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Extension of a predictive substrate model for human cytochrome P4502D6

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1. Metoprolol, indoramine, codeine, tamoxifen and prodipine, compounds which are clinically used, and MDMA (ecstasy) were fitted in a small molecule model for substrates of human cytochrome P4502D6.

2. For both the *R*- and *S*-enantiomer of metoprolol, the *R*- and *S*-enantiomer of MDMA, and for indoramine and codeine (all proven substrates of cytochrome P4502D6) an acceptable fit in the substrate model was obtained.

3. For tamoxifen, for which the involvement of cytochrome P4502D6 in the 4-hydroxylation is uncertain, no acceptable fit could be obtained in the substrate model.

4. For prodipine, a competitive inhibitor of P4502D6, for which the involvement of P4502D6 in the metabolism is uncertain, no acceptable fit in the substrate model could be obtained.

5. The substrate model was extended in a direction in which two large known substrates extend from the original substrate model. This extension did not change the flat hydrophobic region of the original substrate model.

Introduction

Cytochromes P450 (Nelson *et al.* 1996) constitute a large superfamily of haem-containing enzymes, capable of oxidizing and in some cases reducing a variety of substrates, both of endogenous and exogenous origin (Koymans *et al.* 1993a, Goeptar *et al.* 1995). Human P4502D6 is a polymorphic member of the P450 superfamily and is absent in 5–9% of the Caucasian population as a result of a recessive inheritance of gene mutations. Several inactivating alleles have been reported (Daly *et al.* 1996) resulting in a higher percentage of the Caucasian population having one (heterozygous) or two (homozygous) inactivating mutant alleles (Meyer *et al.* 1990, Armstrong *et al.* 1994, Panserat *et al.* 1994). The mutations result in a deficiency in drug oxidations, also known as the debrisoquine/sparteine polymorphism, which also affects the oxidative metabolism of numerous other drugs (Eichelbaum and Gross 1990, Kroemer and Eichelbaum 1995). A diminished metabolism of these drugs is found in poor metabolizers, who have two non-functional 2D6 alleles compared with so-called extensive metabolisers with at least one functional allele. Today, various antiarrhythmics, ß-adrenoceptor antagonists, antidepressants, opiates and neuroleptics have been reported to be substrates for the human cytochrome P4502D6 (Eichelbaum and Gross 1990, Korzekwa and Jones 1993, Koymans *et al.* 1993a, Kroemer and Eichelbaum 1995). These compounds represent a variety of chemical structures, the common

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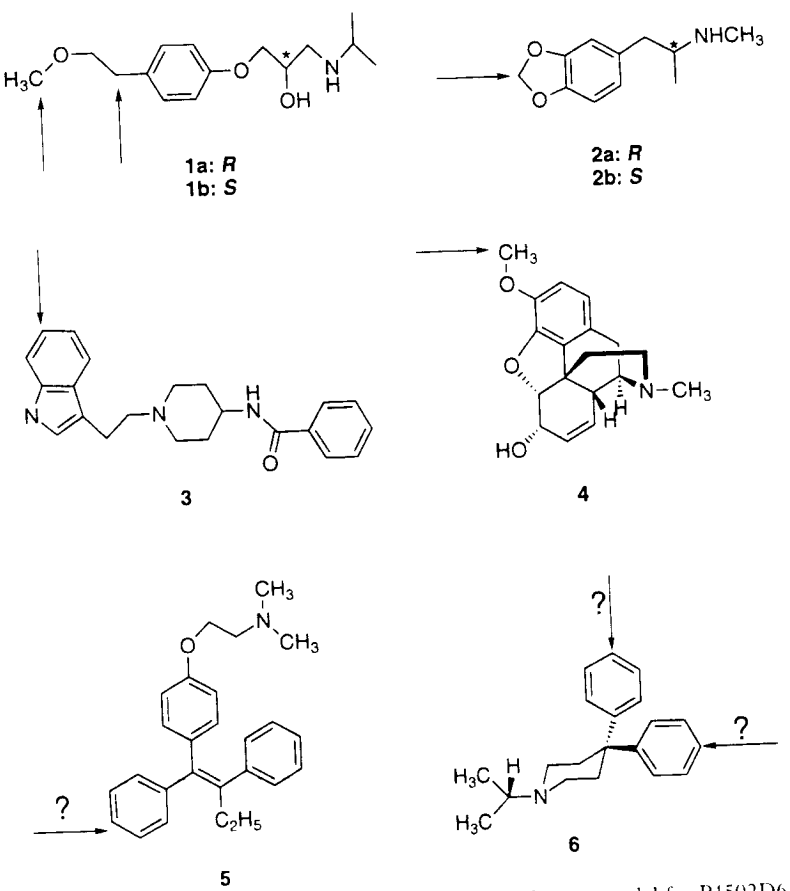

Figure 1. New compounds incorporated in the predictive substrate model for P4502D6 as derived by Koymans *et al.* (1992). Arrows indicate (possible) sites of oxidation.

characteristics being one or more basic nitrogen atoms, a distance of 5 or 7 Å between the site of oxidation and the basic nitrogen atoms, a flat hydrophobic region and a negative molecular electronic potential above the planar part of the molecule (Koymans *et al.* 1992, Strobl *et al.* 1993).

Several small molecule models have been derived for P4502D6 using computational chemistry techniques and a variety of substrates (Wolff *et al.* 1985, Meyer *et al.* 1986, Islam *et al.* 1991, Koymans *et al.* 1992, de Groot *et al.* 1997) or inhibitors (Strobl *et al.* 1993). Based on crystal structures of bacterial P450s (P450101 [Poulos *et al.* 1985], P450102 [Ravichandran *et al.* 1993, Li and Poulos 1995] and P450108 [Hasemann *et al.* 1994]), also protein models for P4502D6 have been derived confirming specific details of the small molecule models (Koymans *et al.* 1993b, Lewis 1995, de Groot *et al.* 1996, Ellis *et al.* 1996, Modi *et al.* 1996).

The aim of the present study was, first, to evaluate the utility of the small molecule model for substrates of P4502D6 (Koymans *et al.* 1992, de Groot *et al.* 1995) toward several structurally different drugs known to be substrates for P4502D6, and, second, to predict the potential involvement of P4502D6 in the metabolism of two other drugs. The compounds under investigation are depicted in figure 1: (**1a, b**) *R*- and *S*- metoprolol, (**2a, b**) *R*- and *S*-MDMA (methylenedioxymethamphetamine, ecstasy), (**3**) indoramine (3-(2-(4-benzamidopiperid-1-

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yl)ethyl)-indole hydrochloride), (**4**) codeine, (**5**) tamoxifen (*Z*-(1-[4-(2-dimethylaminoethoxy)-phenyl]-1,2-diphenyl-1-butene)), and (**6**) prodipine (1-*i*-propyl-4,4-diphenylpiperidine).

Computational methods

The initial conformations of the compounds were generated using the molecular modelling package ChemX (Chemical Design 1993) with the exception of **1** and **4** for which the initial conformation was retrieved from the Cambridge Structural Database (CSD [Allan and Kennard 1993]).

The parallel version of the density functional† program package ADF (Department of Theoretical Chemistry Vrije Universiteit Amsterdam 1995, Fonseca Guerra *et al.* 1995) implemented on IBM/RS6000 workstations, on a cluster of six IBM/RS6000/250 workstations and on an 8-node IBM/9076-SP1 was used for the calculations described. The local density approximation (LDA‡) adapted by Vosko, Wilk and Nusair (Vosko *et al.* 1980) was used. The geometries of all substrates were optimized using the single zeta (SZ) basis set. On the resulting single zeta geometries, an energy calculation was performed using the double zeta (DZ) basis set including non-local gradient corrections according to Becke (1988) and Perdew (1986).

The compounds under investigation were fitted onto the template molecules of the substrate model (Koymans *et al.* 1992) (debrisoquine and dextromethorphan) by using a rigid fitting procedure, followed by a flexible fitting procedure using ChemX (Chemical Design 1993). In the rigid fitting procedure used, compounds were fitted onto the template molecules only allowing global rotations and translations. In the flexible fitting procedure, using the ChemX non-bonded energy force field, user-defined exocyclic dihedral angles were also minimized with respect to restraints set by the user on the one hand, and the non-bonded energy of the fitted molecule on the other hand.

The oxidation sites of the compound and the templates were matched onto each other, assuming one fixed oxidation site within the P4502D6 protein. Furthermore, the basic nitrogen atoms in compounds **1–6** were fitted onto the basic nitrogen atom of the template molecule dextromethorphan (7 Å template), assuming a similar environment for each substrate in the active site. When the distance between fitted nitrogen atoms was $< 0{\cdot}5$ Å, and the distance between the oxidation sites was $< 0{\cdot}001$ Å, the fitted conformation was accepted. Furthermore, a fit was accepted when the energy difference between the minimal energy conformation and the energy of the conformation of the molecule fitted into the model (ΔE), both calculated using ADF, was generally < 10 kcal mol^{-1}. This is an arbitrarily chosen limit, which has been used for the original substrate model based on semiempirical calculations (Koymans *et al.* 1992). In the early stages of our P4502D6 substrate modelling, it turned out that conformations of substrates which gave acceptable energy differences (e.g. < 10 kcal mol^{-1}) when calculated with semiempirical methods, resulted in larger energy differences when calculated with density functional methods as used for the present refined small molecule model (data not shown).

The limit of ΔE is most likely also dependent on the number of bonding interactions between the protein and the substrate. A large substrate (e.g. indoramine (**3**)) will in principle be capable of forming a larger number of favourable interactions with the protein, than a relative small substrate (e.g. MDMA (**2**)). The reverse, however, holds for unfavourable interactions. As the number of interactions remains uncertain, we usually use a limit of 10 kcal mol^{-1}, but allow deviations depending on the size and nature of the substrate. Based on receptor binding data (Andrews *et al.* 1984), however, we effectively changed our limit for several compounds to 30 kcal mol^{-1} as described below.

Molcular electrostatic potentials (MEPs) based on Coulomb point charge interactions between the compound and a positive charge were calculated with ChemX on the van der Waals surface for a series of compounds using Mulliken charges derived from the ADF-calculations (DZ basis set).

Results

The primary aim of the present study was to extend the predictive substrate model of P4502D6 (Koymans *et al.* 1992, de Groot *et al.* 1995) with four drugs (metoprolol (**1a, b**), MDMA (**2a, b**), indoramine (**3**) and codeine (**4**), comprising

† Density functional theory (DFT) is an alternative to *ab initio* theory. In contrast with *ab initio* methods, no attempt is made in DFT to solve the Schrödinger equation. DFT uses the one-electron density (ρ) as the basic variable instead of the wavefunction (ψ) to calculate properties (Parr and Yang 1989). Like in *ab initio* methods no parametrization is necessary.

‡ In the local density approximation (LDA) electronic properties are determined as functionals of the electron density by applying local relations appropriate for a homogeneous electronic system (Parr and Yang 1989).

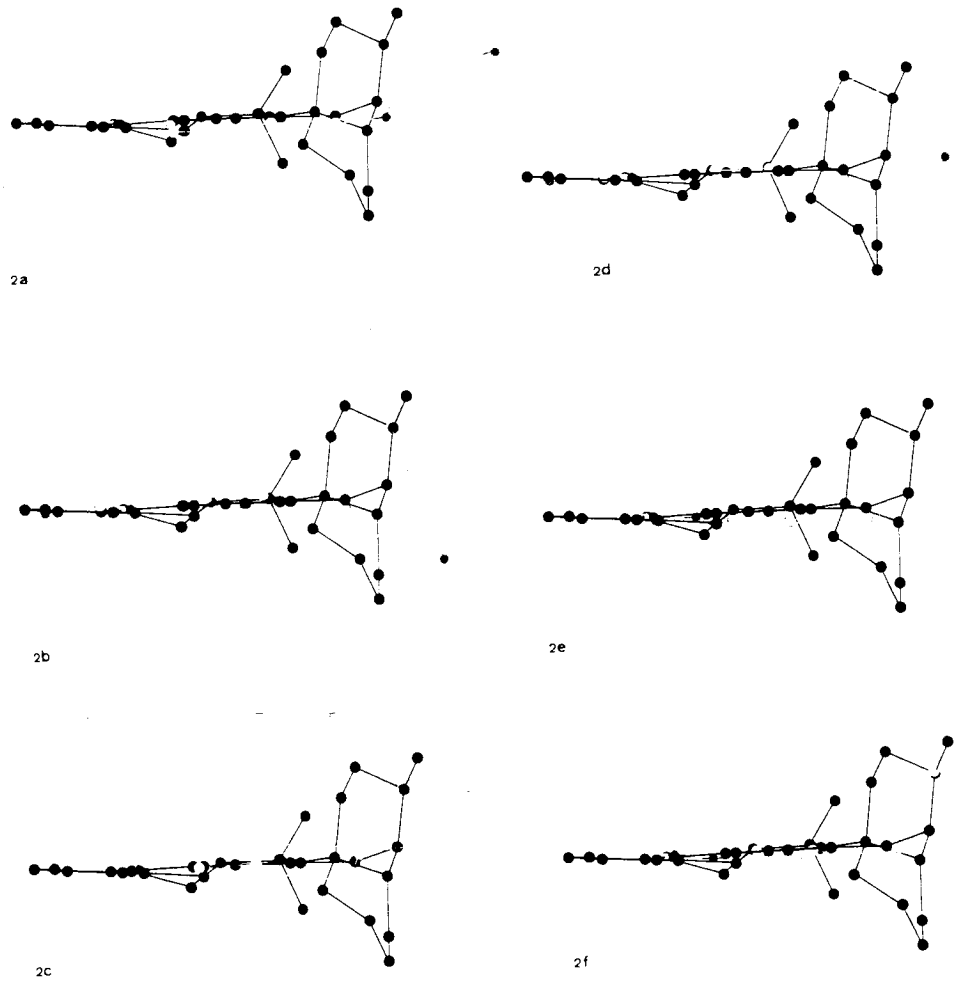

Figure 2. For legend see opposite.

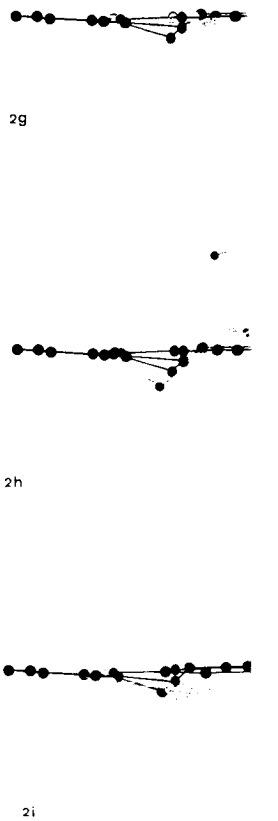

Figure 2. Fits of compou
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eight metabolic pathways) known to be mediated by P4502D6, and, second, to examine two metabolic reactions (4-hydroxylation of tamoxifen (**5**), and aromatic hydroxylation of prodipine (**6**) (figure 1), of which possible involvement of P4502D6 has been suggested. The chemical structure, the chiral centers and the (assumed) sites of metabolism as mediated by P4502D6 of the above mentioned drugs are shown in figure 1. The results obtained will be discussed below for each drug separately.

A fit was accepted when the energy difference between the minimal energy conformation and the energy of the conformation of the molecule fitted into the model (ΔE), both calculated using ADF, was generally < 10 kcal mol^{-1} (an arbitry limit). Observed binding energies to receptors have been compared with predicted binding energies for a wide variety of compounds, based on the number of functional groups (Andrews *et al.* 1984). The observed (receptor) binding energies ranged from 3·1 to 20·5 kcal mol^{-1}, with the majority of observed binding energies

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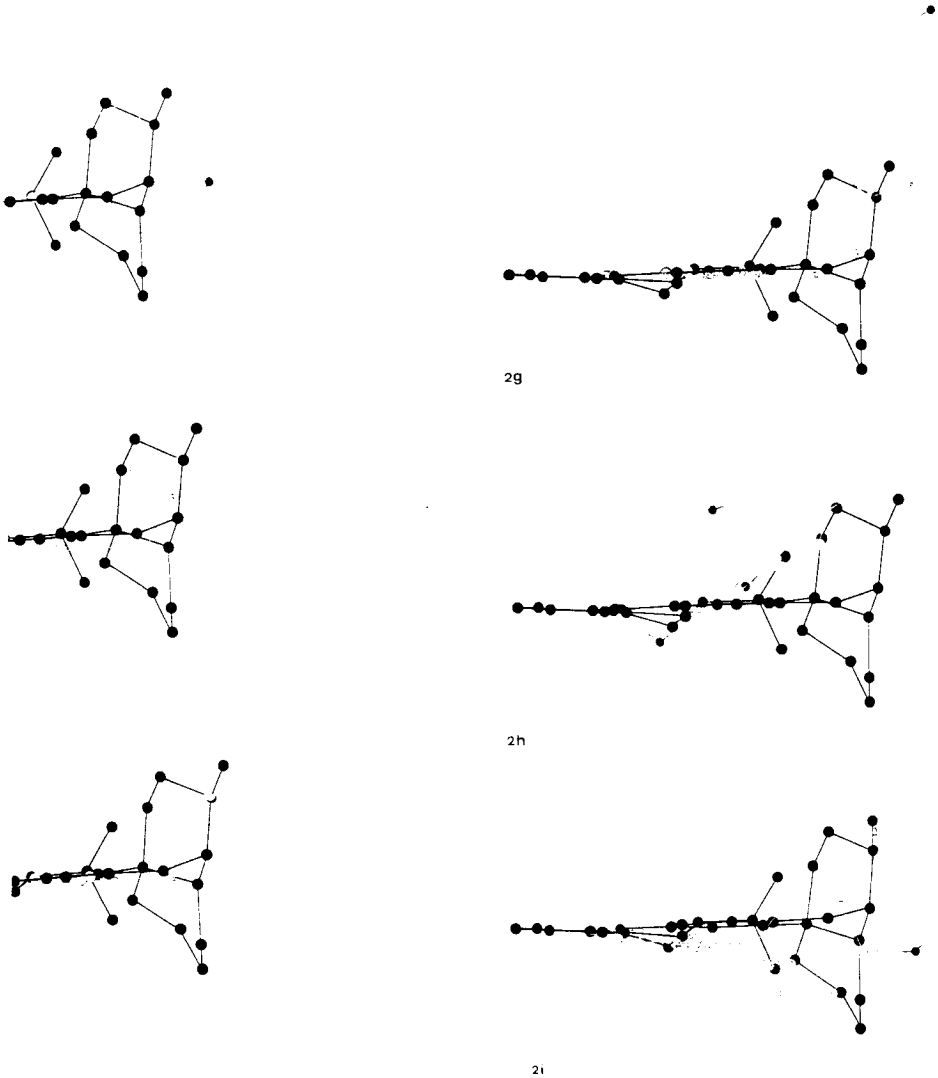

Figure 2. Fits of compounds (gray) on the template molecules (debrisoquine [black] and dextromethorphan [black]); (a) **1a** *O*-demethylation, (b) **1a** benzylic hydroxylation, (c) **1b** *O*-demethylation, (d) **1b** benzylic hydroxylation, (e) **2a**, (f) **2b**, (g) **3**, (h) **4**, (i) **5**, (j) **6**.

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between 8 and 14 kcal mol^{-1} (Andrews *et al.* 1984). Apart from changes in the conformations, other unfavourable terms (like changes in rotational and conformational free energy and a reduction of internal rotations in both the substrate and the protein [Williams *et al.* 1991]) have to be compensated by favourable terms associated with binding (hydrogen bond formation, van der Waals interactions and hydrophobic effects [Williams *et al.* 1991]). Based on these results, in the case of enzymes such as P450s energy differences up to 20–30 kcal mol^{-1} (caused by conformational changes in the substrate) can probably be compensated by substrate–protein interactions as well.

Table 1. Computational results.

Compound‡	Reaction	$d_{N\cdots Ox}$ (Å)	d_1 (10^{-3} Å)	d_2 (Å)	ΔE (kcal mol^{-1})	Figure
1a (*R*)	*O*-demethylation	7·63	0·02	0·32	24·2	2a
1a (*R*)	benzylic hydroxylation	7·40	0·02	0·02	24·4	2b
1b (*S*)	*O*-demethylation	7·41	0·16	0·11	28·0	2c
1b (*S*)	benzylic hydroxylation	7·10	0·29	0·38	25·5	2d
2a (*R*)	*O*-demethylation	7·43	0·05	0·05	5·6	2e
2b (*S*)	*O*-demethylation	7·38	0·06	0·06	8·1	2f
3	6-hydroxylation	7·26	0·59	0·11	1·3	2g
4	*O*-demethylation	8·02	0·64	0·64	2·7	2h
5	4-hydroxylation	7·89	0·13	0·13	27·6	2i
6	4-hydroxylation	7·09	0·35	0·34	3·2	2j

‡ See figure 1 for structure of compounds.
$d_{N\cdots Ox}$, distance between basic nitrogen atom and (proposed) site of oxidation.
d_1, distance between oxidation site and oxidation site in template.
d_2, distance between basic nitrogen and basic nitrogen atom in the template.

Metoprolol (1)

Metoprolol (**1a, b**) is a ß-adrenoceptor antagonist, which is generally administered as a racemate, although the ß-blocking effects are thought to reside in the *S*-enantiomer (Lennard *et al.* 1986). Metoprolol is known to be *O*-demethylated (65 % of total metabolism [$K_m(R) = 10{\cdot}5\ \mu$M, $V_{max}(R) = 7{\cdot}40$ nmol·h^{-1}·(mg protein)$^{-1}$, $K_m(S) = 14{\cdot}8\ \mu$M, $V_{max}(S) = 6{\cdot}22$ nmol·h^{-1}·(mg protein)$^{-1}$]) and hydroxylated at the benzylic carbon atom (10 % of total metabolism [$K_m(R) = 12{\cdot}0\ \mu$M, $V_{max}(R) = 4{\cdot}50$ nmol·h^{-1}·(mg protein)$^{-1}$, $K_m(S) = 12{\cdot}8\ \mu$M, $V_{max}(S) = 4{\cdot}96$ nmol·h^{-1}·(mg protein)$^{-1}$]) by P4502D6 (Mautz *et al.*1995).

We fitted both stereoisomers (**1a, b**) in the substrate model for P4502D6 in orientations leading to both metabolic products, as shown in figures 2a–d. Two cDNAs of P4502D6 heterologously expressed in a yeast system (Mautz *et al.* 1995, Ellis *et al.* 1996), resulting in a single amino acid difference in P4502D6 (Val^{374}/Met^{374}), have been used to distinguish between the stereo- and regioselectivity of metoprolol resulting in different ratios of *O*-demethylation versus benzylic hydroxylation.

The present computational results are summarized in table 1. Both the *R*- (**1a**) and the *S*-enantiomer (**1b**) of metoprolol gave acceptable fits for both the *O*-demethylation (figure 2a and c respectively) and the benzylic hydroxylation reaction (figure 2b and d respectively). The energy differences for both metabolic pathways of *R*- and *S*-metoprolol were relatively high (around 25 kcal mol^{-1}), however, the fits (for the flat hydrophobic region, the site of oxidation and the basic nitrogen atom) were all acceptable. The calculated MEP of **1** revealed a highly negative region (< -45 kcal mol^{-1}) in the flat area near the oxidation site, in agreement with the original substrate model (Koymans *et al.* 1992). Assuming sufficient interactions compensate the extra conformational energy required, we accepted the energy differences between the minimized geometry and fitted geometry for both enantiomers of this relative large compound.

MDMA (2)

MDMA (**2**), also known as ecstasy, is a drug whose use has led to exaggerated responses and deaths (Screaton *et al.* 1992). The major metabolic pathway of **2** is *O*-demethylation, which is catalysed by P4502D6 ($K_m(R) = 1{\cdot}72\ \mu$M, $V_{max}(R) = 6{\cdot}45$ nmol·min^{-1}·(nmol P450)$^{-1}$ [Tucker *et al.* further metabolized 1994).

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Table 1 show pathway of **4**. The

ΔE (kcal mol^{-1})	Figure
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24·4	2b
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nmol·min^{-1}·(nmol P450)$^{-1}$, $K_m(S)$ = 2·90 μM, $V_{max}(S)$ = 7·61 nmol·min^{-1}·(nmol P450)$^{-1}$ [Tucker *et al.* 1994]) forming a reactive catechol intermediate, which can be further metabolized *via* an *ortho*-quinone to neurotoxic products (Tucker *et al.* 1994).

Both the *R*- (**2a**) and *S*-enantiomer (**b**) were fitted into the substrate model and are shown in figure 2e–f. Distances between the fitted nitrogen atoms and oxidation sites of the substrates and the template were well within the criteria for the model. Energy differences between optimized and fitted geometries also indicated that both **2a** and **b** fitted well in the model (5·6 and 8·1 kcal mol^{-1} respectively; table 1). Evaluation of the MEP of **2** again revealed a highly negative region (< -45 kcal mol^{-1}) in the flat area near the oxidation site, qualitatively in agreement with the original substrate model (Koymans *et al.* 1992). All computational results indicate P4502D6 to be involved in the *O*-demethylation of both stereoisomers of **2**.

Indoramine (3)

Indoramine (**3**), an α_1-adrenoceptor antagonist, is a very large molecule that has been shown to be hydroxylated by P4502D6 at the 6-position (figure 1) (Pierce *et al.* 1987). The molecule extended significantly from the substrate model into a direction of which as yet little is known (figure 2g). A recent study on the dopamine uptake inhibitor GBR 12909 (de Groot *et al.* 1995) indicated that there is an area into which large substrates of P4502D6 may extend beyond the boundaries of the present substrate model for P4502D6. The extending part of **3** appeared to occupy the same area as GBR 12909 (data not shown). Both the distance between the fitted atoms (site of oxidation and basic nitrogen atom) and the energy difference between optimized and fitted geometry were within the criteria stated in Computational Methods (table 1), indicating that the experimentally verified 6-hydroxylation of **3** could be accommodated in the substrate model for P4502D6, except for an extension outside the boundaries of the present substrate model (Koymans *et al.* 1992). However, an extension of the present substrate model in the direction into which both **3** and GBR 12909 (de Groot *et al.* 1995) extend from the original substrate model (Koymans *et al.* 1992) is probably allowed, as these two compounds are known substrates of P4502D6 and are both larger than any substrate added to the substrate model so far. This makes an extension of the substrate model necessary. This was further supported by evaluation of the MEP of **3**, which revealed a highly negative region (< -45 kcal mol^{-1}) in the flat area near the oxidation site.

Codeine (4)

Codeine (**4**) is widely used as an analgesic drug for the relief of mild pain, for the relief of unproductive cough and as an anti-diarrhoeal agent (Mikus *et al.* 1991). Codeine is mainly metabolized by conjugation to glucuronic acid. Minor metabolic routes, however, comprise *N*-demethylation and *O*-demethylation (Yue and Säwe 1992). The *O*-demethylation pathway is catalysed by P4502D6 (K_m = 149 μM, V_{max} = 17·6 nmol·h^{-1}·(mg protein)$^{-1}$)), and results in the formation of morphine (Dayer *et al.* 1988, Yue and Säwe 1992, Mikus *et al.* 1994).

Table 1 shows the computational results concerning the *O*-demethylation pathway of **4**. The fit of **4** into the substrate model is shown in figure 2h. The energy

difference between optimized and fitted geometries was well within the criteria, although the distances between fitted atoms were slightly larger than the criteria mentioned in the Methods. This indicates that the substrate model in principle can accommodate the *O*-demethylation of **4**, although the fit results were not very good. This might in part explain the relatively low rate at which **4** is metabolized *via* this pathway ($V_{max}/K_m = 0{\cdot}12$ ml·h^{-1}·(mg protein)$^{-1}$ [Dayer *et al.* 1988]) when compared with dextromethorphan ($V_{max}/K_m = 0{\cdot}98$ ml·h^{-1}·(mg protein)$^{-1}$ [Jaruratanasirikul and Hortiwakul 1994]). Evaluation of the MEP of **4**, revealed a highly negative region (< -45 kcal mol^{-1}) in the flat area near the oxidation site. Despite the fact that dextromethorphan (the 7 Å-template molecule of the model ($K_m = 8{\cdot}16$ μM, $V_{max} = 7{\cdot}96$ nmol·h^{-1}·(mg protein)$^{-1}$) [Jaruratanasirikul and Hortiwakul 1994]) and codeine are rather similar in shape, the orientation of **4** in the substrate model differs considerably from the orientation of dextromethorphan.

Tamoxifen (5)

The *trans*-isomer of the triphenylethylene tamoxifen (**5**) is an oestrogen receptor antagonist, which is widely used in the treatment of breast cancer (Mani and Kupfer 1991, Mani *et al.* 1993). Tamoxifen was selected for this study because recent findings, using P4502D6 heterologously expressed in a yeast system (Ellis 1995 personal communication, Crew *et al.* 1996), indicated P4502D6 to be involved in the 4-hydroxylation of **5** (along with P4503A4 and 2C9), while in human livers 4-hydroxylation of **5** correlated with P4502C9 although 2C8 and 2D6 might also be involved (White *et al.* 1995). Previously, based on experiments with genetically engineered cell lines, P4502B1/2 (Mani and Kupfer 1991), 2E1 and 2D6 (Styles *et al.* 1994) were suggested to be involved in the metabolism of tamoxifen to 4-hydroxytamoxifen. However, inhibition studies using human liver microsomes indicated P4502D6 not to be involved in this reaction (Blankson *et al.* 1991). Wiseman and Lewis rationalized the metabolic routes of **5** using protein models of P4503A4, 2D6 and 2C9 (Wiseman and Lewis 1996). In case of the P4502D6 protein model, however, a different orientation of **5**, not having an interaction with Asp^{301}, was necessary (Wiseman and Lewis 1996).

In the present study, we attempted to fit **5** into the substrate model for P4502D6. The resulting fit is shown in figure 2i. The energy difference between optimized and fitted geometries was of the same order as those obtained for **1a** and **b**, and also the distance criteria for the fitted atoms (site of oxidation and basic nitrogen atoms) was met, as indicated in table 1. The calculated MEP of **5** revealed a highly negative region (< -45 kcal mol^{-1}) in the flat area near the oxidation site, in analogy with substrates **1–4**. However, **5** extended significantly from the substrate model in the flat hydrophobic region in a direction not occupied by any other P4502D6 substrate (figure 2i). At present, according to the substrate model results, this compound is probably not hydroxylated at the 4-position by P4502D6. In another substrate model for P4502D6, **5** could also not be accommodated (Islam *et al.* 1991).

A fit using a different orientation of **5** in the active site, as suggested by Wiseman and Lewis (1996) which does not have an interaction with Asp^{301}, cannot be modelled in the present substrate model. Incorporation of orientations of substrates (like **5**) not interacting with Asp^{301} (an interaction which is explicitly assumed in the present substrate model) would require incorporation of parts of the

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Prodipine (6)

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Discussion

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protein in the substrate model. Work to incorporate protein parts, as derived from a recent protein model for P4502D6 based on P450s 101, 102 and 108 (de Groot *et al.* 1996), into the substrate model (de Groot *et al.* 1997) is in progress and might give new insights concerning **5**.

Prodipine (6)

The anti-depressant prodipine (**6**) is used for the treatment of Parkinson patients, and has been shown to be a competitive inhibitor of P4502D6 (K_i = 4·8 nM versus (+)-bufuralol [Fonne-Pfister and Meyer 1988]). So far, however, no experimental data are available concerning the metabolism of **6** by P4502D6. Owing to the two phenyl rings connected to an sp^3-hybridized carbon atom, **6** could not be accommodated appropriately into the P4502D6 substrate model (figure 2j). When the most probable site of oxidation (in either one of the two phenyl rings) is superimposed onto the site of oxidation of the substrate model, the other phenyl ring extends significantly from the model in a region where all other compounds in the substrate model are planar. This indicates that **6** is not likely to be a substrate for P4502D6, despite the fact that the fit of the basic nitrogen atom and the oxidation site is acceptable, the energy difference between minimum energy conformation and fitted conformation is well within the criteria stated above (table 1) and evaluation of the MEP of **6** revealed a highly negative region (< -45 kcal mol^{-1}) in the flat area near the oxidation site.

Discussion

Metoprolol (**1**), MDMA (**2**) and codeine (**4**) could be fitted well within the boundaries of the original P4502D6 substrate model, whereas indoramine (**3**) extended beyond the known boundaries into the same direction as GBR 12909 was recently found to extend (de Groot *et al.* 1995), thus accommodating metabolic oxidations of these compounds (**1–4**) as indicated in figure 1. In contrast, tamoxifen (**5**) and prodipine (**6**) extended beyond the boundaries of the original substrate model into areas not occupied by any other known substrate. Therefore, **5** and **6** were predicted not to be metabolized by P4502D6, despite the fact that some experimental findings indicate **5** to be metabolized by 2D6 and **6** is a known competitive inhibitor of 2D6.

In order to accept a compound as a P4502D6 substrate four criteria have to be met: (1) the sites of oxidation must be superimposed while (2) the basic nitrogen atom of the compound is within 0·5 Å from the basic nitrogen atom of either dextromethorphan (7 Å-template molecule) or debrisoquine (5 Å-template molecule), (3) no parts of the compound extend into regions, not defined by the substrate model and (4) the energy difference between minimum energy conformation and fitted conformation is < 30 kcal mol^{-1} (or preferably < 10 kcal mol^{-1}). Based on the present computational findings, it can be concluded that the energy difference between optimized and fitted geometries of potential P4502D6 substrates should be not too large. However, extension into certain (restricted) regions is a more compelling criterion to assess the question whether or not a compound is a substrate for P4502D6.

The present fit data, in combination with the original data used to derive the substrate model (Koymans *et al.* 1992) and the data on GBR 12909 (de Groot *et al.* 1995), indicated that the original substrate model can be extended in a specific region, thereby accommodating large known substrates like GBR 12909 and indoramine.

Work to incorporate protein parts, as derived from a recent protein model for P4502D6 based on P450s 101, 102 and 108 (de Groot *et al.* 1996), into the substrate model is in progress (de Groot *et al.* 1997).

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