

Inhibition studies: Testing the predictive power of SimCYP®

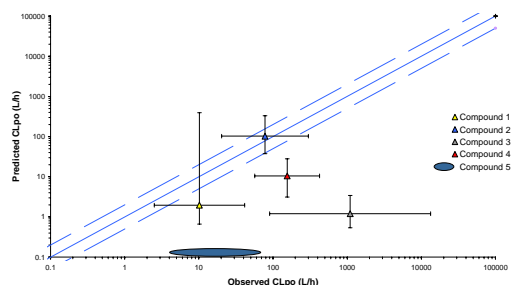
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Introduction

For the first time Neurosciences CEDD have used SimCYP® to predict clinically relevant interactions in man from *in vitro* data. We selected 5 commercially available compounds which have previously been shown to bring about a ≥2-fold difference in AUC based on literature publications, either as a result of direct inhibition and/or time-dependent inhibition at various CYPs. These compounds have previously been characterised to predict CLb with varying degrees of success (see Figure 1) by determining parameters such as CLi (HLM, S9 and rCYP), $F_{u,inc}$, permeability and plasma protein binding using in-house methods.

Figure 1 SimCYP® predictions of human CLb



Data generated from expressed isoform CLi, Fumic, Permeability and PPB results
Compound 5 was not metabolised to any measurable extent by rCYP and the prediction was based on data generated using HLM/S9 CLi results

Methods

The compounds had previously been characterised from a metabolism perspective, for these estimates the expressed isoforms characterised were as follows: 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4 and 3A5 (supplied by CYPEX Ltd, Dundee, UK). CLi were determined at 3 different protein concentrations (0.1, 0.5 and 1.5 mg/mL) and if <15% turnover was noted at the highest protein level, it was assumed that the compound was not a substrate for that isoform. Hybrid ISEFs (abundances from SimCYP whilst K_m and V_{max} values were determined in-house after correcting for $F_{u,inc}$) were used for all predictions.

The compounds were then assayed in standard in-house CYP inhibition assays taking into account direct inhibition and time-dependent inhibition using fluorimetric probes against 5 isoforms only (1A2, 2C9, 2C19, 2D6 and 3A4). Briefly, the methodology was as follows when the substrate concentration used was equivalent to the K_m unless stated otherwise:

Direct inhibition The inhibitors were diluted in methanol (MMC), DMSO (BMC) or acetonitrile (ER, DEF and FCA) which comprised 2% (v/v) of the final incubation volume and were serially diluted to final concentrations ranging from 0.1 μ M to 100 μ M. 0.1 mg/mL of each of the individual isoforms was added to the incubation mixture in buffer and pre-incubated for 10 minutes prior to starting the reaction by adding an NADPH-regenerating system. The rate of formation of metabolites derived from Ethoxymisofurin (ER by CYP1A2), 7-Methoxy-4-trifluoromethylcoumarin-3-acetic acid (FCA by CYP2C9), 3-Butyryl-7-methoxycoumarin (BMC by CYP2C19), 4-Methylaminomethyl-7-methoxycoumarin (MMC by CYP2D6) and Diethoxyfluorescein (DEF by CYP3A4) was monitored fluorimetrically over the following 10 minutes taking readings every minute. Data was normalised against a solvent control (100% activity) before IC_{50} 's were calculated using Grafit software (version 5.0.8, Erithacus Software Ltd).

Time-dependent inhibition The methods for this assay were identical to that for the direct inhibition assay above with the exception that the substrate concentrations used were 1/3rd K_m , K_m , 3x K_m and 10x K_m and the experiment was left to incubate for 30 minutes taking readings every minute. In addition to IC_{50} values (determined at K_m substrate concentrations only), K_i values were also determined using GraphPad Prism 5.0 (GraphPad Software) using 0-10 minute data only. Fold differences in the rate of metabolite formation were compared between 0-10 minute data and 20-30 minute data and if differences were noted over the timecourse, K_{app} and K_{inact} were subsequently determined using macros developed in-house based on XLfit4.

Predictions Simulations were performed using V8 of SimCYP® where the effect of the inhibitors as perpetrators of an interaction were based on AUC changes for a number of probe substrates (taken from SimCYP library) after 7 days dosing (steady state was reached for Compounds 1, 2 and 4 but not for Compounds 3 or 5 within this dosing period). Simulations were performed to incorporate the effect of direct (using K_i values only) and time-dependent inhibition (using K_i , K_{app} and K_{inact} values). The following substrates were used as probes on the effect of specific isoforms from the SimCYP® library of compounds: Caffeine, Bupropion, Rosiglitazone, Diclofenac, Omeprazole, Bufuralol, Chlorzoxasone, Midazolam and Simvastatin.

Results

Table 1 Accuracy of inhibition predictions using SimCYP®

		Inhibition of							
Compound 1		1A2	2B6	2C8	2C9	2C19	2D6	2E1	3A4/5
IC50 (uM)	<1 1-10 10+								
Ki (uM)	<1 1-10 10+								
TDI noted						B	B		Ki
K_{app}/K_{inact}	0.001-0.1 0.1-1 1+								
AUC sub/sub + inhib fold change	1-2 2-10 >10					D	D		Mid D
Literature AUC change									Sim + Mid TDI 1.7/3
Metabolised by									X

		Inhibition of							
Compound 2		1A2	2B6	2C8	2C9	2C19	2D6	2E1	3A4/5
IC50 (uM)	<1 1-10 10+								
Ki (uM)	<1 1-10 10+								
TDI noted						Ki			
K_{app}/K_{inact}	0.001-0.1 0.1-1 1+								
AUC sub/sub + inhib fold change	1-2 2-10 >10								Mid + Sim
Literature AUC change									1.3-1.7 1.2-3.75
Metabolised by									

B= borderline TDI
IC50 = IC50 method used
K_i= Ki method used
D= result from Direct inhibition simulation
Mid= midazolam used as substrate
2 batches of enzyme were used for the determination of IC_{50} values, these gave different fold changes in the predictions for Compound 5 only
X = major isoform contributing to metabolism
Ki= Ki method used
TDI= result from Time-dependent inhibition simulation
Sim= Simvastatin used as substrate

		Inhibition of							
Compound 3		1A2	2B6	2C8	2C9	2C19	2D6	2E1	3A4/5
IC50 (uM)	<1 1-10 10+								
Ki (uM)	<1 1-10 10+								
TDI noted						Ki	IC50 + Ki		IC50 + Ki
K_{app}/K_{inact}	0.001-0.1 0.1-1 1+								
AUC sub/sub + inhib fold change	1-2 2-10 >10						D		Mid
Literature AUC change									1.3 Sim
Metabolised by		X	X	X	X	X		X	X

		Inhibition of							
Compound 4		1A2	2B6	2C8	2C9	2C19	2D6	2E1	3A4/5
IC50 (uM)	<1 1-10 10+								
Ki (uM)	<1 1-10 10+								
TDI noted						Ki	IC50 + Ki	IC50	
K_{app}/K_{inact}	0.001-0.1 0.1-1 1+								
AUC sub/sub + inhib fold change	1-2 2-10 >10						D	IC50	Mid + Sim
Literature AUC change									1.9-8.2 1.9-2 0.67-1.5
Metabolised by									X

		Inhibition of							
Compound 5		1A2	2B6	2C8	2C9	2C19	2D6	2E1	3A4/5
IC50 (uM)	<1 1-10 10+								Batch 1 Batch 2
Ki (uM)	<1 1-10 10+								
TDI noted								IC50	IC50 + Ki
K_{app}/K_{inact}	0.001-0.1 0.1-1 1+								
AUC sub/sub + inhib fold change	1-2 2-10 >10								Mid Sim D Sim TDI
Literature AUC change									0.79-8.8 0.15 0.18 0.04 1.7 0.74-8.3
Metabolised by									

Conclusions

- Midazolam is less sensitive to 3A4/5 inhibition than simvastatin in SimCYP® predictions.
- All IC_{50}/K_i values <0.1 μ M in the direct assay formats were predicted to be clinically relevant by SimCYP® unless CYP2D6 was the affected isoform.
- Even if multiple isoforms are inhibited by a compound to what is considered a reasonable extent (IC_{50} >10 μ M) this can lead to predicted interaction for multiple isoforms involved in its metabolism (eg. Compound 2).
- All K_{app}/K_{inact} values >0.1 were predicted to be clinically relevant by SimCYP® and this threshold was lower still for TDI against 3A4.
- The results show that the data generated with screening assays is sufficient to progress compounds during the lead optimisation phase of drug discovery.

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