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Design, synthesis and evaluation of flavonoid derivatives as potent AChE inhibitors

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ABSTRACT

A new series of flavonoid derivatives have been designed, synthesized and evaluated as potent AChE inhibitors. Most of them showed more potent inhibitory activities to AChE than rivastigmine. The most potent inhibitor isoflavone derivative **10d** inhibit AChE with a IC_{50} of 4 nM and showed high BChE/AChE inhibition ratio (4575-fold), superior to donepezil (IC_{50} = 12 nM, 389-fold). Molecular docking studies were also performed to explore the detailed interaction with AChE.

1. Introduction

Since being introduced into clinical practice, acetylcholinesterase (AChE) inhibitors (tacrine, donepezil, galantamine, huperzine A and rivastigmine) have been the standard approach to the symptomatic treatment of Alzheimer's disease (AD).[1] In recent year, mounting evidences proved that AChE may own its 'nonclassical' functions, that is, colocalizing with amyloid-β peptide (Aβ) and promoting amyloid fibril formation through its peripheral anionic site (PAS).[2] The crystallographic structure of AChE reveals that it possesses two separate ligand binding sites—catalytic site and peripheral anionic site (PAS). It implied that molecules that can interact with both binding sites of AChE could not only inhibit AChE, but prevent the aggregation of AChE with Aβ. Therefore, dual-site binding AChE inhibitors have been presented as a new therapeutic strategic option for AD in recent years.[3–5]

In our previous study, a series of indanone derivatives (**1c**, Fig. 1) were synthesized and evaluated as dual-site binding AChE inhibitors. These compounds contain an indanone moiety (from donepezil, **1a**, Fig. 1) and a dialkylbenzyl amine moiety (from rivastigmine, **1b**, Fig. 1). The molecular docking study reveals that these series of compounds can concurrently bind to both the active and peripheral site.[6,7] Flavonoids are well known natural compounds possessing a broad range of pharmacological properties related to Alzheimer's disease, such as neuro-protective effect,[8] AChE inhibitory activities,[9] Aβ fibril formation inhibitory activity,[10] H_2O_2-induced ROS formation reduction effect, and so on.[11] In our previous work, we have also designed and synthesized a series of flavonoid derivatives by linking a benzyl piperidine moiety to the flavonoid moiety through an oxygen atom or a OCH_2 group. Most of these compounds showed low micromolar inhibition to AChE.[12] Modeling study of **1d** (Fig. 1) with AChE indicated that ring B (phenyl ring of phenoxy group) got close to the crucial amino acid Trp 279 in PAS, ring A (dimethoxyphenyl ring), which is designed to bind to PAS, was away from Trp279.[12] In this paper, we reported the design, synthesis and evaluation of a series of new flavonoid derivatives (**6a–f**, **7a–f**, **8a–f**, **10a–f**) by replacing the 2-phenoxy

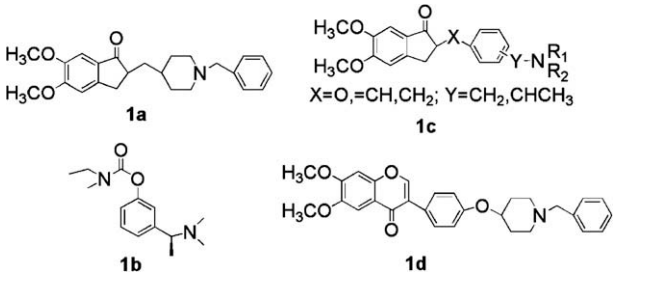

Figure 1. The Structures of donepezil **1a**, rivastigmine **1b**, indanone derivatives **1c** and flavonoid derivative **1d**.

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(or benzyl, benzylidiene)-indan-1-one moiety of **1c** with different flavonoids (chalcone, flavone, flavanone and isoflavone) and introduction of various amino alkyl groups at the *para*- or *meta*-position of ring B with the purpose to alter the binding mode of ring A, ring B with AChE. In the designed compounds, the dimethoxyphenyl (chalcone), dimethoxy-chroman-4-one (flavanone), and dimethoxy-4*H*-chromen-4-one (flavone, isoflavone) moiety, was expected to bind the PAS of AChE, and the nitrogen atom of benzyl amino groups would interact with the catalytic center of AChE through a cation–π interaction, which was presented in the docking study of indanone derivatives.[6,7] Moreover, we described the preliminary structure–activity relationship (SAR) and molecular docking studies for the interactions of these flavonoid derivatives with AChE.

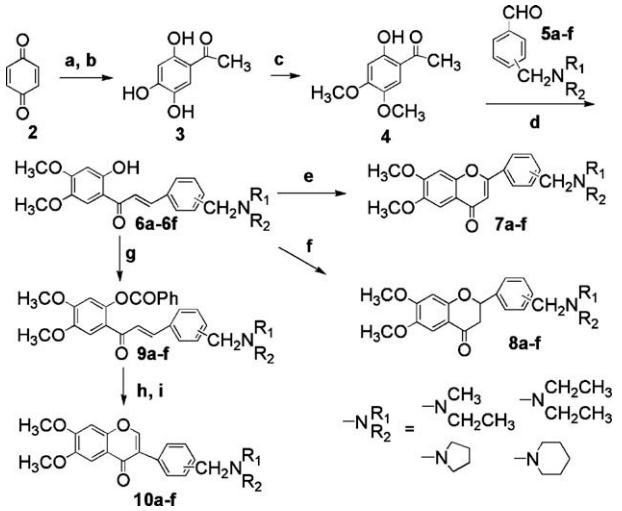
2. Results and discussion

2.1. Chemistry

The synthesis of flavonoid derivatives **6a–f**, **7a–f**, **8a–f**, **10a–f** was outlined in Scheme 1. Acetylation of 1,4-benzoquinone **2** with acetic anhydride in the presence of sulfuric acid, followed by hydrolysis of the acetyl groups with *p*-toluenesulfonic acid (PTS) and water yielded 2,4,5-trihydroxy acetophenone **3**.[13] Methylation of 3 with dimethyl sulfate provided 2-hydroxy-4,5-dimethoxy acetophenone **4**.[14] Condensation of **4** with alkylamino-methyl-benzaldehyde **5a–f**[7] in the presence of KOH afforded chalcone **6a–f**. Oxidation of **6a–f** with iodine in DMSO furnished flavones **7a–f**.[15] Refluxing **6a–f** with NaOAc in alcohol–H_2O gave flavanones **8a–f**. For the preparation of isoflavone derivatives **10a–f**, protection of the hydroxyl groups in chalcones **6a–f** with benzoyl group afforded 2′-benzoyloxychalones **9a–f** which were reacted with DIB/PTS and then treated with NaOH to get target compounds.[16]

2.2. Biological activities

The AChE and BuChE inhibitory activities of all the synthesized flavonoid derivatives were tested according to the modified Ellman method using rat cortex homogenate (AChE) and rat serum (BuChE), with donepezil and rivastigmine as the reference standard.[17]



Scheme 1. Synthesis of flavonoid derivatives **6**, **7**, **8**, **10**. Reagent and conditions (a) Ac_2O, H_2SO_4, H_2O, 135 °C, 1 h; (b) PTS, H_2O, reflux 1 h; (c) $(CH_3)_2SO_4$, K_2CO_3, acetone, reflux 1 h; (d) KOH, CH_3OH, 50–55 °C, 48 h; (e) I_2, DMSO, 80–85 °C, 24 h; (f) NaOAc, CH_3CH_2OH–H_2O, reflux 24 h; (g) PhCOCl, K_2CO_3, DMF, 60 °C, 4 h; (h) DIB, PTS, CH_3OH, rt 24 h; (i) NaOH, CH_3OH–H_2O, rt 24 h.

As shown in Table 1, most of the tested compounds demonstrated higher inhibitory activities against AChE than rivastigmine, and seven of them (**6d**, **6e**, **7d**, **7f**, **10d**, **10e**, **10f**, IC_{50} = 0.070–0.004 μM) exhibit similar activity to donepezil (IC_{50} = 0.012 μM). The preliminary structure–activity relationships could be drawn from the results as follows:

(1) The variation of flavonoid scaffold affected the AChE inhibitory activities dramatically. Isoflavone derivatives, which showed sub-micromolar or nanomolar AChE inhibitory activities, were more potent than other series. For example, compound **10d** (IC_{50} = 0.004 μM) displayed potent AChE inhibitory activity than that of donepezil (IC_{50} = 0.012 μM), and compound **10e**, **10f** (IC_{50} = 0.070, 0.068 μM, respectively) exhibited comparable inhibitory activities to donepezil. The compounds of chalcone and flavone series demonstrated sub-micromolar AChE inhibitory activities except compound **6c** and **7c**, with IC_{50} values ranging from 0.037 to 1.61 μM and 0.034 to 5.99 μM, respectively. The flavanone series displayed weaker inhibitory activity than others, most of them demonstrated low micromolar AChE inhibitory activities.

(2) From the IC_{50} values of the tested compounds, it appeared that compounds with aminomethyl group substituted in the *para*-position of ring B (i.e., **6b**, **7d**, **10d)** were more potent than those *meta*-position substituted ones (i.e., **6c**, **7e**, **10e**), which was consistent with the SAR of previous synthesized indanone derivatives.[6,7]

(3) The sort of aminomethyl group on ring B affected the activity of AChE inhibition, compounds containing pyrrolidine or piperidine group (i.e., **6d**, **6e**, **7f**, **8f**, **10d**) showed higher activity than those with methylethylamino or diethylamino group (i.e., **6a**, **6b**, **7b**, **8a**, **10c**), which indicated that a conformational constrained hydrophobic moiety would be favorable in this position.

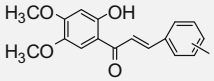
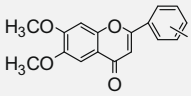
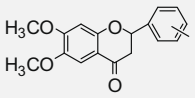
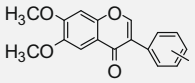
(4) Almost all the compounds showed very weak activity against BChE, and several of them demonstrated higher selectivity (i.e., **6d**, **6e**, **7f**, **10d**) for AChE over BChE than donepezil. Especially, the most potent AChE inhibitors **10d** was 4575-fold more active inhibiting AChE than BuChE, being much more selective than donepezil (389-fold).

2.3. Molecular docking

To gain insight into the molecular determinants that modulate the inhibitory activity of these compounds, molecular docking simulations for **10c**, **10d**, **10e** to TcAChE were performed using the FLEXIDOCK program in Sybyl 6.9 software based on the X-ray crystal structure of TcAChE-E2020 complex.[18]

As seen in Figure 2, compound **10d** has a nice fit along the active-site gorge of AChE, it can bind to the central and the peripheral anionic site concurrently. The Figures 3a and 3b show that **10d** makes several principal interactions along the active-site gorge of AChE with different mode in comparison with indanone derivatives and donepezil.[6,7] Near the top of the gorge (the PAS), besides the expected hydrophobic interaction between dimethoxy-phenyl moiety with Trp279, the dimethoxy-phenyl ring of isoflavone formed classical π–π stacking with the phenyl ring of Tyr334, with the distance of 2.8 Å, more over, the oxygen of one methoxy group makes a hydrogen bond with the NH of indole ring in Trp279, with the distance of 2.42 Å. Noteworthy, in the middle of the gorge, the oxygen of carbonyl group of **10d** formed a highly specific hydrogen bond with OH group in Tyr70 with close distance of 1.46 Å, which imply that it make some contribution on its high activity. Near the bottom of the gorge (the central site), just as expected, the charged

Table 1
Cholinesterase inhibitory activity and selectivity of flavonoid derivatives

	Flavonoids scaffold	Position	$-NR_1R_2$	IC_{50} (μM)		Selectivity for AChE[a]
				AChE	BChE	
6a		*Para*	Methylethylamino	0.126	>100	>794
6b		*Para*	Diethylamino	0.103	>100	>970
6c		*Meta*		1.61	>100	>62.1
6d		*Para*	Pyrrolidine-1-yl	0.037	>100	>2702
6e		*Meta*		0.047	77.3	1645
6f		*Para*	Piperidine-1-yl	0.122	>100	>820
7a		*Para*	Methylethylamino	0.336	>100	>297
7b		*Para*	Diethylamino	0.639	64.6	101
7c		*Meta*		5.99	>100	>16.7
7d		*Para*	Pyrrolidine-1-yl	0.055	89.7	1630
7e		*Meta*		0.704	>100	>142
7f		*Para*	Piperidine-1-yl	0.034	75.2	2211
8a		*Para*	Methylethylamino	4.67	>100	>21.4
8b		*Para*	Diethylamino	4.97	>100	>20.1
8c		*Meta*		172	>100	>0.58
8d		*Para*	Pyrrolidine-1-yl	1.24	68.2	55
8e		*Meta*		5.36	>100	>18.7
8f		*Para*	Piperidine-1-yl	0.248	48.0	194
10a		*Para*	Methylethylamino	0.171	61.0	357
10b		*Para*	Diethylamino	0.599	>100	>167
10c		*Meta*		0.560	>100	>178
10d		*Para*	Pyrrolidine-1-yl	0.004	18.3	4575
10e		*Meta*		0.070	>100	>1428
10f		*Para*	Piperidine-1-yl	0.068	>100	>1471
Donepezil				0.012	4.67	389
Rivastigmine				2.25	1.66	0.74

[a] Selectivity for AChE is defined as IC_{50} (BuChE)/IC_{50} (AChE).

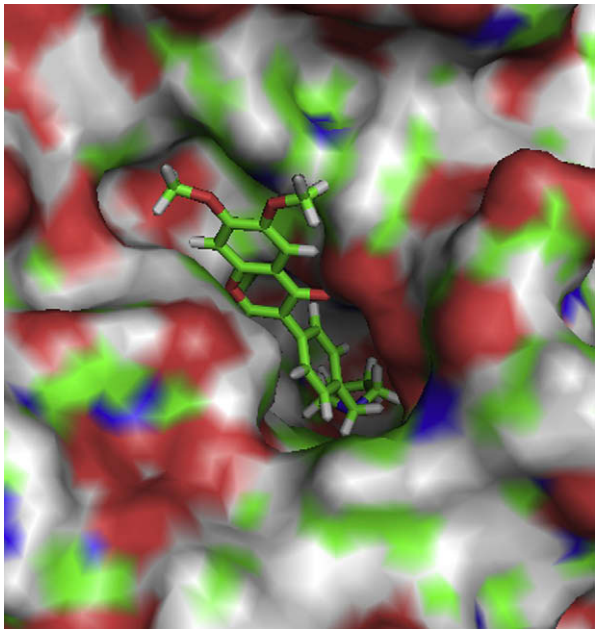


Figure 2. Compound **10d** in the gorge of AChE.

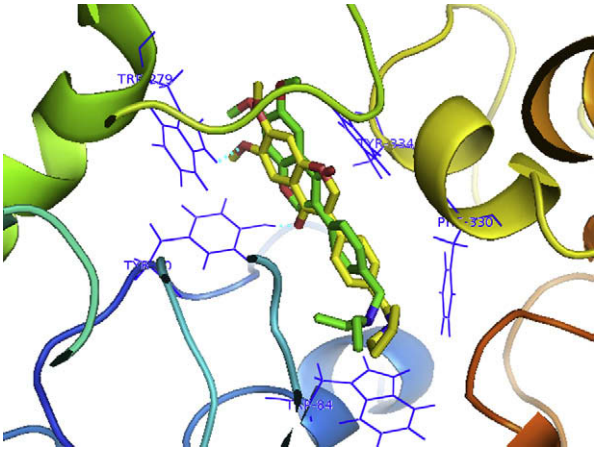


Figure 3a. Predicted binding model of compound **10c** (carbon atoms depicted in green) and **10d** (carbon atoms depicted in yellow) to AChE. The carbon atoms in several key residues in AChE are colored in blue, nitrogen and oxygen atoms are colored in blue and red, respectively.

nitrogen makes a cation–π interaction with the phenyl ring of Phe330, with the distance is 3.10 Å, and the pyrrolidine moiety also display hydrophobic contacts with the indole group of Trp84.

Although there is only little difference between compounds **10c** and **10d**, diethyl amino moiety in **10c** and pyrrolidine moiety in **10d**, the molecular docking (Fig. 3a) shows that there is obvious difference in the conformation of dimethoxy-4*H*-chromen-4-one between two compounds, the carbonyl in **10d** is direct to Tyr70 and make a close hydrogen band with it, while for **10c**, it points to the opposite direction, which result to the lost of hydrogen band, and it could be account for its lower activity.

The molecular docking (Fig. 3b) shows that although **10e** has similar bound conformation of **10d** to AChE, especially has the same hydrogen band with Tyr70 at same distance, the *meta*-position substituted pyrrolidine methyl moiety causes some deviation. At the PAS, it lost the hydrogen band with Trp279 because of the different direction of methoxy moiety in comparison of **10d**. All these facts could explain its sub-micromolar AChE inhibitory activity, IC_{50} = 0.070 μM.

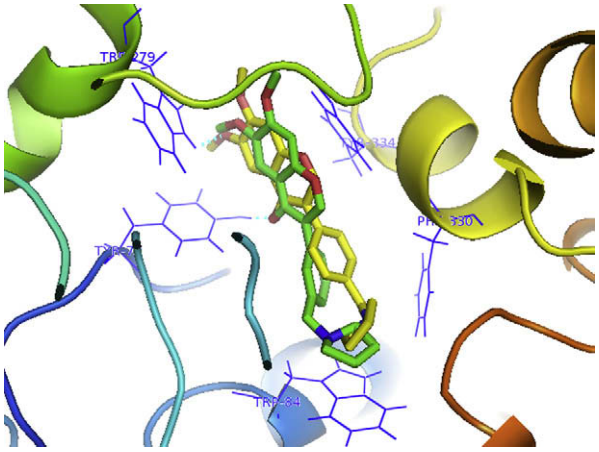


Figure 3b. Predicted binding model of compound **10e** (carbon atoms depicted in green) and **10d** (carbon atoms depicted in yellow) to AChE. The carbon atoms in several key residues in AChE are colored in blue, nitrogen and oxygen atoms are colored in blue and red, respectively.

3. Conclusion

In summary, based upon the SAR study of indanone derivatives and the structural characteristic of flavonoids, a new series of flavonoid derivatives were designed and synthesized as dual-site binding AChE inhibitors and their inhibitory activities against AChE and BChE were evaluated. The results indicate that isoflavone derivatives were potent AChE inhibitors with higher BChE/AChE selectivity, and the molecular Docking simulations of compounds **10c**, **10d** and **10e** with TcAChE gave clear interpretation of the detail interactions at the gorge of the AChE, and give some explanation on SAR. According to the obtained results, we think isoflavone skeleton would be a promising structural template for the development of novel AChE inhibitors, and the preliminary SAR and molecular modeling study results will shed light on the further study.

4. Experimental

4.1. Chemistry

All Reagents and solvents used were purchased from common commercial of analytical grade. Melting points were recorded on a B-540 Buchi melting-point apparatus and uncorrected, IR spectra were recorded on a Brüker VECTOR 22 FTIR spectrophotometer. ^{1}H NMR spectra were recorded on a Brucker Advance DMX 400 MHz spectrometer with TMS as the internal standard. Proton Chemical shifts are expressed in parts per million (ppm) and coupling constants in hertz. Mass spectra (ESI-MS, positive) were recorded on an Esquire-LC-00075 spectrometer.

4.1.1. 2,4,5-Trihydroxy acetophenone (3)

To a mixture of acetic anhydride (55.5 mL, 0.59 mol) and concd H_2SO_4 (0.9 mL, 17 mmol), 1,4-benzoquinone (21 g, 0.194 mol) was added in portions with stirring at 50 °C in 15 min. Then, the mixture was heated to 135 °C and H_2O (30 mL) was added in drop wise with distilling off the formed acetic acid in the meanwhile. After the addition, H_2O (50 mL) and *para*-toluenesulfonic acid (PTS, 2.0 g, 11 mmol) were added to the residue. The resulting mixture was refluxed for 1 h, and then it was cooled to rt and poured into a beaker with 300 mL ice-water. The formed brown solid was filtered and washed with ice-water, followed by recrystallization with H_2O to get 10.1 g pale yellow solid, yield 30.6%, mp 188–190 °C (lit.,[19] 201 °C).

4.1.2. 2-Hydroxy-4,5-dimethoxy acetophenone (4)

To a refluxed solution of compound **3** (5.37 g, 32 mmol) and potassium carbonate (13.25 g, 96 mmol) in 70 mL anhydrous acetone, $(CH_3)_2SO_4$ (6.64 mL, 70 mmol) was added in drop wise. The mixture was stirred for another 1 h after addition, cooled to rt and filtered. The filtrate was concentrated under reduced pressure to get brown solid, which was recrystallized with 95% alcohol twice to afford 3.67 g pale purple solid, yield 58.5%, mp 112–115 °C. (lit.,[14] 114–116 °C).

4.1.3. General procedure for preparation of chalcone derivatives 6a–6f

To a solution of potassium hydroxide (220 mg, 3.93 mmol) in 5 mL methanol and 0.22 mL H_2O, compound **4** (1.02 mmol) and substituted benzaldehyde **5** (1.02 mmol) were added in a portion. The mixture was warmed to 50–55 °Cand stirred for 48 h, then, the solvent was removed. H_2O (15 mL) was added to the residue and extracted with ethyl acetate (15 mL × 3). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4. The solvent was evaporated to give crude product, which was purified by silica gel column chromatography (eluent, petroleum ether/EtOAc/Et_3N = 40:10:1) to afford **6**.

4.1.3.1. (*E*)-3-{4-[(Ethylmethylamino)-methyl]-phenyl}-1-(2-hydroxy-4,5-dimethoxyphenyl)-prop-2-en-1-one (6a). Orange solid, yield 57.8%, mp 74–75 °C; IR (KBr) ν 3583, 3060, 2968, 2937, 2835, 2789, 1675, 1635, 1612, 1504, 1265, 1158, 833 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 13.39 (s, 1H, OH), 7.88 (d, 1H, J = 15.6 Hz, H-3), 7.61 (d, 2H, J = 8.0 Hz, Ar–H), 7.49 (d, 1H, J = 15.2 Hz, H-2), 7.40 (d, 2H, J = 8.0 Hz, Ar–H), 7.27 (s, 1H, Ar–H), 6.52 (s, 1H, Ar–H), 3.94 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.54 (s, 2H, benzylic-CH_2), 2.45 (q, 2H, J = 7.2 Hz, CH_2CH_3), 2.22 (s, 3H, NCH_3), 1.10 (t, 3H, J = 7.2 Hz, CH_2CH_3); MS (ESI) m/z = 356.6 [M+H]$^+$.

4.1.3.2. (*E*)-3-{4-[(Diethylamino)-methyl]-phenyl}-1-(2-hydroxy-4,5-dimethoxyphenyl)-prop-2-en-1-one (6b). Orange solid, yield 46.6%, mp 84–86 °C; IR (KBr) ν 3584, 3057, 2966, 2873, 2786, 1671, 1635, 1582, 1510, 1250,1158, 832 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 13.38 (s, 1H, OH), 7.86 (d, 1H, J = 15.2 Hz, H-3), 7.58 (d, 2H, J = 8.0 Hz, Ar–H), 7.46 (d, 1H, J = 15.6 Hz, H-2), 7.39 (d, 2H, J = 8.0 Hz, Ar–H), 7.25 (s, 1H, Ar–H), 6.49 (s, 1H, Ar–H), 3.92 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.59 (s, 2H, benzylic-CH_2), 2.49 (q, 4H, J =6.8 Hz, $2CH_2CH_3$), 1.02 (t, 6H, J = 6.8 Hz, $2CH_2CH_3$); MS (ESI) m/z = 370.5 [M+H]$^+$.

4.1.3.3. (*E*)-3-{3-[(Diethylamino)-methyl]-phenyl}-1-(2-hydroxy-4,5-dimethoxyphenyl)-prop-2-en-1-one (6c). Orange oil, yield 59.3%; IR (KBr) ν 3585, 3050, 2967, 2933, 2801, 1674, 1635, 1574, 1512, 1250, 1157, 841 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 13.40 (s, 1H, OH), 7.87 (d, 1H, J = 15.2 Hz, H-3), 7.64 (s, 1H, Ar–H), 7.50–7.53 (m, 2H, H-2 and Ar–H), 7.36–7.39 (m, 2H, Ar–H), 7.27 (s, 1H, Ar–H), 6.50 (s, 1H, Ar–H), 3.92 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.60 (s, 2H, benzylic-CH_2), 2.52 (q, 4H, J = 7.2 Hz, $2CH_2CH_3$), 1.04 (t, 6H, J = 7.2 Hz, 2 CH_2CH_3); MS (ESI) m/z = 370.5 [M+H]$^+$.

4.1.3.4. (*E*)-1-(2-Hydroxy-4,5-dimethoxyphenyl)-3-[4-(pyrrolidin-1-ylmethyl)-phenyl]-prop-2-en-1-one (6d). Orange solid, yield 52.5%, mp 104–108 °C; IR (KBr) ν 3585, 3054, 2964, 2876, 2785, 1676, 1636, 1576, 1507, 1267,1159, 836 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 13.39 (s, 1H, OH), 7.87 (d, 1H, J = 15.2 Hz, H-3), 7.60 (d, 2H, J = 8.4 Hz, Ar–H), 7.47 (d, 1H, J = 15.2 Hz, H-2), 7.40 (d, 2H, J = 8.4 Hz, Ar–H), 7.26 (s, 1H, Ar–H), 6.51 (s, 1H, Ar–H), 3.93 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.66 (s, 2H, benzylic-CH_2), 2.53–2.55 (m, 4H, pyrrolidine-CH_2), 1.79–1.81 (m, 4H, pyrrolidine-CH_2); MS (ESI) m/z = 368.5 [M+H]$^+$.

4.1.3.5. (*E*)-1-(2-Hydroxy-4,5-dimethoxyphenyl)-3-[3-(pyrrolidin-1-ylmethyl)-phenyl]-prop-2-en-1-one (6e). Orange oil, yield 53.4%; IR (KBr) ν 3584, 3066, 2962, 2786, 1635, 1574, 1512, 1248, 1156, 844 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.40 (s, 1H, OH), 7.87 (d, 1H, J = 15.2 Hz, H-3), 7.69 (s, 1H, Ar–H), 7.52–7.56(m, 2H, H-2 and Ar–H), 7.35–7.39 (m, 2H, Ar–H), 7.28 (s, 1H, Ar–H), 6.50 (s, 1H, Ar–H), 3.93 (s, 6H, $2OCH_3$), 3.68 (s, 2H, benzylic-CH_2), 2.53–2.56 (m, 4H, pyrrolidine-CH_2), 1.80–1.83 (m, 4H, pyrrolidine-CH_2); MS (ESI) m/z = 368.5$[M+H]^+$.

4.1.3.6. (*E*)-1-(2-Hydroxy-4,5-dimethoxyphenyl)-3-[4-(piperidin-1-ylmethyl)-phenyl]-prop-2-en-1-one (6f). Orange solid, yield 55.0%, mp 118–120 °C; IR (KBr) ν 3585, 3057, 2926, 2852, 2787, 1636, 1574, 1510, 1442, 1248, 840 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.40 (s, 1H, OH), 7.88 (d, 1H, J = 15.6 Hz, H-3), 7.60 (d, 2H, J = 8.0 Hz, Ar–H), 7.47 (d, 1H, J = 15.2 Hz, H-2), 7.39 (d, 2H, J = 8.0 Hz, Ar–H), 7.26 (s, 1H, Ar–H), 6.51 (s, 1H, Ar–H), 3.94 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.50 (s, 2H, benzylic-CH_2), 2.39(m, 4H, piperidine-NCH_2), 1.56–1.61 (m, 4H, piperidine-CH_2), 1.44–1.45 (m, 2H, piperidine-CH_2); MS (ESI) m/z = 382.5 $[M+H]^+$.

4.1.4. General procedure for preparation of flavone derivatives 7a–7f

Compound **6** (0.23 mmol) and iodine (9.3 mg, 0.037 mmol) was added to a solution of concd H_2SO_4 (0.042 mL) in DMSO (3 mL), the mixture was warm to 80–85 °C and stirred for 24 h. When the reaction was over (TLC analysis), a mixture of 1 N HCl/ethyl acetate (20 mL:20 mL) was added at rt, the organic layer was discarded, and concentrated ammonium hydroxide was added into the aqueous solution until it was clearly basic (pH > 9), the product was extracted with ethyl acetate (20 mL × 3). The extract was washed with brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (eluent, petroleum ether/EtOAc/Et_3N = 20:10:1) to afford **7**.

4.1.4.1. 6,7-Dimethoxy-2-{4-[(ethylmethylamino)-methyl)-phenyl}-4*H*-chromen-4-one (7a). Pale yellow solid, yield 44.4%, mp 128–130 °C; IR (KBr) ν 3054, 2967, 2930, 2838, 2786, 1632, 1598, 1506, 1430, 1262, 1079, 826 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.85 (d, 2H, J = 8.0 Hz, Ar–H), 7.57 (s, 1H, H-5), 7.47 (d, 2H, J = 8.4 Hz, Ar–H), 7.00 (s, 1H, H-3), 6.78 (s, 1H, H-8), 4.02 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 3.56 (s, 2H, benzylic-CH_2), 2.45 (q, 2H, J = 7.2 Hz, CH_2CH_3), 2.22 (s, 3H, NCH_3), 1.10 (t, 3H, J = 7.2 Hz, CH_2CH_3); MS (ESI) m/z = 354.4 $[M+H]^+$.

4.1.4.2. 2-[4-(Diethylamino-methyl)-phenyl]-6,7-dimethoxy-4*H*-chromen-4-one (7b). Pale yellow solid, yield 55.0%, mp 125–126 °C; IR (KBr) ν 3057, 2972, 2932, 2808, 1631, 1601, 1507, 1431, 1266, 1110, 836 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.84 (d, 2H, J = 8.4 Hz, Ar–H), 7.56 (s, 1H, H-5), 7.51 (d, 2H, J = 8.0 Hz, Ar–H), 7.00 (s, 1H, H-3), 6.78 (s, 1H, H-8), 4.02 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 3.68 (s, 2H, benzylic-CH_2), 2.57 (q, 4H, J = 6.8 Hz, $2CH_2CH_3$), 1.07 (t, 6H, J = 6.8 Hz, $2CH_2CH_3$); MS (ESI) m/z = 368.5 $[M+H]^+$.

4.1.4.3. 2-{3-[(Diethylamino)-methyl]-phenyl}-6,7-dimethoxy-4*H*-chromen-4-one (7c). Pale yellow solid, yield 19.0%, mp 124–127 °C; IR (KBr) ν 3066, 2967, 2929, 2804, 1635, 1603, 1507, 1430, 1354, 1263, 1082, 826, 699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.96 (s, 1H, Ar–H), 7.78 (d, 1H, J = 7.6 Hz, Ar–H), 7.56 (s, 1H, H-5), 7.46–7.51 (m, 2H, Ar–H), 7.06 (s, 1H, H-3), 6.81 (s, 1H, H-8), 4.03 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 3.70 (s, 2H, benzylic-CH_2), 2.59 (q, 4H, J = 7.2 Hz, $2CH_2CH_3$), 1.08 (t, 6H, J = 7.2 Hz, $2CH_2CH_3$); MS (ESI) m/z = 368.5 $[M+H]^+$.

4.1.4.4. 6,7-Dimethoxy-2-[4-(pyrrolidin-1-ylmethyl)-phenyl]-4*H*-chromen-4-one (7d). Pale yellow solid, yield 33 %, mp 172–174 °C; IR (KBr) ν 3053, 2926, 2800, 1630, 1598, 1506, 1431, 1262, 1080, 826 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.84 (d, 2H, J = 8.4 Hz, Ar–H), 7.56 (s, 1H, H-5), 7.48 (d, 2H, J = 8.8 Hz, Ar–H), 7.00 (s, 1H, H-3), 6.78 (s, 1H, H-8), 4.02 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 3.70 (s, 2H, benzylic-CH_2), 2.55–2.57 (m, 4H, pyrrolidine-CH_2), 1.81–1.83 (m, 4H, pyrrolidine-CH_2); MS (ESI) m/z = 366.5 $[M+H]^+$.

4.1.4.5. 6,7-Dimethoxy-2-[3-(pyrrolidin-1-ylmethyl)-phenyl]-4*H*-chromen-4-one (7e). Pale yellow solid, yield 35.0%, mp 128–130 °C; IR (KBr) ν 3060, 2953, 2930, 2788, 1632, 1600, 1505, 1428, 1347, 1261, 1079, 822 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.94 (s, 1H, Ar–H), 7.79 (d, 1H, J = 8.0 Hz, Ar–H), 7.57 (s, 1H, H-5), 7.47–7.51 (m, 2H, Ar–H), 7.06 (s, 1H, H-3), 6.82 (s, 1H, H-8), 4.03 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 3.76 (s, 2H, benzylic-CH_2), 2.62–2.65 (m, 4H, pyrrolidine-CH_2), 1.83–1.87 (m, 4H, pyrrolidine-CH_2); MS (ESI) m/z = 366.5 $[M+H]^+$.

4.1.4.6. 6,7-Dimethoxy-2-[4-(piperidin-1-ylmethyl)-phenyl]-4*H*-chromen-4-one (7f). Pale yellow solid, yield 60.3%, mp 135–137 °C; IR (KBr) ν 3057, 2934, 2853, 2795, 1646, 1603, 1509, 1434, 1272, 1082, 840 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.88 (d, 2H, J = 8.0 Hz, Ar–H), 7.58 (s, 1H, H-5), 7.55 (d, 2H, J = 8.4 Hz, Ar–H), 7.01 (s, 1H, H-3), 6.80 (s, 1H, H-8), 4.03 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 3.71 (s, 2H, benzylic-CH_2), 2.57–2.60 (m, 4H, piperidine-CH_2), 1.68–1.74 (m, 4H, piperidine-CH_2), 1.51–1.52 (m, 2H, piperidine-CH_2); MS (ESI) m/z = 380.5 $[M+H]^+$.

4.1.5. General procedure for preparation of flavanone derivatives 8a–8f

Compound **6** (0.11 mmol) and sodium acetate (163 mg, 1.99 mmol) was added to a solution of ethyl alcohol (2.2 mL) and H_2O (0.08 mL), the mixture was heated to reflux, and keep the temperature for 24 h, the solvent was evaporated under vacuum, 10 mL H_2O was added to the residue and extracted with ethyl acetate (10 mL × 3), the combined organic layer was washed with 2 N NaOH, brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (eluent, petroleum ether/EtOAc/Et_3N = 40:10:1) to afford **8**.

4.1.5.1. 6,7-Dimethoxy-2-{4-[(ethylmethylamino)-methyl]-phenyl}-chroman-4-one (8a). Pale yellow solid, yield 64.3%, mp 118–119 °C; IR (KBr) ν 2965, 2834, 2778, 1672, 1614, 1503, 1470, 1441, 1264, 1056, 900, 829 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.42 (d, 2H, J = 8.0 Hz, Ar–H), 7.38 (d, 2H, J = 8.0 Hz, Ar–H), 7.33 (s, 1H, H-5), 6.53 (s, 1H, H-8), 5.43 (dd, 1H, J_1 = 13.6 Hz, J_2 = 3.2 Hz, H-2), 3.91 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.51 (s, 2H, benzylic-CH_2), 3.02 (dd, 1H, J_1 = 16.8 Hz, J_2 = 13.2 Hz, H-3a), 2.79 (dd, 1H, J_1 = 16.8 Hz, J_2 = 3.2 Hz, H-3b), 2.43 (q, 2H, J = 7.2 Hz, CH_2CH_3), 2.21 (s, 3H, NCH_3), 1.09 (t, 3H, J = 7.2 Hz, CH_2CH_3); MS (ESI) m/z = 356.4 $[M+H]^+$.

4.1.5.2. 2-{4-[(Diethylamino)-methyl]-phenyl}-6,7-dimethoxy-chroman-4-one (8b). Pale yellow solid, yield 54.5%, mp 120–122 °C; IR (KBr) ν 2967, 2833, 2785, 2713, 1671, 1615, 1502, 1469, 1441, 1264, 1056, 845, 828 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.42–7.46 (m, 4H, Ar–H), 7.33 (s, 1H, H-5), 6.53 (s, 1H, H-8), 5.43 (dd, 1H, J_1 = 13.6 Hz, J_2 = 3.2 Hz, H-2), 3.91 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.61 (s, 2H, benzylic-CH_2), 3.02 (dd, 1H, J_1 = 17.2 Hz, J_2 = 13.6 Hz, H-3a), 2.79 (dd, 1H, J_1 = 16.8 Hz, J_2 = 3.2 Hz, H-3b), 2.52 (q, 4H, J = 7.2 Hz, $2CH_2CH_3$), 1.04 (t, 6H, J = 7.2 Hz, $2CH_2CH_3$); MS (ESI) m/z = 370.5 $[M+H]^+$.

4.1.5.3. 2-{3-[(Diethylamino)-methyl]-phenyl}-6,7-dimethoxy-chroman-4-one (8c). Pale yellow oil, yield 54.0%; IR (KBr) ν 2967, 2933, 2873, 2800, 1677, 1612, 1504, 1468, 1424, 1267, 1056, 870, 843 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.43 (s, 1H, Ar–H), 7.33 (m, 3H, Ar–H), 7.29 (s, 1H, H-5), 6.50 (s, 1H, H-8), 5.40 (dd, 1H, J_1 = 13.6 Hz, J_2 = 2.8 Hz, H-2), 3.87 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.57 (s,

2H, benzylic-CH_2), 2.98 (dd, 1H, J_1 = 17.2 Hz, J_2 = 13.6 Hz, H-3a), 2.76–2.81 (dd, 1H, J_1 = 16.8 Hz, J_2 = 2.8 Hz, H-3b), 2.48 (q, 4H, J = 6.8 Hz, 2$CH_2$$CH_3$), 1.00 (t, 6H, J = 6.8 Hz, 2$CH_2$$CH_3$); MS (ESI) m/z = 370.5 $[M+H]^+$.

4.1.5.4. 6,7-Dimethoxy-2-[4-(pyrrolidin-1-ylmethyl)-phenyl]-chroman-4-one (8d). Pale yellow solid, yield 57.5%, mp 125–126 °C; IR (KBr) ν 2961, 2876, 2809, 1667, 1611, 1503, 1469, 1421, 1262, 1053, 861 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.44–7.48 (m, 4H, Ar–H), 7.33 (s, 1H, H-5), 6.53 (s, 1H, H-8), 5.43 (dd, 1H, J_1 = 13.6 Hz, J_2 = 2.8 Hz, H-2), 3.91 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.69 (s, 2H, benzylic-CH_2), 3.01 (dd, 1H, J_1 = 16.8 Hz, J_2 = 13.6 Hz, H-3a), 2.79 (dd, 1H, J_1 = 17.2 Hz, J_2 = 2.8 Hz, H-3b), 2.59–2.63 (m, 4H, pyrrolidine-CH_2), 1.83–1.85 (m, 4H, pyrrolidine-CH_2); MS (ESI) m/z = 368.5 $[M+H]^+$.

4.1.5.5. 6,7-Dimethoxy-2-[3-(pyrrolidin-1-ylmethyl)-phenyl]-chroman-4-one (8e). Pale yellow oil, yield 52.8%; IR (KBr) ν 2962, 2875, 2833, 2787, 1678, 1612, 1503, 1468, 1267, 1056, 870, 843 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.44 (s, 1H, Ar–H), 7.32–7.37 (m, 3H, Ar–H), 7.31 (s, 1H, H-5), 6.52 (s, 1H, H-8), 5.41 (dd, 1H, J_1 = 13.6 Hz, J_2 = 2.8 Hz, H-2), 3.89 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.64 (s, 2H, benzylic-CH_2), 2.99 (dd, 1H, J_1 = 16.8 Hz, J_2 = 13.6 Hz, H-3a), 2.78 (dd, 1H, J_1 = 17.2 Hz, J_2 = 2.8 Hz, H-3b), 2.51–2.52 (m, 4H, pyrrolidine-CH_2), 1.77–1.80 (m, 4H, pyrrolidine-CH_2); MS (ESI) m/z = 368.5 $[M+H]^+$.

4.1.5.6. 6,7-Dimethoxy-2-[4-(piperidin-1-ylmethyl)-phenyl]-chroman-4-one (8f). Pale yellow solid, yield 77.8%, mp 137–140 °C; IR (KBr) ν 2934, 2851, 2752, 1667, 1611, 1503, 1468, 1436, 1264, 1053, 835 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.43 (d, 2H, J = 8.0 Hz, Ar–H), 7.39 (d, 2H, J = 8.0 Hz, Ar–H), 7.34 (s, 1H, H-5), 6.54 (s, 1H, H-8), 5.43 (dd, 1H, J_1 = 13.6 Hz, J_2 = 3.2 Hz, H-2), 3.92 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.51 (s, 2H, benzylic-CH_2), 3.03 (dd, 1H, J_1 = 16.8 Hz, J_2 = 13.6 Hz, H-3a), 2.80 (dd, 1H, J_1 = 16.8 Hz, J_2 = 3.2 Hz, H-3b), 2.40–2.43 (m, 4H, piperidine-CH_2), 1.56–1.61 (m, 4H, piperidine-CH_2), 1.45–1.46 (m, 2H, piperidine-CH_2); MS (ESI) m/z = 382.5 $[M+H]^+$.

4.1.6. General procedure for preparation of compounds 10a–10f

Compound **6** (0.16 mmol), anhydrous potassium carbonate (110 mg, 0.40 mmol), and benzoyl chloride (75 mg, 0.53 mmol) were mixed with anhydrous DMF (1.5 mL), and the mixture was warmed to 60 °C and stirred for 4 h. H_2O (20 mL) was added to the mixture and extracted with ethyl acetate (20 mL × 3), the organic layer was washed with brine, dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was purified by silica gel column chromatography (eluent, petroleum ether/EtOAc/Et_3N = 40:10:1) to afford benzoate derivatives **9**. To a solution of **9** (0.16 mmol) in methanol (3 mL), a mixture of iodosobenzene diacetate (DIB, 105 mg, 0.33 mmol) and PTS (124 mg, 0.65 mmol) in 2.5 mL methanol was added in dropwise, stirred for 24 h at.rt Then, a solution of NaOH (41 mg, 1.02 mmol)in CH_3OH–H_2O (6.8 mL: 1.4 mL) was added, and stirred for 24 h at rt. The solvent was evaporated under vacuum and the residue was extracted with ethyl acetate (20 mL × 3), washed with brine, and dried over anhydrous Na_2SO_4. The solvent was removed and the residue was purified by silica gel column chromatography (eluent, petroleum ether/EtOAc/Et_3N = 30:10:1) to afford **10**.

4.1.6.1. 6,7-Dimethoxy-3-{4-[(ethylmethylamino)-methyl]-phenyl}-4*H*-chromen-4-one (10a). Pale yellow solid, yield 22.8%, mp 102–104 °C; IR (KBr) ν 2968, 2936, 2837, 2789, 1635, 1605, 1507, 1471, 1432, 1273, 1048, 827 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.97 (s, 1H, H-2), 7.64 (s, 1H, H-5), 7.53 (d, 2H, J = 8.0 Hz, Ar–H), 7.38 (d, 2H, J = 8.0 Hz, Ar–H), 6.89 (s, 1H, H-8), 4.00 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 3.54 (s, 2H, benzylic-CH_2), 2.46 (q, 2H, J = 7.2 Hz, CH_2CH_3), 2.23 (s, 3H, NCH_3), 1.10 (t, 3H, J = 7.2 Hz, CH_2CH_3); MS (ESI) m/z = 354.4 $[M+H]^+$.

4.1.6.2. 3-{4-[(Diethylamino)-methyl]-phenyl}-6,7-dimethoxy-4*H*-chromen-4-one (10b). Pale yellow solid, yield 48.7%, mp 121–123 °C; IR (KBr) ν 2964, 2933, 2869, 2796, 1636, 1600, 1508, 1473, 1420, 1273, 1045, 830 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.98 (s, 1H, H-2), 7.64 (s, 1H, H-5), 7.52 (d, 2H, J = 8.0 Hz, Ar–H), 7.41 (d, 2H, J = 8.0 Hz, Ar–H), 6.89 (s, 1H, H-8), 4.00 (s, 6H, 2OCH_3), 3.63 (s, 2H, benzylic-CH_2), 2.54 (q, 4H, J =7.2 Hz, 2$CH_2$$CH_3$), 1.06 (t, 6H, J = 7.2 Hz, 2$CH_2$$CH_3$); MS (ESI) m/z = 368.5 $[M+H]^+$.

4.1.6.3. 3-{3-[(Diethylamino)-methyl]-phenyl}-6,7-dimethoxy-4*H*-chromen-4-one (10c). Pale yellow oil, yield 33.5%; IR (KBr) ν 2965, 2926, 2853, 1638, 1606, 1506, 1471, 1432, 1272, 1057, 826, 801 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.18 (s, 1H, H-2), 7.78 (s, 1H, H-5), 7.62 (m, 2H, Ar–H), 7.44–7.47 (m, 2H, Ar–H), 6.90 (s, 1H, H-8), 4.00 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 3.92 (s, 2H, benzylic-CH_2), 2.85 (q, 4H, J = 6.8 Hz, 2$CH_2$$CH_3$), 1.25 (t, 6H, J = 6.8 Hz, 2$CH_2$$CH_3$). MS (ESI) m/z = 368.5 $[M+H]^+$.

4.1.6.4. 6,7-Dimethoxy-3-[4-(pyrrolidin-1-ylmethyl)-phenyl]-4*H*-chromen-4-one (10d). Pale yellow solid, yield 54.0%, mp 126–128 °C; IR (KBr) ν 2952, 2926, 2855, 2784, 1629, 1599, 1507, 1462, 1431, 1276, 1047, 834 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.97 (s, 1H, H-2), 7.64 (s, 1H, H-5), 7.53–7.55 (d, 2H, J = 8.0 Hz, H-2′ and H-6′), 7.41–7.43 (d, 2H, J = 8.4 Hz, H-3′ and H-5′), 6.89 (s, 1H, H-8), 4.00 (s, 6H, 2 × OCH_3), 3.67 (s, 2H, benzylic-CH_2), 2.56 (m, 4H, pyrrolidine-CH_2, H-2″ and H-5″), 1.81 (m, 4H, H-3″ and H-4″); MS (ESI) m/z = 366.5 $[M+H]^+$.

4.1.6.5. 6,7-Dimethoxy-3-[3-(pyrrolidin-1-ylmethyl)-phenyl]-4*H*-chromen-4-one (10e). Pale yellow solid, yield 20.4%, mp 123–124 °C; IR (KBr): 2955, 2903, 2877, 2784, 1643, 1621, 1601, 1519, 1473, 1432, 1275, 1054, 820, 796 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.99 (s, 1H, H-2), 7.64 (s, 1H, H-5), 7.55 (s, 1H, H-2′), 7.46 (d, 1H, J = 7.2 Hz, Ar–H), 7.35–7.41 (m, 2H, Ar–H), 6.89 (s, 1H, H-8), 4.00 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 3.68 (s, 2H, benzylic-CH_2), 2.55 (m, 4H, pyrrolidine-CH_2), 1.79–1.80 (m, 4H, pyrrolidine-CH_2); MS (ESI) m/z = 366.5 $[M+H]^+$.

4.1.6.6. 6,7-Dimethoxy-3-[4-(piperidin-1-ylmethyl)-phenyl]-4*H*-chroman-4-one (10f). Pale yellow solid, yield 35.2%, mp 151–152 °C; IR (KBr) ν 2935, 2854, 2761, 1636, 1599, 1508, 1456, 1423, 1271, 1044, 828 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.97 (s, 1H, H-2), 7.63 (s, 1H, H-5), 7.51 (d, 2H, J = 8.0 Hz, Ar–H), 7.38 (d, 2H, J = 8.0 Hz, Ar–H), 6.89 (s, 1H, H-8), 4.00 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 3.51 (s, 2H, benzylic-CH_2), 2.40 (m, 4H, piperidine-CH_2), 1.57–1.61 (m, 4H, piperidine-CH_2), 1.43–1.44 (m, 2H, piperidine-CH_2); MS (ESI) m/z = 380.5 $[M+H]^+$.

4.2. Pharmacology and molecular docking

4.2.1. AChE and BuChE inhibition assays

AChE and BuChE activities were measured by the spectrophotometric method with slight modification, rat cortex homogenate and rat serum were used as the resource of AChE and BuChE, respectively. The brain homogenate was preincubated for 5 min with tetraisopropyl pyrophosphoramido (isoOMPA, selective inhibitor of BuChE, 0.04 mmol/L). For assay of AChE or BuChE activity, a reaction mixture of 200 μl containing acetylthiocholine iodide 0.3 mmol/L or butyrylthiocholine iodide 0.4 mmol/L, sodium phosphate buffer (0.1 mmol/L, pH 7.4) 100 μl, homogenate or serum 20 μl and different concentrations of test compounds 20 μl was incubated at 37 °C for 15 min. The reaction was terminated by adding 50 μl 3% sodium lauryl sulface, then, 50 μl 0.2% 5,5′-

dithio-bis-(2-nitrobenzoic acid) was added to produce the yellow anion of 5-thio-2-nitro-benzoic acid. The values of IC50 were calculated by UV spectroscopy from the absorbance changes at 450 nm. Donepezil and rivastigmine were applied as positive drugs. All samples were assayed in duplicate.

4.2.2. Molecular docking of compound 10d

Molecular docking studies were performed using FLEXIDOCK module of Sybyl 6.9. The X-ray crystal structure of Tc-AChE/E2020 (donepezil) was used (PDB ID: 1EVE). A **10d**-binding pocket was defined to cover all residues within 3.0 Å of the ligand in the complex. All bound waters and ligands were removed from the complex, followed by adding hydrogen atoms. All the single bonds of residue side chains inside the defined pocket and the ligand were allowed to rotate on all single bonds. Kollman all-atom charges were loaded to the protein, and Gasteiger–Huckel charges were assigned to the ligand atoms. The structure optimization was performed for 100,000 generations using a genetic algorithm and the 20 best scoring ligand–protein complexes were kept for further analyses.

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