Bioorganic & Medicinal Chemistry Letters 22 (2012) 6540–6548

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

BMCL Digest

How hydrogen bonds impact P-glycoprotein transport and permeability

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ARTICLE INFO

Article history:
Received 4 June 2012
Revised 3 August 2012
Accepted 14 August 2012
Available online 23 August 2012

Keywords:
Hydrogen bond
Intramolecular hydrogen bond
P-Glycoprotein
Permeability
HBA
HBD
TPSA
ATP-binding cassette

ABSTRACT

The requirement to cross a biological membrane can be a complex process especially if multidrug transporters such as P-gp must be considered. Drug partitioning into the lipid membrane and efflux by P-gp are tightly coupled processes wherein H-bonding interactions play a key role. All H-bond donors and acceptors are not equal in terms of the strength of the H-bonds that they form, hence it is important to consider their relative strength. Using various examples from literature, we illustrate the benefits of considering the relative strengths of individual H-bonds and introducing intramolecular H-bonds to increase membrane permeability and/or decrease P-gp efflux.

During preclinical development, medicinal chemists design compounds for effective delivery to the appropriate tissue or site of action. This requires, in many instances, crossing a lipid membrane or cellular barrier. Therefore, the compounds need to have the appropriate balance of physiochemical properties to cross these barriers preferably by passive diffusion. In addition, there are active efflux transporters located in these membranes whose goal is to 'pump out' xenobiotics or potentially toxic substances including drugs, which can negatively impact attaining target engagement at a desired dose. Consequently, structure–activity relationships (SAR) that improve biological activity and maintain appropriate physicochemical properties to provide an acceptable pharmacokinetic profile can be a daunting task for the medicinal chemist.

The human ATP-binding cassette (ABC) transporters belong to a superfamily of 51 human genes classified into seven subfamilies.[1] These transporters use the energy of ATP hydrolysis to extrude compounds across a lipid membrane by a complex translocation process.[2] Nine of these proteins are involved in multi-drug transport. The most studied transporters are ABCB1, better known as P-glycoprotein (P-gp) or MDR1, ABCG2, known as breast cancer resistance protein (BCRP), and several multidrug resistance-associated proteins (MRPs) from the ABCC subfamily comprised of 13 proteins.[3] Of all the known efflux pumps, P-gp is the one most widely studied and understood.[4] Because P-gp is localized at the surface of endothelial or epithelial cells, it can pump drugs back to the external space or to the blood depending upon the orientation of expression (Fig. 1), which can affect absorption, distribution, and elimination of substrates. Therefore, knowledge of successful SAR strategies to circumvent, or conversely to introduce P-gp efflux if CNS exposure might need to be reduced, could be valuable learning for the medicinal chemist. For example, a decrease in unbound CNS exposure might be achieved such that a sufficient margin of safety is realized against an unwanted CNS side effect as suggested by Polli et al. for differentiating sedating (P-gp substrates) from non-sedating (non-P-gp substrates) antihistamines.[5] A similar differentiation was proposed recently to explain the CNS-sparing, clinical outcome of antimuscarinic agents that are P-gp substrates.[6]

In the last few years, the medicinal chemistry field has seen a tremendous initiative to link successful marketed drugs to drug-like attributes and specific physicochemical properties with the ultimate goal of improved medicinal chemistry design.[7,8] In the CNS area, retrospective analysis of marketed drugs has shown that P-gp efflux and passive permeability are two crucial parameters to evaluate.[9,10] Recently, Wager et al. highlighted a set of six physicochemical properties, for example, *c*log*P* (calculated logarithm of the octanol/water partition coefficient), *c*log*D* (calculated logarithm of the octanol/water distribution coefficient at pH 7.4), molecular weight (MW), topological Polar Surface Area (TPSA), number of hydrogen bond (H-bond) donors (HBD; NH and OH groups), and most basic center (pK_a, logarithm of the acid dissociation constant), as the most relevant descriptors to take into

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0960-894X
http://dx.doi.org/10.1016/j.bmcl.2012.08.059

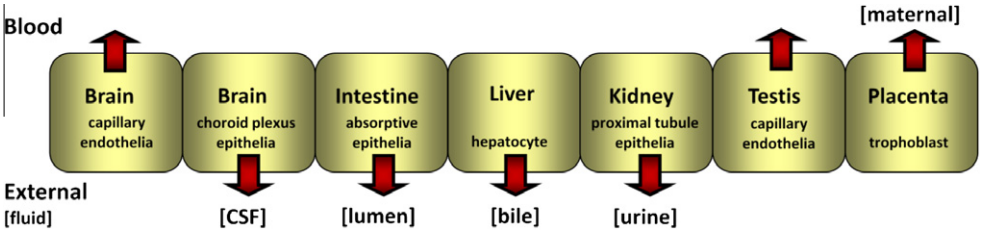


Figure 1. Schematic representation of P-gp expression and direction of net transport at different cellular barriers.

account for designing CNS molecules inclusive of modulating P-gp efflux and permeability.[8,11] These molecular descriptors are readily predicted with reasonable accuracy to guide SAR design. Interestingly and contrary to popular understanding based on the total brain-to-plasma concentration ratio, hydrophobicity does not appear to be a dominant determining factor of unbound brain exposure. The molecular descriptors related to polarity such as H-bond acceptors (HBA; N and O atoms), HBD and TPSA have been reported by many to be key factors for CNS exposure and especially determining unbound brain exposure.[8,12] The additive effect of increasing TPSA (through increase in HBA/HBD count) on decreasing passive permeability and simultaneously increasing P-gp transport efficiency, is likely to be responsible for a decrease in free drug exposure in brain.[13]

In our analysis of unbound brain exposure at five minutes after an intravenous bolus dose in mice[14] for a set of approximately 1000 structurally-diverse compounds from the Eli Lilly collection, it appeared that compounds with *c*log*P* less than 4.0 and with TPSA between 40–80 Å^2 had greater propensity to achieve relatively higher unbound brain exposure. In another internal analysis of ~2000 compounds tested for P-gp efflux using bidirectional flux across MDCK-MDR1 cell monolayers, we observed that compounds with TPSA less than 60 Å^2 and a calculated most basic pk_a of less than 8.0 had a much higher likelihood of being identified as non-P-gp substrates (94%) (unpublished data). Similar P-gp non-substrate behavior was observed if we replaced TPSA with HBA counts of less than 7. This was not surprising since TPSA is mainly dependent upon the total number of oxygen and nitrogen atoms. We also observed that increasing passive permeability is aligned with relatively lower likelihood of identifying a compound as a P-gp substrate in vitro. Because P-gp most likely binds its substrates in the lipid membrane, specifically in the cytosolic membrane leaflet, and then flips them to the outer leaflet, compounds must first cross the membrane by passive diffusion to reach the P-gp binding site.[15] If a compound diffuses rapidly across the membrane it can partially escape to the cytosol before being 'caught' by P-gp. Thus, P-gp efflux transport efficiency might be decreased by increasing passive permeability and could be one possible strategy for circumvention although success is chemotype dependent upon the net effects of compound-transporter binding site affinity, passive diffusion rate, and concentration of compound at the binding site.[16]

Comparing the data from these two analyses, it was apparent that factors such as TPSA (or HBA/HBD counts) affecting P-gp efflux and passive permeability also appeared to impact overall unbound brain exposure. Also, specifically considering the molecular properties affecting recognition by P-gp at a global level across the large set of structurally-diverse compounds, contribution of HBA count also was aligned with the recent data from Blatter and Seeling.[17] They conducted a systematic study of polyoxyethylene alkyl ethers that varied in the number of methylene and ethoxyl residues as model P-gp substrates. This study offered better understanding of the hydrophobic and electrostatic contributions to the process of substrate binding from water to P-gp within the lipid membrane environment. The lipid-water partitioning step preceding substrate binding to the P-gp cavity appeared to be driven by hydrophobic interactions whereas binding from the lipid membrane to the P-gp cavity seemed to be primarily driven exclusively by H-bonding, or specifically HBA.

While most publications describing key physicochemical properties impacting passive permeability and P-gp substrate recognition have focused on HBD and HBA counts and TPSA, relatively limited attention has been paid to detailed understanding of how differences in the HBD and HBA strengths contribute to these key parameters impacting CNS exposure. Thus, for a chemical series where the total counts of H-bonds and TPSA remain constant while observing significant changes in their P-gp recognition and/or permeability, it might be valuable to consider H-bond strength as potential descriptor affecting the outcome. Insights from this analysis then can be applied to guide structural changes that decrease or overcome P-gp efflux and/or increase passive permeability. Some case studies from the literature that exemplify this approach are described in this review. The reader is referred to additional recent examples where increased CNS exposure was achieved by reducing P-gp efflux efficiency[18–20] or where CNS exposure was decreased by decreasing passive diffusion or increasing P-gp efflux efficiency.[21,22]

Hydrogen bond strength and impact on p-gp and passive permeability

H-bond interactions between a ligand and its target protein are known to play a critical role in determining the overall affinity between the two. Not surprisingly, such interactions also have been considered to significantly influence recognition of compounds as substrates by P-gp.[13,16,23] In many cases, especially when comparing different chemical series to each other, a count of the number of HBD and HBA groups or calculated TPSA may serve as simple tools to differentiate scaffolds for relative likelihood of P-gp recognition. However, a more rigorous approach also can be desirable for the description of molecules as has been advocated by Abraham et al.[24] Not all donors and acceptors are 'equal' in terms of the strength of the H-bond that they form with the corresponding partner and hence parameters that can differentiate or rank order relative H-bond strengths are valuable. Among some of the experimental sources of H-bond scales, measurement of the H-bond equilibrium constants of a diverse set of proton donors and acceptors by Morris et al. is considered to provide an important reference set.[25] In this approach, 4-nitrophenol was employed as the standard donor and *N*-methylpyrrolidinone as the acceptor in presence of 1,1,1-trichloroethane as solvent with high dipolarity ($\varepsilon = 7.53$; making it a better model than tetrachloro methane/tetrachloro ethane for simulating biological membranes). Moreover, this data set provided good coverage of common HBD/HBA groups including several heterocyclic compounds. The HBD and HBA strengths were reported in terms of $\log K_\alpha$ and $\log K_\beta$ values, 'respectively'. The acidity '$\log K_\alpha$' term for a given functional group relates to the strength of H-bond formed by the solute donor when it interacts with lone pairs of acceptor groups in solvent molecules.

The basicity '$\log K_\beta$' term of a group relates to the strength of H-bond formed by the lone pairs of an acceptor (the solute) when it interacts with donor solvents.[26] Using such information, it is now possible to predict the relative strengths of individual H-bonds, or dipole–dipole attractions, and thus to quantify the various intermolecular forces between a drug molecule and its surrounding solvent molecules and/or receptors.

Several authors have reported surrogate in silico methods to calculate Abraham's H-bond strength parameters to enable use of these parameters as descriptors for SAR analysis. Japertas et al.[27] reported a fragment-based method while a more rigorous method based on quantum mechanics calculations has been described by Gancia et al.[28] The latter method was based on the premise that Abraham's H-bond scales could be related to parameters derivable from quantum-mechanical calculations such as atom self-polarizability and electrophilic superdelocalizability. These are, broadly speaking, parameters that include terms relating to the degree to which a given molecular orbital receives a contribution from an atomic orbital, and the energy of the orbital. The calculations were performed using semi-empirical quantum mechanics methods such as Austin Model 1 (AM1) [29] or Parametrization Method 3 (PM3).[30] In some of the examples described in this review, we have used $\log K_\alpha$ and $\log K_\beta$ values (as HBD/HBA strengths) calculated using an internal implementation of the quantum mechanical approach described by Gancia et al.[28]

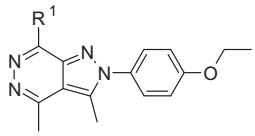
An example of a systematic assessment of HBD/HBA strength of a larger set of diverse compounds is the classification analysis of P-gp substrates by Didziapetris et al.[31] The authors used a fragment-based approach[27] to calculate Abraham's HBD/HBA strength parameters, α and β, 'respectively'.[25] The authors compiled a data set of 1000 compounds from various sources and based on careful assessment classified the same into P-gp substrates and non-substrates. The overall analysis highlighted HBA strength based on Abraham's β parameter (a measure of HBA strength) as one of the key descriptors, along with other physicochemical parameters such as MW, molecular volume, and ionization constants. Three different types of models were reported—global, probabilistic and class-specific. The global model suggested that simple, conformation-independent properties listed above could be used as indicators to assess the likelihood for a compound to be a P-gp substrate. Based on this model, compounds with Abraham's $\beta > 1.7$, MW > 400 and most acidic $pK_a > 4$ are likely to be P-gp substrates while those with $\beta < 1.7$, MW < 400 and most basic $pK_a < 8$ are likely to be non-substrates. Abraham's β parameter was also reported as one of the key descriptors for the probabilistic model along with volume and indicator variables for acidic or basic nature of the compounds.

In an interesting case study of voltage-gated calcium channel inhibitors for the potential treatment of neuropathic pain, an amino-substituted pyrazolopyridazine **1** (Table 1) was identified as the key starting point; however, it suffered from high clearance in rats.[32] In an attempt to reduce the clearance, the authors designed and synthesized compounds with lower hydrophobicity through the introduction of polar functional groups, exemplified by **2–4** (Table 1). They realized that while **1** was not a P-gp substrate, inclusion of additional donors/acceptors such as hydroxyl and primary amine groups, led to recognition by P-gp. The authors then employed an in-house in silico 'global' model for P-gp substrate recognition that was based on Abraham's descriptors for HBD/HBA strengths, total charge, $c\log D$ at pH 7.4 and $c\log P$. The model correctly identified the P-gp liability for **2–4** and indicated low probability for **1** to be a P-gp substrate. While using this model prospectively to mitigate P-gp substrate recognition, the authors designed and synthesized methoxy derivatives **5** and **6**, predicted to be marginal P-gp substrates, which were later confirmed to have lower P-gp efflux ratios (<2). Further optimization of this series led to **7** having a balanced profile with desirable potency and ADME characteristics, including lack of recognition by P-gp. In fact, both **7** and a very close analog **8**, showed improved in vivo Kp,brain values exceeding 0.9 (Kp,brain refers to brain:blood ratio) compared to **2** with Kp,brain value of 0.17. Compounds **7** and **8** also exhibited desirable activity in the in vivo pain model. Thus, the authors successfully utilized an in silico model incorporating HBD/HBA strengths (along with other descriptors) to guide the SAR to overcome P-gp liability and identified compounds with greater total CNS exposure in vivo.

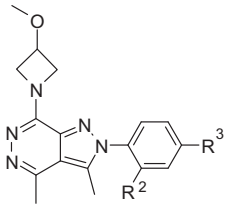
The next set of examples were selected to illustrate the potential role of HBD/HBA strengths in driving P-gp recognition within a highly homologous series where the 'traditional' physicochemical parameters such as HBD/HBA count or calculated TPSA remain constant. The first set of compounds are from the biphenylaminocyclopropane carboxamide series reported by Kuduk et al. as part of their effort to identify potent bradykinin B1 receptor antagonists with good pharmacokinetic properties including sufficient CNS exposure for efficacy.[33] As shown in Table 2, **9a–d**, which only differ in the R groups attached to the terminal amide, were found to have similar potency against B1 receptor and acceptable passive permeability while exhibiting a range of efflux ratios (1.4–8.6) by human P-gp. The dramatic effect of the polyhaloacetamide on P-gp transport cannot be explained based on the HBD/HBA counts or TPSA since these values are constant across these compounds. The authors proposed that the halogens alpha to the amide group result in a strong electron withdrawing effect thereby making the amide a poorer HBA that consequently make it less prone to recognition by P-gp. This hypothesis is also supported by our analysis of the HBD/HBA strengths calculated using the abovementioned quantum mechanical method (Table 2). The HBA strength of the terminal amide appears to increase from 1.3 to 2.1 (about 10-fold increase given that these values are in log scale) as the number of halogens attached to the amide are gradually removed from **9a–d**. The authors made additional minor structural changes to **9b** given its very poor bioavailability in dog and monkey. Thus, **10** was identified that maintained the trifluoroacetamide group with minimal P-gp efflux, acceptable CNS exposure in accord with receptor occupancy, along with higher bioavailability and lower clearance in both species.

H-bonding is known to be an important parameter for describing drug permeability[34–36] H-bonding of the solute with water predominates over H-bonds involving polar atoms of the phospholipid head groups, which is consistent with the number of water molecules present in this membrane region exceeding the number of lipid molecules.[37] However, the degree to which a compound interacts with the solvent water phase is not simply determined by the total number of H-bonds it is able to form with water molecules, but by the relative strength of these H-bonds.[38] The size and nature of the non-polar portion of a molecule, which depend upon the conformation assumed in that region of the membrane, also has a significant influence on the strength of the H-bonds the molecule is able to form.[37,38] During transmembrane diffusion, movement of the solute from the region of minimum free energy (at ~15 Å from the bilayer center) into the ordered chain region within the bilayer interior is energetically disfavored and this is largely due to the progressive breakage of H-bonds.[37] This concept of desolvation originally proposed by Stein[39] was reintroduced by Burton's laboratory in the early 1990s with the demonstration that passive diffusion of a congener series of purposefully designed, small neutral peptides correlated with the number of H-bond groups.[40] As the number of solute-solvent H-bonds increased, permeability decreased and removal of the peptide bonds by N-methylation resulted in predictable increases in permeability.[41,42] As described in the case of P-gp, and also true for permeability, consideration of the HBD/HBA strength would be critical when

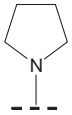
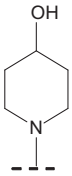
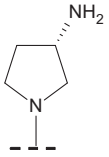
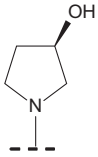
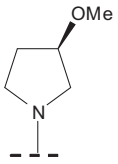
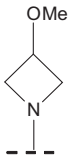
Table 1
Summary of potency and P-glycoprotein data for compound examples from Myatt et al.[32]



1, 2 - 6



7 - 8

Compound	R^1	R^2	R^3	$\alpha_2\delta_1$ IC_{50} (nM)	Predicted P-gp score[a]	P-gp Efflux ratio[b]
1		—	—	2	0.38	1.4
2		—	—	32	−0.40	9.1
3		—	—	2	−0.68	29
4		—	—	5	−0.36	9
5		—	—	6	−0.01	1.8
6		—	—	5	−0.03	1.8
7	—	OCF_3	Cl	8	NA	Non-substrate
8	—	F	OEt	8	NA	Non-substrate

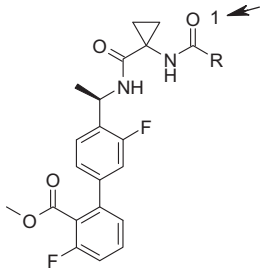
[a] P-gp score calculated by an unpublished Pfizer method used by the authors: <−0.20 predicts a substrate with high confidence; −0.20–0.00 predicts a borderline substrate; 0.00–0.20 predicts a borderline non-substrate and >0.20 predicts a non-substrate with high confidence.
[b] Bi-directional transport ratio of P_{app} (B–A)/(A–B) across MDCK-MDR1 cell monolayer overexpressing human P-gp.

the total number of HBD/HBA are constant across a series of homologous compounds. In the subsequent text, we provide a few examples where changes in H-bond strength can explain changes in passive diffusion of matched pairs.

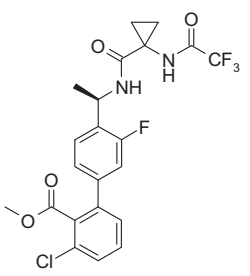
The first example compares compounds **11** and **12** from Cox et al.[43] These dihydropyrrole inhibitors of Kinesin Spindle Protein were optimized to circumvent P-gp efflux (see also[44,45]). In this matched pair, α-difluoromethyl substitution on the C2-aminopropyl side chain not only decreased apparent P-gp efflux, but also increased passive permeability nearly 10-fold (Table 3). β-fluorination reduced the pK_a of the amine from 10.3 to 7.0 and increased log*P* from 1.2 to 3.2, which are consistent with the increase in passive diffusion. In this case, neither the number of H-bonds nor the TPSA changed at all; however, the predicted HBA strength of the amine decreased by 6.9-fold for **12** versus **11** (Table 3). The change in HB accepting strength also is consistent with the change in passive permeability. It is expected that these H-bond effects will be less useful in cases for ionic species where electrostatic interactions may dominate.[40]

The second example involves piperidine-based renin inhibitors that were optimized for faster passive diffusion to improve oral absorption.[46] Bi-directional transport in LLC-PK1 cells with and without rat P-gp expression showed the pyridone series to have slow (<30 nm/sec) permeability that agreed with poor rat oral bioavailability (Table 4). It was established that intestinal P-gp also contributed to low rat oral bioavailability by measuring a 10-fold

Table 2
Summary of potency, passive permeability, P-glycoprotein efflux and calculated H-bond accepting strength for selected compounds from Kuduk et al.[33]



9a-d

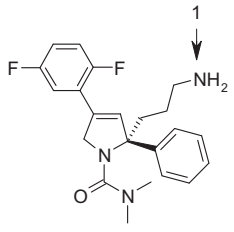


10

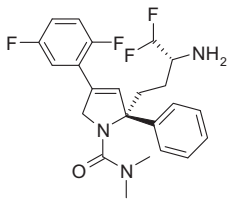
Compound[a]	R	hB1K_i[b] (nM)	P-gp Efflux ratio[c]	HBA (log) Strength of atom 1[d]	P_{app}[e] (nm/sec)
9a	CF_2CF_3	1.6	1.4	1.35	310
9b	CF_3	0.57	2.3	1.39	280
9c	CHF_2	0.4	3.2	1.63	310
9d	CH_3	0.93	8.6	2.12	210
10	—	0.44	1.9	1.39	340

[a] HBD/HBA count and calculated PSA are 2, 6 and 84.5, respectively for all compounds.
[b] Bradykinin B1 receptor binding affinity.
[c] Bi-directional transport ratio of P_{app} (B–A)/(A–B) across MDCK-MDR1 cell monolayer overexpressing human P-gp.
[d] HBA strength was calculated based on method described in the manuscript.[28]
[e] Passive apparent permeability coefficient with P-gp inhibited.

Table 3
Summary of physicochemical values, passive permeability, P-glycoprotein efflux and calculated H-bond accepting strength for two compounds from Cox et al.[43]



11

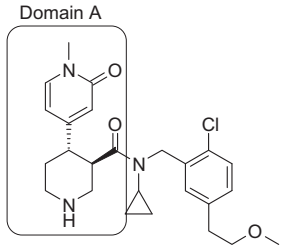


12

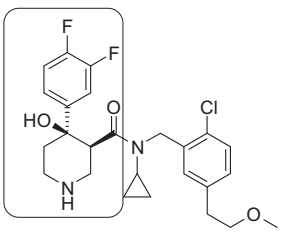
Compound	**11**	**12**
$P_{passive}$[a], nm/sec	40	380
MDR ratio[b]	1200	5
P-gp efflux ratio[c]	18.5	2.5
log*P*	1.2	3.2
pK_a	10.3	7.0
PSA	46	42
No. of HBA	1	1
No. of HBD	4	4
HBA (log) Strength of amine[d] (atom 1)	2.82	1.98

[a] Passive apparent permeability coefficient with P-gp inhibited.
[b] Ratio of cell uptake between parent cell line and cell line over-expressing P-gp.
[c] Bi-directional transport ratio of P_{app} (B–A)/(A–B) across MDCK-MDR1 cell monolayer overexpressing human P-gp.
[d] HBA strength was calculated based on method described in the manuscript.[28]

Table 4
Summary of physicochemical values, passive permeability, oral bioavailability and calculated H-bond accepting strength for two compounds from Levesque et al.[46]



13



14

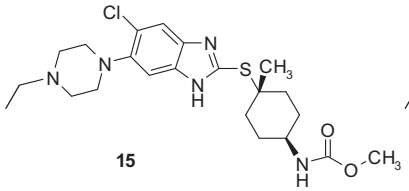
Compound	**13**	**14**
$P_{passive}$[a], nm/sec	10	110
%F, po[b]	1	16
log*D*	1.0	3.1
PSA	66	58
No. of HBA	6	5
No. of HBD	1	2
HBA (log) Strength domain A[c]	4.92	3.63
HBA (log) Strength total[c]	9.24	8.16

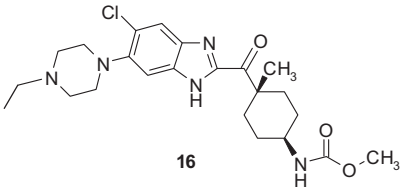
[a] Passive apparent permeability coefficient across LLC-PK1 cell monolayers without rat P-gp expression.
[b] Rat bioavailability after oral dose.
[c] HBA strength was calculated based on method described in the manuscript.[28]

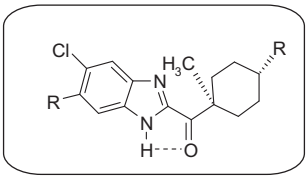
increase in systemic exposure after co-dosing pyridone analogs with the P-gp inhibitor elacridar (GF120918). They determined that substitution of the pyridone moiety at the 4-position of the piperidine ring with a (3,4-difluorophenyl)-piperidin-4-ol aryl group increased passive permeability, which led to improved oral absorption by overwhelming gut P-gp efflux. They concluded that the increase in rat oral bioavailability was likely caused by increased passive permeability since there was minimal effect on P-gp efflux. Compound **14** had 11-fold faster passive permeability than **13**, which corresponds to an increase in hydrophobicity, but a decrease in number of HBA and an increase in the number of HBD. The modest decrease in PSA from 66 to 58 does not explain more than 10-fold increase in passive permeability (Table 4). However, considering the calculated H-bond strengths, it appears that eliminating the pyridine and introducing the hydroxyl resulted in a 12-fold decrease in total HBA strength with only a modest increase in HBD strength, which is more in line with the extent of increase in passive permeability (Table 4). This example once again supports the use of predicted H-bond strength to help drive SAR.

In summary, it appears that when considering a more diverse set of compounds ('global data'), the HBD/HBA strength-related parameters have been identified as key descriptors, but would require additional physicochemical parameters to provide a reasonable indication for P-gp liability across the larger set. On

Table 5
Example of an intramolecular H-bond decreasing P-glycoprotein transport from Kobayashi et al.[53]







Compound	Linker	ORL1 binding IC50 (nM)	MW	*c*Log P[a]	Basic *cp*K_a of benzimidazole N[a]	PSA	No. of HBA	No. of HBD	P-gp efflux ratio[b]
15	S	2.4	466	4.2	4.64	74	7	2	16
16	C=O	5.7	462	3.7	3.55	90	8	2 (1)[c]	1.9

[a] Calculated using Chemaxon.
[b] Bi-directional transport ratio (B/A)/(A/B) across MDCK-MDR1 cell monolayers over-expressing human P-gp.
[c] The intramolecular H-bond can neutralize the benzimidazole NH, effectively leaving only one HBD as well as masking the additional carbonyl acceptor.

the other hand, there are numerous examples involving homologous series where the HBD/HBA strength appears to have direct impact on recognition by P-gp and passive permeability. Various in silico tools to calculate $\log K_\alpha$ and $\log K_\beta$ provide a reasonable estimation of the relative HBD/HBA strengths. At the same time, it should be noted that most tools to calculate these parameters would face some limitations in handling tautomers and conformational flexibility. In such cases, care should be taken to ensure that the most relevant tautomer/conformation is used for calculations. Also, steric effect or accessibility of the donor/acceptors may not be differentiated by these methods. Thus, these descriptors may have limited application when comparing compounds spanning a range of structural diversity.

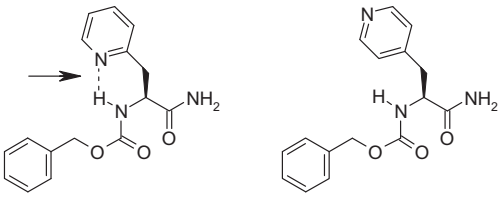
Intramolecular hydrogen bonds and impact on P-gp substrate recognition and passive permeability

An attractive alternative to the permanent removal of HBD, to maintain target activity, is the introduction of a complementary HBA that is able to assume a conformation where the two groups can adopt a suitable geometry and distance to form a temporary ring or conformation where an intramolecular H-bond is formed. This temporary ring is different than the intermolecular conformations with solvent resulting in a thermodynamic equilibrium between the closed (intramolecular H-bond) and open (intermolecular H-bond) conformation. Intramolecular H-bonds, though often weaker than their intermolecular counterparts, have significant influence on properties such as charge distribution within molecules, the relative stability of conformers, and reactivity.[47] The closed form, masking polarity from the environment and effectively removing one donor and one acceptor atom from the molecule, may then be less recognized as a P-gp substrate. Additionally, the closed form tends to be more lipophilic and might display faster membrane permeability.[48,49] Indeed, Wright and Painter used uptake into red blood cells as a surrogate for membrane permeability to show that introduction of an intramolecular H-bond increased permeability of positional isomers of hydroxybenzoic acid.[50]

Kuhn et al. reported a systematic analysis of the Cambridge Crystallographic Database in terms of propensity for intramolecular H-bond formation of five- to eight-membered ring systems.[49] The highest frequency of intramolecular H-bonds was observed for planar, six-membered rings stabilized by conjugation with a π-system. Infantes et al. investigated the competition between inter- and intramolecular H-bonding and concluded that intramolecular H-bonds are preferred when five- or six-membered conjugated rings are formed.[51] A variety of far less-explored topologies were identified by Kuhn et al., such as six-membered ring H-bonds containing one sp3 center (non-planar) and a number of non-planar, seven- and eight-membered rings.[49] They also synthesized a series of closely-related compounds where the changes in membrane permeability, water solubility and hydrophobicity were directly related to the potential to form an intramolecular H-bond. In this section, we will highlight a few recent examples showing how the formation of an intramolecular H-bond can reduce P-gp efflux and improve target exposure, such as in the brain. The reader is referred to the decade-old paper by Ashwood et al., which elegantly demonstrates how introduction of an intramolecular H-bond in the SAR was used to effectively increase CNS exposure.[52]

To evaluate the biological role of the opioid-related (ORL1) receptor and assess the therapeutic potential of the ORL1 antagonists, it was important to develop orally bioavailable and brain penetrant compounds.[53] An initial medicinal chemistry effort by the Tsukuba researchers led to identification of potent and highly-selective ORL1 antagonists represented by **15** (Table 5). Further testing revealed that most compounds with a hydrophilic group on a thioether moiety (like **15**) exhibited markedly low brain exposure. They demonstrated that **15** was a substrate for human P-gp, so SAR was directed to overcome P-gp substrate recognition to increase brain exposure. As part of a systematic SAR, substitution of the thioether linkage by a ketone (**16**) was tolerated in terms of binding affinity in contrast to replacement with methylene, a nitrogen atom, or an oxygen atom that resulted in complete loss of potency (Table 5). Interestingly, **16** was not a P-gp substrate. To explain this unexpected result, the authors speculated that it might correlate with the decrease in the calculated pK_a value of the benzimidazole. However, an alternative explanation might be the possibility of intramolecular H-bond formation between the introduced carbonyl and the benzimidazole NH. Analogous to the strategy described by Kuhn et al.,[49] we conducted a systematic analysis of relative energies, which were calculated using quantum mechanical calculations, of open and closed conformations of **16** in

Table 6
Example of an intramolecular H-bond decreasing P-glycoprotein transport from Rafi et al.[54]



17 **18**

Compound	MW	*c*Log*P*[a]	PSA	No. of HBD	*cp*K$_a$[a]	A–B P_{app}[b] (nm/sec)	P-gp efflux ratio[c]
17	299	1.2	94	2 (1[d])	4.5	177	1.1
18	299	1.2	94	2	5.1	43	3.1

[a] Calculated using Chemaxon.
[b] Apparent permeability coefficient across MDCK-MDR1 cell monolayers in the A-to-B direction.
[c] Bi-directional transport ratio (B/A)/(A/B) across MDCK-MDR1 cell monolayers overexpressing human P-gp.
[d] The intramolecular H-bond can effectively neutralize one donor (carbamate NH) and one acceptor (pyridyl *N*).

both polar and non-polar media. It was estimated that in both environments, the closed conformation with an intramolecular H-bond between the carbonyl oxygen and the benzimidazole NH would be significantly more stable than the open form without such interactions. One can hypothesize that this is likely to result in loss of potential key interaction between the benzimidazole NH and P-gp resulting in a significant decrease in P-gp substrate recognition.

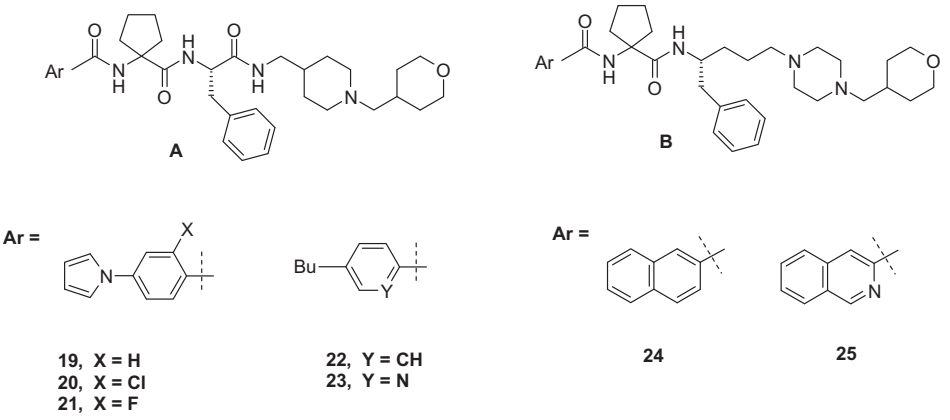
To systematically explore the impact of intramolecular H-bonds on passive permeability, Jacobsen and colleagues have been designing and synthesizing short peptides and peptidic small molecules in which a HBA is positioned within proximity of backbone NH groups.[54,55] Matched pairs of compounds containing either HBA-bearing or unmodified amino acids were tested for permeability by employing MDR1-MDCK cell monolayers expressing human P-gp. In the particular pair of compounds shown in Table 6 (**17** and **18**), the 2-pyridyl analogue (**17**) was both predicted and found experimentally to be more permeable than **18** (177 versus 43 nm/sec, respectively).[54] The ability of **17** to form an intramolecular H-bond reduced the number of exposed HBD and attenuated the *p*K$_a$ resulting in increased permeability. There also was a reduction, albeit modest, in the P-gp efflux ratio although this cannot be attributed directly to a decrease in compound-P-gp interaction (Table 6). This study shows that robust conformational analysis, such as an all-atom force-field based method, is a key component of their analysis making it suitable for intramolecular H-bonding predictions to optimally refine drug-like properties within a congener series.

The utility of using intramolecular H-bonds to increase permeability also is nicely captured by a congener series of tripeptidic neurokinin-2 (NK2) receptor antagonists.[56] The N-terminal amide proton is masked by terminally capping two similar scaffolds with ortho-substituted heteroatoms capable of forming an intramolecular H-bond. Membrane permeability was measured in vitro using both a Parallel Artificial Membrane Permeability Assay (PAMPA) and Caco-2 cell monolayers (Table 7). Compound **19** of scaffold A had very slow permeability. Aromatic chlorine (**20**) is generally known to be a poor HBA, but a study by Huque et al. demonstrated that it decreases the HBD capability of its partner when involved in an intramolecular interaction. Similarly, fluorine's role as a HBA is also debated.[57] A fluorine atom bonded to carbon needs very short contacts (~2.6 Å) with acidic hydrogen atoms in order to act as a HBA, so this distance criterion is not reliable to infer F···H H-bond formation.[58,59] But, aromatic fluorine as in **21** can participate in significant H-bonds albeit weaker than those involving HBAs such as oxygen and nitrogen.[60] Both halogenated compounds increased permeability in both assays with fluorine yielding the greater effect (Table 7). A similar, but more dramatic, effect was achieved in scaffolds A and B using ortho pyridine (**23** vs **22**) and isoquinoline (**25** vs **24**) (Table 7). Isoquinoline **25** proved the most shielded in DMSO and water, as determined by NMR studies on temperature dependence of the NH resonance value, which agrees with the permeability data.

Labby et al. introduced an intramolecular H-bond within selective *syn*-3,4-substituted pyrrolidine inhibitors of neuronal nitric oxide synthase (nNOS) to increase their membrane permeability for improved oral and blood–brain barrier absorption (Fig. 2).[61] They had tried different strategies like replacing the high *p*K$_a$ amino groups with neutral functionalities such as ethers and amides or by inserting electron-withdrawing groups, such as ether, monofluoromethylene, difluoromethylene, and cyclopropyl, to inductively decrease the *p*K$_a$ of the amino group, but despite improved membrane permeability, in vitro potency and selectivity were adversely compromised. Compounds **26a–c** have benzyl-like aryl substituents so that 6-membered intramolecular H-bonds would form. The 5-membered intramolecular HB (**26d**) was found to be the least likely to form, which is consistent with Kuhn et al.[49] Using NMR spectroscopy, H-bonding was observed in water at physiological pH and above 37 °C suggesting that the intramolecular H-bonds of **26b** and **26c** are stable in solution and that this approach would be amenable to enhancing membrane permeability in vivo. Interestingly, they found, using an indirect in vitro measure to represent relative permeability, **26a** and **26b** had the highest permeability despite NMR evidence that compounds **26b** and **26c** formed an intramolecular H-bond. In spite of the dogma that fluorine-mediated H-bonds are weak, the results with **26a** were inconsistent with this interpretation and contrary to the NMR evidence provided. The authors suggested that **26a** with a fluorophenyl has higher lipophilicity plus fluorine decreased the *p*k$_a$ of the basic nitrogen, where as **26c** has polar hydroxyl group. It is known that a single, aromatic H/F exchange raises log*D* by ~0.25 and larger increases in log*D* are usually seen when fluorine is introduced nearby a basic N increasing the ratio of neutral to protonated molecules.[62] These F···H–N intramolecular H-bonds or dipolar interactions could be made stronger depending upon the functional group in the beta position with respect to the F atom to effect its shielding.[63]

The above-mentioned examples clearly highlight the potential impact of introducing an intramolecular H-bond to address P-gp efflux and/or passive permeability. It should be noted that when designing compounds to incorporate intramolecular H-bonds, the arrangement of donor–acceptor pair in a suitable location may not be a sufficient indicator that such interactions will occur. As described in detail elsewhere, it is critical to consider relative

Table 7
Example of an intramolecular H-bond increasing passive permeability[56]



Compound	19	20	21	22	23	24	25
PAMPA[a] (nm/sec)	—	1.6	6.6	16	72	60	155
Caco-2[b] (nm/sec)	<10	42	138	93	176	146	252

[a] Apparent permeability coefficient from the Parallel Artificial Membrane Permeability Assay (PAMPA).
[b] Permeability across Caco-2 cell monolayers.

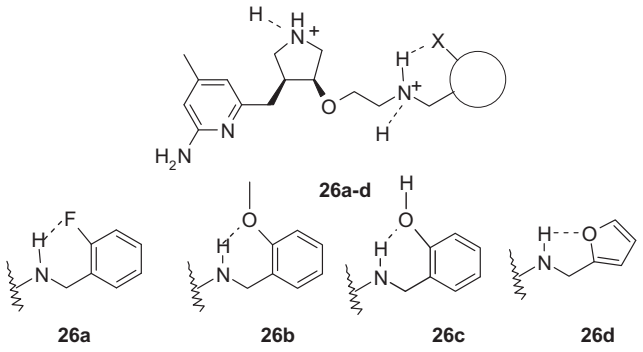


Figure 2. Impact of different intramolecular H-bonds to increase membrane permeability.[61]

stability of the open versus closed conformations of the molecule in both water and the membrane environment, and the impact of intramolecular H-bond formation on changes in the free energy associated with transfer of the compound from water to membrane or to P-gp within the membrane.[49,54,61,63] It is also important to consider how the intramolecular H-bond might impact other attributes beyond intrinsic activity such as solubility. If solubility is affected negatively, such as in a difference in crystal packing, then dissolution rate in the GI tract could decrease and result in lower oral bioavailability.[64]

In summary, the balancing of multiple key attributes during optimization of a chemical series can be challenging and is sometimes under-supported, or even misled, by application of a handful of easily computed molecular properties that broadly describe global characteristics and trends. The requirement to cross a biological membrane can be a complex process especially if ABC multidrug transporters such as P-gp must be considered. For example, use of a popular PSA descriptor alone accounts for only 50–60% of total absorption variance[65] despite its undeniable impact on medicinal chemistry.[66] Drug partitioning into the lipid membrane and drug binding to P-gp within the lipid membrane are tightly coupled processes where too many H-bonding groups in a drug molecule can have a deleterious effect on membrane penetration, due to energetically unfavorable water desolvation and binding of a drug molecule to P-gp is favored primarily through H-bond accepting groups. Using a number of examples from the recent literature, we have illustrated the benefits of predicting the relative strengths of individual H-bonds and introducing intramolecular H-bonds to increase membrane permeability and/or decrease P-gp transport. Despite potential ramifications for potency, removal of H-bonding groups is a much more effective and efficient strategy than adding more hydrocarbon and, in lieu of permanently removing H-bonds to maintain target activity, introduction of a intramolecular H-bond to mask groups that are required for potency may be the preferred strategy to achieve improved target engagement.

Acknowledgments

The authors are thankful to Dr. Howard Broughton (Eli Lilly) for providing the tool used to calculate hydrogen bond strength based on semi-empirical quantum mechanical methods. We also appreciate valuable suggestions on the manuscript by Dr. Kevin Gardinier (Eli Lilly).

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