Inhibitory effects of antihypertensive drugs on human cytochrome P450 2J2 activity: potent inhibition by azelnidipine and manidipine

Noriaki Ikemura[a,b], Satoshi Yamaori[a,b,*], Chinatsu Kobayashi[c], Shinobu Kamijo[b], Norie Murayama[d], Hiroshi Yamazaki[d], Shigeru Ohmori[a,b]

[a] *Department of Pharmacy, Shinshu University Hospital, 3-1-1 Asahi, Matsumoto 390-8621, Japan*

[b] *Department of Biochemical Pharmacology and Toxicology, Graduate School of Medicine, Shinshu University, 3-1-1 Asahi, Matsumoto 390-8621, Japan*

[c] *School of Medicine, Shinshu University, 3-1-1 Asahi, Matsumoto 390-8621, Japan*

[d] *Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, 3-3165 Higashi-tamagawa Gakuen, Machida, Tokyo 194-8543, Japan*

*To whom correspondence should be addressed

Satoshi Yamaori, Ph.D.
Department of Pharmacy
Shinshu University Hospital
3-1-1 Asahi, Matsumoto 390-8621, Japan
Tel: +81 263-37-3021, Fax: +81 263-37-3021
E-mail: syamaori@shinshu-u.ac.jp

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CYP, cytochrome P450; DCCB, dihydropyridine calcium channel blocker; EET, epoxyeicosatrienoic acid; K_I, half-maximal inhibitory concentration; k_{inact}, maximal inactivation rate constant; k_{obs}, observed rate of inactivation; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.

ABSTRACT

The inhibitory effects of antihypertensive drugs (dihydropyridine calcium channel blockers, angiotensin II receptor blockers, and angiotensin-converting enzyme inhibitors) on cytochrome P450 2J2 (CYP2J2) activity were examined. Amlodipine, azelnidipine, barnidipine, benidipine, cilnidipine, efonidipine, felodipine, manidipine, nicardipine, nifedipine, nilvadipine, nisoldipine, nitrendipine, telmisartan, delapril, and quinapril inhibited luciferin-2J2/4F12 *O*-dealkylase activity of recombinant human CYP2J2 in a concentration-dependent manner (IC_{50} = 0.116 – 9.19 μM). Kinetic analyses of the inhibition indicated that azelnidipine, barnidipine, benidipine, cilnidipine, efonidipine, manidipine, nicardipine, telmisartan, delapril, and quinapril competitively inhibited CYP2J2 activity, while amlodipine, felodipine, nifedipine, nilvadipine, nisoldipine, and nitrendipine showed mixed inhibition. Among these drugs, manidipine showed the strongest reversible inhibition with K_i value of 0.0294 μM. The docking simulation data supported the potent inhibition of CYP2J2 by these drugs. Next, the effect of preincubation on CYP2J2 inhibition was investigated to determine whether these antihypertensive drugs inhibited CYP2J2 activity in a metabolism-dependent manner. A 20-min preincubation of azelnidipine and felodipine in the presence of NADPH potentiated the inhibition of CYP2J2. Furthermore, kinetic analysis of the inactivation showed that azelnidipine caused a preincubation time- and concentration-dependent decrease in CYP2J2 activity yielding k_{inact}/K_I value of 105 l/mmol/min, although felodipine showed no preincubation time-dependent inhibition. The azelnidipine-mediated inactivation required NADPH. These results indicated that manidipine is a potent competitive reversible inhibitor while azelnidipine is a potent mechanism-based inactivator of human CYP2J2.

Keywords: cytochrome P450 2J2; antihypertensive drugs; inhibition; inactivation; docking simulation.

1. Introduction

Cytochrome P450 2J2 (CYP2J2) was first characterized as predominantly expressed in the human heart, although it has also been detected in the liver, gastrointestinal tract, skeletal muscle, and placenta [1 – 3]. CYP2J2 was recently reported to be highly expressed in a variety of cancer cells and tumors [4 – 6]. CYP2J2 converts arachidonic acid to produce four epoxyeicosatrienoic acids (EETs), which have diverse biological effects on the cardiovascular system and endothelial cells [1,7]. Furthermore, it has recently been shown that EETs enhance tumor angiogenesis like vascular endothelial growth factor (VEGF) and promote primary tumor growth and metastasis [4,5,8,9]. Interestingly, it has been reported that intratumoral EETs increased by overexpression of CYP2J2 in carcinoma cell lines and murine xenograft tumor model accelerate tumor growth and metastasis [4,5,8]. Conversely, reduced production of EETs by suppression of CYP2J2 expression and chemical inhibition of CYP2J2 activity in cancer cells has been shown to have an antitumorigenesis effect [4,5,8]. These findings suggest that the chemical inhibition of intratumoral CYP2J2 activity could be a novel strategy for the treatment of cancer.

In addition to the metabolism of arachidonic acid, an endogenous compound, CYP2J2 also oxidizes a large number of drugs, including ebastine [10], astemizole [11], and terfenadine [12]. It has recently been reported that CYP2J2 metabolizes several tyrosine kinase inhibitors (TKIs), such as sorafenib and sunitinib [6]. Sorafenib and sunitinib are molecular targeted drugs that inhibit tyrosine kinase activities of VEGF receptors and exert antitumor effects by suppressing tumor angiogenesis [13,14]. However, these VEGF

receptor-targeted TKIs commonly cause hypertension as a major side effect because they also inhibit tyrosine kinase activity of the VEGF receptor in normal vascular endothelial cells [15]. In this case, the administration of antihypertensive drugs is recommended to treat hypertension induced by VEGF receptor-targeted TKIs. The inclusion of potent inhibitors of CYP2J2 among the concomitantly administered antihypertensive drugs may contribute not only to a reduction of the side effect but also to potentiation of the antitumor effects of TKIs and/or suppression of EET-induced tumor cell proliferation. It has been reported that certain antihypertensive drugs, including nicardipine and telmisartan, inhibit CYP2J2 activity [16 – 18]. However, there have been no detailed studies regarding the inhibitory effects of many other antihypertensive drugs.

In the present study, the inhibitory effects of antihypertensive drugs on CYP2J2 activity were examined using recombinant CYP2J2 as an enzyme source and dihydropyridine calcium channel blockers (DCCBs), angiotensin II receptor blockers (ARBs), and angiotensin-converting enzyme inhibitors (ACEIs) (Supplemental Fig. 1) as chemical inhibitors. Here, we report that manidipine is a potent competitive reversible inhibitor of CYP2J2 and azelnidipine is a potent mechanism-based inactivator of CYP2J2.

2. Materials and methods

2.1. Materials

Microsomes from baculovirus-infected insect cells expressing CYP2J2 with NADPH-CYP reductase and cytochrome b_5 (Supersomes™) were purchased from Corning

Incorporated (Woburn, MA). Luciferin-2J2/4F12, luciferin detection reagent with esterase, and beetle luciferin were purchased from Promega (Madison, WI). Other chemicals were obtained from the following sources: amlodipine from Cayman Chemical (Ann Arbor, MI); azelnidipine, cilnidipine, felodipine, nisoldipine, benazepril hydrochloride, and delapril hydrochloride from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); barnidipine hydrochloride, benazeprilat, cilazapril, imidapril hydrochloride, perindoprilat, quinaprilat, temocaprilat, and trandolaprilat monohydrate from Toronto Research Chemicals (North York, ON, Canada); benidipine hydrochloride, efonidipine hydrochloride monoethanolate, nilvadipine, azilsartan, irbesartan, losartan potassium, telmisartan, and valsartan from Sigma-Aldrich (St. Louis, MO); manidipine dihydrochloride from Adooq Bioscience (Irvine, CA); nicardipine hydrochloride, nifedipine, nitrendipine, alacepril, and captopril from Wako Pure Chemical Ind. (Osaka, Japan); candesartan and olmesartan from Tocris Bioscience (Bristol, UK); danazol, enalapril, enalaprilat monohydrate, perindopril erbumine, quinapril hydrochloride, temocapril hydrochloride, and trandolapril from LKT Laboratories (St. Paul, MN); NADP, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase from Oriental Yeast Co. Ltd. (Tokyo, Japan). All other chemicals and solvents used were of the highest quality commercially available.

2.2. Inhibition studies

The luciferin-2J2/4F12 *O*-dealkylase activity of recombinant CYP2J2 was measured as described previously [19]. The incubation mixture consisted of recombinant CYP2J2 (1

pmol), 3 μM luciferin-2J2/4F12 (a substrate concentration near to the K_m value), each test compound (up to 100 μM), an NADPH generating system (0.5 mM NADP, 10 mM glucose 6-phosphate, 10 mM magnesium chloride, and 1 unit/ml glucose 6-phosphate dehydrogenase), and 100 mM potassium phosphate buffer (pH 7.4) in a final volume of 200 μl. After pre-warming at 37°C for 5 min, reactions were initiated by addition of the NADPH-generating system. Incubations were carried out at 37°C for 20 min. An aliquot (50 μl) of the incubation mixture was transferred to a luminometer tube containing 50 μl of luciferin detection reagent with esterase. After allowing to stand at room temperature for 20 min, luminescence was measured using a Sirius luminometer (Berthold Detection Systems GmbH, Pforzheim, Germany). All test compounds used were dissolved in dimethylsulfoxide and added to the incubation mixture at a final dimethylsulfoxide concentration of 0.5%. The IC_{50} values were calculated by nonlinear regression analysis with Origin 7.5J Software (OriginLab, Northampton, MA), using the logistic dose-response.

The effects of four different inhibitor concentrations on luciferin-2J2/4F12 *O*-dealkylation were examined at four or five substrate concentrations (1.25 – 12.5 μM) to characterize the enzyme kinetics for CYP2J2 inhibition by antihypertensive drugs. The apparent K_i values and the mode of inhibition were determined by nonlinear regression analysis with GraphPad Prism 5.02 (GraphPad Software Inc., San Diego, CA). Akaike's information criterion was used as a measure of goodness of fit. The Lineweaver-Burk plots for CYP2J2 inhibition by antihypertensive drugs and the second plots obtained from slope of the Lineweaver-Burk plots versus inhibitor concentration were depicted for visual inspection.

2.3. Inactivation studies

To identify potential metabolism-dependent inhibition of CYP2J2 by antihypertensive drugs, inhibition experiments were conducted as described below. The preincubation mixture consisted of recombinant CYP2J2 (1 pmol), each test compound (up to 100 μM), the NADPH-generating system, and 100 mM potassium phosphate buffer (pH 7.4) in a final volume of 180 μl. After pre-warming at 37°C for 5 min, reactions were initiated by addition of the NADPH-generating system. Following 20-min preincubation, 20 μl of luciferin-2J2/4F12 solution was added to the preincubation mixtures (final substrate concentration 3 μM). Incubations were performed and the luciferin-2J2/4F12 *O*-dealkylase activity was measured in the same manner as described above. Coincubations in which luciferin-2J2/4F12 was added together with the NADPH-generating system at the end of pre-warming were also carried out.

The kinetics of CYP2J2 inactivation by azelnidipine and felodipine was measured as described below. The preincubation mixture consisted of recombinant CYP2J2 (10 pmol), each test compound (up to 9 μM), the NADPH-generating system, and 100 mM potassium phosphate buffer (pH 7.4) in a final volume of 200 μl. Preincubation was carried out at 37°C for various amounts of time up to 15 min. An aliquot (20 μl) of the preincubation mixture was transferred to 180 μl of enzyme assay mixture containing 3 μM luciferin-2J2/4F12 and the NADPH-generating system, and the reaction was initiated. Incubations were performed and the luciferin-2J2/4F12 *O*-dealkylase activity was measured in the same manner as described

above. The observed rates of CYP2J2 inactivation (k_{obs}) were calculated from the initial slopes of the linear regression lines of semilogarithmic plots (remaining activity of luciferin-2J2/4F12 *O*-dealkylation versus preincubation time). The obtained k_{obs} values were plotted against the inhibitor concentrations. The maximal inactivation rate constant (k_{inact}) and half-maximal inhibitory concentration (K_I) values were calculated by nonlinear regression analysis using GraphPad Prism 5.02 (GraphPad Software Inc.).

To determine the effect of NADPH on azelnidipine-mediated CYP2J2 inactivation, the preincubation mixture was prepared as described for examination of the inactivation kinetics. Preincubation was carried out with or without the NADPH-generating system in both the absence and presence of azelnidipine for 9 min.

2.4. Docking simulation

The structure of human CYP2J2 was modeled in this study using MOE software (ver. 2018.0101; Computing Group, Montreal, QC, Canada) according to Lafite et al. [20]. The primary sequence of human CYP2J2 (Genbank AB080265.1) was aligned with the crystal structures of human CYP2C9 (Protein Data Bank code 1OG5), CYP2C8 (1PQ2), rabbit CYP2B4 (2BDM), and human CYP3A4 (1TQN). Prior to docking simulation, the energy of CYP2J2 was minimized using the CHARM22 force field. Chemical structures were taken from PubChem (an open chemistry database at the National Institutes of Health) and were optimized in MOE. Docking simulations were carried out for chemical binding to CYP2J2 using Amber 10 force field in the MOE software. Twenty solutions were generated

for each docking experiment and ranked according to ligand-interaction energies (*U* values, kcal/mol) using the program ASEdock in MOE. The lower the *U* value, the more effective the chemical is at the docking into CYP2J2.

2.5. Statistical analysis

The significance of differences between the means of the various groups was evaluated by one-way analysis of variance followed by Dunnett's or Bonferroni's post hoc tests. The correlations between the K_i and *U* values of antihypertensive drugs were determined by Pearson's correlation coefficient. All statistical analyses were carried out with GraphPad Prism 5.02 (GraphPad Software).

3. Results

3.1. Inhibition of human CYP2J2 by antihypertensive drugs

In this study, luciferin-2J2/4F12 was used as a luminogenic probe substrate of CYP2J2. Among the 21 recombinant human CYP enzymes examined, CYP2J2 has the most prominent *O*-dealkylase activity of luciferin-2J2/4F12, although CYP1A1, CYP1B1, CYP3A4, and CYP4F12 show only modest activity (https://www.promega.jp/Resources/PubHub/eNotes/Cytochrome%20P450%202J2%20Enzyme%20Assay%20Using%20a%20Novel%20Bioluminescent%20Probe%20Substrate/?fq=cyp2j2). Furthermore, this substrate is more highly sensitive and specific for CYP2J2 as compared with other luminogenic substrates for P450 enzymes [21]. At first, we investigated

the inhibitory effect of danazol, the most potent CYP2J2 inhibitor reported so far [16], on the luciferin-2J2/4F12 *O*-dealkylase activity of recombinant human CYP2J2. Danazol strongly inhibited this activity with an IC_{50} value (mean ± S.D.) of 0.0434 ± 0.0040 μM (Supplemental Fig. 2). The inhibitory potency of danazol against CYP2J2-mediated luciferin-2J2/4F12 *O*-dealkylation was comparable to the previous finding [16], in which terfenadine *t*-butyl methyl hydroxylation (IC_{50} = 0.077 μM) and astemizole *O*-demethylation (IC_{50} = 0.019 μM) were used as marker activities of recombinant human CYP2J2.

Next, the effects of antihypertensive drugs on CYP2J2 activity were examined with recombinant human CYP2J2. All of the DCCBs tested, telmisartan, delapril, and quinapril effectively inhibited CYP2J2 activity (Fig. 1). To determine the IC_{50} values of these antihypertensive drugs, more detailed inhibition experiments were further conducted. These drugs inhibited CYP2J2 activity in a concentration-dependent manner (Fig. 2). In particular, azelnidipine and manidipine potently inhibited the catalytic activity with IC_{50} values of 0.137 and 0.116 μM, respectively (Table 1). The other drugs also showed relatively strong inhibition with IC_{50} values of 1.09 – 9.19 μM (Table 1).

Kinetic analyses were carried out to clarify the modes of inhibition by these antihypertensive drugs. Azelnidipine, barnidipine, benidipine, cilnidipine, efonidipine, manidipine, nicardipine, telmisartan, delapril, and quinapril competitively inhibited CYP2J2 activity (Table 1, Supplemental Fig. 3). On the other hand, amlodipine, felodipine, nifedipine, nilvadipine, nisoldipine, and nitrendipine showed mixed-type inhibition with a relatively high component of competitive inhibition (α = 4.39 – 9.74) (Table 1, Supplemental Fig. 3). Among

the antihypertensive drugs examined, manidipine was the most potent reversible inhibitor of CYP2J2 with an apparent K_i value of 0.0294 μM (Table 1).

3.2. Metabolism-dependent inhibition of human CYP2J2 by antihypertensive drugs

To determine whether antihypertensive drugs inhibit CYP2J2 activity in a metabolism-dependent manner, the effects of preincubation on CYP2J2 inhibition were investigated with the drugs whose IC_{50} values were determined in the inhibition study. When preincubated in the presence of NADPH for 20 min, azelnidipine potentiated CYP2J2 inhibition; the IC_{50} value (mean ± S.D.) was shifted from 0.170 ± 0.022 μM without preincubation to 0.102 ± 0.010 μM with preincubation (Fig. 3A). Felodipine also increased CYP2J2 inhibition; the IC_{50} value was changed from 3.05 ± 0.08 μM without preincubation to 2.37 ± 0.11 μM with preincubation (Fig. 3B). In contrast, no potentiated inhibition was seen with preincubation of manidipine (IC_{50} values of 0.104 ± 0.005 μM without preincubation and 0.138 ± 0.010 μM with preincubation, Fig. 3C) as well as any other drugs (data not shown).

We analyzed the enzyme kinetics to determine the parameters for inactivation of CYP2J2 by azelnidipine and felodipine. The preincubation of azelnidipine in the presence of NADPH caused a time- and concentration-dependent reduction in CYP2J2 activity (Fig. 4A). The k_{inact} and K_I values (mean ± S.D.) were 0.0824 ± 0.0072 min^{-1} and 0.795 ± 0.109 μM, respectively, yielding a k_{inact}/K_I value of 105 ± 18 l/mmol/min (Table 2). In contrast, the preincubation of felodipine did not show time-dependent inhibition of CYP2J2 activity (Fig. 4B).

Preincubation of azelnidipine was performed in the presence and absence of NADPH to determine whether the observed CYP2J2 inactivation required metabolism of azelnidipine. Preincubation of recombinant CYP2J2 with azelnidipine or NADPH alone did not cause any reduction in CYP2J2 activity (Supplemental Fig. 4). However, the presence of both azelnidipine and NADPH led to a significant decrease in this activity.

3.3. Docking simulation of antihypertensive drugs into human CYP2J2

Docking simulation of DCCBs into CYP2J2 was performed to examine the mechanism underlying the potent reversible inhibition of CYP2J2 by manidipine. The top-rank docking models for CYP2J2 were adopted; a *U* energy value (kcal/mol) of manidipine (−56.9) was lower compared with those of the other DCCBs tested (−29.3 for amlodipine, −36.8 for barnidipine, −28.0 for benidipine, −47.3 for cilnidipine, −42.1 for efonidipine, −36.0 for felodipine, −39.3 for nicardipine, −20.4 for nifedipine, −20.1 for nilvadipine, and −14.2 for nisoldipine) except for nitrendipine (−56.9). These *U* energy values were significantly correlated with the IC_{50} values for inhibition of CYP2J2 ($r = 0.877$, $P < 0.001$, Supplemental Fig. 5).

Docking simulation of ARBs into CYP2J2 was also conducted to examine the mechanism underlying the potent inhibition of CYP2J2 by telmisartan. The *U* energy value (kcal/mol) of telmisartan (−68.3) was much lower than those of the other ARBs tested (−20.8 for azilsartan, −23.4 for candesartan, −35.5 for irbesartan, −21.4 for losartan, −13.9 for olmesartan, and −32.7 for valsartan).

Figure 5 shows models of possible interactions of azelnidipine, manidipine, telmisartan, and luciferin2J2/4F12 with CYP2J2.

4. Discussion

Recently, the chemical inhibition of intratumoral CYP2J2 function has attracted attention as a new strategy for chemotherapy [27]. We focused on antihypertensive drugs, because these drugs are administered concomitantly to treat hypertension induced by VEGF receptor-targeted TKIs. However, it remains unclear which drugs can strongly inhibit CYP2J2 activity. Therefore, we investigated the inhibitory effects of antihypertensive drugs (DCCBs, ARBs, and ACEIs) on CYP2J2 activity.

Inhibition of certain human P450 enzymes by DCCBs with different structures and by different mechanisms have been reported [28 – 30]. In this study, all of the DCCBs tested except for azelnidipine showed efficient reversible inhibition of CYP2J2 activity. The results of kinetic analyses for the inhibition suggested that barnidipine, benidipine, cilnidipine, efonidipine, manidipine, and nicardipine could bind to the catalytic site of CYP2J2 and inhibit its activity. On the other hand, amlodipine, felodipine, nifedipine, nilvadipine, nisoldipine, and nitrendipine were suggested to bind to either the catalytic site or another site (putative allosteric site) within the CYP2J2 molecule and inhibit its activity. Interestingly, the DCCBs exhibiting competitive inhibition contain bulky aromatic side chains in structures, whereas the DCCBs showing mixed-type inhibition possess short side chains (Supplemental Fig. 1A). Thus, smaller DCCB molecules are suggested to be exactly accommodated in a putative

allosteric site of CYP2J2. Among the DCCBs examined, manidipine was identified as the strongest reversible CYP2J2 inhibitor. These results were well supported by the docking simulation data. It should be noted that the structure of manidipine, in contrast to the other DCCBs, contains a diphenylmethylamino group (Supplemental Fig. 1A). Therefore, the potent reversible CYP2J2 inhibition by manidipine may be explained by the presence of the diphenylmethylamino group.

Several studies on CYP2J2 inhibition have been conducted [16 – 18,22,23,31 – 37]. Among the CYP2J2 inhibitors identified, danazol was the most potent reversible inhibitor with the K_i value of 0.020 μM [16]. The inhibitory potency of manidipine against CYP2J2 in the present study was comparable to that of danazol. Therefore, manidipine could be categorized as a potent reversible inhibitor of CYP2J2.

Among the ARBs tested, only telmisartan effectively inhibited CYP2J2 activity. The inhibitory potency of telmisartan was similar to that reported previously (K_i = 0.19 μM) [17]. In addition, telmisartan showed competitive inhibition against CYP2J2. However, Ren et al. [17] reported that this drug showed mixed inhibitory activity against CYP2J2. This discrepancy may have been due to the differences in substrates used, because they used astemizole as a substrate. A previous molecular modeling study of CYP2J2 with telmisartan proposed that the nitrogen on the distal benzimidazole ring of telmisartan forms a hydrogen bond with the side chain of Arg-484 in CYP2J2, leading to potent inhibition of CYP2J2 by this drug [17]. Interestingly, such a distal benzimidazole ring is included only in the structure of telmisartan among the ARBs used in this study (Supplemental Fig. 1B). Furthermore,

telmisartan has a carboxyl group instead of tetrazole or oxadiazole group present in the structures of the other ARBs (Supplemental Fig. 1B). These findings suggest that these structural differences between telmisartan and the other ARBs may influence the inhibitory effects of these drugs on CYP2J2 activity. Our molecular docking results also supported the potent inhibition of CYP2J2 by telmisartan.

The results of inhibition study with ACEIs indicated that delapril and quinapril efficiently inhibited CYP2J2 activity. The whole structures of both drugs closely resemble each other, i.e. 1) N-(N-(1-(ethoxycarbonyl)-3-phenylpropyl)alanyl)glycine of delapril and the corresponding structure of quinapril and 2) a benzene ring of the dihydroindenyl group of delapril and benzene ring of the tetrahydroisoquinoline group of quinapril, as compared with the other ACEIs (Supplemental Fig. 1C). These structural characteristics may be one of the factors determining the degree of CYP2J2 inhibition. On the other hand, quinaprilat, an active metabolite of quinapril, showed no inhibition against CYP2J2. These results indicated that at least the ester structure of quinapril could play an important role in CYP2J2 inhibition.

In this study, we demonstrated that azelnidipine inactivated CYP2J2 activity. This inactivation was preincubation time- and concentration-dependent and required the presence of NADPH during preincubation. These results suggest that the inactivation by azelnidipine may proceed via metabolism of this drug. The dihydropyridine ring of azelnidipine would be oxidized by CYP2J2 to yield the pyridine ring, resulting in a reactive moiety with an amino group. However, this precise metabolite identification should be left to a future chemical study. Although various inhibitors of CYP2J2 have been identified, as described above, there

are few mechanism-based inactivators (Table 2). The inactivation potency (k_{inact}/K_I) of azelnidipine against CYP2J2 was high next to that of compound 13, a terfenadone derivative with a benzo-1,3-dioxole terminal group (Table 2). These results indicated that azelnidipine is a potent mechanism-based inactivator of CYP2J2. On the other hand, felodipine did not inactivate CYP2J2 activity. In this study, the inactivation experiments including a dilution step was performed to circumvent reversible inhibition as a confounding factor. However, we could not exclude the possibility that a substrate concentration in the secondary incubation was not high enough to completely avoid the confounding effect of reversible inhibition, because this concentration was near to the K_m value (approximately 2.5 μM) for luciferin-2J2/4F12 *O*-dealkylation catalyzed by recombinant CYP2J2.

There are some previous studies investigating antitumor effects of compounds showing inhibitory effects on CYP2J2 activities [32,33,38]. Compounds 4, 5, 11, and 26, synthesized terfenadone derivatives, repressed tumor growth and metastasis in human carcinoma cell lines and/or murine xenograft tumor model [38]. Decursin, a coumarin derivative isolated from *Angelica gigas N.*, and tanshinone IIA, a diterpene quinone derivative isolated from *Salvia miltiorrhiza*, decreased viability of human hepatoma HepG2 cells, while these compounds did not induce cytotoxicity against mouse hepatocytes [32,33]. These findings indicated that CYP2J2 inhibitors were potential anticancer drugs, as commented by Karkhanis et al. [27]. Thus, it would be worth investigating antitumor effects of certain potent CYP2J2 inhibitors identified in this study. In particular, azelnidipine is considered to be promising, because this drug caused a mechanism-based inhibition against CYP2J2. Once a

mechanism-based inhibitor inactivated a target enzyme, its inhibitory effect would be sustained until the enzyme is newly synthesized. Therefore, it is possible that mechanism-based inactivators of CYP2J2, such as azelnidipine, exhibit more potent and long-lasting antitumor effects than reversible CYP2J2 inhibitors.

A previous study showed that recombinant CYP2J2 expressed in HepG2 cells is capable of metabolizing TKIs, i.e., sorafenib, sunitinib, dasatinib, imatinib, and nilotinib [6]. Furthermore, this metabolic capability is similar to or greater than those of CYP1A1 and CYP1B1 [6], which are highly expressed in various tumors [39 – 41]. These findings suggest that intratumoral CYP2J2 activity may greatly influence metabolic degradation of the molecular targeted drugs and these anticancer effects. If CYP2J2 inhibitors characterized in the present study could reduce CYP2J2 activity in tumor cells, intratumoral concentrations of these molecular targeted drugs should be increased, leading to potentiated antitumor activities. To our knowledge, however, there is no evidence for the enhancement of antitumor effects in patients with the concomitant administration of certain VEGF receptor-targeted TKIs and antihypertensive drugs. Further investigations are needed to clarify the supportive role of antihypertensive drugs inhibiting CYP2J2 activity in antitumorigenesis.

5. Conclusion

We demonstrated that manidipine is a potent competitive reversible inhibitor of human CYP2J2. Furthermore, our results indicated that azelnidipine could act as a potent mechanism-based inactivator of CYP2J2.

Acknowledgements

We thank Mrs. Kaori Aikawa (Department of Biochemical Pharmacology and Toxicology, Graduate School of Medicine, Shinshu University) for technical support.

Conflict of interest

There is no conflict of interests related to this research.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] S. Wu, C.R. Moomaw, K.B. Tomer, J.R. Falck, D.C. Zeldin, Molecular cloning and expression of CYP2J2, a human cytochrome P450 arachidonic acid epoxygenase highly expressed in heart. J. Biol. Chem. 271 (1996) 3460–3468.

[2] D.C. Zeldin, J. Foley, S.M. Goldsworthy, M.E. Cook, J.E. Boyle, J. Ma, C.R. Moomaw, K.B. Tomer, C. Steenbergen, S. Wu, CYP2J subfamily cytochrome P450s in the gastrointestinal tract: expression, localization, and potential functional significance, Mol. Pharmacol. 51 (1997) 931–943.

[3] I. Bièche, C. Narjoz, T. Asselah, S. Vacher, P. Marcellin, R. Lidereau, P. Beaune, I. de Waziers, Reverse transcriptase-PCR quantification of mRNA levels from cytochrome (CYP)1, CYP2 and CYP3 families in 22 different human tissues, Pharmacogenet. Genomics 17 (2007) 731–742.

[4] J.G. Jiang, C.L. Chen, J.W. Card, S. Yang, J.X. Chen, X.N. Fu, Y.G. Ning, X. Xiao, D.C. Zeldin, D.W. Wang, Cytochrome P450 2J2 promotes the neoplastic phenotype of carcinoma cells and is up-regulated in human tumors, Cancer Res. 65 (2005) 4707–4715.

[5] C. Chen, X. Wei, X. Rao, J. Wu, S. Yang, F. Chen, D. Ma, J. Zhou, R.T. Dackor, D.C. Zeldin, D.W. Wang, Cytochrome P450 2J2 is highly expressed in hematologic malignant diseases and promotes tumor cell growth, J. Pharmacol. Exp. Ther. 336 (2011) 344–355.

[6] C. Narjoz, A. Favre, J. McMullen, P. Kiehl, M. Montemurro, W.D. Figg, P. Beaune, I. de

Waziers, B. Rochat, Important role of CYP2J2 in protein kinase inhibitor degradation: a possible role in intratumor drug disposition and resistance. PLoS One, 9 (2014) e95532.

[7] K.L. Jamieson, T. Endo, A.M. Darwesh, V. Samokhvalov, J.M. Seubert, Cytochrome P450-derived eicosanoids and heart function, Pharmacol. Ther. 179 (2017) 47-83.

[8] J.G. Jiang, Y.G. Ning, C. Chen, D. Ma, Z.J. Liu, S. Yang, J. Zhou, X. Xiao, X.A. Zhang, M.L. Edin, J.W. Card, J. Wang, D.C. Zeldin, D.W. Wang, Cytochrome P450 epoxygenase promotes human cancer metastasis, Cancer Res. 67 (2007) 6665–6674.

[9] D. Panigrahy, M.L. Edin, C.R. Lee, S. Huang, D.R. Bielenberg, C.E. Butterfield, C.M. Barnés, A. Mammoto, T. Mammoto, A. Luria, O. Benny, D.M. Chaponis, A.C. Dudley, E.R. Greene, J.A. Vergilio, G. Pietramaggiori, S.S. Scherer-Pietramaggiori, S.M. Short, M. Seth, F.B. Lih, K.B. Tomer, J. Yang, R.A. Schwendener, B.D. Hammock, J.R. Falck, V.L. Manthati, D.E. Ingber, A. Kaipainen, P.A. D'Amore, M.W. Kieran, D.C. Zeldin, Epoxyeicosanoids stimulate multiorgan metastasis and tumor dormancy escape in mice, J. Clin. Invest. 122 (2012) 178–191.

[10] T. Hashizume, S. Imaoka, M. Mise, Y. Terauchi, T. Fujii, H. Miyazaki, T. Kamataki, Y. Funae, Involvement of CYP2J2 and CYP4F12 in the metabolism of ebastine in human intestinal microsomes, J. Pharmacol. Exp. Ther. 300 (2002) 298–304.

[11] S. Matsumoto, T. Hirama, T. Matsubara, K. Nagata, Y. Yamazoe, Involvement of CYP2J2 on the intestinal first-pass metabolism of antihistamine drug, astemizole, Drug Metab. Dispos. 30 (2002) 1240–1245.

[12] M. Xu, W. Ju, H. Hao, G. Wang, P. Li, Cytochrome P450 2J2: distribution, function,

regulation, genetic polymorphisms and clinical significance, Drug Metab. Rev. 45 (2013) 311–352.

[13] S.M. Wilhelm, C. Carter, L. Tang, D. Wilkie, A. McNabola, H. Rong, C. Chen, X. Zhang, P. Vincent, M. McHugh, Y. Cao, J. Shujath, S. Gawlak, D. Eveleigh, B. Rowley, L. Liu, L. Adnane, M. Lynch, D. Auclair, I. Taylor, R. Gedrich, A. Voznesensky, B. Riedl, L.E. Post, G. Bollag, P.A. Trail, BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis, Cancer Res. 64 (2004) 7099–7109.

[14] D.B. Mendel, A.D. Laird, X. Xin, S.G. Louie, J.G. Christensen, G. Li, R.E. Schreck, T.J. Abrams, T.J. Ngai, L.B. Lee, L.J. Murray, J. Carver, E. Chan, K.G. Moss, J.O. Haznedar, J. Sukbuntherng, R.A. Blake, L. Sun, C. Tang, T. Miller, S. Shirazian, G. McMahon, J.M. Cherrington, In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship, Clin. Cancer Res. 9 (2003) 327–337.

[15] J.M. Roodhart, M.H. Langenberg, E. Witteveen, E.E. Voest, The molecular basis of class side effects due to treatment with inhibitors of the VEGF/VEGFR pathway, Curr. Clin. Pharmacol. 3 (2008) 132–143.

[16] C.A. Lee, J.P. Jones, J. Katayama, R. Kaspera, Y. Jiang, S. Freiwald, E. Smith, G.S. Walker, R.A. Totah, Identifying a selective substrate and inhibitor pair for the evaluation of CYP2J2 activity, Drug Metab. Dipos. 40 (2012) 943–951.

[17] S. Ren, J. Zeng, Y. Mei, J.Z.H. Zhang, S.F. Yan, J. Fei, L. Chen, Discovery and characterization of novel, potent, and selective cytochrome P450 2J2 inhibitors. Drug Metab. Dispos. 41 (2013) 60–71.

[18] M. Sisignano, C. Angioni, C.K. Park, S. Meyer Dos Santos, H. Jordan, M. Kuzikov, D. Liu, S. Zinn, S.W. Hohman, Y. Schreiber, B. Zimmer, M. Schmidt, R. Lu, J. Suo, D.D. Zhang, S.M.G. Schäfer, M. Hofmann, A.S. Yekkirala, N. de Bruin, M.J. Parnham, C.J. Woolf, R.R. Ji, K. Scholich, G. Geisslinger, Targeting CYP2J to reduce paclitaxel-induced peripheral neuropathic pain, Proc. Natl. Acad. Sci. USA 113 (2016) 12544–12549.

[19] S. Yamaori, N. Araki, M. Shionoiri, K. Ikehata, S. Kamijo, S. Ohmori, K. Watanabe, A specific probe substrate for evaluation of CYP4A11 activity in human tissue microsomes and a highly selective CYP4A11 inhibitor: luciferin-4A and epalrestat, J. Pharmacol. Exp. Ther. 366 (2018) 446–457.

[20] P. Lafite, F. André, D.C. Zeldin, P.M. Dansette, D. Mansuy, Unusual regioselectivity and active site topology of human cytochrome P450 2J2, Biochemistry 46 (2007) 10237–10247.

[21] J.J. Cali, D. Ma, M. Sobol, D.J. Simpson, S. Frackman, T.D. Good, W.J. Daily, D. Liu, Luminogenic cytochrome P450 assays, Expert Opin. Drug Metab. Toxicol. 2 (2006) 629-645.

[22] A. Karkhanis, H.Y. Lam, G. Venkatesan, S.K. Koh, C.L. Chai, L. Zhou, Y. Hong, P. Kojodjojo, E.C. Chan, Multiple modes of inhibition of human cytochrome P450 2J2 by

dronedarone, amiodarone and their active metabolites, Biochem. Pharmacol. 107 (2016) 67–80.

[23] P. Lafite, S. Dijols, D.C. Zeldin, P.M. Dansette, D. Mansuy. Selective, competitive and mechanism-based inhibitors of human cytochrome P450 2J2, Arch. Biochem. Biophys. 464 (2007) 155–168.

[24] R. Kaspera, B.J. Kirby, T. Sahele, A.C. Collier, E.D. Kharasch, J.D. Unadkat, R.A. Totah, Investigating the contribution of CYP2J2 to ritonavir metabolism in vitro and in vivo, Biochem. Pharmacol. 91 (2014) 109–118.

[25] H.L. Lin, H. Zhang, V.J. Walker, J. D'Agostino, P.F. Hollenberg, Heme modification contributes to the mechanism-based inactivation of human cytochrome P450 2J2 by two terminal acetylenic compounds, Drug Metab. Dispos. 45 (2017) 990–999.

[26] H.L. Lin, H. Zhang, P.F. Hollenberg, Formation of both heme and apoprotein adducts contributes to the mechanism-based inactivation of human CYP2J2 by 17α-ethynylestradiol, Drug Metab. Dispos. 46 (2018) 813–822.

[27] A. Karkhanis, Y. Hong, E.C.Y. Chan, Inhibition and inactivation of human CYP2J2: implication in cardiac pathophysiology and opportunities in cancer therapy, Biochem. Pharmacol. 135 (2017) 12–21.

[28] M. Katoh, M. Nakajima, N. Shimada, H. Yamazai, T. Yokoi, Inhibition of human cytochrome P450 enzymes by 1,4-dihydropyridine calcium antagonists: prediction of in vivo drug-drug interactions, Eur. J. Clin. Pharmacol. 55 (2000) 843–852.

[29] M. Štěpánková, K. Krasulová, A. Dořičáková, O. Kurka, P. Anzenbacher, Z. Dvořák,

Optical isomers of dihydropyridine calcium channel blockers display enantiospecific effects on the expression and enzyme activities of human xenobiotics-metabolizing cytochromes P450, Toxicol. Lett. 262 (2016) 173–186.

[30] K. Krasulova, O. Holas, P. Anzenbacher, Influence of amlodipine enantiomers on human microsomal cytochromes P450: stereoselective time-dependent inhibition of CYP3A enzyme activity, Molecules 22 (2017) 1879.

[31] E.A. Evangelista, R. Kaspera, N.A. Mokadam, J.P. Jones, R.A. Totah, Activity, inhibition, and induction of cytochrome P450 2J2 in adult human primary cardiomyocytes, Drug Metab. Dispos. 41 (2013) 2087–2094.

[32] B. Lee, Z. Wu, S.H. Sung, T. Lee, K.S. Song, M.Y. Lee, K.H. Liu, Potential of decursin to inhibit the human cytochrome P450 2J2 isoform, Food Chem. Toxicol. 70 (2014) 94–99.

[33] Y.J. Jeon, J.S. Kim, G.H. Hwang, Z. Wu, H.J. Han, S.H. Park, W. Chang, L.K. Kim, Y.M. Lee, K.H. Liu, M.Y. Lee, Inhibition of cytochrome P450 2J2 by tanshinone IIA induces apoptotic cell death in hepatocellular carcinoma HepG2 cells, Eur. J. Pharmacol. 764 (2015) 480–488.

[34] E. Lee, Z. Wu, J.C. Shon, K.H. Liu, Danazol inhibits cytochrome P450 2J2 activity in a substrate-independent manner, Drug Metab Dispos. 43 (2015) 1250–1253.

[35] N.M. Phuc, Z. Wu, Y. O, J.H. Lee, S. Oh, G.Y. Song, K.H. Liu, LKY-047: first selective inhibitor of cytochrome P450 2J2, Drug Metab. Dispos. 45 (2017) 765–769.

[36] S.H. Park, N.M. Phuc, J. Lee, Z. Wu, J. Kim, H. Kim, N.D. Kim, T. Lee, K.S. Song, K.H.

Liu, Identification of acetylshikonin as the novel CYP2J2 inhibitor with anti-cancer activity in HepG2 cells, Phytomedicine 24 (2017) 134–140.

[37] M.J. Xu, L.F. Jiang, T. Wu, J.H. Chu, Y.D. Wei, J.Y. Aa, G.J. Wang, H.P. Hao, W.Z. Ju, P. Li, Inhibitory effects of Danshen components on CYP2C8 and CYP2J2, Chem. Biol. Interact. 289 (2018) 15–22.

[38] C. Chen, G. Li, W. Liao, J. Wu, L. Liu, D. Ma, J. Zhou, R.H. Elbekai, M.L. Edin, D.C. Zeldin, D.W. Wang, Selective inhibitors of CYP2J2 related to terfenadine exhibit strong activity against human cancers in vitro and in vivo, J. Pharmacol. Exp. Ther. 329 (2009) 908–918.

[39] Y.K. Leung, K.M. Lau, J. Mobley, Z. Jiang, S.M. Ho, Overexpression of cytochrome P450 1A1 and its novel spliced variant in ovarian cancer cells: alternative subcellular enzyme compartmentation may contribute to carcinogenesis, Cancer Res. 65 (2005) 3726–3734.

[40] T. Oyama, K. Sugio, T. Isse, A. Matsumoto, N. Nose, H. Uramoto, T. Nozoe, M. Morita, N. Kagawa, T. Osaki, M. Muto, K. Yasumoto, T. Kawamoto, Expression of cytochrome P450 in non-small cell lung cancer, Front. Biosci. 13 (2008) 5787–5793.

[41] G.I. Murray, M.C. Taylor, M.C. McFadyen, J.A. McKay, W.F. Greenlee, M.D. Burke, W.T. Melvin, Tumor-specific expression of cytochrome P450 CYP1B1, Cancer Res. 57 (1997) 3026–3031.

Table 1

Kinetic parameters for inhibition of CYP2J2 activity by DCCBs, telmisartan, delapril, and quinapril.

Inhibitors	IC_{50} (μM)	K_i (μM)	α	Mode of inhibition
Amlodipine	5.07 ± 0.54	2.58 ± 0.23	9.74 ± 0.93	Mixed
Azelnidipine	0.137 ± 0.014	0.0671 ± 0.0029	–	Competitive
Barnidipine	2.87 ± 0.30	0.651 ± 0.036	–	Competitive
Benidipine	2.16 ± 0.22	0.435 ± 0.012	–	Competitive
Cilnidipine	1.09 ± 0.03	0.279 ± 0.018	–	Competitive
Efonidipine	1.45 ± 0.03	0.315 ± 0.019	–	Competitive
Felodipine	2.78 ± 0.36	1.23 ± 0.15	5.81 ± 0.82	Mixed
Manidipine	0.116 ± 0.017	0.0294 ± 0.0023	–	Competitive
Nicardipine	2.36 ± 0.35	0.566 ± 0.082	–	Competitive
Nifedipine	7.34 ± 0.05	2.76 ± 0.29	8.92 ± 0.94	Mixed
Nilvadipine	4.55 ± 0.67	1.66 ± 0.03	5.55 ± 0.89	Mixed
Nisoldipine	6.56 ± 0.34	2.38 ± 0.29	5.45 ± 0.23	Mixed
Nitrendipine	1.45 ± 0.16	0.689 ± 0.104	4.39 ± 0.38	Mixed
Telmisartan	2.04 ± 0.23	0.345 ± 0.002	–	Competitive
Delapril	9.01 ± 0.49	2.63 ± 0.13	–	Competitive
Quinapril	9.19 ± 0.72	2.55 ± 0.11	–	Competitive

Values are represented as mean ±S.D. of triplicate determinations.

Table 2

Kinetic parameters for mechanism-based inhibition of CYP2J2 by its known inactivators.

Inactivators	Substrates	k_{inact} (/min)	K_I (μM)	k_{inact}/K_I (l/mmol/min)	References
Ritonavir	Astemizole	0.034	0.030	1100	[22]
Dronedarone	Astemizole	0.034	0.050	680	[22]
Compound 5	Ebastine	0.080	0.45	180	[23]
Compound 13	Ebastine	0.47	2.9	160	[23]
Azelnidipine	Luciferin-2J2/4F12	0.0824	0.795	105	Present study
Ritonavir	Terfenadine	0.042	0.51	82	[24]
Amiodarone	Astemizole	0.015	0.21	69	[22]
N-Desbutyldronedarone	Astemizole	0.024	0.48	49	[22]
MS [a]	Hydroxyebastine	0.22	6.1	36	[25]
17-Ethynylestradiol	Hydroxyebastine	0.080	3.6	22	[26]
17-Octadecynoic acid	Hydroxyebastine	0.050	2.5	20	[25]

[a] *N*-(Methylsulfonyl)-6-(2-propargyloxyphenyl)-hexanamide.

Figure legends

Fig. 1. Effects of DCCBs, ARBs, and ACEIs on CYP2J2 activity. Recombinant CYP2J2 was incubated with luciferin-2J2/4F12 (3 μM) in the presence of DCCBs (A), ARBs (B), and ACEIs (C). Each column and bar represent mean ± S.D. of triplicate determinations. ** $p < 0.01$, *** $p < 0.001$ vs control (Dunnett's test).

Fig. 2. Inhibitory effects of DCCBs, telmisartan, delapril, and quinapril on CYP2J2 activity. Recombinant CYP2J2 was incubated with luciferin-2J2/4F12 (3 μM) in the presence of various amounts of DCCBs (A – D), ARB (E), and ACEIs (F). Each point and bar represent mean ± S.D. of triplicate determinations.

Fig. 3. Effect of preincubation on inhibition of CYP2J2 activity by azelnidipine, felodipine, and manidipine. Recombinant CYP2J2 was preincubated with azelnidipine (A), felodipine (B), and manidipine (C) in the presence of NADPH for 0 min (closed symbols) or 20 min (open symbols). Incubations were conducted after the addition of luciferin-2J2/4F12 (3 μM). Each point and bar represent mean ± S.D. of triplicate determinations.

Fig. 4. Inactivation of CYP2J2 activity by azelnidipine and felodipine. Recombinant CYP2J2 was preincubated with azelnidipine (A) and felodipine (B) (0 – 9 μM) in the presence of NADPH for up to 15 min. Aliquots were removed from the preincubation mixtures at the indicated time points and diluted 10-fold for measurement of the residual activity. Each point

and bar represent mean ± S.D. of triplicate determinations.

Fig. 5. Docking of azelnidipine, manidipine, telmisartan, and luciferin-2J2/4F12 into the active site cavity of modeled human CYP2J2. The interactions of azelnidipine (A), manidipine (B), telmisartan (C), and luciferin-2J2/4F12 (D) with the active site of CYP2J2 are depicted.

Figure 1

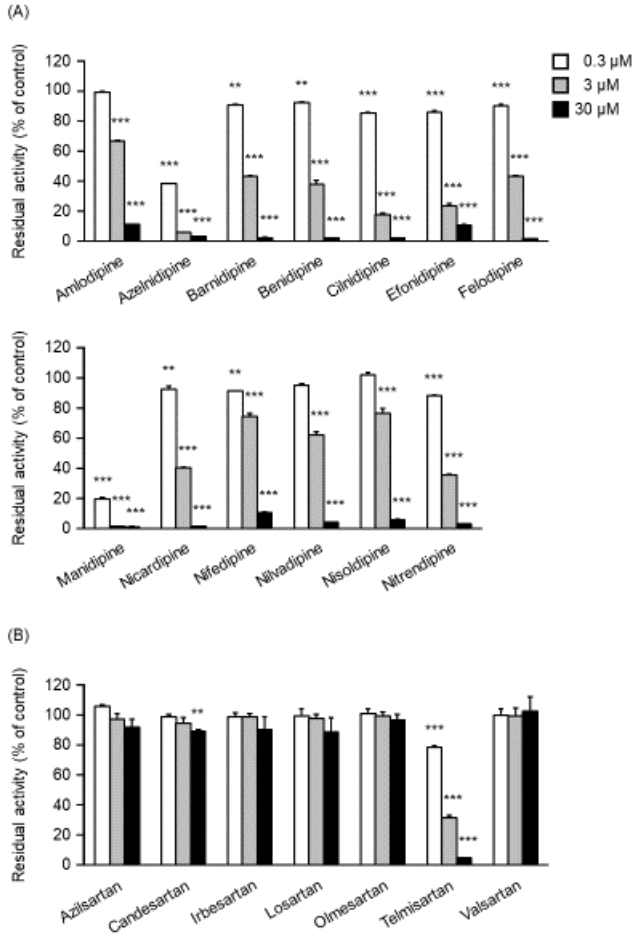

(A)
Residual activity (% of control)
0.3 μM
3 μM
30 μM
Amlodipine
Azelnidipine
Barnidipine
Benidipine
Cilnidipine
Efonidipine
Felodipine
Manidipine
Nicardipine
Nifedipine
Nilvadipine
Nisoldipine
Nitrendipine
(B)
Azilsartan
Candesartan
Irbesartan
Losartan
Olmesartan
Telmisartan
Valsartan

Figure 1 (Continued)

(C)

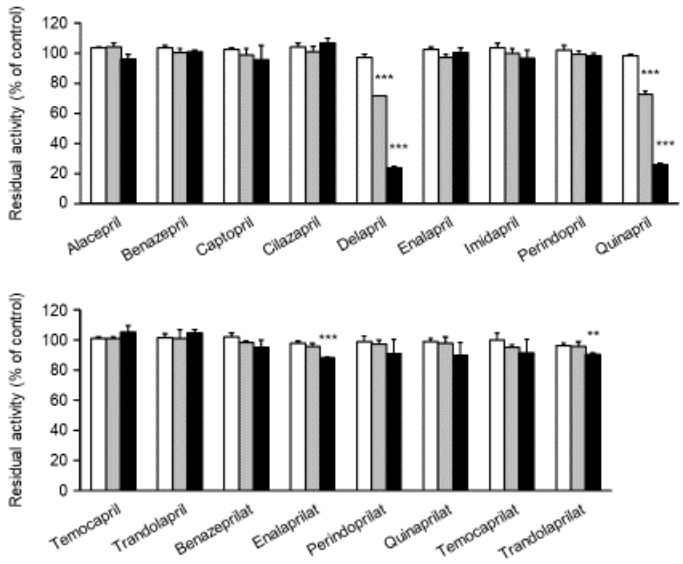


Figure 2

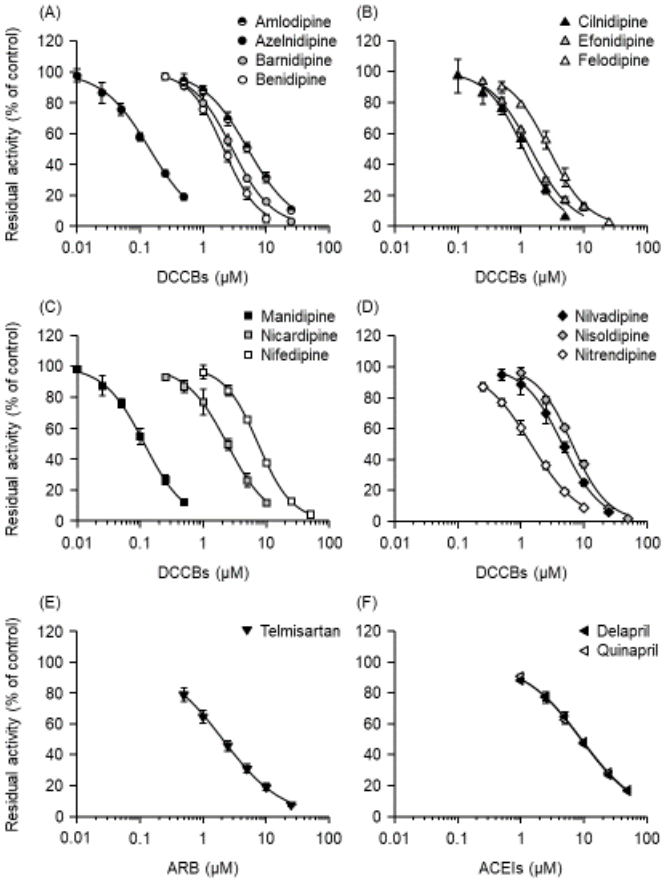

(A)
Amlodipine
Azelnidipine
Barnidipine
Benidipine
(B)
Cilnidipine
Efonidipine
Felodipine
(C)
Manidipine
Nicardipine
Nifedipine
(D)
Nilvadipine
Nisoldipine
Nitrendipine
(E)
Telmisartan
(F)
Delapril
Quinapril
Residual activity (% of control)
DCCBs (μM)
ARB (μM)
ACEIs (μM)

Figure 3

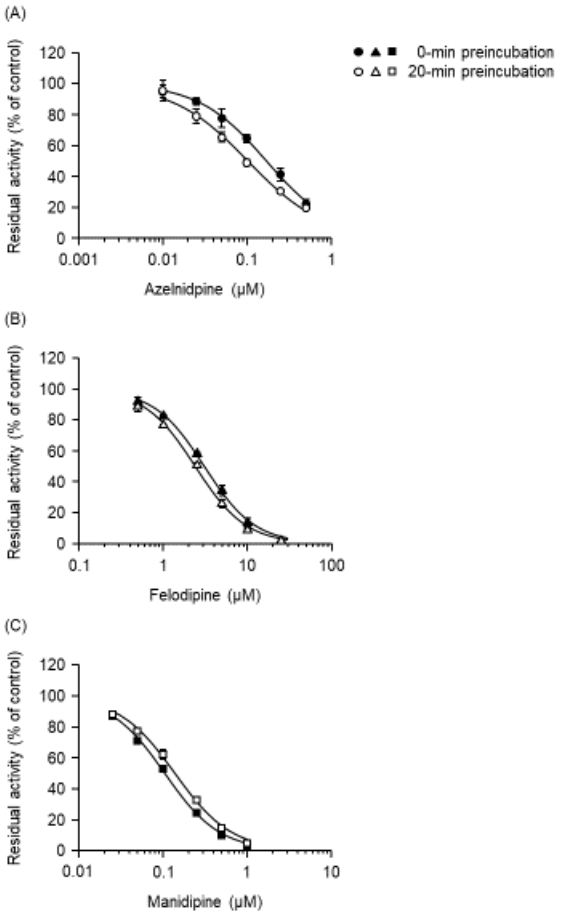

(A)
0-min preincubation
20-min preincubation
Residual activity (% of control)
120
100
80
60
40
20
0
0.001
0.01
0.1
1
Azelnidpine (μM)
(B)
Residual activity (% of control)
120
100
80
60
40
20
0
0.1
1
10
100
Felodipine (μM)
(C)
Residual activity (% of control)
120
100
80
60
40
20
0
0.01
0.1
1
10
Manidipine (μM)

Figure 4

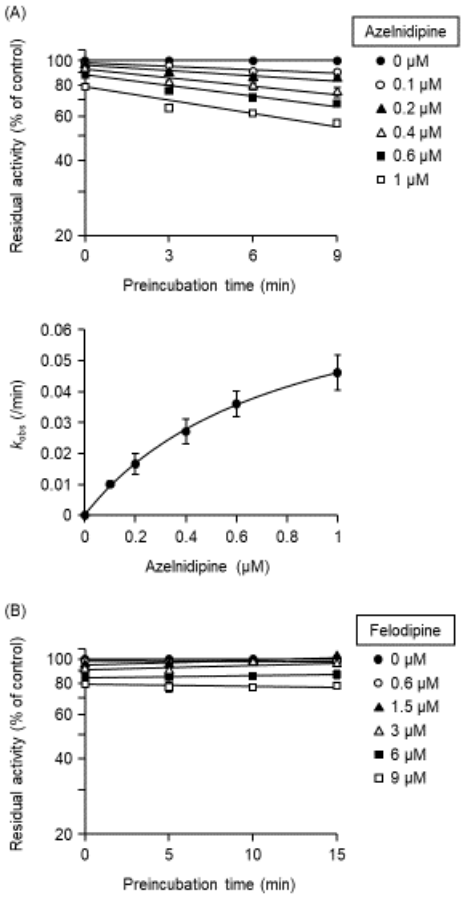
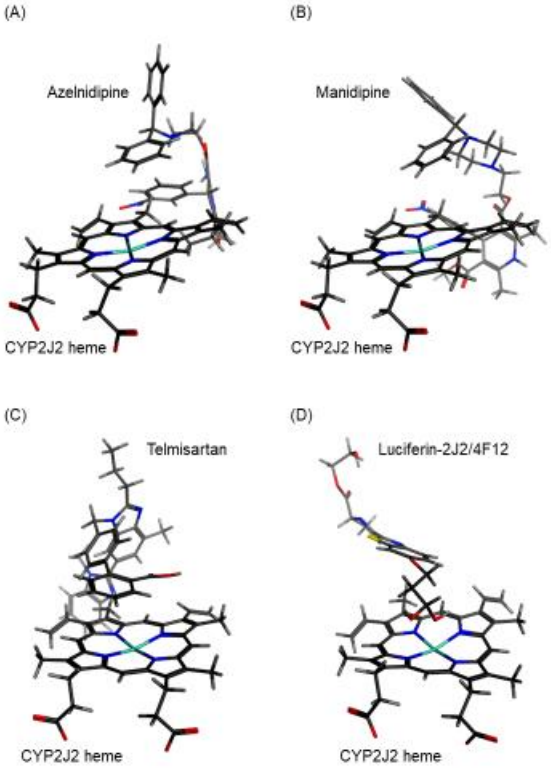

(A)
Azelnidipine
0 μM
0.1 μM
0.2 μM
0.4 μM
0.6 μM
1 μM
Residual activity (% of control)
Preincubation time (min)
k_{obs} (/min)
Azelnidipine (μM)
(B)
Felodipine
0 μM
0.6 μM
1.5 μM
3 μM
6 μM
9 μM
Residual activity (% of control)
Preincubation time (min)

Figure 5


(A)
Azelnidipine
CYP2J2 heme
(B)
Manidipine
CYP2J2 heme
(C)
Telmisartan
CYP2J2 heme
(D)
Luciferin-2J2/4F12
CYP2J2 heme

Inhibitory effects of antihypertensive drugs on human cytochrome P450 2J2 activity: potent inhibition by azelnidipine and manidipine

Noriaki Ikemura[a,b], Satoshi Yamaori[a,b], Chinatsu Kobayashi[c], Shinobu Kamijo[b], Norie Murayama[d], Hiroshi Yamazaki[d], Shigeru Ohmori[a,b]

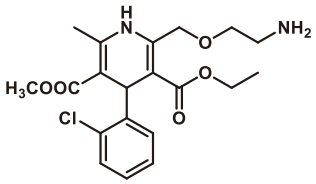
[a] *Department of Pharmacy, Shinshu University Hospital, 3-1-1 Asahi, Matsumoto 390-8621, Japan*

[b] *Department of Biochemical Pharmacology and Toxicology, Graduate School of Medicine, Shinshu University, 3-1-1 Asahi, Matsumoto 390-8621, Japan*

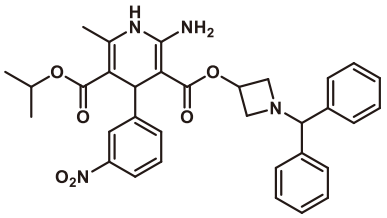
[c] *School of Medicine, Shinshu University, 3-1-1 Asahi, Matsumoto 390-8621, Japan*

[d] *Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, 3-3165 Higashi-tamagawa Gakuen, Machida, Tokyo 194-8543, Japan*

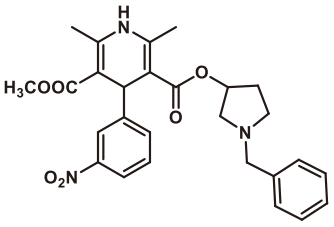
(A)



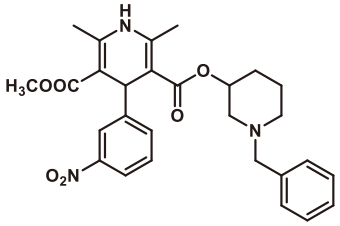
Amlodipine



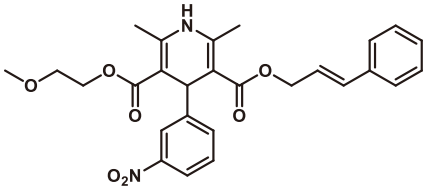
Azelnidipine



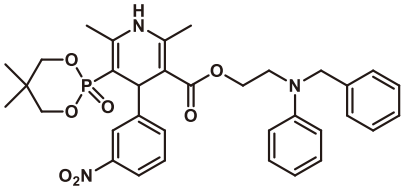
Barnidipine



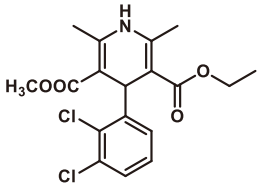
Benidipine



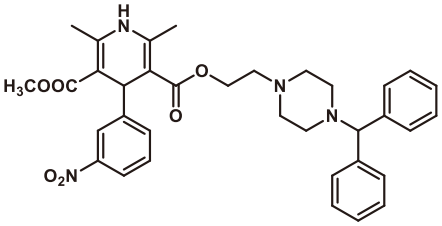
Cilnidipine



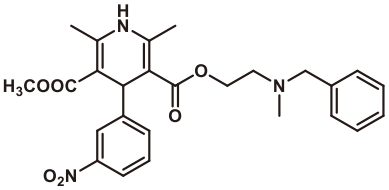
Efonidipine



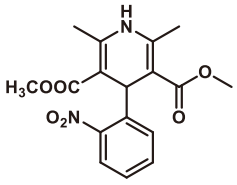
Felodipine



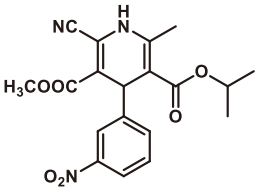
Manidipine



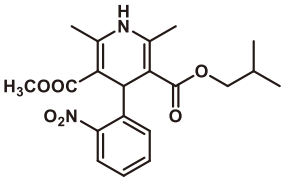
Nicardipine



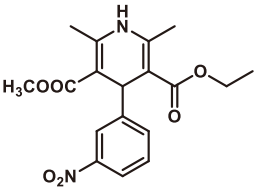
Nifedipine



Nilvadipine

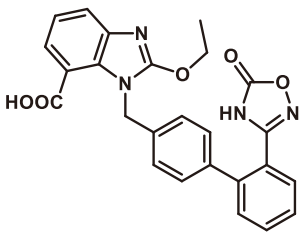


Nisoldipine

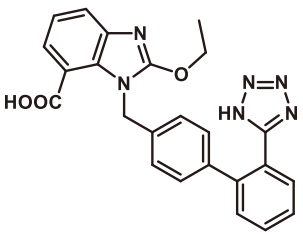


Nitrendipine

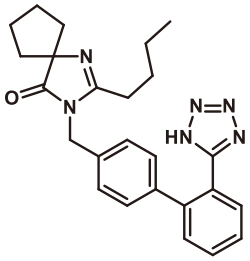
(B)



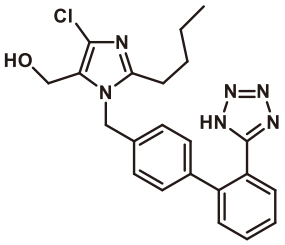
Azilsartan



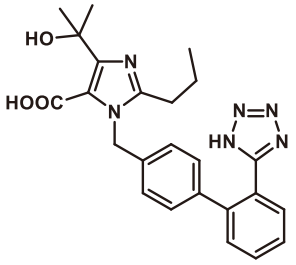
Candesartan



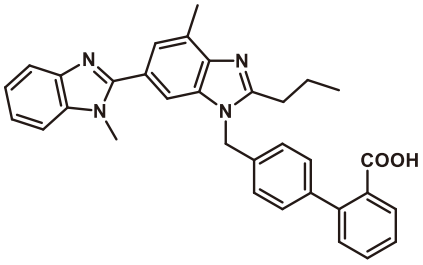
Irbesartan



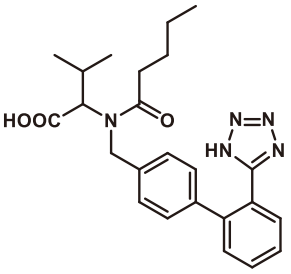
Losartan



Olmesartan

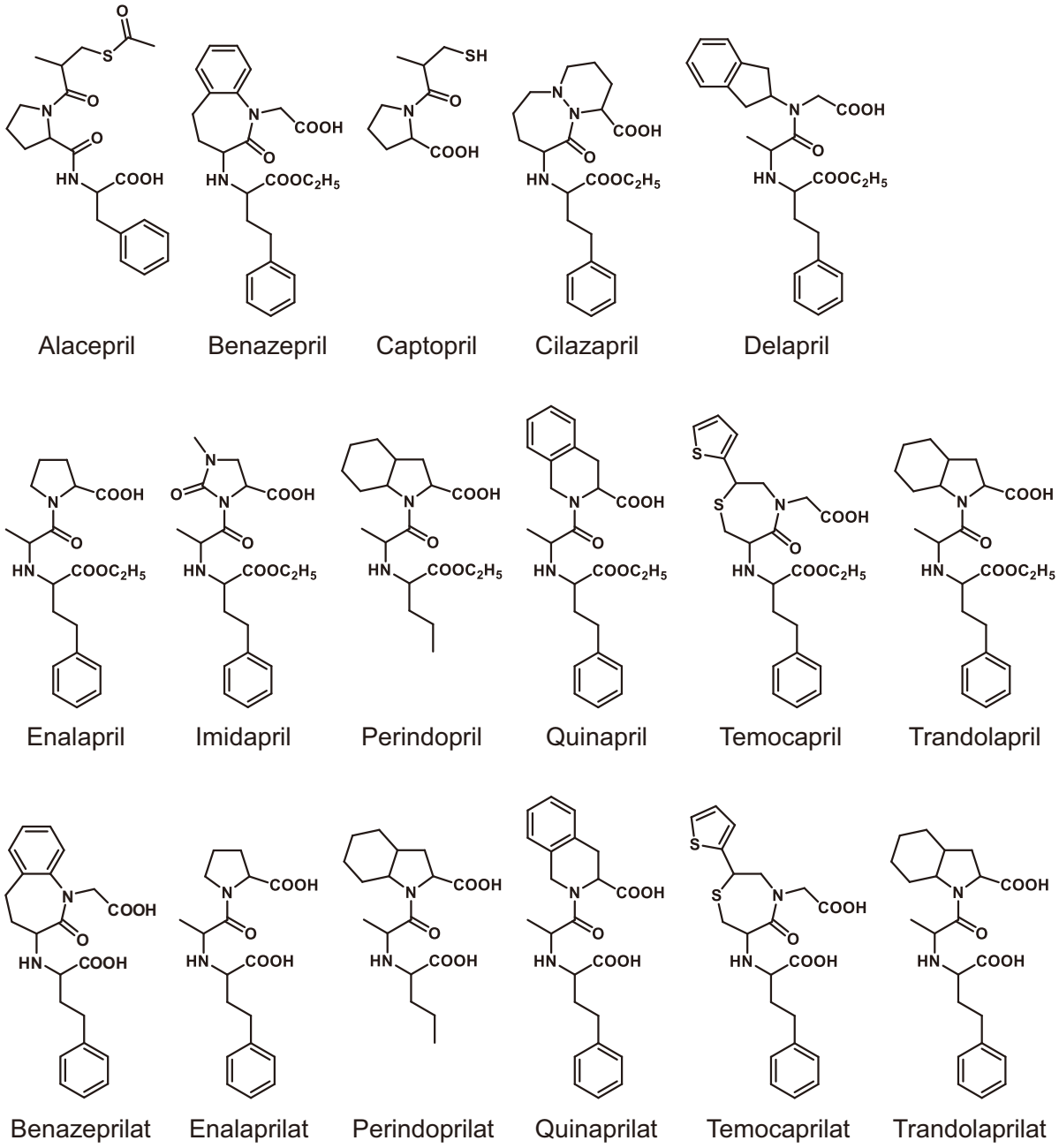


Telmisartan

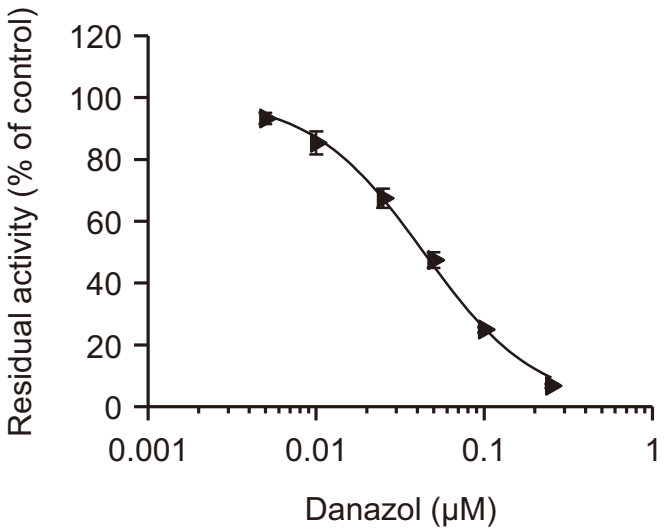


Valsartan

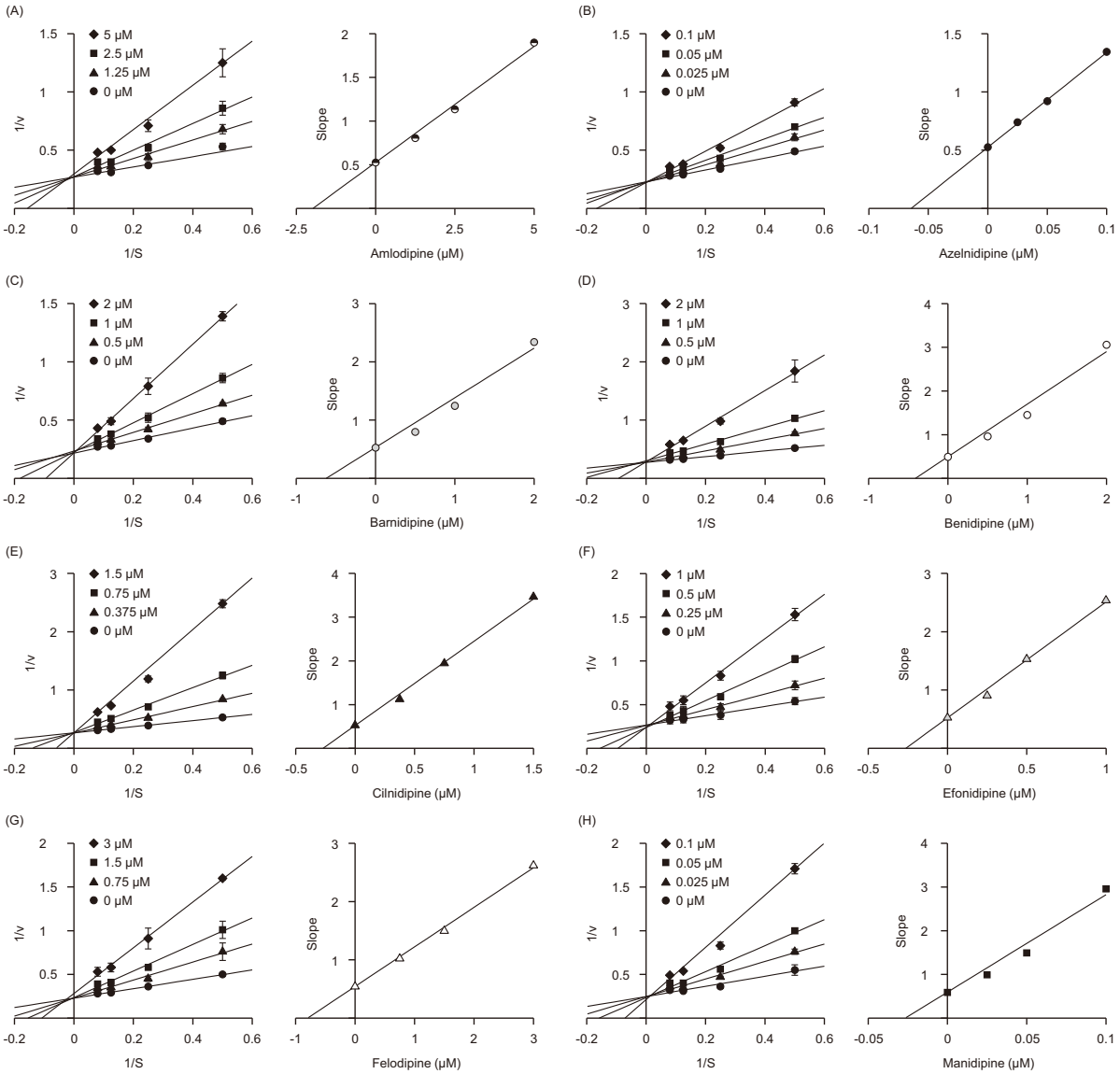
(C)

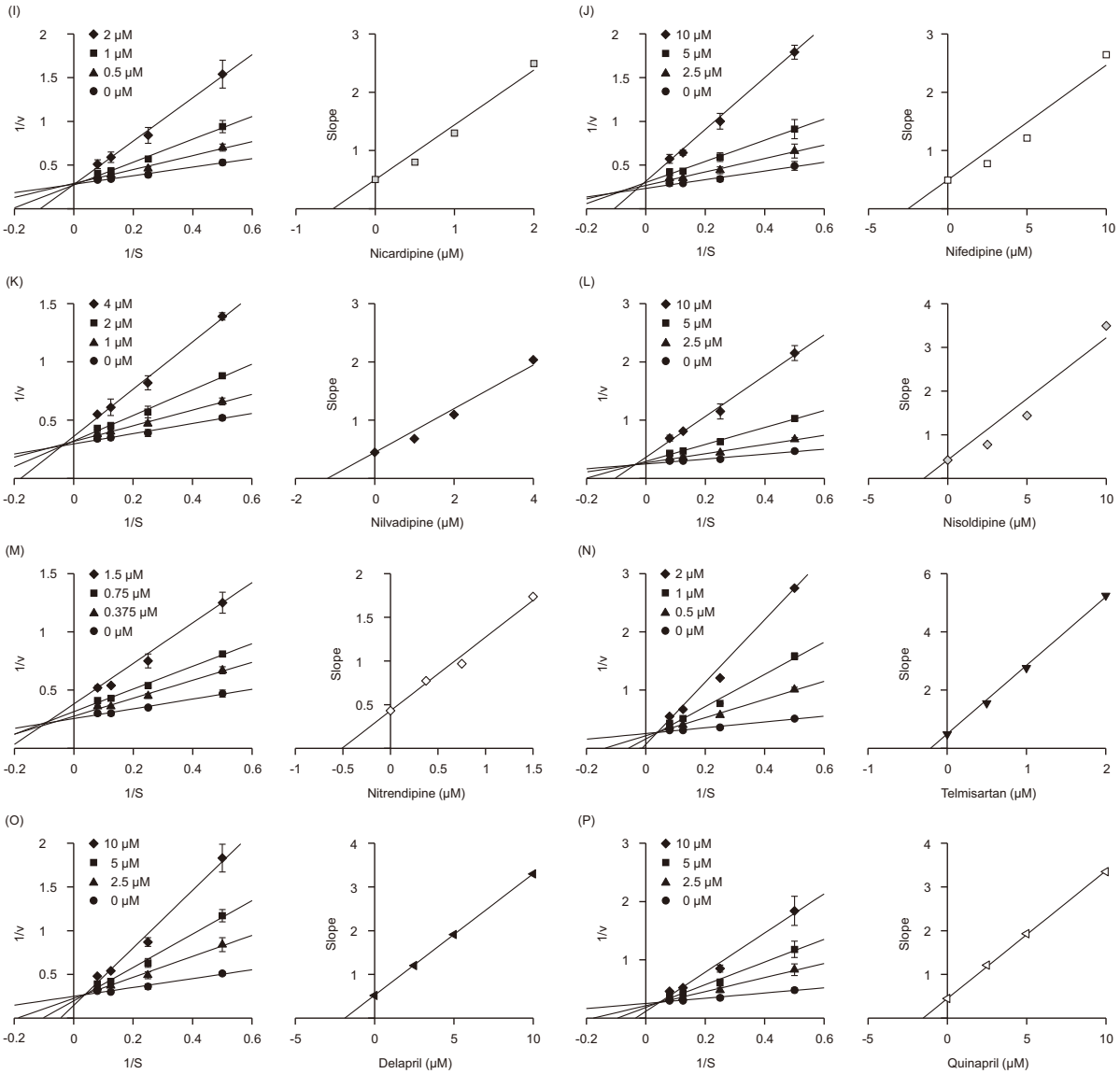


Supplemental Fig. 1. Chemical structures of DCCBs, ARBs, and ACEIs used in this study. (A) DCCBs, (B) ARBs, and (C) ACEIs.

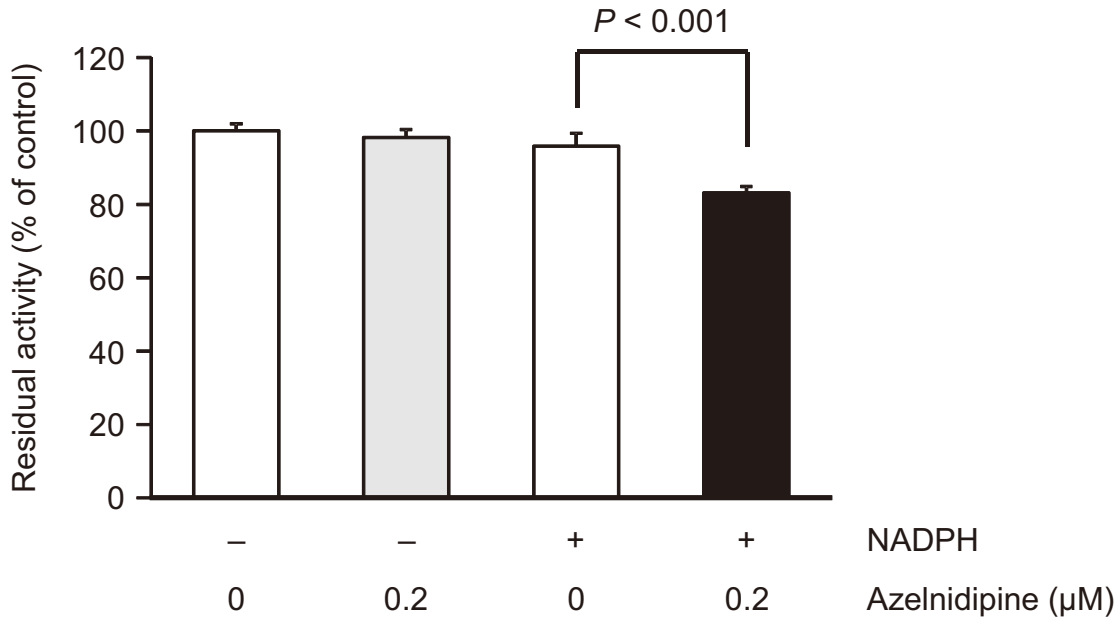


Supplemental Fig. 2. Inhibitory effect of danazol on CYP2J2 activity. Recombinant CYP2J2 was incubated with luciferin-2J2/4F12 (3 μM) in the presence of various amounts of danazol. Each point and bar represent mean ± S.D. of triplicate determinations.

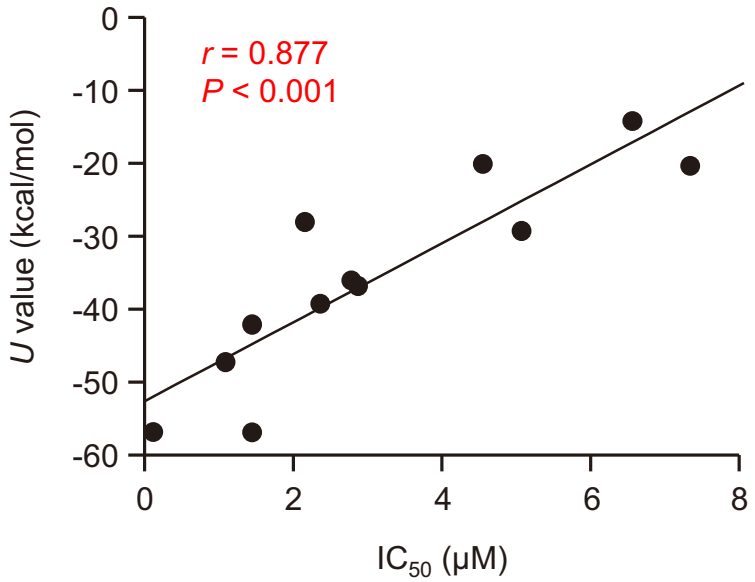

(A)
5 μM
2.5 μM
1.25 μM
0 μM
1/v
1/S
Slope
Amlodipine (μM)
(B)
0.1 μM
0.05 μM
0.025 μM
0 μM
1/v
1/S
Slope
Azelnidipine (μM)
(C)
2 μM
1 μM
0.5 μM
0 μM
1/v
1/S
Slope
Barnidipine (μM)
(D)
2 μM
1 μM
0.5 μM
0 μM
1/v
1/S
Slope
Benidipine (μM)
(E)
1.5 μM
0.75 μM
0.375 μM
0 μM
1/v
1/S
Slope
Cilnidipine (μM)
(F)
1 μM
0.5 μM
0.25 μM
0 μM
1/v
1/S
Slope
Efonidipine (μM)
(G)
3 μM
1.5 μM
0.75 μM
0 μM
1/v
1/S
Slope
Felodipine (μM)
(H)
0.1 μM
0.05 μM
0.025 μM
0 μM
1/v
1/S
Slope
Manidipine (μM)



Supplemental Fig. 3. Kinetic analyses for inhibition of CYP2J2 activity by DCCBs, telmisartan, delapril, and quinapril. Recombinant CYP2J2 was incubated with luciferin-2J2/4F12 in the presence of various amounts of DCCBs (A – M), telmisartan (N), delapril (O), and quinapril (P). The left graphs depict the Lineweaver-Burk plots. S and v indicate substrate concentration (μM) and luciferin-2J2/4F12 *O*-dealkylase activity (nmol/min/nmol P450), respectively. Each point and bar represent mean ± S.D. of triplicate determinations. The right graphs depict the second plots obtained from slope of the Lineweaver-Burk plots versus inhibitor concentration.



Supplemental Fig. 4. Requirement for NADPH in inactivation of CYP2J2 by azelnidipine. Recombinant CYP2J2 was preincubated with azelnidipine (0 or 0.2 μM) in the presence or absence of NADPH for 9 min. Data are expressed as the mean ± S.D. (n = 4). The statistical significance was evaluated by Bonferroni's test.



Supplemental Fig. 5. Relationship between *U* energy values and IC_{50} valu