In Silico Identification and Evaluation of Novel Paroxetine Analogues as Potential Serotonin Transporter (SERT) Inhibitors

A Dissertation

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CANDIDATE'S DECLARATION

I, Ram Avtar Singh, confirm that the work is part of my main project in the thesis titled " In Silico Identification and Evaluation of Novel Paroxetine Analogues as Potential Serotonin Transporter (SERT) Inhibitors " to meet the requirements for the degree of Master of Science in Biotechnology. The work is submitted to the Department of Biotechnology, Delhi Technological University, Delhi. I swear this record of my work, done from January 2025 to June 2025, under the guidance of Prof. Yasha Hasija.

I also state that I have not shared the work in this report for any other degree or with any other school or university.

Place: Delhi

Ram Avtar Singh

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CERTIFICATE BY THE SUPERVISOR

Certified that Ram Avtar Singh (23/MSCBIO/40) did his research work shown in this thesis titled "In Silico Identification and Evaluation of Novel Paroxetine Analogues as Potential Serotonin Transporter (SERT) Inhibitors" for the degree of Master of Science in Biotechnology. The work was done by him and given to the Department of Biotechnology, Delhi Technological University, Delhi, under my watch. This thesis shows original work, and the study was done by the student alone. The contents do not form the base for giving any other degree to him or anyone else from this or any other school or group.

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Ram Avtar Singh

ABSTRACT

Major depression, anxiety, and obsessive-compulsive disorder(OCD) are mental health problems that are linked to the poor work of the serotonin transporter (SERT). SERT is a protein that helps move serotonin (a brain chemical) back into nerve cells. This process is key for controlling how serotonin signals in the brain. When this system does not work well, it can cause unbalanced brain signals and lead to these conditions. Drugs called SSRIs, such as Paroxetine, are often used to block SERT and help fix these problems. However, these drugs can have side effects and may not help everyone. Because of this, new and better treatment options are needed.

In this study, a computer-based method was used to find better SERT blockers that are similar in structure to Paroxetine. Using the SwissSimilarity tool, 327 similar compounds were found. These compounds were checked for how well they might work as drugs-like using SwissADME. This filter compounds Based on Molecular Charecterstics like lipophilicity,size, Hydrogen Bonding and blood-brain barrier. Out of these, 203 compounds with good drug-like traits were chosen for docking tests.

The docking was done with AutoDock Vina on the PyRx platform. The 3D crystal structure of SERT (PDB ID: 5I6X) was prepared by removing water molecules and unnecessary chains. The binding site where Paroxetine fits was marked. The Docking results showed five similar compounds that had better binding strength than Paroxetine. showing stable interactions with Key amino acid residues such as TYR95, ASP98, ILE172, SER438, TYR176, and.

Further ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling using pkCSM identified three compounds (PubChem IDs: 11697676, 4470577, and 11100995) as non-mutagenic and pharmacokinetically favorable. These candidates demonstrated high gastrointestinal absorption, acceptable solubility, and compliance with Lipinski's Rule of Five.

Overall, the study presents promising Paroxetine-like inhibitors with improved binding to SERT and better safety profiles. These findings support their potential development as new antidepressant agents, though additional laboratory validation in biological models is necessary to confirm their therapeutic effectiveness.

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LIST OF ABBREVIATION

MDD	Major Depressive Disorder
SERT	Serotonin Transporter
SSRI	Selective Serotonin Reuptake Inhibitor
5-HT	5-hydroxytryptamine(Serotonin)
ADMET	Adsorption,Distribution,Metabolism,Excretion, and Toxicity
CNS	Central Nervous System
BBB	Blood-Brain Barrier
GIA	Gastrointestinal Absorption
PDB	Protein Data Bank
SMILES	Simplified Molecular Input Line Entry System
CSV	Comma-Separated Values
SDF	Structure Data File
PDBQT	Protein Data Bank, Partial Charges & Torsions format
PyRx	Python Prescription Docking Tool
pkCSM	Pharmacokinetics and Chemical Structure Modeling

Vina	AutoDock Vina (Molecular Docking Software)
Lipinski's Rule	Lipinski's Rule of Five (Drug-likeness criteria)
SwissADME	Swiss Absorption, Distribution, Metabolism, and Excretion predictor
SwissSimilarity	Swiss ligand-based virtual screening tool
2D/3D	Two-Dimensional / Three-Dimensional
H-bond	Hydrogen Bond
ESOL	Estimated Solubility
TPSA	Topological Polar Surface Area
OCD	Obsessive-Compulsive Disorder
SMILES	Simplified Molecular Input Line Entry System
CSV	Comma-Separated Values
SDF	Structure Data File
PDBQT	Protein Data Bank, Partial Charges & Torsions format
PyRx	Python Prescription Docking Tool
pkCSM	Pharmacokinetics and Chemical Structure Modeling
Vina	AutoDock Vina (Molecular Docking Software)
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SwissSimilarity	Swiss ligand-based virtual screening tool
2D/3D	Two-Dimensional / Three-Dimensional
H-bond	Hydrogen Bond
ESOL	Estimated Solubility
TPSA	Topological Polar Surface Area

INTRODUCTION

Major depressive disorder (MDD) is a significant mental health issue with more than 280 million patients globally experiencing life-long sadness, lack of energy, sleep disturbance, and suicidal ideation [1]. MDD is recognized by WHO as a major global neuropsychiatric public health problem, as well as a condition that acutely affects the risk of premature mortality and the burden of disability [2]. The dysregulation of key neurotransmitters, including serotonin, dopamine, and norepinephrine, in particular, is a fundamental neurobiological constituent of MDD [3].

One of these is serotonin, which is critical for regulating mood. The reuptake is mediated through SERT, a member of the family of solute carriers 6 (SLC6)[4]. For the purpose of terminating the serotonergic signal and maintaining neurotransmitter homeostasis, SERT assists to reuptake serotonin (5-HT) from the synaptic cleft into axonal varicosities [5]. The SERT activity dysfunction is indeed an excellent pharmacological target as it is associated to anxiety, neurodegenerative and MDD diseases [6].

To further extend serotonin half-life in the synaptic cleft, reuptake 28 of the released serotonin into the presynaptic neuron is inhibited by first-line drugs like selective serotonin reuptake inhibitors (SSRIs), e.g., fluoxetine, sertraline, and paroxetine by their pharmacological interference with SERT activity [7]. But their clinical applications often are restricted by side effects of nausea, sexual dysfunction, and sleep disorder [8], and the overly simplified theory of serotonin deficiency in depression is still controversial . These drawbacks underline the requirement of new therapeutic compounds with better pharmacological characteristics and less toxicity. Computational drug discovery has revolutionized early stage of drug development with the help of in silico estimation of pharmacokinetic property and high throughput virtual screening in recent years [4]. By being this approach, the lead compounds are being quickly identified with low toxicity profile, good ADME rules, and high bondage affinity [5].

In order to find and assess new SSRI paroxetine analogues, the current study makes use of this computational method. Structurally similar molecules were chosen using SwissADME for pharmacokinetic profiling and SwissSimilarity for ligand-based virtual screening . Molecular docking studies targeting the active binding site of SERT (PDB ID: 5I6X) were conducted using AutoDock Vina in PyRx to further assess these . The best candidates were then examined for their mutagenic potential, binding interactions, and ADME characteristics in order to find potent SERT inhibitors that might be used as antidepressants.

This study advances our knowledge of structure-activity relationships (SAR), which are essential for the inhibition of the serotonin transporter (SERT), in addition to introducing new paroxetine analogues with advantageous pharmacokinetic and drug-likeness profiles. In order to find derivatives with a higher binding affinity, better gastrointestinal absorption, and less mutagenic potential, the study refines a clinically validated SSRI scaffold using virtual screening, molecular docking, and ADME-based filtering. The results of this study demonstrate the efficiency of in silico approaches in speeding up the development of antidepressant medications and help to develop safer and more effective therapeutic candidates for Major Depressive Disorder (MDD).

CHAPTER 2

REVIEW OF LITERATURE

2.1 Major Depressive Disorder

Major Depressive Disorder (MDD) is a persistent and life-interfering mood disorder that affects emotional and psychological states. MDD is known to be one of the most common mental health issues around the world and is one of the top 5 causes of disease burden globally by 2030[1] . The prevalence of MDD affects around 280 million people worldwide and is more likely to be seen in women and older adults [2]. Depressive symptoms are well recognized and can include: constant sadness, anhedonia, tiredness, difficulty concentrating, poor sleep, hopelessness, and suicidal thoughts [9]. These symptoms lead to marked impairment in personal, social, and work functioning.

The pathophysiology of the disorder is based on altered neurotransmission primarily of serotonin (5-HT), norepinephrine (NE) and dopamine (DA). Of these three biogenic amines, serotonin is the most contributory molecule for regulating the processes of mood, cognition and emotional functions. The serotonin transporter (SERT), which is encoded by the SLC6A4 gene, is responsible for the reuptake of serotonin from the synaptic cleft. Any impairment of an SERT can change serotonergic signaling leading to depressive behaviours [10]. Most pharmacological treatment options attempt to return the monoamine levels with similar agents known as selective serotonin reuptake inhibitors (SSRIs) including fluoxetine and paroxetine. While SSRIs are among the most commonly used clinical treatments for depression and behavioural disturbances, no SSRI or clinically released antidepressant (Zyban and Wellbutrin are marketed as antidepressants; however they function differently) has been able to bypass time progressing before therapeutic effects are realized, and may also possess side effects of nausea, sexual dysfunction and emotional blunting [11]. This does indicate the need for novel antidepressant interventions that are tolerated with a shorter onset of therapeutic effect.

2.2 Molecular and Genetic Pathogenesis of Major Depressive Disorder

Major Depressive Disorder (MDD) is a complex illness caused by the interplay of neurochemical, genetic, inflammatory and environmental factors. The core neurobiological feature of MDD is dysregulation of monoaminergic transmission, especially serotonin, dopamine and norepinephrine. But evidence is mounting that the molecular pathology of MDD goes beyond neurotransmitter deficiency [12]. One of the key mechanisms involved in the disorder is mitochondrial dysfunction. Mitochondria, the powerhouses of the cell and responsible for ATP generation and energy metabolism, are structurally and functionally abnormal in depression. Reduced mitochondrial respiration, impaired oxidative phosphorylation and increased production of reactive oxygen species (ROS) have been found in the brains and peripheral cells of MDD patients [13][14]. The excess ROS leads to oxidative stress which damages cellular macromolecules—DNA, lipids and proteins—making neurons and synapses vulnerable [15].

Another big one is neuroinflammation. Pro-inflammatory cytokines like IL-6, IL-1β and TNF-α are elevated in the cerebrospinal fluid and serum of depressed people. These cytokines can alter neurotransmission, reduce neurogenesis and impair glial function which all contribute to depression pathology [16]. Neuroinflammation also exacerbates oxidative stress creating a feedback loop that promotes neuronal degeneration. Meanwhile the autophagy-lysosomal pathway and ubiquitin-proteasome system are compromised in MDD leading to accumulation of dysfunctional proteins and organelles in neurons [17]. Disruption of these cellular clearance systems impairs neuroplasticity and cognitive function which are often affected in depressive episodes.

From a genetic perspective many polymorphisms are linked to MDD. SLC6A4 (serotonin transporter gene), BDNF (brain derived neurotrophic factor) and FKBP5 (glucocorticoid receptor regulator) have a link to stress response, neuroplasticity and how well antidepressants work [18]. The short allele of the 5-HTTLPR polymorphism in SLC6A4 has a correlation with lower SERT expression and higher risk of depression when exposed to environmental stress [19]. Another hallmark of MDD is the decrease of BDNF in the hippocampus which is involved in synaptic shrinkage and reduced neurogenesis [20].

As a result, oxidative stress, inflammation, impaired protein degradation, and genetic susceptibility all coexist in the pathophysiology of MDD. These results highlight the necessity of multi-targeted treatment strategies that target mitochondrial repair, inflammation management, neurotrophic support, and neurotransmitter balance.

Table 1: summarizes the key molecular pathways disrupted in Depression and highlights major proteins and genes implicated in Major Depressive Disorder.

Pathway	Role	Effect in Depression	Key Molecules involved	Ref.
Monoaminergic signalling	Mood, motivation sleep	Decreased neurotransmitter	Serotonin(5HT),NE,DA,SERT, MAO-A	[21]
HPA Axis Regulation	Stress response, Cortisol regulation	Hyperactivity Cortisol increase	CRH, ACTH, cortisol, GR	[22]
Neurotrophic Support	Neuronal survival And plasticity	↓neurogenesis& synaptic plasticity	BDNF,Trkb	[23]
Glutamanergic Signalling	Excitatory transmission	NMDA receptor dysregultion	NMDA-R,AMPA-R, Glutamate, mGluRs	[24]
GABAergic inhibition	Inhibitory transmission	Reduce GABA-tone, anxiety	GABA-A,GABA-B receptor	[25]
Inflammatory Pathway	Immune balance	Pro-inflammatory Cytokine increase	IL-6,TNF-alpha,iL-1beta, microglia	[26]

Mitochondrial function	Energy production, ROS regulation	Mytochondrial dysfuntion,ROS↑	ATP synthase,COX,SOD	[27]
Circadian Rhythem Rgulation	Sleep wake cycle mood	Dysrupted sleep & Mood cycle	CLOCK,BMAL1,PER,CRY gene	[28]
Dopaminergic Reward Pathway	Motivation, reward,pleasure	Anhedonia and Reduce dopamine	DA,D1/D2 receptor,VTA,NAC	[29]

2.3 Serotonin Transporter (SERT): Structure and Function

5-HTT Gene 5-HTT, encoded by the SLC6A4 gene, is the main regulator of serotonergic neurotransmission, which by the reuptake of serotonin (5-HT) from the synaptic cleft into presynaptic neuron. This reuptake is necessary for the cessation of serotonin neurotransmission and synaptic homeostasis. SERT is a pharmacological target for selective serotonin reuptake inhibitors (SSRIs) including paroxetine, fluoxetine, and sertraline, which act by inhibiting the clearance of serotonin [30].

Structurally, SERTbelongs to the solute carrier 6 (SLC6) family and is formed of 12 transmembrane domains, while the N- and C-termini are situated in the cytoplasm. The pocket containing serotonin and inhibitor molecules (Asp98, Tyr95 and Ser438) is also the central binding pocket. Such stru ctural information has been revealed by X-ray crystallography studies of the human SERT protein [31].

Genetic polymorphism in SLC6A4 also serves to modulate SERT activity. The most frequently investigated polymorphism is the serotonin-transporter linked polymorphic-region (5-HTTLPR) in the promoter. The short (S) allele of 5-HTTLPR is characterized of lower transcriptional activity, facing gene expression and greater risks of MDD[32].

The structural and genetic role of serotonin transporter make it suitable molecular target for making new antidepressent with higher efficacy and lower side effet compared to previous SSRIs.

2.4 Pharmacological Targeting of SERT: SSRIs and Mechanism of Action

Selective serotonin reuptake inhibitors (SSRIs) make up most of the drugs that we give for depression, and they are used mostly because they seem to work and they don’t cause many terrible side effects. The main key to how SSRIs work is the way they change the way the brain uses a thing called the serotonin transporter (SERT), accces mechanism and recycling between outward facing,occluded, and inward facing confermations to transport serotonin across membrane powered by theThis transporter work via alternating and operate by sodium & chloride gredient which is the thing that takes in serotonin (5-HT) back into the part of the brain that caused a nerve impulse to happen, and so it stops the nerve from doing its work [33].

Drugs such as paroxetine, fluoxetine, and sertraline stick to the part of SERT that is in the middle of the cell. They lie on top of the part that serotonin would normally stick to in order to work this way. When these drugs stick to SERT, they keep it in a Fixing out face interaction. This stops serotonin from doing its job [34]. When there is more serotonin in the space between nerve cells, and it works like it is supposed to, the depressed, anxious, or worried person is less likely to feel like that anymore.

Despite their work, SSRIs have many faults. They take time to work, may only partly help some people, and may make some side effects. Such as, sex trouble, wakefulness, and upset stomach. The time delay is due to the and times needed for the changes in nerve, including the becoming less sensitive of how they react and the growth of new nerve cells in the brain's hippocampus [35].

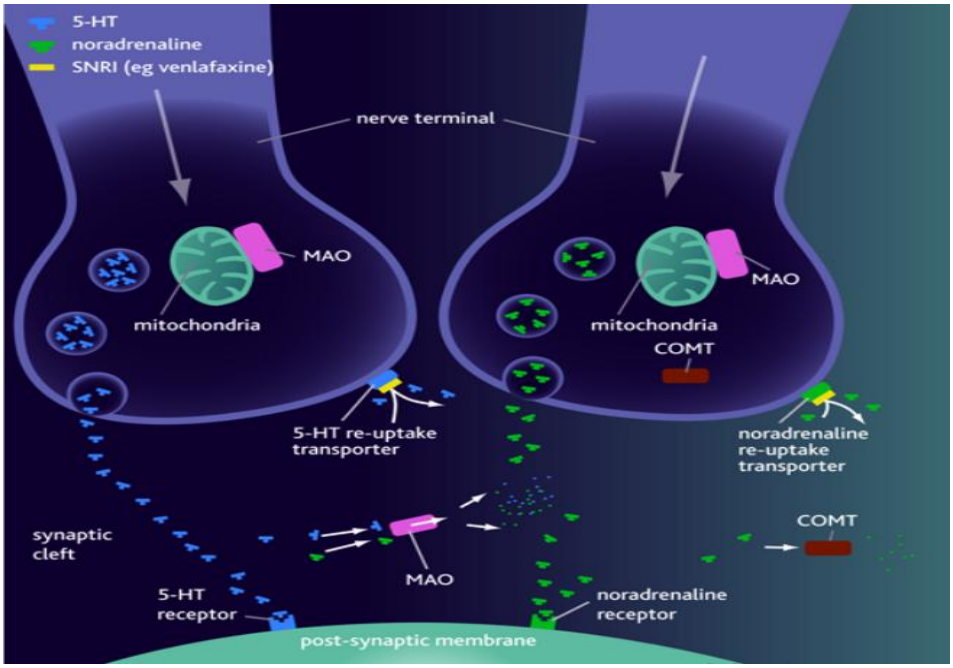


Figure 1. Target of antidepressant action (Mechanism) on noradrenergic and serotonergic neuron

2.5 Existing Serotonin transporter inhibitors/Blocker

The most widely prescribed class of antidepressants for the treatment of major depressive disorder/depression are currently available SERT Inhibitors (SSRIs). By specifically blocking the serotonin transporter (SERT), that involve in serotonin reuptake these drugs increase the quantity of serotonin found in the synaptic cleft. examples of some common SSRIs are Fluvoxamine, vilazodone, paroxetine, citalopram, escitalopram, fluoxetine, sertraline, and vortioxetine . Notwithstanding their effectiveness, they frequently have drawbacks such side effects, treatment resistance, and a delayed commencement of action. To address these problems, new agents with improved pharmacokinetic and pharmacodynamic characteristics are being investigated [36] .

Table 2. Comparative Table of Major Selective serotonin inhibitors(SSRIs)

Drug	**metabolism**	**mechanism**	**Half-life**	**feature**	**References**
Fluoxetine	CYP2D6	Blocks serotonin reuptake	4-6 days	Long half-life,can accumulates	[37]
Paroxetine	CYP2D6(I	Selective SERT inhibitors	~24 hrs	Strong CY P2D6 Inhibitors	[38]
sertraline	CYP2B6,CYP2C19	Selective SERT inhibitors	24-32 hrs	Used in PSTD & anxiety	[39]
Escitalopram	CYP2C19,CYP3A4	Highly Selective SERT inhibitors	27-33 hrs	Risk of QT prolongation In high dose	[40]
citalopram	CYP2C19,CYP3A4	Selective SERT inhibitors	~35 hrs	Better tolerated then citalopram	[41]
fluvoxamine	CYP1A2,CYP2D6	Selective SERT inhibitors	12-15 hrs	Sedating,strong CYP1A2 inhibitors	[42]
vilazodone	CYP3A4	Partial agonist at5-HT1A receptors	~25 hrs	Faster onset,lower sexual dysfunction	[43]
vortioxetine	CYP2D6	Modulation of multiple serotonin receptors	~66 hrs	Improves cognitive function in MDD	[44]
depoxetine	CYP2A4,CYP2D6	Selective SERT inhibitors	1.3-1.5 hrs	Used in premature ejeculation	[45]

2.6 Limitation of selective serotonin reuptake inhibitors

1.Delayed onset of action

This may pose a challenge to patients in acute state, as the beneficial effects of SSRIs usually take 2 – 6 weeks to come up. Experiencing lack of efficacy on day four is of high concern, as premature drop-out and non-compliance are fostered. It also decreases their worth in urgent clinical situation[46].

2.Treatment Rasistence

30 to 40% of patients do not respond to SSRI's, even after trying several different drugs. Frequently this practice results in a polypharmacy, switch, or escalation of therapy. This opposition points to A depression all the time and a Bad Quality of life[47].

3.Side Effect

"SSRIs are notorious for causing weight gain, gastrointestinal complaints, insomnia, and sexual dysfunction." These side effects could have substantial impact on patientThe adherence and discontinuation. Chronic users may also have disordered metabolism[48].

4.Discontinuation Syndrome

Withdrawal symptoms from SSRIs and in particular, short half-life SSRIs might include dizziness, anxiety, and flu-like symptoms upon abrupt discontinuation. This withdrawal syndrome is vigorous and should be slowly tapered. It complicates the treatment of changes in therapy[49].

5. Low Effectiveness in severe depression

Meta-analyses suggest that SSRIs are less effective in patients with more severe symptoms at baseline (including both very severe and melancholic patients). In these cases the degree of separation of SSRI over and above placebo is minimal. This questions their status as first-line agents across all forms of depression[50].

CHAPTER-3

METHODLOGY

3.1 Selection of Protein SERT & ligand molecules

Information on the target protein and reference molecule is obtained by reviewing the literature. Initially, the literature was collected from webserver like PubMed (https://pubmed.ncbi.nlm.nih.gov/).

The membrane bound protein known as serotonin transporter(SERT) was selected as the therapeutic target based on its central role in serotonin reuptake. Swisssimilarity search (http://www.swisssimilarity.ch/)

is used to collect all the compounds that are similar to paroxetine from the DRUG Bank (https://go.drugbank.com/).

The Drug database is mainly used for virtual screening. The database yielded a list of 327 chemicals, which were then further filtered using several criteria like medication bioavailability, BBB permeability, and the Lipinski rule of five. After applying the filter, we obtained 203 molecules. There were notable structural similarities between these compounds and Paroxetine.

3.2 Retrieval of target protein and ligand

The 3D Structure of the SERT crystal was taken from the Protein Data Bank (PDB ID: 5i6X) in .pdb format. The Structure was checked and visualized for full details using BIOVIA Discovery Studio Visualizer. The 3D Structure of the small molecules were downloaded from PubChem as SDF files (https://pubchem.ncbi.nlm.nih.gov/).

3.3 Protein & Ligand preparations

Protein is prepared using BIOVIA Discovery Studio , which removes water,heteroatom and adds Kollman charges. Only hydrogens are then added to the polar. The .pdbqt format is used to store the protein structure.

All ligand molecules in .sdf format were converted into.pdb format using Open Babel in order to perform docking within PyRx. open Bebel in PyRx adds polar hydrogen, computes gasteier charges, combines non-polar hydrogen,Minimize all . The receptor and ligand grid map has been created with the following dimensions, x = 25; y = 25; z = 25)and the center :(x = -32.60; y = 21.38; z = 1.8)

3.4 Active Site Prediction:

It is important to identify protein's active site for understanding molecular interactions, as the active site plays a important role in the protein's function. correct prediction of these binding

sites allows for efficient drug design by guiding the selection of potential ligands that can interact with the protein's active site. There are multiple computational techniques and tools available to predict these binding sites.

The BIOVIA Discovery Studio was used to predict the probable active site residues of the SERT protein. The PDB structure was uploaded, and the top-ranked binding pocket based on ligandability score was selected for molecular docking. Predicted residues were noted and matched with the ligand binding residues post-docking to validate specific interactions.

3.5 Molecular Docking

Molecular docking is a computer technique that predicts how well ligands will attach to receptor proteins. Docking is done with PyRx. It is a molecular docking tool that is openly accessible. Compared to other docking systems, it was thought to be Easy to use and open source molecular Docking Tool .it has graphical user interface for AutoDock Vina and AutoDock. It not requiring command-line usage.It set Grid box for docking quickly and provides docking results. Docking parameters are configured via graphical user interface. target protein is loaded and converted into macromolecule. Subsequently, ligand is also loaded and their energy is minimized and converted into .pdbqt in open babel of pyrx software. Then for specific docking area of the grid represented on the software in the image is set according to specif centre XYZ. Then proceeded for docking.

Once Docking is done. a log file is generated showing the docking results and binding Affinity . An out.pdbqt file containing the binding mode/pose. When the docking process is complete, a list of the best binding molecules is generated based on the binding affinity, and the best molecule is recommended for further study. The reference molecule, paroxetine, has a binding affinity of 10.6 kcal/mol.

3.6 Protein-ligand Interactions analysis

After the docking process was completed, all of the target-ligand interaction structures were captured in the out Pdb file . To evaluate every encounter, BIOVIA Discovery Studio (version v25.1.0.24284) was utilized.

3.7 Pharmacokinetic and Toxicity Prediction (ADME/PkCSM)

SwissADME (http://www.swissadme.ch/), an open-access online software, was used for analyzing pharmacokinetic and Drug likeliness properties of compounds. ADME analysis is used for each of the 203 drug compounds that were selected from 327 drug candidate. The main criteria used in the evaluation was water solubility, lipophilicity, high GI absorption, blood-brain barrier permeability, violations of Lipinski's Rule, and bioavailability.

Toxicity profiling was conducted using PkCSM, focusing on hepatotoxicity, carcinogenicity, and LD_{50} class prediction to assess safety and tolerability.

3.8 Protein–Protein Interaction (PPI) Network Analysis

To understand the broader biological context of PINK1, STRING database (v12.0) was used to generate a protein–protein interaction network. The query was set as “SERT” (Homo sapiens) with a high-confidence score threshold (≥0.9). A maximum of 10 interactors was selected to visualize the core network. Evidence sources included experimental data, curated databases, and co-expression.

CHAPTER 4

Result

4.1 Molecular docking Result

When the 201 compounds tested against the SERT protein were moved with each other in the blind docking, the binding power test of the molecular docking showed a wide spread. Paroxetine, which is the reference chemical, had a binding energy of 10.5 kcal/mol. In the data, some drugs that can cross the BBB or ones will cross over did not bind too well with the part where the chemical dopamin interacts with the target protein. Those with high expected binding powers were found with binding energies as low as –11.5 kcal/mol. These changes in the binding scores show that the ones we picked bind better to the binder than the medicine we used as a base.

The structural analogues of Paroxetine that were examined were chosen for their drug-likeness and capacity to alter Serotonin transport associated with SERT activity. To ensure the docking site's biological relevance, all ligands were docked into the top-ranked active site pocket determined via Biovia Discovery Studio.

Table 3 summarizes the docking data, which include the chemical Name , Docking score, and PubChem CID for each ligand. Interestingly, a number of potential compounds showed binding energies that were better than Paroxetine, indicating improved interaction with the SERT binding pocket. Stronger thermodynamic stability inside the SERT active site was shown by the DrugBank-screened compounds, such as CID 11697676,CID 448642 and CID 444031, which showed binding energies of –11.5 kcal/mol, –11.4 kcal/mol and –11.4 kcal/mol, respectively. In order to develop prospective treatment leads, our results provide a solid foundation for further examination of pharmacokinetic characteristics, toxicity, and molecular interaction profiles.

Table 3. I: Top five compounds along with pub chem IDs, binding energy, interacting amino acid residues and other possible interactions. (N=number of hydrogen bonds)

Compound Name	PUBCHEM ID	BINDING ENERGY (KJ/MOL)	N	Intersecting residue
7-[4-(4-naphthalen-1-ylpiperazin-1-yl)butoxy]-3,4-dihydro-1*H*-1,8-naphthyridin-2-one/Pf-00217830	11697676	11.5	1	ARG104,ALA331, TYR176, PHE335, ILE172, SER438

3-(1H-indol-3-yl)-4-{1-[2-(1-methylpyrrolidin-2-yl)ethyl]-1H-indol-3-yl}-1H-pyrrole-2,5-dione	448642	11.4	1	TYR176,TYR95,ILE172,ASP98, GLU493,THR497,PHE335,ALA173
Darifenacin	444031	11.4	2	TYR176, ILE172, VAL501, PHE341, PHE335, ALA331, ARG104
1,3,4,9-Tetrahydro-2-(Hydroxybenzoyl)-9-[(4Hydroxyphenyl)Methyl]-6-Methoxy-2h-Pyrido[3,4-B]Indole	4470577	11.0	2	GLU493, ARG104, ASP98, PHE335, TYR95, TRP103
1'-[2-(2,4-difluorophenyl)ethyl]-4-oxospiro[3H-chromene-2,4'-piperidine]-6-carbonitrile	11100995	11.0	1	TYR176, SER438, ARG104, GLU493, PHE335, ASP98, and ILE172

4.1.1 Top-Ranked Compounds Based on Binding Affinity

Out of a total of 201 compounds docked against the SERT protein, the top 5 molecules exhibiting the most favorable binding affinities were shortlisted based on their docking scores. Out of these compounds PubChem CID: 11697676 is selected for further analysis due to their significantly lower binding energies -11.5 kcal/mol. compared to the reference drug, Paroxetine(PubChem CID: 43815), which recorded a binding energy of –10.5 kcal/mol.

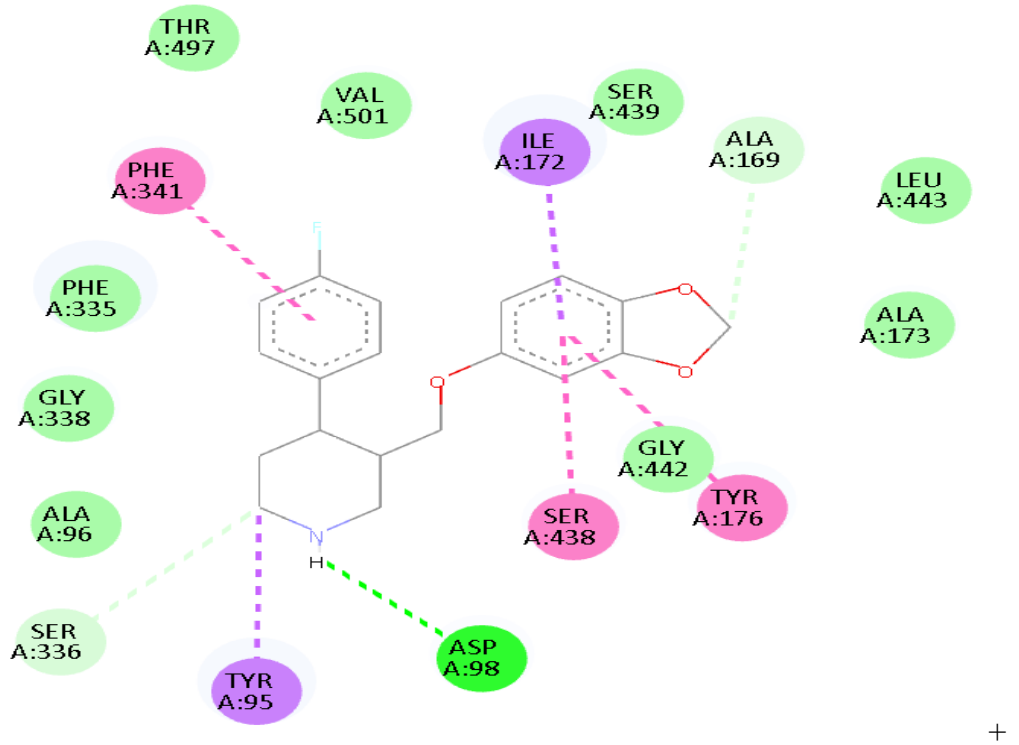


Fig.2. 2D representation of the binding interaction between PubChem CID: 43815 and SERT. Binding interactions includes Van der Waals forces, pi-alkyl, alkyl, hydrogen bonds, and pi-anion-like interactions

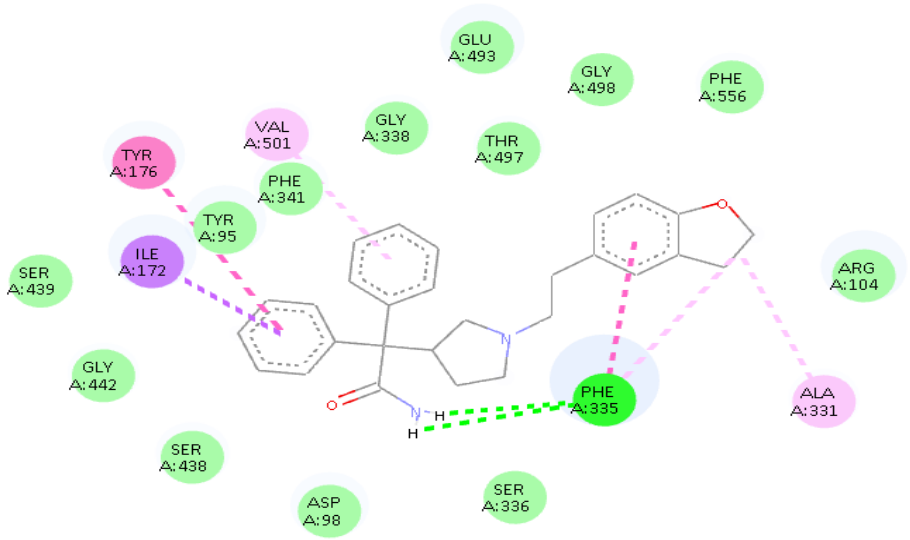


Fig.3. 2D representation of the binding interaction between PubChem CID: 444031 and SERT. Binding interactions includes Van der Waals forces, pi-alkyl, alkyl, hydrogen bonds, and pi-anion-like interactions

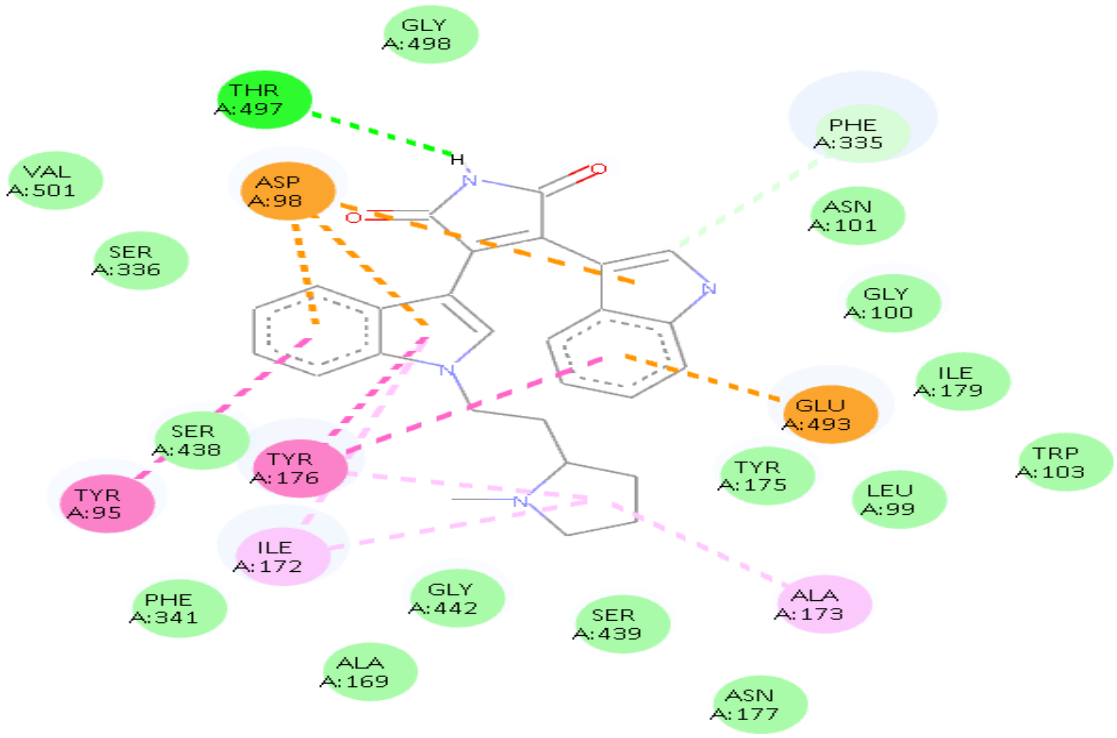


Fig.4. 2D representation of the binding interaction between PubChem CID: 448642 and SERT. Binding interactions includes Van der Waals forces, pi-alkyl, alkyl, hydrogen bonds, and pi-anion-like interactions

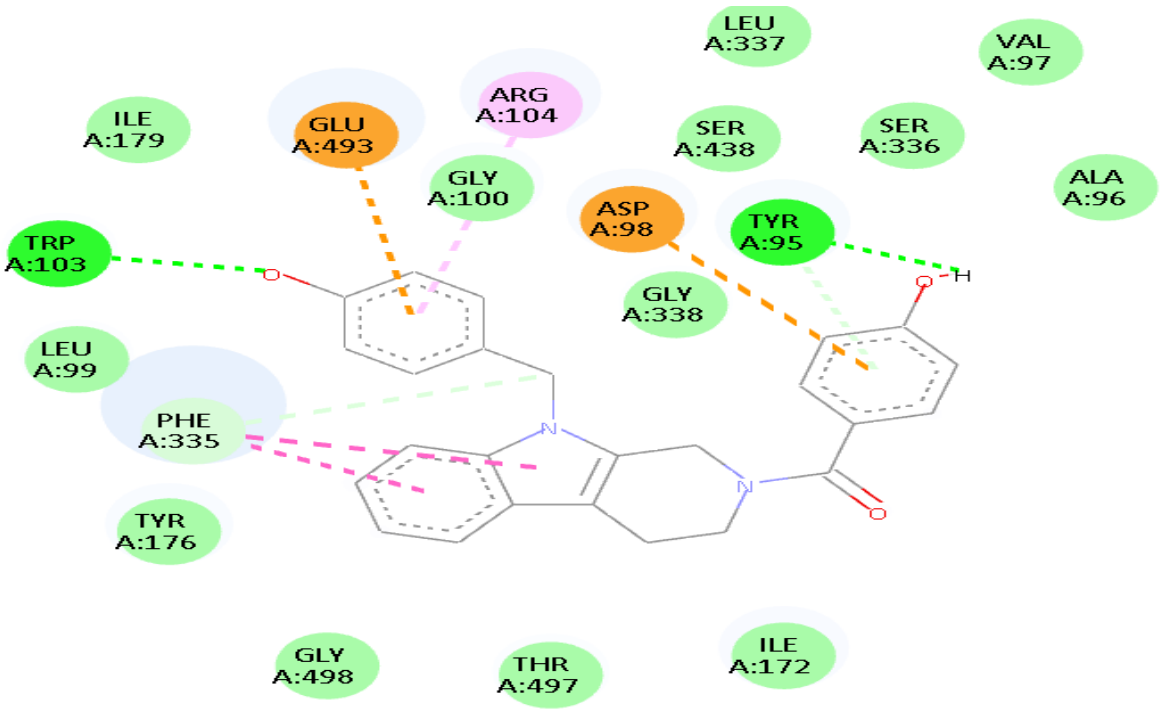


Fig.5. 2D representation of the binding interaction between PubChem CID: 4470577 and SERT. Binding interactions includes Van der Waals forces, pi-alkyl, alkyl, hydrogen bonds, and pi-anion-like interactions

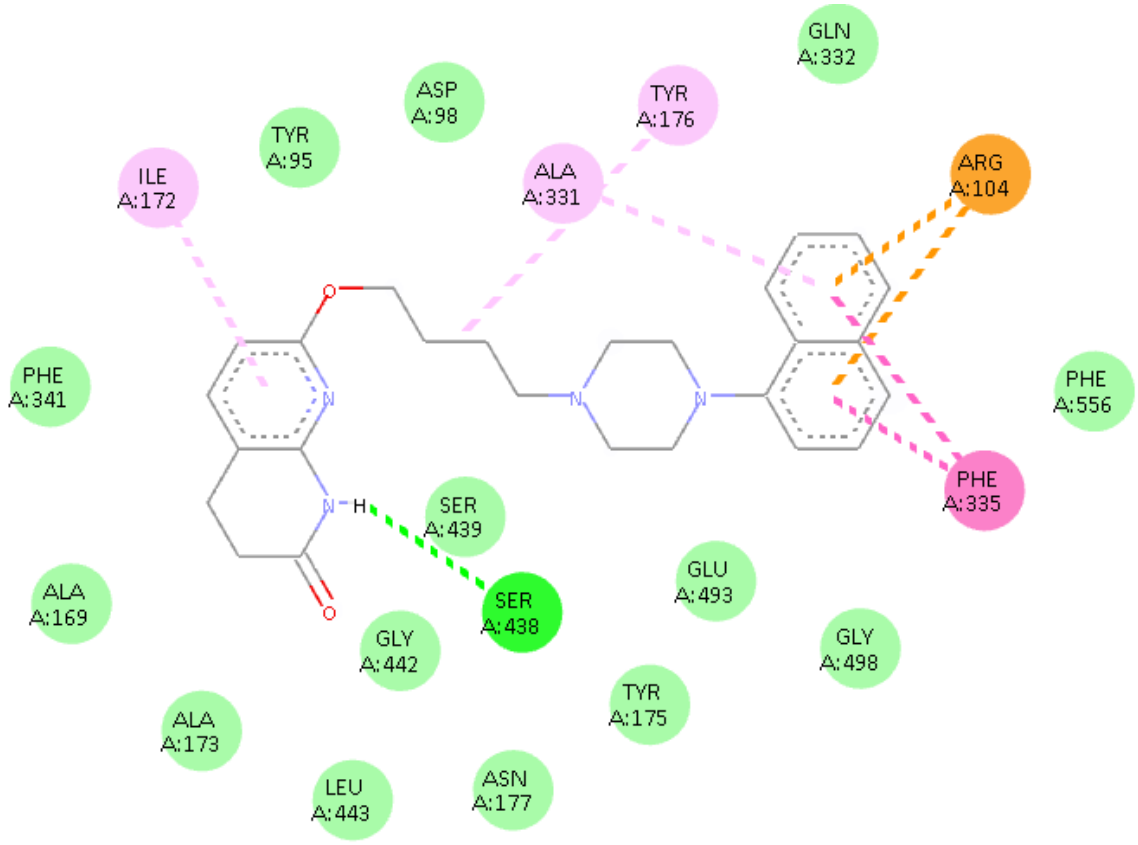


Fig.6. 2D representation of the binding interaction between PubChem CID: 11697676 and SERT. Binding interactions includes Van der Waals forces, pi-alkyl, alkyl, hydrogen bonds, and pi-anion-like interactions

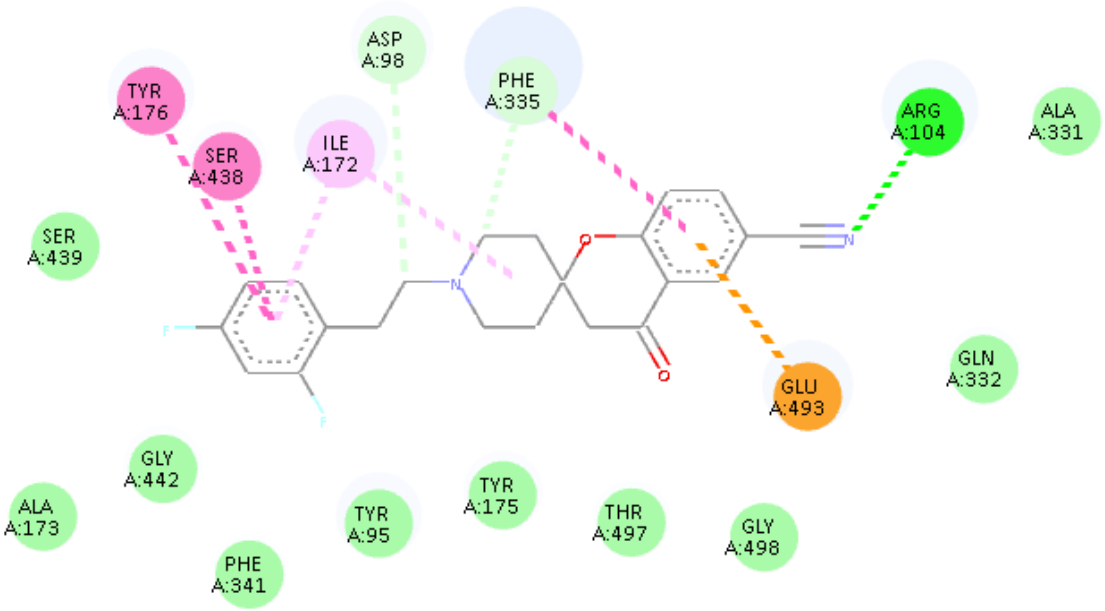
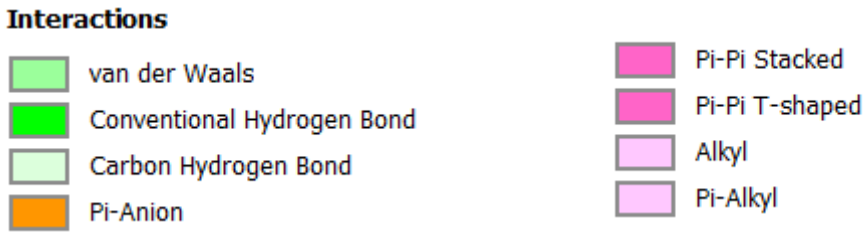


Fig.7. 2D representation of the binding interaction between PubChem CID: 1100995 and SERT. Binding interactions includes Van der Waals forces, pi-alkyl, alkyl, hydrogen bonds, and pi-anion-like interactions



(All the above figures represent 2D and 3D interaction of SERT with 5 compounds)

4.2. ADMET properties analysis:

ADME analysis of leading compounds of FDA-approved drugs shows significant results shown in the table below. All properties of ADME such as pharmacokinetics, drug likeliness, water solubility, and other properties mentioned**.**

Table 4: ADMET properties of pharmacokinetics and Drug likeliness of top affinity FDA-approved Drug using SWISS ADME and pkcsm

PUB CHEM IDs of compounds	ESOL (logS)	GIA	BBB permeant	Bioavailbility	Ames Test (+)
11697676	-5.08	HIGH	YES	0.55	NO
448642	-4.94	HIGH	YES	0.55	YES
444031	-5.36	HIGH	YES	0.55	YES
4470577	-5.03	HIGH	YES	0.55	NO
11100995	-4.64	HIGH	YES	0.55	NO
43815	-4.20	HIGH	YES	0.55	YES

4.3 Protein-Protein Interaction (PPI) Analysis

The STRING database (v12.0) was used to build a protein–protein interaction (PPI) network with a confidence score threshold of >.900 in order to evaluate the functional landscape of SERT and its interacting partners in Depression.

The result shows a highly interconnected and biologically significant connections network in (Figure 8), which had 21 nodes and 168 edges—much more than the predicted number of edges 23. As seen by the average node degree of 16 in the network, the majority of proteins were linked to a significant number of other proteins. It is highly likely that nearby proteins interact with one another, as indicated by the average local clustering coefficient of 0.853. Crucially, the PPI enrichment p-value was less than 1.0e-16, indicating that the discovered network is heavily enriched for functional relationships rather than being the result of chance.

4.3.1 PPI Network Statistics for SERT via STRING-DB

Table 5: Represent the network stats of SERT

Parameter	**value**
Number of node	21
Number of edges	168
Expected number of edges	23
Average node degree	16
Average clustering coefficient	0.853
PPI Enrichment p-value	1.0e-16

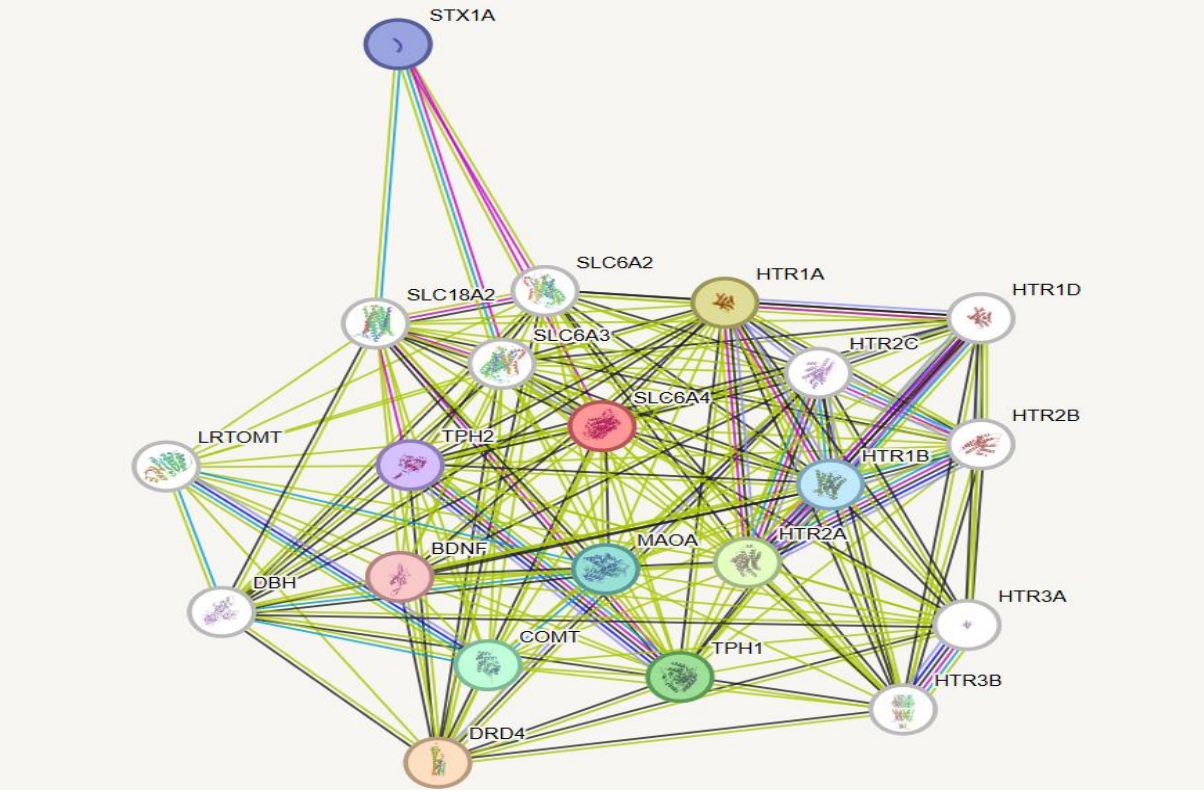


Fig.8. PPI analysis of SERT using STRING-DB

CHAPTER-5

CONCLUSION AND DISCUSSION

Major Depressive Disorder (MDD) is one of the most known and harmful neuro-behav illnesses around the world, and more than 280 million people suffer from it, say the WHO. To treat it first, doctors often give out medicines called high level (or potent) selective serotonin reuptake inhibitors (SSRIs), which work by raising the amount of serotonin (or 5-HT) in the space two nerve cells meet, by stopping the reuptake of that substance at the releasing cell. Of these, Paroxetine has been used the most because it binds strongly and keeps high the level of SERT. But there are worries about its bad effects, which can be reduced or stopped, and about the tests, which seem to show Paroxetine could cause mutations, and so we need to find drugs that are both safer and better.

So, this work used a scheme to find new analogs of Paroxetine that we could use in the real world, and that could be both safer and have different, but not worse, effects. From SwissSimilarity, we looked through a library of 327 drug-like (or similar) compounds, then filtered on their drug-like and pharmacokinetic effects to meet the rules of SwissADME

This high-throughput screen whittled the list down to 203 on the list based on Lipinski's Rule of five rule, blood-brain barrier (BBB) passability, high safe use (GIA), and the right set of solubility rules. The abstract filters made sure that only drugs that had a good chance of being used as oral drugs and that looked like they might work as a drug were considered for the next round of research.

The next step used AutoDock Vina in the PyRx box to do the work of binding the best drugs onto the shape of the 3D form of human SERT (PDB ID: 5I6X). The goal was to see how well each of the candidate drugs fit in the Sandwich Binding Site. As a group, five of the drugs that were looked at (PubChem IDs: 11697676, 4470577, 11100995, 448642, and 444031) fit in the site better than Paroxetine did (−11.0 to −11.5 kcal/mol; Paroxetine had −10.6 kcal/mol). .

The molecules had stepped on to key bodies that hold the SERT back. We saw by the use of Visuals that the drugs cased the molecules to be very deep down in the SERT site leading to good bonds with other molecules like hydrogen, π–π and things that stick to each other. We used pkCSM and SwissADME to look at how these drugs could act in an animal's body. Three of the drugs (11697676, 4470577, and 11100995), could do a lot of good things in work and body. Only one drug (11697676) showed the best comp of all three. It made the strongest bonds with the side by side with other s and scored tight to the body by passing three tests (–11.5 kcal/mol), made said bonds (ARG104, ALA331, PHE335, SER438), and could go through a body with a quick tour around the blood train. It did these by being right and not having any bad effects.

Our results show how in silico models can be very useful for early drug research. These models let us test many new drugs fast and at low cost. We can do this before performing the more costly tests in lab and in animals. We got good results by combining models that rely on how known drugs look with models that use the timing of how tests are so fast. We found many new things when compared to the drug Paroxetine, which is very well used for drugs

that stop the extrasensation of high pressure in the brain. The good points of our work must be taken with caution. First, the estimates of count of bonds and ADMET points are only guesses. They need to be checked in the lab. Second, we did not look at what side effects can happen when new drugs work with the body. Third, though we looked at many similar drugs, there could be other drugs like these.

Finally, things like how flexible the compound or cell is and how they work freely in the body are not taken into account.

As we look ahead, it is necessary to do real lab tests on the best compounds so that we can see how they stop SERT from working, if they go into the cell, and if they are safe. We should then use animal models to test how they can change behavior, get into the body, and if there are any side effects. Also, using machine learning, there are QSAR models we could use in the future to predict how active they might be over more compounds.

In the end, this work points to find good Paroxetine-like compounds that might work better by binding to SERT, and be easier on the body. copound 11697676 is strong candidate to move ahead as a new anti-depressant. This work gives a start for more lab work and shows how using computers for drug work can lead to a safer and better work on mental health drugs.

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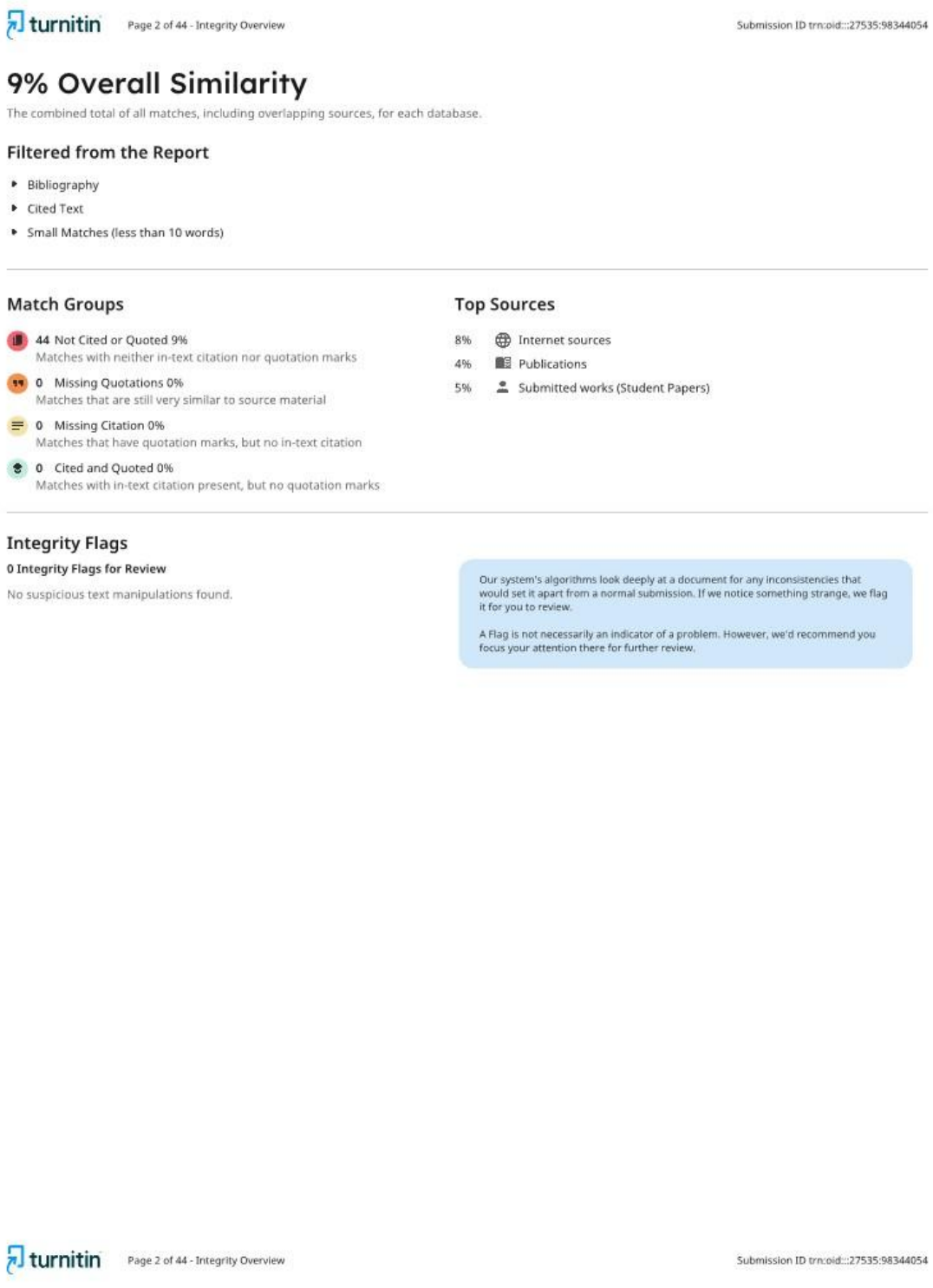

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