# SUBSTRATES OF HUMAN CYTOCHROMES P450 FROM FAMILIES CYP1 AND CYP2: ANALYSIS OF ENZYME SELECTIVITY AND METABOLISM

David F.V. Lewis[1]*, Brian G. Lake[1,2] and Maurice Dickins[3]

*[1]School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, [2]BIBRA International Limited, Carshalton, Surrey and [3]Pfizer UK Limited, Sandwich, Kent, UK*

**SUMMARY**

A compilation of information relating to substrate metabolism via human cytochromes P450 (CYP) from the CYP1 and CYP2 families is reported. The data presented include details of preferred sites of metabolism and $K_m$ values (usually for the expressed enzymes) for each reaction for selected substrates of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP2E1. Although other P450 databases are available, they do not provide such information as is collated here, and which can prove useful for comparing P450 substrate characteristics. This information can be employed in analysing the structural requirements for human P450 enzyme selectivity and for establishing various rules regarding preferred site of metabolism for selective P450 substrates. For example, in most cases it would appear that there is a set number of intervening 'heavy' atoms (atoms other than hydrogen) between sites of metabolism and key hydrogen bond acceptors (or donors) for human P450 substrates, with the number of intervening atoms being dependent upon the type of P450 involved.

* Author for correspondence:
David F.V. Lewis
School of Biomedical and Molecular Sciences
University of Surrey
Guildford
Surrey, GU2 7XH, UK
e-mail: d.lewis@surrey.ac.uk

**KEY WORDS**

cytochrome P450, drug metabolism, structure-activity relationships

## INTRODUCTION

The cytochromes P450 (CYP*) constitute a superfamily of haemthiolate enzymes that are involved in the oxidation of a large number of organic compounds, both endogenous and exogenous /1-3/. P450 enzymes are found in most species and across all five biological kingdoms; the number of P450s identified to date exceeds 2,000 individual enzymes, and it is thought that about 60 P450s are present in the human genome /4,5/. The majority of Phase 1 metabolic reactions for drugs and other xenobiotics are catalysed by P450s /6/ from families CYP1, CYP2 and CYP3 /7,8/. Of these, enzymes of the CYP2 family appear to be most important overall (50%), especially for the CYP2C subfamily and CYP2D6, followed by CYP3 family enzymes of which CYP3A4 represents the main component, this being associated with the Phase 1 metabolism of about 30% of known P450 substrates investigated so far /8/. Of lesser importance are enzymes from the CYP1 family, although CYP1A2 plays a role in the Phase 1 metabolism of a significant number (about 12%) of drug substrates /8/. This compilation of metabolic data focuses on substrates of the CYP1 and CYP2 families, and information on their physicochemical properties (e.g. log P, $pK_a$, log $D_{7.4}$ and molecular weight values) has been published recently /9/. Routes of metabolism mediated by particular P450s and apparent Michaelis constant ($K_m$ values) data for metabolism mediated by the P450 enzyme involved in each case is provided such that it is possible to appreciate the differing binding affinities for various substrates of the same P450. Data on substrates of the less important P450s, such as CYP1A1, CYP1B1 and CYP2F1, have not been included as these represent relatively minor components of the hepatic P450 complement and, consequently, their role in drug metabolism is minor. The purpose of this exercise is to provide a framework for evaluating structure-activity relationships within human P450 substrates that are either drugs in current clinical use or

* CYP = cytochrome P450 when referring to particular families, subfamilies and individual enzymes; otherwise P450 is used for referring to the enzymes in general.

other xenobiotics of interest. This information can also be employed for examining factors responsible for P450 enzyme selectivity which can, therefore, aid novel compound development in which metabolic pathways are of major importance to clearance in *Homo sapiens* /10-12/.

## METHODS

The metabolic data on substrates of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP2E1 have been collated from the current literature /3,7,8,13,14/. Physicochemical data in the form of log P and $pK_a$ values were either obtained from the literature /6,15-19/ or calculated using the Pallas Software System (CompuDrug Limited, Budapest). Values for relative molecular masses ($M_r$ data) of compounds were also obtained from the literature /20/ or calculated using the Pallas system.

The ClogP (BioByte Corporation, Pomona, California) database of experimental log P values was also accessed in some cases, although ClogP was not employed in deriving calculated values.

## RESULTS AND DISCUSSION

Tables 1-8 present the collated data on typical substrates of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP2E1, respectively. These provide $K_m$ values and details relating to the site of metabolism mediated by the relevant P450 enzyme concerned. In some instances, the $K_D$ values are provided, where $K_D$ is the spectroscopically-determined enzyme-substrate dissociation constant, such as in the case of certain CYP2D6 substrates (see Table 7). It is thought that although the $K_D$ value is preferred, the $K_m$ value also provides an adequate description of the substrate binding affinity from which free energy values may be calculated /21/. Although $K_m$ is a parameter derived from enzyme kinetics, it is regarded as a ratio of individual rate constants /22/ and, consequently, represents an equilibrium constant for the enzyme-substrate binding interaction. In most cases, $K_m \cong K_D$ because the rate-determining step for the reaction is usually the formation of the enzyme-substrate complex /21,22/. Although this may not necessarily

**TABLE 1**

Human CYP1A2 substrates and their metabolism

| Compound | log P | Metabolic pathway | $K_m$ (μM) | Ref. |
|---|---|---|---|---|
| **1. Caffeine** | 0.08 | $N^3$ demethylation | 180 | /31,32/ |
| **2. PhIP** | 2.23 | *N*-hydroxylation | 55 | /33/ |
| **3. 7-Methoxyresorufin** | 3.15 [c] | *O*-demethylation | 0.21 | /34/ |
| **4. Phenacetin** | 1.57 | *O*-deethylation | 48 | /35/ |
| **5. IQ** | 1.47 | *N*-hydroxylation | 33 | /36/ |
| **6. MeIQ** | 1.98 | *N*-hydroxylation | 13 | /35/ |
| **7. 4-Aminobiphenyl** | 2.86 | *N*-hydroxylation | 30 | /36/ |
| **8. 7-Ethoxyresorufin** | 3.61 [c] | *O*-deethylation | 1.7 | /35/ |
| **9. Aflatoxin $B_1$** | 2.20[c] | 8,9-epoxidation | 31 | /37/ |
| **10. Theophylline** | 0.95 | $N^3$ demethylation | 455 | /13/ |
| **11. Tacrine** | 2.71 | 7-hydroxylation | 14 | /38/ |
| **12. Naproxen** | 3.34 | *O*-demethylation | 160 | /39/ |
| **13. Estradiol** | 2.69 | 2-hydroxylation | 20 | /40/ |
| **14. Melatonin** | 1.45 | 6-hydroxylation | 85.5 | /41/ |
| **15. Propranolol** | 2.98 | *N*-desisopropylation | 87 | /42/ |
| **16. MeIQx** | 1.01 | *N*-hydroxylation | 19 | /43/ |

c = calculated value obtained via the Pallas software (CompuDrug, Ltd., Budapest);
IQ = 2-amino-3-methylimidazo[4,5-f]quinoline;
MeIQ = 2-amino-3,8-dimethylimidazo[4,5-f]quinoline;
MeIQx = 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline;
PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine.

**TABLE 2**

Human CYP2A6 substrates and their metabolism

| Compound | log P | Metabolic pathway | $K_m$ (μM) | Ref. |
|---|---|---|---|---|
| **1. Coumarin** | 1.39 | 7-hydroxylation | 2.1 | /44/ |
| **2. SM-12502** | 1.06 | *S*-oxidation | 4.7 | /45/ |
| **3. S-Nicotine** | 1.17 | 5′-oxidation | 25.5 | /46/ |
| **4. Losigamone** | 1.46 [c] | 3-hydroxylation | 10.0 | /47/ |
| **5. 4-Nitroanisole** | 2.03 | *O*-demethylation | 9.0 | /48/ |
| **6. Cotinine** | 0.07 | 3′-hydroxylation | 265.0 | /49,50/ |
| **7. Paracetamol** | 0.51 | 3-hydroxylation | 2170 | /51/ |
| **8. Quinoline** | 2.03 | 1-oxidation | 540 | /52/ |
| **9. Halothane** | 2.30 | reduction | 14 | /53/ |
| **10. Hexamethylphosphoramide** | 0.28 | demethylation | 2900 | /54/ |

c = calculated value obtained via the Pallas software (CompuDrug Ltd., Budapest); SM-12502 = (+)-*cis*-3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one.

**TABLE 3**

CYP2B6 substrates and their metabolism

| Compound | log P | Metabolic pathway | $K_m$ (μM) | Ref. |
|---|---|---|---|---|
| **1.** **7-Ethoxy-4-trifluoromethylcoumarin** | 3.31 [c] | *O*-deethylation | 2.9 | /55/ |
| **2.** **7-Benzyloxyresorufin** | 4.75 [c] | *O*-debenzylation | 1.28 | /55/ |
| **3.** **S-Mephenytoin** | 1.90 | *N*-demethylation | 564 | /56/ |
| **4.** **Benzphetamine** | 4.35 [c] | *N*-demethylation | 93.4 | /55/ |
| **5.** **Testosterone** | 3.32 | 16β-hydroxylation | 50.5 | /55/ |
| **6.** **Bupropion** | 2.54 | *t*-butyl oxidation | 107.5 | /55/ |
| **7.** **PNU 249173** | 4.89 [c] | cyclopentyl hydroxylation | 19.6 | /57/ |
| **8.** **Diazepam** | 2.86 | *N*-demethylation | 113 | /55/ |
| **9.** **7-Ethoxycoumarin** | 2.30 | *O*-deethylation | 115 | /55/ |
| **10.** **4-Chloromethyl-7-ethoxycoumarin** | 2.94 [c] | *O*-deethylation | 33.7 | /55/ |
| **11.** **3-Cyano-7-ethoxycoumarin** | 1.74 | *O*-deethylation | 71.3 | /55/ |
| **12.** **Methoxychlor** | 4.30 | 3-hydroxylation | N/A | /55/ |

c = calculated value obtained via the Pallas software (CompuDrug, Ltd., Budapest);
PNU 249173 = 2-cyclopentyloxy-*N*-(3,5-dichloro-4-pyridyl)-4-methoxybenzamide (also reported as RP 73401 in the literature); N/A = data not available.

**TABLE 4**

CYP2C8 substrates and their metabolism

| Compound | log P | Metabolic pathway | $K_m$ (μM) | Ref. |
|---|---|---|---|---|
| **1. Rosiglitazone** | 3.20 | *N*-demethylation | 10 | /59/ |
| **2. Retinoic acid** | 6.61 | 4-hydroxylation | 9 | /60/ |
| **3. Taxol** | 2.28[c] | 6α-hydroxylation | 5 | /61/ |
| **4. Carbamazepine** | 2.28 | 10,11-epoxidation | N/A | /62/ |
| **5. Zopiclone** | 1.50 [c] | *N*-demethylation | 71 | /63/ |
| **6. Cerivastatin** | 5.20 | *O*-demethylation | N/A | /64/ |
| **7. Arachidonic acid** | 6.98 | 14,15-epoxidation | 20.5 | /65/ |
| **8. Retinol** | 5.68 | 4-hydroxylation | 71 | /66/ |
| **9. Diclofenac** | 4.40 | 4′-hydroxylation | 630 | /67/ |
| **10. Amiodarone** | 2.54 | *N*-demethylation | 8.6 | /68/ |

c = calculated value obtained via the Pallas software (CompuDrug, Ltd., Budapest); N/A = data not available.

**TABLE 5**

CYP2C9 substrates and their metabolism

| Compound | log P | Metabolic pathway | $K_m$ (μM) | Ref. |
|---|---|---|---|---|
| **1. Diclofenac** | 4.40 | 4′-hydroxylation | 6 | /99/ |
| **2. Tolbutamide** | 2.34 | 4-methyl hydroxylation | 132 | /99/ |
| **3. Tienilic acid** | 3.15 | *S*-oxidation | 6 | /99/ |
| **4. S-Warfarin** | 2.70 | 7-hydroxylation | 4 | /99/ |
| **5. S-Ibuprofen** | 3.51 | isobutyl hydroxylation | 53 | /99/ |
| **6. Mefenamic acid** | 5.12 | 3′-methyl hydroxylation | 7 | /68/ |
| **7. Naproxen** | 3.34 | *O*-demethylation | 126 | /69/ |
| **8. 58C80** | 5.18 | *t*-butyl oxidation | 141 | /99/ |
| **9. Piroxicam** | 3.06 | 5′-hydroxylation | 40 | /70/ |
| **10. Phenytoin** | 2.47 | 4-hydroxylation | 45 | /71/ |
| **11. Losartan** | 4.48 | hydroxylmethyl oxidation | 20 | /72/ |
| **12. Lornoxicam** | 2.62 | 5′-hydroxylation | 3.6 | /73/ |

58C80 = 2-(4-*t*-butylcyclohexyl)-3-hydroxy-1,4-naphthoquinone.

**TABLE 6**

CYP2C19 substrates and their metabolism

| Compound | log P | Metabolic pathway | $K_m$ (μM) | Ref. |
|---|---|---|---|---|
| **1. Omeprazole** | 2.23 | 5-methyl hydroxylation | 8.6 | /74/ |
| **2. S-Mephenytoin** | 1.90 | 4′-hydroxylation | 50 | /75/ |
| **3. Proguanil** | 2.53 | isopropyl oxidation | 96 | /76/ |
| **4. Diazepam** | 2.86 | *N*-demethylation | 20 | /77/ |
| **5. LY307640** | 1.79 | *O*-demethylation | 19 | /78/ |
| **6. Phenytoin** | 2.47 | 4-hydroxylation | 70 | /71/ |
| **7. R-Hexobarbital** | 1.49 | 3′-hydroxylation | 740 | /79/ |
| **8. R-Mephobarbital** | 1.84 | 4-hydroxylation | 65 | /80/ |
| **9. Moclobemide** | 2.13 | morpholine oxidation | 770 | /81/ |
| **10. Melatonin** | 1.45 [c] | *O*-demethylation | 282 | /41/ |
| **11. Imipramine** | 4.44 | *N*-demethylation | 24.7 | /82/ |
| **12. R-Warfarin** | 2.70 | 8-hydroxylation | 330 | /83/ |

c = calculated value obtained via the Pallas software (CompuDrug, Ltd., Budapest); LY307640 = 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole (also known as Rabeprazole).

**TABLE 7**

CYP2D6 substrates and their metabolism

| Compound | | log P | Metabolic pathway | $K_D$ (μM) | Ref. |
|---|---|---|---|---|---|
| **1.** | **Propranolol** | 2.98 | 4-hydroxylation | 2.73 | /14/ |
| **2.** | **Debrisoquine** | 0.75 | 4′-hydroxylation | 13 | /14/ |
| **3.** | **R-Bufuralol** | 3.50 | 1′-hydroxylation | 8.6 | /14/ |
| **4.** | **Metoprolol** | 1.88 | *O*-demethylation | 46 * | /84/ |
| **5.** | **Dextromethorphan** | 3.70 | *O*-demethylation | 2.76 | /14/ |
| **6.** | **Codeine** | 1.14 | *O*-demethylation | 15 | /14/ |
| **7.** | **MDMA** | 2.28 [c] | *O*-demethylation | 1.72 [†] | /85/ |
| **8.** | **Imipramine** | 4.44 | 2-hydroxylation | 2.4 | /14/ |
| **9.** | **Desipramine** | 4.90 | 2-hydroxylation | 20 | /14/ |
| **10.** | **Nortriptyline** | 4.04 | 10-hydroxylation | 47 | /14/ |
| **11.** | **MPTP** | 2.14 | *N*-demethylation | 130 [†] | /14,86/ |
| **12.** | **Ondansetron** | 2.14 | 7-hydroxylation | 102 | /14/ |

c = calculated value obtained via the Pallas software (CompuDrug, Ltd., Budapest);
MDMA = methylenedioxy-*N*-methylamphetamine;
MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.
Methodology for determination of $K_D$ values is given in reference /14/.
* $K_s$ value quoted which is probably of the same order of magnitude as the $K_D$ value (not determined).
† $K_m$ values quoted in these references are likely to be close to the $K_D$ value (not determined).

**TABLE 8**

Human CYP2E1 substrates and their metabolism

| Compound | log P | Metabolic pathway | $K_m$ (μM) | Ref. |
|---|---|---|---|---|
| **1. 4-Nitrophenol** | 2.04 | 2-hydroxylation | 21 | /87/ |
| **2. Chlorzoxazone** | 2.36 [c] | 6-hydroxylation | 39 | /88/ |
| **3. Aniline** | 0.90 | 4-hydroxylation | 873 | /89/ |
| **4. Paracetamol** | 0.51 | *N*-oxidation | 1290 | /51/ |
| **5. Lauric acid** | 4.20 | $\omega^{-1}$ hydroxylation | 130 | /90/ |
| **6. Butadiene** | 1.99 | 1,2-epoxidation | 200 | /91/ |
| **7. Halothane** | 2.30 | dehalogenation | 35 | /72/ |
| **8. Ethanol** | -0.32 | 2-hydroxylation | 11200 | /93/ |
| **9. Benzene** | 2.13 | epoxidation | 25 | /94/ |
| **10. Tetrachloromethane** | 2.83 | dehalogenation | 57 | /95/ |
| **11. Dimethylnitrosamine** | -0.57 | *N*-demethylation | 2000 | /96/ |
| **12. Salicylic acid** | 2.25 | 5-hydroxylation | 280 | /97/ |

c = calculated value obtained via the Pallas software (CompuDrug, Ltd., Budapest).

be the case in all examples, it appears to hold reasonably well for P450-mediated reactions for which both determinations are available. However, $K_m$ values are frequently reported in the literature, whereas $K_D$ values are rarely available from published work. In the current work, $K_D$ data have been determined for several substrates using a spectroscopic method, as has been described previously /14/.

From previously published data relating to P450 substrates /9/, it is possible to delineate certain characteristics (Table 9) which tend to be common for substrates of each P450 enzyme. For example, the physicochemical data (in the form of log P, log $D_{7.4}$ and $pK_a$ values) usually lie within a certain well-defined range of values, especially log

**TABLE 9**

Numbers of heavy atoms between hydrogen bond donor/acceptor atoms and sites of metabolism in human P450 substrates

| CYP | Primary site | Secondary site | Other characteristics |
|---|---|---|---|
| **1A2** | 6 | 9 | Planar molecules |
| **2A6** | 6 | – | Relatively planar molecules |
| **2B6** | 8 | – | Medium-sized molecules |
| **2C8** | 12 | – | Relatively large molecules |
| **2C9** | 10 | – | Acidic group in molecules |
| **2C19** | 7 | 8 | Medium-sized molecules |
| **2D6** | 9 | – | Basic group in molecules |
| **2E1** | 3 | 5 | Relatively small molecules |

*Note:* The primary and secondary sites refer to hydrogen bond donor/acceptor atoms in the molecule and their distances from the preferred site of metabolism in terms of numbers of intervening 'heavy' atoms (atoms other than hydrogen). These represent the averages for a relatively narrow range of values (± 1) in terms of numbers of atoms between hydrogen bond donor/acceptors and sites of metabolism. The location of these sites is presented in Figures 1-7 which relate to substrates of CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP2E1, respectively /98/.

P (Table 10) for substrates of each P450, and selective substrates frequently take values close to the average for each enzyme /23/. For example, substrates of CYP2D6 tend to be basic, whereas those of CYP2C9 are usually weakly acidic in character. Moreover, many substrates of CYP2E1 are neutral molecules of relatively small molecular weight. In the case of CYP2B6, the substrates tend to be basic in character but it is thought that they are likely to bind in the non-ionised state. However, for CYP2D6 substrates, binding as the protonated base is almost certainly essential for interaction with acidic residues in the active site, particularly with aspartate-301, although

**TABLE 10**

Ranges and average log P values for substrates of human CYP1 and CYP2 families

| CYP | Range | No. of compounds | Average | Typical substrate (log P) | |
|---|---|---|---|---|---|
| **1A2** | 0.08 to 3.61 | 18 | 2.01 (± 1.93) | MeIQ | (1.98) |
| **2A6** | 0.07 to 2.79 | 18 | 1.44 (± 1.37) | Losigamone | (1.46) |
| **2B6** | 0.23 to 4.89 | 16 | 2.54 (± 2.35) | Bupropion | (2.54) |
| **2C8** | 0.06 to 6.98 | 12 | 3.38 (+ 3.60) | Rosiglitazone | (3.20) |
| **2C9** | 0.89 to 5.18 | 18 | 3.20 (± 2.31) | Naproxen | (3.18) |
| **2C19** | 1.49 to 4.42 | 16 | 2.56 (± 1.86) | Proguanil | (2.53) |
| **2D6** | 0.75 to 5.04 | 16 | 3.08 (± 2.33) | Propranolol | (3.09) |
| **2E1** | -1.35 to 3.63 | 20 | 2.07 (± 3.42) | 4-Nitrophenol | (2.04) |

It is important to note that the log P values of typical substrates /23/ are close to the average value for each individual enzyme's substrates, thus indicating that there may be an optimal log P for substrates binding to individual P450s.

recent studies indicate a role for another cationic residue glutamate-216 /24/. Similarly, CYP2C19 substrates are also basic, although they tend to be weaker bases than those of CYP2D6, but hydrogen bond donor/acceptor atoms in the molecule at specific sites tend to confer CYP2C19 substrate selectivity. Similar relationships exist for substrates of CYP1A2 and CYP2A6, some of which are basic in character - although many are neutral molecules. However, an overall high degree of molecular planarity is characteristic of CYP1A2 substrates. Although CYP2C8 substrates also tend to be acidic compounds, like those of CYP2C9, their molecular structures are somewhat different in terms of size and shape /9/. In the latter respect, there is some degree of overlap with CYP3A4 substrate selectivity and, moreover, compounds such as rosiglitazone, trimethoprim and amiodarone are substrates of both enzymes. Possible rationales for human P450

substrate selectivities are, therefore, highlighted by a consideration of their physicochemical properties and also their molecular structures, especially the disposition of hydrogen bond donor/acceptors in the molecule. Figures 1-7 show structures of typical substrates for CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP2E1, respectively. The similarities between these compounds for each class and the location of their sites of metabolism are now discussed for each class of substrate, under subheadings for each P450 enzyme.

**CYP1A2 substrates**

Figure 1 shows a total of 12 CYP1A2 substrates, including the mechanism-based inhibitor furafylline, and the preferred sites of metabolism and location of hydrogen bond donors and acceptors are presented in each case. All of the compounds possess relatively planar molecules by virtue of their fused aromatic ring systems, especially 7-methoxyresorufin which is a very good substrate for CYP1A2. All of the substrates contain hydrogen bond donor/acceptor atoms and these are located at fairly specific distances from the sites of metabolism in terms of the number of intervening 'heavy' atoms (atoms other than hydrogen). The number and disposition of these groupings is critical for CYP1A2 selectivity and binding affinity, together with the presence of aromatic ring systems which facilitate interaction with the enzyme's binding site. In fact, there would appear to be a direct relationship between the numbers of hydrogen bonded and $\pi$-$\pi$ stacking interactions for CYP1A2 substrates and their binding affinity towards the enzyme /25/ although the desolvation energy also makes an important contribution to the overall binding energy.

**CYP2A6 substrates**

In Figure 2, a number of typical CYP2A6 substrate structures are presented, together with that of the inhibitors, pilocarpine and methoxsalen. Although some CYP2A6-selective compounds have relatively planar structures (e.g. coumarin) as has the inhibitor methoxsalen, there are many that are non-planar, such as nicotine, losigamone and SM-12502. The preferred site of metabolism is indicated for each substrate in Figure 2, as is the most likely hydrogen bond contact (or contacts) within the CYP2A6 active site. It can be

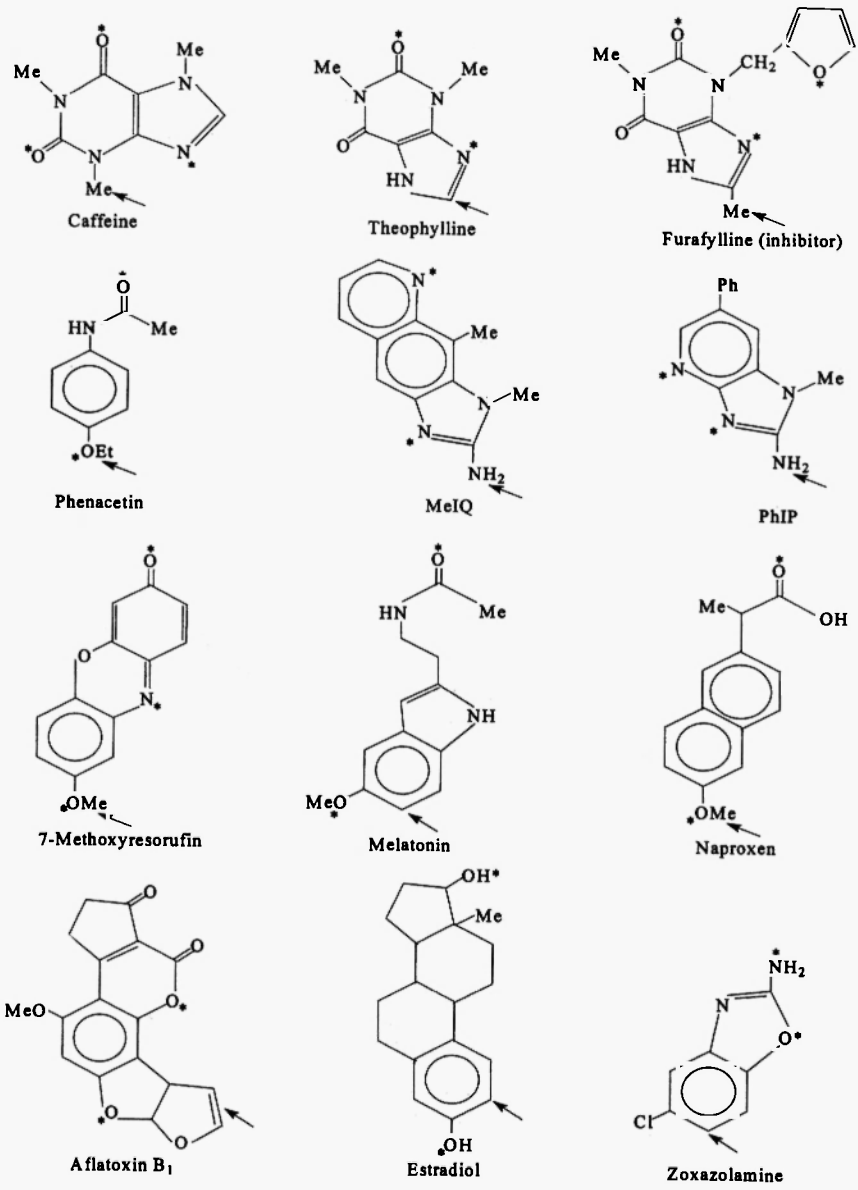


**Fig. 1:** Some typical CYP1A2 substrates showing sites of metabolism (↑) and hydrogen bonding (*). The selective inhibitor furafylline is also shown.

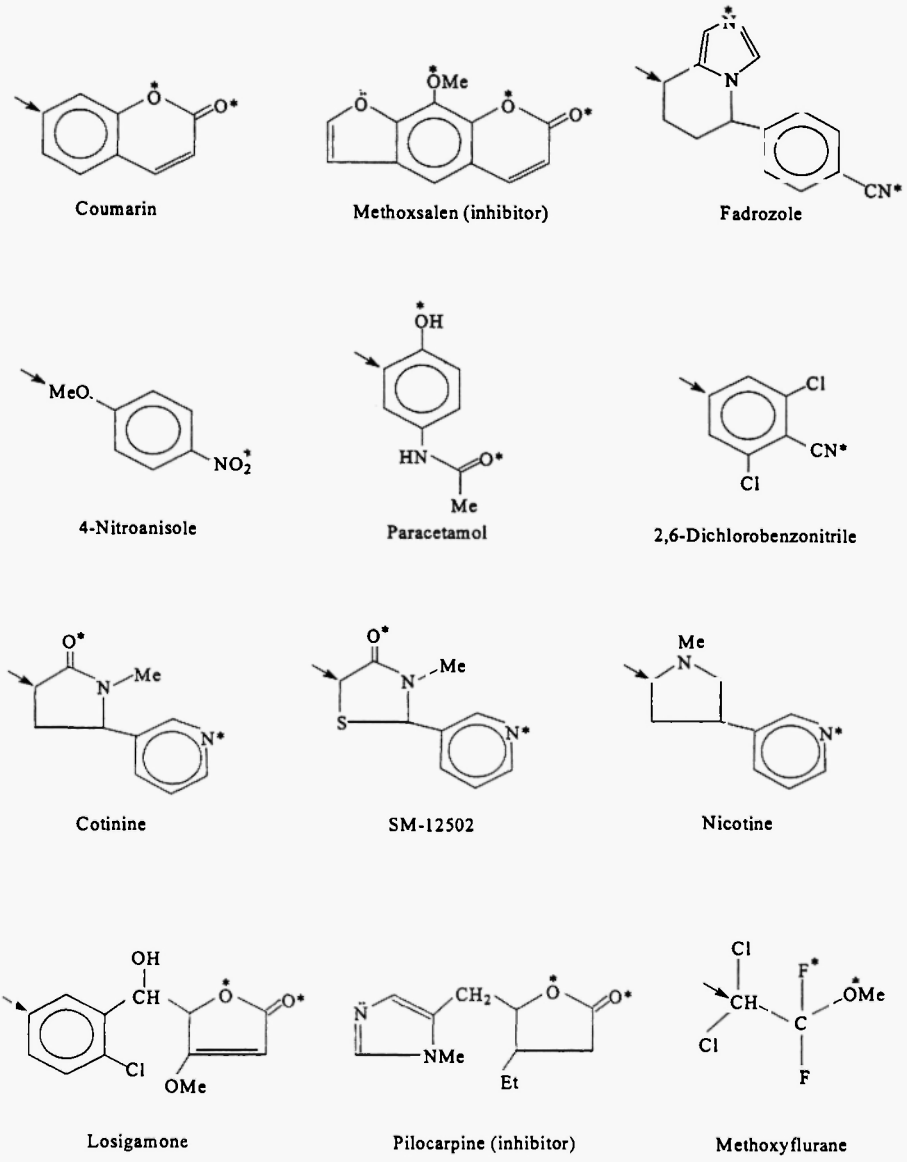


**Fig. 2:** Some typical CYP2A6 substrates showing sites of metabolism (↑) and hydrogen bonding (*). The inhibitors pilocarpine and methoxsalen are also shown.

appreciated, from inspection of the information presented in Figure 2, that the site of CYP2A6-mediated metabolism lies about six heavy atoms distance from the hydrogen bond site, thus indicating the presence of a hydrogen bond donor residue relatively close to the haem iron in CYP2A6. This is supported by homology modelling investigations of the enzyme itself /25/.

**CYP2B6 substrates**

Figure 3 presents a number of typical CYP2B6 substrates, and shows their preferred sites of metabolism together with the locations of hydrogen bond acceptors that appear to be binding with the enzyme's active site. In general, the site of metabolism lies about seven heavy atoms distance from a hydrogen bond acceptor atom, although there appears to be a range of values about this average. Some of these compounds are also metabolised by other P450s, sometimes at other positions, although bupropion appears to be metabolised primarily via CYP2B6. There is a good correlation between compound lipophilicity and substrate binding affinity for many CYP2B6 substrates, although the highly selective compounds such as 4-trifluoromethyl-7-ethoxycoumarin and PNU 249173 exhibit stronger affinity than expected due to the formation of additional hydrogen bond interactions within the CYP2B6 active site region /26/.

**CYP2C9 substrates**

In Figure 4, the structures of several typical substrates of CYP2C9 are presented, together with indications of the known sites of metabolism and hydrogen bond donor/acceptor atoms. The majority of these compounds are weakly acidic in character and some actually contain a carboxylic acid group /27/. The location of the sites of metabolism and hydrogen bond interactions tends to lie approximately 10 heavy atoms apart, as listed in Table 9. Usually, the acidic grouping tends to be associated with hydrogen bond interactions as opposed to ionic pairing with a basic residue within the CYP2C9 active site; although this is a second possibility, as basic amino acid residues are indeed present in the active site region. Molecular modelling of typical CYP2C9 substrates within the putative active site of CYP2C9 appears to be consistent with experimental findings from site-directed mutagenesis and substrate metabolism. There is a degree of overlapping

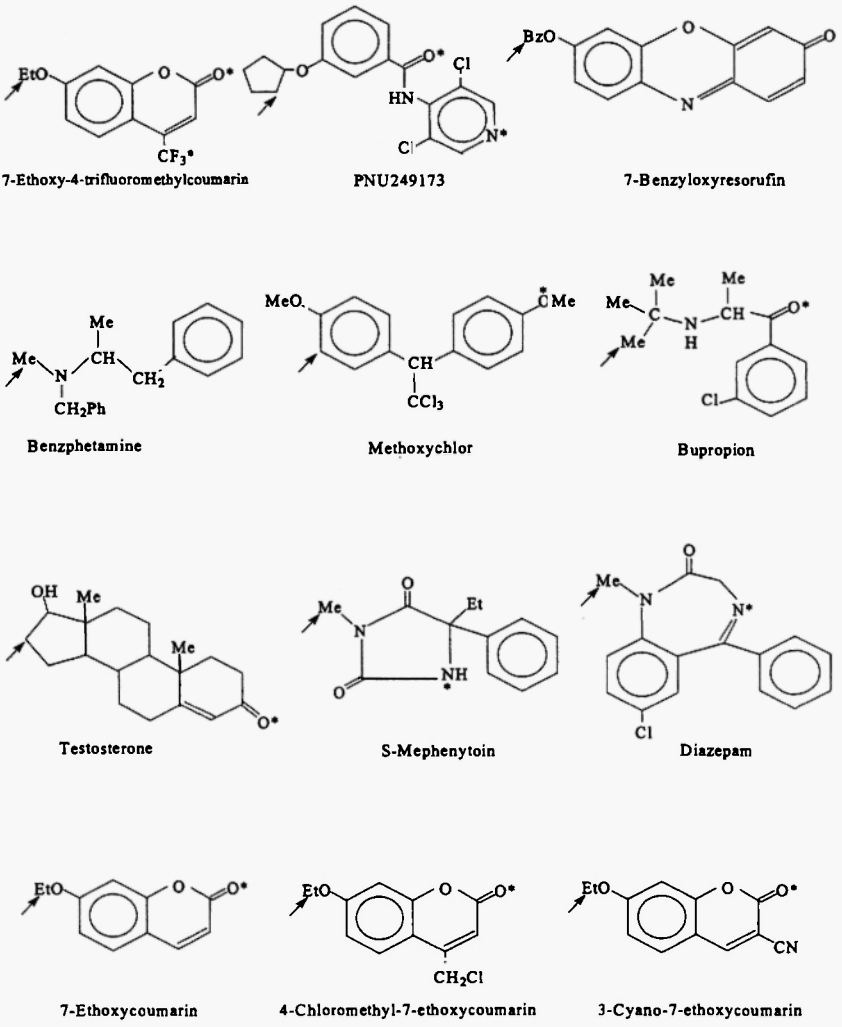


**Fig. 3:** Some typical CYP2B6 substrates showing sites of metabolism (↑) and hydrogen bonding (*).

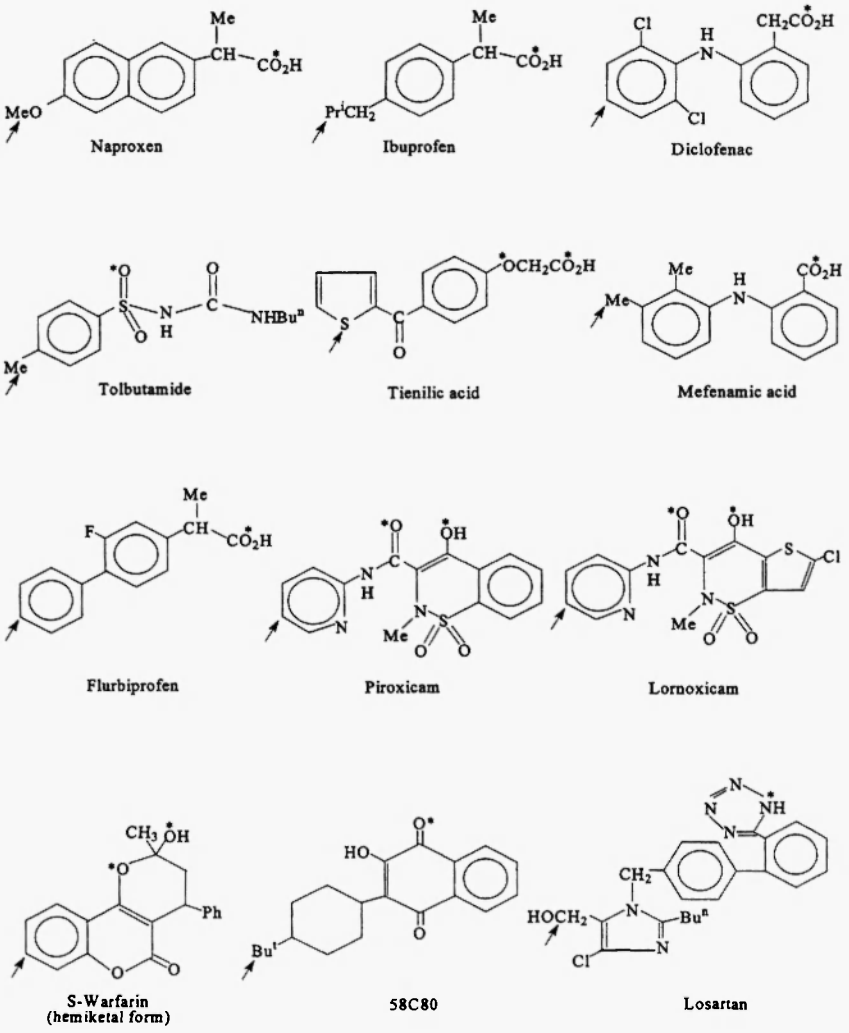


**Fig. 4:** Some typical CYP2C9 substrates showing sites of metabolism (↑) and hydrogen bonding (*).

substrate selectivity towards CYP2C9 and CYP2C8, although the latter can be somewhat larger sized molecules and, as indicated in Table 9, there tends to be a greater distance between the acidic group and site of metabolism for CYP2C8 substrates. It is interesting to note that the CYP2C5-derived model of CYP2C9 agrees closely with the reported crystal structure of CYP2C9 /28/, as has been published recently /29/. However, the (*S*)-warfarin substrate, which is located several Ångstroms from the haem in the CYP2C9 crystal structure, is likely to move closer to the haem moiety for oxygenation to occur during the final stages of the catalytic cycle.

**CYP2C19 substrates**

The structures of several CYP2C19-selective substrates are shown in Figure 5. In each case, the sites of metabolism and likely hydrogen bonding to the active site are indicated. Unlike those of CYP2C9, substrates of CYP2C19 are not usually acidic in character: some are amides and amines, as exemplified by moclobemide and propranolol, respectively. Furthermore, there are usually two hydrogen bond donor/acceptor atoms in the CYP2C19 substrates as opposed to the situation in those compounds that are selective for CYP2C9 which normally possess only one hydrogen bond contact site. As presented in Table 9, the sites of metabolism for CYP2C19 substrates tend to lie about seven or eight heavy atoms from the hydrogen bond donor/ acceptor sites. Molecular modelling of these compounds within the putative active site of CYP2C19, generated by homology with CYP2C5, is consistent with known information regarding positions of metabolism and site-directed mutagenesis experiments within the CYP2C subfamily, in which one or more hydrogen bond contacts between substrates and key amino acid residues are apparent.

**CYP2D6 substrates**

The structures of some typical substrates of CYP2D6 are presented in Figure 6, and it is characteristic that these compounds exhibit basicity by virtue of a protonatable nitrogen atom in the molecule. In general, the site of CYP2D6-mediated metabolism lies at a distance of between 5 and 7 Å from the basic nitrogen atom, although some exceptions do exist. Some CYP2D6 substrates also indicate the possibility for hydrogen bonding within the enzyme's active site and

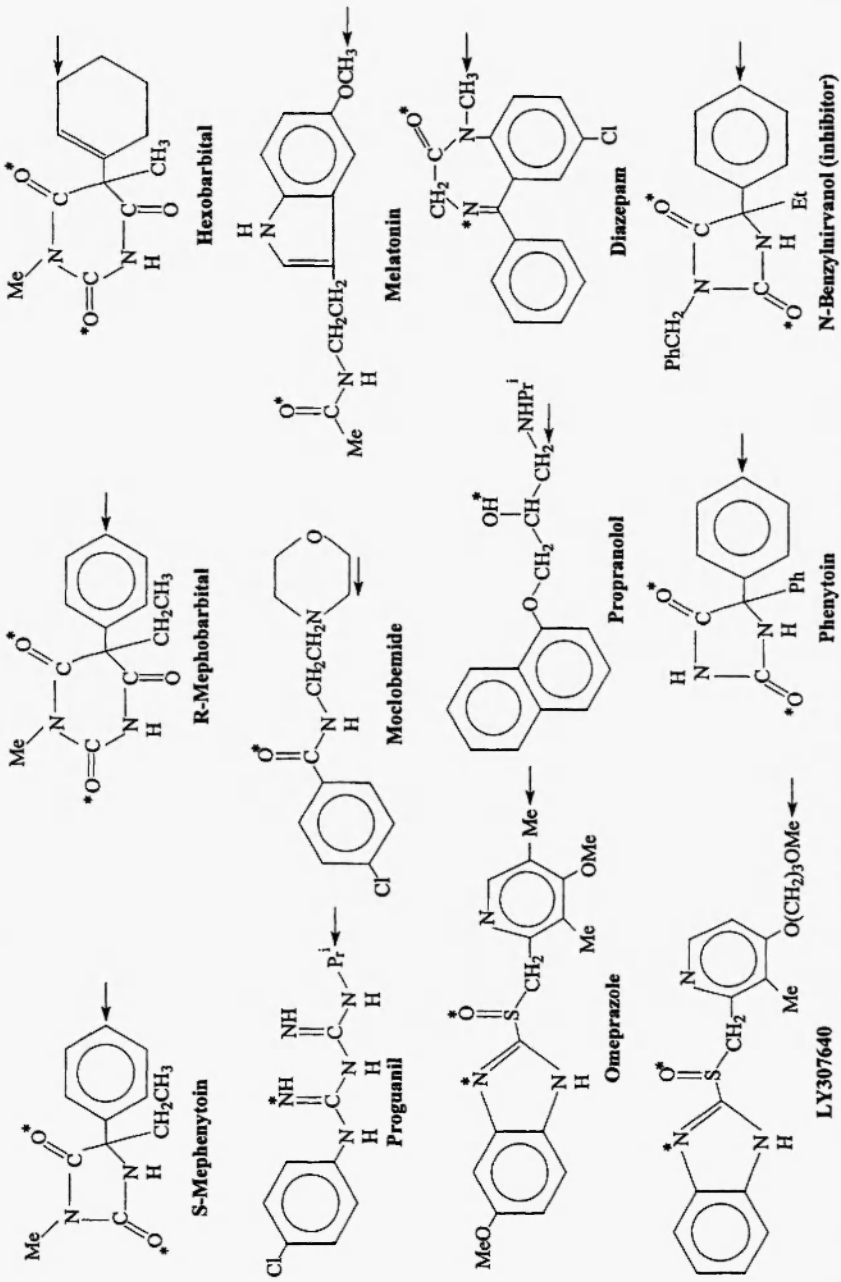


**Fig. 5:** Some typical CYP2C19 substrates showing sites of metabolism (↑) and hydrogen bonding (*). The inhibitor *N*-benzylnirvanol is also shown.

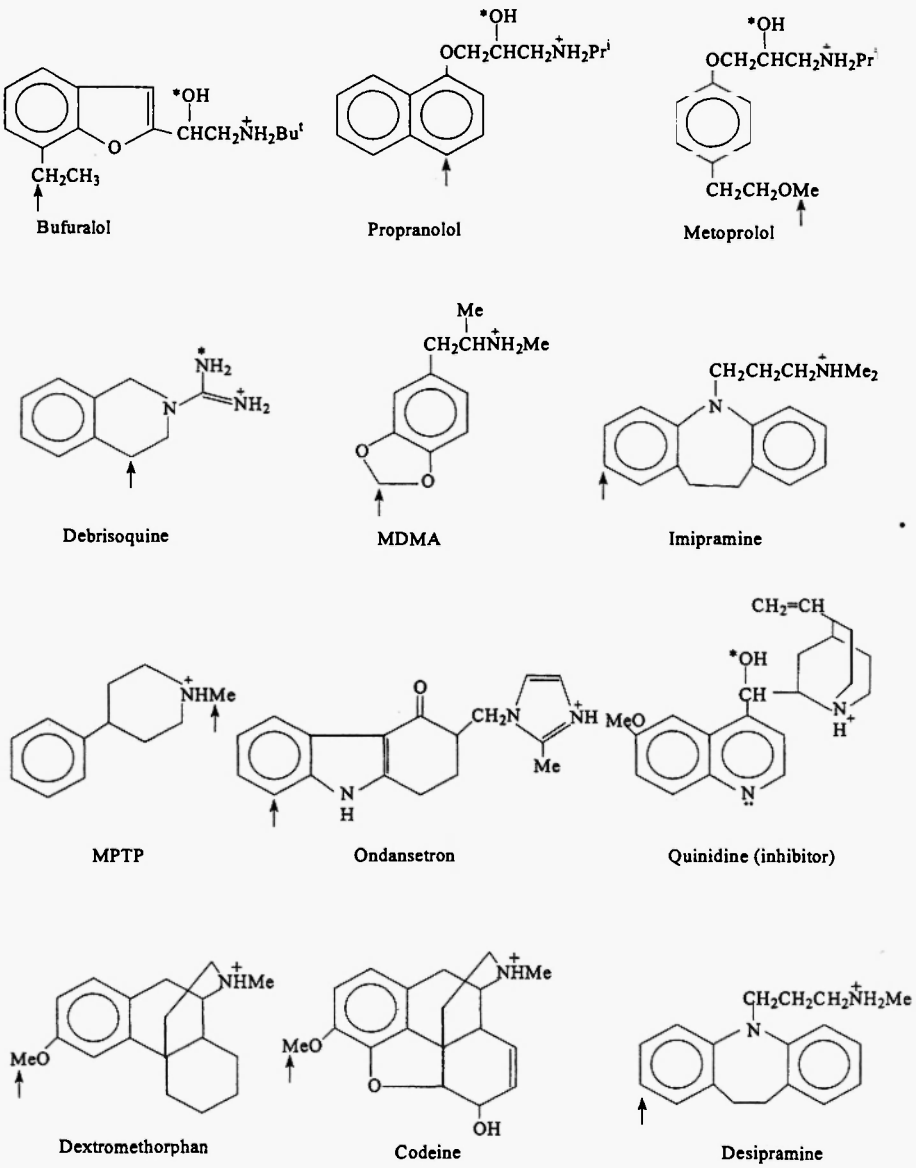


**Fig. 6:** Some typical CYP2D6 substrates showing sites of metabolism (↑) and hydrogen bonding (*), together with the protonated nitrogen atom (+). The selective inhibitor quinidine is also shown.

the possession of at least one aromatic ring is a common feature, as can be appreciated from an inspection of the structures in Figure 6. The likely amino acid residues involved in ionic and π-π stacking interactions with typical substrates have been identified by site-directed mutagenesis, and molecular modelling studies appear to show consistency with the results of experimental work, in terms of mutagenesis and sites of CYP2D6-mediated metabolism. Although the general characteristic is one of an essentially hydrophobic active site, there are key acidic residues (such as aspartate-301) that appear to form ion-pair interactions with a protonated nitrogen that is present in the majority of CYP2D6 substrates.

**CYP2E1 substrates**

Figure 7 shows the structures of typical CYP2E1 substrates. Although structurally diverse, such compounds tend to possess relatively small-sized molecules. Moreover, there is usually a degree of hydrogen bond-forming potential with the preferred site of CYP2E1-mediated metabolism lying about three heavy atoms from a hydrogen bond donor or acceptor in the substrate molecule. In some cases, however, the hydrogen bond donor/acceptor atom is five heavy atoms distant from the site of metabolism, as can be appreciated by inspection of the structures presented in Figure 7. There is also a good correlation between binding affinity and lipophilicity, as determined by the log P value, for CYP2E1 substrates /30/. Very little site-directed mutagenesis has been performed on CYP2E1 enzymes, but molecular modelling studies exhibit consistency with current information on CYP2E1-mediated metabolism.

## CONCLUSIONS

Consideration of human P450 substrate properties can aid in the understanding of enzyme selectivity within the CYP1 and CYP2 families. In particular, the disposition of hydrogen bond donors and acceptors in the substrate molecule appears to be important for determining the site of metabolism. Furthermore, the molecular shape and lipophilic character play a role in substrate selectivity, although there may be overlapping lipophilicity ranges for different human P450s, together with an associated finding that some compounds may

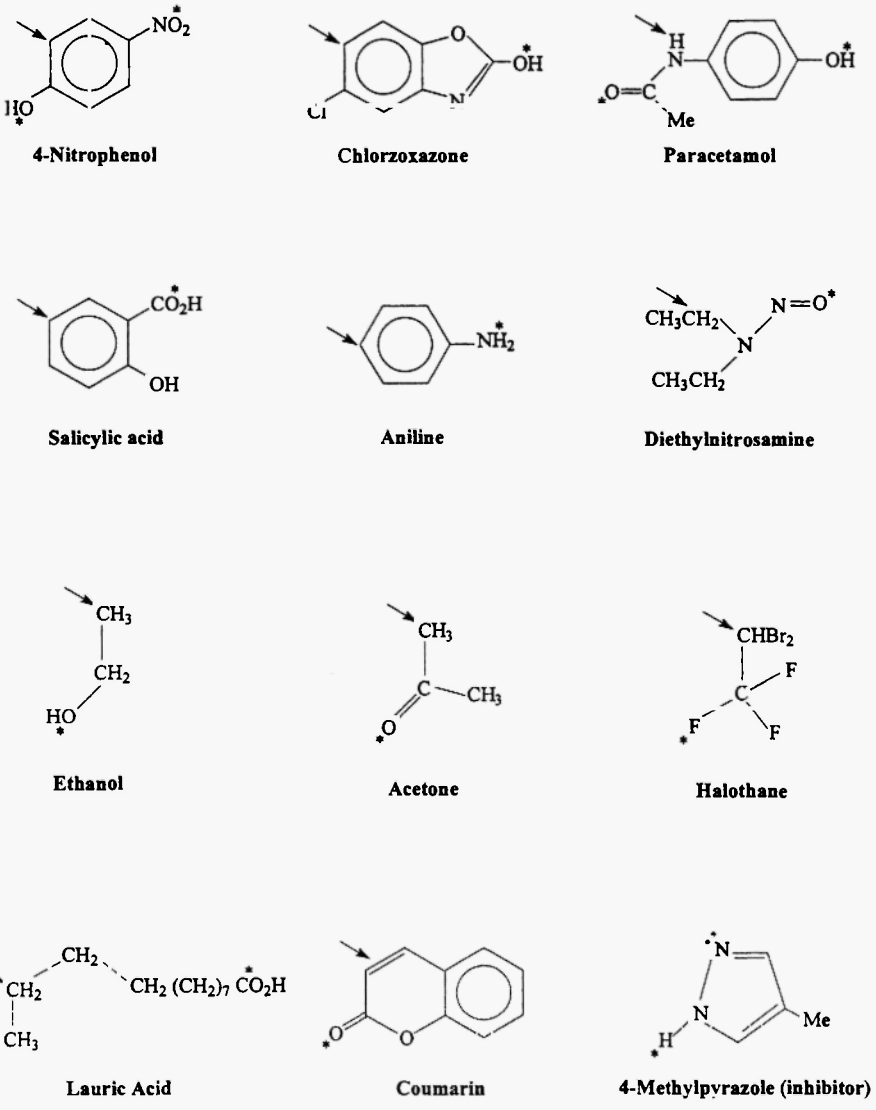


**Fig. 7:** Some typical CYP2E1 substrates showing sites of metabolism (↑) and hydrogen bonding (*). The inhibitor 4-methylpyrazole is also shown.

be metabolised by different P450s but sometimes (although not always) at the same site in the molecule.

**ACKNOWLEDGEMENTS**

The financial support of GlaxoSmithKline Research and Development Limited, Merck Sharp & Dohme Limited and the University of Surrey Foundation Fund is gratefully acknowledged by one of us (DFVL).

**REFERENCES**

1. Ortiz de Montellano PR, ed. Cytochrome P450, 2nd Ed. New York: Plenum, 1995.
2. Lewis DFV. Cytochromes P450: Structure, Function and Mechanism. London: Taylor & Francis, 1996.
3. Lewis DFV. Guide to Cytochromes P450 Structure and Function. London: Taylor & Francis, 2001.
4. Nelson DR. Introductory remarks on human CYPs. Drug Metab Rev 2002; 34: 1-5.
5. Guengerich FJ. Update information on human P450s. Drug Metab Rev 2002; 34: 7-15.
6. Dollery C. Therapeutic Drugs, 2nd Ed. Edinburgh: Churchill-Livingstone, 1999.
7. Rendic S, DiCarlo FJ. Human cytochrome P450 enzyme: a status report summarizing their reactions, substrates, inducers and inhibitors. Drug Metab Rev 1997; 29: 413-580.
8. Rendic S. Summary of information on human CYP enzymes: human P450 metabolism data. Drug Metab Rev 2002; 34: 83-448.
9. Lewis DFV. Human cytochromes P450 associated with the phase 1 metabolism of drugs and other xenobiotics: a compilation of substrates and inhibitors of the CYP1, CYP2 and CYP3 families. Curr Med Chem 2003; 10: 1955-1972.
10. Carlile DJ, Hakooz N, Bayliss MK, Houston JB. Microsomal prediction of in vivo clearance of CYP2C9 substrates in humans. Br J Clin Pharmacol 1999; 47: 625-635.
11. Tucker GT, Houston JB, Huang S-M. Optimizing drug development: strategies to assess drug metabolism/transporter interaction potential - towards a consensus. Br J Clin Pharmacol 2001; 52: 107-117.
12. Lu AYH, Wang RW, Lin JH. Cytochrome P450 in vitro reaction phenotyping: a re-evaluation of approaches used for P450 isoform identification. Drug Metab Dispos 2003; 31: 345-350.

13. Pelkonen O, Maenpaa J, Taavitsainen P, Raunio H. Inhibition and induction of human cytochrome P450 (CYP) enzymes. Xenobiotica 1998; 28: 1203-1253.
14. Lewis DFV, Modi S, Dickins M. Structure-activity relationships for human cytochrome P450 substrates and inhibitors. Drug Metab Rev 2002; 34: 69-82.
15. Hansch C, Leo AJ. Substituent Constants for Correlation Analysis in Chemistry and Biology. New York: Wiley, 1979.
16. Hansch C, Leo A, Hoekman D. Exploring QSAR: Hydrophobic, Electronic and Steric Constants. Washington, DC: American Chemical Society, 1995.
17. Newton DW, Kluza RB. pKa values of medicinal compounds in pharmacy practice. Drug Intell Clin Pharm 1978; 12: 546-554.
18. Sangster J. Octanol-water partition coefficients of simple organic compounds. J Phys Chem Ref Data 1989; 18: 1111-1229.
19. Suzuki T, Kudo Y. Automatic log P estimation based on combined additive modeling methods. J Comput Aid Mol Design 1990; 4: 155-198.
20. Elks J, Ganellin CR. Dictionary of Drugs. London: Chapman and Hall, 1990.
21. Bauer C, Osman AM, Cercignani G, Gialluca N, Paolini M. A unified theory of enzyme kinetics based upon the systematic analysis of the variations of $k_{cat}$, $K_m$ and $k_{cat}/K_m$ and the relevant $\Delta G^{o\ddagger}$ values–possible implications in chemotherapy and biotechnology. Biochem Pharmacol 2001; 61: 1049-1055.
22. Northrop DB. On the meaning of $K_m$ and V/K in enzyme kinetics, Journal of Chem Educ 1998; 75: 1153-1157.
23. Lewis DFV. Essential requirements for substrate binding affinity and selectivity toward human CYP2 family enzymes. Arch Biochem Biophys 2003; 409: 32-46.
24. Guengerich FP, Hanna IH, Martin MV, Gillam EMJ. Role of glutamic acid 216 in cytochrome P450 2D6 substrate binding and catalysis. Biochemistry 2003; 42: 1245-1253.
25. Lewis DFV. Homology modelling of human CYP2 family enzymes based on the CYP2C5 crystal structure. Xenobiotica 2002; 32: 305-323.
26. Lewis DFV, Lake BG, Dickins M, Goldfarb PS. Molecular modelling of CYP2B6 based on homology with the CYP2C5 crystal structure: analysis of enzyme-substrate interactions. Drug Metab Drug Interact 2003; 19: 115-135.
27. Smith DA, Jones BC. Speculations on the substrate structure-activity relationship (SSAR) of cytochrome P450 enzymes. Biochem Pharmacol 1992; 44: 2089-2098.
28. Williams PA, Cosme J, Ward A, Angove HC, Vinkovic DM, Jhoti H. Crystal structure of human cytochrome P450 2C9 with bound warfarin. Nature 2003; 424: 464-468.
29. Lewis DFV. 57 varieties: the human cytochromes P450. Pharmacogenomics 2004; 5: 305-318.
30. Lewis DFV, Lake BG, Bird MG, Loizou GD, Dickins M, Goldfarb PS. Homology modelling of human CYP2E1 based on the CYP2C5 crystal structure: investigation of enzyme-substrate and enzyme-inhibitor interactions. Toxicol Vitro 2003; 17: 93-105.
31. Tassaneeyakul W, Birkett DJ, McManus ME, Tassaneeyakul W, Veronese ME, Andersson T, Tukey RH, Miners JO. Caffeine metabolism by human

hepatic cytochromes P450: contributions of 1A2, 2E1 and 3A isoforms. Biochem Pharmacol 1994; 47: 1767-1776.
32. Tassaneeyakul W, Mohamed Z, Birkett DJ, McManus ME, Veronese ME, Tukey RH, Quattrochi LC, Gonzalez FJ, Miners JO. Caffeine as a probe for human cytochrome P450: validation using cDNA expression, immuno-inhibition and microsomal kinetic and inhibitor techniques. Pharmacogenetics 1992; 2: 173-183.
33. Zhao K, Murray S, Davies DS, Boobis AR, Gooderham NJ. Metabolism of the food derived mutagen and carcinogen 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) by human liver microsomes. Carcinogenesis 1994; 15: 1285-1288.
34. Turesky RJ, Constable A, Richoz J, Varga N, Markovic J, Martin MV, Guengerich FP. Activation of heterocyclic aromatic amines by rat and human liver microsomes and by purified rat and human cytochrome P4501A2. Chem Res Toxicol 1998; 11: 925-936.
35. Parikh A, Josephy PD, Guengerich FP. Selection and characterization of human cytochrome P4501A2 mutants with altered catalytic properties. Biochemistry 1999; 38: 5283-5289.
36. Hammons GJ, Milton D, Stepps K, Guengerich FP, Tukey RH, Kadlubar FF. Metabolism of carcinogenic heterocyclic and aromatic amines by recombinant human cytochrome P450 enzymes. Carcinogenesis 1997; 18: 851-854.
37. Gallagher EP, Kunze KL, Stapleton PL, Eaton DL. The kinetics of aflatoxin $B_1$ oxidation by human cDNA-expressed and human liver microsomal cytochromes P4501A2 and 3A4. Toxicol Appl Pharmacol 1996; 141: 595-606.
38. Becquemont L, Le Bot MA, Riche C, Funck-Brentano C, Jaillon P, Beaune P. Use of heterologously expressed human cytochrome P4501A2 to predict tacrine-fluvoxamine drug interaction in man. Pharmacogenetics 1998; 8: 101-108.
39. Rodrigues AD. Integrated cytochrome P450 reaction phenotyping: attempting to bridge the gap between cDNA-expressed cytochromes P450 and native human liver microsomes. Biochem Pharmacol 1999; 57: 465-480.
40. Yamazaki H, Shaw PM, Guengerich FP, Shimada T. Roles of cytochrome P450 1A2 and 3A4 in the oxidation of estradiol and estrone in human liver microsomes. Chem Res Toxicol 1998; 11: 659-665.
41. Facciolá G, Hidestrand M, von Baher C, Tybring G. Cytochrome P450 isoforms involved in melatonin metabolism in human liver microsomes. Eur J Clin Pharmacol 2001; 56: 881-888.
42. Ching MS, Bichara N, Blake CL, Ghabrial H, Tukey RH, Smallwood RA. Propranolol 4- and 5-hydroxylation and *N*-desisopropylation by cloned human cytochrome P4501A1 and P4501A2. Drug Metab Dispos 1996; 24: 692-694.
43. Guengerich FP, Parikh A, Turesky RJ, Josephy PD. Inter-individual differences in the metabolism of environmental toxicants: cytochrome P4501A2 as a prototype. Mutat Res 1999; 428: 115-124.
44. Shimada T, Yamazaki H, Guengerich FP. Ethnic-related differences in coumarin 7-hydroxylation activities catalyzed by cytochrome P4502A6 in

liver microsomes of Japanese and Caucasian populations. Xenobiotica 1996; 26: 395-403.

45. Nunoya K-I, Yokoi Y, Kimura K, Kodama T, Funayama M, Inoue K, Nagashima K, Funae Y, Shimada N, Green C, Kamataki T. (+)-*Cis*-3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one hydrochloride (SM-12502) as a novel substrate for cytochrome P4502A6 in human liver microsomes. J Pharmacol Exp Ther 1996; 277: 768-774.
46. Messina ES, Tyndale RF, Sellers EM. A major role for CYP2A6 in nicotine C-oxidation by human liver microsomes. J Pharmacol Exp Ther 1997; 282: 1608-1614.
47. Torchin CD, McNeilly PJ, Kapetanovic IM, Strong JM, Kupferberg HJ. Stereoselective metabolism of a new anticonvulsant drug candidate, losigamone, by human liver microsomes. Drug Metab Dispos 1996; 24: 1002-1008.
48. Jones BC, Tyman CA, Smith DA. Identification of the cytochrome P450 isoforms involved in *O*-demethylation of 4-nitroanisole in human liver microsomes. Xenobiotica 1997; 27: 1025-1037.
49. Nakajima M, Tainamoto T, Nunoya K-I, Yokoi T, Nagashima K, Inoue K, Funae Y, Shimada N, Kamataki T, Kuroiwa Y. Role of human cytochrome P4502A6 in C-oxidation of nicotine. Drug Metab Dispos 1996; 24: 1212-1217.
50. Nakajima M, Tamamoto T, Nunoya K-I, Yokoi T, Nagashima K, Inoue K, Funae Y, Shimada N, Kamataki T, Kuroiwa Y. Characterization of CYP2A6 involved in 3′-hydroxylation of cotinine in human liver microsomes. J Pharmacol Exp Ther 1996; 277: 1010-1015.
51. Chen W, Koenigs LL, Thompson SJ, Peter RM, Rettie AE, Trager WF, Nelson SD. Oxidation of acetamidophen to its toxic quinone imine and nontoxic catechol by Baculovirus-expressed and purified human cytochromes P4502E1 and 2A6. Chem Res Toxicol 1998; 11: 295-301.
52. Reigh G, McMahon H, Ishizaki M, Ohara T, Shimane K, Esumi Y, Green C, Tyson C, Ninomiya S-I. Cytochrome P450 species involved in the metabolism of quinoline. Carcinogenesis 1996; 17: 1989-1996.
53. Spracklin DK, Kharasch ED. Human halothane reduction in vitro by cytochrome P450 2A6 and 3A4: identification of low and high $K_m$ isoforms. Drug Metab Dispos 1998; 26: 605-607.
54. Liu C, Zhuo X, Gonzalez FJ, Ding X. Baculovirus-mediated expression and characterization of rat CYP2A3 and human CYP2A6: role in metabolic activation of nasal toxicants. Mol Pharmacol 1996; 50: 781-788.
55. Ekins S, Wrighton SA. The role of CYP2B6 in human xenobiotic metabolism, Drug Metab Rev 1999; 31: 719-754.
56. Heyn H, White RC, Stevens JC. Catalytic role of cytochrome P4502B6 in the *N*-demethylation of *S*-mephenytoin. Drug Metab Dispos 1996; 24: 948-954.
57. Stevens J, Hauer M, Beck J, Voorman R. Further characterization of human liver CYP2B6 expression and catalytic function. Drug Metab Rev 2000; 32 (Suppl 2): 218.

58. Dehal SS, Kupfer D. Metabolism of the proestrogenic pesticide methoxychlor by hepatic P450 monooxygenases in rats and humans. Drug Metab Dispos 1994; 22: 937-946.
59. Baldwin SJ, Clarke SE, Chenery RJ. Characterization of the cytochrome P450 enzymes involved in the in vitro metabolism of rosiglitazone. Br J Clin Pharmacol 1999; 48: 424-432.
60. Nadin L, Murray M. Participation of CYP2C8 in retinoic acid 4-hydroxylation in human hepatic microsomes. Biochem Pharmacol 1999; 58: 1201-1208.
61. Crespi CL, Chang TKH, Waxman DJ. High-performance liquid chromatographic analysis of CYP2C8-catalyzed paclitaxel 6α-hydroxylation. In: Phillips IR, Shephard EA, eds. Cytochrome P450 Protocols. Totowa, NJ: Humana Press, 1998; 123-127.
62. Kerr BM, Thummel KE, Wurden CJ, Klein SM, Kroetz DL, Gonzalez FJ, Levy RH. Human liver carbmazepine metabolism: role of CYP3A4 and CYP2C8 in 10,11-epoxide formation. Biochem Pharmacol 1994; 47: 1969-1979.
63. Becquemont L, Mouajjah S, Escaffre O, Beaune P, Funck-Bretano C, Jaillon P. Cytochrome P4503A4 and 2C8 are involved in zopiclone metabolism. Drug Metab Dispos 1999; 27: 1068-1073.
64. Mück W, Ochmann K, Rohde G, Unger S, Kuhlmann J. Influence of erythromycin pre- and co-treatment on single-dose pharmacokinetics of the HMG-CoA reductase inhibitor cerivastatin. Eur J Clin Pharmacokinet 1998; 53: 469-473.
65. Zeldin DC, Moomaw CR, Jesse N, Tomer KB, Beetham J, Hammock BD, Wu S. Biochemical characterization of the human liver cytochrome P450 arachidonic acid epoxygenase pathway. Arch Biochem Biophys 1996; 330: 87-96.
66. Leo MA, Lasker JM, Raucy JL, Kim C-I, Black M, Lieber CS. Metabolism of retinol and retinoic acid by human liver cytochrome P450IIC8. Arch Biochem Biophys 1989; 269: 305-312.
67. Mancy A, Antignac M, Minoletti C, Dijols S, Mouries V, Ha Duong N-T, Battioni P, Dansette PM, Mansuy D. Diclofenac and its derivatives as tools for studying human cytochromes P450 active sites: particular efficiency and regioselectivity of P4502Cs. Biochemistry 1999; 38: 14264-14270.
68. Bonnabry P, Leeman T, Dayer P. Biotransformation by hepatic $P450_{TB}$ (CYP2C) controls mefenamic acid elimination. Clin Pharmacol Ther 1994; 55: 139.
68. Ohyama K, Nakajima M, Nakamura S, Shimada N, Yamazaki H, Yokoi T. A significant role of human cytochrome P450 CYP2C8 in amiodarone *N*-deethylation: an approach to predict the contribution with relative activity factor. Drug Metab Dispos 2000; 28: 1303-1310.
69. Miners JO, Coulter S, Tukey RH, Veronese ME, Birkett DJ. Cytochromes P4501A2 and 2C9 are responsible for the human hepatic *O*-demethylation of *R*- and *S*-naproxen. Biochem Pharmacol 1996; 51: 1003-1008.
70. Leeman TD, Transon C, Bonnabry P, Dayer P. A major role for cytochrome $P450_{TB}$ (CYP2C subfamily) in the actions of non-steroidal anti-inflammatory drugs. Drugs Exp Clin Res 1993; 19: 189-195.

71. Bajpai M, Roskos LK, Shen DD, Levy RH. Roles of cytochrome P4502C9 and cytochrome P4502C19 in the stereoselective metabolism of phenytoin to its major metabolite. Drug Metab Dispos 1996; 24: 1401-1403.
72. Stearns RA, Chakravarty PK, Chen R, Chiu S-H. Biotransformation of losartan to its active carboxylic acid metabolite in human liver microsomes. Drug Metab Dispos 1995; 23: 207-215.
73. Bonnabry P, Leeman T, Dayer P. Role of human liver microsomal CYP2C9 in the biotransformation of lornoxicam, Eur J Clin Pharmacol 1996; 49: 305-308.
74. Andersson T, Miners JO, Veronese ME, Tassaneeyakul W, Tassaneeyakul W, Meyer UA, Birkett DJ. Identification of human liver cytochrome P450 isoforms mediating omeprazole metabolism. Br J Clin Pharmacol 1993; 36: 521-530.
75. Tsao C-C, Wester MR, Ghanayem B, Coulter SJ, Chanas B, Johnson EF, Goldstein JA. Identification of human CYP2C19 residues that confer *S*-mephenytoin 4′-hydroxylase activity to CYP2C9. Biochemistry 2001; 40: 1937-1944.
76. Birkett DJ, Rees D, Andersson T, Gonzalez FJ, Miners JO, Veronese ME. In vitro proguanil activation to cycloguanil by human liver microsomes is mediated by CYP3A isoforms as well as by *S*-mephenytoin hydroxylase. Br J Clin Pharmacol 1994; 37: 413-420.
77. McGinnity DF, Griffin SJ, Moody GC, Voice M, Hanlon S, Freidberg T, Riley RJ. Rapid characterization of the major drug-metabolizing human hepatic cytochrome P450 enzymes expressed in *Escherichia coli*. Drug Metab Dispos 1999; 27: 1017-1023.
78. VandenBranden M, Ring BJ, Binkley SN, Wrighton SA. Interaction of human liver cytochromes P450 in vitro with LY307640, a gastric proton pump inhibitor. Pharmacogenetics 1996; 6: 81-91.
79. Knodell RG, Dubey RK, Wilkinson GR, Guengerich FP. Oxidative metabolism of hexobarbital in human liver: relationship to polymorphic *S*-mephenytoin 4-hydroxylation. J Pharmacol Exp Ther 1988; 245: 845-849.
80. Kobayashi K, Kogo M, Tani M, Shimada N, Ishizaki T, Numazawa S, Yoshida T, Yamamoto T, Kuroiwa Y, Chiba K. Role of CYP2C19 in stereoselective hydroxylation of mephobarbital by human liver microsomes. Drug Metab Dispos 2001; 29: 36-40.
81. Hoskins J, Shenfield G, Murray M, Gross A. Characterization of moclobemide *N*-oxidation in human liver microsomes. Xenobiotica 2001; 31: 387-397.
82. Koyama E, Chiba K, Tani M, Ishizaki T. Reappraisal of human CYP isoforms involved in imipramine *N*-demethylation and 2-hydroxylation: a study using microsomes obtained from putative extensive and poor metabolizers of *S*-mephenytoin and eleven recombinant human CYPs. J Pharmacol Exp Ther 1997; 281: 1199-1210.
83. Wienkers LC, Wurden CJ, Storch E, Kunze KL, Rettie AE, Trager WF. Formation of (R)-8-hydroxywarfarin in human liver microsomes. Drug Metab Dispos 1996; 24: 610-614.
84. Ellis SW, Rowland K, Ackland MJ, Rekka E, Simula AP, Lennard MS, Wolf CR, Tucker GT. Influence of amino acid residue 374 of cytochrome P4502D6

(CYP2D6) on the regio- and enantio-selective metabolism of metoprolol. Biochem J 1996; 316: 647-654.
85. Tucker GT, Lennard MS, Ellis SW, Woods HF, Cho AK, Lin LY, Hiratsuka A, Schmitz DA, Chu TYY. The demethylation of methylenedioxymethanphetamine ('Ecstasty') by debrisoquine hydroxylase (CYP2D6). Biochem Pharmacol 1994; 47: 1151-1156.
86. Modi S, Gilham DE, Sutcliffe MJ, Lian L-Y, Primrose WU, Wolf CR, Roberts GCK. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine as a substrate of cytochrome P4502D6: allosteric effects of NADPH-cytochrome P450 reductase. Biochemistry 1997; 36: 4461-4470.
87. Tassaneeyakul W, Veronese ME, Birkett DJ, Gonzalez FJ, Miners JO. Validation of 4-nitrophenol as an in vitro substrate probe for human liver CYP2E1 using cDNA expression and microsomal kinetic techniques. Biochem Pharmacol 1993; 46: 1975-1981.
88. Peter R, Bocker R, Beaune PH, Iwasaki M, Guengerich FP, Yang CS. Hydroxylation of chlorzoxazone as a specific probe for human liver cytochrome P450IIE1. Chem Res Toxicol 1990; 3: 566-573.
89. Iwata H, Fujita K-I, Kushida H, Suzuki A, Konno Y, Nakamura K, Fujino A, Kamataki T. High catalytic activity of human cytochrome P450 co-expressed with human NADPH-cytochrome P450 reductase in *Escherichia coli*. Biochem Pharmacol 1998; 55: 1315-1325.
90. Clarke SE, Baldwin SJ, Bloomer JC, Ayrton AD, Sozio RS, Chenery RJ. Lauric acid as a model substrate for the simultaneous determination of cytochrome P4502E1 and 4A in hepatic microsomes. Chem Res Toxicol 1994; 7: 836-842.
91. Duescher RJ, Elfarra AA. Human liver microsomes are efficient catalysts of 1,3-butadiene oxidation: evidence for major roles by cytochromes P4502A6 and 2E1. Arch Biochem Biophys 1994; 311: 342-349.
92. Spracklin DK, Hankins DC, Fisher JM, Thummel KE, Kharasch ED. Cytochrome P450 2E1 is the principal catalyst of human oxidative halothane metabolism in vitro. J Pharmacol Exp Ther 1997; 281: 400-411.
93. Bell LC, Guengerich FP. Oxidation kinetics of ethanol by human cytochrome P450 2E1. J Biol Chem 1997; 272: 29643-29651.
94. Nedelcheva V, Gut I, Soucek P, Tichavska B, Tynkova L, Mraz J, Guengerich FP, Ingelman-Sundberg M. Metabolism of benzene in human liver microsomes: individual variations in relation to CYP2E1 expression. Arch Toxicol 1999; 73: 33-40.
95. Zangar RC, Benson JM, Burnett VL, Springer DL. Cytochrome P4502E1 is the primary enzyme responsible for low-dose carbon tetrachloride metabolism in human liver microsomes. Chem-Biol Interact 2000; 125: 233-243.
96. Guengerich FP, Kim D-H, Iwasaki M. Role of human cytochrome P450IIE1 in the oxidation of many low molecular weight cancer suspects. Chem Res Toxicol 1991; 4: 168-179.
97. Dupont I, Berthou F, Bodenez P, Bardou L, Guirriec C, Stephan N, Dreano Y, Lucas D. Involvement of cytochromes P450 2E1 and 3A4 in the 5-hydroxylation of salicylate in humans. Drug Metab Dispos 1999; 27: 322-326.

98. Lewis DFV, Dickins M. Substrate SARs in human P450s. Drug Discov Today 2002; 7: 918-925.
99. Mancy A, Broto P, Dijols S, Dansette PM, Mansuy D. The substrate binding site of human liver cytochrome P4502C9: an approach using designed tienilic acid derivatives and molecular modelling. Biochemistry 1995; 34: 10365-10375.