Properties of cytochrome P450 isoenzymes and their substrates

Part 2: properties of cytochrome P450 substrates

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Cytochrome P450 isoenzymes are pivotal in drug clearance. Part 1 of this two-part review, published in the October issue of *Drug Discovery Today*, described the active site characteristics of members of the P450 superfamily. This article describes the great increase in our understanding of the substrate requirements of the human cytochrome P450 family and highlights the relevance of this knowledge for the design of new therapeutic agents.

The movement of xenobiotic molecules into and out of the body is to a large extent governed by their physicochemistry. While portals of entry include the nasal mucosa and lung, many xenobiotics, particularly pharmaceuticals, gain access via the gastrointestinal tract. Some penetration via this route is available for small water-soluble molecules by passage through aqueous pores or tight junctions (paracellular absorption); however, rapid transfer is only achieved by traversing the lipid core of the membranes of the gastrointestinal tract. This transcellular absorption requires, by definition, that molecules have positive lipophilicity. Once present in the system, such molecules are clearly free to diffuse through organs and tissues to reach receptors and enzymes, etc., and influence these targets in a beneficial or harmful manner.

The kidney is a key organ in the elimination of xenobiotic molecules. Compounds circulating in the blood can be filtered at the glomerulus (glomerular filtration). In addition, anionic and cationic compounds can be actively transported into the kidney tubule by carrier systems. The membranes of the kidney, like those described above, allow the passage of lipophilic compounds, and compounds concentrated in the tubule can diffuse back (passive reabsorption) to re-establish equilibrium with the blood. Thus, only water-soluble molecules show substantial rates of renal elimination[1].

Xenobiotic compounds that are too lipophilic to be filtered by the kidney are directly metabolized by the body to more hydrophilic compounds, which can undergo renal elimination. Xenobiotic metabolism is generally divided into phase I and phase II reactions. Phase I metabolism normally involves oxidative attack. The most important enzyme system involved in phase I metabolism is the cytochrome P450 enzyme system. This enzyme system has been shown to metabolize a diverse range of substrates from cyclosporin to acetone[1].

P450 is a superfamily of similar proteins (isoenzymes) with the same porphyrin–haem complex as the catalytic centre, but with different amino acid sequences, altering the topography of the active site. The identity of each protein is based on amino acid sequence homology such that all members of a particular family or subfamily are at least 40%

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similar to all the other members of that family or subfamily. Analysis of the literature indicates that six major forms of P450 isoenzymes are involved in the metabolism of pharmaceuticals in man: CYP1A2, CYP2D6, CYP2C9, CYP2C19, CYP3A4 and CYP2E1 (Ref. 2). These isoenzymes form the subject of this review.

Metabolism of a compound by each isoenzyme is determined by three generic rules:

- The topography of the active site.
- The degree of steric hindrance of the access of the iron–oxygen complex to the possible sites of metabolism.
- The possible ease of electron or hydrogen abstraction from the various carbons or heteroatoms of the substrate.

The topography of the active site has been described in Part 1 of this review, and hydrophobic, ion-pair and hydrogen bond interactions were key features. The physicochemical properties of the substrates therefore play a key role in determining which of the P450 isoenzymes metabolize a particular molecule.

Understanding the structure and physicochemistry of the substrates of each of the P450 isoenzymes can reveal what part chemical functionality, desolvation, lipophilicity, etc., play in recognition, access and actual active site binding of P450 substrates and inhibitors.

CYP1A2

Traditionally CYP1A2 has been associated with the activation of promutagens and carcinogens such as 2-acetylaminofluorene[3]. However, an increasing number of drug substrates have been identified for this isoform, including caffeine, phenacetin, tacrine, clozapine and propranolol (Figure 1).

CYP1A2 is able to accommodate neutral molecules such as ethoxyresorufin and basic molecules such as propranolol ($pK_a \approx 10$). The wide range of physicochemical parameters, including lipophilicity, for CYP1A2 substrates and inhibitors is illustrated in Table 1.

It has long been noted that substrates for the CYP1A family tend to be flat, aromatic lipophilic molecules such as benzo[*a*]pyrene[4]. Since this early description of the substrate structure–activity relationship (SSAR) of the isoforms, little has been done to define the physicochemistry of CYP1A2 substrates. The increase in the number of CYP1A2 substrates/inhibitors has allowed an SSAR to be developed and with it an understanding of the physicochemistry of these molecules.

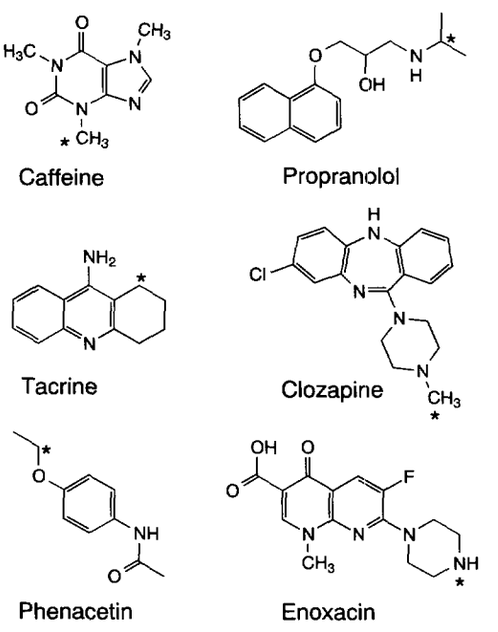

Figure 1. *Structures of a selection of CYP1A2 substrates/inhibitors. *The site of CYP1A2-mediated metabolism or, in the case of enoxacin, the site of interaction with CYP1A2.*

Table 1. Physicochemical properties of CYP1A2 substrates

Compound	pK_a	Log P (calculated)	Log $D_{7.4}$ in octanol
Caffeine	0.6, 14.0	–0.1	–0.1
Phenacetin	2.2	1.8	1.7
Tacrine	9.8	3.5	0.5
Enoxacin	6.3, 8.7	–1.3	–2.2
Clozapine	7.5	4.2	3.0
Propranolol	9.6	2.8	1.1

Enoxacin is an inhibitor that is directly coordinated to the haem iron of CYP1A2 via the 4′-nitrogen atom on the piperazine function (Figure 1)[5]. Considering the substrates for CYP1A2, it is clear that the majority contain planar aromatic regions. This feature is a clear determinant of interaction with the isoform. The aromatic regions could be important in forming π–π interactions with aromatic residues in the enzyme active site[6]. This interaction may be considered different from lipophilic interactions since it involves the π-electron clouds of aromatic systems. If a π–π interaction is

the main determinant of binding with CYP1A2, then it is not surprising that octanol/buffer partition coefficients or overall lipophilicity are not reflective of the interaction between CYP1A2 and its substrates *per se.*

CYP2C9

An increasing number of drug molecules have been identified as being metabolized by CYP2C9, including phenytoin, tolbutamide, naproxen, ibuprofen, diclofenac, Δ^1-tetrahydrocannabinol and (*S*)-warfarin[7] (Figure 2). The SSAR described here for CYP2C9 does not apply to CYP2C19, which has somewhat different substrate requirements[8]. In terms of physicochemistry, the majority of the CYP2C9 substrates are acidic and ionized at physiological pH.

The physicochemical parameters of some of the CYP2C9 substrates are detailed in Table 2. These molecules are chemically diverse, but examination of the structures suggests that all the substrates have a region that is anionic or can act as a hydrogen bond donor (see preceding section).

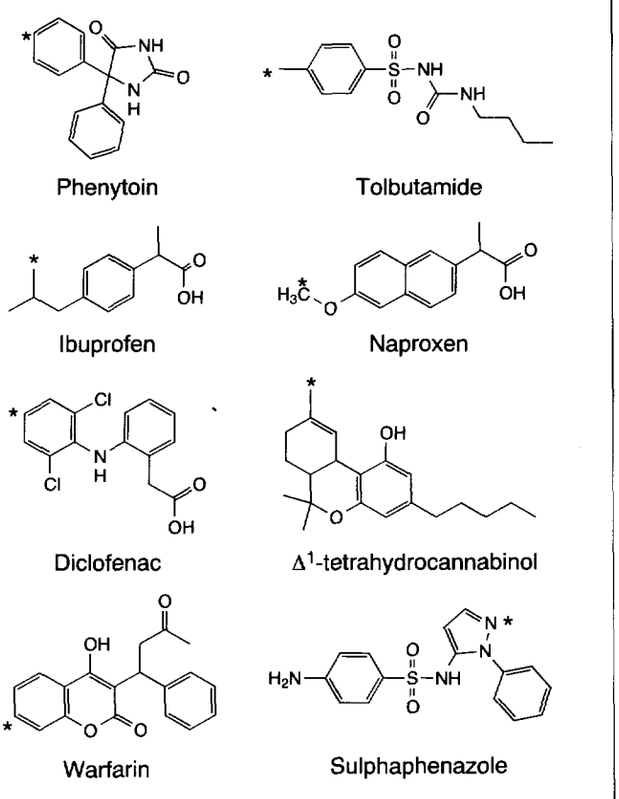

Figure 2. *Structures of a selection of CYP2C9 substrates/inhibitors. *Sites of CYP2C9-mediated metabolism.*

Table 2. Physicochemical properties of CYP2C9 substrates

Compound	pK_a	Log P (calculated)	Log $D_{7.4}$ in octanol
Phenytoin	8.3	2.1	2.4
Tolbutamide	5.3	2.5	0.5
Naproxen	4.6	2.8	0.3
Ibuprofen	4.6	3.7	1.7
Diclofenac	4.5	4.7	1.1
Δ^1-THC	–	7.2	7.0
(*S*)-Warfarin	5.0	2.9	1.2
Sulphaphenazole	5.9	2.1	0.2

Δ^1-THC, Δ^1-tetrahydrocannabinol.

Octanol contains an alcohol function that is able to support hydrogen bond formation. Moreover, in partitioning experiments, the octanol phase was found to contain a significant proportion of water. Hence, it is possible for compounds to partition into the octanol phase of an octanol/buffer mixture with their hydration sheath. Lipophilicity can be determined using solvents such as cyclohexane that do not support hydrogen bond formation. In this case, only the dehydrated drug molecules can partition into the organic phase. Comparison of cyclohexane/buffer partition coefficients for CYP2C9 substrates/inhibitors with CYP3A4 substrates/inhibitors shows that CYP2C9 compounds have markedly lower partition coefficients than CYP3A4 compounds[9] (Table 3).

The data demonstrate that CYP3A4 substrates/inhibitors have higher partitioning than CYP2C9 substrates/inhibitors when the assessment is made in a nonhydrogen bonding solvent such as cyclohexane. This also supports the contention that hydrogen bonding plays an important part in the octanol/buffer partitioning of CYP2C9 substrates/inhibitors since phenytoin (CYP2C9) and erythromycin (CYP3A4) have similar octanol/buffer partition coefficients (2.4 and 2.0, respectively) but markedly different cyclohexane/buffer partition coefficients (–2.0 and 2.2, respectively).

The active site of cytochrome P450 is thought to contain a number of water molecules in its resting state (e.g. CYP101; Ref. 10). It has been suggested that the expulsion of water from the active site by the substrate provides the entropic driving force behind the substrate binding. This would certainly be the case for lipophilic molecules binding to a lipophilic active site such as CYP3A4 (see previous section). In addition, any water molecules associated with the substrate

Table 3. Comparison of octanol and cyclohexane partitioning for CYP2C9 and CYP3A4 substrates/inhibitors

Compound	Isoform	Substrate/ inhibitor	Log $D_{7.4}$ in octanol	Log $D_{7.4}$ in cyclohexane	Δlog D
Phenytoin	CYP2C9	Substrate	2.4	–2.0	4.4
Tolbutamide	CYP2C9	Substrate	0.5	–2.4	2.9
Sulphaphenazole	CYP2C9	Inhibitor	0.2	–4.1	4.3
Terfenadine	CYP3A4	Substrate	5.2	3.5	1.7
Erythromycin	CYP3A4	Substrate	2.0	2.2	–0.2
Ketoconazole	CYP3A4	Inhibitor	3.9	–0.6	4.5

would have to be removed before the substrate binding. Many of the substrates for CYP2C9 contain functional groups that would tightly associate with solvent water, producing a water sheath around portions of the molecule such as the sulphonylurea of tolbutamide. To allow binding to an active site comprising mainly hydrophobic residues, this water sheath would have to be stripped away. The energy requirement for this process may be too great to be compensated for by a lipophilic binding interaction. However, if the active site were able to support hydrogen bond formation, such as proposed for CYP2C9, these water molecules could play an important part in the binding of the molecule to the enzyme[8]. In such a process, the lipophilic region of each molecule would still be capable of water displacement and the resultant entropic binding effects leading to spin state change and formation of the $(FeO)^{3+}$ unit.

CYP2C19

The metabolic activity of the related CYP2C19 has been probed both *in vivo* and *in vitro* using (*S*)-mephenytoin as a marker for this activity. Substrates for this isoform include (*R*)-mephobarbital, moclobemide, proguanil, diazepam, omeprazole and imipramine[11,12] (Figure 3), but these compounds do not show obvious structural or physicochemical similarities. Some patterns emerge when the differences between the CYP2C9 substrate phenytoin and the CYP2C19 substrate (*S*)-mephenytoin are considered. Phenytoin is *para*-hydroxylated on the *pro*-(*S*)-phenyl ring by CYP2C9. The *S*-enantiomer of mephenytoin is *para*-hydroxylated by CYP2C19. While (*S*)-mephenytoin is close to phenytoin in structure, the *N*-methyl function in mephenytoin makes donation of a hydrogen bond (as required by CYP2C9) impossible. This would explain why mephenytoin is not a substrate for CYP2C9. In the absence of a detailed SSAR for CYP2C19 it is not clear why phenytoin is not a substrate for this isoenzyme. One possibility is that, like CYP1A2, CYP2C19 binds substrates by donating hydrogen bonds to atoms of the substrate molecule that have a negative electrostatic potential (for mephenytoin this would be the carbonyl oxygens). CYP2C19 can bind compounds that are weakly basic (e.g. diazepam, $pK_a = 3.4$), strongly basic (e.g. imipramine, $pK_a = 9.5$) or acidic compounds (e.g. (*R*)-warfarin, $pK_a = 5.0$). Hence, unlike CYP2C9 and CYP2D6, it has no preference for acidic or basic molecules, supporting the hypothesis that substrate binding is via a hydrogen bond acceptor mechanism.

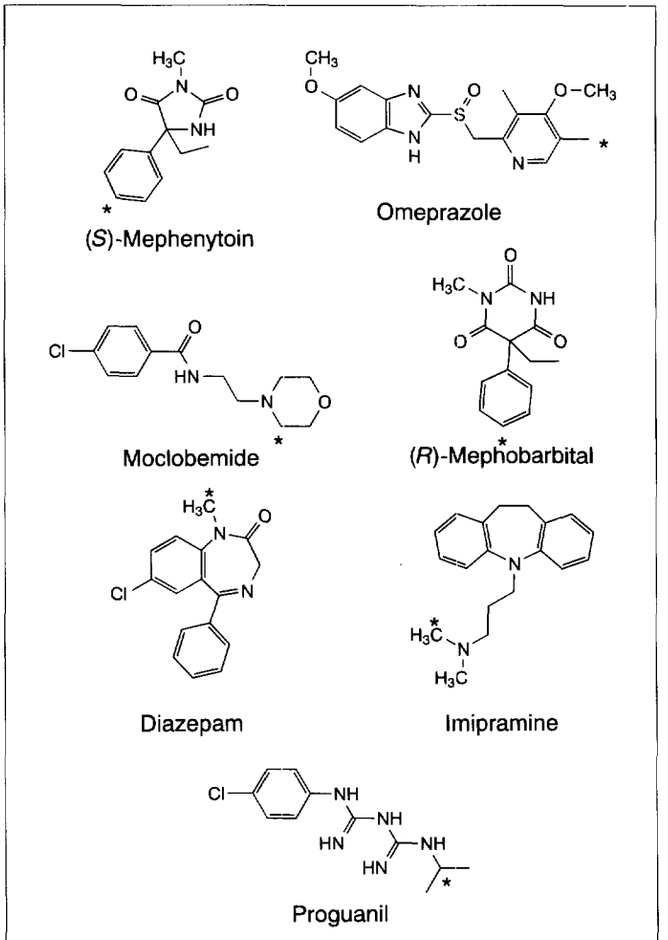

Figure 3. *Structures of a selection of CYP2C19 substrates. *Sites of CYP2C19-mediated metabolism.*

CYP2D6

Substrates for CYP2D6 include tricyclic antidepressants, β-blockers, class I antiarrhythmics, and selective serotonin reuptake inhibitors (SSRIs)[7]. All the substrates contain a basic nitrogen atom ($pK_a > 8$), which is ionized at physiological pH (Ref. 7). The interaction that governs the substrate selectivity of CYP2D6 is an ion-pair interaction between a protonated nitrogen atom and an aspartic acid residue (see previous section). Because most of the substrates for this isoform meet this criterion ($pK_a > 8$), any difference observed in binding affinities can be attributed to other π–π or hydrophobic interactions (see previous section). Thus, despite the dominance of the ion-pair interaction, a correlation between the intrinsic lipophilicity of the molecule and its affinity for CYP2D6 should be observed. One study has investigated this using a series of β-blockers[13]. In this case, the intrinsic lipophilicity was measured as the octanol/buffer partition coefficient (log $D_{7.4}$). Since all the compounds examined have approximately the same pK_a (between 9 and 10), differences in the partition coefficient equate directly to differences in intrinsic lipophilicity. The interaction with CYP2D6 was assessed as the inhibitory potential (K_i) against dexthorphan *O*-demethylation, a well-characterized CYP2D6 metabolic pathway (Figure 4). The lipophilicity of the molecules correlated with their inhibitory potency, supporting the hypothesis that, in addition to the ion-pair interaction, there are other interaction(s) between CYP2D6 and its substrates that may be hydrophobic rather than exclusively π–π. Such interactions explain why perhexilene, a compound in which the alicyclic ring is the site of attack, is a substrate.

It has been suggested that, for very potent CYP2D6 inhibitors such as ajmalicine (K_i = 3 nM), there is, in addition to the ion-pair and hydrophobic/lipophilic interactions, a hydrogen acceptor site which increases the inhibitory potency[14]. This is supported by the strong interaction of the HIV-1 protease inhibitor ritonavir (Figure 5). While this molecule does have a weakly basic centre in the thiazole moiety ($pK_a \approx 3$), this does not explain its interaction with CYP2D6 (IC_{50} = 2.5 μM)[15]. The molecule does have a number of hydrogen bonding groups which, if there are complementary hydrogen bonding sites in the CYP2D6 active site, may explain the inhibitory potency. Ritonavir is a substrate for CYP2D6 as well as an inhibitor, with the isoform being partly responsible for oxidation on the tertiary carbon atom of the isopropylthiazole moiety, as indicated in Figure 5 (Ref. 15). This suggests that the SSAR involving the hydrogen bond acceptor may be transferable from the very potent inhibitors to nonbasic substrates.

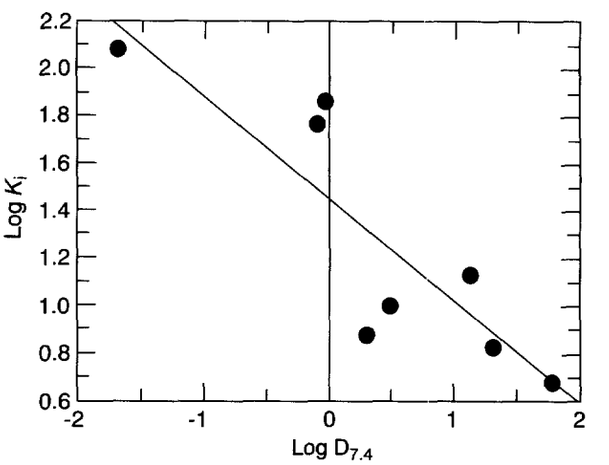

Figure 4. *Plot of relative lipophilicity versus affinity for CYP2D6 for a series of β-blockers illustrating the role of lipophilicity in binding to CYP2D6 in a series in which the degree of ionization is constant.*

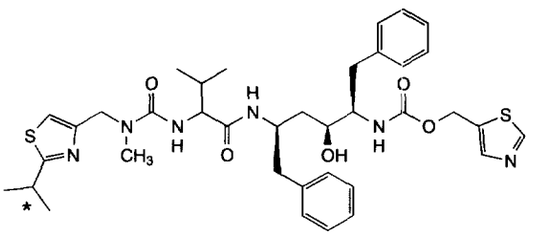

Figure 5. *Structure of the HIV-1 protease inhibitor ritonavir, which is a moderately potent inhibitor of CYP2D6 despite being a neutral molecule. *Site of CYP2D6-mediated metabolism.*

CYP3A4

CYP3A4 appears to metabolize lipophilic drugs in positions largely dictated by the ease of hydrogen abstraction in the case of carbon hydroxylation, or of electron abstraction in the case of *N*-dealkylation reactions[7]. Among the CYP3A4 substrates are erythromycin, lidocaine, diltiazem, tamoxifen, amiodarone and terfenadine[7], all of which undergo *N*-dealkylation. In addition, the allylic and benzylic positions, which are present in molecules such as steroids (e.g. progesterone, testosterone and budesonide), quinidine,

zatosetron, indinavir, cyclosporin, salmeterol and lovastatin, are also metabolized by CYP3A4 (Ref. 7).

As with conventional radical chemistry, reactivity needs to be combined with probability. Thus, in molecules such as terfenadine (Figure 6), the tertiary butyl group will be liable to oxidation because of its 'maximum number' of equivalent primary carbons. Thus, although not an especially labile function, the site of metabolism becomes dominated by statistical probability.

Binding of substrates seems to be essentially due to lipophilic forces (see previous section) and results in the expulsion of water from the active site. As already described for CYP2C9, this expulsion of water provides the driving force for the spin state change and hence the formation of the catalytic $(FeO)^{3+}$ unit. Comparison of the octanol and cyclohexane partition coefficients for CYP3A4 and CYP2C9 substrates and their calculated Δlog D values[9] shows that CYP3A4 molecules have much lower values than CYP2C9 substrates (Table 3). This indicates that the CYP3A4 substrates favour a lipophilic environment devoid of water, in contrast to the more hydrated environment favoured by CYP2C9 substrates.

The principle of extension of lipophilic functions normally hidden from solvent is further supported by a study of the soluble bacterial $P450_{BM-3}$ (CYP102). In this case, the substrates are fatty acids, which in aqueous solution adopt a 'globular' conformation. Upon entering the lipophilic access channel, the fatty acid opens out into an extended conformation, with the lipophilic headgroup directed at the haem and the polar acid function directed at the solvent. This principle can be extended to CYP3A4 substrates such as cyclosporin[7]. In aqueous solution cyclosporin adopts a conformation in which the allylic site of CYP3A4 metabolism is internalized, shielded from the solvent. However, in aprotic (lipophilic) solvents, cyclosporin adopts a conformation that allows the primary site of CYP3A4 metabolism to extend out, away from the bulk of the molecule. This 'spreading out' of apparently sterically hindered molecules, as judged by X-ray or aqueous solution structure, may help in understanding the selectivity of CYP3A4.

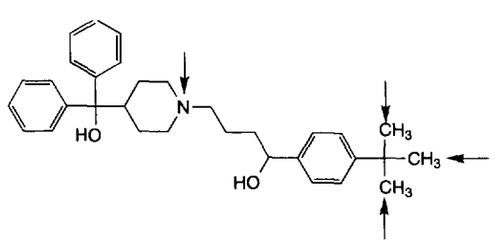

Figure 6. *Structure of terfenadine, a CYP3A4 substrate which undergoes metabolism by the enzyme at more than one site. Arrows indicate the sites of CYP3A4 metabolism.*

Binding based solely on hydrophilic interactions is relatively weak, and without specific hydrogen bond or ion-pair interactions allows motion of the substrate in the active site. The substrate is therefore able to adopt more than one orientation in the active site and the eventual product of the reaction is a result of the interaction between one of these orientations and the $(FeO)^{3+}$ unit. This is illustrated by the metabolism of midazolam by CYP3A4, which produces two products – 4-hydroxymidazolam and 1′-hydroxymidazolam[16]. Moreover, simultaneous binding of two substrate molecules, phenanthrene and 7,8-benzoflavone, to the CYP3A4 active site can occur[17]. Since both molecules are metabolized simultaneously, they must have access to the active oxygen. Further studies into the effect of substrates on the binding of carbon monoxide to the CYP3A4 active site have suggested that the active site can adopt different conformations depending on the substrate[18]. The authors suggest that it is the ability of the active site of CYP3A4 to adopt different conformations that contributes to its wide range of substrates.

CYP2E1

The principal pharmaceutical substrates halothane and enflurane[19] are lipophilic (log D or log P in octanol, hydrocarbons or oils, of 2.3 and 2.1, respectively), suggesting a predominant hydrophobic interaction, as with CYP3A4. However, other substrates include ethanol, which is moderately hydrophilic (log D or log P in octanol and cyclohexane of –0.3 and –2.4, respectively), suggesting that hydrogen bonding interactions can also occur. These two apparent contradictions probably represent the reality that each of the P450 isoenzymes can bind substrates or inhibitors by more than one mechanism (although one may dominate), leading to relative selectivity rather than specificity.

Knowledge of cytochrome P450 isoenzymes and drug design

The foregoing review has illustrated the large increase in our knowledge about the substrate requirements of the human cytochrome P450 family. In summary, the major forms possess aromatic amino acid residues close to the catalytic

haem centre that are capable of π–π interactions with substrates. Other residues more distant from the catalytic centre partake in further substrate binding, including further hydrophobic (CYP3A4), hydrogen bonding (CYP1A2 and CYP2C9) or ion-pair (CYP2D6, CYP2C9) interactions. In general, therefore, P450 substrates are lipophilic (log D > 0). It has been suggested that lipophilic interactions are long-range interactions[20] and, therefore, may be responsible for the attraction of substrate molecules to P450 molecules. Once the molecule has been attracted to the P450, its own physicochemical properties determine which of the isoenzymes will metabolize it. Box 1 lists the broad selectivity patterns described in the previous sections.

The oxidative process involved with cytochrome P450 can also be modelled in many cases as initial abstraction of electrons or hydrogen atoms from the substrate. It is therefore possible to designate molecules as likely substrates (or inhibitors) of individual isoenzymes based on key structural and physicochemical features. Moreover, it is feasible to design molecules or derivatives of molecules that overcome problems posed by interactions with these isoenzymes. Such problems include metabolism by a single variable isoenzyme, too rapid a metabolism giving unrealistic dosage regimens, and the potential for interactions with likely coadministered drugs.

Box 1. Summary of substrate/selectivity rules for P450 isoenzymes

CYP1A2 Neutral or basic, lipophilic, planar molecules with at least one putative hydrogen bond donating site. Principal substrate is theophylline.
CYP2D6 Aryl-alkyl amines (basic), with site of oxidation a discrete distance from a protonated nitrogen. Substrates are lipophilic, particularly when measured or calculated for the neutral form. Principal substrates are β-adrenoceptor blockers, class I antiarrhythmics and tricyclic antidepressants. Often hydroxylation occurs in an aromatic ring or an accompanying short alkyl side-chain.
CYP2C9 Neutral or acidic molecules with site of oxidation a discrete distance from hydrogen bond donor or possibly anionic heteroatom. Molecules tend to be amphipathic, with a region of lipophilicity at the site of hydroxylation and an area of hydrophobicity around the hydrogen bond forming region. Principal substrates are nonsteroidal anti-inflammatory agents. Oxidation often occurs in an aromatic ring or an accompanying short alkyl side-chain.
CYP3A4 Lipophilic, neutral, or basic molecules with site of oxidation often nitrogen (*N*-dealkylation) or allylic positions. Wide range of substrates covering all fields of pharmaceuticals.
CYP2E1 Small (molecular weight of 200 or less), normally lipophilic, linear and cyclic molecules. Principal pharmaceutical compounds are volatile anaesthetics.

Strategies to overcome metabolism by P450 isoenzymes

Metabolism by polymorphic isoenzymes such as CYP2D6 can be problematic in drug development because of the wide variation in the pharmacokinetics of the patient population. Molecules of the aryl-alkyl amine type are likely CYP2D6 substrates. The regioselectivity of CYP2D6 towards its substrate and indeed actual substrate binding are governed by ion-pair binding of the protonated nitrogen of the amine and the hydrophobic or π–π interaction of the aryl function. Substrates must have an alkyl chain of the correct dimensions to allow these key interactions to take place. Synthetic strategies to overcome metabolism by CYP2D6 include disruption of the hydrophobic or π–π interaction by adding functionality to the aryl group, altering the aryl-amine separation by changing alkyl chain length, or abolishing the ion-pair interaction by attenuating the basicity of the nitrogen. An example of altering metabolism by adding functionality to the aryl group is provided by betoxolol. Here, the *para* position of the aryl grouping of a β-adrenoceptor antagonist was substituted with a bulky metabolically stable function to give much improved pharmacokinetics and an absence of polymorphic metabolism[7]. Figure 7 illustrates synthetic strategies to lower the interaction between compounds and CYP2D6 based on imipramine. The length and rigidity of the alkyl chain has been altered or the basic nature of the nitrogen abolished, to disrupt the key interactions with the CYP2D6 isoenzyme. These changes result in marked lower affinities, as measured by the ability to inhibit the metabolism of other substrates by the enzyme.

The dominance of hydrophobic interactions with CYP3A4 means that reduction in overall lipophilicity of the substrate will usually result in a decrease in metabolism by this isoenzyme. CYP3A4 has the ability to accommodate molecules in multiple orientations, and metabolism often occurs at labile sites for electron or hydrogen abstraction. Strategies to overcome metabolism by CYP3A4 can also, therefore, involve removal or alteration of functions to decrease the lability to hydrogen or electron abstraction. Figure 8 shows a number of Na^+ channel antagonists (local anaesthetics, class I antiarrhythmic agents) for which the rapid metabolism by CYP3A4 of lidocaine (*N*-dealkylation) can be attenuated by reducing the functionality around the tertiary amine (replacement with a secondary or primary amine), or lowering overall

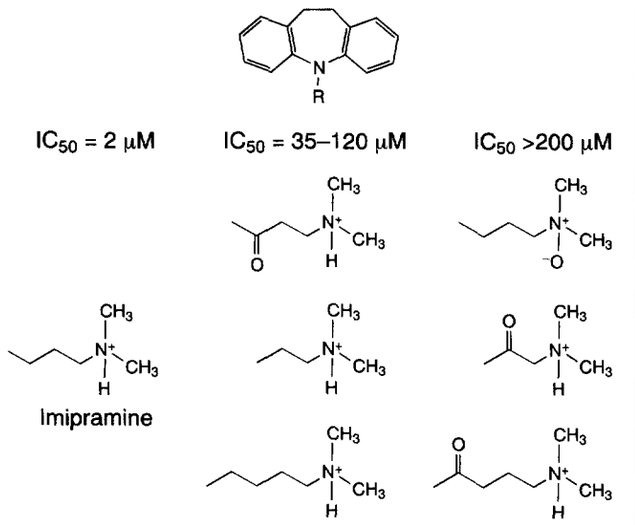

Figure 7. *Alterations in chain length or rigidity, or attenuation of the basicity of the alkylamine nitrogen, reduce the affinity of imipramine analogues for CYP2D6.*

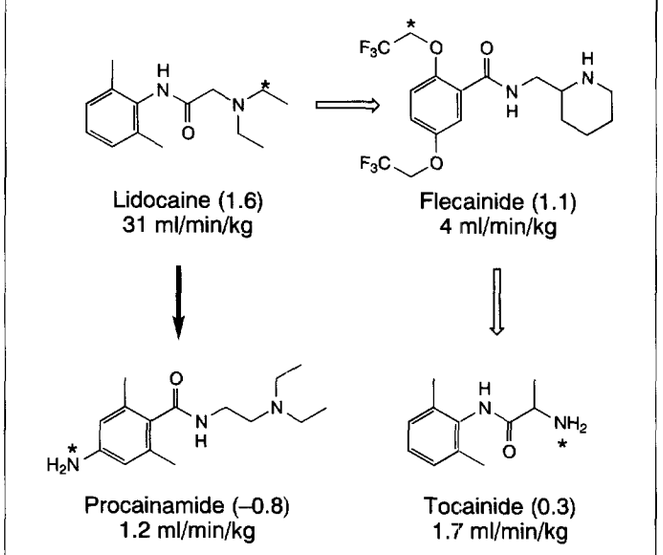

Figure 8. *Altering the functionality around the nitrogen centre of lidocaine (⇒), or decreasing the lipophilicity (→), results in lowered metabolic clearance by CYP3A4.*

lipophilicity by incorporation of a more polar function (aryl to aniline) at a site remote from the site of metabolism.

A further key binding interaction with P450 can occur for compounds containing unsaturated, nitrogen-containing heterocycles such as imidazole and pyridine. Such functionality is often added to aid solubility, but can form a ligand interaction with the haem of P450 via the nitrogen lone pair. Calculations suggest that the presence of such a functionality will add 6 kcal/mol binding energy to existing interactions. Such affinity explains the exquisite potency of certain inhibitors of P450s (e.g. ketoconazole and sulphaphenazole for CYP3A4 and CYP2C9, respectively). Moreover, the binding is of such strength that compounds containing these functions may become 'general' P450 inhibitors rather than inhibitors of only one or two isoenzymes. Where this type of P450 binding is not essential for activity, as for aromatase or C-14 demethylase inhibitors, steric addition around the nitrogen or substitution of the function with a bioisostere can overcome this problem. For instance, cimetidine inhibits the metabolism of a wide variety of drugs, including phenytoin, cyclosporin and propranolol, substrates that are normally ascribed to CYP2C9, CYP3A4 and CYP2D6, respectively. Cimetidine incorporates an imidazole grouping in its structure. In contrast, ranitidine, in which the imidazole ring is replaced by a furan ring, shows little potential for drug interactions.

In summary, our expanding knowledge of P450 enzymes allows favourable metabolism and inhibition properties to be designed into new chemical entities. Such compounds facilitate development and have greater potential to be successful drugs.

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