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Supporting Information

SUPPORTING INFORMATION FOR

Remarkable Influence of the Side Chain of Scyphostatin on the Mode of Inhibition of Neutral Sphingomyelinase

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Biology

Materials:

Frozen stripped rat brains were purchased from Pel-Freez Biologicals (Rogers, AK) and stored at -80°C.

An Amersham Biosciences HPLC System (Basic 100) was used. All chemicals were obtained from Sigma.

N-[methyl-^{14}C]sphingomyelin from bovine brain were obtained from Amersham Biosciences.

Chemical Synthesis

General: All reactions were carried out under a dry argon atmosphere with anhydrous, freshly distilled solvents under anhydrous conditions unless otherwise noted. All reactions were magnetically stirred with Teflon stir bars, and temperatures were measured externally. Reactions requiring anhydrous conditions were carried out in oven dried (120°C, 24 h) or flame dried (vacuum < 0.5 Torr) glassware. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. All reagents were obtained from Aldrich Chemical Co. Inc. and used without further purification. All reactions were monitored by thin layer chromatography (TLC) carried out on 0.25-mm E.Merck silica gel plates (60F-254). E.Merck silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography. Mass spectra were measured on a Finnigan MAT MS 70 (EI) spectrometer or on a Bruker Daltonics Apex II (ESI). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM-250 or a Bruker Advance DRX-500 instrument as noted individually. Chemical shifts are measured in parts per million (δ) relative to the deuterated solvent used in the experiment. Multiplicities are designated as singlet (s), doublet (d), triplet (t), or multiplet (m). Broad peaks are indicated as “b”.

2,2,2-trichloro-*N*-(3,4-dihydro-7-hydroxy-2H-chromen-3-yl)acetamide (7)

To a stirred solution of 7-hydroxy-3-amino-3,4-dihydro-2*H*-1-benzopyran (**2**) (2.1 g, 12.7 mmol) in THF (100 mL) was added at ambient temperature hexachloroacetone (1.9 mL, 12.7 mmol). The mixture was heated to 60 °C for 6 h and was then and concentrated under reduced pressure. The residue thus obtained was purified by flash column chromatography (8:2 hexane-ethyl acetate) to produce 2,2,2-trichloro-*N*-(3,4-dihydro-7-hydroxy-2H-chromen-3-yl)acetamide (**7**) (3.8 g, 95%) as amorphous white solid: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.04 (d, *J* = 7.0 Hz, 1 H, N*H*CO), 6.90 (d, *J*

= 8.3 Hz, 1 H, Ar*H*), 6.48 (dd, *J* = 8.3, 2.4 Hz, 1 H, Ar*H*), 6.42 (d, *J* = 2.4 Hz, 1 H, Ar*H*), 6.07 (bs, 1 H, ArO*H*), 4.45–4.41 (m, 1 H, C*H*NHCO), 4.22 (ddd, *J* = 11.2, 3.8, 2.2 Hz, 1 H, C*H*HO), 4.11 (dd, *J* = 11.2, 1.6 Hz, CH*H*O), 3.12 (dd, *J* = 16.6, 5.2 Hz, 1 H, ArC*H*H), 2.78 (bd, *J* = 16.6 Hz, 1 H, ArCH*H*); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 162.2, 155.7, 154.1, 131.0, 109.9, 109.6, 103.6, 92.0, 66.9, 44.9, 29.3; HR-ESI: *m*/z: 331.96173 [*M*+Na^+], $C_{32}H_{47}NO_3Na$ requires 494.3629.

Data for compound 8. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.61 (bd, *J* = 5.6 Hz, 1 H, N*H*CO), 7.56 (d, *J* = 8.6 Hz, 2 H, Ar*H*), 6.92 (d, *J* = 8.6 Hz, 2 H, Ar*H*), 6.58 (dd, *J* = 10.0, 3.8 Hz, 1 H, =C*H*), 6.33 (s, 1 H, OC*H*O), 6.07 (d, *J* = 10.0 Hz, 1 H, =C*H*), 4.16 (dt, *J* = 12.5, 2.3 Hz, 1 H, C*H*HO), 4.03–3.99 (m, 1 H, C*H*NHCO), 3.81 (s, 3 H, OCH_3), 3.58 (dt, *J* = 3.5, 1.3 Hz, 1 H C*H*(O)CH), 3.53 (d, *J* = 12.4 Hz, 1 H, CH*H*O), 3.47 (d, *J* = 3.8 Hz, 1 H, CH(O)C*H*), 2.51 (dt, *J* = 15.3, 2.1 Hz, 1 H, C*H*H), 1.82 (dd, *J* = 15.3, 4.5 Hz, 1 H, CH*H*); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.4, 160.9, 133.1, 130.9, 129.0, 127.7, 113.9, 101.8, 99.5, 92.5, 78.1, 64.9, 57.2, 55.3, 49.0, 44.3, 31.0; HR-ESI: m/z: 484.00903 [*M*+Na^+], $C_{19}H_{18}Cl_3NO_6Na$ requires 484.00919.

Preparation of acetamide 9. A solution of trichloroacetamide **8** (12.6 mg, 27 μmol) in toluene (5 mL) was cooled to –78 °C and DIBAL-H (1.0 M solution in toluene; 54 μL, 54 μmol) was added dropwise. The mixture was stirred at the same temperature until TLC analysis indicated complete consumption of the starting material (ca. 10 min) and then excess reagent was quenched by careful addition of cooled acetone (0.3 mL). The mixture was allowed to warm up to 0 °C and then acetic anhydride (0.3 mL, 3 mmol) and pyridine (0.5 mL, 6 mmol) were added. The mixture was stirred for 2 h, during which period it was allowed to gradually warm up to ambient temperature. The reaction mixture was then poured in a saturated aqueous solution of potassium sodium tartrate (10 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic phases were washed sequentially with a saturated aqueous solution of $CuSO_4$ (3 × 10 mL) and brine (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue thus obtained was purified by flash column chromatography (4:1 ethyl acetate/acetone) to produce acetamide **9** as colorless glass. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, *J* = 8.7 Hz, 2 H, Ar*H*), 6.92 (d, *J* = 8.7 Hz, 2 H, Ar*H*), 6.57 (bd, *J* = 6.4 Hz, 1 H, N*H*CO), 6.53 (dd, *J* = 10.0, 3.8 Hz, 1 H, =C*H*), 6.28 (s, 1 H, OC*H*O), 6.04 (dd, *J* = 10.0, 1.6 Hz, 1 H, =C*H*), 4.08–4.04 (m, 1 H, C*H*NHCO), 4.05–4.02 (m, 1 H, C*H*HO), 3.81 (s, 3 H, OCH_3), 3.55 (dt, *J* = 3.8, 1.7 Hz, 1 H C*H*(O)CH), 3.47 (dd, *J* = 11.8, 1.0 Hz, 1 H, CH*H*O), 3.46 (d, *J*

= 3.7 Hz, 1 H, CH(O)C*H*), 2.37 (dt, *J* = 15.3, 2.4 Hz, 1 H, C*H*H), 2.03 (s, 3 H, COCH_3), 1.75 (dd, *J* = 15.3, 5.0 Hz, 1 H, CH*H*); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 169.4, 160.6, 132.4, 131.1, 128.8, 128.0, 113.7, 101.7, 99.2, 78.2, 65.8, 57.5, 55.1, 48.9, 41.8, 31.7, 23.3; HR-ESI: m/z: 382.12588 [*M*+Na^+], $C_{19}H_{21}NO_6Na$ requires 382.12611.

General procedure for the preparation of amides 10–12. A solution of trichloroacetamide **8** (30.0 mg, 65 μmol) in toluene (7 mL) was cooled to –78 °C and DIBAL-H (1.0 M solution in toluene; 150 μL, 0.15 mmol) was added dropwise. The mixture was stirred at the same temperature until TLC analysis indicated complete consumption of the starting material (ca. 10 min) and then excess reagent was quenched by careful addition of cooled acetone (2 mL). To the resulting solution was added solid $Na_2SO_4{\cdot}10H_2O$ (large excess) at 0 °C. After stirring for 30 min at 0 °C, the mixture was allowed to warm up to ambient temperature, the insoluble materials were filtered off and the filtrate was concentrated under reduced pressure to give crude amine.

To a solution of the crude amine in a mixture of dichloromethane (7 mL) and DMF (0.5 mL) cooled to 0 °C were added *i*Pr_2EtN (30 μL, 0.17 mmol), sorbic (11 mg, 98 μmol) or hexanoic (10 μL, 80 μmol) acid and PyBop (70 mg, 0.13 mmol). The mixture was stirred at 0 °C for 3 h and was then allowed to gradually warm up to ambient temperature. After 8 h, the reaction mixture was poured in water (10 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue thus obtained was purified by flash column chromatography (4:1 ethyl acetate/acetone) to produce amide **10** or **11**. The same experimental procedure except from the temperature at which DIBAL-H was added (–20 °C instead of –78 °C) produced amide **12**.

Amide 10. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, *J* = 8.7 Hz, 2 H, Ar*H*), 6.92 (d, *J* = 8.7 Hz, 2 H, Ar*H*), 6.59 (bd, *J* = 7.1 Hz, 1 H, N*H*CO), 6.53 (dd, *J* = 10.0, 3.8 Hz, 1 H, =C*H*), 6.27 (s, 1 H, OC*H*O), 6.04 (dd, *J* = 10.0, 1.6 Hz, 1 H, =C*H*), 4.09–4.01 (m, 2 H, C*H*NHCO + C*H*HO), 3.81 (s, 3 H, OCH_3), 3.55 (dt, *J* = 3.8, 1.6 Hz, 1 H C*H*(O)CH), 3.47 (dd, *J* = 12.0, 1.2 Hz, 1 H, CH*H*O), 3.46 (d, *J* = 3.8 Hz, 1 H, CH(O)C*H*), 2.37 (dt, *J* = 15.2, 2.5 Hz, 1 H, C*H*HCHNH), 2.23 (m, 2 H, COCH_2), 1.75 (dd, *J* = 15.2, 5.0 Hz, 1 H, CH*H*CHNH), 1.69–1.63 (m, 2 H, $COCH_2CH_2$), 1.38–1.28 (m, 4 H, CH_2CH_2), 0.90 (t, *J* = 7.0 Hz, 3 H, CH_2CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.7, 160.8, 132.7,

131.3, 129.0, 128.2, 113.9, 101.7, 99.4, 78.2, 66.0, 57.6, 55.3, 49.1, 41.7, 36.8, 31.6, 31.4, 25.3, 22.4, 13.9; HR-ESI: m/z: 438.18849 [*M*+Na^+], $C_{23}H_{29}NO_6Na$ requires 438.18871.

Amide 11. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, *J* = 8.7 Hz, 2 H, Ar*H*), 7.19 (dd, *J* = 15.0, 10.6 Hz, 1 H, =C*H*), 6.93 (d, *J* = 8.7 Hz, 2 H, Ar*H*), 6.58 (bd, *J* = 7.3 Hz, 1 H, N*H*CO), 6.53 (dd, *J* = 10.0, 3.8 Hz, 1 H, =C*H*), 6.29 (s, 1 H, OC*H*O), 6.21–6.07 (m, 2 H, =C*H*), 6.04 (dd, *J* = 10.0, 1.6 Hz, 1 H, =C*H*), 5.76 (d, *J* = 15.0, 1 H, =C*H*), 4.18–4.13 (m, 1 H, C*H*NHCO), 4.07 (dt, *J* = 12.2, 2.2 Hz, 1 H, C*H*HO), 3.81 (s, 3 H, OCH_3), 3.55 (dt, *J* = 3.9, 1.6 Hz, 1 H C*H*(O)CH), 3.50 (dd, *J* = 12.2, 1.5 Hz, 1 H, CH*H*O), 3.46 (d, *J* = 3.9 Hz, 1 H, CH(O)C*H*), 2.39 (dt, *J* = 15.2, 2.3 Hz, 1 H, C*H*H), 1.84 (d, *J* = 6.4 Hz, 3 H, CH_3), 1.78 (dd, *J* = 15.3, 5.0 Hz, 1 H, CH*H*); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 165.8, 160.8, 141.6, 138.2, 132.7, 131.4, 129.6, 129.0, 128.2, 121.2, 114.0, 101.7, 99.4, 78.3, 66.0, 57.6, 55.3, 49.1, 41.9, 31.7, 18.6; HR-ESI: m/z: 434.15703 [*M*+Na^+], $C_{23}H_{25}NO_6Na$ requires 434.15741.

Amide 12. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.6 Hz, 2 H, Ar*H*), 6.91 (d, *J* = 8.7 Hz, 2 H, Ar*H*), 6.40 (s, 1 H, OC*H*O), 6.18 (bd, *J* = 7.0 Hz, 1 H, N*H*CO), 5.99 (dt, *J* = 10.2, 3.8 Hz, 1 H, =C*H*), 5.77 (bd, *J* = 10.2, 1 H, =C*H*), 4.09–4.03 (m, 1 H, C*H*NHCO), 3.88 (ddd, *J* = 11.7, 4.4, 1.5 Hz, 1 H, C*H*HO), 3.81 (s, 3 H, OCH_3), 3.79 (dd, *J* = 8.5, 2.3 Hz, 1 H C*H*OH), 3.65 (dd, *J* = 11.8, 2.0 Hz, 1 H, CH*H*O), 2.48–2.40 (m, 2 H, C*H*HCHOH + CHHCHO*H*), 2.22 (t, *J* = 7.6 Hz, 2 H, COCH_2), 2.16 (dd, *J* = 15.4, 4.2 Hz, 1 H, C*H*HCHNH), 2.07–1.99 (m, 2 H, CH*H*CHNH + CH*H*CHOH), 1.69–1.58 (m, 2 H, $COCH_2CH_2$), 1.36–1.23 (m, 4 H, CH_2CH_2), 0.89 (t, *J* = 6.9 Hz, 3 H, CH_2CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.7, 160.5, 129.9, 128.6, 127.7, 124.7, 113.9, 101.8, 100.5, 79.6, 70.2, 64.7, 55.3, 42.4, 36.9, 31.4, 31.2, 30.8, 25.4, 22.4, 13.9; HR-ESI: m/z: 440.20410 [*M*+Na^+], $C_{23}H_{31}NO_6Na$ requires 440.20436.