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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of novel carbamate-substituted flavanone derivatives as potent acetylcholinesterase inhibitors and anti-amnestic agents

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Abstract This study was designed to synthesize and evaluate flavanone derivatives with phenylcarbamate moiety as potent acetylcholinesterase (AChE) inhibitors and anti-amnestic agents for management of AD. The synthesis of carbamate-substituted flavanone derivatives involved base-catalysed Claisen-Schmidt condensation reaction of 2-hydroxy acetophenone/2-hydroxy-4,6-dimethoxyacetophenone with differently substituted benzaldehydes to yield differently substituted chalcones that underwent intramolecular oxidative cyclization on refluxing with glacial acetic acid to yield flavanone compounds. Thereafter, refluxing of flavanone compounds with phenyl isocyanate in the presence of petroleum-ether and triethylamine provided phenyl carbamate-substituted flavanone derivatives. The synthesized compounds were screened in vitro for AChE inhibitory activity with donepezil as the standard drug. The most potent test compound (**5f′**) was evaluated in vivo for memory restorative actions in scopolamine (0.4 mg/kg)-induced amnesia in mice by Morris water maze test. All the compounds exhibited AChE inhibitory activity with carbamate substituted 5,7-dimethoxyflavanone derivatives (**5a′**–**5g′**) being the most potent compounds with IC_{50} ranging from 21.5 ± 1.8 to 9.9 ± 1.6 nM. Compound **5f′** also ameliorated scopolamine-induced amnesia in mice in terms of restoration of time spent in target quadrant and escape latency time. It may be concluded that phenylcarbamate-substituted 5,7-dimethoxyflavanones may be a promising structural template for the development of novel AChE inhibitors in managing amnestic disorders including AD.

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Keywords Flavanone · Carbamate · Acetylcholinesterase · Alzheimer's disease

Introduction

Alzheimer's disease (AD), characterised by Alois Alzheimer in 1907, is a progressive neurodegenerative disorder of the brain and is the most common form of dementia among the elderly. It affects over 20 million individuals worldwide and this number will substantially increase in the future along with the increase of the number of elderly in the population. Its prevalence increases with age, from 10 % at 65 years to nearly 50 % at 85 years (Bachurin, 2003; Colombres *et al.*, 2004). According to the 'cholinergic hypothesis', impairment in the cholinergic function is of critical importance in AD especially the brain areas dealing with learning, memory, behaviour and emotional responses that include the neocortex and the hippocampus. Brain atrophy is the most obvious clinical finding in AD in which the levels of acetylcholine (ACh) are decreased due to its rapid hydrolysis by acetylcholinesterase (AChE) enzyme (Ladner and Lee, 1998). Furthermore, a broad range of evidences have shown that enzyme AChE produces secondary non-cholinergic functions that include promotion in beta-amyloid (Aβ) deposition in the form senile plaques/neurofibrillary tangles in the brain of afflicted individuals (Castro and Martinez, 2001; Selkoe 2002; Bartolini *et al.*, 2003; Rees *et al.*, 2003). Thus, AChE inhibition has been documented as a critical target for the effective management of AD by an increase in the availability of ACh in the brain regions and decrease in the Aβ deposition (De Ferrari *et al.*, 2001).

Flavonoids are natural phytochemicals that are distributed in fruits, vegetables and whole grains with a wide

range of pharmacological properties including anti-inflammatory, -tumour, hepatoprotective, anti-viral, -fungal, -microbial and -oxidant (Kim *et al.*, 2004). The studies have also shown that natural as well as the chemically synthesised flavonoid analogues exhibit neuroprotective effects (Wang *et al.*, 2001), AChE inhibitory (Jung and Park, 2007) along with Aβ fibril formation inhibitory activities (Kim *et al.*, 2005; Zhu *et al.*, 2007). Several studies have also documented the anti-amnestic and memory restorative functions of flavonoid derivatives in different experimental models of amnesia along with prevention/slowing of progressive neurodegeneration in AD (Richetti *et al.*, 2011). Furthermore, the studies have shown that addition of different chemical moieties to flavonoid scaffold such as benzyl piperidine (Shen *et al.*, 2009) and amino alkyl groups at *para* and *meta* positions enhance the effectiveness of flavonoid nucleus for AChE inhibition (Sheng *et al.*, 2009).

A number of studies have revealed that carbamate is an important structural constituent imparting AChE inhibitory properties. Infact, carbamate is an integral part of clinically employed AChE inhibitor, rivastigmine. The various research groups have synthesised carbamate derivatives by incorporating other chemical moieties to produce potent AChE inhibitors that included phenyl ring (Lin *et al.*, 1999), benzopyrano [4,3-*b*] pyrroles (Bolognesi *et al.*, 2004), 1,2-phenylalkyl-substituted piperidines (Mustazza *et al.*, 2002), *N*-propargylaminoindan and *N*-propargylphenethylamine (Sterling *et al.*, 2002). Based on these, it may be proposed that the addition of carbamate moiety to flavonoid molecule may produce potent AChE inhibitors (Fig. 1) that in turn may be beneficial for memory restoration in amnesia of diverse aetiology including AD. Therefore, this study was designed to synthesise novel carbamate derivatives of flavanone followed by their screening for AChE inhibitory activity with in vitro testing along with in vivo evaluation of the most potent AChE inhibitor for the memory restorative effect in a mice model of amnesia.

Results and discussion

Chemistry

The synthesis of carbamate substituted flavanone derivatives is illustrated as a representative case in Figs. 2 and 3. The base-catalysed Claisen-Schmidt condensation reaction of 2-hydroxyacetophenone (**1**) or 2-hydroxy-4,6-dimethoxyacetophenone (**1′**) with differently substituted benzaldehydes (**2a**–**2g**) in the presence of ethyl alcohol and 60 % KOH followed by neutralisation in the presence of cold acetic acid yielded differently substituted chalcones

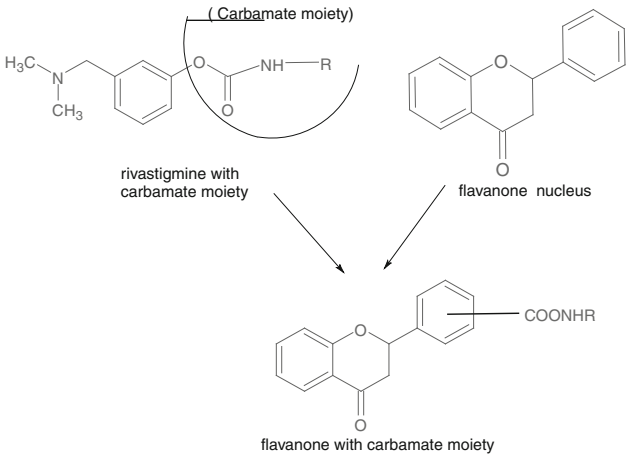
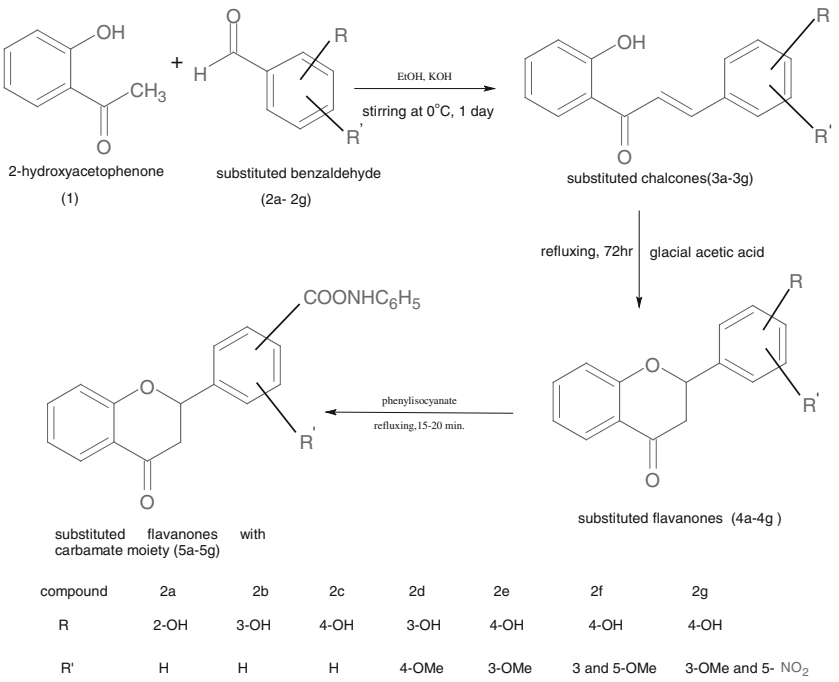


Fig. 1 Design strategy of carbamate derivatives of flavanone

(**3a**–**3g**) and (**3a′**–**3g′**), respectively (Lin *et al.*, 2002). Subsequently, the differently substituted chalcones (**3a**–**3g**) and (**3a′**–**3g′**) underwent intra-molecular oxidative cyclization on refluxing with glacial acetic acid to yield flavanone compounds (**4a**–**4g**) and (**4a′**–**4g′**), respectively (Cabrera *et al.*, 2007). Thereafter refluxing of flavanone compounds (**4a**–**4g**) and (**4a′**–**4g′**) with phenyl isocyanate in the presence of petroleum-ether and triethylamine (2 or 3 drops) provided phenyl carbamate-substituted flavanone derivatives (**5a**–**5g**) and (**5a′**–**5g′**), respectively.

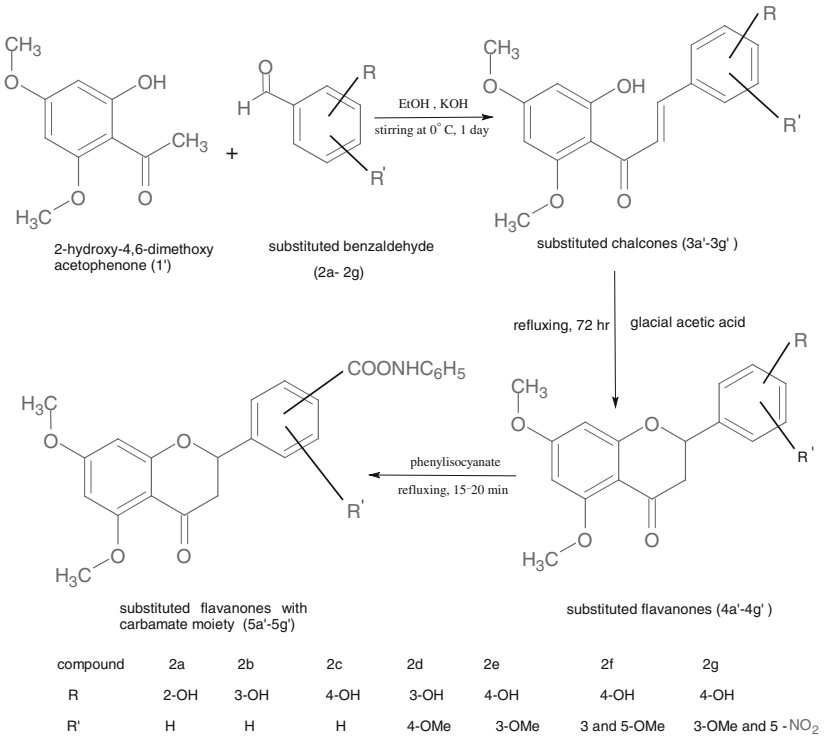
Biological activity

All the synthesized flavanone derivatives were screened for AChE inhibitory activity in the rat cortex homogenate using modified Ellman method with donepezil as the standard AChE inhibitor (Ellmann *et al.*, 1961; Cheng and Tang, 1998). All the compounds exhibited AChE inhibitory activity with carbamate substituted 5,7-dimethoxyflavanone derivatives (**5b′**–**5g′**) being the most potent compounds with IC_{50} ranging from 19.6 ± 1.8 to 9.9 ± 1.6 nM (Tables 1, 2). Furthermore, the compound **5f′** was found to be the most potent AChE inhibitor with IC_{50} 9.9 ± 1.6 nM. The replacement of –OH group of ring B of flavanone scaffold (**4a**–**4g**, **4a′**–**4g′**) with the phenyl carbamate moiety (**5a**–**5g**, **5a′**–**5g′**) led to dramatic increase in AChE inhibitory activity suggesting phenyl carbamate as an important moiety that may influence the AChE activity. The presence of two electron releasing methoxy groups at 5th and 7th position of phenyl ring A of carbamate-substituted flavanones conferred greater AChE inhibitory activity in compound **5a′**–**5g′** as compared to compound **5a**–**5g** without dimethoxy groups at 5th and 7th positions of carbamate-substituted flavanones. Furthermore, the position of carbamate moiety linked to ring B of flavanone scaffold also influenced the AChE inhibitory activity with higher



compound	2a	2b	2c	2d	2e	2f	2g
R	2-OH	3-OH	4-OH	3-OH	4-OH	4-OH	4-OH
R'	H	H	H	4-OMe	3-OMe	3 and 5-OMe	3-OMe and 5- NO_2

Fig. 2 Schematic diagram describing the steps in the synthesis of substituted flavanone derivatives (**4a**–**4g**) and substituted flavanone derivatives with carbamate moiety (**5a**–**5g**) from 2-hydroxyacetophenone and substituted benzaldehyde

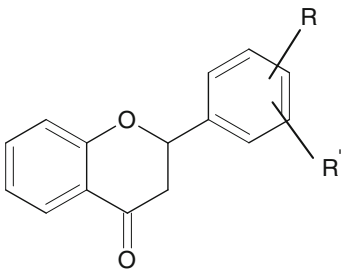


compound	2a	2b	2c	2d	2e	2f	2g
R	2-OH	3-OH	4-OH	3-OH	4-OH	4-OH	4-OH
R'	H	H	H	4-OMe	3-OMe	3 and 5-OMe	3-OMe and 5 - NO_2

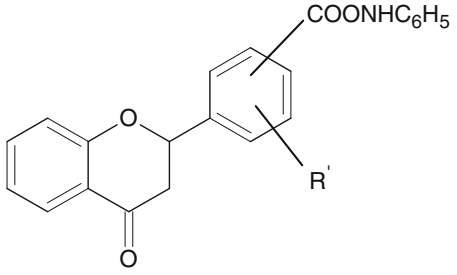
Fig. 3 Schematic diagram describing the steps in the synthesis of substituted 5,7-dimethoxyflavanone derivatives (**4a′**–**4g′**) and substituted 5,7-dimethoxyflavanone derivatives with carbamate moiety (**5a′**–**5g′**) from 2-hydroxy-4,6-dimethoxyacetophenone and substituted benzaldehyde

potency for compounds with carbamate attached to flavanone at *para* position (**5e′**) as compared to corresponding compounds with carbamate moiety at *meta* positions (**5d′**). The nature of substituent, i.e. electron releasing/withdrawing groups attached to ring B of flavanone moiety also influenced the AChE inhibitory activity. The compound with two $-OCH_3$ groups (electron releasing) at ring B (**5f′**, IC_{50} 9.9 ± 1.6 nM) demonstrated higher AChE inhibition as compared to corresponding compounds with one $-OCH_3$ group (**5d′**, IC_{50} 16.3 ± 2.1 nM; **5e′**, IC_{50} 12.8 ± 1.8 nM) and $-NO_2$ group (electron withdrawing) (**5g′** IC_{50} 18.2 ± 1.9 nM). The compound (**5f′**) was also evaluated for memory restoration in scopolamine-induced amnesia in mice. Administration of scopolamine significantly decreased

Table 1 AChE inhibitory activity of flavanone derivatives (**4a**–**4g**) and flavanone derivatives with carbamate moiety (**5a**–**5g**)



substituted flavanones (4a-4g)



substituted flavanones with carbamate moiety (5a-5g)

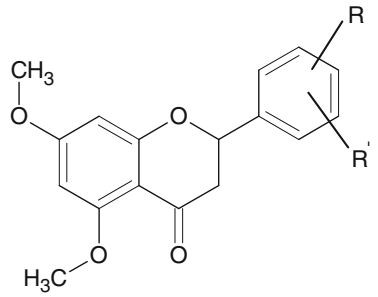
Sr. no.	Compound	R	R′	–COONHC$_6$H$_5$ (carbamate moiety)	IC$_{50}$ (nM)
1	**4a**	2′-OH	H	–	149 ± 1.2
2	**4b**	3′-OH	H	–	147 ± 1.1
3	**4c**	4′-OH	H	–	145 ± 1.6
4	**4d**	3′-OH	4′-OMe	–	140 ± 1.5
5	**4e**	4′-OH	3′-OMe	–	138 ± 1.6
6	**4f**	4′-OH	3′,5′-di–OMe	–	131 ± 1.1
7	**4g**	4′-OH	3′-OMe and 5′-NO$_2$	–	144 ± 2.1
8	**5a**	–	H	*ortho*	39.9 ± 2.5
9	**5b**	–	H	*meta*	38.8 ± 1.8
10	**5c**	–	H	*para*	36.7 ± 1.9
11	**5d**	–	4′-OMe	*meta*	27.6 ± 1.2
12	**5e**	–	3′-OMe	*para*	21.9 ± 1.3
13	**5f**	–	3′,5′-di-OMe	*para*	19.3 ± 1.8
14	**5g**	–	3′-OMe and 5′-NO$_2$	*para*	30.5 ± 1.2
15	Donepezil	–	–	–	21.5 ± 3.2

day 4 ELT and TSTQ on day 5 indicating an impairment of memory as assessed on Morris water maze as compared to normal mice. However, treatment with test compound **5f′** (5 and 10 mg/kg) along with donepezil (25 mg/kg) attenuated scopolamine-induced decrease in day 4 ELT and TSTQ on day 5 in a significant manner (Table 3; Fig. 4).

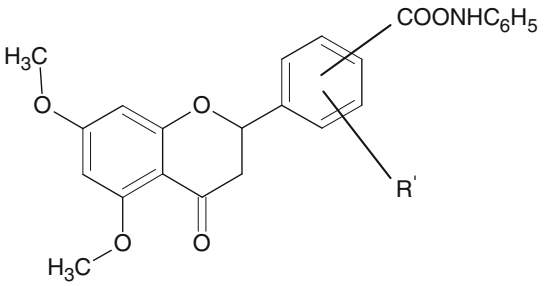
Molecular docking

To disclose a possible binding mode of compound **5f′** with human AChE enzyme's binding pockets, the docking simulation were performed using the available crystallographic structure of enzyme (PDB code 1B41) using Molegro Virtual Docker. The docking simulation revealed that the enzyme and compound **5f′** interacted through π–π aromatic interactions and hydrogen bonding (Fig. 5). The oxygen atom of methoxy group attached to the ring B of flavonoid may interact with –NH– group of Gly 121; –NH– of Gly 122 and –OH group of Ser 203 through hydrogen bonding with a distance of 3.46, 3.15 and 2.77 Å, respectively. Gly 121 and Gly 122 are important groups of 'oxy-anionic hole' which in turn provide hydrogen bond donors to stabilize tetrahedral transition state of substrate. Ser 203 is an important constituent of catalytic site lying deep with in the molecule at the base of an narrow 20 Å deep gorge. The oxygen of other methoxy group attached to ring B of flavonoid scaffold may show hydrogen bonding with hydroxyl group of Tyr 337 (a constituent of 'anionic' sub-site in the gorge and involved in optimally positioning the ester at the acylation site along with binding to trimethylammonium choline through π-cation interactions) at the distance of 3.20 Å. The heterocyclic oxygen of ring C of flavonoid scaffold may interact with hydroxyl group of Tyr 124 (one of the five residues of peripheral anionic site clustered around the entrance to the active site gorge) at a distance of 2.89 Å. The docking results also revealed the potential π–π aromatic interactions between compound **5f′** and amino acid residues of human AChE. The heterocyclic phenyl C-ring of the compound **5f′** may show π–π interactions with Phe 338 (constitute 'anionic

Table 2 AChE inhibitory activity of 5,7-dimethoxyflavanone derivatives (**4a′–4g′**) and 5,7-dimethoxyflavanone derivatives with carbamate moiety (**5a′–5g′**)



substituted flavanones (4a'-4g')



substituted flavanones with carbamate moiety (5a'-5g')

Sr. no.	Compound	R	R′	–$COONHC_6H_5$	IC_{50} (nM)
1	**4a′**	2′-OH	H	–	120 ± 1.7
2	**4b′**	3′-OH	H	–	119 ± 2.1
3	**4c′**	4′-OH	H	–	118 ± 1.2
4	**4d′**	3′-OH	4′-OMe	–	114 ± 1.1
5	**4e′**	4′-OH	3′-OMe	–	112 ± 1.3
6	**4f′**	4′-OH	3,′5′-di-OMe	–	107 ± 1.8
7	**4g′**	4′-OH	3′-OMe and 5′-NO_2	–	116 ± 1.2
8	**5a′**	–	H	*ortho*	21.5 ± 1.3
9	**5b′**	–	H	*meta*	19.6 ± 1.8
10	**5c′**	–	H	*para*	18.8 ± 1.6
11	**5d′**	–	4′-OMe	*meta*	16.3 ± 2.1
12	**5e′**	–	3′-OMe	*para*	12.8 ± 1.8
13	**5f′**	–	3′,5′-di-OMe	*para*	9.9 ± 1.6
14	**5g′**	–	3′-OMe and 5′-NO_2	*para*	18.2 ± 1.9

Table 3 Effect of different interventions on escape latency time (ELT) using Morris water maze for memory evaluation

S. no	Group	Dose	Day 1 ELT	Day 4 ELT
1.	Normal	–	86.2 ± 5.5	37.2 ± 5.2[a]
2.	Scopolamine	0.4	89.8 ± 4.8	79.3 ± 6.3[b]
3.	Compound **5f′** in scopolamine	2 mg/kg (i.p)	84.3 ± 2.8	72.1 ± 3.6
4.	Compound **5f′** in scopolamine	5 mg/kg (i.p)	87.5 ± 4.1	66.4 ± 5.9
5.	Compound **5f′** in scopolamine	10 mg/kg (i.p)	83.1 ± 3.9	45.4 ± 3.7[c]
6.	Vehicle in scopolamine	5 ml/kg (i.p.)	89.7 ± 3.8	78.7 ± 5.7
7.	Donepezil	25 mg/kg (i.p.)	84.8 ± 4.1	52.6 ± 4.0[c]

Values are expressed as mean ± SEM for six animals

[a] $p < 0.05$ vs. day 1 ELT in normal

[b] $p < 0.05$ vs. day 4 ELT in normal

[c] $p < 0.05$ vs. day 4 ELT in scopolamine

sub-site' along with constituents of peripheral anionic site Tyr 341 and Tyr 72). The phenyl A-ring of flavonoid scaffold may also show π–π interactions with Phe 295 (a part of acyl pocket, which is responsible for substrate selectivity by preventing access of other larger members of choline ester series) and Trp 286 (one of the five residues of peripheral anionic site clustered around the entrance to the active site gorge).

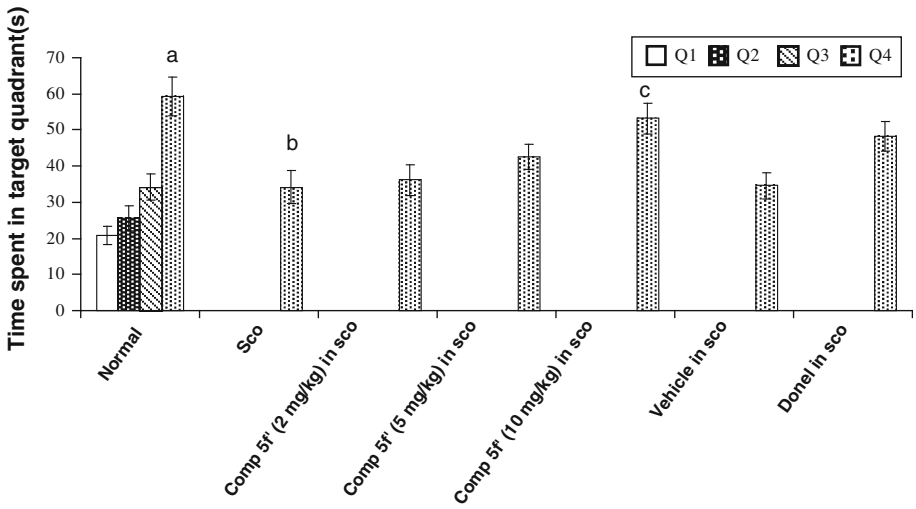


Fig. 4 Effect of different interventions on time spent in target quadrant (TSTQ), i.e. Q4 in Morris water maze test for memory evaluation. Values are expressed as mean ± SEM for six animals. *Sco* scopolamine, *Donel* donepezil. *a* $p < 0.05$ versus time spent in other quadrants (Q1, Q2, Q3) in normal. *b* $p < 0.05$ versus TSTQ in normal. *c* $p < 0.05$ versus TSTQ in scopolamine treated. The data were analysed using One-way ANOVA followed by Tukey's multiple range test

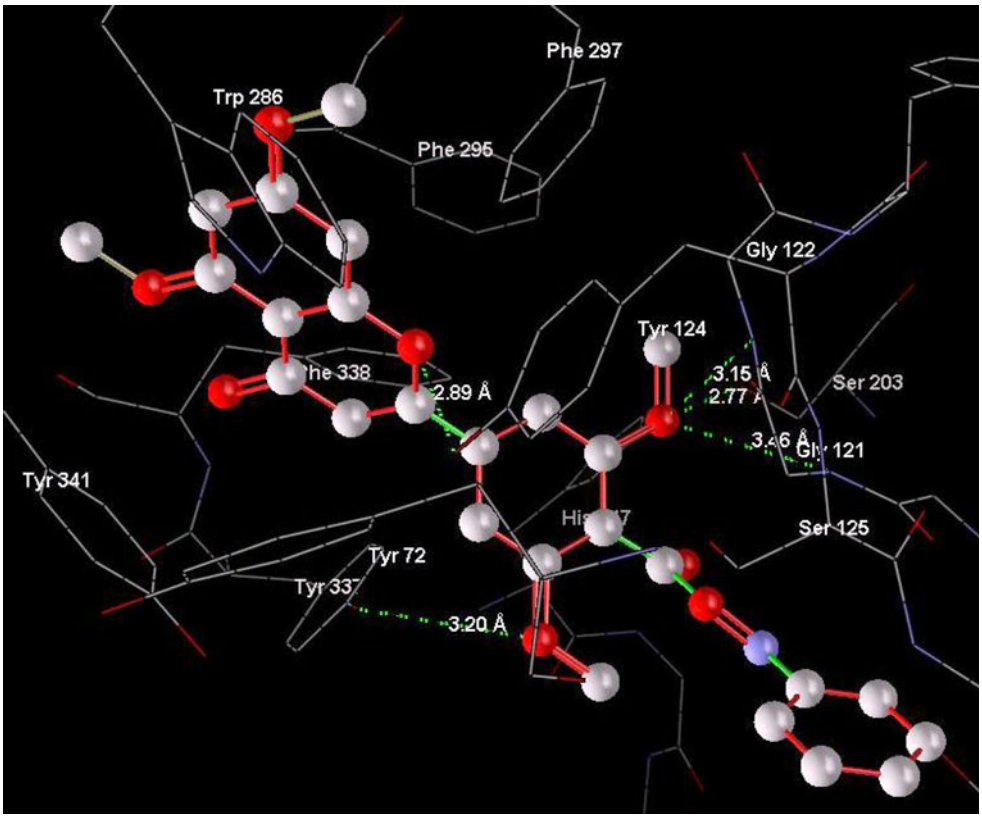


Fig. 5 The docking view of compound **5f'** with AChE (PDB code 1B41) showing five hydrogen-bond interactions (shown by *broken lines*) among the different amino acid residues and structural parts of compound. The different atoms are shown in *different colours*, i.e. nitrogen with *blue*, oxygen with *red* and carbon with *white* (Color figure online)

Conclusion

A new series of carbamate derivatives of flavanone were designed and synthesized as AChE inhibitors and anti-amnestic agents. The results of the present study suggests that phenylcarbamate substituted 5,7-dimethoxyflavanones may be a promising structural template for the development of novel AChE inhibitors in managing amnesic disorders including AD.

Experimental

Chemistry

All the reagents and the solvents used were of analytical grade. Melting points were determined on Gallen–Kamp apparatus and uncorrected. H^1 NMR spectra were recorded on Bruker Avance II 400 MHz spectrometer in $CDCl_3$ with TMS (Me_4Si) as an internal standard and chemical shifts

were recorded in parts per million (ppm) along with coupling constants (*J* values) in Hertz. Multiplicities were designated as singlet (s), doublet (d), triplet (t), quadruplet (q) and multiplet (m). IR spectra were recorded on a Perkin Elmer spectrum RX IFT-IR system. Mass spectra were recorded using Thermo Scientific, LTQ-XL LCMS with ionization current of 70 eV. Mass spectra (MS) signals were given in *m/z*. Elemental analysis (EA) was performed on an Elementar Analysensysteme GmbH. All the reactions were monitored using TLC.

*General procedure for the synthesis of substituted chalcones (**3a–3g** or **3a′–3g′**)*

To a solution of 2-hydroxyacetophenone (11 mmol) (**1**) or 2-hydroxy-4,6-dimethoxyacetophenone(11 mmol) (**1′**) and substituted benzaldehydes (9 mmol) in absolute ethyl alcohol (10 ml) was added a 60 % of KOH (10 g) solution dropwise at 0 °C. The reaction was stirred at 0 °C for 1 day. The cold water was added and the reaction mixture was neutralised with cold acetic acid. The yellow precipitates were collected, washed with water and recrystallized from ethyl alcohol to yield substituted chalcones, respectively (**3a**–**3g** or **3a′**–**3g′**).

*General procedure for the synthesis of substituted flavanones (**4a–4g** or **4a′–4g′**)*

A mixture of the corresponding substituted chalcones (1 equiv.) (**3a**–**3g** or **3a′**–**3g′**) in glacial acetic acid (25 ml/mmol of substituted chalcones) was refluxed during 72 h. Thereafter, the mixture was poured into water and extracted with ethyl acetate (3 × 25 ml). The organic layer was washed with brine until neutrality and dried with $MgSO_4$ anhydrous. The solvent was evaporated in vacuo to afford solid product that was recrystallized from ethyl alcohol to yield flavanones, respectively (**4a**–**4g** or **4a′**–**4g'**).

*2-(2-Hydroxyphenyl)chroman-4-one (**4a**)* Pale yellow solid, yield 54.5 %, mp 118–120 °C; IR (Nujol) υ 3510, 1680, 1607, 1603, 1273, 1238, 1213 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.80 (dd, 1H, $J_1 = 12.48$ and $J_2 = 3.44$ Hz, H-2), 3.03 (dd, 1H, $J_1 = 16.8$ and $J_2 = 13.64$ Hz, H_{3a}), 2.58 (dd, 1H, $J_1 = 16.8$ and $J_2 = 3.2$ Hz, H_{3b}), 7.52–6.88 (m, 8H, aromatic H), 9.39 (s, 1H, OH); ESI–MS (positive mode) *m/z* 241 $(M + H)^+$; Anal. Cald for $C_{15}H_{12}O_3$: C, 74.99; H, 5.03; O, 19.98. Found: C, 74.67; H, 5.02; O, 19.58.

*2-(3-Hydroxyphenyl)chroman-4-one (**4b**)* Yellow solid, yield 57.8 %, mp 120–122 °C; IR (Nujol) υ 3507, 1681, 1608,1603 1273,1240, 1210, 1124 cm^{-1}; 1H NMR ($CDCl_3$, 400 MHz) δ 5.83 (dd, 1H, $J_1 = 13.61$ Hz and $J_2 = 3.42$ Hz, H-2), 3.01 (dd, 1H, $J_1 = 17.2$ and $J_2 = 13.70$ Hz, H_{3a}), 2.59 (dd, 1H, $J_1 = 16.7$ and $J_2 = 3.1$ Hz, H_{3b}), 7.51–6.87 (m, 8H, aromatic H), 9.38 (s, 1H, OH); ESI–MS (positive mode) *m/z* 241 $(M + H)^+$; Anal. Cald for $C_{15}H_{12}O_3$: C, 72.88; H, 5.13; O, 18.98. Found: C, 72.64; H, 5.03; O, 18.68.

*2-(4-Hydroxyphenyl)chroman-4-one (**4c**)* Yellow solid, yield 60.2 %, mp 121–122 °C; IR (Nujol) υ 3510, 1678, 1610,1605 1274,1241, 1211,1126 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.81 (dd, 1H, $J_1 = 12.48$ Hz and $J_2 = 3.22$ Hz, H-2), 3.03 (dd, 1H, $J_1 = 16.7$ and $J_2 = 13.60$ Hz, H_{3a}), 2.58 (dd, 1H, $J_1 = 16.8$ and $J_2 = 3.1$ Hz, H_{3b}), 7.52–6.85 (m, 8H, aromatic H), 9.39 (s, 1H, OH); ESI–MS (positive mode) *m/z* 241 $(M + H)^+$; Anal. Cald for $C_{15}H_{12}O_3$: C, 73.89; H, 5.24; O, 20.98. Found: C, 72.79; H, 5.21; O, 20.88.

*2-(3-Hydroxy-4-methoxyphenyl)chroman-4-one (**4d**)* Yellow solid, yield 64.5 %, mp 125–126 °C; IR (Nujol) υ 3508, 1679, 1610,1605 1270,1239, 1211, 1125, 1025 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.81 (dd, 1H, $J_1 = 12.44$ Hz and $J_2 = 2.98$ Hz, H-2), 3.03 (dd, 1H, $J_1 = 16.8$ and $J_2 = 13.62$ Hz, H_{3a}), 2.60 (dd, 1H, $J_1 = 16.7$ and $J_2 = 3.2$ Hz, H_{3b}), 7.53–6.81 (m, 7H, aromatic H), 10.01 (s, 1H, OH), 3.83 (s, 3H, 4′-OCH_3); ESI–MS (positive mode) *m/z* 271 $(M + H)^+$; Anal. Cald for $C_{16}H_{14}O_4$: C, 71.19; H, 5.22; O, 23.68. Found: C, 70.97; H, 5.18; O, 23.68.

*2-(4-Hydroxy-3-methoxyphenyl)chroman-4-one (**4e**)* Yellow solid, yield 63.7 %, mp 128–130 °C; IR (Nujol) υ 3520, 1680, 1607,1603 1273,1239, 1213, 1126, 1025 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.82 (dd, 1H, $J_1 = 12.40$ Hz and $J_2 = 2.99$ Hz, H-2), 3.02 (dd, 1H, $J_1 = 16.8$ and $J_2 = 13.70$ Hz, H_{3a}), 2.59 (dd, 1H, $J_1 = 17.2$ and $J_2 = 2.9$ Hz, H_{3b}), 7.54–6.75 (m, 7H, aromatic H), 10.00 (s, 1H, OH), 3.82 (s, 3H, 3′-OCH_3); ESI–MS (positive mode) *m/z* 271 $(M + H)^+$; Anal. Cald for $C_{16}H_{14}O_4$: C, 71.29; H, 5.10; O, 19.98. Found: C, 70.99; H, 5.01; O, 18.79.

*2-(4-Hydroxy-3,5-dimethoxyphenyl)chroman-4-one (**4f**)* Yellow solid, yield 56.8 %, mp 135–137 °C; IR (Nujol) υ 3510, 1681, 1606,1603 1273,1235, 1210, 1026 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.80 (dd, 1H, $J_1 = 12.80$ Hz and $J_2 = 3.10$ Hz, H-2), 3.01 (dd, 1H, $J_1 = 17.2$ and $J_2 = 13.6$ Hz, H_{3a}), 2.58 (dd, 1H, $J_1 = 16.8$ and $J_2 = 3.1$ Hz, H_{3b}), 7.51–6.53 (m, 6H, aromatic H), 9.40 (s, 1H, OH), 3.81 (s, 3H, 3′-OCH_3), 3.78 (s, 3H, 5′-OCH_3); ESI–MS (positive mode) *m/z* 301 $(M + H)^+$; Anal. Cald for $C_{17}H_{16}O_5$: C, 67.29; H, 5.39; O, 25.87. Found: C, 66.67; H, 4.92; O, 24.85.

*2-(4-Hydroxy-3-methoxy 5-nitrophenyl)chroman-4-one (**4g**)* Yellow solid, yield 55.6 %, mp 133–135 °C; IR (Nujol) υ 3510, 1682, 1607,1603 1274,1239, 1213,1027 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.82 (dd, 1H, $J_1 = 12.52$ Hz and $J_2 = 3.12$ Hz, H-2), 3.03 (dd, 1H, $J_1 = 16.8$ and $J_2 = 13.7$ Hz, H_{3a}), 2.80 (dd, 1H, $J_1 = 17.2$ and $J_2 = 3.2$ Hz, H_{3b}), 7.59–6.83 (m, 6H, aromatic H), 9.35 (s, 1H, OH), 3.82 (s, 3H, 3′-OCH_3); ESI–MS (positive mode) *m/z* 316 $(M + H)^+$; Anal. Cald for $C_{16}H_{13}NO_6$: C, 60.29; H, 4.39; O, 27.78; N, 4.44. Found: C, 60.24; H, 4.25; O, 26.86; N, 4.35.

*2-(2-Hydroxyphenyl)-5,7-dimethoxychroman-4-one (**4a′**)* White solid, yield 53.3 %, mp 130–132 °C; IR (Nujol) υ 3511, 1678, 1608, 1604, 1275, 1239, 1214 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.75 (dd, 1H, $J_1 = 12.45$ and $J_2 = 3.46$ Hz, H-2), 3.02 (dd, 1H, $J_1 = 16.6$ and $J_2 = 13.65$ Hz, H_{3a}), 2.57 (dd, 1H, $J_1 = 16.77$ and $J_2 = 3.15$ Hz, H_{3b}), 7.32–6.30 (m, 6H, aromatic H), 9.40 (s, 1H, 2′-OH), 3.82 (s, 3H, 5-OCH_3), 3.86 (s, 3H, 7-OCH_3); ESI–MS (positive mode) *m/z* 301 $(M + H)^+$; Anal. Cald for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37; O, 25.96. Found: C, 66.77; H, 5.27; O, 25.23.

*2-(3-Hydroxyphenyl)-5,7-dimethoxychroman-4-one (**4b′**)* Colourless solid, yield 55.5 %, mp 134–135 °C; IR (Nujol) υ 3509, 1683, 1605,1601 1274,1237, 1211, 1123 cm^{-1}; 1H NMR ($CDCl_3$, 400 MHz) δ 5.85 (dd, 1H, $J_1 = 13.63$ Hz and $J_2 = 3.40$ Hz, H-2), 3.01 (dd, 1H, $J_1 = 17.5$ and $J_2 = 13.65$ Hz, H_{3a}), 2.54 (dd, 1H, $J_1 = 16.7$ and $J_2 = 3.10$ Hz, H_{3b}), 7.38–6.28 (m, 6H, aromatic H), 9.58 (s, 1H, 3′-OH), 3.85 (s, 3H, 5-OCH_3), 3.89 (s, 3H, 7-OCH_3); ESI–MS (positive mode) *m/z* 301 $(M + H)^+$; Anal. Cald for $C_{17}H_{16}O_5$: C, 67.88; H, 5.35; O, 25.95. Found: C, 67.77; H, 5.22; O, 23.58.

*2-(4-Hydroxyphenyl)-5,7-dimethoxychroman-4-one (**4c′**)* White solid, yield 60 %, mp 136–138 °C; IR (Nujol) υ 3508, 1680, 1611,1605, 1275,1240, 1209,1124 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.82 (dd, 1H, $J_1 = 12.50$ Hz and $J_2 = 3.24$ Hz, H-2), 3.04 (dd, 1H, $J_1 = 16.5$ and $J_2 = 13.58$ Hz, H_{3a}), 2.59 (dd, 1H, $J_1 = 16.78$ and $J_2 = 3.12$ Hz, H_{3b}), 7.35–6.30 (m, 6H, aromatic H), 9.54 (s, 1H, 4′-OH), 3.81 (s, 3H, 5-OCH_3), 3.84 (s, 3H, 7-OCH_3); ESI–MS (positive mode) *m/z* 301 $(M + H)^+$; Anal. Cald for $C_{17}H_{16}O_5$: C, 67.89; H, 5.36; O, 26.01. Found: C, 67.34; H, 5.01; O, 25.85.

*2-(3-Hydroxy-4-methoxyphenyl)-5,7-dimethoxychroman-4-one (**4d′**)* White solid, yield 59.7 %, mp 140–142 °C; IR (Nujol) υ 3505, 1680, 1608,1604 1269,1240, 1210, 1123, 1020 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.82 (dd, 1H, $J_1 = 12.48$ Hz and $J_2 = 3.04$ Hz, H-2), 3.01 (dd, 1H, $J_1 = 16.7$ and $J_2 = 13.60$ Hz, H_{3a}), 2.64 (dd, 1H, $J_1 = 16.5$ and $J_2 = 3.23$ Hz, H_{3b}), 7.37–6.31 (m, 5H, aromatic H), 10.05 (s, 1H, 3′-OH), 3.80 (s, 3H, 5-OCH_3), 3.86 (s, 3H, 7-OCH_3), 3.83 (s, 3H, 4′-OCH_3); ESI–MS (positive mode) *m/z* 331 $(M + H)^+$; Anal. Cald for $C_{18}H_{18}O_6$: C, 65.45; H, 5.50; O, 29.16. Found: C, 64.76; H, 5.22; O, 28.38.

*2-(4-Hydroxy-3-methoxyphenyl)-5,7-dimethoxychroman-4-one (**4e′**)* White solid, yield 61.2 %, mp 145–146 °C; IR (Nujol) υ 3519, 1678, 1605,1602 1274,1238, 1211, 1123, 1022 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.81 (dd, 1H, $J_1 = 12.44$ Hz and $J_2 = 3.01$ Hz, H-2), 3.01 (dd, 1H, $J_1 = 16.75$ and $J_2 = 13.68$ Hz, H_{3a}), 2.60 (dd, 1H, $J_1 = 17.3$ and $J_2 = 2.94$ Hz, H_{3b}), 7.32–6.35 (m, 5H, aromatic H), 10.02 (s, 1H, 4′-OH), 3.79 (s, 3H, 5-OCH_3), 3.87 (s, 3H, 7-OCH_3), 3.82 (s, 3H, 3′-OCH_3); ESI–MS (positive mode) *m/z* 331 $(M + H)^+$; Anal. Cald for $C_{18}H_{18}O_6$: C, 65.46; H, 5.48; O, 30.10. Found: C, 64.34; H, 4.72; O, 29.86.

*2-(4-Hydroxy-3,5-dimethoxyphenyl)-5,7-dimethoxychroman-4-one (**4f′**)* White solid, yield 53.4 %, mp 158–160 °C; IR (Nujol) υ 3511, 1680, 1605,1602 1275,1232, 1205, 1024 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.78 (dd, 1H, $J_1 = 12.78$ Hz and $J_2 = 3.12$ Hz, H-2), 3.04 (dd, 1H, $J_1 = 17.21$ and $J_2 = 13.62$ Hz, H_{3a}), 2.59 (dd, 1H, $J_1 = 16.79$ and $J_2 = 3.12$ Hz, H_{3b}), 7.31–6.34 (m, 4H, aromatic H), 9.58 (s, 1H, 4′-OH), 3.84 (s, 3H, 5-OCH_3), 3.88 (s, 3H, 7-OCH_3), 3.81 (s, 3H, 3′-OCH_3), 3.78 (s, 3H, 5′-OCH_3); ESI–MS (positive mode) *m/z* 361 $(M + H)^+$; Anal. Cald for $C_{19}H_{20}O_7$: C, 63.33; H, 5.59; O, 31.08. Found: C, 63.32; H, 5.57; O, 31.02.

*2-(4-Hydroxy-3-methoxy-5-nitrophenyl)-5,7-dimethoxychroman-4-one (**4g′**)* White solid, yield 55 %, mp 151–153 °C; IR (Nujol) υ 3514, 1680, 1604,1602 1272,1234, 1215,1024 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.81 (dd, 1H, $J_1 = 12.54$ Hz and $J_2 = 3.11$ Hz, H-2), 3.01 (dd, 1H, $J_1 = 16.78$ and $J_2 = 13.6$ Hz, H_{3a}), 2.81 (dd, 1H, $J_1 = 17.4$ and $J_2 = 3.22$ Hz, H_{3b}), 7.34–6.29 (m, 4H, aromatic H), 9.39 (s, 1H, 4′-OH), 3.80 (s, 3H, 5-OCH_3), 3.86 (s, 3H, 7-OCH_3), 3.82 (s, 3H, 3′-OCH_3); ESI–MS (positive mode) *m/z* 376 $(M + H)^+$; Anal. Cald for $C_{18}H_{17}NO8$: C, 57.62; H, 4.56; O, 34.10; N, 3.72. Found: C, 57.59; H, 4.52; O, 34.05; N, 3.67.

*General procedure for the synthesis of flavanone derivatives with carbamate moiety (**5a–5g** and **5a′–5g′**)*

The dissolved mixture of 0.01 mol of corresponding flavanones (**4a–4g** or **4a′–4g′**) and 0.01 mol of phenyl isocyanate in 20 ml of petroleum-ether and 2–3 drops of

triethylamine was refluxed for 15–20 min. Thereafter, the reaction mixture was allowed to stand for crystallisation followed by filtration to get target compounds (**5a**–**5g** or **5a′**–**5g′**) which was further recrystallized in ethyl alcohol till a single spot was observed on TLC plate with solvent mixture of diethylether:petroleum-ether in 9:1 ratio.

*2-(4-Oxochroman-2-yl)phenyl phenylcarbamate (**5a**)* White solid, yield 51.6 %, mp 138–140 °C; IR (Nujol) υ 3510, 3157, 1760, 1680, 1672, 1606,1602 1272,1238, 1212 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.43 (dd, 1H, $J_1 = 13.60$ Hz and $J_2 = 2.8$ Hz, H-2), 3.01 (dd, 1H, $J_1 = 16.7$ and $J_2 = 13.03$ Hz, H_{3a}), 2.76 (dd, 1H, $J_1 = 16.8$ and $J_2 = 3.2$ Hz, H_{3b}), 7.93–6.88 (m, 13H, aromatic H), 8.40 (s, 1H, NH); ESI–MS (positive mode) *m/z* 360 $(M + H)^+$; Anal. Cald for $C_{22}H_{17}NO_4$: C, 73.53; H, 4.77; O, 17.81; N, 3.90. Found: C, 72.95; H, 4.72; O, 17.78; N, 3.85.

*3-(4-Oxochroman-2-yl)phenyl phenylcarbamate (**5b**)* White solid, yield 53.2 %, mp 143–145° C; IR (Nujol) υ 3513, 3156, 1762, 1682, 1607,1603 1273,1240, 1210 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.42 (dd, 1H, $J_1 = 13.58$ Hz and $J_2 = 3.1$ Hz, H-2), 3.02 (dd, 1H, $J_1 = 16.8$ and $J_2 = 13.6$ Hz, H_{3a}), 2.79 (dd, 1H, $J_1 = 16.8$ and $J_2 = 3.1$ Hz, H_{3b}), 7.88–6.81 (m, 13H, aromatic H), 8.37 (s, 1H, NH); ESI–MS (positive mode) *m/z* 360 $(M + H)^+$; Anal. Cald for $C_{22}H_{17}NO_4$: C, 72.53; H, 4.67; O, 18.28; N, 3.70. Found: C, 72.48; H, 4.65; O, 18.24; N, 3.61.

*4-(4-Oxochroman-2-yl)phenyl phenylcarbamate (**5c**)* White solid, yield 58.6 %, mp 141–142 °C; IR (Nujol) υ 3512, 3158, 1761,1673, 1606, 1603 1273,1241, 1210 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.41 (dd, 1H, $J_1 = 13.59$ Hz and $J_2 = 2.9$ Hz, H-2), 3.03 (dd, 1H, $J_1 = 16.7$ and $J_2 = 13.6$ Hz, H_{3a}), 2.80 (dd, 1H, $J_1 = 16.9$ and $J_2 = 3.2$ Hz, H_{3b}), 7.87–6.81 (m, 13H, aromatic H), 8.39 (s, 1H, NH); ESI–MS (positive mode) *m/z* 360 $(M + H)^+$; Anal. Cald for $C_{22}H_{17}NO_4$: C, 74.33; H, 3.97; O, 18.28; N, 3.39. Found: C, 73.95; H, 3.78; O, 18.21; N, 3.25.

*2-Methoxy-5-(4-oxochroman-2-yl)phenyl phenylcarbamate (**5d**)* White solid, yield 60.1 %, mp 146–148 °C; IR (Nujol) υ 3510, 3157, 1763, 1674, 1607,1604 1273,1238, 1027 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.41 (dd, 1H, $J_1 = 13.58$ Hz and $J_2 = 2.9$ Hz, H-2), 3.01 (dd, 1H, $J_1 = 16.8$ and $J_2 = 13.68$ Hz, H_{3a}), 2.77 (dd, 1H, $J_1 = 17.2$ and $J_2 = 2.9$ Hz, H_{3b}), 7.85–6.82 (m, 12H, aromatic H), 8.12 (s, 1H, NH), 3.81 (s, 3H, 4′-OCH_3); ESI–MS (positive mode) *m/z* 390 $(M + H)^+$; Anal. Cald for $C_{23}H_{19}NO_5$: C, 70.33; H, 4.94; O, 20.28; N, 3.46. Found: C, 70.23; H, 4.72; O, 19.78; N, 3.39.

*2-Methoxy-4-(4-oxochroman-2-yl)phenyl phenylcarbamate (**5e**)* White solid, yield 61.7 %, mp 143–145 °C; IR (Nujol) υ 3514, 3158, 1763, 1675, 1607, 1602, 1273,1237, 1028 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.40 (dd, 1H, $J_1 = 13.61$ Hz and $J_2 = 3.1$ Hz, H-2), 2.99 (dd, 1H, $J_1 = 16.8$ and $J_2 = 13.52$ Hz, H_{3a}), 2.78 (dd, 1H, $J_1 = 16.7$ and $J_2 = 3.1$ Hz, H_{3b}), 7.76–6.81 (m, 12H, aromatic H), 8.23 (s, 1H, NH), 3.82 (s, 3H, 3′-OCH_3); ESI–MS (positive mode) *m/z* 390 $(M + H)^+$; Anal. Cald for $C_{23}H_{19}NO_5$: C, 70.63; H, 4.90; O, 20.32; N, 3.38. Found: C, 70.58; H, 4.74; O, 20.29; N, 3.14.

*2,6-Dimethoxy-4-(4-oxochroman-2-yl)phenyl phenylcarbamate (**5f**)* White solid, yield 54.9 %, mp 152–154 °C; IR (Nujol) υ 3515,3159, 1760, 1673, 1608,1604 1273,1237, 1027 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.43 (dd, 1H, $J_1 = 13.7$ Hz and $J_2 = 3.0$ Hz, H-2), 3.02 (dd, 1H, $J_1 = 16.8$ and $J_2 = 13.6$ Hz, H_{3a}), 2.79 (dd, 1H, $J_1 = 16.8$ and $J_2 = 2.98$ Hz, H_{3b}), 7.70–6.91 (m, 11H, aromatic H), 8.15 (s, 1H, NH), 3.82 (s, 3H, 3′-OCH_3), 3.78 (s, 3H, 5′-OCH_3); ESI–MS (positive mode) *m/z* 420 $(M + H)^+$; Anal. Cald for $C_{24}H_{21}NO_6$: C, 68.63; H, 5.05; O, 22.89; N, 3.34. Found: C, 67.95; H, 4.87; O, 21.78; N, 3.25.

*2-Methoxy-6-nitro-4-(4-oxochroman-2-yl)phenyl phenylcarbamate (**5g**)* White solid, yield 52.7 %, mp 150–152 °C; IR (Nujol) υ 3510,3157, 1761, 1678, 1607,1603 1275,1238, 1026 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.41 (dd, 1H, $J_1 = 13.6$ Hz and $J_2 = 2.88$ Hz, H-2), 3.03 (dd, 1H, $J_1 = 16.8$ and $J_2 = 13.7$ Hz, H_{3a}), 2.80 (dd, 1H, $J_1 = 17.2$ and $J_2 = 3.2$ Hz, H_{3b}), 7.86–6.81 (m, 11H, aromatic H), 8.32 (s, 1H, NH), 3.82 (s, 3H, 3′-OCH_3); ESI–MS (positive mode) *m/z* 435 $(M + H)^+$; Anal. Cald for $C_{23}H_{18}N_2O_7$: C, 63.63; H, 4.19; O, 25.32; N, 6.45. Found: C, 62.95; H, 4.05; O, 24.88; N, 6.39.

*2-(5′-7′-Dimethoxy-4-oxochroman-2-yl)phenyl phenylcarbamate (**5a′**)* White solid, yield 50.3 %, mp 153–155 °C; IR (Nujol) υ 3503, 3158, 1762, 1682, 1671, 1605,1603 1275,1234, 1211 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.41 (dd, 1H, $J_1 = 13.62$ Hz and $J_2 = 2.81$ Hz, H-2), 3.03 (dd, 1H, $J_1 = 16.8$ and $J_2 = 13.5$ Hz, H_{3a}), 2.79 (dd, 1H, $J_1 = 16.7$ and $J_2 = 3.3$ Hz, H_{3b}), 7.94–6.29 (m, 11H, aromatic H), 8.47 (s, 1H, NH), 3.83 (s, 3H, 5-OCH_3), 3.85 (s, 3H, 7-OCH_3); ESI–MS (positive mode) *m/z* 420 $(M + H)^+$; Anal. Cald for $C_{24}H_{21}NO_6$: C, 68.74; H, 5.05; O, 22.89; N, 3.34. Found: C, 68.55; H, 4.97; O, 22.76; N, 3.15.

*3-(5′-7′-Dimethoxy-4-oxochroman-2-yl)phenyl phenylcarbamate (**5b′**)* White solid, yield 51.6 %, mp 160–162 °C; IR (Nujol) υ 3510, 3158, 1760, 1681, 1608,1602 1274,1248, 1207 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz)

δ 5.43 (dd, 1H, $J_1 = 13.59$ Hz and $J_2 = 3.14$ Hz, H-2), 3.01 (dd, 1H, $J_1 = 16.78$ and $J_2 = 13.5$ Hz, H_{3a}), 2.80 (dd, 1H, $J_1 = 16.7$ and $J_2 = 3.10$ Hz, H_{3b}), 8.02–6.35 (m, 11H, aromatic H), 8.40 (s, 1H, NH), 3.84 (s, 3H, 5-OCH_3), 3.87 (s, 3H, 7-OCH_3); ESI–MS (positive mode) *m/z* 420 $(M + H)^+$; Anal. Cald for $C_{24}H_{21}NO_6$: C, 68.73; H, 5.04; O, 22.87; N, 3.30. Found: C, 67.95; H, 5.02; O, 22.78; N, 3.12.

*4-(5′-7′-Dimethoxy-4-oxochroman-2-yl)phenyl phenylcarbamate (**5c′**)* White solid, yield 54.8 %, mp 165–166 °C; IR (Nujol) υ 3510, 3159, 1760,1674, 1605, 1602 1274,1243, 1204 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.40 (dd, 1H, $J_1 = 13.64$ Hz and $J_2 = 2.92$ Hz, H-2), 3.04 (dd, 1H, $J_1 = 16.75$ and $J_2 = 13.62$ Hz, H_{3a}), 2.84 (dd, 1H, $J_1 = 16.8$ and $J_2 = 3.17$ Hz, H_{3b}), 7.92–6.32 (m, 11H, aromatic H), 8.38 (s, 1H, NH), 3.79 (s, 3H, 5-OCH_3), 3.85 (s, 3H, 7-OCH_3); ESI–MS (positive mode) *m/z* 420 $(M + H)^+$; Anal. Cald for $C_{24}H_{21}NO_6$: C, 68.72; H, 5.07; O, 22.88; N, 3.33. Found: C, 68.69; H, 4.73; O, 22.78; N, 3.23.

*2-Methoxy-5-(5′-7′-dimethoxy-4-oxochroman-2-yl)phenyl phenylcarbamate (**5d′**)* White solid, yield 59.9 %, mp 171–172 °C; IR (Nujol) υ 3503, 3156, 1761, 1671, 1604,1601 1271,1234, 1025 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.39 (dd, 1H, $J_1 = 13.53$ Hz and $J_2 = 2.97$ Hz, H-2), 3.02 (dd, 1H, $J_1 = 16.78$ and $J_2 = 13.69$ Hz, H_{3a}), 2.79 (dd, 1H, $J_1 = 17.1$ and $J_2 = 2.89$ Hz, H_{3b}), 7.94–6.35 (m, 10H, aromatic H), 8.32 (s, 1H, NH), 3.82 (s, 3H, 5-OCH_3), 3.88 (s, 3H, 7-OCH_3), 3.81(s, 3H, 4′-OCH_3); ESI–MS (positive mode) *m/z* 450 $(M + H)^+$; Anal. Cald for $C_{25}H_{23}NO_7$: C, 66.80; H, 5.17; O, 24.92; N, 3.10. Found: C, 66.65; H, 4.76; O, 23.78; N, 3.03.

*2-Methoxy-4-(5′-7′-dimethoxy-4-oxochroman-2-yl)phenyl phenylcarbamate (**5e′**)* White solid, yield 58.6 %, mp 169–170 °C; IR (Nujol) υ 3505, 3159, 1762, 1674, 1606, 1601, 1274,1236, 1025 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.38 (dd, 1H, $J_1 = 13.62$ Hz and $J_2 = 3.12$ Hz, H-2), 2.98 (dd, 1H, $J_1 = 16.7$ and $J_2 = 13.54$ Hz, H_{3a}), 2.80 (dd, 1H, $J_1 = 16.5$ and $J_2 = 3.12$ Hz, H_{3b}), 7.96–6.38 (m, 10H, aromatic H), 8.33 (s, 1H, NH), 3.90 (s, 3H, 5-OCH_3), 3.87 (s, 3H, 7-OCH_3), 3.82 (s, 3H, 3′-OCH_3); ESI–MS (positive mode) *m/z* 450 $(M + H)^+$; Anal. Cald for $C_{25}H_{23}NO_7$: C, 66.81; H, 5.18; O, 24.91; N, 3.11. Found: C, 66.71; H, 5.14; O, 23.78; N, 3.15.

*2,6-Dimethoxy-4-(5′,7′-dimethoxy-4-oxochroman-2-yl)phenyl phenylcarbamate (**5f′**)* White solid, yield 51.2 %, mp 178–179 °C; IR (Nujol) υ 3513,3156, 1760, 1673, 1610,1605, 1274,1240, 1023 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.40 (dd, 1H, $J_1 = 13.70$ Hz and $J_2 = 3.05$ Hz, H-2), 3.04 (dd, 1H, $J_1 = 16.84$ and $J_2 = 13.61$ Hz, H_{3a}), 2.77 (dd, 1H, $J_1 = 16.88$ and $J_2 = 3.04$ Hz, H_{3b}), 7.88–6.34 (m, 9H, aromatic H), 8.31 (s, 1H, NH), 3.85 (s, 3H, 5-OCH_3), 3.90 (s, 3H, 7-OCH_3), 3.82 (s, 3H, 3′-OCH_3), 3.78 (s, 3H, 5′-OCH_3); ESI–MS (positive mode) *m/z* 480 $(M + H)^+$; Anal. Cald for $C_{26}H_{25}NO_8$: C, 65.13; H, 5.25; O, 26.68; N, 2.93. Found: C, 63.97; H, 5.22; O, 25.85; N, 2.91.

*2-Methoxy-6-nitro-4-(5′,7′-dimethoxy-4-oxochroman-2-yl)phenyl phenylcarbamate (**5g′**)* White solid, yield 51.8 %, mp 174–175 °C; IR (Nujol) υ 3505, 3159, 1759, 1678, 1603, 1602, 1275, 1240, 1026 cm^{-1}; H^1 NMR ($CDCl_3$, 400 MHz) δ 5.40 (dd, 1H, $J_1 = 13.60$ Hz and $J_2 = 2.99$ Hz, H-2), 3.03 (dd, 1H, $J_1 = 16.84$ and $J_2 = 13.72$ Hz, H_{3a}), 2.85 (dd, 1H, $J_1 = 17.42$ and $J_2 = 3.24$ Hz, H_{3b}), 8.01–6.33 (m, 9H, aromatic H), 8.32 (s, 1H, NH), 3.79 (s, 3H, 5-OCH_3), 3.84 (s, 3H, 7-OCH_3), 3.82 (s, 3H, 3′-OCH_3); ESI–MS (positive mode) *m/z* 495 $(M + H)^+$; Anal. Cald for $C_{25}H_{22}N_2O_9$: C, 60.73; H, 4.49; O, 29.14; N, 5.65. Found: C, 60.69; H, 4.42; O, 27.88; N, 5.55.

Biological evaluation

Experimental animals

Swiss albino mice of either sex, weighing 20–25 g were employed for in vivo evaluation of learning and memory. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No. 107/1999/CPCSEA).

Drugs and chemicals

Scopolamine was dissolved in normal saline and the test drug was dissolved in 10 % dimethylsulfoxide (DMSO). All other reagents used in the present study were of analytical grade and freshly prepared.

In vitro estimation of cholinesterase inhibitory activity

The brain AChE inhibitory activity of the test compounds was assessed spectrophotometrically at 420 nm (Ellman *et al.,* 1961). The potency of test compounds were expressed IC_{50} with donepezil as the standard AChE inhibitor.

Assessment of learning and memory by Morris water maze

Morris water maze is one of the most commonly used animal models to test memory (Morris, 1984). It consists of

large circular pool and is divided into four quadrants (Q1, Q2, Q3, Q4). Each animal was subjected to trial of 120 s in the water maze for five consecutive days and the memory was assessed in terms of (i) escape latency time (ELT), i.e. the time taken by the animal to locate the hidden platform in the target quadrant (Q4) for the first 4 days of training (ii) time spent in target quadrant (Q4) on 5th day of trial, i.e. the day of retrieval.

Experimental protocol

Seven groups, each group comprising six Swiss albino mice, were employed in this study.

Group I: normal control Normal mice, without any treatment, were subjected to trials on the water maze for 5 days to note escape latency time (ELT) for first 4 days (an index of learning) and time spent in target quadrant (TSTQ) on 5th day of trial (an index of retrieval).

Group II: scopolamine control Scopolamine (0.4 mg/kg i.p.) was administered to each mouse, 30 min prior to each trial, for first 4 days of trials. In scopolamine-treated mice, the ELT and TSTQ were noted as described in group I.

*Group III, IV and V: compound **5f'** (2, 5 and 10 mg/kg) in scopolamine control* The test compound with the most potent AChE, i.e. compound **5f'** was administered by intraperitoneal route in different doses (2, 5 and 10 mg/kg) in scopolamine-treated mice (30 min before scopolamine administration) to each mouse before subjecting to first four trials and rest of the procedure was same as described in group I.

Group VII: vehicle in scopolamine control 10 % DMSO (1 ml/kg) was administered in scopolamine-treated mice (30 min before scopolamine administration) to each mouse before subjecting to first four trials and rest of the procedure was same as described in group I.

Group VII: donepezil in scopolamine control Donepezil (25 mg/kg) was administered in scopolamine-treated mice (30 min before scopolamine administration) to each mouse before subjecting to first four trials and rest of the procedure was same as described in group I.

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