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Structure Based Drug Designing and *In Silico* Screening of Monosubstituted 2-{[(*E*)-{5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentylidene}amino]oxy} Ethanamine (fluvoxamine) as a Potential Drug for Obsessive-Compulsive Disorder

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Abstract

2-{[(*E*)-{5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentylidene}amino]oxy}ethanamine (fluvoxamine)is a sigma-1 receptor (σ_1R), agonist used primarily for the treatment of obsessive-compulsive disorder (OCD). We carried out molecular docking for ten analogues structurally diverse 2-{[(*E*)-{5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentylidene}amino]oxy}ethanamine (fluvoxamine) with σ_1 receptors using patchdock and firedock online docking server. Extensive structure activity relationship work was carried out with these molecules, compared with the non-substituted fluvoxamine by performing the docking studies on crystal structure of σ_1 receptors (PDB ID: 1AGN). These molecules were designed by substituting NH_2 group in fluvoxamine with different chemical groups. The scoring function (empirical binding free energy) of the firedock was used to estimate the free binding energy of the protein-ligand complex. The binding energy of fluvoxamine/σ_1 receptorswas -31.73 kcal/mol. $CONH_2$, CH_2CH_3, CN, NO_2, COOH, SO_2NH_2 and C_6H_5 analogues lead to a decrease in the binding affinity, meaning that, they have better functional activity. The free binding energies were higher in CF_3, CH_3 and NO_2 analogues, meaning that, they have lesser functional activity. These results clearly indicated that the new agonist have very good binding affinity towards σ_1 receptors like fluvoxamine. Synthesis and pre-clinical studies of these monosubstituted derivatives with σ_1 receptorsis recommended.

Keywords

Fluvoxamine, Receptors, Docking, Obsessive-Compulsive Disorder

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1. Introduction

2-{[(*E*)-{5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentylidene}amino]oxy}ethanamine (fluvoxamine) is a σ_1 receptoragonist used primarily for the treatment of obsessive-compulsive disorder (OCD) [1]. Itis a drug which functions as a selective serotonin reuptake inhibitor (SSRI). It can also used to treat major depressive disorder (MDD), and anxiety disorders such as panic disorder and post-traumatic stress disorder (PTSD) [2]. Fluvoxamine CR (controlled release) is approved to treat social anxiety disorder [3]. It has also been found to possess some analgesic properties in line with other

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tricyclic antidepressants [4-6]. Some evidence shows fluvoxamine may be a helpful adjunct in the treatment of schizophrenia, improving the depressive, negative, and cognitive symptoms of the disorder [7]. Its actions at the sigma receptor may afford it a unique advantage among antidepressants in treating the cognitive symptoms of schizophrenia [8].

The sigma-1 receptor (σ_1R), one of two sigma receptor subtypes, is a chaperone protein at the endoplasmic reticulum (ER) that modulates calcium signaling through the IP3 receptor [9]. In humans, the σ_1 receptor is encoded by the *SIGMAR1*gene [10, 11]. The receptor is an integral membrane protein with 223 amino acids. The σ_1 receptor is a transmembrane protein expressed in many different tissue types. It is particularly concentrated in certain regions of the central nervous system [12]. It has been implicated in myriad phenomena, including cardiovascular function, schizophrenia, clinical depression, the effects of cocaine abuse, and cancer [13, 14]. Much is known about the binding affinity of hundreds of synthetic compounds to the σ_1 receptor. The wide scope and effect of ligand binding on σ_1 receptors has led some to believe that σ_1 receptors are intracellular signal transduction amplifiers [14].

Structure based drug design plays crucial role in drug design and discovery. A vital tool for structure based drug design is *in silico*screening, in which small molecules virtually docked into a drug target and the binding free energy estimated using simplified calculations. Modern approaches to finding new leads for therapeutic targets are increasingly based on 3-dimensional information about receptors. An effective way to predict the binding structure of a substrate in its receptor is docking simulation, which has been successfully used in many applications [15, 16]. Docking procedures basically aim to identify the correct conformation of ligands in the binding pocket of a protein and to predict the affinity between the ligand and the protein. In other words, it describes a process by which two molecules fit together in a 3-dimensional space [17].

2. Methodology

2.1. Protein Preparation

The three dimensional structure of σ_1 receptors(Figure 1) was obtained from the Protein Data Bank [18], PDB ID – 1AGN. The protein structure was subjected to a refinement protocol using molegro molecular viewer [23].

2.2. Designing of Structural Analogues of Fluvoxamine

The structure of fluvoxamine was drawn with ACD/Chem Sketch software. The structural analogues of fluvoxamine were developed with structural modifications with different substituents. The NH_2 group at 14^{th}position in fluvoxamine was replaced with CF_3, $CONH_2$, CH_3, CH_2CH_3, NO_2, CN, COOH, SO_2NH_2, C_6H_5 groups. The structures were built with ACD/ChemSketch software and minimized with Arguslab software [19].

2.3. Molecular Docking

Molecular docking was performed using patchdock online server [20]. Patchdock is a molecular docking algorithm based on shape complementarity principles. The Receptor and ligand molecule were uploaded in PDB format in Patchdock server, an automatic server for molecular docking. Clustering RMSD was chosen as 1.5 Å. The docking job was submitted to the Patchdock server, refined in firedock online server [21, 22] and processed with molegro molecular viewer [23].

2.4. Lipinski Rule of 5

Lipinski rule of 5 was evaluated using Sanjeevini, a freely accessible web-server for target directed lead molecule discovery [24].

2.5. Threshold of Toxicological Concern (TTC) Was Performed Using Toxtree

Toxtree is a full-featured and flexible user-friendly open source application, which is able to estimate toxic hazard by applying adecision tree approach. Currently it includes the following plugins: Cramer rules [25] and Verhaar scheme for predicting toxicity mode of actions [26].

3. Results

Estimated free energy of binding (FEB) and Cramer's toxicology scheme of fluvoxamine and its analogues is shown in Table 1. Assessment of drug-likeness of the prescreened ligand is presented in Table 2. Crystal structure σ_1 receptor is shown in Figure 1, while the docked fluvoxamine analogues with σ_1 receptors are presented in Figures 2–11.

Table 1. Estimated free energy of binding (FEB) of fluvoxamine and its analogues (Kcal/mol) and Cramer's toxicology scheme.

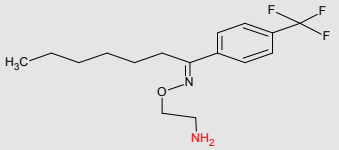
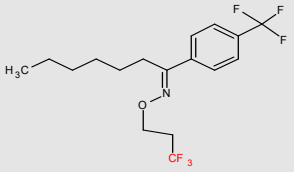
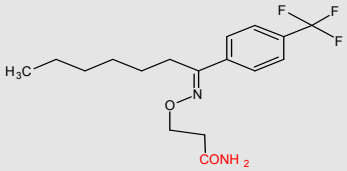
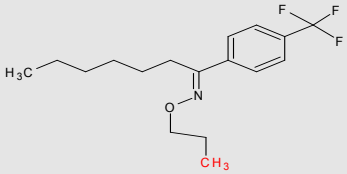
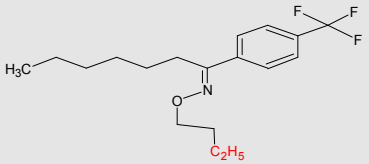
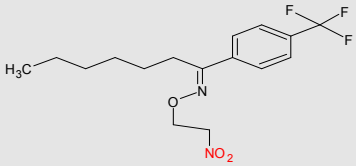
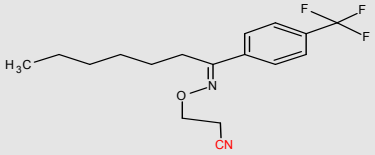
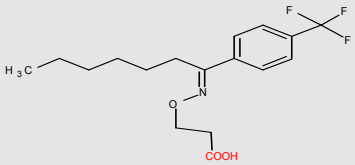
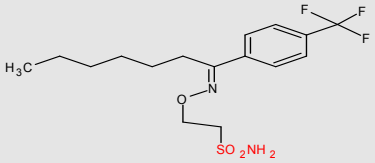
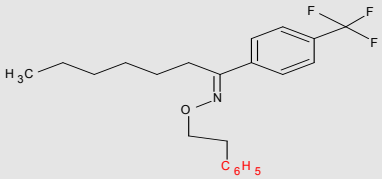
Ligand	Structure	Docking score (Kcal/mol)	Cramer's toxicology scheme
NH_2		-31.73	Class III
CF_3		-26.05	Class III
$CONH_2$		-32.70	Class III
CH_3		-31.13	Class III
CH_2CH_3		-33.53	Class III
NO_2		-31.41	Class III
CN		-32.20	Class III
COOH		-32.13	Class III
SO_2NH_2		-34.93	Class III
C_6H_5		-34.18	Class III

Table 2. Assessment of drug-likeness of the prescreened ligand.

Ligand	Molecular weight (g/mol)	Number ofhydrogen bondacceptor(s)	Number ofhydrogen bonddonor(s) BD	lipophilicityLogP	Molar Refractivity
NH_2	311.000000	2	3	3.024300	80.078094
CF_3	365.000000	2	0	5.958802	83.088989
$CONH_2$	340.000000	4	2	3.881799	86.228386
CH_3	311.000000	2	0	5.416401	82.707985
CH_2CH_3	325.000000	2	0	5.806501	87.324982
NO_2	340.000000	2	0	4.059099	84.184395
CN	325.000000	2	3	3.414400	84.695091
COOH	338.000000	4	0	2.922399	81.946991
SO_2NH_2	373.000000	5	2	4.077498	89.660896
C_6H_5	371.000000	2	0	6.025102	102.468987

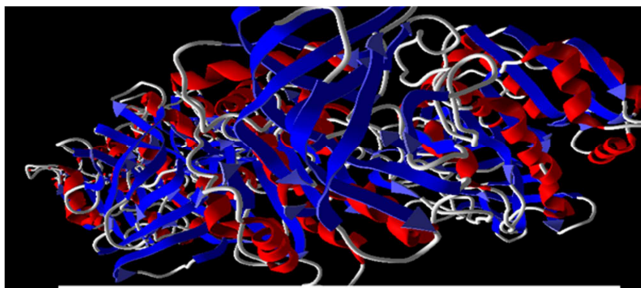


Figure 1. Crystal structure of sigma-1 receptor (σ_1R), PDB 1AGN.

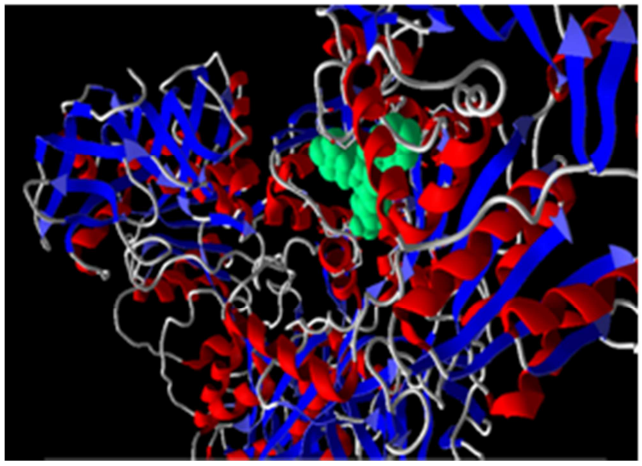


Figure 2. Sigma-1 receptor (σ_1R) docked with CF_3 substituted fluvoxamine.

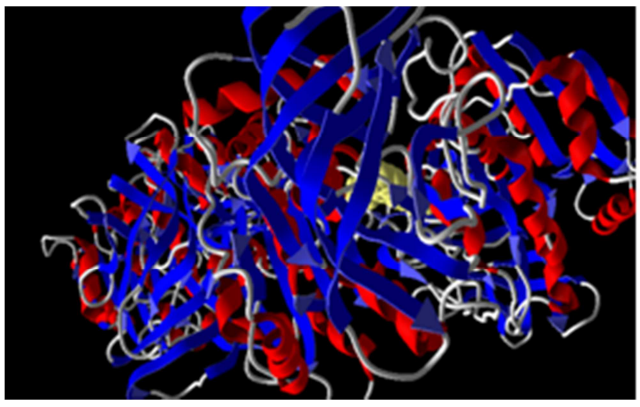


Figure 3. Sigma-1 receptor (σ_1R) docked with $CONH_2$ substituted fluvoxamine.

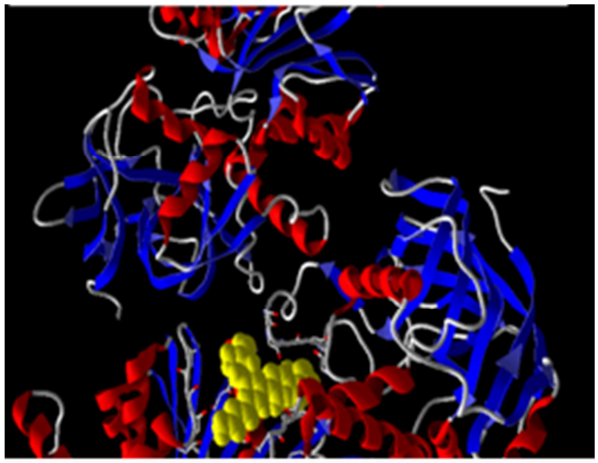


Figure 4. Sigma-1 receptor (σ_1R) docked with CH_3 substituted fluvoxamine.

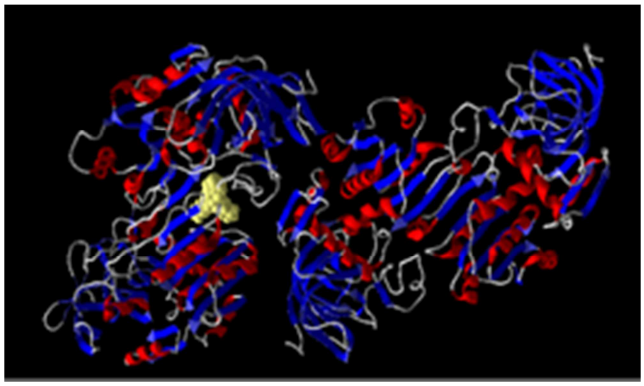


Figure 5. Sigma-1 receptor (σ_1R) docked with CH_2H_3 substituted fluvoxamine.

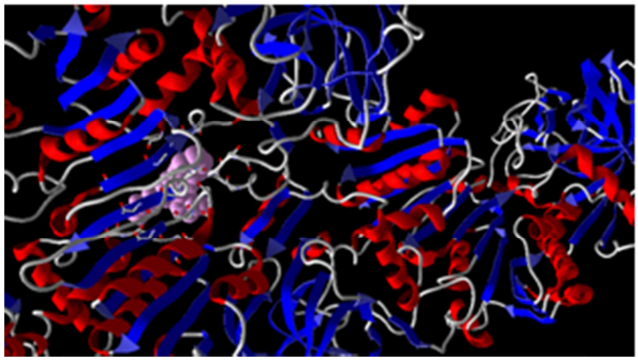


Figure 6. Sigma-1 receptor (σ_1R) docked with NO_2 substituted fluvoxamine.

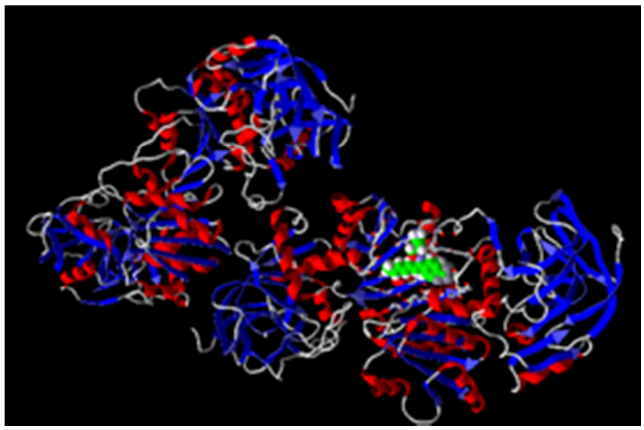


Figure 7. Sigma-1 receptor (σ_1R) docked with CN substituted fluvoxamine.

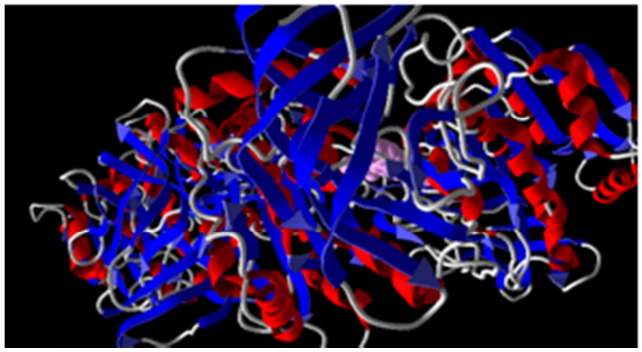


Figure 8. Sigma-1 receptor (σ_1R) docked with COOH substituted fluvoxamine.

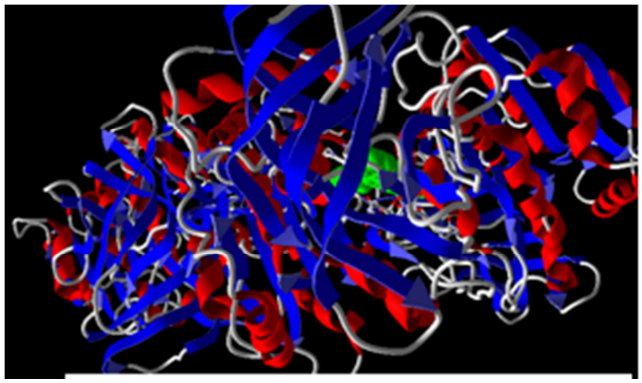


Figure 9. Sigma-1 receptor (σ_1R) docked with SO_2NH_2substituted fluvoxamine.

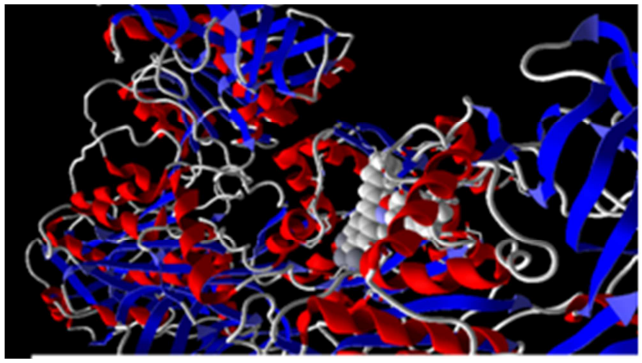


Figure 10. Sigma-1 receptor (σ_1R) docked with C_6H_5 substituted fluvoxamine.

Figure 11. Sigma-1 receptor (σ_1R) docked with fluvoxamine.

4. Discussion

The docking structures of all the compounds showed that they bind in a very similar pattern with the active site of σ_1 receptors,as is evident from the superposition of all the 10 analogues in Figures 2-11. The calculated free energy of binding of the 10 fluvoxamine analogues are shown in Table 1. This confirms that the structural modification implemented in this study is significantly related to their activity. Also, this proved the reasonability and reliability of the docking results. It can be seen that substitution of NH_2 functional group of fluvoxamine with $CONH_2$, CH_2CH_3, CN, NO_2, COOH, SO_2NH_2 and C_6H_5 at positions 14 lead to an decrease in the binding affinity of modified analogues, meaning that, they have better functional activity. The free binding energy of the fluvoxamine and its analogues are tabulated (Table. 1). The binding energy of fluvoxamine was -31.73 kcal/mol. The free binding energies were higher in CF_3, CH_3 and NO_2 analogues, meaning that, they have lesser functional activity. These results clearly indicated that before synthesis and biochemical testing of new analogues, one can use molecular docking based methods for qualitative assessment of relative binding affinities for speeding up drug discovery process by eliminating less potent compounds from synthesis. Synthetic studies followed by pre-clinical studies are further recommended.

Lipinski rule of 5[27] helps in distinguishing between drug like and non drug like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules

- Molecular mass less than 500 Dalton

- High lipophilicity (expressed as LogP less than 5)

- Less than 5 hydrogen bond donors

- Less than 10 hydrogen bond acceptors

- Molar refractivity should be between 40-130

We analysed 5 physically significant descriptors and

pharmaceutically relevant properties of fluvoxamine and its analogues. The properties were molecular weight, H-bond donors, H-bond acceptors, log P (octanol/water) and molar refractivity according to Lipinski's rule of 5 (Tables 2). Lipinski's ruleof 5 [27] is a rule of thumb to evaluate drug likeness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule describes molecular properties important for drug's pharmacokinetics in the human body, including its ADME. However, the rule does not predict if a compound is pharmacologically active [27]. In this study, NH_2, $CONH_2$, NO_2, CN, COOH and SO_2NH_2 structures showed allowed values for the properties analysed and exhibited drug- likeness characteristics based on Lipinski's rule of 5.

In the application of the Threshold of Toxicological Concern (TTC) concept to non-cancer endpoints, the decision tree proposed by Cramer, Ford and Hall in 1978 [25], commonly referred to as the Cramer scheme, is probably the most widely used approach for classifying and ranking chemicals according to their expected level of oral systemic toxicity. The decision tree categorises chemicals, mainly on the basis of chemical structure and reactivity, into three classes indicating a high (Class III), medium (Class II) or low (Class I) level of concern. Each Cramer class is associated with a specified human exposure level, below which chemicals are considered to present a negligible risk to human health. Class I represent substances with simple chemical structures and for which efficient modes of metabolism exist, suggesting a low order of oral toxicity. Class II are substances which possess structures that are less innocuous than class I substances, but do not contain structural features suggestive of toxicity like those substances in class III. Class III are substances with chemical structures that permit no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups. Munro and coworkers [28] proposed TTC values of 1800, 540 and 90 µg/person/day for class I, II and III, respectively. All the fluvoxamine analogues fell into Cramer class III. Their TTC values should be 90 µg/person/day.

Recently structure based drug designing of new acetyl cholinesterase inhibitors for Alzheimer's disease was reported [29]. The results of a comprehensive screening based on structural similarity and docking simulation on the surface of enzymes for possible substitutes for paracetamol have been reported by László and Daniela [30]. Design and *in silico* analysis of ring-a monosubstituted chalcones as potential anti-inflammatory agents was studied by Ranganathanand Rachana [31]. Virtual screening, in which small molecules virtually docked into a drug target and the binding affinities are estimated using simplified free energy calculation methods is a promising tool for structure based drug design.

5. Conclusion

We carried out molecular docking for ten analogous structurally diverse 2-{[(*E*)-{5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentylidene}amino]oxy}ethanamine (fluvoxamine)with σ_1 receptor using patchdock and firedock online docking server. Substitution of NH_2 functional group of fluvoxamine with $CONH_2$, CH_2CH_3, CN, NO_2, COOH, SO_2NH_2 and C_6H_5 at positions 14 lead to an decrease in the binding affinity of modified analogues, meaning that, they have better functional activity. The free binding energies were higher in CF_3, CH_3 and NO_2 analogues, meaning that, they have lesser functional activity. Synthesis and pre-clinical studies of these monosubstituted derivatives with σ_1 receptorsis recommended

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