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Ortho-Methylarylamine as Time-Dependent Inhibitors of Cytochrome P450 1A1 Enzyme

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Abstract

Members of the cytochrome P450 1A family metabolize many procarcinogens such as polycyclic aromatic hydrocarbons and heterocyclic amines. Inactivation of these enzymes is a prerequisite for cancer prevention and treatment in certain cases. Mechanism-based inhibition (time and co-factor dependent) is an effective method for the inactivation of these enzymes. Our recent study on emodin analogs revealed an anthraquinone with *ortho*-methylarylamine moiety that exhibited time-dependent inhibition of P450 enzymes 1A1 and 1A2. To determine whether the amino group or the methyl group or both were responsible for the time-dependent inhibition of these enzymes, a set of eleven compounds containing the *ortho*-methylarylamine moiety were identified through a database search, and studied for the inhibition of the P450 enzymes 1A1, 1A2, 2A6 and 2B1. Four compounds were found to cause selective time-dependent inhibition of P450 1A1 with KI values ranging from 0.24 to 8.25 μ M. These compounds exhibited only direct inhibition of P450 1A2. Molecular modeling studies of these molecules indicated that the shapes of the molecules, their binding modes, and the methyl substituent in close proximity (4.5–5.7 Å) to the heme-Fe all contributed to their selective time-dependent inhibition activity on P450 1A1. Our earlier studies on carbazole derivatives provided us with highly selective P450 1A2 inhibitors. Glycine scanning studies were performed on the docked protein-ligand complexes of compounds **1–20** in order to understand the contribution of different protein residues towards the ligand binding.

Keywords

Carbazoles; cytochrome P450; docking; glycine scanning; *ortho*-methylarylamine; time-dependent inhibition

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

NOTES

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1. INTRODUCTION

The cytochrome P450 (P450) superfamily of hemecontaining enzymes is involved in the metabolism of a wide variety of xenobiotics, including drugs and carcinogens as well as endobiotics [1, 2]. These enzymes catalyze the oxidation of a wide range of compounds with great structural diversity. The cytochrome P450 1A family enzymes, P450 1A1 (cytochrome P450 1A1) and P450 1A2 (cytochrome P450 1A2), are known to metabolize a large number of procarcinogens resulting in cancer initiation and tumor induction [3–6]. P450 1A1 mainly metabolizes polycyclic aromatic hydrocarbons, whereas P450 1A2 activates aminofluorenes and nitrosamines by N-hydroxylation, and metabolizes important drugs such as theophylline, caffeine, imipramine, and propranolol. Additionally, P450s 2A6 and 2B1 have also been shown to oxidize polycyclic aromatic hydrocarbons to procarcinogens [1, 7–10]. Cytochrome P450 2A6 is the predominant enzyme to metabolize nicotine into its procarcinogen cotinine by C-oxidation [11, 12]. Inhibitors of P450s 1A1, 1A2, 2A6, and 2B1 can have a direct impact on cancer prevention and drug metabolism [13]. Our study is focused on the identification of selective inhibitors for the cytochrome 1A family. To substantiate the selectivity of our inhibitors, our compounds were analyzed for their inhibition of two additional cytochrome P450 enzymes belonging to other P450 families, namely, P450 2A6 belonging to the 2A family and P450 2B1 belonging to the 2B.

Cooking and grilling of meat products result in the formation of amines belonging to the quinoline, quinoxaline, and pyridine classes of heterocyclic compounds [14]. Heterocyclic amines have been shown to be potent carcinogens in F344 rats inducing the formation of tumors in liver, lung, Zymbal's gland, mammary tissue, and colon [14, 15]. Aromatic amines and heterocyclic amines are converted into genotoxins through metabolic activation by a series of enzymatic processes, the initial step of which is N-hydroxylation by the P450 enzymes 1A1 and 1A2 [15, 16].

Strategic deactivation of these enzymes leads to the prevention of the initial oxidation of the arylamines, thereby leading to reduced genotoxicity. Our strategy involves the design and development of *ortho*-methylarylamine analogs as time-dependent inhibitors of P450 1A1 and/or 1A2 enzymes. This approach is based on the fact that arylamines have high affinity for the P450 enzymes 1A1 and 1A2, making them ideal inhibitors if they can covalently bind to the enzyme's active site. Such compounds when optimally designed can serve dual purposes of inactivating the enzyme permanently as well as decreasing the potential genotoxicity of other arylamines.

At present, there are two prevalent methods used for the deactivation of cytochrome P450 enzymes. The first method involves direct inhibition of the enzyme using small molecule competitive inhibitors. To be effective, these molecules need to have higher affinity toward the enzyme than the native ligands. The second method is to inhibit the enzymes by a process called time-dependent inhibition (TDI) [17–19]. If the inhibitor is incubated with the enzyme prior to the addition of the substrate and when this results in an increase in the inhibition, it is called Time-Dependent Inhibition. It is a kinetic phenomenon. Mechanism-Based Inactivation is a subset of TDI wherein the process of inactivation of the enzyme through the initial oxidation of the compound by the P450 enzyme into a highly reactive

intermediate that covalently binds to reactive amino acid residues in the active site, thereby completely inactivating the enzyme [20]. A mechanism-based inhibitor is time- and cofactor-dependent. A number of methyl-substituted aromatic compounds (such as furafylline), as well as allenes and acetylenes (such as propargyl and ethynyl derivatives) are well-known as mechanism-based inhibitors of these enzymes. The aromatic methyl carbon-hydrogen bond cleavage is a crucial event leading to inhibition, as evidenced by the scale of the isotope effect for the cytochrome P450-catalyzed oxidation of toluene to benzyl alcohol [21] ($DV = 1.9$, $DV/K = 3.51$), and the 2-fold effect on K_{inact} as shown for deuterated furafylline for cytochrome P450 1A2, systems that provide an aromatic methyl for comparison [22]. During our recent study on anthraquinones as P450 inhibitors, we came across an inhibitor which exhibited time-dependent inhibition of P450 enzymes 1A1 and 1A2 (compound **1** in Fig. 1) [23]. A database search was performed for compounds with a methyl and an amino group that are adjacent (*ortho*) to each other. A total of eleven compounds (Fig. 1) were identified and obtained for analysis of their inhibition activities on P450 enzymes 1A1, 1A2, 2A6, and 2B1. In our recent studies we had found that certain carbazoles (Supplementary Material, Table S1) selectively inhibited P450 1A2 over P450 1A1, however, none of the carbazoles studied showed time-dependent inhibition of the P450 enzymes [24]. To investigate whether the presence of an *ortho*-methylarylamine moiety on the carbazole skeleton would result in time-dependent inhibition of P450 1A2, compound **12** was purchased and assessed for P450 1A1, 1A2, 2A6 and 2B1 inhibition. Computational molecular modeling studies such as docking studies followed by glycine scanning studies on the docked complexes were performed on the compounds **1–20** to understand the contribution of different protein residues in the binding pocket towards ligand binding.

2. MATERIALS AND METHODS

The eleven compounds used in this study were obtained from the NCI/DTP Open Chemical Repository (<http://dtp.cancer.gov>; NSC13973, NSC71947, NSC115905, NSC608636, NSC3092, NSC12454, NSC10466, NSC36758, NSC47719, NSC326051, NSC628945). Compound **1** was purchased from Specs Chemical Services (Wakefield, RI).

2.1. Assays

The P450 1A1-, 1A2-, 2A6- and 2B1-dependent activities as well as the pre-incubation assays in the presence and absence of NADPH were performed in a same manner as described in earlier publications [7, 9–12, 25].

2.2. Database Search

Based on the docking studies carried out on compound **1** with P450s 1A1 and 1A2 in our earlier studies, it was concluded that the presence of a methyl group *ortho* to the amino group on an aromatic compound would present favorable structural features for time-dependent inhibition, and 2-methylaniline was chosen as the pharmacophore. A 2D-database search was performed in the NCI database of compounds using the UNITY module of SYBYLX1.3. A total of 1071 hits were obtained. Our earlier studies on P450 1A1 and 1A2 inhibitors revealed that compounds with 2–3 fused aromatic rings in the core structure showed good inhibition potency. The structures of the hits were visually inspected and

molecules that had 2–3 fused aromatic rings that also contained a methyl group *ortho* to the amino group on the aromatic ring were selected. Eleven of these compounds were procured from NIH for analysis.

2.3. Binding Modes by Docking Simulations

Docking studies were performed using the methods that have been outlined in our earlier publications [7, 12]. The non-standard forcefield parameters for the heme and the cysteine-iron bond were adapted from the literature [26]. MOE energy minimization method was used [27].

2.4. Computational Glycine Scanning

In order to gain an understanding of the mechanisms by which inhibitors bind within the P450 1A1 and 1A2 binding cavities we have carried out computational glycine scanning studies. Glycine scanning is used to estimate the strengths of all ligand-residue interactions within a receptor's binding cavity, indicating which of these interactions is most critical to stabilization of the protein-ligand complex. In glycine scanning, binding pocket residues are individually mutated to glycine, that is to say that residue sidechains are deleted and replaced by a single bound hydrogen, the structure of the mutated complex is minimized, and an estimated binding free energy is computed using a scoring function [28, 29]. The contribution that the mutated residue makes to overall binding stability is determined assessing the degree to which the complex is destabilized by that particular mutation.

In order to limit the number of binding pocket residues to be mutated in our studies, for each receptor we have determined the amino acids that are within 4Å of the ligand for the entire set of inhibitors. That is to say that an amino acid is added to the list of residues to be mutated if it is within 4Å of any of the inhibitors studied here. For obvious reasons, glycine itself was excluded from the list. Lists of mutated residues for each receptor are provided in (Figs.3 and 5). For each of the receptor/inhibitor combinations studied here, the structure of the unmutated complex was optimized using the Amber 99 force field [30] and the estimated binding free energy was computed using the Autodock scoring function [31]. Explicit TIP3P water was incorporated into all models in order to account for solvation effects. Single point mutations were then made to each of the residues on the mutation list (as described above), the structure was reminimized (Amber 99), and the binding free energy recalculated (Autodock). The destabilization resulting from that mutation was computed as a percent of the binding energy for the native receptor. This process was repeated for each of the residues on the mutation list. All minimization and scoring computations were performed using the YASARA suite of molecular mechanics software.

3. RESULTS

We recently determined that ortho-aminomethyl anthroquinone is a time-dependent inhibitor of P450 enzymes 1A1 and 1A2 [23]. Our hypothesis was that due to the presence of the primary amino group and a methyl group in close proximity, the mechanism of action could involve the formation of a benzylic carbon radical intermediate via a hydrogen abstraction by an iron-oxo species, which could rearrange to produce inactivated complexes. For such a

time-dependent inhibition, the binding mode of the molecule to the P450 enzyme heme site would need to bring the amino group and the methyl group of the molecule to an optimal distance from the heme center. This brought us to the question as to whether polycyclic aromatic hydrocarbons bearing an amino group and a methyl group on adjacent carbons would behave the same way. To answer this, we performed a database search of NCI-database using 2-methylaniline as the pharmacophore as outlined in the Methods Section. These compounds were tested for their ability to inhibit P450 enzymes 1A1, 1A2, 2A6, and 2B1. The compounds in the present study were evaluated for two types of inhibition (direct inhibition and time-dependent inhibition). A direct inhibitor is described as simple competitive inhibitor of the enzyme. An inhibitor that is metabolized by the enzyme into a reactive intermediate that further reacts with the enzyme to form a complex that is no longer enzymatically active is termed as a mechanism-based inhibitor which is time- and cofactor-dependent.

All of the compounds showed direct inhibition of P450 1A1 enzyme (Table 1). The potency ranged from $IC_{50} = 0.1 \mu M$ to $IC_{50} = 13.23 \mu M$. Compounds 1, 3 and 4 showed submicromolar inhibition potency with compound 3 exhibiting the best potency with $IC_{50} = 0.10 \mu M$. All of the compounds except for compounds 8 and 10 also inhibited P450 1A2 enzyme directly (Table 1). The range of inhibition showed a greater variation from $IC_{50} = 0.032 \mu M$ to $IC_{50} = 16.90 \mu M$. Compounds 1, 2, and 3 showed similar inhibition potencies for P450 1A2 ($IC_{50} = 0.53 \mu M$, $0.53 \mu M$ and $0.43 \mu M$ respectively). Compound 12 showed 50 and 134 fold selectivity for P450 1A2 ($IC_{50} = 0.032 \mu M$) over P450 1A1 ($IC_{50} = 1.61 \mu M$) and P450 2B1 ($IC_{50} = 4.31 \mu M$). This was in-line with our earlier observations that 9H-carbazole analogs selectively inhibited P450 1A2 enzyme over P450 1A1 and P450 2B1 enzymes. Only four compounds (compounds 2, 3, 7 and 12) directly inhibited P450 2B1 enzyme with a potency range of $IC_{50} = 0.17 \mu M$ to $IC_{50} = 17.34 \mu M$ (Table 1). Compound 7 had the best inhibition potency for P450 2B1 ($IC_{50} = 0.17 \mu M$). None of the compounds showed direct inhibition of P450 2A6.

Compounds 3, 4, 6 and 9 along with the lead structure compound 1 showed time-dependent inhibition of P450 1A1 (Table 2). Only the lead structure compound 1 showed time-dependent inhibition of the enzyme P450 1A2. Figure S1 depicts the plots of the results of the NADPH-dependency tests of compounds 1, 3, 4, 6, and 9 for these enzymes. The NADPH-dependency test allows affirmation of time-dependent inhibition versus direct inhibition of the compound. The results of the NADPH-dependency tests for P450s 1A1 and 1A2 at different concentrations of the compound 1 are illustrated in (Fig. 2A and Fig. 2B). For P450 1A1, the rate constant for maximal inactivation at saturation ($K_{inactivation}$) was 0.57 min^{-1} and the concentration required to produce one-half of the maximal rate of inactivation (KI) was $5.38 \mu M$. In the case of P450 1A2, the KI and $K_{inactivation}$ values were $0.50 \mu M$ and 0.08 min^{-1} . For compounds 3, 4, 6 and 9, the rate constants for maximal inactivation of P450 1A1 at saturation ($K_{inactivation}$) were 0.6, 0.04, 0.2 and 0.29 min^{-1} respectively, and the concentrations required to produce one-half of the maximal rate of inactivation (KI) were 0.24, 0.37, 0.47 and $8.25 \mu M$ respectively (Figs. 2C – Figs. 2F).

Docking studies were performed using three different software MOE, Tripos-SYBYL and GOLD. The consensus binding posture shown by these docking results was obtained for

each of the compounds (Fig. 2). The aromatic rings of all of the compounds 1–11 made π - π interactions with the aromatic residues Phe224 and Phe258 of P450 1A1. The distance between the aromatic methyl group closest to the heme was between 4.39–5.50 Å. The binding poses also showed that the methyl group was in closer proximity to the heme residue than the amino group in most of the binding poses. The amino group of compound 4 made an additional H-bond with the Asn222 residue. All of Carbazoles 12–20 docked with good score in the binding pocket of P450 1A2 enzyme. The amino group that is part of ring structure in compounds 13–20 made a H-bonding interaction with the residue Asp320. Compound 12 which had *ortho*-methyl and amino substituents on the carbazole ring exhibited a flipped structure with the ring amino group now making a H-bond with the hydroxyl group of Thr124 residue. All of the carbazoles make additional π -H interactions with Thr321 and Ala317 and/or π - π interactions with Phe226 and Phe280.

4. DISCUSSION

P450s 1A1 and 1A2 belong to the same family of cytochrome P450 enzymes (1A) and have larger binding cavities relative to other cytochrome P450 enzymes. The binding sites of these enzymes are lined with several phenylalanine residues capable of forming π - π interactions with the bound ligands [32, 33], and also have comparable number of polar residues and hydrophobic residues [34]. In P450 1A1, there are two polar residues capable of forming such covalent bonds in close proximity to the heme and on either side of it - Ser122 and Thr321. In P450 1A2, residues Thr124 and Thr321 can have similar properties. Our earlier studies on the anthroquinone analogs, compounds 1 and 2, showed that the presence of a methyl group in an *ortho* position to the aromatic amino group could have contributed to the time-dependent inhibition of P450s 1A1 and 1A2 by compound 1 [23], as compound 2 (lacking this methyl group) did not exhibit time-dependent inhibition of either of these enzymes. Our speculation on the mechanism of action for this method of inhibition of P450s 1A1 and 1A2 by compound 1 involved hydrogen abstraction by an iron-oxo species to produce the benzylic carbon radical intermediate, that probably rearranged to produce inactivated complexes. This would mean that other compounds with similar structural features, a methyl group in *ortho* position to an amino group on a polycyclic aromatic core structure, could also exhibit time-dependent inhibition of these P450 enzymes. Compounds 3–12 had a methyl group in *ortho* position to the amino group, and compounds 4, 6, 10 and 11 had two sets of these groups in *ortho* positions at both ends of the molecules which should double their probability of exhibiting time-dependent inhibition based on their binding mode to the enzyme. Four of these compounds, compounds 3, 4, 6 and 9, showed time-dependent inhibition of P450 1A1 but not P450 1A2. Compound 3 when compared to compound 1 has one additional methyl group that potentially could be an additional oxidation site. Compound 3 showed similar binding mode as compound 1, and compound 3 had additional hydrophobic interactions with the meta-methyl group and hydrophobic residues in the active site of P450 1A1 (Fig. 2A). The *meta* methyl group of compound 3 was closer to the heme-Fe (4.39 Å) than the methyl group that is *ortho* to the aromatic amino group (5.5 Å). This provides the potential for both methyl groups acting as oxidation sites on compound 3, which could account for its increased potency. Compounds 4, and 6 showed similar binding modes in the active site of P450 1A1 (Fig. 2B). Compound 12 had similar

binding mode to P450 1A2 as exhibited by the Carbazoles 13–20 (Table 3A) from our earlier studies (Fig. 2C) [24]. The *ortho*-methyl group of the carbazole 12 was pointing away from the heme, which could have resulted in compound 12 being only a direct inhibitor and not a time-dependent inhibitor (Fig. 2d).

The shapes of binding pockets in P450s 1A1 and 1A2 greatly impact the shapes of the optimal inhibitors. Our earlier studies on a series of probe molecules, α -naphthoflavone-like and β -naphthoflavone-like derivatives, clearly demonstrated that P450 1A1 has a long and narrow cavity capable of accommodating a planar long strip molecule (the maximum width of the strip in P450 1A1 was less than that of P450 1A2) [35]. On the contrary, P450 1A2 possesses a compact active site cavity, capable of accommodating triangular-shaped molecules. Compounds 3–6 and 9 that are planar long strip molecules show good inhibition of P450 1A1 enzyme with more selectivity for P450 1A1 over 1A2. Compounds 10 and 11 show a non-planar branching on the central ring of the polycyclic aromatic core structure leading to their non-conformation to the required dimensions for inhibitors of P450 enzymes 1A1 and 1A2, which could have contributed to their reduced potency for these enzymes. Compounds 7, 8 and 11 are charged molecules in the salt form, which probably contributed to their reduced potency for both P450 enzymes 1A1 and 1A2. The increased inhibition potency of compound 12 for P450 enzyme 1A2 follows our inferred paradigm that triangular molecules show selectivity for P450 1A2.

Docking studies in P450 enzymes 1A1 and 1A2 were carried out with the compounds in Table 2. The binding modes of compounds 1, 3, 4 and 6 that showed excellent time-dependent inhibition of P450 1A1 were scrutinized to determine their structural features responsible for such activity. In our earlier work on compound 1, we looked at two different possible mechanisms- (1) one that is similar to tacrine where the authors have suggested the formation of a benzylic carbon radical intermediate by the abstraction of a hydrogen from an adjacent methylene group by an iron-oxo species [21, 36], or (2) one that is similar to furafylline, wherein the initiating event leading to the inactivation of P450 1A2 has been shown to be the abstraction of a hydrogen atom from the C-8 methyl group of furafylline by the enzyme through isotope effect studies [22]. Four probable pathways for the bioactivation of compound 3 are shown in the supplementary materials section as Figure S2. The binding mode of compound 3 was similar to that of compound 1 with the methyl substituents *ortho* to the amino groups being in the proximity of the heme-Fe atom (3.63 Å). This suggests that the mechanism in play could be similar to that of furafylline. The binding modes of compounds 4 and 6 also showed the presence of an aromatic methyl group close to the heme-Fe atom (3.24–3.34 Å). These molecules have similar potency to compound 3 and probably follow a similar mechanism for inhibition of P450 1A1.

To determine the contribution of the different active site residues to the binding of the ligands (compounds 3–6) with nanomolar inhibition potency and increased selectivity for P450 1A1, a Glycine scan was performed for the docked structures of the protein-ligand complexes of the compounds 1, 3, 4, 5 and 6 (Fig. 3). There are three residues, Phe123, Phe224 and Phe258, which make particularly strong contributions to ligand binding. Residue Phe224 plays an especially pronounced role in the binding of compounds 3, 4 and 6 with sidechain deletion of each of these amino acids resulting in a destabilization by

approximately 8–12%. For all five compounds (1, 3, 4, 5 and 6) a strong parallel-displaced aromatic-aromatic interaction exists between the Phe224 ring and a ligand ring. In the case of compound 3, a parallel-displaced “sandwich” configuration, involving the Phe224 and Phe258 rings and a ligand aromatic ring, occurs, with the ligand ring occupying the central position in the “sandwich” motif (Fig. 4). The “sandwich” configuration has been noted to be a particularly stable arrangement of the benzene trimer [37]. Glycine scanning results also indicated that interaction involving Phe123 play important roles in stabilizing these protein-ligand complexes, with sidechain deletion resulting in destabilization by up to approximately 4–7% for most compounds. Contacts between ligand and Phe123 do not generally represent any particular type of aromatic binding motif (ie T-shape, sandwich, parallel-displaced), but can better be described as general van der Waals contacts. It should be noted that, in the case of compound 5, the contributions to binding made by residues Phe224 and Phe258 are substantially diminished (compared with the other 4 compounds). Additionally, among the five compounds considered here, Phe123 makes its largest binding contribution for compound 5.

Carbazoles (Table S1, compounds 13–20) from our earlier studies and compound 12 have exhibited high selectivity and potency for P450 1A2 over 1A1 enzyme. All of these molecules have the classic triangular shape that fits our earlier hypothesis that such molecules are ideally suited for the active site of P450 1A2 enzyme. To look into the residues in the active site of P450 1A2 that contribute to their binding potency, a Glycine scan was performed for each of the docked structures of the protein-ligand complexes of the carbazoles in Table 3B and compound 12 (Fig. 5).

There are four residues within the P450 1A2 active site that seem to be of particular importance in ligand binding, these are Glu225, Phe226, Phe260 and Leu497. The two phenylalanine residues make particularly strong contributions to binding for many of the carbazole ligands, most notably compounds 13 and 19. In many cases, the Phe226 aromatic ring forms a near-ideal parallel-displaced configuration with a ligand carbazole ring. For compounds 13 and 19, Phe260 forms a close contact with the ring section of the ligand, forming a strong aromatic-aromatic interaction. For the other carbazole compounds, Phe260 is either relatively far from the ligand or forms a relatively weak interaction with an aliphatic ligand sidechain. Glu225 does not tend to participate in any directional interactions involving its carboxylic group, but is involved in nonspecific aliphatic contacts with the ligand. The large aliphatic Leu497 sidechain is generally located above a ligand aromatic ring, forming strong contacts through aromatic-aliphatic interactions. It has been noted in several benchmark studies that the strengths of aromatic-aliphatic interactions can approach those of aromatic-aromatic interactions [38, 39].

None of the compounds studied here showed inhibition of the cytochrome P450 2A6 enzyme. Docking studies revealed that the compounds did not dock in the active site and steric hindrance was shown to be the reason for poor docking results. Four of the compounds inhibited cytochrome P450 2B1 with micromolar potencies, except for compound 7 which had an inhibition potency of 0.17 μ M. Our docking studies did not reveal any clear reason for the inhibition potency of compound 7 with P450 2B1.

5. CONCLUSION

Based on our earlier studies, a database search for *ortho* methyl aromatic amino groups produced a set of commercially available compounds that were purchased and assessed for their ability to cause time-dependent inhibition of P450 enzymes 1A1, 1A2, 2A6, and 2B1. Four of the compounds indeed showed time-dependent inhibition of P450 1A1 but not 1A2. A carbazole derivative, compound 12, with a methyl substituent *ortho* to the aromatic amino group was also assessed since carbazoles show more specificity for P450 1A2 over 1A1. Compound 12 showed similar specificity for P450 1A2 over p450 1A1, but the presence of the *ortho*-methylanino groups did not lead to the compound being a time-dependent inhibitor. The reason for the absence of such activity could be due to greater distance between the sidechain methyl group and heme-Fe atom. The binding modes revealed that the proximity of the methyl group to the heme-Fe enables the abstraction of a hydrogen atom leading to a reactive intermediate that inactivates the P450 1A1 enzyme. Further studies will focus on confirmation of the involvement of the methyl group in enzyme inactivation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. (1).
Structures of compounds 1–12.

Fig. (2). Binding mode of compounds (A) 1 And 3 (B) 4 and 6 to the active site of P4501A1, and compounds (C) 12–20 and (D) compound 12 to the active site of P4501A2. The protein residues are shown as thin-stick models, the heme residue is shown as a ball and stick model and the ligand molecules is shown as a thick-stick model.

Fig. (3).

Glycine scanning protein-ligand interaction destabilization for compounds **1,3,4,5** and **6** bound to P450 1A1.

Fig. (4).
Interactions of Phe 224 and Phe258 with compound 3 within the P450 1A1 active site.

Fig. (5).
Glycine scanning protein-ligand interaction destabilization for compounds 12–20 bound to P450 1A2.

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Inhibition data in supersomes for compounds 1–12 measured using human P450 enzymes (1A1, 1A2, and 2A6) and rat P450 enzyme (2B1).

Table 1.

Compound	IC ₅₀ (μ M)			
	1A1	1A2	2B1	2A6
1	0.4	0.53	>50	>50
2	2.09	0.53	2.15	>50
3	0.1	0.43	17.34	>50
4	0.5	1.59	>50	>50
5	0.58	3.51	>50	>50
6	1.52	1.27	>20	>20
7	3.67	7.15	0.17	>50
8	13.23	>50	>50	>50
9	1.85	16.9	>50	>50
10	7.7	>50	>50	>50
11	11	8.17	>50	>50
12	1.61	0.032	4.31	>10

Table 2. Mechanism-based inhibition of human P450 enzymes 1A1 and 1A2 by compound 1, 3, 4, 6 and 9.

Compound	1A1	
	$K_I(\mu\text{M})$	Limit $K_{\text{inact}}(\text{min}^{-1})$
1	5.38	1.57
3	0.24	0.6
4	0.37	0.04
6	0.47	0.20
9	8.25	0.29
Compound	1A2	
	$K_I(\mu\text{M})$	Limit $K_{\text{inact}}(\text{min}^{-1})$
1	0.50	0.08

Structures of carbazoles and their inhibition potencies for P450s 1A1, 1A2 and 2B1 from our earlier studies.

Table 3.

A	Compound	B		
		IC ₅₀ (μ M)		
		1A1	1A2	2B1
	13	21.500	0.350	1.830
	14	10.300	0.206	1.140
	15	8.730	0.302	0.659
	16	6.640	0.110	1.780
	17	35.000	0.375	13.300
	18	15.600	0.309	8.430
	19	26.400	6.350	10.600
	20	8.650	2.700	5.260