Journal of Medicinal Chemistry

Article

Benzylpiperidine-linked diarylthiazoles as potential anti-Alzheimer's agents-synthesis and biological evaluation

Mahesh Shidore, Jatin Machhi, Kaushik Shingala, Prashant Murumkar, Mayank Kumar Sharma, Neetesh Agrawal, Ashutosh Tripathi, Zalak Parikh, Prakash Pillai, and Mange Ram Yadav

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.6b00426 • Publication Date (Web): 02 Jun 2016

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Benzylpiperidine-linked diarylthiazoles as potential anti-Alzheimer's agents-synthesis and biological evaluation

Mahesh Shidore[a], Jatin Machhi[a], Kaushik Shingala[a], Prashant Murumkar[a], Mayank Kumar Sharma[a], Neetesh Agrawal[a], Ashutosh Tripathi[b], Zalak Parikh[b], Prakash Pillai[b], and Mange Ram Yadav[a]*

[a]*Faculty of Pharmacy,*
Kalabhavan Campus,
The Maharaja Sayajirao University of Baroda,
Vadodara – 390 001, India

[b]*Zoology Department,*
Faculty of Science,
The Maharaja Sayajirao University of Baroda
Vadodara – 390 001, India

KEYWORDS: Alzheimer's disease, $A\beta_{1-42}$, *p*-Tau, 2′,7′-Dichlorofluorescine diacetate assay.

ABSTRACT

A novel series of hybrid molecules were designed and synthesized by fusing the pharmacophoric features of cholinesterase inhibitor donepezil and diarylthiazole as potential multi-target-directed ligands for the treatment of Alzheimer's disease (AD). The compounds showed significant *in vitro* anti-cholinesterase (anti-ChE) activity; the most potential compound (**44**) among them showing the highest activity (IC_{50} value: 0.30±0.01 μM) for AChE and (1.84±0.03 μM) for BuChE. The compound (**44**) showed mixed inhibition of AChE in the enzyme kinetic studies. Some compounds exhibited moderate to high inhibition of AChE-induced $A\beta_{1-42}$ aggregation, and noticeable *in vitro* anti-oxidant and anti-apoptotic properties. Compound (**44**) showed significant *in vivo* anti-ChE and anti-oxidant activities. Furthermore, compound (**44**) demonstrated *in vivo* neuroprotection by decreasing $A\beta_{1-42}$-induced toxicity by attenuating abnormal levels of $A\beta_{1-42}$, *p*-Tau, cleaved-caspase-3 and cleaved-PARP proteins. Compound (**44**) exhibited good oral absorption and was well tolerated up to 2000 mg/kg, p.o. dose without showing toxic effects.

INTRODUCTION

In the current era of innovative therapeutics, Alzheimer's disease (AD) has emerged as a challenging disease to treat among the neurodegenerative disorders. As on today almost 46 million people worldwide live with dementia.[1] It is expected that by 2050, AD affected population would multiply three fold, especially in the developed countries like U.S. and Europe.[2, 3] Although the exact cause of AD is not yet fully known, reports suggest the involvement of several factors for the development of the disease, including low levels of acetylcholine (ACh) in the hippocampus and cortex area of the brain. The deposition of amyloid β (Aβ) peptide, neurofibrillary tangles (*p*-Tau) and oxidative stress are also known to play vital roles in the pathogenesis of AD.[4, 5] As per the cholinergic hypothesis, the disease is attributed to the reduced levels of ACh, an important neurotransmitter involved in memory and learning in the brain.[6] Acetylcholinesterase (AChE) is a hydrolase enzyme that hydrolyzes ACh and quickly terminates the cholinergic synaptic transmission.[7] Studies also suggest that butyrylcholinesterase (BuChE), another cholinesterase present in the brain is also responsible for hydrolysis of ACh, and its inhibition therefore may further enhance cholinergic transmission in AD.[8-10] Thus, the use of acetylcholinesterase inhibitors (AChEIs) and butyrylcholinesterase inhibitors (BuChEIs) has become currently the foundation for the management of AD.[11]

Researchers have also widely explored the amyloid hypothesis in AD.[12-14] As per this hypothesis, accumulation of Aβ peptide aggregates leads to numerous pathophysiological changes causing cognitive dysfunction. Secretase proteases are responsible for the formation of Aβ in the brain, leading to increased levels of Aβ in senile plaques which ultimately lead to development of AD.[15] Studies have also indicated that AChE increased the formation of Aβ fibrils and Aβ plaques in the cerebral cortex of transgenic mouse models of AD.[16, 17] Depleting

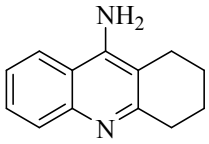
the Aβ peptide from the human brain is considered as a rational therapeutic approach for the treatment of AD.[18, 19]

Pathogenic role of oxidative damage in the progression of neurodegeneration has also been well reported.[20-22] Oxidative stress leads to deposition of senile plaques, neurofibrillary tangles,[21, 23] and the deposition of Aβ in the brain.[24] Some studies have suggested that the neurotoxicity of aggregated Aβ is mediated through its ability to induce oxidative stress via spontaneous generation of free radicals and reactive oxygen species (ROS).[25-27] Oxidative and nitrative stress resulting in the formation of ROS and reactive nitrogen species (RNS)[28] cause oxidative damage to the proteins leading to cellular dysfunction and cell death.[29, 30] Thus, successful protection of the neuronal cells from oxidative damage could probably prevent AD.[31] Oxidative stress and apoptosis are closely linked physiological phenomena which are implicated in various pathological conditions including AD.[32] Apoptotic cell death comprises of a sequence of events leading to the activation of caspase cascade which initiates the fragmentation of cellular proteins and DNA, leading to the disintegration of the cell ultimately.[33] It is now well accepted that massive neuronal and glial cell death in the brain due to apoptosis is a common characteristic feature in patients suffering from AD.[34, 35] Thus, a significant level of neuroprotection could be achieved by employing chemical entities exhibiting anti-oxidant and anti-apoptotic potentials in the brain.

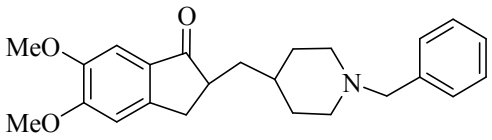
Various drugs have been used over the past several years for the treatment of AD.[36] But, currently, there is no drug which can completely cure AD.[37] Primary therapeutic option available for the treatment of AD is the use of US-FDA approved cholinesterase inhibitors (**Fig. 1**) like tacrine (**1**), donepezil (**2**), rivastigmine (**3**) and galantamine (**4**). Tacrine (**1**) exhibited hepatotoxicity via elevation of serum alanine aminotransferase levels, forcing its limited clinical

use and consequent withdrawal from the market shortly after its approval.[38] Donepezil (**2**) is the most effective pharmacological agent among the list of approved drugs to treat AD efficiently.[39]

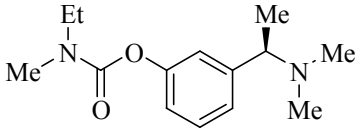
Development of multi-target-directed ligands is considered one of the most promising drug discovery approaches for diseases with complex etiology like AD. Single-targeted drugs might not always alter the complex diseased system adequately, even if they modulate their targets with high affinity and selectivity. In contrast, in case of targets connected in a network, a multi-target-directed ligand interacting with lower but balanced affinities can still exert superior effects compared to a single-targeted molecule.[40, 41] Multi-target-directed drugs have a better ability to affect the complex equilibrium of whole cellular network than single-targeted drug because of their simultaneous effects on several therapeutic targets. Low-, but balanced-affinity multi-target-directed drugs demonstrate weak links with several targets to stabilize the complex networks of a biological system. A multi-target-directed drug can actually have a better efficacy/safety ration than a mono-targeted drug. A balanced, mild activity for multiple therapeutic targets might have better safety and reduces the risk of therapeutic resistance.[40-43] Hence, there is a need to design such compounds that can act on different causative targets of AD simultaneously with balanced affinity. This can probably be achieved by linking together various active moieties having affinity for different targets. The resulting hybrids with balanced affinity for different targets could be beneficial to take care of a complex disease like AD.[5, 42, 44-46]



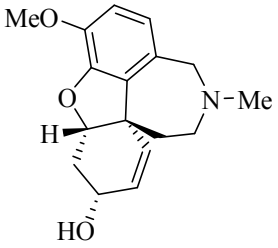
Tacrine **(1)**



Donepezil **(2)**



Rivastigmine **(3)**



Galantamine **(4)**

Figure 1. AChE inhibitors (**1-4**) used for the management of AD.

Recently various bispharmacophore/bivalent ligands[47-49] have been reported in the literature wherein two of the same or different pharmacophoric moieties like donepezil-propargylamine, donepezil-pyridyl, donepezil-ebselen, tacrine-ferulic acid, huprine-tacrine etc are linked together via a spacer for the treatment of AD.[31, 39, 44-46, 50] Taking a cue from these studies, it was planned to synthesize some novel diarylthiazol-benzylpiperidine hybrids and to assess their potential use in the treatment of AD by performing various *in vitro* and *in vivo* experiments.

RESULTS AND DISCUSSION

Designing considerations. Thiazole ring is present in different chemical entities having a broad spectrum of biological properties such as antimicrobial, anticonvulsant, antituberculor, antiinflammatory, antiviral, antimalarial, anticancer, antihypertensive, anti-HIV, anti-schizophrenia, hypnotic, antinociceptive, and bacterial DNA gyrase B inhibitory activity.[51-54] Acotiamide is a thiazole ring containing drug having potent AChE inhibitory activity.[55, 56] There are several other reports which suggest that the thiazole containing compounds can be used for the ChE inhibiting activity.[54, 57-62]

Some vicinal diaryl triazine derivatives have been reported as potent neuroprotective agents.[63] Our laboratory has also reported recently the design and development of vicinally substituted diaryltriazines (**5**)[64] as potential multi-target-directed therapeutics for the treatment of AD. It was inferred from the study that the multipotent activity of these diaryltriazines (**5**) resided in the diaryltriazine scaffold, and not in the morpholino/piperazinoethyl side chain. A preliminary molecular modeling study demonstrated that replacement of the morpholino/piperazinoethyl side chain of compound (**5**) by benzylpiperidine tail of donepezil offered a hybrid molecule in which the diaryltriazine and benzylpiperidine portions were bound to the peripheral anionic site (PAS) and the catalytic active site (CAS) respectively with a G-score of -9.21. In a parallel study, replacement of the diaryltriazine moiety with the diarylthiazole scaffold exhibited a slightly better G-score of -9.38 in the active site of AChE. Considering these preliminary results and the earlier reports on AChE inhibitory potential of thiazole-containing derivatives, it was persumed that diarylthiazole scaffold could probably be a better CNS-active moiety for the development of novel multi-target-directed ligands against AD. Based upon these preliminary studies, it was planned to link the diarylthiazole ring with the benzylpiperidine tail of donepezil to obtain novel diarylthiazol-benzylpiperidine hybrids (**Fig. 2**). It was considered that the diarylthiazole core of the designed hybrids would mimic the dimethoxyindanone scaffold of donepezil (**2**) into the PAS of AChE and the benzylpiperidine fragment of the designed compounds would interact with the CAS similar to benzylpiperidine tail of donepezil, retaining the same π-stacking interactions. Thus, the designed diarylthiazol-benzylpiperidine hybrids could show multi-target-directed potentials for the management of AD. The designed molecules are novel because such vicinal diarylthiazoles have not yet been reported as AD-responsive agents.

The resulting compounds were planned to be evaluated for their anti-Alzheimer's potentials. Hence, we report herewith an approach wherein a vicinal diarylthiazole scaffold is linked to a benzylpiperidine moiety to get novel hybird molecules of type (A) (**Fig. 2**) as potential multi-target-directed anti-Alzheimer's agents.

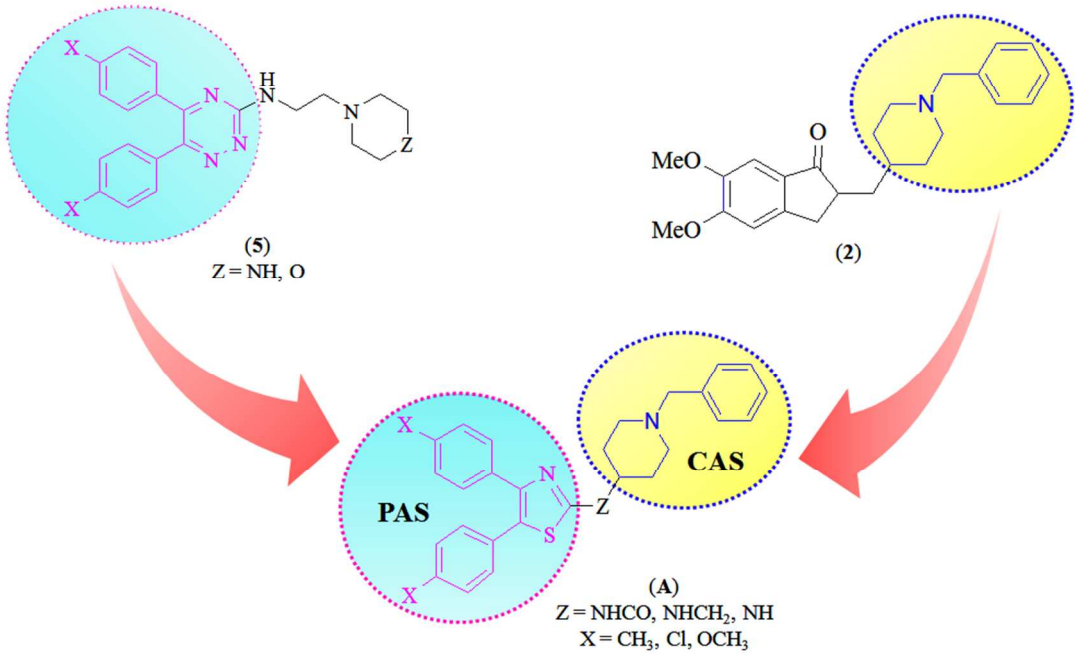
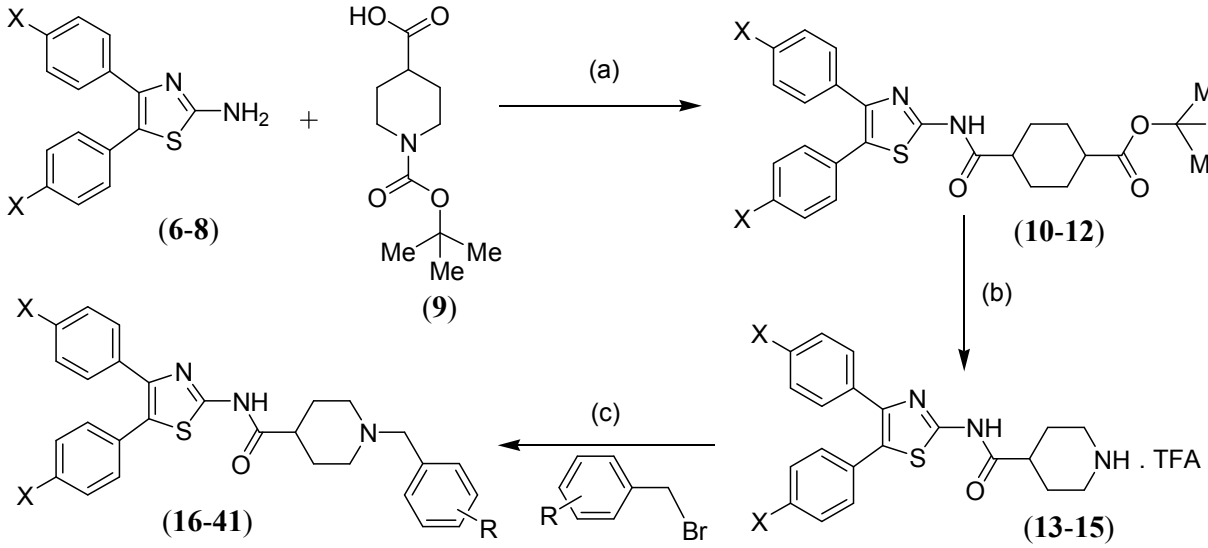


Figure 2. Designing of a novel series of compounds as multi-target-directed potential anti-Alzheimer's agents.

Chemistry. The targeted compounds were synthesized as per **Scheme 1-3**. In **Scheme 1**, the amines[65, 66] (**6-8**) were treated with 1-(*t*.butoxycarbonyl)piperidine-4-carboxylic acid[67] (**9**) in presence of *N,N*-di*iso*propylethylamine and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) as the coupling reagent to get the amides (**10-12**). Deprotection of the *tert*-butoxycarbonyl (BOC) group was performed using trifluoroacetic acid (TFA) in dichloromethane (DCM) to get the amine salts (**13-15**) which were treated further with substituted benzyl bromides using potassium carbonate and dimethylformamide (DMF) to yield the desired compounds (**16-41**).

Scheme 1. Synthesis of 1-substituted benzyl-*N*-[4,5-bis(substituted phenyl)thiazol-2-yl]piperidine-4-carboxamides (16-41)



X			R	X			R
Me	**Cl**	**OMe**		**Me**	**Cl**	**OMe**	
16	**26**	**34**	2-Me	**23**	-	-	4-CN
17	**27**	**35**	2-CF_3	**24**	-	-	4-NO_2
18	**28**	**36**	3-F	**25**	-	-	4-CF_3
19	**29**	**37**	3,5-DiF	-	**31**	**40**	2-CN
20	**30**	-	2-Cl, 4-F	-	**32**	**41**	4-F, 2-CF_3
21	-	**38**	4-F	-	**33**	-	4-OCF_3
22	-	**39**	2-Cl, 6-F				

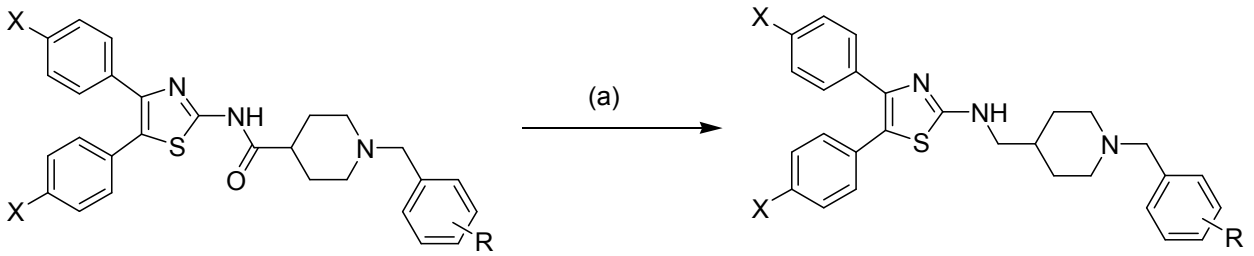
Compd	X
6, 10, 13	Me
7, 11, 14	Cl
8, 12, 15	OMe

Reagents and Conditions: a) BOP reagent, *N,N*,-di*iso*propylethylamine, dry acetonitrile, ^{o}C to rt; b) TFA:DCM (70:30), rt; c) Potassium carbonate, dry DMF, substituted benzyl bromides, rt to heating 60 ^{o}C.

N-[(1-Substituted benzyl)piperidin-4-yl]methyl-4,5-diaryl-2-amines **(42-52)** were prepared by reduction of 1-substituted benzyl-*N*-[4,5-bis(substituted phenyl)thiazol-2-yl]piperidine-4-carboxamides (**16**, **17**, **19**, **21**, **26**, **29**, **30**, **32**, **34**, **35** and **39**) in presence of borane-dimethyl sulphide complex in THF at 0 ^{o}C as shown in **Scheme 2**.

4-Piperidinecarboxamide (**53**) was treated with substituted benzyl bromides (**54-59**) using potassium carbonate in methanol to get 1-(1-substituted phenyl)piperidine-4-carboxamides (**60-65**), which were treated further with bis(triacetoxyiodobenzene) and benzoyl isothiocyanate to obtain 1-(substituted benzyl)piperidin-4-ylthioureas (**66-71**). The thiourea derivatives (**66-71**) were then reacted with 2-bromo-1,2-diarylethanone (**72a-c**) in dry ethanol to obtain the desired compounds (**73-82**) as shown in **Scheme 3.**

Scheme 2. Synthesis of *N*-[(1-substitued benzyl)piperidin-4-ylmethyl]-4,5-bis(substituted phenyl)-2-ylamines (42-52)

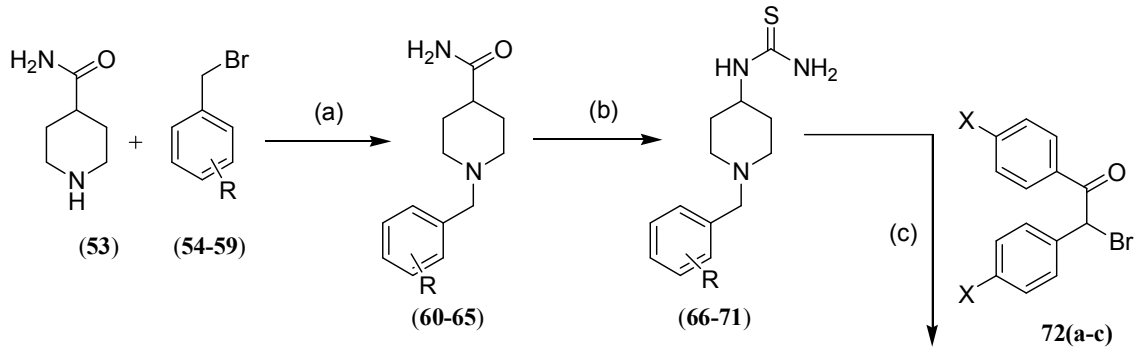


(**16**, **17**, **19**, **21**, **26**, **29**, **30**, **32**, **34**, **35**, **39**) (**42-52**)

X			R
Me	**Cl**	**OMe**	
16, 42	**26, 46**	**34, 50**	2-Me
17, 43	**-**	**35, 51**	2-CF_3
19, 44	**29, 47**	**-**	3,5-diF
21, 45	**-**	**-**	4-F
-	**30, 48**	**-**	2-Cl, 4-F
-	**32, 49**	**-**	2-CF_3, 4-F
-	**-**	**39, 52**	2-Cl, 6-F

Reagents and Conditions: a) i) BH_3-DMS, dry THF, 0 ^oC to rt; ii) 1N HCl, Reflux 4 hrs; iii) 5 % sodium bicarbonate, rt to reflux, 2 hrs.

Scheme 3. Synthesis of 1-(substituted benzyl)-*N*-[4,5-bis(substituted phenyl)thiazol-2yl]-piperidin-4-ylamines (73-82)

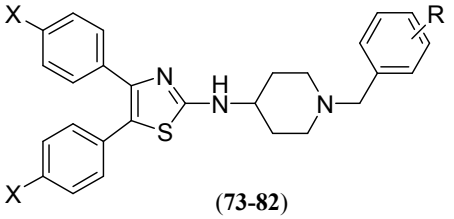


(**53**) (**54-59**) (**60-65**) (**66-71**) **72(a-c)**

Compd			R
54	**60**	**66**	H
55	**61**	**67**	4-Me
56	**62**	**68**	4-OMe
57	**63**	**69**	2-Cl, 4-F
58	**64**	**70**	4-CN
59	**65**	**71**	4-CF_3

Compd	X
72a	Me
72b	Cl
72c	OMe

X			R
Me	**Cl**	**OMe**	
73	**75**	**-**	H
74	**76**	**80**	4-Me
-	**77**	**81**	4-OMe
-	**-**	**82**	4-CF_3
-	**78**	**-**	4-CN
-	**79**	**-**	2-Cl, 4-F



(**73-82**)

Reagents and Conditions: a) Potassium carbonate, methanol, reflux; b) i) Bis(triacetoxyiodobenzene), Potassium carbonate, ACN/water, rt to reflux; ii) Benzoyl isothiocyanate, dry DCM iii) THF/1N NaOH, reflux, 4 hrs; c) Dry ethanol, reflux.

Biological Evaluation. *In vitro* AChE and BuChE inhibition assays. The synthesized compounds (**16-52** and **73-82**) were assessed to determine their anti-cholinesterase (anti-ChE) activity. AChE and BuChE inhibition activities were evaluated by the method described by Ellman[68] wherein tacrine and donepezil were used as reference standards (**Table 1**). Compounds (**43-45**, **48** and **52**) having benzylpiperidine and 4,5-disubstituted thiazolyl-2-amine linked with aminomethylene spacer exhibited higher AChE inhibitory activity [IC_{50} values (μM): 0.40±0.01, 0.30±0.01, 0.37±0.01, 0.52±0.01 and 0.36±0.01 respectively] than the rest of the derivatives.

Compounds (**16-18**, **20** and **25**) having 4,5-bis(*p*-tolyl)thiazol-2-ylamine and substituted benzyl piperidines linked through the carboxamide group were found to be inactive [IC_{50} value (μM): >10]. Compounds (**80-82**) having 4,5-bis(4-methoxypheny)thiazol-2-ylamine and substituted benzylpiperidines without a carbon spacer showed good inhibitory activities [IC_{50} values (μM): 0.83±0.03, 0.77±0.03, 0.75±0.03 respectively].

It has been reported that BuChE level remains constant or increases in advanced AD while AChE concentration in certain brain regions decreases.[8-10] These reports concluded that balanced inhibition of both AChE and BuChE could be beneficial for the treatment of patients with cognitive deficit observed in AD. Thus, it was decided to check the test compounds for BuChE inhibitory activities also. Majority of the synthesized compounds exhibited good BuChE inhibitory activity. Compounds (**16**, **26**, **28**, **36** and **39**) showed better BuChE inhibitory activity [IC_{50} value (μM): 1.50±0.02, 1.04±0.02, 0.72±0.01, 1.54±0.03 and 1.57±0.04 respectively] than the rest.

The two test compounds (**44**, **48**) which showed the best AChE inhibitory activities [IC_{50} values (μM): 0.30±0.01 and 0.52±0.01 respectively] also showed reasonably good BuChE inhibitory activities [IC_{50} values (μM): 1.84±0.03 and 0.74±0.01 respectively].

To get an insight into the mechanism of AChE enzyme inhibition, the most potent compound (**44**) was subjected to a kinetic study. Lineweaver-Burk reciprocal plots were generated by plotting the reciprocal of the reaction rates versus the reciprocal of substrate concentrations using different concentrations of the test compound (inhibitor, **44**). The plots showed that with increasing concentrations of the inhibitor (**44**) there occurred increase in slope (decreased V_{max}) and the intercept (higher K_m) (**Fig. 3**). This characteristic pattern indicated a mixed type of an inhibition which supported binding of the compound (**44**) to AChE at both the sites (CAS and PAS).[39, 69, 70]

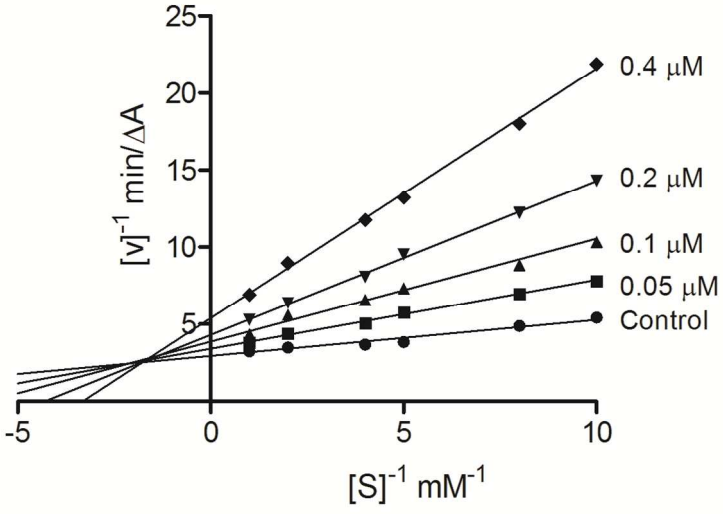


Figure 3. Kinetic study of the mechanism of AChE inhibition by compound **(44)**. Lineweaver-Burk reciprocal plots of the AChE initial velocity at increasing substrate concentrations (0.1-1 mM) in the absence and presence of **44** (0.05-0.4 μM) are shown.

Table 1. AChE and BuChE inhibitory activities (IC_{50} values), inhibition of AChE-induced $A\beta_{1-42}$ aggregation and selectivity ratio of the compounds (16-52 and 73-82)

Compound	AChE	BuChE	Selectivity ratio[b]	$A\beta_{1-42}$ aggregation[c]
	(IC_{50} μM) ± SEM[a]	(IC_{50} μM) ± SEM[a]		% Inhibition
16	>10	1.50±0.02	7.0	nd[d]
17	7.57±0.03	1.88±0.03	4.0	nd[d]
18	>10	1.97±0.02	5.1	nd[d]

19	2.50±0.03	3.51±0.03	0.7	nd[d]
20	>10	1.60±0.03	7.8	nd[d]
22	2.88±0.02	2.77±0.03	1.0	nd[d]
23	1.28±0.02	5.32±0.04	0.2	nd[d]
24	2.32±0.02	>10	0.1	nd[d]
25	>10	2.78±0.03	10.3	nd[d]
26	1.10±0.02	1.04±0.02	1.1	nd[d]
27	2.77±0.02	3.36±0.03	0.8	nd[d]
28	0.65±0.01	0.72±0.01	0.9	18.82±2.23
29	0.64±0.01	3.03±0.04	0.2	12.02±2.35
30	3.70±0.02	>10	0.3	nd[d]
31	1.77±0.02	1.59±0.03	1.1	nd[d]
33	1.38±0.03	2.18±0.03	0.6	nd[d]
34	1.34±0.03	1.85±0.03	0.7	nd[d]
35	0.68±0.01	1.92±0.02	0.4	14.46±2.15
36	0.57±0.01	1.54±0.03	0.4	21.17±1.21
37	2.45±0.01	>10	0.1	nd[d]
38	1.16±0.03	1.73±0.03	0.7	nd[d]
39	0.41±0.02	1.57±0.04	0.3	16.56±1.75
40	2.80±0.01	3.58±0.01	0.8	nd[d]
41	1.23±0.02	1.78±0.03	0.7	nd[d]
42	0.50±0.01	2.51±0.01	0.2	16.17±1.96
43	0.40±0.01	2.20±0.03	0.2	26.35±2.46
44	**0.30±0.01**	**1.84±0.03**	0.2	**27.65±2.91**
45	0.37±0.01	1.95±0.03	0.2	25.79±3.53
46	0.87±0.01	1.43±0.02	0.6	23.26±2.51
47	0.63±0.01	0.82±0.01	0.8	19.39±2.65

48	0.52±0.01	0.74±0.01	0.7	15.00±2.13
49	1.24±0.04	3.30±0.02	0.4	nd[d]
50	0.59±0.01	2.21±0.03	0.3	25.64±2.77
51	0.69±0.01	8.01±0.02	0.1	20.60±2.35
52	**0.36±0.01**	**2.15±0.01**	0.2	**27.20±3.15**
73	1.28±0.03	3.59±0.01	0.4	nd[d]
74	1.09±0.03	6.03±0.04	0.2	nd[d]
75	1.38±0.02	3.30±0.03	0.4	nd[d]
76	3.19±0.01	>10	0.3	nd[d]
77	1.12±0.01	1.57±0.02	0.7	nd[d]
78	1.02±0.03	1.72±0.03	0.6	nd[d]
79	**0.37±0.01**	**1.97±0.02**	0.2	**27.47±2.85**
80	0.83±0.03	2.25±0.03	0.4	19.24±2.38
81	0.77±0.03	2.53±0.03	0.3	11.42±1.43
82	0.75±0.03	1.68±0.03	0.4	18.93±2.85
Tacrine	0.05±0.01	0.01±0.00	4.9	10.63±1.28
Donepezil	0.01±0.00	1.26±0.01	0.01	37.26±2.69

[a]Results are the mean ± SEM of at least three determinations. [b]Selectivity ratio = (IC_{50} of AChE)/(IC_{50} of BuChE). [c]Inhibition of AChE-induced $A\beta_{1-42}$ aggregation produced by the test compounds at 10 μM. [d]Not determined.

Molecular docking studies on AChE and BuChE. All the compounds were docked in the active site of AChE (PDB ID: 4EY7). Donepezil was used as a reference drug for the docking studies showing similar binding interactions as reported in the literature.[71] The most active compound (**44**) in the series exhibited G-scores of -10.80 for AChE (**see supplementary information, Table S1**). The energy of the complex formed by compound (**44**) with AChE was -57.22 kcal/mol indicating that compound (**44**) formed a stable complex with AChE. It was observed that compound (**44**) exhibited a binding mode similar to that of donepezil. The theoretical binding energy and the G-scores were a pointer to a stronger binding affinity of the

test compound (**44**) to AChE than the standard drug donepezil. Phenyl ring of the benzylpiperidine group of compound (**44**) showed strong π-π interactions with the anionic site residue W86 (centroid-centroid = 3.94 Å) similar to the benzylpiperidine tail of donepezil. The piperidine ring of compound (**44**) packed against the hydrophobic portion of Y337, F338 and H447 residues. The diphenyl thiazole scaffold of compound (**44**) was oriented towards the entrance of the PAS gorge interacting favourably with Y72, Y124, W286, S293 and Y341 residues indicating strong binding interactions of the diphenyl thiazole ring system in the PAS. Thus, the aromatic diarylthiazole scaffold of compound (**44**) was disposed in PAS gorge similar to the dimethoxyindanone ring of donepezil. NH group of compound (**44**) formed a hydrogen bond with Y124 residue, a part of PAS, and the fluorine on the phenyl ring in compound (**44**) formed a hydrogen bond with the anionic site residue Y133 (**Fig. 4**). This interaction study demonstrated that compound (**44**) was strongly bound to both the binding sites CAS as well as PAS of AChE, supporting potentially high inhibitory activity of compound (**44**) which was supported by the kinetic study exhibiting binding of the compound (**44**) to the dual sites.

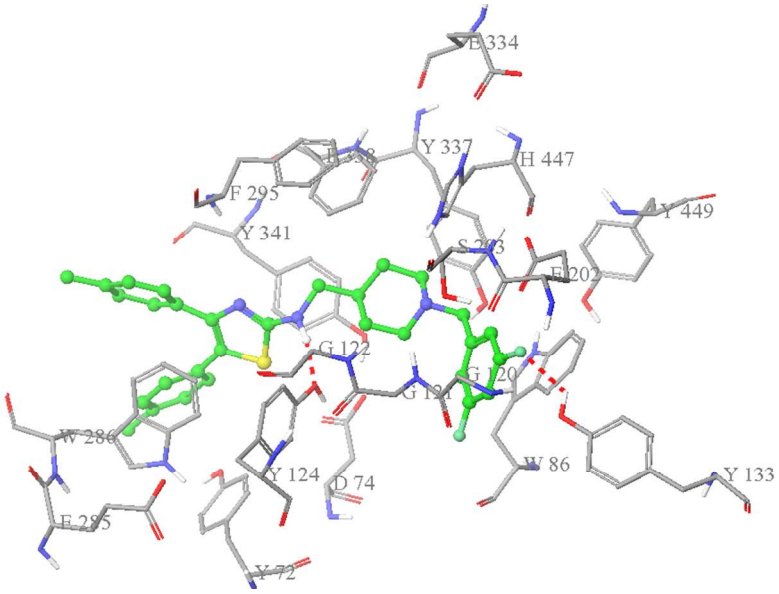


Figure 4. Orientation of compound **(44)** in the active sites of AChE.

Inhibition of AChE-induced $A\beta_{1-42}$ aggregation. There are reports suggesting that localized AChE and Aβ promoted the assembly of Aβ into fibrils, and accelerated the deposition of Aβ peptide in senile plaques. AChE directly binds to Aβ through its PAS to promote the aggregatory effect.[72, 73] Blockade of PAS could attenuate the AChE-induced Aβ peptide aggregation.[74] Those compounds which showed potent AChE inhibitory activity in the *in vitro* ChE inhibition studies were selected for thioflavin T (ThT) assay[75, 76] (**Table 1**) to evaluate their potential to inhibit AChE-induced $A\beta_{1-42}$ aggregation. Results of the study showed that three compounds (**44**, **52** and **79**) among the series exhibited potent inhibition (~27 %) of AChE-induced $A\beta_{1-42}$ aggregation at 10 μM concentration. These compounds (**44**, **52** and **79**) demonstrated potent AChE inhibitory activity in submicromolar range in the earlier experiment also.

***In vitro* blood-brain barrier permeation assay.** Successful crossing of blood−brain barrier (BBB) by a drug is a pre-requisite for treatment of CNS disorders. To explore whether the synthesized hybrid diarylthiazol-benzylpiperidine derivatives have the ability to penetrate into the brain, a parallel artificial membrane permeation assay of the blood-brain barrier (PAMPA-BBB) was performed as described by Di *et al*.[77-79] This model has the advantage of predicting passive diffusion of a molecule through BBB with high accuracy. The *in vitro* permeability (P_e) of the selected hybrid diarylthiazol-benzylpiperidine derivatives (**Table 2**) and nine commercially available drugs (**see the supplementary information, Table S2**) was determined through a lipid extract of porcine brain lipid in PBS/ethanol (70:30). The assay was validated by comparing the experimentally obtained permeability [P_e(exp)] of these nine drugs with the reported values of permeation [P_e(ref)] offering a linear relationship *i.e.* $P_e(\text{exp.}) = 1.171P_e(\text{ref.}) + 1.489$ ($R^2 = 0.983$) (**see the supplementary information, Figure S1**). From this equation and considering the limits established by Di *et al*,[77] for BBB permeation, it was concluded that

compounds with permeability values (P_e) greater than 5.9×10^{-6} cm s^{-1} (**see the supplementary information, Table S3**) were capable of crossing the BBB. All the tested compounds demonstrated permeability values above this limit. Therefore, the experimentally determined permeability values (P_e) of the test compounds were a pointer towards their potential to comfortably cross the BBB by passive diffusion.

Table 2. Permeability (P_e, 10^{-6} cm s^{-1}) results from the PAMPA-BBB assay for some of the test compounds with their predictive penetration in CNS

Compd	P_e (10^{-6} cm s^{-1})[a]	Prediction[b]	Compd	P_e (10^{-6} cm s^{-1})[a]	Prediction[b]
28	15.9±1.2	CNS+	47	12.5±1.3	CNS+
29	8.8±0.4	CNS+	48	13.2±1.5	CNS+
35	12.3±0.8	CNS+	50	12.3±0.8	CNS+
36	12.5±0.8	CNS+	51	8.4±0.8	CNS+
39	8.2±0.4	CNS+	52	12.2±0.6	CNS+
42	10.6±1.1	CNS+	79	8.4±0.8	CNS+
43	12.8±0.8	CNS+	80	9.6±1.1	CNS+
44	**14.5±1.5**	**CNS+**	81	10.3±0.6	CNS+
45	10.1±0.6	CNS+	82	8.6±0.2	CNS+
46	8.8±0.4	CNS+			

[a]Data expressed as mean ± SEM of three independent experiments. [b]CNS+ indicates good passive CNS permeation.

Cell viability and neuroprotective studies. To check the therapeutic suitability of the selected hybrid diarylthiazole-benzylpiperidine derivatives, their effect on cell viability and neuroprotective potential against oxidative stress were evaluated using the human neuroblastoma SH-SY5Y cell line. For the assessment of cytotoxicity of the test compounds, cells were exposed to considerably high concentrations of the test compounds (40 μM and 80 μM) for 24 hr followed by determination of the cell viability using 3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyltetrazolium bromide (MTT) assay. In this experiment, the selected test compounds caused negligible cell death even at such high concentrations (**Table 3**).

In another set of experiments, neuroprotective potential of the selected hybrid diarylthiazole-benzylpiperidine derivatives was assessed. Oxidative stress-like condition in SH-SY5Y cells was induced using H_2O_2 as a toxic insult.[78, 80] In this assay,[78] addition of H_2O_2 (100 μM) to the growth media caused significant cell death as evidenced by reduction in cell viability (52-56 %) compared to the control. To assess the neuroprotective potential of the test compounds against the toxic onslaught of H_2O_2, the cells were treated for 2 hr with the test compounds (5 μM, 10 μM and 20 μM) prior to H_2O_2 insult for 24 hr. At 5 μM concentration, the compounds did not show significant protective effect (data not shown). However, as shown in (**Table 3**), the selected derivatives exhibited significant neuroprotective effect at 10 μM and 20 μM concentrations. Amongst all of the hybrid derivatives, compound (**44**) showed the highest neuroprotection against the H_2O_2 insult. The results revealed that compounds (**28**, **29**, **35**, **36**, **39**, **42-48**, **50-52**, **77** and **80-82**) can protect neuronal cells against oxidative stress-associated cell death.

Table 3. Cell viability, neuroprotection and free radical scavenging activity of some of the test compounds in the human neuroblastoma SH-SY5Y cell line and DPPH assay

Compound	Cell viability (%)[a]		Neuroprotection (%)[b]		RP of DPPH (%)[c]	
	40 μM	80 μM	10 μM	20 μM	10 μM	20 μM
28	93.6±2.5	91.1±2.9	29.2±2.4	50.8±3.4	40.6±3.1	61.7±2.8
29	95.6±2.4	92.8±3.1	31.5±3.7	53.4±2.9	43.5±2.4	64.6±2.8
35	92.4±3.7	93.1±2.1	21.9±3.2	38.7±2.7	43.0±2.9	63.6±2.9
36	94.0±2.7	90.3±3.9	33.3±2.3	39.9±3.0	40.5±3.3	62.6±3.1
39	92.9±2.2	91.6±4.3	32.9±2.6	55.7±2.9	46.0±2.7	63.6±2.4
42	91.4±3.1	90.1±3.1	26.9±2.9	52.7±2.8	49.0±2.8	58.9±2.7
43	93.5±2.8	91.7±3.6	29.3±2.8	48.8±3.3	48.9±2.1	59.4±3.1

44	**95.2±2.6**	**91.3±2.9**	**39.6±2.3**	**59.5±2.4**	**54.6±2.7**	**70.5±2.6**
45	91.8±2.0	89.7±3.2	28.8±1.4	42.5±2.6	51.2±2.7	56.5±3.3
46	95.4±1.9	90.9±2.4	23.6±2.8	49.7±3.1	45.4±2.9	63.4±2.4
47	92.4±3.7	91.7±2.8	29.8±2.2	42.9±3.2	44.8±2.2	60.9±2.2
48	92.7±2.6	88.9±3.6	31.5±2.7	53.7±2.6	46.3±3.2	57.4±2.3
50	91.6±3.3	87.2±3.9	34.2±2.1	41.4±2.2	42.5±3.1	63.4±2.7
51	94.2±3.6	90.1±2.5	33.2±3.1	48.4±2.2	51.8±2.8	66.9±2.6
52	94.2±2.2	87.1±3.6	28.8±2.4	41.5±2.9	48.3±2.5	57.1±2.8
79	94.2±3.2	92.5±1.9	32.0±3.3	53.2±2.7	42.2±2.4	63.2±3.2
80	95.4±2.7	89.5±2.7	29.9±2.1	47.4±2.3	47.2±3.1	63.4±2.3
81	92.1±3.6	89.5±3.2	25.5±2.8	40.1±3.4	49.3±2.2	65.5±2.9
82	94.5±2.6	90.4±2.4	30.4±2.4	42.0±2.2	50.0±2.9	66.2±2.4
Tacrine	89.4±2.3	90.0±3.4	44.7±3.3	57.9±2.1	54.9±2.1	68.8±3.7
Donepezil	96.9±1.4	92.3±2.4	48.2±2.4	62.0±2.5	59.9±2.6	75.5±2.9
Ascorbic acid	nd	nd	nd	nd	56.4±2.7	67.7±2.7

[a]Percentage cell viability of SH-SY5Y cells exposed at relatively high concentrations (40 μM and 80 μM) of test compounds. [b]Percentage neuroprotection of SH-SY5Y cells at relatively lower concentrations (10 μM and 20μM) of the test compounds against H_2O_2 (100 μM) insult. Results are the mean ± SEM of at least three independent experiments. [c]RP of DPPH **(%)** = reduction percentage of DPPH. Results are the mean ± SEM of at least three independent experiments. nd = not determined.

Anti-oxidant activity. Diphenyl-1-picrylhydrazyl (DPPH) is one of the few stable and commercially available organic nitrogen radicals. DPPH assay is commonly used for preliminary screening of compounds capable of scavenging activated oxygen species.[81] The selected hybrid diarylthiazole-benzylpiperidine derivatives were evaluated for their anti-oxidant potential using DPPH assay. All of the test compounds were found to exhibit significant free radical scavenging activity. At a concentration of 10 μM, all of the test compounds showed free radical scavenging activity in the range of 40-55 % with compounds (**44**) showing the highest activity (55 %). Moreover, at 20 μM concentration, the test compounds exhibited free radical scavenging activity ranging from 56-70 % with compound (**44**) showing 70 % scavenging activity which was more than what was shown by the standard drug ascorbic acid.

ROS estimation in primary hippocampal neuronal culture. Hippocampal region of brain is considered as the memory origin centre having ACh as the major neurotransmitter. Therefore, degradation of hippocampal neurons causes impairment of memory and ultimately leads to degeneration of the neurons.[82, 83] Elevated levels of ROS is a sign of heightened oxidative stress in various pathological conditions.[84] $A\beta_{1-42}$ toxicity is known to induce oxidative stress in the pathogenesis of AD.[85] The anti-oxidant potential of the most potent test compound (**44**) was evaluated by estimating intracellular levels of ROS in primary rat hippocampal neuronal cells using 2′,7′-dichlorofluorescine diacetate (DCFH-DA) assay.[31, 86] DCFH-DA crosses cell membrane and gets readily hydrolyzed by intracellular esterases to nonfluorescent DCFH. In the presence of ROS, DCFH is rapidly oxidized to the highly fluorescent 2′,7′-dichlorofluorescine (DCF). As shown in **Figure 5A**, $A\beta_{1-42}$ insult elevated the ROS level significantly as compared to the control cells (p<0.001). However, the test compound (**44**) (10 μM and 20 μM) (**Fig. 5A**, p<0.001) like the standard drug donepezil (**Fig. 5A**, p<0.001) dose dependently attenuated the ROS generation caused by $A\beta_{1-42}$ toxicity. These results revealed excellent ROS scavenging ability of compound (**44**).

Flow cytometry. Apoptosis is a key cellular event which plays an important role in the etiology of various neurodegenerative diseases.[84, 87] It has been reported that $A\beta_{1-42}$, a pathological hallmark of AD, causes apoptosis leading to neurodegeneration ultimately.[88-90] Apoptosis was assessed using flow cytometry by staining the primary rat hippocampal neurons with annexin V-FITC and propidium iodide (PI). Annexin V enters into the cells in both, the early and the late stages of apoptosis while the PI stains the cells only in the late stage of apoptosis or the necrosis stage. $A\beta_{1-42}$ (10 μM) toxicity increased the percentage of early apoptotic cells significantly (**Fig. 5B**, 38.80 %) as compared to the control cells (**Fig. 5B**, 1.78 %). Compound (**44**) (20 μM) (**Fig.**

5B, 13.40 %) significantly abrogated the percentage of early apoptotic cells induced by $A\beta_{1-42}$ insult in a manner similar to donepezil (**Fig. 5B**, 7.84 %). The results demonstrated anti-apoptotic potential of compound (**44**) against $A\beta_{1-42}$-induced toxicity which further supported its anti-Alzheimer's potential.

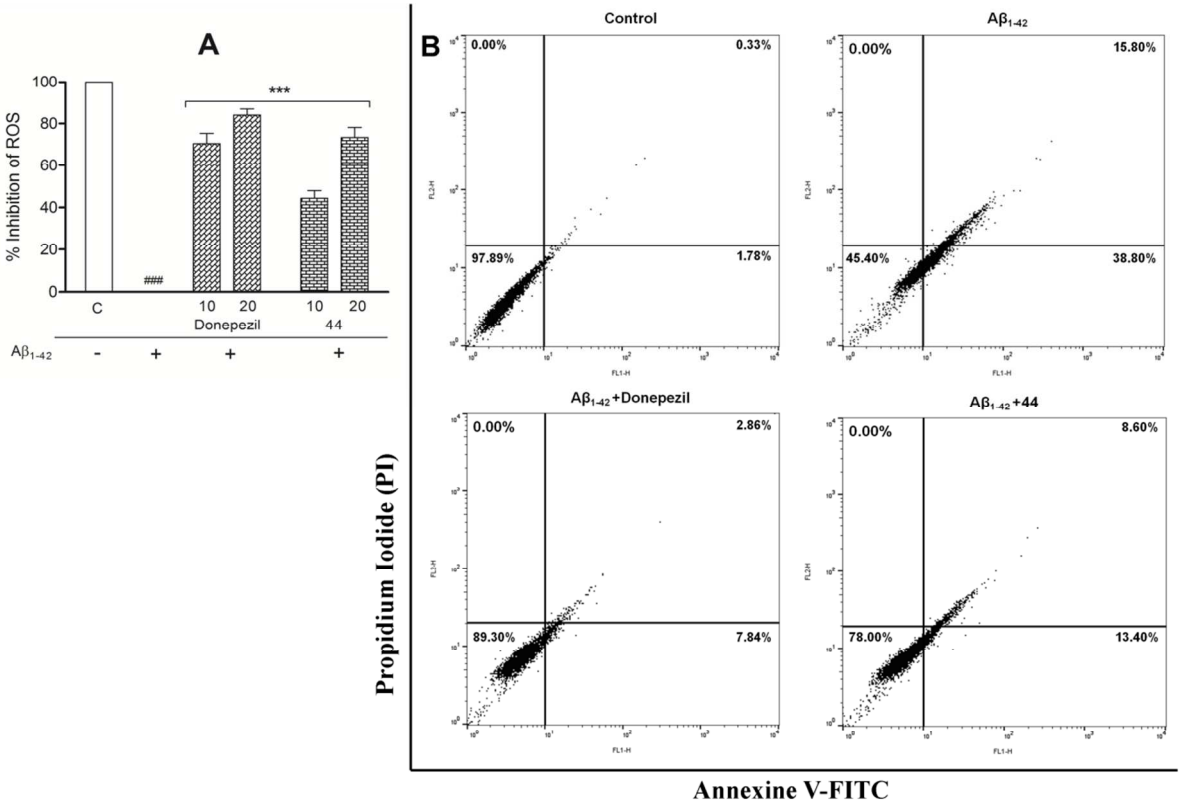


Figure 5. *In vitro* ROS scavenging and anti-apoptotic potential of compound **(44)** against $A\beta_{1-42}$ (10 μM) insult in primary rat hippocampal neuronal culture. (A) Percentage inhibition of ROS generation as assessed by DCFH-DA assay. (B) Flow cytometric assessment of apoptosis using Annexin V-FITC and PI staining. Cells in the lower left quadrant are viable (Annexin V-FITC-/PI-). Cells in the lower right quadrant are early apoptotic (Annexin V-FITC+/PI-) and those in the upper right quadrant are late apoptotic or necrotic (Annexin V-FITC+/PI+). Data are expressed as mean ± SEM. $^{###}p<0.001$ *vs.* control cells. *** $p<0.00$ *vs.* $A\beta_{1-42}$-treated control cells. C=control cells.

Behavioural studies. To determine the *in vivo* anti-ChE activity of the most potent compound (**44**) in the series, scopolamine model was adopted.[91-93] Scopolamine-induced amnesia in rodents is an accepted standard model in behavioural pharmacology for evaluation of ChE inhibiting potential of drug candidates to be developed for the anti-Alzheimer's therapy. Scopolamine blocks the muscarinic cholinergic receptors distinctly, that lead to impairment of the cognitive

functions. To evaluate the anti-amnesic effect of compound (**44**), Morris Water Maze (MWM) test was utilized. During the last five days of the treatment period, escape latency time (ELT) and number of platform area crossings were recorded for the animals of different experimental groups. The escape latencies were significantly prolonged (**Fig. 6A**, $p<0.001$) while the number of platform area crossings (**Fig. 6B**, $p<0.001$) were significantly reduced by the scopolamine treatment (1.4 mg kg^{-1}, i.p.). Donepezil (at 5 mg kg^{-1}, p.o.) significantly reduced ELT (**Fig. 6A**, $p<0.001$) and increased platform area crossings (**Fig. 6B**, $p<0.01$) as compared to the scopolamine-treated control group. At an equivalent dose corresponding to donepezil, compound (**44**) significantly shortened ELT (**Fig. 6A**, $p<0.001$) and increased the number of platform area crossings (**Fig. 6B**, $p<0.05$) as compared to the scopolamine-treated control group. The results revealed anti-amnesic effect of compound (**44**) against the scopolamine-induced AD-like phenotype.

In further experimentation, the effect of compound (**44**) on the AChE and BuChE levels of brain was estimated in mice using Ellman's method.[68, 92] As described in our previous report,[64] scopolamine treatment significantly elevated AChE (**Fig. 6C**, $p<0.001$) and BuChE (**Fig. 6D**, $p<0.001$) levels compared to the vehicle-treated control group. However, the elevated levels of AChE (**Fig. 6C**, $p<0.001$) and BuChE (**Fig. 6D**, $p<0.001$) were significantly attenuated by compound (**44**) at a dose equivalent to that of donepezil.

In order to further elucidate the mechanism of anti-amnesic effect of compound (**44**), brain levels of malondialdehyde (MDA - a byproduct of lipid peroxidation) and catalase (CAT - which catalyzes H_2O_2 decomposition) were determined following completion of the MWM test. Scopolamine treatment significantly elevated the MDA level (**Fig. 6E**, $p<0.001$) while reduced the CAT level in the brains of the treated animals (**Fig. 6F**, $p<0.001$) compared to the vehicle-

treated control group animals. However, treatment of the amnesic mice with compound (**44**) significantly attenuated the MDA level (**Fig. 6E**, $p<0.001$) while elevated the CAT level (**Fig. 6F**, $p<0.01$). The results therefore revealed the *in vivo* ChE inhibitory and anti-oxidant activities of compound (**44**) similar to donepezil, which further substantiated anti-Alzheimer's potential of this most promising test compound (**44**).

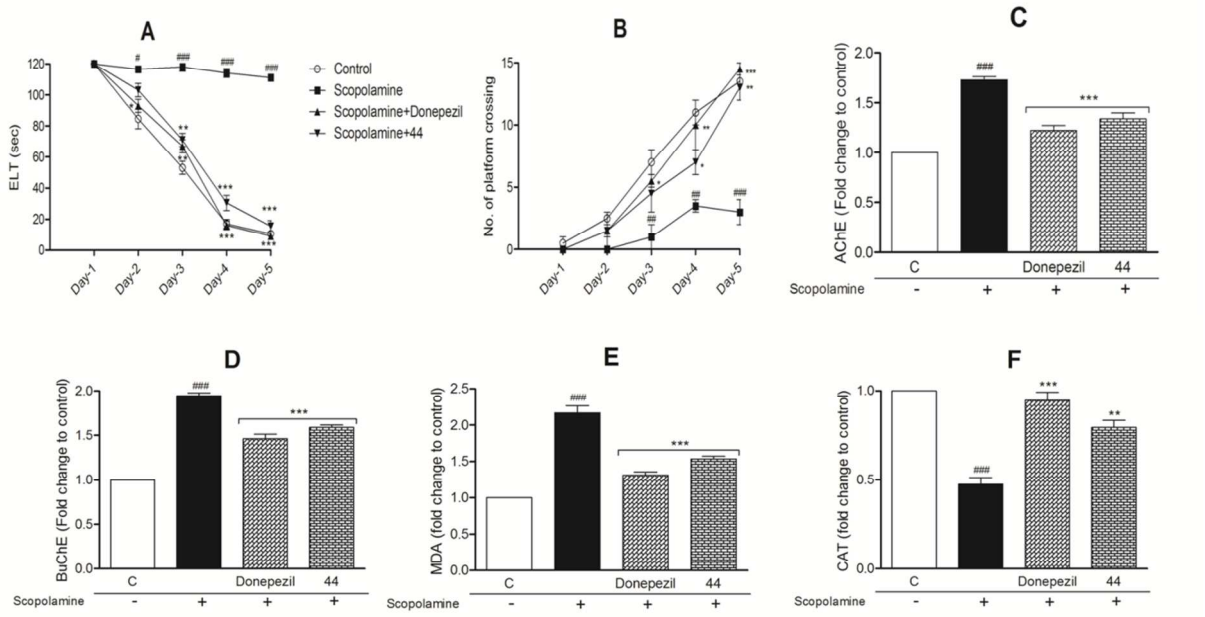


Figure 6. Compound (**44**) improved spatial learning and memory in scopolamine-treated mice through anti-ChE and anti-oxidant activities. In MWM test, scopolamine (1.4 mg kg^{-1}, i.p.) increased (A) ELT while (B) reduced number of platform area crossings. This pattern was significantly reversed by **44** similar to donepezil at 5 mg kg^{-1}. (C) AChE and (D) BuChE levels elevated by scopolamine treatment were significantly attenuated by **44**. Oxidative stress parameters are represented as (E) MDA and (F) CAT levels. Altered MDA and CAT levels after scopolamine treatment were significantly reversed by treatment with compound (**44**). Data are expressed as mean ± SEM (n=6). $^{###}p<0.001$, $^{##}p<0.01$, $^{#}p<0.05$ *vs.* vehicle-treated control group.*** $p<0.001$, ** $p<0.01$, * $p<0.05$ *vs.* scopolamine-treated control group. C=vehicle-treated control group.

Y maze test. To develop AD-like phenotype, animals were subjected to intracerebroventricular (ICV) injection of $A\beta_{1-42}$ in the hippocampal region of brain. This is a promising model to evaluate the neuroprotective potential of any drug candidate in AD-like condition.[64, 94] Impairment of the working memory in the animals was assessed using Y maze test. "Spontaneous alteration" is indicative of the working memory. $A\beta_{1-42}$-treated control animals exhibited significantly reduced percentage of "spontaneous alterations" throughout the experimental session as compared to the vehicle-treated control animals (**Fig. 7A**, $p<0.001$).

Animals treated with compound (**44**) displayed increased percentage of "spontaneous alterations" (**Fig. 7A**, $p<0.01$). However, the mean number of arm entries remained unchanged across all the experimental groups which indicated that the general locomotor activity was not hampered by $A\beta_{1-42}$ toxicity (**Fig. 7B**). Thus, $A\beta_{1-42}$-worsened hippocampal-dependant working memory was improved by the treatment with compound (**44**).

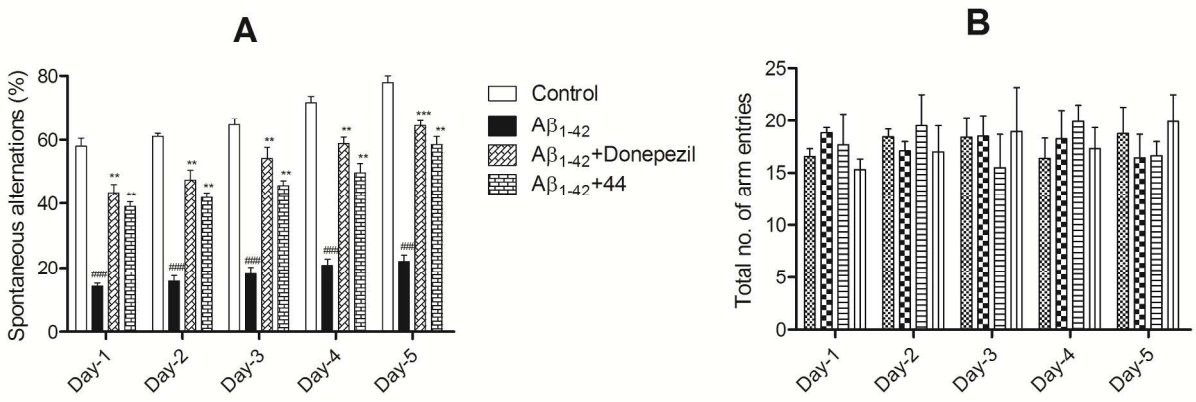


Figure 7. Compound **(44)** improved immediate working memory in rats which received ICV injection of $A\beta_{1-42}$ in Y maze test. (A) Percentage of spontaneous alterations and (B) total number of arm entries, as recorded by a blind observer. Data are expressed as mean±SEM (n=6). $^{###}p<0.001$, $^{##}p<0.01$, $^{#}p<0.05$ *vs.* vehicle-treated control group.*** $p<0.001$, ** $p<0.01$, * $p<0.05$ *vs.* scopolamine-treated control group. C=vehicle-treated control group.

Western blot analysis. The *in vivo* neuroprotective potential of compound (**44**) was assessed by determining the levels of pathological hallmarks of AD in the hippocampal region of rat brain which was subjected to ICV injection of $A\beta_{1-42}$. Elevated levels of $A\beta_{1-42}$ and *p*-tau are the key indicators of AD progression.[95] At the end of the ICV experimental model, animals from different groups were scarified to estimate $A\beta_{1-42}$ and *p*-Tau levels using Western blot analysis (**Fig. 8A**). A significant rise in the levels of $A\beta_{1-42}$ and *p*-Tau was observed in the $A\beta_{1-42}$-treated control group as compared to the vehicle-treated control group. Supporting the results of the *in vitro* $A\beta_{1-42}$ aggregation inhibition (ThT) assay, treatment of the animals with compound (**44**) significantly attenuated the levels of $A\beta_{1-42}$ (**Fig. 8B**, $p<0.01$) and *p*-Tau (**Fig. 8C**, $p<0.05$) similar to donepezil. The results confirmed the anti-$A\beta_{1-42}$ aggregatory and anti-Alzheimer's

potential of compound (**44**). Additionally, to assess the *in vivo* anti-apoptotic potential of compound (**44**), cleaved-caspase-3 and cleaved-PARP levels were also determined in the hippocampal region of the rat brain which received $A\beta_{1-42}$ insult. It has been well documented that cleavage/activation of caspase-3 is a key event in cell apoptosis.[87] It has been demonstrated that caspase-3 is either partially or fully responsible for the proteolytic cleavage of poly(ADP-ribose) polymerase-1 (PARP), a nuclear-DNA binding protein[96, 97] involved in numerous cellular events including DNA repair.[98] PARP overactivation is associated with various neurodegenerative conditions including AD.[99] It has been reported earlier that Aβ peptide caused induction of PARP activity in the hippocampal region of adult rat brain.[100, 101] Cleavage of PARP is followed by the caspase-3 activation which subsequently leads to apoptosis and ultimately neuronal cell death. Thus, cleaved-caspase-3 and cleaved-PARP are the two significant key markers of apoptosis.[102] In this study, the levels of cleaved-caspase-3 (**Fig. 8D**, $p<0.001$) and cleaved-PARP (**Fig. 8E**, $p<0.001$) were found to be significantly elevated in the hippocampal region of the brains of $A\beta_{1-42}$-treated control animals as compared to the vehicle-treated control group. Supporting the *in vitro* results, compound (**44**) demonstrated significant anti-apoptotic potential by lowering the levels of cleaved-caspase-3 (**Fig. 8D**, $p<0.001$) and cleaved-PARP (**Fig. 8E**, $p<0.05$) in the treated group similar to donepezil. In conclusion, compound (**44**) has exhibited multi-target-directed actions highlighting its potential as a possible therapeutic alternative for the treatment of AD.

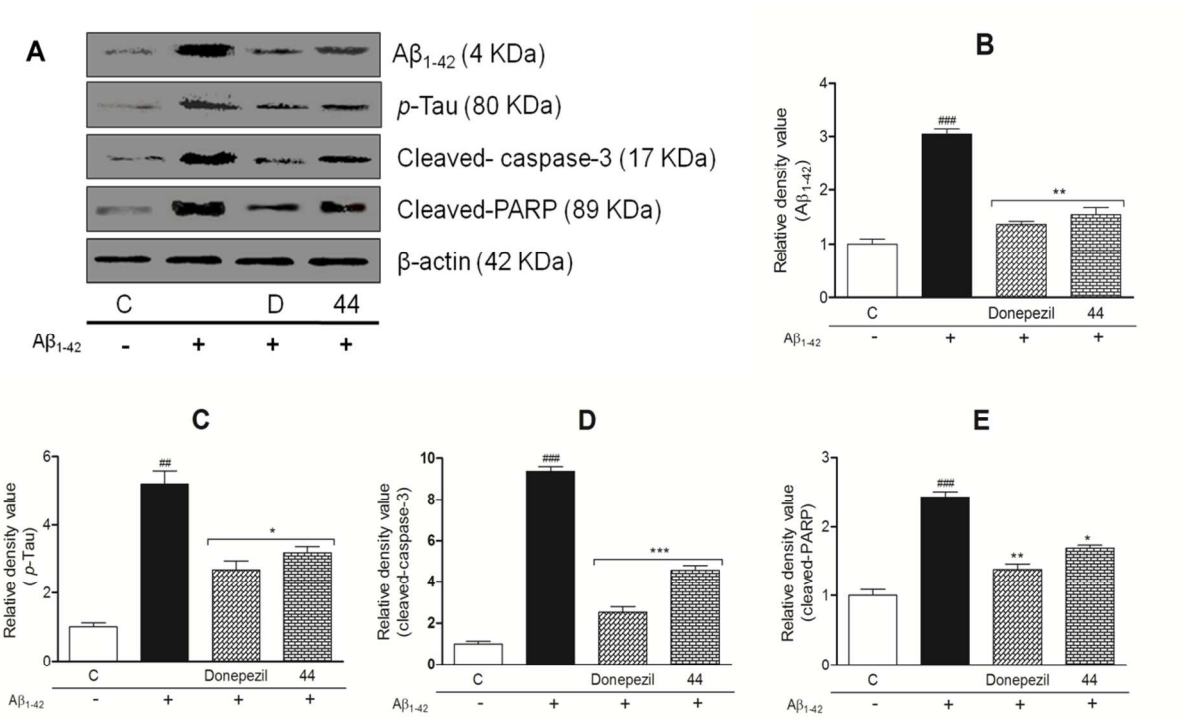


Figure 8. *In vivo* neuroprotective and anti-apoptotic potential of compound **(44)**. (A) The expression of $A\beta_{1-42}$, *p*-Tau, cleaved-caspase-3 and cleaved-PARP was assessed by Western blot analysis in the hippocampal region of rat brain which was given ICV injection of $A\beta_{1-42}$. Densitometric analysis revealed attenuation of (B) $A\beta_{1-42}$, (C) p-Tau, (D) cleaved-caspase-3 and (E) cleaved-PARP levels by compound **(44)** which were elevated by $A\beta_{1-42}$ toxicity. Data are expressed as mean±SEM (n=6). $^{###}p<0.001$, $^{##}p<0.01$, $^{#}p<0.05$ *vs.* vehicle-treated control group.*** $p<0.001$, ** $p<0.01$, * $p<0.05$ *vs.* $A\beta_{1-42}$-treated control group. C=vehicle-treated control group. D=donepezil-treated group.

Acute toxicity study. For the development of a compound as a new drug, determination of its acute toxicity profile is considered to be an important criterion. Acute toxicity of compound (**44**), the most promising candidate of the current study was determined in male Swiss Albino mice at doses of 0, 677, 1333 and 2000 mg kg^{-1} (n = 5 per group) by oral administration. The animals were regularly observed for 14 days after treatment. After the observation period of 14 days, all the animals remained alive and appeared healthy in terms of fur sleekness, water and food consumption and body weight. On the 15^{th} day, all the animals were sacrificed for macroscopic examination of the heart, liver and kidneys for any damage. No damage was observed to these organs. The results of the study showed that mice treated with compound (**44**) did not produce

any acute toxicity or mortality immediately or during the post-treatment period. Therefore, compound (**44**) can be considered to be non-toxic and well tolerated at doses up to 2000 mg kg^{-1}.

Pharmacokinetic analysis. Preliminary pharmacokinetic analysis of compound (**44**) was performed in male Wistar rats. Compound (**44**) was administered orally to the animals at a dose of 5 mg kg^{-1} followed by blood sampling at the indicated time points (see experimental section). Pharmacokinetic parameters summarized in **Table 4** were calculated from the concentration-time profile using noncompartmental extravascular analysis. The mean concentration-time course of compound (**44**) after single oral administration is shown in **Figure 9**. An asymmetric curve of the compound (**44**) deviating from the Guaussian distribution curve suggested a prolonged elimination phase as compared to the absorption phase. Peak plasma concentration (C_{max}) of the compound (**44**) was achieved approximately at 3.66±0.57 hr after single dose oral administration. The terminal elimination half-life ($t_{1/2}$) was observed to be 19.42±4.69 hr. Overall, the results suggested that the compound (**44**) exhibited good oral absorption and was eliminated at a rate which is moderate as compared to the absorption phase. However, *in vivo* human clinical trials are required to be performed to prove the actual utility of the test compound (**44**).

Table 4. Pharmacokinetic parameters of compound (44) after administration of single oral dose (5 mg kg^{-1}).

Pharmacokinetic parameters	Compound (44)[a]
C_{max} (ng/ml)	21.20±1.99
t_{max} (hr)	3.66±0.57
$AUC_{(0-8)}$ (ng.hr/ml)	587.16±62.09
$t_{1/2}$ (hr)	19.42±4.69

[a]Date are expressed as mean±SD (n=4).

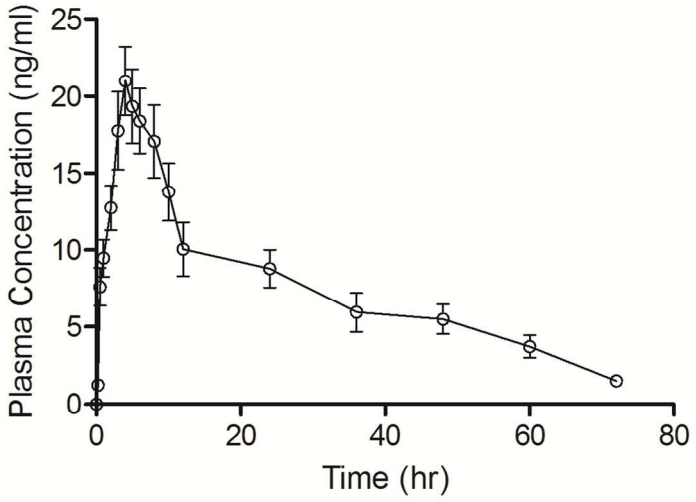


Figure 9. Mean plasma concentration vs. time curve of single oral dose (5 mg kg^{-1}) of compound **(44)** in rats. Error bars represents the standard deviation of the mean (n=4).

CONCLUSION

A novel series of hybrid molecules have been synthesized by combining the benzylpiperidine fragment of donepezil and vicinal diarylthiazole as a privileged scaffold to obtain chemical entities exhibiting anti-Alzheimer's potentials. The synthesized hybrids were evaluated for their *in vitro* AChE and BuChE inhibitory activities. Some of the test compounds (**28**, **29**, **35**, **36**, **39**, **42-48**, **50-52**, **79**, **81** and **82**) showed moderate to high AChE inhibitory activity. These derivatives also exhibited good binding affinity for the active sites of AChE in the molecular modeling studies. Among the series, compound (**44**) exhibited the most promising inhibitory potential for AChE (IC_{50} = 0.30 ± 0.01 μM) and BuChE (IC_{50} = 1.84 ± 0.03 μM). Enzyme kinetics proved that compound (**44**) caused a mixed type of AChE inhibition by binding to both the active sites (PAS and CAS) of AChE. Those derivatives which showed good AChE inhibitory activities also exhibited reasonably good inhibition of AChE-induced $A\beta_{1-42}$ aggregation. Among these, three compounds (**44**, **52** and **79**) demonstrated the highest level of anti-amyloid activity (~ 27 % inhibition) at a concentration of 10 μM. Although these molecules

(**44**, **52** and **79**) showed submicromolar AChE inhibitory potencies and their concurrent $A\beta_{1-42}$ aggregation inhibitory activities were in the single-digit micromolar range, they exhibited noticeable neuroprotective activities *in vitro* and *in vivo*. In particular, compound (**44**) showed ROS scavenging and anti-apoptotic properties against $A\beta_{1-42}$ insult in the primary rat hippocampal neuron cultures. In the $A\beta_{1-42}$-induced Alzheimer's rat model, compound (**44**) improved the spatial learning and memory. Compound (**44**) significantly reversed the $A\beta_{1-42}$-induced enhancement of ChEs levels and favourably altered the oxidative stress parameters (MDA and CAT) in the hippocampal region of rat brain similar to the standard drug donepezil. Results of the *in vivo* studies revealed the anti-ChE and anti-oxidant potential of compound (**44**) which further substantiated the results of the *in vitro* experiments. Compound (**44**) also exhibited significant neuroprotection in rats subsequent to intra-hippocampal injection of $A\beta_{1-42}$ by attenuating the abnormal levels of $A\beta_{1-42}$, *p*-Tau, cleaved-caspase-3 and cleaved-PARP proteins as assessed by Western blot analysis. Over and above, compound (**44**) was found to be well tolerated and nontoxic up to 2000 mg/kg, p.o. dose. Pharmacokinetic analysis revealed that compound (**44**) exhibited good oral absorption, and was eliminated at a relatively moderate rate compared to the absorption phase. Thus, compound (**44**) has shown its real potential as a multi-target-directed ligand by interacting with different pathophysiological targets of AD demonstrating significant multiple effects like anti-ChE, anti-Aβ aggregatory, neuroprotective, ROS scavenging, anti-oxidant and anti-apoptotic activities in different *in vitro* and *in vivo* experiments. All put together, the beneficial effects of the novel diarylthiazole-benzylpiperidine hybrid molecules qualified them as potential lead candidates to be developed as new drugs for the treatment of AD, and the most promising multi-target-directed ligand (**44**) amongst them could be considered as a potential drug molecule for further development.

EXPERIMENTAL PROCEDURE

Chemistry. All the reagents and solvents required for synthesis of the compounds were purified by general laboratory techniques[103] before use. Melting points were determined using silicon oil bath type (Veego) and heating block type (Lab India) melting point apparatus and are uncorrected. Completion of the reactions were monitored by thin layer chromatography (TLC) using silica gel pre-coated plates ($60F_{254}$, Merck, 0.25 mm thickness) visualizing in ultraviolet light (254 nm) or iodine vapours. Yields reported here are un-optimized. The IR spectra (wave numbers in cm^{-1}) were recorded on a BRUKER ALPHA-T (Germany) FT-IR spectrophotometer using potassium bromide discs. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on Bruker Advance-II 400 MHz spectrometer in $CDCl_3$ or DMSO-d_6 solvents; chemical shift has been expressed as δ ppm and coupling constant (*J*) in Hz. Mass spectra were recorded using Thermo Fisher mass spectrometer with EI ion source or Advion mass spectrometer with ESI ion source and also using AB Sciex 3200 Q Trap mass analyzer for the compounds. Elemental analyses were performed on a Thermo Fisher FLASH 2000 organic elemental analyzer. Chromatographic separations were performed on columns using silica gel (100–200) or neutral alumina, activity grade I. All reagents used were of analytical reagent grade obtained from S. d. fine chemicals, Spectrochem, Qualigens or Sigma-Aldrich or Avera chemicals.

For determining purity of the compounds, HPLC analysis was performed with an Ekspert ultraLC -100XL (As a part of Sciex) quaternary pump which delivered the gradient mobile phase at a flow rate of 0.8 ml/min, the mobile phase composition was phase A - water with 0.1 % formic acid and B- methanol:acetonitrile (50:50) in different ratios. The gradient program followed was, 0 min to 3 min (B 10 %), 12 min (B 80 %), 16 min (B 10 %) with an Ekspert auto-sampler, degasser and column compartment. The auto-sampler was equipped with a 108 well

plate and was used to inject 20 µl samples onto the HPLC column. The Ekspert autosasmpler cooling device was set at 15°C. The column used was a Phenomenex® Luna C18 (100 × 2.0 mm id, 5 µm) analytical column fitted with a Phenomenex® Security Guard™ System containing a C18 (4 × 3 mm) pre-column. The column was kept at 40°C with an Ekspert 100 column oven compartment. Purity of the compounds was found to be higher than 98 %.

Mass analysis was performed on an AB SCIEX API 3200 triple quadrupole mass spectrometer (AB SCIEX,) equipped with an electrospray ionization (ESI^+) source operated at 550°C and set in the positive ion mode for ion production. Multiple Reaction Monitoring (MRM) method, a gold standard method for quantitation and gives better selectivity and sensitivity was used. Transition of the protonated precursor ions (Q1MS) and their fragment ions (Q2MS) were monitored at unit resolution in the MRM mode with a dwell time in ms as per the number of transitions. The curtain, nebulizer, turbo, and collision gases were set at 25, 50, 50 and 5 psi, respectively, while the ion spray voltage and the source temperature were set at 5500 V and 550°C, respectively. The declustering potential, collision energy, entrance potential, and collision cell exit potential were optimized. The instrument was interfaced to a workstation running Analyst™ software version 1.6.2.

General procedure A. Synthesis of *t*.butyl 4-[4,5-bis(substituted phenyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylate (10-12). 1-(*t*.Butoxycarbonyl)piperidine-4-carboxylic acid[67] (**9**) (0.85 gm, 3.73 mmol) and BOP reagent (2.4 gm, 3.73 mmol) were dissolved in dry acetonitrile (20 ml) maintaining a temperature of 0 ^oC. To this stirred reaction mixture, DIPEA (1.5 ml, 5.59 mmol) was added slowly followed by addition of 4,5-bis(substituted phenyl)thiazol-2-ylamine[65, 66] (**6-8**) and the stirring was continued overnight. TLC analysis (EtOAc:*n*-hexane 2:8) was used to confirm completion of the reaction. Acetonitrile was

removed, cold water added and the reaction mixture basified with sodium hydroxide followed by extraction with DCM to get the pure compounds.

***t.*Butyl 4-[4,5-bis(*p*-tolyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylate (10).** Synthesized as per the general procedure (A) using 4,5-bis(*p*-tolyl)thiazol-2-ylamine (**6**) (0.5 gm) to get compound (**10**) as a white solid (0.38 gm, 79 %); Mp: 184-186 ^{o}C; IR (KBr, cm^{-1}): 3435, 3134, 1688, 1536, 1161, 825; MS (m/z): 491.07 $(M)^{+}$.

***t.*Butyl 4-[4,5-bis(4-chlorophenyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylate (11).** Synthesized as per the general procedure (A) using 4,5-bis(4-chlorophenyl)lthiazol-2-ylamine (**7**) (0.5 gm) to get compound (**11**) as a white solid (0.43 gm, 85 %); Mp: 188-190 ^{o}C; IR (KBr, cm^{-1}): 3438, 3141, 1686, 1565, 979; MS (m/z): 532.00 $(M)^{+}$.

***t.*Butyl 4-[4,5-bis(4-methoxyphenyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylate (12).** Synthesized as per the general procedure (A) using 4,5-bis(4-methoxyphenyl)thiazol-2-ylamine (**8**) (0.5 gm) to get compound (**12**) as a white solid (0.35 gm, 83 %); Mp: 138-140 ^{o}C; IR (KBr, cm^{-1}): 3435, 1690, 1543, 1248; MS (m/z): 523.14 $(M)^{+}$.

General procedure B. Synthesis of *N*-[4,5-bis(substituted phenyl)thiazol-2-yl] piperidine-4-carboxamide (13-15). To an RBF containing *t.*butyl 4-[4,5-bis(substituted phenyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylate (**10-12**), a mixture of trifluoroacetic acid: DCM (70:30) (3 ml) was added and allowed to stir for 2 hrs. DCM was distilled off and diethyl ether was added to the reaction mixture in cold conditions slowly with continuous stirring to get a white solid compound (**13-15**).

***N*-[4,5-Bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (13).** Synthesized as per the general procedure (B) using *t.*butyl 4-[4,5-bis(*p*-tolyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylate

(**10**) (0.5 gm) to get compound (**13**) as a white solid (0.35 gm, 88 %); Mp: 186-188 ^{o}C ; IR (KBr, cm^{-1}): 3193, 3027, 1674, 1547, 1132; MS (m/z): 391.33 $(M)^{+}$.

***N*-[4,5-Bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (14).** Synthesized as per the general procedure (B) using *t*.butyl 4-[4,5-bis(4-chlorophenyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylate (**11**) (0.5 gm) to get compound (**14**) as a white solid (0.38 gm, 90 %); Mp: 240-242 ^{o}C; IR (KBr, cm^{-1}): 3162, 2856, 1677, 1552, 832; MS (m/z): 431.70 $(M)^{+}$.

***N*-[4,5-Bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (15).** Synthesized as per the general procedure (B) using *t*.butyl 4-[4,5-bis(4-methoxyphenyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylate (**12**) (0.5 gm) to get compound (**15**) as a white solid (0.40 gm, 95 %); Mp: 168-170 ^{o}C; IR (KBr, cm^{-1}): 3425, 3176, 1675, 1549, 833; MS (m/z): 423.17 $(M)^{+}$.

General procedure C. Synthesis of 1-(substituted benzyl)-*N*-[4,5-bis(4-substituted phenyl) thiazolyl]piperidine-4-caroxamide (16-41). In a round-bottomed flask, *N*-[4,5-bis(4-substituted phenyl)thiazol-2-yl]piperidine-4-carboxamide (0.5 gm) and anhydrous K_2CO_3 (0.36 gm, 3.15 mmol) were dissolved in Dry DMF. Suitable quantity of the substituted benzyl bromide derivative was added to the contents of the flask. The mixture was stirred at 60 ^{o}C for 1 hr and then poured into water. The precipitated off-white solid was filtered, washed with cold water and dried. Further purification was carried by column chromatography with the help of petroleum ether and ethyl acetate as eluents.

1-(2-Methylbenzyl)-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (16). Synthesized as per the general procedure (C) using 2-methylbenzyl bromide (0.15 ml) and *N*-[4,5-bis(*p*-

tolyl)thiazol-2-yl]piperidine-4-carboxamide (**13**) (0.5 gm) to get compound (**16**) as a white solid (0.42 gm, 80 %); Mp: 156-158 °C; IR (KBr, cm^{-1}): 3152, 3024, 2858, 1683, 1541, 1445, 1266, 816; ^{1}H-NMR: 11.05 (s, 1H), 7.40-7.38 (d, 2H, *J* = 8.1 Hz), 7.26-7.24 (d, 2H, *J* = 8.1 Hz), 7.18-7.07 (m, 8H), 3.33 (s, 2H), 2.72-2.70 (d, 2H, *J* = 9.3 Hz), 2.36 (s, 3H), 2.33 (s, 6H), 1.68-1.65 (m, 5H), 1.49-1.46 (d, 2H, *J* = 9.3 Hz); ^{13}C-NMR: 173.37, 156.54, 143.60, 137.69, 137.66, 137.54, 131.95, 130.26, 129.72, 129.49, 129.35, 129.20, 129.13, 128.80, 127.01, 126.60, 125.45, 60.94, 52.62, 29.72, 28.51, 21.26, 19.23; LC-MS/MS: t_R 5.95 min, 496.29 (M+H); MS (m/z): 495.85 $(M)^+$; Anal. Calcd for $C_{31}H_{33}N_3OS$: C, 75.12; H, 6.71; N, 8.48. Found: C, 74.95; H, 6.87; N, 8.29 %.

1-(2-Trifluoromethylbenzyl)-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (17). Synthesized as per the general procedure (C) using 2-trifluoromethylbenzyl bromide (0.35 gm) and *N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (**13**) (0.5 gm) to get compound (**17**) as a white solid (0.34 gm, 79 %); Mp: 194-196 ^{o}C; IR (KBr, cm^{-1}): 3159, 3029, 2858, 1685, 1541, 1266, 1312, 1118, 769; ^{1}H-NMR: 11.74 (s, 1H), 7.74-7.72 (d, 1H, *J* = 8.0 Hz), 7.59-7.57 (d, 1H, *J* = 8.0 Hz), 7.49-7.42 (m, 3H), 7.30-7.25 (m, 3H), 7.15-7.13 (d, 2H, *J* = 8.0 Hz), 7.11-7.09 (d, 2H, *J* = 8.0 Hz), 3.49 (s, 2H), 2.64-2.62 (d, 2H, *J* = 8.6 Hz), 2.37 (s, 3H), 2.28 (s, 3H), 1.67-1.58 (m, 4H), 1.54-1.51 (m, 1H), 1.38-1.36 (d, 2H, *J* = 8.6 Hz); ^{13}C-NMR: 173.84, 157.52, 143.44, 138.09, 137.85, 137.77, 137.77, 131.86, 131.77, 130.13, 129.54, 129.04, 128.93, 128.22, 126.61, 126.54, 125.55, 125.50, 58.13, 52.55, 41.94, 28.45, 21.22; LC-MS/MS: t_R 7.01 min, 550.23 (M+H); MS (m/z): 549.80 $(M)^+$; Anal. Calcd for $C_{31}H_{30}F_3N_3OS$: C, 67.74; H, 5.50; N, 7.64. Found: C, 67.46; H, 5.67; N, 7.83 %.

1-(3-Fluorobenzyl)-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (18). Synthesized as per the general procedure (C) using 3-fluorobenzyl bromide (0.14 ml) and *N*-[4,5-bis(*p*-

tolyl)thiazol-2-yl]piperidine-4-carboxamide (**13**) (0.5 gm) to get compound (**18**) as a white solid (0.37 gm, 75 %); Mp: 165-167 ^{o}C; IR (KBr, cm^{-1}): 3147, 2926, 2816, 1680 , 1542, 1445, 1263, 814; ^{1}H-NMR: 11.68 (s, 1H), 7.39-7.37 (d, 2H, *J* = 8.0 Hz), 7.26-7.24 (d, 2H, *J* = 8.1 Hz), 7.21-7.20 (m, 1H), 7.15-7.13 (d, 2H, *J* = 8.0 Hz), 7.07-7.05 (d, 2H, *J* = 8.1 Hz), 6.98-6.95 (m, 2H), 6.88-6.86 (m, 1H), 3.35 (s, 2H), 2.66-2.64 (d, 2H, *J* = 11.5 Hz), 2.37 (s, 3H), 2.34 (s, 3H), 1.65-1.49 (m, 5H), 1.39-1.36 (d, 2H, *J* = 11.5 Hz); ^{13}C-NMR: 173.72, 164.12, 161.67, 157.50, 143.37, 141.02, 140.95, 137.86, 131.86, 129.60, 129.33, 129.28, 129.05, 128.90, 126.50, 124.43, 115.76, 113.74, 62.43, 52.22, 41.90, 28.27, 21.25; LC-MS/MS: t_R 5.93 min, 500.25 (M+H); MS (m/z): 499.47 $(M)^+$; Anal. Calcd for $C_{30}H_{30}FN_3OS$: C, 72.12; H, 6.05; N, 8.41. Found: C, 72.35; H, 6.18; N, 8.27 %.

1-(3,5-Difluorobenzyl)-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (19). Synthesized as per the general procedure (C) using 3,5-difluorobenzyl bromide (0.16 ml) and *N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (**13**) (0.5 gm) to get compound (**19**) as a white solid (0.29 gm, 78 %); Mp: 153-155 ^{o}C; IR (KBr, cm^{-1}): 3155, 2924, 2806, 1686, 1547, 1452, 1268, 1117, 810; ^{1}H-NMR: 11.48 (s, 1H), 7.40-7.38 (d, 2H, *J* = 8.1 Hz), 7.26-7.24 (d, 2H, *J* = 8.0 Hz), 7.17-7.13 (d, 2H, *J* = 8.1 Hz), 7.09-7.07 (d, 2H, *J* = 8.0 Hz), 6.81-6.77 (m, 2H), 6.67-6.62 (m, 1H), 3.45 (s, 2H), 2.66-2.63 (d, 2H, *J* = 9.8 Hz), 2.39 (s, 3H), 2.30 (s, 3H), 1.70-1.54 (m, 5H), 1.43-1.37 (d, 2H, *J* = 9.8); ^{13}C-NMR: 173.69, 164.20, 161.74, 157.59, 143.35, 142.92, 142.78, 137.80, 131.87, 130.80, 129.32, 129.02, 128.54, 126.54, 111.16, 102.30, 62.13, 52.26, 41.79, 29.37, 21.28; LC-MS/MS: t_R 6.21min, 518.21 (M+H); MS (m/z): 517.10 $(M)^+$; Anal. Calcd for $C_{30}H_{29}F_2N_3OS$: C, 69.61; H, 5.65; N, 8.12; Found: C, 69.74; H, 5.78; N, 8.25 %.

1-(2-Chloro-4-fluorobenzyl)-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (20). Synthesized as per the general procedure (C) using 2-chloro-4-fluorobenzyl bromide (0.27 gm) and *N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (**13**) (0.5 gm) to get compound

(**20**) as a white solid (0.45 gm, 89 %); Mp: 178-180 ^{o}C; IR (KBr, cm^{-1}): 3144, 2926, 2857, 1680, 1543, 1444, 1264, 815; ^{1}H-NMR: 11.16 (s, 1H), 7.40-7.38 (d, 3H, *J* = 8.1 Hz), 7.26-7.24 (d, 2H, *J* = 8.0 Hz), 7.15-7.13 (d, 2H, *J* = 8.0 Hz), 7.09-7.07 (m, 3H), 6.94-6.90 (m, 1H), 3.45 (s, 2H), 2.72-2.70 (d, 2H, *J* = 9.8 Hz), 2.37 (s, 3H), 2.30 (s, 3H), 1.75-1.67 (m, 7H); ^{13}C-NMR: 173.52, 157.15, 143.47, 137.77, 134.59, 134.48, 131.89, 131.59, 131.50, 129.52, 129.33, 129.05, 128.87, 126.58, 116.66, 116.41, 113.94, 113.73, 58.48, 52.34, 42.07, 28.37, 21.25; LC-MS/MS: t_R 2.68 min, 534.27 (M+H); MS (m/z): 533.04 $(M)^+$; Anal. Calcd for $C_{30}H_{29}ClFN_3OS$: C, 67.46; H, 5.47; N, 7.87; Found: C, 67.62; H, 5.57; N, 7.81 %.

1-(4-Fluorobenzyl)-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (21). Synthesized as per the general procedure (C) using 4-fluorobenzyl bromide (0.20 ml) and *N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (**13**) (0.5 gm) to get compound (**21**) as a white solid (0.23 gm, 85 %); Mp: 167-169 ^{o}C; IR (KBr, cm^{-1}): 3201, 2852, 1675, 1539, 1401, 1190, 809, 655.

1-(2-Chloro-6-fluorobenzyl)-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (22). Synthesized as per the general procedure (C) using 2-chloro-6-fluorobenzyl bromide (0.36 gm) and *N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (**13**) (0.5 gm) to get compound (**22**) as a white solid (0.35 gm, 85 %); Mp: 194-196 ^{o}C; IR (KBr, cm^{-1}): 3144, 2930, 2854, 1680, 1543, 1449, 1267, 815; ^{1}H-NMR: 11.35 (s, 1H), 7.35-7.33 (d, 2H, *J* = 8.0 Hz), 7.26-7.24 (d, 2H, *J* = 8.0 Hz),7.15-7.13 (d, 4H, *J* = 8.0 Hz), 7.04-7.02 (d, 2H, *J* = 8.0 Hz), 6.95-6.91(m, 1H), 3.60 (s, 2H), 2.77-2.74 (d, 2H, *J* =11.1 Hz), 2.37(s, 3H), 2.29 (s, 3H), 1.77-1.52 (m, 3H), 1.65- 1.62 (m, 2H), 1.42-1.39 (d, 2H, *J* = 11.1 Hz); ^{13}C-NMR: 173.66, 163.31, 160.84, 157.39, 143.39, 137.75, 137.66, 136.73, 136.67, 131.86, 129.49, 129.07, 128.86, 126.45, 125.40, 123.91, 114.01, 113.77, 52.34, 51.87, 41.90, 28.27, 21.25; LC-MS/ MS: t_R 2.69 min, 534.27 (M+H); MS (m/z):

533.24 $(M)^+$; Anal. Calcd for $C_{30}H_{29}ClFN_3OS$: C, 67.46; H, 5.47; N, 7.87; Found: C, 67.58; H, 5.34; N, 7.94 %.

1-(4-Cyanobenzyl)-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (23). Synthesized as per the general procedure (C) using 4-cyanobenzyl bromide (0.32 gm) and *N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (**13**) (0.5 gm) to get compound (**23**) as a white solid (0.41 gm, 85 %); Mp: 92-94 ^oC; IR (KBr, cm^{-1}): 3148, 3029, 2924, 2227, 1659, 1559, 1444, 1266, 822; ^{1}H-NMR: 11.46 (bs, 1H), 7.58-7.56 (d, 2H, *J* = 8.0 Hz), 7.39-7.37 (d, 2H, *J* = 8.0 Hz), 7.38-7.36 (d, 2H, *J* = 8.0 Hz), 7.26-7.24 (d, 2H, *J* = 8.0 Hz), 7.15-7.13 (d, 2H, *J* = 8.0 Hz), 7.08-7.06 (d, 2H, *J* = 8.0 Hz), 3.42 (s, 2H), 2.66-2.64 (d, 2H, *J* = 8.6 Hz), 2.38 (s, 3H), 2.29 (s, 3H), 1.50-1.44 (m, 5H), 1.50-1.42 (d, 2H, *J* = 8.6 Hz); ^{13}C-NMR: 173.24, 156.83, 143.48, 137.82, 137.77, 132.11, 131.86, 129.54, 129.35, 129.30, 129.23, 129.00, 128.82, 118.90, 116.25, 111.01, 62.44, 52.46, 42.03, 29.72, 28.30, 21.29; LC-MS/MS: t_R 5.97 min, 507.24 (M+H); MS (m/z): 506.92 $(M)^+$; Anal. Calcd for $C_{31}H_{30}N_4OS$: C, 73.49; H, 5.97; N, 11.06; Found: C, 73.24; H, 5.76; N, 10.83 %.

1-(4-Nitrobenzyl)-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (24). Synthesized as per the general procedure (C) using 4-nitrobenzyl bromide (0.26 gm) and *N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (**13**) (0.5 gm) to get compound (**24**) as a white solid (0.36 gm, 81 %); Mp: 203-205 ^oC; IR (KBr, cm^{-1}): 3267, 3023, 2858, 1680, 1552, 1448, 1270, 815; ^{1}H-NMR: 11.75 (s, 1H), 8.14-8.12 (d, 2H, *J* = 8.6 Hz), 7.43-7.38 (d, 4H, *J* = 8.0 Hz), 7.26-7.24 (d, 2H, *J* = 8.6 Hz), 7.16-7.14 (d, 2H, *J* = 8.0 Hz), 7.08-7.06 (d, 2H, *J* = 8.0 Hz), 3.47 (s, 2H), 2.64-2.62 (d, 2H, *J* = 6.8 Hz), 2.38 (s, 3H), 2.29 (s, 3H), 1.67-1.62 (m, 4H), 1.54-1.52 (d, 1H, *J* = 7.7 Hz), 1.42-1.39 (d, 2H, *J* = 6.8 Hz); ^{13}C-NMR: 173.44, 157.22, 147.13, 146.39, 143.39, 137.86, 137.82, 131.84, 129.56, 129.32, 129.29, 128.97, 128.87, 125.24, 126.61, 123.51, 62.14, 52.42, 41.80, 28.28,

21.29; LC-MS/MS: t_R 6.06 min; MS (m/z): 527.2 $(M+1)^+$; Anal. Calcd for $C_{30}H_{30}N_4O_3S$: C, 68.42; H, 5.74; N, 10.64; Found: C, 68.73; H, 5.63; N, 10.76 %.

1-(4-Trifluoromethylbenzyl)-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (25). Synthesized as per the general procedure (C) using 4-trifluoromethylbenzyl bromide (0.36 gm) and *N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (**13**) (0.5 gm) to get compound (**25**) as a white solid (0.27 gm, 71 %); Mp: 130-132 ^oC; IR (KBr, cm^{-1}): 3160, 2926, 2823, 1686, 1544, 1446, 1263, 852; ^{1}H-NMR: 11.48 (s, 1H), 7.38-7.36 (d, 2H, J = 8.0 Hz), 7.26-7.24(d, 4H, J = 8.0 Hz), 7.15-7.10 (d, 4H, J = 8.0 Hz), 7.05-7.03 (d, 2H, J = 8.0 Hz), 3.37 (s, 2H), 2.66-2.64 (d, 2H, J = 9.7 Hz), 2.37 (s, 3H), 2.28 (s, 3H), 1.26-1.55 (m, 5H), 1.41-1.39 (m, 2H, J = 9.7 Hz); ^{13}C-NMR: 173.68, 157.47, 148.23, 143.36, 137.79, 136.92, 131.85, 130.18, 129.53, 129.31, 129.26, 129.04, 128.90, 126.50, 120.68, 119.21, 62.10, 52.13, 41.89, 28.27, 21.23; LC-MS/MS: t_R 7.01 min, 500.23 (M+H); MS (m/z): 549.52 $(M)^+$; Anal. Calcd for $C_{31}H_{30}F_3N_3OS$: C, 67.74; H, 5.50; N, 7.64; Found: C, 67.87; H, 5.62; N, 7.56 %.

1-(2-Methylbenzyl)-*N*-[4,5-bis(4-chlorophenylthiazol-2-yl]piperidine-4-carboxamide (26). Synthesized as per the general procedure (C) using 2-methylbenzyl bromide (0.39 ml) and *N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (**14**) (0.5 gm) to get compound (**26**) as a white solid (0.42 gm, 84 %); Mp: 179-181 ^oC; IR (KBr, cm^{-1}): 3158, 3047, 2927, 1688, 1545, 1297, 825; ^{1}H-NMR: 10.13 (bs, 1H), 7.41-7.38 (d, 2H, J = 8.5 Hz), 7.32-7.30 (d, 2H, J = 8.5 Hz), 7.28-7.26 (d, 4H, J = 8.5 Hz), 7.21-7.19 (d, 1H, J = 6.7 Hz), 7.16-7.12 (m, 3H), 3.39 (s, 2H), 2.84-2.81 (d, 2H, J = 11.2 Hz), 2.33 (s, 3H), 1.99-1.93 (m, 1H), 1.85-1.77 (m, 2H), 1.75-1.65 (m, 4H); ^{13}C-NMR: 173.08, 156.48, 143.29, 137.57, 136.36, 134.15, 134.03, 132.92, 130.72, 130.32, 130.24, 130.12, 129.75, 129.22, 128.81, 127.09, 126.23, 125.48, 60.97, 52.71,

42,95, 28.54, 19.23; LC-MS/MS: t_R 6.04 min; MS (m/z): 535.93 $(M)^+$; Anal. Calcd for $C_{29}H_{27}Cl_2N_3OS$: C, 64.92; H, 5.07; N, 7.83; Found: C, 65.04; H, 5.22; N, 7.75 %.

1-(2-Trifluoromethylbenzyl)-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (27). Synthesized as per the general procedure (C) using 2-trifluoromethylbenzyl bromide (0. 44 ml) and *N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl] piperidine-4-carboxamide (**14**) (0.5 gm) to get compound (**27**) as a white solid. (0.27 gm, 83 %); Mp: 162-164 ^oC; IR (KBr, cm^{-1}): 3155, 2927, 2855, 1690, 1540, 1373, 1113, 825; ^1H-NMR: 10.62 (s, 1H), 7.75-7.73 (d, 1H, *J* = 7.8 Hz), 7.61-7.59 (d, 1H, *J* = 7.8 Hz), 7.51-7.43 (m, 1H), 7.41-7.40 (m, 2H), 7.34-7.24 (m, 7H), 3.56 (s, 2H), 2.79-2.76 (d, 2H, *J* = 10.64 Hz), 1.89-1.71 (m, 5H), 1.62-1.58 (d, 2H, *J* = 12.64 Hz); LC-MS/MS: t_R 5.30 min; MS (m/z): 589.33 $(M)^+$; Anal. Calcd for $C_{28}H_{24}ClFN_3OS$: C, 58.99; H, 4.10; N, 7.12; Found: C, 59.13; H, 4.32; N, 7.28 %.

1-(3-Fluorobenzyl)-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (28). Synthesized as per the general procedure (C) using 3-fluorobenzyl bromide (0.52 gm) and *N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (**14**) (0.5 gm) to get compound (**28**) as a white solid. (0.30 gm, 90 %); Mp: 166-168 ^oC; IR (KBr, cm^{-1}): 3144, 2925, 2763, 1681, 1547, 1297, 1092, 826; ^1H-NMR: 12.25 (bs, 1H), 7.42-7.39 (d, 2H, *J* = 8.5 Hz), 7.38 (s, 1H), 7.35-7.33(d, 2H, *J* = 8.5 Hz), 7.29-7.28 (d, 2H, *J* = 6.5 Hz), 7.27-7.26 (d, 2H, *J* = 6.5 Hz), 7.18-7.13 (m, 2H), 7.03-6.99 (m, 1H), 3.78 (s, 2H), 3.02-3.00 (d, 2H, *J* = 10.8 Hz), 2.64 (s, 1H), 2.00 (bs, 2H), 1.92-1.80 (d, 4H, *J* = 10.8 Hz); ^{13}C-NMR: 173.34, 163.40, 160.97, 156.21, 142.99, 133.22, 132.84, 132.55, 130.69, 129.97, 129.84, 128.88, 128.22, 124.98, 124.48, 115.77, 114.20, 114.00, 60.93, 51.87, 29.01, 27.40; MS (m/z): 539.50 $(M)^+$; Anal. Calcd for $C_{28}H_{24}ClFN_3OS$: C, 62.22; H, 4.48; N, 7.77; Found: C, 62.48; H, 4.26; N, 7.89 %.

1-(3,5-Difluorobenzyl)-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (29). Synthesized as per the general procedure (C) using 3,5-difluorobenzyl bromide (0.35 ml) and *N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (**14**) (0.5 gm) to get compound (**29**) as a white solid (0.42 gm, 88 %). Mp: 196-198 ^{o}C; IR (KBr, cm^{-1}): 3138, 3023, 2852, 1688, 1534, 1264, 1093, 828. ^{1}H-NMR: 10.35 (bs, 1H), 7.37-7.35 (d, 2H, *J* = 8.5 Hz), 7.33-7.31 (d, 2H, *J* = 8.5 Hz), 7.26-7.24 (d, 4H, *J* = 8.5 Hz), 7.19-7.17 (m, 2H), 6.99-6.94 (m, 1H), 3.66-3.65 (s, 2H), 2.91-2.88 (d, 2H, *J* = 11.4 Hz), 2.04-1.94 (m, 2H), 1.76-1.62 (m, 5H); ^{13}C-NMR: 173.41, 163.31, 160.84, 157.61, 142.97, 136.75, 134.21, 132.76, 130.71, 130.17, 129.25, 129.11, 128.92, 126.13, 125.46, 114.05, 113.82, 52.36, 42.38, 29.71, 28.31; MS (m/z): 557.06 (M^{+}) ; Anal. Calcd for $C_{28}H_{23}Cl_2F_2N_3OS$: C, 60.22; H, 4.15; N, 7.52 10.23; Found: C, 60.05; H, 4.28; N, 7.69 %.

1-(2-Chloro-4-fluorobenzyl)-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (30). Synthesized as per the general procedure (C) using 2-chloro-4-fluorobenzyl bromide (0.61 gm) and *N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (**14**) (0.5 gm) to get compound (**30**) as a white solid (0.52 gm, 78 %). Mp: 179-181 ^{o}C; IR (KBr, cm^{-1}): 3155, 2948, 2763, 1686, 1536, 1488, 1262, 1093, 828; ^{1}H-NMR: 10.94 (bs, 1H), 7.42-7.40 (d, 2H, *J* = 8.5 Hz), 7.38 (s, 1H), 7.34-7.31 (d, 2H, *J* = 8.5 Hz), 7.29-7.25 (d, 4H, *J* = 8.5 Hz), 7.09-7.06 (d, 1H, *J* = 8.4 Hz), 6.96-6.91 (d, 1H, *J* = 8.4 Hz), 3.47(s, 2H), 2.79-2.77 (d, 2H, *J* = 8.8 Hz), 1.89-1.70 (m, 4H), 1.55-1.53 (m, 2H), 1.30-1.25 (m, 1H); ^{13}C-NMR: 173.20, 164.32, 160.84, 157.28, 143.10, 134.26, 134.20, 132.83, 130.69, 130.18, 130.11, 129.27, 128.92, 126.25, 116.74, 116.50, 114.50, 113.79, 58.52, 52.46, 42.44, 28.40; LC-MS/MS: t_R 6.38 min, 574.10 (M+H); MS (m/z): 573.54 $(M)^{+}$; Anal. Calcd for $C_{28}H_{23}Cl_3FN_3OS$: C, 58.49; H, 4.03; N, 7.31; Found: C, 58.78; H, 3.89; N, 7.23 %.

1-(2-Cyanobenzyl)-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (31). Synthesized as per the general procedure (C) using 2-cyanobenzyl bromide (0.60 gm) and *N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (**14**) (0.5 gm) to get compound (**31**) as a white solid (0.31 gm, 85 %); Mp: 196-198 ^{o}C; IR (KBr, cm^{-1}): 3249, 3213, 2953, 2923, 2231, 1648, 1544, 1291, 824; ^{1}H-NMR: 10.89 (bs, 1H),7.63-7.61(d, 1H, *J* = 7.6 Hz), 7.54 7.52 (d, 2H, *J* = 8.0 Hz), 7.42-7.40 (d, 2H, *J* = 8.0 Hz), 7.34-7.32 (m, 3H), 7.29-7.27 (d, 4H, *J* = 8.0 Hz), 3.61 (s, 2H), 2.79-2.76 (d, 2H, *J* = 10.56 Hz), 1.85-1.68 (m, 5H), 1.57-1.55 (d, 2H, *J* = 8.6 Hz); ^{13}C-NMR: 172.99, 156.79, 143.21, 142.43, 134.21, 134.09, 132.85, 132.67, 130.72, 130.15, 129.87, 129.25, 128.86, 127.60, 126.25, 117.80, 112.84, 60.46, 52.54, 42.51, 29.71, 28.41; LC-MS/MS: t_R 5.72 min; MS (m/z): 546.82 $(M)^+$; Anal. Calcd for $C_{29}H_{24}Cl_2N_4OS$: C, 63.62; H, 4.42; N, 10.23; Found: C, 63.74; H, 4.31; N, 10.18 %.

1-(4-Fluoro-2-trifluoromethylbenzyl)-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (32). Synthesized as per the general procedure (C) using 4-fluoro-2-trifluoromethylbenzyl bromide (0.35 ml) and *N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (**14**) (0.5 gm) to get compound (**32**) as a white solid (0.87 gm, 73 %); Mp: 154-156 ^{o}C; IR (KBr, cm^{-1}): 3213, 2917, 2754, 2764, 1665, 1578, 1232, 1121, 863.

1-(4-Trifluoromethoxybenzyl)-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (33). Synthesized as per the general procedure (C) using 4-trifluoromethoxybenzyl bromide (0. 47 ml) and *N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (**14**) (0.5 gm) to get compounds (**33**) as a white solid (0.89 gm, 63 %); Mp: 134-136 ^{o}C; IR (KBr, cm^{-1} : 3154, 2924, 2853, 2764, 1688, 1540, 1259, 1092, 827; ^{1}H-NMR: 9.92 (bs, 1H), 7.31-7.29 (d, 2H, *J* = 8.5 Hz), 7.25-7.23 (d, 4H, *J* = 8.0 Hz), 7.20-7.16 (m, 4H), 7.09-7.07 (d, 2H, *J* = 8.5 Hz), 3.39 (s, 2H), 2.79-2.76 (d, 2H, *J* = 11.16 Hz), 2.02-1.97 (m, 1H), 1.85-1.78 (m, 6H); ^{13}C-NMR:

173.14, 157.02, 148.28, 143.13, 136.87, 134.23, 132.81, 130.68, 130.19, 130.16, 130.13, 129.25, 128.87, 126.21, 121.76, 120.78, 119.21, 62.15, 52.43, 42.52, 31.94; LC-MS/MS: t_R 2.62 min, 606.13 (M+H); MS (m/z): 607.1$(M)^+$; Anal. Calcd for $C_{29}H_{24}Cl_2F_3N_3O_2S$: C, 57.43; H, 3.99; N, 6.93; Found: C, 57.61; H, 3.65; N, 6.82 %.

1-(2-Methylbenzyl)-*N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (34). Synthesized as per the general procedure (C) using 2-methylbenzyl bromide (0. 40 ml) and *N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (**15**) (0.5 gm) to get compound (**34**) as a white solid (0.65 gm, 54 %); Mp: 158-160 ^oC; IR (KBr, cm^{-1}): 3162, 3010, 2939, 2836, 1664, 1537, 1250, 1176, 836; ^{1}H-NMR: 11.11 (bs, 1H), 7.58 (s, 1H), 7.44-7.42 (d, 2H, *J* = 8.5 Hz), 7.19-7.13 (m, 6H), 6.87-6.82 (m, 3H), 3.92 (s, 3H), 3.78 (s, 3H), 3.34 (s, 2H), 2.73-2.71(d, 2H, *J* = 5.5 Hz), 2.31 (s, 3H), 1.68-1.63(m, 5H), 1.49 (bs, 2H); ^{13}C-NMR: 173.69, 159.29, 159.25, 156.90, 142.92, 137.53, 136.48, 130.74, 130.25, 130.16, 129.72, 127.28, 126.99, 125.55, 125.42, 124.34, 114.29, 113.95, 60.93, 55.32, 52.58, 42.44, 28.50, 19.23; LC-MS/MS: t_R 5.59 min, 528.23 (M+H); MS (m/z): 527.82 $(M)^+$; Anal. Calcd for $C_{31}H_{33}N_3O_3S$: C, 70.56; H, 6.30; N, 7.96; Found: C, 70.78; H, 6.61; N, 7.87 %.

1-(2-Trifluoromethylbenzyl)-*N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (35). Synthesized as per the general procedure (C) using 2-trifluoromethylbenzyl bromide (0. 45 ml) and *N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (**15**) (0.5 gm) to get compound (**35**) as a white solid (0.91 gm, 70 %); Mp: 194-196 ^oC; IR (KBr, cm^{-1}): 3150, 2949, 2843, 1685, 1540, 1311, 1255, 836; ^{1}H-NMR: 11.32 (bs, 1H), 7.76-7.75 (m, 1H), 7.60-7.56 (m, 2H), 7.51-7.43 (m, 3H), 7.32-7.24 (m, 3H), 6.90-6.81(m, 3H), 3.92(s, 3H), 3.78 (s, 3H), 3.59 (s, 2H) 2.7 (bs, 2H), 1.87-1.84 (m, 4H), 1.49-1.42 (m, 2H), 1.25-0.97 (m, 1H); ^{13}C-NMR: 173.59, 159.50, 159.30, 157.18, 155.59, 143.66, 134.12, 131.83, 130.74, 130.15, 129.73,

128.24, 126.83, 125.80, 123.89, 114.32, 113.99, 112.03, 111.92, 56.29, 55.29, 52.65, 42.23, 28.44; LC-MS/MS: t_R 2.39 min, 582.18 (M+H); MS (m/z): 581.46 $(M)^+$; Anal. Calcd for $C_{31}H_{30}F_3N_3O_3S$: C, 64.01; H, 5.20; N, 7.22; Found: C, 64.38; H, 5.29; N, 7.13 %.

1-(3-Fluorobenzyl)-*N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (36). Synthesized as per the general procedure (C) using 3-fluorobenzyl bromide (0. 35 ml) and *N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (**15**) (0.5 gm) to get compound (**36**) as a white solid (0.89 gm, 74 %); Mp: 158-160 ^oC; IR (KBr, cm^{-1}): 3141, 3034, 2929, 1677, 1609, 1539, 1298, 1250, 1032, 832; ^{1}H-NMR: 11.46 (s, 1H), 7.42-7.40 (d, 2H, *J* = 8.7 Hz), 7.29-7.26 (d, 2H, *J* = 8.7 Hz), 7.23-7.20 (m, 1H) 7.02-6.99 (m, 2H), 6.97-6.91 (m, 1H), 6.89-6.87 (d, 2H, *J* = 8.7 Hz), 6.82-6.80 (d, 2H, *J* = 8.7 Hz), 3.84 (s, 3H), 3.77 (s, 3H), 3.39 (s, 2H), 2.36 (bs, 2H), 1.67 (bs, 5H), 1.47 (bs, 2H); ^{13}C-NMR: 173.50, 164.11, 161.67, 159.33, 157.01, 142.84, 130.72, 130.14, 129.64, 129.56, 127.21, 125.58, 124.50, 124.26, 115.83, 115.62, 114.31, 113.95, 62.37, 55.31, 52.26, 42.02, 28.25; LC-MS/MS: t_R 5.59 min, 532.22 (M+H); MS (m/z): 532.3 (M+1) ; Anal. Calcd for $C_{30}H_{30}FN_3O_3S$: C, 67.78; H, 5.69; N, 7.90; Found: C, 67.64; H, 5.95; N, 7.98 %.

1-(3,5-Difluorobenzyl)-*N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (37). Synthesized as per the general procedure (C) using 3,5-difluorobenzyl bromide (0.36 ml) and *N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (**15**) (0.5 gm) to get compound (**37**) as a white solid (0.97 gm, 80 %); Mp: 175-177 ^oC; IR (KBr, cm^{-1}): 3141, 3032, 2929, 1680, 1542, 1251, 1176, 1032, 833; ^{1}H-NMR: 11.45 (s, 1H), 7.45-7.43, (d, 2H, *J* = 8.7 Hz), 7.29-7.27 (d, 2H, *J* = 8.7 Hz), 6.89-6.87 (d, 2H, *J* = 8.7 Hz), 6.82-6.80 (d, 2H, *J* = 8.7 Hz), 6.79-6.77 (m, 2H), 6.68-6.63 (m, 1H), 3.84 (s, 3H), 3.78 (s, 3H), 3.34 (s, 2H), 2.68-2.65 (d, 2H, *J* = 8.2 Hz), 1.65-1.60 (bs, 5H), 1.45-1.43 (d, 2H, *J* = 8.7 Hz); ^{13}C-NMR: 173.35, 164.27, 161.80,

161.08, 159.36, 157.16, 142.81, 130.72, 130.15, 127.23, 125.61, 124.23, 114.33, 113.98, 111.38, 111.20, 102.58, 102.07, 62.13, 55.31, 52.33, 41.95, 28.29; LC-MS/MS: t_R 2.57 min, 550.18 (M+H); MS (m/z): 549.73$(M)^+$; Anal. Calcd for $C_{30}H_{29}F_2N_3O_3S$: C, 65.56; H, 5.32; N, 7.65; Found: C, 65.73; H, 5.39; N, 7.49 %**.**

1-(4-Fluorobenzyl)-*N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (38). Synthesized as per the general procedure (C) using 4-fluorobenzyl bromide (0. 35 ml) and *N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (**15**) (0.5 gm) to get compound (**38**) as a white solid (0.95 gm, 79 %); Mp: 124-126 ^oC; IR (KBr, cm^{-1}): 3153, 2939, 2762, 1688, 1541, 1176, 1033, 832; ^{1}H-NMR: 10.95 (s, 1H), 7.34-7.32 (d, 2H, *J* = 8.5 Hz), 7.21-7.19 (d, 2H, *J* = 8.5 Hz), 7.16-7.12 (m, 2H), 6.91-6.87 (m, 2H), 6.81-6.79 (d, 2H, *J* = 8.5 Hz), 6.75-6.73 (d, 2H, *J* = 8.5 Hz), 3.71 (s, 6H), 3.32 (s, 2H), 2.66 (bs, 2H), 1.69-1.61 (m, 4H), 1.48 (bs, 3H); ^{13}C-NMR: 173.59, 163.28, 160.85, 159.32, 157.04, 142.92, 133.76, 130.78, 130.56, 127.29, 125.65, 124.34, 115.14, 114.93, 114.13, 112.12, 62.22, 55.38, 52.26, 42.22, 29.78; LC-MS/MS: t_R 5.56 min, 532.22 (M+H); MS (m/z): 531.23$(M)^+$; Anal. Calcd for $C_{30}H_{30}FN_3O_3S$: C, 67.78; H, 5.69; N, 7.90; Found: C, 67.43; H, 5.87; N, 7.75 %.

1-(2-Chloro-6-fluorobenzyl)-*N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (39). Synthesized as per the general procedure (C) using 2-chloro-6-fluorobenzyl bromide (0. 40 ml) and *N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (**15**) (0.5 gm) to get compound (**39**) as a white solid (0.8 gm, 62 %); Mp: 187-189 ^oC; IR (KBr, cm^{-1}): 3157, 2931, 2841, 1683, 1539, 1255, 1112, 805; ^{1}H-NMR: 11.08 (bs, 1H), 7.493-7.490 (d, 2H), 7.29-7.16 (m, 2H), 6.84-6.74 (m, 6H), 6.64-6.57(m, 1H), 3.85 (s, 2H), 3.71 (s, 6H), 3.31 (bs, 2H), 2.65 (bs, 3H), 1.18 (bs, 4H) ; MS (m/z): 565.75 $(M)^+$; Anal. Calcd for $C_{30}H_{29}ClFN_3O_3S$: C, 63.65; H, 5.16; N, 7.42; Found: C, 63.58; H, 5.29; N, 7.27 %.

1-(2-Cyanobenzyl)-*N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (40). Synthesized as per the general procedure (C) using 2-cyanobenzyl bromide (0. 55 gm) and *N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (**15**) (0.5 gm) to get compound (**40**) as a white solid (0.75 gm, 62 %); Mp: 187-189 ^{o}C; IR (KBr, cm^{-1}): 3228, 3176, 3006, 2953, 2219, 1684, 1531, 1290, 1029, 830; ^{1}H-NMR: 11.34 (bs, 1H), 7.61-7.59 (d, 1H, *J* = 7.6 Hz), 7.51-7.49 (d, 2H, *J* = 8.6 Hz), 7.45-7.42(d, 2H, *J* = 8.6 Hz), 7.34-7.26 (m, 3H), 6.89-6.87 (d, 2H, *J* = 8.6 Hz), 6.83-6.81 (d, 2H, *J* = 8.6 Hz), 3.84 (s, 3H), 3.78 (s, 3H), 3.59 (s, 2H), 2.73-2.71 (d, 2H, *J* = 9.72 Hz), 1.78-1.70 (m, 5H), 1.50-1.29 (m, 2H); ^{13}C-NMR: 173.31, 159.31, 159.26, 156.72, 142.92, 142.52, 132.73, 132.65, 130.73, 130.13, 129.82, 127.48, 127.20, 125.64, 124.21, 117.78, 114.31, 113.96, 112.70, 60.39, 55.29, 52.44, 42.13, 29.70, 28.38; LC-MS/MS: t_R 6.18 min; MS (m/z): 538.44 $(M)^+$; Anal. Calcd for $C_{31}H_{30}N_4O_3S$: C, 69.12; H, 5.61; N, 10.40; Found: C, 69.35; H, 5.45; N, 10.63 %.

1-(4-Fluoro-2-trifluoromethylbenzyl)-*N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (41). Synthesized as per the general procedure (C) using 4-fluoro-2-trifluorobenzyl bromide (0. 40 ml) and *N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (**15**) (0.5 gm) to obtain compound (**41**) as a white solid (0.75 gm, 55 %); Mp: 166-168 ^{o}C; IR (KBr, cm^{-1}): 3145, 3004, 2948, 1682, 1543, 1251, 1038, 834; ^{1}H-NMR: 11.60 (s, 1H), 7.74-7.71 (m, 1H), 7.47-7.45 (d, 2H, *J* = 8.7 Hz), 7.30-7.24 (m, 3H), 7.20-7.18 (d, 1H, *J* = 8.3 Hz), 6.89-6.87 (d, 2H, *J* = 8.7 Hz), 6.84-6.82 (d, 2H, *J* = 8.3 Hz), 3.84 (s, 3H), 3.77 (s, 3H), 3.46 (s, 2H), 2.65 (s, 2H), 1.67-1.57 (m, 5H), 1.49 (bs, 2H); ^{13}C-NMR: 173.56, 162.15, 159.49, 157.25, 156.99, 155.60, 142.91, 134.12, 133.70, 132.33, 130.73, 129.72, 127.24, 125.66, 124.23, 118.81, 114.22, 113.99, 112.04, 57.53, 55.29, 52.56, 42.13, 28.47; LC-MS/MS: t_R 6.83 min,

600.29 (M+H); MS (m/z): 599.29$(M)^+$; Anal. Calcd for $C_{31}H_{29}F_4N_3O_3S$: C, 62.09; H, 4.87; N, 7.01; Found: C, 62.42; H, 4.68; N, 7.23 %.

General procedure D. Synthesis of 1-(substituted benzyl)piperidin-4-yl-4,5-bis(substituted phenyl)thiazol-2-ylamines (42-52). 1-(Substituted benzyl)-*N*-[4,5-bis(4-substituted phenyl)thiazol-2-yl]piperidine-4-caroxamide was dissolved in dry THF under nitrogen atmosphere. Borane-dimethyl sulfide solution (BH_3-DMS) was added to the reaction mixture in ice-cold conditions and allowed to stirr for overnight. After completion of the reaction, THF was removed and the reaction mixture was acidified with conc. HCl and the reaction mixture was refluxed again for 2-3 hrs. The reaction mixture was basified using $NaHCO_3$. The title compounds were extracted by DCM and further purification was carried out by column chromatography using chloroform and methanol as eluents and silica gel (100-200) as the adsorbent.

***N*-[(1-(2-Methylbenzyl)piperidin-4-yl)methyl]-4,5-bis(*p*-tolyl)thiazol-2-ylamine (42).** Synthesized as per the general procedure (D) using 1-(2-methylbenzyl)-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (**16**) (1 gm) to get compound (**42**) as a white solid (0.87 gm, 89 %); Mp: 142-144 ^oC; IR (KBr, cm^{-1}): 3186, 3023, 2942, 1557, 1271, 819; ^{1}H-NMR: 7.36-7.34 (d, 2H, J = 8.00 Hz), 7.27-7.25 (d, 2H, J = 8.00 Hz), 7.16-7.11 (m, 4H), 7.06-7.04 (d, 4H, J = 8.0 Hz), 5.70 (bs, 1H), 3.44 (s, 2H), 3.09 (s, 2H), 2.90-2.87 (d, 2H, J = 11.8 Hz), 2.34-2.31 (s, 9H), 2.00-1.94 (m, 3H), 1.71-1.62 (d, 2H, J = 11.8 Hz), 1.32-1.25 (t, 2H, J = 11.08 Hz); ^{13}C-NMR: 167.81, 145.73, 137.51, 137.14, 136.71, 132.85, 130.29, 130.17, 129.84, 129.27, 129.16, 128.91, 128.78, 127.12, 125.55, 119.69, 60.99, 53.49, 36.15, 30.16, 21.34, 19.34; MS (m/z): 481.61 (M^+); Anal. Calcd for $C_{31}H_{35}N_3S$: C, 77.30; H, 7.32; N, 8.72; Found: C, 77.58; H, 7.13; N, 8.85 %.

***N*-[(1-(2-Trifluoromethylbenzyl)piperidin-4-yl)methyl]-4,5-bis(*p*-tolyl)thiazol-2-ylamine (43).** Synthesized as per the general procedure (D) using 1-(2-(trifluoromethyl)benzyl)-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (**17**) (1 gm) to get compound (**43**) as a white solid (0.65 gm, 67 %); Mp: 142-144 ^{o}C; IR (KBr, cm^{-1}) : 3200, 3023, 2957, 1584, 1313, 1161, 1109, 818; ^{1}H-NMR: 7.81-7.80 (d, 1H, *J* =7.7 Hz), 7.62-7.60 (d, 2H, *J* = 7.7 Hz), 7.53-7.49 (m, 1H), 7.36-7.29 (m, 3H), 7.17-7.15 (d, 2H, *J* = 8.1 Hz), 7.06-7.04 (d, 4H, *J* = 8.1 Hz), 3.64 (s, 2H), 3.17-3.15 (d, 2H, *J* = 6.28 Hz), 2.89-2.86 (d, 2H, *J* =11.4 Hz), 2.32-2.28 (s, 6H), 2.09-2.03 (m, 2H), 1.74-1.67 (m, 5H) ; MS (m/z): 536.10 (M^{+}) ; Anal. Calcd for $C_{31}H_{32}F_3N_3S$: C, 69.51; H, 6.02; N, 7.84; Found: C, 69.67; H, 6.11; N, 7.74 %.

***N*-[(1-(3,5-Difluorobenzyl)piperidin-4-yl)methyl]-4,5-bis(*p*-tolyl)thiazol-2-ylamine (44).** Synthesized as per the general procedure (D) using 1-(3,5-difluorobenzyl)-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (**19**) (1 gm) to get compound (**44**) as a white solid (0.85 gm, 88 %); Mp: 152-154 ^{o}C; IR (KBr, cm^{-1}): 3198, 2925, 2856, 1583, 1459, 1331, 1117, 851; ^{1}H-NMR: 7.29-7.27 (d, 2H, *J* = 7.6 Hz), 7.08-7.06 (d, 2H, *J* = 7.6 Hz), 6.99-6.95 (d, 4H, *J* = 7.6 Hz), 6.77-6.76 (d, 2H, *J* = 6.5 Hz), 6.60-6.56 (m, 1H), 6.20 (bs, 1H), 3.33 (s, 2H), 2.95 (s, 2H), 2.73-2.70 (d, 2H, *J* = 12.0 Hz), 2.23 (s, 6H), 1.85-1.79 (m, 2H), 1.59-1.56 (d, 2H, *J* = 12.0 Hz), 1.18-1.10 (m, 3H); ^{13}C-NMR: 167.97, 164.29, 161.83, 145.69, 143.35, 137.11, 136.63, 132.86, 130.10, 129.21, 128.89, 119.40, 111.39, 111.21, 102.49, 101.98, 62.36, 53.32, 52.16, 35.85, 30.01, 21.29; LC-MS/MS: t_R 2.61 min, 504.20 (M+H); MS (m/z): 503.84 (M^{+}); Anal. Calcd for $C_{30}H_{31}F_3N_3S$: C, 71.54; H, 6.20; N, 8.34; Found: C, 71.48; H, 6.35; N, 8.45 %.

***N*-[(1-(4-Fluorobenzyl)piperidin-4-yl)methyl]-4,5-bis(*p*-tolyl)thiazol-2-ylamine (45).** Synthesized as per the general procedure (D) using 1-(4-fluorobenzyl)-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidine-4-carboxamide (**21**) (1 gm) to get compound (**45**) as a white solid (0.76 gm, 78

%); Mp: 178-180 ^{o}C; IR (KBr, cm^{-1}): 3196, 3091, 2818, 2791, 1582, 1425, 1330, 818; ^{1}H-NMR: 7.36-7.34 (d, 2H, *J* = 8.1 Hz), 7.26-7.24 (m, 2H), 7.15-7.14 (d, 2H, *J* = 8.1 Hz), 7.06-7.04 (d, 4H, *J* = 8.1 Hz), 7.01-6.96 (m, 2H), 5.58 (s, 1H), 3.45 (s, 2H), 3.13-3.10 (m, 2H), 2.88-2.86 (d, 2H, *J* = 12.0 Hz), 2.35-2.28 (s, 6H), 1.91-1.90 (m, 3H), 1.64-1.62 (d, 2H, *J* = 12.0 Hz), 1.36-1.25 (m, 2H); ^{13}C-NMR: 166.02, 144.65, 136.19, 135.97, 132.76, 130.35, 129.96, 129.01, 128.67, 128.36, 128.31, 117.73, 114.73, 114.52, 61.45, 52.76, 40.20, 39.37, 38.95, 29.61, 20.78; MS (m/z): 485.01(M)$^{+}$; Anal. Calcd for $C_{30}H_{32}FN_3S$: C, 74.19; H, 6.64; N, 8.65; Found: C, 74.05; H, 6.56; N, 8.57 %.

***N*-[(1-(2-Methylbenzyl)piperidin-4-yl)methyl]-4,5-bis(4-chlorophenyl)thiazol-2-ylamine (46).** Synthesized as per the general procedure (D) using 1-(2-methylbenzyl)-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (**26**) (1 gm) to get compound (**46**) as a white solid (0.75 gm, 77 %); Mp: 155-157 ^{o}C; IR (KBr, cm^{-1}): 3200, 2919, 2799, 2756, 1580, 1330, 1092, 824; ^{1}H-NMR: 7.39-7.36 (m, 2H), 7.26-7.21 (m, 5H), 7.18-7.13 (m, 5H), 5.53 (bs, 1H), 3.44 (s, 2H), 3.15- 3.12 (t, 2H, *J* = 12.1 Hz), 2.91-2.89 (d, 2H, *J* = 11.3 Hz), 2.35 (s, 3H), 2.01-1.96 (t, 2H, *J* = 11.1 Hz), 1.70-1.64 (m, 2H), 1.35-1.25 (m, 3H); ^{13}C-NMR: 167.90, 145.26, 137.44, 133.57, 133.50, 133.05, 131.09, 130.44, 130.24, 130.21, 129.79, 128.87, 128.48, 127.02, 125.50, 119.16, 60.82, 53.35, 51.97, 36.08, 30.02, 19.27; LC-MS/MS: t_R 6.04 min, 522.15 (M+H); MS (m/z): 521.86 (M)$^{+}$; Anal. Calcd for $C_{29}H_{29}Cl_2N_3S$: C, 66.66; H, 5.59; N, 8.04; Found: C, 66.42; H, 5.66; N, 8.22 %.

***N*-[(1-(3,5-Difluorobenzyl)piperidin-4-yl)methyl]-4,5-bis(4-chlorophenyl)thiazol-2-ylamine (47).** Synthesized as per the general procedure (D) using 1-(3,5-difluorobenzyl)-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (**29**) (1 gm) to get compound (**47**) as a white solid (0.79 gm, 79 %); Mp: 134-136 ^{o}C; IR (KBr, cm^{-1}): 3195, 3087, 2924, 2845, 1543, 1331,

1091, 824; ^{1}H-NMR: 7.38-7.35 (m, 2H), 7.26- 7.14 (m, 8H), 7.00-6.95 (m, 1H), 5.53 (s, 1H), 3.70 (s, 2H), 3.14-3.11 (t, 2H, *J* = 10.8 Hz), 2.99-2.96 (d, 2H, *J* = 11.5 Hz), 2.17-2.13 (m, 2H), 1.74-1.71 (d, 2H, *J* = 12.0 Hz), 1.37-1.27 (m, 3H); LC-MS/MS: t_R 2.47 min, 545.36 (M+H); MS (m/z): 544.32 $(M)^+$; Anal. Calcd for $C_{28}H_{25}Cl_2N_3S$: C, 61.76; H, 4.63; N, 7.72; Found: C, 61.94; H, 4.89; N, 8.02 %.

***N*-[(1-(2-Chloro-4-fluorobenzyl)piperidin-4-yl)methyl]-4,5-bis(4-chlorophenyl)thiazol-2-ylamine (48).** Synthesized as per the general procedure (D) using 1-(2-chloro-4-fluorobenzyl)-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidine-4-carboxamide (**30**) (1 gm) to get compound (**48**) as a white solid (0.76 gm, 78 %); Mp: 134-136 ^{o}C; IR (KBr, cm^{-1}): 3198, 3091, 2940, 1579, 1490, 1327, 1046, 819; ^{1}H-NMR: 7.93-7.90 (m, 1H), 7.43-7.39 (m, 2H), 7.28-7.23 (m, 4H), 7.22-7.19 (m, 2H), 7.16-7.15 (m, 1H), 6.96-6.93 (m, 1H), 5.62 (bs, 1H), 3.57 (s, 2H), 3.16-3.14 (m, 2H), 2.93-2.90 (d, 2H, *J* = 11.2 Hz), 2.17-2.08 (m, 2H), 1.76-1.69 (d, 2H, *J* = 12.8 Hz), 1.66-1.64 (m, 1H), 1.37-1.36 (m, 2H); LC-MS/MS: t_R 2.66 min, 562.08 (M+H); MS (m/z): 560.2 (M+1); Anal. Calcd for $C_{28}H_{25}Cl_3FN_3S$: C, 59.95; H, 4.49; N, 7.49; Found: C, 59.78; H, 4.57; N, 7.63 %.

***N*-[(1-(4-Fluoro-2-trifluoromethylbenzyl)piperidin-4-yl)methyl]-4,5-bis(4chlorophenyl)thiazol-2-ylamine (49).** Synthesized as per the general procedure (D) using *N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]-1-(4-fluoro-2-trifluoromethylbenzyl)piperidine-4-carboxamide (**32**) (1 gm) to get compound (**49**) as a white solid (0.94 gm, 91 %); Mp: 172-174 ^{o}C; IR (KBr, cm^{-1}): 3221, 3191, 2838, 1556, 1494, 1327, 2144, 1046, 836; ^{1}H-NMR: 7.79-7.76 (m, 1H), 7.39-7.36 (m, 2H), 7.33-7.30 (d, 1H), 7.26-7.20 (m, 5H), 7.18-7.15 (m, 2H), 5.61 (bs, 1H), 3.58 (s, 2H), 3.17-3.14 (t, 2H, *J* = 9.5 Hz), 2.85-2.82 (d, 2H, *J* = 11.5 Hz), 2.17-2.08 (t, 2H, *J* = 11.5 Hz), 1.75-1.72 (d, 2H, *J* = 12.5 Hz), 1.43-1.27 (m, 3H); ^{13}C-NMR: 168.14, 162.13,

145.30, 134.03, 133.66, 133.56, 133.06, 131.09, 130.59, 130.26, 129.82, 128.96, 128.53, 124.42, 122.21, 119.07, 118.71, 113.22, 57.75, 53.38, 52.09, 35.95, 29.71; LC-MS/MS: t_R 2.93 min, 594.12 (M+H); Anal. Calcd for $C_{29}H_{25}Cl_2F_4N_3S$: C, 58.59; H, 4.24; N, 7.07; Found: C, 58.47; H, 4.17; N, 7.23 %.

***N*-[(1-(2-Methylbenzyl)piperidin-4-yl)methyl]-4,5-bis(4-methoxyphenyl)thiazol-2-ylamine (50).** Synthesized as per the general procedure (D) using 1-(2-methylbenzyl)-*N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (**34**) (1 gm) to get compound (**50**) as a white solid (0.86 gm, 88 %); Mp: 138-140 ^oC; IR (KBr, cm^{-1}): 3200, 2957, 2900, 1579, 1456, 1295, 1243, 1035, 829; ^{1}H-NMR: 7.40-7.38 (m, 2H), 7.20-7.19 (m, 2H), 7.18-7.17 (m, 4H), 6.81-6.75 (m, 4H), 5.49 (s, 1H), 3.79 (s, 3H), 3.78 (s, 3H) 3.44 (s, 2H), 3.12 (bs, 2H), 2.91-2.88 (d, 2H, J = 11.4 Hz), 2.35 (s, 3H), 2.04-1.98 (m, 2H), 1.71-1.67 (m, 2H), 1.36-1.22 (m, 3H); ^{13}C-NMR: 167.49, 158.82, 144.93, 137.40, 136.67, 133.82, 130.49, 130.18, 129.71, 129.48, 128.65, 128.18, 126.89, 125.44, 118.67, 113.95, 111.74, 60.92, 55.22, 53.43, 52.09, 36.09, 30.13, 19.25; LC-MS/MS: t_R 2.51 min, 514.21(M+H); MS (m/z) : 513.62 $(M)^+$; Anal. Calcd for $C_{31}H_{35}N_3O_2S$: C, 72.48; H, 6.87; N, 8.18; Found: C, 72.73; H, 6.39; N, 8.32 %.

***N*-[(1-(2-Trifluoromethylbenzyl)piperidin-4-yl)methyl]-4,5-bis(4-methoxyphenyl)thiazol-2-ylamine (51).** Synthesized as per the general procedure (D) using 1-(2-trifluoromethylbenzyl)-*N*-[4,5-bis(4-methoxyphenyl]thiazol-2-yl]piperidine-4-carboxamide (**35**) (1 gm) to get compound (**51**) as a white solid (0.89 gm, 91 %); Mp: 179-181 ^oC; IR (KBr, cm^{-1}): 3207, 2998, 2842, 1583, 1313, 1221, 836; ^{1}H-NMR: 7.81-7.79 (d, 1H, J = 7.8 Hz), 7.62-7.60 (d, 1H, J = 7.7 Hz), 7.53-7.49 (m, 2H), 7.41-7.37 (m, 2H), 7.33-7.26 (m, 1H), 7.15-7.13 (m, 1H), 6.82-6.76 (m, 4H), 5.70 (s, 1H), 3.89 (s, 3H), 3.79(s, 3H), 3.64 (s, 2H), 3.15-3.13 (m, 2Hz), 2.88-2.85 (d, 2H, J = 11.4 Hz), 2.07-2.20 (m, 2H), 1.75-1.67 (m, 3H), 1.42-1.25 (m, 2H); ^{13}C-NMR: 167.64, 159.09,

154.85, 145.82, 133.90, 131.74, 130.20, 129.61, 128.61, 128.31, 127.75, 126.96, 125.66, 116.95, 114.01, 113.66, 111.83, 67.92, 58.30, 56.25, 55.25, 35.97, 30.14; LC-MS/MS: t_R 2.58 min, 568.22 (M+H); MS (m/z): 568.4(M+1); Anal. Calcd for $C_{31}H_{32}F_3N_3S$: C, 65.59; H, 5.68; N, 7.40; Found: C, 65.76; H, 5.49; N, 7.23 %.

***N*-[(1-(2-Chloro-6-fluorobenzyl)piperidin-4-yl)methyl]-4,5-bis(4-methoxyphenyl)thiazol-2-ylamine (52).** Synthesized as per the general procedure (D) using 1-(2-chloro-6-fluorobenzyl)-*N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidine-4-carboxamide (**39**) (1 gm) to get compound (**52**) as a white solid (0.85 gm, 84 %); Mp: 145-147 ^oC; IR (KBr, cm^{-1}): 3206, 3099, 2929, 2799, 1596, 1506, 1250, 1113, 836; ^{1}H-NMR: 7.49-7.48 (d, 1H), 7.39-7.37 (m, 2H), 7.13-7.12 (m, 1H), 6.88-6.87 (d, 2H, *J* = 6.2 Hz), 6.81-6.76 (m, 4H), 6.70-6.65 (m, 1H), 5.68 (s, 1H), 3.89-3.88 (s, 3H), 3.79-3.78 (s, 3H), 3.47 (s, 2H), 3.16-3.14 (d, 2H, *J* = 5.9 Hz), 2.90-2.84 (d, 2H, *J* = 12.2 Hz), 2.03-1.98 (m, 2H), 1.77-1.74 (d, 2H, *J* = 12.2 Hz), 1.64-1.61 (m, 3H); MS (m/z): 551.29 $(M)^+$; Anal. Calcd for $C_{30}H_{31}ClFN_3O_2S$: C, 65.26; H, 5.66; N, 7.61; Found: C, 65.08; H, 5.73; N, 7.93 %.

General procedure E. Synthesis of 1-(substituted benzyl)piperidine-4-carboxamides[104] **(60-65).** To a solution of 4-piperidinecarboxamide (**53**) (2 gm, 1.28 mM) in methanol, potassium carbonate (4.3 gm, 2.56 mM) and substituted benzyl bromide (**54-59**) was added and the reaction mixture was refluxed for 5-6 hrs. Solvent was distilled off and addition of crushed ice to the residue led to formation of white colored precipitates of 1-(substituted benzyl)piperidine-4-carboxamides (**60-65**).

1-Benzylpiperidine-4-carboxamide (60). Synthesized as per the general procedure (E) using benzyl bromide (**54**) (0.89 ml) to get compound (**60**) as a white solid (0.89 gm, 85 %); Mp: 161-163 ^{o}C (Lit[105] Mp: 162 ^{o}C); IR (KBr, cm^{-1}): 3348, 2946, 2756, 1635, 1463, 1043, 810.

1-(4-Methylbenzyl)piperidine-4-carboxamide (61). Synthesized as per the general procedure (E) using 4-methylbenzyl bromide (**55**) (0.64 ml) to get compound (**61**) as a white solid (3 gm, 83 %); Mp: 169-170 ^{o}C; IR (KBr, cm^{-1}): 3342, 3170, 2921, 1630, 1430, 1145; MS (m/z): 232.01 $(M)^{+}$.

1-(4-Methoxybenzyl)piperidine-4-carboxamide (62). Synthesized as per the general procedure (E) using 4-methoxybenzyl bromide (**56**) (1.05 ml) to get compound (**62**) as a white solid (2.9 gm, 75 %); Mp: 144-146 ^{o}C; IR (KBr, cm^{-1}): 3343, 3160, 3012, 2790, 1636, 1511, 1245, 814; MS (m/z): 248.92 $(M)^{+}$.

1-(2-Chloro-4-flurobenzyl)piperidine-4-carboxamide (63). Synthesized as per the general procedure (E) using 2-chloro-4-fluorobenzyl bromide (**57**) (1.6 gm) to get compound (**63**) as a white solid (3.2 gm, 76 %); Mp: 156-157 ^{o}C; IR (KBr, cm^{-1}): 3381, 3188, 2938, 1654, 1491, 1041; MS (m/z): 270.19 $(M)^{+}$.

1-(4-Cyanobenzyl)piperidine-4-carboxamide (64). Synthesized as per the general procedure (E) using 4-cyanobenzyl bromide (**58**) (1.53 ml) to get compound (**64**) as a white solid (3.21 gm, 85 %); Mp: 162-164 ^{o}C; IR (KBr, cm^{-1}): 3445, 3197, 2943, 2224, 1673, 1503, 1045; MS (m/z): 243.94 $(M)^{+}$.

1-(4-Trifluoromethylbenzyl)piperidine-4-carboxamide (65). Synthesized as per the general procedure (E) using 4-trifluoromethylbenzyl bromide (**59**) (1.20 ml) to get compound (**65**) as a

white solid (4.2 gm, 93 %); Mp: 132-134 ^{o}C; IR (KBr, cm^{-1}): 3334, 3163, 2949, 2793, 1636, 1439, 1247, 932, 830; MS (m/z): 286.46 $(M)^{+}$.

General procedure F. Synthesis of 1-(substituted benzyl)piperidin-4-ylthiourea[104, 106] **(66-71).** A solution of bis(trifluoroacetoxy)iodobenzene (2.07 gm, 4.82 mM) in acetonitrile (8 ml) and water (4 ml) was added to 1-(substituted benzyl)piperidine-4-carboxamide (**60-65**). This mixture was heated overnight at 65 ^{o}C. After addition of water (10 ml) the mixture was cooled in an ice bath. Conc HCl (2 ml) was added and the mixture was washed twice with diethyl ether. The aqueous layer was concentrated *in vacuo*, and the residue was dissolved in water (40 ml). The resulting solution was saturated with solid potassium carbonate and the resulting mixture was extracted with dichloromethane. The combined organic extract was dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to get a yellow coloured oil of the amine. The amine was dissolved in dry dichloromethane and benzoyl isothiocyanate (0.72 ml, 4.11 mM) was added to this solution. The reaction mixture was stirred overnight at room temperature, the solvent removed and the residue so obtained was dissolved in THF/1N sodium hydroxide (1:1) and refluxed for 5 hr. The organic solvent was removed under reduced pressure to get the desired thioureas.

1-(Benzylpiperidin-4-yl)thiourea (66). Synthesized as per the general procedure (F) using 1-benzylpiperidine-4-carboxamide (**60**) (1 gm) to get compound (**66**) as a brown solid (0.64 gm, 58 %); Mp: 124-126 ^{o}C; IR (KBr, cm^{-1}): 3406, 3195, 1620, 1440, 795.

1-(4-Methylbenzyl)piperidin-4-ylthiourea (67). Synthesized as per the general procedure (F) using 1-(4-methylbenzyl)piperidine-4-carboxamide (**61**) (1 gm) to get compound (**67**) as a brown solid (0.78 gm, 71 %); Mp: 130-132 ^{o}C; IR (KBr, cm^{-1}): 3298, 3181, 2940, 1630, 1566, 1437.

1-(4-Methoxybenzyl)piperidin-4-ylthiourea (68). Synthesized as per the general procedure (F) using 1-(4-methoxybenzyl)piperidine-4-carboxamide (**62**) (1 gm) to get compound (**68**) as a white solid (0.89 gm, 79 %); Mp: 144-146 ^{o}C; IR (KBr, cm^{-1}): 3394, 3296, 2939, 1630, 1565, 1437, 1102.

1-(2-Chloro-4-fluorobenzyl)piperidin-4-ylthiourea (69). Synthesized as per the general procedure (F) using 1-(2-choro-4-flurobenzyl)piperidine-4-carboxamide (**63**) (1 gm) to get compound (**69**) as a white solid (0.77 gm, 71 %); Mp: 114-116 ^{o}C; IR (KBr, cm^{-1}): 3405, 3293, 2825, 1627, 1491, 840; MS (m/z): 301.17 $(M)^{+}$.

1-(4-Cyanobenzyl)piperidin-4-ylthiourea (70). Synthesized as per the general procedure (F) using 1-(4-cyanobenzyl)piperidine-4-carboxamide (**64**) (1 gm) to get compound (**70**) as a white solid (0.86 gm, 76 %); Mp: 151-153 ^{o}C; IR (KBr, cm^{-1}): 3405, 3163, 2230, 1686, 1564, 977; MS (m/z): 274.90 $(M)^{+}$.

1-(4-Trifluoromethylbenzyl)piperidin-4-ylthiourea (71). Synthesized as per the general procedure (F) using 1-(4-trifluoromethylbenzyl)piperidine-4-carboxamide (**65**) (1 gm) to get compound (**71**) as a semisolid (0.76 gm, 69 %); IR (KBr, cm^{-1}) : 3394, 3296, 2939, 1630, 1565, 1437, 1102, 830.

General procedure G. Synthesis of 1-(substituted benzyl)-*N*-[4,5-bis(4-substituted phenyl)thiazol-2-yl]piperidin-4-ylamine (73-82). Substituted 2-bromo-1,2-diphenyethanone[107, 108] (**72a-c**) (1 gm 3.29 mM) was dissolved in sufficient quantity of methanol in a 25 ml round bottom flask. 1-(Substituted benzyl)piperidin-4ylthiourea (**66-71**) (3.95 mM) and 3-4 drops of water were added into the reaction mixture and refluxed for 4-6 hrs. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured onto ice-cold water

and the resulting solution was basified with ammonia. The product so precipitated was filtered, dried and purified using column chromatograph using *n*-hexane: ethyl acetate (30 %) as the eluent.

1-Benzyl-*N*-[4,5-bis(*p*-tolyl)thiazol-2-yl]piperidin-4-ylamine (73). Synthesized as per the general procedure (G) using 1-benzylpiperidin-4-ylthiourea (**66**) (0.98 gm) and 2-bromo-1,2-bis(*p*-tolyl)ethanone (**72a**) (0.5 gm) to get compound (**73**) as a white solid (1.2 gm, 85 %); Mp: 158-160 ^{o}C; IR (KBr, cm^{-1}): 3396, 3197, 2944, 1534, 1208, 818; ^{1}H-NMR: 7.29-7.27 (d, 2H), 7.23-7.21 (d, 2H), 7.19-7.15 (m, 2H), 7.09-7.07 (m, 3H), 6.97-6.95 (m, 4H), 5.21-5.19 (d, 1H, *J* = 6.76 Hz), 3.42 (s, 2H), 3.30 (bs, 1H), 2.74-2.71 (d, 2H, *J* = 11.68 Hz), 2.33 (s, 3H), 2.22 (s, 3H), 2.11-2.06 (t, 2H, *J* = 10.48 Hz), 2.02-1.99 (d, 2H, *J* = 12.48 Hz), 1.54-1.49 (m, 2H); ^{13}C-NMR: 165.91, 145.61, 138.19, 137.08, 136.76, 132.71, 130.10, 129.25, 129.18, 128.84, 128.80, 128.28, 127.13, 119.93, 63.68, 52.96, 51.97, 32.20, 21.30, 21.23; LC-MS/MS: t_R 2.57 min, 454.21 (M+H); MS (m/z): 453.05 $(M)^+$; Anal. Calcd for $C_{29}H_{31}N_3S$: C, 76.78; H, 6.89; N, 9.26; Found: C, 76.64; H, 6.76; N, 9.35 %.

4-Methylbenzyl-*N*-[4,5-bis(*p*-tolyl)thiazol-2yl]piperidin-4-ylamine (74). Synthesized as per the general procedure (G) using 1-(4-methylbenzyl)piperidin-4ylthiourea (**67**) (0.86 gm) and 2-bromo-1,2-bis(*p*-tolyl)ethanone (**72a**) (0.5 gm) to get compound (**74**) as a white solid (0.98 gm, 63 %); Mp: 179-181 ^{o}C; IR (KBr, cm^{-1}): 3201, 2918, 2849, 1553, 1209, 818; ^{1}H-NMR: 7.37-7.35 (d, 2H, *J* = 8.0 Hz), 7.19-717 (d, 2H, *J* = 8.0 Hz), 7.17-7.15 (d, 2H, *J* = 8.0 Hz), 7.13-7.11 (d, 2H, *J* = 8.0 Hz), 7.05-7.03 (d, 4H, *J* = 8.0 Hz), 5.17-5.15 (d, 1H, *J* = 7.5 Hz), 3.47 (s, 2H), 3.38 (bs, 1H), 2.81-2.78 (d, 2H, *J* = 11.4 Hz), 2.33 (s, 3H), 2.31 (s, 6H), 2.18-2.13 (m, 2H), 2.10-2,07 (d, 2H, *J* = 12.9 Hz), 1.62-1.54 (m, 2H); ^{13}C-NMR: 165.84, 145.63, 137.06, 136.75, 135.01, 132.71, 130.10, 129.19, 128.95, 128.82, 119.96, 62.79, 52.96, 51.88, 32.23, 29.73, 14.15; LC-

MS/MS: t_R 5.97 min, 468.29 (M+H); MS (m/z): 467.17 $(M)^+$; Anal. Calcd for $C_{30}H_{33}N_3S$: C, 77.05; H, 7.11; N, 8.99; Found: C, 77.14; H, 7.26; N, 8.86 %.

1-Benzyl-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidin-4-ylamine (75). Synthesized as per the general procedure (G) using 1-benzylpiperidin-4-ylthiourea (**66**) (0.87gm) and 2-bromo-1,2-bis(4-chlorophenyl)ethanone (**72b**) (0.5 gm) to get compound (**75**) as a white solid (1.22 gm, 85 %); Mp: 169-171 ^oC; IR (KBr, cm^{-1}): 3394, 3191, 2934, 1560, 1330, 1089; ^1H-NMR: δ 7.32-7.31 (m, 3H), 7.25-7.23 (m, 2H), 7.17-7.14 (m, 6H), 7.08-7.07 (m, 2H), 5.18-5.16 (d, 1H, J = 7.2 Hz), 3.46 (s, 2H), 3.32 (bs, 1H), 2.78-2.75 (d, 2H, J = 11.6 Hz), 2.15-2.09 (t, 2H, J = 10.6 Hz), 2.05-2.01 (d, 2H, J = 13.5 Hz), 1.58-1.49 (m, 2H); ^{13}C-NMR: 166.30, 145.22, 138.00, 133.57, 133.51, 133.12, 131.11, 130.51, 130.23, 129.19, 128.92, 128.49, 128.30, 127.19, 119.30, 63.04, 51.90, 32.12; LC-MS/MS: t_R 2.53 min, 494.12 (M+H); MS (m/z): 494.62 $(M)^+$; Anal. Calcd for $C_{27}H_{25}Cl_2N_3S$: C, 65.58; H, 5.10; N, 8.50; Found: C, 65.41; H, 5.34; N, 8.57 %.

4-Methylbenzyl-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidin-4-ylamine (76). Synthesized as per the general procedure (G) using 1-(4-methylbenzyl)piperidin-4-ylthiourea (**67**) (0.91 gm) and 2-bromo-1,2-bis(4-chlorophenyl)ethanone (**72b**) (0.5 gm) to get compound (**76**) as a white solid (1.15 gm, 78 %); Mp: 182-184 ^oC; IR (KBr, cm^{-1}): 3182, 2919, 2827, 1551, 1090, 826; ^1H-NMR: 7.38-7.36 (d, 2H, J = 8.5 Hz), 7.25-7.23 (m, 5H), 7.22-7.20 (d, 2H, J = 8.2 Hz), 7.14-7.12 (m, 3H), 5.22-5.20 (d, 1H, J = 7.9 Hz), 3.49 (s, 2H), 3.39-3.37 (bs, 1H), 2.83-2.80 (d ,2H, J = 11.5 Hz), 2.34 (s, 3H), 2.19-2.14 (m, 2H), 2.11-2,07 (d, 2H, J = 12.8 Hz), 1.63-1.55 (m, 2H); ^{13}C-NMR: 166.29, 145.25, 136.79, 134.88, 133.60, 133.50, 133.12, 131.14, 130.51, 130.22, 129.17, 128.96, 128.91, 128.47, 119.30, 62.75, 53.05, 32.14, 29.72, 21.13; LC-MS/MS: t_R 2.56 min, 508.12 (M+H); MS (m/z): 507.15 $(M)^+$; Anal. Calcd for $C_{28}H_{27}Cl_2N_3S$: C, 66.13; H, 5.35; N, 8.26; Found: C, 66.02; H, 5.71; N, 8.42 %.

4-Methoxybenzyl-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidin-4-ylamine (77). Synthesized as per the general procedure (G) using 1-(4-methoxybenzyl)piperidin-4-yl thiourea (**68**) (0.97 gm) and 2-bromo-1,2-bis(4-chlorophenyl)ethanone (**72b**) (0.5 gm) to get compound (**77**) as a white solid (1.20 gm, 79 %); Mp: 138-140 ^{o}C; IR (KBr, cm^{-1}): 3398, 3207, 2928, 1565, 1242, 1088; ^{1}H-NMR: 7.49-7.47 (d, 2H, *J* = 7.8 Hz), 7.25-7.21 (m, 6H), 7.15-7.14 (d, 2H), 6.87-6.85 (d, 2H, *J* = 8.5 Hz), 5.27 (bs, 1H), 3.80 (s, 3H), 3.49-3.47 (s, 2H), 3.40 (bs, 1H), 2,85-2.83 (d ,2H, *J* = 11.4 Hz), 2.21-2.16 (m, 2H), 2.12-2.09 (d, 2H, *J* = 12.8 Hz), 1.66-1.58 (m, 2H); LC-MS/MS: t_R 2.59 min, 524.10 (M+H); MS (m/z): 523.13 $(M)^+$; Anal. Calcd for $C_{28}H_{27}Cl_2N_3OS$: C, 64.12; H, 5.19; N, 8.01; Found: C, 64.38; H, 5.10; N, 7.94 %.

4-Cyanobenzyl-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidin-4-ylamine (78). Synthesized as per the general procedure (G) using 1-(4-cyanobenzyl)piperidin-4-ylthiourea (**70**) (0.97 gm) and 2-bromo-1,2-bis(4-chlorophenyl)ethanone (**72b**) (0.5 gm) to get compound (**78**) as a white solid (1.13 gm, 75 %); Mp: 166-168 ^{o}C; IR (KBr, cm^{-1}): 3394, 3352, 2921, 2222, 1598, 1541, 1090; ^{1}H-NMR: 7.62-7.60 (d, 2H, *J* = 8.3 Hz), 7.46-7.44 (d, 2H, *J* = 8.1Hz), 7.38-7.36 (d, 2H, *J* = 8.3 Hz), 7.26-7.24 (d, 2H, *J* = 8.1 Hz), 7.23-7.21 (d, 2H, *J* = 8.1 Hz), 7.17-7.15 (d, 2H, *J* = 8.3Hz), 5,36 (bs, 1H), 3.57 (s, 2H), 3.43 (bs, 1H), 2.82-2.79 (d, 2H, *J* =11.6 Hz), 2,26-2.24 (m ,2H), 2.11-2.08 (m, 2H), 1.67-1.59 (m, 2H); LC-MS/MS: t_R 2.68 min, 521.22 (M+H); MS (m/z): 519.84 $(M)^+$; Anal. Calcd for $C_{28}H_{24}Cl_2N_4S$: C, 64.74; H, 4.66; N, 10.79; Found: C, 64.63; H, 4.38; N, 10.92 %.

1-(2-Chloro-4-fluorobenzyl)-*N*-[4,5-bis(4-chlorophenyl)thiazol-2-yl]piperidin-4-ylamine (79). Synthesized as per the general procedure (G) using 1-(2-chloro-4-fluorobenzyl) piperidin-4-ylthiourea (**69**) (1.05 gm) and 2-bromo-1,2-bis(4-chlorophenyl)ethanone (**72b**) (0.5 gm) to get compound (**79**) as a white solid (1.25 gm, 79 %); Mp: 151-153 ^{o}C; IR (KBr, cm-1): 3210, 2925,

2852, 1601, 1488, 828; ^{1}H-NMR: 7.50-7.44 (m, 1H), 7.37-7.35 (m, 2H), 7.25-7.21 (m, 4H), 7.18-7.16 (m, 2H), 7.12-7.09 (d, 1H), 6.99-6.94 (m, 1H), 5.31-5.30 (d, 1H, *J* = 6.7 Hz), 3.59 (s, 2H), 3.44 (bs, 1H), 2.86-2.83 (d, 2H, *J* = 11.7 Hz), 2.31-2.26 (m, 2H), 2.12-2.10 (d, 2H, *J* = 10.5 Hz), 1.65-1.56 (m, 2H); ^{13}C-NMR: 166.23, 162.68, 160.21, 145.16, 134.78, 134.68, 133.50, 131.11, 130.46, 129.49, 128.89, 128.45, 119.30, 116.80, 113.98, 58.48, 51.88, 31.56, 29.68; MS (m/z): 545.70 $(M)^{+}$; Anal. Calcd for $C_{27}H_{23}Cl_3FN_3OS$: C, 59.29; H, 4.24; N, 7.68; Found: C, 59.46; H, 4.69; N, 7.78 %.

4-Methylbenzyl-*N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidin-4-ylamine (80). Synthesized as per the general procedure (G) using 1-(4-methylbenzyl)piperidin-4-ylthiourea (**67**) (0.94 gm) and 2-bromo1,2-bis(4-methoxyphenyl)ethanone (**72c**) (0.5 gm) to get compound (**80**) as a white solid (1.02 gm, 68 %); Mp: 110-112 ^{o}C; IR (KBr, cm^{-1}): 3391, 2924, 2850, 1550, 1249, 1023, 808; ^{1}H-NMR: 7.54-7.52 (d, 1H, *J* = 8.4 Hz), 7.48-7.47 (d, 2H), 7.21-7.19 (d, 2H, *J* = 7.8 Hz), 7.14-7.12 (d, 3H, *J* = 8.0 Hz), 6.79-6.75 (m, 3H), 5.24 (bs, 1H), 3.85 (s, 3H), 3.73 (s, 3H), 3.52 (s, 2H), 3.39 (bs , 1H), 2.87-2.84 (d, 2H, *J* = 11.0 Hz), 2.30 (s, 3H), 2.28-2.12 (m, 2H), 2.10-2,072 (d, 2H), 1.67-1.60 (m, 2H); LC-MS/MS: t_R 5.92 min, 500.25 (M+H); MS (m/z): 499.58 $(M)^{+}$; Anal. Calcd for $C_{30}H_{33}N_3O_2S$: C, 72.11; H, 6.66; N, 8.41; Found: C, C, 72.32; H, 6.43; N, 8.52 %.

4-Methoxybenzyl-*N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidin-4-ylamine (81). Synthesized as per the general procedure (G) using 1-(4-methoxybenzyl)piperidin-4-yl thiourea (**68**) (1 gm) and 2-bromo-1,2-bis(4-methoxyphenyl)ethanone (**72c**) (0.5 gm) to get compound (**81**) as a white solid (1.02 gm, 73 %); Mp: 135-137 ^{o}C; IR (KBr, cm^{-1}): 3398, 3207, 2928, 1565, 1242, 1088; ^{1}H-NMR: 7.49-7.48 (d, 1H), 7.40-7.36 (m, 2H), 7.26-7.22 (m, 3H), 7.15-7.12 (d, 1H, *J* = 8.5 Hz), 6.87-6.85 (d, 2H, *J* = 8.5 Hz), 6.79-6.75 (m, 3H), 5.27 (bs, 1H), 3.88 (s, 3H),

3.80 (s, 6H), 3.49 (s, 2H), 3.39 (bs ,1H), 2.85-2.83 (d, 2H), 2.28-2.16 (m, 2H), 2.13-2.10 (d, 2H), 1.66-1.61 (m, 2H); ^{13}C-NMR: 165.81, 159.15, 154.89, 145.64, 133.92, 132.49, 130.82, 130.60, 129.61, 128.99, 127.60, 126.83, 117.19, 114.40, 113.80, 111.82, 62.02, 56.25, 55.29, 52.28, 51.49, 31.94; LC-MS/MS: t_R 2.34 min, 516.21 (M+H); MS (m/z): 515.01 $(M)^+$; Anal. Calcd for $C_{30}H_{33}F_3N_3O_2S$: C, 69.87; H, 6.45; N, 8.15; Found: C, 69.71; H, 6.57; N, 8.07 %.

4-Trifluoromethoxybenzyl-*N*-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]piperidin-4-ylamine (82). Synthesized as per the general procedure (G) using 1-(4-trifluromethylbenzyl)piperidin-4-ylthiourea (**71**) (1.12 gm) and 2-bromo-1,2-bis(4-methoxyphenyl)ethanone (**72c**) (0.5 gm) to get compound (**82**) as a white solid (1.25 gm, 76 %); Mp: 102-103 ^{o}C; IR (KBr, cm^{-1}): 3384, 3210, 2926, 2799, 1550, 1250, 833; ^{1}H-NMR: 7.49-7.48 (d, 1H), 7.40-7.36 (m, 2H), 7.29-7.27 (m, 2H), 7.15-7.12 (m, 1H), 7.03-6.97 (m, 3H), 6.81-6.75 (m, 3H), 5.27 (bs, 1H), 3.86 (s, 3H), 3.79 (s, 3H), 3.48 (s, 2H), 3.39 (bs ,1H), 2.82-2.79 (d, 2H), 2.20-2.17 (d, 2H), 2.14-2.10 (t, 2H), 1.64-1.55 (m, 2H); ^{13}C-NMR: 165.94, 160.85, 159.06, 154.88, 145.69, 133.92, 132.49, 130.69, 129.20, 113.63, 111.83, 62.51, 56.25, 52.91, 51.83, 32.65, 29.72; MS (m/z): 553.40 $(M)^+$; Anal. Calcd for $C_{30}H_{30}F_3N_3O_2S$: C, C, 65.22; H, 5.57; N, 7.41 %.

BIOLOGICAL

***In vitro* AChE and BuChE inhibition assays**. The enzyme inhibition activity of the test compounds was performed adopting the method of Ellman et al[68] as described in our earlier report.[109] AChE from human erythrocytes and BuChE from equine serum, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB-Ellman's reagent), acetylthiocholine iodide (ATCI) and butyrylthiocholine iodide (BTCI) were purchased from Sigma. Tacrine and donepezil were used as reference compounds (sigma). All the experiments were conducted in 50 mM Tris-HCl buffer

at *p*H 8. Five different concentrations (0.001–100 μM) of each test compound were used to determine the enzyme inhibition activity. Briefly, 50 μl of AChE (0.22 U ml^{-1}) or 50 μl of BuChE (0.06 U ml^{-1}) and 10 μl of the test or standard compound were incubated in 96-well plates at room temperature for 30 min. Further, 30 μl of the substrate viz. ATCI (15 mM) or BTCI (15 mM) was added and the solution was incubated for additional 30 min. Finally 160 μl of DTNB (1.5 mM) was added to it and the absorbance was measured at 415 nm wavelength using microplate reader 680 XR (BIO-RAD, India). The IC_{50} value was calculated from the absorbance obtained for various concentrations of the test and the standard compounds. The IC_{50} value depicts the concentration of the drug resulting in 50 % inhibition of the enzyme activity. All the determinations were performed in triplicate and at least in three independent runs.

The enzyme kinetic study was done to determine the mechanism of AChE inhibition by compound (**44**) in a similar manner as described above. Relatively low concentrations of substrate (ATCI; 0.1-1 mM) were incubated with AChE in absence and presence of different concentrations of compound (**44**; 0.05-0.4 μM) and the activities were measured at different times. V_{max} and K_m values of Michaelis-Menten kinetics were estimated by nonlinear regression from the substrate-velocity curves using Graph Pad Prism 5. Linear regression was used to calculate the Lineweaver-Burk plots.[39, 69]

Thioflavin T (ThT) assay. Inhibition of AChE-induced $A\beta_{1-42}$ aggregation was evaluated using thioflavin T (ThT) fluorescence assay as described earlier.[75, 76] $A\beta_{1-42}$ (Sigma) was dissolved in phosphate buffer saline (PBS) and was further diluted with 0.215 M sodium phosphate buffer (*p*H 8). Test compounds were dissolved in DMSO and diluted further with 0.215 M sodium phosphate buffer (*p*H 8). Briefly, 2 μl of $A\beta_{1-42}$ was incubated with 16 μl AChE in the presence of 2 μl of the test compound to obtain final concentrations of 50 μM of $A\beta_{1-42}$, 230 μM of AChE

and 10 μM of the test compound. The mixture was incubated at room temperature for 24 hr. After incubation, 180 μl of 20 μM ThT (prepared in 50 mM glycine-NaOH buffer; *p*H 8.5) was added. Fluorescence intensity of the solution was read at 442 nm excitation and 490 nm emission wavelengths using Synergy HTX fluorescence microplate reader. The percentage inhibition of the AChE-induced $A\beta_{1-42}$ aggregation was calculated using the following formula: 100-($IF_i/IF_o \times 100$), where IF_i and IF_o are fluorescence intensities in the presence and absence of the test compound respectively. Each assay was conducted in triplicate and each experiment was repeated at least three times independently.

***In vitro* blood-brain barrier permeation assay.** To predict the possible *in vivo* blood-brain barrier (BBB) permeation of the selected hybrid diarylthiazole-benzylpiperidine derivatives, a parallel artificial membrane permeation assay of the blood-brain barrier (PAMPA-BBB) was performed as described by Di et al.[77-79] Commercial drugs and dodecane were obtained from Sigma. Porcine brain lipid (PBL) was purchased from Avanti Polar Lipids. The donor microplates (PVDF membrane, pore size 0.45 μm) and the acceptor microplates were obtained from Millipore. The acceptor microplate was filled with 200 μl of phosphate buffer saline (PBS):ethanol (70:30) and the filter surface of the donor microplate was impregnated with 4 μl of porcine brain lipid in dodecane (20 mg ml^{-1}). The test compounds (5 mg ml^{-1}) were dissolved in DMSO and diluted with PBS/ethanol (70:30) to get a final concentration of 100 μg ml^{-1}. 200 μl of the solution was filled in the donor well and the donor plate was carefully placed on the acceptor plate to form a sandwich, keeping it undisturbed for 120 min at 25°C. After the incubation period, the donor plate was removed and the concentration of the test compounds in the acceptor wells was determined using UV spectroscopy. Each sample was analysed at five different wavelengths, in four wells, and at least in three independent runs. The results are

expressed as mean ± SEM. To validate the experiment, nine standard quality commercial drugs of known BBB permeability[31] were given the same treatment as described above, their permeability (P_e) determined and the experimentally determined P_e values regressed against the P_e(ref.) to obtain a linear correlation.

Cell culture. The human neuroblastoma SH-SY5Y cell line was obtained from National Centre for Cell Science (NCCS) (Pune, India). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 1 mM glutamine, 50 U ml^{-1} penicillin and 50 µg ml^{-1} streptomycine (reagents from Gibco) at 37°C in a humidified incubator at 5 % CO_2. All the cells used in the study were of low passage number (< 15).

Determination of cell viability and neuroprotection. To determine the cytotoxicity of the selected test compounds, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed. SH-SY5Y cells were seeded in 96-well plate at density of 5×10^4 cells per well. After 24 hr, the medium was replaced with relatively higher concentrations of test compounds (40 µM and 80 µM) for another 24 hr at 37°C. After incubation period, the cell viability was determined using MTT assay. In another set of experiments, the test compounds were assessed for their ability to protect SH-SY5Y cells against oxidative damage induced by H_2O_2.[78, 80] The cells were exposed to the test compounds at relatively lower concentrations (5 µM, 10 µM and 20 µM) and incubated for 2 hr. After the incubation period, the test compounds were replaced with a medium containing cytotoxic insult, i.e. H_2O_2 (100 µM)[78] which was left for an additional 24 hr period. Thereafter, the cell viability was assessed using MTT assay. Briefly, the medium was replaced with 80 µl of fresh medium and 20 µl of MTT (0.5 mg ml^{-1}, final concentration; Sigma) in PBS. After 4 hr, MTT was removed and the crystals of formazan were dissolved in DMSO. Formazan concentrations were quantified at 570 nm with 630 nm

reference wavelengths using a microplate reader 680 XR (BIO-RAD, India). Percentage protection against H_2O_2 insult was calculated by considering absorbance of the control cells as 100 % of the cell viability.

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) assay. The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay is based on the reduction of DPPH, a purple colored stable free radical. DPPH gets paired and reduced to a yellow colored diphenylpicrylhydrazine by anti-oxidants. Thus the assay measures an electron (or hydrogen atom) donating ability and hence provides assessment of anti-oxidant activity of a compound attributed to its free radical scavenging ability.[70, 81] The spectrophotometric DPPH assay was carried out as described earlier.[110] Concentrations of the selected test derivatives that showed promising neuroprotective effects against H_2O_2 insult were selected for the DPPH assay. In brief, 10 µl of a test compound (10 and 20 µM, in Tris-HCl buffer-*p*H 7.4) was mixed with 20 µl of DPPH (from 10 mM stock, in methanol) (Hi-Media) in the 96 well plate. Finally, the volume was adjusted to 200 µl using methanol. After 30 sec incubation at room temperature and protection from light, the absorbance was read at 520 nm wavelength using a microplate reader 680 XR (BIO-RAD, India). The free radical scavenging activity was determined as the reduction percentage (RP) of DPPH using the equation: $RP= 100[(A_0 - A_C)/A_o]$, where A_0 is the untreated DPPH absorbance and A_C is the absorbance value for added sample concentration $_C$. Ascorbic acid was used as the standard anti-oxidant.

ROS estimation using primary rat hippocampal culture. The intracellular ROS level was estimated in primary rat hippocampal neuronal culture using 2′,7′-dichlorofluorescin diacetate (DCFH-DA) assay.[31, 86] The most potent hybrid diarylthiazole-benzylpiperidine (**44**) was assessed for its ROS scavenging ability. $A\beta_{1\text{-}42}$ was used to induce ROS generation in the

primary rat hippocampal neuronal culture which was prepared as described earlier.[111] Briefly, hippocampal tissues were dissected out from 18 days old rat foetuses, washed with cold HBSS, minced and incubated in 0.1 % trypsin for 30 min at 37°C. A single cell suspension was prepared by trituration. Cells were plated in 96 well plates at a density of 5×10^4 viable cells ml^{-1}. Cells were grown as neurons in serum free neurobasal medium (Invitrogen) containing N-2 supplement (1 %) (Invitrogen), B-27 supplement (2 %) (Invitrogen) and antibiotic-antimycotic solution (1 %) (Sigma). Cultures of the neurons were placed in a humidified incubator at 37°C and 5 % CO_2. Medium was changed every 3 days. Formation of small proliferating neurons started after 1 week, and mature neurons were observed after the twentieth day. On the twenty first day, the neuronal cells were exposed to the compound (**44**) (10 μM and 20 μM) for 2 hr, followed by $A\beta_{1\text{-}42}$ (10 μM; Sigma)[112] treatment for 24 hr. Later on, the cells were incubated with 10 μM DCFH-DA in PBS (Sigma) at 37ºC for 30 min. After rinsing with PBS, the plates were read at 485 nm excitation and 530 nm emission wavelengths using the Synergy HTX multi-mode microplate reader. The fluorescence intensities in the presence and absence of the test/ standard compound were compared using appropriate controls and the percentage inhibition of ROS generation was determined.

Assessment of apoptosis by flow cytometry. Flow cytometric assessment of apoptosis was performed using Annexin V-FITC and propidium iodide (PI) staining. Briefly, the rat hippocampal neuronal cells were seeded in six well plate which were exposed to $A\beta_{1\text{-}42}$ (10 μM) for 24 hr. To determine anti-apoptotic potential of the test compound, cells were pre-treated with compound (**44**) (20 μM) for 2 hr followed by $A\beta_{1\text{-}42}$ treatment. After the incubation period of 24 hr, cells were harvested and suspended in 500 μl Annexin V binding buffer. Later, 5 μl Annexin V-FITC (BD Biosciences) and 10 μl PI (Sigma) were added and incubated with the cells for 5

min in the dark. Untreated cells were used as the control for double staining. The stained cells were directly analysed using a FAC Scan flow cytometer.

Behavioural study. The experiments were performed in adult male Swiss Albino mice weighing 20-25 gm. The study protocol was approved by IAEC (Institutional Animal Ethics Committee) and the experiments were performed as per CPCSEA (Committee for the purpose of Supervision of Experiments on Animals) guidelines (Approval No. MSU/IAEC/2014-15/1401). Scopolamine hydrochloride and donepezil hydrochloride were purchased from Sigma.

Scopolamine rodent model was adopted to induce AD-like phenotype especially amnesia. Mice were divided into four experimental groups of six animals each as per the given treatment: (i) vehicle, (ii) scopolamine, (iii) scopolamine plus donepezil and (iv) scopolamine plus compound (**44**). Scopolamine hydrochloride (1.4 mg kg^{-1})[92, 93] was dissolved in saline and administered intraperitoneally (i.p.) to all the groups except the vehicle-treated control group that received an equal volume of saline. Donepezil hydrochloride was suspended in 0.5 % sodium carboxymethyl cellulose (CMC-Na) and was given at a dose of 5 mg kg^{-1} orally 30 min prior to administration of scopolamine.[64, 113] Equivalent dose of the test compound (**44**) corresponding to donepezil was also administered orally as a suspension in CMC-Na, 30 min prior to the scopolamine treatment. All these treatments were continued for nine consecutive days. During the last five days of treatment period, spatial learning and memory was assessed using the Morris Water Maze (MWM) test.[64, 92]

The maze consisting of a circular pool (65 cm diameter; 30 cm height) was filled with water (26±1°C) up to 20 cm depth. The inside walls of the pool were painted black. The pool was divided into four quadrants and the escape platform was placed 1 cm below the water surface in the middle of any one quadrant. Experiments on the individual animals were carried

out to determine the time required by the animal reaching the hidden platform (i.e. escape latency time-ELT) and the number of platform area crossings during 2 min of the training session to assess the spatial learning and memory. All the experiments were carried out in a sound proof room and supervised by a blind observer.

Neurochemical analysis. At the end of MWM test, the animals were sacrificed, whole brains isolated from the skull and homogenized in glass teflon homogenizer in 12.5 mM sodium phosphate buffer (*p*H 7). The homogenates were centrifuged at 15,000 rpm for 15 min at 4°C. The supernatants were utilized for estimations of different biochemical parameters.

The cholinergic biomarkers AChE and BuChE, were estimated in the mice brain using Ellman's method.[68, 92] 100 µl of the supernatant was incubated with 2.7 ml of phosphate buffer and 100 µl of freshly prepared ATCI or BTCI (15 mM) for 5 min. Finally, 100 µl of DTNB (1.5 mM) was added and the absorbance was read at 415 nm wavelength spectrophotometrically.

MDA, an indicator of lipid peroxidation, was estimated using thiobarbituric acid reacting substance (TBARS) method as described earlier.[93, 114] MDA reacted with thiobarbituric acid in acidic medium at high temperature and formed a red complex TBARS which was read spectrophotometrically. Briefly, 200 µl of the supernatant was mixed with 1 ml of 50 % trichloroacetic acid in 0.1 M HCl and 1 ml of 26 mM thiobarbituric acid. After vortex mixing, samples were heated at 95°C for 20 min. Later on the samples were centrifuged at 15,000 rpm for 10 min and the supernatants were read at 532 nm wavelength.

Catalase (CAT) is an enzyme mediating breakdown of H_2O_2, a toxic form of oxygen metabolite into oxygen and water. CAT activity was determined following the method described by Sinha[115] Briefly, 100 µl of the supernatant was mixed with 150 µl of 0.01 M phosphate buffer (*p*H 7). Reaction was started by addition of 250 µl of H_2O_2 (0.16 M), the medium incubated at

37°C for one min and the reaction was stopped by addition of 1 ml of dichromate:acetic acid reagent (5 % $K_2Cr_2O_7$:glacial acetic acid; 1:3; v/v). The reaction mixture was immediately kept on a boiling water bath for 15 min that resulted in development of a green color. Finally, the mixture was read at 570 nm wavelength spectrophotometrically.

ICV rat model of AD. Adult male Wister rats (200-250 gm) were divided into four experimental groups of six animals each as per the given treatment: (a) vehicle, (b) $A\beta_{1-42}$, (c) $A\beta_{1-42}$ plus donepezil and (d) $A\beta_{1-42}$ plus compound (**44**). The animals were anaesthetized with ketamine (100 mg/kg, i.p.) and xylazine (30 mg/kg, i.p.) and mounted on a stereotaxic apparatus (Stoelting, USA). All the groups (except the vehicle-treated control group which received equal volume of normal saline) were injected with 4 µl of $A\beta_{1-42}$ (2 µM/µl in normal saline) unilaterally at the following co-ordinates: -4.0 mm anteroposterior, -2.5 mm mediolateral and -3.5 mm dorsoventral from Bregma. Compound (**44**) was administered at an equivalent dose of donepezil (5 mg/kg, p.o. in 0.1 % CMC) to the respective experimental group animals for 15 consecutive days after five days of surgical recovery.[116, 117]

Y maze test. Y-maze test was adopted for the assessment of immediate working memory.[118] The test was carried out during the last five days of the treatment period in the animals which underwent ICV injection of $A\beta_{1-42}$. Each animal from the treated groups was kept at the end of any one arm of the maze and allowed to explore all the three arms. The sequence and the number of arm entries were recorded visually for each rat over a period of 5 min. An actual "alteration" was defined as entries in all three arms in consecutive choices (i.e. ABC, BCA or CAB but not BAB). Repeat arm entry was considered as a sign of memory impairment. The number of arm entries indicated locomotor activity. The "alteration score" for each rat was calculated using the equation:

$$\% \text{ Alternation} = [(\text{Number of alternations}) / (\text{Total arm entries} - 2)] \times 100$$

Western blot analysis. Hippocampal regions from different experimental rat brains were homogenized in tissue lysis buffer supplemented with protease and phosphatase inhibitors (Sigma). Homogenized samples were sonicated for 5 s, and centrifuged at 4 °C at 15,000 rpm for 30 min. Equal amounts of proteins (100 µg) were loaded on 10 % Tris-glycine gel. Membranes were blocked for 1 hr at room temperature using Tris-buffered saline/Tween-20 (TBST) (50 mM Tris-HCl, 150 mM NaCl, *p*H 7.4, 1 % Tween-20) containing 5 % non-fat-dried milk. Membranes were incubated overnight at 4°C with rabbit anti-$A\beta_{1-42}$ (1:500, Santa Cruz), goat anti-*p*-Tau (1:500, Santa Cruz), rabbit anti-cleaved caspase-3 (active) (1:500, Sigma) and rabbit anti-cleaved poly (ADP-ribose) polymerase-1 (PARP) (1:1000, Cell Signalling) primary antibodies. After incubation, membranes were washed thrice with TBST and incubated for 1 hr with HRP–conjugated secondary antibody (Sigma). Immunoreactive proteins were detected using the ECL Plus chemiluminescent kit (Invitrogen) according to the manufacturer's instructions. Protein bands were quantified using Scion Image for Windows.

Acute toxicity study. A total of twenty male Swiss Albino mice (20-25 gm) were used to determine acute toxicity of the test compound (**44**). During the experiment, animals were maintained with free access of food and water *ad libitum*. Compound (**44**) was suspended in 0.5 % CMC-Na and given orally to the divided experimental groups (at 0, 677, 1333 and 2000 mg kg^{-1}, n = 5 per group). After administration of the test compound, the animals were observed continuously for the first 4 hr for any abnormal behaviour and mortality. Later on the animals were observed intermittently for the next 24 hr and occasionally for 14 consecutive days after administration of compound (**44**). After 14 days, the animals were sacrificed and macroscopically examined for possible damage to the heart, liver and kidneys.[31, 39]

Pharmacokinetic studies. Healthy male Wistar rats (200-250 gm, n=4) were used for the pharmacokinetic analysis. The animals were fasted overnight with free-access to water and pre-dosed blood samples were withdrawn. Compound (**44**) suspended in Na-CMC was administered orally to the animals at a dose of 5 mg kg^{-1}. After administration, the animals were anesthetized with ether and blood samples were collected from retro-orbital plexus at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48, 60 and 72 hr into the heparinised tubes. The tubes were centrifuged at 1000×g for 10 min at 4 ºC to separate the plasma. Later, 400 µl of acetonitrile was added to 100 µl of the rat plasma to extract the compound (**44**). The mixture was vortex-mixed for 5 min and centrifuged for 10 min at 1000×g. The organic layer was taken in separate Eppendorf tube and evaporated to dryness under a stream of nitrogen gas. The residue was reconstituted with 100 µl of the mobile phase, vortexed briefly and transferred to pre-labelled autosampler vials. From there, 20 µl of the sample was injected into HPLC system with photo-diode array detector (Waters Corporation, USA) for analysis. The samples were analysed using the mobile phase consisting of 0.05 M phosphate buffer (*p*H 4.8) (27 %) and acetonitrile (63 %) at a flow rate of 1 ml/min using a Sunfire® C18 column (4.6 mm×150 mm, particle size 5µm). The pharmacokinetic parameters were calculated using the plasma concentration data of the test compound (**44**) at different time points using extravascular analysis of non-compartmental model.

Docking studies. Molecular modeling studies were performed using Maestro 9.0 software of Schrödinger, LLC, New York, NY, USA.[119] The structures of the compounds were drawn and cleaned up in Maestro 9.0 software. Energy minimizations of the structures were carried out using OPLS 2005 force field in LigPrep tool of Maestro 9.0.[120] These minimized structures were used further for molecular modeling purposes. The molecular docking studies were carried out

by using standard option Glide 5.5 in Maestro 9.0.[121] The 3D crystallographic structure for AChE (PDB Code: 4EY7) was obtained from RCSB Protein Data Bank[122] and prepared for docking with protein preparation wizard within Schrödinger by removing of all water molecules. The grid was generated over the active site considering the ligand. The generated grid was validated by re-docking the co-crystallized ligand again in the active site. Docking calculations for the minimized 3D ligand structures were performed in extraprecision (XP) mode within the active sites of the receptor structure. The docking protocol was validated by observing the interactions of the docked conformer of donepezil in the active site of AChE which was found to be in consonance with the reported literature.[71]

ASSOCIATED CONTENT

Supporting Information

^{1}H-NMR spectra of compounds (**16-20**, **22-31** and **33-52**), ^{13}C-NMR spectra of compounds (**16-20**, **22-26**, **28-31**, **33-38**, **40-43**, **44-46**, **49-51**, and **81-82**), molecular modelling data and experimental validation of PAMPA-BBB assay.

AUTHOR INFORMATION

Corresponding Author

Phone: +91-0265-2434187. E-mail: mryadav11@yahoo.co.in

Author contributions

M.S. and J.M. contributed equally to this work.

Notes

The authors declare no competing financial interests.

ACKNOWLEDGEMENT

MS, NA and MKS were supported by UGC-BSR-RFMS fellowship. JM, thanks UGC project (F.No. 41-716/2012-SR). The authors acknowledge Mr Pradeep Sarkate for providing LS-MS-MS data. Analytical facilities provided by Dr. Vikram Sarabhai Research Centre, The Maharaja Sayajirao University of Baroda, Vadodara, India are thankfully acknowledged. The authors are thankful to Mr. Karan Mittal for his generous help in carrying out pharmacokinetic analysis.

ABBREVIATIONS USED

AD, Alzheimer's disease; ACh, acetylcholine; AChE, Acetylcholinesterase; ROS, reactive oxygen species; RNS, reactive nitrogen species; BOP, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DPPH, Diphenyl-1-picrylhydrazyl; DCFH-DA, 2′,7′-dichlorofluorescine diacetate.

REFERENCES

1. Alzheimer's Disease International, World Alzheimer Report. http://www.alz.co.uk/research/files/WorldAlzheimerReport2015ExecutiveSummary.pdf (accessed April 24, 2016).
2. Hebert, L. E.; Scherr, P. A.; Bienias, J. L.; Bennett, D. A.; Evans, D. A. Alzheimer disease in the US population: prevalence estimates using the 2000 census. *Arch. Neurol.* **2003,** *60*, 1119-1122.
3. Wancata, J.; Musalek, M.; Alexandrowicz, R.; Krautgartner, M. Number of dementia sufferers in Europe between the years 2000 and 2050. *Eur. Psychiatry* **2003,** *18*, 306-313.
4. Goedert, M.; Spillantini, M. G. A century of Alzheimer's disease. *Science* **2006,** *314*, 777-781.
5. Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. Multi-target-directed ligands to combat neurodegenerative diseases. *J. Med. Chem.* **2008,** *51*, 347-372.
6. Harel, M.; Sussman, J. L.; Krejci, E.; Bon, S.; Chanal, P.; Massoulie, J.; Silman, I. Conversion of acetylcholinesterase to butyrylcholinesterase: modeling and mutagenesis. *Proc. Natl. Acad. Sci. U S A* **1992,** *89*, 10827-10831.
7. Savini, L.; Gaeta, A.; Fattorusso, C.; Catalanotti, B.; Campiani, G.; Chiasserini, L.; Pellerano, C.; Novellino, E.; McKissic, D.; Saxena, A. Specific targeting of acetylcholinesterase and butyrylcholinesterase recognition sites. Rational design of novel, selective, and highly potent cholinesterase inhibitors. *J. Med. Chem.* **2003,** *46*, 1-4.

8. Eskander, M. F.; Nagykery, N. G.; Leung, E. Y.; Khelghati, B.; Geula, C. Rivastigmine is a potent inhibitor of acetyl- and butyrylcholinesterase in Alzheimer's plaques and tangles. *Brain Res.* **2005,** *1060*, 144-152.
9. Giacobini, E. Cholinesterase inhibitors: From the Calabar bean to Alzheimer therapy. In *Cholinesterases and cholinesterase inhibitors*. Martin Dunitz Ltd.: London, **2000**; pp 181-226.
10. Mesulam, M.; Guillozet, A.; Shaw, P.; Quinn, B. Widely spread butyrylcholinesterase can hydrolyze acetylcholine in the normal and Alzheimer brain. *Neurobiol. Dis.* **2002,** *9*, 88-93.
11. Mesulam, M. M.; Guillozet, A.; Shaw, P.; Levey, A.; Duysen, E. G.; Lockridge, O. Acetylcholinesterase knockouts establish central cholinergic pathways and can use butyrylcholinesterase to hydrolyze acetylcholine. *Neuroscience* **2002,** *110*, 627-639.
12. De Strooper, B.; Vassar, R.; Golde, T. The secretases: enzymes with therapeutic potential in Alzheimer disease. *Nat. Rev. Neurol.* **2010,** *6*, 99-107.
13. Sisodia, S. S.; Koo, E. H.; Beyreuther, K.; Unterbeck, A.; Price, D. L. Evidence that beta-amyloid protein in Alzheimer's disease is not derived by normal processing. *Science* **1990,** *248*, 492-495.
14. Weidemann, A.; Paliga, K.; Durrwang, U.; Reinhard, F. B.; Schuckert, O.; Evin, G.; Masters, C. L. Proteolytic processing of the Alzheimer's disease amyloid precursor protein within its cytoplasmic domain by caspase-like proteases. *J. Biol. Chem.* **1999,** *274*, 5823-5829.
15. Seubert, P.; Oltersdorf, T.; Lee, M. G.; Barbour, R.; Blomquist, C.; Davis, D. L.; Bryant, K.; Fritz, L. C.; Galasko, D.; Thal, L. J.; Lieberburg, I.; Schenk, D. B. Secretion of beta-amyloid precursor protein cleaved at the amino terminus of the beta-amyloid peptide. *Nature* **1993,** *361*, 260-263.

16. Golde, T. E.; Estus, S.; Younkin, L. H.; Selkoe, D. J.; Younkin, S. G. Processing of the amyloid protein precursor to potentially amyloidogenic derivatives. *Science* **1992,** *255*, 728-730.

17. Haass, C.; Koo, E. H.; Mellon, A.; Hung, A. Y.; Selkoe, D. J. Targeting of cell-surface beta-amyloid precursor protein to lysosomes: alternative processing into amyloid-bearing fragments. *Nature* **1992,** *357*, 500-503.

18. Walsh, D. M.; Selkoe, D. J. A beta oligomers - a decade of discovery. *J. Neurochem.* **2007,** *101*, 1172-1184.

19. Conde, S. β-amyloid peptide as a target for treatment of Alzheimer's disease. *Expert Opin. Ther. Pat.* **2002,** *12*, 503-512.

20. Nunomura, A.; Moreira, P. I.; Takeda, A.; Smith, M. A.; Perry, G. Oxidative RNA damage and neurodegeneration. *Curr. Med. Chem.* **2007,** *14*, 2968-2975.

21. Su, B.; Wang, X.; Nunomura, A.; Moreira, P. I.; Lee, H. G.; Perry, G.; Smith, M. A.; Zhu, X. Oxidative stress signaling in Alzheimer's disease. *Curr. Alzheimer Res.* **2008,** *5*, 525-532.

22. Zhu, X.; Raina, A. K.; Perry, G.; Smith, M. A. Alzheimer's disease: the two-hit hypothesis. *Lancet Neurol.* **2004,** *3*, 219-226.

23. Wang, J. Z.; Liu, F. Microtubule-associated protein tau in development, degeneration and protection of neurons. *Prog. Neurobiol.* **2008,** *85*, 148-175.

24. Smith, M. A.; Taneda, S.; Richey, P. L.; Miyata, S.; Yan, S. D.; Stern, D.; Sayre, L. M.; Monnier, V. M.; Perry, G. Advanced Maillard reaction end products are associated with Alzheimer disease pathology. *Proc. Natl. Acad. Sci. U S A* **1994,** *91*, 5710-5714.

25. Smith, M. A.; Hirai, K.; Hsiao, K.; Pappolla, M. A.; Harris, P. L.; Siedlak, S. L.; Tabaton, M.; Perry, G. Amyloid-beta deposition in Alzheimer transgenic mice is associated with oxidative stress. *J. Neurochem.* **1998,** *70*, 2212-2215.

26. Rottkamp, C. A.; Raina, A. K.; Zhu, X.; Gaier, E.; Bush, A. I.; Atwood, C. S.; Chevion, M.; Perry, G.; Smith, M. A. Redox-active iron mediates amyloid-beta toxicity. *Free Radical Biol. Med.* **2001,** *30*, 447-450.

27. Hensley, K.; Carney, J. M.; Mattson, M. P.; Aksenova, M.; Harris, M.; Wu, J. F.; Floyd, R. A.; Butterfield, D. A. A model for beta-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer disease. *Proc. Natl. Acad. Sci. U S A* **1994,** *91*, 3270-3274.

28. Butterfield, D. A.; Stadtman, E. R. Protein oxidation processes in aging brain. *Adv. Cell Aging Gerontol.* **1997,** *2*, 161-191.

29. Butterfield, D. A.; Poon, H. F.; St Clair, D.; Keller, J. N.; Pierce, W. M.; Klein, J. B.; Markesbery, W. R. Redox proteomics identification of oxidatively modified hippocampal proteins in mild cognitive impairment: insights into the development of Alzheimer's disease. *Neurobiol. Dis.* **2006,** *22*, 223-232.

30. Castegna, A.; Aksenov, M.; Aksenova, M.; Thongboonkerd, V.; Klein, J. B.; Pierce, W. M.; Booze, R.; Markesbery, W. R.; Butterfield, D. A. Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part I: creatine kinase BB, glutamine synthase, and ubiquitin carboxy-terminal hydrolase L-1. *Free Radical Biol. Med.* **2002,** *33*, 562-571.

31. Lu, C.; Guo, Y.; Yan, J.; Luo, Z.; Luo, H. B.; Yan, M.; Huang, L.; Li, X. Design, synthesis, and evaluation of multitarget-directed resveratrol derivatives for the treatment of Alzheimer's disease. *J. Med. Chem.* **2013,** *56*, 5843-5859.

32. Kannan, K.; Jain, S. K. Oxidative stress and apoptosis. *Pathophysiology* **2000,** *7*, 153-163.

33. Behl, C. Apoptosis and Alzheimer's disease. *J. Neural. Transm.* **2000,** *107*, 1325-1344.

34. Kitamura, Y.; Taniguchi, T.; Shimohama, S. Apoptotic cell death in neurons and glial cells: implications for Alzheimer's disease. *Jpn. J. Pharmacol.* **1999,** *79*, 1-5.

35. Shimohama, S. Apoptosis in Alzheimer's disease--an update. *Apoptosis* **2000,** *5*, 9-16.

36. Young, A. B. Four decades of neurodegenerative disease research: how far we have come! *J. Neurosci.* **2009,** *29*, 12722-12728.

37. Bartolucci, C.; Haller, L. A.; Jordis, U.; Fels, G.; Lamba, D. Probing Torpedo californica acetylcholinesterase catalytic gorge with two novel bis-functional galanthamine derivatives. *J. Med. Chem.* **2009,** *53*, 745-751.

38. Watkins, P. B.; Zimmerman, H. J.; Knapp, M. J.; Gracon, S. I.; Lewis, K. W. Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. *JAMA* **1994,** *271*, 992-998.

39. Luo, Z.; Sheng, J.; Sun, Y.; Lu, C.; Yan, J.; Liu, A.; Luo, H. B.; Huang, L.; Li, X. Synthesis and evaluation of multi-target-directed ligands against Alzheimer's disease based on the fusion of donepezil and ebselen. *J. Med. Chem.* **2013,** *56*, 9089-9099.

40. Korcsmaros, T.; Szalay, M. S.; Bode, C.; Kovacs, I. A.; Csermely, P. How to design multi-target drugs. *Expert Opin. Drug Discovery* **2007,** *2*, 799-808.

41. Csermely, P.; Agoston, V.; Pongor, S. The efficiency of multi-target drugs: the network approach might help drug design. *Trends Pharmacol. Sci.* **2005,** *26*, 178-182.

42. Morphy, J. R. and Harris, C. J. The challenges of multi-target lead optimization. *Designing Multi-Target Drugs.* The Royal Society of Chemistry, Cambridge, UK **2012**, pp 141-154.

43. Bolognesi, M. L.; Bartolini, M.; Tarozzi, A.; Morroni, F.; Lizzi, F.; Milelli, A.; Minarini, A.; Rosini, M.; Hrelia, P.; Andrisano, V.; Melchiorre, C. Multitargeted drugs discovery: balancing anti-amyloid and anticholinesterase capacity in a single chemical entity. *Bioorg. Med. Chem. Lett.* **2011,** *21*, 2655-2658.

44. Huang, L.; Lu, C.; Sun, Y.; Mao, F.; Luo, Z.; Su, T.; Jiang, H.; Shan, W.; Li, X. Multitarget-directed benzylideneindanone derivatives: anti-beta-amyloid (Abeta) aggregation, antioxidant, metal chelation, and monoamine oxidase B (MAO-B) inhibition properties against Alzheimer's disease. *J. Med. Chem.* **2012,** *55*, 8483-8492.

45. Nepovimova, E.; Uliassi, E.; Korabecny, J.; Pena-Altamira, L. E.; Samez, S.; Pesaresi, A.; Garcia, G. E.; Bartolini, M.; Andrisano, V.; Bergamini, C.; Fato, R.; Lamba, D.; Roberti, M.; Kuca, K.; Monti, B.; Bolognesi, M. L. Multitarget drug design strategy: quinone–tacrine hybrids designed to block amyloid-β aggregation and to exert anticholinesterase and antioxidant effects. *J. Med. Chem.* **2014,** *57*, 8576-8589.

46. Guzior, N. Recent development of multifunctional agents as potential drug candidates for the treatment of Alzheimer's disease. *Curr. Med. Chem.* **2015,** *22*, 373-404.

47. Yerdelen, K. O.; Koca, M.; Anil, B.; Sevindik, H.; Kasap, Z.; Halici, Z.; Turkaydin, K.; Gunesacar, G. Synthesis of donepezil-based multifunctional agents for the treatment of Alzheimer's disease. *Bioorg. Med. Chem. Lett.* **2015,** *25*, 5576-5582.

48. Zhang, X.; Wang, J.; Hong, C.; Luo, W.; Wang, C. Design, synthesis and evaluation of genistein-polyamine conjugates as multi-functional anti-Alzheimer agents. *Acta Pharm. Sin. B* **2015,** *5*, 67-73.

49. Bajda, M.; Jonczyk, J.; Malawska, B.; Czarnecka, K.; Girek, M.; Olszewska, P.; Sikora, J.; Mikiciuk-Olasik, E.; Skibinski, R.; Gumieniczek, A.; Szymanski, P. Synthesis, biological evaluation and molecular modeling of new tetrahydroacridine derivatives as potential multifunctional agents for the treatment of Alzheimer's disease. *Bioorg. Med. Chem.* **2015,** *23*, 5610-5618.

50. Zanaletti, R.; Bettinetti, L.; Castaldo, C.; Cocconcelli, G.; Comery, T.; Dunlop, J.; Gaviraghi, G.; Ghiron, C.; Haydar, S. N.; Jow, F.; Maccari, L.; Micco, I.; Nencini, A.; Scali, C.; Turlizzi, E.; Valacchi, M. Discovery of a novel alpha-7 nicotinic acetylcholine receptor agonist series and characterization of the potent, selective, and orally efficacious agonist 5-(4-acetyl[1,4]diazepan-1-yl)pentanoic acid [5-(4-methoxyphenyl)-1H-pyrazol-3-yl] amide (SEN15924, WAY-361789). *J. Med. Chem.* **2012,** *55*, 4806-4823.

51. Gouda, M. A.; Berghot, M. A.; Abd El-Ghani, G. E.; Khalil, A. M. Synthesis and antimicrobial activities of some new thiazole and pyrazole derivatives based on 4,5,6,7-tetrahydrobenzothiophene moiety. *Eur. J. Med. Chem.* **2010,** *45*, 1338-1345.

52. Raslan, M.; Khalil, M. Heterocyclic synthesis containing bridgehead nitrogen atom: Synthesis of 3-[(2H)-2-oxobenzo [b] pyran-3-yl]-s-triazolo [3, 4-b]-1, 3, 4-thiadiazine and thiazole derivatives. *Heteroat. Chem.* **2003,** *14*, 114-120.

53. Milinkevich, K. A.; Ye, L.; Kurth, M. J. Synthesis of 5-(thiazol-5-yl)-4,5-dihydroisoxazoles from 3-chloropentane-2,4-dione. *J. Comb. Chem.* **2008,** *10*, 521-525.

54. Yurtta; , L.; Ozkay, Y.; Gencer, H. K.; Acar, U. Synthesis of some new thiazole derivatives and their biological activity evaluation. *J. Chem.* **2015,** *2015*, 1-7.

55. Nagahama, K.; Matsunaga, Y.; Kawachi, M.; Ito, K.; Tanaka, T.; Hori, Y.; Oka, H.; Takei, M. Acotiamide, a new orally active acetylcholinesterase inhibitor, stimulates gastrointestinal motor activity in conscious dogs. *Neurogastroenterol. Motil.* **2012,** *24*, 566-574.

56. Tack, J.; Janssen, P. Acotiamide (Z-338, YM443), a new drug for the treatment of functional dyspepsia. *Expert Opin. Invest. Drugs* **2011,** *20*, 701-712.

57. Yurttas, L.; Kaplancikli, Z. A.; Ozkay, Y. Design, synthesis and evaluation of new thiazole-piperazines as acetylcholinesterase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2013,** *28*, 1040-1047.

58. Rahim, F.; Javed, M. T.; Ullah, H.; Wadood, A.; Taha, M.; Ashraf, M.; Khan, M. A.; Khan, F.; Mirza, S.; Khan, K. M. Synthesis, molecular docking, acetylcholinesterase and butyrylcholinesterase inhibitory potential of thiazole analogs as new inhibitors for Alzheimer disease. *Bioorg. Chem.* **2015,** *62*, 106-116.

59. Fengchao Jiand , P. Z., Jianguo Chen , Yue Wang, Baoshuai Cao ,Jia Yan. 2-aminothiazole derivative, preparation method, and use. **2014**, EP 2682390 A1, Jan 8, 2014.

60. Ozkay, Y.; Yurttas, L.; Mohsen, U. A.; Sever, B.; Hussein, W.; Ozturk, O.; Saglik, B. N.; Acar, U.; Erdogan, O. N.; Pekbag, A. Study on thiazolyl-hydrazone derivatives as acetylcholinesterase inhibitors. *Marmara Univ. Saglik Bilimleri Enst. Derg.* **2014,** *4*, 38.

61. Turan-Zitouni, G.; Ozdemir, A.; Kaplancikli, Z. A.; Altintop, M. D.; Temel, H. E.; Çiftçi, G. A. Synthesis and biological evaluation of some thiazole derivatives as new cholinesterase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2013,** *28*, 509-514.

62. Kurt, B. Z.; Gazioglu, I.; Sonmez, F.; Kucukislamoglu, M. Synthesis, antioxidant and anticholinesterase activities of novel coumarylthiazole derivatives. *Bioorg. Chem.* **2015,** *59*, 80-90.

63. Irannejad, H.; Amini, M.; Khodagholi, F.; Ansari, N.; Tusi, S. K.; Sharifzadeh, M.; Shafiee, A. Synthesis and in vitro evaluation of novel 1,2,4-triazine derivatives as neuroprotective agents. *Bioorg. Med. Chem.* **2010,** *18*, 4224-4230.

64. Sinha, A.; Tamboli, R. S.; Seth, B.; Kanhed, A. M.; Tiwari, S. K.; Agarwal, S.; Nair, S.; Giridhar, R.; Chaturvedi, R. K.; Yadav, M. R. Neuroprotective role of novel triazine derivatives by activating wnt/beta catenin signaling pathway in rodent models of Alzheimer's disease. *Mol. Neurobiol.* **2015,** *52*, 638-652.

65. Kamal, A.; Balakrishna, M.; Nayak, V. L.; Shaik, T. B.; Faazil, S.; Nimbarte, V. D. Design and synthesis of imidazo[2,1-b]thiazole-chalcone conjugates: microtubule-destabilizing agents. *ChemMedChem* **2014,** *9*, 2766-2780.

66. Dash, B.; Patra, M.; Mohapatra, P. Synthesis of some thiazole schiff-bases and their derivatives. *J. Ind. Chem. Soc.* **1985,** *62*, 460-464.

67. Nimbarte, V. D.; Murtuza, H.; Phaniraj, S.; Shrivastava, S.; Naidu, V.; Kumar, N. S.; Atcha, K. R. Design, synthesis and biological evaluation of 4-(1-(4 (sulphanilamide) phenyl)-3-(methyl)-1H-pyrazol-5-yl) dine urea and N-acyl derivatives as a soluble epoxide hydrolase inhibitors. *Med. Chem. Res.* **2014,** *23*, 2178-2197.

68. Ellman, G. L.; Courtney, K. D.; Andres, V., Jr.; Feather-Stone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961,** *7*, 88-95.

69. Wang, Y.; Guan, X. L.; Wu, P. F.; Wang, C. M.; Cao, H.; Li, L.; Guo, X. J.; Wang, F.; Xie, N.; Jiang, F. C. Multifunctional mercapto-tacrine derivatives for treatment of age-related neurodegenerative diseases. *J. Med. Chem.* **2012,** *55*, 3588-3592.

70. Chen, Y.; Sun, J.; Fang, L.; Liu, M.; Peng, S.; Liao, H.; Lehmann, J.; Zhang, Y. Tacrine-ferulic acid-nitric oxide (NO) donor trihybrids as potent, multifunctional acetyl- and butyrylcholinesterase inhibitors. *J. Med. Chem.* **2012,** *55*, 4309-4321.

71. Cheung, J.; Rudolph, M. J.; Burshteyn, F.; Cassidy, M. S.; Gary, E. N.; Love, J.; Franklin, M. C.; Height, J. J. Structures of human acetylcholinesterase in complex with pharmacologically important ligands. *J. Med. Chem.* **2012,** *55*, 10282-10286.

72. Alvarez, A.; Bronfman, F.; Perez, C. A.; Vicente, M.; Garrido, J.; Inestrosa, N. C. Acetylcholinesterase, a senile plaque component, affects the fibrillogenesis of amyloid-beta-peptides. *Neurosci. Lett.* **1995,** *201*, 49-52.

73. Alvarez, A.; Opazo, C.; Alarcon, R.; Garrido, J.; Inestrosa, N. C. Acetylcholinesterase promotes the aggregation of amyloid-beta-peptide fragments by forming a complex with the growing fibrils. *J. Mol. Biol.* **1997,** *272*, 348-361.

74. Inestrosa, N. C.; Alvarez, A.; Perez, C. A.; Moreno, R. D.; Vicente, M.; Linker, C.; Casanueva, O. I.; Soto, C.; Garrido, J. Acetylcholinesterase accelerates assembly of amyloid-beta-peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. *Neuron* **1996,** *16*, 881-891.

75. Kwon, Y. E.; Park, J. Y.; No, K. T.; Shin, J. H.; Lee, S. K.; Eun, J. S.; Yang, J. H.; Shin, T. Y.; Kim, D. K.; Chae, B. S.; Leem, J. Y.; Kim, K. H. Synthesis, in vitro assay, and molecular modeling of new piperidine derivatives having dual inhibitory potency against acetylcholinesterase and Abeta1-42 aggregation for Alzheimer's disease therapeutics. *Bioorg. Med. Chem.* **2007,** *15*, 6596-6607.

76. Mohamed, T.; Zhao, X.; Habib, L. K.; Yang, J.; Rao, P. P. Design, synthesis and structure-activity relationship (SAR) studies of 2,4-disubstituted pyrimidine derivatives: dual activity

as cholinesterase and Abeta-aggregation inhibitors. *Bioorg. Med. Chem.* **2011,** *19*, 2269-2281.

77. Di, L.; Kerns, E. H.; Fan, K.; McConnell, O. J.; Carter, G. T. High throughput artificial membrane permeability assay for blood-brain barrier. *Eur. J. Med. Chem.* **2003,** *38*, 223-232.
78. Porcal, W.; Hernandez, P.; Gonzalez, M.; Ferreira, A.; Olea-Azar, C.; Cerecetto, H.; Castro, A. Heteroarylnitrones as drugs for neurodegenerative diseases: synthesis, neuroprotective properties, and free radical scavenger properties. *J. Med. Chem.* **2008,** *51*, 6150-6159.
79. Fernandez-Bachiller, M. I.; Perez, C.; Gonzalez-Munoz, G. C.; Conde, S.; Lopez, M. G.; Villarroya, M.; Garcia, A. G.; Rodriguez-Franco, M. I. Novel tacrine-8-hydroxyquinoline hybrids as multifunctional agents for the treatment of Alzheimer's disease, with neuroprotective, cholinergic, antioxidant, and copper-complexing properties. *J. Med. Chem.* **2010,** *53*, 4927-4937.
80. Blat, D.; Weiner, L.; Youdim, M. B.; Fridkin, M. A Novel iron-chelating derivative of the neuroprotective peptide NAPVSIPQ shows superior antioxidant and antineurodegenerative capabilities. *J. Med. Chem.* **2008,** *51*, 126-134.
81. Kedare, S. B.; Singh, R. P. Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol.* **2011,** *48*, 412-422.
82. Greicius, M. D.; Krasnow, B.; Boyett-Anderson, J. M.; Eliez, S.; Schatzberg, A. F.; Reiss, A. L.; Menon, V. Regional analysis of hippocampal activation during memory encoding and retrieval: fMRI study. *Hippocampus* **2003,** *13*, 164-174.
83. Hitti, F. L.; Siegelbaum, S. A. The hippocampal CA2 region is essential for social memory. *Nature* **2014,** *508*, 88-92.
84. Hengartner, M. O. The biochemistry of apoptosis. *Nature* **2000,** *407*, 770-776.

85. Butterfield, D. A. Amyloid beta-peptide (1-42)-induced oxidative stress and neurotoxicity: implications for neurodegeneration in Alzheimer's disease brain. A review. *Free Radical Res.* **2002,** *36*, 1307-1313.

86. Sai, Y.; Wu, Q.; Le, W.; Ye, F.; Li, Y.; Dong, Z. Rotenone-induced PC12 cell toxicity is caused by oxidative stress resulting from altered dopamine metabolism. *Toxicol. In Vitro* **2008,** *22*, 1461-1468.

87. Roth, K. A. Caspases, apoptosis, and Alzheimer disease: causation, correlation, and confusion. *J. Neuropathol. Exp. Neurol.* **2001,** *60*, 829-838.

88. Dickson, D. W. Apoptotic mechanisms in Alzheimer neurofibrillary degeneration: cause or effect? *J. Clin. Invest.* **2004,** *114*, 23-27.

89. Muirhead, K. E.; Borger, E.; Aitken, L.; Conway, S. J.; Gunn-Moore, F. J. The consequences of mitochondrial amyloid beta-peptide in Alzheimer's disease. *Biochem. J.* **2010,** *426*, 255-270.

90. Yao, M.; Nguyen, T. V.; Pike, C. J. Beta-amyloid-induced neuronal apoptosis involves c-Jun N-terminal kinase-dependent downregulation of Bcl-w. *J. Neurosci.* **2005,** *25*, 1149-1158.

91. Chen, J.; Long, Y.; Han, M.; Wang, T.; Chen, Q.; Wang, R. Water-soluble derivative of propolis mitigates scopolamine-induced learning and memory impairment in mice. *Pharmacol. Biochem. Behav.* **2008,** *90*, 441-446.

92. Lee, M. R.; Yun, B. S.; Park, S. Y.; Ly, S. Y.; Kim, S. N.; Han, B. H.; Sung, C. K. Anti-amnesic effect of Chong-Myung-Tang on scopolamine-induced memory impairments in mice. *J. Ethnopharmacol.* **2010,** *132*, 70-74.

93. Goverdhan, P.; Sravanthi, A.; Mamatha, T. Neuroprotective effects of meloxicam and selegiline in scopolamine-induced cognitive impairment and oxidative stress. *Int. J. Alzheimer's Dis.* **2012,** *2012*, 974013.

94. Cetin, F.; Yazihan, N.; Dincer, S.; Akbulut, G. The effect of intracerebroventricular injection of beta amyloid peptide (1-42) on caspase-3 activity, lipid peroxidation, nitric oxide and NOS expression in young adult and aged rat brain. *Turk. Neurosurg.* **2013,** *23*, 144-150.

95. Aguzzi, A.; O'Connor, T. Protein aggregation diseases: pathogenicity and therapeutic perspectives. *Nat. Rev. Drug Discovery* **2010,** *9*, 237-248.

96. Lee, D. H.; Park, T.; Kim, H. W. Induction of apoptosis by disturbing mitochondrial-membrane potential and cleaving PARP in Jurkat T cells through treatment with acetoxyscirpenol mycotoxins. *Biol. Pharm. Bull.* **2006,** *29*, 648-654.

97. Oliver, F. J.; de la Rubia, G.; Rolli, V.; Ruiz-Ruiz, M. C.; de Murcia, G.; Murcia, J. M. Importance of poly(ADP-ribose) polymerase and its cleavage in apoptosis. Lesson from an uncleavable mutant. *J. Biol. Chem.* **1998,** *273*, 33533-33539.

98. Berger, N. A. Poly(ADP-ribose) in the cellular response to DNA damage. *Radiat. Res.* **1985,** *101*, 4-15.

99. Chaitanya, G. V.; Steven, A. J.; Babu, P. P. PARP-1 cleavage fragments: signatures of cell-death proteases in neurodegeneration. *Cell Commun. Signaling* **2010,** *8*, 31.

100. Adamczyk, A.; Jesko, H.; Strosznajder, R. P. Alzheimer's disease related peptides affected cholinergic receptor mediated poly(ADP-ribose) polymerase activity in the hippocampus. *Folia Neuropathol.* **2005,** *43*, 139-142.

101. Strosznajder, J. B.; Jesko, H.; Strosznajder, R. P. Effect of amyloid beta peptide on poly(ADP-ribose) polymerase activity in adult and aged rat hippocampus. *Acta Biochim. Pol.* **2000,** *47*, 847-854.

102. Sairanen, T.; Szepesi, R.; Karjalainen-Lindsberg, M. L.; Saksi, J.; Paetau, A.; Lindsberg, P. J. Neuronal caspase-3 and PARP-1 correlate differentially with apoptosis and necrosis in ischemic human stroke. *Acta Neuropathol.* **2009,** *118*, 541-552.

103. Armarego, W. L. F.; Chai C. L. L. Prification of biochemicals and related products. In *Purification Of Laboratory Chemicals* 5th Ed. Elsevier Science: Burlington, MA. **2003**, pp 500-577.

104. Shum, P. W.; Peet, N. P.; Weintraub, P. M.; Le, T. B.; Zhao, Z.; Barbone, F.; Cashman, B.; Tsay, J.; Dwyer, S.; Loos, P. C.; Powers, E. A.; Kropp, K.; Wright, P. S.; Bitonti, A.; Dumont, J.; Borcherding, D. R. The design and synthesis of purine inhibitors of CDK2. III. *Nucleosides Nucleotides Nucleic Acids* **2001,** *20*, 1067-1078.

105. Contreras, J. M.; Rival, Y. M.; Chayer, S.; Bourguignon, J. J.; Wermuth, C. G. Aminopyridazines as acetylcholinesterase inhibitors. *J. Med. Chem.* **1999,** *42*, 730-741.

106. Stieber, F.; Hellmuth, K.; Waldmann, H.; Mazitschek, R.; Giannis, A. 4-(Hetero-)aryl substituted (Thia/Oxa/Pyra)zoles for inhibitor TIE-2. WO 03/062215A1. July 31, **2003**.

107. Schoening, K. U. Hartwig, J. Preparation process for atorvastatin, an inhibitor of HMG-CoA reductase, process for the preparation of the key intermediate 2-[2-(4- fluorophenyl)-2-oxo-1-phenylethyl]-4-methyl-3-oxopentanoic acid phenylamide. PCT Int. WO 2003004457 A2. Jan. 26, **2003**.

108. Rynbrandt, R. H.; Nishizawa, E. E.; Balogoyen, D. P.; Mendoza, A. R.; Annis, K. A. Synthesis and platelet aggregation inhibitory activity of 4,5-bis(aryl)-2-substituted-thiazoles. *J. Med. Chem.* **1981,** *24*, 1507-1510.

109. Kanhed, A. M.; Sinha, A.; Machhi, J.; Tripathi, A.; Parikh, Z. S.; Pillai, P. P.; Giridhar, R.; Yadav, M. R. Discovery of isoalloxazine derivatives as a new class of potential anti-Alzheimer agents and their synthesis. *Bioorg. Chem.* **2015,** *61*, 7-12.

110. Mathew, M.; Subramanian, S. In vitro screening for anti-cholinesterase and antioxidant activity of methanolic extracts of ayurvedic medicinal plants used for cognitive disorders. *PloS One* **2014,** *9*, e86804.

111. Kaech, S.; Banker, G. Culturing hippocampal neurons. *Nat. Protoc.* **2006,** *1*, 2406-2415.

112. Sanchez, M.; Gastaldi, L.; Remedi, M.; Caceres, A.; Landa, C. Rotenone-induced toxicity is mediated by Rho-GTPases in hippocampal neurons. *Toxicol. Sci.* **2008,** *104*, 352-361.

113. Kim, M.-S.; Jeon, W. K.; Lee, K. W.; Park, Y. H.; Han, J.-S. Ameliorating effects of ethanol extract of fructus mume on scopolamine-induced memory impairment in mice. *Evid. Based Complement. Alternat. Med.* **2015,** *2015*, 102734.

114. Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **1979,** *95*, 351-358.

115. Sinha, A. K. Colorimetric assay of catalase. *Anal. Biochem.* **1972,** *47*, 389-394.

116. Colaianna, M.; Tucci, P.; Zotti, M.; Morgese, M. G.; Schiavone, S.; Govoni, S.; Cuomo, V.; Trabace, L. Soluble beta amyloid(1-42): a critical player in producing behavioural and biochemical changes evoking depressive-related state? *Br. J. Pharmacol.* **2010,** *159*, 1704-1715.

117. Tucci, P.; Mhillaj, E.; Morgese, M. G.; Colaianna, M.; Zotti, M.; Schiavone, S.; Cicerale, M.; Trezza, V.; Campolongo, P.; Cuomo, V.; Trabace, L. Memantine prevents memory consolidation failure induced by soluble beta amyloid in rats. *Front. Behav. Neurosci.* **2014,** *8*, 332.

118. Yan, J. J.; Cho, J. Y.; Kim, H. S.; Kim, K. L.; Jung, J. S.; Huh, S. O.; Suh, H. W.; Kim, Y. H.; Song, D. K. Protection against beta-amyloid peptide toxicity in vivo with long-term administration of ferulic acid. *Br. J. Pharmacol.* **2001,** *133*, 89-96.

119. *Maestro, version 9.0*, Schrodinger, LLC, New York, NY, USA **2009**.

120. *LigPrep. version 2.3*, Schrodinger, LLC, . New York, NY, **2009**.

121. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem.* **2004,** *47*, 1739-1749.

122. Protein data bank. www.rcsb.org/pdb/home/home.do. Accessed on Sept. 09, **2015**.

Captions

Figure 1. AChE inhibitors (**1**-**4**) used for the management of AD.

Figure 2. Designing of a novel series of compounds as multi-target-directed potential anti-Alzheimer's agents.

Figure 3. Kinetic study of the mechanism of AChE inhibition by compound (**44**). Lineweaver-Burk reciprocal plots of the AChE initial velocity at increasing substrate concentrations (0.1-1 mM) in the absence and presence of **44** (0.05-0.4 μM) are shown.

Figure 4. Orientation of compound (**44**) in the active sites of AChE.

Figure 5. *In vitro* ROS scavenging and anti-apoptotic potential of compound (**44**) against $A\beta_{1-42}$ (10 μM) insult in primary rat hippocampal neuronal culture. (A) Percentage inhibition of ROS generation as assessed by DCFH-DA assay. (B) Flow cytometric assessment of apoptosis using Annexin V-FITC and PI staining. Cells in the lower left quadrant are viable (Annexin V-FITC-/PI-). Cells in the lower right quadrant are early apoptotic (Annexin V-FITC+/PI-) and those in the upper right quadrant are late apoptotic or necrotic (Annexin V-FITC+/PI+). Data are expressed as mean ± SEM. ###$p<0.001$ vs. control cells. *** $p<0.00$ vs. $A\beta_{1-42}$-treated control cells. C=control cells.

Figure 6. Compound (**44**) improved spatial learning and memory in scopolamine-treated mice through anti-ChE and anti-oxidant activities. In MWM test, scopolamine (1.4 mg kg-1, i.p.) increased (A) ELT while (B) reduced number of platform area crossings. This pattern was significantly reversed by **44** similar to donepezil at 5 mg kg-1. (C) AChE and (D) BuChE levels elevated by scopolamine treatment were significantly attenuated by **44**. Oxidative stress parameters are represented as (E) MDA and (F) CAT levels. Altered MDA and CAT levels after scopolamine treatment were significantly reversed by treatment with compound (**44**). Data are expressed as mean ± SEM (n=6). ###$p<0.001$, ## $p<0.01$, # $p<0.05$ vs. vehicle-treated control group.*** $p<0.001$, ** $p<0.01$, * $p<0.05$ vs. scopolamine-treated control group. C=vehicle-treated control group.

Figure 7. Compound (**44**) improved immediate working memory in rats which received ICV injection of $A\beta_{1-42}$ in Y maze test. (A) Percentage of spontaneous alterations and (B) total number

of arm entries, as recorded by a blind observer. Data are expressed as mean±SEM (n=6). ###p<0.001, ## p<0.01, # p<0.05 vs. vehicle-treated control group.*** p<0.001, ** p<0.01, * p<0.05 vs. scopolamine-treated control group. C=vehicle-treated control group.

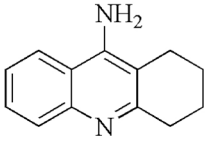
Figure 8. *In vivo* neuroprotective and anti-apoptotic potential of compound (**44**). (A) The expression of $A\beta_{1-42}$, p-Tau, cleaved-caspase-3 and cleaved-PARP was assessed by Western blot analysis in the hippocampal region of rat brain which was given ICV injection of $A\beta_{1-42}$. Densitometric analysis revealed attenuation of (B) $A\beta_{1-42}$, (C) p-Tau, (D) cleaved-caspase-3 and (E) cleaved-PARP levels by compound (**44**) which were elevated by $A\beta_{1-42}$ toxicity. Data are expressed as mean±SEM (n=6). ###p<0.001, ## p<0.01, # p<0.05 vs. vehicle-treated control group.*** p<0.001, ** p<0.01, * p<0.05 vs. $A\beta_{1-42}$-treated control group. C=vehicle-treated control group. D=donepezil-treated group.

Figure 9. Mean plasma concentration vs. time curve of single oral dose (5 mg/kg) of compound (**44**) in rats. Error bars represent the standard deviation of the mean (n=4).

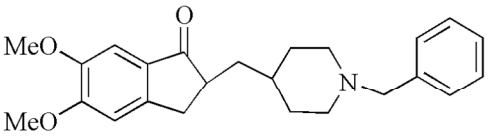
Scheme 1. Synthesis of 1-substituted benzyl-*N*-[4,5-bis(substituted phenyl)thiazol-2-yl]piperidine-4- carboxamides (16-41)

Scheme 2. Synthesis of *N*-[(1-substitued benzyl)piperidin-4-ylmethyl]-4,5-bis(substituted phenyl)-2-ylamines (42-52)

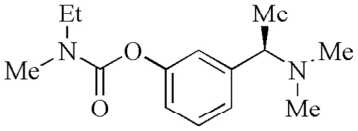
Scheme 3. Synthesis of 1-(substituted benzyl)-*N*-[4,5-bis(substituted phenyl)thiazol-2-yl]piperidin-4-ylamines (73-82)



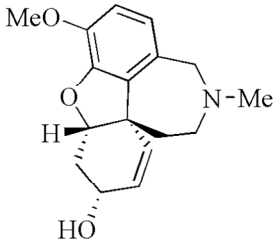
Tacrine **(1)**



Donepezil **(2)**



Rivastigmine **(3)**



Galantamine **(4)**

Figure 1. AChE inhibitors (1-4) used for the management of AD.
133x66mm (300 x 300 DPI)

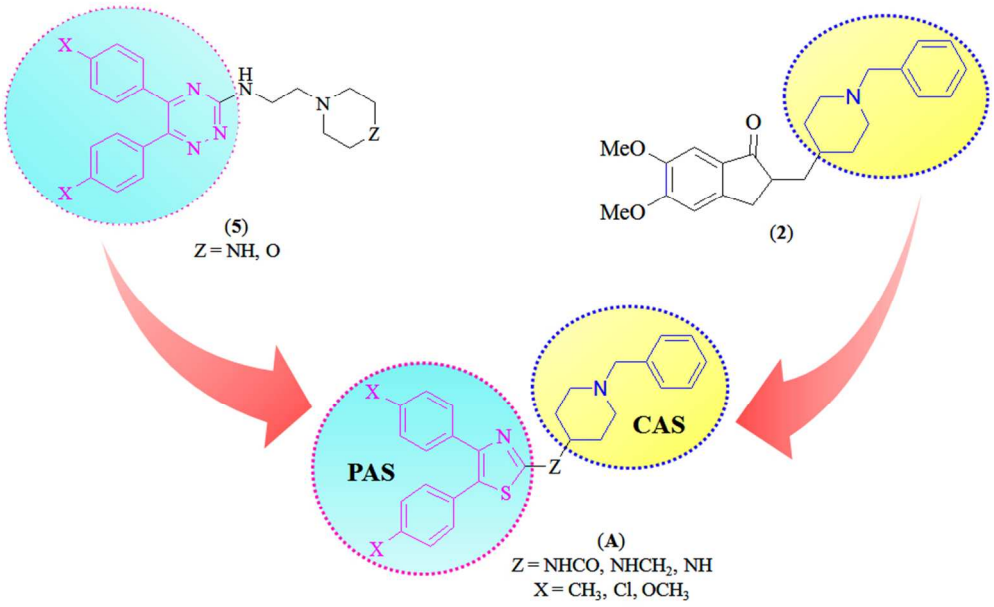


Figure 2. Designing of a novel series of compounds as multi-target-directed potential anti-Alzheimer's agents.
262x162mm (96 x 96 DPI)

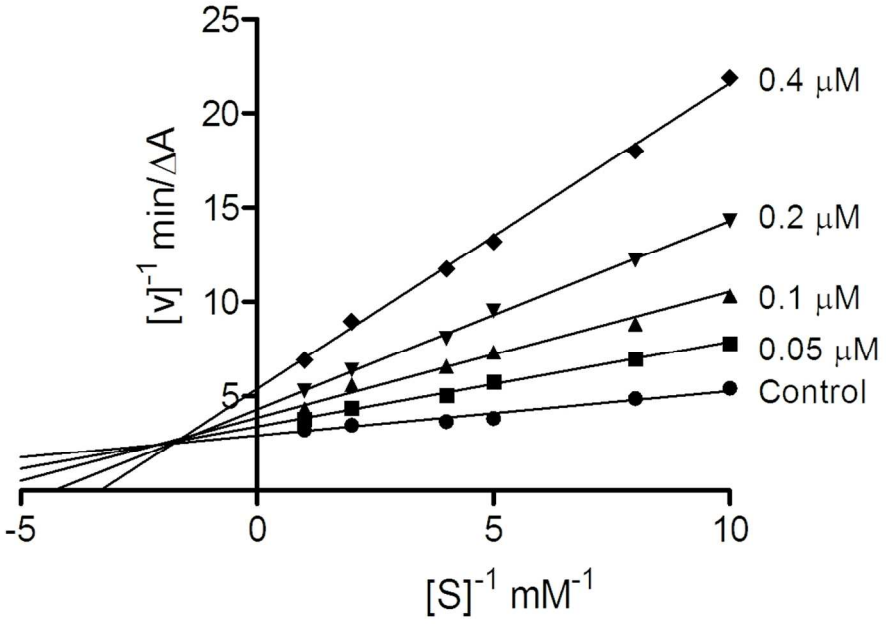


Figure 3. Kinetic study of the mechanism of AChE inhibition by compound (44). Lineweaver-Burk reciprocal plots of the AChE initial velocity at increasing substrate concentrations (0.1-1 mM) in the absence and presence of 44 (0.05-0.4 μM) are shown.
107x75mm (300 x 300 DPI)

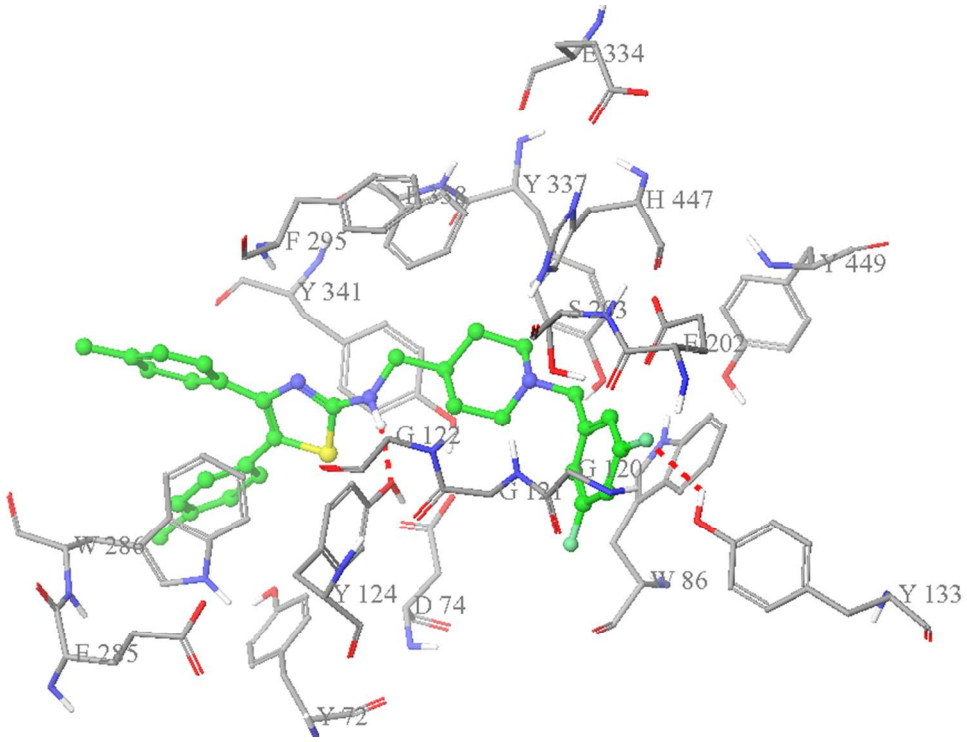


Figure 4. Orientation of compound (44) in the active sites of AChE.
238x174mm (96 x 96 DPI)

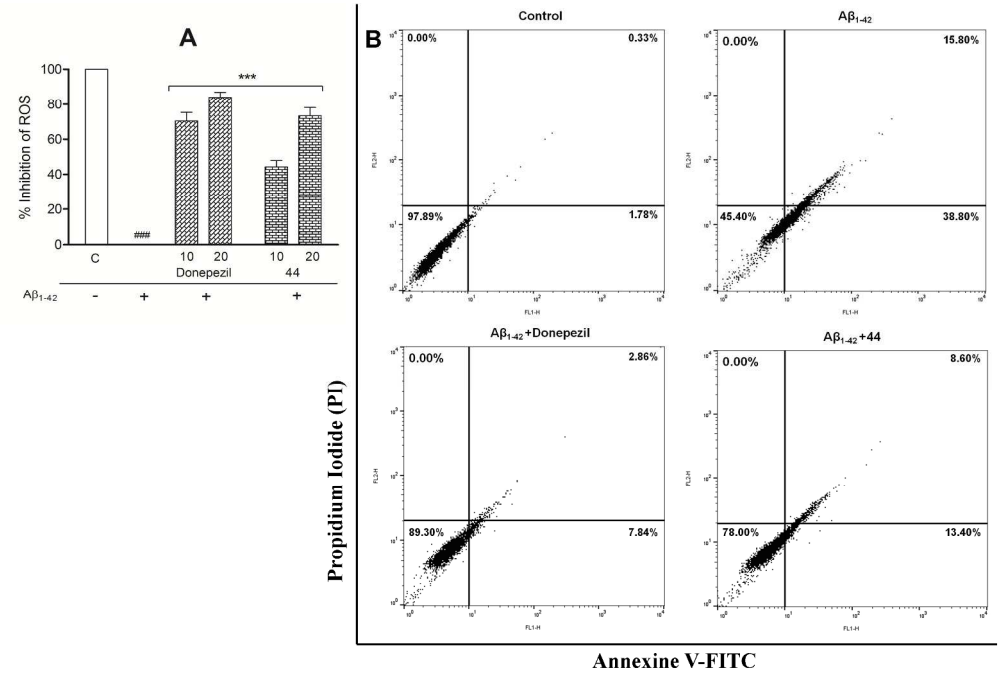


Figure 5. In vitro ROS scavenging and anti-apoptotic potential of compound (44) against Aβ1-42 (10 μM) insult in primary rat hippocampal neuronal culture. (A) Percentage inhibition of ROS generation as assessed by DCFH-DA assay. (B) Flow cytometric assessment of apoptosis using Annexin V-FITC and PI staining. Cells in the lower left quadrant are viable (Annexin V-FITC-/PI-). Cells in the lower right quadrant are early apoptotic (Annexin V-FITC+/PI-) and those in the upper right quadrant are late apoptotic or necrotic (Annexin V-FITC+/PI+). Data are expressed as mean ± SEM. ###p<0.001 vs. control cells. *** p<0.00 vs. Aβ1-42-treated control cells. C=control cells.
926x626mm (96 x 96 DPI)

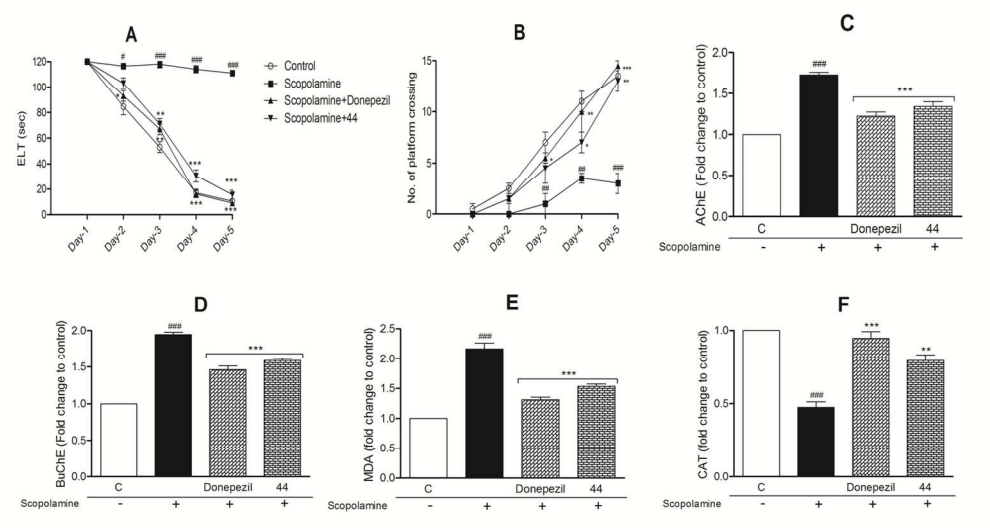


Figure 6. Compound (44) improved spatial learning and memory in scopolamine-treated mice through anti-ChE and anti-oxidant activities. In MWM test, scopolamine (1.4 mg kg-1, i.p.) increased (A) ELT while (B) reduced number of platform area crossings. This pattern was significantly reversed by 44 similar to donepezil at 5 mg kg-1. (C) AChE and (D) BuChE levels elevated by scopolamine treatment were significantly attenuated by 44. Oxidative stress parameters are represented as (E) MDA and (F) CAT levels. Altered MDA and CAT levels after scopolamine treatment were significantly reversed by treatment with compound (44). Data are expressed as mean ± SEM (n=6). ###p<0.001, ## p<0.01, # p<0.05 vs. vehicle-treated control group.*** p<0.001, ** p<0.01, * p<0.05 vs. scopolamine-treated control group. C=vehicle-treated control group.
455x245mm (96 x 96 DPI)

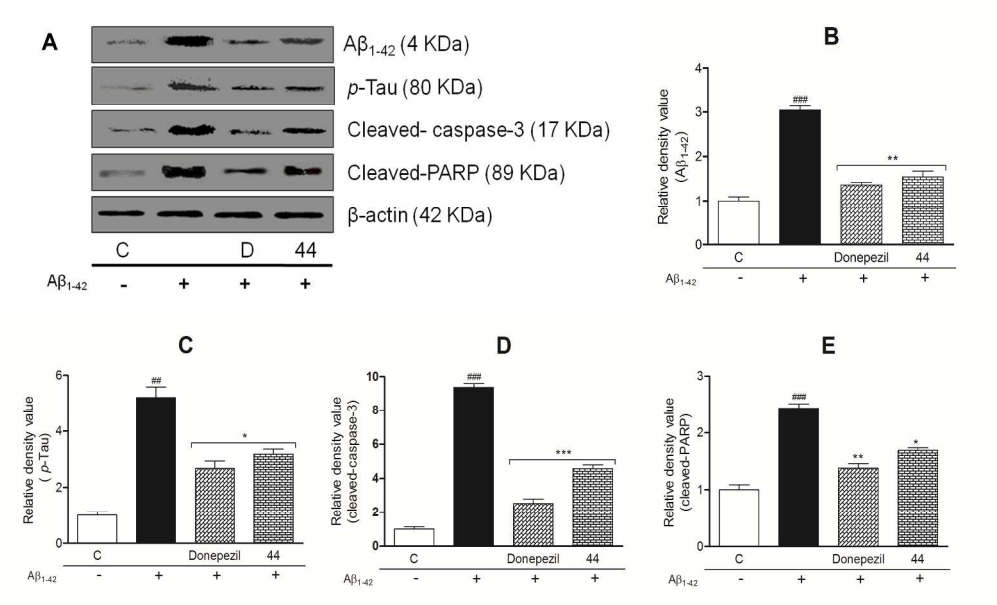


Figure 8. In vivo neuroprotective and anti-apoptotic potential of compound (44). (A) The expression of Aβ1-42, p-Tau, cleaved-caspase-3 and cleaved-PARP was assessed by Western blot analysis in the hippocampal region of rat brain which was given ICV injection of Aβ1-42. Densitometric analysis revealed attenuation of (B) Aβ1-42, (C) p-Tau, (D) cleaved-caspase-3 and (E) cleaved-PARP levels by compound (44) which were elevated by Aβ1-42 toxicity. Data are expressed as mean±SEM (n=6). ###p<0.001, ## p<0.01, # p<0.05 vs. vehicle-treated control group.*** p<0.001, ** p<0.01, * p<0.05 vs. Aβ1-42-treated control group. C=vehicle-treated control group. D=donepezil-treated group.
1237x756mm (96 x 96 DPI)

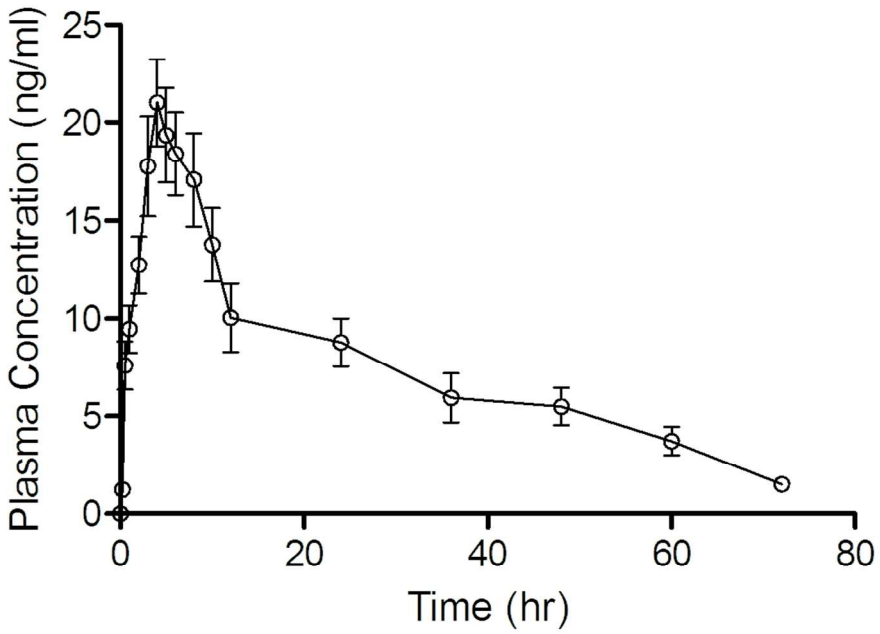
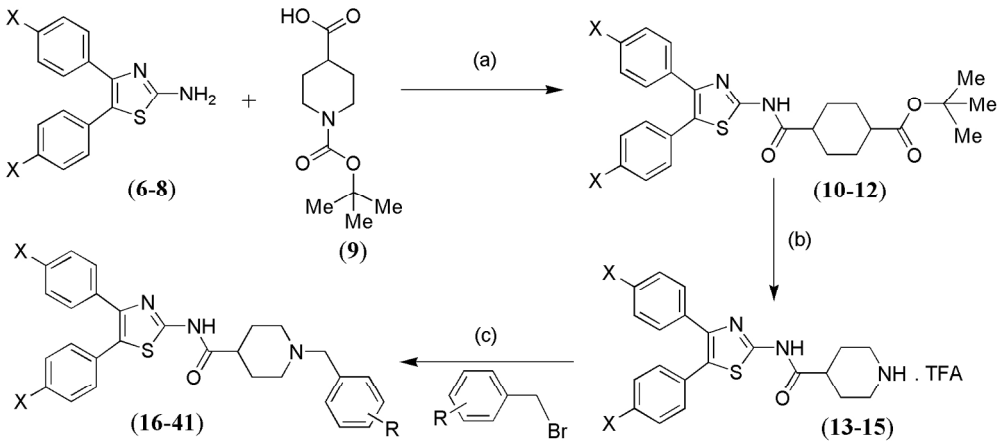


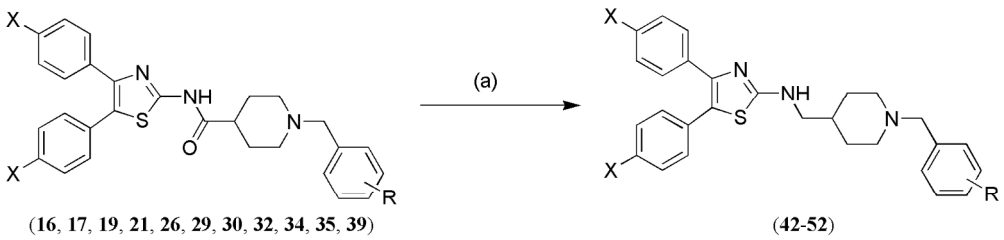
Figure 9. Mean plasma concentration vs. time curve of single oral dose (5 mg kg-1) of compound (44) in rats. Error bars represents the standard deviation of the mean (n=4).
104x73mm (300 x 300 DPI)



X			R	X			R
Me	**Cl**	**OMe**		**Me**	**Cl**	**OMe**	
16	**26**	**34**	2-Me	**23**	-	-	4-CN
17	**27**	**35**	2-CF_3	**24**	-	-	4-NO_2
18	**28**	**36**	3-F	**25**	-	-	4-CF_3
19	**29**	**37**	3,5-DiF	-	**31**	**40**	2-CN
20	**30**	-	2-Cl, 4-F	-	**32**	**41**	4-F, 2-CF_3
21	-	**38**	4-F	-	**33**	-	4-OCF_3
22	-	**39**	2-Cl, 6-F				

Compd	X
6, 10, 13	Me
7, 11, 14	Cl
8, 12, 15	OMe

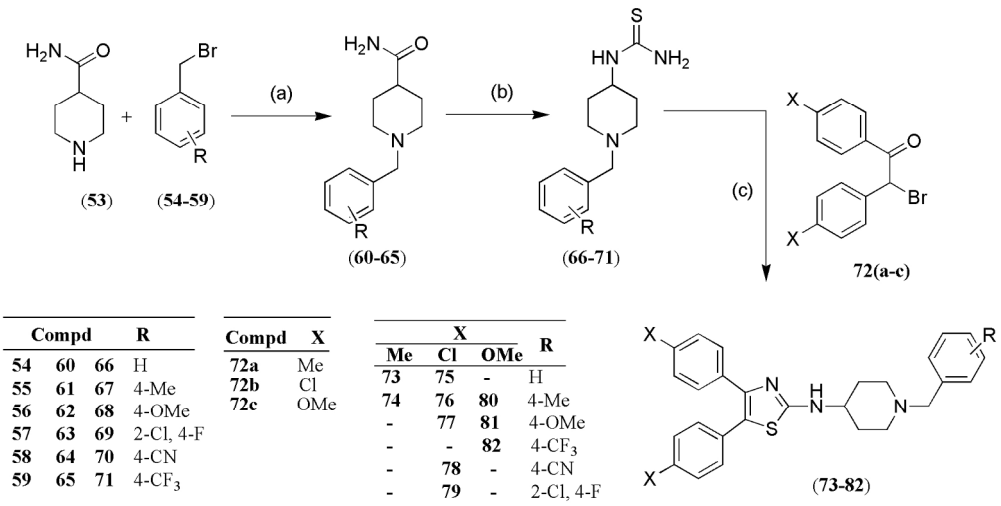
Scheme 1. Synthesis of 1-substituted benzyl-N-[4,5-bis(substituted phenyl)thiazol-2-yl]piperidine-4-carboxamides (16-41)
173x116mm (300 x 300 DPI)



(16, 17, 19, 21, 26, 29, 30, 32, 34, 35, 39) (42-52)

X			R
Me	Cl	OMe	
16, 42	**26, 46**	**34, 50**	2-Me
17, 43	-	**35, 51**	2-CF_3
19, 44	**29, 47**	-	3,5-diF
21, 45	-	-	4-F
-	**30, 48**	-	2-Cl, 4-F
-	**32, 49**	-	2-CF_3, 4-F
-	-	**39, 52**	2-Cl, 6-F

Scheme 2. Synthesis of N-[(1-substitued benzyl)piperidin-4-ylmethyl]-4,5-bis(substituted phenyl)-2-ylamines (42-52)
170x80mm (300 x 300 DPI)



Compd			R
54	**60**	**66**	H
55	**61**	**67**	4-Me
56	**62**	**68**	4-OMe
57	**63**	**69**	2-Cl, 4-F
58	**64**	**70**	4-CN
59	**65**	**71**	4-CF_3

Compd	X
72a	Me
72b	Cl
72c	OMe

X			R
Me	**Cl**	**OMe**	
73	**75**	-	H
74	**76**	**80**	4-Me
-	**77**	**81**	4-OMe
-	-	**82**	4-CF_3
-	**78**	-	4-CN
-	**79**	-	2-Cl, 4-F

Scheme 3. Synthesis of 1-(substituted benzyl)-N-[4,5-bis(substituted phenyl)thiazol-2yl]-piperidin-4-ylamines (73-82)
181x91mm (300 x 300 DPI)

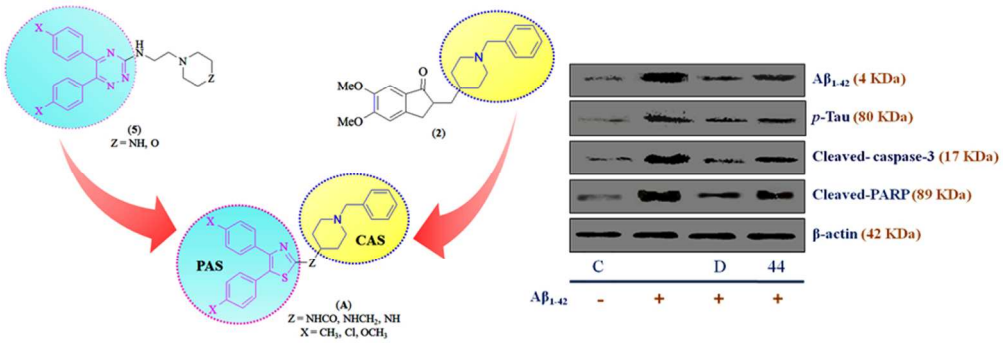


Table of Contents Graphic
266x92mm (96 x 96 DPI)