In Silico Study of the Interaction Between Human 5-HT2C Receptor and Antidepressant Drug Candidates

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Abstract. *Depression is a major public health concern affecting 300 million people worldwide, according to WHO. The mechanism of depression is presumed to be related to the role of serotonin (5-hydroxytryptamine - 5-HT) and its receptors in the central nervous system. In this work a human 5-HT2C receptor model was created by homology modeling, and molecular docking studies were performed with the ligands fulvic acid, paroxetine, citalopram, and serotonin itself. Fulvic acid had similar affinity parameters to paroxetine and s-citalopram, which are widely used drugs for the treatment of psychiatric disorders such as depression and anxiety.*

1. Introduction

Depression represents an important public health concern, affecting 300 million people worldwide, according to World Health Organization [WHO 2018]. The mechanism of depression is presumed to be strongly related to the role of serotonin (5-hydroxytryptamin – 5-HT) and its receptors in the central nervous system, especially in the dorsal raphe nucleus [Borroto-Escuela et al. 2018; Grandjean et al. 2019; Marek 2017; Yohn et al. 2017]. Antidepressant drugs like paroxetine, fluoxetine, and s-citalopram act by selectively inhibiting serotonin reuptake, and therefore making serotonin available inside synaptic ambient for a longer time [Grandjean et al. 2019]. It is known to exist 14 different types of serotonin receptors [Palacios 2016], and that 5-HT2C is a prominent subject of studies on mental diseases like anxiety, major depression disorder, and bipolar disorder, and on neuro-degenerative diseases like Alzheimer and Parkinson [Tohda 2014].

In traditional ayurvedic medicine a mineral called Shilajit, whose main compounds are fulvic acids (50–60% of the total) and humic acids, have been used for many centuries [Bhavsar et al. 2016]. Fulvic and humic acids are two of three fractions that can be obtained from the filtration of humic substances, which are components of humus, resulted from the degradation of organic matter in the soil and the water [Qin et al. 2019; Saleh et al. 1989; Thurman and Malcolm 1981]. Some beneficial activities are already related to fulvic acid, like antidiarrheal [Qin et al. 2019], anti-inflammatory [Chien et al. 2015], humoral immune stimulation [Vucskits et al. 2010], as potential drug against Aβ17–42 mediated cytotoxicity and neurodegeneration [Verma et al. 2013], a promising topical remedy for drug-resistant wound infections [Zhao et al. 2015], and therapy for the management of oral biofilm infections [Sherry et al. 2012].

In silico studies play a major role in the process of drug discovery and development. Through computational calculations it is possible to evaluate stereochemical characteristics of the molecules involved in the biological functions and to predict the binding affinity of receptors and ligands. However, to the best of our knowledge, no in silico studies on the molecular interaction between 5-HT2C and fulvic and humic acids were previously made.

Thus, the aim of this work is to in silico study the interaction of fulvic acid and humic acid ligands with a human 5-HT2C receptor model created by homology modeling, and to compare them with those of known antidepressants and serotonin, so that it may be possible to indicate antidepressant drug candidate for further in silico, in vitro, and in vivo studies.

2. Methods

2.1 Softwares

All softwares used in this study were chosen because of their high acceptance and referencing in the scientific community and because they are free. All of them were designed based on mathematical models of the biochemical properties of the atoms involved in the proteins constitution. The conformation of the proteins are mainly conceived by the means of genetic algorithm computation, especially that of molecular docking calculations.

2.2 Homology modeling

Structural models of 5-hydroxytryptamine 2C receptor isoform – a precursor (5-HT2C) of *Homo sapiens* – were generated by homology modeling using the software MODELLER 9v21 (https://salilab.org/modeller/) [Eswar et al. 2006], through its the graphical interface integrated to the software UCSF Chimera (https://www.cgl.ucsf.edu/chimera/) [Pettersen et al. 2004].

The search for homolog proteins to 5-HT2C (amino acids sequences with accession code NP_000859.1 in NCBI's database) was done among experimental structures available in the Protein Data Bank (PDB) (https://www.rcsb.org/) [Berman 2000], from which three templates (Table 1) were chosen: 5-HT2C, 5-HT2AR, and 5-HT2B-BRIL. One hundred independent models were generated and the model presenting the most negative value for the function of DOPE energy was chosen [Plácido et al. 2017].

Table 1. Details of the protein models used for homology modeling.

Template proteins	PDB code	Resolution	Sequence Identity	Species
5-HT2C	6BQG	3.0Å	76.5%	*Escherichia coli*, *Homo sapiens*
5-HT2AR	6A93	3.0Å	56.4%	*Escherichia coli*, *Homo sapiens*
5-HT2B-BRIL	4IB4	2.7Å	47.7%	*Escherichia coli*, *Homo sapiens*

The 3D structures of fulvic acid (CID 5359407), humic acid (CID 90472028), paroxetine (CID 43815), and serotonin (CID 5202) were downloaded from PubChem Open Chemistry Database (https://pubchem.ncbi.nlm.nih.gov/) [Kim et al. 2019]. The structures of the ligands are shown in Figure 1.

2.3 Molecular Docking

The molecular docking calculations were done with the software AutoDock Vina (https://vina.scripps.edu/) [Trott and Olson 2010]. The ligand and the protein were prepared for the calculations with AutoDock Tools (ADT) 1.5.6 [Morris et al. 2009]. 5-HT2C model was set as rigid and all ligands was considered as flexible, with their torsion being added during the preparation process. Hydrogens were added to both receptor and ligands individually, Gasteiger charges were then calculated by ADT, and non-polar hydrogens were merged. The grid box was sized as 22.5 x 22.5 x 22.5 units of 1 Å for each axis. The grid box was centered in the coordinates of the oxygen of the residue Asp95, in the active site of 5-HT2C. The number of modes were set to 100, and

exhaustiveness was set to 64. All other parameters were left in default. The conformations with the highest affinity were ranked by their root mean square deviation (RMSD). The conformation with the highest affinity was chosen to further visual inspection and analysis.

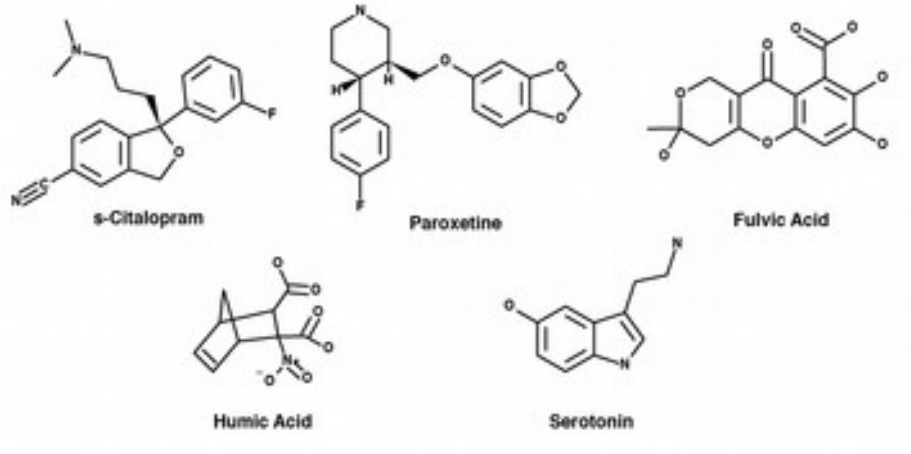

Figure 1. Structure of the ligands used in the study

3. Results and discussion

The results of the molecular docking of the template-based model of 5-HT2C with fulvic acid, humic acid, paroxetine, s-citalopram, and serotonin are shown in Table 2.

Serotonin is the element used in this study to represent, alongside with paroxetine and s-citalopram, the standards to assess the results obtained with fulvic and humic acids. Trans-membranes proteins of the same family as 5-HT2C do the transportation of serotonin from the synaptic cleft back to the interior of neuron's cytoplasm. The binding energy showed by the complex of serotonin with the 5-HT2C model was -6.5 kcal mol^{-1}. Therefore, these values were considered as reference for the typical physiological pattern for this enzyme and its main ligand in the context of this template-based model of 5-HT2C.

Table 2. Parameters affinity of the molecular docking with 5-HT2C

Complex (Protein-Ligand)	ΔG_{bind} [a] (kcal/mol)	Amino acids interacting through hydrogen bonds [b]	Amino acids exerting hydrophobic interactions [b]
Paroxetine	-8.7	None	Asp95, Val96, Leu170, Trp91, Trp285, Phe288, Ile92, Ser99, Phe175, Val315
Fulvic Acid	-8.4	Ser99, Ser180, Thr100	Phe288, Leu170, Val96, Val176, Gly179, Phe289, Trp91, Ile92, Asp95, Ala183
S-citalopram	-8.0	Asn292, Ser71	Leu170, Val315, Trp91, Val96, Tyr79, Ile92, Asp95, Phe288, Ala74, Ile75, Leu311
Serotonin	-6.5	Asp95, Ser180, Val176, Ser99	Val96, Gly179, Phe289, Ile92, Leu170
Humic Acid	-6.3	Ser180, Ser99	Val96, Phe289, Gly179, Phe288, Leu170, Phe175, Val176, Ala183

[a]Estimated free binding energy [b] Obtained with the Ligplot software.

The complex of paroxetine with the 5-HT2C model showed highest estimated free binding energy (-8.7 kcal mol^{-1}) of all the complexes tested. Paroxetine formed no

hydrogen bonds, but made hydrophobic interactions with 12 residues (Table 2). S-citalopram showed a biding affinity of -8.0 kcal mol^{-1} in complex with 5-HT2C. This ligand formed hydrogen bonds with the Asn292, and Ser71 residues of 5-HT2C, and made 13 hydrophobic interactions. These results are consistent with the fact that paroxetine and s-citalopram are well-known selective serotonin reuptake inhibitors (SSRI) [Matthäus et al. 2016], which are widely used for the treatment of several psychiatric disorders like depression and anxiety, with paroxetine being one of the most potent SSRI known [Davis et al. 2016].

Testing fulvic acid and humic acid was the main objective of this study. The complex of humic acid with 5-HT2C presented way less estimated binding affinity than any other compound in study (-6.3 kcal mol^{-1}). It formed hydrogen bonds with Ser180 and Ser99, and made hydrophobic interactions with 10 residues. As for fulvic acid, it showed similar estimated free binding energy values of that of paroxetine (-8.7 kcal mol^{-1} versus -8.4 kcal mol^{-1}), and higher affinity than serotonin (-6.5 kcal mol^{-1} versus -8.4 kcal mol^{-1}). Fulvic acid made hydrogen bonds with Ser99, Ser180, Thr100 residues of 5-HT2C model, and made other 12 hydrophobic interactions within the active site. These results indicate that fulvic acid may be considered a potential drug candidate to the inhibition of 5-HT2C receptor, acting in similar way as paroxetine and s-citalopram, and having higher affinity than s-citalopram.

All hydrogen bonds and hydrophobic interactions are shown in Figure 2.

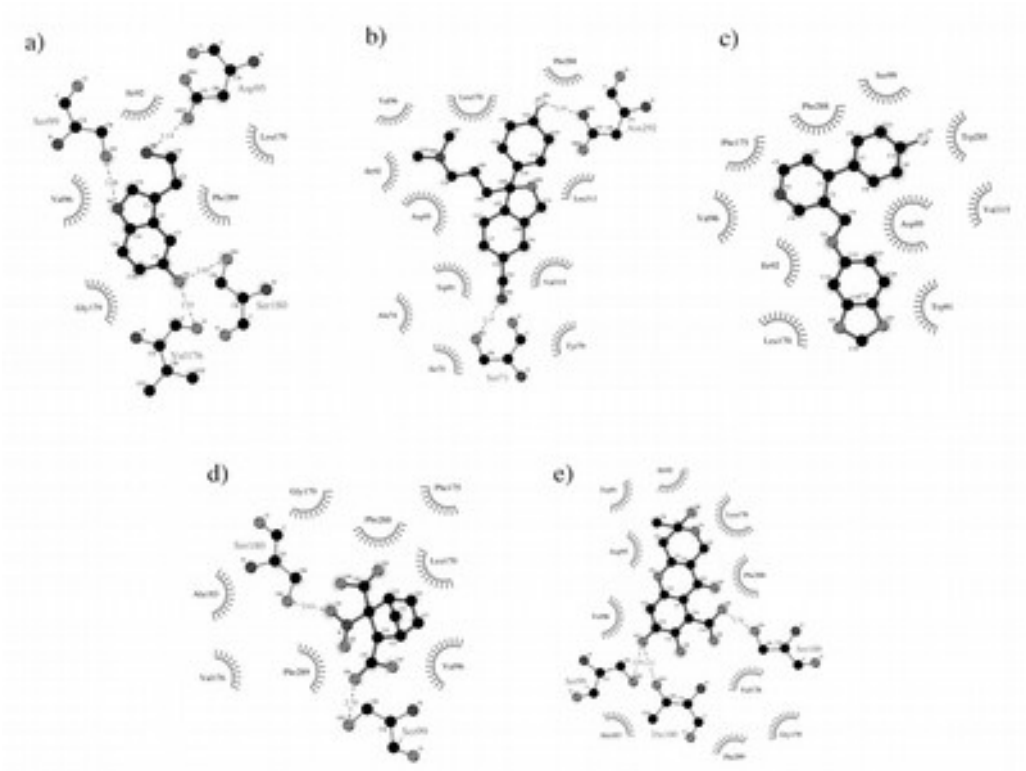

Figure 2. hydrogen bonds and hydrophobic interactions obtained with Ligplot+: a) Serotonin, b) s-citalopram, c) paroxetine, d) humic acid, and e) fulvic acid.

5. Conclusion

Fulvic acid may be considered a potential drug candidate to the inhibition of 5-HT2C receptor, once it showed similar binding affinity to paroxetine and s-citalopram, which are widely used drugs in the treatment of psychiatric disorders like depression and anxiety. On the other hand, humic acid had the lowest binding affinity of all ligands tested and may not have as great potential as fulvic acid.

Further studies, such as molecular dynamics simulation, in vitro, and in vivo studies are necessary to prove the efficacy of fulvic acid as an antidepressant drug.

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