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In Silico Prediction of Drug Binding to Cytochrome P450 2D6: Identification of a new Metabolite of Metoclopramide

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Abbreviations used: CYP2D6, cytochrome P450 2D6; CYPs, cytochromes P450; P450 reductase, NADPH cytochrome P450 oxidoreductase; HLMs, human liver microsomes; CID, collision induced dissociation; HPLC-TOFMS, high performance liquid chromatography-time of flight mass spectrometry.

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ABSTRACT

Patients with cancer are often taking many different classes of drugs to treat the effects of their malignancy and the side effects of treatment, as well as their co-morbidities. The potential for drug-drug interactions which may affect the efficacy of anti-cancer treatment is high and a major source of such interactions is competition for the drug-metabolising enzymes cytochromes P450. We have examined a series of 20 drugs commonly prescribed for cancer patients looking for potential interactions via cytochrome P450 2D6. We used a homology model of cytochrome P450 2D6 together with molecular docking techniques to perform an *in silico* screen for binding to CYP2D6. Experimental IC_{50} values were determined for these compounds and compared with the model predictions to reveal a correlation with a regression coefficient of $r^2 = 0.61$. Importantly, the docked conformation of the commonly prescribed anti-emetic metoclopramide predicted a new site of metabolism that was further investigated through *in vitro* analysis with recombinant CYP2D6. An aromatic *N*-hydroxy metabolite of metoclopramide – consistent with predictions from our modelling studies – was identified by high performance liquid chromatography mass spectrometry. This metabolite was found to represent a major product of metabolism in human liver microsomes and CYP2D6 was identified as the main P450 isoform responsible for catalyzing its formation. In view of the prevalence of inter-individual variation in the CYP2D6 genotype and phenotype, we suggest that those experiencing adverse reactions with metoclopramide, *e.g.* extra-pyramidal syndrome, are likely to have a particular CYP2D6 genotype/phenotype. This warrants further investigation.

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INTRODUCTION

Cytochrome P450's (CYPs) are a superfamily of heme-containing enzymes that are responsible for the oxidation of a structurally diverse range of xenobiotic compounds. The human genome contains 55 CYPs, but the vast majority (~90%) of therapeutic drugs are metabolised by five CYPs: 1A2, 2C9, 2C19, 2D6 and 3A4 (Wolf et al., 2000). Inhibition and/or induction of these isoforms is probably the most common mechanism underlying drug-drug interactions *in vivo*. Patients with cancer are potentially vulnerable to significant drug-drug interactions arising from drug metabolism by CYPs since they are subject to polypharmacy. These patients are often taking drugs to relieve symptoms of their malignancy (*e.g.* analgesics, anti-convulsants for neuropathic pain), side effects of their treatment (*e.g.* anti-emetics) as well as drugs to treat co-morbidities (*e.g.* anti-hypotensives and lipid lowering agents). Adverse drug interactions involving CYP2D6 are likely to be significant since up to 30% of drugs are substrates of this enzyme, including opioids, antidepressants, neuroleptics and various cardiac medications.

Various methods have been developed to predict interactions between drugs and CYP isoforms, and these can be broadly divided into data modelling or molecular modelling approaches (van de Waterbeemd and Gifford, 2003). The former involving statistical correlations between molecular and structural descriptors are relatively fast and can provide a useful computational filter in early drug discovery (Ekins et al., 2003). We have used the latter molecular approach, combining experimental analysis of substrate binding with protein modelling, to study CYP2D6 active-site-ligand interactions. This has helped us (Kirton et al., 2002; Paine et al., 2003; Flanagan et al., 2004; Kemp et al., 2004; McLaughlin et al., 2005) and others [*e.g.* (Venhorst et al., 2003; Keizers et al., 2004; Keizers et al., 2005)] to identify residues that play a key role in metabolism, and to determine the binding orientation and affinity of ligands in the active site (Kirton et al., 2002; Kemp et al., 2004). The recently determined crystal structure of CYP2D6

(Rowland et al., 2006) has confirmed the correctness of many of the features of the active site of our model of the enzyme.

Here we have used this approach to investigate interactions of CYP2D6 with a small set of 20 drugs that are commonly used by our cancer patients. As well as showing a useful correlation between our *in silico* predictions and the corresponding experimental results, this has uncovered the correct metabolic route for metoclopramide, a drug frequently used to prevent the nausea and vomiting associated with cancer chemotherapy (Harrington et al., 1983).

MATERIALS AND METHODS

Materials

Terrific Broth (TB), chloramphenicol, δ–aminolevulinic acid (ALA), dithiothreitol, glucose 6-phosphate, $NADP^+$, phenylmethylsulfonyl fluoride (PMSF), sodium dithionite, cytochrome *c*, dextromethorphan and all the co-administered drugs were purchased from Sigma (Poole, UK). Ampicillin was obtained from Beecham Research (Welwyn Garden City, UK) and isopropyl β-D-thiogalactopyranoside (IPTG) from Melford Laboratories (Ipswich, UK). Glucose 6-phosphate dehydrogenase (type VII) was purchased from Roche Molecular Biochemicals (Lewes, UK). Library efficient competent *E.coli* JM109 was purchased from Promega. Pooled human liver microsome was purchased from Gentest (Woburn, USA). HPLC grade solvents were purchased from Rathburn Chemicals (Walkerburn, UK), and HPLC columns from Phenomenex (Cheshire, UK). Dextrorphan was purchased from Ultra Fine Chemicals (Manchester, UK). All other chemicals were from BDH (Poole, UK).

Coexpression of the P450s and P450 reductase in *E. coli*

Expression was carried out essentially as previously described (Kemp et al., 2004). Briefly, pB81 plasmid was co-transfected with pJR7 into *E. coli* JM109. Cultures were grown in TB at 30 °C until the $O.D._{600}$ reached >0.8 whereupon the haem precursor ALA was added to a final concentration of 1mM. Induction was initiated with the addition of IPTG to a final concentration of 1mM. Cultures were grown until the appearance of P450 in the CO-reduced spectra of whole cells (usually 24 hrs), at which point cells were harvested. Sphaeroplasts were prepared, sonicated and the membrane fraction pelleted by ultracentrifugation at 100,000 g. Membranes were resuspended in TSE buffer (50 mM Tris; pH 7.6, 250 mM sucrose, 10 % glycerol) and the P450 content determined by P450 (Fe^{2+})-CO *vs*. P450 (Fe^{2+}) difference

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spectra. P450 reductase activity was estimated by NADPH-dependent cytochrome *c* reduction (Kemp et al., 2004). Membranes were stored at –70 °C until required.

Analytical Assays

Fluorescence assay The fluorogenic substrate 3-[2-(N, N-diethy-N-Methylamino)ethyl]-7-methoxy-4methylcoumarin (AMMC) was used and assays were conducted in 96 well microtitre plates using a final volume of 200 µl. IC_{50} determination requires the serial dilution of test compounds across a concentration range that optimally covers 0 to 100% inhibition of CYP2D6 activity. The compounds tested were serially diluted in 3-fold steps in a final volume of 100 µl of 2x enzyme/substrate stock solution (0.1 pmol/µl P450 in 100 mM KPB pH 7.4 with 2 mM AMMC). A solvent control was included to correct for any solvent effects across the dilution range. Plates were then pre-incubated for 3 min at 37 ^{o}C, and the enzyme reaction was initiated by the addition of a 100 µl aliquot of pre-warmed 160x NADPH generating system (1.3 mM $NADP^{+}$, 66 mM glucose 6-phosphate, 66 mM $MgCl_2$, 0.8 U/ml glucose 6-phosphate dehydrogenase) in 100 mM potassium phosphate buffer, pH 7.4). The reaction was maintained at 37 ^{o}C, and the activities of test compound were determined by Fluoroskan Ascent FL microtitre plate reader (λ_{ex} = 390, λ_{em} = 460 nm). Activity was expressed as a percentage of the corresponding solvent-only control, and IC_{50} values were calculated using GraFit 5.0.4 (Erithacus Software).

HPLC-MS assay I Metoclopramide (0-150 µM) was incubated in 50 µM potassium phosphate buffer (pH 7.4) with 20 pmol P450 2D6 or 0.1 mg human liver microsomes (HLM; protein concentration of 8.9 mg/ml) and an NADPH regenerating system (0.41 mM glucose 6-phosphate, 0.41 mM magnesium chloride, 0.4 U glucose 6-phosphate dehydrogenase and 8.2 µM $NADP^{+}$) in a total volume of 200 µl. After 3 minutes pre-incubation at 37 °C, reactions

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were initiated with the addition of the NADPH regenerating system, and incubated for up to 30 minutes with recombinant CYP2D6, or 60mins with HLMs before being stopped with the addition of 200 µl ice cold methanol. All reactions were carried out in duplicate. After incubation, samples were centrifuged at 16,000 × *g*, 5 min to remove particulate material. Typically, 25 µl of the reaction supernatant was analysed by HPLC/MS. The analytes were separated on a Hyperclone BDS C_{18} column (5 µm; 150 x 2.0 mm) at a flow rate of 0.15 ml /min for metabolite screening and 0.2 ml/min for enymatic determinations. A linear gradient was applied using water (A) and acetonitrile (B), both containing 0.1% (v / v) formic acid. The gradient for metabolite screening ran from 10% to 100% B in 10 min and was held for 3min before returning to the initial conditions. The run time was 20 min. The gradient for enzyme assays ran from 15% to 100% B over 6 min before returning to the initial conditions. The total run was 10 min. HPLC/MS analyses were carried out using a Waters 2695 HPLC coupled with a Micromass Q-ToF Micro mass spectrometer (MS) (Waters, Hertsfordshire, UK). The major MS parameters were: capillary = 4.5 kV, sample cone = 15 V, extraction cone = 5.0 V, desolvation temperature = 350 °C, source temperature = 120 °C, cone and desolvation gases were nitrogen at 80 and 500 L / hr respectively. For MS/MS experiments, the collision energy was 25 eV and the collision gas was argon.

Modeling and Docking. The homology model of CYP2D6 was produced as described previously (Kirton et al., 2002), using the comparative modelling programme Modeller (Sali and Blundell, 1993) with five structural templates: P450s cam (Poulos et al., 1986), terp (Hasemann et al., 1994), eryF (Cupp-Vickery et al., 2000), BM3 (Ravichandran et al., 1993), and 2C5 (Williams et al., 2000). Docking studies of the 20 drug compounds listed in Table 1 were performed as previously described (Kemp et al., 2004), using the program GOLD v3.0 (Jones et al., 1997) – a program consistently found to perform well, e.g. (Kellenberger et al.,

2004; Kontoyianni et al., 2004) – with the ChemScore (Eldridge et al., 1997; Verdonk et al., 2003; Kirton et al., 2005) fitness function. Ten solutions were generated for each ligand and ranked according to the value of the ChemScore fitness function, and the best ranked orientation of each compound was analysed further.

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RESULTS

In silico docking.

The CYP2D6 structural model (Kirton et al., 2002; Kemp et al., 2004) was used to screen *in silico* 20 drugs often taken as medication by patients with cancer undergoing chemotherapy. Each drug was docked into the CYP2D6 model, and the value of the ChemScore fitness function for the best-ranked solution is given in Table 1. All 20 drugs were screened experimentally for inhibition of AMMC demethylase activity and compared with the *in silico* data (Table 1). We obtained a good correlation between the ChemScore values and the experimental log IC_{50} values (Figure 1), with a regression coefficient of $r^2 = 0.61$ ($q^2 = 0.59$). The three drugs predicted by *in silico* analysis to be the strongest binders (Table 2) are loperamide, domperidone, and amitriptyline, which are three of the four tightest binding compounds, with experimental IC_{50} values of 0.7 µM, 2.2 µM, and 3.6 µM, respectively. Of the 13 drugs predicted to inhibit CYP2D6 [ChemScore values < -30 kJ/mol (Kemp et al., 2004)], 11 are found experimentally to be inhibitors with IC_{50} < 100 µM. Of the 7 drugs predicted not to inhibit CYP2D6 [ChemScore values > -30 kJ/mol (Kemp et al., 2004)], 5 have experimental IC_{50} values > 100 µM.

Metoclopramide Metabolism

In examining the orientations of the compounds in the active site predicted by our docking procedure, metoclopramide appeared to adopt a position in the active site that was inconsistent with the *N*-deethylation reaction previously reported (Desta et al., 2002). When docked into the active site of our CYP2D6 model, metoclopramide adopts two main orientations. In one of these (ChemScore = -30.5 kJ/mol for the most energetically favourable docking), the aromatic amino group of the drug lies in close proximity to the heme (Figure 2a). This orientation suggests

possible oxidative reactions at C_3, C_4, or the aromatic amino group (Figure 3). In most of the solutions occupying this orientation a salt bridge is observed between the basic nitrogen of metoclopramide and the sidechain carboxyl group of Glu-216 (Figure 2a). In the second docked orientation (ChemScore = -26.0 kJ/mol for the most energetically favourable docking), the basic nitrogen of metoclopramide is in close proximity to the iron and the aromatic moiety forms a good π- π interaction with the sidechain of Phe-120 (Figure 2b). This orientation is consistent with oxidation of the tertiary amine concomitant with formation of the *N*-deethylated metabolite(s) described by Desta *et al.* (Desta et al., 2002). The relatively small difference in predicted ChemScore values between these two orientations suggests that CYP2D6 can catalyse oxidation at both the basic nitrogen and the aromatic amino moities of metoclopramide.

To investigate the possibility of hydroxylation of the aromatic amino group of metoclopramide by CYP2D6, we analysed the metabolism of metoclopramide by human liver microsomes (HLMs) and recombinant CYP2D6 using HPLC/mass spectroscopy. Extracted ion chromatograms of a test sample (metoclopramide incubated with HLMs or CYP2D6), a control sample (without NADPH regenerating system) and a test sample in the presence of quinidine, a specific CYP2D6 inhibitor, are shown in Figure 4. A metabolite peak (m/z 316) with a retention time of 8.7 min was formed following incubation with both HLMs and CYP2D6. Its appearance was dependent on the presence of NADPH and was inhibited by quinidine (5 μM), a diagnostic inhibitor of CYP2D6. The formation of the m/z 316 metabolite followed Michaelis-Menten kinetics with a K_M value of 6.41 ± 0.42 μM. The V_{max} could not be calculated due to the absence of a standard. This low K_M is consistent with the measured IC_{50} value of 9.2 μM and the K_i value of 4.7 μM reported for the inhibition of CYP2D6-mediated dextromethorphan *O*-demethylation by metoclopramide (Desta et al., 2002).

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We also located an m/z 272 ion with the same retention time as the m/z 316 ion, which is presumably the deethylated product described by Desta *et al* (Desta et al., 2002) . However, the intensity of this ion was approximately six times lower than that of the m/z 316 ion. In addition, we found the formation of the m/z 272 ion was independent of NADPH, an essential cofactor for CYP2D6 catalysis (Figure 5). Importantly, we found the 316 m/z ion to be relatively unstable in comparison with the m/z 272 ion (the 316 m/z ion peak disappeared from samples left at room temperature for ~32 hours, while no significant change was found in the m/z 272 signal). Taken together these data indicate that the m/z 316 ion is the primary metabolite produced following the metabolism of metoclopramide by CYP2D6.

Identification of the m/z 316 metabolite

The 316 m/z metabolite represents a 16 mass unit increase over metoclopramide, indicative of the addition of an oxygen atom. To identify the location of the reaction, we compared the collision induced dissociation (CID) mass spectra of metoclopramide and the metabolite. The CID of metoclopramide (m/z 300) produced 4 major fragments at m/z 227.03, 212.02, 184.00 and 156.01 (Figure 6A). These correspond to the progressive loss of the following functional groups, as shown in Figure 6A: m/z 227.03 ion – loss of the diethylamino group (73 mass units); m/z 212.02 - additional loss of a methyl group (15 mass units); m/z 184.00 – additional loss of -CHNH (28 mass units), and m/z 156.01 – additional loss of carbon monoxide (28 mass units).

The CID of the m/z 316 metabolite produced four major fragments at m/z 243.02, 226.01, 199.99 and 182.97 (Figure 6B). The m/z 243.02 fragment corresponds to a 16 mass unit increase relative to the m/z 227.03 fragment seen in the CID spectrum of metoclopramide, corresponding to the addition of a hydroxyl group. The m/z 226.01 and 182.97 fragments

appear to be derived from loss of 17 mass units from the m/z 243.02 and 199.99 fragments respectively (Fig 6b), corresponding to the facile loss of OH from a hydroxylamino group. Taken together, these data point to oxidation of the aromatic amino group to a hydroxylamino group by CYP2D6, consistent with the prediction from Figure 2a.

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DISCUSSION

CYP2D6 metabolises many drugs commonly taken by patients undergoing anti-cancer chemotherapy. We previously reported the validation of this CYP2D6 model by the successful prediction of relative affinities (Kemp et al., 2004) and sites of metabolism (Kirton et al., 2002; Kemp et al., 2004). Indeed the recent publication of a CYP2D6 crystal structure (Rowland et al., 2006) shows good agreement with the global structure and the positioning of key active site residues predicted by our modelling data. We have now used the same method to screen a small but diverse range of concomitant medications used by patients with cancer; we recently performed a similar study of the interaction of co-medication compounds with CYP3A4 (Marechal et al., 2006). The *in silico* screen successfully discriminated between compounds which bound to CYP2D6 and those which do not. Taking (as we have previously; (Kemp et al., 2004)) a ChemScore of -30 kJ/mol and an IC_{50} of 100 μM as the dividing lines between 'binders' and 'non-binders', the docking calculations produced two false positives and two false negative out of the 20 compounds examined. In terms of a quantitative comparison, the experimental data for these 20 compounds correlated reasonably well with the values predicted from the model, with a regression coefficient of $r^2 = 0.61$ – this is significantly better than random.

In addition to predicting binding affinity, our modelling approach can also be used to predict the sites of metabolism of a wide range of drugs (Kirton et al., 2002; Kemp et al., 2004). In the present work, this has proved useful in predicting a novel metabolic pathway for metoclopramide, a commonly prescribed anti-emetic. The arylhydroxyamino metabolite of metoclopramide was identified from urine analysis (Maurich et al., 1994), and our study now identifies CYP2D6 as mediating the oxidative metabolism of metoclopramide to this hydroxylamino derivative rather than to the *N*-deethylated product previously reported (Desta et

al., 2002). The discrepancy may be associated with the instability of the hydroxylamino metabolite, allied to differences in sample handling prior to mass spectrometry analysis (the analysis of Desta *et al.* was based on off line mass spectrometry), which creates the potential for the degradation of the genuine metabolite.

With respect to the clinical significance of this new finding, it is unlikely that P450-mediated metabolism determines the overall clearance of metoclopramide (Teng et al., 1977; Bateman et al., 1980). However, minor metabolic pathways could be clinically relevant if linked to the generation of a toxic metabolite. In this respect, it is well documented that metoclopramide induces movement disorders, such as parkinsonism, dystonia, tardive dyskinesia, and akathisia (Jimenez-Jimenez et al., 1997), as well as arrhythmias (Malkoff et al., 1995; Baguley et al., 1997), hypertension (Harrington et al., 1983) and disorders involving haemoglobin oxidation (Grant et al., 1994; Langford and Sheikh, 1999; Kuehl et al., 2001). Thus, since toxic aromatic amines are known to be generated from related compounds such as procainamide (Radomski, 1979), further investigation into the potential toxicity of the metoclopramide metabolite is warranted. Furthermore, patients experiencing extra-pyramidal or other side effects from metoclopramide should be screened to see whether the adverse effect is related to their CYP2D6 genotype. If this is the case then there may be implications for the patients who may be particularly prone to adverse effects from other drugs using this as the major metabolic pathway.

In cancer treatment drug-drug interactions involving CYP2D6 are probably most significant with tamoxifen, an oestrogen receptor antagonist that is widely used to treat breast cancer. CYP2D6 has been shown to play an important role in the metabolism of this compound, increasing potency through conversion to the antiestrogen 4'-hydroxytamoxifen (Dehal and

Kupfer, 1997; Crewe et al., 2002), and we measured a relatively low IC_{50} for tamoxifen of 6.8 μM. Importantly, the efficacy of tamoxifen varies widely, with recent evidence linking this to variation in CYP2D6 activity (Jin et al., 2005). Thus rationalising the molecular basis for drug-drug interactions with co-medications in relation to CYP2D6 may help shed light on the inter-individual variation associated with tamoxifen treatment.

Overall this study demonstrates that molecular modelling and docking provides a useful *in silico* tool for predicting CYP2D6 inhibition. Incorporation of experimental CYP2D6 structures will lead to further improvements in predictive power and hence in the usefulness of *in silico* screening in warning of potential drug-drug interactions in clinical practice. Importantly, examination of the docked orientations of individual compounds provides a useful insight into the metabolism of CYP2D6 substrates, as proven here with metoclopramide where new metabolites have been predicted and confirmed to suggest that adverse reactions with metoclopramide might be linked with the CYP2D6 genotype/phenotype of an individual.

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FOOTNOTES

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FIGURE LEGENDS

Figure 1. Correlation between the value of the ChemScore fitness function for the best ranked docked solution and the experimental log IC_{50} value for each of the co-medication compounds listed in Table 2,

Figure 2. Models of the CYP2D6: metoclopramide complex. Docked solutions illustrating orientations consistent with (a) hydroxylation of the aromatic amino group and (b) deethylation of the tertiary amine. Ovals denote sites of reaction suggested by the dockings, and dashed lines denote salt bridges.

Figure 3. Structure of metoclopramide. The arrows depict the arylhydroxyamino and *N*-deethylated metabolites.

Figure 4. (a) Extracted chromatograms (m/z 316) of metoclopramide incubated with HLMs, a control sample (without NADPH regenerating system) and a test sample with quinidine (5 μM). **(b)** Extracted chromatograms (m/z 316) of metoclopramide incubated with CYP2D6, a control sample (without NADPH regenerating system) and a test sample with quinidine (5 μM).

Figure 5. (a) Extracted chromatograms (m/z 272) of metoclopramide incubated with HLMs and a control sample (without NADPH regenerating system). **(b)** Extracted chromatograms (m/z 272) of metoclopramide incubated with 2D6 and a control sample (without NADPH regenerating system).

Figure 6. CID mass spectra of **(a)** metoclopramide and **(b)** its m/z 316 metabolite.

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Table 1. Experimental IC_{50} and calculated ChemScore values for 20 co-administered drugs in our set of common co-medication drugs which bind most tightly to CYP2D6.

Drug name	**Drug type**	**IC_{50} (μM)[a]**	**ChemScore (kJ/mol)[b]**
Fluoxetine[c,f]	Anti-depressant	0.4 ± 0.1	-38.3
Loperamide[c,d]	Anti-diarrhoea	0.7 ± 0.1	-41.8
Domperidone[c,e]	Anti-emetic	2.2 ± 0.3	-42.2
Amitriptyline[c,e]	Anti-depressant	3.6 ± 0.3	-44.6
Tamoxifen[c,d]	Anti-oestrogen	6.8 ± 0.6	-34.2
Metoclopramide[c,d]	Anti-emetic	9.2 ± 1.4	-30.5
Citalopram[c,f]	Anti-depressant	9.7 ± 0.6	-36.1
Ondansetron[c,f]	Anti-emetic	11.6 ± 1.3	-31.2
Lansoprazole	Anti-ulcer	13.3 ± 0.6	-31.1
Simvastatin	Anti-cholesterol	23.1 ± 3.0	-27.5
Diltiazem[c,d]	Anti-hypertensive	27.4 ± 1.5	-28.2
Omeprazole	Anti-ulcer	53.1 ± 2.1	-34.0
Lorazepam	Anxiolytic	94.3 ± 9.9	-30.5
Cimetidine	Anti-ulcer	164.4 ± 26.1	-22.0
Docetaxel	cytotoxic	230.1 ± 56.0	-22.6
Oxazepam	Anxiolytic	233.0 ± 56.0	-31.8
Prednisolone	Steroid	451.7 ± 59.3	-22.9
Ranitidine	Anti-ulcer	680.0 ± 28.9	-27.9
Dexamethasone	Steroid	707.8 ± 162.5	-24.9
Atenolol	Anti-hypotensive	737.4 ± 72.5	-30.2

[a]For inhibition of AMMC oxidation. Errors are goodness in fit to concentration-effect curve.

[b]Best ranked of 10 docked orientations.

[c]Drugs that are substrates of CYP2D6. Details of metabolites, and K_M data where available, are as follows – amitriptyline: 10-hydroxylation, K_M 5-13 μM (Olesen and Linnet, 1997); citalopram: *N*-demethylation, K_M 18-22 μM (Rochat et al., 1997); diltiazem: *O*-demethylation, K_M 5 μM (Molden et al., 2000); domperidone: 5-hydroxylation (Ward et al., 2004); fluoxetine: *N*-demethylation (Margolis et al., 2000); loperamide: *N*-demethylation (Kim et al., 2004); metoclopramide: *N*-hydroxylation, 6 μM (this study); ondansetron: 7- and 8-hydroxylation (Dixon et al., 1995); tamoxifen: 4-hydroxylation (Desta et al., 2004).

[d]Best ranked docking consistent with known metabolite[c].

[e]At least one of the ten dockings consistent with known metabolite[c].

[f]Known metabolite[c] not consistent with any of the ten docked orientations.

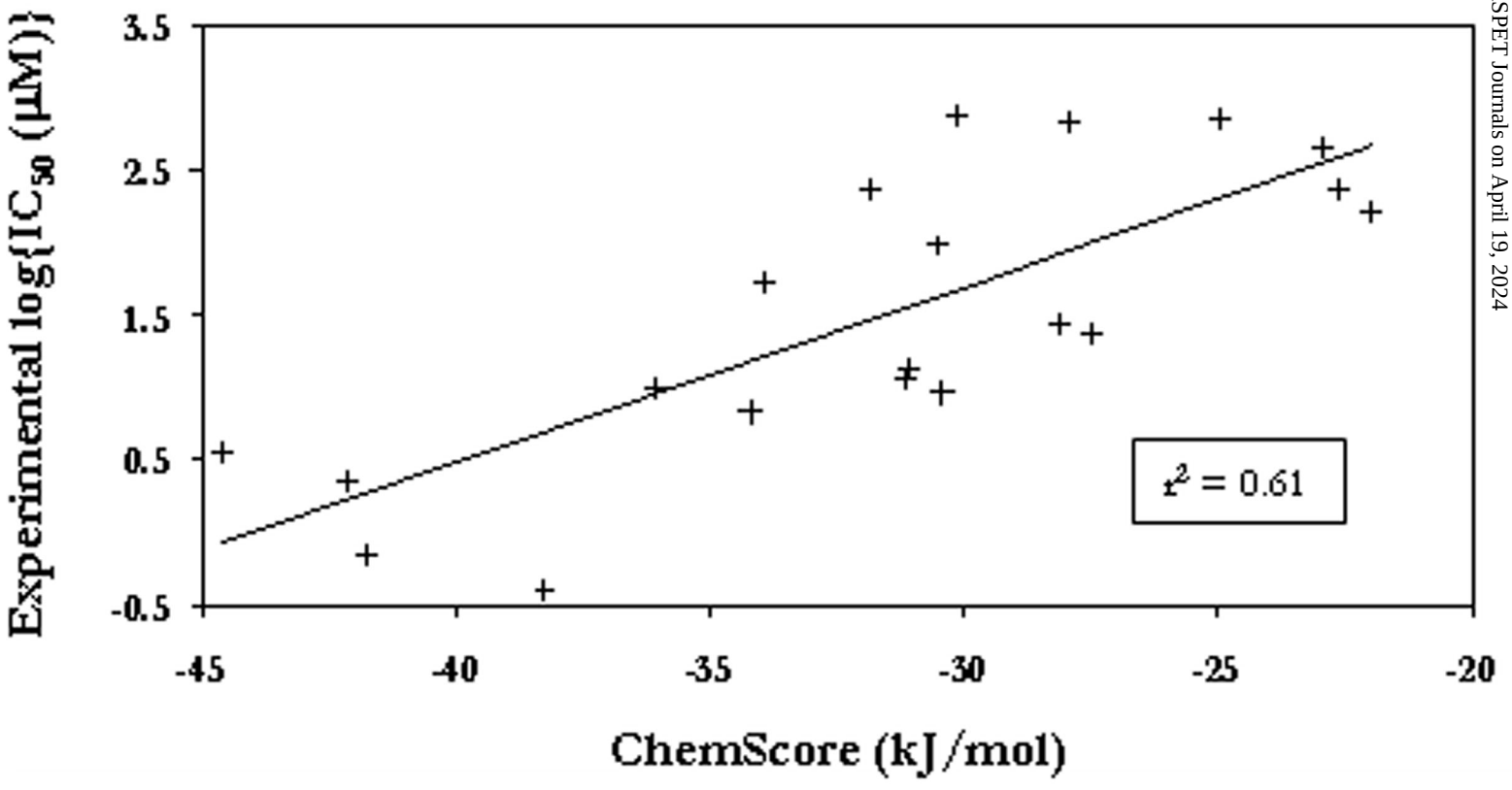

Fig 1

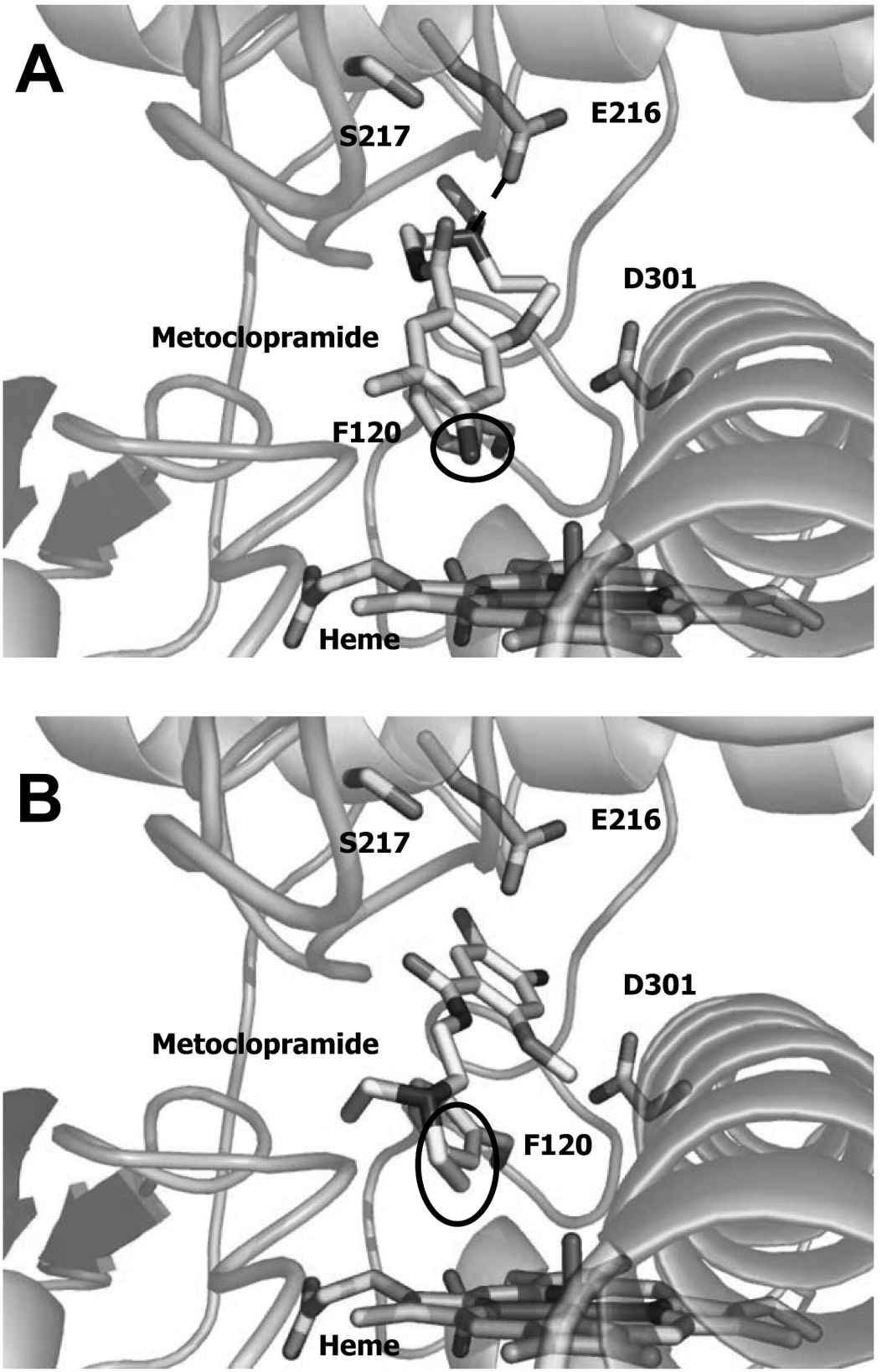
A
S217
E216
D301
Metoclopramide
F120
Heme
B
S217
E216
D301
Metoclopramide
F120
Heme

Fig. 2

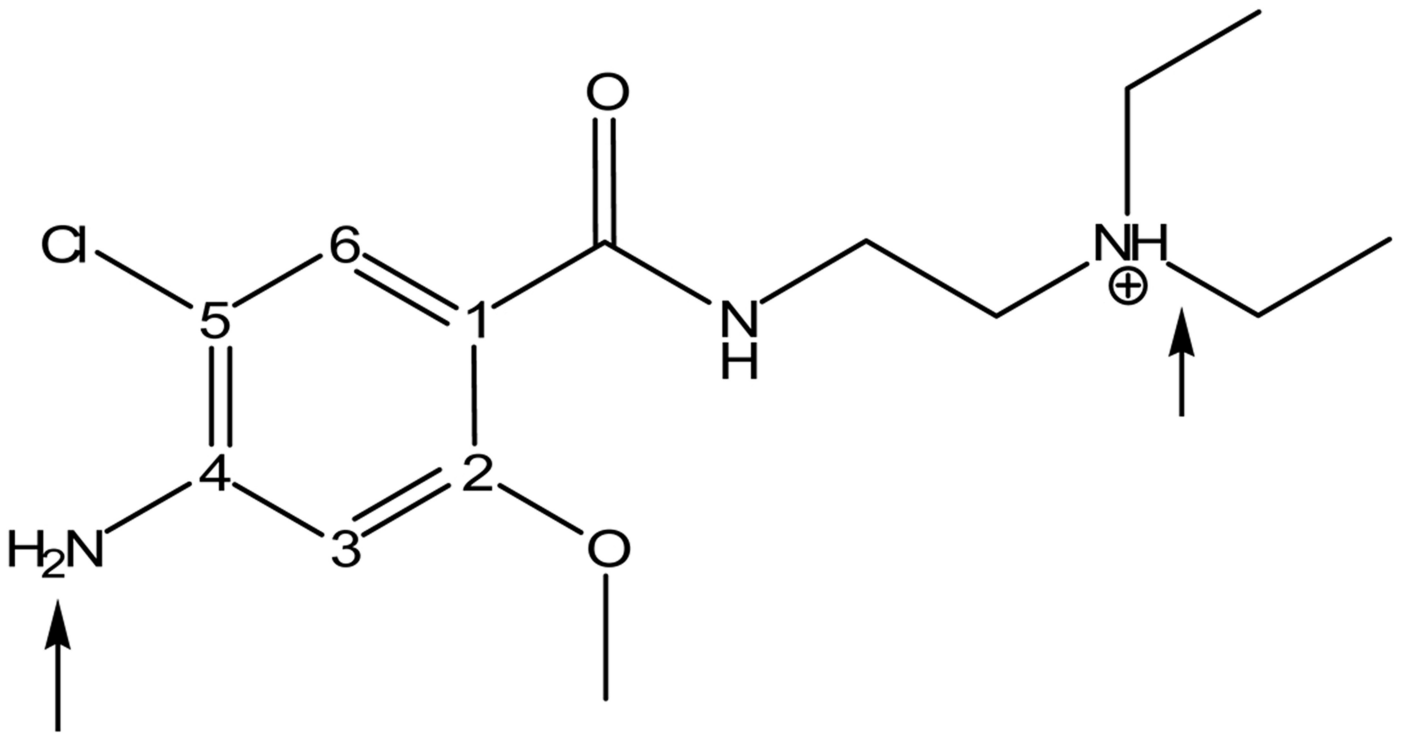

Figure 3

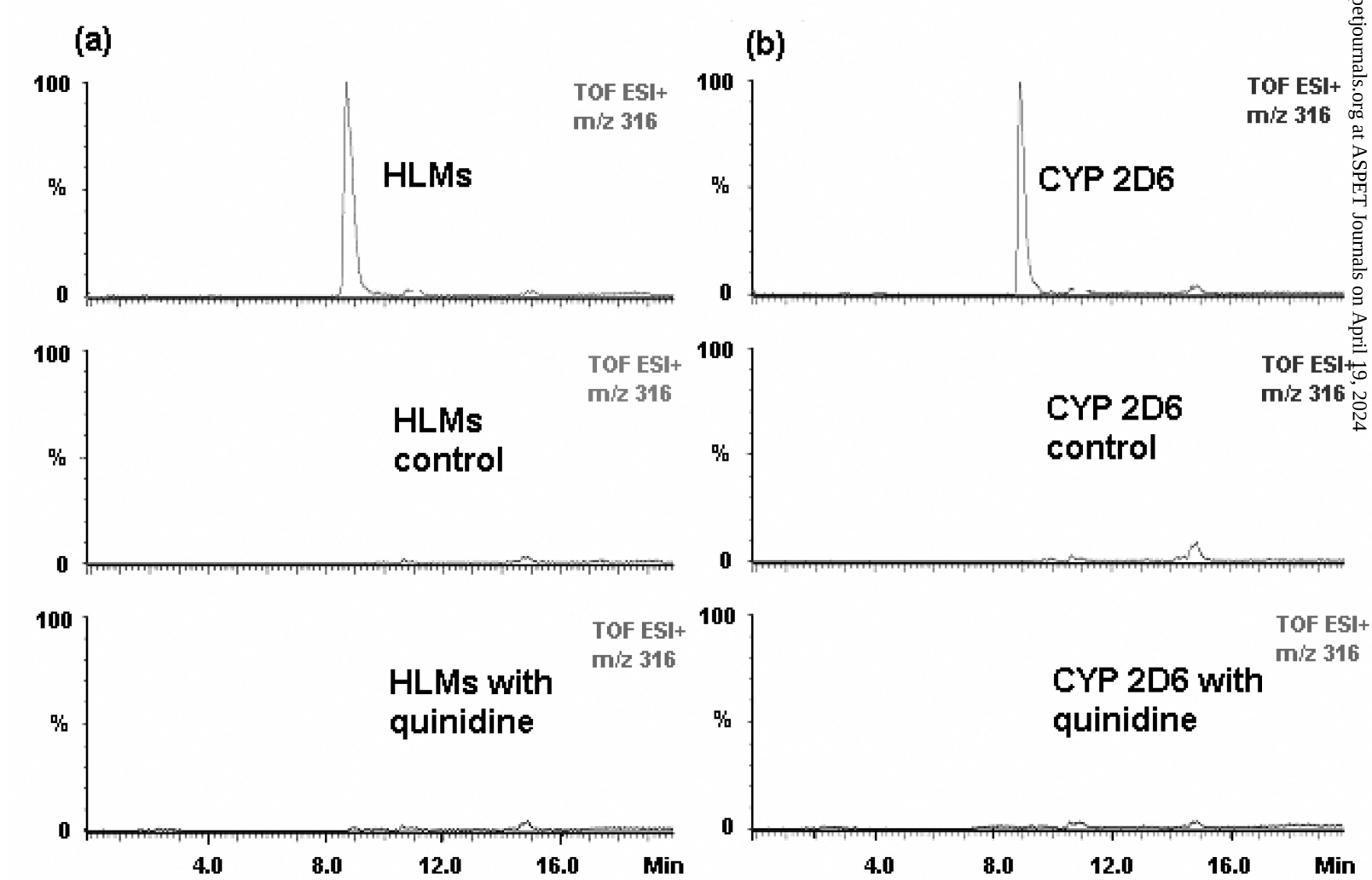

Figure 4.

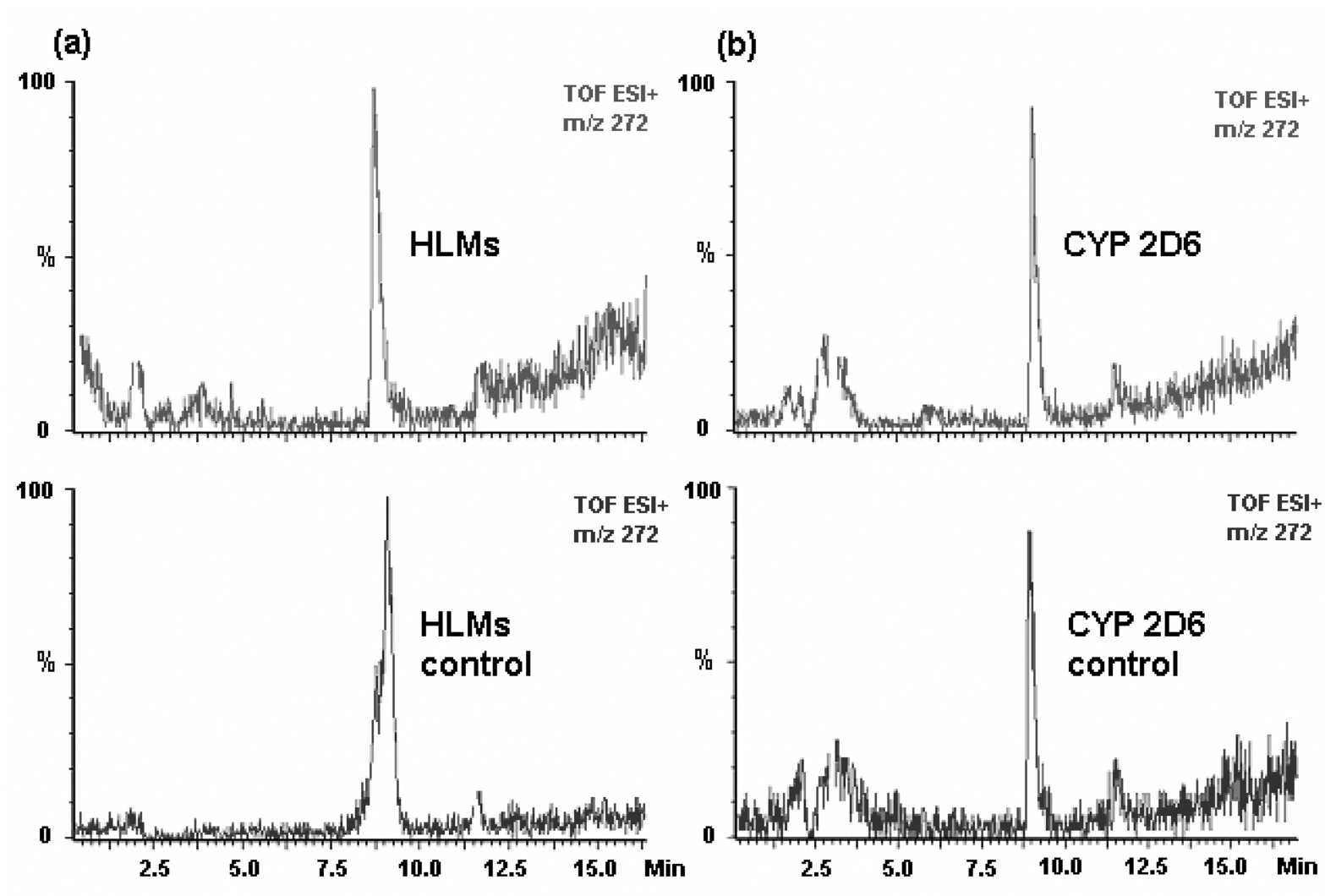

Figure 5.

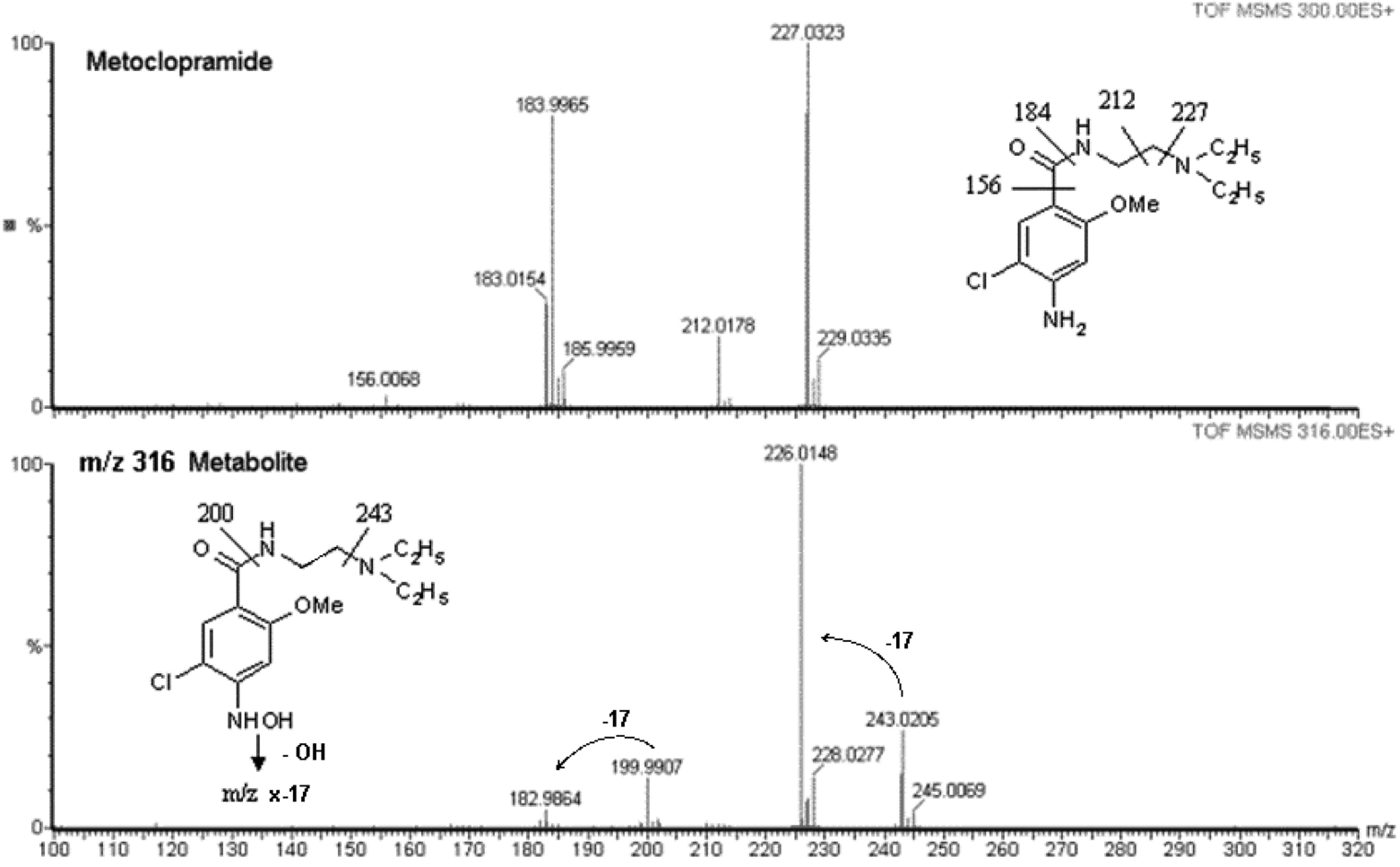

Figure 6.