European Journal of Medicinal Chemistry 93 (2015) 196–201

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Benzofuran-derived benzylpyridinium bromides as potent acetylcholinesterase inhibitors

Farzaneh Baharloo [a], Mohammad Hossein Moslemin [a], Hamid Nadri [b], Ali Asadipour [c], Mohammad Mahdavi [d], Saeed Emami [e], Loghman Firoozpour [f], Razieh Mohebat [a], Abbas Shafiee [d], Alireza Foroumadi [c, d, *]

[a] Department of Chemistry, Faculty of Chemistry, Islamic Azad University, Yazd, Iran
[b] Department of Medicinal Chemistry, Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[c] Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medicinal Sciences, Kerman, Iran
[d] Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medicinal Sciences, Tehran, Iran
[e] Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran
[f] Drug Design & Development Research Center, Tehran University of Medicinal Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 29 December 2014
Received in revised form
4 February 2015
Accepted 6 February 2015
Available online 7 February 2015

Keywords:
Acetylcholinesterase
Alzheimer's disease
Benzofuran
Docking study
Pyridinium

ABSTRACT

A series of benzofuran-based *N*-benzylpyridinium derivatives **5a**–**o** were designed and synthesized as novel AChE inhibitors. The synthetic pathway of the compounds involved the preparation of 4-(benzofuran-2-yl)pyridine intermediates via the reaction of different salicylaldehyde derivatives and 4-(bromomethyl)pyridine, followed by intramolecular cyclization. Subsequently, the 4-(benzofuran-2-yl) pyridines were *N*-benzylated by using appropriate benzyl bromide to afford the final product **5a**–**o**. The results of in vitro AChE activity evaluation of synthesized compounds revealed that all compound had potent anti-AChE activity comparable or more potent than standard drug donepezil. The *N*-(3,5-dimethylbenzyl) derivative **5e** with IC_{50} value of 4.1 nM was the most active compound, being 7-fold more potent than donepezil.

1. Introduction

Alzheimer's disease (AD) is the most prevalent chronic neurodegenerative disorder which characterized by memory loss, language problems, disorientation, loss of motivation, and behavioral impairment [1]. AD is a multifactorial disease in which a complex of proteins, enzymes, or receptors is involved [2]. The pathogenesis of AD is not completely clear; however, the typical pathological hallmarks are amyloid-β (Aβ) deposits, τ-protein aggregation, oxidative stress, and decreased levels of acetylcholine (ACh) in the brain [3].

Since the AD is associated with a loss of cholinergic neurotransmission with decreased levels of ACh in the brain areas dealing with learning, memory, and behavioral responses, a majority of investigations has been focused on the basal forebrain cholinergic system [4]. Accordingly, one strategy in the treatment of AD can be the increase of synaptic levels of ACh in the brain by inhibiting the acetylcholinesterase (AChE) enzyme, which is mainly responsible for hydrolysis of ACh [5]. Nowadays, the symptomatic management of patients with AD is based on utilizing acetylcholinesterase inhibitors (AChEIs) such as donepezil, rivastigmine, and galantamine, as well as the NMDA-antagonist memantine [6].

The crystal structure of AChE indicates that the catalytic triad is located at the bottom of a narrow gorge [7]. The entrance of the gorge is termed the peripheral anionic site (PAS) in which the aromatic residues of amino acids interact with cationic ligands. At the bottom of the gorge, there is the catalytic active site (CAS) [8]. The AChE inhibition by AChE inhibitors maybe occurs via a competitive interaction with CAS, via a non-competitive binding with PAS, or via both interactions. It was demonstrated that AChE also interacts with Aβ by a hydrophobic environment close to the PAS. The latter

* Corresponding author. Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medicinal Sciences, Tehran, Iran
E-mail address: aforoumadi@yahoo.com (A. Foroumadi).

http://dx.doi.org/10.1016/j.ejmech.2015.02.009
0223-5234/

interaction can promote the deposition and aggregation of Aβ in the brain [9].

Accordingly, the AChE inhibitors with dual action on PAS and CAS could be more effective agents for the management of AD [10]. Despite the purely symptomatic mode of action of AChE inhibitors, they still represent the first-line treatment of AD [11]. Considering this information and looking at the high prevalence of AD, discovery and development of a highly effective AChE inhibitor is urgently needed.

Among the various compounds developed as AChE inhibitors, donepezil analogs are studied more extensively. The X-ray crystallography and docking studies demonstrated that donepezil has dual-binding mode of action. It was found that the presence of benzyl piperidine contributes its inhibitory activity by interacting with the CAS, while the indanone moiety of the molecule as a hydrophobic aromatic part binds to the PAS [12,13].

In the search for finding new AChE inhibitors, we have recently reported benzofuranone-based compounds containing benzylpyridinium moiety as dual-binding inhibitors (Fig. 1) [14]. It was postulated that the presence of *N*-benzylpyridinium moiety contributes to inhibitor activity by interacting with the catalytic site, and the aromatic part of benzofuranone ring engages in π–π stacking with the PAS of AChE. In continue of the work, we simplified the primary lead structure by replacing benzofuranone moiety with benzofuran ring which directly connected to the *N*-benzylpyridinium moiety. Thus we described here, the synthesis and anti-AChE activity of new benzofuran-derived compounds **5a**–**o** bearing *N*-benzylpyridinium scaffold (Fig. 1). It is interesting to highlight that a series of 2-arylbenzofurans [15] and pyridinium-type [16] AChE inhibitors have received much attention as multi-potent AD modifying agents.

2. Chemistry

The synthesis of compounds **5** was illustrated in Scheme 1. The reaction of salicylaldehyde derivatives **1** with 4-(bromomethyl) pyridine **2** in the presence of K_2CO_3 produced *O*-substituted salicylaldehydes **3**. Cyclization of compounds **3** by using *t*-BuOK as base in DMF afforded 4-(benzofuran-2-yl)pyridine derivatives **4** [17]. The *N*-benzylation of the latter compounds with appropriate benzyl bromide in acetonitrile gave final compounds as benzyl pyridinium bromide salts **5**.

3. Results and discussion

3.1. AChE inhibitory activity

The anti-AChE activity of compounds **5a**–**o** was assessed in vitro by using reported method [14]. The obtained IC_{50} values of compounds are presented in Table 1, in comparison with reference drug donepezil. All compounds showed very potent inhibitory activity against AChE at nano-molar level. A survey on IC_{50} values of compounds revealed that all compounds with exception of **5i** were more potent than donepezil. However, compound **5i** with IC_{50} value of 31.5 nM was as potent as donepezil. Among the tested compounds, 3,5-dimethylbenzyl derivative **5e** with IC_{50} value of 4.1 nM was the most potent compound. Its inhibitory activity was 7-times more than that of donepezil.

The limited structure-activity relationships study on the benzofuran ring indicated that the introduction of 5-bromo group diminished the activity (compare compounds **5i** and **5c** or **5j** and **5d**). Moreover, by comparing the IC_{50} values of 7-methoxy compounds **5n** and **5o** with those of corresponding congeners **5c** and **5d**, it could be concluded that the insertion of methoxy group on C-7 position decreased the anti-AChE activity. Compounds **5g**, **5h**, and **5l** containing fluorobenzyl moiety were more potent than related benzyl derivatives **5f** and **5k**, respectively. Therefore, the presence of fluorine atom on benzyl pendent group had positive effect on inhibitory activity. In contrast, 4-bromobenzyl derivatives **5i** and **5n** showed less activity when compared to their corresponding benzyl analogs **5f** and **5k**. Thus, the 4-bromo substitution on benzyl group diminished the activity. Also, the introduction of 4-nitro on the benzyl group of compounds **5f** and **5k** resulted in more active compounds **5j** and **5o**.

3.2. Docking study

To get insight into whether and how the binding and inhibition profile of synthesized compounds are influenced by the structure, the docking simulation was performed. Therefore, all the compounds **5a**–**o** were investigated computationally to define their binding profile. For instance, the most active compound **5e** was

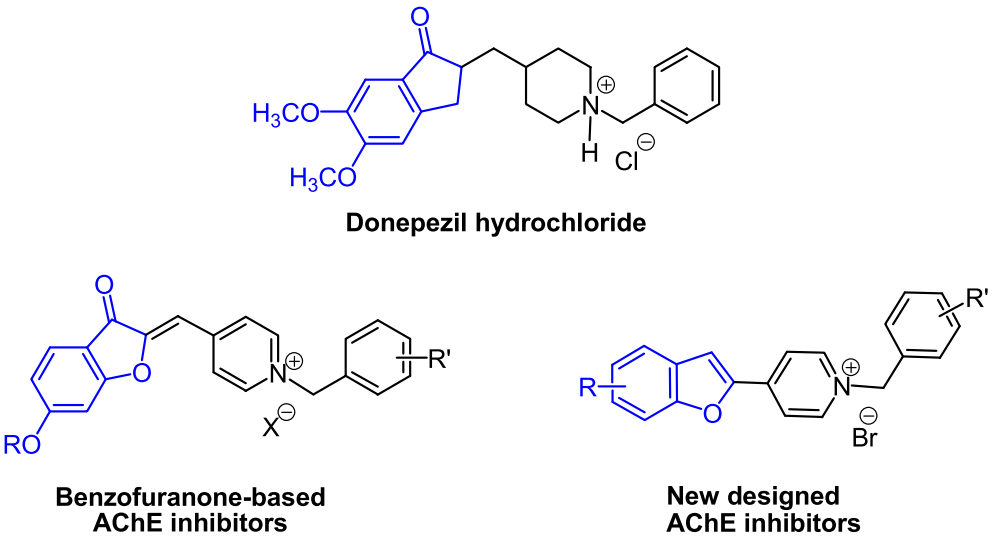
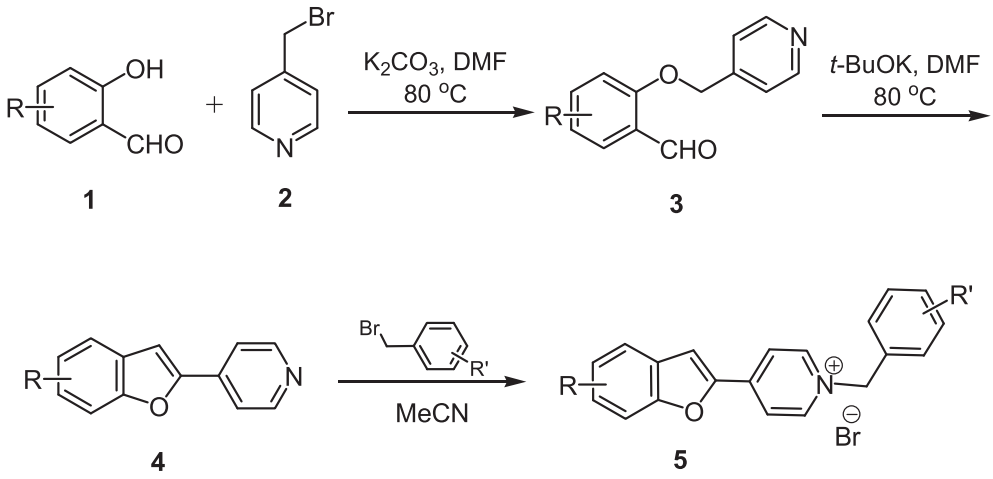
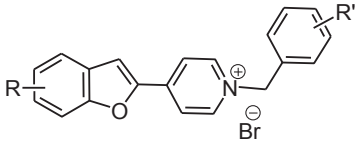


Fig. 1. Structures of donepezil hydrochloride as a well-known inhibitor of AChE, benzofuranone-based compounds reported as anti-AChE agents [14] and benzofuran-derived benzylpyrininium analogs as newly designed AChE inhibitors.



Scheme 1. Synthesis of *N*-benzyl-4-(benzofuran-2-yl)pyridinium bromide derivatives **5**.

Table 1
The IC_{50} values (nM) of compounds **5a**–**o** against AChE.



Compounds	R	R′	IC_{50} (nM)
5a	H	2-Me	6.8 ± 1.2
5b	H	2-NO_2	4.9 ± 1.1
5c	H	4-Br	16.0 ± 3.8
5d	H	4-NO_2	5.8 ± 2.4
5e	H	3,5-$(Me)_2$	4.1 ± 1.5
5f	5-Br	H	13.5 ± 2.8
5g	5-Br	3-F	9.6 ± 1.8
5h	5-Br	4-F	8.7 ± 2.1
5i	5-Br	4-Br	31.5 ± 5.3
5j	5-Br	4-NO_2	8.1 ± 2.5
5k	7-MeO	H	26.5 ± 4.2
5l	7-MeO	3-F	18.9 ± 3.7
5m	7-MeO	3-Br	22.4 ± 4.6
5n	7-MeO	4-Br	29.5 ± 4.6
5o	7-MeO	4-NO_2	10.6 ± 3.9
Donepezil			31.0 ± 5.2

subjected for further analysis. The detailed picture and key interaction table of the docking pose of compound **5e** is displayed in Fig. 2 and Table 2, respectively. From the results, it is demonstrated that the inhibitor is well-fitted in the active site. As expected, the aromatic interactions are involved in the binding of inhibitor to the active site. The benzyl pyridinium part of the molecule locates around Trp84 in the vicinity of catalytic site. The positively charged nitrogen contributes in forming π-cation interaction with aromatic residues (Phe330 and Tyr334) at mid-gorge recognition site. Moreover, pyridinium ring is engaged in T-shape π–π stacking with Tyr121. Another stacking stabilizes the inhibitor conformation through making a π–π interaction of benzofuran moiety with Trp279 in the PAS.

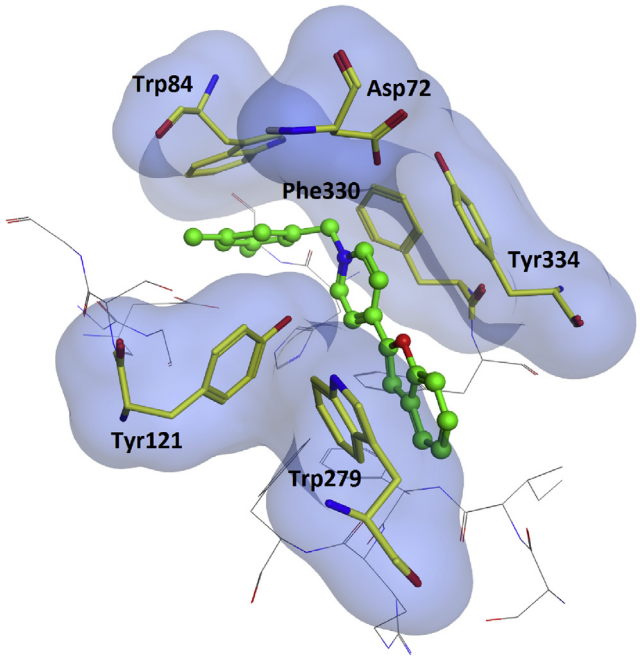


Fig. 2. Illustrative interaction of compound **5e** with AChE active site. The key residues are showed by yellow color. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
The ligand-protein contacts on the basis of distance at 5 Å.

Residue	Type of contact	Distance (Å)	Angle (degree)
Asp72	Ionic	4.65	–
Trp84	Parallel stacking	4.01	12
Tyr121	T-shape stacking	3.85	63
Trp279	Parallel stacking	3.9	17
Phe330	π-cation	4.40	–
Phe331	Lipophilic	4.30	–
Tyr334	π-cation	4.52	–

4. Conclusion

We have designed and synthesized a novel series of benzofuran-based *N*-benzylpyridinium derivatives as AChE inhibitors. The multistep sequence for synthesis of the compounds involved the initial preparation of 4-(benzofuran-2-yl)pyridine precursors via a reaction between different salicylaldehyde derivatives with 4-(bromomethyl)pyridine followed by intramolecular cyclization in the presence of potassium *tert*-butoxide. The 4-(benzofuran-2-yl)

pyridines were subsequently subjected to an *N*-benzylation reaction by using appropriate benzyl bromide, providing the final product. The results of in vitro AChE activity evaluation of synthesized compounds revealed that all compound had potent anti-AChE activity comparable or more potent than standard drug donepezil. Compound **5e** containing *N*-(3,5-dimethylbenzyl) moiety was the most active compound, being 7-fold more potent than donepezil. The synthetic feasibility and highly potent activity of compound **5e** prototypes make them as promising lead compounds for further investigation in the field of AD management and therapy.

5. Experimental

5.1. Chemistry

5.1.1. General information

The melting points of synthesized compounds were taken on a Kofler hot-stage apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Bruker FT-500 using TMS as an internal standard. Fig. 3 presenting the atom numbering of the general formula of compounds **5a**–**o** was used for easy interpretation of NMR data. The IR spectra were obtained on a Nicolet Magna FTIR 550 spectrometer (KBr disks). Mass spectra were recorded with an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV.

5.1.2. General procedure for the preparation of 2-((pyridin-4-yl) methoxy)benzaldehydes **3**

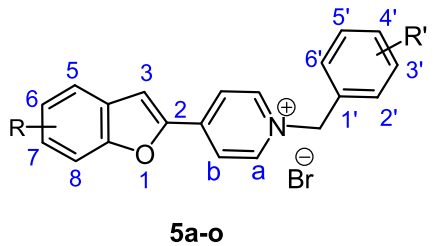
A mixture of appropriate salicylaldehyde **1** (5.0 mmol), 4-(bromomethylpyridine (**2**, 850 mg, 5.0 mmol) and K_2CO_3 (691 mg, 5.0 mmol) in DMF (5 ml) was stirred at 50 °C for 3 h. The reaction mixture was cooled down to room temperature and poured into ice-cold water, and the white precipitates were filtered off and recrystallized from EtOH to give pure 2-((pyridin-4-yl)methoxy) benzaldehyde derivative **3**.

5.1.3. General procedure for the preparation of 4-(benzofuran-2-yl) pyridine derivatives **4**

A mixture of compound **3** (639 mg, 3.0 mmol) and potassium *tert*-butoxide (561 mg, 5.0 mmol) in DMF (5 ml) was stirred at 80 °C for 3 h. The reaction mixture was cooled down to room temperature and poured into ice-cold water, and the white precipitates were filtered off and recrystallized from EtOH to give 4-(benzofuran-2-yl)pyridine derivative **4**.

5.1.4. General procedure for the preparation of N-benzyl-4-(benzofuran-2-yl)pyridinium bromides **5a**–**o**

Dry acetonitrile (7 ml) was added to 4-(benzofuran-2-yl)pyridine **4** (1 equiv), and the mixture was dissolved by heating under reflux. Then the appropriate benzyl bromide derivative (1.2 equiv) was added thereto. After heating under reflux condition for 2–3 h, the solvent was evaporated under reduced pressure. Then *n*-hexane (15 ml) was added to the residue, the precipitated crystals were separated by filtration and washed with *n*-hexane to give compounds **5**.



5a-o

Fig. 3. Atom numbering of compounds **5a**–**o**, only used for NMR spectra interpretation.

*5.1.4.1. N-(2-Methylbenzyl)-4-(benzofuran-2-yl)pyridinium bromide (**5a**).* Quantitative yield, mp 317–320 °C, IR ν_{max}/cm^{-1} (KBr): 1633 (C=N), 1574 (C=C), 2930 (CH_3), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.1 (d, 2H, H_a, J = 6.8 Hz), 8.7 (d, 2H, H_b, J = 6.8 Hz), 8.35 (s, 1H, H_3), 7.87 (d, 1H, H_5, J = 7.8 Hz), 7.77 (d, 1H, H_8, J = 8.4 Hz), 7.55 (t, 1H, H_7, J = 7.8 Hz), 7.22–7.41 (m, 5H, $H_{6,2',3',4',5'}$), 5.94 (s, 2H, CH_2N), 2.4 (s, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 18.8 (CH_3), 60.7 (CH_2N), 111.9, 113.2, 118, 121.9, 123.1, 124.4, 126.7, 128.4, 129.0, 129.3, 130.9, 132.4, 136.9, 143.7, 145.2, 149.4, 155.8. MS, *m*/*z* (%): 301 (M+1, <1), 105 (96), 139 (35), 166 (21), 195 (100).

*5.1.4.2. N-(2-Nitrobenzyl)-4-(benzofuran-2-yl)pyridinium bromide (**5b**).* Quantitative yield, mp 322–225 °C, IR ν_{max}/cm^{-1} (KBr): 1636 (C=N), 1577 (C=C), 1342,1525 (NO_2), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.17 (d, 2H, H_a, J = 5.7 Hz), 8.63 (d, 2H, H_b, J = 5.7 Hz), 8.39 (s, 1H, H_3), 8.27 (d, 1H, $H_{5'}$, J = 8.2 Hz), 7.88 (d, 1H, H_5, J = 8.0 Hz), 7.82 (t, 1H, $H_{3'}$, J = 7.6 Hz), 7.73–7.78 (m, 2H, $H_{8,4'}$), 7.56 (t, 1H, H7, J = 8.0 Hz), 7.4 (t, 1H, H_6, J = 7.8 Hz), 7.31 (d, 1H, $H_{2'}$, J = 7.6 Hz) 6.27 (s, 2H, CH_2N). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 59.8 (CH_2N), 111.9, 113.5, 122, 123.2, 124.4, 125.6, 127.8, 128.5, 129.4, 130.3, 130.4, 134.9, 144, 145.8, 147.5, 149.5, 155.8.

*5.1.4.3. N-(4-Bromobenzyl)-4-(benzofuran-2-yl)pyridinium bromide (**5c**).* Quantitative yield, mp 311–315 °C, IR ν_{max}/cm^{-1} (KBr): 1635 (C=N), 1578 (C=C), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.25 (d, 2H, H_a, J = 6.6 Hz), 8.55 (d, 2H, H_b, J = 6.6 Hz), 8.33 (s, 1H, H_3), 7.86 (d, 1H, H_5, J = 7.8 Hz), 7.78 (d, 1H, H_8, J = 8.0 Hz),7.68 (d, 2H, $H_{3',5'}$, J = 8.3 Hz), 7.57 (d, 2H, $H_{2',6'}$, J = 8.3 Hz), 7.55 (t, 1H, H_7, J = 7.8 Hz), 7.39 (t, 1H, H_6, J = 7.5 Hz),5.85 (s, 2H, CH_2N). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 61.6 (CH_2N), 111.9, 113.3, 122.1, 122.8, 123.2, 124.4, 127.8, 128.4, 131.1, 132.1, 133.7, 143.7, 145.1, 149.4, 155.8.

*5.1.4.4. N-(4-Nitrobenzyl)-4-(benzofuran-2-yl)pyridinium bromide (**5d**).* Quantitative yield, mp 310–314 °C, IR ν_{max}/cm^{-1} (KBr): 1636 (C=N), 1575 (C=C), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.29 (d, 2H, H_a, J = 5.7 Hz), 8.6 (d, 2H, H_b, J = 5.7 Hz), 8.36 (s, 1H, H_3), 8.3 (d, 2H, $H_{3',5'}$, J = 7.55 Hz), 7.87 (d, 1H, H_5, J = 7.6 Hz), 7.83 (d, 2H, $H_{2',6'}$, J = 7.8 Hz), 7.77 (d, 1H, H_8, J = 7.8 Hz), 7.56 (t, 1H, H_7, J = 7.5 Hz), 7.4 (t, 1H, H_6, J = 7.2 Hz), 6.04 (s, 2H, CH_2N). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 61.3 (CH_2N), 112, 113.5, 122.2, 123.2, 124.1, 124.5, 127.8, 130, 141.4, 143.9, 145.5, 147.9, 149.4, 155.8.

*5.1.4.5. N-(3,5-dimethylbenzyl)-4-(benzofuran-2-yl)pyridinium bromide (**5e**).* Quantitative yield, mp 216–219 °C, IR ν_{max}/cm^{-1} (KBr): 1634 (C=N), 1576 (C=C), 2906 (CH_3), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.21 (d, 2H, H_a, J = 6.6 Hz), 8.54 (d, 2H, H_b, J = 6.6 Hz), 8.32 (s, 1H, H_3), 7.87 (d, 1H, H_5, J = 7.8 Hz), 7.77 (d, 1H, H_8, J = 8.3 Hz), 7.55 (t, 1H, H_7, J = 7.6 Hz), 7.4 (t, 1H, H_6, J = 7.8 Hz), 7.18 (s, 2H, $H_{2',6'}$), 7.06 (s, 1H, $H_{4'}$), 5.75 (s, 2H, CH_2N), 2.3 (s, 6H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 20.8 (CH_3), 62.6 (CH_2N), 111.9, 113.1, 122, 123.1, 124.4, 126.3, 127.8, 128.4, 130.6, 134.2, 138.4, 143.6, 145, 149.5, 155.8. MS, *m*/*z* (%): 314 (M^+, 15), 264 (96), 239 (48), 195 (75), 119 (58), 83(74), 69 (87), 57 (100).

*5.1.4.6. N-Benzyl-4-(5-bromobenzofuran-2-yl)pyridinium bromide (**5f**).* Quantitative yield, mp 307–310 °C, IR ν_{max}/cm^{-1} (KBr): 1634 (C=N), 1577 (C=C), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.44 (bs, 2H, H_a), 8.32 (bs, 2H, H_b), 7.81 (s, 1H, H_5), 7.78 (s, 1H, H_3), 7.66 (d, 2H, $H_{2',6'}$, J = 6.4 Hz), 7.51 (d, 1H, H_8, J = 8.6 Hz), 7.36–7.37 (m, 4H, $H_{7,3',4',5'}$), 6.24 (s, 2H, CH_2N). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 63.9 (CH_2N), 112.4, 113.4, 117.6, 122.1, 125.5, 129.6, 129.7, 130,

131.5, 132.8, 144, 145.1, 149.8, 155.

*5.1.4.7. N-(3-Fluorobenzyl)-4-(5-bromobenzofuran-2-yl)pyridinium bromide (**5g**).* Quantitative yield, mp 311–316 °C, IR ν_{max}/cm^{-1} (KBr): 1640 (C=N), 1574 (C=C), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.33 (d, 2H, H_a, J = 6.4 Hz), 8.57 (d, 2H, H_b, J = 6.4 Hz), 8.26 (s, 1H, H_3), 8.08 (s, 1H, H_5), 7.73 (d, 1H, H_8, J = 8.8 Hz), 7.63 (d, 1H, H_7, J = 8.8 Hz), 7.46–7.56 (m, 3H, $H_{2',3',6'}$), 7.26–7.29 (m, 1H, $H_{4'}$), 5.9 (s, 2H, CH_2N). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 61.7 (CH_2N), 112.2, 113.9, 115.9 (d, $^2J_{CF}$ = 22.36 Hz, $C_{4'}$), 116.2 (d, $^2J_{CF}$ = 20.68 Hz, $C_{6'}$), 116.6, 122.1, 125, 125.4, 129.9, 130.8, 131.3 (d, $^3J_{CF}$ = 7.72 Hz, $C_{3'}$), 136.7 (d, $^3J_{CF}$ = 6.85 Hz, $C_{1'}$), 143.3, 145.2, 150.6, 154.5, 162.1 (d, $^1J_{CF}$ = 243.68 Hz, $C_{5'}$).

*5.1.4.8. N-(4-Fluorobenzyl)-4-(5-bromobenzofuran-2-yl)pyridinium bromide (**5h**).* Quantitative yield, mp 305–310 °C, IR ν_{max}/cm^{-1} (KBr): 1634 (C=N), 1579 (C=C), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.35 (bs, 2H, H_a), 8.58 (bs, 2H, H_b), 8.28 (s, 1H, H_3), 8.10 (s, 1H, H_5), 7.7–7.74 (m, 3H, H $_{8,3',5'}$), 7.66 (d, 1H, H_7, J = 8.4 Hz), 7.31 (m, 2H, $H_{3',5'}$), 6.05 (s, 2H, CH_2N). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 61.6 (CH_2N), 112.2, 114, 116.1 (d, $^2J_{CF}$ = 21.75 Hz, $C_{3',5'}$), 116.6, 122.4, 125.4, 129.9, 130.7, 130.8, 131.5 (d, $^3J_{CF}$ = 8.02 Hz, $C_{2',6'}$), 143.2, 145.2, 150.7, 154.5, 162 (d, $^1J_{CF}$ = 243 Hz, $C_{4'}$).

*5.1.4.9. N-(4-Bromobenzyl)-4-(5-bromobenzofuran-2-yl)pyridinium bromide (**5i**).* Quantitative yield, mp 308–312 °C, IR ν_{max}/cm^{-1} (KBr): 1633 (C=N), 1574 (C=C), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.22 (d, 2H, H_a, J = 6.7 Hz), 8.56 (d, 2H, H_b, J = 6.7 Hz), 8.33 (s, 1H, H_3), 7.87 (d, 1H, H_8, J = 7.7 Hz), 7.77 (d, 1H, H_5, J = 8.5 Hz), 7.68 (d, 2H, $H_{3',5'}$, J = 8.4 Hz), 7.55 (d, 2H, $H_{2',6'}$, J = 8.4 Hz),7.41 (t, 1H, H_6, J = 7.6 Hz), 5.82 (s, 2H, CH_2N). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 61.7 (CH_2N), 112, 122.1, 122.8, 123.2, 124.4, 127.8, 128.4, 131.1, 132.1, 133.6, 143.7, 145.1, 149.4, 155.8.

*5.1.4.10. N-(4-Nitrobenzyl)-4-(5-bromobenzofuran-2-yl)pyridinium bromide (**5j**).* Quantitative yield, mp 318–322 °C, IR ν_{max}/cm^{-1} (KBr): 1633 (C=N), 1575 (C=C), 1349, 1534 (NO_2), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.33 (d, 2H, H_a, J = 6.2 Hz), 8.61 (d, 2H, H_b, J = 6.3 Hz), 8.29–8.31 (m, 3H, $H_{3,3',5'}$), 8.13 (d, 1H, H_5, J = 1.4 Hz), 7.83 (d, 2H, $H_{2',6'}$, J = 8.5 Hz), 7.76 (d, 1H, H_8, J = 8.9 Hz), 7.69 (dd, 1H, H_7, J = 8.9, 1.4 Hz), 6.05 (s, 2H, CH_2N). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 61.5 (CH_2N), 112.4, 114, 116.6, 122.5, 124.1, 125.5, 129.9, 130, 130.9, 141.3, 143.5, 145.6, 147.8, 150.6, 154.6.

*5.1.4.11. N-Benzyl-4-(7-methoxybenzofuran-2-yl)pyridinium bromide (**5k**).* Quantitative yield, mp 316–320 °C, IR ν_{max}/cm^{-1} (KBr): 1640 (C=N), 1582 (C=C), 1351, 1513 (NO_2), 2982 (CH_3), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.24 (d, 2H, H_a, J = 6.3 Hz), 8.52 (d, 2H, H_b, J = 6.3 Hz), 8.29 (s, 1H, H_3), 7.59 (d, 2H, H $_{2',6'}$, J = 7.0 Hz), 7.43–7.47 (m, 3H, $H_{3',4',5'}$), 7.38 (d, 1H, H_5, J = 7.8 Hz), 7.29 (t, 1H, H_6, J = 7.8 Hz), 7.13 (d, 1H, H_7, J = 7.8 Hz), 5.87 (s, 2H, CH_2N), 3.9 (s, 3H, OMe). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 55.9 (OCH_3), 62.5 (CH_2N), 110.1, 113.4, 114.7, 122, 125.2, 128.7, 129.2, 129.3, 134.4, 143.5, 145.1, 145.2, 149.4. MS, *m/z* (%): 316 (M^+, <1), 225(100), 195 (20), 182 (28), 154 (36), 91(95).

*5.1.4.12. N-(3-Fluorobenzyl)-4-(7-methoxybenzofuran-2-yl)pyridinium bromide (**5l**).* Quantitative yield, mp 317–322 °C, IR ν_{max}/cm^{-1} (KBr): 1634 (C=N), 1585 (C=C), 2971 (CH_3), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.25 (d, 2H, H_a, J = 6.5 Hz), 8.54 (d, 2H, H_b, J = 6.5 Hz), 8.31 (s, 1H, H_3), 7.43–7.54 (m, 3H, $H_{2',3',6'}$), 7.39 (d, 1H, H_5, J = 7.8 Hz), 7.31 (m, 2H, $H_{6,4'}$), 7.15 (d, 1H, H_7, J = 7.8 Hz), 5.88 (s, 2H, CH_2N), 4.00 (s, 3H, OMe). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 55.96 (OCH_3), 61.7 (CH_2N), 110.1, 113.5, 114.7, 115.8 (d, $^2J_{CF}$ = 22.38 Hz, $C_{4'}$), 116.2 (d, $^2J_{CF}$ = 20.72 Hz, $C_{6'}$), 122.1, 124.9, 125.2, 129.3, 131.3 (d, $^3J_{CF}$ = 7.73 Hz, $C_{3'}$), 136.8 (d, $^3J_{CF}$ = 7.3 Hz, $C_{1'}$), 143.7, 145.1, 149.4, 162.2 (d, $^1J_{CF}$ = 243.52 Hz, $C_{5'}$).

*5.1.4.13. N-(3-Bromobenzyl)-4-(7-methoxybenzofuran-2-yl)pyridinium bromide (**5m**).* Quantitative yield, mp 319–324 °C, IR ν_{max}/cm^{-1} (KBr): 1635 (C=N), 1579 (C=C), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.27 (d, 2H, H_a, J = 6.7 Hz), 8.52 (d, 2H, H_b, J = 6.7 Hz), 8.29 (s, 1H, H_3), 7.91 (s, 1H, $H_{6'}$), 7.63–7.64 (m, 2H, $H_{2',4'}$), 7.43 (t, 1H, $H_{3'}$, J = 7.9 Hz), 7.36 (d, 1H, H_5, J = 7.9 Hz), 7.27 (t, 1H, H_6, J = 7.9 Hz), 7.11 (d, 1H, H_7, J = 7.9 Hz), 5.88 (s, 2H, CH_2N), 4.0 (s, 3H, OMe). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 55.9 (OCH_3), 61.4 (CH_2N), 110.1, 113.5, 114.6, 122.1, 122.2, 125.2, 128, 129.1, 129.3, 131.3, 131.7, 132.2, 136.8, 143.6, 145.1, 145.2, 149.4.

*5.1.4.14. N-(4-Bromobenzyl)-4-(7-methoxybenzofuran-2-yl)pyridinium bromide (**5n**).* Quantitative yield, mp 314–320 °C, IR ν_{max}/cm^{-1} (KBr): 1638 (C=N), 1583 (C=C), 2955 (CH_3) ^{1}H NMR (DMSO-d_6, 500 MHz), 9.19 (d, 2H, H_a, J = 6.7 Hz), 8.54 (d, 2H, H_b, J = 6.7 Hz), 8.3 (s, 1H, H_3), 7.68 (d, 2H, $H_{3',5'}$, J = 8.4 Hz), 7.54 (d, 2H, $H_{2',6'}$, J = 8.4 Hz), 7.4 (d, 1H, H_5, J = 7.9 Hz), 7.32 (t, 1H, H_6, J = 7.9 Hz), 7.17 (d, 1H, H_7, J = 7.9 Hz), 5.82 (s, 2H, CH_2N), 4.0 (s, 3H, OMe). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 56 (OCH_3), 63.9 (CH_2N), 110.15, 113.5, 114.7, 122.1, 122.8, 125.3, 129.3, 131, 132.1, 133.6, 143.7, 145.1, 145.3, 149.4. MS, *m/z* (%): 394 (M^+, <1), 250 (8), 225 (63), 169 (61), 69 (100), 41 (94).

*5.1.4.15. N-(4-Nitrobenzyl)-4-(7-methoxybenzofuran-2-yl)pyridinium bromide (**5o**).* Quantitative yield, mp 312–316 °C, IR ν_{max}/cm^{-1} (KBr): 1636 (C=N), 1584 (C=C), 1348, 1520 (NO_2), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.26 (d, 2H, H_a, J = 6.8 Hz), 8.58 (d, 2H, H_b, J = 6.8 Hz), 8.33 (s, 1H, H_3), 8.3 (d, 2H, $H_{3',5'}$, J = 8.7 Hz), 7.82 (d, 2H, $H_{2',6'}$, J = 8.7 Hz), 7.4 (d, 1H, H_5, J = 7.8 Hz), 7.31 (t, 1H, H_6, J = 7.9 Hz), 7.16 (d, 1H, H_7, J = 7.9 Hz), 6.04 (s, 2H, CH_2N), 3.95 (s, 3H, OMe). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C (ppm) 55.9 (OCH_3), 61.3 (CH_2N), 110.2, 113.7, 114.7, 122.2, 124.1, 125.3, 129.3, 130, 141.4, 143.8, 145.2, 145.3, 145.5, 147.8, 149.4. MS, *m/z* (%): 361 (M^+, <1), 339 (11), 313 (20), 225 (68), 195 (28), 154 (32), 136 (36), 83 (52), 69 (74), 55 (94), 43 (100).

5.2. AChE inhibition test

AChE (E.C.3.1.1.7, Type V–S, from *Electric eel*), Ellman's reagent (DTNB), and acetylthiocholine (ATC) were purchased from Sigma–Aldrich. Donepezil (Sigma) was used as reference drug for AChE inhibition. The AChE inhibitory activities of compounds **5a**–**o** were determined by using previously reported method [14].

5.3. Molecular modeling

The structure of AChE containing co-crystalized ligand E2020 (PDB code: 1EVE) was retrieved from RCSB Protein Data Bank (PDB, http://www.rcsb.org/pdb/home/home.do) and docking studies were performed by Autodock Vina (1.1.1) as previously described method [18].

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.02.009.

References

[1] H.W. Querfurth, F.M. LaFerla, N. Engl. J. Med. 362 (2010) 329–344.
[2] C. Ballard, S. Gauthier, A. Corbett, C. Brayne, D. Aarsland, E. Jones, Lancet 377 (2011) 1019–1031.

[3] M. Prinz, S. Parlar, G. Bayraktar, V. Alptüzün, E. Erciyas, A. Fallarero, D. Karlsson, P. Vuorela, M. Burek, C. Förster, E. Turunc, G. Armagan, A. Yalcin, C. Schiller, K. Leuner, M. Krug, C.A. Sotriffer, U. Holzgrabe, Eur. J. Pharm. Sci. 49 (2013) 603–613.
[4] G.L. Wenk, J. Clin. Psychiatry 64 (2003) 7–10.
[5] A.V. Terry, J.J. Buccafusco, J. Pharmacol. Exp. Ther. 306 (2003) 821–827.
[6] F. Massoud, S. Gauthier, Curr. Neuropharmacol. 8 (2010) 69–80.
[7] J.L. Sussman, M. Harel, F. Frolow, C. Oefner, A. Goldman, et al., Science 253 (1991) 872–879.
[8] D.A. Dougherty, D.A. Stauffer, Science 250 (1990) 1558–1560.
[9] M. Pera, A. Martinez-Otero, L. Colombo, M. Salmona, D. Ruiz-Molina, A. Badia, et al., Mol. Cell. Neurosci. 40 (2009) 217–224.
[10] E. Giacobini, Neurochem. Res. 28 (2003) 515–522.
[11] V. Jelic, T. Darreh-Shori, Clin. Med. Insights Ther. 2 (2010) 771–788.
[12] G. Koellner, G. Kryger, C.B. Millard, I. Silman, J.L. Sussman, T. Steiner, J. Mol. Biol. 296 (2000) 713–735.
[13] A. Saxena, J.M. Fedorko, C.R. Vinayaka, R. Medhekar, Z. Radić, P. Taylor, O. Lockridge, B.P. Doctor, Eur. J. Biochem. 270 (2003) 4447–4458.
[14] H. Nadri, M. Pirali-Hamedani, M. Shekarchi, M. Abdollahi, V. Sheibani, M. Amanlou, A. Shafiee, A. Foroumadi, Bioorg. Med. Chem. 18 (2010) 6360–6366.
[15] S. Rizzo, A. Tarozzi, M. Bartolini, G. Da Costa, A. Bisi, S. Gobbi, F. Belluti, A. Ligresti, M. Allarà, J.-P. Monti, V. Andrisano, V. Di Marzo, P. Hrelia, A. Rampa, Eur. J. Med. Chem. 58 (2012) 519–532.
[16] P. Kapková, V. Alptüzün, P. Frey, E. Erciyas, U. Holzgrabe, Bioorg. Med. Chem. 14 (2006) 472–478.
[17] W. Chen, X.-Y. Deng, Y. Li, L.-J. Yang, W.-C. Wan, X.-Q. Wang, H.-B. Zhang, X.-D. Yang, Bioorg. Med. Chem. Lett. 23 (2013) 4297–4302.
[18] M. Khoobi, F. Ghanoni, H. Nadri, A. Moradi, M. Pirali Hamedani, F. Homayouni Moghadam, S. Emami, M. Vosooghi, R. Zadmard, A. Foroumadi, A. Shafiee, Eur. J. Med. Chem. 89 (2015) 296–303.