# Effect of solvent choice when performing Fluorimetric $IC_{50}$ assays with Bactosomes can influence the end result.

Caroline F. Peet, Anna W. Kaaz, Michael W. Voice and Michael P. Pritchard
Cypex Ltd, 6 Tom McDonald Avenue, Dundee, DD2 1NH, UK

## Introduction

As part of the validation of our fluorimetric CYP inhibition screening assay to support drug discovery and lead optimization, we investigated the use of 3 solvents for suitability for inclusion in this assay format (methanol [MeOH], dimethyl sulfoxide [DMSO] and acetonitrile [MeCN]). Initially the assays were performed in the absence of any potential inhibitors and up to 2% (v/v) solvent to assay kinetic parameters, linearity with time and protein for the substrates listed in the table. After these experiments had been completed it became apparent that MeCN was not suitable for use in these assays as the rates were not linear for sufficiently long either due to reduced or potentiated rates of reaction and no further studies were conducted with MeCN. Due to known solvent effects at increasing compositions [1,2], the assays were subsequently performed using both 1.5% and 2.5% (v/v) MeOH or DMSO; 0.5% (v/v) was associated with the substrate and up to an additional 2% (v/v) was associated with potential inhibitor dissolution.

## Methods

The fluorogenic substrates listed in the table were incubated at their apparent $K_m$ or $S_{50}$ (for reactions which did not obey Michaelis-Menten first order kinetics) for the reaction (or the lowest value calculated if there was a solvent effect noted in kinetic experiments). Metabolite production was monitored at excitation and emission wavelengths listed in the table. Rates of metabolite formation were linear for a minimum of 20 min in each assay but slowed down at later time-points during a 30 min incubation period at 37°C for CYP2B6 and CYP2C8, only.

27 compounds were investigated to calculate $IC_{50}$ values against each enzymatic reaction; potential inhibitors were assayed at final incubation concentrations over the 100-0.14 µM range, or lower, depending where the lower limit of quantification (LLQ) for the assay lay.

Both DMSO and MeOH were present as solvents accounting for either 1.5% or 2.5% (v/v) of the final incubation volume of 250 µL (solvents were matched between substrate and potential inhibitors).

Assays were carried out in black polypropylene 96 well plates in 250 µl final volume in 45 mM potassium phosphate pH 7.4, 4.5 mM $MgCl_2$. After pre-incubation at 37°C for 10 min 25 µL of NADPH regenerating system (1.7 mg/mL $NADP^+$, 7.8 mg/mL G-6-P and 6U/mL G-6-P DH in 0.2% [v/v] $NaHCO_3$) was added to start the reaction. CYP2C8 and CYP2C9 showed an initial lag in the rate of reaction so co-factor was added at the start of the preincubation. CYP3A4 and CYP3A5 had an additional 5 min incubation after co-factor addition before readings commenced, again, to overcome an initial lag period in the rate of reaction.

Fluorescent readings were taken every 1.5 min for 30 min. The rate of formation of fluorescent metabolite was plotted as a function of time and mean slopes in 8 solvent matched, uninhibited control wells on each plate were used to represent 100% of activity. Slopes of wells containing potential inhibitors were expressed as a % activity of control wells and $IC_{50}$ values were determined using XLfit software. Each plate also included a positive control inhibitor, Miconazole, which was incubated over the concentration range 10-0.01 µM. All experiments were repeated with 2 different batches of enzymes and mean $IC_{50}$ values are presented.

## Results

The $IC_{50}$ results of the 27 compounds (and Miconazole) investigated using DMSO and MeOH at final solvent concentrations of 1.5% (v/v) and 2.5% (v/v) are detailed in Tables 1-8.

Pearson Rank Coefficients were highest across all isoforms when DMSO was used as the solvent, with the final solvent % having negligible effect on the value determined (r>0.94 for 27 compounds). If the results in other solvents were more than 1.9-fold higher or lower than the 1.5% DMSO results these have been highlighted in red (higher) or green (lower) in Tables 1-8.

It should be noted that very steep curves which did not fit the Hill equation were noted for Fluoxetine, Cinacalcet, Montelukast, 8-MOPS and Ticlopidine and solubility was limited with Troglitazone, TAO, Furafylline and Montelukast. As such results for these compounds should be treated with caution (denoted by *). Activation of the uninhibited control rates was noted with various isoforms and Amiodarone, Montelukast, Cinacalcet, Tranylcypromine, and Ketoconazole where values >120% of control values were excluded from the data fit, but nevertheless, these $IC_{50}$ values may be overestimated as a result in this assay format.

Where calculated $IC_{50}$ values were <0.1 µM using the normal dilution range, these were repeated at lower concentration ranges to assign an accurate value to the determination. Irrespective of the concentration range used, this had very little effect on the $IC_{50}$ value determined eg CYP2C9 inhibitors in Table 6.

All of the prototypical inhibitors recommended for *in vitro* interaction studies by the FDA[3] were tested during validation of this assay and were found to be comparable or lower than their expected $K_i$ values against respective isoforms. See our adjacent poster 143 for full results.

There was only one exception to this which was Furafylline *vs* CYP1A2 which did not include a pre-incubation step during the incubation and as such gave a higher $IC_{50}$ value as a result; It should be noted that Furafyllines $IC_{50}$ values did decrease over the incubation period indicating that our assay design can be used to observe this phenomenon but cannot differentiate between time-dependent (TDI) and metabolism-based (MBI) elements of this inhibition. It should also be noted that various inhibitors have been documented to have additional TDI/MBI mechanisms which contribute to their inhibition kinetic parameters, but mainly these have been documented in human liver microsomal studies- not with individual recombinant CYP isoforms. As a result, the metabolite/mechanism forming this additional element to the decrease in activity may not be formed in this single isoform assay format, which could result in false negatives.

It is likely that MeOH gave more variable results in the test set of compounds in 1.5% (v/v) MeOH due to precipitation occurring when diluted in $H_2O$ during the dilution step for compounds with lower aqueous solubility (e.g. Troglitazone) and this is the single largest factor affecting results in this assay as DMSO is a better solvent than MeOH for sparingly soluble compounds.

Evaporation may also have been a contributing factor to the reproducibility of the data sets between 2 batches of enzyme as MeOH is more volatile than DMSO, especially when small volumes (typically ≤5 µL) are added to the incubation well as was the case especially when used at 2.5% (v/v). This probably explains why the DMSO results are more robust and reproducible with correspondingly higher Pearson Rank Coefficients.

Table 1. Effect of different solvents on the $IC_{50}$ value determined for 27 compounds and Miconazole on the activity of recombinant CYP1A2

| | 1.5% DMSO | 2.5% DMSO | 1.5% MeOH | 2.5% MeOH |
|---|---|---|---|---|
| Furafylline | 62.95 | 0.2 | 1.2 | 0.3 |
| Furafylline (Batch 1) | 43.20 | 1.1 | 2.3 | 2.3 |
| Furafylline (Batch 2) | 57.36 | 0.6 | 1.7 | 1.3 |
| Fluvoxamine | 0.13 | 0.9 | 0.6 | 0.7 |
| Fluvoxamine 10-0.01 | 0.09 | 1.0 | 0.8 | 0.6 |
| Fluvoxamine 5-0.005 | 0.09 | 1.1 | 1.3 | 1.2 |
| Amiodarone | 98.17 | 1.0 | 0.6 | 0.6 |
| Mibefradil | 100.00 | 1.0 | 1.0 | 1.0 |
| Isoniazid | 100.00 | 1.0 | 1.0 | 1.0 |
| Clopidogrel | 6.50 | 1.2 | 0.8 | 0.8 |
| Diltiazem | 100.00 | 1.0 | 1.0 | 1.0 |
| 8-MOPS | 0.59 | 0.9 | 0.4 | 0.3 |
| 8-MOPS 10-0.01 | 0.37 | 1.0 | 1.5 | 2.3 |
| Ticlopidine | 28.75 | 1.0 | 0.5 | 0.5 |
| Montelukast | 19.16 | 0.7 | 1.5 | 1.0 |
| Gemfibrozil | 100.00 | 1.0 | 1.0 | 1.0 |
| Fluoxetine | 100.00 | 1.0 | 1.0 | 1.0 |
| Troglitazone | 52.28 | 0.7 | 1.9 | 0.6 |
| Sulphafenazole | 100.00 | 1.0 | 1.0 | 1.0 |
| Tienilic acid | 100.00 | 1.0 | 1.0 | 1.0 |
| Tranylcypromine | 76.77 | 0.9 | 0.6 | 0.4 |
| Chloramphenicol | 100.00 | 1.0 | 1.0 | 1.0 |
| N-Benzylphenobarbital | 100.00 | 1.0 | 1.0 | 1.0 |
| Quinidine | 100.00 | 1.0 | 1.0 | 1.0 |
| Citalopram | 100.00 | 1.0 | 1.0 | 1.0 |
| Paroxetine | 29.79 | 1.4 | 0.9 | 0.9 |
| Cinacalcet | 16.67 | 1.1 | 1.1 | 1.0 |
| Ketoconazole | 66.34 | 0.6 | 0.7 | 0.4 |
| TAO | 100.00 | 1.0 | 1.0 | 1.0 |
| Erythromycin | 100.00 | 1.0 | 1.0 | 1.0 |
| Verapamil | 100.00 | 1.0 | 1.0 | 1.0 |
| Fluconazole | 100.00 | 1.0 | 1.0 | 1.0 |
| Pearson rank | - | 0.97 | 0.91 | 0.91 |
| Uninhibited control rate | 891 | 913 | 736 | 672 |
| Miconazole $IC_{50}$ (µM) | 1.09 | 0.88 | 1.96 | 0.67 |

Table 2. Effect of different solvents on the $IC_{50}$ value determined for 27 compounds and Miconazole on the activity of recombinant CYP2B6

| | 1.5% DMSO | 2.5% DMSO | 1.5% MeOH | 2.5% MeOH |
|---|---|---|---|---|
| Furafylline | 100.00 | 1.0 | 1.0 | 1.0 |
| Fluvoxamine | 3.97 | 1.1 | 1.9 | 1.0 |
| Fluvoxamine 10-0.01 | 7.33 | 1.0 | 1.4 | 1.4 |
| Amiodarone | 89.47 | 0.7 | 1.1 | 1.1 |
| Mibefradil | 96.00 | 1.0 | 1.0 | 0.5 |
| Isoniazid | 100.00 | 1.0 | 1.0 | 1.0 |
| Clopidogrel | 0.08 | 1.0 | 1.1 | 0.9 |
| Clopidogrel 1-0.001 | 0.06 | 0.9 | 0.7 | 0.5 |
| Diltiazem | 100.00 | 1.0 | 0.9 | 0.7 |
| 8-MOPS | 4.15 | 0.8 | 1.0 | 0.6 |
| Ticlopidine | 0.10 | 0.8 | 1.1 | 0.8 |
| Ticlopidine 1-0.001 | 0.12 | 0.9 | 1.0 | 0.8 |
| Montelukast | 9.51 | 1.2 | 1.1 | 1.2 |
| Gemfibrozil | 100.00 | 1.0 | 1.0 | 1.0 |
| Fluoxetine | 96.50 | 1.0 | 1.0 | 1.0 |
| Troglitazone | 6.25 | 1.2 | 16.0 | 1.0 |
| Sulphafenazole | 100.00 | 1.0 | 1.0 | 1.0 |
| Tienilic acid | 100.00 | 1.0 | 1.0 | 1.0 |
| Tranylcypromine | 2.72 | 1.2 | 1.9 | 1.1 |
| Chloramphenicol | 39.68 | 1.1 | 2.5 | 2.0 |
| N-Benzylphenobarbital | 45.13 | 1.1 | 1.9 | 1.0 |
| Quinidine | 100.00 | 1.0 | 1.0 | 1.0 |
| Citalopram | 100.00 | 1.0 | 1.0 | 1.0 |
| Paroxetine | 0.49 | 1.2 | 0.9 | 0.5 |
| Cinacalcet | 6.20 | 1.2 | 1.6 | 1.3 |
| Ketoconazole | 1.18 | 1.0 | 1.2 | 1.1 |
| Ketoconazole 10-0.01 | 1.73 | 1.3 | 1.9 | 1.5 |
| TAO | 100.00 | 1.0 | 1.0 | 1.0 |
| Erythromycin | 100.00 | 1.0 | 1.0 | 1.0 |
| Verapamil | 100.00 | 1.0 | 1.0 | 1.0 |
| Fluconazole | 100.00 | 1.0 | 1.0 | 1.0 |
| TAO (Biotrend) | 100.00 | 1.0 | 1.0 | 1.0 |
| Pearson rank | - | 0.99 | 0.91 | 0.96 |
| Uninhibited control rate | 823 | 750 | 1062 | 814 |
| Miconazole $IC_{50}$ (µM) | 0.035 | 0.028 | 0.054 | 0.029 |

Table 3. Effect of different solvents on the $IC_{50}$ value determined for 27 compounds and Miconazole on the activity of recombinant CYP3A4

| | 1.5% DMSO | 2.5% DMSO | 1.5% MeOH | 2.5% MeOH |
|---|---|---|---|---|
| Furafylline | 100.00 | 1.0 | 1.0 | 0.8 |
| Fluvoxamine | 8.44 | 1.0 | 0.8 | 0.4 |
| Fluvoxamine 10-0.01 | 7.47 | 1.1 | 0.7 | 1.0 |
| Amiodarone | 1.70 | 1.0 | 0.4 | 0.3 |
| Mibefradil | 0.10 | 0.9 | 0.8 | 0.6 |
| Mibefradil 5-0.005 | 0.03 | 1.4 | 0.9 | 0.5 |
| Isoniazid | 71.14 | 1.0 | 0.0 | 0.0 |
| Clopidogrel | 15.73 | 1.4 | 0.6 | 0.4 |
| Diltiazem | 2.66 | 1.2 | 0.9 | 0.6 |
| 8-MOPS | 98.94* | 1.0 | 0.1 | 0.0 |
| Ticlopidine | 91.69* | 1.0 | 2.8 | 0.3 |
| Montelukast | 17.38* | 1.0 | 2.3 | 0.4 |
| Gemfibrozil | 100.00 | 1.0 | 1.0 | 1.0 |
| Fluoxetine | 2.34 | 1.1 | 2.7 | 1.0 |
| Troglitazone | 11.45 | 0.3 | 8.7 | 0.0 |
| Sulphafenazole | 3.73 | 1.3 | 4.0 | 0.6 |
| Tienilic acid | 100.00 | 1.0 | 1.0 | 1.0 |
| Tranylcypromine | 65.89 | 1.2 | 1.2 | 0.5 |
| Chloramphenicol | 100.00 | 1.0 | 1.0 | 1.0 |
| N-Benzylphenobarbital | 43.49 | 1.2 | 2.3 | 0.8 |
| Quinidine | 5.75 | 1.2 | 1.9 | 0.7 |
| Citalopram | 46.38 | 1.3 | 1.6 | 0.6 |
| Paroxetine | 10.73 | 1.7 | 0.2 | 0.2 |
| Cinacalcet | 0.64 | 2.3 | 0.5 | 0.4 |
| Ketoconazole | 0.19 | 1.0 | 0.4 | 0.4 |
| Ketoconazole 10-0.01 | 0.05 | 1.0 | 0.4 | 0.2 |
| TAO (USP) | 0.10 | 0.9 | 5.1 | 2.1 |
| TAO (Biotrend) | 0.12 | 0.9 | 8.6 | 3.3 |
| Erythromycin | 0.67 | 1.2 | 1.1 | 0.8 |
| Verapamil | 2.96 | 1.5 | 0.6 | 0.4 |
| Fluconazole | 2.43 | 0.9 | 1.0 | 0.6 |
| Pearson rank | - | 0.99 | 0.81 | 0.92 |
| Uninhibited control rate | 627 | 453 | 915 | 656 |
| Miconazole $IC_{50}$ (µM) | 0.020 | 0.020 | 0.143 | 0.088 |

Table 4. Effect of different solvents on the $IC_{50}$ value determined for 27 compounds and Miconazole on the activity of recombinant CYP3A5

| | 1.5% DMSO | 2.5% DMSO | 1.5% MeOH | 2.5% MeOH |
|---|---|---|---|---|
| Furafylline | 81.82 | 1.2 | 1.2 | 0.9 |
| Fluvoxamine | 19.63 | 1.2 | 1.6 | 0.6 |
| Fluvoxamine 10-0.01 | 10.00 | 1.0 | 0.5 | 1.0 |
| Amiodarone | 5.79 | 1.2 | 1.2 | 1.1 |
| Mibefradil | 1.04 | 1.5 | 0.6 | 0.3 |
| Mibefradil 10-0.01 | 2.15 | 1.5 | 0.4 | 0.3 |
| Isoniazid | 61.83 | 0.4 | 0.9 | 0.5 |
| Clopidogrel | 17.51 | 1.4 | 0.9 | 0.4 |
| Diltiazem | 33.68 | 1.4 | 0.7 | 0.5 |
| 8-MOPS | 19.00 | 2.6 | 0.1 | 0.0 |
| Ticlopidine | 95.07 | 1.0 | 0.9 | 0.7 |
| Montelukast | 2.28 | 2.5 | 0.6 | 0.2 |
| Gemfibrozil | 100.00 | 1.0 | 1.0 | 1.0 |
| Fluoxetine | 12.86* | 1.1 | 7.8 | 4.8 |
| Troglitazone | 12.25 | 1.1 | 8.2 | 0.3 |
| Sulphafenazole | 19.57 | 1.1 | 5.1 | 0.9 |
| Tienilic acid | 100.00 | 1.0 | 1.0 | 1.0 |
| Tranylcypromine | 22.86 | 1.4 | 1.1 | 0.6 |
| Chloramphenicol | 100.00 | 1.0 | 1.0 | 1.0 |
| N-Benzylphenobarbital | 50.07 | 2.0 | 2.0 | 1.4 |
| Quinidine | 100.00 | 1.0 | 0.0 | 0.0 |
| Citalopram | 100.00 | 1.0 | 1.0 | 1.0 |
| Paroxetine | 22.22 | 1.3 | 1.5 | 0.3 |
| Cinacalcet | 48.30* | 1.0 | 0.1 | 0.1 |
| Ketoconazole | 0.11 | 0.9 | 0.7 | 0.7 |
| Ketoconazole 10-0.01 | 0.06 | 1.0 | 0.6 | 0.3 |
| TAO (USP) | 1.16 | 0.7 | 1.8 | 0.8 |
| TAO (Biotrend) | 0.94 | 0.6 | 1.4 | 0.9 |
| Erythromycin | 9.08 | 1.1 | 1.0 | 0.7 |
| Verapamil | 9.36 | 1.7 | 0.3 | 0.1 |
| Fluconazole | 17.03 | 1.2 | 1.1 | 0.8 |
| Pearson rank | - | 0.94 | 0.71 | 0.88 |
| Uninhibited control rate | 893 | 764 | 1226 | 1094 |
| Miconazole $IC_{50}$ (µM) | 0.00 | 1.45 | 0.64 | 0.15 |

Table 5. Effect of different solvents on the $IC_{50}$ value determined for 27 compounds and Miconazole on the activity of recombinant CYP2C19

| | 1.5% DMSO | 2.5% DMSO | 1.5% MeOH | 2.5% MeOH |
|---|---|---|---|---|
| Furafylline | 65.86 | 0.8 | 1.0 | 0.8 |
| Fluvoxamine | 0.16 | 13.8 | 0.5 | 0.5 |
| Fluvoxamine 10-0.01 | 0.09 | 1.1 | 0.8 | 0.5 |
| Fluvoxamine 5-0.005 | 0.05 | 1.1 | 1.1 | 0.9 |
| Fluvoxamine 2-0.002 | 0.07 | 0.9 | 1.4 | 1.3 |
| Amiodarone | 18.80 | 3.0 | 0.2 | 0.1 |
| Mibefradil | 11.34 | 0.5 | 0.4 | 0.1 |
| Isoniazid | 100.00 | 0.8 | 0.7 | 0.5 |
| Clopidogrel | 1.21 | 1.8 | 0.4 | 0.2 |
| Diltiazem | 100.00 | 1.0 | 1.0 | 0.5 |
| 8-MOPS | 5.37 | 0.8 | 0.5 | 0.2 |
| Ticlopidine | 0.52 | 97.4 | 0.4 | 0.3 |
| Montelukast | 3.94 | 13.5 | 0.6 | 1.2 |
| Gemfibrozil | 87.71 | 1.1 | 0.6 | 0.2 |
| Fluoxetine | 0.12 | 0.9 | 0.5 | 0.6 |
| Fluoxetine 10-0.01 | 0.06 | 1.6 | 1.1 | 0.6 |
| Fluoxetine 5-0.005 | 0.08 | 1.3 | 0.5 | 0.4 |
| Fluoxetine 2-0.002 | 0.08 | 1.1 | 0.5 | 0.3 |
| Troglitazone | 2.90 | 0.8 | 21.6 | 0.1 |
| Sulphafenazole | 100.00 | 1.0 | 0.6 | 0.3 |
| Tienilic acid | 96.32 | 0.9 | 1.0 | 0.5 |
| Tranylcypromine | 4.00 | 0.8 | 0.3 | 0.3 |
| Chloramphenicol | 5.93 | 0.8 | 0.3 | 0.3 |
| N-Benzylphenobarbital | 0.26 | 0.8 | 0.5 | 0.4 |
| N-Bpb 10-0.01 | 0.29 | 0.9 | 0.6 | 0.5 |
| Quinidine | 49.18 | 1.1 | 1.2 | 0.2 |
| Quinidine 10-0.01 | 10.00 | 1.0 | 1.0 | 0.5 |
| Citalopram | 100.00 | 1.0 | 0.6 | 0.1 |
| Paroxetine | 0.95 | 2.5 | 0.9 | 0.6 |
| Cinacalcet | 23.59* | 1.3 | 0.1 | 0.1 |
| Ketoconazole | 2.13 | 1.8 | 0.6 | 0.3 |
| TAO | 100.00 | 1.0 | 1.0 | 0.6 |
| Erythromycin | 100.00 | 1.0 | 1.0 | 0.6 |
| Verapamil | 100.00 | 1.0 | 1.0 | 0.3 |
| Fluconazole | 1.21 | 1.7 | 1.1 | 0.4 |
| Pearson rank | - | 0.94 | 0.93 | 0.88 |
| Uninhibited control rate | 205 | 120 | 391 | 320 |
| Miconazole $IC_{50}$ (µM) | 0.017 | 0.045 | 0.020 | 0.022 |

Table 6. Effect of different solvents on the $IC_{50}$ value determined for 27 compounds and Miconazole on the activity of recombinant CYP2C9

| | 1.5% DMSO | 2.5% DMSO | 1.5% MeOH | 2.5% MeOH |
|---|---|---|---|---|
| Furafylline | 100.00 | 1.0 | 1.0 | 0.6 |
| Fluvoxamine | 1.01 | 1.4 | 1.2 | 1.4 |
| Fluvoxamine 10-0.01 | 1.01 | 1.2 | 2.4 | 3.8 |
| Amiodarone | 100.00 | 1.0 | 1.0 | 1.0 |
| Mibefradil | 13.71 | 1.3 | 0.4 | 0.2 |
| Isoniazid | 100.00 | 1.0 | 1.0 | 1.0 |
| Clopidogrel | 5.27 | 1.3 | 0.5 | 0.2 |
| Diltiazem | 100.00 | 1.0 | 0.6 | 0.1 |
| 8-MOPS | 3.39 | 1.1 | 1.3 | 0.5 |
| Ticlopidine | 31.24 | 1.2 | 0.3 | 1.6 |
| Montelukast | 0.41 | 1.2 | 1.4 | 0.8 |
| Montelukast 5-0.005 | 0.32 | 1.0 | 0.8 | 0.5 |
| Montelukast 2-0.002 | 0.24 | 0.9 | 0.7 | 0.2 |
| Gemfibrozil | 26.39 | 1.2 | 2.7 | 3.0 |
| Fluoxetine | 3.61 | 1.2 | 1.1 | 0.5 |
| Fluoxetine 10-0.01 | 4.42 | 1.2 | 2.3 | 2.3 |
| Troglitazone | 0.21 | 1.6 | 479.9 | 0.7 |
| Troglitazone 2.5-0.0025 | 0.17 | 1.6 | 0.4 | 0.3 |
| Troglitazone 1-0.001 | 0.12 | 1.4 | 0.3 | 0.2 |
| Sulphafenazole | 0.36 | 1.3 | 1.1 | 0.7 |
| Sulphaphenazole 5-0.005 | 0.27 | 1.4 | 0.9 | 0.8 |
| Sulphaphenazole 2-0.002 | 0.21 | 1.4 | 1.1 | 0.3 |
| Tienilic acid | 0.22 | 1.6 | 1.0 | 5.9 |
| Tienilic acid 5-0.005 | 0.23 | 1.0 | 1.5 | 1.0 |
| Tienilic acid 2-0.002 | 0.13 | 1.3 | 1.3 | 0.5 |
| Tranylcypromine | 4.13 | 1.1 | 0.8 | 0.3 |
| Chloramphenicol | 100.00 | 1.0 | 1.0 | 0.6 |
| N-Benzylphenobarbital | 3.08 | 1.2 | 2.4 | 0.2 |
| Quinidine | 100.00 | 1.0 | 1.0 | 0.7 |
| Quinidine 10-0.01 | 10.00 | 1.0 | 1.0 | 1.0 |
| Citalopram | 100.00 | 1.0 | 0.8 | 0.6 |
| Paroxetine | 9.40 | 1.9 | 0.5 | 0.6 |
| Cinacalcet | 7.75 | 1.5 | 0.5 | 0.6 |
| Ketoconazole | 1.26 | 1.4 | 0.6 | 0.8 |
| TAO | 100.00 | 1.0 | 1.0 | 1.0 |
| Erythromycin | 100.00 | 1.0 | 1.0 | 1.0 |
| Verapamil | 64.48 | 1.4 | 0.6 | 0.3 |
| Fluconazole | 5.66 | 1.5 | 0.8 | 1.2 |
| Pearson rank | - | 0.99 | 0.88 | 0.89 |
| Uninhibited control rate | 573 | 805 | 720 | 499 |
| Miconazole $IC_{50}$ (µM) | 0.10 | 0.11 | 0.43 | 0.84 |

Table 7. Effect of different solvents on the $IC_{50}$ value determined for 27 compounds and Miconazole on the activity of recombinant CYP2D6

| | 1.5% DMSO | 2.5% DMSO | 1.5% MeOH | 2.5% MeOH |
|---|---|---|---|---|
| Furafylline | 100.00 | 0.7 | 0.3 | 0.5 |
| Fluvoxamine | 0.61 | 1.2 | 0.7 | 0.9 |
| Fluvoxamine 10-0.01 | 0.42 | 0.8 | 1.0 | 1.0 |
| Fluvoxamine 5-0.005 | 0.44 | 1.0 | 1.2 | 1.3 |
| Amiodarone | 3.80 | 1.2 | 2.0 | 4.1 |
| Mibefradil | 0.07 | 1.3 | 1.1 | 0.9 |
| Mibefradil 5-0.005 | 0.03 | 0.8 | 3.0 | 1.7 |
| Isoniazid | 100.00 | 1.0 | 0.7 | 0.8 |
| Clopidogrel | 23.21 | 1.1 | 1.0 | 0.9 |
| Diltiazem | 35.39 | 0.9 | 2.1 | 2.2 |
| 8-MOPS | 2.21 | 1.1 | 1.6 | 1.3 |
| Ticlopidine | 0.27 | 1.2 | 1.6 | 1.6 |
| Montelukast | 2.64 | 1.5 | 1.6 | 3.2 |
| Gemfibrozil | 100.00 | 1.0 | 1.0 | 1.0 |
| Fluoxetine | 0.19 | 1.1 | 1.1 | 0.8 |
| Fluoxetine 10-0.01 | 0.15 | 1.0 | 1.7 | 1.0 |
| Fluoxetine 5-0.005 | 0.15 | 0.9 | 1.4 | 1.0 |
| Troglitazone | 57.15 | 0.3 | 1.7 | 0.4 |
| Sulphafenazole | 100.00 | 1.0 | 0.9 | 0.8 |
| Tienilic acid | 100.00 | 1.0 | 1.0 | 1.0 |
| Tranylcypromine | 6.22 | 0.9 | 1.6 | 1.3 |
| Chloramphenicol | 100.00 | 1.0 | 1.0 | 1.0 |
| N-Benzylphenobarbital | 100.00 | 1.0 | 1.0 | 1.0 |
| Quinidine | 0.06 | 0.5 | 0.3 | 0.9 |
| Quinidine 10-0.01 | 0.01 | 1.2 | 1.0 | 1.1 |
| Quinidine 2-0.002 | 0.003 | 0.9 | 2.1 | 2.2 |
| Citalopram | 2.26 | 1.4 | 1.0 | 0.8 |
| Paroxetine | 0.16 | 1.4 | 1.1 | 0.8 |
| Cinacalcet | 0.05 | 0.4 | 1.0 | 0.9 |
| Cinacalcet 5-0.005 | 0.01 | 0.7 | 1.1 | 0.8 |
| Ketoconazole | 7.04 | 0.6 | 1.7 | 0.9 |
| TAO | 81.96 | 1.0 | 1.1 | 1.2 |
| Erythromycin | 89.03 | 1.0 | 1.0 | 1.1 |
| Verapamil | 24.07 | 0.5 | 0.8 | 0.3 |
| Fluconazole | 85.06 | 0.8 | 1.2 | 1.0 |
| Pearson rank | - | 0.98 | 0.92 | 0.94 |
| Uninhibited control rate | 875 | 540 | 480 | 523 |
| Miconazole $IC_{50}$ (µM) | 0.11 | 0.14 | 0.25 | 0.22 |

Key to tables:

$IC_{50}$ ≤ 0.5 fold 1.5% DMSO value

$IC_{50}$ ≥ 1.9 fold 1.5% DMSO value

The uninhibited control rate is expressed as the change in RFU / min. 1.5% DMSO values are the $IC_{50}$ in µM, values for the other solvents are the fold difference compared to the 1.5% DMSO value.

Table 8. Effect of different solvents on the $IC_{50}$ value determined for 27 compounds and Miconazole on the activity of recombinant CYP2C8

| | 1.5% DMSO | 2.5% DMSO | 1.5% MeOH | 2.5% MeOH |
|---|---|---|---|---|
| Furafylline | 100.00 | 1.0 | 1.0 | 1.0 |
| Fluvoxamine | 100.00 | 1.0 | 1.0 | 1.0 |
| Amiodarone | 0.89 | 1.1 | 3.6 | 3.4 |
| Mibefradil | 4.91 | 0.9 | 1.7 | 1.7 |
| Isoniazid | 100.00 | 1.0 | 1.0 | 1.0 |
| Clopidogrel | 44.57 | 1.2 | 1.3 | 1.3 |
| Diltiazem | 100.00 | 1.0 | 1.0 | 1.0 |
| 8-MOPS | 58.25 | 0.5 | 0.2 | 0.1 |
| Ticlopidine | 78.25 | 1.1 | 1.2 | 1.0 |
| Montelukast | 0.23 | 0.4 | 0.5 | 0.3 |
| Montelukast 2-0.002 | 0.15 | 0.7 | 1.0 | 0.9 |
| Gemfibrozil | 100.00 | 1.0 | 1.0 | 1.0 |
| Fluoxetine | 100.00 | 1.0 | 1.0 | 1.0 |
| Troglitazone | 0.91 | 1.1 | 110.5 | 1.4 |
| Sulphafenazole | 68.00 | 1.3 | 1.5 | 1.5 |
| Tienilic acid | 100.00 | 1.0 | 1.0 | 1.0 |
| Tranylcypromine | 2.42 | 0.9 | 3.5 | 2.0 |
| Chloramphenicol | 100.00 | 1.0 | 1.0 | 1.0 |
| N-Benzylphenobarbital | 31.37 | 1.1 | 2.7 | 1.3 |
| Quinidine 100-0.1 | 51.00 | 1.3 | 2.0 | 1.9 |
| Citalopram | 100.00 | 1.0 | 1.0 | 1.0 |
| Paroxetine | 72.50 | 1.0 | 1.4 | 1.4 |
| Cinacalcet | 50.22 | 0.7 | 1.2 | 1.0 |
| Ketoconazole | 35.91 | 0.3 | 0.5 | 0.5 |
| Ketoconazole 10-0.01 | 9.05 | 1.1 | 1.1 | 1.1 |
| TAO (USP) | 100.00 | 1.0 | 1.0 | 1.0 |
| TAO (Biotrend) | 100.00 | 1.0 | 1.0 | 1.0 |
| Erythromycin | 100.00 | 1.0 | 1.0 | 1.0 |
| Verapamil | 31.00 | 0.9 | 2.5 | 2.1 |
| Fluconazole | 100.00 | 1.0 | 1.0 | 1.0 |
| Pearson rank | - | 0.98 | 0.81 | 0.92 |
| Uninhibited control rate | 464 | 764 | 315 | 369 |
| Miconazole $IC_{50}$ (µM) | 1.41 | 1.45 | 3.28 | 2.33 |

## Conclusion

The prototypical inhibitors of each isoform as recommended by the FDA [3] in 2006 were investigated in this study and were found to be some of the most potent inhibitors of the reactions monitored.

Selective concentrations of inhibitors for inclusion in this assay format have been determined for the first time especially against the more recently commercially available isoforms e.g. CYPs 2B6, 2C8 and 3A5, where fewer studies have been performed to date.

In future experiments, we recommend the use of 1.5% (v/v) DMSO as a) being the most reproducible and least volatile solvent, b) having the least inhibitory effect on control rates of metabolite formation and c) being superior to MeOH for its dissolution properties with precipitation being less of a confounding factor.

## Fluorescent $IC_{50}$ Assay Details

| CYP Isoform | Cypex Product code | Substrate | Final [S] (µM) | Detection wavelength (nm) | |
|---|---|---|---|---|---|
| | | | | Excitation | Emission |
| 1A2 | CYP/EZ012 | 7-ER | 1 | 572 | 604 |
| 2B6 | CYP/EZ041 | 7-EFC | 1.5 | 431 | 535 |
| 2C8 | CYP/EZ049 | DEF | 0.5 | 485 | 530 |
| 2C9 | CYP/EZ037 | MFC | 40 | 431 | 535 |
| 2C19 | CYP/EZ008 | CEC | 25 | 410 | 460 |
| 2D6 | CYP/EZ007 | MAMC | 6.4 | 429 | 470 |
| 3A4 | CYP/EZ005 | DEF | 1 | 485 | 530 |
| 3A5 | CYP/EZ048 | DEF | 2.5 | 485 | 530 |

7-ER = 7-ethoxyresorufin (Cypex CYP510) 7-EFC = 7-ethoxy-4-trifluoromethylcoumarin (Invitrogen E2882)
DEF = Diethoxyfluorescein (Cypex CYP531) MFC = 7-methoxy-4-trifluoromethylcoumarin (Cypex CYP517)
CEC = 3-Cyano-7-ethoxycoumarin (Sigma UC455)
MAMC = 7-methoxy-4-aminomethylcoumarin (Cypex CYP515)

## References

1. Chauret N, Gauthier A, Nicoll-Griffith DA. Drug Metab Dispos. 1998, Jan;26 (1):1-4.
2. Hickman D, Wang JP, Wang Y, Unadkat JD. Drug Metab Dispos. 1998, Mar;26 (3):207-15.
3. www.FDA.gov/cder/drug/druginteraction/default.htm