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4,6-Diphenylpyrimidine Derivatives as Dual Inhibitors of Monoamine Oxidase and Acetylcholinesterase for the Treatment of Alzheimer's Disease

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Conflict of Interest Statement: A provisional patent application has been filed containing these compounds (Indian patent application number **201811008301)**.

Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder with multifactorial pathogenesis. Monoamine oxidase (MAO) and acetylcholinesterase enzymes (AChE) are potential targets for the treatment of AD. A total of 15 new propargyl containing 4,6-diphenylpyrimidine derivatives were synthesized and screened for the MAO and AChE inhibition activities along with ROS production inhibition and metal chelation potential. All the synthesized compounds were found selective and potent inhibitors of MAO-A and AChE enzymes at nano molar concentrations. **VB1** was found to be the most potent MAO-A and BuChE inhibitor with IC_{50} values of 18.34 ± 0.38 nM and 0.666 ± 0.03 μM, respectively. It also showed potent AChE inhibition with IC_{50} value of 30.46 ± 0.23 nM. Compound **VB8** was found to be the most potent AChE inhibitor with IC_{50} value of 9.54 ± 0.07 nM and displayed IC_{50} value of 1010 ± 70.42 nM against MAO-A isoform. In the cytotoxic studies, these compounds were found non-toxic to the human neuroblastoma SH-SY5Y cells even at 25 μM concentration. All the compounds were found reversible inhibitors of MAO-A and AChE enzymes. In addition, these compounds also showed good neuroprotective properties against 6-OHDA and H_2O_2 induced neurotoxicity in SH-SY5Y cells. All the compounds accommodate nicely to the hydrophobic cavity of MAO-A and AChE enzymes. In the molecular dynamics simulation studies, both **VB1** and **VB8** were found stable in the respective cavities for 30 ns. Thus, 4,6-diphenylpyrimidine derivatives can act as promising leads in the development of dual acting inhibitors targeting MAO-A and AChE enzymes for the treatment of Alzheimer's disease.

Key words: Alzheimer's disease, MAO inhibitors, Diphenylpyrimidine, Acetylcholinesterase inhibitors, Dual inhibitors, Neuroprotective agents

Introduction

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by the progressive memory loss, dementia and other cognitive impairments.[1] It is estimated that one in every three senior persons dies with AD and one in ten persons over 65 years of age has AD.[2] According to Alzheimer's association, in recent years' deaths due to stroke, heart disease, and HIV decreased 21%, 14%, and 54%, respectively, whereas deaths from AD increased 89%.[2] The exact molecular pathogenesis of AD is not clear however, in most of the cases the disease state is linked with the degeneration of neurons and glial cells or inclined metabolism of signaling neurotransmitters. Various biochemical and histopathological studies indicated that over expressed monoamine oxidase (MAO) enzyme, low acetylcholine levels, amyloid-β (Aβ) deposits,[3] hyper-phosphorylated tau-protein aggregation and oxidative stress play crucial roles in the pathophysiology of the disease. Depressive symptoms occur in patients suffering with AD and these may be associated with the decreased serotonergic and noradrenergic transmission in the limbic system,[4, 5] Hyper activation of MAO enzyme has been observed in patients suffering with AD that decreases the concentration of dopaminergic and serotoninergic neurotransmitters. Some clinical trials have shown that deprenyl, a potential MAO-B inhibitor, alleviate the symptoms of AD.[6-8] Acetylcholine is an important enzyme in the regulation of learning and memory processes. It is hydrolyzed by acetylcholinesterase (AChE) and cholinesterase inhibitors enhance the level of acetylcholine. Thus, cholinergic system has been explored as an important target for the treatment of AD. Currently, tacrine, donepezil,[9] galantamine,[10] and rivastigmine[11] are FDA (Food and Drug Administration US) approved drugs that improve AD symptoms by inhibiting AChE.[11-13] Apart from the beneficial palliative properties of AChE inhibitors in AD,[14] cholinergic drugs have shown little efficacy to prevent the progression of the disease. Consequently, there is no efficient drug to cure, stop or even slow the progression of the disease therefore, effective therapeutics are sought for the permanent treatment of AD.[15, 16]

The pathogenesis of AD is complex and targeting single pathway may not be an effective strategy for the complete treatment of the disease. Multi-targeted ligands design strategy involves the incorporation of two or more distinct pharmacophores of different drugs in the single structure to develop hybrid molecules.[17, 18] Hence, the multitargeting ligands which can simultaneously inhibit MAO, AChE and BuChE enzymes are being developed as drugs for the management/treatment of AD.[19, 20] A number of multitargeting ligands have been reported as potential therapeutic agents for the treatment of AD.[21, 22] Although many newly developed MAO,

AChE and BuChE inhibitors are under different phases of clinical trials but none of these still reached to clinical use because of the number of adverse effects. Recently, we have reported various phenyl-/benzhydrylpiperazine derivatives[23] as selective ligands for MAO-A and MAO-B isoforms. In another study, we have synthesized and screened a number of pyrimidine bridged biaryls[24] for their MAO inhibition potential. Most of these compounds were found reversible and selective inhibitors of the MAO-B isoform. There is a high structural resemblance between the two isoforms of MAO enzyme. The volume of entrance cavity of MAO-B is smaller (~300 $Å^3$) as compared to the active cavity of MAO-A (~400 $Å^3$). [6, 21]Thus, MAO-B accommodates smaller molecules while bulkier ligands selectively bind to the MAO-A isoform. A small alteration in the structure of ligand can change its preference for either of the isoforms (MAO-A or MAO-B). Taking leads from these studies, we got interested in the design and synthesis of dual acting ligands that are equipotent to both MAO and AChE enzymes for the treatment of AD. From the literature search, we noted that the propargylamino group play an important role in providing the neuronal and mitochondrial protective properties.[25, 26] The propargyl group is also involved in the crucial covalent bond formation with FAD co-factor of MAO enzyme. Similarly, in the literature survey it has been observed that most of the AChE inhibitors such as donepezil, rivastigmine, pyridostigmine, phenserine galantamine etc. contained a tertiary amino group in the scaffold as a part of ring or open chain.[9-13, 27] Hence, we presumed that a tertiary nitrogen atom might be playing crucial role for the AChE inhibition activity and introduced a piperidine/morpholine ring in the scaffold as potential pharmacophore for the AChE enzyme. The chain length between the propargyl and piperidine moieties is reported to control the dual interaction of these moieties with both the catalytic active site and peripheral anionic site of the AChE enzyme.[28] It is envisaged that incorporation of a piperidine/morpholine ring in the scaffold would make the molecules bulkier and these compounds might show selectivity towards MAO-A isoform.

Thus, on the basis of our previous experience and with the aim of developing dual inhibitors, a series of 4,6-diphenylpyrimidine derivatives (**VB1-VB15**) have been designed with a propargyl group and a piperidine/morpholine moiety as potential pharmacophores for the MAO and AChE enzymes. These compounds were found to be potent inhibitors of both MAO and AChE enzymes with IC_{50} values in nano molar range. All the compounds were found reversible inhibitors of the MAO-A and AChE enzymes and displayed no toxicity to the human neuroblastoma SH-SY5Y cells. Most of these compounds were also found to be inhibitors of BuChE in the micro molar

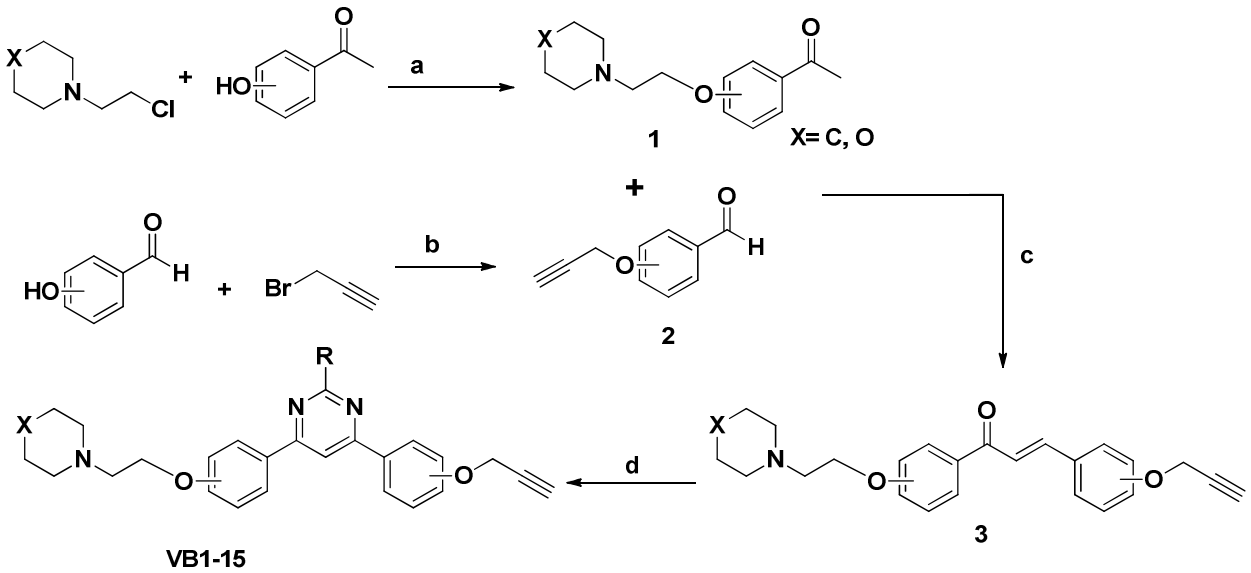
range. In the molecular dynamics simulation studies of 30 ns, the most potent MAO inhibitor (**VB1**) and most potent AChE inhibitor (**VB8**) were found quite stable in the active sites of the enzymes. These compounds also displayed neuroprotective potential against 6-OHDA and H_2O_2 induced neurotoxicity in SH-SY5Y cells.

Results and discussion

Chemistry

All the compounds were synthesized as per reaction procedures described in Scheme-1. Briefly, *O*-alkylated acetophenones **(1)** and benzaldehydes **(2)** were synthesized from corresponding hydroxy acetophenones or benzaldehydes by refluxing these with suitable alkyl halides in the presence of potassium carbonate and acetone as solvent. *O*-alkylated acetophenones **(1)** and benzaldehydes **(2)** were reacted through aldol condensation to get the intermediate chalcones (**3**). In the final step chalcones (**3)** were reacted with various amidines in the presence of sodium carbonate to obtain the target compounds 4,6-diphenylpyrimidine derivatives **(VB1-VB15).** All the final products were characterized by IR, ^{1}H NMR, ^{13}C NMR, ESI-MS and HRMS.

Scheme-1: Reaction scheme for the synthesis of target molecules

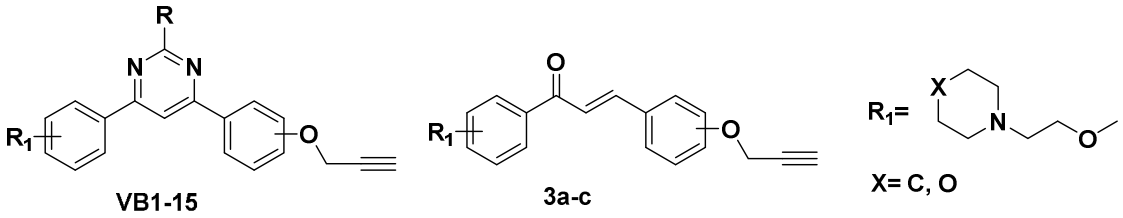


Reagents and Conditions: a) K_2CO_3, KI, acetone, reflux, 6 h; **b)** K_2CO_3, acetone, reflux, 6 h; **c)** CH_3OH, 10% NaOH aq., rt, stirring, 3 h; and **d)** Na_2CO_3, CH_3CN, amidine/guanidine/benzamidine, reflux, 24h

MAO, AChE and BuChE enzyme inhibition studies

MAO inhibition potential of the synthesized 4,6-diphenylpyrimidine derivatives (**VB1-VB15**) was evaluated through fluorimetric method using recombinant human MAO-A and MAO-B enzymes and Amplex® Red assay kit.[29] Similarly, acetylcholinesterase inhibition activity was determined using Amplex Red Acetylcholine/Acetylcholinesterase assay kit (A12217) purchased from the Molecular probes Inc. Invitrogen. The results of MAO inhibition studies are described in **Table 1** in terms of IC_{50} values expressed in nano molar concentrations. Morpholine or piperidine ethyl chains (R_1) were used as meta or para substituents at one of the phenyl ring. To develop structure activity relationship profile of 4,6-diphenylpyrimidines, three amidines were used with R as $-NH_2$, $-CH_3$ or $-C_6H_5$. Similarly, second phenyl ring was substituted with a propargyl group at ortho/meta/para positions. MAO-A, MAO-B and AChE inhibitory activities of some of the intermediate chalcones were also evaluated to compare them with the final products. Clorgyline, pargyline and donepezil were used as standard inhibitors for MAO-A, MAO-B and AChE enzymes, respectively. All the compounds were found selective towards MAO-A isoform with IC_{50} values varying from 18 nM to 2430 nM. MAO-B inhibition activities of these compounds were found in the range of 7313 nM to 14850 nM. These compounds were also found to be the potent inhibitors of AChE with IC_{50} values in nano molar range. However, most of the compounds displayed BuChE inhibitory activity in the micro molar range (0.66 μM to 161.6 μM). **VB1** was found to be the most potent MAO-A inhibitor with IC_{50} value of 18.34 ± 0.38 nM and selectivity index (SI) of 540 over MAO-B. **VB1** was also found to be the most potent BuChE inhibitor in the series with IC_{50} value of 0.666 ± 0.03 μM**.** Similarly, **VB8** was found to be the most potent AChE inhibitor with IC_{50} value of 9.54 ± 0.07 nM. **VB3** was also found potent inhibitor of both AChE and MAO-A with IC_{50} values of 18.92 ± 0.29 nM and 28.33 ± 3.22 nM, respectively. All the chalcone intermediates were found less active as compared to the corresponding final products.

Table 1. Results of the MAO, AChE and BuChE inhibition studies of synthesized compounds



Entry Name	R_1	R	Propargyl group position	X	IC_{50} values (mean ± S.E. nM)			IC_{50} values (mean±S.E. µM)	MAO SI
					hMAO-A	hMAO-B	*ee*AChE	*eq*BuChE	
VB1	C4	NH_2	C4	O	18.34 ± 0.38	9910.14 ± 32.83	30.46 ± 0.23	0.666 ± 0.03	540
VB2	C4	C_6H_5	C4	O	86.35 ± 0.42	6915.62 ± 26.72	39.83 ± 0.39	14.84 ± 0.27	80
VB3	C4	CH_3	C4	O	28.33 ± 3.22	8409.53 ± 25.69	18.92 ± 0.29	14.98 ± 0.31	297
VB4	C4	C_6H_5	C4	C	103.42 ± 11.43	8376.28 ± 23.73	24.69 ± 0.76	19.72 ± 0.27	81
VB5	C4	CH_3	C4	C	752.63 ± 14.34	12140.12 ± 100.64	765.14 ± 3.51	54.46 ± 1.42	16
VB6	C4	C_6H_5	C3	O	2140.12± 50.43	14850.34 ± 80.62	1841.56 ± 27.67	8.216 ± 0.10	7
VB7	C4	NH_2	C3	O	230.12 ± 7.22	7313.76 ± 65.32	20.45. ± 0.17	16.65 ± 0.17	32
VB8	C4	CH_3	C3	O	1010 ± 70.42	12185.94 ± 190.86	9.54 ± 0.07	13.75 ± 0.23	12
VB9	C4	NH_2	C2	O	890.45 ± 8.32	8875.37 ± 110.52	2390.23 ± 20.72	161.6 ± 5.88	10
VB10	C4	C_6H_5	C2	O	690.34 ± 20.32	12305.63 ± 87.72	30105.12 ± 70.14	22.68 ± 0.36	18
VB11	C3	C_6H_5	C4	O	2430.23 ± 45.33	8974.73 ± 93.73	2269.82 ± 32.01	14.66 ± 0.20	4
VB12	C3	NH_2	C4	O	360.33 ± 2.34	8738.97 ± 51.73	1438.54 ± 27.67	23.48 ± 0.18	24
VB13	C3	CH_3	C4	O	450.44 ± 5.13	8469.63 ± 83.72	717.88 ± 7.54	15.27 ± 0.11	19
VB14	C4	C_6H_5	C3	C	1320.22 ± 30.22	9565.83 ± 120.53	2093.71 ± 15.69	15.95 ± 0.12	7
VB15	C4	NH_2	C3	C	1040.12 ± 40.12	7706.23 ± 95.73	1441.42 ± 20.33	34.79 ± 0.27	7

3a	C4	----	C4	O	260.12 ± 13.12	9320.12 ± 24.22	20427.18 ± 22.15	**----**	36
3b	C4	----	C2	O	313.23 ± 17.22	8110.11 ± 40.11	**	----	26
3c	C3	----	C4	O	532.42 ± 19.23	7809.02 ± 35.13	**	**----**	15
Clorgyline					4.39 ± 1.02	**-----**	**----**	**----**	MAO-A
Pargyline					----	0.15 ± 0.02	----	----	MAO-B
Donepezil					----	----	11.32 ± 0.001	1.28 ± 0.04	----

**Inactive or showed less than 50% inhibitory activity at 50 μM concentration and precipitated at higher concentrations. IC_{50} values of BuChE are expressed in μM. MAO SI= IC_{50} of MAO-B / IC_{50} of MAO-A.

Reversibility inhibition studies

First generation MAO inhibitors were irreversible in nature and associated with severe side effects. Reversibility of the target compound is frequently considered in designing and development of new class of inhibitors. Thus, to determine the reversible inhibition of the enzyme by the most active and selective compounds i.e. **VB1, VB3** and **VB8**, reversibility inhibition studies were performed using earlier reported protocol by us and others.[23, 30, 31] All the tested compounds were found reversible inhibitors of both MAO-A and AChE enzymes. Upon treatment of MAO-A and AChE with the test compounds at concentrations of $10xIC_{50}$ and $100xIC_{50}$ activity was reduced to minimum. Upon 100 times dilution with the substrate solution, recovery of more than 75% enzymatic activity was achieved as shown in Fig. 1A and Fig. 1B for MAO-A and AChE respectively. Thus, it can be concluded that the tested compounds were found to be reversible inhibitors of MAO-A and AChE.

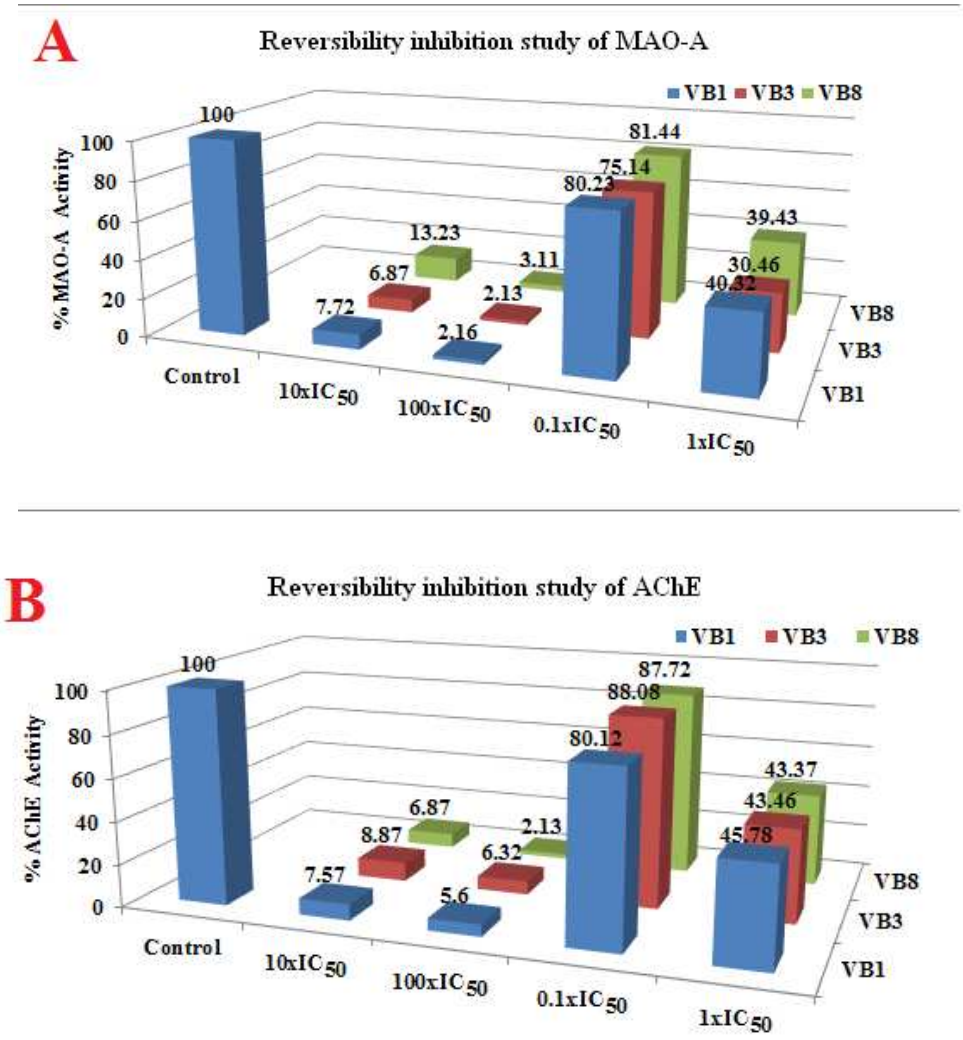


Fig. 1- (A) Reversibility inhibition studies of the most potent and selective inhibitors with MAO-A enzyme; (B) Reversibility inhibition studies with AChE enzyme.

Intracellular ROS determination

It is a well-known fact that monoamine oxidase mediated oxidative metabolism of mono amines lead to the production of H_2O_2 as a byproduct.[32, 33] Subsequently, H_2O_2 get converted to the free radicals ($\dot{O}H$, $\dot{O}_2$) through Fenton's reaction which contribute to the oxidative stress. Uncontrolled increase in the concentrations of the free radicals, initiate free radical-mediated chain reactions that causes oxidative damage to the cell membranes, lipid peroxidation and DNA strand breakdown. Thus, prevention of ROS generation along with the MAO inhibition is important strategy to reduce or eliminate neurotoxicity in neurodegenerative disorders.

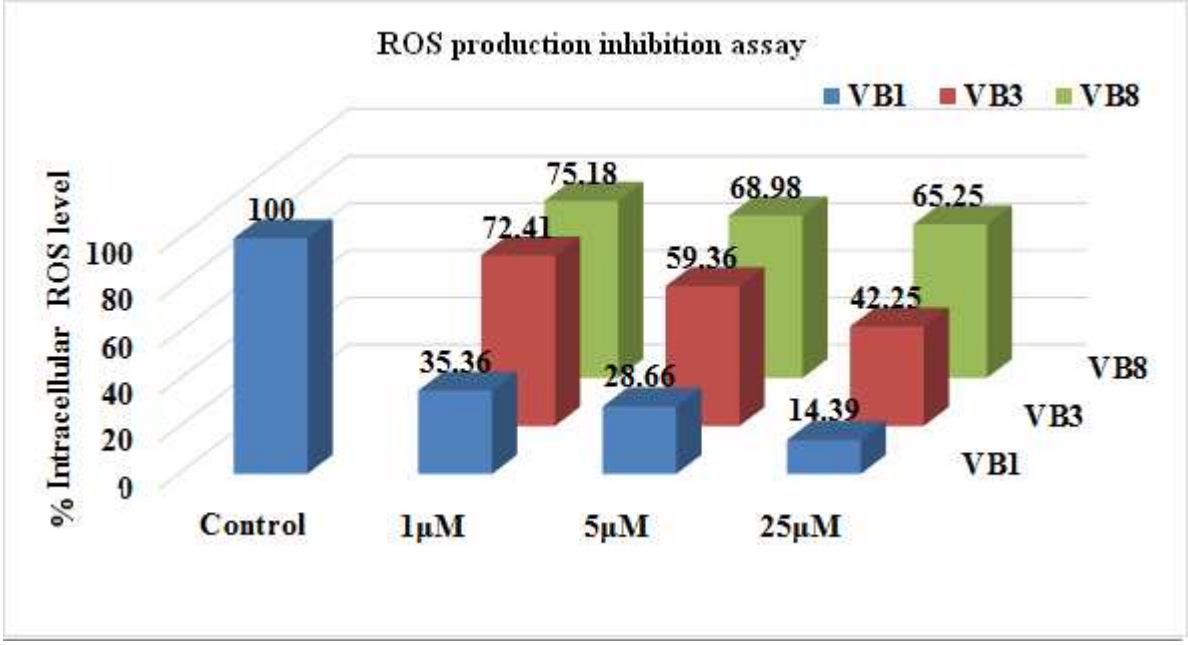


Fig. 2- ROS production inhibition studies of VB1, VB2 and VB3 against SH-SY5Y cell lines

Intracellular ROS level of SH-SY5Y cell lines was determined using non-fluorescent compound 2,7-dichlorofluorescein diacetate (DCF-DA). It is permeable and oxidized by ROS to a fluorescent compound 2,7-DCF. **VB1** was found to be the most potent ROS inhibitor and reduced the intracellular ROS level to 35.36 % and 14.39 % at 1 µM and 25 µM concentration respectively (Fig. 2). **VB3** and **VB8** also reduced the ROS levels to 42.25 % and 65.25 % respectively at 25 µM concentrations.

Neuroprotection studies

The most potent compounds (**VB1**, **VB3** and **VB8**) were evaluated for their neuroprotective potential against 6-hydroxydopamine (6-OHD) neurotoxin in the SH-SY5Y cell lines. But none of the tested compounds showed promising neuroprotective potential at low concentrations (Fig. 3). **VB8** was found to be the most potent amongst the tested compounds and displayed recovery of cells up to 61.77% at 25 µM concentration as compared to the 6-OHD which reduced the cell viability to less than 50 % at 12.5 µM.

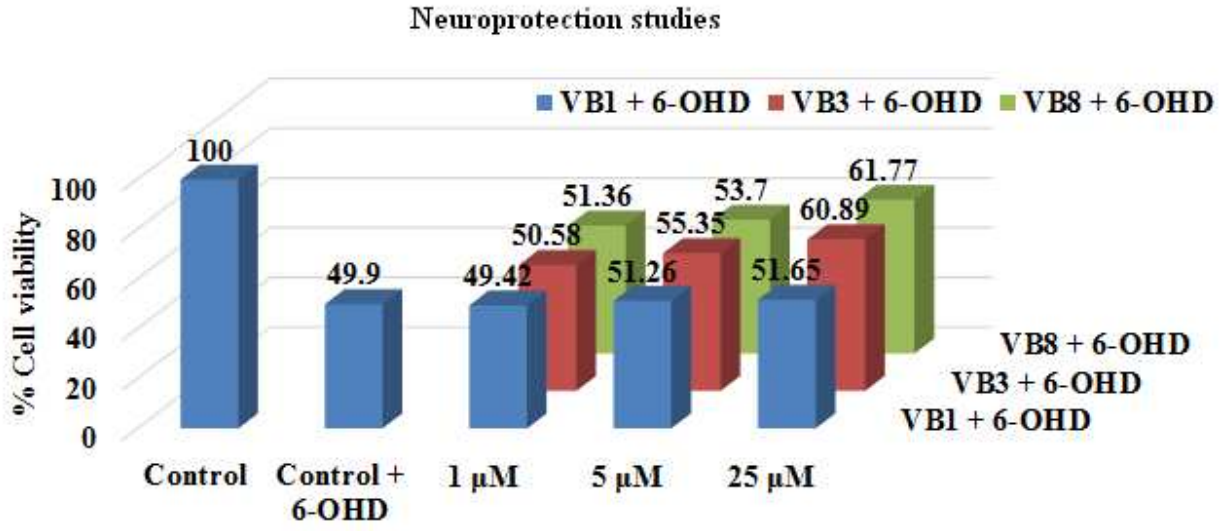


Fig. 3- Neuroprotection studies of the most potent and selective MAO-A and AChE inhibitors

Cytotoxicity studies

The cytotoxic effects of the most active compounds (**VB1, VB3 and VB8**) were evaluated against human neuro-blastome (SH-SY5Y) cell lines because of their similarity to the dopaminergic neurons.[34] The test compounds were incubated at 1 µM, 5 µM and 25 µM concentrations and were analyzed after 24h treatment time. The percentage cell viability was measured using MTT assay. As depicted in Fig. 4, the compounds were found non-toxic against the tested cells even at 25 µM concentrations. It has been observed that as concentration of the compounds increases, the percentage cell viability also increases. Least cell viability of 80% was observed with **VB8** at 1 µM concentration. Thus, keeping in view the nano molar IC_{50} values obtained for enzyme inhibition, the current series of compounds were found to be non-toxic to the tissue cells.

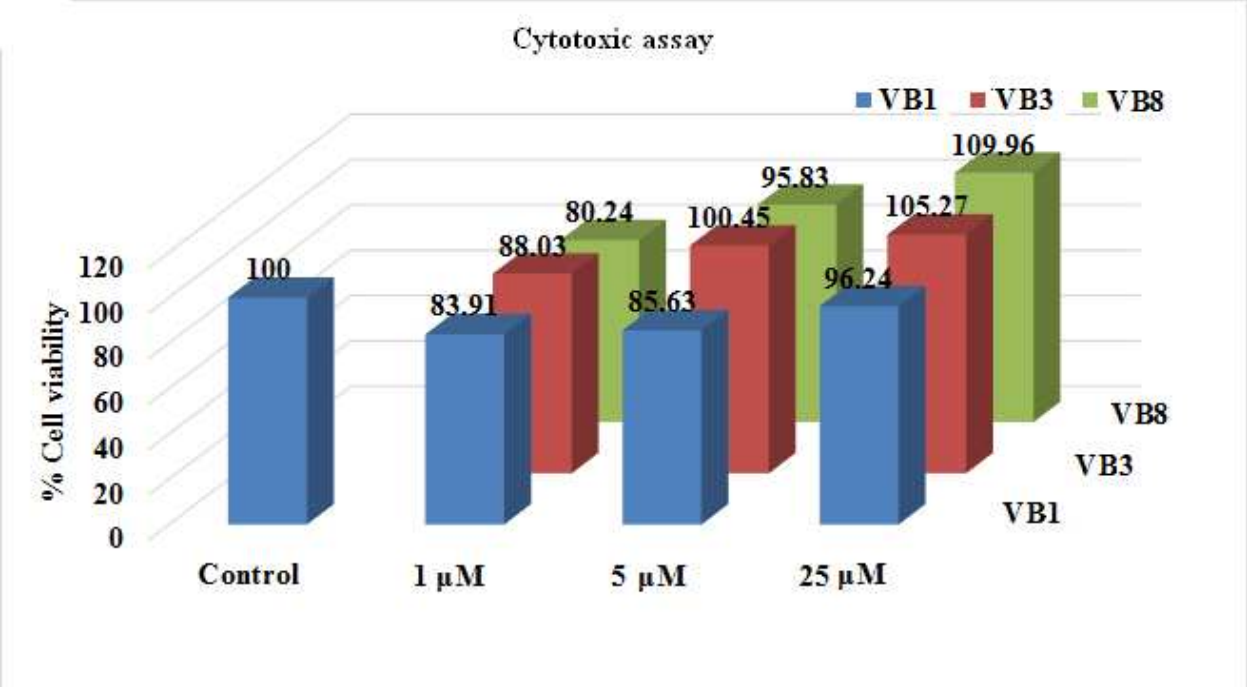


Fig. 4- Cytotoxicity studies of **VB1**, **VB3** and **VB8** against SH-SY5Y cell lines at 1 µM, 5 µM and 25 µM.

Metal chelating studies

Metal chelating studies of the most potent compounds i.e. **VB1, VB3** and **VB8** were performed with a UV−vis spectrophotometer. The absorption spectra of each compound (50 µM, final concentration) alone or in the presence of $CuSO_4$, $FeSO_4$, and $FeCl_3$ (50 µM, final concentration) was recorded. In metal chelating studies, compounds were found ineffective against metals and does not form any chelates with the metal salts.

Kinetic studies of AChE inhibition

To determine the mechanism of inhibition of AChE, kinetic study was carried out with the most potent inhibitor of AChE i.e. **VB8** using eeAChE. The reciprocal Lineweaver-Burk plots (Fig. 5) illustrate increased slope (decreased V_{max}) and higher intercepts (K_m) with the increasing concentration of **VB8.** The intersection point of the Lineweaver-Burk reciprocal plots was located in the second quadrant, which indicate that the inhibition mode of VB8 was mixed-type inhibition. Thus, it can be concluded that VB8 binds to both CAS and PAS of AChE simultaneously.

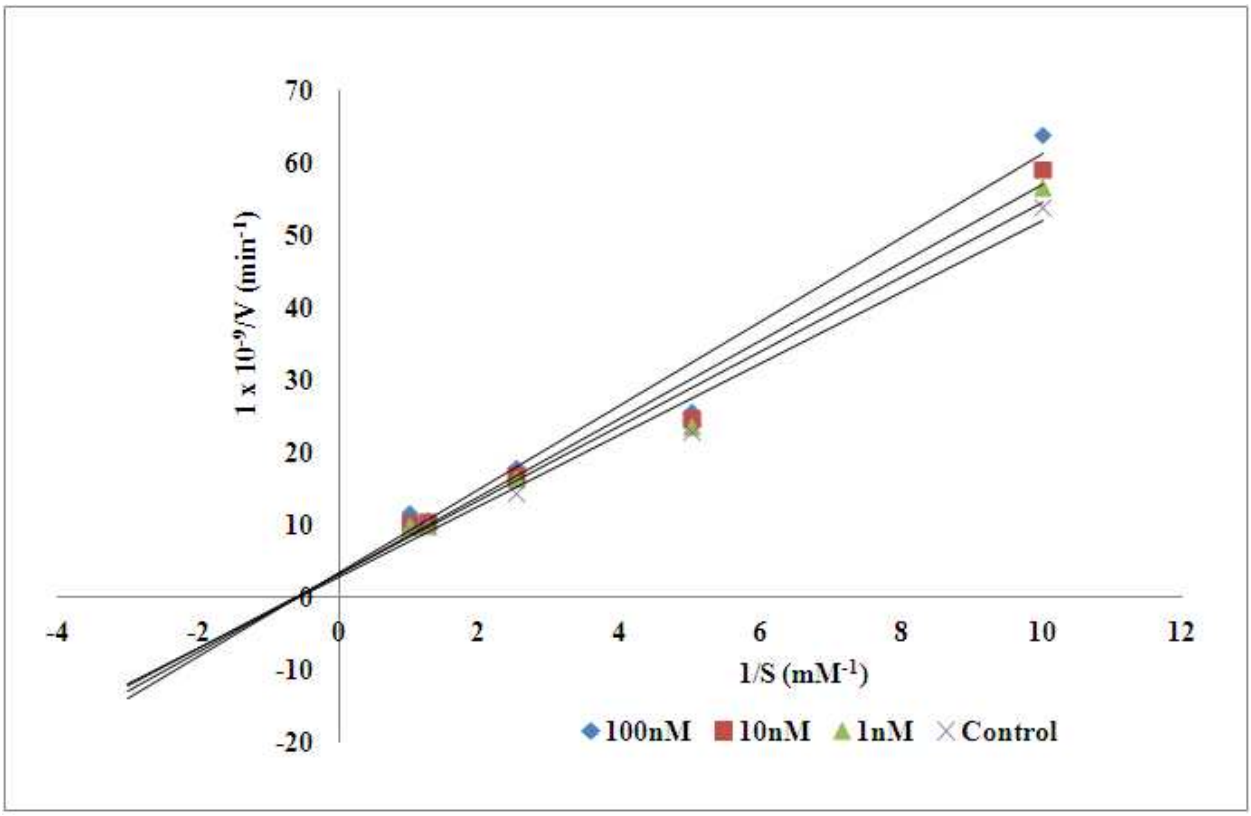


Fig. 5: Kinetic study on the mechanism of *ee*AChE inhibition by **VB8**. Overlaid Lineweaver−Burk reciprocal plots of AChE initial velocity at increasing substrate concentration (0.1−1 mM) in the absence or presence of **VB8** are shown.

Molecular docking studies

The most potent compounds (**VB1, VB3** and **VB8**) were subjected to molecular docking studies to find the interaction pattern of the molecules with the amino acid lining and their orientation at the active site of the receptors. Compounds were docked at the active site of hMAO-A (PDB ID- 2BXR and 2Z5X)[35, 36] and AChE (PDB ID- 1EVE).[37] The X-ray crystal structures were imported from the protein data bank using Maestro 11.1 (Schrödinger LLC). The docking procedure employed was first validated by redocking the co-crystallized ligands into the MAO and AChE models and the process was found suitable for the current study. Three most potent compounds (**VB1, VB3** and **VB8**) were docked at the structure of MAO-A (2BXR and 2Z5X) enzymes co-crystallized with clorgyline and harmine.

All the compounds were found to accommodate nicely to the active site of MAO-A delineated by hydrophobic residues Tyr69, Phe208, Arg296, Ile335, Leu337, Phe352, Tyr407, Trp441,

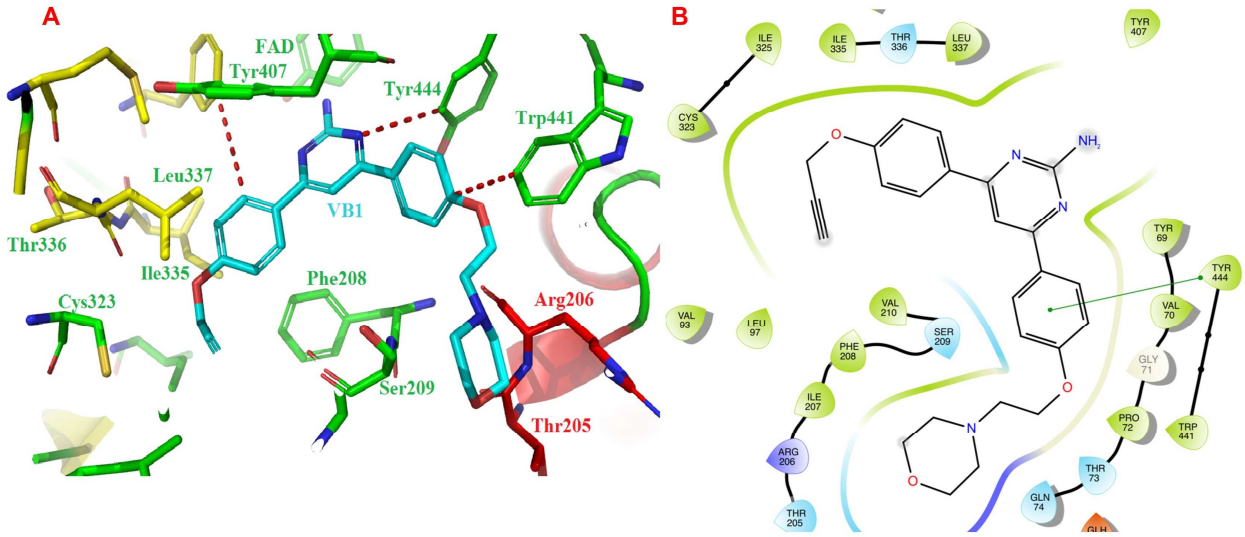


Fig. 6- A) Binding pattern (3D) of **VB1** with the amino acid residues at the active site of MAO-A (2BXR) and B) Binding interactions (2D) of **VB1** with various amino acids of MAO-A active cavity,

Tyr444 and FAD. Most of the interactions observed were hydrophobic and π-π aromatic stacking which showed hydrophobic nature of the MAO-A active site. Pyrimidine moiety was aligned towards the FAD co-factor while 4-(2-phenoxyethyl)morpholine fragment was exposed to the outer part of the active site cavity lined by the polar amino acid residues Thr205 and Arg206. The (prop-2-yn-1-yloxy)benzene fragment of **VB1** showed π-π aromatic interaction with Phe352 while pyrimidine ring showed π-π aromatic interaction with Tyr444. 4-(2-phenoxyethyl)morpholine showed π-π aromatic interaction with Trp441. **VB3** and **VB8** also showed interactions with the same set of amino acids at the active cavity of the enzyme (Fig. S1). The only difference observed in the conformation of **VB8** was the orientation of propargyl group. In case of **VB1** and **VB3** propargyl group was oriented towards rest of the molecule to the inner side of the cavity while in **VB8**, it was directed away from the rest of the molecule towards outer side of the cavity (Fig. 6 and Fig. S1). This difference in the conformation of **VB8** might be responsible for its lower activity towards MAO-A (Fig. 6 and S1). Propargylamino group of clorgyline is reported to interact with FAD co-factor and form strong covalent bond. These strong interactions might be responsible for the irreversible nature of clorgyline. As evident from Fig. 6 and Fig. S1, in **VB1**, **VB3** and **VB8** pyrimidine ring is aligned close to the FAD while propargyl group is oriented away from the FAD co-factor. These conformations of the ligands at the active site negate any possibility of covalent bond formation between the propargyl group and FAD. Thus, these ligands may not form strong covalent bond with the enzyme and hence

display reversible inhibition activity. Son *et al.*,[36] reported a very high resolution structure of human MAO-A co-crystallized with a reversible MAO-A inhibitor harmine which was different from the structure reported by Colibus *et al.* [35] On the advice of one of the reviewer of this manuscript, we have also performed docking studies of reversible MAO-A inhibitor VB1 with PDB-2Z5X (Fig. S2). In this case too, it has been observed that pyrimidine ring was aligned towards the FAD co-factor and the propargyl group was directed towards outer part of the cavity aligned with amino acid resides Val484 and Thr487 (Fig. S2). These observations further strengthen our claim that the propargyl group may not be able to form strong covalent bond with the FAD and hence current series of compounds behave as reversible inhibitors.

Similarly, the most potent compounds (**VB1, VB3** and **VB8**) were docked in the crystal structure of AChE (1EVE) enzyme co-crystallized with donepezil (Fig. 7 and S3). All the compounds were accommodating nicely to the active site as well as the peripheral anionic site (PAS) of AChE as done by the standard inhibitor donepezil. The AChE anionic site, composed of Trp84, Tyr130, Phe330 and Phe331 residues while peripheral anionic site (PAS) consists of five amino acids-Tyr70, Asp72, Tyr121, Trp279 and Tyr334. The increased chain length between the phenyl and morpholine ring, as compared to the N-benzylpiperidine moiety of donepezil, pushed the 4-(2-phenoxyethyl)morpholine fragment of **VB1** and **VB3** inside the CAS of AChE. The morpholine moiety was aligned towards the narrowest part of the active site cavity. However, for **VB8,** the binding alignment was inverted and 4-(2-phenoxyethyl)morpholine fragment was aligned towards the 2,3-dihydroinden-1-one fragment of the donepezil (Fig. 8). Thus the (prop-2-yn-1-yloxy)benzene fragment of **VB8** was oriented towards CAS of AChE.

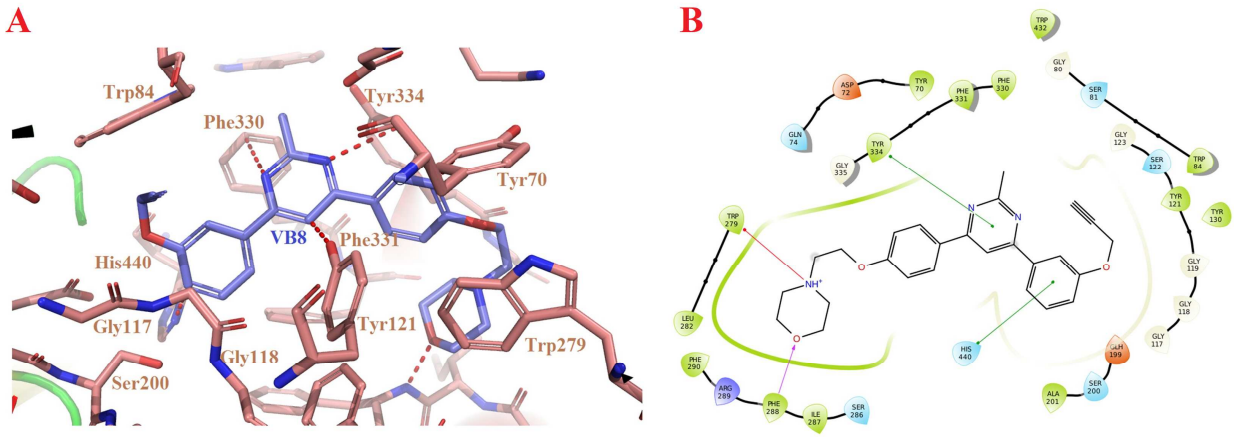


Fig. 7 A) Binding pattern (3D) of **VB8** with the amino acid residues at the active site of AChE (1EVE), and B) Binding interactions (2D) of **VB8** with various amino acids of AChE active cavity.

Comparison of the binding patterns (predicted) of donepezil and **VB8** reveals the plausible reason of its high potency for AChE. Donepezil showed π–π aromatic interactions of N-benzylpiperidine fragment with Trp84 residue and cation–π interactions with Phe331 and Tyr334 residues. The 2,3-dihydroinden-1-one fragment of the donepezil showed π–π stacking with Trp279. **VB1** and **VB3** preserved most of the interactions of the donepezil with the same set of amino acid residues. These compounds showed cation–π interactions with Trp84 and π–π stacking with Trp279, Phe331 and Tyr334 residues. Similarly**,** pyrimidine fragment of **VB8** showed π–π aromatic stacking with Phe331 and Tyr334. However, 4-(2-phenoxyethyl)morpholine fragment was aligned in the opposite direction as compared to the benzylpiperidine moiety of donepezil and displayed cation–π interactions with the Trp279 residue and hydrogen bonding interactions with the Phe288 residue through oxygen atom of the morpholine ring (Fig. 7 and S3). Furthermore, the (prop-2-yn-1-yloxy)benzene fragment of **VB8** showed additional aromatic π–π stacking with His440 residue, not observed for **VB1**, **VB3** and donepezil.

VB1, **VB3** and **VB8** were superimposed with donepezil at the active site of AChE (Fig. 8). The (prop-2-yn-1-yloxy)benzene fragments of **VB1** and **VB3** were oriented towards 2,3-dihydroinden-1-one fragment of the donepezil in PAS of AChE while 4-(2-phenoxyethyl)morpholine fragment overlap with the N-benzylpiperidine moiety of donepezil and oriented towards CAS. Pyrimidine rings of **VB1** and **VB3** showed good overlap with the dihydroindenone part of donepezil. Surprisingly, **VB8** showed reverse orientation and its (prop-2-yn-1-yloxy)benzene fragment overlapped with the benzylpiperidine moiety of the donepezil aligned towards CAS of AChE. The 4-(2-phenoxyethyl)morpholine moiety of **VB8** oriented towards 2,3-dihydroinden-1-one fragment of donepezil and accommodate in the PAS of AChE. The pyrimidine ring of **VB8** overlapped with the piperidine ring of donepezil while (prop-2-yn-1-yloxy)benzene fragment overlapped with the benzylic ring. Thus, altered orientation and additional interactions at the active site might be responsible for the high potency of **VB8** towards AChE enzyme.

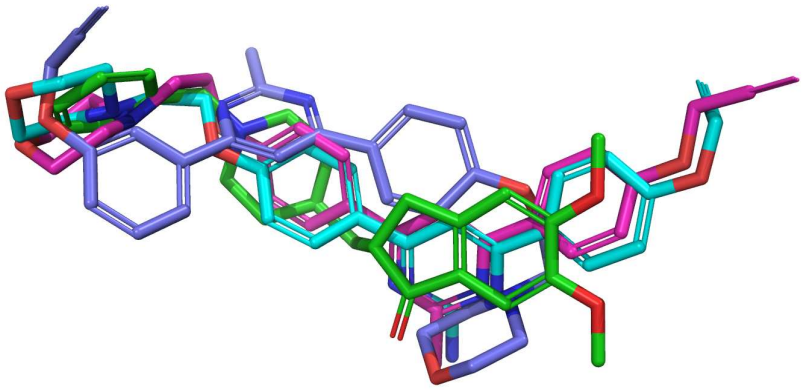


Fig. 8- Superimposed poses of **VB1** (cyan), **VB3** (pink) and **VB8** (violet) with donepezil (green) at the active site of AChE (1EVE).

Molecular dynamics simulation studies

Molecular dynamics simulation (MD) were performed to study the protein–ligand interactions and to determine the thermodynamic stability of the docked compounds at the active pocket of the enzymes. The protein–ligand docked complexes of the most active compounds **VB1** with MAO-A and **VB8** with AChE were used for MD simulations. The MD simulation studies were conducted for 30 ns and the interaction pattern of the test compound with different amino acids was analyzed. MD simulations yielded stable trajectories for **VB1** with MAO-A in the first 20 ns, as noted by the time evolution of the potential energy and the root-mean-square deviation (RMSD) of the protein backbone, which ranged from 0.6 to 1.0 Å (Fig. 9). There were no major structural alterations and the pyrimidine moiety adopted similar orientation in the active site of MAO-A (Fig. 9) as obtained during the docking studies. In particular, the 4-(2-phenoxyethyl)morpholine fragment of **VB1** was stacked against Tyr444 and Arg206. After the time interval of 22 ns, there was a sudden hike in the RMSD and thereafter it showed stable trajectory up to 30 ns. RMSD oscillates in the range of 2 Å to 3 Å in this time interval. In the MD simulations studies it has been observed that **VB1** preserved most of the interactions observed during the docking studies. In addition, some new interactions with various amino acid residues were also observed (Fig 9). In the MD simulations, π-π aromatic interaction with Tyr444 was retained. The NH_2 group present on the pyrimidine fragment move more towards

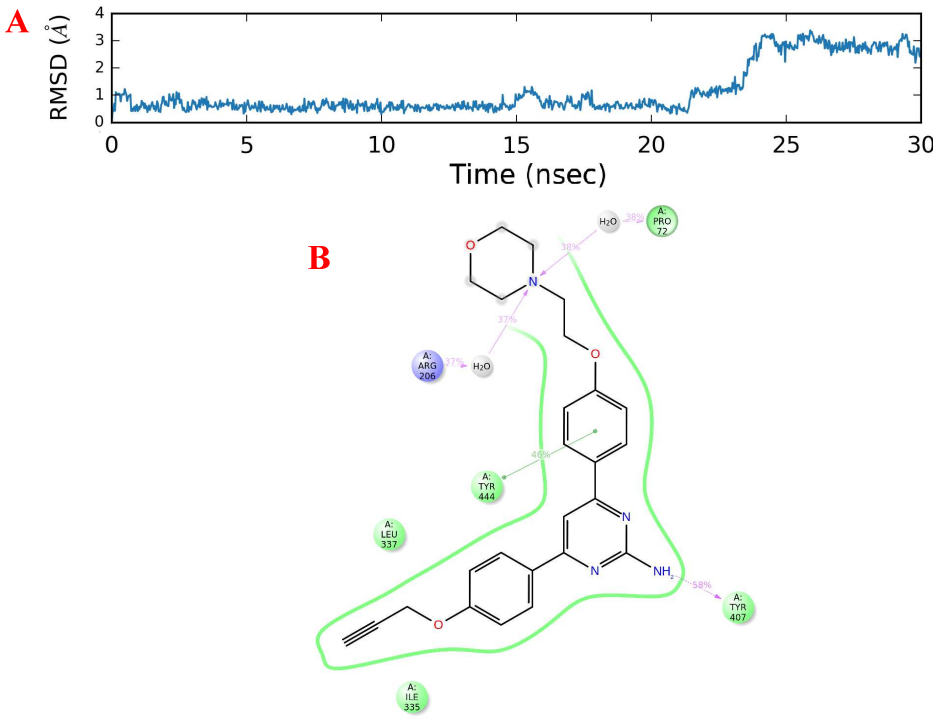


Fig 9- A) RMSD graph of MD studies of **VB1** with MAO-A for 30 ns, and **B**) interactions of **VB1** with the active site residues of MAO-A after 30 ns MD simulation studies

Tyr407 and formed a hydrogen bond with it. The nitrogen atom of the morpholine ring formed hydrogen bonds with Pro72 and Arg206 through water bridge formation. It demonstrates the structural integrity of the MAO-A-VB1 complex, and displayed similar binding features as done by clorgyline.

The MD simulations of AChE-VB8 complex was performed to determine the binding stability of **VB8** at CAS and PAS of AChE. The complex yielded stable trajectories from 2 ns to 30 ns, as noted by the time evolution of the potential energy and the root-mean-square deviation (RMSD) of the protein backbone. The RMSD values for AChE-VB8 complex ranged from 1.6 to 2.0 Å (Fig. 10). For the initial 2s, the RMSD value increase from 0.8 to 1.6 and there after it remained stable up to 30 ns. Analyzing the MD trajectory of AChE-VB8 complex, it can be concluded that **VB8** showed strong tendency to be localized in the binding region of AChE. Nitrogen atom of the pyrimidine ring form hydrogen-bond with Tyr121. In the PAS of AChE, the protonated nitrogen atom of morpholine moiety maintained the initial cation–π interaction (from comparison of Fig. 7 and Fig. 10) throughout the MD simulations interval, indicating stable binding of **VB8** with AChE. In addition, morpholine ring formed hydrogen bonding with the

Glu278 residue through water bridge formation. The (prop-2-yn-1-yloxy)benzene fragment aligned towards CAS of AChE and showed aromatic π-π interaction of **VB8** with Tyr330 and Trp84. The MD simulations of **VB8** does not indicate any abrupt local force that could potentially break up the inhibitor or can even delocalize it from the binding site of AChE. Thus, from the MD simulations studies of AChE-VB8 complex, it can be concluded that the complex is stable and this stability might be responsible for the high *in vitro* potency of **VB8** for AChE.

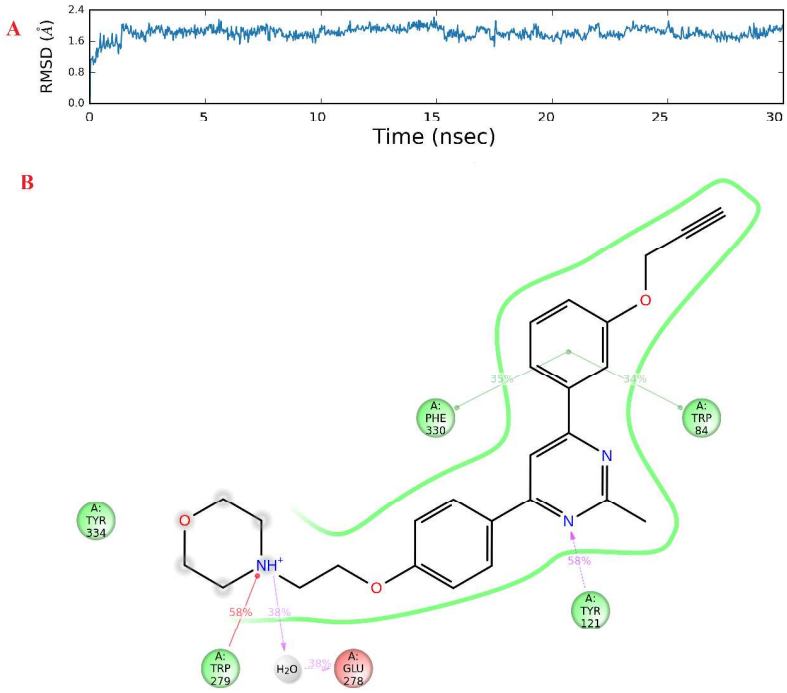


Fig 10- A) RMSD graph of MD simulations studies of **VB8** with AChE for 30 ns, and **B**) interactions of **VB8** with the active site residues of AChE during 30 ns MD simulation studies

ADME properties

To determine the drug like characteristics of the synthesized compounds, ADME parameters of these compounds were determined using Qikprop application of Schrodinger. **VB1** displayed very good drug-like profile with LogP value less than 5 and QPlogBB value as -0.65. In addition, **VB1** showed optimum oral absorption and blood-brain barrier permeability. **VB3** and **VB8** also showed 100 % oral absorption and QPlogBB in the optimum range to cross the blood brain barrier.

Table 2: Physiochemical properties of the most potent and selective MAO-A and AChE inhibitors

Name	Mol. Wt.	Log P	HB donor	HB acceptor	% human oral absorption	QPlogBB (Optimum range -3.0 – 1.2)	BBB permeability predicted
VB1	430.505	3.94	3	8	96	-0.65	+ve
VB3	429.518	5.06	1	7	100	0.22	+ve
VB8	429.518	5.09	1	7	100	0.28	+ve

+ve = high blood-brain barrier permeability, HB = Hydrogen bond, QPlogBB = qualitatively predicted logarithmic ratio between the concentration of a compound in the brain and blood, LogP = partition coefficient of a molecule between an aqueous and lipophilic phase (octanol and water)

SAR studies

In the current studies, three different amidines have been used with R as $-NH_2$, $-CH_3$ or $-C_6H_5$ (Table 1). One of the ring of diphenylpyrimidines is optionally substituted with the morpholine or piperidine ethyl chain while other phenyl ring is functionalized with a propargyl group at ortho, meta or para positions. The effect of different substituents on the activity is analyzed as reported in the Table 1. The compounds were evaluated for MAO-A, MAO-B, *ee*AChE and eqBuChE inhibitory activities using enzymatic assays. All the compounds were found selective for MAO-A isoform with IC_{50} values in nano molar range and with moderate to very high selectivity index. It has been observed that the compounds with morpholine ethyl chain and propargyl group at para positions of both the phenyl rings showed high potency for MAO-A isoform and *ee*AChE. **VB1** with R as $-NH_2$ was found to be the most potent MAO-A inhibitor with IC_{50} value of 18.34 nM and it also showed potent *ee*AChE inhibitory activity with IC_{50} value of 30.46 nM. **VB1** was also found to be the most potent BuChE inhibitor in the series with IC_{50} value of 0.666 ± 0.03 μM. **VB3** with R as -CH3 also showed strong inhibition potential against MAO-A and *ee*AChE with IC_{50} values of 28.33 nM and 18.92 nM respectively. Replacement of $-NH_2$ with C_6H_5 in **VB2** reduces MAO-A activity by 5-folds. Shifting of propargyl group from para position to meta position (**VB7**) reduces MAO-A inhibitory activity by more than 12-folds but there was no effect on the *ee*AChE inhibitory activity. Similarly, in

VB8 meta propargyl group reduces MAO-A inhibitory activity by 35-folds when compared to **VB3**. However, **VB8** was found to be the most potent *ee*AChE inhibitor in the series with IC_{50} values of 9.54 nM. In general, replacement of morpholine ring with the piperidine ring reduces MAO-A inhibitory activity (**VB4** and **VB5**). Shifting of morpholine substituent from para (**VB1**) to meta position (**VB12**) decreases MAO-A activity by 19-folds and *ee*AChE activity by 47-folds. It has been found that compounds with a propargyl substitution at the ortho and meta positions were less active towards MAO-A as compared to the para substituents. The current series of compounds displayed high selectivity index towards AChE as compared to BuChE (Table 1). **VB1** was found to be the most potent BuChE inhibitor however, it showed SI of 22-folds for AChE. In the current series of compounds, VB8 displayed highest SI of about 10^3 for AChE. Replacement of amino group with methyl or phenyl groups (**VB2** and **VB3**) decreases BuChE inhibitory activity by about 22-folds. Shifting of propargyl group from para (**VB1**) to ortho (**VB9**) position drastically reduced the activity by about 240-folds. Similarly, shifting of morpholine ring from para (**VB1**) to meta position (**VB12**) decreases BuChE activity by 35-folds.Thus, **VB1, VB3** and **VB8** were found to be the most promising compounds amongst the reported series of compounds.

Conclusion

AD is multifactorial in nature and different enzymes including MAO, AChE and amyloid beta are implicated in its pathogenesis. Pathomechanism of AD is complex in nature and single target drugs proved ineffective for the treatment of the disease. Thus, multi-target directed approach is being explored for the development of effective drug candidates for the treatment of AD. A drug active on multiple targets may be characterized by an improved efficacy when compared to a highly selective pharmacological agent. Multi-target activities may potentiate efficacy either additively or synergistically and be less prone to the drug resistance. In the current study, a series of 4,6-diphenylpyrimidines has been rationally designed that can simultaneously target MAO enzymes and acetylcholinesterases for the treatment of AD. The phenyl rings were optionally substituted with a morpholine or piperidine ethyl chain and *O*-propargylated groups at ortho, meta and para positions and structure activity relationship profile has been generated. Most of the compounds were found potent dual inhibitors of MAO-A isoform and AChE with IC_{50} values in nano molar range. In the current series, **VB1** was found to be the most potent MAO-A and BuChE inhibitor with IC_{50} values of 18.34 ± 0.38 nM and 0.66 ± 0.03 μM. It also displayed

potent AChE inhibitory activity with IC_{50} value of 30.46 ± 0.23 nM. **VB3** was another promising compound in the series with IC_{50} values of 28.33 ± 3.22 nM and 18.92 ± 0.29 nM against MAO-A and AChE respectively. **VB8** was found to be the most potent AChE inhibitor with IC_{50} value of 9.54 ± 0.07 nM and displayed very high SI (10^3) for AChE over BuChE. In the reversibility inhibition studies, **VB1**, **VB3** and **VB8** were found reversible inhibitors of MAO-A and AChE. These compounds also displayed neuroprotective potential against 6-OHDA and H_2O_2 induced neurotoxicity in SH-SY5Y cells. Cytotoxicity studies were also performed with the three lead compounds using SH-SY5Y cell lines and the compounds were found non-toxic even at 25 μM concentration. In the MD simulation studies of 30 ns, **VB1** and **VB8** were found stable in the active site of MAO-A and AChE respectively. In the physicochemical evaluation studies, the most active compounds were found to possess drug like characteristics with optimum oral absorption and good permeability to blood brain barrier. Thus, some of these dual acting compounds have the potential to be developed as drug candidates for the treatment of AD.[30]

Experimental

General procedure for the synthesis of 1 and 2

To hydroxy substituted acetophenone (0.5g) or benzaldehydes (0.5g), morpholine or piperidine ethylchloride (1.2eq) was added in the presence of potassium carbonate as base (2.4eq.) and acetone (30 ml) as solvent. The reaction mixture was refluxed for 12 h at 60˚ C. The progress of reaction was monitored via TLC. After completion of the reaction, excess of solvent was evaporated from the mixture using vacuum rotary evaporator, water (10 mL) was added and aqueous phase was extracted with ethyl acetate (10 mL × 3), and washed with brine, dried over anhydrous Na_2SO_4 and the organic solvent was concentrated under vacuum using rotary evaporator.

General procedure for the synthesis of 3

To a mixture of **1** (1 eq.) and **2** (1 eq.) in methanol (20 ml), aqueous sodium hydroxide (20 %) was added slowly with continuous stirring. The reaction mixture was stirred for 3 h at room temperature. The completion of reaction was monitored via TLC. After completion of reaction, excess of solvent was evaporated from the mixture using rotary evaporator. Chilled water was poured into the reaction mixture and precipitates of **3** were filtered and dried.

General procedure for the synthesis of VB1-VB15

To a mixture of **3** (500 mg), and amidine (1.2 eq.), anhydrous sodium carbonate (2.4 eq.) was added in acetonitrile (5 mL) as solvent. The reaction mixture was refluxed for 24 h at 85˚ C. The progress of the reaction was monitored via TLC. After completion of the reaction, excess of solvent was evaporated under vacuum using rotary evaporator, water (10 mL) was added aqueous phase was extracted with ethyl acetate (3×10 mL), washed with brine, dried over anhydrous Na_2SO_4, and the organic solvent was concentrated under vacuum using rotary evaporator and purified via column chromatography (EtOAc:Pet ether). The final products were characterized by NMR spectroscopy and HRMS.

Spectral analysis

4-(4-(2-morpholinoethoxy)phenyl)-6-(4-(prop-2-yn-1-yloxy)phenyl)pyrimidin-2-amine (**VB1**): Yield 59%, ^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.05-8.00 (4H, m), 7.35 (1H, s), 7.07 (2H, d, *J*= 8Hz), 6.99 (2H, d, *J*= 8Hz), 5.16 (2H, s), 4.76 (2H, d, *J*= 2.4Hz), 4.18 (2H, t, *J*= 4Hz), 3.75 (4H, t, *J*= 4Hz), 2.83 (2H, t, *J*= 4Hz), 2.60 (4H, t, *J*= 4Hz), 2.55 (1H, t, *J*= 2.4Hz), ^{13}C NMR **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 165.40, 165.28, 163.49, 160.71, 159.41, 131.13, 130.36, 128.36, 128.58, 115.00, 114.70, 102.86, 78.19, 75.89, 66.93, 65.89, 57.58, 55.85, 54.13 **HRMS**: for $C_{25}H_{26}N_4O_3$, calculated $[M+H]^+$: 431.2083; observed $[M+H]^+$: 431.2060

4-(2-(4-(2-phenyl-6-(4-(prop-2-yn-1-yloxy)phenyl)pyrimidin-4-yl)phenoxy)ethyl)morpholine (**VB2**): Yield 63%, ^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.68 (2H, d, *J*= 8Hz), 8.24 (4H, t, *J*= 8Hz), 7.86 (1H, s), 7.53-7.49 (3H, m), 7.12 (2H, d, *J*= 8Hz), 7.04 (2H, d, *J*= 8Hz), 4.77 (2H, d, *J*= 2.4Hz), 4.19 (2H, t, *J*= 4Hz), 3.75 (4H, t, *J*= 4Hz), 2.84 (2H, t, *J*= 4Hz), 2.60 (4H, t, *J*= 4Hz), 2.56 (1H, t, *J*= 2.4Hz), ^{13}C NMR **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 164.01, 163.89, 161.02, 159.73, 138.40, 130.59, 130.28, 128.83, 128.48, 115.20, 114.89, 108.75, 78.19, 75.89, 66.99, 65.93, 57.66, 55.95, 54.20 **HRMS**: for $C_{31}H_{29}N_3O_3$, calculated $[M+H]^+$: 492.2287; observed $[M+H]^+$: 492.2271

4-(2-(4-(2-methyl-6-(4-(prop-2-yn-1-yloxy)phenyl)pyrimidin-4-yl)phenoxy)ethyl)morpholine (**VB3**): Yield 58%, ^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.11-8.07 (4H, m), 7.76 (1H, s), 7.10 (2H, d, *J*= 8Hz), 7.02 (2H, d, *J*= 8Hz), 4.77 (2H, d, *J*= 2.4Hz), 4.19 (2H, t, *J*= 4Hz), 3.75 (4H, t, *J*= 4Hz), 2.84 (2H, t, *J*= 4Hz), 2.81 (3H, s), 2.61 (2H, t, *J*= 4Hz), 2.56 (1H, t, *J*= 2.4Hz), **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 168.32, 164.07, 163.96, 160.85, 159.55, 130.94, 130.17,

128.72, 115.18, 114.87, 108.49, 78.13, 75.93, 66.91, 65.90, 57.57, 55.87, 54.13, 26.53 **HRMS**: for $C_{26}H_{27}N_3O_3$, calculated $[M+H]^+$: 430.2131; observed $[M+H]^+$: 430.2079

2-phenyl-4-(4-(2-(piperidin-1-yl)ethoxy)phenyl)-6-(4-(prop-2-yn-1-yloxy)phenyl)pyrimidine (**VB4**): Yield 61%, ^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.69 (2H, dd, J_1= 8Hz, J_2= 4Hz), 8.23 (4H, t, *J*= 8Hz), 7.84 (1H, s), 7.52-7.42 (3H, m) 7.11 (2H, d, *J*= 8Hz), 7.03 (2H, d, *J*= 8Hz), 4.76 (2H, d, *J*= 2.4Hz), 4.19 (2H, t, *J*= 4Hz), 2.82 (2H, t, *J*= 4Hz), 2.57-2.55 (5H, m), 1.66-1.60 (4H,m) 1.46 (2H, bd), **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 164.26, 164.04, 163.82, 161.12, 159.70, 138.43, 131.04, 130.56, 130.11, 128.80, 128.71, 128.48, 115.18, 114.89, 108.71, 78.26, 76.04, 66.02, 57.83, 55.94, 55.14, 29.81, 25.88, 24.19 **HRMS**: for $C_{32}H_{31}N_3O_2$, calculated $[M+H]^+$: 490.2495; observed $[M+H]^+$: 490.2470

2-methyl-4-(4-(2-(piperidin-1-yl)ethoxy)phenyl)-6-(4-(prop-2-yn-1-yloxy)phenyl)pyrimidine (**VB5**): Yield 67%,^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.07 (4H, t, *J*= 8Hz), 7.74 (1H, s), 7.08 (2H, d, *J*= 8Hz), 7.00 (2H, d, *J*= 8Hz), 4.75 (2H, d, *J*= 4Hz), 4.17 (2H, t, *J*= 4Hz), 2.82-2.79 (5H, m), 2.53 (4H, b), 1.61 (4H, b), 1.44 (2H, b), 1.23 (1H, b), **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 168.35, 164.18, 163.98, 161.03, 159.59, 131.01, 130.79, 130.35, 130.11, 130.06, 129.47, 128.77, 115.33, 115.23, 114.95, 108.53, 78.21, 76.01, 66.07, 57.85, 55.92, 55.13, 29.79, 25.89, 24.17, **HRMS**: for $C_{27}H_{29}N_3O_2$, calculated $[M+H]^+$: 428.2338; observed $[M+H]^+$: 428.2293

4-(2-(4-(2-phenyl-6-(3-(prop-2-yn-1-yloxy)phenyl)pyrimidin-4-yl)phenoxy)ethyl)morpholine (**VB6**): Yield 62%,^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.70 (2H, dd, J_1= 8Hz, J_2= 4Hz), 8.25 (2H, d, *J*= 8Hz), 7.97 (1H,s), 7.91 (1H,s), 7.86 , (1H, d, *J*= 8Hz), 7.54-7.56 (4H, m), 7.15 (1H, dd, J_1= 8Hz, J_2= 4Hz), 7.06 (2H, d, *J*= 8Hz), 4.82 (2H, d, *J*= 4Hz), 4.20 (2H, t, *J*= 4Hz), 3.76 (4H, t, *J*= 4Hz), 2.85 (2H, t, *J*= 4Hz), 2.60 (5H, m), **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 164.37, 164.27, 164.06, 161.13, 158.17, 139.32, 138.25, 130.70, 130.10, 130.01, 128.88, 128.51, 120.50, 117.11, 114.93, 113.99, 109.61, 78.50, 75.94, 67.01, 65.98, 57.65, 56.13, 54.21, **HRMS**: for $C_{31}H_{29}N_3O_3$, calculated $[M+H]^+$: 492.2287; observed $[M+H]^+$: 492.2256

4-(4-(2-morpholinoethoxy)phenyl)-6-(3-(prop-2-yn-1-yloxy)phenyl)pyrimidin-2-amine (**VB7**): Yield 59%,^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.00 (2H, d, *J*= 8Hz), 7.90 (1H, dd, J_1= 8Hz, J_2= 4Hz), 7.65 (1H, m), 7.39 (1H, t, *J*= 8Hz), 7.36 (1H, s), 7.08 (1H, dd, J_1= 8Hz, J_2= 4Hz), 6.98 (2H, d, *J*= 8Hz), 6.90 (1H, t, *J*= 8Hz), 5.22 (2H, s), 4.77 (2H, d, *J*= 4Hz), 4.16 (4H, t, *J*=

4Hz), 3.73 (2H, t, *J*= 4Hz), 2.82 (4H, t, *J*= 4Hz), 2.53 (2H, b), **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 165.71, 163.56, 160.86, 158.01, 139.49, 130.69, 129.89, 128.70, 120.44, 117.08, 114.79, 114.48, 114.29, 113.53, 103.73, 78.49, 75.82, 66.97, 65.93, 57.63, 56.04, 54.18, **HRMS**: for $C_{25}H_{26}N_4O_3$, calculated $[M+H]^+$: 431.2083; observed $[M+H]^+$: 431.2060

4-(2-(4-(2-methyl-6-(3-(prop-2-yn-1-yloxy)phenyl)pyrimidin-4-yl)phenoxy)ethyl)morpholine (**VB8**): Yield 59%,^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.08 (2H, d, *J*= 8Hz), 7.78 (1H, m), 7.72-7.69 (2H, m), 7.43 (1H, t, *J*= 8Hz), 7.10 (1H, d, *J*= 8Hz), 7.01 (2H, t, *J*= 8Hz), 4.78 (2H, d, *J*= 4Hz), 4.18 (2H, t, *J*= 4Hz), 3.74 (4H, t, *J*= 4Hz), 2.83 (2H, t, *J*= 4Hz), 2.83 (3H, s), 2.59 (4H, t, *J*= 4Hz), 2.54 (1H, t, *J*= 4Hz), **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 168.50, 164.37, 164.29, 161.01, 158.14, 139.31, 131.03, 130.07, 128.83, 120.56, 117.13, 114.97, 113.77, 109.45, 78.44, 75.84, 66.98, 65.97, 57.64, 56.08, 54.20, 29.79, **HRMS**: for $C_{26}H_{27}N_3O_3$, calculated $[M+H]^+$: 430.2131; observed $[M+H]^+$: 430.2086

4-(4-(2-morpholinoethoxy)phenyl)-6-(2-(prop-2-yn-1-yloxy)phenyl)pyrimidin-2-amine (**VB9**): Yield 55%,^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.00 (2H, d, *J*= 8Hz), 7.85 (1H, dd, J_1= 8Hz, J_2= 4Hz), 7.60 (1H, m), 7.40 (1H, dd, J_1= 8Hz, J_2= 4Hz), 7.12 (2H, t, *J*= 8Hz), 6.97 (2H, d, *J*= 8Hz), 5.12 (2H, s), 4.75 (2H, d, *J*= 4Hz), 4.16 (2H, t, *J*= 4Hz), 3.73 (4H, t, *J*= 4Hz), 2.82 (2H, t, *J*= 4Hz), 2.59 (4H, b), 2.52 (1H, t, *J*= 4Hz), **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 164.69, 164.30, 163.39, 160.70, 155.69, 130.94, 128.81, 122.22, 114.70, 114.16, 113.54, 109.19, 108.56, 78.53, 75.92, 66.99, 65.92, 57.66, 56.69, 54.18, **HRMS**: for $C_{25}H_{26}N_4O_3$, calculated $[M+H]^+$: 431.2083; observed $[M+H]^+$: 431.2036

4-(2-(4-(2-phenyl-6-(2-(prop-2-yn-1-yloxy)phenyl)pyrimidin-4-yl)phenoxy)ethyl)morpholine (**VB10**): Yield 63%,^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.67 (1H, dd, J_1= 8Hz, J_2= 4Hz), 8.27 (1H, s), 8.26 (2H, d, *J*= 8Hz), 7.53-7.44 (4H, m), 7.21 (1H, t, *J*= 8Hz), 7.13 (1H, d, *J*= 8Hz), 7.04 (2H, d, *J*= 8Hz), 4.80 (2H, d, *J*= 4Hz), 4.19 (2H, t, *J*= 4Hz), 3.75 (4H, t, *J*= 4Hz), 2.84 (2H, t, *J*= 4Hz), 2.60 (4H, b), 2.56 (1H, t, *J*= 4Hz), **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 164.19, 163.29, 162.79, 160.97, 156.23, 138.60, 131.59, 131.33, 130.56, 130.40, 128.97, 128.44, 128.41, 126.56, 122.35, 114.86, 114.72, 113.53, 78.50, 76.00, 67.02, 66.05, 57.68, 56.82, 54.22, **HRMS**: for $C_{31}H_{29}N_3O_3$, calculated $[M+H]^+$: 492.2287; observed $[M+H]^+$: 492.2256

4-(2-(3-(2-phenyl-6-(4-(prop-2-yn-1-yloxy)phenyl)pyrimidin-4-yl)phenoxy)ethyl)morpholine (**VB11**): Yield 62%,^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.68 (2H, dd, J_1= 8Hz, J_2=

4Hz), 8.26 (2H, dd, J_1= 8Hz, J_2= 4Hz), 7.90 (1H, d, *J*= 8Hz), 7.85 (1H, s), 7.81 (1H, d, *J*= 8Hz), 7.54-7.50 (3H, m), 7.44 (1H, d, *J*= 8Hz), 7.13 (2H, dd, J_1= 8Hz, J_2= 4Hz), 7.01 (1H, dd, J_1= 8Hz, J_2= 4Hz), 4.78 (2H, d, *J*= 4Hz), 4.26 (2H, t, *J*= 4Hz), 3.77 (4H, t, *J*= 4Hz), 2.90 (2H, t, *J*= 4Hz), 2.67 (4H, b), 2.56 (1H, t, *J*= 4Hz), **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 166.67, 163.80, 161.95, 160.94, 147.89, 138.06, 130.75, 129.60, 128.88, 128.62, 128.42, 119.49, 116.86, 114.91, 114.58, 112.04, 110.66, 80.96, 76.80, 71.58, 66.75, 65.72, 57.56, 54.09, **HRMS**: for $C_{31}H_{29}N_3O_3$, calculated $[M+H]^+$: 492.2287; observed $[M+H]^+$: 492.2256

4-(3-(2-morpholinoethoxy)phenyl)-6-(4-(prop-2-yn-1-yloxy)phenyl)pyrimidin-2-amine (**VB12**): Yield 72%, ^1H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.01 (2H, d, *J*= 8Hz), 7.60 (1H, s), 7.57 (1H, d, *J*= 8Hz), 7.36 (2H, t, *J*= 8Hz), 7.04 (2H, d, *J*= 12Hz), 7.00 (1H, d, *J*= 8Hz), 5.35 (2H, s), 4.73 (2H, d, *J*= 2.4Hz), 4.18 (2H, t, *J*= 4Hz), 3.73 (4H, t, *J*= 4Hz), 2.82 (2H, t, *J*= 4Hz), 2.59 (4H, t, *J*= 4Hz), 2.54 (1H, t, *J*= 2.4Hz), **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 165.71, 163.56, 160.86, 158.01, 139.49, 130.69, 129.89, 128.70, 120.44, 117.08, 114.79, 114.48, 114.29, 113.53, 103.73, 78.49, 75.82, 66.97, 65.93, 57.63, 56.04, 54.18, **HRMS**: for $C_{25}H_{26}N_4O_3$, calculated $[M+H]^+$: 431.2083; observed $[M+H]^+$: 431.2018

4-(2-(3-(2-methyl-6-(4-(prop-2-yn-1-yloxy)phenyl)pyrimidin-4-yl)phenoxy)ethyl)morpholine (**VB13**): Yield 54%, ^1H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.09 (2H, d, *J*= 8Hz), 7.78 (1H, s), 7.64 (1H, d, *J*= 8Hz), 7.51-7.47 (1H, m), 7.38 (1H, t, *J*= 8Hz), 7.07 (2H, d, *J*= 12Hz), 7.02 (1H, dd, J_1= 8Hz, J_2= 4Hz), 4.74 (2H, d, *J*= 4Hz), 4.28 (2H, t, *J*= 4Hz), 4.19 (2H, t, *J*= 4Hz), 3.72 (4H, t, *J*= 4Hz), 2.81 (3H, s), 2.59 (4H, t, *J*= 4Hz), 2.54 (1H, t, *J*= 2.4Hz), **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 168.47, 164.44, 164.25, 159.72, 159.33, 139.14, 131.02, 130.02, 128.91, 128.83, 118.83, 116.96, 115.27, 113.29, 109.49, 78.18, 76.07, 66.98, 65.88, 65.66, 57.75, 55.92, 54.17, **HRMS**: for $C_{26}H_{27}N_3O_3$, calculated $[M+H]^+$: 430.2131; observed $[M+H]^+$: 430.2101

2-phenyl-4-(4-(2-(piperidin-1-yl)ethoxy)phenyl)-6-(3-(prop-2-yn-1-yloxy)phenyl)pyrimidine (**VB14**): Yield 53%, ^1H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.70 (2H, dd, J_1= 8Hz, J_2= 4Hz), 8.24 (2H, d, *J*= 8Hz), 7.96 (1H, t, *J*= 8Hz), 7.91 (1H, s), 7.85 (1H, dd, J_1= 8Hz, J_2= 4Hz), 7.53-7.45 (4H, m), 7.13 (1H, dd, J_1= 8Hz, J_2= 4Hz), 7.05 (2H, d, *J*= 8Hz), 4.81 (2H, d, *J*= 8Hz), 4.20 (2H, t, *J*= 4Hz), 2.83 (2H, t, *J*= 4Hz), 2.58 (4H, t, *J*= 4Hz), 1.66-1.60 (4H, m), 1.47-1.40 (3H, m), **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 164.35, 164.37, 164.05, 161.16, 158.17, 139.36, 138.29, 130.70, 130.01, 128.85, 128.51, 120.52, 117.11, 114.56, 113.96, 109.61, 78.39, 75.83,

57.92, 56.16, 75.83, 29.72, 25.86, 24.13, **HRMS**: for $C_{32}H_{31}N_3O_2$, calculated $[M+H]^+$: 490.2495; observed $[M+H]^+$: 490.2480

4-(4-(2-(piperidin-1-yl)ethoxy)phenyl)-6-(3-(prop-2-yn-1-yloxy)phenyl)pyrimidin-2-amine (**VB15**): Yield 58%, ^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 7.99 (2H, d, *J*= 8Hz), 7.65-7.62 (2H, m), 7.39 (1H, t, *J*= 8Hz), 7.35 (1H, s), 7.08 (1H, dd, J_1= 8Hz, J_2= 4Hz), 6.97 (2H, d, *J*= 8Hz), 5.26 (2H, s), 4.75 (2H, d, *J*= 4Hz), 4.16 (2H, t, *J*= 4Hz), 2.80 (2H, t, *J*= 4Hz), 2.53 (5H, b), 1.62-1.58 (4H, m), 0.97-0.92 (2H, m), **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 165.75, 165.55, 163.60, 160.96, 158.07, 139.53, 131.02, 129.87, 128.67, 120.45, 117.08, 114.79, 113.50, 103.68, 78.51, 75.83, 66.01, 57.85, 56.03, 55.11, 25.87, 34.17, **HRMS**: for $C_{26}H_{28}N_4O_2$, calculated $[M+H]^+$: 429.2291; observed $[M+H]^+$: 429.2238

(E)-1-(4-(2-morpholinoethoxy)phenyl)-3-(4-(prop-2-yn-1-yloxy)phenyl)prop-2-en-1-one (3a): Yield 85%, ^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0):8.01 (2H, d, *J*= 8Hz), 7.66 (1H, d, *J*= 16Hz), 7.60 (2H, d, *J*= 8Hz), 7.43 (1H, d, *J*= 16Hz), 7.02-6.95 (4H, m), 4.73 (2H, d, *J*= 4Hz), 4.18 (2H, t, *J*= 4Hz), 3.73 (4H, t, *J*= 4Hz), 2.83 (2H, t, *J*= 4Hz), 2.58 (2H, t, *J*= 4Hz), 2.55 (1H, t, *J*= 4Hz), ^{13}C NMR **(**$CDCl_3$, 100 MH_Z, δ with TMS=0): 188.72, 162.50, 159.37, 143.68, 131.45, 130.80, 130.12, 128.68, 120.01, 115.34, 114.42, 77.46, 76.12, 66.99, 66.09, 57.55, 55.90, 54.19 **MS**: for $C_{24}H_{25}NO_4$, calculated $[M]^+$: 391.17; observed $[M]^+$: 391

(E)-1-(4-(2-morpholinoethoxy)phenyl)-3-(2-(prop-2-yn-1-yloxy)phenyl)prop-2-en-1-one (3b): Yield 83%, ^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 8.02-8.09 (3H, m), 7.63-7.69 (2H, m), 7.34 (1H, t, *J*= 8H_Z), 7.03 (2H, t, *J*= 8H_Z), 6.96 (2H, d, *J*= 8H_Z), 4.79 (2H, d, *J*= 2.4 H_Z), 4.18 (2H, t, *J*= 4H_Z), 3.73 (4H, t, *J*= 4H_Z), 2.82 (2H, t, *J*= 4H_Z), 2.56 (4H, t, *J*= 4H_Z), 2.55 (1H, t, *J*= 4H_Z), ^{13}C NMR **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 189.25, 162.48, 156.72, 139.33, 131.39, 130.93, 129.57, 124.76, 123.17, 121.77, 114.38, 112.80, 78.14, 76.07, 66.99, 66.06, 57.55, 56.26, 54.18 **MS**: for $C_{24}H_{25}NO_4$, calculated $[M]^+$: 391.17; observed $[M]^+$: 391

(E)-1-(3-(2-morpholinoethoxy)phenyl)-3-(4-(prop-2-yn-1-yloxy)phenyl)prop-2-en-1-one (**3c**): Yield 87%, ^{1}H NMR ($CDCl_3$, 400 MH_Z, δ with TMS=0): 7.76 (1H, d, *J*= 16Hz), 7.58 (3H, t, *J*= 8Hz), 7.52 (1H, s), 7.40-7.36 (2H, m), 7.11 (1H, d, *J*= 8Hz), 7.00 (2H, dd, J_1= 8Hz, J_2= 4Hz), 4.72 (2H, t, *J*= 4Hz), 3.72 (4H, t, *J*= 4Hz), 2.81 (2H, t, *J*= 4Hz), 2.57 (4H, b), 2.54 (1H, t, *J*= 4Hz), ^{13}C NMR **(**$CDCl_3$, 100 MH_Z, δ with TMS=0**)** δ: 190.25, 159.57, 159.07, 144.61, 139.86, 130.27, 129.66, 128.47, 121.23, 120.23, 119.70, 115.39, 113.67, 78.06, 76.15, 66.97, 65.95, 57.67, 55.91, 54.15 **MS**: for $C_{24}H_{25}NO_4$, calculated $[M]^+$: 391.17; observed $[M]^+$: 391

Biological studies

Determination of hMAO inhibition activity

The synthesized compounds were evaluated against MAO-A and MAO-B isoforms for their inhibitory potential using Amplex® Red assay kit through fluorimetric method described by us and others.[23]

Briefly, 100 µl of sodium phosphate buffer (0.05 M, pH 7.4) containing the test drug and reference inhibitors, in various concentrations along with adequate amounts of recombinant hMAO (hMAO-A: 1.1 µg protein; specific activity: 150 nmol of p-tyramine oxidized to p-hydroxyphenylacetaldehyde/min/mg protein; hMAO-B: 7.5 µg protein; specific activity: 22 nmol of p-tyramine transformed/min/mg protein) enzyme, were incubated for 15 min at 37 °C in a flat-black-bottom 96-well plates (Tarsons) in a incubator. After the incubation period, the reaction was started by adding (final concentrations) 200 µM Amplex® Red reagent, 1 U/mL horseradish peroxidase and 1 mM p-tyramine. After 30 min incubation in dark, the production of H_2O_2 was quantified at 37 °C in a multi-detection microplate fluorescence reader (Synergy^{HI}, Bio-Tek® Instruments) based on the fluorescence generated at excitation wavelength of 545 nm, and emission wavelength of 590 nm. Control experiments were carried out simultaneously by replacing the test drug with the vehicle. A direct reaction of test drug with the Amplex® Red reagent in a sodium phosphate buffer was performed to minimize the possibility of the test drugs to influence the fluorescence generated in the reaction mixture due to non-enzymatic inhibition. However, no significant modulation in the fluorescence was observed. The specific final fluorescence emission was calculated after subtraction of the background activity determined from vials containing all the components except the hMAO enzymes replaced by a sodium phosphate buffer solution.

Acetylcholinesterase inhibition assay

Acetylcholinesterase inhibition activity was determined using Amplex Red Acetylcholine/Acetylcholinesterase assay kit (A12217) purchased from the Molecular probes Inc. Invitrogen.[38] Briefly, 100 µl of Tris-HCl buffer (0.05 M, pH 8.0) containing the synthesized test drugs and reference inhibitors, in various concentrations along with adequate amounts of recombinant AChE (0.2U/mL) enzyme, were incubated for 15 min at 37 °C in a flat-black-bottom 96-well plates (Tarsons). Reaction was started by adding 100 µl of working solution of

400 μM Amplex Red reagent containing 2 U/mL horseradish peroxidase, 0.2 U/mL choline oxidase and 100 μM acetylcholine. After 30 min incubation in dark, the production of H_2O_2 and subsequent formation of resorufin from Amplex red dye was quantified at 37 °C in a multi-detection microplate fluorescence reader (Synergy^{HI}, Bio-Tek® Instruments) based on the fluorescence generated at excitation wavelength of 545 nm, and emission wavelength of 590 nm. Positive control experiment was carried out simultaneously by replacing the test drugs with the vehicle. Second positive control was carried out by using 20 mM H_2O_2. 1X reaction buffer without acetylcholinesterase was used as negative control. The specific final fluorescence emission was calculated after subtraction of the background activity, determined from wells containing all components except the AChE replaced by a 1X buffer solution. Each experiment was performed in triplicate (n=3).

BuChE inhibition assay

The procedure described by Ellman et al. was used for BuChE inhibition assays with minor modifications.[39] BuChE was purchased from sigma Aldrich (CAS No. 9001-80-5). Butyrylthiocholine iodide (BTCI), 5.5'-dithiobis (2-nitrobenzoic acid) (DTNB - Ellman's reagent) were purchased from Himedia. The assays were performed tris-HCl buffer (pH-8) and donepezil was used as standard compound. Six different concentrations of 20 μM, 10 μM, 1μM, 0.1 μM, 0.01 μM, 0.001 μM of test compound were used to determine the IC_{50}. 50 μL (0.6 Uml^{-1}) and 20 μL of test or standard compounds were incubated in 96 well plates for 30 min. 100 μL (1.5 mM) of DTNB was added in the above solution. The substrate i.e. BTCI (30 mM, 10 μL) was added into it and absorbance was recorded immediately at 415 nm for 20 min at 1 min interval using Biotek well plate reader. The IC_{50} values were calculated using absorbance obtained from the test and standard compounds. The assays were performed in triplicate and in three independent runs.

Reversibility inhibition studies

For reversibility inhibition studies protocol was adopted from the literature.[30, 31] Briefly, the test inhibitors were incubated with the MAO enzymes at concentrations of 10 × IC_{50} and 100 × IC_{50} at 37 °C for 30 min (negative control performed in the absence of inhibitor), and 4% DMSO was added as co-solvent to all incubations. After 30 min incubation period, the samples were subsequently diluted to 100–fold with the addition of tyramine substrate to achieve final inhibitor concentrations of 0.1 × IC_{50} and 1 x IC_{50} value, respectively. As positive controls, MAO-A and

MAO-B were incubated with the irreversible inhibitors, clorgyline and pargyline respectively, at 10 × IC_{50} concentrations and then diluted 100-fold to achieve final inhibitor concentrations of 0.1 × IC_{50}. The residual MAO activities after dilutions were measured (n=3) and the residual enzyme activities were expressed as mean ± SD.

ROS production inhibition studies

Intracellular levels of ROS were determined using protocol described elsewhere,[40] using non-fluorescent compound 2-7-dichlorofluorescein diacetate (DCF-DA). It is permeable to cell membrane where it is hydrolyzed by intracellular esterases and further oxidized by ROS to a fluorescent compound 2-7-DCF. Cells (SH-SY5Y) were seeded in 96 well plates ($1x10^4$ cells/ well) and left for 24 h in complete media at 37 ^oC. Then media was removed, washed with PBS and cells were treated with the test compounds (without FBS) for 24 h and 48 h at different concentrations (1 μM, 5 μM and 25 μM). After completion of experiment, cells were rinsed with PBS thrice and then treated with H_2DCF-DA (50μM) and incubated for 30 min at 37 ^oC. Following incubation, cells were rinsed with PBS and fluorescence was detected at wavelength of 478 nm excitation and 518 nm emission.

Neuroprotective studies

The neuroprotective potential of compounds was determined against 6-OHDA neurotoxin using MTT assay.[41] For this SH-SY5Y cells were plated in 96 wells, at 10^6 cells/well density. The cells were cultured for 24h in DMEM/F-12 media containing 10% FBS and horse serum supplemented 1% penicillin antibiotic solution. Then cells were treated with the target compounds (at concentrations of 1-25Mm), 4h before 6-OHDA (12.5 μM). After 24 h incubation in the oven, at 37 °C and a 5% CO_2, 95% O_2 atmosphere, the tested compounds were replaced with 80 mL of medium and 20 mL of MTT in PBS (0.5 mg/mL, final concentration). The cells were incubated for another 4h. After the removal of MTT, the formazan crystals were dissolved in DMSO. The amount of formazan was measured using a microculture plate reader with a test wavelength of 570 nm. Results were expressed as the mean ± SD of three independent experiments.

Cytotoxicity studies

With an aim to test the cytotoxicity of the synthesized compounds on neuronal cells, MTT assays were carried out with human neuroblastoma SH-SY5Y cell lines.[23] Approximately 10,000 cells

were seeded per well of 96 well plate in DMEM/F-12 media containing 10% FBS and horse serum supplemented 1% penicillin antibiotic solution for 24h and treated as indicated in the experimental design. Cells were treated with synthesized compounds at concentration of 1 μM, 5 μM and 25 μM for 24h in humidified CO_2 incubator, maintained at 37 ^oC with 5% CO_2 and 95% humidity under serum-free conditions.

Metal-chelating study

Metal chelating studies were performed with a UV−vis spectrophotometer. The absorption spectra of each compound (50 μM, final concentration) alone or in the presence of $CuSO_4$, $FeSO_4$, and $FeCl_3$ (50 μM, final concentration) for 30 min in 20% (v/v) methanol/buffer (20 mM HEPES, 150 mM NaCl, pH = 7.4) were recorded at room temperature.[42]

Kinetic studies of AChE inhibition

To determine the mechanism of action of the most potent inhibitor of AChE, **VB8,** kinetic study was performed using *ee*AChE with the help of earlier reported protocols.[25, 43] Linewevaer-Burk double reciprocal graph was plotted at different concentrations of substrate ACh (0.1mM- 1mM) by using same methodology reported for the *in vitro* inhibition study of AChE. Progress curves were analyzed by steady state turnover of substrate and values of linear regression were fitted according to Lineweaver-Burk replots using excel software (2016). Three concentrations of VB8 (1 nM, 10 nM and 100 nM) were used for kinetic study. The plots were assessed by weighted least-square analysis that assumed the variance of ν to be constant for whole data set. Slopes of the reciprocal of ν were then plotted against the reciprocal of the substrate concentration.

Molecular docking studies

Docking studies can provide some valuable information on ligand's orientation and its interactions at the receptor site. To determine the mode of interaction of the synthesized ligands at the active site of hMAO-A and AChE enzymes, molecular docking studies were performed using Maestro 11.1 (Schrödinger LLC).[44] X-ray crystal structures of hMAO-A (PDB ID- 2BXR and 2Z5X)[35, 36] and AChE (PDB ID- 1EVE)[37] enzymes were imported from the protein data bank (www.rscb.org). Protein was prepared using "protein preparation wizard" application of Schrödinger suite 2017. Energy was minimized using OPLS2005 force field. Ligands were drawn in ChemBio Draw Ultra-12 and prepared using ligand preparation application in Schrödinger suite 2017. For each compound, the top-score docking poses were chosen for final

ligand-target interaction analysis employing XP interaction visualizer of Maestro 11.1 software. Validation of the docking procedure was done by re-docking the co-crystalized ligand into the active site of the enzymes. Qikprop application of Schrodinger suit was used to determine the drug like and ADME properties of the compounds.[45]

Molecular dynamics simulation studies

In order to investigate the behavior and stability of the potent inhibitors into the active site of the MAO and AChE, molecular dynamic (MD) simulation was utilized. For this docking complex of **VB1** with MAO-A and **VB8** with AChE were used. MD simulations were performed using Desmond standard protocol.[46] Complex was solvated by TIP3P water model and then naturalized by adding 0.15M Na^+ and Cl^- ions. The thickness of water layer was set to 10 Å. Before the MD simulations the systems were minimized with a maximum iteration of 2000 steps. Then, the systems were submitted to 30 ns MD simulation for equilibration and production MD run. Temperature and pressure were assigned on 300 K and 1.01325 bar, respectively using Isothermal–isobaric (NPT) ensemble. Cut-off radios of 9 Å was used for Coulomb interactions.

Supplementary Information

NMR spectral data and HRMS graphs of the final compounds are provided in the supplementary information file.

Conflict of Interest Statement

A provisional patent application has been filed containing these compounds (Indian patent application number **201811008301)**.

Author Contributions

Bhupinder Kumar was involved in the design and synthesis of most of the compounds. He also performed hMAO and *ee*AChE inhibitory assays, performed *in silico* studies and drafted the manuscript. Ashish Ranjan Dwivedi was also involved in the synthesis of some of the compounds, column purification and spectral analysis of the compounds. He also assisted in the molecular modeling studies and drafting of the manuscript. Bibekananda Sarkar under the supervision of Anil K. Mantha, performed other *in vitro* biological studies like cytotoxic studies, ROS production inhibition potential and neuroprotective studies. Sukesh Kumar Gupta and Sairam Krishnamurthy conducted BuChE inhibition studies of the compounds. Jyoti Prakash

helped in the evaluation of hMAO and *ee*AChE inhibition activities and reversibility inhibition studies. Vinod Kumar conceptualized, supervised and coordinated all the studies and prepared the final draft of the manuscript. All authors read and approved the final manuscript.

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Abbreviations

QPlogBB: qualitatively predicted logarithmic ratio between the concentration of a compound in brain and blood; LogP: partition coefficient of a molecule between an aqueous and lipophilic phase (octanol and water); CAS: Catalytic active site; PAS: Peripheral active site; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; 6-OHDA: 6-hydroxydopamine; DMEM: Dulbecco's Modified Eagle's medium; HEPES: (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)

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4,6-Diphenylpyrimidine Derivatives as Dual Inhibitors of Monoamine Oxidase and Acetylcholinesterase for the Treatment of Alzheimer's Disease

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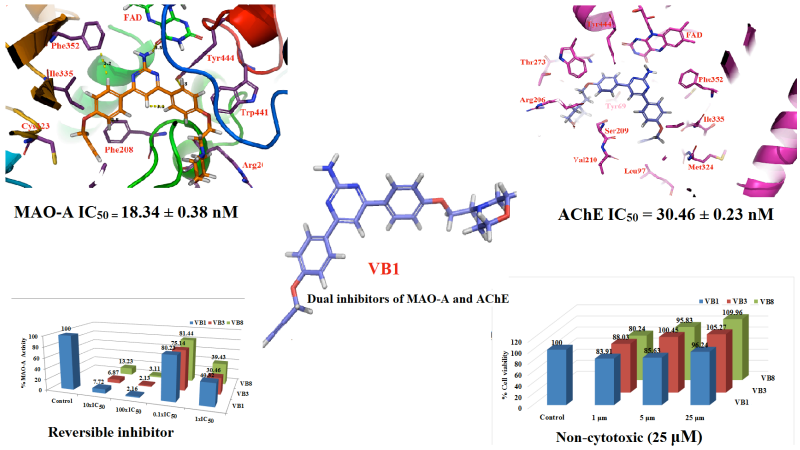
Graphical abstract



4,6-diphenylpyrimidine derivatives as dual inhibitors of monoamine oxidase and acetylcholinesterase