Donepezil: A review of the recent structural modifications and their impact on anti-Alzheimer activity

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Alzheimer disease (AD) is characterized by a low level of acetylcholine, beta-amyloid (Aβ) aggregation and oxidative stress. Donepezil is the core medicine used for the treatment of AD. Various structural modifications of donepezil have been carried out. Benzylpiperidine part of donepezil has been replaced with benzylpyridine, pyridyl methylpiperidine, benzylpiperazine, pyrimidyl piperazine. These derived molecules showed promising activities as anti-Alzheimer agents. Replacement of indanone part by other heterocyclic rings such as pyridine resulted in the formation of compounds which exhibited monoamine oxidase (MAO) as well as acetylcholinesterase (AChE) inhibition. Propargylamine containing derivatives displayed AChE as well as MAO inhibition properties. Attachment of donepezil with natural compounds like ferulic acid, flavonoids, and curcumin showed antioxidant activities in addition to inhibition of the AChE. Benzylpiperidine and benzylpiperazine have also been combined with condensed heterocyclic rings and these compounds displayed promising anti-Alzheimer properties. This review highlights the important structural modifications of donepezil and their influence on biological activities as anti-Alzheimer agents.

Keywords: Acetylcholinesterase (AChE). Butyrylcholinesterase (BuChE). Benzylpiperidine. Indanone. Hybrid compounds.

INTRODUCTION

The Alzheimer disease (AD) is a neurodegenerative disorder and is characterized by premature senile mental deterioration. It mainly affects the elder people aged above 65 years (Naj et al., 2011). At present, 36 million people are affected due to this disease all over the world and according to some estimates, this number will increase up to 115 million in 2050 (Bolea et al., 2011). Characteristic features of this disease are memory loss, cognitive impairment, behavioural changes and eventual death (Scarpini, Scheltens, Feldman, 2003). Low level of acetylcholine is one of the major causes in the development and progress of this disease (Talesa, 2001). Alteration in the dopaminergic, serotonergic and monoaminergic neurotransmission also contribute to some symptoms of Alzheimer disease. Beta-amyloid (Aβ) aggregation and oxidative stress are the other causes of this disease (Gella, Durany, 2009; Selkoe, Podlisny, 2002). Accumulation of metal ions also results in fatal neurologic disorders and it is closely associated with abnormal Aβ plaques (Bush, 2008). Donepezil, rivastigmine, and galanthamine are the drugs which increase acetylcholine concentration by inhibiting cholinesterases and are effective in the treatment of AD (Birks, Harvey, 2006; Leon, Garcia, Marco-Contelles, 2013). An N-methyl-D-aspartate receptor antagonist, memantine is also approved for the treatment of this disease (David et al., 2012). Donepezil is a selective inhibitor of AChE and this drug was approved for the treatment of AD in 1996 (Doody, Cummings, Farlow, 2012). Donepezil is a well tolerated and is most effective AChE inhibitor without causing hepatotoxicity. The benzylic portion of donepezil combines with the catalytic anionic site (CAS) and dimethoxy indanone part combines with the peripheral anionic site (PAS) of AChE enzyme (Cheung et al., 2012). The AD is very complex in nature and involves many factors. Therefore, a drug molecule which interacts with a single receptor or enzyme is not sufficient for the treatment of this disease. Hybrid molecular strategy, in which the pharmacophoric parts of two drugs are combined in a single molecule,
is widely being used for designing the new donepezil derivatives (Leon et al., 2013). Over the last couple of years, a number of donepezil derivatives have been prepared and evaluated as anti-Alzheimer agents. To find the new derivatives, N-benzyl piperidinium and 5,6-dimethoxyindanone parts of donepezil have been attached as well as replaced by various other pharmacophores. In this review, an attempt has been made to summarize these derivatives on the basis of chemical structures and their biological activities as anti-Alzheimer agents.

DONEPEZIL DERIVED COMPOUNDS

Donepezil molecule comprises benzylpiperidine part which is connected to dimethoxy indanone through methylene group (Figure 1). It is used as a racemic mixture. Various structural modifications of this drug have been carried out and they are described in the following sections.

FIGURE 1 - Structural formula of donepezil.

Benzylpiperidine, benzylpyridine, pyridyl methylpiperidine fragments attached to indanone

Costanzo et al. (2016) carried out the synthesis of donepezil derivatives in which benzylpiperidine and indanone part were connected through the double bond. Compounds 2 (Figure 2) and 3 showed the most active inhibition of human erythrocytes AChE having IC$_{50}$ values of 0.058 $\pm$ 0.033 $\mu$M and 0.043 $\pm$ 0.007 $\mu$M respectively. These compounds were found to be selective for AChE as compared to butrylcholinesterase (BuChE) with selectivity values of 81 and 132 for compounds 2 and 3 respectively (Costanzo et al., 2016). Azzouz et al. synthesized bioxidiazable prodrugs of donepezil in which N-benzylpyridinium quaternary amine fragment was attached to indanone, tetralone and acetophenone fragments. Prominent activity was displayed by N-benzylpyridinium quaternary amine having the indanone ring. Compound 4 showed prominent inhibition of human AChE enzyme having an IC$_{50}$ value of 0.36 nM. Docking studies of compound 4 showed that indanone part interacted with PAS while N-benzyl group interacted with CAS of AChE. Carbonyl group of indanone part formed hydrogen bonding with Phe295 of AChE (Azzouz et al., 2018). Lan et al. carried out the synthesis of new donepezil derivatives in which 5,6-dimethoxy indanone part of donepezil was conjugated with N-benzylpyridinium moiety. Compound 5 presented activity as cholinesterase inhibitor having IC$_{50}$ values of 1.9 nM and 0.8 nM for electric eel AChE and human AChE respectively. Aβ aggregation inhibitory activity (53.7%) and antioxidant activity (0.54 Trolox equivalent) were also demonstrated by this compound. Compound 5 was also found to be neuroprotective at the dose of 1.56-12.5 $\mu$M. Compound 5 showed interaction with the CAS as well as with the PAS of AChE and showed the mixed type of inhibition (Lan et al., 2017). Wang et al. modified the benzyl group of donepezil with various other heterocyclic rings such as pyridine, quinoline and hydroxyquinoline and small groups were also introduced at these heterocyclic rings. Compound 6 exhibited prominent inhibition of the AChE having IC$_{50}$ values of 85 nM and 73 nM for electric eel AChE and human AChE respectively. Compound 6 also inhibited the Aβ$_{1,42}$ aggregation (46.3%) induced by copper (Cu$^{2+}$) and showed neuroprotective effects. Molecular modeling studies showed a strong binding interaction of this compound with the CAS and PAS of AChE (Wang et al., 2016). Renou et al. (2016) synthesized four donepezil derivatives in which benzyl group of donepezil was replaced by 3-hydroxy pyridinaldoxime. Newly synthesized oxime derivatives showed the capacity to re-activate VX (an organophosphorous nerve reagent) and inhibited the human AChE better than the standard drug pralidoxime. Compound 7 showed the prominent activation of human BuChE (5 to 11 fold higher) as compared to pralidoxime (Renou et al., 2016). Żurek, Szymański and Mikiciuk-Olasik (2013) synthesized donepezil derivatives by incorporating hydrazine nicotinate to the indanone part. Derivatives in this series showed a higher affinity for AChE than BuChE. Compound 9 was the most active AChE inhibitor (IC$_{50}$ = 1.087 x 10$^{-5}$ $\mu$M) and also showed selectivity (selectivity index= 248.776) for this enzyme. An increased AChE inhibition was observed by replacing piperidine with benzylpiperidine and the resulting compound 9 appeared to be more active than compound 8. Docking studies of compound 9 showed a binding score of -181.939 with AChE using cache software (Żurek, Szymański, Mikiciuk-Olasik, 2013).
Benzylpiperidine attached to aromatic rings other than indanone

Özer et al. attached benzylpiperidine with the aromatic rings through an acylhydrazone group. These compounds were moderately active and nonselective as AChE and BuChE inhibitors. Compound 10 (Figure 3) was the most active in this series having IC\textsubscript{50} values of 53.1 ± 4.56 μM and 67.3 ± 5.24 μM for AChE and BuChE enzymes respectively. Docking studies also showed the π-π interaction with PAS and CAS site of human AChE. These compounds showed excellent inhibition (69-80%) of the Aβ aggregation and have the potential for further modifications (Özer et al., 2013). N-benzylpiperidine-aryl-acylhydrazone derivatives were prepared by Viegas et al. (2018) and among these, compound 12 presented excellent inhibition of electrophorus electricus AChE with an IC\textsubscript{50} value of 8.65 μM. Compounds 11 and 12 also exhibited the inhibition of COX-1 (51% and 64% for 11 and 12) and COX-2 (65% and 53% for 11 and 12) enzymes. The interaction with COX enzymes was also confirmed by molecular docking studies. These compounds also showed neuroprotective ability (Viegas et al., 2018). N-benzylpiperidine part of donepezil was attached with the beta-secretase (BACE-1) inhibitor through isophthalamide group to produce the new hybrid molecules. New molecules showed dual inhibition of AChE and BACE-1 enzymes and compound 13 was most prominent having IC\textsubscript{50} values of 1.83 ± 0.12 μM, 0.567 ± 0.159 μM for AChE and BACE-1 respectively. This compound also reduced the production of Aβ (IC\textsubscript{50} = 98.7 nM) and showed protection against hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) induced cytotoxicity (Zhu et al., 2009). In 2017 attached the optical isomers of glutamic acid (D and L glutamic acid) with N-benzylpiperidine part of donepezil. L-glutamine derivatives were found to be more active than D-glutamine. Compound 14 showed prominent activity having IC\textsubscript{50} values of 4.99 ± 0.16 μM and 0.40 ± 0.02 μM for AChE and BuChE respectively. Compound 14 contains carboxy benzyl side chain (Monjas et al., 2017).

Benzylpiperidine attached with heterocyclic rings

Samadi et al. (2012) synthesized new pyridonepezil derivatives by linking the N-benzylpiperidine part
of donepezil and 2-aminopyridine heterocyclic ring. Compounds 15 (IC$_{50}$ = 0.0094 ± 0.0004 μM) (Figure 4) and 16 (IC$_{50}$ = 0.070 ± 0.005 μM) showed prominent AChE inhibitory activity as well as selectivity (S.I. = 703, 24 for compound 15 and 16 respectively) for human AChE than human BuChE (Samadi et al., 2012). An year later, Samadi et al. (2013) synthesized 6-chloropyridonepezil derivatives and compound 17 displayed the most active inhibition of human AChE (IC$_{50}$ = 0.013 ± 0.002 μM) and human BuChE (IC$_{50}$ = 8.13 ± 0.0004 μM). These compounds showed the ability to cross the BBB. Docking studies revealed that compound 17 interacted with the CAS and PAS of the AChE (Samadi et al., 2013). Li et al. (2016) synthesized donepezil derivatives by linking the benzylpiperidine part of donepezil with benzamide and picolinamide moiety from an MAO inhibitor lazabemide. These compounds displayed AChE, BuChE, MAO-A and MAO-B inhibitory activities. Compound 18 presented the most potent inhibition of cholinesterases having IC$_{50}$ values of 0.220 μM, 1.23 μM and 0.454 μM for electric eel AChE, equine BuChE and human AChE respectively. This compound also showed inhibition of MAO-A and MAO-B having IC$_{50}$ values of 3.14 μM and 13.4 μM. Compound 18 showed the capacity to cross the BBB and also exhibited metal chelating property. Docking studies of compound 18 with MAO-B demonstrated that 5-chloropicolinamide interacted with hydrophobic pocket while the N-benzylpiperidine bound via π-cation interaction with MAO-B (Li et al., 2016).

**Indanone derivatives having aromatic and heterocyclic rings**

Yerdelen et al. (2015) attached the indanone part of donepezil with substituted aromatic rings through the amide group. Compound 19 (Figure 5) was the most prominent...
inhibitor ($IC_{50} = 0.08 \pm 1.83 \mu M$) of AChE and it was most selective (SI = 40.1) for this enzyme. Compound 19 exhibited the most prominent inhibition of Aβ (55.3% at 25 μM) aggregation. Compound 19 also showed bonding interactions with CAS and PAS in the AChE binding sites (Yerdelen et al., 2015). Meng et al. synthesized new donepezil derivatives in which the indanone part was connected to the pyridine ring through the double bond. Upon evaluation as AChE and BuChE inhibitor, compound 20 showed the most prominent inhibition of AChE having an $IC_{50}$ value of 0.0018 μM and selectivity (5248.6) for AChE as compared to BuChE. It was more potent than the standard drugs donepezil and tacrine. Compound 20 also showed prominent metal chelating ability (Meng et al., 2012). Nan DD et al. synthesized 6-methoxy indanone derivatives and evaluated them as probes for β amyloid plaques. Iodine ($^{125}$I) radiolabelled derivative (21) showed better binding to the β amyloid plaque, higher uptake in the brain and rapid clearance. Compound 21 also showed better penetration across BBB having a partition coefficient of 3.45 (Nan et al., 2016). Rizzo et al. (2010) attached indanone part of donepezil to the phenyl-N-methylbenzylamino fragment from AP2238. Compound 22 showed prominent inhibition (46.8 ± 2.0%) of AChE induced Aβ aggregation. Derivatives 23 and 24 showed prominent AChE inhibitory activities having $IC_{50}$ values of 0.056 ± 0.003 μM and 0.052 ± 0.002 μM respectively. These compounds contain pentamethylene chain connecting diethylamine and piperidine group with the tetralone part. Compounds 23 and 24 also showed the Aβ aggregation inhibition induced by AChE having the inhibitory values of 42.9 ± 0.8% and 48.3 ± 0.9%. Compounds 23 and 24 also inhibited the self-aggregation of Aβ. The long alkyl chain of these compounds having amino group showed a better interaction with PAS (Rizzo et al., 2010). Mishra et al. carried out the synthesis of new 5,6-dimethoxy indanone-piperazine derivatives in which
structural parts of donepezil, curcumin and piperazine ring were combined to produce new molecules. Compounds 25 and 26 showed excellent AChE inhibitory potential having IC\textsubscript{50} values of 0.034 ± 0.002 μM and 0.025 ± 0.001 μM and showed selectivity (SI = 28.5 and 97.3 for 25 and 26 respectively) for AChE than BuChE. Compounds 25 and 26 showed excellent inhibition of the Aβ aggregation (80.4 ± 1.08%, 81.6 ± 0.78% for 25 and 26 respectively) and antioxidant (3.89 ± 0.01, 2.34 ± 0.03 for 25 and 26 respectively) activities. Molecular docking studies showed that these compounds have good interaction with AChE and Aβ peptide. Pyrimidine part of 26 binds through π-π interaction with PAS of AChE while the indanone part forms the hydrophobic interaction with CAS (Mishra et al., 2017). Synthesis of new donepezil derivatives was carried out by Sağlık et al. and their inhibitory effect on AChE was investigated. Compounds 27-30 displayed prominent inhibition of this enzyme (89.14-90.31% inhibition) and were found to be nontoxic (IC\textsubscript{50} = 78-320 μM) on NH/3T3 cell line. Side chains having another basic centre at the position # 4 of piperazine ring produced compounds with significant activity. Molecular docking studies also showed the interaction between these derivatives and targeted enzyme. Long chains of these compounds increased the van der Waals interactions with target enzyme (Sağlık, Ilğun, Özkay, 2016). Ismail et al. used indole molecule as the bioisosteric replacement of indanone part of donepezil. These compounds exhibited prominent AChE inhibitory activity and compound 31 was the most active (55% inhibition) derivative in this series. Molecular docking studies of this compound also showed good energy score (-23.4711 kcal/mol) in the active site of AChE. This was related to the additional 3-oxo and 2-fluorobenzyl groups in this compound (Ismail et al., 2012a).

**Phthalimide derivatives**

Mohammadi-Farani et al. (2013) carried out the synthesis of donepezil derivatives in which the phthalimide ring was used in place of indanone part of donepezil. It was connected to benzylpiperazine through an alkyl chain. In this series, compound 32 (Figure 6) was the most prominent inhibitor of AChE having an IC\textsubscript{50} value of 0.91 μM. This compound contains ortho chloro substituent attached to the phenyl ring. Therefore electron withdrawing groups increased the activity of phthalimide derivatives. The molecular modeling study of this compound showed that piperazine, phthalimide and benzyl group occupied the same position at the AChE as the piperidine, indanone and benzyl group of donepezil (Mohammadi-Farani et al., 2013). Ignasik et al. (2012) synthesized 2-(diethylaminoalkyl)-isoindoline-1,3-dione derivatives in which the phthalimide fragment was used as a substituent to indanone part of donepezil. Dialkylamine was used as a part which binds with the CAS. Compound 35 was the most prominent derivative in this series having

![FIGURE 6 - Phthalimide derived compounds.](image-url)
an IC\textsubscript{50} value of 0.9 μM for AChE. It contains an alkyl chain of eight methylene units. Compounds 33 and 34 also showed the promising and selective inhibition of AChE having IC\textsubscript{50} values of 1.2 μM and 1.1 μM. Docking studies of these compounds also showed interaction with AChE only. Compounds 33 and 34 also exhibited the inhibition (33.8% and 39.4% for 33 and 34) of Aβ aggregation (Ignasik et al., 2012). Sang et al. (2017) carried out the synthesis of new molecules comprising phthalimide and 4-amino tetrahydrothienoquinoline. The reaction was done by direct alkylation of 37a-e with 36 and 38. These compounds have the potential for evaluation as AChE inhibitors (Song, Jo, 2009).

### Donepezil and ferulic acid hybrid molecules

Ferulic acid is a phenolic compound which is found abundantly in plants. Donepezil and ferulic acid hybrids compounds were synthesized by Benchekroun et al. Resultant molecules showed prominent antioxidant activity and also showed selective inhibition of equine BuChE. Compounds 49 (Figure 7) and 50 were most active inhibitors of the BuChE having IC\textsubscript{50} values of 10.39 ± 0.48 nM and 20.1 ± 1.9 nM respectively. Compounds 49 and 50 exhibited oxygen radical absorbing capacity of 8.71 ± 0.20 μmol and 8.48 ± 0.33 μmol respectively. These compounds contain ethylene and propylene linkers respectively and both of them contain melatonin as part of their structure (Benchekroun et al., 2015). Dias et al. also synthesized donepezil-ferulic acid hybrid molecules by combining their pharmacophores. Compounds 49 and 50 exhibited anti-inflammatory potential by inhibiting COX-1, COX-2 and lipooxygenase (5-LOX) enzymes. This compound also showed metal chelating capacity with certain metals along with neuroprotective effects (Dias et al., 2017). Sang et al. carried out the synthesis of ferulic acid-O-alkylamine derivatives by attaching the benzylpiperidine part of donepezil with ferulic acid and secondary amines by using methylene spacer. These compounds showed BuChE inhibitory and antioxidant activity. Compound 52 appeared as the most excellent inhibitor of BuChE (IC\textsubscript{50} = 0.021 μM) and AChE (IC\textsubscript{50} = 2.13 μM). Compound 52 also showed the inhibition of Aβ (50.8 ± 0.82%) aggregation. It also showed the ability to cross the BBB as well as less toxicity. Molecular modeling studies of compound 52 presented cation-π interaction, π-π interactions and hydrophobic interactions with BuChE but only hydrophobic interactions were observed with AChE which may be the reason for its selectivity for BuChE (Sang et al., 2017). Xu et al. (2016) combined the pharmacophores of donepezil and ferulic acid to produce new hybrid molecules. Compound 53 displayed the prominent inhibition of electric eel AChE and equine BuChE having IC\textsubscript{50} values of 0.398 μM and 0.976 μM respectively. Significant antioxidant activity (1.78 Trolox equivalents) was also observed for this compound. Molecular docking studies showed that compound 53 binds with PAS by polar contacts of phenyl propanoid group and with CAS by the π-π interaction of the phenyl ring (Xu et al., 2016).

### Donepezil and tacrine hybrid compounds

Tacrine was the first compound used for the treatment of AD but it was withdrawn from the market due to the toxic side effects. 7-Methoxytacrine is a less toxic derivative of tacrine (Soukup et al., 2013). Alonso et al.
reported the synthesis of donepezil and tacrine hybrid compounds in which indanone part of donepezil was replaced by phthalimide ring. These derivatives showed more selectivity for AChE as compared to BuChE. Compounds 54 and 55 (Figure 8) displayed excellent inhibition of the AChE enzyme having IC\textsubscript{50} values of 2.8 nM and 2.4 nM respectively. These compounds have the linker chain length of 9 and 12 methylene units between 9-aminoacridine and phthalimide scaffold. Molecular modeling of compounds 54 and 55 revealed that tetrahydroacridine/acridine rings of these compounds interacted with the catalytic site. Phthalimide ring attached with the peripheral site of AChE. Aromatic ring nitrogen formed hydrogen bonding while the chlorine atom interacted with hydrophobic pocket (Alonso et al., 2005).

Synthesis of donepezil and tacrine hybrid molecules was carried out by Camps et al. in which the 5,6-dimethoxy-2-(4-piperidylmethyl) 1-indanone piperidine part of donepezil was attached to the tacrine. These compounds showed prominent inhibition of AChE and BuChE enzymes. Compound 56 was most potent having an IC\textsubscript{50} value of 0.27 ± 0.03 nM for human AChE. Compound 57 was the most prominent inhibitor (65.9 ± 2.5% inhibition) of A\textbeta aggregation. Tetrahydroacridine ring of compound 56 attached with the catalytic site of AChE while ring nitrogen formed hydrogen bonding with the carbonyl oxygen of His447 of enzyme and chlorine atom occupies the hydrophobic pocket (Camps et al., 2008).

Sepsova et al. (2015) evaluated 7-methoxytacrine and donepezil hybrid molecules for their interaction with AChE, with muscarinic and nicotinic acetylcholine receptors and these compounds presented activity in the micromolar range. They also showed the capacity to penetrate into CNS. Compound 58 (IC\textsubscript{50} = 1.38 ± 0.15 μmol/litre) was the most potent

![FIGURE 8 - Hybrid molecules comprising donepezil and tacrine fragments.](image-url)
AChE inhibitor and showed a mixed type of inhibition i.e. competitive and non-competitive (Sepsova et al., 2015). Hybrid molecules comprising 7-methoxytacrine and donepezil were synthesized by Korabecny et al. (2014) in which benzylpiperazine acted as isostere of the benzylpiperidine fragment of donepezil. The synthesized compounds exhibited excellent cholinesterase inhibitory activity against human and animal cholinesterases. Compounds 59, 60, 61 were prominent having IC\textsubscript{50} values of 1.12 $\pm$ 0.11 μM, 1.16 $\pm$ 0.22 μM and 1.94 $\pm$ 0.26 μM against human AChE while 62 was the most active inhibitor of human BuChE with an IC\textsubscript{50} value of 0.42 $\pm$ 0.01 μM. Molecular modeling investigations showed the interaction of these derivatives with the CAS and PAS of the AChE. 4-Bromophenyl group of compound 59 forms a new π-π interaction with enzyme and showed a binding energy of -13.8 kcal/mol (Korabecny et al., 2014). Misik et al. (2015) evaluated 7-methoxytacrine and N-benzylpiperazine hybrid molecules as AChE inhibitors and compound 63 showed prominent inhibition (11.3%) of this enzyme as compared to donepezil (41%). Compound 63 contains benzylpiperazine group in place of benzylpiperidine fragment of donepezil (Misik et al., 2015).

**Donepezil hybrids with natural compounds**

Compounds isolated from natural sources serve as lead compounds for new drug development (Koehn, Carter, 2005). Natural compounds have been combined with benzylpiperidine part of donepezil and these molecules demonstrated anti-Alzheimer activities. Xie et al. combined N-benzylpiperidine part of donepezil with coumarin to produce new molecules having cholinesterase and MAO-B inhibitory activities. Compound 64 (Figure 9) appeared as the most active inhibitor of human AChE and human BuChE with IC\textsubscript{50} values of 1.37 $\pm$ 0.05 μM and 1.98 $\pm$ 0.08 μM respectively. Compound 64 also showed inhibition of the MAO-B with an IC\textsubscript{50} value of 2.62 $\pm$ 0.81 μM. Molecular modeling studies showed that phenyl group of coumarin ring binds with PAS through π-π interaction and carbonyl oxygen forms hydrogen bonding with PAS. N-benzylpiperidine forms π-π interaction with CAS of AChE. Compound 64 showed the capacity to cross the BBB and was found non-toxic for HepG2 cell line (Xie et al., 2016). Yan et al. synthesized new molecules by combining pharmacophores of donepezil and curcumin. Compound 65 exhibited the prominent inhibition of AChE and it also showed selectivity for AChE (IC\textsubscript{50} = 0.187 $\pm$ 0.015 μM) than BuChE (IC\textsubscript{50} = 12.4 $\pm$ 1.03 μM). Compound 65 also showed inhibition (45.3 $\pm$ 3.58%) of Aβ aggregation. This compound also penetrated BBB (Pe = 11.85 $\pm$ 1.00) and showed antioxidant activity (3.07 $\pm$ 0.04 μmol) (Yan et al., 2017). Shen et al. (2009) linked N-benzylpiperidine part of donepezil to various flavonoids to produce the new hybrid molecules. Among these derivatives, Compound 66 exhibited the most effective inhibition of AChE with an IC\textsubscript{50} value of 0.093 μM. Compound 66 showed increased selectivity for AChE as compared to BuChE. Compounds having oxygen linker were found to be more active as compared to the methoxy group. Molecular docking investigations of this compound with AChE showed that isoflavone part

*FIGURE 9* - Donepezil hybrids molecules with natural compounds.
of compound 66 joined the peripheral pocket through hydrophobic interactions. Two hydrogen bonds were also observed for this compound with Ser286 and Arg289. Compound 66 showed a binding free energy of -22.28 kcal/mol (Shen et al., 2009). Rodriguez-Franco et al. (2005) also linked benzylpiperidine and benzylpyridine to 8-substituted xanthine derived from theophylline. Methoxymethyl group was used as a linker group between these two pharmacophores. These compounds exhibited AChE and BuChE inhibitory activities. Compound 67 showed prominent activity in this series presenting an IC\textsubscript{50} value of 0.1 ± 0.01 μM for AChE. Compound 68 was the most active inhibitor of the BuChE with an IC\textsubscript{50} value of 0.4 ± 0.01 μM. Therefore it was noted that compounds having benzylpyridine part showed better interaction with AChE. The additional aromatic ring interacted with the aromatic amino acid of AChE (Rodríguez-Franco et al., 2005).

**Donepezil derivatives having propargylamine group**

The presence of propargylamine group in certain drugs such as rasagiline and ladostigil confer neuroprotective, antiapoptotic and Aβ aggregation inhibitory properties (Bar-Am et al., 2010). Bautista-Aguilera et al. carried out the synthesis of new donepezil and indole hybrid molecules as anti-Alzheimer agents. In this compound, benzylpiperidine was attached to the position # 5 of indole through three methylene units and propargylamine part was attached at position # 2 of the indole ring. Compound 69 (Figure 10) was found to be the most potent agent in this class. It showed potent human MAO-A (IC\textsubscript{50} = 0.0055 ± 0.0014 μM) inhibitory activity as well as selectivity as compared to human MAO-B (IC\textsubscript{50} = 0.15 ± 0.031nM). Compound 69 also exhibited AChE (IC\textsubscript{50} = 0.19 ± 0.01 μM) and BuChE (IC\textsubscript{50} = 0.83 ± 0.16 μM) inhibitory activities. Docking studies with AChE showed that propargylamine interacted via π-π bonding with Trp86. Phenyl ring interacted with the PAS and compound showed the binding free energy of -11.9 kcal/mol (Bautista-Aguilera et al., 2014). Bolea et al. synthesized some multitarget molecules in which benzylpiperidine part of donepezil and indole propargylamine part of MAO inhibitor N-[5-benzyloxy-1-methyl-1H-indol-2-yl)methyl]-N-methylprop-2-yn-1-amine were conjugated. Resultant compounds showed the capacity to interact with cholinesterases and MAO enzymes. Compound 70 exhibited prominent activity having MAO-A (IC\textsubscript{50} = 5.2 ± 1.1 nM), MAO-B (IC\textsubscript{50} = 43 ± 8 nM), AChE (IC\textsubscript{50} = 0.35 ± 0.01 nM), BuChE (IC\textsubscript{50} = 0.46 ± 0.06 nM) inhibitory activities. Compound 70 also showed the inhibition (32.4 ± 7.0%) of Aβ aggregation. Molecular docking studies revealed that benzylpiperidine fragment attached with catalytic site and indole propargylamine part is stacked between aromatic rings with Trp279 and Tyr334. Propargylamine formed van der Waals interaction with phenyl ring of Tyr70 (Bolea et al., 2011). Wang et al. (2014) synthesized hybrid compounds in which structural parts of donepezil, propargylamine and 8-hydroxyquinoline were combined. Compound 71 was the most active compound and showed simultaneous inhibition of MAO-A and MAO-B presenting IC\textsubscript{50} values of 6.2 μM and 10.2 μM respectively. Significant AChE (IC\textsubscript{50} = 1.8 ± 0.1 μM) and BuChE (IC\textsubscript{50} = 1.6 ± 0.25 μM) inhibitory values were also observed for this compound. Compound 71 also showed excellent pharmacokinetic properties and displayed less toxicity to HepG2 cell line. Compound 71 contains 8-hydroxyquinoline and N-benzylpiperidine attached to the central propargylamine part having a nitrile group. This compound is a racemic mixture and both isomers showed binding at PAS and CAS of electric eel AChE (Wang et al., 2014). Later on, Wu et al. (2016) also

![FIGURE 10 - Propargylamine derivatives of donepezil.](image-url)
combined propargylamine group with benzylpiperidine and quinoline to produce new hybrid molecules. Among these derivatives, compound 72 showed excellent inhibition of AChE ($IC_{50} = 0.029 \pm 0.003$ μM), BuChE ($IC_{50} = 0.039 \pm 0.003$ μM) and selective MAO-A inhibition ($IC_{50} = 10.1 \pm 1.1$ μM). Compound 72 also showed metal chelating and antioxidant properties. The potency of compound 72 for cholinesterases was linked to the appropriate distance between the aromatic part and basic centre which are necessary for interaction with PAS, mid-gorge and CAS of AChE. Compound 72 is the bigger derivative in this series and cyano group can interact with mid gorge because of the correct length (Wu et al., 2016).

**Attachment of benzyl piperidine and benzyl piperazine with condensed heterocyclic rings**

Vila et al. (2016) carried out the synthesis of donepezil analogues in which the indanone part of donepezil was replaced by the phthalazinone nucleus and the phthalazine was connected to the N-benzylpiperidine fragment through the alkyl chain of two to three carbons. Compound 73 was prominently active and found to be the equipotent inhibitor of AChE and BuChE having $IC_{50}$ values of $3.45 \pm 0.23$ μM and $5.50 \pm 0.37$ μM. Compound 73 (Figure 11) contains p-tolyl group at position # 4 of the phthalazine ring which gives an additional hydrophobic interaction with cholinesterases. Docking studies also showed dual binding of this compound with AChE as well as with BuChE (Vila et al., 2016). Pudlo et al. connected quinoline carboxamide to the benzylpiperidine to produce the new hybrid molecules. These compounds exhibited the better inhibition of AChE as compared to BuChE. Compounds 74 and 75 were most active having $IC_{50}$ values of $0.11 \pm 0.03$ μM and $0.48 \pm 0.14$ μM. Compound 75 also showed highest antioxidant activity ($EC_{50} = 12.2 \pm 0.4$ μM) and it contains catechol ring. Catechol ring was found to be important for antioxidant activity. Docking studies of compound 75 showed that phenyl group formed $\pi-\pi$ interactions with CAS, piperidinium interacted with gorge and quinoline part was attached with PAS of AChE (Pudlo et al., 2014). Synthesis of donepezil-Trolox hybrid molecules was reported by Cai et al. (2017). These compounds demonstrated activity against AChE and MAO-B enzymes. The most potent compound 76 showed inhibition of human AChE and MAO-B with $IC_{50}$ values of $0.54$ μM and $4.3$ μM respectively. This compound also showed significant antioxidant activity ($IC_{50} = 41.33$ μM) and less hepatotoxicity to cell lines PC12, HePG2 and BV-2 (Cai et al., 2017). $N$-benzylpiperidine fragment of donepezil and melatonin were fused together to produce the new molecules. Most of the compounds showed higher inhibition of BuChE as compared to AChE. Compound 77 was the excellent inhibitor of electric AChE and equine BuChE having $IC_{50}$ values of $193$ nM and $73$ nM. This compound also displayed Aβ aggregation (56.3%) inhibition and antioxidant activities (3.28 Trolox equivalent). It also showed the capacity to penetrate into CNS. Molecular modeling studies showed that compound 77 interacted with CAS and PAS of AChE (Wang et al., 2016).

Ismail et al. (2012b) carried out the synthesis of donepezil derivatives in which the indanone part of donepezil was substituted by tetrahydrobenzo[b] thiophene ring having carbonyl group at position # 3 of tetrahydrobenzo[b]thiophene. Acetamide group was introduced as a linker between the tetrahydrobenzo[b] thiophene and benzylpiperidine. Compounds 78 and 79

![FIGURE 11 - Donepezil derivatives having condensed heterocyclic rings.](image-url)
(Figure 12) exhibited prominent inhibition (56.67%, 60% for 78 and 79) of AChE which was better than the standard drug donepezil (40%). In molecular docking studies, compound 78 presented strong hydrogen bonding than donepezil, which may be due to the amide group (Ismail et al., 2012b). Omran et al. (2005) carried out the synthesis of new indanones and thiaindanones derivatives and evaluated their AChE inhibitory activity. Compound 80 exhibited excellent inhibition of AChE having an IC$_{50}$ value of 0.06 μM as compared to donepezil (IC$_{50}$ = 0.02 μM). It contains oxime group in the thiaindanone part and thiaindanone is attached to 2-fluorobenzyl piperazine through acetamide linker. Molecular modeling studies were also carried out and it was observed that fluorine atom at the ortho position of the benzyl group amplified the π-π interaction with the AChE enzyme (Omran et al., 2005). Omran et al. (2008) carried out further modification and compound 81 was the most active inhibitor of AChE showing an IC$_{50}$ value of 0.5 μM. It was observed that the replacement of the piperazine ring leads to the formation of inactive compounds. Replacement of hydroxyl group of this molecule with the bromine or fluorine produced less active compounds (Omran et al., 2008).

**DISCUSSION**

A literature review of donepezil showed that benzylpiperidine part of donepezil can be replaced with benzylpyridine, benzylpiperazine, pyridyl methylpiperidine, aniline and phenylpiperazine motifs with the retention of activity as anti-Alzheimer agents. Replacement of benzylpiperidine with unsubstituted piperidine produced less active compounds. 3-Hydroxy-6-(piperidin-1-ylmethyl)picolinaldehyde oxime derivatives were found to be reactivator of the AChE. Alkylamine, carboxamide and acylhydrazone are most commonly used linkers in these molecules. Attachment of benzylpiperidine with heterocyclic rings such as pyridine showed AChE and BuChE inhibitory activity while the picolinamide derivative showed MAO inhibition in addition to cholinesterase inhibitory activity. Benzylpiperidine part of donepezil has also been attached with condensed heterocyclic rings such as phthalazine, quinoline, tetrahydrobenzothiophene, indole and Trolox and these molecules showed anti-Alzheimer activities. Propargylamine derived compounds also exhibited MAO inhibition in addition to cholinesterase inhibition properties. In some derivatives, indanone part has been replaced with tetralone, oxindole, and phthalimide with retention of activity. Hybrid molecules of donepezil with ferulic acid showed higher BuChE inhibitory activity as compared to AChE inhibitory activity. These compounds also showed antioxidant as well as Aβ aggregation inhibitory activities. Hybrid molecules of donepezil with tacrine produced compounds having AChE and BuChE inhibition. Donepezil hybrid molecules with natural compounds like flavonoids, coumarins, and curcumin presented antioxidant and MAO inhibitory activities in addition to cholinesterase inhibition. Amino acid derived compounds of donepezil showed dual inhibition of AChE.
and BuChE enzymes. In this review, we have summarized the recent research progress of donepezil derived anti-Alzheimer agents. This review can help to provide some useful information for organic and medicinal chemists working in this field.

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