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Proposed structural basis of interaction of piperine and related compounds with monoamine oxidases

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ABSTRACT

Several studies have revealed piperine and a few related compounds as potent inhibitors of monoamine oxidases without delineating the underlying mechanism. Using in silico modelling, we propose a structural basis of such activity by showing that these compounds can successfully dock into the inhibitor binding pockets of human monoamine oxidase isoforms with predicted affinities comparable to some known inhibitors. The results therefore suggest that piperine can be a promising lead for developing novel monoamine oxidase inhibitors.

Piperine (*trans trans* isomer of 1-piperolylpiperidine) is an alkamide present as a major constituent in the fruits of black pepper (*Piper nigrum* Linn.), long pepper (*Piper longum* Linn.) and many other *Piper* species belonging to the plant family Piperaceae.[1] It shares structural similarity with capsaicin—the pungent principle in red chilli peppers and like capsaicin, piperine also serves as a natural agonist of the vanilloid receptor (TRPV1 channel), the latter being crucially involved in the neurotransmission of thermal and nociceptive stimuli.[2,3] Existing literature on piperine suggests that this molecule is endowed with a diverse array of biological activities, notably including anti-oxidant, anti-inflammatory, analgesic, anti-platelet,[4] anti-hyperlipidemic,[5] anti-hypertensive,[6] cytoprotective, anti-tumour, antimicrobial,[7,8] hepatoprotective,[9] antidepressant and bioavailability-enhancing activity.[10] The underlying molecular mechanisms of such broad range of activities of piperine are rather poorly understood and hitherto its molecular interaction has been shown only with bovine β-lactoglobulin[11] and human serum albumin[12] whilst its precise modes of interaction with other suitable macromolecular targets remain to be elucidated.

Amidst the vast spectrum of piperine's activities, it was particularly intriguing to notice the consistent outcome from several independent studies, suggesting significant antidepressant-like activity of this compound. For example, piperine and its shorter analog, antiepilepsirine exhibited antidepressant-like activity in classical behavioral models such as forced swimming test and tail suspension tests in rodents.[1,13,14] Piperine treatment also led to increased level of biogenic amines such as noradrenaline and serotonin in some regions of mouse brain, which indirectly suggests its ability to interfere with the metabolism of these amines.[13,14] Lastly, piperine and its analog—methylpiperate have been directly shown to inhibit the relevant enzymes that is, monoamine oxidases (MAOs; EC 1.4.3.4) in vitro.[15–17]

The mammalian MAO family comprises of two flavin-dependent isozymes (MAO-A and MAO-B) that remain bound to the mitochondrial outer membrane and catalyze the oxidative deamination of the endogenous (e.g., dopamine, serotonin, noradrenaline) and exogenous amines (e.g., phenylethylamine and tyramine).[18] Selective human MAO-A (hMAO-A) inhibitors are useful in anxiety and depression, while selective human MAO-B (hMAO-B) inhibitors can be potentially beneficial in neurodegenerative disorders such as Alzheimer's and Parkinson's disease, either alone or in combination with other drugs.[19,20] The availability of several high-resolution structures of MAO-A and MAO-B[18,21,22] has advanced our understanding of the molecular mechanism of action of these enzymes and these can be exploited in rational design of therapeutically effective, selective MAO inhibitors with minimum or preferably no side effects. In the present study, computational docking experiments of piperine together with few related compounds (Fig. 1) against hMAO-A and hMAO-B were attempted to assess the possible mode of interaction of these compounds with hMAO isoforms and thereby finding a rationale for their reported antidepressant action.

For the present study, crystal structures of hMAO-A (PDB: 2Z5X)[23] and hMAO-B (PDB: 2V5Z)[21] were obtained from the protein databank (www.pdb.org) and the ligands (Fig. 1) were

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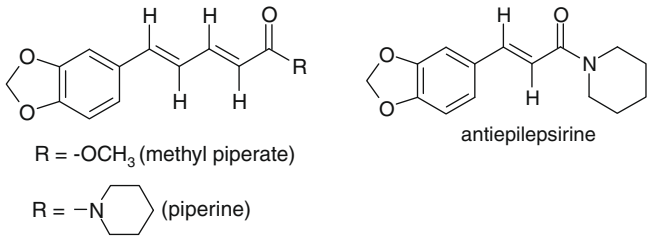

Figure 1. Structure of piperine and related alkamides.

obtained from the PubChem database (http://pubchem.ncbi.nlm.nih.gov). The protein structures were first pretreated as described previously.[20] Then they were subjected to 0.1 ps molecular dynamic simulation (MD) followed by flexible docking performed in NAMD version 2.7b1 and AutoDock 4.0, respectively in accordance with previously described protocols.[24] From the estimated free energy of ligand binding ($\Delta G_{binding}$, kcal/mol), the inhibition constant (K_i) for each ligand was calculated. Only the best pose (i.e., the one with the lowest $\Delta G_{binding}$) was considered for each ligand. Finally, the best poses were analyzed for hydrogen bonding interactions using Ligplot.[25] PyMOL (http://pymol.sourceforge.net/) was used for representing the docked structures. It is worthy of mentioning that the correlations between the apparent and experimental values of K_i were significantly improved (data not shown) by the pre-treatment of the protein structures[20] and by relaxing the protein structures through brief MD simulation prior docking.

To evaluate the accuracy of AutoDock 4.0 as an appropriate docking tool for the present purpose, the co-crystalized ligands (harmine and safinamide for 2Z5X.pdb and 2V5Z.pdb, respectively) were redocked within the inhibitor binding cavity (IBC) of hMAOs and the procedure was repeated at least three times. As shown in Supplementary Fig. 1, the best poses of redocked harmine and safinamide were within reasonable proximity (root mean square deviation, RMSD ≤1.5 Å) of the original poses in the crystal structures of cognate hMAOs and the poses were obtained reproducibly. AutoDock 4.0 therefore deemed reliable for docking piperine and related compounds into the IBC of hMAOs.

The piperine structure (Fig. 1) can be split into three fragments namely methylenedioxyphenyl (MDP) ring, side chain with conjugated double bonds and a basic piperidine moiety attached through a carbonylamide linkage to side chain. As illustrated in Fig. 2, piperine docked into the IBC of hMAO-A outlined by residues such as Tyr 69, Ile 180, Asn 181, Ile 207, Gln 215, Cys 323, Ile 335, Leu 337, Tyr 407 and the isoalloxazine ring of FAD.[22] In this pose, the two oxygen atoms of the MDP ring appear to remain hydrogen bonded to three buried water molecules (726th, 746th and 805th in 2Z5X.pdb). At the other end of the molecule, the carbonyl oxygen of the carbonylamide linkage seems to be engaged in similar interaction with the thiol group of Cys 323 (Fig. 2B). These hydrogen bonding interactions therefore are likely to contribute to the placement of piperine within the IBC of hMAOA. However, the MDP ring of piperine fails to be appropriately positioned between the phenolic side chains of Tyr407 and Tyr444—the residues that constitute the 'aromatic cage' of this hydrophobic pocket[18] and thus does not permit any π–π stacking interaction with these residues. It is worth mentioning that in semi-flexible docking experiments where hMAO-A structure was held rigid (i.e., without any 0.1 ps MD simulation prior docking), the MDP ring was indeed placed within the aromatic cages (data not shown). But, the present pose obtained through fully flexible docking approach indicates that such orientation of piperine within the IBC of hMAO-A was not stable.

Unlike hMAO-A, the IBC of hMAO-B represents a bipartite hydrophobic space consisting of a 'substrate-binding cavity'

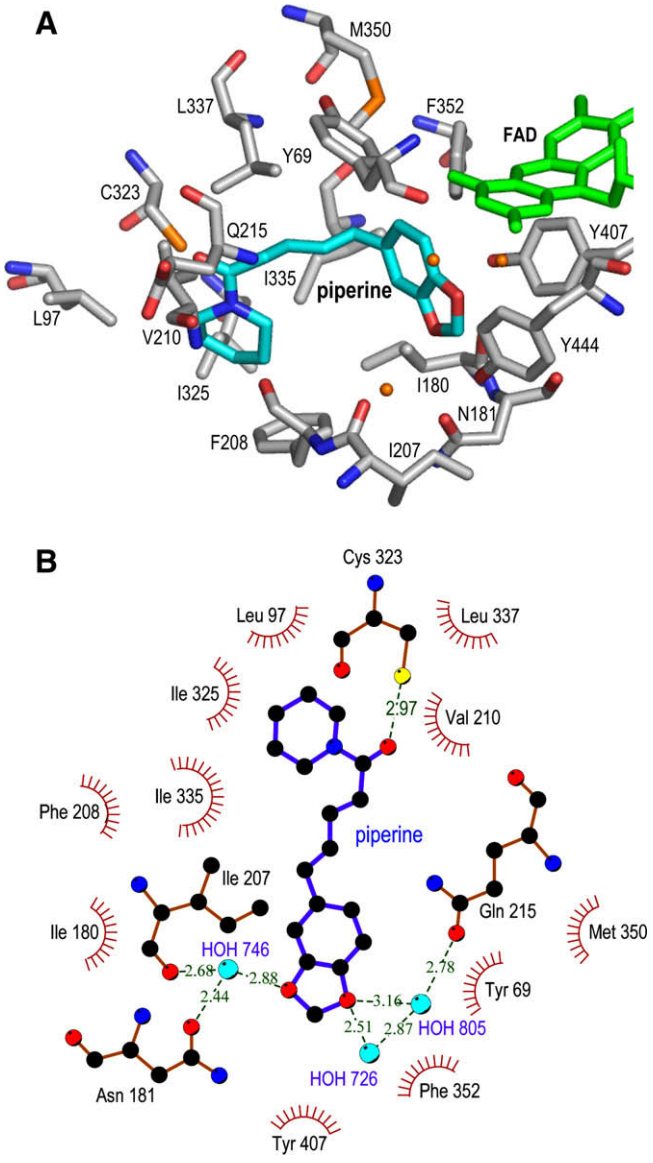

Figure 2. Docking result of piperine with human MAO-A. The lowest energy configuration of piperine and hMAO-A complex is shown in PyMol (A) and Ligplot (B) presentations. Residues identified in Ligplot are labelled and shown in PyMol as sticks along with piperine (cyan) and FAD (green). Dashed lines in PyMol (A) and Ligplot illustration (B) indicate H-bonds. Carbons are in black, nitrogens in blue and oxygens in red. Water molecules are shown as orange spheres.

separated by a smaller 'entrance cavity' with Ile 199 effectively serving as a 'gate' between these cavities.[26] From close inspection of piperine docked into the IBC of hMAO-B (Fig. 3), the molecule appears to span both the entrance and substrate-binding cavities, largely through hydrophobic interaction. The MDP ring of piperine was placed inside the substrate-binding cavity lined by residues including Tyr 398, Tyr 435, Tyr 188, Gln 206, Gly 434, Cys 172 and isoalloxazine ring of FAD. The basic piperidine moiety was accommodated within the entrance cavity lined by Ileu 199, Tyr326, Leu 171, Thr 201, Phe 168, Leu 164 and Leu 167 (Fig. 3). Two buried water molecules within the IBC of hMAO-B appear to be involved in hydrogen bonding with the piperine structure. One such interaction involves an oxygen atom of the MDP ring and a water molecule (1155th in 2V5Z.pdb) to which Cys 172 and Tyr 188 also seem to form hydrogen bonding. The second hydrogen bonding involves the carbonyl oxygen of the carbonylamide linkage of piperine and another water molecule (1229th in

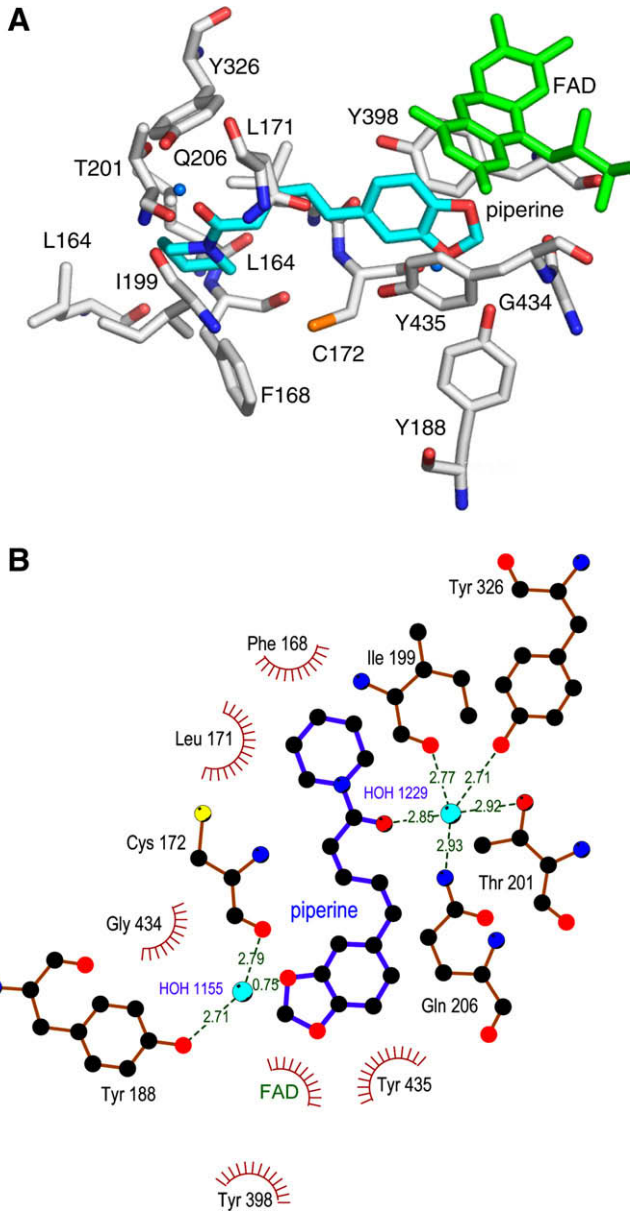

Figure 3. Docking result of piperine with human MAO-B. The lowest energy configuration of piperine hMAO-B complex is shown in PyMol (A) and Ligplot (B) presentations. Residues identified in Ligplot are labelled and shown in PyMol as sticks along with piperine (cyan) and FAD (green). Dashed lines in Ligplot illustration (B) indicate H-bonds. Carbons are in black, nitrogens in blue, oxygens in red, and sulfurs in yellow. Water molecules are shown as cyan spheres.

2V5Z.pdb) to which Ileu 199, Tyr 326, Thr 201 and Gln 206 seem to interact in similar way. The overall orientation of piperine within the inhibitor binding cavity of hMAO-B was broadly similar to its pose within the IBC of hMAO-A. The hydrogen bonding interactions could promote such binding orientation of piperine into hMAOs and for hMAO-B. It was noteworthy that the overall binding pose of piperine to the IBC of hMAO-B remained relatively unchanged regardless of whether the protein structure was held flexible or not, suggesting a more stable complex of piperine with hMAO-B compared to that with hMAO-A. This may account for the apparent hMAO-B selectivity of piperine observed in the present study (Table 1). Previously, piperine was found to be more MAO-A selective for rat[15] while it was more MAO-B selective for mouse.[16] The reasons for such discrepancy are not clear but species difference is likely to be among plausible explanations. Our flexible docking

Table 1
Predicted and experimentally observed values of inhibition constants (K_i) of the studied inhibitors

Compound	K_i (μM) (apparent)[a]		K_i (μM) (experimental)[b]		Ref.
	MAO-A	MAO-B	MAO-A	MAO-B	
Harmine	0.008	—	0.005 ± 0.0002	—	22
Safinamide	—	0.60	365 ± 18.7	0.45 ± 0.13	20
Piperine	21.81	1.98	49.3	91.3	15
			19 ± 0.9	3.19 ± 0.5	16
Methyl piperate	23.27	2.82	27.1	1.6	17
Antiepilepsirine	0.42	0.97	—	—	

[a] Calculated from the free energies of binding (ΔG_b, kcal/mol) obtained through flexible docking with AutoDock 4.0 (temperature = 300 K). For the latter, protein structures at the end of 0.1 ps MD simulation were used.
[b] Experimentally obtained values from human (for harmine and safinamide) and mouse/rat brain mitochondrial membrane preparation. The corresponding references are given in the right column.

experiment with hMAO isoforms offer better correlation of K_i values with data from mouse brain MAO activity, suggesting selectivity towards hMAO-B for piperine (Table 1). In this regard, it is intriguing to note that the overall piperine structure somewhat resembles to 1,4-diphenyl-1,3 butadiene which was found to have selective MAO-B inhibitory activity.[27]

While inhibition of mouse and rat brain-derived MAO-B by piperine has been shown to be competitive in previous studies, the mode of its inhibitory action on rodent brain-derived MAO-A was found to be either competitive or mixed type for mouse and rat MAO-A, respectively.[15,16] Irreversible inhibition of hMAO isoforms by piperine in our docking experiments was difficult to envisage as there was no obvious chemical features within the MDP ring of piperine that could potentially lead to the formation of covalent adduct with the N5 of the isoalloxazine ring of FAD and this was even less likely for hMAO-A as the MDP ring was further away from the isoalloxazine ring of FAD (Figs. 2 and 3). However, as proposed for some imidazoline (I_2) ligands,[28] the reported mix mode of rat MAO-A by piperine[15] could also involve an allosteric inhibition site. But for piperine, we failed to detect in silico any additional binding site within hMAOs.

In the present study, we have refrained from a detailed analysis of structure–activity relationship for piperine in the context of its inhibition of MAO activity. However, with a view to having some preliminary idea, the docking protocols were repeated with two related alkamides namely methyl piperate and antiepilepsirine (also known as ilepcimide) (Fig. 1). Methyl piperate has a methoxy group in place of the piperidine moiety of piperine and it has recently been shown to inhibit both MAO-A and MAO-B with more selectivity for the latter (Table 1).[17] The preliminary docking results suggest that methyl piperate can also be successfully docked into the IBC of hMAO-A and hMAO-B (Supplementary Fig. 2 and Table 1). Based on the values of apparent K_i, methyl piperate seems to be more hMAO-B selective in general like piperine, albeit with lower inhibitory potential. However, its selectivity towards hMAO-A appears to be comparable to that of piperine (Table 1). Thus, the piperidine moiety of piperine seems to aid in achieving better MAO-B selectivity while its omission barely affects the MAO-A selectivity. It is interesting to note that piperidine itself was shown to have some MAO inhibitory effect[29] and thus the piperidine moiety of piperine and its congeners could be an important structural feature for inhibition of MAO in general.

In order to have some idea about the minimum length of piperine analogs that can act as an effective inhibitor of MAOs, docking was carried out with antiepilepsirine which is a shorter analog of piperine (Fig. 1) and which has been reported to have antidepressant activity.[13] As shown in Supplementary Fig. 3, antiepilepsirine could also be docked into the IBC of both hMAO-A and hMAO-B

successfully. Interestingly, the apparent K_i values of this compound for hMAOs were markedly less compared to that of piperine and methyl piperate with more apparent selectivity towards hMAO-A (Table 1). This suggests that shortening the length of piperine scaffold may dramatically improve the overall selectivity of the compound for hMAOs while relative selectivity towards hMAO-A can still be retained. However, it should be noted that this data requires validation from in vitro assays of MAO with antiepilepsirine.

All compounds screened in the present study had the intact MDP ring (Fig. 1) and compounds having such moiety seem to offer wide range of bioactivities with particular propensity for being psychoactive and inhibitory to hepatic mixed function oxidases.[30–32] Therefore, the MDP ring of piperine, methyl piperate and antiepilepsirine is likely to be important for imparting MAO-inhibitory activity. Interestingly, such moiety when attached to different scaffolds, seems to improve the MAO-inhibitory potential of the synthesized compounds.[33,34] Some nitrogen containing metabolites of safrole[29] as well as myristicin[35] both of which are archetypical MDP-ring containing substances, were found to inhibit MAOs of rat liver, kidney, brain and mitochondrial origin. Besides, the MDP moiety of piperine and its congeners may also broadly mimic the structure of eugenol which has been shown to inhibit MAOs.[36] All these evidences indirectly suggest that the MDP moiety is likely to be an essential feature for piperine and related alkamides to exhibit MAO-inhibitory activity.

MAO-A has been suggested to act preferentially on serotonin and norepinephrine, while both MAO isoforms degrade dopamine with almost equal efficacy. Selective inhibition of MAO-A activity has been considered as an effective antidepressant strategy.[19] However, several studies have also suggested a deficiency of dopamine in depression.[37] Therefore, the antidepressant-like effect of piperine and its congeners may be mediated through the enhancement of not only serotonergic and norepinephrinegic neurotransmission but also of dopaminergic neurotransmission.[16] Selective upregulation of MAO-B has been implicated in age-related neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease.[19] The seemingly greater selectivity of piperine towards MAO-B as we and others[16] have observed, could make it a better lead for designing neuroprotective and cognitive-enhancing drugs. Indeed piperine treatment has been found to promote neurogenesis and cognition as well as to provide neuroprotection.[1,13,14]

In conclusion, we have shown the possible interactions of piperine—a major alkamide of many common spices and few of its congeners such as methyl piperate and antiepilepsirine with the IBC of both hMAO-A and hMAO-B. This could provide a molecular basis for the existing evidences for antidepressant-like as well as few other neuroactive properties of piperine and related compounds. Such observations can also help to consider piperine as an effective scaffold for rational design of novel and potential drugs against diseases precipitated by increased metabolism of biogenic amines within some key areas of the central nervous system.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.11.106.

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