Supporting Information

Naturally Occurring Anti-TB Agents: Isolation, Chemical Transformations and In Vitro Antitubercular Activities of Secondary Metabolites of Rhizomes of Alpinia galanga

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Materials and Methods

General

All melting points were recorded on a Büchi melting point apparatus in open capillaries and are uncorrected. Optical rotations were recorded on a JASCO P-1020 polarimeter. NMR spectra were recorded on Bruker AV200 (200 MHz for $^1$H NMR and 50 MHz for $^{13}$C NMR), AV400 (400 MHz for $^1$H NMR and 100 MHz for $^{13}$C NMR) and AV500 (500 MHz for $^1$H NMR and 125 MHz for $^{13}$C NMR) spectrometers using CDCl$_3$ as the solvent. Tetramethylsilane (0.00 ppm) served as an internal standard in $^1$H NMR and CDCl$_3$ (77.0 ppm) in $^{13}$C NMR, respectively. Chemical shifts are expressed in parts per million (ppm). Mass spectra were recorded on LC-MS/MS-TOF API QSTAR PULSAR spectrometer, and samples were introduced by an infusion method using the ESI. HRMS (ESI) of the samples was taken on Orbitrap (quadrupole plus ion trap) and TOF mass analyzers. Flash chromatography was performed with CombiFlash R$_f$ 200i with UV/VIS and ELSD (Isco Teledyne Inc.) using RediSep$^\text{®}$ as the column (SiO$_2$). All other chemicals and reagents were of analytical grade. Optical density was measured using a microplate reader (SpectraMax Plus 384 plate reader, Molecular Devices Inc.) at 470 nm.

Plant material
The rhizomes of *A. galanga* were collected and identified by Prof. Kornkanok Ingkainan, Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Thailand from Phitsanulok, Thailand in May 2008. A herbarium specimen (003566) is being maintained at Department of Biology, Faculty of Pharmaceutical Sciences, Naresuan University, Thailand.

**Extraction and isolation**

Air-dried and grounded rhizomes (1.150 kg) of *A. galanga* were extracted with acetone (5 × 2.0 L) at room temperature for 5 days. After completion of the extraction, the solvent was evaporated under reduced pressure to afford the acetone extract (27.9 g). The remaining plant material was re-extracted with MeOH (8 × 1.5 L) at room temperature and after completion of the extraction, the solvent was evaporated under reduced pressure to furnish the methanolic extract (82.6 g). A portion of the acetone extract (25.0 g) was fractionated on a SiO$_2$ (250 g, 100-200 mesh) column eluting with petroleum ether:ethyl acetate (0→10%) to furnish nine fractions (A-I). Fraction B (3.63 g) was flash chromatographed using a RediSep® column (SiO$_2$, 12 g) and eluted with petroleum ether:ethyl acetate (1→6%) to furnish 2-acetoxy-1,8-cineole (1, 16 mg). Fraction C (5.3 g) was chromatographed on silica gel column eluting with petroleum ether:ethyl acetate (1→6%) furnishing 12 subfractions (C1 to C12). These subfractions were further flash chromatographed using RediSep® column (SiO$_2$, 12 g) and eluted with petroleum ether:ethyl acetate (1→6%) afforded four compounds, 1'S-1'-acetoxychavicol acetate (2, 562 mg), *trans*-p-coumaryl diacetate (3) (140 mg), 1'S-1'-acetoxyeugenol acetate (4, 150 mg), and *trans*-coniferyl diacetate (5) (5 mg). Further, fraction D (312 mg) was flash chromatographed using RediSep® column (SiO$_2$, 12 g) and eluted with
petroleum ether:ethyl acetate (1–10%) to furnish compound 1’S-1’-hydroxychavicol acetate (6, 196 mg).

2-Acetoxy-1,8-cineole (1): pale yellow viscous liquid; \( R_t \) 0.43 (EtOAc-petroleum ether, 1:3); \([\alpha]_D^{25}\) +0.64 (c 0.5, CHCl\(_3\)); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta_H\): 5.32 (d, \(J = 9.8\) Hz, 1H), 2.23-2.17 (m, 1H), 2.07 (s, 3H), 1.96-1.88 (m, 1H), 1.86-1.79 (m, 1H), 1.74 (q, \(J = 3.2\) Hz, 1H), 1.71-1.63 (m, 1H), 1.54-1.47 (m, 1H), 1.42 (d, \(J = 2.7\) Hz, 1H), 1.29 (s, 3H), 1.27 (s, 3H), 1.08 (s, 3H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta_C\): 170.8, 73.3, 70.5, 69.4, 40.0, 37.1, 30.8, 28.7, 28.4, 26.9, 21.3, 14.8; ESI-MS: \(m/z\) 213.2 \([\text{M} + \text{H}]^+\); HRMS (ESI): calcd. for C\(_{12}\)H\(_{21}\)O\(_3\) \([\text{M} + \text{H}]^+\) 213.1485, found 213.1484.

1’S-1’-Acetoxychavicol acetate (2): viscous liquid; \( R_t \) 0.68 (EtOAc-petroleum ether, 1:3); \([\alpha]_D^{25}\) -47.7 (c 1, CHCl\(_3\)); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta_H\): 7.38-7.35 (d, \(J = 8.5\) Hz, 2H), 7.09-7.06 (d, \(J = 8.5\) Hz, 2H), 6.26 (d, \(J = 5.8\) Hz, 1H), 5.98 (ddd, \(J = 5.8, 10.6, 16.9\) Hz, 1H), 5.32-5.23 (m, 2H), 2.29 (s, 3H), 2.10 (s, 3H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta_C\): 169.9, 169.4, 150.4, 136.4, 136.0, 128.4, 121.7, 117.1, 75.5, 21.2, 21.1; ESI-MS: \(m/z\) 257.1 \([\text{M} + \text{Na}]^+\); HRMS (ESI): calcd. for C\(_{13}\)H\(_{14}\)O\(_4\)Na \([\text{M} + \text{Na}]^+\) 257.0784, found 257.0776.

trans-p-Coumaryl diacetate (3): viscous liquid; \( R_t \) 0.56 (EtOAc-petroleum ether, 1:3); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta_H\): 7.43-7.35 (d, \(J = 8.6\) Hz, 2H), 7.10-7.00 (d, \(J = 8.6\) Hz, 2H), 6.63 (d, \(J = 15.9\) Hz, 1H), 6.23 (dt, \(J = 6.4, 15.9\) Hz, 1H), 4.72 (d, \(J = 6.4\) Hz, 2H), 2.29 (s, 3H), 2.10 (s, 3H); \(^{13}\)C NMR (100 MHz, DCl\(_3\)) \(\delta_C\): 170.8, 169.4, 150.4, 134.0, 133.1, 127.6, 123.5, 121.8, 64.9, 21.1, 21.0; ESI-MS: \(m/z\) 257.0 \([\text{M} + \text{Na}]^+\); HRMS (ESI): calcd. for C\(_{13}\)H\(_{14}\)O\(_4\)Na \([\text{M} + \text{Na}]^+\) 257.0784, found 257.0774.

1’S-1’-Acetoxyeugenol acetate (4): viscous liquid; \( R_t \) 0.50 (EtOAc-petroleum ether, 1:3); \([\alpha]_D^{25}\) -26.8 (c 1, CHCl\(_3\)); \(^1\)H NMR (200 MHz, CDCl\(_3\)) \(\delta_H\): 7.06-6.89 (m, 3H), 6.25 (d, \(J = 5.6\) Hz, 1H), 6.09-5.88 (m, 1H), 5.41-5.19 (m, 2H), 3.83 (s, 3H), 2.31 (s, 3H), 2.11 (s, 3H); \(^{13}\)C NMR (50 MHz, CDCl\(_3\)) \(\delta_C\):
trans-Coniferyl diacetate (5): viscous liquid; \( R_f \) 0.42 (EtOAc-petroleum ether, 1:3); \(^1\)H NMR (400 MHz, CDCl\(_3\) \( \delta \): 7.03-6.93 (m, 3H), 6.62 (d, \( J = 15.7 \) Hz, 1H), 6.24 (dt, \( J = 6.4, 15.8 \) Hz, 1H), 4.72 (d, \( J = 6.4 \) Hz, 2H), 3.85 (s, 3 H), 2.31 (s, 3H), 2.11 (s, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\) \( \delta \): 170.9, 169.0, 151.1, 139.6, 135.3, 133.6, 123.5, 122.9, 119.4, 110.2, 64.9, 55.9, 21.0, 20.7; ESI-MS: \( m/z \) 287.1 \([\text{M + Na}]^+\); HRMS (ESI): calcd. for C\(_{14}\)H\(_{16}\)O\(_5\)Na \([\text{M + Na}]^+\) 287.0890, found 287.0881.

\( \alpha \)-S-\( \alpha \)-Hydroxychavicol acetate (6): viscous liquid; \( R_f \) 0.30 (EtOAc-petroleum ether, 1:3); \([\alpha]_D^{25} +0.30 \) (c 1, CHCl\(_3\) ); \(^1\)H NMR (400 MHz, CDCl\(_3\) \( \delta \): 7.40-7.33 (d, \( J = 8.3 \) Hz, 2H), 7.10-7.02 (d, \( J = 8.6 \) Hz, 2H), 6.00 (ddd, \( J = 6.1, 10.5, 16.9 \) Hz, 1H), 5.33 (d, \( J = 17.1 \) Hz, 1H), 5.21-5.14 (m, 2H), 2.28 (s, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\) \( \delta \): 169.6, 150.0, 140.2, 140.0, 127.5, 121.6, 115.3, 77.4, 77.1, 76.8, 74.7, 21.1; ESI-MS: \( m/z \) 215.0 C\(_{11}\)H\(_{12}\)O\(_3\)Na \([\text{M + Na}]^+\).

**Synthesis of compound 7**

A mixture of \( \alpha \)-S-\( \alpha \)-acetoxychavicol acetate (2; 40 mg, 0.5 mmol), Pd(OAc)\(_2\) (10 mol%), and ligand PPh\(_3\) (25 mg) in DMSO (2 mL) was stirred at room temperature for 5 min. Next, cyclohexanone (1.5 mmol, 3 equiv.) and pyrrolidine (30 mol%) were added and the reaction mixture was further stirred at room temperature for 3 h. After completion of the reaction (TLC), the reaction mixture was quenched with H\(_2\)O (5 mL) and was extracted with EtOAc (3 × 25 mL). The organic layers were pooled together and washed with brine solution (1 × 25 mL). The organic layer was dried over anhydrous Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. The crude reaction mixture was flash chromatographed using a RediSep\textsuperscript{®} column (SiO\(_2\), 12 g) and eluted with petroleum ether:ethyl acetate (0→10%) to furnish pure compound 7 as a viscous liquid (17 mg, 37%); \( R_f \) 0.30.
(EtOAc-petroleum ether, 1:4); \([\alpha]_D^{25} +0.23 \text{ (c 1, CHCl}_3\); \({}^1\)H NMR (200 MHz, CDCl\(_3\)) \(\delta_H\): 7.33 (d, \(J = 8.6\) Hz, 2H), 7.00 (d, \(J = 8.6\) Hz, 2H), 6.44-6.29 (m, 1H), 6.24-6.05 (m, 1H), 2.75-2.57 (m, 1H), 2.52-2.32 (m, 3H), 2.29 (s, 3H), 2.24-2.01 (m, 4H), 1.88 (dd, \(J = 3.4, 8.3\) Hz, 1H), 1.74-1.59 (m, 2H); \({}^{13}\)C NMR (50 MHz, CDCl\(_3\)) \(\delta_C\): 212.5, 169.6, 149.6, 135.4, 130.7, 128.7, 126.9, 121.6, 77.7, 77.0, 76.4, 50.7, 42.2, 33.6, 33.0, 27.9, 25.1, 21.2; ESI-MS: \(m/z\) 295.1 \([\text{M} + \text{Na}]^+\); HRMS (ESI): calcd. for C\(_{17}\)H\(_{20}\)O\(_3\)Na \([\text{M} + \text{Na}]^+\) 295.1305, found 295.1298.

**Synthesis of compound 8**

A stirred solution of 1'S-1'-acetoxychavicol acetate (2, 40 mg) was dissolved in dry CH\(_2\)Cl\(_2\) (2 mL) and degassed for 15 min. Then, Grubb's I\(^{\text{st}}\) generation catalyst (15 mol\%) was added to the reaction mixture and stirring was continued for a further 16 h at room temperature under an argon atmosphere. After the completion of reaction (TLC), the solvent was removed under reduced pressure. The crude reaction mixture was flash chromatographed using a RediSep\textsuperscript{®} column (SiO\(_2\), 12 g) and eluted with petroleum ether:ethyl acetate (0→20\%) to furnish pure homodimer 8 as a colorless solid (68 mg, 91\%); \(R_f\) 0.30 (EtOAc-petroleum ether, 3:7); m.p. 83-85°C; \([\alpha]_D^{25} -36.6 \text{ (c 1, CHCl}_3\); \({}^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta_H\): 7.34 (d, \(J = 8.7\) Hz, 4H), 7.07 (d, \(J = 8.2\) Hz, 4H), 6.27-6.32 (m, 2H), 5.90 (dd, \(J = 2.7, 1.4\) Hz, 2H), 2.29 (s, 6H), 2.09 (s, 6H); \({}^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta_C\): 169.9, 169.5, 150.6, 136.3, 130.6, 130.6, 128.6, 121.9, 74.4, 21.3, 21.2; ESI-MS: \(m/z\) 463.1 \([\text{M} + \text{Na}]^+\); HRMS (ESI): calcd for C\(_{24}\)H\(_{24}\)O\(_8\)Na \([\text{M} + \text{Na}]^+\) 463.1363, found 463.1351.

**Antitubercular assay using the XRMA protocol**

Crude extracts and pure compounds 1-8 were evaluated for their *in vitro* effects against the active and dormant phase of *M. tuberculosis* H37Ra (MTB) using the XRMA protocol [51]. *M.*
tuberculosis H37Ra (ATCC 25177) was obtained from MTCC, Chandigarh, India. MTB (ATCC No. 25177) were grown to the logarithmic phase (O.D. 1.0) in *M. phlei* medium. The stock culture was maintained at -70°C and subcultured once in *M. phlei* medium before inoculation into the experimental culture. All experiments were performed in triplicate, and IC$_{50}$ and MIC values were calculated from their dose-response curves. %Inhibition = 100 − (A$_1$ − blank)/(A$_2$ − blank) × 100, where A$_1$ is the culture absorbance at 470 nm in the presence of the compound after the addition of menadione, A$_2$ is the culture absorbance at 470 nm (DMSO solvent control) after the addition of menadione, and blank is the culture absorbance at 470 nm of the respective data points before the addition of XTT/menadione [51].

**XRMA protocol**

Activity against MTB was determined through the XTT reduction menadione assay (XRMA), reading absorbance at 470 nm as per the protocol [51]. A compound solution (2.5 μL) was added in a total volume of 250 μL of *M. phlei* medium consisting of the MTB, sealed with plate sealers, and allowed to incubate for 8 (active phase) and 12 (dormant phase) days at 37°C. The XRMA was then carried out to estimate viable cells present in different wells of the assay plate. To all wells, 200 μM XTT were added and incubated at 37°C for another 20 min. It was followed by the addition of 60 μM of menadione and incubated at 37°C for 40 min. The optical density was measured using a microplate reader (SpectraMax Plus 384 plate reader, Molecular Devices Inc.) at 470 nm filter against a blank prepared from a well free of cells. Absorbance obtained from the cells treated with 1% DMSO alone was considered 100% cell growth. The %Inhibition in the presence of test material is calculated by using the following formula: %inhibition = (Average of control-Average of compound)/ (Average of control-Average of blank) × 100, where control is culture medium with
cells and DMSO and blank are culture medium without cells. For all samples, each compound concentration was tested in triplicate in a single experiment and the quantitative value is expressed as the mean ± standard deviation (S.D.).
**Fig. 1S** $^1$H NMR (500 MHz, CDCl$_3$) of 1.

**Fig. 2S** $^{13}$C NMR (125 MHz, CDCl$_3$) of 1.
Fig. 3S DEPT (125 MHz, CDCl$_3$) of 1.

Fig. 4S LC-MS of 1.
**Fig. 5S** HR-MS of 1.

**Fig. 6S** ¹H NMR (500 MHz, CDCl₃) of 2.
Fig. 7S $^{13}$C NMR (125 MHz, CDCl$_3$) of 2.

Fig. 8S DEPT (125 MHz, CDCl$_3$) of 2.
**Fig. 9S** LC-MS of 2.

AGA-C-BC-1 #89

RT: 0.47

AV: 1

NL: 2.83E9

T: FTMS + p ESI Full ms [100.00-1500.00]

**Fig. 10S** HR-MS of 2.

Fig. 11S $^1$H NMR (400 MHz, CDCl$_3$) of 3.

Fig. 12S $^{13}$C NMR (100 MHz, CDCl$_3$) of 3.
Fig. 13S DEPT (100 MHz, CDCl$_3$) of 3.

Fig. 14S LC-MS of 3.
Fig. 15S HR-MS of 3.

Fig. 16S $^1$H NMR (200 MHz, CDCl$_3$) of 4.
Fig. 17S $^{13}$C NMR (50 MHz, CDCl$_3$) of 4.

Fig. 18S DEPT (50 MHz, CDCl$_3$) of 4.
Fig. 19S LC-MS of 4.

AGA-C-GH-1 #87 RT: 0.45 AV: 1 NL: 3.24E9
T: FTMS + p ESI Full ms [100.00-1500.00]

Fig. 20S HR-MS of 4.
Fig. 21S $^1$H NMR (400 MHz, CDCl$_3$) of 5.

Fig. 22S $^{13}$C NMR (100 MHz, CDCl$_3$) of 5.
Fig. 23S DEPT (100 MHz, CDCl₃) of 5.

Fig. 24S LC-MS of 5.
Fig. 25S HR-MS of 5.

Fig. 26S $^1$H NMR (500 MHz, CDCl$_3$) of 6.
Fig. 27S $^{13}$C NMR (125 MHz, CDCl$_3$) of 6.

Fig. 28S DEPT (125 MHz, CDCl$_3$) of 6.
Fig. 29S LC-MS of 6.

Fig. 30S $^1$H NMR (200 MHz, CDCl$_3$) of 7.
**Fig. 31S** $^{13}$C NMR (50 MHz, CDCl$_3$) of 7.

**Fig. 32S** DEPT (50 MHz, CDCl$_3$) of 7.
Fig. 33S LC-MS of 7.

TRV-01-06 #114 RT: 0.51 AV: 1 NL: 4.62E7
T: FTMS + p ESI Full ms [100.00-1500.00]

Fig. 34S HR-MS of 7.
Fig. 35S $^1$H NMR (400 MHz, CDCl$_3$) of 8.

Fig. 36S $^{13}$C NMR (100 MHz, CDCl$_3$) of 8.
Fig. 37S DEPT (50 MHz, CDCl₃) of 8.

Fig. 38S LC-MS of 8.
Fig. 39S HR-MS of 8.