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ORIGINAL ARTICLE **OPEN ACCESS**

A Study on Molecular interaction analysis of 3-[2-(methylamino)ethyl]-1H-Indol-5-ol and Oxaserotonin by using 5-HT_{2A} receptor with special reference to Schizophrenia

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ABSTRACT

5-HT (5-hydroxytryptamine) receptors except 5-HT_3 found inside the central and peripheral nervous systems are a bunch of G protein-coupled receptors (GPCRs). These receptors have an eccentric attribute to trigger the several types of signaling pathways depend on the binding of ligand. These ligands show various impact levels to initiate a receptor. The first drug targets for designing the new drugs are the GPCRs. Drug designing for these receptors are additionally exigent thanks to their intrinsic property in the recognition of ligand and resulting in increase to many side effects of current drugs. However,In-Silico approach has provided a practical representation needed to know the molecular structure of a serotonin receptor. Therefore, a three dimensional structure of 5-HT_{2A} was built via homology modeling so as to explicate the modes of 5-HT_{2A}-Ligand binding by the analysis of Molecular Docking and Simulations by Molecular Dynamics (MD) . 5-HT_{2A} has evolved as an important therapeutic target for the treatment of disease like Schizophrenia.
Keywords: 5-hydroxytryptamine, GPCRs, Molecular Docking, Molecular Dynamic Simulation

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INTRODUCTION

Serotonin (5-HT) is one of the important neurotransmitters which is present in many peripheral tissues an also present in central nervous system. Its several organic features are facilitated by using various kinds of serotonin receptors [1]. These serotonin receptors as well referred to as 5-HT receptors are a collection of G protein-coupled receptors (GPCRs) besides most effective 5-$HT_3$is ionotropic receptors. These receptors mediate both excitatory as well as inhibitory neurotransmission [2]. The serotonin receptors influence many organic and neural strategies inclusive of aggression, anxiety, cognition, memory, sleep, mood, sickness, knowledge etc. In the last few decades, the subtypes of 5-HT receptor had been categorized starting from 5-HT_1 to 5-HT_7 [3]. The 5-HT_2 receptor family specifically includes three types - 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors, and these can be comparable in relations of their molecular form, pharmacology, and transduction pathways [4]. In a majority of 5-HT_2 subtypes, the receptor 5-HT_{2A} has revealed wide scientific effects meanwhile it is concerned in neurological disorder starting from depression, interest deficit-hyperactivity disorder, Schizophrenia and obsessive to ingesting syndromes which include anorexia, nervosa and autism spectrum disorders [5]. This specific receptor has been determined for the binding to a number of agonists, antagonists, and inverse agonists, which in result change the signaling thru G-proteins to deal with different disorders [6]. For example, when the level of 5-HT receptor changes within the brain and result to Schizophrenia and Depression [7].

In silico examine of this receptor can offer a foundation for expertise its shape and purposeful prospect. There is a full-size break within the tempo of sequence of protein and experimental purpose of the 3D structure. When there is no crystal structure available of a receptor, homology modeling gives an exceptional manner to get a structural insight into proteins. The shape of 5-Hydroxytryptamine 2A receptor generated using a comparative modeling approach. These generated models were subjected to

Molecular Dynamics Simulations to observe the time progression in addition to time averaged values of structural properties. The resulted model can be used for different experimental purposes.

MATERIAL AND METHODS

Sequence analysis

The protein sequence and data of 5-HT_{2A} was collected from the National Centre of Biotechnology (NCBI), in FASTA format, with the accession number NP >AAH96839.1 5. The sequence length reported to be 471 amino acids.

Molecular Modeling

In the direction of search for homologous sequence of 5-HT_{2A}, BLASTP was performed contrary to the databases of NCBI. Sequences were selected from the search of sequence similarity method supported the identity percentage (cut of 95% identity). The 471 aminoacid residue of 5-HT_{2A} receptor was subjected to BLASTP against Protein Data Bank to spot suitable template for comparative study of the protein structure for modeling. The 5-HT_{2A} receptor was model by using Swiss model.Identification of these amino acid Threonine, Phenylalanine, Isoleucine, Methionine, Histidine, Alanine, Arginine, Aspartic acid, Cystine, Glutamic acid, Glycine, Proline, Serine and Tyrosine identified by this method.

Validation of the Structures

The generated5-HT_{2A} structure was validated by the phi/psi distributions of Ramachandran plot obtained through RAMPAGE analysis. Further ERRAT program was used to analyze the quality of5-HT_{2A} structure. The secondary structure analysis, sequence motifs, matching folds, nest analysis; cleft analysis was done by ProfuncHydropathy analysis was done by MPEx Protein Explorer. Protein identification and characterization of channel in transmembrane was identified by porewalker.

Pharmacophore Generation/ Ligand Selection for 5-HT2A receptor

Ligand-based pharmacophore model was selected by knowing the common features of the three-dimensional structures of compounds which are known to interact with the targeted protein i.e, ligand. A Pharmacophore model of serotonin was produced by using the BIOVIA Discovery studio where the collections of 3D conformations were sieved supported the annotation points derived from every conformation. A pharmacophore was created from training the set molecules inobservation of annotation points likeH-bond donors, aromatic center, acceptors and hydrophobic centroids of the molecule.

Preparation of the protein

The protein preparation was done by using the wizard tool in Maestro version 11.5. The water molecules occupying the protein structure weren't acceptable the docking study and so, it absolutely was removed followed by minimization and optimization using OPLS_2005 field of force of Schrodinger.

Preparation of the ligand

On the idea of pharmacophore modeling of serotonin, we identify four different ligands. The Ligands 3D structures were created with the Ligprep2.5 in Schrödinger Suite with an OPLS_2005 field of force. Further, maximum possible tautomeric, stereochemical and ionization variants of those molecules were generated.

Docking grid generation

Schrödinger Suite was used to prepare the optimized three-Dimensional structure of 5-$HT_{2A}$by using the "Protein Preparation Wizard" workflow before molecular docking. After that the allocation of Bond orders and addition of hydrogen atoms to the protein has been done. Then the structure was minimized to succeed to the value 0.30 Å the converged root means square deviation (RMSD) by the OPLS_2005 field of force. Possible binding sites of ligand were searched using SiteMap2.6 in Schrödinger Suite. After that the contour maps of hydrophilic and hydrophobic fields were made. Further these hydrophilic maps were divided into donor, acceptor, and metal-binding regions. Finally, by the calculation of various properties, the measuring of all the sites has been done. Afterward, Schrödinger Suite was used to define docking grid by using "Receptor Grid Generation. The grid enclosing box was placed in 5-HT_{2A} with a size of 30 × 30 × 30 (x × y × z, Å). All the possible binding sites of ligand within the protein were made hide by using the adequate size of the grid.

Molecular Docking

Molecular Docking was disbursed by using Glide module for the receptor-ligand docking. The prepared and optimized ligands were docked within the grid box of the protein. The optimized 3D structures of the 5-HT_{2A} binders were docked into the docking grid within the 3D structure of 5-HT_{2A} using Glide5.8 in Schrödinger Suite with standard precision (SP).

RESULTS AND DISCUSSION

Homology modeling of Human 5-HT_{2A} receptor

The 5-HT_{2A}protein sequence was retrieved from NCBI (Accession no.NP >AAH96839.1 5.)and it comprises 471 amino acid residues. A sequence similarity seek for the protein against other sequences with existing structural information was performed using the NCBI BLAST. Crystal structure of 5-HT_{2A}PDB ID: was selected as template, having 62% sequence identity with target. The 5-HT_{2A} receptor was modeled by SWISS-MODEL.

Validation of the Model

The Protein has been modeled by using several types of homology modeling tools, and this gives a conclusion after comparing all these results that the SWISSMODEL generate more accurate model than the other modeling programs. Ramachandran plot was used to validate the generated model via RAMPAGE. The Angles, phi and psi were distributed for the residue of amino acids was symbolized by using Ramachandran plot. The model which was generated through this process was clearly founded to be very acceptable so, Ramachandran plot outlier region shows 0.0% (Zero residues). Afterwards ERRAT PLOT was used to measure every residue of the amino acids in the 3Dstructuralmodel and the computed model gives 94.218 %, the overall quality factor. The validated structure model is further analyzed using Profunc. The result found 8 motifs that were scanned and matched comparatively with PROSUTE, PRINTS, PFam-A, TIGRFAM, PROFILES and PRODOM motifs. Functionally important residues of 5-HT_{2A} were predicted by profunc through Nest analysis. Nest analysis located 4 nests in the structure. A score above 2.0 is suggestive of the nest being a functionally significant; there is only one significant hit in the structure. In the secondary structure of 5-HT_{2A} consists of 18 helices, 7 beta turns, 7 gamma turns and 1 disulphide.

Known ligands: Serotonin acting as 5-HT2A inhibitor was loaded in BIOVIA discovery studio. It gave pharmacophore features of serotonin as (Figure 1) [A=Acceptor, H=Hydrophobic, R=Aromatic Rings].

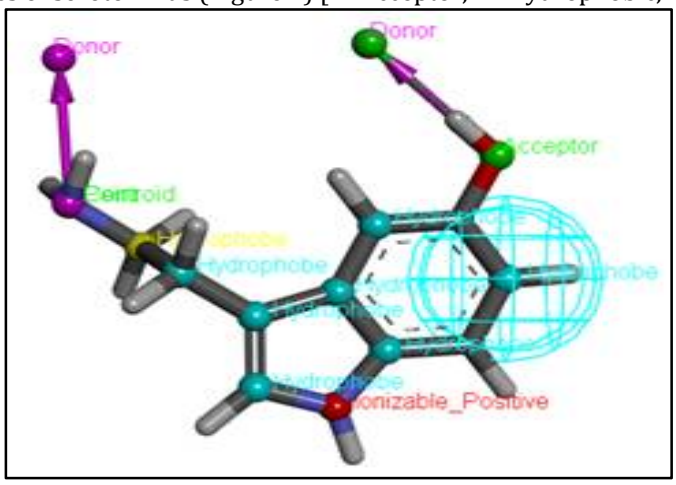
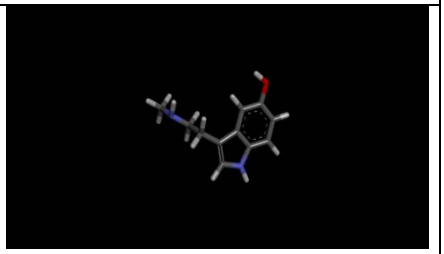


Figure 1: Pharamacophore Features of serotonin

The image of Serotonin Pharmacophores: atoms showing Donor, Acceptor, Hydrophobes, Centroid, Ionizable positive etc. These are some common parameters for Pharmacophore modeling.

These pharmacophore features matched with the following compounds:

	Ligands	Chemical structure
Compound 1	3-[2-(methylamino)ethyl]-1H-Indol-5-ol	

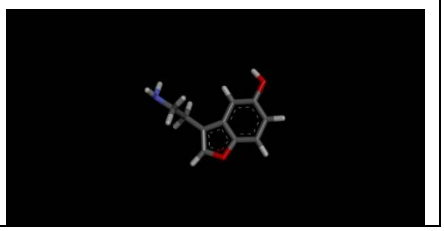
Compound 2	Oxa serotonin	

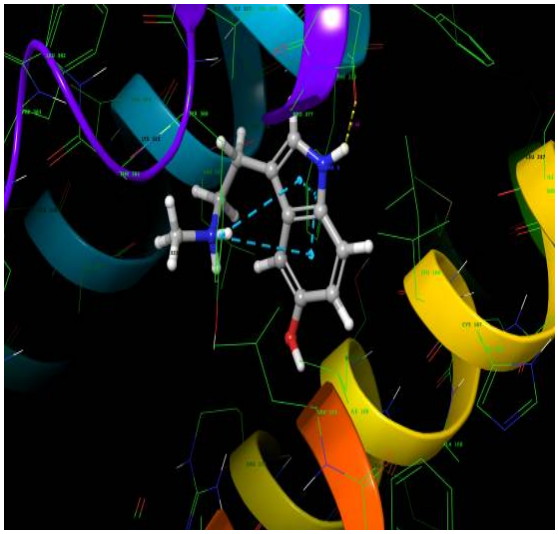
Table 1: Chemical Structure of designed compounds.

Molecular Docking

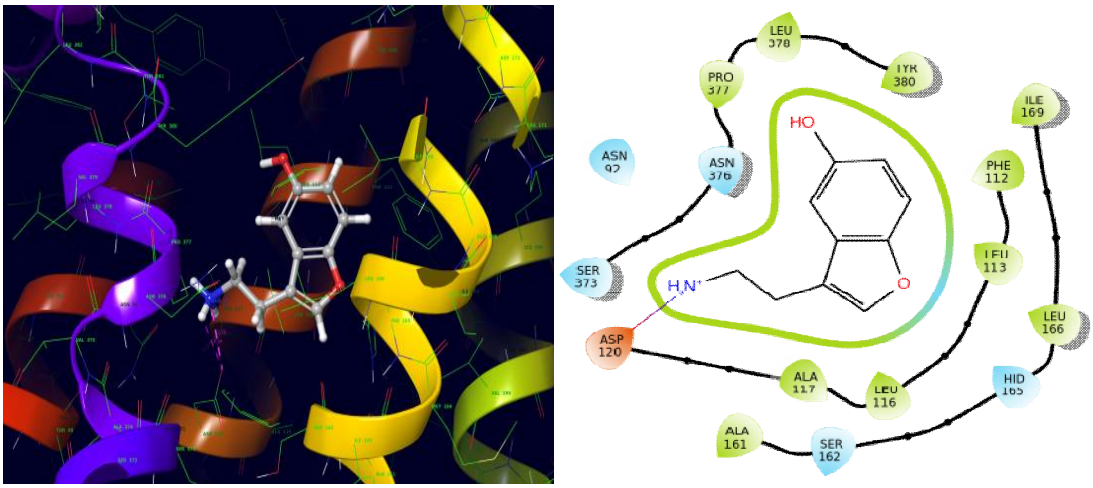
The analysis of docked complexes depends on basis of lowest binding energy values (Kcal/mol) and analysis of hydrogen/hydrophobic interaction. The binding modes of the Inhibitors with 5-HT_{2A} receptor give information about the amino acid residues that are involved during the binding.A comparative study of the binding energies of all the compounds indicates that oxaserotonin has the least binding energy among all the inhibitors and also show the maximum affinity for binding towards the 5-HT_{2A} receptor (Table-2).We performed molecular docking of 3 different compounds using the GLIDE tool from Schrodinger maestro to identify the binding mode at the 5-HT_{2A}receptor.Finally, we have selected the best inhibitor for targeted 5-HT_{2A}receptoron the basis of lowest binding energy from GLIDE scores (Table-2).

Entry	Compounds	Glide XP G-Sore	Glide energy	XP H bond
1	**3-[2-(methylamino)ethyl]-1H-Indol-5-ol**	-0.626	-248.773	0
2	**Oxaserotonin**	-3.864	-270.644	-0.087

Table 2: Binding energy of different inhibitors with 5-HT_{2A} receptor.



(a) 5HTR$_{2A}$with 3-[2-(methylamino)ethyl]-1H-Indol-5-ol(a) 2D representation of 5HTR$_{2A}$ with 3-[2-(methylamino)ethyl]-1H-Indol-5-ol



(b) 5HTR$_{2A}$withoxaserotonin(b) 2D representation of 5HTR$_{2A}$ with oxaserotonin

Figure 2: Docking results of 5-HT$_{2A}$ with ligands

Figure 2 (a) shows the result of docking analysis of 3-[2-(methylamino)ethyl]-1H-Indol-5-ol with 5-HT$_{2A}$ receptor. Figure 2 (a) 1: The predicted amino acids in the active site region are: Tyr 380, Leu 325, Pro 377, Phe 329, Ile 169.Figure 2 (a) 2: showed the 2D representation or Interaction of of3-[2-(methylamino)ethyl]-1H-Indol-5-ol with 5-HT$_{2A}$ receptor; this compound establishes hydrogen bond with Asn 376. Phe 329 and Tyr 380 shows Pi Cation interaction.

Figure 2 (b) shows the result of docking analysis of Oxaserotonin with 5-HT$_{2A}$ receptor.

Figure 2 (b) 1: The predicted amino acids in the active site region are: Leu113, Met 114, Ser115, Ala117, Asn376, Phe112, Leu166. Figure 2 (a) 2: showed the 2D representation or Interaction of Oxaserotonin with 5-HT$_{2A}$ receptor; the compound forms hydrogen bond with Asp 120.

Molecular Dynamic Simulation

For the evaluation of overall stability and flexibility of docked complexes or to confirm the exact binding of the ligand, we have to perform MD simulation with the Desmond program. The stability of 5-HT$_{2A}$ receptor with molecule oxaserotonin complex was evaluated through 10 ns molecular dynamic simulations The MD simulations provided the exact interaction or binding of the docked complex with system embedded water molecules, temperature and pressure. The complex was created in all proper binding poses with an acceptable RMSD value (< 3 Å).The deviations of residual and fluctuations in the complex was determined by using the graph of RMSD and RMSF. The RMSD of backbone atoms of the 5-HT$_{2A}$ receptor relative to initial structures indicates that stable molecular systems were obtained during MD (Figure 3). The RMSD plots indicated that 5-HT$_{2A}$oxa-serotonin complex was more stable in the simulations study of 10ns. For the characterization of local changes in the protein chain we also obtained the protein RMSF Plot for the local changes (Figure 4).To check the stability of ligand protein complex we have used solvating water molecules by the MD simulation study. This simulation study gives the better binding conformation for docked complexes.

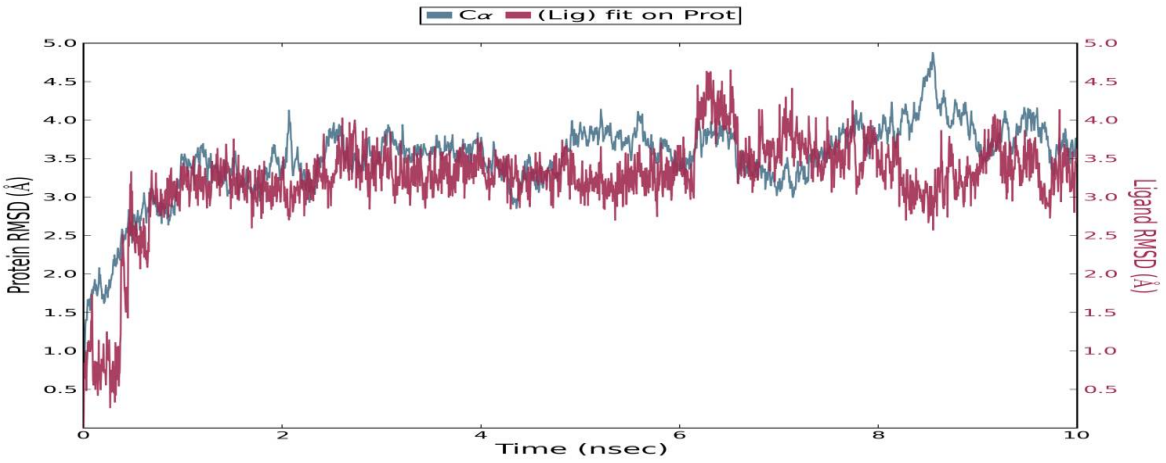


Figure 3: The Root Mean Square Deviations (RMSD) of backbone atoms relative to the starting complexes during 10 ns MD

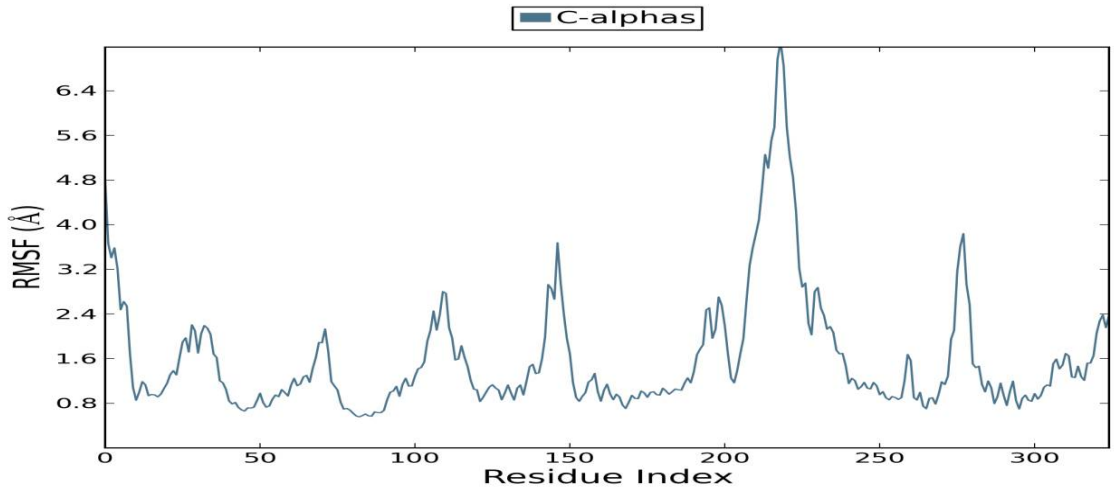


Figure 4: Protein RMSF plot, the peak indicate the most fluctuating area of the protein during the simulation and residues of the protein molecule with ligand.

CONCLUSION

The Interpretation through computational method for the newly designed compounds to identify the binding analysis in the active site of protein gives an idea to the pharmaceutical industries to check the efficacy of the molecules before the experimental work.

The study would help in deriving a comparative account of already existing molecules being used in drugs as well as the comparative account of *in-silico* designed molecules of these known drugs.The data generated in the present study would help in deriving, designing and synthesis of more potent drugs being specific for 5-HT_{2A}, than the drugs which are in practice. The site specific drug which will be revealed from the study could be more efficient for inhibiting the 5-HT_{2A} receptor and hence better cure for neurological disorder like Schizophrenia. The data generated could be submitted to pharmacoinformatics data banks that would be beneficial for future research in drug discovery.

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