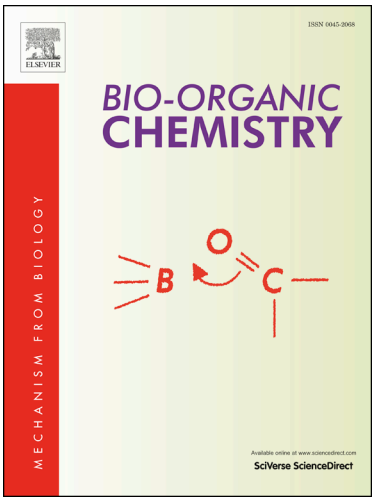


New classes of carbazoles as potential multi-functional anti-Alzheimer's agents

Niloufar Choubdar, Mostafa Golshani, Leili Jalili-Baleh, Hamid Nadri, Tuba Tüylü Kü ç ükkilinç, Beyza Ayazgök, Alireza Moradi, Farshad Homayouni Moghadam, Zahra Abdolahi, Alieh Ameri, Fatemeh Salehian, Alireza Foroumadi, Mehdi Khoobi

PII: S0045-2068(19)30555-3
DOI: https://doi.org/10.1016/j.bioorg.2019.103164
Reference: YBIOO 103164

To appear in: *Bioorganic Chemistry*

Received Date: 13 April 2019
Revised Date: 20 July 2019
Accepted Date: 29 July 2019

Please cite this article as: N. Choubdar, M. Golshani, L. Jalili-Baleh, H. Nadri, T. Tüylü Kü ç ükkilinç, B. Ayazgök, A. Moradi, F. Homayouni Moghadam, Z. Abdolahi, A. Ameri, F. Salehian, A. Foroumadi, M. Khoobi, New classes of carbazoles as potential multi-functional anti-Alzheimer's agents, *Bioorganic Chemistry* (2019), doi: https://doi.org/10.1016/j.bioorg.2019.103164

New classes of carbazoles as potential multi-functional anti-Alzheimer's agents

Niloufar Choubdar[a], Mostafa Golshani[b], Leili Jalili-Baleh[b], Hamid Nadri[c], Tuba Tüylü Küçükkilinç[d], Beyza Ayazgök[d], Alireza Moradi[c], Farshad Homayouni Moghadam[e], Zahra Abdolahi[c], Alieh Ameri[f], Fatemeh Salehian[b], Alireza Foroumadi[b], Mehdi Khoobi[b,*]

[a]*Department of Organic Chemistry, Faculty of Pharmaceutical Chemistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran*

[b]*Biomaterials Group, The Institute of Pharmaceutical Sciences (TIPS), Tehran University of Medical Sciences, Tehran, 1417614411, Iran*

[c]*Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

[d]*Hacettepe University, Faculty of Pharmacy, Department of Biochemistry, Ankara, Turkey*

[e]*Department of Cellular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran*

[f]*Department of Medicinal Chemistry, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran*

Abstract

Multi-Target approach is particularly promising way to drug discovery against Alzheimer's disease. In the present study, we synthesized a series of compounds comprising the carbazole backbone linked to the benzyl piperazine, benzyl piperidine, pyridine, quinoline, or isoquinoline moiety through an aliphatic linker and evaluated as cholinesterase inhibitors. The synthesized compounds showed IC_{50} values of 0.11-36.5 μM and 0.02-98.6 μM against acetyl- and butyrylcholinesterase (AChE and BuChE), respectively. The ligand-protein docking simulations and kinetic studies revealed that compound **3s** could bind effectively to the peripheral anionic binding site (PAS) and anionic site of the enzyme with mixed-type inhibition. Compound **3s** was the most potent compound against AChE and BuChE and showed acceptable inhibition potency for self- and AChE-induced $A\beta_{1-42}$ aggregation. Moreover, compound **3s** could significantly protect PC12 cells against H_2O_2-induced toxicity. The results suggested that the compounds **3s** could be considered as a promising multi-functional agent for further drug discovery development against Alzheimer's disease.

Keywords: Alzheimer's disease, acetylcholinesterase, butyrylcholinesterase, carbazoles, docking study, β-amyloid aggregation.

* Corresponding author: The Institute of Pharmaceutical Sciences (TIPS), Tehran University of Medical Sciences, P.O. Box: 14155-6451, Tehran, Iran; Tel/Fax: ++98-21-64121510; E-mails: mehdi.khoobi@gmail.com

1. Introduction

Alzheimer's disease (AD), the most common cause of dementia, is considered as a progressive and degenerative disorder of the brain which affects approximately 10% of the population over the age of 65 [1,2]. A complex series of neurochemical factors involve in the initiation and progression of the disease. It is associated with loss of presynaptic markers of the cholinergic system such as acetylcholine (ACh) and other related neurotransmitters presenting in hippocampus. The presence of neurofibrillary tangles and amyloid depositions in the brain are also some important signs of the disease [3,4].

Over the past two decades, several hypotheses have been proposed for AD mechanism including cholinergic hypothesis, amyloid hypothesis, and oxidative hypothesis leading to the generation of different therapeutic approaches [5-7].

Although there have been several theories concerning the pathogenesis of AD, the molecular cause of the disease has remained unknown. Cholinergic hypothesis suggests that the decline in cognitive and mental functions is associated with the loss of cortical cholinergic neurotransmission. Therefore, inhibition of acetylcholinesterase (AChE) activity could be helpful to increase the cholinergic neurotransmission. According to the cholinergic hypothesis, AChE inhibitors (AChEIs) increase the level of synaptic ACh, leading to improve the cholinergic function in the central nervous system [8-10]. Consequently, AChEIs slow down the progression of AD and therefore postpone the cognitive deterioration of patients suffering from AD [11].

Galantamine, rivastigmine, donepezil, and tacrine are four clinically approved AChEIs for the treatment of AD. These drugs are able to enhance cholinergic neurotransmission by increasing acetylcholine in the synaptic cleft [12]. Although, tacrine a former AChE inhibitor has been discontinued due to the high incidence of hepatoxicity [13, 14], attempts have been continuing to prepare modified tacrine analogues without hepatoxicity [15, 16].

The X-ray analysis of AChE reported by Sussman *et al*. has revealed that the active site of AChE comprises at least four active sites including peripheral anionic binding site (PAS), esteratic site (ES), aromatic cation-binding site (ACS) and anionic substrate binding site (AS) [17-19]. AChEIs with the ability to interact exclusively with PAS or with both catalytic and peripheral binding sites can prevent the AChE-induced amyloid-beta (Aβ) aggregation [20]. Dual catalytic site and PAS binding inhibitors control the disease through cholinesterase inhibition as well as the prevention of AChE-Aβ complex formation, which is more toxic than self-induced Aβ aggregation. Several studies have

shown that some AChEIs have a dual action thereby facilitate the cholinergic transmissions and interfere in the deposition and aggregation of amyloid peptides [21, 22].

Carbazoles are naturally occurring phytochemicals in many plants with a wide range of biological activities. Naturally occurring carbazoles and their synthetic derivatives have demonstrated wide range of pharmacological activities [23-25]. Carbazole derivatives have increasingly gained interest as lead scaffold in the design of anti-AD agents. Carbazole-tacrine hybrids could interact simultaneously with the CAS and PAS of AChE and showed appropriate antioxidant activity. They could efficiently protect neuroblastoma cells against toxicity induced by $A\beta_{1-40}$ and H_2O_2 (compound **a**, Fig. 1) [26]. Additionally, carbazole derivatives bearing pyridinium or quinolinium salts showed perfect inhibitory activity against self-induced and AChE-induced Aβ aggregation [27] and potent neuroprotective activity against A*β* in human neuronal-like cells (compound **b**, Fig. 1) [25]. *β*-carbolinium salts also showed potent inhibition activity against both AChE/BChE. The most potent derivative showed IC_{50} values of 0.5 μM for AChE and 5.7 μM for BChE, respectively (compound **c**, Fig. 1) [28].

Figure 1

In the light of the above findings and in pursuit of searching for multifunctional drug candidates as potent anti-AD [29-33], in the present study we introduce a new class of carbazole derivatives bearing benzyl piperazine, piperidine, pyridinium, quinolinium, or isoquinolinium part as well-known pharmacophores inhibiting CAS of AChE linked through aliphatic carbon chain length together. The synthesized compounds were evaluated as multifunctional agents against AChE and BuChE, self- and AChE-induced amyloid-beta protein 1-42 ($A\beta_{1-42}$) peptide aggregating, and hydrogen peroxide-induced neurotoxicity. Kinetic study and ligand-AChE docking simulation were also done to elucidate the mechanism of the ligand interaction with enzyme.

2. Results and Discussion

2.1. Chemistry

Target compounds were easily synthesized from commercially available carbazole. Compound **1** was synthesized via the reaction of carbazole with different alkyl dibromides in the presence of NaH in DMF. The intermediate **2** was then reacted with excess amount of piperazine, KI in EtOH under reflux condition followed by reaction with different substituted benzyl halides in the presence of

catalytic amounts of K_2CO_3 in dry acetonitrile under reflux condition to afford the desired final products **3a-r** (Scheme 1). Also, products **3s-w** were directly synthesized from the reaction of compound **1** with quinoline, isoquinoline, pyridine, piperidine or 4-benzylpiperidine in dry acetonitrile under reflux condition.

All reactions were monitored by TLC and the products were purified by flash column chromatography using petroleum ether/ethyl acetate (10:1- 3:1) as the eluent. ^1H, ^{13}C NMR and MS spectroscopy were utilized for products characterization.

Scheme 1

2.2. Anti-cholinesterase activity

The inhibitory activities of the synthesized compounds **3a-w** were evaluated *in vitro* against AChE and BuChE enzymes based on Ellman procedure [34]. The results are presented as IC_{50} values in Table 1 comparing to donepezil as a standard drug. IC_{50} values for the tested compounds were in the range of 0.11-36.5 μM against AChE and in the range of 0.02-98.6 μM against BuChE indicating the potential anti-cholinesterase activity of the designed compounds (Table 1).

Based on the results, it has been concluded that different factors such as type and position of the substituents with the length of the linker could modulate the activity of the compounds. In the series of the compounds bearing *N*-benzylpiperazine, five carbon chain length as cross-linker demonstrated better inhibitory activity comparing to the corresponding linker with three carbon chain length. All the tested compounds showed activity in the range of micro to nano molar. Compounds **3s-u** showed higher activity than donepezil as the reference drug against BuChE (IC_{50} = 0.02-1.4 μM).

Comparing the inhibitory activity of *N*-benzylpiperazine **3f** with 4-benzylpiperidine **3w**, demonstrated that replacing the piperazine ring with piperidine, could decrease the activity against both AChE and BuChE. According to this finding, it could be suggested that the nitrogen atom at position 4 of the piperazine ring play an important role in interaction with the enzyme. By removing the benzyl moiety in compound **3w**, the anticholinesterase activity improved (see the IC_{50} value of compound **3v)**.

The *N*-benzylpiperazine derivatives with five carbon chain lengths (**3f-r**) exhibited inhibitory activities in the range of 1.4 to 22.0 μM. The meta-brominated *N*-benzylpiperazine derivative **3k** was the most active compound against both AChE and BuChE with the IC_{50} value of 1.4 μM and 5.1 μM, respectively. 3,4-Dichlorobenzyl substituted derivative **3m** showed the least activity against both

enzymes. Considering the potency of meta-methoxy substituted compound **3l** (IC_{50} AChE=19.0, IC_{50} BuChE=67.9), it has been revealed that the more lipophilic substituent such as Br is necessary for optimum activity.

Among fluorinated derivatives, compound with 4-F substituent **3r** showed a better inhibitory activity comparing to its 2-F analogue **3h**. In contrast, among chlorinated benzyl derivatives, 2-chlorobenzylpiperazine derivative **3g** showed better inhibition comparing to its para congener **3p**. In another analogy, the order of the activity for 4-substituted benzyl derivatives having five carbon chain length was 4-Br > 4-F > 4-OMe > H > 4-Me > 4-Cl (IC_{50} values of 1.7, 4.4, 9.4, 10.1, 10.7, 10.9 μM, respectively). Considering these results, it could be concluded that the inhibitory activities of the 4-substituted benzylpiperazine derivatives are not significantly affected by the electron withdrawing or electron donating properties of the substituents at the para position. Alternatively, the ortho-substituted benzyl piperazine derivatives with five carbon chain linker showed relative inhibitory activity of 2-Cl~2-Me > 2-NO_2 > 2-F with IC_{50} values of 5.8, 5.9, 9.8, 11.5 μM, respectively (Table 1). Among compounds **3a-3e** except for **3b**, by shortening the linker to three atoms the activity against acetylcholinesterase diminished due to the shrinking of the molecule. Replacing the cyclic aliphatic amine with the aromatic amines such as pyridine or quinoline led to the superior activity implying the fact that aromatic stacking interactions has crucial role in the enzyme inhibition. Furthermore, bicyclic aromatic amines (quinoline and isoquinoline) expressed higher activity owing to the extra π-stacking interactions. According to the calculated BuChE/AChE selectivity, the best selectivity (4.00) is related to the compound **3q** possessing 4-bromo-benzylpiperazine group connected to carbazolzole moiety via 5 carbon spacer.

Table 1

2.3. Kinetic study of AChE inhibition

To determine the inhibition type and steady-state inhibition constant, enzyme kinetic studies were carried out on compound **3s** as the most active compound using AChE and BuChE. The results proposed that compound **3s** exhibited mixed type-inhibition on AChE and BuChE with Ki values of 48 and 6.6 μM, respectively. The dual mode of inhibition (competitive and uncompetitive) for compound **3s** advocates the fact that compound **3s** could simultaneously bind to both catalytic active site (CAS) and peripheral anionic site (PAS). The Lineweaver–Burk double reciprocal plot and secondary plot of compound **3s** for both AChE and BuChE are represented in Figure 2. The kinetic

studies were carried out as described above using different concentrations of the substrate and the compounds. Compound **3s** with concentrations of 216, 108, 54 and 0 μM were used for AChE kinetic analysis while the concentrations of 48, 24, 12 and 0 μM were used for BuChE kinetic analysis.

Figure 2

2.4. Inhibition of self- and AChE-induced $A\beta_{1-42}$ aggregation

Inhibitory properties of compound **3s** as the most active compound and **3k** as the best active compound among carbazole derivatives conjugated to the benzylpiperazine moiety on self-induced and AChE-induced aggregation of $A\beta_{1-42}$ were determined using a thioflavin T (ThT)-based fluorescence assay and compared with donepezil and rifampicin as reference compounds [35,36]. Based on the results (Table 2), both compounds **3s** and **3k** showed acceptable potency for inhibition of self-induced Aβ aggregation of 27.4 and 19.5%, respectively, relative to the reference compounds rifampicin (27.5%) and donepezil (22%). Compound **3s** bearing quinoline moiety was more active to inhibit Aβ self-aggregation in comparison to compound **3k** with benzylpiperazine. Moreover, both compounds showed moderate inhibitory activity toward AChE-induced Aβ aggregation at 100 μM (24.9% and 19.2% respectively) which are less than their Aβ self-aggregation inhibitory activity, while biologic data analysis and molecular docking study confirmed the binding affinity of the compounds to PAS as a dual AChE inhibitor. This result indicated that there was no correlation between AChE-induced Aβ aggregation inhibitory activity of the compounds and their high binding affinity to PAS.

Table 2

2.5. Neuroprotection against H_2O_2-induced cell death in PC12 cells

The neuroprotective effect of compound **3s** against H_2O_2-induced PC12 cell injury at the concentrations of 1, 10, and 100 μM, prior to treatment with H_2O_2 (375 μM) was evaluated via MTT-based colorimetric assay. Compound **3s** showed significant effect on cell viability at a concentration of 10 μM (Cell violability = 1.054, $P < 0.0098$) which is comparable to the Quercetin (Cell violability = 1.092, $P < 0.001$) as standard control suggesting acceptable neuroprotective activity of compound **3s (**Fig. 3).

Figure 3

2.6. Ligand-protein docking simulation

According to the IC_{5o} values, compound **3s** demonstrated the highest potency for interaction with the active site of the enzyme. Based on the docking data, compound **3s** binds effectively to the PAS and anionic site of the enzyme with the assistance of hydrophobic and π stacking interactions. π-Stacking interaction is formed between Tyr-332 and quinoline fragment of the compound (Fig. 4). As a result, compound **3s** is fitted quite well into the PAS and anionic site of the AChE demonstrating higher therapeutic activity.

Figure 4

3. Conclusion

We have designed and synthesized a new class of carbazole derivatives bearing benzyl piperazine, benzyl piperidine, pyridinium, quinolinium, or isoquinolinium salts linked through a 3 or 5 carbon atom chain length. All of the tested compounds showed reasonable activity in the range of micro to nano molar; among them the best candidate was quinolinium salt derivative **3s** with IC_{50} = 0.11 μM and 0.02 μM against AChE and BuChE, respectively. Replacing the benzyl piperazine moiety with quinolinium or isoquinolinium salts (**3s** and **3t**) improved the enzyme inhibition quite remarkably. Two factors might have been contributed in the high inhibitory activity of these compounds including formation of the stable cationic part and the improved π-stacking interactions of the quinoline or isoquinoline moieties with the PAS of the enzyme. Kinetic studies for compound **3s** exhibited mixed-type inhibition on AChE and BuChE. Docking data also confirmed the dual mode of inhibition for compound **3s** advocating the fact that compound **3s** could simultaneously bind to both CAS and PAS. Above all, compound **3s** demonstrated good potency for inhibition of self-induced Aβ aggregation and revealed acceptable neuroprotective activity against PC12 cells injury induced by H_2O_2. Owing to the high AChE and BuChE inhibition activity, acceptable neuroprotective effects, satisfactory self- and AChE-induced Aβ aggregation inhibition, compound **3s** could be suggested as a promising multifunctional compound against Alzheimer's disease.

4. Materiarls and Methods

4.1. Chemistry

All commercially available chemicals were purchased from Merck, Sigma, and across without further purification. ^{1}H NMR and ^{13}C NMR were recorded on Brucker 500 MHz instrument with frequencies of 500 and 125 MHz, respectively. Solvents used for NMR analyses were $CDCl_3$ and DMSO and tetramethylsilane were used as an internal standard. The chemical shifts (δ) and coupling constants (*J*) were expressed in parts per million and Hertz, respectively. Mass spectra were obtained by HP Agilent Technologies 5937 at ionization potential of 70 eV. Elemental analyses were performed with CHN-Rapid Heraeus. Melting points were measured by Electrothermal 1A9100. Elemental Analyzer and the results (C, H, N) were within ± 0.4% of the calculated values. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with silica gel 60F-254 as the adsorbent. Column chromatography was performed using silica gel 60 (230-400 mesh).

*4.1.1. General procedure for the synthesis of compound **1***

The commercially available carbazole (10 mmol) was dissolved in dry DMF (15 mL). The mixture was cooled to 0 °C and NaH (10 equiv.) was added followed by dropwise addition of the appropriate dibromoalkane (15 equiv.). The mixture was stirred at room temperature for 15 h and quenched by addition of ice. The crude product was diluted with ethyl acetate (25 mL) and washed with brine (100 mL). The organic phase was dried over Na_2SO_4 and filtered. The concentrated crude product was purified by flash chromatography using petroleum ether to afford compound **1** as a colorless oil [37].

*4.1.1.1. 9-(3-bromopropyl)-9H-carbazole **1a** (n =3))* Yield 85%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.13 (d, 2H, J = 7.5 Hz), 7.58 (d, 2H, J = 8.0 Hz), 7.44 (t, 2H, J = 7.0 Hz), 7.19 (t, 2H, J = 7.0 Hz), 4.38 (t, 2H, J = 7.0 Hz), 3.40 (t, 2H, J = 6.0 Hz), 1.88 (m, 2H).

*4.1.1.2. 9-(5-bromopentyl)-9H-carbazole **1b** (n = 5)* Yield 82%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.13 (d, 2H, J = 7.7 Hz), 7.57 (m, 2H), 7.44 (m, 2H), 7.19 (m, 2H), 4.36 (t, 2H, J = 7.0 Hz), 3.42 (m, 2H), 1.76 (m, 4H), 1.43 (m, 2H); ^{13}C NMR (DMSO- d_6, 125 MHz) δ 139.9, 125.6, 122.0, 120.2, 118.6, 109.2, 42.0, 34.8, 31.9, 28.3, 25.2.

*4.1.2. General procedure for the synthesis of piperazine intermediate **2***

Compound **1** (5 mmol) was dissolved in anhydrous ethanol (30 mL). Anhydrous piperazine (75 mmol) and KI (2.86 mmol) were added to the mixture and refluxed for 1h. The solvent was removed

under reduced pressure and the crude product was diluted with ethyl acetate (25 mL) and washed with brine (100 mL). The organic phase was dried over Na_2SO_4 and filtered. The concentrated crude product was purified by flash chromatography (petroleum ether/EtOAc 3:1) to afford piperazine intermediate **2** as a pale yellow oil [38].

4.1.2.1. 9-(3-(piperazin-1-yl)propyl)-9H-carbazole ***2a*** *(n = 3)* Yield 85%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.13 (d, 2H, J = 8.0 Hz), 7.59-7.56 (m, 2H), 7.43 (t, 2H, J = 7.5 Hz), 7.18 (t, 2H, J = 7.5 Hz), 4.38 (t, 2H, J = 7.0 Hz), 2.73 (bs, 4H), 2.16-2.13 (m, 6H), 1.90-1.86 (m, 2H); ^{13}C NMR (DMSO- d_6, 125 MHz) δ 140.0, 125.4, 122.0, 120.0, 118.5, 109.1, 56.0, 54.9, 52.8, 45.0, 25.1.

4.1.2.1. 9-(5-(piperazin-1-yl)pentyl)-9H-carbazole ***2b*** *(n = 5)* Yield 80%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.11 (d, 2H, J = 7.0 Hz), 7.57 (d, 2H, J = 8.5 Hz), 7.43 (t, 2H, J = 7.5 Hz), 7.20-7.17 (m, 2H), 4.38 (t, 2H, J = 6.5 Hz), 2.73 (bs, 2H), 2.26 (bs, 4H), 2.16-2.14 (m, 2H), 1.78-1.76 (m, 2H), 1.42-1.27 (m, 4H).

4.1.3. General procedure for the synthesis of compound ***3a-r***

A mixture of piperazine intermediate **2** synthesized above (1 mmol), anhydrous K_2CO_3 (1.1 mmol) and appropriate benzyl bromide (1.2 mmol) was refluxed in dry acetonitrile (25 mL) for 3 h. The mixture was cooled to room temperature and filtered. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (petroleum ether/EtOAc 5:1) to afford compound **3a-r** [38].

4.1.3.1. 9-(3-(4-benzylpiperazin-1-yl)propyl)-9H-carbazole ***3a****:* [39] Yield 80%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.08 (d, 1H, J = 7.6 Hz), 7.79 (d, 1H, J = 8.0 Hz), 7.64 (d, 1H, J = 8.1 Hz), 7.57-7.47 (m, 6H) 7.36-7.11 (m, 4H), 4.63-4.34 (m, 2H), 3.62 (m, 2H), 2.41-2.22 (m, 10H), 1.93 (m, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 139.9, 137.4, 133.7, 132.6, 130.4, 129.0, 128.9, 128.4, 127.5, 127.3, 125.9, 122.3, 119.1, 118.6, 109.3, 60.3, 57.2, 54.4, 53.1, 45.1, 30.7. Anal. Calcd. for $C_{26}H_{29}N_3$(383.53): C, 81.42; H, 7.62; N, 10.96. Found: C, 81.40; H, 7.78; N, 11.02.

4.1.3.2. 9-(3-(2-(2-fluorobenzyl)piperazin-1-yl)propyl)-9H-carbazole ***3b****:* Yield 70%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (m, 2H), 7.60 (m, 2H), 7.44-7.35 (m, 2H), 7.30 (m, 2H), 7.15-7.12 (m, 4H), 4.39 (t, 1H, J = 6.5 Hz), 3.50 (bs, 2H), 2.51-2.15 (m, 10H), 1.89 (t, 2H, J = 6.5 Hz); ^{13}C

NMR (DMSO-d_6, 125 MHz) δ161.8 and 159.8 (C-F, J = 242.5 Hz), 140.0, 131.6, 131.6, 129.1, 129.0, 125.6, 124.8, 124.6, 122.0, 120.2, 118.6, 115.2, 115.0, 109.3, 54.5, 54.4, 52.6, 52.5, 25.4. Anal. Calcd. for $C_{26}H_{28}FN_3$(401.53): C, 77.77; H, 7.03; N, 10.47. Found: C, 77.54; H, 7.32; N, 10.33.

4.1.3.3. 9-(3-(4-fluorobenzyl)piperazin-1-yl)propyl)-9H-carbazole ***3c****:* Yield 60%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (d, 2H, J = 7.5 Hz), 7.59 (d, 2H, J = 8.0 Hz), 7.45-7.42 (m, 2H), 7.31-7.29 (m, 2H), 7.28-7.11 (m, 4H), 4.40 (t, 2H, J = 7.0 Hz), 3.40 (s, 2H), 2.34-2.17 (m, 8H), 2.17-2.15 (m, 2H), 1.98 (t, 2H, J = 5.0 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 162.2 and 160.3 (C-F, J = 237.5 Hz), 140.1, 135.2, 134.4, 130.7, 130.6, 125.8, 125.4, 122.2, 122.1, 120.2, 118.9, 118.8, 115.2, 114.9, 114.8, 109.3, 61.2, 54.4, 52.6, 25.5. Anal. Calcd. for $C_{26}H_{28}FN_3$(401.53): C, 77.77; H, 7.03; N, 10.47. Found: C, 77.38; H, 7.32; N, 10.67.

4.1.3.4. 9-(3-(4-bromobenzyl)piperazin-1-yl)propyl)-9H-carbazole ***3d****:* Yield 63%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (d, 2H, J = 8.0 Hz), 7.58-7.42 (m, 6H), 7.29-7.17 (m, 4H), 4.39 (t, 2H, J = 6.5 Hz), 3.45 (s, 2H), 2.35-2.17 (m, 10H), 1.92-1.88 (m, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 140.0, 137.7, 131.0, 130.9, 128.6, 125.6, 122.0, 120.2, 119.9, 118.6, 109.2, 61.2, 54.4, 54.3, 50.0, 25.4. Anal. Calcd. for $C_{26}H_{28}BrN_3$(462.44): C, 67.53; H, 6.10; N, 9.09. Found: C, 67.81; H, 6.38; N, 9.33.

4.1.3.5. 9-(3-(4-methoxybenzy)lpiperazin-1-yl)propyl)-9H-carbazole ***3e****:* Yield 61%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (d, 2H, J = 7.7 Hz), 7.60 (d, 2H, J = 8.5 Hz), 7.43 (t, 2H, J = 8.0 Hz), 7.19-7.16 (m, 4H), 6.87 (d, 2H, J = 8.5 Hz), 4.40 (t, 2H, J = 7.0 Hz), 3.72 (s, 3H, OMe), 3.37 (bs, 2H), 2.35-2.19 (m, 10H), 1.91-1.89 (m, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 158.4, 130.2, 129.5, 129.4, 129.2, 129.2, 127.9, 125.6, 122.0, 118.7, 113.7, 113.6, 113.6, 113.4, 109.3, 73.3, 70.8, 62.5, 61.2, 55.0, 54.3, 52.2, 25.3. Anal. Calcd. for $C_{27}H_{31}N_3O$(413.57): C, 78.42; H, 7.56; N, 10.16. Found: C, 78.15; H, 7.46; N, 10.32.

4.1.3.6. 9-(5-(4-benzylpiperazin-1-yl)pentyl)-9H-carbazole ***3f****:* Yield 78%; White solid; m.p. 108-110 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.15 (d, 1H, J = 7.6 Hz), 7.64 (d, 1H, J = 8.1 Hz), 7.55-7.44 (m, 5 H) 7.32-7.18 (m, 6H), 4.59-4.45 (m, 2H), 3.55 (bs, 2H), 3.34 (bs, 2H), 3.09 (bs, 3H), 2.83-2.79 (m, 2H), 2.63 (bs, 3H), 1.90-1.80 (m, 4H), 1.29 (bs, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 140.0, 137.3, 133.7, 132.9, 130.3, 129.0, 128.8, 128.4, 127.4, 127.3, 125.7, 122.0, 120.3, 118.7, 109.3, 60.4,

56.8, 45.4, 40.8, 28.2, 24.2, 20.6. Anal. Calcd. for $C_{27}H_{31}N_3O$(413.57): C, 78.42; H, 7.56; N, 10.16. Found: C, 78.15; H, 7.46; N, 10.32.

4.1.3.7. 9-(5-(4-(2-chlorobenzyl)piperazin-1-yl)pentyl)-9H-carbazole ***3g****:* Yield 80%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (d, 2H, *J* = 7.5 Hz), 7.58 (d, 2H, *J* = 8.5 Hz), 7.44-7.41 (m, 4H), 7.30-7.28 (m, 2H), 7.17 (t, 2H, *J* = 7.5 Hz), 4.37 (t, 2H, *J* = 7.0 Hz), 3.50 (s, 2H), 2.49-2.15 (m, 10H), 1.76 (bs,2H), 1.39 (bs, 2H), 1.23 (bs, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 140.0, 135.6, 133.2, 130.8, 129.2, 128.5, 127.0, 125.6, 122.0, 120.2, 118.6, 109.2, 58.6, 57.7, 52.7, 42.2, 28.3, 26.0, 24.4. Anal. Calcd. for $C_{28}H_{32}ClN_3$(446.04): C, 75.40; H, 7.23; N, 9.42. Found: C, 75.33; H, 7.40; N, 9.21.

4.1.3.8. 9-(5-(4-(2-fluorobenzyl)piperazin-1-yl)pentyl)-9H-carbazole ***3h****:* Yield 60%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (d, 2H, *J* = 8.0 Hz), 7.57 (d, 2H, *J* = 8.0 Hz), 7.43 (t, 2H, *J* = 7.0 Hz), 7.42-731 (m, 2H), 7.20-7.15 (m, 4H), 4.35 (t , 2H, *J* = 7.0 Hz), 3.51 (bs, 2H), 2.37-2.31 (m, 8H), 2.17-2.14 (m, 2H), 1.77-1.74 (m, 2H), 1.39-1.36 (m, 2H), 1.26-1.23 (m, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.8 and 159.9 (C-F, *J* = 242.5 Hz), 140.0, 131.6, 129.3, 129.1, 129.1, 125.7, 124.6, 124.4, 124.2, 122.1, 120.3, 118.7, 115.3, 115.1, 109.3, 57.6, 54.5, 52.6, 52.3, 42.2, 28.0, 25.0, 25.9, 24.4. Anal. Calcd. for $C_{28}H_{32}FN_3$(429.58): C, 78.29; H, 7.51; N, 9.78. Found: C, 78.13; H, 7.20; N, 9.41.

4.1.3.9. 9-(5-(4-(2-methylbenzyl)piperazin-1-yl)pentyl)-9H-carbazole ***3i****:* Yield 81%; White solid; m.p. 130-132 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.17 (d, 2H, *J* = 5 Hz), 7.65 (d, 2H, *J* = 8.0 Hz), 7.47-7.37 (m, 6H), 7.31-7.29 (m, 2H), 7.25-7.21 (m, 2H), 4.61-4.47 (m, 2H), 3.52 (bs, 2H), 3.15-3.12 (m, 2H), 2.73-2.66 (m, 8H), 2.37 (s, 3H, Me), 1.91-1.76 (m, 4H), 1.36 (bs, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 140.3, 140.0, 137.1, 135.1, 134.5, 131.6, 130.3, 130.1, 129.6, 127.2, 126.0, 125.9, 125.6, 125.4, 122.0, 120.2, 118.6, 109.2, 62.8, 58.3, 55.8, 54.6, 45.3, 41.9, 28.1, 23.4, 20.9, 19.7, 18.7. Anal. Calcd. for $C_{29}H_{35}N_3$(425.62): C, 81.84; H, 8.29; N, 9.87. Found: C, 82.15; H, 8.02; N, 9.51.

4.1.3.10. 9-(5-(4-(2-nitrobenzyl)piperazin-1-yl)pentyl)-9H-carbazole ***3j****:* Yield 70%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.12 (d, 2H, *J* = 8.0 Hz), 7.83 (d, 1H, *J* = 5.0 Hz), 7.63-7.40 (m, 7H), 7.17 (t, 2H, *J* = 7.5 Hz), 4.34 (t, 2H, *J* = 7.0 Hz), 3.72 (t, 2H, *J* = 7.5 Hz), 2.25-2.11 (m, 10H), 1.76-1.73 (m, 2H), 1.37-1.24 (m, 4H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 149.6, 139.9, 132.7, 132.5, 131.0, 128.5, 125.5, 124.0, 122.0, 120.1, 118.5, 109.1, 58.1, 57.4, 52.5, 52.4, 42.1, 28.2, 25.8, 24.3,

21.0. Anal. Calcd. for $C_{28}H_{32}N_4O_2$(456.59): C, 73.66; H, 7.06; N, 12.27. Found: C, 73.43; H, 7.36; N, 12.31.

4.1.3.11. 9-(5-(4-(3-bromobenzyl)piperazin-1-yl)pentyl)-9H-carbazole ***3k****:* Yield 88%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (bs, 2H), 7.60 (bs, 2H), 7.48-7.43 (m, 4H), 7.29 (bs, 2H), 7.19 (bs, 2H), 4.40 (bs, 2H), 3.58 (m, 2H), 3.47-3.35 (m, 4H), 3.25 (m, 1H), 2.66 (bs, 2H), 1.98 (bs, 2H), 1.58 (bs, 2H), 1.29 (bs, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 139.9, 131.3, 130.5, 130.4, 130.1, 130.0, 127.8, 125.6, 122.0, 121.7, 121.6, 120.3, 118.7, 118.6, 109.2, 59.5, 57.6, 45.2, 42.0, 28.2, 23.4, 20.5. Anal. Calcd. for $C_{28}H_{32}BrN_3$(490.49): C, 68.57; H, 6.58; N, 8.57. Found: C, 68.33; H, 6.87; N, 8.65.

4.1.3.12. 9-(5-(4-(3-methoxybenzyl)piperazin-1-yl)pentyl)-9H-carbazole ***3l****:* Yield 73%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (d, 2H, J = 7.7 Hz), 7.58 (d, 2H, J = 8.2 Hz), 7.43 (t, 2H, J = 7.0 Hz), 7.20-7.16 (m, 3H), 6.84-6.79 (m, 2H), 4.37 (t, 2H, J = 7.0 Hz), 3.72 (s, 3H, OMe), 3.38 (s, 2H), 2.30-2.18 (m, 5H), 1.78-1.75 (m, 2H), 1.40 (bs, 2H), 1.27 (bs, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 159.2, 139.9, 129.1, 125.6, 122.0, 120.9, 120.2, 118.6, 114.2, 112.2, 109.2, 61.9, 57.6, 54.9, 52.7, 52.5, 42.1, 28.3, 25.9, 24.4. Anal. Calcd. for $C_{29}H_{35}N_3O$(441.62): C, 78.87; H, 7.99; N, 9.52. Found: C, 79.12; H, 6.97; N, 9.35.

4.1.3.13. 9-(5-(4-(3,4-dichlorobenzyl)piperazin-1-yl)pentyl)-9H-carbazole ***3m****:* Yield 76%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.13 (d, 2H, J = 7.6 Hz), 7.58-7.47 (m, 6H), 7.27-7.16 (m, 3H), 4.37 (t, 2H, J = 6.5 Hz), 3.41 (s, 2H), 2.29-2.17 (m, 8H), 1.90 (bs, 2H), 1.39 (bs, 2H), 1.26 (bs, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 140.0, 139.7, 130.4, 130.3, 129.3, 129.0, 125.6, 122.0, 120.2, 60.4, 57.6, 52.6, 52.3, 42.2, 28.3, 25.9, 24.4, 21.0. Anal. Calcd. for $C_{28}H_{31}Cl_2N_3$(480.48): C, 69.99; H, 6.50; N, 8.75. Found: C, 70.22; H, 6.67; N, 8.95.

4.1.3.14. 9-(5-(4-(4-methoxybenzyl)piperazin-1-yl)pentyl)-9H-carbazole ***3n****:* Yield 65%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.13 (d, 2H, J = 8.0 Hz), 7.58 (d, 2H, J = 8.0 Hz), 7.43 (t, 2H, J = 7.5 Hz), 6.86-6.84 (m, 6H), 4.36 (bs, 2H), 3.71 (s, 3H, OMe), 2.34 (bs, 2H), 2.30-2.10 (m, 10H), 1.76 (bs, 2H), 1.39 (bs, 2H), 1.26 (bs, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 158.2, 139.9, 130.0, 125.6, 122.0, 120.2, 118.6, 113.5, 109.2, 61.4, 57.6, 55.0, 52.6, 52.4, 52.3, 42.1, 28.3, 25.8, 24.4. Anal. Calcd. for $C_{29}H_{35}N_3O$(441.62): C, 78.87; H, 7.99; N, 9.52. Found: C, 78.77; H, 7.71; N, 9.81.

*4.1.3.15. 9-(5-(4-(4-methylbenzyl)piperazin-1-yl)pentyl)-9H-carbazole **3o**:* Yield 67%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.13 (d, 2H, J = 7.6 Hz), 7.57 (d, 2H, J = 8.0 Hz), 7.42 (t, 2H, J = 8.0 Hz), 7.19-7.09 (m, 6H), 4.35 (t, 2H, J = 7.0 Hz), 3.37 (s, 2H), 2.25-2.21 (m, 8H), 1.95 (bs, 2H), 1.90 (3H, Me), 1.77 (bs, 2H), 1.39 (bs, 2H), 1.25 (bs, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 140.0, 136.0, 134.8, 128.8, 128.7, 125.6, 122.0, 120.2, 118.6, 109.2, 61.7, 57.4, 52.5, 52.1, 42.1, 28.3, 25.7, 24.3, 21.1, 20.7. Anal. Calcd. for $C_{29}H_{35}N_3$(425.62): C, 81.84; H, 8.29; N, 9.87. Found: C, 81.98; H, 8.46; N, 9.65.

*4.1.3.16. 9-(5-(4-(4-chlorobenzyl)piperazin-1-yl)pentyl)-9H-carbazole **3p**:* Yield 73%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.13 (bs, 2H), 7.59 (bs, 2H), 7.43 (bs, 2H), 7.37 (bs, 2H), 7.30 (bs, 2H), 7.19 (bs, 2H), 4.37 (bs, 2H), 3.44 (bs, 2H), 2.45-2.25 (bs, 8H), 1.17 (bs, 2H), 1.47 (bs, 2H), 1.22-1.16 (m, 4H), 1.16 (m, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 140.0, 139.7, 130.8, 130.4, 130.3, 129.3, 129.0, 125.6, 122.0, 120.2, 118.6, 109.2, 60.4, 57.6, 52.6, 52.3, 54.1, 28.3, 25.9, 24.4, 21.0; MS m/z: 445.60 $(M-H)^+$, 265.15 ($C_{15}H_{22}ClN_2^{\bullet}$), 223.10 ($C_{12}H_{16}ClN_2^{\bullet}$), 180.08 ($C_{13}H_{10}N^{\bullet}$), 125.02 ($C_7H_6Cl^{\bullet}$); Anal. Calcd. for $C_{28}H_{32}ClN_3$(446.04): C, 75.40; H, 7.23; N, 9.42. Found: C, 75.43; H, 7.16; N, 9.33.

*4.1.3.17. 9-(5-(4-(4-bromobenzyl)piperazin-1-yl)pentyl)-9H-carbazole **3q**:* Yield 93%; White solid; m.p. 128-129 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.16 (bs, 2H), 7.64-7.39 (m, 10H), 4.60-4.45 (m, 2H), 3.53 (bs, 2H), 2.95 (bs, 2H), 2.74 (bs, 2H), 2.63 (bs, 2H), 1.98-1.79 (m, 4H), 1.28-1.17 (m, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 140.0, 136.9, 135.0, 132.0, 131.2, 131.0, 126.7, 125.7, 124.2, 122.0, 120.3, 118.7, 109.3, 59.5, 56.9, 45.2, 42.0, 28.2, 23.2, 20.5. Anal. Calcd. for $C_{28}H_{32}BrN_3$(490.49): C, 68.57; H, 6.58; N, 8.57. Found: C, 68.33; H, 6.24; N, 8.21.

*4.1.3.18. 9-(5-(4-(4-fluorobenzyl)piperazin-1-yl)pentyl)-9H-carbazole **3r**:* Yield 86%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.13 (d, 2H, J = 7.6 Hz), 7.57 (d, 2H, J = 8.02 Hz), 7.42 (t, 2H, J = 7.0 Hz), 7.28 (t, 2H, J = 6.0 Hz), 7.18-7.09 (m, 4H), 4.36 (t, 2H, J = 7.0 Hz), 3.49 (bs, 2H), 2.30-2.11 (m, 10H), 1.75 (bs, 2H), 1.40 (bs, 2H), 1.25-1.16 (m, 4H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 162.2 and 160.2 (C-F, J = 240.85 Hz) , 139.9, 134.2, 130.6, 125.6, 122.0, 120.2, 118.6, 114.9, 114.7, 109.2, 61.0, 57.4, 52.5, 52.1, 42.1, 29.0, 25.7, 24.3. Anal. Calcd. for $C_{28}H_{32}FN_3$(429.58): C, 78.29; H, 7.51; N, 9.78. Found: C, 78.41; H, 7.67; N, 9.87.

*4.1.4. General procedure for the synthesis of compound **3s-w***

Compound **1** (1 mmol) was dissolved in dry acetonitrile (20 mL). The desired amine **2** (1.2 equiv.) was added to the mixture and refluxed for 6-8 h. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (petroleum ether/EtOAc 5:1) to afford compound **3s-w** [38].

*4.1.4.1. 9-(5-(quinolin-1(2H)-yl)pentyl)-9H-carbazole **3s**:* Yield 61%; Amber oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.18 (d, 1H, *J* = 5.5 Hz), 8.87 (d, 1H, *J* = 8.5 Hz), 8.15 (d, 1H, *J* = 8.5 Hz), 8.10 (d, 1H, *J* = 9.0 Hz), 7.99-7.99 (m, 5H), 7.37-7.15 (m, 3H), 7.15 (t, 2H, *J* = 7.0 Hz), 5.18 (t, 2H, *J* = 7.5 Hz), 4.25 (t, 2H, *J* = 7.0 Hz), 2.03 (bs, 2H), 1.96-1.87 (m, 4H), 1.52 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 149.9, 147.0, 140.1, 137.3, 135.8, 131.0, 129.8, 125.7, 122.5, 122.1, 120.1, 118.8, 118.0, 108.8, 57.4, 42.6, 29.8, 28.2, 24.0; MS m/z: 235.14 ($C_{17}H_{17}N^{\bullet}$), 180.08 ($C_{13}H_{10}N^{\bullet}$), 166.07 ($C_{12}H_8N^{\bullet}$), 129.07 ($C_9H_7N^{\bullet}$); Anal. Calcd. for $C_{25}H_{26}N_2$(366.50): C, 85.21; H, 7.15; N, 7.64. Found: C, 85.45; H, 7.53; N, 7.71.

*4.1.4.2. 9-(5-(isoquinolin-2(3H)-yl)pentyl)-9H-carbazole **3t**:* Yield 60%; Amber oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.68 (s, 1H), 8.49 (d, 2H, *J* = 9.0 Hz), 8.02-7.88 (m, 5H), 7.74 (t, 1H, *J* = 7.0 Hz), 7.34-31 (m, 3H), 7.09 (d, 2H, *J* = 7.5 Hz), 4.79 (t, 2H, *J* = 7.0 Hz), 4.21(t, 2H, *J* = 7.0 Hz), 2.45 (bs, 2H), 2.01-1.98 (m, 2H), 1.82-1.79 (m, 2H), 1.41-1.40 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 149.6, 140.0, 136.9, 136.6, 134.2, 130.8, 127.4, 126.7, 125.8, 125.6, 122.4, 120.0, 118.7, 108.7, 60.7, 42.4, 31.0, 27.9, 23.5. Anal. Calcd. for $C_{26}H_{26}N_2$(366.51): C, 85.21; H, 7.15; N, 7.64. Found: C, 85.33; H, 7.16; N, 7.81.

*4.1.4.3. 9-(5-(pyridin-1(2H)-yl)pentyl)-9H-carbazole **3u**:* Yield 55%; Amber oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.11 (d, 2H, *J* = 6.0 Hz), 8.18 (t, 1H, *J* = 8.0 Hz), 8.00 (d, 2H, *J* = 7.5 Hz), 7.75 (t, 2H), 7.41-7.28 (m, 4H), 7.17 (t, 1H, *J* = 7.5 Hz), 4.65 (t, 2H, *J* = 7.5 Hz), 4.24 (t, 1H, *J* = 7.0 Hz), 2.40 (bs, 2H), 1.90-1.81 (m, 4H), 1.34-1.28 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 144.7, 144.6, 140.1, 128.2, 125.8, 122.5, 120.2, 118.9, 108.8, 61.1, 42.4, 31.0, 27.8, 23.3. Anal. Calcd. for $C_{22}H_{24}N_2$(316.45): C, 83.50; H, 7.64; N, 8.85. Found: C, 85.67; H, 7.82; N, 9.22.

4.1.4.4. 9-(5-(piperidin-1-yl)pentyl)-9H-carbazole ***3v****:* Yield 52%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (d, 2H, *J* = 8.0 Hz), 7.63 (d, 2H, *J* = 8.5 Hz), 7.45 (t, 2H, *J* = 7.5 Hz), 7.19 (t, 2H, *J* = 7.0 Hz), 4.41 (t, 2H, *J* = 6.5 Hz), 2.98-2.75 (m, 6H), 180-1.55 (m, 8H), 1.31 (bs, 4H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 139.8, 125.5, 121.9, 120.0, 118.5, 109.1, 55.3, 51.6, 43.3, 41.8, 27.8, 22.5, 22.0, 21.9, 21.5, 21.2. Anal. Calcd. for $C_{22}H_{28}N_2$(320.48): C, 82.45; H, 8.81; N, 8.74. Found: C, 82.21; H, 8.91; N, 8.66.

4.1.4.5. 9-(5-(4-benzylpiperidin-1-yl)pentyl)-9H-carbazole ***3w****:* Yield 61%; Amber oil; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (d, 2H, *J* = 7.5 Hz), 7.57 (d, 2H, *J* = 8.0 Hz), 7.44 (t, 2H, , *J* = 7.0 Hz), 7.28-7.25 (m, 2H), 7.20-7.12 (m, 5H), 4.37 (t, 2H, *J* = 7.0 Hz), 2.96-2.93 (m, 2H), 2.50-2.40 (m, 4H), 2.14-2.10 (m, 2H), 1.91 (s, 1H), 1.79-1.76 (m, 2H), 1.55-148 (m, 4H), 1.29-1.22 (m, 4H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 139.9, 128.9, 128.0, 125.7, 125.5, 122.0, 120.1, 118.5, 109.1, 56.8, 52.4, 42.0, 41.8, 36.2, 30.2, 28.8, 28.1, 24.8, 24.1, 21.1. Anal. Calcd. for $C_{29}H_{34}N_2$(410.60): C, 84.83; H, 8.35; N, 6.82. Found: C, 85.11; H, 8.13; N, 6.54.

4.1.5. In vitro AChE/BuChE inhibition assay

The AChE and BuChE inhibitory activities were determined by Ellman's method [34]. AChE (*electric eel*), BuChE (*equine serum*), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), acetyl- and butyrylthiocholine iodides were purchased from Sigma-Aldrich. Five different concentrations of the corresponding compounds in Ethanol-DMSO (9:1) were prepared. For assay, 20 µL of the corresponding enzyme (0.2 units/mL in Phosphate-pH 8.0 containing 25% v/v glycerol) was added in a 24-well plate containing 2000 µL of PBS, 30 µL of the tested compound solution and 60 µL of DTNB. After 3 min of incubation, 20 µL of the substrate solution (ATC/BTC) was added and then incubated for at least 1 min at 25 °C. The absorbance was measured at 412 µM using a microplate reader (BioTek synergy HT). The inhibition curve was obtained by plotting percentage of enzyme activity (100% for the reference) versus logarithm of the tested compound concentration. Results are reported as the mean ± SD for at least three different experiments.

For the kinetic analysis, the rate of ChE inhibition by different concentrations of the potent inhibitor **3s** was measured in the presence of substrate (ATCh and/or BTCh). The cholinesterase inhibition was determined as describedin the experimental section. For each inhibitor concentration, the initial velocity was measured at different substrate concentrations (S) and the reciprocal of the initial velocity (1/v) was plotted respect to the reciprocal of substrate concentration (1/[S]). Also the Lineweavere-

Burk secondary plot for the inhibition of AChE/BuChE through re-plotting of slope versus at different concentrations of the potent inhibitor **3s** was measured in the presence of substrate (ATCh and/or BTCh) for calculation of steady-state inhibition constant (Ki).

4.1.6. Determination of the inhibitory potency on $A\beta_{1-42}$ self-induced and AChE-induced aggregation

Inhibitory activity of our compounds on self-induced and AChE induced aggregation of $A\beta_{1-42}$ were measured using a thioflavin T (ThT)-based fluorescence assay [36]. $A\beta_{1-42}$ (Sigma A9810) was dissolved in Phosphate Buffer Saline (PBS, pH 7.4, HyClone Thermo Scientific) containing 1% ammonium hydroxide. This solution (50 μM) was incubated for 72 h at 37 ^{o}C for pre-fibrillation. For the assay, $A\beta_{1-42}$ (10 μL) ± human recombinant AChE (0.01 u/mL, Sigma C1682) was added to 0.05 M KP buffer (pH 7.4) and incubated at 37 ^{o}C for 48 h in the absence and presence of 100 μM solution of the target compound. 100 μL of incubated mixture was mixed with 50 μL of thioflavin T (ThT, 200 μM) in 50 mM glycine-NaOH buffer (pH 8.5). The ThT excitation and emission were measured at 448 μM and 490 μM, respectively (SpectraMax® Microplate Reader). Self or AChE-induced aggregation percentages due to the presence of target compounds were calculated by the following equation:

$[(IF_i/IF_o) \times 100]$ where IF_i and IF_o are the fluorescence intensities for Aβ ± AChE in the presence and in the absence of inhibitors, respectively. Rifampicin (100 μM, Sigma R-3501) and donepezil (100 μM, Sigma D-6821) were used as the reference agents.

4.1.7. Neuroprotection assay against H_2O_2-induced cell death in PC12 cells

Cell viability of rat differentiated PC12 cells (provided by the Pasteur Institute, Tehran, Iran) was examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [40]. All cultured media and supplements were purchased from Gibco. Cells were cultivated in DMEM supplemented with 10% fetal calf serum, 5% horse serum and antibiotics (100 units/mL penicillin, 100 μg/mL streptomycin). To induce neuronal differentiation, PC12 cells were re-suspended using trypsin/EDTA (0.25%) and seeded in 96 well culture plate (4000 cells/well) and cultured for 1 week in differentiation medium (DMEM + 2% horse serum + NGF (100 ng/mL) + penicillin & streptomycin). To evaluate the effect of the compounds on survival rate of neurons, the cultured medium was changed to NGF free medium and different concentrations of compound **3s** (1, 10, 100 μM) were applied on the cells. Quercetin (10 μM) was used as a positive control. Compound **3s** was diluted in DMEM and added to each well in the volume of

10 μL. Induction of ROS mediated apoptosis was initiated by adding H_2O_2 (375 μM) to their medium after three hours and MTT assay was performed after 12 hours. MTT solution (5 mg/mL) was added to each well in a volume of 10 μL, and after 3 h 100 μL of the solution (10% SDS in 0.01 M HCl (v/v)) was added into each well. The plates were allowed to stand overnight in the incubator in a humidified atmosphere. Using an ELISA reader, absorbance at 570 μM was recorded for each well. Each experiment was performed in triplicate.

4.1.8. Docking simulation

Ligand-protein docking was performed using Autodock Vina (1.1.2) [41]. The binding poses of the ligand to the active site of AChE was retrieved from the earlier mentioned application. 3D coordination of the AChE was obtained from Protein Data Bank (PDB). The crystal structure of AChE (PDB ID: 1eve) in complex with donepezil was chosen. All of non-protein atoms were omitted during the protein. (RMSD = 0.3 Å) the protein was minimized. A grid box with the size of 25×25×25 was determined and the center of box was fixed on the co-crystal ligand. After docking the best pose was selected for further analysis.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

Acknowledgments

This work was supported by a grant from the Research Council of Tehran University of Medical Sciences and National Institute for Medical Research Development (NIMAD, grant number: 971370).

References

[1] M. Racchi, M. Mazzucchelli, E. Porrello, C. Lanni, S. Govoni, Acetylcholinesterase inhibitors: novel activities of old molecules, Pharma. Res. 50 (2004) 441-451.

[2] A. Cavalli, M.L. Bolognesi, A. Minarini, M. Rosini, V. Tumiatti, M. Recanatini, C.J. Melchiorre, Multi-target-directed ligands to combat neurodegenerative diseases, J. Med. Chem. 51 (2008) 347-372.

[3] H.W. Querfurth, F.M. LaFerla, Alzheimer's disease, N. Engl. J. Med. 362 (2010) 329-344.

[4] R. Jakob-Roetne, H. Jacobsen, Alzheimer's disease: from pathology to therapeutic approaches, Angew. Chem. Int. Ed. 48 (2009) 3030-3059.

[5] M.B. Youdim, T. Amit, M. Falach-Yogev, O. Bar Am, W. Maruyama, M. Naoi, The essentiality of Bcl-2, PKC and proteasome-ubiquitin complex activations in the neuroprotective-antiapoptotic action of the anti-Parkinson drug, rasagiline, Biochem. Pharmacol. 66 (2003) 1635-1641.

[6] A. Castro, S. Conde, Rodriguez-Franco, M.I. Martinez, A. Non-cholinergic pharmacotherapy approaches to the future treatment of Alzheimer's disease, Mini Rev. Med. Chem. 2 (2002) 37-50.

[7] P. Sharma, P. Srivastava, A. Seth, P.N. Tripathi, A.G. Banerjee, S.K. Shrivastava, Comprehensive review of mechanisms of pathogenesis involved in Alzheimer's disease and potential therapeutic strategies, Prog. Neurobiol. 174 (2019) 53–89.

[8]P.N. Tripathi, P. Srivastava, P. Sharma, M.K. Tripathi, A. Seth, A. Tripathi, S.N. Rai, S.P.Singh, S .K. Shrivastava, Biphenyl-3-oxo-1, 2, 4-triazine linked piperazine derivatives as potential cholinesterase inhibitors with anti-oxidant property to improve the learning and memory Bioorg. Chem. 85 (2019) 82-96.

[9] P. N.Tripathi, P. Srivastava, P. Sharma, A. Seth, S. K. Shrivastava, Design and development of novel *N*-(pyrimidin-2-yl)-1,3,4-oxadiazole hybrids to treat cognitive dysfunctions, Bioorg. Med. Chem. 27 (2019) 1327-1340.

[10]P. Srivastava, P.N. Tripathi, P. Sharma, S.N. Rai, S.P. Singh, R.K. Srivastava, S. Shankar, S.K. S hrivastava, Design and development of some phenyl benzoxazole derivatives as a potent acetylcholinesterase inhibitor with antioxidant property to enhance learning and memory, Eur. J. Med. Chem. 163 (2018) 116-135.

[11] A.V. Terry, J.J. Buccafusco, The Cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development, J. Pharmacol. Exp. Ther. 306 (2003) 821-827.

[12] F. Massoud, S. Gauthier, Update on the pharmacological treatment of Alzheimer's disease, Curr. Neuropharmacol. 8 (2010) 69-80.

[13] B.J. Sahakian, A.M. Owen, N.J. Morant, S.A. Eagger, S. Boddington, L. Crayton, H.A. Crockford, M. Crooks, K. Hill, R. Levy, Further analysis of the cognitive effects of

tetrahydroaminoacridine (THA) in Alzheimer's disease: assessment of attentional and mnemonic function using CANTAB, Psychopharmacology 110 (1993) 395-401.

[14] P.B. Watkins, H.J. Zimmerman, M.J. Knapp, S.I. Gracon, K.W. Lewis, Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease, JAMA, 271 (1994) 992-998.

[15] B. Sameem, M. Saeedi, M. Mahdavi, A. Shafiee, A review on tacrine based scaffolds as multi target drugs (MTDLs) for Alzheimer's disease, Eur. J. Med. Chem. 128 (2017) 332-345.

[16] L. Pourabdi, M. Khoobi, H. Nadri, A. Moradi, F.H. Moghadam, S. Emami, M.M. Mojtahedi, I. Haririan, H. Forootanfar, A. Ameri, A. Foroumadi, A. Shafiee, Synthesis and structure-activity relationship study of tacrine-based pyrano[2,3-c]pyrazoles targeting AChE/BuChE and 15-LOX, Eur. J. Med. Chem. 123 (2016) 298-308.

[17] J. L. Sussman, M. Harel, F. Frolow, C. Oefner, A. Goldman, L. Toker, I. Silman, Atomic structure of acetylcholinesterase from Torpedo californica: a prototypic acetylcholine-binding protein, Science 253 (1991) 872-879.

[18] M. Harel, L.K. Sonoda, I. Silman, J.L. Sussman, T.L. Rosenberry, Crystal structure of thioflavin T bound to the peripheral site of torpedo californica acetylcholinesterase reveals how thioflavin T acts as a sensitive fluorescent reporter of ligand binding to the acylation site, J. Am. Chem. Soc. 130 (2008) 7856-7861.

[19] M. Harel, D.M. Quinn, H.K. Nair, I. Silman, J.L. Sussman, The X-ray structure of a transition State analog complex reveals the molecular origins of the catalytic power and substrate specificity of acetylcholinesterase, J. Am. Chem. Soc. 118 (1996) 2340-2346.

[20] N.C. Inestrosa, A. Alvarez, C.A. Perez, R.D. Moreno, M. Vicente, C. Linker, O.I. Casanueva, C. Soto, J. Garrido, Acetylcholinesterase accelerates assembly of amyloid-beta-peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme, Neuron 16 (1996) 881-891.

[21] E. Giacobini, Cholinesterases: new roles in brain function and in Alzheimer's disease, Neurochem. Res. 28 (2003) 515-522.

[22] A. Castro, A. Martı’nez, Peripheral and dual binding site acetylcholinesterase inhibitors: implications in treatment of Alzheimer's disease, Mini-Rev. Med. Chem. 3 (2001) 267-272.

[23] H. Akrami, B. F. Mirjalili, M. Khoobi, A. Moradi, H. Nadri, S. Emami, A. Foroumadi, M. Vosooghi, A. Shafiee, 9H-Carbazole derivatives containing the N-benzyl-1,2,3-triazole moiety as new acetylcholinesterase inhibitors, Arc. Pharm. Chem. Life Sci. 348 (2015) 366-374.

[24] L. Fang, M. Chen, Z. Liu, X. Fang, S. Gou, L. Chen, Ferulic acid–carbazole hybrid compounds: combination of cholinesterase inhibition, antioxidant and neuroprotection as multifunctional anti-Alzheimer agents, Bioorg. Med. Chem. 24 (2016) 886-93.

[25] X. Wu, J. Kosaraju, W. Zhou, K.Y. Tam, Neuroprotective effect of SLM, a novel carbazole-based fluorophore, on SH-SY5Y cell model and 3xTg-AD mouse model of Alzheimer's disease, ACS Chem. Neurosci. 8 (2017) 676-685.

[26] S. Thiratmatrakul, C. Yenjai, P. Waiwut, O. Vajragupta, P. Reubroycharoen, M. Tohda, C. Boonyarat, Synthesis, biological evaluation and molecular modeling study of novel for the treatment of Alzheimer's disease, Eur. J. Med. Chem. 75 (2014) 21-30.

[27] W. Yang, Y. Wong, O.T. Ng, L. Bai, D.W. Kwong, Y. Ke, Z. Jiang, H. Li, K.K. Yung, M.S. Wong, Inhibition of betaamyloid peptide aggregation by multifunctional carbazole based fluorophores, Angew. Chem. Int. Ed. Engl. 51 (2012) 1804-1810.

[28] Y. Rook, K.U. Schmidtke, F. Gaube, D. Schepmann, B. Wunsch, J. Heilmann, J. Lehmann, T. Winckler, Bivalent β-carbolines as potential multitarget anti-alzheimer agents, J. Med. Chem. 53 (2010) 3611-3617.

[29] L. Jalili-Baleh, H. Nadri, H. Forootanfar, A. Samzadeh-Kermani, Novel 3-phenyl coumarin-lipoic acid conjugates as multi-functional agents for potential treatment of AD, Bioorg. Chem. 79 (2018) 223-234.

[30] L. Jalili-Baleh, H. Forootanfar, T.T. Küçükkılınç, H. Nadri, Z. Abdolahi, A. Ameri, M. Jafari, B. Ayazgok, M. Baeeri, M. Rahimifard, S.N. Abbas Bukhari, M. Abdollahi, M.R. Ganjaali, S. Emami, M. Khoobi, A. Foroumadi, Design, synthesis and evaluation of novel multi-target-directed ligands for treatment of Alzheimer's disease based on coumarin and lipoic acid scaffolds, Eur. J. Med. Chem. 152 (2018) 600-614.

[31] L. Jalili-Baleh, E. Babaei, Sh. Abdpour, S.N.A. Bukhari, A. Foroumadi, A. Ramazani, M. Sharifzadeh, M. Abdollahi, M. Khoobi, A review on flavonoid-based scaffolds as multi-target-directed ligands (MTDLs) for Alzheimer's disease, Eur. J. Med. Chem. 152 (2018) 570-589.

[32] S.K. Shrivastava, S.K. Sinha, P. Srivastava, P.N. Tripathi, P. Sharma, M.K. Tripathi, A. Tripathi, P.K. Choubey, D.K. Waiker, L.M. Aggarwal, Design and development of novel p-aminobenzoic acid derivatives as potential cholinesterase inhibitors for the treatment of Alzheimer's disease, Bioorg. Chem. 82 (2019) 211-223.

[33] P. Sharma, A. Tripathi, P.N. Tripathi, S.K. Prajapati, A. Seth, M.K. Tripathi, P. Srivastava, V. Tiwari, S. Krishnamurthy, S.K. Shrivastava, Design and development of multitarget-directed N-

benzylpiperidine analogs as potential candidates for the treatment of Alzheimer's disease. Eur. J. Med. Chem. 167 (2019) 510–524.

[34] G.L. Ellman, K.D. Courtney, V. Andres, R.M. Featherstone, A new and rapid colorimetric determination of acetyl cholinesterase activity, Biochem. Pharmacol. 7 (1961) 88-95.

[35] P. Camps, X. Formosa, C. Galdeano, T. Gómez, D. Muñoz-Torrero, M. Scarpellini, E. Viayna, A. Badia, M.V. Clos, A. Camins, M. Pallàs, M. Bartolini, F. Mancini, V. Andrisano, J. Estelrich, M. Lizondo, A. Bidon-Chanal, F.J. Luque, Novel donepezil-based inhibitors of acetyl- and butyrylcholinesterase and acetylcholinesterase-induced β-amyloid aggregation, J. Med. Chem. 51 (2008) 3588–3598.

[36] H. Levine, Thioflavine T interaction with synthetic Alzheimer's disease β-amyloid peptides: Detection of amyloid aggregation in solution, Protein Sci. 20 (1993) 404-410.

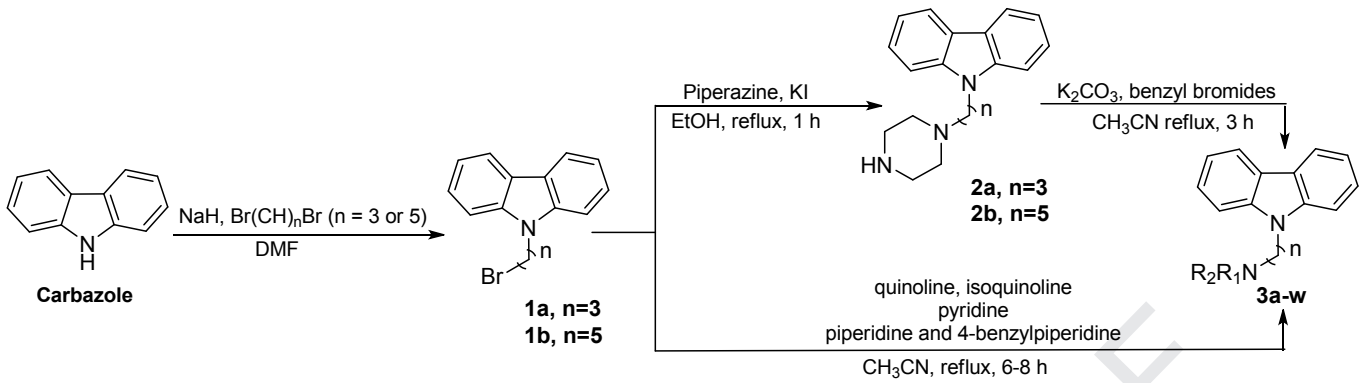
[37] D. Hesek, M. Lee, B.C. Noll, J.F. Fisher, S. Mobashery, Complications from Dual Roles of Sodium Hydride as a Base and as a Reducing Agent, J. Org. Chem. 74 (2009) 2567–2570.

[38] S.S. Xie, X.B. Wang, J.Y. Li, *et al*. Design, synthesis and evaluation of novel tacrine–coumarin hybrids as multifunctional cholinesterase inhibitors against Alzheimer's disease, Eur. J. Med. Chem. 64 (2013) 540–53.

[39] K. Youngjae, Y. Miyoung; L. Soyeon, T. Jinsung, K. Hak Joong, R. Hyewhon, S. Jihye, C. Kyung Il, M. Sun-Joon, C. Hyunah, Synthesis of N-Alkyl-Carbazole Derivatives as 5-HT7R Antagonists, Bull. Korean Chem. Soc. 39 (2018) 1083-1089.

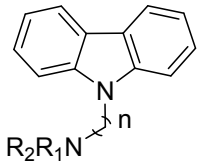
[40] Z. Datki, A. Juhász, M. Gálfi, K. Soós, R. Papp, D. Zádori, B. Penke, Method for measuring neurotoxicity of aggregating polypeptides with the MTT assay on differentiated neuroblastoma cells, Brain Res. Bull. 62 (2003) 223-229.

[41] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K.; Belew, D.S. Goodsell, A.J. Olson, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, J. Comput. Chem. 30 (2009) 2785-2791.



Scheme 1. Synthesis of target compounds **3a-w**.

Table 1. Inhibitory activity of the target compounds **3a-w** against AChE and BuChE.



3a-w

Compound	N[c]	NR_1R_2	AChE IC_{50} (μM)[a]	BuChE IC_{50} (μM)[b]	SI[d]
3a	3	benzylpiperazine	15.9±0.12	32.1±0.11	2.01
3b	3	2-F-benzylpiperazine	6.8±0.12	17.1±0.13	2.51
3c	3	4-F-benzylpiperazine	36.5±0.11	98.6±0.11	2.70
3d	3	4-Br-benzylpiperazine	11.8±0.15	27.2±0.15	2.30
3e	3	4-OMe-benzylpiperazine	23.3±0.13	58.3±0.17	2.50
3f	5	benzylpiperazine	10.1±0.12	31.2±0.12	3.08
3g	5	2-Cl-benzylpiperazine	5.8±0.11	24.5±0.11	4.22
3h	5	2-F-benzylpiperazine	11.5±1.02	36.4±0.15	3.16
3i	5	2-Me-benzylpiperazine	5.9±0.16	22.5±0.15	3.81
3j	5	2-NO_2-benzylpiperazine	9.8±1.01	38.3±0.14	3.91
3k	5	3-Br-benzylpiperazine	1.4±0.19	5.1±1.02	3.64
3l	5	3-OMe-benzylpiperazine	19.0±0.13	67.9±0.16	3.57
3m	5	3,4-dichlorobenzylpiperazine	22.0±1.00	80.6±0.13	3.66
3n	5	4-OMe-benzylpiperazine	9.4±0.15	30.2±0.11	3.21
3o	5	4-Me-benzylpiperazine	10.7±0.12	46.3±0.13	4.32
3p	5	4-Cl-benzylpiperazine	10.9±0.11	32.1±0.12	2.94
3q	5	4-Br-benzylpiperazine	1.7±0.14	6.8±1.02	4.00
3r	5	4-F-benzylpiperazine	4.4±0.13	11.8±0.15	2.68
3s	5	quinoline	0.11±0.18	0.02±0.11	0.18
3t	5	isoquinoline	0.44±0.12	0.08±0.15	0.18
3u	5	pyridine	6.4±0.11	1.4±0.15	0.22
3v	5	piperidine	11.9±0.12	46.2±0.18	3.88
3w	5	4-benzylpiperidine	24.2±0.19	73.2±0.15	3.02
Donepezil		-	0.016±0.12	4.5±0.11	281.25

[a] Inhibitor concentration (mean ± SEM of three experiments) required for 50% inactivation of AChE (electric eel).
[b] Inhibitor concentration (mean ± SEM of three experiments) required for 50% inactivation of BuChE (equine serum).
[c] Length of linker (n = 3 or 5).
[d] Selectivity index = IC_{50} (BChE)/IC_{50} (AChE).

Table 2. Inhibition of AChE-induced and self-induced Aβ aggregation by the compounds **3k** and **3s.**

Compound	Inhibition of Aβ aggregation (%)	
	Self-induced[a]	**AChE-induced[b]**
3k	19.5±1.3	19.2±3.4
3s	27.4±1.8	24.9±6.4
Rifampicin	27.5±4.3	12.2±3.0
Donepezil	22.0±5.4	26.1±2.5

[a]Inhibition of self-induced $A\beta_{1-42}$ aggregation (10 μM) produced by the tested compound at 100 μM concentration. Values are expressed as means ± SEM of three experiments.

[b] Co-aggregation inhibition of $A\beta_{1-42}$ and AChE (0.01 u/mL) by the tested compound at 100 μM concentration was detected by ThT assay. Values are expressed as means ± SEM of three experiments.

[c] Not Active

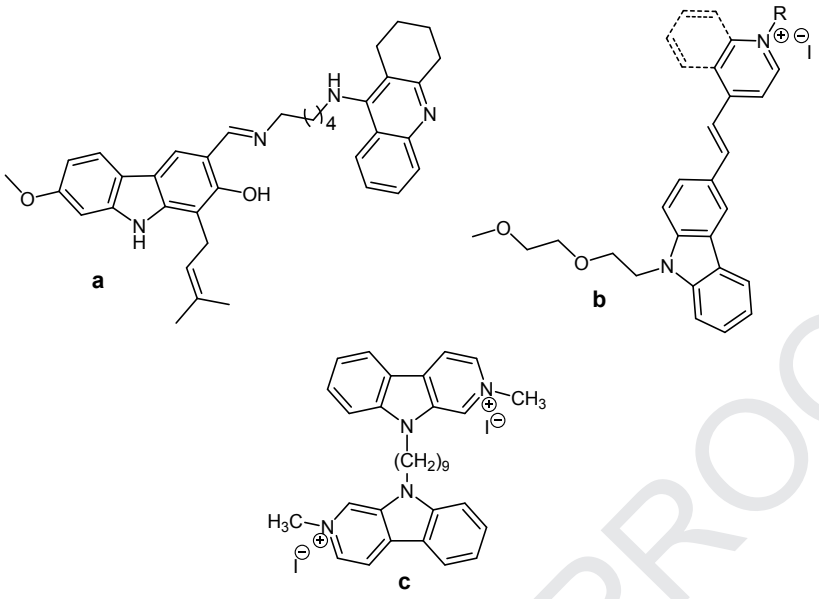
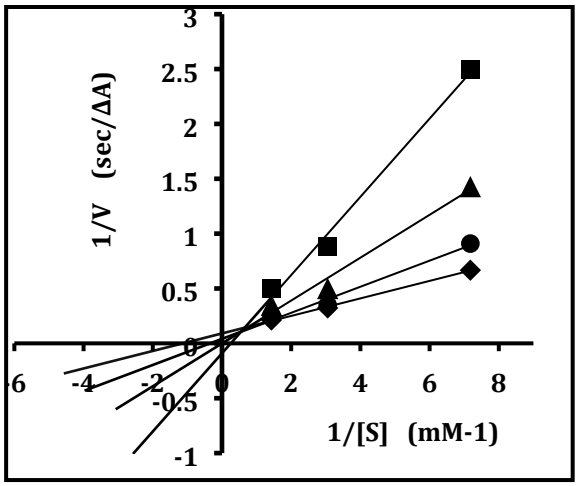
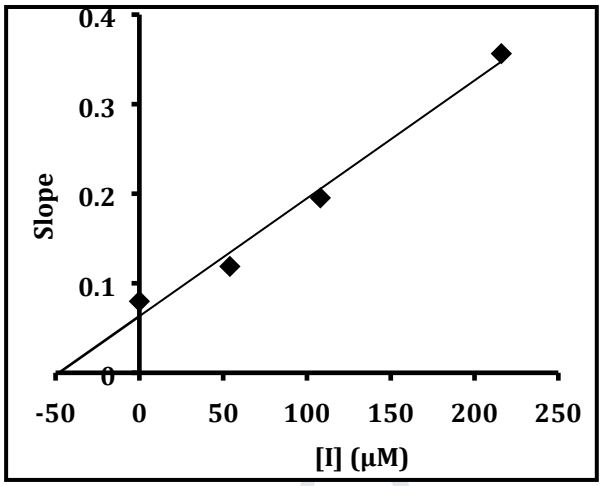


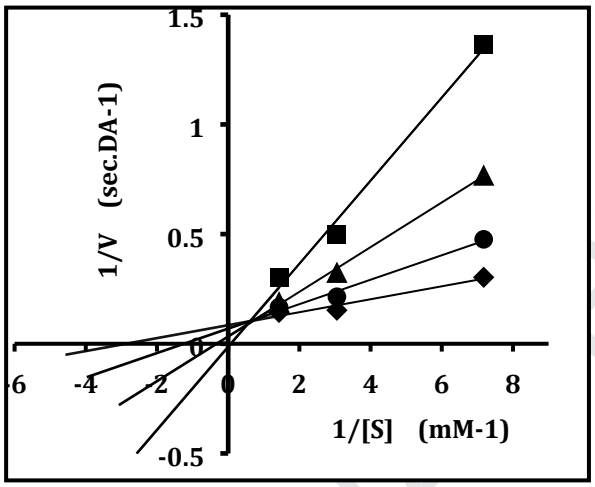
Figure 1. Biologically active carbazoles.



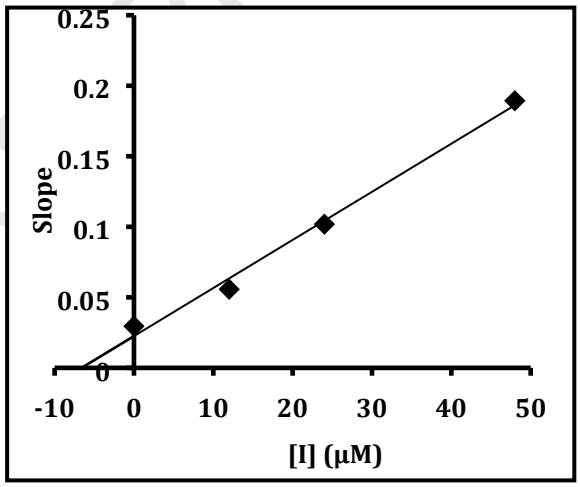
2-1: Lineweaver-Burk plot for the inhibition of AChE by compound **3s**. ♦: control, ■ : 216 µM, ▲ 108 µM, • 54 µM.



2-2: Lineweaver-Burk secondary plot of compound **3s** for AChE Ki calculation.



2-3: Lineweaver-Burk plot for the inhibition of BuChE by compound **3s**. ♦ : control, ■: 48 µM, ▲: 24 µM, •: 12 µM.



2-4: Lineweaver-Burk secondary plot of compound **3s** for BuChE Ki calculation.

Figure 2. Lineweaver-Burk plot for the inhibition of AChE and BuChE with compound **3s**.

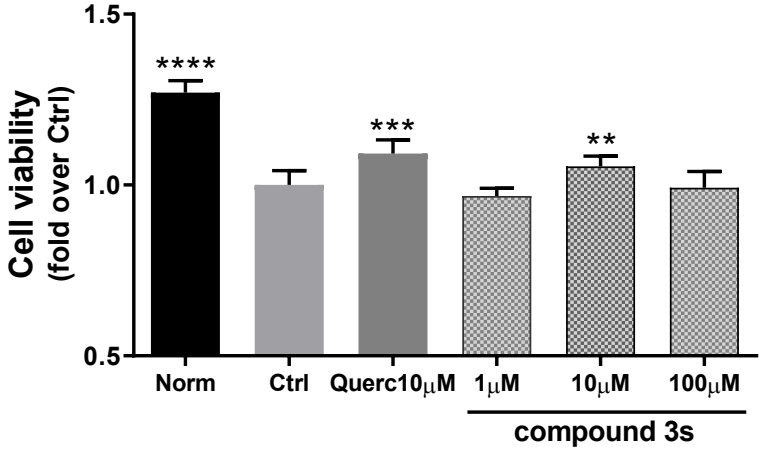


Figure 3. The protective effect of selected compound **3s** against H_2O_2 induced apoptosis in PC12 cells at different concentrations. ****: $P<0.0001$, ***: $P<0.001$, **: $P<0.0098$ all versus control (Ctrl) group. Quercetin (Querc) 10 μM was used as a positive control group.

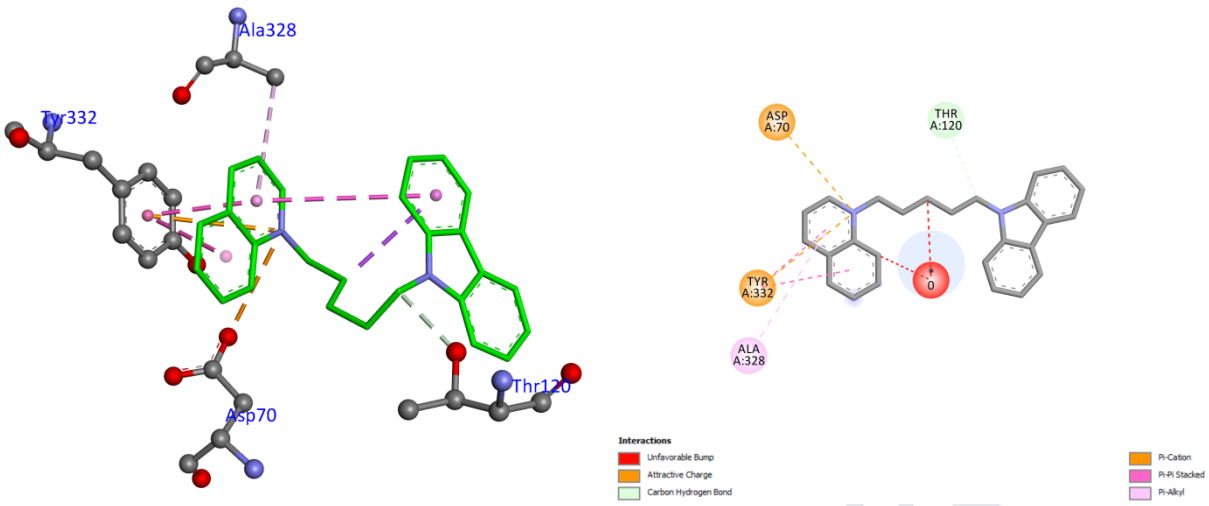
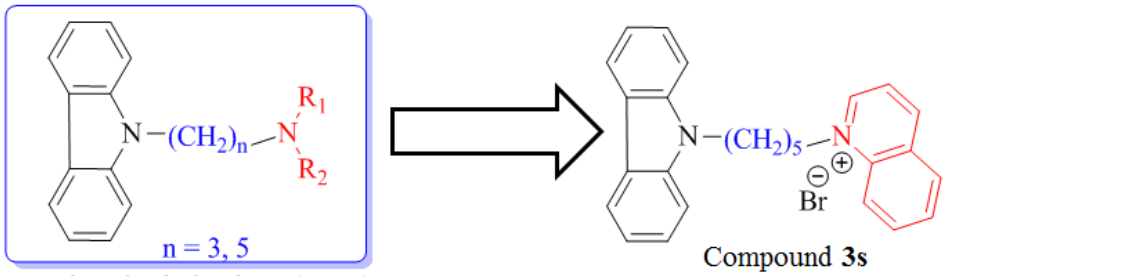


Figure 4. Binding modes of compound **3s** with AChE.

Highlights

- Novel carbazoles were introduced as multifunctional anti-Alzheimer's disease
- Compound **3s** having quinolinium part showed high AChE and BuChE inhibition activity
- Compound **3s** showed significant anti-Aβ aggregation activity
- Compound **3s** exhibited high neuroprotective activity

Graphical Abstract



23 carbazole derivatives (**3a**-**w**)

Compound **3s**

IC_{50} (AChE)= 0.11 µM

IC_{50} (BuChE)= 0.02 µM

Self-induced Inhibition of Aβ aggregation= 27.4%

AChE-induced Inhibition of Aβ aggregation= 24.9%

Protective against H_2O_2 induced apoptosis in PC12 cells

(Cell violability= 1.054-fold over Ctrl at concentration of 10 µM)