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Synthesis and Evaluation of Chroman-4-One Linked to *N*-Benzyl Pyridinium Derivatives as New Acetylcholinesterase Inhibitors

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A novel series of chroman-4-one derivatives containing the *N*-benzyl pyridinium moiety were designed, synthesized, and evaluated for their acetylcholinesterase (AChE) inhibitory activities. Among the various synthesized compounds, (*E*)-1-(2,3-dibromobenzyl)-4-((7-ethoxy-4-oxochroman-3-ylidene)methyl)pyridinium bromide (**8l**) depicted the most potent anti-AChE activity ($IC_{50} = 0.048\ \mu M$). In addition, the molecular modeling study allowed us to detect possible binding modes that are in full compliance with the observed results through *in vitro* experiments.

Keywords: Acetylcholinesterase / Alzheimer's disease / Chroman-4-one / Docking study / *N*-Benzyl pyridinium

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Introduction

Alzheimer's disease (AD) is an age-related disease leading to progressive decline in cognition and memory [1]. There are two main hypotheses in the pathogenesis of AD: reduced levels of acetylcholine and the polymerization of the amyloid β-peptide (Aβ) in the brain [2]. Therefore, increasing the synaptic level of acetylcholine by means of acetylcholinesterase inhibitors such as donepezil, rivastigmine, and galantamine could be beneficial to ameliorate the symptoms of AD without any effect in the course of the disease [3]. The suitable route to prevent the disease progression has been reported by the assistance of various compounds with anti-amyloid potency such as melatonin, rifampicin, benzofuran, and acridinone derivatives which are in clinical trial [4].

It is well known that AChE, in addition to the hydrolysis of acetylcholine, interacts with Aβ through peripheral anionic site (PAS) and accelerates the polymerization of Aβ peptides [5–7]. Thus, applying dual binding site AChE inhibitors which interact with both active site and PAS, would be a new therapeutic approach for seeking new drug candidates against AD [8, 9].

According to molecular and biological studies on donepezil (Fig. 1A), the most popular approved therapies for AD, this drug could be regarded as a dual binding site AChE inhibitor [10, 11]. Docking study evidences have also shown that donepezil interacts with the catalytic site and PAS through benzyl piperidine moiety and indanone ring, respectively [12].

In recent years, our research group has reported the synthesis of various new AChE inhibitors [13–16]. In this study, we decided to design efficient dual AChE inhibitors based on our previous reports on the potent donepezil analogs (Fig. 1B and C) [17–19]. Accordingly, a novel series of *N*-benzyl-

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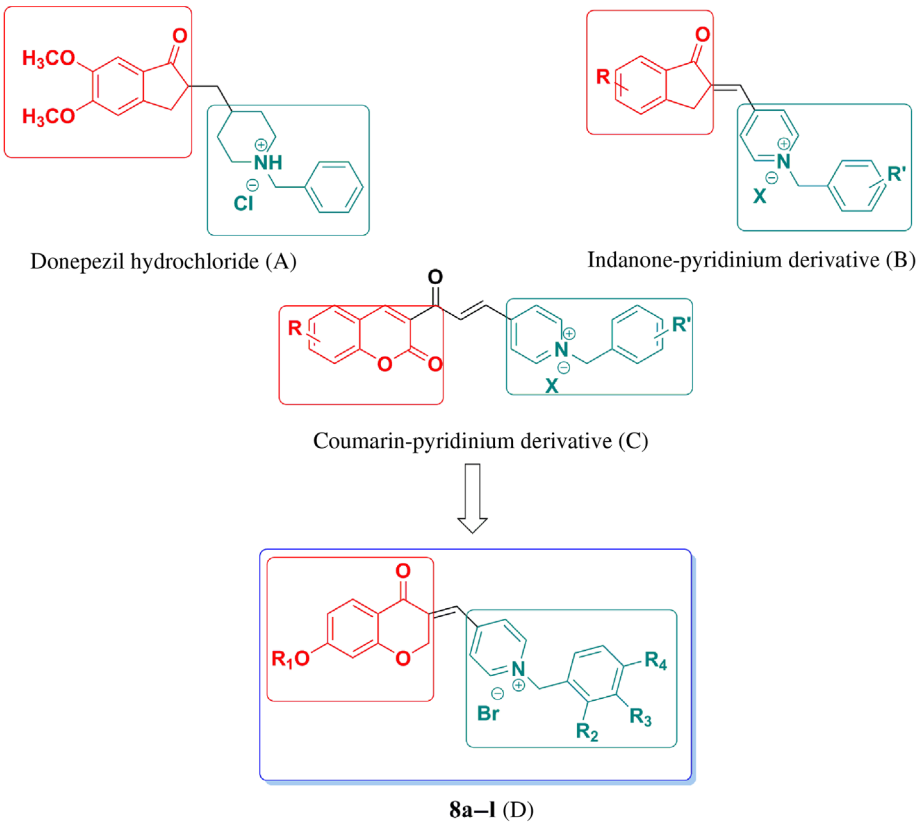


Figure 1. Structures of donepezil hydrochloride (A), pyridinium containing AChE inhibitors (B, C), and designed compounds **8a–l** (D).

4-((7-alkoxy-4-oxochroman-3-ylidene)methyl)pyridinium bromide derivatives **8a–l** (Fig. 1D) have been designed, synthesized, and evaluated as AChE inhibitors.

Results and discussion

Chemistry

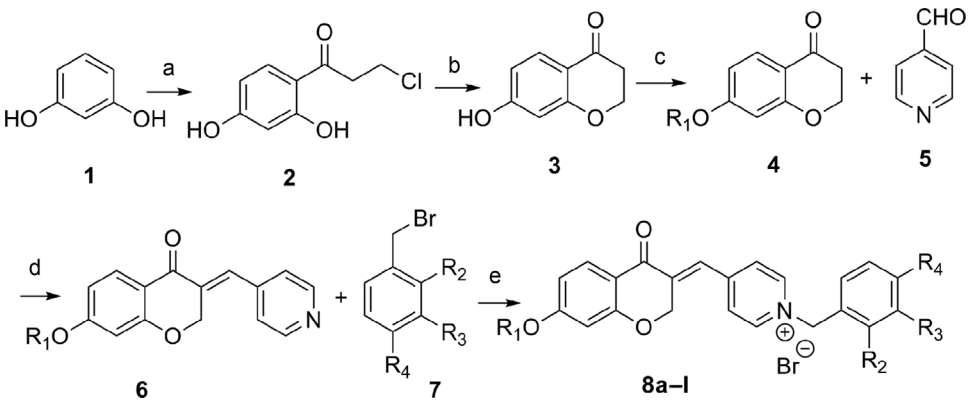
The reaction of resorcinol **1** with 3-chloropropionic acid using trifluoromethane sulfonic acid produced 3-chloro-1-(2,4-dihydroxyphenyl)propan-1-one **2** which upon cyclization in basic solution afforded 7-hydroxychroman-4-one **3** [20]. The *O*-alkylation step by the action of alkyl halide in dry *N,N*-dimethylformamide (DMF) produced **4** in good yield. Then, compound **4** was subjected to the condensation with isonicotinic aldehyde **5** in the presence of *p*-toluenesulfonic acid (PTSA) followed by *N*-alkylation using benzyl bromide derivatives resulting in the formation of **8a–l** in good yields (Scheme 1).

Inhibitory activity against AChE

The *in vitro* activity of all compounds **8a–l** for AChE inhibitory activity was evaluated by Ellman's method and compared with donepezil as the standard drug [21]. All data were expressed as mean ± SE of three independent experiments. The anticholinesterase activities (μM) of two series 7-methoxy and 7-ethoxy chroman-4-one derivatives (**8a–f** and **8g–l**, respectively) are shown in Table 1. Based on the enzyme inhibition data, the target compounds showed significant inhibitory activities toward acetylcholinesterase at concentrations less than 7.24 μM. Among **8l**, **8k**, **8h**, **8a**, **8f**, and **8e** with satisfactory results, **8l** from the second series of compounds with IC_{50} value of 0.048 μM was found as the most potent compound. Interestingly, 7-ethoxy derivatives like **8l** and **8k** showed better effects with respect to their corresponding 7-methoxy analogs (**8f** and **8e**).

In the first series of target compounds, **8f** with two bromine substituents at *ortho*- and *meta*-position of pendent benzyl group revealed the most inhibitory effect against AChE (IC_{50} = 0.128 μM). In this category, un-substituted benzyl **8a** (IC_{50} = 0.194 μM) and 2-bromo benzyl derivative **8e** (IC_{50} = 0.502 μM) exhibited good inhibitory effects, respectively. On the other hand, presence of fluorine or methyl group in position 4 of pendent benzyl group (**8b**, **8d)** led to the reduction in anti-AChE activity (IC_{50} = 3.814 and 7.242 μM, respectively) compared to other compounds in this category.

In the second series (7-ethoxy derivatives, **8g–l**), the compound **8l** with 2,3-dibromo substituents on pendent

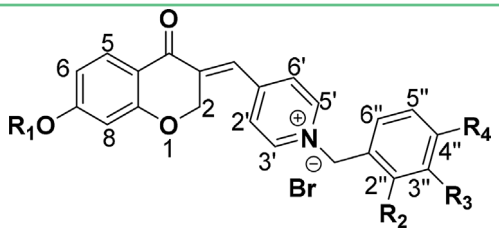


Scheme 1. Reagents and conditions: (a) 3-Chloropropionic acid, CF_3SO_3H, 80°C, 30 min; (b) 2.0 M NaOH, 5°C→r.t., 2 h; (c) alkyl halide, K_2CO_3, DMF, 80°C, 3 h; (d) PTSA, toluene, reflux Dean–Stark, 6 h; (e) acetonitrile, reflux, 1–3 h.

benzyl group exhibited considerable inhibitory activity (IC_{50} = 0.046 μM) compared to donepezil (IC_{50} = 0.022 μM). As well, the presence of a bromine substituent in position 2 of pendent benzyl group in **8k** was found beneficial (IC_{50} = 0.058 μM), while **8j** with fluorine substituent in the same position was the weakest halo-substituted compound in this series (IC_{50} = 0.463 μM). Moreover, the introduction of 4-chloro on *N*-benzyl ring showed good anti-AChE potency as observed in compound **8h** (IC_{50} = 0.091 μM). The presence of 4-nitro substituent on the *N*-benzyl group instead of chlorine in **8i** led to the decreased inhibitory activity in comparison to the compound **8h**.

The results showed that inhibitory effects of designed compounds **8a–l** against AChE enzyme were dependent on the type of alkoxy group in the position 7 of chroman-4-one as well as type and the substitution pattern on the pendent benzyl group of pyridinium ring. The presence of the ethoxy group in second series imparted higher activity in comparison

Table 1. Acetylcholinesterase inhibition IC_{50} (nM) of compounds 8a–l.



Entry	R_1	R_2	R_3	R_4	IC_{50} (μM)[a]
8a	CH_3	H	H	H	0.194 ± 0.43
8b	CH_3	H	H	F	3.81 ± 0.003
8c	CH_3	H	H	Br	N.d
8d	CH_3	H	H	CH_3	7.24 ± 0.02
8e	CH_3	Br	H	H	0.502 ± 0.031
8f	CH_3	Br	Br	H	0.128 ± 0.001
8g	CH_2CH_3	H	H	H	N.d
8h	CH_2CH_3	H	H	Cl	0.091 ± 0.012
8i	CH_2CH_3	H	H	NO_2	2.713 ± 0.34
8j	CH_2CH_3	F	H	H	0.463 ± 0.017
8k	CH_2CH_3	Br	H	H	0.058 ± 0.23
8l	CH_2CH_3	Br	Br	H	0.048 ± 0.052
Donepezil hydrochloride					0.022 ± 0.00

[a] Values are the mean ± SD. All experiments were performed at least three times.

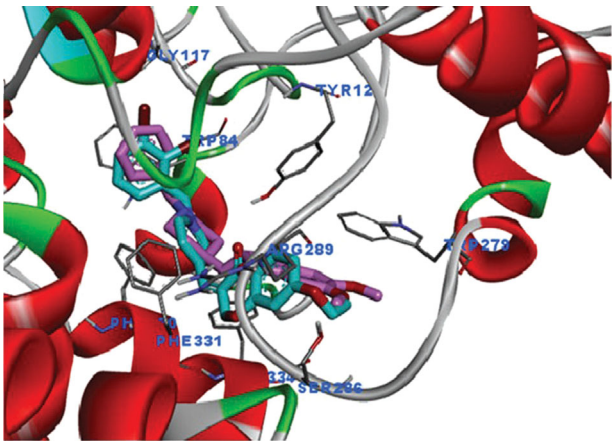


Figure 2. Superposed structure of **8l** (cyan) and donepezil (pink) inside the active site of AChE.

to methoxy analogs. On the other hand, it seems that the bromine substituent had a positive effect on anti-AChE activity of these compounds.

Docking studies

To gain a perspective on the interaction of the target compounds with the active site of the AChE enzyme, docking studies were performed by Autodock Tools (1.5.6) and Discovery Studio 4.0 Client. In this study, among several available crystal structures of the acetylcholine esterase enzyme in the RCSB protein data bank (http://www.rcsb.org/pdb/home/home.do), PDB structure of 1EVE complexed with donepezil was retrieved for docking purpose. Among the synthesized compounds, compound **8l** as the most potent compound was subjected to docking studies. Figure 2 shows a superposition of the best pose of **8l** and the co-crystallized donepezil in the active site of AChE.

The possible interactions of **8l** with amino acid residues in the active site of enzyme obtained from molecular modeling are depicted in Fig. 3. Trp84, Phe330, and Tyr334 are responsible for the interactions of **8l** with CAS. The π–π stacking interactions between the pendant benzyl group and pyridinium ring with aromatic moieties of Trp84 and Phe330, respectively, and π-cation interaction of the positively charged nitrogen with Tyr334 are considered as important interactions in CAS. Hydrogen bonding between carbonyl moiety of chroman-4-one and hydroxyl group of Tyr121 accompanied by the hydrophobic interaction between 7-ethoxy group of chroman-4-one and Trp279 was detected in the PAS [22]. Compounds **8l** and **8f** with 2,3-dibromo substituents on pendent benzyl group showed the best effect in each series. According to Fig. 3, it is clear that 3-bromine substituent in **8l** shows a weak interaction with carbonyl group of active site which is in accordance with the obtained results through *in vitro* experiments [23].

Conclusion

In this study, we found some new derivatives of the chroman-4-one linked to benzyl pyridinium group as new acetylcholinesterase inhibitors with moderate to high effects. According to the available biological data, anti-AChE activity of these compounds was sensitive to the type of alkoxy group in position 7 of chroman-4-one as well as the type and pattern of substitution on the pendent benzyl group of pyridinium ring. Structure–affinity relationships of synthesized compounds were explained by docking simulations.

Experimental

Chemistry

Melting points were determined with a Kofler hot stage apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker FT-500, using TMS as an internal standard. IR spectra were recorded on a Nicolet Magna FTIR 550 spectrophotometer using KBr disks. Mass spectra were obtained with an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. Elemental analysis for C, H, and N was determined with an Elementar Analysensysteme GmbH VarioEL CHNS mode.

*General procedure for the synthesis of 7-alkoxychroman-4-one (**4**)*

To a mixture of 7-hydroxychroman-4-one **3** (1 mmol) and potassium carbonate (1.5 mmol) in DMF (5 mL), alkyl halide (1 mmol) was added and the mixture was stirred for 4 h at 80°C. After cooling, water (20 mL) was added and the residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried with Na_2SO_4 and the solvent was removed under reduced pressure to afford **4**.

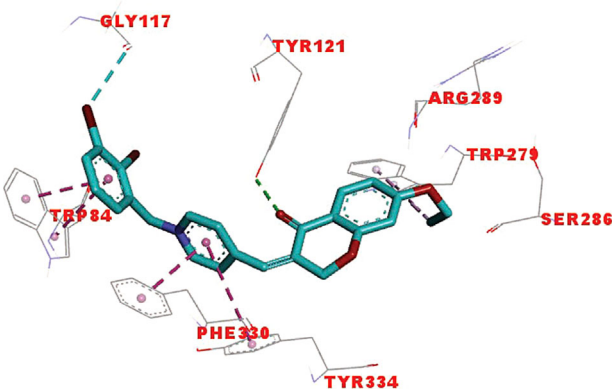


Figure 3. Docking of **8l** in the active site of AChE. The interactions are shown by dashed line.

*General procedure for the synthesis of 7-alkoxy-3-(pyridin-4-ylmethylene)chroman-4-one (**6**)*
7-Alkoxychroman-4-one **4** (1 mmol), isonicotinaldehyde **5** (1.4 mmol), and PTSA (1.5 mmol) 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. The wet solid was suspended in aqueous solution of sodium hydrogen carbonate (50 mL, 10%) and stirred for 30–60 min at room temperature. The resulting solid was filtered, washed with water (50 mL), and dried. The crude solid was purified by crystallization from acetonitrile.

*General procedure for the synthesis of (E)-1-benzyl-4-((7-alkoxy-4-oxochroman-3-ylidene)methyl)pyridin-1-ium bromide (**8**)*
To the stirred solution of 7-alkoxy-3-(pyridin-4-ylmethylene)-chroman-4-one **6** (1 mmol) in dry acetonitrile (7 mL) under reflux, benzyl bromide derivatives **7** (1.2 mmol) were added and the reaction was continued for 2–3 h. Upon completion, the solvent was removed and *n*-hexane (15 mL) was added to the residue. The precipitated crystals were separated by filtration, washed with *n*-hexane, and purified if needed by flash chromatography using chloroform/methanol (99:1) as the mobile phase to afford compounds **8a–l**.

*(E)-1-Benzyl-4-((7-methoxy-4-oxochroman-3-ylidene)-methyl)pyridinium bromide (**8a**)*
Yellow solid; yield: 76%; mp > 250°C; IR (KBr): 1708 (C=O), 1635 (C=C alkene) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) 9.18 (d, $J = 6.4$ Hz, 2H, $H_{2'}$, $H_{6'}$), 8.19 (s, 1H, CH), 8.09 (d, $J = 6.4$ Hz, $H_{3'}$, $H_{5'}$), 7.88 (d, $J = 7.8$ Hz, 1H, H_5), 7.56 (m, 2H, $H_{2''}$, $H_{6''}$), 7.41 (m, 3H, $H_{3''}$, $H_{4''}$, $H_{5''}$) 7.17 (t, $J = 7.8$ Hz, 1H, H_6), 7.05 (d, $J = 2.6$ Hz, 1H, H_8), 5.85 (s, 2H, N–CH_2), 4.47 (s, 2H, O–CH_2), 3.89 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) 194.8, 158.4, 155.8, 151.4, 144.5, 134.9, 129.7, 129.6, 129.3, 128.3, 126.8, 125.9, 120.2, 117.4, 115.4, 111.2, 62.7, 56.6, 51.5; Anal. elem. calcd.: C: 63.02, H: 4.60, N: 3.20. Found: C: 63.22, H: 4.34, N: 3.08.

*(E)-1-(4-Fluorobenzyl)-4-((7-methoxy-4-oxochroman-3-ylidene)methyl)pyridinium bromide (**8b**)*
Yellow solid; yield: 73%; mp > 250°C; IR (KBr): 1693 (C=O), 1640 (C=C alkene) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 8.97 (d, $J = 5.6$ Hz, 2H, $H_{2'}$, $H_{6'}$), 7.23 (d, $J = 5.6$ Hz, 2H, $H_{3'}$, $H_{5'}$), 8.06 (s, 1H, CH), 7.94 (d, $J = 7.9$ Hz, 2H, $H_{3''}$, $H_{5''}$), 7.91 (d, $J = 8.9$ Hz, 1H, H_5), 7.48 (d, $J = 7.9$ Hz, 2H, $H_{2''}$, $H_{6''}$), 7.18 (d, $J = 1.5$ Hz, 1H, H_8), 7.11 (d, $J = 8.9$ Hz, 1H, H_6), 5.87 (s, 2H, N–CH_2), 4.61 (s, 2H, O–CH_2), 3.98 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6): 193.1, 163.0, 158.9, 155.2, 151.6, 149.0, 142.6, 131.8, 131.0, 129.9, 128.1, 126.9, 125.9, 119.2, 114.6, 110.5, 60.0, 55.5, 52.0; Anal. elem. calcd.: C: 60.54, H: 4.20, N: 3.07. Found: C: 60.14, H: 4.38, N: 3.24.

*(E)-1-(4-Bromobenzyl)-4-((7-methoxy-4-oxochroman-3-ylidene)methyl)pyridinium bromide (**8c**)*
Yellow solid; yield: 72%; mp > 250°C; IR (KBr): 1700 (C=O), 1633 (C=C alkene) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 9.04 (d, $J = 5.2$ Hz, 2H, $H_{2'}$, $H_{6'}$), 8.36 (d, $J = 5.2$ Hz, 2H, $H_{3'}$, $H_{5'}$), 8.03 (s, 1H, CH), 7.81 (d, $J = 7.6$ Hz, 1H, H_5), 7.77 (d, $J = 8.3$ Hz, 2H, $H_{3''}$, $H_{5''}$), 7.34 (d, $J = 8.3$ Hz, 2H, $H_{2''}$, $H_{6''}$), 7.28 (d, $J = 3.3$ Hz, 1H, H_8), 7.25 (d, $J = 7.6$ Hz, 1H, H_6), 5.78 (s, 2H, N-CH_2), 4.45 (s, 2H, O-CH_2), 3.86 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6): 194.3, 158.2, 155.1, 148.7, 139.1, 136.6, 134.3, 131.6, 130.6, 129.4, 128.9, 128.3, 127.4, 126.9, 119.2, 111.9, 58.5, 55.3, 51.4; Anal. elem. calcd.: C: 53.41, H: 3.70, N: 2.71. Found: 53.72, H: 3.25, N: 3.06.

*(E)-4-((7-Methoxy-4-oxochroman-3-ylidene)methyl)-1-(4-methylbenzyl)pyridinium bromide (**8d**)*
Yellow solid; yield: 79%; mp > 250°C; IR (KBr): 1703 (C=O), 1646 (C=C alkene) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 8.79 (d, $J = 5.4$ Hz, 2H, $H_{2'}$, $H_{6'}$), 8.09 (s, 1H, CH), 7.98 (d, $J = 5.4$ Hz, 2H, $H_{3'}$, $H_{5'}$), 7.92 (d, $J = 8.9$ Hz, 1H, H_5), 7.49 (d, $J = 7.9$ Hz, 2H, $H_{3''}$, $H_{5''}$), 7.10–7.17 (m, 3H, $H_{2''}$, $H_{6''}$, H_6), 7.07 (d, $J = 3.3$ Hz, 1H, H_8), 5.76 (s, 2H, N–CH_2), 4.58 (s, 2H, O–CH_2), 3.89 (s, 3H, OCH_3), 2.31 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): 193.1, 158.4, 155.1, 148.7, 138.2, 136.6, 134.2, 131.5, 130.5, 129.4, 128.9, 128.3, 126.2, 119.2, 111.7, 58.0, 55.2, 52.7, 21.3; Anal. elem. calcd.: C: 63.73. H: 4.90. N: 3.10. Found: 63.67, H: 4.52, N: 2.94.

*(E)-1-(2-Bromobenzyl)-4-((7-methoxy-4-oxochroman-3-ylidene)methyl)pyridinium bromide (**8e**)*
Yellow solid; yield: 73%; mp > 250°C; IR (KBr): 1704 (C=O), 1637 (C=C alkene) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 9.10 (d, $J = 6.5$ Hz, 2H, $H_{2'}$, $H_{6'}$), 8.21 (d, $J = 6.4$ Hz, $H_{3'}$, $H_{5'}$), 8.05 (s, 1H, CH), 8.03 (d, $J = 8.5$ Hz, 1H, H_5), 7.72 (d, $J = 7.8$ Hz, $H_{3''}$), 7.35 (t, $J = 7.5$ Hz, 1H, $H_{5''}$), 7.32 (t, $J = 7.8$ Hz, H4″), 7.26 (d, $J = 7.6$ Hz, 1H, $H_{6''}$), 7.19 (d, $J = 8.5$ Hz, 1H, H_6), 7.11 (d, $J = 2.3$ Hz, 1H, H_8), 5.67 (s, 2H, N–CH_2), 4.52 (s, 2H, O–CH_2), 3.97 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6): 195.0, 158.1, 155.9, 151.2, 148.7, 139.0, 136.6, 134.2, 131.6, 130.5, 129.4, 128.9, 128.3, 127.4, 126.9, 126.2, 119.2, 117.9, 116.4, 111.3, 57.3, 55.9, 52.9; MS *m/z* (%) 439 ([M^++2], 8), 437 (M^+, 8), 241 (12), 228 (11), 169 (15), 161 (25), 83 (39), 69 (19), 55 (97), 41 (100); Anal. elem. calcd.: C: 53.41, H: 3.70, N: 2.71. Found: 53.26, H: 3.48, N: 2.83.

*(E)-1-(2,3-Dibromobenzyl)-4-((7-methoxy-4-oxochroman-3-ylidene)methyl)pyridinium bromide (**8f**)*
Yellow solid; yield: 74%; mp > 250°C; IR (KBr): 1705 (C=O), 1640 (C=C alkene) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 9.15 (d, $J = 5.7$ Hz, 2H, $H_{2'}$, $H_{6'}$), 8.28 (d, $J = 5.7$ Hz, $H_{3'}$, $H_{5'}$), 8.25 (s, 1H, CH), 8.04 (d, $J = 7.7$ Hz, 1H, H_5), 7.84 (t, $J = 9.0$ Hz, 1H, $H_{5''}$), 7.76 (d, $J = 9.0$ Hz, 1H, $H_{4''}$), 7.73 (d, $J = 9.0$ Hz, 1H, $H_{6''}$), 7.30 (d, $J = 7.7$ Hz, 1H, H_6), 7.14 (d, $J = 3.5$ Hz, 1H, H_8), 5.70 (s, 2H, N–CH_2), 4.60 (s, 2H, O–CH_2), 3.88 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6): 193.4, 157.5, 155.5, 149.4, 138.0, 131.8, 131.5, 130.9, 129.5, 128.3, 128.1, 127.9, 127.6, 125.7, 119.1, 114.5, 114.2, 111.3, 58.9, 55.4, 52.4; MS *m/z* (%) 517 ([M^++2], 6), 515 ($M^{+\cdot}$, 6), 268 (28), 267 (100), 266 (93), 250 (30), 151 (77), 117 (22), 79 (23), 63 (38), 51 (28); Anal. elem. calcd.: C: 46.34, H: 3.04, N: 2.35. Found: 46.64, H: 2.86, N: 2.62.

*(E)-1-Benzyl-4-((7-ethoxy-4-oxochroman-3-ylidene)-methyl)pyridinium bromide (**8g**)*
Yellow solid; yield: 76%; mp > 250°C; IR (KBr): 1705 (C=O), 1642 (C=C alkene) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 9.21 (d, $J = 5.8$ Hz, 2H, $H_{2'}$, $H_{6'}$), 8.26 (s, 1H, CH), 8.19 (d, $J = 5.8$ Hz, $H_{3'}$, $H_{5'}$), 7.88 (d, $J = 8.9$ Hz, 1H, H_5), 7.71 (m, 2H, $H_{2''}$, $H_{6''}$), 7.16 (m, 3H, $H_{3''}$, $H_{4''}$, $H_{5''}$) 7.10–7.12 (m, 2H, H_6, H_8), 5.79 (s, 2H, N–CH_2), 4.63 (s, 2H, O–CH_2), 3.91 (q, $J = 9.45$ Hz, 2H, O–**CH_2**–CH_3), 1.21 (t, $J = 9.85$ Hz, 3H, O–CH_2–**CH_3**); ^{13}C NMR (125 MHz, DMSO-d_6): 193.7, 158.7, 155.3, 148.7, 138.8, 136.8, 134.4, 131.9, 130.6, 129.4, 129.2, 128.8, 126.3, 123.6, 119.6, 119.3, 113.2, 111.5; Anal. elem. calcd.: C: 63.73, H: 4.90, N: 3.10. Found: 63.92, H: 5.11, N: 3.67.

*(E)-1-(4-Chlorobenzyl)-4-((7-ethoxy-4-oxochroman-3-ylidene)methyl)pyridinium bromide (**8h**)*
Yellow solid; yield: 78%; mp > 250°C; IR (KBr): 1706 (C=O), 1644 (C=C alkene) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 9.14 (d, $J = 4.8$ Hz, 2H, $H_{2'}$, $H_{6'}$), 8.20 (s, 1H, CH), 8.10 (d, $J = 4.8$ Hz, 2H, $H_{3'}$, $H_{5'}$), 7.86 (d, $J = 7.5$ Hz, 1H, H_5), 7.57 (d, $J = 8.1$ Hz, 2H, $H_{3''}$, $H_{5''}$), 7.40 (d, $J = 8.1$ Hz, 2H, $H_{2''}$, $H_{6''}$), 7.14 (d, $J = 2.4$ Hz, 1H, H_8), 7.04 (dd, $J = 7.5$, 2.5 Hz, 1H, H_6), 5.83 (s, 2H, N–CH_2), 4.58 (s, 2H, O–CH_2), 4.16 (q, $J = 9.8$ Hz, 2H, O–**CH_2**–CH_3), 1.36 (t, $J = 9.8$ Hz, 3H, O–CH_2–**CH_3**); ^{13}C NMR (125 MHz, DMSO-d_6): 194.4, 159.0, 155.3, 152.7, 144.7, 138.5, 136.5, 133.9, 133.1, 130.7, 128.4, 126.5, 124.2, 123.1, 116.5, 111.7, 58.6, 55.8, 51.5, 17.7; Anal. elem. calcd.: C: 59.22, H: 4.35, N: 2.88. Found: C: 59.43, H: 4.66, N: 3.16.

*(E)-4-((7-Ethoxy-4-oxochroman-3-ylidene)methyl)-1-(4-nitrobenzyl)pyridinium bromide (**8i**)*
Yellow solid; yield: 69%; mp > 250°C; IR (KBr): 1708 (C=O), 1642 (C=C alkene), 1370–1546 (NO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 9.11 (d, $J = 4.8$ Hz, 2H, $H_{2'}$, $H_{6'}$), 8.30 (d, $J = 4.8$ Hz, 2H, $H_{3'}$, $H_{5'}$), 8.23 (s, 1H, CH), 8.12 (d, $J = 7.6$ Hz, 2H, $H_{3''}$, $H_{5''}$), 7.88 (d, $J = 8$ Hz, 1H, H_5), 7.88 (d, $J = 7.6$ Hz, 2H, $H_{2''}$, $H_{6''}$), 7.16 (d, $J = 1.8$ Hz, 1H, H_8), 7.05 (dd, $J = 8.0$, 1.9 Hz, 1H, H_6), 5.97 (s, 2H, N–CH_2), 4.52 (s, 2H, O–CH_2), 4.17 (q, $J = 9.4$ Hz, 2H, O–**CH_2**–CH_3), 1.37 (t, $J = 9.4$ Hz, 3H, O–CH_2–**CH_3**); ^{13}C NMR (125 MHz, DMSO-d_6): 194.3, 158.6, 155.2, 150.0, 144.6, 144.1, 138.4, 134.1, 130.7, 129.8, 126.4, 125.4, 113.2, 112.8, 111.9, 59.2, 55.0, 52.8, 17.8; Anal. elem. calcd.: C: 57.96, H: 4.26, N: 5.63. Found: C: 58.21, H: 4.57, N: 5.39.

*(E)-4-((7-Ethoxy-4-oxochroman-3-ylidene)methyl)-1-(2-fluorobenzyl)pyridinium bromide (**8j**)*
Yellow solid; yield; 72%; mp > 250°C; IR (KBr): 1709 (C=O), 1633 (C=C alkene) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 9.15 (d, $J = 4.8$ Hz, 2H, $H_{2'}$, $H_{6'}$), 8.25 (s, 1H, CH), 8.10 (d, $J = 6.4$ Hz, $H_{3'}$, $H_{5'}$), 7.87 (d, $J = 7.8$ Hz, 1H, H_5), 7.50 (d, $J = 7.8$ Hz, $H_{3''}$, $H_{4''}$), 7.42 (d, $J = 8.9$ Hz, 1H, $H_{6''}$), 7.26 (t, $J = 8.9$ Hz, 1H, $H_{5''}$), 7.14 (d, $J = 2.3$ Hz, 1H, H_8), 7.04 (dd, $J = 8.0$, 2.9 Hz, 1H, H_6), 5.85 (s, 2H, N–CH_2), 4.55 (s, 2H, O–CH_2), 4.16 (q, $J = 9.1$ Hz, 2H, O–**CH_2**–CH_3), 1.36 (t, $J = 9.1$ Hz, 3H, O–CH_2–**CH_3**); ^{13}C NMR (125 MHz, DMSO-d_6): 194.5, 162.9, 158.7, 152.9, 147.9, 145.1, 143.7, 141.0, 136.7, 133.1, 130.2, 128.5, 124.1, 123.2, 116.5, 114.8, 111.1, 58.8, 56.8, 51.5, 17.8; Anal. elem. calcd.: C: 61.29, H: 4.50, N: 2.98. Found: C: 61.51, H: 4.74, N: 3.25.

*(E)-1-(2-Bromobenzyl)-4-((7-ethoxy-4-oxochroman-3-ylidene)methyl)pyridinium bromide (**8k**)*
Yellow solid; yield: 75%; mp > 250°C; IR (KBr): 1697 (C=O), 1643 (C=C alkene) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 8.96 (d, $J = 5.1$ Hz, 2H, $H_{2'}$, $H_{6'}$), 8.23 (s, 1H, CH), 8.11 (d, $J = 6.4$ Hz, $H_{3'}$, $H_{5'}$), 7.88 (d, $J = 7.6$ Hz, 1H, H_5), 7.75 (dd, $J = 6.8$, 1.9 Hz, $H_{3''}$), 7.50 (t, $J = 7.0$ Hz, 1H, $H_{5''}$), 7.42 (t, $J = 6.8$ Hz, 1H, $H_{4''}$), 7.36 (dd, $J = 7.0$, 1.5 Hz, $H_{6''}$), 7.16 (d, $J = 1.8$ Hz, 1H, H_8), 7.01 (dd, $J = 7.6$, 2.2 Hz, 1H, H_6), 5.92 (s, 2H, N–CH_2), 4.61 (s, 2H, O–CH_2), 4.18 (q, $J = 9.4$ Hz, 2H, O–**CH_2**–CH_3), 1.37 (t, $J = 9.4$ Hz, 3H, O–CH_2–**CH_3**); ^{13}C NMR (125 MHz, DMSO-d_6): 192.4, 158.3, 156.3, 149.2, 148.4, 136.3, 133.6, 132.2, 131.5, 131.3, 129.9, 129.7, 129.2, 128.1, 126.6, 124.5, 113.1, 111.1, 58.2, 55.0, 50.1, 19.2; Anal. elem. calcd.: C: 54.26, H: 3.98, N: 2.64. Found: C: 54.58, H: 4.32, N: 2.87.

*(E)-1-(2,3-Dibromobenzyl)-4-((7-ethoxy-4-oxochroman-3-ylidene)methyl)pyridinium bromide (**8l**)*
Yellow solid; yield: 68%; mp > 250°C; IR (KBr): 1704 (C=O), 1645 (C=C alkene) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 9.16 (d, $J = 5.1$ Hz, 2H, $H_{2'}$, $H_{6'}$), 8.10 (d, $J = 5.1$ Hz, $H_{3'}$, $H_{5'}$), 8.01 (s, 1H, CH), 7.89 (d, $J = 8.4$ Hz, 1H, H_5), 7.66 (d, $J = 7.1$ Hz, $H_{4''}$), 7.28 (t, $J = 7.1$ Hz, 1H, $H_{5''}$), 7.26 (d, $J = 7.1$ Hz, 1H, $H_{6''}$), 7.13 (d, $J = 2.5$ Hz, 1H, H_8), 7.02 (dd, $J = 8.5$, 2.8 Hz, 1H, H_6), 5.82 (s, 2H, N–CH_2), 4.66 (s, 2H, O–CH_2), 4.17 (q, $J = 9.4$ Hz, 2H, O–**CH_2**–CH_3), 1.21 (t, $J = 9.4$ Hz, 3H, O–CH_2–**CH_3**); ^{13}C NMR (125 MHz, DMSO-d_6): 194.2, 158.9, 155.3, 152.8, 144.8, 143.1, 136.5, 133.1, 130.9, 129.5, 128.3, 126.7, 124.2, 123.0, 116.5, 111.2, 59.8, 55.8, 51.8; Anal. elem. calcd.: C: 47.24, H: 3.30, N: 2.30. Found: C: 47.69, H: 3.51, N: 2.07.

AChE inhibition assay

Colorimetric Ellman's method was used to evaluate the inhibitory potency of target compounds toward AChE [20]. Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from electric eel, 1000 unit) was obtained from Sigma–Aldrich. 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB), potassium dihydrogen phosphate, dipotassium hydrogen phosphate, potassium hydroxide, sodium hydrogen carbonate, and acetylthiocholine iodide were purchased from Fluka. Donepezil hydrochloride was obtained from Merck, Darmstadt, Germany. In short, to determine IC_{50} values, 50 μL of the five different concentrations of the test compounds that produced inhibition in the range of 20–80% was added to the mixture of 3 mL phosphate buffer 0.1 M, pH = 8.0, and 100 μL of DTNB solution (0.1 M) and 50 μL AChE. Ten microliters solution of acetylthiocholine iodide (0.15 M) as substrate was added following 10 min incubation at 25°C. The progress curve was plotted by measuring the absorbance at 412 nm for 6 min. The IC_{50} values were determined graphically from inhibition curves (inhibitor concentration vs. percent of inhibition). UNICO double beam spectrophotometer 2100 was used for colorimetric measurements.

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Molecular docking study

Docking studies were performed using the AUTODOCK 4.2 program. For this purpose, the crystal structure of acetylcholinesterase enzyme (pdb code: 1EVE) was taken from the Brookhaven protein database (http://www.rcsb.org) as a complex bound with inhibitor E2020 (donepezil). Subsequently, the water molecules and the original inhibitors were removed from the protein structure. The 3D structure of the compound **8l** was provided using Marvine Sketch 5.8.3, 2012, ChemAxon (http: //www. chemaxon.com) and converted to pdbqt coordinate by AUTODOCK 4.2 program. Also, the autodock format of protein was provided using the same software. Polar hydrogen atoms were added to amino acid residues using Autodock Tools (ADT; version 1.5.6), Koullman charges were assigned to all atoms of the enzyme, and the obtained enzyme structure was used as an input for the AUTOGRID program. AUTOGRID performed a pre-calculated atomic affinity grid maps for each atom type in the ligand, plus an electrostatics map, and a separate desolation map presented in the substrate molecule. All maps were calculated with 0.375 Å spacing between grid points. The center of the grid box was placed at the center of donepezil with coordinates $x = 1.75$, $y = 63.1$, $z = 67.11$. The dimensions of the active site box were set at $36 \times 34 \times 56$ that covered all binding sites occurred in active site of the enzyme. Flexible ligand docking was accomplished for the compound **8l**. Each docked system was carried out by 100 runs of the AUTODOCK search by the Lamarckian genetic algorithm (LGA). Other than the above-mentioned parameters, the other parameters were accepted as default. The lowest energy conformation of ligand–enzyme complex was considered for analyzing the interactions between AChE and the inhibitor. The results were visualized using Discovery Studio 4.0 Client.

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The authors have declared no conflicts of interest.

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