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ORIGINAL ARTICLE

Structure–activity relationship investigation of coumarin–chalcone hybrids with diverse side-chains as acetylcholinesterase and butyrylcholinesterase inhibitors

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Abstract

Chalcones containing tertiary amine side-chains have potent activity as acetylcholinesterase (AChE) inhibitors. However, the effects of the location of the tertiary amine groups as well as of other groups on AChE and butyrylcholinesterase (BChE) activity have not been reported. Here, we report the synthesis and testing of 36 new coumarin–chalcone hybrids (**5d–7j**, **9d–11f**, **12k–13m**) against AChE and BChE. The nature and position of the chalcone substituents had major effects on inhibitory activity as well as selectivity for AChE over BChE. Compounds with *para*-substituted chalcone fragments in which the substituents were choline-like had potent activity against AChE and poor activity against BChE, while *ortho*-substituted analogs exhibited an opposite effect. Replacement of the terminal amine groups by amide, alkyl or alkenyl groups abrogated activity. Compound **5e** showed potent inhibitory activity ($IC_{50} = 0.15 \pm 0.01$ μmol/L) and good selectivity for AChE over BChE (ratio 27.4), and a kinetic study showed that **5e** exhibited mixed-type inhibition against AChE. Computational docking results indicate that **5e** binds to Trp 279, Tyr334 and Trp 84 in AChE, but only to Trp 82 in BChE. Overall, the results show that coumarin–chalcone hybrids with choline-like side-chains have promising activity and selectivity against AChE and be promising therapeutic leads for Alzheimer's disease.

Keywords Coumarin · Chalcone · Cholinesterase inhibitors · Structure–activity relationship · Tertiary amine group

Introduction

Alzheimer's disease (AD) is one of most common nervous system diseases and is characterized by impaired memory, cognition and self-care ability [1]. At present, treatment of AD is mainly provided with acetylcholinesterase (AChE) inhibitors, compounds which increase the level of acetylcholine in the brain [2]. However, since there are frequent adverse side-effects including hepatic injury, diarrhea or cardiovascular damage [3–5], the discovery of new, selective AChE inhibitors is of interest. In this context, many natural products or their derivatives have been found to have AChE inhibitory activity [6–9]. Chalcones, for example, are promising natural product scaffolds for drug development and exhibit a wide variety of bioactivities including anti-obesity, anti-inflammatory and anti-gout activities. For example, the chalcones metochalcone and sofalcone, Fig. 1, have been used in the clinic as choleretic and anti-ulcer agents, respectively [10]. Flavokawain B, first isolated from *Piper methysticum Forst* [11], is another natural product containing the chalcone scaffold. In our laboratory, we found that flavokawain B Mannich base derivatives had AChE inhibitory effects [12] and, following this discovery, we synthesized a series of tertiary amine chalcone derivatives,

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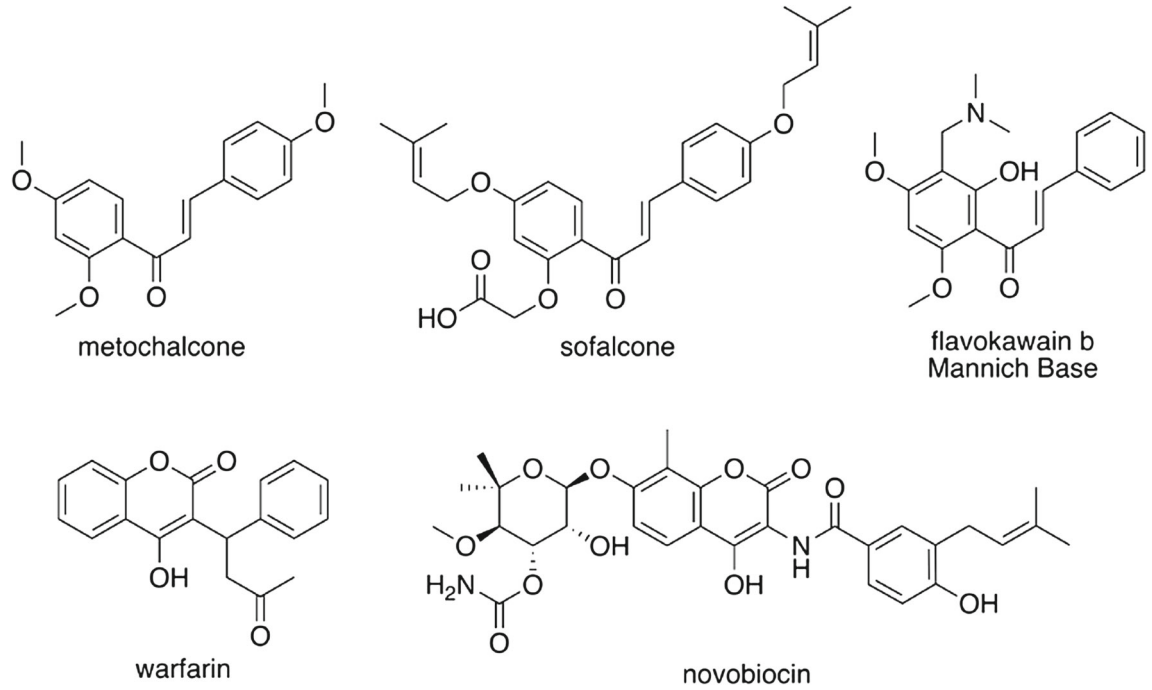


Fig. 1 Examples of chalcones and coumarins

some of which had quite potent AChE inhibitory activity (IC_{50} = 0.21−4.68 μmol/L) [13–16]. In our studies, we investigated AChE inhibitory effects of various tertiary amine groups, the nature of the spacer between the chalcone scaffold and the tertiary amine side-chain, as well as the effects of other substituents in the chalcone scaffold, but no investigations into the influence of different substituent positions of tertiary amine groups were reported.

A second interesting class of natural products for scaffold building are coumarins. This class of compound includes anticoagulants such as warfarin [17], choleretics [18] and even antibiotics such as novobiocin, and several structures are shown in Fig. 1. Coumarins are also used extensively as starting materials in drug discovery, and some coumarin derivatives have potent enzyme inhibition activity [19–21]. For example, the hybrids containing coumarin and other moieties have been used as histone deacetylase inhibitors [22], cyclo-oxygenase inhibitors [23] and monoamine oxidase inhibitor [24]. Coumarin–chalcone hybrids, are reported as anti-parasitics agents against *Plasmodium* spp. [25], malaria parasites, as well as trypanosomatids. Such hybrids offer multiple opportunities for derivatization on both aromatic rings.

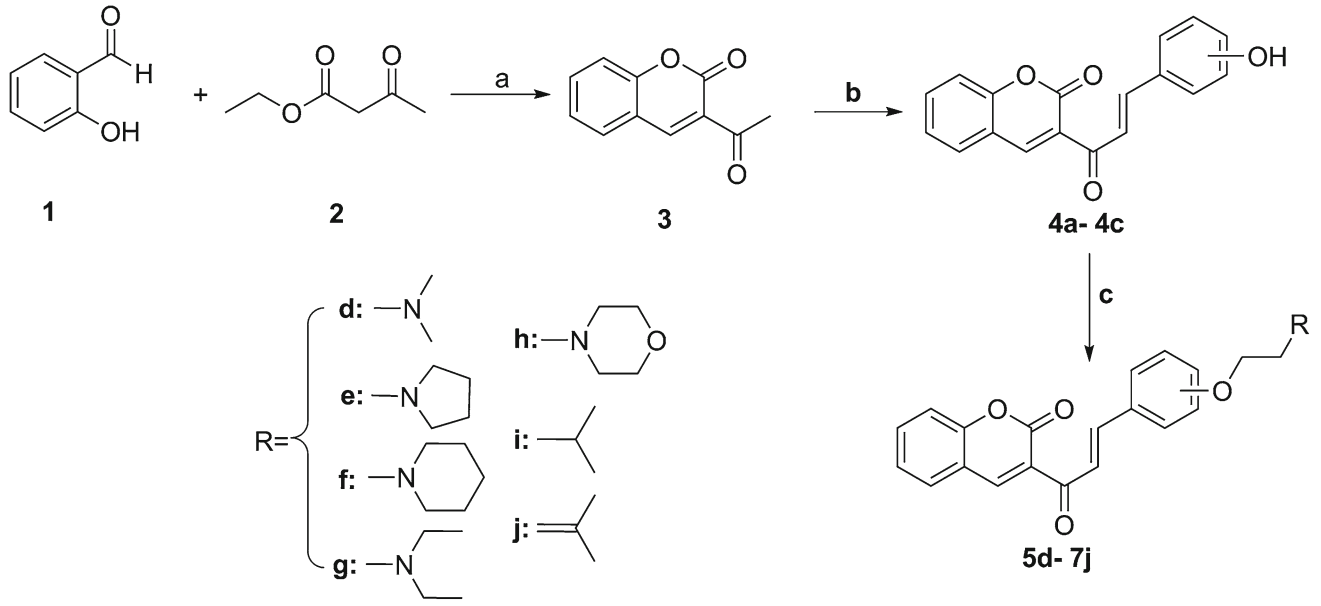
In this work, we synthesized and tested a broad range of coumarin–chalcone hybrids focusing on derivatizing the chalcone ring with both neutral and basic groups (Scheme 1). Then, we determined IC_{50} values of these compounds against AChE and BChE, evaluated AChE inhibition kinetics of the most potent inhibitors and used computational docking to elucidate AChE/BChE selectivity.

Results and discussion

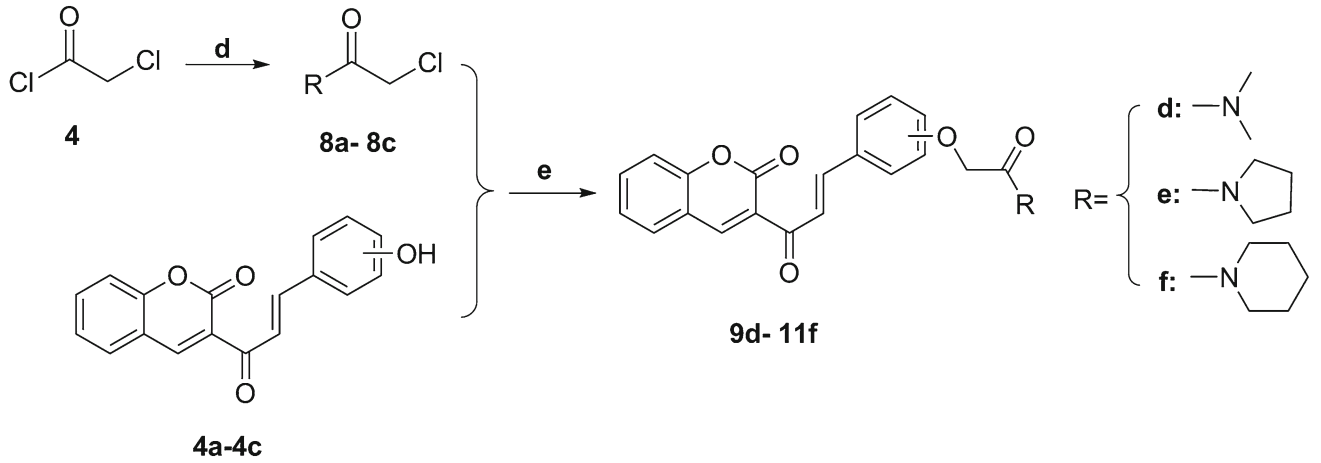
Chemistry

Coumarin–chalcone hybrids were synthesized as follows. Briefly, 3-acetyl coumarin was prepared by the condensation of salicylic acid with ethyl acetoacetate using piperidine as catalyst [26]. Compounds **4a**–**4c** were synthesized from 3-acetyl coumarin and hydroxybenzaldehyde via Claisen–Schmidt condensation in the presence of piperidine [27]. Compounds **5d**–**7j** were generated from compounds **4a**–**4c** with one of the following: (chloroethyl dimethylamine hydrochloride, chloroethyl diethylamine hydrochloride, chloroethyl piperidine hydrochloride, chloroethyl pyrrolidine hydrochloride, chloroethyl morpholine hydrochloride, 1-bromo-3-methylbutane, or 3,3-dimethylallyl bromide), all in the presence of K_2CO_3 and NaI [28] (Scheme 1).

Compounds **9d**–**11f** were synthesized via the routes illustrated in Scheme 2. First, chloroacetyl chloride was converted into compounds **8a**–**8c** using a secondary amine (dimethylamine, pyrrolidine or piperidine) in the presence of anhydrous sodium acetate [29]. Next, compounds **8a**–**8c** were condensed with compounds **4a**–**4c** (in the presence of K_2CO_3 and NaI as catalysts) to afford compounds **9d**–**11f** (Scheme 2). Compounds **5** and **6** were generated by condensing 3-acetyl coumarin with vanillic aldehyde or syringaldehyde using *p*-toluenesulfonic acid as catalyst. Then, compounds **12k**–**13m** were synthesized from compound **5** or **6**, chloroethyl dimethylamine hydrochloride, chloroethyl piperidine hydrochloride, or chloroethyl pyrroli-



Scheme 1 Synthesis of compounds **5d**–**7j**. Reagents and conditions: (a) piperidine, room temperature; (b) piperidine, hydroxybenzaldehyde, ethanol, reflux; (c) $Cl(CH_2)_2NR/Br(CH_2)_2R$, K_2CO_3, Nal, DMF, 56 °C



Scheme 2 Synthesis of compounds **9d**–**11f**. Reagents and conditions: (d) HNR_1R_2, NaAc, DCM, 0 °C to room temperature; (e) K_2CO_3, NaI, DMF, 56 °C

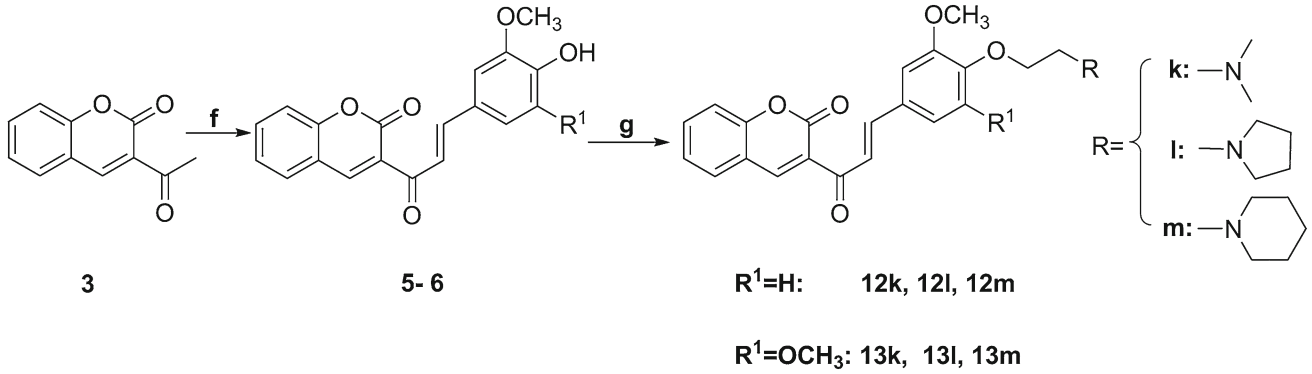
dine hydrochloride, again in the presence of K_2CO_3 and NaI (Scheme 3).

The final compounds were purified by silica gel chromatography, and structures were confirmed by using nuclear magnetic resonance spectroscopy (^{1}H NMR, ^{13}C NMR) and high-resolution mass spectrometry (HRMS). Representative ^{1}H, ^{13}C NMR and mass spectra are given in the supplementary materials.

In vitro inhibition of AChE and BChE

The inhibitory effects of the newly synthesized compounds on AChE and BChE activity were evaluated by using the Ellman method using rivastigmine as positive control. Half-maximal inhibitory concentrations (IC_{50} values) for AChE and BChE as well as the selectivity for AChE are summarized in Table 1. All assays were conducted in triplicate. These results indicate that many of the compounds show inhibition activity against AChE and BChE, and some of them have far more potent inhibitory activity than that of the control, rivastigmine.

The chemical nature of the substituents as well as their positions in the chalcone benzene ring greatly influenced both activity and selectivity. Compounds **5d**, **5e** and **5f** with IC_{50} values of 0.37, 0.15 and 0.69 μmol/L, respectively, showed more potent activity than the positive control rivastigmine (IC_{50} = 10.54 μmol/L) with compound **5e** exhibiting the most potent AChE inhibitory activity (IC_{50} = 0.15 μmol/L) as well as higher selectivity for AChE versus BChE (an IC_{50} ratio of ratio 27.4:1) than rivastigmine



Scheme 3 Synthesis of compounds **12k**–**13m**. Reagents and conditions: (f) Acetic acid, TsOH, 70 °C; (g) $Cl(CH_2)_2NR_1R_2$, K_2CO_3, NaI, acetone, reflux

(a ratio of 0.02:1). In general, the inhibitory potency of the coumarin–chalcone derivatives against AChE was as follows: *para*-substituted > *meta*-substituted > *ortho*-substituted. However, the inhibitory potency against BChE was reversed: *ortho*-substituted > *meta*-substituted > *para*-substituted. In addition, the inhibitory activity against AChE was highly dependent on the nature of the ring substituent. Compounds containing a dimethylamine, pyrrolidine or piperidine group (compounds **5d**, **5e**, **5f**, **6d** and **6f**) possessed much higher activity than did those compounds containing isopropyl or isopropenyl groups (compounds **5i**–**7j)** (IC_{50} > 500 μmol/L) (Table 1), while all compounds containing amide groups (**9d**–**11f**) showed very weak inhibition activity against both AChE and BChE. It can be observed that the presence of choline-like dimethylethanolamine (**5d**) or analogous pyrrolidine species (**5e**) make major contributions to activity.

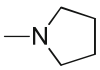
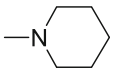
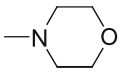
Since compounds **5d**, **5e** and **5f** had potent activity and high selectivity in inhibiting AChE, we next investigated several analogs of these compounds. Interestingly, compounds **13k**, **13l**, **13m**, containing two methoxyl substituents, had much weaker inhibitory activity against AChE than did the parent compounds, **5d**, **5e**, **5f**. However, compounds **12k**, **12l**, **12m**, which contained just a single methoxyl group, had comparable inhibitory activity to that of the parent compounds with compound **12m** ($IC_{50} = 0.37 \pm 0.02$ μmol/L) actually having slightly more potent activity that the parent compound **5f** ($IC_{50} = 0.69 \pm 0.06$ μmol/L). Thus, addition of the second methoxyl group clearly decreases activity, presumably due to a steric repulsion in the AChE active site.

Compound **5e** was then selected for a kinetic assay since it had the most potent activity against AChE. A Lineweaver–Burk analysis of the steady-state inhibition data of compound **5e** is shown in Supplementary Fig. 2 and shows that increasing the concentration of compound **5e** results in different slopes and intercepts. The K_m values show an increase with increasing concentrations of compound **5e**, but the V_{max} values exhibit a decrease, indicating a mixed-type inhibition mechanism. As shown in Supplementary Table 2, the competitive inhibition constant (K_i) of compound **5e** is 0.11 μmol/L, while the non-competitive constant (K_i') is 0.19 μmol/L.

Molecular modeling studies

To explore the molecular mechanism of AChE and BChE inhibition, representative compounds (**5e**, **6e**, **7e**, **5h**) were selected for a molecular docking investigation using MOE2014 (Montreal, Quebec, Canada: Chemical Computing Group Inc., www.chemcomp.com). As shown in Supplementary Fig. 3, compound **5e** exhibited multiple interactions with AChE (with a total binding energy of − 34.6 kJ/mol). In the top of the AChE "gorge," the phenyl ring bound to the peripheral anionic site (PAS) via a π–π stacking interaction with Trp279 (4.03 Å) and Tyr334 (4.32 Å). In the bottom of the gorge, the protonated nitrogen of the pyrrolidine ring bound to the catalytic active site (CAS) via a cation-π interaction with Trp84 (4.33 Å), while compound **5e** bound to BChE (− 20.08 kJ/mol) via a $\pi - H^+$ interaction in the CAS with Trp84 (3.11 Å). Compound **6e** exhibited binding to multiple sites in AChE (− 35.7 kJ/mol). In the top of the gorge, the phenyl ring bound to the PAS via a π–π stacking interaction with Trp279 (3.44 Å). In the bottom of the gorge, the protonated nitrogen of the pyrrolidine ring bound to the CAS via a cation-π interaction with Trp84 (4.06 Å). Compound **6e** bound to BChE (− 20.9kJ/mol) via a π–H interaction with Phe329 (3.77 Å) in the PAS and a cation-π interaction with Trp82 (3.76 Å), in the CAS, providing a partial explanation for the similar inhibition for AChE and BChE. However, compound **7e** exhibited multiple binding interactions with BChE (− 20.5 kJ/mol). In the bottom of the gorge, the 2-*H*-chromen-2-one moiety was observed to bind to the CAS via a π–π stacking interaction with Trp82 (3.81 Å), and a π–H interaction with His438 (4.47 Å). Compound **7e** bound to AChE (− 28.9 kJ/mol) via a $\pi - H^+$ interaction with Trp84 (3.29 Å) in the CAS. Compound **5h** exhibited very

Table 1 Effects of new synthesized compounds on AChE and BChE

Compounds	Position	*R*	IC_{50}^{a} (μmol/L)		Selectivity for AChE[b]
			AChE	BChE	
5d	*Para*		0.37 ± 0.03	> 500	> 1340
6d	*Meta*		0.92 ± 0.07	15.32 ± 1.16	16.64
7d	*Ortho*		16.40 ± 0.22	15.16 ± 0.60	0.92
9d	*Para*		18.61 ± 1.06	> 500	> 26.86
10d	*Meta*		> 500	44.02 ± 2.37	< 0.09
11d	*Ortho*		> 500	> 500	–
12k	*Para*		1.65 ± 0.09	8.48 ± 0.29	5.15
13k	*Para*		7.07 ± 0.46	13.22 ± 0.32	1.87
5e	*Para*		0.15 ± 0.01	4.11 ± 0.16	27.40
6e	*Meta*		3.25 ± 0.14	4.62 ± 0.16	1.42
7e	*Ortho*		230.34 ± 14.82	3.76 ± 0.35	0.02
9e	*Para*		> 500	> 500	–
10e	*Meta*		> 500	> 500	–
11e	*Ortho*		> 500	> 500	–
12l	*Para*		1.67 ± 0.04	0.46 ± 0.01	0.28
13l	*Para*		28.95 ± 1.68	13.61 ± 0.53	0.47
5f	*Para*		0.69 ± 0.06	1.03 ± 0.05	1.49
6f	*Meta*		0.61 ± 0.02	4.01 ± 0.14	6.61
7f	*Ortho*		29.86 ± 0.98	4.50 ± 0.22	0.15
9f	*Para*		> 500	> 500	–
10f	*Meta*		152.49 ± 12.82	222.84 ± 11.54	1.46
11f	*Ortho*		> 500	106.14 ± 3.09	0.21
12m	*Para*		0.37 ± 0.02	0.52 ± 0.01	1.41
13m	*Para*		9.58 ± 0.80	6.41 ± 0.36	0.67
5g	*Para*		4.34 ± 0.29	6.21 ± 0.19	1.43
6g	*Meta*		51.30 ± 1.04	1.12 ± 0.11	0.02
7g	*Ortho*		202.70 ± 2.50	1.96 ± 0.14	0.01
5h	*Para*		> 500	> 500	–
6h	*Meta*		> 500	> 500	–
7h	*Ortho*		260.95 ± 4.17	28.98 ± 1.23	0.11
5i	*Para*		> 500	> 500	–
6i	*Meta*		> 500	> 500	–
7i	*Ortho*		> 500	> 500	–
5j	*Para*		> 500	> 500	–
6j	*Meta*		> 500	> 500	–
7j	*Ortho*		> 500	100.33 ± 6.84	< 0.20
Rivastigmin e*			10.54 ± 0.86	0.26 ± 0.08	0.02

[a]IC_{50}: 50% inhibitory concentration (means ± SD of three assays)
[b]Selectivity for AChE is defined as IC_{50} (BChE)/IC_{50} (AChE)
*Used for control

weak binding to both AChE and BChE, consistent with its IC_{50} > 500 μM against both AChE and BChE (see Table 1).

Compound **5e** thus has potent inhibitory activity against AChE together with a high selectivity over BChE; compound **6e** has moderate inhibitory activity against AChE with low selectivity over BChE, while compound **7e** has poor inhibitory activity against AChE as well as poor selectivity over BChE, and compound **5h** has almost has no inhibitory activity against either AChE or BChE, indicating that having a hydrophilic morpholine ring abrogates essentially all

activity, demonstrating that hydrophobic interactions in this region are essential for activity [35].

Conclusions

In summary, we synthesized a series of coumarin–chalcone hybrids (**5d**–**7j**, **9d**–**11f**, **12k**–**3m**) containing amino-alkyl, alkyl, alkenyl and amide side-chains. The nature of the side-chain as well as its location on the chalcone phenyl ring markedly influenced activity in inhibiting AChE and BChE. In general, compounds with *para*-substituted amino-alkyl groups had the most potent activity and were selective for AChE. Most of compounds with amide, alkyl or alkenyl groups had very poor activity, IC_{50} values of them against AChE are more than 500 μmol/L. Addition of a single methoxyl group into the benzene ring resulted in activity comparable to that of the parent compounds, but addition of two methoxyl groups again blocked activity. Compound **5e** with an IC_{50} value of 0.15 ± 0.01 μmol/L against AChE exhibited the best activity as well as a high selectivity for AChE over BChE, and our kinetics results indicate that compound **5e** has a mixed-type inhibition against AChE. The molecular docking results show that compound **5e** can bind to both the CAS and the PAS of AChE, contributing to its potent activity. In conclusion, compound **5e** may serve as a potential lead for the development of novel compounds as AD therapeutics.

Experimental

Chemistry

All chemicals and reagents were of analytical reagent grade and were used without further purification. Melting points were measured on a WRS-lA melting point detector (uncorrected). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz instrument using tetramethylsilane (TMS) as the internal standard (s = singlet, d = doublet, t = triplet, m = multiplet). Mass spectra were obtained on Waters XEVO-G2XSQTOF Liquid chromatography–mass spectrometry with electrospray ionization (ESI-MS) method. The purity of compounds was checked using a Shimadzu LC-20A high-performance liquid chromatography.

Preparation of 3-acetyl-2*H*-chromen-2-one (3)

2-Hydroxybenzaldehyde (15 mmol, 1.56 mL) and ethyl acetoacetate (15 mmol, 1.89 mL) were added into a flask (25.0 mL) at room temperature. A small amount of piperidine (4 drops) was added as catalyst, and the solution was stirred continuously until the reaction was complete. Then, the solvent was removed, and the resulting residue was dissolved in ethanol. The target product obtained was recrystallized from ethanol. Pale yellow solid, Yield: 90%, mp: 118−119 °C.

General procedure for the synthesis of compounds 4a–4c

Compound **3** (5.00 mmol, 0.94 g) was added into absolute ethanol (30 mL) under stirring, followed by the addition of an appropriate hydroxybenzaldehyde (15.00 mmol, 1.83 g). Then, a catalytic amount of piperidine (4 drops) was added into the solution, and the reaction mixture was refluxed for 6–8 h. The mixture was cooled, and the solid obtained by filtration. The crude products were recrystallized from ethanol to afford compounds **4a**–**4c** with yield 82–90%.

General procedure for the synthesis of compounds 5d–7j

Compounds **4a**–**4c** (1.00 mmol, 0.292 g), together with aminoethyl chloride or aminoethyl bromide (3.00 mmol) and anhydrous potassium carbonate (3 mmol, 0.415 g) in 8 mL DMF, were stirred for 20 minutes. Then, a small amount of NaI (0.005 g, 0.02 mmol) was added as catalyst. The reaction mixture was stirred for 10–15 h at 56 °C, then cooled to room temperature and filtered. The filtrate was poured into a saturated saline solution and filtered. The solid obtained was purified using column chromatography with dichloromethane/methanol (70:1) to yield compounds **5d**–**7j**, respectively.

(E)-3-(3-(4-(2-(dimethylamino)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (5d)

Yellow solid, yield: 85.6%, mp: 137.5–139.2 °C; ^{1}H NMR (400MHz, $DCCl_3$): δ (ppm)2.29 (6H, s, 2 × $NC\underline{H}_3$), 2.70 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 4.06 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$), 6.86 (2H, d, J = 8 Hz, $H_{3,5}$ phenyl), 7.26–7.34 (2H, m, $H_{6,8}$ coumarin), 7.55–7.61 (4H, m, $H_{2,6}$ phenyl and $H_{5,7}$ coumarin), 7.74 (1H, d, J = 16 Hz, H_α), 7.79 (1H, d, J = 16 Hz, H_β), 8.51(1H, s, H_4 coumarin)^{13}C NMR (100 MHz, $CDCl_3$) δ: 45.54, 57.46, 65.73, 114.94, 116.68, 118.64, 121.47, 124.91, 125.60, 127.37, 129.95, 130.83, 134.04, 145.23, 147.75, 155.18, 159.36, 161.61, 186.36. HRMS(ESI) m/z: 364.1549 $[M+H]^+$. Purity: 98.9% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one(5e)

Yellow solid, yield: 81.5%, mp: 135.9–137.3 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.88 (4H, m, pyrrolidine-H), 2.77 (4H, m, pyrrolidine-H), 3.02 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 4.23 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$), 6.93–6.95 (2H, d, J = 8 Hz, $H_{3,5}$ phenyl), 7.34–7.41 (2H, m, $H_{6,8}$ coumarin), 7.62–7.68(4H, m, $H_{2,6}$ phenyl and $H_{5,7}$ coumarin), 7.80–7.89 (2H, m, H_α and H_β), 8.58(1H, s, H_4 coumarin)^{13}C NMR (100 MHz, $CDCl_3$) δ:23.50, 54.75, 67.13, 114.99, 116.68, 118.62, 121.63, 124.93, 125.54, 127.65, 129.97, 130.81, 134.08, 145.10, 147.82, 155.18, 159.36, 161.21, 186.30. HRMS(ESI) m/z: 390.1698 $[M+H]^+$. Purity: 98.8% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(4-(2-(piperidin-1-yl)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (5f)

Brown solid, yield: 85.9%, mp: 144.1–144.9 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ(ppm), 1.18 (5H, m, piperidine-H), 2.61 (4H, m, piperidine-H), 2.89 (2H, m, $OCH_2C\underline{H}_2$), 4.19 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$), 6.86 (2H, d, J = 8 Hz, $H_{3,5}$ phenyl), 7.26–7.34 (2H, m, $H_{6,8}$ coumarin), 7.55–7.61 (4H, m, $H_{2,6}$ phenyl and $H_{5,7}$ coumarin), 7.73–7.81 (2H, m, H_α and H_β), 8.51(1H, s, H_4 coumarin).^{13}C NMR (100 MHz, $CDCl_3$) δ:24.01, 25.28, 54.63, 57.30, 65.34, 114.96, 116.68, 118.62, 121.63, 124.93, 125.54, 127.65, 129.97, 134.18, 145.16, 147.82, 155.18, 159.36, 161.21, 186.34. HRMS(ESI) m/z: 404.1860 $[M+H]^+$. Purity: 99.1% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(4-(2-(diethylamino)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (5g)

Brown solid, yield: 85.6%, mp: 159.3–160.1 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.14 (6H, t, J = 8 Hz, $2\times NCH_2C\underline{H}_3$), 2.75 (4H, m, $2\times NC\underline{H}_2CH_3$), 2.99 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 4.17 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$), 6.93 (2H, d, J = 8 Hz, $H_{3,5}$ phenyl), 7.33–7.41 (2H, m, $H_{6,8}$ coumarin), 7.60–7.68(4H, m, $H_{2,6}$phenyl and $H_{5,7}$ coumarin), 7.81 (1H, d, J = 16 Hz, H_α), 7.86 (1H, d, J = 16 Hz, H_β), 8.58(1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:11.34, 47.78, 51.45, 66.13, 114.96, 116.69, 118.62, 121.72, 124.95, 125.52, 127.76, 129.98, 130.83, 134.11, 145.05, 147.85, 155.18, 159.39, 161.00, 186.33. HRMS(ESI) m/z: 392.1855 $[M+H]^+$. Purity: 98.3% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(4-(2-morpholinoethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (5h)

Yellow solid, yield: 80.8%, mp: 167.8–169.2 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 2.63 (4H, s, morpholine-H), 2.87 (2H, s, J = 8 Hz, $OCH_2C\underline{H}_2$), 3.77(4*H*, s, morpholine-H), 4.18(2*H*, *t*, J = 8 Hz, $OC\underline{H}_2CH_2$), 6.94 (2H, d, J = 8 Hz, $H_{3,5}$ phenyl), 7.34–7.41 (2H, m, $H_{6,8}$ coumarin), 7.63–7.68 (4H, m, $H_{2,6}$ phenyl and $H_{5,7}$ coumarin), 7.81–7.89 (2H, m, H_α and H_β), 8.59 (1H, s, H_4 coumarin), ^{13}C NMR (100 MHz, $CDCl_3$) δ:54.05, 57.47, 66.76, 114.98, 116.70, 118.62, 121.79, 124.96, 125.50, 127.85, 129.99, 130.82, 134.13, 144.96, 147.89, 155.20, 159.39, 160.99, 186.29. HRMS(ESI) m/z: 406.1660 $[M+H]^+$. Purity: 98.2% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(4-(isopentyloxy)phenyl)acryloyl)-2*H*-chromen-2-one (5i)

Yellow solid, yield: 80.5%, mp: 161.7–164.3 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 0.97 (6H, d, J = 8 Hz, $2\times CHC\underline{H}_3$), 1.71 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 1.81–1.88(1H, m, $C\underline{H}(CH_3)_2$), 4.04 (2H, t, J = 12 Hz, $OC\underline{H}_2CH_2$), 6.92 (2H, d, J = 8 Hz, $H_{3,5}$ phenyl), 7.33–7.41 (2H, m, $H_{6,8}$ coumarin), 7.62–7.68(4H, m, $H_{2,6}$ phenyl and $H_{5,7}$ coumarin), 7.81 (1H, d, J = 16 Hz, H_α), 7.86 (1H, d, J = 16 Hz, H_β), 8.57 (1H, s, H_4 coumarin), ^{13}C NMR (100 MHz, $CDCl_3$) δ:22.58, 25.04, 37.85, 66.59, 114.91, 116.68, 118.64, 121.47, 124.91, 125.60, 127.37, 129.95, 130.83, 134.04, 145.23, 147.75, 155.18, 159.36, 161.63, 186.30. HRMS(ESI) m/z: 363.2079 $[M+H]^+$. Purity: 98.5% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(4-(3-methylbut-2-enyloxy)phenyl)-prop-2-enoyl)-2*H*-chromen-2-one (5j)

Yellow solid, yield: 89%, mp:119–121 °C. This is a known compound but without reports regarding inhibiting cholinesterases [27].

(E)-3-(3-(3-(2-(dimethylamino)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (6d)

Yellow solid, yield: 85.5%, mp:127.9–128.7 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 2.41 (6H, s, $2\times NC\underline{H}_3$), 2.83 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$,), 4.15 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$,), 6.99 (2H, d, J = 8 Hz, H_4 phenyl), 7.21 (1H, s, H_2 phenyl), 7.24–7.42 (4H, m, $H_{5,6}$ phenyl and $H_{6,8}$ coumarin), 7.65–7.69 (2H, m, $H_{5,7}$ coumarin), 7.82 (1H, d, J = 16 Hz, H_α), 7.92 (1H, d, J = 16 Hz, H_β), 8.59 (1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:45.70, 58.10, 65.75, 114.23, 116.74, 117.38, 118.56, 122.01, 124.20, 125.01, 125.29, 129.92, 130.06, 134.28, 136.15, 144.98,

148.13, 155.26, 159.02, 159.32, 186.49. HRMS(ESI) m/z: 364.1297 $[M+H]^+$. Purity: 98.5% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(3-(2-(pyrrolidin-1-yl)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (6e)

Pale yellow solid, yield: 80.8%, mp: 136.1–138.2 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.91 (4H, m, pyrrolidine-H), 2.85 (4H, t,J = 8 Hz, pyrrolidine-H), 2.97 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 4.23 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$), 7.03 (2H, d, J = 8 Hz, H_4 phenyl), 7.21 (1H, s, H_2 phenyl), 7.29–7.42 (4H, m, $H_{5,6}$ phenyl and $H_{6,8}$ coumarin), 7.63–7.69 (2H, m, $H_{5,7}$ coumarin), 7.82 (1H, d, J = 16 Hz, H_α), 7.91 (1H, d, J = 16 Hz, H_β), 8.59 (1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:23.54, 54.75, 67.05, 114.38, 116.72, 117.34, 118.53, 121.88, 124.18, 125.00, 125.27, 129.90, 130.05, 134.28, 136.13, 144.97, 148.08, 155.23, 159.07, 159.26, 186.43. HRMS(ESI) m/z: 390.1704 $[M+H]^+$. Purity: 98.6% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(3-(2-(piperidin-1-yl)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (6f)

Pale yellow solid, yield: 85.6%, mp: 151.8–153.1 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.47–1.67 (6H, m, piperidine-H), 2.58 (4H, m, piperidine-H), 2.4 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 4.18 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$), 6.97 (2H, d, J = 8 Hz, H_4 phenyl), 7.20 (1H, s, H_2 phenyl), 7.29–7.41 (4H, m, $H_{5,6}$ phenyl and $H_{6,8}$ coumarin), 7.66 (2H, m, $H_{5,7}$ coumarin), 7.81 (1H, d, J = 16 Hz, H_α), 7.90 (1H, d, J = 16 Hz, H_β), 8.58(1H, s, H_4 coumarin)^{13}C NMR (100 MHz, $CDCl_3$) δ: 24.05, 25.73, 55.01, 57.80, 66.85, 114.33, 116.72, 117.34, 118.54, 121.88, 124.18, 125.00, 125.27, 129.90, 130.05, 134.27, 136.13, 144.97, 148.08, 155.23, 158.26, 159.28, 186.49. HRMS(ESI) m/z: 404.1874 $[M+H]^+$. Purity: 99.3% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(3-(2-(diethylamino)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (6g)

Brown viscous liquid, yield: 82.5%; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.14 (6H, t, J = 8 Hz, $2\times NCH_2C\underline{H}_3$), 2.72–2.77 (4H, m, $2\times NC\underline{H}_2CH_3$), 3.00 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$,), 4.16 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$,), 6.97 (2H, d, J = 8 Hz, H_4 phenyl), 7.20 (1H, s, H_2 phenyl), 7.29–7.41 (4H, m, $H_{5,6}$ phenyl and $H_{6,8}$ coumarin), 7.64–7.67(2H, m, $H_{5,7}$ coumarin), 7.82 (1H, d, J = 16 Hz, H_α), 7.90 (1H, d, J = 16 Hz, H_β), 8.57 (1H, s, H_4 coumarin)^{13}C NMR (100 MHz, $CDCl_3$) δ:11.85, 47.51, 57.38, 65.90, 114.23, 116.72, 117.34, 118.54, 121.88, 124.18, 125.00, 125.27, 129.90, 130.05, 134.27, 136.13, 144.97, 148.08, 155.23, 159.07, 159.34, 186.42. HRMS(ESI) m/z: 392.1702 $[M+H]^+$. Purity: 98.8% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(3-(2-morpholinoethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (6h)

Yellow solid, yield: 80.5%, mp: 134.1–135.0 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 2.62 (4H, t, J = 8 Hz, morpholine-H), 2.85 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 3.76 (4H, t, J = 8 Hz, morpholine-H), 4.18 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$), 6.967 (2H, d, J = 8 Hz, H_4 phenyl),7.21 (1H, s, H_2phenyl), 7.28–7.42 (4H, m, $H_{5,6}$ phenyl and $H_{6,8}$ coumarin), 7.65–7.69 (2H, m,$H_{5,7}$ coumarin), 7.82 (1H, d, J = 16 Hz, H_α), 7.92 (1H, d, J = 16 Hz, H_β), 8.59(1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ: 54.13, 57.72, 66.85, 114.35, 116.72, 117.34, 118.54, 121.82, 124.18, 125.00, 125.27, 129.90, 130.05, 134.27, 136.13, 144.97, 148.08, 155.23, 159.07, 159.22, 186.45. HRMS(ESI) m/z: 406.1645 $[M+H]^+$. Purity: 98.9% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(3-(isopentyloxy)phenyl)acryloyl)-2*H*-chromen-2-one (6i)

Yellow solid, yield: 86.8%, mp: 150.7–152.2 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 0.96 (6H, d, J = 8 Hz,2× $CHC\underline{H}_3$), 1.71 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 1.81–1.89 (1H, m, $C\underline{H}$ $(CH_3)_2$), 4.17 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$), 6.98 (2H, d, J = 8 Hz, H_4 phenyl), 7.21 (1H, s, H_2 phenyl), 7.29–7.42 (4H, m, $H_{5,6}$ phenyl and $H_{6,8}$ coumarin), 7.65–7.69 (2H, m, $H_{5,7}$ coumarin), 7.82 (1H, d, J = 16 Hz, H_α), 7.92 (1H, d, J = 16 Hz, H_β), 8.52 (1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, CDCl3) δ:22.91, 25.04, 37.18, 66.75, 114.23, 116.74, 117.38, 118.56, 122.01, 124.20, 125.01, 125.29, 129.92, 130.06, 134.28, 136.15, 144.98, 148.13, 155.26, 159.02, 159.32, 186.51. HRMS(ESI) m/z: 363.1548 $[M+H]^+$. Purity: 98.0% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(3-(3-methylbut-2-enyloxy)phenyl)-prop-2-enoyl)-2*H*-chromen-2-one (6j)

Pale yellow solid, yield: 88.7%, mp: 144.4–146.3 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.77 (3H, s, $C\underline{H}_{a3}$), 1.81 (3H, s, $C\underline{H}_{b3}$), 4.56 (2H, d, J = 8 Hz, $OC\underline{H}_2CH$), 5.50 (1H, t,J = 8 Hz, $OCH_2C\underline{H}$), 6.97 (2H, d, J = 8 Hz, H_4 phenyl), 7.20 (1H, s, H_2 phenyl), 7.29–7.39 (4H, m, $H_{5,6}$ phenyl and $H_{6,8}$ coumarin), 7.63–7.67(2H, m, $H_{5,7}$ coumarin), 7.82 (1H, d, J = 16 Hz, H_α), 7.91 (1H, d, J = 16 Hz, H_β), 8.56 (1H, s, H_4 coumarin). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 54.13, 57.72, 66.85, 114.35, 116.72, 117.34,

118.54, 121.82, 124.18, 125.00, 125.27, 129.90, 130.05, 134.27, 136.13, 144.97, 148.08, 155.23, 159.07, 159.22, 186.45. HRMS(ESI) m/z: 361.3237 $[M+H]^+$. Purity: 98.5% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(2-(2-(dimethylamino)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (7d)

Yellow solid, yield: 81.8%, mp: 128.7–130.3 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 2.28 (6H, s, 2×$NC\underline{H}_3$), 2.89 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 4.18 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$), 6.92–7.00 (2H, m, $H_{3,5}$ phenyl),7.32–7.39 (3H, m, H_4 phenyl and $H_{6,8}$ coumarin), 7.62–7.69 (3H, m, H_6 phenyl and $H_{5,7}$ coumarin), 8.00 (1H, d,J = 16 Hz, H_α), 8.20 (1H, d,J = 16 Hz, H_β), 8.54(1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:45.96, 57.98, 66.94, 112.17, 116.64, 118.58, 120.94, 123.90, 124.46, 124.90, 125.65, 129.81, 129.94, 132.17, 134.04, 140.73, 147.66; 155.15, 158.28, 159.18, 186.91. HRMS(ESI) m/z: 364.1552 $[M+H]^+$. Purity: 98.7% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(2-(2-(pyrrolidin-1-yl)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (7e)

Yellow solid, yield: 80.6%, mp: 137.7–138.5 °C;^{1} H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.82–1.85 (4H, m, pyrrolidine-H), 2.61–2.68 (4H, m, pyrrolidine-H), 2.94 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 4.16 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$), 6.91–7.00 (2H, m, $H_{3,5}$ phenyl), 7.32–7.39 (3H, m, H_4 phenyl and $H_{6,8}$ coumarin), 7.61–7.69 (3H, m, H_6 phenyl and $H_{5,7}$ coumarin), 8.00 (1H, d,J = 16 Hz, H_α), 8.19 (1H, d,J = 16 Hz, H_β), 8.51 (1H, s, H_4 coumarin)^{13}C NMR (100 MHz, $CDCl_3$) δ:23.53, 54.72, 67.17, 112.25, 116.68, 118.61, 123.89, 124.48, 124.92, 125.75, 129.83, 129.93, 132.19, 134.03, 140.81, 147.85, 155.18, 158.26, 159.23, 186.94. HRMS(ESI) m/z: 390.1707 $[M+H]^+$. Purity: 99.1% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(2-(2-(piperidin-1-yl)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (7f)

Brown solid, yield: 80.9%, mp: 150.3–151.6 °C;^{1} H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.45–1.64 (6H, m, piperidine-H), 2.58–2.61 (4H, m, piperidine-H), 2.94 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 4.24 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$), 6.93–7.01(2H, m, $H_{3,5}$ phenyl), 7.33–7.41 (3H, m, H_4 phenyl and $H_{6,8}$ coumarin), 7.63–7.69 (3H, m, H_6 phenyl and $H_{5,7}$ coumarin), 8.00 (1H, d,J = 16 Hz, H_α), 8.19 (1H, d,J = 16 Hz, H_β), 8.55 (1H, s, H_4 coumarin)^{13}C NMR (100 MHz, $CDCl_3$) δ:24.01, 25.82, 54.97, 57.62, 66.63, 112.24, 116.68, 118.61, 120.92, 123.89, 124.48, 124.92, 125.75, 129.83, 129.93, 132.19, 134.03, 140.81, 147.84, 155.15, 158.26, 159.21, 186.97. HRMS(ESI) m/z: 404.1870 $[M+H]^+$. Purity: 98.7% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(2-(2-(dimethylamino)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (7g)

Pale red solid, yield: 85.8%, mp: 135.7–136.1 °C;^{1} H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.13 (6H, t,J = 8 Hz, 2×$NCH_2C\underline{H}_3$), 2.72–2.77 (4H, m, 2×$NC\underline{H}_2CH_3$), 2.99 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 4.16 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$), 6.91–7.00 (2H, m, $H_{3,5}$ phenyl), 7.32–7.39 (3H, m, H_4 phenyl and $H_{6,8}$ coumarin), 7.61–7.69 (3H, m, H_6 phenyl and $H_{5,7}$ coumarin), 8.00 (1H, d,J = 16 Hz, H_α), 8.20 (1H, d,J = 16 Hz, H_β), 8.54(1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:11.36, 47.71, 57.44, 66.16, 112.25, 116.68, 118.61, 123.89, 124.48, 124.92, 125.75, 129.83, 129.93, 132.19, 134.03, 140.81, 147.64; 155.18, 158.29, 159.25, 186.92. HRMS(ESI) m/z:392.4354 $[M+H]^+$. Purity: 98.1% by HPLC (MeOH /0.1% TEA 85:15 (v/v)).

(E)-3-(3-(2-(2-morpholinoethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (7h)

Brown viscous liquid, Yield:85.5%; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 2.61 (4H, m, morpholine-H), 2.95 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 3.72–3.74 (4H, m, morpholine-H), 4.24 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$), 6.98–7.05 (2H, m, $H_{3,5}$ phenyl), 7.29–7.39 (3H, m, H_4 phenyl and $H_{6,8}$ coumarin), 7.82–7.86 (3H, m, H_6 phenyl and $H_{5,7}$ coumarin), 8.01 (1H, d, J = 16 Hz, H_α),8.18 (1H, d, J = 16 Hz, H_β), 8.55 (1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ: 54.94, 57.68, 66.68, 67.62, 112.24, 116.68, 118.61, 123.89, 124.48, 124.92, 125.75, 129.83, 129.93, 132.19, 134.03, 140.81, 147.64; 155.18, 158.29, 159.25, 186.99. HRMS(ESI) m/z: 406.1639 $[M+H]^+$. Purity: 98.9% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(2-(isopentyloxy)phenyl)acryloyl)-2*H*-chromen-2-one (7i)

Yellow solid, yield: 85.6%, mp: 153.4–155.2 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 0.98 (6H, d,J = 8 Hz, 2×$CHC\underline{H}_3$), 1.71 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 1.80–1.88 (1H, m, $C\underline{H}(CH_3)_2$), 4.04 (2H, t, J = 8 Hz, $OC\underline{H}_2CH_2$), 6.92–7.01(2H, m, $H_{3,5}$ phenyl), 7.32–7.41 (3H, m, H_4 phenyl and $H_{6,8}$ coumarin), 7.63–7.69 (3H, m, H_6 phenyl and $H_{5,7}$ coumarin), 8.00 (1H, d,J = 16 Hz, H_α), 8.19 (1H, d,J = 16 Hz, H_β), 8.57 (1H, s, H_4 coumarin)^{13}C NMR (100 MHz, $CDCl_3$) δ:22.59, 25.06, 37.87, 66.62, 112.26, 116.68, 118.61, 123.89, 124.48, 124.92, 125.75, 129.83, 129.93, 132.19, 134.03, 140.81, 147.83, 155.18, 158.26, 159.28, 186.93.

HRMS(ESI) m/z: 363.1596 $[M+H]^+$. Purity: 98.6% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(2-(3-methylbut-2-enyloxy)phenyl)-prop-2-enoyl)-2*H*-chromen-2-one (7j)

Yellow solid, yield: 80%, mp: 92–95 °C; This is a known compound but without reports regarding inhibiting cholinesterases [27].

General procedure for the synthesis of compounds 8a–8c

Secondary amine (dimethylamine, pyrrolidine, piperidine, 5.00 mmol) and anhydrous sodium acetate (5.5 mmol, 0.451 g) were sequentially added into CH_2Cl_2 (10 mL). The reaction mixture stirred for 20 min at 0 °C. Chloroacetyl chloride (6.00 mmol, 0.477 mL) was then added into the mixture, and the reaction was stirred for 5–6 h at ambient temperature. The resulting mixture was extracted thrice with 60 mL CH_2Cl_2. The separated organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. Then, compounds **8a**–**8c** were isolated. Yield: 80–89%.

General procedure for the synthesis of compounds 9d–11f

Compounds **8a**–**8c** (3.00 mmol) were added into a solution containing intermediate compounds **4a**–**4c** (1.00 mmol), K_2CO_3 (3.00 mmol, 0.43 g,) and NaI (0.05 mmol, 0.012 g) in DMF (6 mL). The reaction mixture was stirred for 10–12 h at 56 °C, then cooled to room temperature and filtered. The filtrate was poured into a saturated saline solution and filtered. The crude product was purified by column chromatography using dichloromethane/methyl alcohol (100:1) as eluent to give the pure product compounds **9d**–**11f**. Yield: 90–95%.

(E)-N,N-dimethyl-2-(4-(3-oxo-3-(2-oxo-2*H*-chromen-3-yl)prop-1-en-1-yl) phenoxy)acetamide (9d)

Yellow solid, yield: 90.8%, mp: 193.7–195.1 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 2.99 (3H, s, $NC\underline{H}_{a3}$), 3.10 (3H, s, $NC\underline{H}_{b3}$), 4.75 (2H, s, $OC\underline{H}_2CO$), 6.98 (2H, d, J = 8 Hz, $H_{3,5}$ phenyl), 7.34–7.41 (2H, m, $H_{6,8}$ coumarin), 7.63–7.68 (4H, m, $H_{2,6}$ phenyl and $H_{5,7}$ coumarin), 7.80–7.88 (2H, m, H_α and H_β), 8.58 (1H, s, H_4 coumarin) ^{13}C NMR (100MHz, $CDCl_3$) δ:35.75, 36.58, 67.35, 115.11, 116.70, 118.61, 122.13, 124.95, 125.48, 128.46, 129.99, 130.82, 134.13, 144.76, 147.90, 155.21, 159.36, 160.23, 167.29, 186.30. HRMS(ESI) m/z: 378.2458 $[M+H]^+$. Purity: 98.6% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (9e)

Yellow solid, yield: 90.3%, mp: 225.7–227.2 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.85–2.01 (4H, m, pyrrolidine-H), 3.53 (4H, m, pyrrolidine-H), 4.68 (2H, s, $OC\underline{H}_2CO$), 6.98 (2H, d, J = 8 Hz, $H_{3,5}$ phenyl), 7.33–7.41 (2H, m, $H_{6,8}$ coumarin), 7.63–7.68 (4H, m, $H_{2,6}$ phenyl and $H_{5,7}$ coumarin), 7.79–7.87 (2H, m, H_α and H_β), 8.58 (1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:23.82, 26.28, 46.02, 46.27, 67.82, 115.09, 116.69, 118.60 122.10, 124.96, 125.46, 128.40, 130.00, 130.82, 134.14, 144.76, 147.90, 155.19, 159.35, 160.31, 165.91, 186.32. HRMS(ESI) m/z: 404.1502 $[M+H]^+$. Purity: 98.9% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(4-(2-oxo-2-(piperidin-1-yl)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (9f)

Yellow solid, yield: 92.5%, mp: 217.4–219.6 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.56–1.65 (6H, m, piperidine-H), 3.48–3.57 (4H, m, piperidine-H), 4.74 (2H, s, $OC\underline{H}_2CO$), 6.98 (2H, d, J = 8 Hz, $H_{3,5}$ phenyl), 7.33–7.40 (2H, m, $H_{6,8}$ coumarin), 7.62–7.68 (4H, m, $H_{2,6}$ phenyl and $H_{5,7}$ coumarin), 7.78–7.86 (2H, m, H_α and H_β), 8.57 (1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:24.42, 25.53, 26.50, 43.27, 46.41, 67.56, 115.09, 116.67, 118.59 122.08, 124.96, 125.45, 128.35, 130.00, 130.81, 134.14, 144.74, 147.87, 155.17, 159.33, 160.32, 165.57, 186.30. HRMS(ESI) m/z: 418.1854 $[M+H]^+$. Purity: 98.7% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-N,N-dimethyl-2-(3-(3-oxo-3-(2-oxo-2*H*-chromen-3-yl)prop-1-en-1-yl)phenoxy)acetamide (10d)

Pale yellow solid, yield: 94.6%, mp: 127.8–129.1 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 2.89 (3H, s, $NC\underline{H}_{a3}$), 3.12 (3H, s, $NC\underline{H}_{b3}$), 4.78 (2H, s, $OC\underline{H}_2CO$), 7.03 (1H, d, J = 8 Hz, H_4 phenyl), 7.21 (1H, s, H_2 phenyl), 7.30–7.40 (4H, m, $H_{5,6}$ phenyl and $H_{6,8}$ coumarin), 7.65–7.69 (2H, m,$H_{5,7}$ coumarin), 7.81 (1H, d, J = 16 Hz, H_α), 7.90 (1H, d, J = 16 Hz, H_β), 8.54(1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:35.66, 36.78, 67.65, 114.29, 116.69, 117.64, 118.55, 121.69, 124.11, 125.28, 130.04, 134.22, 136.10, 145.09, 148.03, 155.24, 158.71, 159.39, 166.25, 186.45. HRMS(ESI) m/z: 378.2412 $[M+H]^+$. Purity: 98.6% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(3-(2-oxo-2-(pyrrolidin-1-yl)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (10e)

Pale yellow solid, yield: 93.2%, mp: 246.7–248.3 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.86–2.02 (4H, m,

pyrrolidine-H), 3.55 (4H, t, J = 8 Hz, pyrrolidine-H), 4.68 (2H, s, OC$\underline{H}_2$CO), 7.03 (2H, d, J = 8 Hz, H_4 phenyl), 7.22 (1H, s, H_2 phenyl), 7.30–7.41 (4H, m, $H_{5,6}$ phenyl and $H_{6,8}$ coumarin), 7.65–7.69 (2H, m, $H_{5,7}$ coumarin), 7.81 (1H, d, J = 16 Hz, H_α), 7.90 (1H, d, J = 16 Hz, H_β), 8.58 (1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:23.83, 26.28, 46.05, 46.27, 67.84, 114.60, 116.72, 117.24, 118.53, 122.37, 124.41, 125.03, 125.21, 130.08, 134.31, 136.29, 144.71, 148.17, 155.24, 158.41, 159.30, 166.20, 186.48. HRMS(ESI) m/z: 404.2765 $[M+H]^+$. Purity: 98.9% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(3-(2-oxo-2-(piperidin-1-yl)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (10f)

Yellow solid, yield: 90.7%, mp: 152.6–154.3 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.56–1.65 (6H, m, piperidine-H), 3.50–3.58 (4H, m, piperidine-H), 4.73 (2H, s, OC$\underline{H}_2$CO), 7.03 (1H, d, J = 8 Hz, H_4 phenyl), 7.21 (1H, s, H_2 phenyl), 7.30–7.40 (4H, m, $H_{5,6}$ phenyl and $H_{6,8}$ coumarin), 7.64–7.69 (2H, m, $H_{5,7}$ coumarin), 7.81 (1H, d, J = 16 Hz, H_α), 7.90 (1H, d, J = 16 Hz, H_β), 8.57(1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:24.46, 25.55, 26.52, 43.25, 46.43, 67.61, 114.80, 116.70, 117.10, 118.52, 122.25, 124.42, 125.02, 125.22, 130.07, 134.29, 136.28, 144.68, 148.12, 155.23, 158.52, 159.27, 165.78, 186.47. HRMS(ESI) m/z: 418.1599 $[M+H]^+$. Purity: 98.7% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-N,N-dimethyl-2-(2-(3-oxo-3-(2-oxo-2*H*-chromen-3-yl)prop-1-en-1-yl)phenoxy)acetamide (*11d*)

Pale yellow solid, yield: 92.3%, mp: 161.7–163.4 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 3.00 (3H, s, NC$\underline{H}_{a3}$), 3.15 (3H, s, NC$\underline{H}_{b3}$), 4.82 (2H, s, OC$\underline{H}_2$CO), 6.98–7.06 (2H, d, J = 8 Hz, $H_{3,5}$ phenyl), 7.33–7.40 (3H, m, H_4 phenyl and $H_{6,8}$ coumarin), 7.64–7.71 (3H, m, H_6 phenyl and $H_{5,7}$ coumarin), 8.04 (1H, d,J = 16 Hz, H_α), 8.19 (1H, d,J = 16 Hz, H_β), 8.59 (1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:35.77, 36.91, 68.20, 112.55, 116.68, 118.61, 121.76, 124.06, 124.93, 125.63, 130.00, 130.12, 132.21, 134.10, 140.21, 147.83; 155.20, 157.40, 159.21, 167.55, 186.92. HRMS(ESI) m/z: 378.1331 $[M+H]^+$. Purity: 98.3% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(2-(2-oxo-2-(pyrrolidin-1-yl)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (11e)

Yellow solid, yield: 92.8%, mp: 288.8–231.0 °C;^{1} H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.84–1.99 (4H, m, pyrrolidine-H), 3.52–3.59 (4H, m, pyrrolidine-H), 4.76 (2H, s, OC$\underline{H}_2$CO), 6.98–7.05 (2H, m, $H_{3,5}$ phenyl), 7.35–7.40 (3H, m, H_4 phenyl and $H_{6,8}$ coumarin), 7.63–7.70 (3H, m, H_6 phenyl and $H_{5,7}$ coumarin), 8.04 (1H, d,J = 16 Hz, H_α), 8.19 (1H, d,J = 16 Hz, H_β), 8.60 (1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:23.77, 26.32, 46.28, 46.33, 68.68, 112.54, 116.66, 118.61, 121.71, 124.01, 124.89, 124.93, 125.61, 130.01, 130.08, 132.22, 134.10, 140.20, 147.83; 155.18, 157.49, 159.18, 166.23, 186.93. HRMS(ESI) m/z: 404.1311 $[M+H]^+$. Purity: 99.1% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(2-(2-oxo-2-(piperidin-1-yl)ethoxy)phenyl)acryloyl)-2*H*-chromen-2-one (11f)

Pale yellow solid, yield: 90.5%, mp: 148.7–150.3 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.54–1.62 (6H, m, piperidine-H), 3.57 (4H, m, piperidine-H), 4.82 (2H, s, OC$\underline{H}_2$CO), 7.01–7.05 (2H, m, $H_{3,5}$ phenyl), 7.35–7.40 (3H, m, H_4 phenyl and $H_{6,8}$ coumarin), 7.63–7.71 (3H, m, H_6 phenyl and $H_{5,7}$ coumarin), 8.02 (1H, d,J = 16 Hz, H_α), 8.20 (1H, d,J = 16 Hz, H_β), 8.58 (1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:24.45, 25.58, 26.51, 43.31, 46.62, 68.29, 112.48, 116.66, 118.59, 121.66, 123.92, 124.77, 124.94, 125.57, 129.90, 130.00, 132.27, 134.11, 140.15, 147.82; 155.17, 157.43, 159.20, 165.83, 186.82 HRMS(ESI) m/z: 418.1647 $[M+H]^+$. Purity: 98.5% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

General procedure for the synthesis of compounds 5 and 6

Compound **3** (5.00 mmol, 0.94 g) was added into acetic acid (30 mL) under stirring, followed by the addition of an appropriate vanillic aldehyde or syringaldehyde (5.00 mmol) and *p*-toluenesulfonic acid (4.5 mmol). The reaction was maintained at 70 °C for 5–7 h, then cooled to room temperature and filtered. The filtrate was neutralized with a sodium bicarbonate solution, and the resulting mixture was extracted thrice with 90 mL CH_2Cl_2. The organic layer was separated and dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methyl alcohol (90:1) as eluent to give pure products **5** and **6**.

General procedure for the synthesis of compounds 12k–13m

Compounds **5** or **6** (1.00 mmol), together with aminoethyl chloride (3.00 mmol) and anhydrous potassium carbonate (3.00 mmol, 0.415 g), were dissolved into 15 mL acetone in a flask, and stirred for 20 minutes. Then, a little NaI (0.005 g, 0.02 mmol) was added into the reaction solution as a catalyst. The reaction solution kept at 40 °C for 10–12 h, then cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure, and the crude prod-

uct was purified by silica gel column chromatography using dichloromethane/methyl alcohol (40:1) as eluent to give the pure product compounds **12k**–**3m**. Yield: 80–85%.

(E)-3-(3-(4-(2-(dimethylamino)ethoxy)-3-methoxyphenyl) acryloyl)-2*H*-chromen-2-one (12k)

Brownish yellow solid, yield: 85.6%, mp: 124.9–126.3 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 2.62 (6H, s, 2×NC$\underline{H}_3$), 3.15 (2H, t, J = 8 Hz, $OCH_2C\underline{H}_2$), 3.92 (3H, s, OC$\underline{H}_3$), 4.34 (2H, t, J = 8 Hz, O$\underline{C}H_2CH_2$), 6.92 (1H, d, J = 8 Hz, H_5 phenyl), 7.20(1H, s, H_2 phenyl), 7.25 (1H, d, J = 8 Hz, H_6 phenyl), 7.35–7.42 (2H, m, $H_{6,8}$ coumarin), 7.65–7.69 (2H, m, $H_{5,7}$ coumarin), 7.78–7.86 (2H, m, H_α and H_β), 8.59 (1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:45.46, 55.98, 57.46, 65.77, 110.75, 112.83, 116.67, 118.59, 122.03, 123.88, 124.99, 125.47, 128.31, 130.00, 134.15, 145.17, 147.85, 149.46, 150.63, 155.15, 159.41, 186.25. HRMS(ESI) m/z: 394.1651 $[M+H]^+$. Purity: 98.5% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(3-methoxy-4-(2-(pyrrolidin-1-yl)ethoxy) phenyl)acryloyl)-2*H*-chromen-2-one (12l)

Brownish yellow solid, yield: 75.2%, mp: 158.9–160.7 °C; ^{1} H NMR (400 MHz, $DCCl_3$): δ (ppm) 2.22–2.26 (4H, m, pyrrolidine-H), 3.15–3.20 (4H, m, pyrrolidine-H), 3.62 (2H, s, $OCH_2C\underline{H}_2$), 3.92 (3H, s, OC$\underline{H}_3$), 4.65 (2H, s, O$\underline{C}H_2CH_2$), 6.94 (1H, d, J = 8 Hz, H_5 phenyl), 7.20–7.24 (1H, m, $H_{2,6}$ phenyl), 7.35–7.41 (2H, m, $H_{6,8}$ coumarin), 7.65–7.70 (2H, m, $H_{5,7}$ coumarin), 7.81 (2H, m, H_α and H_β), 8.59 (1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:23.29, 53.78, 54.60, 55.98, 64.37, 110.80, 114.18, 116.70, 118.57, 122.74, 123.66, 125.05, 125.34, 129.67, 130.07, 134.25, 144.69, 148.03, 149.06, 149.71, 155.18, 159.44, 186.30. HRMS(ESI) m/z: 420.1808 $[M+H]^+$. Purity: 98.9% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(3-methoxy-4-(2-(piperidin-1-yl)ethoxy) phenyl)acryloyl)-2*H*-chromen-2-one (12m)

Yellow solid, yield: 80.6%, mp: 270.9–272.1 °C; ^{1} H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.26–1.52 (6H, m, piperidine-H),1.74 (4H, s, piperidine-H), 3.01 (2H, t, J = 8 Hz , $OCH_2C\underline{H}_2$), 3.92 (3H, s, OC$\underline{H}_3$), 4.31 (2H, t, J = 8 Hz, O$\underline{C}H_2CH_2$), 6.91 (1H, d, J = 8 Hz, H_5 phenyl), 7.19 (1H, s, H_2 phenyl), 7.25 (1H, d, J = 8 Hz, H_6 phenyl), 7.34–7.40 (2H, m, $H_{6,8}$ coumarin), 7.64–7.69 (2H, m, $H_{5,7}$ coumarin), 7.77–7.85 (2H, m, H_α and H_β), 8.57 (1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:23.55, 25.01, 29.70, 54.68, 55.98, 57.12, 65.77, 110.76, 112.80, 116.67, 118.59, 122.03, 123.88, 124.99, 125.47, 128.31, 130.00, 134.15, 145.17, 147.85, 149.46, 150.60, 155.14, 159.41, 186.27 HRMS(ESI) m/z: 434.1960 $[M+H]^+$. Purity: 98.2% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(4-(2-(dimethylamino)ethoxy)-3,5-dimethoxyph enyl)acryloyl)-2-chromen-2-one (13k)

Yellow solid, yield: 80.2%, mp: 183.9–185.6 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 2.62 (6H, s, 2×NC$\underline{H}_3$), 3.14 (2H, s, J = 8 Hz, $OCH_2C\underline{H}_2$), 3.82 (6H, s, 2×OC$\underline{H}_3$), 4.33 (2H, t, J = 8 Hz, O$\underline{C}H_2CH_2$), 6.83 (2H, s, $H_{2,6}$ phenyl), 7.36–7.43 (2H, m, $H_{6,8}$ coumarin), 7.65–7.70 (2H, m, $H_{5,7}$ coumarin), 7.77 (1H, d, J = 16 Hz, H_α), 7.85(1H, d, J = 16 Hz, H_β), 8.65 (1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:45.67, 52.46, 56.12, 67.93, 105.68, 116.79, 118.54, 123.71, 125.08, 125.87, 128.73, 130.09, 134.35, 139.91, 142.35, 144.78, 148.18, 153.28, 155.29, 159.44, 186.29 HRMS(ESI) m/z: 424.1759 $[M+H]^+$. Purity: 98.8% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(3,5-dimethoxy-4-(2-(pyrrolidin-1-yl)ethoxy)phe nyl)acryloyl)-2*H*-chromen-2-one (13l)

Brownish yellow solid, yield: 80.3%, mp: 274.1–276.4 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 2.22–2.26 (4H, m, pyrrolidine-H), 3.12–3.20 (4H, m, pyrrolidine-H), 3.62 (2H, s, J = 8 Hz, $OCH_2C\underline{H}_2$), 3.90 (6H, s, 2×OC$\underline{H}_3$), 4.62 (2H, t, J = 8 Hz, O$\underline{C}H_2CH_2$), 6.88 (2H, s, $H_{2,6}$ phenyl), 7.35–7.41 (2H, m, $H_{6,8}$ coumarin),7.65–7.74 (2H, m, $H_{5,7}$ coumarin), 7.77 (1H, d, J = 16 Hz, H_α), 7.85(1H, d, J = 16 Hz, H_β), 8.57 (1H, s, H_4 coumarin) ^{13}CNMR(100MHz, $CDCl_3$) δ:23.75, 52.46, 56.05, 67.93, 105.35, 116.75, 118.54, 123.71, 125.08, 125.87, 128.75, 130.09, 134.35, 139.91, 142.35, 144.76, 148.18, 153.28, 155.20, 159.35, 186.25 HRMS(ESI) m/z: 450.1915 $[M+H]^+$. Purity: 98.2% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

(E)-3-(3-(3,5-dimethoxy-4-(2-(piperidin-1-yl)ethoxy)phenyl) acryloyl)-2*H*-chromen-2-one (13m)

Yellow solid, yield: 78.3%, mp: 214.9–216.7 °C; ^{1}H NMR (400 MHz, $DCCl_3$): δ (ppm) 1.26 (6H, s, piperidine-H), 1.63 (4H, s, piperidine-H), 2.33 (2H, s, $OCH_2C\underline{H}_2$), 3.88 (6H, s, 2×OC$\underline{H}_3$), 4.36 (2H, s, O$\underline{C}H_2CH_2$), 6.87 (2H, s, $H_{2,6}$ phenyl), 7.35–7.41 (2H, m, $H_{6,8}$ coumarin), 7.65–7.70 (2H, m, $H_{5,7}$ coumarin), 7.77(1H, d, J = 16 Hz, H_α), 7.85(1H, d, J = 16 Hz, H_β), 8.59(1H, s, H_4 coumarin) ^{13}C NMR (100 MHz, $CDCl_3$) δ:21.33, 23.34, 29.70, 54.00, 56.11, 57.14, 67.93, 105.66, 116.71, 118.54, 123.71, 125.08, 125.87, 128.75, 130.09, 134.35, 139.91, 142.35, 144.76, 148.18, 153.28, 155.20, 159.44, 186.26. HRMS(ESI) m/z: 464.2076 $[M+H]^+$. Purity: 99.1% by HPLC (MeOH/0.1% TEA 85:15 (v/v)).

AChE and BChE inhibition assay

The effects of newly synthesized compounds in AChE or BChE inhibition were measured by using a modified *Ellman* method [30]. The individual compound was dissolved in Tween 80 (final concentration was 0.06% in each reaction) and diluted with water to different concentrations immediately before use. Five different concentrations were tested for each compound in triplicate. The reaction mixture containing 2.76 mL of Na_2HPO_4/NaH_2PO_4 buffer (pH 8.0, 0.1mol/L), 100 μL of the different concentrations of tested compounds, and 100 μL of AChE or BChE (100 μL) was incubated for 30 min (30 °C). Then, the reaction was terminated by the addition of 100 μL of 20% sodium dodecylsulfate (SDS), 100 μL 10 mmol/L 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) was added as chromogenic agent to generate a yellow solution. The absorbance of each assay solution was measured at 412 nm by UV spectroscopy. The IC_{50} values were calculated using Bliss method and expressed as mean ± SD.

Kinetic studies

Kinetic studies of AChE were performed by using a reported method [31,32]. Compound was added into the assay solution and preincubated with the enzyme at 30 °C, followed by the addition of 100 μL acetylthiocholine iodide including five concentrations. The assay solution contained 100 μL compound, 100 μL DTNB, 2.79 mL 0.1 mol/L Na_2HPO_4/NaH_2PO_4 buffer (pH 7.4). Kinetic assay of the hydrolysis of acetylthiocholine iodide catalyzed by AChE was conducted spectrometrically at 412 nm. The parallel control experiment was carried out without compound in the mixture.

Molecular docking

Molecular docking study was carried out with Molecular Operating Environment (MOE) software package (Chemical Computing Group, Montreal, Canada), and structure models of AChE/BChE X-ray crystal structures (PDB ID: 1EVE/1P0I) were gained from protein data bank [33,34]. The 3D structures of the compounds were built with virtue of the builder interface of MOE program, and docked into the active site of the protein after energy being minimized. The Dock scoring in MOE software was done by ASE scoring function.

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