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Synthesis and evaluation of 4-substituted coumarins as novel acetylcholinesterase inhibitors

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ABSTRACT

A series of 4-hydroxycoumarin derivatives were designed and synthesized as new acetylcholinesterase (AChE) inhibitors which could be considered for Alzheimer's disease therapeutics. Among the 19 coumarin-derived compounds tested toward *Electrophorus electricus* acetylcholinesterase (*eel*AChE) and horse serum butyrylcholinesterase (*eq*BChE), *N*-(1-benzylpiperidin-4-yl)acetamide derivative **4m** displayed highest AChE inhibitory activity ($IC_{50} = 1.2$ μM) and good selectivity (37 times). The docking study of the most potent compound **4m**, indicated that Phe330 is responsible for ligand recognition and trafficking by forming π-cation interaction with benzylpiperidine moiety. Furthermore, the formation of an additional π–π interaction between coumarin moiety and Trp279 of peripheral anionic site could stabilize the ligand in the active site resulting in more potent inhibition of the enzyme.

1. Introduction

Alzheimer's disease is a chronic and progressive neurodegenerative disorder of the central nervous system which is associated with memory loss, cognitive impairment and decline in language [1]. Neuropathological evidence has shown that the reduced levels of acetylcholine, β-amyloid senile plaques and neurofibrillary tangles formation within the brain of afflicted individuals play a crucial role in the pathogenesis of Alzheimer's disease [2]. Accordingly, the enhancement of cholinergic neurotransmission and the inhibition of β-amyloid peptide formation are considered as main approaches for effective treatment of Alzheimer's disease [3–5].

Since acetylcholinesterase (AChE) plays a pro-aggregating (non-catalytic) role to accelerating β-amyloid peptide aggregation and deposition into the fibrils [6], thus inhibition of AChE is still the most successful therapeutic strategy for the symptomatic treatment of Alzheimer's disease and its progression [7,8].

Several AChE inhibitors (Fig. 1) such as donepezil, rivastigmine, galantamine, ensaculine, propidium, and tacrine have been developed for symptomatic treatment of Alzheimer's disease in the early to moderate stages [9,10]. The study of donepezil–TcAChE complex by X-ray crystallography has revealed that the indanone and benzylpiperidine moieties of donepezil interact with the peripheral (non-catalytic) and central (catalytic) binding site of AChE, respectively [11]. Accordingly, agents able to bind both the peripheral and catalytic sites of AChE are proposed as a better choice for Alzheimer's disease therapy.

Structurally, AChE inhibitors belong to different classes of compounds. Among them, ensaculine is a coumarin derivative containing piperazine ring with three atom linker (Fig. 2), which slows down or prevents the progression of the neurodegradation and Alzheimer's disease [12,13]. Furthermore, Piazzi et al. have designed AP2238, a coumarin derivative as dual binding site AChE inhibitor (Fig. 2), which is able to simultaneously interact with both the central and the peripheral anionic sites [14]. Coumarins are naturally occurring compounds with wide range of biological activities including AChE inhibition. Previously, several studies with coumarin derived AChE inhibitors have demonstrated that coumarin ring primarily interacts with peripheral anionic site of

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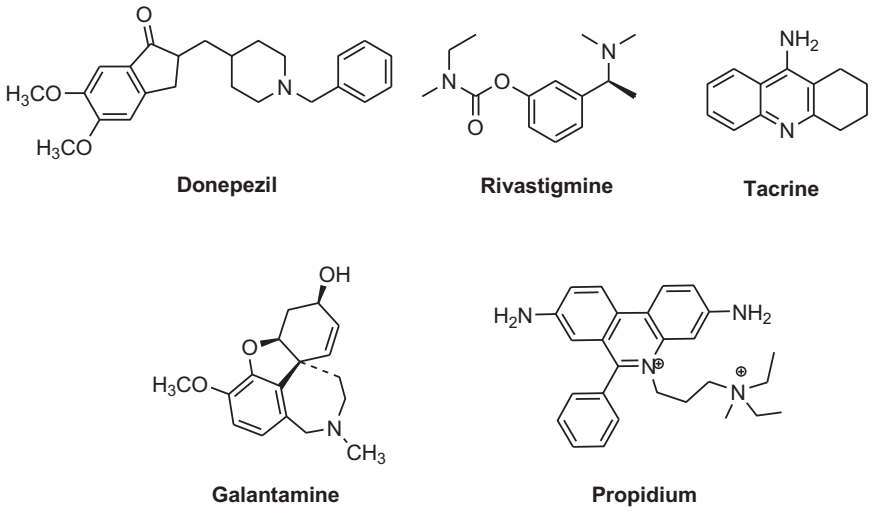


Fig. 1. Structures of several AChE inhibitors, have been developed for symptomatic treatment of Alzheimer's disease.

AChE and amine functional moiety including benzylamino, phenylpiperazine or anilino which linked to coumarin heterocycle using appropriate spacer, interacts with catalytic site of AChE [15]. Moreover, the fact that chemical substitutions can occur at many positions of this core structure, have made coumarins interesting molecules for drug discovery in the field of AChE inhibitors [15].

In continuation of our previous study for developing new AChE inhibitors [16], in this work we focused our attention on 4-hydroxycoumarin derivatives bearing an amine functional group on alkyl side chain in which the presence of lipophilic moiety and often a tertiary amino group represents the key requirement for a good AChE inhibition. Thus, we report herein synthesis, biological evaluation and molecular docking study of 4-substituted coumarins **4a**–**s** as novel AChE inhibitors (Fig. 2).

2. Chemistry

The synthetic route to the target compounds **4a**–**s** starting from commercially available 4-hydroxycoumarin (**1**) was shown in Scheme 1. *O*-Alkylation of compound **1** with ethyl 2-bromoacetate or ethyl 4-bromobutanoate in the presence of K_2CO_3 in DMF afforded ethyl ester derivatives **2a** or **2b**, respectively. The esters **2a** and **2b** were hydrolyzed with aqueous solution of sodium hydroxide in dioxane to yield the corresponding acids **3a** and **3b**. The condensation of the carboxylic acids (**3a**, **3b**) with appropriate amines were attempted by various reagents and conditions such as carbonyldiimidazole (CDI) and dicyclohexylcarbodiimide (DCC), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and hydroxybenzotriazole (HBT) in different solvents, but the best result was obtained by EDC/HBT in acetonitrile.

3. Pharmacology

3.1. In vitro inhibition studies of cholinesterases

The modified Ellman's method [17] was utilized to determine the inhibitory activity of compounds **4a**–**s** against acetylcholinesterase and butyrylcholinesterase using *Electrophorus electricus*

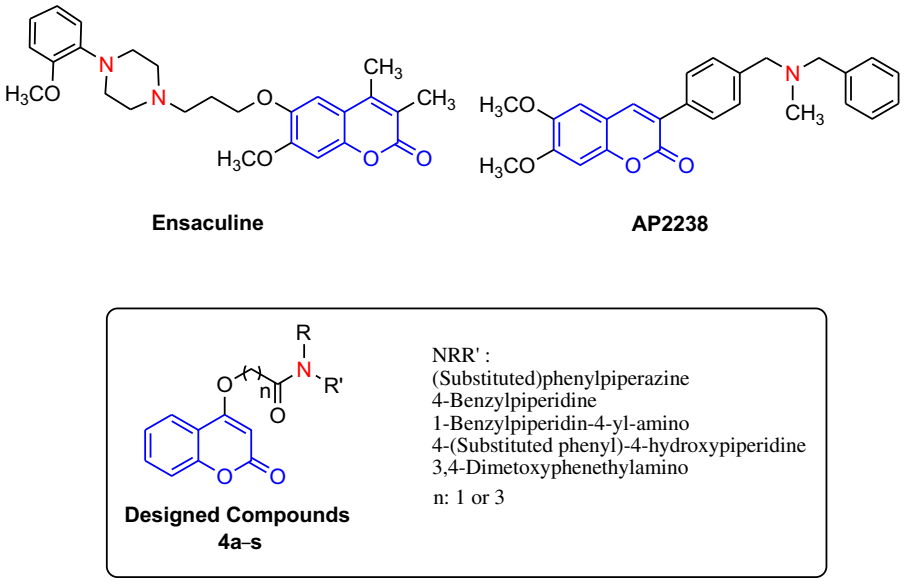
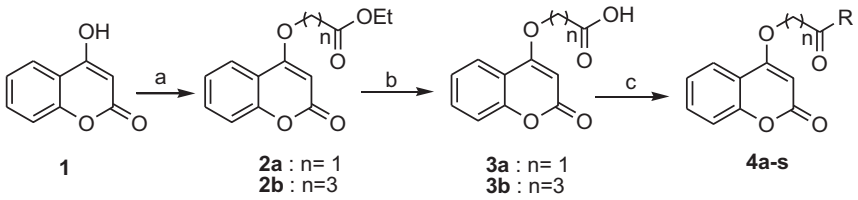


Fig. 2. AChE inhibitors ensaculine and AP2238 belong to coumarin class and designed compounds **4a**–**s**.



Scheme 1. Synthesis of compounds **4a**–**s**. Reagents and conditions: (a) Ethyl 2-bromoacetate or ethyl 4-bromobutanoate, K_2CO_3, DMF; (b) Dioxane, NaOH (aq); (c) appropriate amine, EDC, HBT, CH_3CN.

acetylcholinesterase (*eel*AChE) and horse serum butyrylcholinesterase (*eq*BChE). In brief, five different concentrations of target compounds which produce inhibition in a range of 20%–80%, were tested to determine the IC_{50} values. The final concentration of substrate and 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) was 467 μM and 0.003 M, respectively. All experiments were carried out in triplicate and the temperature of all solutions was set to 25 °C prior to use. Donepzil hydrochloride was used as reference drug. The same protocol was used for butyrylcholinesterase assay.

The modified Ellman's method as described by Kapkova et al. [18] is modified such as preparation step of the enzyme stock solution in glycerin 25% instead of gelatin 1%. This modification led to a more storage time of the enzyme aliquots. Furthermore, in the original Ellman method, 50 μl of 5 unit/ml solution of enzyme was added while in the current method a 2.5 unit/ml enzyme solution was used. Finally, the absorbance reading time was 6 min in the original Ellman method, while through our studies; it was observed that the reading time of 1 min would be sufficient.

3.2. Ferric reducing/antioxidant power (FRAP) assay

The total antioxidant activity of target compounds was evaluated by FRAP assay. The FRAP assay represents the potential of compound to reduce the ferric complex of 2,4,6-tripyridyl-*s*-triazine to the colored ferrous complex [19]. The results are expressed as mmol Fe (II)/g dry mass of test compound. Ascorbic acid was used as standard antioxidant.

4. Results and discussion

4.1. Biology

4.1.1. Cholinesterase inhibitory activity

All the newly synthesized compounds **4a**–**s** were evaluated for their in vitro inhibitory activities toward AChE and BChE in comparison with commercially available donepezil as standard drug. The anti-cholinesterase activities are summarized in Table 1.

The IC_{50} values in Table 1 revealed that compound **4m** with an *N*-(1-benzylpiperidin-4-yl)acetamide pendent group displayed highest AChE inhibitory activity (IC_{50} = 1.2 μM) among all the tested compounds. Besides, compounds **4a**, **4b**, **4d**, **4k** and **4n** with IC_{50} values less than 3.5 μM showed significant inhibition against AChE. Generally, in both 2-oxoethoxy and 4-oxobutoxy series ($n = 1$ or 3), *N*-(1-benzylpiperidin-4-yl) derivatives **4m** and **4n** exhibited the most potent inhibitory activity toward AChE. Moreover, in *N*-phenylpiperazine derivatives, the elongation of oxoalkoxy linker (2C to 4C) resulted in the lack of inhibitory activity. However, in the cases of *N*-(1-benzylpiperidin-4-yl) and 4-benzylpiperidin-1-yl derivatives, this modification did not remarkably affect the AChE inhibitory potency (**4n** vs. **4m** and **4l** vs. **4k**).

The lack of inhibitory activity of 4-phenyl-4-hydroxypiperidin-1-yl derivatives **4o**–**4r** may be attributed to the absence of distal nitrogen. However, 4-benzylpiperidin-1-yl compounds **4k** and **4l** without distal nitrogen showed significant activity against AChE. By comparing the IC_{50} values of *N*-phenylpiperazine derivatives **4a**–**e**, it could be concluded that the substitution on phenyl ring was not beneficial for AChE inhibition. Also, the substitution on *ortho*-position of phenylpiperazine moiety decreased AChE inhibitory activity (compounds **4c** and **4e** vs. **4a**). These results may be due to the steric hindrance of *ortho*-substitution on phenyl ring.

All compound showed no BChE inhibition at concentrations less than 41 μM. Remarkably, the BChE inhibition, of at least 34 orders of magnitude lower than that against AChE, revealed an extremely selective inhibitory profile for compounds **4a**, **4b**, **4d** and **4m**.

Recently, we have designed and described a series of coumarin AChE inhibitors namely (*E*)-1-benzyl-4-(3-(2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)pyridinium halides. Some of these compounds have shown higher activity compared with donepezil hydrochloride as standard drug [16]. In the present study, among the synthesized coumarin derivatives, compound **4m** displayed the highest AChE inhibitory activity with IC_{50} value of 1.2 μM. Although the activity of compound **4m** was less than standard drug donepezil, but it had a fairly good inhibitory activity. In addition, it could be synthesized by a simple and affordable synthesis. Therefore, it could be considered as a new lead for further optimization.

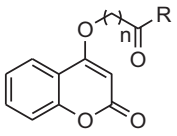
4.1.2. Ferric reducing/antioxidant power (FRAP)

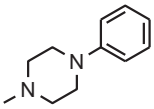
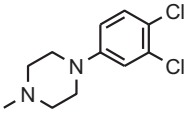
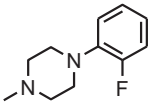
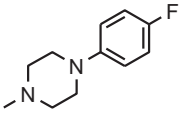
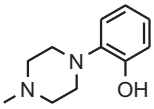
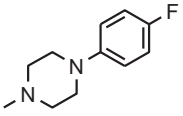
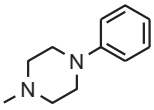
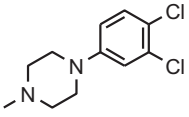
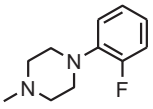
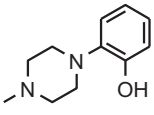
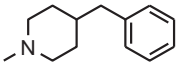
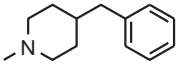
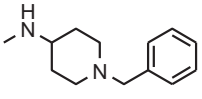
Numerous synthetic and naturally occurring coumarins have potential antioxidant activity. Thus, the antioxidant capacity of coumarin derivatives **4a**–**s** were evaluated by using FRAP assay in comparison with ascorbic acid. The results are summarized in Table 2. According to Table 2, compounds **4e** and **4j** exhibited significant antioxidant activity compared to ascorbic acid. However, the remaining compounds did not show significant antioxidant activity. The antioxidant potential of compounds **4e** and **4j** could be due to the presence of phenolic hydroxyl group in their structures.

4.2. Molecular docking study

Molecular docking study was performed with the most active compound **4m** to clarify the binding mode of the inhibitor. Crystal structure of *Torpedo californica* acetylcholinesterase had been retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) as the receptor model and was finally prepared using Autodock Tools (1.5.4) [20]. Since the structure of *T. californica* acetylcholinesterase (*Tc*AChE) (PDB code: 3I6Z) is fundamentally similar to *E. electricus* AChE (*eel*AChE), it was used as the receptor for molecular docking studies. The interaction of the best docking pose of compound **4m** and amino acids in the active site of *Tc*AchE was shown in Fig. 3. The compound **4m** was spanning along the bottom of the active site to peripheral anionic site (PAS). The ligand was anchored through formation of a π–π interaction between the phenyl ring of *N*-benzylpiperidine moiety and Trp84 and a π-cation interaction between quaternary nitrogen of piperidine moiety and Phe330. One interesting feature of AChE inhibitors is their ability of making sandwich with Phe330 and Trp84 by the contribution of

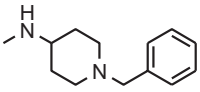
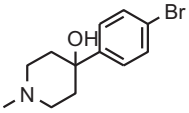
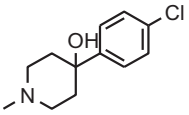
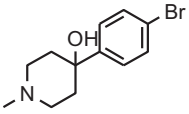
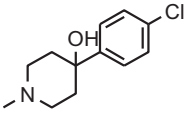
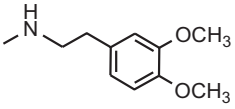
Table 1
AChE and BChE inhibitory activities of compounds **4a**–**s**.



Compound	R	n	AChE (μM)	BChE (μM)
4a		1	2.9	>100
4b		1	2.3	89
4c		1	12.1	>100
4d		1	2.1	76
4e		1	21.2	>100
4f		3	>100	>100
4g		3	>100	>100
4h		3	>100	>100
4i		3	>100	>100
4j		3	>100	>100
4k		1	3.4	65
4l		3	8.0	76
4m		1	1.2	45

(*continued on next page*)

Table 1 (*continued*)

Compound	R	n	AChE (μM)	BChE (μM)
4n		3	2.37	41
4o		1	>100	>100
4p		1	>100	>100
4q		3	>100	>100
4r		3	>100	>100
4s		3	>100	>100
Donepezil			0.014	5.38

planar aromatic ring linked to ionizable nitrogen as observed in compound **4m**. It was reported that Phe330 is responsible for ligand recognition and trafficking by forming π-cation interaction with the ligand at the bottom of the active site. Furthermore, the formation of an additional π–π interaction between coumarin moiety and Trp279 of PAS could stabilize the ligand in the active site resulting in more potent inhibition of the enzyme.

As presented in Table 1, by increasing the length of the linker to $n = 3$, both anti-AChE and BChE activity of the compounds were

Table 2
Antioxidant activity of target compounds **4a**–**s** determined by FRAP assay.

Compound	FRAP (mmol Fe(II)/g) [a]
4a	<100
4b	145 ± 16
4c	161 ± 25
4d	207 ± 11
4e	**840 ± 15**
4f	<100
4g	183 ± 31
4h	<100
4i	143 ± 23
4j	**770 ± 32**
4k	114 ± 14
4l	<100
4m	<100
4n	<100
4o	132 ± 9
4p	<100
4q	<100
4r	<100
4s	126 ± 26
Ascorbic acid	2246 ± 34

[a] The data are expressed as Mean ± SD of three experiments.

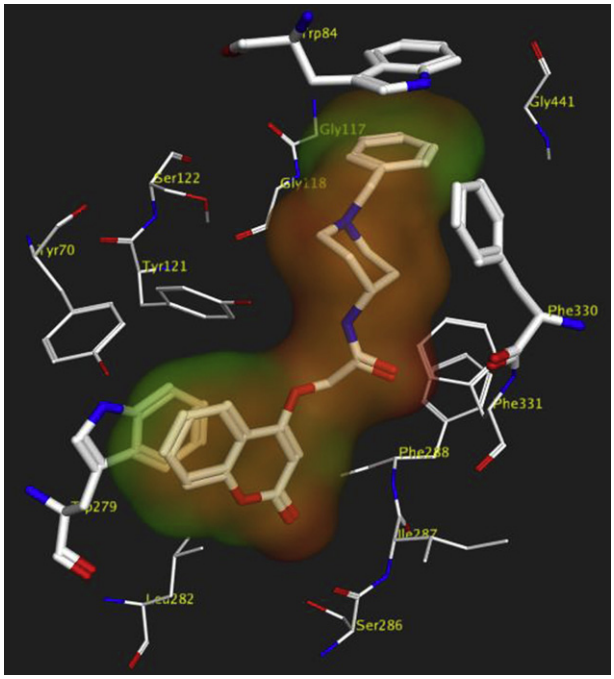


Fig. 3. The proposed binding mode of compound **4m** within the active site of *Tc*AChE (PDB code: 3I6Z) constructed by UCSF Chimera package [22].

affected. In the case of BChE, the change in the activity was of little importance, while for anti-AChE the activity significantly decreased as seen in compounds **4k** and **4l** or **4m** and **4n**. Compounds **4a**, **4b**, **4d** and **4m** ($n = 1$) exhibited superior anti-AChE activity in comparison with their counterparts with $n = 3$. According to docking study, a π–π interaction between coumarin ring of the target compounds and Trp84 of AChE might enhance the potency of the enzyme inhibition via stabilization of the molecule in the gorge of the enzyme. As the length of the linker between coumarin and anionic binding site moiety was increased ($n = 3$), the activity had significantly decreased due to interruption of the aforementioned π–π stacking. The described π–π stacking was not observed in case of BChE since no Trp was present in the peripheral anionic site of BChE. As a result, this kind of interactions might be responsible for the activity against AChE but not against BChE.

5. Conclusion

A series of 4-hydroxycoumarin derivatives with potential anti-acetylcholinesterase activity were designed and synthesized. Particularly, we focused our efforts on *N*-phenylpiperazine and *N*-benzylpiperidine derivatives connected with an alkoxy amide spacer to the coumarin scaffold in order to binding with the catalytic and peripheral sites of AChE. Among the 19 coumarin-derived compounds, compound **4m** with an *N*-(1-benzylpiperidin-4-yl) acetamide pendent group displayed highest AChE inhibitory activity ($IC_{50} = 1.2$ μM) and good selectivity (AChE relative to BChE) of 37 times. Structure–activity relationship studies showed that the anti-AChE activity of compounds was influenced by the type of the cyclic amine attached to the 2-oxo- or 4-oxoalkoxycoumarin backbone. The docking study of the most potent compound **4m**, indicated that Phe330 is responsible for ligand recognition and trafficking by forming π-cation interaction with benzylpiperidine moiety. Furthermore, the formation of an additional π–π interaction between coumarin moiety and Trp279 of PAS could stabilize the ligand in the active site resulting in more potent inhibition of the enzyme. The obtained results with compound **4m** prototype are considerable for further study focusing on investigating the potential candidates for Alzheimer's disease therapeutics.

6. Experimental

All starting material and reagents were purchased from Sigma–Aldrich, Fluka, Acros Organics and Merck, and used without further purification. Melting points were measured on a Kofler hot stage apparatus and are uncorrected. Column chromatography was carried out on silica gel (70–230 mesh). TLC was conducted on silica gel 250 micron, F254 plates. The IR spectra were taken using Nicolet FT-IR Magna 550 spectrometer (KBr disks). ^{1}H NMR spectra were recorded on a Bruker 500 MHz NMR instrument. The chemical shifts (δ) and coupling constants (J) are expressed in parts per million (ppm) and Hertz (Hz), respectively. Data are reported as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; br, broad; m, multiplet), integration and coupling constants. Mass spectra of the products were obtained with an HP (Agilent technologies) 5937 Mass Selective Detector. Elemental analyzes were carried out with a Perkin–Elmer model 240-C apparatus. The results of elemental analyzes (C, H, N) were within ±0.4% of the calculated values.

6.1. General procedure for the synthesis of compounds *2*

4-Hydroxycoumarin (5 mmol) and potassium carbonate (5.5 mmol) were dissolved in DMF (5 ml). The solution was stirred at room temperature for several minutes and then ethyl 2-bromoacetate or ethyl 4-bromobutanoate (5.2 mmol) was added dropwise to the mixture. The solution was heated to 90 °C for 5 h. After completion of the reaction (monitored by TLC), the mixture was cooled to room temperature and diluted with water. The precipitate was filtered and washed with water, and used without further purifications.

6.2. General procedure for the synthesis of compounds *3*

Compound **2a** or **2b** (2 mmol) was dissolved in 1,4-dioxane (5 ml) and then NaOH 5% (2 ml) was added dropwise to the mixture at room temperature. The solution was reflux for 2 h. After cooling, the mixture was extracted with ethyl acetate. The aqueous solution was acidified with HCl 6% and the white solid was filtered off and subsequently washed with water to neutralize.

6.3. General procedure for the synthesis of compounds *4a–s*

Compound **3** (1 mmol), EDC (1 mmol) and HBT (1 mmol) were dissolved in dry acetonitrile (5 ml). The solution was stirred at room temperature for 30 min and then appropriate amine (1 mmol) was added to the mixture. The solution was stirred at room temperature for 24 h. After completion of the reaction (monitored by TLC), the mixture was diluted with water and the precipitate was filtered and washed with saturated sodium carbonate. The resulting crude product was purified by flash chromatography (Silica gel) eluting with ethyl acetate/petroleum ether (1:1) to give compounds **4a–s**.

6.4. Characterization data for compounds

6.4.1. 2-(2-Oxo-2H-chromen-4-yloxy)acetic acid (*3a*)

White solid; yield 80%; mp 198–200 °C (Lit [21] mp 219–220); IR (KBr, cm^{-1}) ν_{max}: 1722 (C=O), 1623 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 (d, $J = 8.0$ Hz, 1H, H_5 coumarin), 7.46 (t, $J = 8.0$ Hz, 1H, H_7 coumarin), 7.26–7.17 (m, 2H, $H_{6,8}$ coumarin), 5.50 (s, 1H, H_3 coumarin), 4.65 (s, 2H, CH_2O).

*6.4.2. 4-(2-Oxo-2H-chromen-4-yloxy)butanoic acid (**3b**)*

White solid; yield 79%; mp 164–166 °C; IR (KBr, cm^{-1}) ν_{max}: 1701 (C=O), 1607 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, $J = 7.5$ Hz, 1H, H_5 coumarin), 7.57 (t, $J = 7.5$ Hz, 1H, H_7 coumarin), 7.35–7.28 (m, 2 H, H_6 and H_8 coumarin), 5.71 (s, 1H, H_3 coumarin), 4.23 (t, $J = 6.0$ Hz, 2H, CH_2O), 2.66 (t, $J = 6.0$ Hz, 2H, CH_2CO), 2.30–2.18 (m, 2H, -CH_2-). Anal. Calcd for $C_{13}H_{12}O_5$ (248.23): C, 62.90; H, 4.87. Found: C, 62.79; H, 4.49.

*6.4.3. 4-(2-Oxo-2-(4-phenylpiperazin-1-yl)ethoxy)-2H-chromen-2-one (**4a**)*

White solid; yield 90%; mp 190–192 °C; IR (KBr, cm^{-1}) ν_{max}: 1714 (C=O), 1677 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 7.90 (d, $J = 7.5$ Hz, 1H, H_5 coumarin), 7.58 (t, $J = 7.5$ Hz, 1H, H_7 coumarin), 7.35–7.28 (m, 4H, $H_{6,8}$ coumarin, $H_{3,5}$ phenyl), 6.95–6.93 (m, 3H, $H_{2,4,6}$ phenyl), 5.71 (s, 1H, H_3 coumarin), 4.94 (s, 2H, CH_2O), 3.84 (br s, 2H, CH_2N), 3.68 (br s, 2H, CH_2N), 3.23 (br s, 4H, $2CH_2N$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.3, 163.1, 161.9, 152.8, 150.1, 132.2, 128.8, 123.6, 122.5, 120.4, 116.3, 114.7, 91.0, 66.3, 49.4, 48.8, 44.5, 41.5. Anal. Calcd for $C_{21}H_{20}N_2O_4$ (364.39): C, 69.22; H, 5.53; N, 7.69. Found: C, 69.48; H, 5.32; N, 7.81.

*6.4.4. 4-(2-(4-(3,4-Dichlorophenyl)piperazin-1-yl)-2-oxoethoxy)-2H-chromen-2-one (**4b**)*

White solid; yield 70%; mp 216–218 °C; IR (KBr, cm^{-1}) ν_{max}: 1710 (C=O), 1678 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 7.88 (d, $J = 7.1$ Hz, 1H, H_5 coumarin), 7.58 (t, $J = 7.1$ Hz, 1H, H_7 coumarin), 7.35–7.28 (m, 3H, H_6 coumarin, $H_{5,6}$ phenyl), 6.97 (s, 1H, H_2 phenyl), 6.75 (d, $J = 7.1$ Hz, 1H, H_8 coumarin), 5.71 (s, 1H, H_3 coumarin), 4.93 (s, 2H, CH_2O), 3.82 (br s, 2H, CH_2N), 3.67 (br s, 2H, CH_2N), 3.21 (br s, 4H, $2CH_2N$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.2, 163.2, 161.9, 152.8, 149.4, 132.3, 130.2, 123.6, 123.1, 122.4, 117.6, 116.3, 115.5, 114.7, 91.0, 66.3, 48.8, 48.3, 44.2, 41.2. Anal. Calcd for $C_{21}H_{18}Cl_2N_2O_4$ (433.28): C, 58.21; H, 4.19; N, 6.47. Found: C, 58.34; H, 4.32; N, 6.11.

*6.4.5. 4-(2-(4-(2-Fluorophenyl)piperazin-1-yl)-2-oxoethoxy)-2H-chromen-2-one (**4c**)*

Yellow solid; yield 79%; mp 160–162 °C; IR (KBr, cm^{-1}) ν_{max}: 1717 (C=O), 1675 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 7.90 (dd, $J = 7.8$ and 1.5 Hz, 1H, H_5 coumarin), 7.59 (dt, $J = 7.8$ and 1.5 Hz, 1H, H_7 coumarin), 7.36–7.30 (m, 2H, $H_{3,5}$ phenyl), 7.11–6.99 (m, 3H, H_6 coumarin and $H_{4,6}$ phenyl), 6.94 (dd, $J = 7.8$ and 1.5 Hz, 1H, H_8 coumarin), 5.70 (s, 1H, H_3 coumarin), 4.93 (s, 2H, CH_2O), 3.86 (br s, 2H, CH_2N), 3.69 (br s, 2H, CH_2N), 3.14 (br s, 4H, $2CH_2N$); Anal. Calcd for $C_{21}H_{19}FN_2O_4$ (382.38): C, 65.96; H, 5.01; N, 7.33. Found: C, 65.62; H, 5.29; N, 7.17.

*6.4.6. 4-(2-(4-(4-Fluorophenyl)piperazin-1-yl)-2-oxoethoxy)-2H-chromen-2-one (**4d**)*

Yellow solid; yield 87%; mp 185–187 °C; IR (KBr, cm^{-1}) ν_{max}: 1711 (C=O), 1671 (C=O); ^1H NMR (500 MHz, DMSO-d_6) δ 7.86 (d, $J = 7.6$ Hz, 1H, H_5 coumarin), 7.66 (t, $J = 7.6$ Hz, 1H, H_7 coumarin), 7.43–7.38 (m, 2H, $H_{6,8}$ coumarin), 7.08–7.05 (m, 2H, $H_{3,5}$ phenyl), 7.00–6.97 (m, 2H, $H_{2,6}$ phenyl), 5.98 (s, 1H, H_3 coumarin), 5.23 (s, 2H, CH_2O), 3.61–3.58 (m, 4H, $2CH_2N$), 3.15 (br s, 2H, CH_2N), 3.07 (br s, 2H, CH_2N); Anal. Calcd for $C_{21}H_{19}FN_2O_4$ (382.38): C, 65.96; H, 5.01; N, 7.33. Found: C, 65.73; H, 5.27; N, 7.19.

*6.4.7. 4-(2-(4-(2-Hydroxyphenyl)piperazin-1-yl)-2-oxoethoxy)-2H-chromen-2-one(**4e**)*

White solid; yield 96%; mp 157–159 °C; IR (KBr, cm^{-1}) ν_{max}: 3316 (OH), 1718 (C=O), 1669 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 7.91 (d, $J = 7.7$ Hz, 1H, H_5 coumarin), 7.59 (t, $J = 7.7$ Hz, 1H, H_7 coumarin), 7.36–7.28 (m, 2H, $H_{6,8}$ coumarin), 7.15–7.11 (m, 2H, $H_{3,5}$ phenyl), 7.00 (d, $J = 7.8$ Hz, 1H, H_6 phenyl), 6.90 (t, $J = 7.8$ Hz, 1H, H_4 phenyl), 6.82 (br s, 1H, OH), 5.72 (s, 1H, H_3 coumarin), 4.95 (s, 2H, CH_2O), 3.86 (br s, 2H, CH_2N), 3.69 (br s, 2H, CH_2N), 2.95 (br s, 4H, $2CH_2N$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.8, 163.7, 162.4, 153.3, 151.1, 137.8, 132.7, 127.1, 124.1, 123.0, 121.3, 120.4, 116.8, 115.2, 114.6, 91.5, 66.7, 52.6, 52.1, 45.6, 42.7. Anal. Calcd for $C_{21}H_{20}N_2O_5$ (380.39): C, 66.31; H, 5.30; N, 7.36. Found: C, 66.15; H, 5.53; N, 7.19.

*6.4.8. 4-(4-(4-(4-Fluorophenyl)piperazin-1-yl)-4-oxobutoxy)-2H-chromen-2-one (**4f**)*

Yellow solid; yield 79%; mp 141–143 °C; IR (KBr, cm^{-1}) ν_{max}: 1710 (C=O), 1656 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 7.80 (dd, $J = 7.9$ and 1.5 Hz, 1H, H_5 coumarin), 7.53 (dt, $J = 7.9$ and 1.5 Hz, 1H, H_7 coumarin), 7.33–7.24 (m, 2H, $H_{6,8}$ coumarin), 6.98–6.94 (m, 2H, $H_{3,5}$ phenyl), 6.85–6.83 (m, 2H, $H_{2,6}$ phenyl), 5.67 (s, 1H, H_3 coumarin), 4.22 (t, $J = 6.4$ Hz, 2H, CH_2O), 3.78 (br s, 2H, CH_2N), 3.63 (br s, 2H, CH_2N), 3.08–3.04 (m, 4H, $2CH_2N$), 2.60 (t, $J = 6.4$ Hz, 2H, CH_2CO), 2.30 (quintet, $J = 6.4$ Hz, 2H, $\underline{CH_2}CH_2CO$). Anal. Calcd for $C_{23}H_{23}FN_2O_4$ (410.44): C, 67.31; H, 5.65; N, 6.83. Found: C, 67.15; H, 5.92; N, 6.69.

*6.4.9. 4-(4-Oxo-4-(4-phenylpiperazin-1-yl)butoxy)-2H-chromen-2-one (**4g**)*

White solid; yield 95%; mp 133–135 °C; IR (KBr, cm^{-1}) ν_{max}: 1715 (C=O), 1645 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 7.82 (dd, $J = 8.0$ and 1.5 Hz, 1H, H_5 coumarin), 7.56 (dt, $J = 8.0$ and 1.5 Hz, 1H, H_7 coumarin), 7.35–7.28 (m, 4H, $H_{6,8}$ coumarin and $H_{3,5}$ phenyl), 6.95–6.93 (m, 3H, $H_{2,4,6,}$ phenyl), 5.72 (s, 1H, H_3 coumarin), 4.25 (t, $J = 7.1$ Hz, 2H, CH_2O), 3.82 (br s, 2H, CH_2N), 3.66 (br s, 2H, CH_2N), 3.19–3.17 (m, 4H, $2CH_2N$), 2.62 (t, $J = 7.1$ Hz, 2H, CH_2CO), 2.33 (quintet, $J = 7.1$ Hz, 2H, $\underline{CH_2}CH_2CO$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.4, 164.9, 162.3, 152.8, 150.3, 131.9, 128.7, 123.3, 122.3, 120.1, 116.3, 116.2, 115.2, 90.1, 68.1, 49.2, 48.9, 44.8, 41.1, 28.6, 23.5. Anal. Calcd for $C_{23}H_{24}N_2O_4$ (392.45): C, 70.39; H, 6.16; N, 7.14. Found: C, 70.16; H, 6.38; N, 7.20.

*6.4.10. 4-(4-(4-(3,4-Dichlorophenyl)piperazin-1-yl)-4-oxobutoxy)-2H-chromen-2-one (**4h**)*

Yellow solid; yield 45%; mp 152–155 °C; IR (KBr, cm^{-1}) ν_{max}: 1715 (C=O), 1652 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 7.80 (dd, $J = 7.5$ and 1.5 Hz, 1H, H_5 coumarin), 7.54 (dt, $J = 6.9$ and 1.5 Hz, 1H, H_7 coumarin), 7.31–7.26 (m, 3H, H_6 coumarin, $H_{5,6}$ phenyl), 6.93 (d, $J = 2.8$ Hz, 1H, H_2 phenyl), 6.72 (dd, $J = 7.5$ and 2.8 Hz, 1H, H_8 coumarin), 5.69 (s, 1H, H_3 coumarin), 4.22 (t, $J = 6.5$ Hz, 2H, CH_2O), 3.77 (br s, 2H, CH_2N), 3.62 (br s, 2H, CH_2N), 3.15–3.14 (m, 4H, $2CH_2N$), 2.63 (t, $J = 6.5$ Hz, 2H, CH_2CO), 2.30 (quintet, $J = 6.5$ Hz, 2H, $\underline{CH_2}CH_2CO$). Anal. Calcd for $C_{23}H_{22}Cl_2N_2O_4$ (461.34): C, 59.88; H, 4.81; N, 6.07. Found: C, 59.72; H, 4.92; N, 6.30.

*6.4.11. 4-(4-(4-(2-Fluorophenyl)piperazin-1-yl)-4-oxobutoxy)-2H-chromen-2-one (**4i**)*

Yellow solid; yield 89%; mp 143–145 °C; IR (KBr, cm^{-1}) ν_{max}: 1716 (C=O), 1632 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 7.80 (dd, $J = 8.2$ and 1.5 Hz, 1H, H_5 coumarin), 7.56 (dt, $J = 8.2$ and 1.5 Hz, 1H, H_7 coumarin), 7.34–7.28 (m, 2H, $H_{3,5}$ phenyl), 7.09–6.97 (m, 3H, H_8 coumarin and $H_{4,6}$ phenyl), 6.92 (dt, $J = 8.2$ and 1.5 Hz, 1H, H_6 coumarin), 5.71 (s, 1H, H_3 coumarin) 4.25 (t, $J = 6.5$ Hz, 2H, CH_2O), 3.83 (br s, 2H, CH_2N), 3.67 (br s, 2H, CH_2N), 3.09–3.07 (m, 4H, $2CH_2N$), 2.62 (t, $J = 6.5$ Hz, 2H, CH_2CO), 2.32 (quintet, $J = 6.5$ Hz, 2H, $\underline{CH_2}CH_2CO$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9, 165.4, 162.8, 156.7, 154.7, 153.3, 139.4, 132.3, 124.5, 123.8, 123.2, 122.8, 119.1, 116.8, 116.3, 116.1, 115.6, 90.6, 68.6, 50.8, 50.3, 45.4, 41.7, 29.0, 24.0. Anal. Calcd for $C_{23}H_{23}FN_2O_4$ (410.44): C, 67.31; H, 5.65; N, 6.83. Found: C, 67.12; H, 5.72; N, 6.59.

*6.4.12. 4-(4-(4-(2-Hydroxyphenyl)piperazin-1-yl)-4-oxobutoxy)-2H-chromen-2-one (**4j**)*

White solid; yield 73%; mp 138–140 °C; IR (KBr, cm^{-1}) ν_{max}: 3393 (OH), 1715 (C=O), 1620 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 7.83 (dd, J = 7.7 and 1.5 Hz, 1H, H_5 coumarin), 7.57 (dt, J = 7.7 and 1.5 Hz, 1H, H_7 coumarin), 7.34–7.28 (m, 2H, $H_{6,8}$ coumarin), 7.22 (dt, J = 6.5 and 2.2 Hz, 1H, H_4 phenyl), 7.11 (dt, J = 6.5 and 2.2 Hz, 1H, H_5 phenyl), 7.07–7.02 (m, $H_{3,6}$ phenyl), 5.72 (s, 1H, H_3 coumarin), 4.25 (br s, 2H, CH_2O), 3.76 (br s, 2H, CH_2N), 3.59 (br s, 2H, CH_2N), 2.99–2.97 (m, 4H, $2CH_2N$), 2.61 (t, J = 6.5 Hz, 2H, CH_2CO), 2.33–2.31 (m, 2H, $\underline{CH_2}CH_2CO$). Anal. Calcd for $C_{23}H_{24}N_2O_5$ (408.45): C, 67.63; H, 5.92; N, 6.86. Found: C, 67.75; H, 5.79; N, 6.68.

*6.4.13. 4-(2-(4-Benzylpiperidin-1-yl)-2-oxoethoxy)-2H-chromen-2-one (**4k**)*

White solid; yield 82%; mp 149–151 °C; IR (KBr, cm^{-1}) ν_{max}: 1721 (C=O), 1671 (C=O). ^1H NMR (500 MHz, $CDCl_3$) δ 7.90 (d, J = 7.5 Hz, 1H, H_5 coumarin), 7.58 (t, J = 7.5 Hz, 1H, H_7 coumarin), 7.34 (d, J = 7.5 Hz, 1H, H_8 coumarin), 7.34–7.29 (m, 3H, $H_{3,4,5}$ phenyl), 7.24–7.21 (t, J = 7.5 Hz, 1H, H_6 coumarin), 7.14 (d, J = 7.3 Hz, 2H, $H_{2,6}$ phenyl), 5.64 (s, 1H, H_3 coumarin), 4.86 (s, 2H, CH_2O), 4.58–4.57 (m, 1H, CHN), 3.73 (m, 1H, CHN), 3.09–3.07 (m, 1H, CHN), 2.66–2.56 (m, 3H, CHN and CH_2 benzyl), 1.88–1.76 (m, 3H, CH piperidine, $2\underline{CH}CH_2N$), 1.25–1.20 (m, 2H, $2\underline{CH}CH_2N$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.4, 162.6, 162.0, 152.8, 139.0, 132.1, 128.5, 127.8, 125.7, 123.5, 122.6, 116.2, 114.8, 90.9, 66.3, 44.7, 42.2, 42.0, 37.5, 32.0, 31.0. MS, (*m*/*z*, %) 377 (M^+, 72), 216 (21), 187 (51), 145 (5), 117 (19), 91 (100), 69 (27). Anal. Calcd for $C_{23}H_{23}NO_4$ (377.43): C, 73.19; H, 6.14; N, 3.71. Found: C, 73.31; H, 6.37; N, 3.54.

*6.4.14. 4-(4-(4-Benzylpiperidin-1-yl)-4-oxobutoxy)-2H-chromen-2-one (**4l**)*

Orange solid; yield 53%; mp 114–116 °C; IR (KBr, cm^{-1}) ν_{max}: 1723 (C=O), 1624 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, J = 7.7 Hz, 1H, H_5 coumarin), 7.53 (t, J = 7.7 Hz, 1H, H_7 coumarin), 7.30–7.18 (m, 5H, $H_{6,8}$ coumarin and $H_{3,4,5}$ phenyl), 7.12–7.10 (m, 2H, $H_{2,6}$ phenyl), 5.68 (s, 1H, H_3 coumarin), 4.63–4.62 (m, 1H, CHN), 4.20 (t, J = 5.0 Hz, 2H, CH_2O), 3.85–3.84 (m, 1H, CHN), 2.95–2.94 (m, 1H, CHN), 2.56–2.53 (m, 5H, CHN, CH_2 benzyl and CH_2CO), 2.27–2.25 (m, 2H, $\underline{CH_2}CH_2CO$), 1.75–1.68 (m, 3H, CH piperidine and $2\underline{CH}CH_2N$), 1.25–1.20 (m, 2H, $2\underline{CH}CH_2N$). Anal. Calcd for $C_{25}H_{27}NO_4$ (405.49): C, 70.05; H, 6.71; N, 3.45. Found: C, 70.22; H, 6.63; N, 3.46.

*6.4.15. N-(1-Benzylpiperidin-4-yl)-2-(2-oxo-2H-chromen-4-yloxy) acetamide (**4m**)*

White solid; yield 84%; mp 192–194 °C; IR (KBr, cm^{-1}) ν_{max}: 3303 (NH), 1728 (C=O), 1657 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, J = 6.9 Hz, 1H, H_5 coumarin), 7.61 (t, J = 6.9 Hz, 1H, H_7 coumarin), 7.37–7.28 (m, 7H, $H_{6,8}$ coumarin, 5H phenyl), 6.41 (br s, 1H, NH), 5.72 (s, 1H, H_3 coumarin), 4.64 (s, 2H, CH_2O), 4.09–3.97 (m, 1H, CH–NHCO), 3.60 (s, 2H, CH_2 benzyl), 2.96–2.94 (m, 2H, 2CHN), 2.28–2.18 (m, 2H, 2CHN), 2.05–2.00 (m, 2H, $2\underline{CH}CH_2N$), 1.73–1.71 (m, 2H, $2\underline{CH}CH_2N$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.3, 163.4, 161.5, 152.8, 132.4, 128.9, 128.7, 127.9, 127.1, 123.7, 121.9, 116.6, 114.3, 91.5, 67.0, 62.1, 51.4, 45.6, 31.0, 30.4. Anal. Calcd for $C_{23}H_{24}N_2O_4$ (392.45): C, 70.39; H, 6.16; N, 7.14. Found: C, 70.56; H, 6.19; N, 7.27.

*6.4.16. N-(1-Benzylpiperidin-4-yl)-4-(2-oxo-2H-chromen-4-yloxy) butanamide (**4n**)*

White solid; yield 88%; mp 156–158 °C; IR (KBr, cm^{-1}) ν_{max}: 3300 (NH), 1718 (C=O), 1628 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, J = 7.3 Hz, 1H, H_5 coumarin), 7.56 (t, J = 7.3 Hz, 1H, H_7 coumarin), 7.33–7.28 (m, 7H, $H_{6,8}$ coumarin, 5H phenyl), 5.68 (s, 1H, H_3 coumarin), 5.37 (br s, 1H, NH), 4.76 (s, 2H, CH_2O), 4.19 (br s, 2H, CH_2 benzyl), 3.82 (br s, 1H, $\underline{CH}$–NHCO), 3.48 (s, 2H, CH_2CO), 2.80–2.79 (m, 2H, 2CHN), 2.28 (t, J = 6.6 Hz, 2H, $\underline{CH_2}CH_2CO$), 2.11–2.10 (m, 2H, 2CHN), 1.88–1.87 (m, 2H, $2\underline{CH}CH_2N$), 1.44–1.42 (m, 2H, $2\underline{CH}CH_2N$). Anal. Calcd for $C_{25}H_{28}N_2O_4$ (420.5): C, 71.41; H, 6.71; N, 6.66. Found: C, 71.29; H, 6.88; N, 6.58.

*6.4.17. 4-(2-(4-(4-Bromophenyl)-4-hydroxypiperidin-1-yl)-2-oxoethoxy)-2H-chromen-2-one (**4o**)*

Yellow solid; yield 71%; mp 233–235 °C; IR (KBr, cm^{-1}) ν_{max}: 3455 (OH), 1710 (C=O), 1658 (C=O); ^1H NMR (500 MHz, DMSO-d_6) δ 7.92 (d, J = 7.4 Hz, 1H, H_5 coumarin), 7.58 (t, J = 7.4 Hz, 1H, H_7 coumarin), 7.48–7.45 (m, 2H, $H_{3,5}$ phenyl), 7.42–7.30 (m, 4H, $H_{2,6}$ phenyl and $H_{6,8}$ coumarin), 5.72 (s, 1H, H_3 coumarin), 4.96 (s, 2H, CH_2O), 4.62 (br s, 1H, OH), 4.52–4.50 (m, 1H, CHN), 3.68–3.66 (m, 1H, CHN), 3.22–3.20 (m, 2H, 2CHN), 1.94–1.73 (m, 4H, $2\underline{CH_2}CH_2N$). Anal. Calcd for $C_{22}H_{20}BrNO_5$ (458.3): C, 57.66; H, 4.40; N, 3.06. Found: C, 57.82; H, 4.19; N, 3.19.

*6.4.18. 4-(2-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-2-oxoethoxy)-2H-chromen-2-one (**4p**)*

White solid; yield 78%; mp 224–226 °C; IR (KBr, cm^{-1}) ν_{max}: 3459 (OH), 1712 (C=O), 1659 (C=O). ^1H NMR (500 MHz, DMSO- d_6) δ 7.88 (d, J = 7.6 Hz, 1H, H_5 coumarin), 7.68 (t, J = 7.6 Hz, 1H, H_7 coumarin), 7.52–7.51 (m, 2H, $H_{3,5}$ phenyl), 7.44–7.38 (m, 4H, $H_{6,8}$ coumarin, $H_{2,6}$ phenyl), 5.97 (s, 1H, H_3 coumarin), 5.30–5.18 (m, 3H, CH_2O and OH), 4.31–4.30 (m, 1H, CHN), 3.69–3.68 (m, 1H, CHN), 3.45–3.44 (m, 1H, CHN), 3.04–3.03 (m, 1H, CHN), 2.09–2.02 (m, 1H, $\underline{CH}CH_2N$), 1.82–1.78 (m, 1H, $\underline{CH}CH_2N$), 1.64–1.61 (m, 2H, $2\underline{CH}CH_2N$). Anal. Calcd for $C_{22}H_{20}ClNO_5$ (413.85): C, 63.85; H, 4.87; N, 3.38. Found: C, 63.68; H, 4.99; N, 3.49.

*6.4.19. 4-(4-(4-(4-Bromophenyl)-4-hydroxypiperidin-1-yl)-4-oxobutoxy)-2H-chromen-2-one (**4q**)*

White solid; yield 43%; mp 146–149 °C; IR (KBr, cm^{-1}) ν_{max}: 3361 (OH), 1720 (C=O), 1617 (C=O). ^1H NMR (500 MHz, $CDCl_3$) δ 7.82 (dd, J = 8.0 and 1.5 Hz, 1H, H_5 coumarin), 7.58 (dt, J = 8.0 and 1.5 Hz, 1H, H_7 coumarin), 7.51–7.69 (m, 2H, $H_{3,5}$ phenyl), 7.35–7.30 (m, 4H, $H_{6,8}$ coumarin and $H_{2,6}$ phenyl), 5.72 (s, 1H, H_3 coumarin), 4.64–4.62 (m, 1H, CHN), 4.25 (t, J = 6.5 Hz, 2H, CH_2O), 3.78–3.77 (m, 1H, CHN), 3.58–3.57 (m, 1H, CHN), 3.13–3.12 (m, 1H, CHN), 2.61 (t, J = 6.5 Hz, 2H, CH_2CO), 2.32 (quintet, J = 6.5 Hz, 2H, $\underline{CH_2}CH_2CO$), 2.01–1.80 (m, 4H, $4\underline{CH}CH_2N$). Anal. Calcd for $C_{24}H_{24}BrNO_5$ (486.36): C, 59.27; H, 4.97; N, 2.88. Found: C, 59.45; H, 4.58; N, 2.69.

*6.4.20. 4-(4-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-4-oxobutoxy)-2H-chromen-2-one (**4r**)*

White solid; yield 67%; mp 166–168 °C; IR (KBr, cm^{-1}) ν_{max}: 3419 (OH), 1717 (C=O), 1618 (C=O); ^1H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, J = 6.6 Hz, 1H, H_5 coumarin), 7.56 (t, J = 6.6 Hz, 1H, H_7 coumarin), 7.41–7.31 (m, 6H, $H_{6,8}$ coumarin, 4H phenyl), 5.71 (s, 1H, H_3 coumarin), 4.64–4.63 (m, 1H, CHN), 4.25 (br s, 2H, CH_2O), 3.79–3.78 (m, 1H, CHN), 3.59–3.58 (m, 1H, CHN), 3.13–3.12 (m, 1H, CHN), 2.61 (br s, 2H, CH_2CO), 2.01–1.79 (m, 6H, 4H $\underline{CH_2}CH_2CO$ and 2H $\underline{CH_2}CH_2N$). MS, (*m*/*z*, %) 441 (M^+, 1), 279 (48), 250 (8), 222 (3), 193 (19), 162 (83), 141 (10), 120 (100), 98 (73), 77 (10), 56 (21). Anal. Calcd for $C_{24}H_{24}ClNO_5$ (441.9): C, 65.23; H, 5.47; N, 3.17. Found: C, 65.07; H, 5.71; N, 3.31.

*6.4.21. N-(3,4-Dimethoxyphenethyl)-4-(2-oxo-2H-chromen-4-yloxy)butanamide (**4s**)*

White solid; yield 86%; mp 110–112 °C; IR (KBr, cm^{-1}) ν_{max}: 3303 (NH), 1721 (C=O), 1645 (C=O). ^1H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, J = 7.5 Hz, 1H, H_5 coumarin), 7.56 (t, J = 7.5 Hz, 1H, H_7 coumarin), 7.34–7.27 (m, 2H, $H_{6,8}$ coumarin), 6.77 (d, J = 7.5 Hz, 1H, H_6 phenyl), 6.76–6.70 (m, 2H, $H_{2,5}$ phenyl), 5.67 (s, 1H, H_3 coumarin), 5.46 (br s, 1H, NH), 4.17 (br s, 2H, CH_2O), 3.86 (s, 3H,

OCH_3), 3.85 (s, 3H, OCH_3), 3.55–3.52 (m, 2H, CH_2NH), 2.78–2.75 (m, 2H, CH_2 benzyl), 2.38–2.28 (m, 4H, CH_2CH_2CO). Anal. Calcd for $C_{23}H_{25}NO_6$ (411.45): C, 67.14; H, 6.12; N, 3.40. Found: C, 67.22; H, 6.49; N, 3.51.

6.5. Determination of inhibitory potency on eelAChE and eqBChE

The inhibitory potency of target compounds on AChE and BChE was determined using slightly modified Ellman's method [17,18]. Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from *electric eel,* 1000 unit), butylcholinesterase (BChE, E.C. 3.1.1.8, from equine serum) and butylthiocholine iodode (BTC) were 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. In brief, 50 μl of five different concentration of the test compounds was added to the mixture of 3 ml phosphate buffer 0.1 M, pH = 8.0 and 100 μl of DTNB solution. After 10 min of incubation at 25 °C, 10 μl solution of acetylthiocholine iodide as substrate was added. The change of absorbance was measured at 412 nm (UV-2100 Rayleigh Double Beam Spectrophotometer) for 6 min. The IC_{50} values were determined graphically from inhibition curves (log inhibitor concentration vs. percent of inhibition). The same method was used for BChE inhibition assay.

6.6. FRAP assay

The modified method of Benzie and Strain was employed to evaluate the antioxidant activity of the synthesized compounds by using the FRAP assay [19]. The complete procedure was described in our previous work [16].

6.7. Docking study

Crystal structure of *T. californica* acetylcholinesterase had been retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) as the receptor model and was finally prepared using Autodock Tools (1.5.4) [20]. The ligands were sketched using MarvineSketch 5.8.3, 2012, ChemAxon (http://www.chemaxon.com), converted to 3D structure and minimized by Openbabel (2.3.1) [23]. Autodock vina (1.1.1) [24] was used for molecular docking simulation and the docking parameters were set as follow: Center_x = 6.231, Center_y = 67.871, Center_z = 58.888, size_x = 30, size_y = 30, size_z = 40, exhaustiveness = 100. Other parameters for vina were retained unchanged. Finally, the best conformation of the ligand with the lowest binding energy was selected to analyze the probable interaction with the receptor.

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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.2013.03.021.

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