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Short Communication

Selective Inhibition of Cytochrome P450 2D6 by Sarpogrelate and its Active Metabolite, M-1, in Human Liver Microsomes

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Running title: CYP2D6 inhibition by sarpogrelate and its active metabolite

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Abbreviations:

CYP, cytochrome P450; LC-MS/MS, liquid chromatography-tandem mass spectrometry; IC_{50}, the 50% inhibitory concentration; K_i, inhibition constant; C_{max}, maximum plasma concentration.

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ABSTRACT

The present study was performed to evaluate the *in vitro* inhibitory potential of sarpogrelate and its active metabolite, M-1, on the activities of nine human cytochrome (CYP) isoforms. Using a cocktail assay, the effects of sarpogrelate on nine CYP isoforms and M-1 were measured by specific marker reactions in human liver microsomes. Sarpogrelate potently and selectively inhibited CYP2D6-mediated dextromethorphan *O*-demethylation with an IC_{50} (K_i) value of 3.05 μM (1.24 μM), in a competitive manner. M-1 also markedly inhibited CYP2D6 activity; its inhibitory effect with an IC_{50} (K_i) value of 0.201 μM (0.120 μM) was more potent than that of sarpogrelate, and was similarly potent as quinidine (K_i, 0.129 μM), a well-known typical CYP2D6 inhibitor. In addition, sarpogrelate and M-1 strongly inhibited both CYP2D6-catalyzed bufuralol 1'-hydroxylation and metoprolol α-hydroxylation activities. However, sarpogrelate and M-1 showed no apparent inhibition of the other eight CYPs: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, or CYP3A4/5. Upon 30-min preincubation of human liver microsomes with sarpogrelate or M-1 in the presence of NADPH, no obvious shift in IC_{50} was observed in terms of inhibition of the nine CYP activities, suggesting that sarpogrelate and M-1 are not time-dependent inactivators. Sarpogrelate strongly inhibited the activity of CYP2D6 at clinically relevant concentrations in human liver microsomes. These observations suggest that sarpogrelate could have an effect on the metabolic clearance of drugs possessing CYP2D6-catalyzed metabolism as a major clearance pathway, thereby eliciting pharmacokinetic drug-drug interactions.

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Introduction

Sarpogrelate ((R,S)-1-{2-[2-(3-methoxyphenyl)ethyl]phenoxy}-3-(dimethyl amino)-2-propyl hydrogen succinate hydrochloride; Fig. 1) is a highly specific 5-HT_{2A} receptor antagonist widely used in China, Japan and South Korea to treat peripheral arterial disease (Rashid et al., 2003, Doggrell, 2004). Sarpogrelate has inhibitory effects on serotonin-induced platelet aggregation (Hara et al., 1991a, Nakamura et al., 1999), thrombus formation (Hara et al., 1991b, Yamashita et al., 2000), vasoconstriction and vascular smooth muscle cell proliferation (Sharma et al., 1999), all of which are mediated by 5-HT_{2A} receptors, and consequently reduces the ischemic symptoms associated with peripheral arterial disease. Additionally, sarpogrelate beneficial effects in restenosis after coronary stenting (Fujita at al., 2003; Saini et al., 2004), pulmonary hypertension (Saini et al., 2004), angina pectoris (Kinugawa et al., 2002), and diabetes mellitus (Pietraszek et al., 1993; Ogawa et al., 1999), although the precise mechanisms remain unknown. Sarpogrelate is metabolized to (±)-1-{2-[2-(3-methoxyphenil)ethyl]-phenoxy}-3-(dimethylamino)-2-propanol hydrochloride (M-1; Fig.1), formed by hydrolysis from sarpogrelate (Saini et al., 2004; Nagatomo et al., 2004). The M-1 is an active sarpogrelate metabolite, which has inhibitory effects exceeding those of sarpogrelate *in vitro* (Pertz & Elz, 1995).

General sarpogrelate dosing in patients is one 100-mg tablet taken three times per day after meals (Shinohara et al., 2008). After oral administration of 100-mg sarpogrelate to healthy male subjects, sarpogrelate is rapidly absorbed from the gastrointestinal tract with a mean maximum plasma concentration (C_{max}) of 856.3 ng/mL at 0.7 h and is rapidly eliminated from plasma with a half-life of 0.8 h (Kim et al., 2013a). The active

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metabolite, M-1, reaches a C_{max} of 49.3 ng/mL at 0.9 h and exhibits slower elimination than sarpogrelate, with a half-life of 4.4 h (Kim et al., 2013a). After absorption, sarpogrelate and M-1 further undergo glucuronide conjugations to form several metabolites, which are mainly excreted in bile (Kim et al., 2013b). Despite the wide use and excellent pharmacological properties of sarpogrelate, to date there is no information regarding the potential inhibitory effects of sarpogrelate and M-1 on human P450s isozymes.

In the present study, the inhibitory effects of sarpogrelate and M-1 on the nine CYP isozymes were evaluated using a cocktail assay to assess the potential of sarpogrelate to cause drug-drug interactions with other concomitantly administered drugs. We report herein that especially, M-1 is a selective competitive inhibitor of CYP2D6 *in vitro*.

Materials and Methods

Chemicals and Reagents. Pooled human liver microsomes from a mixed pool of 24 donors (male: 17 and female: 7), *S*-benzylnirvanol, and 1’-hydroxybufuralol were purchased from BD Gentest (Woburn, MA). Sarpogrelate and M-1 were obtained from Kunwha pharmaceutical company (Seoul, Republic of Korea). Acetaminophen, bufuralol, chlorpropamide, chlorzoxazone, coumarin, dextrorphan, diethyldithiocarbamate, furafylline, α-hydroxymetoprolol, ketoconazole, metoprolol, phenacetin, propranolol, quercetin, quinidine, rosiglitazone, *S*-mephenytoin, sulfaphenazole, tolbutamine, 1,1′,1″-phosphinothioylidynetrisaziridine, potassium fluoride (KF), β-nicotinamide adenine dinucleotide phosphate (NADP), glucose 6-phosphate, glucose 6-phosphate dehydrogenase, and $MgCl_2$ were purchased from

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Sigma–Aldrich Corporation (St. Louis, MO). Bupropion, dextromethorphan, 6-hydroxy bupropion, 6-hydroxychlorzoxazone, 7-hydroxycoumarin, 4'-hydroxymephenytoin, 1'-hydroxymidazolam, *p*-hydroxy rosiglitazone, 4-hydroxytolbutamide, and midazolam were obtained from Toronto Research Chemicals (North York, ON, Canada). Solvents were high-performance liquid chromatographic (HPLC) grade (Burdick & Jackson Company, Morristown, NJ) and other chemicals were of the highest quality available.

Screening of Reversible Inhibitory Effects of Sarpogrelate and M-1 on the Activities of Nine Cytochrome P450 Enzymes. So-called cocktail assays in which several enzyme activities are determined in parallel by liquid chromatography-tandem mass spectrometry (LC-MS/MS) are particularly useful. The inhibitory potencies of sarpogrelate and M-1 were determined as described previously with slight modification. (Bae et al., 2013). Phenacetin *O*-deethylase, coumarin 7-hydroxylase, bupropion 6-hydroxylase, rosiglitazone *p*-hydroxylase, tolbutamide 4-hydroxylase, *S*-mephenytoin 4-hydroxylase, dextromethorphan *O*-demethylase, chlorzoxazone 6-hydroxylase, and midazolam 1'-hydroxylase activities were determined as probe activities in human liver microsomes for CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5, respectively. Sarpogrelate or M-1 (concentration: 0−50 μM), and all substrates were dissolved in acetonitrile and serially diluted with acetonitrile to the required concentrations to give a final organic solvent concentration of 1.0% in the incubation mixture. Concentrations of P450-selective substrates were used close to their reported K_m values (Table S1; Kim et al., 2005; Bae et al., 2013).

Briefly, the incubation mixtures containing pooled human liver microsomes (final concentrations: 0.25 mg/ml), each P450-selective substrate, and an NADPH-generating system (1.3 mM $NADP^+$, 3.3 mM glucose 6-phosphate, 3.3 mM $MgCl_2$, and 0.4 unit/ml glucose-6-phosphate dehydrogenase) were preincubated for 5 min at 37°C. The reaction was initiated by adding an aliquot of sarpogrelate or M-1 (concentration range: 0−50 μM) and incubated for 15 min at 37°C in a shaking water bath. When sarpogrelate as an inhibitor was incubated, a 10-μL aliquot of 1 M KF in 0.1 M phosphate buffer (pH 7.4) was added before incubation to inhibit esterase activity (Clarke & Waskell, 2003). After incubation, reactions were stopped by addition of 50 μL of ice-cold acetonitrile containing 2 μM chlorpropamide as an internal standard, and they were chilled and centrifuged (13,000 rpm, 8 min, 4°C). The supernatant was then diluted 100-fold with acetonitrile and then injected into the LC-MS/MS system. All incubations were performed in triplicate, and mean values were used for analysis. Additionally, identical parallel incubation samples containing well-known reversible CYP inhibitors were included as positive controls. Two different microsomal protein concentrations, 0.05 and 0.1 mg/mL, were also used to evaluate the inhibitory potential for CYP2D6 activities.

Additionally, sarpogrelate or M-1 was tested as an inhibitor of bufuralol 1'-hydroxylase (Kronbach et al., 1987; Boobis et al., 1985) and metoprolol α-hydroxylase (Otton et al., 1988), other CYP2D6-speccific biotransformation pathways. Concentrations of bufuralol (5 μM) and metoprolol (20 μM) were used in this study. Other procedures were similar to those used in the cocktail assays.

Determination of the K_i of Sarpogrelate and M-1 for CYP2D6. Based on the IC_{50} values, the K_i values of sarpogrelate and M-1 for CYP2D6 were determined. Briefly, dextromethorphan, a specific substrate for CYP2D6, was incubated with sarpogrelate, M-1 or quinidine, a well-known typical CYP2D6 inhibitor. For determination of K_i values, dextromethorphan concentrations used were 2.5, 5, and 10 μM. The concentrations of quinidine, sarpogrelate, and M-1 were as follows; 0−1 μM for

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quinidine, and 0−10 μM for sarpogrelate and M-1. All incubations were performed in triplicate, and mean values were used for the analysis. Other procedures were similar to those of the reversible inhibition studies.

Time-Dependent Inhibitory Effects of Sarpogrelate and M-1 on the Activities of Nine Cytochrome P450 Enzymes. The IC_{50} shift assay is one of most efficient and convenient methods of evaluating the time-dependent inhibitory effects of sarpogrelate and M-1. Changes in enzymatic activity are usually detected with and without preincubation of the test compound for a defined period. A shift in IC_{50} to a lower value ("shift") following preincubation indicates time-dependent inactivation (Obach et al., 2006a).

Pooled human liver microsomes (1 mg/mL) were incubated with sarpogrelate or M-1 (0−50 μM) in the absence or presence of an NADPH-generating system for 30 min at 37°C (*i.e.*, the "inactivation incubation"). After inactivation incubation, aliquots (10 μL) were transferred to fresh incubation tubes (final volume 100 μL) containing an NADPH-generating system and each P450-selective substrate cocktail set. When sarpogrelate was studied, a 10-μL aliquot of 1 M KF was added into both inactivation and incubation mixtures. The reaction system (100-μL total volume) was incubated for 15 min at 37°C in a shaking water bath. After incubation, reactions were stopped by addition of 50-μL ice-cold acetonitrile containing 2 μM chlorpropamide, as an internal standard, and they were chilled and centrifuged (13,000 rpm, 8 min, 4°C). The supernatant was then diluted 10-fold with acetonitrile and injected into the LC-MS/MS system.

Determination of the Unbound Fraction of Sarpogrelate or M-1 in Human Liver Microsomes and Human Plasma. Equilibrium dialysis was conducted to assess

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the unbound fraction of sarpogrelate or M-1 in human liver microsomes and human plasma using a single-use plate rapid equilibrium dialysis device with dialysis membranes with a molecular weight cut-off of ~8,000 Da (Thermo Scientific, Rockford, IL) (Bae et al., 2013). Human liver microsome samples containing sarpogrelate or M-1 at concentrations of 0.5 and 10 μM, respectively (100 μl), were dialyzed against 50 mM phosphate buffer (300 μl) at pH 7.4. The loaded dialysis plate was covered with sealing tape, placed on an orbital shaker at approximately 500 rpm and incubated at 37°C for 4 h. All incubations were performed in triplicate, and mean values were used for the analysis. Nonspecific binding in microsome/buffer mixed matrix was evaluated for sarpogrelate or M-1 concentrations using the LC-MS/MS method. In plasma protein binding studies, the final concentrations of sarpogrelate or M-1 were both 0.5 and 10 μM. The LC-MS/MS conditions for determination of sarpogrelate and M-1 were optimized based on the conditions used in a previous study (Kim et al., 2013a).

LC-MS/MS Analysis. Metabolites of nine P450-selective substrates were analyzed using a tandem quadrupole mass spectrometer (QTrap 5500 LC-MS/MS; Applied Biosystems, Foster City, CA) equipped with an electrospray ionization interface, as reported previously (Bae et al., 2013). Single reaction monitoring mode using specific precursor/product ion transition was used for quantification. The mass transitions of the metabolites of the nine P450-selective substrates and collision energies are listed in the supplemental data (Supplemental Table 1). Peak areas for all of the analytes were integrated automatically using the Analyst software (version 1.5.2; Applied Biosystems, Foster City, CA).

The mass transitions used for quantification of 1’-hydroxybufuralol or α-hydroxymetoprolol were optimized based on the conditions used in a previous study

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(VandenBrink et al., 2012).

Data Analysis. For reversible inhibition and time-dependent inhibition screening, the P450-mediated activities in the presence of the inhibitor, sarpogrelate or M-1, were expressed as percentages of the corresponding control values at 0 μM of sarpogrelate or M-1. From plots of percent inhibition *versus* inhibitor concentrations, corresponding IC_{50} values were calculated by nonlinear regression using the WinNonlin software (version 4.0 Pharsight, Mountain View, CA). The apparent kinetic parameters for inhibitory potential (K_i values) were estimated from the fitted curves using the WinNonlin software. The inhibition data were fit to different models of enzyme inhibition (competitive, non-competitive, uncompetitive or mixed) by nonlinear least-squares regression analysis (WinNonlin software). The most appropriate inhibition model selected based upon the goodness of fit criteria of a visual inspection of the data, correlation of determination (R^2) and corrected Akaike's Information Criterion. For visual inspection, data are presented as Dixon plots and Lineweaver–Burk plots.

Results

The inhibitory effects of sarpogrelate and M-1 on the activities of nine CYP isozymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5) at microsomal protein concentrations of 0.25 mg/mL are shown in Figs. 2 and 3, respectively. The IC_{50} values of sarpogrelate and M-1 at a microsomal protein concentration of 0.25 mg/mL are listed in Table 1. The IC_{50} values for the positive controls used in the reversible inhibition studies were in good agreement with published values to an acceptable degree of accuracy (data not shown). Of the nine P450 isoforms

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tested, CYP2D6-catalyzed dextromethorphan hydroxylation was most strongly inhibited by sarpogrelate and M-1, with IC_{50} values of 3.05 and 0.201 μM, respectively (Table 1). Similar inhibitory potencies of sarpogrelate and M-1 at the different microsomal protein concentrations (0.05 and 0.1 mg/mL) were observed (data not shown), indicating that nonspecific microsomal binding of sarpogrelate and M-1 did not affect the inhibitory potencies. However, sarpogrelate and M-1 showed no apparent inhibition of the other eight CYPs tested (Table 1, Figs. 2 and 3); the remaining activities at the tested highest concentration (50 μM) were greater than 90%.

To determine whether the inhibitory activities of sarpogrelate and M-1 were substrate specific, we examined the inhibitory effects on other CYP2D6-specific biotransformation pathways (*i.e.*, bufuralol 1'-hydroxylation and metoprolol α-hydroxylation) and found that sarpogrelate also markedly inhibited their activities, with IC_{50} values of 4.02 and 3.37 μM, respectively (data not shown). M-1 also potently inhibited CYP2D6 activity; corresponding IC_{50} values were 0.360 and 0.545 μM, respectively (data not shown).

To characterize the type of reversible inhibition of CYP2D6 by sarpogrelate or M-1 based on the IC_{50} values, enzyme kinetic assays were conducted with varying concentrations of sarpogrelate or M-1 and dextromethorphan. Identical parallel incubation samples containing a known potent inhibitor of CYP2D6, quinidine, were included. The K_i values of sarpogrelate, M-1 and quinidine are listed in Table 2. Representative Dixon plots for the inhibition of CYP2D6 by sarpogrelate, M-1 and quinidine in human liver microsomes are shown in Fig. 4. Sarpogrelate Sarpogrelate and M-1 strongly and selectively inhibited CYP2D6 with K_i values of 1.24 μM and 0.120 μM, respectively. Specifically, inhibition of CYP2D6 by M-1 was more potent

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than that of sarpogrelate, and was similarly potent as quinidine (K_i, 0.129 μM) (Table 3). Visual inspection of the Dixon plots and further analysis of the enzyme inhibition modes suggested that the inhibition data of sarpogrelate, M-1 and quinidine all fit well to a competitive inhibition type.

A shift in the inhibition curve to a lower IC_{50} value by 30-min preincubation in the presence of NADPH is an indicator of time-dependent inhibition. After 30-min preincubation of sarpogrelate or M-1 with human liver microsomes in the presence of NADPH, no obvious shift in IC_{50} was observed for inhibition of the nine CYPs (data not shown). Representative IC_{50} shift plots for CYP2D6 activity by sarpogrelate or M-1 are shown in supplementary Fig. S1 (Supplemental Figure 1). These suggest that sarpogrelate and M-1 are not time-dependent inhibitors.

The free fractions of sarpogrelate at concentrations of 0.5 and 10 μM in human plasma were 96.8 ± 4.29% and 95.1 ± 3.12%, respectively (n = 3, each). However, incubation in human liver microsomes for 4 h prohibited the measurement of microsomal binding for sarpogrelate due to its instability in microsomes. When M-1 was added at concentrations of 0.5 and 10 μM to human liver microsomes (human plasma), the free fractions of M-1 were 72.0 ± 6.12% (97.3 ± 4.28%) and 68.1 ± 5.18% (97.8 ± 3.09%), respectively (n = 3 each). The free fractions of sarpogrelate and M-1 were not affected by the concentrations added.

Discussion

To our knowledge, there are no reports of *in vitro* drug interactions of sarpogrelate via CYP isozymes. In this study we demonstrated that sarpogrelate is a potent and selective

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competitive inhibitor of CYP2D6 *in vitro*. Additionally, M-1, an active metabolite of sarpogrelate, significantly inhibited CYP2D6 activities; its inhibitory effects with an IC_{50} (K_i) value of 0.201 μM (0.120 μM) was more potent than those of sarpogrelate, with an IC_{50} (K_i) value of 3.05 μM (1.24 μM). Sarpogrelate and M-1 strongly inhibited other CYP2D6-catalyzed bufuralol 1'-hydroxylation and metoprolol α-hydroxylation activities. However, sarpogrelate and M-1 showed no apparent inhibition of the other eight CYPs: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP2E1. Preincubation of sarpogrelate or M-1 with human liver microsomes and an NADPH-generating system did not alter the inhibition potencies against the nine CYPs, suggesting that sarpogrelate or M-1 are not time-dependent inactivators.

Generally, alterations in the activities of hepatic CYPs, inhibition or induction, *in vivo* represent the major mechanisms underlying pharmacokinetic drug-drug interactions (Clarke & Jones, 2002; Leucuta & Vlase, 2006). While it accounts for only 2–5% of all hepatic CYP isozymes, CYP2D6 metabolizes approximately 25% of all clinically used medications, such as some cytotoxins, tamoxifen, and many agents used to treat associated complications such as antiarrhythmics, antiemetics, antidepressants, antipsychotics, and analgesics (Wolf & Smith, 1999; Ingelman-Sundberg & Evans, 2001). In addition, the CYP2D6 gene is highly polymorphic with more than 112 variants described to date (http://www.imm.ki.se/CYPalleles/cyp2d6.htm) and that such variations in CYP2D6 expression is thought to increase the potential for drug-drug interactions (Bernard et al., 2006).

The *in vitro* inhibition potency alone does not dictate the likelihood of pharmacokinetic drug interactions because the *in vivo* concentration of the inhibitor should also be considered. For reversible inhibitors, the magnitude of the increase in

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exposure is related to the inhibitory potency (K_i), the concentration of inhibitor ($[I]_{in\ vivo}$), and the fraction of the affected drug that that ordinarily goes through the inhibited enzyme (f_m) (Yao & Levy, 2002; Ito et al., 2004; Obach et al., 2006b). As stated in the Introduction, the C_{max} of sarpogrelate was 856.3 ng/mL (1.99 μM) following a single 100-mg sarpogrelate oral dose in healthy subjects. In addition, our clinical trial data indicate that the C_{max} values of sarpogrelate and M-1 in steady state are 657 ± 302 ng/ml (1.56 μM) at 0.9 h with a half-life of 0.64 h and 53.0 ± 16.1 ng/ml (0.161 μM) at 1.08 h with a half-life of 4.98 h, respectively (our unpublished data).

In human *in vivo* interaction studies, the degree of interaction is usually expressed as the ratio of the area under the plasma concentration–time curve (AUC) in the presence (AUC_i) and absence of an inhibitor. When the *in vivo* inhibition potency of sarpogrelate against completely CYP2D6-cleared drug (f_m = 1) is determined from the plasma concentration of sarpogrelate described above, the K_i values of sarpogrelate (1.24 μM) for CYP2D6, and the unbound fractions in both human liver microsomes and plasma by the methods of Obach et al. (2006b), the AUC_i to AUC ratio is estimated to be 1.17–11.5 (Table 3). These estimates of the magnitude of drug-drug interactions for a CYP2D6-cleared drug range from 1.17 to 11.5, largely due to whether the unbound or total sarpogrelate concentrations are most relevant to enzyme inhibition *in vivo*. However, all the AUC_i to AUC ratios had > 1.1 when either total or unbound concentrations were used for the calculation of ratios. Thus, we cannot exclude the possibility that the *in vivo* inhibitory potency of CYPD6 by sarpogrelate.

There are some limitations to our calculations. First, the effects of M-1 were not considered although its inhibitory effect was more potent than that of sarpogrelate. Second, the free fraction of sarpogrelate in human liver microsomes is assumed to be

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same as that of M-1. Instability of sarpogrelate in microsomes for 4 h incubation did not allow for an accurate measurement. Finally, from a clinical drug-drug interactions point of view, a meaningful inhibitory effect of sarpogrelate might not be observed despite high inhibitory potencies of sarpogrlate (K_i 1.24 μM) and M-1 (K_i 0.120 μM) for CYP2D6 due to their considerable short half-lives (sarpogrlate, 0.64 h and M-1, 4.98 h) and T_{max} (sarpogrlate, 0.9 h and M-1, 1.08 h). This may explain previously observed interactions between paroxetin as a potent CYP2D6 inhibitor and clozapine as a CYP2D6 substrate (Hiemke & Härrter, 2000). Applying dosages above 20 mg paroxetine per day produced a substantial increase in clozapine plasma levels (Centorrino et al., 1996), while a fixed dose of 20 mg/day could not observe increased plasma levels of clozapine (Wetzel et al., 1998). The magnitude of CYP2D6 inhibition correlates with its plasma concentrations (Hiemke & Härrter, 2000). Therefore, further investigations are required to clarify the *in vivo* interactions of CYP2D6-targeted drugs with sarpogrelate.

In conclusion, these observations suggest that sarpogrelate and M-1 are potent and selective competitive inhibitors of CYP2D6 *in vitro*. Especially, inhibition of CYP2D6 by M-1 was 10-fold more potent than that of sarpogrelate, and was similarly potent as quinidine, a well-known typical CYP2D6 inhibitor. The data support the use of M-1 as a well-known inhibitor of CYP2D6 instead of quinidine for routine screening of P450 reversible inhibition when human liver microsomes are used as the enzyme source. Finally, it would be expected that sarpogrelate could have an effect on the metabolic clearance of drugs possessing CYP2D6-catalyzed metabolism as a major clearance pathway, thereby eliciting pharmacokinetic drug-drug interactions.

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Authorship Contributions

Participated in research design: Cho, Y.W. Kim, B.-T. Kim, S.H. Bae, and S.K. Bae

Conducted experiments: S.H. Bae and Lee

Contributed analytic tools: S.H. Bae and Lee

Performed data analysis: Cho, S.H. Bae, Y.W. Kim, B.-T. Kim, and S.K. Bae

Wrote or contributed to the writing of the manuscript: Cho, S.H. Bae, Y.W. Kim, B.-T. Kim, and S.K. Bae

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Footnotes

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Doo-Yeoun Cho and Soo Hyeon Bae contributed equally to this work.

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Legends for Figures

Fig. 1. Chemical structures of sarpogrelate (A) and its active metabolite, M-1, (B)

Fig. 2. IC_{50} curves of sarpogrelate for human P450 activities using the cocktail substrate including CYP1A2 for phenacetin *O*-deethylase (A), CYP2A6 for coumarin 7-hydroxylase (B), CYP2B6 for bupropion hydroxylase (C), CYP2C8 for rosiglitazone *p*-hydroxylase (D), CYP2C9 for tolbutamide 4-hydroxylase (E), CYP2C19 for *S*-mephenytoin 4-hydroxylase (F), CYP2D6 for dextromethorphan *O*-demethylase (G), CYP2E1 for chlorzoxazone 6-hydroxylase (H), and CYP3A4/5 for midazolam 1′-hydroxylase (I). Data are the mean ± SD of triplicate determinations. The dashed lines represent the best fit to the data using non-linear regression.

Fig. 3. IC_{50} curves of M-1 for human P450 activities using the cocktail substrate including CYP1A2 for phenacetin *O*-deethylase (A), CYP2A6 for coumarin 7-hydroxylase (B), CYP2B6 for bupropion hydroxylase (C), CYP2C8 for rosiglitazone *p*-hydroxylase (D), CYP2C9 for tolbutamide 4-hydroxylase (E), CYP2C19 for *S*-mephenytoin 4-hydroxylase (F), CYP2D6 for dextromethorphan *O*-demethylase (G), CYP2E1 for chlorzoxazone 6-hydroxylase (H), and CYP3A4/5 for midazolam 1′-hydroxylase (I). Data are the mean ± SD of triplicate determinations. The dashed lines represent the best fit to the data using non-linear regression.

Fig. 4. Dixon plots to determine K_i values for CYP2D6 of sarpogrelate (A), M-1 (B) and quinidine (C). The concentrations of dextromethorphan were determined 2.5 (●), 5 (○), and 10 (▾) μM, respectively. *V* represents formation rate of dextrophan (pmol/min/mg protein). Data are the mean values of triplicate determinations. The solid lines of sarpogrelate, M-1 and quinidine fit well to all competitive inhibition types.

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Table 1. Values of IC_{50} (μM) of sarpogrelate and M-1 for each CYP isozymes in human liver microsomes

CYPs	Reactions	IC_{50} (μM)	
		Sarpogrelate	M-1
CYP1A2	Phenacetin *O*-deethylation	> 50	> 50
CYP2A6	Coumarin 7-hydroxylation	> 50	> 50
CYP2B6	Bupropion hydroxylation	> 50	> 50
CYP2C8	Paclitaxel 6α-hydroxylation	> 50	> 50
CYP2C9	Tolbutamide 4-hydroxylation	> 50	> 50
CYP2C19	*S*-Mephenytoin 4'-hydroxylation	> 50	> 50
CYP2D6	Dextromethorphan *O*-demethylation	3.05	0.201
CYP2E1	Chlorzoxazone 6-hydroxylation	> 50	> 50
CYP3A4/5	Midazolam 1'-hydroxylation	> 50	> 50

The assay conditions are described in *Methods*.
Data are expressed as the mean of triplicate determinations.

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Table 2. K_i values of the inhibition for CYP2D6 by sarpogrelate, M-1, and quinidine at microsomal protein concentrations of 0.25 mg/mL

CYPs	K_i (μM)		
	Sarpogrelate	M-1	Quinidine
CYP2D6	1.24[a]	0.120[a]	0.129[a]

Concentrations of sarpogrelate, M-1, and quinidine were as following; 0–10 μM for sarpogrelate and M-1, and 0–1 μM for quinidine, respectively.

[a] Inhibition type was determined by the best fit to competitive mode based on AICs.

Table 3. Prediction of maximum interactions with CYP2D6-cleared drugs caused by sarpogrelate

$[I]_{in\ vivo}$	Equation used to estimate the $[I]_{in\ vivo}$	Fold increase in exposure (AUC_i/AUC) $1 + ([I]_{in\ vivo} / K_i)$
Systemic total C_{max}	$[I]_{in\ vivo} = C_{max}$ at steady state	5.19
Systemic free C_{max}	$[I]_{in\ vivo} = fu \cdot C_{max}$ at steady state	1.17
Estimated total portal C_{max}	$[I]_{in\ vivo} = C_{max} + k_a \cdot F_a \cdot D/Q_h$	11.5
Estimated total free C_{max}	$[I]_{in\ vivo} = fu \cdot (C_{max} + k_a \cdot F_a \cdot D/Q_h)$	1.42

[I], concentrations of sum of inhibitors; AUC_i, the area under the plasma concentration–time curve in the presence of an inhibitor; C_{max}, maximum plasma concentration, fu, unbound fraction of sarpogrelate in plasma; k_a, absorption rate constant; F_a; fraction absorbed from the gastrointestinal tract; D, dose of inhibitor; Q_h, human hepatic blood flow rate.

Values used for sarpogrelate include: D = 100 mg; k_a = 0.0167/min; fu = 0.04, C_{max} = 1.56 μM (our unpublished data); Q_h = 1470 ml/min (Obach et al., 2006b); adjusted K_i (as free fraction in microsomes) = 0.372 μM. The free fraction of sarpogrelate in microsomes is assumed to be same as that of M-1. The fraction of the affected drug cleared by CYP2D6 and F_a are assumed to be unity. The estimate for k_a was calculated from the expression $T_{max} = (\ln(k_a/k_e))/(k_a-k_e)$, where k_e is the elimination rate constant. The value of k_e was calculated from half-life, 0.64 h, and T_{max} value was 0.9 h (our unpublished data).

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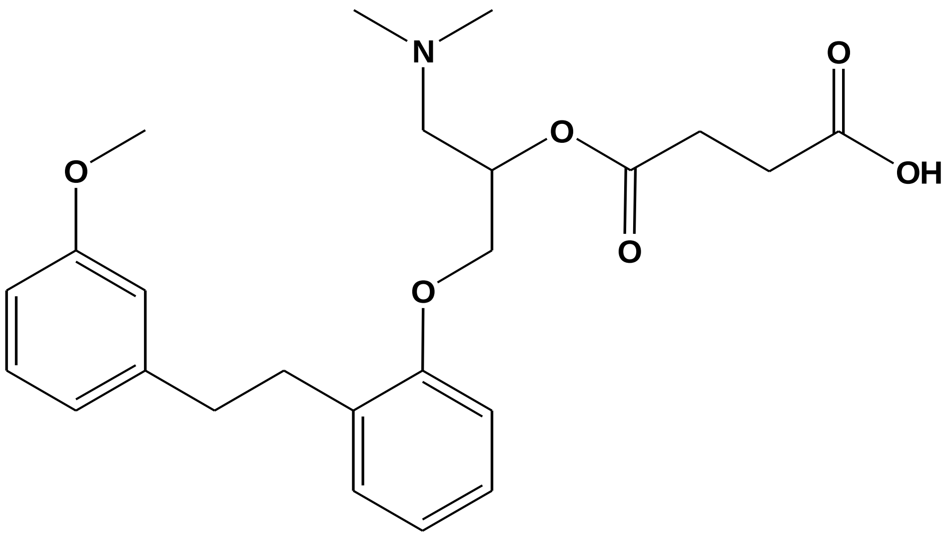
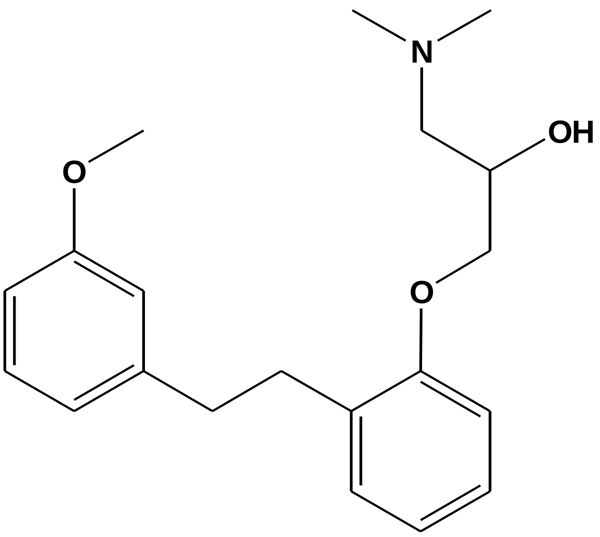
Fig. 1

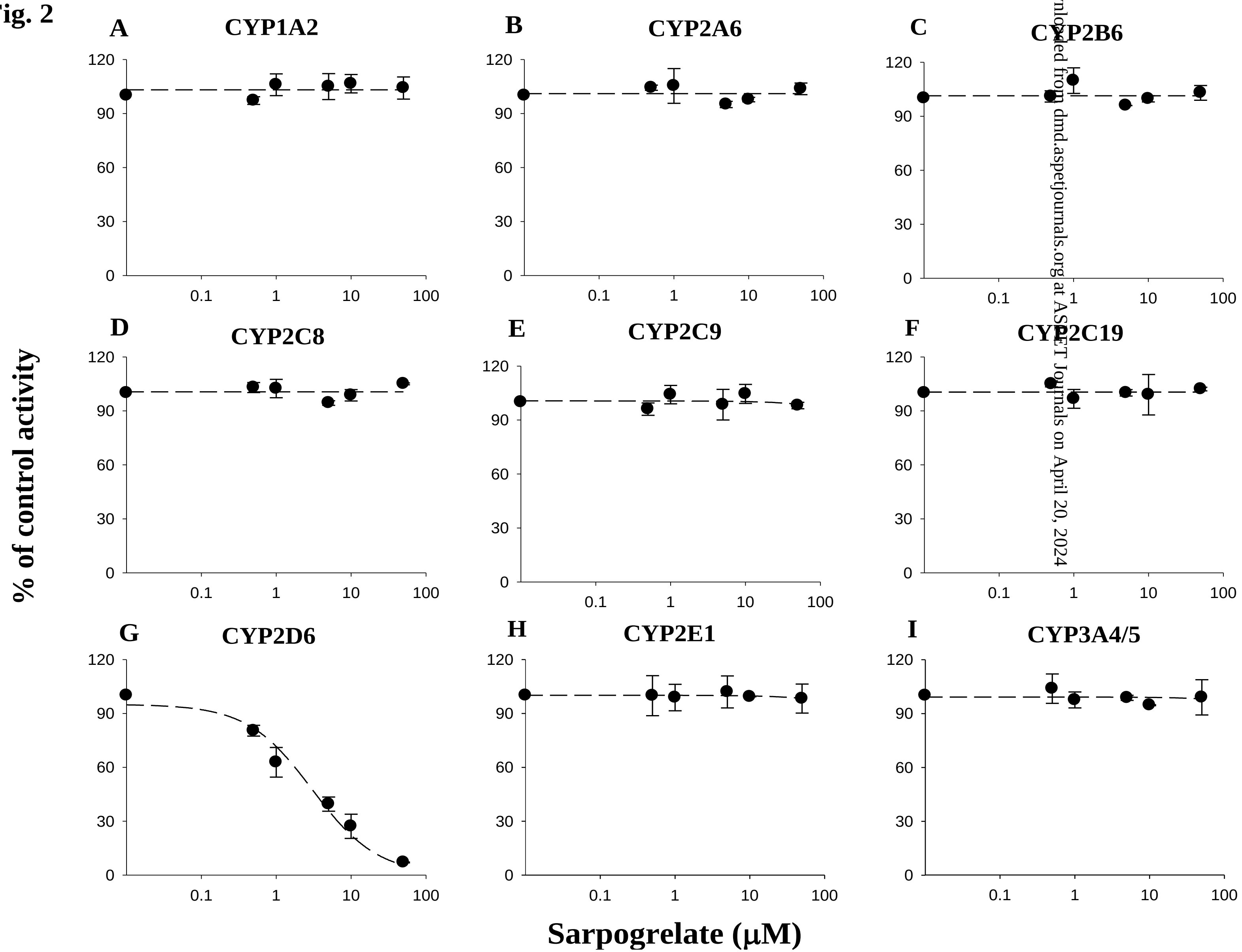


(A)



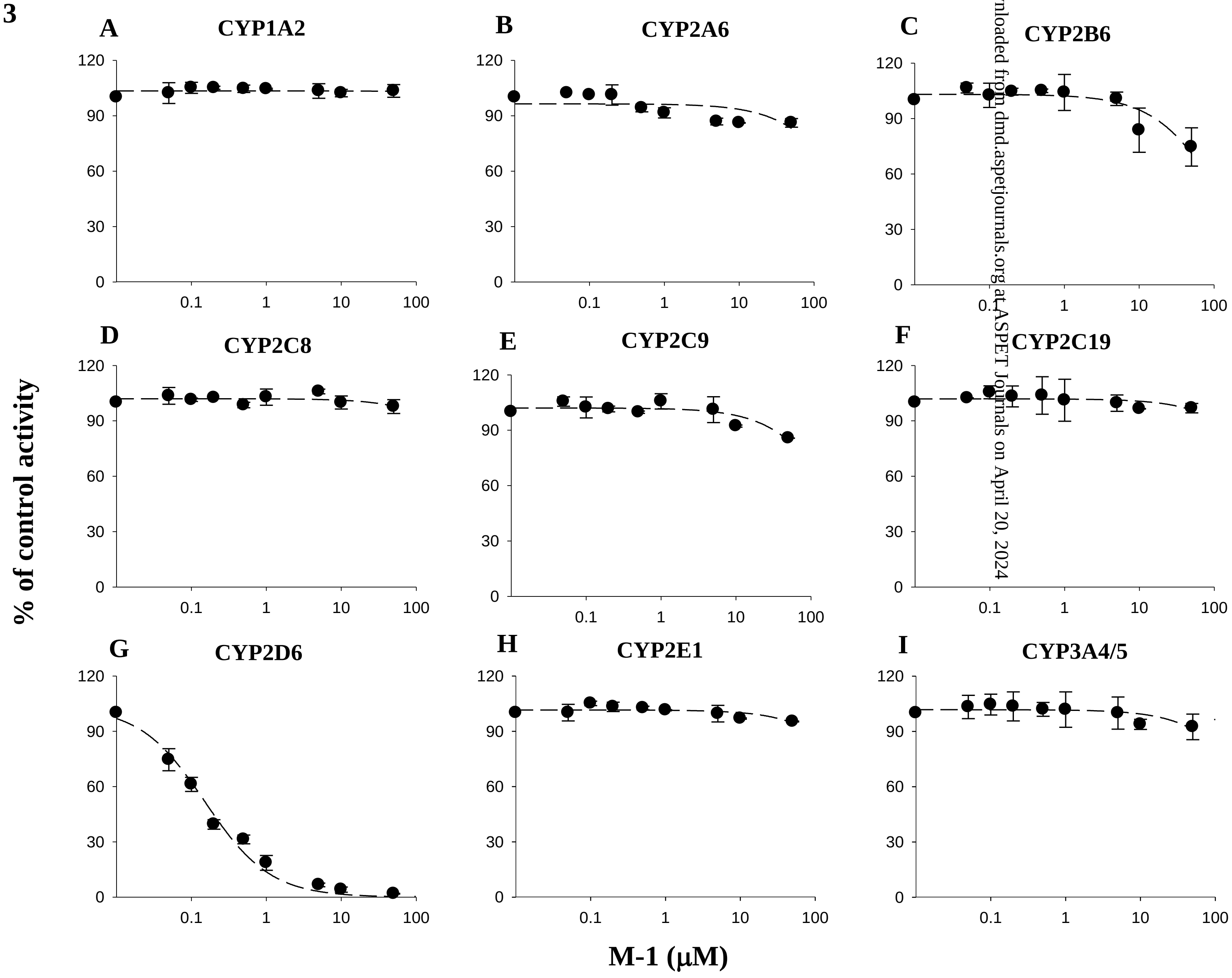
(B)

Fig. 2



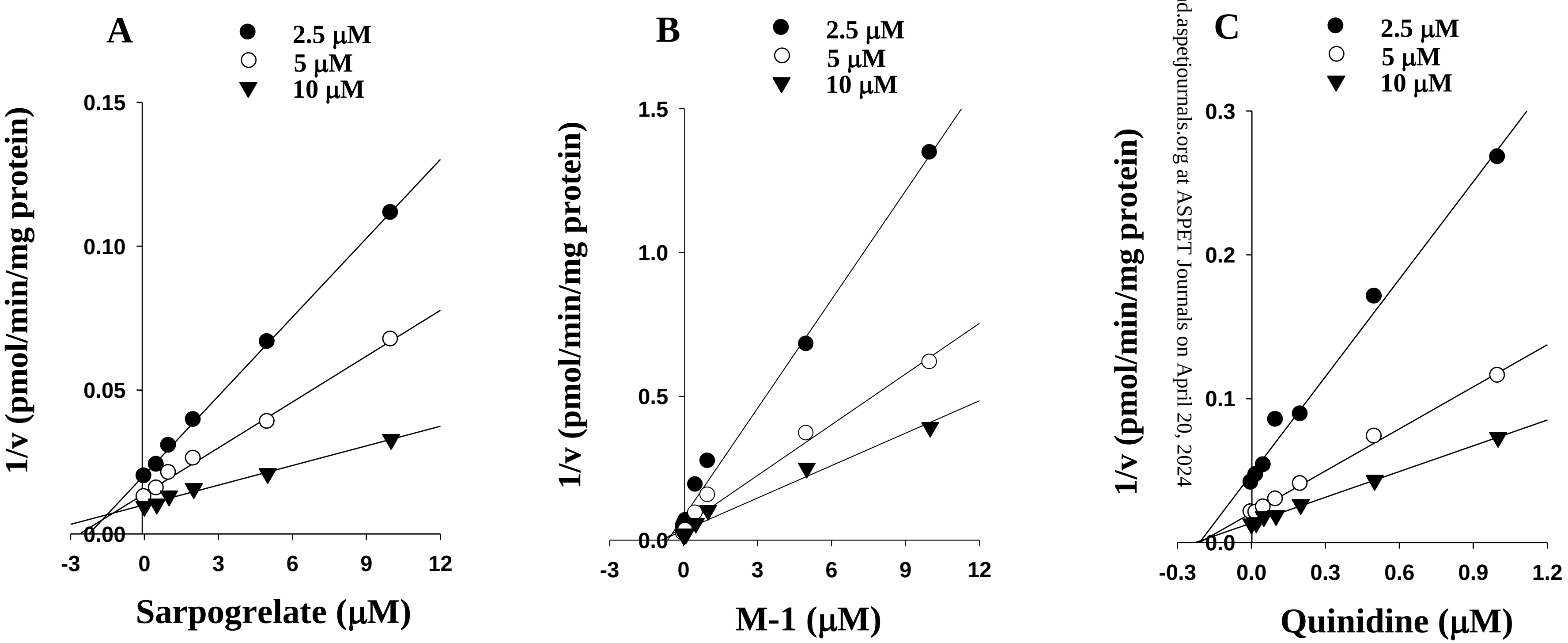
A
CYP1A2
B
CYP2A6
C
CYP2B6
D
CYP2C8
E
CYP2C9
F
CYP2C19
G
CYP2D6
H
CYP2E1
I
CYP3A4/5
% of control activity
Sarpogrelate (μM)

Fig. 3



A
CYP1A2
B
CYP2A6
C
CYP2B6
D
CYP2C8
E
CYP2C9
F
CYP2C19
G
CYP2D6
H
CYP2E1
I
CYP3A4/5
0
30
60
90
120
0.1
1
10
100
% of control activity
M-1 (μM)

Fig. 4



A
2.5 μM
5 μM
10 μM
1/v (pmol/min/mg protein)
0.15
0.10
0.05
0.00
-3
0
3
6
9
12
Sarpogrelate (μM)
B
2.5 μM
5 μM
10 μM
1/v (pmol/min/mg protein)
1.5
1.0
0.5
0.0
-3
0
3
6
9
12
M-1 (μM)
C
2.5 μM
5 μM
10 μM
1/v (pmol/min/mg protein)
0.3
0.2
0.1
0.0
-0.3
0.0
0.3
0.6
0.9
1.2
Quinidine (μM)