. Ho et al. disclosed in 2015 their studies to identify inhibitors of porcupine (PORCN), a membrane-bound O-acyltransferase that is essential for the initial step in the activation and secretion of Wnt proteins (Figure 16).⁶² A cell-based HTS campaign followed by an SAR study included the evaluation of pyrrole **26**, which compared to the imidazole screening hit **27** displays >3300-fold lower cellular potency (HEK293-STF3A IC₅₀ > 50 μ M for **26** and IC₅₀ = 0.015 μ M for **27**). An overlay of a low energy conformation of **27** (gray) and two other known PORCN inhibitors (green and lavender) suggests that they fit into a common pharmacophore, in which the imidazole N atom of **27** serves as a HBA in PORCN. Further optimization of **27** was required to ameliorate CYP inhibition, increase permeability, and improve solubility, among other properties, for which the strategic substitution of several CH groups with N atoms played a key role.

Hodgetts and collaborators revealed in 2010 their investigations into antagonists of the transient receptor potential cation channel, subfamily V, member 1 (TRPV1), a non-selective cation channel that can be activated by a wide variety of stimuli (Figure 17).⁶³ To attempt to improve oral exposure, reduce mutagenicity risks, and increase structural novelty, a N-scan SAR study of a quinazoline-based lead compound bearing an aniline moiety was undertaken. Remarkably, the substitution of a single CH group with a N atom in [1,5]naphthyridine analogue **28** to give pyrido[2,3-*b*]pyrazine **29** resulted in a 1300-fold improvement in cellular potency (hTRPV1 IC₅₀ = 310 and 0.23 nM for **28** and **29**, respectively). Interestingly, **29** displays significantly lower solubility than **28** in 0.10 N HCl (S_{0.10 N HCl} = 38 and 8.0 μ g/mL for **28** and **29**, respectively).

Dandapani and co-workers reported in 2014 their efforts to find growth inhibitors of *Trypanosoma cruzi* (*T. cruzi*), the parasite that causes Chagas disease (Figure 18).⁶⁴ A phenotypic HTS campaign of ~100000 compounds prepared by diversity-oriented synthesis (DOS) followed by SAR studies led to the evaluation of benzamide **30**, which was found to exhibit 500-fold lower cellular potency as compared to its 4-pyridylamide analogue **31** (*T. cruzi* IC₅₀ = 500 and 1.0 nM for **30** and **31**, respectively). The 2- and 3-pyridylamide analogues of **30** also show significantly reduced cellular potency compared to **31** (*T. cruzi* IC₅₀ = 340 and 45 nM, respectively). Interestingly, only the 3-pyridylamide analogue displays good solubility in phosphate-buffered saline solution (S_{PBS} < 1.0 μ M for **30**, **31**, and the 2-pyridylamide analogue; S_{PBS} = 57 μ M for the 3-pyridylamide analogue).

Ai, Geng, Duan et al. disclosed in 2016 their studies to identify selective inhibitors of fibroblast growth factor receptors (FGFRs), a family of four highly conserved transmembrane tyrosine kinase receptors that upon fibroblast growth factor (FGF) binding undergo dimerization and autophosphorylation, resulting in the activation of several downstream signaling pathways that have roles in cell survival, differentiation, proliferation, and migration (Figure 19).⁶⁵ A scaffold-hopping approach based on a known FGFR inhibitor led to the identification of indazole **32**, which was found to exhibit 11-fold

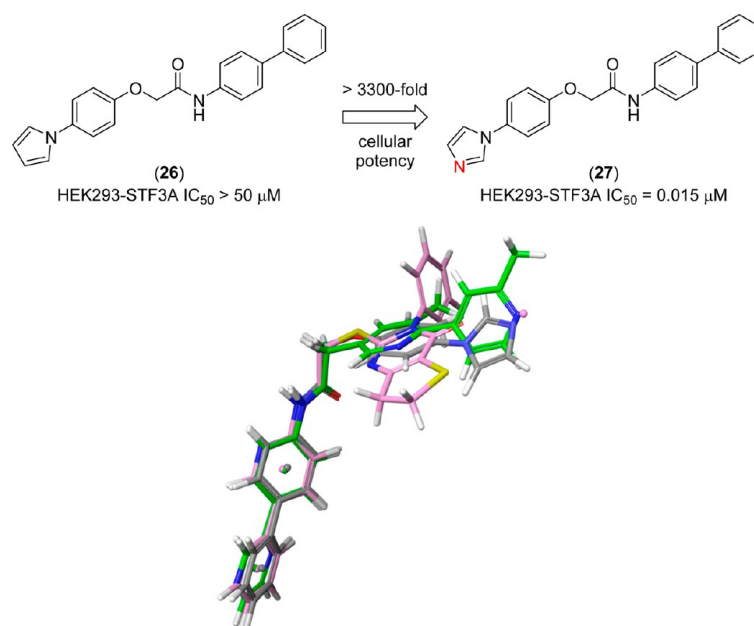


Figure 16.

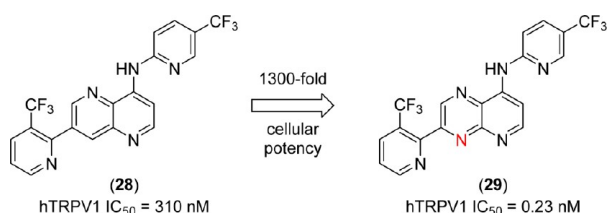


Figure 17.

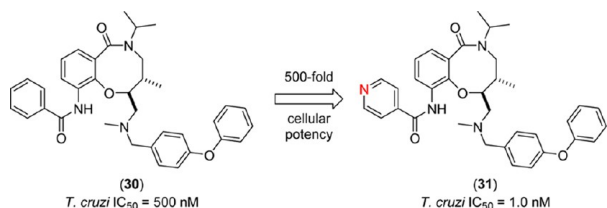


Figure 18.

lower enzymatic potency than pyrazolo[3,4-*b*]pyridine 33 (FGFR1 $IC_{50} = 3.3$ and 0.30 nM for 32 and 33, respectively). Even more remarkable was the finding of a 190-fold difference in cellular potency between 32 and 33 (H1581 cell $IC_{50} = 320$ and 1.7 nM for 32 and 33, respectively). The modeled binding pose of 33 in the ATP site of FGFR1 using a reported crystal structure of the FGFR1 kinase domain is shown in Figure 19. It is unclear from this model what role the additional N atom plays in the improved potency observed for 33.

Fox and collaborators revealed in 2015 their investigations into selective antagonists of the prostaglandin E_2 (PGE₂) receptor subtype 2 (EP2), the activation of which by PGE₂ is believed to be involved in inhibiting microglial-mediated phagocytosis and clearance of amyloid- β plaques in Alzheimer's disease (AD) as well as increasing inflammation and oxidative damage in AD and other neurodegenerative diseases (Figure 20).⁶⁶ An HTS campaign resulted in the identification of 3-

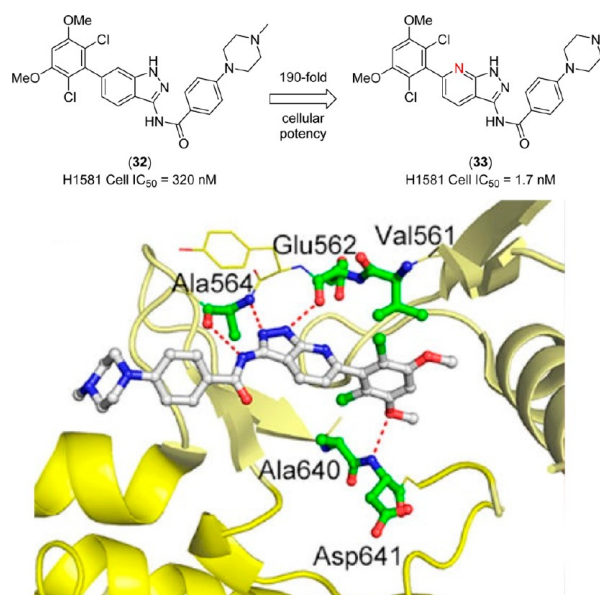


Figure 19.

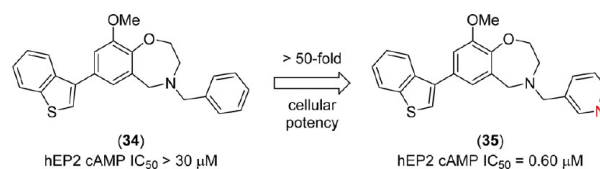


Figure 20.

pyridyl-substituted benzodioxepine 35, which was found to display 140-fold improved binding affinity versus phenyl-substituted analogue 34 in a scintillation proximity assay (SPA) using ³H-PGE₂ and membrane preparations from ECV304 cells overexpressing hEP2 (hEP2 SPA $IC_{50} = 10$ and

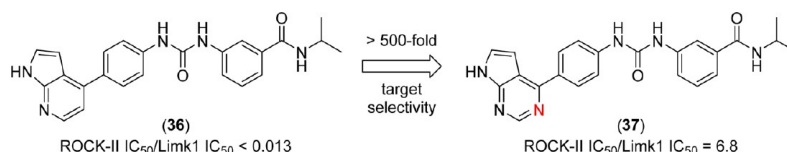


Figure 21.

0.070 μ M for **34** and **35**, respectively). Using the same cells in a functional assay measuring inhibition of PGE₂-stimulated cAMP, **35** exhibits >50-fold improved potency versus **34** (hEP2 cAMP IC_{50} > 30 μ M and IC_{50} = 0.60 μ M for **34** and **35**, respectively). Further optimization of **35** was required to ablate its potent inhibition of CYPs 3A4 and 2D6.

Target Selectivity. LoGrasso, Feng, and co-workers reported in 2015 their efforts to find selective inhibitors of LIM kinase (Limk), a serine–threonine protein kinase involved in the dynamic regulation of actin cytoskeleton and cellular motility by phosphorylating and inactivating its substrate cofilin (Figure 21).⁶⁷ SAR studies stemming from a previously reported Rho kinase (ROCK) inhibitor included the evaluation of pyrrolopyrimidine **36**, which displays selective inhibition of ROCK-II over Limk1 (ROCK-II IC_{50} = 130 nM; Limk1 IC_{50} > 10000 nM). Incredibly, pyrrolopyrimidine analogue **37** was found to exhibit dramatically reversed selectivity (ROCK-II IC_{50} = 1400 nM; Limk1 IC_{50} = 200 nM). Further optimization efforts preserving the hinge-binding pyrrolopyrimidine moiety of **37** led to the identification of even more potent and selective Limk inhibitors. Docking studies of lead compounds using the X-ray crystal structure of Limk1 revealed that they are all type I ATP-competitive kinase inhibitors and that a hydrogen bonding interaction between the newly installed N atom and the side chain hydroxyl group of Thr413 is plausible and could explain the ROCK-II/Limk1 selectivity switch but would require rotation of the side chain.

Li et al. disclosed in 2014 their studies to identify selective dual inhibitors of FMS-like tyrosine kinase 3 (FLT3), a member of the receptor tyrosine kinase class III family, and cyclin-dependent kinase 4 (CDK4), as these two kinases share similar binding domains and synergistic activity had been observed previously by inhibiting both enzymes in disease-relevant cell lines (Figure 22).⁶⁸ An SAR effort starting with a

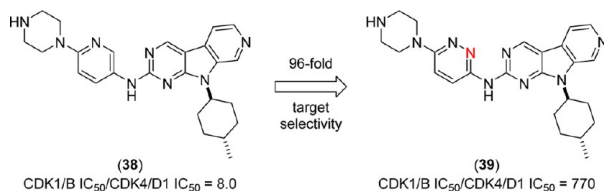


Figure 22.

previously disclosed inhibitor of CDK4/6 and including a partial N-scan led to the identification of aminopyridine **38** (CDK4/D1 IC_{50} = 0.0030 μ M; FLT3 IC_{50} = 0.00030 μ M), which was found to display undesirably poor selectivity against CDK1 (CDK1/B IC_{50} = 0.024 μ M). Remarkably, aminopyridazine analogue **39** was discovered to exhibit not only excellent potency toward CDK4 and FLT3 (CDK4/D1 IC_{50} = 0.0020 μ M; FLT3 IC_{50} = 0.0060 μ M) but also dramatically improved selectivity against CDK1 (CDK1/B IC_{50} = 1.5 μ M).

No explanation was provided for the improved selectivity against CDK1.

Henry and collaborators revealed in 2015 their investigations into pan-RAF inhibitors (BRAF V600E, BRAF, and CRAF), which unlike previously reported selective inhibitors of BRAF V600E should possess minimal paradoxical activation and activity against wild-type BRAF or mutant RAS tumor cells (Figure 23).⁶⁹ As a surrogate indicator of broader kinase selectivity, activity against the tyrosine kinase KDR was also monitored. Beginning with a previously identified dual inhibitor of BRAF V600E and CRAF, an SAR investigation led to the identification of naphthyridine **40** (BRAF V600E IC_{50} = 0.018 μ M; KDR IC_{50} = 0.17 μ M). Additional modification of the hinge binder moiety afforded pyridopyrimidine **41**, which was found to exhibit better potency toward BRAF V600E and profoundly improved selectivity against KDR compared to **40** (BRAF V600E IC_{50} = 0.0058 μ M; KDR IC_{50} = 3.9 μ M). Fortuitously, both compounds were shown to display similar potency toward wild-type BRAF and CRAF (BRAF IC_{50} = 0.028 and 0.015 μ M for **40** and **41**, respectively; CRAF IC_{50} = 0.0056 and 0.0043 μ M for **40** and **41**, respectively). Although an X-ray crystal structure of **41** bound to BRAF V600E was obtained and shows a bidentate hydrogen bonding interaction with a water molecule near Phe583 involving the newly installed N atom, no explanation was provided for the improved selectivity that **41** exhibits against KDR.

Morales, Durden, and co-workers reported in 2013 their efforts to find selective inhibitors of phosphatidylinositol-3-kinase α (PI3K α), one of several PI3K isoforms that convert phosphatidylinositol(4,5)-bisphosphate (PIP2) into phosphatidylinositol(3,4,5)-trisphosphate (PIP3; Figure 24).⁷⁰ An SAR effort commencing with the bioisosteric replacement of a fused phenyl ring in a known pan-PI3K inhibitor with a fused thiophene ring gave **42**, which was found to display similar potency and selectivity across the PI3K isoforms (PI3K α IC_{50} = 300 nM; PI3K β IC_{50} = 380 nM; PI3K δ IC_{50} = 780 nM; PI3K γ IC_{50} = 1600 nM). Interestingly, 3-aza-analogue **43** was shown to be equipotent toward PI3K α but to exhibit much better selectivity against PI3K δ as well as modestly improved selectivity against PI3K β and PI3K γ (PI3K α IC_{50} = 290 nM; PI3K β IC_{50} = 1000 nM; PI3K δ IC_{50} > 50000 nM; PI3K γ IC_{50} = 8800 nM). The 4-aza-analogue of **42** was found to display similar, but less favorable, potency and selectivity profiles as compared to **43** (PI3K α IC_{50} = 680 nM; PI3K β IC_{50} = 2600 nM; PI3K δ IC_{50} > 23000 nM; PI3K γ IC_{50} = 5500 nM). An overlay of the modeled binding poses of the progenitor inhibitor (green) and its thienyl bioisostere **42** (gray) in a PI3K γ -based homology model of the PI3K α binding site (gray) does not provide a clear explanation for the observed selectivity differences of **42** and **43**.

O'Donnell, Rogers et al. disclosed in 2010 their studies to identify selective agonists of the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR), a homopentameric ligand-gated ion channel composed of five $\alpha 7$ subunits (Figure 25).⁷¹ Bioisosteric replacement of an aryl carbamate group with a

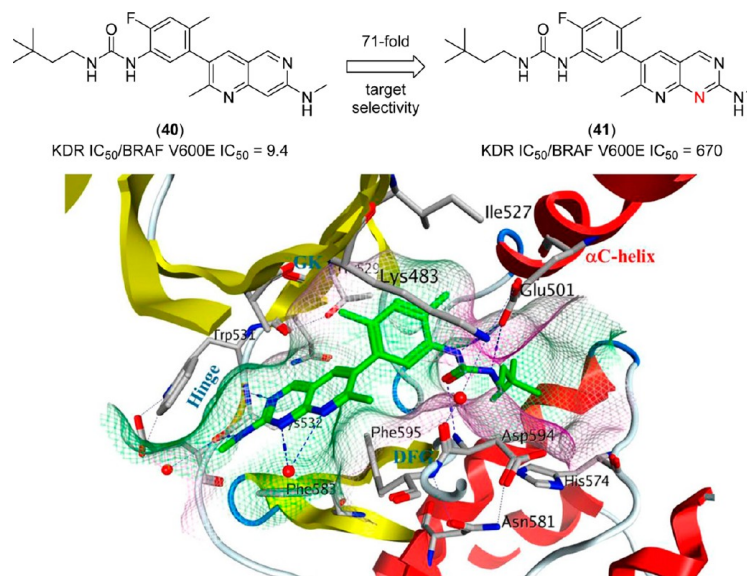


Figure 23.

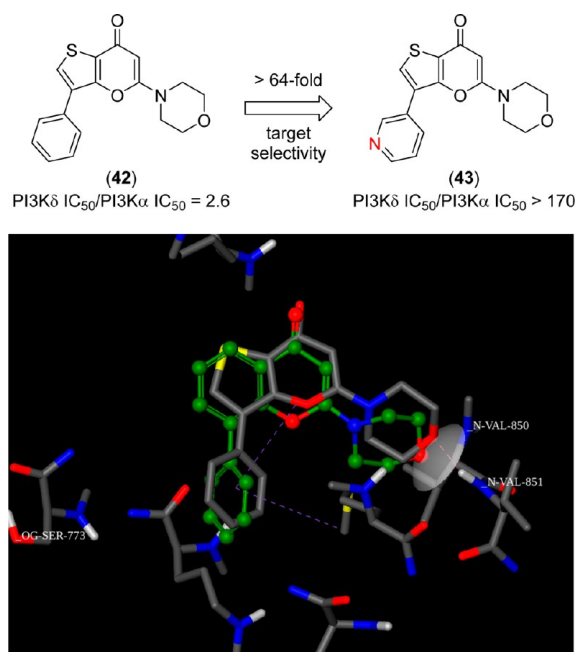


Figure 24.



Figure 25.

benzoxazole ring system in a previously explored 1,4-diazabicyclo[3.2.2]nonane series of agonists gave compound 44, which was shown to exhibit good affinity and full agonism for α₇ nAChR but with undesirable equal affinity for the 5-HT₃

receptor (α₇ nAChR K_i = 23 nM; α₇ nAChR E_{max} = 180%; 5-HT₃R K_i = 13 nM). A N-scan revealed that 4-azabenzoxazole analogue 45 maintains good affinity and full agonism for α₇ nAChR but shows dramatically improved selectivity against 5-HT₃R (α₇ nAChR K_i = 30 nM; α₇ nAChR E_{max} = 220%; 5-HT₃R K_i = 580 nM). The other aza-analogues of 44 were found to display reduced affinity for α₇ nAChR and/or lower selectivity against 5-HT₃R.

Functional Activity Switch.⁷² Fabis and collaborators revealed in 2012 their investigations into selective high-affinity antagonists of the serotonin 4 receptor subtype (5-HT₄R), a class A GPCR expressed both in the periphery and the CNS, where it is involved in a variety of neurological processes (Figure 26).⁷³ An SAR effort aimed at identifying ligands suitable for the brain imaging of 5-HT₄R started with evaluating substituted derivatives of a previously studied phenanthridine scaffold and led to the identification of fluoro-analogue 46, which displays good binding affinity and inverse agonist activity (h5-HT₄R K_i = 3.1 nM; h5-HT₄R EC₅₀ = 63 nM). Substitution of the C10 CH group with a N atom gave aza-analogue 47, which shows profoundly improved binding affinity compared to 46 as well as potent antagonist activity (h5-HT₄R K_i = 0.040 nM; h5-HT₄R K_B = 0.025 nM). Aza-analogue 47 was also found to display excellent selectivity against a panel of 11 different 5-HT receptor subtypes (≥3400-fold). The authors cautioned that the different intrinsic activity observed for 46 and 47 could be due to the different constitutive activity of the cloned 5-HT₄ receptors used in the functional activity measurements, as each compound was evaluated at a separate location using a different engineered cellular system. It is also worth noting that the 5-HT₄R competitive binding assays for 46 and 47 were conducted at separate locations using different engineered cellular systems, although both laboratories used the same radioligand. The modeled binding pose of aza-analogue 47 (green) in a β₂-adrenergic receptor-based homology model of the 5-HT₄R binding site (multiple colors) suggests that the improved binding affinity of 47 versus 46 may arise from a hydrogen bond that the newly installed N atom of 47 can make with Ser197.

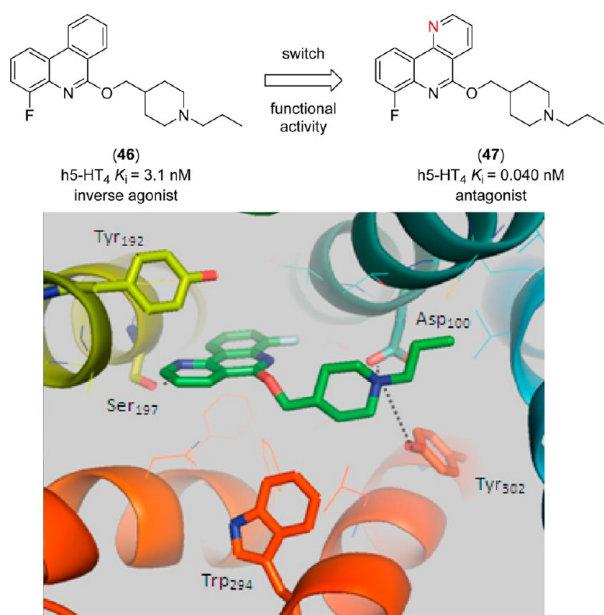


Figure 26.

Reid, Fairlie, and co-workers reported in 2014 their efforts to find agonists and antagonists with suitable properties for use as chemical probes of the human complement component 3a receptor (C3aR), a class A GPCR that is a key element of immune, inflammatory, and metabolic processes (Figure 27).⁷⁴

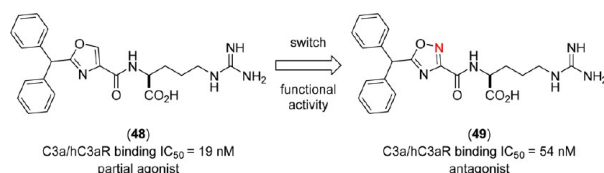


Figure 27.

Their SAR investigation commenced from a previously reported antagonist for which an embedded ether O atom was speculated to be a critical HBA for binding affinity and functional activity. Five-membered heteroaromatic rings were proposed as bioisosteres of the O atom, with the potential benefit of reducing the number of rotatable bonds and tuning the basicity of the HBA atoms in the heteroaromatic rings. Interestingly, oxazole **48** was found to exhibit 2.8-fold better binding affinity than oxadiazole analogue **49** but also to display partial agonist activity rather than the antagonist activity exhibited by **49** (C3a/hC3aR $IC_{50} = 19$ and 54 nM for **48** and **49**, respectively; hC3aR Ca^{2+} flux $E_{max} \sim 50\%$ for **48**; hC3aR Ca^{2+} flux $IC_{50} = 1.2$ μ M for **49**). These findings were in line with their predictions based on ab initio calculations of the relative hydrogen bond interaction energies of the oxazole and oxadiazole N atoms of **48** and **49** with a molecule of water ($IE = -5.7$ and -5.1 kcal/mol, respectively). These trends were also observed for the other heteroaromatic rings examined in this study.

Zhang et al. disclosed in 2009 their studies to identify potent and selective naltrexamine-based antagonists of the μ -opioid receptor (μ -OR), a class A GPCR (Figure 28).⁷⁵ Docking studies with naltrexone using a bovine rhodopsin-based μ -OR

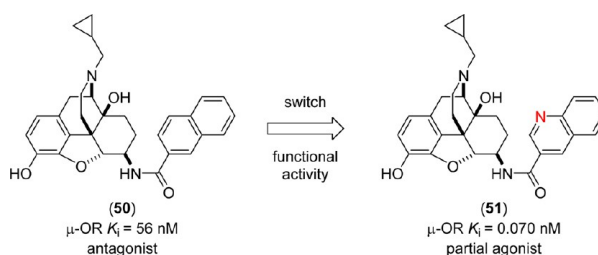


Figure 28.

homology model suggested that heteroaromatic amide derivatives at C6 of the naltrexone scaffold might increase binding affinity and selectivity through hydrogen bonding and/or π -stacking interactions with aromatic residues in a binding pocket formed by extracellular loops II and III and trans-membrane helix VII. SAR efforts including partial N-scans found that naphthalene amide **50** displays 800-fold lower binding affinity than quinoline amide **51** in an assay using membrane preparations from CHO cells stably expressing μ -OR (μ -OR $K_i = 56$ and 0.070 nM versus [³H]-naltrexone for **50** and **51**, respectively). Quinoline amide **51** was also shown to exhibit partial agonist activity using the same membrane preparations (μ -OR GTP γ S $EC_{50} = 0.29$ nM; $E_{max} = 200\%$ versus basal and 53% versus DAMGO). The functional activity of naphthalene amide **50** was not assessed in this study.⁷⁵ A separate report from another laboratory determined that naphthalene amide **50** displays high binding affinity (μ -OR $K_i = 1.3$ pM versus [³H]-DAMGO), exhibits potent antagonism of DAMGO-stimulation (μ -OR Ca^{2+} flux POC $\sim 80\%$ with 0.10 pM **50**), and shows negligible agonist activity (μ -OR Ca^{2+} flux $EC_{50} > 1000$ nM) in this series of assays using HEK293 cells stably expressing human μ -OR.⁷⁶ Interestingly, in an assay using membrane preparations from the same HEK293 cells stably expressing human μ -OR, naphthalene amide **50** was found to display some agonist activity (μ -OR GTP γ S $EC_{50} = 0.30$ nM; $E_{max} \sim 100\%$ versus basal). The evaluation of these compounds at separate laboratories using different engineered cellular systems and reference standards makes it difficult to determine if this example represents a true functional activity switch.

In Vitro PK/ADME Profile. Contemporary drug design requires simultaneous optimization of molecular, physicochemical, pharmacokinetic, and metabolite properties alongside binding affinity and functional activity profiles.^{26,77} Although the effects of substituting a CH group with a N atom on basicity, lipophilicity, polar surface area, and hydrogen bonding capacity are relatively predictable,^{7,13–18} effects on aqueous solubility, passive permeability, active transport, protein binding, and metabolic stability can be more capricious.^{26,44,77} These properties are particularly important to consider for therapeutic targets located in the central nervous system (CNS) behind the blood–brain barrier (BBB).⁷⁸ A number of approaches for overcoming pharmacological challenges arising from problematic structural elements or suboptimal properties of bioisosteres have been developed.¹² For example, high PSA and multiple HBAs and HBDs can be detrimental for passive permeability and Pgp-mediated efflux.^{38,78,79} Perhaps counter-intuitively, the substitution of a CH group with a N atom can actually improve permeability and/or efflux profiles in some instances if the newly installed N atom can properly tune lipophilicity or HBA/HBD strength, or form an intramolecular

hydrogen bond with a HBD in another part of the ligand.^{29,78,80} The case studies below illustrate the dramatic impact that the substitution of a CH group with a N atom can have on aqueous solubility, cellular permeability, protein binding, and metabolic stability.

Aqueous Solubility. Wagner and collaborators revealed in 2013 their investigations into inhibitors of cytokine-mediated STAT1 signal transduction in β -cells, with the aim of suppressing β -cell apoptosis (Figure 29).⁸¹ An HTS campaign

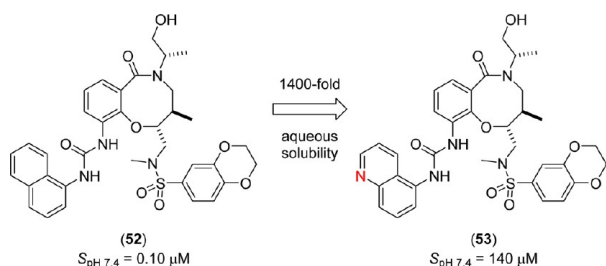


Figure 29.

using a phenotypic assay measuring β -cell viability in the presence of three different cytokines identified a hit molecule initially prepared by DOS. A lead optimization effort resulted in the discovery of naphthyl urea **52** ($\text{IC}_{50} = 1.7 \mu\text{M}$; $I_{\text{max}} = 71\%$), which was found to exhibit poor solubility in a thermodynamic solubility assay using phosphate-buffered saline solution ($S_{\text{pH } 7.4} = 0.10 \mu\text{M}$). A N-scan around the naphthyl ring of **52** led to the discovery of quinolyl urea **53** ($\text{IC}_{50} = 1.6 \mu\text{M}$; $I_{\text{max}} = 41\%$), which was shown to display a remarkable 1400-fold improvement in aqueous solubility ($S_{\text{pH } 7.4} = 140 \mu\text{M}$). Interestingly, the aqueous solubility at pH 7.4 of the seven aza-analogues spanned a broad range from $0.70 \mu\text{M}$ for the 1-isoquinolyl analogue to $140 \mu\text{M}$ for 5-quinolyl analogue **53**.

Traquandi and co-workers reported in 2010 their efforts to find inhibitors of cyclin-dependent kinases (CDKs), which phosphorylate a wide variety of proteins involved in cell cycle and function regulation, as well as transcriptional regulation (Figure 30).⁸² Unfortunately, a lead compound **54** exhibiting

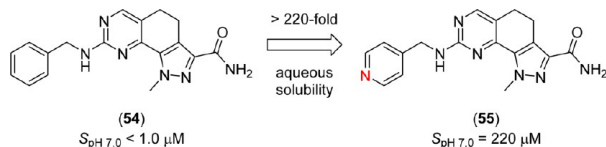


Figure 30.

interesting biochemical and cellular CDK activity (CDK2/CyA $\text{IC}_{50} = 0.051 \mu\text{M}$; A2780 cell $\text{IC}_{50} = 0.93 \mu\text{M}$) was found to exhibit poor solubility in a nonequilibrium solubility assay using buffered aqueous solution ($S_{\text{pH } 7.0} < 1.0 \mu\text{M}$). An SAR effort including a focus on improving physicochemical properties was undertaken. A N-scan on the phenyl ring of **54** afforded 4-pyridyl analogue **55**, which was found to exhibit similar biochemical and cellular potency as **54** (CDK2/CyA $\text{IC}_{50} = 0.021 \mu\text{M}$; A2780 cell $\text{IC}_{50} = 1.2 \mu\text{M}$) but to show >220-fold better aqueous solubility ($S_{\text{pH } 7.0} = 220 \mu\text{M}$). The aqueous solubility at pH 7.0 of the 2- and 3-pyridyl analogues of **54** was found to be 120 and $140 \mu\text{M}$, respectively.

Sugane et al. disclosed in 2013 their studies to identify selective inhibitors of glycine transporter 1 (GlyT1), a specific

sodium/chloride-dependent transporter that terminates glycine activity by mediating its uptake in synapses (Figure 31).⁸³ An

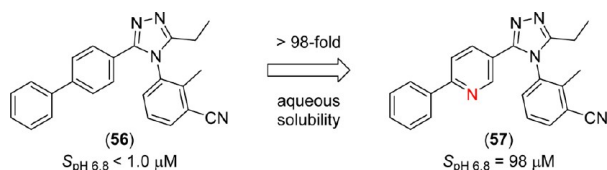


Figure 31.

HTS campaign followed by a lead optimization effort led to the identification of cyano-substituted analogue **56** (rGlyT1 $\text{IC}_{50} = 0.49 \mu\text{M}$; rGlyT2 $\text{IC}_{50} > 30 \mu\text{M}$), which was shown to display poor solubility in buffered aqueous solution ($S_{\text{pH } 6.8} < 1.0 \mu\text{M}$). A N-scan study on a precursor of **56** suggested that inserting a N atom into the internal phenyl ring of the biphenyl moiety should impart improved aqueous solubility. Fortunately, aza-analogue **57** was found to exhibit both improved GlyT1 activity (rGlyT1 $\text{IC}_{50} = 0.16 \mu\text{M}$; rGlyT2 $\text{IC}_{50} > 30 \mu\text{M}$) and markedly better aqueous solubility ($S_{\text{pH } 6.8} = 98 \mu\text{M}$). Further studies revealed that under physiological conditions **57** exists as a stable pair atropisomers at the biaryl linkage off the N atom of the triazole ring, with the *R* isomer being the eutomer (rGlyT1 $\text{IC}_{50} = 0.064 \mu\text{M}$; rGlyT2 $\text{IC}_{50} > 30 \mu\text{M}$) and *S* isomer being the distomer (rGlyT1 $\text{IC}_{50} = 20 \mu\text{M}$; rGlyT2 $\text{IC}_{50} > 30 \mu\text{M}$). As expected, the aqueous solubility of the separated atropisomers was identical to the racemate ($S_{\text{pH } 6.8} = 95$ and $94 \mu\text{M}$ for the *R* and *S* isomers, respectively).

Yokokawa, Jiricek, and collaborators revealed in 2013 their investigations into compounds effective against *Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis (Figure 32).⁸⁴ A whole-cell HTS campaign using *Mycobacterium bovis*

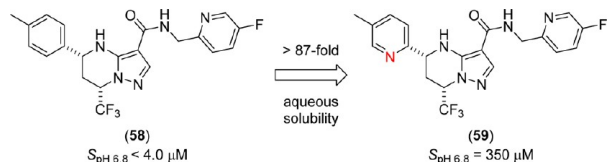


Figure 32.

BCG as a surrogate for Mtb followed by an SAR effort against Mtb included the evaluation of tetrahydropyrazolo[1,5-*a*]-pyrimidine **58** (Mtb MIC = $0.83 \mu\text{M}$), which was found to exhibit poor solubility in an equilibrium solubility assay using buffered aqueous solution ($S_{\text{pH } 6.8} < 4.0 \mu\text{M}$). Unfortunately, although aza-analogue **59** was found to show a >87-fold improvement in aqueous solubility ($S_{\text{pH } 6.8} = 350 \mu\text{M}$), it was also shown to display a dramatic loss of inhibitory potency (Mtb MIC = $52 \mu\text{M}$). These results were somewhat surprising given that the analogue bearing a 2-pyridyl ring on the left-hand side and a phenyl ring on the right-hand side and the analogue with a phenyl ring on both the left-hand side and the right-hand side were shown to exhibit good potency against Mtb but low aqueous solubility (Mtb MIC = 0.44 and $0.15 \mu\text{M}$, respectively; $S_{\text{pH } 6.8} = 9.0 \mu\text{M}$ and $S_{\text{pH } 6.8} < 4.0 \mu\text{M}$, respectively).

Cushman and co-workers reported in 2011 their efforts to find inhibitors of topoisomerase I (Top1), a ubiquitous cellular enzyme that produces reversible single-strand cuts in DNA during vital processes including replication, transcription, and repair (Figure 33).⁸⁵ An SAR effort stemming from a previously

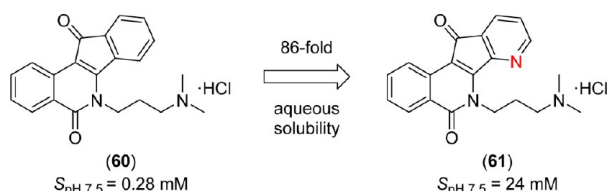


Figure 33.

identified indenoisoquinoline series of inhibitors was undertaken with the aim of simultaneously improving inhibitory activity and aqueous solubility. Ab initio calculations suggested that increasing aromatic ring electron affinity by the substitution of a CH group with a N atom in the upper right-hand ring of indenoisoquinoline **60** (Top1 cleavage = ++; $S_{\text{pH } 7.5} = 0.28 \text{ mM}$) would facilitate charge transfer complex formation and improve π -stacking interactions with DNA base pairs. Gratifyingly, 7-aza-indenoisoquinoline analogue **61** was found to display similar inhibitory activity and 86-fold improved solubility in an equilibrium solubility assay using buffered aqueous solution (Top1 cleavage = +++; $S_{\text{pH } 7.5} = 24 \text{ mM}$). This finding is particularly remarkable given the presence of the solubilizing hydrochloride salt of the dimethylamino group in another part of the molecule.

Cellular Permeability. Doller et al. disclosed in 2011 their studies to identify positive allosteric modulators (PAMs) of metabotropic glutamate receptor 4 (mGlu4), a class C GPCR (Figure 34).⁸⁶ An SAR effort inspired by previously reported

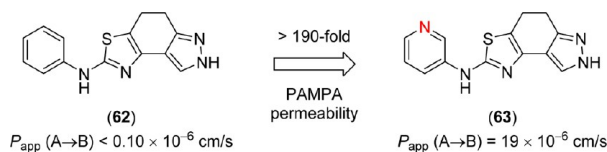


Figure 34.

thiazolopyrazole PAMs sought to explore the effects of conformationally constraining the two heterocyclic rings by cyclization using either an ethylidene or propylidene linkage. Ethylidene-linked analogue **62** was shown to display weak PAM activity in addition to poor passive permeability in a parallel artificial membrane permeability assay (mGlu4 $\text{EC}_{50} = 2.2 \mu\text{M}$, $E_{\text{max}} = 160\%$; PAMPA $P_{\text{app}} \text{ A} \rightarrow \text{B} < 0.10 \times 10^{-6} \text{ cm/s}$). Incredibly, 3-pyridyl analogue **63** was found to exhibit a 190-fold improvement in permeability but at the expense of a complete loss of measurable PAM activity (mGlu4 $\text{EC}_{50} > 10 \mu\text{M}$; PAMPA $P_{\text{app}} \text{ A} \rightarrow \text{B} = 19 \times 10^{-6} \text{ cm/s}$). The 2-pyridyl analogue of **62** was shown to display a >30-fold improvement in permeability as well as a 10-fold increase in potency (mGlu4 $\text{EC}_{50} = 0.22 \mu\text{M}$, $E_{\text{max}} = 150\%$; PAMPA $P_{\text{app}} \text{ A} \rightarrow \text{B} = 3.0 \times 10^{-6} \text{ cm/s}$); unfortunately, the kinetic solubility of the 2-pyridyl analogue was found to be similarly low as **62** using buffered aqueous solution ($S_{\text{pH } 7.4} = 0.10 \mu\text{M}$). Adding another N atom to give the 2-pyrimidinyl analogue resulted in the best balance of PAM activity, solubility, and permeability (mGlu4 $\text{EC}_{50} = 0.065 \mu\text{M}$, $E_{\text{max}} = 180\%$; $S_{\text{pH } 7.4} = 20 \mu\text{M}$; PAMPA $P_{\text{app}} \text{ A} \rightarrow \text{B} = 3.4 \times 10^{-6} \text{ cm/s}$).

Pennington and collaborators revealed in 2012 their investigations into selective agonists of the sphingosine-1-phosphate-1 receptor (S1P_1), a class A GPCR (Figure 35).³³ Starting from a quinolinone core embodied by **64**, which

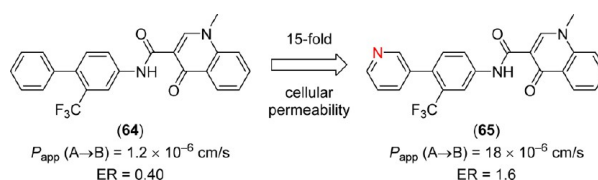


Figure 35.

stemmed from bioisosteric replacement of a previously identified carbamoylbenzamide scaffold, a systematic N atom scan (N-scan) SAR strategy was devised to probe the effect on potency and tune molecular and physicochemical properties. Although quinolinone **64** was found to exhibit reasonable agonist activity toward S1P_1 ($\text{S1P}_1 \text{ EC}_{50} = 0.070 \mu\text{M}$, $E_{\text{max}} = 110\%$), it was also shown to display poor cellular permeability (LLC-PK1 $P_{\text{app}} \text{ A} \rightarrow \text{B} = 1.2 \times 10^{-6} \text{ cm/s}$, ER = 0.40). In contrast, aza-analogue **65** was found to exhibit a 15-fold improvement in permeability (LLC-PK1 $P_{\text{app}} \text{ A} \rightarrow \text{B} = 18 \times 10^{-6} \text{ cm/s}$, ER = 1.6) but at the expense of lower S1P_1 agonist activity ($\text{S1P}_1 \text{ EC}_{50} = 0.31 \mu\text{M}$, $E_{\text{max}} = 110\%$). Other aza-analogues of **64** were found to possess improved agonist activity as well as a better balance of physicochemical properties and cellular permeability, which translated to improved activity in vivo. Interestingly, none of the aza-analogues was shown to have measurable activity against S1P_3 , an undesired activity that **64** was found to exhibit ($\text{S1P}_3 \text{ EC}_{50} = 1.5 \mu\text{M}$, $E_{\text{max}} = 92\%$).

Protein Binding. Huang, Degnan, and co-workers reported in 2016 their efforts to find PAMs of metabotropic glutamate receptor subtype 5 (mGluR5), a class C GPCR (Figure 36).⁸⁷

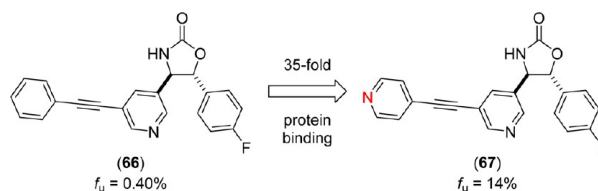


Figure 36.

An HTS campaign followed by an SAR effort, including a N-scan around one of three phenyl rings present in the hit molecule, led to the identification of oxazolidinone **66** (mGluR5 PAM $\text{EC}_{50} = 0.40 \text{ nM}$; E_{max} fold shift = 8.4). Oxazolidinone **66** was shown to exhibit high protein binding in human serum using high-throughput dialysis ($f_u = 0.40\%$). A N-scan on the terminal left-hand side phenyl ring of **66** resulted in the discovery of 4-pyridyl analogue **67** (mGluR5 PAM $\text{EC}_{50} = 14 \text{ nM}$; E_{max} fold shift = 4.4), which was found to display dramatically reduced protein binding ($f_u = 14\%$). The 2- and 3-pyridyl analogues of **66** also were shown to exhibit lower protein binding ($f_u = 6.4$ and 8.2% , respectively), along with modified functional activity profiles (mGluR5 PAM $\text{EC}_{50} = 25$ and 3.3 nM , respectively; E_{max} fold shift = 2.7 and 7.0, respectively).

Bell et al. disclosed in 2013 their studies to identify high-affinity antagonists of the calcitonin gene-related peptide receptor (CGRP receptor), a heterodimeric GPCR (Figure 37).⁸⁸ This effort to identify analogues with enhanced CNS penetration and functionality suitable for introduction for a radiolabel to make a positron emission tomography (PET) tracer included the evaluation of indene **68** (CGRP $K_i = 0.50$

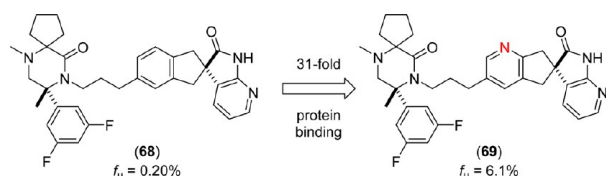


Figure 37.

nM), an analogue of a previously identified CGRP receptor antagonist. Unfortunately, **68** was shown to suffer suboptimal physicochemical properties and high protein binding in monkey plasma using equilibrium dialysis ($\log D_{7.0} = 4.3$; $f_u = 0.20\%$). Aza-analogue **69** was also evaluated and found to possess superior binding affinity, physicochemical properties, and lower protein binding (CGRP $K_i = 0.045$ nM; $\log D_{7.0} = 3.2$; $f_u = 6.1\%$). These in vitro findings were recapitulated in vivo (rhesus monkey) for the [^{11}C]-tracers of **68** and **69**; a low specific signal was found for [^{11}C]-**68** and a high specific signal was shown for [^{11}C]-**69**.

Dzierba and collaborators revealed in 2012 their investigations into antagonists of the corticotropin-releasing factor-1 receptor (CRFR1), a class B GPCR (Figure 38).⁸⁹ Pyridopyr-

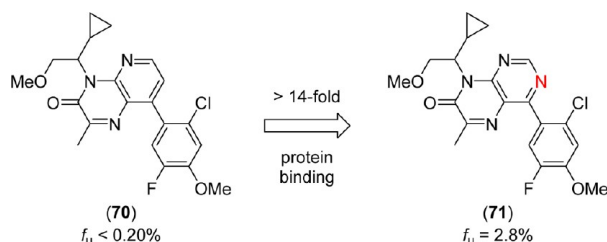


Figure 38.

azinone-based antagonist **70** was shown to exhibit excellent potency toward CRFR1 (rCRFR1 $\text{IC}_{50} = 1.5$ nM) but to display high lipophilicity and extensive protein binding in rat plasma ($\text{cLogP} = 3.4$; $f_u < 0.20\%$). In contrast, pteridinone-based analogue **71** was found to display not only excellent potency (rCRFR1 $\text{IC}_{50} = 7.5$ nM) but also to exhibit lower lipophilicity and protein binding ($\text{cLogP} = 2.6$; $f_u = 2.8\%$). Interestingly, the protein binding differences observed for the pyridopyrazinone-based and pteridinone-based antagonists did not show a consistent trend across all of the matched pairs tested.

Govek and co-workers reported in 2015 their efforts to find selective, nonsteroidal degraders of the estrogen receptor (ER), a nuclear hormone receptor (Figure 39).⁹⁰ A program to further explore the SAR of a clinical candidate was undertaken with the aims of improving potency, lowering steady-state ER levels, and increasing oral exposure. Phenyl analogue **72** was

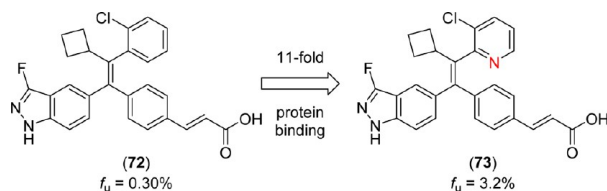


Figure 39.

found to display excellent potency toward lowering steady-state ER α levels (MCF-7 ER α $\text{EC}_{50} = 0.40$ nM, $E_{\text{max}} = 90\%$) but to display high protein binding in diluted mouse plasma ($f_u = 0.30\%$). Interestingly, 2-pyridyl analogue **73** was shown to exhibit not only excellent potency (MCF-7 ER α $\text{EC}_{50} = 0.30$ nM, $E_{\text{max}} = 90\%$) but also to display 11-fold lower protein binding ($f_u = 3.2\%$). The 4-pyridyl analogue of **72** also was found to exhibit excellent potency as well as 4-fold lower protein binding (MCF-7 ER α $\text{EC}_{50} = 0.30$ nM, $E_{\text{max}} = 90\%$; $f_u = 1.2\%$).

Metabolic Stability. Zheng et al. disclosed in 2013 their studies to identify inhibitors of nicotinamide phosphoribosyl-transferase (Nampt), the rate-limiting enzyme in the biosynthesis of nicotinamide adenine dinucleotide (NAD) from nicotinamide (Figure 40).⁹¹ A virtual screening effort identified

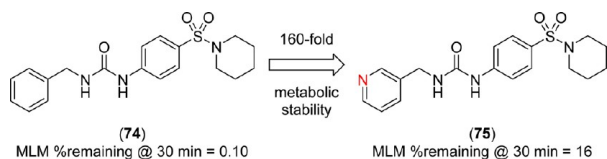


Figure 40.

a thiourea-based inhibitor. Subsequent structure-guided optimization led to the discovery of a urea analogue that was found to display identical potency but with improved aqueous solubility. SAR studies included the evaluation of inhibitor **74** (Nampt $\text{IC}_{50} = 0.47$ μM), which was shown to suffer poor stability in mouse liver microsomes (MLM) in addition to modest aqueous solubility (MLM % remaining @ 30 min = 0.10; $S_{\text{H}_2\text{O}} = 2.0$ μM). Aza-analogue **75** was found to display dramatically improved potency, microsomal stability, and aqueous solubility compared to **74** (Nampt $\text{IC}_{50} = 0.0070$ μM ; MLM % remaining @ 30 min = 16; $S_{\text{H}_2\text{O}} = 82$ μM). Further optimization of **75** focused on the examination of novel N heterocycles of the sulfonamide moiety.

Thomas and collaborators revealed in 2015 their investigations into inhibitors of phosphodiesterase 10A (PDE10A), an enzyme that hydrolyzes cAMP and cGMP to their inactive phosphates (Figure 41).⁹² Starting with an inactive compound

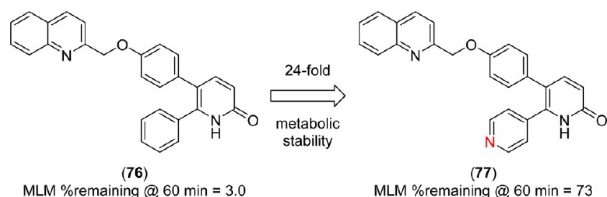


Figure 41.

from an internal library that was selected for screening due to its structural similarity to known PDE10A inhibitors, substitution of a methyl ether group with a quinolinemethyl ether moiety gave rise to an analogue with measurable inhibitory activity (PDE10A $\text{IC}_{50} = 2400$ nM). Conversion of the aminopyridine ring of this compound to the corresponding pyridone ring resulted in analogue **76**, which was found to exhibit a remarkable 2200-fold improvement in biochemical potency (PDE10A $\text{IC}_{50} = 1.1$ nM) but also to display high turnover in monkey liver microsomes (MLM % remaining @ 60 min = 3.0). 4-Pyridyl analogue **77** was shown to exhibit

nearly identical potency (PDE10A IC_{50} = 1.2 nM) but to display 24-fold better microsomal stability versus **76** (MLM % remaining @ 60 min = 73). Interestingly, this dramatic microsomal stability difference was also observed for mouse liver microsomes, but not for rat, dog, or human liver microsomes. Other findings of note included that the 3-pyridyl analogue of **76** was shown to display 15-fold lower potency (PDE10A IC_{50} = 19 nM) and the 2-naphthyl derivative of **76** was found to exhibit only 5.8% inhibition at a concentration of 10 μ M (>8900-fold potency loss).

Fukaya and co-workers reported in 2013 their efforts to find improved ligands of translocator protein (TSPO), which functions as a heterotrimeric complex with the voltage-dependent anion channel (VDAC) and the adenine nucleotide transporter (ANT) and is believed to be involved in the transport of cholesterol into mitochondria to promote the synthesis of neurosteroids (Figure 42).⁹³ Included in this new

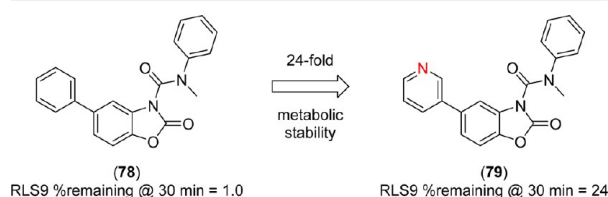


Figure 42.

study were two previously reported compounds: phenyl-substituted benzoxazolone **78** (TSPO K_i = 1.6 nM) and its 3-pyridyl analogue **79** (TSPO K_i = 11 nM). Although **78** displays better binding affinity than **79**, it also exhibits less stability in rat liver S-9 fraction and lower aqueous solubility (RLS9% remaining @ 30 min = 1.0 and 24 for **78** and **79**, respectively; $S_{pH\ 7.4}$ < 1.0 μ g/mL and $S_{pH\ 7.4}$ = 5.0 μ g/mL for **78** and **79**, respectively). A new compound found to have a favorable balance of efficacy and safety in vivo bears a trifluoromethyl group at the para position of the upper right-hand phenyl ring of **79** (TSPO K_i = 8.6 nM; RLS9% remaining @ 30 min = 38; $S_{pH\ 7.4}$ = 1.0 μ g/mL).

Norman et al. disclosed in 2007 their studies to identify antagonists of the transient receptor potential cation channel subfamily V member 1 (TRPV1), an ion channel implicated in the transmission of pain signaling (Figure 43).⁹⁴ SAR efforts

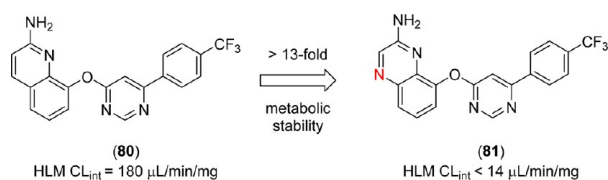


Figure 43.

stemming from a previously identified 4-oxypyrimidine series of antagonists were directed toward improving pharmacokinetic profiles. A metabolite identification study of the lead compound using human liver microsomes revealed two mono-oxidation metabolites of its quinolinyl ether substituent. An examination of several fused heteroaromatic ring systems led to the identification of 2-aminoquinoline analogue **80** (rTRPV1 CAP IC_{50} = 11 nM). Unfortunately, **80** was found to exhibit high turnover in both human and rat liver microsomes (HLM

and RLM CL_{int} = 180 and 110 μ L/min/mg, respectively). A metabolite identification study of **80** using human liver microsomes showed that oxidation occurred on the 2-aminoquinoline ring system. 2-Aminoquinoxaline analogue **81** was found to display good potency toward TRPV1 (rTRPV1 CAP IC_{50} = 63 nM) along with improved stability in human and rat liver microsomes (HLM CL_{int} < 14 μ L/min/mg; RLM CL_{int} = 15 μ L/min/mg). Surprisingly, it was discovered that **81** exhibits high clearance in rat (CL = 4.3 L/h/kg), being converted to its 3-oxo metabolite. This metabolite was also prepared and evaluated, revealing that it shows dramatically improved potency compared to **81** (rTRPV1 CAP IC_{50} = 0.64 nM) as well as lower clearance both in vitro and in vivo (HLM and RLM CL_{int} < 14 μ L/min/mg; CL = 0.26 L/h/kg).

Cid and collaborators revealed in 2012 their investigations into PAMs of the metabotropic glutamate 2 receptor (mGlu2), a class C GPCR (Figure 44).⁹⁵ An imidazopyridine series

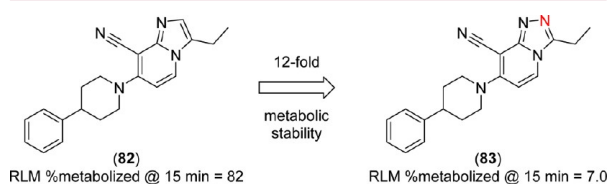


Figure 44.

represented by **82** was previously identified through a sequential combination of HTS and pharmacophore modeling (mGlu2 EC_{50} = 500 nM, E_{max} = 230%). It was speculated that the relatively high lipophilicity of **82** contributed to its rapid turnover in human and rat liver microsomes (cLogP = 4.4; HLM % metabolized @ 15 min = 74; RLM % metabolized @ 15 min = 82). Indeed, aza-analogue **83** possessing lower lipophilicity was shown to display not only similar potency but also slower microsomal turnover (mGlu2 EC_{50} = 700 nM, E_{max} = 250%; cLogP = 3.4; HLM % metabolized @ 15 min = 11; RLM % metabolized @ 15 min = 7.0). Further optimization of **83** to give a molecule with the best pharmacological profile included exchanging a trifluoromethyl group for the cyano functionality and a cyclopropylmethyl group for the ethyl substituent (mGlu2 EC_{50} = 17 nM, E_{max} = 290%; cLogP = 5.3; HLM % metabolized @ 15 min = 23; RLM % metabolized @ 15 min = 25).

In Vitro Safety Profile. Modern drug design also necessitates concomitant consideration of potential off-target effects as part of the multiparameter optimization process, including drug–drug interactions and other safety and tolerability liabilities. This aspect of drug design often presents the greatest challenge due to the large number of known and unknown off-target effects that are possible. Several strategies for circumventing pharmacological challenges stemming from the most common antitargets have been developed. For example, shared strategies for avoiding CYP inhibition, PXR activation, and hERG activity include introducing polarity, reducing lipophilicity, attenuating basicity, and biasing conformation.^{30–32} The substitution of a CH group with a N atom is a discrete structure modification that is well-suited to address all of these properties. The case studies below illustrate the profound impact that the substitution of a CH group with a N atom can have on CYP inhibition, PXR activation, hERG activity, and Ames mutagenicity.

CYP Inhibition. Cioffi, Petrukhin and co-workers reported in 2014 their efforts to find antagonists of retinol binding protein 4 (RBP4), a lipocalin serum protein that forms a ternary complex with retinol and transthyretin and serves to transport retinol from circulation to retinol-dependent tissues (Figure 45).⁹⁶ An SAR effort was undertaken to improve a previously

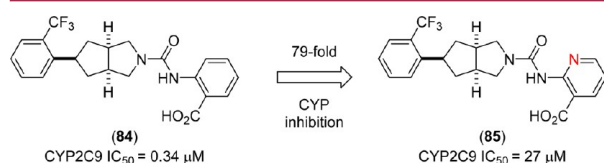


Figure 45.

reported antagonist of RBP4 that was shown to suffer poor stability in human liver microsomes (RBP4 IC_{50} = 15 nM; HLM % remaining @ 30 min = 3.0%). Bioisosteric replacement of a piperidine ring in the known antagonist with a bicyclic [3.3.0]-octahydrocyclopenta[*c*]pyrrolo ring system gave **84** (RBP4 IC_{50} = 73 nM), which was found to display improved stability in human liver microsomes (HLM % remaining @ 30 min = 100%). Unfortunately, **84** was also shown to exhibit potent inhibition of CYP2C9 (CYP2C9 IC_{50} = 0.34 μ M). Although 2-pyridyl analogue **85** was demonstrated to have a dramatically lower CYP2C9 liability (CYP2C9 IC_{50} = 27 μ M), it also was found to possess no measurable activity toward RBP4 (RBP4 IC_{50} > 3000 nM). Interestingly, the 4-pyridyl analogue of **84** was found to display good potency toward RBP4 and only moderate activity against CYP2C9 (RBP4 IC_{50} = 19 nM; CYP2C9 IC_{50} = 5.6 μ M) but also to exhibit potent inhibition of CYP3A4 (CYP3A4 IC_{50} < 0.046 μ M).

Johnson et al. disclosed in 2011 their studies to identify inhibitors of fatty acid amide hydrolase (FAAH), an integral membrane serine hydrolase that degrades fatty acid amide signaling lipids including anandamide (Figure 46).⁹⁷ Speculation that the potency of a known methylpiperidine-based inhibitor could be improved by conformational restriction led to the identification of unsaturated methylenepiperidine analogue **86**, which was found to display ~2-fold improved hydrolase activity (hFAAH k_{inact}/K_i = 13000 and 22000 $M^{-1} s^{-1}$, respectively). Compound **86** was also demonstrated to exhibit potent inhibition of CYP3A4 (CYP3A4 IC_{50} = 0.80 μ M). The less basic pyridazine analogue **87** was shown to possess both improved activity toward FAAH as well as dramatically reduced inhibition of CYP3A4 (hFAAH k_{inact}/K_i = 40,000 $M^{-1} s^{-1}$; CYP3A4 IC_{50} = 30 μ M). Compound **87** was advanced into human clinical trials.

Savall and collaborators revealed in 2015 their investigations into antagonists of the P2X7 receptor (P2X7R), a ubiquitously expressed trimeric ATP-gated cation channel that is predominantly found on immune cells (Figure 47).⁹⁸ Inspired by a report of 1,2,4-triazolopiperazines as P2X7R antagonists, an

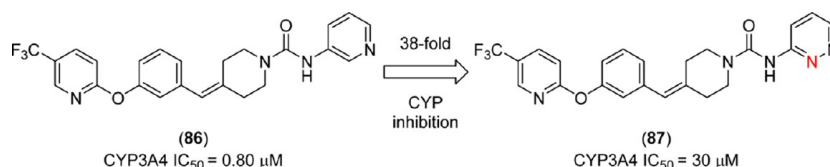


Figure 46.

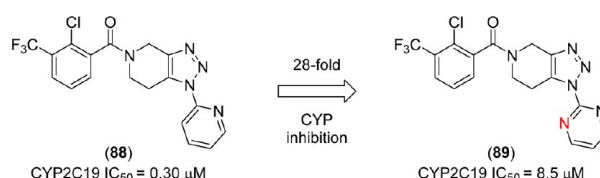


Figure 47.

SAR effort was initiated on a related series of 1,2,3-triazolopiperidines. Although 2-pyridinyl analogue **88** was shown to display excellent potency toward the human receptor (hP2X7R IC_{50} = 3.8 nM), it was also found to exhibit poor activity toward the rat receptor as well as potent inhibition of CYP2C19 (rP2X7R IC_{50} = 1400 nM; CYP2C19 IC_{50} = 0.30 μ M). In contrast, 2-pyrimidinyl analogue **89** was found to exhibit potent inhibition toward both the human and rat receptors (hP2X7R IC_{50} = 4.2 nM; rP2X7R IC_{50} = 6.8 nM) and dramatically reduced inhibition of CYP2C19 (CYP2C19 IC_{50} = 8.5 μ M). 2-Pyrimidinyl analogue **89** also displays improved stability in human liver microsomes (HLM ER = 0.90 and 0.61 for **88** and **89**, respectively).

Safina and co-workers reported in 2012 their efforts to find selective inhibitors of phosphoinositide 3-kinase δ (PI3K δ), a member of a lipid kinase family that catalyzes the phosphorylation of PIP2 to PIP3 (Figure 48).⁹⁹ A previously

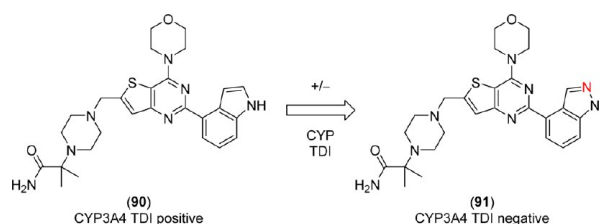


Figure 48.

reported class of indole-substituted morpholinyl thienopyrimidine inhibitors of PI3K δ represented by **90** was found to suffer time-dependent inhibition (TDI) of CYP3A4 (PI3K δ IC_{50} = 1.8 nM; CYP3A4 TDI positive). Interestingly, indazole-substituted analogue **91** was shown to maintain excellent potency toward PI3K δ with no evidence of TDI of CYP3A4 (PI3K δ IC_{50} = 3.1 nM; CYP3A4 TDI negative), albeit with 2–14-fold reduced selectivity against the other PI3K isoforms. A N-scan on the phenyl ring-portion of the indole moiety of **90** revealed that while all three aza-analogues maintained good potency toward PI3K δ (PI3K δ IC_{50} = 0.67–3.5 nM), only the 5-azaindole analogue did not display evidence of TDI of CYP3A4 (PI3K δ IC_{50} = 3.5 nM; CYP3A4 TDI negative).

Barsanti et al. disclosed in 2015 their studies to identify selective inhibitors of ataxia telangiectasia and Rad3-related protein (ATR), a serine/threonine-specific protein kinase that

is a member of the phosphatidylinositol 3-kinase-related kinase protein family (Figure 49).¹⁰⁰ A focused HTS campaign

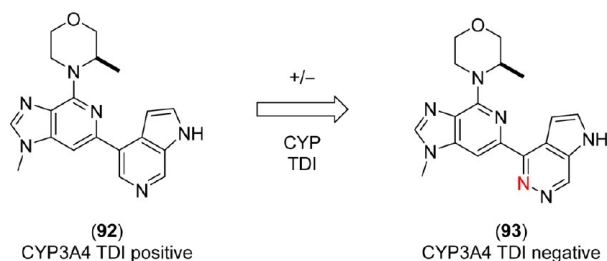


Figure 49.

followed by a structure-guided SAR effort led to the identification of 6-azaindole-substituted azabenzimidazole **92** that was subsequently found to exhibit TDI of CYP3A4 (ATR IC_{50} = 0.40 nM; CYP3A4 TDI positive). As the 6-azaindole region of **92** was shown to be highly sensitive to structural modification and critical for maintaining ATR activity, as well as kinase selectivity, a focus on electronic perturbations to this moiety was made. Fortuitously, pyrrolo[2,3-*d*]pyridazine analogue **93** was demonstrated to possess excellent activity toward ATR and no TDI of CYP3A4 (ATR IC_{50} = 0.50 nM; CYP3A4 TDI negative). Pyrrolo[2,3-*d*]pyridazine analogue **93** was also found to have the added benefit of a reduced hERG liability compared to **92** (hERG IC_{50} = 13 μ M and hERG IC_{50} > 30 μ M for **92** and **93**, respectively).

PXR Activation. McMinn and collaborators revealed in 2009 their investigations into inhibitors of 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), a member of the oxidoreductase enzyme family (Figure 50).¹⁰¹ A previously

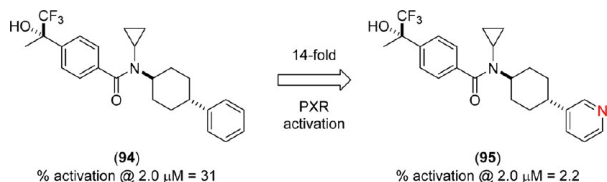


Figure 50.

reported inhibitor **94** was found to display excellent potency toward 11 β -HSD1 (h11 β -HSD1 IC_{50} = 1.3 nM) but also to exhibit activation of PXR (PXR % activation @ 2.0 μ M = 31). 3-Pyridyl analogue **95** was shown to retain excellent potency toward 11 β -HSD1 (h11 β -HSD1 IC_{50} = 1.7 nM) with negligible activation of PXR (PXR % activation @ 2.0 μ M = 2.2). Although both **94** and **95** were demonstrated to possess good stability in human liver microsomes (HLM % turnover @ 30 min <5.0), 3-pyridyl analogue **95** was found to display appreciable cytotoxicity (HeLa IC_{50} = 2.5 μ M), a liability that **94** was shown not to suffer (HeLa IC_{50} > 10 μ M).

Sun and co-workers reported in 2011 their efforts to find inhibitors of 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), which converts the inactive glucocorticoid cortisone to the active form, cortisol (Figure 51).¹⁰² Stemming from a previously reported series of triazole-based inhibitors of 11 β -HSD1, an SAR effort was initiated with the aim of optimizing a number of pharmacological parameters including PXR activation. Docking studies of the 11 β -HSD1 inhibitors with PXR suggested that substitution of a distal phenyl ring with

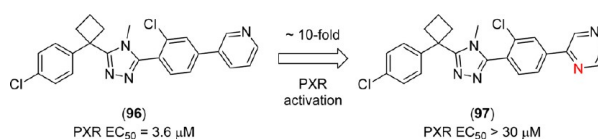


Figure 51.

polar groups might reduce PXR activity by destabilizing interactions in a hydrophobic region of the ligand binding pocket. 3-Pyridyl inhibitor **96** was found to have identical potency toward 11 β -HSD1 as its phenyl analogue (h11 β -HSD1 IC_{50} = 1.0 nM) but to possess reduced activation of PXR (PXR EC_{50} = 1.7 μ M for the phenyl analogue and 3.6 μ M for **96**). Impressively, 2-pyrazinyl analogue **97** was shown to exhibit almost equipotent activity toward 11 β -HSD1 and approximately 10-fold lower activation of PXR compared to **96** (h11 β -HSD1 IC_{50} = 2.3 nM; PXR EC_{50} > 30 μ M).

hERG Activity. Bregman et al. disclosed in 2012 their studies to identify antagonists of the voltage-gated sodium-channel type IX α subunit (Na $_v$ 1.7), an integral membrane protein that plays an important role in action potential production in peripheral neurons (Figure 52).¹⁰³ An HTS campaign followed

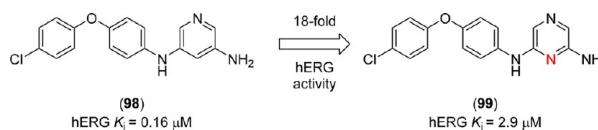


Figure 52.

by an SAR effort led to the evaluation of 3-aminopyridine **98** (hNa $_v$ 1.7 IC_{50} = 2.9 μ M), which was found to display high binding affinity against hERG (hERG K_i = 0.16 μ M). Interestingly, 2-pyrazinyl analogue **99** was shown to exhibit 4.7-fold better potency toward Na $_v$ 1.7 as well as 18-fold lower binding affinity against hERG (hNa $_v$ 1.7 IC_{50} = 0.62 μ M; hERG K_i = 2.9 μ M). The optimized compound from this study arose from substitution of the Cl atom with a trifluoromethoxy group on the distal left-hand side phenyl ring of **99** (hNa $_v$ 1.7 IC_{50} = 0.24 μ M; hERG % inhibition @ 30 μ M = 33).

Deng and collaborators revealed in 2015 their investigations into antagonists of the adenosine A $_{2A}$ receptor (A $_{2A}$), a class A GPCR (Figure 53).¹⁰⁴ Lead compound **100** was found to display excellent potency toward A $_{2A}$ (hA $_{2A}$ K_i = 5.5 nM) but also to exhibit inhibition of hERG (hERG IC_{50} = 1.6 μ M). An overlay of a low energy conformation of **100** with that for a previously reported potent inhibitor of hERG that had been subjected to docking studies in a homology model of hERG suggested that the terminal fluorophenyl ring of **100** may form favorable π -stacking interactions with the four Phe656 residues of the tetrameric ion channel. Docking studies of **100** with A $_{2A}$ indicated that the terminal fluorophenyl ring resides at the opening of the binding pocket, which is spacious, flexible, and exposed to solvent, allowing ample opportunities for structural modification. 2-Pyridinyl analogue **101** was shown to retain good potency toward A $_{2A}$ and to display 14-fold lower inhibition of hERG (hA $_{2A}$ K_i = 13 nM; hERG IC_{50} = 22 μ M). An unsubstituted 2-pyrimidinyl analogue of **101** was demonstrated to exhibit good potency toward A $_{2A}$ and no measurable activity against hERG (hA $_{2A}$ K_i = 27 nM; hERG IC_{50} > 60 μ M).

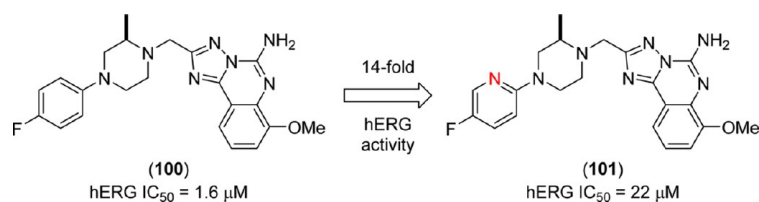


Figure 53.

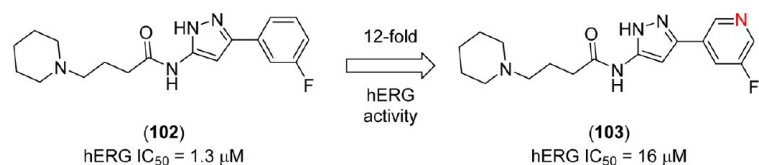


Figure 54.

Zanaletti and co-workers reported in 2012 their efforts to find agonists of the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$), a homopentameric ligand-gated ion channel composed of five $\alpha 7$ subunits (Figure 54).¹⁰⁵ An SAR effort starting from a previously reported agonist of $\alpha 7$ was initiated to try to improve plasma stability and brain exposure profiles. 3-Fluorophenyl agonist **102** was found to exhibit reasonable activity toward $\alpha 7$ ($\alpha 7$ pEC₅₀ = 6.1) but also to display inhibition of hERG (hERG IC₅₀ = 1.3 μM). 3-Pyridinyl analogue **103** was shown to retain good potency toward $\alpha 7$ and to display 12-fold lower inhibition of hERG ($\alpha 7$ pEC₅₀ = 6.1; hERG IC₅₀ = 16 μM). Lead compound **103** was further evaluated in vivo and found to exhibit good pharmacokinetic, efficacy, and safety profiles.

Ames Mutagenicity.¹⁰⁶ Boldron et al. disclosed in 2014 their studies to identify novel antagonists of the platelet P2Y purinoceptor 12 (P2Y12), a class A GPCR (Figure 55).¹⁰⁷ An

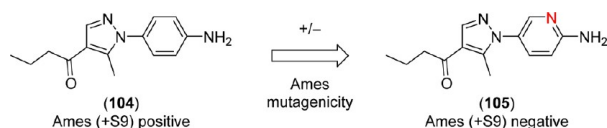


Figure 55.

HTS campaign followed by an SAR effort identified a promising antagonist containing a 1-(1-(4-aminophenyl)-5-methyl-1H-pyrazol-4-yl)butan-1-one moiety (**104**), which was found to display mutagenic activity in an Ames assay in the presence of liver S9 fraction. Although the 2-aminopyridine fragment **105** was shown to exhibit no mutagenic activity, the requisite amide derived from it for inclusion into the final molecule appeared to be chemically unstable under both slightly acidic and basic conditions. The 3-aminopyridazine analogue of **105** was demonstrated to be nonmutagenic and the amide derived from it was shown to be chemically stable; it was this moiety that was ultimately included in the preclinical candidate molecule.

Beaulieu and collaborators revealed in 2015 their investigations into inhibitors of hepatitis C virus nonstructural protein 5B polymerase thumb pocket 1 (HCV NSSB), a viral protein that has the key function of replicating HCV RNA (Figure 56).¹⁰⁸ An SAR effort stemming from a previously identified inhibitor was undertaken to replace an (*E*)-3-(4-amino-2-ethoxyphenyl)acrylic acid moiety (**106**) in the

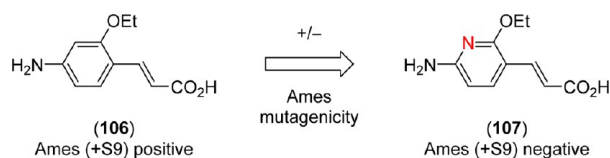


Figure 56.

molecule, which was found to display mutagenic activity in an Ames assay in the presence of liver S9 fraction. The 2-aminopyridine analogue **107** was demonstrated to be non-mutagenic; however, the final molecule derived from **107** suffered from a poorer pharmacokinetic profile versus the final molecule derived from its aniline progenitor **106**, which led to an unacceptably high predicted dose in human to achieve a strong antiviral response.

In Vivo Pharmacological Profile. Occasionally, the full benefit of substituting a CH group with a N atom does not clearly manifest itself until the in vivo setting, wherein the effects observed in vitro can be further magnified. In vitro measures of the binding affinity, functional activity, target selectivity, aqueous solubility, cellular permeability, active transport, protein binding, metabolic stability, and safety of a molecule are put to the ultimate test in vivo. It is in the whole-organism context that a drug molecule meets all of the biological barriers, metabolic machinery, biochemical competition, and other anatomical features and physiological processes that may preclude its desired pharmacological profile. Importantly, however, inherent species differences in anatomy and physiology must be considered in addition to therapeutic target and animal model validity. The case studies below illustrate the dramatic impact that the substitution of a CH group with a N atom can have on in vivo pharmacological profiles.

Li et al. disclosed in 2008 their studies to identify inhibitors of matrix metalloproteinase-13 (MMP-13), a member of a large family of zinc- and calcium-dependent endopeptidases that are involved in the degradation of the extracellular matrix (Figure 57).¹⁰⁹ An HTS campaign followed by an SAR effort led to the identification of quinazolinone **108** (MMP-13 IC₅₀ = 11 nM) and its aza-analogue pyrido[3,4-*d*]pyrimidin-4-one **109** (MMP-13 IC₅₀ = 31 nM), which was designed to block the CYP-mediated metabolism at C7 that had been observed for **108**. In a pharmacokinetic study in rats, **109** displayed dramatically improved bioavailability, clearance, and exposure compared to

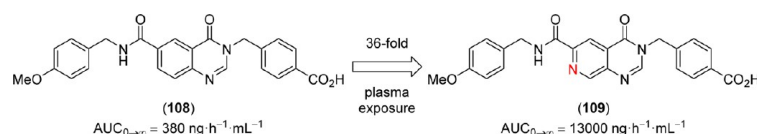


Figure 57.

108 (%F = 9.0, CL = 20 mL/min/kg, $AUC_{0 \rightarrow \infty}$ = 380 ng/h/mL for **108**; %F = 73, CL = 7.6 mL/min/kg, $AUC_{0 \rightarrow \infty}$ = 13000 ng/h/mL for **109**).

Chibale and collaborators revealed in 2012 their investigations into compounds effective against *Plasmodium falciparum* (*P. falciparum*), one of the two parasites that are the primary cause of malaria in humans (Figure 58).¹¹⁰ An SAR

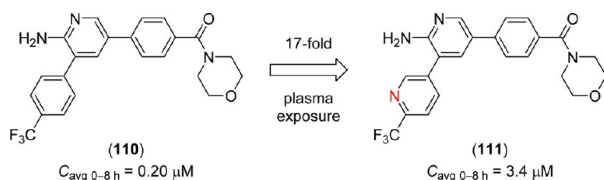


Figure 58.

effort starting with a previously reported aminopyridine-based scaffold was undertaken to attempt to reduce a potential cardiotoxicity risk due to the hERG inhibition activity observed for a lead compound. This effort identified compound **110** and its aza-analogue **111** as promising leads (*P. falciparum* K1 IC₅₀ = 19 and 12 nM for **110** and **111**, respectively). Aza-analogue **111** was found to exhibit a moderately improved pharmacological profile in vitro compared to **110** ($S_{\text{pH } 6.5}$ = 9.4 $\mu\text{g/mL}$, HLM CL_{int} = 9.0 $\mu\text{L/min/mg}$, hERG IC₅₀ = 3.0 μM for **110**; $S_{\text{pH } 6.5}$ = 38 $\mu\text{g/mL}$, HLM CL_{int} < 7.0 $\mu\text{L/min/mg}$, hERG IC₅₀ = 20 μM for **111**). These findings in vitro were further extended in vivo, with **111** shown to display higher plasma concentrations after a single 30 mg/kg oral dose and greater efficacy after multiple 50 mg/kg oral doses in mice ($C_{\text{avg } 0-8 \text{ h}}$ = 0.20 μM , MSD = 12 d for **110**; $C_{\text{avg } 0-8 \text{ h}}$ = 3.4 μM , MSD = 24 d for **111**).

Zou and co-workers reported in 2015 their efforts to find inhibitors of dengue virus (DENV), the causative agent of dengue fever (Figure 59).¹¹¹ A phenotypic HTS campaign

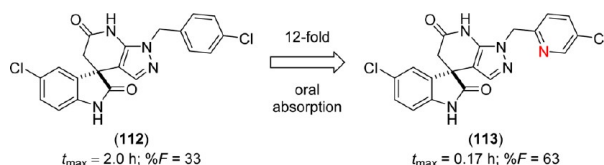


Figure 59.

against DENV serotype 2 (DENV-2) identified a racemic spiro[pyrazolo]pyridone inhibitor that was resolved to give the eutomer **112** (DENV-2 EC₅₀ = 12 nM). A subsequent cell-based SAR effort starting from **112** led to the identification of aza-analogue **113** (DENV-2 EC₅₀ = 42 nM). In vitro, aza-analogue **113** was found to exhibit improved lipophilicity and solubility compared to **112**, as well as similar stability in mouse liver microsomes (logP = 4.9, $S_{\text{pH } 6.8}$ = 11 μM , MLM $t_{1/2}$ = 31 min for **112**; logP = 2.8, $S_{\text{pH } 6.8}$ = 500 μM , MLM $t_{1/2}$ = 24 min for **113**). After a 25 mg/kg oral dose in rats, **113** was shown to

display more rapid and greater absorption than **112** (t_{max} = 2.0 h, C_{max} = 0.78 $\mu\text{g/mL}$ for **112**; t_{max} = 0.17 h, C_{max} = 3.9 $\mu\text{g/mL}$ for **113**), as well as better bioavailability and higher exposure versus **112** (%F = 33, $AUC_{0 \rightarrow 24 \text{ h}}$ = 9.5 $\mu\text{g} \cdot \text{h/mL}$ for **112**; %F = 63, $AUC_{0 \rightarrow 24 \text{ h}}$ = 14 $\mu\text{g} \cdot \text{h/mL}$ for **113**).

Horne et al. disclosed in 2014 their studies to identify antagonists of the transient receptor potential melastatin 8 (TRPM8), a nonselective cation channel that is activated by cold temperatures or cooling compounds such as menthol or icilin (Figure 60).¹¹² An SAR effort using a previously reported

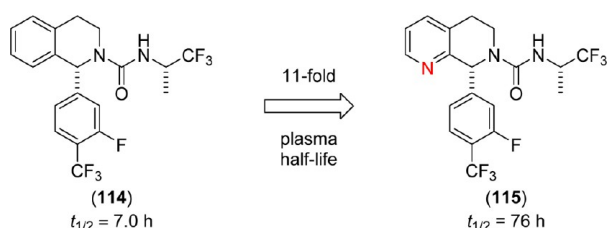


Figure 60.

tetrahydroisoquinoline-based scaffold was undertaken toward improving the potency and pharmacokinetic profiles observed for a lead compound. This investigation led to compound **114** and its aza-analogue **115** as promising leads (hTRPM8 IC₅₀ = 10 nM, rTRPM8 IC₅₀ = 18 nM, HLM CL_{int} < 14 $\mu\text{L/min/mg}$, RLM CL_{int} = 47 $\mu\text{L/min/mg}$ for **114**; hTRPM8 IC₅₀ = 12 nM, rTRPM8 IC₅₀ = 23 nM, HLM CL_{int} < 14 $\mu\text{L/min/mg}$, RLM CL_{int} < 14 $\mu\text{L/min/mg}$ for **115**). After a 2.0 mg/kg intravenous dose in rats, **115** was shown to display a much longer half-life and reduced clearance compared to **114** ($t_{1/2}$ = 7.0 h, CL = 0.64 L/h/kg for **114**; $t_{1/2}$ = 76 h, CL = 0.090 L/h/kg for **115**). After a 5.0 mg/kg oral dose in rats, **115** was found to exhibit comparable bioavailability, but to achieve higher plasma concentration and exposure versus **114** (%F = 67, C_{max} = 1.7 μM , $AUC_{0 \rightarrow \infty}$ = 12 $\mu\text{M} \cdot \text{h}$ for **114**; %F = 51, C_{max} = 3.9 μM , $AUC_{0 \rightarrow \infty}$ = 72 $\mu\text{M} \cdot \text{h}$ for **115**). Finally, aza-analogue **115** was also shown to display efficacy at a lower dose than **114** in the rat wet-dog shake (WDS) model (ED₉₀ = 4.7 and 1.1 mg/kg for **114** and **115**, respectively), albeit with a higher unbound plasma concentration (unbound IC₉₀ = 0.010 and 0.062 μM for **114** and **115**, respectively).

Shin and collaborators revealed in 2016 their investigations into inhibitors of phosphoinositide 3-kinase δ (PI3K δ), a member of a lipid kinase family that regulates numerous biological functions (Figure 61).¹¹³ An SAR effort stemming from the bioisosteric replacement of a quinoline ring system in a previously identified inhibitor with a benzimidazole ring system led to the identification of compound **116** and its aza-analogue **117** (PI3K δ IC₅₀ = 0.0022 and 0.016 μM for **116** and **117**, respectively; RLM % turnover @ 30 min = 26 and RLM % turnover @ 30 min < 10 for **116** and **117**, respectively; rPPB f_u = 9.0 and 36% for **116** and **117**, respectively). After an intravenous 0.50 mg/kg dose of **116** or 0.71 mg/kg dose of **117** in rats, **117** was shown to display much lower total and

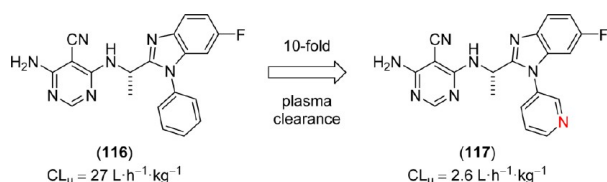


Figure 61.

unbound clearance compared to **116** ($CL = 2.4 \text{ L/h/kg}$, $CL_u = 27 \text{ L/h/kg}$ for **116**; $CL = 0.93 \text{ L/h/kg}$, $CL_u = 2.6 \text{ L/h/kg}$ for **117**). Aza-analogue **117** was also found to exhibit efficacy in a keyhole limpet hemocyanin (KLH) assay in rats ($\text{IgG ED}_{50} = 0.080 \text{ mg/kg}$; $\text{IgM ED}_{50} = 0.12 \text{ mg/kg}$).

CONCLUSION

In this Perspective, the “necessary nitrogen atom” has been shown to be a versatile high-impact design element for multiparameter optimization, wherein ≥ 10 -, 100-, or 1000-fold improvement in a variety of key pharmacological parameters can be realized through beneficial effects on molecular and physicochemical properties and intra- and intermolecular interactions. Although only instances of dramatic pharmacological improvements incurred upon the substitution of a CH group with a N atom were discussed, many more examples of similarly important, but more incremental, pharmacological improvements abound in the literature. To increase the likelihood of effecting dramatic pharmacological improvements, one should strive to select the best CH group to replace with a N atom; however, strategic approaches that open opportunities for serendipitous improvements should also be exploited when appropriate, for example, through the use of the systematic N atom scan (N-scan). Some recommendations for choosing the best CH group to replace with a N atom include where the newly installed N atom can:

1. Engage in a hydrogen bond with specific residues of the target receptor or receptor-bound water molecules that need to be satisfied
2. Remove unfavorable van der Waals interactions the replaced CH group made with the target receptor
3. Form unfavorable electrostatic interactions with an antitarget receptor
4. Have a positive effect on the binding conformation of the ligand
5. Mask a hydrogen bond donor in the ligand
6. Reduce the basicity or HBA strength of an existing N atom in the ligand
7. Evenly distribute the polar surface area of the ligand
8. Properly tune the lipophilicity of the ligand
9. Be shielded by other substituents or functionality in the ligand
10. Stabilize chemically labile functionality in the ligand
11. Replace a metabolically labile CH group in the ligand

It should be noted that the synthetic tractability of the resulting N analogues can often be more difficult than for their CH progenitors. The case studies discussed in this Perspective, however, have demonstrated that this greater synthesis challenge can be well worth the extra effort. It should also be noted that including proposed explanations for dramatic pharmacological differences resulting from discrete structure modifications in the biomedical research literature would help to inform hypothesis-driven multiparameter optimization

efforts by other researchers. Finally, the reporting of more pharmacological profiling data in the biomedical research literature would facilitate a deeper understanding of the broader pharmacological impacts of discrete structure modifications, which may serve to help streamline multiparameter optimization efforts in the future, as well.

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Demetri T. Moustakas is in the Modeling and Informatics group at Alkermes in Waltham, Massachusetts. Previously, from 2008 to 2012, he worked in Computational Chemistry supporting Infection Discovery at AstraZeneca in Waltham, Massachusetts. Prior to that, he was a postdoctoral fellow in George Whitesides' group at Harvard University from 2004 to 2008, where he studied the thermodynamic contributions of binding site waters to protein–ligand binding. He pursued his Ph.D. studies in the UC Berkeley/UC San Francisco Graduate Group in Bioengineering, where he studied under Tack Kuntz developing small molecule docking methods, and under Bob Stroud investigating the structure of HIV Integrase. He earned a B.S. in Biomedical Engineering from the Boston University College of Engineering in 1994, under the mentorship of Professor Charles M. DeLisi.

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ABBREVIATIONS USED

S_{SBF} , solubility in a simulated biological fluid; P_{app} , apparent permeability; P_{app} ($A \rightarrow B$), P_{app} from the apical to the basolateral side of a membrane; ER, efflux ratio; PPB, plasma

protein binding; CL_{int} , intrinsic clearance; PK, pharmacokinetics; ADME, absorption, distribution, metabolism, and excretion; PD, pharmacodynamics; MW, molecular weight; FG, functional group; AR, aromatic ring; F_{sp^3} , fraction of sp^3 -hybridized carbon atoms; RB, rotatable bond; HBD, hydrogen bond donor; HBA, hydrogen bond acceptor; PSA, polar surface area; $\log P$, logarithm of the octanol/water partition coefficient; $\log D_{pH}$, logarithm of the octanol/water distribution coefficient at a specified pH; pK_a , negative logarithm of the acid dissociation constant; S_{H_2O} , solubility in water; B3LYP, Becke, three-parameter, Lee–Yang–Parr hybrid density functional; CLogP, calculated $\log P$; IC_{50} , molar concentration of compound that produces half-maximal inhibition; pK_{BHX} , negative logarithm of the 1:1 complexation constant of an HBA with 4-fluorophenol in carbon tetrachloride; IP, ionization potential; ΔE , interaction energy of the parallel-displaced π -stacking configuration; R , vertical intermonomer separation; H , horizontal displacement of the parallel-displaced π -stacking configuration; BE, binding energy; IE, relative interaction energy between a specified CH group with water versus a methane CH group with water; CD, charge demand (fraction of π -charge transferred from a trigonal carbanion to an adjacent electron withdrawing group); N-scan, N atom scan; Pgp, P-glycoprotein; CYP, cytochrome P450; PXR, pregnane X receptor; hERG, human ether-à-go-go-related gene product ($K_{11.1}$ potassium ion channel); MMPA, matched molecular pair analysis; SASA, solvent-accessible surface area; ΔG_{trans} , difference in the free energies of solvation in hexadecane and in water; K_i , inhibition constant; pK_i , negative logarithm of the K_i ; K_B , inhibition constant for antagonist functional activity; K_d , dissociation constant; GPCR, G-protein coupled receptor; PAM, positive allosteric modulator; EC_{50} , molar concentration of compound that produces half-maximal activation; pEC_{50} , negative logarithm of the EC_{50} ; E_{max} , percent maximal activation versus basal or a reference standard; I_{max} , percent maximal inhibition versus basal or a reference standard; MIC, minimum inhibitory concentration; POC, percent-of-control; HTS, high-throughput screening; DOS, diversity-oriented synthesis; SAR, structure–activity relationship; CNS, central nervous system; BBB, blood–brain barrier; HLM, human liver microsomes; RLM, rat liver microsomes; MLM, mouse or monkey liver microsomes; f_w , fraction unbound; TDI, time-dependent inhibition; CL, clearance of compound from plasma; CL_w , clearance of unbound compound from plasma; C_{avg} , average concentration of compound in plasma; V_{dss} , volume of distribution at steady state; $t_{1/2}$, half-life of compound in plasma; C_{max} , maximal compound concentration in plasma; t_{max} , time at which maximal compound concentration in plasma was achieved; AUC, area under the plasma concentration–time curve; F , bioavailability

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