SYNTHESIS AND EVALUATION OF PHOSPHODIESTERASE IV INHIBITORY POTENCY OF ISOCARBOSTYRIL DERIVATIVES OF NATURALLY OCCURRING ISOCOUMARINS, SCOPARINES A AND B

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Abstract – Eight isocarbostyril derivatives bearing n-propyl substituent at the 3-position were prepared from the naturally occurring isocoumarins, scoparines A and B. Their phosphodiesterase IV (PD-4) inhibitory potency were evaluated by the vascular relaxation response in the mesenteric artery. Eight derivatives showed vascular relaxation response, probably relating with PD-4 inhibitory. However, these responses were weaker than the known PD-4 inhibitor (Ro201724).

The isocoumarins, naturally occurring lactones with a wide range of biological activities,1,2 have drawn the attention of medicinal chemists because of their attractive potency for medicinal purpose. Their nitrogen analogues, isocarbostyril derivatives, are recognized as bioactive natural products,3 and also as potential medicinal compounds.4

![Figure 1. Structures of scoparines A and B, and ruprechstylr](image)

Recently, we have established5 the first syntheses of natural isocoumarins, scoparines A and B, and an
isocarbostyril, ruprechstyryl, respectively, where the Sonogashira coupling \(^6\) of aromatic halides and alkynes, followed by regioselective 6-endo-dig cyclization \(^2\) of oxygen functions to carbon-carbon triple bonds were involved as key steps, as shown in Scheme 1.

**Scheme 1. Previous synthesis of natural isocoumarins, scoparines A and B**

As an extension of this work, we are interested in the synthesis and the biological activity of isocarbostyril derivatives of the natural isocoumarins, scoparines A and B, since it is recognized that ruprechstyryl-type compounds with a hydrophilic substituent at the 2-position inhibit phosphodiesterases, especially phosphodiesterase IV (PD-4), to be useful for treatment of asthma, bronchitis, angina pectoris, arrhythmia, allergy, rheumatism, etc. \(^4\)

For the derivatization of scoparines A and B, we decided to introduce a hydrophilic substituent at the 2-position based on the consideration of the structural features of ruprechstyryl-type compounds. \(^8\) Thus, eight isocarbostyril derivatives of the natural isocoumarins, scoparines A and B, were prepared as follows.

Scoparine A methoxymethyl ether (4), derived from scoparine A, \(^5\) was treated with ammonium hydroxide solution in DMF to give the corresponding isocarbostyril (5), which on alkylation with ethyl bromoacetate or methyl 2-bromopropionate in DMF in the presence of sodium hydride (50% in oil) afforded the N-alkylated products (6) and (7) in 49 and 80% yields, respectively. Deprotection of the methoxymethyl group at the 6-position of the former ester (6) with concentrated HCl and subsequent hydrolysis of the ester function with 15N NaOH in MeOH provided the phenolic acid (8) in 27% yield from 6. The ester (7) was also transformed into the acid (9), in 66% yield from 7, by the same procedures as described for the preparation of 8. Next, we attempted to introduce a primary alcohol function at the 2-position as an alternative hydrophilic substituent. Treatment of 5 with 2-bromoethanol
tert-butyldimethylsilyl ether in DMF in the presence of NaH (50% in oil) gave the alkylation product (10) in 64% yield, which on hydrolysis with concentrated HCl furnished the desired alcohol (12) in 76% yield. Similar treatment of 5 with 3-bromopropanol tert-butyldimethylsilyl ether, and subsequent acid hydrolysis of the resulting ether (11) with concentrated HCl afforded 13 in 17% yield from 5.

Scheme 2. Synthesis of isocarbostyril derivatives of scoparines A and B with an acid moiety

Applying the same synthetic strategy as above, scoparine B methoxymethyl ether (14), derived from scoparine B,5 was first converted to the corresponding isocarbostyril (15), which was further transformed to the acids (18 and 19) via the esters (16 and 17), and the primary alcohols (22 and 23) via the silyl ethers (20 and 21), as shown in Schemes 2 and 3.
With the requisite materials available, a biological study on PD-4 inhibitory activity was carried out by the vascular relaxation response in the mesenteric artery of rats. In the vascular system, adenosine 3', 5'-cyclic monophosphate (cAMP) plays important roles in the regulation of vascular tone and the intracellular levels of cAMP are tightly regulated by rate of control of its hydrolysis (by cyclic nucleotide phosphodiesterases (PDs)); i.e. cAMP signaling in mammalian cells is terminated by cyclic nucleotide PDs, a multifamily class of enzymes that catalyze the hydrolysis of cyclic nucleotides to 5'-nucleotide monophosphates (which do not activate cAMP effector proteins). Among the various PDs, the two of greatest are PD-3 and PD-4 because they preferentially hydrolyze cAMP. Consequently, to investigate cAMP-mediated relaxation in the rat mesenteric artery, we tested Ro201724 (a specific PD-4 inhibitor), when it was added cumulatively to rings precontracted by phenylephrine (0.1 μM). Ro201724 caused concentration-dependent relaxations (ED₅₀; 215 nM). Next, to determine whether our synthesized compounds really did act as a PD-4 inhibitor, we performed experiments in which each compound (1 nM to 10 μM) was added cumulatively to mesenteric artery rings. The results are summarized in Figure 2. Each compounds caused a concentration-dependent relaxation. But those compounds-induced relaxation responses were significantly decreased. However, the derivatives such as 12, 13, 22 and 23 were found to be more potent than those such as 8, 9, 18 and 19. Moreover, the derivatives such as 22 and 23 were found to be more potent than those such as 12 and 13.

**Figure 2.** Concentration-response curves for the relaxations induced by Ro201724 (PD-4 inhibitor) and the synthesized compounds in isolated rings of superior mesenteric artery. Ordinate shows relaxation of the superior mesenteric artery as a percentage of the contraction induced by phenylephrine. Each data point represents the mean ± SE from 5 experiments. ***P<0.001 vs Ro201724

In conclusion, these results suggested that the synthesized compounds had the vasorelaxant effects. Among them, it was found that 7-methoxy derivatives (22 and 23)-induced maximal response almost
were no different to Ro201724, suggesting that those compounds might be PD-4 inhibitors. Furthermore, we found that 7-methoxy derivatives showed the stronger vasorelaxant activity than those of the corresponding 7-hydrogen derivatives, and the derivatives with a primary alcohol moiety at the 2-position showed stronger than those with a carboxylic acid moiety. Further studies on 22 or 23 are necessary to develop a novel PD-4 inhibitor with controlling vascular tone.

EXPERIMENTAL
Melting points were measured with a Yanagimoto MP apparatus and are uncorrected. IR spectra were obtained as thin films on sodium chloride plates using a JASCO FT/IR-200 spectrophotometer. $^1$H and $^{13}$C NMR spectra were obtained on Bruker AV-400 ($^1$H NMR: 270 MHz, $^{13}$C NMR: 67.8 MHz) instrument for solutions in CDCl$_3$, and chemical shifts are reported on the $\delta$ scale from internal TMS. MS spectra were measured with a JEOL JMS-D 300 spectrometer. Elemental analyses were performed on a Yanaco-MT5.

Materials and methods
Reagents
Phenylephrine was purchased from Sigma Chemical Co. (St. Louis, MO, USA). 4-(3-Butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro201724) was from Calbiochem-Novabiochem (La Jolla, CA). Drugs were dissolved in dimethyl sulfoxide except phenylephrine (which was dissolved in saline). All concentrations are expressed as the final molar concentration of the base in the organ bath.

Animals and experimental design
Male Wistar control rats were obtained at the age of 4 weeks (Clea, Tokyo, Japan). All animals were allowed a standard laboratory diet (MF; Oriental Yeast Industry, Tokyo, Japan) and water ad libitum in a controlled environment (room temperature 21-22 °C, room humidity 50 ± 5%) until they were 36-38 weeks old. This study was approved by the Hoshi University Animal Care and Use Committee, and all studies were conducted in accordance with “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health, and with “Guide for the Care and Use of Laboratory Animals” adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University (which is accredited by the Ministry of Education, Culture, Sports, Science, and Technology, Japan).

Measurement of isometric force
At 36-38 weeks of age, rats were anesthetized with diethyl ether, then euthanized by decapitation. The superior mesenteric artery was rapidly removed and immersed in oxygenated, modified Krebs-Henseleit solution (KHS). This solution consisted of (in mM) 118.0 NaCl, 4.7 KCl, 25.0 NaHCO$_3$, 1.8 CaCl$_2$, 1.2 NaH$_2$PO$_4$, 1.2 MgSO$_4$, and 11.0 glucose. The artery was carefully cleaned of all fat and connective tissue,
and ring segments 2 mm in length were placed in a bath containing 10 mL of KHS at 37 °C. The rings were stretched until an optimal resting tension of 1.0 g was loaded, and then allowed to equilibrate for at least 60 min. Force generation was monitored by means of an isometric transducer (model TB-611T; Nihon Kohden, Tokyo, Japan). For the relaxation studies, mesenteric rings were precontracted with an equally effective concentration of phenylephrine (0.1 μM). When the phenylephrine-induced contraction had reached a plateau level, Ro201724 or our new synthesized compounds (1 nM - 10 μM) was added in a cumulative manner.

5-Methoxymethylscoparine A (4). To a stirred solution of scoparaine A (0.8 g 3.42 mmol) in CH₂Cl₂(7 mL) in the presence of iPr₂NEt (0.78 mL, 4.45 mmol) was added MOMCl (0.31 ml, 4.10 mmol) at 0 °C under argon. The mixture was stirred at the same temperature for 30 min, and then treated with saturated aqueous NH₄Cl solution. The mixture was extracted with CHCl₃, and the extract was washed with brine and dried over Na₂SO₄. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane:AcOEt (5:1, v/v) gave the ether (4) (0.89 g, 94%) as a pale yellow oil. IR νmax: 2962, 1730, 1655, 1600 cm⁻¹; ¹H NMR δ: 1.01 (3H, t, J = 7.4 Hz), 1.70-1.80 (2H, m), 2.51 (2H, t, J = 7.4 Hz), 3.53 (3H, s), 3.92 (3H, s), 5.34 (2H, s), 6.54 (1H, s), 7.25 (1H, d, J = 8.9 Hz), 7.98 (1H, d, J = 8.9 Hz); ¹³C NMR δ: 13.3, 20.1, 35.5, 56.4, 61.1, 94.6, 97.2, 114.6, 115.3, 126.3, 132.7, 142.3, 154.5, 158.1, 162.4; HRMS (EI) Calcd for C₁₅H₁₈O₅ (M⁺) 278.1154. Found 278.1167.

5-Methoxy-6-(methoxymethoxy)-3-propylisoquinolin-1(2H)-one (5). A mixture of the ether (4) (0.77 g, 2.8 mmol) and 25% NH₄OH (15 mL) in DMF (15 mL) was heated at 45 °C for 22 h. The mixture was concentrated to leave a residue, which was subjected to column chromatography on silica gel. Elution with hexane:AcOEt (5:1, v/v) gave the amide (5) (0.63 g, 82%) as a colorless solid. Mp. 124-125 °C; IR νmax: 2961, 1656, 1638, 1604 cm⁻¹; ¹H NMR δ: 1.02 (3H, t, J = 7.3 Hz), 1.75-1.84 (2H, m), 2.64 (2H, J = 7.6 Hz), 3.55 (3H, s), 3.94 (3H, s), 5.34 (2H, s), 6.59 (1H, s), 7.28 (1H, d, J = 8.9 Hz), 8.12 (1H, d, J = 8.9 Hz), 11.23 (1H, br s); ¹³C NMR δ: 13.6, 21.7, 35.6, 56.4, 61.2, 94.9, 98.0, 114.8, 119.9, 124.1, 134.3, 142.2, 142.7, 152.5, 164.3; HRMS (Cl) Calcd for C₁₅H₂₀NO₄ (M+H⁺) 278.1392. Found 278.1414. Anal Calcd for C₁₅H₁₉NO₄: C, 64.97; H, 6.91; N, 5.05. Found: C, 64.70; H, 6.86; N, 5.00.

Ethyl [5-methoxy-6-(methoxymethoxy)-1-oxo-3-propylisoquinolin-2(1H)-yl]acetate (6). To a stirred solution of isocarbostyril (5) (100 mg, 0.36 mmol) in DMF (1 mL) was added a suspension of NaH (50% in oil) (39.8 mg, 0.83 mmol) in DMF (1.0 mL) at 0 °C, and the resulting mixture was stirred at rt for 1 h. To this mixture was added a solution of ethyl bromoacetate (95.3 mg, 0.54 mmol) in DMF (0.5 mL) at rt, and the whole was stirred at the same temperature for 1 h. After treatment with saturated aqueous NH₄Cl solution, the mixture was extracted with Et₂O. The ethereal layer was washed with brine and dried over
Na$_2$SO$_4$. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane:AcOEt (5:1, v/v) gave the ester (6) (63.8 mg, 49%) as a colorless solid.

Mp. 99-100 °C; IR $\nu$ max: 2963, 1743, 1658, 1623 cm$^{-1}$. $^1$H NMR $\delta$: 1.05 (3H, t, $J = 7.3$ Hz), 1.29 (3H, t, $J = 7.1$ Hz), 1.67-1.74 (2H, m), 2.55 (2H, t, $J = 7.5$ Hz), 3.54 (3H, s), 3.93 (3H, s), 4.25 (2H, q, $J = 7.1$ Hz), 4.81 (2H, s), 5.32 (2H, s), 6.64 (1H, s), 7.26 (1H, d, $J = 8.9$ Hz), 8.09 (1H, d, $J = 8.9$ Hz); $^{13}$C NMR $\delta$: 13.7, 14.1, 21.6, 35.5, 45.0, 56.4, 61.2, 61.6, 88.9, 94.9, 99.3, 115.2, 119.6, 124.8, 132.3, 142.5, 152.4, 162.9, 168.7; HRMS (CI) Calcd for C$_{19}$H$_{25}$NO$_6$ (M$^+$) 363.1682. Found 363.1704.

Methyl 2-[5-methoxy-6-(methoxymethoxy)-1-oxo-3-propylisoquinolin-2(1H)-yl]propanoate (7). Alkylation of 5 (150 mg, 0.54 mmol) with methyl 2-bromopropanoate (542.8 mg, 3.25 mmol) and NaH (50% in oil) (57.2 mg, 1.19 mmol) in DMF (3.5 mL) was carried out by the same procedure as described for the preparation of 6 to give the ester (7) (157.4 mg, 80%) as a pale yellow oil. IR $\nu$ max: 2957, 1758, 1628 cm$^{-1};$ $^1$H NMR $\delta$: 0.93 (3H, t, $J = 7.4$ Hz), 1.70 (3H, d, $J = 7.0$ Hz), 1.71-1.79 (2H, m), 2.61-2.74 (2H, m), 3.55 (3H, s), 3.71 (3H, s), 3.96 (3H, s), 5.41 (2H, s), 5.44 (1H, q, $J = 7.0$ Hz), 7.26 (1H, s), 7.35 (1H, d, $J = 9.1$ Hz), 8.00 (1H, d, $J = 9.1$ Hz); $^{13}$C NMR $\delta$: 13.8, 17.6, 22.2, 39.8, 51.9, 56.4, 61.2, 70.2, 95.4, 106.6, 114.0, 116.6, 120.9, 135.0, 142.4, 150.0, 152.6, 158.6, 173.1; HRMS (EI) Calcd for C$_{19}$H$_{25}$NO$_6$ (M$^+$) 363.1682. Found 363.1657.

(6-Hydroxy-5-methoxy-1-oxo-3-propylisoquinolin-2(1H)-yl)acetic acid (8). A solution of the ester (6) (61.8 mg, 0.17 mmol) and conc. HCl (0.1 mL) in MeOH (1.5 mL) was stirred at rt for 17 h. After evaporation of the solvent, the residue was dissolved into MeOH (1.5 mL) and then treated with 15N NaOH solution (0.2 mL). The mixture was stirred at rt for further 1.5 h, and then concentrated in vacuo. The residue was dissolved into water, which was washed with Et$_2$O. The aqueous layer was acidified with 2N HCl and extracted CHCl$_3$. The organic layer was washed with brine and dried over Na$_2$SO$_4$. Evaporation of the solvent gave a colorless solid, which was recrystallized from EtOH to give the acid (8) (27.1 mg, 55%). Mp. 271-273 °C; IR v max: 3198, 2359, 1715, 1645, 1620, 1577 cm$^{-1};$ $^1$H NMR $\delta$: 1.07 (3H, t, $J = 7.3$ Hz), 1.70-1.76 (2H, m), 2.58 (2H, t, $J = 7.7$ Hz), 3.89 (3H, s), 4.81 (2H, s), 6.65 (1H, s), 7.02 (1H, d, $J = 8.7$ Hz), 7.96 (1H, d, $J = 8.7$ Hz); $^{13}$C NMR $\delta$: 11.5, 19.1, 32.5, 42.9, 58.4, 95.7, 114.7, 114.8, 122.0, 130.0, 137.5, 131.4, 150.8, 160.0, 168.3; HRMS (EI) Calcd for C$_{15}$H$_{17}$NO$_5$ (M$^+$) 291.1106. Found 291.1087. Anal. Calcd for C$_{15}$H$_{17}$NO$_5$: C, 61.85; H, 5.88; N, 4.81. Found: C, 61.74; H, 6.12; N, 4.65.

2-(6-Hydroxy-5-methoxy-1-oxo-3-propylisoquinolin-2(1H)-yl)propanoic acid (9). The ester (7) (157.5 mg, 0.43 mmol) was hydrolyzed by the same procedure as described for the preparation of 8 to afford the acid as a colorless solid, which was recrystallized from benzene to provide the acid (9) (110 mg, 83%). Mp. 135-136 °C; IR v max: 2961, 1724, 1626 cm$^{-1};$ $^1$H NMR $\delta$: 0.95 (3H, t, $J = 7.4$ Hz), 1.74 (3H, d, $J = ...
6.9 Hz), 1.75-1.82 (2H, m), 2.75 (2H, t, J = 7.5 Hz), 3.94 (3H, s), 5.41 (1H, q, J = 6.9 Hz), 7.19 (1H, d, J = 8.9 Hz), 7.19 (1H, s), 7.99 (1H d, J = 8.9 Hz); $^{13}$C NMR δ: 13.4, 17.2, 22.0, 39.5, 60.8, 70.3, 106.1, 112.4, 117.3, 121.2, 134.6, 138.9, 149.8, 152.2, 158.9, 174.9; HRMS (CI) Calcd for C$_{16}$H$_{20}$NO$_5$ (M+H$^+$) 306.1341. Found 306.1357. Anal. Calcd for C$_{16}$H$_{19}$NO$_5$: C, 62.94; H, 6.27; N, 4.59. Found: C, 63.05; H, 6.36; N, 4.55.

2-(2-[[tert-Butyl(dimethyl)silyl]oxy]ethyl)-5-methoxy-6-(methoxymethoxy)-3-propylisoquinolin-1(2H)-one (10). To a stirred solution of 5 (150 mg, 0.54 mmol) in DMF (1 mL) was added a suspension of NaH (50% in oil) (57.1 mg, 1.19 mmol) in DMF (1.0 mL) at 0 °C, and the mixture was stirred at rt for 1 h. To this mixture was added a solution of the bromide (777 mg, 3.25 mmol) in DMF (1.0 mL) at rt, and the whole was stirred at the same temperature for further 48 h. After treatment with saturated aqueous NH$_4$Cl solution, the mixture was extracted with Et$_2$O. The ethereal layer was washed with brine and dried over Na$_2$SO$_4$. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane:AcOEt (7:1, v/v) gave the ether (10) (149.8 mg, 64%) as a pale yellow oil. IR $\nu$ max: 2957, 1654, 1622, 1597 cm$^{-1}$; $^1$H NMR δ: -0.10 (6H, s), 0.82 (9H, s), 1.26 (3H, t, J=7.1 Hz), 1.66-1.75 (2H, m), 2.81 (2H, t, J = 7.6 Hz), 3.54 (3H, s), 3.92 (3H, s), 3.94 (2H, t, J = 5.3 Hz), 4.22 (2H, t, J = 5.3 Hz), 5.32 (2H, s), 6.59 (1H, s), 7.24 (1H, d, J = 9.0 Hz), 8.10 (1H, d, J = 9.0 Hz); $^{13}$C NMR δ: -5.7, 13.8, 18.1, 21.8, 25.7, 35.8, 45.7, 56.4, 61.12, 61.14, 94.9, 98.6, 114.9, 120.1, 124.5, 132.3, 142.2, 144.5, 152.1, 162.8; HRMS (CI) Calcd for C$_{23}$H$_{37}$NO$_5$Si (M+H$^+$) 435.2441. Found 435.2464.

2-(2-[[tert-Butyl(dimethyl)silyl]oxy]propyl)-5-methoxy-6-(methoxymethoxy)-3-propylisoquinolin-1(2H)-one (11). By the same procedure as described for the preparation of 10, compound 5 (200 mg, 0.72 mmol) was alkylated with the bromide (1.1 g, 4.34 mmol) to give the ether (11) (66.4 mg, 21%). IR $\nu$ max: 2956, 1654, 1622, 1597 cm$^{-1}$; $^1$H NMR δ: 0.08 (6H, s), 0.92 (9H, s), 1.06 (3H, t, J=7.4 Hz), 1.68-1.78 (2H, m), 1.89-1.95 (2H, m), 2.73 (2H, t, J = 7.6 Hz), 3.54 (3H, s), 3.72 (2H, t, J = 5.6 Hz), 3.92 (3H, s), 4.19 (2H, t, J = 7.7 Hz), 5.32 (2H, s), 6.59 (1H, s), 7.24 (1H, d, J = 9.0 Hz), 8.09 (1H, d, J = 9.0 Hz); $^{13}$C NMR δ: -5.4, 13.8, 18.2, 22.4, 25.9, 31.8, 35.2, 41.1, 56.4, 60.5, 61.2, 95.0, 99.0, 115.1, 120.2, 124.5, 132.2, 142.2, 143.7, 152.0, 162.8; HRMS (CI) Calcd for C$_{24}$H$_{40}$NO$_5$Si (M+H$^+$) 450.2675. Found 450.2666.

6-Hydroxy-2-(2-hydroxyethyl)-5-methoxy-3-propylisoquinolin-1(2H)-one (12). A solution of the ether (10) (141.0 mg, 0.32 mmol) in MeOH (2.0 mL) in the presence of conc. HCl (0.1 mL) was stirred at rt for 9 h. After concentration of the mixture, the residue was subjected to column chromatography on silica gel. Elution with hexane:AcOEt (1:1, v/v) gave a colorless solid, which was recrystallized from CHCl$_3$ to afford the alcohol (12) (52.5 mg, 76%). Mp. 187-189 °C; IR $\nu$ max: 3234, 2963, 1640, 1590 cm$^{-1}$; $^1$H NMR δ: 1.07 (3H, t, J = 7.3), 1.68-1.77 (2H, m), 2.74 (2H, t, J = 7.6 ), 3.28 (2H, br s), 3.86 (2H,
t, J = 5.9 Hz), 3.88 (3H, s), 4.25 (2H, t, J = 5.9 Hz), 6.62 (1H, s), 7.02 (1H, d, J = 8.8 Hz), 7.98 (1H, d, J = 8.8 Hz); \(^{13}\)C NMR δ: 13.3, 21.8, 35.1, 45.6, 60.2, 60.6, 99.8, 116.5, 117.4, 124.3, 132.1, 139.3, 143.2, 152.5, 163.8; HRMS (CI) Calcd for C\(_{15}\)H\(_{19}\)NO\(_4\) (M\(^+\)) 277.1314. Found 277.1310. Anal. Calcd for C\(_{15}\)H\(_{19}\)NO\(_4\): C, 64.97; H, 6.91; N, 5.05. Found: C, 64.80; H, 7.01; N, 5.07.

**6-Hydroxy-2-(2-hydroxypropyl)-5-methoxy-3-propylisoquinolin-1(2H)-one (13).** By the same procedure as described for the preparation of 12, the ether (11) (66.4 mg, 0.15 mmol) was hydrolyzed with conc. HCl to give the alcohol (13) (35.5 mg, 82%) (recrystallized from AcOEt). Mp. 144-146 °C; \(^{1}H\) NMR δ: 1.09 (3H, t, J = 7.3), 1.68-1.81 (2H, m), 1.88-1.94 (2H, m), 2.69 (2H, t, J = 7.5 Hz), 3.53 (2H, t, J = 5.3 Hz), 3.90 (3H, s), 4.31 (2H, t, J = 5.6 Hz), 4.31 (1H, br s), 6.46 (1H, br s), 6.56 (1H, s), 7.08 (1H, d, J = 8.8 Hz), 8.11 (1H, d, J = 8.8 Hz); \(^{13}\)C NMR δ: 13.8, 22.5, 32.7, 34.9, 39.3, 58.1, 61.8, 99.7, 115.8, 118.2, 125.8, 131.3, 139.2, 143.4, 151.8, 164.0; HRMS (CI) Calcd for C\(_{16}\)H\(_{21}\)NO\(_4\) (M\(^+\)) 291.1470. Found 291.1452. Anal. Calcd for C\(_{16}\)H\(_{21}\)NO\(_4\): C, 65.96; H, 7.27; N, 4.81. Found: C, 65.89; H, 7.35; N, 4.79.

**6-Methoxymethylscoparine B (14).** To a stirred solution of scoparine B (1.1 g, 4.02 mmol) in CH\(_2\)Cl\(_2\) (8 mL) in the presence of \(\text{iPr}_2\)NEt (1.13 mL, 6.43 mmol) at 0 °C under argon. The mixture was stirred at the same temperature for 30 min, and then treated with saturated aqueous NH\(_4\)Cl solution. The mixture was extracted with CHCl\(_3\), and the extract was washed with brine and dried Na\(_2\)SO\(_4\). Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane:AcOEt (6:1, v/v) gave the ether (14) (1.2 g, 94%) as a colorless oil. IR \(\nu_{\text{max}}\): 2962, 1727, 1654, 1601 cm\(^{-1}\); \(^{1}\)H NMR δ: 1.69-1.79 (2H, m), 2.54 (2H, t, J = 7.7 Hz), 3.63 (3H, s), 3.95 (3H, s), 3.97 (3H, s), 5.25 (2H, s), 6.48 (1H, s), 7.52 (1H, s); \(^{13}\)C NMR δ: 13.5, 20.3, 35.5, 56.2, 57.4, 61.5, 97.3, 98.5, 106.3, 116.0, 127.2, 144.8, 147.5, 153.1, 156.5, 162.8; HRMS (EI) Calcd for C\(_{16}\)H\(_{21}\)O\(_6\) (M\(^+\)) 309.1338. Found 309.1344.

**5,7-Dimethoxy-6-(methoxymethoxy)-3-propylisoquinolin-1(2H)-one (15).** A mixture of the ether (14) (1.3 g, 4.1 mmol) and 25% NH\(_4\)OH (20 mL) in DMF (25 mL) was stirred at rt for 12 h and then at 95 °C for 12 h. The mixture was concentrated to leave a solid, which was recrystallized from EtOH to give the amide (15) (0.78 g, 62%) as a colorless solid. Mp. 159-160 °C; IR \(\nu_{\text{max}}\): 2961, 1641, 1605, 1474 cm\(^{-1}\); \(^{1}\)H NMR δ: 1.01 (3H, t, J = 7.3 Hz), 1.68-1.80 (2H, m), 2.54 (2H, t, J = 7.7 Hz), 3.63 (3H, s), 3.95 (3H, s), 3.97 (3H, s), 5.25 (2H, s), 6.52 (1H, s), 7.61 (1H, s); \(^{13}\)C NMR δ: 13.6, 21.7, 35.5, 56.1, 57.3, 61.4, 98.1, 98.6, 103.7, 120.9, 128.5, 139.6, 143.1, 147.7, 152.3, 163.5; HRMS (EI) Calcd for C\(_{16}\)H\(_{21}\)NO\(_5\) (M\(^+\)) 307.1419. Found 307.1422. Anal. Calcd for C\(_{16}\)H\(_{21}\)NO\(_5\): C, 62.53; H, 6.89; N, 4.56. Found: C, 62.36; H, 6.98; N, 4.62.

**Ethyl [5,7-dimethoxy-6-(methoxymethoxy)-1-oxo-3-propylisoquinolin-2(1H)-yl]acetate (16).** To a
stirred solution of 15 (134 mg, 0.44 mmol) in DMF (1 mL) was added a suspension of NaH (50% in oil) (42.0 mg, 0.87 mmol) in toluene (1 mL), and the resulting mixture was stirred at rt for 1 h. The mixture was treated with ethyl bromoacetate (119 mg, 0.71 mmol) and the whole was heated at 110 °C for 4 h. After treatment with saturated aqueous NH₄Cl solution, the mixture was extracted with Et₂O. The ethereal layer was washed with brine and dried over Na₂SO₄. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane:AcOEt (2:1, v/v) gave the ester (16) (148 mg, 86%) as a pale yellow oil. IR \( \nu_{\text{max}} \): 2963, 1748, 1654, 1597 cm⁻¹; \(^1\)H NMR \( \delta \): 1.05 (3H, t, \( J = 7.3 \) Hz), 1.30 (3H, t, \( J = 7.1 \) Hz), 1.64-1.76 (2H, m), 2.55 (2H, t, \( J = 7.6 \) Hz), 3.62 (3H, s), 3.95 (6H, s), 4.25 (2H, q, \( J = 7.1 \) Hz), 5.24 (2H, s), 6.59 (1H, s), 7.61 (1H, s); \(^{13}\)C NMR \( \delta \): 13.7, 14.1, 21.6, 35.3, 45.3, 56.1, 57.3, 61.4, 61.6, 98.6, 99.4, 104.4, 120.5, 126.6, 140.3, 143.0, 147.5, 152.4, 162.5, 168.6; HRMS (EI) Calcd for C₂₀H₂₇NO₇ (M⁺) 393.1787. Found 393.1791.

Methyl 2-[5,7-dimethoxy-6-(methoxymethoxy)-1-oxo-3-propylisoquinolin-2(1H)-yl]propanoate (17). Alkylation of 15 (50.0 mg, 0.16 mmol) with methyl 2-bromopropanoate (40.6 mg, 0.24 mmol) and NaH (50% in oil) (15.6 mg, 0.32 mmol) was carried out by the same procedure as described for the preparation of 16 to give the ester (17) (56.4 mg, 89%) as a pale yellow oil. IR \( \nu_{\text{max}} \): 2957, 1759, 1597 cm⁻¹; \(^1\)H NMR \( \delta \): 0.92 (3H, t, \( J = 7.4 \) Hz), 1.72 (3H, d, \( J = 7.0 \) Hz), 1.69-1.81 (2H, m), 2.59-2.75 (2H, m), 3.63 (3H, s), 3.72 (3H, s), 3.98 (3H, s), 5.25 (2H, s), 5.44 (1H, q, \( J = 7.0 \) Hz), 7.21 (1H, s), 7.38 (1H, s); \(^{13}\)C NMR \( \delta \): 13.7, 17.6, 22.2, 39.6, 51.8, 56.0, 57.3, 61.4, 70.2, 98.7, 99.2, 106.8, 114.2, 130.2, 141.4, 147.1, 150.4, 152.2, 157.5, 173.2; HRMS (Cl) Calcd for C₂₀H₂₈NO₇ (M⁺H⁺) 394.1866. Found 394.1892.

(6-Hydroxy-5,7-dimethoxy-1-oxo-3-propylisoquinolin-2(1H)-yl)acetic acid (18). By the same procedure as described for the preparation of 8, hydrolysis of 16 (98.3 mg, 0.25 mmol) with a trace amount of conc. HCl in MeOH (1.0 mL) was carried out to give the acid (18) (36.8 mg, 37%) (from AcOEt) as a colorless solid. Mp. 220-222 °C; IR \( \nu_{\text{max}} \): 2959, 1726, 1691, 1588 cm⁻¹; \(^1\)H NMR \( \delta \): 1.06 (3H, t, \( J = 7.3 \) Hz), 1.66-1.78 (2H, m), 2.60 (2H, t, \( J = 7.5 \) Hz), 2.97 (2H, br s), 3.94 (3H, s), 3.98 (3H, s), 4.83 (2H, s), 6.63 (1H, s), 7.56 (1H, s); \(^{13}\)C NMR \( \delta \): 13.6, 21.5, 35.2, 45.2, 56.2, 61.0, 99.6, 103.3, 116.4, 127.0, 139.7, 140.5, 143.0, 147.7, 163.0, 170.4; HRMS (Cl) Calcd for C₁₆H₁₉NO₆ (M⁺H⁺) 322.1290. Found 322.1288. Anal Calcd for C₁₆H₁₉NO₆: C, 59.81; H, 5.96; N, 4.36. Found: C, 59.80; H, 6.01; N, 4.32.

2-(6-Hydroxy-5,7-dimethoxy-1-oxo-3-propylisoquinolin-2(1H)-yl)propanoic acid (19). Hydrolysis of 17 (62.8 mg, 0.16 mmol) was carried out by the same procedure as described for the preparation of 18 to give the acid (19) (18.1 mg, 34%) (from AcOEt) as a colorless solid. Mp. 156-157 °C; IR v max: 3370, 2929, 1702, 1614 cm⁻¹; \(^1\)H NMR \( \delta \): 0.87 (1H, br s), 0.94 (3H, t, \( J = 7.4 \) Hz), 1.28 (1H, br s), 1.76 (3H, d, \( J = 6.9 \) Hz), 1.71-1.82 (2H, m), 2.47 (2H, t, \( J = 7.5 \) Hz), 4.01 (3H, s), 4.04 (3H, s), 5.40 (1H, q, \( J = 6.9 \) Hz),
7.28 (1H, s), 7.30 (1H, s); $^{13}$C NMR δ: 13.7, 17.2, 22.4, 39.1, 56.4, 61.1, 73.3, 98.2, 107.6, 111.4, 130.8, 139.0, 141.5, 147.9, 149.5, 159.0, 174.3; HRMS (EI) Calcd for C$_{17}$H$_{21}$NO$_6$ (M$^+$) 335.1369. Found 335.1380. Anal Calcd for C$_{17}$H$_{21}$NO$_6$: C, 60.89; H, 6.31; N, 4.18. Found: C, 60.87; H, 6.26; N, 4.20.

2-(2-[[tert-Butyl(dimethyl)silyl]oxy]ethyl)-5,7-dimethoxy-6-(methoxymethoxy)-3-propylisoquinolin-1(2H)-one (20). To a stirred solution of 15 (100 mg, 0.33 mmol) in DMF:toluene (1:2, 1 mL) was added a solution of NaH (50% in oil) (34.3 mg, 0.72 mmol) in DMF (0.5 mL) at 0 °C, and the mixture was stirred at rt for 1 h. To this solution was added a solution of the bromide (311.0 mg, 1.3 mmol) in DMF (1.0 mL), and the resulting mixture was stirred at the same temperature for further 48 h. After treatment with saturated aqueous NH$_4$Cl solution, the mixture was extracted with Et$_2$O. The ethereal layer was washed with brine and dried over Na$_2$SO$_4$. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane:AcOEt (17:1, v/v) gave the ether (20) (71.8 mg, 47%) as a colorless solid. Mp. 99-100 °C; IR $\nu$ max: 2957, 1651, 1598 cm$^{-1}$; $^1$H NMR δ: -0.09 (6H, s), 0.82 (9H, s), 1.06 (3H, t, $J = 7.3$ Hz), 1.65-1.74 (2H, m), 2.80 (2H, t, $J = 7.6$ Hz), 3.63 (3H, s), 3.93 (3H, s), 3.94 (2H, t, $J = 5.4$ Hz), 3.96 (3H, s), 4.24 (2H, t, $J = 5.44$ Hz), 5.25 (2H, s), 6.53 (1H, s), 7.61 (1H, s); $^{13}$C NMR δ: -5.7, 13.8, 18.1, 21.9, 25.8, 35.6, 46.0, 56.1, 57.3, 61.1, 61.4, 98.6, 98.7, 104.1, 120.9, 126.6, 142.2, 142.7, 147.3, 152.2, 162.5; HRMS (CI) Calcd for C$_{24}$H$_{39}$NO$_6$Si (M$^+$) 465.2546. Found 465.2541. Anal Calcd for C$_{24}$H$_{39}$NO$_6$Si: C, 61.90; H, 8.44; N, 3.01. Found: C, 61.78; H, 8.42; N, 3.03.

2-(2-[[tert-Butyl(dimethyl)silyl]oxy]propyl)-5,7-dimethoxy-6-(methoxymethoxy)-3-propylisoquinolin-1(2H)-one (21). The ether (21) (66.4 mg, 21%), as a pale yellow oil, was obtained from 15 (200 mg, 0.72 mmol) and the bromide (1.1 g, 4.34 mmol) in DMF in the presence of NaH (50% in oil) (47.5 mg, 0.99 mmol) by using the same procedure as described for the preparation of 20. IR $\nu$ max: 2957, 1650, 1598 cm$^{-1}$; $^1$H NMR δ: 0.08 (6H, s), 0.92 (9H, s), 1.05 (3H, t, $J = 7.3$ Hz), 1.67-1.76 (2H, m), 1.90-1.96 (2H, t, $J = 7.6$ Hz), 2.71 (2H, t, $J = 7.6$ Hz), 3.62 (3H, s), 3.72 (2H, t, $J = 5.6$ Hz), 3.94 (3H, s), 3.95 (3H, s), 4.20 (2H, t, $J = 7.6$ Hz), 5.23 (2H, s), 6.55 (1H, s), 7.62 (1H, s); $^{13}$C NMR δ: -5.7, 13.8, 18.1, 21.9, 25.8, 35.6, 46.0, 56.1, 57.3, 61.1, 61.4, 98.6, 98.7, 104.1, 120.9, 126.6, 142.2, 142.7, 147.3, 152.2, 162.5; HRMS (Cl) Calcd for C$_{24}$H$_{39}$NO$_6$Si (M$^+$-56(isobutene)) 423.2077. Found 423.2047.

6-Hydroxy-2-(2-hydroxyethyl)-5,7-dimethoxy-3-propylisoquinolin-1(2H)-one (22). A solution of the ether (20) (71.0 mg, 0.15 mmol) and a trace amount of conc. HCl in MeOH (2.0 mL) was stirred at rt for 5 h. After concentration of the mixture, the residual solid was recrystallized from AcOEt to give the alcohol (22) (29.8 mg, 64%). Mp. 144-146 °C; IR $\nu$ max: 3111, 2961, 1635, 1574 cm$^{-1}$; $^1$H NMR δ: 1.06 (3H, t, $J = 7.3$ Hz), 1.67-1.76 (2H, m), 2.68 (2H, t, $J = 7.8$ Hz), 3.70 (1H, br s), 3.95 (3H, s), 3.98 (2H, t, $J = 4.6$ Hz), 4.00 (3H, s), 4.34 (2H, t, $J = 4.4$ Hz), 6.25 (1H, br s), 6.62 (1H, s), 7.57 (1H, s); $^{13}$C NMR δ:
6-Hydroxy-2-(2-hydroxypropyl)-5,7-dimethoxy-3-propylisoquinolin-1(2H)-one (23). Hydrolysis of the ether (21) (66.4 mg, 0.15 mmol) was carried out with conc. HCl by the same procedure as described for the preparation of 22 to afford the alcohol (23) (35.5 mg, 82%) (from AcOEt) as a colorless solid. Mp. 144-146 °C; IR \( \nu_{\text{max}} \): 3306, 2961, 1640, 1587 cm\(^{-1}\); \(^1\)H NMR \( \delta \): 1.07 (3H, t, \( J = 7.3 \) Hz), 1.70-1.80 (2H, m), 1.89-1.95 (2H, m), 2.69 (2H, \( t, J = 7.5 \) Hz), 3.51 (2H, \( t, J = 5.4 \) Hz), 3.97 (3H, s), 4.00 (3H, s), 4.33 (2H, br s), 4.49 (1H, br s), 6.46 (1H, br s), 6.65 (1H, s), 7.61 (1H, s); \(^{13}\)C NMR \( \delta \): 13.8, 22.5, 32.7, 34.7, 39.4, 56.4, 57.9, 61.0, 100.3, 103.3, 116.7, 126.9, 139.4, 141.0, 142.5, 147.4, 163.6; HRMS (CI) Calcd for C\(_{17}\)H\(_{24}\)NO\(_5\) (M\(^+\)) 322.1654. Found 322.1655. Anal Calcd for C\(_{17}\)H\(_{23}\)NO\(_5\): C, 63.54; H, 7.21; N, 4.36. Found: C, 63.41; H, 7.15; N, 4.30.

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