ABSTRACT:
The inhibitory effects of six commonly used calcium channel blockers on three major cytochrome P-450 activities were examined and characterized in human liver microsomes. All six compounds reversibly inhibited CYP2D6 (bufuralol 1'-hydroxylation) and CYP2C9 (tolbutamide methyl hydroxylation) activities. The IC₅₀ values for the inhibition of CYP2D6 and CYP2C9 for nicardipine were 3 to 9 μM, whereas those for all others ranged from 14 to >150 μM. Except for nifedipine, all calcium channel blockers showed increased inhibitory potency toward CYP3A activities (testosterone 6β-hydroxylation and midazolam 1'-hydroxylation) after 30-min preincubation with NADPH. IC₅₀ values for the inhibition of testosterone 6β-hydroxylation obtained in the NADPH-preincubation experiment for nicardipine (1 μM), verapamil (2 μM), and diltiazem (5 μM) were within 10-fold, whereas those for amiodipine (5 μM) and felodipine (13 μM) were >200-fold of their respective plasma concentrations reported after therapeutic doses. Similar results also were obtained based on midazolam 1'-hydroxylase activity. Unlike the observations with mibefradil, a potent irreversible inhibitor of CYP3A, the NADPH-dependent inhibition of CYP3A activity by nicardipine and verapamil was completely reversible on dialysis, whereas that by diltiazem was partially restored (80%). Additional experiments revealed that nicardipine, verapamil, and diltiazem formed cytochrome P-450 iron (II)-metabolite complex in both human liver microsomes and recombinant CYP3A4. Nicardipine yielded a higher extent of complex formation (~30% at 100 μM), and was a much faster-acting inhibitor (maximal inhibition rate constant ~2 min⁻¹) as compared with verapamil and diltiazem. These present findings that the CYP3A inhibition caused by nicardipine, verapamil, and diltiazem is, at least in part, quasi-irreversible provide a rational basis for pharmacokinetically significant interactions reported when they were coadministered with agents that are cleared primarily by CYP3A-mediated pathways.

Calcium channel blockers (CCBs)¹ have been used widely for the treatment of hypertension, angina pectoris, and other cardiovascular diseases since first introduced in the 1960s. With such widespread use, there have been a number of reports on significant pharmacokinetic and pharmacodynamic drug interactions associated with CCBs (Hunt et al., 1989; Kirch et al., 1990; Schlanz et al., 1991; Rosenthal and Ezra, 1995; Lamberg et al., 1998). Most recently, numerous cases have been reported in patients receiving mibefradil, a newly introduced CCB, which ultimately motivated the voluntary withdrawal of the compound from the market (Welker et al., 1998). Inhibition of cytochrome P-450 (P-450) activities by CCBs has been suggested as one of possible explanations for such interactions. However, little has been reported in the literature on inhibitory effects of CCBs on human P-450 activities other than those of CYP3A. Close examination of the data available on CYP3A indicated that, except for mibefradil, CCBs are not very potent P-450 inhibitors. Values for IC₅₀ or Kᵢ for inhibition of CYP3A activities in human liver microsomes ranged from ~10 μM for nicardipine to 100 μM for diltiazem (Tjia et al., 1989; Pichard et al., 1990; Wrighton and Ring, 1994; Zhao and Ishizaki, 1997). These values are over 100-fold greater than typical plasma concentrations of CCBs reported after clinical doses (Kelly and O’Malley, 1992). Recently, diltiazem has been shown to be a quasi-irreversible inhibitor of CYP3A both in vitro and in vivo in rats (Bensoussan et al., 1995). Although these results have not been confirmed with human liver microsomes, they appeared consistent with several significant drug interactions reported for diltiazem in vivo (Lin and Lu, 1998). To date, there have been limited data on mechanisms of CYP3A inhibition by other CCBs in animals or humans.

Chemically, CCBs (Fig. 1) are classified into three classes, benzothiazepines (e.g., diltiazem), dihydropyridines (e.g., amiodipine, felodipine, nicardipine, and nifedipine), and phenylalkylamines (e.g., verapamil). Like diltiazem, most of these CCBs contain an amine functional group and undergo N-dealkylation; both features are common for metabolic intermediate (MI) complexing agents such as diltiazem and several other amine-containing compounds (Pershing and Franklin, 1982; Bensoussan et al., 1995). However, possible formation of such a complex has not been reported for any of the amine-containing phenylalkylamines and dihydropyridines.

In this study, we examined and characterized the in vitro inhibition profiles of six commonly used CCBs (amiodipine, diltiazem, felodipine, nicardipine, nifedipine, and verapamil) on three major P-450 isozymes (CYP3A, CYP2D6, and CYP2C9) in human liver microsomes. Mibefradil, the CCB recently shown to be a potent mechanism-based inhibitor of CYP3A (Prueksaritanont et al., 1999), also was included in the study for comparison. In addition, the ability of

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¹Abbreviations used are: CCBs, calcium channel blockers; P-450, cytochrome P-450; MI, metabolic intermediate; TAO, troleandomycin.
diltiazem, as well as the amine-containing dihydropyridine nicardipine and phenylalkylamine verapamil, to form the MI complex with human P-450 enzymes was examined using human liver microsomes and recombinant P-450 enzymes.

Materials and Methods

Chemicals and Reagents. Testosterone, midazolam, tolbutamide, diltiazem, nicardipine, nifedipine, verapamil, trofandomycin (TAO), 17α-ethylnylestradiol, quinidine, sulfaphenazole, and NADPH were purchased from Sigma Chemical Co. (St. Louis, MO), whereas 6β-hydroxytestosterone and ketoconazole were purchased from Steraloids Inc. (Wilton, NH) and Research Diagnostics Inc. (Flanders, NJ), respectively. 3-Methylhydroxytolbutamide, 1′-hydroxymidazolam, bufuralol, and 1′-hydroxybufuralol were obtained from Ultrafine Chemicals (Manchester, England). Felodipine and amlopidine were obtained in house (Merck Research Laboratories, Rahway, NJ). Human liver microsomes pooled from 10 or 15 subjects were obtained from the International Institute for the Advancement of Medicine (Exton, PA) and In Vitro Technologies Inc. (Baltimore, MD). Human liver microsomes known to contain high levels of CYP3A activity (used in the P-450-iron (II)-metabolite complex formation experiment) were obtained from Gentest Corp. (Woburn, MA). Microsomes prepared from insect cells with cDNAs of human CYP3A4 and NADPH-dependent reductase coexpressed were prepared and characterized at Merck Research Laboratories (West Point, PA).

Assays for P-450 Activities. Assays for CYP3A (testosterone 6β-hydroxylation and midazolam 1′-hydroxylation), CYP2D6 (bufuralol 1′-hydroxylation), and CYP2C9 (tolbutamide methyl hydroxylation) activities were described previously (Prueksaritanont et al., 1996). Testosterone, midazolam, tolbutamide, and bufuralol were used at concentrations (50, 100, and 10 μM, respectively) comparable to their reported K_m values. Human liver microsomes were preincubated with CCBs for 30 min at 37°C, either in the presence or absence of 1 mM NADPH, before assaying for P-450 activities. Known selective inhibitors for P-450 CYP3A (TAO and ketoconazole), CYP2D6 (quinidine), and CYP2C9 (sulfaphenazole) were included as positive controls. Time- and concentration-dependent inhibition of testosterone 6β-hydroxylation was performed by preincubating human liver microsomes with CCBs in the presence of 1 mM NADPH for up to 45 min at 37°C. The reaction mixtures were then diluted 5-fold for the determination of testosterone 6β-hydroxylase activity, using 250 μM testosterone.

Dialysis Experiment. Human liver microsomes (0.5 mg) were incubated with 1 mM NADPH and CCBs or known CYP3A inhibitors for 30 min at 37°C, in a final volume of 0.5 ml. Incubation mixtures were immediately transferred in to “slide-a-lyzer” dialysis cassettes (10,000 molecular weight cutoff; Pierce, Rockford, IL) and dialyzed against 1.5 liters of 0.1 M sodium phosphate buffer containing 5 mM sodium EDTA, pH 7.4, for 16 h. The dialysis buffer was changed once after 4 to 6 h. Undialyzed and dialyzed samples were diluted 5-fold and determined for their testosterone 6β-hydroxylase activities using 250 μM testosterone. Protein contents of dialyzed samples were subsequently measured using Lowry’s method (Lowry et al., 1951).

Formation of P-450-Iron (II)-Metabolite Complexes. Spectral differences (400 to 500 nm) between the reference and sample cuvettes were obtained using Perkin Elmer UV 20 double-beam UV-visible spectrophotometer. Incubation mixtures containing 1 mg/ml (P-450 content = 0.53 nmol/mg protein) human liver microsomes or 0.25 μM insect cell expressed recombinant CYP3A4, 0.1 M sodium phosphate, pH 7.4, 10 mM magnesium chloride, and 1 mM NADPH were placed in both cuvettes. CCBs were added at a final concentration of 0.1 mM to the sample cuvette and incubated at room temperature. Spectral differences were monitored every 4 min for up to 28 min. To prevent carbon monoxide complex formation (Franklin, 1991), the incubation mixture in the sample cuvette was bubbled with air briefly every 10 min. The extent of P-450-iron (II)-metabolite complex formed was quantified based on a previously reported extinction coefficient (455–490 nm) value of 64,000 M⁻¹ cm⁻¹ (Pershing and Franklin, 1982; Franklin, 1991). In the experiment with CYP3A4, spectral differences also were monitored after the addition of potassium ferricyanide (50–200 μM).

Data Analysis. The concentration of CCBs producing a 50% decrease in the activities of P-450 (IC50) values were estimated using nonlinear regression analysis (PCNONLIN; Scientific Consulting, Cary, NC), based on the following relationship:

\[ E = E_{\text{max}} \times \left[ 1 - \left( \frac{C}{C + IC_{50}} \right) \right] \]

where E and E_{\text{max}} are the effects measured in the presence of CCBs (at concentration C) and in the absence of CCBs, respectively.

Results

Inhibitory Effects of CCBs on CYP3A Activity. Concentration-dependent inhibitory effects of the six CCBs as well as those of known inhibitors of testosterone 6β-hydroxylation in the absence and presence of NADPH during the 30-min preincubation period are shown in Table 1. Both ketoconazole and TAO produced inhibitory profiles consistent with their mechanisms of inhibition. The potent reversible inhibitor ketoconazole did not show an increased inhibitory effect after preincubation with NADPH (Table 1). In fact, a decreased inhibition was observed (~3-fold, Table 1). This was consistent with the fact that ketoconazole is a substrate for CYP3A. Also as expected, the quasi-irreversible inhibitor TAO showed increased inhibitory potency when preincubated in the presence of NADPH (Fig. 2, A and B; Table 1). In addition, IC50 values obtained in the present study for both ketoconazole and TAO agreed well with those reported previously (Wrighton and Ring, 1994; Ealing et al., 1998; McKillop et al., 1998).

Among the six CCBs examined and after preincubation with NADPH, nicardipine was the most potent inhibitor (IC50 = 1.7 μM) of testosterone 6β-hydroxylation activity. All other CCBs showed comparable inhibitory potencies, with IC50 values ranging from ~20 to ~80 μM (Fig. 2A, Table 1). The IC50 values of verapamil, diltiazem, and nifedipine for 6β-testosterone hydroxylase were comparable to those reported earlier using cyclosporin, quinine, or midazolam as a CYP3A3 probe (Tjia et al., 1989; Pichard et al., 1990; Wrighton and Ring, 1994; Sutton et al., 1997; Zhao and Ishizaki, 1997). With the exception of nifedipine, the inhibition effects of all CCBs were increased (from 2-fold for nicardipine to >10-fold for verapamil and diltiazem) when preincubated in the presence of NADPH, similar to the observation with mibefradil (Fig. 2B, Table 1). In the preincubation experiment with NADPH, nicardipine also was the most potent inhibitor (IC50 = ~0.9 μM), whereas nifedipine was the least potent inhibitor (IC50 = ~30 μM). Under similar conditions, the IC50 value for mibefradil was 0.3 μM (Prueksaritanont et al., 1999).

The inhibitory profiles of these CCBs and the known inhibitors on midazolam 1′-hydroxylation activity, another commonly used CYP3A3 marker, were very similar to those of testosterone 6β-hydroxylase activity (Table 1). In most cases, values for IC50 obtained for the two
markers were within 2-fold of each other (Table 1). This similarity in the IC_{50} values, which were obtained at their \( K_m \) values, were consistent with the fact that both markers are substrates for CYP3A. As was observed with testosterone 6β-hydroxylation, nicardipine also was more potent than the other CCBs tested, but was less potent than mibefradil in inhibiting midazolam 1'9-hydroxylation.

### Inhibitory Effects of CCBs on CYP2D6 Activity

Unlike the observations on CYP3A activity, none of the CCBs examined showed increases in inhibitory potencies or decreases in IC_{50} values toward CYP2D6 activity (bufuralol 1'9-hydroxylase) after preincubation with NADPH (Table 2). Among the CCBs studied, the most potent inhibitor was nicardipine (IC_{50} = 2.8 μM) and diltiazem (IC_{50} = 6.8 μM), respectively (Table 2). The inhibitory potencies of verapamil, amlodipine, felodipine, and nifedipine were comparable, with IC_{50} values ranging between 40 and 70 μM. The results obtained with nicardipine were similar to those reported previously (Fonne-Pfister and Meyer, 1988). Under the present conditions, the IC_{50} value (0.06 μM) of quinidine, a known CYP2D6-selective inhibitor, on CYP2D6 activity also was comparable to that reported earlier (Holland et al., 1995).

### Inhibitory Effects of CCBs on CYP2C9 Activity

Similar to the above findings on CYP2D6 activity, inhibitory effects of the CCBs on CYP2C9 activity (tolbutamide methyl hydroxylase) were not increased by preincubation in the presence of NADPH (Table 3). In either experiment, nicardipine was the most potent inhibitor (IC_{50} = 0.5 μM). The known CYP2C9-selective inhibitor sulfaphenazole yielded an IC_{50} value of 0.5 μM.

### Characterization of CYP3A Inhibition

To characterize the apparent NADPH-dependent inhibition observed on CYP3A activity, nicardipine, verapamil, and diltiazem were chosen for additional studies. As shown in Fig. 3, the inhibition of testosterone 6β-hydroxylation by nicardipine, verapamil, and diltiazem was both time- and
concentration-dependent. Nicardipine was a fast-acting inhibitor, whereas diltiazem was a much slower inhibitor. Values for the maximal inhibition rate constant and apparent $K_i$ of nicardipine, verapamil, and diltiazem were estimated, based on reciprocal plots of the initial inhibition rate constant and CCB concentration, to be 2, 0.03, and 0.01 min$^{-1}$ and 0.6, 0.5, and 0.5 μM, respectively.

Reversibility of CYP3A Inhibition. Reversibility of the inhibition of CYP3A activity by nicardipine, verapamil, and diltiazem was examined by dialysis. As expected, the activity of CYP3A that was inhibited by all of the inhibitors examined after preincubation without NADPH was virtually restored by dialysis (Table 4). In the experiment with NADPH present during preincubation, CYP3A activity inhibited by nicardipine and verapamil also was fully recovered after dialysis (Table 4). However, dialysis only partially (~80%) restored the inhibited CYP3A activity by diltiazem (Table 4). In a parallel experiment with mibebradil and ethynylestradiol, both potent irreversible inhibitors of CYP3A (Guengerich, 1988; Pruksaritanont et al., 1999), only 14 to 31% of the control activity was recovered after dialysis (Table 4).

Formation of P-450-Metabolite Complex. In vitro studies were further conducted to investigate whether the NADPH-dependent inhibition of CYP3A activity by nicardipine, verapamil, and diltiazem was via the formation of MI complex. The three CCBs all contain an amine functional group, formed P-450-iron (II)-metabolite complex, as evident by a Soret peak at around 455 nm (Buening and Franklin, 1976; Pershing and Franklin, 1982; Bensoussan et al., 1995) when incubated with human liver microsomes in the presence of NADPH (Fig. 4, A–C). Under the conditions used (which yielded ~40% complex formation for TAO, data not shown), the extent of complex formation was estimated to be ~30, 20, and ~6% for nicardipine, diltiazem, and verapamil, respectively. Similar results also were observed with recombinant CYP3A4 (Fig. 4, A–C), but not CYP2D6 or CYP2C9 (data not shown). Addition of ferrocyanide reduced absorbance at the maxima, consistent with dissociation of the complex and regeneration of P-450-iron (III) (Fig. 4, A–C). These results are in agreement with the aforementioned NADPH-dependent inhibition of nicardipine, diltiazem, and verapamil on CYP3A, and not CYP2D6 or CYP2C9 activity, and supported that this inhibition was due, in part, to the complex formation between their metabolites and CYP3A.

Discussion

Mechanisms of CYP inhibition of a compound can be divided into three categories: reversible, quasi-irreversible, and irreversible (Lin and Lu, 1998). Quasi-irreversible and irreversible inhibitors require at least one cycle of the CYP catalytic process, and are thus signified by both NADPH- and time-dependent inhibition. Experimentally, mechanisms of inhibition of inhibitors could be assessed initially by comparing their inhibitory effects obtained in the presence and absence of NADPH during a preincubation period. In the present study, the IC$_{50}$ values obtained for all CCBs tested on CYP2D6 or CYP2C9 activity in the presence of NADPH were not lower than those observed in the absence of NADPH during preincubation. This NADPH-independent inhibition suggests that all six CCBs are not quasi-irreversible or irreversible, but rather reversible inhibitors for these isozymes. In contrast, except for nifedipine, all CCBs tested showed increased inhibitory potencies (up to 16-fold) toward CYP3A activities when preincubated in the presence of NADPH. The results suggest that these CCBs may be converted, at least in part, to reactive intermediates or products that contribute to the overall inhibition, either reversible, quasi-irreversible, or irreversible, of CYP3A activities.

To characterize this apparent NADPH-dependent CYP3A inhibition, nicardipine, verapamil, and diltiazem, representatives of dihydropyridines, phenylalkylamines, and benzothiazepines, respectively, were selected for additional studies. Based on the finding that NADPH-dependent inhibition was completely reversible on dialysis (Table 4), both nicardipine and verapamil are not irreversible inactivators for human CYP3A. The finding of ~20% irreversible inhibition on dialysis after preincubation of diltiazem with NADPH (Table 4) suggests that diltiazem might act in part as an irreversible inhibitor of CYP3A. A possibility also exists that highly potent metabolite(s) of diltiazem, such as N-desmethyl diltiazem and N, N-didesmethyl diltiazem (Sutton et al., 1997), due possibly to tight binding, could remain in the incubation mixture after the 16-h dialysis period.

Subsequent studies showed that nicardipine, verapamil, and diltiazem were able to form P-450-iron (II)-metabolite complex in human liver microsomes and in recombinant human CYP3A4, indicating that they are quasi-irreversible inhibitors of CYP3A4. These results were not unexpected considering that N-desmethylated metabolites, all shown to be mediated by CYP3A (Kroemer et al., 1993; Sutton et al., 1997; Fukunaga et al., 1998), have been observed after administration of the three CCBs, and that N-dealkylation of a secondary or tertiary amine has been suggested as the first step leading to formation of P-450-iron (II)-metabolite complex. The smaller extent of the complex formation observed with verapamil (~6%) as compared with that obtained with nicardipine (30%) or diltiazem (20%) suggests that the MI complex formation contributed relatively less to the overall NADPH-dependent inhibition for verapamil than for nicardipine or diltiazem. Considering that verapamil exhibited lower IC$_{50}$ values (Table 1) and higher maximal inhibition rate constant/K$_{i}$ ratio than diltiazem, the relatively low level of MI complex observed for verapamil suggests that metabolite(s) of verapamil might also be potent, but reversible inhibitor(s). It is also interesting to point out that the
metabolite complexes observed with these CCBs were relatively unstable on dialysis because virtually complete restoration of testosterone 6β-hydroxylase activity was observed after dialysis in the case of nicardipine and verapamil (Table 4). A similar observation was also obtained in our laboratory with TAO, a known P-450-metabolite complex-forming agent (Table 4). It is presently unclear whether the P-450-iron (II)-metabolite complex formed by diltiazem was more stable than those complexes formed by nicardipine, verapamil, or TAO.

Based on the above observations and considering typical plasma concentrations of ~0.1 to 0.4 μM for nicardipine, verapamil, diltiazem, and nifedipine, and of ~5 to 50 nM for amlodipine and felodipine (Kelly and O’Malley, 1992), all CCBs tested, with the exception of nicardipine, could be considered as weak reversible inhibitors for CYP2D6 and CYP2C9. Significant degree of metabolic inhibition on CYP2D6 and CYP2C9 activities may not be expected after a therapeutic dose of verapamil, diltiazem, nifedipine, amlodipine, or felodipine. However, the present study suggested, based on their IC_{50} values (0.9–5 μM) obtained in the presence of NADPH preincubation relative to their therapeutic concentrations (0.1–0.4 μM), that nicardipine, verapamil, and diltiazem are relatively potent inhibitors of CYP3A in humans. Inhibition of CYP3A activities likely contributed, at least in part, to the previously observed decreased clearances or increased plasma concentrations of CYP3A substrates after concomitant administration with nicardipine, diltiazem, and verapamil (Kirch et al., 1990; Schlanz et al., 1991; Rosenthal and Ezra, 1995; Azie et al., 1998; Kantola et al., 1998; Lamborg et al., 1998). The above conclusion is additionally supported by the present finding that the three CCBs are quasi-irreversible inhibitors of CYP3A, eliciting inhibitory effects, in part, via MI complex. In vivo, such a complex is known to be so stable that the CYP involved in the complex formation would be unavailable for drug metabolism. As a result, the inhibitory effects of quasi-irreversible inhibitors are more prominent after multiple dosing and last longer than those of reversible inhibitors (Murray and Reidy, 1990; Lin and Lu, 1998). Consistent with these, there have been several cases of significant interactions noted after repeated doses of nicardipine, verapamil, or diltiazem with CYP3A substrates as compared with after other CCBs (Kirch et al., 1990; Schlanz et al., 1991; Rosenthal and Ezra, 1995; Azie et al., 1998; Kantola et al., 1998; Lamborg et al., 1998). The relatively fewer cases of pharmacokinetic interactions reported for felodipine and amlodipine also appeared to be consistent with the present finding that their IC_{50} values obtained in the presence of NADPH were >200-fold higher than reported plasma concentrations after a therapeutic dose. Noteworthy, the potent and irreversible CYP3A inhibitor mibefradil, which was recently withdrawn from the market due to drug-interaction potential, exhibited lower IC_{50} value (0.3 μM) relative to its therapeutic concentrations (0.5–1 μM) than all six CCBs studied (Prueksaritanont et al., 1999).

To conclude, the present results revealed that among six CCBs tested, only nifedipine was a reversible inhibitor of CYP3A, CYP2D6, and CYP2C9. All other CCBs reversibly inhibited CYP2D6 and CYP2C9, but not CYP3A activities. Based on ratios between IC_{50} values obtained in the presence of NADPH during preincubation and the respective therapeutic concentrations, nicardipine, verapamil, and diltiazem are relatively potent inhibitors of CYP3A. In addition, the three CCBs inhibited CYP3A activities, at least in part, through the formation of MI complex, and thus are classified as quasi-irreversible inhibitors. The results provide a rational basis for significant pharmacokinetic interactions reported between these CCBs and P-450 substrates, and support the notion that an understanding of the underlying

### Table 4: Inhibitory effects of CCBs on testosterone 6β-hydroxylase activity in human liver microsomes before and after dialysis

<table>
<thead>
<tr>
<th>Initial Concentration</th>
<th>μM</th>
<th>Without NADPH</th>
<th>With NADPH</th>
<th>Without NADPH</th>
<th>With NADPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>100 ± 0.5</td>
<td>100 ± 1</td>
<td>100 ± 0.8</td>
<td>100 ± 1</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>2</td>
<td>66 ± 0.8</td>
<td>20 ± 0.5</td>
<td>91 ± 0.8</td>
<td>116 ± 1</td>
</tr>
<tr>
<td>Verapamil</td>
<td>10</td>
<td>91 ± 1</td>
<td>24 ± 0.4</td>
<td>101 ± 2</td>
<td>111 ± 1</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>25</td>
<td>98 ± 1</td>
<td>47 ± 0.3</td>
<td>101 ± 3</td>
<td>79 ± 1</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>1</td>
<td>22 ± 1</td>
<td>32 ± 0.4</td>
<td>85 ± 0.2</td>
<td>93 ± 3</td>
</tr>
<tr>
<td>Mibefradil</td>
<td>2</td>
<td>87 ± 2</td>
<td>5 ± 0.4</td>
<td>96 ± 1</td>
<td>14 ± 0.4</td>
</tr>
<tr>
<td>Ethynylestradiol</td>
<td>50</td>
<td>54 ± 1</td>
<td>8 ± 0.3</td>
<td>93 ± 1</td>
<td>31 ± 0.2</td>
</tr>
<tr>
<td>TAO</td>
<td>50</td>
<td>86 ± 1</td>
<td>14 ± 0.2</td>
<td>98 ± 0.7</td>
<td>102 ± 2</td>
</tr>
</tbody>
</table>

![Figure 4](image-url)
mechanism of inhibition is important to provide valuable insights into drug-drug interactions observed in vivo (Lin and Lu, 1998).

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References


