2. REVIEW OF LITERATURE

In 1907, Dr. Alois Alzheimer, a German scientist, reported a neurodegenerative disease characterized by decreased cognitive ability and severe behavioural abnormalities like disorientation, depression, restlessness, irritability and anxiety, now known as Alzheimer's disease (AD). The progression rate of AD can vary and the average life expectancy subsequent to diagnosis is approximately three to nine years.[102]

Literature has supported the role of both the enzymes AChE (E.C. 3.1.1.7) as well as BuChE (E.C. 3.1.1.8) in AD. Literature has supported the evidence of gradual fall in the levels of AChE in the brain of AD patients, while there occurs slight increase in the activity of BuChE as the condition progresses. Further, postmortem tissue analysis of AD patients showed a high level of BuChE in the hallmark lesions of AD. In rats, selective BuChE inhibitor cymserine was found to elevate ACh levels and enhanced long-term CNS potentiation and learning.[104,105]

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P22303 ACES_HUMAN          1    MRPPQCLLHTPSLASPLLLLLLWLL-GGGVGAEGREDAELLVTVRGGRLRGIRLKTPGGP      59
PO6276 CHLE_HUMAN          1    -------MHSKVT-IICIRFLFWFLLLCMLIGKSHTEDDIIIATKNGKVRGMNLTVFGGT      52
                                       :*:       : :*:*:*    : .:.: : ::::::.: *::**:.*.. **

P22303 ACES_HUMAN          60   VSAFLGIPFAEPPMGPRRFLPPEPKQPWSGVVDATTFQSVCYQYVDTLYPGFEGTEMWNP      119
PO6276 CHLE_HUMAN          53   VTAFLGIPYAQPPLGRLRFKKPQSLTKWSDIWNATKYANSCCQNIDQSFPGFHGSEMWNP      112
                                *:*******:*:**:*  **  *:    ** : :**.: . * * :*  :***.*:*****

P22303 ACES_HUMAN          120  NRELSEDCLYLNVWTPYPRPTSPTPVLVWIYGGGFYSGASSLDVYDGRFLVQAERTVLVS      179
PO6276 CHLE_HUMAN          113  NTDLSEDCLYLNVWIPAPKPKNA-TVLIWIYGGGFQTGTSSLHVYDGKFLARVERVIVVS      171
                                * :*********** * *:*..   **:******* :*:***.****:**.:.**.::**

P22303 ACES_HUMAN          180  MNYRVGAFGFLALPGSREAPGNVGLLDQRLALQWVQENVAAFGGDPTSVTLFGESAGAAS      239
PO6276 CHLE_HUMAN          172  MNYRVGALGFLALPGNPEAPGNMGLFDQQLALQWVQKNIAAFGGNPKSVTLFGESAGAAS      231
                                *******:*******. *****:**:**:*******:*:*****:*.**************

P22303 ACES_HUMAN          240  VGMHLLSPPSRGLFHRAVLQSGAPNGPWATVGMGEARRRATQLAHLVGCPPGGTGGNDTE      299
PO6276 CHLE_HUMAN          232  VSLHLLSPGSHSLFTRAILQSGSFNAPWAVTSLYEARNRTLNLAKLTGCSR----ENETE      287
                                *.:***** *:.** **:****: *.***...: ***.*: :**:*.**       *:**

P22303 ACES_HUMAN          300  LVACLRTRPAQVLVNHEWHVLPQESVFRFSFVPVVDGDFLSDTPEALINAGDFHGLQVLV      359
PO6276 CHLE_HUMAN          288  IIKCLRNKDPQEILLNEAFVVPYGTPLSVNFGPTVDGDFLTDMPDILLELGQFKKTQILV      347
                                :: ***.:  * :: .* .*:*  : : ..* *.******:* *: *:: *:*:  *:**

P22303 ACES_HUMAN          360  GVVKDEGSYFLVYGAPGFSKDNESLISRAEFLAGVRVGVPQVSDLAAEAVVLHYTDWLHP      419
PO6276 CHLE_HUMAN          348  GVNKDEGTAFLVYGAPGFSKDNNSIITRKEFQEGLKIFFPGVSEFGKESILFHYTDWVDD      407
                                ** ****: **************:*:*:* **  *::: .* **::. *::::*****:.

P22303 ACES_HUMAN          420  EDPARLREALSDVVGDHNVVCPVAQLAGRLAAQGARVYAYVFEHRASTLSWPLWMGVPHG      479
PO6276 CHLE_HUMAN          408  QRPENYREALGDVVGDYNFICPALEFTKKFSEWGNNAFFYYFEHRSSKLPWPEWMGVMHG      467
                                : * . ****.*****:*.:**. ::: :::  * ..: * ****:*.* ** **** **

P22303 ACES_HUMAN          480  YEIEFIFGIPLDPSRNYTAEEKIFAQRLMRYWANFARTGDPNEPRDPKAPQWPPYTAGAQ      539
PO6276 CHLE_HUMAN          468  YEIEFVFGLPLERRDNYTKAEEILSRSIVKRWANFAKYGNPNETQN-NSTSWPVFKSTEQ      526
                                *****:**:**:   ***  *:*::: ::: *****: *:*** :: :: .** :.:  *

P22303 ACES_HUMAN          540  QYVSLDLRPLEVRRGLRAQACAFWNRFLPKLLSATDTLDEAERQWKAEFHRWSSYMVHWK      599
PO6276 CHLE_HUMAN          527  KYLTLNTESTRIMTKLRAQQCRFWTSFFPKVLEMTGNIDEAEWEWKAGFHRWNNYMMDWK      586
                                :*::*: .  .:   **** * **. *:**:*. * .:**** :*** ****..**:.**

P22303 ACES_HUMAN          600  NQFDHYSK-QDRCSDL                                          614
PO6276 CHLE_HUMAN          587  NQFNDYTSKKESCVGL                                          602
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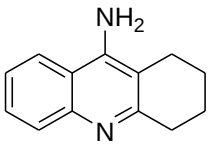
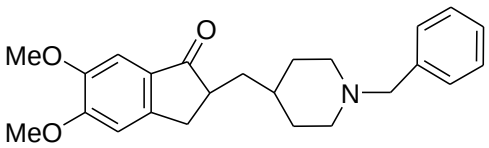
Figure 4: Alignment of AChE and BuChE amino acid sequence (catalytic triad marked in red color).

From the literature it was clearly observed that although AChE and BuChE are produced by different genes they are highly homologous with more than 65% similarity in conserved region. AChE has two major binding sub-sites, a peripheral anionic site (PAS) and the other a catalyatic anionic site (CAS), located in the deep gorge of the enzyme structure and is assigned to Ser-His-Glu catalytic triad. The gorge is lined by around 14 aromatic amino acids making the active site more hydrophobic, leading to better interaction with hydrophobic substrates. The gorge goes through half way in the enzyme and is roughly 20 Å long. Common to AChE, BuChE also has a catalytic triad consisting of Ser–His–Glu. Majority of the important features of the active site of BuChE like a triad of Ser-His-Glu, a p-cation-binding site, an oxyanion hole, and an acyl-binding pocket are similar to AChE. The acyl binding pocket of BuChE is obviously larger than that of AChE. The active sites of both the enzymes acting as nucleophiles are situated at the base of a cavity to attack the carbonyl group of the substrates.[109]

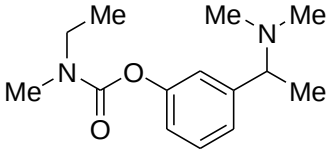
There are several molecules under study for AD. In 1993, FDA approved tacrine as the first drug against Alzheimer's but later on it was discarded because of its hepatotoxicity. Tacrine was a dual acting first approved drug for AD that was marketed under the trade name of Cognex. It inhibits both AChE as well as BuChE enzymes.[106]



Tacrine



Donepezil



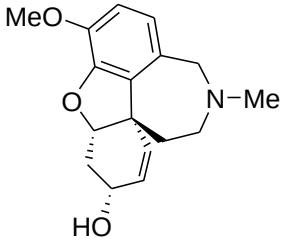
Rivastigmine

Currently four drugs are approved for Alzheimer's treatment by FDA and these are discussed below.

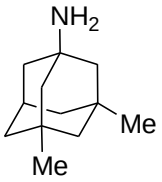
Donepezil was approved in 1996 for AD treatment and it is a centrally acting reversible and selective AChE inhibitor. It was developed by developer Eisai and partner Pfizer, and is marketed under the trade name Aricept.[112]

Rivastigmine[113] was developed by Marta Weinstock-Rosin of the Pharmacology Department, the Hebrew University of Jerusalem. Then it was sold to Novartis for commercial development. It is a semi-synthetic derivative of physostigmine and it is a dual AChE and BuChE inhibitor.

Galantamine[113] is an alkaloid used for the treatment of mild to moderate AD. It is competitive and reversible nicotinic AChE inhibitor approved by US FDA in 2001 for the treatment of AD.



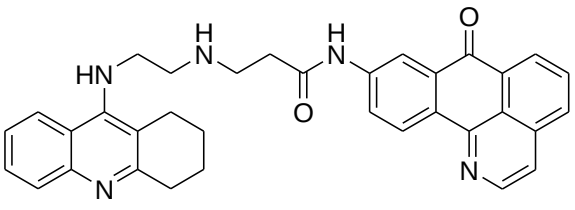
Galantamine



Memantine

Memantine[114] is noncompetitive Glutaminergic NMDA receptor blocker used in AD. It was first synthesized by Eli Lilly in 1968. It is marketed under various trade names like Axura, Akatinol, Namenda etc for the treatment of AD.

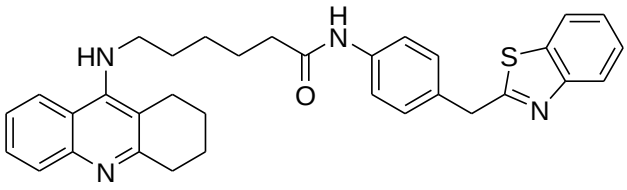
Apart from these drugs, recently many research groups have reported diverse scaffolds against AD. These include cholinesterase inhibitors, ChEIs combined with Aβ aggregation inhibitors, β-secretase inhibitors or multifunctional agents. It is reported that in healthy brain tissue, AChE is the primary enzyme accountable for acetylcholine hydrolysis, while BuChE plays a supportive role whereas in AD, the AChE activity decreases and BuChE shows a progressive and considerable increase in its action. In the following section various recently developed ChEIs with different scaffolds are summarized.



(**1**)

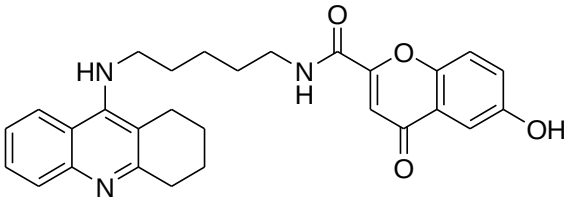
The structure of tacrine is widely used as a pharmacophore for the development of agents against AD. Tang et al. reported oxoisoaporphine-tacrine heterodimers causing a combined action on both the sites i.e. CAS and PAS as well as inhibition of Aβ aggregation. The compound (**1**) reported by this research group showed IC_{50} value on *Ee*AChE = 3.4 nM and *Ee*BuChE 110 nM. The Aβ aggregation inhibition was observed to be 79.8% at 10μM.[115]

Benzothiazole derivatives are used as Alzheimer's brain imaging agents. The use of benzothiazole moiety connected to tacrine with different linkers as heterodimers are reported to be neroprotective in nature. The representative compound (**2**) exhibited *Ee*AChE IC_{50} = 0.57 μM. The Aβ aggregation inhibition was observed to be 61.3% at 50μM.[116]

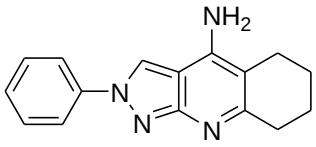


(**2**)

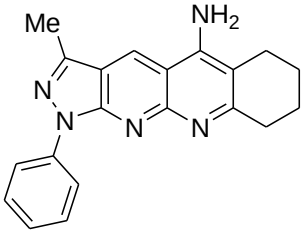
The heterodimers of substituted tacrine with chromone derivatives were reported by Fernandez-Bachiller et al. as potent AChE, BuChE and BACE1 inhibitors. The representative compound (**3**) showed very good activity against all the three targets. The IC_{50} values against *h*AChE = 8.0 nM, *h*BuChE = 1.5 nM and *h*BACE1 = 2.8 μM was reported for compound (**3**).[117]



(**3**)



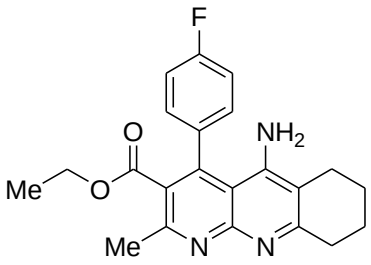
(**4**)



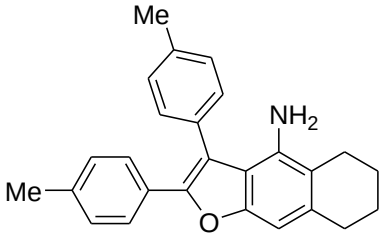
(**5**)

Barreiro et al. reported the design and synthesis of a series of selective AChE inhibitors containing azaheterocyclic pyrazolo[4,3-*d*]pyridine or pyrazolo[3,4-*b*][1,8]naphthyridine systems as isosteres of the quinoline ring of tacrine. The most active compounds (**4** and **5**) obtained from this study showed IC_{50} values of 6.0 μM and 6.4 μM respectively against AChE, while, the inhibition of BuChE was found to be poor with the selectivity indices of 5.3 and 20.9 respectively.[118]

Marco et al. synthesized novel class of tacrine analogs comprising [1,8]naphthyridine (**6**) as dual cholinesterase inhibitors. Compound (**6**) was found to be selective AChE inhibitor with IC_{50} value of 52 nM. The aromatic ring of tacrine was replaced with furo-aromatic five membered ring which gave compound (**7**) with IC_{50} against AChE = 0.377 μM, and BuChE = 100 μM.[119]

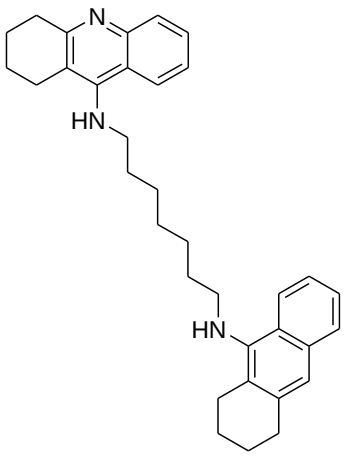


(**6**)

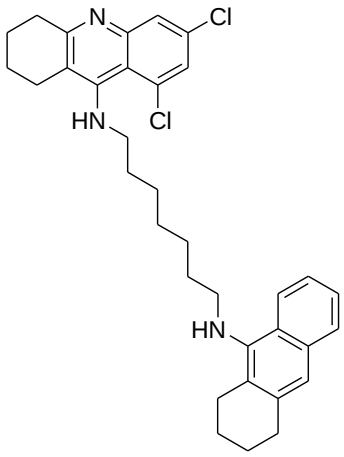


(**7**)

Savini et al. have reported some novel homo- and hetero- dimers of tacrine (**8**) (IC_{50}: AChE = 1.3 nM and BuChE = 2 nM) and dichlorotacrine (**9**) analog (IC_{50}: AChE = 6 nM and BuChE = 180 nM).[120]

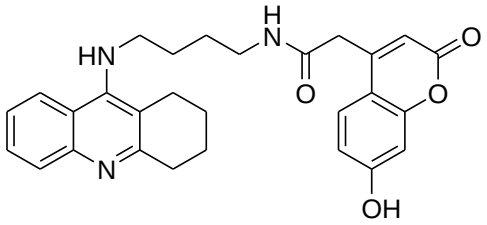


(**8**)



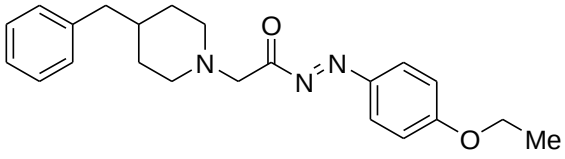
(**9**)

Heterodimer of tacrine with coumarin was reported by Hamulakova et al. Compound (**10**) was found to be active against both AChE and BuChE with IC_{50} values of 0.015 µM and 0.32 µM, respectively.[121]



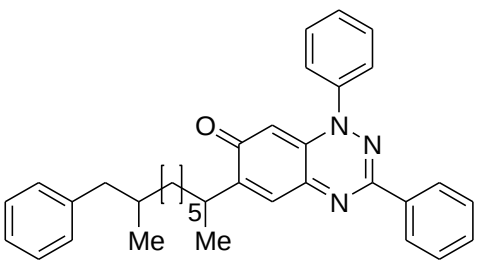
(**10**)

Ozer et al. developed a new series of compounds based on the structure of donepezil. The representative molecule (**11**) was found to be active against the cholinesterases as well as against Aβ aggregation. It was found to inhibit *h*AChE and *Eq*BuChE with IC_{50} of 53.1µM and 67.3 µM respectively. The Aβ aggregation inhibition was observed to be 80% at 100µM.[122]

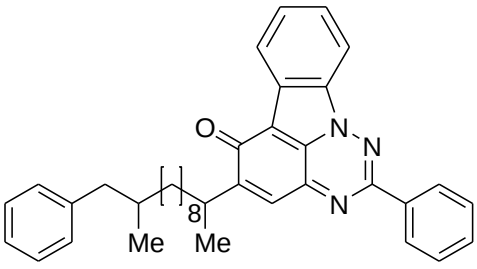


(**11**)

Catto et al. reported benzo[*e*][1,2,4]triazin-7(1*H*)-one derivatives and [1,2,4]triazino[5,6,1-*j*,*k*]carbazol-6-one derivatives as ChE and Aβ aggregation inhibitors. Compound (**12**) was observed to be having balanced activity against *Ee*AChE as well as *Eq*BuChE with IC_{50} value of 1.5 µM and 1.9 µM. Comparatively, compound (**13**) was observed to be a selective BuChE inhibitor with *Eq*BuChE IC_{50} = 25 nM. Both the compounds showed Aβ aggregation inhibition at IC_{50} 1.4 µM.[123]

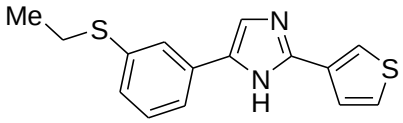


(**12**)

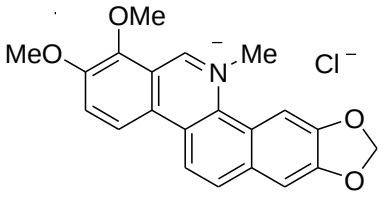


(**13**)

Karlsson et al. reported the diarylimidazole derivatives as a potential class of selective BuChE and Aβ aggregation inhibitors. The representative compound (**14**) inhibits *h*BuChE (IC_{50} = 1.11 μM) and Aβ aggregation (IC_{50} = 5.8 μM).[124]



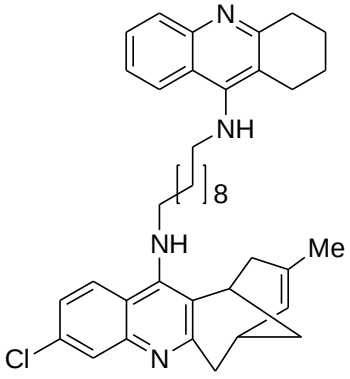
(**14**)



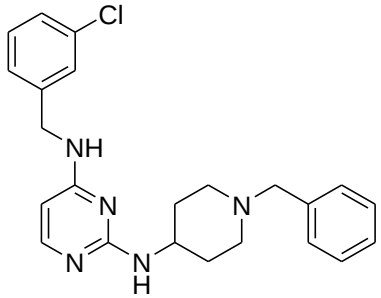
(**15**)

Chelerythrine (**15**) (an isoquinoline alkaloid) was reported to be moderately active AChE/BuChE as well as Aβ aggregation inhibitor by Brunhofer et al. This compound inhibits *h*AChE and *h*BuChE with IC_{50} values of 1.54 μM and 10.34 μM, respectively. Its Aβ aggregation inhibition IC_{50} value was 4.2 μM.[125]

Munoz-Torrero group developed hybrid compounds of huprine and tacrine connected by alkyl or alkylamine linkers. These compounds are potential inhibitors of AChE, BuChE and BACE1. The representative compound (**16**) shows IC_{50} values of 1.32 nM, 35.1 nM and 4.9 μM against *h*AChE, *h*BuChE and BACE1 respectively.[126]

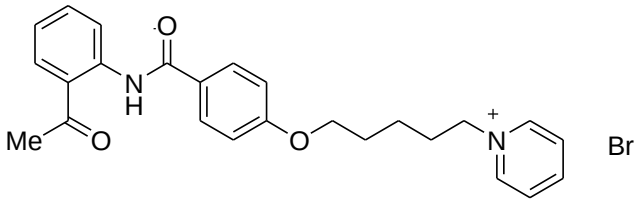


(**16**)



(**17**)

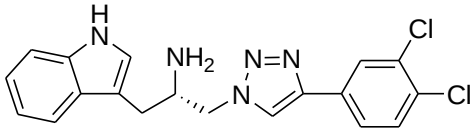
Mohamed et al. reported the design and synthesis of pyrimidine derivatives as ChEs and BACE1 inhibitors. The representative compound (**17**) shows IC_{50} values of 7.7 μM, 2.5 μM and 0.6 μM against *h*AChE, *Eq*BuChE and *h*BACE1, respectively.[127,128]



(**18**)

By using structure based approach Peng et al. reported a series of benzamide derivatives as ChEI as well as BACE1 inhibitors. The representative compound (**18**) exhibited potent activity with IC_{50} values of 81 nM, 93 nM and 0.31 μM against *h*AChE, *h*BuChE and BACE1, respectively.[129]

Jiaranaikulwanitch et al. developed a new series of tryptoline and tryptamine triazole derivatives as potential BACE1 and Aβ aggregation inhibitors. The representative compound (**19**) showed IC_{50} values of 20.75 μM against BACE1 and 83.23 μM for anti-aggregation activities.[130]



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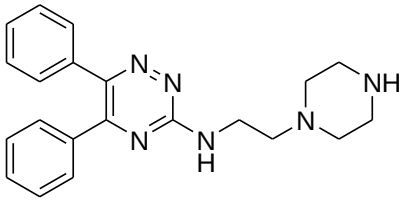
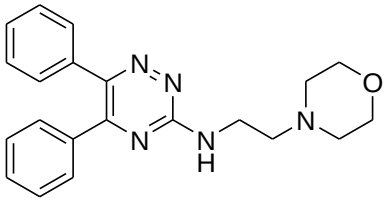


(**20**)



(**21**)

Sinha et al. reported piperazinoethyl and morpholinoethyl substituted triazine derivatives as novel ChEIs. Representative compounds (**20**, IC_{50}= 4.23 μM and 13.3 μM) and (**21**, IC_{50}= 5.79 μM and 163.4 μM) were found to be potent AChE and BuChE inhibitors, respectively.[131]