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Anxiolytic effects of newly synthesized derivatives in mice and molecular docking studies as serotonin $5HT_{2A}$ receptor inhibitor

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ABSTRACT

Obsessive-Compulsive Disorder (OCD) is an anxiety disorder featuring disturbing and troubling thoughts, which are perceived as the products of one's own mind unlike schizophrenia. Marble burying in mice has been used to model anxiety disorders including obsessive–compulsive disorder (OCD) due to the extreme nature of the behavior and due to the pharmacological effects of clinical standards. Test groups (7a-k) and fluoxetine administration decreased the number of marbles buried compared with the control group (all $P<0.01$) which suggests the anxiolytic-like effect of it. Further compounds 7 (a-k) did not show any neurotoxic effects at dose administered (40 mg/kg). Moreover in the present study molecular docking studies was conducted on serotonin 5-HT_{2A} receptor (2VT4) and synthesized derivatives 7 (a-k). The docking method describes the binding interaction with the ligand and receptor 2VT4. In conclusion the previously synthesized derivatives 7 (a-k) showed marked anxiolytic activity similar to that of fluoxetine in the marble burying behavior test in mice.

Keywords: Obsessive-compulsive disorder, anxiolytic, marble burying behavior, molecular docking.

INTRODUCTION

According to the World Health organization report approximately 450 million people experience a mental or behavioral disorder, yet only a small minority of them receives even the most basic treatment. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020.[1] Anxiety and mental depression are the most common mental illnesses in the world and a prominent health care problem. The World Health Organization (WHO) estimates that the prevalence of these diseases ranges from 0.1 to 16.9%[2]. Depression is a very common psychiatric condition. It is the most common mood affective disorder which refers to a pathological change in mood; it may range from very mild to severe psychotic depression and is accompanied by hallucinations and delusions. Depression is characterized by sad mood low and loss of interest in activities previously enjoyed. From world health organization, depression is currently the fourth major cause of disease burden worldwide. An estimated 340 million people suffer from mental depression. It is predicted that depressive disorder will be the second leading cause of disease burden worldwide by the year 2020[3].

Obsessive-Compulsive Disorder (OCD) is an anxiety disorder featuring disturbing and troubling thoughts, which are perceived as the products of one's own mind unlike schizophrenia. OCD may be looked upon as a condition which

affects the person's frequently experiences irresistible urges to perform repetitive rituals (compulsions). The obsessive thoughts about cleanliness, perfectness and household tools responsible for anxiety are apparently neutralized by repetitive rituals such as excessive and repetitive cleaning, arranging, checking and rechecking. OCD is a depressive disorder characterized by intrusive thoughts that produce anxiety, by repetitive behaviors aimed at reducing anxiety, or by combinations of such thoughts (obsessions) and behaviors (compulsions)[4]

Rats and mice bury the unpleasant object to cause aversion stimuli and fearful thoughts. Marble burying in mice has been used to model anxiety disorders including obsessive–compulsive disorder (OCD) due to the extreme nature of the behavior and due to the pharmacological effects of clinical standards[5]. Only potent serotonin reuptake inhibitors (SSRIs) are consistently effective in patients of obsessive–compulsive disorder[6]. The mice and rats bury the unpleasant object able to cause aversion stimuli and fearful thoughts. An acute administration of certain classes of antidepressants like selective tricyclic antidepressants (TCAs), serotonin reuptake inhibitors (SSRIs) and serotonin been shown to dose-dependently inhibit marble-burying in mice.[7]

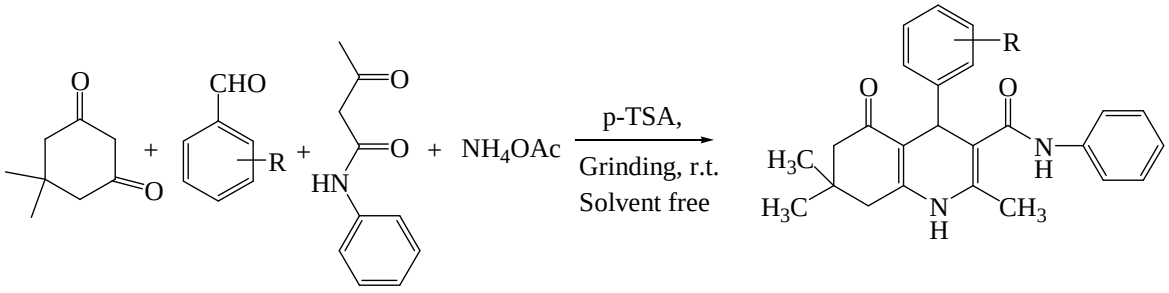
The marble-burying behavior is a well-accepted model to screen anti-compulsive activity in mice. The single-dose administration of certain classes of antidepressants like tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs) has been shown to dose dependently inhibit marble burying behavior of mice[8]. The anxiolytic agent chlordiazepoxide, decreased the number of marbles buried. However, there is no proper remedy for OCD[9]. The marble burying test is a useful model of neophobia, anxiety and obsessive compulsive behavior. It has also been proposed that the test may have predictive validity for the screening of novel antidepressants, anxiolytics and antipsychotics[10].

Quinoline and their derivatives performing as a nucleus in several natural products and drugs attributing to their diverse applications in the pharmaceutical industries uphold a remarkable place among the heterocyclic compounds[11]. Quinolines having 1,4-DHP (1,4-dihydro pyridine) nucleus have been reported as significant compounds due to their therapeutic and pharmacological properties such as vasodilator, antitumor, geroprotective, bronchodilator, antimalarial, anti-inflammatory, antiasthematic, and antibacterial activities[12],[13]

In particular hexahydro-quinolines are a class of hetrocycles that possess anxiolytic property[14], antidepressant [15] and antihistaminic activity[16], antispermatogenic agents[17], calcium antagonistic activity[18] and anticancer activity[19].

Serotonin is a neurotransmitter frequently associated with anxiety and anxiolytic drugs. 5-HT_{1A} and 5-HT_{2A} receptors are proposed to play an important role in anxiety. Moreover, preclinical studies have shown that 5-HT_{1A} agonists exhibit anxiolytic-like effects in animal models, and 5-HT_{1A} antagonists block the anxiolytic-like effects of some drugs, such as cannabidiol[10].

In the present study, we have used 6 compounds which were previously synthesized[20] in the chemistry laboratory of our college (scheme 1). The synthesized molecules were subjected to docking study by using 'Glide 2.5' (a molecular docking programme). Standard anxiolytic drug fluoxetine was also docked to validate the docking model. In this communication the objective was to evaluate the anti-anxiety effect of synthesized compound 7 (a-k) using marble burying behavior and also to study the molecular docking of new compounds on the crystal structures of Serotonin 5-HT_{2A} receptor (PDB ID: 2VT4) retrieved from Protein Data Bank (www.rcsb.org).



4 2 (a-k) 5 7 (a-k)

Scheme 1 General reaction scheme for the synthesis 7 (a-k)

MATERIALS AND METHODS

Test animals

The experimental animals were swiss albino mice (20-25 gm) of either sex used. They were housed in groups in polypropylene cages with wood shavings as bedding, under a controlled 12 h/12 h light/dark cycle (lights on at 7:00 a.m.) and controlled temperature. The animals were given standard laboratory feed and water *ad-libitum* with the exception of 1 h before and during the experiments. The experiments were performed between 8.00 am to 1.00 pm. The experiments were conducted in a sound proof laboratory. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee.

Marble burying behaviour

Marble burying behavior behavior belongs is probably a type of defensive burying typical of rodents. This behavior in mice and rats is based on the behavior of burying harmless objects. Anxiolytic like drugs decrease the number of marbles buried during a session at doses that do not alter locomotor activity[21]. The apparatus consisted of an opaque plastic chamber (40cm×25cm×12cm) similar to the home cage. The floor of the chamber was evenly covered with 5cm deep sawdust. 24 clean glass marbles (2cm diameter) were equally spaced against the wall. No food or water was present. The mice was singly placed in the cage and left undistributed. The number of marbles buried (at least 2/3rd) by the mice in the 30-min observation period was recorded.

Experimental design

Mice were divided in eight groups and each group consisted of minimum of three animals. Separate animals were used for each experiment.

Group I: Control group of mice (n=3)
Group II: Standard group, treated with fluoxetine 40mg/kg i.p.
Group III: Test group, treated with 7a 40mg/kg i.p.
Group IV: Test group, treated with 7c 40mg/kg i.p.
Group V: Test group, treated with 7e 40mg/kg i.p.
Group VI: Test group, treated with 7f 40mg/kg i.p.
Group VII: Test group, treated with 7g 40mg/kg i.p.
Group VIII: Test group, treated with 7k 40mg/kg i.p.

Rota-rod test

The Rota-rod equipment was used to evaluate motor coordination produced by drugs in animals[22]. Further the neurotoxicity of the compounds was measured in mice by the rotarod test. The mice were trained before the experiment to acquire the capacity to remain on a rotating rod. Two or three trials were sufficient for the animals to learn the task. The mice were trained to stay on an accelerating rota-rod of diameter 3.2 cm that rotates at 10 rpm. Trained mice were given an intraperitoneal (i.p.) injection of the test compounds 30 min before the test in each of the experimental groups. Then, the mice were placed in the four paws on the rotating bar, which is 25 cm high from the floor. The mice were observed for a period of one minute. The ability of the mice to remain on the rota-rod for 1 min was recorded. After each test, the equipment was washed with soap and water, cleaned with ethanol 70% and dried. Then, the subsequent mouse was tested. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1min in each of the trials.

Molecular docking

Computer Aided Drug Design (CADD) is a specialized discipline that utilizes computational methods to simulate drug-receptor interactions. CADD methods are mainly dependent on bioinformatics tools, applications and databases[23].

Using docking, scientist virtually screen database of compounds and predict the strongest binders based on various scoring functions. Molecular docking explores ways in which two molecules, such as drugs and an enzyme receptor fit together and dock to each other well. The molecules after binding to a receptor, inhibit its function, and thus act as drug. The drug and receptor complex was identified via docking and their relative stabilities were evaluated using molecular dynamics and their binding affinities, using free energy simulations.

Glide (grid-based ligand docking with energetics) has been designed to perform as close to an exhaustive search of the positional, orientational, and conformational space available to the ligand as is feasible while retaining sufficient computational speed to screen large libraries. Glide-Score, is used for predicting binding affinity and rank-ordering ligands in database screens.

The computational studies were carried out using HP Desktop PC, (Core 2 Duo Processor; 1GB RAM) running on Windows XP using Maestro 9.0. The synthesized molecules were evaluated in silico (docking) using the homology models of serotonin 5-HT_{2A} (2VT4). Various parameters including docking score, glide score and glide emodel were calculated. Similar data emphasizing the degree of interaction between the test compounds and receptor were deduced additionally.

Serotonin 5-HT_{2A} receptor structure

The model of serotonergic 5-HT_{2A} receptor employed for the in silico evaluation was retrieved from Protein Data Bank (www.rcsb.org). The model selected was based on modified turkey beta(1)- adrenergic receptor in complex (PDB ID: 2VT4) with the high-affinity antagonist cyanopindolol, whose crystal structure was determined at 2.7 Å. The ligand-binding pocket comprises 15 side chains from amino acid residues in 4 trans-membrane alpha-helices and extracellular loop 2. This loop defines the entrance of the ligand-binding pocket and is stabilized by two disulphide bonds and a sodium ion. Binding of cyanopindolol to the beta(1)-adrenergic receptor and binding of carazolol to the beta(2)-adrenergic receptor involve similar interactions

The pre-processing of the protein was carried out by assigning the bond orders, adding hydrogen atoms to the crystal structure, creating disulfide bonds, filling missing side chains and loops (using Prime). The water molecules beyond 5 Å were deleted straightaway. This was followed by reviewing and modifying the pre-processed protein, where the workspace protein was analyzed for multiple chains and associated ligands. In the next step (refinement), hydrogen bonds were optimized using PROPKA at pH of 7. The amino acids residues lying close to the active site were allowed to flip. Finally, the strain was minimized using OPLS2005.

The receptor grid for docking was prepared by Receptor Grid generation tool, under glide menu. The receptor was defined by default settings as the workspace structure. The van der Waals radii were scaled by a factor of 1.0 for all those atoms carrying partial atomic charge. As suggested in literature, the receptor grid site was selected as the centroid of residues Asp120 and Asp155. No constraints and excluded volumes were added. The default settings were applied for grid generation

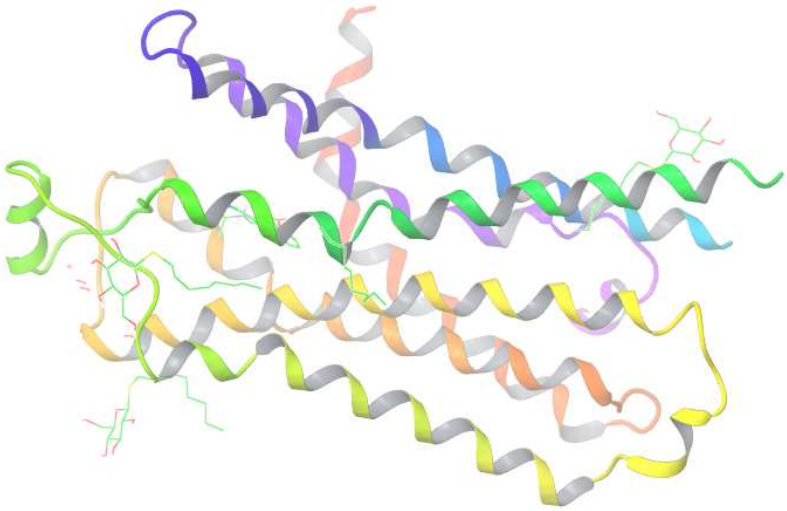


Fig 1: Structure of Serotonin 5-HT$_{2A}$ receptor (PDB ID: 2VT4)

RESULTS AND DISCUSSION

Marble Burying Behaviour

Mice shows a spontaneous behavior of burying aversive materials present in their environment, such as objects, nasty food, and small predators, being characterized as a defensive behavior reflecting the anxiety state of the animals. The marble burying test (MBT) is characterized by evaluating the rodent behavior in relation to attempt to bury potentially dangerous objects, and this behavior is reduced or suppressed by the action of anxiolytic drugs[24].
In the marble-burying test (Table 1) effects were observed after acute treatment. Test groups 7 (a-k) and fluoxetine administration reduced the number of marbles buried compared with the control group (all *P<0.01*) which suggests the anxiolytic-like effect of it. Test group 7a and 7k decreased the numbers of marbles buried (*P<0.01*) compared to control. Moreover test group 7b, 7c, 7d and 7e had superior anxiolytic like effect as indicated by significant (*P<0.001*); decreased the number of marbles buried compared to control group.

Table 1 Antianxiety activity of the compounds in marble-burying behavior.

Groups	Number of buried marbles
Control	19.67 ± 1.45
Phenytoin	5.00 ± 1.16***
7a	10.33 ± 1.76**
7c	7.67 ± 1.76***
7e	8.33 ± 1.86***
7f	8.0 ± 1.53***
7g	8.0 ± 1.73***
7k	11.0 ± 1.53**

*Values represent means ±S.E.M. (n =3). *P < 0.05, **P< 0.01, ***P < 0.001 compared with vehicle (One-way ANOVA followed by Dunnett's post hoc test).*

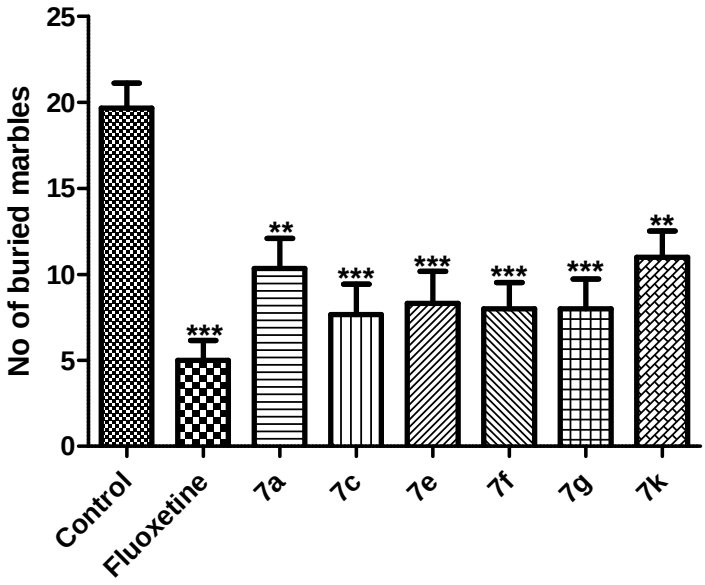


Fig. 2 Effects of different synthesized derivatives 7 (a-k) in marble burying model in mice. Results are expressed as means ± S.E.M. (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with vehicle (One-way ANOVA followed by Dunnett's post hoc test)

Rota-rod test

Rota-rod test were used to evaluate if the probable action of test compounds on the CNS is related to sedative and muscular relaxation effects. The effect of a single dose of test compounds on the CNS was compared with single doses of Fluoxetine, which is a standard drug to treat anxiety disorders. The neurotoxicities of the synthesized compounds 7 (a-k) in the current study were determined using the minimal motor impairment-rotarod screen. As shown in Table 2 compounds 7 (a-k) did not show any neurotoxic effects at dose administered (40 mg/kg). Further fluoxetine as a standard anxiolytic did not affect the motor activity in the present investigation as depicted in table 2.

Table 2 Neurotoxicity screening of the synthesized compounds in rotarod test

Groups	Rota rod test
Control	_
Fluoxetine	_
7a	0/4
7c	0/4
7e	0/4
7f	0/4
7g	0/4
7k	0/4

Rotarod toxicity (number of animals exhibiting toxicity/number of animals tested).
Compounds prepared were administered at 40 mg/kg; fluoxetine was administered at 40 mg/kg.

Table 3 Docking Score of synthesized derivatives

Entry	R	Product	Glide Score (2VT4)	Interacting residues
1	H	7a	-6.93	CYS 199, ASN 329
2	m-Cl	7c	-7.28	CYS 199, PHE 201, ASN 329
3	3,4-Cl_2	7e	-7.17	CYS 199, PHE 201, ASN 329
4	o-OH	7f	-7.69	CYS 199, PHE 201, ASN 329
5	p-OH	7g	-7.73	CYS 199, ASN 310, ASN 329,
6	o-NO_2	7k	-6.18	CYS 199, PHE 201, ASN 329
7	Fluoxetine	Standard	-7.11	CYS 199, ASN 329, PHE 325

Molecular Docking

Docking results between Serotonin 5-HT_{2A} receptor and synthesized derivatives 7 (a-k) are reported in Table 3. Computational strategies for structure based drug discovery offer a valuable alternative to the costly and time consuming process of random screening. Glide 2.5 is employed to study the docking molecules within active site region of 2VT4. At the end of each run, docked orientations are saved and the resultant molecules are checked for geometry. The newly synthesized molecules were docked against the protein 2VT4. The standard chemotherapeutic agent fluoxetine on docking with 2VT4 has glide score of -7.11. When the synthesized derivatives were docked against the same receptor the energy values are greater than the standards for some derivatives. Derivative 7g has glide score of -7.73. It was observed that the Derivative 7g containing hydroxyl group at para position of benzene, is showing better binding nature, which resulted in a decrease in the energy value. This particular compound showed a decreased in energy values which means it was more compatible with the receptor than the standard and other derivatives.

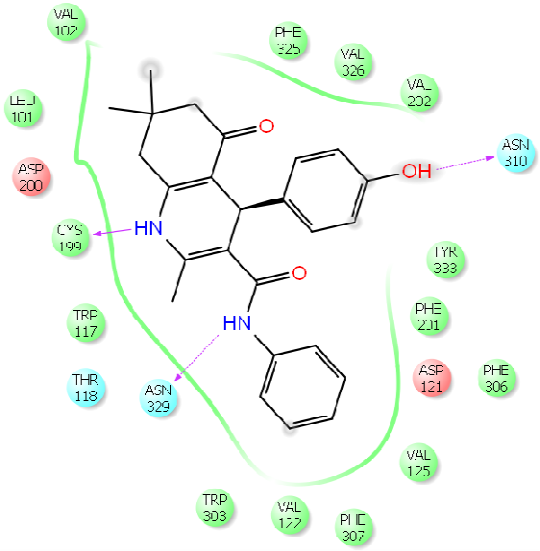


Fig 3: 2D representation of docking interaction of 7g with 2VT4 active site

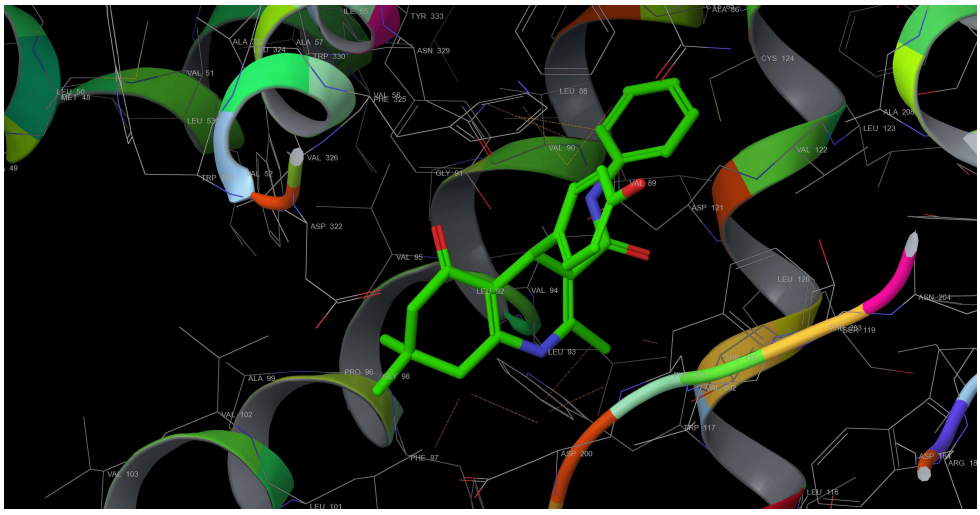


Fig 4: Three dimensional binding mode of 7g in the active site of 2VT4 along with interacting amino acids

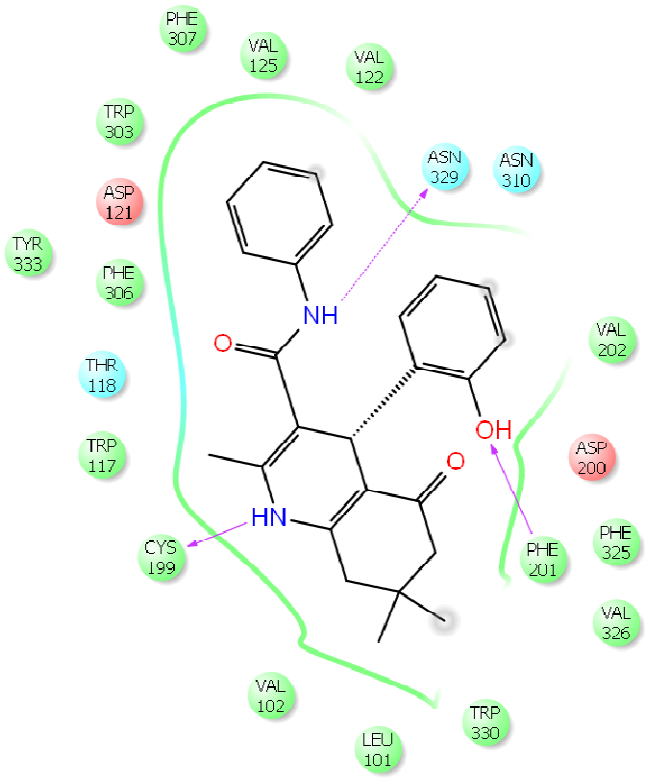


Fig 5: 2D representation of docking interaction of 7f with 2VT4 active site

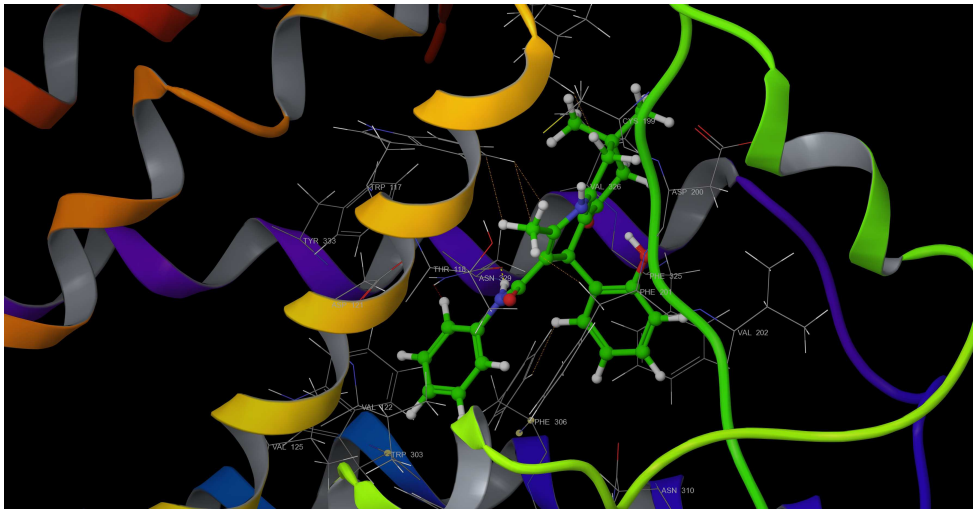


Fig 6: Three dimensional binding mode of 7f in the active site of 2VT4 along with interacting amino acids

CONCLUSION

In OCD, senseless, repetitive rituals serve to counteract the anxiety precipitated by obsessive thoughts e.g. symmetry and exactness preoccupations. Obsessive-Compulsive disorder can impair all areas of brain functioning and produce upsetting effects on patients and their families.

The rodents bury the unpleasant object able to cause aversion stimuli and fearful thoughts. Marble burying behavior of mice is a well established paradigm to screen anticompulsive activity. An acute administration of certain classes

of anxiolytics like selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) has been shown to dose dependently inhibit marble burying in mice.

In the present study molecular docking studies was conducted on serotonin 5-HT_{2A} receptor and synthesized derivatives 7 (a-k). The docking method describes the binding interaction with the ligand and receptor 2VT4. The result shows CYS 199, PHE 201 and ASN329 are important amino acids in the active site to bind with the ligands. Based on docking studies synthesized derivatives are good serotonin 5-HT_{2A} receptor inhibitors.

In conclusion the previously synthesized derivatives 7 (a-k) showed marked anxiolytic activity similar to that of fluoxetine in the marble burying behavior test in mice.

REFERENCES

[1] P.B. Vikas Gupta, Pawan Kumar, Richa Shri, *Asian J. Pharm. Clinical Res.*, **2010,** *3*, 98-100.
[2] C.A.R.d.A. Costa, D.O. Kohn, V.M. de Lima, A.C. Gargano, J.C. Flório, M. Costa, *Journal of Ethnopharmacology*, **2011**, *137*, 828-836.
[3] S.L.B. Vaishali M. Mute, *Int. J. Pharm. Pharmaceutical Sci.*, **2015**, *7*, 407-410.
[4] M.P. Uday Gaikwad, *Acta Poloniae Pharmaceutica. Drug Research*, **2011,** *68*, 435-440.
[5] M.P. Uday Gaikwad, *Asian J. Pharm. Clin. Res*., 2010, *3*, 101-103.
[6] T. Archer, A. Fredriksson, T. Lewander, U.L.F. SÖDerberg, *Scandinavian Journal of Psychology*, **1987**, *28*, 242-249.
[7] M.V.K. R. P. Prajapati, V. P. Karkare, S. K. Parmar, and N. R. Sheth, *Pharmacognosy Research*, **2011**, *3*, 62-66.
[8] M.J. Millan, A. Gobert, F. Lejeune, A. Newman-Tancredi, J.M. Rivet, A. Auclair, J.L. Peglion, *The Journal of Pharmacology and Experimental Therapeutics*, **2001**, *298*, 565-580.
[9] I. Gyertyan, *Behavioural Pharmacology*, **1995**, *6*, 24-31.
[10] L.R. Chioca, M.M. Ferro, I.P. Baretta, S.M. Oliveira, C.R. Silva, J. Ferreira, E.M. Losso, R. Andreatini, *Journal of Ethnopharmacology*, **2013**, *147*, 412-418.
[11] D. Patil, D. Chandam, A. Mulik, S. Jagdale, P. Patil, M. Deshmukh, *Journal of Saudi Chemical Society*, **2014.**
[12] S. Kumar, P. Sharma, K.K. Kapoor, M.S. Hundal,*Tetrahedron*, **2008**, 64, 536-542.
[13] R. Surasani, D. Kalita, A.V.D. Rao, K. Yarbagi, K.B. Chandrasekhar, *Journal of Fluorine Chemistry*, **2012**, *135*, 91-96.
[14] J. Wichmann, G. Adam, S. Röver, M. Hennig, M. Scalone, A.M. Cesura, F.M. Dautzenberg, F. Jenck, *European Journal of Medicinal Chemistry*, **2000**, *35*, 839-851.
[15] R. Kunstmann, G. Fischer, *Journal of Medicinal Chemistry*, **1984**, *27*, 1312-1316.
[16] J. Augstein, A.L. Ham, P.R. Leeming, *Journal of Medicinal Chemistry*, **1972**, *15*, 466-470.
[17] C.E. Cook, M.C. Wani, J.M. Jump, Y.-W. Lee, P.A. Fail, S.A. Anderson, Y.Q. Gu, V. Petrow, *Journal of Medicinal Chemistry*, **1995**, *38*, 753-763.
[18] R. Şimşek, U.B. İsmailoğlu, C. Şafak, İ. Sahin-Erdemli, *Il Farmaco*, **2000**, *55*, 665-668.
[19] M.S. Al-Said, M.M. Ghorab, M.S. Al-Dosari, M.M. Hamed, *European Journal of Medicinal Chemistry*, **2011**, *46*, 201-207.
[20] K. Ahmed, A.K. Jain, B. Dubey, B. Shrivastava, P.S. and, S. Nadeem, *Der Pharma Chemica*, **2015**, *7*, 52-65.
[21] I.P. Baretta, R.A. Felizardo, V.F. Bimbato, M.G.J.d. Santos, C.A.L. Kassuya, A. Gasparotto Junior, C.R. da Silva, S.M. de Oliveira, J. Ferreira, R. Andreatini, *Journal of Ethnopharmacology*, **2012**, *140*, 46-54.
[22] L.F. Pires, L.M. Costa, O.A. Silva, A.A.C. de Almeida, G.S. Cerqueira, D.P. de Sousa, R.M. de Freitas,*Pharmacology Biochemistry and Behavior*, 112 (**2013**) 42-48.
[23] K.S. shiny George, Meena Chandran, Pallavi Gangwar, M. Gururagavan, *Asian Journal of Pharmaceutical and Clinical Research*, 5 (**2012**) 94-96.
[24] A.A.C. de Almeida, R.B.F. de Carvalho, O.A. Silva, D.P. de Sousa, R.M. de Freitas, *Pharmacology Biochemistry and Behavior*, 118 (**2014**) 69-78.