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Quantitative structure-activity relationships (QSARs) in inhibitors of various cytochromes P450: The importance of compound lipophilicity

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Abstract
The results of extensive quantitative structure-activity relationship (QSAR) analyses on 15 series of cytochrome P450 inhibitors, covering a total of 7 enzymes and 199 compounds, are reported. In general, it is found that lipophilicity represents the most important single factor in describing differences in inhibitory potency towards P450 enzymes. In two instances, this relationship is parabolic in nature but, by and large, the logarithm of inhibitory activity relates linearly with log P, where P is the octanol-water partition coefficient. On occasions, other parameters are involved in the QSAR expressions but there are many examples where either log P or its ionization-corrected equivalent, log $D_{7.4}$, are the sole structural descriptors of inhibition. The correlations presented exhibit a range in R value from 0.85 to 0.99, where R is the correlation coefficient, and it is found that R is greater than 0.9 in 80% of the QSARs presented. It is apparent from these findings, therefore, that compound lipophilicity plays a major role in the ability of xenobiotics to inhibit enzymes of the cytochrome P450 superfamily, presumably due to the essentially hydrophobic nature of the active site region.

Keywords: *Cytochrome P450, inhibitors, QSAR, lipophilicity*

Introduction

The cytochromes P450 (CYP or P450) constitute a superfamily of over 4500 individual haem-thiolate enzymes which play an important role in the metabolism (primarily oxidative) of a large number of structurally diverse organic compounds, both endogenous and exogenous [1–4]. In mammalian species, including *Homo sapiens*, P450s of the CYP1, CYP2 and CYP3 families are primarily associated with the oxidative (and sometimes reductive) metabolism of drugs and other xenobiotics, whereas several highly selective P450s from other families are involved in endogenous functions such as steroid biosynthesis and lipid metabolism [5]. Inhibitors of some P450 enzymes have important roles in the treatment of several conditions, such as fungal infections, and in disease states like prostate cancer and breast cancer. However, in these cases, it is one of the steroidogenic P450s (eg. aromatase) that are the targets for selective inhibition. Furthermore, selective inhibitors of enzymes from the CYP1, CYP2 and CYP3 families are important in the drug development process for establishing the likely P450s involved in novel compound metabolism. For example, furafylline is a theophylline analogue which is employed as a selective inhibitor of CYP1A2, whereas ketoconazole is known to inhibit CYP3A4 at relatively low concentrations (reviewed in [2]). One method for investigating the key features involved in inhibitory potency involves the use of quantitative structure-activity relationships (QSARs) as we have reported previously [6,7]. This study represents the results of several QSAR investigations on P450 inhibitors which covers almost 200 compounds and 7 different P450 enzymes.

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Methods

Biological data on the compounds from various series of P450 inhibitors were obtained from the published literature, apart from one dataset of CYP1A2 inhibitors which were provided by an industrial sponsor (GlaxoSmithKline) and can, therefore, be regarded as unpublished findings. Physicochemical and structural descriptors were either obtained from the existing literature or calculated using appropriate software, Sybyl, Pallas and ClogP. The Pallas Software (CompuDrug Limited, Budapest) was employed in the calculation of log P and pK_a values, whereas the ClogP program (BioByte Corporation, Pomona, California) was used for both log P calculation and extraction of some experimental log P values. Molecular structural and electronic parameters were calculated using the Sybyl molecular modelling software (Tripos Associates, St. Louis, Missouri). Statistical analyses were carried out using multiple linear regression or single regression via the GraphPad suite of programs (GraphPad Software Inc., San Diego, California).

Results and discussion

The expressions derived from quantitative structure-activity relationship (QSAR) analysis on various series of P450 inhibitors are presented in Table I. The correlation coefficients (R values) when expressed to two decimal places, as shown here, range from 0.85 to 0.99 (with corresponding R^2 values ranging from 0.72 to 0.98), and these can be considered as corresponding to statistically significant correlations for the relevant number of compounds involved in each case. In the majority of examples presented, there are good correlations between inhibition of P450 and compound lipophilicity derived from partition

Table I. QSARs for P450 Inhibitors of CYP1, CYP2, CYP3 and CYP154 families.

	n	S	R	F
CYP1A1				
1. $-\log IC_{50} = 0.506\ \log P + 0.156\ a/d^2 + 1.815\ E_H + 11.810$ (± 0.156) (± 0.056) (± 1.2353)	23	0.3948	0.892	24.74
CYP1A2				
2. $-\log K_i = 0.875\ \log D_{7.4} + 3.794$ (± 0.046)	16	0.3035	0.982	369.97
3. $-\log K_i = 0.030\ a/d^2 - 0.04\ M_r + 8.035$ (± 0.019) (± 0.001)	16	0.2619	0.912	32.11
4. $-\log K_i = 0.105\ a/d^2 - 0.001\ M_r + 0.584\ l/w + 7.293$ (± 0.022) (± 0.00081) (± 0.421)	18	0.2674	0.916	24.32
CYP1B1				
5. $\Delta G_{bind} = 0.471\ \Delta G_{part} - 6.708$ (± 0.066)	12	0.1612	0.915	51.66
CYP2A6				
6. $\Delta G_{bind} = 0.586\ \Delta G_{part} - 2.225$ (± 0.032)	16	0.2476	0.980	334.36
7. $\Delta G_{bind} = 0.475\ \Delta G_{part} - 3.502$ (± 0.020)	5	0.1383	0.997	553.31
CYP2E1				
8. $-\log K_i = 0.932\ \log P - 0.654\ \log P^2 - 0.689\ HB_A - 3.513$ (± 0.231) (± 0.174) (± 0.163)	12	0.3315	0.854	7.17
9. $-\log K_I = 0.983\ \log P - 0.203\ \log P^2 - 1.789$ (± 0.107) (± 0.026)	10	0.2177	0.963	89.4
10. $\log IC_{50} = 0.113SA - 9.812\ Q_C^L - 2.014\ N_c - 0.504$ (± 0.018) (± 1.523) (± 0.355)	22	0.4428	0.894	23.98
11. $-\log K_i = 1.047\ \log D_{7.4} + 0.244\ \log D_2^{7.4} - 2.659$ (± 0.096) (± 0.024)	11	0.1689	0.968	119.0
CYP3A4				
12. $\Delta G_{inh} = 0.915\ \Delta G_{part} - 2.522$ (± 0.069)	6	0.1600	0.989	174.00
13. $\Delta G_{bind} = 0.926\ \Delta G_{part} - 4.990$ (± 0.055)	13	0.3047	0.981	279.73
14. $\Delta G_{bind} = 0.579\ \Delta G_{part} - 3.889$ (± 0.011)	13	0.0873	0.998	304.73
CYP154				
15. $\Delta G_{bind} = 0.707\ \Delta G_{part} - 1.854$ (± 0.044)	7	0.1489	0.991	259.41
16. $-\log MIC = 0.707\ \log D_{7.4} + 1.306$ (± 0.044)	7	0.2952	0.991	259.41

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

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coefficients (log P values). These will now be discussed for each P450 enzyme family.

CYP1 Family QSARs

For a series of 23 compounds, of diverse structure although all possessing relatively planar molecules, there appears to be a fairly good correlation ($R = 0.89$) between CYP1A1 inhibitory activity ($-\log IC_{50}$) and a combination of log P, planarity (area/depth^2) and energy of the highest occupied molecular orbital (E_H) as presented in Equation (1), Table I. This finding tends to suggest that a number of structural and electronic factors combine for explaining the variation in CYP1A1 inhibitory potency for the compounds investigated. It is possible that the planarity term (area/depth^2) relates to their ability to fit the active site of CYP1A1, whereas the E_H term may be describing electron donor ability of the molecule either for hydrogen bond acceptance from active site donors, such as threonine serine and allelic variants, or for π-π stacking with aromatic amino acid residues in the enzyme's hydrophobic pocket where the heme is located. There is evidence from site-directed mutagenesis experiments in CYP1 family enzymes that at least one aromatic residue, and probably two, makes contact with substrates and inhibitors as they bind relatively close to the heme moiety for metabolism or inhibition to occur.

For the CYP1A2 inhibitors, it is found that molecular planarity in the form of the area/depth^2 ratio, is again important, and this agrees with reported findings from homology modelling [8]. Table I also indicates that the combination of area/depth^2 with relative molecular mass is required for explaining the variation in inhibitory potency for the compounds studied. Furthermore, inclusion of the rectangularity factor, length/width, has a moderate effect on the correlation with the above variables, especially where 18 compounds are considered. However, this may result in too many independent variables (3 in total) for the number of compounds involved as there would be 6 compounds per variable used, and it is interesting to note that the variance ratio (F-value) is somewhat lower than that of the preceding correlation equation where two independent variables are employed for a regression involving 16 compounds. Consequently, although interesting, it is likely that Equation (2) for CYP1A2 inhibitors (in Table I) represents the more significant correlation, as this is utilizing two descriptor variables instead of three, albeit for a slightly smaller dataset of 16 compounds. Figure 1 shows a plot of the lipophilicity relationship for these compounds.

Turning to the inhibitors of CYP1B1, there is a good correlation ($R = 0.91$) between binding affinity and log P-derived partitioning energy for 12 compounds, as shown in Table I. The value of the intercept for this relationship indicates the presence of two

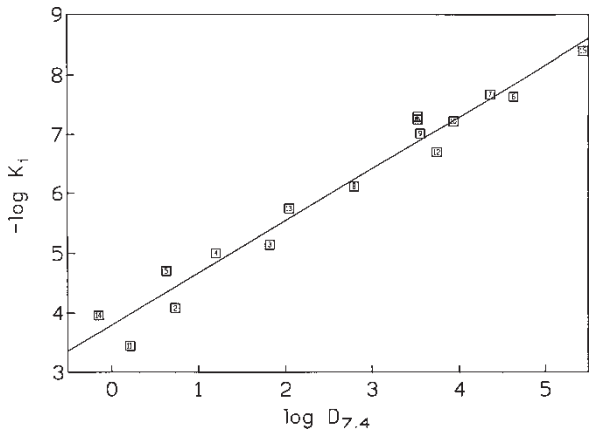

Figure 1. Lipophilicity relationship between $-\log K_i$ and $\log D_{7.4}$ for a series of 16 structurally diverse inhibitors of CYP1A2, shown in Equation (2), Table I.

hydrogen bonds and two π-π stacking interactions within the CYP1B1 substrate binding site, and this is consistent with molecular modelling studies on substrates and inhibitors of this enzyme [9]. Figure 2 displays this relationship graphically to enable visual analysis of the information.

CYP2 Family QSARs

For two series of CYP2A6 inhibitors, it is found that their corresponding ΔG_{bind} values correlate linearly with the free energy of partitioning derived from log P data, as shown in Table I. Although the number of compounds in the second series is relatively small, there is a similarity between the slopes for the two equations relating to CYP2A6 inhibitors, which is indicative of the lipophilicity in the CYP2A6 active site. There is, however, a difference between the two intercepts of these equations and this indicates that the average binding interaction shown by the two series of compounds changes from -2.2 to -3.5 kcalmol^{-1}. The most likely source of this difference is an additional hydrogen bond of average energy -1.3

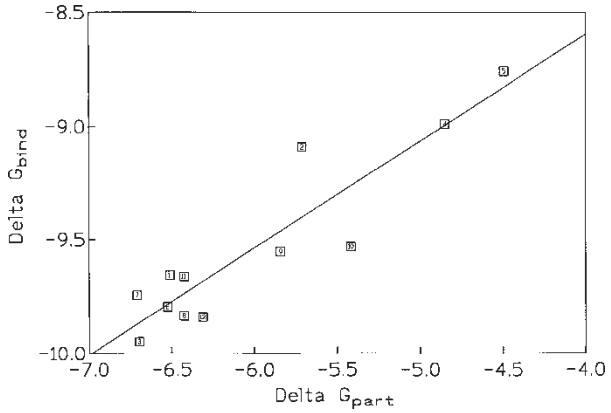

Figure 2. Lipophilicity relationship between ΔG_{bind} and ΔG_{part} for a series of 12 inhibitors of CYP1B1, shown in Equation (5), Table I.

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kcalmol^{-1} formed in the latter case, although the possibility of a π-π stacking interaction cannot be ruled out. Molecular modelling studies of the CYP2A6 active site region indicate the presence of both hydrogen bond-forming and π-π stacking amino acid residues [10], thus suggesting that these two alternatives may explain the energy differences between the two lipophilicity relationships. Figure 3 shows a plot of the lipophilicity relationship exemplified by Equation (6) in Table I.

For CYP2E1 inhibitors, there is again a strong indication of hydrophobic interactions playing an important role, as shown in the relevant expressions presented in Table I. This is due to the presence of log P and log $D_{7.4}$ hydrophobicity/lipophilicity parameters in most expressions, whereas the surface area (SA) of the molecule in one equation also suggests a desolvation component to the binding. Interestingly, the QSARs involving lipophilicity descriptors (log P or log $D_{7.4}$) show a quadratic function which indicates that there would be an optimal value for compounds binding to the CYP2E1 active site. Furthermore, the possibility of hydrogen bonding between substrate and enzyme is indicated by the number of hydrogen bond acceptors (HB_A) term in the first equation of this section, whereas the LUMO density (Q_CL) on the α-carbon of the hydroxyl group in a series of alcohols inhibiting CYP2E1 activity, shows that electronic effects are also in operation, and it is possible that this relates to a specific interaction between the alcohol's C-OH moiety and the enzyme active site. A plot of the lipophilicity relationship from Equation (11) is provided as a graphical representation in Figure 4.

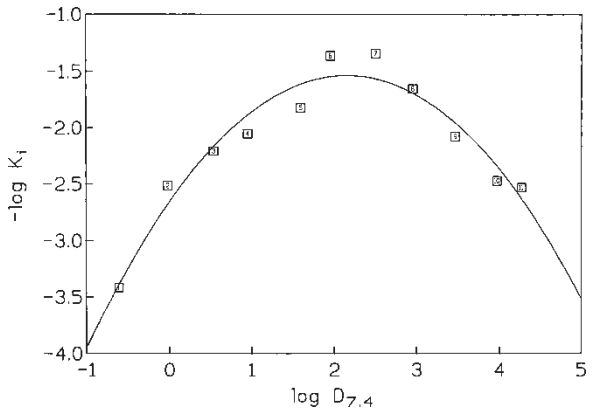

Figure 4. Lipophilicity relationship between $-\log K_i$ and $\log D_{7.4}$ for a series of 11 inhibitors of CYP2E1, shown in Equation (11), Table I.

CYP3 Family QSARs

For several series of CYP3A4 inhibitors, lipophilicity relationships are apparent for the compounds investigated thus far [11]. Some of these results are shown in Table I, where the correlation equations for three series are presented. The first series represents a small group of statins, whereas the remaining two series are for structurally diverse compounds. However, the slopes for the first two equations are very close in value, which indicates that the compounds probably occupy the same region of the CYP3A4 active site. The ΔG_{part} quantity, shown in these expressions, is related to compound lipophilicity in the form of log P, where P is the octanol-water partition coefficient. The partitioning energies are derived as follows:

$$\Delta G_{part} = -RT\ln P$$

where R is the gas constant and T is the absolute temperature (usually taken as 310°K).

By and large, experimental log P values have been used in the calculation of ΔG_{part}. However, where these values were unavailable, calculated log P data was employed. In most cases, there is a good agreement between calculated and experimental log P values [12] thus providing confidence in the use of calculated log P in the absence of experimental values. Overall, therefore, the lipophilicity relationships in CYP3A4 inhibitors can be rationalized in terms of typical active site interactions such as hydrogen bonding and π-π stacking; whereas the variation in gradients could be due to the presence of multiple binding sites in the heme environment and this would accord with experimental observations which indicate allosteric binding between substrates and, consequently, the likelihood of more than one substrate binding site [13,14]. Figure 5 gives a graphical representation of the lipophilicity relationship expressed as Equation (12) in Table I.

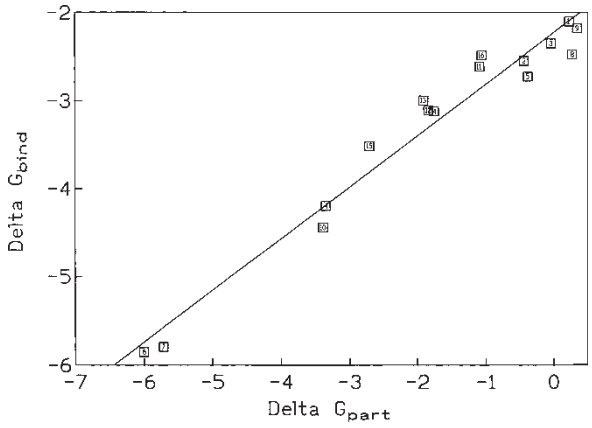

Figure 3. Lipophilicity relationship between ΔG_{bind} and ΔG_{part} for a series of 16 inhibitors of CYP2A6, shown in Equation (6), Table I.

CYP154 QSARs

In addition to mammalian P450 inhibition, it is also interesting to note that the inhibition of the bacterial enzyme CYP154, from *Streptomyces coelicolor*, also

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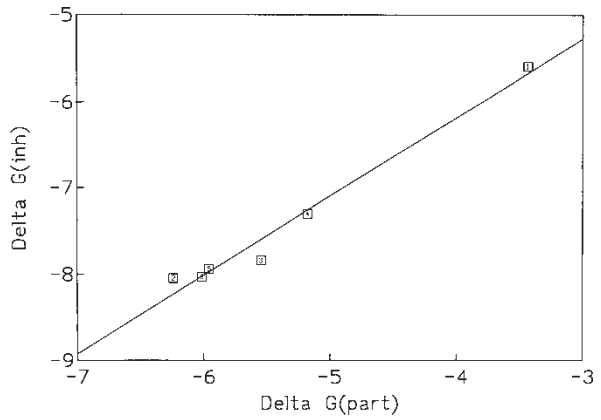

Figure 5. Lipophilicity relationship between ΔG_{bind} and ΔG_{part} for a series of 6 statins acting as CYP3A4 inhibitors, shown in Equation (12), Table I.

displays a lipophilicity relationship [15]. Equations (15) and (16) in Table I provide the relevant QSAR expressions which exhibit a very high correlation ($R = 0.991$) for 7 antifungal agents, such as econazole and fluconazole, which can also act as inhibitors of CYP154 and certain other bacterial P450s. A plot of the relationship shown in Equation (16) is also presented as Figure 6, and thus makes a useful comparison with the other graphical plots, whereas Equation (15) represents the same initial data expressed in terms of the relevant free energies. Consequently, it would appear that lipophilicity is an important factor for explaining, in general terms, the particular inhibitory potency differences encountered in cytochrome P450 inhibition across various enzyme families.

Conclusions

Inhibition of P450 enzymes from the CYP1, CYP2 and CYP3 families is related to compound lipophilicity in the form of log P or log $D_{7.4}$, where P is the octanol/water partition coefficient and $D_{7.4}$ is the distribution coefficient at pH 7.4, which is an ionization-corrected partition coefficient. In two cases, the relationship is quadratic (ie. parabolic) rather than linear (although this is the usual situation) and other factors can also come into play, including: planarity (area/depth^2), rectangularity (length/width ratio), energy of the highest occupied molecular orbital (E_H), number of hydrogen bond acceptors (HB_A) and number of carbon atoms (N_C), together with relative molecular mass (M_r) and solvent-accessible surface area (SA) which possibly relate to desolvation energy in the same way as lipophilic character.

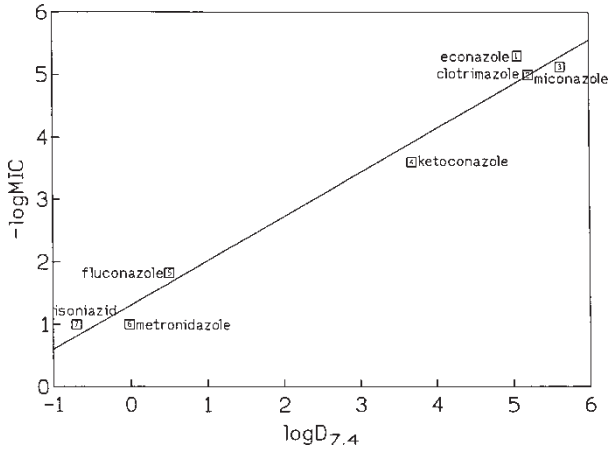

Figure 6. Lipophilicity relationship between −log MIC and log $D_{7.4}$ for a series of 7 inhibitors of the bacterial enzyme, CYP154, shown in Equation (16), Table I. MIC is the minimum concentration giving rise to inhibition of the enzyme.

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