A Synopsis on

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL VICINAL DIARYL SUBSTITUTED HETEROCYCLIC COMPOUNDS

Submitted

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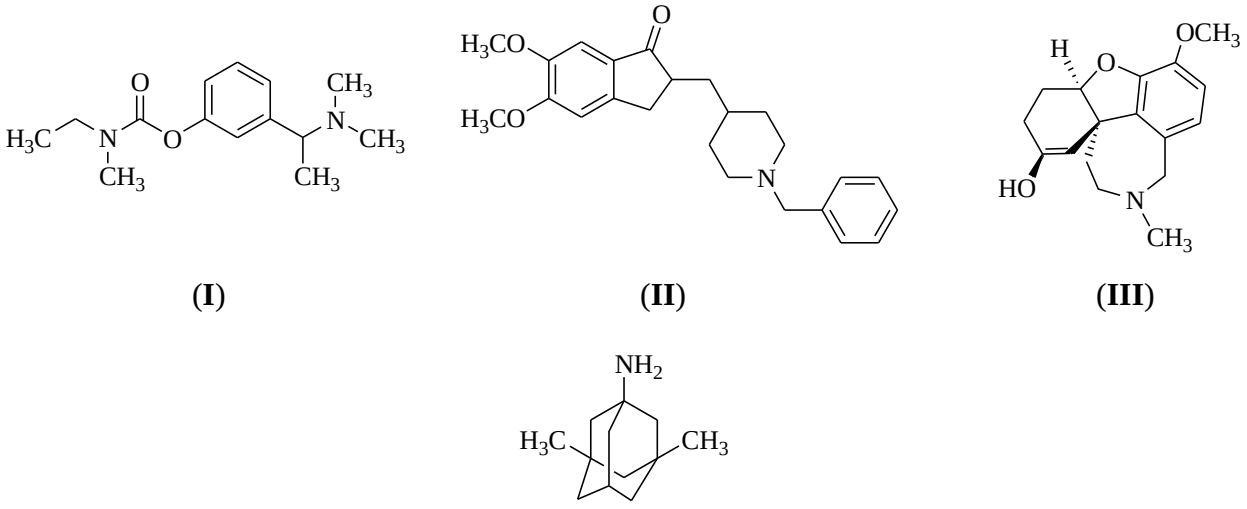
1. Introduction

Alzheimer's disease (AD), one of the major brain diseases, is a complex and progressive neurodegenerative disease which worsens with time. Reports suggest that AD starts 20 years or more prior to appearance of its symptoms, which are generally the small changes in the brain that remain unnoticeable to the affected persons.[1] AD is more prevalent in elderly populations, usually characterized by cognitive impairment with loss of memory, language and learning skills.[2] AD is ranked as the fifth leading cause of death affecting almost 47 million population worldwide, and the number is still rising and is estimated to grow up to 130 million or more by 2050.[3] Since the discovery of Alzheimer's disease by a German psychiatrist Alois Alzheimer in the year 1906, researchers had undertaken great efforts to understand and unfold the pathophysiology of AD. However the exact cause of AD still remains uncertain but various causative factors such as misfolding and aggregation of amyloid-β protein, tau protein hyperphosphorylation, oxidative stress, metal ion dyshomeostasis and deficit of acetylcholine have been recognized to play important roles in pathophysiology of the disease.



(**I**) (**II**) (**III**)

(**IV**)

Figure 1: Currently marketed drugs for the treatment of AD.

Till date no single drug has been clinically effective to prevent or stop the progress of AD. Currently available drugs in the market for the treatment of AD (**Figure 1**) include three acetylcholinesterase inhibitors (AChEIs) viz. rivastigmine (**I**), donepezil (**II**) and galantamine (**III**), and one *N*-methyl-*D*-aspartate receptor (NMDAR) antagonist, memantine (**IV**). These AD treatments are mainly effective to treat mild cognitive impairments (MCI) providing temporary relief from symptoms; however they fail to cure or reverse the progression of AD.[4]

1.1 Pathophysiology of AD

Due to the complexity of AD, the etiology of the disease is not yet understood completely. It is mostly caused by genetic, environmental and endogenous factors. Inheritance from the parents has been believed to be one of the major risk factors of the disease. Several hypotheses have also been suggested to elucidate the cause of the disease which mainly include cholinergic hypothesis, amyloid β-cascade hypothesis, tau hypothesis, oxidative stress and metal ion dyshomeostasis.[5]

1.1.1 Cholinergic hypothesis

According to the cholinergic hypothesis, degeneration of neuronal cells, low levels of neurotransmitter acetylcholine (ACh) and related decrease in neurotransmission in the hippocampus and cortex region of the brain mostly causes the cognitive impairments seen in AD patients.[4,5] Cholinesterases (ChEs), serine hydrolase enzymes are responsible for the decreased level of ACh in the brain as they rapidly hydrolyze the neurotransmitter into acetate and choline. ChEs are of two types viz. acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). To terminate the ACh-mediated response in human brain, ACh is mainly hydrolyzed by AChE rather than BuChE.[6,7] Therefore, ChEs have been considered as the potential therapeutic targets to develop newer ChEIs for the management of AD.

1.1.2 Amyloid β-cascade and Tau (τ) hypothesis

The major histophathological characteristics of AD are the senile plaques (SPs) and neurofibrillary tangles (NFTs) which are the aggregates of the amyloid β-peptides and hyperphosphorylated tau proteins, respectively. [8-10] Amyloid hypothesis suggested that Aβ, the building block of the amyloidogenic pathway, is produced by the abnormal proteolysis of amyloid precursor protein (APP) which further on aggregation in various parts of the brain form amyloid fibrils causing neuritic injury and cell death. [11]

NFTs mainly comprise of paired helical filaments (PHFs) of atypical hyperphosphorylated Tau (τ) protein. τ-Proteins belonging to the group of microtubules associated proteins (MAPs) are crucial in the normal functioning of neurons as they stabilize microtubules and carry out neuronal trafficking. [12] In the process of formation of NFTs, hyperphosphorylated τ-proteins develop as amorphous tangles and oligomers first, which then form PHFs. These PHFs within the nerve cells combine with other proteins including normal τ-

proteins and MAPs to form NFTs.[13,14] Deposition of NFTs in the neuronal cells causes microtubule depolymerization [10,15,16] and interruption in the neuronal transport system leading to the death of neuronal cells. [17-19]

Some of the reports indicate the association of amyloid and tau hypotheses suggesting that Aβ promotes the formation of NFTs and also the oligomers of Aβ and hyperphosphorylated τ collectively causes neurotoxicity.[20-23] Overall, inhibition of Aβ aggregation has been established as the potential therapeutic target to develop clinical agents for AD therapy.

1.1.3 Oxidative stress

Reactive oxygen species (ROS) are inevitably produced endogenously in various biological processes at the cellular level.[24] Increasing evidences indicate that the oxidative stress generated from ROS and reactive nitrogen species (RNS) is involved in the neurodegeneration processes occurring in AD.[25] Oxidative stress is responsible to increase the activity of β- and γ-secreatases and to decrease the activity of α-secreatase which subsequently causes increase in production of Aβ.[26, 27] Oxidative stress can also leads to mitochondrial dysfunction causing increase in concentration of ROS which react with biomolecules such as lipids, proteins, nucleic acids and carbohydrates. [28-30] Thus, efforts have been devoted to develop newer multifunctional antioxidants to manage or target the oxidative stress along with other pathological factors.

1.1.4 Metal ion dyshomeostasis

Metal ions play a vital role in various biological processes like metabolism, catalysis and signal transmission to name a few. [31,32] A number of reports have appeared in the literature describing the roles and functions of first-row transition metals such as iron (Fe), copper (Cu) and zinc (Zn). It has been observed that the deregulation of these active metal ions generally lead to increase in the oxidative stress in the brain of AD patients.[33,34] Metal ions have also been involved in producing Aβ toxicity and hyperphosphorylation of τ-proteins.[35,36] Interaction of metal ions with Aβ at lower physiological concentration causes metal-induced Aβ aggregation.[37] Therefore, clinical candidates having the metal chelating ability would be an additional benefit to combat AD.

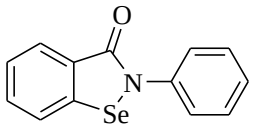
2. Literature review

Acetylcholinesterase (AChE) is an important target for the design of novel therapeutics for the treatment of AD. AChE possessed two binding sites i.e. catalytic active site (CAS) and peripheral anionic site (PAS). Along with the hydrolysis of acetylcholine, AChE also induces the aggregation of amyloid-β protein. The interaction of Aβ peptides with PAS of the AChE resulted in the fibril formation. Inhibition of both the active sites simultaneously could be an effective clinical strategy to control the progression of AD.[38] Therefore compounds with dual binding affinity (CAS & PAS) would be of great interest to design and develop multi-target-directed ligands (MTDLs).

Donepezil, one of the marketed drugs for AD treatment, inhibits AChE effectively interacting with both the active sites of the enzyme. Owing to this dual-binding mode of donepezil, many researchers worldwide modified and explored the structure of donepezil to obtain various donepezil-based MTDLs as multifunctional cholinesterase inhibitors.[39]

Donepezil-based multifunctional cholinesterase inhibitors

Luo *et al.* described the synthesis and biological evaluation of some MTDLs for the treatment of AD. In their efforts to develop a novel series of MTDLs, the authors combined structural pharmacophores of donepezil and ebselen (**V**), an antioxidant. Among the series, compound (**VI**) was found to be the most potent inhibitor of AChE (IC_{50} value of 42 nM for *ee*AChE and 97 nM for *h*AChE) and AChE-induced Aβ aggregation (21.4%).[40]



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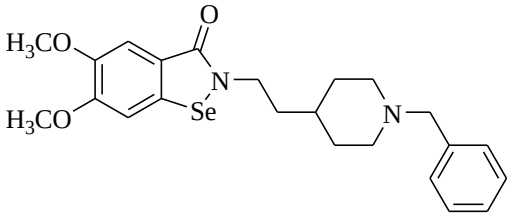
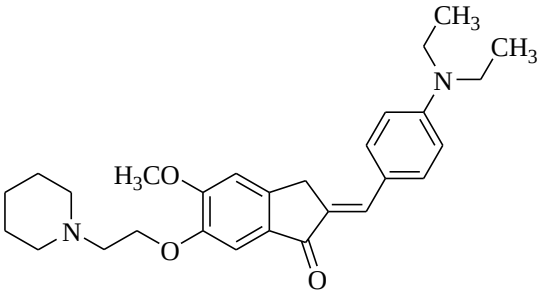
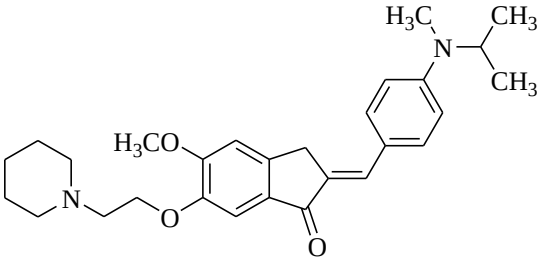


VI

In another report, Huang *et al.* disclosed a series of some indanone derivatives as anti-AD agents wherein compound (**VII** and **VIII**) were observed to inhibit *ee*AChE with an IC_{50} value of 14.8 nM and 18.6 nM, respectively. Both of these compounds (**VII** and **VIII**) also showed 85.5% and 83.8% inhibition of self-induced $A\beta_{1-42}$ aggregation,respectively.[41]

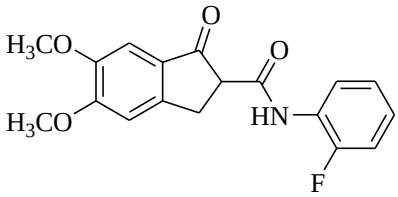


VII



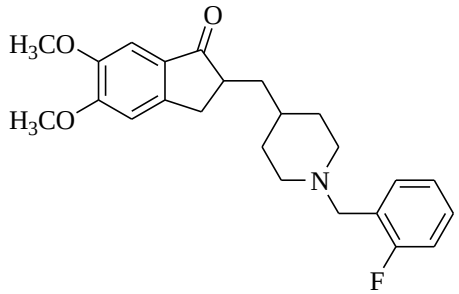
VIII

A series of substituted carboxamide analogs of donepezil have been reported by Yerdelen *et al*. as anti-Alzheimer Agents. Compound (**IX**) was found to be the most potent showing the highest AChE inhibition with an IC_{50} value of 80 nM (*ee*AChE) and about 55% inhibition of self-induced $A\beta_{1\text{-}42}$ aggregation. It also showed promising antioxidant characteristics.[42]

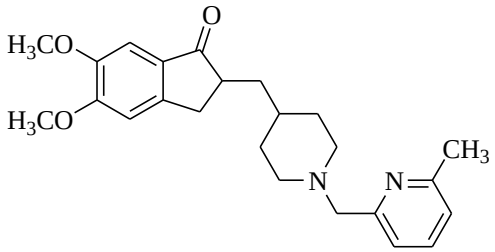


IX

Wang et al. synthesized some donepezil derivatives having substituted phenyl and heteroaryl rings. Various substituents (CH_3, Cl, F, CF_3 and NO_2) at different positions (2nd, 3rd and 4th) of the phenyl ring of donepezil have been employed to improve the AChE inhibitory activity of donepezil. Biological screening of these compounds revealed that compounds with fluoro substituent at 2nd and 3rd positions of the phenyl ring offered more potent AChE inhibitory activity and higher selectivity towards AChE compared to BuChE. Compound (**X**) showed inhibition of both *human* AChE (*h*AChE) with an IC_{50} value of 32 nM and *electric eel* AChE (*ee*AChE) with an IC_{50} value of 43 nM.[43]



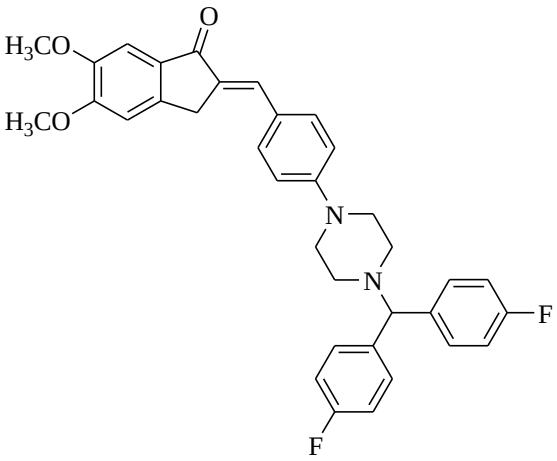
X



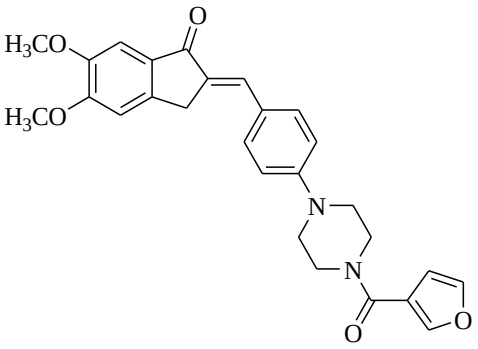
XI

Further the metal chelating property was introduced into the compounds in addition to AChE inhibition by replacing the phenyl ring with the pyridyl ring. Compound (**XI**) exhibited the most potent AChE inhibitory activity in the series with an IC_{50} value of 73 nM (*h*AChE) and 85 nM (*ee*AChE) comparable to the standard, donepezil having IC_{50} value of 48 nM (*h*AChE) and 51 nM (*ee*AChE). It has also displayed moderate potency to inhibit 18.5% of self-induced, 46.3% of Cu^{2+}-induced and 72.4% of AChE-induced $A\beta_{1-42}$ aggregation.

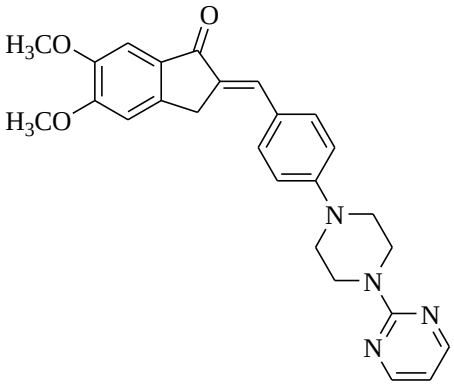
Mishra *et al.* designed and synthesized some substituted benzylidene derivatives of donepezil as MTDLs for AD therapy. Amongst the series, compounds (**XII-XIV**) showed excellent AChE inhibitory activity against *ee*AChE with an IC_{50} value of 45 nM, 34 nM and 25 nM, respectively. Additionally, the most active compound (**XIV**) displayed prominent (81%) inhibition of self-induced $A\beta_{1-42}$ aggregation.[44]



XII

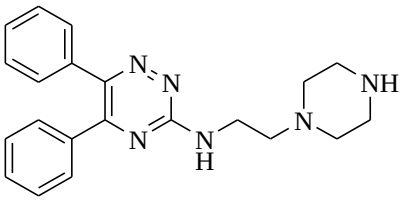


XIII

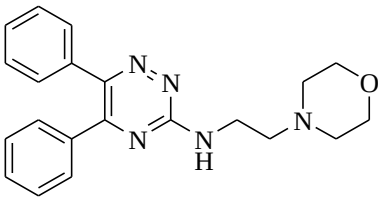


XIV

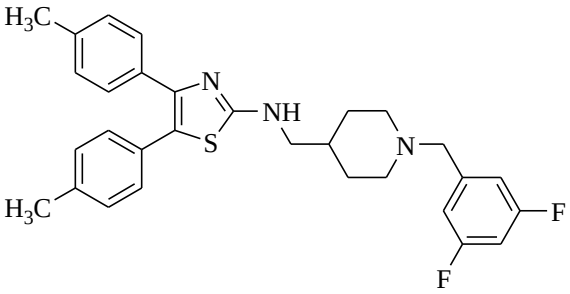
Our research group at The Maharaja Sayajirao University of Baroda reported vicinal diaryltriazines as potent MTDLs for AD. Compound (**XV** and **XVI**) showed good AChE inhibitory activity and prevented Aβ aggregation.[45]



XV



XVI



XVII

Based on the previous research work carried out in the lab, Shidore *et al*. designed and synthesized some novel benzylpiperidine-linked vicinal diarylthiazole derivatives as promising AChE inhibitors. Compound (**XVII**) displayed potent AChE inhibition with IC_{50} value of 0.30 μM for *h*AChE and also demonstrated good *in vivo* protection.[46]

3. Aim and objectives

Despite its exceptional pharmacological profile, safety and tolerance, donepezil still has some serious issues. Donepezil could be effective for the treatment of early to intermediate stages of AD. It can be useful only to delay the progression of AD or manage the symptoms, it unfortunately failed to terminate or eliminate the cognitive impairments completely. It had been reported that donepezil causes moderate improvements in the quality of life of patients suffering from AD. Moreover, there is no single report available claiming the long-term clinical efficacy and safety of donepezil. Due to its limited potential, use of donepezil becomes inadequate to treat severe and advanced stages of AD.[47] This is the reason why so many efforts have continuously been made by the medicinal chemists involved in AD research to develop novel AChE inhibitors with long-term efficacy and safety.

As the literature survey depicted, a number of new compounds have been designed by the hybrid approach wherein the two pharmacophores with different biological activities on different targets are fused together to form one hybrid structure or molecule which can be called as multi-target-directed ligands (MTDLs). These MTDLs have been synthesized by modifying either the 5,6-dimethoxy indanone part or the benzylpiperidine pharmacophore of the donepezil.

Our research group has also been actively involved in the designing and development of various therapeutically active compounds and successfully reported some vicinal diaryl heterocyclic systems as anti-Alzheimer agents.

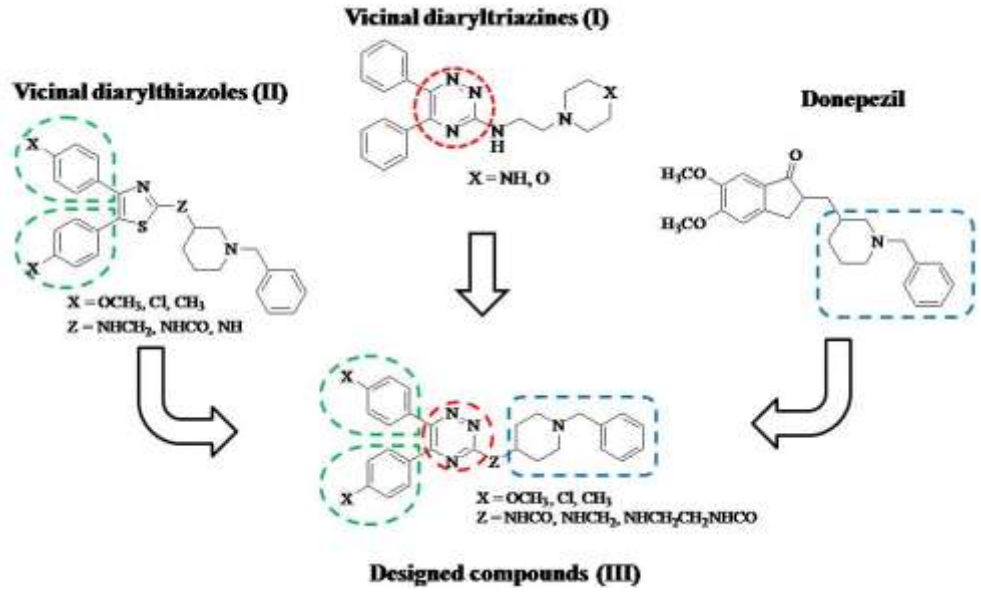


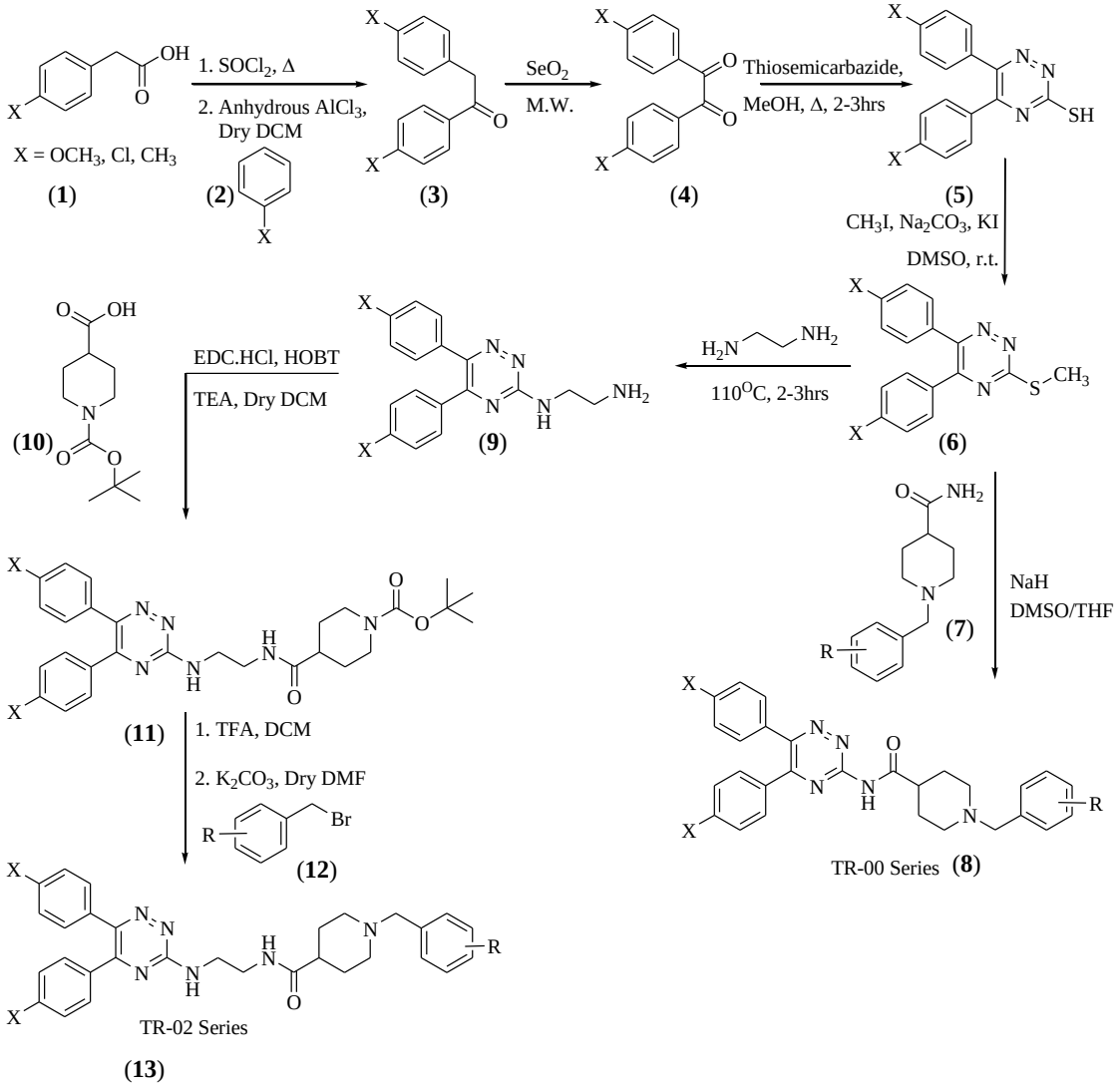
Figure 3.1: Designing of novel vicinal diaryltriazines (**III**) as anti-Alzheimer agents.

Earlier we have disclosed the anti-Alzheimer activity of compounds (**I**) containing vicinal diaryltriazine scaffold.[45] Some benzylpiperidine-linked vicinal diarylthiazole derivatives (**II**) as MTDLs for the treatment of AD have also been reported from the lab.[46] Based on these previous research works, it was planned to explore the structure of vicinal diaryltriazine fused with the substituted benzylpiperidines to develop a novel series of compounds (**III**) as anti-Alzheimer agents (**Figure 3.1**)

4. Result and discussion

4.1 Chemical Work

Synthesis of the designed vicinal diaryltriazine containing compounds was carried out using the following synthetic scheme 4.1.



Scheme 4.1: Synthesis of vicinal diaryltriazine containing compounds (**8** and **13**)

4.1.1 Synthesis of *N*-(5,6-diaryl-1,2,4-triazin-3-yl)-1-benzylpiperidine-4-carboxamides (8):

Synthesis of *N*-(5,6-diaryl-1,2,4-triazin-3-yl)-1-benzylpiperidine-4-carboxamide (**8**) was carried out as shown in the scheme 4.1. First, acid chlorides were prepared from substituted phenylacetic acid (**1**) in the presence of thionyl chloride and the subsequent Friedel-Crafts acylation using substituted benzene (**2**) produced 1,2-diarylethanones (**3**). Further oxidation of (**3**) was carried out using selenium dioxide to get 1,2-diarylethan-1,2-diones (**4**) which on refluxing with thiosemicarbazide undergo cyclization to give 5,6-diaryl-1,2,4-triazine-3-thiol (**5**). In the next step, **5** was methylated by methyl iodide under basic conditions to obtain 3-(methylthio)-5,6-diphenyl-1,2,4-triazine (**6**). Finally 3-(methylthio)-5,6-diphenyl-1,2,4-triazine (**6**) was treated with 1-substituted benzylpiperidines-4-carboxamides (**7**) in presence of sodium hydride/DMSO at R.T. to obtain the desired *N*-(5,6-diaryl-1,2,4-triazin-3-yl)-1-benzylpiperidine-4-carboxamides (**8**).

- **1,2-Bis(4-methoxyphenyl)ethanone (3a)**

  M. P. : 108-110 ^{o}C (Reported 110-112 ^{o}C)[48]

  IR (KBr, cm^{-1}) : 3028, 2963, 1675, 1597, 1166, 825

- **1,2-Bis(4-methoxyphenyl)ethane-1,2-dione (4a)**

  M. P. : 133-135 ^{o}C

  IR (KBr, cm^{-1}) : 2951, 1655, 1599, 1460, 1160, 833

- **5,6-Bis(4-methoxyphenyl)-1,2,4-triazine-3-thiol (5a)**

  M. P. : 237-239 ^{o}C

  IR (KBr, cm^{-1}) : 3120, 2837, 1656, 1561, 1256, 1167, 831

- **3-(Methylthio)-5,6-bis(4-methoxyphenyl)-1,2,4-triazine (6a)**

  M. P. : 133-135 ^{o}C

  IR (KBr, cm^{-1}) : 2960, 1602, 1460, 1252, 1176

- ***N*-(5,6-Bis(4-methoxyphenyl)-1,2,4-triazin-3-yl)-1-(2-chloro)benzylpiperidine-4-carboxamide (08)**

  M.P. : 181-183 ^{o}C

  IR (KBr, cm^{-1}) : 3204, 3078, 2925, 1723, 1603, 1460, 1255

^{1}H-NMR : 9.94 (s,1H), 7.67-7.65 (d,2H), 7.51-7.49 (d,2H), 7.33-7.31 (d,1H), 7.26-7.14 (m,3H), 6.95-6.93 (d,2H), 6.84-6.82 (d,2H), 3.85 (s,1H), 3.82 (s,1H), 3.56 (s,2H), 2.96-2.92 (m,3H), 2.00-1.94 (m,6H)

Mass (m/z) : 544.4 (M+1)

Other compounds having various R and X groups were synthesized on the similar lines.

4.1.2 Synthesis of *N*-(2-(5,6-diaryl-1,2,4-triazin-3-ylamino)ethyl)-1-benzylpiperidine-4-carboxamides (13):

3-(Methylthio)-5,6-diaryl-1,2,4-triazine (**6**) on refluxing with ethylenediamine at 110 ^{o}C for 2-3 hrs gave *N*-(2-aminoethyl)-5,6-diaryl-1,2,4-triazin-3-amine (**9**) which in the next step was treated with 1-(*t*.butyloxycarbonyl)piperidine-4-carboxylic acid (**10**) in presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) and hydroxybenzotriazole (HOBt) under basic condition to prepare *tert*-butyl 4-(2-(5,6-diaryl-1,2,4-triazin-3-ylamino)ethylcarbamoyl)piperidine-1-carboxylate (**11**). In the last step (**11**) was deprotected using trifluoroacetic acid (TFA) and refluxed with substituted benzyl bromides (**12**) to obtain final *N*-(2-(5,6-diaryl-1,2,4-triazin-3-ylamino)ethyl)-1-benzylpiperidine-4-carboxamides (**13**).

- ***N*-(2-Aminoethyl)-5,6-bis(4-methoxyphenyl)-1,2,4-triazin-3-amine (10a)**

  M. P. : 153-155 ^{o}C

  IR (KBr, cm^{-1}) : 3239, 3072, 2928, 1600, 1437, 1250, 1174, 833

- ***tert*-Butyl-4-(2-(5,6-bis(4-methoxyphenyl)-1,2,4-triazin-3-ylamino)ethylcarbamoyl) piperidine-1-carboxylate (11a)**

  M. P. : 160-162 ^{o}C

  IR (KBr, cm^{-1}) : 3269, 3077, 2935, 1686, 1520, 1430, 1253

- ***N*-(2-(5,6-Bis(4-methoxyphenyl)-1,2,4-triazin-3-ylamino)ethyl)-1-(2-chloro)benzyl piperidine -4-carboxamide (13)**

  M. P. : 178-180 ^{o}C

  IR (KBr, cm^{-1}) : 3259, 3125, 2922, 1670, 1460, 1253

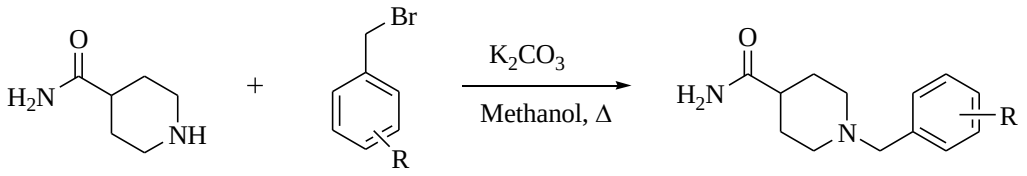
  ^{1}H-NMR ($CDCl_3$) : 7.51-7.49 (d,2H), 7.42-7.41 (d,1H), 7.36-7.34 (d,2H), 7.31-7.29 (d,1H), 7.20-7.12 (m,2H), 6.88-6.80 (m,4H),

3.81 (s,1H), 3.79 (s,1H), 3.76-3.72 (q,2H), 3.63-3.59 (q,2H),
3.49 (s,1H), 2.78-2.75 (d,2H), 1.69-1.61 (m, 7H)

Other compounds having different R and X groups were synthesized in a similar way.

4.1.3 Synthesis of 1-substitutedbenzylpiperidine-4-carboxamides (7):

Piperidine-4-carboxamide (**A**) was refluxed with substituted benzyl bromides (**B**) in methanol and potassium carbonate to produce 1-substituted benzylpiperidine-4-carboxamide (**7**) as shown in scheme 4.2.



(**A**) (**B**) (**7**)

Scheme 4.2: Synthesis of 1-substituted benzylpiperidine-4-carboxamides (7)

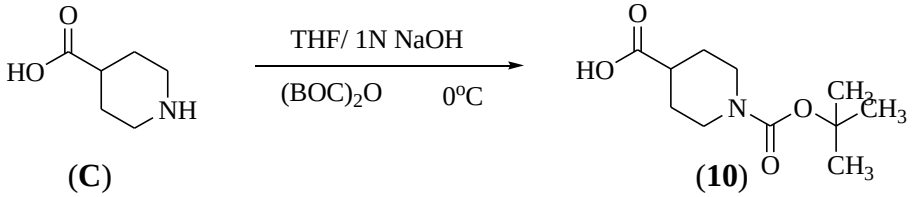
- **1-(2-Chloro)benzylpiperidine-4-carboxamide** (**B2**)

M. P. : 155-157 ^{o}C

IR (KBr, cm^{-1}) : 3334, 3164, 3008, 2945, 1632, 1488, 1264

4.1.4 Synthesis of 1-(*t*.butoxycarbonyl)piperidine-4-carboxylic acid (10):

Amine of the piperidine-4-carboxylic acid (**C**) was protected by di-*t*.butyl dicarbonate using 1N/NaOH and THF at 0 ^{o}C to prepare 1-(*t*.butoxycarbonyl)piperidine-4-carboxylic acid (**10**) (**Scheme 4.3**). M. P. – 150-152 ^{o}C (reported 150-152 ^{o}C).[49] Its IR spectra showed peaks at 3212, 2974, 1735, 1661, 1242, 1163, 825 cm^{-1}.



(**C**) (**10**)

Scheme 4.3: Synthesis of 1-(*t*.butoxycarbonyl)piperidine-4-carboxylic acid (**10**)

4.2 Biological Evaluation

4.2.1 AChE/BuChE Assay

The AChE and BuChE inhibitory activity of the synthesized compounds was evaluated using the Ellman assay.[50]

4.2.2 Thioflavin T Assay

Thioflavin T assay was carried out as per the previously reported protocol to evaluate the $A\beta_{1-42}$ aggregation inhibition of the synthesized compounds.[51,52]

The results of the biological screening of the compounds will be discussed in detail in the thesis.

5. References

1. Alzheimer's Association. 2019 Alzheimer's disease facts and figures. *Alzheimer's & Dementia*, **2019**, 15(3), 321-387.
2. Mucke, L. Neuroscience: Alzheimer's disease. *Nature*, **2009**, 461(7266), 895.
3. Alzheimer's Association. 2017 Alzheimer's disease facts and figures. *Alzheimer's & Dementia*, **2017**, 13(4), 325-373.
4. Savelieff, M. G., Nam, G., Kang, J., Lee, H. J., Lee, M. and Lim, M. H. Development of multifunctional molecules as potential therapeutic candidates for alzheimer's disease, parkinson's disease, and amyotrophic lateral sclerosis in the last decade. *Chemical reviews*, **2018**, 119(2), 1221-1322.
5. Cavalli, A., Bolognesi, M. L., Minarini, A., Rosini, M., Tumiatti, V., Recanatini, M. and Melchiorre, C. Multi-target-directed ligands to combat neurodegenerative diseases. *Journal of medicinal chemistry*, **2008**, 51(3), 347-372.
6. Perry, E. K., Perry, R. H., Blessed, G. and Tomlinson, B. E. Changes in brain cholinesterases in senile dementia of Alzheimer type. *Neuropathology and applied neurobiology*, **1978**, 4(4), 273-277.
7. Arendt, T., Bigl, V., Walther, F. and Sonntag, M. Decreased ratio of CSF acetylcholinesterase to butyrylcholinesterase activity in Alzheimer's disease. *The Lancet*, **1984**, 323(8369), 173.
8. Hardy, J. A.; Higgins, G. A. Alzheimer's Disease: The Amyloid Cascade Hypothesis. *Science* **1992**, 256, 184−185.
9. Hardy, J. and Selkoe, D. J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *science*, **2002**, 297(5580), 353-356.
10. Grundke-Iqbal, I., Iqbal, K., Tung, Y. C., Quinlan, M., Wisniewski, H. M. and Binder, L. I. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proceedings of the National Academy of Sciences*, **1986**, 83(13), 4913-4917.
11. Singh, M., Kaur, M., Kukreja, H., Chugh, R., Silakari, O. and Singh, D. Acetylcholinesterase inhibitors as Alzheimer therapy: from nerve toxins to neuroprotection. *European Journal of Medicinal Chemistry*, **2013**, 70, 165-188.
12. Ballatore, C., Lee, V. M. Y. and Trojanowski, J. Q. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nature Reviews Neuroscience*, **2007**, 8(9), 663.
13. Daebel, V., Chinnathambi, S., Biernat, J., Schwalbe, M., Habenstein, B., Loquet, A., Akoury, E., Tepper, K., Müller, H., Baldus, M. and Griesinger, C. β-Sheet core of tau paired helical

filaments revealed by solid-state NMR. *Journal of the American Chemical Society*, **2012**, 134(34), 13982-13989.

14. Hall, G.F., Chu, B., Lee, G. and Yao, J. Human tau filaments induce microtubule and synapse loss in an in vivo model of neurofibrillary degenerative disease. *J Cell Sci*, **2000**, 113(8), 1373-1387.
15. Baudier, J. and Cole, R. D. Phosphorylation of tau proteins to a state like that in Alzheimer's brain is catalyzed by a calcium/calmodulin-dependent kinase and modulated by phospholipids. *Journal of Biological Chemistry*, **1987**, 262(36), 17577-17583.
16. Goedert, M., Spillantini, M.G., Jakes, R., Rutherford, D. and Crowther, R. A. Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron*, **1989**, 3(4), 519-526.
17. Goedert, M., Spillantini, M. G. and Crowther, R. A. Tau proteins and neurofibrillary degeneration. *Brain pathology*, **1991**,1(4), 279-286.
18. Iqbal, K., Alonso, A. D. C., Chen, S., Chohan, M. O., El-Akkad, E., Gong, C. X., Khatoon, S., Li, B., Liu, F., Rahman, A. and Tanimukai, H. Tau pathology in Alzheimer disease and other tauopathies. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, **2005**, 1739(2-3), 198-210.
19. Chun, W. and Johnson, G.V. The role of tau phosphorylation and cleavage in neuronal cell death. *Frontiers in bioscience: a journal and virtual library*, **2007**, 12, 733-756.
20. Takashima, A., Noguchi, K., Sato, K., Hoshino, T. and Imahori, K. Tau protein kinase I is essential for amyloid beta-protein-induced neurotoxicity. *Proceedings of the National Academy of Sciences*, **1993**, 90(16), 7789-7793.
21. Roberson, E. D., Scearce-Levie, K., Palop, J. J., Yan, F., Cheng, I. H., Wu, T., Gerstein, H., Yu, G. Q. and Mucke, L. Reducing endogenous tau ameliorates amyloid ß-induced deficits in an Alzheimer's disease mouse model. *Science*, **2007**, 316(5825), 750-754.
22. Ittner, L. M. and Götz, J. Amyloid-β and tau—a toxic pas de deux in Alzheimer's disease. *Nature Reviews Neuroscience*, **2011**, 12(2), 67.
23. Guerrero-Muñoz, M. J., Gerson, J. and Castillo-Carranza, D. L. Tau oligomers: The toxic player at synapses in Alzheimer's disease. *Frontiers in cellular neuroscience*, **2015**, 9, 464.
24. Petersen, R. B., Nunomura, A., Lee, H. G., Casadesus, G., Perry, G., Smith, M. A. and Zhu, X. Signal transduction cascades associated with oxidative stress in Alzheimer's disease. *Journal of Alzheimer's Disease*, **2007**, 11(2), 143-152.
25. Praticò, D. Evidence of oxidative stress in Alzheimer's disease brain and antioxidant therapy: lights and shadows. *Annals of the New York Academy of Sciences*, **2008**, 1147(1), 70-78.
26. Zhao, Y. and Zhao, B. Oxidative stress and the pathogenesis of Alzheimer's disease. *Oxidative medicine and cellular longevity*, **2013**, 2013. http://dx.doi.org/10.1155/2013/316523.
27. Ganguly, G., Chakrabarti, S., Chatterjee, U. and Saso, L. Proteinopathy, oxidative stress and mitochondrial dysfunction: cross talk in Alzheimer's disease and Parkinson's disease. *Drug design, development and therapy*, **2017**, 11, 797.

28. Moreira, P. I., Nunomura, A., Nakamura, M., Takeda, A., Shenk, J. C., Aliev, G., Smith, M. A. and Perry, G. Nucleic acid oxidation in Alzheimer disease. *Free Radical Biology and Medicine*, **2008**, 44(8), 1493-1505.
29. Fukuda, M., Kanou, F., Shimada, N., Sawabe, M., Saito, Y., Murayama, S., Hashimoto, M., Maruyama, N. and Ishigami, A. Elevated levels of 4-hydroxynonenal-histidine Michael adduct in the hippocampi of patients with Alzheimer's disease. *Biomedical research*, **2009**, 30(4), 227-233.
30. Sultana, R., Mecocci, P., Mangialasche, F., Cecchetti, R., Baglioni, M. and Butterfield, D.A. Increased protein and lipid oxidative damage in mitochondria isolated from lymphocytes from patients with Alzheimer's disease: insights into the role of oxidative stress in Alzheimer's disease and initial investigations into a potential biomarker for this dementing disorder. *Journal of Alzheimer's Disease*, **2011**, 24(1), 77-84.
31. Gschwind, A., Zwick, E., Prenzel, N., Leserer, M. and Ullrich, A. Cell communication networks: epidermal growth factor receptor transactivation as the paradigm for interreceptor signal transmission. *Oncogene*, **2001**, 20(13), 1594.
32. Kepp, K.P., 2012. Bioinorganic chemistry of Alzheimer's disease. *Chemical reviews*, **2012**, 112(10), 5193-5239.
33. Guglielmotto, M., Giliberto, L., Tamagno, E. and Tabaton, M., 2010. Oxidative stress mediates the pathogenic effect of different Alzheimer's disease risk factors. *Frontiers in aging neuroscience*, **2010**, 2, 3.
34. Lee, H. P., Zhu, X., Casadesus, G., Castellani, R. J., Nunomura, A., Smith, M. A., Lee, H. G. and Perry, G. Antioxidant approaches for the treatment of Alzheimer's disease. *Expert review of neurotherapeutics*, **2010**, 10(7), 1201-1208.
35. Yamamoto, A., Shin, R.W., Hasegawa, K., Naiki, H., Sato, H., Yoshimasu, F. and Kitamoto, T. Iron (III) induces aggregation of hyperphosphorylated τ and its reduction to iron (II) reverses the aggregation: implications in the formation of neurofibrillary tangles of Alzheimer's disease. *Journal of neurochemistry*, **2002**, 82(5), 1137-1147.
36. Duce, J. A., Tsatsanis, A., Cater, M. A., James, S. A., Robb, E., Wikhe, K., Leong, S. L., Perez, K., Johanssen, T., Greenough, M. A. and Cho, H. H. Iron-export ferroxidase activity of β-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. *Cell*, **2010**, 142(6), 857-867.
37. Huang, X., Cuajungco, M. P., Atwood, C. S., Hartshorn, M. A., Tyndall, J. D., Hanson, G. R., Stokes, K. C., Leopold, M., Multhaup, G., Goldstein, L. E. and Scarpa, R. C. Cu (II) potentiation of Alzheimer Aβ neurotoxicity correlation with cell-free hydrogen peroxide production and metal reduction. *Journal of Biological Chemistry*, **1999**, 274(52), 37111-37116.
38. Unzeta, M., Esteban, G., Bolea, I., Fogel, W. A., Ramsay, R. R., Youdim, M. B., Tipton, K. F. and Marco-Contelles, J. Multi-target directed donepezil-like ligands for Alzheimer's disease. *Frontiers in neuroscience*, **2016**, 10, 205.

39. Li, Q., He, S., Chen, Y., Feng, F., Qu, W. and Sun, H. Donepezil-based multi-functional cholinesterase inhibitors for treatment of Alzheimer's disease. *European journal of medicinal chemistry*. **2018**. doi10.1016/j.ejmech.2018.09.031.
40. Luo, Z., Sheng, J., Sun, Y., Lu, C., Yan, J., Liu, A., Luo, H. B., Huang, L. and Li, X. Synthesis and evaluation of multi-target-directed ligands against Alzheimer's disease based on the fusion of donepezil and ebselen. *Journal of medicinal chemistry*, **2013**, 56(22), 9089-9099.
41. Huang, L., Miao, H., Sun, Y., Meng, F. and Li, X. Discovery of indanone derivatives as multi-target-directed ligands against Alzheimer's disease. *European journal of medicinal chemistry*, **2014**, 87, 429-439.
42. Yerdelen, K.O., Koca, M., Anil, B., Sevindik, H., Kasap, Z., Halici, Z., Turkaydin, K. and Gunesacar, G. Synthesis of donepezil-based multifunctional agents for the treatment of Alzheimer's disease. *Bioorganic & medicinal chemistry letters*, **2015**, 25(23), 5576-5582.
43. Wang, Z. M., Cai, P., Liu, Q. H., Xu, D. Q., Yang, X. L., Wu, J. J., Kong, L. Y. and Wang, X. B. Rational modification of donepezil as multifunctional acetylcholinesterase inhibitors for the treatment of Alzheimer's disease. *European journal of medicinal chemistry*, **2016**, 123, 282-297.
44. Mishra, C. B., Kumari, S., Manral, A., Prakash, A., Saini, V., Lynn, A. M. and Tiwari, M. Design, synthesis, in-silico and biological evaluation of novel donepezil derivatives as multi-target-directed ligands for the treatment of Alzheimer's disease. *European journal of medicinal chemistry*, **2017**, 125, 736-750.
45. Sinha, A., Tamboli, R. S., Seth, B., Kanhed, A. M., Tiwari, S. K., Agarwal, S., Nair, S., Giridhar, R., Chaturvedi, R. K. and Yadav, M. R. Neuroprotective role of novel triazine derivatives by activating Wnt/β catenin signaling pathway in rodent models of Alzheimer's disease. *Molecular neurobiology*, **2015**, 52(1), 638-652.
46. Shidore, M., Machhi, J., Shingala, K., Murumkar, P., Sharma, M. K., Agrawal, N., Tripathi, A., Parikh, Z., Pillai, P. and Yadav, M. R. Benzylpiperidine-linked diarylthiazoles as potential anti-Alzheimer's agents: synthesis and biological evaluation. *Journal of medicinal chemistry*, **2016**, 59(12), 5823-5846.
47. Steele, L. S. and Glazier, R. H. Is donepezil effective for treating Alzheimer's disease? *Canadian Family Physician*, **1999**, 45, 917.
48. Schneider, M. R., Von Angerer, E., Schoenenberger, H., Michel, R. T. and Fortmeyer, H. P. 1, 1, 2-triphenylbut-1-enes: relationship between structure, estradiol receptor affinity, and mammary tumor inhibiting properties. *Journal of medicinal chemistry*, **1982**, 25(9), 1070-1077.
49. Nimbarte, V. D., Murtuza, H., Phaniraj, S., Shrivastava, S., Naidu, V. G. M., Kumar, N. S. and 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. *Medicinal Chemistry Research*, **2014**, 23(5), 2178-2197.

50. Ellman, G. L., Courtney, K. D., Andres Jr, V. and Featherstone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology*, **1961**, 7(2), 88-95.
51. 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. and Leem, J. Y. Synthesis, in vitro assay, and molecular modeling of new piperidine derivatives having dual inhibitory potency against acetylcholinesterase and Aβ1–42 aggregation for Alzheimer's disease therapeutics. *Bioorganic & medicinal chemistry*, **2007**, 15(20), 6596-6607.
52. Mohamed, T., Zhao, X., Habib, L. K., Yang, J. and Rao, P. P. Design, synthesis and structure–activity relationship (SAR) studies of 2, 4-disubstituted pyrimidine derivatives: Dual activity as cholinesterase and Aβ-aggregation inhibitors. *Bioorganic & medicinal chemistry*, **2011**, 19(7), 2269-2281.