Design, Synthesis, and Pharmacological Evaluation of Piperazine-Hydrazide Derivatives as Potential CNS-Active Antidepressants: MAO-A Inhibition and In Silico Docking Studies on Protein 2BXR and N1 Neuraminidase (PDB 2HU4) for Alzheimer's Disease

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Abstract
Since piperazine-substituted oxadiazoles have the potential to be kinase inhibitors, antibacterial agents, and medications that target the central nervous system (CNS), their synthesis and evaluation have attracted a lot of attention. In this work, six novel piperazine-oxadiazole derivatives (VSM 1–VSM 6) are designed, synthesised, and biologically evaluated. The compounds were created via condensation processes, hydrazinolysis, and nucleophilic substitution, producing stable heterocyclic structures. Extensive molecular docking studies and spectrum analysis (IR, NMR) were conducted, and in silico predictions suggested promising drug-like qualities. The substances demonstrated minimal toxicity, high oral bioavailability, and blood-brain barrier (BBB) penetration. Furthermore, every drug exhibited favourable pharmacokinetic profiles and passed Lipinski's rule of five. Strong binding affinities to the hMAO-A enzyme were found by docking tests; VSM 3 and VSM 6 had the highest affinities, indicating possible antidepressant action. At dosages of 20–30 mg/kg, VSM 3 and VSM 6 dramatically decreased immobility time, according to biological tests such as the Forced Swim Test (FST) and Tail Suspension Test (TST), which further supports their antidepressant-like effects. These results underscore the potential of the compounds to treat depression and point to the need for further study to optimise their safety and effectiveness profiles. Additionally, VSM 2 and VSM 4 demonstrated strong binding affinities to N1 Neuraminidase, indicating potential uses in Alzheimer's disease. The potential of piperazine-substituted oxadiazoles as a treatment for CNS illnesses, including mood disorders and neurodegenerative diseases, is highlighted by this study.

Keywords: Molecular docking, blood-brain barrier, forced swimming test, antidepressant, piperazine-substituted oxadiazoles, and drug-like characteristics.

Introduction

In order to comprehend how therapeutic drugs work, the interdisciplinary science of medicinal chemistry combines concepts from chemistry and biology. Its main objective is to create novel drugs while enhancing the effectiveness, safety, and half-life of current ones[1-4]. This necessitates a thorough understanding of bioavailability, molecular interactions, and the effects of medicinal substances on biological systems. In order to develop medicines that not only reduce the symptoms of an illness but also improve the patient's quality of life with the fewest

possible side effects, medicinal chemistry is thus essential to drug discovery and optimisation. The class of bioactive chemicals known as piperazine-substituted oxadiazoles, which combines an oxadiazole ring structure with a piperazine ring, is a major focus of study in this field. In contrast to the piperazine ring, which has six members and also contains nitrogen atoms, the oxadiazole ring has five members. These substances are important because they may interact with a variety of biological targets, including ion channels, enzymes, and neurotransmitter receptors[5-9]. These interactions' adaptability makes them promising candidates for drug development, especially for kinase inhibitors, antimicrobial medicines, and drugs active in the central nervous system (CNS). With continued research in non-medical domains including agrochemicals and materials science, their uses stretch beyond medicinal chemistry. The possibility for developing medications with more focused activities and fewer side effects a significant benefit in the development of safe and effective therapies is enhanced by the distinct molecular structure of piperazine-substituted oxadiazoles. With some exhibiting promising biological activity and a melting temperature of 195–198°C, which suggests their durability and potential for more research, these compounds have shown promise in medical applications[10-19]. The treatment of depression, a mental illness that drastically lowers quality of life and raises the risk of suicide, is one area where the creation of such chemicals might have a particularly substantial effect. Most people believe that depression is caused by a neurotransmitter imbalance, specifically with regard to serotonin and norepinephrine. It is thought that this imbalance results from monoamine oxidase enzyme malfunction, which lowers neurotransmitter levels and impairs neuronal transmission. The development of antidepressants, beginning with tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) in the 1950s, was made possible by the comprehension of this process[20-26]. In contrast to the earlier treatments, more focused pharmaceuticals such as selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) were created throughout the decades, providing more precise regulation of neurotransmitter systems with fewer adverse effects. However, many antidepressants have negative side effects include weight gain, impaired vision, dry mouth, and sexual dysfunction despite their effectiveness. These adverse effects emphasise the need of creating medications that are safer and easier to use. This requirement is particularly urgent when treating depression subtypes that may call for more individualised treatment plans, such melancholic depression or atypical depression. Even though modern antidepressants have significantly improved patient outcomes and therapy, additional research is necessary to find novel molecules with better safety profiles and more potent therapeutic choices[27-31].

A reduction in intellectual capacity and memory impairment are hallmarks of dementia, a neurological illness. Alzheimer's disease (AD)[32,33], a progressive neurodegenerative illness involving the death of neurones in particular brain areas, has drawn the most attention among the several types of dementia. The illness is characterised by a persistent deficiency in cholinergic neurotransmission, which mostly affects cholinergic neurones in the basal forebrain, in addition to its characteristic neurofibrillary tangles and neuritic plaques. Decreased levels of high-energy phosphates like ATP and ADP[34,35], impaired glucose metabolism, and decreased glucose utilisation are also linked to Alzheimer's-related dementia. Increased oxidative stress is closely related to this compromised energy metabolism, which causes biomolecules to oxidise and excitotoxic brain damage to begin. Gaining insight into

these processes creates new avenues for investigating organic methods of regaining mental and cognitive ability. There is potential for increasing patient safety and tolerance as well as the overall effectiveness of depression therapies by pursuing antidepressants with fewer side effects and investigating innovative chemical structures such as piperazine-substituted oxadiazoles. In conclusion, the discovery of new chemicals and the improvement of already-approved medications represent further advancements in medicinal chemistry. Piperazine-substituted oxadiazoles are a prime example of how advances in chemistry may result in novel medication discovery, especially in fields like antibacterial treatment and central nervous system disorders. In order to effectively treat complicated disorders like depression, research will continue to concentrate on developing drugs with increased potency, safety, and fewer adverse effects[36-41].

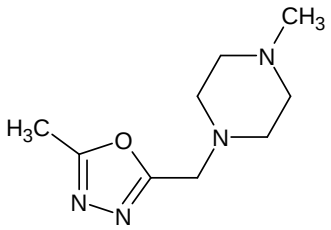
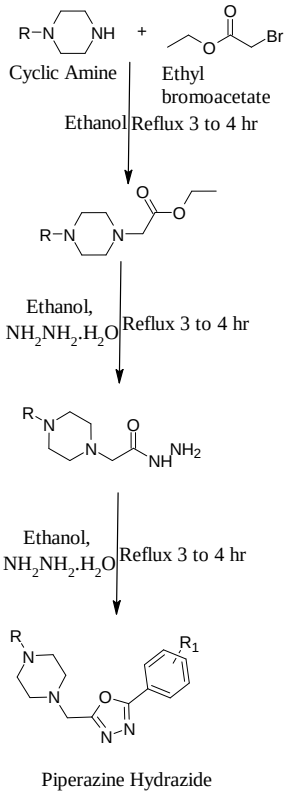


Figure-1. 2-Methyl-5-((4-Methyl- piperazin-1-Yl) Methyl)-1,3,4- Oxadiazole.

Material and Method

All chemicals and solvent employed in the synthesis of compounds, as well as in biological activities and the production of metal complexes, comes from the companies Merck, CDH, Loba, S.D. Fine, Sigma, CGP laboratory, Fisher Scientific, and Molychem. A Bruker Advance III and Bruker Av III HD 500 MHz VT range from -150°C to +150°C nuclear magnetic resonance (NMR) spectrometer with solid state attachment was used to plot 1H-NMR spectra, while a Schimadzu FTIR spectrophotometer was used to obtain FT-IR (Fourier Transform Infrared Spectroscopy) spectra at room temperature. Autoclave for sterilising different biological activities, analytical balance (Virgo), and melting point instrument (Virgo) are used. Simple reflux condensation method is used for synthesis of piperazine hydrazide as mentioned in scheme 1.



Scheme 1. Synthesis of piperazine hydrazide.

Table 1. List of Substitution of R and R_1.

Compound Code	R	R_1
VSM 1	$-CH(C_6H_5)_2$	C_6H_5
VSM 2	$-CH(C_6H_5)_2$	$4\text{-}Cl(C_6H_4)$
VSM 3	$-CH(C_6H_5)_2$	$4\text{-}OCH_3(C_6H_4)$
VSM 4	$-CH(C_6H_5)_2$	$2\text{-}OH(C_6H_4)$
VSM 5	$-CH(C_6H_5)_2$	2,4,6-tri $OCH_3(C_6H_2)$
VSM 6	$-CH(C_6H_5)_2$	-SH

A nucleophilic substitution initiates the reaction pathway, whereby the nitrogen in a cyclic amine (R-NH) attacks the electrophilic carbon of ethyl bromoacetate, dislodging the bromide and forming an ester-linked product. The presence of hydrazine hydrate causes this ester to undergo hydrazinolysis. When hydrazine attacks the carbonyl carbon of the ester, ethanol is released as a byproduct, and a hydrazide ($-CONHNH_2$) is produced. The next step involves the condensation of this hydrazide with carboxylic acid (R_1-COOH). By attacking the carboxylic acid's carbonyl carbon, the hydrazide's terminal amine creates an amide bond (-CONH-NH-CO-) by a condensation process, with water as a byproduct. Through a sequence of nucleophilic substitution, hydrazinolysis, and amide bond production, this reaction produces a stable molecule with a heterocyclic structure including amides.

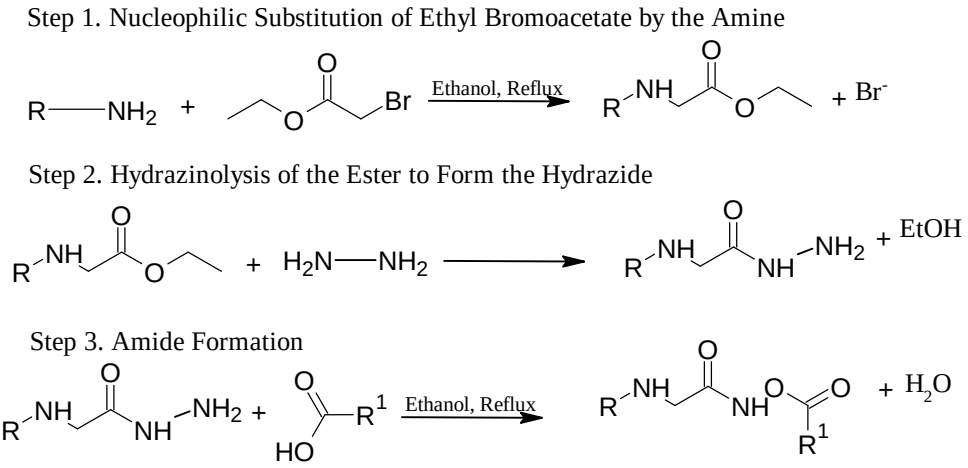


Figure-2. Reaction mechanism of piperazine hydrazide.

In silico studies of compounds of oxadiazole containing piperazine were created. To draw the proposed compounds, Chem Bio draws Ultra 12.0 was used. Every developed chemical was examined using Molegro Virtual Docker 7.0 (MVD 7.0). The hMAO-A enzyme structure from the protein bank (PDB ID: 2BXR) was downloaded in pdd.org format[42-44]. The chosen protein was produced using the MVD 7.0 application. The protein is prepared by removing the ligand (Clorgyline) and adding hydrogen atoms. Polar hydrogen was added, while water molecules were removed. Next, save the file in the.pdbqt format and add the Kollman charges .Figure 3 three-dimensional X-ray structure of hMAO-A complexed with the protein chlorgyline[45-47]. The 3D X-ray structure of the MAO-A enzyme (PDBID: 2BXR) was made available by the Data Bank at a resolution of 3.00 Å (www.rscb.org).

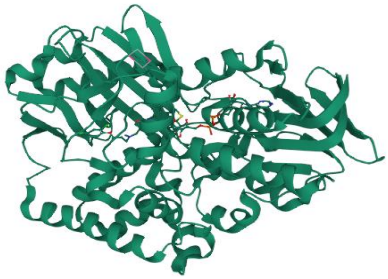


Figure-3. 3D X-ray structure of hMAO-A complexed.

Virtual Screening for Neuroaminidase 1 Inhibitors

All derivatives of oxadiazole containing piperazine were prepared in 2D form using Chem Sketch 2021.2.1, and the files were saved in MOL format. The 3D X-ray crystal structure of the MAO-A enzyme (PDB ID: 2HU4) was obtained from the RCSB Protein Data Bank (www.rscb.org) at a resolution of 2.50 Å. Protein preparation was performed using Discovery Studio 2024, where all heteroatoms, other chains, and ligands were removed, and chain A was isolated and saved in PDB format for further analysis. The protein was loaded into PyRx docking software, converted into a macromolecule, and processed by adding polar hydrogens, Kollman charges, and fixing potentials. The 2D structures of the compounds were loaded using

the Babel option, energy-minimized, and converted into PDBQT format. A grid box was set up with the following parameters: exhaustiveness = 8, center_x = -10.4911, center_y = 70.4291, center_z = 101.7728, size_x = 47.7887, size_y = 54.7245, size_z = 54.2057. The docking process was executed, and docked ligand-protein complexes were visualized using Discovery Studio[48-50].

Behavioural Investigations (MWM Cognitive Test)

The animals' capacity for memory and learning was assessed using the Morris water maze test. A large circular pool (150 cm in diameter by 45 cm in height) filled with water down to 30 cm and kept at 28±1°C is part of the arrangement. In the target pool quadrant, a white, submerged platform (10 x 10 cm) was positioned 1 cm below the water's surface. Every animal had four training trials in a row, separated by five minutes. As an indicator of learning and acquisition, the escape latency time (ELT), which represents the amount of time needed to find the concealed platform, was measured on the fourth day.

Result

The compound VSM 1, with the chemical formula C26H26N4O, was obtained as a yellow solid with a melting point in the range of 193-195°C and a yield of 65%. The Rf value was 0.5. The IR spectrum revealed characteristic absorption peaks at 2927 cm^{-1} (Ar-CH), 2903 cm^{-1} (Ali-CH), 1596 cm^{-1} (C=C), 1631 cm^{-1} (C=N), 1195 cm^{-1} (C–N), and 1085 cm^{-1} (C–O). The ^1H NMR spectrum (400 MHz, $CDCl_3$) showed signals at δ 7.72 - 7.02 (15H, aromatic protons), 5.32 (1H, CH), 3.61 (4H, piperazine CH_2), 3.51 (4H, piperazine CH_2), and 2.02 (2H, CH_2). VSM 2, with the formula C26H25ClN4O, was a pale yellow solid that melted between 196-198°C and gave a yield of 63%. The Rf value was 0.8. The IR spectrum displayed peaks at 3001 cm^{-1} (Ar-C-H), 2923 cm^{-1} (Ali-C-H), 1621 cm^{-1} (C=C), 1619 cm^{-1} (C=N), 1341 cm^{-1} (C-N), 1285 cm^{-1} (C–O), and 751 cm^{-1} (C–Cl). The ^1H NMR spectrum (400 MHz, $CDCl_3$) showed δ values of 7.60 - 7.04 (14H, aromatic protons), 5.32 (1H, CH), 3.61 (4H, piperazine CH_2), 3.51 (4H, piperazine CH_2), and 2.10 (2H, CH_2), VSM 3, with the chemical formula C27H28N4O2, appeared as a light brown solid, with a melting point of 192-194°C and a yield of 68%. Its Rf value was 0.45. The IR spectrum of VSM 3 exhibited peaks at 3011 cm^{-1} (Ar-C-H), 2810 cm^{-1} (Ali-C-H), 1491 cm^{-1} (C=C), 1652 cm^{-1} (C=N), 1263 cm^{-1} (C–N), and 1171 cm^{-1} (C–O). In the ^1H NMR spectrum (400 MHz, $CDCl_3$), the signals appeared at δ 7.50 - 7.08 (14H, aromatic protons), 4.20 (2H, CH_2), 2.92 (3H, CH_3), 2.48 (4H, t, J = 4 Hz, piperazine), 2.29 (4H, piperazine CH_2), and 1.86 (1H, CH), VSM 4, with the chemical formula C26H26N4O2, was obtained as a black solid with a melting point of 184-186°C and a yield of 62%. Its Rf value was 0.48. The IR spectrum displayed absorption peaks at 3237 cm^{-1} (O-H), 3013 cm^{-1} (Ar-C-H), 2923 cm^{-1} (Ali-C-H), 2358 cm^{-1} (C=N), 1681 cm^{-1} (C=C), 1531 cm^{-1} (C–O), and 1180 cm^{-1} (C–N). The ^1H NMR spectrum (400 MHz, $CDCl_3$) showed δ values at 9.73 (1H, O-H), 7.71 - 7.02 (14H, aromatic protons), 5.33 (1H, CH), 3.62 (4H, piperazine CH_2), 3.51 (4H, piperazine CH_2), and 2.22 (2H, CH_2), VSM 5, with the chemical formula C29H32N4O4, was a dark brown solid with a melting point between 199-201°C and a yield of 61%. Its Rf value was 0.6. The IR spectrum showed peaks at 3011 cm^{-1} (Ar-C-H), 2810 cm^{-1} (Ali-C-H), 1491 cm^{-1} (C=C), 1652 cm^{-1} (C=N), 1263 cm^{-1} (C–N), and 1171 cm^{-1} (C–O). The ^1H NMR spectrum (400 MHz, $CDCl_3$) showed δ values of 7.72 - 7.02 (14H, aromatic protons),

5.32 (2H, CH_2), 3.61 (3H, CH_3), 3.51 (4H, t, J = 4 Hz, piperazine), 2.10 (4H, piperazine CH_2), and 1.45 (1H, CH), VSM 6, with the chemical formula C20H22N4OS, was obtained as a yellowish white solid with a melting point of 184-186°C and a yield of 71%. Its Rf value was 0.48. The IR spectrum displayed peaks at 3011 cm^{-1} (Ar-C-H), 2810 cm^{-1} (Ali-C-H), 1491 cm^{-1} (C=C), 1652 cm^{-1} (C=N), 1263 cm^{-1} (C–N), and 1171 cm^{-1} (C–O). The ^1H NMR spectrum (400 MHz, $CDCl_3$) exhibited signals at δ 12.99 (1H, SH), 7.72 - 7.12 (14H, aromatic protons), 5.32 (2H, CH_2), 3.61 (3H, CH_3), 2.31 (4H, t, J = 4 Hz, piperazine), 2.10 (4H, piperazine CH_2), and 1.30 (1H, CH), confirming the structure of synthesised compound.

In silico drug likeness predictions

A complicated balancing act between a number of structural and molecular characteristics, including as stability, oral bioavailability, good pharmacokinetic capabilities, lack of toxicity, and medium additive potential, results in drug similarity. The ADMET prediction of CNS active medicines is an essential method for evaluating the safety and ability to achieve the intended target of produced compounds[51-53]. The complex structure of the complete drug molecule makes correlating efforts difficult, even though many of these traits rely on the biological and physicochemical properties that are intrinsic to the drug molecule. To evaluate the computational drug similarity of various planned and produced derivatives, Swiss ADME prediction technology was used.The results of the prediction data (Table 2) demonstrated that the synthesised chemical met Lipinski's requirements for drug-like compounds. The LogP value of each synthetic material is less than five. The majority of the chemicals have molecular weights of less than 500 g/mol, and the presence of HBD and HBA is also beneficial. The proportion of chemicals that were absorbed orally was promising. All of the compounds were also able to pass across the blood-brain barrier (BBB) to reach their desired location in the blood-brain and show action in the central nervous system. It is possible to draw the conclusion that the synthesised compound has a good chemical skeleton and might be turned into a useful therapeutic molecule without going against Lipinski's requirements.

Table 2. Swiss ADME analysis data (**Abbreviations:** M.W.: Molecular weight (HBA) stands for hydro. bond acceptor, (HBD) for hydro. bond donor, Topological polol surfaces area, or TPSA BBB: permeability of the blood-brain barrier, LogP: the compounds partition coefficient between water and n-octanol, logarithm Rule of Five, or ROF)

S. No.	Compounds ID	M. W.	LogP o/w	HB Donor	HB Acceptor	BBB	TPSA	ROF
1.	VSM 1	411	4.340	0	5	0.10647	45.40	0
2.	VSM 2	445	4.450	0	5	0.12720	45.40	0
3.	VSM 3	441	4.520	0	6	0.07433	54.630	0
4.	VSM 4	427	4.030	1	6	0.04240	65.630	0
5.	VSM 5	501	4.900	0	8	0.70670	73.090	1
6.	VSM 6	366	3.550	0	5	0.65477	84.20	0

To determine the safety of target chemicals and reduce the possibility of failure in pre-clinical research, toxicity prediction is an essential technique. For this objective, the safety profile of several synthesised derivatives was predicted using the Pre-ADME and ProToxweb-based

technologies[54-56]. Since all of the created derivatives are within class 5, where the least lethal dosage (LD50) ranges from 1000 to 2500 mg/kg, they are all non-toxic, as shown in Table 3. Ingestion of Class 5 substances at levels ranging from 2000 to 3000 mg/kg does not result in any adverse consequences. Importantly, the generated compounds exhibited a very low affinity for the human ether-á-go-go (hERG) gene, indicating that there is little chance of the derivatives affecting the cardiac action potential. Consequently, our toxicity prediction findings may suggest that the proposed compounds are safer for future preclinical and development studies.

Table 3. Toxicological properties of compounds VSM 1 - VSM 6

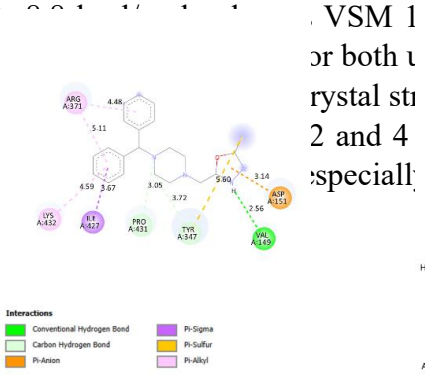
S. No.	Name	HERG	Carcino Mouce	Carceno Rat	ProtoxPredicted LD5*10(mg/kg)
1	VSM 1	Medium - risk	-	+	1260.0
2	VSM 2	Medium-risk	-	+	2000.0
3	VSM 3	Medium-risk	-	+	2412.0
4	VSM 4	Medium-risk	-	+	2032.0
5	VSM 5	Medium-risk	-	+	2032.0
6	VSM 6	Medium-risk	-	+	2000.0

Using the Molegro virtual docker 7.0 tool, all of the produced compounds were put through docking tests Compared with the PDB ID of 2BXR, the MAO-A enzyme. Based on the series, it was discovered that every synthetic chemical fit the MAO-A enzyme's active site as well as the conventional inhibitor clorgyline did. According to the docking studies, VSM 3 and VSM 6possess the greatest affinity for the enzyme *h*MAO-A, scoring -9.6 kcal per mol and -8.6 kcal per mol, respectively. H boding represents by blue color, electrostatic bonding is represented by green color, and stearic interactions were represented by red color. To determine the synthetic compounds' potential for medicinal uses, the binding affinities of VSM 1 and VSM 6 were measured. The following are the affinity values, which were calculated in kcal/mol: The binding affinity of VSM 2 was marginally higher at -8.0 kcal/mol than that of VSM 1, which had an affinity of -7.9 kcal/mol. At -9.6 kcal/mol, VSM 3 showed the strongest affinity, suggesting a substantial possibility for interaction. The affinities of VSM 4 and VSM 5 were -8.2 and -7.8 kcal/mol, respectively. The second-highest affinity of the synthesised compounds, -8.6 kcal/mol, was shown by VSM 6. Clorgyline, a reference chemical, showed an affinity of -7.1 kcal/mol for comparison. These findings imply that, among the synthesised compounds, VSM 3 and VSM 6 had the greatest binding affinities, with VSM 3 demonstrating the highest

affinity overall. The compounds' interactions, which may be essential to their effectiveness for certain therapeutic targets, are revealed by the affinity values.

Docked Neuroaminidase 1 Inhibitors

All of the synthesised oxadiazole derivatives that included piperazine were docked to the Alzheimer's disease-related protein N1 Neuraminidase in complex with oseltamivir (PDB ID: 2HU4) using the PyRx docking program. All of the derivatives had significant binding affinities and fit well into the enzyme's active site, according to the docking tests. With an affinity of -9.9 kcal/mol, VSM 2 had the highest affinity of all the compounds, closely followed by VSM 4. VSM 3's binding affinity was -9.4 kcal/mol, whilst VSM 5's was slightly lower at -9.5 kcal/mol. Although it still exceeded many accepted norms, VSM 6 had the lowest affinity in the series at VSM 1 kcal/mol. All compounds' Rl or both u cating that the docked positio rystal stı s representing respective figu 2 and 4 es, suggesting that they may l especiall



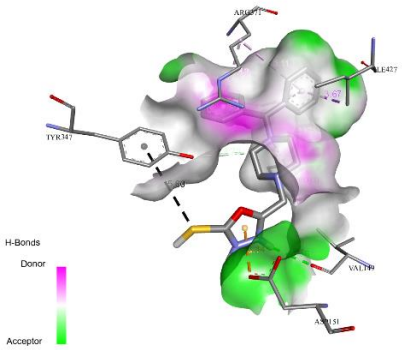
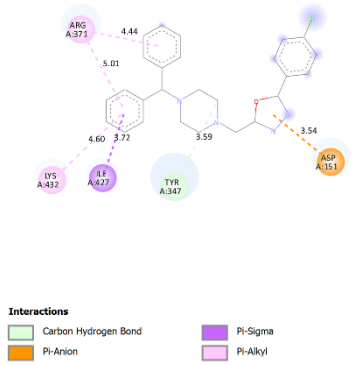


Figure 5. 2d and 3d representation of VSM1 complex with 2HU4 protein.



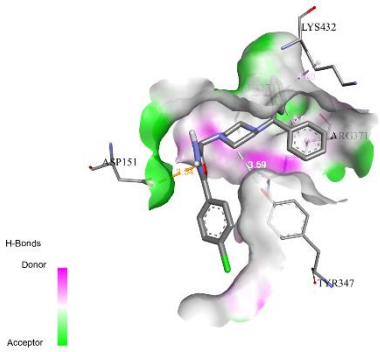


Figure 6. 2d and 3d representation of VSM2 complex with 2HU4 protein.

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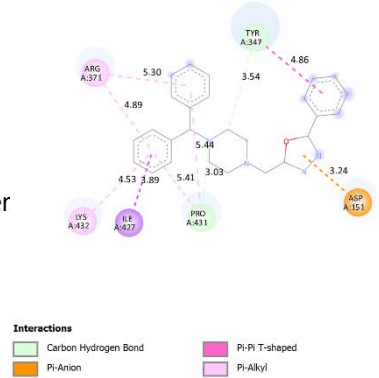

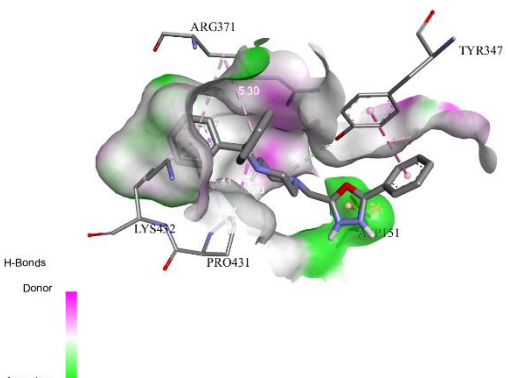

Figure 7. 2d and 3d representation of VSM3 complex with 2HU4 protein.

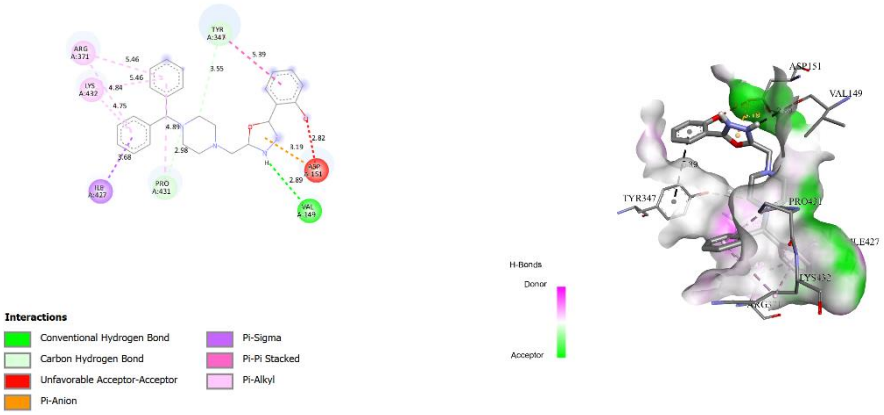

Figure 8. 2d and 3d representation of VSM4 complex with 2HU4 protein.

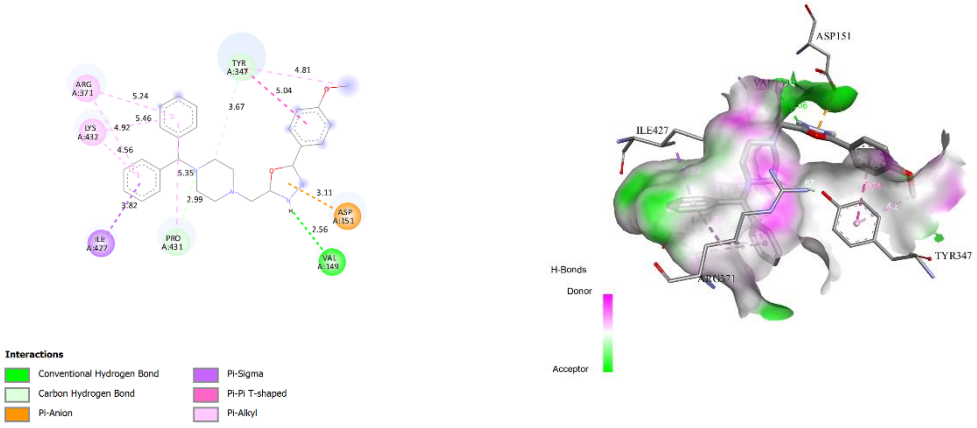

Figure 9. 2d and 3d representation of VSM5 complex with 2HU4 protein.

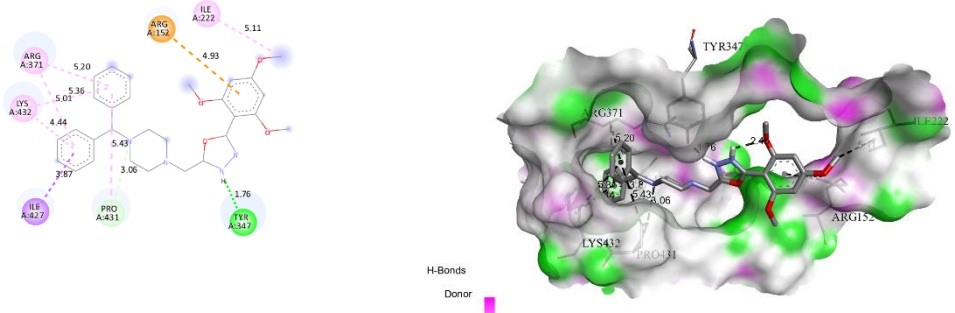

Figure 10. 2d and 3d representation of VSM6 complex with 2HU4 protein.

Antidepressant Model Forced swimming test (FST)

FST, a prominent depression model, was used to investigate the antidepressant effects of the two most effective drugs, VSM 3 and VSM 6. Clorgiline was administered intraperitoneally (i.p.) to mice at a dosage of 20 mg/kg, whereas VSM 3 and VSM 6 were administered orally at doses of 20 and 30 mg/kg, respectively. Mice were administered the medication for two hours, during which time FST was administered and their immobility was recorded[57-59]. When compared to the group under supervision, the conventional medication Clorgiline[60-62] showed the greatest reduction in immobility time. Mice[63] treated with VSM 3 (twenty mg/kg and 30 mg/kg)[64] and VSM 6 (20 and 30 mg/kg) showed significant results, with durations of immobility of 174 seconds, 148 seconds, and 212 seconds, 192 seconds, respectively, in comparison to the control group (265 seconds) Figure 5.

Tail suspension test (TST)

The TST also looked at compounds VSM 3 and VSM 6 (at the same dosage), Examine another model of antidepressant-like activity to further support this effect. It was recorded how long the treated mice hung still with their tails in a horizontal rod. The immobility durations of both compounds were much shorter than those of the control (Fig. 3.17). Immobility time was assessed in TST after a 2-hour administration interval. Compound VSM 3 injections at dosages of 2*10 mg/kg and 3*10 mg/kg resulted in significantly ($p<0.05$) shorter immobilisation times (62 and 45 seconds, respectively). Compound VSM 6 considerably reduced the immobility period in TST at concentrations of 2*10 mg/kg and 3*10 mg/kg (80 and 68 seconds, respectively) as compared to the control group (149 sec)[65-68].

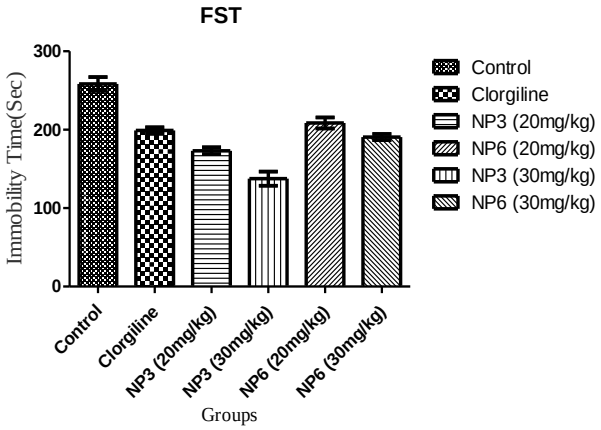

Figure-11. In the FST, Clorgiline (20 mg/kg, i.p.) and VSM 3 and VSM 6 (2*10 mg/kg and 3*10 mg/kg, m-oral) demonstrated antidepressant effect. Comparing the treatment group with the control group are indicated by asterisks.

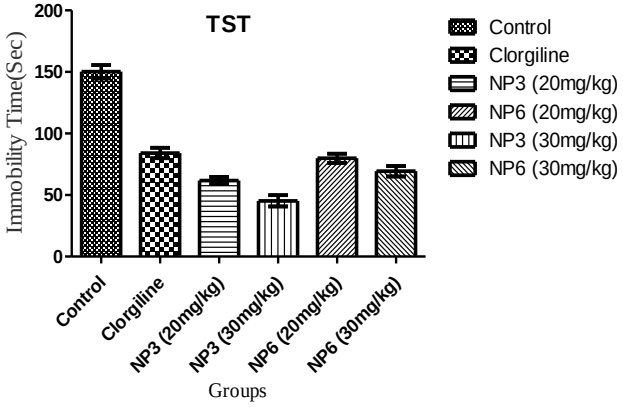

Figure 12. The antidepressant effects of VSM 3 and VSM 6 (2*10 mg/kg and 3*10 mg/kg, mouthly) as well as Clorgiline (20 mg/kg, i.p.) were demonstrated by TST. A comparison between the one receiving treatment group and the control are indicated by asterisks.

Morris water Maze Test

By assessing the Escape Latency Time (ELT) in the Morris water maze, spatial working memory was evaluated. Table 4 displays the impact of the VSM2 and VSM4 compounds on ELT. Group II's ELT increased significantly ($P < 0.001$) after receiving STZ in comparison to Group I. The ELT of Groups III, IV, and V was significantly ($P < 0.001$) lower than that of Group II.

Table 4. VSM 2 & VSM4 Impact on Morris Water Maze Escape Latency Time

Group	Escape Latency (Time in sec)
Group I (Vehicle)	30.63 ± 4.11
Group II (STZ)	141.375 ± 14.98 ***
Group III (Donepezil 5 mg/kg + STZ)	31.425 ± 2.10 &&&
Group IV (LD 20 mg/kg + STZ)	45.875 ± 5.16 ###
Group V (HD 40 mg/kg + STZ)	36.90 ± 3.90 $$$

Results are shown as mean ± SEM for a total of six animals. The control group and the negative control group are compared when the ***P < 0.001 value is present. A comparison between the negative control group and the low-dose group is indicated by ###P < 0.001. The high-dose group and the negative control group are compared when $$$P < 0.001.

Discussion

Piperazine-oxadiazole derivatives have shown promise as remedies for neurological diseases, including Alzheimer's disease, and as antidepressants. These substances are thought to function by blocking monoamine oxidase A (MAO-A), an enzyme that controls the amounts of neurotransmitters like dopamine, serotonin, and norepinephrine—all of which are essential for mood regulation. By inhibiting MAO-A, these compounds may increase neurotransmitter levels, perhaps reducing depressive symptoms. When combined with piperazine, the oxadiazole scaffold also provides a new and perhaps more selective method for creating effective MAO-A inhibitors that could have less side effects than conventional antidepressant treatments. Since these derivatives of piperazine-oxadiazole have the ability to block enzymes like N1 Neuraminidase, which are implicated in the pathophysiology of Alzheimer's disease, they may potentially have therapeutic uses in the condition. In order to investigate this dual potential, docking tests were carried out using the PyRx docking program. These investigations focused on how these chemicals interacted with the Alzheimer's disease-related protein oseltamivir (PDB ID: 2HU4) and N1 Neuraminidase in complex. Each synthetic derivative's binding affinities were evaluated by docking it against the enzyme. Docking experiments showed that all of the piperazine-oxadiazole derivatives had significant binding affinities and fit well into the enzyme's active region. The derivative with the highest binding affinity, VSM 2, was -9.9 kcal/mol, followed by VSM 4, which also had an affinity of -9.8 kcal/mol. At -9.5 kcal/mol, VSM 5's affinity was somewhat lower than VSM 3's, which was -9.4 kcal/mol. VSM 1 had an affinity of -9.2 kcal/mol, while VSM 6 surpassed several traditional criteria even though it had the lowest affinity in the series (-8.8 kcal/mol). For both upper and lower boundaries, the RMSD values for every molecule were zero, suggesting that the docked positions matched the target enzyme's crystal structure. According to these results, compounds with the greatest binding affinities, such VSM 2 and VSM 4, have the potential to be important treatments for Alzheimer's disease because they may inhibit N1 Neuraminidase in addition to acting as antidepressants by blocking MAO-A. Given these chemicals' dual action, piperazine-oxadiazole derivatives may provide a more all-encompassing strategy for treating Alzheimer's

disease and depression. However, further study is required to improve their selectivity for MAO-A over other isoforms like MAO-B, as well as to maximise their metabolic stability, bioavailability, and blood-brain barrier crossing capabilities. The success of these drugs' clinical development and treatment will depend on ensuring their long-term efficacy and safety.

Conclusion

Piperazine hydrazide was used in two different synthetic pathways to create compounds VSM 1–VSM 6. The first technique created piperazine-oxadiazole derivatives by treating piperazine hydrazide with phosphorus oxychloride and several aromatic acids. The second method considerably increased the variety of the produced chemicals by reacting piperazine hydrazide with carbon disulphide and sodium hydroxide. The potential of the resultant piperazine-oxadiazole derivatives to inhibit the human monoamine oxidase A (hMAO-A) enzyme known therapeutic target for antidepressant medications because of its function in the degradation of important neurotransmitters like serotonin, norepinephrine, and dopamine—was then assessed. When compared to clorgyline, a popular MAO-A inhibitor, VSM 3 and VSM 6 showed a higher binding affinity to hMAO-A in computational docking tests. According to these findings, VSM 3 and VSM 6 may have strong inhibitory action on hMAO-A, which might provide a fresh substitute or enhancement of current antidepressant treatments. Their potential as therapeutic drugs targeting hMAO-A was further supported by the docking experiments, which showed that VSM 3 and VSM 6 established stable connections with the enzyme, most likely via hydrogen bonding and other advantageous interactions. The produced compounds were tested using two reputable animal behavioural models, the Forced Swim Test (FST) and the Tail Suspension Test (TST), to verify their antidepressant-like effects. Immobility time, a behavioural indicator often linked to antidepressant effectiveness, was significantly reduced in both experiments by VSM 3 and VSM 6. This decrease was similar to what was seen with clorgyline, which is known to have antidepressant effects via inhibiting MAO-A. Both VSM 3 and VSM 6 showed a significant reduction in the amount of time spent immobile in the FST, suggesting that antidepressants may have an impact via encouraging more activity or coping mechanisms in response to stress. Additionally, VSM 3 and VSM 6 demonstrated a significant decrease in immobility time in the TST, another popular model for depression-like behaviour, which further supports their antidepressant-like qualities. All of these results point to the great potential of piperazine-oxadiazole derivatives as antidepressants, especially VSM 3 and VSM 6. They are positioned as viable candidates for further development into strong antidepressant drugs due to their capacity to inhibit hMAO-A and their display of antidepressant-like effects in the FST and TST. These two behavioural models' encouraging outcomes further highlight the chemicals' applicability in treating depression, a complicated and common mental health condition. The produced piperazine-oxadiazole compounds were evaluated for their ability to bind to N1 Neuraminidase, an enzyme linked to Alzheimer's disease, in addition to their potential as antidepressants. Given that VSM 2 and VSM 4 had the greatest binding affinities for N1 Neuraminidase among the substances examined, it is possible that these derivatives might also be used therapeutically to treat Alzheimer's disease. Because it influences the breakdown of cellular components and aids in the course of the illness, the N1 Neuraminidase enzyme is essential to the pathophysiology of Alzheimer's. The flexibility of the piperazine-oxadiazole derivatives is highlighted by the dual-target action of these compounds, where VSM

2 and VSM 4 exhibit strong binding to N1 Neuraminidase, while VSM 3 and VSM 6 show promise as antidepressants via MAO-A inhibition. These substances are promising candidates for further study and development in the domains of neurology and psychiatry since they may be able to address many therapeutic targets at once. These substances may be developed into multipurpose medications that may treat mood disorders like depression as well as neurodegenerative illnesses like Alzheimer's disease because of their capacity to control both central nervous system targets. All things considered, the results of these investigations highlight the potential of compounds based on piperazine as multipurpose therapeutic agents with wide-ranging applications in the treatment of complicated illnesses including depression and Alzheimer's disease. Further optimisation of these drugs, including enhancement of their pharmacokinetic characteristics, such as metabolic stability, blood-brain barrier penetration, bioavailability, and selectivity for the targeted targets, will be the next stages in this study.

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