

Structural optimization of coumarin-pyridine hybrid derivative salts as potent acetylcholinesterase inhibitors

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Abstract

Coumarins playing multiple roles in the life of plants also exhibit a wide range of positive effects for human health. Their basic skeletons have shared characteristics with the partial structure of donepezil [the US FDA-approved drug used to treat Alzheimer's disease (AD)]. The objective of this study was to elucidate the possible structure-activity relationships of coumarin-type derivative salts incorporating benzylpyridinium units against acetylcholinesterase (AChE) *in vitro*. Our findings highlight the great potential of coumarin- and isocoumarin-pyridine hybrid derivative salts as AChE inhibitors.

Introduction

Coumarins constitute a vast group of phenolics that predominantly found in various plant species [1]. Their carbon skeleton which is known as α -benzopyranone core (C_6-C_3), originate from the phenylpropanoid pathway. From several viewpoints such as plant physiology, chemical defense, and plant-environment interactions, coumarin constituents are of considerable importance in plants [2–4].

Various plant-based medicinal coumarins and their derived analogues have demonstrated prominent therapeutic efficacy against human diseases [5]. Warfarin (brand name: Coumadon) has been utilized for decades as a widely prescribed anticoagulant for the prophylaxis and treatment of venous thrombosis and thromboembolic events [6]. Phenprocoumon (Marcoumar) and acenocoumarol (Acitrom 3), coumarin-type anticoagulants, are counterparts of warfarin used in the clinic [7,8]. Photochemotherapy with psoralens [methoxsalen (Oxsoralen) and trioxsalen (Agilen-25)] is commonly applied to skin diseases including psoriasis, eczema, vitiligo, and some cutaneous lymphomas [9,10].

Coumarin-heterocycle conjugates have attracted extensive scientific attention over the years due to their health benefits. Numerous researches prove that they possess a broad spectrum of favorable biological activities, including antimicrobial, anticancer, antidiabetic, antitubercular, neuroprotective properties, and varying enzyme inhibitions [11–14]. Coumarins are privileged scaffolds in medicinal chemistry, and their hybrid molecules contribute to future drug discovery.

Recently, we designed a series of donepezil-inspired flavonoid derivative salts focusing on their structural similarities [15]. Among them, fluorinated aurone-pyridine hybrid derivative salts remarkably inhibited the enzymic activity of acetylcholinesterase (AChE) and effectively worked on neuroprotection, neurogenesis, and amyloid- β aggregation *in vitro*. Within the scope of our ongoing program aimed to explore phytochemical-based chemotherapeutics from naturally occurring phenolics, we herein report the results of synthesis and structure-activity relationship analysis of coumarin-pyridine hybrid derivative salts as Alzheimer's disease (AD) drug candidates.

Results and discussion

Initially, benzylpyridyl moiety was introduced into the coumarin core in imitation of donepezil. As shown in Scheme 1, Knoevenagel condensation of 2-hydroxy-4,5-dimethoxybenzaldehyde (1), prepared *via*

selective demethylation, and methyl 4-pyridylacetate with piperidine furnished 6,7-dimethoxy-3-pyridylcoumarin (**2**, 70% yield), which upon *N*-benzylation using BnCl afforded the corresponding coumarin-pyridine hybrid derivative salt (**3**) in 89% yield. The next motif isocoumarins abundant in microbes and higher plants are coumarin isomers with a reversed lactone moiety. Methyl 2-iodo-4,5-dimethoxybenzoate (**5**) was prepared through Sandmeyer reaction (88% yield) followed by methylation (75% yield). Sonogashira coupling between **5** and 4-ethynylpyridine hydrochloride in the presence of PdCl₂(PPh₃)₂, CuI, and Et₃N smoothly offered access to the arylalkyne product **6** in 98% yield. The AgOTf-mediated intramolecular cyclization of **6** gave **7** (71% yield) [16], which was salfied using BnCl, to provide the desired isocoumarin-pyridine hybrid derivative salt (**8**) in 71% yield.

According to our previous reports [15,17], pyridine hybridizations of flavone, isoflavone, and aurone were accomplished as outlined in Scheme 2 and their synthetic details are shown in the Experimental section.

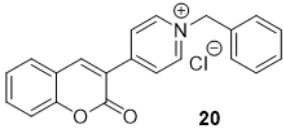
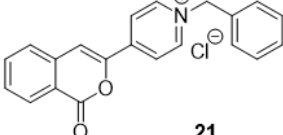
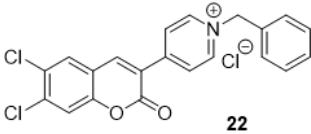
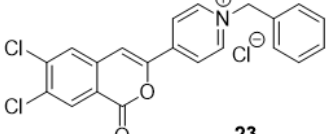
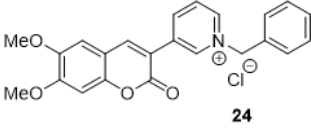
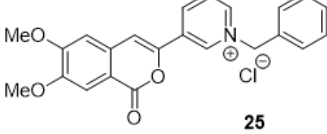
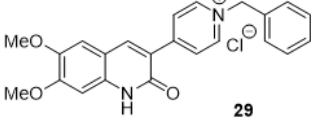
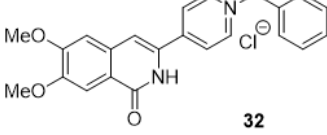
The inhibitory effects of coumarin-type and flavonoid-type pyridine hybrid derivative salts (**3**, **8**, **12**, **15**, and **19**) were assessed against AChE with donepezil as a positive control using the Ellman's method, in which the enzymatic activity is measured using acetylcholine iodide (AChEI) as a substrate and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) as a chromogenic reagent [15,18]. Their IC₅₀ values were calculated as 10.4 ± 0.02 nM for **3**, 6.6 ± 0.1 nM for **8**, 19.4 ± 1.4 nM for **12**, 29.0 ± 0.3 nM for **15**, and 24.0 ± 1.5 nM for **19**, respectively. The AChE inhibitory effects of these compounds were comparable to or more advanced than that of donepezil (IC₅₀: 49.4 ± 0.1 nM). Overall, coumarin-type derivative salts denoted a marked tendency to possess higher inhibition than flavonoid-type counterparts.

For confirming the importance of substructures of **3** and **8** upon ACE inhibition, we synthesized several derivative salts from alternative starting materials. No substitution, dichloro-functionalized, or 3-pyridine hybrid derivative salts (**20–25**) were prepared through the procedures of Scheme 1. The synthetic routes of carbostyryl and isocarbostyryl derivative salts (**29** and **32**) are presented in Scheme 3. Methyl 2-amino-4,5-dimethoxybenzoate was reduced by LiAlH₄ (77% yield) and oxidized mildly by MnO₂ (23% yield), affording the relative benzaldehyde (**27**). Though Knoevenagel condensation (31% yield) and *N*-benzylation (91% yield) described above, the corresponding carbostyryl-pyridine hybrid derivative salt (**29**) was obtained. A primary amide moiety in 2-bromo-4,5-dimethoxybenzamide (**30**) was prepared from the relative benzoic acid via chlorination by SOCl₂ and amidation by aqueous ammonia in 85% yield over two steps. The Cu-catalyzed arylation (27% yield) [19] and *N*-benzylation (72% yield) proceeded to give the desired isocarbostyryl-pyridine hybrid derivative salt (**32**).

To gain additional insights into the structure-activity relationships, we screened for the AChE inhibitory effects of synthetic derivative salts (**20–25**, **29**, and **32**). Results of **20–23** in Table 1 revealed the significance of two methoxy groups on benzene ring. An in-depth analysis of the key binding interactions using the X-ray crystal structure of the [*Torpedo californica* (electric eel) AChE]-donepezil complex (2.5 Å) reported that one of the most important interactions is hydrogen bonding between Glu185, a bridging water molecule, and the *o*-dimethoxy functionality [20]. Given that the reposition of a pyridine nitrogen

atom reduced the effects, 4-pyridine units in **3** and **8** are essential to their AChE inhibitions. Additionally, the substitution to lactam from lactone did not lead to a positive outcome.

Table 1 IC₅₀ values of **20–25**, **29**, and **32** against AChE

Compound	IC ₅₀ (nM)	Compound	IC ₅₀ (nM)
 20	42.6 ± 0.1	 21	21.1 ± 0.01
 22	137.0 ± 2.3	 23	95.4 ± 0.02
 24	150.0 ± 1.2	 25	149.1 ± 1.5
 29	28.6 ± 0.1	 32	139.0 ± 4.2

In an attempt to explore the effects of functional groups on benzyl moiety upon AChE inhibition, a series of coumarin-pyridine hybrid derivative salts was prepared from **2**. As shown in Scheme 4, various *N*-benzylations on pyridine ring succeeded in 59–90% yields. Results of the AChE inhibition assays are summarized in Table 2. As an overall trend, the introduction of various functional groups on benzyl moiety decreased the effectiveness of **3**. In particular, relatively bulky functional groups caused adverse effects on the AChE inhibitory capacity. The IC₅₀ values of **33**, **34**, **37**, **39**, and **40** were over 100 nM. The negative influence of fluorine was limited (IC₅₀: 15.8 ± 0.5 nM). These disappointing results are attributed to the narrowness of AChE catalytic active site.

Table 2
IC₅₀ values of **33–40** against
AChE

Compound	IC ₅₀ (nM)
33	145.1 ± 5.0
34	123.0 ± 4.2
35	75.3 ± 5.4
36	15.8 ± 0.5
37	268.0 ± 9.4
38	34.6 ± 3.3
39	132.2 ± 10.7
40	189.0 ± 2.4

Conclusion

A series of coumarin-type pyridine hybrid derivative salts was synthesized and evaluated for their inhibitory effects on the enzymatic activity of AChE. Compared to donepezil, coumarin and isocoumarin derivative salts (**3** and **8**) turns out to be valuable frameworks for AD drug development. Continuing from these preliminary results, further studies are currently underway and will be reported in the near future.

Experimental section

Materials and methods

All solvents and reagents were purchased from the suppliers and used without further purification. IR spectra were recorded on a JASCO FT/IR-4700 spectrometer. MS spectra were obtained using JEOL JMS-T100TD (AccuTOF). ¹H (500 MHz) and ¹³C (125 MHz) NMR spectra were recorded with a JEOL ECA-500 spectrometer with tetramethylsilane as an internal standard. Silica gel column chromatography (CC) was performed on silica gel 60N (Wakogel, 38–100 µm). Thin-layer chromatography (TLC) spots on plates pre-coated with silica gel 70F₂₅₄ were detected with a UV lamp (254 nm). Fractionations for all CCs were based on TLC analyses.

Synthesis of **1**

BBr₃ solution (1 mol/L in DCM, 10.2 mmol) was slowly added to a solution of 2,4,5-trimethoxybenzaldehyde (10.2 mmol) in DCM (30 mL) at 0°C. After stirring for 16 h, the reactant solution was carefully poured into distilled water, partitioned with EtOAc, washed with sat. NaHCO₃ aq. and brine,

dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluted with *n*-hexane/EtOAc (1/1), to afford **1**.

2-Hydroxy-4,5-dimethoxybenzaldehyde (1): 69% yield. ¹H NMR (500 MHz, CDCl₃): δ 11.40 (1H, s, OH), 9.70 (1H, s, CHO), 6.91 (1H, s, H-3), 6.47 (1H, s, H-6), 3.94 (3H, s, OMe), 3.88 (3H, s, OMe); ¹³C NMR (125 MHz, CDCl₃): δ 194.1, 159.4, 157.3, 143.0, 113.2, 112.9, 100.2, 56.5, 56.4.

Synthesis of 2

After stirring of **1** (1.59 mmol) and methyl 4-pyridylacetate (1.00 mmol) in 2-propanol (12 mL) at 85°C for 8 h, the resultant solution was cooled and stood overnight. The precipitated crystals were filtered, washed using 2-propanol, and dried *in vacuo*, to afford **2**.

6,7-Dimethoxy-3-(4-pyridyl)coumarin (2): 70% yield. ¹H NMR (500 MHz, CDCl₃): δ 8.67 (2H, d, *J* = 5.6 Hz, H-2' and H-6'), 7.91 (1H, s, H-4), 7.67 (2H, d, *J* = 5.6 Hz, H-5' and H-3'), 6.95 (1H, s, H-8), 6.88 (1H, s, H-5), 3.98 (3H, s, OMe), 3.94 (3H, s, OMe); ¹³C NMR (125 MHz, CDCl₃): δ 160.2, 153.8, 150.2, 150.1, 146.8, 142.6, 141.6, 122.6, 122.1, 111.8, 108.2, 99.7, 56.6, 56.5.

Synthesis of 3

After stirring of **2** (0.25 mmol) and BnCl (1.24 mmol) in MeCN (5 mL) at 80°C for 24 h, the resultant solution was cooled, filtered, and dried *in vacuo*, to afford **3**.

6,7-Dimethoxy-3-(4-pyridyl)coumarin N-benzyl Cl salt (3): 89% yield. IR(ATR): 1715, 1556, 1504, 1291, 1244 cm⁻¹; ¹H NMR [500 MHz, DMSO-*d*₆/D₂O (1:1)]: δ 8.82 (2H, d, *J* = 5.8 Hz, H-6' and H-2'), 8.46 (1H, s, H-4), 8.32 (2H, d, *J* = 5.8 Hz, H-3' and H-5'), 7.41–7.39 (5H, m, Bn), 7.10 (1H, s, H-8), 6.92 (1H, s, H-5), 5.64 (2H, s, CH₂), 3.72 (3H, s, OMe), 3.67 (3H, s, OMe); ¹³C NMR [125 MHz, DMSO-*d*₆/D₂O (1:1)]: δ 160.3, 155.3, 151.4, 151.2, 147.1, 146.6, 143.9, 133.5, 130.3, 130.0, 129.2, 126.1, 116.4, 111.9, 109.5, 99.8, 63.8, 56.8, 56.2; HRESIMS: *m/z* 374.1375 [M]⁺ (calcd. for C₂₃H₂₀NO₄, 374.1392).

Synthesis of 4

Conc. HCl was slowly added to a solution of 4,5-dimethoxyanthranilic acid (5.07 mmol) in water (10 mL). Subsequently, NaNO₂/H₂O (5.07 mmol) and KI/H₂O (10.1 mmol) solutions were added to the reactant solution in that order at 0°C. After stirring for 30 min, the precipitated solid was washed with distilled water and dried *in vacuo*, to afford **4**.

2-Iodo-4,5-dimethoxybenzoic acid (4): 88% yield. ¹H NMR (500 MHz, DMSO-*d*₆): δ 7.38 (1H, s, H-6), 7.35 (1H, s, H-3), 3.79 (3H, s, OMe), 3.75 (3H, s, OMe); ¹³C NMR (125 MHz, DMSO-*d*₆): δ 167.5, 151.9, 148.8, 127.7, 123.7, 114.2, 85.1, 56.5, 56.1.

Synthesis of 5

After stirring of **4** (3.25 mmol), MeI (6.49 mmol), and K₂CO₃ (6.49 mmol) in acetone (30 ml) at rt overnight, the resultant solution was poured into 5% K₂CO₃ aq., partitioned with EtOAc, dried over anhydrous Na₂SO₄, and concentrated *in vacuo*, to afford **5**.

Methyl 2-iodo-4,5-dimethoxybenzoate (5): 75% yield. ¹H NMR (500 MHz, CDCl₃): δ 7.44 (1H, s, H-6), 7.39 (1H, s, H-3), 3.92 (3H, s, OMe), 3.91 (3H, s, OMe), 3.90 (3H, s, COOMe); ¹³C NMR (125 MHz, CDCl₃): δ 165.9, 152.0, 148.7, 126.0, 123.8, 113.9, 84.8, 56.3, 56.1, 52.3.

Synthesis of 6

After stirring of **5** (1.43 mmol), 4-ethynylpyridine hydrochloride (2.86 mmol), PdCl₂(PPh₃)₂ (1 mol%), and CuI (2 mol%) in Et₃N/DMF (5/7 mL) at 55°C for 2 h, the resultant solution was poured into 5% Na₂CO₃ aq., partitioned with EtOAc, dried over anhydrous Na₂SO₄, and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluted with *n*-hex/EtOAc (1/3), to afford **6**.

Methyl 4,5-dimethoxy-2-(4-pyridylethynyl)benzoate (6): 98% yield. ¹H NMR (500 MHz, CDCl₃): δ 8.48 (2H, s, H-2' and H-6'), 7.39 (1H, d, *J* = 7.5 Hz, H-6), 7.29 (2H, s, H-3' and H-5'), 6.94 (1H, d, *J* = 7.5 Hz, H-3), 3.82 (6H, s, 2OMe), 3.81 (3H, s, COOMe); ¹³C NMR (125 MHz, CDCl₃): δ 165.7, 151.6, 149.7, 149.3, 131.7, 125.5, 125.0, 116.1, 115.9, 113.0, 93.3, 90.0, 56.1, 56.0, 52.1.

Synthesis of 7

After stirring of **6** (0.50 mmol), TsOH·H₂O (0.67 mmol), and AgOTf (0.05 mmol) in DCE (8 mL) at 60°C for 24 h, the resultant solution was poured into 5% Na₂CO₃ aq., partitioned with CHCl₃, dried over anhydrous Na₂SO₄, and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluted with EtOAc, to afford **7**.

6,7-Dimethoxy-3-(4-pyridyl)isocoumarin (7): 71% yield. ¹H NMR (500 MHz, CDCl₃): δ 8.64 (2H, d, *J* = 4.6 Hz, H-2' and H-6'), 7.62 (2H, d, *J* = 4.6 Hz, H-3' and H-5'), 7.60 (1H, s, H-8), 7.00 (1H, s, H-5), 6.86 (1H, s, H-4), 3.98 (1H, s, OMe), 3.94 (1H, s, OMe); ¹³C NMR (125 MHz, CDCl₃): δ 161.5, 155.4, 150.7, 150.6, 149.8, 139.3, 132.0, 118.6, 114.7, 109.7, 107.0, 104.3, 56.5, 56.4.

Synthesis of 8

the same method as **Synthesis of 3** was used.

6,7-Dimethoxy-3-(4-pyridyl)isocoumarin N-benzyl Cl salt (8): 71% yield. ¹H NMR [500 MHz, DMSO-*d*₆/D₂O (1:1)]: δ 8.87 (2H, s, H-2' and H-6'), 8.13 (2H, s, H-3' and H-5'), 7.56 (1H, s, H-8), 7.42–7.40 (5H, m, Bn), 7.21 (1H, s, H-5), 6.96 (1H, s, H-4), 5.65 (2H, s, CH₂), 3.66 (3H, s, OMe), 3.62 (3H, s, OMe); ¹³C NMR

[125 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 161.1, 155.2, 151.6, 146.6, 145.3, 144.8, 133.5, 131.1, 130.3, 130.0, 129.3, 122.4, 144.9, 111.5, 109.3, 109.2, 63.9, 56.5, 56.3; HRESIMS: m/z 374.1396 [M] $^+$ (calcd. for C $_{23}$ H $_{20}$ NO $_4$, 374.1392).

Synthesis of 9

After stirring of 3,4-dimethoxyphenol (3.24 mmol), chloroacetic acid (6.48 mmol), and K $_2$ CO $_3$ (12.9 mmol) in 10% NaOH aq. (10 mL) at 100°C for 24 h, the resultant solution was poured into 1.0 M HCl aq., partitioned with EtOAc, dried over anhydrous Na $_2$ SO $_4$, and concentrated *in vacuo*, to afford **9**.

2-(3,4-Dimethoxyphenoxy)acetic acid (9): 31% yield. ^1H NMR (500 MHz, CDCl $_3$): δ 10.01 (1H, brs, COOH), 6.76 (1H, d, J = 8.6 Hz, H-5), 6.60 (1H, d, J = 2.9 Hz, H-2), 6.36 (1H, d, J = 8.6 Hz, H-6), 4.63 (2H, s, CH $_2$), 3.84 (3H, s, OMe), 3.82 (3H, s, OMe); ^{13}C NMR (125 MHz, CDCl $_3$): δ 174.4, 152.1, 150.0, 144.4, 111.7, 103.9, 101.4, 65.5, 56.4, 55.9.

Synthesis of 10

After stirring of **9** (0.71 mmol) and TfOH (3.53 mmol) in DCE (6 mL) at 60°C for 6 h, the resultant solution was poured into iced water, partitioned with CHCl $_3$, dried over anhydrous Na $_2$ SO $_4$, and concentrated *in vacuo*, to afford **10**.

5,6-Dimethoxybenzofuran-3(2 H)-one (10): 74% yield. ^1H NMR (500 MHz, CDCl $_3$): δ 6.88 (1H, s, H-4), 6.48 (1H, s, H-7), 4.50 (2H, s, CH $_2$), 3.87 (3H, s, OMe), 3.77 (3H, s, OMe); ^{13}C NMR (125 MHz, CDCl $_3$): δ 198.0, 171.5, 158.5, 145.8, 112.3, 102.8, 95.7, 75.7, 56.5, 56.3.

Synthesis of 11

After stirring of **10** (0.52 mmol), pyridine-4-carboxaldehyde (1.03 mmol), and TsOH·H $_2$ O (0.67 mmol) in toluene (5 mL) at 100°C for 4 h, the resultant solution was poured into 5% Na $_2$ CO $_3$ aq., partitioned with CHCl $_3$, dried over anhydrous Na $_2$ SO $_4$, and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluted with CHCl $_3$ /MeOH (20/1), to afford **11**.

5,6-Dimethoxy-2-(4-pyridylmethylene)benzofuran-3(2 H)-one (11): 75% yield. ^1H NMR (500 MHz, CDCl $_3$): δ 6.89 (2H, d, J = 4.0 Hz, H-2' and H-6'), 7.71 (2H, d, J = 4.0 Hz, H-3' and H-5'), 7.17 (1H, s, H-4), 6.84 (1H, s, CH), 6.69 (1H, s, H-7), 4.04 (3H, s, OMe), 3.92 (3H, s, OMe); ^{13}C NMR (125 MHz, CDCl $_3$): δ 183.1, 163.9, 158.3, 150.5, 150.3, 147.1, 139.7, 124.6, 112.5, 108.3, 104.3, 95.7, 56.9, 56.5.

Synthesis of 12

the same method as **Synthesis of 3** was used.

5,6-Dimethoxy-2-(4-pyridylmethylene)benzofuran-3(2 H)-one N-benzyl Cl salt (12): 65% yield. ^1H NMR [500 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 8.83 (2H, d, J = 6.9 Hz, H-2' and H-6'), 8.25 (2H, d, J = 6.9 Hz, H-3' and H-5'), 7.42–7.40 (5H, m, Bn), 6.98 (1H, s, H-7), 6.96 (1H, s, CH), 6.65 (1H, s, H-4), 5.66 (2H, s, CH $_2$), 3.80 (3H, s, OMe), 3.62 (3H, s, OMe); ^{13}C NMR [125 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 183.1, 164.6, 159.2, 153.1, 148.9, 147.1, 144.3, 133.4, 130.3, 130.0, 129.2, 128.6, 111.2, 104.4, 104.3, 96.8, 64.1, 57.1, 56.2; HRESIMS: m/z 374.1392 $[\text{M}]^+$ (calcd. for C $_{23}$ H $_{20}$ NO $_4$, 374.1392).

Synthesis of 13

After stirring of 2-hydroxy-4,5-dimethoxyacetophenone (1.78 mmol) and prepared isonicotinoyl chloride (3.57 mmol) in Et $_3$ N/DCM (2/5 mL) at rt for 30 min, the resultant solution was poured into 5% Na $_2$ CO $_3$ aq., partitioned with DCM, dried over anhydrous Na $_2$ SO $_4$, and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluted with *n*-hex/EtOAc (1/1), to afford **13**.

2-Acetyl-4,5-dimethoxyphenyl isonicotinate (13): 88% yield. ^1H NMR (500 MHz, CDCl $_3$): δ 8.72 (2H, s, H-2' and H-6'), 7.86 (2H, s, H-3' and H-5'), 7.28 (1H, s, H-3), 6.63 (1H, s, H-6), 3.82 (3H, s, OMe), 3.79 (3H, s, OMe), 2.35 (3H, s, Ac); ^{13}C NMR (125 MHz, CDCl $_3$): δ 195.0, 164.0, 153.3, 150.8, 146.8, 144.2, 136.6, 123.1, 121.9, 112.3, 106.8, 56.3, 56.2, 29.4.

Synthesis of 14

After stirring of **13** (1.16 mmol) and KOH (3.48 mmol) in DMF (10 mL) at rt for 3 h, the resultant solution was poured into sat. NaCl aq., partitioned with EtOAc, dried over anhydrous Na $_2$ SO $_4$, and concentrated *in vacuo*. The residue was dissolved in H $_2$ SO $_4$ /AcOH (0.7/7 mL) and heated at 100 °C for 1 h. The reactant mixture was poured into 5% Na $_2$ CO $_3$ aq., partitioned with DCM, dried over anhydrous Na $_2$ SO $_4$, and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluted with CHCl $_3$ /MeOH (20/1), to afford **14**.

2-(4-Pyridyl)-6,7-dimethoxy-4 H -chromen-4-one (14): 34% yield. ^1H NMR (500 MHz, CDCl $_3$): δ 8.69 (2H, s, H-2' and H-6'), 7.63 (2H, s, H-3' and H-5'), 7.38 (1H, s, H-5), 6.88 (1H, s, H-3), 6.74 (1H, s, H-8), 3.93 (3H, s, OMe), 3.85 (3H, s, OMe); ^{13}C NMR (125 MHz, CDCl $_3$): δ 177.0, 159.6, 154.9, 152.2, 150.7, 148.0, 139.2, 119.6, 117.5, 108.7, 104.2, 99.8, 56.6, 56.3.

Synthesis of 15

the same method as **Synthesis of 3** was used.

2-(4-Pyridyl)-6,7-dimethoxy-4 H -chromen-4-one N-benzyl Cl salt (15): 65% yield. ^1H NMR [500 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 9.03 (2H, d, J = 6.9 Hz, H-2' and H-6'), 8.47 (2H, d, J = 6.9 Hz, H-3' and H-5'), 7.44–7.42 (5H, m, Bn), 7.16 (1H, s, H-5), 7.11 (1H, s, H-3), 7.09 (1H, s, H-8), 5.78 (2H, s, CH $_2$), 3.77 (3H, s, OMe),

3.65 (3H, s, OMe); ^{13}C NMR [125 MHz, $\text{DMSO-}d_6/\text{D}_2\text{O}$ (1:1)]: δ 177.7, 156.2, 155.6, 152.7, 148.2, 146.9, 145.4, 133.0, 130.4, 130.0, 129.4, 124.6, 116.7, 111.3, 103.3, 100.8, 64.5, 56.8, 56.1; HRESIMS: m/z 374.1393 $[\text{M}]^+$ (calcd. for $\text{C}_{23}\text{H}_{20}\text{NO}_4$, 374.1392).

Synthesis of 16

After stirring of 2-hydroxy-4,5-dimethoxyacetophenone (1.02 mmol) in *N,N*-dimethylformamide dimethyl acetal (5 mL) at 100 °C for 1 h, the resultant solution was concentrated *in vacuo* and purified using silica gel column chromatography eluted with *n*-hex/EtOAc (1/4), to afford **16**.

(E)-3-(Dimethylamino)-1-(2-hydroxy-4,5-dimethoxyphenyl)-2-propen-1-one (**16**): 94% yield. ^1H NMR (500 MHz, CDCl_3): δ 7.82 (1H, d, J = 12.0 Hz, H- β), 7.11 (1H, s, H-6), 6.43 (1H, s, H-3), 5.62 (1H, d, J = 12.0 Hz, H- α), 3.88 (3H, s, OMe), 3.86 (3H, s, OMe), 3.11 (3H, s, NMe), 2.92 (3H, s, NMe); ^{13}C NMR (125 MHz, CDCl_3): δ 190.1, 160.0, 154.9, 154.2, 141.2, 111.7, 111.1, 100.8, 89.6, 57.0, 55.9, 45.2, 37.3.

Synthesis of 17

After stirring of **16** (0.80 mmol), I_2 (1.60 mmol), and pyridine (1.60 mmol) in CHCl_3 (10 mL) at rt overnight, the resultant solution poured into sat. $\text{Na}_2\text{S}_2\text{O}_3$ aq., partitioned with CHCl_3 , dried over anhydrous Na_2SO_4 , and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluted with *n*-hex/EtOAc (1/4), to afford **17**.

3-Iodo-6,7-dimethoxy-4 H -chromen-4-one (**17**): 77% yield. ^1H NMR (500 MHz, CDCl_3): δ 8.13 (1H, s, H-2), 7.40 (1H, s, H-8), 6.75 (1H, s, H-5), 3.90 (3H, s, OMe), 3.88 (3H, s, OMe); ^{13}C NMR (125 MHz, CDCl_3): δ 172.4, 156.9, 154.8, 152.3, 148.1, 115.2, 105.0, 99.5, 86.6, 56.6, 56.5.

Synthesis of 18

After stirring of **17** (0.45 mmol), pyridyn-4-boronic acid (0.90 mmol), $\text{Pd}(\text{PPh})_4$ (5 mol%), and Na_2CO_3 (1.36 mmol) in $\text{H}_2\text{O}/\text{THF}$ (1.5/10 mL) at 60°C for 24 h, the resultant solution was poured into distilled water, partitioned with EtOAc, dried over anhydrous Na_2SO_4 , and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluted with *n*-hex/EtOAc (1/4), to afford **18**.

6,7-Dimethoxy-3-(4-pyridyl)-4 H -chlomen-4-one (**18**): 25% yield. ^1H NMR (500 MHz, CDCl_3): δ 8.67 (2H, d, J = 5.7 Hz, H-2' and H-6'), 8.07 (1H, s, H-2), 7.61 (1H, s, H-5), 7.56 (2H, d, J = 5.7 Hz, H-3' and H-5'), 6.90 (1H, s, H-8), 4.00 (3H, s, OMe), 3.99 (3H, s, OMe); ^{13}C NMR (125 MHz, CDCl_3): δ 174.5, 154.8, 153.3, 152.3, 150.0, 148.2, 140.4, 123.4, 122.3, 117.9, 104.9, 99.7, 56.6, 56.5.

Synthesis of 19

the same method as **Synthesis of 3** was used.

2-(4-Pyridyl)-6,7-dimethoxy-4 H -chromen-4-one N-benzyl Cl salt (19): 51% yield. ^1H NMR [500 MHz, $\text{DMSO-}d_6/\text{D}_2\text{O}$ (1:1)]: δ 8.87 (2H, d, J = 6.3 Hz, H-2' and H-6'), 8.69 (1H, s, H-2), 8.28 (2H, d, J = 6.3 Hz, H-3' and H-5'), 7.43 – 7.41 (5H, m, Bn), 7.39 (1H, s, H-5), 7.19 (1H, s, H-8), 3.83 (3H, s, OMe), 3.79 (3H, s, OMe); ^{13}C NMR [125 MHz, $\text{DMSO-}d_6/\text{D}_2\text{O}$ (1:1)]: δ 172.6, 156.7, 153.4, 150.7, 148.2, 146.3, 142.1, 131.6, 128.3, 128.0, 127.2, 125.1, 116.3, 114.9, 102.3, 99.0, 62.0, 54.9, 54.3; HRESIMS: m/z 374.1391 $[\text{M}]^+$ (calcd. for $\text{C}_{23}\text{H}_{20}\text{NO}_4$, 374.1392).

Synthesis of 20, 22, and 24: the same synthetic procedures of **3** were used.

3-(4-Pyridyl)coumarin N-benzyl Cl salt (20): ^1H NMR (500 MHz, $\text{DMSO-}d_6$): δ 8.89 (2H, d, J = 8.6 Hz, H-2' and H-6'), 8.56 (1H, s, H-4), 8.35 (2H, d, J = 8.6 Hz, H-3' and H-5'), 7.72 (1H, d, J = 9.8 Hz, H-5), 7.63 (1H, t, J = 10.9 Hz, H-7), 7.44–7.39 (5H, m, Bn), 7.36–7.31 (2H, m, H-6 and H-8), 5.69 (2H, s, CH_2); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$): δ 159.9, 154.0, 151.2, 147.2, 144.2, 135.1, 133.4, 130.4, 130.3, 130.0, 129.2, 127.0, 125.9, 120.9, 118.8, 116.6, 64.0; HRESIMS: m/z 314.1191 $[\text{M}]^+$ (calcd. for $\text{C}_{21}\text{H}_{16}\text{NO}_2$, 314.1181).

6,7-Dichloro-3-(4-pyridyl)coumarin N-benzyl Cl salt (22): ^1H NMR [500 MHz, $\text{DMSO-}d_6/\text{D}_2\text{O}$ (1:1)]: δ 8.93 (2H, d, J = 6.9 Hz, H-2' and H-6'), 8.46 (1H, s, H-4), 8.34 (2H, d, J = 6.9 Hz, H-3' and H-5'), 7.93 (1H, s, H-5), 7.65 (1H, s, H-8), 7.43–7.40 (5H, m, Bn), 5.71 (2H, s, CH_2); ^{13}C NMR [125 MHz, $\text{DMSO-}d_6/\text{D}_2\text{O}$ (1:1)]: δ 158.9, 152.5, 150.7, 144.9, 144.3, 137.4, 133.4, 130.6, 130.3, 130.0, 129.2, 128.6, 127.2, 122.4, 118.9, 118.8, 64.1; HRESIMS: m/z 382.0419 $[\text{M}]^+$ (calcd. for $\text{C}_{21}\text{H}_{14}\text{Cl}_2\text{NO}_2$, 382.0402).

6,7-Dimethoxy-3-(3-pyridyl)coumarin N-benzyl Cl salt (24): ^1H NMR [500 MHz, $\text{DMSO-}d_6/\text{D}_2\text{O}$ (1:1)]: δ 9.28 (1H, s, H-2'), 8.87 (1H, d, J = 5.7 Hz, H-6'), 8.67 (1H, d, J = 7.5 Hz, H-4'), 8.23 (1H, s, H-4), 8.03–7.95 (1H, m, H-5'), 7.08 (1H, s, H-5), 6.88 (1H, s, H-8), 5.75 (2H, s, CH_2), 3.71 (3H, s, OMe), 3.69 (3H, s, OMe); ^{13}C NMR [125 MHz, $\text{DMSO-}d_6/\text{D}_2\text{O}$ (1:1)]: δ 159.0, 152.2, 148.2, 144.5, 142.8, 142.5, 141.5, 141.2, 133.6, 131.3, 128.4, 128.0, 127.3, 126.3, 114.2, 109.9, 107.2, 97.8, 62.9, 54.6, 54.1; HRESIMS: m/z 374.1378 $[\text{M}]^+$ (calcd. for $\text{C}_{23}\text{H}_{20}\text{NO}_4$, 374.1392).

Synthesis of 21, 23, and 25: the same synthetic procedures of **8** were used.

3-(4-Pyridyl)isocoumarin N-benzyl Cl salt (21): ^1H NMR [500 MHz, $\text{DMSO-}d_6/\text{D}_2\text{O}$ (1:1)]: δ 8.88 (2H, d, J = 6.9 Hz, H-2' and H-6'), 8.25 (2H, d, J = 6.9 Hz, H-3' and H-5'), 8.05 (1H, d, J = 7.5 Hz, H-8), 7.81 (1H, s, H-4), 7.73 (1H, t, J = 7.5 Hz, H-6), 7.67 (1H, d, J = 7.5 Hz, H-5), 7.54 (1H, t, J = 8.0 Hz, H-7), 7.44–7.42 (5H, m, Bn), 5.68 (2H, s, CH_2); ^{13}C NMR [125 MHz, $\text{DMSO-}d_6/\text{D}_2\text{O}$ (1:1)]: δ 159.9, 144.8, 144.5, 142.9, 134.5, 133.5, 131.4, 129.9, 128.4, 128.1, 127.6, 127.3, 126.7, 121.0, 119.4, 109.7, 62.1; HRESIMS: m/z 314.1162 $[\text{M}]^+$ (calcd. for $\text{C}_{21}\text{H}_{16}\text{NO}_2$, 314.1181).

6,7-Dichloro-3-(4-pyridyl)isocoumarin N-benzyl Cl salt (23): ^1H NMR [500 MHz, $\text{DMSO-}d_6/\text{D}_2\text{O}$ (1:1)]: δ 8.94 (2H, d, J = 6.9 Hz, H-2' and H-6'), 8.31 (2H, d, J = 6.9 Hz, H-3' and H-5'), 8.23 (1H, s, H-8), 7.91 (1H, s,

H-5), 7.73 (1H, s, H-4), 7.43–7.40 (5H, m, Bn), 5.70 (2H, s, CH₂); ¹³C NMR [125 MHz, DMSO-*d*₆/D₂O (1:1)]: δ 159.9, 147.7, 146.3, 145.1, 140.0, 135.1, 134.6, 133.2, 131.0, 130.4, 130.0, 129.9, 129.3, 123.2, 121.2, 109.6, 64.3; HRESIMS: *m/z* 382.0425 [M]⁺ (calcd. for C₂₁H₁₄Cl₂NO₂, 382.0402).

6,7-Dimethoxy-3-(3-pyridyl)isocoumarin N-benzyl Cl salt (25): ¹H NMR [500 MHz, DMSO-*d*₆/D₂O (1:1)]: δ 9.25 (1H, s, H-2'), 8.87 (1H, d, *J* = 5.7 Hz, H-6'), 8.70 (1H, d, *J* = 8.0 Hz, H-4'), 8.03–7.92 (1H, m, H-5'), 7.49–7.36 (5H, m, Bn), 7.32 (1H, s, H-8), 7.20 (1H, s, H-5), 6.95 (1H, s, H-4), 5.79 (2H, s, CH₂), 3.69 (3H, s, OMe), 3.62 (3H, s, OMe); ¹³C NMR [125 MHz, DMSO-*d*₆/D₂O (1:1)]: δ 159.6, 153.2, 148.6, 142.8, 142.3, 138.6, 138.4, 131.2, 130.8, 129.8, 128.5, 128.0, 127.3, 126.9, 111.7, 106.9, 106.5, 105.3, 63.1, 54.4, 54.0; HRESIMS: *m/z* 374.1405 [M]⁺ (calcd. for C₂₃H₂₀NO₄, 374.1392).

Synthesis of 26

After stirring of methyl 2-amino-4,5-dimethoxybenzoate (9.45 mmol) and LiAlH₄ (2 mol/L in THF, 14.2 mmol) in THF (60 mL) at rt for 30 min, concentrated ammonia water (3 mL) was added and stirred for 30 min. The resultant solution was filtered through celite, poured into 5% Na₂CO₃ aq., partitioned with EtOAc, dried over anhydrous Na₂SO₄, and concentrated *in vacuo*, to afford **26**.

(2-Amino-4,5-dimethoxyphenyl)methanol (26): 77% yield. ¹H NMR (500 MHz, CDCl₃): δ 6.65 (1H, s, H-6), 6.31 (1H, s, H-3), 4.60 (2H, s, CH₂), 3.84 (3H, s, OMe), 3.80 (3H, s, OMe); ¹³C NMR (125 MHz, CDCl₃): δ 150.0, 141.5, 140.1, 116.5, 114.1, 101.3, 63.9, 56.8, 55.9.

Synthesis of 27

After stirring of **26** (5.46 mmol) and MnO₂ (27.3 mmol) in DCM (30 mL) at rt for 1 h, the resultant solution was filtered through celite and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluted with *n*-hex/EtOAc (1/2), to afford **27**.

2-Amino-4,5-dimethoxybenzaldehyde (27): 23% yield. ¹H NMR (500 MHz, CDCl₃): δ 9.69 (1H, s, CHO), 6.88 (1H, s, H-6), 6.12 (2H, brs, NH₂), 6.12 (1H, s, H-3), 3.89 (3H, s, OMe), 3.85 (3H, s, OMe); ¹³C NMR (125 MHz, CDCl₃): δ 191.5, 156.2, 147.3, 141.1, 116.0, 111.4, 98.5, 56.6, 56.1.

Synthesis of 28

After stirring of **27** (1.38 mmol), methyl 4-pyridylacetate (2.07 mmol), and piperidine (0.69 mmol) in 2-propanol (12 mL) at 85°C for 8 h, the resultant solution was cooled and stood overnight. The precipitated crystals were filtered, washed using 2-propanol, and dried *in vacuo*, to afford **28**.

6,7-Dimethoxy-3-(4-pyridyl)carbostyryl (28): 31% yield. ¹H NMR (500 MHz, DMSO-*d*₆): δ 11.92 (1H, s, NH), 8.60 (2H, d, *J* = 4.6 Hz, H-2' and H-6'), 8.26 (1H, s, H-4), 7.83 (2H, d, *J* = 4.6 Hz, H-3' and H-5'), 7.29

(1H, s, H-8), 6.89 (1H, s, H-5), 3.85 (3H, s, OMe), 3.81 (3H, s, OMe); ^{13}C NMR (125 MHz, DMSO- d_6): δ 160.9, 153.3, 149.9, 145.6, 144.5, 139.3, 135.5, 125.5, 123.2, 113.0, 109.5, 97.7, 56.3, 56.2.

Synthesis of 29

the same method as **Synthesis of 3** was used.

6,7-Dimethoxy-3-(4-pyridyl)carbostyryl N-benzyl Cl salt (29): 88% yield. ^1H NMR [500 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 8.71 (2H, s, J = 5.7 Hz, H-2' and H-6'), 8.30 (2H, d, J = 5.7 Hz, H-3' and H-5'), 8.16 (1H, s, H-4), 7.43–7.40 (5H, m, Bn), 6.89 (1H, s, H-8), 6.51 (1H, s, H-5), 5.61 (2H, s, CH $_2$), 3.57 (3H, s, OMe), 3.58 (3H, s, OMe); ^{13}C NMR [125 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 160.7, 154.8, 152.7, 146.0, 143.6, 143.1, 136.2, 133.8, 130.4, 130.1, 129.3, 125.9, 119.9, 113.4, 109.0, 97.3, 63.8, 56.3, 56.0; HRESIMS: m/z 373.1555 [M] $^+$ (calcd. for C $_{23}$ H $_{21}$ N $_2$ O $_3$, 373.1552).

Synthesis of 30

After stirring of 2-bromo-4,5-dimethoxybenzoic acid (5.75 mmol) and SOCl $_2$ (57.5 mmol) in DCM (40 mL) at reflux for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in DCM (15 mL) and added to concentrated ammonia water (15 mL) slowly. The resultant solution was filtered through celite, poured into distilled water, partitioned with DCM, dried over anhydrous Na $_2$ SO $_4$, and concentrated *in vacuo*, to afford **30**.

2-Bromo-4,5-dimethoxybenzamide (30): 85% yield. ^1H NMR (500 MHz, DMSO- d_6): δ 7.73 (1H, s, NH $_2$), 7.48 (1H, s, NH $_2$), 7.14 (1H, s, H-6), 7.02 (1H, s, H-3), 3.79 (3H, s, OMe), 3.77 (3H, s, OMe); ^{13}C NMR (125 MHz, DMSO- d_6): δ 169.2, 150.3, 148.2, 131.2, 116.3, 112.6, 109.8, 56.5, 56.3.

Synthesis of 31

After stirring of **30** (3.84 mmol), 4-acetylpyridine (3.84 mmol), Cs $_2$ CO $_3$ (7.69 mmol), and CuBr (10 mol%) in DMSO (5 mL) at 80°C for 24 h, the resultant solution was poured into 5% Na $_2$ CO $_3$ aq., partitioned with EtOAc, dried over anhydrous Na $_2$ SO $_4$, and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluted with EtOAc/MeOH (20/1), to afford **31**.

6,7-Dimethoxy-3-(4-pyridyl)isocarbostyryl (31): 27% yield. ^1H NMR (500 MHz, DMSO- d_6): δ 11.55 (1H, s, NH), 8.66 (2H, d, J = 6.3 Hz, H-2' and H-6'), 7.78 (2H, d, J = 6.3 Hz, H-3' and H-5'), 7.59 (1H, s, H-8), 7.25 (1H, s, H-5), 7.12 (1H, s, H-4), 3.90 (3H, s, OMe), 3.89 (3H, s, OMe); ^{13}C NMR (125 MHz, DMSO- d_6): δ 162.5, 153.8, 150.7, 149.9, 141.4, 135.9, 133.1, 120.8, 120.0, 108.1, 107.2, 105.4, 56.3, 56.1.

Synthesis of 32

the same method as **Synthesis of 3** was used.

6,7-Dimethoxy-3-(4-pyridyl)isocarbostyryl N-benzyl Cl salt (32): 72% yield. ^1H NMR [500 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 8.88 (2H, d, J = 5.2 Hz, H-2' and H-6'), 8.20 (2H, d, J = 5.2 Hz, H-3' and H-5'), 7.44–7.41 (5H, m, Bn), 7.40 (1H, s, H-8), 7.27 (1H, s, H-5), 7.11 (1H, s, H-4), 5.68 (2H, s, CH $_2$), 3.77 (3H, s, OMe), 3.71 (3H, s, OMe); ^{13}C NMR [125 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 160.9, 151.7, 148.8, 146.7, 142.6, 131.3, 130.3, 129.7, 128.3, 128.0, 127.3, 122.2, 118.3, 109.7, 106.7, 104.7, 61.9, 54.3, 54.0; HRESIMS: m/z 373.1569 [M] $^+$ (calcd. for C $_{23}$ H $_{21}$ N $_2$ O $_3$, 373.1552).

Synthesis of 33–40: the same method as **Synthesis of 3** was used.

6,7-Dimethoxy-3-(4-pyridyl)coumarin N-(4-cyanobenzyl) Cl salt (33): 85% yield. ^1H NMR [500 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 8.78 (2H, d, J = 6.3 Hz, H-2' and H-6'), 8.50 (1H, s, H-4), 8.38 (2H, d, J = 6.3 Hz, H-3' and H-5'), 7.76 (2H, d, J = 8.6 Hz, H-3'' and H-5''), 7.51 (2H, d, J = 8.6 Hz, H-2'' and H-6''), 7.17 (1H, s, H-5), 6.99 (1H, s, H-8), 5.75 (2H, s, CH $_2$), 3.79 (3H, s, OMe), 3.74 (3H, s, OMe); ^{13}C NMR [125 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 158.7, 153.3, 150.0, 149.2, 145.3, 144.6, 142.1, 136.5, 131.6, 127.6, 124.3, 116.8, 114.6, 110.5, 110.0, 107.6, 97.9, 60.9, 54.7, 54.2; HRESIMS: m/z 399.1354 [M] $^+$ (calcd. for C $_{24}$ H $_{19}$ N $_2$ O $_4$, 399.1345).

6,7-Dimethoxy-3-(4-pyridyl)coumarin N-(4-nitrobenzyl) Cl salt (34): 79% yield. ^1H NMR [500 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 8.81 (2H, d, J = 6.9 Hz, H-2' and H-6'), 8.53 (1H, s, H-4), 8.38 (2H, d, J = 6.9 Hz, H-3' and H-5'), 8.18 (2H, d, J = 8.6 Hz, H-3'' and H-5''), 7.57 (2H, d, J = 8.6 Hz, H-2'' and H-6''), 7.19 (1H, s, H-5), 7.00 (1H, s, H-8), 5.78 (2H, s, CH $_2$), 3.79 (3H, s, OMe), 3.74 (3H, s, OMe); ^{13}C NMR [125 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 160.5, 155.5, 152.1, 151.3, 148.4, 147.4, 146.7, 144.3, 140.4, 130.1, 126.4, 124.7, 116.7, 112.0, 109.7, 100.0, 62.6, 56.9, 56.3; HRESIMS: m/z 419.1471 [M] $^+$ (calcd. for C $_{23}$ H $_{19}$ N $_2$ O $_6$, 419.1443).

6,7-Dimethoxy-3-(4-pyridyl)coumarin N-(4-chlorobenzyl) Cl salt (35): 85% yield. ^1H NMR [500 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 8.82 (2H, d, J = 6.9 Hz, H-2' and H-6'), 8.50 (1H, s, H-4), 8.38 (2H, d, J = 6.9 Hz, H-3' and H-5'), 7.43–7.40 (4H, m, Bn), 7.16 (1H, s, H-5), 6.98 (1H, s, H-8), 5.65 (2H, s, CH $_2$), 3.77 (3H, s, OMe), 3.73 (3H, s, OMe); ^{13}C NMR [125 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 160.3, 155.4, 151.7, 151.2, 147.2, 146.7, 143.9, 135.3, 132.3, 131.0, 129.9, 126.2, 116.5, 111.9, 109.6, 99.9, 62.9, 56.8, 56.2; HRESIMS: m/z 408.0998 [M] $^+$ (calcd. for C $_{23}$ H $_{19}$ ClNO $_4$, 408.1003).

6,7-Dimethoxy-3-(4-pyridyl)coumarin N-(4-fluorobenzyl) Cl salt (36): 90% yield. ^1H NMR [500 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 8.77 (2H, d, J = 5.8 Hz, H-2' and H-6'), 8.41 (1H, s, H-4), 8.30 (2H, d, J = 5.8 Hz, H-3' and H-5'), 7.47–7.44 (2H, m, H-2'' and H-6''), 7.17–7.13 (2H, m, H-3'' and H-5''), 7.07 (1H, s, H-5), 6.90 (1H, s, H-8), 5.63 (2H, s, CH $_2$), 3.72 (3H, s, OMe), 3.68 (3H, s, OMe); ^{13}C NMR [125 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 162.2, 160.3, 158.5, 153.2, 149.4, 149.1, 145.0, 144.5, 141.8, 129.5, 127.3, 124.0, 114.8, 114.7, 114.3, 109.9, 107.4, 97.8, 61.0, 54.7, 54.1; HRESIMS: m/z 392.1283 [M] $^+$ (calcd. for C $_{23}$ H $_{19}$ NO $_4$ F, 392.1298).

6,7-Dimethoxy-3-(4-pyridyl)coumarin N-(4-methoxybenzyl) Cl salt (37): 81% yield. ^1H NMR [500 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 8.81 (2H, d, J = 5.7 Hz, H-2' and H-6'), 8.51 (1H, s, H-4), 8.33 (2H, d, J = 5.7 Hz, H-3' and H-5'), 7.41 (2H, d, J = 7.5 Hz, H-2'' and H-6''), 7.21 (1H, s, H-5), 7.02 (1H, s, H-8), 7.41 (2H, d, J = 7.5 Hz, H-3'' and H-5''), 5.59 (2H, s, CH $_2$), 3.81 (3H, s, OMe), 3.76 (3H, s, OMe), 3.69 (3H, s, OMe); ^{13}C NMR [125 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 160.5, 159.7, 155.7, 151.6, 151.4, 147.2, 147.0, 144.1, 131.2, 126.3, 126.2, 116.9, 115.2, 111.9, 109.9, 100.1, 63.1, 57.1, 56.4, 55.7; HRESIMS: m/z 404.1489 [M] $^+$ (calcd. for C $_{24}$ H $_{22}$ NO $_5$, 404.1498).

6,7-Dimethoxy-3-(4-pyridyl)coumarin N-(4-methylbenzyl) Cl salt (38): 81% yield. ^1H NMR [500 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 8.81 (2H, d, J = 5.2 Hz, H-2' and H-6'), 8.42 (1H, s, H-4), 8.30 (2H, d, J = 5.2 Hz, H-3' and H-5'), 7.33 (2H, d, J = 7.5 Hz, H-2'' and H-6''), 7.22 (2H, d, J = 7.5 Hz, H-3'' and H-5''), 7.06 (1H, s, H-5), 6.87 (1H, s, H-8), 6.00 (2H, s, CH $_2$), 3.70 (3H, s, OMe), 3.66 (3H, s, OMe), 2.22 (3H, s, Me); ^{13}C NMR [125 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 158.2, 153.3, 149.3, 149.1, 145.0, 144.6, 141.8, 138.3, 128.6, 128.5, 127.2, 124.0, 114.3, 109.8, 107.4, 97.8, 61.7, 54.7, 54.1, 18.9; HRESIMS: m/z 388.1550 [M] $^+$ (calcd. for C $_{24}$ H $_{22}$ NO $_4$, 388.1549).

6,7-Dimethoxy-3-(4-pyridyl)coumarin N-(4-trifluoromethylbenzyl) Cl salt (39): 70% yield. ^1H NMR [500 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 8.85 (2H, d, J = 5.7 Hz, H-2' and H-6'), 8.48 (1H, s, H-4), 8.36 (2H, d, J = 5.7 Hz, H-3' and H-5'), 7.73 (2H, d, J = 7.5 Hz, H-3'' and H-5''), 7.60 (2H, d, J = 7.5 Hz, H-2'' and H-6''), 7.11 (1H, s, H-5), 6.92 (1H, s, H-8), 5.76 (2H, s, CH $_2$), 3.74 (3H, s, OMe), 3.69 (3H, s, OMe); ^{13}C NMR [125 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 158.3, 153.4, 149.7, 149.2, 145.2, 144.6, 142.1, 135.8, 128.8, 127.7, 124.7, 124.2, 122.1, 114.3, 109.9, 107.5, 97.8, 61.0, 54.8, 54.2; HRESIMS: m/z 442.1266 [M] $^+$ (calcd. for C $_{24}$ H $_{19}$ NO $_4$ F $_3$, 442.1266).

6,7-Dimethoxy-3-(4-pyridyl)coumarin N-(4-tert-butylbenzyl) Cl salt (40): 56% yield. ^1H NMR [500 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 8.83 (2H, d, J = 6.9 Hz, H-2' and H-6'), 8.42 (1H, s, H-4), 8.30 (2H, d, J = 6.9 Hz, H-3' and H-5'), 7.42 (2H, d, J = 8.6 Hz, H-2'' and H-6''), 7.38 (2H, d, J = 8.6 Hz, H-3'' and H-5''), 7.04 (1H, s, H-5), 6.85 (1H, s, H-8), 5.61 (2H, s, CH $_2$), 3.68 (3H, s, OMe), 3.64 (3H, s, OMe), 1.16 (9H, s, *t*-Bu); ^{13}C NMR [125 MHz, DMSO- d_6 /D $_2$ O (1:1)]: δ 160.1, 155.3, 153.4, 151.2, 151.1, 146.9, 146.6, 143.8, 130.8, 129.0, 126.8, 126.0, 116.1, 111.8, 109.4, 99.7, 63.5, 56.7, 56.1, 35.7, 31.1; HRESIMS: m/z 430.2010 [M] $^+$ (calcd. for C $_{27}$ H $_{28}$ NO $_4$, 430.2018).

Acetylcholinesterase (AChE) inhibitory assay [15,18]: AChE (from *Electrophorus electricus*) was purchased from Sigma-Aldrich Japan. A 100 μL amount of 0.1 M Tris-HCl buffer (pH 8.5), 20 μL of 0.5 U/mL AChE solution in buffer, and 20 μL of tested solution (samples were dissolved in 0.1 M Tris-HCl buffer containing 85% MeOH) were mixed and incubated at 37°C for 15 min in 96-well plates. Then, 40 μL of 0.75 mM DTNB was added followed by 20 μL of 1.5 mM ATCI to initiate the reaction. After 60 min, the hydrolysis of ATCI was detected using a microplate reader (E_{max} precision microplate reader,

Molecular Devices Japan, Tokyo, Japan) at a wavelength of 405 nm. The reaction system with the test solution replaced by equivalent MeOH buffer was used as blank control, and the AChE replaced by buffer was used as background of compound group. The reaction system without compounds and AChE was used as reagent background.

Declarations

Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://XXXXXXXXX>.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution

K.B., S.K., and T.H. conducted the experiments. M.N. and M.K. wrote the main manuscript text and prepared the figures. All authors reviewed the manuscript.

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Scheme

Scheme 1 to 4 are available in the Supplementary Files section.

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