Cytochrome P450 2C9 inhibition by sophoranone in human liver microsomes

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Abstract

Sophora tonkinensis, a traditional medicine is used for the treatment of asthma, allergic dermatitis, and throat inflammation in China and Korea. The present study was performed to evaluate the *in vitro* inhibitory potential of sophoranone, one of marker components of *Sophora tonkinensis*, on the activities of nine human cytochrome (CYP) isoforms. Using an LC-MS/MS cocktail assay, the effects of sophoranone on specific marker reactions of the nine CYP isoforms were measured in human liver microsomes. Sophoranone showed potent inhibition of CYP2C9-mediated tolbutamide 4-hydroxylation with an IC_{50} value of 1.21 μM and K_i value of 0.418 μM in a competitive manner; this was similarly potent as a well-known typical CYP2C9 inhibitor, sulfaphenazole (K_i = 0.398 μM). In addition, sophoranone slightly inhibited CYP2C8 and CYP2C19 activities (IC_{50} values of 17.8 μM and 16.4 μM). These observations indicated 13.6- and 12.5-fold decreases in inhibition potency, respectively, compared with that of CYP2C9 by sophoranone. However, no inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2D6, CYP2E1, or CYP3A activities was observed. These observations suggest that sophoranone is a selective and potent inhibitor of CYP2C9 *in vitro*, whereas inhibition of other CYPs is substantially lower. These *in vitro* data support that *Sophora tonkinensis* extract or sophoranone as a single compound may cause herb-drug interactions via inhibition of CYP2C9, and precautions should be taken when *Sophora tonkinensis* extract or sophoranone is co-administered with drugs that are mainly metabolized by CYP2C9.

Introduction

Drug interactions remain an important concern in both drug development and clinical practice. Most of drug-drug interactions are metabolism-based interactions and are mediated primarily via the cytochrome P450(CYP) family of enzymes, especially, nine CYP isoforms (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5) which are expressed in a human liver micoromes.

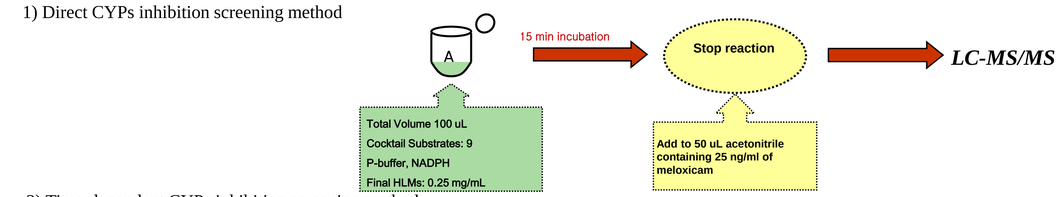
Inhibition of drug-metabolizing enzymes is the most frequent mechanism underlying deleterious drug-drug interactions. Inhibition of CYP isozyme(s) by a xenobiotic may decrease the metabolic clearance of a co-administered drug resulting in the elevation of blood concentrations of the drug. These consequences may result in adverse drug reactions, furthermore, toxicity. Understanding of these underlying metabolic pathways of a drug via CYP isozyme(s) may improve the efficacy of drug and decrease the toxicity of drug.

Recently, we found out that sophoranone could inhibit CYP isozymes mainly CYP2C9 activities and minorly CYP 2C8 and CYP 2C19 activities. We experiment to figure out each IC_{50} and K_i by using HLMs and substrate cocktail model.

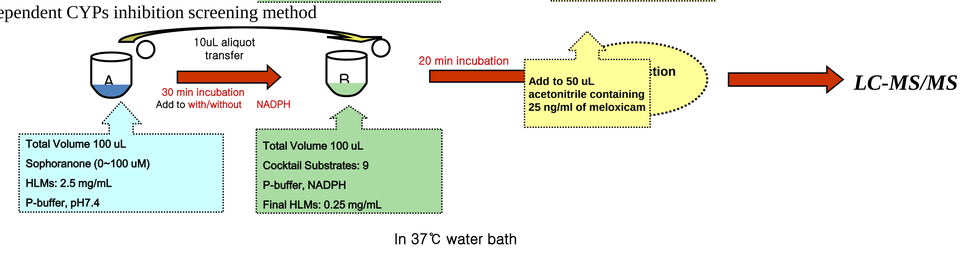
Materials and Methods

We used typical nine CYP substrates (phenacetin for CYP1A2, coumarin for CYP2A6, bupropion for CYP2B6, paclitaxel for CYP2C8, tolbutamide for CYP2C9, *S*-mephenytoin for CYP2C19, dextromethorphan for CYP2D6, chlorzoxazone for CYP2E1 and midazolam for CYP3A4/5) for the evaluation of the direct inhibitory effects using cocktail method and examined the possibility of time-dependent inhibition evaluating for the influence of pre-incubation presence of NADPH with the inhibition. IC_{50} (the inhibitor concentration required to cause 50% inhibition of the enzyme activity) and K_i (apparent inhibitory constant) values were calculated using nonlinear regression analysis with Winnonlin software (Pharsight, CA). Different models of enzyme inhibition (i.e., competitive, noncompetitive, uncompetitive, and mixed-type inhibition) were fitted to the kinetic data. An assessment of goodness of fit of the models was made using the size of the residual sum of squares and the random distribution of the residual sum of squares and the random distribution of the residuals, the standard error, and the 95% confidence interval of the parameter estimates.

1) Direct CYPs inhibition screening method



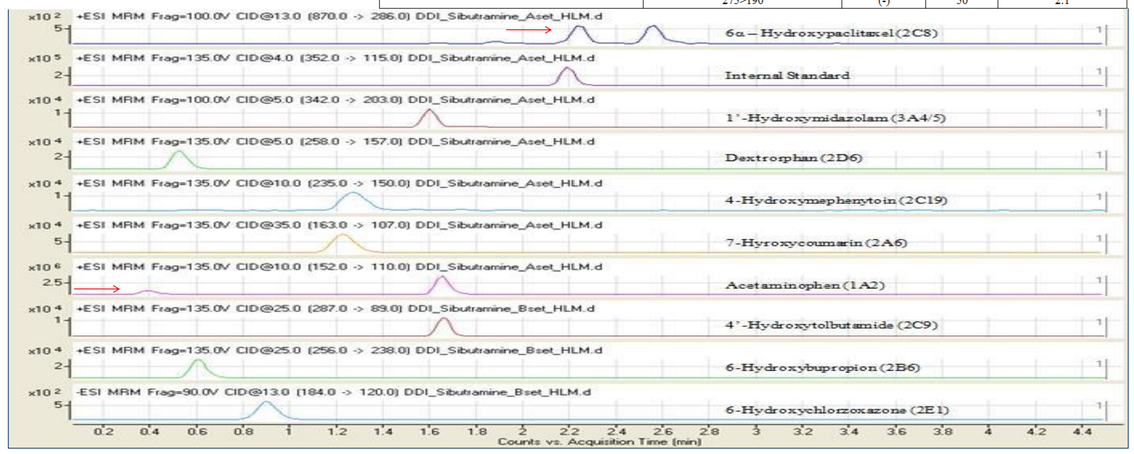
2) Time-dependent CYPs inhibition screening method



a)

CYPs	Substrates	Conc. (uM)	Metabolites	Transition (m/z)	Polarity	CE (eV)	Retention time (min)
1A2	Phenacetin	50	Acetaminophen	152>110.2	(+)	21	0.41
2A6	Coumarin	5	7-Hydroxycoumarin	163>107	(+)	30	1.15
2B6	Bupropion	50	6-Hydroxybupropion	256>238	(+)	19	0.56
2C8	Rosiglitazone	10	p-Hydroxyrosigliaonze	374>151	(+)	33	0.82
2C9	Tolbutamide	100	4-Hydroxytolbutamide	287>89	(+)	42	1.59
2C19	Omeprazole	20	5-Hydroxyomeprazole	362>214	(+)	15	3.09
2D6	Dextromethorphan	5	Dextrorphan	258>157	(+)	60	0.5
2E1	Chlorzoxazone	50	6-Hydroxychlorzoxazone	184>119.9	(-)	25	0.87
3A	Midazolam	5	1'-Hydroxymidazolam	342>203	(+)	25	1.46
IS	Chlorpropamide			277>111	(+)	25	2.1
				275>190	(-)	50	2.1

b)



a) LC-MS/MS anaylsis methods of the substrate cocktail metabolites

b) LC-MS/MS chromatograms from the analysis of a human liver microsomal sample incubated with the substrate cocktails

Results & Discussion

1. Screening of competitive inhibitor of sophoranone using direct CYPs inhibition screening method

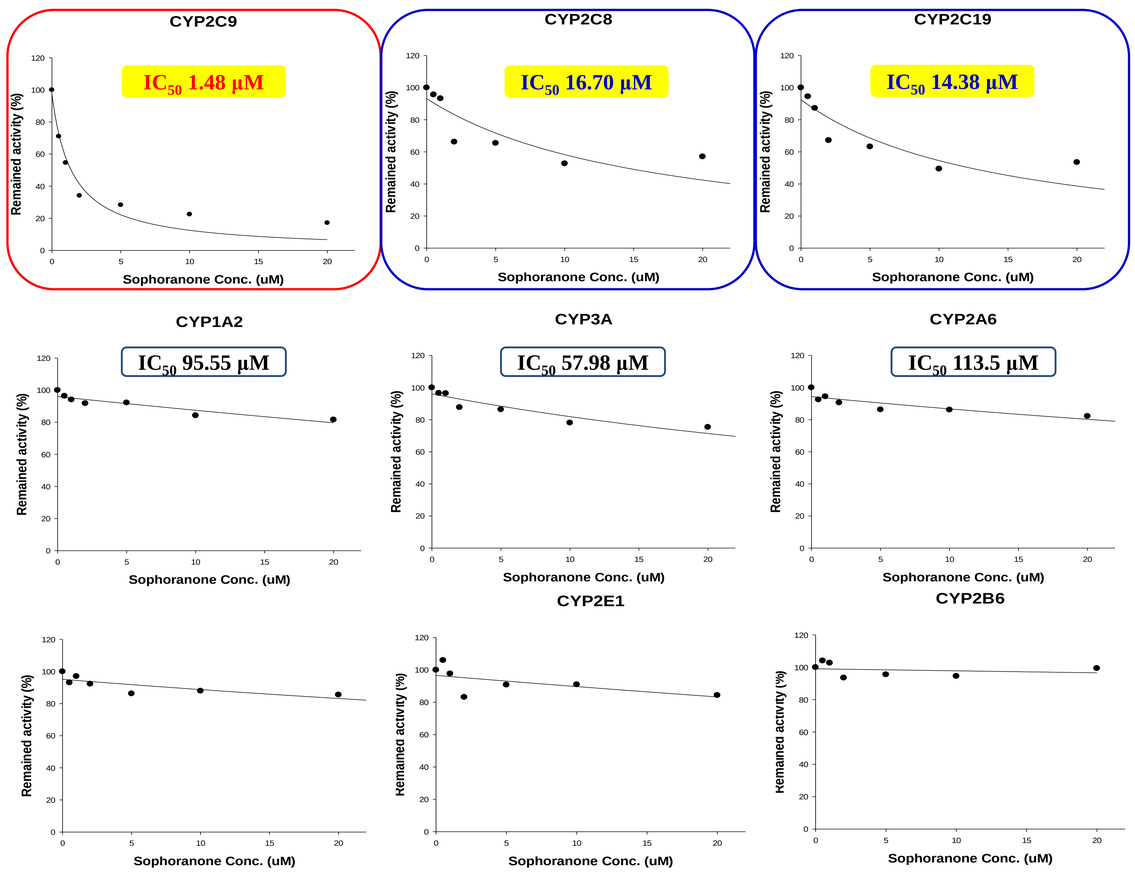


Fig. 1) The results showed that sophoranone inhibited CYP2C9, CYP2C8, CYP2C19 with an IC_{50} of 1.48 μM, 16.70 μM, and 14.38 μM, respectively, while other CYP isoforms negligible influenced. Most of all, sophoranone seems to be a potent inhibitor of CYP2C9.

2. Evaluation of IC_{50} shift of CYP2C9, 2C8 and 2C18 using time-dependent CYPs inhibition screening method

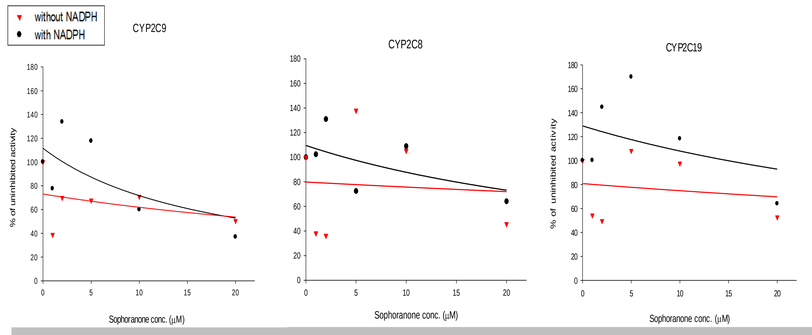


Fig. 2) The results of screening reversible inhibition of sophoranone showed that sophoranone seemed to be a potent inhibitor of CYP2C9. To evaluate whether sophoranone is time-dependent inhibitor of CYP2C9 or not, we checked IC_{50} shift of CYP2C9 detecting the changes of enzymatic activity absence or presence of NADPH when preincubation with sophoranone. IC_{50} shift of CYP2C9 is negligible.

3. K_i determination of sibutramine for CYP2B6, 2C19, and 2D6 using Dixon and Michaelis-menton plot

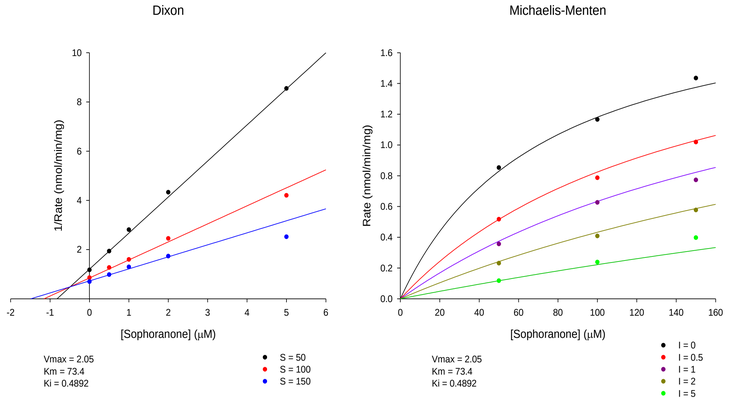
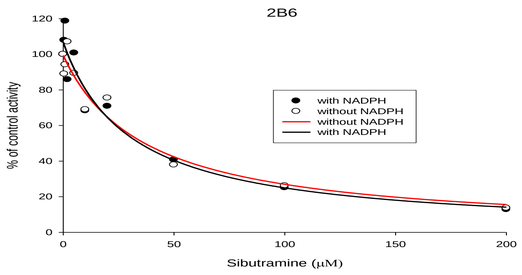


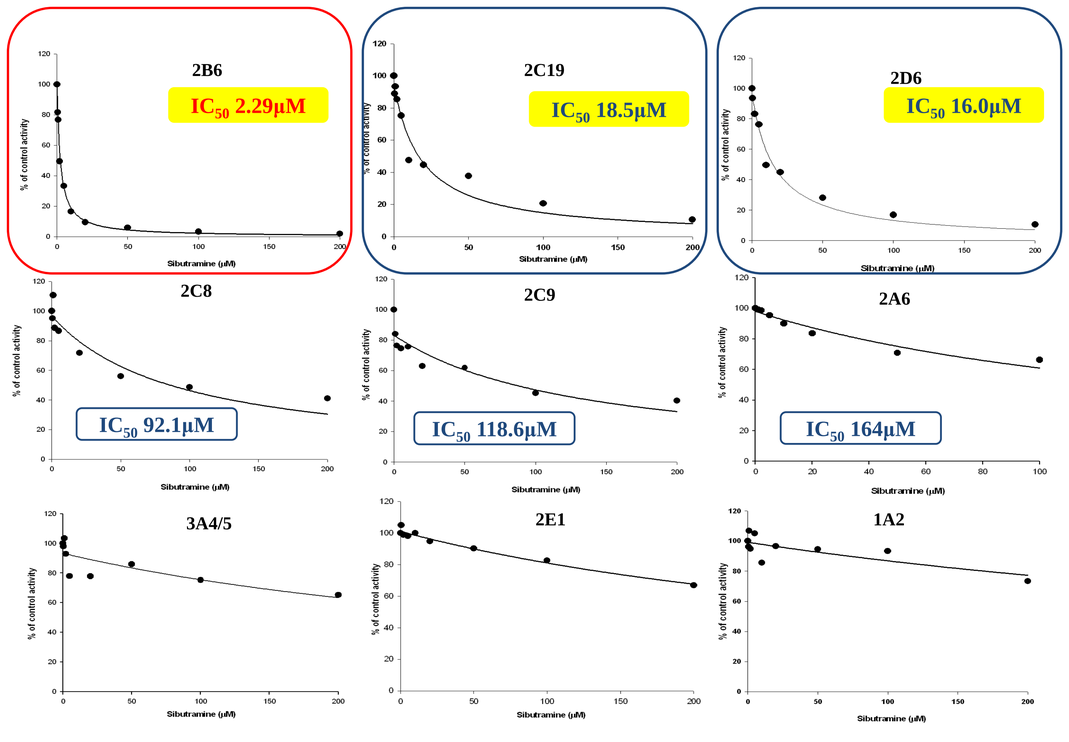
Fig. 3) The results showed that sophoranone inhibited more CYP2C9 when the concentration of sophoranone is raised exhibiting the aspects of competitive inhibitor. And it preseneted that the Vmax is elevated as rising the concentration of substrates

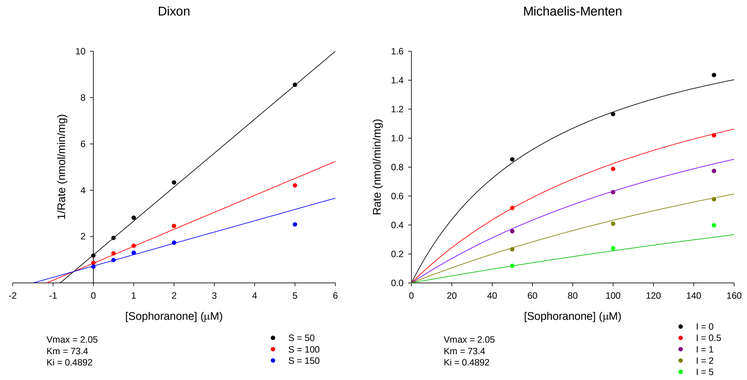
- Competitive inhibitor model
- K_i of sophoranone for CYP2C9: 0.4892 μM
- K_i of sulfaphenazole for CYP2C9: 0.195 μM
  → 0.40-fold
- IC50 of sophoranone for CYP2C9: 1.48 μM
- IC50 of sulfaphenazole for CYP2C9: 0.52 μM
  → 0.35-fold

Conclusion

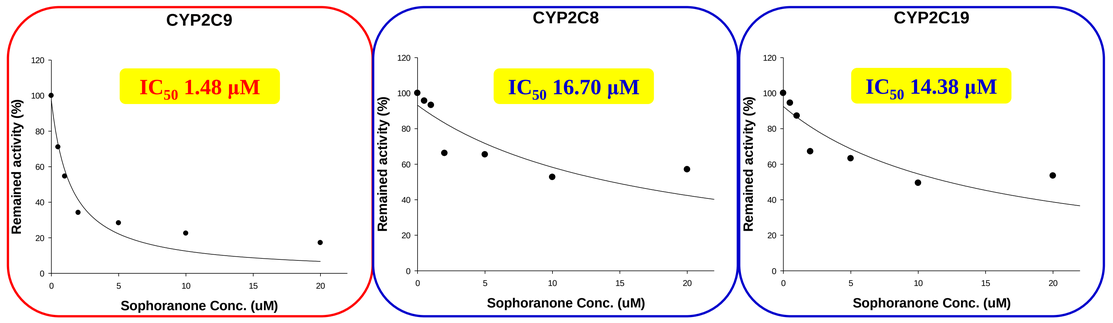
Sophoranone strongly inhibited CYP2C9, in a less extent, CYP2C8 and CYP2C19. In CYP2C9, it didn't show time-dependent CYPs inhibition, but it showed proper Dixon and Michaelis-menton plot. These experiments is conducted using human liver microsomes, and additional experiments with recombinant CYPs and in vivo test are considered necessary for figuring out the efficacy of sophoranone more exactly .

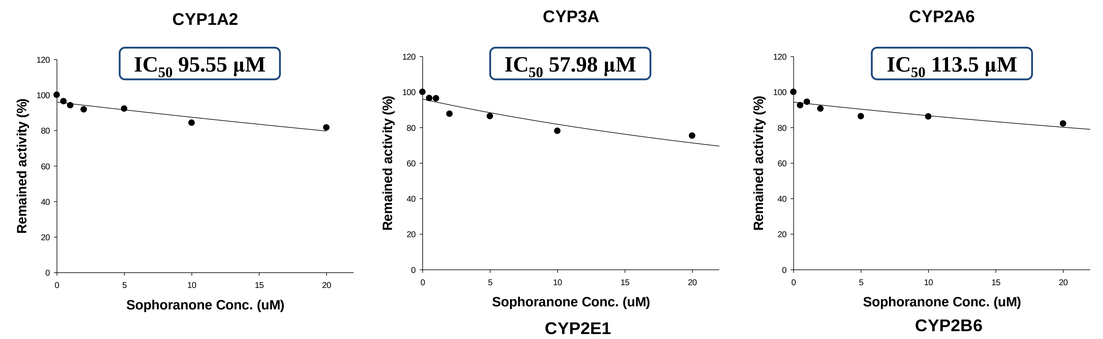

2B6
% of control activity
Sibutramine (μM)
with NADPH
without NADPH
without NADPH
with NADPH

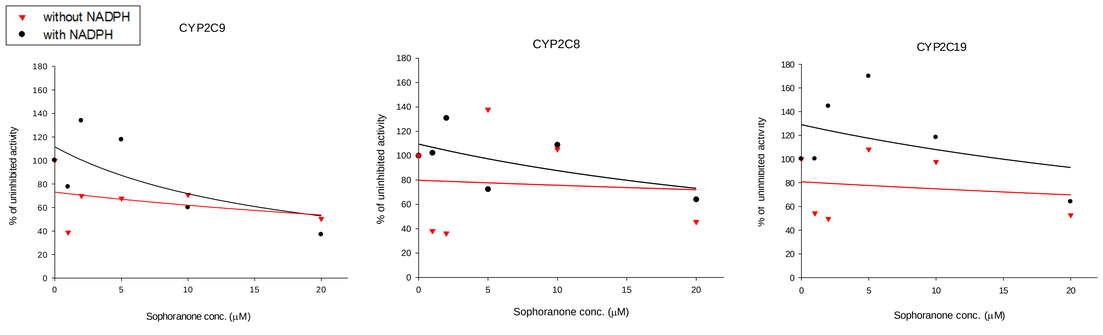

2B6
IC50 2.29μM
2C19
IC50 18.5μM
2D6
IC50 16.0μM
2C8
IC50 92.1μM
2C9
IC50 118.6μM
2A6
IC50 164μM
3A4/5
2E1
1A2
% of control activity
Sibutramine (μM)


Dixon
1/Rate (nmol/min/mg)
[Sophoranone] (μM)
Vmax = 2.05
Km = 73.4
Ki = 0.4892
S = 50
S = 100
S = 150

Michaelis-Menten
Rate (nmol/min/mg)
[Sophoranone] (μM)
Vmax = 2.05
Km = 73.4
Ki = 0.4892
I = 0
I = 0.5
I = 1
I = 2
I = 5


CYP2C9
IC50 1.48 μM
CYP2C8
IC50 16.70 μM
CYP2C19
IC50 14.38 μM
Remained activity (%)
Sophoranone Conc. (uM)


CYP1A2
IC50 95.55 μM
CYP3A
IC50 57.98 μM
CYP2A6
IC50 113.5 μM
CYP2E1
CYP2B6
Remained activity (%)
Sophoranone Conc. (uM)


without NADPH
with NADPH
CYP2C9
CYP2C8
CYP2C19
% of uninhibited activity
Sophoranone conc. (μM)