Evaluation of Cytochrome P450 Inhibition in the 7-in-1 Cocktail Using Preferred Substrates Recommended by Regulatory Agencies

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Abstract

An investigational drug's potential to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A should be evaluated as recommended by regulatory agencies such as FDA and PMDA. The selection of the probe substrate for the aforementioned CYPs is the first step for *in vitro* experiments. Numerous combinations of substrates have been characterized in previously reported substrate cocktails *in vitro* screening assays to evaluate the inhibitory potency of the compound of interest. However, not all of the substrates in the 7-in-1 cocktail were the preferred substrates[1]. The objective of this presentation was to design and introduce a 7-in-1 cocktail approach with each of the substrates being the preferred ones recommended by the regulatory agencies.

Method

The 7-in-1 substrate cocktail with each of the substrates being the preferred ones was incubated with human liver microsomes (HLM). The substrates are as follows: Phenacetin (CYP1A2), bupropion (CYP2B6), Amodiaquine (CYP2C8), Diclofenac (CYP2C9), S-mephenytoin (CYP2C19), dextromethorphan (CYP2D6) and midazolam (CYP3A). Inhibitory profiles of known inhibitors for each of the CYPs were studied. The results were compared to those generated in-house using an individual substrate approach.

Results

To optimize the reaction conditions, the probe substrates and initial concentrations were selected based on the guidelines from the regulatory agencies, literature values[2] and Km values generated in-house. Finally, a cocktail of seven probe substrates with its concentration (25 μM phenacetin, 5 μM bupropion, 0.2 μM amodiaquine, 5 μM diclofenac, 15 μM S-mephenytoin, 5 μM dextromethorphan and 0.2 μM midazolam) was selected for a 10-min incubation with 0.2 mg protein/mL of HLM as the optimized condition (Table 1).

Table 1.Probe substrates, enzyme reactions and substrate concentrations were used in the cocktail assay.

CYP isoform	Probe substrate	CYP-specific reaction	Final substrate concentrations (μM)
1A2	Phenacetin	Phenacetin O-deethylation	25
2B6	Bupropion	Bupropion hydroxylation	5
2C8	Amodiaquine	Amodiaquine N-deethylation	0.2
2C9	Diclofenac	Diclofenac 4'-hydroxylation	5
2C19	S-mephenytoin	Mephenytoin 4'-hydroxylation	15
2D6	Dextromethorphan	Dextromethorphan O-demethylation	5
3A	Midazolam	Midazolam 1'-hydroxylation	0.2

To validate this method, seventeen known inhibitors including α-naphthoflavone, ticlopidine, montelukast, sulfaphenazole, (S)-(+)-N-3-Benzylnirvanol, quinidine, and ketoconazole were evaluated. The IC_{50} values generated were compared with those generated in-house using an individual substrate approach. The IC_{50} values obtained from these two approaches are illustrated in Figure 1. The data from the 7-in-1 substrate cocktail approach were correlated well with the data from the single substrate approach with the R^2 value of 0.995.

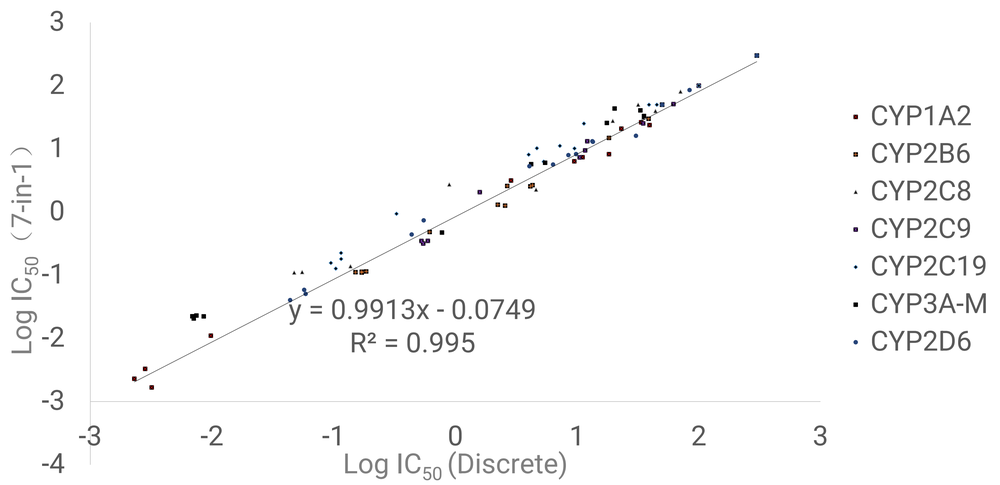

Figure 1. Validation of WuXi AppTec's 7-in-1 CYP inhibition assay against our discrete CYP inhibition assay.

Conclusion

We established a 7-in-1 cocktail approach with each of the preferred substrates for CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A for evaluating CYP inhibition potential of compound of interest in early drug discovery phase.

References

[1] Spaggiari D, et al.. J. Pharm. Biomed. Anal., 2014, 101:221-237.
[2] Chen ZH, et al. Acta Pharmacol Sin. 2016 May;37(5):708-18.

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