QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS (QSARs) WITHIN CYTOCHROMES P450 2B (CYP2B) SUBFAMILY ENZYMES: THE IMPORTANCE OF LIPOPHILICITY FOR BINDING AND METABOLISM

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SUMMARY

The results of qualitative structure-activity relationship (QSAR) analysis are reported for several series of compounds which act as substrates for mammalian CYP2B subfamily enzymes, together with a homologous series of aliphatic primary amines which are known to inhibit CYP2B enzymes. It is found that the compound lipophilicity in the form of the log P value (where P is the octanol/water partition coefficient) is related, either linearly or quadratically, to equilibrium constants of inhibition (K_i), binding (K_s) or metabolism (K_m) depending on the series of compounds in question. In some instances, the difference between frontier orbital energy levels (ΔE) also features in several of the log P expressions with biological activity. Also

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present in a small number of correlations are parameters which are likely to be related to logP: namely, R_m, which is the partitioning factor derived from thin layer chromatography (TLC) retention times, and also the compound molecular weight (M_r). All of these three parameters ((log P, R_m and M_r) are thought to be related to the compound's ability to desolvate the P450 active site when they bind to the enzyme. Although the linear relationships between lipophilicity and CYP2B-related activity point to a major role for desolvation of the enzyme binding site in the overall interaction, it is noted that there may be an optimal log P value displayed by preferred substrates as shown by parabolic relationships with this lipophilic parameter. In addition, there is a remarkable similarity in the coefficients for the log P term of any QSAR expression, which suggests that the hydrophobicity of CYP2B active sites may be broadly equivalent between the various mammalian species.

KEY WORDS

QSARs, cytochromes P450, drug metabolism, CYP2B subfamily

INTRODUCTION

The cytochromes P450 (CYP) constitute a superfamily of haemthiolate enzymes, of which over 3,000 individual members are currently known /1-4/. The function of these enzymes in mammalian systems appears to be associated with the oxidative metabolism of xenobiotics, although some substrate reductions may also be catalysed by P450s, and other P450 enzymes are involved in endogenous processes such as steroid biosynthesis and lipid metabolism /1,5/. Foreign compound metabolism in Mammalia is largely catalysed by P450s from the CYP1, CYP2 and CYP3 families /6,7/. Of these, the CYP2 family comprises several subfamilies designated as CYP2A,

Abbreviations: TLC = thin layer chromatography; CYP = cytochrome P450; QSAR = quantitative structure-activity relationship; TCPOBOP = 1,4-*bis*[2-(3,5-dichloropyridyloxy)]benzene; log P = logarithm of the octanol-water partition coefficient; M_r = relative molecular mass; ΔE = difference between E(LUMO) and E(HOMO), which correspond to the frontier orbital energies for lowest unoccupied and highest occupied molecular orbitals, respectively.

CYP2B, CYP2C, CYP2D and CYP2E, for example. Overall, the CYP2 family makes the largest contribution to P450-mediated metabolism of exogenous compounds in mammalian systems, including human /6-8/. The CYP2B subfamily /9/ is inducible by xenobiotics such as phenobarbital and it is thought that its regulation is mediated primarily by a member of the steroid hormone receptor superfamily known as CAR, the constitutive androstane receptor /10/. The CYP2B subfamily has been well characterised from rodent studies and many substrates and inducers are known, such as phenobarbital, TCPOBOP and various chlorinated hydrocarbons, such as aldrin and DDT. The human orthologue, CYP2B6, however, appears to play only a relatively minor role in xenobiotic metabolism compared to other P450s, such as CYP3A4, CYP2D6 and CYP2C9, and its levels in human liver are relatively low compared with the major components such as CYP3A4 /6/. Nevertheless, it has been established that certain drug substrates are metabolised almost entirely by CYP2B6 mediation, such as bupropion /11/. Consequently, there is current interest in the CYP2B subfamily in the area of xenobiotic metabolism.

It has been known for some time that P450 activities may be related to substrate lipophilicity /12/ and we have shown previously that lipophilicity relationships exist for various P450 substrates /13/. This study focuses on the CYP2B subfamily enzymes to derive correlations between lipophilicity and factors related to the binding and metabolic clearance of known CYP2B substrates. We have reported previously /14-18/ that QSARs can be formulated for series of P450 substrates, including those of CYP2B subfamily enzymes, and molecular modelling of the enzymes appears to be consistent with these findings /15,17,18/. The current work reports on further QSAR analyses conducted for a series of hydrocarbons binding to CYP2B1, the rat orthologue, and compares these with previous findings for CYP2B subfamily enzymes, highlighting the importance of compound lipophilicity in substrate binding and metabolism.

METHODS

Lipophilicity parameters, such as log P (where P is the octanol-water partition coefficient) and R_m (from chromatographic measurements), have been collated from previously published studies or from tabulations in the literature. In some cases, where experimental values

were not available, log P has been calculated using the Pallas Software System (CompuDrug Ltd., Budapest, Hungary). In the case of the alkoxyresorufins series, lipophilicity data in the form of TLC-derived R_m values have been employed /19/ instead of log P as there are significant differences between the values calculated by ClogP (BioByte Corporation, Pomona, California) and Pallas (CompuDrug Ltd., Budapest) although both correlate well with the R_m data. Quantitative structure-activity relationships (QSARs) were generated via single and multiple stepwise regression analysis (MRA) and the correlations obtained were regarded as statistically significant according to standard tests. The InPlot and InStat software packages (GraphPad Software, Inc., San Diego) were employed in the statistical calculations which were executed on a PC operating under MS-DOS within the Windows environment. Biological data in the form of K_i, K_S or K_m values were taken from the original source publications, together with k_{cat}/K_m as a measure of clearance for one series. For barbitals, the clearance ratio has been used in addition to K_S and, for alkoxyresorufins, a metabolic ratio of dealkylation in phenobarbital-induced rats was employed as a measure of activity. In each case, it is assumed that the binding or metabolism data relate primarily to CYP2B enzymes. In general, microsomal preparations have been employed for the generation of the CYP2B activities, as noted in the source publications. For aliphatic primary amines and *p*-substitued toluenes, studies were conducted in the rabbit and the relevant enzyme involved would, therefore, be CYP2B4. The remaining series of compounds were all tested via rat liver microsomes and it is assumed that the P450 involved was primarily CYP2B1. For a number of structurally diverse drugs and other xenobiotics binding to the human orthologue, CYP2B6, data were derived from studies conducted on the expressed enzyme in general, as reported in the published literature. Table 1 provides details of all of the compounds studied, and Table 2 gives information on the data used to generate QSARs for the series of hydrocarbons.

RESULTS AND DISCUSSION

Table 3 shows the correlation data for a total of eight series of compounds which exhibit activity associated with CYP2B enzymes. The sources of the original biological data are provided in this table,

together with previous reports of their QSAR analyses. In each case, good correlations are apparent between measures of their CYP2B activity and lipophilicity parameters, such as log P, R_m (a TLC-partitioning factor) or M_r (relative molecular mass) with correlation coefficients (R values) usually ranging from 0.90 to 0.99, as presented in Table 3, although log P is only fairly well correlated (R = 0.79) with CYP2B binding for a series of 28 hydrocarbons. Interestingly, these factors span the various animal species under consideration, namely, rat, rabbit and human CYP2B subfamily enzymes, which correspond to CYP2B1, CYP2B4 and CYP2B6, respectively. It is likely, therefore, that there are certain similarities between these enzymes which extend to their active site regions. Although only one CYP2B enzyme (CYP2B4) has had its crystal structure determined /20/, sequence analysis indicates that the active sites of mammalian CYP2B enzymes are likely to be fairly similar, and molecular models tend to support this /17/.

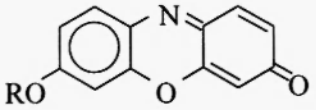
It is apparent that the correlations presented in Table 3 show, in certain cases, that the lipophilicity relationships are quadratic (i.e. parabolic) in nature, although this is not shown in all series of compounds investigated so far. These quadratic relationships with log P are shown by Equations 1, 3, 4, 5a and 5b (Table 3), whereas the n-alkoxyresorufin series correlated in Equation 6 exhibits a parabolic lipophilicity relationship with R_m, which is a TLC-derived partitioning factor related to log P itself /21/, as shown in Figure 1.

However, in other expressions it is clear that log P (or M_r in the case of the hydrocarbons series) is linearly related to binding parameters such as log K_m and log K_s, as shown for Equations 2, 7 and 8 in Table 3. The relationships for Equations 7c and 8 are shown graphically in Figures 2 and 3. The relationship in Figure 2 corresponding to Equation 7c (Table 3) shows the scattering of points where log M_r has been employed as the descriptor variable. In the case of Equation 2, the lipophilicity parameter appears in combination with ΔE, which is the difference between the frontier orbital energy levels (E_{LUMO} - E_{HOMO}), and Equations 3 and 4 also have a ΔE term. We have reported previously /22,23/ that this ΔE parameter may have a role in P450 binding and, consequently, its appearance in these expressions is perhaps not surprising. It is possible that ΔE relates to π-π stacking interactions between enzyme and substrate, although hydrogen bonding may also be a feature in such interactions.

TABLE 1

Series of chemicals involved in QSAR studies with CYP2B enzymes

1.	**Primary aliphatic amines** (8)	RNH_2	R = propyl, butyl, pentyl, hexyl, heptyl, octyl, decyl and dodecyl
2.	***p*-Substituted toluenes** (8)		X = I, Me, Br, H, Cl, F, CN, NO_2, Pr^i and Bu^t
3.	**Haloalkanes** (11)	RHal	CCl_4, CCl_3F, CCl_2F_2, $CClF_3$, CF_4, $CHCl_3$, $CHCl_2F$, $CHClF_2$, CH_2Cl_2, CH_2ClF and $MeCCl_3$
4.	**Dimethylanilines** (9)		X = Me, H, F, Cl, Br, CHO, CN, NO_2 and 3-Me
5.	**Barbitals** (10)		R = propyl, butyl, pentyl, iso-pentyl, sec-pentyl, dimethylbutyl, hexyl, heptyl, octyl and nonyl

6.	**Alkoxyresorufins** (7)		R = methyl, ethyl, propyl, butyl, hexyl, heptyl and octyl
7.	**Hydrocarbons** (28)		hexane, heptane, octane, cyclohexane, cycloheptane, methylcyclohexane, cyclohexene, 1,4-cyclohexadiene, cyclooctatetraene, decalin, perhydrophenanthrene, perhydroanthracene benzene, naphthalene, phenanthrene, anthracene, pyrene, toluene, *o*-xylene, *m*-xylene, *p*-xylene, cumene, biphenyl, diphenylmethane, fluorene, 1,2-diphenylethane, acenaphthene and tetralin
8.	**CYP2B6 substrates** (16)		7-benzyloxyresorufin, testosterone, benzphetamine, 7-ethoxycoumarin, diazepam, bupropion, s-mephenytoin, sm-12502, antipyrine, 4-chloromethyl-7-ethoxycoumarin, deprenyl, propofol, lidocaine, carbamazepine, imipramine and arteether

The number of compounds in each set is shown in parentheses.

TABLE 2

Physicochemical and binding data for hydrocarbons

Compound	log P	M_r	log M_r	K_S (mM)	-log K_S
1. Hexane	4.00	86.18	1.9354	0.26	3.5880
2. Heptane	4.50	100.20	2.0001	0.13	3.8861
3. Octane	5.15	114.23	2.0578	0.091	4.0410
4. Cyclohexane	3.44	84.16	1.9251	0.29	3.5376
5. Cycloheptane	4.00	98.19	1.9921	0.21	3.6778
6. Methylcyclohexane	3.88	98.19	1.9921	0.18	3.7447
7. Cyclohexene	2.86	82.15	1.9146	0.53	3.2757
8. 1,4-Cyclohexadiene	2.47	80.13	1.9038	0.28	3.5528
9. Cyclooctatetraene	3.08	104.16	2.0177	0.11	3.9586
10. Decalin	4.57	138.25	2.1407	0.011	4.9586
11. Perhydrophenanthrene	5.64[c]	192.34	2.2841	0.0053	5.2757
12. Perhydroanthracene	5.64[c]	192.34	2.2841	0.0028	5.5528
13. Benzene	2.13	78.11	1.8927	2.42	2.6162
14. Naphthalene	3.35	128.17	2.1078	0.049	4.3098

15. Phenanthrene	4.52	178.23	2.2510	0.015	4.8239
16. Anthracene	4.52	178.23	2.2510	0.096	4.0177
17. Pyrene	5.00	202.26	2.3059	0.014	4.8539
18. Toluene	2.73	92.14	1.9644	0.43	3.3665
19. o-Xylene	3.12	106.17	2.0260	0.21	3.6778
20. m-Xylene	3.20	106.17	2.0260	0.16	3.7959
21. p-Xylene	3.15	106.17	2.0260	0.26	3.5850
22. Cumene	3.66	120.19	2.0799	0.075	4.1249
23. Biphenyl	3.98	154.21	2.1881	0.018	4.7447
24. Diphenylmethane	4.14	168.24	2.2259	0.012	4.9208
25. Fluorene	4.18	166.22	2.2207	0.018	4.7447
26. 1,2-Diphenylethane	4.70	182.27	2.2607	0.006	5.2218
27. Acenaphthene	3.92	154.21	2.1881	0.025	4.6021
28. Tetralin	3.49	132.21	2.1213	0.040	4.3979

c = calculated values (Pallas System, CompuDrug Ltd., Budapest).
See Table 3 for key to symbols used.
Reference to K_S values: Sipal *et al.* 1261.

TABLE 3

QSAR relationships in CYP2B subfamily substrates and inhibitors involving lipophilicity parameters

QSAR expression and chemical series involved	n	s	R	F
1. Aliphatic primary amines (CYP2B4) (Lewis *et al.* /17/)				
$-\log K_i = 0.93 \log P - 0.19 \log P^2 + 0.88$	8	0.2156	0.99	114.53
2. *p*-Substituted toluenes (CYP2B4) (Lewis *et al.* /28/)				
$-\log K_m = 43.27 - 0.60 \log P - 4.03 \Delta E$	8	0.3940	0.95	23.71
3. Halogenated alkanes (CYP2B1) (Lewis *et al.*/17/)				
$\log K_S = 0.70 \log P - 0.34 \log P^2 + 0.06 \Delta E - 0.44$	11	0.2350	0.93	15.50
4. *N,N*-Dimethylanilines (CYP2B1) (Lewis /27/)				
$\log k_{cat}/K_m = 3.94 \log P - 0.69 \log P^2 - 0.24 \Delta E - 5.47$	9	0.1247	0.96	20.88
5. Barbital derivatives (CYP2B1) (Lewis /27/)				
a) $\log Cl_{ratio} = 2.25 \log P - 0.30 \log P^2 - 4.10$	10	0.1085	0.99	461.42
b) $\log K_S = 1.386 \log P - 0.22 \log P^2 - 0.50$	10	0.1197	0.95	65.22
6. Alkoxyresorufins (CYP2B1) (Lewis *et al.* /21/)				
$\log PB = 1.87 R_m - 1.25 R_m^2 + 0.11$	7	0.9380	0.97	71.09
7. Hydrocarbons (CYP2B1) (Sipa' *et al.* /26/)				
a) $-\log K_S = 0.623 \log P + 1.750$	28	0.4410	0.79	43.61
b) $-\log K_S = 0.016 M_r + 2.112$	28	0.3192	0.90	106.94
c) $-\log K_S = 4.845 \log M - 5.964$	28	0.2973	0.91	126.71

8. Structurally diverse substrates (CYP2B6) (Lewis *et al.* /25/)				
$-\log K_m = 0.88 \log P + 1.68$	16	0.2378	0.97	233.45

Key:

n = number of observations; s = standard error; R = correlation coefficient; F = variance ratio.

The CYP2B isoform involved is given in parentheses for each series of compounds listed above.

CYP2B1 is a rat orthologue, CYP2B4 is a rabbit orthologue and CYP2B6 is a human orthologue.

CL_{ratio} = clearance ratio relative to hepta barbital in phenobarbital-treated rats (Yin and van Rossum /28/).

k_{ca} = catalytic reaction rate constant for CYP2B-mediated metabolism (MacDonald *et al.* /29/).

P = octanol-water partition coefficient.

M_r = relative molecular mass (Da).

K_i = inhibition constant (μM) for aliphatic amines (Jefcoate *et al.* /30/).

K_m = Michaelis constant (μM) (White and McCarthy /31/; Eknes and Wrighton /11/)

K_s = spectroscopic binding constant (μM), also termed the spectral dissociation constant (Eldefrawi /32/; Sipal *et al.* /26/).

PB = ratio of metabolism of alkoxyresorufin in phenobarbital-induced mice relative to control value (Burke and Mayer /19/).

$$R_m = \log\left(\frac{1-R_f}{R_f}\right)$$

where R_f is a TLC-derived partitioning factor related to compound retention time (Burke and Mayer /19/).

In fact R_m correlates with log P with an R value of 0.98 for the alkoxyresorufin series shown above (Lewis *et al.* /21/).

ΔE = difference between frontier orbital energy levels, E_{LUMO} and E_{HOMO} (eV).

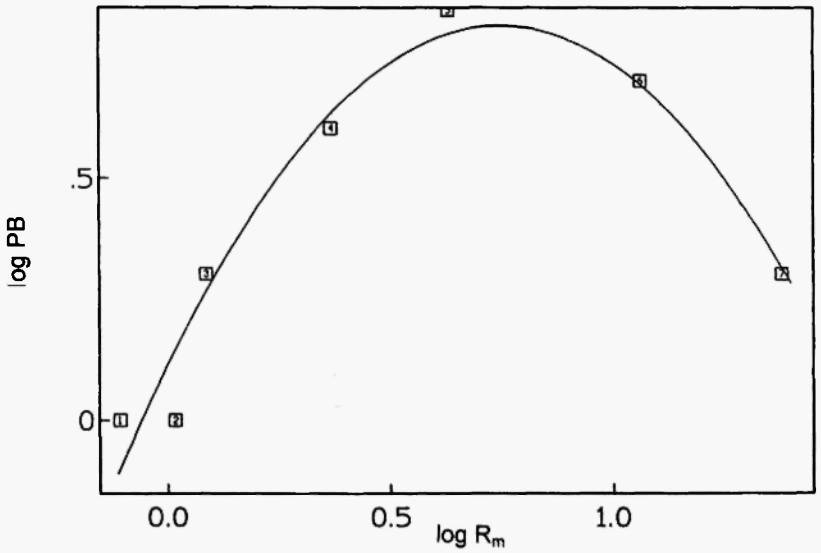


Fig. 1: A graphical plot of Equation 6, Table 3, for seven alkoxyresorufins interacting with CYP2B1, showing a parabolic relationship between log PB and R_m, the TLC-derived partitioning factor.

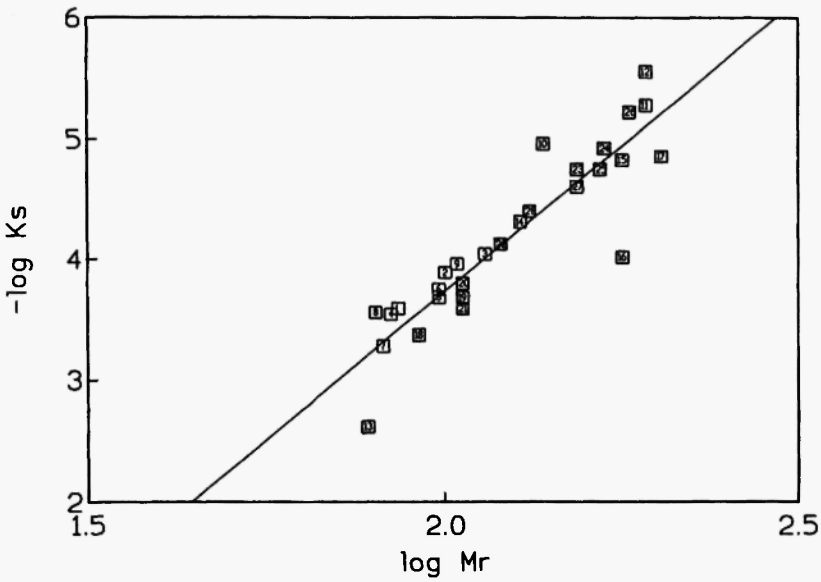


Fig. 2: A graphical plot of Equation 7, Table 3, for hydrocarbons interacting with CYP2B1, showing a linear relationship between -log K_S and log M_r for 28 compounds, where K_S is the spectroscopic binding constant.

However, for the halogenoalkanes there is only a possibility that the latter plays a role in an otherwise lipophilicity-related expression, because none of the compounds in this series contains an aromatic ring system, although the likelihood of charge-transfer interactions would offer an alternative rationale for the appearance of ΔE in such expressions.

For a series of hydrocarbons binding to CYP2B1, which is a rat enzyme, there is a fairly good linear correlation ($R = 0.79$) between the spectroscopic binding constant, K_s, expressed logarithmically and log P (Equation 7a, Table 3). However, it is also found that the relative molecular mass, M_r, gives a statistically more significant correlation ($R = 0.90$) with log K_s than log P. For log M_r, Equation 7c indicates that the correlation is slightly better ($R = 0.91$). This finding may imply that the bulk property of molecular mass is a better means of describing the desolvation component of the binding energy for these compounds than that associated with the lipophilicity parameter, log P, because M_r is unaffected by molecular shape. Figure 2 shows a plot of this latter relationship, where it can be seen that a number of minor outliers are apparent. However, it is possible to explain the existence of such outliers on the grounds of aromatic ring structures in the molecules concerned, which include benzene, anthracene, phenanthrene and pyrene.

The presence of these outliers to the relationship given in Figure 2 probably results from the fact that these compounds possess fused aromatic rings, whereas those which are well described by the correlation tend to constitute molecules which contain aliphatic groupings, although some may exhibit restricted molecular flexibility brought about by ring systems which are not necessarily aromatic. For example, benzene, anthracene and pyrene represent outliers for the lipophilicity relationship based on M_r values (expressed as Equation 7b, Table 3). However, in the case of the log P correlation ($R = 0.79$) it would appear that the more conformationally flexible molecules are represented as outliers to the main lipophilicity relationship (Equation 7a, Table 3). The significantly lower correlation coefficient based on log P values indicates that there is more scattering of points than that shown in the M_r or log M_r expressions, thus suggesting that, in this instance, the use of M_r may be a better reflection of active site desolvation than log P. However, it is possible that there is another explanation for this finding because, in general, log P and M_r data

usually provide close agreement for the estimation of desolvation energy for simple hydrocarbons /24/.

Nevertheless, for CYP2B6 substrates, which are compounds of diverse molecular structure, the log P relationship with log K_m is very satisfactory (R = 0.97) for a dataset of 16 substrates /13,25/, as shown in Equation 8, Table 3, and Figure 3 provides a graphical plot of this relationship. Conversion of these data to free energy values indicates that the common interaction between such substrates and the CYP2B6 active site is either a hydrogen bond or two π-π stacking interactions with complementary amino acid residues, and molecular modelling studies show a good agreement with this finding /15,17/. This has subsequently been confirmed by the recent publication of a CYP2B4 crystal structure /20/ and has led to the construction of a refined model of CYP2B6 which is an improvement on previous models. Figure 4 shows the putative active site of CYP2B6 where there are two phenylalanine residues (Phe-115 and Phe-206) which can enter into π-π stacking interactions with substrates. In addition, several hydrophobic amino acid residues are present in the active site which can be expected to make contact with complementary groupings on substrate molecules. This would appear to provide an explanation of the key role for compound lipophilicity in the QSAR expressions, where it is likely that desolvation of water molecules from the active site occurs on binding.

Consequently, it is apparent that QSAR studies, including the employment of lipophilicity relationships /13,25/, can derive useful expressions to aid in the understanding of determinants for P450 substrate binding and metabolism within the CYP2B subfamily, including the human enzyme CYP2B6 where there is derived information on structurally diverse substrates from experimentally determined K_m values. For many of the series, there is a similarity in the log P coefficients for the QSARs with K_m, K_i or K_S activities, irrespective of the mammalian species involved. If one excludes the barbitals, the log P term has an average coefficient of 0.75 ($\pm$ 0.18), and it is possible this indicates that the hydrophobic character of the respective CYP2B active sites is similar across species. Sequence comparison between the three mammalian CYP2B enzymes does indeed indicate that this may well be the case, especially with regard to the putative active sites obtained from molecular modelling. Examination of the CYP2B4 crystal structure with bound inhibitor /20/ shows the presence of three

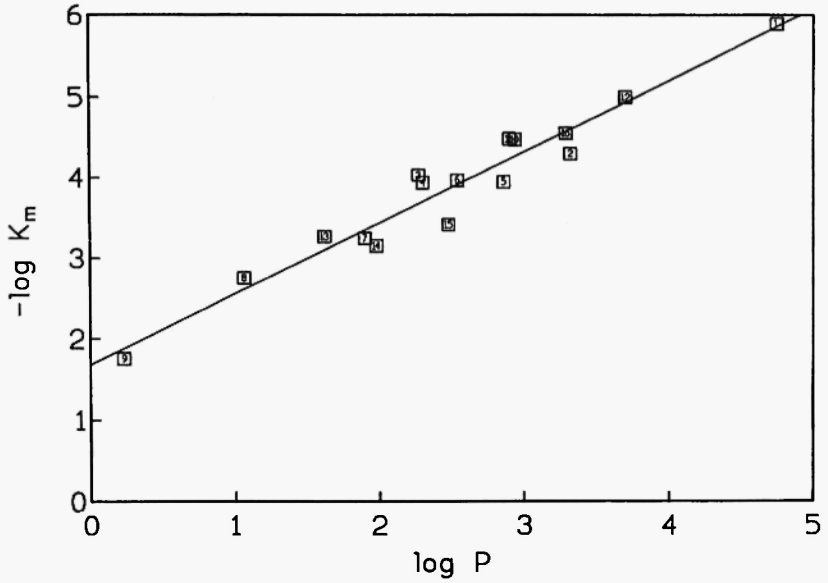


Fig. 3: A graphical plot of Equation 8, Table 3, for structurally diverse CYP2B6 substrates binding to CYP2B6, showing a linear relationship between -log K_m and log P for 16 compounds, where K_m is the apparent Michaelis constant.

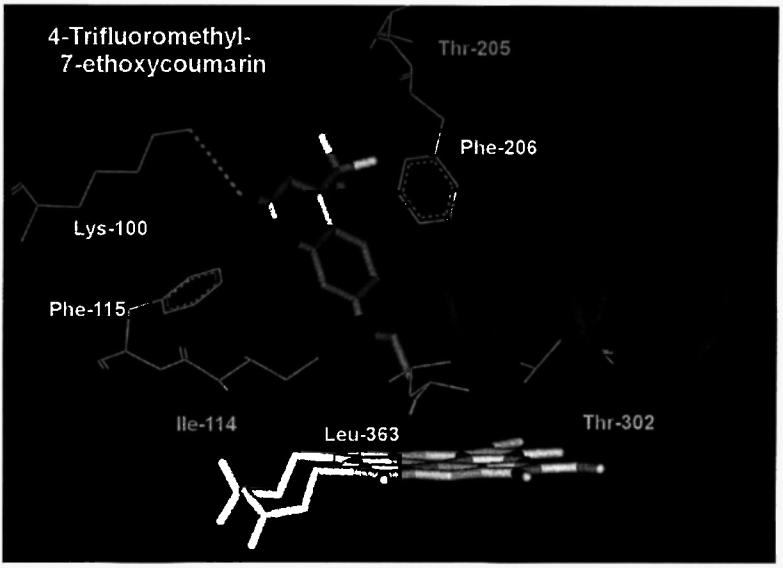


Fig. 4: The putative active site of CYP2B6 modelled by homology with CYPB4, showing a typical substrate, 4-trifluoromethyl-7-ethoxycoumarin, orientated for *O*-deethylation.

phenylalanine residues (Phe-115, Phe-206 and Phe-297) which may enter into π-π stacking with complementary substrates, and a number of other hydrophobic residues (Ile-114, Ile-363, Val-367 and Val-477) are also present for contacting substrates. For the model of CYP2B6, constructed by homology with CYP2B4, it would appear that the conserved residues Phe-115 and Phe-206 could form π-π stacking with a selective substrate, such as 4-trifluoromethyl-7-ethoxycoumarin, as shown in Figure 4, whereas Ile-114 and Leu-363 could represent hydrophobic contacts for typical CYP2B6 substrates. These positions tend to be conserved as hydrophobic residues across the mammalian CYP2B sequences and, therefore, it can be expected that the mode of substrate binding is likely to be similar for this enzyme subfamily.

CONCLUSIONS

Quantitative relationships between structural properties and CYP2B-related activity for several series of compounds suggest that compound lipophilicity is important for substrate binding and, to some extent, metabolic clearance. In a few cases, it is possible to delineate parabolic relationships with the lipophilicity parameter, log P, and there are also instances where additional descriptors contribute to the explanation of CYP2B activity differences irrespective of whether the log P dependency is linear or quadratic in appearance. The QSAR results for the series of hydrocarbons indicate that lipophilicity is an important factor relating to their binding to P450, and the coefficients for the log P term in many of the QSAR expressions are similar in value, thus indicating that the hydrophobic character of the CYP2B active sites is similar across several mammalian species.

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