Bioorganic & Medicinal Chemistry 18 (2010) 6360–6366

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and anticholinesterase activity of a novel series of 1-benzyl-4-((6-alkoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium derivatives

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ARTICLE INFO

Article history:
Received 8 June 2010
Revised 6 July 2010
Accepted 6 July 2010
Available online 30 July 2010

Keywords:
Benzofuranone
Benzylpyridinium
Cholinesterase inhibitor
Ellman's method

ABSTRACT

A novel series of benzofuranone-ylidene-methyl benzylpyridinium derivatives (**6a**–**u**) were synthesized as acetylcholinesterase inhibitors. The anticholinesterase activity of synthesized compounds was measured using colorimetric Ellman's method. It was revealed that some synthesized compounds exhibited high anticholinesterase activity, among them compound **6b** was the most active compound (IC_{50} = 10 ± 6.87 nM).

1. Introduction

Alzheimer's disease (AD) is considered as a common neurodegenerative disorder mostly observed in aged populations.[1] It has been reported that around 10% of the world's population is bothered by AD.[1] Decrease of acetylcholine level in the hippocampus and cortex is the one of the most important causes of AD, therefore some of acetylcholinesterase (AChE) inhibitors are effective agents for treatment of AD's symptoms.[2] On the X-ray crystal structure of AChE (TcAChE), two separate ligand binding sites namely catalytic site (active site) and peripheral anionic site (PAS) could be observed in the long (20 Å), narrow gorge of AChE.[3] Besides, it has been demonstrated that AChE can accelerate formation of β-amyloid fibril by means of peptide deposition in a non-catalytic manner through attaching of nonamyloidogenic form of Aβ protein to peripheral site of AChE.[4–6] Thus, compounds capable of binding to both active and peripheral sites of AChE could prevent Aβ aggregation and AChE activity, simoltaneusly.[7] Furthermore, peripheral AChE has been targeted in the treatment of myasthenia gravis, glaucoma and for the reversal of neuromuscular blockade.[8]

The synthesis and evaluation of AChE inhibitors have been largely described.[9–11] In our study, we have focused on designing compounds with potential ability of interaction towards both binding sites on AChE. In an attempt to find novel agents against AD, a series of aurone derivatives (Fig. 1, structure I) have been reported as compounds able to inhibit acetylcholinesterase enzyme.[12]

It had been proposed in this study that π–π stacking interaction of a aurone planar ring system could be a reason for improved acetylcholinesterase inhibitory activity of the synthesized structures.[12] On the other hand, synthesis and acetylcholinesterase inhibitory activity of benzyl piperidine derivatives (Fig. 1, structure II) have been largely discussed.[13–16] π-Cation interactions of quaternary amine with Tyr 70, Tyr 121 and Phe 330 have been revealed through docking studies.[16] In pursuit of the described studies in this area and to obtain novel AChE inhibitors (AChEIs), a hybrid structure of compounds **I** and **II** has been designed (Fig. 1, structure III). Isosteric replacement of phenyl ring with pyridine has been taken to introduce a quaternary amine site, while keeping the planar conformation of the aurone ring system. Since aurones showed binding affinity to Aβ aggregates in vitro,[17] we have retained the aurone moiety to improve remedial efficiency. The activity of the target compounds has been evaluated through in vitro AChE inhibition assay using an Ellman's colorimetric method.[18]

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doi:10.1016/j.bmc.2010.07.012

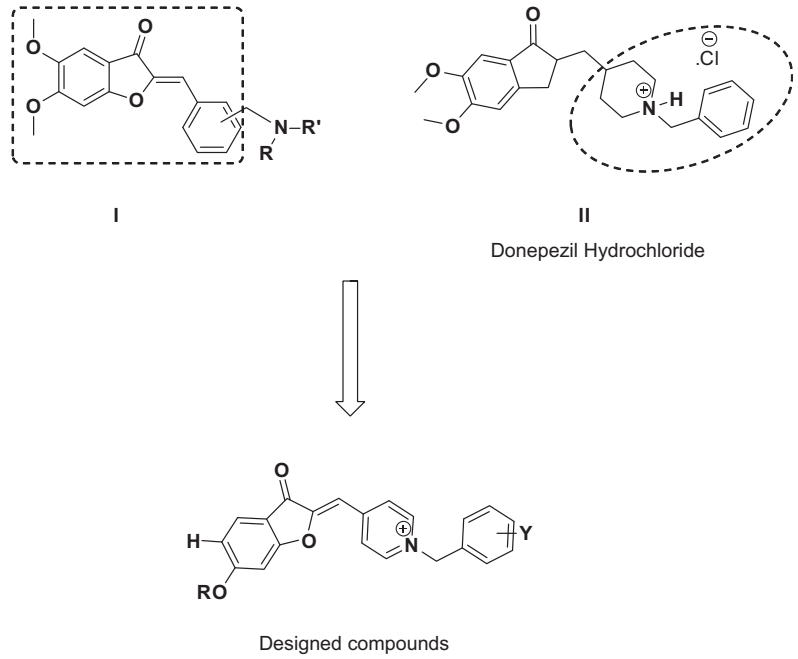


Figure 1. Design strategy of the target compounds.

2. Results and discussion

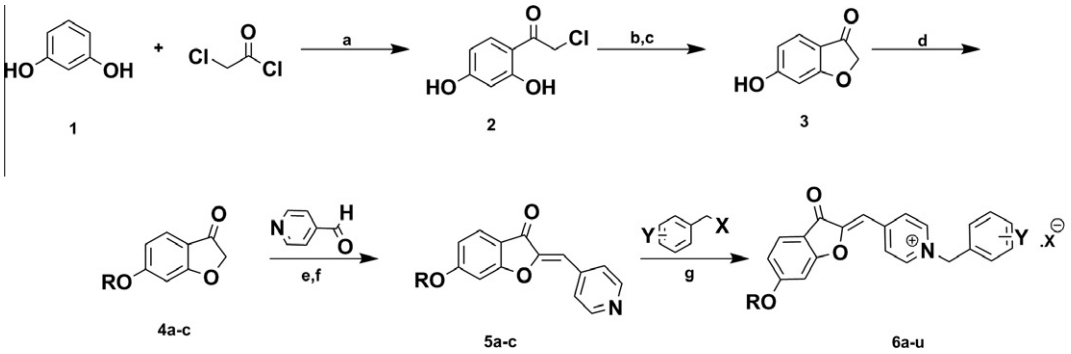
2.1. Chemistry

The synthetic pathway for synthesis of our designed compounds is shown in Scheme 1. Reaction of resorcinol (**1**) with chloroacetyl chloride using $AlCl_3$ as Lewis acid furnished 2-chloro-1-(2,4-dihydroxyphenyl) ethanone (**2**).[19] In the second step, 6-hydroxybenzofuran-3(2*H*)-one (**3**) was prepared followed by intramolecular cyclization with NaOH.[19]

The alkylation of hydroxyl group in compound **3** using proper alkyl halide in dry *N*,*N*-dimethylformamide (DMF) gave compounds **4a**–**c**. The three intermediates were then reacted with pyridine-4-carboxaldehyde in the presence of *p*-toluenesulfonic acid (PTSA) to yield compounds **5a–c** in *Z*-configuration. The exocyclic formed double bond may exist as either *E* (*trans*) or *Z* (*cis*)-configuration. It has been reported that 2-(pyridin-4-ylmethylene) benzofuran-3(2*H*)-one prefer *Z*-configuration as thermodynamically favored structure.[20] This assignment has been made based on bond dipole moment measurements study.[20] According to this study we have deduced that compounds **5a–c** may exist as *Z*-isomers. Furthermore, the configuration of aurones exocyclic double bond has been assigned through measurement of olefinic (β) proton chemical shift.[21] It has been reported that chemical shift of δ 6.70 ppm was mentioned for the *Z*-isomer and δ 7.01 ppm for the *E*-isomer because of anisotropic effect of carbonyl group.[21] The ^{1}H chemical shifts of the vinylic hydrogen in compounds **5a**–**c** emerged at δ 6.66–6.69 ppm, in keeping with a *Z*-configuration of the vinylic hydrogen of aurones. Final compounds (**6a**–**u**) were obtained through addition of proper benzyl bromide or chloride derivatives to compounds **5a**–**c** in dry acetonitrile.

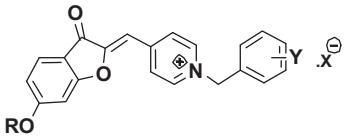
2.2. Biological activity

Modified Ellman's method[22] was used to determine anticholinesterase activity of new series of benzofuranone derivatives **6a**–**u** using freshly prepared AChE from electrophorus electricus as enzyme source and donepezil hydrochloride as reference compound. Table 1 shows anticholinesterase activity of synthesized compounds expressed as IC_{50} values. The data regarding the set of compounds **6a**–**u** allowed us to examine the consequence of changing the benzofuranone moiety and benzyl group substituent. As shown in Table 1, most of the synthesized compounds showed moderate to high inhibitory activity toward acetylcholinesterase in comparison to donepezil as reference drug. The result showed that acetylcholinesterase inhibitory activity was influenced by the type and position of substituent on the benzyl group. Compound **6a** containing unsubstituted benzyl group, exhibited considerable inhibitory



Scheme 1. Reagents and conditions: (a) $AlCl_3$, reflux, overnight; (b) NaOH 5%, 0 °C to rt; (c) HCl 6 M; (d) alkyl halide, anhydrous K_2CO_3, 80 °C, 2 h; (e) PTSA, reflux, 3 h; (f) 10% sodium hydrogen carbonate, 30–60 min; (g) benzyl halide derivatives, reflux, 2–3 h.

Table 1
Acetylcholinesterase inhibition IC_{50} average ± SD of compounds **6a–u** and donepezil hydrochloride



Compound	R	Y	X	AChE Inhibition (IC_{50} ± SD)[a] (nmol/L)
6a	$-CH_3$	H	Br	41 ± 7.94
6b	$-CH_3$	2-F	Cl	**10 ± 6.87**
6c	$-CH_3$	3-F	Cl	60 ± 9.35
6d	$-CH_3$	4-F	Br	**22 ± 6.25**
6e	$-CH_3$	$2-CH_3$	Cl	68 ± 10.34
6f	$-CH_3$	$3-CH_3$	Cl	123 ± 9.28
6g	$-CH_3$	$4-CH_3$	Cl	475 ± 29
6h	$-CH_2CH_3$	H	Br	63 ± 34
6i	$-CH_2CH_3$	2-F	Cl	32 ± 7.75
6j	$-CH_2CH_3$	3-F	Cl	48 ± 6.39
6k	$-CH_2CH_3$	4-F	Br	51 ± 10
6l	$-CH_2CH_3$	$2-CH_3$	Cl	546 ± 56
6m	$-CH_2CH_3$	$3-CH_3$	Cl	399 ± 88
6n	$-CH_2CH_3$	$4-CH_3$	Cl	790 ± 173
6o	$-CH_2CH_2CH_3$	H	Br	80 ± 14
6p	$-CH_2CH_2CH_3$	2-F	Cl	50 ± 9.86
6q	$-CH_2CH_2CH_3$	3-F	Cl	107 ± 23
6r	$-CH_2CH_2CH_3$	4-F	Br	68 ± 13
6s	$-CH_2CH_2CH_3$	$2-CH_3$	Cl	328 ± 44
6t	$-CH_2CH_2CH_3$	$3-CH_3$	Cl	234 ± 72
6u	$-CH_2CH_2CH_3$	$4-CH_3$	Cl	612 ± 80
Donepezil hydrochloride				28 ± 6.62

[a] Data are means ± standard deviation of triplicate independent experiments.

activity (IC_{50} = 41 ± 7.94 nM) in comparison to donepezil (IC_{50} = 28 ± 6.62 nM). Introduction of a fluorine atom at position 2 or 4 of benzyl moiety resulted in increasing of anticholinesterase activity. In fact, among tested compounds, compound **6b** having fluorine atom on C-2 position of benzyl group exhibited the most potent inhibitory activity (IC_{50} = 10 ± 6.87 nM) which was greater than donepezil as reference drug (IC_{50} = 28 ± 6.62). Changing the position of fluorine atom on benzyl group in compound **6b**, from 2- to 3-position resulted a large activity decrease in compound **6c** (60 ± 9.35 nM). However, the 4-fluoro isomer (**6d**) showed a rather slight decrease (22 ± 6.25 nM) in comparison to the most potent compound **6b**. Substitution of the methyl group on benzyl moiety led to the compounds with lower potencies, except for **6e** which showed moderate anticholinesterase activity (IC_{50} = 68 ± 10.34 nM).

It has been reported that the methoxy group at position 6 of indanone moiety in donepezil, significantly enhanced binding to the active site of AChE enzyme.[12] Accordingly, we have introduced the methoxy group at the same position on the benzofuranone ring in our synthesized compounds (**6a–u**). Among tested compounds, compounds **6b** and **6d** which contained methoxy group were shown to be more potent than donepezil as reference compound. Moreover, it was shown that increasing of alkoxy group length was adversely contributed to the AChE inhibitory activity. Furthermore, it was observed that replacement of the methoxy group with ethoxy and propoxy groups at the same position resulted in less active compounds except for compound **6j** which was proved to be more active.

3. Conclusion

A new series of benzofuranone-ylidene-methyl benzylpyridinium derivatives (**6a–u**) were synthesized from substituted benzofuranones as acetylcholinesterase inhibitors. Assuming biological data, all synthesized compounds have shown moderate to high anticholinesterase activity in which compound **6b** was the most potent compound against acetylcholinesterase.

4. Experimental

4.1. Chemistry

The ^1H nuclear magnetic resonance (NMR) spectra were recorded with tetramethylsilane (TMS) as the internal standard on a Bruker FT-500 MHz spectrometer. Coupling constants were given in Hertz (Hz). Chemical shifts are expressed as δ (part per million) downfield from TMS as internal standard. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet) and m (multiplet). The atoms numbering of the target compounds used for ^1H NMR data are depicted in Figure 2. Mass spectroscopy (MS) spectra were recorded on Finigan TSQ-70 spectrometer at 70 eV. Melting points of all compounds were determined using Kofler hot stage apparatus and are uncorrected. Reaction progress and product mixtures were routinely checked by thin-layer chromatography (TLC) on Merck pre-coated Silica Gel F254 plates. Column chromatography was performed with silica gel (70–230 mesh). IR spectra were recorded on a Nicolet FT-IR Magna 550 spectrophotometer. Elemental microanalyses were carried out with a Perkin–Elmer 240-C apparatus for C, H, and N. Chemical reagents and solvents used in this study were purchased from Merck AG, Aldrich or Acros Organics.

4.1.1. General procedure for synthesis of 6-alkoxybenzofuran-3(2*H*)-one (4a–c)

To a mixture of 6-hydroxybenzofuran-3(2*H*)-one (**3**) (1 equiv) and anhydrous potassium carbonate (1 equiv) in 5 ml dry DMF, proper alkyl halide (1 equiv) was added and the mixture was stirred under argon for 2 h at 80 °C. Water (20 ml) was added after which the mixture was cooled and was extracted with ethyl acetate (3 × 30 ml). The combined organic extracts were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The resulted oil was purified by column chromatography using petroleum ether–ethyl acetate (60:40) as the mobile phase to afford O-alkylated compounds **4a–c** in good yield.

4.1.2. 6-Methoxybenzofuran-3(2*H*)-one (4a)

Starting from 6-hydroxybenzofuran-3(2*H*)-one (**3**) (10 mmol, 1.5 g) and methyl iodide (10 mmol, 1.42 g), compound **4a** was obtained, 93% yield, yellow solid: mp 105–108 °C, ^1H NMR ($CDCl_3$, 500 MHz), δ: 7.55 (d, 1H, H_4, J = 8.5 Hz), 6.63 (dd, 1H, H_5, J = 8.5 Hz, J = 2 Hz), 6.52 (d, 1H, H_7, J = 2 Hz), 4.61 (s, 2H, $-CH_2C{=}O$), 3.88 (s, 3H, OMe), EI-MS *m*/*z* (%) 164 (M^+, 100), 135 (91), 32 (67).

4.1.3. 6-Ethoxybenzofuran-3(2*H*)-one (4b)

Starting from 6-hydroxybenzofuran-3(2*H*)-one (**3**) (12 mmol, 1.8 g) and ethyl iodide (12 mmol, 1.872 g), compound **4b** was obtained, 86% yield orange solid: mp 113–115 °C, ^1H NMR ($CDCl_3$, 500 MHz), δ: 7.56 (d, 1H, H_4, J = 8.6 Hz), 6.61 (dd, 1H, H_5, J = 8.6 Hz, J = 2.2 Hz), 6.53 (d, 1H, H_7, J = 2.2 Hz) 4.61 (s, 2H, $-CH_2C{=}O$), 4.1 (q, 2H, OCH_2, J = 6.8 Hz), 1.45 (t, 3H, $-CH_3$, J = 6.8 Hz), EI-MS *m*/*z* (%) 178 (M^+, 100), 135 (88), 47 (79).

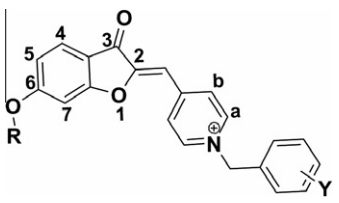


Figure 2. Atom numbering of the target compounds used for ^1H NMR data.

4.1.4. 6-Propoxybenzofuran-3(2*H*)-one (4c)

Starting from 6-hydroxybenzofuran-3(2*H*)-one (**3**) (12 mmol, 1.8 g) and 1-propyl bromide (1.476 g, 12 mmol) in presence of potassium iodide (3 mmol), compound **4c** was obtained, 90% yield yellow solid: mp 119–121 °C, ^{1}H NMR ($CDCl_3$, 500 MHz), δ: 7.55 (d, 1H, H_4, J = 8.5 Hz), 6.64 (dd, 1H, H_5, J = 8.5 Hz, J = 2 Hz), 6.51 (d, 1H, H_7, J = 2 Hz) 4.61 (s, 2H, $-CH_2C{=}O$), 3.98 (t, 2H, OCH_2, J = 6.5 Hz), 1.86–1.82 (m, 2H, $-CH_2-$), 1.04 (t, 3H, $-CH_3$, J = 6.5 Hz), EI-MS m/z (%) 192 (M^+, 100), 135 (91), 60 (81).

4.1.5. General procedure for synthesis of (*Z*)-6-alkoxy-2-(pyridin-4-ylmethylene) benzofuran-3(2*H*)-one (5a–c)

6-Alkoxybenzofuran-3(2*H*)-one **4a**–**c** (1 equiv), pyridine-4-carboxaldehyde (1.4 equiv) and PTSA (1.5 equiv) were suspended in toluene (25 ml) and heated to reflux using water separator for 3 h. The resulting mixture was cooled to 25–40 °C and the solid was filtered. Further the wet solid was suspended in aqueous 10% sodium hydrogen carbonate solution (50 ml) and stirred for 30–60 min at room temperature. The resulting precipitate solid was filtered and washed with water (50 ml) and dried. The crude solid was purified by crystallization from acetonitrile.

4.1.6. (*Z*)-6-Methoxy-2-(pyridin-4-ylmethylene) benzofuran-3(2*H*)-one (5a)

Starting from 6-methoxybenzofuran-3(2*H*)-one **4a** (1.266 g, 5 mmol), pyridine-4-carboxaldehyde (7 mmol, 0.75 g) and *p*-toluenesulfonic acid (7.5 mmol, 1.3 g), compound **5a** was obtained, 77% yield, mp 180–183 °C, IR ν_{max}/cm^{-1} (KBr): 1705 (C=O), 1637 (C=C alkene), ^{1}H NMR ($CDCl_3$, 500 MHz), 8.69 (dd, 2H, H_a-pyridine, J = 4.5 Hz, J = 2 Hz), 7.71 (dd, 2H, H_b-pyridine, J = 5 Hz, J = 1.5 Hz), 7.7 (d, 1H, H_4, J = 8.5 Hz), 6.77 (dd, 1H, H_5, J = 8.5 Hz, J = 2.3 Hz), 6.76 (d, 1H, H_7, J = 2.3 Hz), 6.66 (s, 1H, C=CH), 3.94 (s, 3H, OMe), EI-MS m/z (%) 254 ((M^++1), 100), 225 (90), 34 (63).

4.1.7. (*Z*)-6-Ethoxy-2-(pyridin-4-ylmethylene) benzofuran-3(2*H*)-one (5b)

Starting from 6-ethoxybenzofuran-3(2*H*)-one **4b** (1.336 g, 5 mmol), pyridine-4-carboxaldehyde (7 mmol, 0.75 g) and *p*-toluenesulfonic acid (7.5 mmol, 1.3 g), compound **5b** was obtained, 83% yield, mp 205–207 °C, IR ν_{max}/cm^{-1} (KBr): 1703 (C=O), 1637 (C=C alkene), ^{1}H NMR ($CDCl_3$, 500 MHz), 8.69 (dd, 2H, H_a-pyridine, J = 4.5 Hz, J = 2 Hz), 7.707 (dd, 2H, H_b-pyridine, J = 5 Hz, J = 1.5 Hz), 7.70 (d, 1H, H_4, J = 8.5 Hz), 6.77 (dd, 1H, H_5, J = 8.5 Hz, J = 2.1 Hz), 6.76 (d, 1H, H_7, J = 2.1 Hz), 6.67 (s, 1H, C=CH), 4.17 (q, 2H, $-OCH_2-$, J = 6.8 Hz), 1.53 (t, 3H, $-CH_3$, J = 6.8 Hz). EI-MS m/z (%) 268 ((M^++1), 100), 224 (86), 46 (83).

4.1.8. (*Z*)-6-Propoxy-2-(pyridin-4-ylmethylene) benzofuran-3(2*H*)-one (5c)

Starting from 6-propoxybenzofuran-3(2*H*)-one **4c** (1.4 g, 5 mmol), pyridine-4-carboxaldehyde (7 mmol, 0.75 g) and *p*-toluenesulfonic acid (7.5 mmol, 1.3 g), compound **5b** was obtained, 72% yield, mp 209–212 °C, IR ν_{max}/cm^{-1} (KBr): 1705 (C=O), 1640 (C=C alkene), ^{1}H NMR ($CDCl_3$, 500 MHz), 8.69 (dd, 2H, H_a-pyridine, J = 4.5 Hz, J = 2 Hz), 7.707 (dd, 2H, H_b-pyridine, J = 5 Hz, J = 1.5 Hz), 7.69 (d, 1H, H_4, J = 8.4 Hz), 6.77 (dd, 1H, H_5, J = 8.4 Hz, J = 2.3 Hz), 6.76 (d, 1H, H_7, J = 2.3 Hz), 6.69 (s, 1H, C=CH), 3.97 (t, 2H, OCH_2, J = 6.5 Hz), 1.86–1.82 (m, 2H, $-CH_2-$), 1.05 (t, 3H, $-CH_3$, J = 6.5 Hz), EI-MS m/z (%) 282.11 ((M^++1), 100), 223 (77), 60 (83).

4.1.9. General procedure for synthesis of 1-benzyl-4-((6-alkoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium bromide and chloride (6a–u)

Dry acetonitrile (7 ml) was added to (*Z*)-6-alkoxy-2-(pyridin-4-ylmethylene) benzofuran-3(2*H*)-one (**5a**–**c**) (1 equiv), and the mixture was dissolved by heating under reflux. Then different benzyl bromide or benzyl chloride derivatives (1.2 equiv) were added thereto. After heating under reflux condition for 2–3 h, it was left for cooling to room temperature and then evaporated. *n*-Hexane (15 ml) was added to the residue. The precipitated crystals were separated by filtration, washed with *n*-hexane and dried. The crystals were further purified if needed by flash chromatography using chloroform–methanol (99:1) as the mobile phase to afford compounds **6a**–**u**.

4.1.10. (*Z*)-1-Benzyl-4-((6-methoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium bromide (6a)

Starting from (*Z*)-6-methoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.253 g) and benzyl bromide (1.2 mmol, 0.205 g), compound **6a** was obtained, quantitative yield, mp 252–254 °C, IR ν_{max}/cm^{-1} (KBr): 1708 (C=O), 1635 (C=C alkene), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.21 (d, 2H, H_a-pyridine, J = 6 Hz), 8.52 (d, 2H, H_b-pyridine, J = 6.1 Hz), 7.75 (d, 1H, H_4, J = 8.3 Hz), 7.54–7.44 (m, 5H, Ph), 7.16 (d, 1H, H_7, J = 2.2 Hz), 7.07 (s, 1H, C=CH), 6.94 (dd, 1H, H_5, J = 8.3 Hz, J = 2.2 Hz), 5.86 (s, 2H, $-CH_2N$), 3.95 (s, 3H, OMe). Anal. Calcd for $C_{22}H_{18}BrNO_3$: C, 62.28; H, 4.28; N, 3.30. Found: C, 62.12; H, 4.29; N, 3.29. EI-MS m/z (%) 344 (M^+, 9), 343 (31), 252 (68), 238 (53), 151 (49), 91 (100), 63 (56).

4.1.11. (*Z*)-1-(4-Fluorobenzyl)-4-((6-methoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium bromide (6d)

Starting from (*Z*)-6-methoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.253 g) and 4-fluorobenzyl bromide (1.2 mmol, 0.226 g), compound **6d** was obtained, quantitative yield, mp 216–219 °C, IR ν_{max}/cm^{-1} (KBr): 1697 (C=O), 1643 (C=C alkene), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.22 (d, 2H, H_a-pyridine, J = 6.6 Hz), 8.51 (d, 2H, H_b-pyridine, J = 6.75 Hz), 7.77 (d, 1H, H_4, J = 8.6 Hz), 7.67–7.64 (m, 2H, Ph), 7.32–7.28 (m, 2H, Ph), 7.16 (d, 1H, H_7, J = 2 Hz), 7.04 (s, 1H, C=CH), 6.94 (dd, 1H, H_5, J = 2 Hz, J = 8.6 Hz), 5.85 (s, 2H, $-CH_2N$), 3.95 (s, 3H, OMe). Anal. Calcd for $C_{22}H_{17}BrFNO_3$: C, 59.74; H, 3.87; N, 3.17. Found: C, 59.65; H, 3.68; N, 3.18. EI-MS m/z (%) 362 (M^+, 2), 283 (90), 268 (40), 142 (60), 109 (8), 91 (100).

4.1.12. (*Z*)-1-(4-Fluorobenzyl)-4-((6-ethoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium bromide (6k)

Starting from (*Z*)-6-ethoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.267 g) and 4-fluorobenzyl bromide (1.2 mmol, 0.226 g), compound **6k** was obtained, quantitative yield, mp 232–235 °C, IR ν_{max}/cm^{-1} (KBr): 1693 (C=O), 1640 (C=C alkene), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.25 (d, 2H, H_a-pyridine, J = 6.75 Hz), 8.52 (d, 2H, H_b-pyridine, J = 6.75 Hz), 7.76 (d, 1H, H_4, J = 8.5 Hz), 7.70–7.67 (m, 2H, Ph), 7.33–7.30 (m, 2H, Ph), 7.18 (d, 1H, H_7, J = 2 Hz,), 7.03 (s, 1H, C=CH), 6.92 (dd, 1H, H_5, J = 8.5, Hz J = 2 Hz), 5.88 (s, 2H, $-CH_2N$), 4.25 (q, 2H, OCH_2, J = 6.8 Hz), 1.39 (t, 2H, $-CH_3$, J = 6.8 Hz). Anal. Calcd for $C_{23}H_{19}BrFNO_3$: C, 60.54; H, 4.20; N, 3.07. Found: C, 60.35; H, 4.19; N, 3.09. EI-MS m/z (%) 376 (M^+, 5), 369 (15), 267 (30) 238 (41), 109 (100), 63 (23).

4.1.13. (*Z*)-1-Benzyl-4-((6-ethoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium bromide (6h)

Starting from (*Z*)-6-ethoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.267 g) and benzyl bromide (1.2 mmol, 0.205 g), compound **6h** was obtained, quantitative yield, mp 220–223 °C, IR ν_{max}/cm^{-1} (KBr): 1693 (C=O), 1646 (C=C alkene), ^{1}H NMR (DMSO-d_6, 500 MHz), 9.22 (d, 2H, H_a-pyridine, J = 6.7 Hz), 8.52 (d, 2H, H_b-pyridine, J = 6.65 Hz), 7.78 (d, 1H, H_4, J = 8.6 Hz), 7.56–7.55 (m, 2H, Ph), 7.47–7.46 (m, 3H, Ph), 7.16 (d, 1H, H_7, J = 2 Hz), 7.05 (s, 1H, C=CH), 6.93 (dd, 1H, H_5, J = 8.6 Hz, J = 2 Hz), 5.86 (s, 2H, $-CH_2N$), 4.25 (q, 3H, OCH_2, J = 6.8 Hz),

1.4 (t, 2H, $-CH_3$, J = 6.8 Hz). Anal. Calcd for $C_{23}H_{20}BrNO_3$: C, 63.02; H, 4.60; N, 3.07. Found: C, 63.23; H, 4.62; N, 3.18. EI-MS m/z (%) 358 (M^+, 5), 357 (21), 267 (68), 165 (65), 91 (100), 65 (57).

4.1.14. (*Z*)-1-Benzyl-4-((6-propoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium bromide (6o)

Starting from (*Z*)-6-propoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.281 g) and benzyl bromide (1.2 mmol, 0.205 g), compound **6o** was obtained, quantitative yield, mp 215–218 °C, IR ν_{max}/cm^{-1} (KBr): 1704 (C=O), 1637 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.27 (d, 2H, H_a-pyridine, J = 6.6 Hz), 8.52 (d, 2H, H_b-pyridine, J = 6.6 Hz), 7.75 (d, 1H, H_4, J = 8.6 Hz), 7.58–7.57 (m, 2H, Ph), 7.47–7.44 (m, 3H, Ph), 7.18 (d, 1H, H_7, J = 2.1 Hz), 7.04 (s, 1H, C=CH), 6.92 (dd, 1H, H_5, J = 8.6 Hz, J = 2.1 Hz), 5.9 (s, 2H, $-CH_2N$), 4.15 (t, 2H, OCH_2, J = 6.5 Hz), 1.79 (m, 2H, $-CH_2-$), 1.00 (t, 3H, $-CH_3$, J = 6.5 Hz). Anal. Calcd for $C_{24}H_{22}BrNO_3$: C, 63.73; H, 4.90; N, 3.10. Found: C, 63.92; H, 4.88; N, 3.33. EI-MS m/z (%) 372 (M^+, 2), 211 (22), 281 (18) 137 (36), 91 (100), 43 (45).

4.1.15. (*Z*)-1-(4-Fluorobenzyl)-4-((6-propoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium bromide (6r)

Starting from (*Z*)-6-propoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.281 g) and 4-fluorobenzyl bromide (1.2 mmol, 0.226 g), compound **6r** was obtained, quantitative yield, mp 233–235 °C, IR ν_{max}/cm^{-1} (KBr): 1709 (C=O), 1638 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.27 (d, 2H, H_a-pyridine, J = 6.6 Hz), 8.52 (d, 2H, H_b-pyridine, J = 6.6 Hz), 7.76 (d, 1H, H_4, J = 8.6 Hz), 7.71–7.68 (m, 2H, Ph), 7.33–7.29 (m, 2H, Ph), 7.2 (d, 1H, H_7, J = 1.9 Hz), 7.03 (s, 1H, C=CH), 6.92 (dd, 1H, H_5, J = 8.6 Hz, J = 1.9 Hz), 5.9 (s, 2H, $-CH_2N$), 4.14 (t, 2H, OCH_2, J = 6.5 Hz), 1.79 (m, 2H, $-CH_2-$), 1.00 (t, 3H, $-CH_3$, J = 6.5 Hz). Anal. Calcd for $C_{24}H_{21}BrFNO_3$: C, 61.29; H, 4.50; N, 2.98. Found: C, 61.05; H, 4.51; N, 3.01. EI-MS m/z (%) 390 (M^+, 3), 383 (23), 314 (20), 136 (47), 109 (73), 43 (100).

4.1.16. (*Z*)-1-(3-Methylbenzyl)-4-((6-methoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6f)

Starting from (*Z*)-6-methoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.253 g) and 3-methylbenzyl chloride (1.2 mmol, 0.169 g), compound **6f** was obtained, quantitative yield, mp 233–235 °C, IR ν_{max}/cm^{-1} (KBr): 1704 (C=O), 1645 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.25 (d, 2H, H_a-pyridine, J = 6.40 Hz), 8.51 (d, 2H, H_b-pyridine, J = 6.45 Hz), 7.76 (d, 1H, H_4, J = 8.55 Hz), 7.38–7.31 (m, 3H, Ph), 7.23 (s, 1H, Ph), 7.17 (d, 1H, H_7, J = 2.1 Hz), 7.03 (s, 1H, C=CH), 6.93 (dd, 1H, H_5, J = 8.55 Hz, J =2.1 Hz), 5.83 (s, 2H, $-CH_2N$), 3.94 (s, 3H, OMe), 2.3 (s, 3H, $-CH_3$). Anal. Calcd for $C_{23}H_{20}ClNO_3$: C, 70.14; H, 5.12; N, 3.56. Found: C, 70.56; H, 5.14; N, 3.55. EI-MS m/z (%) 359 (M^+, 4), 321 (90), 150 (100), 105 (60).

4.1.17. (*Z*)-1-(2-Fluorobenzyl)-4-((6-methoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6b)

Starting from (*Z*)-6-methoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.253 g) and 2-fluorobenzyl chloride (1.2 mmol, 0.173 g), compound **6b** was obtained, quantitative yield, mp 247–250 °C, IR ν_{max}/cm^{-1} (KBr): 1712 (C=O), 1635 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.19 (d, 2H, H_a-pyridine, J = 5.80 Hz), 8.53 (d, 2H, H_b-pyridine, J = 5.95 Hz), 7.77 (d, 1H, H_4, J = 8.35 Hz), 7.65–7.62 (m, 1H, Ph), 7.53–7.51 (m, 1H, Ph), 7.34–7.31 (m, 2H, Ph), 7.16 (d, 1H, H_7, J = 2.2 Hz), 7.05 (s, 1H, C=CH), 6.93 (dd, 1H, H_5, J = 8.35 Hz, J = 2.2 Hz), 5.98 (s, 2H, $-CH_2N$), 3.95 (s, 3H, OMe). Anal. Calcd for $C_{22}H_{17}ClFNO_3$: C, 66.42; H, 4.31; N, 3.52. Found: C, 66.25; H, 4.33; N, 3.52. EI-MS m/z (%) 362 (M^+, 3), 283 (93), 268 (35), 142 (56), 109 (11), 91 (100).

4.1.18. (*Z*)-1-(3-Fluorobenzyl)-4-((6-methoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6c)

Starting from (*Z*)-6-methoxy-2-(pyridin-4-ylmethylene) benzofuran-3(2*H*)-one (1 mmol, 0.253 g) and 3-fluorobenzyl chloride (1.2 mmol, 0.173 g), compound **6c** was obtained, quantitative yield, mp 231–234 °C, IR ν_{max}/cm^{-1} (KBr): 1697 (C=O), 1639 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.29 (d, 2H, H_a-pyridine, J = 6 Hz), 8.52 (d, 2H, H_b-pyridine, J = 6.2 Hz), 7.77 (d, 1H, H_4, J = 8.5 Hz), 7.51–7.50 (m, 2H, Ph), 7.43–7.41 (m, 1H, Ph), 7.29–7.26 (m, 1H, Ph), 7.18 (d, 1H, H_7, J = 2.3 Hz), 7.04 (s, 1H, C=CH), 6.93 (dd, 1H, H_5, J = 8.5 Hz, J = 2.3 Hz), 5.9 (s, 2H, $-CH_2N$), 3.95 (s, 3H, OMe). Anal. Calcd for $C_{22}H_{17}ClFNO_3$: C, 66.42; H, 4.31; N, 3.52. Found: C, 66.34; H, 4.33; N, 3.51. EI-MS m/z (%) 362 (M^+, 2), 283 (87), 268 (44), 142 (63), 109 (8), 91 (100).

4.1.19. (*Z*)-1-(2-Methylbenzyl)-4-((6-methoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6e)

Starting from (*Z*)-6-methoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.253 g) and 2-methylbenzyl chloride (1.2 mmol, 0.169 g), compound **6e** was obtained, quantitative yield, mp 227–230 °C, IR ν_{max}/cm^{-1} (KBr): 1712 (C=O), 1637 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.08 (d, 2H, H_a-pyridine, J = 6.85 Hz), 8.52 (d, 2H, H_b-pyridine, J = 6.80 Hz), 7.8 (s, 1H, Ph), 7.74 (d, 1H, H_4, J = 8.6 Hz), 7.42–7.35 (m, 1H, Ph), 7.31 (d, 1H, H_7, J = 1.9 Hz), 7.30–7.29 (m, 2H, Ph), 6.99 (s, 1H, C=CH), 6.9 (dd, 1H, H_5, J = 8.6 Hz, J = 1.9 Hz), 6.01 (s, 2H, $-CH_2N$), 3.99 (s, 3H, OMe), 2.35 (s, 3H, $-CH_3$). Anal. Calcd for $C_{23}H_{20}ClNO_3$: C, 70.14; H, 5.12; N, 3.56. Found: C, 69.87; H, 5.11; N, 3.54. EI-MS m/z (%) 359 (M^+, 5), 321 (93), 150 (100), 105 (60).

4.1.20. (*Z*)-1-(4-Methylbenzyl)-4-((6-methoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6g)

Starting from (*Z*)-6-methoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.253 g) and 4-methylbenzyl chloride (1.2 mmol, 0.169 g), compound **6g** was obtained, quantitative yield, mp 234–237 °C, IR ν_{max}/cm^{-1} (KBr): 1701 (C=O), 1646 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.24 (d, 2H, H_a-pyridine, J = 6.4 Hz), 8.49 (d, 2H, H_b-pyridine, J = 6.5 Hz), 7.77 (d, 1H, H_4, J = 8 Hz), 7.47 (d, 2H, J = 6.3 Hz, Ph), 7.26 (d, 2H, J = 6.3 Hz, Ph), 7.18 (d, 1H, H_7, J = 2 Hz), 7.03 (s, 1H, C=CH), 6.93 (dd, 1H, H_5, J = 8 Hz, J = 2 Hz), 5.83 (s, 2H, $-CH_2N$), 3.95 (s, 3H, OMe), 2.29 (s, 3H, $-CH_3$). Anal. Calcd for $C_{23}H_{20}ClNO_3$: C, 70.14; H, 5.12; N, 3.56. Found: C, 70.42; H, 5.12; N, 3.57. EI-MS m/z (%) 359 (M^+, 3), 321 (88), 150 (100), 105 (63).

4.1.21. (*Z*)-1-(4-Methylbenzyl)-4-((6-ethoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6n)

Starting from (*Z*)-6-ethoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.267 g) and 4-methylbenzyl chloride (1.2 mmol, 0.169 g), compound **6n** was obtained, quantitative yield, mp 242–245 °C, IR ν_{max}/cm^{-1} (KBr): 1704 (C=O), 1645 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.25 (d, 2H, H_a-pyridine, J = 6.50 Hz), 8.51 (d, 2H, H_b-pyridine, J = 6.57 Hz), 7.77 (d, 1H, H_4, J = 7.9 Hz), 7.47 (d, 2H, Ph, J = 7.8 Hz), 7.27 (d, 2H, Ph, J = 7.8 Hz), 7.17 (d, 1H, H_7, J = 1.8 Hz), 7.04 (s, 1H, C=CH), 6.94 (dd, 1H, H_5, J = 7.9 Hz, J = 1.8 Hz), 5.83 (s, 2H, $-CH_2N$), 4.24 (q, 2H, $-OCH_2$, J = 6.8 Hz), 2.5 (s, 3H, $-CH_3$), 1.40 (t, 3H, $-CH_3$, J = 6.8 Hz). Anal. Calcd for $C_{24}H_{22}ClNO_3$: C, 70.67; H, 5.44; N, 3.43. Found: C, 70.43; H, 5.42; N, 3.44. EI-MS m/z (%) 372 (M^+, 5), 321 (40), 255 (35), 199 (50), 105 (12), 83 (36), 55 (86), 43 (100).

4.1.22. (*Z*)-1-(3-Methylbenzyl)-4-((6-ethoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6m)

Starting from (*Z*)-6-ethoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.267 g) and 3-methylbenzyl chloride (1.2 mmol, 0.169 g), compound **6m** was obtained, quantitative

yield, mp 244–247 °C, IR ν_{max}/cm^{-1} (KBr): 1705 (C=O), 1637 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.23 (d, 2H, H_a-pyridine, J = 6.45 Hz), 8.52 (d, 2H, H_b-pyridine, J = 6.45 Hz), 7.77 (d, 1H, H_4, J = 8.5 Hz), 7.36–7.28 (m, 3H, Ph), 7.25 (s, 1H, Ph), 7.16 (d, 1H, H_7, J = 2 Hz), 7.04 (s, 1H, C=CH), 6.95 (dd, 1H, H_5, J = 8.5 Hz, J = 2 Hz), 5.82 (s, 2H, $-CH_2N$), 4.23 (q, 2H, OCH_2, J = 6.8 Hz), 2.3 (s, 3H, $-CH_3$), 1.41 (t, 3H, $-CH_3$, J = 6.8 Hz). Anal. Calcd for $C_{24}H_{22}ClNO_3$: C, 70.67; H, 5.44; N, 3.43. Found: C, 70.92; H, 5.44; N, 3.42. EI-MS m/z (%) 372 (M^+, 6), 321 (46), 255 (40), 199 (50), 105 (12), 83 (33), 55 (88), 43 (100).

4.1.23. (*Z*)-1-(2-Fluorobenzyl)-4-((6-ethoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6i)

Starting from (*Z*)-6-ethoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.267 g) and 2-fluorobenzyl chloride (1.2 mmol, 0.173 g), compound **6i** was obtained, quantitative yield, mp 226–230 °C, IR ν_{max}/cm^{-1} (KBr): 1709 (C=O), 1635 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.18 (d, 2H, H_a-pyridine, J = 6.4 Hz), 8.54 (d, 2H, H_b-pyridine, J = 6.5 Hz), 7.76 (d, 1H, H_4, J = 8.6 Hz), 7.66–7.63 (m, 1H, Ph), 7.54–7.52 (m, 1H, Ph), 7.36–7.33 (m, 2H, Ph), 7.16 (d, 1H, H_7, J = 2.1 Hz), 7.04 (s, 1H, C=CH), 6.91 (dd, 1H, H_5, J = 8.6 Hz, J = 2.1 Hz), 5.99 (s, 2H, $-CH_2N$), 4.25 (q, 2H, OCH_2, J = 6.8 Hz), 1.39 (t, 3H, $-CH_3$, J = 6.8 Hz). Anal. Calcd for $C_{23}H_{19}ClFNO_3$: C, 67.07; H, 4.65; N, 3.40. Found: C, 67.14; H, 4.63; N, 3.42. EI-MS m/z (%) 376 (M^+, 5), 369 (17), 267 (32) 238 (54), 109 (100), 63 (14).

4.1.24. (*Z*)-1-(3-Fluorobenzyl)-4-((6-ethoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6j)

Starting from (*Z*)-6-ethoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.267 g) and 3-fluorobenzyl chloride (1.2 mmol, 0.173 g), compound **6j** was obtained, quantitative yield, mp 230–233 °C, IR ν_{max}/cm^{-1} (KBr): 1707 (C=O), 1643 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.17 (d, 2H, H_a-pyridine, J = 6.8 Hz), 8.53 (d, 2H, H_b-pyridine, J = 6.8 Hz), 7.77 (d, 1H, H_4, J = 8.5 Hz), 7.52–7.50 (m, 2H, Ph), 7.43–7.41 (m, 1H, Ph), 7.30–7.27 (m, 1H, Ph), 7.2 (d, 1H, H_7, J = 2 Hz), 7.04 (s, 1H, C=CH), 6.96 (dd, H_5, 1H, J = 8.5 Hz, J = 2 Hz), 5.91 (s, 2H, $-CH_2N$), 4.25 (q, 2H, OCH_2, J = 6.8 Hz), 1.39 (t, 3H, $-CH_3$, J = 6.8 Hz). Anal. Calcd for $C_{23}H_{19}ClFNO_3$: C, 67.07; H, 4.65; N, 3.40. Found: C, 66.89; H, 4.66; N, 3.41. EI-MS m/z (%) 376 (M^+, 8), 369 (17), 267 (36) 238 (51), 109 (100), 63 (23).

4.1.25. (*Z*)-1-(2-Methylbenzyl)-4-((6-ethoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6l)

Starting from (*Z*)-6-ethoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.267 g) and 2-methylbenzyl chloride (1.2 mmol, 0.169 g), compound **6l** was obtained, quantitative yield, mp 228–231 °C, IR ν_{max}/cm^{-1} (KBr): (C=O), 1637 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.09 (d, 2H, H_a-pyridine, J = 6.85 Hz), 8.55 (d, 2H, H_b-pyridine, J = 6.80 Hz), 7.77 (d, 1H, H_4, J = 8.5 Hz), 7.36–7.35 (m, 2H, Ph), 7.33 (d, 1H, H_7, J = 2.2 Hz), 7.18–7.15 (m, 2H, Ph), 7.08 (s, 1H, C=CH), 6.94 (dd, 1H, H_5, J = 8.5 Hz, J = 2.2 Hz), 5.94 (s, 2H, $-CH_2N$), 4.25 (q, 2H, OCH_2, J = 6.8 Hz), 2.33 (s, 3H, $-CH_3$), 1.39 (t, 3H, $-CH_3$, J = 6.8 Hz). Anal. Calcd for $C_{24}H_{22}ClNO_3$: C, 70.67; H, 5.44; N, 3.43. Found: C, 70.43; H, 5.45; N, 3.43. EI-MS m/z (%) 372 (M^+, 8), 321 (34), 255 (46), 199 (54), 105 (14), 83 (35), 55 (86), 43 (100).

4.1.26. (*Z*)-1-(2-Fluorobenzyl)-4-((6-propoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6p)

Starting from (*Z*)-6-propoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.281 g) and 2-fluorobenzyl chloride (1.2 mmol, 0.173 g), compound **6p** was obtained, quantitative yield, mp 222–225 °C, IR ν_{max}/cm^{-1} (KBr): 1701 (C=O), 1643 (C=C alkene), ^1H NMR (DMSO-d_6 500 MHz), 9.26 (d, 2H, H_a-pyridine, J = 6.80 Hz), 8.52 (d, 2H, H_b-pyridine, J = 6.55 Hz), 7.76 (d, 1H, H_4, J = 8.6 Hz), 7.65–7.62 (m, 1H, Ph), 7.54–7.52 (m, 1H, Ph), 7.34–7.31 (m, 2H, Ph), 7.14 (d, 1H, H_7, J = 2.4 Hz), 7.06 (s, 1H, C=CH), 6.88 (dd, 1H, H_5, J = 8.6 Hz, J = 2.4 Hz), 5.97 (s, 2H, $-CH_2N$), 4.15 (t, 2H, OCH_2, J = 6.5 Hz), 1.79 (m, 2H, $-CH_2-$), 1.00 (t, 2H, $-CH_3$, J = 6.5 Hz). Anal. Calcd for $C_{24}H_{21}ClFNO_3$: C, 67.69; H, 4.97; N, 3.29. Found: C, 67.78; H, 4.99; N, 3.30. EI-MS m/z (%) 390 (M^+, 5), 383 (23), 314 (24), 136 (40), 109 (70), 43 (100).

4.1.27. (*Z*)-1-(2-Methylbenzyl)-4-((6-propoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6s)

Starting from (*Z*)-6-propoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.281 g) and 2-methylbenzyl chloride (1.2 mmol, 0.169 g), compound **6s** was obtained, quantitative yield, mp 225–228 °C, IR ν_{max}/cm^{-1} (KBr): 1705 (C=O), 1644 (C=C alkene),^1H NMR (DMSO-d_6, 500 MHz), 9.09 (d, 2H, H_a-pyridine, J = 6.35 Hz), 8.55 (d, 2H, H_b-pyridine, J = 6.30 Hz), 7.77 (d, 1H, H_4, J = 8.55 Hz), 7.34–7.32 (m, 2H, Ph), 7.30 (d, 1H, H_7, J = 2.1 Hz), 7.18–7.16 (m, 2H, Ph), 7.08 (s, 1H, C=CH), 6.95 (dd, 1H, Hz, H_5, J = 8.55, J = 2.1 Hz), 5.94 (s, 2H, $-CH_2N$), 4.15 (t, 2H, OCH_2, J = 6.5 Hz), 2.33 (s, 3H, $-CH_3$), 1.79 (m, 2H, $-CH_2-$), 1.00 (t, 2H, $-CH_3$, J = 6.5 Hz). Anal. Calcd for $C_{25}H_{24}ClNO_3$: C, 71.17; H, 5.73; N, 3.32. Found: C, 70.90; H, 5.71; N, 3.31. EI-MS m/z (%) 386 (M^+, 6), 281 (48), 238 (82), 105 (100), 79 (22), 43 (16).

4.1.28. (*Z*)-1-(4-Methylbenzyl)-4-((6-propoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6u)

Starting from (*Z*)-6-propoxy-2-(pyridin-4-ylmethylene) benzofuran-3(2*H*)-one (1 mmol, 0.281 g) and 4-methylbenzyl chloride (1.2 mmol, 0.169 g), compound **6u** was obtained, quantitative yield, mp 234–238 °C, IR ν_{max}/cm^{-1} (KBr): 1709 (C=O), 1640 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.19 (d, 2H, H_a-pyridine, J = 6.55 Hz), 8.51 (d, 2H, H_b-pyridine, J = 6.50 Hz), 7.77 (d, 1H, H_4, J = 8 Hz), 7.45 (d, 2H, Ph, J = 7.9 Hz), 7.27 (d, 2H, Ph, J = 7.9 Hz), 7.17 (d, 1H, H_7, J = 2 Hz), 7.04 (s, 1H, C=CH), 6.93 (dd, 1H, H_5, J = 8 Hz, J = 2 Hz), 5.8 (s, 2H, $-CH_2N$), 4.15 (t, 2H, OCH_2, J = 6.5 Hz), 2.31 (s, 3H, $-CH_3$), 1.79 (m, 2H, $-CH_2-$), 1.00 (t, 2H, $-CH_3$, J = 6.5 Hz). Anal. Calcd for $C_{25}H_{24}ClNO_3$: C, 71.17; H, 5.73; N, 3.32. Found: C, 71.34; H, 5.73; N, 3.30. EI-MS m/z (%) 386 (M^+, 9), 281 (50), 238 (84), 105 (100), 79 (22), 43 (15).

4.1.29. (*Z*)-1-(3-Fluorobenzyl)-4-((6-propoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6q)

Starting from (*Z*)-6-propoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.281 g) and 3-fluorobenzyl chloride (1.2 mmol, 0.173 g), compound **6q** was obtained, quantitative yield, mp 224–227 °C, IR ν_{max}/cm^{-1} (KBr): 3011 1705 (C=O), 1642 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.25 (d, 2H, H_a-pyridine, J = 6.8 Hz), 8.53 (d, 2H, H_b-pyridine, J = 6.8 Hz), 7.76 (d, 1H, H_4, J = 8.6 Hz), 7.53–7.50 (m, 2H, Ph), 7.43–7.41 (m, 1H, Ph), 7.29–7.26 (m, 1H, Ph), 7.2 (d, 1H, H_7, J = 2 Hz), 7.04 (s, 1H, C=CH), 6.93 (dd, 1H, H_5, J = 8.6 Hz, J = 2 Hz), 5.91 (s, 2H, $-CH_2N$), 4.15 (t, 2H, OCH_2, J = 6.5 Hz), 1.79 (m, 2H, $-CH_2-$), 1.00 (t, 2H, $-CH_3$, J = 6.5 Hz). Anal. Calcd for $C_{24}H_{21}ClFNO_3$: C, 67.69; H, 4.97; N, 3.29. Found: C, 67.86; H, 4.95; N, 3.30. EI-MS m/z (%) 390 (M^+, 5), 383 (20), 314 (23), 136 (43), 109 (70), 43 (100).

4.1.30. (*Z*)-1-(3-Methylbenzyl)-4-((6-propoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium chloride (6t)

Starting from (*Z*)-6-propoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (1 mmol, 0.281 g) and 3-methylbenzyl chloride (1.2 mmol, 0.169 g), compound **6t** was obtained, quantitative yield, mp 238–241 °C, IR ν_{max}/cm^{-1} (KBr): 1704 (C=O), 1645 (C=C alkene), ^1H NMR (DMSO-d_6, 500 MHz), 9.2 (d, 2H, H_a-pyridine, J = 6.4 Hz), 8.5 (d, 2H, H_b-pyridine, J = 6. 5 Hz), 7.75 (d, 1H, H_4, J = 8.5 Hz), 7.33–7.29 (m, 3H, Ph), 7.21 (s, 1H, Ph), 7.16 (d, 1H, H_7,

J = 2.3 Hz), 7.02 (s, 1H, C=CH), 6.95 (dd, 1H, H_5, J = 8.5 Hz, J = 2.3 Hz), 5.80 (s, 2H, $-CH_2N$), 4.15 (t, 2H, OCH_2, J = 6.5 Hz), 2.3 (s, 3H, $-CH_3$), 1.79 (m, 2H, $-CH_2-$), 1.00 (t, 2H, $-CH_3$, J = 6.5 Hz). Anal. Calcd for $C_{25}H_{24}ClNO_3$: C, 71.17; H, 5.73; N, 3.32. Found: C, 70.91; H, 5.71; N, 3.32. EI-MS m/z (%) 386 (M^+, 4), 281 (54), 238 (80), 105 (100), 79 (24), 43 (17).

4.2. Biological activity

4.2.1. In vitro inhibition studies on AChE

Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from *electric eel*, 1000 unit) was purchased from Sigma-Aldrich (Steinheim, Germany). 5,5′-Dithiobis-(2-nitrobenzoic acid), potassium dihydrogen phosphate, dipotassium hydrogen phosphate, potassium hydroxide, sodium hydrogen carbonate, and acetylthiocholine iodide were obtained from Fluka (Buchs, Switzerland). Compounds were dissolved in a mixture of 20 ml distilled water and 5 ml methanol and then diluted in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0) to afford a final concentration range.

The method of Ellman et al. was carried out. Prior to use all solutions were adjusted to 25 °C. To obtain 20–80% inhibition of AChE activity five different concentrations of each compound were tested. The assay solution consisted of a 0.1 M potassium phosphate buffer pH 8.0, with the addition of 0.01 M 5,5′-dithio-bis(2-nitrobenzoic acid), 2.5 unit/mL of enzyme solution (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from *electric eel*) (Sigma Chemical).

Test compounds were added to the assay solution and preincubated at 25 °C with the enzyme for 15 min followed by adding 0.075 M substrate (acetylthiocholine iodide). After rapid and immediate mixing the change of absorption was measured at 412 nm. In order to justify non-enzymatic reaction assays were carried out with a blank containing all components except AChE. The blank reading contained 3 ml buffer, 200 µl water, 100 µl DTNB and 20 µl substrate. The reaction rates were calculated, and the percent inhibition of test compounds was determined. Each concentration was analyzed in triplicate, and IC_{50} values were determined graphically from inhibition curves (log inhibitor concentration vs percent of inhibition). Spectrophotometric measurements were performed on a Cecil BioAquarius CE 7250 Double Beam Spectrophotometer. Details of the inhibition study are given in Kapková et al.[22]

Acknowledgments

This work was supported by grants from the research council of Tehran University of Medical Sciences and Iran National Science Foundation (INSF).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.07.012. These data include MOL files and InChiKeys of the most important compounds described in this article.

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