Metabolism of 4-Aminopiperidine Drugs by Cytochrome P450s: Molecular and Quantum Mechanical Insights into Drug Design

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Supporting Information

ABSTRACT: 4-Aminopiperidines are a variety of therapeutic agents that are extensively metabolized by cytochrome P450s with CYP3A4 as a major isoenzyme catalyzing their N-dealkylation reaction. However, its catalytic mechanism has not been fully elucidated in a molecular interaction level. Here, we applied theoretical approaches including the molecular mechanics-based docking to study the binding patterns and quantum mechanics-based reactivity calculations. They were supported by the experimental human liver microsomal clearance and P450 isoform phenotyping data. Our results herein suggested that the molecular interactions between substrates and CYP3A4 active site residues are essential for the N-dealkylation of 4-aminopiperidines. We also found that the serine 119 residue of CYP3A4 may serve as a key hydrogen-bonding partner to interact with the 4-amino groups of the studied drugs. The reactivity of the side chain α-carbon hydrogens drives the direction of catalysis as well. As a result, structure-based drug design approaches look promising to guide drug discovery programs into the optimized drug metabolism space.

KEYWORDS: Cytochrome P450, drug metabolism, N-dealkylation, 4-aminopiperidine, CYP3A4, drug design

Acyclic amines are common chemical moieties of small-molecule therapeutic agents. Their metabolic fates include ring α-oxidation to lactams, N-oxidation, the N-dealkylation of the side chain α-carbon, and ring-opening reactions, which have been extensively investigated. In principle, these reactions are catalyzed by cytochrome P450s but sometimes also by other phase I drug-metabolizing enzymes such as flavin-containing monoxygenase. Previous in vitro and clinical studies on the metabolism of the drugs containing a 4-aminopiperidine moiety, the typical acyclic amine serving as a synthetic linker (sometimes also pharmacologically active), pointed out that it is the N-dealkylation reaction that occurred predominantly rather than other piperidine ring-related biotransformation pathways as described above. At the moment, the therapeutic agents sharing a 4-aminopiperidine moiety include astemizole, bami- pine, benperidol, bezitramide, cisapride, clebopride, domperidone, enzastaurin, fentanyl, indoramim, lorcanide, α-methylfentanyl, pimo- zide, sabeluzole, and timiperone (Figure 1), which act on diverse drug targets (Table 1). Our primary objective was to elucidate the molecular mechanisms of their metabolic fate by P450s with a focus on N-dealkylation reactions.

N-Dealkylation is a typical biotransformation pathway in P450-catalyzed oxygenation reactions, which are initiated from the binding of substrates into the enzyme active site pocket. It is followed by exposing the reaction center of substrates to the heme for the abstraction by P450 compound I catalytic species, a rate-limiting step for N-dealkylation reactions. Theoretically, other molecular mechanisms may also exist such as a direct nitrogen atom oxidation. However, no previous studies have focused on the molecular mechanisms of the N-dealkylation reaction of these 4-aminopiperidine drugs. There are good reasons to hope that the mechanistic information extracted from this study may guide us into rational drug design and redesign from the perspective of drug metabolism.

Here, we applied theoretical approaches including the quantum mechanics-based density functional theory (DFT) to calculate the activation energy of the α-carbon radical intermediate formation and the molecular mechanics-based docking method to simulate the substrate binding modes to P450s. In particular, a series of 4-aminopiperidine fragments in simulating their corresponding drugs were designed to calculate the side-chain α-carbon hydrogen atom abstraction to address the impact of the electronic properties of various substitution groups. Likewise, the effects of the molecular interactions between substrates and P450 active site residues on their metabolic fate were investigated by the docking of 4-aminopiperidine drugs directly into CYP3A4, the major isoenzyme for their N-dealkylation reactions. Some key metabolic end points were lacking or not well characterized in previous studies, so experimental methods including the half-life-based human liver microsomal clearance determination and the phenotyping identification of P450 isoforms were incorporated

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in this study as well. The combined approaches led us to elucidate the overall and individual mechanisms of the metabolic fate of the studied 4-aminopiperidine drugs, especially their N-dealkylation reactions.

Overall, the human liver microsomal clearance results indicated that these 4-aminopiperidine drugs have moderate to high clearance, indeed, high clearance for a majority of them. CYP3A4 was found to be a major isoform for the metabolism of these drugs with the exception of indoramin, lorcanide, and sabeluzole. For them, CYP2D6 also contributed significantly. These findings are supported by our in vitro phenotyping experiments (Table 1) that measured the disappearance rate constant of the tested compounds with recombinant enzymes CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 and further confirmed by the CYP inhibitor studies. However, CYP3A4 was indeed the major isoform for the N-dealkylation of all studied 4-aminopiperidines (CYP2D6 catalyzes totally different reactions such as an aromatic hydroxylation as discussed below). Hence, we chose CYP3A4 to model the substrate binding to elucidate the mechanism of the N-dealkylation of 4-aminopiperidines. It demonstrated that the N-dealkylation orientation is one of the lowest energy binding clusters but not necessarily the lowest one. This is quite understandable given the fact that some lowest energy binding clusters may account for other pharmacological and biological processes besides metabolism and catalysis, for example, the enzyme inhibition. It appears that several structural regions of CYP3A4 have close interactions with 4-aminopiperidines, including its B–C loop (R105, R106, P107, F108, S119, and I120), F–G loop (R212, F213, F215, and F241), I helix (I301, F304, A305, and T309), K–β region (I369, A370, M371, R372, L373, and E374), and several residues at its β3 sheets (G481, L482, and L483). In particular, the substituents at the 4-amino end of 4-aminopiperidines tend to bind toward the B–C and F–G loops, and those at the piperidine ring nitrogen side bind toward the K–β region along the I helix of CYP3A4. More importantly, the predicted N-dealkylation binding poses also suggested a crucial role of the 4-amino nitrogen atom, which was found to sit closely (3–4 Å) to the oxygen atom of the serine 119’s hydroxyl group at the

Figure 1. Chemical structures of 4-aminopiperidine therapeutic agents.
Table 1. Therapeutic and Metabolic Profiles of 4-Aminopiperidine Drugs

| 4-aminopiperidine therapeutics | main indication | mechanism | N-dealkylation | active metabolite by N-dealkylation | P450 for N-dealkylation | half-life (t1/2) of parent compound depletion from phenotyping assay | microsomal clearance (µL/min/mg) |
|-------------------------------|---------------|-----------|---------------|----------------------------------|------------------------|-------------------------------------------------|---------------------------|
| astemizole                    | antihistaminic| nonselecting type histamine | minor          | active                           | CYP3A4                 | 3A4 (1.5 m), 2D6 (4.0 m), others (>60 m)          | 136.0 ± 13.5 (n = 8)     | N/A                      |
| bamipine                      | antihistaminic| sedating type histamine receptor antagonist | major          | undetermined                      | unknown                | N/A                                             | N/A                       |
| benperidol                    | antipsychotic | butyrophenone cerebral dopamine receptor blocker | major          | undetermined                      | CYP3A4                 | 3A4 (3.3 m), 2D6 (10.4 m), 2C8 (43.8 m), others (>60 m) | 42.0 ± 1.7 (n = 4)   |
| bezitramide                   | analgesic     | opioid | major          | undetermined                      | unknown                | N/A                                             | N/A                       |
| cisapride                     | gastroprokinetic | serotonin 5-HT4 receptor | major          | 1/6 active                        | CYP3A4                 | 3A4 (<5 m), others (>60 m)                       | 120.7 ± 5.8 (n = 13)    |
| clebopride                    | antiemetic; antiemetic | dopamine receptor antioxidant | major          | active                           | CYP3A4                 | 3A4 (<5 m), others (>60 m)                       | 50.4 ± 3.3 (n = 9)      |
| domperidone                   | antiemetic; antipsychotic | dopamine D2 receptor antagonist | major          | not active                        | CYP3A4                 | 3A4 (<5 m), 2D6 (58 m), others (>60 m)           | 108.6 ± 6.1 (n = 13)    |
| enzastaurin                   | antineoplastic | protein kinase C-β inhibitor | major          | active                           | CYP3A4                 | N/A                                             | N/A                       |
| fentanyl                      | analgesic     | prototype anilidopiperidine opioid | major          | not active                        | CYP3A4                 | N/A                                             | 32.3 ± 3.1 (n = 3)      |
| indoramin                     | antihypertensive | α1-adrenoceptor antagonist | minor          | undetermined                      | CYP3A4                 | 2D6 (<5 m), 3A4 (16.0 m), others (>60 m)          | 46.4 ± 7.6 (n = 9)      |
| lorcaainide                   | antiarrhythmic | type IC | minor          | active                           | CYP3A4                 | 2D6 (4.7 m), 3A4 (27.9 m), 2C19 (57.0 m), others (>60 m) | 159.0 ± 58.8 (n = 12)  |
| α-t-methylfenatyl fentanyl    | analgesic     | designer drug of fentanyl, opioid | major          | not active                        | unknown                | N/A                                             | N/A                       |
| pimozide                      | antipsychotic | dihydrobenzylpiperidine class | major          | undetermined                      | CYP3A4                 | 3A4 (2.7 m), 2D6 (3.2 m), others (>60 m)          | 57.0 ± 24.0 (n = 12)    |
| sabeluzole                    | nootropic     | N-methyl D-aspartate receptor antagonist | minor          | undetermined                      | CYP3A4                 | 2D6 (1.5 m), 3A4 (<5.0 m), others (>60 m)         | 37.0 ± 1.2 (n = 3)      |
| timiperone                    | antipsychotic | butyrophenone cerebral dopamine receptor blocker | major          | not active                        | unknown                | N/A                                             | N/A                       |

B–C loop of CYP3A4 in an energetically favored orientation to form a strong hydrogen bond. With this key force, 4-aminopiperidines are juxtaposed above the heme porphyrin ring to expose their α-carbon hydrogens to P450 compound I (3–4 Å between the α-carbon and the heme iron of CYP3A4). For a comprehensive analysis of these findings in the hope of elucidating their catalytic mechanisms individually, 4-aminopiperidines were categorized into several structural subgroups according to the type and size of these substituents connected to the piperidine ring nitrogen atom.

4-Aminopiperidines in the first group are those equipped with small-sized aliphatic substituents such as the methyl, ethyl, and isopropyl moieties. Metabolic studies of the antihistamine drug bamipine found that its major metabolites are isopropyl moieties. Metabolic studies of the antihistamine drug astemizole and a para-bamipine found that its major metabolites are isopropyl moieties. Metabolic studies of the antihistamine drug astemizole and a para-bamipine found that its major metabolites are isopropyl moieties. Metabolic studies of the antihistamine drug astemizole and a

The K–β loop of CYP3A4 (Figure 2). On the other hand, the DFT calculations demonstrated that the α-carbon hydrogen activation energy of the methyl, ethyl, and isopropyl 4-aminopiperidines are 98.47, 96.64, and 96.55 kcal/mol (Table 2), respectively, a reasonable range for P450-catalyzed oxygenation reactions.6,7

Clebopride and enzastaurin are a group of 4-aminopiperidines with a methylene linker connecting the piperidine ring nitrogen atom and a bulky aromatic moiety, that is, phenyl and pyridine, respectively. Our docking results indicated that these aromatic rings appear to slide along the I helix toward the K–β loop and hence to drag the α-carbons above the heme iron (Figure 2). The activation energy of clebopride’s α-carbon hydrogen is dramatically decreased (−13.17 kcal/mol) as compared to its methylated analogue, which suggests that its radical form is stabilized by the resonance effect, that is, the electron donating from the p system of the adjacent aromatic ring. Clebopride is mainly metabolized via an N-dealkylation pathway to N-desbenzyloclebopride8 and extensively metabolized by CYP3A4 and human liver microsomes (50 µL/min/mg). Likewise, the replacement of the benzene moiety with a pyridine ring (like enzastaurin) further lowers the activation potential (−4.70 kcal/mol to the benzene and −17.87 kcal/mol to N-methyl analogues) (Table 2). It is expected that the developing anticancer drug enzastaurin is predominantly metabolized via an N-demethylenedepipiperidine pathway.9

Astemizole, fentanyl/α-t-methylfentanyl, and indoramin are another group of 4-aminopiperidine drugs. They differentiate from the rest because of their ethylene linker. Likewise, to expose their α-carbon hydrogens for N-dealkylation, the derivatives at the other end of the ethylene linker are subject to certain torsional angle restrictions that arise from the hindrance of the
neighboring I helix and heme porphyrin. It is highly unlikely that the trans conformation of these substrates exists, which otherwise would push the aromatic end groups penetrating into the heme porphyrin ring. It turned out that the measured

Figure 2. Proposed N-dealkylation binding poses of 4-aminopiperines, (A) astemizole, (B) bamipine, (C) benperidol, (D) bezitramide, (E) cisapride, (F) clebopride, (G) domperidone, (H) enzastaurin, (I) fentanyl, (J) indoramin, (K) lorcainide, (L) α-methylfentanyl, (M) pimozide, (N) sabeluzole, and (O) timiperone. Conformation and orientation are generated by AutoDock and selected from one of the lowest energy binding poses and illustrated using PyMOL. Essential active site regions are colored and marked.

Table 2. Quantum Chemical Calculation of the α-Carbon Hydrogen Atom Abstraction of 4-Aminopipyridine Fragments Using the DFT/B3LYP 6-31G** Method

| 4-Aminopiperidine Fragments | Original Energy (hartree) | Radical Energy (hartree) | Activation Energy (kcal/mol) |
|-----------------------------|---------------------------|--------------------------|----------------------------|
| 4-Aminopiperidine Fragments | -616.911006 | -655.575714 | 85.30 |
| 4-Aminopiperidine Fragments | -616.274801 | -655.229485 | 96.66 |
| 4-Aminopiperidine Fragments | -616.536906 | -655.575714 | 94.42 |
| 4-Aminopiperidine Fragments | -616.536906 | -655.575714 | 93.85 |
| 4-Aminopiperidine Fragments | -616.911006 | -655.575714 | 80.60 |
| 4-Aminopiperidine Fragments | -616.911006 | -655.575714 | 96.28 |
Dihedral angles tend to adopt a gauche-like conformation (but not exactly 60 degrees), that is, an eclipsed conformation close to 90 degrees (Figure 2). The activation energy of their α-carbon hydrogen(s) is very close to those of direct aliphatic chain substitution and hence much less affected by the aromatic ring’s resonance effects (Table 2). Previous studies pointed out that CYP3A4-catalyzed N-dealkylation of indoramin, which has an indole moiety connected to the ethylenic linker, is a relatively minor metabolic pathway, as compared to CYP2D6-catalyzed indole 6-hydroxylation reaction.10 This may be explained by the unfavorable torsional rotation by the bulky indole substitution. Indeed, this phenomenon also applies to the second-generation antihistamine astemizole, a drug for the treatment of allergic rhinitis. Its major metabolite is O-desmethylastemizole (67% conversion from astemizole) that is catalyzed by CYP2D6 and 2J2. However, its N-dealkylated metabolite norastemizole (or tecastemizole) and the benzimidazole hydroxylated metabolite are only 9 and 25% converted from astemizole, respectively, which are catalyzed by CYP3A4 predominantly.11 On the other hand, for smaller end group substitution at the ethylenic linker, such as fentanyl, the typical binding mode described above is energetically favored consistently; hence, its N-dealkylated metabolite norfentanyl was found to be a major metabolite by CYP3A4 (~50% turned over from the parent compound),12 but its human liver microsomal clearance is moderate to high (32 μL/min/mg, Table 1). To further explore the effect of other steric variation of the ethylene linker, for example, to add a methyl group to its α-carbon, we found that its activation energy is lowered (−2.81 kcal/mol, Table 2), but this effect may be concealed since the methyl group per se hinders the exposure of the α-carbon hydrogen presumably. Take α-methylfentanyl, a designer drug of fentanyl by the methylation of its α-carbon, it was demonstrated that its N-dealkylated metabolite (nor-fentanyl) is a major metabolite like fentanyl but has a lower turnover rate (24%).13

With three or more “linear” aliphatic carbons connected to the piperidine ring nitrogen atom, it appears that the steric hindrance of the bulky substituents of 4-aminopiperidines may be avoided because of these flexible linkers. Their N-dealkylation orientations fit well into those CYP3A4 functional regions such as the B−C loop, F−G loop, I helix, and K−β loop in an energetically favored manner (Figure 2). Take timiperone and benperidol, both of which are the butyrophenone class interact with the hydroxyl group of the serine 119 residue at the active site especially those direct interactions from the F loop, G loop, K/C0 loop, and I helix are essential to orient substrates at an appropriate position for catalysis. Yet, despite that serine 119 is not a flexible active site residue as observed during the X-ray co-crystallization with structurally different substrates bound,14 it is indeed a key residue to control the catalysis of 4-aminopiperidines.

Furthermore, 4-aminopiperidine drugs are high-clearance compounds because of their reactive side chain α-carbon atoms. For example, the human liver microsomal clearances of astemizole and cisapride are 136 and 121 μL/min/mg, respectively. It is noticeable that astemizole was recalled in 1999 because of drug–drug interactions, a direct result of its high hepatic clearance by P450s. Other withdrawn drugs for a similar reason were terfenadine (in 1998), mibefradil (in 1998), and cisapride (in 2000). Interestingly, the N-dealkylated active metabolite of astemizole, tecastemizole, was further developed, but unfortunately, the program was terminated after the new drug application with a “not approvable” decision from FDA for additional concerns of pharmacokinetics and safety issues. In addition, two indoramin analogues were designed and synthesized in this study by blocking either the piperidine side chain α-carbon or its 5-position aromatic carbon at the indole ring, with a hydroxyl group. We found that their human liver microsomal clearance was dropped from 46, down to 11 and 16 μL/min/mg, respectively. As expected, physicochemical properties (such as lipophilicity), electronic properties, and substrate binding orientations are all important factors to determine the microsomal clearance. Therefore, combining these factors into drug design schemes should help us achieve better pharmacokinetic and metabolic profiles to expand the chemical space of lead compounds. Hopefully, structure-based drug design approaches are reliable tools to fulfill these goals in the near future.

**ASSOCIATED CONTENT**

*Supporting Information.* Experimental procedures and supporting results. This material is available free of charge via the Internet at http://pubs.acs.org.

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