A Synopsis on

DESIGN AND SYNTHESIS OF NOVEL CHEMICAL ENTITIES FOR THE TREATMENT OF ALZHEIMER'S DISEASE

Submitted

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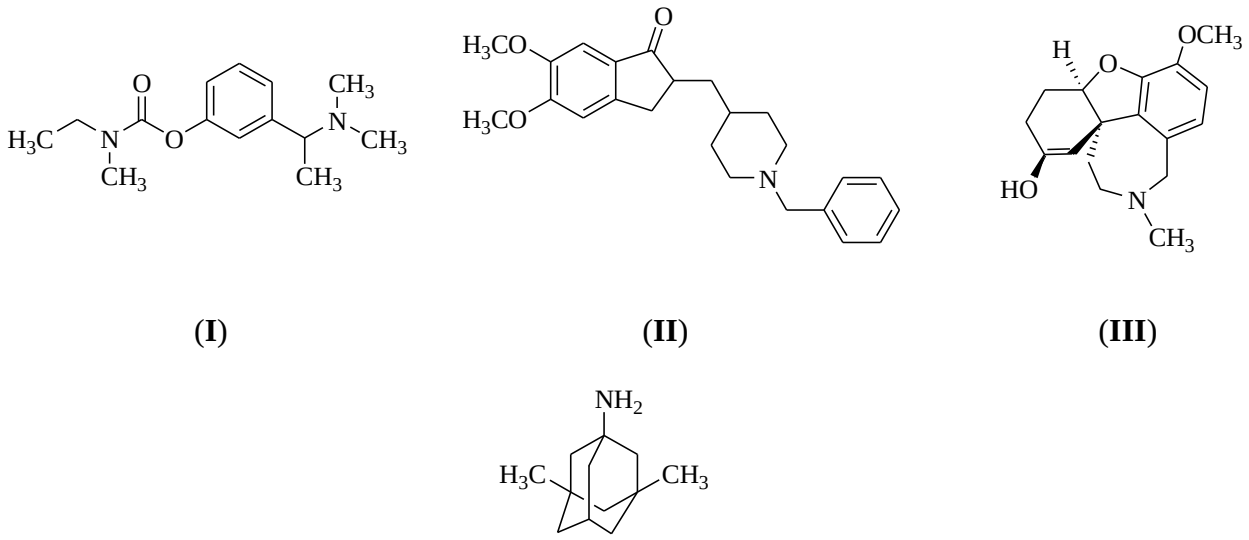
1. Introduction

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 efficactive 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.

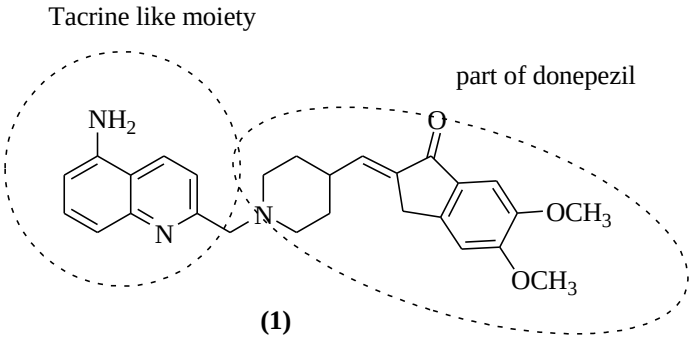
1.1.4 Metal ion dyshomeostasis

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

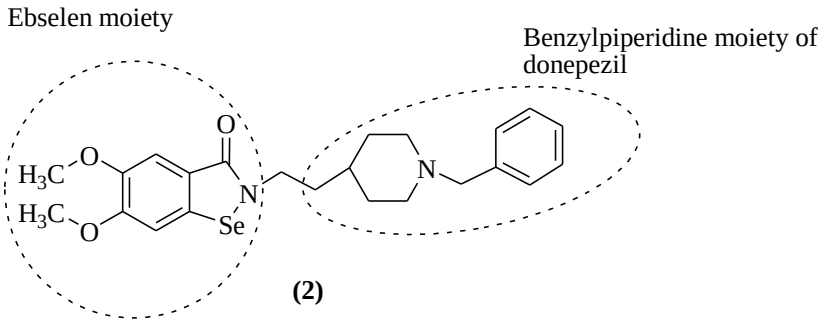
More recently a number of studies have been focused on the development of multitarget drugs to fight AD. Combining of different compounds with potent activity as hybrids are considered as one of the most explored and successful drug design strategies. Development of hybrids that combine the ChEs inhibition role, for the enhancement of the cholinergic neurotransmission, with other relevant roles (e.g., reduction the Aβ fibril self-aggregation, antioxidant and metal depletion) have been reported. Structure of donepezile has been widely used as scaffold to provide new molecules endowed with additional properties beyond simple AChE inhibition[34-35]. Some interesting hybrids of donepezil with an anti-oxidant and other relavent compounds are described below:

Talita *et al.* have reported series of donepezil-tacrine hybrids related derivatives as dual AChEIs, that could bind simultaneously to the peripheral and to the catalytic sites of the enzyme. Among the reported series compound **(1)** shows most potent activity with an IC_{50} value of 0.014 μM. [36]



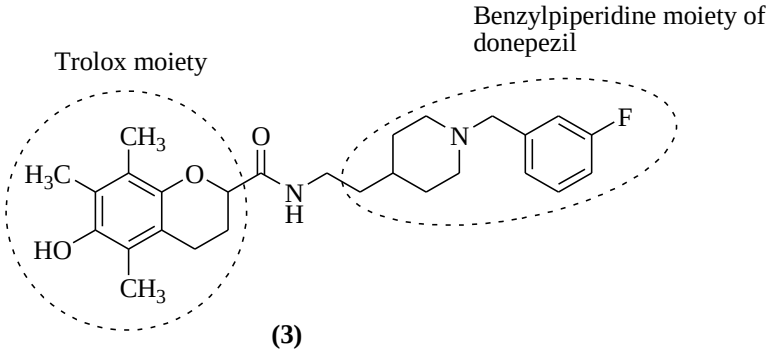
(1)

Luo *et al.* designed fusion of donepezil and ebselen using multi-targeted-directed ligand approach as a anti-Alzheimer agent. Among the reported series compound **(2)** showed highly activity with an IC_{50} value of 0.042 μM. [37]



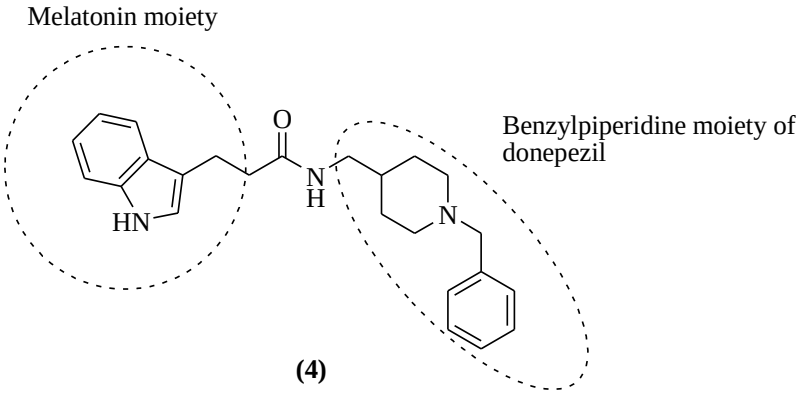
(2)

Pei Cai *et al.* successfully reported series of donepezil and trolox hybrides against Alzheimer's disease with cholinergic, anti-oxident, neuroprotective, and cognition enhancing properties. Compound **(3)** showed good activity with an IC_{50} value of 0.054 μM. [38]



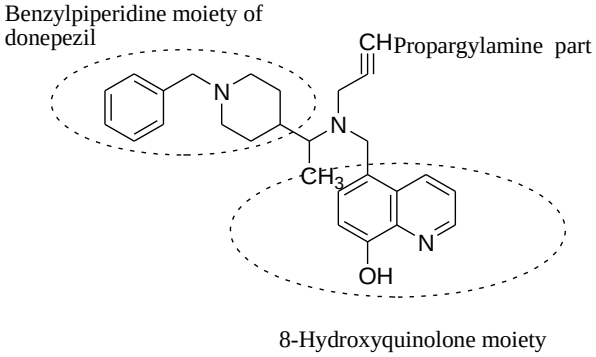
(3)

Wang *et al.* reported series of the compound based on the fusion of donepezil (AChE inhibitor) and melatonin(anti-oxident) as anti-alzheimer agent. Among the reported series compound **(4)** showed good activity with an IC_{50} value of 0.193 μM. [39]



(4)

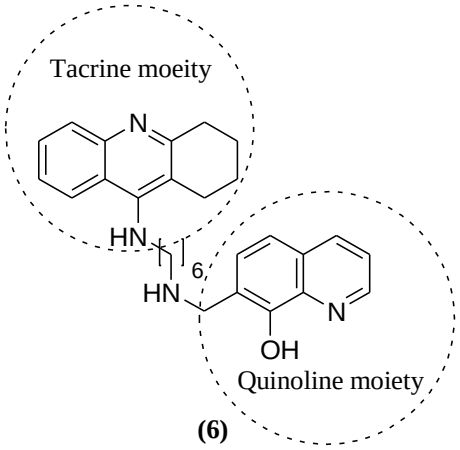
Li wang *et al.* reported the novel scaffold of donepezil, propargylamine and 8-hydroxyquinoline hybrid as a new multifunctional metal-chelator, AChE inhibitor and MAO inhibitors. Among the reported series, compound **(5)** showed highest activity with IC_{50} value of 0.1 μM[40].



(5)

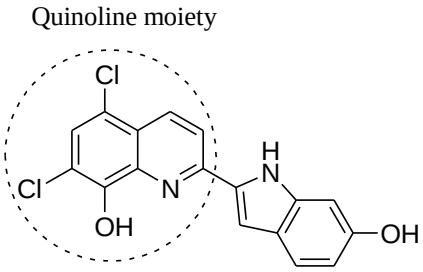
Maria *et al.*Synthesized Novel Tacrine-8-Hydroxyquinoline Hybrids as Multifunctional Agents for the Treatment of Alzheimer's Disease, with Neuroprotective,

Cholinergic, Antioxidant, and Copper-Compl\]exing Properties. Among the reported series, compound **(8)** showed good activity with IC_{50} value of 0.02 μM[41].



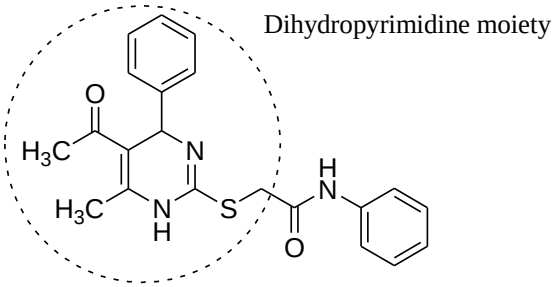
(6)

Wang et al. Designed Orally Bioavailable Quinoline−Indole Derivatives as Innovative Multitarget-Directed Ligands:Promotion of Cell Proliferation in the Adult Murine Hippocampus for the Treatment of Alzheimer's Disease. Among the series Compound **(7)** showed good activity.[42]



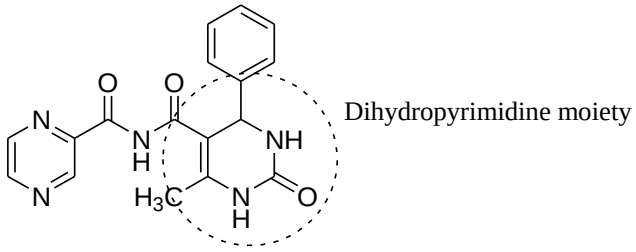
(7)

Ahmad *et al.* synthesized and designed dihydropyrimidine based dual binding site acetylcholinesterase inhibitors. Among the reported series, compound **(8)** showed good activity with IC_{50} value of 0.17 ± 0.01 μM[43].



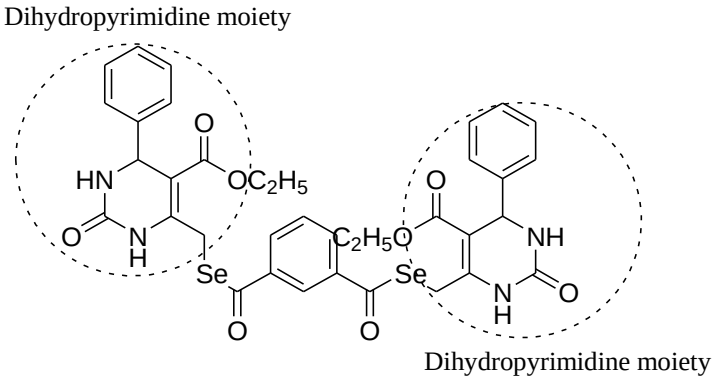
(8)

Karthikeyan *et al.* reported Acetylcholinesterase enzyme inhibitor activity of some novel pyrazinamide condensed 1,2,3,4-tetrahydropyrimidine. Compound **(9)** showed good activity with an IC_{50} value of 0.11 μM. [44]



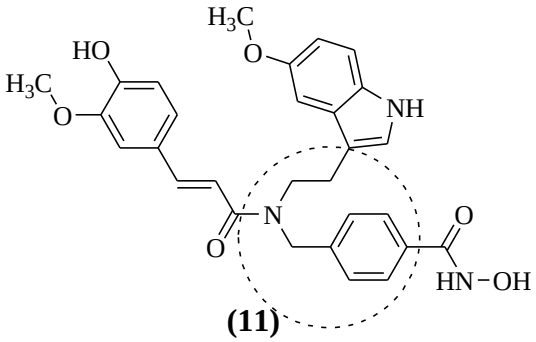
(9)

Flavio et al. reported dihydropyrimidinone-derived selenoesters as multi-targeted directed compounds against Alzheimer's disease. Among the reported series, compound **(10)** showed good activity with IC_{50} value of 0.30 ± 0.1 μM[45].



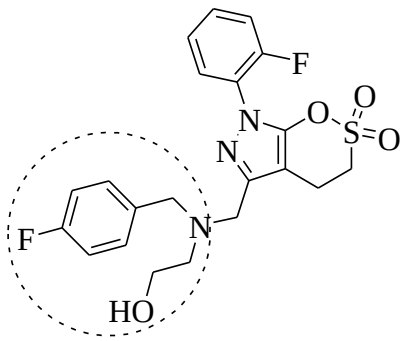
(10)

Feng *etal*. merged melatonin and ferulic acid into tertiary amide-based histone deacetylase 6 (HDAC6) inhibitors to develop MTDL of AD. Compound **(11)** shows pronounced and selective inhibition of HDAC6 with IC_{50} value of 30.7 nM. It also shows comparable DPPH radical scavenging ability to ferulic acid, comparable ORAC value to melatonin and comparable Cu^{2+} chelating ability to EDTA.[46]

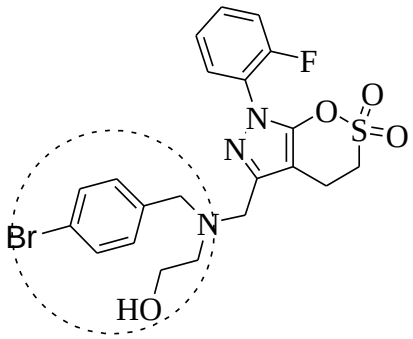


(11)

Z. Zhang *etal.* carried out structure-based optimization of someδ-sulfonolactone-fused pyrazole derivatives to improve the potency and selectivity of BuChE enzymes. Among the reported series, compounds **(12)** and **(13)** with the substitution of benzyl amines were identified as highly selective for BuChE enzyme with the IC_{50} value of 8.3 and 7.7 nM respectively. Both compounds exhibited mild antioxidant activity, nontoxicity, lipophilicity and neuroprotective activity. Kinetic studies showed that BuChE inhibition of compound **(16)** was mixed-type against BuChE (Ki = 24 nM) and > 2000-fold selectivity for BuChE over AChE.[47]

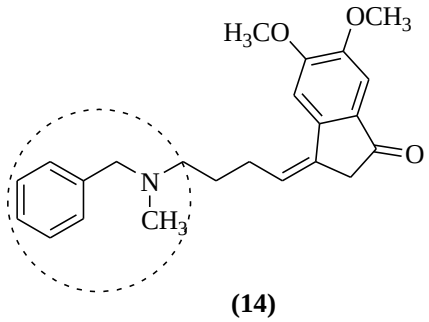


(12)



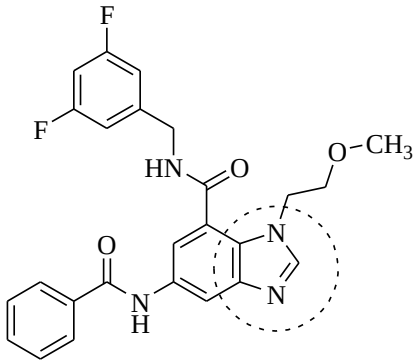
(13)

Luo *et al***(2020)** designed, synthesized and evaluated series of phthalide alkyl tertiary amine derivatives having potential to treat for AD. Among the synthesized compounds, compound **(17)** having displayed the most excellent activity on AChE with IC_{50} value of 2.66 nM, which was significantly better than the control drug Donepezil. [48]



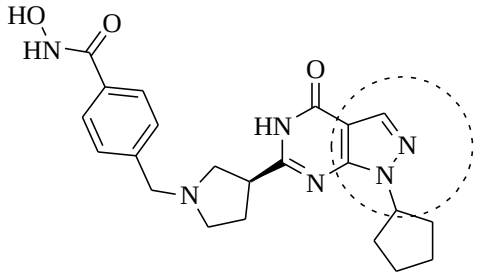
(14)

M.T. Gabr *et al.* designed of multitargeted therapeutics for AD through dual inhibition of acetylcholinesterase (AChE) and microRNA-15b biogenesis. Compound **(15)** was identified by HTS which was the first dual inhibitor of AChE and microRNA-15b biogenesis. These was also showed protection of neuroblastoma cell from Aβ-induced cytotoxicity. [49]



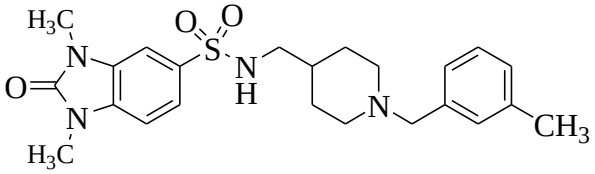
(15)

Rabal *et al*. designed a series of dual-target phosphodiesterase 9 (PDE9) and histone deacetylase (HDAC) inhibitors for the treatment of AD. In the reported series, compound **(32)** showed selectivity for HDAC6 with acceptable brain permeability. [50]

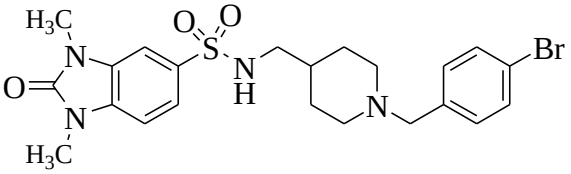


(16)

J. Mo *etal*. designed and synthesized a novel series of 1,3-dimethylbenzimidazolinone derivatives as cholinesterase inhibitors. Among the series, compounds **(17)** and **(18)** showed good inhibitory activity in sub micromolar concentration for *eeAChE* and *eqBuChE* with IC_{50} value of compound **(17)** for AChE was found to be 0.39 µM whereas an IC_{50} value of compound **(18)** towards BuChE was found to be 0.16 µM. [51]



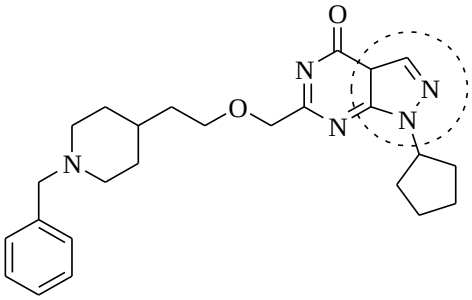
(17)



(18)

J. Hu *et al*. designed, synthesized and evaluated a series dual-target AChE/PDE9A inhibitors as anti-AD. Among the reported series,compound **(19)** showed good activity against AChE and PDE9A with IC_{50} value of 0.048 µM and 0.530 µM respectively and compound **(20)** showed good activity against AChE and PDE9A with IC_{50} value of 0.223 µM

and 0.285 μM respectively. In additionally, these both compounds having good BBB penetrability and low neurotoxicity. [52]



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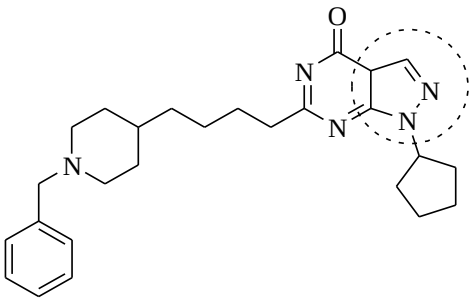
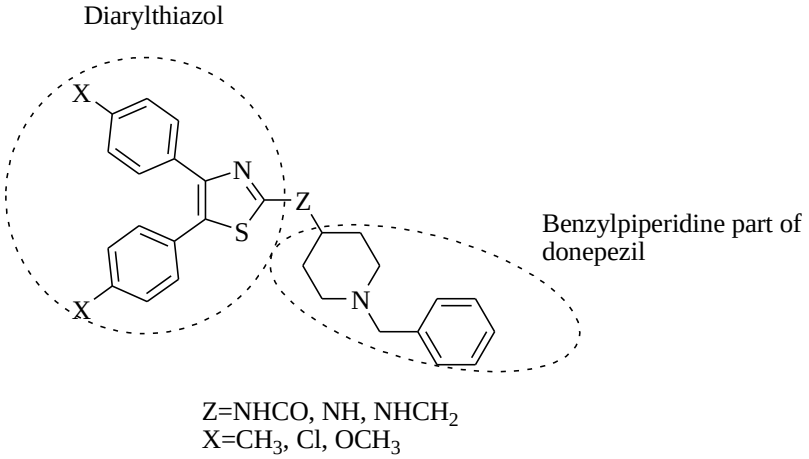


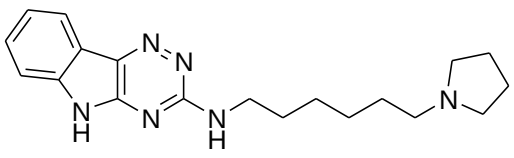
20

Work from our own lab has also been reported wherein benzylpiperidine moiety of donepezil was linked with diarylthiazole to give some of the highly active hybrid compounds. Among the reported series, compound **(21)** showed good activity with IC_{50} value of 0.30 ± 0.1 μM[53].



21

Our lab has recently reported some triazinolindole derivatives as a multi-targeted directed ligand as anti-alzheimer's agent. Among the series compound **(12)** showed highly potent activity with IC_{50} value of 0.56 ± 0.02 μM for AChE and IC_{50} value of 1.17 ± 0.09 μM for BChE. These derivatives also endowed with potent anti-oxidant activity [54].



(22)

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.

Based on the literature review we thought to design a new multifunctional ChEIs endowed with additional properties such as a MAO inhibition, anti-oxidant, matal chealation and amyloid inhibition. As per literature pyrazole moiety was reported as a reported as AChE and MAO-B inhibitor while benzyl amine moiety was also reported with the AChE and BuChE inhibitory activity as well and quinoline was reported as a well known anti-oxidant. So, based on these findings, we thought it was logical to combining the quinoline with the pyrazolone pharmacophore and benzyl amine moiety to develop novel pyrazolone based inhibitors of AD(**Fig 3.1**).

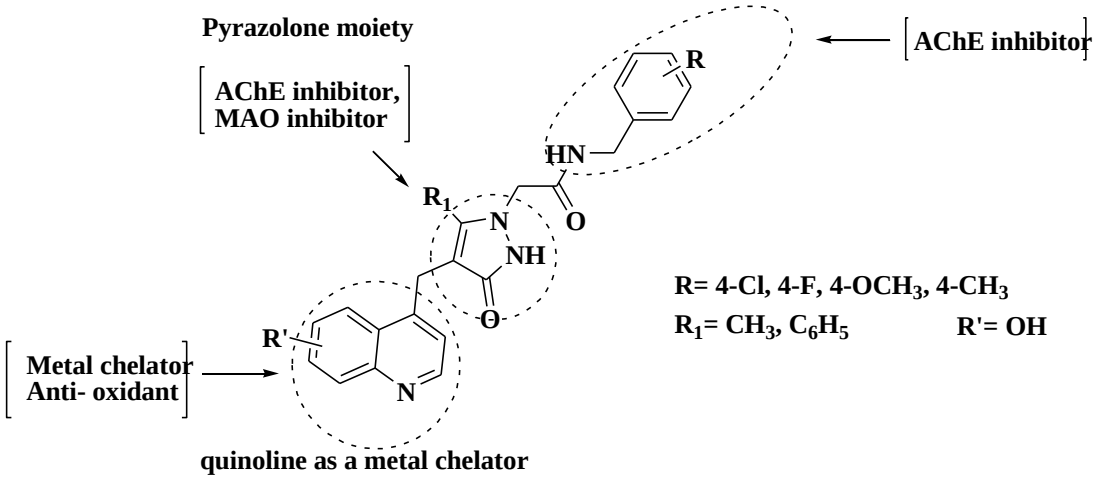
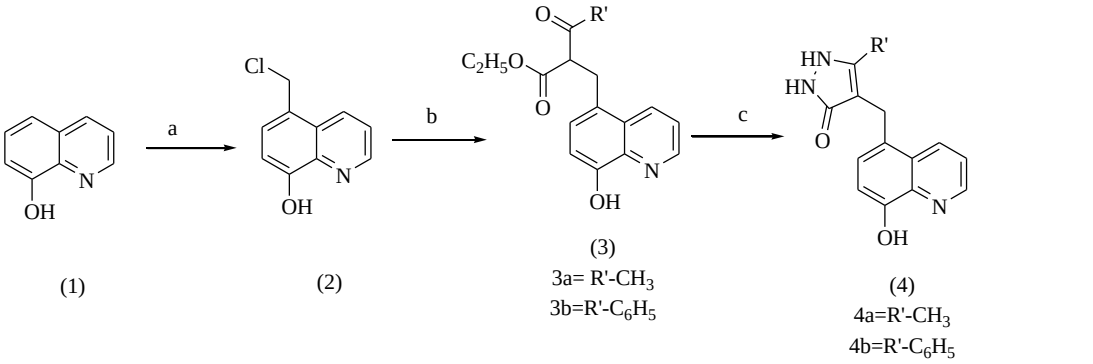


Figure 3.1: Design of anti- Alzheimer's agents

4. Result and Discussion

4.1 Chemical Work

Synthesis of the novel pyrazolone based inhibitors was carried out using the following synthetic scheme 4.1.1.



(7, 8)

7a=R'-CH_3,R= 4-CF_3	8a=R'C_6H_5, R=2-CF3
7b=R'-CH_3,R=3,5-diF	8b=R'C_6H_5, R=4-NO_2
7c=R'-CH_3,R=4-Cl	8c=R'C_6H_5, R=3-OCH_3
7d=R'-CH_3,R=2,6-diCl	8d=R'C_6H_5, R=4-F
7e=R'-CH_3,R=2-CF_3	8e=R'C_6H_5, R=4-CH_3
7f=R'-CH_3,R=2,5-diCH_3	8f=R'C_6H_5, R=3-Cl
7g=R'-CH_3,R=3-Cl	8g=R'C_6H_5, R=4-Cl
7h=R'-CH_3,R=3,5-diCH_3	8h=R'C_6H_5, R=2,6-diF
7i=R'-CH_3,R=4-CH_3	8i=R'C_6H_5, R=H
7j=R'-CH_3,R=H	8j=R'C_6H_5, R=3-F

(5, 6)

5a=R'-C_6H_5,R_1-4F
5b=R'-C_6H_5,R_1-4Cl
5c=R'-C_6H_5,R_1-4OCH_3
6a=R'-CH_3,R_1-F
6b=R'-CH_3,R_1-4OCH_3
6c=R'-CH_3,R_1-4CH_3

Reagents and conditions: (a) HCl, formaldyhide, HCl gas; (b) aryloyl ethyl acetoacetate, DMF, NaH, (c) hydrazine hydrate, EtOH, reflux, 2h; (d) Substituted benzyl amine, DMF, K_2CO_3, RT, 2 h, (e) Substituted benzyl halide, DMF, K_2CO_3, RT, 2 h,

Scheme 4.1.1: Synthesis of novel pyrazolone based inhibitors of AD.

4.1.1 Synthesis of *substituted 4-((8-hydroxyquinolin-5-yl)methyl)-5-methyl-1H-pyrazol-3(2H)-one (5-8)*

Synthesis of substituted *N*-benzyl-2-(3-methyl-5-oxo-4-((quinolin-5-yl))-*1H*-pyrazol-2(*5H*)-yl)acetamide (5 & 6) was carried out as shown in the scheme 4.1. First, 8-hydroxyquinoline **(1)** was treated with conc. HCl and formaldehyde in presence of HCl gas to obtain 5-(chloromethyl)-8-hydroxyquinoline hydrochloride **(2)**. Further nucleophilic substitution reaction was carried out using ethyl acetoacetate and NaH to get the compound **(3)** which on refluxing with hydrazine hydrate undergo cyclization to give 1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-methylpyrazol-5-one **(4).** In the next step, **(4)** was treated with *N*-substituted benzyl-2-chloroacetamide under the basic condition to obtain the desired compound **(5)** and **(6)**. Further, compound **(4)** was further treated with substituted benzyl halides under the basic condition to get the desired compounds **(7)** and **(8).**

- **5-(Chloromethyl)-8-hydroxyquinoline hydrochloride (2)**

  M. P. : 295-296 ºC decomposed [**Reported 294-296ºC**][55]

- **Ethyl 2-((8-hydroxyquinolin-5-yl)methyl)-3-oxobutanoate (3a)**

  M. P. : 74-78 ºC

  IR (KBr, cm^{-1}) : 3332, 2970, 1709, 1225, 840

  ^{1}H-NMR (DMSO) : 0.99-1.02 (t, 3H), 2.17 (s, 3H), 3.31-3.37 (q, 1H), 3.43-3.48 (q, 1H), 3.95-4.04 (m, 3H), 6.97-6.99 (d, 1H), 7.23-7.25 (d, 1H), 7.56-7.59 (q, 1H), 8.47-8.49 (d, 1H), 8.84-8.85 (d, 1H), 9.69 (s, 1H)

  Mass (m/z) : 288.46 $[M+H]^+$

- **Ethyl 2-((8-hydroxyquinolin-5-yl)methyl)-3-oxo-3-phenylpropanoate (3b)**

  M. P. : 76-80 ºC

  IR (KBr, cm^{-1}) : 3334, 1734, 1687, 1276, 840

  ^{1}H-NMR (DMSO) : 0.89-0.92 (t, 3H), 3.51-3.60 (t, 2H), 3.88-3.96 (m, 2H), 4.96-4.99 (t,- 1H), 6.94-6.96 (d, 1H), 7.27-7.29 (s, 1H), 7.45-7.49 (t, 2H), 7.57-7.63 (m, 2H), 7.86-7.88 (d, 2H), 8.55-8.58 (d, 1H), 8.84-8.85 (d, 1H), 9.63 (s, 1H)

  Mass (m/z) : 350.59 $[M+H]^+$

- **4-((8-Hydroxyquinolin-5-yl)methyl)-5-methyl-1*H*-pyrazol-3(2*H*)-one (4a)**

M. P. : 235-237 ºC

IR (KBr, cm^{-1}) : 3333, 3048, 1653, 1174, 840

^{1}H-NMR (DMSO) :1.04(s, 3H), 3.89 (s, 1H), 6.96-6.98(d, 1H), 7.16-7.18 (d, 1H), 7.53-7.56 (m, 1H), 8.60-8.62 (d, 1H), 8.82-8.83 (d, 1H), 9.58 (s, 1H), 10.43 (s, 1H).

Mass (m/z) : 256.39 $[M+H]^+$

- **4-((8-Hydroxyquinolin-5-yl)methyl)-5-phenyl-1*H*-pyrazol-3(2*H*)-one (4b)**

M. P. : 212-215 ºC

IR (KBr, cm^{-1}) : 3324, 2359, 1679, 1198, 833

^{1}H-NMR (DMSO) :3.18 (s, 2H), 6.93-6.94 (d, 2H), 6.99-7.00 (d, 1H), 7.27-7.29 (t, 1H), 67.31-7.34 (t, 2H), 7.39-7.41 (t, 2H), 7.54-7.57 (q, 1H), 8.56-8.58 (d, 1H), 8.85-8.87 (s, 1H), 9.52 (s, 1H), 11.57 (s, 1H)

Mass (m/z) : 318.50 $[M+H]^+$

- **(4-Fluorophenyl)methanamine**

M. P. : 108-112 ºC(Reported 112 ºC) [56]

IR (KBr, cm^{-1}) : 3270, 3092, 1675

- **(4-Chlorophenyl)methanamine**

M. P. : 115-117ºC [Reported 112-116 ºC] [57]

IR (KBr, cm^{-1}) : 3265, 3086, 1645

- **(4-Methoxyphenyl)methanamine**

M. P. : 98-102 ºC (Reported 96-101 ºC) [56]

IR (KBr, cm^{-1}) : 3265, 3086,1645

- **(4-Methylphenyl)methanamine**

M. P. : 123-127 ºC (Reported 122 ºC) [57]

IR (KBr, cm^{-1}) : 3250, 3022, 1655

- ***N-(4-fluorobenzyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)-3-oxo-5-phenyl-2,3-dihydro-1H-pyrazol-1-yl)acetamide (5a)***

M.P. : 180-183 ºC

IR (KBr, cm^{-1}) : 3281, 1679, 1475, 1377, 1124, 827

^{1}H-NMR (DMSO) : 4.26 (s, 2H), 4.29-4.30 (d, 2H), 4.672 (s, 2H), 6.88-6.90 (d, 2H), 7.01-7.03 (d, 1H), 7.07-7.12 (t, 1H), 7.24-7.28 (t, 1H), 7.33-7.42 (m, 5H), 7.52-7.55 (m, 1H), 8.26-8.29 (t, 1H), 8.54-8.56 (d, 1H), 8.85-8.86 (d, 1H), 9.54 (s, 1H), 12.30 (s, 1H)

Mass (m/z) : 483.22 $[M+H]^+$

- ***N-(4-chlorobenzyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)-5-oxo-3-phenyl-1H-pyrazol-2(5H)-yl)acetamide (5b)***

  M.P. : 185-189 ºC

  IR (KBr, cm^{-1}) : 3288, 2958, 2926, 1679, 1439, 822

  Mass (m/z) : 499.19 $[M+H]^+$

- ***N-(4-methoxybenzyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)-5-oxo-3-phenyl-1H-pyrazol-2(5H)-yl)acetamide (5c)***

  M.P. : 176-180 ºC

  IR (KBr, cm^{-1}) : 3306, 3067, 2924, 1664, 1547, 1247, 1074, 818

  ^{1}H-NMR (DMSO) : 3.74-3.59 (s, 3H), 4.16-4.18 (t, 1H), 4.25-4.28 (t, 2H), 4.69 (s, 2H), 6.85-6.86 (d, 1H), 6.91-6.93 (d, 1H),7.04-7.06 (d, 1H), 7.15-7.16 (d, 1H), 7.34-7.45 (m, 5H), 7.34-7.45 (q, 1H), 7.70-7.75 (t, 1H), 8.16-8.18 (t, 1H), 8.56-8.58 (d, 1H), 8.88-8.89 (d, 1H), 9.57 (s, 1H), 12.34 (s, 1H)

  Mass (m/z) : 495.26 $[M+H]^+$

- ***N-(4-fluorobenzyl)-2-(4-((8-hydroxyquinolin-5-yl)methyl)-5-methyl-3-oxo-2,3-dihydro-1H-pyrazol-1-yl)acetamide (6a)***

  M.P. : 192-195 ºC

  IR (KBr, cm^{-1}) : 3358, 3305, 2924, 1638, 1487, 1281, 1113, 886

  ^{1}H-NMR : 1.19-2.02 (s, 3H), 3.90-4.05 (s, 2H), 4.17-4.19 (t, 1H), 4.30-4.36 (d, 2H), 4.66 (d, 2H), 7.02-7.12 (d, 1H), 7.13-7.18 (t, 1H), 7.19-7.24 (d, 1H), 7.54-7.56 (t, 1H), 7.70-7.72 (q, 1H), 7.75-7.77 (m, 1H), 8.32-8.34 (t, 1H), 8.64-8.67 (d, 1H), 8.86-8.89 (d, 1H), 9.57 (s, 1H), 11.59 (s, 1H).

  Mass (m/z) : 421.22 $[M+H]^+$

- ***2-(4-((8-Hydroxyquinolin-5-yl)methyl)-5-methyl-3-oxo-2,3-dihydro-1H-pyrazol-1-yl)-N-(4-methylbenzyl)acetamide (6b)***

  M.P. : 184-187ºC

  IR (KBr, cm^{-1}) : 3377, 2959, 1654, 1412, 1210, 896

  ^{1}H-NMR : 1.19-2.03 (s, 3H), 2.31-2.32 (s, 3H), 4.05 (s, 2H), 4.28-4.33 (d, 2H), 4.65 (s, 2H), 6.90-7.02 (d, 1H), 7.13-7.19 (m, 4H), 7.24-7.26 (d, 1H), 7.54-7.56 (q, 1H), 8.24-8.26 (s, 1H), 8.64-8.66 (d, 1H), 8.86-8.87 (d, 1H), 9.56 (s, 1H), 11.58 (s, 1H)

Mass (m/z) : 417.25 $[M+H]^+$

- ***2-(4-((8-Hydroxyquinolin-5-yl)methyl)-5-methyl-3-oxo-2,3-dihydro-1H-pyrazol-1-yl)-N-(4-methoxybenzyl)acetamide (6c)***

M.P. : 188-192 ^oC

IR (KBr, cm^{-1}) : 3279, 2959, 1658, 1462, 1274, 783

^{1}H-NMR : 1.95 (s, 3H), 3.73 (s, 3H), 4.00 (s, 2H), 4.10-4.15 (q, 2H), 4.25-4.26 (d, 2H), 4.59 (s, 2H), 6.84 -7.22 (m, 4H), 7.49-7.52 (q, 1H), 7.60-7.70 (m, 1H), 8.18-8.21(t, 1H), 8.82-8.83 (d, 1H), 9.54 (s, 1H), 11.54 (s, 1H)

Mass (m/z) : 433.23 $[M+H]^+$

- ***2-(4-(Trifluoromethyl)benzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-methylpyrazol-5-one (7a)***

M.P. : 205-207 ^oC

IR (KBr, cm^{-1}) : 3391, 2958, 1696, 1473, 1254, 919

^{1}H-NMR :δ 11.53 (s, 1H), 9.56 (s, 1H), 8.83 (dd, *J* = 4.1, 1.6 Hz, 1H), 8.54 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.68 (d, *J* = 8.1 Hz, 3H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.19 (d, *J* = 7.8 Hz, 1H), 6.98 (d, *J* = 7.8 Hz, 1H), 5.26 (s, 2H), 3.96 (s, 3H), 2.00 (s, 3H)

Mass (m/z) : 414.23 $[M+H]^+$

- ***2-(3,5-Difluorobenzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-methylpyrazol-5-one(7b)***

M.P. : 210-213 ^oC

IR (KBr, cm^{-1}) : 3306, 2920, 1627, 1452, 1222, 987

^{1}H-NMR : 11.53 (s, 1H), 9.56 (s, 1H), 8.83 (dd, *J* = 4.1, 1.6 Hz, 1H), 8.54 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.68 (d, *J* = 8.1 Hz, 3H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.19 (d, *J* = 7.8 Hz, 1H), 6.98 (d, *J* = 7.8 Hz, 1H), 5.26 (s, 2H), 3.96 (s, 3H), 2.00 (s, 3H)

Mass (m/z) : 382.23 $[M+H]^+$

- ***2-(4-Chlorobenzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-methylpyrazol-5-one(7c)***

M.P. : 212-214 ^oC

IR (KBr, cm^{-1}) : 3356, 2997, 1695, 1499, 1214, 994

^{1}H-NMR : 11.51 (s, 1H), 9.56 (s, 1H), 8.83 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.53 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.48 (dd, *J* = 8.6, 4.1 Hz, 1H), 7.37 (q, *J* = 8.8 Hz, 4H), 7.17 (d, *J* = 7.9 Hz, 1H), 6.97 (d, *J* = 7.8 Hz, 1H), 5.15 (s, 2H),3.93 (s, 2H),1.99 (s, 3H)

Mass (m/z) : 380.23 $[M+H]^{+}$, 382.20 $[M+2]^{+}$

- ***2-(2,6-Dichlorobenzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-methylpyrazol-5-one(7d)***

M.P. : 208-211 ^{o}C

IR (KBr, cm^{-1}) : 3357, 2945, 1626, 1473, 1276, 822

^{1}H-NMR : 11.55 (s, 1H), 9.56 (s, 1H), 8.83 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.52 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.63 (s, 1H), 7.36 (d, *J* = 1.6 Hz, 2H), 7.19 (d, *J* = 7.9 Hz, 1H), 6.98 (d, *J* = 7.9 Hz, 1H), 5.20 (s, 2H), 3.95 (s, 2H), 2.00 (s, 3H)

Mass (m/z) : 414.28 $[M+H]^{+}$, 416.15 $[M+2]^{+}$

- ***2-(2-(Trifluoromethyl)benzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-methylpyrazol-5-one(7e)***

M.P. : 211-214 ^{o}C

IR (KBr, cm^{-1}) : 3347, 2922, 1588, 1452, 1226, 821

^{1}H-NMR : 11.53 (s, 1H), 9.53 (s, 1H), 8.82 (dd, *J* = 4.1, 1.6 Hz, 1H), 8.52 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.74 (d, *J* = 7.6 Hz, 1H), 7.60 (t, *J* = 6.9 Hz, 1H), 7.54 (s, 1H), 7.51 (d, *J* = 7.0 Hz, 1H), 7.46 (dd, *J* = 8.6, 4.1 Hz, 1H), 7.19 (d, *J* = 7.9 Hz, 1H), 6.98 (d, *J* = 7.8 Hz, 1H), 5.33 (s, 2H), 3.96 (s, 2H), 2.00 (s, 3H)

Mass (m/z) : 414.13 $[M+H]^{+}$

- ***2-(2,5-Dimethylbenzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-methylpyrazol-5-one(7f)***

M.P. : 217-220 ^{o}C

IR (KBr, cm^{-1}) : 3311, 2956, 1684, 1473, 1218, 836

^{1}H-NMR : 12.24 (s, 1H), 9.57 (s, 1H), 8.85 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.51 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.51 (dd, *J* = 8.6, 4.2 Hz, 1H), 7.45 – 7.31 (m, 4H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.92 (d, *J* = 7.9 Hz, 1H), 5.13 (s, 2H), 4.14 (s, 2H), 2.19 (s, 6H)

Mass (m/z) : 374.18 $[M+H]^{+}$

- ***2-(3-chlorobenzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-methylpyrazol-5-one(7g)***

  M.P. : 214-216 ^{o}C

  IR (KBr, cm^{-1}) : 3327, 2924, 1686, 1421, 1225, 822

  ^{1}H-NMR : 11.52 (s, 1H), 9.55 (s, 1H), 8.83 (dd, *J* = 4.1, 1.5 Hz, 1H), 8.55 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.49 (dd, *J* = 8.6, 4.2 Hz, 1H), 7.41 – 7.31 (m, 4H), 7.19 (d, *J* = 7.9 Hz, 1H), 6.97 (dd, *J* = 7.8, 2.9 Hz, 2H), 5.18 (s, 2H), 3.94 (s, 2H), 2.00 (s, 3H)

  Mass (m/z) : 380.28 $[M+H]^{+}$, 382.10 $[M+2]^{+}$

- ***2-(3,5-Dimethylbenzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-methylpyrazol-5-one(7h)***

  M.P. : 218-222 ^{o}C

  IR (KBr, cm^{-1}) : 3314, 2971, 1604, 1583, 1471, 837

  ^{1}H-NMR : 11.47 (s, 1H), 9.54 (s, 2H), 8.83 (dd, *J* = 4.3, 1.6 Hz, 1H), 8.56 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.47 (dd, *J* = 8.6, 4.1 Hz, 1H), 7.17 (dd, *J* = 12.3, 7.8 Hz, 2H), 6.97 (dd, *J* = 7.8, 2.9 Hz, 2H), 6.91 (d, *J* = 3.7 Hz, 3H), 5.07 (s, 2H), 3.92 (s, 2H), 2.22 (s, 6H)

  Mass (m/z) : 374.18 $[M+H]^{+}$

- ***2-(4-Methylbenzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-methylpyrazol-5-one(7i)***

  M.P. : 213-216 ^{o}C

  IR (KBr, cm^{-1}) : 3139, 2921, 1592, 1496, 1269, 941

  ^{1}H-NMR : 11.48 (s, 1H), 9.54 (s, 1H), 8.82 (dd, *J* = 4.1, 1.6 Hz, 1H), 8.54 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.46 (dd, *J* = 8.6, 4.1 Hz, 1H), 7.24 (d, *J* = 8.1 Hz, 2H), 7.19 – 7.12 (m, 3H), 6.96 (d, *J* = 7.8 Hz, 1H), 5.11 (s, 2H), 3.91 (s, 2H), 2.29 (s, 3H), 1.98 (s, 3H)

  Mass (m/z) : 360.1 $[M+H]^{+}$

- ***2-Benzyl-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-methylpyrazol-5-one(7j)***

  M.P. : 208-211 ^{o}C

  IR (KBr, cm^{-1}) : 3180, 2897, 1650, 1485, 1290, 886

  ^{1}H-NMR : 9.51 (s, 1H), 8.85 (dd, *J* = 4.2, 1.6 Hz, 1H), 8.62 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.57 (dd, *J* = 8.5, 4.2 Hz, 1H), 7.36 – 7.24 (m, 5H), 6.98 (dd, *J* = 7.8, 2.5 Hz, 2H), 5.04 (s, 2H), 3.93 (s, 2H), 1.97 (s, 3H)

Mass (m/z) : 346.39 $[M+H]^+$

- ***2-(2-(Trifluoromethyl)benzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-phenylpyrazol-5-one (8a)***

M.P. : 242-246 ºC

IR (KBr, cm^{-1}) :3329, 1623, 1474, 1270, 971

^{1}H-NMR : 12.31 (s, 1H), 9.58 (s, 1H), 8.85 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.50 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.74 – 7.69 (m, 1H), 7.60 – 7.33 (m, 10H), 7.02 (d, *J* = 7.9 Hz, 1H), 6.94 (d, *J* = 7.9 Hz, 1H), 5.40 (s, 2H), 4.18 (s, 2H)

Mass (m/z) : 476.46 $[M+H]^+$

- ***2-(4-Nitrobenzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-phenylpyrazol-5-one(8b)***

M.P. : 238-241 ºC

IR (KBr, cm^{-1}) : 3305, 2944, 1602, 1415, 1270, 971

^{1}H-NMR : 12.30 (s, 1H), 9.60 (s, 1H), 8.86 (dd, *J* = 4.1, 1.5 Hz, 1H), 8.50 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.57 – 7.47 (m, 5H), 7.41 (ddd, *J* = 8.2, 7.1, 5.5 Hz, 4H), 7.20 – 7.13 (m, 1H), 7.02 (d, *J* = 7.9 Hz, 1H), 6.96 (d, *J* = 7.9 Hz, 1H), 5.37 (s, 2H), 4.19 (s, 2H)

Mass (m/z) : 453.14 $[M+H]^+$

- ***2-(3-Methoxybenzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-phenylpyrazol-5-one(8c)***

M.P. : 188-192 ºC

IR (KBr, cm^{-1}) : 3351, 2962, 1704, 1472, 1040

^{1}H-NMR : 12.26 (s, 1H), 9.56 (s, 1H), 8.85 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.53 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.53 – 7.45 (m, 3H), 7.43 – 7.32 (m, 7H), 7.00 – 6.97 (m, 1H), 6.83 (dd, *J* = 8.2, 2.6 Hz, 1H), 5.20 (s, 2H), 4.15 (s, 2H), 3.66 (s, 3H)

Mass (m/z) : 438.17 $[M+H]^+$

- ***2-(4-Fluorobenzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-phenylpyrazol-5-one(8d)***

M.P. : 188-192 ºC

IR (KBr, cm^{-1}) : 3186, 2890, 1598, 1498, 1224

^{1}H-NMR : 9.50 (s, 1H), 8.81 (dd, *J* = 4.2, 1.5 Hz, 2H), 8.35 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.47 (dd, *J* = 8.6, 4.1 Hz, 1H), 7.39 – 7.34 (m,

5H), 7.20 (dd, *J* = 6.6, 3.0 Hz, 2H), 7.01 (d, *J* = 5.5 Hz, 2H), 6.97 (d, *J* = 7.9 Hz, 1H), 6.91 – 6.85 (m, 2H), 4.97 (s, 2H), 3.91 (s, 2H)

Mass (m/z) : 426.25 $[M+H]^+$

- ***2-(4-Methylbenzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-phenylpyrazol-5-one(8e)***

M.P. : 188-192 ^oC

IR (KBr, cm^{-1}) : 3325, 2945, 1642, 1622, 1444, 1272, 757

^{1}H-NMR : 9.56 (s, 1H), 8.85 (dd, *J* = 4.1, 1.6 Hz, 1H), 8.51 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.51 (dd, *J* = 8.6, 4.2 Hz, 1H), 7.43 – 7.29 (m, 6H), 7.20 (d, *J* = 8.1 Hz, 2H), 7.11 (d, *J* = 7.9 Hz, 2H), 6.97 (d, *J* = 7.9 Hz, 1H), 6.91 (d, *J* = 7.9 Hz, 1H), 5.17 (s, 2H), 4.13 (s, 2H), 2.27 (s, 3H)

Mass (m/z) : 422.17 $[M+H]^+$

- ***2-(3-Chlorobenzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-phenylpyrazol-5-one(8f)***

M.P. : 188-192 ^oC

IR (KBr, cm^{-1}) : 3233, 1577, 1221, 998

^{1}H-NMR : 12.28 (s, 1H), 9.56 (s, 1H), 8.85 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.51 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.51 (dd, *J* = 8.6, 4.2 Hz, 1H), 7.44 – 7.32 (m, 8H), 7.28 – 7.24(m, 1H), 6.99 (d, *J* = 7.9 Hz, 1H), 6.93 (s, 1H), 5.23 (s, 2H), 4.16 (s, 2H).

Mass (m/z) : 442.17 $[M+H]^+$, 444.10 $[M+2]^+$

- ***2-(4-Chlorobenzyl)-1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-phenylpyrazol-5-one(8g)***

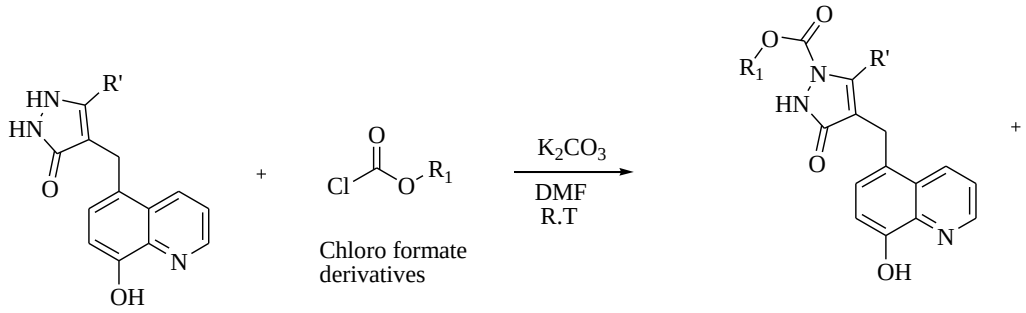
M.P. : 188-192 ^oC

IR (KBr, cm^{-1}) : 3307, 2944, 1675, 1498, 1222, 775

^{1}H-NMR : 12.27 (s, 1H), 9.58 (s, 1H), 8.85 (dd, *J* = 4.1, 1.6 Hz, 1H), 8.50 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.51 (dd, *J* = 8.6, 4.2 Hz, 1H), 7.45 – 7.27 (m, 9H), 6.98 (d, *J* = 8.0 Hz, 1H), 6.93 (d, *J* = 7.9 Hz, 1H), 5.21 (s, 2H), 4.15 (s, 2H)

Mass (m/z) : 442.17 $[M+H]^+$, 444.10 $[M+2]^+$

4.1.2. Scheme 2:



(4)
4a=R'-CH_3
4b=R'-C_6H_5

(9) =R'-CH_3, **(10)** R'-C_6H_5

9a, 10a, R_1, Phenyl Chloroformate
9b,10b, R_1, Methyl Chloroformate
9c,10c, R_1, Benzyl Chloroformate
9d, 10d, R_1, n-Pentyl Chloroformate
9e, 10e, R_1, 2,2,2-Trichloro Chloroformate
9f, 10f, R_1, Flouroenylmethyl Chloroformate
9g, 10g, R_1, Ethyl Chloroformate
9h, 10h, R_1, Isobutyl Chloroformate

4.1.2 Synthesis of *substituted 1,2-dihydro-4-((8-hydroxyquinolin-5-yl)methyl)-3-methyl-2-propylpyrazol-5-one (8)*

Compound **(4)** was further treated with different chlorofarmate derivatives under the basic condition to get the compound **(9)** and **(10).**

- ***Phenyl 4-((8-hydroxyquinolin-5-yl)methyl)-3-methyl-5-oxo-1H-pyrazole-2(5H)-carboxylate (9a)***

  M.P. : 202-204 ^oC

  IR (KBr, cm^{-1}) : 3333, 2943, 1746, 1690, 1421, 1275, 906

  ^{1}H-NMR : 11.32 (s, 1H), 9.62 (s, 1H), 8.87 (dd, *J* = 4.1, 1.5 Hz, 1H), 8.62 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.62 (dd, *J* = 8.6, 4.2 Hz, 1H), 7.50 – 7.43 (m, 2H), 7.35 – 7.28 (m, 3H), 7.15 (d, *J* = 8.0 Hz, 1H), 7.00 (d, *J* = 7.9 Hz, 1H), 4.02 (s, 2H), 2.39 (s, 3H)

  Mass (m/z) : 376.22 $[M+H]^+$

- ***Methyl 4-((8-hydroxyquinolin-5-yl)methyl)-3-methyl-5-oxo-1H-pyrazole-2(5H)-carboxylate(9b)***

  M.P. : 208-211 ^oC

  IR (KBr, cm^{-1}) : 3399, 2943, 1750, 1618, 1210, 934

^{1}H-NMR : 12.27 (s, 1H), 9.61 (s, 1H), 8.59 (dd, *J* = 8.6, 1.6 Hz, 1H), 8.36 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.55 (dd, *J* = 8.6, 4.1 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 6.98 (d, *J* = 7.9 Hz, 2H), 3.95 (s, 3H), 3.84 (s, 2H), 2.07 (s, 3H)

Mass (m/z) : 314.10 $[M+H]^+$

- ***Benzyl 4-((8-hydroxyquinolin-5-yl)methyl)-3-methyl-5-oxo-1H-pyrazole-2(5H)-carboxylate(9c)***

M.P. : 198-201 ºC

IR (KBr, cm^{-1}) : 3334, 2987, 1729, 1630, 1471, 971

^{1}H-NMR : 11.13 (s, 1H), 9.61 (s, 1H), 8.86 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.58 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.59 (dd, *J* = 8.6, 4.2 Hz, 1H), 7.48 – 7.36 (m, 5H), 7.09 (d, *J* = 7.9 Hz, 1H), 6.97 (d, *J* = 7.9 Hz, 1H), 5.31 (s, 2H), 3.96 (s, 2H), 2.35 (s, 3H)

Mass (m/z) : 390.14 $[M+H]^+$

- ***Pentyl 4-((8-hydroxyquinolin-5-yl)methyl)-3-methyl-5-oxo-1H-pyrazole-2(5H)-carboxylate(9d)***

M.P. : 211-214 ºC

IR (KBr, cm^{-1}) : 3335, 2940, 1731, 1624, 1416, 1218

^{1}H-NMR : 11.10 (s, 1H), 9.60 (s, 1H), 8.86 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.59 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.60 (dd, *J* = 8.6, 4.1 Hz, 1H), 7.10 (d, *J* = 7.9 Hz, 1H), 6.98 (d, *J* = 7.9 Hz, 1H), 4.24 (t, *J* = 6.6 Hz, 2H), 3.96 (s, 2H), 2.34 (s, 4H), 1.67 (p, *J* = 6.9 Hz, 2H), 1.35 – 1.30 (m, 4H), 0.90 – 0.85 (m, 4H)

Mass (m/z) : 370.16 $[M+H]^+$

- ***2,2,2-Trichloroethyl 4-((8-hydroxyquinolin-5-yl)methyl)-3-methyl-5-oxo-1H-pyrazole-2(5H)-carboxylate(9e)***

M.P. : 212-116 ºC

IR (KBr, cm^{-1}) : 3279, 2959, 1730,1658, 1462, 1274, 783

^{1}H-NMR : 11.40 (s, 1H), 9.62 (s, 1H), 8.86 (dd, *J* = 4.1, 1.6 Hz, 1H), 8.59 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.61 (dd, *J* = 8.6, 4.2 Hz, 1H), 7.12 (d, *J* = 7.9 Hz, 1H), 6.98 (d, *J* = 7.9 Hz, 1H), 5.11 (s, 2H), 3.99 (s, 2H), 2.39 (s, 3H)

Mass (m/z) : 430.78$[M^+]$, 432.36 $[M+2]^+$

- ***(9H-Fluoren-9-yl)methyl 4-((8-hydroxyquinolin-5-yl)methyl)-3-methyl-5-oxo-1H-pyrazole-2(5H)-carboxylate(9f)***

M.P. : 220-223 ^{o}C

IR (KBr, cm^{-1}) : 3338, 2992, 1742, 1627, 1261, 961

^{1}H-NMR : 11.10 (s, 1H), 9.61 (s, 1H), 8.86 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.57 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.89 (d, *J* = 4.9 Hz, 2H), 7.76 (d, *J* = 7.3 Hz, 2H), 7.60 (dd, *J* = 8.6, 4.1 Hz, 1H), 7.42 (t, *J* = 7.2 Hz, 2H), 7.35 (d, *J* = 7.5 Hz, 2H), 7.05 (d, *J* = 7.9 Hz, 1H), 6.97 (d, *J* = 7.9 Hz, 1H), 4.71 (d, *J* = 6.2 Hz, 2H), 4.40 (t, *J* = 6.3 Hz, 1H), 3.93 (s, 2H), 2.04 (s, 2H)

Mass (m/z) : 478.17 $[M+H]^{+}$

- ***Ethyl 4-((8-hydroxyquinolin-5-yl)methyl)-3-methyl-5-oxo-1H-pyrazole-2(5H)-carboxylate(9g)***

M.P. : 201-204 ^{o}C

IR (KBr, cm^{-1}) : 3359, 2985, 1739, 1620, 1472, 1209, 821

^{1}H-NMR : 11.11 (s, 1H), 9.60 (s, 1H), 8.86 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.59 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.60 (dd, *J* = 8.7, 4.2 Hz, 1H), 7.10 (d, *J* = 7.9 Hz, 1H), 6.98 (d, *J* = 7.9 Hz, 1H), 4.29 (q, *J* = 7.1 Hz, 2H), 3.96 (s, 2H), 2.34 (s, 3H), 1.29 (t, *J* = 7.1 Hz, 3H)

Mass (m/z) : 328.12 $[M+H]^{+}$

- ***Isobutyl 4-((8-hydroxyquinolin-5-yl)methyl)-3-methyl-5-oxo-1H-pyrazole-2(5H)-carboxylate(9h)***

M.P. : 207-210 ^{o}C

IR (KBr, cm^{-1}) : 3315, 2989, 1728, 1636, 1278, 994

^{1}H-NMR : 11.11 (s, 1H), 9.60 (s, 1H), 8.86 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.59 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.60 (dd, *J* = 8.6, 4.2 Hz, 1H), 7.10 (d, *J* = 7.9 Hz, 1H), 6.98 (d, *J* = 7.8 Hz, 1H), 4.04 (d, *J* = 6.7 Hz, 2H), 3.96 (s, 2H), 2.34 (s, 3H), 0.94 (d, *J* = 6.7 Hz, 7H)

Mass (m/z) : 356.15 $[M+H]^{+}$

4.2 Biological Evaluation

4.2.1 AChE/BuChE Assay

- *In vitro* biological analysis of AChE and BuChE inhibition is carried out by adaptation of spectroscopic method described by Ellman.[58]

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.[59, 60]

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

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