Approved Cholinesterase Inhibitor-Based Derivatives: Synthesis and Their Biological Evaluation

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Mange Ram Yadav, Prashant R. Murumkar, Karan Joshi, Rahul Barot, and Rasana Yadav

Abstract

Discovery of therapeutics for multifactorial diseases and disorders involving multiple pathophysiological pathways is still a challenge for the researchers. Alzheimer's disease (AD) is one such disease that involves multiple largely ambiguous pathophysiological pathways, which makes it a challenging thrust area of research around the globe. The current approved drugs for the management of the disease include rivastigmine, donepezil, galantamine (all AChE inhibitors), and memantine (NMDA receptor antagonist). Furthermore, bulk of the research on AD focuses on multi-target directed ligand (MTDL) approach which involves combining together two or more distinct pharmacophores to obtain potent MTDLs. In this chapter, we have discussed synthesis and biological evaluation of the most active novel moieties developed on the basis of the existing anti-Alzheimer's drugs reported in the last 5 years, along with the in silico overview of their interactions with the biological targets.

Keywords

Alzheimer's disease · Multifactorial disease · AChE inhibitor · BuChE inhibitor NMDA blocker · Pharmacophore · Multi-target directed ligand

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7.1 Introduction

Alzheimer's disease (AD) is considered to be a nonreversible chronic neurological disease currently. It has affected almost 55 million people till date and would affect around 78 million people worldwide by 2030 (https://www.Alzint.Org/Resource/World-Alzheimer-Report-2021/). Serious efforts have been made by the scientific community at various levels for the development of newer anti-AD drugs all over the world. Common symptoms of AD include memory loss and challenges in solving day-to-day problems and planning of work, along with decreased judgment and mood swings. Major causes listed for AD include neuron death, diminishing levels of neurotransmitter acetylcholine (ACh), metal dyshomeostasis, oxidative stress, formation of amyloid plaques, and tau protein disruption in the brain (Ashraf et al. 2019; Ge et al. 2022). Recently, activation of choline acetyltransferase (CHAT) activators (Kumar et al. 2019; Darreh-Shori et al. 2019) has been considered as an emerging approach for alleviating the problem, wherein acetylcholine synthesis is increased, thereby increasing the concentration of this neurotransmitter in the brain. A combination of drugs having the ability to increase the synthesis of acetylcholine (ACh) through CHAT activation along with inhibition of AChE and BuChE could be a significant breakthrough to treat the AD (Leng and Edison 2021).

Since AD is a multifactorial disease, there is no singular treatment for the disease (Yadav et al. 2023a). Use of multi-target directed ligands (MTDLs) as drugs is the most commonly advocated approach for the management of AD (Yadav et al. 2018). As far as MTDLs for AChE inhibitors are concerned, they consist of two or more pharmacophores having the potential to bind to the peripheral active site (PAS) and catalytic anionic site (CAS) of AChE fused together to form unique molecules having the potential to exhibit potent effect in inhibiting the breakdown of the neurotransmitter ACh, thereby increasing ACh levels in the brains of the AD patients (Shidore et al. 2016; Barmade et al. 2017). Novel compounds have been synthesized which act on multiple targets, like inhibition of AChE and BuChE enzymes, inhibition of Aβ aggregation, preventing tau hyper-phosphorylation, having metal chelating ability, and ability to scavenge free radicals.

7.2 Current Treatment Available for AD Patients

Currently available treatment for AD includes four major drugs (**1**–**4**). Out of them, three drugs donepezil (**1**), rivastigmine (**2**), and galantamine (**3**) (natural product) are AChE inhibitors, whereas memantine (**4**) is an NMDA receptor blocker. Tacrine (**5**), the first anti-AD drug introduced in the market in 1993, is another AChE inhibitor which had to be withdrawn from the market in 2013 due to its hepatotoxic side effect (Gupta 2014). A new drug in the form of monoclonal antibody Aduhelm was approved by the FDA in 2021 as a β-amyloid aggregation inhibitor, but the drug does not have much presence in the market. Figure 7.1 shows a brief timeline of approved drugs for AD from 1993 till 2021 (Zhang et al. 2022). Thus, discovering new drugs to combat AD remains a vital necessity for the whole world, as there is no

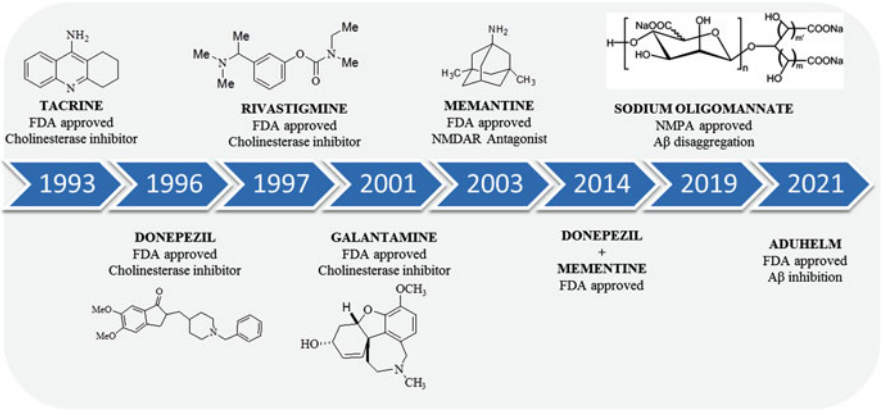
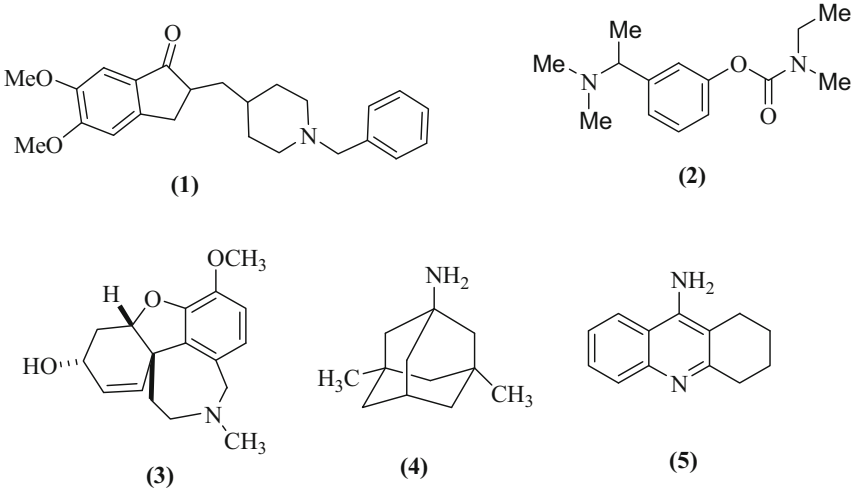


Fig. 7.1 Chronology of approved anti-Alzheimer's drugs

specific drug which can be used to terminate or reverse the progression of AD. Therefore, research for the discovery of newer molecules remains a priority as the number of dementia patients is increasing with every passing day.



Before designing of any new derivative, in silico interactions produced by the standard drugs with acetylcholinesterase and butyrylcholinesterase enzymes would give enough insight to assess the stability of the protein-ligand complexes. Before considering the molecular interactions of the ligands with the enzymes, knowledge of the structures and active sites of the enzymes AChE and BuChE becomes important to see the correlation of the groups, which would be interacting with the enzymes' active sites.

7.3 Structure of Acetylcholinesterase and Butyrylcholinesterase

AChE enzyme hydrolyzes the ACh at the synaptic cleft of the neurons where the inhibition of AChE would result in increased concentration of the neurotransmitter, therefore compensating its deficit, which is a characteristic feature in AD patients (Martorana et al. 2010). The enzyme has ellipsoidal structure and is about 45 Å × 60 Å × 65 Å in size. At the bottom of a deep-narrow gorge of about 20 Å, it contains a catalytic active site (CAS) having 14 aromatic amino acid chains composed of mainly Tyr-70, 121, 334, Trp 84, 279, and Phe-288, 290, and 330 residues, where the hydrolysis of ACh takes place. At the mouth of the enzyme, an additional binding site, the peripheral anionic site (PAS), is present. Between these two binding sites, an array of low-affinity binding sites are present which are spread throughout the gorge that facilitates the binding of the ligands to the CAS site of the enzyme (Harel et al. 1993; Galdeano et al. 2012).

Butyrylcholinesterase (BuChE) is another hydrolyzing enzyme responsible for the hydrolysis of ACh having about 65% homology with the amino acid sequence of AChE. The gorge volume of the BuChE enzyme is about 200 $Å^3$ larger than the cavity of AChE, as it has a deficit of 6 aromatic amino acid residues out of the 14 amino acids present in AChE; therefore, BuChE is supposed to be less stereoselective for the substrate structure, for the same reason as given above (Nachon et al. 2003; Saxena et al. 1997, 1999; Johnson et al. 2003; Szegletes et al. 1999; Lane and He 2009). Moreover, the regulation of ACh hydrolysis in BuChE is done at the peripheral site; hence, it is the main binding site for the substrates, and therefore its binding with higher substrate concentration results in inhibitory activity, while lower concentration may result in accelerated rate of hydrolysis (Johnson et al. 2003; Szegletes et al. 1999). The AChE gorge comprises 14 aromatic residues, which make a substantial portion of the gorge, while the catalytic site at the bottom contains π-electron cloud of Trp84 along with Phe330, which assists in the binding of ACh through π-cation interactions to the enzyme through its quaternary ammonium center. Trp279 is additionally present at the entrance of the peripheral site (PAS), which is a site for binding of the substrate at the entry point. The active site of BuChE is located deep in the narrow gorge at the bottom of the enzyme, into which ACh diffuses and gets hydrolyzed. AChE is the major hydrolyzing enzyme in case of normal brain, and BuChE plays a supportive role, whereas BuChE is the main hydrolyzing enzyme for ACh in the peripheral nervous system. As the disease progresses, the brain AChE level and the ratio of AChE/BuChE gradually decrease in the AD patients, and the role of BuChE becomes more prominent as a vital therapeutic target to treat the AD patients (Lane and He 2009; Giacobini 2004). It is suggested that BuChE and AChE dual inhibition could broaden the scope of treatment by synergizing the effectiveness of medication and offer supplementary neuroprotection (Venneri et al. 2005).

The interaction of standard drugs like donepezil and rivastigmine with the enzyme AChE is important in order to understand the vital interactions that are

responsible for inhibition of the enzyme activity. These interactions would be helpful to have a broader idea for designing new molecules as AChE inhibitors.

7.4 Interactions of Acetylcholine with Acetylcholinesterase

The neurotransmitter acetylcholine is responsible for the functioning of cholinergic or parasympathetic nervous system. Its concentration in the system is controlled by the enzyme acetylcholinesterase, which is responsible for hydrolyzing the neurotransmitter to choline and acetate. Thus, it is important to discuss the interactions between ACh and AChE (Fig. 7.2) to understand their binding pattern.

The neurotransmitter contains two fragments, an ester component and a quaternary ammonium ion component on either side of the ethylene chain. The carbon of the carbonyl group of the ester binds to the enzyme's catalytic triad through the OH of Ser200, while the quaternary ammonium ion binds to the anionic sub-site which consists of Glu199, Trp86, and Phe330 in the gorge. The PAS site does not have any interaction with acetylcholine due to its small structure. It is imperative to understand the interactions of the choline component with the enzyme to get an idea about the active binding sites of the enzyme along with important amino acids taking part in the interaction (Martins et al. 2021).

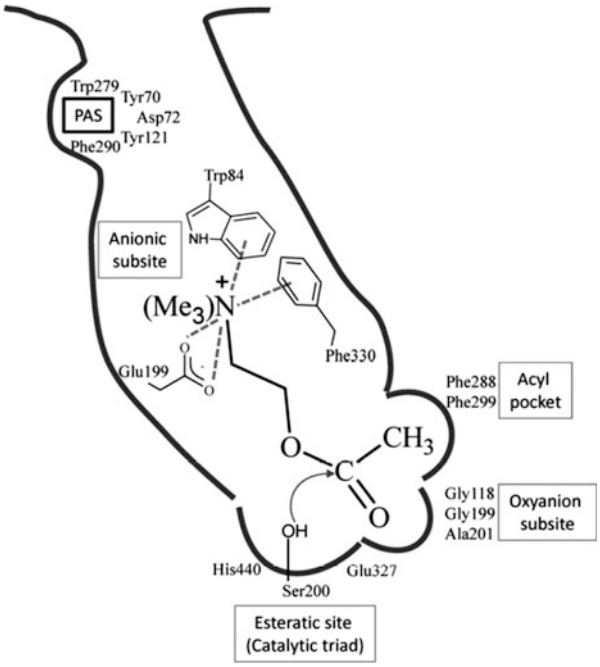


Fig. 7.2 Acetylcholine docked in the binding site of AChE enzyme gorge (Agatonovic-Kustrin et al. 2018)

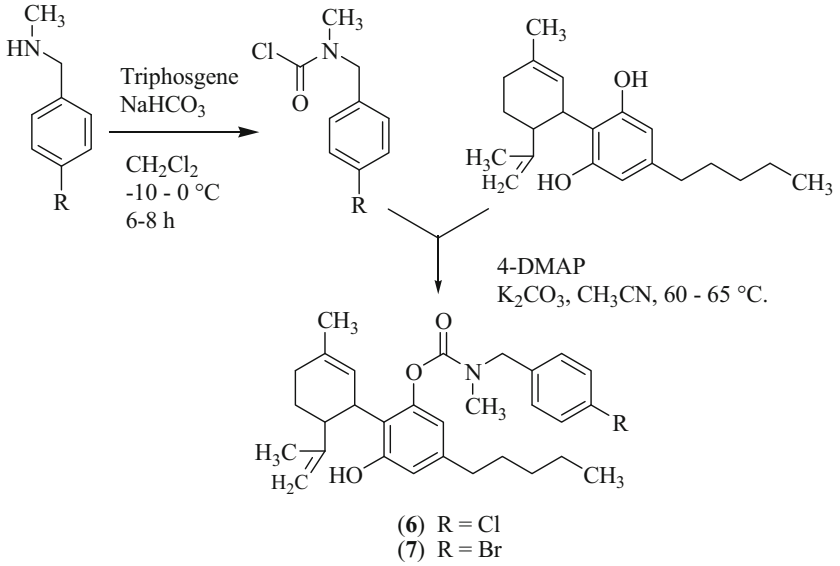
7.5 Interaction of Rivastigmine with Cholinesterase

The structure of rivastigmine consists of two distinct side arms, a tertiary amine and a carbamate moiety at positions 1 and 3 of the central phenyl ring. The carbamate part consists of two fragments, an aliphatic carbamate group and an aromatic residue to which the carbamate is attached. The carbamate group binds to the CAS site of the AChE receptor by interacting with the hydroxyl groups of Ser200 and Ser203, whereas the aryl part of the structure interacts with Trp84 of the PAS site (Lin et al. 2005; Bar-On et al. 2002). It is hypothesized that the CAS is also responsible for hydrolysis of the carbamate group, but the rate of hydrolysis is quite slow. Therefore, rivastigmine along with other carbamate-containing derivatives is considered to be a pseudo-irreversible inhibitor (Mishra et al. 2021; Martins et al. 2021).

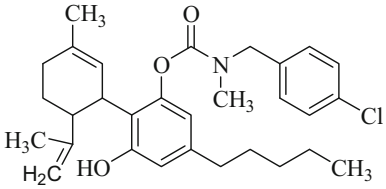
7.5.1 Designing of Rivastigmine-Based New Molecules

On the basis of rivastigmine structure, a plethora of new molecules have been designed, synthesized, and evaluated, which were found to exhibit potent anticholinesterase activity. The following section describes the best of the compounds exhibiting cholinesterase inhibitory activity as reported in literature in recent years along with their synthesis.

It is observed that drugs in general containing amide bond bind to a large number of receptors and enzymes eliciting biological responses. This observation underlines the importance of amide- and carbamate-based scaffolds in drug molecules and makes such scaffolds a dominant choice for the designing of new drugs. Based on fragment reassembly and in silico modeling approaches, Jiang et al. (2021) reported cannabidiol (CBD)-carbamate hybrids as selective BuChE inhibitors. CBD is able to insert itself into the BuChE binding groove, leading to multiple molecular interactions between Trp332 and the phenyl ring via π-π interactions, and hydrophobic interactions among Trp82 and His438, and cyclohexene ring, along with formation of two hydrogen bonds between Asp70 and -OH group, and with the Trp332 fragment. In the carbamate group containing drugs, the carbamate group can be considered as the main interacting group with the enzymes/receptors in the modern-day pharmacotherapy for AD as cholinesterase inhibitors. Based on the in silico studies, Jiang et al. (2021) designed and synthesized novel compounds among which compound **6** exhibited a highly selective and potent BuChE inhibitory activity having an IC_{50} value of 5.3 nM and SI of >4000. Additionally, compound **6** showed good BBB-penetrating ability, antioxidant activity, and pseudo-irreversible inhibition of BuChE with IC_{50} of 13 nM and k_2 value of 0.26 min^{-1}, offering neuroprotection, thus behaving like a good drug entity.



Scheme 7.1 Synthesis of compounds **6** and **7**



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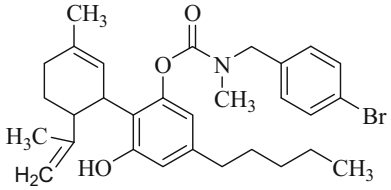
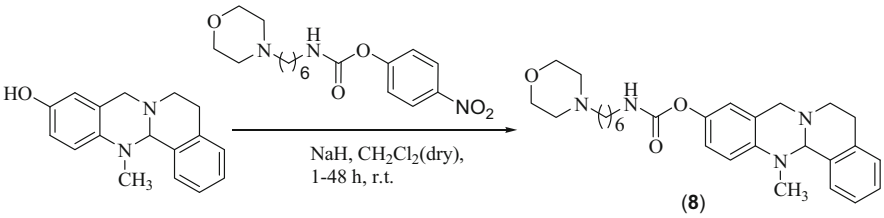


(7)

Compound **7** exhibited inhibition of $_{eq}$BuChE in nanomolar range having an IC_{50} value of 7.3 nM and selectivity index of >4000. Both the compounds **6** and **7** showed much potent hBuChE inhibition (IC_{50} values of 1.77 and 2.15 μM, respectively) in comparison to rivastigmine.

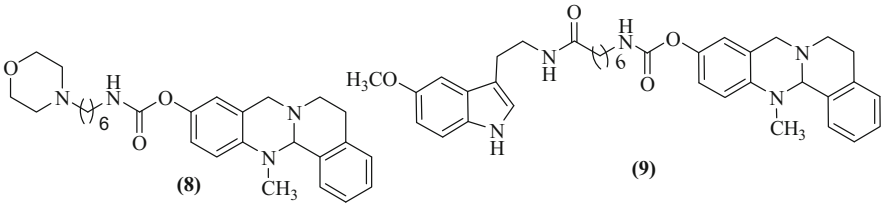
For the synthesis of compounds **6** and **7**, the substituted amines were converted to the respective carbamic chlorides by treating them with triphosgene in cold conditions. The respective carbamic acid chlorides were condensed with the cannabinoid derivative in the presence of 4-(dimethylamino)pyridine (DMAP) as base and acetonitrile as solvent at 65 °C to obtain the desired derivatives (**6**, **7**) (Scheme 7.1).

To study the effect of carbamate binding to BuChE, Scheiner et al. (2021) and Hoffmann et al. (2019) carried out creative modification of carbamate moiety introducing diverse heterocycles and alkyl spacers and performed systematic evaluation of the resulting compounds, including binding affinity to the enzyme and carbamate binding kinetics. A group of compounds having alkyl spacers (2–10 methylenes) between different heterocycles (e.g., tetrahydroquinazoline,



Scheme 7.2 Synthesis of compound **8**

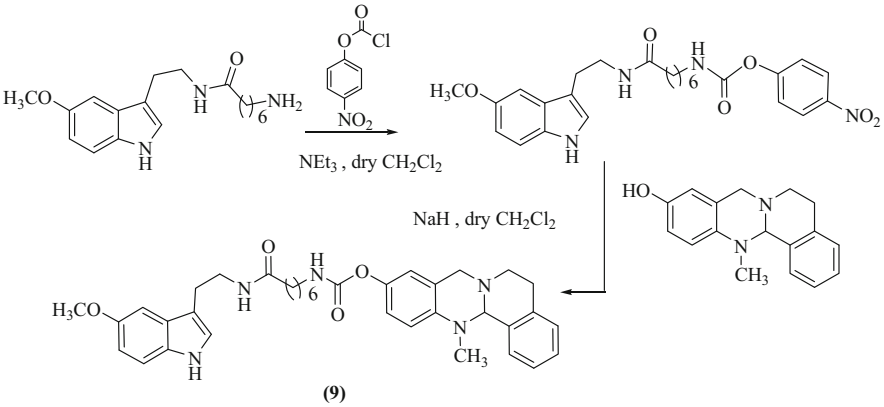
benzimidazole, piperidine, and morpholine) and the carbamate residue were synthesized as per Scheme 7.2, and the resulting compounds were evaluated for their potential to inhibit the enzyme. Compound **8** was found to be the most potent compound among the series on the basis of enzyme inhibition and kinetic studies of the synthesized inhibitors, with BuChE inhibitory activity (IC_{50} value of 49.3 nM). The compounds also displayed neuroprotective activity against glutamate treatment and improved specifically the Aβ-induced memory impairments.



Compound **8** was synthesized by nucleophilic acyl substitution reaction using the tetrahydroquinazoline derivative and 4-nitrophenylcarbamate-containing morpholine derivative in the presence of pyrophoric sodium hydride (NaH) to offer the desired product (**8**) (Scheme 7.2).

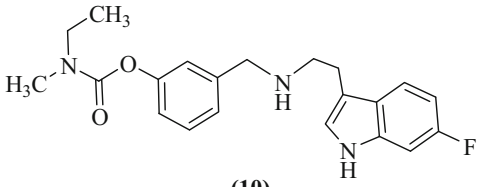
Multifactorial pathogenesis of AD involving multiple features such as metal dyshomeostasis, neuroinflammation, and oxidative stress is considered to be the causative factor for the development of AD. Scheiner et al. (2021) and Hoffmann et al. (2019) incorporated antioxidant moieties such as melatonin, ferulic acid, Trolox, and cinnamic acid into the carbamate-bearing compounds and developed some potent MTDLs. Among the synthesized compounds, compound **9** having melatonin and tetrahydroquinazoline moieties joined through carbamate linkage offered an IC_{50} value of 102 nM with additional neuroprotective and antioxidant activities.

For the synthesis of compound **9**, a suitably substituted indole derivative was reacted with 4-nitrophenyl chloroformate to offer a substituted carbamate derivative, which was further subjected to nucleophilic substitution reaction with tetrahydroquinazoline derivative in the presence of sodium hydride to offer the desired compound **9** (Scheme 7.3).



Scheme 7.3 Synthesis of compound **9**

Serotonin (5-HT), an endogenous neurotransmitter, plays numerous roles in physiological processes like memory and learning and in many pathological conditions. It was observed that the concentration of serotonin receptors (5-HTRs) got reduced in the cortex region in AD patients to prevent the decreasing levels of ACh, as a means of circumventing the changes caused by the pathophysiology of the disease. Substantial proof appeared that activation of 5-HTRs could significantly reduce cholinergic transmission, and ACh production could be stimulated by inhibition of 5-HTRs, indicating that we could probably combat AD by blocking 5-HTRs and inhibiting AChE. Based on this observation, Toublet et al. (2021) reported pleiotropic prodrugs as dual-butyrylcholinesterase inhibitors and 5-HT_6 receptor antagonists. Among the reported series, compound **10** behaves as a covalent inhibitor of BuChE (IC_{50} = 0.97 μM). The synthesis of compound **10** was carried out using Scheme 7.4 employing 6-fluoro-*1H*-indole and converting it into an aldehyde using Vilsmeier-Haack aldehyde synthesis in the presence of $POCl_3$ and DMF. The aldehyde is then reacted with nitromethane in the presence of ammonium acetate under reflux to produce an alkene, which is further reduced to an alkyl amine. The amine portion of indole derivative is reacted with the carbamate-containing aldehyde to produce a Schiff base, which is reduced to an amine in situ, to offer the desired compound **10** (Scheme 7.4).



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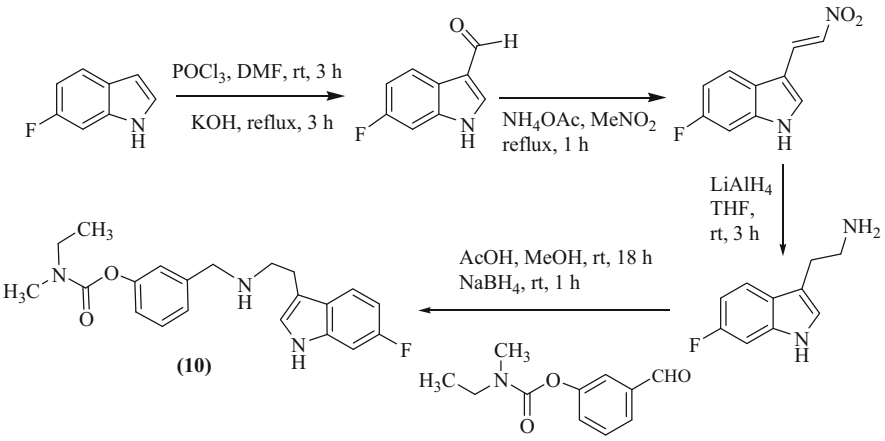
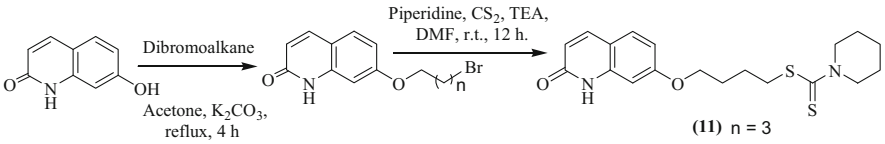
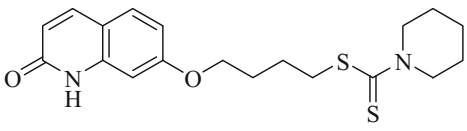


Scheme 7.4 Synthesis of compound **10**



Scheme 7.5 Synthesis of compound **11**

Recently, Fu et al. (2020) reported analogs of quinolinones having a dithiocarbamate group. In vitro evaluation showed that compound **11** inhibited self-induced Aβ aggregation and acted as a mixed type of cholinesterase inhibitor. It was observed to be an effective inhibitor at 25 μM concentration of self-induced Aβ aggregation (30.67%) and the best inhibitor at 100 μM of Aβ aggregation induced by AChE (29.02%). Compound **11** acted as a potent inhibitor of both *hAChE* (IC_{50} value of 0.16 μM) and *eeAChE* (IC_{50} value of 0.22 μM). Kinetic studies and molecular modeling studies showed that the compound **11** caused mixed type of inhibition with the capability to bind simultaneously to both the catalytic active site (CAS) and the peripheral anionic site (PAS) of AChE. Compound **11** was synthesized as per Scheme 7.5 wherein the hydroxyquinoline derivative was reacted with dibromoalkane to produce an ether which was further reacted with appropriate secondary amine in the presence of carbon disulfide in DMF to offer compound **11**.



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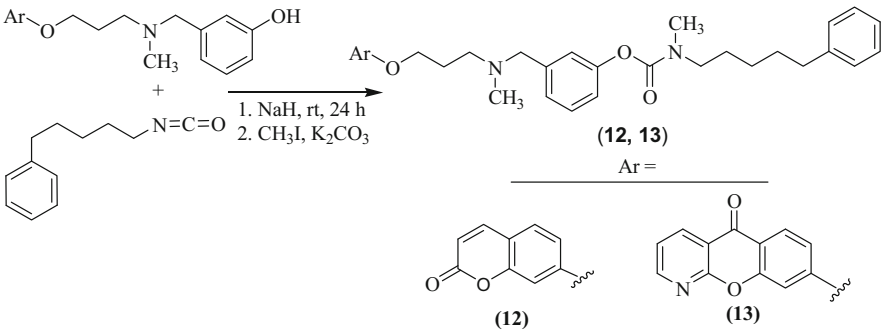
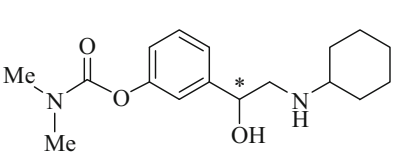


Scheme 7.6 Synthesis of compounds **12** and **13**

Coumarin, a well-known naturally occurring product, is reported to have excellent AChE inhibition potency. The molecule has infused strong interest among the researchers for developing novel agents against AD using this scaffold. Rampa et al. (2012) reported carbamate-based coumarin derivatives as anti-AD agents. Biological evaluation of the prepared derivatives showed that compounds **12** and **13** exhibited inhibitory activity in the nanomolar range against AChE, BChE, and fatty acid amide hydrolase (FAAH). This was a totally new and first-of-its-kind approach adopted by the researchers to develop novel entities exhibiting anti-AD effects, wherein the designed compounds possessed dual-FAAH-cholinesterase inhibitory activities. Compound **12** showed IC_{50} value of 74.9 nM against *h*AChE and 1.57 nM against *h*BuChE. Compound **13** showed IC_{50} value of 89.5 nM against *h*AChE and 1.71 nM against *h*BuChE. Both the compounds **12** and **13** were synthesized as per Scheme 7.6, wherein substituted phenol derivatives for the respective compounds were reacted with 5-isocyanatopentylbenzene in the presence of sodium hydride to offer compounds **12** and **13**, respectively.



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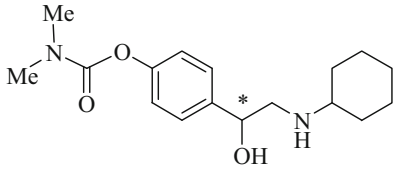
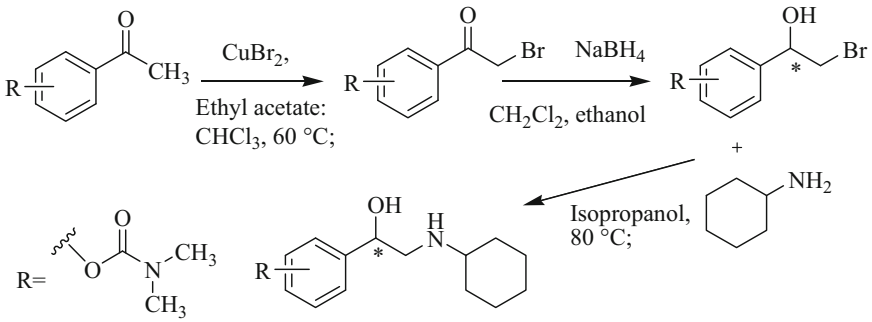


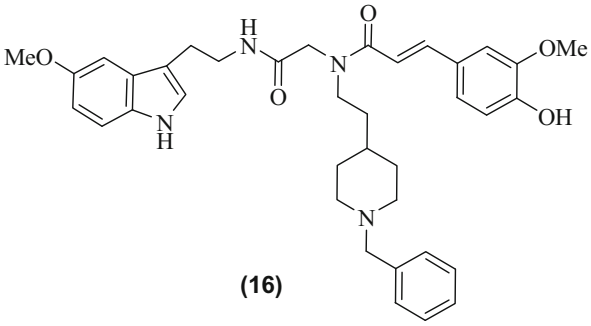
15

Terbutaline, a well-known β_2-adrenergic agonist, is used as an anti-asthmatic drug. Bambuterol (BMB) is its prodrug, which gets hydrolyzed by ChE to terbutaline, the active drug. BMB exhibited weak inhibitory activity (IC_{50} value of 3×10^{-5} M) against AChE but strong inhibitory activity against BuChE (IC_{50} value of 3×10^{-9} M). Based on these observations, Wu et al. (2020) designed and synthesized some carbamate derivatives as AChE and BuChE inhibitors. Among



Scheme 7.7 Synthesis of compounds **14** and **15**

the reported series, compound **14** (IC_{50} values of BuChE 2.2 nM and AChE 792 nM) and compound **15** (IC_{50} values of BuChE = 10.6 nM and AChE = 266 nM) were found to be the most potent compounds. It was interesting to note that both the enantiomers of these compounds **14** and **15** were found to be active against the enzyme BuChE.



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The synthesis of compounds **14** and **15** was carried out (Scheme 7.7) by simple bromination of the substituted acetophenones to convert them into α-haloketones, followed by reduction of the carbonyl group to hydroxyl, ultimately substituting the bromo group with cyclohexylamine.

7.6 Interaction of Donepezil with Cholinesterase Enzymes

Donepezil (**1**) was approved in the year 1997 by the US FDA. It is an AChE inhibitor containing 5,6-dimethoxyindanone and *N*-benzylpiperidine moieties, which are connected through a methylene bridge. The molecular docking of donepezil revealed that benzylpiperidine part of the structure exhibited interactions at the CAS site of the enzyme, binding to the catalytic site composed of amino acids Tyr70, Asp72, Trp86, Tyr121, and Tyr334, while the indanone part of the molecule showed

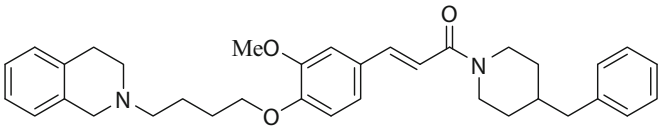
interactions with the PAS site of the receptor eliciting interaction with Trp286 (Saxena et al. 2003).

Donepezil has a very low selectivity for BuChE due to the presence of a larger gorge of BuChE, and therefore it is unable to bind with PAS and CAS simultaneously. The carbonyl oxygen of the indanone moiety shows hydrogen bond interactions in the gorge, thereby increasing the inhibitory activity, while there is not much discussion about the effects of benzylpiperidine and its interaction with the BuChE enzyme (Agatonovic-Kustrin et al. 2018).

On the basis of in silico studies as discussed above, a number of AChE and BuChE inhibitors have been designed and synthesized having structure similarity to donepezil. Efforts have been made to retain one of the two active components of the drug while substituting the other part with some other moiety. The results of the biological evaluation of such compounds have been found to be highly satisfying. In the following section, the most active molecules with the highest potency along with their syntheses are discussed in order to give an idea about the current scenario of research on anti-AD activities of the resulting compounds.

7.6.1 Design and Development of Donepezil-Based Compounds

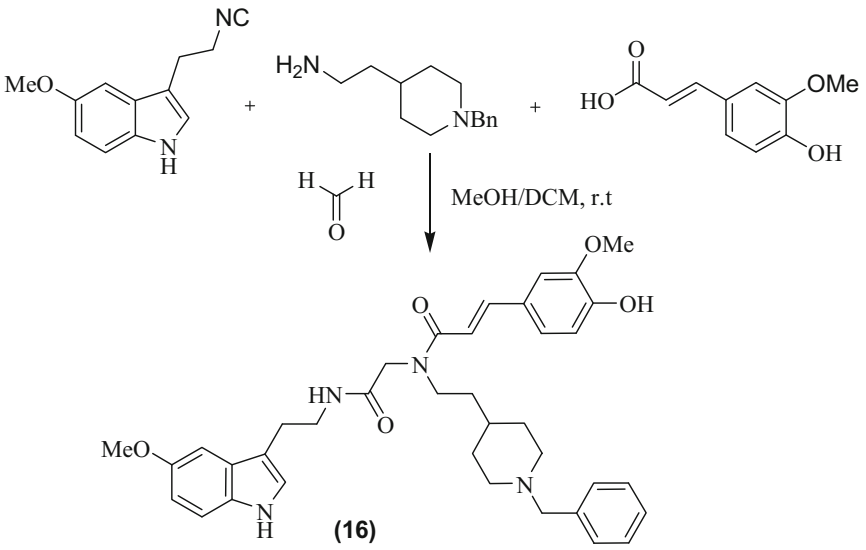
In order to develop a series of multi-targeted drugs for the treatment of Alzheimer's disease, Benchekroun et al. (2015) reported hybrids of donepezil-ferulic acid. The compounds were synthesized by one-pot U-4CR technique at room temperature for 24 h. Out of the 12 derivatives reported, compound **16** has shown the most potent activity against AChE and BuChE. Compound **16** showed activity similar to donepezil having IC_{50} value of 29.3 nM. The derivatives were also tested for their oxygen radical scavenging ability, and it has been observed that compound **16** showed free radical scavenging activity at 8.71 μM (Benchekroun et al. 2015).



(17)

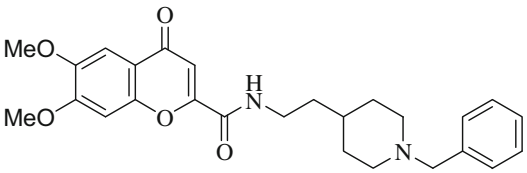
The compound **16** was synthesized using one-pot reaction by stirring a mixture of 3-(2-isocyanoethyl)-5-methoxy-1*H*-indole, ferulic acid, and N-benzylated piperidine derivative in DCM and methanol at room temperature overnight (Scheme 7.8).

Similarly, in 2016, Sang et al. (2017) reported ferulic acid-*O*-alkylamine derivatives as anti-Alzheimer agents. All the derivatives were screened for the AChE and BuChE inhibitory activity along with Aβ self-induced aggregation and antioxidant profiles. Among the synthesized derivatives, compound **17** showed promising results as AChE ($IC_{50} = 0.39$ nM) and BuChE ($IC_{50} = 76$ nM) inhibiting



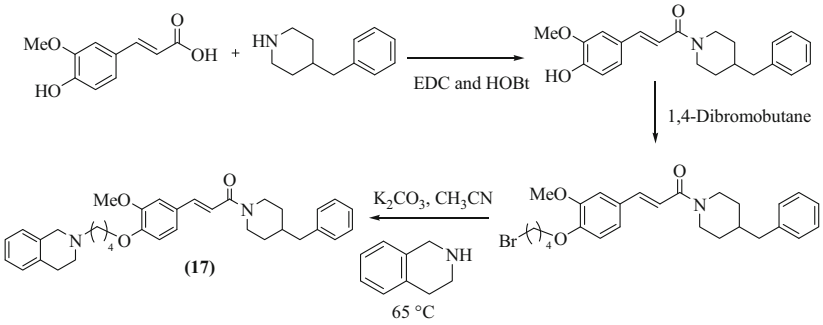
Scheme 7.8 Synthesis of compound **16**

agent. This compound has also shown low toxicity along with the antioxidant activity equivalent to 0.55 eq. of Trolox (Sang et al. 2017).



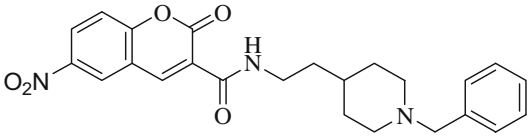
(18)

The most potent compound **17** was synthesized (Scheme 7.9) by reacting ferulic acid with 4-benzylpiperidine in the presence of HOBt and EDC using THF as the solvent. The reaction mixture was kept at room temperature under stirring. The solvent was removed under vacuum after completion of the reaction and diluted with water and the mixture extracted with dichloromethane. The compound so obtained was reacted with 1,4-dibromobutane under an inert atmosphere by heating the reaction mixture for 8–10 h at 60–65 °C in the presence of potassium carbonate as a base and acetonitrile as the solvent. The reaction mixture was extracted with DCM after completion of the reaction mixture and the solvent removed to obtain a compound, which was then reacted with tetrahydroisoquinoline (THIQ) to yield the final product, in the presence of K_2CO_3 and CH_3CN at 60–65 °C and continuous stirring under argon atmosphere.



Scheme 7.9 Synthesis of compound **17**

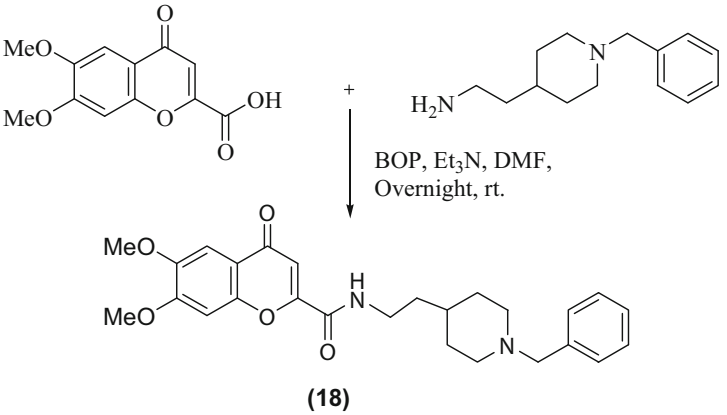
In 2018, donepezil-flavonoid hybrids were explored by Estrada Valencia et al. (2018) and his group with an aim to synthesize multi-targeted derivatives. The compounds were synthesized with an aim to have affinity towards sigma-1 receptor along with inhibition of AChE, 5-lipoxygenase, and monoamine oxidase. Among the synthesized compounds, the benzylpiperidine derivative (**18**) has shown activity in nanomolar range against all the specified targets. This compound also showed good affinity for *h*AChE with IC_{50} value of 46 nM (Estrada Valencia et al. 2018).



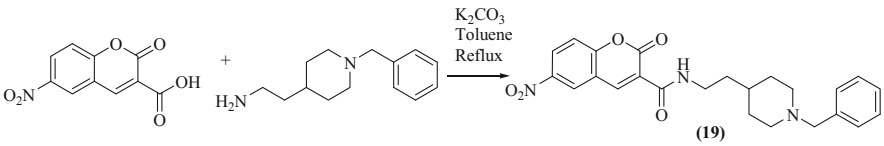
(19)

The most active compound **18** was synthesized (Scheme 7.10) using the chromone derivative 6,7-dimethoxy-4-oxo-4*H*-chromene-2-carboxylic acid as the starting material. The solvent used for the reaction was DMF under nitrogen environment. To the reaction mixture, BOP and triethylamine were added with continuous stirring. After 5 min, 2-(1-benzylpiperidin-4-yl)ethan-1-amine was added to the reaction mixture at room temperature and the reaction mixture stirred overnight. On completion of the reaction, the reaction mixture was sequentially washed with dilute hydrochloric acid and aqueous solution of $NaHCO_3$ followed by brine and purified using column chromatography.

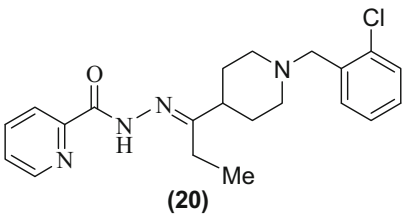
In 2013, potent acetylcholinesterase inhibitors were reported by Asadipour et al. (2013) having coumarin-3-carboxamide and benzylpiperidine moieties. A number of derivatives were synthesized using different linkers. Compound **19** having N-ethylcarboxamide linker and a nitro substituent at sixth position has shown potent activity with high selectivity and 46-fold higher potency against AChE with IC_{50} value of 0.3 nM as compared to the standard drug donepezil (Asadipour et al. 2013).



Scheme 7.10 Synthesis of compound **18**

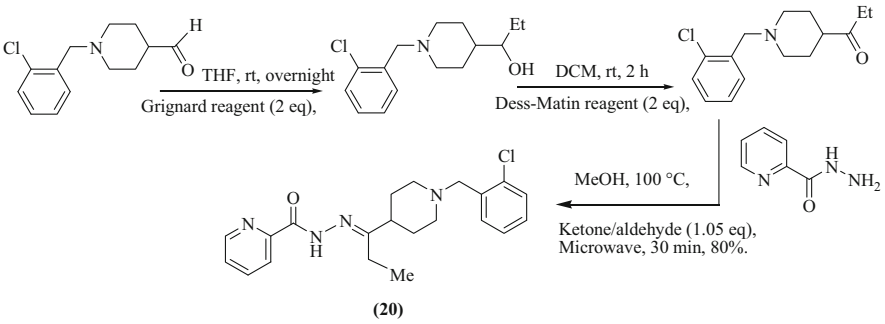


Scheme 7.11 Synthesis of compound **19**



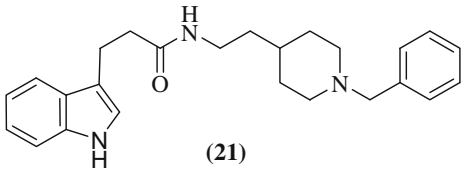
The compound **19** was synthesized (Scheme 7.11) using substituted coumarin-3-carboxylic acid and amine. Firstly, the coumarin-3-carboxylic acid was refluxed with thionyl chloride for 3–5 h. After completion of the reaction, excess thionyl chloride was removed and 2-(1-benzylpiperidin-4-yl)ethanamine was added to the reaction mixture along with anhydrous potassium carbonate using dry toluene as a solvent. The reaction was refluxed for 6–12 h and monitored by TLC for the completion of the reaction. The reaction mixture was processed, the solvent was removed under vacuum, and the crude product so obtained was purified by column chromatography.

In 2019, Zhou et al. (2019) reported that the main cause of dementia and disability in AD patients is cholinergic depletion, and AChE enzyme is mainly responsible for the cholinergic deficit. Thus, a novel series of compounds were designed having different chemical fragments like pyridine, acylhydrazone, and N-benzylpiperidine. Among the synthesized compounds, compound **20** has shown selectivity and



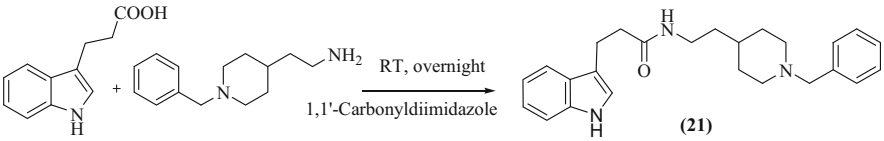
Scheme 7.12 Synthesis of compound **20**

activity against AChE with IC_{50} value of 6.0 nM, while no activity was observed against BuChE. Drug-like property through ADMET predication and PAMPA permeability evaluation has also been reported for the compound (Zhou et al. 2019).

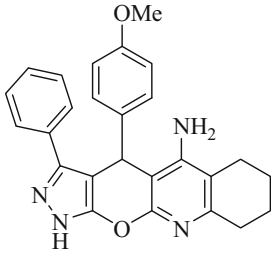


The synthesis of compound **20** involved green synthesis (Scheme 7.12) and was conducted under microwave conditions using methanol as a solvent. All the required chemicals along with the solvent were irradiated in a microwave reactor for 30 min at 100 °C. The excess solvent was removed from the reaction mixture after the completion of the reaction. The residue so obtained was purified by column chromatography to obtain the final product (**20**).

In 2016, Wang et al. (2016) reported a novel series of multi-targeted compounds. Carboxamide-linked N-benzylpiperidine–indole-based hybrids were reported. Among the synthesized compounds, compound **21** has shown effective antioxidant activity at 20 uM and 56.3% inhibition of Aβ aggregation. Compound **21** successfully crossed blood-brain barrier and decreased the death of PC12 cells by chelating the metal ions, which led to decrease in oxidative stress in the cells. It has also shown effective binding affinity with CAS and PAS sites of AChE.



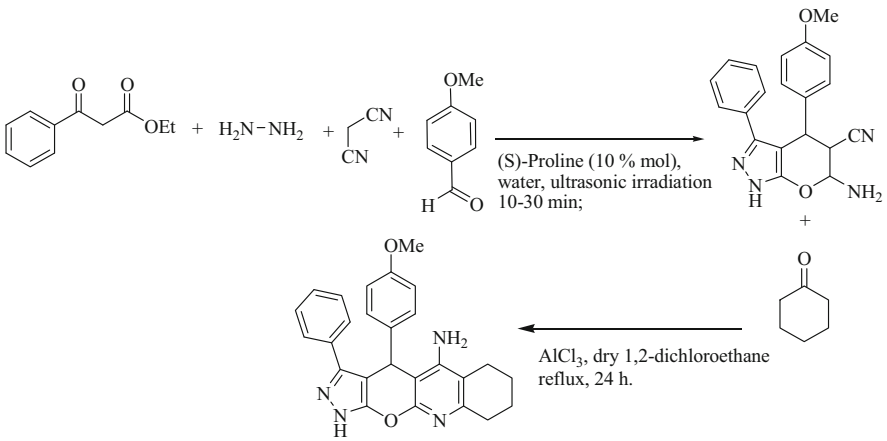
Scheme 7.13 Synthesis of compound **21**



The most potent compound **21** was synthesized using 1,1-carbonyldiimidazole as the dehydrating agent and the acid, 3-(1*H*-indol-3-yl)propanoic acid, as the starting material in tetrahydrofuran as the solvent at room temperature. 1-Benzylpiperidin-4-ethylamine was added to the reaction mixture after 1-h stirring, and the reaction mixture was continued to be stirred overnight. After completion of the reaction, the reaction mixture was diluted with water, extracted with ethyl acetate, and processed further to obtain the desired compound **21** (Scheme 7.13).

7.7 Interaction of Tacrine with Cholinesterases

Tetrahydroaminoacridine or tacrine was the first marketed drug for AD patients, introduced in 1993 under the name of Cognex, which acted as reversible AChE and BuChE inhibitor, but unfortunately it was withdrawn from the market in the year 2013 due to its persistent hepatotoxicity (Heilbronn 1961). From the in silico studies, it has been seen that tacrine is binding to AChE catalytic triad via double π stacking interaction with the aromatic amino acid residues Trp84 and Phe330, which is responsible for binding of Ach to the active site. A π–π stacking interaction was observed between the indole moiety of Trp84 and the phenyl ring of benzylpiperidine, while the tertiary amine of the piperidine moiety interacted with the phenyl ring of Phe330 to yield a cation–π interaction (Sussman et al. 1991). Tacrine was one of the most widely prescribed drugs for AD, but because of its major side effect of hepatotoxicity, it was stopped from clinical use. Tacrine analogs possess a great scope to be used as the potential treatment of choice if the issue of hepatotoxicity is taken care and the potency is maintained. In the following section, efforts have been made to discuss some recently reported potent tacrine derivatives,

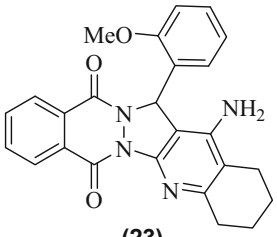


(22)

Scheme 7.14 Synthesis of compound **22**

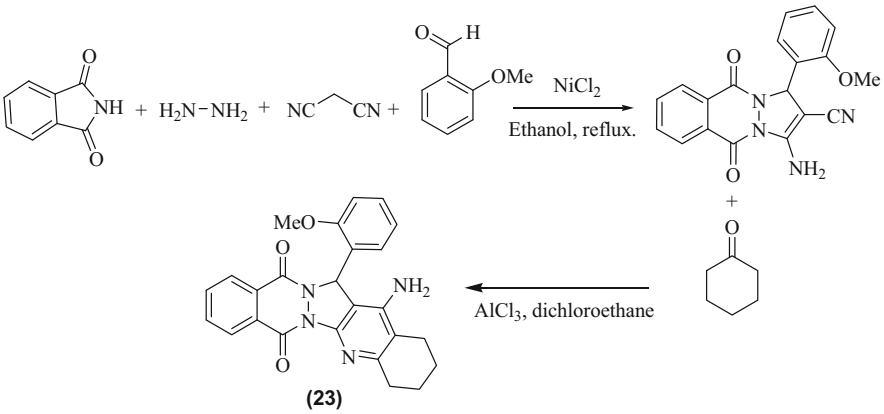
which have shown potency higher than tacrine without the side effect of hepatotoxicity.

Pourabdi et al. (2016) in 2016 designed and synthesized a series of novel pyrano [2,3-*c*]pyrazoles by taking tacrine as the lead molecule. The designed compounds were prepared considering AChE/BuChE as the targets. Among the synthesized compounds, one compound (**22**) produced inhibition in nanomolar concentration against both BuChE and AChE offering IC_{50} values of 0.007 and 0.081 μM, respectively, against the enzymes. Additionally, compound **22** was also found to show potent activity against 15-LOX with IC_{50} value of 31 μM. Most importantly, the compound was found to be nontoxic in comparison to tacrine in cytotoxicity assay on HepG2 cells even at concentrations as high as 50 μM.



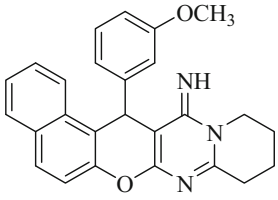
(23)

For the synthesis of compound **22**, the pyrazole-carbonitrile intermediate was synthesized by reacting ethyl benzoylacetate with hydrazine hydrate, *anisaldehyde*, and malononitrile in water under microwave irradiation for 10–30 min. The intermediate so formed is then reacted with cyclohexanone in the presence of anhydrous aluminum chloride under reflux to produce compound **22** (Scheme 7.14).



Scheme 7.15 Synthesis of compound **23**

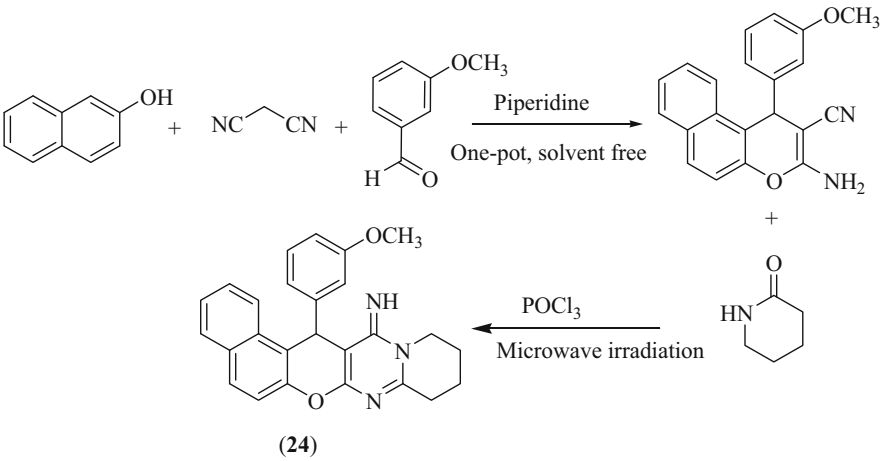
Jalili-Baleh et al. (2017) in 2017 reported fused pyrazolo[1,2-*b*]phthalazines as anti-AD agents. Compound **23** exhibited higher selectivity for AChE over BuChE, and it was found to be sevenfold more potent ($IC_{50} = 49$ nM) than tacrine. Using neuronal cell line (PC12) and hepatocytes (HepG2) for the cell-based assay, compound **23** was found to show significantly low hepatotoxic effect and provided additional neuroprotective activity against hydrogen peroxide-induced cell damage in PC12 cells. Overall, it was seen that the compound was highly potent, selective, lowly toxic, and neuroprotective in comparison to the standard drug tacrine.



(24)

For the synthesis of compound **23**, phthalimide, hydrazine hydrate, malononitrile, and 2-methoxybenzaldehyde were refluxed in ethanol in the presence of nickel chloride to offer an intermediate (Scheme 7.15). The intermediate on refluxing with cyclohexanone in dichloromethane in the presence of anhydrous $AlCl_3$ offered the desired compound **23**.

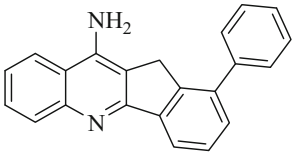
Dgachi et al. (2016) in 2016 prepared analogs of tacrine with an intent to inhibit the cholinesterase enzymes. Compound **24** showed the highest activity with AChE inhibition having IC_{50} value of 5.3 nM. Compound **24** was also found to be possessing good antioxidant and amyloid aggregation inhibitory properties. Six-membered ring attached to the pyrimidine ring when replaced with five- or



(**24**)

Scheme 7.16 Synthesis of compound **24**

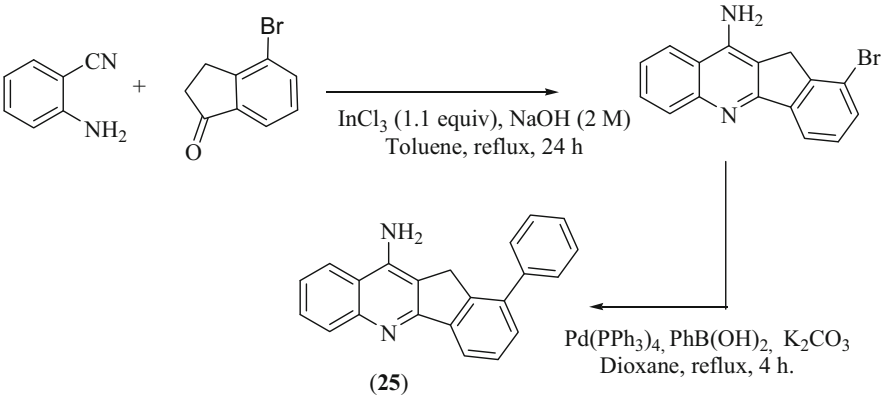
seven-membered rings, and the activity was found to decrease; therefore, it could be inferred that a six-membered ring is essential for good AChE inhibitory activity.



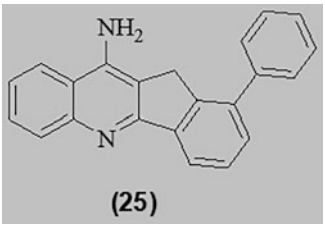
(25)

A one-pot multicomponent reaction starting from malononitrile, 3-methoxybenzaldehyde, and β-naphthol in the presence of piperidine yielded the carbonitrile derivative. Condensation of the intermediate carbonitrile with the lactam for 15 min under microwave conditions in the presence of phosphoryl chloride as the Lewis acid and bromobenzene as the solvent offered the desired compound **24** (Scheme 7.16).

Ekiz et al. (2018) in 2018 synthesized bromoindenoquinolines using Friedlander reaction along with Suzuki coupling to produce compounds with cholinergic inhibitory activity. Out of all the synthesized compounds, compound **25** bearing a phenyl substituent offered the most desirable biological activity yielding BuChE inhibition (IC_{50} value of 93 nM) and AChE inhibition (IC_{50} value of 37 nM), thereby making it a strong cholinesterase enzyme inhibitor. It was also found that the compound was very less hepatotoxic compared to tacrine showing higher potency. Therefore, compound **25** is considered to be a good lead for further drug designing and optimization to offer newer active molecules.



Scheme 7.17 Synthesis of compound **25**



The synthesis of compound **25** was done (Scheme 7.17) using Friedlander reaction in which the carbonitrile compound was taken in toluene and added to bromoindanone to produce the intermediate. The bromo group was replaced with phenyl ring using Suzuki coupling reaction to yield the desired compound **25**.

7.8 Conclusion

Alzheimer's disease, a neurodegenerative disease, has offered multiple targets in its pathophysiology, which could be exploited in order to either slow down or inhibit the progression of the disease. Inhibition of the most likely and popular targets, i.e., AChE and BuChE, has remained a choice for researchers and medicinal chemists, which involved targeting the active sites PAS and CAS of the enzymes. In order to design and synthesize anti-Alzheimer's drugs which act by cholinesterase inhibition, these sites are to be targeted to produce compounds with high potency. Therefore, already existing US FDA-approved marketed drugs donepezil and rivastigmine, which have the potential to bind to both CAS and PAS sites of the cholinesterase enzymes, have been considered to be the primary choice for novel drug designing and for which tremendous amount of work has been done in the past, and research work is still ongoing for the development of potent anticholinesterase inhibitors to treat AD. Furthermore, when we consider rivastigmine as a standard, the carbamate

part along with the tertiary amine group becomes important as these groups bind to the respective CAS and PAS sites of the enzyme, whereas benzylpiperidine (binding to CAS) and 5,6-dimethoxyindanone (binding to PAS) of donepezil are considered to be the other two important moieties for drug designing. On the basis of the information acquired from in silico studies conducted for donepezil and rivastigmine on AChE and BuChE enzymes, a number of reports in literature showed similar interactions which mimic the standard drugs wherein different moieties like ferulic acid, flavonoids, coumarins, quinolines, tetrahydroisoquinoline, and many heterocycles were attached to the active parts of either donepezil or rivastigmine, offering highly potent compounds when compared to the standard drugs (Yadav et al. 2023b).

Among the compounds discussed above, compound **6** which was designed on the basis of rivastigmine moiety containing the active carbamate component was synthesized by Jiang et al. (2021). Compound **6** had shown AChE inhibition with IC_{50} value of 5.3 nM and BuChE with IC_{50} value of 13 nM, along with good neuroprotective and antioxidant activities, with the capacity to permeate through the BBB. Compound **6** possesses drug-like properties, and therefore it could be considered as an important lead molecule to further optimize into novel anticholinergic drug molecules based on carbamate component of rivastigmine. The compounds discussed under donepezil-based designing, coumarin-3-carboxamide derivative attached to benzylpiperidine part of donepezil, compound **19** (Asadipour et al. 2013), showed highly potent activity with high selectivity and 46-fold more potency than its lead molecule donepezil as a standard drug against AChE with IC_{50} value of 0.3 nM. This proves that there is still ample scope for research to produce potent compounds bearing the benzylpiperidine fragment of donepezil. For the tacrine-based derivatives, compound **24** (Dgachi et al. 2016) possessed AChE inhibition activity offering IC_{50} value of 5.3 nM, which was more potent than the lead compound tacrine and was comparatively very less hepatotoxic; therefore, such series of compounds can be considered for further development as anti-AD drugs, where modifications on the tricyclic ring system result in reduced hepatotoxicity and increased potency.

In a nutshell, it can be concluded that rivastigmine and donepezil as lead molecules offer tremendous opportunities for the development of newer molecules to treat AD by targeting the cholinesterase enzymes, wherein the active components of these two marketed drugs are attached to various heterocyclic and other chemical moieties and the resulting compounds are evaluated for anticholinesterase activity. It can also be concluded that MTDL approach is one of the most preferred approaches of structure-based drug designing (Murumkar et al. 2023) when it comes to designing of newer anti-AD drugs.

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