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Design and synthesis of new piperidone grafted acetylcholinesterase inhibitors

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder affecting 35 million people worldwide. A common strategy to improve the well-being of AD patients consists on the inhibition of acetylcholinesterase with the concomitant increase of the neurotransmitter acetylcholine at cholinergic synapses. Two series of unreported *N*-benzylpiperidines **5(a–h)** and thiazolopyrimidines **9(a–q)** molecules were synthesized and evaluated *in vitro* for their acetylcholinesterase (AChE) inhibitory activities. Among the newly synthesized compounds, **5h**, **9h**, **9j**, and **9p** displayed higher AChE enzyme inhibitory activities than the standard drug, galantamine, with IC₅₀ values of 0.83, 0.98, and 0.73 μ M, respectively. Cytotoxicity studies of **5h**, **9h**, **9j**, **9n** and **9p** on human neuroblastoma cells SH-SY5Y, showed no toxicity up to 40 μ M concentration. Molecular docking simulations of the active compounds **5h** and **9p** disclosed the crucial role of π - π -stacking in their binding interaction to the active site AChE enzyme. The presented compounds have potential as AChE inhibitors and potential AD drugs.

Keywords

N-Benzylpiperidines; Thiazolopyrimidinium; AChE activity; Molecular modeling; π - π -Stacking interactions; SH-SY5Y cell; Cytotoxicity

Alzheimer's disease (AD) is a prevalent, irreversible neurodegenerative disorder that affects more than 35 million people worldwide. It is estimated that AD will be the cause of more than 700,000 deaths in 2016, making it one of the leading causes of death in the United States.¹ Despite the tremendous efforts in search for novel and potent therapeutic agents, the pipeline of available treatments is low.^{2,3} The etiology of AD is not completely understood.

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However, the appearance of extracellular β -amyloid plaques and the imbalance of acetylcholine (ACh) neurotransmitters (due to the severe loss of cholinergic neurons) are two major hallmarks of the disease.^{4,5} The cholinergic system is severely affected in AD patients, leading to a reduced concentration of the neurotransmitter acetylcholine (ACh) at the synaptic clefts.⁶ Acetylcholinesterase (AChE), the enzyme responsible for degradation and regulation of acetylcholine in the human body, has been actively investigated as a target for AD treatments.⁷ Previous reports suggest that increasing acetylcholine levels by inhibiting AChE activity partially restores the substantial impairment of memory and cognitive dysfunctions.⁸ In fact, the main AD treatments are cholinesterase inhibitors, such as Donepezil, that work by increasing acetylcholine concentration at synaptic clefts.^{9,10} Therefore, compounds that can inhibit the action of AChE could rival Donepezil as promising AD treatments.

The active site of AChE is located at the bottom of a 20 Å long, narrow gorge comprised of five regions: the catalytic triad (composed of Glu327, His440 and Ser200), the oxyanion hole (Gly121, Gly122 and Ala201), the choline binding site (Trp84 and Phe330), the acyl binding pocket (Phe288 and Phe290), and the peripheral anionic site (PAS) (Trp279, Phe330 and Tyr70).^{11–14} The PAS of AChE catalyzes a series of reactions on the A β fibrils that lead to the formation of β -sheets with high aggregating potential.^{15–17} AChE inhibitors that bind to the PAS of the enzyme decrease aggregation rates of amyloid peptides and facilitate their clearance when compared to inhibitors that only bind the catalytic triad of the enzyme.^{17–19} The AChE active site is highly hydrophobic²⁰ and it is hypothesized molecules with multiple aromatic cores can bind to it more effectively^{21,22} due to hydrophobic interactions.^{23,24} Studies also showed that compounds having α , β -unsaturated moiety or the thiazolopyrimidine core structure are capable of inhibiting AChE.^{23,25–27} Moreover, the *N*-benzylpiperidine moiety is a structure found in donepezil, a FDA-approved drug for treatment of mild to moderate AD.²⁸ The crystal structure of AChE in complex with donepezil, revealed a π - π stacking as well as hydrogen bonding interaction of *N*-benzylpiperidine to the active site of AChE.²⁹ Inspired by these precedents, and the characteristics of the enzyme's binding pocket, we used computer modeling to design potential inhibitors of the AChE enzyme. In this study, we report the synthesis, AChE inhibitory activity, and toxicity towards human neuroblastoma SH-SY5Y cells of these new *N*-benzylpiperidines and thiazolopyrimidines derivatives. Finally, the plausible binding interactions of the most active derivatives were investigated using molecular docking.

Molecular modeling techniques were employed for designing the potential cores of various inhibitors. The energy minimizations of the different molecules lead us to two potential cores; **5** and **9**. The in silico studies showed the critical role of (i) the benzylamine moiety in **5(a–h)** and (ii) sulfur atom in **9(a–q)** to form favorable π -stacking and hydrogen bonding interactions to the active site residues of AChE enzyme, respectively.

The desired molecules can be prepared from the same precursor molecule **3**. The common α,β -unsaturated ketone precursors (**3**) were prepared by the Claisen-Schmidt condensation of 4-piperidone hydrochloride (**1**) with aromatic aldehydes (**2**) in the presence of HCl in acetic acid (Scheme 1).³⁰ The reaction of the **3** with benzyl bromides (**4**) under basic conditions provided *N*-benzylpiperidines **5(a–h)**. Also, the unsaturated ketone **3** was reacted

with thiourea (**6**) in presence of sodium ethoxide²⁴ to yield **7**. These compounds were reacted with α -Bromoacetophenones **8** (**a–d**) in refluxing ethanol for 12 h to afford thiazolopyrimidinium salts **9(a–q)**.

The synthesis of the thiazolopyrimidinium molecules (**9**) can yield two possible isomers (see SI). Thus, we prepared **9q** and studied its spectroscopic properties to assign the correct stereochemistry (Fig. 1). In the ¹H NMR spectrum of **9q**,³¹ the one hydrogen singlet at 6.27 ppm is due to H-5 and assigned to the carbon peak at 63.2 ppm using HMQC. This proton showed HMBC correlations to C-6 at 42.8 ppm besides showing correlation with C-5a and C-3 at 106.6 and 140.0 ppm, respectively. From HMQC, the doublets at 3.38 and 4.08 ppm ($J=16.5$ Hz) are assigned to H-6a and H-6b whilst the other doublets at 4.27 and 4.42 ppm ($J=14.5$ Hz) can be attributed to H-8a and H-8b. The singlet at 7.43 ppm is due to H-12 and H-2, which showed HMBC correlation to C=N at 163.8 ppm. The aromatic protons for ring A showed up as two singlets at 6.01 and 6.40 ppm in proton NMR having HMQC correlation with corresponding carbons at 102.0 and 112.3 ppm in ¹³C NMR. On the same basis, the protons of ring B were elucidated as two singlets at 6.52 and 6.55 ppm in ¹H NMR, having correlation to carbons at 112.3 and 101.6 ppm through HMQC. The ¹H and ¹³C chemical shifts as well as selected ROESY and HMBC correlations of **9q** are depicted in Figs. 1 and 2a/b, respectively. The infrared spectroscopy (IR) for **9q** showed three medium peaks at 3469 cm⁻¹, 1654 cm⁻¹ and 755 cm⁻¹ specific for free NH, C=N and C–S moieties, respectively. This data helped us to conclude the spatial arrangement of the atoms in **9q** (and all the other analogues).

All the newly synthesized *N*-benzylpiperidines **5** and thiazolopyrimidinium bromide salts **9** were evaluated for their inhibitory activity against AChE (Table 1) using the Ellman's method,³² which uses acetylthiocholine and 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB). In presence of AChE enzyme, the reaction produces the yellow compound 5-thio-2-nitrobenzoate. AChE inhibitors decrease the rate of the reaction resulting in a less colored solution, the absorption difference is correlated to inhibitory activity (see SI). Compound **9p** showed the highest inhibitory activity among both series, with an IC₅₀ value of 0.73 μ M, nearly two fold better than the standard drug (IC₅₀ = 1.31 μ M). Other active compounds are: **5h**, **9h**, **9n**, and **9j** with IC₅₀ values of 0.83, 0.98, 1.01 and 1.09 μ M. Compounds bearing 2-naphthyl moiety (except for **5f** and **9d**), showed good IC₅₀ values suggesting the importance of π - π interactions. Based on the above results, it can be deduced that those compounds having extended aromatic systems on either R¹ or R³ positions, may display more van der Waals interactions on the active site of AChE enzyme. It was also noticed that compounds **5h** and **9p**, bearing bromine atoms, showed the highest inhibitory potencies among their series. This may suggest an electronic effect due to the halide. Studies are undergoing to decipher the role electronically diverse substituents have on AChE inhibitory activity.

The cytotoxicity of the most active inhibitors, namely **5h**, **9h**, **9j**, **9n** and **9p**, was evaluated on the neuronal cell line SH-SY5Y using the MTT assay, which measures the cell metabolic activity. The assay was performed after 24 h incubation of neuronal cells with concentrations ranging from 0.2 to 40 μ M. The molecules showed significant cell viability at this range of concentrations (Fig. 3).

The most active compounds from each series (**5h** and **9p**) were docked into the groove of the AChE enzyme. Compound **5h** (Fig. 4) mainly displayed interaction to the amino acids composing the PAS of the enzyme. As predicted, the main interactions occur between Tyr 70, Trp 279 with the 2-naphthyl moiety of **5h** (distances of 3.69 Å and 3.36 Å). The B ring of the molecules also interacts with Phe 330 and Tyr 334 (3.31 Å and 3.67 Å). Compound **9p** also sits on the PAS of the AChE enzyme (Fig. 5). The amide nitrogens of Phe 288 and Arg display a H-bond type interaction to the sulfur of the thiazoline ring with contact distance of 2.02 Å and 2.33 Å. Phe 331 displays a strong “T-shaped” stacking interaction to ring A at the left side of the molecule (3.31 Å). Trp 279 shows a “T-shaped” stacking with ring B of the molecule (3.49 Å). Finally, there is a potential cation- π interaction between the piperidine ring and Tyr 334 (3.58 Å).

In conclusion, two series of piperidone grafted heterocyclic compounds were prepared employing efficient synthetic methodologies in pure form and were tested for their inhibitory activity against AChE enzyme. Compound **5h** and **9p** displayed the highest inhibitory activities among their series with IC₅₀ values of 0.73 and 0.83 μ M, respectively. The most active compounds did not show cell toxicity at the tested concentrations. Molecular modeling disclosed binding interaction patterns to the active site of AChE suggesting the positive effect of aromatic core multiplicity.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2016.11.065>.

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31. General procedure for the synthesis of (3,5-dimethoxybenzylidene)-5-(3,5-dimethoxyphenyl)-3-(4-nitrophenyl)-6,7,8,9-tetrahydro-5H-pyrido[4,3-d]thiazolo[3,2-a]pyrimidine (**9q**): unsaturated ketones (**3**, 5 mmol) were allowed to react with thiourea (**6**, 6.5 mmol) by adding few drops NaOEt over water bath for 15 min. After completion of the reaction, as evident by TLC, the reaction mixture poured into the ice and the solid (**7**) was filtered and washed with water. Thereafter, mixture of dried (**7**, 1 mmol) and *o*-Bromoacetophenones (**8**, 1.2mmol) were refluxed ethanol (15 ml) for 12h. Thereafter, the precipitated salts (**9a-q**) were isolated from reaction media, in pure form, without utilizing any purification technique, as evident by TLC and ¹H NMR spectroscopy. (3,5-Dimethoxybenzylidene)-5-(3,5-dimethoxyphenyl)-3-(4-nitrophenyl)-6,7,8,9-tetrahydro-5H-pyrido[4,3-d]thiazolo[3,2-a]pyrimidine (**9q**): White solid; yield: 65%, C₃₂H₃₁N₄O₆S⁺, Calculated mass: 599.1959, Found mass [MH⁺]: 598.1884; ¹H NMR (500 MHz, MeOD): δ_H 3.38 (d, *J*= 16.5 Hz, 1H, H-6a), 3.63 (s, 6H, O-CH₃), 3.83 (s, 6H, O-CH₃), 4.08 (d, *J*= 16.5 Hz, 1H, H-6b), 4.27 (d, *J*= 14.5 Hz, 1H, H-8a), 4.42 (d, *J*= 14.5 Hz, 1H, H- 8b), 6.03 (s, 2H, H-aromatic), 6.27 (s, 1H, H-5), 6.42 (s, 1H, H-aromatic), 6.53 (2H, s, H-aromatic), 6.56 (s, 1H, H-aromatic), 7.44 (s, 2 H, H-2, H-12), 7.59 (d, *J*= 9 Hz, H-aromatic), 8.36 (d, *J*= 9Hz, H-aromatic). ¹³C NMR (125 MHz, MeOD): δ_C 40.27, 42.80, 55.98, 63.23, 101.6, 102.0, 106.6, 107.8, 108.4, 124.6, 131.7, 132.8, 135.0, 137.1, 140.0, 140.3, 150.3, 162.6, 163.1, 163.8.
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Fig. 1.
Selected ^1H and ^{13}C chemical shifts of **9q**.

Fig. 2.
Selected ROESY (a) and HMBC (b) correlations of **9q**.

Fig. 3.
Effect of compounds **5h**, **9h**, **9j**, **9n** and **9p** on apoptotic neuronal cell death in SH-SY5Y.
MTT assay results are plotted for different concentration of compounds treated for 24 h.

Fig. 4.
Binding interaction of **5h** with major active site residues of AchE enzyme.

Fig. 5.
Binding interaction of **9p** with major active site residues of AChE enzyme.

Scheme 1.

Reagent and conditions: (a) NaOH, EtOH, rt, 24 h; (b) EtOH, K₂CO₃, rt, 2 h; (c) heat, NaOEt, 15 min; (d) EtOH, reflux, 12 h.

Table 1

AChE inhibitory activities of **5(a-h)** and **9(a-q)**.

Entry	Compound	R ¹	R ²	R ³	AChE inhibition % at 5 µg/ml	AChE inhibition (IC ₅₀) (µM)	AChE inhibition (IC ₅₀) (µM)
1	5a	H	3-CH ₃	-	63.1	1.2±0.08	3.18
2	5b	-	2-Naphthyl	-	67.9	0.80±0.05	1.93
3	5c	4-CH ₃	3-CH ₃	-	59.8	1.31±0.10	3.22
4	5d	-	2-Naphthyl	-	72.6	0.71±0.07	1.59
5	5e	4-Cl	3-CH ₃	-	41.6	-	-
6	5f	-	2-Naphthyl	-	42.4	-	-
7	5g	4-Br	3-CH ₃	-	45.7	-	-
8	5h	-	2-Naphthyl	-	67.2	0.47±0.03	0.83
9	9a	H	-	H	48.6	-	-
10	9b	-	-	4-CH ₃	42.1	-	-
11	9c	-	-	4-Br	48.7	-	-
12	9d	-	-	2-Naphthyl	41.8	-	-
13	9e	4-CH ₃	-	H	45.7	-	-
14	9f	-	-	4-CH ₃	60.4	0.86±0.09	1.80
15	9g	-	-	4-Br	64.6	1.23±0.08	2.27
16	9h	-	-	2-Naphthyl	69.6	0.51±0.04	0.98
17	9i	4-Cl	-	H	41.6	-	-
18	9j	-	-	4-CH ₃	74.7	0.57±0.03	1.09
19	9k	-	-	4-Br	41.6	-	-
20	9l	-	-	2-Naphthyl	62.8	0.74±0.07	1.35

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Entry	Compound	R ¹	R ²	R ³	AChE inhibition % at 5 µg/ml	AChE inhibition (IC ₅₀)	
						(µg/ml)	(µM)
21	9m	4-Br	–	H	66.7	0.87 ± 0.09	1.48
22	9n	–	–	4-CH ₃	68.3	0.61 ± 0.04	1.01
23	9o	–	–	4-Br	48.3	–	–
24	9p	–	–	2-Naphthyl	65.7	0.47 ± 0.03	0.73
25	9q	3,5-OCH ₃	–	4-NO ₂	–	–	–
	Galantamine	–	–	–	–	0.48 ± 0.01	1.30