Drug-Like Property Concepts in Pharmaceutical Design

Li Di*, Edward H. Kerns and Guy T. Carter

Chemical Technologies, Chemical Sciences, Wyeth Research, Princeton, NJ 08852, USA

Abstract: The pharmaceutical industry is facing an ever increasing challenge to deliver safer and more effective medicines. Traditionally, drug discovery programs were driven solely by potency, regardless of the properties. As a result, the development of non-drug-like molecules was costly, had high risk and low success rate. To meet the challenges, the bar has been rising higher for drug candidates. They not only need to be active, but also drug-like to be advanced to clinical development. Drug-like properties, such as solubility, permeability, metabolic stability and transporter effects are of critical importance for the success of drug candidates. They affect oral bioavailability, metabolism, clearance, toxicity, as well as *in vitro* pharmacology. Insoluble and impermeable compounds can result in erroneous biological data and unreliable SAR in enzyme and cell-based assays. Rapid metabolism by enzymes and high efflux by transporters can lead to high clearance, short half-life, low systemic exposure and inadequate efficacy. Early property information helps teams make informed decisions and avoids wasting precious resources. Structure-property relationships are essential to guide structural modification to improve properties. High throughput ADME/TOX assays have been implemented and are being widely used to drive drug discovery projects in parallel with activity screening. Property design has become an integrated and inseparable part of the modern drug discovery paradigm. The approach has been proven to be a winning strategy.

Key Words: ADME/TOX, solubility, permeability, metabolism, transporters, P-glycoprotein, MRP2, BCRP.

INTRODUCTION

Discovery and development of new medicines is becoming increasingly challenging for the pharmaceutical industry. The R&D cost has grown dramatically over the years. The development timeline continues to rise due to novel therapeutic targets. The regulatory climate has become more uncertain. The public concern about safety has ever increased. To face the challenges, the pharmaceutical industry is developing and implementing new strategies to increase R&D productivity [1]. One of the strategies is to incorporate drug-like properties into pharmaceutical design early in the drug discovery process.

Traditionally, design of pharmaceuticals was driven solely by potency against therapeutic targets based on pharmacological screening (Fig. **1**). Project teams typically had very little information on drug-like properties during hit-to-lead and lead optimization phases. *In vivo* pharmacokinetic studies were typically conducted on a few potent compounds at later stages of drug discovery before entering into development. As a result, a large number of compounds failed to demonstrate efficacy *in vivo*, although they had good potency *in vitro*. This is mostly because of poor pharmacokinetics caused by inadequate drug-like properties, such as low solubility, poor permeability, high metabolism, and strong efflux by transporters [2]. The drug discovery model in the past was time consuming, costly and had a low success rate. Nowadays, many *in vitro* high throughput ADME/TOX assays have been developed and implemented early in drug discovery. Drug-like properties are being screened in parallel with biological activity (Figs. **1** and **2**), so that issues can be identified and addressed early. This approach has paid off. It has been shown that less than 10% of the drugs failed due to poor drug-like properties in 2000 as compare to more than 40% ten years ago (Fig. **3**) [3].

This review highlights a few key drug-like properties that have the most significant impact on drug discovery programs, including solubility, permeability, metabolism, and transporter effects. For more comprehensive reviews on this topic, readers should consult other references [4-9], as well as articles in this special issue.

KEY DRUG-LIKE PROPERTIES

1. Solubility

Solubility is one of the most challenging properties in drug discovery. It has been reported that over 30% of drug discovery compounds had solubilities in biological assays of less than 10 μM [10, 11]. This can lead to a number of issues for *in vitro* bioassays (Table **1**), since 10 μM is a typical concentration used in primary screens and HTS [11-15]. Compounds that are not fully soluble in bioassays can result in erratic assay results, erroneous SAR, and discrepancies between assays, such as enzyme and cell-based assays. This can appear as artificially low potency, owing to a right shift of the IC_{50} curve, and low HTS hit rates, because the actual concentration in solution is much lower than the target concentration. Low solubility also leads to under-estimation of toxicity, such as CYP450 inhibition or hERG channel blockage. Sometimes, the activities and properties just can not be measured because nothing is in solution. Solubility issues cause a lot of frustration and lost of productivity in drug discovery.

*Address correspondence to this author at the Chemical Technologies, Chemical Sciences, Wyeth Research, Princeton, NJ 08852, USA; E-mail: Tel: 732-274-4489; Fax: 732-274-4505; E-mail: DIL@WYETH.COM

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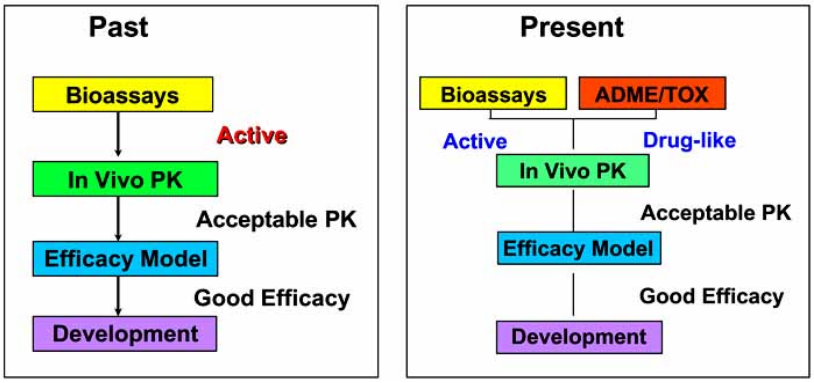


Fig. (1). Past and present screening paradigms of drug discovery

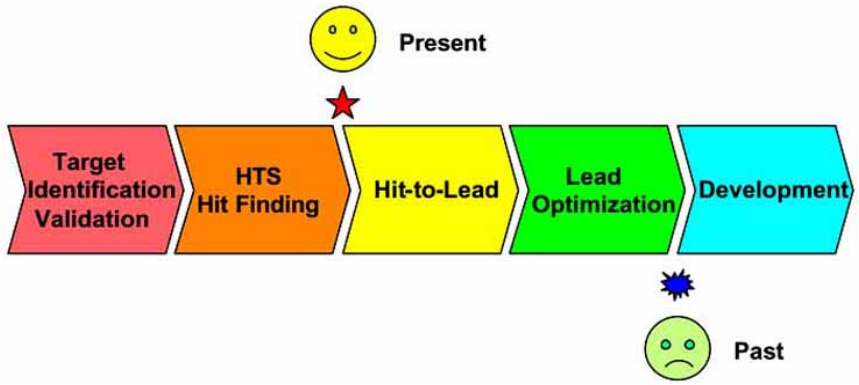


Fig. (2). Timing of profiling drug-like properties

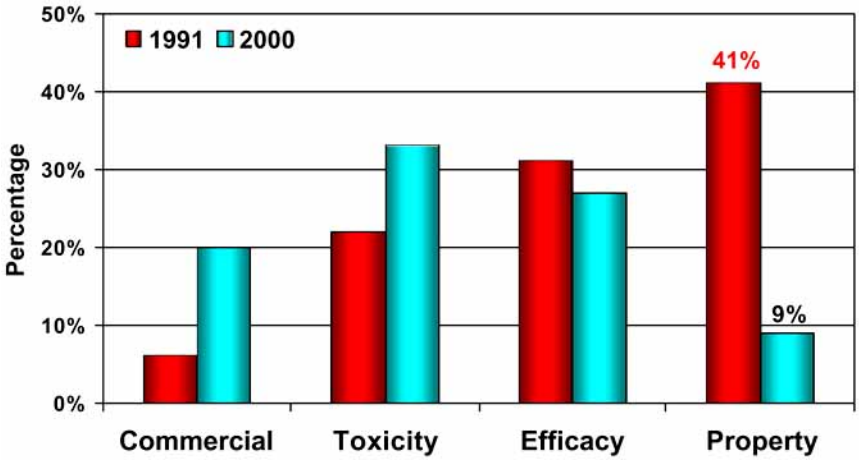


Fig. (3). Impact of incorporating drug-like properties in pharmaceutical design (modified from reference [3]).

It has been reported that over 75% of drug development candidates have low solubility based on BCS classification [16]. Among those, >50% were Class II (low solubility, high permeability) and > 25% were Class IV (low solubility, low permeability). Therefore, solubility is also a major issue for drug development. Poor solubility can significantly affect animal and clinical studies, as well as formulation development (Table **1**). Insoluble compounds tend to have poor oral bioavailability, lack of efficacy owing to low exposure, abnormal PK profiles owing to precipitation at the site of injection and re-dissolution, and have inter-species and inter-subject variations. Formulation of low solubility compounds can be problematic. Sometimes, a high amount of organic solvent has to be used to dissolve the compounds, which can cause toxicity. For example, the IV formulation of taxol contained a large amount of organic solvents (50% Cremophor EL and 50% ethanol). The vehicle was toxic when giving in large volumes, which limited the administration of taxol at high doses [17]. Sometimes, the solubility issues have to be resolved using prodrug approaches, which can be challeng-

ing [18, 19]. Development of insoluble compounds can be expensive and time consuming. The burden has, at times, been transferred to patients, by requiring administration of several large pills several times per day.

Table 1. Impairment of Solubility on *In Vitro* and *In Vivo* Results

In Vitro Impairment	*In Vivo* Impairment
Erratic assay results	Poor oral bioavailability
Erroneous SAR	Lack of efficacy
Discrepancies between assays	Abnormal PK profile
Artificially low potency	Inter-subject, -species variation
Low HTS hit rate	Problematic formulation
Underestimate toxicity	Expensive and prolong development
Can not be measured	Burden to patients

Therefore, optimization of solubility in drug design is critical to enhance discovery and development productivity, reduce cost and increase success rate. Strategies to enhance solubility include structural modification, prodrug approach and formulation development.

Introducing an ionizable center is very effective for increasing solubility. An example is shown in Fig. (**4**) for EP1 receptor antagonists. Compound A had no aqueous solubility. By introducing a basic nitrogen, an ionizable group, the solubility of compound B was enhanced greatly while maintaining good potency [20].

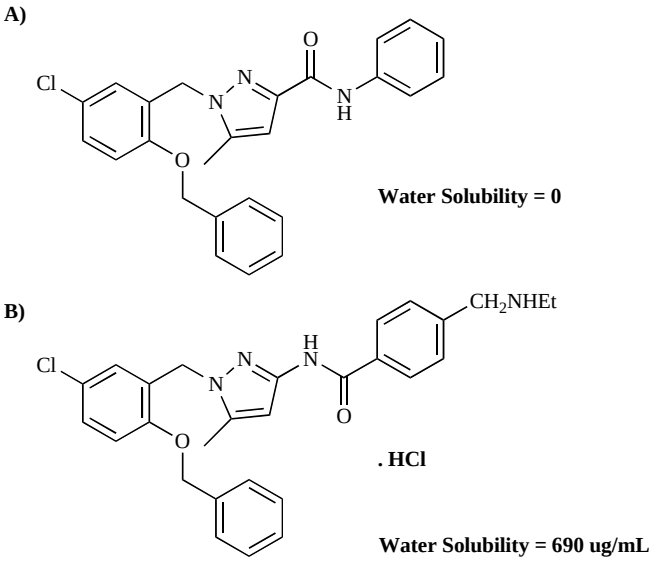


Fig. (4). EP1 receptor antagonists: Enhancement of Solubility By Introducing an Ionizable Group. Compounds **A** and **B** have similar potency. Compound **B** is much more soluble in water due to the basic amine, which ionized under acidic conditions [20].

Decreasing crystal packing energy by introducing out of plane substitution is another powerful strategy to increase solubility for planar molecules. Fig. (**5**) shows two cyclin-dependent kinase (CDK) inhibitors, compounds C and D. Compound D had much higher solubility than compound C, though compound D is more lipophilic (higher Log D). The methyl substituent of compound D disrupted the crystal packing lattice, increased packing energy and improved solubility [21].

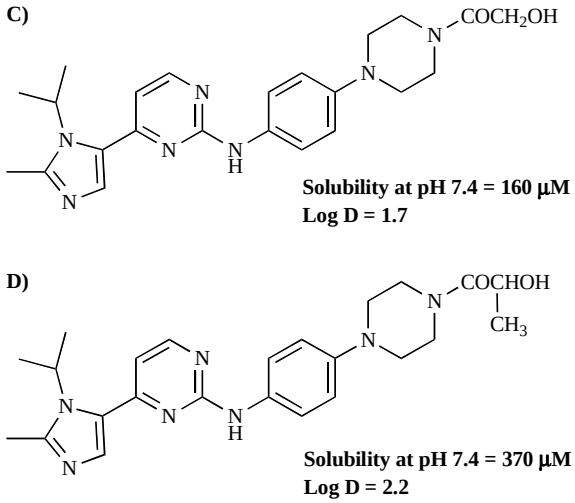


Fig. (5). CDK inhibitors: enhancement of solubility by disrupting crystal packing. Compounds (**C**) and (**D**) have equivalent IC_{50} values. Compound (**D**) is much more soluble than compound (**C**), even though compound (**D**) has higher Log D. The out of plane methyl substitution in compound (**D**) disturbed the crystal packing, reduced packing energy and increased solubility [21].

Prodrug approaches have been quite successful for increasing solubility of insoluble drugs. An example of using a phosphate prodrug to increase solubility is shown in Fig. (**6**). Amprenavir (Agenerase®) is an HIV protease inhibitor with

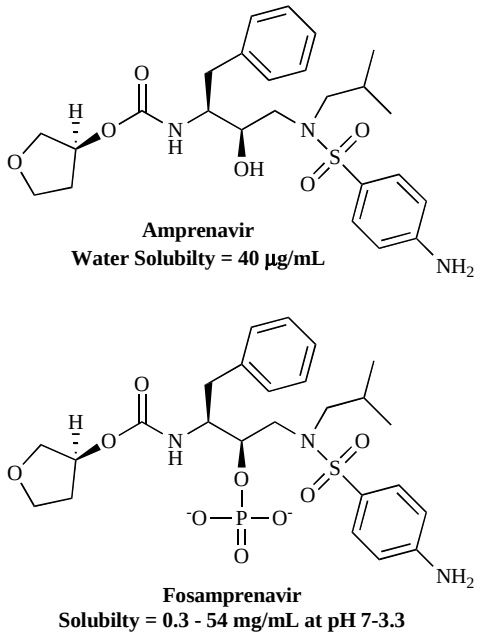


Fig. (6). HIV Protease inhibitors: phosphate prodrug increased solubility [19].

low water solubility (40 μg/mL)[19]. Owing to the poor solubility and high dose (1200 mg), a large amount of excipents was used to formulate the drug, which posed potential toxicity problems and reduced its usage. Fosamprenavir (Telzir® and Lexiva®) is the phosphate prodrug of Amprenavir. The solubility of Fosamprenavir is 0.3 mg/mL at pH 7 and 54 mg/mL at pH 3.3 [19, 22], which is an 8-1400 fold increase over Amprenavir.

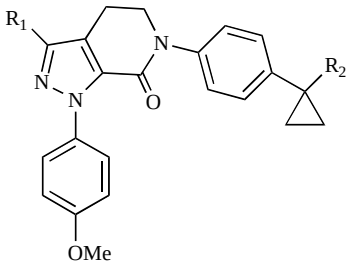
Formulation is another effective approach to improve solubility[23, 24]. Numerous strategies and technologies have been developed to formulate insoluble compounds for optimal *in vivo* exposure [23]. Recently, formulation has also been expanded to optimize *in vitro* bioassay conditions and minimize solubility effects on biological assays [11, 13]. Different additives can be added to bioassay media to maximize solubility and avoid precipitation during experiments. This is of critical importance in generating reliable SAR.

2. Permeability

High throughput screening (***HTS***) leads tend to have higher molecular weight, higher Log P and lower solubility than leads in the pre-***HTS era*** [25]. This paradigm shift leads to more insoluble compounds and fewer impermeable drugs. Since the implementation of HTS in the 90s, permeability has usually not been a major issue for drug discovery programs. Only a small portion (<15%) of development candidates belong to Class III (low permeability, high solubility) [16]. With ~25% in Class IV (low permeability, low solubility), the total number of low permeability development candidates is about 35% [16]. Permeability issues are less serious than solubility issues (>75% of development candidates) in drug discovery, but it is, nevertheless, an important drug-like property to be optimized. For peptides, peptide-mimetics and proteins, permeability is one of the major obstacles for oral delivery.

Compounds with poor permeability tend to have low oral absorption and low oral bioavailability, poor cell membrane penetration in cell-base assays, and low exposure for specific target organs, such as the brain. Permeability increases with lipophilicity and decreases with polarity, hydrogen bonding capacity and size of the molecules [26].

For a series of Factor Xa inhibitors (Fig. **7**), the following trends were observed with increasing Caco-2 permeability: $SO_2Me \sim CONH_2 < CN < CF_3$ [27]. Increasing permeability was, thus, consistent with decreasing polarity and polar surface area (PSA).



R2	R1 (Caco-2 Permeability x10^{-6} cm/s)			
	SO_2Me	$CONH_2$	CN	CF_3
CH_2NHMe	1	1	6	26
CH_2NMe_2	0.83	1.2	46	85

Fig. (7). Factor Xa inhibitors: permeability increased with decreased polarity [27].

Fig. (**8**) shows that increased permeability and reduced Pgp efflux improved oral bioavailability (%F) of CDK2 Inhibitors [28].

Prodrugs are a common strategy to enhance cell membrane penetration and passive absorption [19]. An example of a protein farnesyltransferase (FT) inhibitor prodrug to improve membrane permeability is shown in Fig. (**9**) [29]. The ester prodrug (compound F) increased the lipophilicity and cell membrane penetration. The prodrug was much more active in the cell-based assay and *in vivo* [29].

3. Metabolism

Metabolism affects clearance and oral bioavailability of compounds. Rapid clearance and low oral bioavailability can

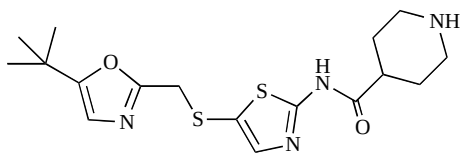
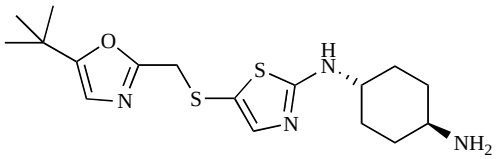
Compounds	IC50 (nM)	MDCK P_{app} (x10^{-6}cm/s)	MDCK Efflux Ratio	% Remaining (HLM/MLM)	CL (mL/min/Kg)	%F
	46	1	15	87/94	55	14
	30	5.8	9.4	53/77	72	62

Fig. (8). CDK2 inhibitor: improved permeability and reduced Pgp efflux enhanced oral bioavailability[28].

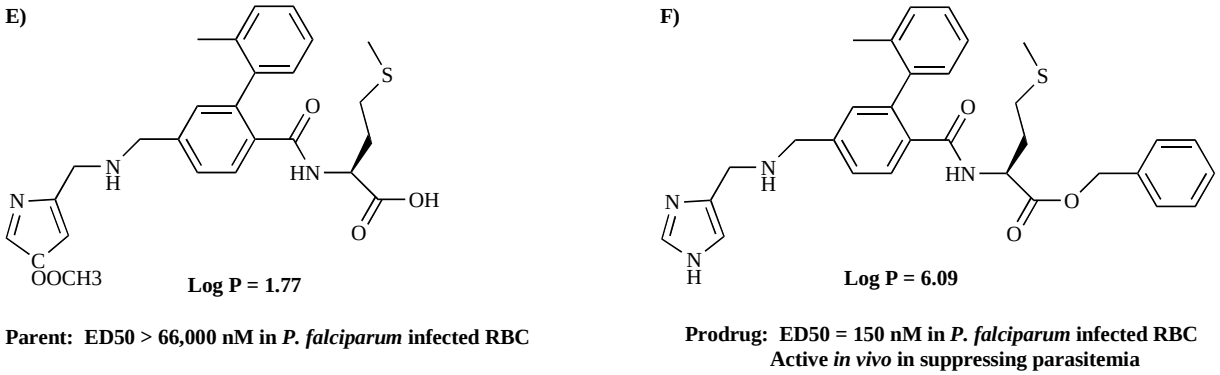


Fig. (9). Ester prodrug improved the cell membrane permeability of FT inhibitors [29].

limit the exposure of drugs to the target tissue and it is less likely to be efficacious. Extremely low metabolism can lead to prolonged half-life and accumulation of the drug in the body, resulting in toxicity. Therefore, identifying compounds with a desirable metabolic profile is critical for discovering drug candidates.

Metabolism is highly species dependent. The metabolic rate can vary significantly among different species, due to the unique metabolizing enzymes in the each species, strain and gender [30]. For example, CYP3A4 is the most important metabolizing enzyme in humans. More than 50% of the marketed drugs are metabolized by this enzyme. However, CYP3A4 is not found in any of the other species, not even monkey (Table **2**) [30]. The closest enzyme to human CYP3A4 is the mouse CYP3A11. Typically, rodents have a higher metabolic rate than dogs, monkeys and humans (Fig. (**10**), Compound G metabolic rate: Rat/Mouse > Dog > Monkey > Humans). However, there are some exceptions. For certain structural series, the rate of metabolism can be totally reversed (Fig. **10**, Compound H metabolic rate: Rat/Mouse < Dog < Monkey < Humans). For these classes of compounds, it is more difficult to judge the impact on *in vivo* PK from *in vitro* data, since clinical trials will be required to evaluate human PK and *in vitro-in vivo* correlation. Screening of metabolic stability in multiple animal species early in drug discovery is very useful to guide structural modification and selection compounds for *in vivo* studies. Metabolite identification is also helpful for teams to know the metabolically labile sites.

Table 2. Species Dependence of CYP3A [30]

Human	Mouse	Rat	Dog	Monkey
3A4	3A11	3A1/3A23	3A12	3A8
3A5	3A13	$3A2^{m}$	3A26	
3A7	3A16	$3A9^{f}$		
3A43	3A25	$3A18^{m}$		
	3A41	3A62		
	3A44			

m: male specific; f: female specific

Several strategies have been developed to improve metabolic stability, including blocking the labile "soft spots", removing the labile sites, reducing lipophilicity and isosteric replacement of the labile groups.

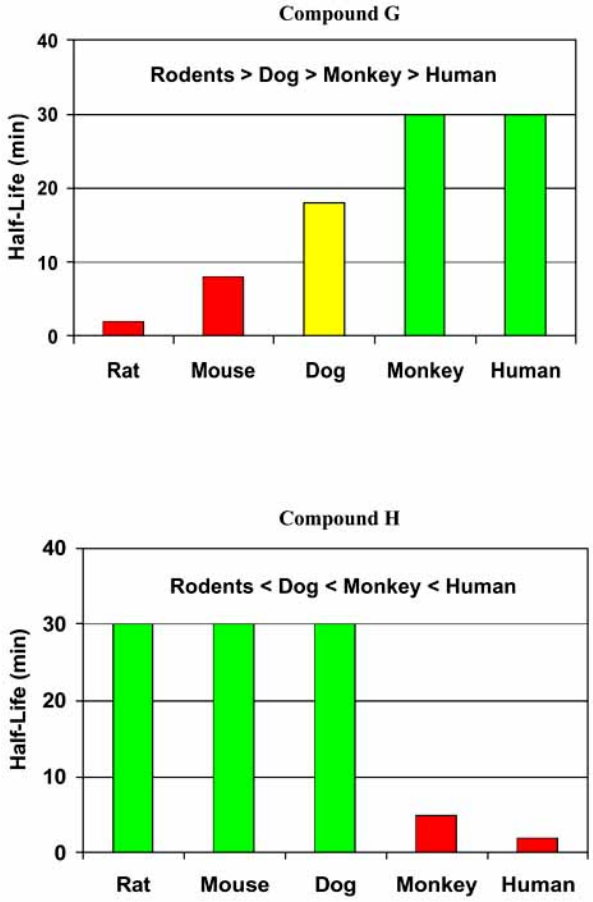
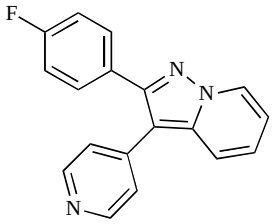


Fig. (10). Comparison of metabolic rates among species.

Fig. (**11**) shows an example of P38 inhibitors. Blocking the metabolically labile site improved the metabolic stability, reduced clearance and enhanced oral bioavailability in dog [31].

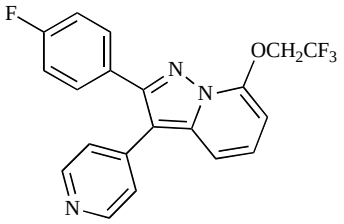
Certain functional groups, such as OH and COOH, are susceptible to Phase II glucuronidation. Isosteric replacement with less labile functional groups is effective to improve metabolic stability. Fig. (**12**) is an example of opioid

I)



% Metabolized = 81 % in Dog S9, 30 min
CL = 34 mL/min/Kg (Dog)
% F = 18 % (Dog)

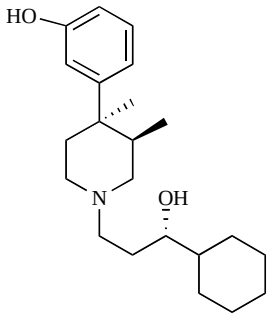
J)



% Metabolized = 0 % in Dog S9, 30 min
CL = 17 mL/min/Kg (Dog)
% F = 32 % (Dog)

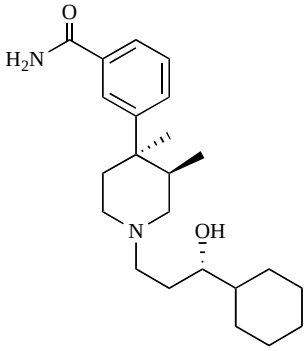
Fig. (11). Metabolic stability of p38 drug candidates: blocking the site of metabolism improved metabolic stability, reduced clearance and enhanced oral bioavailability [31].

K)



ED50 = 0.26 mpk (PO)
ED50 = 0.017 mpk (SC)
% Metabolized = 98 % rat liver slice, 24 h
% F = 2.5 % rat

L)



ED50 = 0.15 mpk (PO)
ED50 = 0.024 mpk (SC)
% Metabolized = 27 % rat liver slice, 24 h
% F = 32 % rat

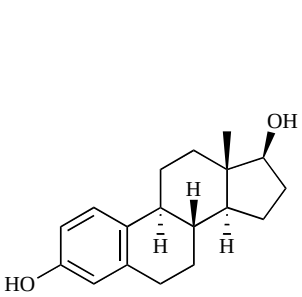
Fig. (12). Phase II glucuronidation of opioid antagonists: isosteric replacement of phenolic alcohol with amide improved Phase II metabolic stability, oral bioavailability and efficacy [32].

receptor antagonists [32]. Replacement of the phenolic alcohol (compound K) with amide (compound L) reduced Phase II conjugation, increased oral bioavailability and improved efficacy.

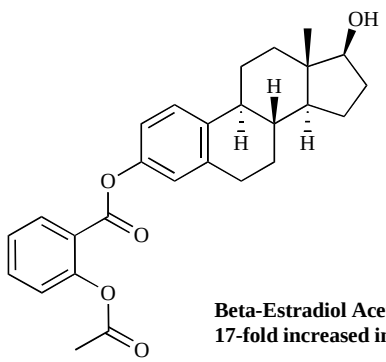
Prodrugs have been developed to reduce Phase II metabolism [19]. They are essentially slow release drugs. Fig. (**13**) shows how a acetylsalicylate prodrug of β-estradiol enhanced the oral bioavailability by 17 fold in dog owing to reduction of pre-systemic Phase II metabolism at the phenolic alcohol [19, 33].

4. Transporters

Effects of transporters on drug absorption, disposition and elimination have been widely studied and knowledge continues to increase [34, 35]. Transporters play an increasingly important role in drug discovery and development, owing to their significance in pharmacokinetics, efficacy and safety. The evolution of the transporter field is similar to the advancement of the cytochrome P450 family field in drug metabolism in the early 1990s. It is an exciting era for transporter research. The critical roles of transporters in absorp-



Beta-Estradiol



Beta-Estradiol Acetylsalicylate Prodrug
17-fold increased in oral bioavailability in dog

Fig. (13). Prodrug approach to reduce phase II conjugation at the phenolic alcohol [19, 33].

tion, blood brain barrier penetration, clearance, drug resistance, drug-drug interaction and safety have been well-recognized in the pharmaceutical industry.

4.1. Influx Transporters

Influx transporters can enhance drug uptake into the systemic circulation and specific tissues, such as brain and tumors. Many active uptake transporters have been targeted to facilitate transport of novel drug molecules into the disease targets. Transport systems for peptides, amino acids, monocarboxylic acids, bile acids, nucleotides and vitamins have been discovered and applied for targeted drug delivery [36, 37].

The uptake of gabapentin into the brain is mediated by L-amino acid transporter 1 (LAT1) [38]. The prodrug of gabapentin (Fig. **14**), XP13512/GSK1838262, is a substrate of monocarboxylate transporter Type 1 (MCT-1) and the sodium-dependent multivitamin transporter (SMVT)[39]. The extended-release dose of the gabapentin prodrug provided more predictable and prolonged exposure, higher oral bioavailability (74.5% vs. 36.6%), and lower dosing frequency than gabapentin [39]. Utilization of transporters enhances the pharmacokinetic profile of gabapentin.

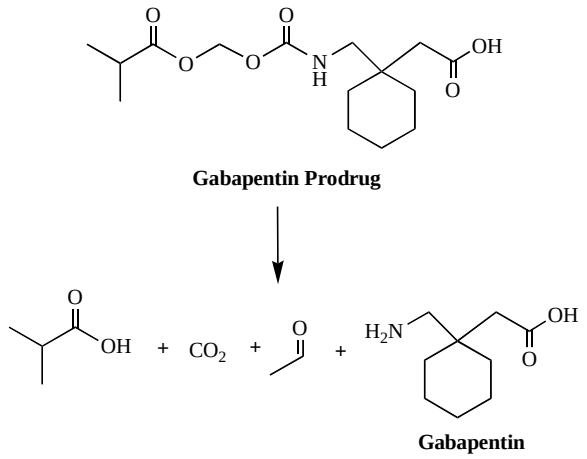


Fig. (14). Enhanced oral absorption of gabapentin prodrug utilizing uptake Transporters, MCT-1 and SMVT [39]. (Decomposition of the prodrug following absorption is shown).

LY544344, a prodrug of LY354740, is a potent group II metabotropic glutamate receptor agonist (Fig. **15**) [40]. LY544344 is a substrate of human intestine peptide transporter, hPEPT1 (SLC15A1). The oral bioavailability of the prodrug was 8.5 fold higher than the parent in rat (85% vs. 10%) [41]. PEPT1 is a very attractive transporter for drug delivery, due to its high capacity, broad substrate specificity, high level of expression in the intestinal epithelium, and low occurrence of functional polymorphisms [42, 43].

Other uptake transporters are also favorable targets for drug delivery, such as bile acid transporters [44, 45] and nucleoside transporters [46].

4.2. Efflux Transporters

Numerous efflux transporters have been discovered. The ATP binding cassette (ABC)-containing family of proteins have the greatest impact in drug discovery and development. They have tremendous impact on oral bioavailability, hepatobiliary and urinary clearance of drugs and metabolites, tissue penetration (brain, testes, uterus, skin, tumor, etc.) and drug resistance [47, 48]. There are 49 human ABC transporters belonging to 7 subfamilies from A to G [35]. Here we will focus the discussion on three apical efflux transporters

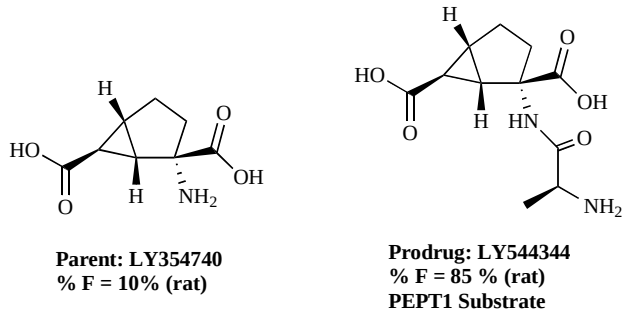


Fig. (15). PEPT1 substrate of LY354740 prodrug, LY544344, has increased oral absorption due to active transport mediated process [40, 41].

that are most relevant for drug discovery: Pgp, MRP2 and BCRP. The specificities of the substrates, inhibitors and stimulators for the transporters are shown in Table **3**. A significant amount of substrate overlap was observed for the three transporters (37-44%) and even higher overlap for inhibitors (67%) [49]. It is not uncommon that drugs can be substrates and inhibitors for multiple transporters and metabolizing enzymes.

Pgp is present in many important protective barriers, such as blood brain barrier, small and large intestines, liver, kidney, adrenal gland, pregnant uterus and skin. Pgp is of great clinical significance. It plays a very important role in multidrug resistance to cancer cells and resistance to antibiotics. It reduces oral bioavailability and brain penetration and increases drug excretion through liver and kidney. Doxorubicin is a Pgp substrate (Fig. **16**). The drug is eliminated through biliary clearance mediated by Pgp efflux (41% in human). Inhibition of Pgp reduced the biliary clearance by 38 fold [50]. Pgp has 4 binding sites. Structure modification strategies to reduce Pgp efflux are: decreasing basicity, reducing H-bond donors, increasing steric hindrance and reducing molecular weight (Table **3**). Fig. (**17**) shows that reducing basicity with decreased pKa overcame Pgp efflux for KSP inhibitors [51].

MRP2 is one of the most extensively expressed ABC transporters in the human liver and is a major determinant of biliary efflux of intrinsically anionic drugs such as methotrexate and pravastatin. The major function of MRP2 is biliary excretion of drugs as part of the hepatic detoxification process. Genetic disorders due to non-functional MRP2 can lead to Dubin-Johnson syndrome, a conjugated hyperbilirubinemia. Inhibition of MRP2 in the hepatocyte can result in disruption of lipid homeostasis and toxic accumulation of compounds in the liver, which is a major cause of withdrawal of drugs from the market [49]. MRP2 substrates include glutathione and other conjugates, organic anions, and leukotriene (LTC4) [52]. Studies of 25 methotrexate analogs

Table 3. Structural Features of Efflux Substrates

Transporter	Pgp	MRP2	BCRP
Substrate Specificity	Basic (pKa > 4, amines) H-bond Acceptors (N+O>8) High MW (> 400) [54, 57]	Negatively Charged (acids, phenols) Hydrophobic Aromatic [52, 58, 59]	Large molecules Positively & Negatively Charged Amiphilic and Lipophilic H-bond Donor [54, 60]
Inhibitor Specificity	Log P ≥ 2.92 Molecular Axis ≥ 18 atoms Tertiary N atom [54, 61]	Positively or Negatively Charged or Neutral High MW and Size Lipophilic and Aromatic [49]	Lipophilic Polarizability* Rich in Nitrogen [55]
Stimulator Specificity		Highly Negatively Charged High H-Bonding capacity [49]	
Binding Sites	4 [62, 63]	2 or 3 [64, 65]	2 or 3 [49, 66]

* Hydrogen bonding and π-π interaction.

showed that hydrophobicity , negatively charged groups and aromatic rings are important for MRP2 transport [52]. Lai, *et al.*, found there is a correlation between MRP2 transport and inhibition of the torsion angle of the biphenyls for a series of biphenyl-substituted heterocycles (compounds O, P, and Q; Fig. **18**) [53]. MRP2 inhibitors have: higher molecular weight, higher lipophilicity and higher aromaticity than non-inhibitors, while the PSA and charge were similar (Table **3**) [49]. MRP2 inhibitors can be positively or negatively charged or neutral, but MRP2 substrates and stimulators are mostly negatively charged. Multiple binding sites (A, B, and C) of MRP2 were proposed [49].

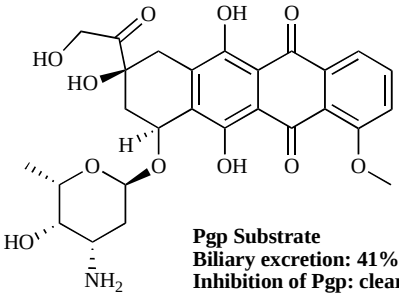
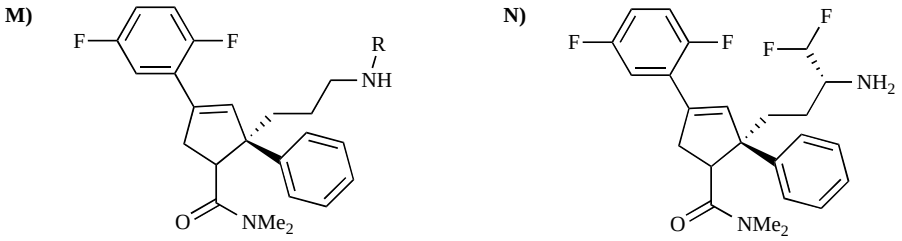


Fig. (16). Effect of Pgp efflux on clearance of doxorubicin [50].

BCRP (Breast Cancer Resistance Protein) is expressed in normal tissues such as small intestine, liver, placenta, kidney, BBB, testes, ovary and colon. It plays an active role in: limiting drug penetration to the central ***nervous*** systems (CNS), limiting oral absorption, secreting compounds from hepatocytes into bile, and secreting compounds from kidney into urine [54]. BCRP has 2-3 binding sites (Table **3**). Membrane partitioning is an important factor for drug interaction with BCRP. The strong influence of lipophilicity probably

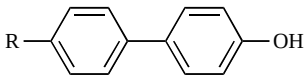


R	KSP IC_{50} (nM)	MDR Ratio	pKa	log P
H	2.2	1200	10.3	1.2
CH_2CH_3	10	> 135	10.7	1.6
CH_2CH_2F	10	32	8.8	2.6
CH_2CHF_2	12	3	7.0	3.4
CH_2CF_3	110	1	5.2	> 3.2
N	5.2	5	7	3.2

Fig. (17). KSP inhibitors: Pgp efflux was overcome by reducing basicity with decreased pKa [51].

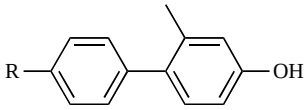
reflects a need for membrane partitioning to occur for the drug to reach the BCRP binding site [55]. Fig. (**19**) shows that increased lipophicity improved the inhibition of BCRP for a series of flavonoids (compounds R, S and T) [56].

O)



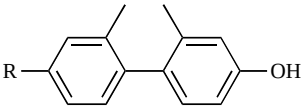
θ = 36.9 neither substrate nor inhibitor of MRP2

P)



θ = 54.2 substrate and mild inhibitor for MRP2

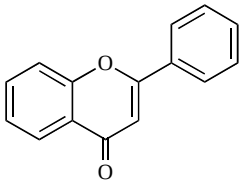
Q)



θ = 87.5 inhibitor for MRP2

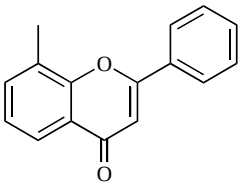
Fig. (18). Effect of substituents on torsion angle and MRP2 activity of bi-phenyls [53].

R)



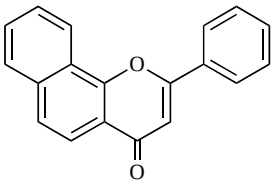
EC_{50} = 3.99 uM Log P =3.07

S)



EC_{50} = 0.61 uM Log P = 3.56

T)



EC_{50} = 0.07 uM Log P = 4.07

Fig. (19). Increased lipophilicity increased BCRP inhibition for flavonoids [56].

CONCLUSIONS

Drug-like properties have become an integrated part of the drug discovery process. They are playing a critical role in the success of drug candidates. Drug-like property information provides an early alert to potential issues, guides structural modification, prioritizes chemical series and diagnoses *in vivo* PK and pharmacology. As new concepts and technologies continue to evolve in the field, we will see more and more applications and impact of drug-like properties in drug discovery.

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