Supporting information for

Structure-inspired design of a sphingolipid mimic sphingosine-1-phosphate receptor agonist from a naturally occurring sphingomyelin synthase inhibitor

Mahadeva M. M. Swamy,‡a Yuta Murai,‡a,b Yusuke Ohno,c Keisuke Jojima,c Akio Kihara,c Susumu Mitsutake,a Yasuyuki Igarashi,a Jian Yu,d Min Yao,d Yoshiko Suga,a Masaki Anetai,a and Kenji Monde *a,b

‡Graduate School of Life Science, Kita 21 Nishi 11, Sapporo 001-0021, Japan
bFrontier Research Center for Advanced Material and Life Science, Faculty of Advanced Life Science, Hokkaido University, Kita 21 Nishi 11, Sapporo 001-0021, Japan
cFaculty of Pharmaceutical Sciences, Hokkaido University, Kita 12 Nishi 6, Sapporo 060-0812, Japan
dFaculty of Advanced Life Science, Hokkaido University, Kita 10 Nishi 8, Sapporo 060-0810, Japan

Email: kmonde@sci.hokudai.ac.jp

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1. General experimental method

$^1$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) spectra were recorded on a Varian Inova instrument at 25 °C in CDCl$_3$ or CD$_3$OD purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, USA). Chemical shifts (δ) are reported in ppm and coupling constant values (J) are in Hertz (Hz) relative to CDCl$_3$ (H, δ 7.26; C, δ 77.00) or CD$_3$OD (H, δ 3.4, 4.8; C, δ 49.3) and tetramethylsilane. The following abbreviations were used for signal multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet. High Resolution Mass spectra (HRMS (ESI)) were obtained on a AB sciex Triple TOF® 5600+ at Platform for Research on Biofunctional Molecules, Hokkaido University. Low-resolution mass spectra (ESI-MS) were obtained by a JEOL JMS-T100LP spectrometer. Analytical TLC was performed on 0.2 mm silica gel plates (Merck 60 F-254). SiO$_2$ gel column chromatography was carried out using silica gel (Wakogel® N60, spherical, 38-100 µm) with air flashing.

2. Construction of plant extracts library

To construct plants extracts library, approximately 650 plants grown wildly or cultivated in Hokkaido area were collected. Each plant was dried by hot air at 50°C for 24 hours. Dried plants were cut into small pieces/powdered by grinder. Each dried plants (20 g) were extracted with 200 ml of MeOH at room temperature for 1 day. The MeOH solutions were filtered, and concentrated in vacuo to dryness. Each of residues and the solutions dissolved in DMSO at the concentrations of 100 mg/ml were stocked at -20°C respectively.
3. Isolation of ginkgolic acid (15:1) from *Ginkgo biloba*

*Ginkgo biloba* stem collected from campus of Hokkaido University, Sapporo on 16th October, 2014 dried for 6 months. Dried *G. biloba* was grinded/powdered well. Five hundred g of powder extracted with MeOH (2 L) at room temperature three times after 24 h each. The combined MeOH extract was concentrated under reduced pressure to give a dark brown residue (15.9 g), which was dissolved in 20% MeOH in water (500 mL) and partitioned with hexane (250 mL × 3), Et₂O (250 mL × 3) and EtOAc (250 mL × 3). After removal of solvent, each of residues was used for SMS assay. We found that hexane fraction was more activity than Et₂O fraction but, EtOAc and water fractions turned out to be inactive. The active hexane fraction (3.8 g) was further purified by silica gel column chromatography. The active component was identified as ginkgolic acid (15:1) confirmed by NMR spectroscopy and HRMS. Yield: 0.006% (27.5 mg). Ginkgolic acid (15:1) is one of the major components of *G. biloba* leaves.

Ginkgolic acid (15:1); light yellow oil; 'H NMR (CDCl₃, 500 MHz): δ = 10.99 (1H, s), 7.37 (1H, t, 7.9 Hz), 6.88 (1H, d, J=8.3 Hz), 6.78 (1H, d, J=7.5 Hz), 5.32-5.38 (2H, m), 2.97-3.00 (2H, t, J=7.5 Hz), 2.02-2.06 (4H, m), 1.60-1.62 (2H, m), 1.26-1.33 (18H, m), 0.88 (3H, t, J=6.6 Hz). ¹³C NMR (125 MHz, CDCl₃): δ = 176.2, 163.6, 147.8, 135.4, 129.9, 129.8, 122.7, 115.8, 110.4, 36.4, 32.0, 31.9, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 27.2, 26.9, 22.3, 14.0. HRMS (m/z): [M+H]+ calculated for C₂₂H₃₄O₃: 347.2580; found 347.2555.

4. Total Synthesis of ginkgolic acid (15:1) and derivatives

Ginkgolic acid (15:1) (1):

Compounds S₁ and S₂ were synthesized as reported method.¹ Compound S₃ was synthesized by palladium catalyzed reaction (heck reaction) of S₂ with hydroxyl group protected 7-octene-l-ol in the presence of K₂CO₃² followed by deprotection of hydroxyl group with TBAF (tetra-N-butyl ammonium fluoride) to get S₄. The hydrogenation of double bond was carried out using Pd/C, H₂ to obtain S₅. Compound S₆ which was synthesized by oxidation of S₅ using IBX (2-iodoxybenzoic acid) followed by Wittig reaction of S₆ and heptyltriphenylphosphonium ion in the presence of NaH to get S₇.³ Finally, ginkgolic acid (15:1) was obtained by hydrolysis of S₇. The natural and synthetic ginkgolic acid (15:1) is equally potent SMS inhibitor.
Scheme S1: Synthesis of ginkgolic acid (15:1)

5-hydroxy-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (S1)

To the stirred solution of 2,6-dihydroxybenzoic acid (8 g, 51.9 mmol) in 1,2-dimethoxyethane (50 mL) at 0 °C was added acetone (4.9 mL, 67.5 mmol), SOCl\(_2\) (4.8 mL, 67.5 mmol) and DMAP (316 mg, 2.6 mmol). The reaction mixture was stirred under Ar atmosphere for 1 h at 0 °C and stirred at room temperature overnight. After completion of the reaction saturated NaHCO\(_3\) solution was added and extracted with Et\(_2\)O (100 mL × 2). The combined organic layer was washed with saturated aqueous NaCl solution, dried over MgSO\(_4\) and the solution was concentrated under vacuum. The residue was purified by silica gel column chromatography using hexane/EtOAc (8:2) to give S1 (9.3 g, Yield 93%) as a white solid.

\(^{1}\)H NMR (CDCl\(_3\), 500 MHz): \(\delta = 10.31\) (1H, s), 7.38 (1H, t, 8.3 Hz), 6.59 (1H, d, 8.35 Hz), 6.41 (1H, d, 8.0 Hz), 1.72 (6H, s). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta = 165.4\), 161.3, 155.5, 137.9, 110.7, 107.2, 107.1, 99.3, 25.5. HRMS (m/z): [M+H]\(^+\) calculated for C\(_{10}\)H\(_{10}\)O\(_4\): 195.0651; found 195.0648
2,2-dimethyl-4-oxo-4\textit{H}-benzo[\textit{d}][1,3]dioxin-5-yl trifluoromethanesulfonate (S2)

![Chemical structure of S2]

To a stirred solution of S1 (5 g, 25.8 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (30 mL) at 0 \degree C was added anhydrous pyridine (7.4 mL, 92.7 mmol) and trifluoromethanesulfonic anhydride (4.4 mL, 30.9 mmol). The reaction mixture was stirred for additional 1 h at same temperature. After completion of the reaction the reaction mixture was extracted with Et\textsubscript{2}O (100 mL \times 2) and the combined organic layer was dried over MgSO\textsubscript{4} and concentrated under vacuum. The residue obtained was purified by silica gel column chromatography using hexane/EtOAc (7:3) to give S2 (7.6 g, Yield 90%) as a white solid.

\textbf{1}^1\text{H} NMR (CDCl\textsubscript{3}, 500 MHz): \(\delta = 7.62\) (1H, t, 8.3 Hz), 7.0 (1H, d, 8.5 Hz), 7.01 (1H, d, 8.3 Hz), 1.76 (6H, s). \textbf{13}^1\text{C} NMR (125 MHz, CDCl\textsubscript{3}): \(\delta = 157.4, 157.0, 148.6, 136.2, 120.0, 117.9, 116.5, 108.2, 106.8, 25.4\). HRMS (m/z): [M+H]\textsuperscript{+} calculated for C\textsubscript{11}H\textsubscript{9}O\textsubscript{6}F\textsubscript{3}S: 327.0144; found 327.0133.

(E)-5-(8-(\textit{tert}-butyldiphenylsilyloxy)oct-1-en-1-yl)-2,2-dimethyl-4\textit{H}-benzo[\textit{d}][1,3]dioxin-4-one (S3)

![Chemical structure of S3]

To a stirred solution of \textit{tert}-butyl(oct-7-en-1-yloxy)diphenylsilane (240 mg, 0.68 mmol) in DMF (3 mL) at room temperature was added K\textsubscript{2}CO\textsubscript{3} (94 mg, 0.68 mmol) and stirred for 0.5 h. Compound S2 (202 mg, 0.62 mmol) and palladium catalyst PdCl\textsubscript{2}(dpff) (15 mg, 0.02 mmol) were added to the reaction mixture. Then the reaction mixture was stirred overnight at 75 \degree C. The reaction mixture was acidified with 2M HCl and extracted with Et\textsubscript{2}O. The organic layer was dried over MgSO\textsubscript{4} and concentrated under vacuum. The residue obtained was purified by silica gel column chromatography using hexane/EtOAc (9:1) to give S3 (227 mg, Yield 67%) as colorless oil.

\textbf{1}^1\text{H} NMR (CDCl\textsubscript{3}, 500 MHz): \(\delta = 7.76\) (4H, d, 7.8 Hz), 7.55 (1H, d, J=9.7 Hz), 7.42-7.45 (7H, m), 7.28 (1H, d, 7.8 Hz), 6.87(1H, d, J=8.0 Hz), 6.27-6.33 (1H, m), 3.76 (2H, t, 6.4 Hz), 2.34 (2H, q, 13.9, 7.0, 6.8 Hz), 1.75 (7H, s), 1.69-1.66 (2H, m), 1.43-1.65 (6H, m), 1.14 (9H, m). \textbf{13}^1\text{C} NMR (125 MHz, CDCl\textsubscript{3}): \(\delta = 160.3, 158.8, 142.7, 136.6, 135.1, 134.2, 129.5, 128.2, 127.7, 121.3, 115.5, 110.6, 105.1, 64.0, 33.2, 32.6, 31.7, 31.6, 29.2, 29.1, 27.0, 25.7, 22.7, 19.3, 19.3, 14.2. HRMS (m/z): [M+H]\textsuperscript{+} calculated for C\textsubscript{34}H\textsubscript{42}O\textsubscript{4}Si: 543.2925; found 543.2929.
(E)-5-(8-hydroxyoct-1-en-1-yl)-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (S4)

To a stirred solution of S3 (50 mg, 0.1 mmol) in THF (4 mL) at room temperature was added TBAF (36 mg, 0.14 mmol) and stirred for 2 h. After completion of the reaction the THF was evaporated and the residue was extracted with EtOAc. The organic layer was dried over MgSO₄ and concentrated under reduced vacuum. The residue obtained was purified by silica gel column chromatography using hexane/EtOAc (6:4) to give S4 (24 mg, Yield 86%) as colorless oil.

1H NMR (CDCl₃, 500 MHz): \( \delta = 7.36-7.41 \) (2H, m), 7.19 (1H, d, \( J=7.8 \) Hz), 6.77 (1H, d, \( J=8.0 \) Hz), 6.15-6.21 (1H, m), 3.59 (2H, t, \( J=6.6 \) Hz), 2.24 (2H, q, \( 13.9, 7.1, 6.8 \) Hz), 1.66 (6H, s), 1.49-1.51 (4H, m), 1.34-1.37 (4H, m).

13C NMR (125 MHz, CDCl₃): \( \delta = 160.4, 156.7, 142.5, 135.4, 135.0, 128.0, 121.2, 115.2, 110.4, 105.0, 62.6, 32.9, 32.6, 28.9, 28.8, 25.5, 25.5. \)

HRMS (m/z): [M+H]⁺ calculated for C₁₈H₂₄O₄: 305.1747; found 305.1772.

5-(8-hydroxyoctyl)-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (S5)

To a stirred solution of S4 (94 mg, 0.31 mmol) in EtOAc (10 ml) was added 10% Pd/C (50 mg, 0.05 mmol). The reaction mixture was stirred overnight at room temperature under H₂ atmosphere. Pd/C was filtered off and the filtrate was concentrated under vacuum to afford a residue that was purified by silica gel column chromatography using hexane/EtOAc (6:4) to give S5 (89 mg, 95%) as colorless oil.

1H NMR (CDCl₃, 500 MHz): \( \delta = 7.74 \) (1H, t, 8.0 Hz), 6.94(1H, d, \( J=7.5 \) Hz), 6.81(1H, d, 8.1 Hz), 3.64 (2H, t, 6.5 Hz), 3.09 (2H, t, 7.8 Hz), 1.71 (6H, s), 1.56-1.62 (4H, m), 1.32-1.41 (6H, m).

13C NMR (125 MHz, CDCl₃): \( \delta = 160.2, 157.0, 148.4, 135.0, 125.0, 115.0, 112.0, 104.9, 62.8, 34.3, 32.7, 31.1, 29.5, 29.3, 29.2, 25.6, 25.6. \)


8-(2,2-dimethyl-4-oxo-4H-benzo[d][1,3]dioxin-5-yl)octanal (S6)
To a stirred solution of S5 (150 mg, 0.49 mmol) in EtOAc (10 ml) was added IBX (412 mg, 1.47 mmol). The reaction mixture was stirred overnight at 40 °C. The white solid was filtered off and the filtrate was concentrated under vacuum. The residue was purified by silica gel column chromatography using hexane/EtOAc (9:1) to give S6 (91 mg, 92%) as colorless oil.

1H NMR (CDCl$_3$, 500 MHz): $\delta$ = 9.06 (1H, s), 7.33 (1H, t, 8.1), 6.86 (1H, d, J=7.5 Hz), 6.73 (1H, d, 8.3 Hz), 3.01 (2H, t, 7.5 Hz), 2.35 (2H, t, 6.1 Hz), 1.63 (6H, s), 1.49-1.56 (4H, m), 1.27-1.31 (6H, m). 13C NMR (125 MHz, CDCl$_3$): $\delta$ = 202.7, 160.1, 157.0, 148.2, 135.6, 125.0, 115.0, 111.9, 111.9, 104.8, 43.7, 34.2, 31.0, 28.9, 28.9, 25.5, 21.9. HRMS (m/z): [M+H]$^+$ calculated for C$_{18}$H$_{24}$O$_4$: 305.1747; found 305.1776.

(Z)-2,2-dimethyl-5-(pentadec-8-en-1-yl)-4H-benzo[d][1,3]dioxin-4-one (S7)

To a stirred solution of bromo(heptyl)triphenylphosphonium ion (50 mg, 0.11 mmol) in THF (5 mL) and DMSO (1 mL) was added NaH (5 mg, 0.23 mmol) at 0 °C under Ar atmosphere. The reaction mixture was stirred at room temperature for 1 h. Compound S6 (17 mg, 0.06 mmol) was added in THF (1 mL) at 0 °C and continued stirring for 1 h at the same temperature. After completion of the reaction, saturated NH$_4$Cl was added to the reaction mixture and extracted with hexane. The organic layer was dried under MgSO$_4$ and concentrated under vacuum. The residue was purified by silica gel column chromatography using hexane/EtOAc (9.75:0.25) to give S7 (42 mg, 66%) as yellow oil.

1H NMR (CDCl$_3$, 500 MHz): $\delta$ = 7.38 (1H, t, 7.8 Hz), 6.92 (1H, d, J=7.8 Hz), 6.79 (1H, d, 8.3 Hz), 5.31-5.35 (2H, m), 3.08 (2H, t, 7.8 Hz), 1.98-2.02 (4H, m), 1.69 (6H, s), 1.55-1.61 (2H, m), 1.27-1.38 (18H, m), 0.87 (3H, t, 6.3 Hz). 13C NMR (125 MHz, CDCl$_3$): $\delta$ = 160.2, 157.1, 148.5, 135.0, 129.8, 125.0, 115.0, 112.2, 104.9, 34.3, 31.9, 31.1, 27.2, 27.2, 27.3, 27.4, 27.6, 27.6 28.9, 28.9, 27.2, 25.6, 22.6, 14.1. HRMS (m/z): [M+H]$^+$ calculated for C$_{25}$H$_{38}$O$_3$: 387.2893; found 387.2911.

Ginkgolic acid (15:1) (1)

The solution of S7 (37 mg, 0.1 mmol) in 50% KOH (0.5 mL) and DMSO (1.5 mL) stirred at 80°C for 1 h. The reaction was cooled to room temperature and acidified with 2M HCl, then extracted with EtOAc. The organic layer was dried under MgSO$_4$ and concentrated under vacuum. The residue was purified by silica gel column chromatography using hexane/EtOAc (9:1) as an eluent to give 1 (30 mg, 91%) as yellow oil.
Synthesis of ginkgolic acid (15:0) (2) and its derivative (3)

Synthesis of ginkgolic acid (15:0) 2 and its derivative 3 were achieved in three steps starting from S2. It involves Heck coupling of S2 with 1-octene and 1-pentadecene using PdCl2(dppf).CH2Cl2 to get compounds S8 and S9, which were subjected to hydrogenation using Pd/C, H2 to get compounds S10 and S11 followed by basic hydrolysis to obtain desired compounds ginkgolic acid (15:0) 2 and 3.

![Scheme S2: Synthesis of ginkgolic acid (15:0) 2 and compound 3](image)

(E)-2,2-dimethyl-5-(pentadec-1-en-1-yl)-4H-benzo[d][1,3]dioxin-4-one (S8)

To a stirred solution of 1-pentadecene (354 mg, 1.68 mmol) in DMF (15 mL) at room temperature was added K2CO3 (231 mg, 1.68 mmol) and stirred for 0.5 h. Compound S2 (500 mg, 1.53 mmol) and palladium catalyst PdCl2(dppf).CH2Cl2 (37 mg, 0.05 mmol) was added to the reaction mixture. Then the reaction mixture was stirred overnight at 75 °C. After completion of the reaction, the reaction mixture was acidified with 2M HCl and extracted with Et2O. The organic layer was dried over MgSO4 and concentrated under vacuum. The residue obtained was purified by silica gel column chromatography using hexane/EtOAc (9:1) to give S8 (410 mg, Yield 69%) as colorless oil.

1H NMR (CDCl3, 500 MHz): δ = 7.38-7.46 (2H, m), 7.22 (1H, d, J=7.8 Hz), 6.80 (1H, d, 7.0 Hz), 6.19-6.25 (1H, m), 2.24-2.29 (2H, m), 1.69 (6H, s), 1.46-1.52 (2H, m), 1.22-1.30 (20H, m), 0.87 (3H, t, 6.8 Hz). 13C NMR (125 MHz, CDCl3): δ = 160.2, 156.6, 142.6, 135.6, 134.8, 127.8, 121.1, 115.3, 110.4, 104.9, 33.1, 31.8, 29.6, 29.6, 29.6, 29.5, 29.4, 29.2, 29.2, 29.1, 25.5, 22.6, 14.0. HRMS (m/z): [M+H]+ calculated for C25H38O3: 387.2893; found 387.2869.
(E)-2,2-dimethyl-5-(oct-1-en-1-yl)-4H-benzo[d][1,3]dioxin-4-one (S9)

![Chemical Structure](image)

To a stirred solution of 1-octene (189 mg, 1.68 mmol) in DMF (15 mL) at room temperature was added K$_2$CO$_3$ (231 mg, 1.68 mmol) and stirred for 0.5 h. Compound S2 (500 mg, 1.53 mmol) and palladium catalyst PdCl$_2$(dppf).CH$_2$Cl$_2$ (37 mg, 0.05 mmol) was added to the reaction mixture. Then the reaction mixture was stirred overnight at 75 °C. After completion of the reaction, the reaction mixture was acidified with 2M HCl and extracted with Et$_2$O. The organic layer was dried over MgSO$_4$ and concentrated under vacuum. The residue obtained was purified by silica gel column chromatography using hexane/EtOAc (9:1) to give S9 (295 mg, Yield 67%) as colorless oil.

$^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ = 7.39-7.46 (2H, m), 7.23 (1H, d, J=8.0 Hz), 6.81(1H, d, 8.0 Hz), 6.20-6.26 (1H, m), 2.25-2.29 (2H, m), 1.69 (6H, s), 1.46-1.52 (2H, m), 1.28-1.39 (6H, m), 0.88 (3H, t, 6.8 Hz).

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ = 160.3, 156.6, 142.6, 135.6, 134.9, 127.8, 121.1, 115.3, 110.4, 104.9, 33.1, 31.6, 29.0, 28.8, 25.5, 22.5, 14.0. HRMS (m/z): [M+H]$^+$ calculated for C$_{18}$H$_{24}$O$_3$: 289.1752; found 289.1747.

2,2-dimethyl-5-pentadecyl-4H-benzo[d][1,3]dioxin-4-one (S10)

![Chemical Structure](image)

To a stirred solution of S8 (100 mg, 0.26 mmol) in EtOAc (10 ml) was added 10% Pd/C (30 mg, 0.026 mmol). The reaction mixture was stirred overnight at room temperature under H$_2$ atmosphere. Pd/C was filtered off and the filtrate was concentrated under vacuum to afford a residue that was purified by silica gel column chromatography using hexane/EtOAc (9:1) as an eluent to give S10 (92 mg, 91%) as colorless oil.

$^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ = 7.39 (1H, t, 8.0 Hz), 6.93 (1H, d, J=7.5 Hz), 6.80 (1H, d, 8.0 Hz), 3.09 (2H, t, 7.6 Hz), 1.70 (6H, s), 1.54-1.62 (2H, m), 1.19-1.31 (24H, m), 0.88 (3H, t, 6.6 Hz).

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ = 159.8, 156.9, 148.2, 134.8, 124.8, 114.8, 111.8, 104.6, 34.2, 31.7, 31.0, 29.5, 29.5, 29.5, 29.4, 29.4, 29.3, 29.2, 22.5, 13.9. HRMS (m/z): [M+H]$^+$ calculated for C$_{25}$H$_{40}$O$_3$: 389.3050; found 387.3046.
To a stirred solution of S9 (100 mg, 0.35 mmol) in EtOAc (10 ml) was added 10% Pd/C (40 mg, 0.035 mmol). The reaction mixture was stirred overnight at room temperature under H₂ atmosphere. The solid was filtered off and the filtrate was concentrated under vacuum to afford a residue that was purified by silica gel column chromatography using hexane/EtOAc (9:1) to give S11 (95 mg, 94%) as colorless oil.

1H NMR (CDCl₃, 500 MHz): \( \delta = 7.38 \) (1H, t, J=8.3 Hz), \( 6.92 \) (1H, d, J=8.8 Hz), \( 6.79 \) (1H, d, 8.0 Hz), \( 3.08 \) (2H, t, J=7.8 Hz), \( 1.68 \) (6H, s), \( 1.55-1.61 \) (2H, m), \( 1.21-1.32 \) (10H, m), \( 0.86 \) (3H, t, 6.8 Hz). ¹³C NMR (125 MHz, CDCl₃): \( \delta = 160.1, 157.0, 148.4, 134.9, 125.0, 115.0, 112.0, 104.8, 34.3, 31.8, 31.1, 29.4, 29.2, 25.6, 22.6, 14.0 \). HRMS (m/z): [M+H]+ calculated for C₁₈H₂₄O₃: 291.1954; found 291.1949.

Ginkgolic acid (15:0) (2)

The solution of S10 (50 mg, 0.13 mmol) in 50% KOH (0.75 mL) and DMSO (2 mL) stirred at 80 °C for 1 h. The reaction was cooled to temperature to room temperature and acidified with 2M HCl then extracted with EtOAc. The organic layer was dried under MgSO₄ and concentrated under vacuum. The residue was purified by silica gel column chromatography using hexane/EtOAc (7:3) as an eluent to give 2 (41 mg, 91%) as a white solid.

1H NMR (CDCl₃, 500 MHz): \( \delta = 11.13 \) (1H, s), \( 7.37 \) (1H, t, 8.0 Hz), \( 6.88 \) (1H, d, J=8.3 Hz), \( 6.73 \) (1H, d, J=7.3 Hz), \( 3.00 \) (2H, t, J=7.8 Hz), \( 1.59-1.65 \) (2H, m), \( 1.29-1.34 \) (24H, m), \( 0.89 \) (3H, t, J=6.8 Hz). ¹³C NMR (125 MHz, CDCl₃): \( \delta = 176.3, 163.6, 147.8, 135.4, 122.7, 115.8, 110.4, 36.4, 32.0, 31.9, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.3, 22.7, 14.1 \). HRMS (m/z): [M+H]+ calculated for C₂₂H₃₆O₃: 349.2737; found 349.2712.

2-hydroxy-6-octylbenzoic acid (3)

The solution of S11 (30 mg, 0.10 mmol) in 50% KOH (0.5 mL) and DMSO (1.5 mL) stirred at 80 °C for 1 h. The reaction was cooled to temperature to room temperature and acidified with 2M HCl then extracted with EtOAc. The organic layer was dried under MgSO₄ and concentrated under vacuum. The
residue was purified by silica gel column chromatography using hexane/EtOAc (7:3) as an eluent to give 3 (25 mg, 96%) as a white solid.

\(^1\)H NMR (CDCl\(_3\), 500 MHz): \(\delta = 10.99\) (1H, s), 7.37 (1H, t, 8.0 Hz), 6.88 (1H, d, J=8.3 Hz), 6.79 (1H, d, J=7.1 Hz), 2.98 (2H, t, J=8.0 Hz), 1.58-1.64 (2H, m), 1.28-1.38 (10H, m), 0.88 (3H, t, J=6.6 Hz). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta = 176.3, 163.6, 147.8, 135.4, 122.8, 115.8, 110.3, 36.4, 31.9, 31.8, 29.7, 29.4, 29.2, 22.6, 14.0\). HRMS (m/z): [M+H]+ calculated for C\(_{15}\)H\(_{22}\)O\(_3\): 251.1641; found 251.1636.

Ginkgolic acid (15:0) (2) from Ginkgolic acid (15:1) (1)

To a stirred solution of ginkgolic acid (15:1) 1 (50 mg, 0.14 mmol) in EtOAc (10 ml) was added 10% Pd/C (30 mg, 0.028 mmol). The reaction mixture was stirred overnight at room temperature under H\(_2\) atmosphere. The solid was filtered off and the filtrate was concentrated under vacuum to afford a residue that was purified by silica gel column chromatography using hexane/EtOAc (9.0:1.0) to give 2 (48 mg, 96%) as colorless oil.

\(^1\)H NMR (CDCl\(_3\), 500 MHz): \(\delta = 11.13\) (1H, s), 7.37 (1H, t, 8.0 Hz), 6.88 (1H, d, J=8.3 Hz), 6.73 (1H, d, J=7.3 Hz), 3.00 (2H, t, J=7.8 Hz), 1.59-1.65 (2H, m), 1.29-1.34 (24H, m), 0.89 (3H, t, J=6.8 Hz). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta = 176.3, 163.6, 147.8, 135.4, 122.7, 115.8, 110.4, 36.4, 32.0, 31.9, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.3, 22.7, 14.1\). HRMS (m/z): [M+H]+ calculated for C\(_{22}\)H\(_{36}\)O\(_3\): 349.2737; found 349.2712

Methyl ester of Ginkgolic ester (4)

To a stirred solution of ginkgolic acid (15:1) 1 (25 mg, 0.1 mmol) in methanol (2 mL) and diethyl ether (2 mL) was added a solution of TMS-CH\(_2\)N\(_2\) in hexane until the color of the solution became yellow at 0 °C. The reaction mixture was stirred at same temperature for 0.5 h. The reaction was quenched with acetic acid and extracted with EtOAc, dried over MgSO\(_4\). The organic layer was concentrated under vacuum to afford a residue that was purified by silica gel column chromatography using hexane/EtOAc (9.75:0.25) as an eluent to give the ester 4 (25 mg, 96%) as colorless oil.

\(^1\)H NMR (CDCl\(_3\), 500 MHz): \(\delta = 11.13\) (1H, s), 7.29 (1H, t, 7.8 Hz), 6.85 (1H, d, J=8.3 Hz), 6.73 (1H, d, J=7.3 Hz), 5.39-5.33 (2H, m), 3.90 (3H s), 2.89 (2H, t, J=7.8 Hz), 2.03-2.08 (4H, m), 1.51-1.56 (2H, m), 1.29-1.34 (18H, m), 0.91 (3H, t, J=7.1 Hz). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta = 171.9, 162.5, 146.1, 134.1, 129.8, 129.8, 122.8, 122.4, 115.5, 111.8, 52.0, 36.6, 32.1, 31.9, 29.9, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5,
Methyl (Z)-2-methoxy-6-(pentadec-8-en-1-yl)benzoate (5)

To a stirred solution of Ginkgolic acid (15:1) 1 (100 mg, 0.29 mmol) in DMF (2mL) was added K$_2$CO$_3$ (0.73 mmol, 2.5 eq) and iodomethane (0.58 mmol, 2 eq) at room temperature. Then the reaction mixture was stirred at 90 °C under Ar atmosphere overnight. After completion of the reaction the reaction mixture was cooled to room temperature and acidified with 2M HCl and extracted with EtOAc, and dried over MgSO$_4$. The organic layer was concentrated under vacuum to afford a residue that was purified by silica gel column chromatography using hexane/EtOAc (9.5:0.5) to give the ester 5 (103 mg, 95%) as colorless oil.

$^1$H NMR (CDCl$_3$, 500 MHz): $\delta = 7.28$ (1H, t, $J=8.3$ Hz), 6.83 (1H, d, $J=7.8$ Hz), 6.77 (1H, d, $J=8.3$ Hz), 5.33-5.37 (2H, m), 3.92 (3H, s), 3.83 (3H, s), 2.55 (2H, t, $J=8.0$ Hz), 2.01-2.04 (4H, m), 1.56-1.61 (2H, m), 1.29-1.34 (18H, m), 0.90 (3H, t, $J=7.0$ Hz). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta =$ 168.9, 156.2, 141.3, 130.2, 129.9, 129.8, 123.4, 121.4, 108.3, 33.4, 31.7, 31.1, 29.7, 29.7, 29.4, 29.3, 29.1, 28.9, 27.2, 27.1, 22.6, 14.0. HRMS ($m/z$): [M+H]$^+$ calculated for C$_{24}$H$_{38}$O$_3$: 375.2893; found 375.2867.

Synthesis of compound 6 and 7

Compound 6 and 7 synthesis was achieved in three steps from the commercially available compound, 3-bromo-2-nitro-anisole. Compounds S12 and S13 were synthesized by coupling of 3-bromo-2-nitro-anisole with 1-pentadecene and 1-octene under palladium catalyst through heck reaction. Reduction of nitro group and an unsaturation in hydrocarbon chain of S12 and S13 were achieved in single step by using H$_2$ and Pd/C at ambient temperature to get S14 and S15, followed by O-demethylation was done by Lewis acid BBr$_3$ at 0 °C to get desired compounds 6 and 7.
(E)-1-methoxy-2-nitro-3-(oct-1-en-1-yl)benzene (S13)

To a stirred solution of 3-bromo-2-nitro-anisole (750 mg, 3.23 mmol) in DMF (10 mL) was added 1-octene (725 mg, 6.46 mmol), Na$_2$CO$_3$ (536 mg, 3.87 mmol) and PdCl$_2$ (28 mg, 0.16 mmol). The reaction mixture was refluxed overnight. The reaction mixture was cooled to room temperature, neutralized with 2M HCl and extracted with Et$_2$O. The organic layer was dried under MgSO$_4$ and concentrated under vacuum. The residue was purified by silica gel column chromatography using hexane/EtOAc (9.5:0.5) to give S13 (410 mg, 48%) as yellow oil.

$^1$H NMR (CDCl$_3$, 500 MHz): $\delta = 7.23$ (1H, t, 8.3 Hz), 6.04(1H, d, J=7.85 Hz), 6.78(1H, d, 8.3 Hz), 6.20-6.26 (1H, m), 5.28- 3.53 (1H, m), 3.87 (3H, s), 2.45 (1H, t, 8.0 Hz), 2.13 (1H, q, 14.4, 7.1, 7.3) 1.65-1.84 (1H, m), 1.37-1.57 (1H, m), 1.20-1.35 (6H, m), 0.80 (3H, t, 6.6 Hz). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta =$ 150.6, 137.5, 130.4, 121.8, 121.6, 117.8, 110.3, 56.3, 56.2, 33.1, 31.6, 29.1, 28.8, 22.5, 14.0. HRMS ($m/z$): [M+H]$^+$ calculated for C$_{15}$H$_{21}$NO$_3$: 264.1594; found 264.1575.

(E)-1-methoxy-2-nitro-3-(pentadec-1-en-1-yl)benzene (S12)

$^1$H NMR (CDCl$_3$, 500 MHz): $\delta = 7.31$ (1H, t, 8.0 Hz), 6.86 (2H, d, 8.2 Hz), 6.21-6.34 (1H, m), 5.34-5.42 (1H, m), 3.87 (3H, s), 2.53(2H, t, 7.8 Hz), 2.15-2.18 (1H, m), 1.96-1.99 (2H, m), 1.57-1.60 (4H, m), 1.25-1.32 (16H, m), 0.87 (3H, t, 6.6 Hz). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta =$ 150.6, 150.5, 137.5, 130.5, 130.4, 121.8, 121.6, 117.8, 110.3, 56.3, 56.2, 43.8, 33.8, 33.2, 31.9, 30.8, 32.9, 30.5, 29.8, 28.7, 29.6, 29.3, 29.1, 28.2, 23.8, 22.6, 14.1. HRMS ($m/z$): [M+Na]$^+$ calculated for C$_{22}$H$_{35}$NO$_3$Na: 384.2509; found 384.2518.

2-methoxy-6-octylaniline (S15)

To a stirred solution of S13 (275 mg, 1.04 mmol) in EtOAc (10 ml) was added 10% Pd/C (220 mg, 0.21 mmol). The reaction mixture was stirred overnight under H$_2$ atmosphere at room temperature. Pd/C was filtered off and the filtrate was concentrated under vacuum. The residue was purified by silica gel column chromatography using hexane/EtOAc (9:1) to give S15 (235 mg, 96%) as yellow oil.
1H NMR (CDCl₃, 500 MHz): δ = 6.67-6.70 (3H, m), 3.84 (3H, s), 3.78(2H, s), 2.49 (2H, t, 7.6 Hz), 1.54-1.64 (2H, m), 1.21-1.37 (24H, m), 0.87 (3H, t, 6.8 Hz). 13C NMR (125 MHz, CDCl₃): δ = 147.2, 133.5, 127.4, 121.6, 117.7, 108.1, 55.5, 55.5, 55.4, 31.9, 31.2, 29.7, 29.7, 29.9, 29.6, 29.4, 28.8, 27.8, 27.6, 22.7, 14.1. HRMS (m/z): [M+H]+ calculated for C₁₅H₂₅NO: 236.2008; found 236.1998.

2-methoxy-6-pentadecylaniline (S14)

2-amino-3-octylphenol (7)

To a stirred solution of s7b (100 mg, 0.426 mmol) in CH₂Cl₂ (5 ml) under Ar atmosphere was added BBr₃ (320 mg, 1.28 mmol) at 0°C. The reaction mixture was stirred at 0 °C for additional 2h. After completion of the reaction, reaction mixture was quenched with EtOAc/H₂O (1:1) and extracted with EtOAc. The organic layer was dried with MgSO₄ and concentrated under vacuum. The residue was purified by silica gel column chromatography using hexane/EtOAc (8:2) to give 7 (410 mg, 96%) as yellow oil.

1H NMR (CDCl₃, 500 MHz): δ = 6.57-6.70 (3H, m), 4.61 (3H, s), 2.50-2.53 (2H, t, 7.6 Hz), 1.60-1.64 (2H, m), 1.22-1.40 (10H, m), 0.91 (3H, t, 6.8 Hz). 13C NMR (125 MHz, CDCl₃): δ = 144.3, 131.7, 129.6, 121.5, 119.1, 112.9, 31.9, 31.6, 29.7, 29.7, 29.3, 29.0, 22.8, 14.1. HRMS (m/z): [M+H]+ calculated for C₁₄H₂₃NO: 222.1852; found 222.1831.

2-amino-3-pentadecylphenol (6)

1H NMR (CDCl₃, 500 MHz): δ = 6.59-6.70 (3H, m), 4.36 (2H, s), 2.55 (2H, t, 7.6 Hz), 1.56-1.64 (4H, m), 1.22-1.39 (25H, m), 0.90 (3H, t, 7.0 Hz). 13C NMR (125 MHz, CDCl₃): δ = 144.2, 131.9, 129.6, 121.6,
118.9, 112.8, 31.9, 31.2, 29.7, 29.7, 29.7, 29.6, 29.5, 29.0, 22.7, 14.1. HRMS (m/z): [M+H]⁺ calculated for C₁₂H₁₇NO: 320.2947; found 320.2967.

7. Synthesis of ginkgolic acid ceramides

General procedure for amidation reaction:

To a stirred solution of acid (0.315 mmol) in DMF under Ar atmosphere was added N,N-diisopropylethylamine (1.1 mmol) at room temperature. To the reaction mixture amine (0.21 mmol) and HATU (0.42 mmol) was added. The reaction mixture was stirred under Ar atmosphere at room temperature for additional 2h. After completion of the reaction, the reaction mixture was diluted with water and extracted with EtOAc. The organic layer was dried with MgSO₄ and concentrated under vacuum. The residue was purified by silica gel column chromatography using hexane/EtOAc to give desired compound.

\[
\text{Scheme S4: Coupling reaction and O-demethylation reaction}
\]

\[\text{N-(2-methoxy-6-octylphenyl)decanamide (S16)}\]

(As a white solid, 92%) \(\text{\textsuperscript{1}H NMR (CDCl}_3, 500 MHz): \delta = 7.17 (1H, t, 7.8 Hz), 6.87 (1H, d, 7.8 Hz), 6.76 (1H, d, 7.8 Hz), 6.68 (1H, s), 3.80(3H, s), 2.56 (2H, t, 7.8), 2.41 (2H, t, 7.3 Hz), 1.73-1.78 (2H, m), 1.54-1.60 (2H, m), 1.41-1.43 (2H, m), 1.21-1.29 (20H, m), 0.84-0.89 (6H, m). HRMS (m/z): [M+H]⁺ calculated for C₂₅H₄₃NO₂: 390.3366; found 390.3363.}

\[\text{N-(2-methoxy-6-pentadecylphenyl)decanamide (S21)}\]

(As white solid, 93%) \(\text{\textsuperscript{1}H NMR (CDCl}_3, 500 MHz): \delta = 7.15 (1H, t, 7.8 Hz), 6.85 (1H, d, 7.8 Hz), 6.74 (1H, d, 7.8 Hz), 6.67 (1H, s), 3.79 (3H, s), 2.55 (2H, t, 7.6), 2.40 (2H, t, 7.3 Hz), 1.73-1.77 (2H, m), 1.55-}
1.51 (2H, m), 1.42-1.44 (1H, m), 1.19-1.26 (36H, m), 0.87 (6H, t, 7.1 Hz). HRMS (m/z): [M+H]^+ calculated for C_{32}H_{57}NO_{2}: 488.4462; found 488.4442.

**N-(2-methoxy-6-octylphenyl)-5-(2-nitrophenyl)furan-2-carboxamide (S17)**

![Chemical structure of S17](image)

(As yellow oil, 87%) ^1^H NMR (CDCl$_3$, 500 MHz): δ = 8.01 (1H, s), 7.76 (2H, t, 8.1 Hz), 6.64 (1H, t, 8.0 Hz), 7.50-7.54 (2H, m), 7.28 (1H, d, 7.8 Hz), 6.89 (1H, d, 7.3), 6.78-6.82 (2H, m), 3.84(3H, s), 2.64 (2H, t, 7.8 Hz), 1.20-1.33 (10H, m), 0.83(3H, t, 6.8 Hz). HRMS (m/z): [M+H]^+ calculated for C$_{26}$H$_{30}$N$_2$O$_5$: 451.2267; found 451.2247.

**N-(2-methoxy-6-pentadecylphenyl)-5-(2-nitrophenyl)furan-2-carboxamide (S22)**

![Chemical structure of S22](image)

(As a yellow solid, 94%) ^1^H NMR (CDCl$_3$, 500 MHz): δ = 7.75-7.79 (2H, m), 7.65 (1H, t, 8.1 Hz), 7.58 (1H, s), 7.52 (1H, t, 8.0 Hz), 7.21-7.3 (2H, m), 6.91 (1H, d, 7.8), 6.79-6.84 (2H, m), 3.85 (3H, s), 2.66 (2H, t, 7.8 Hz), 1.60-1.64 (2H, m), 1.33-1.22 (24H, m), 0.89 (3H, t, 6.8 Hz). HRMS (m/z): [M+H]^+ calculated for C$_{33}$H$_{44}$N$_2$O$_5$: 549.3323; found 549.3316.

**4-(N,N-dipropylsulfamoyl)-N-(2-methoxy-6-octylphenyl)benzamide (S18)**

![Chemical structure of S18](image)

(As a white solid, 88%) ^1^H NMR (CDCl$_3$, 500 MHz): δ = 8.02 (1H, d, 7.8 Hz), 7.90 (2H, d, 7.3 Hz), 7.55 (1H, s), 7.23 (1H, t, 8.0 Hz), 6.92 (1H, d, 7.8 Hz), 6.80 (1H, d, 7.5 Hz), 3.80 (3H, s), 3.10 (4H, t, 7.8 Hz), 2.62 (2H, t, 7.8 Hz), 1.55-1.61 (6H, m), 1.55-1.61(10H, m), 0.83-0.90 (9H, m). HRMS (m/z): [M+H]^+ calculated for C$_{28}$H$_{42}$N$_2$O$_4$S: 503.2938; found 503.2929.
4-(N,N-dipropylsulfamoyl)-N-(2-methoxy-6-pentadecylphenyl)benzamide (S23)

(As a yellow solid, 86%) \(^1\)H NMR (CDCl\(_3\), 500 MHz): \(\delta = 8.01 (1H, d, 5.4 \text{ Hz}), 7.89 (2H, d, 7.1 \text{ Hz}), 7.53 (1H, s), 7.22 (1H, t, 8.0 \text{ Hz}), 6.91 (1H, d, 7.8 \text{ Hz}), 6.79 (1H, d, 7.5 \text{ Hz}), 3.79 (3H, s), 3.10 (4H, t, 7.6 \text{ Hz}), 2.62 (2H, t, 7.8 \text{ Hz}), 1.53-1.62 (6H, m), 1.22-1.30 (25H, m), 0.86-0.90 (9H, m). HRMS (m/z): [M+H]\(^+\) calculated for C\(_{35}\)H\(_{56}\)N\(_2\)O\(_4\)S: 601.4033; found 601.4031.

4-(chloromethyl)-N-(2-methoxy-6-octylphenyl)benzamide (S19)

(As a yellow solid, 90%) \(^1\)HNMR (CDCl\(_3\), 500 MHz): \(\delta = 8.69 (1H, d, 4.3 \text{ Hz}), 8.33 (1H, d, 8.3 \text{ Hz}), 7.9 (1H, s), 7.92 (1H, d, 8.3 \text{ Hz}), 7.61 (2H, d, 7.1 \text{ Hz}), 7.38 (1H, t, 4.0 \text{ Hz}), 6.89 (1H, d, 7.8 \text{ Hz}), 6.76 (1H, d, 8.0 \text{ Hz}), 5.69 (2H, s), 3.76 (3H, s) 2.60 (2H, t, 7.8 Hz) 1.56-1.60 (2H, m), 1.20-1.24 (10H, m), 0.83 (3H, t, 6.8Hz). HRMS (m/z): [M+H]\(^+\) calculated for C\(_{23}\)H\(_{30}\)ClNO\(_2\): 388.2037; found 388.2033.

4-(chloromethyl)-N-(2-methoxy-6-pentadecylphenyl)benzamide (S24)

(As a yellow solid, 93%) \(^1\)H NMR (CDCl\(_3\), 500 MHz): \(\delta = 7.93 (2H, s), 7.48-7.53 (3H, m), 7.23 (1H, t, 8.0 Hz), 6.93 (1H, d, 7.8 Hz), 6.80 (1H, d, 7.8 Hz), 4.65 (2H, s), 3.81 (3H, s), 2.65 (2H, t, 7.5 Hz), 1.59-1.65 (2H, m), 1.24-1.27 (24H, m), 0.90 (3H, t, 6.8Hz). HRMS (m/z): [M+H]\(^+\) calculated for C\(_{30}\)H\(_{44}\)ClNO\(_2\): 48603133; found 486.3133.

N-(2-methoxy-6-octylphenyl)-5-nitrothiophene-2-carboxamide (S20)
(As a light yellow solid, 87%) $^1$H NMR (CDCl$_3$, 500 MHz): $\delta = 7.90$ (1H, s), $7.53$ (1H, s), $7.25$-$7.37$ (2H, m), $6.93$ (1H, d, 7.8 Hz), $6.81$ (1H, d, 8.0 Hz), $3.81$ (3H, s), $2.61$ (2H, t, 7.8 Hz), $1.55$-$1.60$ (2H, m), $1.23$-$1.28$ (10H, m), $0.86$ (3H, t, 6.8 Hz). HRMS ($m/z$): [M+H]$^+$ calculated for C$_{20}$H$_{26}$N$_2$O$_4$S: 391.1686; found 391.1675.

$N$-(2-methoxy-6-pentadecylphenyl)-5-nitrothiophene-2-carboxamide (S25)

(As a yellow solid, 88%) $^1$H NMR (CDCl$_3$, 500 MHz): $\delta = 8.89$ (1H, s), $7.53$ (1H, s), $7.26$-$7.39$ (2H, m), $6.92$ (1H, d, 7.5 Hz), $6.81$ (1H, d, 7.3 Hz), $3.81$ (3H, s), $2.60$ (2H, t, 8.1 Hz), $1.56$-$1.61$ (2H, m), $1.23$-$1.31$ (25H, m), $0.88$ (3H, t, 6.8Hz). HRMS ($m/z$): [M+H]$^+$ calculated for C$_{27}$H$_{40}$N$_2$O$_4$S: 489.2781; found 489.2754.

**General procedure for O-demethylation of compounds S16-S25:**

To a stirred solution of methyl phenyl ethers (0.06 mmol) in CH$_2$Cl$_2$ under Ar atmosphere was added BBr$_3$ (320 mg, 1.28 mmol) at 78°C. The reaction mixture was stirred for 1h at 0°C. After completion of the reaction, the reaction mixture was cooled to room temperature and quenched with EtOAc/H$_2$O (1:1) and extracted with EtOAc. The organic layer was dried with MgSO$_4$ and concentrated under vacuum. The residue was purified by silica gel column chromatography using hexane/EtOAc.

$N$-(2-hydroxy-6-octylphenyl)decanamide (Table 1 entry 1)

(As yellow oil, 90%) $^1$H NMR (CDCl$_3$, 500 MHz): $\delta = 7.31$ (1H, d, 8.3 Hz), $7.20$ (1H, t, 7.8 Hz), $7.11$ (1H, d, 7.3 Hz), $2.91$-$2.98$ (4H, m), $1.83$-$1.89$ (2H, m), $1.71$-$1.77$ (2H, m), $1.27$-$1.43$ (22H, m), $0.87$-$0.89$ (6H, m). HRMS ($m/z$): [M+H]$^+$ calculated for C$_{24}$H$_{41}$NO$_2$: 376.3210; found 376.3198.

$N$-(2-hydroxy-6-pentadecylphenyl)decanamide (Table 1 entry 6)

(As yellow oil, 90%) $^1$H NMR (CDCl$_3$, 500 MHz): $\delta = 7.31$ (1H, d, 8.3 Hz), $7.20$ (1H, t, 7.8 Hz), $7.11$ (1H, d, 7.3 Hz), $2.91$-$2.98$ (4H, m), $1.83$-$1.89$ (2H, m), $1.71$-$1.77$ (2H, m), $1.27$-$1.43$ (22H, m), $0.87$-$0.89$ (6H, m). HRMS ($m/z$): [M+H]$^+$ calculated for C$_{24}$H$_{41}$NO$_2$: 376.3210; found 376.3198.
(As a white solid, 87%) $^1$H NMR (CDCl$_3$, 500 MHz): $\delta = 7.08$ (1H, t, 7.7 Hz), 6.91 (1H, d, 7.0 Hz), 6.75 (1H, d, 8.5 Hz), 2.54 (2H, t, 7.8), 2.47 (2H, t, 7.6 Hz), 1.74-1.78 (2H, m), 1.52-1.58 (2H, m), 1.23-1.41 (40H, m), 0.86-0.89 (6H, m). HRMS (m/z): [M+H]$^+$ calculated for C$_{31}$H$_{55}$NO$_2$: 474.4305; found 474.4286.

$N$-(2-hydroxy-6-octylphenyl)-5-(2-nitrophenyl)furan-2-carboxamide (Table 1 entry 2)

(As a red solid, 95%) $^1$H NMR (CDCl$_3$, 500 MHz): $\delta = 8.34$ (1H, s), 8.10 (1H, d, 3.9 Hz), 7.79 (1H, d, 8.0 Hz), 7.72 (1H, d, 7.8 Hz), 7.67 (1H, t, 7.3 Hz), 7.57 (1H, t, 7.6 Hz), 7.14 (1H, t, 7.8 Hz), 6.97 (1H, s), 6.88 (1H, d, 3.6 Hz), 6.82 (1H, d, 7.6 Hz), 2.69 (2H, t, 7.5 Hz), 6.61-6.66 (2H, m), 1.04-1.40 (2H, m), 1.16-1.30 (10H, m), 0.83 (3H, t, 6.8 Hz). HRMS (m/z): [M+H]$^+$ calculated for C$_{25}$H$_{28}$N$_2$O$_5$: 437.2071; found 437.2056.

$N$-(2-hydroxy-6-pentadecylphenyl)-5-(2-nitrophenyl)furan-2-carboxamide (Table 1 entry 7)

(As an orange solid, 97%) $^1$H NMR (CDCl$_3$, 500 MHz): $\delta = 8.35$ (1H, s), 8.1 (1H, s), 7.81 (1H, d, 6.8 Hz), 7.73 (1H, d, 6.8 Hz), 7.68 (1H, t, 7.3), 7.57 (1H, t, 7.6 Hz), 7.40 (1H, d, 3.6), 7.15 (1H, t, 7.8 Hz), 6.98 (1H, t, 7.3) (2H, m), 6.90 (1H, d, 3.6 Hz), 6.83 (1H, d, 8.3 Hz), 2.70 (2H, t, 7.6 Hz), 1.62-1.67 (2H, m), 1.38-1.41 (2H, s), 1.19-1.31 (24H, m), 0.88 (3H, t, 6.6 Hz). HRMS (m/z): [M+H]$^+$ calculated for C$_{32}$H$_{42}$N$_2$O$_5$: 535.3166; found 535.3162.

$4$-(N,N-dipropylsulfamoyl)-$N$-(2-hydroxy-6-octylphenyl)benzamide (Table 1 entry 3)

(As a yellow solid, 90%) $^1$H NMR (CDCl$_3$, 500 MHz): $\delta = 8.0$ (2H, d, 8.5 Hz), 7.97 (3H, t, 8.3 Hz), 6.99 (1H, d, 8.3 Hz), 6.84 (1H, d, 8.5 Hz), 3.13 (4H, t, 7.8 Hz), 2.67 (2H, t, 7.6 Hz), 1.55-1.61 (6H, m), 1.21-1.30 (10H, m), 0.85-0.90 (9H, m). HRMS (m/z): [M+H]$^+$ calculated for C$_{27}$H$_{40}$N$_2$O$_5$S: 489.2781; found 489.2785.
4-(N,N-dipropylsulfamoyl)-N-(2-hydroxy-6-pentadecylphenyl)benzamide (Table 1 entry 8)

(As yellow oil, 92%) \(^{1}\)H NMR (CDCl\(_3\), 500 MHz): \(\delta = 7.96-8.03\) (4H, m), 7.18 (1H, t, 7.8 Hz), 7.00 (1H, d, 5.6 Hz), 6.85 (1H, d, 7.3 Hz), 3.14 (4H, t, 7.8 Hz), 2.68 (2H, t, 7.3 Hz), 1.55-1.65 (6H, m), 1.25-1.35 (24H, m), 0.89 (9H, t, 7.3 Hz). HRMS (m/z): [M+H]\(^+\) calculated for C\(_{34}\)H\(_{54}\)N\(_2\)O\(_4\)S: 587.3877; found 587.3855.

4-(chloromethyl)-N-(2-hydroxy-6-octylphenyl)benzamide (Table 1 entry 4)

(As yellow oil, 90%) \(^{1}\)H NMR (CDCl\(_3\), 500 MHz): \(\delta = 8.30\) (1H, s), 7.94 (1H, s), 7.88 (2H, d, 8.3 Hz), 7.56 (2H, d, 8.3 Hz), 7.14 (1H, t, 7.8 Hz), 6.8 (1H, d, 8.3 Hz), 6.81 (1H, d, 8.5 Hz), 4.53 (2H, s), 2.66 (2H, t, 7.6 Hz), 1.59-1.65 (2H, m), 1.21-1.39 (12H, m), 0.86 (3H, t, 7.0 Hz). HRMS (m/z): [M+H]\(^+\) calculated for C\(_{22}\)H\(_{28}\)ClNO\(_2\): 374.1881; found 374.1873.

4-(chloromethyl)-N-(2-hydroxy-6-pentadecylphenyl)benzamide (Table 1 entry 9)

(As a white solid, 95%) \(^{1}\)H NMR (CDCl\(_3\), 500 MHz): \(\delta = 8.33\) (1H, s), 7.91-8.00 (3H, m), 7.58 (2H, d, 7.5 Hz) 7.16 (1H, t, 8.0 Hz), 6.99 (1H, d, 7.8 Hz), 6.82 (1H, d, 7.5 Hz), 4.66 (2H, s), 2.67 (2H, t, 7.0 Hz), 1.56-1.68 (2H, m), 1.26-1.42 (25H, m), 0.88 (3H, t, 6.8Hz). HRMS (m/z): [M+H]\(^+\) calculated for C\(_{29}\)H\(_{42}\)ClNO\(_2\): 472.2976; found 472.2973.
**N-(2-hydroxy-6-octylphenyl)-5-nitrothiophene-2-carboxamide (Table 1 entry 5)**

![Structure](https://via.placeholder.com/150)

(As a yellow solid, 92%) \(^1\)H NMR (CDCl\(_3\), 500 MHz): \(\delta = 8.41\ (1H, s),\ 7.4\ (1H, s),\ 7.28\ (1H, d, 7.3),\ 7.12\ (1H, t, 7.8 Hz),\ 6.96\ (1H, d, 8.0 Hz),\ 6.79\ (1H, d, 7.3 Hz),\ 4.55\ (1H, s),\ 2.63\ (2H, t, 7.5 Hz),\ 1.50-1.64\ (2H, m),\ 1.26-1.41\ (10H, m),\ 0.88\ (3H, t, 6.5 Hz). HRMS (m/z): [M+H]+ calculated for C\(_{19}\)H\(_{24}\)N\(_2\)O\(_4\)S: 377.1529; found 377.1528.

**N-(2-hydroxy-6-pentadecylphenyl)-5-nitrothiophene-2-carboxamide (Table 1 entry 10)**

![Structure](https://via.placeholder.com/150)

(As a yellow solid, 92%) \(^1\)H NMR (CDCl\(_3\), 500 MHz) \(\delta = 8.41\ (1H, s),\ 7.45\ (1H, s),\ 7.28\ (1H, s),\ 7.11\ (1H, d, 7.8 Hz),\ 6.96\ (1H, d, 7.6 Hz),\ 6.79\ (1H, d, 7.5 Hz),\ 4.56\ (1H, s),\ 2.63\ (2H, t, 7.3 Hz),\ 1.58-1.63\ (2H, m),\ 1.26-1.40\ (24H, m),\ 0.88\ (3H, t, 6.8Hz). HRMS (m/z): [M+H]+ calculated for C\(_{26}\)H\(_{38}\)N\(_2\)O\(_4\)S: 475.0562; found 475.0548.

**6. Synthesis of ginkgolic acid 2-phosphate**

**Benzyl 2-hydroxy-6-pentadecylbenzoate (S26)**

![Structure](https://via.placeholder.com/150)

To a stirred solution of ginkgolic acid (15:0) 2 (100 mg, 0.29 mmol) in triethylamine (1:1 v/v) was stirred at 90 °C for few minutes. Then benzyl chloride (36 mg, 0.29 mmol) was added to the reaction mixture at 90 °C and stirred for additional 1.5 hours at 90 °C. After completion of the reaction, the reaction mixture was cooled to room temperature and acidified with 2M HCl, extracted with EtOAc and dried over MgSO\(_4\). The organic layer was concentrated under vacuum to afford a residue that was purified by silica gel column chromatography using hexane/EtOAc (9.25:0.75) to give desired compound S26 (118 mg, 94%) as colorless oil.

\(^1\)H NMR (CDCl\(_3\), 500 MHz): \(\delta = 11.21\ (1H, s),\ 7.47\ (2H, d, 6.11 Hz),\ 7.40-7.44\ (3H, m),\ 7.30\ (1H, t, 8.0 Hz),\ 6.86\ (1H, d, J=8.3 Hz),\ 6.72\ (1H, d, J=7.3 Hz),\ 5.41\ (2H, s),\ 2.83\ (2H, t, 9.8 Hz),\ 1.40-1.47\ (2H, m),\ 1.30-1.37\ (18H, m),\ 1.09-1.20\ (6H, m),\ 0.92\ (3H, t, J=6.8 Hz). \(^13\)C NMR (125 MHz, CDCl\(_3\)) \(\delta = 171.3,\)
To a stirred solution of S26 (60 mg, 0.18 mmol) in CH3CN (5 mL) was stirred at 0 °C for few minutes under Ar atmosphere. DIPEA (57 mg, 0.44 mmol), DMAP (catalytic amount 0.05 eq), CCl4 (270 mg, 1.75 mmol) and dibenzylphosphite (55 mg 0.21 mmol) were added to the reaction mixture at 0 °C and stirred at room temperature for 2 h. After completion of the reaction, the reaction mixture was cooled to room temperature and acidified with 2M HCl, extracted with EtOAc and dried over MgSO4. The organic layer was concentrated under vacuum. The residue was purified by silica gel column chromatography using chloroform (100%) as an eluent to get S27 (86 mg, 91%) as colorless oil.

1H NMR (CDCl3, 500 MHz): δ = 7.40 (2H, t, 6.8 Hz), 7.25-7.31 (15H, m), 7.03 (1H, d, 7.5 Hz), 5.29 (2H, s), 5.01-5.10 (4H, m), 2.57 (2H, t, 7.8 Hz), 1.51-1.56 (2H, m), 1.17-1.32 (24H, m), 0.90 (3H, t, J=6.6 Hz). 13C NMR (125 MHz, CDCl3): δ = 166.6, 147.4, 142.5, 135.5, 135.4, 135.3, 130.5, 128.7, 128.5, 128.5, 128.4, 127.9, 127.9, 125.9, 117.2, 70.0, 67.2, 33.6, 31.9, 31.3, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 29.4, 22.7, 14.1. HRMS (m/z): [M+H]+ calculated for C43H55O6P: 699.3809; found 699.3816.

Ginkgolic acid 2-phosphate (GA2P)

To a stirred solution of S27 (40 mg, 57 µmol) in MeOH (5 ml) was added 10% Pd/C (45 mg, 52 µmol). The reaction mixture was stirred overnight at rt under H2 atmosphere. The solid was filtered off and the filtrate was concentrated under vacuum to afford a residue that was washed with non-polar solvents to get pure ginkgolic acid 2-phosphate (23 mg, 96%) as white solid.

1H NMR (CD3OD, 500 MHz): δ = 7.26-7.32 (2H, t, m), 7.01 (1H, d, 7.0 Hz), 2.66 (2H, t, 8.0 Hz), 1.57-1.61 (2H, m), 1.28-1.32 (24H, m), 0.89 (3H, t, J=7.1 Hz). HRMS (m/z): [M+Na]+ calculated for C22H37O6PNa: 451.2220; found 451.2214.
7. SMS assay

Cell lysates were prepared as follows: ZS/SMS1 and ZS/SMS2 cells (protein concentration 0.1 µg/µL) were diluted by Tris-buffer (pH 7.5) 20 mM and sonicated. Aliquots of the cell lysates 100 µL were added 1 µL of inhibitor of desired concentration and incubated at 37°C. After 30 min, the solutions were added 1 µL of C6-NBD-ceramide and incubated for 3 h at 37°C. The reaction was stopped by addition of 400 µL of MeOH/CHCl3 [1/2 (v/v)]; the mixture was shaken and centrifuged (1500 rpm x 5 min). The formation of C6-NBD-sphingomyelin was quantified by determination of the peak area of C6-NBD-sphingomyelin using HPLC. A reverse phase HPLC assay using a JACSO HPLC system was developed for the quantitative analysis of the inhibitory activity. The system was equipped with a PU-2089 Plus and FP-2020 Plus set at $\lambda_{ex} = 470$ nm and $\lambda_{em} = 530$ nm. A 50 x 4.6 YMC-Pack Diol-120-NP column (5-µm particle size) was used with mobile phase (IPA/hexane/water) at a flow rate of 1.0 mL/min.

**SMS inhibition assay of ginkgolic acid (15:1)**

![Figure S1: Inhibition activity of ginkgolic acid (15:1) with IC<sub>50</sub> of 1.5 µM on both SMS1 and SMS2. IC<sub>50</sub> values were measured by cell based assay system: SMS expressing cell lysates (20 mM tris-buffer, 100 mL) and compounds were incubated for 3 hours at 37°C, then fluorescent lipids were extracted from lysates by the Bligh-Dyer method, and directly applied to HPLC.](image-url)
SMS2 inhibition activity of ginkgolic acid derivatives

![Graph showing inhibition activities of ginkgolic acid (15:1) and its derivatives 2-5 on SMS2. IC₅₀ values were measured by cell based assay system: SMS expressing cell lysates (20 mM tris-buffer, 100 mL) and compounds were incubated for 3 hours at 37°C, then fluorescent lipids were extracted from lysates by the Bligh-Dyer method, and directly applied to HPLC.]

Table S1: SMS1 and SMS2 Inhibition activities of intermediates with IC₅₀ values. IC₅₀ values were measured by cell based assay system: SMS expressing cell lysates (20 mM tris-buffer, 100 mL) and compounds were incubated for 3 hours at 37°C, then fluorescent lipids were extracted from lysates by the Bligh-Dyer method, and directly applied to HPLC.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀ in µM</th>
<th>Compounds</th>
<th>IC₅₀ in µM</th>
</tr>
</thead>
</table>
| ![Structure S14](image) | SMS1- >100 µM  
SMS2- >100 µM | ![Structure S15](image) | SMS1- >100 µM  
SMS2- >100 µM |
| ![Structure S16](image) | SMS1- 15 µM  
SMS2- >100 µM | ![Structure S17](image) | SMS1- 5 µM  
SMS2- 20 µM |
| ![Structure S18](image) | SMS1- >100 µM  
SMS2- >100 µM | ![Structure S19](image) | SMS1- 9 µM  
SMS2- 70 µM |
8. $[^{32}\text{P}]$ S1P binding assay

$[^{32}\text{P}]$S1P was synthesized enzymatically using $[^{\text{3}}^{1,32}\text{P}]$ATP, sphingosine and purified sphingosine kinase SPHK1 as described previously (reference 5). S1P$_1$-CHO cells (5.0 x 10$^4$ cells) were seeded in 24-well plate and incubated for 24 h. The cells were incubated for 12 h with F-12 medium containing charcoal-treated FBS, and for another 12 h with F-12 medium. Cells were washed twice with ice cold PBS, and incubated for 30 min at 4 °C in 200 µL of binding buffer [20 mM Tris-HCl (pH 7.4), 100 mM NaCl, 15 mM NaF, 2 mM deoxypyridoxine, 0.2 mM phenylmethylsulfonyl fluoride, protease inhibitor cocktail (cOmplete™, EDTA-free, Sigma), fatty acid–free BSA (4 mg/ml), and 1 nM $[^{32}\text{P}]$S1P, in the absence or presence of the indicated concentration of S1P or GA2P. Cells were then washed twice with wash buffer [20 mM Tris-HCl (pH 7.4), 100 mM NaCl, and fatty acid–free BSA (0.4 mg/ml)], and lysed with RIPA buffer [50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% SDS and 0.1% sodium deoxycholate]. Bound $[^{32}\text{P}]$S1P was quantitated by scintillation counter Microbeta (PerkinElmer).
Figure S3: Competitive binding assay of GA2P with S1P. a) Competition of S1P and [³²P]S1P with S1P is carried out by addition of S1P at different concentrations (0-25 nM) in the presence of 1 nM [³²P]S1P. b) Similarly, competitive assay of GA2P with S1P has been carried out at different concentrations of GA2P (0-25 µM) in the presence of 1 nM [³²P]S1P. Bound S1P was quantitated by scintillation counter Microbeta (PerkinElmer).

9. References:

9. NMR spectra of compounds 1-7 and S1-S27
Chemical structures and NMR spectra are shown.
Table 1 entry 5
Table 1 entry 6

S21

S54
Table 1 entry 7
Table 1 entry 8
Table 1 entry 9
Ginkgolic acid 2-phosphate