SUPPLEMENTARY MATERIAL

Antituberculosis compounds from a deep-sea-derived fungus *Aspergillus* sp. SCSIO Ind09F01

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Experimental

General experimental procedures

The NMR spectra were measured on a Bruker AC 500MHz NMR (Bruker, Fällanden, Switzerland) spectrometer using TMS as an internal standardand chemical shifts were recorded as δ-values. ESIMS were recorded on a Bruker micro TOF-QII mass spectrometer (Bruker, Fällanden, Switzerland). TLC and column chromatography (CC) were performed on plates precoated with silica gel GF254 (10–40 μm) and over silica gel (200–300 mesh) (Qingdao Marine Chemical Factory, China), and Sephadex LH-20 (Amersham Biosciences, Sweden), respectively. Spots were detected on TLC under 254 nm UV light or by heating after spraying with 5% H_2SO_4 in EtOH. All solvents used were of analytical grade (Tianjin Fuyu Chemical and Industry Factory). Semi-preparative HPLC was performed using an ODS column (YMC-pack ODS-A, Kyoto, Japan, 10 × 250 mm, 5 μm, 1.5 mL/min).

Fungal material

The fungal strain SCSIO Ind09F01 was isolated from the deep-sea sediment, which was collected from the Indian Ocean (Lat: 82.04513333' N, Long: 0.497883333' E) at the depth of 4530 m in 2013. The isolates were stored on MB agar (malt extract 15 g, sea salt 10 g and agar 15 g) slants at 4 °C and a voucher specimen was deposited in the CAS Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China. The fungus was identified using a molecular biological protocol by DNA amplification and sequencing of the ITS region (GenBank accession no: AY373869) as described previously (Tian et al. 2015) , the strain was identified as *Aspergillus* sp..

Fermentation and extraction

Aspergillus sp. SCSIO Ind09F01 was cultured on MB-agar plates at 25 °C for 7 days. The seed medium (malt extract 15g and sea salt 2.5g in 1.0 liter tap distilled water, pH 7.4–7.8) was inoculated with strain SCSIO Ind09F01 and incubated at 25 °C for 3 days on a rotating shaker (172 r.p.m.). Then large-scale fermentation of fungal isolate SCSIO Ind09F01 was incubated on a rotary shaker (172 rpm) at 27°C for 15 days in 1000 mL × 72 conical flasks containing the liquid medium (300

mL/flask) composed of mannitol 2.0%, maltose 2.0%, glucose 1.0%, corn steep liquor 0.1%, MSG 1.0%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.03%, yeast extract 0.3% and sea salt 1.5%, pH 7.4. The fermented whole broth (21.6 L) was harvested and extracted with acetone and then filtered through cheesecloth to separate it into filtrate and mycelia. The filtrate was concentrated under vacuum to remove the organic solvents and then extracted three times with EtOAc to give an EtOAc solution, while the mycelia were extracted three times with acetone. The acetone solution was evaporated under reduced pressure to afford an aqueous solution. Then the aqueous solution was extracted three times with EtOAc to produce another EtOAc solution. Both EtOAc solutions were combined and concentrated under vacuum to yeild a brown-colored EtOAc extract (30 g).

Purification

The crude EtOAc extract was subjected to silica gel column chromatography eluted with CH_2Cl_2/MeOH in a gradient eluent (v/v, 100:0, 98:2, 97:3, 95:5, 90:10, 80:20, 50:50) to obtain six fractions (fractions 1–6) based on TLC properties. Fraction 2 was applied to silica gel column chromatography eluted with CH_2Cl_2/MeOH (100:0→0:100) to give six subfractions. Fraction 2.2 was purified by further Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and then by semi-preparative reversed-phase HPLC to give compound **10** (28 mg). Fraction 2.3 was purified by semi-preparative reversed-phase HPLC to produce compounds **3** (20 mg), **6** (11 mg), **7** (22 mg) and **8** (21 mg). In addition, Fraction 2.4 was also applied to semi-preparative reversed-phase HPLC to produce compounds **11** (46 mg) and **12** (20 mg). Fraction 3 was applied to silica gel column chromatography eluted with CH_2Cl_2/MeOH (100:0→0:100) to give four subfractions. Fraction 3.2 was purified by semi-preparative reversed-phase HPLC to produce compounds **1** (8 mg), **2** (300 mg) and **4** (21 mg). Besides, Fraction 3.4 was subjected to semi-preparative reversed-phase HPLC to produce compounds **5** (11 mg) and **9** (16 mg).

Biological activity

Anti-tuberculosis was assayed (Chan et al. 2002; Changsen et al. 2003). Autoluminescent *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 ^{0}C. When the cultures reached an OD600 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 30 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 MIC_{50} value of 2.04 μM.

All the isolated compounds were evaluated against the three human tumor cell lines (K562, Huh-7and A549 cell lines) (Wang et al. 2015). Cell lines, K562, Huh-7 and A549 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_2 Water Jacketed Incubator with 5% CO_2 in air at 37 ^{0}C. Cell viability assay was determined by the CCK-8 (Dojindo, Japan) assay. Cells were seeded at a density of 400-800 cells per 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_{50} values using Prism 5.0 (GraphPad Software Inc., USA). Taxol was used as the positive control with IC_{50} values of 3.12, 6.75, and 1.92 nM, respectively.

The antibacterial activities against *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* were evaluated by an agar dilution method (Wang et al. 2015). The tested strains were cultivated in LB agar plates for bacteria at 37 ^{0}C. Compounds and positive control were dissolved in DMSO at different concentrations from 100 to 0.05 μg /mL by the continuous 2-fold dilution methods. A 5 μL quantity of test solution was absorbed by a paper disk (5 mm diameter) and placed on the assay plates.

After 24 h incubation, zones of inhibition (mm in diameter) were recorded. The minimum inhibitory concentrations (MICs) were defined as the lowest concentration at which no microbial growth could be observed. Ciprofloxacin lactate was used as positive control for *E. coli*, *Salmonella* and *S. aureus* with MIC values of 0.033, 0.054 and 0. 370 μM, respectively.

Compounds were evaluated for COX-2 inhibitory activity *in vitro* by using Cayman's COX Fluorescent Inhibitor Screening Assay Kit (Unsal-Tan et al. 2012) (Cayman Chemical Company, Ann Arbor, MI, USA). Ovine COX-1 and human recombinant COX-2 enzymes were pre-incubated with serially diluted test compounds for 15 min at RT, the heme and fluorometric substrate were added and incubated for another 15 min at RT. The reaction was started by the addition of arachidonic acid and allowed to proceed for 2 min. The fluorescence was measured at a 530 nm excitation wavelength and 595 nm emission wavelength using a micro plate reader (Envision, PerkinElmer), the data were analyzed using Graphpad Prism5 (GraphpadSoftware, Inc.). Celecoxib (Sigma, St. Louis, MO, USA) was used as the positive control.

The antiviral activity against H3N2 was evaluated by the CPE inhibition assay (Wang et al. 2015). Confluent MDCK cell monolayers were firstly incubated with influenza virus at 37 ^{0}C for 1 h. After removing the virus dilution, cells were maintained in infecting media (RPMI 1640, 4 μg/mL of trypsin) containing different concentrations of test compounds. After 48 h incubation at 37 ^{0}C, the cells were fixed with 100 μL 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_{50} was calculated as the compound concentration required inhibiting influenza virus yield at 48 h post-infection by 50%. Oseltamivir was used as the positive control with IC_{50} values of 36.8 nM.

The physicochemical data of the known compounds 1–12

Bisdethiobis(methylthio)-dehydrogliotoxin (**1)**: Pale yellow amorphous power; [α] $^{25}_{D}$= -124.9° (*c* = 0.153, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ_H 7.07 (1H, m, H-8), 6.81 (1H, m, H-9), 6.75 (1H, m, H-7), 4.29 (1H, d, *J* = 11.5Hz, H-3aa), 3.88 (2H, d, *J* = 11.5 Hz, H-3ab), 3.53(1H, d, *J* = 16.6 Hz, H-10a), 3.41(1H, d, *J* = 16.6 Hz, H-10b), 3.09 (3H, s, 2-NCH_3), 2.26 (3H, s, 3-SCH_3), 2.14 (3H, s, 10a-SCH_3). ^{13}C NMR (125 MHz, CD_3OD): δ_C 167.4 (qC, C-4), 166.0 (qC, C-1), 147.8 (qC, C-5a), 133.2 (qC, C-9a), 130.0 (CH, C-8), 118.6 (CH, C-7), 117.6 (CH, C-9), 101.3 (qC, C-6), 74.0 (qC, C-3), 72.9 (qC, C-10a), 64.7 (CH_2, C-3a), 40.6 (CH_2, C-10), 29.2 (2-NCH_3), 14.7 (10a-SCH_3), 13.6 (3-SCH_3).

Bisdethiobis-(methylthio)gliotoxin (**2**): Pale yellow oil; ESIMS *m*/*z* 357.1 $[M + H]^+$; $[\alpha]_D^{25}$ = –49.8° (*c* = 0.123, CH_3OH) ; ^{1}H NMR (500 MHz, CD_3OD): δ_H 5.99 (1H, m, H-9), 5.92 (1H, m, H-8), 5.69 (1H, d, *J* = 9.6Hz, H-7), 4.93 (1H, m, H-5a), 4.85 (1H, m, H-6), 4.27 (1H, d, *J* = 11.5Hz, H-15a), 3.88 (1H, d, *J* = 11.5Hz, H-15b), 3.14 (1H, d, *J* = 15.7 Hz, H-10a), 2.95 (1H, d, *J* = 15.7Hz, H-10b), 3.11 (3H, s, 13-NCH_3), 2.27 (3H, s, 14-SCH_3), 2.24 (3H, s, 12-SCH_3). ^{13}C NMR (125 MHz, CD_3OD): δ_C 168.2 (qC, C-4), 167.6 (qC, C-1), 133.8 (qC, C-9a), 130.3 (CH, C-8), 124.6 (CH, C-7), 120.6 (CH, C-9), 75.5 (CH, C-6), 74.1 (qC, C-3), 73.0 (qC, C-11), 70.3 (CH, C-5a), 64.4 (CH_2, C-15), 39.5 (CH_2, C-10), 28.9 (CH_3, C-13), 15.0 (CH_3, C-12), 13.4 (CH_3, C-14).

Gliotoxin (**3**): White amorphous power; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.98 (1H, brd, H-7), 5.93 (1H, brd, H-8), 5.77 (1H, brd, H-9), 5.85 (1H, s, 6-OH), 4.82 (1H, s, H-6), 4.82 (1H, s, H-5a), 4.42 (1H, s, H-6), 4.42 (1H, d, *J* = 12.8 Hz, H-3aa), 4.23 (1H, d, *J* = 12.8 Hz, H-3ab), 3.71 (1H, brd, *J* = 17.9 Hz, H-10a), 2.96 (1H, brd, *J* = 17.9Hz, H-10b), 3.20 (3H, s, 2-NCH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 166.1 (qC, C-1), 165.2 (qC, C-4), 130.9 (qC, C-9a), 129.9 (CH, C-7), 123.5 (CH, C-8), 120.3 (CH, C-9), 77.4 (qC, C-10a), 75.7 (qC, C-3), 73.2 (CH, C-6), 69.8 (CH, C-5a), 60.5 (CH_2 ,C-3a), 36.7 (CH_2, C-10), 27.7 (CH_3, 2-NMe).

Fumiquinazoline F (**4**)**:** Pale yellow amorphous power; $[\alpha]_D^{25}$ = -317.3° (*c* = 0.148, CH_3OH);^{1}H NMR (500 MHz, CD_3OD): δ_H 8.31 (1H, dd, *J* = 8.1, 1.1Hz, H-10), 7.84 (1H, dt, *J* = 8.0, 1.5Hz, H-8), 7.60 (1H, td, *J* = 8.1, 0.9Hz, H-9), 7.59 (1H, brd, *J* = 8.0

Hz, H-7), 7.31 (1H, brd, *J* = 8.0 Hz, H-24), 7.16 (1H, brd, *J* = 8.0 Hz, H-21)，7.02(1H, t, *J* = 7.0 Hz, H-22), 6.81 (1H, s, H-18), 6.72 (1H, t, *J* =7.0 Hz, H-23), 5.54 (1H, dd, *J* =5.5, 3.3 Hz, H-14), 3.66 (1H, dd, *J* =15, 5.5 Hz, H-15a), 3.62 (1H, dd, *J* = 15, 3..3 Hz, H-15b), 2.99 (1H, q, *J* = 6.6 Hz, H-3), 1.31 (3H, d, *J* = 6.6 Hz, H-16). ^{13}C NMR (125 MHz, CD_3OD): δ_C 171.0 (qC, C-1), 162.5 (qC, C-12), 153.5 (qC, C-4), 148.6 (qC, C-6), 137.8 (qC, C-20), 136.0 (CH, C-8), 128.7 (qH, C-11), 128.3 (CH, C-7), 128.2 (CH, C-10), 127.4 (CH, C-18), 125.1 (CH, C-23), 122.8 (CH, C-9), 121.3 (CH, C-25), 120.2 (CH, C-21), 118.8 (CH, C-22), 112.4 (CH, C-24), 109.2 (qH, C-17), 59.1 (CH, C-14), 50.2 (CH, C-3), 27.8 (CH_2, C-15), 18.6 (CH_3, C-16).

Fumiquinazoline A (**5**): Colorless needle crystals; $[\alpha]_D^{25}$ = –202.4° (*c* = 0.122, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ_H 8.07 (1H, m, H-10), 7.71 (1H, m, H-8), 7.60 (1H, m, H-7), 7.49 (1H, m, H-27), 7.41 (1H, m, H-24), 7.35 (1H, m, H-9), 7.23(1H, m, H-25), 7.09 (1H, m, H-26), 5.86 (1H, m, H-14), 5.31 (1H, s, H-18), 4.62 (1H, m, H-3), 4.12 (1H, m, H-20), 2.41 (1H, m, H-15a), 2.13 (1H, m, H-15b), 1.64 (3H, d, *J* =6.9 Hz, H-16), 1.21 (3H, d, *J* = 6.4 Hz, H-29). ^{13}C NMR (125 MHz, CD_3OD): δ_C 173.5 (qC, C-1), 172.4 (qC, C-21), 162.6 (qC, C-12), 153.7 (qC, C-4), 148.5 (qC, C-6), 140.9 (qC, C-28), 137.6 (qC, C-23), 135.7 (CH, C-8), 130.5 (CH, C-25), 128.5 (CH, C-10), 128.1 (CH, C-7), 127.6(CH, C-9), 126.5 (CH, C-26), 126.2(CH, C-27), 121.5 (qC, C-11), 115.7 (CH, C-24), 87.7 (CH, C-18), 81.1 (qC, C-17), 60.2 (CH, C-20), 54.6 (CH, C-14), 50.3 (CH, C-3), 36.9 (CH_2, C-15), 18.5 (CH_3, C-29), 16.8 (CH_3, C-16).

Fumiquinazoline C (**6**): White amorphous power; ^{1}H NMR (500 MHz, CD_3OD): δ_H 7.84 (1H, dd, *J* = 7.4, 1.7 Hz, H-7), 7.89 (1H, ddd, *J* = 7.4, 6.3, 1.7 Hz,H-8), 7.64 (1H, ddd, *J* =7.4, 6.3, 1.7 Hz,H-6), 8.27 (1H, dd, *J* = 7.4, 1.7 Hz, H-10), 5.54 (1H, dd, *J* = 10.9,6.0 Hz, H-14), 2.94 (1H, dd, *J* = 13.7, 10.9 Hz, H-15a), 2.05 (1H, dd, *J* = 13.7, 6.0 Hz, H-15b), 2.01 (3H, s, H-16), 5.27 (1H, s, H-18), 3.68 (1H, m, H-20), 7.36 (1H, dd, *J* = 7.4, 1.0 Hz, H-24), 7.32 (1H, td, *J* = 7.4, 1.0 Hz, H-25), 7.23 (1H, td, *J* = 7.4, 1.0 Hz, H-26), 7.34 (1H, dd, *J* = 7.4, 1.0 Hz, H-27), 1.01(1H, d, *J* = 6.9 Hz, H-29). ^{13}C NMR (125 MHz, CD_3OD): δ_C 173.6 (qC, C-1), 172.6 (qC, C-21), 161.6 (qC, C-12), 151.8 (qC, C-4), 148.2 (qC, C-6), 140.5 (qC, C-28), 136.8 (qC, C-23), 136.0

(CH, C-8), 130.9 (CH, C-25), 129.6 (CH, C-9), 129.4 (CH, C-7), 127.5 (CH, C-10), 127.4 (CH, C-26), 126.1 (CH, C-27), 122.6 (qC, C-11), 116.2 (CH, C-24), 89.0 (CH, C-18), 87.7 (qC, C-17), 85.4 (qC, C-3), 60.0 (CH, C-20), 52.9 (CH, C-14), 32.3 (CH_2, C-15), 24.4 (CH_3, C-16), 18.9 (CH_3, C-29).

Fumitremorgin B (**7**): White amorphous power; $[\alpha]_D^{25}$ = +9.28° (*c* = 0.125, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) : δ_H 5.95 (1H, d, *J* = 10.0 Hz, H-3), 4.45 (1H, m, H-6), 2.45 (1H, m, H-7a), 2.10 (1H, m, H-7b), 2.08 (1H, m, H-8a), 1.94 (1H, m, H-8b), 3.62 (2H, m, H-9), 5.76 (1H, s, H-13), 7.83 (1H, d, *J* = 8.7 Hz, H-16), 6.78 (1H, d, *J* = 8.7 Hz, H-17), 6.68 (1H, s, H-19), 4.53 (2H, d, *J* = 5.12 Hz, H-21), 5.02 (1H, br, H-22), 1.98 (3H, s, H-24), 1.69 (3H, s, H-25), 4.70 (1H, d, *J* = 10.0 Hz, H-26), 1.63 (3H, s, H-28), 1.84 (3H, s, H-29), 3.83 (3H, s, 30-Me), 4.09 (1H, s, 12-OH), 4.73 (1H, br, 13-OH). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 170.6 (qC, C-5), 166.4 (qC, C-11), 156.3 (qC, C-18), 138.0 (qC, C-20), 135.4 (qC, C-27), 134.7 (qC, C-23), 131.3 (qC, C-2), 123.1 (CH, C-26), 121.5 (CH, C-16), 120.6 (qC, C-15), 120.4 (CH, C-22), 109.4 (CH, C-17), 104.5 (qC, C-14), 94.0 (CH, C-19), 83.1 (qC, C-12), 69.1 (CH, C-19), 58.9 (CH, C-6), 55.8 (CH_3, C-30), 49.2 (CH, C-3), 45.4 (CH_2, C-9), 41.9 (CH_2, C-21), 29.1 (CH_2, C-7), 25.8 (CH_3, C-29), 25.6 (CH_3, C-25), 22.7 (CH_2, C-8), 18.5 (CH_3, C-28), 18.3 (CH_3, C-24).

Fumitremorgin C (**8**): White solid; $[\alpha]_D^{25}$ = -4.09° (c=0.122, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) : δ_H 8.10 (1H, s, 1-NH), 7.40 (1H, d, *J* = 8.6 Hz, H-16), 6.84 (1H, d, *J* = 1.9 Hz, H-19), 6.79 (1H, dd, *J* = 8.6, 2.0 Hz, H-17), 5.99 (1H, d, *J* = 9.5 Hz, H-3), 4.91 (1H, d, *J* = 9.5 Hz, H-21), 4.15 (1H, dd, *J* =11.5, 4.8 Hz, H-12), 4.08 (1H, t, *J* = 8.2 Hz, H-6), 3.81 (3H, s, 18-OCH3), 3.62 (2H, m, H-9), 3.51 (1H, dd, *J* = 15.9, 5.0 Hz, H-13), 3.09 (1H, dd, *J* = 15.7, 11.8 Hz, H-13), 2.38 (1H, m, H-7), 2.23 (1H, m, H-7), 2.02 (1H, m, H-8), 1.97 (3H, s, H-24), 1.92 (1H, m, H-8), 1.63 (3H, s, H-23). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 169.6 (qC, C-5), 165.8 (qC, C-11), 156.5 (qC, C-18), 137.1 (qC, C-20), 134.1 (qC, C-22), 132.3(qC, C-2), 124.2 (CH, C-21), 120.8 (qC, C-15), 118.9 (CH, C-16), 109.4 (CH, C-17), 106.2 (qC, C-14), 95.4 (CH, C-19), 59.3 (CH, C-6), 56.9 (CH, C-12), 55.85 (CH_3, 18-OCH_3), 51.1 (CH, C-3), 45.5 (CH_2, C-9), 28.7 (CH_2, C-7), 25.8 (CH_3, C-23), 23.1 (CH_2, C-8), 22.0 (CH_2, C-13), 18.2 (CH_3,

C-24).

Cyclotryprostatin B (**9**): Pale yellow amorphous power; $[\alpha]_D^{25}$ = +141.5° (*c* = 0.171, CH_3OH); ^1H NMR (500 MHz, CD_3OD): δ_H7.41 (1H, d, *J* = 8.5 Hz, H-16), 6.88 (1H, d, *J* = 2.0 Hz, H-19), 6.70 (1H, dd, *J* = 8.5, 2.0 Hz, H-17), 6.49 (1H, d, *J* = 9.7 Hz, H-3), 5.46 (1H, d, *J* = 9.8 Hz, H-21), 4.59 (1H, s, H-13), 4.26(1H, dd, *J* = 10.6, 6.4 Hz, H-6), 3.78 (1H, s,18-OCH_3), 3.71 (1H, m, H-9a), 3.56 (1H, m, H-9b), 3.36 (1H, s, 13-OCH_3), 2.41 (1H, m, H-7a), 2.06 (1H, m, H-8a), 2.01 (3H, s, H-24), 1.97 (1H, m, H-8b), 1.88 (1H, m, H-7b), 1.75 (3H, s, H-23). ^{13}C NMR (125 MHz, CD_3OD): δ_C 168.4 (qC, C-5), 167.6 (qC, C-11), 157.5 (qC, C-18), 138.7(qC, C-22), 138.1 (qC, C-20), 134.9 (qC, C-2), 125.2 (CH, C-21), 123.7 (qC, C-15), 119.2(CH, C-16), 110.5 (CH, C-17), 105.2 (qC, C-14), 96.0 (CH, C-19), 87.2 (qC, C-12), 77.3(CH, C-13), 60.6 (CH, C-6), 57.2 (13-OCH_3), 56.0 (18-OCH_3), 50.5 (CH, C-3), 46.4 (CH_2, C-9), 30.8 (CH_2, C-7), 26.2 (CH_3, C-23), 22.6 (CH_2, C-8), 18.3 (CH_3, C-24).

13-oxofumitremorgin B (**10**): Colorless needle crystalline sold; ^1H NMR (500 MHz, $CDCl_3$) : δ_H 4.71 (1H, d, *J* = 10.0 Hz, H-26), 5.03 (1H, m, H-22), 7.85 (1H,d, *J* = 8.7 Hz, H-16) 6.79 (1H, dd, *J* = 8.7, 2.25 Hz, H-17), 6.68 (1H, d, *J* = 2.15, H-19), 4.45 (1H, m, H-6), 3.83 (3H, s, 18-OMe), 5.99 (1H, d, *J* = 10.0 Hz, H-3), 3.62 (2H, m, H-9), 4.52 (2H, m, H-21), 2.46 (1H, m, H-7a), 2.09 (1H, m, H-7b), 2.06 (1H, m, H-8a), 1.94 (1H, m, H-8b), 1.98 (3H, s, H-24), 1.63 (3H, s, H-29), 1.84 (3H, s, H-28), 1.69 (3H, s, H-25). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 181.7 (qC, C-13), 173.2 (qC, C-5), 165.3 (qC, C-11), 157.8 (qC, C-18), 147.8 (qC, C-20), 139.1 (qC, C-23), 138.3 (qC, C-27), 136.4 (qC, C-2), 123.2 (CH, C-26), 122.3 (CH, C-22), 118.6 (CH, C-16), 118.4 (qC, C-15), 111.8 (CH, C-17), 108.3 (qC, C-14), 95.0 (CH, C-19), 82.0 (qC, C-12), 60.3 (CH, C-6), 55.7 (CH_3, 18-OMe), 48.8 (CH, C-3), 45.6 (CH_2, C-9), 42.8 (CH_2, C-21), 28.7 (CH_2, C-7), 25.7 (CH_3, C-24), 25.6 (CH_3, C-29), 23.3 (CH_2, C-8), 18.8 (CH_3, C-28), 18.4 (CH_3, C-25).

12,13-dihydroxy-fumitremorgin C (**11**): Pale yellow crystalline sold; ^1H NMR (500 MHz, $CDCl_3$) : δ_H 8.01 (1H, br.s, 1-NH),7.80 (1H, d, *J* = 8.7 Hz, H-16), 6.77 (1H, dd, *J* = 8.5, 2.2Hz, H-17), 6.78 (1H, d, *J* = 2.2 Hz, H-19), 5.85 (1H, dd, *J* = 9.5, 1.2 Hz, H-3), 5.73 (1H, dd, *J* = 2.8, 1.3 Hz, H-13), 4.76 (1H, dm, *J* = 9.5 Hz, H-21), 4.39 (1H,

dd, *J* = 9.1, 6.6 Hz, H-6), 3.78 (3H, s, 18-OCH_3). 3.58 (2H, m, H-9), 2.45, 2.04 (2H, m, H-7), 2.04, 1.96 (2H, m, H-8), 1.96 (3H, s, H-23), 1.64 (3H, s, H-24). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 171.1 (qC, C-11), 166.2 (qC, C-5), 156.6 (qC, C-18), 137.6 (qC, C-20), 134.7 (qC, C-22), 130.2 (qC, C-2), 123.9 (CH, C-21), 121.3 (CH, C-16), 120.7 (qC, C-15), 109.7 (CH, C-17), 105.4 (qC, C-14), 95.1 (CH, C-19), 83.2 (qC, C-12), 68.7 (CH, C-13), 58.8 (CH, C-3), 55.7 (CH_3, C-25), 50.2 (CH, C-6), 45.4 (CH_2, C-9), 29.2 (CH_2, C-7), 25.8 (CH_3, C-23), 22.6 (CH_2, C-8), 18.3 (CH_3, C-24).

Helvolic acid (**12**): Colorless needle crystalline sold; ESIMS *m*/*z* 567.5 $[M–H]^-$; ^{1}H NMR (500 MHz, $CDCl_3$) : δ_H 7.31 (1H, d, *J* = 10.2 Hz, H-1), 5.87 (1H, d, *J* = 10.2 Hz, H-2), 5.88 (1H, d, *J* = 8.3 Hz, H-16), 5.23 (1H, s, H-6), 5.10 (1H, t, *J* = 7.2 Hz, H-24), 2.77 (1H, dq, *J* = 12.5, 6.8 Hz, H-4), 2.62 (1H, m, H-9), 2.59 (1H, m, H-13), 2.48 (2H, m, H-22), 2.43 (1H,m, H-12a), 1.80 (1H, m, H-12b), 2.28 (1H, d, *J* = 12.5 Hz , H-5), 2.25(1H, m, H-15a), 1.90 (1H, m, H-15b), 2.14 (1H, m, H-23a), 2.10 (1H, m, H-23b), 2.11 (3H, s, 6-$OCOCH_3$), 1.98 (1H, m, H-11a), 1.57 (1H, m, H-11b), 1.94 (3H, s, 16-$OCOCH_3$), 1.69 (3H, s, H-27), 1.61 (3H, s, H-26), 1.44 (3H, s, H-19), 1.28 (3H, d, *J* = 6.8 Hz, H-28), 1.18 (3H, s, H-29), 0.92 (3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 208.8 (qC, C-7), 201.5 (qC, C-3), 174.2 (qC, C-21), 170.3 (qC, 16-O$\underline{C}OCH_3$), 169.0 (qC, 6- O$\underline{C}OCH_3$), 157.4 (CH, C-1), 147.9 (qC, C-17), 133.1(qC, C-25), 130.4 (qC, C-20), 127.9 (CH, C-2), 122.9 (CH, C-24), 73.9 (CH, C-6), 73.5 (CH, C-16), 52.8 (qC, C-8), 49.6 (CH, C-13), 47.3 (CH, C-5), 46.7 (qC, C-14), 41.8 (CH, C-9), 40.8 (CH_2, C-15), 40.5 (CH, C-4), 38.3 (qC, C-10), 28.7 (CH_2, C-22), 28.5 (CH_2, C-23), 27.7 (CH_3, C-22), 26.0 (CH_2, C-12), 25.9 (CH_3, C-27), 24.0 (CH_2, C-11), 20.9 (CH_3, 6-OCO$\underline{C}H_3$), 20.6 (CH_3, 16-OCO$\underline{C}H_3$), 18.4 (CH_3, C-29), 18.0 (CH_3, C-18), 17.9 (CH_3, C-26), 13.2 (CH_3, C-28).

Table S1. Anti-tuberculosis activities of **1**-**12** (MIC_{50}, μM)

Compounds	anti-tuberculosis	Compounds	anti-tuberculosis

1	> 50	**7**	> 50
2	> 50	**8**	> 50
3	< 0.03	**9**	> 50
4	> 50	**10**	> 50
5	> 50	**11**	2.41
6	> 50	**12**	0.0894
INH	2.04		

Table S2. Cytotoxicity activities of **1-12** (IC_{50}, μM)

Compounds	K562	A549	Huh-7
1	> 50	> 50	> 50
2	> 50	> 50	> 50
3	0.191	0.115	0.0954
4	> 50	> 50	> 50
5	> 50	> 50	> 50
6	> 50	> 50	> 50
7	> 50	> 50	> 50
8	> 50	> 50	> 50
9	> 50	> 50	> 50
10	> 50	> 50	> 50
11	> 50	> 50	> 50
12	> 50	> 50	> 50

Table S3. Antibacterial activities of **1-12** (MIC_{50}, μM)

Compounds	*Escherichia coli*	*Salmonella*	*Staphylococcus aureus*

1	> 50	> 50	> 50
2	> 50	> 50	> 50
3	>30	>30	32.6
4	> 50	> 50	> 50
5	> 50	> 50	> 50
6	> 50	> 50	> 50
7	> 50	> 50	> 50
8	> 50	> 50	> 50
9	> 50	> 50	> 50
10	> 50	> 50	> 50
11	> 50	> 50	> 50
12	>30	>30	1.85

Table S4. Anti-H3N2 virus and COX-2 inhibitory activities of **1**-**12** (IC_{50}, μM)

Compounds	Anti-H3N2	COX-2 inhibitory
1	> 50	> 50
2	> 50	> 50
3	> 50	> 50
4	> 50	> 50
5	> 50	> 50
6	> 50	> 50
7	> 50	> 50
8	> 50	> 50
9	> 50	> 50
10	> 50	> 50
11	> 50	> 50
12	> 50	> 50

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