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Synthesis of aminoalkyl-substituted coumarin derivatives as acetylcholinesterase inhibitors

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ABSTRACT

Alzheimer's disease, one of the most common forms of dementia, is a progressive neurodegenerative disorder symptomatically characterized by declines in memory and cognitive abilities. To date, the successful therapeutic strategy to treat AD is maintaining levels of acetylcholine by inhibiting acetylcholinesterase (AChE). In the present study, coumarin derivatives were designed and synthesized as AChE inhibitors based on the lead structure of scopoletin. Of those synthesized, pyrrolidine-substituted coumarins **3b** and **3f** showed ca. 160-fold higher AChE inhibitory activities than scopoletin. These compounds also ameliorated scopolamine-induced memory deficit in mice when administered orally at the dose of 1 and 2 mg/kg.

1. Introduction

Alzheimer's disease (AD), one of the most common forms of dementia, is a progressive neurodegenerative disorder symptomatically characterized by declines in memory and cognitive abilities.[1] AD is the fourth leading cause of death in developed countries, and because most AD patients are over 65 years old, numbers are expected to increase in parallel with population aging.[2] The estimated number of AD patients worldwide in 2006 was 26.6 million and is predicted to increase to 106.8 million in 2050.[3] The major pathological features of AD are β-amyloid plaque deposition and the presence of neurofibrillary tangles in the brain.[4] However, the precise causes of AD at the molecular level remain unclear despite many suggested hypotheses. Further effective therapy that can delay the neurodegeneration is not available yet.[5]

A deficit in cholinergic neurotransmission, due to the degeneration of cholinergic neurons, in the brain is believed to be one of the major causes of the memory impairments associated with AD.[6] The maintenance of acetylcholine (ACh) levels in the synaptic cleft by inhibiting acetylcholinesterase (AChE), which is responsible for the degradation of ACh, is the only known means of treating AD. The four AChE inhibitors used in clinical practice for treatment of AD are tacrine, donepezil, galantamine, and rivastigmine.[7] Recently, pharmaceutical agents developed based on the β-amyloid hypotheses such as semagacestat (γ-secretase inhibitor), bapineuzumab and solanezumab (anti-Aβ antibody) were failed in phase III clinical trials.[8,9] Therefore, novel AChE inhibitors possessing less adverse effects and more cognitive enhancing effects could contribute to extend the choices to AD patients and clinical practitioners.

(−)-Galantamine (**1**) is an alkaloid present in the Caucasian snow-drop (*Galanthus woronowii*) and in bulbs of the Amaryllidaceae family, and was recently approved by the US FDA and Europe for the treatment of AD.[10] (−)-Galantamine (**1**) is a selective, reversible, competitive AChE inhibitor and allosteric modulator of neural nicotinic acetylcholine receptors.[11] The clinical experiences of galantamine in AD patients are promising, but it is less potent than other approved AChE inhibitors like tacrine and often causes peripheral side effects.[12] Furthermore, the production of galantamine requires a lengthy series of reactions because of its complex structure.

Pharmacophore modeling provides a useful tool of identifying hit compounds of pharmacologic interest.[13] Recently, scopoletin (**2**) (a widely distributed coumarin derivative found in the Solanaceae family) was identified as a potential AChE inhibitor by virtual screening of a 3D database of natural products based on the complex structure of galantamine-AChE.[14] Furthermore, although scopoletin has much less AChE inhibitory activity (IC_{50} = 168.6 μM) than galantamine (**1**, IC_{50} = 3.2 μM), it was found to increase extracellular ACh concentrations in rat brain to the same level as **1**.

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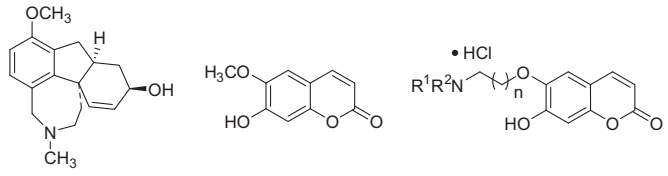
Accordingly, the aim of this study was to develop new coumarin derivatives starting from scopoletin with enhanced AChE inhibitory activities (Fig. 1).

A pharmacophore model generated from the three-dimensional structure of the galantamine-AChE complex and virtual screening based on this information indicated that the C-7 hydroxyl, lactone oxygen, and C-6 methoxy methyl in scopoletin are important features for AChE inhibitory activity, because they probably interact with the catalytic site of AChE as a H-bond donor, a H-bond acceptor, and a hydrophobic group, respectively.[14,15] Accordingly, based on these previous findings, we modified the C-6 methoxy methyl in **2** by incorporating aminoalkyl substituents. Additionally, amino groups were introduced into alkyl chain to increase water solubility, and thus, enhance oral bioavailability, and the effect of the length of the alkyl chain between C-6 oxygen and amino moieties was explored. Resultantly, a series of coumarin derivatives **3a**–**3j** were synthesized via a simple three-step sequence of reactions from scopoletin, and screened for their AChE inhibitory activities. In addition, synthesized compounds were also assessed for their memory ameliorating abilities in a mouse model of scopolamine-induced memory impairment using the passive avoidance task.



1, Galantamine **2**,Scopoletin **3**

Figure 1. Structures of galantamine (**1**), scopoletin (**2**) and target compounds (**3**).

2. Chemistry

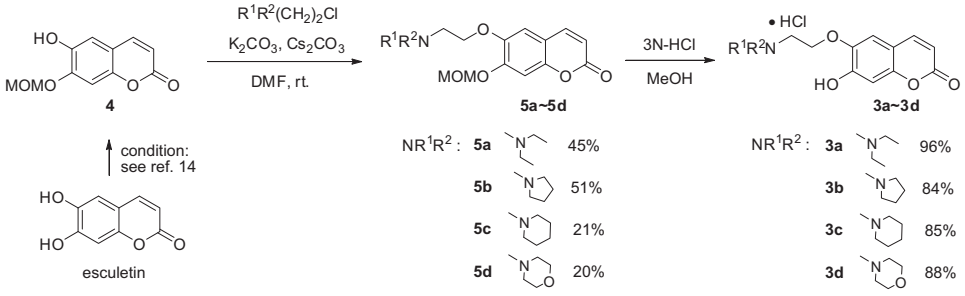
The syntheses of aminoethyl-substituted coumarin derivatives **3a**–**3d** were accomplished using the procedures shown in Scheme 1. C-7 MOM-protected esculetin (**4**), which was obtained from esculetin, as previously described,[16] was used as a starting material. Compound **4** was reacted with four commercially available aminoethyl chlorides in the presence of 1.5 equivalents of K_2CO_3 and 1 equiv of Cs_2CO_3, to provide **5a**–**5d** in 20–51% yields.[17] The MOM group in **5a**–**5d** was removed using 3 N HCl in methanol to afford **3a**–**3d** as hydrochloride salts in 85–95% yields.

Since many aminopropyl chlorides are not available commercially, aminopropyl-substituted coumarin derivatives **3e**–**3j** were prepared by synthesizing 3-halopropyloxy coumarin **6**, as shown in Scheme 2. Compound **4** was reacted with 1-bromo-3-chloropropane to give 3-chloropropyl-substituted coumarin. 3-Bromopropyl-substituted coumarin was also obtained as a minor product and the combined yield of the 3-halopropyl-substituted coumarin **6** was ca. 80%. Because both chloropropyloxy- and bromopropyloxy coumarins give the same products in next step, the mixture of 3-halopropyl-substituted coumarin **6** was reacted with various amines in the presence of K_2CO_3 and Cs_2CO_3 to afford **7**. Subsequent removal of the MOM group of **7** using 3 N HCl in methanol afforded **3e**–**3j** as hydrochloride salts in 16–38% overall yields for two steps from **6**.

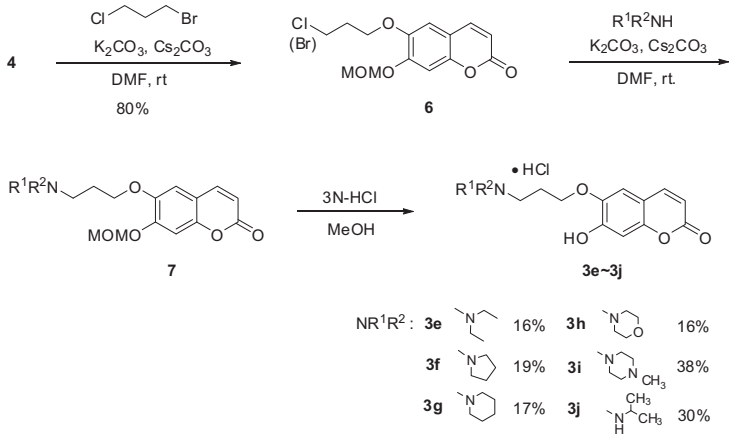
3. Results and discussion

3.1. The AChE inhibitory effects of coumarin derivatives

To determine inhibitory activities of compounds **3a**–**3j** on AChE, in vitro AChE inhibition assays were conducted according to the



Scheme 1.



Scheme 2.

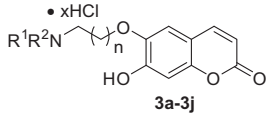
modification of Ellman's method[18,19] using mouse brain homogenates. Scopoletin and galantamine were used as standards, and results are summarized in Table 1. Initially, preliminary screening for AChE inhibitory activities were performed at 100 μM. Selected compounds exhibiting more than 50% inhibition, were re-screened at half-log scale concentrations to elucidate their IC_{50} values. Almost all synthesized compounds were found to be more potent than scopoletin. Furthermore, potency was found to be influenced substantially by amine substituents, but not by alkyl chain length. The pyrrolidine-substituted coumarins **3b** and **3f** were found to have the most potent inhibitory activities with IC_{50} values of 6.85 and 2.87 μM, and the potency of **3f** was ca. 160-fold higher than scopoletin (IC_{50} = 476.37 μM) and nearly equal to that of galantamine (IC_{50} = 2.50 μM). The diethylamine, that is, the open analog of pyrrolidine ring, and piperidine, and isopropylamine-substituted coumarins also showed potent AChE inhibitory activities. However, replacement of the pyrrolidine ring in **3b** or **3f** with a morpholine or a piperazine ring, both of which have additional hetero atoms, significantly reduced AChE inhibition (i.e., **3d**, **3h**–**3j**) implicating the requirement of hydrophobic interactions in this binding region of the enzymes for strong inhibition.

3.2. The amelioration of scopolamine-induced memory deficit in mice

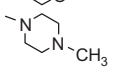
Many efforts have been made, based on the cholinergic hypothesis, to develop AChE inhibitors that improve cognition functions in AD patients.[6] Thus, as the synthesized coumarin derivatives showed potent AChE inhibitory effects in vitro, we sought to determine whether they ameliorate impaired learning and memory functions in vivo. Accordingly, the compounds **3b** and **3f**, which had the greatest AChE inhibitory activities, were tested for their effects on scopolamine-induced learning and memory impairments using the passive avoidance task. The reversal of scopolamine-induced memory deficit in the passive avoidance task has been widely used to screen for agents that ameliorate cognition dysfunction. During the acquisition trial, no differences in latencies were observed among tested groups, which indicated that **3b** and **3f** do not affect the general behavior of mice. The activity profiles of **3b** and **3f** in this model are shown in Figure 2

For retention trials, the mean step-through latency of scopolamine-treated (1 mg/kg, ip) mice was significantly shorter than that of saline-treated controls (P <0.05). Galantamine (1 mg/kg, p.o.) as a positive control significantly increased step-through versus scopolamine treated mice. Compounds **3b** and **3f** also antagonized scopolamine-induced memory deficit in mice when orally administered at 0.25, 0.5 and 5 mg/kg. These compounds reversed clearly the shortened step-through latency induced by scopolamine. Inverted U-shaped dose–response relationships were found with significantly (P <0.05) longer latencies for doses of 2 mg/kg (**3b**) and 1 mg/kg (**3f**) as compared with the scopolamine treated animals. Such inverted U-shaped dose–response relationship which was observed in compound **3b** or **3f** has been well recognized in the research area of learning and memory. Calabrese reviewed a general biological model to attempt to explain this dose–response, namely, the hormetic dose–response model.[20] Under the same conditions, the relative effects of **3b** (2 mg/kg) and **3f** (1 mg/kg) to increase step-through latency were similar to that of galantamine (1 mg/kg, p.o.) (Fig. 2). Finally, coumarin derivatives **3b** and **3f** exhibited memory enhancing effects even higher than galantamine by increasing the latency on passive avoidance response in scopolamine-induced memory deficit model.

Table 1
The acetylcholinesterase (AChE) inhibitory activities of **3a**–**3j**, scopoletin, and galantamine



3a-3j

Compounds	*n*	R_1R_2	% AChE inhibition at 100 (μM)	AChE inhibition IC_{50} (μM)[a]
3a	1		69.42	18.65 ± 2.37
3b	1		93.95	6.85 ± 0.38
3c	1		75.48	12.16 ± 2.22
3d	1		37.68	–
3e	2		70.4	30.53 ± 4.28
3f	2		95.14	2.87 ± 0.17
3g	2		76.29	19.09 ± 1.53
3h	2		2.44	–
3i	2		40.44	–
3j	2		80.79	26.98 ± 2.95
Scopoletin			2.49	476.37 ± 79.54
Galantamine			91.94	2.50 ± 0.14

[a] 50% Inhibitory concentration (means ± SEMs of three experiments).

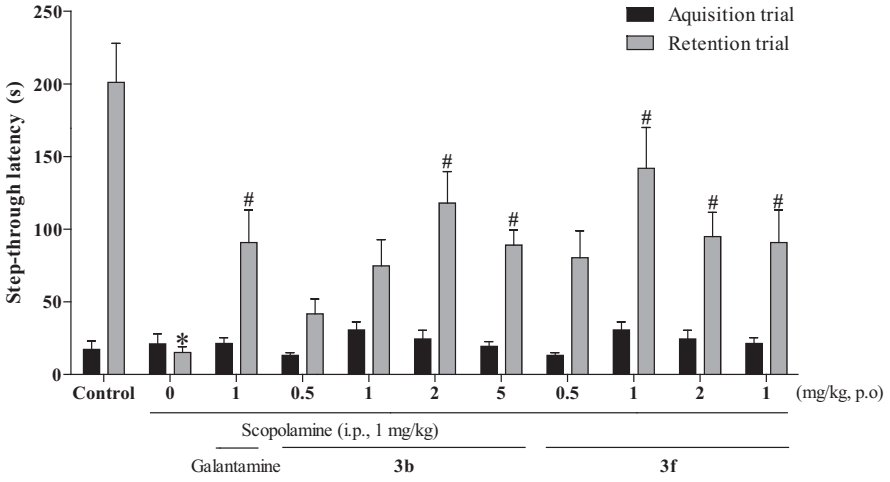


Figure 2. Effects of **3b**, **3f**, and galantamine on the passive avoidance task in scopolamine-induced memory deficit model. *P <0.05 versus vehicle-treated controls. #P <0.05 versus scopolamine-treated group. Data are expressed as mean ± SEM.

4. Conclusions

New coumarin derivatives were designed and synthesized using the structure of scopoletin as a starting point. Almost all synthesized compounds potently inhibited AChE than scopoletin. Of the synthesized, pyrrolidine-substituted coumarins, **3b** and **3f** had the greatest inhibitory effects, and the potency of **3f** was ca. 160-fold higher than scopoletin, and nearly equal to that of galantamine. Oral administrations of **3b** and **3f** also ameliorated scopolamine-induced memory deficit in mice, and the effects of **3b** (2 mg/kg) and **3f** (1 mg/kg) on step-through latency were greater than galantamine (1 mg/kg). These results suggest that **3b** and **3f** have the ability to maintain ACh levels in the synaptic cleft, and thus, to improve learning and memory by inhibiting AChE. Furthermore, the syntheses of **3b** and **3f** are relatively straightforward because these compounds do not have stereogenic centers as galantamine in the structures.

5. Experimental section

5.1. Instrumentation and chemicals

NMR spectra were recorded on a Brucker Avance 400 spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR spectra. Analytical thin-layer chromatography (TLC) was performed on TLC Silica gel (thickness 0.20 mm). Column chromatography was performed with silica gel Merck Silica gel 60 (230–400 mesh). All solvents, chemicals and reagents were purchased from Aldrich, Acros or TCI.

5.2. Syntheses

5.2.1. 6-(2-(Diethylamino)ethoxy)-7-(methoxymethoxy)-2*H*-chromen-2-one (5a)

To a solution of **4**[15] (83 mg, 0.37 mmol) and 2-(diethylamino)ethyl chloride (96 mg, 0.56 mmol) in DMF (5 ml) was added K_2CO_3 (77 mg, 0.56 mmol) and Cs_2CO_3 (120 mg, 0.37 mmol). The reaction mixture was stirred at rt for 3 d. The mixture was concentrated in vacuo and partitioned between H_2O and CH_2Cl_2. The separated organic layer was washed with 10% NaOH and brine, dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure and the residue was solidified from ethyl acetate and *n*-hexane to give **5a** (54 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (1H, d, J = 9.6 Hz), 7.06 (1H, s), 6.97 (1H, s), 6.24 (1H, d, J = 9.6 Hz), 5.21 (2H, s), 4.27 (2H, t, J = 5.6 Hz), 3.42 (3H, s), 3.05 (2H, t, J = 5.6 Hz), 2.82 (4H, q, J = 7.2 Hz), 0.98 (6H, t, J = 7.2 Hz).

5.2.1.1. 7-(Methoxymethoxy)-6-(2-(pyrrolidin-1-yl)ethoxy)-2H-chromen-2-one (5b). The compound **5b** (73 mg) was prepared from **4** (100 mg, 0.45 mmol) and 1-(2-chloroethyl)pyrrolidine (116 mg, 0.68 mmol) using the procedure described for **5a**. Yield 51%. ^{1}H NMR (400 MHz, CD_3OD) δ 7.79 (1H, d, J = 9.6 Hz), 7.13 (1H, s), 7.03 (1H, s), 6.20 (1H, d, J = 9.6 Hz), 5.21 (2H, s), 4.10 (2H, t, J = 5.6 Hz), 3.40 (3H, s), 2.89 (2H, t, J = 5.6 Hz), 2.62–2.65 (4H, m), 1.73–1.79 (4H, m).

5.2.1.2. 7-(Methoxylmethoxy)-6-(2-(piperidin-1-yl)ethoxy)-2H-chromen-2-one (5c). The compound **5c** (16 mg) was prepared from **5** (50 mg, 0.23 mmol) and 1-(2-chloroethyl)piperidine (54 mg, 0.29 mmol) using the procedure described for **5a**. Yield 21%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (1H, d, J = 9.6 Hz), 7.17 (1H, s), 7.00 (1H, s), 6.24 (1H, d, J = 9.6 Hz), 5.27 (2H, s), 4.37 (2H, br s), 3.49 (3H, s), 3.07 (2H, br s), 2.82 (2H, br s), 2.66 (2H, br s), 1.55 (4H, br s), 1.25 (2H, br s).

5.2.1.3. 7-(Methoxylmethoxy)-6-(2-(morpholinoethoxy)-2H-chromen-2-one (5d). The compound **5d** (30 mg) was prepared from **5** (100 mg, 0.45 mmol) and 4-(2-chloroethyl)morpholine (126 mg, 0.68 mmol) using the procedure described for **5a**. Yield 20%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (1H, d, J = 9.6 Hz), 7.14 (1H, s), 6.98 (1H, s), 6.31 (1H, d, J = 9.6 Hz), 5.30 (2H, s), 4.31 (2H, br s), 3.86 (4H, br s), 3.54 (3H, s), 2.66–3.02 (6H, m).

5.2.2. 6-(2-(Diethylamino)ethoxy)-7-hydroxy-2*H*-chromen-2-one hydrochloride (3a)

A solution of **5a** (50 mg, 0.16 mmol) in 3 N HCl (0.5 ml) and MeOH (2 ml) was stirred at 85 °C for 3 h. The solvent was removed, and the residue was recrystallized from ethyl acetate and *n*-hexane to afford **3a** (47 mg, 96%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (1H, d, J = 9.6 Hz), 7.34 (1H, s), 6.92 (1H, s), 6.25 (1H, d, J = 9.6 Hz), 4.38 (2H, br s), 3.53 (2H, br s), 3.21 (4H, q, J = 7.2 Hz), 1.27 (6H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 162.3, 151.5, 150.6, 144.5, 143.6, 111.8, 111.7, 111.3, 103.0, 63.4, 50.8, 48.4, 7.8.

5.2.2.1. 7-Hydroxy-6-(2-(pyrrolidin-1-yl)ethoxy)-2H-chromen-2-one hydrochloride (3b). The compound **3b** (60 mg) was prepared from **5b** (73 mg, 0.23 mmol) using the procedure described for **3a**. Yield 84%. ^{1}H NMR (400 MHz, CD_3OD) δ 7.87 (1H,

d, J = 9.5 Hz), 7.27 (1H, s), 6.85 (1H, s), 6.26 (1H, d, J = 9.5 Hz), 4.40 (2H, t, J = 5.6 Hz), 3.72 (2H, t, J = 5.6 Hz), 3.37–3.38 (2H, m), 3.30–3.34 (2H, m), 2.17–2.21 (4H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 163.6, 152.8, 152.1, 145.8, 145.0, 113.3, 113.2, 112.8, 104.5, 65.8,55.5, 55.0, 49.7, 49.3, 49.2, 23.9.

5.2.2.2. 7-Hydroxy-6-(2-piperidin-1-yl)ethoxy)-2H-chromen-2-one hydrochlroride (3c). The compound **3c** (10 mg) was prepared from **5c** (12 mg, 0.04 mmol) using the procedure described for **3a**. Yield 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.76 (1H, d, J = 9.6 Hz), 7.16 (1H, s), 6.73 (1H, s), 6.14 (1H, d, J = 9.6 Hz), 4.33 (2H, br s), 3.52–3.59 (2H, m), 3.38–3.42 (2H, m), 2.91 (2H, br s), 1.71–2.05 (6H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 163.6, 152.9, 152.2, 145.7, 145.0, 113.4, 113.3, 112.8, 104.5, 64.5, 54.7, 24.2, 22.7.

5.2.2.3. 7-Hydroxy-6-(2-morpholinoethoxy)-2H-chromen-2-one hydrochloride (3d). The compound **3d** (25 mg) was prepared from **5d** (29 mg, 0.09 mmol) using the procedure described for **3a**. Yield 88%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (1H, d, J = 9.6 Hz), 7.33 (1H, s), 6.86 (1H, s), 6.26 (1H, d, J = 9.6 Hz), 4.39 (2H, m), 3.87–4.05 (4H, m), 3.47–3.59 (4H, m), 3.20 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 163.5, 152.8, 152.1, 145.7, 144.9, 113.5, 113.4, 112.8, 104.4, 65.0, 64.2, 57.4, 53.5.

5.2.3. 6-(3-Chloropropoxy)-7-(methoxymethoxy)-2*H*-chromen-2-one (6)

To a mixture of **4** (79 mg, 0.35 mmol) and 1-bromo-3-chloropropane (165 mg, 1.05 mmol) in DMF (5 ml) was added K_2CO_3 (75 mg, 0.54 mmol) and Cs_2CO_3 (114 mg, 0.35 mmol). The reaction mixture was stirred at rt for 1 d. The mixture was concentrated in vacuo and partitioned between CH_2Cl_2 and H_2O. The organic layer was separated and was with 10% NaOH and brine, dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure and the residue was purified by column chromatography (ethyl acetate/n-hexane = 2:1) to give **6** (92 mg, 86%, calculated based on 3-chloropropoxy compound). For major peaks; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 & 7.50 (1H, two d, J = 9.5 Hz), 7.06 & 6.99 (1H, two s), 6.88 & 6.86 (1H, two s), 6.23 & 6.22 (1H, two d, J = 9.5 Hz), 5.20 (2H, s), 4.12–4.16 (2H, m), 3.58–3.72 (2H, m), 3.44 (3H, s), 2.18–2.28 (2H, m).

5.2.4. 6-(3-(Diethylamino)propoxy)-7-hydroxy-2*H*-chromen-2-one hydrochloride (3e)

To a solution of **6** (90 mg, 0.30 mmol) and diethylamine (17 mg, 0.20 mmol) in DMF (4 ml) was added K_2CO_3 (48 mg, 0.40 mmol) and Cs_2CO_3 (75 mg, 0.20 mmol). The reaction mixture was stirred at rt for 3 d. The mixture was concentrated in vacuo and partitioned between H_2O and CH_2Cl_2. The separated organic layer was washed with 10% NaOH and brine, dried over $MgSO_4$, and filtered. After evaporation of solvent, the residue was treated with 3 N HCl (1 ml) in MeOH (2 ml) and stirred at 85 °C for 3 h. The reaction mixture was concentrated and partitioned between H_2O and CH_2Cl_2, and the separated aqueous layer was washed with CH_2Cl_2. The aqueous layer was concentrated and was recrystallized from dichloromethane and ethyl acetate to afford **3e** (12 mg, 16%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.75 (1H, d, J = 9.4 Hz), 7.09 (1H, s), 6.71 (1H, s), 6.12 (1H, d, J = 9.4 Hz), 4.13 (2H, t, J=5.5 Hz), 3.36–3.33 (4H, m), 3.22–3.20 (2H, m), 2.12–2.16 (2H, m), 1.26 (6H, q, J = 7.3 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 163.8, 151.8, 145.9, 145.9, 112.9, 112.6, 112.2, 104.2, 68.2, 51.3, 49.3, 24.9, 9.2.

5.2.4.1. 7-Hydroxy-6-(3-(pyrrolidin-1-yl)propoxy)-2H-chromen-2-one hydrochloride (3f). The compound **3f** (15 mg) was prepared from **6** (92 mg, 0.31 mmol) and pyrrolidine (17.0 mg, 0.24 mmol) using the procedure described for **3e**. Yield 19%. ^{1}H NMR (400 MHz, CD_3OD) δ 7.87 (1H, d, J = 9.4 Hz), 7.20 (1H, s), 6.81 (1H, s), 4.23 (2H, t, J = 5.0 Hz), 3.30–3.42 (6H, m), 2.30–2.32 (2H, m), 1.93 (4H, br s); ^{13}C NMR (100 MHz, CD_3OD) δ 163.7, 151.5, 146.1, 145.9, 145.8, 113.0, 112.7, 112.6, 104.1, 68.5, 56.8, 54.7, 24.8, 24.3.

5.2.4.2. 7-Hydroxy-6-(3-(piperidin-1-yl)propoxy)-2H-chromen-2-one hydrochloride (3g). The compound **3g** (15 mg) was prepared from **6** (100 mg, 0.33 mmol) and piperidine (22 mg, 0.26 mmol) using the procedure described for **3e**. Yield 17%. ^{1}H NMR (400 MHz, CD_3OD) δ 7.87 (1H, d, J = 9.4 Hz), 7.20 (1H, s), 6.83 (1H, s), 6.25 (1H, d, J = 9.4 Hz), 4.24 (2H, t, J = 5.4 Hz), 3.32–3.42 (6H, m), 2.17–2.33 (4H, m), 1.80–2.00 (4H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 162.2, 149.9, 144.5, 144.2, 111.3, 111.1, 110.1, 102.5, 66.7, 55.0, 52.9, 23.3, 22.8, 21.2.

5.2.4.3. 7-Hydroxy-6-(3-morpholinopropoxy)-2H-chromen-2-one hydrochloride (3h). The compound **3h** (14 mg) was prepared from **6** (100 mg, 0.33 mmol) and morpholine (23 mg, 0.26 mmol) using the procedure described for **3e**. Yield 16%. ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (1H, d, J = 9.5 Hz), 7.25 (1H, s), 6.84 (1H, s), 6.25 (1H, d, J = 9.5 Hz), 4.26 (2H, t, J = 5.4 Hz), 3.97–4.07 (4H, m), 3.48–3.54 (4H, m), 3.32–3.33 (2H, m), 2.36–2.42 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 163.9, 152.8, 151.6, 146.2, 145.8, 112.9, 112.7, 111.7, 104.1, 68.1, 65.1, 56.8, 53.4, 24.6.

5.2.4.4. 7-Hydroxy-6-(3-(4-methylpiperazin-1-yl)propoxy)-2H-chromen-2-one dihydrochloride (3i). The compound **3i** (34 mg) was prepared from **6** (53 mg, 0.18 mmol) and 1-methylpiperazine (23 mg, 0.22 mmol) using the procedure described for **3e**. Yield 38%. ^{1}H NMR (400 MHz, CD_3OD) δ 7.87 (1H, d, J = 9.5 Hz), 7.20 (1H, s), 6.83 (1H, s), 6.25 (1H, d, J = 9.5 Hz), 4.26 (2H, t, J = 5.5 Hz), 3.55–3.60 (4H, m), 3.32–3.34 (4H, m), 3.03 (3H, s), 2.36–2.43 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 163.9, 151.6, 146.1, 145.9, 145.8, 113.1, 112.8, 112.7, 104.1, 67.9, 54.1, 51.2, 35.4, 24.9.

5.2.4.5. 7-Hydroxy-6-(3-(isopropylamino)propoxy)-2H-chromen-2-one hydrochloride (3j). The compound **3j** (24 mg) was prepared from **6** (100 mg, 0.33 mmol) and isopropylamine (15 mg, 0.26 mmol) using the procedure described for **3e**. Yield 30%. ^{1}H NMR (400 MHz, CD_3OD) δ 7.86 (1H, d, J = 9.4 Hz), 7.19 (1H, s), 6.80 (1H, s), 6.22 (1H, d, J = 9.4 Hz), 4.25 (2H, t, J = 5.6 Hz), 3.46 (1H, m), 3.32–3.36 (2H, m), 2.22–2.28 (2H, m), 1.40–1.41 (6H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 163.8, 152.9, 151.7, 145.9, 113.0, 112.7, 111.9, 104.0, 68.7, 52.1, 44.7, 27.1, 19.3.

5.3. Acetylcholinesterase inhibition assay

Acetylcholinesterase activity assays were carried out using acetylthiocholine iodide as synthetic substrate based on colorimetric method, as described elsewhere.[18,19] Whole brains of male ICR mice (25–30 g) were homogenized in a glass Teflon homogenizer (Eyela, Japan) containing 50 volumes of phosphate buffer (pH 8.0, 0.1 M), and then centrifuged at 14,000 rpm for 20 min at 4 °C. The supernatant obtained was used as a source of enzyme for the assay. Each drug was initially dissolved in dimethyl sulfoxide (DMSO) and diluted to various concentrations immediately before use. An aliquot of diluted drug solution was then mixed with 640 μl of phosphate buffer (0.1 M, pH 8.0), 25 μl of buffered Ellman's reagent (10 mM 5,5′-dithio-bis[2-nitrobenzoic acid] and 15 mM sodium bicarbonate), and the enzyme source (100 μl) and pre-incubated at room temperature for 10 min. Then 5 μl of acetylthiocholine iodide solution (75 mM) was added to this mixture and mixed. Absorbance was measured at 410 nm after 10 min for the reaction adding 5 μl of acetylthiocholine iodide solution (75 mM) and to the reaction mixtures (OPTIZEN 2120UV, Mecasys Co. Ltd, Korea). The concentration of each drug required to inhibit

acetylcholinesterase activity by 50% (IC_{50}) was calculated using an enzyme inhibition dose response curve. Galantamine was used as a positive control.

5.4. In vivo assay

5.4.1. Animals

Male ICR mice (25–30 g) were purchased from the Orient Co., Ltd, a branch of Charles River Laboratories (Seoul, Republic of Korea). Animals were housed 5 per cage with food and water available ad libitum and maintained under a constant temperature (23 ± 1 °C) and humidity (60 ± 10%) under a 12 h light/dark cycle (light on 07:30–19:30 h). All behavioral tasks were conducted between 10:00 h and 16:00 h. Animal treatment and maintenance were conducted in accordance with the Principle of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Animal Care and the Use Guidelines of Kyung Hee University, Republic of Korea.

5.4.2. Passive avoidance task

Passive avoidance tasks were carried out in identical illuminated and non-illuminated boxes (20 × 20 × 20 cm), separated by a guillotine door (5 × 5 cm) as described elsewhere.[19] The illuminated compartment contained a 50 W bulb, and the floor of the non-illuminated compartment (20 × 20 × 20 cm) was composed of 2 mm stainless steel rods spaced 1 cm apart. For an acquisition trial, mice were initially placed in the illuminated compartment; the door between the two compartments was opened 10 s later. When the mice entered the dark compartment, the door automatically closed and an electrical foot shock (0.5 mA) for 3 s was delivered through the stainless steel rods. One hour before the acquisition trial, mice were orally administered 0.9% vehicle saline or various doses of test compounds dissolved in 0.9% saline (0.5, 1, 2, or 5 mg/kg for **3b**, 0.25, 0.5, 1, 2 mg/kg for **3f**). The galantamine (1 mg/kg) was orally administered as a positive control. Scopolamine (1 mg/kg) was intraperitoneally administered 30 min before the acquisition trial. Twenty-four hours after the acquisition trial, retention trials were conducted by re-placing mice in the illuminated compartment. The time taken for a mouse to enter the dark compartment after opening the door was defined as latency for both acquisition and retention trials. Latency to enter the dark compartment was recorded up to 300 s to avoid ceiling effects.

5.4.3. Statistical analysis

Data from the passive avoidance task was analyzed by Kruskal–Wallis non-parametric test fol lowed by Dunn's post hoc test. All statistical analysis was processed using Sigmastat software (Systat Software, IL 60606). Statistical significance was accepted for P values of <0.05.

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References and notes

1. Pakaski, M.; Kalman, J. *Neurochem. Int.* **2008**, *53*, 103.
2. Farlow, M. R. *Alzheimer Dis. Assoc. Disord.* **1994**, *8*, S58.
3. Brookmeyer, R. E.; Ohnson, J. E.; Ziegler-Graham, K.; Arrighi, H. M. *Alzheimer's Dement.* **2007**, *3*, 186.
4. Perl, D. P. *Mt Sinai J. Med.* **2010**, *77*, 32.
5. Van Marum, R. J. *Fundam. Clin. Pharmacol.* **2008**, *22*, 265.
6. Francis, P. T.; Palmer, A. M.; Snape, M.; Wilcock, G. K. *J. Neurol. Neurosurg. Psychiatry* **1999**, *66*, 137.
7. Francotte, P.; Graindorge, E.; Boverie, S.; de Tullio, P.; Pirotte, B. *Curr. Med. Chem.* **2004**, *11*, 1757.
8. Tayeb, H. O.; Murray, E. D.; Price, B. H.; Tarazi, F. I. *Expert Opin. Biol. Ther.* **2013**, *13*, 1075.
9. Blennow, K.; Zetterberg, H.; Haass, C.; Finucane, T. *Nat. Med.* **2013**, *19*, 1214.
10. Bores, G. M.; Kosley, R. W. *Drugs Future* **1996**, *21*, 621.
11. Lilienfeld, S. *CNS Drug Rev.* **2002**, *8*, 159.
12. Wilcock, G. K.; Lilienfeld, S.; Gaens, E. *BMJ* **2000**, *321*, 1445.
13. Langer, T.; Krovat, E. M. *Curr. Opin. Drug Disc. Dev.* **2003**, *6*, 370.
14. Rollinger, J. M.; Hornick, A.; Langer, T. *J. Med. Chem.* **2004**, *47*, 6248.
15. Nam, S. O.; Yun, Y. D.; Park, D. H.; Ryu, J. H.; Lee, Y. S. *Yakhak Hoeji* **2011**, *55*, 473.
16. Nemoto, T.; Ohshima, T.; Shibasaki, M. *Tetrahedron* **2003**, *59*, 6889.
17. Grice, C. A.; Tays, K. L.; Savall, B. M.; Wei, J.; Butler, C. R.; Axe, F. U.; Bembenek, S. D.; Fouri, A. M.; Dunford, P. J.; Lundeen, K.; Coles, F.; Xue, X.; Riley, J. P.; Williams, K. N.; Karlsson, L.; Edwards, J. P. *J. Med. Chem.* **2008**, *51*, 4150.
18. Ellman, G. L.; Courtney, K. D.; Andres, V., Jr.; Feather-Stone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
19. Kim, D. H.; Hung, T. M.; Bae, K. H.; Jung, J. W.; Lee, S.; Yoon, B. H.; Cheong, J. H.; Ko, K. H.; Ryu, J. H. *Eur. J. Pharmacol.* **2006**, *542*, 129.
20. Calabrese, E. J. *Crit. Rev. Toxicol.* **2008**, *38*, 419.