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Computational Evaluation of Substituted 2-Aminopyrimidine Schiff Bases as Potential Antidepressant Targeting the Muscarinic Acetylcholine M5 Receptor

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ABSTRACT

The effectiveness of existing treatments for depression, a complicated mental health condition that affects people all over the world, is limited. The ability of the muscarinic acetylcholine receptor subtype M5 (M5 mAChR) to modulate dopaminergic and cholinergic pathways has made it a unique target in the search for antidepressant drugs. This work used molecular docking to assess the antidepressant potential of fifteen newly created substituted 2-Aminopyrimidine Schiff base derivatives that target the M5 receptor. For the docking simulations, Auto Dock Tools version 1.5.7 was utilized, and the reference antidepressant was fluoxetine. Out of the fifteen compounds, the four derivatives with the highest binding affinities were SSS-14, SSS-13, SSS-01, and SSS-11. In the ortho steric binding pocket of the M5 receptor, these chemicals showed persistent connections with important amino acid residues through hydrophobic contacts, π–π stacking, and hydrogen bonding. The chosen derivatives' structural characteristics allowed for favourable receptor interaction and spatial orientation. Based on the results, substituted 2-Aminopyrimidine Schiff bases have the potential to be effective antidepressants by selectively modulating the M5 receptor. This study offers a useful computational basis for lead optimization and additional pharmacological validation in the creation of innovative antidepressant treatments

Keywords: *2-Aminopyrimidine, Schiff base derivatives, Muscarinic M5 receptor, Antidepressant activity, Molecular docking, Fluoxetine*

1. INTRODUCTION

A major contributor to the global burden of disease, depression affects approximately 300 million people worldwide and is one of the most common and incapacitating mental illnesses [1]. Even while pharmacotherapies such as serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and selective serotonin reuptake inhibitors (SSRIs) are widely available, a significant percentage of patients do not experience full remission. There is an urgent need

for new therapeutic approaches with increased efficacy and specificity because current antidepressant medications are frequently linked to delayed onset of action, low response rates, and severe side effects.[2]

The development of neuropsychiatric drugs has recently focused on muscarinic acetylcholine receptors (mAChRs), specifically the M5 subtype. Despite having poor central nervous system expression, the M5 mAChR controls mesolimbic dopamine transmission, a critical mechanism associated with mood regulation and reward processing. Selective M5 receptor modulation offers a non-monoaminergic method of antidepressant action that may circumvent the limitations of traditional therapies.[3]

Heterocyclic compounds have demonstrated great pharmacological plasticity in disorders of the central nervous system (CNS), particularly those based on pyrimidine scaffolds. Because they can generate Schiff bases, which can increase bioactivity through electronic delocalization and enhanced receptor affinity, 2-Aminopyrimidine derivatives stand out among the others.[4]

This study uses in silico molecular docking to target the M5 receptor and examine the antidepressant potential of fifteen newly synthesized substituted 2-Aminopyrimidine Schiff base derivatives. As the standard comparison, fluoxetine, a commonly prescribed SSRI, was employed. This study creates a computational basis for further in vitro and in vivo validation and offers important insights into the logical design of M5-targeted antidepressant drugs by discovering compounds with favourable binding affinities and important chemical interactions.[5]

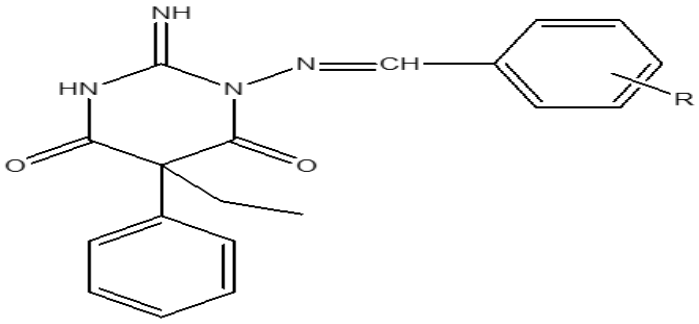


Fig. 1: General structure of selected series of compounds

2. MATERIALS AND METHODS

1. Computational Docking:

I. **Ligand Preparation**: Fifteen substituted 2-Aminopyrimidine Schiff base derivatives were designed using ChemDraw and ChemSketch and their 3D structures were generated using PyMOL. Energy minimisation was performed to optimise geometry [6-8]. The optimised structures were saved in mol format and converted to PDB using Open Babel [9]. These PDB files were further processed in Auto Dock Tools (version 1.5.7) by adding Gasteiger charges, assigning rotatable bonds, and merging non-polar hydrogens to generate PDBQT files. Fluoxetine was used as the reference standard and prepared similarly.[10]

II. **Receptor Preparation**: One protein target associated with Antidepressant activity was identified using the SuperPred web server based on molecular target prediction [11]. Muscarinic acetylcholine receptor M5 (PDB ID: 6OL9). The protein structures were retrieved from the RCSB Protein Data Bank using Auto Dock Tools, all water molecules and heteroatoms were removed, polar hydrogens were added and Kollman charges were assigned. The cleaned structures were saved in PDBQT format.[12]

III. **Selection of Top Derivatives**: Docking scores of all 15 Schiff base derivatives were compared across the target. The four compounds showing the most favourable binding energies and consistent interactions were selected for comparative docking analysis**.**

IV. **Interaction Analysis:** Protein-ligand complexes for the top four compounds were visualised using BIOVIA Discovery Studio Visualizer to analyse hydrogen bonding, hydrophobic contacts and interaction with key amino acid residues in the active site. [13]

2. Drug likeness studies and ADME prediction:

Molecular characteristics and drug-likeness parameters were calculated for each of the designed molecules (SSS-14, SSS-13, SSS-01 and SSS-11). The drug-likeness study is significant since it reveals the molecules that meet the criteria as drug-like molecules. The pharmacokinetic characteristics of pharmacological molecules, their oral bioavailability, cell penetration, metabolism and elimination make the research of ADME qualities essential. Lipinski's rule of five was computed using SWISS ADME tools and additional physicochemical factors such as molecular refractivity, GI absorption, water solubility

and the number of rotatable bonds were anticipated [14,15,16]. Several software tools were employed in this study to guarantee thorough analysis and precise forecasts. Absorption, Distribution, Metabolism, and Excretion (ADME) predictions were made using SWISS ADME, which offered important information on the drug's pharmacokinetic characteristics. To estimate the compounds' toxicity profiles and guarantee their safety for additional consideration, ProTox 3.0 was utilised.[17]

3. Boiled EGG analysis:

The BOILED-Egg model was used to assess the blood–brain barrier penetration and gastrointestinal uptake of the chosen compounds. According to the research, compounds in the yellow zone were anticipated to have more permeability across the blood–brain barrier, whereas substances found in the white region were associated with greater intestinal uptake potential. The study was carried out using the Swiss ADME digital platform. [18,19,20]

4. Toxicity studies:

A toxicity study was performed to assess the safety profile of the synthesised compounds. ProTox-II (version 3.0), an in-silico prediction platform, was used to estimate acute toxicity (LD_{50}), toxicity class, and organ-specific toxicities such as hepatotoxicity, mutagenicity, and carcinogenicity. Based on SMILES input, it enables efficient early-stage screening of potential toxic effects using machine learning and molecular similarity methods. [21,22]

5. Antidepressant Activity: [23-25]

The pharmacological capacity of a substance to reduce the symptoms of depressive illnesses, such as chronic sorrow, anhedonia, exhaustion, and cognitive impairment, is known as antidepressant activity. Conventional antidepressants work by increasing monoaminergic neurotransmission in important brain areas such as the limbic system, hippocampus, and prefrontal cortex, mainly serotonin, norepinephrine, and dopamine. Inhibition of neurotransmitter reuptake (as with SSRIs and SNRIs), inhibition of monoamine oxidase enzymes (MAOIs), or receptor modification (as with 5-HT1A agonists) are common ways that clinically effective antidepressant drugs exhibit their action. Unfortunately, many patients suffer from unpleasant side effects or incomplete remission, and these medications usually take several weeks to reach their full therapeutic impact.

Lately, the focus of research has switched to finding new targets that could result in more potent and quicker antidepressant effects. These include the muscarinic acetylcholine receptors, especially the M5 subtype, which has demonstrated promise in controlling dopaminergic pathways linked to motivation and mood. By decreasing the lag time and side effects connected with traditional medications, compounds that target M5 receptors may provide a non-monoaminergic mechanism of action. Utilizing a mix of in vitro tests (such as receptor binding or signalling studies), in silico molecular docking, and in vivo behavioral models like the Forced Swim Test (FST) and Tail Suspension Test (TST), antidepressant effectiveness can be assessed.

3. RESULT AND DISCUSSION

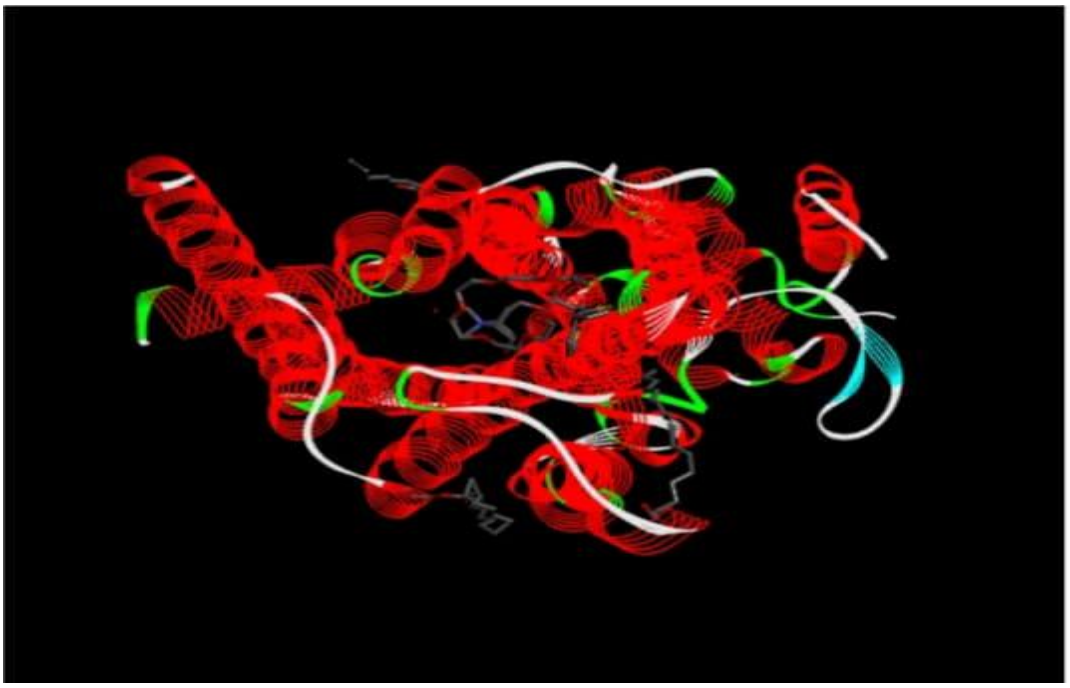
Table 1: Docking Scores of 2-Aminopyrimidine Schiff Base Derivatives with PDBID: 6OL9

Compound Name	R1	Binding Score (Kcal/Mol)
SSS1	Benzaldehyde	-9.7
SSS2	2-Hydroxy Benzaldehyde	-9.4
SSS3	3-Hydroxy Benzaldehyde	-9.1
SSS4	4-Hydroxy Benzaldehyde	-9.2
SSS5	2-Chloro Benzaldehyde	-9.3
SSS6	3-Chloro Benzaldehyde	-9.2
SSS7	3-Bromo Benzaldehyde	-9.3
SSS8	4-Bromo Benzaldehyde	-9.5
SSS9	3-Methoxy Benzaldehyde	-9.5

SSS10	4-Methoxy Benzaldehyde	-9.1
SSS11	3-Nitro Benzaldehyde	-9.6
SSS12	4-Nitro Benzaldehyde	-9.5
SSS13	3,4-Dimethoxy Benzaldehyde	-9.8
SSS14	2,4-Dimethoxy Benzaldehyde	-9.8
SSS15	3,4-Dichloro Benzaldehyde	-9.3

Table 2: Docking score of various synthesised compounds concerning the cross BBB:

SR. NO.	COMPOUNDS	ANTIDEPRESSANT DOCKING SCORE
1	SSS14	-9.8
2	SSS13	-9.8
3	SSS01	-9.7
4	SSS11	-9.6



PDBID: 6OL9

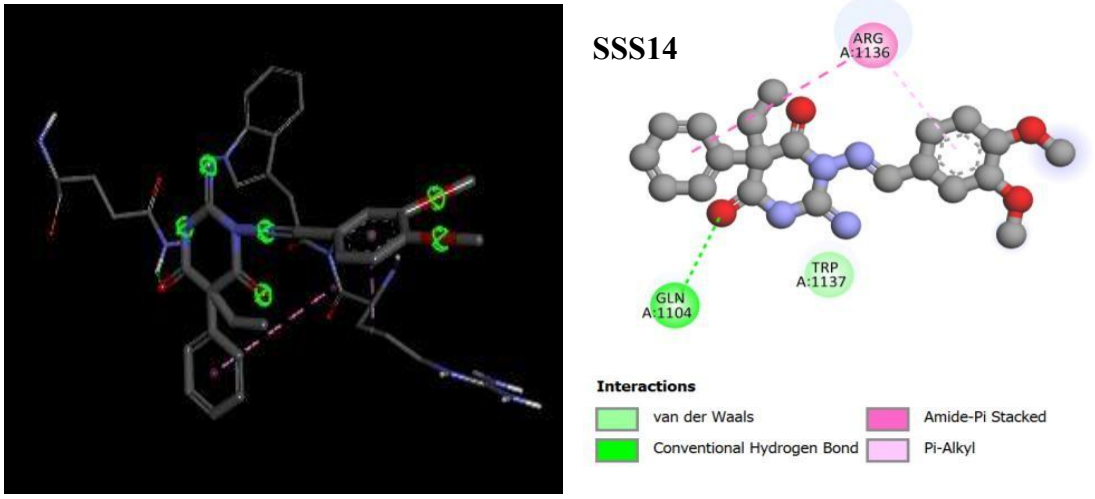


Figure 1: 2D & 3D Representation of compound SSS14 with PDBID: 6OL9

Table 3: Docking interaction of receptor (6OL9) with compound SSS14

Sr. No.	Residue Atom	Distance	Category	Type of Interaction
1	GLN1104	2.54887	Hydrogen Bond	Conventional Hydrogen Bond
2	ARG1136, TRP1137	5.39286	Hydrophobic	Amide-Pi Stacked
3	ARG1136	3.78344	Hydrophobic	Pi-Alkyl

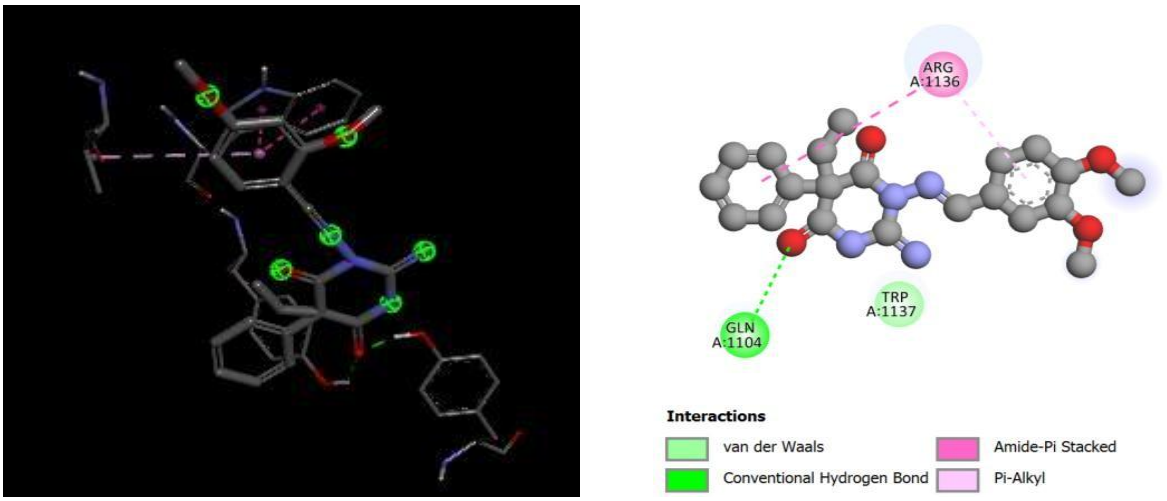


Figure 2: 2D & 3D Representation of compound SSN13 with PDBID: 6OL9

Table 4: Docking interaction of receptor (60L9) with compound SSS13

Sr. No.	Residue Atom	Distance	Category	Type of Interaction
1	TYR111	1.87483	Hydrogen Bond	Conventional Hydrogen Bond
2	TYR481	2.91789	Hydrogen Bond	Conventional Hydrogen Bond
3	TRP477	3.97411	Hydrophobic	Pi-Pi Stacked
4	TRP477	5.30255	Hydrophobic	Pi-Pi Stacked
5	VAL474	4.86276	Hydrophobic	Alkyl
6	TRP477	4.77738	Hydrophobic	Pi-Alkyl
7	TRP477	4.55371	Hydrophobic	Pi-Alkyl
8	VAL474	5.06685	Hydrophobic	Pi-Alkyl

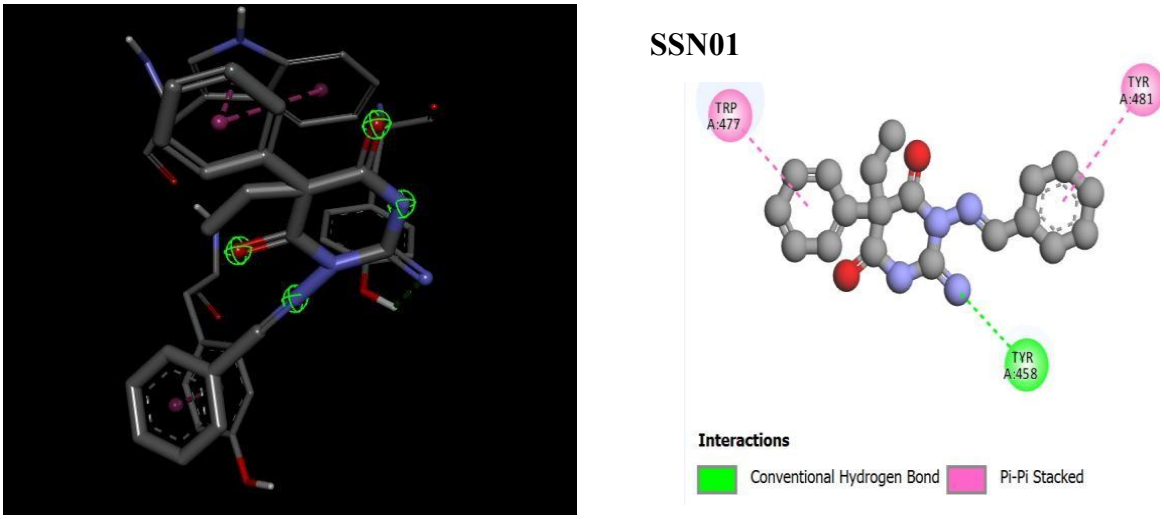


Figure 3: 2D & 3D Representation of compound SSS7 with PDBID: 6OL9

Table 5: Docking interaction of receptor (6OL9) with compound SSS01

Sr. No.	Residue Atom	Distance	Category	Type of Interaction
1	TYR458	2.75756	Hydrogen Bond	Conventional Hydrogen Bond
2	TRP477	3.85435	Hydrophobic	Pi-Pi Stacked
3	TRP477	4.9317	Hydrophobic	Pi-Pi Stacked
4	TYR481	4.14492	Hydrophobic	Pi-Pi Stacked

SSN11

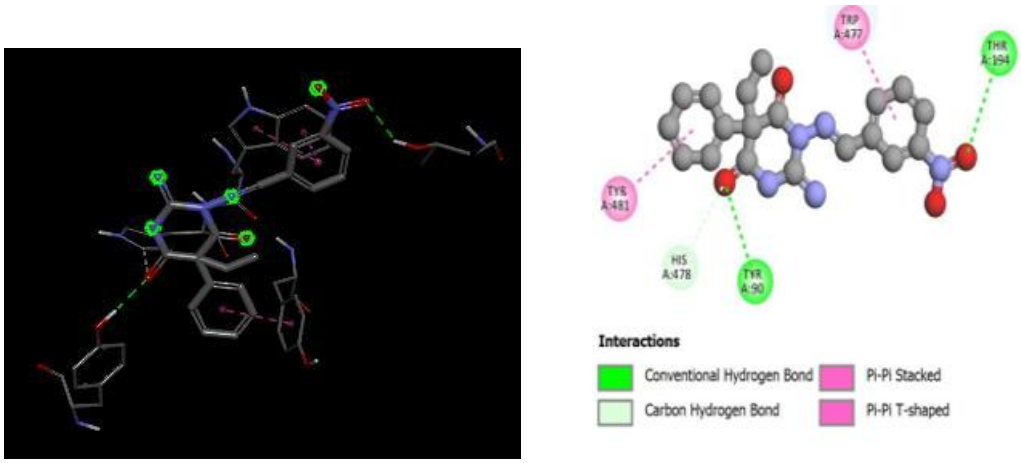


Figure 4: 2D & 3D Representation of compound SSS11 with PDBID: 6OL9

Table 6: Docking interaction of receptor (6OL9) with compound SSS11

Sr. No.	Residue Atom	Distance	Category	Type of Interaction
1	TYR90	2.45462	Hydrogen Bond	Conventional Hydrogen Bond
2	THR194	3.01077	Hydrogen Bond	Conventional Hydrogen Bond
3	HIS478	3.58368	Hydrogen Bond	Carbon-Hydrogen Bond

4	TRP477	4.65388	Hydrophobic	Pi-Pi Stacked
5	TRP477	3.76099	Hydrophobic	Pi-Pi Stacked
6	TYR481	4.79668	Hydrophobic	Pi-Pi T-shaped

Table 7: Screening of Drug-likeness Parameters

Sr. No.	Ligand	MF	Lipinski's Rule of 5				Lipinski's Violation	Lipinski's Rule
			MW (g/mol)	Log P	HBA	HBD		
1	SSS-14	$C_{21}H_{23}N_5O_4$	394.16	2.76	6	2	0	Yes
2	SSS-13	$C_{18}H_{20}N_4O_4$	394.16	3.01	6	2	0	Yes
3	SSS-01	$C_{18}H_{19}N_5O_2$	334.17	2.46	4	2	0	Yes
4	SSS-11	$C_{17}H_{18}N_6O_4$	380.14	2.52	6	3	0	Yes

Ligand	In-silico ADMET							
	Absorption	Distribution	Metabolism					Toxicity
	Water Solubility	BBB permeability	1A2	2C19	2C9	2D6	3A4	Predicted LD50 & Toxicity Class:
SSS-14	-4.14 (Moderately soluble)	2.76	No	No	Yes	No	Yes	250 mg/kg (Class:3)

SSS-13	-4.14 (Moderately soluble)	3.01	No	No	Yes	No	Yes	280 mg/kg (Class: 3)
SSS-01	-4.25 (Moderately soluble)	2.46	No	No	Yes	No	No	800 mg/kg (Class:4)
SSS-11	-3.77 (soluble)	2.52	Yes	No	No	No	No	280 mg/kg (Class: 3)

Table 8: EVALUATION OF IN-SILICO ADMET PARAMETERS

4. CONCLUSION

This study uses computational evaluation to target the muscarinic acetylcholine receptor M5 (M5 mAChR) and demonstrates the promising antidepressant potential of substituted 2-Aminopyrimidine Schiff base derivatives. Four derivatives—SSS-14, SSS-13, SSS-01, and SSS-1—were found by molecular docking studies to have the best binding affinities and stable interactions when compared to the reference medication, fluoxetine. These interactions, which include π–π stacking, hydrogen bonding, and hydrophobic contacts within the receptor's active region, imply that M5 receptor activity a novel non-monoaminergic target implicated in mood regulation can be effectively modulated.

The findings present a compelling case for these derivatives as lead molecules in the search for new antidepressant medications, providing a different strategy from traditional monoaminergic treatments that may be more effective and have fewer adverse effects. Further in vitro functional tests and in vivo behavioral assessments are necessary to confirm their antidepressant efficacy and safety profiles, even if docking studies provide insightful information about receptor-ligand interactions. All things considered, the logical design and optimization of 2-Aminopyrimidine Schiff base derivatives as selective M5 receptor modulators are made possible by this integrated computational study. These developments could have a major impact on the creation of next-generation antidepressants, overcoming the drawbacks of existing therapies and enhancing patient outcomes.

Authors' Contributions:

Smita Sunil Sawalwade and Sonali Sanjay Nikam conceptualised the study, designed the research protocol, and performed the molecular docking analysis. Smita also carried out the in vitro experimentation. All authors contributed to data analysis, manuscript drafting and approved the final version, taking full responsibility for its content and integrity. Bhavna U. Jain supervised the research, provided continuous guidance throughout the study, and critically reviewed the manuscript for intellectual and scientific accuracy.

Future Perspective

The findings support continued optimisation of SSS14 and SSS13 as antidepressant candidates. Future studies should explore pharmacokinetics, chronic toxicity, in vivo studies, and advanced formulation strategies to facilitate clinical translation.

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Conflict of Interest

The author declares no conflict of interest regarding the publication of this manuscript

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