Docking studies of antidepressants against single crystal structure of tryptophan 2, 3-dioxygenase using Molegro Virtual Docker software

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Abstract: Tryptophan 2, 3-dioxygenase (TDO) a heme containing enzyme found in mammalian liver is responsible for tryptophan (Trp) catabolism. Trp is an essential amino acid that is degraded in to N-formylkynurenine by the action of TDO. The protein ligand interaction plays a significant role in structural based drug designing. The current study illustrates the binding of established antidepressants (ADs) against TDO enzyme using in-silico docking studies. For this purpose, Fluoxetine, Paroxetine, Sertraline, Fluvoxamine, Seproxetine, Citalopram, Moclobamide, Hyperforin and Amoxepine were selected. In-silico docking studies were carried out using Molegro Virtual Docker (MVD) software. Docking results show that all ADs fit well in the active site of TDO moreover Hyperforin and Paroxetine exhibited high docking scores of -152.484k cal/mol and -139.706k cal/mol, respectively. It is concluded that Hyperforin and Paroxetine are possible lead molecules because of their high docking scores as compared to other ADs examined. Therefore, these two ADs stand as potent inhibitors of TDO enzyme.

Keywords: Tryptophan, Tryptophan 2, 3-dioxygenase, Molegro Virtual Docker, Antidepressants, Docking,

INTRODUCTION

Tryptophan 2, 3-dioxygenase (TDO, EC 1.13.11.11) is cytosolic heme dioxygenase that catalyzes the oxidative cleavage of the C2-C3 bond of the indole ring of L-tryptophan (Trp). This reaction is first and rate-limiting step of the kynurenine pathway of tryptophan catabolism, which eventually leads to the formation of nicotinamide dinucleotide (NAD^+), a process regarded as the primary biological function of TDO (Stone, 2002). Trp availability to the brain also play important role in central 5-HT synthesis because the rate limiting enzyme of the serotonin–biosynthetic pathway, Trp hydroxylase, is unsaturated with its Trp substrate, brain Trp concentration is the most important single metabolic determinant of the rate of serotonin synthesis (Fernstrom and Wurtman, 1971). Consequently, peripheral factors influencing circulating Trp availability to the brain play important roles in central serotonin synthesis. These include, at the primary level of control, activity of the major Trp degrading enzyme, liver TDO (Badawy, 1977).

Animal's studies have shown that different classes of antidepressants (ADs) increase brain tryptophan (Trp) and thereby enhance 5-HT synthesis by inhibiting tryptophan 2, 3-dioxygenase (TDO) activity (Badawy & Evan 1981; Badawy & Evan 1982; Badawy and Morgan 1991; Bano *et al*., 1999). Earlier we have reported that 20mg/kg acute administration of hypericin in force swimming test rats enhanced TDO activity and normalized brain Trp and serotonin level (Bano & Dawood; 2008). Earlier we have found that acute doses of 10mg/kg of Moclobamide, Tianeptine, Sertraline, Citalopram and Hypericin elevate brain Trp level followed by increase in free plasma Trp level. So the precursor (Trp) availability plays key role for cerebral serotonin synthesis, either for its increased release if required or metabolism (Bano *et al*., 2010). TDO is regulated by numbers of factors like substrate availability, end product inhibition by its metabolites. Cortisol and Trp activate TDO enzyme. Majority of depressed patients were found to have elevated cortisol levels (Salter & Pogson, 1985; Curzon, 1988) and low levels plasma Trp. It is suggested that increased cortisol induce TDO leading to decreased brain serotonin (5-hydroxytrptamine; 5-HT) which is also considered as an etiological factor for depression (Van Pragg, 1978; Wong & Licinio, 2001).

The effectiveness of Trp as an AD alone or in combination is restricted because it is rapidly metabolised through kynurenine pathway in the liver (Moller, 1981; Moller *et al*., 1982). Efficacy of tryptophan may also be limited because of neuronal metabolism by MAO of newly synthesized serotonin (Sharp *et al*., 1992). TDO is rate limiting enzyme of the kynurenine pathway (Salter *et al*., 1986). It is suggested that inhibition of TDO decreases catabolism of endogenous Trp in the body, elevate plasma Trp and thereby increase uptake by the brain. It will in turn increase the saturation of Trp hydroxylase, which remain unsaturated with its substrate under normal physiological conditions thus serotonin availability to the brain increases (Pogson *et al*., 1989). A pharmacokinetically, inhibitor of TDO should also produce a sustained elevation of endogenous Trp (thus

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serotonin), compared to the transient effect of Trp administration, leading to greater antidepressant efficacy. It has been suggested that combined inhibitors of TDO and serotonin reuptake should elevate serotonin to levels greater than those seen with either agent alone (Walinder *et al*., 1976).

Available TDO crystal structures are that of Xanthomonas campestris (xc TDO, PDBid; 2NW8) (Forouhar *et al*., 2007) and Ralstonia metallidurans (rm TDO, PDBid; 2NOX). In both cases the enzyme were found to crystallize in a tetrameric form with several inter subunit contacts. In XcTDO crystal structure, L-Trp binding site consists of residue His 55, Arg 117, Phe 51, Leu 120 and Tyr 113 (Zhang *et al*., 2007).

The stereochemistry of binding of the AD on TDO has not yet been characterized. The aim of the present study is to explore potent TDO inhibitors among established antidepressants by using MVD software.

MATERIALS AND METHODS

In this study, docking of selected ADs against TDO was carried out by MVD soft ware. Differential evolution was introduced by Storn and Price (1995). The docking scoring function of MVD is based on PLP originally introduced by Gehlhaar *et al*., (1995 & 1998) latter modified by Yang *et al.,* (2004) is extended with a new term, taking hydrogen bond directionality into account. Moreover, a re-ranking procedure is applied to the highest ranked poses to further increase docking accuracy. During this study 10 solutions obtained from the 10 independent docking runs and then re-ranked. The antidepressants like Fluoxetine, Paroxetine, Sertraline, Fluvoxamine, Seproxetine, Citalopram, Moclobamide, Hyperforin (phytochemical of Hypericum Perforatum; St. John's Wort) and Amoxepine were selected for this study. The selected ligand structures were built using ChemDraw (fig 1) software and imported to MVD workspace in 'sdf' format. Using the utilities provided in MVD All necessary valency checks and H atom addition were done. For precise docking it is important that the imported structures have been prepared accurately, that is the atom connectivity and bond orders are correct and partial atomic charges are assigned. PDB file often have poor or missing assignment of explicit hydrogens, and the PDB file format cannot accommodate bond order information. MVD automatically detects potential binding sites (cavities) using the cavity detection algorithm. To mechanize benchmarking, cavities within a 30x 30x30$Å^3$ cube centered at the experimentally known ligand position were used. The cavities found by the cavity detection algorithm are actively used by the search algorithm guided differential evolution to focus the search, to that specific area during the docking stimulation.

Several crystal structures of TDO have been solved by X-ray diffraction complexes with different ligands. The crystal structure selected for present study is 2NW8 complexes with tryptophan (fig 2). This crystal structure of TDO (PDB, ID, 2NW8) was downloaded to MVD workspace from protein data bank (htt p://www.rcsb.org/pdb), under the criteria that they had a reasonable resolution ($\leq 2.8A^0$) and involved the non-mutated TDO enzyme- ligands complex.

For each ligand docking, the best orientation for the ligand-protein complex was analyzed and hydrogen bonds were identified and labeled. The ligand energy was inspected and analyzed using MVD score, a linear combination of E-inter (steric, Vander Waals, hydrogen bonding and electrostatic interactions) and E-intra (torsion, sp^2- sp^2, hydrogen bonding, Vander Waals and electrostatic interaction).

RESULTS

Table 1 shows MolDock score, re- rank score and the hydrogen bond energy of selected 9 ADs. Table 2 shows amino acid residues present in the active site of TDO and also ligand binding amino acids. Fig. 1 shows selected ligand structures were built using ChemDraw software. Fig. 2 shows crystal structure of TDO (PDB, ID, 2NW8) complex with ligand (Trp) is represented in ball & stick model prepared by using MVD soft ware. Fig 3 shows detected cavities in TDO. Fig 4 shows docked structure of TDO with nine selected ADs. Ligand 1 (Fluoxetine) bind in to the active site with mole dock score -116.694 k cal/mol & binding site consist of amino acid residues like Ser 123, Gly 121, Arg 117, Tyr 113.Ligand 2 (Paroxetine) binds in to the active site with mole dock score -139 k cal/mol & binding site consist of amino acid residues like Arg 117, Gly 121. Ligand 3 (Sertraline) bind in to the active site with mole dock score -109.796 k cal/mol & binding site consist of amino acid residues like Gly 125, Ser 123,128, Tyr 113. Ligand 4 (Citalopram) bind in to the active site with mole dock score -138.538 k cal/mol & binding site consist of amino acid residues like Thr 254, Leu 120, and Ser 123. Ligand 5 (Fluvoxamine) bind in to the active site with mole dock score -133.451 k cal/mol & binding site consist of amino acid residues like Gly 125. Ligand 6 (Seproxetine) bind in to the active site with mole dock score -138.081 k cal/mol & binding site consist of amino acid residues like Arg 117, Gly 125, Thr 254. Ligand 7 (Hyperforin) bind in to the active site with mole dock score -116.694 k cal/mol & binding site consist of amino acid residues like Tyr 113, Thr 254, Val 119, and His 55. Ligand 8 (Moclobamide) bind in to the active site with mole dock score -121.275 k cal/mol & binding site consist of amino acid residues like Arg 117, Thr 254, Ser 124, Tyr 113. Ligand 9 (Amoxapine) bind in to the active site with mole dock score -116.694 k cal/mol & binding site consist of amino acid residues like Phe 262, Leu 263, Gly 125.

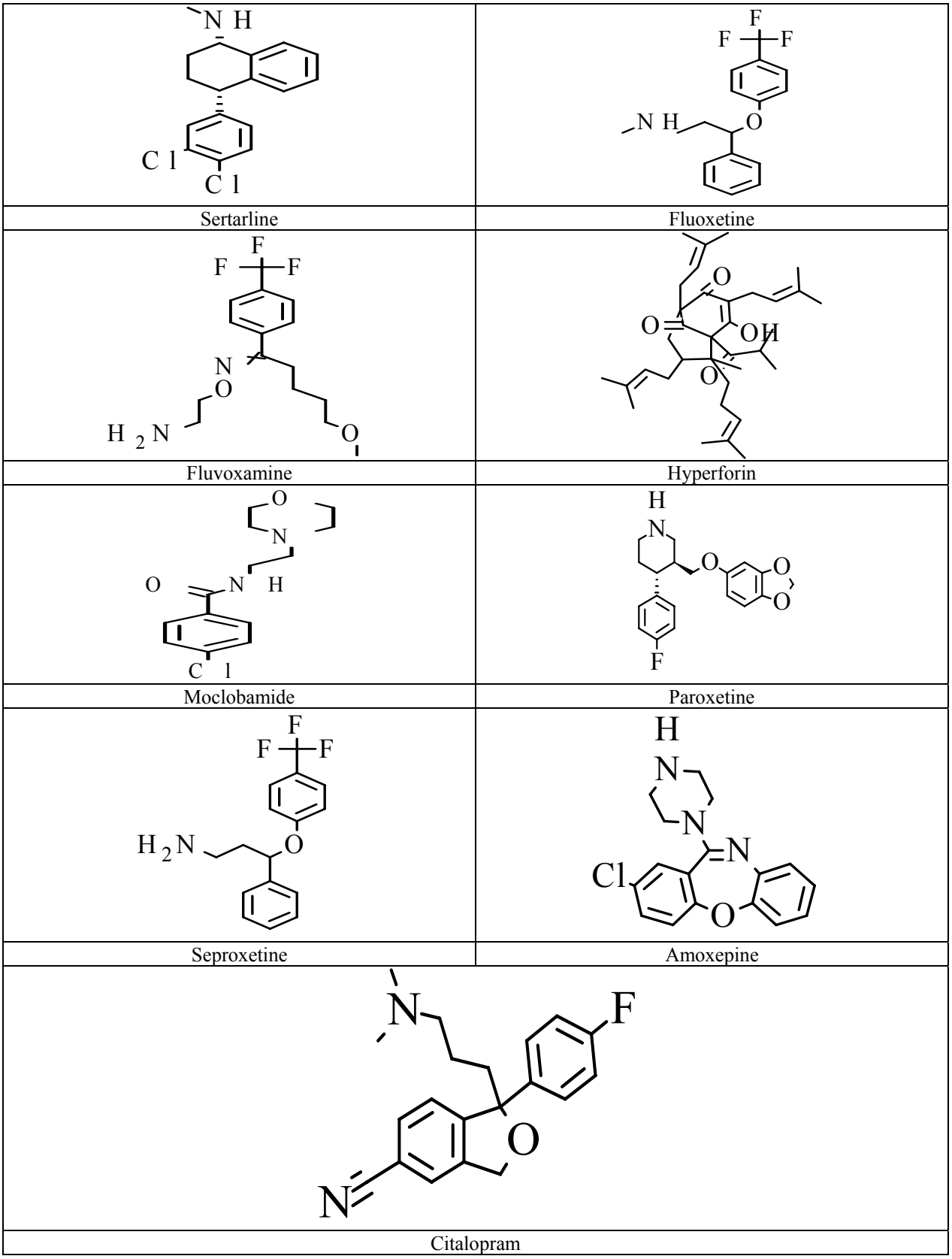


Fig. 1: Antidepressants structures were built using ChemDraw software.

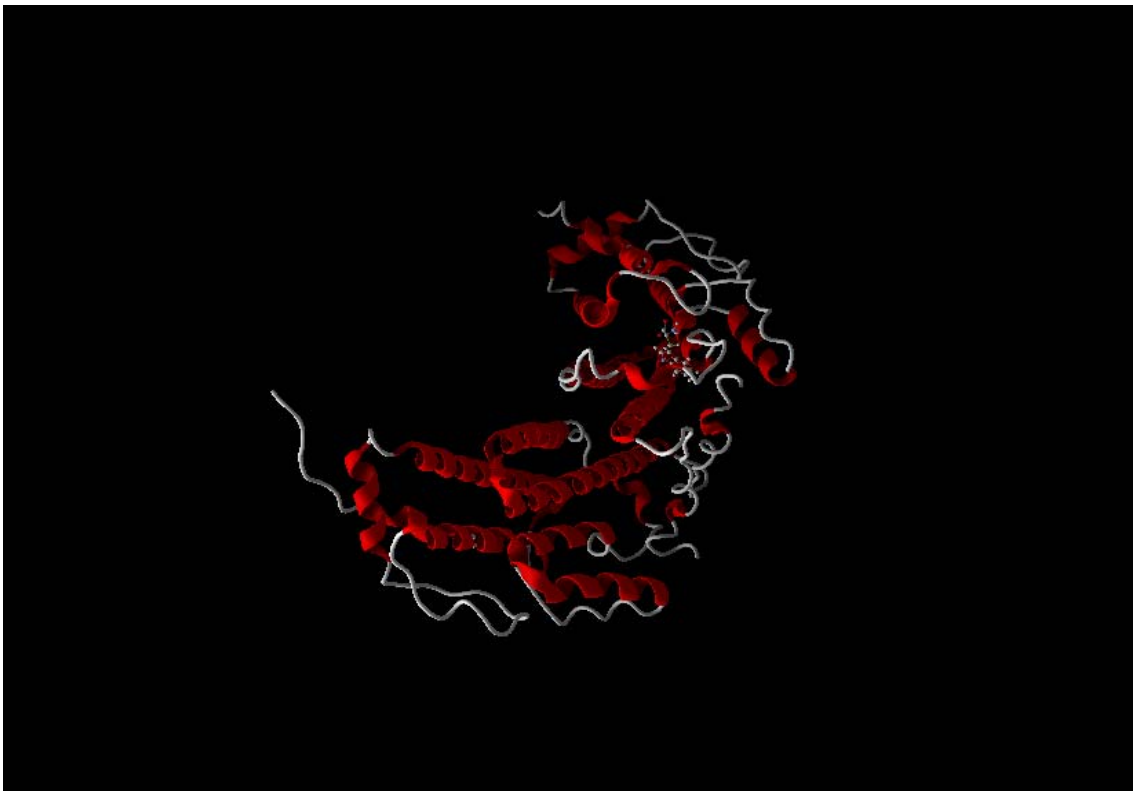


Fig. 2: Structural cartoon of tryptophan 2,3-dioxygenase (PDB code 2NW8; The α helices and β strands are represented as coils (red) and arrows (white), respectively. Tryptophan is represented in ball and stick. Model prepared using MVD.

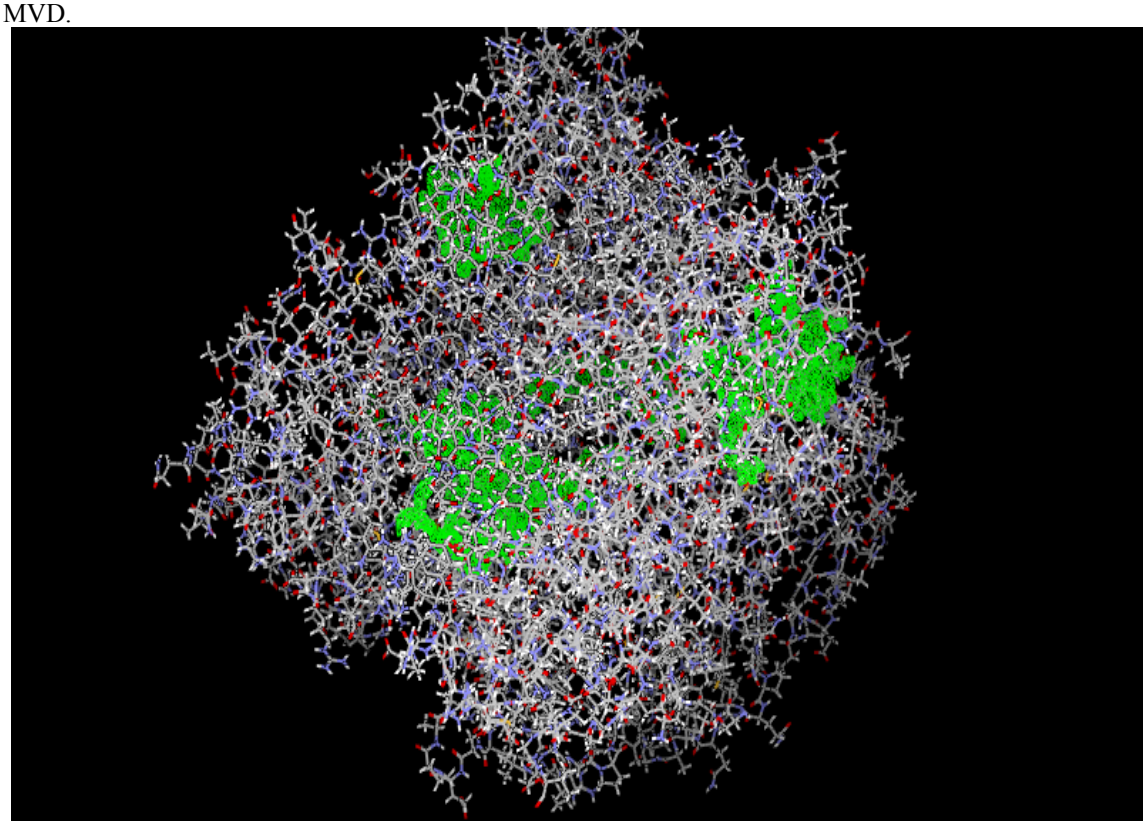
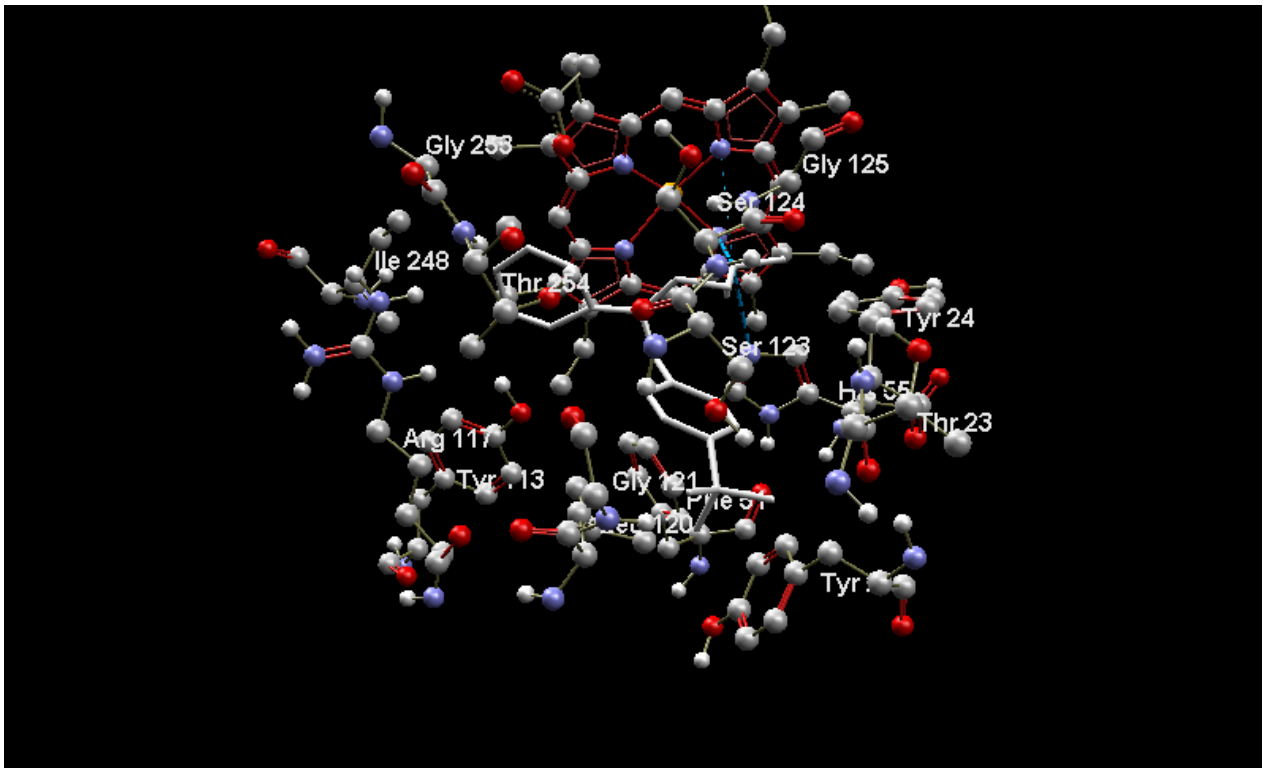
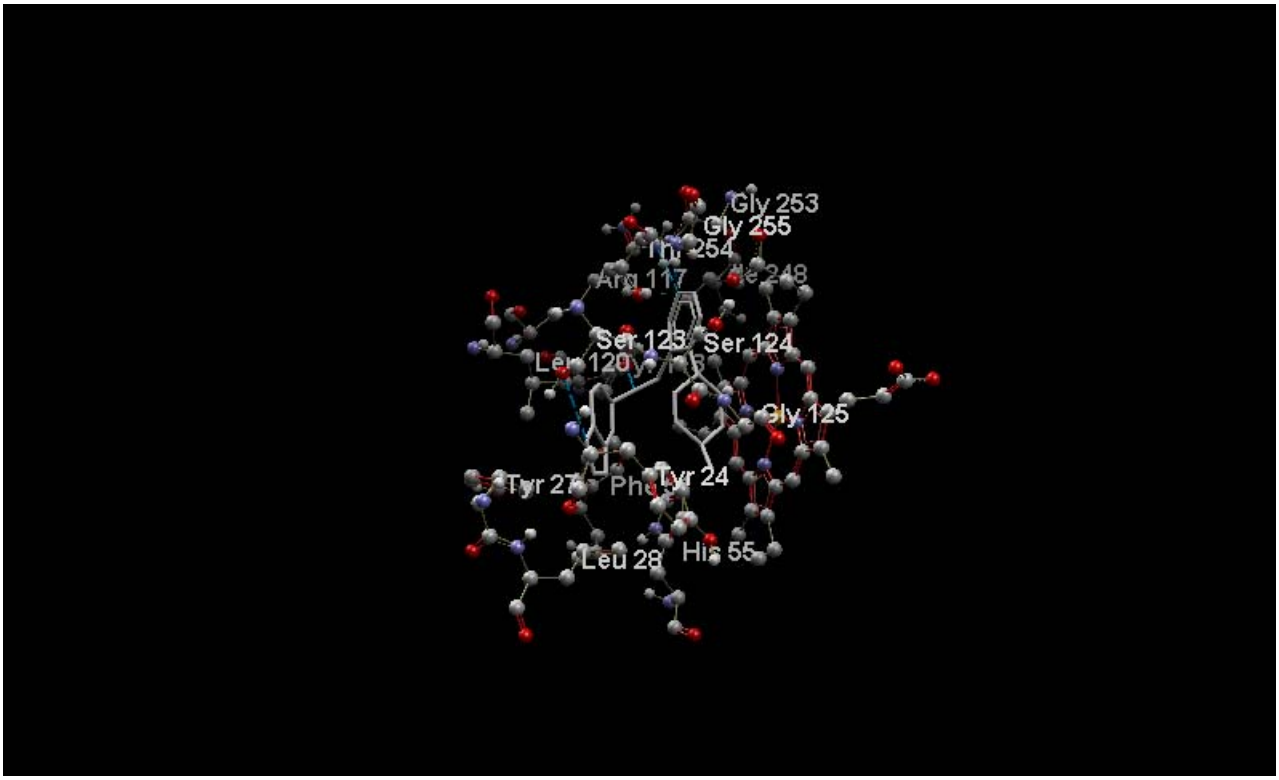


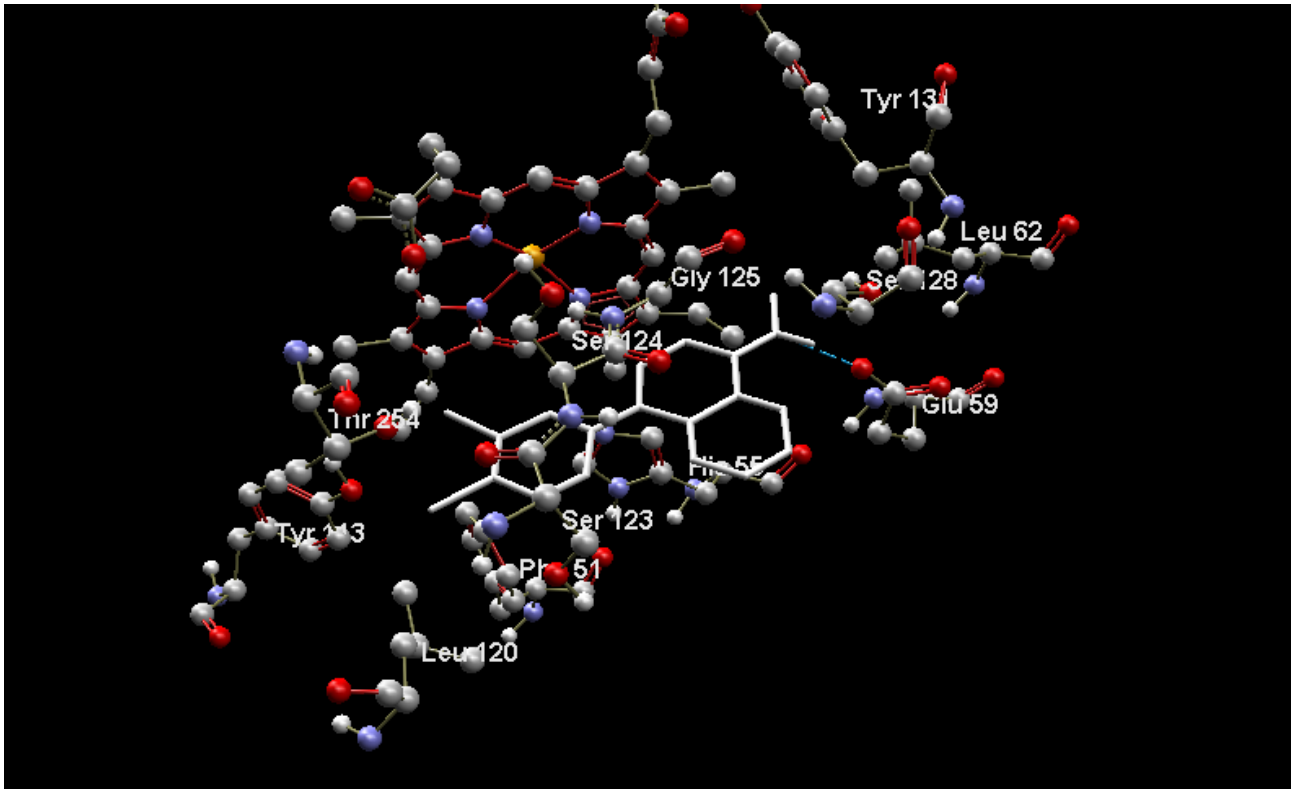
Fig. 3: The five MVD- detected cavities in TDO, PDB code; 2NW8 (Forouhar, 2007). Detected cavities; green; carbon atom; grey; oxygen atoms; red; nitrogen atoms; blue)



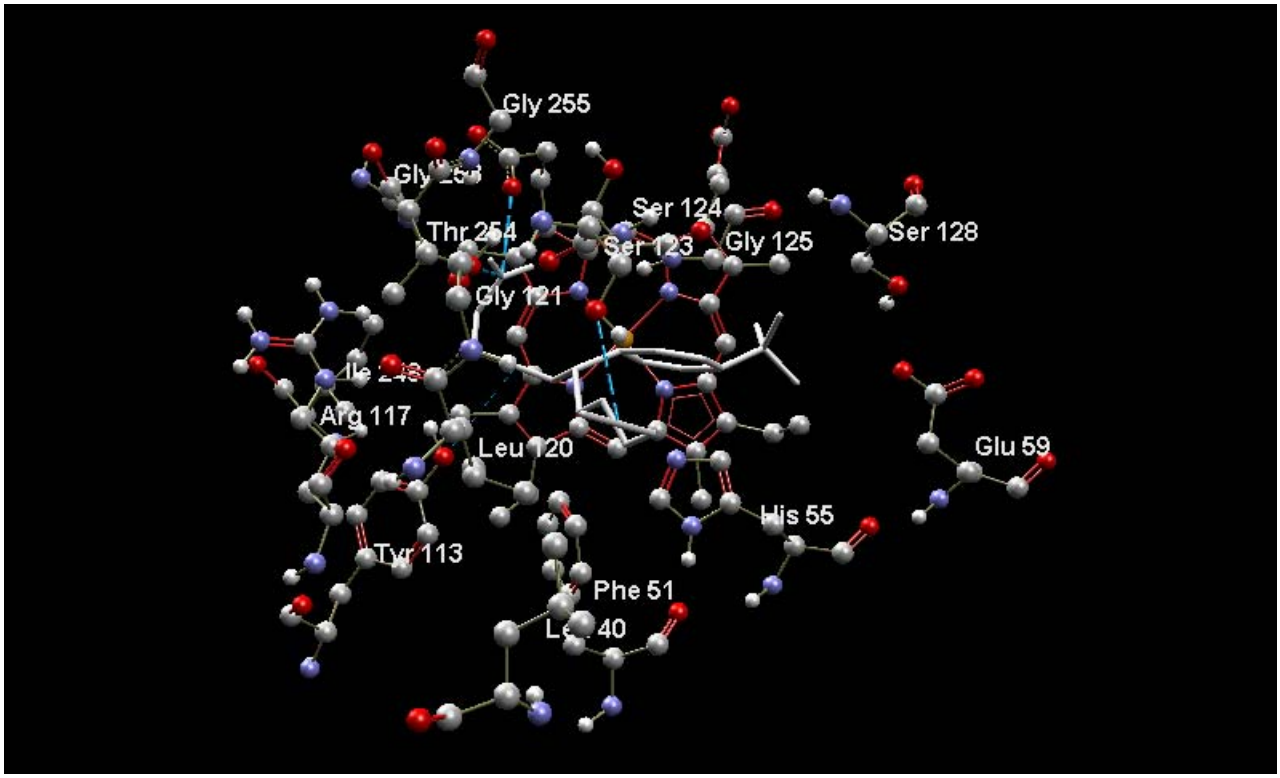
Ligand 1 (Fluoxetine)



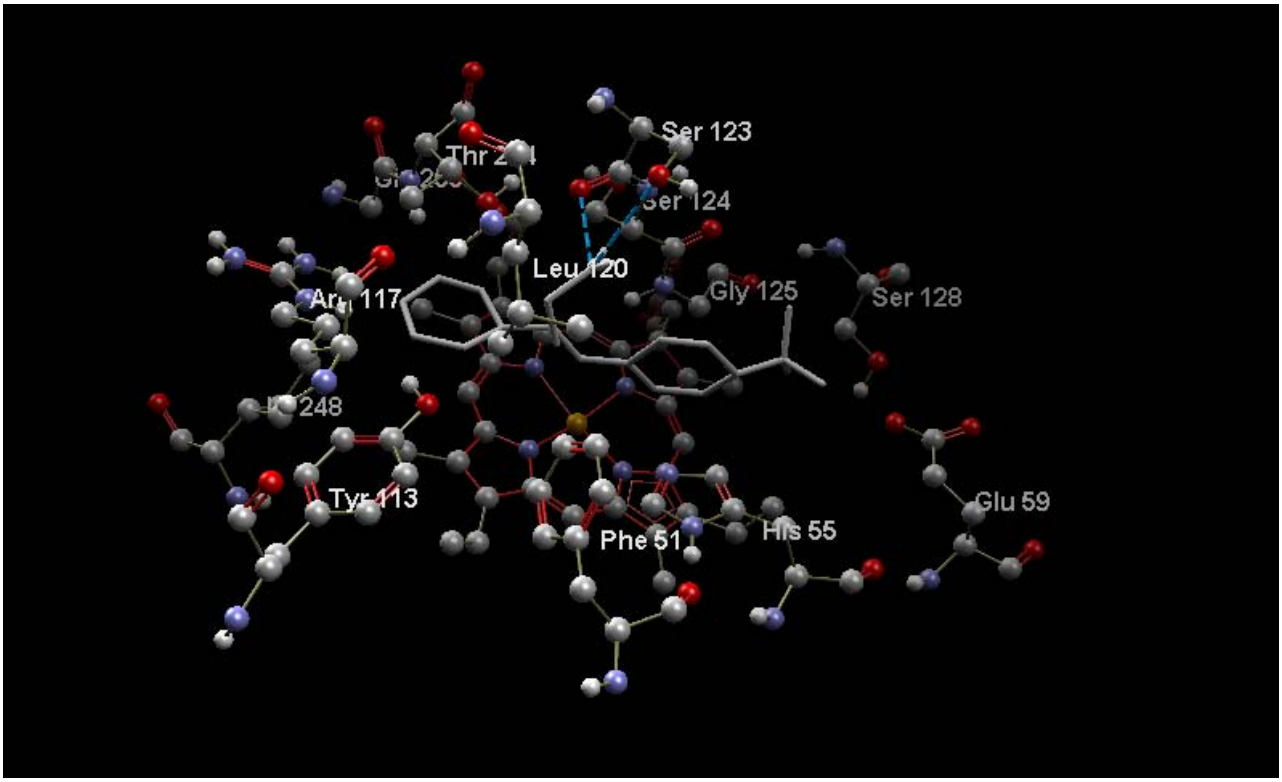
Ligand 2 (Paroxetine)



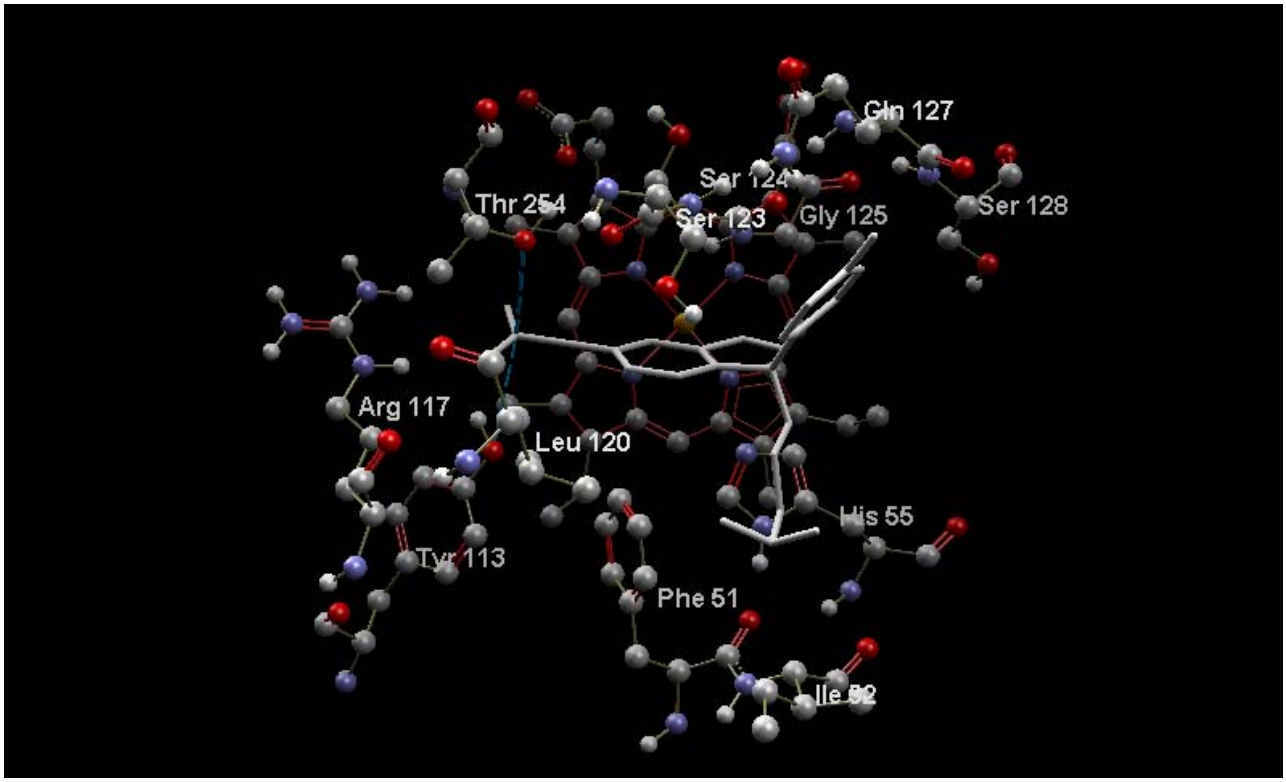
Ligand 3 (Sertraline)



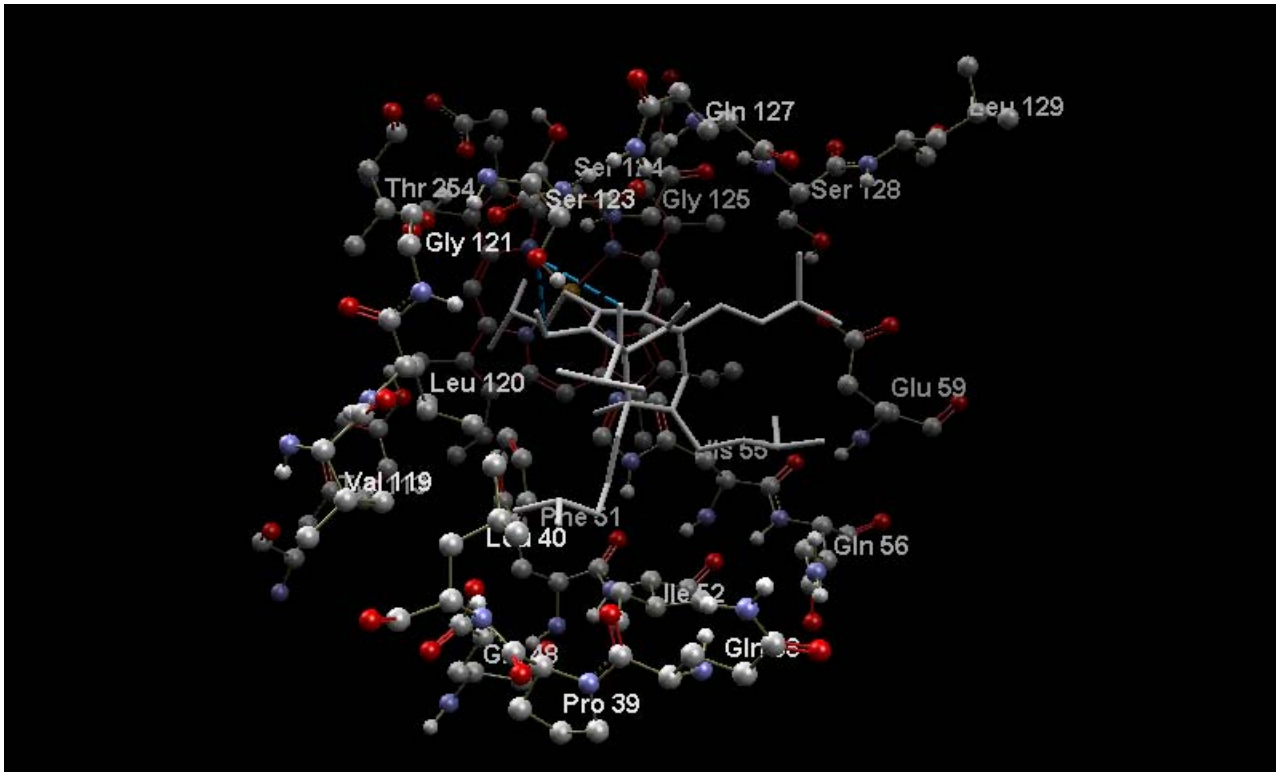
Ligand 4 (Fluvoxamine)



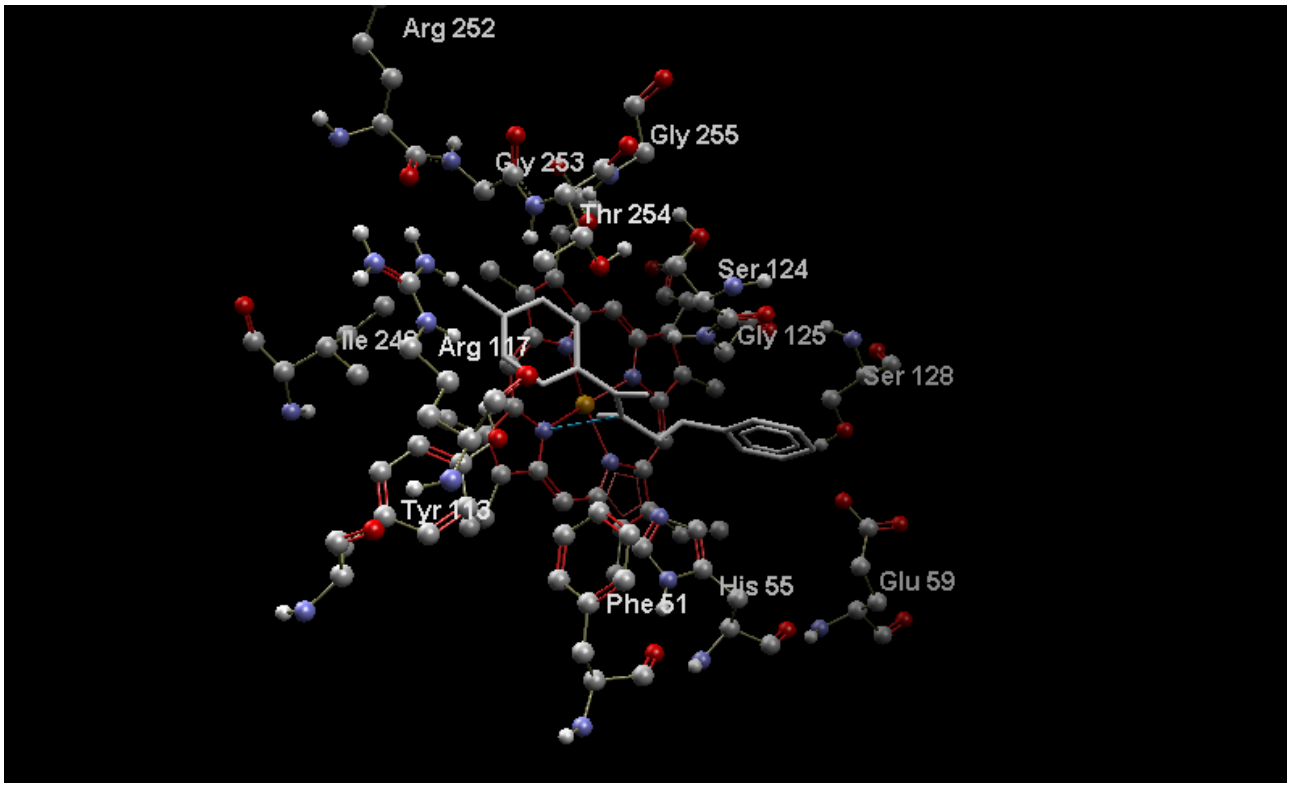
Ligand 5 (Seproxetine)



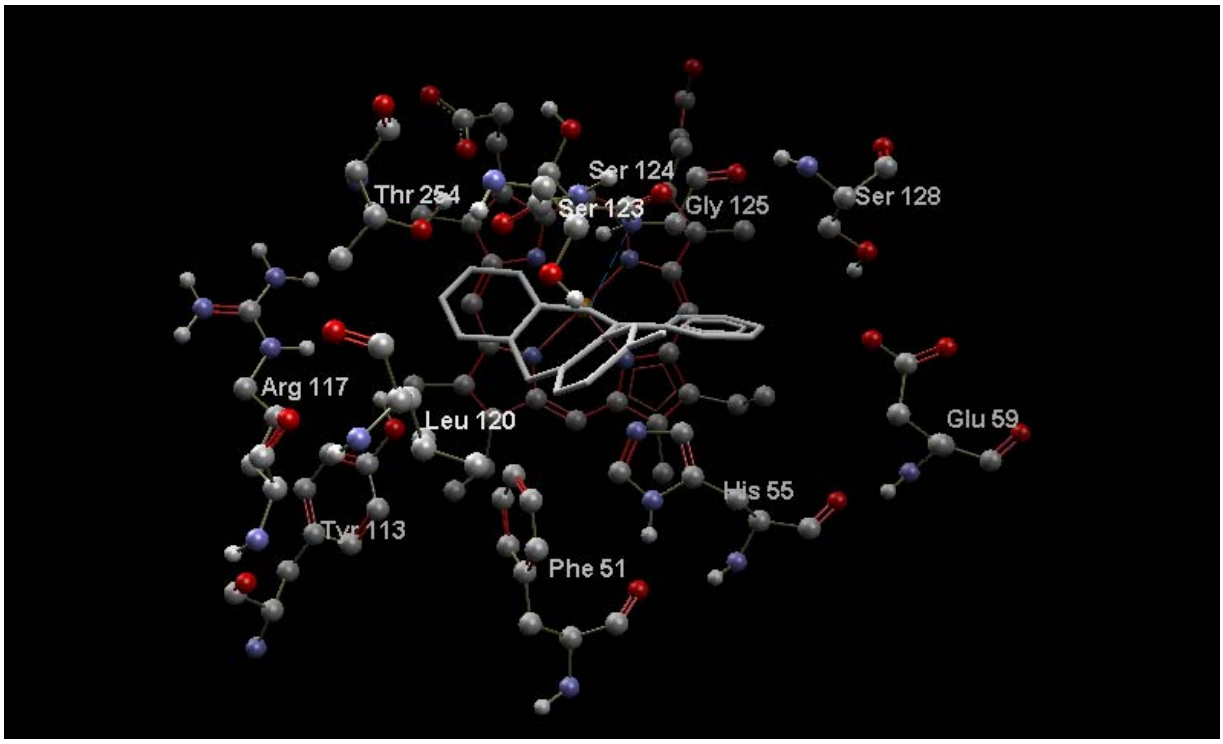
Ligand 6 (Citalopram)



Ligand 7 (Hyperforin)



Ligand 8 (Moclobamide)



Ligand 9 (Amoxapine)

Fig. 4: Docked structure of TDO with ADs (ligands 1 to 9)

Table 1: MolDock score; Re-rank score and the hydrogen bond energy of the docked compounds

S. No	Ligand	MolDock Score kcal/mol	Re-rank Score	H Bond
1	Fluoxetine	-116.694	-90.4309	-1.87188
2	Paroxetine	-139.706	-110.963	-3.31147
3	Sertraline	-109.796	-81.3924	-1.55098
4	Citalopram	-138.538	-95.3585	-0.270029
5	Fluvoxamine	-133.451	-90.2595	-4.60011
6	Seproxetine	-138.081	-94.4274	-3.24063
7	Hyperforin	-152.484	-95.6863	-4.77698
8	Moclobamide	-121.275	-86.5591	-4.06079
9	Amoxapine	-120.086	-87.6386	-0.748501

Table 2: Amino acids residue around active site docked against TDO.

S.NO	Ligand	Amino acid residues	Ligand binding amino acid
1	Fluoxetine	Ser 123, Gly 121, Arg 117, Tyr 113	His 55, Phe 51
2	Paroxetine	Arg 117, Gly 121	Ser 123, Tyr 113, Thr 254
3	Sertraline	Gly 125, Ser 123,128, Tyr 113	Glu 59
4	Citalopram	Thr 254, Leu 120, Ser 123	Tyr 113
5	Fluvoxamine	Gly 125	Ser 123, Tyr 113, Thr 254
6	Seproxetine	Arg 117, Gly 125, Thr 254	Ser 123
7	Hyperforin	Tyr 113, Thr 254, Val 119, His 55	Ser 123
8	Moclobamide	Arg 117, Thr 254, Ser 124, Tyr 113	Thr 254, Gly 125, His 55
9	Amoxapine	Phe 262, Leu 263, Gly 125	Gly 125, Tyr 131, Glu 135

Arg=arginine; Gly= glycine; Glu= glutamate; His= histidine; Leu= leucine; Phe= phenylalanine Ser=serine; Thr= threonine; Tyr= tyrosine

DISCUSSION

Earlier *in vitro* and *in vivo* experimental studies have shown that AD drugs exert a direct inhibitory effect on TDO enzyme activity (Badawy *et al*., 1981; Bano and Sherkheli, 2003; Bano *et al*., 2010). Here using docking studies we have found that in crystal structure of TDO, L Trp (substrate) interacts with heme and L Trp binding site consist of amino acid residues Arg 117, Tyr 113 and Thr 254, Phe 51, Tyr 24, Tyr 27, His 55, Leu 28 our findings are in agreement with Forouhar *et al*., (2007). As the enzyme active site is now known, it provides a good tool to undertake a direct approach of receptor-based drug discovery and design. All of the selected AD when docked with TDO oriented in the same position in the active site as the bound ligand in the crystal structure and also forms hydrogen bond with amino acids Arg 117, Thr 254, Tyr 113, His 55, Ser123, along with few other amino acids with in the active site of protein. On the basis of observed results (table1) it has been shown clearly that the drugs Hyperforin and Paroxetine showed highest docking score -152.484k cal/mol and -139.706k cal/mol respectively.

CONCLUSION

It is concluded that Hyperforin and Paroxetine are probable lead drugs due to their high docking scores they stand as potent inhibitors of TDO enzyme. Present study also proofs TDO as a molecular target of antidepressant drugs.

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