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In vitro **ADME & PK**

PXR and AhR Nuclear Receptor Activation

Background Information

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

[1]Chu V, Einolf HJ, Evers R, Kumar G, Moore D, Ripp S, Silva J, Sinha V, Sinz M, and Skerjanec A (2009) *Drug Metab Dispos* **37**; 1339-1354

- 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 (A*h*R), and the constitutive androstane receptor (CAR) which are known to regulate CYP3A4, CYP1A2 and CYP2B6, respectively.
- An industry survey of current practices and recommendations ([1]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 A*h*R nuclear receptor activation utilising stably-transfected human hepatoma cell lines (DPX2™ for PXR and 1A2-DRE™ for A*h*R) and a luciferase reporter gene assay.

Protocol

Method

Luciferase reporter gene assay

Cell Lines

DPX2™ for PXR and 1A2-DRE™ cells for A*h*R (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 A*h*R)

Number of Replicates

2 replicates per concentration

Test Article Requirements

50µL of a 100mM DMSO solution

Data Delivery

Fold activation relative to vehicle control

EC_{50} and E_{max} (if appropriate)

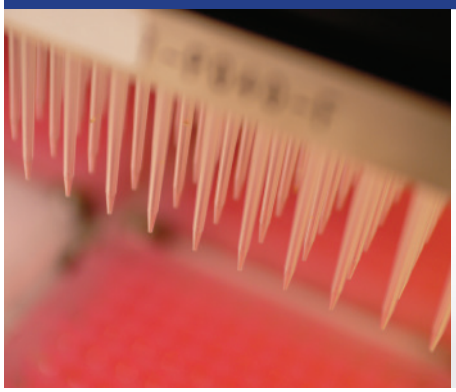
% Cell loss at each concentration and EC_{50} (if appropriate)

Related Services

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

To find out more contact enquiries@cyprotex.com

'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]'

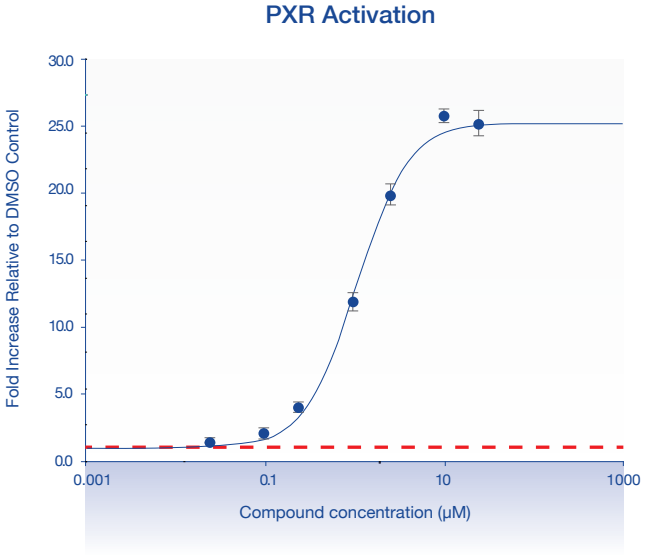


PXR and A*h*R Nuclear Receptor Activation

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

Figure 1

PXR nuclear receptor activation by rifampicin



Data show the mean fold activation relative to the vehicle control. Error bars represent the standard deviation of 3 replicate incubations.

Figure 2

PXR activation data for a set of 6 compounds and comparison against literature data[3]

Compound	Cyprotex EC_{50} (μM)	Cyprotex E_{max} (fold activation)	Literature E_{max} (fold activation)
Rifampicin	1.1	25.7 at 10μM	30.6 at 10μM
Dexamethasone	24.2	9.9 at 100μM	7.1 at 250μM
Troglitazone	3.7	9.4 at 20μM	8.3 at 50μM
Omeprazole	22.4	12.5 at 100μM	25.9 at 100μM
Phenobarbital	323	19.3 at 1000μM	13.5 at 1000μM
Mifepristone	0.8	17.5 at 10μM	9.3 at 10μM

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 EC_{50}.

References

[1] Chu V *et al.*, (2009) *Drug Metab Dispos* **37**; 1339-1354
[2] Youdim KA *et al.*, (2007) *Drug Metab Dispos* **35**; 275–282
[3] Yueh M-F *et al.*, (2005) *Drug Metab Dispos* **33**; 38-48