Cytochrome P450 Inhibition Screen
Using Human Liver Microsomes

1. Objective:

The inhibition screen is used to assess the potential of a test compound to inhibit cytochrome P450 marker reactions for 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4, in human liver microsomes.

2. Introduction:

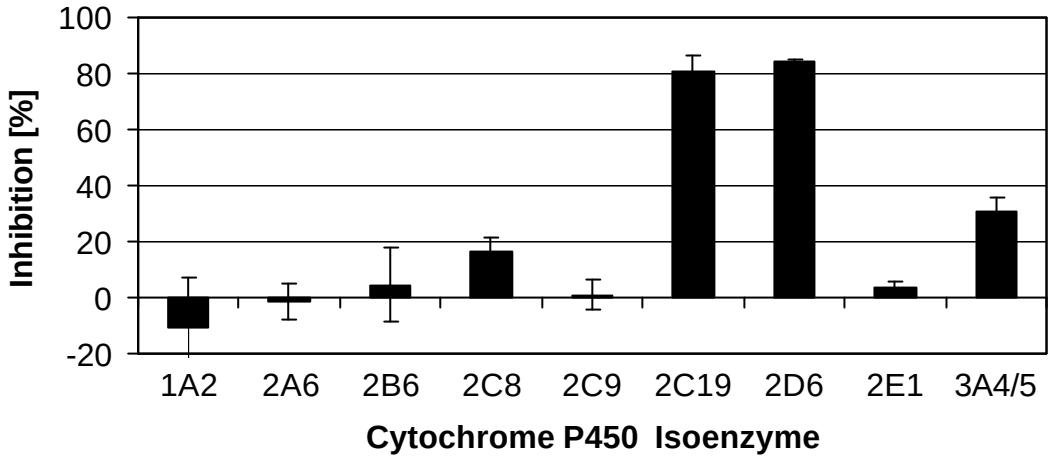
The cytochrome P450s (CYPs) comprise a super family of haem-containing enzymes that play a key role in the metabolism of a variety of chemically diverse compounds including pharmaceutical agents, carcinogens, and environmental pollutants. These enzymes can be inhibited by chemicals and/or antibodies. In drug therapy, CYP inhibition may result in three undesirable consequences:
(1) an increase of pharmacological effects or toxicity caused by decreased drug metabolism,
(2) a decrease of pharmacological effects caused by decreased formation of reactive metabolites,
(3) drug-drug interactions by double medication.
Therefore, CYP inhibition is an important consideration for the development of novel therapeutic agents. Knowledge of the potential for a drug to decrease CYP activity at an early stage of drug discovery and development reduces the risk of failure in the clinic. Liver microsomes are a widely used model system for assessing inhibition of CYP enzymes, because they contain a full complement of CYPs. Incubations with liver microsomes have been shown to retain the most important part of liver function like CYP-mediated metabolism of drugs.

3. Marker Reactions

For the determination of the IC_{50} following marker reactions, marker substrates, reference inhibitors are used:

CYP	Marker Substrate	Marker Reaction	Reference Inhibitor
1A2	7-ethoxyresorufin	7-ethoxyresorufin O-deethylation	furafylline
2A6	coumarin	coumarin 7- hydroxylation	8-methoxypsoralene
2B6	S-mephenytoin	S-mephenytoin N-demethylation	triethylenethiophosphoramide
2C8	paclitaxel	paclitaxel 6a-hydroxylation	ketoconazole
2C9	diclofenac	diclofenac 4'-hydroxylation	sulfaphenazole
2C19	S-mephenytoin	S-mephenytoin 4'-hydroxylation	omeprazole
2D6	bufuralol	bufuralol 1'-hydroxylation	quinidine
2E1	chlorzoxazone	chlorzoxazone 6-hydroxylation	diethyldithiocarbamic acid
3A4	testosterone	testosterone 6ß-hydroxylation	ketoconazole

4. Results



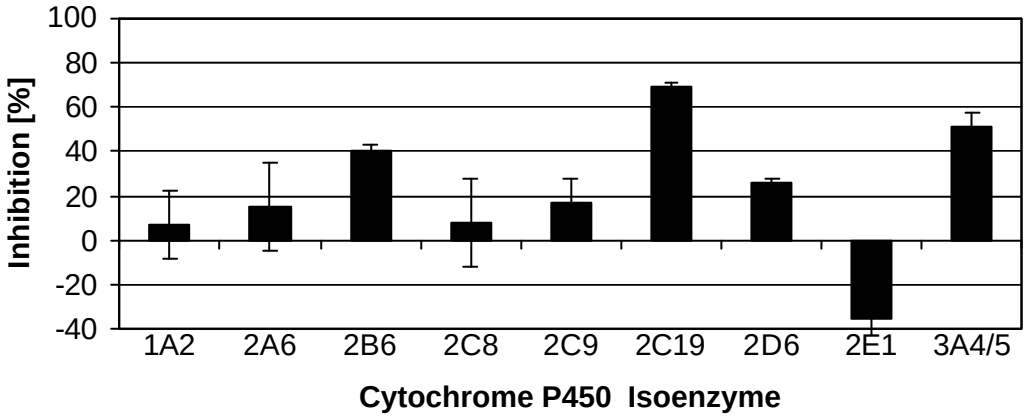


Figure 1 Inhibition Screen Examples