Part 3
Disposition, Metabolism, and Safety

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Transporters

Overview

- *Membrane transporters increase the influx and efflux of substrate compounds.*
- *Transporters are found in many tissues in vivo.*
- *P-glycoprotein efflux in the blood–brain barrier, cancer cells, and intestine is a iability for some compounds.*

Membrane transporters are responsible for two important permeability mechanisms, active uptake and efflux. Carrier mediated transport can contribute significantly to the pharmacokinetics characteristics of a compound.[1,2] Structures can be modified to reduce the deleterious effects of efflux. The possibility of improving absorption or BBB permeation by structure design to enhance uptake transport is a future opportunity.

9.1 Transporter Fundamentals

Passive diffusion is the predominant mechanism for the permeation of drugs throughout the body. A compound must have favorable physicochemical properties (i.e., lipophilicity, hydrogen bonds, molecular weight) to undergo passive diffusion. Many endogenous biochemical compounds that are necessary for life do not have physicochemical properties that allow sufficient passive diffusion, so there are trans-membrane transporters that greatly enhance their permeability. Examples of transporters in the intestine are shown in Table 9.1. For many biochemicals to function properly their concentrations must be significantly higher within a cell compared to the surrounding extracellular fluid. Some compounds, such as bile salts, must be exported to the bile from hepatocytes. Specific transporters move their

TABLE 9.1 ► Transporters Affecting Gastrointestinal Absorption of Some Drugs

Uptake
Oligopeptide transporters (PEPT1, PEPT2)
Organic anion transporters (OATP1, OAT1, OAT3)
Organic cation transporters (OCT1)
Bile acid transporters (NTCP)
Nucleoside transporters
Vitamin transporters
Glucose transporters (GLUT1)
Efflux
P-glycoprotein (Pgp, MDR1)
Breast cancer resistance protein (BCRP)

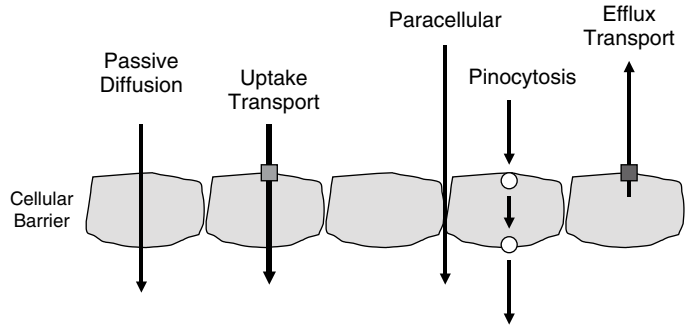


Figure 9.1 ► Uptake and efflux transporters contribute to net permeability.

substrates against a concentration gradient to enhance their accumulation. Uptake (import) transporters provide necessary nutrients and other compounds with physiological functions to tissues that would otherwise not have sufficient concentration for their physiological roles. The process is often referred to as active transport. Other transporters enhance the movement of compounds out of a cell. Efflux (export) transporters assist the bulk movement of compounds. An example is the efflux of potentially toxic xenobiotics (e.g., drugs) from the endothelial cells of the blood-brain barrier by P-glycoprotein (Pgp) before they reach sensitive brain cells. Transporters use energy input, such as from ATP, to perform their function. The roles of uptake and efflux transporters are illustrated in Figure 9.1. A particular transporter is expressed on only one surface of the cell (apical or basolateral). This results in the directional movement of substrates, for example from the blood stream into the bile. There is overlap in the substrate specificity of transporters, which may result in a cooperative effect.

Transporters affect drug pharmacokinetics. New transporters have typically been identified through cloning techniques. Their natural function, substrate specificity, kinetics, expression, and implications for drug development are an active area of research.

9.2 Transporter Effects

Transporters can affect the ADME/Tox characteristics of a compound. Transport occurs when the drug contains a moiety that has a similar moiety to the natural substrate of a transporter, or if it has structural elements that facilitate binding to a transporter with wide substrate specificity (e.g., Pgp). Here are a few examples of how transporters affect ADME/Tox:

- ► Uptake transporters enhance the absorption of some drug molecules in the intestine.
- ► Efflux transporters on the luminal (apical) surface of gastrointestinal epithelial cells oppose the absorption of some molecules.
- ► Transporters assist the uptake of some molecules into hepatocytes to enhance metabolic and biliary clearance.
- ► Efflux transporters oppose the distribution of some drugs from the bloodstream into organs, such as the brain.
- ► Uptake transporters enhance the distribution of certain drugs into some organs.
- ► Elimination of many drugs and metabolites is enhanced by active secretion in the nephrons of the kidney.

► Co-administered drugs can compete for a transporter for which they both have affinity, resulting in drug-drug interactions (DDI) and modification of the pharmacokinetics of one of the compounds.

Owing to the finite number of transporter protein molecules on the cell surface, they can be saturated if the concentration of a substrate is high enough. As the concentration of substrate increases, the flux of molecules increases and then levels off as the maximum capacity of the transporters is reached. Above this concentration the flux is the same. Saturation of drug uptake and efflux transporters in the intestine is observed when the luminal concentration following oral dosing exceeds the saturation concentration of the transporter. Passive diffusion permeation, by contrast, does not saturate.

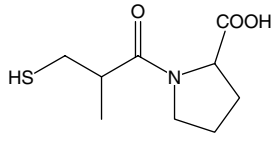
Many commercial drugs are substrates for transporters. Some of these are shown in Table 9.2.[3–5] The effect of the transporters depends on the tissue and substrate concentration. For example, efflux transporters in the blood-brain barrier can exclude some drugs from distributing into the brain, whereas, in the intestine, efflux transporters appear to have less influence on absorption. This is because the drug concentration in the intestine after oral dosing is high (mM) and saturates the efflux transporters, whereas the drug concentration in the blood, thus at the BBB, is much lower (μM) and does not saturate the efflux transporters.

Transporters can be rate limiting in ADME/Tox processes. While passive diffusion is the predominant permeability mechanism for many compounds, transporters can greatly enhance or reduce total permeability for some compounds at certain membranes.

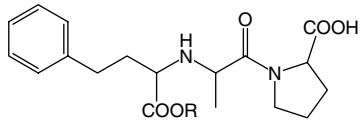
TABLE 9.2 ► Examples of Drugs with Active Uptake Transport

Oligopeptide transporters (PEPT1)

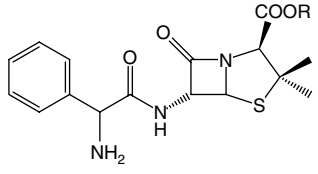
Captopril



Enalapril (R=H)
Enalaprilat (R=C_2H_5)



Ampicillin (R=H)
Pivampicillin
(R=$C_6H_{11}O_2$)



Cephalexin

Also: Ceftibuten
Cefoxitin
Acyclovir
Valacyclovir

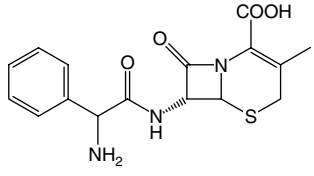


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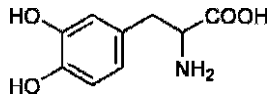
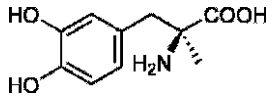
TABLE 9.2 ► *Continued*

Large neutral amino acid transporter (LAT1)

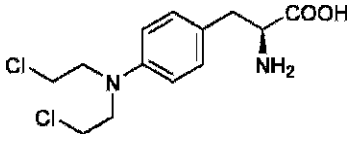
L-dopa



Methyldopa



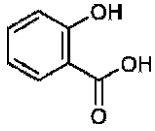
Melphalan



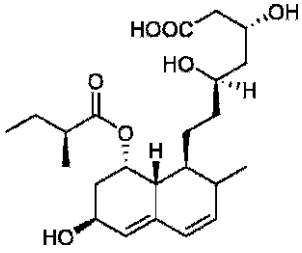
Also:
Phenylalanine
Baclofen

Monocarboxylic acid transporter (MCT1)

Salicylic acid



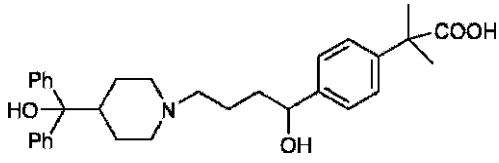
Pravastatin



Also:
Lovastatin acid
Simvastatin acid

Organic anion transporter polypeptide (OATP1)

Fexofenadine



Also:
Enalapril
Temocaprilat
Deltrophin II

Transporters are found at barrier membranes throughout the body. Many of the transporters that are known to affect drug ADME/Tox are listed in Table 9.3 and shown in Figure 9.2. The letter abbreviations are complicated and some transporters have several letter abbreviations. The following sections discuss some of the effects of transporters in different barriers.

TABLE 9.3 ▶ Compilation of Many Transporters and Their ADME Functions

Organ/Barrier	Direction	Function	Transporter	Reference
Intestine/ Epithelial Cells	Absorption	Lumen to Blood	PEPT1	[6]
			ISBT	[6]
			OATP2B1	[6]
			MRP1	[7]
			OSTα	
			OSTβ	
			MRP3	[7]
			OATP1B1	[17]
	Secretion	Blood to Lumen; Epithelium to Lumen	Pgp	[6, 7]
			MRP2	[6, 7]
			BCRP	[6, 7]
Liver/ Hepatocytes	Excretion	Hepatocyte to Bile	Pgp	[6, 7]
			MDR2	
			MDR3	[6]
			BSEP	[6]
			MRP2	[6, 7, 17]
			BCRP	[6, 7]
	Excretion	Blood to Hepatocyte	OATPB	[6,17]
			OATP1B1 (OATPC)	[6, 17]
			OATP1B3 (OATP8)	[6, 17]
			OCT1	[6]
			OAT2	[6]
			NTCP	[6, 17]
			PGT	
			PEPT1	
	Retention/ Recirculation	Hepatocyte to Blood	MRP1	[6, 7]
			MRP3	[6, 7, 17]
			MRP4	[6]
Kidney/Renal Epithelial Cells	Excretion	Blood to Renal Epithelium	OAT1	[6, 17]
			OAT2 [6, 17]	
			OAT3	[6, 17]
			OAT4c1	[6]
			OCT1	[6]
			OCT2	[6]
			OCT3	[6]
	Excretion	Renal Epithelium to Urine	OAT4	[6, 17]
			OAT-K1	[6]
			OAT-K2	[6]
			Pgp	[6]
			MRP2	[6]
			MRP4	[6]

Continued

TABLE 9.3 ► *Continued*

Organ/Barrier	Direction	Function	Transporter	Reference
	Reabsorption	Urine to Renal Epithelium	PEPT1	[6]
			PEPT2	[6]
			OATP1	[17]
	Reabsorption	Renal Epithelium to Blood	MRP1	[6]
			MRP3	[6]
			MRP6	[6]
Blood-Brain Barrier	Into Brain	BBB Endothelium to Brain	MCT1	[18]
			OATP1	[17]
			GLUT1	[18]
			GLUT2	
			SGLT1	
			LAT1	[18]
			CAT1	[18]
			CNT2	[18]
			CHTX	[18]
			NBTX	[18]
			OATP3	[18]
	Elimination from Brain	BBB Endothelium to Blood	Pgp	[18, 28]
			MRP	[18, 28]
			BCRP	[18]
			OATs	[18]
			OATPs	[18]
			EAATs	[18]
			TAUT	[18]

9.2.1 Transporters in Intestinal Epithelial Cells

Transporters in the small intestine can modify absorption of some compounds. This can affect absorption if the substrate compound has low passive permeability and may not be obvious if a compound has high passive permeability. In intestinal epithelial cells, transporters are involved with:

- ► absorptive uptake (from the gastric lumen through epithelial cells and into blood)
- ► efflux (from the epithelial cell membrane back into the gastric lumen)
- ► secretory efflux (from the blood, into the epithelial cells, and into the gastric lumen)

Absorptive uptake increases the concentration of drug in the blood, while secretory efflux lowers the concentration in blood.

9.2.2 Transporters in Liver Hepatocytes

Transporters are important in hepatic clearance. They enhance metabolism by facilitating uptake into hepatocytes, where the molecules encounter metabolizing enzymes. They

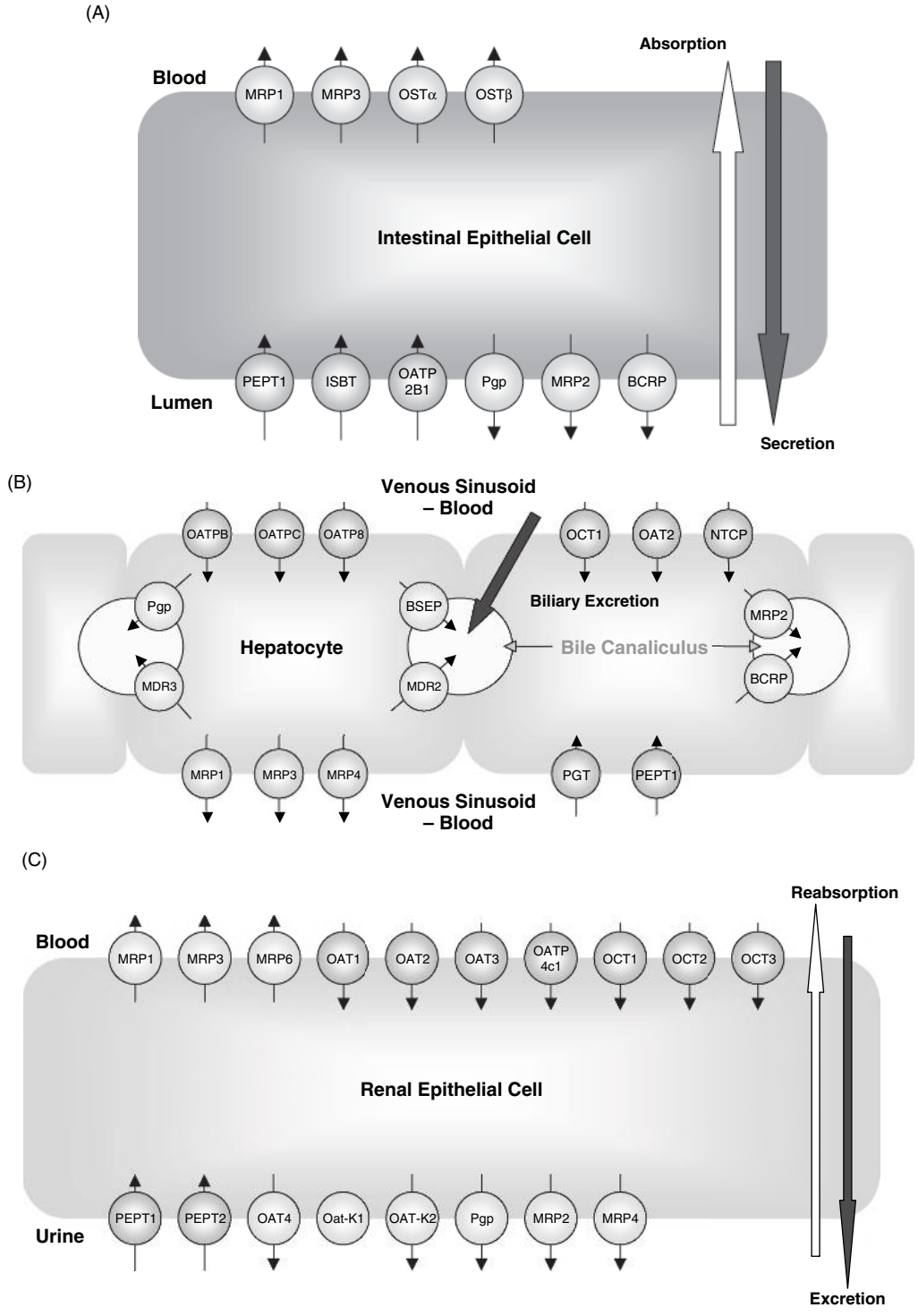


Figure 9.2 ► Diagram of transporters that have been identified in various barrier cell layers. Their roles in transporting drugs continues to be investigated. A) Intestinal epithelium, B) Liver hepatocytes, and C) Kidney epithelial cells[6].

enhance clearance by increasing the flux of the compound and metabolite molecules into the bile canaliculus or back into the blood for renal clearance. Transport rate is more important than metabolic rate in the clearance of some drugs.[6] In liver hepatocytes, transporters are involved with:

- hepatic uptake (from the blood into the hepatocyte)
- biliary clearance (from the hepatocyte into the bile canaliculus)
- hepatocyte efflux (from the hepatocyte into the blood)

9.2.3 Transporters in Kidney Epithelial Cells

In the renal epithelial cells of the nephron, transporters enhance renal clearance for some compounds, which results in a lower compound concentration in the blood. Some compounds are transported from the urine back into the blood. In the kidney, transporters are important for:

- tubular secretion (from blood through renal epithelial cells to urine)
- reabsorption (from urine through renal epithelial cells to blood)

9.2.4 Transporters in Blood–Brain Barrier Endothelial Cells

Chapter 10 discusses BBB permeation. Transporters are involved in keeping compounds out of the brain, but uptake transporters increase the distribution of some compounds into the brain. The important BBB functions of transporters are:

- efflux (from the BBB endothelial cells back into the blood)
- uptake (from the blood, through the BBB endothelial cells and into the brain)

Examples of commercial drugs for which transporter effects on ADME/Tox have been studied are compiled by Shitara, et al.[6]

The following sections discuss some of the transporters that affect drug permeability. The most important efflux transporter for discovery project teams, in general, is Pgp. It is useful for discovery scientists to understand the characteristics and function of Pgp. The effects of other transporters may be recognized (from unexplained pharmacokinetics parameters) during the course of a discovery project. When a project has a lead series that is active *in vitro*, but does not achieve sufficient *in vivo* exposure due to efflux, they might decide to modify the structure to reduce efflux or enhance uptake. For these reasons, the following sections provide introductory information on many of the most important transporters.

9.2.5 Consequences of Chirality on Transporters

Stereoselectivity is observed for transporters. Table 9.4 lists examples of drugs for which transporters have enantiomeric selectivity. This has an effect on any barrier in vivo for which the particular transporter has a significant role.

TABLE 9.4 ► Examples of Stereoselectivity of Transporters

Drug	Enantiomeric ratio	Transporter
Methotrexate	40 (L)	Dipeptide
Cephalexin	>100 (D)	Dipeptide
Dopa	>100 (L)	Amino acid

9.3 Efflux Transporters

Efflux transporters facilitate the export of compounds from the cell. These transporters belong to the ATP-binding cassette (ABC) family.[7]

9.3.1 P-glycoprotein (MDR1, ABCB1) [Efflux]

P-glycoprotein (Pgp) is the most widely known efflux transporter to discovery scientists because it can have a great effect on the success of some drug discovery projects. It is a 170 KD protein with 1280 amino acids and 12 trans-membrane segments (Figure 9.3).[8] Pgp is a member of the ATP Binding Cassette (ABC) family of transporters, of which over 50 are known and have natural transporter functions. Pgp has also been referred to as multi-drug resistant protein 1. Its gene is know as MDR1 or ABCB1. A drug molecule attaches to the binding domain of Pgp, which appears to be within the bilayer membrane. Then two ATPs, bound to the ATP binding regions, become hydrolyzed and induce a conformation change to open a pathway for the drug molecule to pass through into the extracellular fluid.[9]

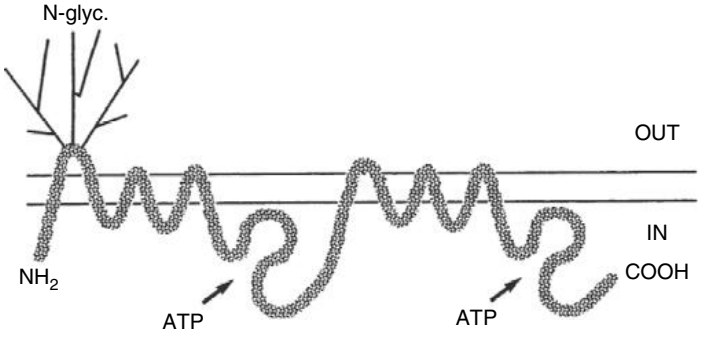


Figure 9.3 ► Schematic diagram of Pgp and its 12 transmembrane segments. Used with permission from [8].

Pgp was initially identified as a major cause of resistance by cancer cells to multiple drugs (e.g., paclitaxel, etoposide) having a variety of structures. With initial chemotherapeutic treatment, many of the cells in tumors died, but some cells lived and continued to grow. These cells were found to express Pgp, or another efflux transporter, which effluxes the cytotoxic cancer drug from the cells, thus allowing them to survive. Oncology research programs have dealt with Pgp for decades. A major oncology discovery strategy has been to test lead compounds for their ability to overcome multidrug resistance in cell lines that are highly drug resistant through the expression of high levels of Pgp and other efflux gransporters

It was also discovered that Pgp is present in many tissues of the body. Pgp is abundant in cell barriers that have a protective function, such as:

- blood-brain barrier
- small and large intestine

- liver
- kidney
- adrenal gland
- pregnant uterus

Pgp is expressed on the luminal surface of gastrointestinal epithelial cells, where it can reduce the total permeability of Pgp substrates. It has been shown to mediate the secretion of some drugs (e.g., digoxin) from the blood into the gastric lumen. In the liver and kidney, Pgp enhances drug and metabolite clearance to the bile and urine, respectively. Pgp attenuates penetration of some compounds into the brain, uterus, testes and other tissues. Pgp knock out animals have been developed. Pgp substrates typically have increased absorption, reduced excretion, increased toxicity, and increased distribution to protected tissues in knock out animals. Efflux by Pgp is a major challenge for some discovery projects, because it affects ADME processes, resulting in reduced exposure of the compound to the therapeutic target.

Efflux appears to have a greater relative effect when the drug concentration on the luminal surface is low (see Section 9.2). For example, the drug concentration circulating in the blood stream and exposed to the luminal surface of the BBB is much lower than the concentration on the luminal surface of intestinal epithelial cells after oral dosing. Therefore, Pgp may have little effect on the oral absorption of a particular Pgp substrate drug at high oral doses, but it may have a major effect on its brain penetration. It is also common to observe a greater effect of Pgp efflux on total permeability when the compound has low passive diffusion compared to high passive diffusion, because passive diffusion can dominate the process.

The substrate specificity for Pgp is very broad. Compounds ranging from a molecular weight of 250 to 1850 are known to be transported by Pgp. There is even evidence that Pgp can efflux peptides Aβ40 and Aβ42 with 40 and 42 amino acids, respectively, which are involved in Alzheimer's disease. Substrates may be aromatic, non-aromatic, linear or circular. The charges on the substrate molecules can be basic, uncharged, zwitterionic, or negatively charged. Some substrates are hydrophobic and some are amphipathic.

It is important to remember that the Pgp binding and efflux of a compound differs between species, owing to the differences in the protein's sequence. *In vivo* Pgp data from one species (e.g., mdr1a knock out mouse) may not properly predict effects in another species (e.g., human). In the same manner, *in vitro* Pgp data from MDR1-MDCKII (transfected with human Pgp-producing gene) cell monolayer efflux assay may not translate well to mouse or rat efficacy species pharmacokinetics.

Owing to the major potential effect on BBB permeability, Pgp efflux has been of particular interest in CNS discovery projects. Industry Pgp research has been greatly motivated by the need to deliver compounds to brain targets.

9.3.1.1 Rules for Pgp Efflux Substrates

As for other properties, rules are useful for the initial assessment of a compound, based on its structure. Rules for Pgp are referred to as "rule of 4". A compound is more likely to be a Pgp substrate if its structure has[10]:

- $N + O \geq 8$
- MW > 400
- Acid with pKa > 4

A compound is more likely to be a Pgp non-substrate if its structure has:

- N+O $\leq$ 4
- MW < 400
- Base with pKa < 8

Increasing numbers of hydrogen bond acceptors (N+O) appear to confer increasing likelihood of Pgp efflux.[11] This may be because binding to Pgp occurs in the lipophilic membrane region. Also, hydrogen bonds afford energetic binding interactions. Another contributor to Pgp binding may be a structural motif involving two H-bond acceptors 4.6 Å apart or three H-bond acceptors 2.5 Å apart.[12]

9.3.1.2 Case Study of Pgp Efflux

For a project lead series, it was shown[11] that compounds with N + O = 4 had a 33% chance of being effluxed by Pgp, compounds with N + O = 6 had a 65% chance, and compounds with N + O = 8 or 9 had a 87% chance. This is consistent with an increasing risk of efflux with increasing hydrogen bond acceptors.

An example of the development of structure-efflux relationships for a lead series is shown in Figure 9.4.[11] The efflux effects of various substituents at two positions in the scaffold were rated for their influence on measured efflux. There was a trend with increasing hydrogen

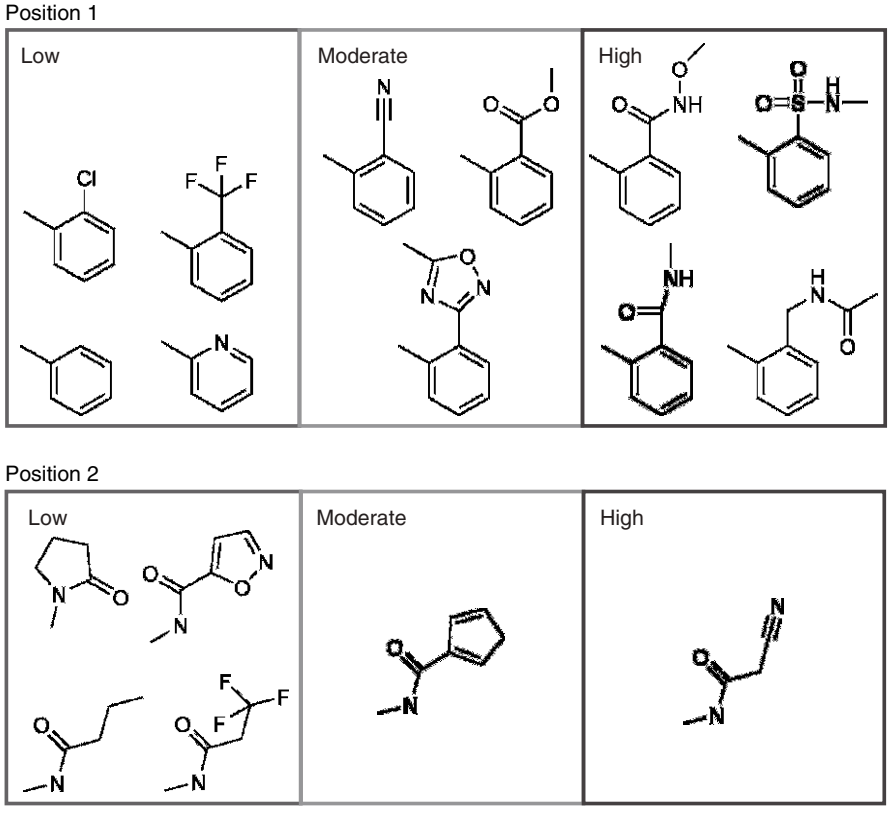


Figure 9.4 ► Influence of substituents at two positions on Pgp transport for a project lead series[11].

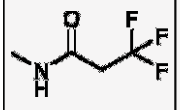
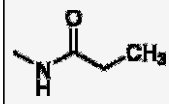
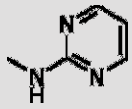
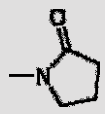
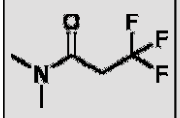
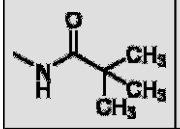
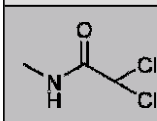
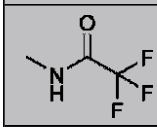
R	MDR1 Transport Ratio (B to A / A to B)	Papp LLC PK1 (cm/s E-6)	Potent	
	8.6	28	Y	Good potency, but high efflux
	8.6	24	Y	
	3.0	12	N	Low efflux, but poor potency
	2.7	35	N	
	2.2	18	N	
	2.6	32	N	
	2.8	22	Y	Low efflux and good potency
	2.2	33	Y	

Figure 9.5 ► In this lead series, reduction of Pgp efflux was achieved while maintaining potency. Used with permission from [11].

bond acceptors. The aromatic amides were especially susceptible to efflux. Substitution of moieties was successful in reducing the Pgp efflux while maintaining potency (Figure 9.5).

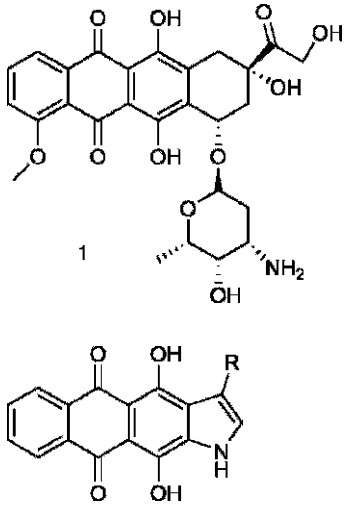
9.3.1.3 Structure Modification Strategies to Reduce Pgp Efflux

Structure modification strategies have been successful in reducing Pgp efflux. First, try to identify hydrogen bond accepting atoms that are shown through structure-efflux relationship studies, or reasonable conjecture, to be involved in the Pgp binding. Then:

1. Introduce steric hindrance to the hydrogen bond donating atoms by:
   a. Attach a bulky group
   b. Methylate the nitrogen

2. Decrease H-bond acceptor potential
   a. Add an adjacent electron withdrawing group
   b. Replace or remove the hydrogen bonding group (e.g., amide)
3. Modify other structural features so that they may interfere with Pgp binding, such as adding a strong acid.
4. Modify the overall structure's Log P to reduce penetration into the lipid bilayer where binding to Pgp occurs.

Steric hindrance can be increased to reduce Pgp efflux. The example in Figure 9.6[13] is for a series of cancer drug candidates for the purpose of overcoming Pgp-induced resistance. A lower Pgp/no Pgp ratio indicates less difference between the resistant (Pgp) cells compared to the normal cells (no Pgp), because the compound is no longer a Pgp substrate. This compound series has increasingly hindered amines at R, resulting in reduced efflux.



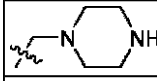
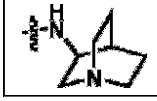
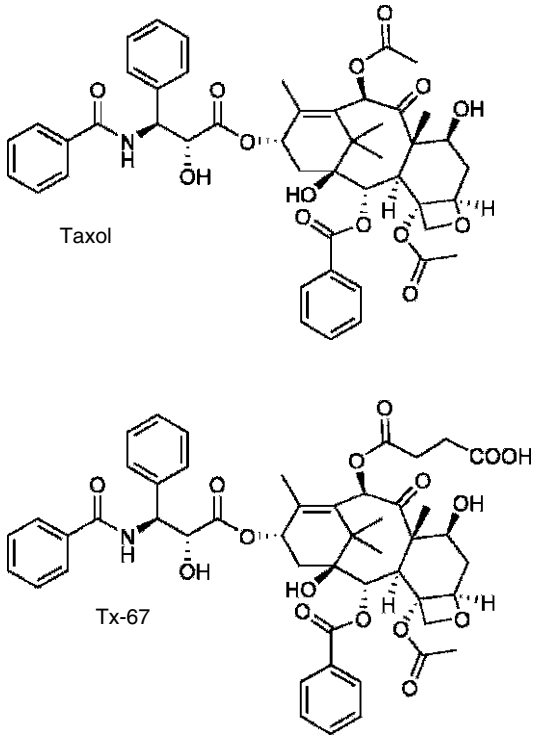
R	IC50 (uM) K562 (no Pgp)	IC50 (uM) K562i/S9 (with Pgp)	Pgp/no Pgp
1	0.2	1.5	8
CH_2NMe_2	1.2	12.5	10
	1.2	1.2	1
	3.2	2.2	1

Figure 9.6 ► Increasing steric hindrance reduces Pgp efflux[13].

Increased acid strength reduces the Pgp substrate affinity for paclitaxel. The structure modification shown in Figure 9.7 introduced a carboxylic acid.[14] This imparted a 10 fold increase in brain penetration by reducing Pgp efflux.

A Pgp inhibitor can chemically knock out efflux, allowing compounds affected by Pgp efflux to reach higher levels at the therapeutic target. Pretreatment or co-dosing with a Pgp inhibitor has been performed for discovery projects as part of pharmacology proof of concept studies. This is also being investigated as a therapeutic strategy in the clinic,[15,16] but safety is a concern.



In Situ Rat Brain Perfusion $P_{app} \times 10^7$ cm/s	
Taxol	0.845
Tx-67	8.47

Figure 9.7 ► Pgp efflux at the BBB was decreased by adding a carboxylic acid moiety [14].

9.3.2 Breast Cancer Resistance Protein (BCRP, ABCG2) [Efflux]

BCPR efflux transporter was identified from chemotherapeutically resistant breast tumor cells. It is expressed normally in many tissues, such as placenta, hepatocytes, and small intestine. BCRP appears to be naturally involved in the efflux of porphyrins and their metabolites. Its role in the elimination of topotecan was demonstrated, and it appears to affect the disposition of several other drugs.[17]

9.3.3 Multidrug Resistance Protein 2 (MRP2, ABCC2) [Efflux]

MRP2 is an efflux transporter that came to light because it can also contribute to cancer multidrug resistance.[17] It has also been termed cMOAT for multispecific organic anion transporter. MRP2 transports glutathione, glucuronide, and sulfate conjugates of lipophilic compounds and some uncongugated compounds. It is expressed in intestinal epithelial cells, where it opposes absorption of substrates, and the canalicular membrane of hepatocytes and on renal tubule cells (kidney), where it enhances elimination of substrates. MRP1 through MRP9 have also been characterized.

9.3.4 Efflux Transporters in the BBB

Efflux transporters at the BBB include: Pgp, MRPs, BCRP, OATs, OATPs, EAATs (glutamic acid/acidic amino acids), and TAUT (taurine). These transporters export their substrates

from the brain and BBB endothelial cells into the blood. They appear to work sequentially in concert: some of the transporters are on the abluminal (toward the brain tissue) membrane and some on the luminal (toward the blood) membrane.[18]

9.4 Uptake Transporters

Uptake transporters facilitate the permeation of compounds into cells.

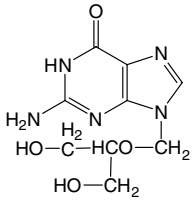
9.4.1 Organic Anion Transporting Polypeptides (OATPs, SLCOs) [Uptake]

OATP1A2 (human) (a.k.a. OATP1, OATP-A) is found in the BBB (uptake), hepatocytes (uptake), and renal epithelium (reabsorption). It is known to transport organic anions (bile acids, steroid glucuronide conjugates, anionic dyes, thyroid hormones), as well as ouabain, cortisol, and large organic cations. It transports the drugs fexofenadine, enalapril, and temocaprilat, N-methyl quinidine, DPDPE and deltrophin II.[17,19]

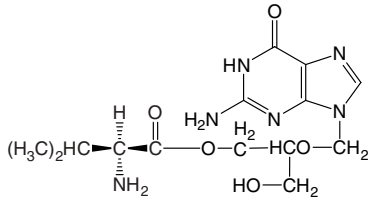
OATP1B1 (human) (a.k.a. OATP2, OATP-C, LST1 [liver specific transporter 1]) is expressed in the liver (and may be in intestine). It has a similar substrate specificity to OATP1A2 and can also transport eicosanoids, benzylpenicillin, methotrexate, rifampin, pravastatin, rosuvastatin, and cerivastatin.[17] There are many other members of the OATP family that have been found in humans and rodents (e.g., OATP3 is found in the kidney, OATP9 in liver transports cardiac glycosides, OATP-K1 in kidney transports methotrexate).

9.4.2 Di/Tri Peptide Transporters (PEPT1, PEPT2) [Uptake]

These transporters enhance the uptake of dipeptides and tripeptides, but not individual amino acids or tetrapeptides.[20] A proton is co-transported with the substrate. Hydrophobicity increases PEPT1 binding and aromatic residues are preferred. Examples in Figures 9.8[21] and 9.9[22] show that prodrugs are transported by PEPT1 when natural amino acid, valine, is attached as a promoiety. Absorption of these prodrugs increased through both passive diffusion and active uptake. PEPT1 is known to transport β-lactam antibiotics and other drugs that contain peptides, as shown in Table 9.2. Peptide transporters have been reviewed.[23]



Ganciclovir
Oral Bioavailability = 6%
Not substrate of PEPT1 or PEPT2



Valganciclovir
Oral Bioavailability = 61%
Substrate of PEPT1 and PEPT2

Figure 9.8 ► Valganciclovir: Enhanced oral absorption by PEPT1 and PEPT2 peptide transporters.

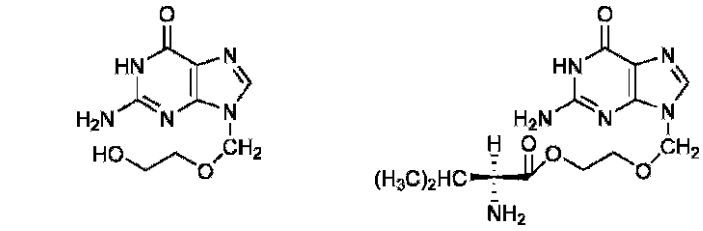


Figure 9.9 ► Valacyclovir: Enhanced oral absorption by PEPT1 and PEPT2 peptide transporters.

9.4.3 Organic Anion Transporters (OATs) [Uptake]

OATs enhance renal clearance of some drugs and drug metabolites by uptake from capillary blood vessels into renal tubule cells.[17,24] OAT1 is known to transport β-lactam antibiotics, NSAIDs, antivirals, AZT, acyclovir, and many other drugs. Other members of the family are: OAT2 through 4, OAT-K1 through 2.

9.4.4 Organic Cation Transporter (OCT) [Uptake]

OCT1 through 3 and OCTN1 through 2 enhance transport into the urine in kidney.[24] Increasing hydrophobicity enhances binding to OCT.

9.4.5 Large Neutral Amino Acid Transporter (LAT1) [Uptake]

LAT1 is present in the apical membrane of the endothelial cells of the BBB. It transports amino acids, such as leucine and phenylalanine. It also transports the drugs L-DOPA, methyl DOPA, daclofen, and melphalan.

9.4.6 Monocarboxylic Acid Transporter (MCT1) [Uptake]

MCT1 is expressed on the apical membrane of the endothelial cells of the BBB and epithelial cells of the intestine. It is involved in uptake of acids. It enhances uptake of salicylic acid, pravastatin, lovastatin, simvastatin acid,[25] and probenecid .[18] MCTs have been reviewed.[26]

9.4.7 Other Uptake Transporters

- **Glucose Transporter (GLUT1) [Uptake]** is present in the apical membrane of the endothelial cells of the BBB and is involved in uptake.
- **Bile Salt Export Pump (BSEP, ABCB11) [Efflux]** is naturally involved in the export (efflux) of bile salts from hepatocytes into bile.
- **Sodium Dependent Taurocholate Co-transporting Polypeptide (NCTP) [Uptake]** assists the enterohepatic circulation of bile acids by transporting bile acids from the blood into hepatocytes, where they are secreted into the bile canaliculus. It may indirectly affect the role of bile acids on nuclear hormone receptors PXR and FXR for regulation of CYP expression and cholesterol metabolism.

- **Uptake transporters in the BBB include:** GLUT1, LAT1, MCT1, CAT1 (cationic amino acids), CNT2 (nucleosides), CHT (choline), and NBT (nucleobase).[18] These enhance the uptake of their substrates into the brain from the blood.

9.4.8 Structure Modification Strategies for Uptake Transporters

Uptake transporters are an attractive option for enhancing the permeability of compounds that are active *in vitro*, but whose passive diffusion is low.[4,18,26–29] Uptake transporters enhance the uptake of many drugs; however, this has often been discovered after the design phase in discovery. This strategy may represent an opportunity in future drug design.

Such an approach would utilize traditional SAR approaches, with informed structure modifications. These would be checked using *in vitro* assays for the specific transporter (see Chapter 27). The relationship of the structural modification to enhanced carrier mediated transport would guide further modifications or decisions to test the pharmacokinetics or tissue uptake *in vivo*.

Problems

(Answers can be found in Appendix I at the end of the book.)

1. Transporters are involved in which of the following: a) GI absorption of nutrients, b) BBB efflux, c) BBB uptake of some drugs, d) GI passive diffusion, e) GI efflux, f) renal secretion, g) GI hydrolysis, h) uptake into hepatocytes, i) biliary clearance.
2. At higher drug concentration, transporters: a) are most effective, b) may be saturated.
3. Which of the following can be affected by transporters: a) absorption, b) distribution, c) metabolism, d) excretion
4. What is the most consistently important transporter in drug discovery and why?
5. Which of the following compounds is more likely to be a substrate for Pgp:

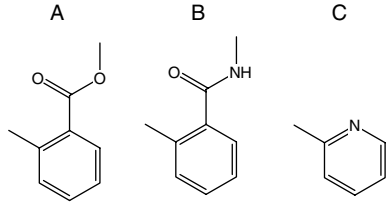
Compound	MW	Ionization	H-Bond acceptors	H-Bond donors	PSA
A	350	pKa = 3	4	1	55
B	520	pKa = 9	10	5	140
C	400	pKa = 4	3	3	60
D	470	pKa = 8	8	2	75

6. What structural modifications may reduce Pgp efflux?
7. Pgp efflux of a compound can be proven in vivo using what?
8. Of the following transporters, which are efflux- and which are uptake-transporters: a) OATP1A2, b) BCRP, c) PEPT1, d) LAT1, e) MRP2, f) MCT1?
9. A particular transporter is found on which cell membrane: a) apical only, b) both apical and basolateral, c) either apical or basolateral, depending on the cell type, d) basolateral only?
10. Which of the following structure classes might have their oral absorption increased by active transport: a) amino acids, b) antibiotics, c) carboxylic acids, d) vitamins, e) di- and tri-peptides?

11. Pgp transporters are present in which of these cells: a) intestinal epithelium, b) blood-brain barrier, c) liver, d) kidney, e) skin?

12. Pgp causes which of the following: a) reduced blood-brain barrier penetration, b) cancer cell drug resistance, c) increased bioavailability?

13. Rank the following groups for increasing potential for Pgp transport:



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Chapter 10

Blood–Brain Barrier

Overview

- *Blood–brain barrier (BBB) is restrictive for some compounds owing to P-glycoprotein efflux, absence of paracellular permeation, and limited pinocytosis.*
- *Brain exposure is assessed in terms of BBB permeability or brain/plasma partition.*
- *Brain exposure is enhanced by reducing H-bonds, molecular weight, P-glycoprotein efflux, metabolism, and plasma protein binding, or by increasing Log P.*

The pharmaceutical treatment of central nervous system (CNS) disorders is the second largest area of therapy, following cardiovascular disease.[1] U.S. sales for CNS drugs exceeded $53 billion in 2002 to 2003 (Figure 10.1). CNS disorders are five of the top 10 causes of disability. Stroke is the third leading cause of death and costs the economy $40 billion annually. Fifteen million people suffer from Alzheimer's disease, which is the second most expensive disease to the economy at $100 billion annually. Many brain diseases do not have satisfactory treatments. Clearly, CNS disorders are an important current and future priority for the pharmaceutical industry.

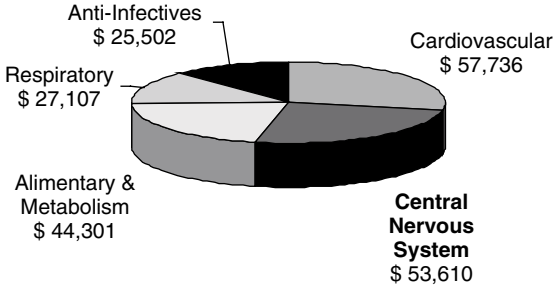


Figure 10.1 ► Central nervous system (CNS) disorders are the second largest pharmaceutical therapeutic area. U.S. pharmacy purchases September 2002 to August 2003 are shown.

In order for CNS drugs to penetrate to the brain tissue, they must pass through the blood–brain barrier (BBB). Many of the compounds that otherwise would be effective in treating CNS diseases are excluded from reaching a sufficient concentration in the brain tissue and producing the desired therapeutic effect (Figure 10.2). It has been estimated that only 2% of the possible CNS therapeutic compounds can pass the BBB.[2]

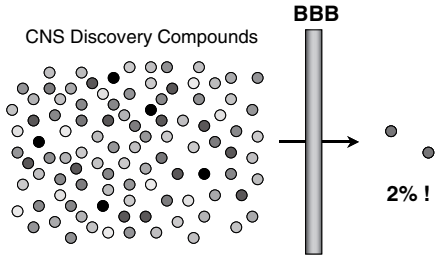


Figure 10.2 ► The BBB excludes as much as 98% of potentially beneficial drugs and is a major challenge for CNS therapy.

10.1 BBB Fundamentals

The term *BBB permeation* is widely used in CNS discovery projects. However, it is important to recognize that the goal is "brain penetration," the *exposure of compound to the therapeutic target in the brain*. *BBB permeation* is a major factor in brain penetration, and a closer look indicates that it is the sum of multiple mechanisms at the BBB (see Section 10.1.1). In addition, *brain distribution* mechanisms (e.g., metabolism, protein binding) also affect brain penetration of drugs (see Section 10.1.2). As with intestinal absorption, various compounds have different mechanisms, or combination of mechanisms, that limit their brain penetration. These depend on the compound properties and target location (e.g., membrane, cytoplasm, brain region). Therefore, it is important that discovery scientists be aware of the many brain penetration mechanisms and determine the mechanism(s) that best correlates with diagnosing and optimizing brain penetration of their leads.

The BBB is associated with the microcapillary blood vessels that run throughout the brain in close proximity to brain cells (Figure 10.3). These vessels naturally provide the nutrients and oxygen needed by the CNS cells and carry away waste. Over 400 miles of blood microcapillaries are present in the brain, with a surface area of approximately 12 m^2.[2]

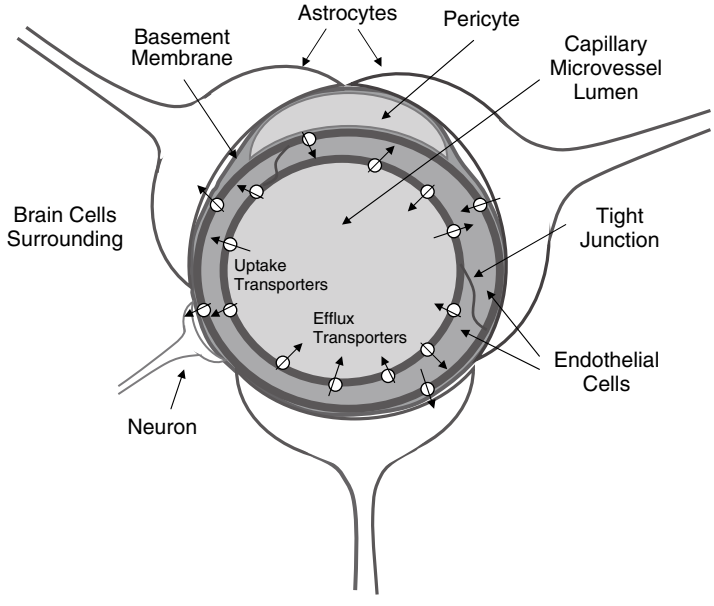


Figure 10.3 ► Schematic diagram of a cross-section of a brain capillary microvessel that constitutes the blood–brain barrier.

The BBB consists of the endothelial cells that form a monolayer lining the inner surface of the capillaries. CNS drugs must permeate through the endothelial cells to penetrate to the brain cells. The endothelial cells are associated with astrocyte and pericyte cells, which do not resist drug penetration but apparently can modify endothelial cell characteristics.

10.1.1 BBB Permeation Mechanisms

The BBB forms a permeation barrier that is more limiting to compound penetration than are most other membrane barriers in the body. Mechanisms affecting BBB permeation are shown in Figure 10.4 and include the following:

- ► Restrictive physicochemical characteristics that limit passive diffusion
- ► High efflux activity
- ► Lack of "leaky" paracellular permeation and capillary wall fenestrations
- ► Limited pinocytosis
- ► Metabolism within endothelial cells
- ► Uptake transport

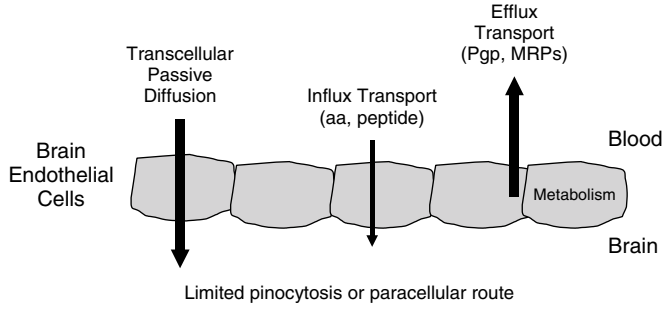


Figure 10.4 ► Permeation mechanisms at the BBB can limit compound exposure to brain cells. Used with permission from [20].

Transport of drugs into the brain is predominantly by transcellular passive diffusion. The same physicochemical properties that affect the permeation of compounds in the GI and other biological membranes also affect BBB permeation. In fact, they are more restrictive for the BBB. In addition, the profile of phospholipids in the BBB endothelial cells tends to have significant negatively charged polarity head groups, which opposes acids. These important limitations are discussed in Section 10.3.

BBB endothelial cells express P-glycoprotein (Pgp) on the apical surface. Efflux is a major limitation to BBB permeation for some compounds. Pgp efflux excludes molecules before they can reach brain cells. Therefore, an important strategy for increasing brain exposure of these compounds is to reduce efflux by Pgp. Endothelial cells also express breast cancer resistance protein (BCRP) and multidrug resistance protein 1 (MRP1) through MRP6, which efflux some compounds.[3] The roles of these efflux transporters are being investigated, but Pgp clearly is the transporter of greatest concern for neuroscience discovery projects.[4,5]

Efflux potential is assessed using an in vitro cell monolayer permeability assay (e.g., MDR1-MDCKII, Caco-2) that expresses Pgp. The term *efflux ratio* (ER) is defined as the permeability in the efflux direction divided by the permeability in the influx direction (Figure 10.4),

as modeled using the in vitro assay (see Section 27.2.1). Generally, ER > 3 indicates significant efflux. Pgp knockout mice also are used for verification of the in vitro conclusions (see Section 27.3.1). A survey of successful CNS drugs indicated ER < 1 for 22%, 1–3 for 72%, and > 3 for 6%.[6] This suggests that many commercial CNS drugs are subject to low-to-moderate levels of Pgp efflux that can be overcome with clinical dosing. However, high Pgp efflux (ER > 3) is uncommon in commercial CNS drugs and should be avoided.

Paracellular permeation is drastically limited in the BBB because the endothelial cells form tight junctions. Endothelial cells of other capillaries in the body do not form such tight junctions and they have fenestrations, which are leaky sections in the vessel. Pinocytosis in BBB endothelial cells is limited.

Metabolism (phases I and II) has been observed in BBB endothelial cells. This structurally modifies compounds before they can reach brain tissue. The role of metabolism at the BBB likely is small.

BBB permeation is enhanced for a few compounds that are substrates for uptake transporters on the endothelial cells. These transporters naturally facilitate the uptake of nutrients (e.g., amino acids, peptides, glucose) and other endogenous compounds. A small number of commercial drugs partially or predominantly penetrate the BBB by active transport. Uptake enhancement is most commonly discovered by serendipity.

10.1.2 Brain Distribution Mechanisms

Several mechanisms limit the access of compounds to brain cells by affecting the distribution of compound to or within the brain (Figure 10.5):

- Metabolic clearance
- Plasma protein binding
- Nonspecific binding to proteins and lipids in brain tissue
- Clearance of compound from the extracellular fluid (ECF) into the blood and cerebrospinal fluid (CSF)

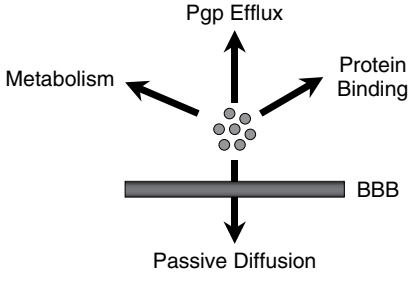


Figure 10.5 ► Mechanisms affecting compound exposure to brain cells are complex. (Reprinted with permission from [21]).

High hepatic clearance limits exposure of compounds to the brain.[7] It restricts compound from systemic circulation. Therefore, an important strategy for increasing brain exposure is to reduce hepatic clearance. For the example shown in Figure 10.6, in vitro assays indicated that passive diffusion (predicted by PAMPA-BBB, see Section 28.2.1.1) of the compound was good (CNS +), and there was no Pgp efflux. However, the compound did not penetrate into the brain in vivo to a significant amount. The reason was found to be a high rate of

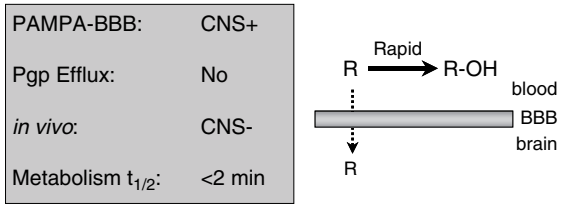


Figure 10.6 ► Example of a CNS project compound for which rapid metabolism resulted in low brain exposure.

liver metabolism ($t_{½}$ < 2 minutes) that greatly reduced the blood concentration and limited access of the compound to the brain.

Plasma protein binding limits penetration to the brain. As shown in Figure 10.7, only free unbound drug permeates the BBB. If the compound is highly bound to plasma protein and the on/off kinetics are moderate to slow, then little free drug is available to penetrate into the brain tissue. However, one must be careful in applying in vitro plasma protein binding data. Figure 10.8 shows that bound drug can release in brain microvessel circulation in vivo much more than with *in vitro* assays.[7] Plasma protein binding is useful in retrospectively diagnosing the causes of low in vivo brain penetration.

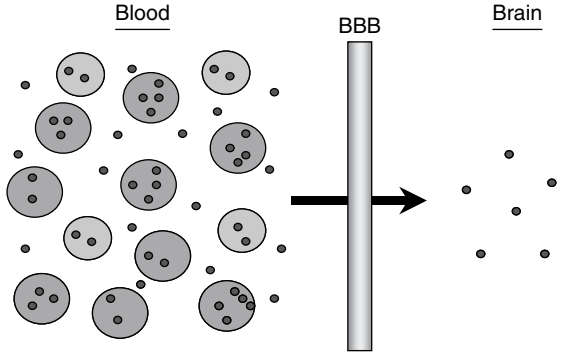


Figure 10.7 ► High plasma protein binding limits BBB permeation.

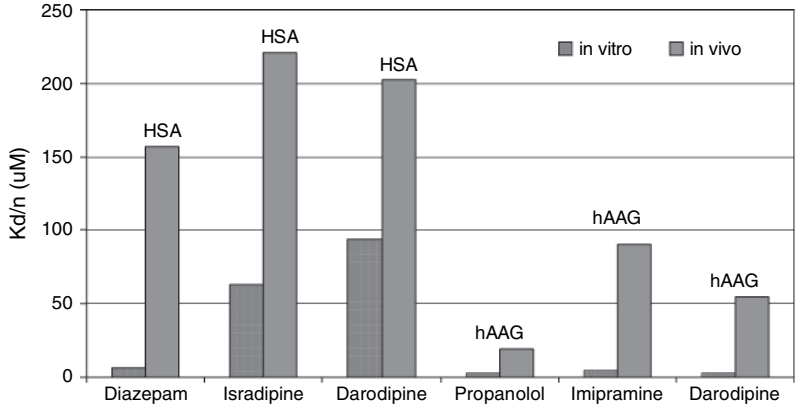


Figure 10.8 ► Plasma protein binding in vitro versus in vivo. Bound drugs release in brain microcirculation in vivo. (Modified from [9]).

Drug molecules that permeate the BBB are subject to *nonspecific binding* in the brain. The "free-drug hypothesis" suggests that a compound's efficacy is determined by the free (unbound) drug concentration in the brain and that binding restricts compound access to the therapeutic target. In this case, analysis of total brain tissue following dosing may indicate good total brain concentration; however, much of the compound may be restricted by nonspecific binding from interacting with the therapeutic target. Measurements of unbound percent in brain for commercial drugs[3] range widely (0.07%–52%). Therefore, some compounds are active in the brain despite high levels of nonspecific binding. This suggests that nonspecific binding correlates with pharmacological activity at some times but not at others. Compound in ECF is cleared into the blood and the CSF. If a compound has low BBB permeation, this clearance may limit the concentration in the ECF.[7] Figure 10.9 summarizes the major mechanisms that affect compound exposure in the brain.

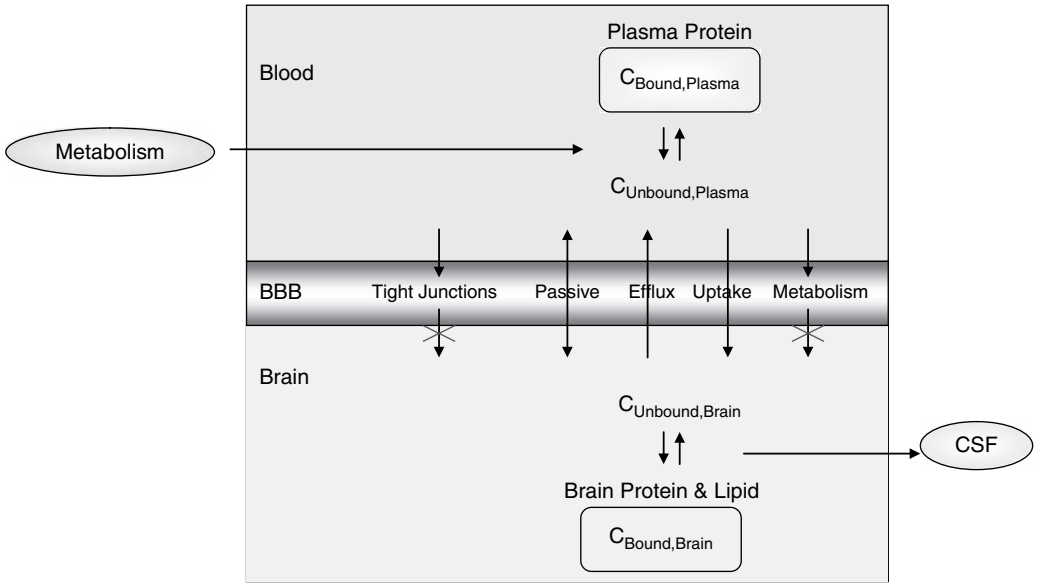


Figure 10.9 ► Compilation of major mechanisms affecting unbound (free) compound concentration in the CNS ECF ($C_{Unbound,Brain}$). $C_{Bound,Brain}$, concentration of compound bound nonspecifically to brain tissue components; $C_{Bound,Plasma}$, concentration of compound bound to plasma proteins; $C_{Unbound,Plasma}$, concentration unbound in blood; *CSF*, cerebrospinal fluid.

10.1.3 Brain–CSF Barrier

A second interface between the blood and the brain is the choroid plexus (Figure 10.10). The BBB interfaces the blood and the ECF of the brain. The choroid plexus interfaces the blood and the CSF and forms the blood–cerebrospinal fluid barrier (BCSFB).The BCSFB is not considered an effective route for drug delivery to the brain because (1) the surface area of the BBB is 5,000-fold larger than the BCSFB, (2) there is little mixing of the CSF components with the ECF, (3) the CSF flows very fast away from the brain tissue toward the arachnoid villi, and (4) CSF is turned over every 5 hours.

Some of the compound in the ECF is cleared into the CSF. Therefore, CSF contains material that penetrated the BBB. CSF has been sampled to study compound penetration into brain.[6] In some cases, CSF concentration correlates with ECF concentration, but it should not be assumed that, in general, compound concentration in CSF is the same as in ECF.[7]

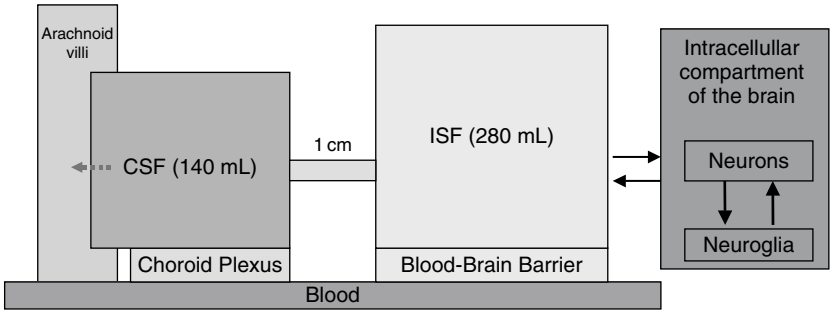


Figure 10.10 ► The BBB is a more effective route for drugs to reach the brain tissue. The BBB has 5,000-fold the surface area of the BCSFB, and CSF flows rapidly away from the brain tissue.[9,22]

10.1.4 Interpreting Data for Brain Penetration

Methods for brain penetration are discussed in Chapter 28. An understanding of how it was generated and its limitations benefits interpretation of these data as discussed in Sections 10.1.4.1 and 10.1.4.2.

10.1.4.1 B/P, Log BB, K_p, and $K_{p,free}$ Indicate the Extent of Brain Distribution

The most commonly used brain penetration data are from in vivo pharmacokinetic (PK) studies, which produce a ratio of drug in brain to drug in plasma or blood. This value is often termed *B/P*; its log is *Log BB*.

The B/P of most prescribed CNS drugs is > 0.3.[6] B/P $= 0.3$ means that the total compound concentration in the brain is 30% of the total compound concentration in the plasma. For many companies, B/P > 0.3 is used as a minimum guideline for CNS discovery projects. Compounds with B/P < 0.1 penetrate poorly into the brain, yet some commercial CNS drugs have B/P < 0.1.

B/P is actually a partition coefficient between total brain tissue and total plasma (just as Log P is a partition coefficient between octanol and water). It is a useful indicator of brain distribution; however, it has limitations as a sole indicator of brain exposure, as discussed below.

The term B/P is sometimes used loosely and is calculated from various data:

- ► AUC_{brain}/AUC_{plasma}
- ► $Concentration_{brain}/Concentration_{plasma}$ at a single time point or at C_{max}
- ► $Concentration_{brain}/Concentration_{plasma}$ at steady state (also termed K_p)

It is important that discovery scientists understand how the B/P values for their compounds were determined in order for the implications and limitations of the value to be properly understood and applied. If B/P is derived at a single time point, it may not accurately reflect brain penetration because C_{max}, t_{max}, or $t_{½}$ for a compound can vary between brain and plasma. Thus, B/P at a single time point can vary with time. Also, B/P frequently varies with dosing level, route of administration, vehicle, and species. For example, if there is significant efflux, the B/P value increases with dose as the efflux transporters are increasingly saturated. Other scenarios can affect B/P, so the project team should think through the effects of other properties of their compounds.

Another limitation of B/P is that it is calculated from total drug in the plasma and brain. A compound with a high B/P value and high nonspecific brain binding has a low free

concentration in the brain. By comparison, a compound with a low B/P and low nonspecific brain binding may actually have a higher free concentration in the brain than the first example. William Pardridge, a noted expert on the BBB, warned against relying heavily on B/P values because they may mislead project teams.[7]

B/P is also limited because it is the result of multiple mechanisms. It does not give medicinal chemists specific guidance on what can be modified in the structure to improve brain penetration. Findings of low B/P values should be followed with investigation of specific mechanisms using in vitro assays to uncover the limiting mechanism(s). The in vitro assay(s) then can be used to monitor new analogs that are synthesized for the purpose of improving the specific mechanism.

It is important to distinguish brain/plasma partitioning (i.e., brain distribution) from BBB permeability. These are distinctly different, and both are significant. The structure modifications strategies for improving distribution differ from those for improving permeability.

A useful ratio is free compound in brain to free compound in plasma[8]:

$$K_{p,free} = C_{Unbound,Brain}/C_{Unbound,Plasma},$$

where $C_{Unbound,Brain}$ = free compound concentration in the brain ECF available to interact with brain cells, and $C_{Unbound,Plasma}$ = free compound concentration in the blood available to permeate the BBB. It is apparent that one strategy for increasing the brain free concentration is to increase the plasma concentration.

10.1.4.2 P_{app}, PS, and $t_{½eq.in}$ Indicate the Rate of BBB Permeability

In vitro, in situ, and in vivo experiments can provide BBB permeability (P). Pardridge[9] demonstrated the value of the permeability surface area coefficient (PS), especially in pharmacokinetic studies. Liu[8] proposed use of "the half-life to reach equilibrium between free drug in brain and plasma" ($t_{½eq.in}$). This value is the result of BBB permeability and brain free compound concentration and is not dependent on plasma free compound concentration.

10.2 Effects of Brain Penetration

BBB penetration is necessary for CNS drugs.[10] However, drugs for therapy in peripheral tissues may cause side effects in the brain; therefore, it is desirable that they have minimal penetration into the brain (Figure 10.11). It may be possible to reduce CNS side effects of peripheral drugs by making structural modifications that reduce the brain penetration.

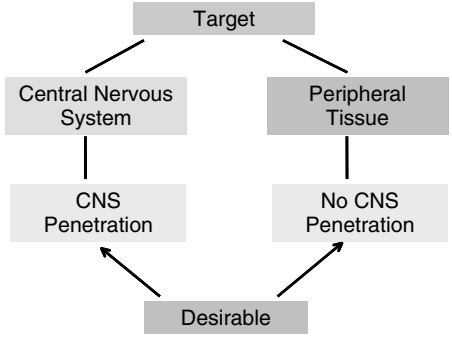


Figure 10.11 ► It is necessary for CNS drugs to penetrate into the brain, but it is undesirable for drugs treating diseases in the peripheral tissues to penetrate into the brain, where they can cause CNS side effects.

10.3 Structure–BBB Penetration Relationships

Physicochemical properties greatly affect the passive transcellular BBB permeation of compounds. Studies have shown the following key structural properties for discovery of CNS drugs[9,11–14]:

- Hydrogen bonds (acceptors and donors)
- Lipophilicity
- Polar surface area (PSA)
- Molecular weight (MW)
- Acidity

These properties are more restrictive at the BBB than at most other membrane barriers in the body. As a group, commercial CNS drugs compared to non-CNS drugs have fewer hydrogen bond donors, higher Log P, lower PSA, and fewer rotatable bonds.[12] A set of physicochemical BBB rules was first proposed by Pardridge.[13] The structure should have the following:

- H-bonds (total) < 8–10
- MW < 400–500
- No acids

Spraklin[3] suggests H-bond donors < 2 and H-bond acceptors < 6. This is in agreement with general consensus that H-bond donors are more limiting than H-bond acceptors.

Another set of BBB rules was compiled by Clark[14] and Lobell et al.[15] The structure should have the following:

- $N + O < 6$
- PSA < 60–70 Å^2
- MW < 450
- Log D $= 1$–3
- ClogP $-$ $(N + O) > 0$

These rules are useful for evaluating BBB permeability prior to synthesis, assessing compounds being brought into a project (e.g., alliance partner), diagnosing poor in vivo brain penetration, and guiding which structural modifications might best improve BBB permeation of a discovery lead series.

Indomethacin is an example of poor BBB permeability by acids (Figure 10.12). CNS drugs tend to contain a basic amine, such as trifluoroperizine. Positively charged amines favorably interact with the negatively charged head groups of phospholipids at the BBB (Figure 10.13). Approximately 75% of the most prescribed CNS drugs are basic, 19% are neutral, and 6% are acids.[11] The amine functional group often is important for CNS activity; however, the basicity also assists BBB influx.

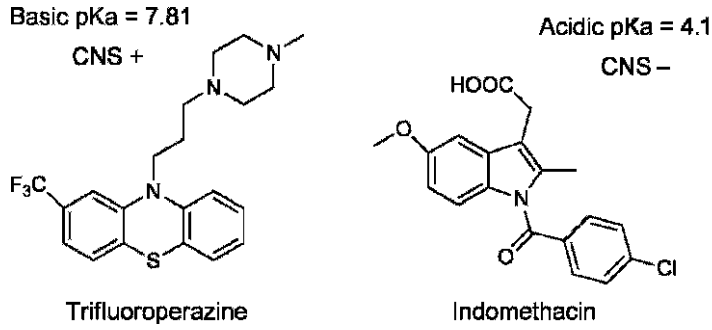


Figure 10.12 ► Acids poorly permeate the BBB (CNS−), whereas bases generally penetrate much better (CNS+). (Reprinted with permission from [21]).

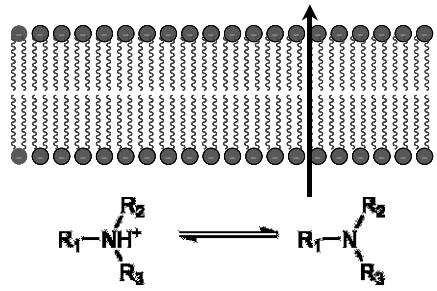


Figure 10.13 ► Amines have favorable interaction with predominantly negatively charged phospholipids head groups at the BBB.

It has been noted that in silico quantitative structure–activity relationship descriptors developed using B/P (Log BB) distribution datasets are similar to those developed using BBB permeability (Log PS) datasets.[16] Thus, the fundamental structural modifications (i.e., H-bonding, lipophilicity, MW, acidity) for brain penetration are consistent.

10.4 Structure Modification Strategies to Improve Brain Penetration

Strategies for modifying structures to improve brain penetration are listed in Table 10.1.

TABLE 10.1 ► Structure Modification Strategies for Brain Penetration Improvement

Structure modification	Section
Reduce P-glycoprotein efflux	10.4.1
Reduce hydrogen bonds	10.4.2
Increase lipophilicity	10.4.3
Reduce molecular weight	10.4.4
Replace carboxylic acid groups	10.4.5
Add an intramolecular hydrogen bond	10.4.6
Modify or select structures for affinity to uptake transporters	10.4.7

10.4.1 Reduce Pgp Efflux

Pgp efflux is the most significant limitation to BBB permeation of some discovery lead series. It is important to assess Pgp efflux early for a series. Structure–efflux relationships can be established by running series examples in an in vitro Pgp assay (see Chapter 28). These relationships will indicate which portions of the molecule might be modified to attempt efflux reduction. Other strategies for structural modifications to reduce Pgp efflux are discussed in Section 9.3.1.3.

10.4.2 Reduce Hydrogen Bonds

Reducing the total number of hydrogen bonds will increase BBB permeation, especially H-bond donors. The effect of reducing the number of total hydrogen bonds on BBB permeation is shown in Figure 10.14 for a series of steroids.[9] As the number of total hydrogen bond donors and acceptors decreases, BBB permeation increases. Functional groups that form hydrogen bonds can be removed, substituted, or blocked to enhance BBB permeation.

An example from a discovery CNS project is shown in Figure 10.15. Reduction of one hydrogen bond donor by blocking with a methyl group greatly increased brain penetration.

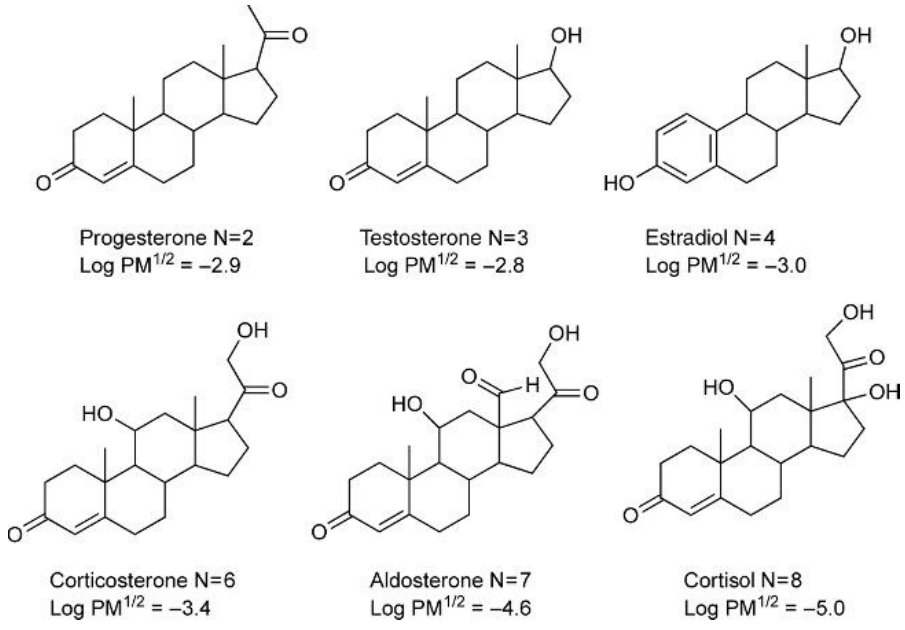


Figure 10.14 ► Effects of H-bonding on BBB permeation.[9]

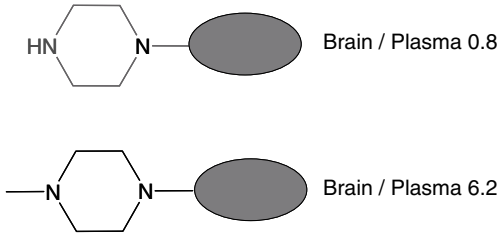


Figure 10.15 ► Example from a discovery CNS project in which the brain penetration was enhanced (B/P) with the reduction of one H-bond donor.

10.4.3 Increase Lipophilicity

Increasing lipophilicity will increase BBB permeation.[9] For example, addition of one methyl group to morphine produces codeine, which has a 10-fold higher BBB permeation (Figure 10.16). Addition of two acetyl groups to produce heroin further increases BBB permeation because of increased lipophilicity, despite increasing the number of H-bond acceptors. Nonpolar groups (e.g., methyl) also can be added to a molecule to enhance lipophilicity.

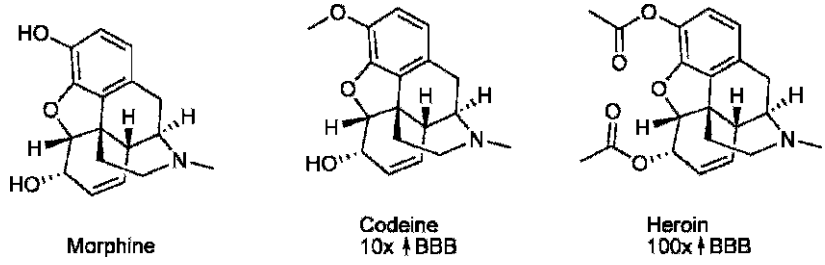


Figure 10.16 ► Effects of lipophilicity on BBB permeation. (Reprinted with permission from [21]).

It should be noted that increasing lipophilicity to improve BBB permeation also can have a negative effect on BBB penetration. According to the "pharmacokinetic rule,"[13] increasing lipophilicity also increases nonspecific binding in the brain. This reduces free compound concentration in ECF at brain cells and decreases activity. Therefore, it is important to balance lipophilicity for optimizing permeability and minimizing brain binding.

10.4.4 Reduce MW

If groups on the structure can be removed without greatly impairing activity, then removing them can be beneficial. This reduces molecular size and improves permeation through lipid bilayer membranes.

10.4.5 Replace Carboxylic Acid Groups

Elimination of an acidic group will increase BBB permeation. An example of carboxylic acid replacement is shown in Figure 10.17.[17]

10.4.6 Add an Intramolecular Hydrogen Bond

An intramolecular hydrogen bond will increase BBB permeation. It reduces the total number of hydrogen bonds with water that must be broken for BBB permeation. In the example shown in Figure 10.18,[18] an amine was added that was reported to form two intramolecular hydrogen bonds and enhanced brain penetration.

10.4.7 Modify or Select Structures for Affinity to Uptake Transporters

Membrane transporters enhance the BBB permeation of some drugs. Carrier-mediated transporters have been advocated as a means of enhancing the penetration of compounds with poor passive BBB permeation.[19] For example, LAT1 (large neutral amino acid transporter) enhances the brain uptake of L-dopa and gabapentin.[8] Other transporters on the luminal side of the BBB endothelial cells are GLUT1 (glucose), MCT1 (monocarboxylic acids),

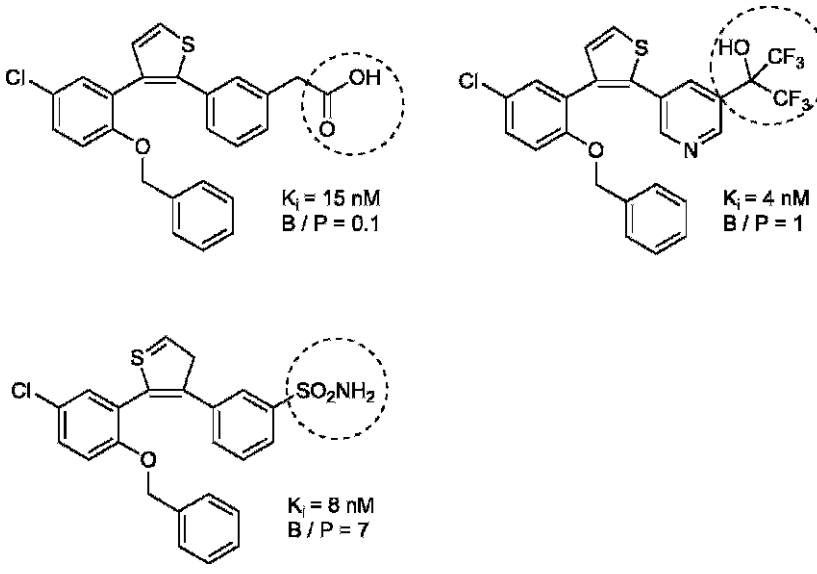


Figure 10.17 ► Replacement of carboxylic acid group in an EP1 receptor antagonist series improved brain penetration.[17]

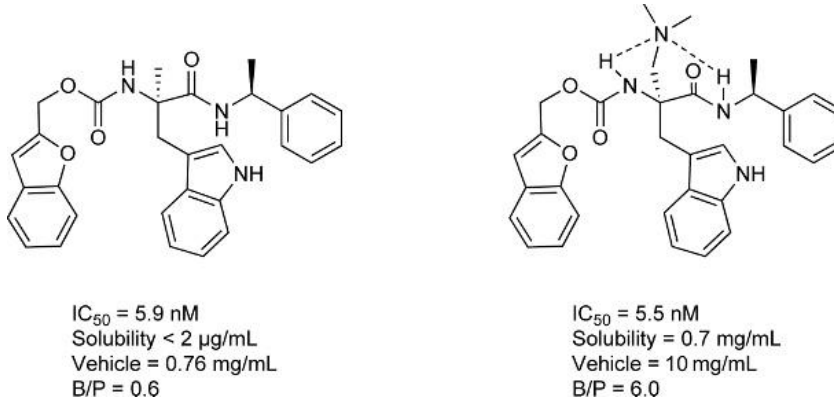


Figure 10.18 ► Addition of an intramolecular hydrogen bond increases brain penetration.[18] (Reprinted with permission from [21]).

CAT1 (cationic amino acids), and CNT2 (nucleosides). The transporter uptake strategy has not been widely implemented but remains an intriguing prospect.

Problems

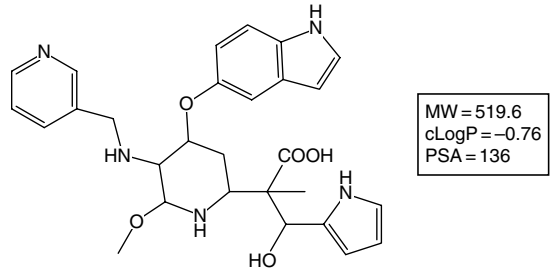
(Answers can be found in Appendix I at the end of the book.)

1. The BBB consists of: (a) a membrane between the skull and the brain, (b) an impermeable membrane surrounding brain cells, (c) the endothelial cells of brain capillaries, (d) membranes that surround each section of the brain.
2. Why is Pgp efflux much more important at the BBB than at the GI endothelium?
3. Uptake of most drugs at the BBB occurs by which mechanism?: (a) Pgp efflux, (b) uptake transport, (c) metabolism, (d) paracellular, (e) passive diffusion, (f) endocytosis, (g) plaque formation.

4. Which of the following compounds are likely to have poor brain penetration and why?

Compound	MW	Ionization	H-bond acceptors	A-bond donors	PSA
A	350	$pK_a = 9$, base	4	1	55
B	520	$pK_a = 5$, acid	10	5	140
C	600	$pK_a = 8$, base	3	3	60
D	470	$pK_a = 8$, base	8	2	75

5. How could the following compound be structurally modified to improve BBB permeation by passive diffusion?



6. Which of the following molecular properties are *not* favorable for BBB permeation?: (a) MW < 450, (b) PSA > 60–$70\,Å^2$ (c) Solubility $> 50\,\mu M$, (d) Log D < 1, (e) (N + O) > 5, (f) ClogP – (N + O) < 0?

7. If low brain concentrations of drug or low B/P are observed in vivo, which of the following approaches can be used to diagnose the cause?: (a) physicochemical/molecular "rules" for brain penetration, (b) thermodynamic solubility, (c) Pgp efflux ratio, (d) metabolic stability, (e) plasma protein binding, (f) CYP inhibition, (g) Pgp knockout animals (lacking Pgp).

8. Which of the following reduce brain penetration?: (a) CSF, (b) Pgp efflux, (c) tight BBB endothelial cell junctions, (d) uptake transporters, (e) high plasma protein binding, (f) carboxylic acid group on molecule, (g) intramolecular H-bonds in molecule.

9. Which of these structural modifications are likely to increase brain penetration?: (a) replace –COOH with $–SO_2NH_2$, (b) increase hydrogen bond donors, (c) increase lipophilicity, (d) increase intramolecular hydrogen bonding, (e) reduce hydrogen bond donors, (f) increase MW, (g) reduce PSA.

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Chapter 11

Metabolic Stability

Overview

- *Metabolism is the enzymatic modification of compounds to increase clearance.*
- *It is a determinant of oral bioavailability, clearance, and half-life in vivo.*
- *Metabolism occurs predominantly in the liver, and some may occur in the intestine.*
- *Metabolic stability is increased by structure modifications that block or sterically interfere with metabolic sites or withdraw electrons.*

Drugs encounter formidable challenges to their stability in vivo. Unfortunately, this imposes significant limitations on the structures of drugs. Structures that are highly active in vitro may not make good drugs because they are susceptible to metabolism in the body. Most drug discovery project teams encounter stability limitations for their lead series. Many pharmacologically interesting molecules must be passed over because they are not sufficiently stable. This and other chapters on stability issues aim to inform discovery scientists about the causes of instability and guide medicinal chemists through successful strategies of structure modification to improve the lead series stability.

An overview of in vivo stability challenges suggests a diverse ensemble of chemical and enzymatic reactions poised to attack various moieties of the molecule (Figure 11.1).[1] In the gut, a molecule risks *intestinal decomposition***,** owing to various pHs and enzymatic hydrolysis reactions. As the molecule moves through the gut wall, enzymes can initiate *intestinal metabolism*. Molecules that reach the portal vein are immediately carried to the liver where they encounter diverse *hepatic metabolism* reactions. Molecules that survive the liver encounter *plasma decomposition* by hydrolytic enzymes in the blood.

In addition to in vivo degradation, stability issues are encountered in drug discovery laboratories. Compounds can encounter *chemical decomposition* due to environmental challenges in the laboratory. It has become apparent that compounds also can undergo in vitro *decomposition* in biological experiments, which confuses the structure–activity relationship (SAR). Medicinal chemists should be aware of all potential in vivo and in vitro stability problems and ensure that their compounds are tested and structurally stabilized against these various challenges. A scheme of in vitro assays that can assess a wide range of stability issues in drug discovery is shown in Figure 11.2. Chapters 12 and 13 discuss the challenges faced as a result of chemical and enzymatic decomposition (e.g., solution and plasma stability). This chapter focuses on metabolic stability, which is widely considered one of the most significant challenges of drug discovery. Methods for predicting stability are discussed in Chapters 29 through 31.

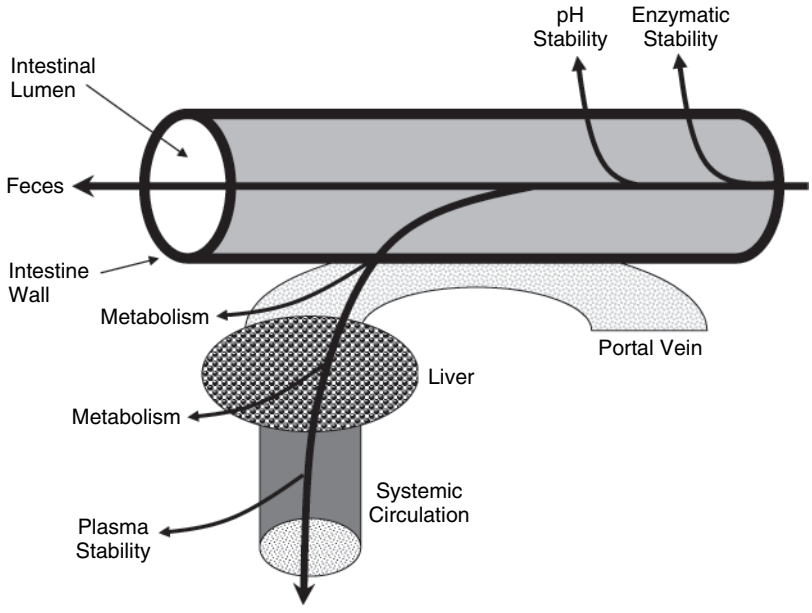


Figure 11.1 ► Stability challenges following oral administration.

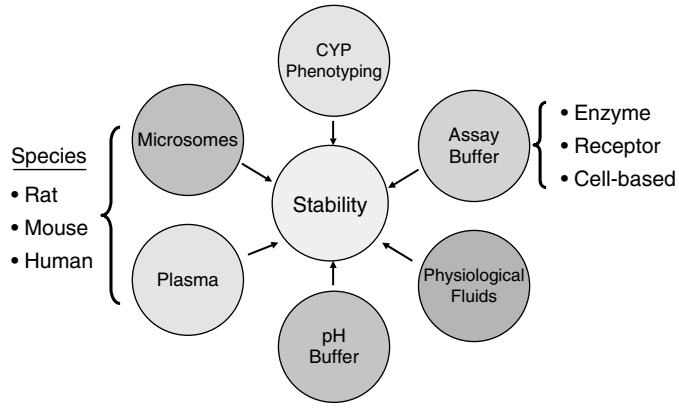


Figure 11.2 ► Scheme for in vitro assessment of diverse stability challenges during drug discovery. (Reprinted with permission from [29].)

11.1 Metabolic Stability Fundamentals

Drug metabolism often is referred to as *biotransformation*, and metabolism reactions have been divided into two phases. Phase I reactions are modifications of the molecular structure itself, such as oxidation or dealkylation. Phase II reactions are additions (conjugations) of polar groups to the molecular structure. Sometimes they are considered sequential: first addition of an attachment point (e.g., hydroxyl) and then addition of a large polar moiety (e.g., glucuronic acid). However, a compound need not undergo a phase I reaction before a phase II reaction if it already has a functional group that is susceptible to conjugation. Both

of these types of reactions produce more polar products that have higher aqueous solubility, so they are more readily excreted from the body via bile and urine. Metabolism increases clearance, reduces exposure, and is a major cause of low bioavailability. The result is a lower concentration of a drug at the therapeutic target than without metabolism. Medicinal chemists often must make structural modifications to lead compounds during drug discovery in an attempt to reduce metabolism.

If the drug is metabolized prior to reaching systemic circulation, it is said to have undergone *presystemic* or *first-pass metabolism*. First-pass metabolism clears absorbed drug before it reaches systemic circulation. First-pass metabolism can occur in both the gut and the liver. By excluding exogenous compounds (e.g., drugs) from the bloodstream by first-pass metabolism and then further reducing drug concentration by metabolism of a portion of the circulating drug with each pass through the liver, metabolic stability is an underlying root property that affects exposure and bioavailability.

Differences in metabolism are observed among species. The metabolic profiles from different species vary in the metabolite structures and their production rates.

11.1.1 Phase I Metabolism

Phase I reactions consist of several mechanisms that modify the compound structure. These include oxidation and reduction.

Several different enzyme families catalyze these reactions. The most prominent are called *monooxygenases*, which include (a) the cytochrome P450 (CYP) family (Figure 11.3) and (b) the flavine monooxygenase (FMO) family. A generalized reaction for monooxygenases is:

$$R-H+O_2+NADPH+H^+ \rightarrow R-OH+NADP^+ + H_2O.$$

Monooxygenases are bound to the endoplasmic reticulum (ER) inside cells and are found in high abundance in hepatocytes. The membrane association of the enzymes is consistent with the correlation of compound metabolism to lipophilicity.

The reaction is catalyzed in CYPs by a heme group containing an iron atom at the active site (Figure 11.3). In CYPs, the iron atom binds oxygen and transfers it to the drug molecule via a series of reactions (Figure 11.4). NADPH provides the electrons for reducing Fe^{III} to Fe^{II} via a second coupled enzyme (NADPH-cytochrome P450 reductase). The CYP family consists of over 400 isozymes. CYP enzymes are found in mammals, insects, plants, yeasts, and bacteria. In mammals, they are distributed in liver, kidney, lung, intestine, colon, brain, skin, and nasal mucosa. The different amino acid sequences of the isozymes result in different binding affinity for compound classes. The rate of the reaction is a result of (1) the affinity of the compound for binding to the CYP enzyme and (2) the reactivity of the position(s) on the molecule that are brought into close proximity with the heme group. In FMOs, a flavine group at the active site catalyzes the reaction. NADPH directly reduces the flavine.

A compound can be metabolized by more than one enzyme or isozyme. If metabolism of a drug at one enzyme is blocked by substrate saturation of the enzyme or by structural modification, then metabolism at another enzyme with weaker binding or lower reactivity may become a more favorable route. This process is called *metabolic switching*. Drug–drug interactions can occur if two or more drugs are coadministered, and one drug inhibits the metabolism of a second drug at a particular isozyme, leading to toxic effects. Drug–drug interactions are discussed in Chapter 15.

Common phase I metabolic reactions are shown in Figure 11.5. These examples indicate many of the potential metabolic reactions that discovery compounds can undergo. The most commonly observed metabolism is hydroxylation of aliphatic and aromatic carbon atoms.

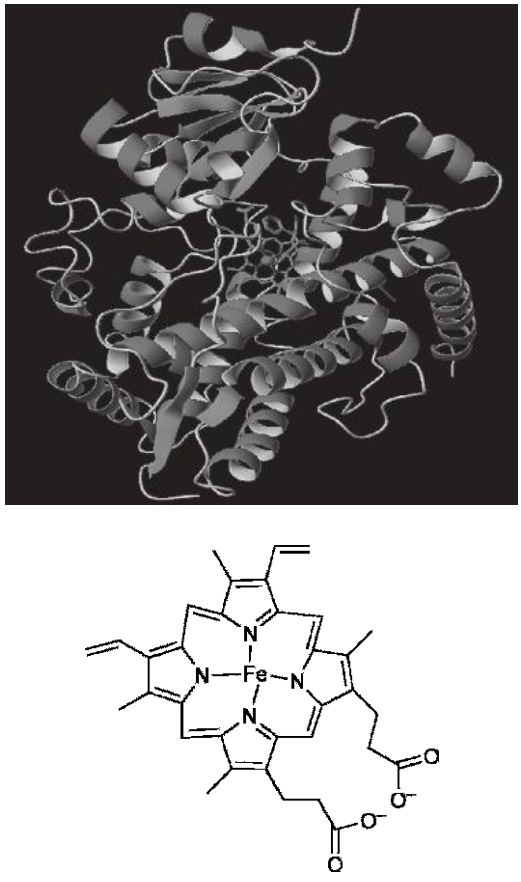


Figure 11.3 ► Structure of human cytochrome P450 3A4 with heme and inhibitor metyrapone.[30] (Drawing courtesy Kristi Fan.) (see Plate 2)

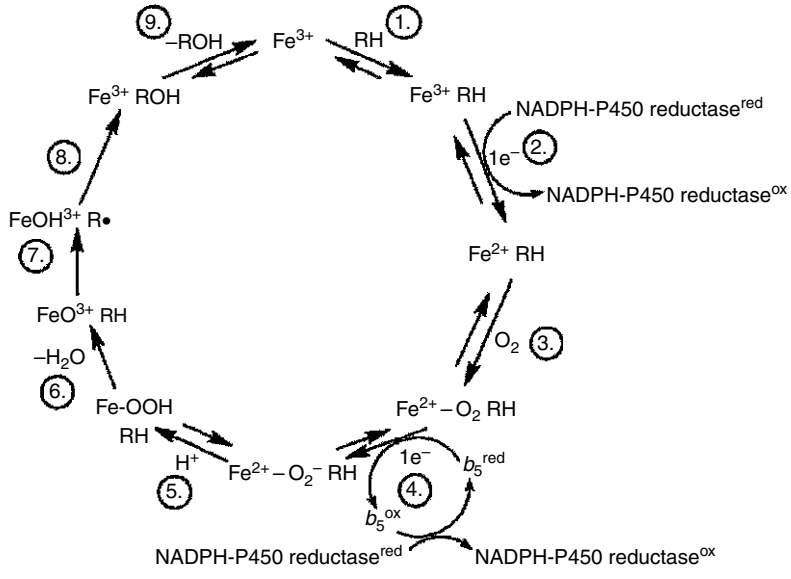
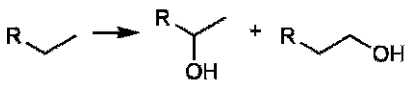
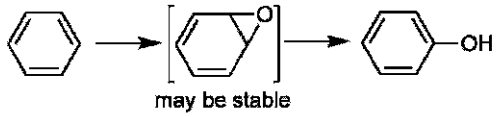


Figure 11.4 ► Mechanism of catalytic cycle for CYP450 reactions. (Reprinted with permission [31].)

Aliphatic Oxidation (Cytochrome P450 (CYP) [Endoplasmic reticulum (ER)])

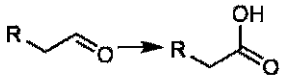


Aromatic Oxidation (CYP [ER])



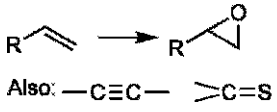
Alcohol Oxidation (Alcohol Dehydrogenase, reversible [Cytosol])

Aldehyde Oxidation (Aldehyde Dehydrogenase [Cytosol, Mitochondria])

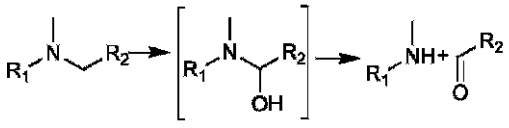


Dehydrogenation (CYP [ER])

Epoxidation (CYP [ER])



N-Dealkylation (CYP [ER])



O-Dealkylation (CYP [ER])

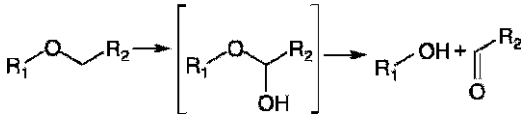
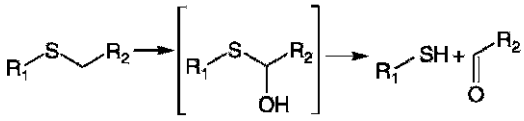
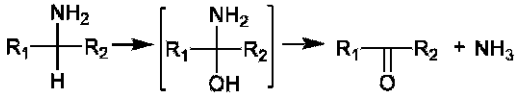


Figure 11.5 ► Examples of major phase I metabolic reactions.

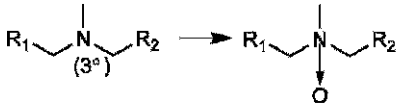
S-Dealkylation (CYP [ER])



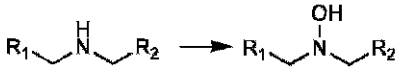
Oxidative Deamination (Monoamine- & Diamine-Oxidases [Mitochondria])



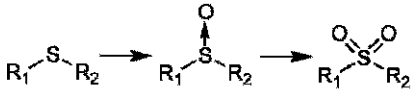
N-Oxidation (Flavin Monooxygenase (FMO) [ER])



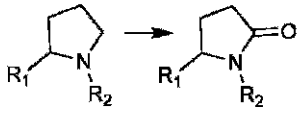
N-Hydroxylation (CYP [ER])



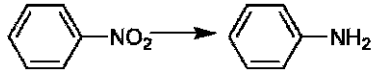
S-Oxidation (FMO [ER])



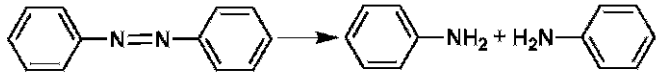
Cyclic Amines to Lactams (Aldehyde oxidase)



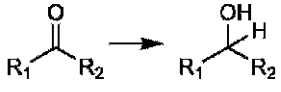
Reductions



([NADPH-CYP450 reductase [ER] and Nitroreductase [cytosol])



(Azoreductase)



(R_2 may be H, Alcohol Dehydrogenase, [cytosol])

Figure 11.5 ► *Continued*

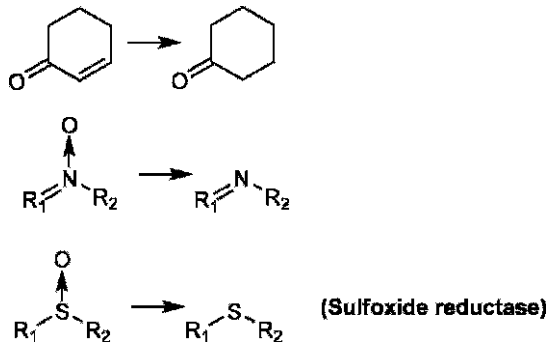


Figure 11.5 ► *Continued*

Aliphatic hydroxyls may be further converted to aldehydes and carboxylic acid. Carbon atoms adjacent to nitrogen atoms are susceptible to oxidation, which leads to dealkylation to form the amine and aldehyde. In the same manner, carbon atoms adjacent to ether oxygens or sulfides can be oxidized and lead to dealkylation. Nitrogen atoms of amines can be oxidized to *N*-oxides. Sulfur atoms of sulfides can be oxidized to sulfoxides and sulfones. Mechanisms of biotransformation reactions are discussed by Magdalou et al.[2] Some software products provide useful predictions of the site of metabolism (see Chapter 29).

11.1.2 Phase II Metabolism

Phase II metabolism is the addition of polar moieties to the molecule. Common additions are shown in Figure 11.6. Glucuronic acid can be added to aromatic or aliphatic hydroxyls, and occasionally carboxylic acids and amines by UDP-glucuronosyltransferases (UGTs) to form glucuronide metabolites. Sulfate can be added to aromatic, aliphatic, or hydroxylamine

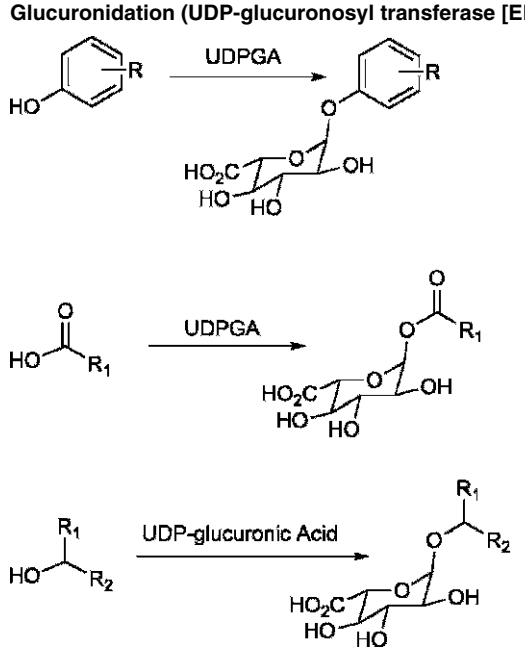
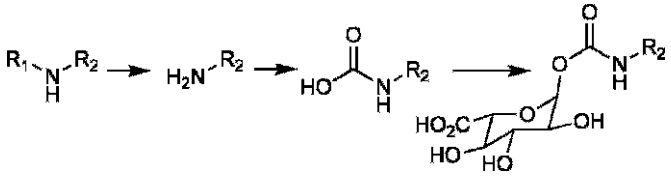


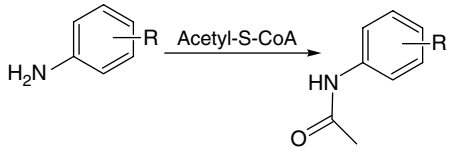
Figure 11.6 ► Examples of major phase II metabolism reactions.

Carbamic Acid Glucuronidation



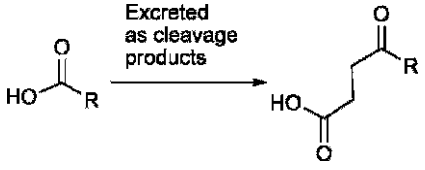
Sulfation (Sulfotranserase [cytosol])

Acetylation (N-Acetyltransferases [cytosol])



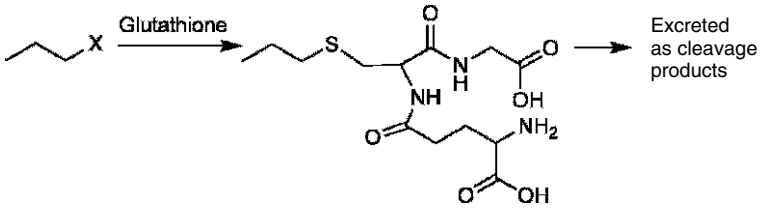
Also: 1°, 2° Amines, Hydrazines, Hydrides; R-NH-OH → -NH-O-$COCH_3$

Glycination [mitochondria]



Also: other amino acid additions (e.g., taurine, glutamine)

Glutathione Conjugation (Glutathione-S-transferases [cytosol])



X: Halogen, Electron-deficient double bond, or epoxide

Figure 11.6 ► *Continued*

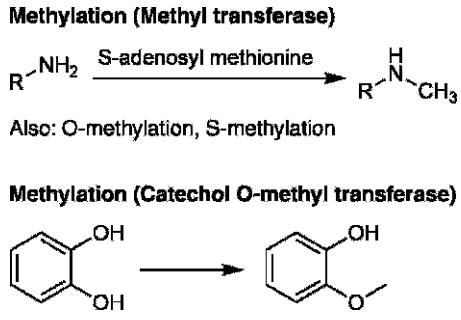


Figure 11.6 ► *Continued*

hydroxyls by sulfotransferases to form sulfate metabolites. This is a rapid, but saturable, reaction. Glutathione can be added to reactive electrophiles (nucleophilic displacement) or to an electron-deficient double bond (nucleophilic addition) by glutathione-*S*-transferases to form glutathione conjugates. This is a major detoxification mechanism for reactive xenobiotics and reactive metabolites. Amines can be acetylated by *N*-acetyltransferases to form amides. Various amino acids, such as glycine, can be added to form conjugates.

The greatly increased hydrophilicity of phase II conjugates enhances their elimination in the bile and urine.

11.2 Metabolic Stability Effects

Metabolic stability affects pharmacokinetics (PK), as shown in Figure 11.7.[3] Metabolic stability has an inverse relationship with clearance (Cl). A decrease in metabolic stability leads to an increase in clearance. Cl and volume of distribution (V_d) directly affect the PK half-life ($t_{½} = 0.693 \times V_d/Cl$), which determines how often the dose must be administered. Cl and absorption, which is determined by intestinal permeability and solubility, directly affect oral bioavailability (F). F determines how much drug must be administered.

This is illustrated with compounds from a discovery project lead series in Table 11.1. The compounds having short in vitro metabolic stability $t_{½}$ tend to have high in vivo clearance (Cl) and low oral bioavailability (%F).

An example of different metabolic stabilities of analogs with the same core template is shown in Table 11.2[4]. Structural differences at R_1, R_2, and R_3 had significant effects on metabolic stability. In addition, the bioavailability increased as the percent metabolized decreased.

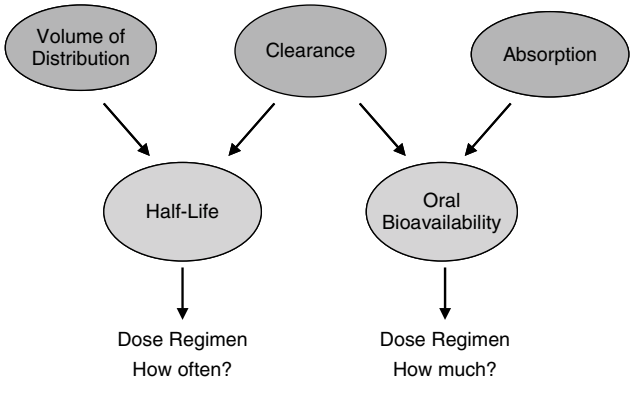
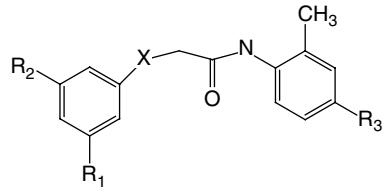


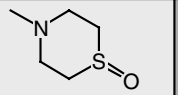
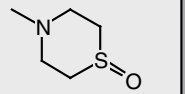
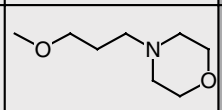
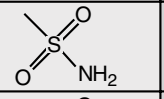
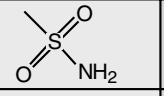
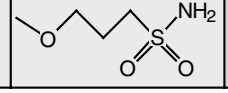
Figure 11.7 ► Effects of metabolic stability on pharmacokinetics. (Reprinted with permission from [3].)

TABLE 11.1 ► Example Compounds from a Discovery Project Lead Series Demonstrating Relationship Between In Vitro Metabolic Stability ($t_{½}$) and In Vivo Cl and F

Compound	In vitro $t_{½}$ (min)	In vivo Cl (ml/min/kg)	% F Rat
1	5	53	3
2	6	55	8
3	7	49	15
4	14	18	20
5	>30	14	41

TABLE 11.2 ► Relationship Among Structure, In Vitro Metabolic Stability, and Oral Bioavailability



				% Metabolized (S9, 1h)			Oral Bioavailability
Compd	R_1	R_2	R_3	Rat	Dog	Human	% F in Rat
A	H	H		96	99	66	14
B	F	H		88	99	63	3
C	H	H		99	99	94	7
D	F	CF_3		7	4	5	40
E	F	F		7	90	21	NA
F	F	F		15	46	43	58

11.3 Structure Modification Strategies for Phase I Metabolic Stability

Before beginning structure modification to improve stability, it is useful to elucidate the specific site of metabolism. In the past, it was necessary to predict the most likely sites of metabolism and then make analogs to counteract possible metabolism at those sites. However,

higher throughput methods for metabolite structure elucidation have been developed and are discussed in Chapter 29.

Several strategies for increasing the metabolic stability of compounds have been successful. These strategies are listed in Table 11.3 for phase I metabolism and Table 11.4 for phase II metabolism. The modifications for phase I metabolism are based on two key characteristics of metabolic reactions: (a) binding of the compound to the metabolic enzyme and (b) reactivity of the site on the molecule that is adjacent to the reactive heme of CYP or reactive site of another metabolic enzyme. Structural changes that reduce compound binding or reactivity at the labile site will increase metabolic stability. These strategies are not always 100% successful, owing to metabolic switching.

TABLE 11.3 ► Structure Modifications Strategies for Phase I Metabolic Stability Improvement

Structure modification	Section
Block metabolic site by adding fluorine	11.3.1
Block metabolic site by adding other blocking groups	11.3.2
Remove labile functional group	11.3.3
Cyclization	11.3.4
Change the ring size	11.3.5
Change chirality	11.3.6
Reduce lipophilicity	11.3.7
Replace unstable groups	11.3.8

11.3.1 Block Metabolic Site By Adding Fluorine

The strategy of blocking the site of hydroxylation is shown in Figure 11.8. The blocking group is less reactive than the hydrogen that was in the site in the metabolized analog. Fluorine is the most commonly used blocking group. Of the possible blockers, it seems to have the least effect on the size of the molecule.

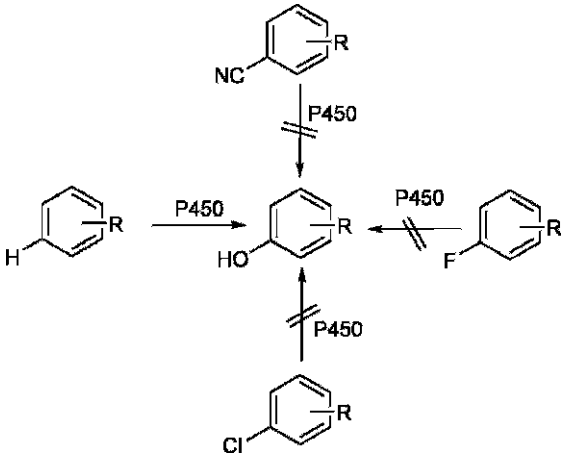


Figure 11.8 ► Blocking the site of metabolism by adding fluorine, chlorine, or nitrile.

Figure 11.9 illustrates the addition of fluorine at the site of metabolism.[5] Hydroxylation of the 5′ position of pyrimidine is a common metabolic reaction for buspirone. By adding

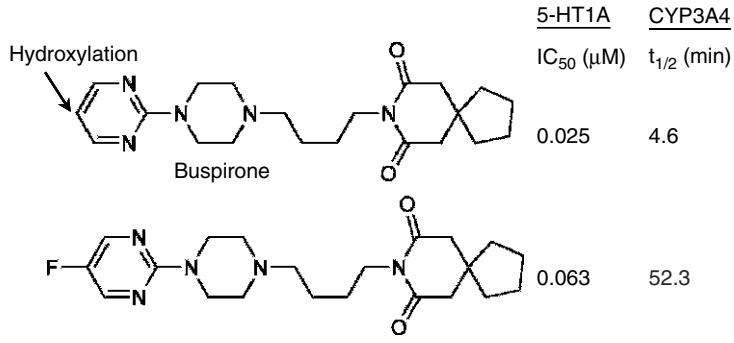
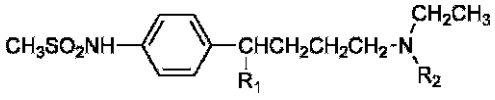


Figure 11.9 ► Fluorination of buspirone at the 5′ position of pyridine blocks metabolism and increases the in vitro $t_{½}$.



R1	R2	Relative Metabolic Stability
(R)-OH	$(CH_2)_6CH_3$	1
OH	$(CH_2)_6CH_2F$	4.2
OH	$(CH_2)_6CHF_2$	>20
OH	$(CH_2)_5CHF_2$	>20
(S)-OH	$(CH_2)_5C(CH_3)_2F_2$	>20
H	$(CH_2)_6CH_2F$	1
H	$(CH_2)_5CH(F)CH_3$	3
H	$(CH_2)_5C(CH_3)_2F$	3

Figure 11.10 ► Substitution of fluorine atoms in R2 increased the metabolic stability.

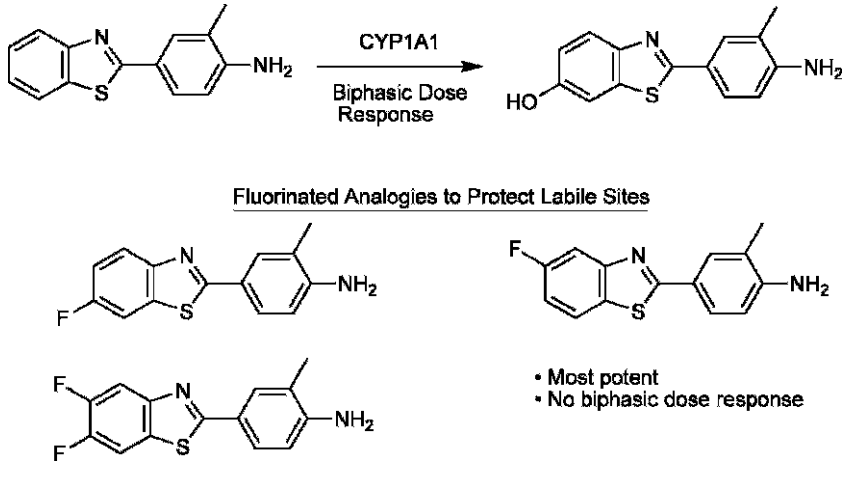


Figure 11.11 ► Metabolism caused a biphasic dose response. Blocking the metabolic site eliminated the biphasic dose response.

fluorine at this site, the in vitro $t_{½}$ with CYP3A4 isozyme increases from 4.6 to 52 minutes, without a major loss of activity. CYP3A4 is the major metabolic enzyme for buspirone.

Various fluorine analogs of ibutilide at the R_2 alkyl moiety are shown in Figure 11.10.[6] Replacement of one or two hydrogens with fluorine atoms increased the relative metabolic stability by 4- and 20-fold, respectively.

The compound in Figure 11.11 demonstrated a biphasic dose response due to metabolism by CYP1A1.[7] Fluorinated analogs blocked metabolism and did not exhibit the biphasic dose response.

11.3.2 Block Metabolic Site By Adding Other Blocking Groups

Groups other than fluorine can be added at the labile site. Substitution with chlorine is illustrated in Figure 11.12. The pharmacokinetic half-life was lengthened from 6 to 33 hours by substitution of the methyl group on tolbutamide with chlorine to make chlorpropamide.

For the compound shown at the top of Figure 11.13, the benzylic methine was found to be a labile site.[8] Additions here resulted in greater metabolic stability and increased in vivo exposure (i.e., area under the curve [AUC]) while improving the activity.

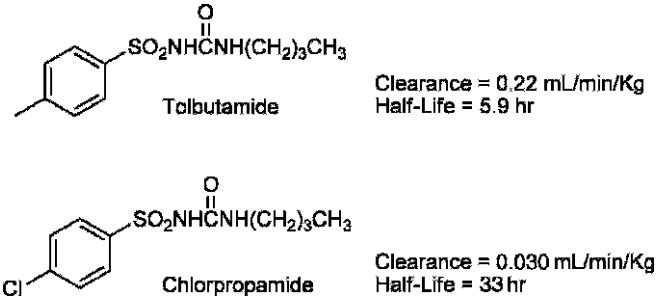


Figure 11.12 ► Substitution of methyl in tolbutamide with chlorine to make chlorpropamide greatly increased stability.

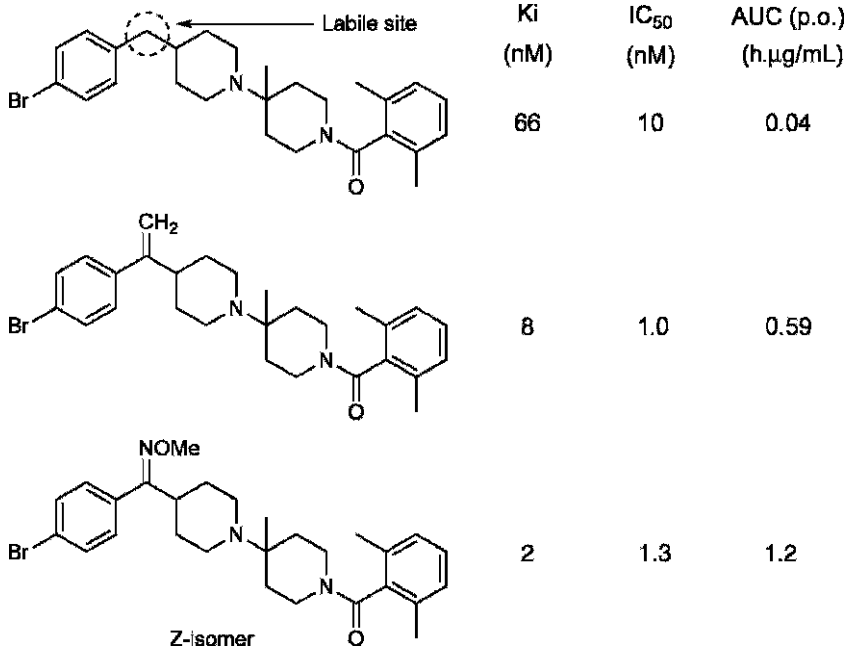


Figure 11.13 ► Blockage of a labile site improved metabolic stability and oral exposure.

More bulky aliphatic groups can also be added adjacent to the labile site. This provides steric hindrance, thus reducing access of the metabolizing enzyme active site to the labile compound site. For example, modification of the methoxy in metoprolol to the cyclopropylmethoxy in betaxolol (Figure 11.14) reduced *O*-dealkylation by CYP2D6 and increased bioavailability.[9]

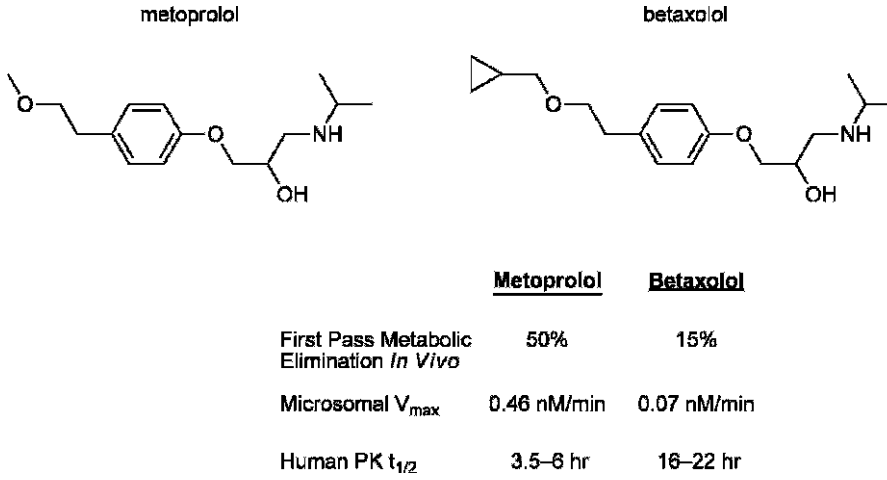


Figure 11.14 ► *O*-Dealkylation of the methoxy of metoprolol was reduced by modification to the cyclopropylmethoxy to make betaxolol, which has improved metabolic stability.

11.3.3 Remove Labile Functional Group

Removing labile groups can improve metabolic stability.[10] For the compound in Figure 11.15, removal of the methoxy methyl increased stability. Further removal of the *N*-propylene group increased stability greatly while maintaining activity. These sites are reactive for dealkylation in the original molecule.

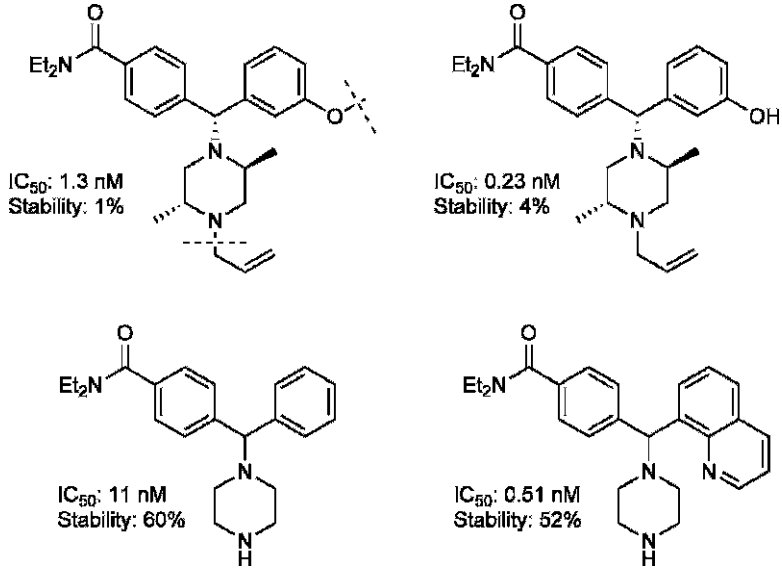


Figure 11.15 ► Removal of labile groups improved metabolic stability (rat, percent remaining after 1-hour incubation).

For the upper compound in Figure 11.16, the methoxy group was found to be metabolically unstable.[11] Removal of the methoxy and substitution with an amide improved the metabolic stability and resulted in a greater C_{max}.

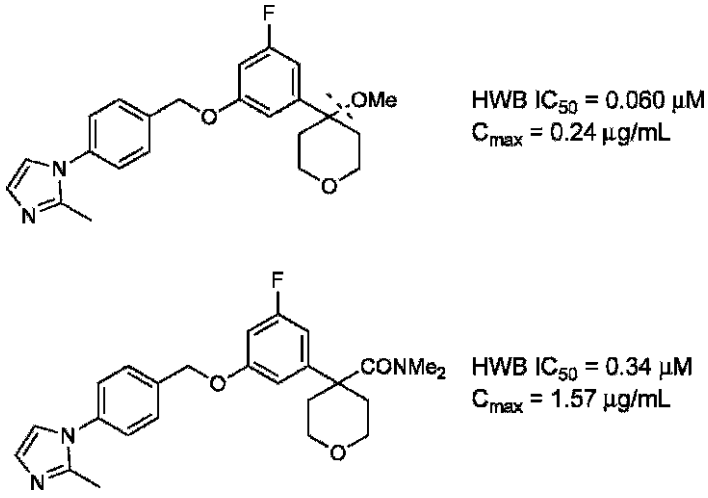


Figure 11.16 ► Remove labile group and replace to improve the metabolic stability (monkey, oral 5 mg/kg dose).

11.3.4 Cyclization

Metabolism of labile groups can be reduced by incorporation into a cyclic structure.[12] For example, the compound in Figure 11.17 was susceptible to *N*-demethylation. Incorporation of the methyl group into a cyclic structure preserved the functionality for the sake of activity while improving metabolic stability.

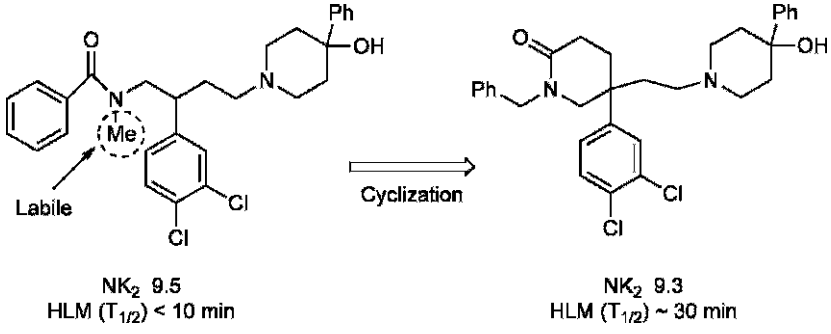


Figure 11.17 ► Cyclization strategy to improve metabolic stability in human liver microsomes (HLM) while maintaining activity (NK2).

11.3.5 Change Ring Size

The size of attached rings can be changed to modify metabolic stability. For the example in Figure 11.18, reducing the ring size increased the metabolic stability ($t_{½}$ in human liver microsomes).[12]

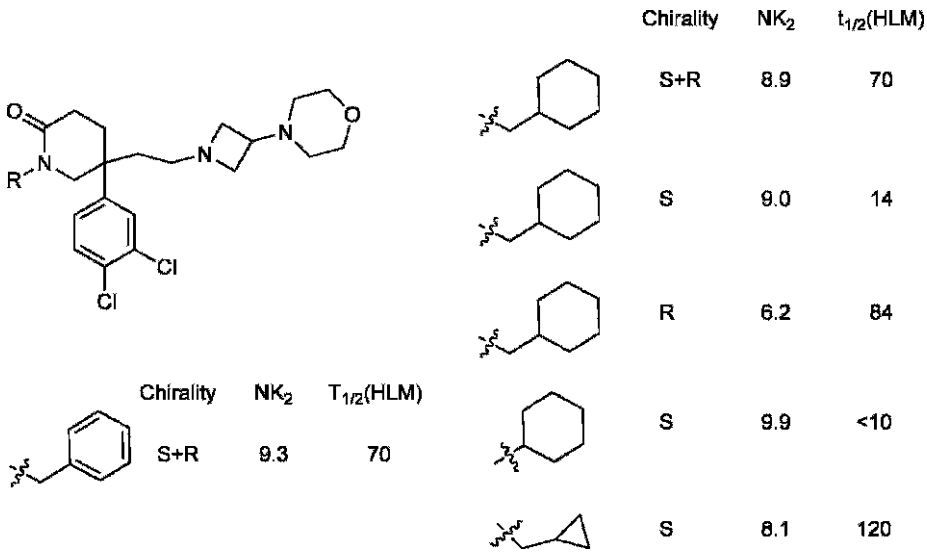


Figure 11.18 ► Reduction of ring size and modification of chirality can increase metabolic stability.

11.3.6 Change Chirality

The chirality of attached groups can affect metabolic stability. Changes in the chirality of the series analogs in Figure 11.18 lead to a significant improvement in metabolic stability.[12] This suggests that the enantiomers bound differently to the metabolic enzyme.

11.3.7 Reduce Lipophilicity

A decrease in lipophilicity often will improve metabolic stability. This reduces binding to metabolic enzymes, which typically have lipophilic binding pockets. The example in Figure 11.19 demonstrates how reduced Log D can lead to improved metabolic stability.[13]

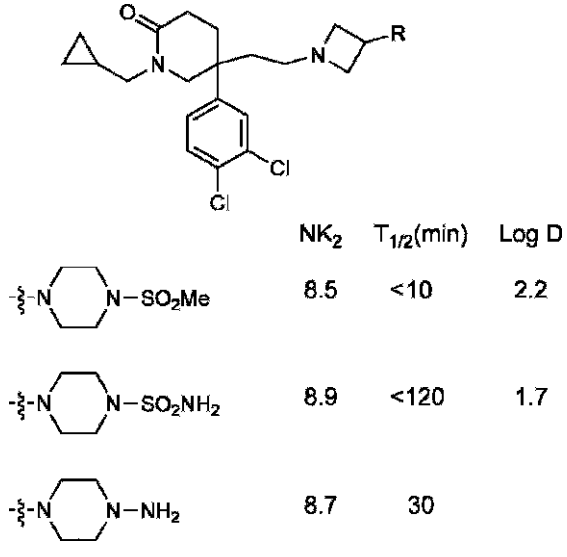


Figure 11.19 ► Reducing the lipophilicity of the R group improved metabolic stability.

11.3.8 Replace Unstable Groups

Groups that cause considerable metabolism of the lead can be substituted to achieve improved stability. The compounds in Figure 11.20 that contained a piperidine group were metabolically unstable. Substitution with a piperazine greatly improved metabolic stability[13].

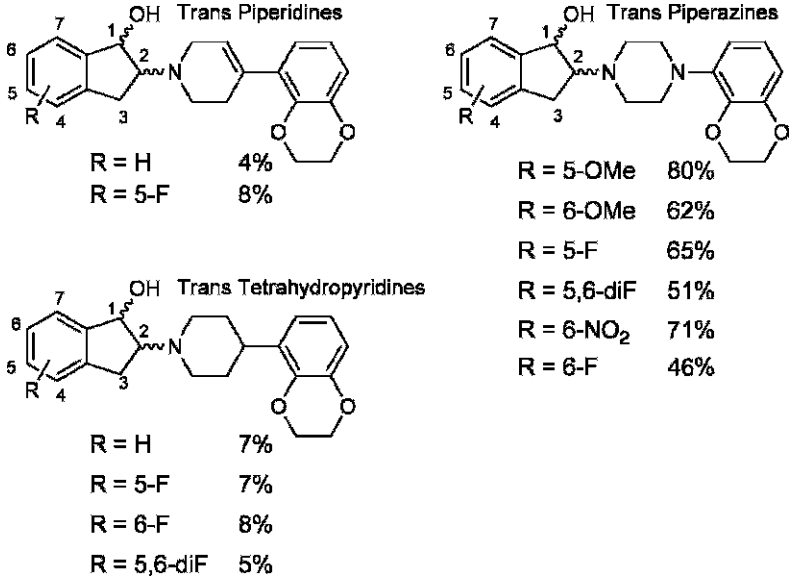


Figure 11.20 ► Substitution of piperazine for piperidine improved the metabolic stability for this series, resulting in increased %F.

The metabolic stability of tiamulin (Figure 11.21) was improved by substitution of the side chain with the carbamate-containing side chain.[14] The new compound has excellent broad-spectrum antibacterial activity and 10-fold slower metabolism.

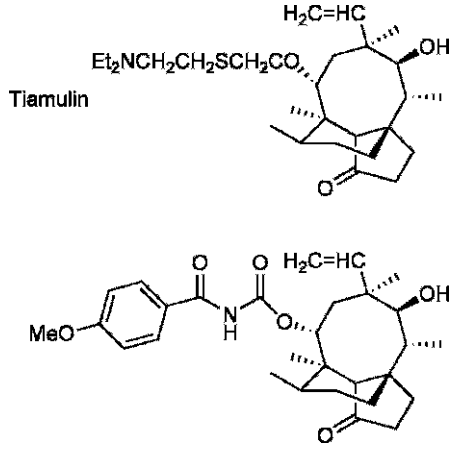


Figure 11.21 ► Substitution of the carbamate side chain improved the metabolic stability while maintaining good antibacterial activity.

11.4 Structure Modification Strategies for Phase II Metabolic Stability

Phase II reactions can be reduced through structure modification (Table 11.4).

TABLE 11.4 ► Structure Modifications Strategies for Phase II Metabolic Stability Improvement

Structure modification	Section
Introduce electron-withdrawing groups or steric hindrance	11.4.1
Change phenolic hydroxyl to cyclic urea or thiourea	11.4.2
Change phenolic hydroxyl to prodrug	11.4.3

11.4.1 Introduce Electron-Withdrawing Groups and Steric Hindrance

Addition of electron withdrawing groups on the aromatic ring will reduce the rate of glucuronidation of a phenol. Figure 11.22 shows an example of the addition of a chlorine atom to the phenol adjacent to the hydroxyl. This modification reduced glucuronidation by reducing the reactivity of the phenol and by steric hindrance.

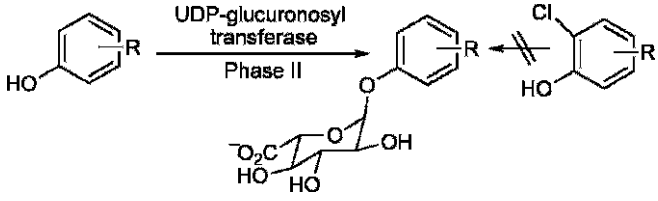
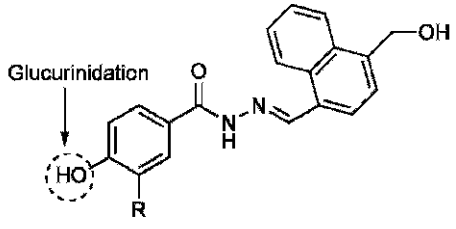
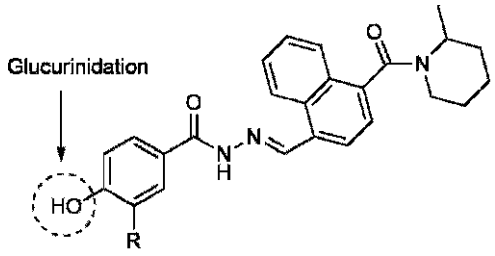


Figure 11.22 ► Addition of a halogen to a phenolic compound reduces glucuronidation by electron withdrawal and steric hindrance.



R	hGluR binding affinity (nM)	Metabolism Clearance (pmol/min/mg)
Cl	41	75
F	29	89
2,3-di-Cl	54	103
CN	30	37

Figure 11.23 ► Addition of a cyano group to the phenolic compound reduced glucuronidation while increasing activity.



R	hGluR binding affinity (nM)	Metabolism Clearance (pmol/min/mg)
Cl	8	267
CN	12	65

Figure 11.23 ► *Continued*

Addition of a cyano group to the phenolic ring adjacent to the hydroxyl will also reduce glucuronidation. Figure 11.23 shows examples of two phenolic groups whose glucuronidation was reduced while improving target activity.[15]

11.4.2 Change Phenolic Hydroxyl to Cyclic Urea or Thiourea

A phenolic group can be replaced with an isostere to reduce glucuronidation. The lead on the left in Figure 11.24 was modified by the addition of a cyclic urea or thiourea. The metabolic stability increased (as indicated by the improved F and AUC) while the activity was maintained.[16]

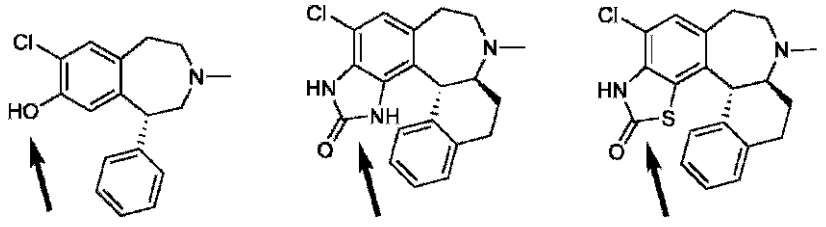


Figure 11.24 ► Isosteric replacement of the phenolic hydroxyl improved metabolic stability against glucuronidation.

11.4.3 Change Phenolic Hydroxyl to Prodrug

A phenolic hydroxyl can be modified to a prodrug (Figure 11.25).[17] The pro-moiety is slowly hydrolyzed to release the free phenol, after it has bypassed first-pass metabolism.

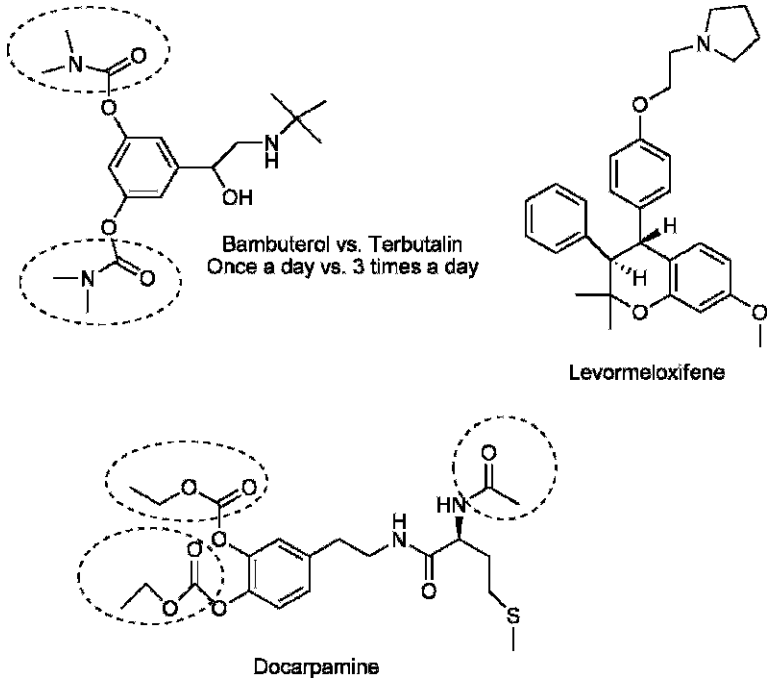


Figure 11.25 ► Phenols can be modified to form prodrugs. The free phenol is slowly released.

11.5 Applications of Metabolic Stability Data

In vitro metabolic stability data are often used to:

- ► Guide structure modifications to improve stability
- ► Select the optimal compound(s) for in vivo PK or activity testing
- ► Prospectively predict in vivo PK performance
- ► Retrospectively diagnose the root causes of poor in vivo PK

Metabolic stability plays a major role in drug clearance. Schemes for the application of metabolic clearance to in vivo PK clearance and bioavailability are shown in Figure 11.26.[3]

Metabolic stability is commonly measured in vitro during drug discovery as soon as a new compound is synthesized. This provides feedback that alerts the project team to metabolic limitations and provides data to guide metabolic stability improvement through structural modifications.

The rate of metabolism of a compound by different metabolizing enzymes is useful to know. During the lead optimization phase, this information can be combined with knowledge of the particular enzyme's substrate specificity. This can guide structural modifications to reduce metabolism. During late discovery, knowledge of the primary metabolizing enzymes can suggest issues with drug–drug interactions (see Chapter 15). When the in vitro experiment studies which CYP isozyme(s) metabolizes the compound, the assay

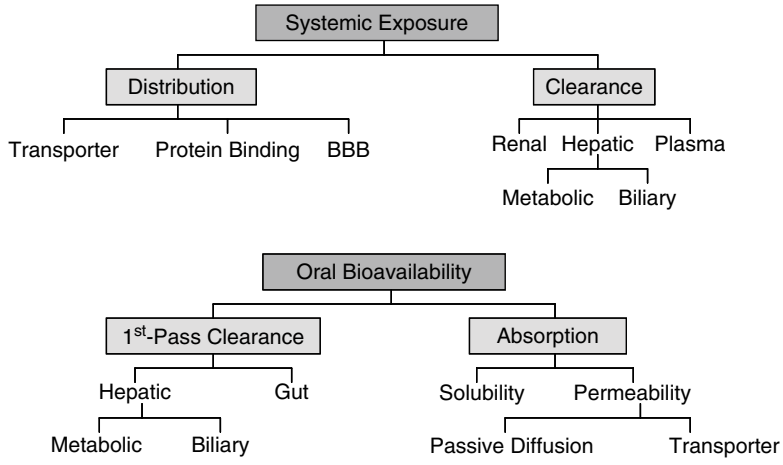


Figure 11.26 ► Schemes for the diagnosis of in vivo PK performance using metabolic clearance. (Reprinted with permission from [3].)

is termed *CYP phenotyping*. This and other metabolic stability assays are discussed in Chapter 29.

Metabolic stability data also can be beneficial in optimizing in vivo PK performance. Improvement of microsomal stability typically leads to lower in vivo Cl and higher F (Table 11.1).

Another example of the application of metabolic stability to a discovery project is shown in Figure 11.27. The compounds in the lead series for this project initially had low metabolic stability. After 3 months of structural optimization, metabolic stability was greatly improved. The keys to this success were as follows:

- ► High-throughput microsomal stability method that assayed hundreds of compounds per month for each of the company's many projects
- ► Fast turnaround of data (1–2 weeks)
- ► Company commitment to parallel optimization of activity and properties

Start, $t_{1/2}$ (min)	
Rat	Mouse
5	10
7	7
5	5
7	8
3	2
8	5
5	3

3 months later, $t_{1/2}$ (min)		
Rat	Mouse	Human
>30	12	>30
>30	29	>30
20	10	18
>30	14	>30
12	30	>30
6	10	>30
>30	13	>30

Figure 11.27 ► Data from a discovery project show that the compounds initially had low metabolic stability. Structural modifications resulted in improved metabolic stability in a short time.

A comparison of the current approach versus the traditional approach is shown in Figure 11.28. The new screening paradigm allows teams to use metabolic stability data to make informed decisions.

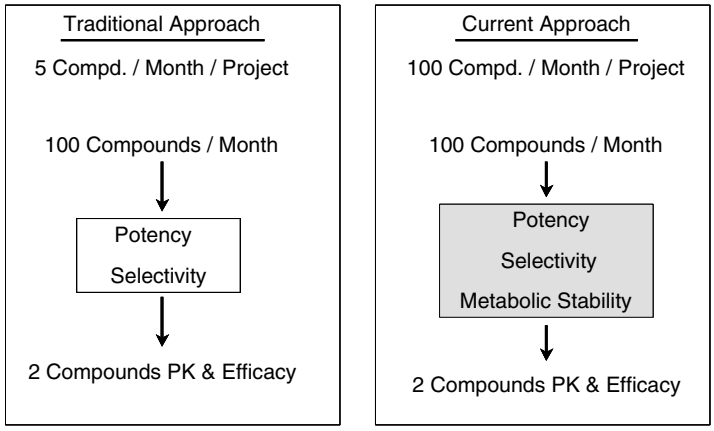


Figure 11.28 ► New approach includes metabolic stability for 100 compounds per month for each project, which enables informed decisions. (Reprinted with permission from [32].)

The crucial nature of metabolic stability and the relatively low cost of in vitro stability assays can be leveraged to assist with more expensive processes in discovery. Metabolic stability of two compounds from a series is shown Figure 11.29. The *R, S*, and racemic compounds in the top table all had the same microsomal stability. For the second compound in the table, the desirable *S*-enantiomer was less stable than the undesirable *R*-enantiomer. These have been consistently observed for this series of compounds. Therefore, using metabolic stability data from the racemate will not underestimate microsomal stability of the desirable enantiomer. Hence, the team uses microsomal stability data along with

Half-Life in minutes

Compounds	Rat	Mouse	Human
Racemate	3	5	18
S	5	4	16
R	3	6	18

Same

Compounds	Rat	Mouse	Human
Racemate	9	6	11
S	2	3	3
R	21	7	19

R > S

Figure 11.29 ► Strategy for triaging chiral compounds using an initial evaluation by the less expensive microsomal stability assay.

potency and selectivity to triage compounds for scaleup, chiral separation, and animal testing (Figure 11.30). In vitro metabolic stability data can be used to help teams make an informed decision on which compounds to take to the more expensive studies (e.g., chirals resolution).

In vitro microsomal stability data can be used to plan in vivo efficacy study designs. The compound in Figure 11.31 had a short microsomal $t_{½}$. Therefore, the in vivo study was planned for a short time window, when there would be a maximum of compound

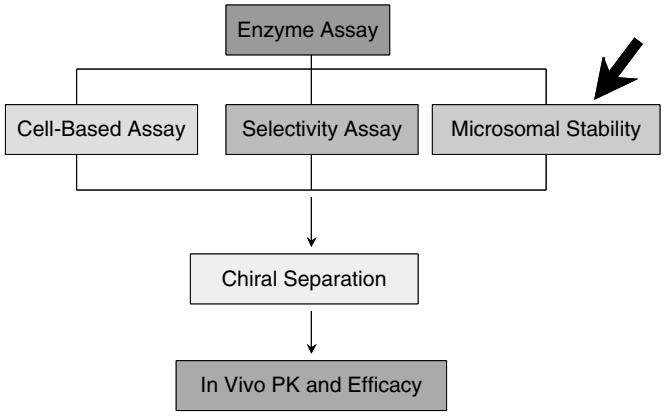


Figure 11.30 ► An efficient discovery project strategy for using microsomal stability for selection of compounds prior to expensive chiral separation and in vivo testing. (Reprinted with permission from [32].)

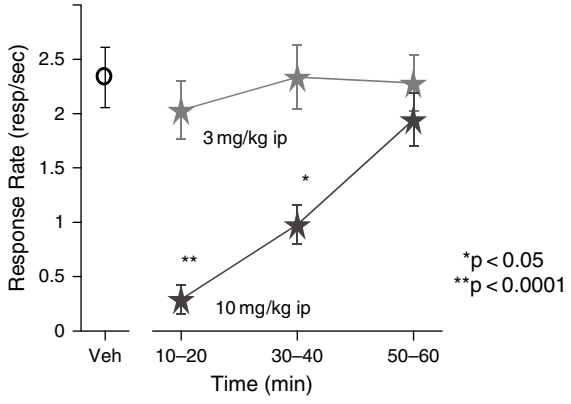


Figure 11.31 ► Microsomal stability indicated low stability, so the in vivo efficacy study time window was set to be short in this behavioral model. Efficacy was observed at 10–20 minutes and 30–40 minutes, whereas insignificant efficacy would have been observed if the window was 50–60 minutes.

concentration. The compound showed significant in vivo efficacy at early time points, which would have been missed if the time window had been 50 to 60 minutes.

An "intrinsic clearance" can be calculated using the in vitro microsomal stability. This is the predicted Cl due to hepatic metabolic reactions. The method for calculating intrinsic clearance was developed by Obach.[18]

$$Cl'_{int} = (0.693/t_{½\ microsomal}) * (\text{mL incubation/mg microsomal protein}) * (\text{mg microsomal protein/g liver}) * (\text{g liver/kg body weight}).$$

For example, if:

- $t_{½\ microsomal} = 15$ minutes
- microsomal protein concentration = 0.5 mg/mL

- mg microsomal protein/g liver = 45 mg/g
- g liver/kg body weight = 20 g/kg (human).

Then:

$$Cl'_{int} = (0.693/15\,\text{min}) * (1\,\text{mL}/0.5\,\text{mg protein}) * (45\,\text{mg protein/g}) * (20\,\text{g/kg weight}) = 83\,\text{mL/min/kg}.$$

Cl'_{int} can be useful in predicting the PK of a compound. This must be used carefully because CYP metabolism may not be the only mechanism of clearance for a compound. Clearance also may be affected by extramicrosomal metabolism, renal clearance, biliary extraction, and hydrolysis in plasma or intestine. Examples of the classification of clearance values are given in Table 11.5.

TABLE 11.5 ► Example of PK Clearance Classifications

Species	HBF	Low Cl (20% HBF)	High Cl (80% HBF)
Mouse	90	18	72
Rat	55	11	44
Monkey	44	9	35
Human	21	4	17

All values are given as mL/min/kg. Cl, Clearance; HBF, hepatic blood flow.

11.6 Consequences of Chirality on Metabolic Stability

Chirality can greatly affect the metabolic stability of compounds. This is because enantiomers bind with different affinity and orientation to metabolizing enzymes. This affects both phase I and II metabolic enzymes. Up to 75% of all chiral drugs show stereoselective metabolism. Differential hepatic clearances are shown for several drugs in Table 11.6. Verapamil clearance shows difference between enantiomers. This is further exemplified

TABLE 11.6 ► Stereospecific Hepatic Metabolism

	Clearance (L/min)		
Drugs	(R)	(S)	Ratio
Propanolol (IV)	1.21	1.03	1.2
Propanolol (PO)	2.78	1.96	1.4
Verapamil (IV)	0.80	1.40	1.8
Verapamil (PO)	1.72	7.46	4.3
Warfarin (PO)	0.23	0.33	1.4
Propafenone (PO)	13.5	32.6	2.4

by the large stereospecific effect between routes of administration, owing to first-pass metabolism following oral administration. Stereoselective metabolism of various compounds is shown in Figures 11.32, 11.33, and 11.34.[19–22] Another example is terbutaline, which has a 1.9 enantiomeric ratio (+/–) for metabolism in the small intestine.

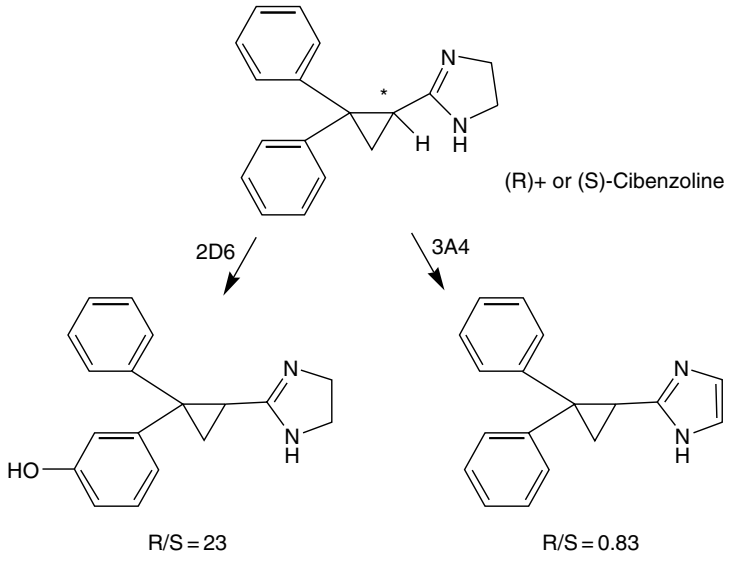


Figure 11.32 ► Stereoselective metabolism of cibenzoline.[19]

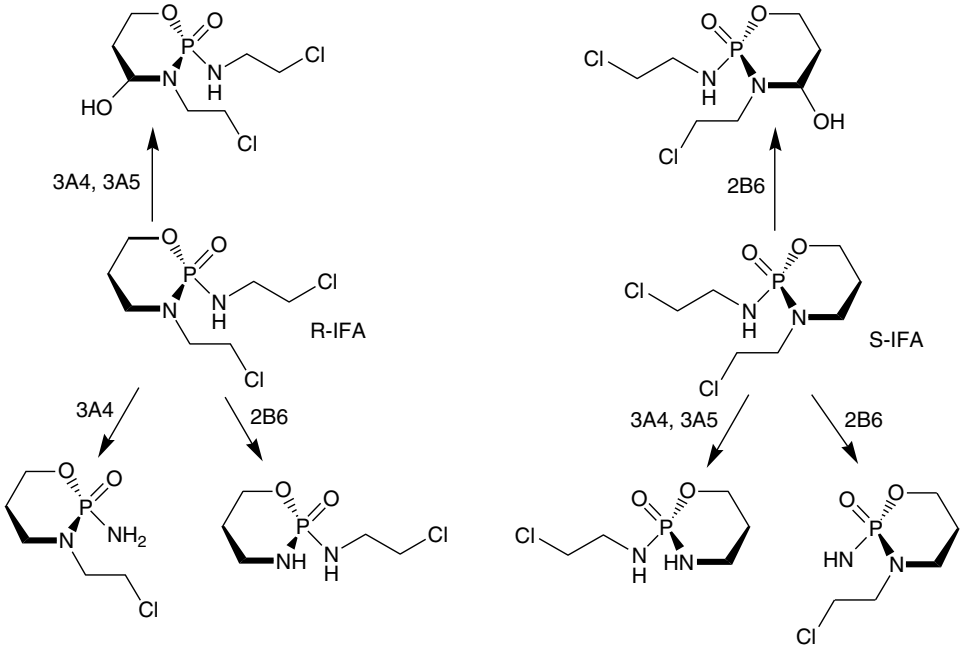


Figure 11.33 ► Stereoselective pathway of ifosfamide metabolism.[20,21]

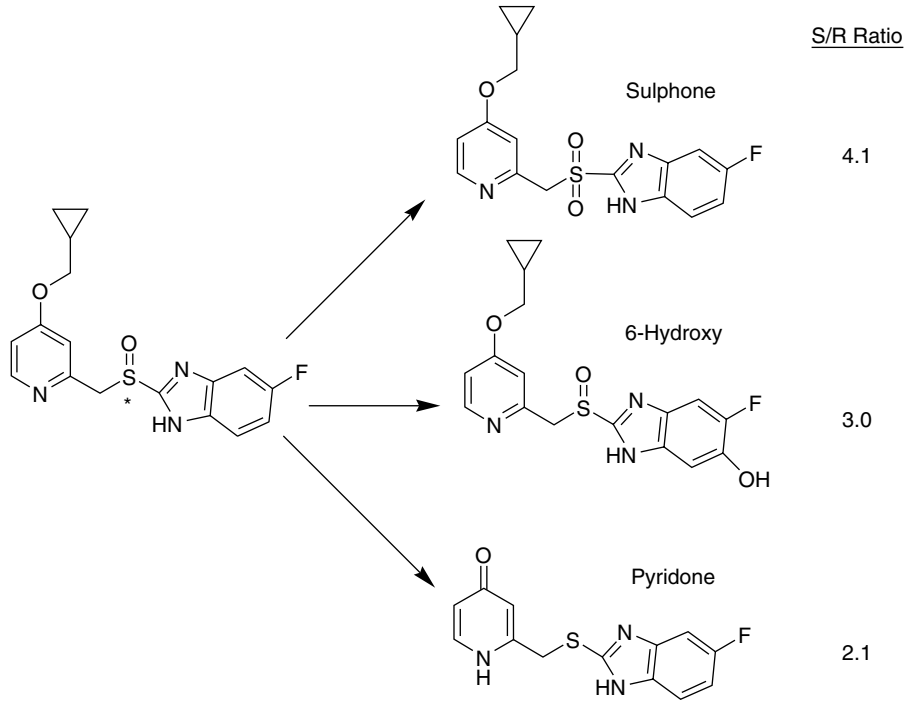


Figure 11.34 ► Stereoselective metabolism by human liver microsomes of a substituted benzimidazole.[22]

11.7 Substrate Specificity of CYP Isozymes

Often a drug is metabolized by more than one CYP isozyme. However, different CYP isozymes have differences in the characteristics of the drugs they tend to metabolize. These differences are listed in Table 11.7.[23] Insights on the structural features that enable binding and the likely sites of oxidation are useful in designing molecules with enhanced metabolic stability.

TABLE 11.7 ► Characteristics of CYP Isozyme Substrates

CYP	Range of Log P	Other characteristics	Typical substrate
3A4	0.97 to 7.54	Large molecules	Nifedipine
2D6	0.75 to 5.04	Basic (Ionized)	Propranolol
2C9	0.89 to 5.18	Acidic (Nonionized)	Naproxen
1A2	0.08 to 3.61	Planar amines and amides	Caffeine

11.7.1 CYP1A2 Substrates

Example CYP1A2 substrates are shown in Figure 11.35. CYP1A2 tends to catalyze the metabolism of planar amines and amides.

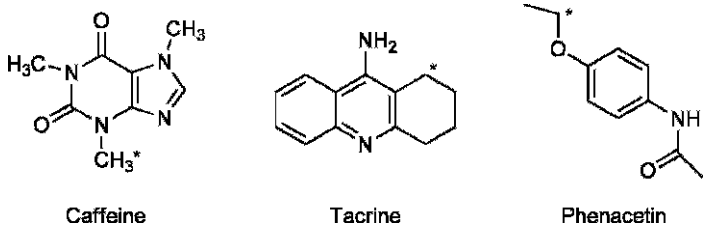


Figure 11.35 ► Example CYP1A2 substrates. The major site of metabolism is labeled.

11.7.2 CYP2D6 Substrates

CYP2D6 tends to metabolize medium size basic amines.[24] The SAR for the active site is shown in Figure 11.36. CYP2D6 substrates have:

- ► ≥1 basic nitrogen atom
- ► Flat hydrophobic area (e.g., planar aromatic) at or near the site of oxidation
- ► 5–7 Å from the basic nitrogen (charged at pH 7.4) to the site of oxidation
- ► Negative molecular electronic potential above the planar part

The cation binds strongly to the anionic aspartic acid in the active site.

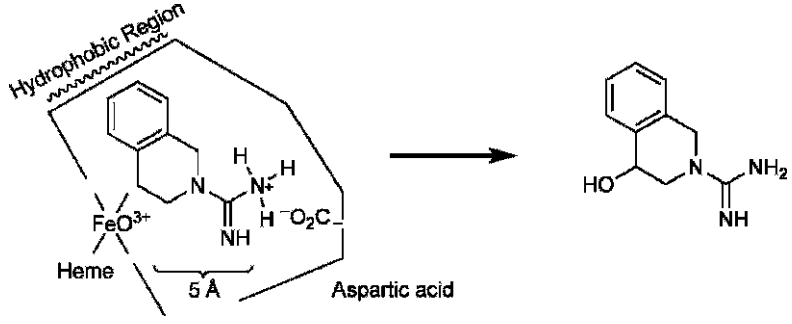


Figure 11.36 ► SAR of the active site for CYP2D6. The site of oxidation is 5–7 Å from the basic nitrogen.[24]

Examples of CYP2D6 substrates are shown in Figure 11.37. A large number of drugs have basic nitrogen atoms. For this reason, CYP2D6 metabolizes about 30% of commercial drugs despite its relatively low abundance in the liver (~2%). This low abundance may be saturable and result in a nonlinear increase in drug concentration with dose. Another problem is that about 7% to 10% of the white population lacks CYP2D6. For these individuals, drugs that are metabolized primarily by CYP2D6 are not cleared as fast and can build up to toxic concentrations.

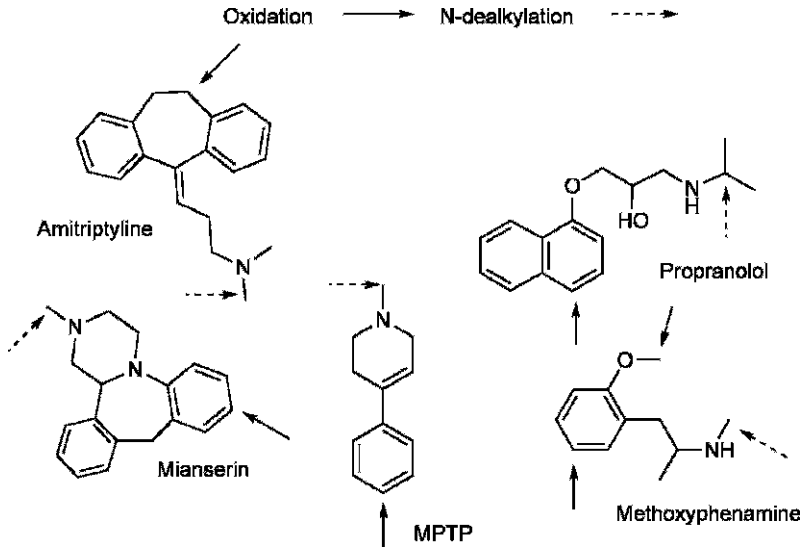


Figure 11.37 ► Examples of CYP2D6 substrates. The typical distance between the basic nitrogen and the site of oxidation is 5–7 Å. The major sites and types of metabolism are labeled.[33]

11.7.3 CYP2C9 Substrates

Substrates for CYP2C9 are less strictly defined. They are characterized as having the following[25–27]:

- ► Large dipole or negative charges
- ► Oxygen-rich (with a carboxylic acid (e.g., nonsteroidal antiinflammatory drugs, sulfonamide, alcohol)), hydrogen-bond acceptors
- ► Aromatic ring or lipophilic interaction

The SAR of the active site is shown in Figure 11.38 and indicates the relationships of atoms in the active site. Examples of CYP2C9 substrates are shown in Figure 11.39.

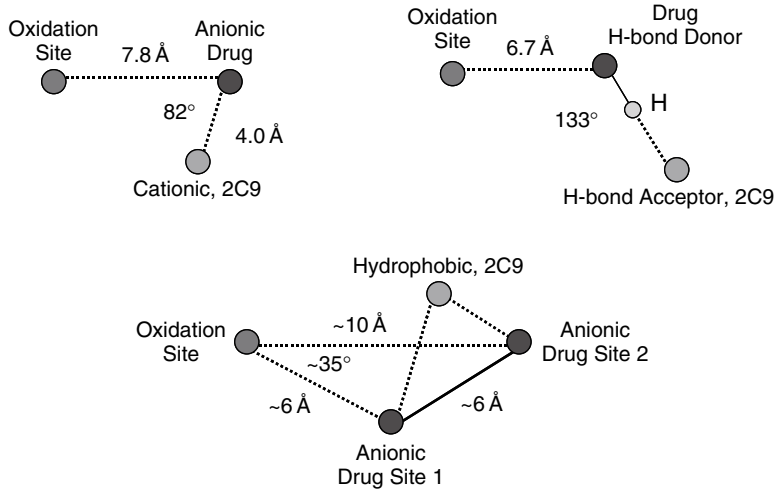


Figure 11.38 ► SAR/active site for CYP2C9, indicating substrate specificity.[27]

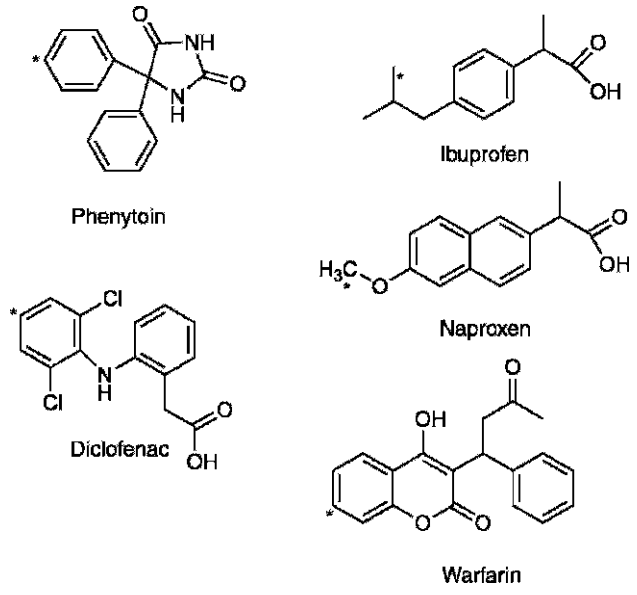


Figure 11.39 ► Examples of CYP2C9 substrates.[25–27] Compounds typically are carboxylic acids or are oxygen-rich (hydrogen-bond acceptors). The major site of metabolism is labeled.

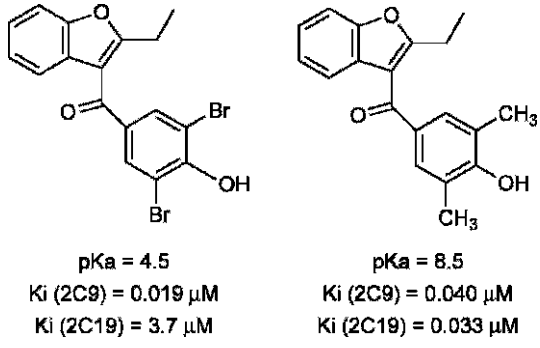


Figure 11.40 ► CYP2C9 and CYP2C19 have similar binding specificity. CYP2C19 prefers neutral substrates.[28]

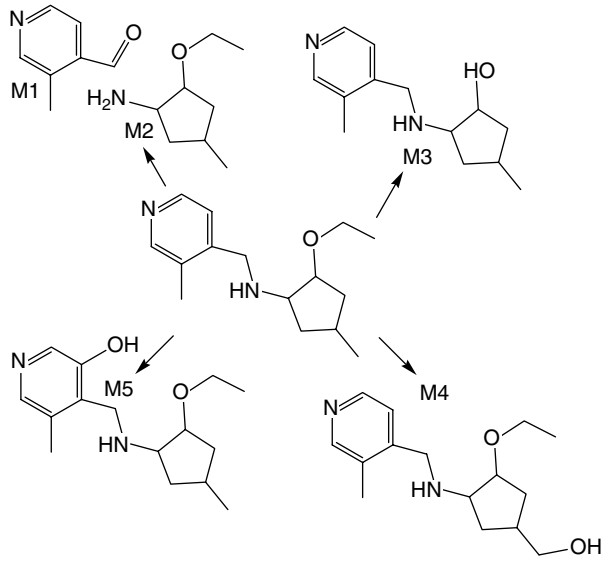
CYP2C9 and CYP2C19 have 91% sequence homology. CYP2C9 has both neutral and anion binding sites. CYP2C19 prefers neutral substrates. The examples in Figure 11.40 [28] show how acidic compounds bind more weakly to CYP2C19.

Problems

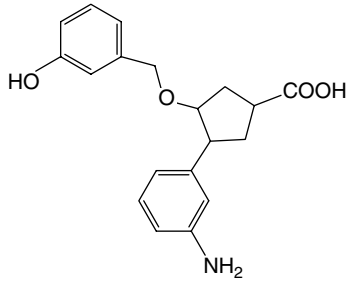
(Answers can be found in Appendix I at the end of the book.)

1. List four types or locations of decomposition/metabolism reactions in living systems.
2. Metabolic reactions make the molecule: (a) more chemically stable, (b) more permeable, (c) more polar, (d) less toxic?

3. Two important aspects of metabolic reactions are: (a) compound solubility, (b) binding to the metabolic enzyme, (c) rotatable bonds, (d) reactivity at molecule position adjacent to the active site?
4. Define metabolic switching.
5. List some major phase I metabolic reactions.
6. List some major phase II metabolic reactions.
7. In vitro metabolic stability is an important property in determining which of the following PK parameters?: (a) C_O, (b) volume of distribution, (c) clearance, (d) t_{max}, (e) bioavailability.
8. The following compound undergoes the major metabolic reactions shown. How could the compound be structurally modified to improve metabolic stability?



9. Which sites on the following molecule might undergo phase II metabolism? What reactions might occur?



10. What can metabolic stability data be used for?: (a) select compounds for PK studies with highest chance of good bioavailability, (b) guide synthetic modification for stability

improvement, (c) diagnose cause of low bioavailability, (d) guide synthetic modification to increase permeability, (e) select compounds for more expensive in vivo efficacy studies.

11. Which of the following structural modifications may increase metabolic stability?: (a) add F or Cl at a site of metabolism, (b) remove labile groups, (c) add hydroxyl groups, (d) cyclization at labile site, (e) reduce ring size, (f) add steric hindrance at labile site, (g) add lipophilic groups.

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Chapter 12

Plasma Stability

Overview

- *Compound decomposition can be catalyzed in plasma by hydrolytic enzymes.*
- *Increased clearance can occur for hydrolyzable substrate compounds.*
- *Plasma stability increases with steric hindrance, electron-withdrawing groups, or replacement with a less reactive group.*

Compounds with certain functional groups can decompose in the bloodstream. Unstable compounds often have high clearance and short $t_{½}$, resulting in poor in vivo pharmacokinetics (PK) and disappointing pharmacological performance. Plasma degradation clearance can be overlooked if discovery project teams focus on microsomal stability. Microsomal enzymes are different than plasma enzymes. Stability in liver microsomes does not imply stability in plasma. Instability in plasma also can cause erroneous PK assay results if the compound degrades in the plasma sample after it is taken from the animal or if quantitative analysis standards are prepared in plasma and degrade. Pharmaceutical companies typically do not develop clinical candidates that are unstable in plasma, unless they are prodrugs or antedrugs. Therefore, it is important for discovery scientists to anticipate and assess this issue early. Plasma degradation can be used to advantage in the development of prodrugs and antedrugs.

12.1 Plasma Stability Fundamentals

Blood contains a large number of hydrolytic enzymes, such as cholinesterase, aldolase, lipase, dehydropeptidase, alkaline and acid phosphatase.[1] The amount of each enzyme is dependent on species, disease state, gender, age, and race.[2] If the compound has affinity for one of these enzymes and it has a hydrolyzable group in the right position, it can be decomposed in the plasma. Many such groups are used to enhance the compound's pharmacological activity at the target protein; thus, medicinal chemists may be reluctant to remove or replace them. However, hydrolysis in plasma can be a major cause of compound clearance, and pharmacologically efficacious concentrations may not be achievable in vivo. For this reason, it is important to assess the liability of potentially unstable moieties in the project's lead series during an early discovery stage and either modify or deprioritize the series before a large amount of effort is expended on activity optimization.

Several functional groups are susceptible to plasma degradation and include the following:

- Ester
- Amide

- Carbamate
- Lactam
- Lactone
- Sulfonamide

Leads containing these groups, especially peptides and peptide mimetics, should be tested for plasma stability.

12.1.1 Consequences of Chirality on Plasma Stability

Plasma stability is affected by chirality, owing to the differential binding of enantiomers to plasma enzymes. For example, in Figure 12.1 the hydrolysis rate constant for *O*-acetyl propranolol is affected by the stereochemistry.[3]

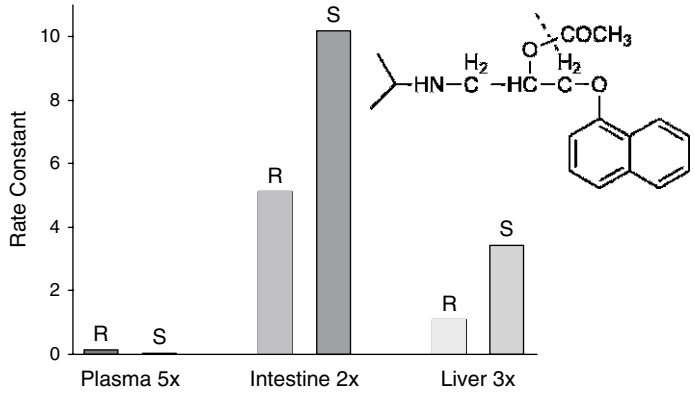


Figure 12.1 ► Hydrolysis rate constant of *O*-acetyl propranolol is affected by chirality.[3]

12.2 Effects of Plasma Stability

Medicinal chemists can take advantage of plasma reactions as part of a prodrug approach. In order to enhance permeation or metabolic stability, a prodrug strategy can be applied. Typically, esters are prepared. The prodrug enhances permeability or metabolic stability so that high concentrations of the prodrug reach the bloodstream. In the blood, a hydrolytic enzyme cleaves the prodrug to release the active drug. Prodrugs are discussed further in Chapter 39.

Antedrugs ("soft drugs") are the opposite of prodrugs.[4,5] These drugs are active locally but rapidly degrade to an inactive compound once they reach the bloodstream. The purpose of this action is to reduce side effects by minimizing the systemic toxicity of the drug. Examples of antedrugs are shown in Figure 12.2.[5] Ciclesonide works locally at the lung for treatment of asthma and chronic obstructive pulmonary disease. Fluocortin-butyl is applied topically as an antiinflammatory agent. Loteprednol etabonate is used as local treatment of eye inflammation. These are esters for local delivery and are readily inactivated systemically through hydrolysis.

In selecting project compounds for in vivo study, plasma stability data can be used to indicate which compounds are most likely to be stable and succeed. For lead series that have a liability for stability, these data can assist selection of the most stable compounds.

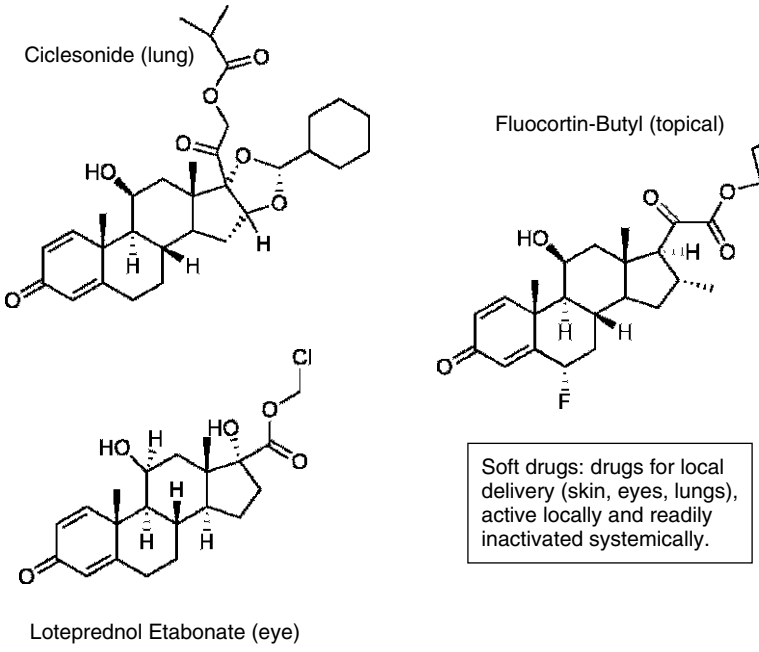
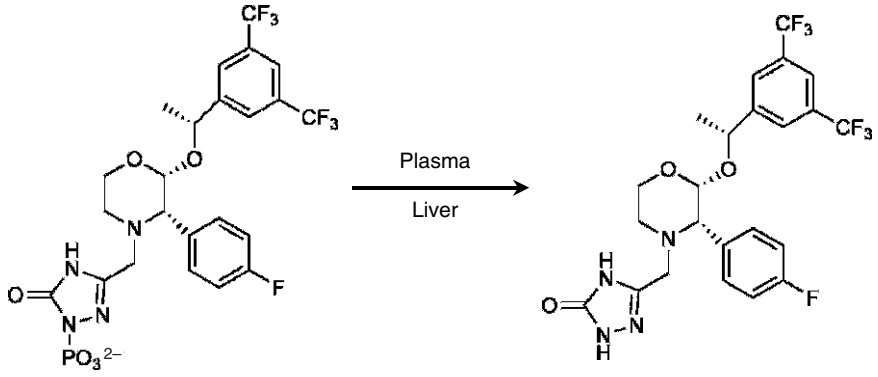


Figure 12.2 ► Antiinflammatory antedrugs (soft drugs).

Plasma stability can vary greatly among species, which makes difficult the prediction of human clinical outcomes of prodrugs from animal data. The plasma stability of the compound in Figure 12.3 increased in stability according to the following species order: rat < dog < human.[6] Typically, compounds are less stable in rodents than in humans.



Time	Rat (μM)		Dog (μM)		Human (μM)	
(min)	Prodrug	Parent	Prodrug	Parent	Prodrug	Parent
0	23	0.6	26	1.2	26	0.8
15	18	5.8	26	1.8	27	1.0
30	13	8.0	25	2.4	26	1.1
60	6.4	11	24	3.9	26	1.4
120	1.9	15	20	6.0	26	2.3

Figure 12.3 ► Species differences in plasma stability.

12.3 Structure Modification Strategies to Improve Plasma Stability

Strategies for improving plasma stability are listed in Table 12.1.

TABLE 12.1 ► Structure Modifications Strategies for Plasma Stability Improvement

Structure modification	Section
Substitute an amide for an ester	12.3.1
Increase steric hindrance	12.3.2
Add electron-withdrawing groups to decrease for antedrug	12.3.3
Eliminate the hydrolyzable group	

12.3.1 Substitute an Amide for an Ester

Amides are more stable against plasma hydrolysis than are esters in the same position. For example, in Figure 12.4, the ester had a half-life <1 minute.[7] An amide at that position increased the half-life to 69 hours. The amide retained the activity of the ester. An ether or amine at the same position, although very stable, greatly reduced activity.

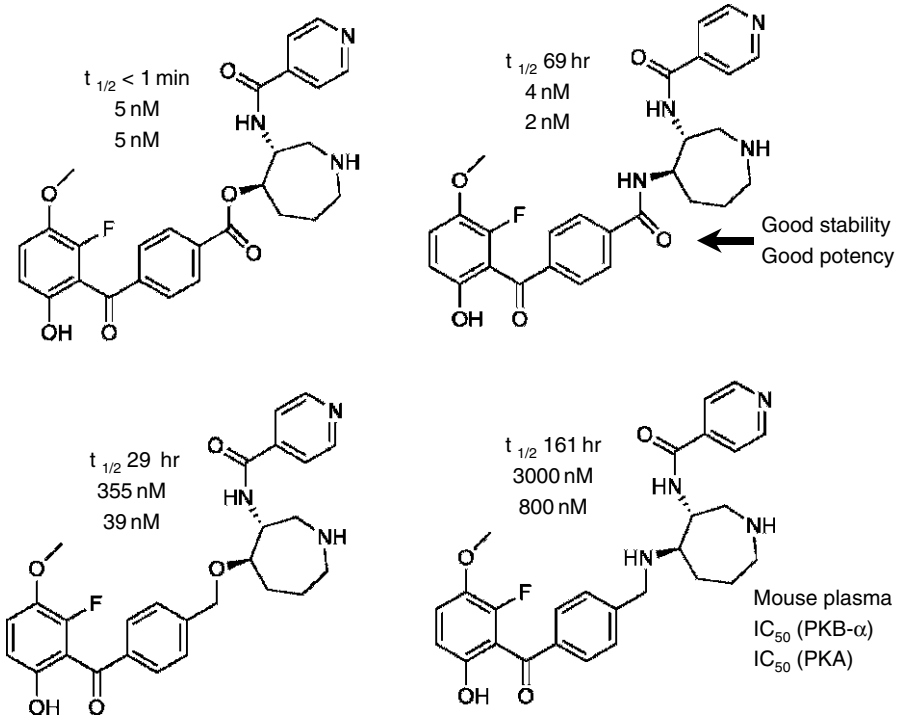
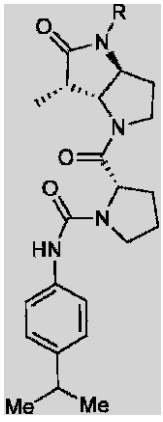


Figure 12.4 ► Substitution of an amide for an ester greatly increased plasma stability and maintained activity. (Reprinted with permission from [8].)

12.3.2 Increase Steric Hindrance

Addition of steric hindrance near a hydrolyzable group can increase plasma stability.[8] The examples in Figures 12.5 and 12.6 demonstrate the stability enhancements achieved with addition of consecutively more bulky *R*-groups attached to the lactam carbonyl. Increasing steric hindrance resulted in longer half-lives in plasma.



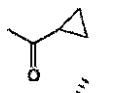
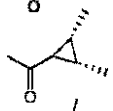
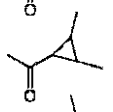
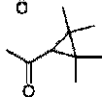
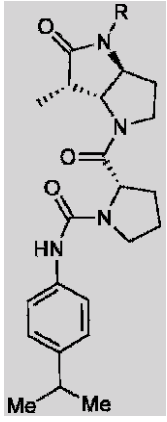
R	Human Plasma Stability $t_{1/2}$ (h)	HCMV Protease IC50 (μM)	HCMV Protease Ki (nM)
	0.5	0.2	2.4
	1.5	1.8	
	6	0.3	16
	16	>20	

Figure 12.5 ► Increased steric hindrance of the lactam carbonyl increased plasma stability. (Reprinted with permission from [8].)



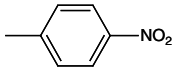
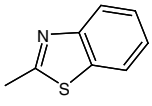
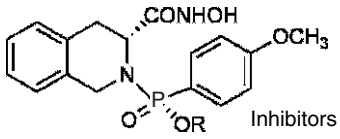
R	Human Plasma Stability $t_{1/2}$ (h)	HCMV Protease IC50 (μM)	HCMV Protease Ki (nM)
	>24	8.7	
	>24	1.5	446
	>24	0.18	10

Figure 12.6 ► Increased steric hindrance of the lactam carbonyl increased plasma stability. (Reprinted with permission from [8].)

12.3.3 Electron-Withdrawing Groups Decrease Plasma Stability for Antedrug

Antedrugs are purposely modified to decrease their plasma stability. This causes the drugs to be cleared rapidly systemically in order to reduce side effects. Figure 12.7 shows an example of modifying the phosphonamide ester of matrix metalloproteinase inhibitors to reduce plasma stability.[4] Including electron-withdrawing groups increased the positive charge on the phosphorous atom and increased the rate of hydrolysis.



Inhibitors for MMP

R	Human Plasma Stab. %Remaining @ 60 min	IC50 (μM) HB-EGF	AR
Et	100	0.23	0.35
CH_2CH_2F	96	0.18	0.47
CH_2CHF_2	0 ($t_{1/2}$ ~ 10 min)	0.51	1.47
CH_2CF_3	0 ($t_{1/2}$ < 1 min)	0.73	0.95
$CH_2CH_2CF_3$	99	0.31	0.97

Figure 12.7 ► Introduce electron-withdrawing group to decrease plasma stability and increase clearance to avoid adverse effects using antedrug approach.

12.4 Applications of Plasma Stability Data

Plasma stability data are used for many purposes in drug discovery.[9] Some of those applications are discussed here.

12.4.1 Diagnose Poor In Vivo Performance

Sometimes the cause of poor in vivo performance of a compound (e.g., low area under the curve, short $t_{½}$, high clearance) cannot be attributed to low metabolic stability. The clearance may be due in part to low plasma stability if the compound has a group(s) that is susceptible to plasma enzyme hydrolysis. Plasma stability data can be used as a custom assay to diagnose this possible cause of poor in vivo performance. When a compound is not stable in plasma, clearance higher than hepatic blood flow can sometimes be observed.

12.4.2 Alert Teams to a Liability

Use of plasma stability as a general screen can alert teams to labile structural motifs. This allows them to recognize problems early and to fix the problem or deprioritize the compound series.

12.4.3 Prioritize Compounds for In Vivo Animal Studies

Information on the plasma stability of compounds adds to the ensemble of data for making informed decisions by teams. This information is used to prioritize compounds for in vivo studies of pharmacology and PK.

For some series of compounds, plasma stability differentiates between compounds and is an effective tool in selecting compounds for further study. For example, Figure 12.8 shows the plasma stability results for 24 of more than 200 compounds in a series.[9] In general, all of the series compounds had similar properties. However, the plasma stability of certain compounds was clearly greater than others. It would not be wise to continue work on the unstable compounds when the stable compounds offer clear advantages. These plasma stability data were combined with other potency and property data to select a small number of compounds for in vivo studies.

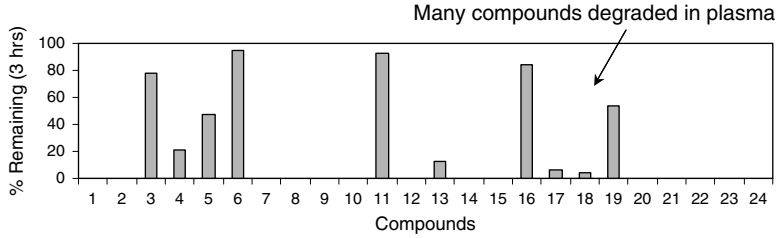


Figure 12.8 ► Plasma stability clearly differentiated series compounds for further study. (Reprinted with permission from [9].)

12.4.4 Prioritize Synthetic Efforts

The example in Figure 12.9 helped to prioritize synthetic efforts. The sulfide series was stable in plasma, but the sulfone series was unstable.[9] Based on this information, the sulfone series was terminated, and the team focused their effort on the sulfide series.

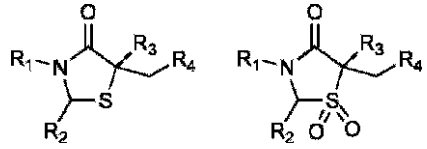


Figure 12.9 ► Plasma stability data provided key insight for continuing the series on the left and terminating the series on the right. (Reprinted with permission from [9].)

12.4.5 Screening of Prodrugs

If the discovery project team is taking a prodrug strategy, in vitro plasma stability can be measured to select a prodrug with the optimal properties. Plasma stability can be used to profile prodrugs. In the example in Table 12.2, a discovery team synthesized different diester prodrugs for the purpose of improving permeability to optimize oral bioavailability.[9] Compound 1 was too stable in plasma to be useful as a prodrug. Compound 6 was stable in gastric intestinal fluid and rapidly converted to diacid in plasma. This is a favorable profile

TABLE 12.2 ► Screening of Diester Prodrugs: Percent of Ester Observed After 3-Hour Incubation in Rat Plasma

Prodrugs	% Diester at 3 Hours	% Monoester at 3 Hours	% Diacid at 3 Hours
1	100	0	0
2	0.0	57.3	42.7
3	0.0	76.7	23.4
4	0.0	77.7	22.3
5	0.0	34.9	65.2
6	0.0	10.4	89.6
7	0.0	0.3	99.8

for a prodrug. In the same manner, antedrugs can be screened in vitro to select compounds for in vivo testing.

12.4.6 Guide Structural Modification

The structures of plasma degradation products often can be obtained using liquid chromatography/mass spectrometry. Figure 12.10 shows an example of a series containing a terminal carbamate and a cyclic carbamate.[9] Based on molecular weight, hydrolysis of the two carbamates could be easily distinguished. The terminal carbamate was found to be unstable, while the cyclic carbamate was stable. Synthetic modifications were undertaken to stabilize or replace the terminal carbamate.

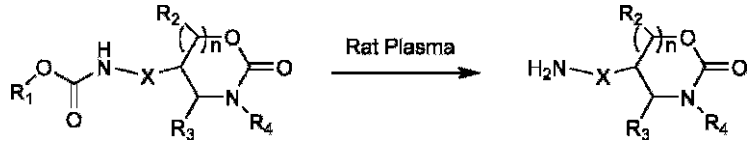
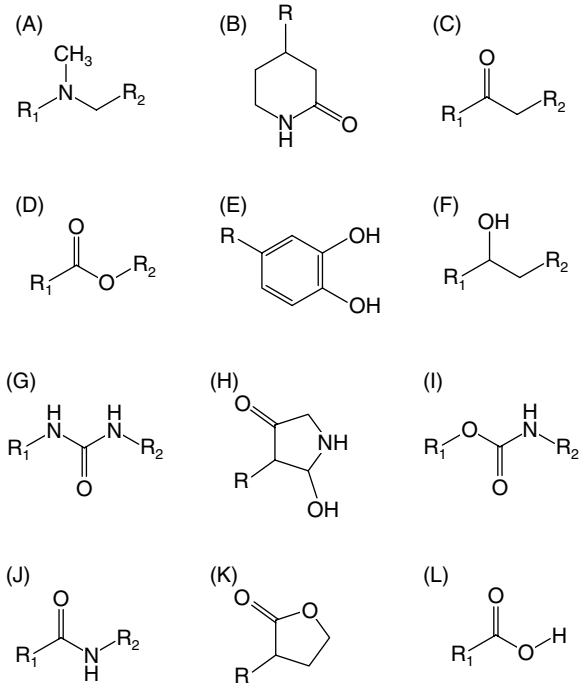


Figure 12.10 ► Identification of degradation products, in addition to quantitative determination of half-life, identified that the terminal carbamate was unstable but the cyclic carbamate was not. Information is used to guide structural modification. (Reprinted with permission from [9].)

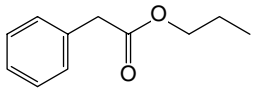
Problems

(Answers can be found in Appendix I at the end of the book.)

1. Which of the following structures might be partially or completely unstable in plasma?



2. List two applications where plasma instability is advantageous.
3. What are some structural modifications that you could try in order to improve the plasma stability of the following compound?



4. Microsomes have hydrolysis activity. Are they useful for assessing potential plasma hydrolysis?
5. Which of the following groups should alert you to potential degradation due to plasma hydrolysis?: (a) phenyl, (b) carboxylic acid, (c) ester, (d) lactone, (e) trifluoromethyl, (f) carbamate, (g) amide.

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Chapter 13

Solution Stability

Overview

- *Compounds can decompose in solution owing to pH, dosing solution excipients, solution components, enzymes, light, oxygen, or temperature.*
- *Instability in solution can cause erroneous in vitro assay or in vivo pharmacokinetic results.*
- *Stability increases by replacing the unstable group, adding steric hindrance, or electron withdrawal.*

Stability of a compound in solution is necessary for success at each stage of discovery and for development of a successful drug product.[1–6] During discovery, compounds must be stable in biological assay buffers in order to produce accurate activity data in enzyme, receptor, and cell-based assays.[7,8] For in vivo oral dosing, compounds must be stable in the acidic, basic, and enzymatic conditions of the gastrointestinal (GI) tract.[9–13] Solution stability also is crucial for prodrugs, which must be stable under certain physiological solution conditions and then hydrolyze to release the active drug under other conditions.[14–17] Parenteral drugs must be stable in the presence of formulations containing various excipients.[18,19] Despite these potential solution stability obstacles, discovery project teams may overlook the assessment of solution stability. Solution instability frequently occurs and can confuse the structure–activity relationship (SAR) and reduce in vivo performance. It is prudent for discovery teams to fully assess the potential for chemical instability of their project compounds.

13.1 Solution Stability Fundamentals

Compounds are exposed to a wide range of solutions during drug discovery. These solutions include the following:

- Organic solvent stocks
- Aqueous buffers
- Bioassay buffers
- Dosing solutions
- GI tract

The specific conditions and components of each of these solutions can cause compound instability. Some of these challenges are as follows:

- pH
- Water
- Counter ions of salts
- Solution components (e.g., dithiotheital (DTT))
- Excipients
- Enzymes
- High-performance liquid chromatography modifiers (especially if concentrated after purification)
- Temperature
- Light
- Oxygen

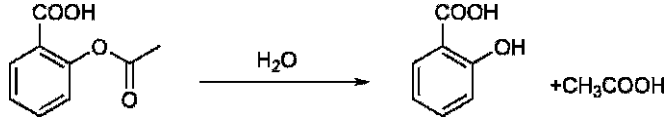
It often is assumed that compounds are stable in stock solutions of organic solvents or in aqueous buffers. Reactions can occur unexpectedly. For example, paclitaxel epimerizes in solutions that are slightly basic. Organic or aqueous buffer solutions can expose compounds to decomposition reactions induced by ambient laboratory light, elevated temperature, and oxygen absorbed from the air.

Bioassay buffers can contain components that promote compound decomposition. pH can promote hydrolysis or hydrate formation. DTT can cause reduction or react as a nucleophile. Solution components can react with discovery compounds. Furthermore, throughout the lifetime of a discovery project, several different assays (binding, cell-based functional assay, selectivity assay, etc.) are used, and each assay buffer solution may introduce new conditions or components that cause compound decomposition. This results in different stability in different assay solutions.

Excipients of dosing solutions for oral, intraperitoneal, or IV administration may promote compound decomposition. For example, lactic acid-containing dosing solutions have a low pH and could potentially cause acid-catalyzed decomposition.

In the GI tract, compounds are exposed to a wide variety of pHs. The pH ranges from acidic in the stomach and upper intestine to basic in the colon. Also, a wide array of hydrolytic enzymes are present in the GI tract, such as pepsin and pancreatin. These enzymes have the natural function of digesting macromolecules to monomers for use as nutrients, but they can also bind and hydrolyze drug compounds.

Hydrolysis probably is the most common reaction causing instability during discovery. It can occur for compounds that contain an ester, amide, thiol ester, imide, imine, carbamic ester, acetal, alkyl chloride, lactam or lactone. For example, aspirin contains an ester that can hydrolyze in water.



Hydrolysis can be catalyzed in solution by acidic or basic conditions, depending on the functional group. The rate of the reaction depends on the functional group, attached chemical constituents, physicochemical nature of the solution, and chemical and enzymatic

components in the solution. Other reactions in solution include oxidation (initiated by trace metal, light, or autoxidation), isomerization, dimerization, and racemization.

13.2 Effects of Solution Instability

Chemical instability in bioassay buffer solution reduces the compound concentration and produces decomposition products that themselves may be active. The lower concentration results in a lower apparent activity of the compound and produces erroneous SAR. Without stability data, the team may interpret stability differences between compounds as real activity differences. If team chemists know that a compound or series is not stable in solution, they can make chemical modifications to improve stability and produce reliable activity data. Alternately, if a buffer component or conditions of the assay are causing degradation and these conditions are not present in vivo, then it may be possible to modify the assay to be more accurate for the compound series.

Compounds can decompose as they are stored in organic solvent solutions. Oxygen, water, trace metals, and materials leached from the glass or plastic containers can react with the compound. Water is rapidly absorbed into dimethylsulfoxide (DMSO) when solutions are exposed to the air, especially with condensations when solutions are cool from refrigerator storage. Absorption of water and residual acidic additives (e.g., trifluoroacetic acid (TFA), formic acid) from chromatographic purification promotes degradation of compound libraries dissolved in DMSO during storage [20–23]. It was found that water is more important in causing compound degradation than oxygen in DMSO stock solution [23]. Light can induce reactions to certain sensitive compounds in a solution that is exposed to laboratory light.

Instability in vivo reduces the compound's PK performance and the in vivo pharmacological activity. This may be overlooked as a cause of low bioavailability and high clearance. The compound may not achieve a sufficiently high concentration to produce in vivo efficacy or achieve pharmacological proof of concept. In drug development, formulation strategies can be developed to enhance stability of dosage forms, which are rarely applied in drug discovery.

13.3 Structure Modification Strategies to Improve Solution Stability

The structure modifications that can improve solution stability depend on the conditions and functional group (Table 13.1). Strategies for overcoming enzymatic hydrolysis are similar to improvement of plasma stability (see Chapter 12).

TABLE 13.1 ► Structure Modifications Strategies for Solution Stability Improvement

Structure modification	Section
Eliminate or modify unstable group	13.3.1
Add an electron-withdrawing group	13.3.2
Isosteric replacement of labile functional group	13.3.3
Increase steric hindrance	13.3.4

13.3.1 Eliminate or Modify the Unstable Group

If the labile functional group does not contribute to binding at the therapeutic target's active site, it can be eliminated without significant loss of activity. Figure 13.1 shows how

elimination of the acetal type group in artemisinin increased stability by 10-fold.[18] The lipoxin analogs in Figure 13.2 were modified to greatly improve solution stability, resulting in significant in vivo exposure improvement.[13]

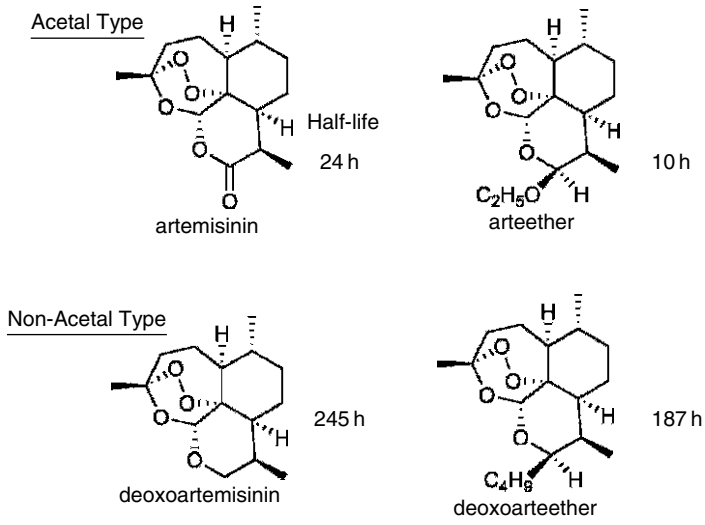


Figure 13.1 ► Improvement of acidic stability of artemisinin analogs. Conditions were pH 2 at 37°C.

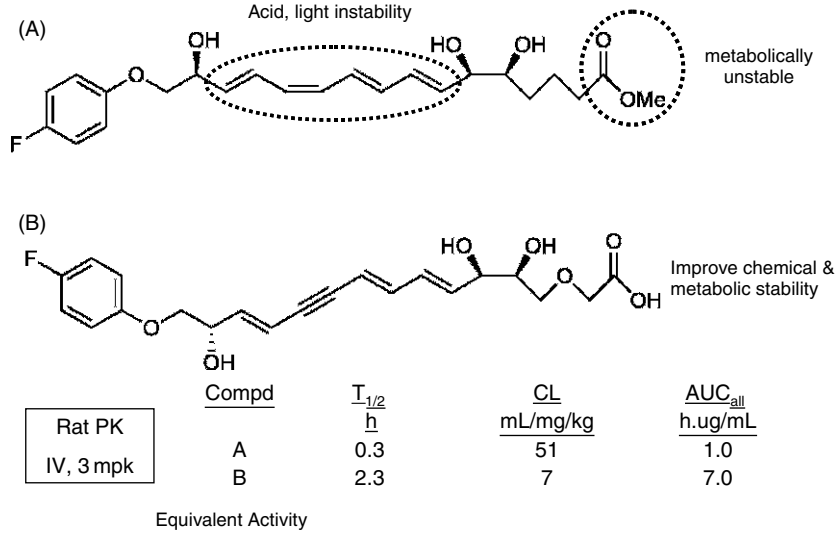
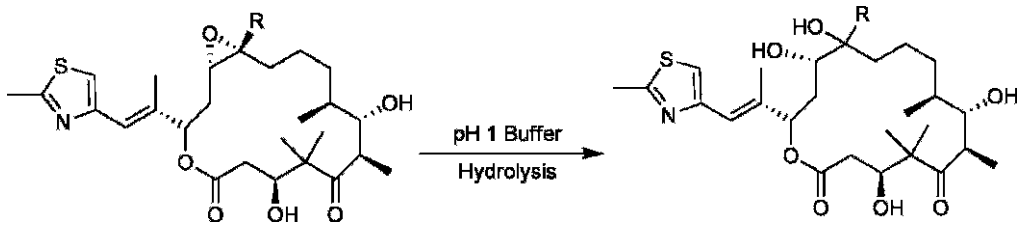


Figure 13.2 ► Modification of the conjugated section and elimination of the ester greatly improved the stability and exposure of these lipoxin analogs.

13.3.2 Add an Electron-Withdrawing Group

Addition of an electron-withdrawing group adjacent to an epoxide can reduce the reaction rate and stabilize the compound. In Figure 13.3, a cyano group was added adjacent to the epoxide, resulting in a 50-fold improvement in stability.[10]



R	$EC_{0.01}$ (tubuline)	IC_{50} (HCT-116)	T_{95} (5% Degrade)
Me (Epothilone B)	2.2 μM	4.4 nM	<0.2 hrs
CN	2.5 μM	4.1 nM	11 hrs

Electron-withdrawing R group slows down S_N1 hydrolysis

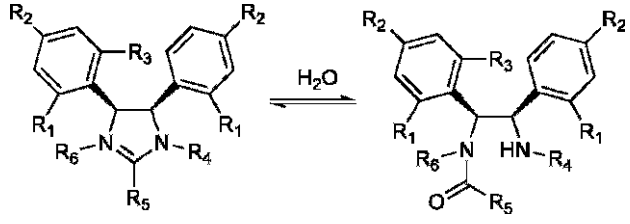
Figure 13.3 ► Addition of an electron-withdrawing cyano group reduced hydrolysis of the adjacent epoxide.

13.3.3 Isosteric Replacement of Labile Functional Group

If the functional group makes a significant contribution to the therapeutic target binding, it is important to know what aspects of the group contribute to active site interactions and attempt to find an isosteric replacement that aids binding without contributing to instability.

13.3.4 Increase Steric Hindrance

Introducing steric hindrance near the site of the reaction can reduce access of the labile functional group and increase stability. Figure 13.4 shows how stability of imidazolines improved at pH 7.4 by introducing bulky substituents adjacent to the labile group.[8] The stability of an amide was enhanced by adding steric hindrance near the amide nitrogen (Figure 13.5).[7]



Compds	R_1	R_2	R_3	R_4	R_5	R_6	half-life ($t_{1/2}$, h)	k_{obs} (h^{-1})	Rel. Act. (% 1uM)
1	Cl	OCH_3					4.58	0.198	
2		OH					6.36	0.122	0
3	Cl	OH					5.94	0.15	67
4	Cl	OH	Cl				13.41	0.052	112
5	Cl	OH			C_2H_4OH		stable	stable	20
6	Cl	OH		C_2H_5			154.03	0.004	103
7	Cl	OH		C_2H_5		C_2H_5	stable	stable	60

Figure 13.4 ► Stability of imidazolines at pH 7.4. Stability improved with increase in steric hindrance.

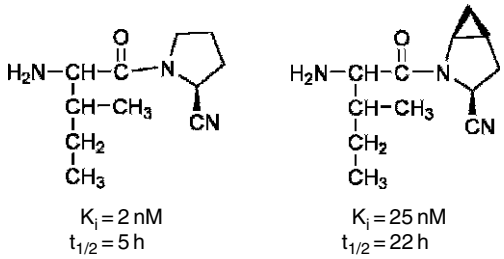


Figure 13.5 ► Dipeptidyl peptidase IV (DPP-IV) inhibitors. Chemical stability in pH 7.2 at 39.5°C. Introducing steric hindrance enhanced stability.

13.4 Applications of Solution Stability Data

Solution stability data can be used in drug discovery for many purposes.

Provide an Early Alert to Liabilities

Early knowledge that a lead series has stability limitations is valuable to a discovery team. Otherwise, the team may synthesize many series analogs that turn out to be wasted effort. Also, biological and property testing may be carried out and result in confusing conclusions and SAR. In Figure 13.6, the pH stability of a β-lactam compound is profiled using the methodology described in Chapter 31. The compound is not stable at low or high pH values. Profiling of solution stability typically is performed during the early exploratory phase to help prioritize different chemical series and alert teams to potential issues in later stages. Information on the stability of compounds at different pH values contributes to selecting the best bioassay conditions, designing synthetic strategies, developing optimal formulations, and predicting oral absorption.[14–17]

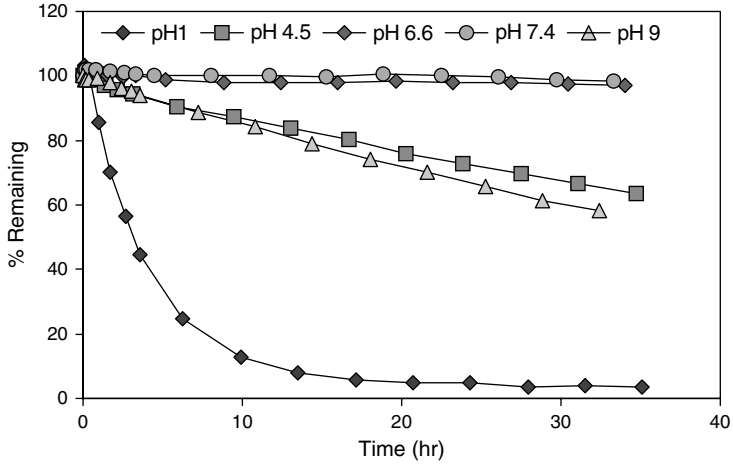


Figure 13.6 ► Stability pH profile of a β-lactam compound. (Reprinted with permission from [4].)

Selection of Conditions for Compound Purification

Early access to information on the stability of a compound at various pHs can suggest conditions for purification at which it is stable. The compound in Figure 13.7 degraded at pH 1. Therefore, acidic conditions (e.g., TFA, formic acid) should be avoided during sample purification.

Buffers	% Remaining at 24 hrs at 37° C
pH 1	14
pH 4.5	96
pH 6.6	101
pH 7.4	100
pH 9	99

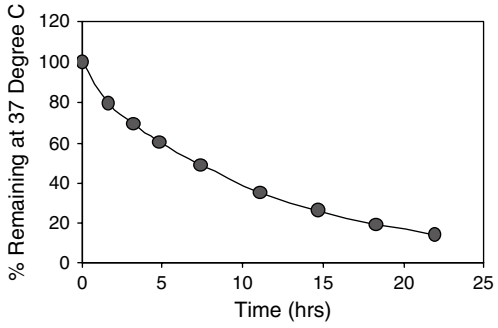


Figure 13.7 ► Screening of compound stability at different pH values.

Develop Structure–Stability Relationships

Solution stability testing of several series analogs can provide structure–stability relationships. This can indicate possible modifications to the series that improve stability.

Diagnose Poor In Vitro Bioassay Performance

A compound will not produce reliable activity in vitro if it is unstable in the bioassay buffer. The assay conditions can be altered to allow for accurate activity measurement. Figure 13.8 shows an example of bioassay buffer screening of 96 compounds. They all were from the same lead series and contained a labile functional group that was susceptible to nucleophilic attack by water. Many compounds were hydrated and rearranged. Stability screening in a 96-well format can quickly evaluate a large number of compounds to diagnose their bioassay stability.

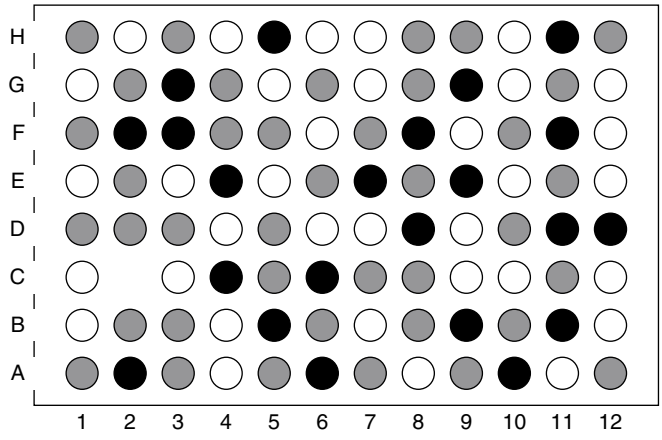


Figure 13.8 ► The stability of 96 compounds in biological assay buffer can be rapidly profiled to indicate which series analogs are stable (white), moderately stable (gray), or unstable (black).

Diagnose Poor In Vivo Performance

Poor PK or pharmacological performance can occur in vivo if the compound is not stable in the GI system. Low pH and enzymatic hydrolysis in the stomach or intestine can reduce in vivo exposure (area under the curve). Little of the dose is absorbed if the compound

is degraded in the stomach or intestine. In vitro testing with simulated gastric fluid and simulated intestinal fluid can help determine if a compound is not stable in the stomach or intestine, respectively (see Chapter 31.

Prioritize Compounds for In Vivo Animal Studies

Knowledge of the stability of compounds in simulated physiological fluids can help evaluate whether they should be tested in vivo. Comparative stability provides a foundation for decisions on which compounds to dose in vivo. An example of screening four compounds for stability in simulated physiological fluids is shown in Figure 13.9. Compounds 1 and 3 are predicted to be the most stable in the GI tract.

Compounds	Buffers	Incubation Time at 37° C	% Remaining
1	SGF	24 hr	106
	SIF	24 hr	102
2	SGF	24 hr	100
	SIF	1 hr	42
3	SGF	24 hr	102
	SIF	24 hr	100
4	SGF	15 min	0
	SIF	15 min	0

SGF: Simulated Gastro Fluid, pH 1.2 + Pepsin
SIF: Simulated Intestinal Fluid, pH 6.8 + Pancreatin

Figure 13.9 ► Compounds can be assayed for solution stability in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) prior to oral dosing.

Structure Elucidation of Solution Stability Products Guides Synthetic Optimization

Evidence for the structures of solution degradants can be readily obtained using liquid chromatography/mass spectometry. This can guide synthetic modifications at the site of the reaction.

Problems

(Answers can be found in Appendix I at the end of the book.)

1. In which of the following solutions might a compound be unstable?: (a) stomach fluid, (b) enzyme assay media, (c) high-throughput screening buffer, (d) pH 7.4 buffer, (e) animal gavage dosing solution, (f) ethanol stock solution, (g) cell assay buffer.
2. If a compound is unstable in solution, wouldn't it be best to eliminate it from further study?

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Plasma Protein Binding

Overview

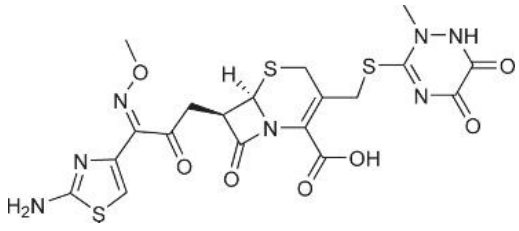
- *Compounds can bind to albumin, α_1-acid glycoprotein, or lipoproteins in blood.*
- *Binding reduces free drug in solution for penetration into tissue to reach the therapeutic target or to the liver and kidney for elimination.*

A majority of pharmaceutical treatment strategies use the bloodstream to deliver the drug to disease targets. Most delivery routes, such as oral dosing, intravenous injection (IV), intraperitoneal (IP), intramuscular (IM), subcutaneous (SC), and transdermal, transfer drug into the bloodstream and distribute it to the different tissues. Once the drug molecules are in the bloodstream, they can bind to a variety of blood constituents, including red blood cells, leukocytes and platelets, as well as proteins such as albumin, α_1-acid glycoprotein (AGP), lipoproteins, erythrocytes and alpha-, beta- and gamma-globulins.[1] Plasma proteins can adsorb a significant percentage of drug molecules. Binding to plasma protein can affect the pharmacokinetics (PK) of the drug substance in tissues and blood as well as the dosing regimen for the drug product.

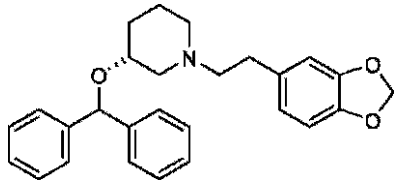
Plasma is the liquid portion of blood (not including cells). Plasma for in vitro studies is obtained when fresh blood is collected in the presence of anticoagulant (e.g., heparin) and centrifuging to remove blood cells. Plasma proteins remain with the liquid. Serum is plasma from which clotting factors (e.g., fibrinogen) have been removed. Serum is collected without anticoagulant. PK studies typically collect plasma, thus retaining both the protein-bound and unbound drug, but discarding cell-bound drug.

14.1 Plasma Protein Binding Fundamentals

Drug compound molecules that are dissolved in the blood are in equilibrium with plasma proteins.[1] The interaction of drug molecules with plasma proteins is electrostatic and hydrophobic. The binding usually is rapid, with an average equilibrium time of 20 ms. The available binding sites on plasma proteins can be saturated. Plasma protein binding (PPB) is reversible. It can vary among species. Plasma protein concentrations can vary in different disease states or with age.[2–4] Figure 14.1 shows species differences in PPB. Ceftriaxone has very different protein binding for human and dog plasma, with 4.6-fold higher percent bound in human.[5] The percent of zamifenacin unbound in human is 10- and 20- fold lower than in dog and rat, respectively. This results in lower clearance in human and C_{max} (total of drug bound to plasma proteins and unbound) in human that is 40 and 74 times higher than in dog and rat.[6] It is important to determine PPB across different species to establish safety margins for human exposure and doses for clinical trials.[6] Subtle concentration variations occur between human individuals. These variations can sometimes affect PK. In most cases, however, changes in free drug concentration do not have a significant impact on



Ceftriaxone
Human Plasma Protein Binding = 90.8% Bound
Dog Plasma Protein Binding = 19.6% Bound



Zamifenacin
Human Plasma Protein Binding = 0.01% Unbound
Dog Plasma Protein Binding = 0.10% Unbound
Rat Plasma Protein Binding = 0.20% Unbound
C_{max} in Humans: 40 and 74 Times Higher than in Dog and Rat, Respectively

Figure 14.1 ► Species differences in plasma protein binding.[5]

clinical exposure and, therefore, do not lead to changes in pharmacological or toxicological responses.[7–9]

Two plasma proteins are most responsible for binding of drug molecules: albumin, which in humans is called human serum albumin (HSA), and AGP. Some lipophilic drugs also bind to plasma lipoproteins (very-high-density lipoprotein [VHDL], high-density lipoprotein [HDL], low-density lipoprotein [LDL], very-low-density lipoprotein [VLDL]).

HSA primarily binds strongly to organic anions (e.g., carboxylic acids, phenols), but it also can bind to basic and neutral drugs. It is the most abundant protein in plasma (60% of the total plasma protein), with a concentration of 500 to 750 μM (35–50 mg/mL). Each molecule circulates throughout the body about once every minute, but of this minute it spends only 1 to 3 seconds in any particular capillary, where it can exchange transported substances with the neighboring cells.[10,11] HSA has 585 amino acids in a single polypeptide chain and a molecular weight (MW) of 66.5 kDa. An x-ray structure was obtained in 1989. HSA has at least six primary binding sites of high specificity. The most common binding sites are I and II.[12] A large number of secondary binding sites with low affinity are nonsaturable. For example, one albumin molecule can weakly bind up to 30 imipramine molecules. Binding is primarily by hydrophobic interactions. The primary natural functions of HSA are to maintain blood pH and osmotic pressure and to transport molecules throughout the body.

AGP primarily binds basic drugs (e.g., amines). It also binds hydrophobic compounds (e.g., steroids). Its concentration in the blood is 15 μM (0.5–1.0 mg/mL). In some disease states it can reach a concentration of 3 mg/mL. AGP consists of 181 amino acids in a single polypeptide chain and has a MW of 44 kDa. It has a very high carbohydrate content (45%) and a very acidic isoelectric point around 3. AGP has one binding site per molecule, which binds compounds primarily by nonspecific hydrophobic interactions. Its primary function is to carry steroids throughout the body.

Acidic drugs that bind to HSA can be classified into different types based on their binding characteristics (Table 14.1). These types demonstrate the wide range of binding

modes of drugs to HSA. Class I drugs, typified by warfarin and diazepam, bind tightly to HSA. There are one to three binding sites per molecule, depending on the compound, and they are saturable. Class II drugs are exemplified by indomethacin, which binds moderately to HSA and has six binding sites per HSA molecule. Phenytoin is an example of a class III drug. It has weak HSA binding but many binding sites per molecule.

TABLE 14.1 ► Classification of Acidic Drugs for HSA Binding

Types of drugs	I	II	III
Reference drugs	Warfarin diazepam	Indomethacin	Phenytoin
Binding proteins	HSA	HSA	HSA
Binding processes	Saturable	Saturable and nonsaturable	Nonsaturable
Association constant (M^{-1})	10^4-10^6	10^3-10^5	10^2-10^3
Binding sites per molecule	1 to 3	6	Many

Basic and neutral drugs that bind to HSA are shown in Table 14.2. Class IV is typified by digitoxin, which binds to HSA and is not saturable. Class V basic drugs, such as erythromycin, bind to HSA and can be saturated. Basic drug imipramine typifies class VI and can bind to HSA, AGP, and lipoproteins (HDL, LDL, VLDL). Lipoproteins tend to bind lipophilic basic and neutral compounds (e.g., probucol, etretinate). Highly water-soluble compounds typically are highly unbound (e.g., caffeine, ketamine).

TABLE 14.2 ► Classification of Non-ionized and Basic Drugs

Types of drug	IV	V	VI
Reference drugs	Digitoxin	Erythromycin	Imipramine
pK_a	–	8.8	9.5
Binding protein	HSA (NS)	HSA (NS)	HSA (NS), α_1-AGP (S), HDL (NS), LDL (NS), VLDL (NS)
Drug plasma saturation	No	Possible	Possible

14.1.1 Consequences of Chirality on PPB

Chirality affects binding of compounds to plasma proteins. Table 14.3 shows examples of stereoselectivity in PPB. This differential PPB affects compound distribution, metabolism, and renal clearance (see Section 14.2). The clinical significance is greater with higher binding and disease state. The stereoselectivity of binding also varies with animal species.

TABLE 14.3 ► Examples of Stereoselectivity in Plasma Protein Binding

	% Unbound		
Drug	+ Enantiomer	– Enantiomer	Enantiomeric ratio
Propranolol	12	11	1.1
Warfarin	1.2	0.9	1.3
Disopyramide	27	39	1.4
Verapamil	6.4	11	1.7
Indacrinone	0.3	0.9	3.0

14.2 PPB Effects

PPB impacts the PK of a drug and exposure to the therapeutic target. This section discusses various PPB effects so that drug discovery project teams can consider all of the possibilities. However, the effects of PPB can vary among compound series and project. Fraction unbound in plasma does not always correlate to in vivo PK parameters. It is advisable to consider the effects for each project and lead series in the context of the other properties of the series. Some organizations take the strategy of considering PPB as a primary property of interest, while others use PPB retrospectively to diagnose poor PK or in vivo pharmacological data.

According to the "free drug hypothesis," the drug–plasma protein complex cannot permeate through cell membranes by passive transcellular or paracellular permeation. Only free drug passes through membranes to reach tissues, and only free drug molecules are available for liver metabolism and renal excretion. Therefore, PPB can confine the compounds in the bloodstream and limit penetration of drug molecules to the target tissue to produce pharmacological effects, into other body tissues, and into clearance organs (e.g., liver, kidney).

There are two complementary factors of PPB:

- Extent of binding at equilibrium (expressed as percent bound or percent unbound in plasma [$f_{u,plasma}$], or equilibrium dissociation constant K_d)
- Rate of association and dissociation (expressed as association and dissociation rate constant k_a and k_d)

In vivo, these factors (Figure 14.2) affect the absorption, distribution, metabolism, and excretion (ADME) of drug molecules (Table 14.4).[10,13,14] If the drug molecules are highly bound (low percent unbound) and tightly bound (slow dissociation) to plasma proteins, the effects of PPB can be as follows:

- Retain drug in plasma compartment
- Restrict distribution of drug into target tissue (reduce volume of distribution [V_d])
- Decrease metabolism, clearance, and prolong $t_{½}$
- Limit brain penetration
- Require higher loading doses but lower maintenance doses

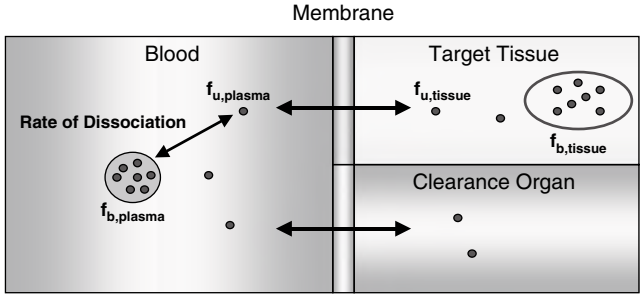


Figure 14.2 ► Penetration of drug into the target tissue or clearance organ depends on the fraction unbound in plasma ($f_{u,plasma}$).

TABLE 14.4 ► Effect of Equilibrium and Rate Constant on In Vivo ADME Properties

% Bound (equilibrium)	k_d (Dissociation rate constant)	In vivo ADME effects
High	Slow	Restrictive
High	Fast	Permissive
Low	Slow	Permissive
Low	Fast	Permissive

Thus, compounds that have high percent bound and slow dissociation may not be able to exert their therapeutic effect before being removed from the site of action by the flow of blood.[10]

On the other hand, if the compound has fast kinetics (high dissociation rate), PPB might not limit compound distribution into tissues, metabolism, excretion, or brain penetration, even if it has apparently high percent bound. Thus, high binding to plasma protein (high percent bound) alone does not itself determine the consequences of plasma binding; the on/off rate of binding can act as a major determining factor.[10]

14.2.1 Impact of PPB on Distribution

PPB can have either a "restrictive" or a "permissive" (nonrestrictive) effect on drug disposition. Examples of drugs for which PPB is restrictive or permissive are given in Table 14.5. For example, furosemide has 4% free drug, which is restrictive of the volume of distribution ($V_d = 0.2$ L/kg). Imipramine has 5% free drug but is permissive of the volume of distribution ($V_d = 30$ L/kg).

TABLE 14.5 ► Restrictive and Permissive Effects of PPB on Drug Disposition

Drug	Free drug in plasma (%)	Volume of distribution (L/kg)
Restrictive		
Furosemide	4	0.2
Ibuprofen	<1	0.14
Nafcillin	10	0.63
Warfarin	<1	0.1
Permissive		
Desipramine	8	40
Imipramine	5	30
Vinblastine	30	35
Vincristine	30	11

PPB also can be restrictive of BBB permeation. Binding keeps the compound in the bloodstream, resulting in reduced permeation (Figure 14.3). Teams often use in vitro PPB in vitro measurements to estimate this effect. However, as shown in Figure 10.8, in vitro measurements of PPB may underestimate the release of drug molecules in the microcirculation environment of brain microvessels.[15] On/off rate also is important.[14]

PPB also affects V_d (see Chapter 19). V_d is determined as follows:

$$V_d = V_{plasma} + V_{tissue}(f_{u,plasma}/f_{u,tissue}),$$

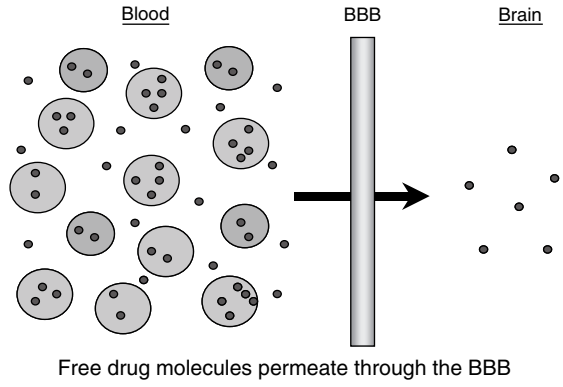


Figure 14.3 ► High PPB can restrict BBB permeation.

where V_{plasma} = volume of plasma in the body, V_{tissue} = volume of tissue in the body, $f_{u,plasma}$ = fraction unbound in the plasma, and $f_{u,tissue}$ = fraction unbound in the tissue. A couple of examples illustrate the possible scenarios:

- If PPB is high, $f_{u,plasma}$ is low, resulting in a low V_d.
- If PPB is low, $f_{u,plasma}$ is high, resulting in a high V_d.
- If nonspecific binding in tissue is high, $f_{u,tissue}$ is low, resulting in a high V_d.
- If nonspecific binding in tissue is low, $f_{u,tissue}$ is high, resulting in a low V_d.

Therefore, in addition to PPB, tissue binding also determines V_d. The ratio $f_{u,plasma}/f_{u,tissue}$ is a balance between the nonspecific binding in the tissue and PPB.[16] As this ratio increases, V_d is greater (more drug in tissue). For V_d much greater than 1 L/kg, high nonspecific tissue binding occurs. High binding to tissue proteins and lipids increases V_d.

14.2.2 Effect of PPB on Clearance

High PPB can be *restrictive* or *permissive* of liver extraction. Table 14.6 shows the case of propranolol, with >90% PPB but is *permissive* of >90% liver extraction. However, warfarin, with 99% PPB, is *restrictive* of liver extraction (<0.3%). Rate constants play an important role in liver clearance.[13]

TABLE 14.6 ► Restrictive and Permissive Effects of PPB on Liver Extraction

Drug	Bound drug in plasma	Liver extraction	Consequence
Propranolol	>90%	>90%	Permissive
Warfarin	>99%	<0.3%	Restrictive

14.2.3 Effect of PPB on Pharmacology

Pharmacology can be affected by PPB. Enzyme inhibition can be reduced if the compound is bound to plasma proteins. For example, inhibition of HIV protease by VX-478 is reduced

two-fold if 45% HSA is added to the in vitro incubation media. PPB reduces vascular receptor occupancy because there are no diffusion barriers for vascular receptors. For example, the activity of angiotensin II is decreased by adding albumin. PPB directly reduces antimicrobial activity, which has not been seen with other groups of agents.

A study of 1,500 frequently prescribed drugs showed 43% of compounds have >90% PPB.[17] PPB shows no significant difference among therapeutic areas: CNS, inflammation, and renal/cardiovascular. An exception is perhaps antiinflammatory drugs, which have a higher percentage (26%) of high protein binding (>99%), and many of them are acids. It is surprising that many CNS drugs possess high PPB characteristics. The most striking finding of this study is that chemotherapeutics, including antibiotic, antiviral, antifungal, and anticancer drugs, have a high percentage (77%) of low binding drugs. In designing drugs for chemotherapeutics, low PPB appears to be advantageous.

14.3 PPB Case Studies

Nonsteroidal antiinflammatory drugs (NSAIDs) are extensively bound to HSA, with a high binding constant. Plasma binding is higher than tissue binding. For this reason, NSAIDs have low tissue distribution.[18]

Christ and Trainor[19] discuss the addition of HSA and AGP to in vitro cell culture assays for HIV reverse transcriptase inhibitors in discovery. The shift in IC_{90} is indicative of the effect of PPB on free compound concentration and has been called a *shift assay*. This experiment was used as part of the strategy for selecting clinical candidates with higher free drug concentration for treatment of mutant HIV strains.

Webster et al.[20] correlated the in vitro IC_{50} that causes hERG blocking (see Chapter 16) to free drug concentration in the plasma that causes QT prolongation (increases the QT ECG interval) and torsades de pointes (TdP) arrhythmia (Figure 14.4). There is a strong correlation, but IC_{50} values overestimate the free drug concentration that causes QT.

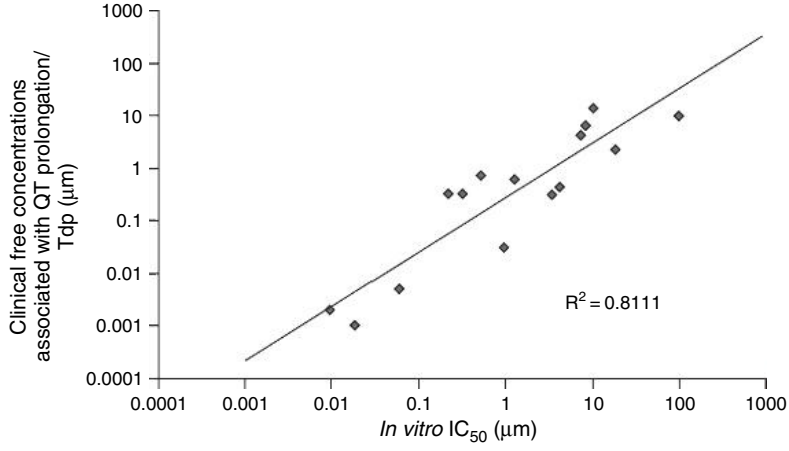


Figure 14.4 ► Relationship of in vitro IC_{50} for hERG blocking and clinical free plasma concentration causing QT prolongation and torsades de pointes (TdP). (Reprinted with permission from [20].)

14.4 Structure Modification Strategies for PPB

Quantitative structure–activity relationship studies have evaluated the physicochemical properties that correlate with PPB.[21] Increasing lipophilicity has the greatest effect on increasing

PPB. This analysis suggests various structure modifications that can be performed to *reduce PPB (*Table 14.7). Synthetic strategies for these structural modifications are found in several other chapters.

TABLE 14.7 ► Structure Modification Strategies to Reduce PPB in Order of Highest to Lowest Potential Effect

Structure Modification Strategy
Reduce lipophilicity (Log P for acids, Log $D_{7.4}$ for nonacids)
Reduce acidity (increase pK_a of the acid)
Increase basicity (increase pK_a of the base)
Reduce nonpolar area
Increase PSA (increasing PSA increases hydrogen bonding)

Urien et al.[16] have suggested that PBB can be used to advantage in restricting the distribution of drugs into general tissues throughout the body where they can cause side effects. It is suggested, as in the case of cetirizine (zwitterion with moderate lipophilicity), that high plasma binding and low tissue binding would restrict the compound to the blood. This may be a useful strategy when the target is in close proximity to the bloodstream.

14.5 Strategy for PPB in Discovery

In general, the prospective use of PPB data for predicting in vivo PK and pharmacodynamics in drug discovery can be misleading. Many commercial drugs have high (>99%) PPB. PPB may be restrictive or permissive for penetration into tissues. This is because PPB is modified by the extent of plasma binding, the plasma protein–drug dissociation rate, and the extent of nonspecific tissue binding. PPB can increase the PK $t_{½}$ (by keeping the compound in the blood and restricting clearance), but it also can restrict exposure to the therapeutic target (by reducing penetration into tissues). PPB alone can be either a positive or a negative aspect of a compound.

However, PPB can be useful, retrospectively, as part of an ensemble of in vitro diagnostic tests to understand the impact of PPB on PK or pharmacological effects. Only when PPB is placed into context with PK parameters can valuable insight be gained into the disposition of the molecule.[6]

14.6 Red Blood Cell Binding

Drug molecules can bind to red blood cells (RBCs). In PK studies, RBCs are immediately removed by centrifugation of blood samples to produce plasma from which drug concentration is quantitated. Therefore, drug bound to RBCs will not be measured in the sample. If in vivo PK studies show only low plasma concentrations or unexplained clearance, especially from IV dosing, red blood cell binding could be one of the causes.

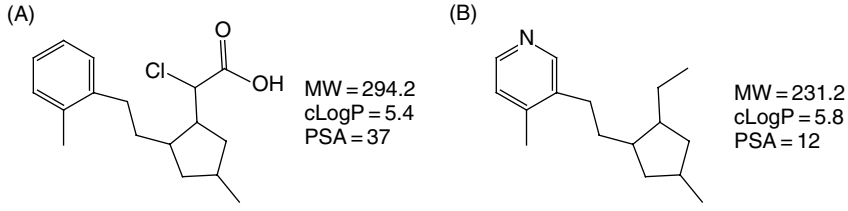
Problems

(Answers can be found in Appendix I at the end of the book.)

1. Would high plasma protein binding of a compound (e.g., 99.9%) and low dissociation rate tend to increase or decrease each of the following, compared to a compound with moderate plasma protein binding (e.g., 50%) and moderate dissociation rate?: (a) metabolic

clearance, (b) tissue concentration, (c) tissue distribution, (d) blood concentration, (e) renal clearance, (f) PK half-life, (g) pharmacological effect for non-bloodstream target, (h) brain penetration.

2. List three plasma proteins to which drugs bind.
3. Why is a shift in activity sometimes observed in vitro when albumin is added to the assay media?
4. For the following compounds, list structure modifications and structural examples that can be synthesized in an attempt to reduce plasma protein binding.



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Cytochrome P450 Inhibition

Overview

- *Drug–drug interactions can occur when two drugs are coadministered and compete for the same enzyme.*
- *In cytochrome P450 (CYP) inhibition, one drug ("perpetrator") binds to the isozyme and the other drug ("victim") is excluded from metabolism, thus increasing to a toxic concentration.*
- *Irreversible binding inactivates CYP and is termed mechanism-based inhibition.*
- *CYP inhibition can cause withdrawal from clinical use or restrictive labeling for a drug.*

Many patients receive more than one drug at a time, and physicians must be careful to avoid drug–drug interactions (DDI). DDI is the interference of one drug with the normal metabolic or pharmacokinetic behavior of a coadministered drug. DDI typically occurs by competition at a specific protein, such as at a metabolizing enzyme, involved in ADME processes. A monograph discusses DDI in detail.[1] A major DDI concern is cytochrome P450 (CYP) inhibition. (Other DDI issues are discussed in Section 15.6.) CYP inhibition has caused withdrawal from clinical use or restricted use of some major drugs. Because of its effects on clearance and half-life, CYP inhibition has become an important concern with the Food and Drug Administration (FDA) and at pharmaceutical companies. CYP inhibition now is assessed for a lead series from the earliest stages of the discovery project and can cause a lead series to be diminished in priority if the issue is uncorrectable. Medicinal chemists often can modify the structure to reduce CYP inhibition.

15.1 CYP Inhibition Fundamentals

CYP inhibition is illustrated in Figure 15.1. When a single drug is administered to a patient, it undergoes normal metabolism at one or more of the CYP isozymes and is eliminated at a predictable clearance (Cl) rate. This clearance is used for calculations of patient pharmacokinetics, dosage levels, and frequency. When the same drug is administered with a second drug that competes for binding at the CYP isozyme that metabolizes the first drug, then the clearance of the first drug is reduced. This can be described as follows:

$$Cl_{int}(i) \approx Cl_{int}/[1 + (I/K_i)],$$

where Cl_{int} = normal intrinsic clearance (without inhibitor), $Cl_{int}(i)$ = intrinsic clearance in the presence of the inhibitor, I = inhibitor concentration at the CYP isozyme, and K_i = inhibitor constant for an isozyme.[2] The result is a reduced clearance rate. Cl decreases

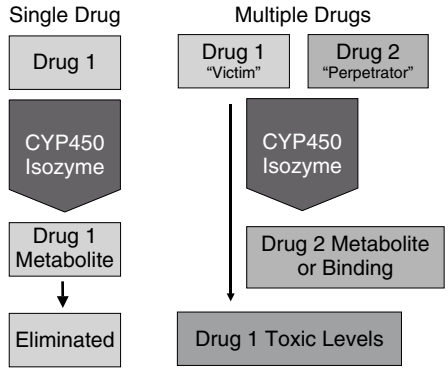


Figure 15.1 ► Administration of drug 1 alone allows a normal rate of metabolism at a particular CYP isozymes. Coadministration of drug 1 and drug 2 can result in drug 2 being an inhibitor of drug 1. Lack of drug 1 clearance can cause drug 1 to increase to a toxic concentration.

with increasing inhibitor concentration. With the reduced clearance, the concentration (e.g., C_{max}) or exposure (i.e., area under the curve [AUC]) of the first drug increases to a higher level than expected. This high concentration may be toxic or cause side effects. The CYP inhibitor is termed the *perpetrator,* and the compound whose metabolism is inhibited is termed the *victim.*

In order to recognize the impact of CYP inhibition, it is useful to understand the CYP isozymes. Many CYP isozymes have been discovered, and their individual contributions to drug metabolism are becoming better understood. The major isozymes present in human liver microsomes (HLM) are shown in Figure 15.2. Some of the major isozymes are the 3A family (28% of total CYP protein) and 2C family (18%).

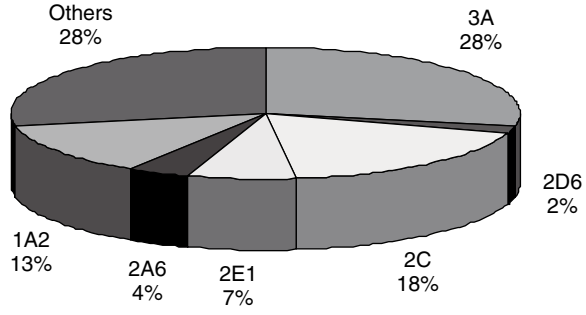


Figure 15.2 ► CYP isoforms in human liver microsomes and their relative abundances.[22]

The percentages of drugs metabolized by different CYP isozymes are shown in Figure 15.3 and summarized in Table 15.1.[3] Although 3A constitutes 28% of the human liver CYP, it is responsible for metabolism of 50% of all drugs. Even more remarkable, 2D6, which constitutes only 2% of the human liver CYP, is responsible for metabolism of 30% of all drugs. 2D6 primarily metabolizes basic amine compounds, of which there are many drugs. Thus, an inhibitor of 3A4 or 2D6 poses significant risk when coadministered with many drugs.

A CYP inhibitor perpetrator itself may be metabolized by the isozyme and, therefore, be a competitive substrate with the victim compound for the isozyme. Alternatively, the CYP inhibitor may bind to the isozyme and itself not be metabolized but inhibit other compounds from binding and being metabolized.

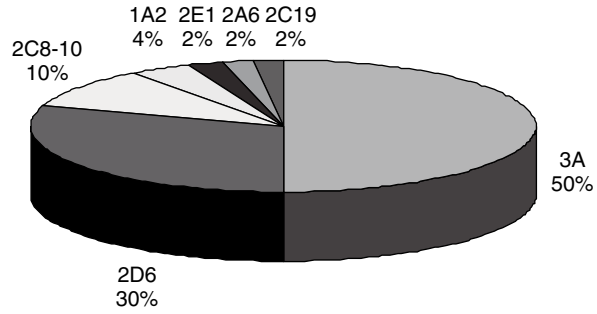


Figure 15.3 ► Percentage of drugs metabolized by different CYP isoforms.[22]

TABLE 15.1 ► Summary of Important CYP Isozymes

Isozyme	Distribution in HLM	Drugs metabolized	Comments
3A family	28%	50%	Most abundant
2D6	2%	30%	Polymorphic, 5% of white males lack isozyme
2C family	18%	10%	Polymorphic
1A2	13%	4%	Enzyme induction

15.2 Effects of CYP Inhibition

An example of CYP inhibition is coadministration of erythromycin and terfenadine (Figure 15.4). Erythromycin inhibits the metabolism of terfenadine by CYP3A4 isozyme. Terfenadine increases to abnormal concentrations and can cause prolongation of the cardiac QT interval and trigger torsades de pointes arrhythmia (see Chapter 16). Coadministered erythromycin is also known to inhibit the metabolism of cyclosporin, carbamazepine, and midazolam.[4]

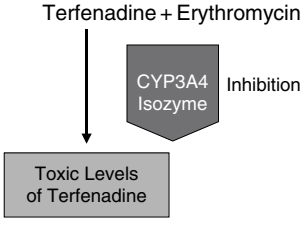


Figure 15.4 ► When erythromycin and terfenadine are coadministered, erythromycin inhibits terfenadine metabolism at CYP3A4 and can result in cardiac arrhythmia.

Pharmaceutical companies must test each clinical candidate for its potential to inhibit CYP isozymes. They also must determine what will happen if the drug's metabolism is inhibited by other drugs. Methods used in CYP inhibition testing are discussed in Chapter 32. In general the assays are conducted by coincubating the CYP isozyme, test compound, and a substrate compound, whose rate of metabolism is well established. Reduction in the rate of metabolism of the substrate is evidence for CYP inhibition by the test compound at the particular isozyme. During initial in vitro CYP inhibition assessment, often at a

test compound concentration of 3 μM, the following guidelines are generally useful in recognizing the potential risk of DDI:

- <15% inhibition @ 3 μM or $IC_{50} > 10\,\mu M$ CYP inhibition *low*
- 15%–50% inhibition @ 3 μM or $3\,\mu M < IC_{50} < 10\,\mu M$ CYP inhibition *moderate*
- >50% inhibition @ 3 μM, $IC_{50} < 3\mu M$ CYP inhibition *high*

Some groups are more conservative and use more stringent guidelines:

- $IC_{50} > 100\,\mu M$ CYP inhibition *low*
- $10\,\mu M < IC_{50} < 100\,\mu M$ CYP inhibition *moderate*
- $IC_{50} < 10\,\mu M$ CYP inhibition *high*

Each organization decides on its own guidelines. This can be determined by using the data obtained from the in-house CYP inhibition assay(s) for known CYP inhibitors from the scientific literature or by using industry consensus.

A useful decision-making tool for discovery project teams is to plot the therapeutic target IC_{50} of project compounds versus the IC_{50} for CYP inhibition.[5] Use of a tool such as Spotfire allows differentiation of compound classes to visualize the trends of different lead series (Figure 15.5).

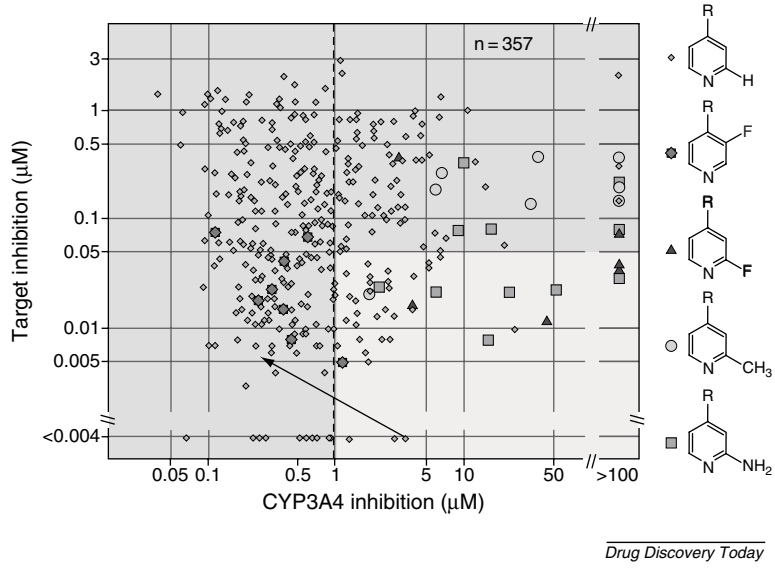


Figure 15.5 ► A plot of activity versus CYP inhibition is useful for multivariable decision making by discovery project teams. Here the CYP3A4 inhibition versus target potency plot shows which pyridine substitutions were effective in reducing CYP3A4 inhibition while maintaining target inhibition. The optimal area for activity with minimal CYP3A4 inhibition is the light area in the bottom right corner. Such plots are useful for all properties. (Reprinted with permission from [5].)

It is important to pay careful attention to compounds having moderate-to-high CYP inhibition. Three options in this case are to attempt structure modification to reduce the inhibition, reduce the priority of that particular lead series, or discuss the ramifications with clinical metabolism experts.

The magnitude of the CYP inhibition problem in vivo is determined by many parameters, in addition to $IC_{50,}$ which should be discussed among drug metabolism experts and the project team scientists. Compound concentration (C_{max}) in vivo relative to K_i is very important. The isozyme that is inhibited is important. For example, a drug that inhibits 3A4, which metabolizes 50% of drugs, has many more drugs it could interact with than if it inhibited 2C19, which metabolizes a low percentage of drugs. Another parameter is plasma protein binding of inhibitor, which reduces its exposure to the liver. Conversely, the inhibitor concentration in liver may be higher than in plasma. For example, hydroxyzine concentration in liver is 10 times the plasma concentration. For drugs that might be inhibited by coadministered drugs, it is useful to consider whether they might switch to another CYP isoform(s) for metabolism if their normal primary isozyme is inhibited, or whether clearance by other routes (renal, other enzyme systems) might increase.

In later stages of discovery[6], an assessment is made of K_i versus drug concentration levels in vivo following dosing. A PhARMA working group published a consensus summary of DDI assessment. Among its findings are that reversible CYP inhibition assessments can be guided by the CYP inhibition results (K_i), and the C_{max} expected at the highest human clinical dose. C_{max} is the highest inhibitor concentration reached in the blood in vivo and is an approximation of the inhibitor concentration (I) at the isozymes in the liver. The guidelines are as follows:

- $C_{max}/K_i < 0.1$ CYP inhibition *not likely*
- $0.1 < C_{max}/K_i < 1$ CYP inhibition *possible*
- $C_{max}/K_i > 1$ CYP inhibition *likely*

These ratios are used in planning early clinical DDI studies.

Data on CYP inhibition is included in regulatory submissions to the FDA. In vitro CYP450 inhibition data showing negligible inhibition can be used to conclude that the compound lacks DDI potential and requires no clinical DDI studies. If in vitro data indicate potential DDI, they are used to plan specific clinical DDI experiments with human volunteers during clinical development.[7] PhARMA has agreed on guidelines for conducting in vitro and in vivo DDI studies. The package insert for a drug contains information on DDI. It states whether the compound is a CYP inhibitor or if its pharmacokinetics is significantly affected by another drug that is an inhibitor. Therefore, strong DDI potential can greatly affect the marketability of the drug. It is good for a new clinical candidate to be metabolized by multiple CYP isozymes, to reduce its potential metabolic inhibition by another drug.

15.3 CYP Inhibition Case Studies

CYP inhibition can lead to toxic effects in patients. Several commercial drugs have been voluntarily withdrawn from the market because of the effects of CYP inhibition (Table 15.2). Terfenadine (Seldane)[8] and cisapride (Propulsid) can produce fatal torsades de pointes

TABLE 15.2 ► Drug Withdrawals due to CYP Inhibition

Drug	Generic name	Date voluntarily withdrawn
Posicor	Mibefradil dihydrochloride	June 1998
Seldane	Terfenadine	February 1998
Hismanal	Astemizole	June 1999

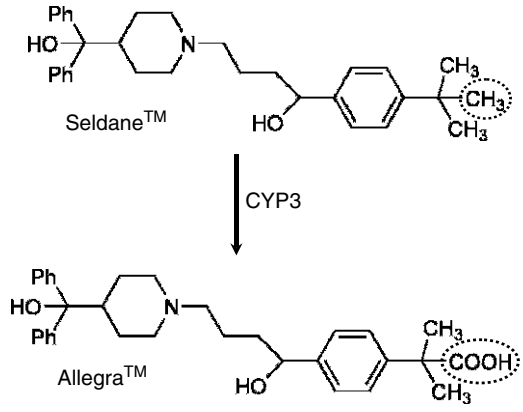


Figure 15.6 ► Seldane was withdrawn from the market because of high C_{max} values when coadministered with a CYP3A4 inhibitor. It was replaced by its active metabolite Allegra.

arrhythmia (see Section 16.2) when their metabolism is inhibited by a coadministered drug. Seldane was voluntarily removed from the market, and its active metabolite Allegra was developed and introduced for clinical use (Figure 15.6). Clinical use of cimetidine (Tagamet) diminished when it was found to cause DDI in the clinic.[9] When cimetidine and disopyramide are coadministered, the plasma C_{max} of disopyramide is greatly increased compared to individual administration.[10]

Reversible competitive inhibition of a CYP isozyme is dependent on the same factors that enhance binding of any ligand to an enzyme active site. These include specific interactions with active site moieties (e.g., lipophilic) and molecular shape.[4] In addition, the presence of a lone electron pair on the inhibitor appears to enhance binding to the heme group of the CYP enzyme. The increased binding energy is on the order of 6 kcal/mol.[11] For example, cimetidine contains an imidazole group and is a CYP3A4 and 2D6 inhibitor, whereas ranitidine has no imidazole group and does not inhibit disopyramide metabolism. Quinoline groups, as contained in quinidine (CYP2D6 inhibitor) and ellipticine (CYP1A inhibitor),[12] and pyridines, as contained in indinavir (CYP3A4 inhibitor),[13] also appear to interact with the heme during competitive binding.

15.3.1 Consequences of Chirality on CYP Inhibition

Chirality affects CYP inhibition. Two examples are shown in Figure 15.7. The (+)-isomer (3R, 5S) of fluvastatin (Figure 15.7A), an antilipidemic drug, more strongly inhibits the CYP2C9 isozyme than its enantiomer.[14] For the structure in Figure 15.7B, the

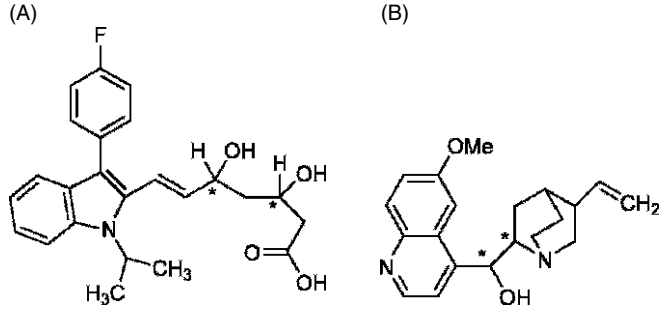
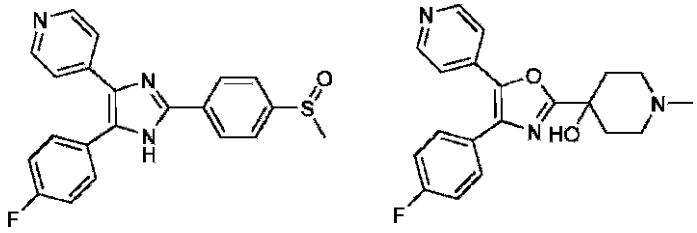


Figure 15.7 ► Stereoselective cytochrome P450 inhibition is observed for **(A)** fluvastatin[14] and **(B)** (+) quinidine and (–) quinine.[15]

(+) enantiomer is quinidine and the (−) enantiomer is quinine. Quinidine is a strong inhibitor of CYP2D6, while quinine has no effect on CYP2D6 metabolism.[15]

15.4 Structure Modification Strategies to Reduce CYP Inhibition

Modification of the structure of the lead series can reduce the IC_{50} of CYP inhibition. In the pyridinyloxazole series shown in Figure 15.8, the IC_{50} values for inhibition of three CYP isozymes were greatly increased with structure modification, while not affecting the activity or selectivity.[16]

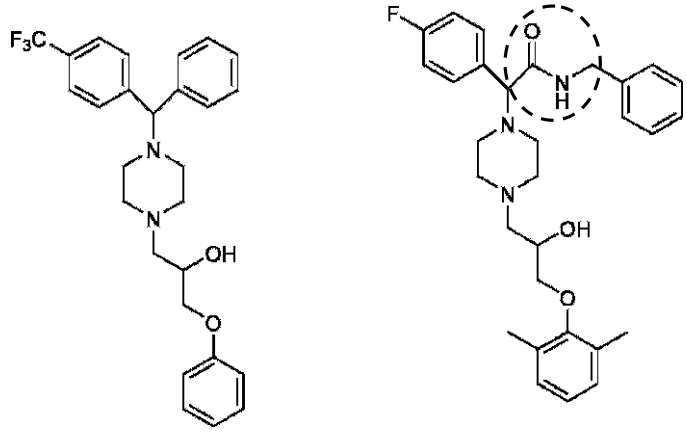


p38α IC_{50} = 0.45 μM
COX-1 IC_{50} = 5 μM (nonselective)
3A4 IC_{50} < 2 μM
2D6 IC_{50} > 100 μM
2C9 IC_{50} < 2 μM
1A2 IC_{50} = 4 μM

p38α IC_{50} = 0.35 μM
COX-1 IC_{50} > 100 μM
3A4 IC_{50} = 100 μM
2D6 IC_{50} = 22 μM
2C9 IC_{50} > 100 μM
1A2 IC_{50} > 100 μM

Figure 15.8 ► Pyridinyloxazole series modification successfully reduced IC_{50} for inhibition of three CYP isozymes without reducing activity and selectivity.

In a sodium channel-blocking project, CYP2D6 inhibition was reduced by structural modification (Figure 15.9). At the same time, the activity of the series was maintained or improved.[17]

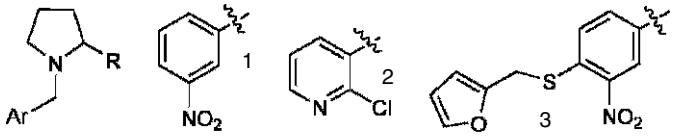


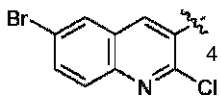
IC_{50} = 893 nm
CYP2D6 (% Inhib. @ 2 μM) = 87%

IC_{50} = 149 nm
CYP2D6 (%Inhib@ 2 μM) = 20%

Figure 15.9 ► CYP2D6 inhibition was reduced while sodium channel-blocking activity was improved by structure modification of this series. The circled modification was primarily responsible for reducing the CYP2D6 inhibition.

A structural series for a G-protein–coupled receptor (GPCR) target showed significant CYP2D6 inhibition (Figure 15.10). This was overcome by structure modification while maintaining the GPCR agonism.[18]





Compd	GPCR IC_{50} (uM)	CYP2D6 IC_{50} (uM)	Selectivity Ratio
1	0.33	<0.05	<0.15
2	0.22	0.02	0.09
3	0.22	2.2	10
4	0.19	22	116

Figure 15.10 ► CYP2D6 inhibition was reduced while maintaining GPCR agonism by structural modifications. Compound 1 was the original lead, which had high CYP2D6 inhibition.

Riley et al.[11] studied CYP3A4 inhibition of pyridine-containing drugs and suggested general rules for predicting CYP3A4 inhibition by nitrogen heterocycle-containing drugs (i.e., triazoles, pyridines, imidazoles, quinolines, thiazoles). Reducing the interaction of the nitrogen loan pair with the 3A4 heme group was beneficial. Riley's rules and related work[5] provide guidance for structure modification (Table 15.3) to reduce CYP3A4 inhibition.

TABLE 15.3 ► Structure Modification Strategies to Reduce CYP Inhibition

Structure Modification Strategy
Decrease the lipophilicity (Log $D_{7.4}$) of the molecule
Add steric hindrance to the heterocycle para to the nitrogen
Add an electronic substitution (e.g., halogen) that reduces the pK_a of the nitrogen

15.5 Reversible and Irreversible CYP Inhibition

CYP inhibition has two general modes. The mode most commonly considered is *reversible inhibition*, in which the inhibitor binds to the CYP enzyme and then releases in a reversible binding scheme. The second mode is *irreversible inhibition*. One irreversible mechanism is the formation of a covalent bond between the inhibitor metabolite and the enzyme (often with the heme). For example, reactive intermediates generated from spironolactone can react with the heme group or with the protein chain. Another irreversible mechanism is tight quasi-irreversible binding. For example, oxidation of the tertiary amine in the amino sugar ring of erythromycin generates a nitroso metabolite that complexes with the CYP3A4 heme.[4] Irreversible binding has been called *mechanism-dependent* (*inhibition* or *mechanism-based inhibition*). It results in permanent inactivation of the enzyme molecule *in vivo*[19].

Irreversible inhibition is diagnosed by time dependency of inhibition. With reversible inhibition, IC_{50} should be the same for any incubation time. However, with irreversible inhibition, IC_{50} decreases with incubation time. This is because the fraction of deactivated

enzyme increases with reaction time, resulting in fewer functional enzyme molecules, making IC_{50} appear to be lower if the starting enzyme concentration is used to calculate IC_{50}. An example of irreversible inhibition diagnosis is shown in Figure 15.11.[20] For R = H in the compound series and for troleandomycin, IC_{50} decreases with increasing incubation time. For the modification of R to F and for ketoconazole, IC_{50} is the same at each incubation time.

Compound	Time-Dependent Inhibition (IC_{50}, μM)			
	5 min	15 min	30 min	45 min
R = H	96	62	33	22
R = F	18	21	19	19
Troleandomycin	61	33	20	16
Ketoconazole	0.016	0.013	0.017	0.022

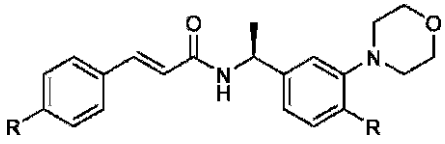


Figure 15.11 ► Examples of reversible and irreversible (red arrows) CYP inhibition. For the structure shown, when R is H irreversible CYP inhibition is observed, as indicated by the decrease in IC_{50} with incubation time. Modification of R to F eliminated the mechanism-dependent inhibition. Examples are also shown for troleandomycin, which exhibits time-dependent inhibition, and ketoconazole, which does not.

Irreversible inhibition is also diagnosed by dialysis or gel filtration. If inhibition is eliminated by dialysis or filtration of the small molecule inhibitor away from the enzyme, then the inhibition is reversible. However, continuation of inhibition after dialysis is evidence of irreversible inhibition.

Irreversible inhibition also may be diagnosed by preincubating the isozymes with inhibitor and cofactor (i.e., NADPH) and then incubating with the test compound. If the inhibitor or inhibitor metabolite is irreversibly bound to the isozymes, then IC_{50} will be lower (apparently more inhibitory) with preincubation versus no preincubation.

The consequences of irreversible inhibition can vary with the conditions. They depend on what fraction of the isozyme is irreversibly inactivated and the replenishment rate of the isozymes.

15.6 Other DDI Issues

The discussion thus far has focused on the important issue of discovery candidates acting as perpetrators by inhibition of the metabolism of other drugs (victims). There are other mechanisms by which a new clinical candidate emerging from drug discovery might have DDI with an existing commercial drug. These mechanisms include (1) the candidate as a victim to a perpetrator commercial drug, (2) the candidate as a victim or perpetrator of DDI at a transporter, and (3) the candidate as a victim or perpetrator of metabolic enzyme induction.

15.6.1 Candidate as Victim to a Metabolism Inhibition Perpetrator

Some drugs in clinical application might act as a perpetrator and inhibit a key metabolic enzyme for the candidate (victim), causing the candidate's concentration to increase to toxic levels. One way to avoid this is to select a candidate that is metabolized by multiple metabolic

isozymes. For such candidates, if a perpetrator inhibits one metabolic pathway, then other pathways will take on a greater portion of the candidate's metabolic clearance. For candidates that are cleared primarily by one isozyme, inhibition of that isozyme likely will lead to an increase in the candidate's concentration. For this reason, the candidate's metabolic stability at specific CYP isozymes and other metabolic enzymes are tested during drug discovery in a process called *metabolic phenotyping* (see Chapter 29). It is preferable for candidates to be metabolized by multiple isozyme pathways. One isozyme should not metabolize more than 50% of the candidate.[1,21] Interestingly, coadministration of an inhibitor also has been purposely performed in the clinic to enhance the blood concentration of drug that is expensive or in short supply.

15.6.2 Candidate as a Victim or Perpetrator at a Transporter

In addition to metabolic enzymes, DDI can occur at transporters. For example, if a candidate for a peripheral disease is a P-glycoprotein (Pgp) substrate, coadministration of a drug that inhibits Pgp can increase the candidate's exposure in protected tissues (e.g., brain, placenta) by inhibiting Pgp at the blood–tissue barrier. This also may cause increased absorption by inhibiting Pgp in the intestine, or it may reduce clearance by inhibiting Pgp active secretion in the nephron or bile canaliculus.

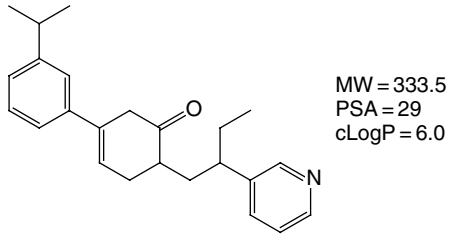
15.6.3 Candidate as a Victim or Perpetrator of Metabolic Enzyme Induction

Some candidates or drugs induce the production of metabolic enzymes and cause DDI. The candidate drug might be repeatedly coadministered or closely after a drug that induces a metabolic enzyme that clears a majority of the candidate. In this case, the candidate will be the victim of elevated enzyme levels, resulting in an enhanced rate of candidate metabolism and lower candidate exposure. This can reduce the pharmacological effect of the candidate, and the disease will not be treated with a concentration of candidate that produces the desired pharmacological effect. A candidate that is primarily metabolized by one enzyme should not be coadministered with a drug that induces that enzyme. Conversely, the candidate may be a metabolic enzyme inducer and perpetrate the enhanced metabolism of another (victim) drug. Such candidates often will fail to advance further in the development process.

Problems

(Answers can be found in Appendix I at the end of the book.)

1. For initial CYP inhibition screening, a useful goal is an IC_{50} greater than what concentration?
2. For human studies, K_i should be what? At what concentration is there likely to be CYP inhibition?
3. Why was Seldane removed from the market?
4. What is the difference between reversible and mechanism-based CYP inhibition? How can you distinguish these mechanisms?
5. How might you modify the following structure to reduce CYP inhibition?



6. What is the risk associated with CYP inhibition?: (a) a coadministered drug is metabolized too quickly, (b) a compound is not stable, (c) a coadministered drug is not metabolized quickly enough, (d) an isozyme may be induced.
7. Should CYP inhibition be used to estimate metabolic stability?

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hERG Blocking

Overview

- *Certain compounds block the cardiac K^+ (hERG) ion channel and induce arrhythmia.*
- *The safety margin for hERG is $IC_{50}/C_{max,unbound}$ >30.*
- *hERG blocking might be decreased by reducing the basicity, reducing lipophilicity, and removing oxygen H-bond acceptors.*

hERG has rapidly emerged as an important safety issue in drug discovery. Awareness about the details of hERG and its impact are growing among discovery scientists. Recent efforts have quickly integrated assessment of hERG risk and solutions into discovery.

hERG is a gene that codes for a cardiac potassium ion channel. If this channel is blocked, a mechanism is initiated that can lead to cardiac arrhythmia. This arrhythmia proceeds to fatality in a small portion of the patient population. In the past, such arrhythmias were observed only after the drug was approved by the Food and Drug Administration (FDA) and was used by a large population of patients. Since the mechanism of this arrhythmia was elucidated, the FDA has carefully reviewed new drug applications for this potential problem. FDA approval or continued clinical use of a drug for a low-risk medical condition (e.g., allergy) is hard to justify if the drug may cause arrhythmia. Drug candidates that are hERG blockers require large clinical trials with many patients in order to demonstrate safety, because arrhythmia caused by hERG channel blockage is a rare event. Thus, the cost of clinical development is elevated if a compound has a potential hERG liability. Therefore, drug companies study this potential problem during discovery. In recent years, hERG blocking has been one of the leading causes for withdrawal from the market of drugs approved by the FDA. Other drugs with this problem have remained on the market, but their use has been severely restricted. Examples of these drugs are shown in Figure 16.1.

16.1 hERG Fundamentals

The full name of the hERG gene is "human ether-a-go-go related gene." The protein product of hERG is the inner pore-forming portion of a critical membrane bound potassium (K^+) channel in heart muscle tissue. It forms a tetramer, with each monomer having six transmembrane regions. It is controlled by voltage (membrane potential) and gates the flow of K^+ ions out of the cell. Movement of K^+ ions across the cell membrane creates the rapidly activating delayed rectifier K^+ current called I_{Kr}.

The potassium channel is part of the ensemble of ion channels that creates the cardiac action potential at the cellular level (Figure 16.2, *A*). The action potential is initiated with the opening of sodium (Na^+) channels. Na^+ ions flow quickly into the cell, causing rapid depolarization of the membrane potential from a resting state of about −90 mV to about

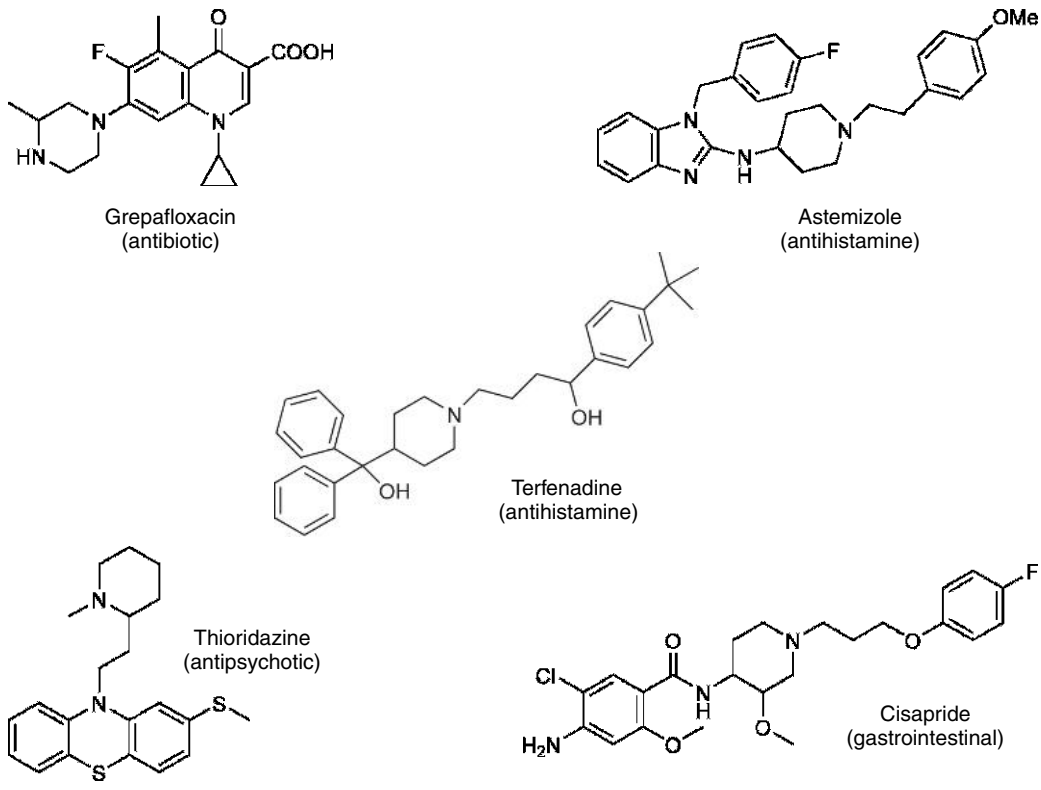


Figure 16.1 ► Commercial drugs that were withdrawn or had major labeling restrictions due to hERG blocking.

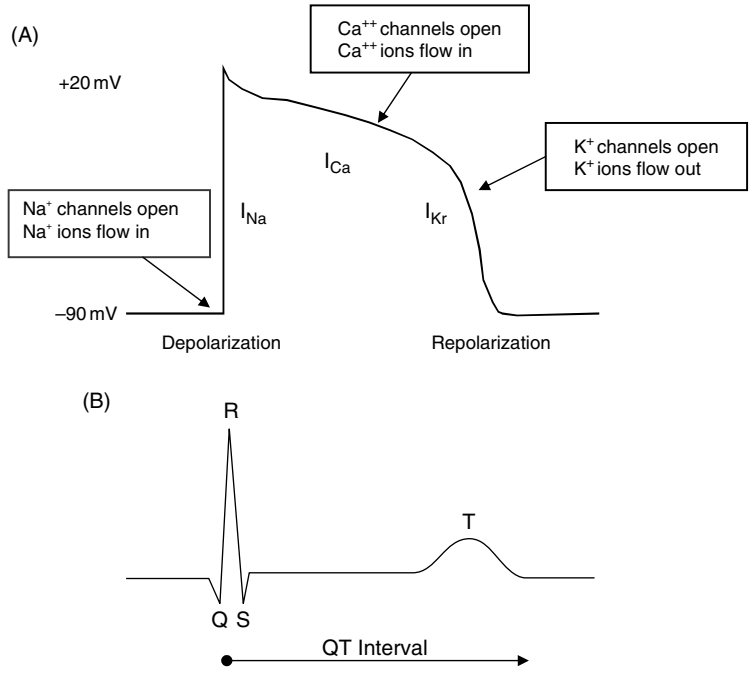


Figure 16.2 ► Cardiac action potential at the cellular level (**A**) and ECG on the surface of the heart (**B**).

+20 mV (voltage inside the cell compared to outside). This depolarization is maintained by subsequent opening of calcium (Ca^{2+}) ion channels, allowing Ca^{2+} ions to flow into the cell. Repolarization to −90 mV occurs by opening of the potassium ion channels, allowing K^+ ions to move out of the cells. The hERG channel is the most important potassium channel for repolarization.

This action potential contributes to the overall electrical activity of the heart, which is measured using an electrocardiogram (ECG) on the surface of the heart tissue (Figure 16.2, *B*). On the ECG, the time from point Q to point T is called the *QT interval* (from depolarization to repolarization). A change in the action potential will change the ECG.

16.2 hERG Blocking Effects

If a compound binds within the hERG K^+ channel, it can obstruct the flow of K^+ ions out of the cell. This causes a slower outflow of K^+ ions, thus lengthening the time required to repolarize the cell (Figure 16.3). From the ECG, it can be seen that the T event is delayed, thus lengthening the QT interval (long QT [LQT]). LQT may trigger life-threatening torsades de pointes (TdP) arrhythmia (Figure 16.4). Although hERG blocking is a triggering factor for TdP, other physiological and genetic factors also increase the chances of LQT.[1] These factors include low serum K^+, slow heart rate, genetic factors (e.g., mutations affecting other ion channels), other cardiac conditions, coadministered drugs that also block hERG, coadministered drugs that inhibit metabolism (e.g., terfenadine), and gender. Some patients can have LQT without progressing to TdP, whereas others progress to TdP with only slight lengthening of the QT interval. TdP arrhythmia leads to ventricular fibrillation, which can cause sudden death. The involvement of the hERG channel in LQT is further supported by a naturally occurring inherited mutation in hERG that leads to LQT, TdP, and ventricular fibrillation.

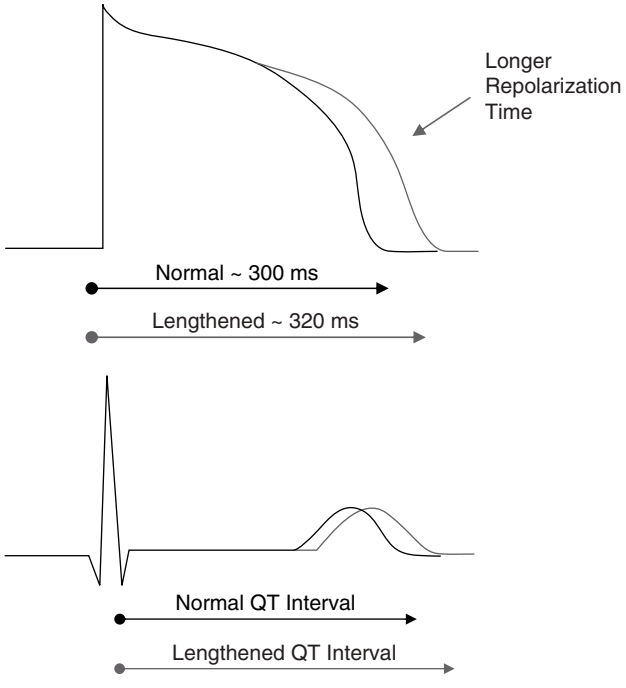


Figure 16.3 ► hERG potassium channel blocking lengthens the time until repolarization, resulting in LQT.

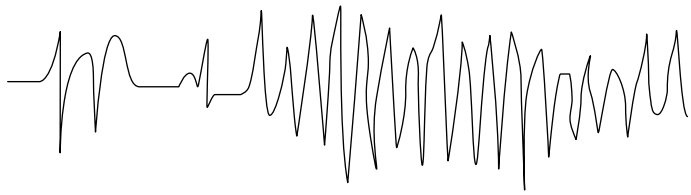


Figure 16.4 ► Torsades de pointes arrhythmia ECG.

The number of drugs that induce TdP is estimated to be higher than the number that induce more rare arrhythmias. TdP was reported to occur in patients taking quinidine (1%–3%), sotalol (1%–5%), dofetilide (1%–5%), and ibutilide (12.5%).[2] Arrhythmia was produced in $1:10^5$ to 10^6 persons taking antihistamines.[1] The incidence of arrhythmia was about 1:50,000 for patients taking terfenadine.[3]

Most drugs that cause LQT and trigger TdP are hERG blockers. However, not all hERG blockers produce TdP. This makes prediction and risk assessment difficult. A three-step approach to assessment of hERG blocking and TdP-induced arrhythmia often is used during drug discovery: (1) in silico structural alert, (2) in vitro hERG channel-blocking screening using a patch-clamp method, and (3) in vivo ECG. Methods for measuring hERG blocking and studying TdP are discussed in Chapter 34.

The safety margin for hERG blocking typically is evaluated as the ratio between the hERG IC_{50} (half maximal inhibition concentration) and $C_{max,unbound}$ (maximal human blood concentration of drug not bound to plasma proteins). A large value (often cited as >30) for the ratio hERG $IC_{50}/C_{max,unbound}$ is recommended. This is based on the experimental observation that for compounds with a ratio <30, 95% produce TdP and only 5% do not, whereas for a ratio >30, 15% produce TdP and 85% do not produce. Another safety margin is the time of QT interval lengthening. Regulatory agencies express major concern about the drug candidate when LQT exceeds an additional 5 ms compared to normal.[4]

Compounds that prolong the QT interval by >20 ms may be approvable if they are for important therapeutic indications, there is a strong benefit compared to the risk, or there is a feasible way to manage the risk. However, these drugs require special restrictive labeling, and their commercial impact is reduced. They may consume considerable resources during development and still not be approved. A human QT study is likely to be required during clinical development and is expensive.[4]

As with many other types of property data, a significant hERG response is not the sole criterion for terminating a compound. hERG-blocking data should be used in combination with all other data and project considerations in a holistic decision-making process.

16.3 hERG Blocking Structure–Activity Relationship

The amino acid residues in the hERG K^+ channel to which blocking drugs bind have been studied by means of single-site mutations.[1] Binding has been isolated to the central cavity of the channel, with Tyr652 and Phe656 of the pore helix being most important in binding. π-stacking appears to occur if the drug has an aromatic group. Alternately, interaction can occur with nonaromatic hydrophobic substructures in the drug. Cation–π interaction occurs between a basic nitrogen in the drug and Tyr652. Interaction with drugs appears to occur mostly when the K^+ channel is in the open position. Trapping of a drug molecule in the central cavity appears to be enhanced in the hERG channel, compared to other ion channels, by certain sequence differences that increase the hERG channel cavity volume, even in the closed state.

Studies agree on several structural features that are common to binding in the hERG channel:

- A basic amine (positively ionizable, pK_a >7.3)
- Hydrophobic/lipophilic substructure(s) (ClogP >3.7)
- Absence of negatively ionizable groups
- Absence of oxygen H-bond acceptors

In one hERG structure–activity relationship (SAR) model, the basic nitrogen is the top of a pyramid, with three or four hydrophobic substructures at the other loci, thus forming a plug of the channel.[1] hERG SAR is an active area of research, and increased data are expected to improve the SAR understanding for hERG.

16.4 Structure Modification Strategies for hERG

Initial suggestions for improving the structures of discovery leads to reduce hERG blocking follow the indications of the SAR and are listed in Table 16.1.

TABLE 16.1 ► Structure Modifications to Reduce hERG Blocking

Structure Modification Strategy
Reduce the pK_a (basicity) of the amine
Reduce the lipophilicity and number of substructures in the binding region
Add acid moiety
Add oxygen H-bond acceptors
Rigidify linkers

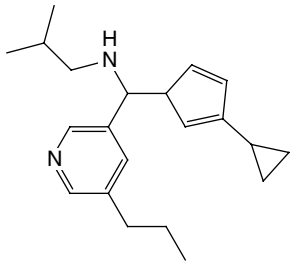
hERG molecular models continue to be improved. These guide structural modifications at points of interaction of the compound and the inner surface of the hERG channel.

Problems

(Answers can be found in Appendix I at the end of the book.)

1. hERG is the gene for what protein?
2. What is the function of the hERG protein?
3. What is LQT?
4. What is TdP?
5. How common in the population is TdP that is triggered by LQT?
6. What safety margin can be used in drug discovery for hERG blocking?
7. Where do most hERG blocking drugs bind?: (a) ATP binding site, (b) hinge region, (c) within the channel cavity, (d) at the allosteric site.

8. Which of the following structural features are favorable toward hERG blocking?: (a) low lipophilicity, (b) carboxylic acid, (c) secondary amine, (d) lipophilic moiety, (e) oxygen H-bond acceptors.
9. What structural modifications might be tried to reduce hERG blocking of the following structure?



MW = 310
PSA = 24
cLogP = 4.9

10. Compounds that cause hERG blocking are at risk for causing which of the following?: (a) K^+ channel opening, (b) myocardial infarction, (c) arrhythmia, (d) metabolic inhibition, (e) QT interval shortening.

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Additional Reading

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Chapter 17

Toxicity

Overview

- *Toxicity remains a significant cause of attrition during development.*
- *Many toxic outcomes are possible, including carcinogenicity, teratogenicity, reproductive toxicity, cytotoxicity, and phospholipidosis.*
- *Toxic mechanisms include reactive metabolites, gene induction, mutagenicity, oxidative stress, and autoimmune response.*
- *The safety window is the concentration range between efficacious response and toxic response.*

Safety is one of the highest goals of pharmaceutical companies. Drugs provide a means of improving the quality and length of patients' lives, but they must minimize possible deleterious side effects and harm. Toxicology studies assess potential toxicities of a drug candidate and determine how drug therapy can be managed to minimize patient risk. As with the pharmacologically beneficial effects of drugs, toxic effects follow a dose–response relationship. Therefore, it is necessary to maximize the safety window (therapeutic index) between efficacy and toxicity (Figure 17.1).

In past years, toxicity studies were performed only during the clinical development phase. However, toxicity remains a major cause of drug candidate attrition during preclinical and clinical development (see Figure 2.4). The Kola-Landis study documented 20% to 30% attrition due to toxicity and clinical safety. A KMR study reported 44% toxicity attrition.[1]

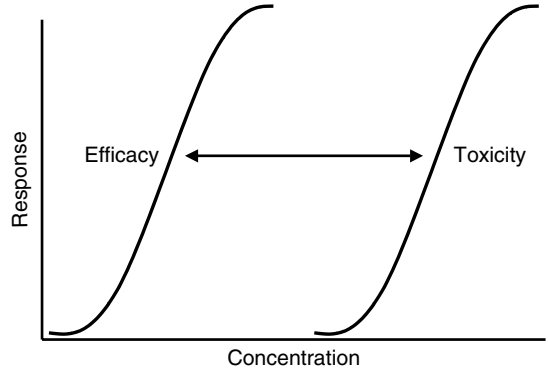

Figure 17.1 ► It is important to maximize the safety window (therapeutic index) of a drug candidate.

Moreover, many drugs have been withdrawn from the market due to human toxicity observed during clinical use in the wider population.

All companies perform Food and Drug Administration (FDA)-prescribed in vivo toxicity studies prior to clinical studies. Most companies also have addressed key safety issues, such as cytochrome P450 (CYP) inhibition and hERG blocking, with in vitro screens during drug discovery. However, these are only two of the potential toxicity challenges that drugs encounter. As with other absorption, distribution, metabolism, excretion, and toxicity (ADME/Tox properties), early screening and correction of toxicity issues can reduce preclinical and clinical attrition. This also can improve the efficiency of drug discovery by reducing the time and effort spent on lead series that are terminated later due to toxicity. In silico and in vitro assays can indicate potential toxicities during discovery. Another opportunity for improving efficiency is the early validation of each discovery target for target-based toxicity associated with modulating the target.[2]

Toxicity studies and safety optimization in discovery follow the same process as other ADME/Tox properties. In silico tools and in vitro higher-throughput screens indicate potential toxicity mechanisms. If performed early, these data are used as part of lead selection. Indications of toxicity from screens are followed with advanced studies that provide definitive data. The data are used to prioritize compounds and lead series and to guide structure optimization to reduced toxicity during discovery. Finally, toxicity data usually are included in the data package that is used to select clinical candidates.

Toxicity is a very broad topic. The purpose of this chapter is to introduce discovery scientists and students to key toxicity concepts and terms. Examples of chemical mechanisms of toxicity are described. The reader is directed to reviews on toxicity and mechanisms for in-depth study.[3–5] Methods for toxicity assessment during drug discovery are discussed in Chapter 35.

17.1 Toxicity Fundamentals

Data from standard animal toxicity studies must be obtained for clinical candidates prior to investigational new drug (IND) filing. Animal studies are designed with consideration to the expected dosing plan for humans so as to distinguish chronic effects (regular dosing over extended time periods) from acute effects (single dose). The *maximum tolerated dose* (MTD), the maximum dose at which no toxic effects are observed, is determined, and dosing proceeds up to a level that produces a toxic effect that can be used as a marker during first in human phase I studies. Animal studies provide clinical researchers with the basis for estimating the safety window (*therapeutic index*), which is the range between the concentration that is predicted to cause human toxicity and the concentration that is predicted to produce human efficacy. For example, it may be determined as the dose at which 50% of individual animals demonstrate a particular toxic response (e.g., death, tumor formation, side effects; i.e., LD_{50}) divided by the dose at which 50% of individual animals demonstrate an efficacious response (i.e., ED_{50}). A large safety window improves the chances of the drug being safe in the clinic. Investigators must evaluate the benefit versus risk to patients of new chemical entities. Pharmacokinetic differences between animals and humans are taken into consideration. In many cases, some toxicity is manageable if the drug provides important benefits to patients. Toxic effects may be reversible by the body (e.g., liver damage) or they may be permanent (e.g., cancer, brain damage, teratogenicity). Differences in absorption, distribution, metabolism, and elimination between people are better tolerated with a wider window. Toxicity may be observed during phase I human studies that was not observed in animals during preclinical safety studies.

17.1.1 Toxicity Terms and Mechanisms

Toxicity can be caused by many different mechanisms. The biochemical mechanism that initiates toxicity might be related to the following:

- Therapeutic target
- Off-target
- Reactive metabolic intermediates

Unintended effects of modulating the therapeutic target can be toxic. These are referred to as *target-* or *mechanism-based toxicity*. The compound also can affect other enzymes, receptors, or ion channels in the body; these are referred to as *off-target*. The effects of target or off-target modulation can be observed as pharmacological (functional effect), pathological (lethal effect), or carcinogenic (producing cancer). Reactive metabolites produce undesirable effects by covalent reaction with endogenous macromolecules, which can lead to cell death, carcinogenicity, or immunotoxicity.

Some of the safety issues and terms of interest in drug discovery are as follows (with examples):

Acute toxicity is the toxic response from a single dose. *Chronic* toxicity is the toxic response from long term dosing.

Carcinogenicity is the production of cancer. There is often a long latency period from compound exposure to tumor appearance.

Cytotoxicity is the production of cell death, such as in liver cells (hepatotoxicity).

Genotoxicity is a mutagenic change of the DNA sequence or chromosome damage. It often leads to cancer.

Idiosyncratic reactions are immune-mediated effects having delayed onset and are caused by reactive metabolites. Proposed mechanisms involve cell damage and individual patient conditions, such as enzyme irregularities or polymorphism.

Immunotoxicity is an immune reaction (e.g., lymphoproliferation, autoimmunity) triggered by the compound (e.g., penicillin) or reaction of a metabolite with endogenous macromolecules to form an antigen.

Organ toxicity deteriorates the function of the organ (e.g., heart, liver, kidney, blood cells, brain).

Phospholipidosis is the accumulation of polar phospholipids in lysosomes as lamellated bodies in response to cationic amphiphilic drugs.

Reproductive toxicology is a change in reproductive systems, normal function, or sexual behavior.

Safety pharmacology studies the effects of a compound on normal physiological functions. Abnormal function is often caused by inhibition of enzymes or antagonism or agonism of receptors or ion channels.

Teratogenicity is embryo toxicity or abnormal development. (The effect of thalidomide on human embryo development is well known.)

17.1.2 Toxicity Mechanisms

17.1.2.1 Therapeutic Target Effects

Modulation of the therapeutic target can have undesirable effects in the target tissue or elsewhere in the body. These effects are observed in animal dosing studies or reports from human clinical use.

17.1.2.2 Off-Target Effects

The lead compound or series might inhibit an unintended enzyme or receptor in the body. This disrupts the normal function and can cause toxicity or side effects. If these are not severe and can be tolerated by the patient, they may be manageable in clinical use of the drug.

One example of off-target effects is drug–drug interaction (DDI), where a drug interferes with the body's absorption, distribution, metabolism, or elimination of a second coadministered drug. DDI can cause a buildup of the second compound to a concentration that causes toxic effects. This is discussed in Chapter 15. DDI also can occur at other metabolic enzymes and at transporters involved in elimination of a drug by the liver or kidney.

Inhibition can occur at a protein that is unrelated to the compound's intended mechanism. For example, inhibition of hERG is discussed in Chapter 16. Compounds having certain physicochemical and structural features can bind in the hERG K^+ ion channel and prevent influx of K^+ ions, resulting in lengthening of the QT interval, which may trigger a fatal arrhythmia.

Just as the lead compound has agonist or antagonist activity at the project's target, it also can have activity at another target in the body. The effects may sometimes be helpful, as other potential positive uses are discovered for the compound. However, the effects can disrupt a biochemical biosynthesis or signaling pathway.

Off-target effects usually are screened in drug discovery by sending the compound to an outside laboratory (e.g., Novascreen, MDS PanLabs). Such laboratories have an array of biochemical assays for diverse targets against which the compound can be rapidly screened for activity. Off-target effects also are revealed in animal dosing studies in which an array of normal physiological functions is monitored.

17.1.2.3 Reactive Metabolites

A major cause of drug toxicity is metabolic bioactivation.[4–11] In most cases, metabolism serves the important function of chemically modifying the compound to make it more polar and, thus, more readily cleared into the bile and urine. An unfortunate consequence of metabolism is that a reactive metabolite or intermediate can cause toxicity. The reactive intermediate or metabolite might be an electrophile, which covalently binds to endogenous nucleophiles (e.g., proteins, DNA). The resulting adduct does not function normally. It can cause DNA mutation, which can lead to cancer. Modifications of proteins can elicit an immune response. Often these reactions occur in the liver, leading to hepatotoxicity, but the reactive metabolites can also cause damage at distal sites. Glutathione is a ubiquitous molecule that acts as a scavenger of electrophiles. However, it does not capture all of the reactive metabolites, especially when the cell is in oxidative stress (see Section 17.1.2.5). Some drugs are activated to toxic or allergenic degradants by exposure to light in the skin (e.g., sulfonamides). Idiosyncratic drug reactions appear to be initiated by reactive metabolites.[6,12]

Excellent reviews are available on the numerous mechanisms of metabolic activation of diverse functional groups and structural classes.[4,5,12] Some compounds are activated to multiple metabolites, and proving which compound or intermediate is responsible for the toxic response can be quite difficult. Table 17.1 lists some substructures that have produced reactive metabolites resulting in toxicity. Medicinal chemists can use this type of information to plan lead series analog synthesis and anticipate issues that might arise if one of these substructures is used in lead series expansion.

TABLE 17.1 ► Partial List of Substructures that may Initiate Toxicity and Their Proposed Reactive Metabolites[4,5,7–10,16]*a*

Substructure	Proposed reactive metabolite
Aromatic amine	Hydroxyl amine, nitroso, quinone-imine (oxidative stress)
Hydroxyl amine	Nitroso (oxidative stress)
Aromatic nitro	Nitroso (oxidative stress)
Nitroso	Nitroso, diazonium ions (oxidative stress)
Alkyl halide	Acylhalide
Polycyclic aromatic	Epoxide
α,β-Unsaturated aldehyde	Michael acceptor
Carboxylic acid	Acyl glucuronide
Nitrogen-containing aromatic	Nitrenium
Bromo aromatic	Epoxide
Thiophene	*S*-oxide, epoxide
Hydrazine	Diazene, diazonium, carbenium ion
Hydroquinones	*p*-Benzoquinone
o- or *p*-Alkylphenols	*o*- or *p*-Quinone methide
Quinone	Quinone (oxidative stress)
Azo	Nitrenium
Furans	α,β-Unsaturated dicarbonyl
Pyrrole	Pyrrole oxide
Acetamide	Radical (oxidative stress)
Nitrogen mustard	Aziridium ions
Ethinyl	Ketene
Nitrosamine	Carbenium ion
Polyhalogenated	Radicals, carbene
Thioamide	Thiourea
Vinyl	Epoxide
Aliphatic amines	Iminium ion
Phenol	Quinine
Arylacetic, arylpropionic acids	
Imidazole	
Medium-chain fatty acids	

[a]These have been termed *toxic alerts*.

Figures 17.2 through 17.6 are examples of substructures that undergo metabolic activation mechanisms that lead to toxicity. Aniline is oxidized by cytochrome P450 (CYP) to form hydroxyl amine and nitroso (Table 17.1). These electrophiles react with proteins to form adducts, which induce an immunologic response. One mechanism for this process is sulfation

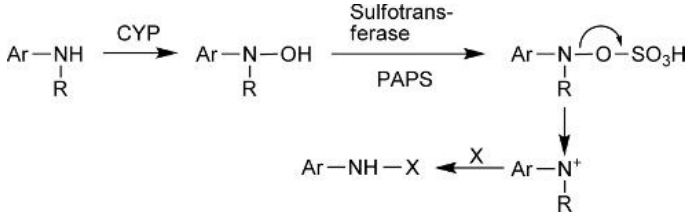

Figure 17.2 ► Primary and secondary amines (especially aromatic amines) form an *N*-hydroxyl amine, which produces a reactive nitrenium intermediate and reacts with a nucleophile "X" (e.g., DNA) to cause genotoxicity.[4,10] Example: acetaminofluorene.

by sulfotransferase, followed by elimination to form the electrophilic nitrenium ion, which reacts with a nucleophile.[10] Hydroxyl amines also can be converted to quinones, etc., which cause oxidative stress.

Carboxylic acids can be glucuronidated in the liver by UDP-glucuronosyltransferase (Figure 17.3). The compound migrates around the glucuronic acid molecule, which then opens to form a reactive center and produces adducts to protein molecules.[13] Alternatively, the drug adducts to the protein following ring opening.

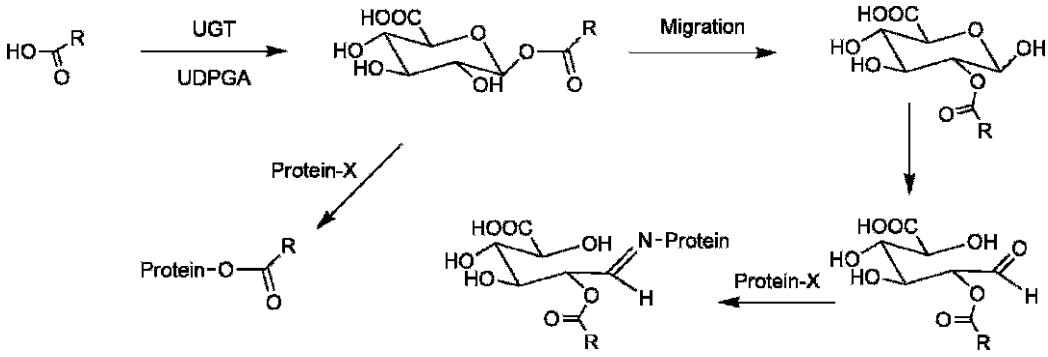

Figure 17.3 ► Carboxylic acids can be glucuronidated to form an acyl glucuronide, followed by rearrangement and reaction with a nucleophile (e.g., DNA, protein) to cause genotoxicity.[10] A proposed mechanism is shown (J. Wang, personal communication, 2006). X is $-NH_2$, –OH, or –SH. Examples: bromfenac, diclofenac.

Unsaturated bonds can be epoxidized by CYP (Figure 17.4). The epoxide readily reacts.[10] This mechanism can lead to adducting to DNA, which can cause mutation and trigger cancer.

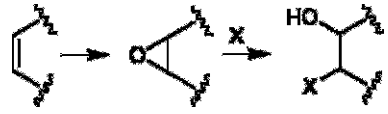

Figure 17.4 ► Epoxidation of unsaturated bonds leads to attachment to DNA and protein. Examples: aflatoxin B_1 with DNA (guanine), bromobenzene with protein (thiol groups).

Nitroaromatics are reduced to form reactive nitro radical, nitroso, nitroxyl radical, and aromatic *N*-oxide (Figure 17.5). These can induce oxidative stress (see 17.1.2.5). Thiophene is oxidized to sulfoxide, which can react with protein sulfhydryl groups (Figure 17.6).

The effects of reactive metabolites can be observed with in vivo dosing studies that include monitoring of normal physiological function and are followed by histopathological examination for abnormal tissue appearance. A large number of in vitro assays are available

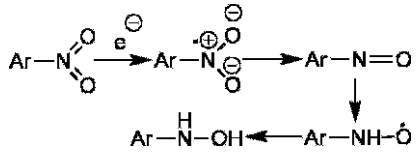

Figure 17.5 ► Nitroaromatics can be reduced to form several reactive intermediates: nitro radical, nitroso, nitroxyl radical, and aromatic *N*-oxide.

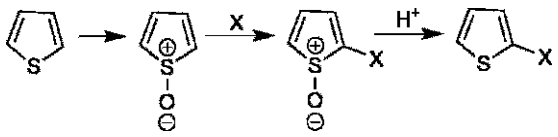

Figure 17.6 ► Thiophene oxidation is followed by attack on protein (sulfhydryl group) to produce covalent binding. Example: tienilic acid. Epoxide formation has also been proposed as a bioactivation mechanism.[4]

(see Chapter 35) to study the effects of mutation (e.g., Ames), chromosome damage (e.g., micronucleus), and cell toxicity (e.g., hepatotoxicity).

17.1.2.4 Gene Induction

A compound can induce the expression of a gene and its protein product. For example, a compound might activate pregnane X-receptor (PXR), which ultimately results in induction of higher levels of CYP3A4. The higher 3A4 levels result in faster metabolism of 3A4 substrates. Rifampicin can induce 3A4, which causes higher metabolism of coadministered oral contraceptives, resulting in their loss of efficacy.[14] Assays have been developed to indicate induction via PXR or aryl hydrocarbon receptor (AHR) activation (see Chapter 35).

17.1.2.5 Oxidative Stress

Some compounds undergo redox cycling (one electron reduction to the radical followed by reoxidation) within the cell and induce an oxidative environment through enhancing the process of oxygen reduction. Normally cells are maintained in a reducing environment by enzyme systems and glutathione. Oxidative stress results in the increase of free radicals and peroxides. These can abstract a hydrogen atom from lipids, glutathione, and DNA, resulting in cell damage and death. Examples of structures that induce oxidative stress are aromatic amines, aromatic nitros, and quinones.

17.2 Toxicity Case Studies

Matrix metalloproteinase (MMP) inhibitors have been studied clinically for treatment of degradation of collagen in arthritis, angiogenesis, and tumor growth. Unfortunately, MMP inhibitor candidates also cause musculoskeletal syndrome (MSS), a tendonitis-like fibromyalgia in humans. Studies have shown that the side effects are not caused by target-specific MMP-1 inhibition but more likely are due to nonselective inhibition of one or more other metalloproteinases. MMP inhibitor compounds that chelate zinc appear to be responsible. Research on new MMP inhibitors is focusing on nonchelators of zinc to reduce this off-target toxicity.[15]

Rofecoxib (Vioxx) was withdrawn from the market after a clinical study showed that patients receiving a 25-mg dose for 19 months had a 3.9-fold increase in thromboembolic adverse events (heart attack). The mechanism remains unclear[16] but may be related to the therapeutic target cyclooxygenase-2 (COX-2).

Troglitazone was withdrawn from the market in 2000 because of human hepatic failure. In vitro studies show that CYP3A4 oxidation of the chromane ring or the thiazolidinedione ring produces an electrophilic intermediate that covalently binds to protein.[4]

In high doses, acetaminophen causes liver damage. As shown in Figure 17.7, oxidation of acetaminophen by CYP produces *N*-acetyl-*p*-benzoquinoneimine (NAPQI), which reacts with nucleophiles, including sulfhydryl groups of proteins.

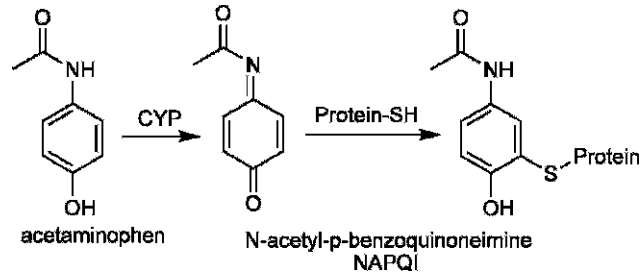

Figure 17.7 ► Proposed mechanism of acetaminophen metabolic activation and covalent binding to protein sulfhydryl groups.[3,4]

17.3 Structure Modification Strategies to Improve Safety

Medicinal chemists take several approaches to avoid or reduce toxicity during the discovery stage. The optimum time to obtain toxicity data for use in redesign of compound structures to reduce toxicity is during the active phase of analog synthesis in lead optimization. Structural strategies for toxicity include the following:

1. Avoid substructures that are known to induce toxic responses. During lead selection, compounds containing potentially toxic substructures (Table 17.1) can receive a lower priority.
2. Early synthetic modifications should be undertaken to remove any potentially toxic substructures from lead series.
3. Potentially toxic substructures should not be added to lead series structures during lead optimization. (If they are made for structure–activity relationship exploration purposes only, they may be acceptable.)
4. Perform reactive metabolite assays (as indicated in Chapter 35) to screen for potentially toxic compounds. In vitro assays range from trapping of glutathione conjugates to screening of DNA mutagenicity following S9 metabolic activation. Data indicating potential toxicity from these tests do not guarantee that toxicity will be observed in vivo, but they provide an early warning that can improve discovery efficiency.
5. Data indicating potential toxicity from in vitro assays can be further investigated by structure elucidation of the metabolites or trapped intermediate using spectroscopy. It can be difficult to definitely assign the toxicity to a specific metabolite; however, the data may point in a direction that can be followed as a possible hypothesis. Knowledge of reactive metabolite structures can suggest structural modifications to reduce metabolism.
6. Utilize the metabolite structural modification strategies discussed in Chapter 11 to attempt to reduce metabolic bioactivation.

Problems

(Answers can be found in Appendix I at the end of the book.)

1. Define therapeutic index (safety window). Is it preferable for this to be small or large?
2. How can reactive and unreactive metabolites be distinguished?

3. Which of the following moieties might themselves be or might form a reactive metabolite: a) aniline, b) thiophene, c) phenol, d) ester, e) Michael acceptor, f) propyl alcohol
4. Define oxidative stress.
5. How is gene induction toxic?
6. Why are off-target effects toxic?

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Integrity and Purity

Overview

- *Data from testing a compound with the incorrect identity or low purity can affect the structure–activity relationship.*
- *The sample may have decomposed, been misidentified, or have significant impurities.*

The quality of materials used in drug discovery plays a crucial role in the success of discovery research. If there is an unknown problem with the quality of the material, the viability of the biological and absorption, distribution, metabolism, excretion, and toxicity (ADME/Tox) property studies will be in question or will confuse the discovery project team. For this reason, integrity and purity profiling of discovery compounds can be as important as other physicochemical and metabolic properties of the compounds.[1]

18.1 Fundamentals of Integrity and Purity

Integrity refers to whether the material is the same as the identity or structure that is recorded in the company's database. *Purity* refers to the percentage of the material that is the correct structure, as opposed to impurities in the material.

Medicinal chemists deal directly with integrity and purity for each new compound and batch that is synthesized. This is commonly accomplished using nuclear magnetic resonance (NMR) and mass spectrometry (MS) for structure verification and high-performance liquid chromatography (HPLC) for purity checking. Despite this early verification of the compound, there are many other stages of discovery where it is wise to requalify the materials.

18.2 Integrity and Purity Effects

Discovery experiments assume that test compounds have the correct identity and are relatively pure. Compounds come into discovery biology and ADME/Tox laboratories from many sources. It is prudent to verify the quality of compounds in order to produce an accurate structure–activity relationship (SAR).

The sources of test compounds include the following.

- Company repository provides the compound:
  - Solid from a storage bottle
  - Solution from a repository solution stock
  - Solution from a well plate used for high-throughput screening (HTS)

- Company collaborator laboratory provides the compound as a solution from a well plate or vial that has been stored in the laboratory
- Partner research company provides the compound
- Purchased from an outside materials sourcing firm
- Solution or solid from a diversity array that was synthesized using parallel (combinatorial) synthesis

Each of these cases has the opportunity for integrity or purity errors. Of particular note are the age and storage conditions of the sample prior to reaching the assay laboratory, human errors in manual handling, and quality of the original structural and purity analysis. Discovery researchers should consider the quality of the material as a variable in the experiment.

The case of HTS is illustrative. For HTS, all the compounds from the corporate screening library are individually placed in solution and transferred to plates. These plates are stored and then retrieved and thawed for HTS runs. In this way, hundreds of thousands of structurally diverse compounds are biologically tested with the therapeutic target protein for activity. When activity is observed for a compound, this "hit" obtains greater interest by medicinal chemists as a possible lead. Verification of the hit identity and purity before further studies is valuable. Experience indicates that some compounds in screening libraries have inaccurate identity or low purity. Misidentification of the HTS hit compound can lead to the wrong SAR conclusions from the beginning of the project. An impurity in the solution can itself be active, whether or not the putative component is active. This can confuse and mislead the research team. In the same way, incorrect assignments of identity or poor purity of compounds from corporate repositories or external sources can lead to unproductive and time-consuming investigations.

In addition to problems with SAR, integrity and purity can affect assessment of the properties of compounds. Impurities can produce false-positive signals in property assay detection methods, such as UV plate readers, light scattering detectors, and fluorescence plate readers. An impurity could be the cause of a response in some assays, such as cytochrome P450 (CYP) inhibition. This will compromise the property results and lead to inaccurate structure–property relationships (SPR), which are used to select leads or improve the properties of leads.

There are several causes for problems with integrity and purity of some materials. Companies often collected compounds over many years. These compounds came from many origins. Some of the compounds were synthesized as long as 30 to 50 years ago and were stored under various conditions that were later found to be unsuitable. Compounds were collected from various companies that merged to form the present company. Compounds came from university laboratories and chemical companies that had various criteria for quality. They may have been synthesized as libraries by contract companies and never individually tested. Usually, the chemist who synthesized the compound is not accessible to the current discovery scientists, so no first-hand knowledge of the material is available.

Another source of error is misidentification. Mistakes in handling or labeling during transfer and weighing occur. Material can be cross-contaminated into another sample. In recent years, automated sample handling has reduced such errors, but manual handling is necessary for some steps.

Compounds may have been incorrectly characterized when they initially were placed in the collection. A spectrum may have been misinterpreted. The compound may have been synthesized before modern NMR and MS techniques were common. Significant levels

of impurities may be present from starting materials or reaction by-products that went undetected.

Compounds may have decomposed. Storage in the solid form exposes the compound to oxygen and water from the air, which can react to form degradants. Counter-ions can react with the compound. Light and elevated temperature accelerate decomposition reactions.

Compounds can degrade in solutions that are stored in the laboratory prior to experiments. Water and oxygen from the air can dissolve in solutions. Cooling of solutions in the refrigerator can cause water from the air to condense and dissolve in the solution. Light from the laboratory can degrade certain compounds. Storage vials can catalyze reactions.

18.3 Applications of Integrity and Purity

It is useful for discovery scientists to check the purity and integrity of compounds at certain times. Here are a few examples of when verification is beneficial:

- Newly synthesized compounds, to assure that they are what was intended and that they have an acceptably low level of impurities
- Compounds that are being collected and formatted into plates for HTS screening, so that early discovery efforts in lead selection are not misled
- Compounds that were identified as potential actives from similarity searching of the corporate collection, so that crucial SAR assignments from a series are accurate
- HTS hits, to assure that they have not degraded, been misidentified, or been mislabeled
- Materials that are being approved for late discovery toxicology, pharmacology, or selectivity testing, to assure that the right compound is being tested and that any negative results (e.g., toxicity, activity at another receptor or enzyme) are not due to impurities

The different tasks require different methodologies, depending on the level of detail that is necessary to answer the research question and the cost that is reasonable at that point. In some cases, the integrity and purity examination may seem like overkill, but without it mistakes may be made if the compounds are assumed to be correct and pure.

18.3.1 Case Study

Popa-Burke et al.[2] provided an example of potential integrity and purity problems of compound libraries. Prior to placing compounds from vendors into a screening library, they were checked for identity using a rapid MS-based method. Approximately 10% of the compounds did not pass the identity check. Compounds from "historical" libraries had lower quality than recently synthesized compounds, suggesting potential degradation or initial misidentification. Furthermore, testing of compound solution concentration showed that the quantity of a compound could vary greatly from the intended concentration, thus compromising the IC_{50} measurements and rank ordering of compounds.

Without integrity and purity profiling, discovery experiments must proceed at risk. If compounds of poor integrity and purity slip into the project, the trends will be made less clear for concluding how the project should proceed.

Problems

(Answers can be found in Appendix I at the end of the book.)

1. What are the negative effects of low purity or inaccurate structural identity?
2. How might a sample have low purity or the wrong identity?
3. What techniques can be used for purity and integrity determination?: (a) HPLC, (b) NMR, (c) LC/MS.
4. Ensuring the integrity and purity of your HTS hits and project compounds does which of the following?: (a) ensures good drug-like properties, (b) avoids erroneous SAR.

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Pharmacokinetics

Overview

- *Pharmacokinetics (PK) studies the concentration time course of compounds and metabolites in vivo.*
- *Key parameters are volume of distribution, area under the curve, clearance, $t_{½}$, C_{max}, and bioavailability.*
- *PK data are heavily used by discovery teams and correlated to pharmacodynamics.*

19.1 Introduction to Pharmacokinetics

After a compound is administered, its concentration in the bloodstream and tissues changes with time, first increasing as it enters systemic circulation and then decreasing as it is distributed to tissues, metabolized, and eliminated. Pharmacokinetics (PK) is the study of the time course of compound and metabolite concentrations in the body.[1]

The PK parameters of a compound (e.g., clearance, half-life, volume of distribution) result from its physicochemical and biochemical properties (see Figure 2.1). These properties are determined by the structure of the compound and the physical and biochemical environment into which the compound is dosed. The dependence of PK parameters on these fundamental properties and on molecular structure is the reason we use property data to predict and improve the PK parameters of discovery compounds. The specific effects of individual physicochemical and biochemical properties on PK parameters are discussed in the chapters on each property and in Chapter 38.

When a compound is administered directly into the bloodstream by intravenous injection (IV), rapid circulation of the blood mixes the compound throughout the entire blood in minutes. Permeation of the compound from the blood capillaries into the tissues begins immediately and is called *distribution*. This rapidly reduces the concentration of compound in the blood during the distribution phase (Figure 19.1). The concentration of free compound in the tissues approaches equilibrium with the concentration of free compound in the blood.

At the same time, compound is being removed from the bloodstream, primarily by the liver and kidneys, in the process termed *elimination*. The elimination phase typically follows first-order kinetics. If the compound is rapidly eliminated, the rate of decrease during the elimination phase is high, and the compound concentration quickly approaches the baseline. As the free drug concentration in the blood drops, the compound permeates back out of the tissues and into the bloodstream.

The time course of compound concentrations is different with other routes of administration. When a compound is administered orally, it must first dissolve, permeate through the gastrointestinal membrane, and pass through the liver before it reaches systemic circulation. As a result, there is a time delay until the compound reaches its peak concentration in the blood (C_{max}). Once it reaches the bloodstream, it undergoes distribution to the tissues and

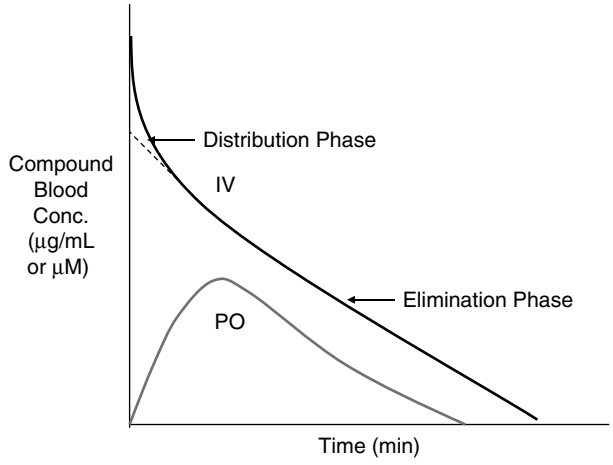


Figure 19.1 ► Hypothetical example of compound concentrations in blood over time following administration by intravenous (IV) and oral (PO) routes.

elimination. Compound exposure is indicated by the area under the time–concentration curve (area under the curve). Oral exposure usually is lower than IV exposure after dose normalization. This is because of the additional barriers (e.g., permeation, solubility, intestinal decomposition) that limit intestinal absorption as well as first-pass metabolism and biliary extraction that eliminate compound material before it reaches systemic circulation. These barriers are not encountered in IV dosing.

19.2 PK Parameters

Several key PK parameters are used in drug discovery, development, and clinical practice. These parameters are reported for discovery PK studies; thus, it is useful to understand their meaning and how they relate to the physicochemical, metabolic, and structural properties of the compound.

19.2.1 Volume of Distribution (V_d)

Volume of distribution (V_d) indicates how widely the compound is distributed in the body. Its units typically are L/kg or mL/kg of body weight. V_d is not a real measurable volume. Instead, it represents the apparent volume into which the compound is dissolved. V_d is a proportionality of compound concentration in plasma to total compound in the body throughout the time course (Figure 19.2).

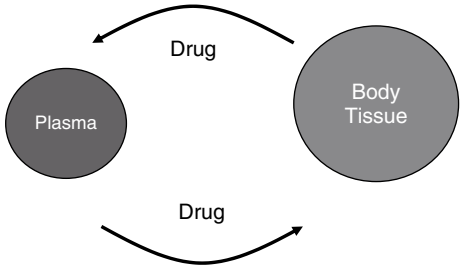


Figure 19.2 ► Volume of distribution (V_d) indicates the equilibrium established by the proportion of compound in the plasma to total compound in the body.

V_d is dependent on the properties of the compound:

- Compounds that are *highly and tightly bound to plasma protein* tend to be restricted to the bloodstream and do not enter the tissues in significant amounts. V_d is close to the volume of blood (approximately 0.07 L/kg).
- Compounds that are *hydrophilic* tend to be restricted to the bloodstream and do not enter the tissues in significant amounts. V_d is close to the volume of blood (approximately 0.07 L/kg).
- Compounds that are *moderately lipophilic and moderately bound to plasma protein and tissue components* tend to distribute evenly throughout the blood and tissues. V_d is in the range of the volume of body water (approximately 0.7 L/kg).
- Compounds that are *highly lipophilic* tend to bind to tissue components (e.g., proteins, lipids), and there is very low blood concentration. V_d exceeds body water volume (0.7 L/kg) and may reach levels as high as 200 L/kg.

Figure 19.3 shows these ranges of V_d. Examples of values of V_d for commercial drugs are listed in Table 19.1. Values range from the V_d of warfarin, which is primarily found in the bloodstream and binds tightly to plasma proteins, to those of imipramine and chloroquine, which are primarily found in the tissues and bind tightly to tissue components.

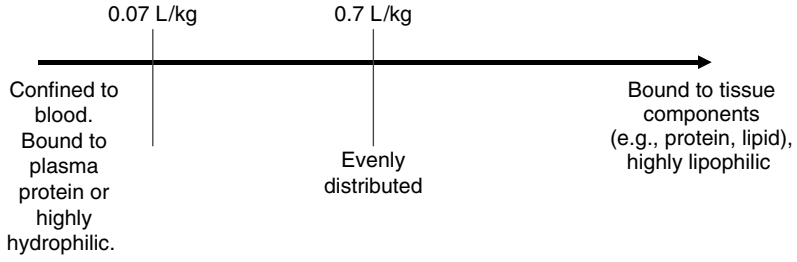


Figure 19.3 ► Diagnosing compound binding locations using V_d.

TABLE 19.1 ► Values of V_d for Some Commercial Drugs

Drug	V_d (L/kg)
Warfarin	0.11
Salicylic acid	0.14
Theophylline	0.5
Atenolol	0.7
Quinidine	2
Digoxin	7
Imipramine	30
Chloroquine	235

The binding of a compound to plasma protein versus tissue components is captured in the expression:

$$V_d = V_{blood} + V_{tissue} \bullet (f_{u,blood}/f_{u,tissue}),$$

where $f_{u,blood}$ = fraction of compound unbound in blood, and $f_{u,tissue}$ = fraction of compound unbound in tissue. This relationship explains the categories of compounds described above. If a compound is highly and tightly bound in blood, $f_{u,blood}$ is low and V_{blood} predominates. If a compound is highly bound in tissue, $f_{u,tissue}$ is low and V_{tissue} predominates. A high V_d does not guarantee high therapeutic target exposure ($f_{u,tissue}$), only that $f_{b,tissue}$ (bound drug in tissue) is high. Increasing tissue binding and increasing V_d are related to increasing lipophilicity.

Compound molecules are carried to the tissues by systemic circulation. Thus, compound distributes fastest to tissues that are highly perfused with blood (high blood flow). These tissues include brain, heart, lung, kidney, and liver. Compound distributes slowest to tissues that have lower perfusion, such as skeletal muscle, bone, and adipose tissue.

V_d is calculated in PK experiments using the following expression:

$$V_d = \text{Dose}/C_0,$$

where dose = compound dosed mass/animal mass (e.g., mg compound/kg body weight), and C_0 = initial blood concentration of compound after an IV dose. C_0 is determined by plotting the log of the blood concentrations versus (linear) time and extrapolating back to time zero.

19.2.2 Area Under the Curve (AUC)

Drug exposure is evaluated using the PK parameter area under the curve (AUC). This is the area under the compound blood concentration–time plot (Figure 19.4). Comparison of AUCs from lead series analogs provides a means to select the compounds that produce the highest exposure levels. It has been reported that, with a 10 mg/kg oral does, if the AUC from 0 to 6 hours is >500 ng•h/mL, then there is an 80% chance of an acceptable bioavailability (>20%F).[2] Structure–AUC (exposure) relationships also can be developed. Another use of AUC is to calculate other PK parameters, such as clearance and bioavailability (see Sections 19.2.3 and 19.2.5).

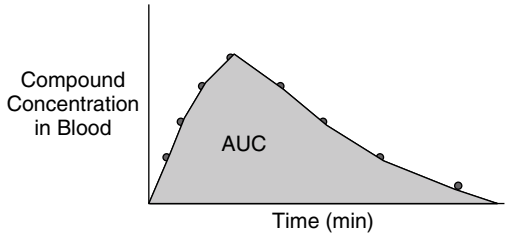


Figure 19.4 ► Area under the curve (AUC) is the area under the compound plasma concentration–time plot.

19.2.3 Clearance (Cl)

Another important PK parameter for discovery scientists is clearance (Cl). It indicates how rapidly the compound is extracted from systemic circulation (bloodstream) and eliminated. Clearance occurs primarily in two organs: kidney and liver.

A portion of cardiac output (i.e., blood flow) goes to the kidney (Figure 19.5). In the kidney, compounds and metabolites in blood are extracted into the urine by glomerular filtration and active secretion by transporters. This results in compound and metabolite elimination. Clearance specifically from the kidney can be determined in detailed PK studies and is termed *renal clearance* (Cl_R).

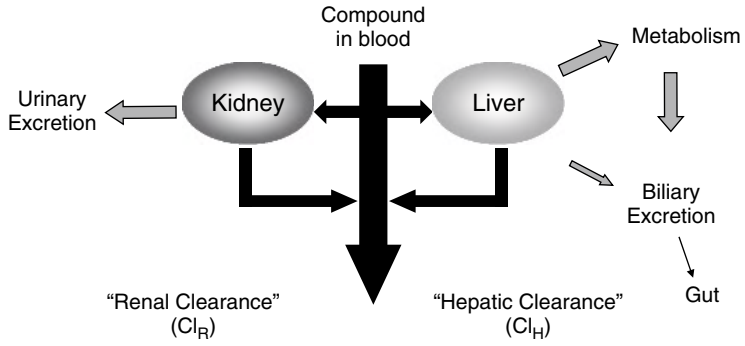


Figure 19.5 ► A significant portion of cardiac blood output flows to the kidney and liver, where a portion of the free compound in solution is extracted and cleared from the body.

The liver also receives a portion of the cardiac output. Compound permeates from blood into hepatocytes by passive diffusion and active transport. Within the hepatocytes, a variety of enzymatic reactions metabolize the compound. Metabolites and a portion of unchanged compound may be extracted into the bile ("biliary extraction") by passive diffusion or active transport into the bile canaliculus. Bile is stored in the gallbladder and excreted into the intestine, from which compounds and metabolites are eliminated in feces. Hepatic clearance (Cl_H) can be determined in detailed PK studies. Metabolites and unchanged compound can also exit the hepatocytes into blood by passive diffusion and active transport and be extracted by the kidney into urine. The amount of compound and metabolites that are extracted into bile or urine depend on their properties (e.g., passive diffusion, transporter affinity, metabolic stability).

The term *systemic clearance* (Cl_S) is used to indicate the total clearance of compound from all sources. Cl_S is the sum of Cl_R and Cl_H. Minor routes of clearance include saliva, sweat, and breath. The units used for clearance typically are mL/min/kg.

Clearance by an organ is determined by two factors: blood flow into the organ (Q) and extraction ratio by the organ (E; Figure 19.6), as follows:

$$Cl = Q \bullet E.$$

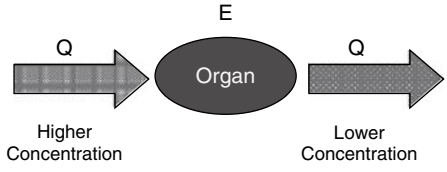


Figure 19.6 ► Extraction of compound by the liver or kidney is dependent on the blood flow to the organ (Q) and the extraction ratio of the organ (E).

Blood flow into the liver and kidney are well characterized in each species used for drug discovery PK studies. The total blood flow from the heart is also known and is termed *cardiac output*. The extraction ratio is the portion of compound removed by the organ with each pass of blood through the organ. For example, if cardiac output to the liver (Q_H) is 55 mL/min/kg and hepatic clearance (Cl_H) is measured as 25 mL/min/kg, then the hepatic extraction ratio (E_H) is 0.45 (i.e., 45% of the compound is removed with each pass through the liver).

E is constant for a specific compound, unless the capacity of the organ is saturated by high compound concentration in blood. The highest value for E is 1 (100%); thus, the highest value for Cl is the blood flow to the organ. Blood flow to organs of some discovery

TABLE 19.2 ► Blood Flow to Organs in Some Species Used in Drug Discovery

	Blood flow (mL/min/kg)		
Species	Hepatic	Renal (GFR)	Total
Mouse	90	15	400
Rat	55	5	300
Monkey	30	2	220
Dog	30	5	120
Human	20	2	80

GFR, Glomerular filtration rate.

species are listed in Table 19.2. The term *glomerular filtration rate* (GFR) is often used with regard to renal clearance. GFR is the flow rate of fluid passing by filtration from the glomerulus into the Bowman's capsule of all the nephrons in the kidney (typically in units of mL/min/kg body weight).

Clearance is calculated from intravenous (IV) PK studies using the dose and total compound exposure from the expression:

$$\mathrm{Cl} = \mathrm{Dose}/\mathrm{AUC}_{\mathrm{IV}}$$

Thus, if clearance is determined, it can be used to estimate the dose that must be administered to provide a level of compound exposure necessary for the therapeutic effect. For example, compounds with higher clearance require higher doses to achieve a certain exposure (AUC) level for in vivo activity. Metabolic stability is a major determinant of hepatic clearance. Thus, medicinal chemists have the opportunity to reduce clearance by structurally modifying the compound to reduce metabolism. Chemists can use in vitro metabolic stability half-life to correlate to Cl.

19.2.4 Half-life ($t_{½}$)

The time for the concentration of a compound in systemic circulation to reduce by half is termed *half-life* ($t_{½}$). Compound clearance typically follows first-order kinetics, and a plot of log compound concentration versus time indicates the elimination rate constant (k) from the slope (Figure 19.7). $t_{½}$ is calculated from k using the expression:

$$t_{½} = 0.693/k.$$

$t_{½}$ also can be calculated from V_d and Cl using the expression:

$$t_{½} = 0.693 \bullet V_d/\mathrm{Cl}.$$

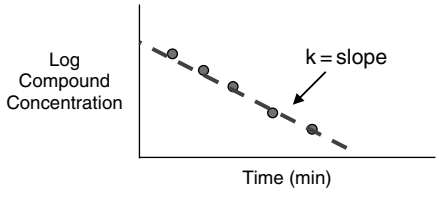


Figure 19.7 ► First-order rate constant (k) for elimination is obtained from a plot of log compound concentration versus time.

Thus, PK half-life is determined by Cl_H (metabolic stability and biliary clearance) Cl_R, and V_d. Chemists should not assume that in vitro metabolic stability half-life correlates to PK half-life. Half-life allows an estimation of how often the compound needs to be redosed to maintain the in vivo therapeutic concentration. Redosing typically is performed every 1 to 3 half-lives.

Another related PK parameter is mean residence time (MRT). This is the time for elimination of 63.2% of the IV dose.

19.2.5 Bioavailability (F)

One of the most commonly used PK parameters is bioavailability (F). It is the fraction of the dose that reaches systemic circulation unchanged. Less than 100% bioavailability typically results from incomplete intestinal absorption or first-pass metabolism. Secondary causes include enzymatic or pH-induced decomposition in the intestine or blood.

Bioavailability is determined by the following experiment. The compound is dosed IV, plasma samples are collected and analyzed, and AUC_{IV} is calculated. After a period of compound washout, or using different living subjects, the compound is dosed PO, plasma samples are collected and analyzed, and AUC_{PO} is calculated. Bioavailability is calculated from the expression:

$$\%F = (AUC_{PO}/AUC_{IV}) \bullet (Dose_{IV}/Dose_{PO}) \bullet 100\%.$$

Often a lower dose is given IV because of the high concentrations that result from IV dosing. Many companies have a goal of at least 20% oral bioavailability for advancement of a candidate to clinical trials. A compound with poor oral bioavailability can have significant patient variability if its metabolizing cytochrome P450 (CYP) isozyme is polymorphic in the population. Bioavailability is sometimes determined for other dosing routes versus IV, in order to diagnose issues such as absorption or first-pass metabolism. A compound with low bioavailability risks high patient-to-patient variability of blood concentrations, especially if the compound is metabolized by an enzyme having expression levels that vary greatly in the population.

19.3 Effects of Plasma Protein Binding on PK Parameters

The binding of compounds to plasma proteins is discussed in detail in Chapter 14. Compound molecules that are bound to plasma protein cannot permeate through membranes. Thus, the percentage of protein-bound compound molecules and their binding affinity determine how much of the compound permeates out of the blood capillaries and into the disease tissues for therapeutic action, other tissues for general distribution, and kidney and liver for clearance.

19.4 Tissue Uptake

Tissue uptake is important for the compound to reach the therapeutic target. Blood–organ barriers limit penetration into some tissues. For example, CNS drugs must penetrate into brain tissue through the blood–brain barrier (BBB; see Chapter 10). The penetration of cancer drugs into tumors may be reduced, compared to other tissues, by reduced blood flow and tumor morphology. Uptake into the target tissue is often measured to determine the exposure of the target to the compound.

19.5 Using PK Data in Drug Discovery

The major PK parameters used in drug discovery and their methods of determination are summarized in Table 19.3 and Figure 19.8. In addition to the PK parameters discussed in previous sections, Figure 19.8 shows the determination of the following parameters:

- C_{max}, the maximum compound concentration from oral dosing
- t_{max}, the time at which C_{max} is reached

Discovery scientists also examine these data in relation to IC_{50} or EC_{50} to estimate how long the plasma concentration is above the effective in vitro concentration. General classifications for PK parameters in discovery are suggested in Table 19.4. These often differ among companies and projects. Table 19.5 lists the PK parameters of some commercial drugs.[3] PK data for many more compounds are given in reference 3.

It is instructive to note in Table 19.5 the relationships of structural properties to PK performance. Compounds having properties that exceed the Lipinski rules tend to have low oral bioavailability and are administered by nonoral routes (e.g., paclitaxel, doxorubicin). Improvement of structural properties can improve bioavailability (e.g., cefuroxime vs cephalexin). High clearance may result in low bioavailability (e.g., buspirone). The prodrug valacyclovir provides improved PK performance for its active drug acyclovir.

This chapter has summarized the fundamental PK parameters used for drug discovery project team support, in order to assist the interpretation of PK data and planning of in vivo experiments for PK and efficacy studies. Chapter 37 discusses the measurement of PK parameters. Chapter 38 discusses how the compound's properties affect PK parameters so

TABLE 19.3 ► Pharmacokinetic Parameters: Definitions, Calculations, and Applications In Discovery

Pharmacokinetic parameter	Symbol	Description	Calculation	Application
Area under the curve	AUC	Area under the concentration vs time curve	Integrate area under curve	Estimate the level of exposure
Initial concentration	C_0	Initial blood concentration after IV dose	Extrapolate plot of log plasma concentration vs time back to zero time	Calculate V_d
Volume of distribution	V_d	Apparent volume in which compound is dissolved	$V_d = Dose/C_0$	Estimate how widely the compound is distributed in body; calculate half-life
Clearance	Cl	How rapidly compound is extracted from systemic circulation	$Cl = Dose/AUC_{IV}$	Calculate the dose needed to achieve a certain exposure (AUC); diagnose mechanisms of compound elimination
Elimination rate constant	*k*	First-order kinetics elimination rate	Slope of log plasma concentration vs time profile	Calculate $t_{½}$

Continued

TABLE 19.3 ► *Continued*

Pharmacokinetic parameter	Symbol	Description	Calculation	Application
Half-life	$t_{½}$	Time for blood concentration to reduce by half	$t_{½} = 0.693/kt_{½} = 0.693 \bullet V_d/Cl$	Calculate how frequently dose must be administered to maintain a therapeutic concentration
Bioavailability	%F	Fraction (percent) of dose reaching systemic circulation unchanged	$\%F = (AUC_{PO}/AUC_{IV}) \bullet (Dose_{IV}/Dose_{PO}) \bullet 100\%$	Diagnose mechanisms limiting compound exposure (e.g., solubility, permeability, first-pass metabolism)
Maximum concentration	C_{max}	Highest concentration of compound in systemic circulation following dose	Interpolate from plot of blood concentration–time profile	Evaluate if pharmacology is driven by C_{max}, time above IC_{50}, or AUC
Time of maximum drug concentration	t_{max}	Time of C_{max}	Interpolate from plot of blood concentration–time profile	Estimate any delay in reaching target tissue from bloodstream

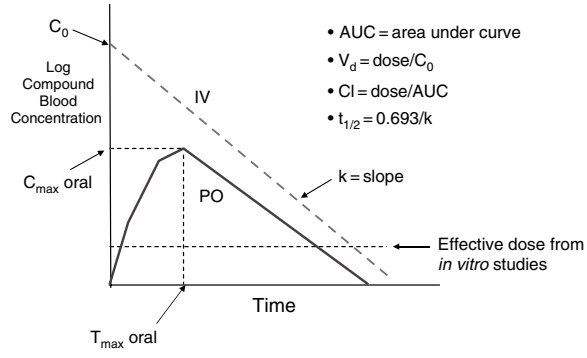
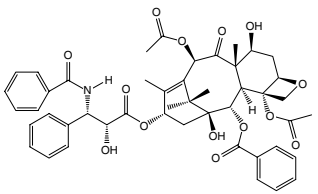
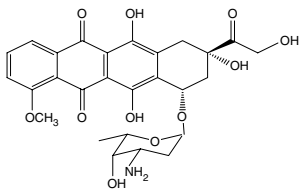
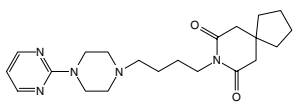


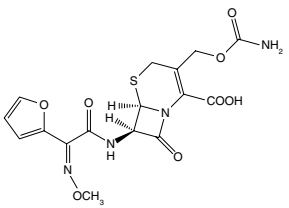
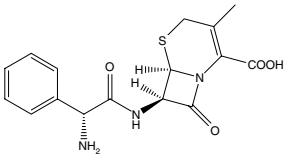
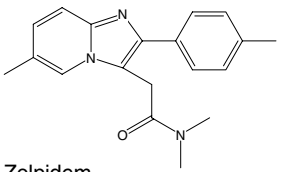
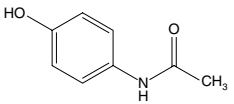
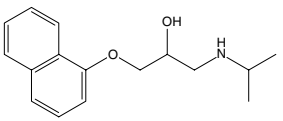
Figure 19.8 ► Pharmacokinetic parameters used in drug discovery and their determination.

TABLE 19.4 ► General PK Parameter Goals for Discovery Compounds

Pharmacokinetic parameter	Symbol	High	Low
Volume of distribution	V_d	>10 L/kg	<1 L/kg
Plasma clearance	Cl	Rat: >45 mL/min/kg Mouse: >70 mL/min/kg Human: >15 mL/min/kg	Rat: <10 mL/min/kg Mouse: <20 mL/min/kg Human: <5 mL/min/kg
Half-life	$T_{½}$	Rat: >3 h Mouse: >3 h Human: >8 h	Rat: <1 h Mouse: <1 h Human: <3 h
Oral bioavailability[2]	%F	>50%	<20%
Oral exposure (10 mpk)[2]	AUC	Rat >2,000 h•ng/mL	Rat <500 h•ng/mL
Time of maximum drug concentration	t_{max}	>3 h	<1 h

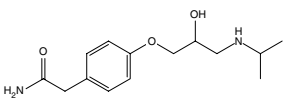
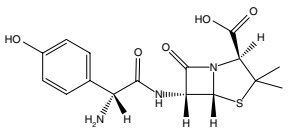
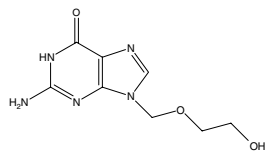
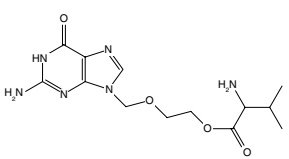
TABLE 19.5 ► Pharmacokinetic Parameters of Selected Drug Compounds[a]

Compound	Oral bioavail-ability (%)	Urinary excretion unchanged (%)	Plasma binding (%)	Cl (ml/min/kg)	Vd (L/kg)	$t_{½}$ (h)	t_{max} (h)	C_{max} (μg/mL)	Dose[b] (mg/kg)	Notes
 Paclitaxel	Low	5	88–98	5.5	2.0	3	—	0.85	250 mg/m^2	IV infusion, oncology
 Doxorubicin	5	<7	76	666	682 L/m^2	26	—	0.95	45 mg/m2	IV infusion, oncology
 Buspirone	3.9	0.1	95	28.3	5.3	2.4	0.71	0.017	0.3	Oral, anxiolytic

 Cefuroxime	32	96	33	0.94	0.2	1.7	2–3	7–10	7.1 oral	Oral, antibiotic
 Cephalexin	90	91	14	4.3	0.26	0.9	1.4	28	7.1 oral	Oral, antibiotic
 Zolpidem	72	<1	92	4.5	0.68	1.9	1–2.6	0.076–0.14	0.14	Oral, sedative
 Acetaminophen	88	3	<20	5	0.95	2	0.33–1.4	20	20 oral	Oral, analgesic
 Propranolol	26	<0.5	87	16	4.3	3.9	1.5	0.049	11	Oral, 4 times per day β-adrenergic-hypertension

Continued

TABLE 19.4 ► *Continued*

 Atenolol	58	94	<5	2.4	1.3	6.1	3.3	0.28	0.7	Oral, β-adrenergic-hypertension
 Amoxicillin	93	86	18	2.6	0.21	1.7	1–2	5	7 oral	Oral, antibiotic
 Acyclovir	15–30	75	15	3.4	0.69	2.4	1.5–2	3.5–5.4 μM	5.7	Oral, 6 times per day, availability increases with dose, antiviral
 Valacyclovir	Val: low Acy: 54%	Val: <1 Acy: 44	Val: 13.5–17.9 Acy: 22–33	—	—	Val: — Acy: 2.5	Val: 1.5 Acy: 1.9	Val: 0.56 Acy: 4.8	14	Oral prodrug for acyclovir, antiviral

[a] Data from reference [3].
[b] mg/kg dose is based on 70-kg average human weight.
Acy, Acyclovir; Val, valacyclovir.

that discovery scientists can diagnose the underlying properties that limit PK and modify the structure to achieve improved PK.

PK data are used throughout drug discovery, development, and clinical treatment. Animal PK data are very useful for discovery projects because they indicate compound behavior in an organism that is used as a model of the human body. Some of the useful applications of PK are as follows:

- Indicate if useful exposure levels can be achieved
- Determine the compound's bioavailability, a key indicator of PK performance
- Reveal PK limitations to prompt structural modifications for improved performance
- Show the dose necessary to produce a concentration at the therapeutic target at which a pharmacological response might be observed in vivo
- Compare lead series analogs to select compounds for more advanced studies
- Establish the relationship of PK parameters to pharmacodynamics (PD; in vivo activity), also known as PK/PD relationship
- Plan dose levels for efficacy and toxicity studies
- Estimate the safety window (therapeutic index; see Chapter 17)
- Measure compound concentration in the target disease tissue to determine penetration through organ barriers (e.g., BBB) and target exposure
- Evaluate potential for drug–drug interaction, based on compound concentration at therapeutic dosing levels
- Extrapolate animal PK parameters to humans to plan initial clinical dosing studies
- Meet company's criteria for advancement

In short, PK data provide key insights that are useful to discovery projects. Chemists optimize structures, pharmacologists plan biological experiments, and team leaders make informed decisions using the data.

Problems

(Answers can be found in Appendix I at the end of the book.)

1. Match the following PK parameters with their definitions:

PK parameter	Definition choices
Cl	a. percentage of the oral dose that reaches systemic circulation unchanged
C_{max}	b. rate a compound is removed from systemic circulation
V_d	c. time for the compound's concentration in systemic circulation to decrease by half
$t_{½}$	d. compound's exposure as determined by blood concentration over time
C_0	e. apparent volume into which the compound is dissolved
AUC	f. highest concentration reached in the blood
%F	g. initial concentration after IV dosing

2. Why does IV bolus administration of a compound have a higher initial concentration than PO administration?
3. What would: (a) enhance volume of distribution, (b) reduce volume of distribution?
4. A low AUC indicates exposure is: (a) high, (b) low?
5. Clearance occurs primarily in what organs?
6. Associate the following V_d values:
   0.1, 1, 100,
   with the following descriptions of distribution:
   evenly distributed throughout the body, highly tissue bound, highly bloodstream restricted
7. Which of the following is a more preferred exposure (ng•h/mL)?: (a) AUC = 45, (b) AUC = 620/
8. Which of the following is a more preferred clearance (mL/min/kg)?: (a) Cl = 20, b) Cl = 60/
9. Which of the following is a more preferred half-life (h) for once-daily dosing?: (a) $t_{½} = 0.5$, (b) $t_{½} = 8$.
10. For the following experiments and data, calculate the bioavailabilities:

IV dose (mg/kg)	PO dose (mg/kg)	AUC PO (ng•h/mL)	AUC IV (ng•h/mL)	Bioavailability
1	10	500	500	
2	10	1000	500	
5	10	300	200	

11. What value of V_d (L/kg)indicates approximately equal distribution between blood and body tissue?: (a) 0.07, (b) 0.7, (c) 7, (d) 70, (e) 700.

References

1. Birkett, D. J. (2002). *Pharmacokinetics made easy*. Sydney, Australia: McGraw-Hill.
2. Mei, H., Korfmacher, W., & Morrison, R. (2006). Rapid in vivo oral screening in rats: reliability, acceptance criteria, and filtering efficiency. *AAPS Journal*, *8*, E493–E500.
3. Thummel, K. E., Shen, D. D., Isoherranen, N., & Smith, H.E. Design and optimization of dosage regimens: pharmacokinetic data, Appendix II. In L.L. Brunton (Ed.). *Goodman and Gilman's the pharmacological basis of therapeutics* (11th ed.). New York: McGraw-Hill.

Chapter 20

Lead-like Compounds

Overview

- *Structure modification during activity optimization often increases H-bonds, molecular weight, and Log P, which deteriorates drug-like properties.*
- *Lead-like compounds have lower initial values for structural properties, allowing increases without becoming non–drug-like.*
- *Fragment screening typically provides more lead-like compounds for optimization.*

The ancient proverb says: "The house built on a foundation of sand will fall, but the house built on rock will prosper." For drug discovery, the foundation is the lead structure. If the foundation is strong, the project team can build a strong drug-like clinical candidate. If the foundation is weak, the team's effort may never advance a drug-like compound to development.

The "hits" that serve as starting places for leads come from high-throughput screening, virtual screening, natural ligands, natural products, and the scientific literature. In the "hit-to-lead" phase, it is important to include properties in the workflow and goals for lead selection. In this evaluation process, some effective concepts have been emerging:

- Lead-likeness
- Template conservation
- Triage
- Fragment-based screening.

The use of one or more of these concepts can increase the chances of success in discovering a strong lead-like structural foundation.

20.1 Lead-likeness

Shortly after introduction of the rule of 5, also known as the Lipinski rules,[1] the value of including property guidelines in the selection of leads was recognized. This was an important step forward in starting with a foundation of leads that are free from major liabilities that would later impede the accomplishment of a viable clinical candidate.

As experience with property guidelines accumulated, it was recognized that the lead optimization phase adds substructures onto the lead template to enhance target affinity and selectivity. Nonpolar groups are added to enhance binding to lipophilic pockets. Other groups are added to increase hydrogen bonding with the binding site. These modifications

add lipophilicity, molecular weight (MW), and hydrogen bonding to the lead. This process can result in compounds that exceed the rule of 5 guidelines and have deleterious properties.

A proposed alternative was "lead-like properties,"[2] in which screening libraries are limited to compounds with:

- MW between 100 and 350
- Clog P between 1 and 3

By selecting leads that have lower MW, lower lipophilicity, and fewer hydrogen bonds, the eventual products of optimization are more likely to have acceptable drug-like properties. It also was proposed that lead-like structures are more likely to bind to the target protein "because they can more easily find a binding mode than larger drug-like molecules" that are commonly included in screening libraries.[2] A separate computational comparison indicated that, on average, lead compounds had lower values for structural properties than drug compounds, including 69-Da lower MW, one fewer ring, one fewer H-bond acceptor, two fewer rotatable bonds, 0.43 lower ClogP, and 0.97 lower $LogD_{7.4}$.[3]

A computational evaluation later suggested the following criteria for inclusion of compounds in lead-like screening libraries[4]:

- MW ≤ 460
- $-4 \leq$ Log P ≤ 4.2
- Log of water solubility (Log S_W) ≤ -5
- Rotatable bonds ≤ 10
- Rings ≤ 4
- Hydrogen-bond donors ≤ 5
- Hydrogen-bond acceptors ≤ 9

Lead-likeness also was suggested as extending to in vivo pharmacokinetic parameters and in vitro property criteria.[4] In many hit-to-lead programs, toxicity and pharmacokinetics are often not studied extensively. These could be used in the final selection of leads that will advance to optimization:

- Bioavailability (%F) $\geq 30\%$
- Clearance (Cl) < 30 mL/min/kg in rat
- $0 \leq$ Log $D_{7.4} \leq 3$
- Binding to cytochrome P450 isozymes = low
- Plasma protein binding $\leq 99.5\%$
- Acute toxicity and chronic toxicity = none (in therapeutic window)
- Genotoxicity, teratogenicity, carcinogenicity = none (at dose 5–10 times therapeutic window)

20.2 Template Conservation

In many cases, a large portion of the lead structure is conserved throughout the lead optimization stage. Structure modifications during optimization are often added onto the lead template, thus retaining much of the original core structure of the lead. The properties associated with the core structure continue to be a primary component of the properties of the analogs and the eventual clinical candidate.

Examples of this are shown in Figure 20.1, and others are found in Proudfoot.[5] Many drugs retain a large portion of the lead core structure. This principle suggests that the greatest opportunity to "lock in" favorable properties is at the lead selection stage. In addition to selecting from among the screening "hits," often a small effort in synthetic modification at the lead selection stage can improve properties and provide a stronger lead for the optimization stage. If the lead coming into the optimization phase does not have good properties, the project team will be required to expend time and resources to improve properties and may never be able to accomplish this at a later time when there are many discovery objectives to complete in a short time. This would be like trying to go back and reconstruct the foundation after the house has already been built. The difficulty and time required are magnified compared to completing this process at the beginning. Also, there is a natural tendency in discovery to maximize target affinity through structure–activity relationship–guided structure design. If the lead already has good properties, then deterioration of properties for the sake of activity optimization still may result in a clinical candidate with acceptable drug-like properties.

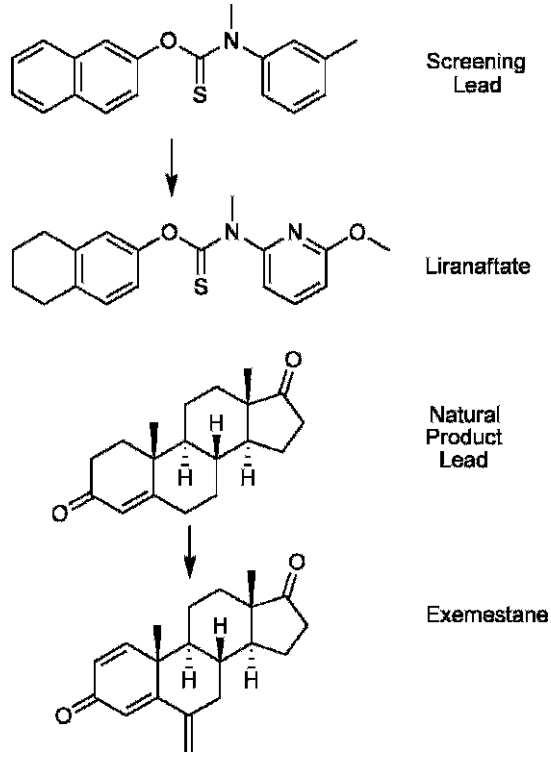


Figure 20.1 ► The lead template often is conserved during lead optimization.[1]

20.3 Triage

Hopefully, the early screening activities of a project provide many hits for consideration. Inclusion of properties in the evaluation criteria, along with activity, selectivity, and novelty, is an important strategy for ensuring that leads will have strong properties. This is consistent with the emergence of "risk" as a major factor in drug discovery decision-making. Selecting compounds that have the greatest chances of success and downgrading compounds that have higher risk of failure is an efficient practice for a drug discovery enterprise.

This process of triage is aided by setting goals for each of the key criteria of the lead.[6] Figure 20.2 is an example of how exploratory medicinal chemists compare the activity, selectivity, and properties of potential leads. This disciplined evaluation process assists triage of hits, guidance of initial synthetic modifications for improvement, and selection of leads for optimization.

	Lead	Analog	Desired Profile
MW	330	445	**<450**
clogP	1.9	5.19	**<4.0**
IC50 (μM)	4.2	>20	**<1.0 μM**
Binding to target (STD, FP, Trp-Fl.)	X-ray		**Yes (NMR, FP)**
MIC			
B. subtilus	>200 μM	50 μM	**<200 μM**
S. aureus MRSA	>200 μM	25 μM	**<200 μM**
S. aureus ATCC	>200 μM	200 μM	**<200 μM**
S. pneumo +	>200 μM	25 μM	**<200 μM**
Selectivity: *C. albicans* (MIC μg/mL)	>200	>200	**>10 fold**
Aqueous Solubility (μg/ml @ pH 7.4)	>100	26.5	**>60**
Permeability (10^{-6} m/s @ pH 7.4)	0	0.15	**>1**
CYP 3A4 (% inhibition @ 3 μM)	11	7	**<15**
CYP 2D6 (% inhibition @ 3 μM)	0	1	**<15**
CYP 2C9 (% inhibition @ 3 μM)	NT	23	**<15**
Microsome stability (% remaining @ 30 min)	NT	NT	**>80**
Definable Series	Yes	Yes	**Yes**
Definable SAR	Yes	Yes	**Yes**

Figure 20.2 ► Example of goals used by Wyeth Research exploratory medicinal chemists for hit selection, initial structural modification, and lead selection in an acyl carrier protein synthase (AcpS) inhibitor project.[6] (see Plate 3)

20.4 Fragment-Based Screening

An emerging strategy in exploring for novel leads has been termed *fragment-based screening*. This approach is based on the theory that screening with larger structures that fit the very specific shape, electrostatic interactions, and hydrophobic contacts of the binding site of the target protein with appreciable affinity is a very low-percentage possibility. Instead, it is suggested that smaller, less complex compounds, or *fragments*, are more likely to

bind to a portion of the binding site. From a fragment core, functionality can be added to enhance binding. Also, by selecting fragments that bind to different portions of the site and then joining them together with a "tether," it may be more likely to find a final lead that binds appreciably to the site. Although fragments bind with low affinity, tethered fragments forming a larger molecule will bind with greater affinity. An example of this method[7] is shown in Figure 20.3.

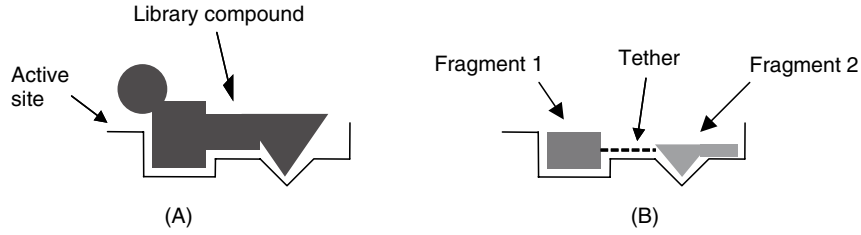


Figure 20.3 ► Fragment-based screening replaces the screening of large libraries containing complex structures to fit a specific active site **(A)** with the screening of a smaller library of smaller molecules that fit a portion of the active site and then are tethered together to form a lead with higher affinity **(B)**.

When fragments bind, they have lower affinity (IC_{50}~50 μM–1 mM)[7] but have a high efficiency of binding for their size.[8] Binding of fragments is difficult to detect with conventional biological assays; however, x-ray crystallography or NMR can be used to detect such weak binding fragments. The binding site and orientation in the pocket can be determined. These techniques are more expensive than conventional screening methods. Fortunately, it appears that a small library of fragments can produce a great deal of structural diversity because they are not encumbered by additional attached structure that would hinder binding. Also, cocktails of small molecules can be tested to accelerate the screening and to observe multiple binding locations. Excellent reviews have been published on fragment screening techniques.[9] library design,[10,11] and examples in drug discovery.[12–14]

The fragment approach nicely complements the goal of selecting leads with good properties. When large screening molecules, which are common in conventional screening libraries, bind to the target protein, they may have a considerable portion of the structure that is not involved in forming binding interactions. This extraneous structure adds to the MW, hydrogen bonds, and lipophilicity that detract from lead-like properties. On the other hand, fragments can minimize the extraneous structure and reduce the useless portions that would be detrimental to absorption. Also, a fragment screening library can be constructed from small molecules having good lead-like properties, allowing expanded lead or the tethered product of two fragments to be minimized to the structural features that are useful for effective binding and, ultimately, produce an active drug-like molecule.

Combination of the ideas of lead-likeness and fragment-based screening has produced guidelines for the properties of molecules used in fragment screening libraries. A set of rules for lead-like compounds has been proposed by Oprea as the "rule of 3"[15]:

- ► MW $\leq$300
- ► Clog P $\leq$3
- ► Rotatable bonds $\leq$3
- ► Hydrogen-bond donors $\leq$3
- ► Hydrogen-bond acceptors $\leq$3
- ► Polar surface area (PSA) $\leq$60 $Å^2$

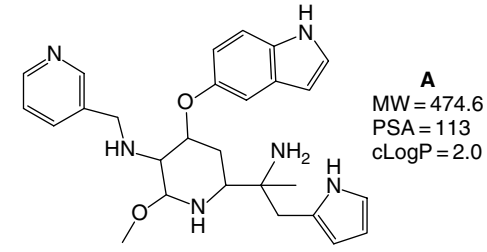
20.5 Lead-like Compounds Conclusions

The well-studied lead-like criteria reinforce the principle that success in discovering drug-like clinical candidates is higher when starting with compounds that have structural properties with lower values, which allow the opportunity for structural modifications to enhance activity and selectivity without condemning the series to unacceptable pharmacokinetic performance. All discovery organizations should consider whether it is useful to include compounds in their screening libraries that have properties falling outside the range guidelines of lead-like, or even drug-like, compounds. The need for efficiency and success in drug discovery adds imperative to a disciplined workflow that bases drug discovery work on a firm foundation from which a strong clinical candidate ("house") can be built.

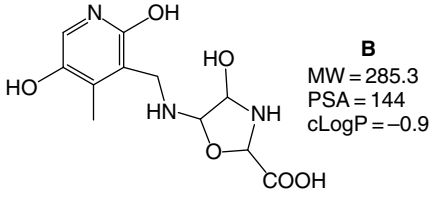
Problems

(Answers can be found in Appendix I at the end of the book.)

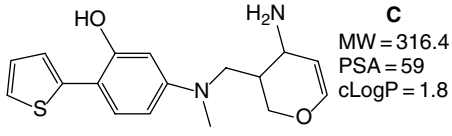
1. If leads are going to be structurally modified during lead optimization, why should compounds that exceed the "rule of 5" guidelines not be included as leads?
2. Evaluate the following compounds for their "lead-likeness" according to the "rule of 3" and list which properties are unfavorable:



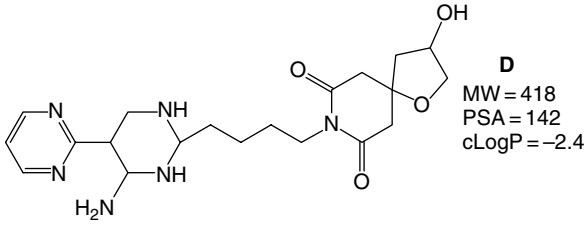
A
MW = 474.6
PSA = 113
cLogP = 2.0



B
MW = 285.3
PSA = 144
cLogP = –0.9



C
MW = 316.4
PSA = 59
cLogP = 1.8



D
MW = 418
PSA = 142
cLogP = –2.4

3. To increase the chances of maintaining good drug-like properties throughout the optimization phase, start with a lead having which of the following "lead-like" properties?: (a) ClogP $\leq$3, (b) MW >400, (c) PSA $\leq$60, (d) H-bond donors >5.

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Chapter 21

Strategies for Integrating Drug-like Properties into Drug Discovery

Overview

- *How and when property data are used can have great impact on a discovery project.*
- *Successful property strategies include assessing properties early, profiling properties rapidly, relating structures to properties, optimizing activity and properties together, using single-property assays, improving bioassays and interpretation, customizing assays for specific issues, and diagnosis.*

The integration of drug-like properties into the workflow of drug discovery deserves thought, creativity, and planning. Different companies may choose different approaches for integration of drug-like properties based on their resources, priorities, and organizational experience. The first decade of drug-like properties in discovery has indicated strategies that are beneficial. Many of these strategies are described in the following sections.

21.1 Assess Drug-like Properties Early

Property assessment can be performed early in the hit-to-lead process (Figure 21.1). This allows properties to be considered along with high-throughput screening (HTS) data, confirmatory in vitro bioassay data, and novelty. The property tools most appropriate for this stage are structural rules, in silico tools, and in vitro assays. If major liabilities are found that are unlikely to be improved by structure modification, then the structural series can be ranked lower in priority, and resources can be used for more promising series. If correctable liabilities are found, then studies can be planned for early structure modifications that attempt to minimize the problem(s) by modifying the lead before committing significant resources to the series. If acceptable or advantageous properties are found, then this series may be ranked higher. As discussed in Chapter 20, the structural template often is conserved during lead optimization, so it is important to advance a reliable core to the optimization phase. This approach emphasizes that absorption, distribution, metabolism, excretion, and toxicity (ADME/Tox) properties are important throughout the entire discovery process. This approach also improves efficiency, because deferring work on drug-like properties until later in discovery can result in series delay or failure after significant resources have already been invested in the series. The assessment of ADME/Tox properties at this stage should use methods having the appropriate level of specificity and resources, as discussed in Chapter 22, and need not be highly resource intensive.

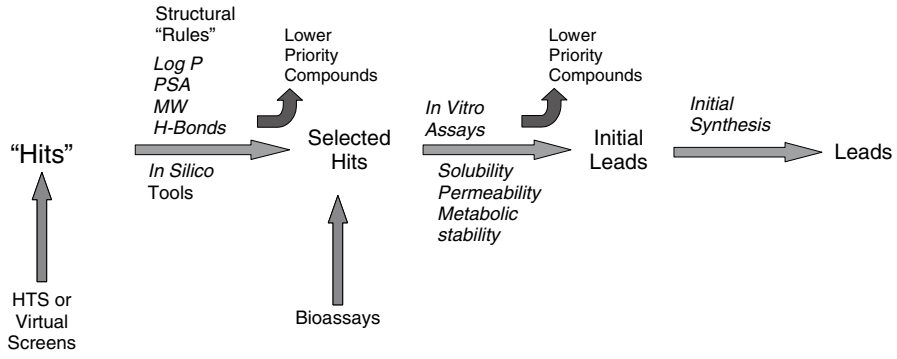

Figure 21.1 ► Early assessment of compound properties during the hit-to-lead phase can remove problematic compounds, augment lead selection using all available data, and help to plan initial synthetic modifications to rescue a series.

21.2 Rapidly Assess Drug-like Properties for All New Compounds

As discovery proceeds and new compounds are synthesized, properties can be rapidly assessed. This provides immediate feedback. If the new compound was synthesized to improve properties, rapid property measurement provides a check on the success of this approach. If the new compound was synthesized to improve activity or selectivity, then rapid property measurement provides a check on whether the properties were affected by the structural modification. Rapid property measurement allows teams to make decisions faster to improve properties. Further modifications can be planned in the same time period, thus providing increased chances of success.

21.3 Develop Structure–Property Relationships

Structure–property relationships (SPRs) can be developed for a series in the same manner as structure–activity relationships (SARs). SAR defines how structure modification at one moiety in the molecule affects activity. SPR defines how structure modification affects properties. An example of a hypothetical series is shown in Figure 21.2. The patterns

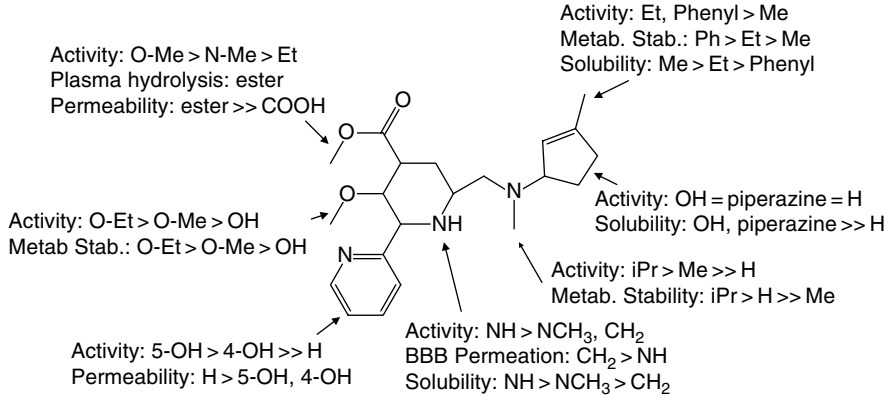

Figure 21.2 ► Develop structure–property relationships (SPR) to complement structure–activity relationships (SAR), as shown in this hypothetical example.

developed from these related processes help medicinal chemists with optimizing activity and properties. Furthermore, through multivariate analysis of SAR, SPR, or both, the interactions of modifications at these sites help to further optimize series performance individually for activity and properties or together in parallel.[1]

21.4 Iterative Parallel Optimization

Activity and properties of new compounds can be tested simultaneously for both activity and properties.[2] The data are fed back to the team for improvement of structure redesign (Figure 21.3). The testing time should be comparable for activity and properties. This approach allows the project teams to most effectively use their resources, make rapid progress, and take a holistic view of optimization. It also ensures that properties do not deteriorate as structural modifications are made to improve activity, and vice versa. A set of automated high-throughput assays can be implemented using an automated parallel process to rapidly provide data for key properties and activity.

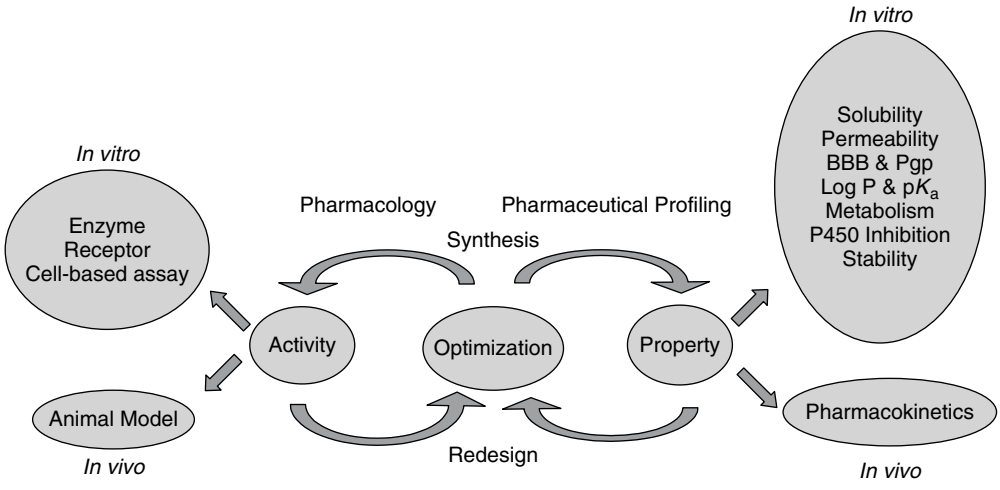

Figure 21.3 ► Iterative parallel optimization by simultaneous assessment of both activity and properties. (Reprinted with permission from [2].) (see Plate 4)

21.5 Obtain Property Data that Relates Directly to Structure

Structure is one of the most important elements of drug discovery. Therefore, property measurement should provide data that are directly related to structure. These data guide medicinal chemists in which structural modifications can be made to adjust the properties. For example, medicinal chemists can make modifications that change pK_a, lipophilicity, molecular weight (MW), and hydrogen bonding, block metabolically labile sites, and remove reactive groups. These modifications affect higher-level properties, such as solubility, permeability, metabolic stability, toxicity, and pharmacokinetic parameters. Rules, in vitro single variable assays, and in silico tools focus on individual properties for which medicinal chemists can readily envision specific structural modifications. Complex assays, such as pharmacokinetics

(PK), Caco-2 permeability, and hepatocyte metabolic stability, are affected by multiple properties and do not provide the specificity needed to make informed structural modification decisions. For example, Caco-2 permeability results are affected by passive, uptake, and efflux permeability as well as solubility and metabolism. Deciding which of these mechanisms is most determinative requires further experiments to obtain the specificity needed to plan specific structural modifications.

21.6 Apply Property Data to Improve Biological Experiments

The study of drug-like properties in discovery originally was implemented to select and optimize leads for in vivo PK. As data for properties have become widely available in discovery, awareness of the effects of these properties on discovery in vitro biological assays has been growing. Chemical stability and solubility in bioassay media, during dilutions, and stored compound solutions affect the concentration of test compound at the biological target (see Chapter 39). Membrane permeability affects compound access to intracellular targets in cell-based bioassays. Applying property data to optimize biological assays has proven to be very valuable in ensuring accurate SAR for discovery.

21.7 Utilize Customized Assays to Answer Specific Project Questions

An ensemble of high-throughput assays for key properties allows rapid access to data. However, the generic assay conditions and the limited number of properties screened with such assays only provide a general understanding of the compounds. It is advantageous to plan customized studies that provide data for specific project team questions that are more sophisticated than the generic assays provide data for. Examples of such studies are chemical stability or solubility in the specific bioassay media, phase II and extramicrosomal metabolic reactions, permeability of low-solubility compounds, and active transport. When an important research question goes beyond the appropriateness of a generic method to adequately answer, a customized assay can be developed and applied to obtain data that are reliable and more definitive for informed decisions.

21.8 Diagnose Inadequate Performance in Complex Systems Using Individual Properties

If inadequate in vivo performance is observed in PK studies, in vitro assays and structural rules can be used to diagnose the limiting property. Custom in vitro assays may be needed to test properties that have not been previously tested for the compound. If a limiting property is found, structure modifications can be made to improve this specific property. The new compound is retested using an in vitro assay to check for improvement of the property. If improvement of the property is observed, the compound can be retested in vivo to see if PK performance is improved. Testing first in vitro saves expensive in vivo resources. This diagnostic process is illustrated in Figure 21.4. This strategy works well as part of the lead optimization phase of discovery.

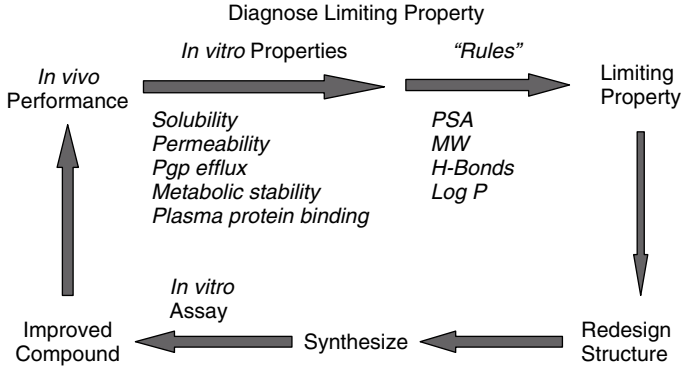

Figure 21.4 ► Diagnose inadequate in vivo performance using in vitro assay data and structural rules to identify the limiting property. Use these data to redesign the structure and test for property improvement.

Problems

(Answers can be found in Appendix I at the end of the book.)

1. Why is it inefficient to wait to optimize properties until after activity is optimized?
2. Having access to property data in 1 week, as opposed to 3 weeks, provides what benefit for project teams?
3. Why is single-property assay data more useful for medicinal chemists than data from assays that involve multiple properties?

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