DESIGN, SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF PIPERAZINE SUBSTITUTED SULPHONYLBENZENE DERIVATIVES AS SELECTIVE MONOAMINO OXIDASE-A INHIBITOR

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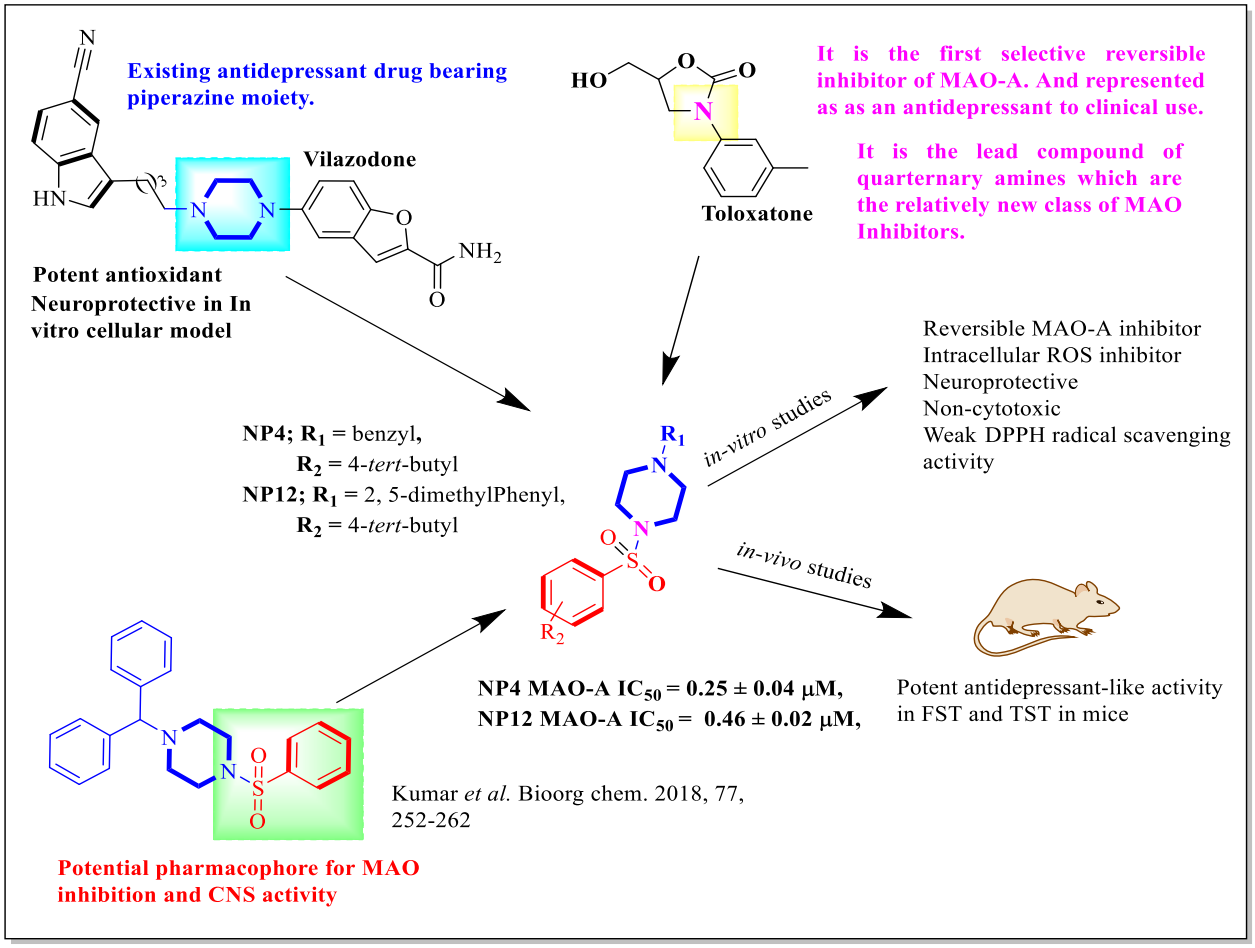
1. Abstract

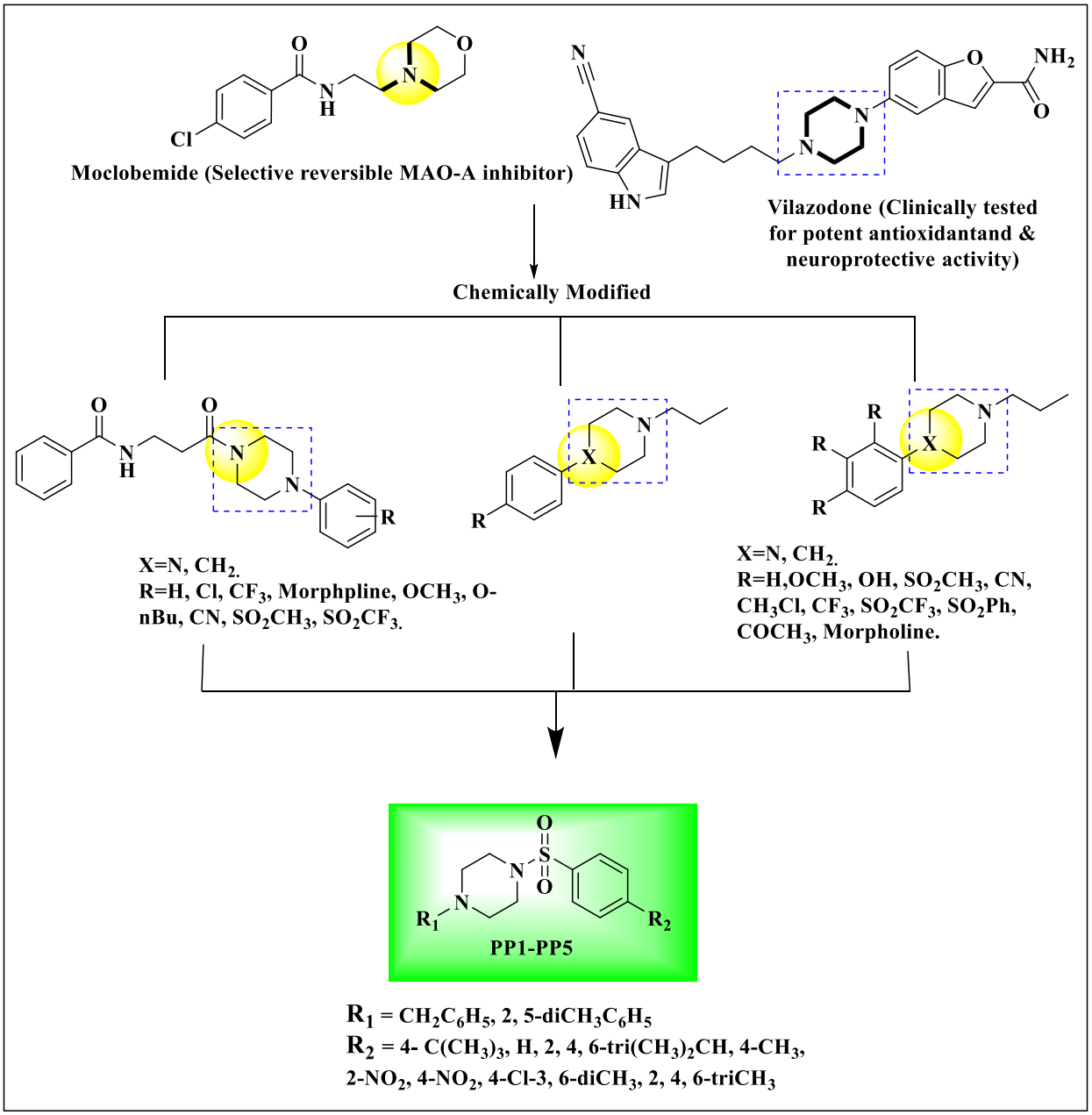
One promising pharmacological option for treating neurological conditions like depression is monoamine oxidase-A inhibitors, or MAO-AIs. The current work used the Amplex Red test to produce and evaluate a series of substituted benzene sulfonyl piperazine
(NP1-NP16) derivatives for their ability to inhibit MAO-A and MAO-B. The MAO-A isoform was found to be selective for the majority of the produced compounds, while compound
was not biased. Complicated NP2 and NP4, with IC50 values of **0.25 ± 0.04** μM and 0.46 ± 0.02 μM, respectively, demonstrated the strongest MAO-A inhibitor activity in the present series. Both compounds were also shown to be reversible inhibitors. As an MAO-A inhibitor, compound NP4 was shown to be the most selective. Every chemical was determined to be non-toxic to SH-SY5Y cells during the cytotoxicity assessment.
and demonstrate qualities that are neuroprotective. Around 50% less absorbance was seen in the DPPH experiments for compounds NP2 and NP4 at a concentration of 1 milligram. Both compounds demonstrated antidepressant-like activity in the Tail Suspension Test (TST), outperforming the conventional treatment fluoxetine, while NP4 and NP12 demonstrated possible antidepressant-like behavior in the in vivo Forced Swimming Test (FST) investigations.
Complementing the findings from the in vitro and in vivo investigations were the molecular docking studies. The functionalized piperazinyl derivatives with substituted benzenesulfonyl was therefore discovered to be a promising ligand and may be used to create novel antidepressant compounds.



Introduction

The flavin adenine dinucleotide (FAD)-containing outer mitochondrial membrane-bound enzyme monoamine oxidase (MAO, EC 1.4.3.4) is found in the brain's neuronal, glial, and other cells as well as in the peripheral (1). At the cysteine residue, a riboflavin bond covalently connects the FAD co-factor to MAO. The amount of biogenic and foreign amines in the brain and other peripheral tissues is regulated by MAO through the catalysis of their oxidative deamination. Among the monoamines it catalyzes during their oxidative deamination are serotonin, dopamine, histamine, adrenaline, and noradrenaline (2). With a sequence similarity of about, the MAO enzyme has two isoforms: MAO-A and MAO-B. 73% Despite variations in substrate specificity and inhibitor selectivity (3, 4). MAO-A deaminates serotonin and is blocked by clorgyline (5), whereas MAO-B deaminates benzylamine and 2-phenylethylamine and is inhibited by (R)-deprenyl. Iproniazid, an anti-tuberculosis drug that elevated mood in sad people, was the first MAO inhibitor used to treat depression (6). Following it were propargylamines, phenelzine, isocarboxazid, tranylcypromine, and imipramine (7, 8). These drugs were all irreversible, non-selective MAO inhibitors that were associated with a number of unfavorable side effects, including as serotonin syndrome and the "cheese effect.", as well as fatal drug-drug interactions (9, 10). New generations of MAO inhibitors were thus developed that were selective for a single MAO isoform, reversible, and free of a number of side effects. It is now recognized that the MAO enzyme is an important

and attractive therapeutic target for the treatment of various neurogenic disorders (11, 12). Due to recent publications, there is a significant deal of interest in investigating the potential of MAO inhibitors for neuroprotection and neuro-recovery. Piperazine, one of the most promising heteroaromatic nuclei, is an essential part of most psychoactive drugs (13, 14). To address depression and other neurodegenerative conditions, many compounds of phenylpiperazine have been developed and tested for their capacity to inhibit MAO (15–18). Pessoa-Mahana et al. (19) identified moclobemide 4-arylpiperazine derivatives as a new class of antidepressants with inhibitory efficacy against MAO-A and an affinity for 5-HT1A. Both 4-phenylpiperazines and para-substituted 4-phenylpiperidines have been synthesized and studied as monoamine oxidase inhibitors. It was found that para substituents with low dipole moments have higher affinity to MAO-A compared to those with high dipole moments (19). A strong correlation was found between striatal DOPAC levels and affinities for the dopamine D2 receptor subtype and MAO-A isoform in recent studies on monosubstituted 4-phenylpiperidines and 4-phenylpiperazines (20). These results suggest that small compounds containing a piperazine nucleus have a substantial MAO inhibitory efficacy; more specifically, the molecules' selectivity towards MAO-A is attributed to the quaternary amine of the piperazine nucleus. In order to evaluate the benylpiperazine and 2, 5-dimethylphenylpiperazine derivatives' potential as MAO inhibitors, we synthesized them for the current study and employed the Amplex Red test. Most compounds exhibited very good inhibitory efficacy against the MAO enzyme as compared to the standard inhibitors. The generated compounds were found to fit snugly into the active cavity of the MAO enzyme after molecular docking tests. Since these compounds were also examined for reversibility, cytotoxicity, and ROS inhibition capacity, it is expected that they could serve as multifunctional agents.



2. Result and Discussion

2.1. Chemistry

Synthesis and docking studies of substituted benzene sulfonyl piperazine derivative have been reviewed in the introduction chapter of this dissertation. In the course of program devoted towards the synthesis of new piperazine derivatives as therapeutically effective agent, we have recently synthesized various substituted benzene sulfonyl piperazine derivatives. Our present research work divided into four parts:

Synthetic study

Docking Study of synthesized compound with *MAO-A* protein

Animal Study

In -vitro, *MAO-A* enz (-) inhibition & antioxidant activity

2.2 Synthetic work

It describes the general steps used to synthesize all of the intermediates and the title compound. TLC tracked the reactions' development. We made sure to indicate the reaction's completion from the TLC. The TlC plates were seen in an iodine chamber and under UV light. Different work-up procedures were used to purify the reaction products in order to get rid of contaminants and unreacted starting material. To get a pure sample of the title compounds, recrystallization, either once or twice, was carried out using the appropriate solvents. Using spectral data (FTIR, H-NMR, and physicochemical characterisation data, melting points and Rf values of all final compounds were verified.

2.3 Synthetic scheme

2.3.1. Genral procedure for the synthesis of PP1-PP5

Potassium car-bonate (2 eq.) and an appropriate alternative to piperazine (g, 1 eq.) were combined in a round-bottom flask with 7 ml. of dichloromethane. At the ice bath, the reaction mixture was agitated for ten to fifteen minutes until the piperazine zero point three (0.3) grms.was entirely dissolved. The reaction mixture was then gradually supplemented with 1 equivalent of substituted benzenesulfonyl chloride, and it was left in an ice bath until it had formed a homogeneous layer. It was then , stir for three to four hrs., in room temp. TLC tracked the course of the reaction. following the conclusion of the response. At the Rota Evaporator, dichloromethane was evaporated. Water was then used to create solid precipitation. To obtain end compound , the solid precipitate were filtered, cleaned with the water, then dried using hexane. final product purification is done by column -chromatography and re-crystallization method.

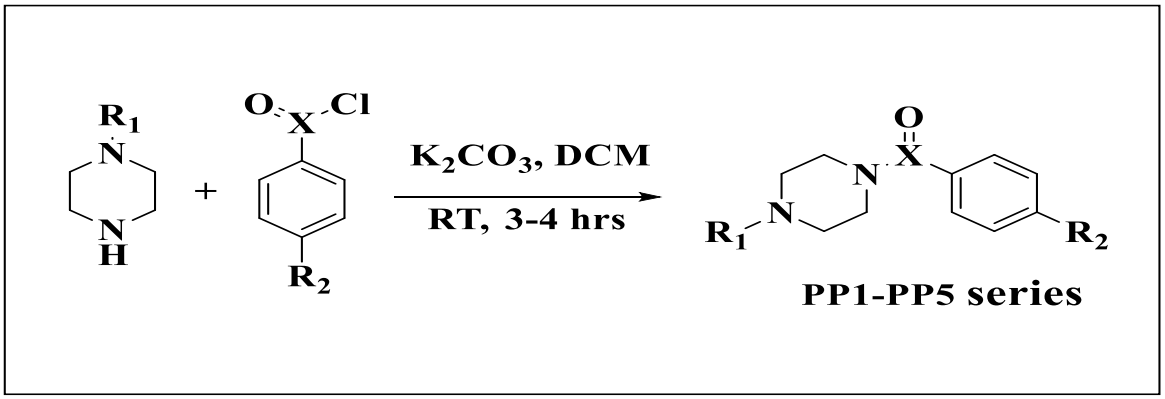


Fig. no. 2.1 Scheme1: synthesis of PP1-PP5

Table 2.1: physicochemical characterization data for the final compound PP1-PP5

S. No.	Compound Name	Chemical Formula	Colour	Molecular Mass	Melting point	% yield	Rf
1.	**PP1**	$C_{23}H_{23}N_3O_4S$	Pale yellow colour solid	437.51	182-184	68%	0.45
2.	**PP2**	$C_{23}H_{24}N_2O_2S$	Pale yellow colour solid	392.52	164-169	84%	0.46
3.	**PP3**	$C_{25}H_{27}ClN_2O_2S$	White yellow colour solid	455.01	183-186	76%	0.53
4.	**PP4**	$C_{23}H_{21}ClF_2N_2O_2S$	White colour solid	462.95	156-158	88%	0.55
5.	**PP5**	$C_{23}H_{23}N_3O_4S$	Yellow colour solid	436.51	162-166	75%	0.48

2.2 *h MAO-A* enzyme inhibition studies

The fluorimetric approach was utilized to assess the MAO-A inhibition capability of the substituted benzenesulfonyl piperazine derivatives (PP1-PP5) by employing recombinants human's MAO- A enzyme and Amplex® Red test kit (148). Table 5.2 provides an explanation of the MAO-A inhibition studies' outcomes in terms of IC50 values presented in micro molar conc. Clorgiline was employed as a standard inhibitor for *MAO-A*, and the most effective inhibitor was determined to be PP2 (1-benzyl-4-tosylpiperazine), the I C ,- 50 val. of 0.25 ± 0 .04 µM.With an IC-50 val of 0.46 ± 0.02 µ M, PP4 (1 - (2 , 5-dimethylphenyl)-4-tosylpiperazine) was also discovered to be the most effective MAO-A inhibitor. Similarly, it was discovered that PP2 and PP4, with the IC-50, val. of 0.56 ± 0 .17 µ M and 0.87 ± 0.07 µM, respect-ively, were most effective MAO-A inhibitors.

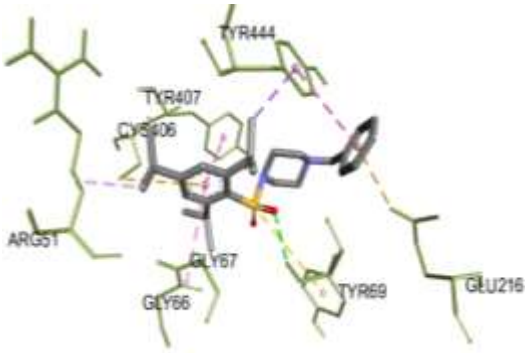
Table 2.2: Result's of the MAO- enzyme inhibition study of synthesized's compounds

Name	**R1**	**R2**	**IC- $_{50}$ value (mean. ± S . E. µM)**
			hMAO-.A
PP1	Benzhydryl	2-nitrobenzenesufonyl chloride	0.55 ± 0.16
PP2	Benzhydryl	Benzene sufonyl chloride	0.48±0.03
PP3	Benzhydryl	4-chloro-2,5-dimethyl benzenesulfonyl chloride	**11.33 ± 0.67**
PP4	Benzhydryl	3,5 Difluorobenzene sulfonyl chloride	0.24 ± 0.06
PP5	Benzhydryl	4-nitrobenzenesulfonyl chloride	**13.85 ± 0.68**
Clorgiline			**-1.02nM**

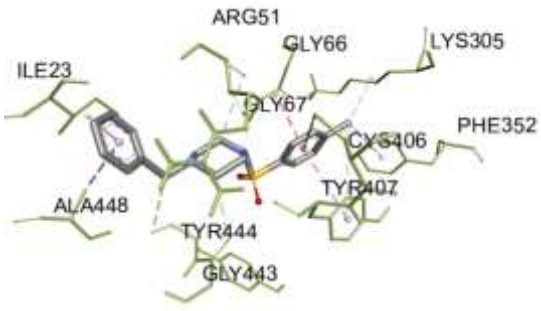
2.3 Molecular docking studies.

signing the Autodock 4.2 tool, all of the produced compounds were put through docking tests against , - the MAO-A, enz (PDB, ID:, 2B-XR). According to series, the synthesized molecule suited the MAO-A active site just as well as the conventional inhibitor clorgyline did. According to the docking studies, PP1 and PP4 had the highest affinity values (-9.2 and -(-9.7), respectively) for MAO- A. Compound PP1 has been shown to interact with the TYR - 444, TYR - 407, FAD - 600, ILE- 335, LEU- 337, CYS 323, and IL E -325 and Compound PP4. with amino's acid residue TYR 444, TYR 407, CYS 406, FAD 600, ARG 51, ALA448, GLY 443, GLY 67, ILE23, PHE352, and GLY66.

S. No.	Compound Name	Affinity (Kcal/mol)
1	**Clorgyline**	-6.6
2	PP1	**-9.6**
3	PP2	-8.3
4	PP3	-8.4
5	**PP4**	**-9.2**
6	PP5	-7.5



PP1



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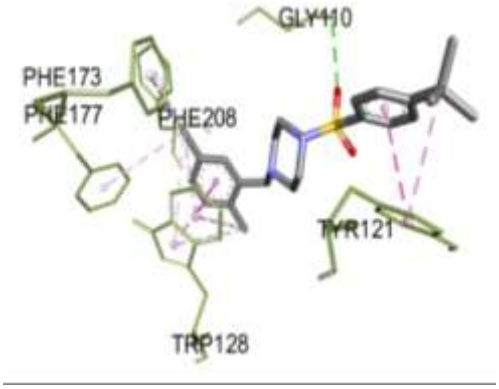

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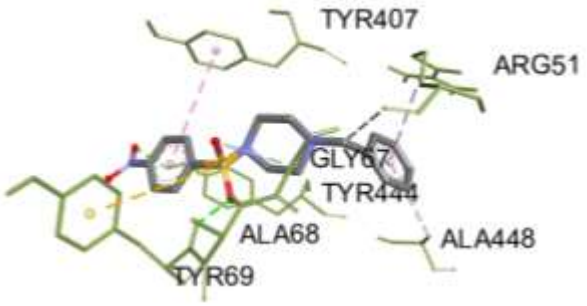
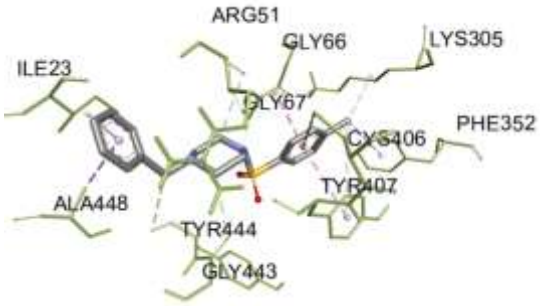
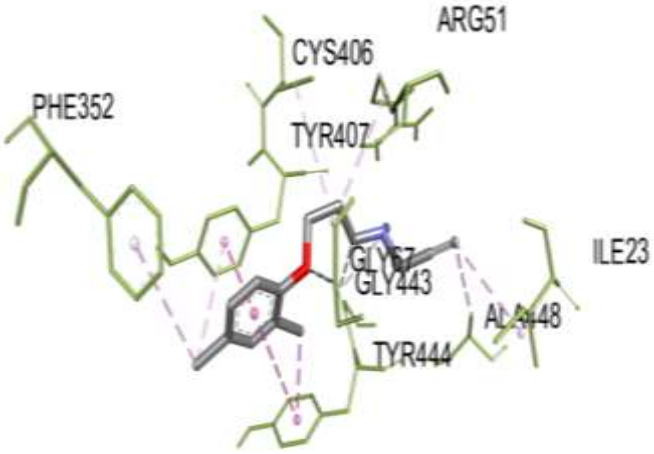


PP4



PP5



PP6

Clorgiline

2.4 Antioxidant activity

In the DPPH test, the produced compounds showed variable degrees of ability to scavenge free radicals. Table 5.3 listed all of the produced compounds' absorbances as well as the percentage decrease in absorbance, using ascorbic acid as a benchmark. At a conc of the 1.0

mm, compound PP4 and PP2 exhibit reductions in absorbance of 45.65% and 50%, respectively.

Table 2.3: Antioxidant activity of compounds (PP1-PP5)

Compound. Code	**Abs. at the 517.0 nm (%,) percentage Reduction in abs.)**		
	0.01 mM	**0.1 mM**	**1.0 mM**
Cont.	**0. 806**	**0. 806**	**0.806**
Ascorbic acid	0.753 (6.35)	0.504 (37.87)	0.083 (89.97)
PP1	0. 779 (3.34)	0.645 (19.97)	0.485 (39.82)
PP2	**0.737 (8.70)**	**0.554 (30.66)**	**0.402 (50.03)**
PP3	0.754 (6.61.)	0.653 (17.84)	0.589 (25.65)
PP4	**0.695 13.52)**	**0.575 (29. 24)**	**0.434 (45. 69)**
PP5	0.792 (1.36)	0.704 (9.32)	0.587 (25.83)

$$\% \boldsymbol{Reduction\ in\ absorbance} = (\boldsymbol{Abscontrol} - \boldsymbol{Abstest})/\boldsymbol{Abscontrol} \times \mathbf{100}$$

2.5 Pharmacological Evaluation

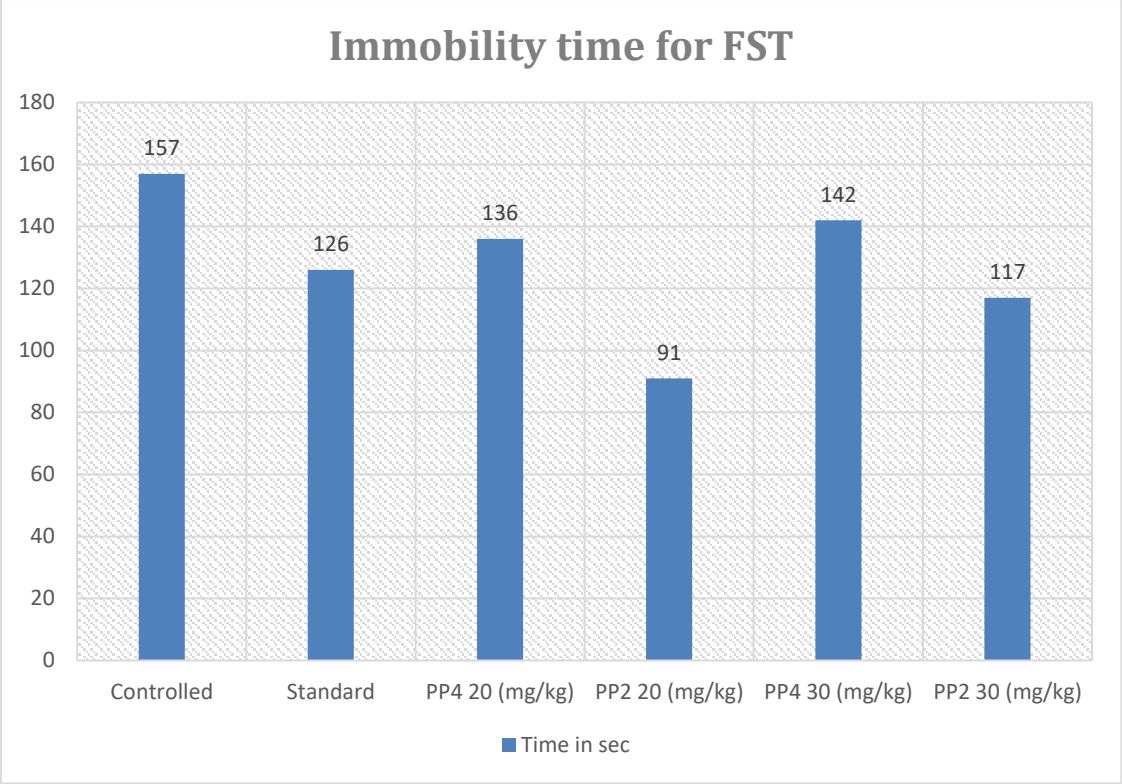
2.5.1 FORCE SWIMMING TEST (FST)

Using the FST, a major model of depression, tests were conducted on the antidepressant-like activity of two of the most effective compounds, PP2 and PP4. The normal medicine

clorgiline was given to the mice dose, of 20.0 mg. / kg, whereas PP2 & PP4 was given a doses of 20.0 mg / kg and 30.0 mg / kg. Every medication was taken orally. Mice were given FST for two hours after injection, and the length of time they remained immobile was noted. The most notable decrease in immobility time was seen with the conventional medication clorgiline when compared to the control group. group under control (157 sec) Compared with the std. group (126.0 sec), mice are treated with compounds PP4 at with a dose of 20 mg/kg and 30 mg/kg demonstrated a substantial ($p <. 0.06$) reductions in the immobilities, period at (139 sec) and (91 sec) Conversely, in in vivo FST investigations, compound PP4 at a drug dose of 20 mg/kg 30 mg/kg were obtained to the active and decreased immobility time (136 sec) in to the cont. group (117 secs) (Figure refer below).

Table no. 2.4 Immobility time for FST

Sr. no.	**Controlled**	**Standard**	**Test 1 (PP4) 30 mg**	**Test 2 (PP2) 20 mg**	**Test 1 (PP4) 20 mg**	**Test 2 (PP2) 30 mg**
1.	156	124	139	122	136	133
2.	182	139	140	116	142	138
3.	187	140	142	96	148	103
4.	174	128	143	104	139	110
5.	180	119	129	92	122	120
6.	163	133	138	113	133	101
Avg.	**157 sec**	**126 sec**	**139 sec**	**91 sec**	**136 sec**	**117 sec**



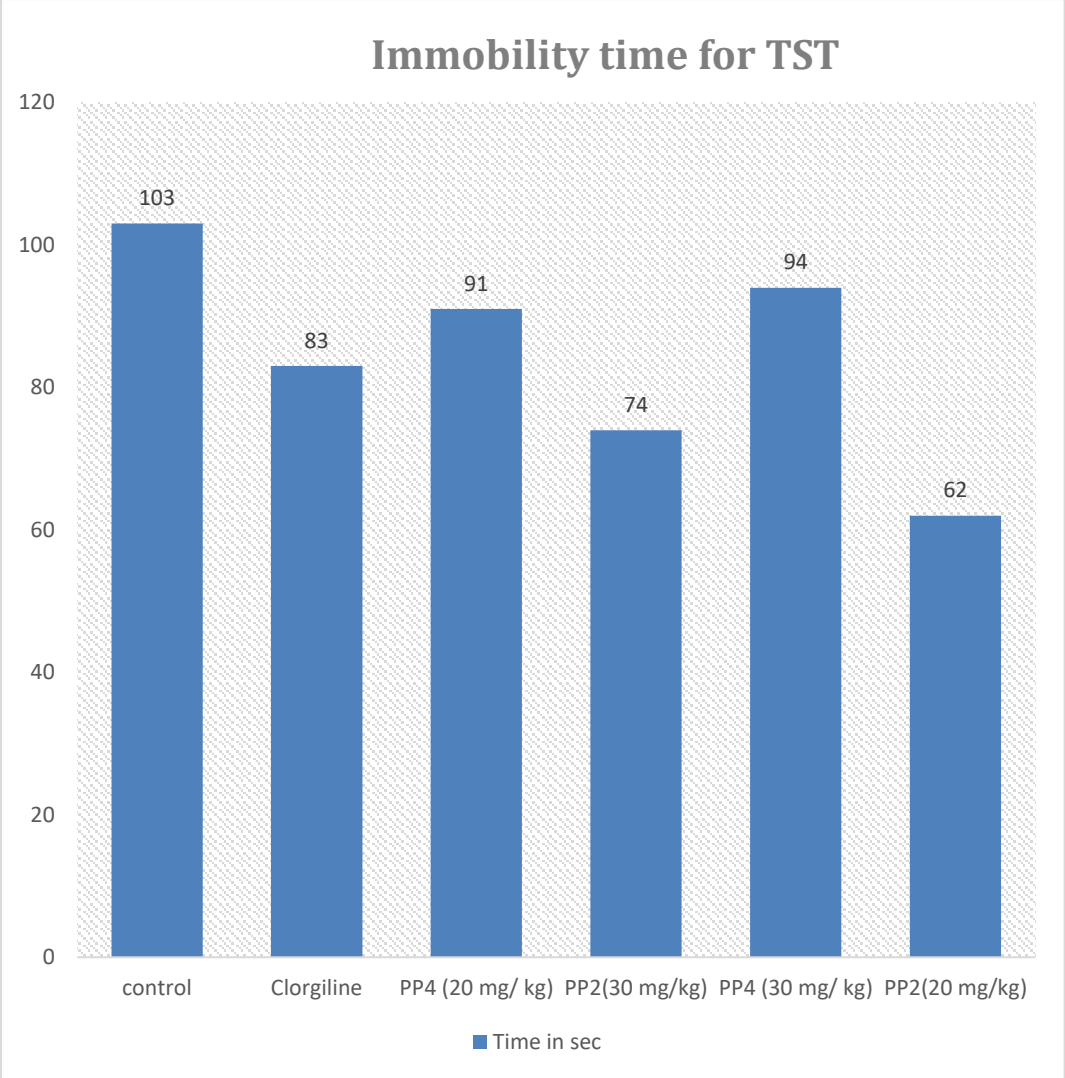
Graph 2.1: In force swimming tests (FST), clorgiline (20 mg / kg , i.p.) and PP2 and PP4 (20.0 mg / kg and 30 mg / kg, orally) shown antidepressant action. Comparison b/w treated group and cont are indicated by asterisks, and statis-tical signi-ficance of p <. 0.06 is shown by the symbol, Control vs. PP).

2.5.2 TAIL SUSPENSION TEST, (TST)

Further corroborate this effect, additional compounds PP2 and PP4 were examined in tail-suspension, test (at, the same dose), which are other model for antidepressants like activities. A horizontal rod was used to hang the treated mice's tails, and the duration of their immobility was noted. Figure 4.8 illustrates a noteworthy decrease in immobility time for both drugs when compared, to- the cont group and clorgiline (20 mg/kg). Two hours after administration, the immobility time was noted in the TST. After compound PP10 was administered a concentrations of 20.0 mg / kg and with 30.0 mg, / kg, the immobility period was observed to be reduced by a highly significant amount ($p<0.06$), measuring 83 sec and 96 seconds, respectively. Compound PP4 was shown to be comparable to the standard medication clorgiline in terms of immobility duration, exhibiting a considerable reduction at with conc. of 20.0 mg, / kg & 30.0 M g / k g (91 sec 74 seconds, respectively).

Table no. 2.5 Immobility time for TST

Sr. no.	**Controlled**	**Standard**	**Test 1 (PP4) 20 mg**	**Test 1 (PP2 20 mg**	**Test 2 (PP4) 30 mg**	**Test 2 (PP2) 30 mg**
1.	89	98 sec	94	67	92	89
2.	113	126 sec	115	60	112	74
3.	107	111 sec	96	57	86	84
4.	95	120 sec	99	62	89	70
5.	101	96 sec	95	68	84	68
6.	114	87 sec	82	61	83	64
Avg.	**103 sec**	**83 sec**	**96 sec**	**62 sec**	**91 sec**	**74 sec**



Graph 2.2 : In which tail- suspension test (TST), clorgiline, (20.0 mg / kg, i.p.) and PP2 and PP4 (20 mg/kg and 30 mg/kg, orally) demonstrated antidepressant effect. Comparision b/w the treated grps and cont. are indicated by asterisks, and statistical signi- ficance of $p < 0.06$ is shown by the symbol Control vs. PP).

2.2. Reversibility studies

Most Several side effects, such as the cheese effect, drug-food interactions, and drug-drug interactions, were caused by the majority of first-generation MAO inhibitors, which were also irreversible. That being said, reversibility is a crucial property of MAO inhibitors and is often pursued in the creation of novel MAO inhibitors. Utilizing established methods, the reversibility of the most powerful molecule, ---- (MAO-A), as well as other compounds, such as ----, ----, ---, and ----, which shown strong selectivity for MAO-A isoform, was assessed (22, 23). Reversible MAO-A isoform inhibitors were discovered in compounds ---- and ----. and up to 67.32% and 63.56%, respectively, of the MAO-A enzyme's activity could be restored (figure 2). Since there was no discernible recovery in the MAO-A activity (8.28%) with the addition of substrate, the chemical ---- (an inhibitor of MAO-A) was determined to be irreversible. On the other hand, the conventional inhibitor, clorgyline (MAO-A), did not exhibit any recovery in activity following substrate treatment, confirming the irreversible nature of these reactions as documented in the literature.

3.3 DPPH Assay

The DPPH experiment, the produced compounds shown variable capabilities for scavenging free radicals (24, 25). Ascorbic acid was used as a reference, and all synthesized compounds' absorbance and percentage reduction in absorbance were listed in Table 2. Compounds NP4 and NP12 exhibit a 50% and 45.65% decrease in absorbance, respectively, at a 1 mM concentration.

% Reduction in absorbance= ($Abs_{control}$-Abs_{test})/$Abs_{control}$ × 100

Table 2: Antioxidant activity of compounds (PP1-PP5)

Compd. Code	**Absorbance at 517nm (%Reduction in absorbance)**		
	0.01mM	**0.1mM**	**1mM**
Control	0.806	0.806	0.806
Ascorbic acid	0.755 (6.32)	0.501 (37.84)	0.081 (89.95)
PP1	0.779 (3.34)	0.645 (19.97)	0.485 (39.82)
PP2	0.769 (4.59)	0.610 (24.31)	0.603 (25.18)
PP3	0.752 (6.69)	0.654 (18.85)	0.599 (25.68)

PP4	0.697 (13.52)	0.570 (29.28)	0.438 (45.65)
PP5	0.795 (1.36)	0.701 (9.30)	0.598 (25.80)

2.3. Neuroprotective studies

Compounds 6-hydroxydopamine (6-OHDA) neurotoxin was used to test the neuroprotective qualities of compounds NP4 and NP12 against SH-SY5Y cells (26). The primary cause of depression's progression is neurodegeneration, and neuroprotective substances can aid in a quick recovery from depression. According to the findings of these in vitro investigations, at concentrations of 25 μM, neuronal cell survival increases and these substances have mild neuroprotective qualities at lower levels (figure 3). Compared to cells treated with 6-OHDA (----%) alone, compound NP4 demonstrated cell viability of -----% at 25 μM. Nevertheless, compound PP5 increased the survival of the cells by ----%, indicating that it has only marginal neuroprotective effects.

2.4. Cytotoxicity

Cytotoxicity of major obstacle in the creation of novel therapeutic molecules is the cytotoxicity of manufactured substances. Using SH-SY5Y cells, MTT experiments were conducted to assess the toxicity of the most promising chemicals against the neuronal cell (27). Figure 4 illustrates that neither NP4 nor NP12 exhibited any cytotoxicity towards these cells when tested at concentrations of 1 μM and 10 μM. When compound NP12 was added at a concentration of 100 μM, the control group's cell viability percentage was 87% lower.

Because there is no neurotoxic present in this instance, the compound's modest neuroprotective qualities may be the reason why cell survival was seen to be higher at lower doses than in the control group. Based on the low submicromolar IC50 value of these drugs, it can be inferred that neither of them is harmful to SH-SY5Y neuronal cells.

Prediction of ADME Parameters

Drug A drug's ability to balance a variety of molecular and structural characteristics, including stability, oral availability, high pharmacokinetic qualities, low propensity for addiction, and minimal toxicity, is known as drug likeness. Determining the safety and target-reaching capability of proposed compounds requires the use of the ADMET prediction for CNS active drugs. Correlating attempts are challenging due to the complicated structure of the entire drug molecule, as many of these features rely on the molecule's intrinsic biological and physicochemical parameters.

In silico drug-likeness A Swiss ADME predictor tool was used to predict the drug-likeness of several designed and manufactured compounds in silico. Lipinski's rule of drug-like

molecules is followed by the synthesized compounds, according to the results derived from prediction data (Table 3). The majority of the synthetic compounds had LogP values that were close to or less than 5. The molecular weight is less than 500, and it is advantageous to have HBD and HBA atoms present.

The compounds' oral absorption percentage (80–100%) was encouraging. Furthermore, each chemical exhibited CNS action and was able to pass through the blood-brain barrier (BBB), which is necessary for reaching the target spot in the brain. The produced compounds have an ideal chemical skeleton that can be developed into possible therapeutic molecules, meaning that Lipinski's criteria has not been broken.

Table 3: Physiochemical properties of designed derivatives

Compounds	**TPSA**	**MW**	**RoB**	**HBD**	**HBA**	**LogP**	**LogS**	**BBB**
PP1	49.00	372.52	5	0	4	3.77	-4.71	Yes
PP2	49.00	316.42	4	0	4	3.11	-3.44	Yes
PP3	49.00	442.66	7	0	4	4.57	-6.03	Yes
PP4	49.00	330.44	4	0	4	3.19	-3.74	Yes
PP5	94.82	361.42	5	0	6	2.54	-3.49	No

2.5. Molecular Docking

All The Autodock 4.2 tool was utilized to conduct docking tests on all the produced compounds against the MAO-A enzyme (PDB ID: 2BXR) (28) in vitro. The produced molecule suited the MAO-A active site well, similar to the conventional inhibitor Clorgyline, according to the series. The results of the docking investigations indicated that, with -9.2 and -9.7, respectively, NP4 and NP12 had the highest affinity towards MAO-A. Compound NP12 interacts with TYR444, TYR407, FAD600, ILE335, LEU337, CYS323, and ILE325 while Compound NP4 interacts with amino acid residues TYR444, TYR407, CYS406, FAD600, ARG51, ALA448, GLY443, GLY67, ILE23, PHE352, and GLY66 (figure 5).

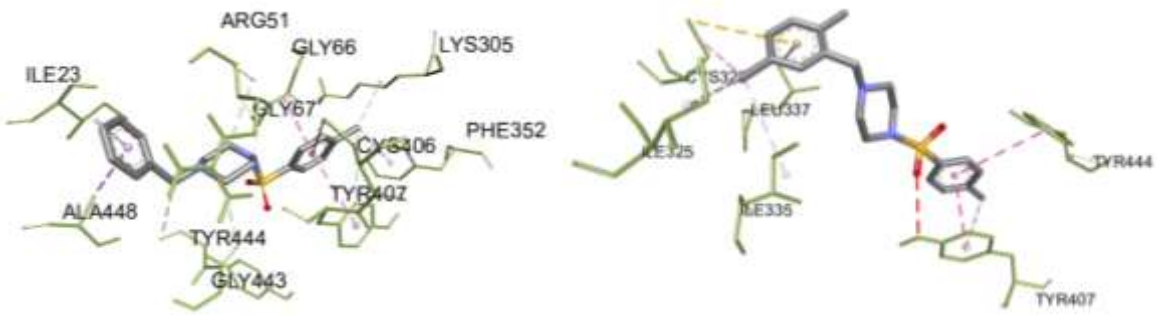


Fig. 5: 2D Binding interactions of compounds PP2 and PP4 with the active site residues of MAO-A (PDB ID: 2BXR);

2.6. Pharmacological evaluation

2.6.1. FST

The Using FST as a major model of depression, antidepressant-like activity investigations were conducted on the two most effective compounds, NP4 and NP12 (29). The normal medicine fluoxetine was given to the mice at a dose of 20 mg/kg, while NP4 and NP12 were given at doses of 20 mg/kg and 30 mg/kg. Every medication was taken orally. Mice were given FST for two hours after injection, and the length of time they remained immobile was noted. Regarding the common medication fluoxetine, The greatest decrease in immobility duration was noted when compared to the control group. Comparing the immobility period (97 seconds) of mice treated with compound NP4 at a dose of 30 mg/kg to that of the control group, there was a substantial ($p<0.05$) reduction. However, compound NP12 was found to be active in in vivo FST trials at a dose of 30 mg/kg, and it shortened the immobility period (67 seconds) compared to 128 seconds for the control group (Figure 6).

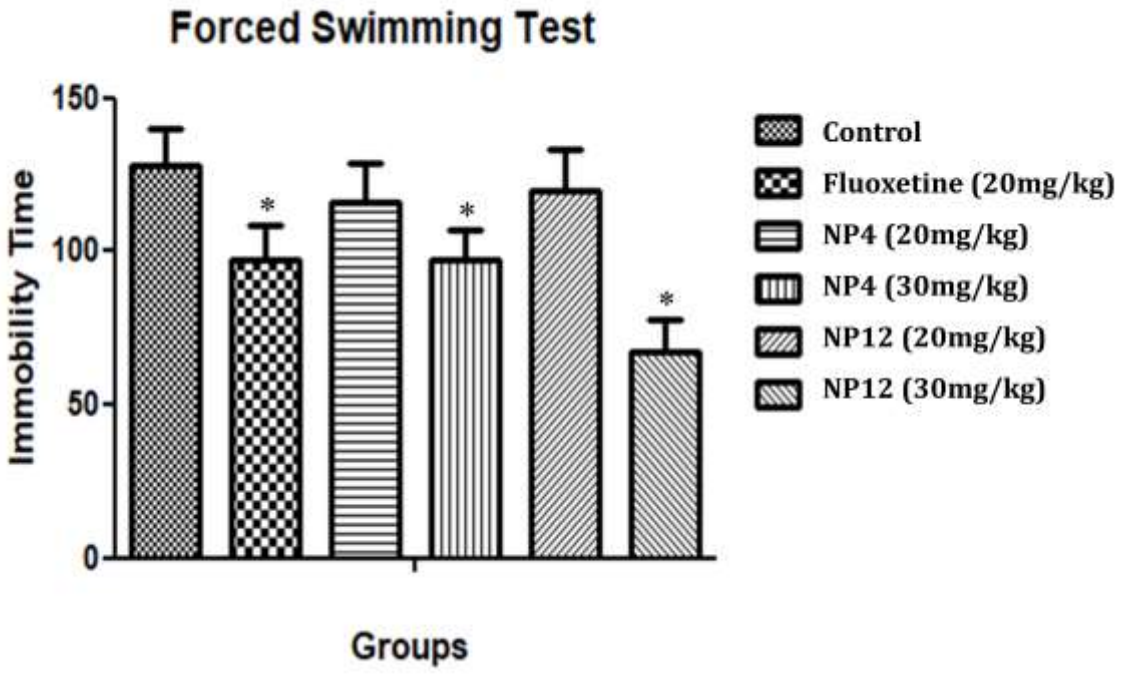


Fig. 6: Antidepressant activity of NP4 and NP12 (20 mg/kg and 30 mg/kg, orally) and fluoxetine (20 mg/kg, i.p.) in the force swimming test (FST). Asterisks represent comparisons between the treatment group and the control. * represent statistical significance of $p < 0.05$ (one-way ANOVA, Control vs NP).

2.6.2. TST

Compounds **NP4** and **NP12** were also tested (at the same dose) in the tail suspension test, which is another model of antidepressant-like activity to further confirm this activity (30). Treated mice were suspended with a tail in a horizontal rod and immobility time was recorded. Both the compounds showed a significant reduction in immobility time (**Figure 7**) as compared to fluoxetine (20mg/kg) and the control group. After 2 hrs of administration, TST immobility time was recorded. The reduction in the immobility time was found to be highly significant ($p<0.05$) after the administration of compound NP12 at concentrations of 20mg/kg and 30mg/kg immobility time was (113 and 34.1 seconds) respectively. In TST, compound NP4 also showed a significant reduction in immobility time at concentrations of 20mg/kg and 30mg/kg (81.5 and 69.6 seconds, respectively) as compared to the control group (211 seconds) which was found comparable to the standard drug fluoxetine.

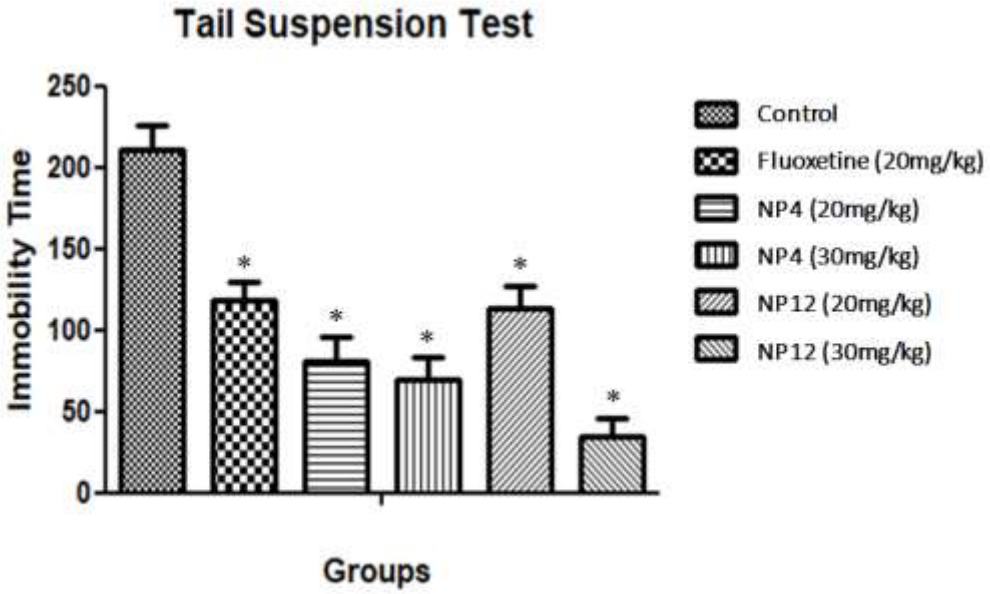


Fig. 7: Antidepressant activity of NP4 and NP12 (20 mg/kg and 30mg/kg, orally) and fluoxetine (20 mg/kg, i.p.) in the tail suspension test (TST). Asterisks represent comparisons between the treatment group and the control. * represent statistical significance of $p < 0.05$ (one-way ANOVA, Control vs NP).

3. Conclusion

DepressionAccording to the WHO, depression ranks as the second most common cause of disability globally. The need for new antidepressants has increased due to the rise in depression cases among young people, adolescents, and the elderly population. Monoamine

oxidase-A (MAO-A) has long been the most promising target for the creation of novel antidepressants. The synthesis and screening of several piperazine derivatives with substituted benzenesulfonyl chlorides have been described. The structural identity of the synthesized compounds was confirmed using spectral investigations including FT-IR, 1HNMR, and 13CNMR, as well as physicochemical tests like melting point and TLC. hMAO-A enzyme inhibition experiments were performed in vitro on all of the produced compounds. NP4 and NP12, with IC50 values of 0.25 ± 0.04 µM and 0.46 ± 0.02 µM, respectively, have the strongest inhibitory action against MAO-A enzymes, according to the results of in vitro enzyme inhibition tests. Additionally, all of the produced compounds were tested for antioxidant activity, and it was discovered that at a concentration of 1 mM, compounds NP4 and NP12 had an absorbance reduction of 45.65% and 50%, respectively. Studies using in vivo FST and TST were conducted on the two most powerful compounds, NP4 and NP10. Compounds NP4 and NP12 have strong antidepressant efficacy ($p<0.05$) in both FST and TST, according to the in vivo findings. The findings support and demonstrate these compounds as strong candidates for additional research and development into antidepressant drugs. Using the Autodock 4.2 tool, all of the produced compounds were put through docking tests against the MAO-A enzyme (PDB ID: 2BXR). It was discovered from the series that every produced molecule suited the MAO-A active site too well, just like the conventional inhibitor clorgyline does. The results of the docking investigations indicate that NP4 and NP12 had the highest affinity for MAO-A, with corresponding values of -9.2 kcal/mol and -9.7 kcal/mol. The current study concluded that a novel class of antidepressant medicines is represented by the tertiary nitrogen of piperazine clubbed with benzenesulfonyl derivatives. Additional research utilizing the discovered lead compounds will be beneficial in the creation and design of the antidepressant activity.

Experimental

3.1. Chemistry

3.1.1. General procedure for the synthesis of the target compounds (NP1-NP16)

A mixture Potassium carbonate (2 eq) and appropriate mono-substituted piperazine (0.5 g, 1 eq) were combined in a round-bottom flask with 5 mL of dichloromethane. A stir was given to the reaction mixture. Subsequently, matching substituted benzene sulfonyl chloride (1 eq) was added gradually to the reaction mixture and placed in an ice bath. After that, stirred for another three to four hours at room temperature. TLC tracked the reaction's development. Dichloromethane was removed from the mixture using a rotating evaporator once the reaction was finished. Next, ethyl acetate and water were used to extract the output. To obtain the final product's crystals, the ethyl acetate layer was filtered, evaporated, and then washed and dried with hexane to yield the crude solution. Column chromatography and re-crystallization were used to purify the finished products. IR, NMR, and physicochemical features were used to characterize the final compounds. TLC was used to assess purity.

1-benzyl-4-((4-(tert-butyl)phenyl)sulfonyl)piperazine (NP1)
Yield: 66%, **M.P.** = 182-184°C, **FTIR (cm^{-1}):** 1242 (CN stretch), 3069 (Ar-CH stretch), 2925 (CH stretch), 946.58 (S=O stretch), 1166 (CC stretch), 1919 (C=C stretch). **^{1}H NMR (400 MHz, $CDCl_3$):** δ = 7.67 (2H, d, J = 8Hz, Ar-H), 7.52 (2H, d, J = 4Hz), (Ar-H) 7.28-7.22 (5H, m, Ar-H), 3.48 (2H, s, CH_2), 3.03 (4H, t, J = 4Hz, Piperazine), 2.53 (4H, t, J = 4Hz, Piperazine), 1.34 (9H, s).

*1-benzyl-4-(phenylsulfonyl)piperazine **(NP2)***
Yield: 84%, **M.P.** = 167-169°C, **FTIR (cm^{-1}):** 1283 (CN stretch), 3069 (Ar-CH stretch), 2922 (CH stretch), 958 (S=O stretch), 1171 (CC stretch), 1612 (C=C stretch). **^{1}H NMR (400 MHz, $CDCl_3$):** δ = 7.74-7.51 (5H, m, Ar-H), 7.29-7.25 (5H, m, Ar-H), 3.59 (2H, s, CH_2), 3.10 (4H, t, J = 4Hz), 2.63 (4H, t, J = 4Hz).

*1-benzyl-4-((2, 4, 6-triisopropylphenyl)sulfonyl)piperazine **(NP3)***
Yield: 77%, **M.P.** = 184-186°C, **FTIR (cm^{-1}):** 1350 (CN stretch), 3069 (Ar-CH stretch), 2813 (CH stretch), 958 (S=O stretch), 1171 (CC stretch), 1598 (C=C stretch). **^{1}H NMR (400 MHz, $CDCl_3$):** δ = 7.34-7.23 (5H, m, Ar-H), 7.16 (1H, s, Ar-H), 7.08(1H, s, Ar-H), 4.14(2H, s, CH_2), 3.30-2.91 (4H, Piperazine), 2.61-2.03 (4H, Piperazine), 1.26 (21H, Isopropyl).

*1-benzyl-4-tosylpiperazine **(NP4)***
Yield: 89%, **M.P.** = 155-157°C, **FTIR (cm^{-1}):** 1293 (CN stretch), 3030 (Ar-CH stretch), 2947 (CH stretch), 945 (S=O stretch), 1169 (CC stretch), 1654 (C=C stretch). **^{1}H NMR (400 MHz, $CDCl_3$):** δ = 7.63-7.61 (4H, m, Ar-H), 7.31-7.22 (5H, m, Ar-H), 3.46 (2H, s, CH_2), 3.00 (4H, Piperazine), 2.50 (4H, Piperazine), 2.42 (1H, s, CH_3
*1-benzyl-4-((2-nitrophenyl)sulfonyl)piperazine **(NP5)***
Yield: 74%, **M.P.** = 147-149°C, **FTIR (cm^{-1}):** 1219 (CN stretch), 2980 (CH stretch), 954 (S=O stretch), 1155 (CC stretch), 1635 (C=C stretch), 1537 (N=O Ass. Stretch), 1358 (N=O Sym. Stretch). **^{1}H NMR (400 MHz, $CDCl_3$):** δ = 7.94-7.58 (4H, m, Ar-H), 7.51-7.23 (5H, m, Ar-H), 3.51 (2H, s, CH_2), 3.30 (4H, t, J = 4Hz), 2.52 (4H, t, J = 4Hz

*1-benzyl-4-((4-nitrophenyl)sulfonyl)piperazine **(NP6)***
Yield: 82%, **M.P.** = 150-151°C, **FTIR (cm^{-1}):** 1200 (CN stretch), 3114 (Ar-CH stretch), 2949 (CH stretch), 954 (S=O stretch), 1133 (CC stretch), 1607 (C=C stretch), 1529 (N=O Ass. Stretch), 1355 (N=O Sym. Stretch). **^{1}H NMR (400 MHz, $CDCl_3$):** δ = 8.35 (2H, d, J = 4Hz, Ar-H), 7.93-7.91 (2H, d, J = 8Hz, Ar-H), 7.25 (5H, m, Ar-H), 3.49 (2H, s, CH_2), 3.08 (4H, t, J = 4Hz), 2.52 (4H, t, J = 4Hz).

*1-benzyl-4-((4-chloro-2, 5-dimethylphenyl)sulfonyl)piperazine **(NP7)***
Yield: 73%, **M.P.** = 183-185°C, **FTIR (cm^{-1}):** , **^{1}H NMR (400 MHz, $CDCl_3$):** δ = ,

3.2. Pharmacological evaluation

3.2.1. Determination of *h*MAO inhibition activity

The As previously reported (31) we evaluated the synthesized compounds' human MAO inhibitory activity using the Amplex® Red test kit. Sodium phosphate buffer (0.05 M, pH 7.4) was used to dissolve standard or test substances at different concentrations. Following the addition of 100 µL of this solution and 1.1 µg or 7.5 µg of hMAO-A or hMAO-B to 96-well plates, the plates were incubated at 37 °C for 30 minutesFollowing the instructions included with kit A12214, Molecular Probes, Invitrogen, 200 µM Amplex® Red reagent was added, and the enzymatic reaction was allowed to incubate in the dark for 30 minutes.

Reversibility studies

We applied the dilution approach, as previously reported by us, to the NP4 and NP12 reversibility investigations (22). For this, NP4 and NP12 at concentrations of 10 × IC50 and 100 × IC50 were incubated with the MAO-A enzyme for 30 minutes at 37 °C (DMSO concentration was less than 4%). Test chemicals were swapped out for dilution solvent in control trials. The enzymatic activity was measured thirty minutes later. Subsequently, the mixture was diluted 100 times with the substrate solution, resulting in inhibitor concentrations of 0.1 × IC50 and 1 x IC50, respectively. Following dilution, the mean ± SD of the three observed residual enzymatic activity was reported.

DPPH free radical scavenging studies

The antioxidant capacity of produced compounds was assessed using the previously described DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging test (24, 32). For these investigations, a solution of 1 mM DPPH in methanol was made and left in the dark for two hours. Following a two-hour incubation period, two milliliters of this one milligram DPPH solution were placed in a tube, and two milliliters of test samples ranging in concentration from 0.01 to 1 millimeter were added. The resultant mixture was well combined, and it was allowed to sit at room temperature for half an hour in the dark. The mixture's absorbance at 517 nm was measured after 30 minutes in order to estimate the target compounds' capacity to scavenge free radicals. Standard compound utilized in all the studies was ascorbic acid, and each study was carried out in triplicate. The mean ± SD was used to express the results.

Neuroprotective studies

Using a previously published procedure (26), the neuroprotective potential of NP4 and NP12 against 6-OHDA neurotoxic against SH-SY5Y cells was also assessed. As previously mentioned, 4-hour prior to 6-OHDA (12.5 µM) treatment, SH-SY5Y cells were treated with NP4 and NP12 at varying doses (1-100 µM). The leftover chemical was taken out after 24 hours, and each well was then filled with 50 µM MTT before being incubated for a further 3 hours. Following a 3-hour duration, the MTT dye was eliminated and 100 µl of DMSO was applied to dissolve the formazan crystal that had formed. With the use of a multi-detection microplate fluorescence reader (SynergyHI, Bio-Tek® Instruments), the absorbance for cell

viability was determined at a wavelength of 570 nm. Every assay, along with the control investigations, was carried out in triplicate and the results were presented as mean ± SD.

Cytotoxicity studies

With SH-SY5Y cells, the cytotoxicity of the most powerful compounds, NP4 and NP12, was assessed using the MTT assay that we previously reported (27). The process outlined in intracellular ROS inhibition research was followed for seeding the cells in 96-well plates. Following a 24-hour period during which the cells adhered to the plate surface, they were subjected to varying concentrations of NP4 and NP12 (1-100 μM) and then incubated at 37°C in a CO2 incubator. The leftover chemical was taken out after 24 hours, and each well was then filled with 50 μM MTT before being incubated for a further 3 hours. After three hours, the MTT dye was taken out and 100 μl of DMSO was injected to dissolve the formazan crystal that had formed. A multi-detection microplate fluorescence reader (SynergyHI, Bio-Tek® Instruments) was used to evaluate the absorbance for cell viability at a wavelength of 570 nm. Each assay and control study was carried out in triplicate, with results reported as mean ± SD.

In vivo Studies

Forced swimming test

To find out if our compounds behaved like antidepressants, in vivo tests were conducted on the most effective MAO-A inhibitors that were screened from in vitro investigations. Swiss albino mice of both sexes, weighing 30-35 grams (n = 6), were used to perform a forced swimming test (FST) in order to assess the initial antidepressant-like response of compounds NP4 and NP12. Following CPCSEA rules (ISFCP/IAEC/CPCSEA/Meeting No. 01/2022 Protocol No. 06) India, the Institutional Animal Ethical Committee (IAEC) accepted the experimental protocol utilized in the study. The creatures came from the Animal House., ISF College of Pharmacy, Moga, India Mice were taught for the same task in order to acclimate them to conditions prior to investigations. In summary, the mice were divided into groups and given oral suspensions (0.5% CMC) of the standard medication fluoxetine (20 mg/kg) and test compounds (30 mg/kg) for comparison. Merely 0.5% CMC was administered orally in an equivalent volume to the control group. For FST tests, mice were placed individually in a 20 × 20 cm plexiglass square tank filled with water at a depth of 14 cm and 25±1 ºC after two hours after dosage administration. With the aid of the camera, the mice's immobility duration was tracked for five minutes following their first one-minute acclimation phase. Mice were only deemed "immobile" when they were floating motionless and there was no movement at all from their hind and paws. The mean immobility time in seconds was used to express the results (29).

Tail suspension test

The procedure for the tail suspension test (TST) followed Steru et al.'s instructions. The TST dosage regimen was administered in the same manner as the FST dose. Following a two-hour dosage delivery period, each mouse was separately suspended 50 cm above the tabletop by its tail attached to the horizontal bar with adhesive tape placed approximately 1.5 cm from the tail tip. Similar to FST, a camera was used to record the mice's immobility time for five minutes following their initial one-minute acclimation phase. When it comes to TST, immobility is defined as the absence of any movements of the limbs or body other than those brought on by breathing (30).

3.2.2. In silico studies

Molecular docking simulation

To Molecular docking studies were conducted using AutoDock4.2 to comprehend the interaction between the potent compound and MAO-A. The X-ray crystal structure of hMAO-A (2BXR) (28) has been docked using the previously published procedure. Preparing protein: Autodock vina has been employed in the process. From www.rcsb.org, the crystallographic model 2BXR (hMAO-A) was downloaded. For FAD, the bond order was adjusted and water and covalently bound ligands were eliminated. The protonation state is finally recorded in the.pdbqt format after charge assignment. Ligand preparation: The 2016 Chemdraw 2D version was used to prepare the ligand structures, which were drawn using a building panel. The 2016 edition of Chemdraw 3D is used to minimize energy use.

4. Conflict of interests

The authors indicate no potential conflicts of interest.

5. Acknowledgment

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