



DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF CHALCONE BASED COMPOUNDS IN ALZHEIMER'S DISEASE

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Abstract

Alzheimer's disease (AD), a complex neurodegenerative brain disorder, a most common cause of dementia among elderly people. To date, the AD is being managed by maintaining the levels of acetylcholine by inhibiting acetylcholinesterase (AChE). Following this approach, a new series of Chalcones were designed, synthesized and biologically evaluated against acetylcholinesterase (AChE) with additional free radical scavenging activity. The *in vitro* studies showed that the majority of synthesized derivatives inhibited acetylcholinesterase (AChE) with IC₅₀ values in the micro molar range. Some of the derivatives strongly inhibited AChE. Besides AChE inhibitory activity, these compounds also exhibited greater ability to scavenge free radicals. Thus, chalcones might be the promising lead compound as potential anti-Alzheimer's agents.

Keywords: AChE inhibitor, Alzheimer's disease, Antioxidants, Chalcones, Acetylcholinesterase

Introduction

Alzheimer's disease (AD), a neurodegenerative disorder associated with a progressive loss of cognitive functions, now emerging as one of the greatest health threat of the twenty-first century affecting almost 50% of adults over the age of 85. With the increase in old age population all over the world, this percentage could reach a very staggering point soon (Bishop *et al.*, 2010)

Progressive decline in cognitive performance observed in AD is accompanied by behavioral and psychological syndromes, such as depression and psychosis (Schmitt *et al.*, 2004). It is an irreversible, progressive brain disorder related to changes in nerve cells that result in the death of brain cells. AD occurs gradually and is not a normal part of the aging process. Many hypotheses described molecular mechanisms which have an important role in the development of AD. The older two of these hypotheses are the "cholinergic hypothesis", which is based on neurochemical findings that suggest a striking decrease in acetylcholine containing neurons in AD brain (Fig. 1), and the "amyloid hypothesis", which is based upon intraneuronal and extracellular deposits of β -amyloid protein in AD brains causing amyloid plaques and neurofibrillary tangles (Upton *et al.*, 2008; Goedert *et al.*, 1991).

The currently available drugs used to treat AD, developed according to the reductionist paradigm of 'one-molecule-one-target,' have turned out to be palliative rather than curative. Thus, drug molecule that can act at multiple targets in neurotoxin cascades offer new hopes toward curing AD neurodegenerative diseases (Singh *et al.*, 2013). The single chemical entities that can modulate multiple targets simultaneously can be developed with superior efficacy and safety profiles (Morphy and Rankovic, 2005; Youdim and Buccafusco, 2005)

Presently, among all hypotheses, the "cholinergic hypothesis" is based on neurochemical findings that suggest a striking decrease in acetylcholine containing neurons in AD brain, and the 'amyloid hypothesis' is based upon

intraneuronal and extracellular deposits of β -amyloid protein in AD brains causing amyloid plaques and neurofibrillary tangles, explored by research scientists (Goedert *et al.*, 1991; Singh *et al.*, 2013). Acetylcholinesterase enzyme is responsible for the depletion of critical neurotransmitter acetylcholine which is plausibly associated with the memory deficits, is one of the most promising targets for treatment of AD. It has also been partially involved in the formation of amyloid plaques and neurofibrillary tangles by binding to β -amyloid, causing aggregation of its fragments. The aggregated complex induces the conformational transition to the amyloidogenic conformer, which are more cytotoxic than β -amyloid fibrils alone (Fig. 1) (Alvarez *et al.*, 1998; Ferrari *et al.*, 2001). Only four cholinesterase inhibitors, Tacrine (1), Donepezil (2), Rivastigmine (3) and Galantamine (4) have been approved by U.S. Food and Drug Administration (FDA) for symptomatic treatment of AD (Fig. 2).

Oxidative stress (OS) has also been proposed as one of the major causes inducing neuronal death in AD. In OS, reactive oxygen species (ROS) like hydroxyl radical ($\cdot\text{OH}$), superoxide ($\text{O}_2^{\cdot-}$), Hydrogen peroxide (H_2O_2) are excessively produced or insufficiently degraded and overcome the protective defense mechanism of cells proceeding the functional disintegration and, finally the cell death.

In particular, oxidative stress-induced injury is observed in the most cellular macromolecules of AD brains, including nucleic acids, proteins, and lipids (Markesbery, 1997). Brain tissue is particularly vulnerable to oxidative damage because of its higher rate of oxidative metabolic activity, intense production of ROS, relatively low antioxidant activity, non-explicative nature of neuronal cells and the high membrane surface to cytoplasm ratio. Despite these, the imbalance between free radical formation and destruction is involved in AD pathogenesis. These findings support the 'oxidative stress' hypotheses of AD, which proposes antioxidants as beneficial therapeutic tools in treatment of such condition.

These inter-related hypotheses contribute to the complex pathogenesis of AD and the compounds that can act at different levels of the neurotoxic cascade or can modulate

multiple targets simultaneously, offer new hopes toward curing AD. In the present study, Chalcones based compound modulating acetylcholine levels, having antioxidant potential have been developed as novel therapeutics for the treatment of AD (Markesbery, 1998).

Chalcones constitute an important class of natural products belonging to the flavonoid family, which have a wide spectrum of biological activities, including Anti-Alzheimer's, AChE inhibitory activities, antibacterial, antifungal, anti-inflammatory, antimicrobial, antitumor, ROS formation reduction effect or antioxidant properties and anti-mutagenic activities (Patil *et al.*, 2009; Bag *et al.*, 2013). The various chalcones were designed by exploring the various positions to improve AChE inhibitory and radical scavenging activity. Chalcones belong to the flavonoid family and display several pharmacological activities which are very important. Chemically it is an α,β -unsaturated ketone having core scaffold of 1,3-diaryl-2-propen-1-one. The phenyl ring attached to the carbonyl group is defined to be the A ring and the other benzene ring is named as the B ring. As discussed earlier chalcone has good antioxidant property due to the presence of a double bond in conjugation with 4-keto functional group. On the basis of these observations, different derivatives were synthesized and evaluated for AChE inhibitory activity along with antioxidant properties.

Material and Methods

All chemicals were procured from Sigma Aldrich Co., SD fine, Loba chemicals and were 99% pure, thus used without any purification. The completion of each reaction was monitored by thin layer chromatography (DC-Alufoleien (20x20 cm) Kieselgel 60 F254 chromatoplates) using hexane:ethylacetate (6:4) as a TLC development solvent system. All final compounds were purified on silica columns while all intermediates were purified by recrystallization from appropriate solvent. The melting point were recorded on a digital automatic melting point apparatus and uncorrected, IR spectra were recorded on a Bruker (Alpha E) FT/IR spectrophotometer, $^1\text{H-NMR}$ spectra were recorded on a Bruker Advance II 400 MHz NMR spectrometer using chloroform (CDCl_3) or dimethylsulfoxide (DMSO-d_6) as solvent and tetramethylsilane (TMS) as an internal standard. Proton chemical shifts are expressed in parts per million (ppm). Mass spectra (ESI-MS, positive) were recorded with a Waters, Q-TOF Micromass (LC-MS).

General procedure for synthesis of compounds

The compounds (CH-1–CH-8) were synthesized by Claisen-Shmidt base catalyzed condensation of appropriate aromatic ketones or substituted aromatic ketones with benzaldehydes or substituted benzaldehydes. The overall synthetic protocol for compounds (CH-1–CH-8) is outlined in Scheme 1. At first, the dissolve 0.01 mol of benzaldehyde and 0.01 mol of the acetophenone in 10 ml of 95% ethanol in a 25 ml Erlenmeyer flask equipped with a magnetic stirring bar. 3.5 ml of 10% NaOH solution was then added to the reaction flask using a pipette. Stir the reaction mixture for 10 minutes. Cool in an ice water bath until crystal formation is complete. Add 8-10 ml of ice-cold water to the flask. The crude products were filtered and allow to air dry. Then, purified on silica columns using hexane: ethyl acetate (6:4) as solvent (Dong *et al.*, 2008; Venkatesan *et al.*, 2010).

1,3-Diphenyl-2-propen-1-one (CH-1):

Yellow, crystalline, yield 82%, mp 55-58°C; $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , δ ppm): 7.08-7.33 (5H, m), 7.48-7.65 (5H, m), 7.70 (1H, d, $J = 7.96$), 8.03 (1H, d, $J = 8.02$). IR: (-CH) 2903 cm^{-1} , (C=O) 1686 cm^{-1} , (-CH=CH-) 1604 cm^{-1} . MS (ESI) $m/z = 209.26$ [$\text{M}+\text{H}$] $^+$, R_f Value: 0.62 (Hexane: Ethyl acetate, 6:4).

3-(3-Chlorophenyl)-1-phenylprop-2-en-1-one (CH-2):

Light yellow, crystalline, yield 70%, mp 118-120 °C; $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , δ ppm): 7.29-7.33 (3H, m), 7.38-7.42 (1H, m), 7.48-7.61 (5H, m), 7.65 (1H, d, $J = 7.80$), 8.11 (1H, d, $J = 8.16$). IR: (-CH) 2915 cm^{-1} , (C=O) 1688 cm^{-1} , (-CH=CH-) 1602 cm^{-1} , (-C-Cl) 815 cm^{-1} . MS (ESI) $m/z = 243.52$ [$\text{M}+\text{H}$] $^+$, R_f Value: 0.74 (Hexane: Ethyl acetate, 6:4).

3-(4-Chlorophenyl)-1-phenylprop-2-en-1-one (CH-3):

Dark grey, amorphous powder, yield: 86%, m.p. 115-118° C. $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , δ ppm): 7.31-7.37 (5H, m), 7.62-7.68 (4H, m), 7.72 (1H, d, $J = 8.0$), 8.07 (1H, d, $J = 7.96$). IR: (-CH) 2907 cm^{-1} , (C=O) 1684 cm^{-1} , (-CH=CH-) 1611 cm^{-1} , (-C-Cl) 820 cm^{-1} . MS (ESI) $m/z = 243.52$ [$\text{M}+\text{H}$] $^+$, R_f Value: 0.68 (Hexane: Ethyl acetate, 6:4).

3-(3-Bromophenyl)-1-phenylprop-2-en-1-one (CH-4):

Light yellowish brown, amorphous powder, yield 75%, mp 111-113 °C; $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm): 7.27-7.42 (4H, m), 7.49-7.60 (5H, m), 7.63 (1H, d, $J = 8.80$), 8.08 (1H, d, $J = 7.90$). IR: (-CH) 2935 cm^{-1} , (C=O) 1665 cm^{-1} , (-CH=CH-) 1606 cm^{-1} , (-C-Br) 713 cm^{-1} . MS (ESI) $m/z = 288.22$ [$\text{M}+\text{H}$] $^+$, R_f Value: 0.62 (Hexane: Ethyl acetate, 6:4).

3-(4-Bromophenyl)-1-phenylprop-2-en-1-one (CH-5):

Yellowish, amorphous powder, yield: 74%, m.p.: 117-119 °C. $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm): 7.25-7.40 (4H, m), 7.45-7.55 (5H, m), 7.67 (1H, d, $J = 8.0$), 8.10 (1H, d, $J = 7.96$). IR: (-CH) 2931 cm^{-1} , (C=O) 1650 cm^{-1} , (-CH=CH-) 1610 cm^{-1} , (-C-Br) 702 cm^{-1} . MS (ESI) $m/z = 288.22$ [$\text{M}+\text{H}$] $^+$, R_f Value: 0.60 (Hexane: Ethyl acetate, 6:4).

3-(4-Nitrophenyl)-1-phenylprop-2-en-1-one (CH-6):

Light yellowish, solid amorphous, yield 80%, m.p.: 157-161°C; $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm): 7.48-7.78 (5H, m), 7.80-7.88 (1H, d, $J = 8.4$), 8.01-8.05 (2H, m), 8.18-8.21 (1H, d, $J = 7.50$), 8.30-8.38 (2H, m). IR: (-CH) 2915 cm^{-1} , (C=O) 1652 cm^{-1} , (-CH=CH-) 1612 cm^{-1} , (N-O) 1552 cm^{-1} . MS (ESI) $m/z = 254.27$ [$\text{M}+\text{H}$] $^+$, R_f Value: 0.56 (Hexane: Ethyl acetate, 6:4).

3-(4-Hydroxyphenyl)-1-phenylprop-2-en-1-one (CH-7):

Yellowish brown, solid amorphous, yield 80%, m.p.: 182-185°C; $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , δ ppm): 6.59-6.62 (2H, m), 7.42-7.46 (2H, m), 7.49-7.82 (5H, m), 7.61-7.62 (1H, d, $J = 8.5$), 8.12 (1H, d, $J = 7.0$), 10.45 (1H, broad s, -OH); IR: (-CH) 2915 cm^{-1} , (-OH), 3352 cm^{-1} broad, (C=O) 1664 cm^{-1} , (-CH=CH-) 1615 cm^{-1} . MS (ESI) $m/z = 225.11$ [$\text{M}+\text{H}$] $^+$, R_f Value: 0.71 (Hexane: Ethyl acetate, 6:4).

1-(2-Hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (CH-8):

Yellowish grey, crystalline solid, yield: 62%, m.p.: 242-246° C. $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , δ ppm): 3.70 (3H, s, -OCH₃), 3.83 (6H, s, -OCH₃), 6.90 (2H, s), 7.44-7.58 (5H, m), 7.63-7.65 (1H, d, $J = 7.96$), 8.10 (1H, d, $J = 8.0$), 12.40

(1H, broad s, -OH); IR (KBr pellets): (-OH) 3375 cm^{-1} (w), (C=C) 1612 cm^{-1} (m), (C=O) 1657 cm^{-1} (s), (C-O-C) 1170 cm^{-1} ; MS: m/z: 315.48 [M+1], R_f Value: 0.45 (Hexane: Ethyl acetate, 6:4).

Biological Evaluation

DPPH radical scavenging activity method

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical scavenging activity of the test compounds. The 0.1 mM solution of DPPH in methanol (39.4 mg in 1000 ml) was freshly prepared. Different concentrations of test compounds were added with an equal volume to methanol solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. Ascorbic acid was used as standard. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. IC_{50} value was determined from the plotted graph of scavenging activity against the different concentrations of test compounds. Ascorbic acid was applied as positive drug (Blois *et al.*, 1958).

Acetylcholinesterase inhibitory Assay

AChE inhibitory activity was measured by the spectrophotometric method with slight modification, rat cortex homogenate was used as the resource of AChE. For assay of AChE activity, a reaction mixture containing 100 μl acetylthiocholine iodide 0.075M/L, 100 μl sodium phosphate buffer (0.1M/L, pH 7.4), 20 μl homogenate or serum and different concentrations of test compounds 20 μl was incubated at 37° C for 15 min. The reaction was terminated by adding 50 μl 3% sodium lauryl sulfate, then, 50 μl , 0.2% of 5,5'-dithio-bis-(2-nitrobenzoic acid) was added to produce the yellow anion of 5-thio-2-nitro-benzoic acid. The values of IC_{50} were calculated by UV spectroscopy from the absorbance changes at 450 nm. Donepezil was applied as positive drug. All samples were assayed in triplicate (Ellman *et al.*, 1961).

Result and Discussion

Chemistry

Chalcones belong to the flavonoid family and display several pharmacological activities which are very important. Chemically it is an α,β -unsaturated ketone having core scaffold of 1,3-diaryl-2-propen-1-one. The phenyl ring attached to the carbonyl group is defined to be the A ring and the other benzene ring is named as the B ring. As discussed earlier chalcone has good antioxidant property due to the presence of a double bond in conjugation with 4-keto functional group. On the basis of these observations, different derivatives were synthesized using different electron withdrawing and electron releasing groups and evaluated for AChE inhibitory activity along with antioxidant properties.

The compounds (CH-1–CH-8) were synthesized by Claisen-Shmidt base catalyzed condensation of appropriate aromatic ketones or substituted aromatic ketones with benzaldehydes or substituted benzaldehydes. The overall synthetic protocol for compounds (CH-1–CH-8) is outlined in Scheme 1. Various substituted benzaldehydes (2) and substituted acetophenones (1) were mixed in 10% NaOH solution and 95% (Dong *et al.*, 2008; Venkatesan *et al.*, 2010). All the compounds were characterized using various spectral techniques like IR, NMR and Mass Spectrophotometer. The completion of each reaction was

monitored by thin layer chromatography (TLC) on pre-coated aluminum plates (DC Kieselgel 60, silica gel 60 F₂₅₄, Merck Millipore). The compounds were purified using column chromatography on normal phase columns or glass columns packed with silica gel (100-200 mesh size) using hexane:ethylacetate (6:4) as a solvent system.

Biological activity

The synthesized chalcones were evaluated for anti-oxidant activity and acetylcholinesterase inhibitory activity.

DPPH radical scavenging activity studies

Synthesized compounds (CH-1–CH-8) were tested for their free radical scavenging activities by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay at 517nm. The IC_{50} (defined as the concentration resulting in 50% scavenging activity) were determined. The DPPH scavenging activities of test compounds are summarized in Table 1. Most of the compounds (CH-3, CH-6, CH-7, and CH-8) exhibited radical scavenging activity comparable to ascorbic acid (Table 1) (Blois *et al.*, 1958).

In vitro AChE inhibition studies

The AChE inhibitory activity of all the synthesized compounds were tested *in-vitro*, according to the modified Ellman method using rat cortex homogenate (Ellman *et al.*, 1961), whereas donepezil was taken as reference drug. The IC_{50} values of all synthesized compounds are summarized in Table 1. All the synthesized compounds have AChE inhibitory activity. Among all, three compounds showed higher (CH-8, IC_{50} =6.4 μM) acetylcholinesterase inhibitory activity than donepezil (IC_{50} =7.5 μM) and compounds CH-3 and CH-6 (IC_{50} =11.8 and 12.5 μM , respectively) have good activity (Table 1). The variation of different substituents at different positions of chalcone scaffolds alters the AChE inhibitory activities dramatically. Chalcones with varied methoxy groups at ring-B and Hydroxyl at ring-A (CH-8) are more potent than chalcones substituted with electron withdrawing groups at ring-B (CH-2, CH-3, CH-4, and CH-5). The compound CH-8 with hydroxyl substituted at 2 position of ring-A and trimethoxy at 3,4,5 position of ring-B of chalcone showed highest AChE activity i.e. IC_{50} 6.4 μM . Removal of hydroxyl group resulted in 2-fold decrease in activity (CH (1-7)). Moreover, the compounds having electron withdrawing groups at *para* position of ring-B (CH-3, CH-5, and CH-6) showed more AChE inhibitory and radical scavenging activity as compared to compounds having electron withdrawing groups at *meta* position (CH-2 and CH-4). *Para* nitro and hydroxyl substituted derivative (CH-6 and CH-7) also showed good activity.

Conclusion

In conclusion, the results of different pharmacological studies have supported the proposed hypothesis that chalcones have the potential to inhibit AChE enzyme with free radical scavenging activity. Among the synthesized chalcones most of the compounds showed the AChE inhibitory activity with good radical scavenging activity. Thus, these compounds may improve cognitive deficits and behave as disease-modifying agents. The synthesized chalcones CH-3, CH-6 and CH-8 have been found to be maximally potent molecules and are potential candidates for the development of drugs for Alzheimer's disease. However, further detailed investigations of mechanisms involved in

these activities may establish their specific therapeutic usefulness.

Conflict of Interest

The authors declare that they have no conflict of interests.

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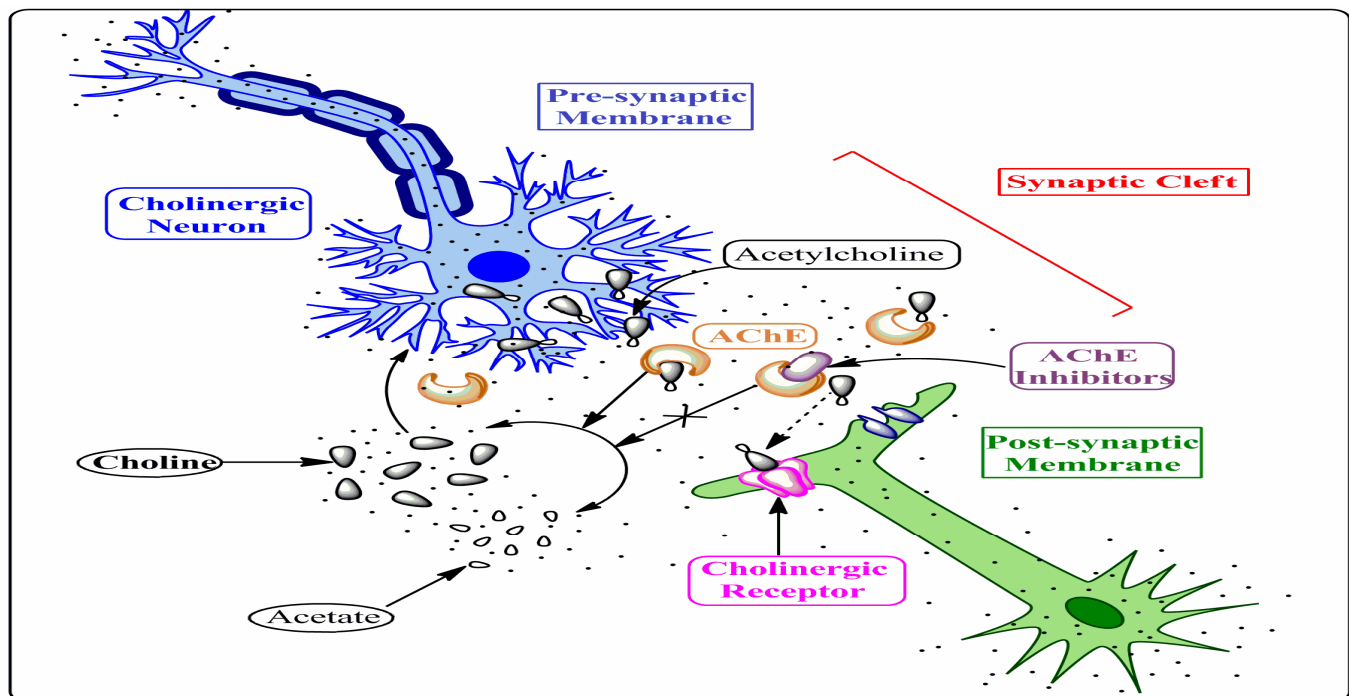


Fig. 1 : Role of Acetylcholinesterase in the pathophysiology of Alzheimer's disease.

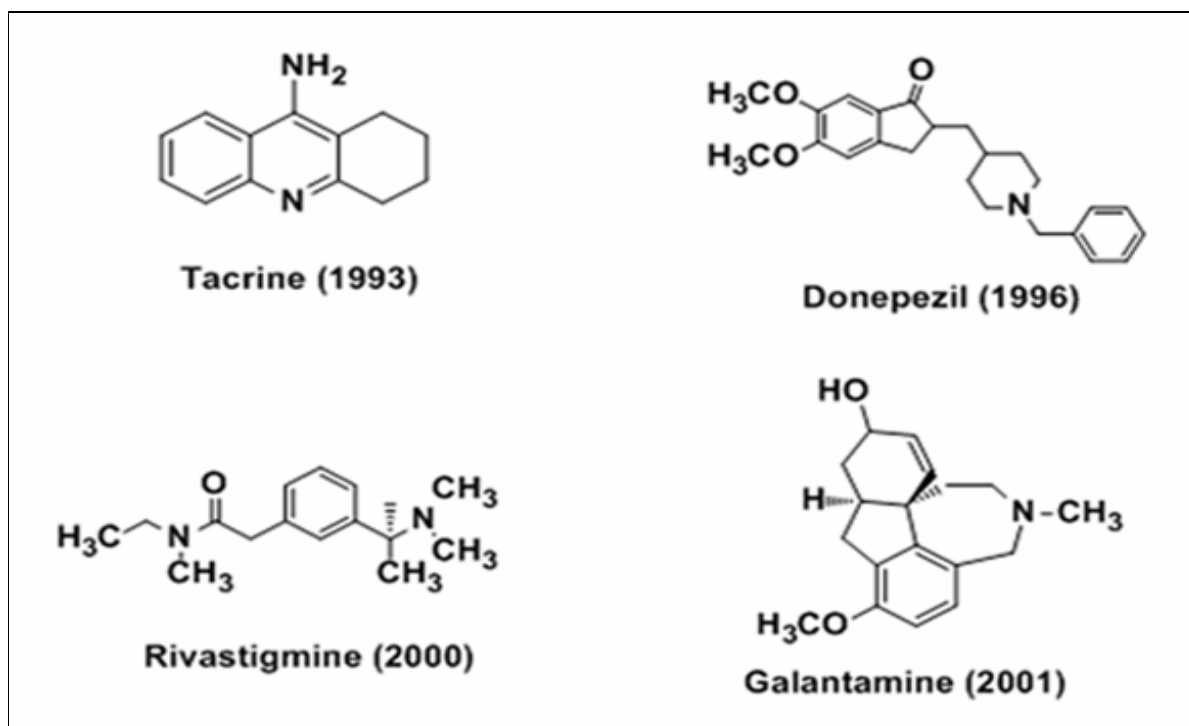
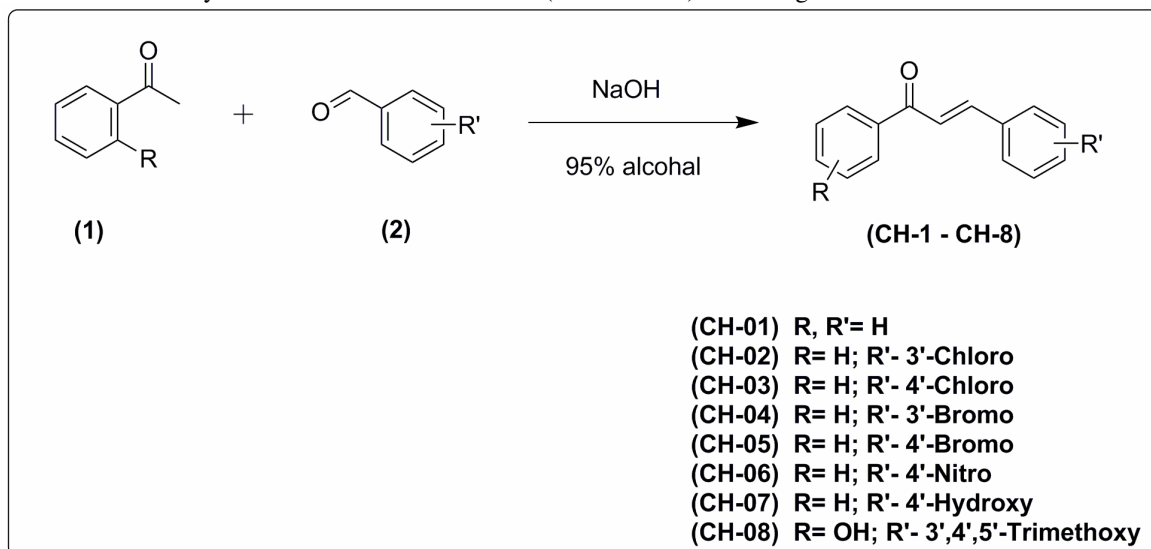


Fig. 2 : FDA approved drugs for the treatment of Alzheimer's disease.

Scheme 1: Synthesis of chalcone derivatives (**CH-1–CH-8**) with Reagents and reaction conditions.**Table 1:** The AChE inhibitory and DPPH radical scavenging activities (IC_{50} , μM) of chalcone derivatives (**CH-1–CH-8**)

C. No.	R	R ₃	R ₄	R ₅	AChE Inhibitory Activity ($IC_{50}^b \pm \text{SEM}^a$, μM)	Radical scavenging activity ($IC_{50}^c \pm \text{SEM}^a$, μM)
CH-1	H	H	H	H	47.5±0.56	62.5±0.37
CH-2	H	-Cl	H	H	16.8±0.42	32.5±0.54
CH-3	H	H	-Cl	H	12.5±0.21	24.0±0.80
CH-4	H	-Br	H	H	18.9±0.48	58.0±0.46
CH-5	H	H	-Br	H	17.5±0.80	42.3±0.80
CH-6	H	H	-NO ₂	H	11.8±0.55	21.5±0.42
CH-7	H	H	-OH	H	15.3±0.62	19.0±0.40
CH-8	OH	-OCH ₃	-OCH ₃	-OCH ₃	6.4±0.32	17.7±0.57
Std.	Donepezil	-	-	-	7.5±0.40	-
Std.	Ascorbic acid	-	-	-	-	18.0± 0.45

^a SEM: Standard Error of the Mean.^b IC_{50} , inhibitor concentration (means \pm SEM of three experiments) for 50% inactivation of AChE.^c IC_{50} was defined as the concentration resulting in 50% scavenging activity.

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