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Validating the Use of Rational Modification of Compounds to Reduce P-gp Efflux

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Abstract

In both the Central nervous system (CNS) as well as Oncology small molecule drug discovery programs, efflux due to P-glycoprotein (P-gp) could be a deterrent during the discovery phase to obtain *in vitro* or *in vivo* pharmacological readouts. Several different strategies have been utilized in the past in order to overcome efflux by P-gp, many of which have been described in a recent article [5] from our labs. We describe the use of Induced-fit docking (IFD) of matched pairs (pairs of molecules modified by a single group) in order to demonstrate that a change in the IFD score, destabilizing the complex, will afford a molecule with less P-gp efflux potential. In a particular discovery program, several iterations might need to be implemented after examining the IFD lowest scored pose to obtain a molecule in that chemical series without P-gp efflux. Examples of such an effort are currently underway and will be published shortly. This summary describes therefore what we consider, a validation of a unifying approach to obtaining molecules with low to no p-gp efflux potential.

Keywords: P-gp, MDR1, Blood-brain barrier, Kp, CNS penetration, P-glycoprotein, Drug interaction, CADD

Background

Successful neurodegenerative disease and central nervous system drugs require exposure in the target tissue. Often limiting drug levels and a major challenge in developing drugs for CNS indications are the efflux transporters expressed at the blood brain barrier (**Figure 1**). One such efflux transporter is permeability glycoprotein or P-glycoprotein, or the ATP-binding cassette subfamily B member 1 (ABCB1) [1]. Avoiding efflux by P-gp is also important for oncology drug discovery where the transporter limits drug exposure in cancer cells. Development of P-gp inhibitors as adjuvants for oncology drugs is an active area of research [2].

Structure based approaches to modelling efflux transporters has been made difficult by the challenge in obtaining atomic resolution structures of membrane bound proteins. Advances in Cryogenic electron microscopy (Cryo-EM) made in the last decade or so have made obtainment of transporter proteins a new possibility. Furthermore, structures of P-Glycoprotein (P-gp) have been available for several years, they are bound with inhibitor rather than substrate. When P-gp operates as an efflux pump the transporter cycles through various conformations with the aid of ATP. The first published structures of human P-gp with the substrate taxol (pdb code 6qex) [3] as well as the drug free structure in a similar conformation (pdb code 7a65) defined the substrate-binding, entrance tunnel and the vestibule regions [4].

The set of cryo-EM structures enabled the article recently published [5] describing a predictive model for P-gp efflux activity. The initial hypothesis that substrates interact more strongly with the transporter when docked into the active site was then evaluated. Test molecules were docked in the substrate binding pocket and an induced-fit docking (IFD) algorithm utilized and the overall binding energy assessed.

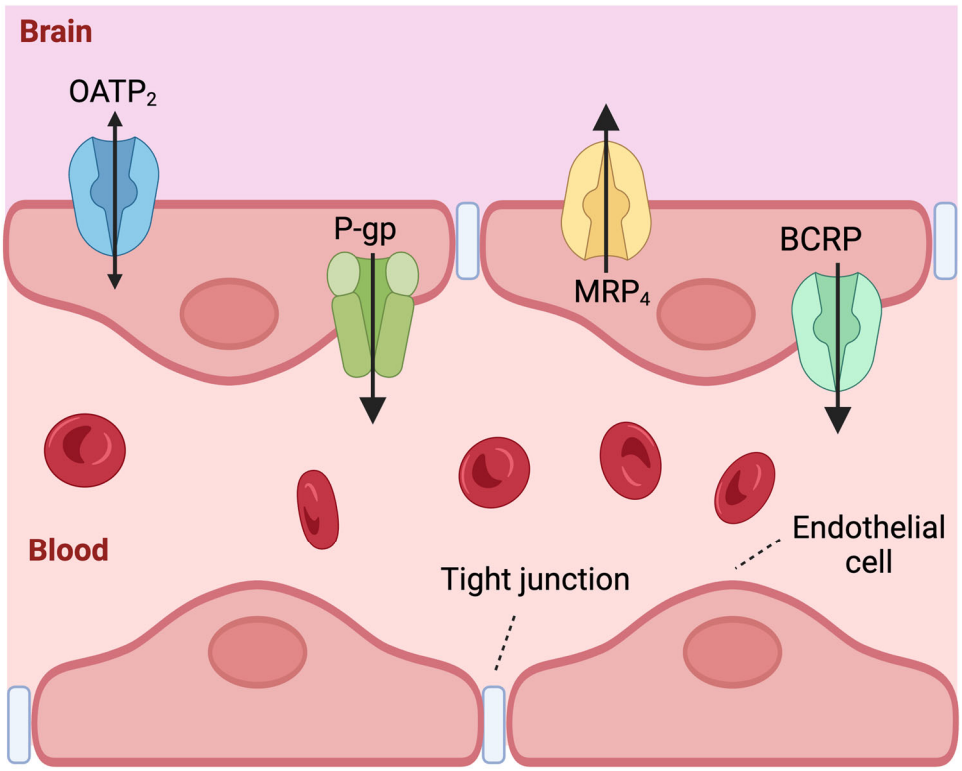


Figure 1. A cartoon representation of the blood brain barrier depicting various transporters present, including P-gp. Created with BioRender.com.

Using matched pairs of compounds with single point changes that are reported to reduce P-gp efflux matched the computed decrease in binding energy. This correlation demonstrated that the computational methodology could be applied to the structure-based design of small molecules with lower P-gp efflux activity.

Five Chemical Structural Modifcations

The molecular changes that affect P-gp efflux described in the CNS and oncology drug discovery literature can be categorized into groups [6,7]. Five examples covering the types of changes will be covered in this summary. The compound names in this commentary are the same used in Conrad *et al.* [5].

Increasing molecular volume

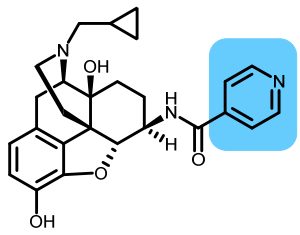
The first example involves increasing the Molecular weight or molecular volume as shown in **Table 1**. This is exemplified by the molecular pair NAP and NAQ. Synthesized as mu-opioid receptor antagonists a 4-pyridyl amide in NAP is replaced with an isoquinoline in NAQ [8]. Both compounds have similar *in vitro* potency towards the opioid receptor. However, only NAQ with an efflux ratio of 1.3, displays *in vivo* efficacy whereas NAP, with an efflux ratio of >10, does not display any pharmacology. This is attributed to significantly lower brain exposure levels for NAP. The IFD pose of NAP is better accommodated by 7 kcal/mol with hydrogen bonds and cation-pi interactions which, due to the increase in size of the second aromatic ring, NAQ cannot form.

Changing conformation

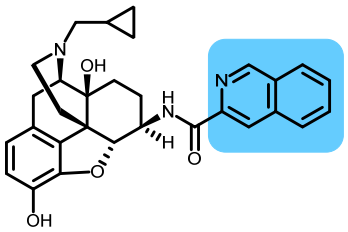
The second type of change, conformation, is shown with the matched pair opioid receptor-like 1 (ORL1) antagonists **1a** and **1b** [9]. In this pair of molecules shown in **Table 2**, a small change from a thio ether in compound **1a** to a ketone group in compound **1b**, a small change in molecular weight, was found to not greatly impact the activity towards ORL1 antagonism. However, the P-gp efflux was reduced drastically. There is a large conformation change for **1a** and **1b** on binding P-pg where compound **1a** was better accommodated in the IFD pose in P-gp by 26 kcal/mol.

Increase pKb

The third type of change is an increase of pKb or decrease of pKa (pKa + pKb = 14) exemplified by the molecules shown in **Table 3** which were synthesized as Kinesin Spindle Protein Inhibitors **2c**-**2f** [10]. In these compounds fluorine was iteratively incorporated, and the efflux measured by an MDR assay [11,12]. Many discovery compounds often contain a basic nitrogen which can also enhance efflux. Therefore, a common



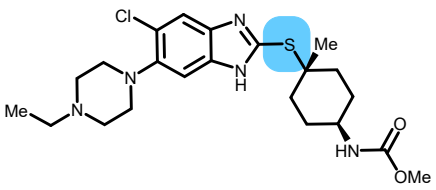
NAP



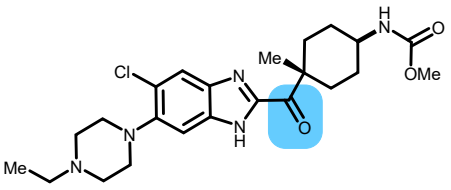
NAQ

Compound	P-gp ER	P_{app} ($x10^{-6}$cm/s)	IFD Score
NAP	>10	<1	-46105
NAQ	1.3	3	-46098

Table 1. Structure of **NAP** and **NAQ**. The highlighted portions show the changes in the pair accounting for the difference in P-gp efflux.



1a



1b

Compound	P-gp ER	IFD Score
1a	16	-46086
1b	1.9	-46050

Table 2. The highlighted single group change in compounds **1a** and **1b** which changes the conformation leading to a change in P-gp efflux.

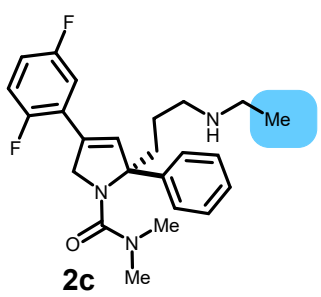
tactic to decrease amine pKa is the replacement of a hydrogen by a fluorine near the basic heteroatom. This can however have several effects. As well as decreasing the hydrogen bond accepting ability of the heteroatom, fluorine has a different size and impact on conformation than hydrogen. In this example the pKa of the N-H will also be lowered by increased fluorine incorporation. In **Table 3**, the pKa decreased progressively from 10.7 to 5.2. On docking these compounds into P-gp, it is clear, that the larger size of fluorine has an impact on the binding pose. Although this is a more complicated case the experimental MDR assay values match the IFD score.

Removal of a hydrogen bond donor

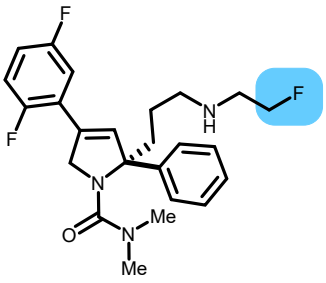
Removal of a hydrogen bond (H-bond) donor can also decrease P-gp efflux and is exemplified by the matched pair in **Table 4**. This pair were synthesized as Cannabinoid-2 agonists for chronic pain [13] with both molecules showing similar *in vitro* CB2 activity. Here the molecular weight of the pair of molecules is identical. The removal of the H-bond donor leads to a decrease in interactions between molecule **5b** with P-gp as the NH to P-gp amide C=O hydrogen bond present in **5a** is lost. A lowering in IFD score for **5b** correlates with a large change in P-gp efflux ratio.

Removal of a hydrogen bond acceptor

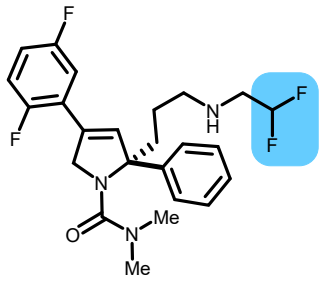
The last example to reduce P-gp efflux is the removal of a H-bond acceptor in dual serotonin and noradrenaline reuptake inhibitors shown in examples **7a** and **7b**, (**Table 5**) [14]. The decrease in molecular weight by deleting acceptors can also improve ligand efficiency and in the example shown in **Table 5**, the molecular weight is reduced by 28 amu. The compounds dock into different pockets with compound **7a** forming stronger interactions even though the tetrahydropyranyl O in compound **7a** is not involved in an H-bond directly.



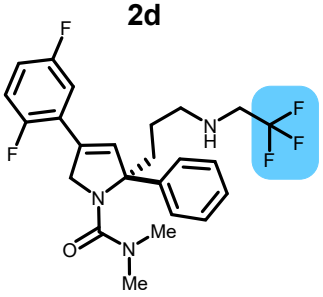
2c



2d



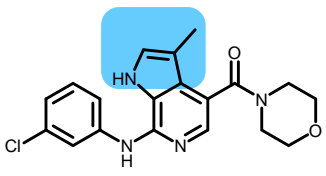
2e



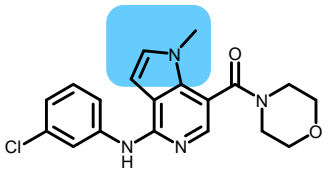
2f

Compound	MDR	pKa	IFD Score
2c	>135	10.7	-45972
2d	32	8.8	-45968
2e	3	7.0	-45968
2f	1	5.2	-45964

Table 3. A series of molecules where the C, beta to the basic N is increasingly substituted with Fluorine (F), decreasing the pKa. The effects of the increasing Fluorine substitution are to make the basic N less basic. Also progressing from **2c** through **2f**, the lowest energy pose for **2c** cannot be achieved by **2d** through **2f** due to the added steric bulk of each added F.



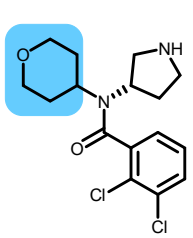
5a



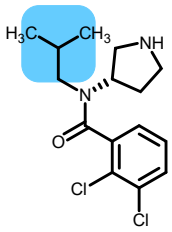
5b

Compound	P-gp ER	pKa	IFD Score
5a	74	5.8	-46077
5b	2.9	6.1	-46057

Table 4. A pair of compounds where removal of an H-bond donor in **5a** leads to a decrease in P-gp Efflux.



7a



7b

Compound	P-gp ER	IFD Score
7a	20	-45891
7b	2.7	-45878

Table 5. A pair of compounds where removal of an H-bond acceptor in **7b** leads to a decrease in P-gp Efflux.

Conclusions

Even though there are several different design strategies to reduce P-gp efflux, the change in the IFD score is unifying. Decrease in IFD score correlates with reduced P-gp efflux and can be employed in modifying a chemical structure from being a strong P-gp substrate to a non-substrate. With this computational tool in hand small molecule drug candidates can be tailored to maintain desired target activity or toxicological properties and minimize P-gp efflux.

Structures of other efflux transporters such as BCRP [15,16] and MRP4 [17,18] as well as *in vitro* data availability for these and other transporters [19] will enable the use of rational design of molecules to overcome efflux from those transporters. These efforts hopefully will lead to compounds with effective pharmacological CNS and oncology compounds with less resistance.

References

1. Schinkel AH. P-Glycoprotein, a gatekeeper in the blood-brain barrier. Adv Drug Deliv Rev. 1999 Apr 5;36(2-3):179-94.

2. Lai JI, Tseng YJ, Chen MH, Huang CF, Chang PM. Clinical Perspective of FDA Approved Drugs With P-Glycoprotein Inhibition Activities for Potential Cancer Therapeutics. Front Oncol. 2020 Nov 16;10:561936.

3. Alam A, Kowal J, Broude E, Roninson I, Locher KP. Structural insight into substrate and inhibitor discrimination by human P-glycoprotein. Science. 2019 Feb 15;363(6428):753-6.

4. Nosol K, Romane K, Irobalieva RN, Alam A, Kowal J, Fujita N, et al. Cryo-EM structures reveal distinct mechanisms of inhibition of the human multidrug transporter ABCB1. Proc Natl Acad Sci USA. 2020 Oct 20;117(42):26245-53.

5. Conrad J, Paras NA, Vaz RJ. Model of P-Glycoprotein Ligand Binding and Validation with Efflux Substrate Matched Pairs. J Med Chem. 2024 Apr 11;67(7):5854-65.

6. Hitchcock SA. Structural modifications that alter the P-glycoprotein efflux properties of compounds. J Med Chem. 2012 Jun 14;55(11):4877-95.

7. Gillis EP, Eastman KJ, Hill MD, Donnelly DJ, Meanwell NA. Applications of Fluorine in Medicinal Chemistry. J Med Chem. 2015 Nov 12;58(21):8315-59.

8. Yuan Y, Li G, He H, Stevens DL, Kozak P, Scoggins KL, et al. Characterization of 6α- and 6β-N-heterocyclic substituted naltrexamine derivatives as novel leads to development of mu opioid receptor selective antagonists. ACS Chem Neurosci. 2011 Jul 20;2(7):346-51.

9. Kobayashi K, Uchiyama M, Takahashi H, Kawamoto H, Ito S, Yoshizumi T, et al. 2-Cyclohexylcarbonylbenzimidazoles as potent, orally available and brain-penetrable opioid receptor-like 1 (ORL1) antagonists. Bioorg Med Chem Lett. 2009 Jun 1;19(11):3096-9.

10. Cox CD, Breslin MJ, Whitman DB, Coleman PJ, Garbaccio RM, Fraley ME, et al. Kinesin spindle protein (KSP) inhibitors. Part V: discovery of 2-propylamino-2,4-diaryl-2,5-dihydropyrroles as potent, water-soluble KSP inhibitors, and modulation of their basicity by beta-fluorination to overcome cellular efflux by P-glycoprotein. Bioorg Med Chem Lett. 2007 May 15;17(10):2697-702.

11. Hochman JH, Yamazaki M, Ohe T, Lin JH. Evaluation of drug interactions with P-glycoprotein in drug discovery: in vitro assessment of the potential for drug-drug interactions with P-glycoprotein. Curr Drug Metab. 2002 Jun;3(3):257-73.

12. Shen DW, Cardarelli C, Hwang J, Cornwell M, Richert N, Ishii S, et al. Multiple drug-resistant human KB carcinoma cells independently selected for high-level resistance to colchicine, adriamycin, or vinblastine show changes in expression of specific proteins. J Biol Chem. 1986 Jun 15;261(17):7762-70.

13. Giblin GM, Billinton A, Briggs M, Brown AJ, Chessell IP, Clayton

NM, et al. Discovery of 1-[4-(3-chlorophenylamino)-1-methyl-1H-pyrrolo[3,2-c]pyridin-7-yl]-1-morpholin-4-ylmethanone (GSK554418A), a brain penetrant 5-azaindole CB2 agonist for the treatment of chronic pain. J Med Chem. 2009 Oct 8;52(19):5785-8.

14. Wakenhut F, Allan GA, Fish PV, Fray MJ, Harrison AC, McCoy R, et al. N-[(3S)-Pyrrolidin-3-yl]benzamides as novel dual serotonin and noradrenaline reuptake inhibitors: impact of small structural modifications on P-gp recognition and CNS penetration. Bioorg Med Chem Lett. 2009 Sep 1;19(17):5078-81.

15. Orlando BJ, Liao M. ABCG2 transports anticancer drugs via a closed-to-open switch. Nat Commun. 2020 May 8;11(1):2264.

16. Kowal J, Ni D, Jackson SM, Manolaridis I, Stahlberg H, Locher KP. Structural Basis of Drug Recognition by the Multidrug Transporter ABCG2. J Mol Biol. 2021 Jun 25;433(13):166980.

17. Huang Y, Xue C, Wang L, Bu R, Mu J, Wang Y, et al. Structural basis for substrate and inhibitor recognition of human multidrug transporter MRP4. Commun Biol. 2023 May 22;6(1):549.

18. Bloch M, Raj I, Pape T, Taylor NMI. Structural and mechanistic basis of substrate transport by the multidrug transporter MRP4. Structure. 2023 Nov 2;31(11):1407-1418.e6.

19. Volpe DA. Transporter assays as useful in vitro tools in drug discovery and development. Expert Opin Drug Discov. 2016;11(1):91-103.