SUPPLEMENTARY MATERIAL

Quinone/hydroquinone meroterpenoids with antitubercular and cytotoxic activities produced by the sponge-derived fungus Gliomastix sp. ZSDS1-F7

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Quinone/hydroquinone meroterpenoids with antitubercular and cytotoxic activities produced by the sponge-derived fungus *Gliomastix* sp. ZSDS1-F7

Fifteen compounds, including six quinone/hydroquinone meroterpenoids, purpurogemutantin (1), macrophorin A (2), 4′-oxomacrophanorin (3), 7-deacetoxyyanuthone A (4), 2,3-hydro-deacetoxyyanuthone A (5), 22-deacetylyanuthone A (6), anicequol (7), three roquefortine derivatives, roquefortine C (8), (16S)-hydroxyroquefortine C (9), (16R)-hydroxyroquefortine C (10), dihydroresorcylic (11), necripyrone (12), together with three fatty acid derivatives, methyl linoleate (13), phospholipase A2 (14), methyl elaidate (15), were isolated from the sponge-derived fungus *Gliomastix* sp. ZSDS1-F7 isolated from the sponge *Phakellia fusca* Thiele collected in the Yongxing island of Xisha. Their structures were elucidated mainly by extensive NMR spectroscopic and mass spectrometric analysis. Among these compounds, compounds 1–3, and 5–7 showed significant *in vitro* cytotoxicities against the K562, MCF-7, Hela, DU145, U937, DU145, U937, H1975, SGC-7901, A549, MOLT-4, and HL60 cell lines, with IC_{50} values ranging from 0.19 to 35.4 μM. And compounds 2–4 exhibited antitubercular activity with IC_{50} values of 22.1, 2.44, and 17.5 μM, respectively. Furthermore, compound 7 had anti-enterovirus 71 activity with MIC value of 17.8 μM. To the best of our knowledge, this is the first report to product two quinone/hydroquinone meroterpenoids skeletons (linear skeleton and drimane skeleton) from the same fungal strain.

**Keywords**: sponge-derived fungus; *Phakellia fusca* Thiele; meroterpenoid; roquefortine; cytotoxic activity; antitubercular activity; anti-enterovirus 71 activity

**Experiment**

**General experimental procedures**

{\textsuperscript{1}H, \textsuperscript{13}C NMR, DEPT, and 2D-NMR spectra were recorded on the Bruker DRX-500 spectrometer (Bruker BioSpin, Fallanden, Switzerland) using TMS as internal standard and chemical shifts were recorded as δ values. HRESI-MS (including ESI-MS) spectra were recorded on an Applied Biosystems Mariner 5140 spectrometer (Life Technologies Ltd, New York, USA). TLC and column chromatography (CC) were performed on plates precoated with silica gel GF_{254} (10–40 μm) and over silica gel (200–300 mesh) (Qingdao Marine Chemical Factory, Qingdao, China), and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden), respectively. All solvents used were of
analytical grade (Tianjin Fuyu Chemical and Industry Factory, Tianjin, China). Semipreparative HPLC was performed using an ODS column (YMC-pack ODS-A, 10 × 250 mm, 5 μm, 4 mL/min).

**Fungal material and culture conditions**

The fungus *Gliomastix* sp. ZSDS1-F7 was obtained from the sponge *Phakellia fusca* Thiele collected in the Yongxing island of Xisha. The fungus was identified using a molecular biological protocol by DNA amplification and sequencing of the ITS region. The sequenced data derived from the fungal strain have been deposited in GenBank (accession no. KT314167). A BLAST search result showed that the sequence was most similar (99%) to the sequence of *Gliomastix* sp. (compared to accession no. AB540563). A reference culture is deposited in at our laboratory at −80 °C. The producing strain was prepared on potato dextrose agar slants at 3.3% salt concentration and stored at 4 °C. The strain *Gliomastix* sp. ZSDS1-F7 was grown in a rice culture medium at 26 °C for 40 days in 1000 mL flasks (rice 200g, sea salt 2.5g, distilled water 200mL)

**Extraction and isolation**

After 40 days, cultures from 30 flasks were harvested and subjected to organic extraction using ethyl acetate (EtOAc). The filtrate was concentrated under vacuum to about a quarter of original volume and then extracted three times with EtOAc to give an EtOAc solution. The EtOAc solution was concentrated under vacuum to give an EtOAc extract (74.8 g). The EtOAc extract (74.8 g) was subjected to VLC on a silica gel column using step gradient elution with MeOH–CH₂Cl₂ (0–100%) to separate into five fractions based on TLC properties. Fraction 1 was divided into six parts (Frs. 1-1–1-6) followed by the reverse phase silica gel (ODS) with MeOH–H₂O (20%–100%). Fr.1-1 was directly separated by HPLC (60% MeOH/H₂O) to yield 12 (8.7 mg, *tₚ* 10.8 min) and 11 (52.3 mg, *tₚ* 18.3 min), respectively. Fr.1-3 was further separated by HPLC (82% MeOH/H₂O) to yield 4 (243.0 mg, *tₚ* 18.2 min), 6 (32.7 mg, *tₚ* 23.3 min), and 3 (13.0 mg, *tₚ* 27.7 min) respectively. Fr. 1-4 was divided into six parts (Frs. 1-4-1–1-4-6) followed by HPLC (90% MeOH/H₂O). Fr.1-4-2 was further separated by HPLC (83% MeOH/H₂O) to yield 14 (22.7 mg, *tₚ* 18.5 min). Fr.1-4-5 was further separated by HPLC (85% CH₃CN/H₂O) to yield 15 (15.2 mg, *tₚ* 13.3 min) and 13 (65.6 mg, *tₚ* 33.5 min), respectively. Fraction 2 was divided into eight parts (Frs. 2-1–2-8) followed by the reverse phase silica gel (ODS) with MeOH–H₂O (30%–100%). Fr. 2-4 was divided into four parts (Frs. 2-4-1–2-4-4) followed by Sephadex LH-20 (MeOH). Fr.2-4-2 was further separated by HPLC (66% CH₃CN/H₂O) to yield 7 (32.0 mg, *tₚ* 13.8 min) and 5 (4.8 mg, *tₚ* 18.2 min), respectively. Fraction 3 was divided into five parts (Frs. 3-1–3-5) followed by the reverse phase silica gel (ODS) with
MeOH–H\textsubscript{2}O (20%–100%). Fr.3-2 was further separated by HPLC (70% MeOH/H\textsubscript{2}O) to yield 9 (4.4 mg, \textit{t} \textsubscript{R} 14.9 min), 10 (3.5 mg, \textit{t} \textsubscript{R} 21.0 min), and 8 (18.1 mg, \textit{t} \textsubscript{R} 23.2 min) respectively. Fr.3-3 was further separated by HPLC (80% MeOH/H\textsubscript{2}O) to yield 1 (21.6 mg, \textit{t} \textsubscript{R} 9.9 min) and 2 (12.2 mg, \textit{t} \textsubscript{R} 13.8 min), respectively.

**Bioassay Protocols**

The antiviral activities against H1N1 and H3N2 were evaluated by the CPE inhibition assay. Confluent MDCK cell monolayers were firstly incubated with influenza virus at 37 °C for 1 h. After removing the virus dilution, cells were maintained in infecting media (RPMI 1640, 4 \( \mu \text{g/mL} \) of trypsin) containing different concentrations of test compounds. After 48 h incubation at 37 °C, the cells were fixed with 100 \( \mu \text{L} \) of 4% formaldehyde for 20 min at room temperature. After removal of the formaldehyde, the cells were stained with 0.1% crystal violet for 30 min. The plates were washed and dried, and the intensity of crystal violet staining for each well was measured in a microplate reader (Bio-Rad, Hercules, CA, USA) at 570 nm. The IC\textsubscript{50} was calculated as the compound concentration required inhibiting influenza virus yield at 48 h post-infection by 50%. Tamiflu was used as the positive control with IC\textsubscript{50} values of 16.9 and 18.5 nM, respectively.

EV71 was assayed on Vero cells with the CCK8 (Dojindo, Japan) method. Vero cells (2\( \times \)10\textsuperscript{3} cells/well) were seeded with DMEM medium (2% FBS) into a 384-well plate. After 24 h, 1000 fold serial dilution of the compound was added in triplicate to the 348-well plate. After incubation at 37 °C for 30 min, a twofolds dilution100 \( \times \) the 50% tissue culture infectious dose (TCID50) of EV71 virus in DMEM supplemented with 2% FBS was added to each well. The plate was incubated at 37 °C for 72–96 h when the viral control cells showed complete CPE, the cell survival was quantified using CCK-8. The A450 of the well was measured with a microtiter platereader (Envision, PerkinElmer). The 50% inhibitory concentration (IC\textsubscript{50}) of the testing compound was calculated using the GraphPad Prism software. Ribavirin was used as the positive control with an IC\textsubscript{50} value of 0.60 \( \mu \text{M} \).

Cytotoxicity was assayed with the CCK-8 (Dojindo, Japan) method. Cell lines, K562, MCF-7, Hela, DU145, U937, H1975, SGC-7901, A549, MOLT-4, and HL60 were purchased from Shanghai Cell Bank, Chinese Academy of Sciences. Cells were routinely grown and maintained in mediums RPMI or DMEM with 10% FBS and with 1% penicillin/streptomycin. All cell lines were incubated in a Thermo/Forma Scientific CO\textsubscript{2} Water Jacketed Incubator with 5% CO\textsubscript{2} in air at 37 °C. Cell viability assay was determined by the CCK-8 (Dojindo, Japan) assay. Cells were seeded at a density
of 400-800 cells/well in 384 well plates and treated with various concentration of compounds or solvent control. After 72 h incubation, CCK-8 reagent was added, and absorbance was measured at 450 nm using Envision 2104 multi-label Reader (Perkin Elmer, USA). Dose response curves were plotted to determine the IC\textsubscript{50} values using Prism 5.0 (GraphPad Software Inc., USA). TSA was used as the positive control with IC\textsubscript{50} values of 0.13, 0.03, 0.06, 0.04, 0.03, 0.02, 0.04, 0.03, 0.04 µM, respectively.

Anti-tubercular was assayed, and autoluminescent \textit{M. tuberculosis} H37Ra were inoculated in a 50 mL centrifuge tube containing 5 mL 7H9 with 0.1% Tween 80 and 10% OADC, then incubated at 37 °C. When the cultures reached an OD\textsubscript{600} nm of 0.3-1.0, the culture was diluted and 50 µL diluted H37Ra were inoculated in sterile 384 well plates, the RLU of which should be between 10000 and 50000 and be recorded as the base luminescent Day0. The compounds and the positive drug were added to the 384 well plates in triplicate by the Echo520 with the final concentration 50 µM. The luminescent value was detected for the following three days. The data were analysis with the Excel compared to the DMSO control to estimate the inhibition activity of the compounds. INH (isoniazid, Sigma) was used as the positive control with IC\textsubscript{50} value of 2.04 µM.

**The physicochemical data of the known compounds 1–15**

purpurogemutatin (1): \textsuperscript{1}H NMR (CD\textsubscript{3}OD, 500 MHz) δ 6.15 (s, H-2'), 4.90 (1H, brs, H-12), 4.37 (2H, dd, J = 18.5 Hz, H-7'), 3.85 (1H, s, H-5'), 3.09 (1H, d, J = 17.6 Hz, H\textsubscript{a}-8'), 2.87 (1H, d, J = 17.5 Hz, H\textsubscript{b}-8'), 2.44 (1H, d, J = 13.0 Hz, H\textsubscript{a}-7), 2.23 (1H, d, J = 15.5 Hz, H\textsubscript{a}-11), 2.11 (1H, td, J = 13.0 Hz, H\textsubscript{b}-7), 2.01 (1H, dd, J = 15.5, 8.5 Hz, H\textsubscript{b}-11), 1.89 (1H, d, J = 8.5 Hz, H-9), 1.79 (1H, brd, J = 13.0 Hz, H-6), 1.76 (1H, brd, J = 13.5 Hz, H\textsubscript{a}-1), 1.62 (1H, m, H\textsubscript{a}-2), 1.50 (1H, m, H\textsubscript{b}-2), 1.39 (1H, m, H\textsubscript{a}-3), 1.35 (1H, qt, J = 13.0 Hz, H\textsubscript{b}-6), 1.23 (1H, m, H-5), 1.20 (2H, m, H\textsubscript{b}-3), 1.17 (2H, td, J = 13.5 Hz, H\textsubscript{b}-1), 0.89 (3H, s, H-13), 0.83 (3H, s, H-14), 0.75 (3H, s, H-15). \textsuperscript{13}C NMR (CD\textsubscript{3}OD, 125 MHz) δ 193.5 (s, C-1'), 169.9 (s, C-9'), 165.5 (s, C-3'), 150.3 (s, C-8), 120.8 (d, C-2'), 108.4 (t, C-12), 86.5 (s, C-6'), 75.3 (d, C-5'), 71.7 (s, C-4'), 60.9 (t, C-7'), 56.9 (d, C-5), 51.2 (d, C-9), 43.5 (t, C-8'), 43.3 (t, C-3), 41.4 (s, C-10), 39.9 (t, C-1), 39.4 (t, C-7), 34.6 (s, C-4), 34.0 (q, C-13), 25.7 (t, C-6), 23.2 (t, C-11), 22.1 (q, C-14), 20.3 (t, C-2), 15.3 (q, C-15).

macrophorin A (2): \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 500 MHz) δ 5.88 (1H, s, H-2'), 4.77 (1H, brs, H-12), 4.57 (1H, brs, H\textsubscript{b}-12), 4.51 (1H, brs, H-4'), 4.27 (2H, s, H-7'), 3.74 (1H, d, J = 1.85 Hz, H-5'), 2.32 (2H, d, J = 14.3 Hz, H-7), 1.92 (1H, td, J = 13.0 Hz, H-11), 1.84 (1H, t, J = 13.0 Hz, H\textsubscript{b}-11), 1.73 (1H, m, H-9), 1.68 (1H, m, H\textsubscript{a}-6), 1.65 (1H, m, H\textsubscript{a}-1), 1.52 (2H, dd, J = 13.5 Hz, H-2), 1.37 (1H, d, J = 13.0 Hz, H\textsubscript{a}-3), 1.28 (1H, qd, J = 12.5 Hz, H\textsubscript{b}-6), 1.18 (1H, m, H-5), 1.15 (1H, m, H\textsubscript{b}-3), 1.09 (1H, m,
H_{2-1}), 0.84 (3H, s, H-13), 0.77 (3H, s, H-14), 0.67 (3H, s, H-15). $^{13}$C NMR (CDCl$_3$, 125 MHz) δ 193.5 (s, C-1’), 157.5 (s, C-3’), 149.4 (s, C-8), 120.7 (d, C-2’), 106.8 (t, C-12), 65.5 (d, C-4’), 62.3 (t, C-7’), 61.1 (s, C-6’), 61.0 (d, C-5’), 55.5 (d, C-5), 51.5 (d, C-9), 42.1 (t, C-3), 39.8 (s, C-10), 38.9 (t, C-1), 38.2 (t, C-7), 33.7 (s, C-4’), 33.6 (q, C-13), 24.5 (t, C-6), 21.8 (q, C-14), 20.9 (t, C-11), 19.5 (t, C-2), 14.6 (q, C-15).

4’-oxomacrophorin (3): $^1$H NMR (CDCl$_3$, 500 MHz) δ 6.64 (1H, s, H-2’), 4.82 (1H, s, H$_2$-12), 4.53 (1H, d, J = 17.5, H$_2$-7’), 4.51 (1H, s, H$_2$-12), 4.35 (1H, d, J = 17.5 Hz, H$_2$-7’), 3.73 (1H, s, H-5’), 2.47 (1H, d, J = 14.9 Hz, H$_6$-11), 2.36 (1H, brd, J = 13.0 Hz, H$_6$-7), 2.01 (1H, m, H$_6$-11), 1.95 (1H, dt, J = 13.0 Hz, H$_6$-7), 1.75 (1H, m, H$_6$-6), 1.71 (1H, m, H$_6$-1), 1.68 (1H, m, H-9), 1.57 (2H, m, H-2), 1.40 (1H, brd, J = 18.0 Hz, H$_6$-3), 1.30 (1H, dd, J = 13.0, 4.1 Hz, H$_6$-6), 1.19 (1H, m, H$_6$-3), 1.18 (1H, m, H$_6$-1), 1.11 (1H, m, H-5), 0.86 (3H, s, H-13), 0.79 (3H, s, H-14), 0.70 (3H, s, H-15). $^{13}$C NMR (CDCl$_3$, 125 MHz) δ 193.7 (s, C-1’), 192.2 (s, C-4’), 148.9 (s, C-8), 147.0 (s, C-3’), 132.2 (d, C-2’), 107.0 (d, C-12), 62.7 (s, C-6’), 59.3 (t, C-7’), 59.2 (d, C-5’), 55.7 (d, C-5), 51.6 (d, C-9), 42.1 (t, C-3), 39.9 (s, C-10), 39.0 (t, C-1), 38.2 (t, C-7), 33.8 (s, C-4), 33.7 (q, C-13), 24.5 (t, C-6), 21.8 (q, C-14), 20.4 (t, C-11), 19.5 (t, C-2), 14.6 (q, C-15).

7-deacetoxyanthonone A (4): $^1$H NMR (CDCl$_3$, 500 MHz) δ 5.70 (1H, s, H-6), 5.02 (1H, t, J = 7.2 Hz, H-10’), 5.01 (H, t, J = 7.2 Hz, H-6’), 4.95 (1H, t, J = 7.2 Hz, H-2’), 4.39 (1H, brs, H-4), 3.64 (1H, d, J = 2.6 Hz, H-3), 2.74 (1H, dd, J = 15.3, 7.4 Hz, H$_3$-1’), 2.39 (1H, dd, J = 15.3, 6.8 Hz, H$_3$-1’), 2.01 (2H, m, H-5’), 1.98 (2H, m, H-9’), 1.95 (2H, m, H-8’), 1.92 (3H, s, H-7), 1.90 (2H, m, H-4’), 1.61 (3H, s, H-9’), 1.57 (3H, s, H-13’), 1.54 (3H, s, H-13) 1.53 (3H, s, H-14’). $^{13}$C NMR (CDCl$_3$, 125 MHz) δ 193.6 (s, C-1), 156.5 (s, C-5), 139.6 (s, C-3’), 135.1 (s, C-7’), 131.2 (s, C-11’), 124.3 (d, C-10’), 123.8 (d, C-6’), 123.3 (d, C-6), 116.2 (d,C-2’), 67.5 (d, C-4), 61.4 (s, C-2), 59.3 (d, C-3), 39.7 (t, C-8’), 39.6 (t, C-4’), 26.7 (t, C-9’), 26.4 (t, C-5’), 26.1 (t, C-1’, 25.6 (q, C-12’), 20.1 (q, C-7), 17.6 (q, C-13’), 16.3 (q, C-15’), 15.9 (q, C-14’).

2,3-hydro-deacetoxyanthonone A (5): $^1$H NMR (CDCl$_3$, 500 MHz) δ 5.96 (1H, s, H-6), 5.09 (1H, m, H-10’), 5.07 (H, m, H-6’), 5.01 (1H,t, J = 7.2 Hz, H-2’), 4.69 (1H, brs, H-4), 4.41 (2H, d, J = 16.5 Hz, H-7), 3.72 (1H, s, H-3), 2.81 (1H, dd, J = 15.4, 8.0 Hz, H$_3$-1’), 2.50 (1H, dd, J = 15.4, 6.7 Hz, H$_3$-1’), 2.07 (2H, m, H-5’), 2.04 (2H, m, H-9’), 2.01 (2H, m, H-8’), 1.97 (2H, m, H-4’), 1.68 (3H, s, H-12’), 1.63 (3H, s, H-15’), 1.60 (3H, s, H-13’), 1.59 (3H, s, H-14’). $^{13}$C NMR (CDCl$_3$, 125 MHz) δ 193.7 (s, C-1), 156.8 (s, C-5), 140.1 (s, C-3’), 135.4 (s, C-7’), 131.6 (s, C-11’), 124.5 (d, C-10’), 123.9 (d, C-6’), 121.1 (d, C-6), 116.1 (d, C-2’), 66.1 (d, C-4), 63.1 (t, C-7), 61.5 (s, C-2), 59.1 (d, C-3), 39.9 (t, C-8’), 39.8 (t, C-4’), 26.8 (t, C-9’), 26.5 (t, C-5’), 26.1 (t, C-1’), 25.8 (q, C-12’), 17.8 (q, C-13’), 16.5 (q, C-15’), 16.2 (q, C-14’).
22-deacetylanthatone A (6): $^1$H NMR (CDCl$_3$, 500 MHz) δ 5.93 (1H, s, H-6), 5.31 (1H, t, $J = 7.0$ Hz, H-2'), 5.09 (1H, m, H-10'), 5.07 (H, m, H-6'), 4.73 (1H, brs, H-4), 4.26 (1H, d, $J = 3.3$ Hz, H-3), 2.82 (2H, m, H$_{a}$-1'), 2.12 (2H, m, H-5'), 2.10 (2H, m, H-4'), 2.05 (3H, s, H-7), 2.04 (2H, m, H-9'), 1.97 (2H, m, H-8'), 1.68 (3H, s, H-15'), 1.67 (3H, s, H-13'), 1.61 (3H, s, H-12'), 1.60 (3H, s, H-14').

$^{13}$C NMR (CDCl$_3$, 125 MHz) δ 191.0 (s, C-1), 158.8 (s, C-5), 140.6 (s, C-3'), 135.6 (s, C-7'), 131.6 (s, C-11'), 124.4 (d, C-10'), 123.9 (d, C-6'), 123.8 (d, C-6), 117.0 (d, C-2'), 74.5 (d, C-3), 68.9 (d, C-4), 68.7 (s, C-2), 40.1 (t, C-4'), 39.83 (t, C-8'), 32.1 (t, C-1'), 26.86 (t, C-9'), 26.5 (t, C-5'), 25.8 (q, C-12'), 20.4 (q, C-7), 17.8 (q, C-13'), 16.7 (q, C-15'), 16.2 (q, C-14').

aniequel (7): $^1$H NMR (DMSO-$_d_6$, 500 MHz) δ 5.15 (1H, m, H-22), 5.15 (1H, m, H-23), 4.87 (1H, m, H-16), 4.55 (2H, m, OH-3,7), 4.26 (1H, s, OH-11), 4.17 (1H, s, H-11), 3.68 (1H, d, $J = 8.8$ Hz, H-7), 2.49 (1H, m, H-20), 2.44 (1H, m, H-15'), 2.29 (1H, dd, $J = 12.1, 2.2$ Hz, H-5), 2.13 (1H, d, $J = 12.1$ Hz, H-12), 1.93 (1H, m, H-8), 1.92 (3H, s, H-30), 1.85 (1H, m, H-1), 1.74 (1H, dd, $J = 13.2, 6.7$ Hz, H-24), 1.63 (1H, d, $J = 9.5$ Hz, H$_a$-4), 1.56 (1H, d, $J = 13.2$ Hz, H$_a$-2), 1.39 (1H, m, H-25), 1.30 (1H, m, H-9), 1.30 (1H, m, H$_b$-15), 1.22 (1H, m, H$_b$-1), 1.22 (1H, m, H$_b$-2), 1.22 (1H, m, H$_b$-4), 1.19 (1H, m, H-17), 1.04 (3H, s, H-18), 1.02 (3H, m, H-21), 0.82 (3H, brd, H-28), 0.80 (3H, s, H-19), 0.78 (3H, brd, H-26), 0.76 (3H, brd, H-27).

$^{13}$C NMR (DMSO-$_d_6$, 125 MHz) δ 210.39 (s, C-6), 169.5 (s, C-29), 135.3 (d, C-22), 131.9 (d, C-23), 78.5 (d, C-7), 74.7 (d, C-16), 68.6 (d, C-3), 66.4 (d, C-11), 59.1 (d, C-17), 55.2 (d, C-14), 54.7 (d, C-9), 53.8 (d, C-5), 47.7 (t, C-12), 42.8 (s, C-13), 42.7 (d, C-24), 41.7(d, C-8), 39.8 (s, C-10), 36.9(t, C-15), 35.3 (t, C-1), 34.1 (d, C-20), 32.5 (d, C-25), 30.4 (t, C-2), 29.7 (q, C-4), 21.3 (q, C-30), 20.8(q, C-21), 19.9(q, C-26), 19.5(q,C-27), 17.8 (q, C-28), 15.3 (q, C-19), 14.9 (q, C-18).

roquefortine C (8): $^1$H NMR (CD$_3$OD, 500 MHz) δ 7.72 (2H, s, NH-1,1'), 7.17 (2H, d, $J = 7.5$ Hz, H-4,4'), 7.04 (2H, t, $J = 7.5$ Hz, H-5,5'), 6.71 (2H, t, $J = 7.8$ Hz, H-6,6'), 6.58 (2H, d, $J = 7.8$ Hz, H-7,7'), 6.05 (2H, dd, $J = 17.4, 10.8$ Hz, H-15,15'), 5.68 (2H, s, H-2,2'), 5.10 (4H, m, H-16,16'), 4.01 (2H, dd, $J = 11.3, 6.0$ Hz, H-11,11'), 2.50 (2H, dd, $J = 12.0, 6.0$ Hz, H$_b$-10,10'), 2.42 (2H, t, $J = 12.0$ Hz, H$_b$-10,10'), 1.12 (6H, s, H-18,18'), 1.00 (6H, s, H-17,17').

$^{13}$C NMR (CD$_3$OD, 125 MHz) δ 168.4 (s, C-12), 160.1 (s, C-12'), 152.3 (s, C-8,8'), 145.2 (d, C-15,15'), 130.2 (s, C-9,9'), 130.0 (d, C-6,6'), 126.1 (d, C-4,4'), 119.5 (d, C-5,5'), 114.8 (t, C-16,16'), 110.1 (d, C-7,7'), 79.3 (d, C-2,2'), 62.6 (s, C-3,3'), 59.9 (d, C-11,11'), 42.1 (s, C-14,14'), 38.4 (t, C-10,10'), 23.4 (q, C-18,18'), 23.0 (q, C-17,17').

(16S)-hydroxyroquefortine C (9): $^1$H NMR (CD$_3$OD, 500 MHz) δ 9.34 (2H, s, NH-1,1'), 8.76 (2H, d, $J = 7.5$ Hz, H-4,4'), 8.59 (2H, t, $J = 7.5$ Hz, H-5,5'), 8.25 (2H, t, $J = 7.5$ Hz, H-6,6'), 8.13 (2H, d, $J = 7.8$ Hz, H-7,7'), 7.60 (2H, dd, $J = 17.0, 10.9$ Hz, H-15,15'), 7.33 (2H, s, H-2,2'), 6.69 (4H, dd, $J = 17.0, 10.9$ Hz, H-16,16'), 4.28 (2H, d, $J = 13.8$ Hz, H$_b$-10,10'), 4.13 (2H, d, $J = 13.8$ Hz, H$_b$-10,10').
2.69 (6H, s, H-18,18'), 2.54 (6H, s, H-17,17'). $^{13}$C NMR (CD$_3$OD, 125 MHz) δ 167.9 (s, C-12,12'), 151.2 (s, C-8,8'), 145.3 (d, C-15,15'), 132.8 (s, C-9,9'), 129.2 (d, C-6,6'), 125.9 (d, C-4,4'), 119.1 (d, C-5,5'), 114.9 (t, C-16,16'), 109.9 (d, C-7,7'), 88.2 (s, C-11,11'), 80.7 (d, C-2,2'), 61.3 (s, C-3,3'), 45.0 (t, C-10,10'), 42.5 (s, C-14,14'), 23.2 (q, C-18,18'), 22.7 (q, C-17,17').

(16R)-hydroxyroquefortine C (10): $^1$H NMR (CD$_3$OD, 500 MHz) δ 7.79 (1H, s, H-20), 7.44 (1H, brs, H-22), 7.19 (1H, d, J = 7.4 Hz, H-12), 7.02 (1H, t, J = 7.4 Hz, H-10), 6.68 (1H, t, J = 7.4 Hz, H-11), 6.56 (d, J = 7.7 Hz, H-9), 6.50 (1H, s, H-17), 6.04 (1H, dd, J = 17.3, 10.9 Hz, H-24), 5.85 (1H, s, H-6), 5.13 (2H, t, J = 11.4 Hz, H-25), 2.81 (3H, s, -OCH$_3$), 2.69 (1H, d, J = 13.8 Hz, H$_2$-15), 2.58 (1H, d, J = 13.8 Hz, H$_3$-15), 1.13 (3H, s, H-26), 0.98 (3H, s, H-27). $^{13}$C NMR (CD$_3$OD, 125 MHz) δ 165.34 (s, C-1), 161.54 (s, C-4), 151.19 (s, C-8), 145.18 (d, C-24), 137.98 (d, C-20), 132.78 (s, C-13), 129.17 (d, C-10), 125.66 (d, C-12), 118.99 (d, C-11), 114.96 (t, C-25), 109.79 (d, C-9), 92.90 (s, C-16), 80.64 (d, C-6), 61.07 (s, C-14), 51.82 (q, C-OCH$_3$), 42.79 (t, C-15), 42.48 (s, C-23), 23.22 (q, C-27), 22.72 (q, C-26).

dihydropyrosolelode (11): $^1$H NMR (CDCl$_3$, 500 MHz) δ 12.04 (1H, s, OH-15), 6.27 (1H, s, H-14), 6.02 (1H, s, H-12), 5.11 (1H, m, H-3), 4.63 (1H, d, J = 18.5 Hz, Ha-10), 3.66 (1H, d, J = 18.5 Hz, Hb-10), 2.58 (1H, dd, J = 14.4, 10.0 Hz, Ha-8), 2.36 (1H, dd, J = 14.4, 10.0 Hz, Hb-8), 1.99 (1H, m, Ha-7), 1.78 (1H, m, Hb-7), 1.63 (1H, m, Ha-4), 1.55 (1H, m, Hb-4), 1.45 (2H, m, H-5), 1.45 (2H, m, H-6), 1.28 (3H, d, J = 6.2 Hz, H-17). $^{13}$C NMR (CDCl$_3$, 125 MHz) δ 210.5(s, C-9), 171.1(s, C-1), 165.5(s, C-15), 161.4(s, C-13), 138.3(s, C-11), 113.1(d, C-12), 105.9(s, C-16), 102.9(d, C-14), 73.7(d, C-3), 50.9(t, C-10), 41.9(t, C-8), 31.7(t, C-4), 27.0(t, C-6), 21.3(t, C-7), 21.1(t, C-5), 19.2(q, C-17).

nectriapryrone (12): $^1$H NMR (CDCl$_3$, 500 MHz) δ 6.68 (1H, q, J = 7.0 Hz, H-8), 6.10 (1H, s, H-4), 3.90 (3H, s, -OCH$_3$), 1.92 (3H, s, H-11), 1.88 (3H, s, H-10), 1.83 (3H, d, J = 7.1 Hz, H-9). $^{13}$C NMR (CDCl$_3$, 125 MHz) δ 166.2(s, C-5), 165.3(s, C-1), 160.3(s, C-3), 129.9(d, C-8), 127.0(s, C-7), 102.0(s, C-6), 91.6(d, C-4), 56.2(q, C-OCH$_3$), 14.4(q, C-10), 12.3(q, C-9), 8.7(q, C-11).

methyl linoleate (13): $^1$H NMR (CDCl$_3$, 500 MHz) δ 5.33 (4H, m, H-9,10,12,13), 3.64 (3H, s, -OCH$_3$), 2.75 (2H, t, J = 6.5 Hz, H-11), 2.28 (2H, t, J = 7.5 Hz, H-2), 2.02 (4H, q, J = 6.9 Hz, H-8,14), 1.60 (2H, m, H-3), 1.30 (14H, m, H-4,5,6,7,15,16,17), 0.87 (3H, t, J = 6.8 Hz, H-18). $^{13}$C NMR (CDCl$_3$, 125 MHz) δ 174.3(s, C-1), 130.2(d, C-13), 130.1 (d, C-9), 128.1 (d, C-10), 127.9 (d, C-12), 51.4 (s, C-OCH$_3$), 34.1 (t, C-2), 31.6 (t, C-16), 29.7 (t, C-7), 29.4 (t, C-15), 29.2 (t, C-4), 29.2 (t, C-5), 29.1 (t, C-6), 27.3 (t, C-8), 27.2 (t, C-14), 25.7 (t, C-11), 25.0 (t, C-3), 22.6 (t, C-17), 14.1 (q, C-18).
phospholipase A₂ (14): \(^1\)H NMR (CDCl₃, 500 MHz) \(\delta\) 5.34 (4H, m, H-9',10',12',13'), 4.14 (2H, m, H-1), 3.91 (1H, m, H-2), 3.68 (1H, brd, \(J = 11.2\) Hz, H₆-3), 3.57 (1H, dd, \(J = 11.2, 5.8\) Hz, H₅-3), 2.76 (2H, t, \(J = 6.5\) Hz, H-11'), 2.33 (2H, t, \(J = 7.6\) Hz, H-2'), 2.04 (4H, dd, \(J = 13.7, 6.8\) Hz, H-8',14'), 1.60 (2H, m, H-3'), 1.30 (14H, m, H-4',5',6',7',15',16',17'), 0.88 (3H,dd, \(J = 8.6, 5.1\) Hz, H-18'). \(^{13}\)C NMR (CDCl₃, 125 MHz) \(\delta\) 174.5(s, C-1'), 130.4(d, C-13'), 130.1(d, C-9'), 128.2(d, C-10'), 128.0(d, C-12'), 70.3(d, C-2), 65.2(t, C-1), 63.4(t, C-3), 34.3(t, C-2'), 31.6(t, C-16'), 29.7(t, C-7'), 29.5(t, C-15'), 29.3(t, C-4'), 29.2(t, C-5'), 27.3(t, C-6'), 27.3(t, C-8'), 27.3(t, C-14'), 25.7(t, C-11'), 24.9(t, C-3'), 22.7(t, C-17'), 14.2(s, C-18').

methyl elaidate (15): \(^1\)H NMR (CDCl₃, 500 MHz) \(\delta\) 5.34 (2H, dt, \(J = 5.8, 3.9\) Hz, H-9,10), 3.66 (3H, s, -OCH₃), 2.29 (2H, t, \(J = 7.6\) Hz, H-2), 2.00 (4H, m, H-8,11), 1.61 (2H, m, H-3), 1.28 (20H, m, H-4,5,6,7,12,13,14,15,16,17), 0.87 (3H, t, \(J = 6.8\) Hz, H-18). \(^{13}\)C NMR (CDCl₃, 125 MHz) \(\delta\) 174.5(s, C-1), 130.1(d, C-10), 129.9(d, C-9), 51.6(s, C-OCH₃), 34.3(t, C-2), 32.1(t, C-16), 29.9 (t, C-6), 29.8 (t, C-13), 29.7(t, C-14), 29.5(t, C-7), 29.5(t, C-12)29.3 (t, C-5), 29.3(t, C-15), 29.2 (t, C-4), 27.4(t, C-8), 27.3(t, C-11), 25.1(t, C-3), 22.8(t, C-17), 14.3(q, C-18).

**Table S1.** Antitubercular activity of 1–15 (MIC, μM).

| Compounds | IC₅₀   | Compounds | IC₅₀   |
|-----------|--------|-----------|--------|
| 1         | > 50   | 9         | > 50   |
| 2         | 22.1   | 10        | > 50   |
| 3         | 2.44   | 11        | > 50   |
| 4         | 17.5   | 12        | > 50   |
| 5         | > 50   | 13        | > 50   |
| 6         | > 50   | 14        | > 50   |
| 7         | > 50   | 15        | > 50   |
| 8         | > 50   | INH       | 2.04   |

**Table S2.** Antiviral activity of 1–15 against H1N1, H3N2 and EV-71 viruses (IC₅₀, μM).

| Compounds | H1N1 | H3N2 | EV-71 |
|-----------|------|------|-------|
| 1         | > 50 | > 50 | > 50  |
| 2         | > 50 | > 50 | > 50  |
| 3         | > 50 | > 50 | > 50  |
| 4         | > 50 | > 50 | > 50  |
| 5         | > 50 | > 50 | > 50  |
|   | > 50 | > 50 | > 50 |
|---|------|------|------|
| 6 |      |      |      |
| 7 | > 50 | > 50 | 17.8 |
| 8 | > 50 | > 50 | > 50 |
| 9 | > 50 | > 50 | > 50 |
|10 | > 50 | > 50 | > 50 |
|11 | > 50 | > 50 | > 50 |
|12 | > 50 | > 50 | > 50 |
|13 | > 50 | > 50 | > 50 |
|14 | > 50 | > 50 | > 50 |
|15 | > 50 | > 50 | > 50 |