In silico and in vitro screening for inhibition of cytochrome P450 CYP3A4 by co-medications commonly used by patients with cancer


Manchester Interdisciplinary Biocentre, School of Chemical Engineering and Analytical Science, University of Manchester, The Mill, PO Box 88, Manchester, M60 1QD, UK (J.-D.M., M.J.S.); Biomedical Research Centre, University of Dundee, Ninewells Hospital & Medical School, Dundee, DD1 9SY, UK (J.Y., S.B., I.K., E.M.R., C.R.W., M.J.I.P.); Department of Biochemistry, University of Leicester, Leicester, LE1 7RH, UK (G.C.K.R.)
Running title: Screening co-medication drugs for CYP3A4 inhibition

Address correspondence to:
Professor Michael J. Sutcliffe, School of Chemical Engineering and Analytical Science, University of Manchester, The Mill, PO Box 88, Manchester, M60 1QD, UK;
Tel: [+44] (0)161 306 5153; FAX: [+44] (0)161 306 9321;
e-mail: michael.sutcliffe@manchester.ac.uk

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ABBREVIATIONS: BFC, 7-benzyloxy-4-trifluoromethylcoumarin; $C_{\text{max}}$, peak plasma concentration; CYP2B6, cytochrome P450 2B6; CYP2C8, cytochrome P450 2C8; CYP2D6, cytochrome P450 2D6; CYP3A4, cytochrome P450 3A4; SRS, substrate recognition site.
ABSTRACT

Cytochrome P450 3A4 (CYP3A4) is the major enzyme responsible for phase I drug metabolism of many anti-cancer agents. It is also a major route for metabolism of many drugs used by patients to treat the symptoms caused by cancer and its treatment as well as their other illnesses, for example cardiovascular disease. To assess the ability to inhibit CYP3A4 of drugs most commonly used by our patients during cancer therapy, we have made in silico predictions based on the crystal structures of CYP3A4. From this set of 33 common co-medicated drugs, ten were predicted to be inhibitors of CYP3A4, with the anti-diarrhoeal drug loperamide predicted to be the most potent. There was significant correlation ($r^2 = 0.75 - 0.66$) between predicted affinity and our measured IC$_{50}$ values, and loperamide was confirmed as a potent inhibitor (IC$_{50}$ of 0.050 ± 0.006 µM). Active site docking studies predicted an orientation of loperamide consistent with formation of the major (N-demethylated) metabolite, where it interacts with the phenylalanine cluster, Arg-212 and Glu-374; experimental evidence for the latter interaction comes from the ~12-fold increase in $K_M$ for loperamide observed for the Glu-374-Gln mutant. The commonly prescribed drugs loperamide, amitryptyline, diltiazem, domperidone, lanzoprazole, omeprazole and simvastatin were identified by our in silico and in vitro screens as relatively potent inhibitors of CYP3A4 which have the potential to interact with cytotoxic agents to cause adverse effects, highlighting the likelihood of drug-drug interactions affecting chemotherapy treatment.
Cytochrome P450 3A4 (CYP3A4) is the major xenobiotic metabolising enzyme in man. A broad specificity coupled with high levels of expression in the liver mean it is responsible for the metabolism of more than half of all prescribed drugs (Guengerich, 1997). When patients receive several medications concurrently, unwanted and life threatening effects can result from competition for the same drug-metabolising enzyme affecting the blood levels of the competing drugs. Cancer patients would appear to be significantly at risk in this respect since CYP3A4 metabolises a large number of anti-cancer drugs and patients are generally prescribed other medications to relieve symptoms (e.g. analgesics) and side-effects (e.g. anti-emetics and anti-diarrhoeals), and to treat co-morbidities. The anti-cancer drugs metabolised by CYP3A4 include docetaxel (Marre et al., 1996), cyclophosphamide (Chang et al., 1993), ifophamide (Walker et al., 1994), etoposide (Kawashiro et al., 1998), tamoxifen (Crewe et al., 1997), irinotecan (Santos et al., 2000), vinblastine (Zhou-Pan et al., 1993) and vinorelbine (Kajita et al., 2000). While there is a marked inter-individual variation of pharmacokinetic parameters between patients (Evans and Relling, 1999), and such variation in patient response is often attributed to polymorphism in P450 genes, CYP3A4 is an exception since only a small percentage of the variation in activity can be attributed to genotype (Lamba et al., 2002a; Lamba et al., 2002b). Interactions with co-medicated compounds are therefore likely to be particularly important in explaining variations in anti-cancer drug pharmacokinetics and side effects. If a patient experiences significant toxicity during chemotherapy, the clinician will usually reduce the dose of the cytotoxic drug, reducing the anti-cancer effect. A more appropriate action might be to substitute a different co-medication that will not interact with the therapy and so maintain dose intensity of the cytotoxic drug. Thus, identifying potential drug-drug interactions involving CYP3A4 is important for improving the treatment of cancer. The development of methods to predict such interactions could lead to the administration of more effective, less toxic drug regimes.
Over recent years, *in silico* methods have proved to be a useful tool for predicting the binding properties of ligands to mammalian cytochromes P450. Studies (for a review, see e.g. (Ekins et al., 2003)) based on quantitative structure-activity relationships and pharmacophore models have generated useful information on ligand binding of CYP3A substrates (Ekins et al., 1999b) and CYP3A4 inhibitors (Ekins et al., 1999a). These methods are relatively fast and have been useful in determining important features such as autoactivation of CYP3A4 (Ekins et al., 2003). We have used an integrated hypothesis-driven structure-based approach to study CYP2D6 and have been able to identify residues that play a key role in metabolism (Smith et al., 1998; Kirton et al., 2002; Paine et al., 2003; Flanagan et al., 2004), reproduced the binding orientation and affinity of ligands in the active site (Kemp et al., 2004) and discriminated between tightly and weakly binding compounds (Kemp et al., 2004).

In this paper, we now describe the use of our structure-based *in silico* approach – which we have used effectively with CYP2D6 – to identify likely drug interactions that would inhibit CYP3A4. We have screened the set of 33 drugs commonly used by our patients with cancer using two independently determined crystal structures of the enzyme (Williams et al., 2004; Yano et al., 2004). This approach correctly predicted that loperamide had the highest affinity for CYP3A4 of all the compounds we tested. This prompted us to investigate its mode of binding. Site directed mutagenesis supported a predicted key interaction between loperamide and CYP3A4. The relative affinities predicted *in silico* correlated well with the experimental IC$_{50}$ values for the 15 co-medication compounds in the set that significantly inhibited the enzyme. Such inhibition may have unwanted effects on the efficacy and toxicity of anticancer agents using the same pathway and this merits further study.
METHODS

Molecular Docking.

We chose a set of 33 drugs based on an analysis of the medications taken by 100 newly-diagnosed patients undergoing chemotherapy for their lung cancer (Table 1). Docking studies for these drugs were carried out as described previously (Kemp et al., 2004). In brief, the program GOLDv2.2 (Jones et al., 1997) was used with the ChemScore (Eldridge et al., 1997; Verdonk et al., 2003) fitness function to generate 10 solutions for each ligand, and the dockings ranked according to the value of the ChemScore fitness function; only the best ranked solution for each ligand was included in further analysis. Dockings were performed into the two available crystal structures of ligand-free CYP3A4 (PDB (Berman et al., 2000) accession codes 1w0e (Williams et al., 2004) and 1tqn (Yano et al., 2004)). The two structures are closely similar with the exception of the region of SRS2 (Gotoh, 1992), where the side chain of Arg-212 is oriented either towards (1tqn) or away from (1w0e) the heme iron. To assess the utility of our approach over the use of relatively fast and simple ligand-based descriptors, we determined the SlogP value, number of hydrogen bond donors, number of hydrogen bond acceptors and molecular weight using MOE (Chemical Computing Group, Montreal, Canada).

Chemicals

The test compounds: 5,5-diphenylhydantoin, allopurinol, amitriptyline, aspirin, atenolol, caffeine, cefuroxime, citalopram, dexamethasone, diclofenac, diltiazem, domperidone, fluoxetine, frusemide, gabapentin, glucosamine, ibuprofen, lansoprazole, loperamide, lorazepam, metformin, metoclopramide, metronidazole, omeprazole, ondansetron, oxazepam, paracetamol, prednisolone, ranitidine, simvastatin, theophyline, R-warfarin were purchased from Sigma-Aldrich (Dorset, UK). Tramadol hydrochloride was purchased from Fluka.
BioChemika (Poole, UK). BFC was obtained from Ultrafine Chemicals (UK). All other reagents used were of the highest available quality.

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

The isolation of the cDNAs and construction of expression plasmids ompA CYP3A4(His$_6$) (pB84) and pJR7 (human NADPH cytochrome P450 oxidoreductase (CPR)) have been described elsewhere (Pritchard et al., 1997). Site-directed mutagenesis was performed using the single stranded DNA template method (Kunkel et al., 1987) using pB84 as a template, the *dut* $^-$ ung $^-*$ *E. coli* strain CJ236 and the oligonucleotide 5'-TTTCAGACCCCTCCTAAGTCTCAGTCAG-3' for E374Q. The presence of the desired mutations was confirmed by DNA sequencing. Co-expression of wild-type CYP3A4 or the E374Q mutant with CPR was carried out as previously described (Pritchard et al., 1997; Smith et al., 1998; Paine et al., 2003; Flanagan et al., 2004), where wild type CYP3A4 and E374Q gave average yields of 250 and 150 nmol P450/l respectively. CPR activity was estimated by NADPH-dependent cytochrome c reduction (Strobel and Dignam, 1978).

**Single point screen and IC$_{50}$ determinations**

Assays were performed in 96 well microtitre plates using the fluorogenic substrate BFC, in a final volume of 200 µl. Compounds dissolved in methanol or H$_2$O (Table 1) were added to a final volume of 100 µl of 2x enzyme/substrate stock solution (0.1 pmol/µl P450 in 50 mM Hepes and 30mM MgCl$_2$ pH 7.4 with 50 µM BFC). 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 °C, and the enzyme reaction was initiated by the addition of a 100 µl aliquot of pre-warmed 2x NADPH-generating system (1.3 mM NADP$^+$, 6.6 mM glucose-6-phosphate, 6.6 mM MgCl$_2$, 0.8 U/ml glucose-6-phosphate dehydrogenase) in 50 mM Hepes buffer (pH 7.4). The reaction was maintained at 37 °C, and production of the fluorescent metabolite 7-
hydroxytrifluoromethyl coumarin (HFC) measured using a Fluoroskan Ascent FL microtitre plate reader ($\lambda_{\text{ex}} = 405$, $\lambda_{\text{em}} = 530$ nm). For the single point screen, the inhibition of BFC hydroxylation was measured using 50 $\mu$M substrate (close to the $K_M$ of 51 $\mu$M measured for BFC) in the presence of 100 $\mu$M of each co-medication compound as described below. Results of the screening were expressed as the percentage activity in the presence of 100$\mu$M co-medication compound compared to activity of the appropriate solvent control. Those co-medication compounds which inhibited enzyme activity to < 70% of control activity in duplicate experiments were evaluated further by measuring the percentage inhibition across a concentration range covering 0 to 100% inhibition of CYP3A4 activity, and IC$_{50}$ values were calculated using GraFit 5.0.4 (Erithacus Software Ltd., Surrey, UK).

**Loperamide kinetics**

The $K_M$s of loperamide as a substrate for wild type CYP3A4 and the E374Q mutant were determined by HPLC-MS/MS. Loperamide (0-100 $\mu$M) was incubated with 20 pmol CYP3A4 or the E374Q mutant in 10 mM Hepes buffer (pH 7.4) containing 6 mM MgCl$_2$ and reactions were initiated by addition of 2x NADPH-regenerating system in a total volume of 200 $\mu$l. After incubation for 8 minutes at 37 °C the reaction was stopped by the addition of 200 $\mu$l ice-cold methanol. After centrifugation at 16,000 × g, 5 min to remove particulate material, 10 $\mu$l of the reaction supernatant was injected into the HPLC-MS/MS system (Micromass Quattro Micro mass spectrometer, Waters, Hertfordshire, UK). The analytes were separated on a Hyperclone BDS C$_{18}$ column (3 $\mu$m; 50 x 2.0 mm) at a flow rate of 0.2 ml /min. A linear gradient was applied using 10mM ammonium formate and 0.1% (v / v) formic acid (A) and acetonitrile (B). The gradient of the mobile phase ran from 25% to 90% B in 5 min followed by 90% B for 3min before returning to the initial conditions. The total run time was 13 min. The major MS parameters were: capillary voltage = 3.6 kV, sample
cone voltage = 25 V, collision energy = 23 eV, desolvation temperature = 350 °C, source temperature = 120 °C, and the transition for the N-demethylated product was: 463 → 252 m/z.
RESULTS

CYP3A4 affinities of co-medication drugs

The 33 co-medication drugs used in this study are shown in Table 1. All these compounds could be successfully docked into the active site of CYP3A4, using either of the two available crystal structures of ligand-free CYP3A4 (Williams et al., 2004; Yano et al., 2004). These dockings yielded ChemScore values ranging from -41.0 kJ/mol to -10.1 kJ/mol (Table 1; note that the more negative the value, the tighter the predicted binding). The ChemScore value for loperamide (< -40 kJ/mol) suggests that it binds tightly to CYP3A4, and this was confirmed by its experimentally determined IC$_{50}$ value of 0.050 ± 0.006 µM. Encouraged by this success with loperamide, we investigated the ability of our other 32 co-medication compounds to inhibit CYP3A4. First, a single point inhibition assay revealed that 18 of the compounds in the set were at best very weak inhibitors (<30% inhibition at 100 µM) – consistent with relatively poor scores for these compounds in our docking studies (ChemScore values > -29 kJ/mol; Table 1). The remaining 15 compounds were selected for IC$_{50}$ determination (Table 1). The ChemScore values for these 15 “binders” correlate significantly with the experimental log(IC$_{50}$) values, with $r^2 = 0.75$ ($q^2 = 0.73$) for docking into the CYP3A4 crystal structure 1tqn and $r^2 = 0.66$ ($q^2 = 0.64$) for docking into the CYP3A4 structure 1w0e. The difference between the results for the two structures should be treated with some caution because only three outliers (citalopram, lansoprazole and omeprazole) led to the slightly poorer regression with 1w0e.

In our previous work on CYP2D6, we defined “tight binders” as those compounds with a ChemScore of < -30 kJ/mol and an IC$_{50}$ value of < 10 µM (Kemp et al., 2004). While these values are somewhat arbitrary, applying the same criteria here reveals that five of the seven compounds with an IC$_{50}$ value of < 10 µM – loperamide, domperidone, simvastin, diltiazam...
and amitriptyline – are correctly predicted, by docking into both crystal structures, as tight binders. The other two compounds with IC$_{50} < 10 \, \mu M$ – omeprazole and lansoprazole – are predicted correctly using the 1tqn, but not the 1w0e, structure. Conversely, all compounds with an IC$_{50}$ value $> 100 \, \mu M$ – metoclopramide, dexamethasone, lorazepam, R-warfarin and prednisolone – are correctly predicted to be weaker binders. The three remaining compounds – fluoxetine, ondansetron and citalopram – with IC$_{50}$ values in the “grey” area between 10 and 100 $\mu M$ are all predicted by docking to be tight binders.

**Loperamide binding**

Our docking studies correctly predicted loperamide to be the tightest binder with a ChemScore value $< -40 \, \text{kJ/mol}$ and an IC$_{50}$ value of 0.05 $\mu M$. This was of interest since at the time this drug was not known to be a CYP3A4 ligand. Two studies reported since this part of our work was carried out have demonstrated that loperamide is a substrate of CYP3A4 (Kalgutkar and Nguyen, 2004; Kim et al., 2004), although the nature of its interactions within the active site has not been investigated. Our docking studies predict that in the favoured (lowest energy) loperamide-CYP3A4 complex, the loperamide is positioned for formation of the major, $N$-demethylated, product (Kalgutkar and Nguyen, 2004; Kim et al., 2004) (Fig. 1). In these models, loperamide interacts with CYP3A4 via (i) non-polar interactions with the phenylalanine cluster (Fig. 1B) and (ii) polar interactions with Arg-212 (the guanidinium moiety of Arg-212 is predicted to hydrogen bond to the amide carbonyl) and Glu-374 (a carboxyl oxygen of Glu-374 is predicted to hydrogen bond to the piperidine hydroxyl). Interestingly, the hydrogen bond acceptor and hydrophobic interactions of loperamide with CYP3A4 (Fig. 1A,B) appears similar to mibefradil fitted to a CYP3A4 pharmacophore (Figure 2a of (Ekins et al., 2003)). The hydrogen bond with Arg-212 is only observed in the 1tqn structure, reflecting the rotation of the Arg-212 side chain towards the heme in this structure. The absence of such an interaction in the dockings into the 1w0e structure does not
change the overall orientation of the ligand in the binding site, but in these models the tertiary amide moiety is positioned closer to the iron atom where it might be more readily oxidised.

To confirm the predicted role of Glu-374 in loperamide binding, mutagenesis experiments were performed. When this negatively charged amino acid was replaced by the polar (uncharged) glutamine, the mutant produced an approximate 50% reduction in the yield of P450. We also noted a three-fold increase in P420 content in the membranes relative to wild type, suggestive of some protein misfolding. We determined a $K_M$ for wild type CYP3A4 of $2.6 \pm 0.3 \, \mu M$ for the production of the $N$-demethylated product, slightly lower than the value of $6.3 \, \mu M$ reported by Kim et al. (Kim et al., 2004). The E374Q mutation has a significant effect on loperamide binding for $N$-demethylation, with a ~12-fold increase in $K_M$ to $31.4 \pm 1.9 \, \mu M$ (i.e. weaker binding). These results indicate a functional role for Glu-374 in the binding of loperamide to CYP3A4, and that our in silico study has correctly identified a significant ligand binding interaction.
DISCUSSION

In this study we have shown that in silico screening, using Gold with the ChemScore scoring function, allows rapid identification of compounds which bind tightly to CYP3A4. The use of two independently-solved crystal structures of CYP3A4 leads to similar predictions (Table 1). The in silico screening correlates well with a single point in vitro experimental screen in identifying which compounds bind to CYP3A4 (Table 1). In addition, it identifies correctly most (7 out of 7 using structure 1tqn and 5 out of 7 using structure 1w0e) of the tightly binding (IC\textsubscript{50} <10\,\mu\text{M}) compounds in our co-medication set. All the compounds predicted to bind weakly (ChemScore > -30 kJ/mol) are found experimentally to have IC\textsubscript{50} > 100 \,\mu\text{M}. The three compounds that are “false positives” (“best” ChemScore < -30 kJ/mol and IC\textsubscript{50} > 10 \,\mu\text{M}) all have IC\textsubscript{50} values in the “grey” area ranging from 10 to 100 \,\mu\text{M}. Significant correlation between theoretical and experimental binding affinities is observed, slightly better when using structure 1tqn (r\textsuperscript{2} = 0.75; q\textsuperscript{2} = 0.73) than structure 1w0e (r\textsuperscript{2}=0.66; q\textsuperscript{2} = 0.64). This difference might seem surprising since in structure 1tqn the side chain of Arg-212 occupies a position in the binding site above the heme while in structure 1w0e it is reoriented away from the binding site. The impact of Arg-212 on substrate binding and catalysis by CYP3A4 remains unclear (Harlow and Halpert, 1997; Yano et al., 2004) and we are currently investigating this further. The results we report here suggest that in many cases the choice of CYP3A4 crystal structure is not a crucial factor; however, in most cases where there is a difference in the predicted ChemScore value between the two structures, the compounds are predicted to bind more tightly to structure 1tqn (Table 1).

The usefulness of relatively fast and simple ligand-based descriptors was investigated. The active site of CYP3A4 is generally regarded as large and hydrophobic – as borne out by a predominantly hydrophobic active site in the crystal structures (Williams et al., 2004; Yano et
Therefore, a “null hypothesis” for ligands binding to CYP3A4 is that ligand binding in the active site of CYP3A4 is due to hydrophobic interactions (Smith et al., 1997). Setting a threshold $S\log P$ value of 2.5 to define tight binders, we were able to correctly identify 6 out of the 7 tight bingers ($IC_{50} < 10 \mu M$ using BFC as a probe substrate). While this simple filter worked well qualitatively, it performed badly quantitatively ($r^2 = 0.37, q^2 = 0.32$ vs $logIC_{50}$), suggesting (as expected) that additional factors over and above hydrophobicity come into play in the selectivity exhibited by 3A4. Using molecular weight ($r^2 = 0.22, q^2 = 0.016$ vs $logIC_{50}$), number of hydrogen bond acceptors ($r^2 = 0.01, q^2 = 0.07$ vs $logIC_{50}$) and number of hydrogen bond donors ($r^2 = 0.06, q^2 = 0.02$ vs $logIC_{50}$) as descriptors also resulted in poor quantitative results.

In this work we have used a structure-based in silico screening approach (with a program consistently found to perform well, e.g. (Kellenberger et al., 2004; Kontoyianni et al., 2004)) in which the structure of the protein remains rigid and a single compound (ligand) is docked at a time. Such an approach might be considered inappropriate for CYP3A4 due to its relatively large active site (Yano et al., 2004), the high degree of flexibility within the active site (Anzenbacherova et al., 2000; Anzenbacher and Hudecek, 2001) and the ability of the enzyme to bind multiple ligands simultaneously (Kenworthy et al., 2001; Khan et al., 2002). Despite these concerns the approach worked well with this set of co-medication compounds, showing that screening for likely drug-drug interactions is possible using a simple approach, without the need for the additional computational overhead and complexity incurred when protein flexibility is invoked.

We predicted (ChemScore, $-45.0$ kJ/mol), and subsequently verified experimentally ($IC_{50}, 0.05 \mu M$), that the anti-diarrhoeal drug loperamide binds tightly to CYP3A4 – consistent with the observation that loperamide probably interacts with CYP3A4 (Tayrouz et al., 2001).
Additionally, the binding orientation predicted by our computational docking is consistent with the production of the major, N-demethylated, metabolite. Soon after we completed this aspect of the work, and consistent with our findings, the major enzymes responsible for the metabolism of loperamide in man were identified as CYP3A4, CYP2C8 and CYP2B6, the major product of its metabolism by CYP3A4 being the N-demethylated compound (Kalgutkar and Nguyen, 2004; Kim et al., 2004).

A number of co-medication drugs have been identified in this work as inhibitors of CYP3A4. Clearly, the most important factor in determining the likelihood of a cytochrome P450-mediated drug-drug interaction is the concentration of a compound to which the P450 enzyme is exposed relative to its inhibitory potency (Ito et al., 2004). It should be noted that the concentration in vivo of a compound is confounded both by intestinal presystemic metabolism by CYP3A4 and by the activity of efflux transporters in the intestine. In the case of loperamide, there appears to be potential to cause clinical problems since the therapeutic levels (C\text{max}) of loperamide (~ 0.04 \mu M (Kim et al., 2004)) are close to the IC\text{50} value of 0.05 \mu M. Indeed, co-administration of loperamide has been shown to reduce exposure to the HIV protease inhibitor saquinavir (Mikus et al., 2004) which is a CYP3A4 substrate (Eagling, 2002). Irinotecan (CPT-11), used as a treatment for colorectal cancer, is converted by carboxylesterases to a potent inhibitor of topoisomerase I. Dose-limiting diarrhoea during irinotecan treatment is commonly treated with loperamide – but irinotecan is a known substrate of CYP3A4 (Haaz, 1998b; Haaz, 1998a) and its metabolism has been shown to be inhibited by loperamide (Haaz, 1998b; Haaz, 1998a) – raising the possibility of significant drug-drug interactions, although the quantitative importance of this remains to be established. In a similar way, the evidence for potent CYP3A4 inhibition by loperamide raises the possibility of interactions with docetaxel, cyclophosphamide and a number of other cytotoxics which can cause troublesome diarrhoea. Several other drugs are highlighted by
this study with low IC<sub>50</sub> values (< 10 µM; i.e. tight binders), including simvastatin, an HMG-CoA reductase inhibitor which is the agent most commonly prescribed in the UK for hypercholesterolaemia, and omeprazole, a gastro-protectant often given to patients on steroids.

To summarise, we have shown that a relatively “simple” in silico method can be used to predict whether drugs commonly taken by patients with cancer as part of a co-medication regime interact with CYP3A4. As validated by our subsequent experimental binding studies, which determined a self-consistent set of IC<sub>50</sub> values, 6 out of 7 tight binding compounds were identified correctly and all the compounds predicted to be weak binders or non-binders to CYP3A4 were correctly identified. The anti-diarrhoeal drug loperamide was identified as a particularly tight binder to CYP3A4, and thus warrants attention for possible involvement in drug-drug interactions. While detailed pharmacokinetic studies will always be required to assess the quantitative importance of such interactions, the work described here demonstrates that our in silico approach is a valuable screen for identification of co-medication compounds that may present problems. There are alternatives to many of the agents we identified as having the potential to cause significant, troublesome interactions with anti-cancer agents. Knowledge of these interactions may lead to more personalised and more appropriate prescribing by oncologists.
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FOOTNOTES

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FIGURE LEGENDS

**Fig. 1.** Predicted binding mode of loperamide in the active site of CYP3A4. **A,B,** Different views of loperamide docked into crystal structure 1tqn (Yano et al., 2004), illustrating that this mode of binding is consistent with N-demethylation of the tertiary amide. To reflect the uncertainty in the position of Arg-212, its position in crystal structure 1w0e (Williams et al., 2004) is also illustrated (light grey for carbon and blue for nitrogen). Predicted hydrogen bonds are shown as dashed lines. (Generated using PyMol (DeLano, 2002)) **C,** Chemical structure of loperamide; the arrow depicts the major (N-demethylated) metabolite.
Table 1.
Calculated ChemScore values, measured single point inhibition data for the 33 common co-
medication drugs screened against CYP3A4, and and measured IC$_{50}$ values for the 15 tightly
binding compounds.

<table>
<thead>
<tr>
<th>Drug</th>
<th>ChemScore values (kJ.mol$^{-1}$)</th>
<th>% residual activity</th>
<th>IC$_{50}$ ($\mu$M)</th>
<th>log(IC$_{50}$, $\mu$M)</th>
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<tr>
<td>5,5-Diphenylhydantoin$^e$</td>
<td>-25.3 a -25.4 b</td>
<td>74</td>
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<td>2.287</td>
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<td>3.6 ± 1.1</td>
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<td>Glucosamide$^f$</td>
<td>-15.4 a -10.1 b</td>
<td>104</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ibuprofen$^e$</td>
<td>-25.9 a -23.5 b</td>
<td>86</td>
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<td></td>
</tr>
<tr>
<td>Lansoprazole$^e$</td>
<td>-31.7 a -26.9 b</td>
<td>2</td>
<td>6.5 ± 0.7</td>
<td>0.813</td>
</tr>
<tr>
<td>Loperamide$^e$</td>
<td>-41.5 a -41.1 b</td>
<td>5</td>
<td>0.050 ± 0.006</td>
<td>-1.301</td>
</tr>
<tr>
<td>Lorazepam$^e$</td>
<td>-29.0 a -25.0 b</td>
<td>33</td>
<td>206 ± 37</td>
<td>2.314</td>
</tr>
<tr>
<td>Metformin$^f$</td>
<td>-14.5 a -15.1 b</td>
<td>102</td>
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<td></td>
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<tr>
<td>Metoclopramide$^f$</td>
<td>-24.7 a -24.2 b</td>
<td>55</td>
<td>107 ± 19</td>
<td>2.027</td>
</tr>
<tr>
<td>Metrodinazole$^f$</td>
<td>-16.4 a -11.5 b</td>
<td>96</td>
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<td></td>
</tr>
<tr>
<td>Omeprazole$^e$</td>
<td>-31.7 a -29.1 b</td>
<td>2</td>
<td>2.3 ± 0.1</td>
<td>0.362</td>
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<tr>
<td>Ondansetron$^e$</td>
<td>-30.8 a -28.5 b</td>
<td>37</td>
<td>68.8 ± 8.1</td>
<td>1.838</td>
</tr>
<tr>
<td>Oxazepam$^e$</td>
<td>-28.5 a -25.7 b</td>
<td>91</td>
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<tr>
<td>Paracetamol$^e$</td>
<td>-25.4 a -19.7 b</td>
<td>76</td>
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<tr>
<td>Prednisolone$^e$</td>
<td>-26.4 a -26.7 b</td>
<td>56</td>
<td>217 ± 100</td>
<td>2.337</td>
</tr>
<tr>
<td>Ranitidine$^f$</td>
<td>-26.2 a -22.5 b</td>
<td>85</td>
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<tr>
<td>Simvastatin$^f$</td>
<td>-34.0 a -34.8 b</td>
<td>2</td>
<td>4.5 ± 0.2</td>
<td>0.653</td>
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<tr>
<td>Theophylline$^e$</td>
<td>-15.1 a -14.0 b</td>
<td>77</td>
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<tr>
<td>Tramadol$^f$</td>
<td>-26.6 a -28.2 b</td>
<td>83</td>
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<tr>
<td>R-Warfarin$^e$</td>
<td>-27.9 a -26.3 b</td>
<td>62</td>
<td>213 ± 29</td>
<td>2.329</td>
</tr>
</tbody>
</table>

$^a$Calculated by docking into the CYP3A4 crystal structure 1tqn (Yano et al., 2004).

$^b$Calculated by docking into the CYP3A4 crystal structure 1w0e (Williams et al., 2004).
Percentage residual BFC oxidation at 100µM test compound; numbers in bold correspond to those compounds (enzyme activity < 70%) for which IC\textsubscript{50} values were measured.

For inhibition of BFC oxidation. Errors are goodness in fit to concentration-effect curve.

Stock solutions dissolved in methanol; all 50 mM apart from ondansetron, oxazepam and theophyline (25 mM) and allopurinol (5 mM).

Stock solutions dissolved in H\textsubscript{2}O; all 50 mM apart from metronidazole (25 mM).