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Molecular Docking and ADME Study for the Identification of Serotonin Transporter Inhibitors from Selected Marine Alkaloids

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ABSRTACT

Depression is among the prevalent mental health conditions affecting individuals globally. People of various ages and ethnicities may be affected. Even with depression meds, only few individuals get the best results from them. The negative effects of antidepressants that are now in use include weight gain, nausea, urine retention, cardiovascular problems, etc. The possibility of natural substances as a therapeutic intervention to eliminate these negative effects is being investigated. Metabolites derived from marine species have a variety of advantageous properties. Compounds with magical abilities to treat mental illnesses are found in a variety of sponges, corals, and seaweeds. The molecular docking of the serotonin transporter (SERT) with a few marine alkaloids is shown in this work. Out of thirteen examined alkaloids, only gelliusine A had a greater binding affinity than the recommended antidepressant paroxetine, according to results obtained by the PyRx virtual screening program. The majority of the chosen alkaloids exhibited improved absorption, distribution, metabolism, and excretion (ADME) characteristics, according to SwissADME. However, gelliusine A does not penetrate the blood-brain barrier (BBB) and has a limited rate of gastrointestinal absorption. In order for these molecules to become more effective antidepressants against serotonin reuptake, further experimental research and optimization are required.

Introduction

Depression affects millions of people globally and is the leading cause of disabilities worldwide. People facing chronic diseases, career failure, financial problems, inferiority complex are more prone to depression. Approximately 3.8% of the population, including 5% of adults (6 and 4% in women and men, respectively) and 5.7% of adults aged over 60 have depression. Around 280 million people globally have depression.[1] Mental healthcare and treatment for depression can vary widely across the world due to limited resources, stigma surrounding mental illness, and insufficient training for healthcare providers. According to community surveys carried by WHO World Mental Health Survey Initiative for 12 months, only 36.8% in high-income countries, 22.0% in upper-middle-income countries and 13.7% in lower-middle-income countries received treatment for depression.[2] The Global Burden of Disease (GBD) Study 2019 found that depressive and anxiety disorders are the two most disabling mental disorders and are ranked among the top 25 leading causes of burden worldwide in 2019. For adolescents it ranked among top 10 causes.[3]There are 9,596 studies in ClinicalTrials.gov database under the field of depression, out of which 324 are in active, not recruiting state, 1418 in recruiting state and 5464 are completed.[4] It is now well known that major depressive disorder (MDD) is highly associated with various chronic physical conditions such as cardiovascular disease, diabetes, cancer, chronic respiratory disease and various chronic pain conditions.[5-9] These conditions are of great personal and public health importance and can be considered representative of the costs of depression.[10] It has been seen that chances of depression rises with the rising age. Studies in older adults also suggest that life incidents, especially financial challenges and death of family members are as important triggers of depression as in young people.[11] Patients' attitude and belief is one of the important factors to influence treatment conformance.[12] Different anti-depressants are used to treat depression like monoamine uptake inhibitors, monoamine oxidase inhibitors, atypical anti-depressants and some other classes. These may inhibit the reuptake of monoamines like noradrenaline, serotonin, serotonin and noradrenaline, noradrenaline and dopamine or by inhibiting monoamine oxidase enzyme. Atypical anti-depressants may act in

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different ways; by increasing uptake of serotonin, by antagonizing the presynaptic alpha2-adrenoreceptor, by antagonizing all serotonin (5-HT) receptors, along with some other different mechanisms.[13] Newer anti-depressants may not be more effective as compared to placebo. Study carried out by Hetrick, S. E., *et al.* suggests that duloxetine, escitalopram, sertraline and fluoxetine could be taken as first-line option for treating depression.[14] Anti-depressants may or may not interact with other receptors but show some adverse effects like reduced salivation, worsening the pre-existing constipation, blurred vision, urinary hesitancy especially in males, drowsiness, weight gain and many more. Some anti-depressants may also have toxic effects like liver damage, hepatic necrosis, hemolytic anemia, arthralgia, fatal systemic vasculitis.[15] 5-hydroxy tryptamine transporter (5-HTT) is the main site of action for various anti-depressants.[16] 5-HTT is an important receptor to study depression and its mechanism. When serotonin is released in synaptic cleft, it binds to different serotonin receptors and activates or represses different signalling cascades. Serotonin transporter reuptakes serotonin and transports it back to presynaptic neuron and is responsible for regulation of released serotonin. Anti-depressants are used to block this reuptake and increases serotonin signalling.[17]

Researchers are focusing on development of antide-pressants which show fewer or no side-effects. It is also necessary to develop drugs which sufficiently act on more than one receptor for same illness. Natural compounds are being evaluated for the same purpose. Most of the plants contain phytochemicals that are effectively showing results against depression and other related mental disorders. Many marine organisms also possess compounds that are used for various diseases. Onchidal is an irreversible inhibitor of acetylcholinesterase (AChE). Manzamine A is anti-inflammatory and has low toxicity. Endogenous peptides in venom of snails are used to treat chronic neuropathic pain.[18] Most of the research carried on marine organisms is related to anti-cancerous, anti-viral or anti-inflammatory compounds. Very little of marine area has been explored regarding anti-depressants. Mollusks, sponges, microorganisms and mostly seaweeds are examined for anti-depressant compounds. Among these promising molecules are alkaloids. Most of the anti-depressant drugs focus on the neurotransmitter systems, i.e. mainly serotonin, noradrenaline, and dopamine. Structurally similar marine alkaloids can be helpful in developing new anti-depressants. Barettin showed interation with serotonin receptors 5-HT2A, 5-HT2C, and 5-HT4 and 8,9-dihydrobarettin solely i nteracted w ith t he 5 -HT2C.[19] 6-bromo-2′-de- N-methylaplysinopsin, 6-bromoaplysinopsin, N-3′- ethylaplysinopsin reported to replace antagonist binding for human serotonin 5-HT2C receptor.[20] Gelliusine A and B possess affinity towards different serotonin receptors.[21] Methylaplysinopsin inhibits monoamine oxidases and removes serotonin form receptor.[22] Phenylethylamine found in marine algae like *Dumontia incrassate, Polysiphonia morrowii, Gelidium crinale* and many more acts as neurotransmitter and neuromodulator and is responsible in rel ieving depression.[23] 5,6-Dibromo-N,N-dimethyltryptamine and aaptamine acted as anti-depressants in forced swim test.[24] 5-bromo-N,N-dimethyltryptamine showed sedative effect by lowering locomotor activity.[25] 5,6-Dibromoabrine and 5,6-dibromotryptamine are also supposed to show anti-depressant activity.[26] Veranamine from *Verongula rigida* demonstrated in-vivo anti-depressant activity and selective affinity for 5HT2B. Hence, can be developed as anti-depressant.[27] As there are multiple targets involved in depression, it is necessary to understand the target-ligand interaction. As well as it is crucial to identify different possible targets for these ligands to develop multi-target anti-depressants. Docking is feasible way to for it. Molecular docking is simple, time-effective, cost-effective tool for generating scores for target and ligand binding.[28] It not only generates binding affinity but also gives structure of target-ligand complex which can be further used for optimizing properties of lead molecule.[29] There are different docking programs out of which GLIDE, AutoDock Vina, GOLD, LeDock are more popular ones. They depend on distinct algorithms for generating score related to binding affinity.[30] It can help researchers to reduce the cost and time required for drug discovery. Unfit or unsuitable molecules can be eliminated earlier. Hence, narrow downs the area when working with large molecular libraries.

Materials and Methods

Retrieval of Receptor

3D structure of Serotonin transporter PDB ID- 6vrh[31] was downloaded from Protein Data Bank (PDB) (https://www. rcsb.org/).[32] Reason for its selection was already bound Paroxetine as a ligand. PDBsum (http://www.ebi.ac.uk/ thornton-srv/databases/pdbsum/) was used to perform PROCHECK analyses to get information about amino acid residues and G-factor.[33]

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Selection and Retrieval of Ligands

Thirteen marine alkaloids were selected as ligands through literature survey. 3D SDF files for ligands along with one anti-depressant Paroxetine were downloaded from PubChem Database (https://pubchem.ncbi.nlm.nih. gov/).[34] PyMOL (https://pymol.org/2/) which is an open source molecular visualization tool was used to convert files into PDB format.[35,36]

Prediction of Binding Pocket

Active Site for SERT was predicted using pocket generation of ProteinPlus server (https://proteins.plus/).[37-39] It is structure-based modeling support server. It is used to predict active binding pockets depending on ligand bound to the protein structure. It can also predict binding site if no ligand is bound. For 6vrh, already bound 8PR (Paroxetine) was selected to find binding pocket.

Molecular Docking

Free version of PyRx (https://pyrx.sourceforge.io/)[40] was used for virtual screening of selected ligands. PyRx uses AutoDock 4 and AutoDock Vina as docking software. PyRx generates binding affinity for single ligand-receptor pair. Out o f w hich f irst o ne w ill b e t he h ighest b inding affinity having root mean square deviation (RMSD) lower bound and upper bound value as 0. Grid box was adjusted according to amino acids present in binding pockets obtained from ProteinPlus.

Ligand-receptor Interaction

For visualizing ligand-receptor interaction Biovia Discovery Studio was used (https://www.3ds.com/ products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/).[41] It is a free visualizer that shows interactions in 2D form. Paid software enables suits like simulation, docking, pharmacophore modelling, quantitative structure-activity relationship (QSAR).

ADME Property Prediction

SwissADME (http://www.swissadme.ch/)[42] was used to predict ADME properties for all selected ligands. It's a free web tool operated by Swiss Institute of Bioinformatics (SIB). Properties like gastrointestinal absorption, blood brain barrier permeability, Lipinski's rule were predicted using SwissADME.

Result and Discussion

Protein Evaluation

6vrh was evaluated for G-factor and amino acid residues using PROCHECK through PDBsum database. Number of residues in disallowed region was 0% and average G-factor was 0.14.

Binding Pocket Prediction

As Paroxet ine (8PR) was already present in the macromolecule, binding pocket was predicted based on it. Fig. 1 shows binding pocket for 6vrh.

Molecular Docking

PyRx was used for molecular docking as there were multiple ligands to evaluate. Among 13 alkaloids only Gelliusine A (-11.4 kca/mol) showed higher binding affinity than Paroxetine (-10.4 kcal/mol). Lowest binding affinity among all ligands was shown by Phenylthylamine (-5.5 kcal/mol). Docking scores for all selected compounds are shown in Table 1.

Ligand-protein Interaction

2D ligand-protein interaction was observed for complex formed by SERT with paroxetine and gelliusine A by using Biovia Discovery Studio visualizer. Paroxetine forms conventional hydrogen bonding with Ser336 and carbon hydrogen bonding with Thr497. Gelliusine A forms no conventional hydrogen bonding but a carbon hydrogen bond with Asp98. Interactions are shown in Fig. 2.

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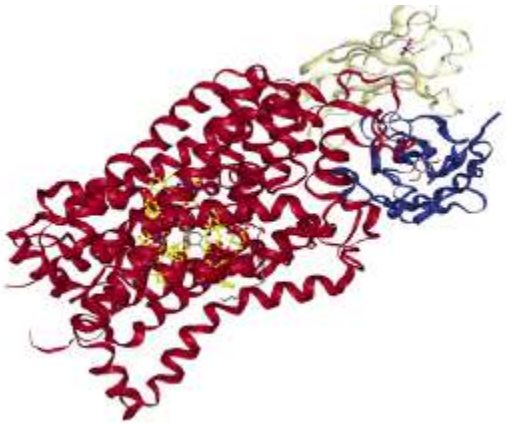
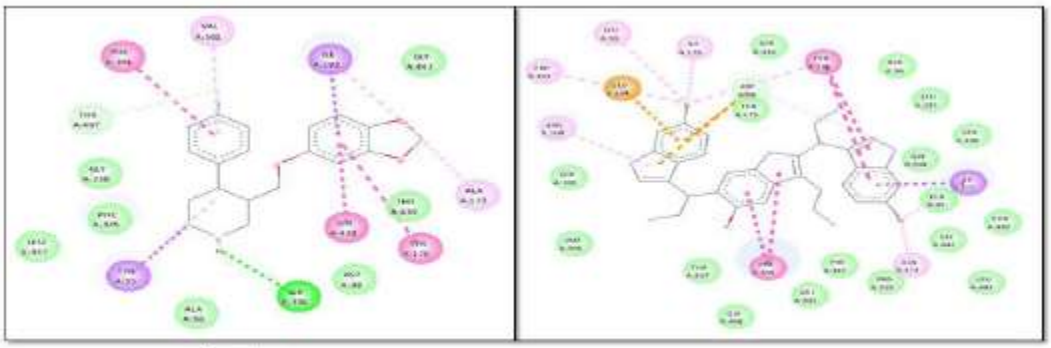


Fig. 1: Active site predicted by ProteinPlus for SERT bound to Paroxetine (6vrh)

Table 1: Binding affinities for paroxetine and selected alkaloids

S. No.	*Ligands*	*Compound ID*	*Predicted Binding Affinity (kcal/mol)*
1	Paroxetine	CID 43815	-10.4
2	5,6-Dibromo-N,N-dimethyltryptamine	CID 360251	-7.3
3	5,6-Dibromoabrine	CID 21776723	-7.5
4	5,6-Dibromotryptamine	CID 309209	-7.1
5	6-bromo-2′-de-N-methylaplysinopsin	CID 135474338	-8.4
6	6-bromoaplysinopsin	CID 135433933	-8.2
7	8,9-dihydrobarettin	CID 12144826	-9.7
8	N-3′-ethylaplysinopsin	CID 135412764	-8.7
9	Aaptamine	CID 122826	-7.2
10	Barettin	CID 11177588	-9.9
11	Gelliusine A	CID 157833	-11.4
12	Methylaplysinopsin	CID 135449090	-8.6
13	Phenylethylamine	CID 1001	-5.5
14	Veranamine	CID 25235287	-8.7



ADME Property Prediction

SwissADME was used to predict pharmacokinetic properties for selected molecules. Table 2 depicts predicted properties. Properties predicted for alkaloids were quite good. All of them except gelliusine A have high GI absorption as well as its topological polar surface area (TPSA) was higher than the required. Excluding 6-bromo-2'-de-N-methylaplysinopsin, 8, 9-dihydrobarettin, barettin and gelliusine A, rest all of the alkaloids are BBB permeant.

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Discussion

Marine alkaloids having structural similarities with serotonin are useful in generating new drug leads for depression.[43] According to previous research these compounds were inhibiting different serotonin receptors as well as other antidepressant targets. There was a possibility that most of them could inhibit serotonin reuptake. In this study, an attempt was made to identify potent leads against SERT from thirteen different alkaloids of marine origin. Paroxetine was selected for pocket generation and to compare docking results as it is most potent among Selective serotonin reuptake inhibitors (SSRIs)[44] and was already bound with target. According to docking results, only Gelliusine A showed more binding affinity than Paroxetine. Predicted pharmacokinetic properties for most alkaloids were satisfactory but some lacked to cross BBB which is an important factor for developing antidepressants. Despite having good affinity Gelliusine A lacked behind to be considered as the best lead as it failed pharmacokinetic properties.

Nevertheless, selected compounds can be checked for binding affinity compared to other anti-depressants for SERT and also for related targets. This computational study may provide a way for designing new anti-depressant and stimulate the process of drug discovery. Results generated from this type of computational analyses must be validated using *in-vitro* and *in-vivo* studies. In drug discovery, it is important to understand the mechanism of action of any compound. This will help to understand the way patients respond to drugs and find better anti-depressants.

Table 2: Properties predicted using swiss adme

S. No.	*Ligands*	*GI absorption*	*BBB*	*Lipinski*	*Leadlikeness*	*TPSA*
1	Paroxetine	High	Yes	Yes	No; 1 violation: XLOGP3>3.5	39.72 Å²
2	5,6-Dibromo-N,N-dimethyltryptamine	High	Yes	Yes	No; 1 no violation: XLOGP3>3.5	19.03 Å²
3	5,6-Dibromoabrine	High	Yes	Yes	No; 1 violation: MW>350	65.12 Å²
4	5,6-Dibromotryptamine	High	Yes	Yes	Yes	41.81 Å²
5	6-bromo-2′-de-N-methylaplysinopsin	High	Yes	Yes	Yes	76.43 Å²
6	6-bromoaplysinopsin	High	Yes	Yes	Yes	66.30 Å²
7	8,9-dihydrobarettin	High	No	Yes	No; 1 violation: MW>350	138.39 Å²
8	N-3′-ethylaplysinopsin	High	Yes	Yes	Yes	54.81 Å²
9	Aaptamine	High	Yes	Yes	No; 1 violation: MW<250	47.14 Å²
10	Barettin	High	No	Yes	No; 1 violation: MW>350	138.39 Å²
11	Gelliusine A	Low	No	No; 2 violations: MW>500, NHorOH>5	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	145.66 Å²
12	Methylaplysinopsin	High	Yes	Yes	Yes	54.81 Å²
13	Phenylethylamine	High	Yes	Yes	No; 1 violation: MW<250	26.02 Å²
14	Veranamine	High	Yes	Yes	No; 1 no violation: XLOGP3>3.5	24.92 Å²

GI: Gastrointestinal absorption, BBB: Blood Brain Barrier, TPSA: Topological Polar Surface Area

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