Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2018; 10(4): 84-89

ISSN- 0975 1556

Research Article

Natural Fungal Compounds as 5-Hydroxytryptamine Receptor 2C Inhibitors: A Homology Modeling and Docking Study

Sowmya Suri*, Bindu Chowhan

Bioinformatics Wing, Prof. G. Ram Reddy Centre for Distance Education, Osmania University, Hyderabad-500007

Received: 8th Oct, 17; Revised: 10th Jan, 18; Accepted: 15th Mar, 18; Available Online:25th April, 18

ABSTRACT

The present study explains computational methods to design 3D structure of "5-hydroxytryptamine receptor 2C of Homo sapiens. Modeling study was performed to generate a 3D model for 5-hydroxytryptamine receptor 2C protein. The model was developed by using Modeler9.18 software. The developed model showed 92.5% of the amino acids in most favored region. The developed model was further docked with 14 natural fungal compounds by using AUTODOCK4.2 software to identify the functional effect of protein. All the compounds exhibited good binding energy and interactions. These studies provide understanding and interpreting the data produced by these methods.

Keywords: Homology modeling, "5-hydroxytryptamine 2C receptor", fungal compounds, docking.

INTRODUCTION

The 5-HT_{2C} (Serotonin) receptor is a key contributor to neurological diseases and autism, psychiatric (schizophrenia), obesity diseases. Lorcaserin is the most advanced 5-HT_{2c} receptor agonist for the treatment of obesity. Vabicaserin is another 5-HT_{2C} receptor agonist for the treatment of psychiatric indications[1]. It is also an important neurotransmitter associated with many psychological processes[2]. 5-HT receptors are divided into 7 families: (5-HT_{1-7}). 5-HT-1, 5-HT-2, 5-HT-3 receptors and are implicated in the control of disinhibition (micturition)[3]. The receptor 5-HT_{2c} has been implicated in eating and mood disorders, it increases anxiety and decreases hypophagia[4]. 5 – Hydroxytryptamine receptor 2C is one of the fourteen members of the 5-hydroxytryptamine receptor family[5]. In hypothalamus and limbic system the 5-HT2c expression level is high, where its activity is implicated in feeding behavior, depression, locomotor, anxiety and sleep disturbace[6].

In the present study, an effort was made to generate the 3D structure of the 5-hydroxytryptamine receptor 2C[7] from Homo sapiens. Modeller9.18 was used for the homology modeling. The model was validated by using PROCHECK. Present study could provide useful information to get the functional characterization of these enzymes. Molecular docking studies were performed by using Autodock4.2 with known inhibitors of 14 natural fungal compounds.

METHODOLOGY

Homology modeling

The amino acid sequence of 5-hydroxytryptamine receptor 2C was retrieved from Uniprot[8]. A sequence similarity search was performed to identify the structural similarity of the query sequence by using Protein BLAST[9] tool by selecting database against Protein Data Bank (PDB) for identifying template for homology model building[10]. The template was identified on the basis of smaller E-value, >30% identity, maximum score. 4IB4 protein was selected as a template for modelled protein. Comparative sequence alignment studies were performed with query and template structure using ClustalX tool and online ClustalW tools[11]. A three dimensional model was generated for " 5-hydroxytryptamine receptor 2C" protein by using modeller.

MODELLER9.18 software was used to develop the model. It is an automated approach to comparative modelling by satisfaction of spatial restrains[12]. To align the query and template sequences manually the input file of alignment.ali was used in MODELLER 9.18. After completion of alignment, twenty models were generated and all the generated models were thermodynamically minimized using molecular dynamics and simulation approach. By implementing MODELLER9.18 auto-model class, calculated 3D models of the target automatically. The best model which is having smallest value was selected on the basis of Lowest Objective Function. It is also known as normalized Discrete Optimized Molecule Energy (DOPE) score. The generated model was then checked in detail for protein structure stereochemistry including Ramachandran plot and Psi/Phi angles using PROCHECK[13].

Molecular docking studies

All the molecules were collected from scientific literature and sketched in SYBYL6.7[14] and the energy was minimized by adding Gasteiger Huckel charges. The molecules were then saved in .mol2 format for molecular docking purpose.

Molecular docking studies were performed to explain the binding mode of proteins and ligands. All the existing

*Author for Correspondence: surisowmya@gmail.com

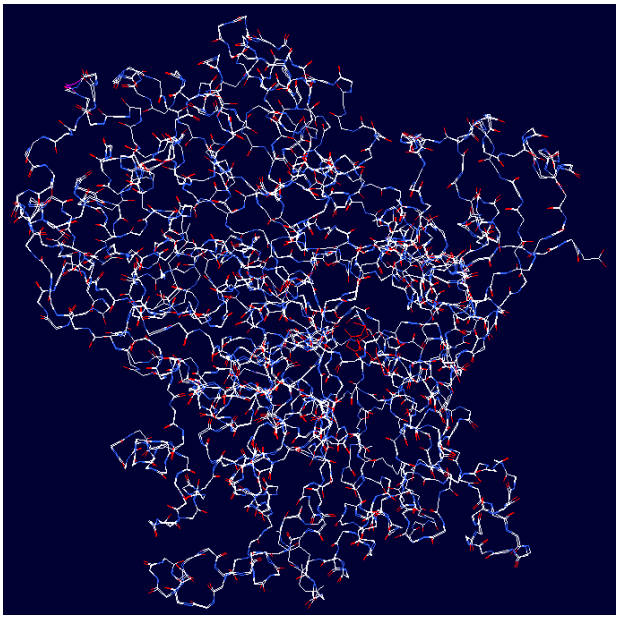


Figure 1: Super pose of model and template structures with backbone trace. The models were superimposed by using swiss pdb viewer (spdbv).

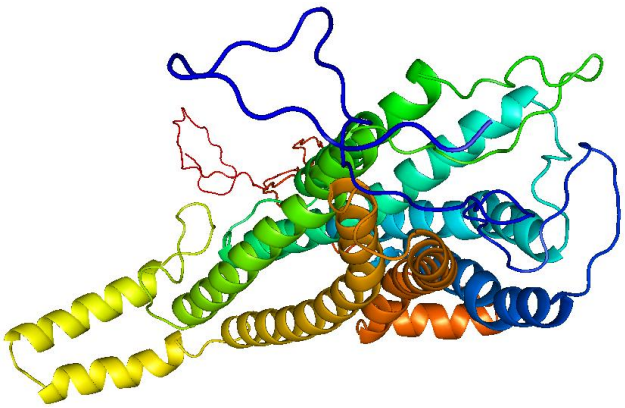


Figure 2: The cartoon of homology derived protein of P28335 modeled protein.

Table 1: Binding energy and interacting residues of natural compounds with modeled P28335 protein

SNo	Compound Name	Interacting Amino acids	Binding Energy (kcal/mol)	Dissociation Constant (KI)
1	Altenuic acid	Arg152, Ser163, Ser167, Lys170, Lys174	-7.38	3.9
2	G7063-2	Arg152, Lys170, Lys174	-5.27	36.09
3	Botralin	Arg152	-6.44	18.98
4	Chokol E	Ser149, Gly314	-6.94	8.15
5	Chokol F	Thr237	-6.60	14.59
6	Coriloxin	Thr237	-4.85	277.49
7	Diboviquinone-3,4	Arg152	-3.83	1.56
8	Enaminomycin B	Thr88, Arg152(2), Lys170, Lys174	-4.81	297.25
9	Enaminomycin C	Thr88, Arg152. Lys174(2), Ser167, Asp151	-6.16	30.76
10	Epoformin	Lys174	-4.57	445.89
11	Epoxydon	Ile160(2)	-3.75	1.78
12	Fusicoccin	Arg157(2)	-3.14	5.02
13	Mediboviquinone-3,3	Arg152	-4.19	24.85
14	Wortmannin	Arg152(2)	-7.67	2.39

compounds were docked by using Autodock 4.2 software[15]. All the molecules were docked individually in Autodock4.2. The modeled three dimensional structure of 5-hydroxytryptamine receptor 2C protein was imported to Autodock 4.2 and structurally optimized by adding hydrogens to protein allocated with kollaman charges[16]. After adding the hydrogens the model was saved in PDBQT format, later ligands were prepared by optimizing the torsion angles and saved them in PDBQT format. A grid was generated around to identify XYZ coordinates (X=29.046, Y=10.614 and Z= -18.598), around binding site of 5-hydroxytryptamine receptor 2C protein. Lamarckian genetic algorithm (LGA) was selected for freezing, docking and default parameters used in autodock4.2.

RESULTS AND DISCUSSION

After sequence alignment and homology modeling of 5-hydroxytryptamine receptor 2C showed highly conserved regions in amino acid sequences. The most homologous template for building a homology model for 5-hydroxytryptamine receptor 2C was identified through protein blast algorithm. Based upon the homology search, Crystallographic structure of "*Chain A, Crystal structure of the chimeric protein of 5-ht2b-bril in complex with Ergotamine (*PDB entry: 4IB4) was selected as a template. The alignment file was tweaked manually to fit excellently in the sequences. Twenty models were generated using Modeler 9.18 program. After the generated models for all the primary sequences, the model with least object function was selected for further protein stereochemistry evaluation (phi and psi angles)

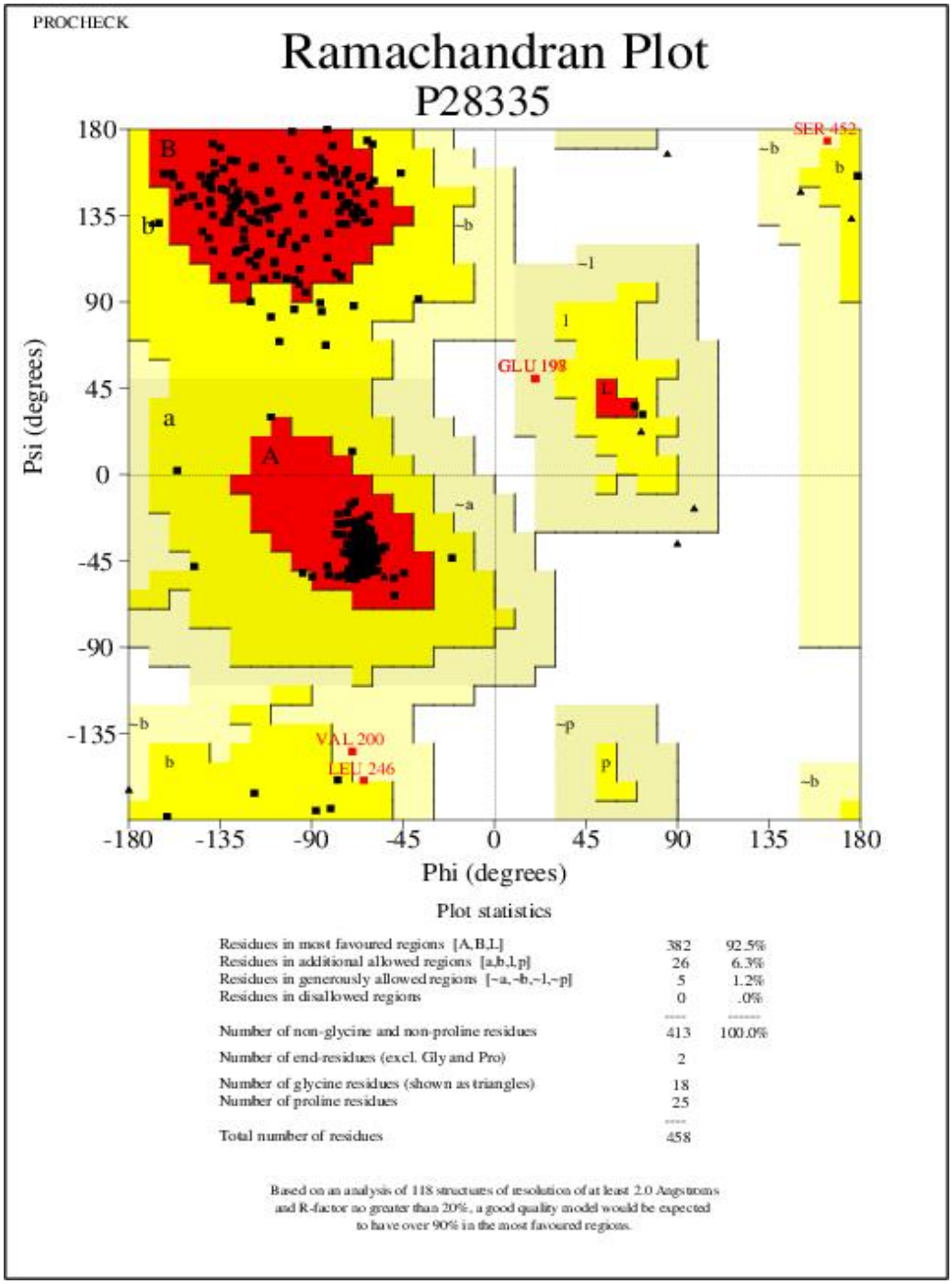


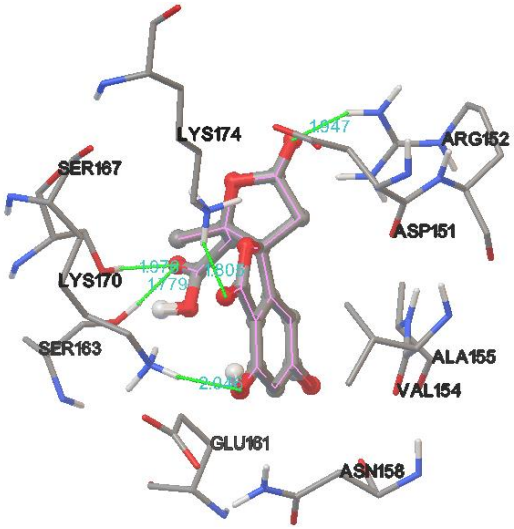
Figure 4: Ramachandran plot of the modeled P28335 protein

with procheck software. Figure1 and 2 shows super pose of model and template structures with backbone trace and the cartoon of homology derived protein of 5-hydroxytryptamine receptor 2C. After model generation RMSD was calculated by superimposing both the models (template and modeled protein) by selecting Cα. The model exhibited RMSD value of 0.48Å.

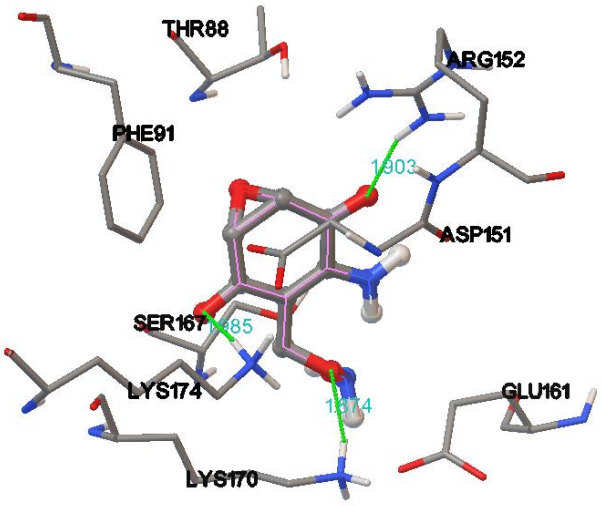
The three (Ø, Ψ and ω) backbone torsion angles are important determinants of a protein fold. PROCHECK software generates a number of scatter plots, known as Ramachandran plots. These plots show complete residue by residue data and the assessment of the general excellence of the producing structure as compared to well refined structures of the same resolution. The Ramachandran plot is the main indicator to check the intrinsic quality of the protein structure. The Ramachandran plot of the template (PDB ID: 4IB4) have 325 amino acid residues (95.0 %) in most favorable regions, 17 amino acid residues (5.0 %) in additionally allowed region and there is no amino acid residues in generously allowed region and disallowed region, whereas for the modeled protein have, 382 amino acid residues (92.5 %) in the most favorable region, 26 amino acid residues in additionally allowed region (6.3 %) and five amino acid residues present in generously allowed region (1.2%). There is no amino acid residue present in

disallowed region. The Ramachandran plot is shown in figure 4. These results clearly indicate that the generated protein model is more conformationally superior to the template structure. The modeled structure was superimposed with the template 4IB4 by using SPDBV. It was observed that RMSD value was of 0.49 Å.

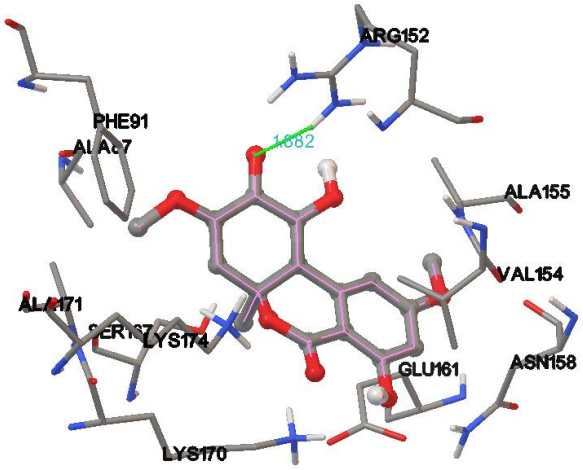
Molecular docking of natural compounds into the binding site of a receptor and estimating the binding affinity of the ligand is a most important part of the structure based drug design process. The molecular docking results



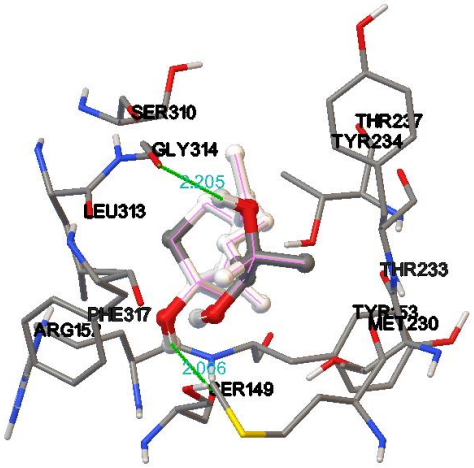
1



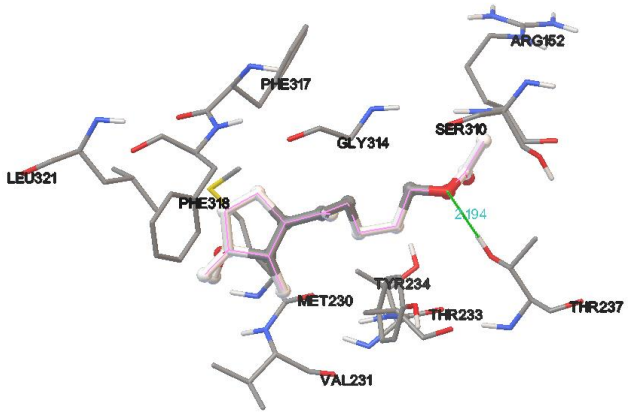
2



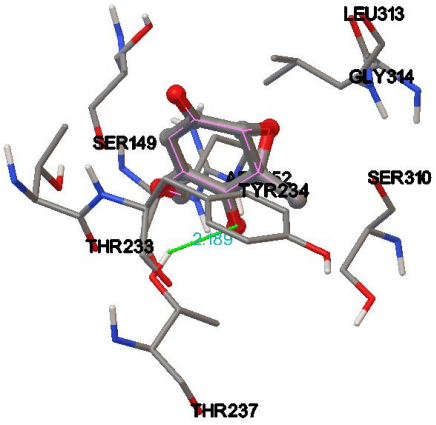
3



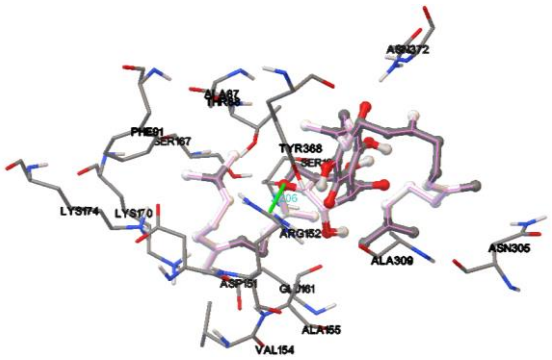
4



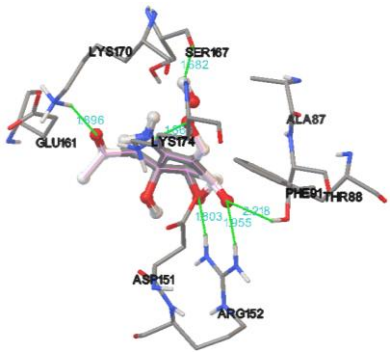
5



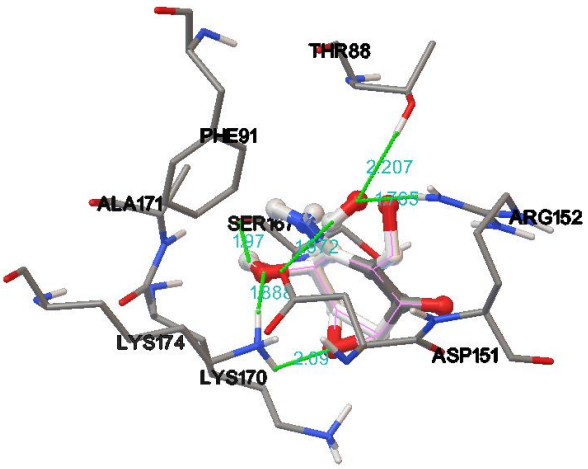
6



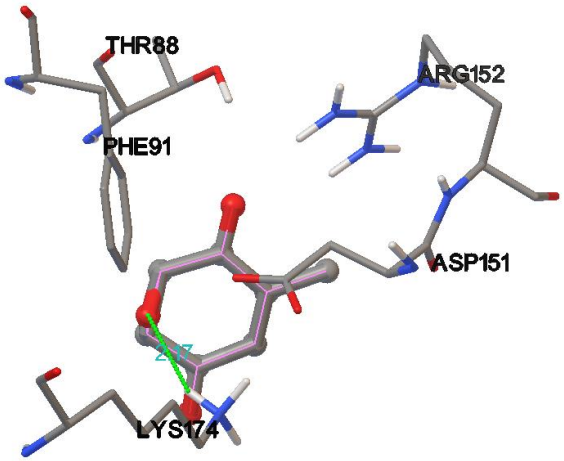
7



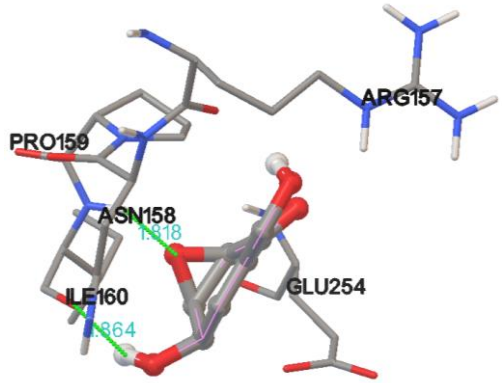
8



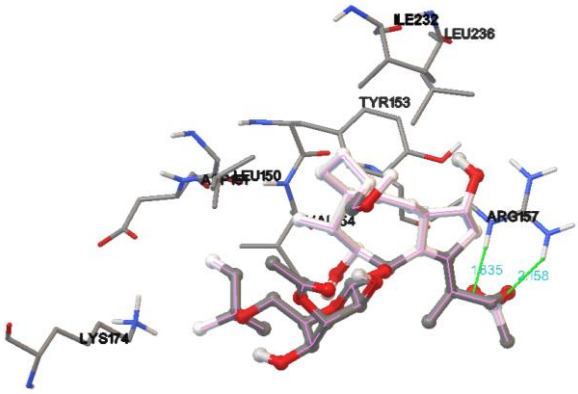
9



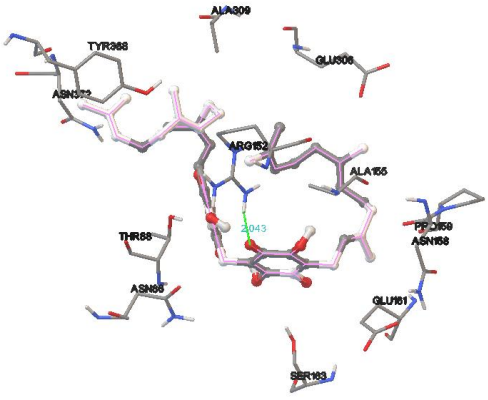
10



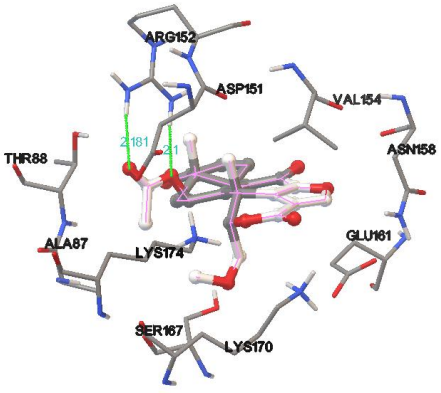
11



12



13



14

Figure 2: Docking interactions of P28335 protein with 14 fungal metabolites

indicate that all the studied natural derivatives occupy an almost similar space in the binding site. Quercetin showed best possible binding mode against modeled 5-hydroxytryptamine receptor 2C protein and is illustrated in Figure 4. During the molecular docking procedure, the program selects only best fit active site pocket of the protein with respect to the ligands in order to dock them. AutoDock 4.2 provides information on the binding orientation of ligands at the active site region. The docking program place both ligand and protein in different orientations, conformational positions and the lowest energy confirmations which are energetically favorable are evaluated and analyzed for interactions. Free energies of binding (ΔGb) and dissociation constants (Ki) as calculated by AutoDock are summarized.

The binding energy and interacting amino acid residues are given in table 1. For all the molecules binding affinity was characterized by binding energy (ΔG) value. Ligand quercetin showed highest binding energy of -6.84 kcal/mol interacting with Met729 and Trp724. Ligands luteolin and riboflavin showed three interactions. Luteolin interacts with Arg173, Met279, His725 with binding energy of -6.59 kcal/mol. Riboflavin interacts with Ans173(2), Thr480, Asn481 with a docking score of -4.62 kcal/mol. All the docking poses of the molecules were shown in Figure 2.

CONCLUSION

In this work, homology modeling and molecular docking studies were performed to explore structural features and binding mechanism of existing quercetin, citric acid, cuminaldehyde, eugenol, luteolin, riboflavin ligands as 5-hydroxytryptamine receptor 2C inhibitors, and to construct a model for designing new 5-hydroxytryptamine receptor 2C protein. Homology derived model statistics are similar to template i.e., crystal structure. Docking the modeled protein with these ligands provided insight into the binding and interaction with the enzyme. Further, the structure based drug discovery process along with protein information of drug targets may improve our understanding towards in-sight of mechanism of protein-ligand interactions and their binding patterns. These studies explain to understand molecular interactions at the active site region.

REFERENCES

1. Sharon Rosenzweig-Lipson. New Horizons for Selective 5-HT2C Receptor Ligands in Psychiatric/Neurological Disorders. Neuropsychopharmacology Reviews (2011) 36, 363–364. doi:10.1038/npp.2010.168.
2. Chen W, Zhang Q, Su W, Zhang H, Yang Y, Qiao J, Sui N, Li M. Effects of 5-hydroxytryptamine 2C receptor agonist MK212 and 2A receptor antagonist MDL100907 on maternal behavior in postpartum female rats. Pharmacol Biochem Behav. 2014 Feb;117:25-33. Doi: 10.1016/j.pbb.2013.11.034.
3. Andrew G. Ramage. The role of central 5-hydroxytryptamine (5-HT, serotonin) receptors in the control of micturition. British Journal of Pharmacology (2006) 147, S120–S131.
4. Kimura A, Stevenson PL, Carter RN, Maccoll G, French KL, Simons JP, Al-Shawi R, Kelly V, Chapman KE, Holmes MC. Overexpression of 5-HT2C receptors in forebrain leads to elevated anxiety and hypoactivity. Eur J Neurosci. 30(2):299-306. Doi: 10.1111/j.1460-9568.2009.06831.x.
5. Martin GR, Humphrey PP.Receptors for 5-hydroxytryptamine: current perspectives on classification and nomenclature. Neuropharmacology. 1994, 33(3-4):261-73.
6. Millan MJ. Serotonin 5-HT2C receptors as a target for the treatment of depressive and anxious states: focus on novel therapeutic strategies. Therapie. 2005, 60(5):441-60.
7. (Niswender CM, Sanders-Bush E, Emeson RB.Identification and characterization of RNA editing events within the 5-HT_{2C} receptor. Ann N Y Acad Sci. 1998; 861:38-48.)
8. http://www.uniprot.org/uniprot/P28335
9. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. LAST+: architecture and applications. BMC Bioinformatics. 2009; 10: 421.
10. http://www.rcsb.org/pdb/home/home.do
11. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. Clustal W and Clustal X version 2.0. Bioinformatics. 2007;23(21):2947-8.
12. Sali A, Blundell TL.Comparative protein modelling by satisfaction of spatial restraints. J MolBiol. 1993;234(3):779-815.
13. Fischmann TO, Hruza A, Niu XD, Fossetta JD, Lunn CA, Dolphin E, Prongay AJ, Reichert P, Lundell DJ, Narula SK, Weber PC. Structural characterization of nitric oxide synthase isoforms reveals striking active-site conservation. Nat Struct Biol. 1999; 6(3):233-42.
14. Gunda SK, Kongaleti SF, Shaik M. Natural flavonoid derivatives as oral human epidermoid carcinoma cell inhibitors. Int J Comput Biol Drug Des. 2015; 8(1):19-39.
15. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009; 30(16):2785-91.
16. Rashidieh B, Madani Z, Azam MK, Maklavani SK, Akbari NR, Tavakoli S, Rigi G. Molecular docking based virtual screening of compounds for inhibiting sortase A in L.monocytogenes. Bioinformation. 2015; 11(11):501-5.