Supporting Information

Seven New Secoiridoids with Anti-Hepatitis B Virus Activity from

*Swertia angustifolia*

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Supporting Information List

1. Characterization data of the new compounds 1–7

► $^1$H NMR, $^{13}$C NMR, DEPT, HSQC, HMBC, COSY, HRESIMS, IR, and UV spectra of swertianglide (1)
► $^1$H NMR, $^{13}$C NMR, DEPT, HSQC, HMBC, COSY, NOESY, HRESIMS, IR, and UV spectra of swertianoside A (2)
► $^1$H NMR, $^{13}$C NMR, DEPT, HSQC, HMBC, COSY, NOESY, HRESIMS, IR, and UV spectra of swertianoside B (3)
► $^1$H NMR, $^{13}$C NMR, DEPT, HSQC, HMBC, COSY, NOESY, HRESIMS, IR, and UV spectra of swertianoside C (4)
► $^1$H NMR, $^{13}$C NMR, DEPT, and HRESIMS spectra of swertianosides D-F (5–7)

2. The procedure for the anti-HBV assay

► Anti-HBV assay
► Assay for HBV antigens secretion
► Assay for HBV DNA replication
► Cytotoxicity assay
► Cell line and cell culture

3. Anti-HBV data of compounds 2, 4–6, and 8–17 from two independent experiments

For swertianglide (1):

**Spectrum 1** $^1$H NMR spectrum of swertianglide (1).
Spectrum 2 $^{13}$C NMR and DEPT spectrum of swertianglide (1).
Spectra 3 HSQC spectrum of swertianglide (1).
Spectra 4 HMBC spectrum of swertiaglde (1).
Spectra 5 COSY spectrum of swertiaglide (1).
Spectra 6 HRESIMS spectrum of swertianglide (1).
Spectra 7 UV and IR spectra of swertianglide (1).
For swertianoside A (2)

Spectra 8 $^1$H NMR spectrum of swertianoside A (2).
Spectra 9 $^{13}$C NMR and DEPT spectra of swertianoside A (2).
**Spectra 10** HSQC spectrum of swertianoside A (2).
Spectra 11 HMBC spectrum of swertianoside A (2).
Spectra 12 COSY spectrum of swertianoside A (2).
Spectra 13 ROESY spectrum of swertianoside A (2).
Spectra 14 HRESIMS data of swertianoside A (2).
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For swertianoside B (3):

Spectra 16 $^1$H NMR spectrum of swertianoside B (3).
Spectra 17 $^{13}$C NMR and DEPT spectra of swertianoside B (3).
Spectra 18 HMOC spectra of swertianoside B (3).
Spectra 19 HMBC spectra of swertianoside B (3).
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Spectra 23 IR and UV spectrum of swertianside B (3).
For swertianoside C (4):

Spectra 24 \(^1\)H NMR spectrum of swertianoside C (4).
Spectra 25 $^{13}$C NMR and DEPT spectra of swertianoside C (4).
Spectra 26 HMQC spectrum of swertianoside C (4).
Spectra 27 HMBC spectrum of swertianoside C (4).
Spectra 28 COSY spectrum of swertianoside C (4).
Spectra 29 ROESY spectrum of swertianoside C (4).
Spectra 30 HRESIMS data of swertianoside C (4).
Spectra 31 IR and UV data of swertianoside C (4).
For swertianoside D (5):

Spectra 32 $^1$H NMR spectrum of swertianoside D (5).
Spectra 33 $^{13}$C NMR and DEPT spectra of swertianoside D (5).
Spectra 34 HRESIMS data of swertianoside D (5).
For swertianoside E (6):

Spectra 35 $^1$H NMR spectrum of swertianoside E (6).
Spectra 36 $^{13}$C NMR and DEPT spectra of swertianoside E (6).
Spectra 37 HRESIMS data of swertianoside E (6).
For swertianoside F (7):

Spectra 38 $^1H$ NMR spectrum of swertianoside F (7).
Spectra 39 $^{13}$C NMR and DEPT spectra of swertianoside F (7).
Spectra 40 HRESIMS data of swertianoside F (7).
2. The procedure for the anti-HBV assay

**Anti-HBV assay**: Compounds 2, 4–6, and 8–17 used in this study were evaluated with the Hep G 2.2.15 cell line, which was stably transfected (Lipofectamine 2000 reagent; Invitrogen) with the HBV genome. The toxicity of the compounds was assayed by using a modified MTT (GIBCO Invitrogen) method. DMSO (GIBCO) alone was added to each culture as a solvent control. All the evaluated compounds were dissolved in DMSO for the anti-HBV activity assays. The concentration of DMSO in the culture was lower than 2.5 µL mL\(^{-1}\), so that the growth of cells was not affected.

**Assay for HBV antigens secretion**: The sub-toxic concentration of the identified compounds was measured with a serial dilution in 96-well microplates in which cells were seeded at a density of 3\times10^4 cells/mL and cultured at 37 °C, 5% CO\(_2\) for 12 days. After incubation, the cells and supernatants were collected. The levels of HBsAg and HBeAg in the supernatants were assayed with an ELISA (AutoBio Diagnostics Co., Ltd.) method. The absorbance (A) of each well was measured at 490 nm using a microplate reader (Model 680; Bio-Rad, Inc.).

**Assay for HBV DNA replication**: HepG 2.2.15 cells were seeded in 24-well culture plates at a density of 5\times10^5 cells/mL. After 2 days, culture medium was replaced with fresh medium supplemented with (or without) the tested compounds; this was repeated every other day for an additional 5 days. Cells were collected, and total DNA was isolated by using a TIANamp Gemomic DNA Kit (TIANGEN, Biotech Co., Ltd.) following the manufacturer’s instructions. The real-time PCR assay was used to detect the HBV DNA. Briefly, 10 µL of DNA sample was amplified in a 25 µL mixture containing 2×SYBR Green PCR Master Mix (Applied Biosystems) and 2 primers specific for HBV: a forward primer (HBV-t1: 5’-CAA GGA ACC TCT ATG TAT CCC TCC-3’) and reverse primer (HBV-t2: 5’-TCC GTC CGA AGG TTT GGT AC-3’) covering the 50-base pair insertion from 541 bp to 591 bp. Amplification and detection were performed in the Mastercycler Ep Realplex System (Eppendorf, Mastercycler Eprealplex) with incubation at 95 °C for 2 min and, subsequently, 40 three-step cycles (20 s at 95 °C; 15 s at 58 °C; 20 min at 72 °C) were performed. The standard was prepared by serial dilutions of a known amount of the cloned HBV plasmid pCP10, carrying two head-to-tail copies of the HBV genome as positive control, which was kindly provided by Professor J. Chen (No. 302 PLA Hospital, Beijing, PR China). The specificity of two primers (HBV-t1 and HBV-t2) was confirmed in every PCR run by dissociation curve analysis. An antiviral agent, Tenofovir (Jiangxi Chenyang Pharmaceutical Co. Ltd.) was used as a positive control.

**Cytotoxicity assay**: The toxicity of compounds 2, 4–6, and 8–17 was assayed by a modified MTT method. In brief, the samples were prepared at different concentrations. After Hep G 2.2.15 cells had been seeded in a 96-well microplate for 4 h, the samples (20 mL) were placed in each well and incubated for 3 days at 37 °C; then, 0.1 mL
MTT [3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide, 400 µg/mL] was added for 4 h. After removal of MTT medium, DMSO (100 µL/well) was added into the microplate for 10 min. The formazan crystals were dissolved, and the absorbance was measured on a microplate reader at 490 nm.

**Cell line and cell culture**: The widely used Hep G 2.2.15 cell line was applied for the assay of anti-HBV activities. In this study, Hep G 2.2.15 cells established from a hepatoma cell line Hep G 2 (ATCC) were cultured in RPMI-1640 (GIBCO) medium supplemented with 10% fetal calf serum (GIBCO), 100 µg/mL G148 (GIBCO), 100 IU/mL penicillin (GIBCO), and 100 IU/mL streptomycin (GIBCO). All cultures were maintained at 37 °C in a moist atmosphere containing 5% CO₂.
3. Anti-HBV activities of compounds 2, 4–6, and 8–17 from two independent experiments

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