

Electronic supporting information for

First Synthesis of the Antiangiogenic Homoisoflavanone, Cremastranone

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Synthetic Methodology

All starting materials and reagents were obtained commercially and were used without further purification. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Dichloromethane and acetonitrile were freshly distilled from calcium hydride. All solvents used for routine product isolation and chromatography were of reagent grade and glass distilled. Reaction glassware was dried at 100 °C before use, and air- and moisture-sensitive reactions were performed under argon. Flash column chromatography was performed using silica gel 60 (230–400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). Mass spectra were obtained using a Waters Auto Purification instrument. ¹H and ¹³C spectra were recorded on either a Bruker 600MHz spectrometer or a Bruker 400MHz spectrometer as solutions in deuteriochloroform (CDCl₃) or methanol-d₄ (CD₃OD). ¹H NMR data are reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet and/or multiple resonances), number of protons, and coupling constant (*J*) in Hertz (Hz).

5,6-dihydroxy-3-(3'-hydroxy-4'-methoxybenzyl)-7-methoxychroman-4-one (2)

SH-11052 (2) and intermediates 5, 6, 7, and 8 were synthesized as previously described.¹

3-(3'-hydroxy-4'-methoxybenzyl)-5,6,7-trimethoxy-4H-chromen-4-one (9)

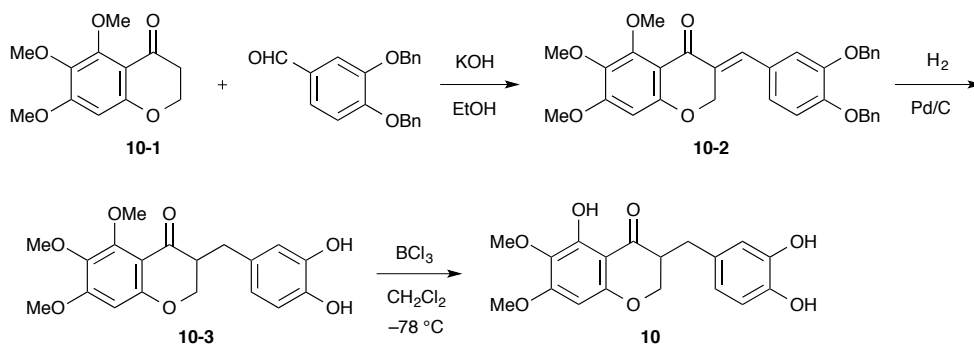
A solution of PCl_5 (180 mg, 0.86 mmol) in DMF (2.5 mL) was stirred at 20 °C for 20 min. To the reaction mixture was added $\text{BF}_3\text{-Et}_2\text{O}$ (0.22 mL, 1.73 mmol) and the dihydrochalcone 1-(6-hydroxy-2,3,4-trimethoxyphenyl)-3-(3'-hydroxy-4'-methoxyphenyl)propan-1-one (**6**) (200 mg, 0.55 mmol) at 20 °C, then the mixture was stirred for 4 h followed by the addition of 1N HCl (2 mL) and dilution with ethyl acetate. The organic layer was washed with water and brine, dried over MgSO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate / *n*-hexane = 1 : 4) to afford the chromone (**9**) (128 mg, 62%). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.34 (s, 1H), 6.83-6.79 (m, 2H), 6.74-6.73 (m, 1H), 6.59 (s, 1H), 5.49 (s, 1H), 3.94 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.67 (s, 2H); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ 175.9, 157.5, 154.7, 152.6, 151.0, 146.5, 144.1, 140.2, 130.6, 125.2, 121.6, 114.3, 112.9, 111.7, 95.9, 62.0, 61.4, 56.2, 55.9, 31.1.; HRMS (ESI): mass calcd for $\text{C}_{20}\text{H}_{20}\text{O}_7$ [$\text{M} + \text{H}$] $^+$, 373.1287; found, 373.1280.

3-(3',4'-dihydroxybenzyl)-5-hydroxy-6,7-dimethoxychroman-4-one (10)

i) For synthesis from compound (8),

To a CH_2Cl_2 (3 mL) solution of the 3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**8**) (42 mg, 0.11 mmol), boron tribromide (1.0 M solution of CH_2Cl_2 , 280 μL , 0.28 mmol) was added at -78 °C. After stirring for 1 h, the reaction mixture was heated to ambient temperature, quenched with methanol, and stirred for 30 min. The organic phase was washed with water and brine, dried over MgSO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate / *n*-hexane = 1 : 2) to afford demethylated compound (**10**) (25 mg, 65%); $^1\text{H-NMR}$ (600 MHz, CD_3OD) δ 6.71 (d, 1H, $J = 6.8$ Hz), 6.67 (d, 1H, $J = 2.4$ Hz), 6.55 (dd, 1H, $J = 7.8$ and 1.8 Hz), 6.14 (s, 1H), 4.29 (dd, 1H, $J = 11.4$ and 4.2 Hz), 4.13 (dd, 1H, $J = 11.4$ and 7.8 Hz), 3.87 (s, 3H), 3.73 (s, 3H), 3.03 (dd, 1H, $J = 13.8$ and 4.8 Hz), 2.82 (m, 1H), 2.59 (dd, 1H, $J = 14$ and 10 Hz); $^{13}\text{C-NMR}$ (150 MHz, CD_3OD) δ 200.6, 162.3, 160.6, 156.2, 146.5, 145.2, 131.3, 130.8, 121.5, 117.1, 116.5, 103.6, 92.5, 70.5, 61.1, 56.8, 33.1.

ii) For synthesis from 5,6,7-methoxy-4-chromanone (**10-1**),



To a solution of 5,6,7-methoxy-4-chromanone (**10-1**) (108 mg, 0.42 mmol) in benzene (5 mL) was added 3',4'-bis(benzyloxy)benzaldehyde (102 mg, 0.45 mmol) and PTSA (9 mg, 0.04 mmol) at 0 °C. The reaction mixture was refluxed for 12 h. After cooling to ambient temperature, the reaction mixture was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 2) to afford (*E*)-3-(3',4'-bis(benzyloxy)benzylidene)-5,6,7-trimethoxychroman-4-one (**10-2**) (117 mg, 51%). And then to an anhydrous MeOH solution of the benzylidene (**10-2**) (78 mg, 0.15 mmol) and 10% Pd/C (8 mg) was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 5) to afford the homoisoflavanone (**10-3**) (42 mg, 77%). ¹H-NMR (600 MHz, CDCl₃) δ 6.78 (d, 1H, *J* = 4.8 Hz), 6.76 (s, 1H), 6.57 (dd, 1H, *J* = 7.8 and 2.4 Hz), 6.22 (s, 1H), 4.24 (dd, 1H, *J* = 11 and 4.2 Hz), 4.09 (dd, 1H, *J* = 12 and 7.2 Hz), 3.90 (s, 3H), 3.84 (s, 3H), 3.77 (s, 3H), 3.05 (dd, 1H, *J* = 7.8 and 4.8 Hz), 2.68 (m, 1H), 2.58 (dd, 1H, *J* = 14 and 10 Hz); ¹³C-NMR (150 MHz, CDCl₃) δ 192.7, 160.0, 159.7, 154.3, 144.1, 142.8, 137.3, 130.5, 121.3, 115.9, 115.3, 108.3, 96.1, 69.0, 61.6, 61.3, 56.1, 48.5, 32.5; HRMS (ESI): mass calcd for C₁₉H₂₀O₇ [M + H]⁺, 361.1287; found, 361.1275. To a CH₂Cl₂ (3 mL) solution of the 3-(3',4'-dihydroxybenzyl)-5,6,7-trimethoxychroman-4-one (**10-3**) (38 mg, 0.1 mmol) boron trichloride (1.0 M solution of CH₂Cl₂, 500 μL, 0.5 mmol) was added at -78 °C. After stirring for 1 h, the reaction mixture was heated to ambient temperature, quenched with methanol, and stirred for 30 min. The organic phase was washed with water and brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate / *n*-hexane = 1 : 2) to afford demethylated compound (**10**) (13 mg, 38%). ¹H-NMR (600 MHz, CD₃OD) δ 6.71 (d, 1H, *J* = 6.8 Hz), 6.67 (d, 1H, *J* = 2.4 Hz), 6.55 (dd, 1H, *J* = 7.8 and 1.8 Hz), 6.14 (s, 1H), 4.29 (dd, 1H, *J* = 11.4 and 4.2 Hz), 4.13 (dd, 1H, *J* = 11.4 and 7.8 Hz), 3.87 (s, 3H), 3.73 (s, 3H), 3.03 (dd, 1H, *J* = 13.8 and 4.8 Hz), 2.82 (m, 1H), 2.59 (dd, 1H, *J* = 14.4 and 10.2 Hz); ¹³C-NMR (150 MHz, CD₃OD) δ 200.6, 162.3, 160.6, 156.2, 146.5, 145.2, 131.3, 130.8, 121.5, 117.1, 116.5, 103.6, 92.5, 70.5, 61.1, 56.8, 33.1; HRMS (ESI): mass calcd for C₁₈H₁₈O₇ [M + H]⁺, 347.1131; found, 347.1122

5-hydroxy-3-(3'-hydroxy-4'-methoxybenzyl)-6,7-dimethoxychroman-4-one (11)

To a solution of 3-(3'-hydroxy-4'-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**8**) (70 mg, 0.187 mmol) in CHCl₃ (2 mL) was added TMSI (53 μ L, 0.374 mmol) at 0 °C and the reaction mixture was heated at 60 °C for 1 h. The mixture was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 2) to afford compound **11** (49 mg, 73%). ¹H-NMR (600 MHz, CD₃OD) δ 6.85 (d, 1H, *J* = 7.8 Hz), 6.71 (d, 1H, *J* = 1.8 Hz), 6.66 (dd, 1H, *J* = 8.4 and 2.4 Hz), 6.14 (s, 1H), 4.29 (dd, 1H, *J* = 11.4 and 4.2 Hz), 4.12 (dd, 1H, *J* = 11.4 and 7.2 Hz), 3.86 (s, 3H), 3.83 (s, 3H), 3.73 (s, 3H), 3.09 (dd, 1H, *J* = 13.8 and 4.2 Hz), 2.87-2.82 (m, 1H), 2.64 (dd, 1H, *J* = 14.4 and 10.2 Hz); ¹³C-NMR (150 MHz, CD₃OD) δ 200.5, 162.4, 160.7, 156.2, 148.0, 147.8, 132.3, 131.4, 121.4, 117.1, 113.0, 103.7, 92.6, 70.6, 61.2, 56.8, 56.5, 48.2, 33.1; HRMS (ESI): mass calcd for C₁₉H₂₀O₇ [M + H]⁺, 361.1287; found, 361.1277.

2'-methoxy-5'-((5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl benzoate (13)

To a solution of 3-(3'-hydroxy-4'-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**8**) (149 mg, 0.39 mmol) in CH₂Cl₂ (2.5 mL) was added Et₃N (0.11 mL, 0.78 mmol), DMAP (10 mg, 0.08 mmol), and benzoyl chloride (0.05 mL, 0.47 mmol) at 0 °C and the reaction mixture was stirred at ambient temperature for 2 h. The reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 2) to afford the benzoate compound (**13**) (182 mg, 80%). ¹H-NMR (400 MHz, CDCl₃) δ 8.18-8.16 (m, 2H), 7.60-7.56 (m, 1H), 7.48-7.44 (m, 2H), 7.06 (d, 1H, *J* = 7.8 Hz), 6.86 (d, 1H, *J* = 1.9 Hz), 6.83 (dd, 1H, *J* = 8.3 and 1.9 Hz), 6.23 (s, 1H), 4.31 (dd, 1H, *J* = 11 and 3.9 Hz), 4.13 (dd, 1H, *J* = 11 and 7.8 Hz), 3.91 (s, 3H), 3.84 (s, 3H), 3.78 (s, 3H), 3.77 (s, 3H), 3.26 (dd, 1H, *J* = 14 and 3.9 Hz), 2.81 (m, 1H), 2.70 (dd, 1H, *J* = 14 and 11 Hz); ¹³C-NMR (100 MHz, CDCl₃) δ 190.9, 164.6, 159.6, 159.2, 154.3, 151.2, 138.5, 137.4, 133.3, 130.1, 129.9, 129.2, 128.3, 128.2, 122.7, 121.2, 113.1, 108.5, 95.9, 68.9, 61.5, 61.1, 56.0, 55.8, 48.2, 32.6.

5'-((5,6-dihydroxy-7-methoxy-4-oxochroman-3-yl)methyl)-2'-methoxyphenyl benzoate (14)

To a solution of benzoate (**13**) (65 mg, 0.136 mmol) in CHCl₃ (1 mL) was added TMSI (77 μ L, 0.54 mmol) at 0 °C and the reaction mixture was heated at 60 °C for 1 h. The mixture was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 2 : 3) to afford the compound **14** (32 mg, 52%). ¹H-NMR (400 MHz, CDCl₃) δ 8.20 (m, 2H), 7.63 (m, 1H), 7.51 (m, 2H), 7.09 (d, 1H, *J* = 7.8 Hz), 6.87 (d, 1H, *J* = 2.0 Hz), 6.85 (dd, 1H, *J* = 7.8 and 2.0 Hz),

6.06 (s, 1H), 4.32 (dd, 1H, $J = 11$ and 3.9 Hz), 4.16 (dd, 1H, $J = 11$ and 7.3 Hz), 3.90 (s, 3H), 3.82 (s, 3H), 3.26 (dd, 1H, $J = 14$ and 3.9 Hz), 2.89-2.87 (m, 1H), 2.78 (dd, 1H, $J = 14$ and 11 Hz); ^{13}C -NMR (100 MHz, CDCl_3) δ 198.4, 164.7, 155.9, 154.7, 151.4, 148.1, 138.8, 136.8, 133.4, 130.2, 129.3, 128.5, 127.3, 123.0, 121.3, 113.2, 102.4, 91.0, 69.2, 56.3, 55.9, 46.8, 32.7, 30.9.

(*E*)-1-(4-(benzyloxy)-6-hydroxy-2,3-dimethoxyphenyl)-3-(3'-hydroxy-4'-methoxyphenyl)prop-2-en-1-one (16)

To a solution of 4'-benzyloxy-6'-hydroxy-2',3'-dimethoxyacetophenone (**15**) (104 mg, 0.34 mmol) in EtOH (6 mL) was added KOH (95 mg, 1.7 mmol) and isovanillin (62 mg, 0.41 mmol) at rt. The reaction mixture was stirred for 48 h at rt. The mixture was concentrated in vacuo. The residue was washed with 2 N HCl solution and brine. Drying over MgSO_4 and removal of the solvent followed by column chromatography on silica gel using (ethyl acetate / *n*-hexane = 1 : 2) gave the chalcone (**16**) (67 mg, 53%) as a yellow solid. ^1H -NMR (600 MHz, CDCl_3) δ 13.68 (s, 1H), 7.81 (d, 1H, $J = 15.6$ Hz), 7.75 (d, 1H, $J = 15.6$ Hz), 7.42 (d, 2H, $J = 6.6$ Hz), 7.38 (t, 2H, $J = 7.8$ Hz), 7.33 (t, 1H, $J = 7.2$ Hz), 7.26 (d, 1H, $J = 2.4$ Hz), 7.11 (dd, 1H, $J = 8.4$ and 2.4 Hz), 6.85 (d, 1H, $J = 8.4$ Hz), 6.32 (s, 1H), 5.12 (s, 2H), 3.92 (s, 3H), 3.91 (s, 3H), 3.83 (s, 3H); ^{13}C -NMR (150 MHz, CDCl_3) δ 192.8, 162.4, 159.1, 155.1, 148.7, 145.9, 143.5, 135.8, 135.5, 129.0, 128.7, 128.2, 127.3, 124.6, 122.8, 113.0, 110.5, 109.0, 97.7, 70.6, 61.9, 61.3, 56.0; HRMS (ESI): mass calcd for $\text{C}_{25}\text{H}_{24}\text{O}_7$ [$\text{M} + \text{H}$] $^+$, 437.1600; found, 437.1620.

1-(4,6-dihydroxy-2,3-dimethoxyphenyl)-3-(3'-hydroxy-4'-methoxyphenyl)propan-1-one (17)

A solution of the chalcone (**16**) (40 mg, 0.11 mmol) and 10% Pd/C (20 mg) in anhydrous MeOH was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the dihydrochalcone (**17**) (30 mg, 94%). ^1H -NMR (600 MHz, CDCl_3) δ 13.23 (s, 1H), 6.81 (d, 1H, $J = 2.4$ Hz), 6.77 (d, 1H, $J = 7.8$ Hz), 6.71 (dd, 1H, $J = 8.4$ and 2.4 Hz), 6.27 (s, 1H), 5.60 (s, 1H), 3.90 (s, 3H), 3.85 (s, 3H), 3.80 (s, 3H), 3.3 (t, 2H, $J = 7.8$ Hz), 2.92 (t, 2H, $J = 7.8$ Hz); ^{13}C -NMR (150 MHz, CDCl_3) δ 207.6, 161.8, 156.2, 154.4, 145.5, 144.8, 134.7, 132.7, 119.8, 114.56, 110.7, 108.5, 99.1, 60.9, 60.6, 56.0, 44.9, 29.8; HRMS (ESI): mass calcd for $\text{C}_{18}\text{H}_{20}\text{O}_7$ [$\text{M} + \text{H}$] $^+$, 349.1287; found, 349.1272.

3-(3'-(benzyloxy)-4'-methoxyphenyl)-1-(4-(benzyloxy)-6-hydroxy-2,3 dimethoxyphenyl)propan-1-one (18)

To an acetone (5 mL) solution of the dihydrochalcone **17** (250 mg, 0.72 mmol) were added benzyl bromide (270 mg, 1.6 mmol) and K₂CO₃ (300 mg, 2.2 mmol). After refluxing for 3 h, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 2) to afford the dibenzylated compound (**18**) (340 mg, 89%). ¹H-NMR (600 MHz, CDCl₃) δ 13.29 (s, 1H), 7.42 (m, 4H), 7.38 (t, 2H, *J* = 7.2 Hz), 7.33 (t, 3H, *J* = 7.2 Hz), 7.26 (t, 1H, *J* = 7.2 Hz), 6.83(d, 1H, *J* = 9.0 Hz), 6.78 (dd, 2H, *J* = 6.0 and 1.8 Hz), 6.27 (s, 1H), 5.12 (s, 2H), 5.10 (s, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 3.76 (s, 3H), 3.25 (t, 2H, *J* = 7.2 Hz), 2.89 (t, 2H, *J* = 7.8 Hz); ¹³C-NMR (150 MHz, CDCl₃) δ 204.8, 159.0, 155.2, 148.0, 137.2, 135.7, 134.9, 134.0, 128.7, 128.5, 128.2, 127.7, 127.3, 127.2, 120.9, 114.7, 111.9, 108.4, 97.3, 71.0, 70.5, 61.1, 61.0, 56.1, 45.0, 29.9 ; HRMS (ESI): mass calcd for C₃₂H₃₂O₇ [M + H]⁺, 529.2226; found, 529.2207.

7-(benzyloxy)-3-(3'-(benzyloxy)-4'-methoxybenzyl)-5,6-dimethoxy-4H-chromen-4-one (19)

To a solution of the dibenzylated dihydrochalcone (**18**) (93 mg, 0.2 mmol) in toluene (5 mL) was added *N,N*-dimethylformamide dimethyl acetal (43 mg, 0.36 mmol). After refluxing for 6 h, the reaction mixture was cooled and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 2) to afford the 4-chromone (**19**) (76 mg, 80%). ¹H-NMR (600 MHz, CDCl₃) δ 7.44 (d, 2H, *J* = 8.4 Hz), 7.40 (t, 4H, *J* = 7.2 Hz), 7.34 (t, 1H, *J* = 6.0 Hz), 7.29 (t, 2H, *J* = 7.2 Hz), 7.22 (m, 2H), 6.80 (s, 2H), 6.77 (s, 1H), 6.63 (s, 1H), 5.16 (s, 2H), 5.09 (s, 2H), 3.95 (s, 3H), 3.89 (s, 3H), 3.93 (s, 3H), 3.62 (s, 2H); ¹³C-NMR (150 MHz, CDCl₃) δ 175.9, 156.6, 154.5, 152.8, 151.0, 148.3, 148.0, 140.6, 137.1, 135.6, 131.2, 128.7, 128.4, 128.3, 127.7, 127.4, 127.2, 125.0, 121.8, 115.3, 113.1, 112.0, 97.4, 71.0, 70.8, 62.1, 61.5, 56.1, 30.8 ; HRMS (ESI): mass calcd for C₃₃H₃₀O₇ [M + H]⁺, 539.2070; found, 539.2049.

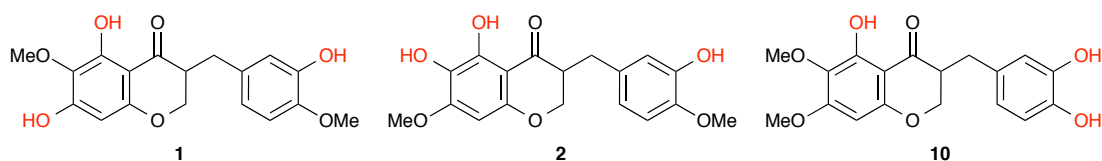
7-hydroxy-3-(3'-hydroxy-4'-methoxybenzyl)-5,6-dimethoxychroman-4-one (20)

A solution of the chromone (**19**) (35 mg, 0.07 mmol) and 10% Pd/C (10 mg) in MeOH was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the 4-chromanone (**20**) (22 mg, 87%). ¹H-NMR (600 MHz, CD₃OD) δ 6.82 (d, 1H, *J* = 14.4 Hz), 6.67 (d, 1H,

$J = 1.8$ Hz), 6.63 (dd, 1H, $J = 8.4$ and 2.4 Hz), 6.16 (s, 1H), 4.21 (dd, 1H, $J = 11.4$ and 4.2 Hz), 4.04 (dd, 1H, $J = 11.4$ and 7.2 Hz), 3.82 (s, 3H), 3.79 (s, 3H), 3.75 (s, 3H), 3.00 (dd, 1H, $J = 13.2$ and 4.2 Hz), 2.66 (m, 1H), 2.58 (dd, 1H, $J = 13.8$ and 10.8 Hz); $^{13}\text{C-NMR}$ (150 MHz, CD_3OD) δ 192.4, 160.0, 158.5, 154.4, 146.3, 146.2, 136.4, 131.2, 119.9, 115.6, 111.5, 107.3, 99.1, 68.6, 60.4, 60.1, 55.0, 48.2, 32.0 ; HRMS (ESI): mass calcd for $\text{C}_{19}\text{H}_{20}\text{O}_7$ $[\text{M} + \text{H}]^+$, 361.1287; found, 361.1270.

Cremastranone (1)

To a solution of 7-hydroxy-3-(3-hydroxy-4-methoxybenzyl)-5,6-dimethoxychroman-4-one (**20**) (113 mg, 0.315 mmol) in CHCl_3 (3 mL) was added TMSI (89 μL , 0.63 mmol) at 0 °C and the reaction mixture was stirred at RT for 30 min. The mixture was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 1) to afford cremastranone (**1**) (95 mg, 87%). $^1\text{H-NMR}$ (600 MHz, CD_3OD) δ 6.85 (d, 1H, $J = 8.4$ Hz), 6.70 (d, 1H, $J = 1.8$ Hz), 6.68 (dd, 1H, $J = 8.4$ and 2.4 Hz), 5.91 (s, 1H), 4.23 (dd, 1H, $J = 11.4$ and 4.2 Hz), 4.06 (dd, 1H, $J = 11.4$ and 7.2 Hz), 3.82 (s, 3H), 3.77 (s, 3H), 3.08 (dd, 1H, $J = 13.8$ and 4.8 Hz), 2.82 (m, 1H), 2.63 (dd, 1H, $J = 13.8$ and 4.2 Hz); $^{13}\text{C-NMR}$ (150 MHz, CD_3OD) δ 200.1, 160.6, 160.1, 156.8, 147.8, 147.6, 132.2, 130.4, 121.3, 117.0, 112.9, 102.9, 95.7, 70.3, 60.9, 56.4, 47.9, 33.1; HRMS (ESI): mass calcd for $\text{C}_{18}\text{H}_{18}\text{O}_7$ $[\text{M} + \text{H}]^+$, 347.1131; found, 347.1118. Purity >95% by LC-MS.

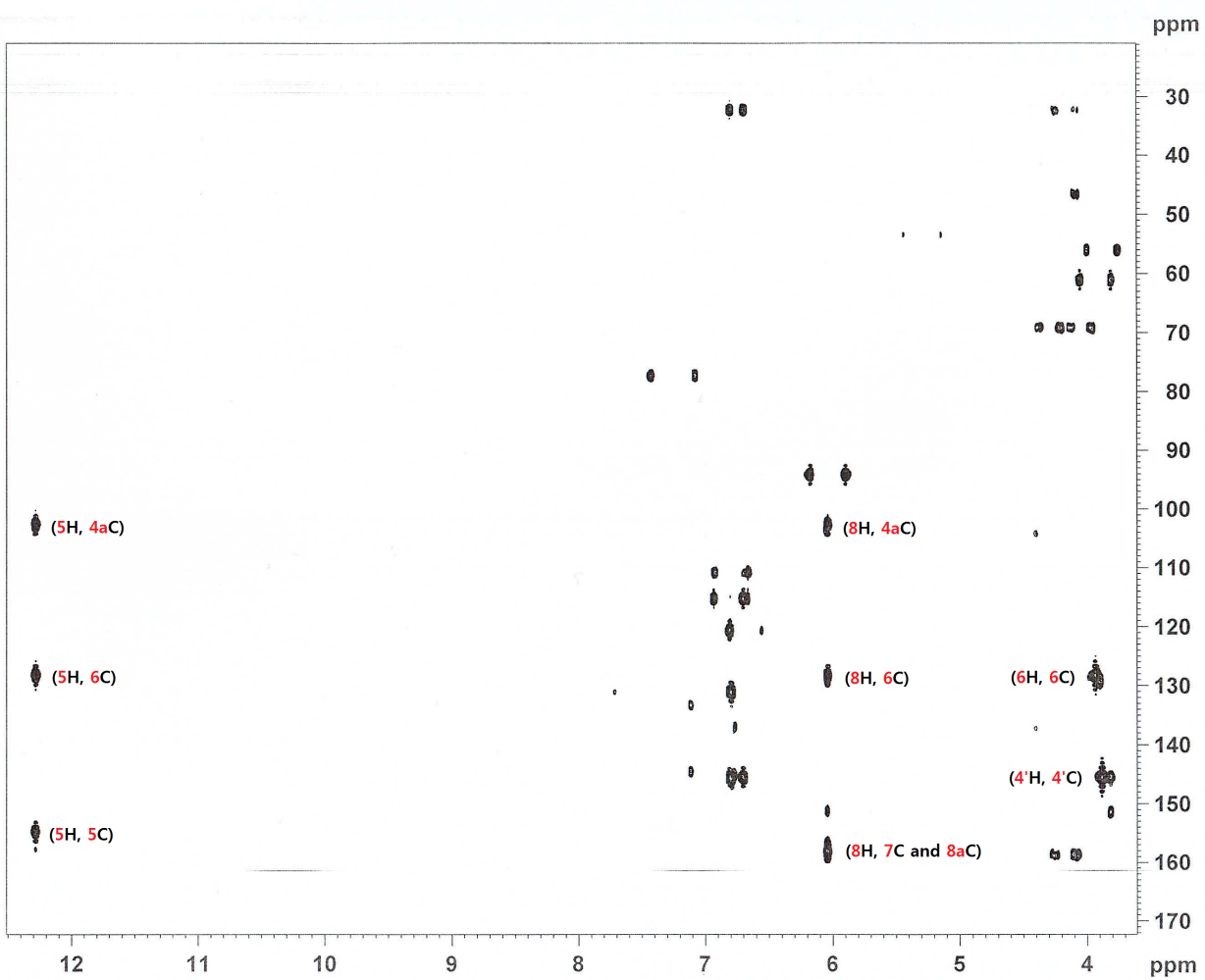
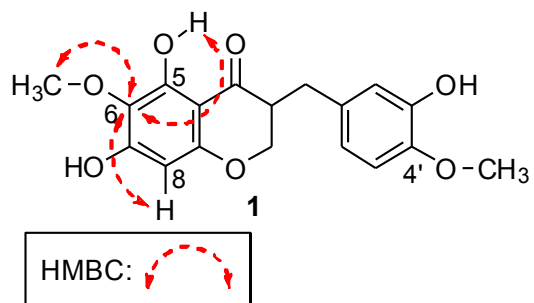


ESI Table 1. Chemical shifts in ^1H - and ^{13}C -NMR for selected compounds.

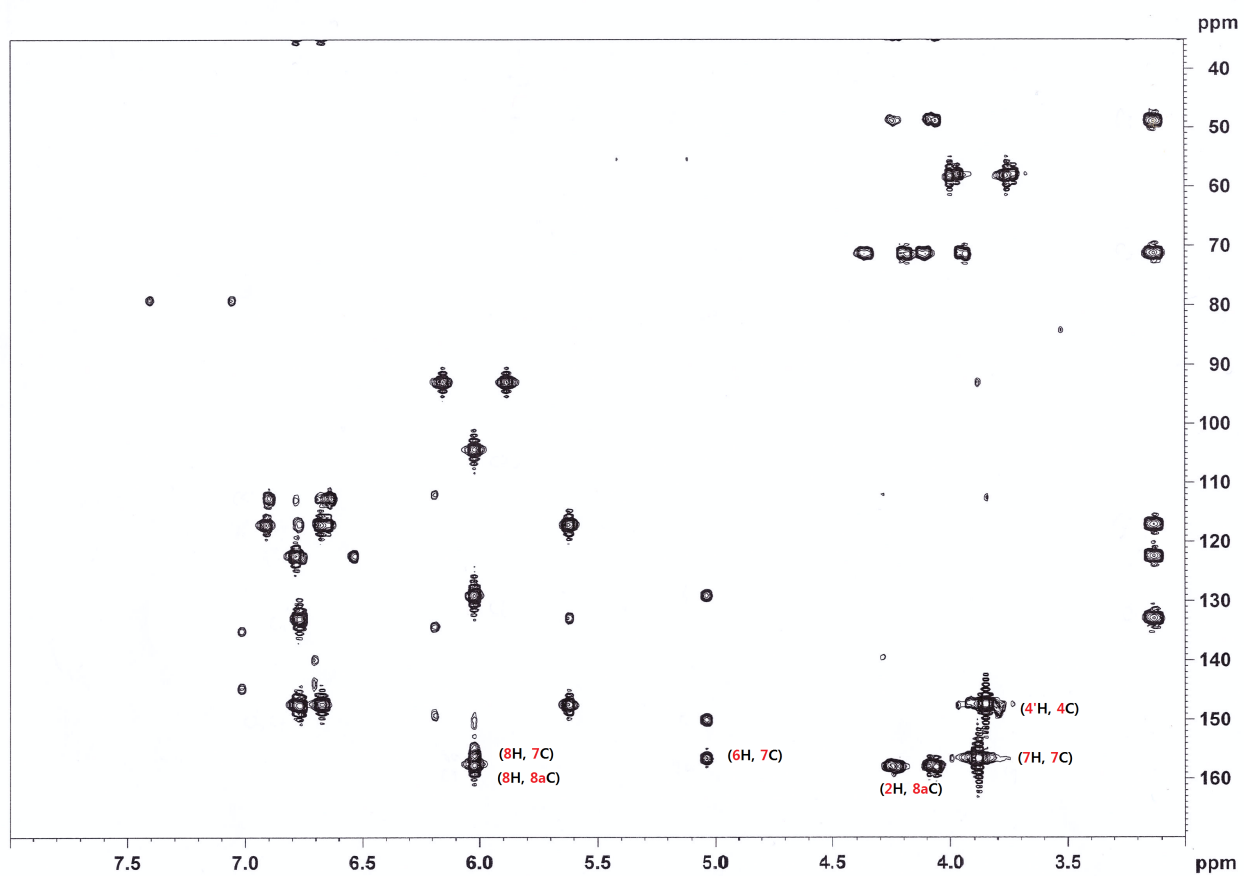
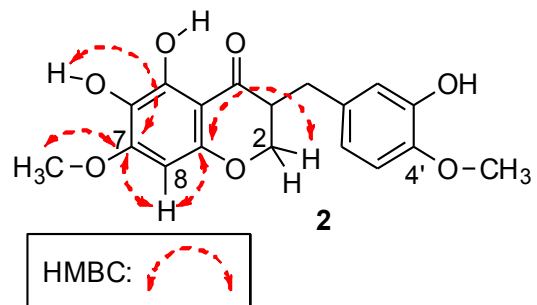
	Reported natural product (1) ²		New synthetic compound (1)		Synthetic compound (2) by TMSI		Synthetic compound (10) by BBr ₃	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
2	4.06, 4.23	70.3	4.07, 4.23	70.3	4.11, 4.25	70.6	4.13, 4.29	70.6
4	2.81	200	2.82	200.1	2.87	200.6	2.85	200.6
4a	-	103		102.9		103.5	-	103.7
5	12.22	156.8		156.8		157.5	-	156.3
6	<u>3.82^a</u>	129.2	<u>3.82^a</u>	130.4	<u>3.87^a</u>	128.7	3.87	
7	9.87	160.9		160.7		149.3	-	162.4
8	5.91	95.8	5.91	95.8	6.14	97	6.15	92.6
8a		160.1		160.1		148.7	-	160.7
9	2.62, 3.06	33.1	2.63, 3.08	33.1	2.69, 3.12	33.2	2.62, 3.06	33.2
1'	-	132.3		132.3		132.4	-	130.8
2'	6.71	117	6.71	117	6.73	117.1	6.67	117.2
3'	8.75	147.8		147.8		147.9	-	146.6
4'	<u>3.77^a</u>	147.8	<u>3.77^a</u>	147.7	<u>3.83^a</u>	147.7	3.74	145.3
5'	6.85	112.9	6.85	112.9	6.8	112.5	6.71	116.6
6'	6.66	121.3	6.66	121.3	6.67	121.4	6.56	121.6
6-OCH₃	-	61		60.9		61.6	-	61.2
7-OCH₃	-						-	
3'-OCH₃	-						-	
4'-OCH₃	-	56.4		56.4		56.5	-	56.8

a : revised assignment by HMBC

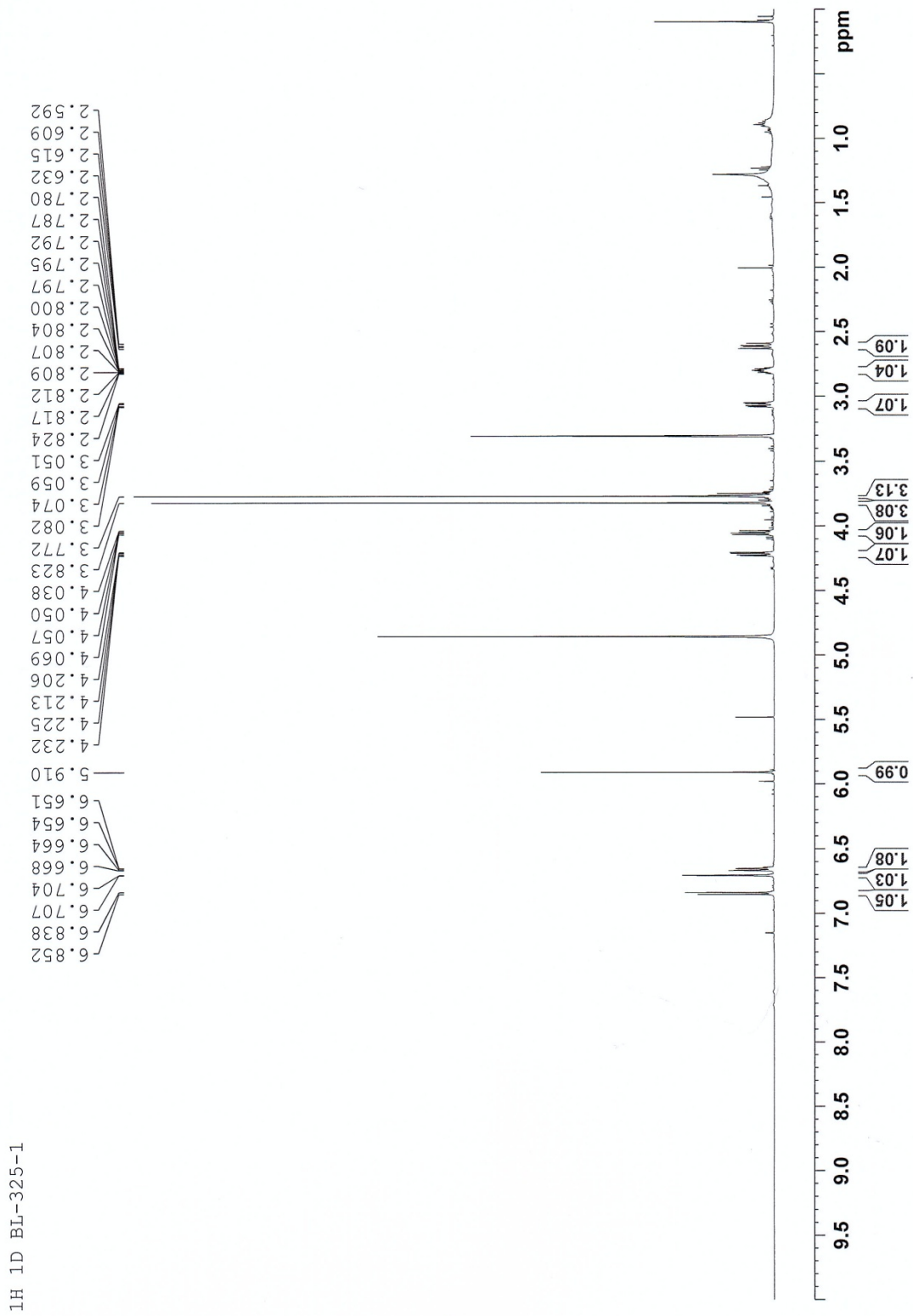
HMBC (600MHz, CD₃OD) of Cremastranone (1)



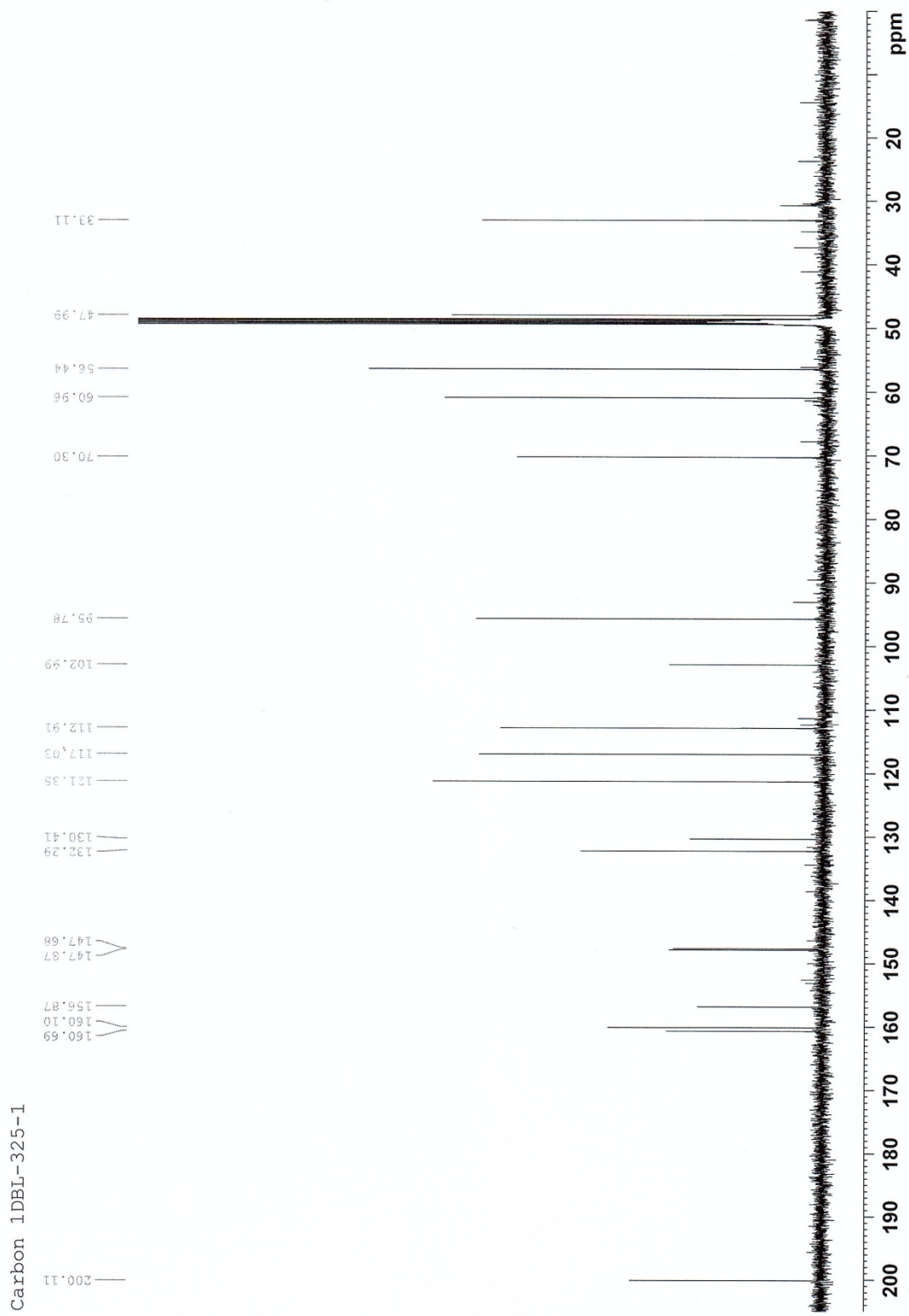
HMBC (600MHz, CDCl₃) of SH-11052 (2)



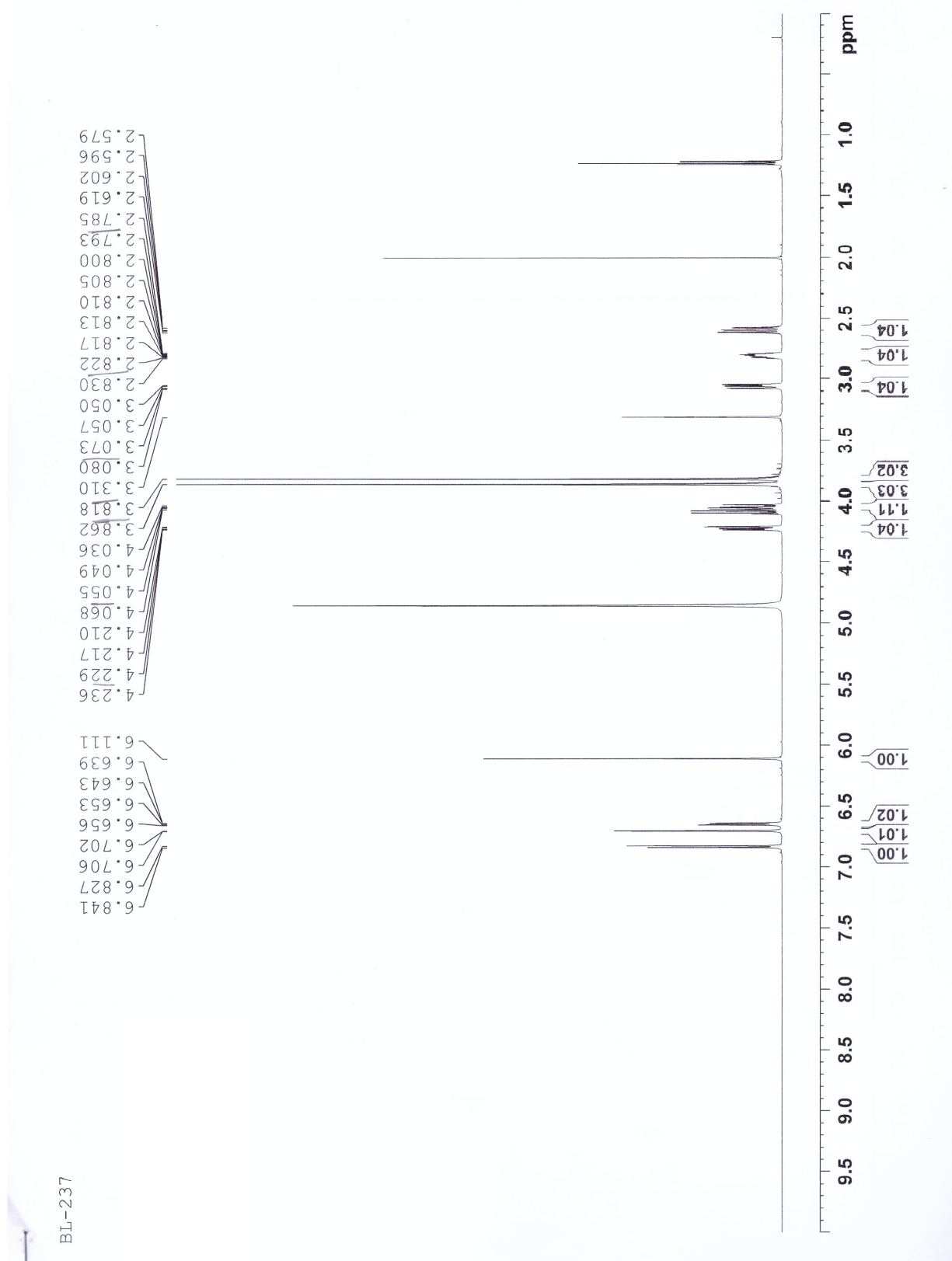
¹H-NMR (600MHz, CD₃OD) of Cremastranone (1)



¹³C-NMR (150MHz, CD₃OD) of Cremastranone (1)

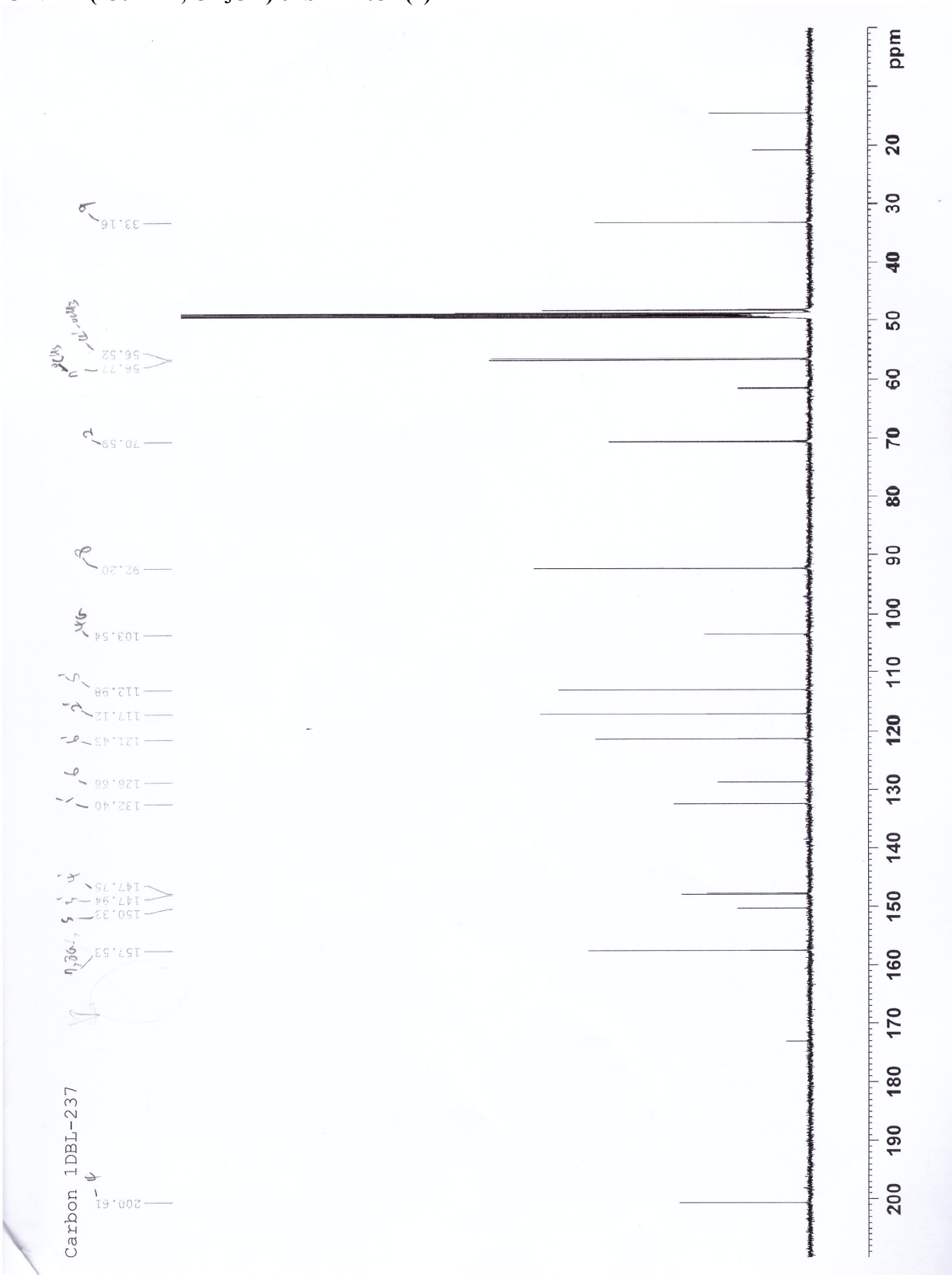


¹H-NMR (600MHz, CD₃OD) of SH-11052 (2)

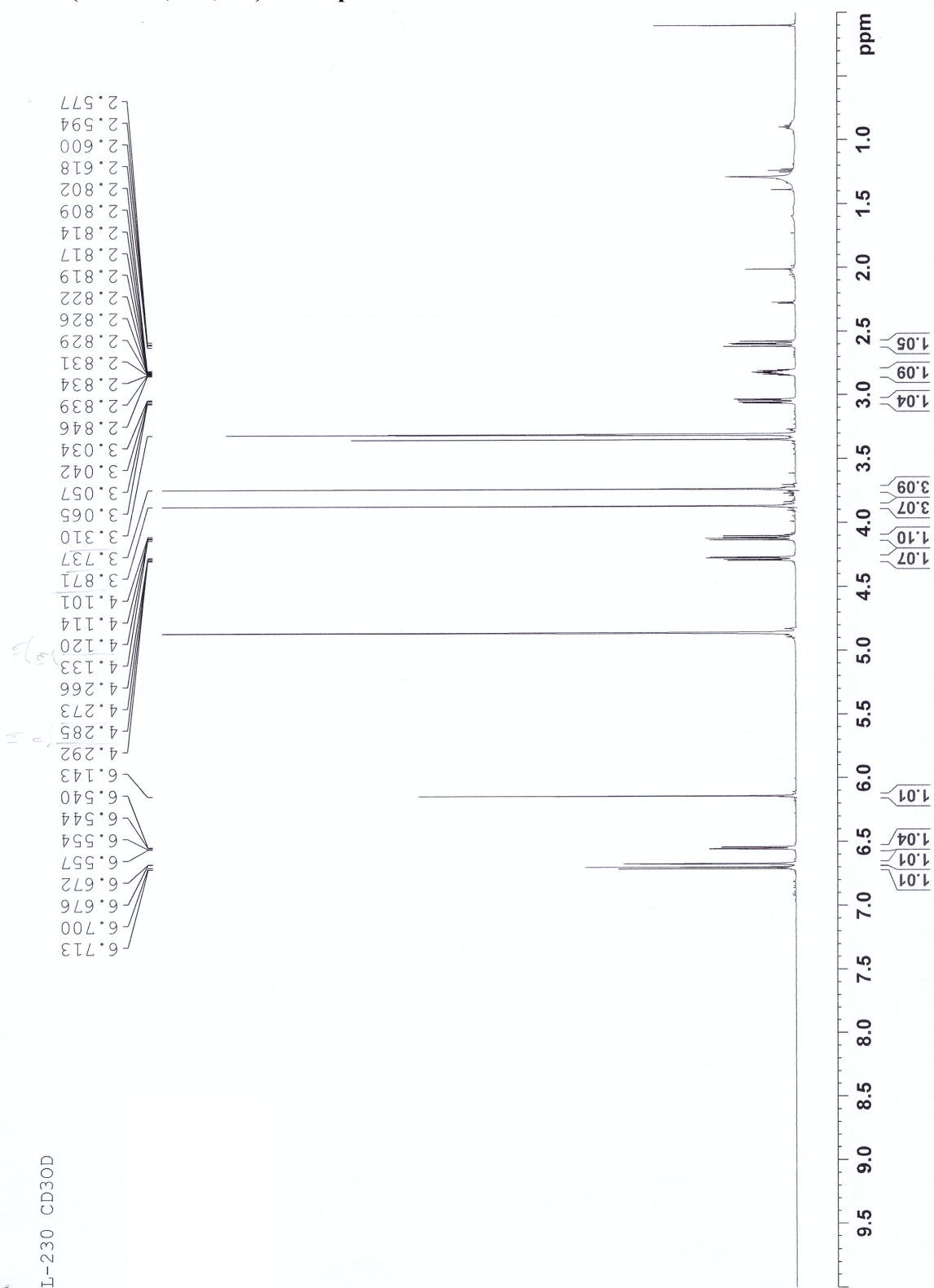


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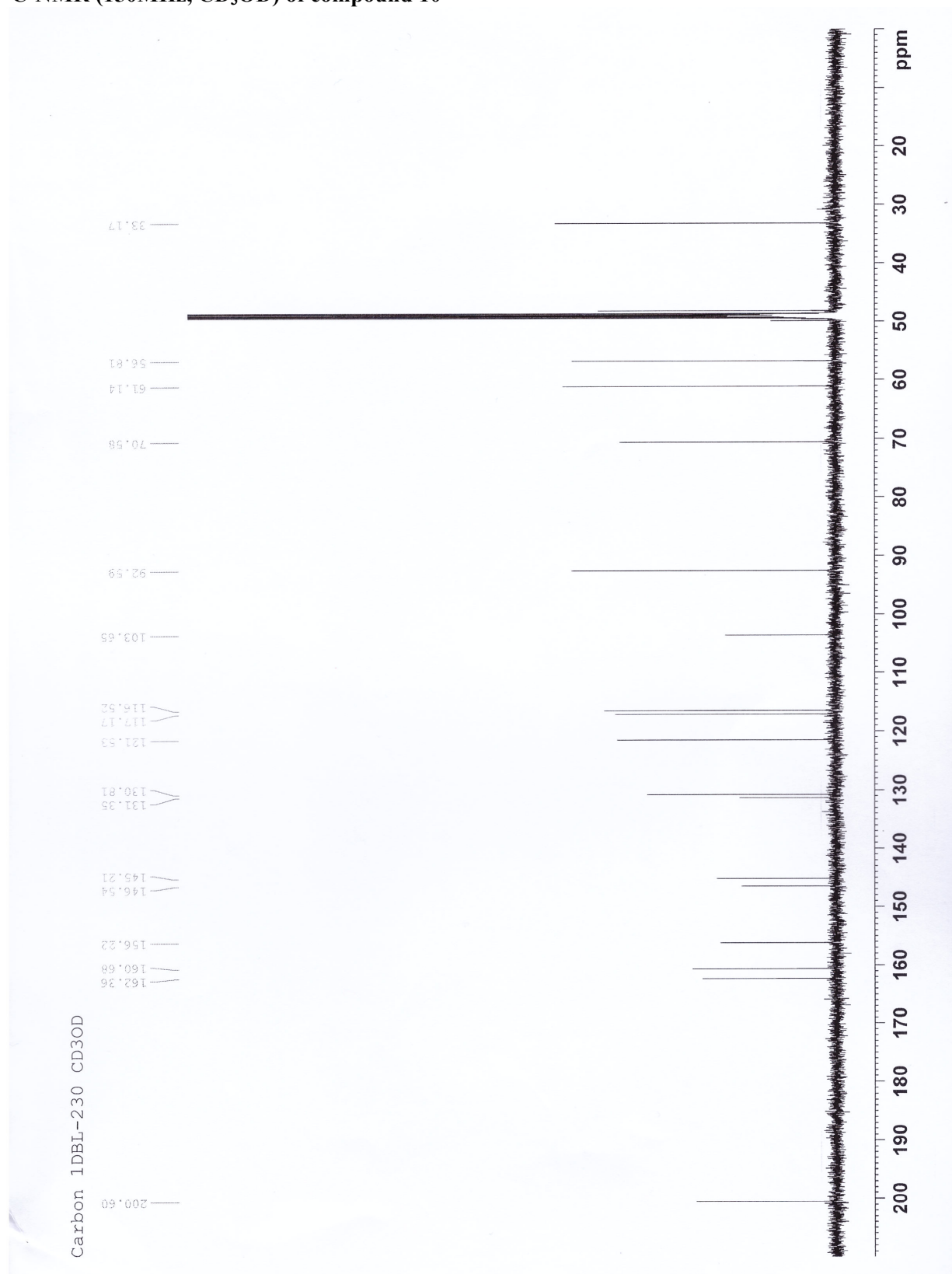
¹³C-NMR (150MHz, CD₃OD) of SH-11052 (2)



¹H-NMR (600MHz, CD₃OD) of compound 10



¹³C-NMR (150MHz, CD₃OD) of compound 10



Biological Methods

Cell proliferation assay

The proliferation of cells was monitored by an alamarBlue based fluorescence assay as described previously.¹ Briefly, 2500 cells in 100 μ L growth medium were incubated in 96-well clear bottom black plates at 37 °C, 5% CO₂ for 24 hours followed by 44 hours incubation with different concentrations of **1** in DMSO (range: 0.5 nM to 500 μ M; final DMSO concentration 1%). Cells used were: Human umbilical vein endothelial cells (HUVECs; Lonza) and human retinal endothelial cells (HRECs; Cell Systems) in EGM-2 medium (Lonza); Y-79 retinoblastoma cells³ (a kind gift of Dr. Brenda L. Gallie, Ontario Cancer Institute) in previously described medium;⁴ 92-1 uveal melanoma cells⁵ (a kind gift of Dr. Martine Jager, University of Leiden) in RPMI-1640, 10% FBS, penicillin/streptomycin; and ARPE-19 human retinal pigment epithelial cells⁶ (a kind gift of Dr. Michael Boulton, Indiana University) in Ham's F10, 10% FBS, penicillin/streptomycin. For VEGF-induced proliferation, HRECs plated as above were starved with EBM-2 medium (Lonza) overnight, then incubated for 44 hours with different concentrations of **1** plus 50 ng/mL human VEGF-165 (BioLegend) in EBM-2. At the end of each incubation, 11.1 μ L of alamarBlue reagent (AbDSerotec) was added and 4 hours after, fluorescent readings were taken on a Synergy H1 plate reader (Biotek) with excitation and emission wavelengths of 560 nm and 590 nm respectively. Data were analysed and dose response curves generated using GraphPad Prism software (v. 6.0).

EdU incorporation assay

The assay was carried out as described before.¹ HRECs (25,000) were seeded onto coverslips coated with attachment factor and grown for 24 hours before starving in serum-free EBM-2 medium. After starvation for 12 hours, the cells were incubated with 10 μ M 5-ethynyl-2'-deoxyuridine (EdU) in the presence of various concentrations of **1** for 8 hours. Then the cells were processed according to the manufacturer's instructions for the click-iT EdU assay kit (Life Technologies). The images were taken using an EVOS microscope (AMG) and data were analysed using ImageJ.

In vitro angiogenesis assay

Matrigel based tube formation assay was performed to monitor the tube-formation ability of HRECs in the presence of **1** as described previously.¹ Briefly, 7500 cells in 100 μ L EGM-2 medium were incubated in the presence or absence of **1** in 96-well clear plates coated with 75 μ L of Matrigel basement

membrane. After 8 hours, images were recorded using the EVOS microscope. Number of polygons was manually counted, and the tube length was measured using Angiogenesis Analyser macros⁷ in ImageJ.

In vitro scratch assay

HRECs (10^5) were seeded in each well of a 6-well plate coated with attachment factor (Cell Systems). The cells were incubated in EGM-2 medium until confluent (~24 hours). The cells were then starved for 12 hours in serum free EBM-2 medium. After starvation, a straight scratch was introduced in the well by a sterile, fine 10 μ L micropipette tip, and the well was rinsed twice using EBM-2 medium to remove unbound cells and debris. Then cells were incubated in EGM-2 medium in the presence of the indicated concentrations of **1** at 37 °C and 5% CO₂. After 8 hours, images were taken using the EVOS microscope and the number of migrated cells into the scratched area was counted.

References

- 1 H. D. Basavarajappa, B. Lee, X. Fei, D. Lim, B. Callaghan, J. A. Mund, J. Case, G. Rajashekhar, S. Y. Seo and T. W. Corson, *PLoS One*, 2014, **9**, e95694.
- 2 M. Adinolfi, M. M. Corsaro, R. Lanzetta, G. Laonigro, L. Mangoni and M. Parrilli, *Phytochemistry*, 1987, **26**, 285-290.
- 3 T. W. Reid, D. M. Albert, A. S. Rabson, P. Russell, J. Craft, E. W. Chu, T. S. Tralka and J. L. Wilcox, *J. Natl. Cancer Inst.*, 1974, **53**, 347-360.
- 4 T. W. Corson, B. C. Samuels, A. A. Wenzel, A. J. Geary, A. A. Riley, B. P. McCarthy, H. Hanenberg, B. J. Bailey, P. I. Rogers, K. E. Pollok, G. Rajashekhar and P. R. Territo, *PLoS One*, 2014, **9**, e99036.
- 5 I. De Waard-Siebinga, D. J. Blom, M. Griffioen, P. I. Schrier, E. Hoogendoorn, G. Beverstock, E. H. Danen and M. J. Jager, *Int. J. Cancer*, 1995, **62**, 155-161.
- 6 K. C. Dunn, A. E. Aotaki-Keen, F. R. Putkey and L. M. Hjelmeland, *Exp. Eye Res.*, 1996, **62**, 155-169.
- 7 C. Gilles, <http://image.bio.methods.free.fr/ImageJ/?Angiogenesis-Analyzer-for-ImageJ.html>, (accessed May 2014).