Supporting Information

Phenolic compounds with in vitro activity against respiratory syncytial virus from the Nigerian lichen Ramalina farinacea

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General experimental procedures

Optical rotations were determined on a Perkin-Elmer-241 MC polarimeter. \(^1\)H, \(^{13}\)C, and 2D NMR spectra were recorded on Bruker ARX 500 or AVANCE DMX 600 NMR spectrometers (500/600 MHz for \(^1\)H, and 125/150 MHz for \(^{13}\)C). Chemical shifts expressing in \(\delta\) (ppm) referred to the solvent peaks \(\delta_H\) 7.26 and \(\delta_C\) 77.0 for CDCl\(_3\), \(\delta_H\) 2.05, and \(\delta_C\) 29.92 for Acetone-\(d_6\), respectively, and coupling constants in Hz. ESI/MS was conducted on a Finnigan LCQ Deca mass spectrometer. Solvents were distilled before use, and spectral grade solvents were used for spectroscopic measurements. HPLC analysis was performed using a HPLC (Dionex P580) system coupled to a photodiode array detector (UVD340S). Routine detection was at 235, 254, 280, and 340 nm. An analytical C\(_{18}\) column (Eurosphere-10, Knauer, Germany) (125×4 mm, L×I.D.) was used by eluting with a linear gradient of MeOH and 0.1% HCOOH in H\(_2\)O and a flow rate of 1 mL/min. A semi-preparative C18 column (Eurosphere-10, Knauer, Germany) (250×10 mm, L×I.D.) was used for purification, with flow rate 5 ml/min, and the detection set at 235 nm, while nanopure water containing 0.1% TFA together with methanol or acetonitrile was used as eluent. UV data (\(\lambda_{max}\)) for individual compounds were extracted from the online UV spectra. Silica gel (230-400 mesh, Macherey-Nagel GmbH & Co. KG, Germany), Sephadex LH-20, (0.25-0.1 mm mesh size, GE Healthcare), and RP-18 (0.04–0.063 mm mesh size, Merck) were used for column chromatography. TLC plates with silica gel F\(_{254}\) (Merck, Darmstadt, Germany) were used for monitoring of fractions. Detection was at 254 and 366 nm or by spraying the plates with anisaldehyde reagent.

Extraction and Isolation

The air-dried lichen of *R. farinacea* (100 g) was powder, and extracted with methanol (400 ml×4) four times at 40°C (3 h×4). After filtration, the methanol in the filtrate was removed under vacuum to give a brown residue (12.5 g), which was suspended in 400 ml distilled water, and partitioned against ethyl acetate(EtOAc) (200 ml×6) to yield a EtOAc extract (7.9 g) after removal of the solvent. The EtOAc extract (7.7 g) was subjected to vacuum liquid chromatography over silica gel (6 cm×8.2 cm, Length (l)×diameter (d)) eluting with a gradient of n-hexane/acetonone [(10:0, 0.8 L), (9:1, 0.8
L), (8:2, 1.2 L), (7:3, 1.2 L), (6:4, 1.2 L), (5:5, 1.2 L), (2.5:7.5, 0.8 L), (0:10, 0.8 L)] to give 18 fractions. Those fractions were analyzed by TLC and HPLC, and similar fractions were pooled to afford seven fractions (F₁-F₇). Fraction F₂ (250 mg), eluted from n-hexane/acetone 9:1, was subjected to column chromatography over Sephadex LH-20 (56.5 × 2.5 cm, l × d) eluting with dichloromethane/methanol (1:1) to give six subfractions (F₂₁–F₂₆). Subfraction F₂₆ (16.5 mg) was separated by semi-preparative HPLC using 75% MeOH as eluent to yield 12 (Rt 10.6 min, 2.5 mg), 10 (Rt 13.0 min, 1.9 mg), and 13 (Rt 19.7 min, 2.8 mg), respectively. Similarly, a portion of fraction F₃ (2.3 g), eluted from n-hexane/acetone 8:2, was rechromatographed over LH-20 (74 × 3.2 cm, l × d) using dichloromethane/methanol (1:1) as mobile phase to give six subfractions (F₃₁–F₃₆). F₃₅ (1.1 g) was rechromatographed over ODS (5.2 × 6 cm, l × d) using a gradient of MeOH in H₂O including 0.1% TFA (from 65% to 90%) as mobile phase, to give subsubfractions F₃₅₁–F₃₅₃. A portion of F₃₅₃ (eluted from 85%MeOH, 1.2 L, 126.3 mg) was further purified by HPLC (74% MeOH) to yield 2 (Rt 7.0 min, 79.4 mg), and 3 (Rt 13.4 min, 23.5 mg). Subfraction F₃₆ (64.5 mg) was separated by semi-preparative HPLC on a C₁₈ column eluting with a gradient of MeOH in H₂O (0.1%TFA) (MeOH%: 0-2 min 50%, 2-3 min 50-65%, 3-12 min 65%, 12-13 min 65-78%, 13-22 min 78%), to successively yield 6 (6.3 min, 19.8 mg), 7 (7.7 min, 6.6 mg), 2 (16.0 min, 18.7 mg), and a mixture (20.0 min) of 3 and 11, which were further purified by HPLC (79% acetonitrile) to give 3 (7.8 min, 6.3 mg) and 11 (9.0 min, 3.5 mg), respectively. A portion of fraction F₆ (123.4 mg), eluted from n-hexane/acetone 5:5, was repeatedly chromatographed over ODS (HPLC) eluting with 73% MeOH to afford 8 (4.2 min, 1.8 mg), 4 (9.9 min, 60.4 mg), and two subfractions F₆-a (2.0-3.5 min), and F₆-b (5.0-6.7 min). F₆-a (4.5 mg) was further purified by HPLC, eluting with 33% acetonitrile, to afford 9 (10.0 min, 1.5 mg); while F₆-b, a mixture of 5 (10.0 min, 13.3 mg), and 1 (11.6 min, 8.9 mg) were purified by HPLC using 57% acetonitrile as eluent. The purities of these compounds were > 95%, as determined by HPLC.

5-hydroxysekiikaic acid (1)
Brown oil; UV (λ_max) 215, 265, 312 nm; ¹H and ¹³C NMR data, see table 1; ESI-MS:
positive m/z 457.0 [M+Na]+, negative m/z 433.1 [M-H]−; HRESIMS: m/z 457.1469 (calculated for C_{22}H_{26}O_{9}Na, 457.1469).

2-dehydro-5-oxysekikaic acid (1a)
UV (λ\text{max}) 214, 264 nm; \textsuperscript{1}H and \textsuperscript{13}C NMR data, see table 1; ESI-MS: positive m/z 432.8 [M+H]+, 455.0 [M+Na]+, negative m/z 431.2 [M-H]−; HRESIMS: m/z 455.1313 (calculated for C_{22}H_{24}O_{9}Na, 455.1313).

Sekikaic acid (2)
Colorless amorphous solid; UV (λ\text{max}) 217, 264, 303; \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 600 MHz) δ\textsubscript{H} 6.39 (2H, s, H-3, and H-5), 6.64 (1H, s, H-5′), 3.00 (2H, t, J= 7.8 Hz, H-2′′), 1.75 (2H, m, H-2′′′), 0.95 (3H, t, J= 7.3 Hz, Me-3′), 2.97 (2H, t, J= 7.8 Hz, H-1′′′), 1.67 (2H, m, H-2′′′), 1.01 (3H, t, J= 7.3 Hz, Me-3′′), 3.83 (3H, s, 4-OMe), 3.90 (3H, s, 4′-OMe), 11.13 (1H, br.s, 2-OH), 11.49 (1H, br.s, 2′-OH); \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 150 MHz) δ\textsubscript{C} 104.4 (C-1, s), 165.5 (C-2, s), 98.9 (C-3, d), 164.4 (C-4, s), 110.9 (C-5, d), 148.7 (C-6, s), 168.8 (C-7, s), 104.6 (C-1′, s), 156.9 (C-2′, s), 124.9 (C-3′, s), 156.4 (C-4′, s), 106.5 (C-5′, d), 147.3 (C-6′, s), 175.4 (C-7′, s), 38.8 (C-1′′, t), 24.8 (C-2′′, t), 14.31 (C-3′, q) \textsuperscript{a}, 39.0 (C-1′′′, t), 25.1 (C-2′′′, t), 14.29 (C-3′′′, q) \textsuperscript{a}, 55.3 (4-OMe, q), 56.0 (4′-OMe, q) (\textsuperscript{a} Assignments maybe interchanged). ESI-MS m/z positive 441.1 [M+Na]+, negative 417.1 [M-H].

Homosekikaic acid (3)
Colorless solid; UV (λ\text{max}) 216, 264, 303; \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 600 MHz) δ\textsubscript{H} 6.39 (2H, s, H-3, and H-5), 6.46 (1H, s, H-5′), 3.00 (2H, overlapped, H-2′′′), 1.75 (2H, m, H-2′′″), 0.95 (3H, t, J= 7.3 Hz, Me-3′), 2.97 (2H, overlapped, H-1′′′), 1.63 (2H, m, H-2′′′), 1.37 (4H, m, H-2′′′, and H-2′′″), 0.92 (3H, t, J= 7.0 Hz, Me-5′′′), 3.83 (3H, s, 4-OMe), 3.90 (3H, s, 4′-OMe), 11.13 (1H, br.s, 2-OH), 11.48 (1H, br.s, 2′-OH); \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 150 MHz) δ\textsubscript{C} 104.4 (C-1, s), 165.5 (C-2, s), 98.9 (C-3, d), 164.4 (C-4, s), 110.9 (C-5, d), 148.7 (C-6, s), 168.8 (C-7, s), 104.6 (C-1′, s), 156.9 (C-2′, s), 124.9 (C-3′, s), 156.4 (C-4′, s), 106.4 (C-5′, d), 147.3 (C-6′, s), 175.4 (C-7′, s), 38.8 (C-1′′, t), 24.8 (C-2′′, t), 14.3 (C-3′, q) \textsuperscript{a}, 39.0 (C-1′′′, t), 25.1 (C-2′′′, t), 14.29 (C-3′′′, q) \textsuperscript{a}, 55.3 (4-OMe, q), 56.0 (4′-OMe, q). ESI-MS m/z positive 469.0 [M+Na]+; negative 445.2 [M-H].
4′-O-Methylnorhomosekikaic acid (4)
Colorless amorphous powder; UV ($\lambda_{\text{max}}$) 217, 265, 303; $^1$H NMR (CDCl$_3$, 600 MHz) $\delta_{\text{H}}$ 6.31 (1H, d, $J$= 2.5 Hz, H-3), 6.30 (1H, d, $J$= 2.5 Hz, H-5), 6.45 (1H, s, H-5′), 2.97 (2H, overlapped, H$_2$-1′′′), 1.73 (2H, m, H$_2$-2′′), 0.94 (3H, t, $J$= 7.3 Hz, Me-3′), 2.96 (2H, overlapped, H$_2$-1′′), 1.61 (2H, m, H$_2$-2′′′), 1.36 (4H, m, H$_2$-3′′, and H$_2$-4′′′), 0.91 (3H, t, $J$= 7.0 Hz, Me-5′′), 3.90 (3H, s, 4′-OMe), 11.01 (1H, br.s, 2-OH), 11.56 (1H, br.s, 2′-OH); $^{13}$C NMR (CDCl$_3$, 150 MHz) $\delta_{\text{C}}$ 104.9 (C-1, s), 165.1 (C-2, s), 101.5 (C-3, d), 160.8 (C-4, s), 111.0 (C-5, d), 149.6 (C-6, s), 168.8 (C-7, s), 104.6 (C-1′, s), 156.8 (C-2′, s), 124.8 (C-3′, s), 156.3 (C-4′, s), 106.3 (C-5′, d), 147.6 (C-6′, s), 174.9 (C-7′, s), 38.7 (C-1′′, t), 24.7 (C-2′′, t), 14.3 (C-3′′, q), 37.0 (C-1′′′, t), 31.6 (C-2′′′, t), 32.0 (C-3′′′, t), 22.4 (C-4′′′, t), 14.0 (C-5′′′, q), 56.0 (4′-OMe, q); ESI-MS m/z positive 455.0 [M+Na]$^+$, negative 431.1 [M-H]$^-$. 

4′-O-Methylnorsekikaic acid (5)
Light yellow oil; UV ($\lambda_{\text{max}}$) 216, 265, 303 nm; $^1$H and $^{13}$C NMR data, see table 1; ESI-MS: $m/z$ positive 426.9 [M+Na]$^+$, 404.7 [M+H]$^+$, negative 403.1[M-H]$^-$. 

Divaricatinic acid (7)
Colorless amorphous powder; UV ($\lambda_{\text{max}}$) 215, 262, 300 nm; $^1$H NMR (CDCl$_3$, 500MHz) $\delta_{\text{H}}$ 6.36 (1H, d, $J$=2.6 Hz, H-3), 6.34 (1H, d, $J$=2.6 Hz, H-5), 2.92 (2H, t, $J$= 7.7 Hz, H$_2$-1′), 1.63 (2H, m, H$_2$-2′), 0.98 (3H, t, $J$=7.3 Hz, Me-3′), 3.83 (3H, s, 4-OMe), 11.56 (1H, s, 2-OH); $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta_{\text{C}}$ 103.1 (C-1, s), 166.7 (C-2, s), 98.9 (C-3, d) 164.9 (C-4, s), 111.2 (C-5, d), 149.2 (C-6, s), 175.1(C-7, s), 38.7 (C-1′, t), 24.8 (C-2′, t), 14.2 (C-3′, q), 55.4 (4-OMe, q); ESI-MS: $m/z$ positive 211.0 [M+H]$^+$, negative 209.2[M-H]$^-$. EI-MS (rel., %): $m/z$ 210 [M$^+$] (37.3), 193 (12.8), 192 [M-H$_2$O]$^+$ (100.0), 177 [M-H$_2$O-CH$_3$]$^+$ (5.7), 166 [M-CO$_2$]$^+$ (14.8), 164 [M-HCOOH]$^+$ (37.3), 151 (5.9), 138 (25.7), 137 (9.2), 136 (7.0), 135 (49.7), 121 (4.0), 107 (5.0), 91 (4.5), 79 (4.7), 77 (7.8), 69 (6.2). 

2,3-dihydroxy-4-methoxy-6-pentylenzoic acid (8)
Colorless solid; UV ($\lambda_{\text{max}}$) 216, 226, 271, 315 nm; $^1$H NMR (CDCl$_3$, 500MHz) $\delta_{\text{H}}$ 6.37 (1H, s, H-5), 2.91 (2H, br.t, $J$= 7.8 Hz, H$_2$-1′), 1.58 (2H, br.m, H$_2$-2′), 1.34 (4H, m, H$_2$-3′, and H$_2$-4′), 0.90 (3H, br.t, $J$= 6.9 Hz, Me-5′), 3.95 (3H, s, 4-OMe), 11.44
(1H, br.s, 2-OH); $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta_{C}$ 103.9 (C-1, s), 151.1 (C-2, s), 131.5 (C-3, s), 150.9 (C-4, s), 106.4 (C-5, d), 139.7 (C-6, s), 174.4 (C-7, s), 36.5 (C-1', t), 31.9 (C-2', t)$^a$, 32.0 (C-3', t)$^a$, 22.5 (C-4', t), 14.1 (C-5', q), 56.0 (4-OMe, q) ($^a$ Assignments may be interchanged); ESI-MS: positive $m/z$ 255.0 [M+H]$^+$, 277.0 [M+Na]$^+$, 530.9 [2M+Na]$^{2+}$, negative $m/z$ 253.3 [M-H]$^-$; HRESIMS: $m/z$ 255.1230 (calculated for C$_{13}$H$_{19}$O$_5$, 255.1227), 277.1049 (calculated for C$_{13}$H$_{18}$O$_5$Na, 277.1046).

2,3-dihydroxy-4-methoxy-6-propylbenzoic acid (9)
Colorless oil; UV ($\lambda_{max}$) 215, 224, 270, 314; $^1$H NMR (CDCl$_3$, 500MHz) $\delta_H$ 6.37 (1H, s, H-5), 2.90 (2H, t, $J$ = 7.7 Hz, H$_2$-1'), 1.61 (2H, m, H$_2$-2'), 0.97 (3H, t, $J$=7.3 Hz, Me-3'), 3.95 (3H, s, 4-OMe), 11.46 (1H, br.s, 2-OH); $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta_{C}$ 103.9 (C-1, s), 151.2 (C-2, s), 131.5 (C-3, s) 150.8 (C-4, s), 106.4 (C-5, d), 139.3 (C-6, s), 174.2 (C-7, s), 38.5 (C-1', t), 25.3 (C-2', t), 14.2 (C-3', q), 56.0 (4-OMe, q); ESI-MS: positive $m/z$ 227.0 [M+H]$^+$, 249.0 [M+Na]$^+$, 474.8 [2M+Na]$^{2+}$, negative $m/z$ 225.2 [M-H]$^-$.

3,4-methylenedioxy-3'-methoxybibenzyl (13)
Colorless amorphous solid. UV ($\lambda_{max}$) 201, 281; $^1$H NMR (CDCl$_3$, 400MHz): $\delta$ 6.67 (1H, d, $J$=1.8 Hz, H-2), 6.711(1H, d, $J$ = 7.8 Hz, H-5), 6.61 (1H, dd, $J$=7.8, 1.8 Hz, H-6), 2.83 (4H, s, H$_2$-7, and H$_2$-7'), 5.91 (2H, s, H$_2$-8), 6.709 (1H, t, $J$ = 1.7 Hz, H-2'), 6.73 (1H, dt, $J$= 7.8, 1.7 Hz, H-4'), 7.19 (1H, t, $J$ = 7.8, H-5'), 6.76 (1H, dt, $J$ = 7.8, 1.7 Hz, H-6'), 3.78 (3H, s, 3'-OMe); $^{13}$C NMR (CDCl$_3$, 100MHz): $\delta$ 135.6 (C-1, s), 109.0 (C-2, d), 145.7 (C-3, s),147.6 (C-4, s), 108.2 (C-5, d), 121.2 (C-6, d), 37.6 (C-7, t), 100.8 (C-8, t), 143.3 (C-1', s), 114.3 (C-2', d), 159.7 (C-3', s), 111.3 (C-4', d), 129.3 (C-5', d), 120.9 (C-6', d), 38.3 (C-7', t), 55.2 (3'-OMe, q); EI-MS (rel., %): $m/z$ 256 [M$^+$] (34.6), 149 (2.0), 136 (8.2), 135 (100.0), 121 (2.1), 105 (2.6), 97 (2.9), 83 (2.5), 77 (8.0), 69 (5.4), 57 (4.6), 51 (3.9).
Viability of Hep 2 cells after drug treatment

**Fig. 1S** Effect on HEp2 cell viability by sekikaic acid and crude extract of *R. farinacea*

HEp2 cells were incubated with various concentrations of sekikaic acid and crude extract of *R. farinacea*. After 48 hrs of infection, MTT was added for 1 hr, and formazan crystal dissolved overnight, and absorbance measured at 550 nm. The results are expressed as percentage of the activity (cell viability) of cells cultured in the absence of the compounds. The mean and the standard deviation of at least triplicate observations are given.
Viability of Vero cells after drug treatment

**Fig. 2S** Effect on Vero cell viability by sekikaic acid and crude extract of *R. farinacea*

Vero cells were incubated with various concentrations of sekikaic acid and crude extract of *R. farinacea*. After corresponding 48hrs of infection, MTT was added for 1hr, and formazan crystal dissolved overnight, and absorbance measured at 550nm. The results are expressed as percentage of the activity (cell viability) of cells cultured in the absence of the compounds. The mean and the standard deviation of at least triplicate observations are given.
Fig. 3S $^1$H NMR spectrum of 1 (Acetone-$d_6$, 500MHz)

Fig. 4S $^1$H NMR spectrum of 1+1a (Acetone-$d_6$, 500MHz)
Fig. 5S Peak assignments for 1 and 1a in the expanded $^1$H NMR spectrum of 1+1a
Fig. 6S $^{13}$C NMR spectrum of 1 (Acetone-$d_6$, 125MHz)
Fig. 7S $^1$H-$^1$H COSY spectrum of 1

Fig. 8S HSQC spectrum of 1
Fig. 9S HMBC spectrum of 1

Fig. 10S HMBC spectrum of 1+1a
Fig. 11S HRESIMS spectrum of 1+1a
Fig. 12S $^1$H NMR spectrum of 8 (CDCl$_3$, 500MHz)

Fig. 13S $^{13}$C NMR spectrum of 8 (CDCl$_3$, 125MHz)
Fig. 14S $^1$H--$^1$H COSY spectrum of 8
Fig. 15S: HRESIMS spectrum of 8.