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RESEARCH ARTICLE

In Silico Studies on 5-Hydroxytryptamine Receptor 1A: Modeling and Docking Studies

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ABSTRACT

Schizophrenia is a chronic mental disorder affecting approximately 1% of the population. It is characterized by the inability to think clearly, make decisions, and form social relationships with others. There are many factors affecting the causation of this disease, but the serotonergic system and 5-hydroxytryptamine receptors (HTRs) are most commonly associated with it. Three-dimensional structure of the protein HTR 1A was built using Modeller 9.20 using crystal structure of the chimeric protein of 5-HT1B-BRIL in complex with ergotamine (PSI Community Target) (PDB ID: 4IAR) as a template. The generated model was validated using Ramachandran plot, which showed a model of good quality having 95.1% of amino acid residues in the most favored region. Molecular docking studies also showed low binding energy for all the compounds. Morusin exhibited the lowest binding energy of value −8.52 K.cal/mol while interacting with Ala289, Ser269, and Gly273.

Keywords: Homology modeling, molecular docking, natural compounds, schizophrenia

INTRODUCTION

Schizophrenia is a mental disorder having an effect on the cognition and behavior of a person. It is associated with anxiety, depression, and withdrawal from society. Although the incidence of schizophrenia is not high, it develops into a chronic disease and patients have a life-long vulnerability to it. It is curable through pharmacological and psychological treatment. While there are other possible factors influencing the disease, the genetic component is a major causative factor[1] and is most commonly associated with the serotonergic system.[2,5,6] Serotonin or 5-hydroxytryptamine (5-HT) is a neurotransmitter. The neurobiological working of the cognitive ability is still under research and is not completely understood, but it is known that the modulation of the serotonergic neural network is responsible for various cognitive functions. It is involved in memory, learning, attention, decision-making, and formation of social relationships. This variety of serotonergic functions are accomplished by the interactions of serotonin with the 5-HT receptors which are of 14 Types.[2] 5-HT receptors (5-HTRs) are GI/o-coupled metabotropic receptors that, in their active state, suppress cyclic adenosine monophosphate levels and ultimately inhibit neuronal activity.[3] The 5-HTR 1A is a receptor which is most investigated, and its importance has been acknowledged due to its role in the regulation of the entire serotonergic system.[2] 5-HTR1A heteroreceptors located on postsynaptic 5-HTergic and non-5-HTergic neurons are involved in the control of cognitive functions, mood, and emotional states.[4] 5-HTR1A receptors have an effect on memory functions by exerting their influence on the activity of glutamatergic, cholinergic, and GABAergic neurons in the cerebral cortex, hippocampus, and the septo-hippocampal projection. The activation of 5-HTR1A releases dopamine into the prefrontal cortex, striatum, and hippocampus. 5-HTR1A regulate the G-protein dependent and independent signaling pathways that target immediate early genes implicated in memory formation.[2] 5-HTR1A has been directly linked with the etiology of depression and clinical effects of antidepressants.[3,7] Despite conflicting reports about the relevance of 5-HTR1A in schizophrenia,[5,6] meta-analysis has shown that there is increased action of 5-HTR1A in schizophrenia.[7] Several SNPs have been studied,

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and the research indicates that abnormalities in the 5-HTR1A, which can be transcriptional/ translational errors, cause alternations in the serotonergic neural transmissions which could be responsible for schizophrenia.[8] Antipsychotic drugs such as clozapine, olanzapine, risperidone, quetiapine, and amisulpride are used in the pharmacological treatment of this disease.[1] Although numerous medications are available, they can be categorized into typical, atypical, and dopamine partial agonistic antipsychotics and have different mechanisms of action.[9] Despite being widely used for treatment, these drugs have extrapyramidal side effects[10] and have adverse effects such as weight gain, hypotension, sedation, glucose intolerance, and many more.[1] This prompts the discovery and invention of alternate methods of therapy and treatment. The aim of the present study was to construct a three-dimensional (3D) model of P08908_Human and explore the binding interactions of natural compounds through molecular docking studies. Homology modeling or comparative modeling of proteins refers to the construction of an atomic model of the chosen protein based on a template protein with which it shares a similarity. In this process, only those templates that show sequence similarities with that of the protein are chosen to obtain a model that most closely resembles that of the template. Molecular docking is a structure-based drug designing tool used to produce ligands with specific electrostatic and stereochemical attributes to achieve a higher binding efficiency.[11] It is a key tool in structural molecular biology and computer-assisted drug design.[12-14] It aims to predict affinity between proteins and ligands at the receptor target site. With the discovery of new proteins and ligands, it becomes important to study the various protein-ligand and protein-protein interactions to better understand cell dynamics. Docking tools have had a great impact on the study of such interactions.[14] This is achieved through position prediction, virtual screening, and binding affinity prediction. In these steps, the possible ligand conformations are generated with respect to the receptor binding site, and the subsequent physical and chemical molecular interactions are predicted.[12] Numerous possible "positions" are tried and evaluated. The scoring function assigns positions with their lowest binding energy are selected as the best match or binding mode, as it is the most stable of the positions.[13] The energy values, reflect the affinity between the given ligand and its receptor binding site. Thus, a good scoring function can distinguish between active and inactive ligands and identify the most potent ligands.[12] From ancient to modern times, plants and other herbs have been used as medicinal agents.[15] Natural products or natural compounds and their derivatives as chemotherapeutic agents, including plant-derived antimalarial, antifungal, antitumor, antiviral, anticancer, antidiabetic, antialzheimer's, antiarthritic, and anti-inflammatory agents, herbal medicine is the primary source of new lead compounds. Most plants contain polyphenols, flavonoids, alkaloids, tannins, saponins, terpenoids, lignans, sterols, iridoids, and fatty acids.

METHODOLOGY

Sequence alignment and structure prediction

The amino acid sequence of 5-HTR1A (UniProt accession number: P08908) from the species Homo sapiens was retrieved from the UniProt KB database.[16] A blast (Basic Local Alignment Search Tool) search was performed to select the template. The Chain A, the crystal structure of the chimeric protein of 5-HT1B-BRIL in complex with ergotamine (PSI Community Target) (PDB ID: 4IAR_A)[17] was selected considering that it had 41% similarity and a resolution of 2.70 Å making it an excellent template. The 3D structure was generated using Modeller 9.20. The respective templates were retrieved from protein databases like PDB.[18,19] When choosing the template, it is important to consider the sequence identity and resolution of the template. When both parameters are high, the resulting model would be sufficiently good to allow structural and functional research.[20] MODELLER 9.20 was then used to generate satisfactory models; an automated approach to homology modeling by the satisfaction of spatial restraints. Sequence alignments using the protein and template sequences were then carried out using platforms such as ClustalX and ClustalW[21] [Figure 1]. Homology models for the chosen protein were then constructed using modeler programs like Modeller 9.20.[19,21] After manually modifying the alignment input file in MODELLER 9.20 to match the query and template sequence,

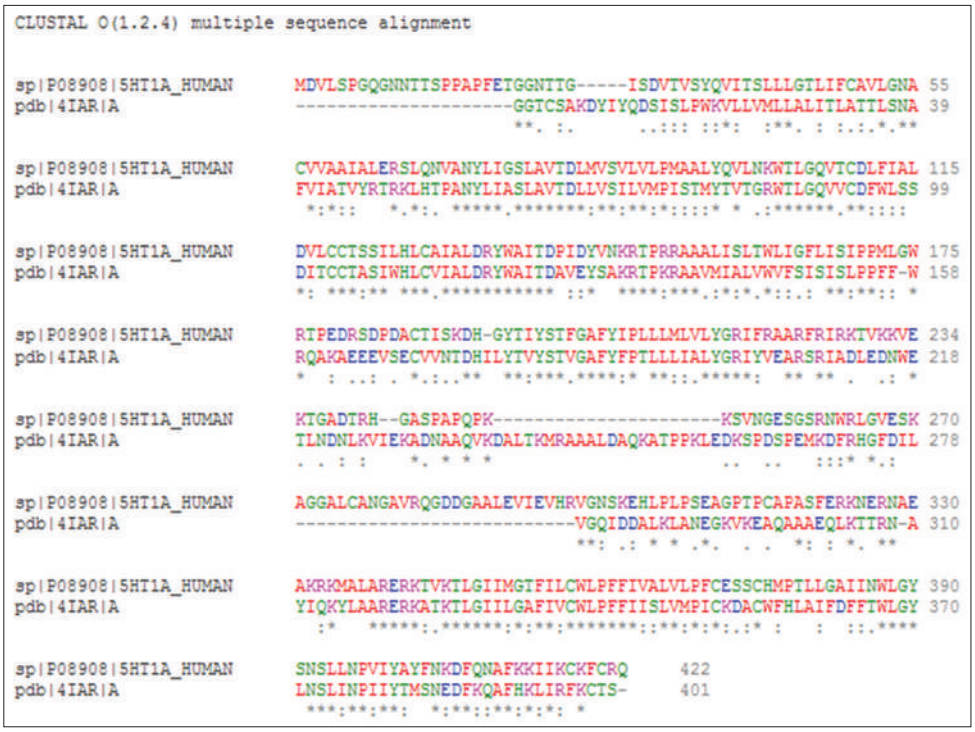

Figure 1: Sequence alignment of 5-hydroxytryptamine receptor 1A protein and template 4IAR

20 models were generated. The best model is determined by the lowest value of the Modeller objective function.[20] The stereochemical quality of the given models was then evaluated using software like PROCHECK,[19] and the model can be used for the further structural or functional study. PROCHECK generated a Ramachandran plot which explains residue by residue listing that facilitates the in-depth calculation of Psi/Phi angles and the backbone conformation of the models. The root mean square deviation (RMSD) was calculated by superimposing (4IAR_A) over the generated model to access the accuracy and reliability of the generated model.

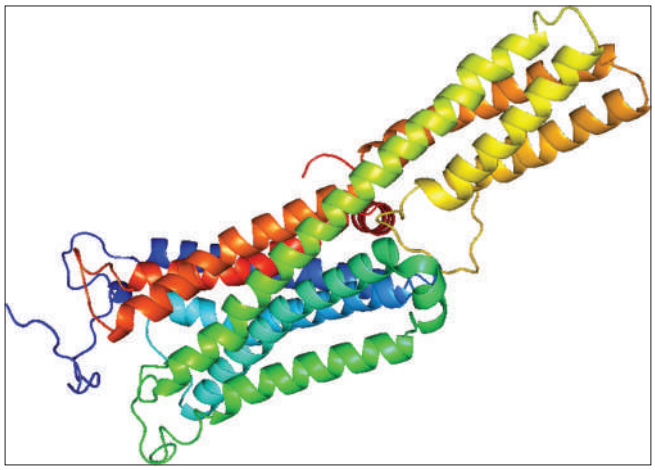

Figure 2: The cartoon model of 5-hydroxytryptamine receptor 1A modeled protein

Docking methodology

Identification of active site pockets

The active site prediction was carried out using Tripo's Sybyl6.7. It showed three active site pockets. The amino acids in pocket one were Ala137, Ile138, Ile218, Ala222, Thr229, Val233, Val267, Glu268, Gly272, Gly273, Ala274, Leu275, Cys276, Ala277, Ala289, Leu290, Glu330, Lys334, Ala338, Lys342, Thr343, Val344, Leu347, Ile350, Ile399, Tyr400, Phe407, Ala71, Asn72, Leu74, Ile75, Leu127, Ile130, Ala131, and Arg134.

The amino acids present in the pocket two and three are Met138, Ile142, Leu82, Phe91, Leu96, Leu98, and Met99 and Tyr172, Phe116, and Phe123, respectively. These were used to identify the best binding site in the protein for the ligands. In total, 35 phytochemicals were downloaded from NCBI and saved in mol^2 format. Molecular docking studies were performed on all the natural ligands separately using AutoDock4.2 program, using the Lamarckian Genetic Algorithm and empirical free energy function was implemented. Initially, the modeled HTR1A protein was loaded, and hydrogens were added before saving it in PDBQT format. Later the ligand was loaded and conformations were set and saved in PDBQT format. The grid parameters were selected and calculated using AutoGrid. For all the dockings, a grid-point spacing of 0.375 Å was applied, and

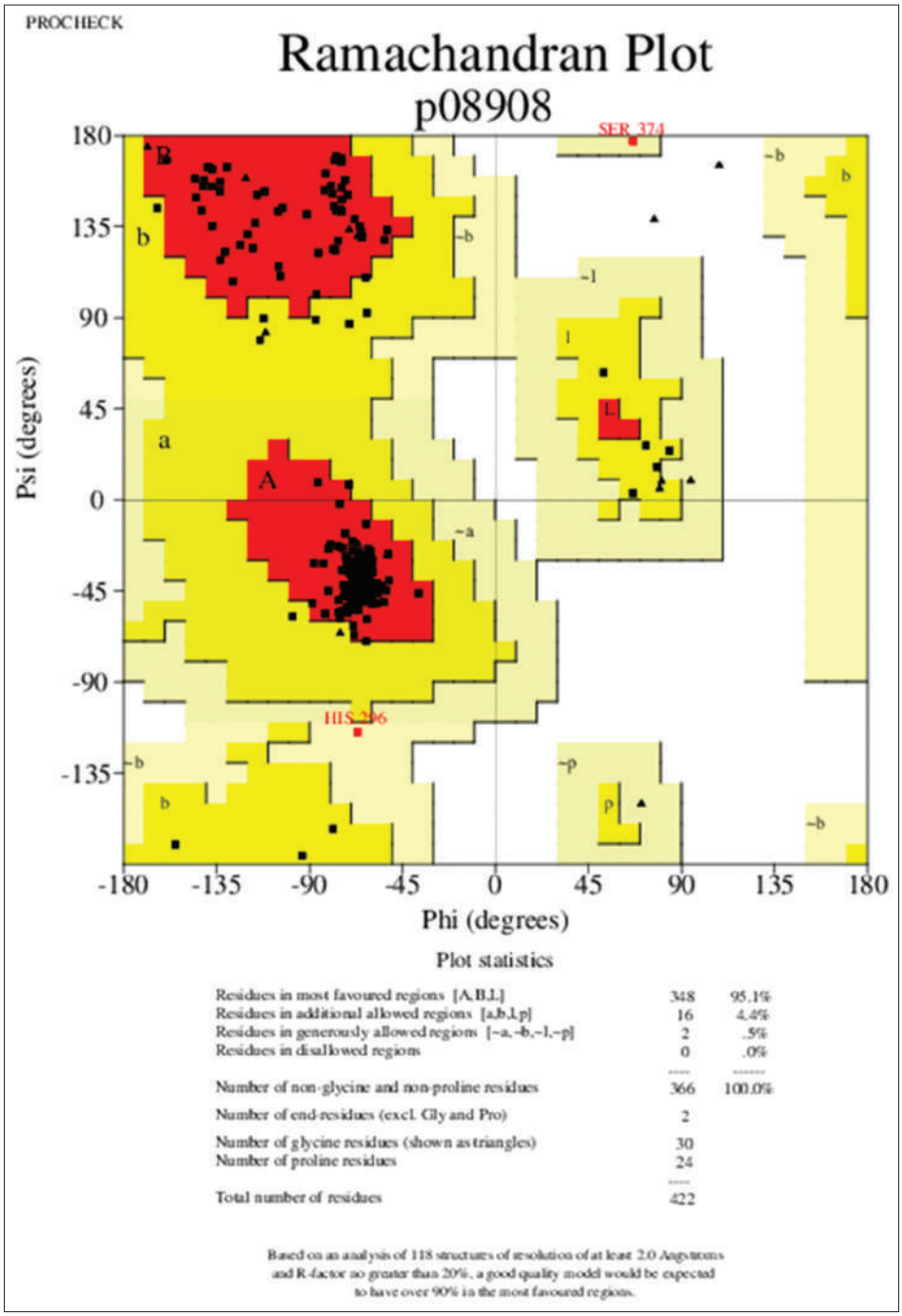

Figure 3: Ramachandran plot of the modeled 5-hydroxytryptamine receptor 1A protein exhibited 95.1% amino acid residues in the most favored region

grid map with 60 × 60 × 60 points was used. X, Y, and Z Coordinates were selected on the basis of the amino acids present in the active site predicted in sybyl6.7 biopolymer module. Default parameters were used to run the AutoDock.

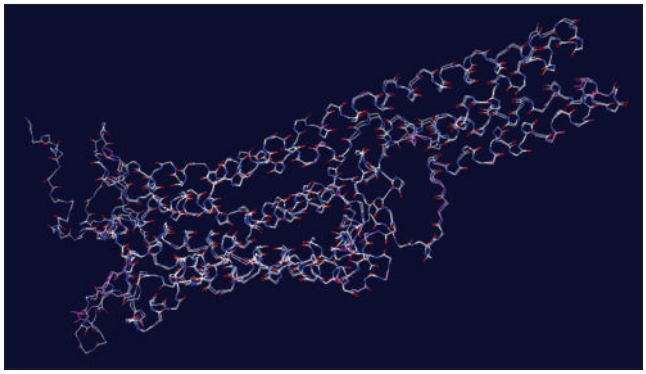

Figure 4: Superimposed model of modeled 5-hydroxytryptamine receptor 1A protein and templated protein

RESULTS AND DISCUSSION

Homology modeling and model evaluation

The present study reports that the template protein (PDB ID: 4IAR_A) having a high degree

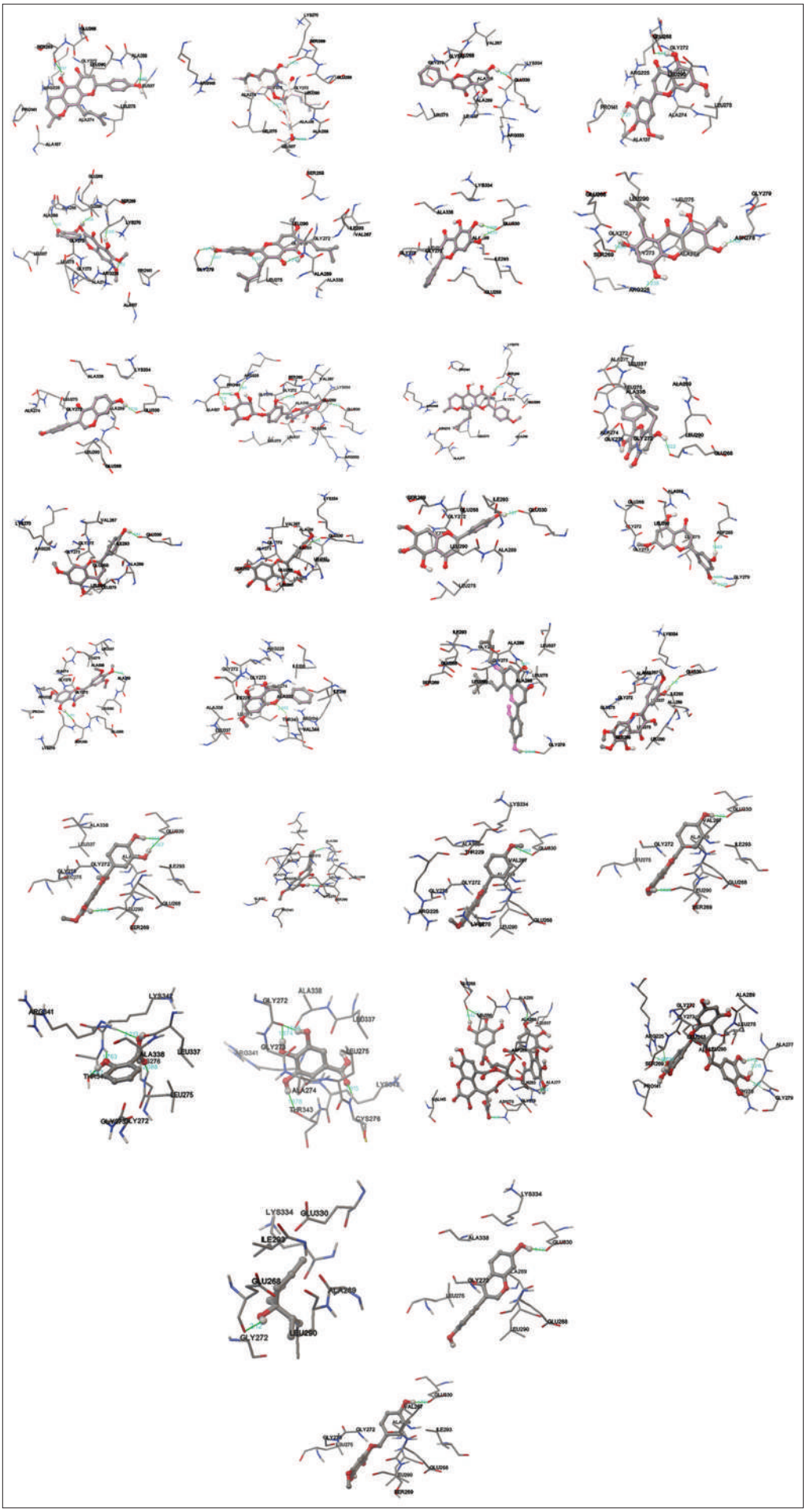

Figure 5: Protein-ligand interactions of 5-hydroxytryptamine receptor 1A against natural compounds

of homology with P08908 protein was used as a template with good atomic resolution of its crystal structure. The target sequence of 5-HTR1A (UniProt accession number: P08908) from Homo sapiens having 422 amino acid residues was retrieved from the UniProt protein sequence database with Accession No. P08908. Using BLAST, PDB ID 4IAR_A was identified and selected as a template for having 41% identity. The structure was modeled using Modeller 9.20. The generated structure was validated using the protein structure and by PROCHECK. The generated model showed 95.1% of amino acid residues in the core region, 4.4% of amino acid residues in additionally allowed region, 0.3% of the amino acid residues in the generously allowed region and there is no amino acids present in the disallowed region. The template PDB shows

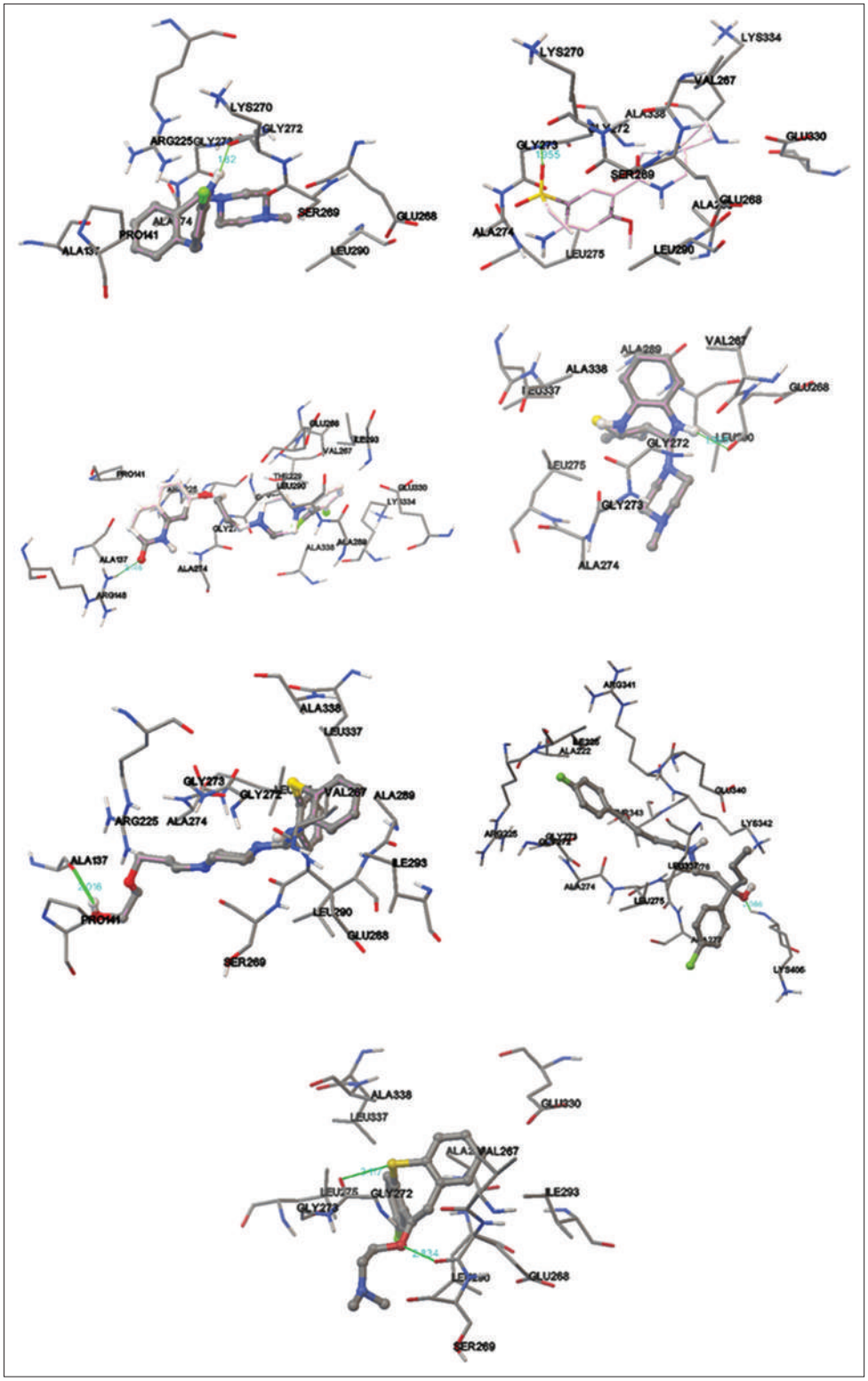

Figure 6: Protein-ligand interactions of 5-hydroxytryptamine receptor 1A against standard compounds

Table 1: Molecular docking results of (binding energy, dissociation constant, and interacting amino acids) natural compounds against modeled 5-hydroxytryptamine receptor 1A

Sl. No.	Ligand	Interacting amino acids	Binding energy ΔG (Kcal/Mol)	Dissociation constant (kI)
1.	Lupinifolin	Ala289, Ser269	−7.83	1.83 uM
2.	Morusin	Ala289, Ser269, Gly273	−8.52	568.15 nM
3.	Chrysin	Glu330	−6.91	8.6 uM
4.	Lethedocin	Ala137, Glu268	−6.53	16.36 uM
5.	Artemetin	Ser269, Lys270, Arg225, Ala289	−7.05	6.76 uM
6.	Artocarpin	Leu275, Gly279, Leu290	−8.25	898.11 nM
7.	Baicalein	Glu330	−7.15	5.78 uM
8.	Cratoxyarborenone	Arg225, Ser269, Asn278	−6.09	34.07 uM
9.	Formononetin	Glu330	−6.94	8.15 uM
10.	Luteolin-7-methyl	Ala137, Arg225, Gly272, Glu330	−8.17	1.02 uM
11.	Khonklonginol_A	Ser269	−7.25	4.84 uM
12.	Glepidotin	Glu266	−6.21	28.05 uM
13.	Genkwanin	Glu330	−6.92	8.41 uM
14.	Casticin	Glu330	−6.77	10.99 uM
15.	Cirsimaritin	Glu330	−6.78	10.71 uM
16.	Blumeatin	Asp285, Gly279	−6.46	18.33 uM
17.	Corymbosin	Ser269	−6.9	8.82 uM
18.	Tectochrysin	Thr343	−6.93	8.33 uM
19.	Sophoraflavanone	Ala289, Gly279	−7.14	5.83 uM
20.	Vitexin	Glu330	−6.69	12.41 uM
21.	3,6-O-dimethoxy-5,7,3’.4’-tetrahydroxy-flavone	Leu337, Glu330	−7.36	4.01 uM
22.	7,4’-o-dimethoxy-5-hydroxy-flavone	Ala 289	−6.68	12.78 uM
23.	7,4’-o-dimethoxy-flavone	Glu 330	−6.75	11.18 uM
24.	Apigenin	Glu330, Leu290	−7.24	4.93 uM
25.	Biochanin A	Glu330	−6.38	21.03 uM
26.	Geinstein	Lys342, Thr343	−7.84	1.8 uM
27.	Phycocyanobilin	Leu275, Cys276, Leu290	−7.3	4.49 uM
28.	Lupeol	Arg148	−7.57	2.81 uM
29.	Salicylic acid	Cys276, Lys342, Thr343	−6.68	12.67 uM
30.	Gallic acid	Cys276	−6.61	14.3 uM
31.	Chebulagic acid	Asn278, Gln283, Ala289	−7.14	5.86 uM
32.	Epigallocatechin-3-gallate	Arg225, Gly279	−6.64	13.58 uM
33.	Terpinen-4-ol	Gly272	−5.43	104.65 uM
34.	Daidzein	Glu330	−6.73	11.72 uM
35.	7-O-methyl naringenin	Glu330	−6.93	8.31 uM

Table 2: Molecular docking results of (binding energy, dissociation constant, and interacting amino acids) standard drugs against modeled 5-hydroxytryptamine receptor 1A

Sl. No.	Standard	Interacting amino acids	Binding energy ΔG (Kcal/Mol)	Dissociation constant (kI)
1.	Clozapine	GLY272	−8.19	995.18 nM
2.	Amisulpride	GLY273	−7.44	3.5 uM
3.	Aripiprazole	ALA148	−8.15	1.07 uM
4.	Olanzapine	GLU268	−7.53	3.0 uM
5.	Quetiapine	ALA137	−7.71	2.23 uM
6 .	Haloperidol	LYS405	−6.66	13.03 uM
7.	Zotepine	SER269, GLY272	−7.89	1.65 uM

93.7% of amino acids in the core region, 6.0% of the amino acid residues in additionally allowed region, and 0.3% of the amino acid residues in the generously allowed region and there are no amino acid residues in disallowed region. Cartoon model of the secondary structure of the modeled protein

is shown in Figure 2 and Ramachandran plot is shown in Figure 3. RMSD was calculated for the template and generated a model using SPDBV. Both the models were loaded and superimposed using the alpha carbon, and RMSD was calculated. It showed RMSD of 1.77Å, which indicates that the generated model shows similarity to the template [Figure 4].

Molecular docking results

Molecular docking is the most extensively used method for the calculation of protein-ligand interactions. It is an efficient method to predict potential ligand interactions. In this study, the native plant secondary metabolites (ligands) have been identified as potent 5-HTR 1A inhibitors. AutoDock4.2 uses binding free energy assessment to assign the best binding conformation**.** Further, the activity of docked ligand molecules was compared to that of standard drugs which were controls. In total, 35 natural compounds were docked against modeled 5-HTR 1A. However, the compounds Morusin and Artocarpin showed better interactions and lower free energy values, indicating more thermodynamically favored interactions. Both the compounds exhibited binding energy of <−8.0 Kcal/mol. Specifically, Morusin exhibited the highest binding energy of value −8.52 K.cal/mol while interacting with Ala289, Ser269, and Gly273, when compared to the standard drugs: Clozapine, Amisulpride, Aripiprazole, olanzapine, quetiapine, haloperidol, and zotepine. Clozapine exhibited binding energy of −8.19 Kcal/mol while interacting with Gly272. Artocarpin exhibited binding energy of −8.25 Kcal/mol while interacting with Leu275, Gly279, and Leu290. The compound Luteolin-7-methyl exhibited binding energy of −8.17 K.cal/mol with interacting four amino acids Ala137, Arg225, Gly272, and Glu330.

Three compounds exhibited binding energy <−8.00 Kcal/mol, 11 compounds exhibited binding energy of <−7.00 Kcal/mol, and 20 compounds exhibited binding energy of <−6.00 Kcal/mol. Luteolin-7-methyl and artemetin showed four interactions, six compounds showed three interactions. The natural compounds with their corresponding interactions and binding energies are shown in Table 1 and Figure 5. The standard drugs used as controls with their corresponding interactions and binding energies are shown in Table 2 and Figure 6.

Standard drugs used for docking studies against modeled protein

Clozapine, Aripriprazole, Olanzapine and Haloperidol are anti-psychotic drugs which were taken for this study from FDA approved list for treatment of schizophrenia22. Zotepine, Quetiapine and Amisulpride are drugs being studied for their anti-psychotic activity.[23, 24, 25]

CONCLUSION

In the present study, we have generated 3D structure of 5-HTR 1A. The generated model can be used to understand the conserved structural patterns. The model exhibited 95.1% of amino acid residues in core region and docking studies revealed that all the compounds are potent inhibitors for schizophrenia. All the molecules have interacted in the same receptor area. The compounds, Morusin, Artocarpin, and Luteolin-7-methyl, showed binding energy <−8.0 Kcal/mol. Morusin showed interactions with Ala289, Ser269, and Gly273, Artocarpin interacted with Leu275, Gly279, and Leu290, and Luteolin-7-methyl interacted with Ala137, Arg225, Gly272, and Glu330. All these compounds have interacted with amino acids and are identified as potent inhibitors for schizophrenia.

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