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Anti-hepatitis B virus active constituents from *Swertia chirayita*

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ABSTRACT

Four new compounds swertiachiralatone A (1), swertiachoside A (2), swertiachirdiol A (3) and swertiachoside B (4), together with twenty-six known ones were isolated from the ethanol extract of *Swertia chirayita*. Their structures were elucidated by extensive spectroscopic analyses (1D- and 2D-NMR, HRESIMS, UV, IR and $[\alpha]_D$). All compounds were evaluated for anti-hepatitis B virus (anti-HBV) activities on HepG 2.2.15 cells line *in vitro*, of which compounds **14** and **19** showed inhibitory activity on hepatitis B surface antigen (HBsAg) secretion with IC_{50} values of 0.31 ± 0.045 and 1.49 ± 0.033 mM; compounds **14** and **28** exhibited activity against hepatitis B e antigen (HBeAg) secretion with IC_{50} values of 0.77 ± 0.076 and 5.92 ± 1.02 mM; and eight compounds (8,9,13,14,24–26,29) possessed activity against HBV DNA replication with IC_{50} values of 0.07–0.33 mM. In particular (+)-cycloolivil-4′-*O*-β-D-glucopyranoside (14) exhibited inhibition not only on the secretions of HBsAg and HBeAg with IC_{50} values of 0.31 ± 0.045 mM (SI = 4.29) and 0.77 ± 0.076 mM (SI = 1.75), respectively, but also on HBV DNA replication with an IC_{50} value of 0.29 ± 0.034 mM (SI = 4.66).

1. Introduction

Swertia chirayita belonging to the family Gentianaceae is distributed throughout the temperate Himalaya area (Kashmir to Bhutan, and Kashia hills) between 1200 and 3000 m above sea level. The whole plant is widely used by local people for the treatment of hepatitis, inflammation and digestive diseases [1]. Previous studies on this species led to the isolation of twenty-one xanthones, eleven triterpenoids, five secoiridoids, as well as eight other compounds including alkaloids, phenolic compounds and steroids [2–18]. Our previous investigation on *Swertia mileensis*, the congener plant of *S. chirayita*, resulted in a series of novel lactones with unprecedented structures and promising anti-hepatitis B virus (anti-HBV) activity [19–22]. According to our preliminary bioassay *in vitro*, the 50% EtOH–H_2O extract of *S. chirayita* could inhibit the secretions of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) with IC_{50} values of 1.08 ± 0.23 mg/mL (SI > 2.0) and 0.11 ± 0.031 mg/mL (SI > 19.6), respectively, as well as HBV DNA replication with an IC_{50} value of 0.17 ± 0.025 mg/mL (SI = 12.9). However, the components responsible for anti-HBV activity in *S. chirayita* were still unclear. In order to clarify the active constituents, bioassay-guided fractionation of the 50% EtOH–H_2O extract resulted in four new compounds (Fig. 1), swertiachiralatone A (1), swertiachoside A (2), swertiachirdiol A (3) and swertiachoside B (4), together with twenty-six known ones, isoorientin (5) [23], 2-*C*-β-D-glucopy-ranosyl-1, 3, 7-trihydroxyxanthane (6) [24], mangiferin (7) [25], 8-*O*-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-1, 7-dihydroxyl-3-methoxyxanthone (8) [26], 8-*O*-[β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl]-1-hydroxyl-3, 7-dimethoxy-xanthone (9) [27], 1-*O*-β-D-glucopyranosyl-3, 5, 8-trihydroxyxanthone (10) [28], 7-*O*-[β-D-xylopyranosyl-(1 → 2)-β-D-xylopyranosyl]-1, 8-dihydroxy-3-methoxyxanthone (11) [29], 1, 5, 8-trihydroxyl-3-methoxyxanthone (12) [30], 1-hydroxy-3, 7-

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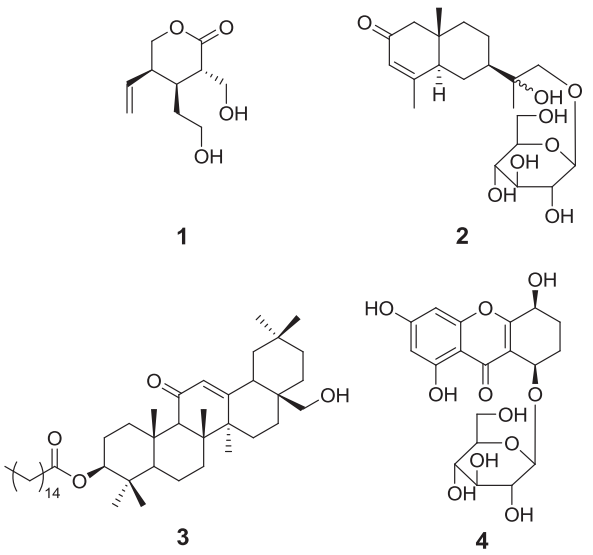

Fig. 1. Structures of compounds **1**–**4**.

dimethoxyxanthone (13) [31], (+)-cycloolivil-4′-*O*-β-D-glucopyranoside (14) [32], 8′-α-hydroxyl-lariciresinol-4-*O*-β-D-glucopyranoside (15) [33], 8′-α-hydroxyllariciresinol-4′-*O*-β-D-glucopyranoside (16) [34], 3, 4′-dihydroxy-3′-methoxypropiophenone 3-*O*-β-D-glucopyranoside (17) [35], *epi*-syringaresinol-4″-*O*-β-D-glucopyranoside (18) [36], syringaresinol 4″-*O*-β-D-glucopyranoside (19) [37], 6′-*O*-β-D-glucopyranosylgentiopicroside (20) [38], 6′-*O*-β-D-glucopyranosylsweroside (21), djalonenol (22) [39], swerbimalactone B (23), 3β-hydroxy-11-oxo-olean-12-enyl-3-palmitate (24) [40], erythrodiol-3-*O*-palmitate (25) [41], olean-12-ene-28-carboxy-3β-hexadecanoate (26) [42], cholest-4-en-3-one (27) [43], 3, 3′, 5-trihydroxybiphenyl (28) [44], bridelionoside B (29) [45], and (6*R*, 7*E*, 9*R*)-9-hydroxymegasigma-4, 7-dien-3-one-9-*O*-β-D-glucopyranoside (30) [46]. All the known compounds were isolated from this plant for the first time. Isolates **1**–**30** were evaluated for their anti-HBV activities on HepG 2.2.15 cells line *in vitro*, of which compounds **14** and **19** showed inhibitory activity on HBsAg secretion with IC_{50} values of 0.31 ± 0.045 and 1.49 ± 0.033 mM; compounds **14** and **28** showed activity against HBeAg secretion with IC_{50} values of 0.77 ± 0.076 and 5.92 ± 1.02 mM; and eight compounds (8,9,13,14,24–26,29) exhibited activity against HBV DNA replication with IC_{50} values of 0.07–0.33 mM. In particular (+)-cycloolivil-4′-*O*-β-D-glucopyranoside (14) exhibited inhibition not only on the secretions of HBsAg and HBeAg with IC_{50} values of 0.31 ± 0.045 mM (SI = 4.29) and 0.77 ± 0.076 mM (SI = 1.75), respectively, but also on HBV DNA replication with an IC_{50} value of 0.29 ± 0.034 mM (SI = 4.66).

2. Experimental

2.1. General experimental procedures

Mass spectra were run on LCMS-IT-TOF spectrometer (Shimadzu, Kyoto, Japan). Optical rotations were obtained on a JASCO model 1020 polarimeter (Horiba, Tokyo, Japan). UV spectra were taken on a Shimadzu UV-2401PC spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were measured on a Bio-Rad FTS-135 spectrometer (Bio-Rad, Hercules, California, USA) with KBr pellets. 1D and 2D-NMR spectra were recorded on Bruker AM-400 or DRX-500 spectrometers (Bruker, Bremerhaven, Germany). Silica gel (200–300 mesh) for column chromatography was obtained from Qingdao Makall Chemical Company (Makall, Qingdao, China). Semi-preparative HPLC was conducted on Waters Alliance 2695 liquid chromatography with a ZORBAX SB-C_{18} (5 μm, 9.4 × 250 mm) column (Agilent, USA). Sephadex LH-20 (20–150 μm, Pharmacia, Sweden) and ODS (45–70 μm, Merck, Germany) were used for column chromatography. Fractions were monitored by thin-layer chromatography (TLC), and spots were visualized by heating silica gel plates after sprayed with 10% H_2SO_4 in EtOH.

2.2. Plant material

The whole plant of *Swertia chirayita* (Roxb. ex Fleming) H. Karst. was collected in Nepal, in September 2008 and identified by Prof. Ju-Le Wang, Medical School of Tibet University. A voucher specimen (No. 2008-11-30) was deposited at the Laboratory of Antivirus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The air-dried and powdered whole plants of *S. chirayita* (5 kg) were extracted with 50% EtOH–H_2O (50 L) at room temperature for 2 times, 24 h for each time. The combined EtOH extracts were concentrated *in vacuo* to yield a brown-yellow gum (1.47 kg), and suspended in H_2O (6 L). The suspension was partitioned with petroleum ether (PE) (5 L × 3), EtOAc (5 L × 3) and *n*-butanol (5 L × 3), successively. The PE part (A, 50 g) was subjected to a silica gel column (7 × 35 cm, 400 g) eluted with H_2O–MeOH–$CHCl_3$ (0:0:100, 0:5:95, 1:10:90, 2:20:80, 4:40:60, 5:50:50, *v*/*v*, each 1 L). The collected fractions were combined based on their TLC characteristics to yield 7 fractions (Frs. A1–A7). Fr. A2 (3.8 g) was further separated into six subfractions (Frs. A2-1–A2-6), by chromatography over a silica gel column (3 × 33 cm, 100 g) using EtOAc–PE (0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 500 mL each) as the eluent. Fr. A2-4 (1.8 g) was applied to a silica gel column (3 × 28 cm, 80 g, Me_2CO–PE, 1:100, 800 mL), to give three subfractions (Frs. A2-4-1–A2-4-3). Fr. A2-4-3 (293 mg) was purified by a column chromatography (CC) over silica gel (2 × 31 cm, 30 g) with the elution of EtOAc–PE (5:95, 500 mL) yielding **9** (40 mg), **22** (30 mg) and **23** (15 mg). Fr. A2-5 (1.2 g) was subjected to a silica gel CC (2 × 30 cm, 40 g; EtOAc–PE, 5:95–50:50 gradient, 1 L) to afford three fractions (Frs. A2-5-1–A2-5-3). Compound **8** (69 mg) was obtained from Fr. A2-5-3 (260 mg) by a silica gel CC (2 × 32 cm, 30 g) using EtOAc–PE (25:75, 800 mL) as the eluent. Fr. A4 (4 g) was chromatographed on a silica gel column (3 × 40 cm, 100 g; EtOAc–PE, 5:95–50:50 gradient, 2 L), to obtain 5 subfractions (Frs. A4-1–A4-5). Fr. A4-5 (1 g) was subjected to silica gel CC (2 × 29 cm, 35 g) eluted with Me_2CO–PE (2:98, 500 mL) to give compounds **10** (600 mg), **24** (200 mg), **25** (80 mg) and **26** (50 mg). Fr. A7 (7.8 g) was subjected to a silica gel CC (4 × 23 cm, 120 g) using EtOAc–PE (5:95–50:50, each 1 L) as the eluent and further purified by a sephadex LH-20 column

(1.3 × 135 cm, 53 g) eluted with MeOH to give compounds **4** (20 mg), **27** (20 mg) and **28** (30 mg).

The EtOAc part (B, 185 g) was chromatographed on a silica gel column (10 × 34 cm, 1000 g) eluted with H_2O–MeOH–$CHCl_3$ (0:5:95, 1:10:90, 2:20:80, 4:40:60, 1 L each) to yield six subfractions (Fr. B1–B6). Fr. B2 (1.2 g) was subjected to a silica gel column (2 × 30 cm, 30 g) eluted with MeOH–$CHCl_3$ (1:200, 800 mL) to give compound **11** (20 mg) and Fr. B2-2 (600 mg). Compounds **13** (10 mg), **20** (15 mg), **21** (20 mg) and Fr. B2-2-2 (200 mg) were obtained from Fr. B2-2 by a silica gel CC (2 × 25 cm, 20 g) using EtOAc–PE (30:70, 500 mL) as the eluent. Fr. B2-2-2 was chromatographed on a silica gel CC (2 × 23 cm, 18 g, Me_2CO–PE, 20:80, 500 mL) to yield compound **12** (50 mg). Fr. B3 (1.6 g) was further divided into three subfractions (Frs. B3-1–B3-3), by a silica gel CC (2 × 35 cm, 35 g) using Me_2CO–PE (20:80, 1 L) as the eluent. Fr. B3-3 was subjected to a silica gel CC (2 × 30 cm, 30 g) eluted with H_2O–CH_3OH–$CHCl_3$ (1:10:90, 800 mL) and further purified over a sephadex LH-20 CC (1.3 × 135 cm, 53 g) with an isocratic solvent system of MeOH–$CHCl_3$ (50:50) to yield compounds **5** (70 mg), **17** (5 mg) and **19** (20 mg). Fr. B4 (11 g) was chromatographed on a silica gel column (4 × 39 cm, 200 g) successively eluted with H_2O–MeOH–$CHCl_3$ system (0:5:95, 1:10:90, 2:20:80, 4:40:60, each 1 L), to yield four sub-fractions (Fr. B4-1–B4-5). Fr. B4-2 (1 g) was chromatographed on a silica gel CC (2 × 30 cm, 30 g, EtOAc-PE, 10:90, 1.2 L) to yield compounds **6** (7 mg), **14** (15 mg), **15** (30 mg) and **16** (20 mg). Fr. B4-3 (700 mg) was purified by HPLC on a Rp-18 column (9.4 × 250 mm) eluted with MeOH–H_2O (30:70, 800 mL) to obtain compounds **2** (20 mg), **18** (15 mg) and **30** (40 mg). Fr. B5 (8 g) was subjected to silica gel CC (4 × 25 cm, 130 g) with the eluent of MeOH–EtOAc (10:90, 1 L) to yield compounds **4** (80 mg), **7** (40 mg) and **29** (40 mg).

2.3.1. Swertiachiralatone A (1)

Colorless gum; $[\alpha_D^{24}]$: +24.0 (MeOH, c = 0.12,); IR (KBr): ν_{max} = 3417, 1712, 1639, 1476, and 1405 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; positive HRESIMS: m/z = 223.0921 (calcd for $C_{10}H_{16}O_4Na$, $[M + Na]^+$, −2.0 mDa).

2.3.2. Swertiachoside A (2)

Colorless gum; $[\alpha_D^{24}]$: −24.9 (MeOH, c = 0.25,); UV (MeOH): λ_{max} (log ε) = 240 (4.8) and 194 (2.5) nm; IR (KBr): ν_{max} = 3423, 1650, 1436, 1411, 1380, 1349, 1316, 1076, and 1039 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; positive HRESIMS: m/z = 415.2343 (calcd for $C_{21}H_{35}O_8$, $[M + H]^+$, 1.7 mDa).

2.3.3. Swertiachirdiol A (3)

White powder, $[\alpha_D^{24}]$: +43.9 ($CHCl_3$, c = 0.07); UV (MeOH): λ_{max} (log ε) = 250 (4.0) nm; IR (KBr): ν_{max} =3348, 1700, 1653, 1466, 1387, and 1364 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 3; positive HRESIMS: m/z = 717.5763 (calcd for $C_{46}H_{78}O_4Na$, $[M + Na]^+$, −2.9 mDa).

2.3.4. Swertiachoside B (4)

Light yellow powder, $[\alpha_D^{24}]$: +10.5 (MeOH, c = 0.11); UV (MeOH): λ_{max} (log ε) = 298 (3.6), 259 (4.1), and 207 (4.2) nm; IR (KBr): ν_{max} = 3440, 1653, 1644, 1633, 1627, 1471, 1464, 1456, 1419, 1386, 1375, 1167, 1096, 1070, and 1038 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 4; positive HRESIMS: m/z = 429.1162 (calcd for $C_{19}H_{25}O_{11}$, $[M + H]^+$, 3.2 mDa).

*2.4. Acid hydrolysis of compounds **2** and **4***

Compounds **2** (2 mg) and **4** (2 mg) was each refluxed with 2 M HCl (5 mL) at 80 °C for 5 h. After neutralization with $NaHCO_3$ and extraction with CH_2Cl_2, the aqueous layer was concentrated and detected by TLC over silica gel (H_2O–MeOH–$CHCl_3$, 4:40:60). The presence of glucose was confirmed by comparison with authentic samples (R_f 0.40). The glucose hydrolyzed from compounds **2** and **4** was purified by Si CC and identified to be D-glucose based on its $[\alpha]_D$ value ($[\alpha_D^{25}]$: +45.5, c 0.16, H_2O; $[\alpha_D^{25}]$: +55.7, c 0.14, H_2O, respectively) [47].

2.5. Anti-HBV assay on HepG 2.2.15 cell line in vitro

The anti-HBV assay was performed according to our previous report [22], with tenofovir (Jiangxi Chenyang Pharmaceutial Co. Ltd, China, purity > 97.6%) as the positive control.

3. Results and discussion

3.1. Structural elucidation

Compound **1** colorless gum, had a molecular formula of $C_{10}H_{16}O_4$ based on positive HRESIMS (223.0921 $[M + Na]^+$, calcd. 223.0941) indicating 3° of unsaturation. The IR absorptional bands at 3417, 1712 and 1639 cm^{-1} suggested the existence of hydroxyl, carbonyl and olefinic groups. The ^1H NMR spectrum (Table 1) displayed signals ascribed to one ethenyl unit [δ_H 5.90 (1H, m, H-7), 5.17 (2H, m, H-9)], three oxygenated methylenes [δ_H 4.59 (1H, dd, J = 10.5, 3.0 Hz, H-11α) and 4.10 (1H, dd, J = 10.5, 3.0 Hz, H-11β); δ_H 4.48 (1H, dd, J = 10.8, 1.8 Hz, H-6α), and 4.31 (1H, dd, J = 10.5, 3.1 Hz, H-6β); δ_H 3.91 (2H, m, H-10)]. Its ^{13}C NMR (DEPT) spectrum (Table 1) revealed the presence of ten carbons including five CH_2, four CH and one quaternary carbon, of which one terminal double bond [δ_C 134.6, (C-7), 118.7 (C-9)] and one carbonyl group [δ_C 172.9 (C-2)] were characterized. From the above analyses, two of the three degrees of unsaturation

Table 1
NMR data of swertiachiralatone A (1) in C_5D_5N (500 Hz for ^1H and 125 Hz for ^{13}C).

Position	δ_C	δ_H (J in Hz)	Position	δ_C	δ_H (J in Hz)
2	172.9 (s)	–	8α	34.7 (t) 1.71, tt, 17.2, 8.6	1.72 (td, 13.1, 6.8)
3	48.9 (d)	2.66 (td, 9.3, 2.7)	8β		1.91 (td, 13.1, 6.8)
4	33.9 (d)	2.98 (tt, 9.2, 4.6)	9	118.7 (t)	5.17 (m)
5	39.5 (d)	2.84 (dd, 7.2, 3.3)	10	59.5 (t)	3.91 (m)
6α	72.6 (t)	4.48 (dd, 10.8, 1.8)	11α	61.7 (t)	4.59 (dd, 10.5, 3.0)
6β		4.31 (dd, 10.8, 3.1)	11β		4.10 (dd, 10.5, 3.0)
7	134.6 (d)	5.90 (m)			

Table 2
NMR data of swertiachoside A (2) in CD_3OD (500 Hz for ^1H and 100 Hz for ^{13}C).

Position	δ_C	δ_H (*J* in Hz)	Position	δ_C	δ_H (*J* in Hz)
1α	55.2 (d)	2.24 (d, 16.0)	11	75.1 (s)	–
1β		2.15 (d, 16.0)	12α	76.9 (t)	3.97 (d, 10.0)
2	202.3 (s)	–	12β		3.34 (d, 10.0)
3	127.0 (d)	5.85 (s)	13	20.5 (q)	1.11 (s)
4	167.9 (s)	–	14	17.0 (q)	0.85 (s)
5	49.0 (d)	2.42 (d, 12.3)	15	22.3 (q)	1.96 (s)
6α	24.1 (t)	2.15 (overlapped)	1′	105.0 (d)	4.25 (d, 8.0)
6β		1.23 (d, 12.6)	2′	75.2 (d)	3.20 (m)
7	45.7 (d)	1.81 (tt, 12.0, 2.5)	3′	77.9 (d)	3.34 (m)
8α	23.0 (t)	1.62 (d, 12.2)	4′	71.6 (d)	3.26 (m)
8β		1.36 (d, 12.9)	5′	78.0 (d)	3.29 (m)
9α	41.0 (t)	1.53 (d, 12.6)	6′α	62.7 (t)	3.84 (d, 11.5)
9β		1.44 (m)	6′β		3.63 (d, 11.5)
10	38.7 (s)	–			

were deduced, and the leaving one unsaturation requires one ring contained in the structure. A δ-lactone moiety was constructed from the ^1H–^1H COSY correlations of H-3/H-4/H-5/H-6 and HMBC correlations form H-6 to C-2 and from H-4 to C-2. The ^1H–^1H COSY correlations of H-5/H-7, H-4/H-8, and H-3/H-11 suggested the direct linkages of C-5 with C-7, C-4 with C-8, and C-3 with C-11. In the ROESY spectrum, the cross-peaks of H-4 with H-5 and H-11, and H-3 and H-7 with H-8 suggested the α-orientation of H-4 and H-5 and β-orientation of H-3 (Fig. 2). Thus, the structure of compound **1** was characterized as (3*S*, 4*S*, 5*R*)-4-(2-hydroxyethyl)-3-(hydroxymethyl)-5-vinyltetrahydro-2*H*-pyran-2-one and named as swertiachiralatone A (1).

Compound **2** was deduced with the molecular formula $C_{21}H_{34}O_8$ by HRESIMS (*m/z* 415.2343 $[M + H]^+$, calcd. 415.2326). The IR spectrum showed the presence of hydroxyl (3423 cm^{-1}), double bond (1650 cm^{-1}) and glycosidic linkage (1076 cm^{-1}) in the structure. The ^{13}C NMR (DEPT) spectrum exhibited 21 carbons, including 4 quaternary carbons, 9 methines, 5 methylenes and 3 methyl groups. Its NMR spectral data (Table 2) were similar to those of isopterocarpolone [48], except that one methyl in isopterocarpolone was changed to be O-substituted methylene [δ_C 76.9 (C-12)] in **2**, together with an additional glucosyl moiety [δ_C 105.0 (C-1′), 75.2 (C-2′), 77.9 (C-3′), 71.6 (C-4′), 78.0 (C-5′), 62.7 (C-6′)]. Hydrolysis of compound **2** yielded D-glucose which was identified by comparing with the authentic sample on TLC and $[\alpha]_D$ experiment ($[\alpha_D^{25}]$: +45.5, *c* 0.16, H_2O). In the HMBC spectrum, the correlations from H-12 to C-1′ and from H-1′ to C-12 suggested the glycosidation at C-12. The relative stereocenters of C-5, C-7 and C-10 were deduced to be identical with those of isopterocarpolone based on the correlations of H-14 with H-1β, H-5 with H-1α and H-7, and H-8β with H-12 in the ROESY spectrum (Fig. 2). Similar to the previously reported atractyloside I [49], the stereochemistry of C-11 was not determined according to the current experimental data. Up to now, only a few cases of sesquiterpenoids with the 11, 12-dihydroxylation pattern were isolated from natural sources, which faced the common problem of the configuration of C-11. Therefore, the structure of **2** was determined as

Table 3
NMR data of swertiachirdiol A (3) in $CDCl_3$ (400 Hz for ^1H and 100 Hz for ^{13}C).

Position	δ_C	δ_H (*J* in Hz)	Position	δ_C	δ_H (*J* in Hz)
1α	38.8 (t)	2.75 (dt, 11.0, 2.8)	20	31.1 (s)	–
1β		1.01 (dt, 11.0, 2.8)	21	31.9 (t)	–
2	25.2 (t)	1.63 (overlapped)	22	30.7 (t)	1.42 (overlapped)
3	80.3 (d)	4.49 (dd, 11.7, 4.6)	23	28.1 (q)	0.87 (overlapped)
4	38.1 (s)	–	24	16.4 (q)	1.15 (s)
5	55.1 (d)	0.80 (overlapped)	25	16.8 (q)	0.87 (overlapped)
6	17.4 (t)	1.42 (overlapped)	26	18.6 (q)	1.10 (s)
7	32.6 (t)	–	27	23.4 (q)	1.38 (s)
8	43.4 (s)	–	28α	69.7 (t)	3.45 (d, 10.9) 3.22, d, 12.0
9	61.7 (d)	2.35 (s)	28β		3.19 (d, 10.9) 3.22, d, 12.0
10	37.0 (s)	–	29	33.0 (q)	0.92 (s)
11	199.9 (s)	–	30	23.4 (q)	0.87 (overlapped)
12	128.3 (d)	5.57 (s)	1′	173.7 (s)	–
13	169.4 (s)	–	2′	34.9 (t)	2.27 (t, 7.5)
14	45.5 (s)	–	3′	33.8 (t)	–
15	25.8 (t)	1.63 (overlapped)	4′–13′	29.7–29.2 (t)	–
16	21.5 (t)	–	14′	23.6 (t)	–
17	36.9 (s)	–	15′	22.7 (t)	1.94 (m)
18	42.7 (d)	2.13 (dd, 10.5, 3.0)	16′	14.2 (q)	0.87 (overlapped)
19	44.9 (t)	1.72 (d, 13.5)			

Table 4
NMR data of swertiachoside B (4) in CD_3OD (400 Hz for ^1H and 400 Hz for ^{13}C).

Position	δ_C	δ_H (J in Hz)	Position	δ_C	δ_H (J in Hz)
1	163.4 (s)	–	8a	117.8 (s)	–
2	100.2 (d)	6.17 (d, 2.0)	8b	105.3 (s)	–
3	166.2 (s)	–	9	183.0 (s)	–
4	95.1 (d)	6.35 (d, 2.0)	1′	105.2 (d)	4.68 (d, 7.6)
4a	159.3 (s)	–	2′	71.7 (d)	3.19 (dd, 12.0, 2.0)
4b	168.4 (s)	–	3′	78.0 (d)	–
5	67.6 (d)	4.58 (dd, 9.5, 7.0)	4′	75.8 (d)	–
6	27.4 (t)	2.06 (m)	5′	78.2 (d)	–
7α	27.9 (t)	2.29 (dq, 13.3, 3.0)	6′α	62.9 (t)	3.88 (dd, 12.0, 2.0)
7β		1.76 (t, 13.3)	6′β		3.69 (dd, 12.0, 2.0)
8	71.3 (d)	4.94 (overlapped)			

isopterocarpolone 12-O-β-D-glucopyranoside and named as swertiachoside A (2).

Compound **3** had a molecular formula of $C_{46}H_{78}O_4$ from HRESIMS at m/z 717.5763 $[M + Na]^+$ (calcd for 717.5792). Its IR spectrum showed absorptional bands at 3448 and 1653 cm^{-1} ascribed to hydroxyl and carbonyl functionalities. Its ^1H NMR spectrum (Table 3) displayed signals of several methyl groups and one axial proton [$\delta_H = 4.49$ (1H, dd, $J =$ 11.7, 4.6 Hz, H-3)]. The ^{13}C NMR (DEPT) data showed 46 carbon signals ascribed to 8 methyls [δ_C 28.1 (C-23), 16.4 (C-24), 16.8 (C-25), 18.6 (C-26), 23.4 (C-27), 33.0 (C-29), 23.4 (C-30), 14.2 (C-16′)], 24 methylenes, 5 methines and 9 quaternary carbon atoms, from which one olean-type triterpene motif and one long-chain fatty acid moiety were easily recognized. The ^1H

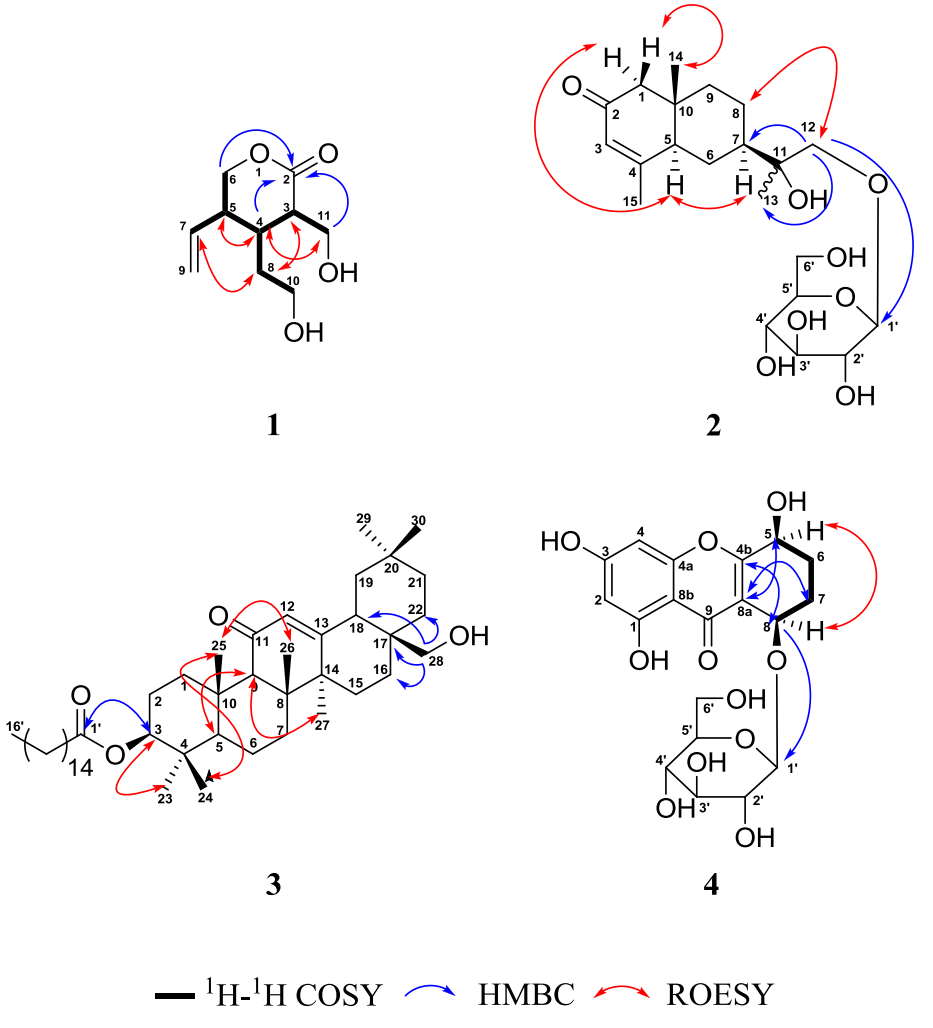

Fig. 2. Key 2D NMR correlations of compounds **1**–**4**.

and ^{13}C NMR (DEPT) data of compound **3** were almost identical to those of 3β-hydroxy-11-oxo-olean-12-enyl-3-palmitate (24) [40], except that one methyl (C-28) in **24** was changed to hydroxymethyl group [δ_C 69.7 (C-28)] in **3**, which was supported by the HMBC correlations of H-28 with C-16, C-17, C-18 and C-22 (Fig. 2). The palmityloxy group was proposed at C-3 position from the HMBC correlation of H-3 to C-1′. The H-3 was determined to be an α-orientation from the splitting and coupling constants (*J*) of the proton signal at 4.49 (dd, $J = 11.7$, 4.6 Hz), and the ROESY correlation of H-3 with H-23. Similarly, the ROESY correlations of H-25 with H-24 and H-26, H-9 with H-5 and H-27 indicated the α-orientation of H-5, H-9 and Me-27 and the β-orientation of Me-24, Me-25 and Me-26. Consequently, the structure of compound **3** was determined as 3β, 28-dihydroxy-11-oxo-olean-12-enyl-3-palmitate and named as swertiachirdiol A (3).

Compound **4** possessed a molecular formula of $C_{19}H_{22}O_{11}$ determined by HRESIMS at *m/z* 425.1081 $[M - H]^+$ (calcd for 425.1084). The IR spectrum suggested the presence of hydroxyl (3440 cm^{-1}) and hydrogen bonded ketone (1653 cm^{-1}) groups. Hydrolysis of compound **4** with 2 M HCl in methanol to yield D-glucose which was identified by comparing with the authentic sample on TLC, as well as the $[\alpha]_D$ experiment ($[\alpha_D^{25}]$: +55.7, *c* 0.14, H_2O). Its ^1H and ^{13}C NMR (DEPT) spectral data (Table 2) were similar to those of tetrahydroswertianolin [50], except for the absence of the methoxy group at C-3, from which compound **4** was proposed to be the 3-O-demethylated derivative of tetrahydroswertianolin. The full NMR data assignments of compound **4** were performed with the aid of the HSQC, ^1H–^1H COSY and HMBC data (Fig. 2). In the HMBC spectrum, the correlation of H-1′ with C-8 indicated that the glucosyl group was attached to C-8. The cross-peaks of H-5 with H-8 in the ROESY spectrum suggested that H-5 and H-8 were located at the same side. Its absolute configuration was determined to be identical with tetrahydroswertianolin by their similar $[\alpha]_D$ values (tetrahydroswertianolin: $[\alpha_D^{24}]$: +8.0, *c* 0.2, MeOH; compound **4**: $[\alpha_D^{24}]$: +10.5, *c* 0.11, MeOH). Hence, compound **4** was defined to be 3-nortetrahydroswertianolin and named as swertiachoside B (4) as shown in Fig. 1.

3.2. In vitro anti-HBV activity

Compounds **1**–**30** was evaluated for their anti-HBV activity on HepG 2.2.15 cells *in vitro*, namely inhibiting the secretions of HBsAg and HBeAg, and HBV DNA replication using tenofovir as the positive control [22]. As shown in Table 5, compounds **10**, **14**, **17**, **19** showed activity inhibiting HBsAg secretion; compounds **14** and **28** showed activity inhibiting HBeAg

Table 5
Anti-HBV activities of compounds **1**–**30**[a].

Compounds	CC_{50} [mM]	HBsAg[b]		HBeAg[c]		DNA[d]	
		IC_{50} [mM]	SI[e]	IC_{50} [mM]	SI	IC_{50} [mM]	SI
1	>4.75	>4.75	–	>4.75	–	>1.19	–
2	>2.04	>2.04	–	>2.04	–	>0.51	–
3	>1.88	>1.88	–	>1.88	–	>0.47	–
4	>2.49	>2.49	–	>2.49	–	>0.62	–
5	3.43 ± 0.55	>4.14	–	>4.14	–	>1.03	–
6	1.34 ± 0.12	>2.15	–	>2.15	–	>0.54	–
7	>3.03	>3.03	–	>3.03	–	>0.76	–
8	>1.81	>1.81	–	>1.81	–	0.30 ± 0.051	>6.03
9	1.38 ± 0.21	>2.56	–	>2.56	–	0.070 ± 0.010	19.71
10	0.02 ± 0.0038	0.40 ± 0.035	<1	>1.47	–	>0.47	–
11	>0.88	>0.88	–	>0.88	–	>0.22	–
12	4.25 ± 0.55	>4.53	–	>4.53	–	>1.13	–
13	>1.98	>1.98	–	>1.98	–	0.16 ± 0.021	>12.38
14	1.35 ± 0.18	0.31 ± 0.045	4.29	0.77 ± 0.076	1.75	0.29 ± 0.034	4.66
15	>3.41	>3.41	–	>3.41	–	>0.85	–
16	>3.79	>3.79	–	>3.79	–	>0.95	–
17	0.30 ± 0.043	1.48 ± 0.23	0.20	>2.25	–	0.33 ± 0.055	<1
18	>3.04	>3.04	–	>3.04	–	>0.76	–
19	1.83 ± 0.36	1.49 ± 0.33	1.23	>2.43	–	>0.61	–
20	2.73 ± 0.44	>3.20	–	>3.20	–	>0.80	–
21	>3.28	>3.28	–	>3.28	–	>0.82	–
22	>10.49	>10.49	–	>10.49	–	>2.62	–
23	>1.67	>1.67	–	>1.67	–	>0.42	–
24	>0.65	>0.65	–	>0.65	–	0.22 ± 0.045	>4.57
25	>1.19	>1.19	–	>1.19	–	0.13 ± 0.021	>5.07
26	>1.00	>1.00	–	>1.00	–	0.24 ± 0.045	>4.85
27	>3.22	>3.22	–	>3.22	–	>0.80	–
28	>7.18	>7.18	–	5.92 ± 1.02	>1.21	>1.80	–
29	0.66 ± 0.076	>1.45	–	>1.45	–	0.22 ± 0.037	2.97
30	>2.68	>2.68	–	>2.68	–	>0.67	–
Tenofovir[f]	>1.74	1.25 ± 0.025	>1.39	1.21 ± 0.031	>1.44	0.0011 ± 0.00043	>1581.82

[a] All values are the mean of two independent experiments.
[b] HBsAg: HBV surface antigen.
[c] HBeAg: HBV e antigen.
[d] DNA: HBV DNA replication.
[e] CC_{50} = 50% cytotoxic concentration, IC_{50} = 50% inhibition concentration, SI (selectivity index) = CC_{50} / IC_{50}.
[f] Tenofovir, an antiviral agent used as a positive control.

secretion; and compounds **8**, **9**, **13**, **14**, **17**, **24**–**26** and **29** exhibited activity inhibiting HBV DNA replication. In particular, compound **13** displayed obviously inhibition on HBV DNA replication with IC_{50} value of 0.16 ± 0.021 mM, with high SI value of >12.38. (+)-Cycloolivil-4′-*O*-β-D-glucopyranoside (14) exhibited inhibition not only on the secretions of HBsAg and HBeAg with IC_{50} values of 0.31 ± 0.045 mM (SI = 4.29) and 0.77 ± 0.076 mM (SI = 1.75), respectively, but also on HBV DNA replication with an IC_{50} value of 0.29 ± 0.034 mM (SI = 4.66).

4. Conclusions

Four new compounds, involving one unusual eudesmane sesquiterpene and one tetrahydroxanthone, together with twenty-six known compounds were firstly isolated from *S. chirayita*. Eleven compounds showed anti-HBV activities on HepG 2.2.15 cells line *in vitro* (four compounds showed activity inhibiting HBsAg secretion, two compounds showed activity inhibiting HBeAg secretion, and nine compounds exhibited activity inhibiting HBV DNA replication). This work will provide valuable information for revealing the anti-HBV active constituents of *S. chirayita*.

5. Statement

The authors declare no conflict of interest.

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Appendix A. Supporting information

1D and 2D NMR, HREIMS/HRESIMS, IR, UV, $[\alpha]_D$ spectra of compounds **1**–**4** are available as Supporting information. Supplementary data related to this article can be found online at doi: http://dx.doi.org/10.1016/j.fitote.2014.11.011

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