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Design, synthesis, and acetylcholinesterase inhibitory activity of novel coumarin analogues

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ABSTRACT

Three series (series A–C) of coumarin analogues with phenylpiperazine functions as substitution were designed and synthesized for studying their potential for treating Alzheimer's (AD) disease. Their anti-cholinesterase activities were assayed according to Ellmann's method against freshly prepared acetylcholinesterase (AChE) from Electrophorus electricus using donepezil as the reference compound. Pharmacological study and preliminary structure–activity relationships showed that coumarins with substitution on positions 3 and/or 4 have parallel anti-AchE activities compared with the reference compound.

1. Introduction

Alzheimer's disease (AD), a progressive and degenerative disorder of the brain, is believed to be the most common cause of dementia among the elderly. AD is associated with a loss of the presynaptic markers of the cholinergic system in the brain areas related to memory and learning, and is characterized by the presence of amyloid deposits and neurofibrillary tangles in the brain of afflicted individuals.[1,2] Up to now, medicines to cure Alzheimer's disease have received significant attention for many years. The molecular causes of this condition remain unknown in spite of the existence of several theories regarding the pathogenesis of AD.[3–6] As a result, most of the existing medicines can only delay the development of the state of AD, but cannot cure the disease in nature. Now, the most widely accepted biochemical theory of the disease, known as the cholinergic hypothesis,[7–10] is that the decline in cognitive and mental functions associated with AD is related to the loss of cortical cholinergic neurotransmission. One rational way to enhance cholinergic neurotransmission is to inhibit an enzyme[11–13] responsible for the metabolic breakdown of acetylcholine (ACh). So up to now most of the drugs approved for AD treatment are acetylcholinesterase (AChE) inhibitors (AChEIs) which can enhance cholinergic neurotransmission by increasing acetylcholine (ACh) availability in the synaptic cleft. Considering the mechanism of AChEIs, they are not expected to interfere with the neurodegenerative cascade of the disease, but only mitigate some of the symptoms temporarily.

Recently, studies have shown that AChEIs can be effective over a longer period[14] for that AChE plays an important role in Aβ deposition.[15–17] It seems likely that AChE interacts with Aβ and promotes the formation of amyloid fibril through a pool of amino acids located in proximity of the peripheral anionic binding site (PAS) of the enzyme.[15] Moreover, it has been shown that molecules interact either exclusively with PAS or with both catalytic and peripheral binding sites, which can also prevent the pro-aggregation of AChE toward Aβ.[16] Compounds showing dual bindings with AChE are intriguing, and they represent a new type of therapeutic agents through the prevention of Aβ aggregation. Recent research revealed that several AChEIs not only facilitate cholinergic transmission, but also interfere with the synthesis, deposition and aggregation of toxic amyloid-β-peptides (Aβ).[18,19] On this basis, AChEIs have become the leading strategy for the development of anti-AD agents. The current interest in these drugs has received considerable attention too.[20,21] Some anti-AChE agents, such as tacrine, donepezil, rivastigmine, and ensaculin (Fig. 1), show a modest induce of the improvement in memory and cognitive functions,[22] and have been used to treat AD clinically for a long time. But only ensaculin, a coumarin derivative, has appeared to prevent or slow down the progressive neurodegeneration in these compounds.[22]

At the same time, the three-dimensional (3-D) X-ray structure of AChE from Torpedo California electric organ has been reported for further study of the enzyme and corresponding inhibitors.[23]

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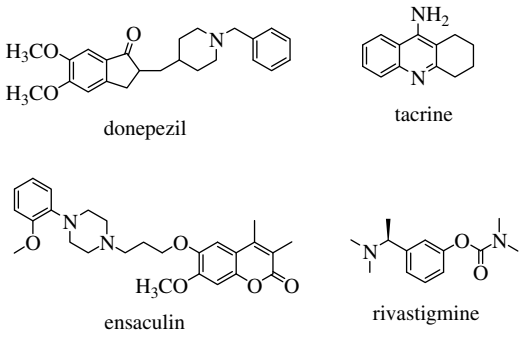


Figure 1. Structures of the acetyl cholinesterase inhibitors as FDA approved Alzheimer's disease therapeutics.

The X-ray structure of a transition state analogue complex of AChE has also been reported.[24] The active sites of AChE were shown in Figure 2. The figure suggested that the active sites of AChE consist at least the following binding sites: (1) an anionic substrate (AS) binding site, such as Trp84, Glu199, and Phe330, which contains a small number of negative charges where the quaternary ammonium part of ACh and various active sites bind through a preferential interaction of quaternary nitrogens with the p electrons of aromatic groups; (2) an ecstatic site (ES), which contains the catalytic triad Ser200-His440-Glu327[18]; (3) an acyl binding site (ABS), Phe288, and Phe299, which binds to the acetyl group of ACh.[19] Besides these, AChE also has a peripheral anionic site (PAS), such as Trp279, Tyr70, Tyr121, Asp72, Glu199, and Phe290, which may bind to 9-aminoacridine and 9-amino-1,2,3,4-tetrahydroacridine (tacrine).[25–28]

As mentioned above, ensaculin composed of a benzopyran with a piperazine substituted moiety (Fig. 1) has been used clinically as its HCl salt with trade name of KA-672 HCl for treating AD as AChEI for a long time.[29] So ensaculin, a coumarin analogue, was chosen to be the parent compound in this study, and the analogues designed were expected to have anti-AChE activity. Coumarin, naturally occurring phytochemicals with fragrance found in many plant species with a wide range of biological activities, such as anti-inflammatory, anti-tumour, anti-allergic and anti-HIV-1 activities, has received significant attention for many years.[30–32] So considering the active sites of AChE and ensaculin structure, three series of coumarin analogues with phenylpiperazine substituted at position 6 of coumarin as series A, at position 3 as series B and at position 4 as series C were designed and synthesized. In the designed compounds, the following hypothesis was expected: (1) the coumarin ring, 2*H*-chromen-2-one heterocycle, a heterocyclic moiety comprising in ensaculin with cognitive functions, demonstrated to be compatible with a high anti-AChE potency,[33,34] acted as the peripheral anonic site, which can interact with the peripheral binding site;[24] (2) the nitrogen atom from the phenylpiperazine groups acted as the positive charge center presented in many potent AChE inhibitors,[35] which can interact with the catalytic center of AChE demonstrated by the X-ray crystallographic studies of the AChE/donepezil and AChE/galantamine complexes;[36,37] and (3) the phenyl ring connecting with the piperazine ring acted as the choline binding site as shown in Figure 3. Furthermore, a linking chain bearing different amounts of carbon atoms might have the chance to line the wall of the AChE gorge.[23]

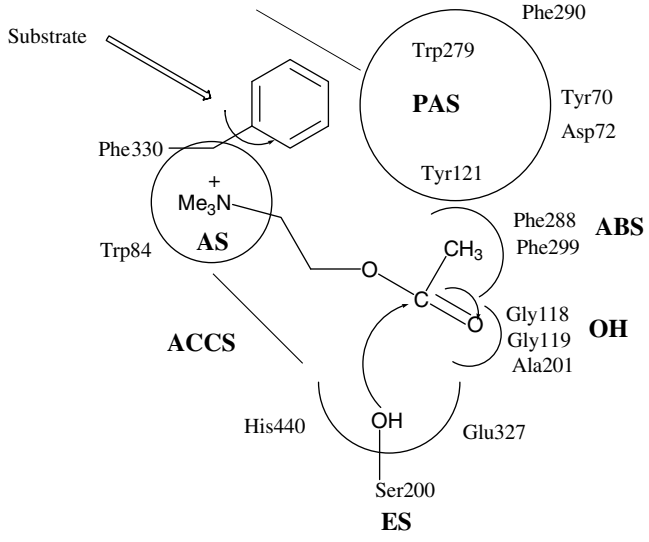


Figure 2. The active site and the PAS of AchE. Numbers refer to residue positions in Torpedo California AchE.[16]

2. Results and discussion

2.1. Chemistry

Series A, B, and C were designed and synthesized as shown in Schemes 1–3, respectively. The key immediate **1** was prepared by *p*-cresol and malonic acid using $ZnCl_2$ as catalyst and $POCl_3$ as solvent. Then, **1** reacted with $(CH_3)_2SO_4$ to give methylated product **2** or reacted with $POCl_3$ to give chlorinated product **4**. **2** or **4** was bromided with NBS to give **3** or **5**. **3** or **5** reacted with corresponding substituents and the target compounds **6**–**20** were obtained (Scheme 1).

As shown in Scheme 2, series B were prepared through the reaction of salicylic aldehyde and diethyl malonate to give the intermediate **21**. Then, **21** was hydrolyzed with NaOH and **22** was obtained. **22** was chlorided with $SOCl_2$ to give the key intermediate **23**. At last, **23** reacted with corresponding substituents, and the target compounds **24**–**37**were obtained.

Series C were prepared from the key intermediate **1** (Scheme 3). Then **1** was chlorided with $POCl_3$ to give **4**. Lastly, **4** reacted with

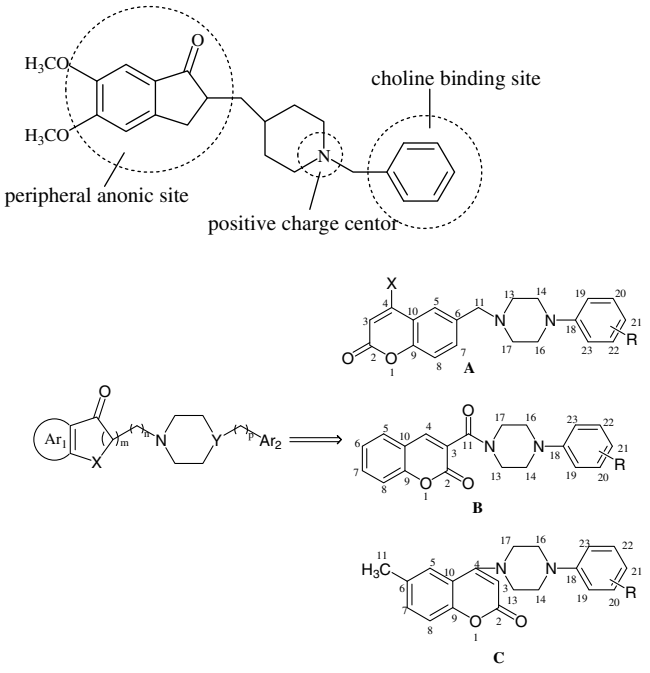
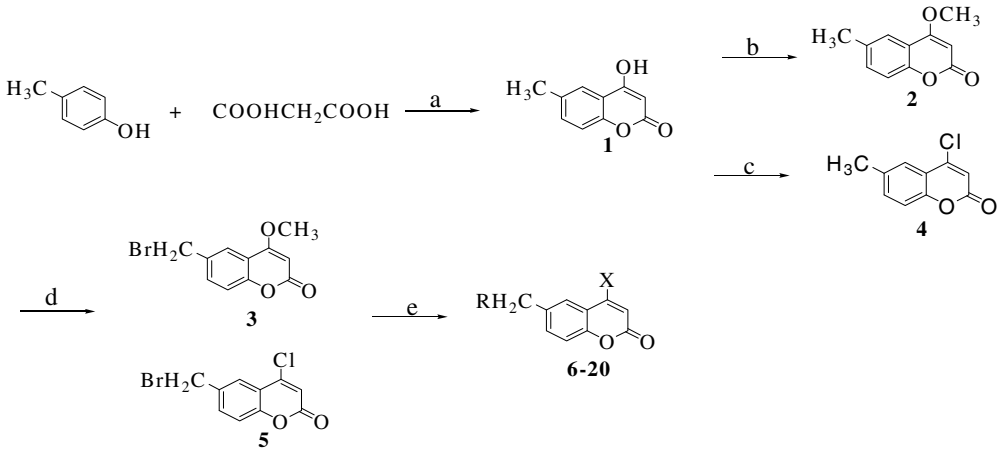
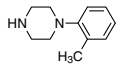
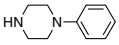
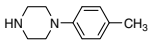
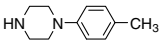
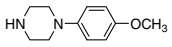
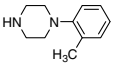
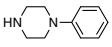
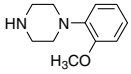
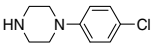
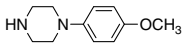
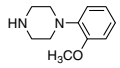
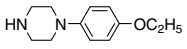
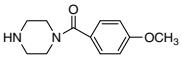
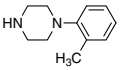
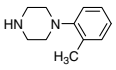
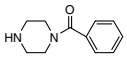


Figure 3. Design strategy of the target compounds.



Compound	R	X	Compound	R	X
6		OCH_3	**14**		Cl
7		OCH_3	**15**		Cl
8		OCH_3	**16**		Cl
9		OCH_3	**17**		Cl
10		OCH_3	**18**		Cl
11		OCH_3	**19**		Cl
12		OCH_3	**20**		
13		OCH_3			

Scheme 1. The preparation of series A. Reagents and conditions: (a) $ZnCl_2$, $POCl_3$, 60 °C; (b) $(CH_3)_2SO_4$, K_2CO_3, reflux; (c) $POCl_3$, reflux; (d) NBS, BPO, reflux; (e) RH, $NaHCO_3$, reflux.

the corresponding substituents to give the target compounds **38**–**50**.

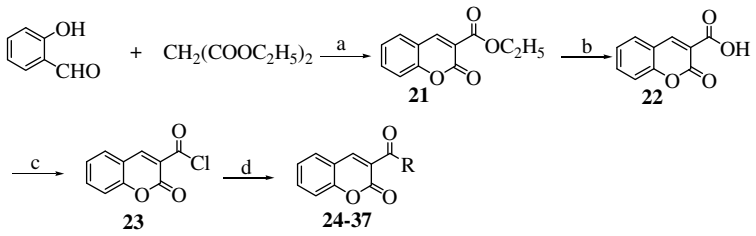
The structures of the target compounds were characterized by ^{1}H NMR, IR, and ESI-MS. One compound of each series was characterized by ^{13}C NMR, too. The purity of the target compounds was analyzed by HPLC. Besides, the structure of compound **16** was further confirmed by single crystal X-ray diffraction.

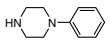
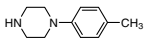
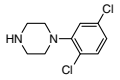
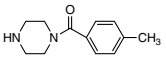
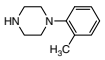
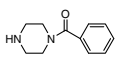
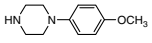
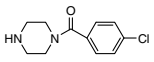
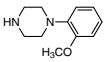
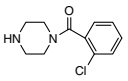
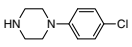
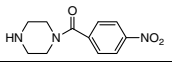
2.2. Inhibition of AChE

To determine the therapeutic potency of the new coumarin derivatives for treating AD, their anticholinesterase activities (compounds **6**–**20**, **24**–**50**) were assayed according to Ellmann's method[38] against freshly prepared AChE from electrophorus electricus using donepezil as reference compound. Inhibition of AChE activities of the synthesized compounds is shown in Table 1.

The data listed in Table 1 clearly show that most of the designed compounds exhibited moderate inhibitory activities toward the cholinesterase. In general, the AChE inhibitory activities of compounds with phenylpiperazine substitutions on the positions 3 and/or 4 of coumarin ring are better than that of the 6-substituted coumarins. Most of the 6-substituted coumarins did not show anti-AChE activities. On comparison of the target 6-substituted coumarins and ensaculin, we found that there is one atom in the linking chain between coumarin backbone and phenylpiperazine in the 6-substituted coumarins, while there are four atoms in ensuculin (Fig. 3). So, we can conclude that the amounts of the atoms in the linking chain are important for anti-AChE activities of 6-phenylpiperazine coumarin analogues for that one atom cannot reach the requirement for the gorge.[19]

The 4-phenylpiperazine substituted coumarin compounds **45** 6-methyl-4-(4-phenylpiperazin-1-yl)2*H*-chromen-2-one and **47** 6-methyl-4-(4-(4-methyl-benzoyl)piperazin-1-yl)2*H*-chromen-2-one showed significant activities with IC_{50} 4.5 μmol/L and 5.3 μmol/L, respectively. There are two carbones between the carbonyl-carbon atom (C2) and piperazine ring in the 4-substituted coumarins (Fig. 3), which is in agreement with donepezil, the positive compound. It suggests that the distance between carbonyl-carbon atom and nitrogen atom of piperazine ring is important for the anti-AChE activities.



Compound	R	Compound	R
24		**31**	
25		**32**	
26		**33**	
27		**34**	
28		**35**	
29		**36**	
30		**37**	

Scheme 2. The preparation of series B. Reagents and conditions: (a) piperidine, CH_3COOH, reflux; (b) NaOH, ethol, reflux; (c) $SOCl_2$, reflux; (d) RH, K_2CO_3, reflux.

All compounds with benzoylpiperazine groups as substitutions, **33–37**, **47**–**50**, showed some anti-AChE activities (Table 1). It suggests that benzoylpiperazine groups are helpful for AChE inhibitory activities.

3. Conclusion

Three series of conformationally restricted coumarin derivatives by linking a substituted phenylpiperazine moiety to the coumarin backbone as potential inhibitors of AChE for curing PAD were designed and synthesized. Preliminary structure–activity relationships revealed that the substitutions on the positions 3 and/or 4 of coumarin ring are more helpful than that on the position 6 for inhibiting AChE. The stronger inhibitory activity of the target compound having smaller substitution on the phenylpiperazine proves that the AChE's active sites can apparently be reached only through a deep and narrow catalytic gorge.[11] So we can conclude that coumarins having phenylpiperazine substitution on the positions 3 and/or 4 with a suitable linking chain show significant anti-AChE activities. They can be considered as interesting inhibitors in the search of new therapies for curing AD. The study of their anti-AChE activities led to a novel family of potent anti-AChE ligands, which are coumarin analogues having substitution on positions 3 and/or 4.

4. Experimental

4.1. Chemistry

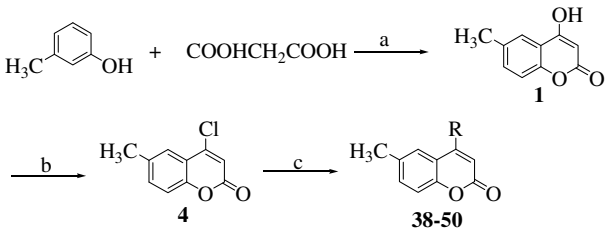
All purchased starting materials and reagents were used without further purification unless noted. Reaction progress was monitored using analytical thin layer chromatography (TLC) on precoated Merck silica gel Kiesegel 60 F254 plates, and the spots were detected under UV light (254 nm). The flash chromatography was conducted using silica gel 230–400 mesh. IR spectra were measured on a Jasco FT/IR-430 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded at 500 MHz on a Bruker ARX 300 spectrometer. The chemical shifts are reported downfield in ppm (δ) relative to internal TMS, and coupling constants are reported in Hertz (Hz). Splitting patterns describe apparent multiplicities, and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). Mass spectra analysis was performed on a Quattro microMS Micromass UK mass spectrometer, and was recorded on an electrospray ionization mass spectrometer as the value *m*/*z*. The HPLC spectra were recorded on a Agilent 1100 HPLC system equipped with an analytical ODS column (406 × 150 mm).

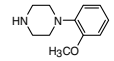
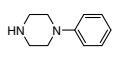
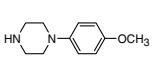
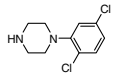
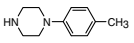
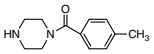
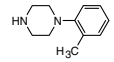
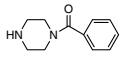
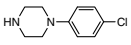
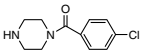
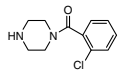
4.1.1. 4-Hydroxy-6-methylcoumarin (1)

The compound **1** was prepared by the classic synthetic way of 4-hydroxy coumarin. 0.07 mol *p*-cresol, 0.07 mol malonic acid and 30 g $ZnCl_2$ were dissolved in 20 ml $POCl_3$. The mixture was kept for 24 h at 60 °C. Then, the reaction mixture was poured into 250 ml ice water and deposited overnight. The precipitation was filtered and recrystallized in 5% ethanol to give **1**, 5.91 g, yield 48%.

4.1.2. 4-Methoxy-6-methyl coumarin (2)

The compound **2** was prepared with 0.02 mol **1** and 0.04 mol $(CH_3)_2SO_4$ dissolved in 200 ml acetone with 0.06 mol K_2CO_3. The mixture was refluxed for 24 h, and then the solvent was removed on vacuum. The residue was treated with flash silica gel (cyclohexane/acetone = 7:3) to give **2**, 3.30 g, yield 86.8%.



Compound	R	Compound	R
38		45	
39		46	
40		47	
41		48	
42		49	
43		50	
44			

Scheme 3. The preparation of series C. Reagents and conditions: (a) $ZnCl_2$, $POCl_3$, 60 °C; (b) $POCl_3$, reflux; (c) RH, K_2CO_3, reflux.

4.1.3. 6-Bromomethyl-4-methoxycoumarin (3)

0.003 mol **2** was added to 34 ml anhydrous CCl_4 with the reaction system protected by N_2. When the mixture was refluxed for 0.5 h, 0.0028 mol NBS (1-bromopyrrolidine-2,5-dione) and 100 mg BPO (methyl benzoperoxoate) were added to the reaction mixture. After the reaction mixture was refluxed for 5 h, the additional 100 mg BPO was added again, and the reaction continued for 3 h. The reaction mixture was cooled down to room temperature and filtered. The filtrate was evaporated under reduced pressure. The flash chromatography (cyclohexane/acetone = 7:3) was performed to give **3**, 0.32 g, yield 39.6%.

4.1.4. 4-Chloro-6-methylcoumarin (4)

0.005 mol **1** and 0.005 mol triethylamine were added to 5 ml $POCl_3$. The mixture was refluxed for 30 min, and the resultant was poured into water. Then the solution was extracted with methylene chloride. The methylene chloride layer was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. The flash chromatography (cyclohexane/acetone = 10:1) was performed to give **4**, 0.8 g, yield 82.3%.

4.1.5. 6-Bromomethyl-4-chlorocoumarin (5)

The compound **5** was prepared from 0.003 mol **4** and 0.003 mol NBS by the same way of synthesis of **3**. The target compound **5**, 0.22 g was obtained, yield 26.3%.

4.2. General procedure for the preparation of compounds 6–13

0.001 mol **3**, 0.001 mol substituted phenylpiperazine derivatives, and 0.002 mol potassium carbonate were added to a mixed solvent containing 15 ml acetone and 15 ml alcohol. The mixture was refluxed for 48 h, then the resultant was evaporated. The flash chromatography (EtOAc/hexane) was performed to give the target compounds, respectively.

4.2.1. 4-Methoxy-6-((4-(2-methylphenyl)piperazin-1-yl)methyl)coumarin (6)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **6**, yield 19%, colorless powder, mp 131–133 °C; MS (ESI) *m/z* 365.2 $(M+H)^+$; IR (KBr) γcm^{-1}: 2949, 1747, 1456, 1376, 1121, 770. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.78 (s, 1H, H-5), 7.57 (m, 1H, H-7), 7.29 (m, 1H, H-8), 7.14–7.17 (m, 2H, Ph), 6.95–7.03 (m, 2H, Ph), 5.69 (s, 1H, H-3), 4.00 (s, 3H, OCH_3), 3.62 (m, 2H, $N_1(CH_2)$), 2.94 (m, 4H, $N_4(CH_2)_2$), 2.62 (m, 4H, $N_1(CH_2)_2$), 2.29 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.9 (C-2), 90.1 (C-3), 166.4 (C-4), 126.5 (C-5), 133.4 (C-6), 131.0 (C-7), 123.1 (C-8), 152.5 (C-9), 115.3 (C-10), 56.3 (OCH_3), 62.4 (C-11), 53.6 (C-13), 51.7 (C-14), 53.6 (C-16), 51.7 (C-17), 151.4 (C-18), 118.9 (C-19), 126.5 (C-20), 116.7 (C-21), 123.2 (C-22), 123.1 (C-23), 17.8 (CH_3). HPLC analysis 96.4% (*V*(Methol): *V*(H_2O) = 65:35, t_R = 6.71 min).

4.2.2. 4-Methoxy-6-((4-*p*-tolylpiperazin-1-yl)methyl)coumarin (7)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **7**, yield 16%, colorless powder, mp 123–125 °C; MS (ESI) *m/z* 365.1 $(M+H)^+$; IR (KBr) γcm^{-1} 2959, 1756, 1610, 1513, 1456, 1260, 802. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.78 (s, 1H, H-5), 7.57 (d, 1H, H-7, *J* = 8.10 Hz), 7.27 (d, 1H, H-7, *J* = 8.46 Hz), 7.05–7.07 (m, 2H, Ph), 6.82–6.86 (m, 2H, Ph), 5.69 (s, 1H, H-3), 3.99 (s, 3H, OCH_3), 3.61 (m, 2H, $N_1(CH_2)$), 3.16 (m, 4H, $N_4(CH_2)_2$), 2.62 (m, 4H, $N_1(CH_2)_2$), 2.26 (s, 3H, CH_3). HPLC analysis 97.0% (*V*(Methol): *V*(H_2O) = 65:35, t_R = 9.67 min).

4.2.3. 4-Methoxy-6-((4-(4-methoxyphenyl)piperazin-1-yl)methyl)coumarin (8)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **8**, yield 20%, colorless powder, mp 126–128 °C; MS (ESI) *m/z* 381.1 $(M+H)^+$; IR (KBr) γcm^{-1} 2959, 1756, 1610, 1513, 1456, 1103, 802. ^1H NMR ($CDCl_3$, 500 MHz)δ 7.78 (s, 1H, H-5), 7.57 (d, 1H, H-7, *J* = 8.10 Hz), 7.27 (d, 1H, H-8, *J* = 8.46 Hz), 7.05–7.07 (m, 2H, Ph), 6.82–6.86 (m, 2H, Ph), 5.69 (s, 1H, H-3), 3.99 (s, 3H, OCH_3), 3.61 (m, 2H, $N_1(CH_2)$), 3.16 (m, 4H, $N_4(CH_2)_2$), 2.62 (m, 4H, $N_1(CH_2)_2$), 2.26 (s, 3H, CH_3). HPLC analysis 99.0% (*V*(Methol): *V*(H_2O) = 65:35, t_R = 4.35 min).

4.2.4. 4-Methoxy-6-((4-phenylpiperazin-1-yl)methyl)coumarin (9)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **9**, yield 20%, colorless powder, mp 136–137 °C; MS (ESI) *m/z* 351.2 $(M+H)^+$; IR (KBr) γcm^{-1} 2938, 1716, 1629, 1458, 1373, 934. ^1H NMR ($CDCl_3$, 500 MHz)δ 7.78 (s, 1H, H-5), 7.57 (d, 1H, H-7, *J* = 8.1 Hz), 7.24–7.31 (m, 3H, Ph, H-8), 6.85–6.93 (m, 3H, Ph), 5.69 (s, 1H, H-3), 3.99 (s, 3H, OCH_3), 3.61 (m, 2H, $N_1(CH_2)$), 3.22 (m, 4H, $N_4(CH_2)_2$), 2.62 (m, 4H, $N_1(CH_2)_2$). HPLC analysis 97.6% (*V*(Methol): *V*(H_2O) = 65:35, t_R = 7.92 min).

4.2.5. 4-Methoxy-6-((4-(4-chlorophenyl)piperazin-1-yl)methyl)-coumarin (10)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **10**, yield 14%, colorless powder, mp 130–132 °C; MS (ESI) *m/z* 384.7 $(M+H)^+$; IR (KBr) γcm^{-1} 2823, 1709, 1603, 1379, 1248, 1217, 821. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.77 (s, 1H, H-5), 7.57 (m, 1H, H-7), 7.28 (m, 1H, H-8), 7.17–7.20 (m, 2H, Ph), 6.80–6.84 (m, 2H, Ph), 5.69 (s, 1H, H-3), 3.99 (s, 3H, OCH_3), 3.60 (m, 2H, $N_1(CH_2)$), 3.17 (m, 4H, $N_4(CH_2)_2$), 2.62 (m, 4H, $N_1(CH_2)_2$), 2.29 (s, 3H, CH_3). HPLC analysis 95.6% (*V*(Methol): *V*(H_2O) = 65:35, t_R = 12.45 min).

4.2.6. 4-Methoxy-6-((4-(2-methoxyphenyl)piperazin-1-yl)methyl)coumarin (11)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **11**, yield 20%, colorless powder, mp

Table 1
Inhibition of AchE activities of the synthesized compounds

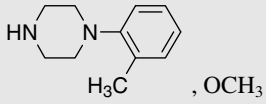
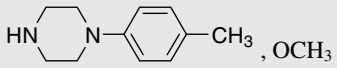
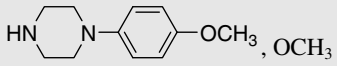
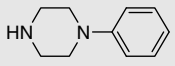
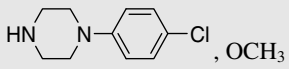
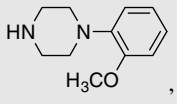
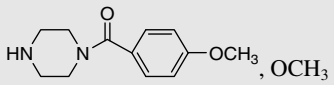
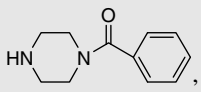
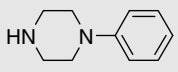
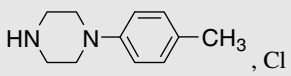
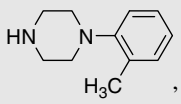
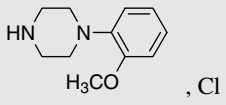
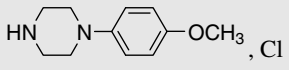
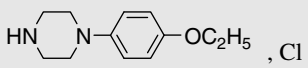
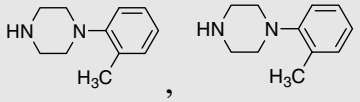
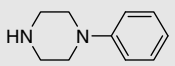
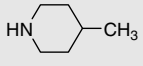
Compound	Series	R	AchE inhibition (IC_{50} ± SD)[a] (μmol/L)
6	A		Inactive
7	A		Inactive
8	A		Inactive
9	A		Inactive
10	A		Inactive
11	A		Inactive
12	A		Inactive
13	A		Inactive
14	A		19 ± 0.1
15	A		Inactive
16	A		Inactive
17	A		Inactive
18	A		Inactive
19	A		Inactive
20	A		Inactive
24	B		**9.3 ± 0.05**
25	B		97 ± 0.2

Table 1 (*continued*)

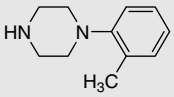
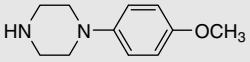
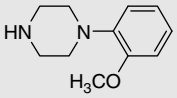
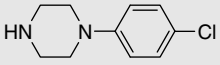
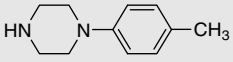
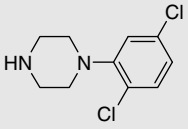
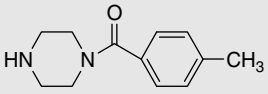
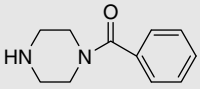
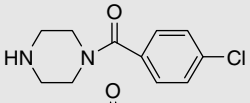
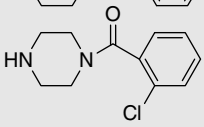
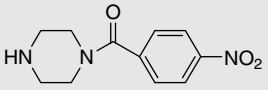
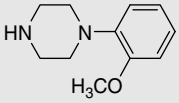
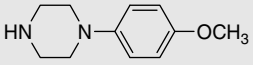
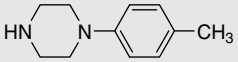
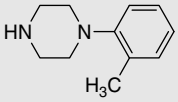
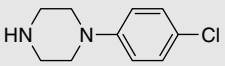
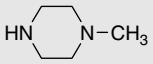
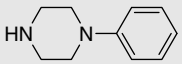
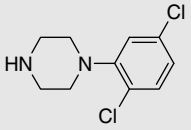
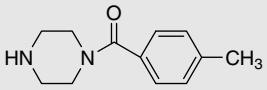
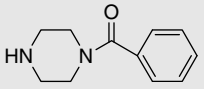
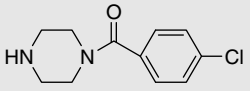
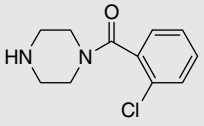
Compound	Series	R	AchE inhibition (IC_{50} ± SD)[a] (μmol/L)
26	B		101 ± 0.1
27	B		**6.7 ± 0.02**
28	B		**7.5 ± 0.03**
29	B		11 ± 0.05
30	B		98 ± 0.1
31	B		**7.6 ± 0.04**
32	B		14 ± 0.04
33	B		18 ± 0.02
34	B		15 ± 0.03
35	B		21 ± 0.1
36	B		26 ± 0.2
37	B		34 ± 0.3
38	C		249 ± 0.2
39	C		18 ± 0.2
40	C		61 ± 0.03
41	C		**7.9 ± 0.02**
42	C		808 ± 0.1

Table 1 (*continued*)

Compound	Series	R	AchE inhibition (IC_{50} ± SD)[a] (μmol/L)
43	C		901 ± 0.1
44	C		875 ± 0.3
45	C		**4.5 ± 0.03**
46	C		827 ± 0.1
47	C		**5.3 ± 0.02**
48	C		**7.4 ± 0.04**
49	C		16 ± 0.2
50	C		21 ± 0.1
Donepezil			0.11 ± 0.01

The bold font stands for that the corresponding compounds exhibited inhibitory some activities toward the cholinesterase.

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

128–130 °C; MS (ESI) *m/z* 381.2 $(M+H)^+$; IR (KBr) γcm^{-1} 3075, 1707, 1629, 1604, 1575, 1456, 1378, 986. ^1H NMR ($CDCl_3$, 500 MHz)δ 7.78 (s, 1H, H-5), 7.57 (d, 1H, H-7, J = 8.10 Hz), 7.28 (m, 1H, H-8), 6.85–6.99 (m, 4H, Ph), 5.69 (s, 1H, H-3), 3.99 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.63 (m, 2H, $N_1(CH_2)$), 3.11 (m, 4H, $N_4(CH_2)_2$), 2.67 (m, 4H, $N_1(CH_2)_2$), 2.26 (s, 3H, CH_3). HPLC analysis 96.1% (V(Methol): $V(H_2O)$ = 65:35, t_R = 5.65 min).

4.2.7. 4-Methoxy-6-((4-(4-methoxybenzoyl)piperazin-1-yl)methyl)coumarin (12)

Purified by flash silica gel column chromatography (EtOAc:hexane = 1:5) to afford compound **12**, yield 21%, colorless powder, mp 124–126 °C; MS (ESI) *m/z* 409.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3075, 1707, 1629, 1604, 1575, 1456, 1378. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.99 (s, 1H, H-5), 7.51 (br d, 1H, H-7), 7.23 (m, 1H, H-8), 7.11–7.23 (m, 4H, Ph), 5.63 (s, 1H, H-3), 3.98 (s, 3H, OCH_3), 2.37 (s, 3H, CH_3), 3.63 (m, 2H, $N_1(CH_2)$), 3.54 (m, 6H, $N_4(CH_2)_2$, CH_2), 2.56 (m, 4H, $N_1(CH_2)_2$). HPLC analysis 96.2% (V(Methol): $V(H_2O)$ = 65:35, t_R = 6.06 min).

4.2.8. 4-Methoxy-6-((4-benzoylpiperazin-1-yl)methyl)coumarin (13)

Purified by flash silica gel column chromatography (EtOAc:hexane = 1:5) to afford compound **13**, yield 16%, colorless powder, mp 131–134 °C; MS (ESI) *m/z* 379.2 $(M+H)^+$; IR (KBr) γcm^{-1} 3075, 1707, 1629, 1604, 1378, 1249, 986. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.73 (s, 1H, H-5), 7.53 (d, 1H, H-7, J = 9.00 Hz), 7.26 (m, 1H, H-8), 7.38–7.40 (m, 5H, Ph), 5.69 (s, 1H, H-3), 3.99 (s, 3H, OCH_3), 3.57 (m, 2H, $N_1(CH_2)$), 3.79 (m, 2H, $N_4(CH_2)$), 3.44 (m, 2H, $N_4(CH_2)$), 2.53 (m, 4H, $N_1(CH_2)_2$). HPLC analysis 97.6% (V(Methol): $V(H_2O)$ = 65:35, t_R = 6.48 min).

4.3. General procedure for the preparation of compounds 14–20

0.001 mol **5**, 0.001 mol substituted phenylpiperazine derivatives, and 0.002 mol potassium carbonate were added to a mixed solvent containing 15 ml acetone and 15 ml alcohol. The mixture was refluxed for 48 h, and then the resultant was evaporated. The flash chromatography (EtOAc/hexane) was performed to give the target compounds, respectively.

4.3.1. 4-Chloro-6-((4-phenylpiperazin-1-yl)methyl)coumarin (14)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **14**, yield 10%, colorless powder, mp 113–115 °C; MS (ESI) *m/z* 355.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3440, 1726, 1500, 1454, 1241, 921, 753. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.84 (s, 1H, H-5), 7.64 (m, 1H, H-7), 7.34 (m, 1H, H-8), 7.24–7.27 (m, 2H, Ph), 6.86–6.93 (m, 3H, Ph), 6.61 (s, 1H, H-3), 3.65 (m, 2H, $N_1(CH_2)$), 3.22 (m, 4H, $N_4(CH_2)_2$), 2.64 (m, 4H, $N_1(CH_2)_2$). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.0 (C-2), 115.4 (C-3), 152.2 (C-4), 125.5 (C-5), 134.0 (C-6), 129.1 (C-7), 119.8 (C-8), 151.2 (C-9), 129.1 (C-10), 61.9 (C-11), 53.1 (C-13), 49.1 (C-14), 53.1 (C-16), 49.1 (C-17), 149.6 (C-18), 117.8 (C-19), 129.1 (C-20), 117.0 (C-21), 129.1 (C-22), 116.1 (C-23). HPLC analysis 97.0% (V(Methol): $V(H_2O)$ = 65:35, t_R = 6.78 min).

4.3.2.4-Chloro-6-((4-*p*-tolylpiperazin-1-yl)methyl)coumarin (15)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **15**, yield 10%, colorless powder, mp 127–129 °C; MS (ESI) *m/z* 369.2 $(M+H)^+$; IR (KBr) γcm^{-1} 3087, 1778, 1614, 1571, 1290, 1240, 1223, 812. ^1H NMR ($CDCl_3$, 500 MHz) δ7.84 (s, 1H, H-5), 7.65 (d, 1H, H-7, J = 8.10 Hz), 7.34 (d, 1H, H-8, J = 8.45 Hz), 7.06–7.08 (m, 2H, Ph), 6.82–6.85 (m, 2H, Ph), 6.61 (s, 1H, H-3), 3.65 (m, 2H, $N_1(CH_2)$), 3.17 (m, 4H, $N_4(CH_2)_2$), 2.64 (m, 4H, $N_1(CH_2)_2$), 2.26 (s, 3H, CH_3). HPLC analysis 96.7% (V(Methol): $V(H_2O)$ = 65:35, t_R = 13.78 min).

4.3.3. 4-Chloro-6-((4-*o*-tolylpiperazin-1-yl)methyl)coumarin (16)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **16**, yield 13%, colorless powder, mp 103–105 °C; MS (ESI) *m/z* 369.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3054, 2818, 1724, 1615, 1454, 1354, 1011, 843. ^1H NMR ($CDCl_3$, 500 MHz) δ7.85 (s, 1H, H-5), 7.68 (m, 1H, H-7), 7.34 (m, 1H, H-8), 7.15–7.26 (m, 2H, Ph), 6.62–7.04 (m, 2H, Ph), 6.61 (s, 1H, H-3), 3.67 (m, 2H, $N_1(CH_2)$), 3.19 (m, 4H, $N_4(CH_2)_2$), 2.65 (m, 4H, $N_1(CH_2)_2$), 2.29 (s, 3H, CH_3). Crystal data: empirical formula, $C_{21}H_{21}Cl_1N_2O_2$; molecular weight, 368.85; crystal dimensions, 0.50 × 0.48 × 0.27 mm; triclinic; $P\bar{1}$; a = 6.010(2) Å, b = 11.126(3) Å, c = 13.829(3) Å; α = 86.026(3)°, β = 87.900(3)°, γ = 85.155(2)°; V = 918.7(5) $Å^3$; Z = 2; D_x = 1.333 mg m^{-3}; R_1 = 0.0462; ωR_2 = 0.1104; GOOF = 1.019; MoKα radiation cell parameters from 3199 reflections; θ = 1.48–25.01°; μ = 0.226 mm^{-1}; T = 298(2) K. HPLC analysis 97.3% (V(Methol): $V(H_2O)$ = 65:35, t_R = 6.31 min).

4.3.4. 4-Chloro-6-((4-(2-methoxyphenyl)piperazin-1-yl)methyl)-coumarin (17)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **17**, yield 12%, colorless powder, mp 116–118 °C; MS (ESI) *m/z* 385.2 $(M+H)^+$; IR (KBr) γcm^{-1} 3440, 1727, 1614, 1570, 1121, 1012, 921. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.84 (s, 1H, H-5), 7.68 (m, 1H, H-7), 7.34 (m, 1H, H-8), 6.85–6.98

(m, 4H, Ph), 6.61 (s, 1H, H-3), 3.86 (s, 3H, OCH_3), 3.67 (m, 2H, $N_1(CH_2)$), 3.11 (m, 4H, $N_4(CH_2)_2$), 2.68 (m, 4H, $N_1(CH_2)_2$). HPLC analysis 95.3% (V(Methol): $V(H_2O)$ = 65:35, t_R = 8.04 min).

4.3.5. 4-Chloro-6-((4-(4-methoxyphenyl)piperazin-1-yl)methyl)-coumarin (18)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **18**, yield 8%, colorless powder, mp 121–124 °C; MS (ESI) m/z 385.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3090, 2950, 1779, 1512, 1180, 1142, 831. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.84 (s, 1H, H-5), 7.65 (d, 1H, H-7, J = 8.17 Hz), 7.34 (d, 1H, H-8, J = 8.46 Hz), 6.82–6.91 (m, 4H, Ph), 6.61 (s, 1H, H-3), 3.76 (s, 3H, OCH_3), 3.65 (m, 2H, $N_1(CH_2)$), 3.11 (m, 4H, $N_4(CH_2)_2$), 2.64 (m, 4H, $N_1(CH_2)_2$). HPLC analysis 96.5% (V(Methol): $V(H_2O)$ = 65:35, t_R = 8.18 min).

4.3.6. 4-Chloro-6-((4-(4-ethoxyphenyl)piperazin-1-yl)methyl)-coumarin (19)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **19**, yield 19%, colorless powder, mp 121–123 °C; MS (ESI) m/z 399.1 $(M+H)^+$; IR (KBr) γcm^{-1} 2939, 1717, 1563, 1342, 1323, 1300, 1198. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.65 (s, 1H, H-5), 7.54 (m, 1H, H-7), 7.49 (m, 1H, H-8), 7.45 (m, 1H, Ph), 7.18–7.20 (m, 2H, Ph), 6.81–6.84 (m, 2H, Ph, H-3), 4.45 (q, 2H, OCH_2, J = 7.13 Hz), 3.65 (m, 2H, $N_1(CH_2)$), 3.17 (m, 4H, $N_4(CH_2)_2$), 2.64 (m, 4H, $N_1(CH_2)_2$), 1.43 (t, 3H, CH_3, J = 7.13 Hz). HPLC analysis 95.9% (V(Methol): $V(H_2O)$ = 65:35, t_R = 9.67 min).

4.3.7. 4-(4-*o*-Tolylpiperazin-1-yl)-6-((4-*o*-tolylpiperazin-1-yl)-methyl)coumarin (20)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:3) to afford compound **20**, yield 8%, colorless powder, mp 169–171 °C; MS (ESI) m/z 509.3 $(M+H)^+$; IR (KBr) γcm^{-1} 2952, 1715, 1617, 1433, 1371, 1028, 765. ^1H NMR ($CDCl_3$, 500 MHz)δ 7.69 (s, 1H, H-5), 7.51 (m, 1H, H-7), 7.32 (m, 1H, H-8), 6.96–7.23 (m, 8H, Ph), 5.79 (s, 1H, 3-H), 3.65 (m, 2H, $N_1(CH_2)$), 3.18 (m, 4H, $N_4(CH_2)_2$), 2.64 (m, 4H, $N_1(CH_2)_2$), 3.44 (m, 4H, $N'_1(CH_2)_2$), 3.18 (m, 4H, $N'_4(CH_2)_2$), 2.36 (s, 3H, CH_3), 2.29 (s, 3H, CH_3). HPLC analysis 97.4% (V(Methol): $V(H_2O)$ = 65:35, t_R = 2.16 min).

4.3.8. Ethyl-2-oxo-2H-chromene-3-carboxylate (21)

0.005 mol salicylaldehyde, 0.005 mol malonic acid, 0.05 ml piperidine, and 0.005 ml glacial acetic acid were added to 20 ml anhydrous alcohol. The mixture was refluxed for 3 h, and then the resultant was poured into 50 ml hot water. The water was cooled down to room temperature, and the residue was filtered and recrytallized with 95% alcohol to give **21**, 0.7 g, yield 64.3%.

4.3.9. 2-Oxo-2H-chromene-3-carboxylic acid (22)

0.02 mol **21** and 250 ml of 0.5% NaOH solution were added to 50 ml alcohol. The mixture was refluxed for 2 h, and then it was acidified with HCl to pH 2. The mixture was cooled down to 0 °C and crystallized with ethanol to give **22**, 3.29 g, yield 86.7%.

4.3.10. 2-Oxo-2H-chromene-3-carbonyl chloride (23)

0.002 mol **22** was added to 5 ml sulfurous oxychloride. The mixture was refluxed for 3 h, and then the resultant was removed with simple distillation to give simple **23**, 3.95 g. The simple compound can be used directly without purification.

4.4. General procedure for the preparation of compounds 24–37

0.001 mol **23**, 0.001 mol substituted phenylpiperazine derivatives, and 0.002 mol potassium carbonate were added to a mixed solvent containing 15 ml acetone and 15 ml alcohol. The mixture was refluxed for 6 h, then the resultant was evaporated. The flash chromatography (EtOAc/hexane) was performed to give the target compounds, respectively.

4.4.1. 3-(4-Phenylpiperazin-1-carbonyl)coumarin (24)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **24**, yield 22%, colorless powder, mp 232–234 °C; MS (ESI) m/z 335.1 $(M+H)^+$; IR (KBr) γcm^{-1} 2831, 1718, 1469, 1234, 754. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.00 (s, 1H, H-4), 7.64 (m, 1H, H-7), 7.58 (m, 1H, H-5), 7.41 (m, 1H, H-8), 7.32 (m, 1H, H-6), 3.99 (m, 2H, $N_4(CH_2)$), 3.60 (m, 2H, $N_4(CH_2)$), 3.33 (m, 2H, $N_1(CH_2)$), 3.26 (m, 2H, $N_1(CH_2)$). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.9 (C-2), 116.8 (C-3), 132.9 (C-4), 125.2 (C-5), 124.9 (C-6), 128.6 (C-7), 118.4 (C-8), 154.2 (C-9), 120.7 (C-10), 163.5 (C-11), 47.1 (C-13), 49.5 (C-14), 49.8 (C-16), 42.1 (C-17), 143.4 (C-18), 116.8 (C-19), 129.3 (C-20), 116.9 (C-21), 129.3 (C-22), 116.9 (C-23). HPLC analysis 97.3% (V(Methol): $V(H_2O)$ = 65:35, t_R = 3.36 min).

4.4.2. 3-(4-Methylpiperazin-1-carbonyl)coumarin (25)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **25**, yield 28%, colorless powder, mp 220–222 °C; MS (ESI) m/z 273.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3423, 1726, 1622, 1476, 1285, 1127, 762. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.90 (s, 1H, H-4), 7.58 (ddd, 1H, H-7, J = 1.59 Hz, 7.37, 1.18 Hz), 7.53 (dd, 1H, H-5, J = 1.53, 7.63 Hz), 7.35 (m, 1H, H-8), 7.31 (ddd, 1H, H-6, J = 1.49, 7.53, 1.85 Hz), 3.79 (m, 2H, $N_4(CH_2)$), 3.41 (m, 2H, $N_4(CH_2)$), 2.51 (m, 2H, $N_1(CH_2)$), 2.45 (m, 2H, $N_1(CH_2)$), 2.33 (s, 3H, CH_3). HPLC analysis 99.3% (V(Methol): $V(H_2O)$ = 65:35, t_R = 2.42 min).

4.4.3. 3-(Morpholino-4-carbonyl)coumarin (26)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **26**, yield 29%, colorless powder, mp 180–182 °C; MS (ESI) m/z 260.0 $(M+H)^+$; IR (KBr) γcm^{-1} 3424, 1721, 1646, 1270, 1238, 1109, 1065, 995, 773. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.95 (s, 1H, H-4), 7.58 (m, 1H, H-7), 7.54 (m, 1H, H-5), 7.33–7.37 (m, 2H, H-6, H-8), 3.79 (m, 2H, OCH_2), 3.72 (m, 2H, $N_1(CH_2)$), 3.41 (m, 2H, $N_1(CH_2)$). HPLC analysis 98.1% (V(Methol): $V(H_2O)$ = 65:35, t_R = 3.63 min).

4.4.4. 3-(4-*o*-Tolylpiperazine-1-carbonyl)coumarin (27)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **27**, yield 25%, colorless powder, mp 164–166 °C; MS (ESI) m/z 349.2 $(M+H)^+$; IR (KBr) γcm^{-1} 2821, 1726, 1631, 1369, 1243, 994, 724. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.95 (s, 1H, H-4), 7.55–7.59 (m, 2H, H-7, H-5), 7.33–7.37 (m, 2H, H-6, H-8), 7.00–7.18 (m, 4H, Ph), 3.93 (m, 2H, $N_4(CH_2)$), 3.55 (m, 2H, $N_4(CH_2)$), 2.99 (m, 2H, $N_1(CH_2)$), 2.94 (m, 2H, $N_1(CH_2)$), 2.32 (s, 3H, CH_3). HPLC analysis 96.3% (V(Methol): $V(H_2O)$ = 65:35, t_R = 2.62 min).

4.4.5. 3-(4-(4-Methoxyphenyl)piperazine-1-carbonyl)coumarin (28)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **28**, yield 23%, colorless powder, mp 140–142 °C; MS (ESI) m/z 365.2 $(M+H)^+$; IR (KBr) γcm^{-1} 2918, 1716, 1636, 1446, 1243, 1036, 763. ^1H NMR ($CDCl_3$, 500 MHz) δ7.95 (s, 1H, H-4), 7.60 (m, 1H, H-7), 7.55 (m, 1H, H-5), 7.33–7.38 (m, 2H, H-6, H-8), 6.91 (m, 2H, Ph), 6.84 (m, 2H, Ph), 3.93 (m, 2H, $N_4(CH_2)$), 3.55 (m, 2H, $N_4(CH_2)$), 3.16 (m, 2H, $N_1(CH_2)$), 3.09 (m, 2H, $N_1(CH_2)$), 3.77 (s, 3H, OCH_3). HPLC analysis 95.3% (V(Methol): $V(H_2O)$ = 65:35, t_R = 2.63 min).

4.4.6. 3-(4-(2-Methoxyphenyl)piperazine-1-carbonyl)coumarin (29)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **29**, yield 29%, colorless powder, mp 146–147 °C; MS (ESI) m/z 365.1 $(M+H)^+$; IR (KBr) γcm^{-1} 2944, 2904, 1738, 1633, 1237, 930, 753. ^1H NMR ($CDCl_3$, 500 MHz) δ

7.95 (s, 1H, H-4), 7.54–7.59 (m, 2H, H-7, H-5), 7.33–7.37 (m, 2H, H-6, H-8), 6.88–6.93 (m, 4H, Ph), 3.97 (m, 2H, $N_4(CH_2)$), 3.59 (m, 2H, $N_4(CH_2)$), 3.16 (m, 2H, $N_1(CH_2)$), 3.11 (m, 2H, $N_1(CH_2)$), 3.87 (s, 3H, OCH_3). HPLC analysis 96.3% (*V*(Methol): *V*(H_2O) = 65:35, t_R = 2.63 min).

4.4.7. 3-(4-(4-Chlorophenyl)piperazine-1-carbonyl)coumarin (30)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **30**, yield 23%, colorless powder, mp 128–130 °C; MS (ESI) *m/z* 369.2 $(M+H)^+$; IR (KBr) γcm^{-1} 3425, 1718, 1635, 1443, 1284, 997, 692. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.96 (s, 1H, H-4), 7.54–7.60 (m, 2H, H-7, H-5), 7.33–7.38 (m, 2H, H-6, H-8), 7.26–7.30 (m, 2H, Ph), 6.91–6.95 (m, 2H, Ph), 3.94 (m, 2H, $N_4(CH_2)$), 3.56 (m, 2H, $N_4(CH_2)$), 3.29 (m, 2H, $N_1(CH_2)$), 3.22 (m, 2H, $N_1(CH_2)$). HPLC analysis 96.4% (*V*(Methol): *V*(H_2O) = 65:35, t_R = 2.92 min).

4.4.8. 3-(4-(4-Methylphenyl)piperazine-1-carbonyl)coumarin (31)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **31**, yield 22%, colorless powder, mp 156–157 °C; MS (ESI) *m/z* 349.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3030, 2825, 1726, 1630, 1476, 1151, 996, 970, 754. ^1H NMR ($CDCl_3$, 500 MHz) δ7.95 (s, 1H, H-4), 7.54–7.59 (m, 2H, H-7, H-5), 7.32–7.37 (m, 2H, H-6, H-8), 6.88–7.32 (m, 4H, Ph), 3.94 (m, 2H, $N_4(CH_2)$), 3.56 (m, 2H, $N_4(CH_2)$), 3.23 (m, 2H, $N_1(CH_2)$), 3.17 (m, 2H, $N_1(CH_2)$), 2.28 (s, 3H, CH_3). HPLC analysis 98.3% (*V*(Methol): *V*(H_2O) = 65:35, t_R = 2.63 min).

4.4.9. 3-(4-(2,5-Dichlorophenyl)piperazine-1-carbonyl)coumarin (32)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **32**, yield 18%, colorless powder, mp 104–106 °C; MS (ESI) *m/z* 403.0 $(M+H)^+$; IR (KBr) γcm^{-1} 3421, 1722, 1639, 1363, 1231, 995, 751. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.97 (s, 1H, H-4), 7.61 (m, 1H, H-7), 7.56 (m, 1H, H-5), 7.37 (m, 1H, H-8), 7.34 (m, 1H, H-6), 7.29 (m, 1H, Ph), 7.05 (m, 1H, Ph), 6.99 (m, 1H, Ph), 3.98 (m, 2H, $N_4(CH_2)$), 3.59 (m, 2H, $N_4(CH_2)$), 3.16 (m, 2H, $N_1(CH_2)$), 3.13 (m, 2H, $N_1(CH_2)$). HPLC analysis 98.2% (*V*(Methol): *V*(H_2O) = 65:35, t_R = 3.36 min).

4.4.10. 3-(4-(4-Methylbenzoyl)piperazine-1-carbonyl)coumarin (33)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **33**, yield 13%, colorless powder, mp 92–94 °C; MS (ESI) *m/z* 377.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3436, 1727, 1627, 1286, 1711, 1050, 751. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.96 (s, 1H, H-4), 7.59 (m, 1H, H-7), 7.55 (m, 1H, H-5), 7.33–7.37 (m, 2H, H-6, H-8), 7.32–7.37 (m, 2H, Ph), 7.21–7.30 (m, 2H, Ph), 3.41–3.77 (m, 8H, $N_1(CH_2)_2$, $N_4(CH_2)_2$), 2.37 (s, 3H, CH_3). HPLC analysis 96.3% (*V*(Methol): *V*(H_2O) = 65:35, t_R = 4.54 min).

4.4.11. 3-(4-Benzoylpiperazine-1-carbonyl)coumarin (34)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **34**, yield 19%, colorless powder, mp 110–112 °C; MS (ESI) *m/z* 363.0 $(M+H)^+$; IR (KBr) γcm^{-1} 3427, 1720, 1627, 1429, 1245, 1172, 1009, 563. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.96 (s, 1H, H-4), 7.60 (m, 1H, H-7), 7.54 (m, 1H, H-5), 7.31–7.41 (m, 7H, H-6, H-8, Ph), 3.42–3.78 (m, 8H, $N_1(CH_2)_2$, $N_4(CH_2)_2$). HPLC analysis 97.9% (*V*(Methol): *V*(H_2O) = 65:35, t_R = 6.34 min).

4.4.12. 3-(4-(4-Chlorobenzoyl)piperazine-1-carbonyl)coumarin (35)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **35**, yield 12%, colorless powder, mp 128–130 °C; MS (ESI) *m/z* 397.0 $(M+H)^+$; IR (KBr) γcm^{-1} 3034, 1719, 1629, 1246, 1172, 748. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.97 (s, 1H, H-4), 7.60 (m, 1H, H-7), 7.55 (m, 1H, H-5), 7.26–7.41 (m, 6H, H-6, H-8, Ph), 3.42–3.78 (m, 8H, $N_1(CH_2)_2$, $N_4(CH_2)_2$). HPLC analysis 97.5% (*V*(Methol): *V*(H_2O) = 65:35, t_R = 3.16 min).

4.4.13. 3-(4-(2-Chlorobenzoyl)piperazine-1-carbonyl)coumarin (36)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **36**, yield 16%, colorless powder, mp 200–203 °C; MS (ESI) *m/z* 397.0 $(M+H)^+$; IR (KBr) γcm^{-1} 3041, 1705, 1641, 1434, 1290, 1241, 1175. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.97 (s, 1H, H-4), 7.56 (m, 1H, H-7), 7.40 (m, 1H, H-5), 7.26–7.38 (m, 6H, H-6, H-8, Ph), 3.32–4.07 (m, 8H, $N_1(CH_2)_2$, $N_4(CH_2)_2$). HPLC analysis 98.1% (*V*(Methol): *V*(H_2O) = 65:35, t_R = 2.76 min).

4.4.14. 3-(4-(4-Nitrobenzoyl)piperazine-1-carbonyl)coumarin (37)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:5) to afford compound **37**, yield 25%, colorless powder, mp 224–226 °C; MS (ESI) *m/z* 408.0 $(M+H)^+$; IR (KBr) γcm^{-1} 3433, 1710, 1633, 1348, 1286, 1241, 1175, 1093, 840. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.30 (m, 2H, Ph), 7.99 (s, 1H, H-4), 7.56–7.64 (m, 4H, H-5, H-7, Ph), 7.34–7.38 (m, 2H, H-6, H-8), 3.36–3.91 (m, 8H, $N_1(CH_2)_2$, $N_4(CH_2)_2$). HPLC analysis 97.7% (*V*(Methol): *V*(H_2O) = 65:35, t_R = 3.45 min).

4.5. General procedure for the preparation of compounds 38–50

0.001 mol **4**, 0.001 mol substituted phenylpiperazine derivatives, and 0.002 mol potassium carbonate were added to a mixed solvent of 15 ml acetone and 15 ml alcohol. The mixture was refluxed for 10 h, and then the resultant was evaporated. The flash chromatography (EtOAc/hexane) was performed to give the target compounds, respectively.

4.5.1. 6-Methyl-4-(4-(2-methoxyphenyl)piperazin-1-yl)coumarin (38)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **38**, yield 19%, colorless powder, mp 131–133 °C; MS (ESI) *m/z* 351.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3441, 2832, 1713, 1596, 1376, 1242, 1029, 936. ^1H NMR ($CDCl_3$, 500 MHz)δ 7.46 (s, 1H, H-5), 7.33 (m, 1H, H-7), 7.28 (m, 1H, 8-H), 6.94–7.09 (m, 4H, Ph), 5.80 (s, 1H, H-3), 3.49 (m, 4H, $N_4(CH_2)_2$), 3.36 (m, 4H, $N_1(CH_2)_2$), 3.93 (s, 3H, OCH_3), 2.45 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.6 (C-2), 98.2 (C-3), 161.2 (C-4), 124.5 (C-5), 132.5 (C-6), 123.7 (C-7), 121.2 (C-8), 152.4 (C-9), 116.1 (C-10), 21.1 (CH_3), 51.3 (C-13), 50.3 (C-14), 51.3 (C-16), 50.3 (C-17), 152.4 (C-18), 132.9 (C-19), 111.7 (C-20), 117.6 (C-21), 118.6 (C-22), 55.9 (C-23), 55.6 (OCH_3). HPLC analysis 98.3% (*V*(Methol): *V*(H_2O) = 70:30 t_R = 8.52 min).

4.5.2. 6-Methyl-4-(4-(4-methoxyphenyl)piperazin-1-yl)coumarin (39)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **39**, yield 17%, colorless powder, mp 146–148 °C; MS (ESI) *m/z* 351.2 $(M+H)^+$; IR (KBr) γcm^{-1} 3441, 2829, 1696, 1561, 1374, 1236, 1030, 938, 823. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.43 (s, 1H, H-5), 7.32 (m, 1H, H-7), 7.25 (m, 1H, 8-H), 6.92–6.99 (m, 4H, Ph), 5.78 (s, 1H, H-3), 3.48 (m, 4H, $N_4(CH_2)_2$), 3.34 (m, 4H, $N_1(CH_2)_2$), 3.91 (s, 3H, OCH_3), 2.42 (s, 3H, CH_3). HPLC analysis 99.1% (*V*(Methol): *V*(H_2O) = 70:30, t_R = 4.81 min).

4.5.3. 6-Methyl-4-(4-(4-methylphenyl)piperazin-1-yl)coumarin (40)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **40**, yield 15%, colorless powder, mp 126–128 °C; MS (ESI) *m/z* 335.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3448,

1688, 1557, 1516, 1425, 1384, 1238, 1204, 937, 840. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.45 (s, 1H, H-5), 7.36 (m, 1H, H-7), 7.29 (m, 1H, H-8), 7.16–7.17 (m, 2H, Ph), 6.96–6.97 (m, 2H, Ph), 5.81 (s, 1H, H-3), 3.41–3.44 (m, 8H, $N_1(CH_2)_2$, $N_4(CH_2)_2$), 2.46 (s, 3H, CH_3), 2.34 (s, 3H, CH_3). HPLC analysis 96.3% (*V*(Methol): *V*(H_2O) = 70:30, t_R = 4.71 min).

4.5.4. 6-Methyl-4-(4-*o*-tolylpiperazin-1-yl)coumarin (41)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **41**, yield 16%, colorless powder, mp 132–134 °C; MS (ESI) *m/z* 335.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3431, 2831, 1713, 1596, 1375, 1242, 1212, 936, 816. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.48 (s, 1H, H-5), 7.35 (m, 1H, H-7), 7.29 (m, 1H, 8-H), 7.24–7.27 (m, 2H, Ph), 7.17 (m, 1H, Ph), 7.09 (m, 1H, Ph), 5.81 (s, 1H, H-3), 3.47 (m, 4H, $N_4(CH_2)_2$), 3.21 (m, 4H, $N_1(CH_2)_2$), 2.46 (s, 3H, CH_3), 2.41 (s, 3H, CH_3). HPLC analysis 97.1% (*V*(Methol): *V*(H_2O) = 70:30, t_R = 2.15 min).

4.5.5. 6-Methyl-4-(4-(4-chlorophenyl)piperazin-1-yl)coumarin (42)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **42**, yield 19%, colorless powder, mp 133–135 °C; MS (ESI) *m/z* 355.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3443, 2835, 1709, 1596, 1426, 1379, 1023, 938. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.44 (s, 1H, H-5), 7.36 (m, 1H, H-7), 7.28–7.31 (m, 3H, H-8, Ph), 6.95–6.97 (m, 2H, Ph), 5.81 (s, 1H, H-3), 3.42–3.45 (m, 8H, $N_1(CH_2)_2$, $N_4(CH_2)_2$), 2.46 (s, 3H, CH_3). HPLC analysis 96.5% (*V*(Methol): *V*(H_2O) = 70:30, t_R = 2.43 min).

4.5.6. 6-Methyl-4-(4-methylpiperazin-1-yl)coumarin (43)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **43**, yield 17%, colorless powder, mp 126-129 °C; MS (ESI) *m/z* 259.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3084, 2930, 1695, 1613, 1371, 1240, 1101, 1006, 934, 833. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36 (s, 1H, H-5), 7.21–7.30 (m, 2H, H-7, H-8), 5.71 (s, 1H, H-3), 3.29 (m, 4H, $N_1(CH_2)_2$), 2.66 (m, 4H, $N_4(CH_2)_2$), 2.41 (s, 6H, 2 × CH_3). HPLC analysis 96.2% (*V*(Methol): *V*(H_2O) = 70:30, t_R = 5.12 min).

4.5.7. 6-Methyl-4-morpholino-coumarin (44)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **44**, yield 23%, colorless powder, mp 106–108 °C; MS (ESI) *m/z* 246.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3441, 2964, 1693, 1562, 1448, 1424, 1202, 948. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36 (s, 1H, H-5), 7.31 (m, 1H, H-7), 7.24 (m, 1H, H-8), 5.71 (s, 1H, H-3), 3.94 (m, 4H, $N_1(CH_2)_2$), 3.24 (m, 4H, $N_4(CH_2)_2$), 2.42 (s, 3H, CH_3). HPLC analysis 97.6% (*V*(Methol): *V*(H_2O) = 70:30, t_R = 3.52 min).

4.5.8. 6-Methyl-4-(4-phenylpiperazin-1-yl)coumarin (45)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **45**, yield 15%, colorless powder, mp 125–127 °C; MS (ESI) *m/z* 321.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3432, 1710, 1596, 1560, 1203, 1024, 939. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41 (s, 1H, H-5), 7.32–7.37 (m, 2H, H-7, Ph), 7.25 (m, 1H, 8-H), 7.01–7.26 (m, 3H, Ph), 5.79 (s, 1H, H-3), 3.48 (m, 8H, $N_1(CH_2)_2$, $N_4(CH_2)_2$), 2.43 (s, 3H, CH_3). HPLC analysis 98.6% (*V*(Methol): *V*(H_2O) = 70:30, t_R = 4.70 min).

4.5.9.6-Methyl-4-(4-(2,5-dichlorophenyl)piperazin-1-yl)coumarin (46)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **46**, yield 22%, colorless powder, mp 141–143 °C; MS (ESI) *m/z* 389.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3441, 1715, 1700, 1581, 1426, 1374, 1229, 1025, 954. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.44 (s, 1H, H-5), 7.33–7.35 (m, 2H, H-7, H-8), 7.27 (m, 1H, Ph), 7.12 (m, 1H, Ph), 7.03 (m, 1H, Ph), 5.80 (s, 1H, H-3), 3.46 (m, 4H, $N_4(CH_2)_2$), 3.32 (m, 4H, $N_1(CH_2)_2$), 2.45 (s, 3H, CH_3). HPLC analysis 97.7% (*V*(Methol): *V*(H_2O) = 70:30, t_R = 2.92 min).

4.5.10.6-Methyl-4-(4-(4-methylbenzoyl)piperazin-1-yl)coumarin (47)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **47**, yield 18%, colorless powder, mp 123–125 °C; MS (ESI) *m/z* 363.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3425, 2832, 1718, 1635, 1443, 1284, 1234. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38 (m, 3H, H-5, Ph), 7.26–7.35 (m, 4H, H-7, H-8, Ph), 5.75 (s, 1H, H-3), 3.90 (m, 4H, $N_4(CH_2)_2$), 3.27 (m, 4H, $N_1(CH_2)_2$), 2.42–2.45 (m, 6H, 2× CH_3). HPLC analysis 95.6% (*V*(Methol): *V*(H_2O) = 70:30, t_R = 3.91 min).

4.5.11. 6-Methyl-4-(4-benzoylpiperazin-1-yl)coumarin (48)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **48**, yield 19%, colorless powder, mp 136–138 °C; MS (ESI) *m/z* 349.1 $(M+H)^+$; IR (KBr) γcm^{-1} 3441, 1694, 1629, 1444, 1424, 1372, 1187, 707. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.45 (m, 5H, Ph), 7.35 (s, 1H, H-5), 7.32 (m, 1H, H-7), 7.24 (m, 1H, H-8), 5.73 (s, 1H, H-3), 3.25–3.98 (m, 8H, $N_1(CH_2)_2$, $N_4(CH_2)_2$), 2.41 (s, 3H, CH_3). HPLC analysis 97.1% (*V*(Methol): *V*(H_2O) = 70:30, t_R = 3.35 min).

4.5.12.6-Methyl-4-(4-(4-chlorobenzoyl)piperazin-1-yl)coumarin (49)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **49**, yield 22%, colorless powder, mp 126–128 °C; MS (ESI) *m/z* 383.0 $(M+H)^+$; IR (KBr) γcm^{-1} 3434, 1720, 1644, 1626, 1430, 1261, 1204, 1011. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.43 (m, 4H, Ph), 7.34 (s, 1H, H-5), 7.32 (m, 1H, H-7), 7.24 (m, 1H, H-8), 5.72 (s, 1H, H-3), 3.25–3.89 (m, 8H, $N_1(CH_2)_2$, $N_4(CH_2)_2$), 2.41 (s, 3H, CH_3). HPLC analysis 96.2% (*V*(Methol): *V*(H_2O) = 70:30, t_R = 4.71 min).

4.5.13.6-Methyl-4-(4-(2-chlorobenzoyl)piperazin-1-yl)coumarin (50)

Purified by flash silica gel column chromatography (EtOAc/hexane = 1:7) to afford compound **50**, yield 26%, colorless powder, mp 166–168 °C; MS (ESI) *m/z* 383.0 $(M+H)^+$; IR (KBr) γcm^{-1} 3434, 1720, 1644, 1626, 1568, 1430, 1011. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.43 (m, 1H, H-5), 7.32–7.89 (m, 4H, Ph), 7.31 (m, 1H, H-7), 7.24 (m, 1H, H-8), 5.73 (s, 1H, H-3), 3.25–3.89 (m, 8H, $N_1(CH_2)_2$, $N_4(CH_2)_2$), 2.41 (s, 3H, CH_3). HPLC analysis 97.2% (*V*(Methol): *V*(H_2O) = 70:30, t_R = 4.52 min).

4.6. In vitro AChE inhibition assay

AChE activity was measured repeated twice by the spectrophotometric method reported by Ellman et al.[25] with some modifications. Brain homogenate was used as the enzyme source. The whole brain except for the cerebellum was homogenized in 9 volumes of 100 mM sodium phosphate buffer (pH 7.0). The test compounds were dissolved in dimethyl sulfoxide (DMSO). The AChE activity was expressed as a change in OD at 412 nm.

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