In vitro ADME & PK

PXR and AhR Nuclear Receptor Activation

Background Information

The primary mechanism of cytochrome P450 induction is via increased gene transcription which typically occurs through nuclear receptor activation.

The most common nuclear receptors involved in the induction of drug metabolising enzymes include the pregnane X receptor (PXR), the aryl hydrocarbon receptor (AhR), and the constitutive androstane receptor (CAR) which are known to regulate CYP3A4, CYP1A2 and CYP2B6, respectively.

An industry survey of current practices and recommendations (Chu et al., 2009 Drug Metab Dispos 37; 1339-1354) indicates 64% of survey respondents routinely use nuclear receptor transactivation assays to assess the potential of test compounds to cause enzyme induction.

Cyprotex can evaluate PXR and AhR nuclear receptor activation utilising stably-transfected human hepatoma cell lines (DPX2™ for PXR and 1A2-DRE™ for AhR) and a luciferase reporter gene assay.

Method
Luciferase reporter gene assay

Cell Lines
DPX2™ for PXR and 1A2-DRE™ cells for AhR (supplied by Puracyp)

Test Article Concentrations
0.1, 0.4, 1, 4, 10, 40, 100µM (different concentrations available)

Quality Controls
Vehicle control
Positive control (rifampicin for PXR and omeprazole for AhR)

Number of Replicates
2 replicates per concentration

Test Article Requirements
50µL of a 100mM DMSO solution

Data Delivery
Fold activation relative to vehicle control EC₅₀ and E_max (if appropriate)
% Cell loss at each concentration and EC₅₀ (if appropriate)

Related Services
Cyprotex also offers cytochrome P450 induction using human hepatocytes to meet with regulatory recommendations.

Protocol

To find out more contact enquiries@cyprotex.com

‘Because reporter assays are relatively high throughput and cost effective, they can be a valuable tool in drug discovery.’

In brief, CAR and PXR regulate distinct but overlapping sets of target genes, which include certain phase I P450 enzymes (e.g., CYP2B, CYP3A, and CYP2C), phase II conjugation enzymes such as UDP glucuronosyltransferase UGT1A1 and sulfotransferase SULT2A, and phase III transporters such as P-glycoprotein (MDR-1). The AhR receptor has been shown to regulate the expression of CYP1A.²

PXR and AhR Nuclear Receptor Activation

Known activators of PXR and AhR were selected and screened in the PXR and AhR nuclear receptor activation assay. Data generated were compared to those published in the literature.

Figure 1
PXR nuclear receptor activation by rifampicin

Figure 2
PXR activation data for a set of 6 compounds and comparison against literature data²

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cyprotex EC₅₀ (µM)</th>
<th>Cyprotex E_max (fold activation)</th>
<th>Literature E_max (fold activation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin</td>
<td>1.1</td>
<td>25.7 at 10µM</td>
<td>30.6 at 10µM</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>24.2</td>
<td>9.9 at 100µM</td>
<td>7.1 at 250µM</td>
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<tr>
<td>Troglitazone</td>
<td>3.7</td>
<td>9.4 at 20µM</td>
<td>8.3 at 50µM</td>
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<tr>
<td>Omeprazole</td>
<td>22.4</td>
<td>12.5 at 100µM</td>
<td>25.9 at 100µM</td>
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<td>Phencobarbital</td>
<td>323</td>
<td>19.3 at 1000µM</td>
<td>13.5 at 1000µM</td>
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<td>Mifepristone</td>
<td>0.8</td>
<td>17.5 at 10µM</td>
<td>9.3 at 10µM</td>
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</tbody>
</table>

The results show that data generated at Cyprotex compare well with data generated in the literature. No cytotoxicity was observed for any of the compounds at the concentration range tested with the exception of troglitazone for which cytotoxicity was observed at the highest concentration of 50µM. This data point was excluded in this instance and not used for calculating the E_max or EC₅₀.

References