Efffect of solvent choice when performing Fluorimetric IC50 assays with Bactosomes can influence the end result.

Introduction

As part of the validation of our fluorometric 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 acetone (MeCN)). Initially, the assays were performed in the absence of any potential inhibitors and up to 2% (v/v) solvent to assay kinetic parameters, linearly 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 times due to reduced or potentiated rates of reaction and no further studies were conducted with MeCN. Due to known solvent effects at increasing compositions (5), the assays were subsequently performed using both 1.5% and 2.5% (v/v) MeOH or DMSO. 0.15% (v/v) solvent 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 Km or Km + 5% (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 as noted in kinetic experiments). Metabolite production was monitored at excitation and emission wavelengths listed in the table. Ratios of metabolic 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 CYP3A4 only. 27 compounds were investigated to calculate IC50 values against each isoform.

Results

The IC50 results of the 27 compounds (and Miconazole) investigated using DMSO and MeCN at final solvent concentrations of 1.5% (v/v) and 2.5% (v/v) are detailed in Table 1.4

Pearson R Coefficient values 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 comparable or lower than their expected Kᵢ values against respective isoforms, this would have indicated a confounding factor.

Table 1. Effect of different solvents on the IC50 values determined for 27 compounds and Miconazole on the activity of recombinant CYP1A2

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µM) 1.5% MeOH</th>
<th>IC50 (µM) 2.5% MeOH</th>
<th>IC50 (µM) 1.5% DMSO</th>
<th>IC50 (µM) 2.5% DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>2-0.002</td>
<td>0.07</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Cinacalcet</td>
<td>0.01</td>
<td>4.42</td>
<td>1.2</td>
<td>2.3</td>
</tr>
<tr>
<td>Montelukast</td>
<td>0.24</td>
<td>0.90</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>8-MOPS</td>
<td>0.32</td>
<td>1.00</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Ticlopidine</td>
<td>13.80</td>
<td>0.50</td>
<td>0.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Effect of solvent choice when performing Fluorimetric IC50 assays with Bactosomes can influence the end result.

Conclusions

The prototypical inhibitors of each isoform as recommended by the FDA in 2008 were investigated for their inhibition kinetic parameters, linearity with time and protein for the substrates listed in the table. All IC50 values were determined (r>0.94 for 27 compounds). If the results in other solvents were more comparable or lower than their expected Kᵢ values against respective isoforms, this would have indicated a confounding factor. Specific concentrations of inhibitors for inclusion in this assay format have been determined for the first time especially for the more recently commercially available isoforms e.g. CYP 2B6, 2C8 and 3A4, where fewer studies have been performed to date.

In future applications, we recommend the use of DMSO as a solvent for the most potent and least volatile solvents, b) having the least inhibitory effect on control rates of metabolic formation and c) being superior to MeOH for its dissolution properties with precipitation being less of a confounding factor.

References