# 1 When and How to Apply ADMET Principles to Drug Discovery and Development

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## 1.1 SUMMARY

The oral delivery of small drug molecules is still the favored target of most drug discovery programs. Medicinal chemistry practices are skilled in the introduction of potency and selectivity against a desired target; however, an oral drug has to combine solubility and permeability, and rates of elimination low enough to sustain therapeutic concentrations. To achieve this screening cascades need to be introduced to select molecules with the right pharmacokinetic properties and to guide structure–activity relationships (SAR). Although there appears considerable freedom to target the right properties, many of the pharmacokinetic properties are dictated by the characteristics of the target and its active/binding site. Open solvent exposed binding sites often lead to larger drug molecules with high polar surface areas (PSAs), whereas smaller hydrophilic pockets lead to smaller molecules with lower PSA. This dictates the intrinsic permeability of a molecule that determines the fate of its overall disposition. Highly permeable molecules are usually cleared exclusively by metabolism, whereas complex

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processes involving metabolism and transporters clear less permeable molecules. With these molecules it is difficult to unravel the rate-limiting step.

Designing the screening cascade correctly is vital since information may not be helpful or may be misleading at the wrong stage. Moreover, even the basic absorption, distributions, metabolism and toxicity (ADMET) screens for solubility, permeability, and metabolic vulnerability can give results compromised by factors such as cosolvents (DMSO), the presence of transporters or membrane binding. Other systems, such as induction screening, may not provide information that can be put into context until the potency and likely dose of the candidate molecules can be estimated. As such these screens probably should be confined to the final phases of the discovery program. For instance, plasma protein binding information is only valuable to determine free fraction in *in vivo* experiments and thereby calculate unbound drug concentrations. Unbound drug concentrations allow understanding of the relationship between *in vitro* pharmacology measurements and *in vivo* activity. They also allow calculation of intrinsic clearance, which relates directly to *in vitro* clearance experiments such as microsomal metabolism rate. Protein binding does not determine *in vivo* unbound drug concentration, which is determined by intrinsic clearance. Attempts to modify drug series to reduce protein binding to achieve better efficacy are scientifically incorrect and may lead discovery programs down false alleys.

## 1.2 INTRODUCTION: THE STAGES OF DRUG DISCOVERY

Around 90% of newly approved drugs are small molecules. Within small molecules the oral route is the most favored with nearly 90% of drugs administered this way. Other routes include intravenous (5%) inhaled and transdermal. The oral route is considered the optimum for small molecule delivery as it allows a wide range of doses to be administered, allows convenient patient self-administration, is adaptable to varying dosage regimens, allows convenient pack size and needs no special equipment. To provide an oral drug the molecule must undergo dissolution in the gastrointestinal tract (GIT), absorption across the GIT, and penetration to the target, if it is intracellular or in the central nervous system (CNS). Moreover, the drug has to be present in the circulation and body for sufficient time and at concentration which activate or inhibit the pharmacological target, to provide a convenient dosage interval.

Whether we will see a change to new modalities in the next decade, in 2010 drug discovery and development programs were still mainly directed toward oral drugs. The methods used to discover drugs have gradually changed to what can be termed *strictly rationale led*. In these programs, a drug is developed against a known target, and usually the drug is optimized to only have affinity and selectivity toward this target. Many technologies including genomics, molecular biology methods of protein expression, microplate high throughput screening technology (HTS), X-ray crystallography have helped facilitate this direction.

A simple scheme for the stages of drug discovery and development is illustrated below:

*Stage I: Target Identification*. The protein of interest becomes apparent in the disease process. The identification may be triggered by genomics and proteomics. For instance, a group may be particularly resistant or sensitive to a disease and share a common genetic mutation, or a protein may be consistently overexpressed

or lacking in a disease. Both clearly are connected but the technology used for identification varies. Validation of the target is also important not only in terms of efficacy, but also safety. This will be discussed in more detail in Section 1.6.1.

*Stage II:* Series/lead identification comprising HTS leading to groups of compounds often termed *HITS*. These are compounds active in the HTS screen but need to be validated in terms of more rigorous pharmacology, structural inspection, purity, and so on. Some of the HITS may be deemed leads and trigger a chemical series. Although used in different ways, the term lead and lead series usually describes the compounds validated from HITS and seen as having sufficient chemical attractiveness to be predicted as capable of being modified into a potential drug. Lead series may also be assembled from fragment-based approaches (which assemble the lead from fragments shown to interact with the target) followed by chemistry follow up in literally joining these molecules together. This approach uses early examination of the modes and forms of interaction of the molecule with the target such as X-ray crystallography.

*Stage III:* Compound identification comprising series/lead development generating SAR, leading to compounds with most of the desirable properties. This is the time-consuming process of turning a potential lead, which may have low affinity for the target, low selectivity against some other proteins, and pharmacokinetic issues such as high metabolic instability. The process culminates in the production or nomination of a development candidate (and back up selection). Often, all the problems identified in the lead series are not completely resolved, and most drug candidates and even drugs are a compromise rather than a "perfect candidate."

*Stage IV: Candidate Development*. The drug candidate is profiled in human volunteers (phase 1) for tolerance and pharmacokinetics (adequate oral drug properties to activate/inhibit the pharmacological target with an acceptable dosage regimen). The drug is then administered in increasing numbers (phases 2 and 3) to patients to demonstrate safety and efficacy. Each stage is supported by preclinical toxicology, pharmacy (formulations), and so on.

Following Stage III, the properties of a molecule are fixed, and while ADMET work is an essential part of drug development, it cannot improve the intrinsic properties of a molecule. It does of course optimize the use of the drug in patients in terms of efficacy and side effects. Dosage regimens can be optimized for patients particularly where con meds are likely to be administered and ADMET preclinical work in combination with clinical pharmacokinetic studies is pivotal.

The statement concerning most drugs being a compromise is largely due to two factors.

1. Humans (and animals) have evolved to have multiple defense mechanisms against foreign molecules. Like many biological systems, there is considerable overlap and compensation. Moreover, these systems are usually promiscuous and able to accept a wide range of chemical forms and structures.
2. The drug targets chosen dictate to a considerable degree the nature of the chemical matter that interacts with them. Thus the medicinal chemist is constrained in his choices as to what molecules he can synthesize and what properties he wants in those molecules.

Even before a single molecule is synthesized or screened certain ADMET properties are therefore defined. The nature of the target largely determines the type of chemical matter and its complexity.

## 1.3 TARGET SPACE DEFINES THE BROAD PROPERTIES OF THE DRUG

Targets for drug discovery programs such as aminergic GPCRs, some ion channels, and certain enzymes such as cycloxygenase and acetylcholinesterase have small cavity volumes (less than 1000 $A^3$ buried within the protein target) and a large hydrophobic surface (representing 30% or greater of the total area). Polar ionic interactions are restricted and confined to this buried active site. Lead series, development candidates and drugs against these targets are small with moderate or low PSA and lipophilicities. These properties are conducive to discovering oral drugs. Some targets (usually lipid or lipid acid enzymes and carriers) require high lipophilicities to interact with the target. For instance, cholesterol ester transfer protein was an attractive target based on initial genomic data. Cholesterol esters have extremely high lipophilicities and it is not surprising, therefore, that inhibitors of the protein are all of very high lipophilicity. The development of these compounds has been hampered by poor solubility, inadequate dissolution, and the need for complex formulations. For some the natural substrate is a protein a considerable portion of which interacts with the active site. Aspartyl proteases, the family of which includes HIV protease, rennin and β-secretase targets for AIDS, hypertension and Alzheimer's respectively, have open binding cavities of greater dimensions, a smaller proportional hydrophobic surface and the need for more polar ionic interactions. Lead series, development candidates and drugs against these targets are large (MW >500) with high PSAs and lipophilicities. The consequences of these properties are explained below.

## 1.4 ADMET SPACE LIMITS THE POSSIBILITIES OF SUCCESS FOR AN ORAL DRUG

Membrane permeability is pivotal to ADMET properties defining absorption, rapid equilibrium between tissues, cell interiors and the circulating unbound drug in plasma, and the route of drug clearance and elimination. Drugs of high membrane permeability are not cleared renally because of tubular absorption. Permeable drugs rapidly establish equilibrium across membranes. This means an absence of influence by active transport processes since the passive flux is faster than any active transport flux. Such drugs are normally cleared by metabolism, whether this is by default, or that the groupings and structure giving good permeability are also amenable to metabolism by enzymes such as CYP450s is an introduction into how metabolism may be attenuated but is unlikely to be abolished. The influence of permeability on clearance is shown in Table 1.1. Drugs with lower membrane permeability may have their disposition influenced by transporters, both efflux and influx. The rate of transport flux now is (much) greater than the intrinsic passive flux so these molecules move in a largely unidirectional way across membranes with the requisite transporters present. Efflux transporters (e.g., Pgp) are particularly important in the GIT and the blood brain barrier. These transporters also play a role in active renal excretion and biliary excretion. The effects in the GIT

**TABLE 1.1 Classification of the Disposition Fate of a Compound Based on Its Permeability Across a Biological Membrane**

| Permeability | Low | Medium | High |
|---|---|---|---|
| PSA/Log *P* | High | Medium | Low |
| Absorption | Low (e.g., aliskeran) unless MW is less than 250 Da and absorbed by paracellular route (e.g., atenolol) | Variable Influenced by permeability and transporters (e.g., nelfinavir) | High via transcellular route (e.g., propranolol) |
| Bioavailability | As for absorption | As for absorption and metabolism | Variable. Influenced by metabolism |
| Clearance | Renal or biliary (possible transporter involvement) | Transporters and metabolism | Metabolism |

Permeability of a biomembrane is favored by lipophilicity and attenuated by polar functionality (PSA). Cells have tight junctions, which act as pores. The pore size is large in most capillaries allowing polar drugs access to extracellular receptors such as GPCRs. Pores are small in the capillaries of the blood brain barrier and moderate in the gastrointestinal tract.

are somewhat attenuated by the effects of saturation of the transporter by the high concentrations of drug present after the dissolution process. Most calculations suggest that the volume of gastrointestinal fluid in the small intestine is around 250 mL in which the entire administered dose can dissolve. In contrast, saturation of those present in the blood brain barrier is unlikely to occur at normal efficacious concentrations. Thus, drugs of lower membrane permeability may have reasonable oral absorption and bioavailability but not penetrate the CNS to a marked degree.

Membrane permeability depends on the drug being able to cross the lipoidal core of the membrane and is dependant therefore on the drug having affinity for lipid (lipophilicity). Lipophilicity (log *P* or log *D*) can be measured or calculated and can be conceived as being composed of two components that of molecular weight and polar surface area (PSA). Transfer through a membrane involves the complete removal of the molecule from water. Lipophilic groups have a positive solvation free energy and are readily soluble in the hydrocarbon core of a membrane. In contrast, the desolvation of polar groups is unfavorable and the groupings do not readily dissolve or penetrate the hydrocarbon core of the membrane. PSA is recognized as one of the most important measurements incorporating both structure and the ability of a molecule to form hydrogen bonds with solvent. A cutoff value for poorly permeable and (therefore, poorly orally absorbed) compounds has been set at $>140-150$ $A^2$. Expressing the above concept as a virtual formula log *P* equates to molecular weight—PSA. This virtual formula defines ADMET space as a conceptual boundary that encloses the properties most likely to be associated with successful drugs. The dimensions of such a space are illustrated in Fig. 1.1. The formula explains the interconnectivity of the physicochemical properties. If we consider absorption, lipophilicity will always give high permeability across the membranes but, at its upper limits, solubility is so low that adequate dissolution is not achieved at clinical doses. The interconnection with molecular weight has spawned a belief that this characteristic is important per se.

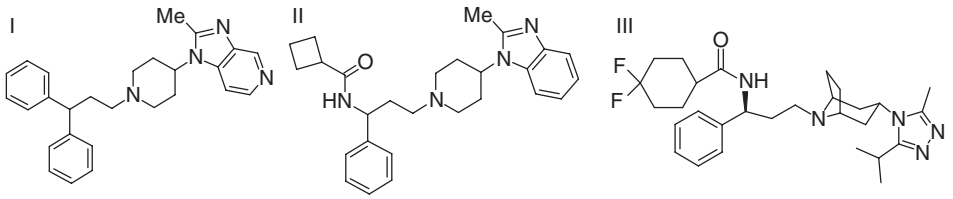


**Figure 1.1** Structures of I the initial HIT/lead compound in the CCR5 project leading to the discovery of maraviroc and illustrating the unhindered pyridine nitrogen leading to potent CYP450 inhibition and its replacement with benzimidazole II. The other structural changes produced a compound antivirally *active in vitro*. Changes to decrease lipophilicity used the triazole group and resulted eventually in maraviroc III.

Most likely, it is the fact that drug molecules are made from carbon (lipophilicity), oxygen, and nitrogen (PSA) and that as molecular weight approaches 500, the chances of too high a lipophilicity or too great a PSA increase so that a molecule does not dissolve in the aqueous media of the GIT or the molecule has low permeability to membranes.

When a lead is being advanced it is usual for the lipophilicity of the newly synthesized analogs to show an increase in lipophilicity particularly as the medicinal chemist tries to increase the potency of a molecule to low nanomolar from often low micromolar. The loss of solvent is a major contributor to the binding free energy. Lipophilic groups have a positive solvation free energy; this, together with favorable direct van der Waals interactions, make lipophilic interactions a key force in drug binding. In contrast, the desolvation of polar groups is unfavorable even though hydrogen bonds between the substrate and enzyme or receptor, are favorable. Overall, the contribution of polar contacts to the binding free energy, is likely to be variable owing to the balance of desolvation and actual binding. Lipophilic groups often, therefore, guarantee potency increases usually to the extent of 0.7 kcal/mol and compounds become increasingly lipophilic to achieve drug like (low nanomolar) potencies.

## 1.5 PROPERTIES NEEDED IN AN ORAL DRUG

### 1.5.1 Solubility

Material in the early phases of drug discovery is amorphous. Moreover, most screening systems are run with the compound handling systems using DMSO stock solutions. Thus, the usual measurement is kinetic solubility in which a (DMSO) stock solution of compound is added to aqueous solvent and the solubility rapidly determined. This method has inaccuracies due to the presence of DMSO and the rapid nonequilibrium conditions [1]. Crystallizing a compound (by definition) produces a less soluble form rendering the kinetic solubility measurements redundant and possibility misleading. More accurate measures are provided by thermodynamic solubility, which refers to the measurement of solubility by addition of the solid material, to aqueous media under conditions of equilibrium and requiring a long incubation time [1]. Such solubility methods are not adaptable to high speed screening and material handling systems. The dilemma then is how useful early measurements of kinetic solubility are on the

amorphous material and how timely are tedious thermodynamic measures of crystalline material. A final problem is the appearance of new polymorphs of the crystal, which can emerge anytime in the discovery, development, and even product cycle.

### 1.5.2 Permeability

During the discovery phase most screening systems rely on Caco-2 cells, MDCK cells, or artificial membranes. The cell lines provide a more realistic model of membrane systems, but usually have the assessment complicated by the presence of transporters systems. Thus the output is often not true passive flux (for poorly permeable compounds) but a composite of permeability and transport. The transporters are saturable rendering the choice of concentrations critical in making intercompound comparisons. It is noticeable also that interlaboratory variation is very high [2] making the data very difficult to apply across compounds sets from different laboratories.

### 1.5.3 Metabolic Stability

The rate of metabolism of compounds can be measured using liver microsomes, human hepatocytes, or recombinant enzymes. Microsomes offer a robust oxidative metabolism screen when fortified with NADPH regenerating systems. Consideration needs to be given to microsomal binding, particularly with lipophilic basic molecules and for comparative data a correction is needed for this [3]. Owing to the large variation in CYP450 enzyme content screening usually uses microsomes prepared from a pool (5–10 individuals) of livers.

Hepatocytes provide the full complement of oxidative and conjugation metabolism. Cryopreserved hepatocytes are normally utilized in compound screening, but caution should be exercised in comparing pathways as the freeze thawing process can lead to loss of cofactors. While microsomal binding is relatively easy to predict or measure, hepatocyte binding is much more difficult to estimate, and to scale, into an intrinsic clearance prediction.

Apart from obvious differences, in the metabolic pathways supported, microsomes and hepatocytes may give conflicting results for compounds of low cell permeability, where the rate of metabolism in microsomes is attenuated by the slow rate of flux across the hepatocyte cell membrane [4]. Much of the work supporting the use of *in vitro* systems such as microsomes to predict *in vivo* clearance used series largely populated with permeable compounds.

### 1.5.4 Drug–Drug Interactions

The effect of the emerging candidate drugs on existing concomitant therapy (drug–drug interactions, DDIs) can be examined by the use of fluorigenic substrates with recombinant enzymes, particularly CYP450s or human liver microsomes and selective substrates [5]. Often the screens are run in two phases with most compounds synthesized submitted to a high throughput screen using recombinant enzymes with relatively nonselective fluorescent probe substrates (the selectivity being gained by use of the individual isoforms). These screens tend to use a single substrate concentration (around $20–50\,\mu M$) and look for percentage of inhibition of the reaction against control. Incubations are conducted with and without preincubation

to look for competitive and time-dependent "mechanism-based" irreversible or quasi irreversible inhibitors. A second phase assay will often be used to examine compounds of high interest. This assay uses human liver microsomes and selective drug substrates. CYPs screened are generally 1A2, 2 C9, 2 C19, 2D6, 2E1, and 3A4. The selective substrates for these are universally recognized and are often phenacetin (1A2), diclofenac (2 C9), *S*-mephenytoin (2 C19), bufuralol (2D6), chlorzoxazone (2E1), and testosterone (3A4). These screens are now firmly established as selectivity screens.

### 1.5.5 Induction

Induction screening can use reporter gene assays, cultured human hepatocytes, tissue slices, or immortalized cells such as HepaRG [6,7]. Reporter gene assays offer the closest to a general screening system but may give false negatives due to the absence of interplay with other receptors involved in the induction process. This criticism also applies to cell lines. Hepatocytes, while a comprehensive system, are variable from donor to donor. The concern around induction varies greatly from company to company and is often related to intracompany experiences and choice of therapeutic area. Clinical induction is actually quite rare and is seen almost exclusively (not absolutely) with high dose drugs. The high doses of these drugs are necessary because of weak affinity for their primary pharmacology target (antiepilectics such as phenytoin and carbemazepine, $30-50\,\mu M$ against their $Na^+$ channel target and around $10\,\mu M$ against the PXR receptor mostly involved in CYP3A4 induction) or the need to maintain plasma concentrations above the $IC^{90}$ (anti-infectives such as nonnucleoside reverse transferase inhibitors for HIV). The only compound identified with high potency is hyperforin (low nanomolar) which cannot really be termed *drug like*.

### 1.5.6 Plasma Protein Binding

Methods to determine protein binding and free drug largely rely on the separation of the unbound drug from the bound drug by dialysis, filtration, or centrifugation or a combination of these methods. During development, traditionally the drug was radiolabeled to make the assay simpler. The methods work well for moderately bound drugs but loose accuracy when drugs are more than 95% bound. Much of the accuracy concerns the variable nonspecific binding to the apparatus used. The accuracy was also confounded, traditionally, by the purity of radiolabeled compound available. Thus, many drugs are categorized as greater than 99% bound. This applies typically to acidic drugs such as nonsteroidal anti-inflammatory agents.

BIACORE SPR is a biosensor-based technique using surface plasmon resonance (SPR) technology and can be used to determine the kinetics of protein–ligand interactions including albumin and other plasma proteins [8]. This technique gives actual affinity data rather than just percentage unbound. From affinity the fraction unbound can of course be calculated. For instance, warfarin binds to albumin (c99%) bound). Using BIACORE technology, it was shown that at high concentrations warfarin bound at more than one site on human serum albumin. The kinetics of the high affinity site were $K_d = 3.7 \pm 1.2\,\mu M$ and a dissociation rate constant of 1.2 $s^{-1}$. From the $K_d$ value, binding of 99.4% was calculated.

## 1.6 HIERARCHICAL AND HORIZONTAL *IN VITRO* SCREENING SEQUENCES

Two process models of screening cover most approaches to drug discovery screening. The hierarchical model places the various screens in a sequence such that only compounds possessing certain criteria move to the next phase. For instance, a newly synthesized compound or series of compounds could be screened for target binding, close neighbors to target binding (selectivity), functional activity (particularly if an agonist), and then for solubility, permeability, metabolic stability, and DDIs. Appropriate compounds may be further progressed into *in vivo* screens for pharmacology and pharmacokinetics. In the hierarchical model, therefore, ADMET data is collected only on compounds with adequate potency and selectivity, and even the collection of this can be staged.

In the horizontal model, ADMET data is collected on all compounds synthesized in a single screening phase incorporating *in vitro* potency and selectivity and the suite of ADMET screens. The horizontal model may be considered optimum for looking for exceptions to druglike property rules and can build SAR streams rapidly so potentially allowing real time SAR of the type normally reserved for retrospective analysis.

These two models indicate a divergence of how the data is handled. The hierarchical model means that full data is available on a few compounds, can be manipulated on a spreadsheet and can be interpreted easily by a medicinal chemist. Certain screens can be moved up in priority as the project SAR reveal key flaws and problems. The horizontal model could result in more than 5000 data points to collate as SAR. This immediately requires computational systems and complex analysis to process and optimize. Moreover, the cost of providing this number of data points makes screening a very expensive process.

### 1.6.1 When are ADMET Properties Critical in Influencing Compound Progression

At any stage, ADMET arguments could be advanced for the progression or cancellation of a project. At target identification the structure of the protein, close family members, nature of the possible drug binding site can provide information on whether the target is druggable. Targets such as aspartic acid proteases may be considered undruggable based on the information, but while being difficult have yielded drugs for hypertension (aliskerin, renin inhibitor [9]), and HIV (various [10]). β-Secretase as a target for alzheimers [11] is a possible higher hurdle since the target of interest is now located in the CNS and drugs will have to cross the blood brain barrier. Perhaps, rather than discard targets on ADMET space grounds, appropriate pathways to a drug should be identified at this stage such as a biological or small chemical approach after the key points of

1. Pivotal point in pathway of disease effects or symptoms.
2. Effects of inhibition or activation of target not likely to be attenuated by competing pathways.
3. Likely overall beneficial effect influenced by interaction with drug molecule in most (all) patients.

4. Sufficient diversity for selection against other receptors including subtypes.

Target identification and selection (1–4) are exemplified in the postgenomic era by CCR5 and its role in AIDS [12,13]. A deletion in the CCR5 gene called *CCR5* Δ32, results in a nonfunctional CCR5 protein that is not expressed on cells. People with two copies of the *CCR5* Δ32 gene are resistant to HIV infection. The CCR5 protein allows a pathway for virus to enter the cell and a rational is therefore to produce an antagonist to mimic deletion of the gene. Encouragement for possible success with a small molecule is provided by CCR5 being a cell surface receptor of the family of nonaminergic GPCRs (G-protein-coupled receptors) which have been successful targets in a number of drug discovery programs. The genomic information also allows an indication of likely safety outcomes in this approach since those carrying the CCR5Δ32 gene are otherwise healthy. This target has produced a recent drug maraviroc (III in Fig. 1.1) in the fight against AIDS. Points 3 and 4 are listed above, unfortunately are often only realized late in the discovery/development/product cycle. For instance, alosetron [14] was a selective 5-$HT_3$ antagonist. The rationale for the drug was the role 5-$HT_3$ antagonists have in suppressing the reflex colonic motor function in response to food ingestion. This reflex in diarrhea-predominant irritable bowel syndrome (IBS) is exaggerated resulting in cramping and diarrhea. The drugs side effects were noted as constipation. Wider use in patients after marketing produced several cases of ischemic colitis. Constipation can facilitate the development of ischemic colitis by increased colonic intraluminal pressure compressing the mucosal vessels and impeding circulation. Cilansetron, another 5-$HT_3$ antagonist, shows identical rates of ischemic colitis indicating that the effects are target related and not drug molecule specific. Although withdrawn, alosetron has been reinstated using very strict labeling and prescriber/patient practices. The antiobesity agents [14] were withdrawn for heart defects. Fenfluramine and dexfenfluramine bind weekly to 5-HT receptors and the major *N*-deethylated metabolite norfenfluramine is responsible for the pharmacological activity. The drugs reduce appetite by activating 5-$HT_{2C}$ receptors via the metabolite. The metabolite is not selective and also activates the 5-$HT_{2B}$ receptors on heart valves and pulmonary artery interstitial cells causing the formation of proliferative fibromyxoid plaques. While antagonists of subtypes of receptors are "routinely" possible there are considerably more demands to obtain selective agonists and the lack of a selective 5-$HT_{2C}$ receptor agonist for obesity treatment may testify to this.

When a number of targets are being prioritized then it seems appropriate to consider the possibility of the binding site yielding molecules fitting within the confines of ADMET space.

Series/lead identification comprising HTS or fragment-based approaches followed by chemistry follow up is the first opportunity to gain actual experimental data, rather than theoretical. Considerable debate occurs as to how useful ADMET data is at this stage rather than physicochemical properties. The principal concern is that the molecular template, while giving potency and selectivity is flawed from an ADMET viewpoint and despite manipulation of the structure will not give favorable oral drug properties. Should lead molecules, therefore, be screened for ADMET properties to exclude them as leads or merely to show areas of improvement. Should the areas of improvement be prioritized early on

**TABLE 1.2 Comparison of the Hydrophilic Drug Atenolol (log *D* 7.4–1.7) and the Lipophilic Drug Propranolol (log *D* 7.4–1.2) Illustrating the Need to Compare All Aspects of Screening Data to Choose Compounds**

| | Affinity $pA_2$ | Renal $CL_i(u)$ (ml/min/kg) | Metabolic $CL_i(u)$ (ml/min/kg) | Vd(u) (L/kg) | Half-life (h) | Daily dose (mg) |
|---|---|---|---|---|---|---|
| Atenolol | 6.5 | 2 | — | 1 | 3–5 | 50–100 |
| Propranolol | 8.3 | — | 470 | 50 | 3–5 | 30–90 |

or should selectivity and potency be paramount until reasonable improvements in this area are seen.

5. Compound identification comprising series/lead development followed by development candidate selection. At this stage, the use of ADMET data is unarguable. Most compounds will be close to the required potency and selectivity and usually one or two ADMET properties are being optimized. Potency and selectivity values should not be divorced from ADMET values and probably each compound should be evaluated in a horizontal manner obtaining full data. This is exemplified in Table 1.2 where two very successful β-adrenoceptor antagonists, with very different physicochemical, biological, and clearance properties, are compared. Owing to the compensatory nature of some of the processes the dose and dosing frequency of both drugs are very similar.

### 1.6.2 Is Drug Discovery a Linear or Nonlinear Process?

It is impossible to change one aspect of a compound's properties and not affect other properties. This interdependence of processes for the delivery and performance of an oral drug is the reason that drug discovery takes a long and somewhat circuitous and meandering route to the goal of a drug candidate. While many of the changes turn out to be deleterious, there is a counterside. Occasionally, this process works for the drug discoverer and a single change can turn an unproductive series to one that produces a drug candidate. The discoveries of amlodipine and fluconazole [15] contained a single chemical change or concept that led to very successful drugs. Unlike most other dihydropyrines calcium channel antagonists amlodipine has a basic primary amine side chain. Differentiating fluconazole was the introduction of a second triazole. Much of the thinking was to mainly to address solubility issues in the lead compounds nifedipine and ketoconazole. These were intuitive steps, based on the knowledge of the physicochemical nature of the new group and some freedom to retain activity by substitution in the selected region. These groupings dramatically enhanced the volume of distribution for amlodipine against other dihydropyridines, leading to a much longer half-life suitable for once a day administration. The bis-triazole series, of which fluconazole is member, had greatly improved solubility and at the lipophilicity of fluconazole (Log *P* 0.6) very little metabolism. Clearance was by glomerular filtration, with about 60–70% tubular reabsorption by passive reabsorption, again leading to a long half-life due to the low clearance value. However, there is an important corollary to this in that the lead compounds and lead series always were reasonably placed in ADMET versus target space.

Marviroc's discovery followed a more linear approach as the drug's discoverers worked through balancing permeability for oral absorption, rate of metabolism, and IKr

channel activity. The lead series and eventual drug are close to the edges of ADMET space versus target space. Screening in terms of permeability and metabolism was provided on almost all compound synthesized once sufficient potency had been attained in the lead series to see actual antiviral activity against the HIV virus (preclinical proof of concept).

The dilemma of all discovery projects is that the future is not predictable and the question is how far should progress be made before termination of a compound series or even a project. *N*-(1-(3,5-Dichlorobenzenesulfonyl)-2S-methyl-azetidine-2-carbonyl)-L-4-(2′,6′-dimethoxyphenyl)phenylalanine was optimized to be a potent inhibitor of the (VLA) antigen-4 [16]. In terms of ADMET, space versus target space the properties dictated were not promising and the compound has physicochemical properties consistent with poor ADMET characteristics: molecular weight 607, PSA 131, log *P*5.6. The compound was rapidly cleared in rat with values consistent with very high extraction by the liver (clearance equal to hepatic blood). In this particular case, further experimentation continued to show that the clearance of the compound was a result of the multiple processes of metabolism by oxidation and glucuronidation and biliary excretion of the parent molecule. The major contributor was the active hepatic uptake of parent compound (the compound was shown to be a substrate of the rat organic anion transporter, Oatp1b2) and metabolism did not influence clearance but determined excreted fate. The lead series was abandoned after these experiments.

## 1.7 TOO EARLY OR TOO LATE

The dilemma is how easy is it to remove a particular ADMET liability of a compound late on in the discovery process. In general, most discovery programs of major pharmaceutical companies have a screening cascade incorporating ADMET data into the earliest stage of compound synthesis and these screens tend to be comprehensive and the data arrives in a hierarchical manner. Even in this situation, a question of weighting needs to be applied.

### 1.7.1 Induction Screening

Induction screening is of low value until some understanding of the likely potency and concentration response of the project compounds is established. Many compounds including the reference standards such as rifampicin, show effects at concentrations approaching or exceeding $1\,\mu M$ in *in vitro* systems. A detailed comparison of *in vitro* screening data and clinical results confirms that $EC_{50}$ or maximum responses to an agent are of little value in determining which compounds will cause induction in the clinic. Only when *in vivo* exposure in humans can be determined ($C_{max}$ or AUC plasma concentration data) can the compounds be correctly ranked. For instance, verapamil, which is not a clinical inducer, has an $EC_{50}$ of $3.2\,\mu M$ while phenytoin and carbamazepine, which are clinical inducers, have $EC_{50}$ values of 8 and $15.6\,\mu M$, respectively. The difference between the compounds is due to the clinical dose and resultant plasma concentrations which for the anticonvulsants are some two orders of magnitude greater than the calcium channel blocker. The structures of clinical P450 inducers of CYP3A4 show no obvious SAR and even detailed analysis suggests that the only common feature is some degree of lipophilicity. This is perhaps to be expected

from what is essentially a weak interaction between the compounds and the receptor [6,7]. In the absence of any other guidance, it is suggested that any induction screening is postponed if target potencies are below 50 n*M*. Because of the promiscuity of the receptors and the higher doses used in preclinical safety assessment, induction is a common finding and should not be viewed as a failure in screening. Moreover, owing to differences in receptors animal findings are not extrapolatable to the clinic.

### 1.7.2 Inhibition of CYP450 Enzymes

Similar comments can be made about the value of the data until an *understanding* of the likely potency and concentration response of the project compounds is established. However, unlike CYP3A4 induction, some structural features are associated with highly potent CYP inhibitors, both reversible and to a lesser extent irreversible. The presence of unhindered nitrogen in a saturated ring system (pyridine, imidazole, triazole) may result in the lone pair of the nitrogen being able to form a ligand interaction with the heme of the CYP450. Many of the potent CYP450 inhibitors bind in this manner and the interaction adds 6 kcal to the binding energy. This interaction is the basis for the action of azole antifungals and a number of aromatase inhibitors. As this interaction is commonplace and invariably leads to highly potent inhibitors such functionality is best avoided from the outset. In the case of the discovery of maraviroc, the original HTS hit and lead compound possessed an imidazopyridine [12] leading to very potent CYP450 inhibition. Replacement of the functionality occurred early on with benzimidazole (Fig. 1.1) and eventually a substituted triazole.

Mechanism-based time-dependent inhibitors form complexes with the heme moiety of CYP450 or bind irreversibly to the protein. Tertiary amines and their metabolism products classically are heme interactants, while irreversible protein binding inhibitors (suicide substrates) are classically formed by conversion to highly reactive short-lived metabolites.

Imidazole ring systems are also prevalent in mechanism-based time-dependent CYP450 inhibitors, although the relationships are more complex and are not accompanied by a mechanistic understanding [15]. Sufficient information is available to allow *in silico* filtering of compound structures to determine possible avenues which may lead to this problem [17].

### 1.7.3 Solubility, Permeability, and Metabolic Stability

Although a molecule has different properties, the three key factors that characterize an oral drug are intertwined by the physicochemical properties that define them. Increasing lipophilicity [which will often provide potency increases (see above)] will usually increase permeability and also increase the rate of metabolism while lowering solubility. This balancing act is a pivotal part of SAR development of a project and starts at the time of the lead series identification. Maraviroc [12] exemplifies the route from a lipophilic metabolically unstable lead to molecules with groupings such as triazole incorporated to counter the high rate of metabolism. The lower lipophilicities gave better metabolic stability but demonstrated low rates of flux in Caco-2 cells and resultant poor oral absorption. The eventual and successful development candidate maraviroc was a compromise of these properties but had sufficiently good pharmacokinetics to promise an $IC^{90}$ on a reasonable oral drug regiment (300 mg od or bd).

As a general rule, lead compounds show rapid metabolism and hence high clearance is a widespread problem. The above example used the lowering of overall lipophilicity as a method to get stability; the other common tactic is the removal of vulnerable functionality or the introduction of blocking groups, particularly halogens, after identifying the sites of metabolism. This tactic forms an almost classical screening synthesis cycle, with potency, selectivity, flux, and overall metabolic rate being provided alongside and site of metabolism. This cycle is possible now because of the development of mass spectrometry technology. The cycle can be extended to use computational prediction of likely metabolites and then monitoring of these in high throughput manner [18]. The software, in this case MetaSite, is only 55% predictive of the compounds primary site of metabolism. However, by analyzing in addition 2nd and 3rd order rank predictions, the combination of *in silico* selection of likely metabolites and then monitoring these *in vitro* the method provides a favorable screening performance. The data on site of metabolism can usually not be provided on the same number of compounds. Typically, labile sites are explored alongside other sites in the generation of SAR. Intrinsic clearance data are calculated by screening compounds in human liver microsomes. This process is shown in the work on corticotropin-releasing factor-1 (CRF1) receptor antagonists [19]. The major sites of metabolism in the initial lead compound (potency 0.26 n$M$) were O-demethylation of both the methoxy groups (Fig. 1.2). These two positions were found to be the major sites of metabolism in all compounds generated and intrinsic clearance was influenced mainly by substitution at these sites. The 1-(1-cyclopropyl)ethyl group was selected as a stable (but less potent) alternative offering scope for potency improvements. The other labile $O$-methoxy position showed lower intrinsic clearance when replaced with groups such as trifluoromethoxy, trifluoromethyl, difluoromethoxy, chloro, cyano, and ethoxy. One of the difluoromethoxy substituted compounds combined potency ($IC_{50} = 1$ n$M$), functional antagonism, selectivity, and metabolic stability (Fig. 1.2).

### 1.7.4 Plasma Protein Binding

There is no common consensus and there are many misconceptions around protein binding and unbound drug. This arises because in a static *in vitro* test, the addition of

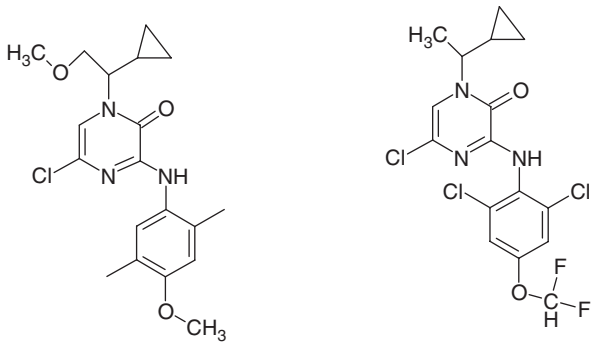


**Figure 1.2** Corticotrophin-releasing factor-1 (CRF1) receptor antagonists. The major sites of metabolism in the initial lead compound were O-demethylation of both the methoxy groups. These positions were stabilized by incorporations of the groupings shown.

albumin of plasma will often decrease the apparent potency of a drug. In this case, the change in free fraction directly relates to the concentration of free (unbound) drug. Because the addition of serum or plasma proteins is common in many assays, a biased view of compound affinity and potency may arise. Moreover, the static *in vitro* situation is then directly extrapolated to the dynamic *in vivo* situation and a belief formed that protein binding will similarly attenuate the effects of the drug. These beliefs drive a discovery project to attempt to lower plasma protein binding and this in turn requires plasma protein binding data to be available alongside data on potency, metabolic stability, permeability, and so on. If we concentrate on oral drugs, dosed to steady state unlike the static *in vitro* system, free concentration *in vivo* is not dependent on free fraction but on intrinsic clearance. The simple analogy is that if plasma protein was added to a clearance assay, and the drug is plasma protein bound, then there will a lower the rate of drug disappearance directly proportional to the reduction in potency. When the drug binds to proteins in a reversible manner the aqueous concentration of drug will be lowered by the amount bound. This will of course reflect the concentration of drug interacting at the receptor, but it will also lower the amount of drug interacting with the clearance enzymes: fewer drugs will be transformed per unit time. Thus, reversible interactions with proteins (or the phospholipids of membranes) will proportionately give lower drug concentrations than no interactions, but those concentrations will be sustained for proportionately longer. In pharmacokinetic terms over a defined period (the dosing interval) reversible interactions with proteins will lower maximum concentrations ($C_{max}$) or aqueous drug but raise minimum concentrations ($C_{min}$) to the exact extent that the average concentration ($C_{av}$) will be unchanged. Because the enzyme kinetics are essentially unchanged, providing first order is observed, daily dose is unchanged. Changing protein binding will not usually be reflected in changes in daily dose size to achieve the same average free drug plasma concentrations (although frequency of administration could be affected) [20]. Essentially, when all the processes of drug metabolism are modeled then $AUC_u = F_{abs} \cdot F_{gut} \cdot \text{Dose}/CL_{int}$, where $AUC_u$ is the unbound drug area under the plasma concentration curve, $F_{abs}$ is the fraction absorbed, $F_{gut}$ is the fraction metabolized by passage through the gut wall (gut first pass) and $Cl_{int}$ is the intrinsic clearance. In the discovery setting, the latter is routinely assessed during metabolic stability screening (see above). Note that the fraction unbound is not present in the equation. It needs to be determined, however, at the stage when *in vivo* experiments are performed to complete the right-hand side. $AUC_u$ is easily converted to $C_{av}$ (average plasma concentration over specified time) by dividing $AUC_u$ by time. $C_{av}$ is a more useful parameter as it allows direct comparison with *in vitro* potency measurements [21].

## 1.8 INTEGRATION OF *IN VIVO* AND *IN VITRO* DATA

While *in vitro* data provides most of the ADMET data to a discovery project *in vivo* data is vital to ensure that what is being generated *in vitro* is consistent with what is observed *in vivo*. A major concern is around clearance processes. Table 1.1 indicates that highly permeable compounds are cleared metabolically and microsome data can be reliably applied. Exceptions to this include compounds with functionality likely to be metabolized by systems not present in microsomes such as aldehyde oxidase. This is one of several enzymes besides cytochrome P450 involved in the

oxidative metabolism of drugs [22]. Aldehyde oxidase [23] catalyzes the oxidation of aldehydes to carboxylic acids. Most importantly for drug discovery it catalyzes the oxidation of carbon–nitrogen double bonds present in aromatic azaheterocyclic compounds. Reliance on microsomal systems may give false indications of stability since the enzyme is soluble, and not bound to the endoplasmic reticulum, and thus appears in the supernatant. Aldehyde oxidase activities in dog are unusually low and strain dependent in rats so *in vivo* experiments may also be misleading.

Similarly, microsomes are normally fortified for oxidative metabolism only and direct conjugation of a substrate will not be observed. This suggests that for any compounds containing functionality likely to be labile to conjugation enzymes (hydroxyl, phenols, etc.) hepatocyte screening should also take place.

For less permeable compounds, the possibility of transporter involvement in clearance becomes likely. This interplay unless understood may lead to false assumptions about project priorities. For instance, the compound *N*-(1-(3,5-dichlorobenzenesulfonyl)-2*S*-methyl-azetidine-2-carbonyl)-L-4-(2′,6′-dimethoxyphenyl)phenylalanine referred to above [16] was metabolized by oxidation and glucuronidation but when examined *in vivo* the major contributor to clearance was the active hepatic uptake of parent compound by a liver transport system. Reliance on microsomal or hepatocyte data may have led the project to attempt to stabilize the metabolic lability of the compound. The fate of the series, however, was largely determined by the physicochemical properties and their effects on permeability and transporter affinity.

## 1.9 WHEN ALL ELSE FAIL—USE AND ABUSE OF PRODRUGS

Often in medicinal chemistry programs, a stage is reached where many criteria for a molecule have been met, but certain elusive properties are unobtainable in the current series. Rather than search for an alternative series, attempts are made to produce a labile chemical derivative that yields the active drug at efficacious concentrations. These labile forms are called *prodrugs* [24] and have been attempted in a variety of forms to address solubility, permeability, clearance, and selectivity issues. Prodrug moieties have been added to molecules to

1. increase water solubility to aid dissolution, or to allow parental formulation;
2. increase permeability to aid absorption;
3. improve systemic pharmacokinetics by decreasing maximum concentrations and increasing drug half-life;
4. improve therapeutic index by targeting a drug to a tissue or cell type.

Actual examples of prodrugs successfully designed, developed, and marketed are largely limited to the first two categories.

The actual form of the prodrug varies. Testa [25] defined the chemical criteria that may be used to classify prodrugs:

Bioprecursors, which do not contain a promoiety, yet are activated by oxidation, reduction, or hydrolysis.

Carrier-linked prodrugs, where the drug is linked to a carrier and activation occurs by hydrolysis, oxidation, or reduction.

Macromolecular prodrugs, where the carrier is a macromolecule such as polyethyleneglycol.

Drug–antibody conjugates, where the carrier is an antibody, for instance, one with affinity for a particular tumor cell.

A number of drugs are prodrugs and termed *bioprecursors* but are not actually created by design including omeprazole, simvastatin, lovastatin, and clopidogrel. Many of these types of drug were discovered and developed without knowledge of the bioprecursor role. Clopidogrel's mechanism of action, as an example was not discovered until 2000 although US approval by the FDA was granted in 1997. The bioprecursor role of clopidogrel is the generation of its 2-oxo metabolite a metabolite, which hydrolyzes to a reactive thiol that can bind covalently to platelet ADP receptors and inhibit platelet aggregation [24].

To increase water solubility addition of water soluble functionality by pegylation and phosphate esters has been used. A recently marketed example of phosphate ester prodrugging is Fosfluconazole, a prodrug of fluconazole, an azole antifungal agent. The hydrolysis of fosfluconazole to fluconazole is catalyzed by alkaline phosphatases, which reside in most tissues especially the kidney and liver. Fosfluconazole is over 40 times more soluble than fluconazole, allowing it to be administered by bolus injection. The water solubility increase is due to a combination of intrinsic hydrophilicity and ionization [24].

The vast majority of successful prodrugs increase membrane permeability to allow oral absorption. These drugs conform to the ADMET space requirements in terms of polar surface area but are not lipophilic enough.

Prodrugging and acid function does little to mask polar functionality (same for alcohol groups) since the esterification of a carboxylic acid reduces PSA by only 11 $A^2$. Thus, potential prodruggable molecules need to conform to PSA requirements prior to prodrugging. While this may be critical in some cases, this is a relatively small reduction and not what is envisaged in the scope of "masking" of polar groups. Major effect of esterification is to render the ionizable carboxylic acid neutral. The overall effect on lipophilicity is a combination of the loss of ionization and the intrinsic lipophilicity of the promoiety. For an acid of p$K_a$ 3.4, approximately 4 log units of lipophilicity will be added to the log $D_{7.4}$. As a guide, therefore, the potential candidate needs a PSA less than 150 $A^2$ to render a possible successful campaign [24].

Targeting a drug to a particular protein usually involves synthesizing a peptide or antibody delivery conjugate. These can show success *in vitro*, but usually fail *in vivo* for a number of reasons. Critical to success is the interplay of rates including [24]:

1. clearance of the drug conjugate from the systemic circulation;
2. rate of uptake by target;
3. rotency of drug;
4. release of drug in or at the target;
5. disappearance from target of the drug;
6. systemic clearance of the drug

Each of these factors is critical and needs to be precisely defined. For instance, for 2 and 3 many attempted delivery systems rely on an antibody conjugate and internalization. The antibody will often have subnanomolar affinity, whereas the drug may only

have micromolar activity against the target. This type of approach places unrealistic demands on the other factors even allowing that the antibody may be able to deliver more than one molecule of drug [24].

## 1.10 DEVELOPMENT: HUMAN METABOLISM THE PIVOTAL STUDY

Once a candidate is submitted for development a well-documented series of studies are conducted linking preclinical and clinical studies together. A pivotal study that causes much debate is the human metabolism study. This normally uses a radiolabeled version of the candidate drug. The emphasis of this study has changed with time but can be defined as a part of understanding the PK/PD (pharmacokinetics/pharmacodynamics) of the drug. It can be broken down into two elements: circulating and excreted metabolites. Smith and Obach [26] have suggested the primary purpose of these:

Circulating metabolites are of interest primarily because they can directly and probably reversibly interact with macromolecules, particularly proteins and cause a change in conformation and function of the protein to elicit a biological effect (beneficial or hazardous). These effects can be similar and additive to the parent molecule or may in some cases be different. Identifying and analyzing these metabolites in the same matrix as the parent allows concentrations to be measured and thereby assessment of PK/PD.

Excreted metabolites are of interest primarily, in human, because they allow the proportion of the parent converted to a particular metabolite to be determined and thereby support the *in vitro* enzymological evaluations for population variations and drug–drug interactions. In addition, they allow the detection of the downstream products of reactive metabolites and, moreover, allow an estimation of the amount (mass) formed. From a technical viewpoint, excreted metabolites often allow an easier workup of sample (than circulating metabolites) for detailed structural analysis and in some cases even yield sufficient sample for biological testing.

The species used in preclinical safety studies are also studied in this manner to allow comparisons with the human data. A variety of standards have been suggested for the comparison but clearly a metabolite present in much higher concentrations than seen in preclinical testing raises concerns and needs careful and thoughtful consideration. To obviate a late stage problem, there is considerable benefit in conducting the human metabolism study early; however, sufficient information needs to be available to make this expensive study pivotal. The most important factor is the clinical dose range, until this is known metabolism studies may be of a limited value. This information begins to emerge at the end of phase 2A or "proof of concept" studies, and it may be that the most opportune time is following these studies in parallel with phase 2B clinical studies. Various designs are employed but the following considerations need to be incorporated:

1. Polymorphically expressed enzymes: does the design need to incorporate representative variants in the volunteer population.
2. Single or multiple does: can single dose results be extrapolated or does the design need to incorporate a steady state regime. An example would be a clinical enzyme inducer which could modify its own metabolism.

3. Unlabeled fragments of the molecule: depending on the site of label novel chemical matter may be liberated as unlabeled material. Should cold assay technology be used?
4. Does the radiolabel become incorporated into intermediary metabolism, does this render the use of a label unethical.

## 1.11 SUMMARY AND FUTURE PERSPECTIVE

The component parts of ADMET screening and their relative importance will continue to be discussed and debated in the foreseeable future. Most of the screening systems are now relatively generally established, but as this chapter tries to emphasize may be applied with the wrong emphasis or timing. There are considerable gaps in the systems to understand fully drug transport, something becoming more important as target and ADMET space ensure less permeable compounds. A part of the problem is the promiscuous SAR of the proteins, the difficulty of establishing how much of the protein is present, and the complexity of transport kinetics [27].

A second factor is the outsourcing and packaging of drug screens. As cost of research becomes an issue it is possible to design compounds in one place, synthesize them in another and screen them in a third. These places may represent different countries and even continents. The concept of a drug discovery team with representatives of the various disciplines intimately supervising the practical aspects becomes lost. Screening becomes a checklist, rather than a bespoke science and the danger of inappropriate data is maximized. Project objectives need to be agreed and careful examination of the appropriate screens and screening sequence conducted. Data from screens becomes available at different times and it is possible that the most rapid screens may disproportionately influence a project rather than the most important screens.

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