

In Vitro Evaluation of Cytochrome-P450 Inhibition

Purpose

Cytochromes P450 (CYP) are the principal xenobiotic metabolizing enzymes in the liver. Many drug-drug interactions involve CYP inhibition. By testing potential drug candidates at an early stage on CYP-inhibition, new chemical entities leading to adverse side effects can be sorted out very quickly thus saving time and resources. This preselection can be done in a very efficient way by measuring the inhibitory effects of drug leads on baculovirus/insect cell-expressed human cytochromes P450 and a detection method measuring fluorescent metabolite formation.

Assay protocol

Inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 can be measured (further isozymes are available on request). Test compounds dissolved in acetonitrile, DMSO, or methanol are serially diluted in a 96-well-plate already containing Cofactors and a NADPH-regenerating system. The reaction is started by addition of the corresponding isozym and the CYP-specific substrate. The fluorescent metabolite formation is measured after incubation. Next, the IC₅₀ is calculated by comparing the signal in presence and in absence of the potential inhibitor. Known positive inhibitors are used as a positive control for each isozyme and are included in each assay.

Model validation

The inhibitory potency of Ketoconazol, Verapamil and Carbamazepine, three known CYP3A4 inhibitors, were tested. The deduced IC₅₀ values were 0.09 µM for the most potent inhibitor Ketoconazole, 5.9 µM for Verapamil and 646 µM for the weakest inhibitor in this study, Carbamazepine.

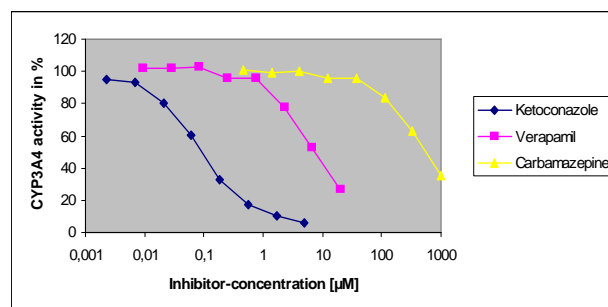


Figure 1: Concentration-response curves of CYP3A4 inhibitors

Table 1: IC₅₀ determination for various control inhibitors

Inhibitor	Enzyme	Average IC ₅₀ in our studies (µM)	Published IC ₅₀ * (µM)
Tranylcypromine	CYP2C19	6.6	5.8
Sulfaphenazole	CYP2C9	1.5	0.23
Quinidine	CYP2D6	0.013	0.009
Ketoconazole	CYP3A4	0.14	0.08

* Anal. Biochem. 1997, 248, 188-190

K_i determination and induction studies

Using this test system, it is also possible to determine a K_i value for an identified inhibitor using multiple substrate concentrations near the apparent K_m of the isozyme and multiple inhibitor concentrations.

As the structure of this assay is not restricted to inhibition studies, induction of Cytochrome P450 isozymes by different substances for a specific CYP can be shown as well.

Please don't hesitate to contact us for a customized quotation

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