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Research paper

Design, synthesis and anticholinesterase activity of novel benzylidenechroman-4-ones bearing cyclic amine side chain

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ABSTRACT

A series of 3-(4-(aminoalkoxy)benzylidene)-chroman-4-ones **7a-r** were designed and synthesized as analogs of homoisoflavonoids which are well known natural products with diverse pharmacological properties related to Alzheimer's disease. The in vitro anti-cholinesterase activity of designed compounds **7a-r** against AChE and BuChE, revealed that compounds bearing piperidinylethoxy residue showed potent activity against AChE at sub-micromolar level (IC_{50} values = 0.122–0.207 μM), more potent than reference drug tacrine. The structure-activity relationships study of piperidinylethoxy series demonstrated that the selectivity and physicochemical properties of compounds could be optimized by selection of a proper substituent on the C-7 position of chroman ring, while the high potency of the molecule against AChE was reserved.

1. Introduction

Dementia is one of the most rapidly growing diseases. The number of people living with dementia worldwide is currently estimated at 35.6 million. This number will double by 2030 and more than triple by 2050 [1,2]. Alzheimer's disease (AD), the most common type of dementia is a progressive neurodegenerative disorder which is regarded the most common illness of over-60-year old people [3]. Although the exact cause of the disease is unknown, several factors are thought to play a role in the cause of AD. These include amyloid β (Aβ) deposits, τ-protein aggregation, oxidative stress and low levels of acetylcholine (ACh) in the hippocampus and cortex areas [4,5]. Based on the cholinergic hypothesis, a possible cause of AD is the reduced synthesis of ACh, a neurotransmitter which is involved in memory and learning [6]. Acetylcholinesterase (AChE), is a hydrolase enzyme that hydrolyzes the neurotransmitter ACh, and serves to terminate synaptic transmission more quickly. Application of acetylcholinesterase inhibitors (AChEIs) has become the foundations for the treatment of AD. Up to now, several AChEIs have been approved for the treatment of symptomatic AD such as tacrine, donepezil, rivastigmine and galantamine (Fig. 1) [7]. However some of the limitations that exist for this drugs, including hepatoxicity, non-selectivity, poor bioavailability, adverse cholinergic side effects in the peripheral, necessitate further research in this area [8,9].

Structurally, AChE possesses two binding sites: the catalytic active site (CAS) and the peripheral anionic site (PAS) connected by a gorge [10]. In addition, AChE interacts with β-amyloid through PAS and accelerates the formation of stable β-amyloid aggregates [11].

According to these findings, designing the new agents that are able to interact with both sites (CAS and PAS) of AChE would be an

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effective approach for management of AD's symptoms [12].

Flavonoids (including chalcones, flavones, flavanones, and isoflavone), and homoisoflavonoid (3-benzylidenechroman-4-ones) are well known natural products possessing a diverse pharmacological properties related to AD, such as anti-AChE activity [13], Aβ fibril formation inhibitory activity [14], MAO-B inhibitory effect [15] and neuroprotection capability [16].

In continuation of our previous studies for developing new AChE inhibitors [17–19], we designed new homoisoflavonoid derivatives by introduction of various amino alkyl groups at the *para* position of 7-substituted 3-benzylidenechroman-4-one. In the designed compounds, the 3-benzylidenechroman-4-one scaffold was expected to bind the PAS of AChE, and the nitrogen atom of amine side chain would interact with the catalytic site of AChE. The cyclic aminoalkoxyphenyl part of designed molecule is found in some reported AChE inhibitors as exemplified by ebselen analog (Fig. 2) [20].

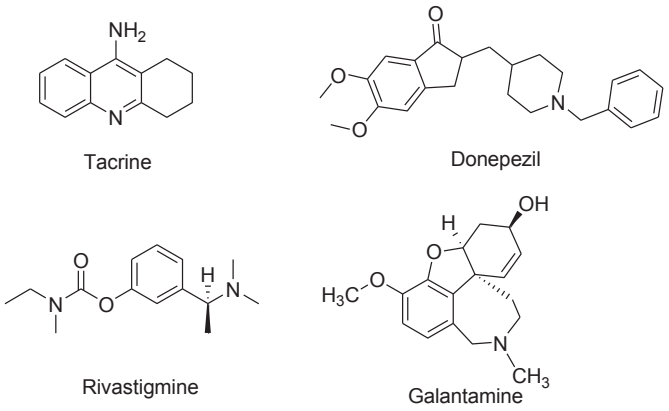


Fig. 1. Several AChE inhibitors that have been approved by FDA for the treatment of AD.

2. Chemistry

The synthesis of target compounds **7a-r** was accomplished using the pathways illustrated in Scheme 1. The aldehyde intermediates **2a**,**b** and **4a-c** were prepared starting from 4-hydroxybenzaldehyde (**1**). The reaction of compound **1** with proper aminoethyl chloride in the presence of K_2CO_3 and KI in CH_3CN afforded corresponding *O*-alkylated derivatives **2a**,**b**. On the other hand, *O*-alkylation of compound **1** with 1-bromo-3-chloropropane gave 3-chloropropoxy analog **3**. Subsequently, the reaction of appropriate cyclic amine with intermediate **3** resulted in compounds **4a-c** [21].

The key intermediate 7-hydroxychroman-4-one (**5**) was synthesized from resorcinol, as previously described method [22]. Compound **5** was *O*-alkylated by proper alkyl halides in the presence of K_2CO_3 in DMF to obtain 7-alkoxychroman-4-one **6a-c**. Condensation of 4-chromanones **5** or **6a-c** with aldehyde intermediates **2** or **4** in the presence of HCl in ethanol gave the hydrochloride salts of compounds **7a-r** [23,24]. The structures of all the newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy.

3. Results and discussion

3.1. Cholinesterase inhibitory activity

The in vitro anti-cholinesterase activity of designed compounds **7a-r** was evaluated against AChE and BuChE, in comparison with tacrine as reference drug. The IC_{50} values of compounds are listed in Table 1. The obtained IC_{50} values against AChE demonstrated that most compounds had potent inhibitory activity. Among them, the piperidinylethoxy derivatives **7d**, **7f**, **7j** and **7p** with IC_{50} values of 0.122–0.207 μM were more potent than standard drug tacrine against AChE.

In general, compounds with two carbons linker (n = 1) were more potent than corresponding compounds bearing three carbons linker (n = 2). The modification of cyclic amine connected to the alkoxybenzylidene part significantly affected the activity. The piperidine was more favorable than morpholine and pyrrolidine for anti-AChE activity. Although the hydroxyl, methoxy, ethoxy and benzyloxy derivatives of piperidinylethoxy series showed same inhibitory activity, however in the morpholine analogs, the methoxy, ethoxy and benzyloxy derivatives were more potent than their hydroxy congeners. The comparison of IC_{50} values of ethoxy compounds **7j-m** to those of related methoxy derivatives **7f-h** revealed that the ethoxy group is more favorable than the methoxy group for anti-AChE activity. While the anti-AChE activity of benzyloxy derivative **7r** was greater than that of its methoxy analog **7e**, but other benzyloxy derivatives **7n-q** were less potent than their corresponding methoxy analogs. These findings indicate that the effect of C-7 substituent on the chroman ring depends on the type of pendent group on the benzylidene moiety.

In the terms of inhibitory activity against BuChE, piperidinylethoxy derivative **7j** showed superior activity, possessing IC_{50} value of 0.854 μM. Nevertheless, compounds **7d**, **7f**, **7i**, **7m** and **7p** exhibited remarkable activity against BuChE (IC_{50} values <10 μM). The activity of piperidine derivatives was higher than corresponding morpholine analogs toward BuChE. Similar to AChE, aminoethoxy derivatives inhibited BuChE more potently compared to aminopropoxy homologues.

While the standard drug tacrine showed low selectivity for AChE in our experiments (selectivity index = 1.25), but all synthesized compound had SI more than 4.2 (Table 1). The results of in vitro anti-cholinesterase evaluation of piperidine derivatives **7d**, **7f**, **7j** and **7p** revealed that while these compounds showed potent anti-AChE activity with closed range of IC_{50}s (0.122–0.207 μM), but they exhibited varied range of activities against BuChE (0.854–8.2 μM). Thus, the AChE/BuChE selectivity of the piperidinylethoxy series could be regulated between 7 and 47, by alteration of C-7 substituent on the chroman ring.

3.2. Docking study

To get information about binding mode and inhibition profile of synthesized compounds, the docking simulation study was performed. Primarily, all the compounds were investigated computationally to define their binding profile. For instance, the most active compound **7j** was subjected for further analysis. The detailed picture of the docking pose of compound **7j** is displayed in Fig. 4. It is established 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 nitrogen containing heterocyclic part of the molecule locates around Trp83 in the vicinity of catalytic site. The positively charged nitrogen contributes in forming a π-cation interaction with aromatic tryptophan. Moreover, compound **7j** has three remarkable π–π stacking interactions with Phe330, Tyr333 and Trp278. Two T-shaped stacking take place in the mid gorge recognition site between benzylidene moiety and Phe330 and Tyr333 having the angles of 41 and 58°, respectively. Another stacking stabilizes the inhibitor conformation through making a π–π interaction of chroman ring with Trp278 in the PAS.

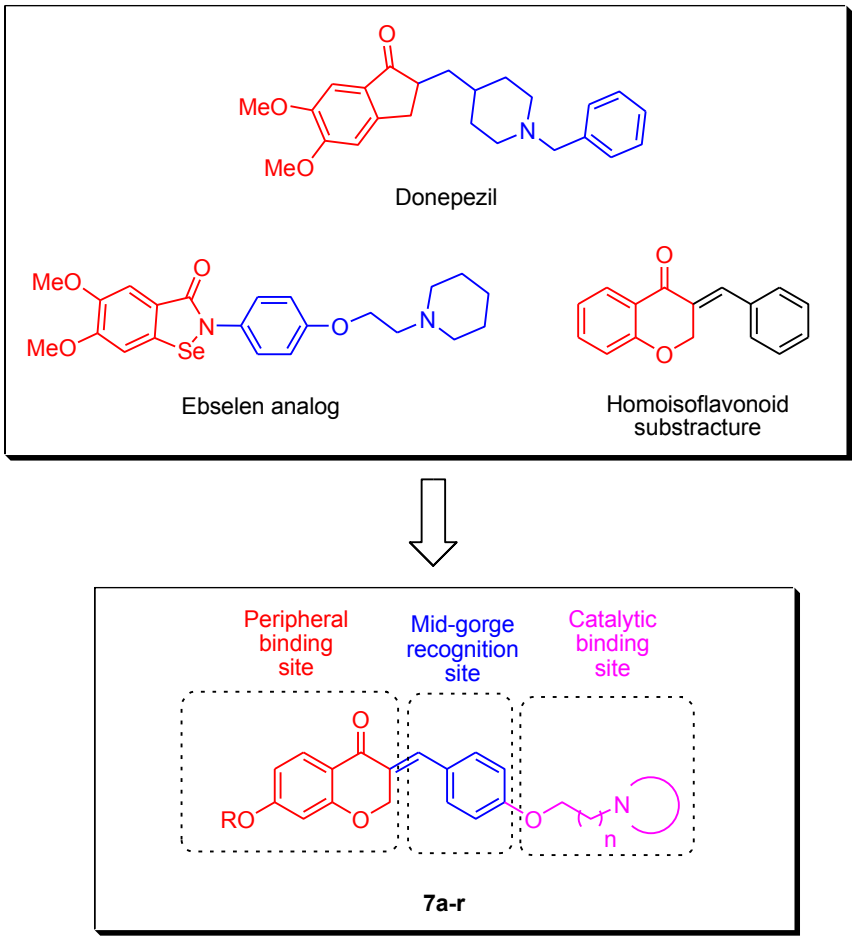


Fig. 2. Design strategy leading to compounds **7a-r** as new AChE inhibitors.

3.3. Kinetic study of AChE inhibition

To get better insight of AChE inhibition by target compounds, the most active compound **7j** was subjected to kinetic studies as previously reported method [17]. For this purpose, the rate of enzyme activity was measured at four different concentrations of inhibitor **7j** (0, 0.061, 0.122 and 0.244 μM) in the presence of different concentrations of substrate (ATCh) using cholinesterase inhibition test mentioned in the experimental section. For each inhibitor concentration, the initial velocity was measured at different substrate concentrations (S) and the reciprocal of the initial velocity (1/v) was plotted versus the reciprocal of substrate concentration (1/[s]). As depicted in Fig. 5, the obtained double reciprocal (Lineweavere–Burk) plot showed a mixed-type inhibition pattern for compound **7j**, which is in agreement with docking studies that shows the target compound occupies both PAS and CS sites. The inhibitory constant (K_i) of compound **7j** was also calculated using the secondary plot as shown in Fig. 5 ($K_i = 0.27$ μM). The K_i value of 4.24 nM was also obtained for donepezil as a reference drug.

4. Conclusion

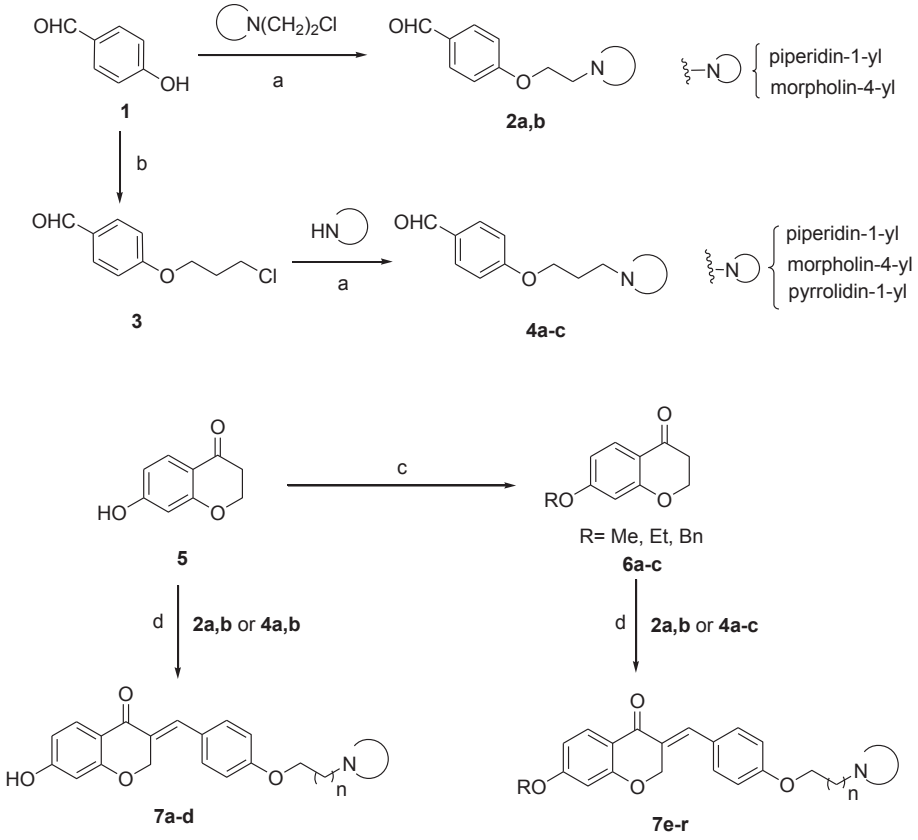
Since the homoisoflavonoids (3-benzylidenechroman-4-ones) were well known natural products with diverse pharmacological properties related to AD, we designed a series of 3-(4-(aminoalkoxy)benzylidene)-chroman-4-ones **7a-r** as potent AChE inhibitors. The in vitro anti-cholinesterase activity of designed compounds **7a-r** against AChE and BuChE, revealed that compounds bearing piperidinylethoxy residue showed high activity against AChE at sub-micromolar level (IC_{50} values = 0.122 −0.207 μM), more potent than reference drug tacrine.

The structure-activity relationships study demonstrated that the 4-(aminoalkoxy) residue on the 3-benzylidene moiety offers the potential of anti-cholinesterase activity of designed compounds. The best results were obtained with piperidine as cyclic amine, and ethoxy (n = 1) as a linker. In the piperidinylethoxy derivatives, various oxygenated functional groups including hydroxyl, methoxy, ethoxy and benzyloxy on the 7-position of chroman ring was tolerated. Therefore, the selectivity and physicochemical properties of the piperidinylethoxy derivatives could be optimized by selection of a proper substituent on the C-7 position of chroman ring, while the high potency of the molecule against AChE was reserved.

5. Experimental

5.1. Chemistry

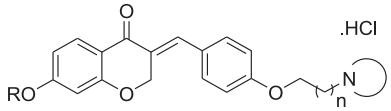
Melting points of compounds are determined using Kofler hot stag apparatus and are uncorrected. IR spectra were taken using Nicolet FT-IR Magna 550 spectrographs (KBr disks). The NMR spectra were recorded by using Bruker 400 or 500 spectrometers. The chemical shifts (δ) are reported in part per million (ppm) down field from TMS as internal standard. Coupling constant (J) values are presented in Hz and spin multiplicities are given as follows: s



Scheme 1. Synthesis of compounds **7a-r**. *Reagents and conditions*: (a) K_2CO_3, KI, CH_3CN, 80 °C; (b) 1-bromo-3-chloropropane, K_2CO_3, CH_3CN, 80 °C; (c) RX, K_2CO_3, DMF, 80 °C; (d) HCl, EtOH.

(singlet), d (doublet), t (triplet), m (multiplet), q (quartet) and br (broad). The numbering of atoms in the target compounds for interpretation of NMR data is represented in Fig. 3. Mass spectra were recorded by LC-Mass Agilent Technologies. All reagents and solvents used in this study were commercially available and purchased from Merck AG, Aldrich or Acros Organics. Thin-layer chromatography (TLC) with pre-coated Silica Gel F254 plates was routinely used for checking the reactions.

Table 1
The IC_{50} values (μM) of compounds **7a-r** against AChE and BuChE.



Compound	R	n	N	AChE	BuChE	SI[a]
7a	OH	2	Morpholino-	158 ± 8	NA	–
7b	OH	2	Piperidin-1-yl	2.38 ± 0.19	27.37 ± 1.2	11.7
7c	OH	1	Morpholino-	8.16 ± 0.32	96 ± 4.25	11.7
7d	OH	1	Piperidin-1-yl	0.192 ± 0.007	2.16 ± 0.35	11.4
7e	OMe	2	Piperidin-1-yl	3.29 ± 0.1	26.35 ± 1.15	8.0
7f	OMe	1	Piperidin-1-yl	0.173 ± 0.008	8.2 ± 0.7	4.0
7g	OMe	1	Morpholino-	1.9 ± 0.056	11.59 ± 0.98	6.1
7h	OMe	2	Morpholino-	13.9 ± 0.77	101 ± 5.2	7.3
7i	OMe	2	Pyrrolidin-1-yl	1.1 ± 0.078	6.85 ± 0.86	6.2
7j	OEt	1	Piperidin-1-yl	0.122 ± 0.007	0.854 ± 0.04	7.0
7k	OEt	2	Morpholino-	6.84 ± 0.29	28.72 ± 1.36	4.2
7l	OEt	1	Morpholino-	1.00 ± 0.045	10.2 ± 1.19	10.2
7m	OEt	2	Piperidin-1-yl	0.562 ± 0.021	3.1 ± 0.56	5.5
7n	OBn	1	Morpholino-	6.44 ± 0.38	83.72 ± 3.96	13.0
7o	OBn	2	Morpholino-	50 ± 1.72	NA	–
7p	OBn	1	Piperidin-1-yl	0.207 ± 0.008	2.58 ± 0.65	12.5
7q	OBn	2	Pyrrolidin-1-yl	3.5 ± 0.22	49 ± 2.64	14
7r	OBn	2	Piperidin-1-yl	2.7 ± 0.31	32.4 ± 2.19	12.0
Tacrine	–	–	–	0.28 ± 0.02	0.35 ± 0.2	1.25

[a] SI: selectivity index = IC_{50} (BuChE)/IC_{50} (AChE).

5.1.1. *General procedure for the synthesis of 4-(2-aminoethoxy) benzaldehydes **2a,b** [21b]*

In a single-neck round bottom flask, in an oil bath, 4-hydroxybenzaldehyde (**1**, 40 mmol, 5.49 g), potassium carbonate (122 mmol, 16.8 g) and acetonitrile (160 ml) were taken and the contents refluxed for 2 h. The reaction mixture was brought to room temperature and catalytic amount of potassium iodide was added, followed by the gradual addition of appropriate aminoethyl chloride hydrochloride (45 mmol) and the reaction mixture was allowed to reflux again. The contents were regularly monitored for reaction progress by TLC using 10% methanol/dichloromethane as the solvent system. The reaction was generally complete in 24 h. At this point, reaction mixture was filtered under suction and the solid inorganic salts were washed with acetonitrile (3 × 60 ml). The

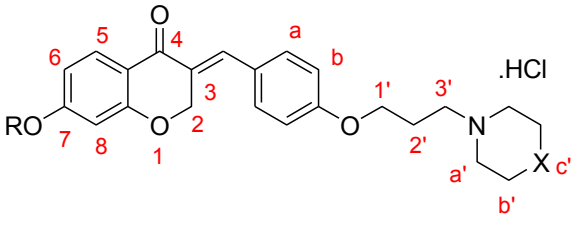


Fig. 3. Atom numbering of the target compounds used for NMR data.

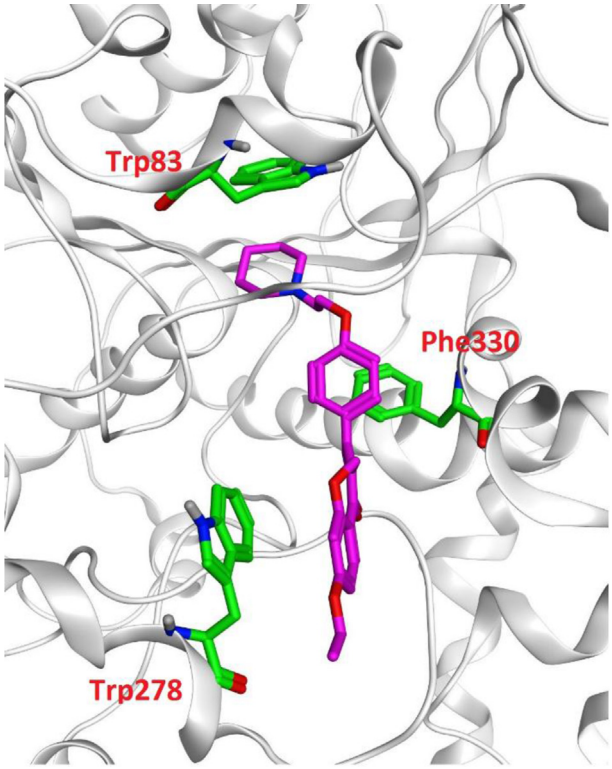


Fig. 4. Illustrative interaction of compound **7j** with AChE active site. The key residues are shown by green color. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

solvent was evaporated under reduced pressure and the residue was purified by column chromatography over silica gel (1–9 % methanol in dichloromethane, v/v as eluent) to afford the pure aldehydes **2a**,**b** as brown syrup in 60–80 % yields.

*5.1.2. General procedure for the synthesis of 4-(3-aminopropoxy) benzaldehydes **4a-c***

Treatment of 4-hydroxybenzaldehyde (**1**, 1 mmol) with potassium carbonate (1.5 mmol) and 1-bromo-3-chloropropane (1 mmol) in acetonitrile (10 ml) gave intermediate **3**. Subsequent displacement of chloride in compound **3** with cyclic secondary amines afforded corresponding compounds **4a-c** in moderate yield as described in the literature [21].

*5.1.2.1. 4-(3-(Piperidin-1-yl)propoxy)benzaldehyde (**4a**).* Orange syrup, yield 66%; IR (KBr, cm^{-1}) ν_{max}: 2966, 2872 (CHO), 1692 (C=O), 1601 (C=C); ^1H NMR ($CDCl_3$, 500 MHz), 9.84 (s, 1H, H aldehyde), 7.79 (d, 2H, $J = 8.75$ Hz), 6.98 (d, 2H, $J = 8.75$ Hz), 4.07 (t, 2H, OCH_2, $J = 7.0$ Hz), 2.45 (t, 2H, CH_2N, $J = 7.0$ Hz), 2.37 (br s, 4H), 1.98 (quintet, 2H, $CH_2CH_2CH_2$, $J = 6.50$ Hz), 1.56 (quintet, 4H, $J = 5.65$ Hz), 1.41 (m, 2H).

*5.1.2.2. 4-(3-Morpholinopropoxy)benzaldehyde (**4b**).* This compound was prepared according to the reported procedure [21].

*5.1.2.3. 4-(3-(Pyrrolidin-1-yl)propoxy)benzaldehyde (**4c**).* This compound was prepared according to the reported procedure [21].

*5.1.3. General procedure for the synthesis of 7-alkoxychroman-4-ones **6a-c***

To a mixture of 7-hydroxychroman-4-one (**5**, 1 equiv) and potassium carbonate (1.5 equiv) in DMF (5 ml), proper alkyl halide (1 equiv) was added and the mixture was stirred for 4 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 with Na_2SO_4 and the solvent was removed under reduced pressure to afford compounds **6a-c** in good yields [24].

*5.1.4. General procedure for the synthesis of (E)-3-(4-(aminoalkoxy)benzylidene)-2,3-dihydro-7-hydroxychromen-4-one hydrochloride derivatives (**7a-r**)*

A solution of 7-hydroxychroman-4-one (**5**) or 7-alkoxychroman-4-one **6a-c** (1 mmol), and 4-substituted benzaldehydes **2a**,**b** or **4a-c** (1 mmol) in EtOH (5 ml) was stirred at room temperature for 5 min, while a stream of HCl gas was introduced. After 24 h stirring at room temperature, the precipitated solid was separated by filtration and crystallized from EtOH to give compounds **7a-r**.

*5.1.4.1. Synthesis of (E)-3-(4-(3-morpholinopropoxy)benzylidene)-2,3-dihydro-7-hydroxychromen-4-one hydrochloride (**7a**).* Starting from 7-hydroxychroman-4-one (**5**, 5 mmol, 0.82 g) and 4-

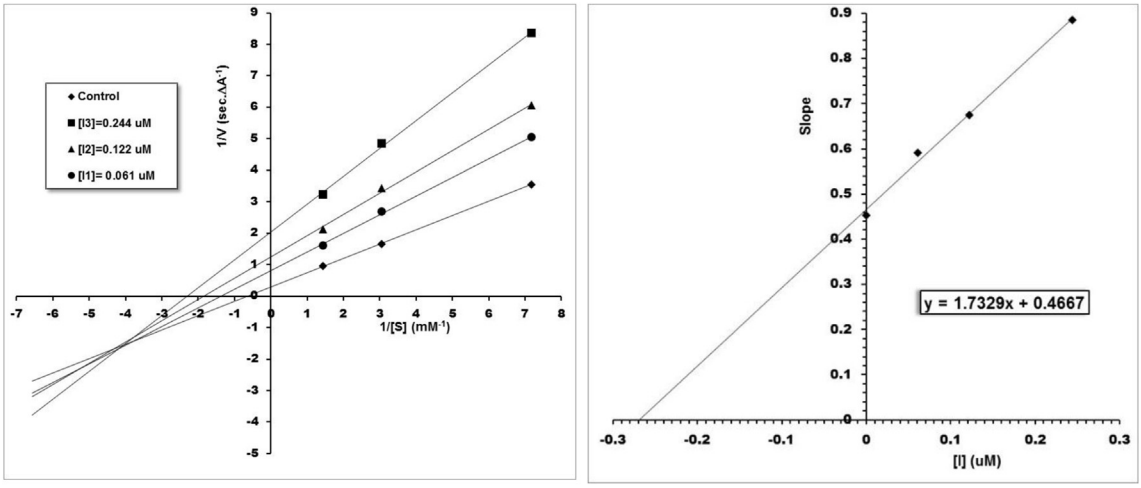


Fig. 5. *Left*: Lineweavere–Burk plot for the inhibition of AChE by compound **7j** at different concentrations of substrate (ATCh), *Right*: Secondary plot of the enzyme for calculation of steady-state inhibition constant (K_i) of **7j**.

(3-morpholinopropoxy) benzaldehyde (5 mmol, 1.245 g), compound **7a** was obtained as purple crystals in 71% yield; mp 198–200 °C; IR (KBr, cm^{-1}) ν_{max}: 3416 (O–H), 1659 (C=O), 1588 (C=C alkene); ^{1}H NMR (DMSO-d_6, 500 MHz), 7.74 (d, 1H, H-5, J = 8.6 Hz), 7.64 (s, 1H, H-8), 7.38 (d, 2H, H-a, J = 8.5 Hz), 7.03 (d, 2H, H-b, J = 8.5 Hz), 6.56 (d, 1H, H-6, J = 8.6 Hz), 6.34 (s, 1H, vinylic-H), 5.36 (s, 2H, H-2), 4.06 (t, 2H, H-1′, J = 6.18), 3.57 (br s, 4H, H-b′), 2.42 (t, 2H, H-3′, J = 7.0 Hz), 2.37 (br s, 4H, H-a′), 1.89 (quintet, 2H, H-2′, J = 6.65); ^{13}C NMR (125 MHz, DMSO-d_6) δ 179.96, 165.10, 162.93, 160.13, 135.70, 132.69, 129.86, 129.23, 126.89, 115.20, 114.71, 111.59, 102.89, 68.01, 66.64, 66.45, 55.22, 53.81, 26.22. LC/MS (ESI): m/z 396.2 [M^+ + H]. Anal. Calcd for $C_{23}H_{25}NO_5$.HCl (431.91): C, 63.96; H, 6.07; N, 3.24. Found: C, 63.73; H, 6.19; N, 3.43.

*5.1.4.2. Synthesis of (E)-3-(4-(3-(piperidin-1-yl)propoxy)benzylidene)-2,3-dihydro-7-hydroxychromen-4-one hydrochloride (**7b**).* Starting from 7-hydroxychroman-4-one (**5**, 5 mmol, 0.82 g) and 4-(3-(piperidin-1-yl)propoxy)benzaldehyde (5 mmol, 1.235 g), compound **7b** was obtained as pinkish crystals in 74% yield; mp 246–248 °C; IR (KBr, cm^{-1}) ν_{max}: 3365 (O–H), 1712 (C=O), 1593 (C=C alkene); ^{1}H NMR (DMSO-d_6, 500 MHz), 10.74 (s, 1H, OH), 10.10 (br s, 1H, NH), 7.74 (d, 1H, H-5, J = 8.6 Hz), 7.65 (s, 1H, H-8), 7.42 (d, 2H, H-a, J = 8.6 Hz), 7.06 (d, 2H, H-b, J = 8.6 Hz), 6.57 (d, 1H, H-6, J = 8.6 Hz), 6.35 (s, 1H, vinylic-H), 5.37 (s, 2H, H-2), 4.14 (t, 2H, H-1′, J = 6.18 Hz), 3.46 (m, 2H, H-a′), 3.17 (m, 2H, H-a′), 2.88 (m, 2H, H-3′), 2.21 (m, 2H, H-2′), 1.79–1.39 (m, 6H, H-b′ and H-c′). ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.98, 165.20, 162.93, 159.72, 135.52, 132.58, 129.75, 129.47, 127.24, 115.18, 114.67, 111.57, 102.85, 67.95, 65.67, 53.89, 52.61,48.95, 23.81, 22.96, 21.89. LC/MS (ESI): m/z 394.2 [M^++H]. Anal. Calcd for $C_{24}H_{27}NO_4$.HCl (429.94): C, 67.05; H, 6.56; N, 3.26. Found: C, 67.11; H, 6.43; N, 3.37.

*5.1.4.3. Synthesis of (E)-3-(4-(2-morpholinoethoxy)benzylidene)-2,3-dihydro-7-hydroxy chromen-4-one hydrochloride (**7c**).* Starting from 7-hydroxychroman-4-one (**5**, 5 mmol, 0.82 g) and 4-(2-(morpholinoethoxy)benzaldehyde (5 mmol, 1.175 g), compound **7c** was obtained as purple crystals in 70% yield; mp 257–259 °C; IR (KBr, cm^{-1}) ν_{max}: 3374 (O–H), 1699 (C=O), 1598 (C=C alkene); ^{1}H NMR (DMSO-d_6, 400 MHz), 11.42 (br s, 1H, NH), 10.79 (s, 1H, OH), 7.74 (d, 1H, H-5, J = 8.6 Hz), 7.65 (s, 1H, H-8), 7.43 (d, 2H, H-a, J = 8.8 Hz), 7.11 (d, 2H, H-b, J = 8.8 Hz), 6.57 (dd, 1H, H-6, J = 8.6 and 2.0 Hz), 6.35 (d, 1H, vinylic-H, J = 2.0 Hz), 5.36 (d, 2H, H-2, J = 2.0 Hz), 4.52 (t, 2H, H-1′, J = 5.0 Hz), 3.91 (br s, 4H, H-b′), 3.56 (t, 2H, H-2′, J = 5.0 Hz), 3.46–3.16 (br s, 4H, H-a′). ^{13}C NMR (125 MHz, DMSO-d_6) δ 179.99, 165.19, 162.94, 158.90, 135.50, 132.64, 129.87, 129.72, 127.78, 115.48, 114.66, 111.68, 102.90, 67.96, 63.67, 62.77, 55.31, 52.15. LC/MS (ESI): m/z 382.2 [M^++H]. Anal. Calcd for $C_{22}H_{23}NO_5$.HCl (417.89): C, 63.23; H, 5.79; N, 3.35. Found: C, 63.10; H, 5.93; N, 3.18.

*5.1.4.4. Synthesis of (E)-3-(4-(2-(piperidin-1-yl)ethoxy)benzylidene)-2,3-dihydro-7-hydroxy chromen-4-one hydrochloride (**7d**).* Starting from 7-hydroxychroman-4-one (**5**, 5 mmol, 0.82 g) and 4-(2-(piperidin-1-yl)ethoxy)benzaldehyde (5 mmol, 1.165 g), compound **7d** was obtained as pinkish crystals in 76% yield; mp 234–236 °C; IR (KBr, cm^{-1}) ν_{max}: 3114 (O–H), 1669 (C=O), 1608 (C=C alkene); ^{1}H NMR (DMSO-d_6, 400 MHz), 10.83 (s, 1H, OH), 10.74 (br s, 1H, NH), 7.75 (d, 1H, H-5, J = 8.6 Hz), 7.65 (s, 1H, H-8), 7.43 (d, 2H, H-a, J = 8.8 Hz), 7.10 (d, 2H, H-b, J = 8.8 Hz), 6.58 (dd, 1H, H-6, J = 8.6 and 2.4 Hz), 6.37 (d, 1H, vinylic-H, J = 2.2 Hz), 5.36 (d, 2H, H-2, J = 2.2 Hz), 4.51 (t, 2H, H-1′, J = 5.0 Hz), 3.48 (m, 4H, H-a′), 3.04 (m, 2H, H-2′), 1.85–1.33 (m, 6H, H-b′, H-c′). ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.98, 165.27, 162.94, 158.91, 135.47, 132.65, 129.83, 129.73, 127.71, 115.45, 114.64, 111.70, 102.93, 67.98, 62.91, 55.03, 53.05, 22.76, 21.65. LC/MS (ESI): m/z 380.2 [M^++H]. Anal. Calcd for $C_{23}H_{25}NO_4$.HCl (415.91): C, 66.42; H, 6.30; N, 3.37. Found: C, 66.56; H, 6.23; N, 3.24.

*5.1.4.5. Synthesis of (E)-3-(4-(3-(piperidin-1-yl)propoxy)benzylidene)-2,3-dihydro-7-methoxychromen-4-one hydrochloride (**7e**).* Starting from 7-methoxychroman-4-one (**6a**, 5 mmol, 0.89 g) and 4-(3-(piperidin-1-yl)propoxy)benzaldehyde (5 mmol, 1.235 g), compound **7e** was obtained as light brown crystals in 84% yield; mp 212–214 °C; IR (KBr, cm^{-1}) ν_{max}: 3380 (O–H), 1669 (C=O), 1613 (C=C alkene); ^{1}H NMR ($CDCl_3$, 500 MHz) 12.25 (br s, 1H, NH), 7.97 (d, 1H, H-5, J = 8.8 Hz), 7.79 (s, 1H, H-8), 7.26 (d, 2H, H-a, J = 8.6 Hz), 6.93 (d, 2H, H-b, J = 8.6 Hz), 6.64 (dd, 1H, H-6, J = 8.8 and 2.4 Hz), 6.41 (d, 1H, vinylic-H, J = 2.0 Hz), 5.35 (d, 2H, H-2, J = 2.0 Hz), 4.15 (t, 2H, H-1′, J = 5.5 Hz), 3.85 (s, 3H, OMe), 3.60 (m, 2H, H-3′), 3.19 (m, 2H, H-a′), 2.67 (m, 2H, H-a′), 2.50 (m, 2H, H-2′), 2.32–1.45 (m, 6H, H-b′ and H-c′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.96, 165.97, 162.94, 159.12, 136.29, 131.93, 129.61, 129.18, 127.67, 115.72, 114.60, 110.39, 100.75, 67.95, 65.14, 55.66, 55.20, 53.52, 23.80, 22.57, 22.09. LC/MS (ESI): m/z 408.2 [M^++H]. Anal. Calcd for $C_{25}H_{29}NO_4$.HCl (443.96): C, 67.63; H, 6.81; N, 3.15. Found: C, 67.92; H, 6.57; N, 3.21.

*5.1.4.6. Synthesis of (E)-3-(4-(2-(piperidin-1-yl)ethoxy)benzylidene)-2,3-dihydro-7-methoxychromen-4-one hydrochloride (**7f**).* Starting from 7-methoxychroman-4-one (**6a**, 5 mmol, 0.89 g) and 4-(2-(piperidin-1-yl)ethoxy)benzaldehyde (5 mmol, 1.165 g), compound **7f** was obtained as yellow crystals in 72% yield; mp 192–194 °C; IR (KBr, cm^{-1}) ν_{max}: 3426 (N–H), 1664 (C=O), 1603 (C=C alkene); ^{1}H NMR ($CDCl_3$, 400 MHz) 12.49 (br s, 1H, NH), 7.96 (d, 1H, H-5, J = 9.0 Hz), 7.78 (s, 1H, H-8), 7.27 (d, 2H, H-a, J = 8.6 Hz), 6.98 (d, 2H, H-b, J = 8.6 Hz), 6.64 (d, 1H, H-6, J = 9.0 Hz), 6.41 (s, 1H, vinylic-H), 5.34 (s, 2H, H-2), 4.65 (br s, 2H, H-1′), 3.86 (s, 3H, OMe), 3.67 (br, 2H, H-a′), 3.44 (br s, 2H, H-a′), 2.84 (br s, 2H, H-2′), 2.28–1.80 (m, 6H, H-b′ and H-c′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.89, 166.00, 162.98, 157.93, 135.99, 131.93, 129.66, 128.43, 115.73, 114.82, 110.44, 100.76, 67.90, 62.95, 56.24, 55.66, 54.09, 22.73, 21.88. LC/MS (ESI): m/z 394.2 [M^++H]. Anal. Calcd for $C_{24}H_{27}NO_4$.HCl (429.93): C, 67.05; H, 6.56; N, 3.26. Found: C, 66.97; H, 6.73; N, 3.31.

*5.1.4.7. Synthesis of (E)-3-(4-(2-morpholinoethoxy)benzylidene)-2,3-dihydro-7-methoxychromen-4-one hydrochloride (**7g**).* Starting from 7-methoxychroman-4-one (**6a**, 5 mmol, 0.89 g) and 4-(2-morpholinoethoxy)benzaldehyde (5 mmol, 1.175 g), compound **7g** was obtained as cream crystals in 64% yield; mp 194–196 °C; IR (KBr, cm^{-1}) ν_{max}: 3374 (N–H), 1699 (C=O), 1598 (C=C alkene); ^{1}H NMR ($CDCl_3$, 400 MHz) 13.36 (br s, 1H, NH), 7.96 (d, 1H, H-5, J = 8.8 Hz), 7.78 (s, 1H, H-8), 7.28 (d, 2H, H-a, J = 8.8 Hz), 7.00 (d, 2H, H-b, J = 8.8 Hz), 6.64 (dd, 1H, H-6, J = 8.8 and 2.4 Hz), 6.41 (d, 1H, vinylic-H, J = 2.2 Hz), 5.33 (d, 2H, H-2, J = 2.2 Hz), 4.67 (t, 2H, H-1′, J = 4.2 Hz), 4.26 (br, 4H, H-b′), 4.03 (br, 2H, H-b′), 3.85 (s, 3H, OMe) 3.60 (br, 2H, H-a′), 3.51 (t, 2H, H-2′, J = 4.0 Hz), 3.12 (br, 2H, H-a′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.89, 166.04, 162.99, 157.75, 135.92, 131.95, 129.79, 129.67, 128.61, 115.71, 114.81, 110.47, 100.77, 67.88, 63.72, 62.86, 56.72, 55.66, 52.82. LC/MS (ESI): m/z 396.2 [M^++H]. Anal. Calcd for $C_{23}H_{25}NO_5$.HCl (431.91): C, 63.96; H, 6.07; N, 3.24. Found: C, 63.66; H, 6.19; N, 3.34.

*5.1.4.8. Synthesis of (E)-3-(4-(3-morpholinopropoxy)benzylidene)-2,3-dihydro-7-methoxychromen-4-one hydrochloride (**7h**).* Starting from 7-methoxychroman-4-one (**6a**, 5 mmol, 0.89 g) and 4-(3-morpholinopropoxy)benzaldehyde (5 mmol, 1.245 g), compound **7h** was obtained as orange crystals in 68% yield; mp 229–233 °C; IR (KBr, cm^{-1}) ν_{max}: 3416 (N–H), 1659 (C=O), 1588 (C=C alkene); ^{1}H NMR (DMSO-d_6 + $CDCl_3$, 400 MHz) 11.12 (br s, 1H, NH) 7.80 (d, 1H, H-5, J = 8.8 Hz), 7.66 (s, 1H, H-8), 7.40 (d, 2H, H-a,

$J = 8.6$ Hz), 7.04 (d, 2H, H-b, $J = 8.6$ Hz), 6.66 (dd, 1H, H-6, $J = 8.8$ and 2.4 Hz), 6.52 (d, 1H, vinylic-H, $J = 2.0$ Hz), 5.39 (d, 2H, H-2, $J = 2.0$ Hz), 4.15 (t, 2H, H-1′, $J = 7.0$ Hz), 3.91 (br s, 4H, H-b′), 3.83 (s, 3H, OMe), 3.44 (br, 2H, H-a′), 3.26 (t, 2H, H-3′, $J = 7.0$ Hz), 3.11 (br, 2H, H-b′), 2.24 (m, 2H, H-2′). ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.11, 166.01, 162.95, 159.75, 135.99, 132.64, 129.42, 129.14, 127.18, 115.64, 115.22, 110.75, 101.26, 68.16, 65.59, 63.73, 56.21, 53.99, 51.61, 23.47. LC/MS (ESI): m/z 410.2 [M^++H]. Anal. Calcd for $C_{24}H_{27}NO_5$.HCl (445.94): C, 64.64; H, 6.33; N, 3.14. Found: C, 64.90; H, 6.11; N, 3.27.

*5.1.4.9. Synthesis of (E)-3-(4-(3-(pyrrolidin-1-yl)propoxy)benzylidene)-2,3-dihydro-7-methoxychromen-4-one hydrochloride (**7i**).* Starting from 7-methoxychroman-4-one (**6a**, 5 mmol, 0.89 g) and 4-(3-(pyrrolidin-1-yl)propoxy)benzaldehyde (5 mmol, 1.165 g), compound **7i** was obtained as brown crystals in 54% yield; mp 234–236 °C; IR (KBr, cm^{-1}) ν_{max}: 3389 (N–H), 1669 (C=O), 1640 (C=C alkene); ^{1}H NMR ($CDCl_3$, 400 MHz) 12.54 (br s, 1H, NH), 7.95 (d, 1H, H-5, $J = 8.6$ Hz), 7.78 (s, 1H, H-8), 7.26 (d, 2H, H-a, $J = 8.6$ Hz), 6.95 (d, 2H, H-b, $J = 8.6$ Hz), 6.63 (d, 1H, H-6, $J = 8.6$ Hz), 6.40 (s, 1H, vinylic-H), 5.34 (s, 2H, H-2), 4.16 (br s, 2H, H-1′), 3.85 (br s, 3H, OMe and 2H, H-a′), 3.32 (br s, 2H, H-a′), 2.87 (br s, 2H, H-3′), 2.48 (br s, 2H, H-2′), 2.28 (br s, 2H, H-b′), 2.12 (br s, 2H, H-b′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.92, 165.97, 162.94, 159.09, 136.23, 131.95, 129.64, 129.27, 127.77, 115.76, 114.66, 110.38, 100.77, 67.98, 65.04, 55.66, 54.04, 53.31, 25.84, 23.54. LC/MS (ESI): m/z 394.2 [M^++H]. Anal. Calcd for $C_{24}H_{27}NO_4$.HCl (429.94): C, 67.05; H, 6.56; N, 3.26. Found: C, 67.29; H, 6.27; N, 3.14.

*5.1.4.10. Synthesis of (E)-3-(4-(2-(piperidin-1-yl)ethoxy)benzylidene)-2,3-dihydro-7-ethoxychromen-4-one hydrochloride (**7j**).* Starting from 7-ethoxychroman-4-one (**6b**, 5 mmol, 0.96 g) and 4-(2-(piperidin-1-yl)ethoxy)benzaldehyde (5 mmol, 1.165 g), compound **7j** was obtained as cream crystals in 42% yield; mp 195–198 °C; IR (KBr, cm^{-1}) ν_{max}: 3426 (N–H), 1664 (C=O), 1603 (C=C alkene); ^{1}H NMR ($CDCl_3$, 400 MHz) 12.12 (br s, 1H, NH), 7.90 (d, 1H, H-5, $J = 8.8$ Hz), 7.74 (s, 1H, H-8), 7.21 (d, 2H, H-a, $J = 8.8$ Hz), 6.89 (d, 2H, H-b, $J = 8.8$ Hz), 6.58 (dd, 1H, H-6, $J = 8.8$ and 2.4 Hz), 6.35 (d, 1H, vinylic-H, $J = 2.0$ Hz), 5.30 (d, 2H, H-2, $J = 2.0$ Hz), 4.11 (t, 2H, H-1′, $J = 5.6$ Hz), 4.04 (q, 2H, OCH_2, $J = 7.0$ Hz), 3.57 (d, 2H, H-a′, $J = 11.6$ Hz), 3.17 (m, 2H, H-2′), 2.68 (m, 2H, H-a′), 2.33–1.83 (m, 6H, H-b′ and H-c′), 1.40 (t, 3H, CH_3, $J = 7.0$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.85, 165.35, 163.00, 159.09, 136.12, 131.89, 129.54, 129.56, 127.68, 115.56, 114.58, 110.72, 101.16, 67.91, 65.13, 63.99, 55.17, 53.47, 22.56, 22.09, 14.56. LC/MS (ESI): m/z 408.2 [M^++H]. Anal. Calcd for $C_{25}H_{29}NO_4$.HCl (443.97): C, 67.63; H, 6.81; N, 3.15. Found: C, 67.52; H, 6.92; N, 3.17.

*5.1.4.11. Synthesis of (E)-3-(4-(3-morpholinopropoxy)benzylidene)-2,3-dihydro-7-ethoxychromen-4-one hydrochloride (**7k**).* Starting from 7-ethoxychroman-4-one (**6b**, 5 mmol, 0.96 g) and 4-(3-morpholinopropoxy)benzaldehyde (5 mmol, 1.245 g), compound **7k** was obtained as orange crystals in 64% yield; mp 216–218 °C; IR (KBr, cm^{-1}) ν_{max}: 3316 (N–H), 1679 (C=O), 1598 (C=C alkene); ^{1}H NMR ($CDCl_3$, 400 MHz) 13.23 (br s, 1H, NH), 7.95 (d, 1H, H-5, $J = 9.0$ Hz), 7.78 (s, 1H, H-8), 7.26 (d, 2H, H-a, $J = 8.8$ Hz), 6.93 (d, 2H, H-b, $J = 8.8$ Hz), 6.62 (dd, 1H, H-6, $J = 9.0$ and 2.4 Hz), 6.39 (d, 1H, vinylic-H, $J = 1.8$ Hz), 5.34 (d, 2H, H-2, $J = 1.8$ Hz), 4.34 (t, 2H, H-b′, $J = 12.0$ Hz), 4.14 (t, 2H, H-1′, $J = 5.4$ Hz), 4.08 (q, 2H, OCH_2, $J = 7.0$ Hz), 4.04 (t, 2H, H-b′, $J = 12.0$ Hz), 3.52 (m, 2H, H-a′), 3.26 (t, 2H, H-a′, $J = 7.6$ Hz), 2.96 (br s, 2H, H-3′), 2.51 (m, 2H, H-2′), 1.44 (t, 3H, CH_3, $J = 7.0$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.90, 165.40, 162.92, 158.95, 136.08, 131.92, 129.62, 129.41, 127.89, 115.60, 114.57, 110.75, 101.19, 67.92, 64.88, 64.00, 63.60, 55.75, 52.17, 23.45, 14.58. LC/MS (ESI): m/z 424.2 [M^++H]. Anal. Calcd for $C_{25}H_{29}NO_5$.HCl (459.97): C, 65.28; H, 6.57; N, 3.05. Found: C, 65.33; H, 6.41; N, 3.19.

*5.1.4.12. Synthesis of (E)-3-(4-(2-morpholinoethoxy)benzylidene)-2,3-dihydro-7-ethoxychromen-4-one hydrochloride (**7l**).* Starting from 7-ethoxychroman-4-one (**6b**, 5 mmol, 0.96 g) and 4-(2-morpholinoethoxy)benzaldehyde (5 mmol, 1.175 g), compound **7l** was obtained as yellow crystals in 64% yield; mp 180–182 °C; IR (KBr, cm^{-1}) ν_{max}: 3384 (N–H), 1691 (C=O), 1594 (C=C alkene); ^{1}H NMR ($CDCl_3$, 400 MHz), 13.39 (br s, 1H, NH), 7.95 (d, 1H, H-5, $J = 8.8$ Hz), 7.78 (s, 1H, H-8), 7.25 (d, 2H, H-a, $J = 8.8$ Hz), 6.98 (d, 2H, H-b, $J = 8.8$ Hz), 6.63 (dd, 1H, H-6, $J = 8.8$ and 2.4 Hz), 6.39 (d, 1H, vinylic-H, $J = 2.2$ Hz), 5.33 (d, 2H, H-2, $J = 2.2$ Hz), 4.67 (br s, 2H, H-1′), 4.29 (t, 2H, H-b′, $J = 11.8$ Hz), 4.07 (q, 2H, OCH_2, $J = 7.0$ Hz) 4.04 (dd, 2H, H-b′, $J = 10.0$ and 3.0 Hz), 3.62 (d, 2H, H-a′, $J = 11.8$ Hz), 3.51 (br s, 2H, H-2′), 3.09 (m, 2H, H-a′), 1.44 (t, 3H, CH_3, $J = 7.0$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.89, 165.46, 162.97, 157.72, 135.82, 131.93, 129.88, 129.64, 128.65, 115.56, 114.79, 110.82, 101.19, 67.85, 64.02, 63.71, 62.84, 56.73, 52.82, 14.58. LC/MS (ESI): m/z 410.2 [M^++H]. Anal. Calcd for $C_{24}H_{27}NO_5$.HCl (445.94): C, 64.64; H, 6.33; N, 3.14. Found: C, 64.91; H, 6.21; N, 3.23.

*5.1.4.13. Synthesis of (E)-3-(4-(3-(piperidin-1-yl)propoxy)benzylidene)-2,3-dihydro-7-ethoxychromen-4-one hydrochloride (**7m**).* Starting from 7-ethoxychroman-4-one (**6b**, 5 mmol, 0.96 g) and 4-(3-(piperidin-1-yl)propoxy)benzaldehyde (5 mmol, 1.235 g), compound **7m** was obtained as orange crystals in 68% yield; mp 228–230 °C; IR (KBr, cm^{-1}) ν_{max}: 3326 (N–H), 1664 (C=O), 1603 (C=C alkene); ^{1}H NMR ($CDCl_3$, 400 MHz) 12.12 (br s, 1H, NH), 7.90 (d, 1H, H-5, $J = 8.8$ Hz), 7.74 (s, 1H, H-8), 7.21 (d, 2H, H-a, $J = 8.8$ Hz), 6.89 (d, 2H, H-b, $J = 8.8$ Hz), 6.58 (dd, 1H, H-6, $J = 8.8$ and 2.4 Hz), 6.35 (d, 1H, vinylic-H, $J = 2.0$ Hz), 5.30 (d, 2H, H-2, $J = 2.0$ Hz), 4.11 (t, 2H, H-1′, $J = 5.6$ Hz), 4.04 (q, 2H, OCH_2, $J = 7.0$ Hz), 3.57 (d, 2H, H-a′, $J = 11.6$ Hz), 3.17 (quintet, 2H, H-a′, $J = 5.20$ Hz), 2.68 (m, 2H, H-a′), 2.46 (m, 2H, H-2′), 2.33–1.83 (m, 6H, H-b′ and H-c′), 1.40 (t, 3H, CH_3, $J = 7.0$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.86, 165.36, 162.90, 159.09, 136.12, 131.89, 129.54, 129.56, 127.68, 115.56, 114.58, 110.72, 101.16, 67.91, 65.13, 63.99, 55.17, 53.48, 23.77, 22.57, 22.09, 14.57. LC/MS (ESI): m/z 422.2 [M^++H]. Anal. Calcd for $C_{26}H_{31}NO_4$.HCl (457.99): C, 67.98; H, 7.04; N, 3.06. Found: C, 67.98; H, 7.12; N, 3.11.

*5.1.4.14. Synthesis of (E)-3-(4-(2-morpholinoethoxy)benzylidene)-2,3-dihydro-7-benzyloxychromen-4-one hydrochloride (**7n**).* Starting from 7-benzyloxychroman-4-one (**6c**, 5 mmol, 1.27 g) and 4-(2-morpholinoethoxy)benzaldehyde (5 mmol, 1.175 g), compound **7n** was obtained as cream crystals in 46% yield; mp 200–202 °C; IR (KBr, cm^{-1}) ν_{max}: 3434 (N–H), 1669 (C=O), 1598 (C=C alkene); ^{1}H NMR (DMSO-d_6, 400 MHz) 11.55 (br s, 1H, NH), 7.82 (d, 1H, H-5, $J = 8.8$ Hz), 7.68 (s, 1H, H-8), 7.46–7.42, 7.36–7.33 (m, 5H, Ph) 7.40 (d, 2H, H-a, $J = 8.8$ Hz), 7.12 (d, 2H, H-b, $J = 8.8$ Hz), 6.77 (dd, 1H, H-6, $J = 8.8$ and 2.4 Hz), 6.65 (d, 1H, vinylic-H, $J = 2.0$ Hz), 5.41 (d, 2H, H-2, $J = 2.0$ Hz), 5.19 (s, 2H, OCH_2), 4.53 (t, 2H, H-1′, $J = 5.0$ Hz), 3.91 (br s, 4H, H-b′), 3.56 (br s, 2H, H-2′), 3.46 (br, 2H, H-a′), 3.23 (br s, 2H, H-a′). ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.15, 165.09, 162.89, 159.01, 136.69, 135.97, 132.75, 129.52, 129.39, 128.99, 128.57, 128.30, 127.64, 115.76, 115.51, 111.45, 102.29, 70.27, 68.19, 63.65, 62.90, 55.22, 52.13. LC/MS (ESI): m/z 472.2 [M^++H]. Anal. Calcd for $C_{29}H_{29}NO_5$.HCl (508.01): C, 68.57; H, 5.95; N, 2.76. Found: C, 68.91; H, 5.63; N, 2.82.

*5.1.4.15. Synthesis of (E)-3-(4-(3-morpholinopropoxy)benzylidene)-2,3-dihydro-7-benzyloxychromen-4-one hydrochloride (**7o**).* Starting from 7-benzyloxychroman-4-one (**6c**, 5 mmol, 1.27 g) and 4-(3-morpholinopropoxy)benzaldehyde (5 mmol, 1.245 g), compound **7o** was obtained as cream crystals in 58% yield; mp 228–230 °C; IR (KBr, cm^{-1}) ν_{max}: 3414 (N–H), 1669 (C=O), 1598 (C=C alkene); ^{1}H NMR (DMSO-d_6, 400 MHz) 11.09 (br s, 1H, NH) 7.81 (d, 1H, H-5, $J = 8.6$ Hz), 7.67 (s, 1H, H-8), 7.47–7.42, 7.36–7.35

(m, 5H, Ph), 7.39 (d, 2H, H-a, $J = 8.8$ Hz), 7.08 (d, 2H, H-b, $J = 8.8$ Hz), 6.77 (dd, 1H, H-6, $J = 8.8$ and 2.4 Hz), 6.65 (d, 1H, vinylic-H, $J = 2$ Hz), 5.41 (d, 2H, H-2, $J = 2.0$ Hz), 5.20 (s, 2H, CH_2O) 4.15 (t, 2H, H-1′, $J = 7.0$ Hz), 3.91 (br s, 4H, H-b′), 3.45 (br, 2H, H-a′), 3.24 (t, 2H, H-3′, $J = 7.0$ Hz), 3.09 (br s, 2H, H-a′), 2.21 (m, 2H, H-2′). ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.15, 165.07, 162.88, 159.83, 136.70, 136.09, 132.19, 129.51, 129.13, 128.99, 128.57, 128.30, 127.15, 115.78, 115.29, 111.43, 102.30, 70.27, 68.21, 65.66, 63.74, 53.89, 51.27, 23.46. LC/MS (ESI): m/z 486.2 [M^++H]. Anal. Calcd for $C_{30}H_{31}NO_5$.HCl (522.04): C, 69.02; H, 6.18; N, 2.68. Found: C, 68.98; H, 6.21; N, 2.71.

*5.1.4.16. Synthesis of (E)-3-(4-(2-(piperidin-1-yl)ethoxy)benzylidene)-2,3-dihydro-7-benzyloxychromen-4-one hydrochloride (**7p**).* Starting from 7-benzyloxychroman-4-one (**6c**, 5 mmol, 1.27 g) and 4-(2-(piperidin-1-yl)ethoxy)benzaldehyde (5 mmol, 1.165 g), compound **7p** was obtained as cream crystals in 42% yield; mp 206–208 °C; IR (KBr, cm^{-1}) ν_{max}: 3431 (N–H), 1654 (C=O), 1598 (C=C alkene); ^{1}H NMR ($CDCl_3$, 400 MHz) 12.47 (br s, 1H, NH), 7.98 (d, 1H, H-5, $J = 9.0$ Hz), 7.79 (s, 1H, H-8), 7.45–7.36 (m, 5H, Ph), 7.27 (d, 2H, H-a, $J = 8.4$ Hz), 6.98 (d, 2H, H-b, $J = 8.4$ Hz), 6.72 (dd, 1H, H-6, $J = 9.0$ and 2.2 Hz), 6.50 (d, 1H, vinylic-H, $J = 2.2$ Hz), 5.34 (d, 2H, H-2, $J = 2.2$ Hz), 5.12 (s, 2H, OCH_2) 4.65 (br s, 2H, H-1′), 3.67 (d, 2H, H-a′, $J = 11.6$ Hz), 3.44 (br s, 2H, H-2′), 2.84 (m, 2H, H-a′), 1.92–1.40 (br m, 6H, H-b′ and H-c′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.89, 165.09, 162.91, 157.94, 136.06, 135.90, 131.94, 129.72, 129.66, 128.72, 128.43, 128.32, 127.52, 115.93, 114.82, 110.98, 101.79, 70.32, 67.90, 62.96, 56.26, 54.11, 22.73, 21.88. LC/MS (ESI): m/z 470.2 [M^++H]. Anal. Calcd for $C_{30}H_{31}NO_4$.HCl (506.04): C, 71.21; H, 6.37; N, 2.77. Found: C, 71.43; H, 6.51; N, 2.49.

*5.1.4.17. Synthesis of (E)-3-(4-(3-(pyrrolidin-1-yl)propoxy)benzylidene)-2,3-dihydro-7-benzyloxy chromen-4-one hydrochloride (**7q**).* Starting from 7-benzyloxychroman-4-one (**6c**, 5 mmol, 1.27 g) and 4-(3-(pyrrolidin-1-yl)propoxy)benzaldehyde (5 mmol, 1.165 g), compound **7q** was obtained as brown crystals in 56% yield; mp 210–213 °C; IR (KBr, cm^{-1}) ν_{max}: 3436 (N–H), 1664 (C=O), 1623 (C=C alkene); ^{1}H NMR ($CDCl_3$, 400 MHz) 12.43 (br s, 1H, NH), 7.95 (d, 1H, H-5, $J = 8.8$ Hz), 7.77 (s, 1H, H-8), 7.44–7.35 (m, 5H, Ph), 7.24 (d, 2H, H-a, $J = 8.8$ Hz), 6.92 (d, 2H, H-b, $J = 8.8$ Hz), 6.70 (dd, 1H, H-6, $J = 8.8$ and 2.4 Hz), 6.48 (d, 1H, vinylic-H, $J = 2.2$ Hz), 5.33 (d, 2H, H-2, $J = 2.2$ Hz), 5.09 (s, 2H, OCH_2) 4.14 (t, 2H, H-1′, $J = 5.6$ Hz), 3.84 (br s, 2H, H-a′), 3.30 (t, 2H, H-a′, $J = 7.2$ Hz), 2.86 (br s, 2H, H-3′), 2.45 (m, 2H, H-2′), 2.27 (m, 2H, H-b′), 2.10 (br m, 2H, H-b′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.91, 165.04, 162.87, 159.11, 136.30, 135.90, 131.95, 129.68, 129.21, 128.71, 128.31, 127.72, 127.51, 115.94, 114.61, 110.92, 101.79, 70.30, 67.95, 64.89, 53.86, 53.11, 25.74, 23.44. LC/MS (ESI): m/z 470.2 [M^++H]. Anal. Calcd for $C_{30}H_{31}NO_4$.HCl (506.03): C, 71.21; H, 6.37; N, 2.77. Found: C, 71.47; H, 6.19; N, 2.99.

*5.1.4.18. Synthesis of (E)-3-(4-(3-(piperidin-1-yl)propoxy)benzylidene)-2,3-dihydro-7-benzyloxychromen-4-one hydrochloride (**7r**).* Starting from 7-benzyloxychroman-4-one (**6c**, 5 mmol, 1.27 g) and 4-(3-(piperidin-1-yl)propoxy)benzaldehyde (5 mmol, 1.235 g), compound **7r** was obtained as cream crystals in 50% yield; mp 215–217 °C; IR (KBr, cm^{-1}) ν_{max}: 3472 (N–H), 1659 (C=O), 1608 (C=C alkene); ^{1}H NMR ($CDCl_3$, 500 MHz) 12.03 (br s, 1H, NH), 7.91 (d, 1H, H-5, $J = 8.8$ Hz), 7.73 (s, 1H, H-8), 7.38–7.31 (m, 5H, Ph), 7.20 (d, 2H, H-a, $J = 7.7$ Hz), 6.88 (d, 2H, H-b, $J = 7.7$ Hz), 6.66 (d, 1H, H-6, $J = 8.8$ Hz), 6.44 (s, 1H, vinylic-H), 5,29 (s, 2H, H-2), 5.05 (s, 2H, OCH_2), 4.09 (s, 2H, H-1′), 3.55 (br s, 2H, H-a′), 3.17 (br s, 2H, H-a′), 2.69 (br s, 2H, H-3′), 2.45 (br s, 2H, H-2′), 2.26–1.83 (m, 6H, H-b′ and H-c′). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.82, 164.99, 162.83, 159.13, 136.25, 135.87, 132.94, 131.93, 129.61, 129.13, 128.67, 128.27, 127.59, 127.49, 115.89, 114.61, 110.88, 101.76, 70.27, 67.94, 65.17, 55.17, 53.49, 23.79, 22.58, 22.08. LC/MS (ESI): m/z 484.2 [M^++H]. Anal. Calcd for $C_{31}H_{33}NO_4$.HCl (520.07): C, 71.33; H, 6.59; N, 2.69. Found: C, 71.33; H, 6.67; N, 2.97.

5.2. Cholinesterase inhibition test

AChE (E.C.3.1.1.7, Type V–S, from *Electric eel*), BuChE (E.C.3.1.1.8, from equine serum), Ellman's reagent (DTNB), acetylthiocholine (ATCh) and butyrylthiocholine (BTCh) were purchased from Sigma–Aldrich. The test was done as defined in the following procedure [25,26]. Five different concentrations of the test compound were tested at 25 °C in triplicate. To determine the percent of inhibition, 50 μl of test compound, 50 μl of enzyme solution and 100 μl DTNB 0.01 M were mixed in 3 ml buffer phosphate 0.1 M (pH = 8). After 3 min of incubation, 20 μl substrate (0.075 M) was added. Afterwards, absorption was measured using Unico spectrophotometer at 412 nm. The inhibition curve was attained by plotting percentage enzyme activity versus logarithm of test compound concentration.

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). To prepare the protein structure, the water molecules, ligand and all unbound molecules were removed. Then ADT/AutoDockTools (1.5.4) [27] was utilized to make pdbqt format of protein using default parameters. Ligands were drawn in Marvine Sketch 5.8.3, 2012, ChemAxon (http://www.chemaxon.com) and then the 3D structures were generated using Openbabel (ver. 2.3.1) and saved as pdbqt file [28]. Before running molecular docking simulation, ligands were optimized in HyperChem 7.5 (Hypercube, inc.). Docking simulation was accomplished by Autodock Vina (1.1.1) [29]. Docking was done in a search space that was defined as a box with dimensions of 40 × 40 × 40. The midpoint of the box was adjusted on the geometrical center of co-crystallized ligand using these parameters: center_x = 2.023, center_y = 63.295, center_z = 67.062. The exhaustiveness was set 80 and other parameters were left unchanged. Finally, the best poses were chosen based on the free energy of binding and further analyzed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.04.055.

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