Short Communication

ASSESSMENT OF THE CONTRIBUTIONS OF CYP3A4 AND CYP3A5 IN THE METABOLISM OF THE ANTIPLATFORMIC AGENT HALOPERIDOL TO ITS POTENTIALLY NEUROTOXIC PYRIDINIUM METABOLITE AND EFFECT OF ANTIDEPRESSANTS ON THE BIOACTIVATION PATHWAY

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ABSTRACT:

As a plausible explanation for the large interindividual variability in the pharmacokinetics of the neuroleptic agent haloperidol, the contributions of CYP3A isoforms (CYP3A4 and the polymorphic CYP3A5) predominantly involved in haloperidol bioactivation to the neurotoxic pyridinium species 4-(4-(chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-pyridinium (HPP⁺) were assessed in human liver microsomes and heterologously expressed enzymes. Based on recent reports on drug-drug interactions between haloperidol and antidepressants including selective serotonin reuptake inhibitors, the inhibitory effects of antidepressants on the CYP3A4/5-mediated haloperidol bioactivation were also evaluated. HPP⁺ formation followed Michaelis-Menten kinetics in microsomes, recombinant CYP3A4, and CYP3A5 with Kₘ values of 24.4 ± 8.9 μM, 18.3 ± 4.9 μM, and 200.2 ± 47.6 μM, respectively, and Vₘₐₓ values of 157.6 ± 13.2 pmol/min/mg of protein, 10.4 ± 0.6 pmol/min/mg P450, and 5.16 ± 0.6 pmol/min/pmol P450, respectively. The similarity in Kₘ values between human liver microsomal and recombinant CYP3A4 incubations suggests that polymorphic CYP3A may not be an important genetic contributor to the interindividual variability in CYP3A-mediated haloperidol clearance pathways. Besides HPP⁺, a novel 4-fluorophenyl-ring-hydroxylated metabolite of haloperidol in microsomes/CYP3A enzymes was also detected. Its formation was consistent with previous reports on the detection of O-sulfate and -glucuronide conjugates of a fluorophenyl ring-hydroxylated metabolite of haloperidol in human urine. Finally, all antidepressants except buspirone inhibited the CYP3A4/5-catalyzed oxidation of haloperidol to HPP⁺ in a concentration-dependent manner. Based on the estimated IC₅₀ values for inhibition of HPP⁺ formation in microsomes, the antidepressants were ranked in the following order: fluoxetine, nefazodone, norfluoxetine, trazodone, and fluvoxamine. These inhibition results suggest that clinically observed drug-drug interactions between haloperidol and antidepressants may arise via the attenuation of CYP3A4/5-mediated 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinol biotransformation pathways.

Although, the neuroleptic agent haloperidol [HP, 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinol] is one of the most widely used antipsychotic drugs, a narrow therapeutic index and a large interindividual and intertrial variability in pharmacokinetics results in the requirement of individualized HP dose optimization (Ulrich et al., 1998). The narrow therapeutic index is associated with the frequent occurrence of extrapyramidal side effects including acute dystonic reactions, akathisia, Parkinsonism, and, following chronic treatment, tardive dyskinesias (TD) that are slow to develop and often irreversible (Wright et al., 1998). The persistence of TD in many patients after discontinuation of HP therapy suggests that this condition may be related to a neuronal lesion induced by HP or a reactive metabolite(s) derived from it.

HP is extensively metabolized in the liver with only ~1% of the administered dose excreted in the urine (Forsman et al., 1977). Major biotransformation pathways of HP in humans have been extensively characterized (see Kudo and Ishizaki, 1999 for a review) and are summarized in Fig. 1. These include 1) glucuronidation of the 30 alcohol moiety (Someya et al., 1992); 2) reduction of the carbonyl alcohol moiety (Someya et al., 1992); 2) reduction of the carbonyl moiety by cytosolic carbonyl reductase, which leads to reduced HP; 3) reverse oxidation of reduced HP back to HP(Eyles and Pond, 1992); 3) reverse oxidation of reduced HP back to HP (Pan et al., 1998); 4) N-dealkylation leading to the formation of 4-(4-chlorophenyl)-4-hydroxypiperidine (CPHP) (Fang et al., 2001); 5) dehydrogenation of 30 alcohol moiety to 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-pyridinium (MPP⁺, 1-methyl-4-phenylypyridinium; P450, cytochrome P450; DMSO, dimethoxy sulfide; SSRI, selective serotonin reuptake inhibitor; LC-MS/MS, high performance liquid chromatography-tandem mass spectrometry; HPLC, high performance liquid chromatography; Rₜ, retention time; CID, collision-induced dissociation; NPP⁺, N-nonyl-4-phenylypyridinium).

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**Abbreviations used are:** HP, haloperidol; TD, tardive dyskinesias; CPHP, 4-(4-chloropheny1)-4-hydroxypiperidine; HPTP, 4-(4-Chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-2,3,6-tetrahydropiperidine; HPP⁺, 4-(4-Chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-pyridinium; RHPP⁺, 4-(4-Chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-pyridinium; MPP⁺, 1-methyl-4-phenylypyridinium; P450, cytochrome P450; DMSO, dimethoxy sulfide; SSRI, selective serotonin reuptake inhibitor; LC-MS/MS, high performance liquid chromatography-tandem mass spectrometry; HPLC, high performance liquid chromatography; Rₜ, retention time; CID, collision-induced dissociation; NPP⁺, N-nonyl-4-phenylypyridinium.
al., 1997). In contrast to earlier studies (Llerena et al., 1992; Viala et al., 1996) that suggested the involvement of cytochrome P450 (P450) 2D6 in HP metabolism to CPHP, HPTP, and HPP⁺, recent reports have indicated that these pathways are almost exclusively catalyzed by CYP3A4 (Usuki et al., 1996; Fang et al., 2001).

In view of the neurotoxic properties of 1-methyl-4-phenylpyridinium (MPP⁺), the monoamine oxidase-B-generated metabolite of the Parkinsonian agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Kalgutkar et al., 2001), the identification of MPP⁺-like HPP⁺ and RHPP⁺ metabolites in significant quantities in the urine (Subramanyam et al., 1991b), plasma (Avent et al., 1997), and post-mortem brain samples (Eyles et al., 1997) in schizophrenic patients treated with HP is of neurotoxicological importance especially in the pathogenesis of HP-induced TD. Additional support for this proposal is evident from the observations that HPP⁺ displays MPP⁺-like neurotoxicity in vivo as well as in vitro (Bloomquist et al., 1994).

Although glucuronidation and reduced HP formation constitute the major routes of HP clearance in humans, the wide interindividual variations in HP oral clearance have been mainly attributed to the “minor” P450-catalyzed biotransformation pathways (30% of overall HP metabolism) (Kudo and Ishizaki, 1999). These proposals are derived from in vitro observations on the lack of variations in HP glucuronidation or reduction activities in vitro and 10- to 15-fold interindividual variation in CYP3A4-mediated HP metabolism. For instance, Usuki et al. found that the rate of formation of HPP⁺ varied 10-fold among 14 liver samples (Usuki et al., 1996), similar to the variation reported for CPHP formation (Pan et al., 1998) and the back-oxidation of reduced HP to HP (Kudo and Odomi, 1998; Fang et al., 2001). In this context, the contribution of polymorphic CYP3A5 (Kuehl et al., 2001) in HP metabolism relative to CYP3A4 remains undetermined.

Since HP is widely used in the treatment of schizophrenia and other psychiatric disorders, HP is commonly coadministered with other antipsychotics and antidepressants. As a consequence, there are several documented reports on drug-drug interactions between HP and antidepressants including selective serotonin reuptake inhibitors (SSRIs) (Vandel et al., 1995; Barbhaiya et al., 1996; Avenoso et al., 1997). Biochemical mechanisms of these interactions were thought to involve the attenuation of CYP2D6-mediated HP metabolism by antidepressants (Nemeroff et al., 1996), based on the well established CYP2D6 inhibitory properties of antidepressants and earlier reports that suggested the involvement of CYP2D6 in HP metabolism. However, recent findings on the exclusive involvement of CYP3A4 and not CYP2D6 in HP metabolism and on the CYP3A4 inhibitory effects of antidepressants including fluoxetine (Schmider et al., 1995), fluvoxamine (Schmider et al., 1995), and nefazodone (von Moltke et al., 1999) on benzodiazepine metabolism suggest that attenuation of the CYP3A4/5-catalyzed metabolism of HP in vivo may also represent a viable mechanism for HP/antidepressant drug-drug interactions. Based on these proposals and our general interest in the bioactivation of cyclic tertiary amines, specific studies were designed to evaluate the relative contributions of CYP3A4 and the polymorphic CYP3A5 in HP bioactivation to the potentially neurotoxic HPP⁺ as a plausible explanation for the observed interindividual variability in HP metabolism. We also characterized the inhibitory effects of antidepressants including SSRIs on the CYP3A4/5-mediated bioactivation pathway.

**Experimental Procedures**

**Materials.** Haloperidol, buspirone, trazodone, fluoxetine, norfluoxetine, and ketoconazole were purchased from Sigma-Aldrich (St. Louis, MO). 4-(4-Chlorophenyl)-4-hydroxypiperidine, 1-(2-pyrimidyl)piperazine·2HCl were purchased from Aldrich (Milwaukee, WI). 4-(4-Chlorophenyl)-1,2,3,6-tetra-
hydroxyprogicetine - HCl, 1-(3-chlorophenyl)piperazine - HCl, and fluvoxamine maleate were obtained from Across Organics (Pittsburgh, PA), Tocris Cookson Inc. (Ballwin, MO), and Avocado Research (Heysham, Lancs, UK), respectively. All other chemicals and reagents were obtained from Aldrich. 3H NMR spectra in DMSO-d6 or CD3CN were recorded on a Varian Unity M-400 MHz spectrometer (Varian Medical Systems, Palo Alto, CA); chemical shifts are expressed in parts per million (ppm, δ) relative to tetramethylsilane as internal standard. Spin multiplicities are given as d (doublet), dd (doublet of doublet), t (triplet), and m (multiplet). Baculovirus-expressed human CYP3A4 and CYP3A5 were purchased from PanVera Corp. (Madison, WI) and BD Gentest Corp. (Woburn, MA). Human liver microsomes were generated at Pfizer using human Liver Microsomes and in the Presence of Recombinant CYP3A4 and CYP3A5. In all experiments, human liver microsomes (P450 concentration 0.1 M) was incubated with pooled human liver microsomal incubation mixtures in the present study have been extensively studied, routine analysis of HP (50 μM) per human liver microsomal incubation mixtures in the present study revealed the presence of two predominant metabolites eluting at Rf = 12.3 and 13.4 min. The identity of the major metabolite (M1 = 354; Rf = 12.3 min) was established as HPP* by comparison of its HPLC retention time and mass spectral properties with the synthetic CYP3A4/5-CATALYZED BIOACTIVATION OF HALOPERIDOL 245

HPP* formation was monitored on a Sciex API model 2000 or 3000 LC-MS/MS triple quadrupole mass spectrometer (PerkinElmerSciex Instruments, Boston, MA). Standard curves containing HPP* in 0.2 ml of control matrix (liver microsomes plus buffer, without cofactors) and 0.2 ml of acetoni trile containing HPP* as internal standard were constructed to estimate HPP* concentrations in incubation mixtures. Analytes were chromatographically separated using a Hewlett Packard Series 1100 HPLC system (Hewlett Packard, Palo Alto, CA). An autosampler was programmed to inject 20 μl on a Phenomenex Primesphere 5 μ C18-HC 30 × 2.0 mm column (Phenomenex, Torrance, CA) using a mobile phase consisting of 10 m ammonium acetate buffer-acetoni trile (60:40 v/v) containing 0.2% (v/v) triethylamine and 0.1% (v/v) acetic acid at a flow rate varying from 1 to 1.5 ml/min. Ionization was conducted in the positive ion mode at the ionspray interface temperature of 400°C, using nitrogen for nebulizing and heating gas. The ion spray voltage was 5.0 kV, and the orifice voltage was optimized at 30 eV. HPP* and NPP* were analyzed using multiple reaction monitoring at mass ranges m/z 354 → 165 and m/z 282 → 156, respectively. For HPP*, this reaction followed the protonated parent mass M1 = 354 to its corresponding collision-induced dissociated fragment at m/z 165, which corresponded to the 4-fluorophenyl-4-oxobutyl side chain.

Quantitation. Enzyme kinetic analyses were performed by nonlinear regression of substrate concentration (S) → velocity (v) data using the Michaelis-Menten Equation:

\[
v = \frac{v_{max} S}{K_m + S}
\]

Model selection was based on examination of Eadie-Hofstee-transformed plots.

Inhibitor potencies (IC50 values) were determined by nonlinear regression of residual velocity (R; as a percent of uninhibited control value) versus inhibitor concentration (I) data using the following equation (where A is an exponent).

\[
R = 100 \left(1 - \left(\frac{I^A}{IC_{50} + I}\right)\right)
\]

Results and Discussion

Metabolism of Haloperidol in Human Liver Microsomes and Recombinant CYP3A4 and CYP3A5. Although the biotransformation pathways of HP in liver microsomes and recombinant CYP3A4 have been extensively studied, routine analysis of HP (50 μM) per human liver microsomal incubation mixtures in the present study revealed the presence of two predominant metabolites eluting at Rf = 12.3 and 13.4 min. The identity of the major metabolite (M1 = 354; Rf = 12.3 min) was established as HPP* by comparison of its HPLC retention time and mass spectral properties with the synthetic standard. The LC-MS/MS spectrum of the second more polar metabolite (MH+) at 392, i.e., 16 mass units higher than those observed for HP, was found to contain 0.2% (v/v) triethylamine and 0.1% (v/v) acetic acid at a flow rate varying from 1 to 1.5 ml/min. Ionization was conducted in the positive ion mode at the ionspray interface temperature of 400°C, using nitrogen for nebulizing and heating gas. The ion spray voltage was 5.0 kV, and the orifice voltage was optimized at 30 eV. HPP* and NPP* were analyzed using multiple reaction monitoring at mass ranges m/z 354 → 165 and m/z 282 → 156, respectively. For HPP*, this reaction followed the protonated parent mass M1 = 354 to its corresponding collision-induced dissociated fragment at m/z 165, which corresponded to the 4-fluorophenyl-4-oxobutyl side chain.

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\[
R = 100 \left(1 - \left(\frac{I^A}{IC_{50} + I}\right)\right)
\]
tion in our studies is consistent with published evidence by Oida and coworkers on the detection of O-sulfate- and -glucuronide conjugates derived from phase II conjugation of a fluorophenyl ring-hydroxylated metabolite of HP in human urine (Oida et al., 1989). Likewise, Van der Schyf and coworkers have also detected the 4-fluorophenyl ring-hydroxylated metabolite of HPP\(^+\) in mouse urine and brain tissue extracts following administration of HP or its dehydrated HPTP metabolite (Van der Schyf et al., 1994). The positional assignment of hydroxylations on the 4-fluorophenyl ring in M1, however, remains to be determined.

Besides HPP\(^+\) and M1, the formation of trace levels of CHPH (MH\(^+\) = 212, \(R_t = 9.8\) min) and HPTP (MH\(^+\) = 358, \(R_t = 14.5\) min) was also discernible in the microsomal incubations. The structural identity of these metabolites was confirmed via comparison of their HPLC retention times and mass spectral properties to those of the authentic standards. Consistent with previous reports (Usuki et al., 1996; Fang et al., 2001), pretreatment of the liver microsomal incubation mixtures with ketoconazole (3 \(\mu\)M) completely prevented HP metabolism suggesting the exclusive involvement of CYP3A4/5 in HPP\(^+\), HPTP, CHPH, and M1 formation.

The metabolic profile of HP following incubation with recombinant CYP3A4 or CYP3A5 was similar to that observed following incubation in human liver microsomes, except for the formation of 4-(4-chlorophenyl)-1,2,3,6-tetrahydroxypyridine (M3, MH\(^+\) = 194, \(R_t = 11.1\) min) and 4-(4-chlorophenyl)pyridine (M4, MH\(^+\) = 190, \(R_t = 14.9\) min) as additional metabolites in the recombinant enzyme incubation (confirmed with authentic standards). The formation of M3 and M4 could presumably occur via CYP3A4-catalyzed N-dealkylation of HPTP and HPP\(^+\), respectively; a proposal that was partially confirmed upon incubation of HPTP in recombinant CYP3A4 or CYP3A5 that generated 4-(4-chlorophenyl)-1,2,3,6-tetrahydroxypyridine (M3) as a major metabolite.

**Enzyme Kinetics for the Reaction Sequence HP \(\rightarrow\) HPP\(^+\) in Human Liver Microsomes and in Recombinant CYP3A4 and CYP3A5.** The contribution of CYP3A5 (relative to CYP3A4) toward HP bioactivation to HPP\(^+\) was assessed in human liver microsomes and in a heterologously expressed enzyme system, especially since this isozyme represents \(\sim 50\%\) of the total hepatic CYP3A content in individuals polymorphically expressing it (Kuehl et al., 2001). HPP\(^+\) formation in human liver microsomes and recombinant CYP3A4 and CYP3A5 followed Michaelis-Menten kinetics (Figs. 3 and 4). The \(K_m\) values for HPP\(^+\) formation in human liver microsomes, recombinant CYP3A4, and CYP3A5 were 24.4 \(\pm\) 8.9 \(\mu\)M, 18.3 \(\pm\) 4.9 \(\mu\)M, and 200.2 \(\pm\) 47.6 \(\mu\)M, respectively; whereas \(V_{max}\) values in liver microsomes, CYP3A4, and CYP3A5 were 157.6 \(\pm\) 13.2 pmol/min/mg of protein, 10.4 \(\pm\) 0.6 pmol/min/\(\mu\)mol P450, and 5.16 \(\pm\) 0.6 pmol/min/\(\mu\)mol P450, respectively. These \(K_m\) values are several orders of magnitude higher than the typical therapeutic plasma concentrations of HP (5–25 ng/ml or 13–66 nM total plasma concentration that represents an unbound plasma concentration range of 1–5 nM) (Javaid et al., 1996) suggesting that hepatic metabolism via this pathway is not expected to be saturated in vivo. Examination of the enzyme kinetic parameters for CYP3A4 and 3A5 indicates that the CYP3A4 \(K_m\) is an order of magnitude lower than the CYP3A5 \(K_m\) and the CYP3A4 \(V_{max}\) is twice that estimated for CYP3A5, resulting in an intrinsic clearance for CYP3A4 that is 22 times greater than that calculated for CYP3A5. The significantly lower intrinsic clearance of CYP3A5 for this metabolic pathway relative to CYP3A4 suggests that CYP3A5 may only play a minor role in HPP\(^+\) formation in human liver microsomes, with CYP3A4 being the major contributor. This is consistent with the similarity in \(K_m\) values for this metabolic pathway determined in human liver microsomal and recombinant CYP3A4 incubations and suggests that polymorphic differences in the CYP3A5 gene may not be important contributors to the interindividual and interracial differences in HP clearance as well as its bioactivation to the potentially neurotoxic pyridinium species. In this context, the effect of polymorphic CYP3A4 expression and its subsequent effects on HP pharmacokinetics remain to be determined. For instance, recent reports have described three genetic variants of the CYP3A4 gene including CYP3A4*1B, CYP3A4*2, and CYP3A4*3. The allelic frequency for the CYP3A4*1B allele, which contains an A(\(-290\))G substitution in the promoter region of CYP3A4, ranges from 0% in Chinese and Japanese Americans to \(>54\%\) in African Americans; American and European Caucasians were reported to have an allelic frequency of \(\sim 4\%\) (Sata et al., 2000; Lamba et al., 2002).

**Effect of Antidepressants on the CYP3A-Catalyzed HP Bioactivation.** The ability of antidepressants and their major metabolites to inhibit HP bioactivation was investigated in human liver microsomes and recombinant CYP3A4 and CYP3A5. HP concentrations in these incubations reflected its approximate \(K_m\) (30 \(\mu\)M for human liver microsomes and recombinant CYP3A4 and 200 \(\mu\)M for recombinant CYP3A5). The use of a single substrate concentration around \(K_m\) was rationalized on the basis of the relationship between the IC\(_{50}\) and \(K_m\) as described by the Cheng-Prusoff equation (Craig, 1993). Thus at \(K_m\), the IC\(_{50}\) value is equal to \(K_m\) for competitive inhibition as has been previously documented for the inhibition of CYP3A4 activity by antidepressants (von Molk et al., 1999). Under these conditions, ketoconazole inhibited the reaction sequence HP \(\rightarrow\) HPP\(^+\) in human liver microsomes and recombinant CYP3A4 and CYP3A5 with IC\(_{50}\) values of 0.02, 0.02, and 0.19 \(\mu\)M, respectively (Table 1).

Although nefazodone was a potent inhibitor of the CYP3A-catalyzed HP bioactivation to HPP\(^+\) in human liver microsomes (IC\(_{50}\) = 9.6 \(\mu\)M), recombinant CYP3A4 (IC\(_{50}\) = 0.7 \(\mu\)M), and CYP3A5 (IC\(_{50}\) = 9.6 \(\mu\)M) (Fig. 5), its major metabolite 1-(3-chlorophenyl)piperazine did not inhibit the CYP3A-catalyzed HP bioactivation in microsomes or recombinant enzyme systems (IC\(_{50}\) > 100 \(\mu\)M). LC-MS/MS analysis of these incubation mixtures revealed that nefazodone not only inhibited HPP\(^+\) formation but also attenuated the formation of fluorophenyl-hydroxylated HP metabolite M1. Compared with nefazodone, the structurally related trazodone was less potent as an inhibitor of HP bioactivation in human liver microsomes and recombinant enzymes, whereas buspirone and its major metabolite 1-(2-pyrimidyl)piperazine had no effect on HP bioactivation (see Table 1). Our findings on the inhibition of CYP3A4/5-catalyzed HP metabolism by nefazodone are consistent with previously published reports on the selective inhibitory properties of nefazodone metabolism against CYP3A4-catalyzed alprazolam and triazolam hydroxylations (von Molk et al., 1999). They strengthen the hypothesis that the impairment in oral HP clearance (36 and 13% increase in the AUC and \(C_{max}\) by nefazodone results from the inhibition of CYP3A4/5-mediated HP metabolism. Likewise, our results on the inhibition of HP bioactivation by trazodone suggest that there is a potential for drug-drug

![CID Fragment Ions](cid.png)

<table>
<thead>
<tr>
<th>HP</th>
<th>13.1</th>
<th>376</th>
<th>Fragment A: 105 (R = H)</th>
<th>Fragment B: 123 (R = H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>12.3</td>
<td>392</td>
<td>Fragment C: 181 (R = OH)</td>
<td>Fragment D: 139 (R = OH)</td>
</tr>
</tbody>
</table>

**Fig. 2.** Collision-induced dissociation fragment ions for HP and the hydroxylated-4-fluorophenyl metabolite of HP.
### TABLE 1

*Effect of antidepressants on the CYP 3A catalyzed bioactivation of HP to HPP$^+$ in human liver microsomes and recombinant enzymes*

$IC_{50}$ values represent an average value from two individual determinations and were determined by nonlinear regression of residual velocity (R as a percent of uninhibited value) versus inhibitor concentration using SigmaPlot 2000.

<table>
<thead>
<tr>
<th>Antidepressant</th>
<th>Liver Microsomes</th>
<th>CYP 3A4</th>
<th>CYP 3A5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoconazole</td>
<td>0.02</td>
<td>0.02</td>
<td>0.19</td>
</tr>
<tr>
<td>Nefazodone</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>1-(3-Chlorophenyl)piperazine</td>
<td>16.3</td>
<td>22.7</td>
<td>192</td>
</tr>
<tr>
<td>Trazodone</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Buspirone</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>1-(2-Pyrimidyl)piperazine</td>
<td>58.6</td>
<td>10.7</td>
<td>56.9</td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>8.1</td>
<td>18.4</td>
<td>265</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>10.1</td>
<td>14.7</td>
<td>39.8</td>
</tr>
<tr>
<td>Norfluoxetine</td>
<td></td>
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</tr>
</tbody>
</table>
interactions with these two drugs. It is noteworthy that the CYP3A4 inhibitory potential of trazodone for HPP formation is substantially greater than for CYP3A4-mediated alprazolam-4-hydroxylation (IC_{50} > 100 \mu M) (von Moltke et al., 1999). Our findings on the lack of detectable CYP3A inhibitory activity of buspirone are consistent with previous reports indicating that the structurally related 1-(2-pyrimidinyl)-piperazine gepirone is not a CYP3A inhibitor (von Moltke et al., 1998).

Besides clinical interactions between HP and antidepressants such as nefazodone, several reports on drug-drug interactions between HP and SSRIs have also been noted in the recent literature. For instance, fluoxetine administration to patients undergoing HP treatment is associated with ~20 to 35% increase in plasma HP concentrations (Viala et al., 1996). Therefore, the ability of several SSRIs to inhibit HP metabolism to HPP^{+} was also assessed. Fluvoxamine, fluoxetine and its N-desmethyl metabolite norfluoxetine inhibited HP bioactivation to HPP^{+} with IC_{50} values (see Table 1) consistent with those reported for their inhibition of the CYP3A4-catalyzed N-demethylation of the tricyclic antidepressant amitriptyline (Schmider et al., 1995) and alprazolam hydroxylation (von Moltke et al., 1999). Overall, these in vitro results on CYP3A inhibition by SSRIs suggest that the clinically observed drug-drug interactions between HP and SSRIs might be partly attributable to the attenuation of CYP3A4/5-mediated HP biotransformation by SSRIs. Attempts to use in vitro HP/antidepressant interactions to quantitatively predict observed in vivo drug-drug interactions are currently underway and will involve the determination of IC_{50} values at low HP concentrations, since for competitive inhibition, IC_{50} values are substrate concentration-dependent, and the IC_{50} determined at low substrate concentrations that reflect actual in vivo plasma concentrations are usually very close to the inhibition constant K_{i} (Venkatakrishnan et al., 2001; Yao and Levy, 2002). Finally the possibility that drug-drug interactions between HP and antidepressants could arise via the inhibition of the major HP clearance pathways including glucuronidation and carbonyl reduction needs to be examined.

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