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Synthesis and evaluation of 2,3,4,9-tetrahydro-1*H*-carbazole derivatives as selective acetylcholinesterase inhibitors: Potential anti-Alzheimer's agents

Hitesh Kukreja[a], Rajan Chugh[a], Jatinder Singh[a], Ramanpreet Shah[a], Dhandeep Singh*[a], Nirmal Singh[a], Dimple Sethi Chopra[a] & Mandeep Singh[b]

[a] Department of Pharmaceutical Sciences and Drug Research, Punjabi University
Patiala 147 002, India

[b] Thapar Institute of Engineering and Technology, Patiala 147 004, India

E-mail: ddd300@gmail.com

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Alzheimer's disease is an irreversible, progressive brain disorder that slowly destroys memory and cognition skills. Dysfunction of acetylcholine containing neurons in the brain contributes substantially to the cognitive decline observed in Alzheimer's disease. Hence, our focus is to synthesize cholinesterase inhibitors. A series (22 compounds) of 6- and 9-substituted derivatives of 2,3,4,9-tetrahydro-1*H*-carbazole have been prepared and *in vitro* evaluated for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition by Ellman's method. By comparing selectivity with standard drug donepezil, amino derivative **3**, methylamino derivative **4** and butyl nitro derivative **17** have been found to be selective AChE inhibitors. Borsche-Drechsel cyclization reaction has been carried out to synthesize 2,3,4,9-tetrahydrocarbazole ring followed by nitration, reduction and derivative synthesis.

Keywords: Alzheimer's disease, acetylcholinesterase (AChE), Borsche-Drechsel cyclization reaction, donepzil, 2,3,4,9-tetrahydro-1*H*-carbazoles

Alzheimer's disease (AD) is irreversible, progressive brain disorder related to changes in nerve cells that result in the death of brain cells delineated by an incessantly decline in cognitive performance accompanied by behavioural and psychological syndromes, such as depression and psychosis[1]. Worldwide, about 50 million people are believed to be living with Alzheimer's disease. These alarming rates could exceed 152 million by 2050[2]. Currently there are many hypotheses that describe molecular mechanisms which may play a role in the development of AD. The two of main hypotheses are the ''cholinergic hypothesis'', which is based on neurochemical findings that suggest a discernible decrease in acetylcholine containing neurons in AD brain. The second hypothesis is the ''amyloid hypothesis'', which revealed intraneuronal deposits of tau-protein derived neurofibrillary tangles and extracellular deposits of β-amyloid protein in AD brains. These deposits are present in ''senile plaques'', which was confirmed during earlier diagnosis of AD. The neurochemical correlates of these clinical manifestations appear to involve in multiple neurotransmitter pathways dysfunctions[3,4].

The main stress of the cholinergic hypothesis is on the enhanced activity of the enzyme acetylcholinesterase. In Alzheimer's disease individuals, the activity of the acetylcholinesterase increases and leads to augmented breakdown of the neurotransmitter acetylcholine and causes the plummeting of the acetylcholine level in brain. Other relation between the enzyme and AD has been the partial involvement of the enzyme in the formation of amyloids plaques and neurfibrillary tangles. It has been shown that acetylcholinesterase (AChE) promotes the aggregation of β-amyloid peptide fragments by forming a complex with the growing fibrils[5]. These complexes have been shown to be more cytotoxic than β-amyloid fibrils alone. The beneficial role of cholinergic manipulation is presumably through the amplification of ACh agonist actions by inhibiting its metabolizing enzymes *i.e.* AChE inhibition. It has been mediated by the effects of direct agonists, both muscarinic and nicotinic. Butyrylcholinesterase (BChE) is abundant in plasma and interstitial fluids of peripheral tissues,and it's inhibition exhibits peripheral side effects like salivation and gastrointestinal complaints[6]. Peripheral

anionic site (PAS) of AChE is involved in converting soluble β-amyloid to insoluble amyloid[7]. Selectivity for acetylcholinesterase is desired for Alzheimer's treatment drug. Four cholinesterase inhibitors, Tacrine(I), Donepezil (II), Rivastigmine(III) and Galantamine(IV) (Figure 1) have been used for treating symptomatic treatment of AD[8].

Literature survey shows that 9H-carbazole drivatives[9] and 2,3,4,4a-yetrahydro-1*H*-carbazoles[10] have anti-cholinesterase activity. Hence, tetrahydro-carbazole is chosen as a central motif and substituted the basic moiety at 6 and 9 position. Therefore, aim of present investigation is to focus on the synthesis and evaluation of 2,3,4,9-tetrahydrocarbazole derivatives as AChE inhibitors. Donepezil is selected as standard drug as italso binds on PAS of AChE[11].

Results and Discussion

Chemistry

Synthesis of tetrahydrocarbazole (compound 1) as shown in Scheme I is Borsche-Drechsel cyclization reaction which involves the condensation of phenylhydrazine with cyclohexanone in presence of glacial acetic acid[12,13]. Nitration of tetrahydrocarbazole involves reaction with sodium nitrate and sulphuric acid[14]. Reduction of compound 2 involves reaction with sodium hydroxide and zinc dust[15]. Synthesis of sulphonamide derivatives compounds 11, 12, 13, 19 and 20 is modified Schotten Baumann type reaction which involves the reaction of compound 2 and 3 with alkyl/aryl sulphonyl chlorides in presence of pyridine[16]. Third type of reaction is nucleophilic substitution of alkyl/aryl halides which involves reaction of compound 2 and 3 with alkyl halides in presence of N,N-Dimethylformamide and potassium carbonate[17] to yield compounds 4-10, 14-18, 21, 22as indicated in Scheme I.

In-vitro AChE and BChE inhibitory activity

The 2,3,4,9-tetrahydro-1*H*-carbazole and its derivatives are evaluated for AChE and BChE inhibition by Ellman's method[18,19] using donepezil as standard drug. All compounds showed AChE and BChE inhibitory activitiesas shown in Table I. Compound 3, 4 and 17 were found to be more selective towards AChE inhibition. Increase in alkyl chain length at 6-amino tetrahydrocarbaole and 6-nitro tetrahydrocarbaole derivativeslead to decrease in AChE selectivity. Furthermore, introduction of bulky group like benzyl, methane sulfonyl, benenesulfonyl and tosyl group of both 6-aminotetrahydrocarbaole and 6-nitrotetrahydrocarbaole derivativeslead to diminished activity.

Docking studies

The structure of the E20-TcAChE complex (1EVE) has 2.5A° resolution. The peripheral anionic site of acetylcholinestrase is chosen for docking analysis. The peripheral anionic site lies on the surface of acetylcholinesterase, approximately 20A° distant from the active site. It mainly consist of five residues Tyr70(72), Asp72(74), Tyr121(124), Trp279(286), and Tyr334(341) clustered around the entrance to the active site gorge. Torpedo numbering is given first,

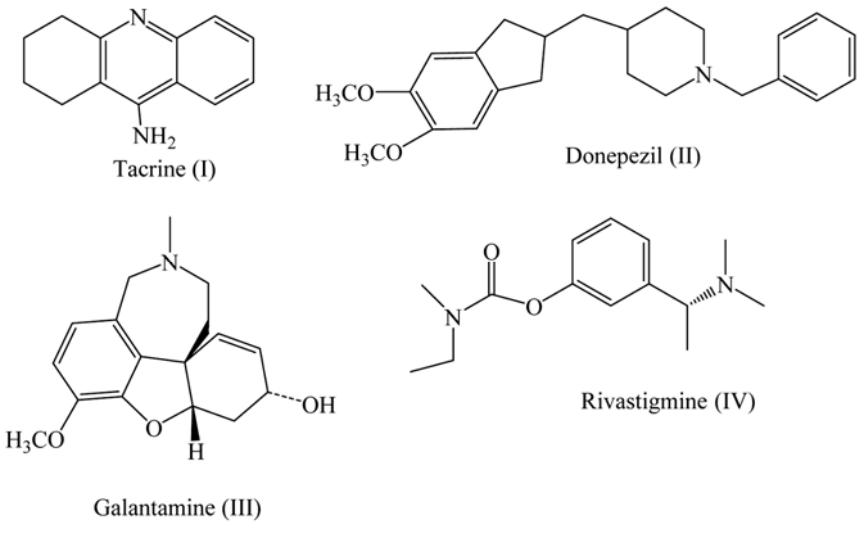
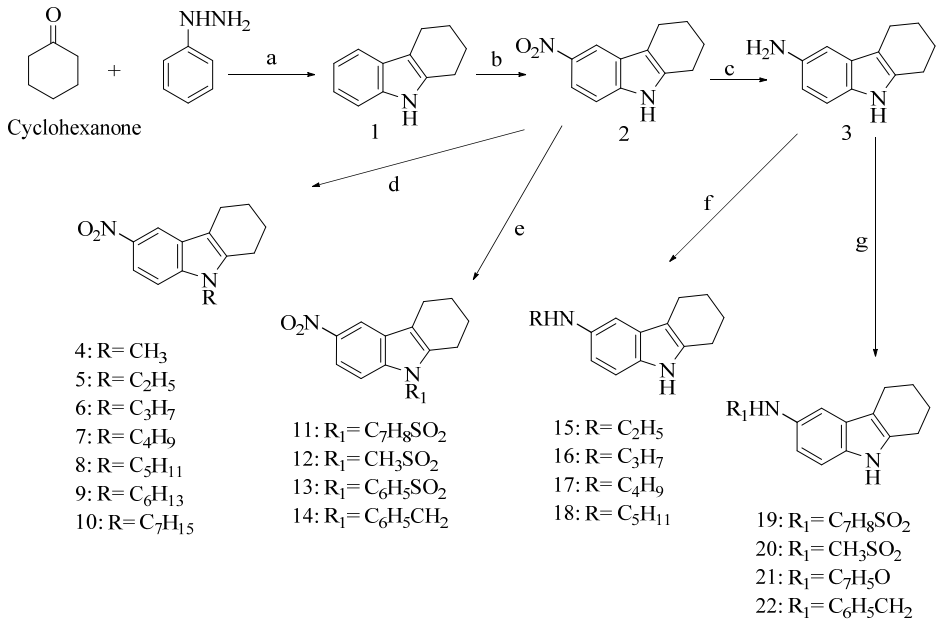


Figure 1 — Structures of compounds **I–IV** used in AD treatment



Scheme I — Reagents and conditions: (a) Cyclohexanone, Glacial acetic acid, reflux, 5 min.; (b) Sodium nitrate in sulfuric acid, stirring 1.5 h; (c) Zinc, Sodum hydroxide, reflux, 1 h; (d) N,N-dimethyl formamide, potassium carbonate, reflux, 24 h, alkylbromides: CH_3Br (4), C_2H_5Br (5), C_3H_7Br (6) C_4H_9Br (7), $C_5H_{11}Br$ (8), $C_6H_{13}Br$ (9), $C_7H_{15}Br$ (10); (e) Dry pyridine, reflux, 30 min., alkyl chloride, aryl chlorides: $C_7H_8SO_2Cl$ (11), CH_3SO_2Cl (12), $C_6H_5SO_2Cl$ (13), $C_6H_5CH_2Cl$ (14); (f) N,N-dimethyl formamide, potassium carbonate, reflux, 24 h, alkyl bromides: C_2H_5Br (15), C_3H_7Br (16) C_4H_9Br (17), $C_5H_{11}Br$ (18); (g) Dry pyridine, reflux, 30 min., alkyl chloride, aryl chlorides: $C_7H_8SO_2Cl$ (19), CH_3SO_2Cl (20), C_7H_5OCl (21), $C_6H_5CH_2Cl$ (22).

Table I — Acetylcholinesterase and butyrylcholinesterase inhibitory activities of compounds **1-22**

Compd	IC_{50} (μM) AChE	IC_{50} (μM) BChE
Donepezil	0.0427 ± 0.078	1.216 ± 0.045
1	2.02 ± 0.479	8.97 ± 0.450
2	2.68 ± 0.652	7.31 ± 0.890
3	0.0388 ± 0.008	0.482 ± 0.180
4	0.0324 ± 0.004	2.553 ± 0.770
5	1.94 ± 0.400	6.04 ± 0.610
6	4.19 ± 0.630	14.38 ± 0.150
7	4.50 ± 0.560	11.21 ± 0.780
8	5.24 ± 0.873	13.29 ± 0.520
9	4.88 ± 0.578	12.05 ± 0.320
10	12.43 ± 0.698	9.16 ± 0.310
11	6.90 ± 0.810	7.63 ± 0.880
12	8.41 ± 0.566	9.03 ± 0.650
13	13.64 ± 0.829	11.98 ± 0.300
14	2.78 ± 0.326	10.0 ± 0.800
15	1.64 ± 0.528	1.82 ± 0.326
16	2.21 ± 0.823	1.79 ± 0.681
17	0.203 ± 0.020	4.13 ± 0.268
18	2.27 ± 0.400	7.82 ± 0.427
19	3.63 ± 0.946	4.68 ± 0.589
20	5.75 ± 0.750	6.14 ± 0.457
21	11.28 ± 0.622	13.24 ± 0.482
22	5.40 ± 0.382	9.45 ± 0.726

followed by mammalian numbering in brackets[20]. All the compounds fit into the PAS gorge as shown in Figure 2 and its interaction with residues is summarised in Table II. Further, most active (most stable based on energy) compound 9 overlays on donepezil and interact with most of residues are indicated in Figure 3.

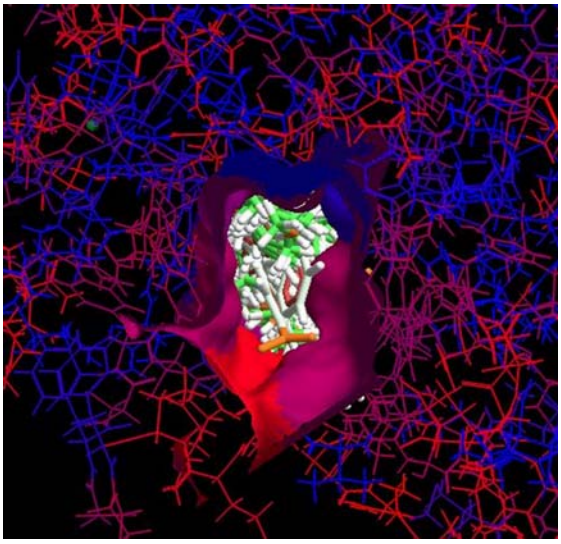


Figure 2 — Figure representing all the ligands together fit in the PAS gorge cavity

For any ligand to bind to PAS of AChE interaction with Tyr70(72), Tyr121(124) and Trp279(286) is critical. All compound except 1 and 3 show interaction with Trp279. Hence all compounds are selective towards AChE than butyrylcholinesterase.

Experimentally compound 3, 4 and 17 are more active and selective towards AChE amongst the

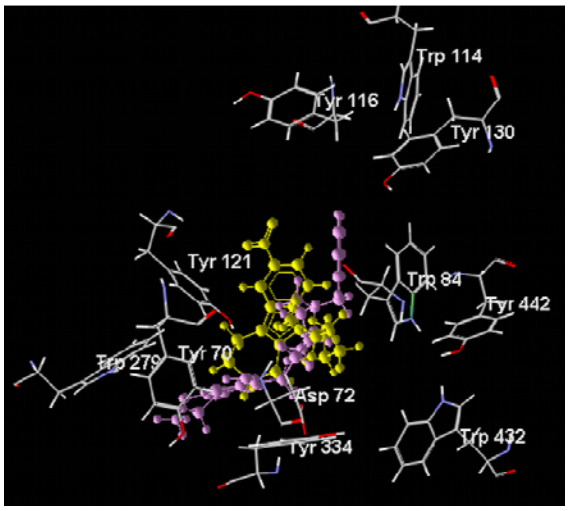


Figure 3 — Interaction of residues with active site (PAS). Donepezil (purple color) and compound with minimum energy (compound no 9) is shown in yellow color

Table II — Table showing total energy and interacting residue of compounds **1-22**

Compd	Total energy	Interaction Residues
Donepezil	-120.7	Tyr121, Tyr70, Asp72, Trp279, Tyr334
1	-74.2	Tyr70, Asp72, Trp84, Tyr121
2	-93.8	Tyr70, Asp72, Trp84, Tyr121, Trp279, Tyr334
3	-82.2	Trp84, Tyr121, Tyr70, Asp72
4	-90.7	Trp84, Tyr121, Asp72, Trp279, Tyr334
5	-89.6	Trp84, Tyr121, Tyr70, Trp279, Tyr334
6	-93.2	Trp84, Tyr121, Tyr70, Trp279, Tyr334
7	-105.6	Trp84, Tyr121, Asp72, Trp279, Tyr334
8	-90.8	Trp84, Tyr121, Asp72, Trp279, Tyr334
9	-114.9	Trp84, Tyr121, Tyr70, Asp72, Trp279, Tyr334
10	-112.8	Trp84, Tyr121, Tyr70, Asp72, Trp279, Tyr334
11	-104.4	Trp84, Tyr121, Tyr70, Asp72, Trp279, Tyr334
12	-108.9	Trp84, Tyr121, Tyr70, Asp72, Trp279, Tyr334
13	-99.3	Trp84, Tyr121, Tyr70, Asp72, Trp279, Tyr334
14	-112.4	Trp84, Tyr121, Tyr70, Asp72, Trp279, Tyr334
15	-90.5	Trp84, Tyr121, Tyr70, Asp72, Trp279, Tyr334
16	-91.9	Trp84, Tyr121, Tyr70, Asp72, Trp279
17	-91.3	Trp84, Tyr121, Tyr70, Asp72, Trp279, Tyr334
18	-91.4	Trp84, Tyr121, Asp72, Trp279, Tyr334
19	-86.1	Trp84, Tyr121, Trp279, Tyr334
20	-88.5	Trp84, Tyr121, Tyr70, Trp279, Tyr334
21	-101.3	Trp84, Tyr121, Tyr70, Asp72, Trp279, Tyr334
22	-96.3	Trp84, Tyr121, Tyr70, Asp72, Trp279, Tyr334

synthesized compounds indicating their binding to PAS. Although, compound 9 is predicted to be most active by docking studies but it is less potent in the experimental *in vitro* activity determination. Experimentally compounds 3, 4 and 17 were found to be more potent and selective AChE inhibitors.

Experimental Section

Chemistry

Melting points reported are uncorrected. Synthetic procedures employed were monitored for completion as well as purity of product by Thin Layer Chromatography (TLC) employing 7 cm×2.5 cm Silica gel 60 F_{254} precoated TLC plates by Merck. Nuclear Magnetic Resonance Spectra were recorded on BrukerAvance DPX-200 (400 MHz). In ^{1}H NMR chemical shifts (δ) are reported in parts per million (ppm) using tetramethylsilane as internal standard. Coupling constants (*J*) are reported in Hz (Hertz). Multiplicities are reported using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m =multiplet, dd = double doublet. Mass Spectra were recorded on a Bruker Esquire 3000_00037. IR spectra were measured on a Hitachi 270-30 infrared and Bruker Vector 22 Spectrophotometers. IR spectra were recorded as KBr pellets.

General procedure for the synthesis of tetrahydrocarbazole

Refluxing of phenylhydrazine and cyclohexanone in glacial acetic acid for 5 min, leads the conversion of cyclohexanone into phenylhydrazone, without its isolation, (Scheme I) being converted into 2,3,4,9-tetrahydrocarbazole. The solid was filtered *in vacuo* and recrystallized with ethanol instantly[12,13].

General procedure for the nitration of tetrahydrocarbazole, 2

To a solution of sodium nitrate (1.70 g, 0.0200M) in concentrated sulphuric acid (50 mL) (H_2SO_4), was added drop wise with stirring, over a period of 1h, a solution of 2,3,4,9-tetrahdrocarbazole (3.18g, 0.0200 M) in 25 mL of concentrated sulphuric acid(H_2SO_4) and keep at 5°C in an ice bath. The solution was stirred for an additional 5 min and then poured onto crushed ice. The crude product was recrystallized from methylene chloride-petroleum ether (1:1). The orange yellowish blunt needles of the pure 6-nitro-2,3,4,9-tetrahydrocarbazole **2** were obtained as crystals[14].

General procedure for the reduction of nitro-2,3,4,9-tetrahydrocarbazole, 3

To a 30 mL solution of ethanol 95% containing sodium hydroxide (3.6 mL, 20%), was added to compound **2** (1g) and dissolved. The mixture was heated to boiling in a two necked round bottom flask fitted with condenser. The source of heating was removed and zinc dust (2.9 g, 44.61mM) was added in portions to keep the solution boiling. After complete addition, the refluxing was continued for 1h until the solution became transparent. Hot mixture was filtered at pump; zinc residue returned to flask and extracted with three 20 mL portions of hot rectified spirit. The extracts were recombined, 2 g of sodium dithionite ($Na_2S_2O_4$) was added and solvent was removed under pressure using rotary evaporator. The dried solid was washed with 20 mL of diethyl ether twice. The product was recrystallized from ethanol giving 6-amino-2,3,4,9-tetrahydrocarbazole **3** as brownish red crystals[15].

General procedure for preparation of sulphonyl derivatives

To 0.4 g (1.16 mM) of compound **2** and **3** in 3 mL dry pyridine, 0.267g (2.33 mM) of methane sulphonyl chloride, benzene sulphonyl chloride and tosyl chloride were added in small proportions and resultant mixture was heated to boiling for 30 min. After completion of the reaction, the reaction mixture was acidified with 2M HCl and poured on crushed ice. Precipitated product was filtered and washed well with water. The product was recrystallized using ethanol to yield compounds **11**, **12**, **13**, **19**, **20** [16].

General procedure for coupling reactions

To a solution of 0.4 g (1.16 mM) of compound **2** and **3**, 1.74 mM of alkyl halides in 4 mL N,N-dimethylformamide, potassium carbonate (0.2g) was added and refluxed for 24 h. The mixture was poured on crushed ice. The products were recrystallized with ethanol to yield compounds **4-10**, **14-18**, **21** and **22** [17].

2,3,4,9-Tetrahydro-1*H*-carbazole, 1: Yield 88%. m.p.118°C. IR (KBr): 3397, 3049, 2927, 1645, 1619, 1233, 737 cm^{-1}; ^{1}H NMR (400MHz, DMSO-d_6): δ 8.14 (s, 1H, N-H)*, 7.42 (d, 1H, Ar H), 7.32 (dd, *J*= 8.2, 2.4 Hz, 1H,Ar H), 7.15 (dd, *J*= 10.6, 2.4 Hz, 1H, Ar H), 7.05 (d,*J*= 2.4Hz, 1H, Ar H), 2.67 (t, *J*= 6.2, 2H, Aliphatic H), 2.60 (t, *J*= 6.2, 2H, Aliphatic H), 1.85-1.78 (m, 4H, Aliphatic H); MS (ESI): 172.03 [M+1]; Mass calcd for $C_{12}NH_{13}$ 171.10 [*m/z*].

6-Nitro-2,3,4,9-tetrahydro-1*H*-carbazole, 2: Yield 80%. m.p.157°C. IR (KBr): 3373, 3046, 2933, 1630, 1516 and 1324, 1473, 754 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 8.41 (d, *J*=2.1, 1H, N-H), 8.23 (s, 1H, Ar H), 8.03 (s, *J*= 7.3, 2.1 Hz, 1H,Ar H), 7.26 (d, *J*= 7.2, 1H, Ar H), 2.74-2.68 (m, 4H, Aliphatic H), 1.92-1.79 (m, 4H,Aliphatic H); MS (ESI): 217.05 [M+1]; Mass calcd for $C_{12}H_{12}N_2O_2$ 216.09 [*m/z*].

6-Amino-2,3,4,9-tetrahydro-1*H*-carbazole, 3: Yield 74%. m.p.134°C. IR (KBr): 3389 and 3214, 2917, 1420, 1325, 735, 686 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 7.4 (s, 1H, N-H)*, 7.28 (s, 2H, N-H)*, 7.01 (d, 1H, Ar H), 6.77 (d, 1H,Ar H), 6.57 (s, 1H, Ar H), 2.71-2.43 (m, 4H, Aliphatic H), 1.92-1.90 (m, 4H, Aliphatic H); MS (ESI): 187.04 [M+1]; Mass calcd for $C_{12}H_{14}N_2$ 186.11 [*m/z*].

9-Methyl-6-nitro-2,3,4,9-tetrahydro-1*H*-carbazole, 4: Yield 71%. m.p.104°C. IR (KBr): 3048, 2921, 1625, 1536 and 1318, 1473, 870, 689 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 7.03 (d, *J* = 8.36 Hz, 1H, Ar H), 6.82 (d, *J* = 2.25 Hz, 1H, Ar H), 6.64 (s, 1H, Ar H), 3.60 (s, 3H, N-CH_3), 2.78 (t, 2H, Aliphatic H), 2.52 (t, 2H, Aliphatic H), 1.90-1.78 (m, 4H, Aliphatic H); MS (ESI): 231.08 [M+1]; Mass calcd for $C_{13}H_{14}N_2O_2$ 230.10 [*m/z*].

9-Ethyl-6-nitro-2,3,4,9-tetrahydro-1*H*-carbazole, 5: Yield 64%. m.p.110°C. IR (KBr): 3041, 2917, 1634, 1528and 1310, 1469, 890, 672 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 7.26 (d, *J* = 8.36 Hz, 1H,Ar H), 6.77 (d, *J* = 2.25 Hz, 1H,Ar H), 6.57 (s, 1H, Ar H), 3.89 (q, 2H, N--CH_2), 2.59 (t, 2H,Aliphatic H), 2.49 (t, 2H,Aliphatic H), 1.56-1.47 (m, 4H, Aliphatic H), 1.43 (t, 3H, N-CH_3); MS (ESI): 245.10 [M+1]; Mass calcd for $C_{14}H_{16}N_2O_2$ 244.11 [*m/z*].

9-Propyl-6-nitro-2,3,4,9-tetrahydro-1*H*-carbazole, 6: Yield 46%. m.p.121°C. IR (KBr): 3048, 2924, 1625, 1516 and 1324, 1473, 870, 686 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 7.18 (d, *J* = 8.36 Hz, 1H, Ar H), 6.83 (d, *J* = 2.25 Hz, 1H,Ar H), 6.69 (s, 1H,Ar H), 3.78 (t, 2H, N--CH_2), 2.66 (t, 2H, Aliphatic H), 2.35 (t, 2H, Aliphatic H), 1.56-1.42 (m, 4H, Aliphatic H), 1.81-1.76 (m, 2H, N-CH_2), 0.96 (t, 3H, N-CH_3); MS (ESI): 259.11 [M+1]; Mass calcd for $C_{15}H_{18}N_2O_2$ 258.12 [*m/z*].

9-Butyl-6-nitro-2,3,4,9-tetrahydro-1*H*-carbazole, 7: Yield 71%. m.p.115°C. IR (KBr): 3048, 2921, 1625, 1516 and 1324, 1473, 870, 686 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 7.48 (d, *J* = 6.1 Hz, 1H, Ar H), 6.97 (d, *J* = 2.4 Hz, 1H,Ar H), 6.74 (s, 1H,Ar H), 3.76 (t, 2H, N--CH_2), 2.48 (t, 2H, Aliphatic H), 2.31 (t, 2H, Aliphatic H), 1.77-1.69 (m, 2H, N--CH_2), 1.51-1.43 (m, 4H, Aliphatic H), 1.33-1.25 (m, 2H, N--CH_2), 0.83 (t, 3H, N-CH_3); MS (ESI): 273.12 [M+1]; Exact mass calcd for $C_{16}H_{20}N_2O_2$272.15 [*m/z*].

9-Pentyl-6-nitro-2,3,4,9-tetrahydro-1*H*-carbazole, 8: Yield 55%. m.p.127°C. IR (KBr): 3048, 2921, 1625, 1516 and 1324, 1473, 870, 686 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 7.92 (d, *J* = 8.36 Hz, 1H, Ar H), 6.99 (d, *J* = 2.5 Hz, 1H, Ar H), 6.83 (s, 1H, Ar H), 3.85 (t, 2H, N-CH_2), 2.68 (t, 2H, Aliphatic H), 2.53 (t, 2H, Aliphatic H), 1.86-1.80 (m, 2H, N-CH_2), 1.62-1.53 (m, 4H,Aliphatic H), 1.48-1.41 (m, 2H, N-CH_2), 1.29-1.24 (m, 2H, N-CH_2), 0.99 (t, 3H, N-CH_3); MS (ESI): 287.14 [M+1]; Mass calcd for $C_{17}H_{22}N_2O_2$286.15 [*m/z*].

9-Hexyl-6-nitro-2,3,4,9-tetrahydro-1*H*-carbazole, 9: Yield 68%. m.p.135°C. IR (KBr): 3048, 2921, 1625, 1516 and 1324, 1473, 870, 686 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 8.20 (d, *J* = 6.1 Hz, 1H,Ar H), 7.26 (s, 1H, Ar H), 6.89 (d, *J* = 2.4 Hz, 1H, Ar H), 3.79 (t, 2H, N-CH_2), 2.52 (t, 2H, Aliphatic H), 2.48 (t, 2H, Aliphatic H), 1.81-1.75 (m, 2H, N--CH_2), 1.48-1.36 (m, 4H, Aliphatic H), 1.21-1.17 (m, 2H, N-CH_2), 1.19-1.13 (m, 2H, N--CH_2), 1.08-1.02 (m, 2H,N--CH_2), 0.83 (t, 3H, N-CH_3); MS (ESI): 301.16 [M+1]; Mass calcd for $C_{18}H_{24}N_2O_2$300.18 [*m/z*].

9-Heptyl-6-nitro-2,3,4,9-tetrahydro-1*H*-carbazole, 10: Yield 58%. m.p.142°C. IR (KBr): 3048, 2921, 1625, 1516 and 1324, 1473, 870, 686 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 9.32 (d, *J* = 6.1 Hz, 1H,Ar H), 7.89 (s, 1H, Ar H), 6.64 (d, *J* = 2.4 Hz, 1H, Ar H), 4.37 (t, 2H, H), 2.96 (t, 2H, Aliphatic H), 2.72 (t, 2H, Aliphatic H), 1.96-1.88 (m, 2H, N-CH_2), 1.74-1.58 (m, 4H, Aliphatic H), 1.42-1.37 (m, 2H, N-CH_2), 1.29-1.18 (m, 2H, N-CH_2), 1.09-1.02 (m, 2H, N-CH_2), 0.92-0.85 (m, 2H, N--CH_2), 0.68 (t, 3H, N-CH_3); MS (ESI): 315.13 [M+1]; Mass calcd for $C_{19}H_{26}N_2O_2$314.20 [*m/z*].

9-(4-Methylphenyl)sulfonyl)-6-nitro-2,3,4,9-tetrahydro-1*H*-carbazole, 11: Yield 65%. m.p.162°C. IR (KBr): 3048, 2921, 1625, 1517 and 1373, 1473, 1321 and 1155, 1124, 870, 686, 667 and 614 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 9.82 (d, *J* = 3.2 Hz, 2H, Ar-Ts H), 8.85 (d,*J* = 4.7 Hz, 2H, Ar-Ts H), 7.89 (s, 1H, Ar H), 7.53 (d, *J* = 5.1 Hz, 1H, Ar H), 7.31 (d, *J* = 2.1 Hz, 1H,Ar H), 2.9 (t, 2H, Aliphatic

H), 2.58 (t, 2H, Aliphatic H), 2.28 (s, 3H, Ar-Ts-CH_3), 1.56-1.34 (m, 4H, Aliphatic H); MS (ESI): 371.08[M+1]; Mass calcd for $C_{19}H_{18}N_2O_4S$ 370.10 [*m/z*].

9-(Methylsulfonyl)-6-nitro-2,3,4,9-tetrahydro-1*H*-carbazole, 12: Yield 54%. m.p.123°C. IR (KBr): 2976, 2817, 1621, 1508 and 1416, 1386, 1085 and 1017, 1226, 870, 786, 667 and 614 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 7.96 (s, 1H, Ar H), 7.62 (d, *J* = 5.1Hz, 1H, Ar H), 6.35 (d, *J* = 2.1Hz, 1H, Ar H), 2.59 (s, 3H, Ms-CH_3), 2.54 (t, 2H, Aliphatic H), 2.27 (t, 2H, Aliphatic H), 1.09-0.82 (m, 4H, Aliphatic H); MS (ESI): 295.02 [M+1]; Mass calcd for $C_{13}H_{14}N_2O_4S$ 294.07 [*m/z*].

6-Nitro-9-(phenylsulfonyl)-2,3,4,9-tetrahydro-1*H*-carbazole, 13: Yield 45%. m.p.143°C. IR (KBr): 3058, 2912, 1658, 1518 and 1463, 1341, 1324 and 1017, 1143, 870, 749, 648 and 618 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 7.90 (d, *J* = 6.7 Hz, 2.1Hz,2H, Ar-Bs H), 7.54 (d, 2H, Ar-Bs H), 7.30 (t, 1H, Ar-Bs H), 7.23 (s, 1H, Ar H), 7.05 (d, *J* = 8.35 Hz, 1H,Ar H), 6.77 (d, *J* = 2.8 Hz, 1H, Ar H), 2.59 (t, 2H, Aliphatic H), 2.43 (t, 2H, Aliphatic H), 1.01-0.80 (m, 4H, Aliphatic H); MS (ESI): 357.07 [M+1]; Mass calcd for $C_{18}H_{16}N_2O_4S$356.08 [*m/z*].

9-Benzyl-6-nitro-2,3,4,9-tetrahydro-1*H*-carbazole, 14: Yield 35%. m.p.122°C. IR (KBr): 3026, 2917, 1607, 1591 and 1481, 1162, 870, 762 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 7.41 (s, 1H, Ar H), 7.34 (dd, *J* = 7.4 Hz, 2.2 Hz, 2H, Bz-Ar H), 7.23 (d, *J* = 8.35 Hz, 1H,Ar H), 7.13 (t, 1H, Bz-Ar H), 7.02 (dd, *J* = 3.2 Hz, 2H,Bz-Ar H), 6.82 (d, *J* = 2.3 Hz, 1H,Ar H), 5.21 (s, 2H, Bz-CH_2), 2.94 (t, 2H,Aliphatic H), 2.72 (t, 2H, Aliphatic H), 1.58-1.43 (m, 4H, Aliphatic H); MS (ESI): 307.11 [M+1]; Mass calcd for $C_{19}H_{18}N_2O_2$306.14 [*m/z*].

N-Ethyl-2,3,4,9-tetrahydro-1*H*-carbazol-6-amine, 15: Yield 64%. m.p.110°C. IR (KBr):3406, 2966, 1629, 1595, 1349 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 8.45 (s, 1H, Ar-NH H)*, 7.50 (d, *J* = 8.12 Hz, 1H, Ar H), 6.90 (d, *J* = 8.14 Hz, 1H, Ar H), 6.75 (dd, *J* = 2.12 Hz, Ar 1H,), 3.02 (s, 1H, Indole-NH)*, 2.84-2.78 (m, 2H, CH_2), 2.59 (q, 4H, Aliphatic H), 2.16 (q, 4H, Aliphatic H), 1.25 (t, 3H, CH_3); MS (ESI): 215.12 [M+1]; Mass calcd for $C_{14}H_{18}N_2$214.15 [*m/z*].

N-Propyl-2,3,4,9-tetrahydro-1*H*-carbazol-6-amine, 16: Yield 46%. m.p.121°C. IR (KBr):3406, 2966, 1629, 1595, 1349 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 8.6 (s, 1H, Ar-NH), 7.01 (d, *J* = 8.10 Hz, 1H, Ar H), 6.82 (d, *J* = 8.14 Hz, 1H,Ar H), 6.68 (dd, *J* = 2.32 Hz, 1H, Ar H), 3.31 (s, 1H,Indole-NH), 3.14 (m, 4H, Aliphatic H and CH_2), 2.98 (t,2H, CH_2), 1.64-1.48 (m, 6H, Aliphatic H), 1.02 (t, 3H, CH_3); MS (ESI): 229.13 [M+1]; Mass calcd for $C_{15}H_{20}N_2$228.16 [*m/z*].

N-Butyl-2,3,4,9-tetrahydro-1*H*-carbazol-6-amine, 17: Yield 71%. m.p.115°C. IR (KBr):3042, 2924, 1631, 1595, 1349, 673 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 8.07 (s, 1H, Ar-NH H), 7.49 (d, *J* = 8.22 Hz, 1H, Ar H), 6.95 (d, *J* = 8.24 Hz, 1H,Ar H), 6.80 (dd, *J* = 2.23 Hz, 1H, Ar H), 3.48 (s, 1H, Indole-NH), 3.23 (q, 2H, CH_2), 2.81 (m, 4H, Aliphatic H), 1.67-1.56 (m, 4H, Aliphatic H), 1.43-1.35 (m, 4H, $2CH_2$), 0.92 (t, 3H, CH_3); MS (ESI): 243.15 [M+1]; Exact mass calcd for $C_{16}H_{22}N_2$242.18 [*m/z*].

N-Pentyl-2,3,4,9-tetrahydro-1*H*-carbazol-6-amine, 18: Yield 55%. m.p.127°C. IR (KBr):3014, 2966, 1618, 1582, 1337, 664 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 7.68 (s, 1H, Ar-NH), 7.14 (d, *J* = 8.48 Hz, 1H,Ar H), 6.63 (d, *J* = 8.41 Hz, 1H, Ar H), 6.75 (dd, *J* = 2.50 Hz, 1H, Ar H), 3.43 (s, 1H,Indole-NH), 3.08 (q, 2H,CH_2), 2.58-2.49 (m, 4H, Aliphatic H), 2.16-2.03 (m, 4H, Aliphatic H), 1.98-1.92 (m, 2H, CH_2), 1.37-1.23 (m, 4H, $2CH_2$), 1.25 (t, 3H, CH_3); MS (ESI): 257.18 [M+1]; Mass calcd for $C_{17}H_{24}N_2$256.19 [*m/z*].

4-Methyl-N-(2,3,4,9-tetrahydro-1*H*-carbazol-6-yl) benzenesulfonamide, 19: Yield 65%. m.p.162°C. IR (KBr): 3373, 2918, 1321 and 1155, 659 and 614 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 8.27 (s, 1H, Ar-NH), 7.57 (d, *J* = 7.24 Hz, 2H,Ar-Ts H), 7.21-7.14 (m, 3H,Ar and Ar-Ts H), 7.03 (d, *J* = 2.35 Hz, 1H,Ar H), 6.78 (dd, *J* = 8.46, 2.35 Hz, 1H,Ar H), 4.20 (s, 1H,,Indole-NH), 2.78 (t, 2H,Aliphatic H), 2.60 (t, 2H,Aliphatic H), 2.19 (s, 3H, CH_3), 1.91-1.79 (m, 4H, Aliphatic H); MS (ESI): 341.09 [M+1]; Mass calcd for $C_{19}H_{20}N_2O_2S$340.12 [*m/z*].

N-(2,3,4,9-Tetrahydro-1*H*-carbazol-6-yl) methanesulfonamide, 20: Yield 54%. m.p.123°C. IR (KBr): 3217, 2786, 1364 and 1128, 657 and 604 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 8.9 (s, 1H, Ar-NH), 7.36 (d, *J* = 8.46 Hz, 1H, Ar H), 7.11 (d, *J* = 8.36 Hz, 1H,Ar H), 6.9 (dd, *J* = 2.46 Hz, 1H,Ar H), 3.12 (s, 1H, Indole-NH), 2.84 (s, 3H,CH_3), 2.74 (t, 2H, Aliphatic H), 2.66 (t, 2H, Aliphatic H), 1.25-1.14 (m, 4H, Aliphatic H); MS (ESI): 265.03 [M+1]; Mass calcd for $C_{13}H_{16}N_2O_2S$264.08 [*m/z*].

N-(2,3,4,9-Tetrahydro-1*H*-carbazol-6-yl)benzamide, 21: Yield 45%. m.p.143°C. IR (KBr): 3258, 1646, 1474, 1319, 1136, 798 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 8.80 (s, 1H,Ar-NH H), 8.23 (s, 1H, Indole-NH), 7.82 (dd, *J* = 7.28 Hz, 2H, Benzoyl H), 7.22-7.15 (m, 4H, Benzoyl H and Ar H), 7.11 (s, 1H, Ar H), 7.04 (d, *J* = 8.26 Hz, 1H, Ar H), 2.66-2.60 (m, 2H, Aliphatic H), 2.54-2.48 (m, 2H, Aliphatic H), 1.25-1.15 (m, 4H, Aliphatic H); MS (ESI): 291.13 [M+1]; Mass calcd for $C_{19}H_{18}N_2O$290.14 [*m/z*].

N-Benzyl-2,3,4,9-tetrahydro-1*H*-carbazol-6-amine, 22: Yield 35%. m.p.122°C. IR (KBr): 3394, 2811, 1573, 1350, 1108, 798 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ 8.01 (s, 1H, Ar-NH), 7.45-7.28 (m, 5H, Benzyl H), 7.01 (d, *J* = 7.46 Hz, 1H), 6.59 (d, *J* = 7.47 Hz, 1H, Ar H), 6.50 (dd, *J* = 2.21 Hz, 1H, Ar H), 3.56 (s, 2H,Benzyl H), 3.21 (s, 1H,Indole-NH), 2.94-2.81 (m, 4H, Aliphatic H), 1.22-1.07 (m, 4H, Aliphatic H); MS (ESI): 276.11 [M+1]; Mass calcd for $C_{18}H_{20}N_2$276.15 [*m/z*].

* Disappeared in D_2O exchange

Biological Activity

The experimental protocol was approved by Institutional Animal Ethics committee (IAEC) and care of animals were taken as per guidelines of committee for the purpose of control and supervision of Experiment on animals (CPCSEA), Ministry of Environment and Forest Government of India, (Reg. No. 107/1999/CPCSEA).

The 2,3,4,9-tetrahydrocarbazole derivatives (from 1 to 22) were synthesized and evaluated for their biological activity as acetylcholinestrase inhibitors to be used as possible Anti-Alzheimer drug candidates, by employing an *in-vitro* acetylcholinesterase assay on brain tissue of swiss albino mice, based on the colorimetric Acetylcholinesterase assay method developed by Ellman. The results are summarized in Table I and compared to Donepezil.

Assay for AChE and BChE inhibitory activity

Miceacetylcholinesterasewas obtained frombrain and butyrylcholinesterasefrom serum. The AChE activity was measured by the method of Ellmanspectrophotometric method with slight modification[18,19]. This was measured on basis of the formation of yellow color due to the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from Acetylthiocholine Iodide in the presence of cholinesterase was measured using a spectrophotometer. 0.5 mL of clear supernatant liquid of the brain homogenate was pipetted out into 25 mL volumetric flask and dilution was made with a freshly prepared 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) solution (10 mg DTNB in 100 mL of Sorenson phosphate buffer, *p*H 8.0). From the volumetric flask, two 4 mL portions were pipetted out into test tubes. Into one of the test tube, 2 drops of donepezil solution was added. 1 mL of substrate solution (75 mg of Acetylthiocholine iodide per 50 mL of distilled water or 75 mg of Butyrylthiocholine iodide per 50 mL of distilled water) was pipetted out into both of the test tubes. The test tube containing donepezil was taken as blank and the absorbance of the test sample was read spectrophotometrically (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) at 420 nm and IC_{50}values have been calculated. All experiments have been performed in triplicate.

Docking and Screening

In order to carry out docking simulations, iGEMDOCK was used as molecular docking tool. iGEMDOCK is an accurate and validated software based on GEMDOCK which uses a genetic evolutionary method for molecular docking and an empirical scoring function[21]. The crystal structure of *Torpedo californica* acetylcholinesterase (1EVE) was retrived from PDB (www.rscb.org)[11]. The defined binding site was set to 'By bounded ligand' and standard parameters were set automatically by the software. The binding site center (Ligand name) was set to E20. Binding site radius was adjusted to 20A° including 'retain reference ligand'. Different compounds were prepared using ChemSketch software. After that structure was cleaned and explicit hydrogens were added. Finally structure was optimised to 3D so that it can be used in iGEMDOCK. At the end structure was saved in MDL MolFile [V2000, (*.mol)] format. All the ligands were loaded in iGEMDOCK software and GA parameters were set. Default setting was set to standard docking; where population size, generation and no of solution were 200, 70 and 2 respectively. Output path was set and then docking was started. After completion of docking, 'view docked pose and post analysis' was done. Under interaction profile, energy of each compound was obtained and under interaction analysis interacting residues were obtained. The results were viewed in 'Molegro Molecular Viewer' software.

Conclusion

In the present investigations 2,3,4,9-tetrahydro-1H-carbazole and its derivatives were designedby substitution at 6 and 9 position as selective acetylcholinesterase inhibitors. These derivatives were tested by Ellman's method for the estimation of acetylcholinesterase and butyrylcholinesterase inhibitory activity. All compounds were found to inhibit both AChE and BChE.The 6-Amino-2,3,4,9-tetrahydro-1*H*-carbazole (compound 3),9-Methyl-6-nitro-2,3,4,9-tetrahydro-1*H*-carbazole(compound 4) and N-Butyl-2,3,4,9-tetrahydro-1*H*-carbazol-6-amine (compound 17) were found to be selective AChE inhibitors. Hence, Compounds 3, 4 and 17 can be taken as lead for future studies as potential anti-Alzheimer's agents.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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Conflict of Interest

Authors declare that they do not have conflict of interest.

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