Decreased Susceptibility of the Cytochrome P450 2B6 Variant K262R to Inhibition by Several Clinically Important Drugs

Jyothi C. Talakad, Santosh Kumar, and James R. Halpert

Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, California

Received July 30, 2008; accepted December 5, 2008

ABSTRACT:

Cytochrome P450 (P450) 2B6 metabolizes a number of clinically relevant drugs and is one of the most highly polymorphic human P450 enzymes, with the Lys262->Arg substitution being especially common in several genetic variants. Therefore, K262R (2B6*4) was created in the CYP2B6dH background (N-terminal-modified and C-terminal His-tagged) and expressed in Escherichia coli. The recombinant CYP2B6dH and K262R were purified and studied to investigate the effect of the Lys262->Arg substitution with six of the most potent drug inhibitors of CYP2B6, namely, clopidogrel, clotrimazole, itraconazole, raloxifene, sertraline, and ticlopidine. K262R showed a >3-fold increase in the \( K_I \) values with clopidogrel, clotrimazole, itraconazole, raloxifene, and ticlopidine, and 6 to 7-fold increase in \( K_I \) compared with CYP2B6dH. Likewise, K262R showed 2-, 4-, and >20-fold higher \( K_I \) values than CYP2B6dH with clotrimogrel, sertraline, and itraconazole, respectively. In contrast, when tested with several known type II inhibitors of CYP2B enzymes, K262R showed a 10-fold lower \( IC_{50} \) with 4-(phenyl)pyridine and 2- to 5-fold lower \( IC_{50} \) with 1-(4-nitrobenzyl)pyridine or 1-(4-phenyl)benzimidazole than CYP2B6dH. Subsequent analysis predicted possible in vivo drug-drug interactions between the CYP2B6 substrate efavirenz and drug inhibitors clopidogrel, clotrimazole, itraconazole, sertraline, and ticlopidine. Furthermore, Q172H/K262R (2B6*6), which is the most common genetic variant of CYP2B6 harboring K262R, was created in CYP2B6dH, expressed, purified, and characterized for inhibition. Q172H/K262R showed a >6-fold increase in \( K_I \) with sertraline and clopidogrel compared with CYP2B6dH. The results suggest that individuals, especially homozygotes, with the 2B6*4 or 2B6*6 allele might be less susceptible to drug interactions resulting from P450 inhibition.

Although cytochrome P450 (P450) 2B6 (CYP2B6) is expressed at relatively low levels in the liver (Guengerich, 2005), the enzyme metabolizes important pharmaceuticals including cyclophosphamide, propofol, promazine, methadone, S-mephénytoïn, efavirenz, bupropion, imipramine, midazolam, artemisinin, and tamoxifen (Rendic, 2002; Lewis et al., 2004; Zanger et al., 2007). In addition, CYP2B6 possesses several important genetic variants; among them the most common are K262R (2B6*4), Q172H/K262R (2B6*6), and R487C (2B6*5). Frequencies of the three most common single nucleotide polymorphisms range from 14 to 49% for Q172H, 17 to 63% for K262R, and 0 to 14% for R487C depending on the ethnicity of the population studied (Lang et al., 2004). For example, studies in German man males have found a K262R allele frequency of approximately 5% and a single nucleotide polymorphism frequency of 30% (Lang et al., 2001; Kirchheiner et al., 2003). Single-dose bupropion pharmacokinetic data obtained from 121 individuals showed 1.3-fold increased clearance by individuals with the 2B6*1/*4 genotype (Kirchheiner et al., 2003). A similar study of 169 individuals with efavirenz showed a 17% reduced area under plasma concentration in *1/*4 heterozygotes (Rotger et al., 2007). In vitro, K262R has been incorporated into the engineered CYP2B6dH (N-terminal-deleted and C-terminal His-tag) by Hollenberg’s group to study structure function. Compared with CYP2B6dH, K262R shows a >2-fold increased \( k_{cat} \) for the metabolism of bupropion to hydroxybupropion (Bumpus et al., 2005) and >2-fold increased catalytic efficiency for the metabolism of efavirenz to 8-hydroxyefavirenz (Bumpus et al., 2006). It is interesting to note that, in contrast to CYP2B6dH, K262R is refractory to mechanism-based inactivation by 17α-ethynylestradiol or efavirenz, whereas susceptibility of the variant to inactivation is preserved with bergamottin, \( N,N',N''-\)triethylenethiophosphoramide, and 8-hydroxyefavirenz (Bumpus et al., 2005, 2006).

Drug-drug interactions (DDIs), especially through inhibition of P450-mediated drug metabolism by a coadministered drug, are one of the primary causes of serious adverse events occurring in clinical practice (Dambró and Kallgren, 1988). The presence of polymorphic variants of P450 further complicates the prediction of in vivo DDIs. To address the issues of genotype-dependent DDIs as a result of

ABBREVIATIONS: P450, cytochrome P450; DDI, drug-drug interaction; 7-MFC, 7-methoxy-4-(trifluoromethyl)coumarin; CYMAL-5, 5-cyclohexylpentyl-β-D-maltoside; CSM, conserved sequence motif.
enzyme inhibition, Kumar et al. (2006) have used five substrates and a battery of inhibitors with CYP2C9.1 and CYP2C9.3 variants. In a recent study, several clinically relevant drugs, such as clopidogrel, clotrimazole, itraconazole, ticlopidine, sertraline, and raloxifene, have been found to be potent inhibitors of CYP2B6 (Walsky et al., 2006). Therefore, in the present study we have studied the effect of the Lys²⁶²→Arg substitution in K262R (2B6*4) and Q172H/K262R (2B6*6) genetic variants on the susceptibility of CYP2B6dH to inhibition by important clinical drugs, and predicted the effect on metabolism of the marker CYP2B6 substrate efavirenz in vivo.

Materials and Methods

Materials. 7-Methoxy-4-(trifluoromethyl) coumarin (7-MFC) and 7-hydroxy-4-trifluoromethylcoumarin were purchased from Invitrogen (Carlsbad, CA). NADPH, drug compounds, and most of the pyridine and imidazole inhibitors were bought from Sigma-Aldrich (St. Louis, MO). 5-Cyclohexylpentyl-β-d-maltoside (CYMAL-5) was from Anatrace (Maumee, OH). Recombinant NADPH P450 reductase and cytochrome b5 from rat liver were prepared as described previously (Harlow et al., 1997). Oligonucleotide primers for polymerase chain reaction were obtained from Sigma-Genosys (The Woodlands, TX). The molecular chaperone plasmid pGro7, which expresses GroES/EL (Nakajima et al., 1994), was obtained from TAKARA BIO (Shiba, Japan). The QuiChange Site-Directed Mutagenesis kit was obtained from Stratagene (La Jolla, CA). Nt-AFFinity resin was purchased from QIA-GEN (Valencia, CA). All of the other chemicals were of the highest grade available and were obtained from standard commercial sources.

Mutagenesis, Expression, and Purification. To create K262R, CYP2B6dH was used as the template, and the forward and reverse primers were 5′-CCCCGGGCCCCGGAGTTATCCTACGAC-3′ and 5′-GTGGATGAGGTC-TCTTGCAGGGTGGC-3′, respectively. For Q172H/K262R, K262R was used as the template, and the forward and reverse primers were 5′-ACCTTCCTTCATTTCATTCCAGG-3′ and 5′-CCGCGGGAATTCGGATGAGGTC-GAGGAAAGT-3′, respectively. The resulting constructs were sequenced to verify the desired mutations and absence of unintended mutations (K262R and K272H/K262R were analyzed at Protein Chemistry Laboratory, University of Texas Medical Branch, Galveston, TX and Retrogen, Inc., San Diego, CA, respectively). CYP2B6dH, K262R, and Q172H/K262R were coexpressed with GroES/EL in JM109 cells (Stratagene) as described previously (Kumar et al., 2007). The proteins were then extracted and purified by modifying the recently described procedure (Kumar et al., 2007). In brief, the cell extract was loaded onto Ni-NTA resin in the presence of the detergent CYMAL-5. Protein was eluted with 10 mM KPi, pH 7.4, containing 100 mM NaCl, 20% glycerol, 10 mM β-mercaptoethanol, 0.5 mM phenylmethylsulphonyl fluoride, and 40 mM histidine. K262R was added to 4.8 mM, and the sample was subsequently loaded onto a CM-Sepharose column. After washing the CM-Sepharose column using 10 mM KPi buffer containing 0.2 mM DTT, 1 mM EDTA, 20% glycerol, and 100 mM NaCl, the protein was eluted using 500 mM NaCl in the above buffer. Eluted protein was dialyzed against 10 mM KPi buffer containing 10% glycerol and 1 mM EDTA with three changes. The P450 content was measured by reduced CO-difference spectra. Protein concentrations were determined using the Bradford protein assay kit (Bio-Rad, Hercules, CA).

Enzyme Inhibition. 7-MFC O-deethylation was measured in a final reaction volume of 100 μl as described earlier (Osegwen et al., 2008). In brief, the reaction mixture contained 150 μM 7-MFC in the standard reconstitution system (P450/NADPH P450 reductase/cytochrome b5, 1:4:2) at 5 pmol of P450 in 50 mM Hepes, pH 7.4, 15 mM MgCl₂, and 2% MeOH. The reaction was performed at 37°C for 5 min using 1 mM NADPH. Nonlinear regression analysis was performed to fit the data using a four-parameter logistic function to derive the IC₅₀ values for all the imidazole and pyridine derivatives. The IC₅₀ values were determined using the 7-MFC O-deethylation assay in a final reaction volume of 100 μl at 0.5 to 5.0 μM drug concentrations and 0 to 50 μM substrate concentrations. For all the inhibition studies, 10 pmol of P450 was used. The Kᵢ was determined using global fit for competitive inhibition from SpectraLab (Davydov et al., 1995). Inhibition and Spectral Binding of CYP2B6dH and K262R by Drugs. To investigate the effect of the Lys²⁶²→Arg substitution on the interaction with the most potent drug inhibitors of CYP2B6 (Walsky et al., 2006), we selected clopidogrel, ticlopidine, clotrimazole, itraconazole, sertraline, and raloxifene (Supplemental Fig. 2). The results are presented in Fig. 1 and Table 1. The Kᵢ values were determined for competitive inhibition of CYP2B6dH and K262R by the six drugs using 7-MFC at concentrations up to 50 μM and 0 to 20 μM inhibitor concentrations. The Kᵢ and Kₚ values for 7-MFC oxidation were 3.8/min and 5.1 μM for CYP2B6dH and 4.9/min and 5.0 μM for K262R, respectively. CYP2B6dH and K262R showed similar Kᵢ values with clotrimazole and ticlopidine (Fig. 1; Table 1). However, K262R showed a >3-fold increase in the Kᵢ values with clopidogrel, itraconazole, and raloxifene compared with CYP2B6dH. In addition, K262R showed ~6-fold increase in Kᵢ with sertraline compared with CYPB6dH.

Clotidogrel, ticlopidine, itraconazole, and sertraline induced type I difference spectra with a peak at ~388 nm and a trough at ~420 nm (Fig. 2, inset). CYP2B6dH and K262R showed similar ΔAₘₐₓ values with clotidogrel, itraconazole, and ticlopidine, whereas the ΔAₘₐₓ with sertraline was 3-fold higher in K262R than the wild-type. Compared with CYPB6dH, K262R showed approximately 2-, 4-, and 20-fold higher Kᵢ values with clopidogrel, sertraline, and itraconazole, respectively (Fig. 2; Table 1). No significant changes were observed in the Kᵢ values of ticlopidine between CYPB2B6dH and K262R. However, the experiment could not be performed with raloxifene because it interfered with the measurement of the type I spectral

Results

Expression and Thermal Stability of CYP2B6dH and K262R. Heterologous expression of CYP2B6 and genetic variants such as M46V, G99E, K139E, Q172H, K262R, R140Q, and I391N in COS-1 cells yielded lower P450 expression, suggesting decreased P450 stability (Lang et al., 2004). Therefore, we investigated P450 expression of K262R (2B6*4) as described previously (Kumar et al., 2007). The expression of K262R in Escherichia coli under our standard conditions was ~1.5-fold higher than CYP2B6dH. However, the thermal stability (Tₘ₀) of K262R was 2°C lower than CYP2B6dH (Supplemental Fig. 1). The results suggest no major difference in the expression or stability of K262R compared with the wild-type. In this study, we used the dH construct because it shows much higher bacterial expression and solubility and more facile purification than the full-length wild-type.

Inhibition and Spectral Binding of CYP2B6dH and K262R by Drugs. To investigate the effect of the Lys²⁶²→Arg substitution on the interaction with the most potent drug inhibitors of CYP2B6 (Walsky et al., 2006), we selected clopidogrel, ticlopidine, clotrimazole, itraconazole, sertraline, and raloxifene (Supplemental Fig. 2). The results are presented in Fig. 1 and Table 1. The Kᵢ values were determined for competitive inhibition of CYP2B6dH and K262R by the six drugs using 7-MFC at concentrations up to 50 μM and 0 to 20 μM inhibitor concentrations. The Kᵢ and Kₚ values for 7-MFC oxidation were 3.8/min and 5.1 μM for CYP2B6dH and 4.9/min and 5.0 μM for K262R, respectively. CYP2B6dH and K262R showed similar Kᵢ values with clotrimazole and ticlopidine (Fig. 1; Table 1). However, K262R showed a >3-fold increase in the Kᵢ values with clotidogrel, itraconazole, and raloxifene compared with CYP2B6dH. In addition, K262R showed ~6-fold increase in Kᵢ with sertraline compared with CYPB6dH.

Clotidogrel, ticlopidine, itraconazole, and sertraline induced type I difference spectra with a peak at ~388 nm and a trough at ~420 nm (Fig. 2, inset). CYP2B6dH and K262R showed similar ΔAₘₐₓ values with clotidogrel, itraconazole, and ticlopidine, whereas the ΔAₘₐₓ with sertraline was 3-fold higher in K262R than the wild-type. Compared with CYP2B6dH, K262R showed approximately 2-, 4-, and 20-fold higher Kᵢ values with clotidogrel, sertraline, and itraconazole, respectively (Fig. 2; Table 1). No significant changes were observed in the Kᵢ values of ticlopidine between CYPB2B6dH and K262R. However, the experiment could not be performed with raloxifene because it interfered with the measurement of the type I spectral...
FIG. 1. Determination of \( K_i \) for inhibition of 7-MFC \( O \)-deethylation by CYP2B6dH and K262R in the presence of inhibitors (A–H). 7-MFC concentrations included in the assay were 2.5, 5, 10, and 50 \( \mu \)M, and the concentrations of the inhibitors used are provided in the plot. Global fitting of all the data from each experiment was used to obtain \( K_i \). The fitting was done using SpectraLab as described under Materials and Methods.
change. At lower concentration of clotrimazole, the majority of CYP2B6dH and K262R P450 was converted into P420 (data not shown).

Analysis of DDIs in CYP2B6dH and K262R. Clopidogrel, sertraline, and raloxifene are among the top 100 prescribed drugs in seniors (http://www.marylandspdap.com), who often use multiple drugs simultaneously, suggesting possible DDIs. Therefore, we analyzed possible DDIs between the established marker drug substrate of CYP2B6, efavirenz, and drug inhibitors of CYP2B6—clopidogrel, ticlopidine, clotrimazole, itraconazole, sertraline, and raloxifene. The analysis was carried out as described under Materials and Methods. The results predicted that whereas raloxifene would not alter efavirenz metabolism in vivo, clopidogrel or clotrimazole would almost completely abolish the metabolism of efavirenz (remaining activity \( \frac{1}{11005} \) and 6%, respectively) by CYP2B6 (Table 2). In addition, the metabolism of efavirenz would be reduced to 15, 48, and 57% in the presence of ticlopidine, sertraline, and itraconazole, respectively (Table 2). Although the Lys\(^{262}\) → Arg substitution would not alter the

<table>
<thead>
<tr>
<th>Drugs</th>
<th>( K_i ) CYP2B6dH</th>
<th>( K_i ) K262R</th>
<th>( K_s ) CYP2B6dH</th>
<th>( K_s ) K262R</th>
<th>( \Delta A_{max} ) CYP2B6dH</th>
<th>( \Delta A_{max} ) K262R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clopidogrel</td>
<td>0.07, 0.12(^a)</td>
<td>0.36, 0.47</td>
<td>0.16 ± 0.11(^b)</td>
<td>0.33 ± 0.14</td>
<td>0.026 ± 0.002</td>
<td>0.030 ± 0.002</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>0.15, 0.11</td>
<td>0.00, 0.17</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>1.42, 1.31, 1.13</td>
<td>4.40, 3.84, 4.34</td>
<td>0.07 ± 0.13</td>
<td>1.73 ± 0.44</td>
<td>0.022 ± 0.003</td>
<td>0.021 ± 0.002</td>
</tr>
<tr>
<td>Raloxifene</td>
<td>5.59, 2.60</td>
<td>15.8, 15.8</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Sertraline</td>
<td>0.00, 0.10</td>
<td>0.10, 0.12</td>
<td>0.51 ± 0.16</td>
<td>0.26 ± 0.61</td>
<td>0.008 ± 0.0005</td>
<td>0.02 ± 0.002</td>
</tr>
<tr>
<td>Ticlopidine</td>
<td>0.11, 0.16</td>
<td>0.00, 0.12</td>
<td>0.28 ± 0.13</td>
<td>0.32 ± 0.11</td>
<td>0.023 ± 0.001</td>
<td>0.025 ± 0.001</td>
</tr>
</tbody>
</table>

N.D., not determined.

\(^a\) \( K_i \) values are shown from each independent determination.

\(^b\) Standard errors for fit to the tight ligand binding equation. The data are representative of at least two independent determinations. The variations between the experiments are ±20%.

![Fig. 2. Representative type I difference spectra of ticlopidine, clopidogrel, sertraline, and itraconazole binding (A–D inset). The data were fit to the tight-binding equation, as described under Materials and Methods, to derive the \( K_i \) values as listed in Table 1.](image-url)
have an Arg residue at 262, suggesting a possible specific role for Gln-172 or Lys-262 in CYP2B6. A molecular model of CYP2B6dH does not predict a role of residue 262 in substrate binding, except that the side chains of Lys and Arg have different orientations. Further in silico analysis suggests that Arg-262 interacts with His-252 (G-helix), Thr-255 (G-helix), and Asp-266 (H-helix) through H-bonds. These H-bonds are not found in Lys-262, suggesting that the additional interactions in K262R contribute to altered drug binding. We have recently shown the importance of H-bonds in CYP2B4dH among the nonactive site residues Glu-149, Asn-177, Arg-187, and Tyr-190 in substrate specificity, inhibitor selectivity, and protein stability (Oezguen et al., 2008).

**Discussion**

In this study the recombinant CYP2B6dH, K262R, and Q172H/K262R provided new insights into the interactions of the enzyme with clinically relevant drugs. First, by determining Kᵢ values, we verified and extended previous findings of potent inhibition of CYP2B6 derived from IC₅₀ values in liver microsomes or S9 cells. Second, the competitive nature of the inhibition shown in our experiments was substantiated by spectral binding assays with four of the drugs, which showed typical type I spectra. Third, decreased inhibitor potency of clopidogrel, itraconazole, raloxifene, and sertraline for K262R is in contrast to increased potency of three small type II inhibitors. Fourth, the role of the nonactive site residue at position 262 in CYP2B6 is consistent with our recent conserved sequence motif analysis of P450 family 2 enzymes. Finally, the lower inhibitor potency of sertraline and clopidogrel for K262R (CYP2B6*4) alone and especially in combination with Q172H (CYP2B6*6) suggests the real possibility of a diminished genotype-dependent drug interaction in vivo.

An earlier report showed that itraconazole yields a type II spectrum with CYP3A4 and can adopt multiple orientations within the CYP3A4 active site, including a catalytically productive mode (type I) and a slowly dissociating inhibitory mode (type II) (Pearson et al., 2006). However, a type I spectrum is observed with CYP2B6dH and K262R, suggesting that the orientation of itraconazole is such that the nitrogen of the ligand is not able to coordinate with the heme iron of the protein. The difference in the mode of binding of itraconazole in CYP3A4 and CYP2B6dH may contribute to the differences in their Kᵢ values (0.019 versus 0.07 µM, respectively). A relatively large and flexible active site of CYP3A4 may facilitate the multiple modes of interactions with itraconazole.

Although molecular modeling suggests that Lys-262 in CYP2B6 does not contact ligands directly, this residue is found within a conserved sequence motif (CSM) in P450 family 2 enzymes (261PRD-FIDVY268). This motif (CSM 11) is only present in the CYP2B and CYP2C subfamilies, where it has a very high rank order of conservation (Oezguen et al., 2008). In addition, analysis of the individual residues showed that Arg-262 is among the most conserved residues within CSM 11, further suggesting its functional and/or structural importance. It is interesting to note that Leu-264 is also among the most conserved residues within the motif (Oezguen et al., 2008), and a Leu²⁶⁴→Phe substitution in CYP2B6dH enhances P450 expression and thermal stability (Kumar et al., 2007).

Because ticlopidine and clopidogrel have been shown to be mechanism-based inactivators of CYP2B6-catalyzed efavirenz hydroxylation (Richter et al., 2004; Walsky and Obach, 2007), an in vivo DDI between efavirenz and the inhibitors might be even more pronounced than indicated based on competitive inhibition alone. The area under plasma concentration ratio of hydroxybupropion to bupropion was reduced by 68 and 90% in the presence of clopidogrel and ticlopidine, respectively, compared with the control, which suggests that both

---

**TABLE 2**

Potential in vivo DDIs between efavirenz with drug inhibitors

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Estimated in Vivo C₅₀ *</th>
<th>Predicted in Vivo Activity *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CYP2B6dH</td>
<td>K262R</td>
</tr>
<tr>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIL</td>
<td>---</td>
<td>2.05(100)</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>9.3</td>
<td>0.04(1.9)</td>
</tr>
<tr>
<td>Clofibrate</td>
<td>3.7</td>
<td>0.13(6.3)</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>1.9</td>
<td>1.16(57)</td>
</tr>
<tr>
<td>Raloxifene</td>
<td>0.003</td>
<td>2.06(100)</td>
</tr>
<tr>
<td>Sertraline</td>
<td>0.62</td>
<td>0.99(48)</td>
</tr>
<tr>
<td>Ticlopidine</td>
<td>1.6</td>
<td>0.30(15)</td>
</tr>
</tbody>
</table>

* C₅₀ of the inhibitors and substrate were obtained from the following literature sources: www.medscapes.com/ (raloxifene); www.pharmkgb.org/ (itraconazole, clopidogrel, and efavirenz); Burgess and Bodey (1972) (clofibrate).

* Predicted in vivo activity was determined using the equation for competitive inhibition. The values for [S] and [I] correspond to the estimated in vivo C₅₀ of efavirenz (13.0 µM) and drug inhibitor, respectively. The Kᵢ values were taken from Table 1, whereas Kₑ and Kᵢₑ values were taken from a previous study (Bumps et al., 2006). Predicted activities for K262R assume that both CYP2B6 alleles are the variant.

* The values in parenthesis indicate the percentage activity.
in the assay were 25, 50, 100, and 150 μM, and the concentrations of the inhibitors used are provided in the plot. Global fitting of all the data from each experiment was used to obtain $K_i$. Experiments were done in duplicate. The individual $K_i$ values were: clopidogrel (0.59, 0.63 μM) and sertraline (2.20, 2.16 μM).

Acknowledgments. We thank Dr. Surendra Negi from the University of Texas Medical Branch (UTMB) for providing the model of CYP2B6dH. We also thank Ling Sun (Pharmacology and Toxicology, UTMB) for creating K262R.

References

Bumpus NN, Kent UM, and Hollenberg PF (2006) Metabolism of efavirenz and 8-hydroxyefa-

virenz by P450 2B6 leads to inactivation by two distinct mechanisms. J Pharmacol Exp Ther 318:345–351.


Burgess MA and Bodey GP (1972) Clotrimazole (Bay b 5097): in vitro and clinical pharmaco-


**Address correspondence to:** Santosh Kumar, Division of Pharmacology and Toxicology, School of Pharmacy, University of Missouri-Kansas City, 2464 Charlotte Street, Kansas City, MO 64108, E-mail: kumarsa@umkc.edu