



ORIGINAL ARTICLE

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Silvaticusins A–D: *ent*-kaurane diterpenoids and a cyclobutane-containing *ent*-kaurane dimer from *Isodon silvaticus*

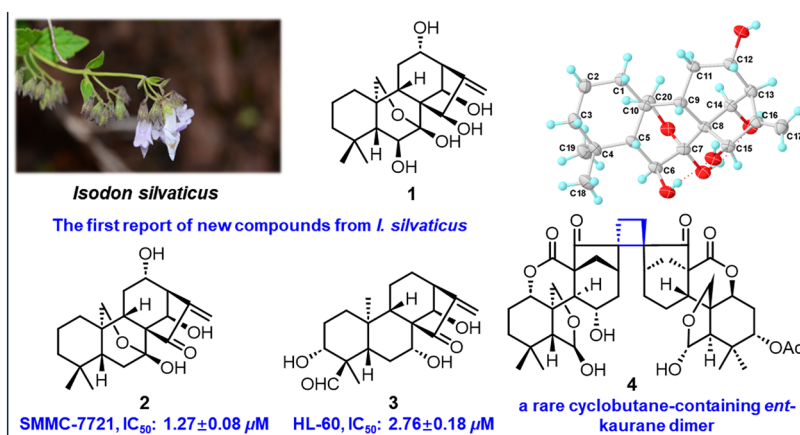
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Abstract

Three new *ent*-kaurane diterpenoids, silvaticusins A–C (**1**–**3**), along with a new *ent*-kaurane dimer silvaticusin D (**4**) were isolated from the aerial parts of *Isodon silvaticus*. The structures of these new compounds were established mainly by comprehensive analysis of their NMR and MS data. The absolute configuration of compounds **1** and **4** were determined using a single-crystal X-ray diffraction and computational methods, respectively. Compounds **2** and **3** were found to exhibit remarkable cytotoxic effects against five human tumor cell lines (HL-60, A-549, SMMC-7721, MDA-MB-231, and SW-480), with IC₅₀ values spanning from 1.27 ± 0.08 to 7.52 ± 0.33 μM.

Keywords *Isodon silvaticus*, *ent*-Kaurane diterpenoid, *ent*-Kaurane dimer, Cyclobutane moiety, Cytotoxicity

Graphical Abstract



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1 Introduction

Isodon species, highly esteemed for their medicinal virtues, harbor a rich array of diterpenoids, with *ent*-kauranoids being the most prominent class [1, 2]. The recent discovery of labdane-based meroditerpenoids with cyclobutane moieties, including isoscopariusins B and C [3], along with clerodane-based dimers scopiosins A and B [4], and scoparicacids A–C [5] from *I. scoparius*, further highlights the chemical versatility of these plants. Furthermore, the bioactivities of these compounds are particularly notable. For example, eriocalyxin B, derived from *I. eriocalyx* var. *laxiflora*, has recently been found to demonstrate significant therapeutic potential in oncology by effectively inhibiting cell migration in triple-negative breast cancer [6].

In a concerted effort to uncover natural products with distinctive structures and potent bioactivities from the genus *Isodon*, a comprehensive phytochemical study was conducted on *Isodon silvaticus* collected from Changdu in the Tibet Autonomous Region. To date, there has been a scarcity of information regarding the chemical composition of *I. silvaticus*, except that a reported synthetic derivative acetyl-macrocalin B [7] has been isolated from this plant. This compound has undergone an in-depth pharmacological assessment, revealing its remarkable capacity to inhibit the growth of non-small cell lung cancer

and esophageal squamous cell carcinoma in both in vitro and in vivo models [8, 9]. The current study resulted in the isolation of silvaticusins A–D (1–4) (Fig. 1), involving two new C-20-oxygenated-*ent*-kaurane diterpenoids (1 and 2), a new C-20 non-oxygenated-*ent*-kaurane diterpenoid (3), and a new dimeric diterpenoid (4) characterized by a cyclobutane moiety formed through a [2+2] cycloaddition between two 6,7-seco-*ent*-kaurane diterpenoids. Meanwhile, 37 miscellaneous known diterpenoids were characterized from the same plant material (Fig. S44 and Table S13). This study presents the first characterization of new compounds from *I. silvaticus*. Notably, the discovery of silvaticusin D (4) enriches the limited array of *ent*-kaurane dimers with a cyclobutane ring. This group now includes maoecrystal M (7,20-epoxy-*ent*-kaurane dimer) [10], bisjaponins A and B (6,7-seco-*ent*-kaurane dimer, 6,7-seco-/7,20-epoxy-*ent*-kaurane dimer, respectively) [11], and bistenuifolin L and M (C-20 non-oxygenated *ent*-kaurane dimers) [12]. The cyclobutane moiety in these compounds, formed through [2+2] cycloaddition, is a key structural feature that may confer unique biological properties and synthetic challenges, underscoring their importance in the field of natural product chemistry [13, 14]. The structures of silvaticusins A–D (1–4) were determined through a combinatorial use of spectroscopic analysis, X-ray diffraction, and quantum

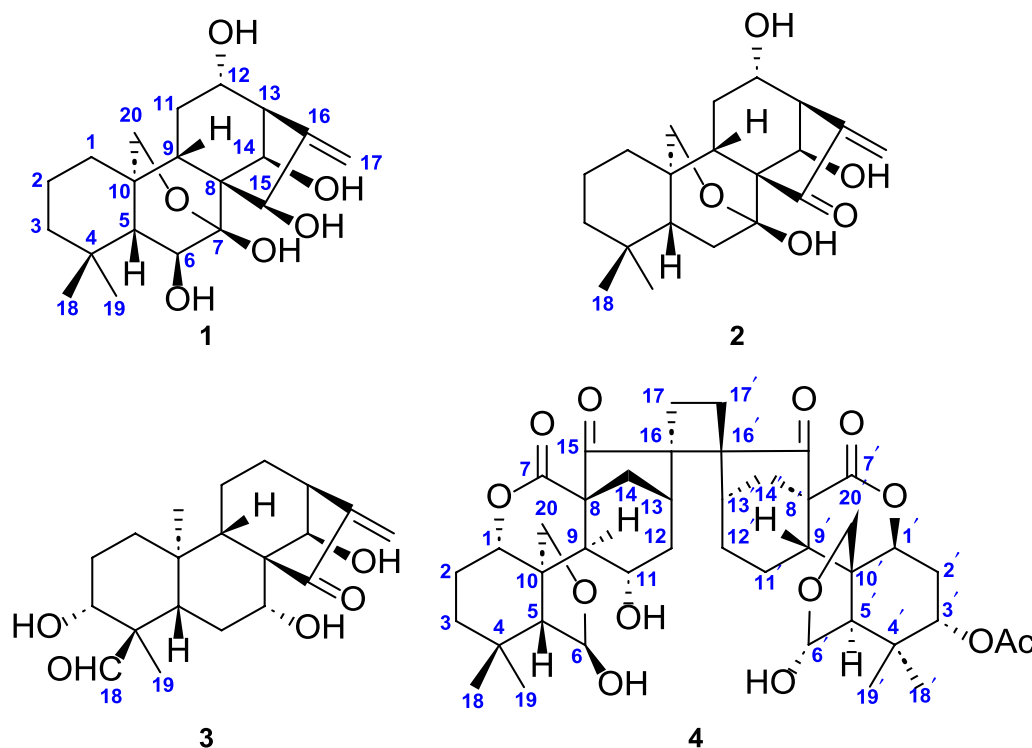


Fig. 1 Chemical structures of silvaticusins A–D (1–4)

chemical calculations. Notably, compounds **2** and **3** were found to exhibit remarkable cytotoxic activities against HL-60, A-549, SMMC-7721, MDA-MB-231, and SW-480 human tumor cell lines with IC_{50} ranging from 1.27 ± 0.08 to $7.52 \pm 0.33 \mu\text{M}$. Herein, the structure elucidation and bioactivity evaluation of silvaticusins A–D (**1–4**) were reported.

2 Results and discussion

Silvaticusin A (**1**), which was obtained as colorless crystals, had the molecular formula $\text{C}_{20}\text{H}_{30}\text{O}_6$ as inferred from the (+)-HRESIMS ion at m/z 389.1936 $[\text{M}+\text{Na}]^+$ (calcd 389.1535) and supported by ^{13}C NMR data. The IR spectrum exhibited characteristic absorption bands indicative of hydroxy (3391 cm^{-1}), and olefinic (1633 cm^{-1}) functionalities. Its ^1H NMR spectrum showed resonances of two methyl groups at δ_{H} 1.05 (s) and 1.15 (s), four oxygenated methines at δ_{H} 4.21 (d, $J=5.3 \text{ Hz}$), 4.32 (t, $J=8.2 \text{ Hz}$), 5.34 (br s), and 5.72 (br s), and two olefinic protons at δ_{H} 5.36 (d, $J=1.9 \text{ Hz}$) and 5.74 (d, $J=1.9 \text{ Hz}$) (Table 1). Its ^{13}C NMR and DEPT spectra featured twenty carbon resonances, including two methyl groups, six methylenes (one olefinic, one oxygenated), seven methines (four oxygenated), five non-protonated carbons (three quaternary) (Table 2). Comprehensive analysis of the 1D and 2D NMR data of **1** manifested that its structure resembled that of the 7,20-epoxy-*ent*-kauranoid, enmenol [15]. The $\text{H}_2\text{-1}/\text{H}_2\text{-2}/\text{H}_2\text{-3}$, $\text{H-5}/\text{H-6}$, and $\text{H-9}/\text{H}_{12}\text{-11}/\text{H-13}/\text{H-14}$ correlations in the ^1H – ^1H COSY spectrum, in tandem with the $\text{H}_2\text{-1}/\text{C-20}$, $\text{H-6}/\text{C-4}$, $\text{H-6}/\text{C-7}$, $\text{H-6}/\text{C-8}$ correlations in the HMBC spectrum (Fig. 2) indicated that silvaticusin A (**1**) possesses a hydroxyl group at the C-12 position, differing from the C-1 hydroxylation in enmenol, while the rest of their structures were consistent.

As for the configurational determination of **1**, assigning H-5 as β -oriented, the $^3J_{\text{H-5}/\text{H-6}}$ (5.3 Hz) indicated that H-6 was α -oriented. The $\text{H-9}/\text{H-5}\beta$, $\text{H-12}/\text{H-9}$, $\text{H}_3\text{-18}/\text{H-5}\beta$, $\text{H-20b}/\text{H}_3\text{-19}$, and $\text{H-14}/\text{H-20a}$ correlations in the ROESY spectrum indicated that H-9, H-12, HO-14 adopted β orientation, while C-20 adopted α orientation. But the orientation of HO-15 could not be conclusively assigned. Fortunately, well-formed crystals of compound **1** were successfully procured using methanol and subsequently underwent X-ray crystallographic analysis. The refined Cu $K\alpha$ data yielded a Flack parameter of $-0.13(15)$, which enabled the determination of its absolute configuration as $5R$, $6S$, $7R$, $9R$, $10R$, $12S$, $13R$, $14R$, and $15R$ (Fig. 3). Thus, the structure of silvaticusin A (**1**) was defined as $6\beta,7\beta,12\alpha,14\beta,15\beta$ -pentahydroxy-7,20-epoxy-*ent*-kaur-16-ene.

Silvaticusin B (**2**), which was obtained as a white amorphous powder, had the molecular formula $\text{C}_{20}\text{H}_{28}\text{O}_5$ as

established by the (+)-HRESIMS ion at m/z 349.2010 $[\text{M}+\text{H}]^+$ (calcd 349.2010). The ^1H and ^{13}C NMR data of compound **2** (Tables 1 and 2) were similar to those of **1**. The ^1H – ^1H COSY spectrum exhibited correlations of $\text{H}_2\text{-1}/\text{H}_2\text{-2}/\text{H}_2\text{-3}$, $\text{H-5}/\text{H}_2\text{-6}$, $\text{H-9}/\text{H}_2\text{-11}/\text{H-12}$, and $\text{H-13}/\text{H-14}$ (Fig. 2). And the HMBC spectrum displayed correlations from H-5 to C-6, C-7, C-10, and C-19, from H-13 to C-8, C-12, C-14, C-15, C-16 and C-17. These findings demonstrated that compound **2** features a carbonyl at C-15 instead of a hydroxy group as in **1**, and a methylene at C-6 instead of the oxygenated methine in **1**. Analysis of the ROESY spectrum of **2** uncovered the β orientations of H-12, HO-14. Thus, the structure of silvaticusin B (**2**) was assigned as $7\beta,12\alpha,14\beta$ -trihydroxy-7,20-epoxy-*ent*-kaur-16-en-15-one.

Silvaticusin C (**3**) was isolated as a white amorphous powder. The molecular formula $\text{C}_{20}\text{H}_{28}\text{O}_5$ was deduced from HREIMS and ^{13}C NMR data. A thorough examination of the ^1H and ^{13}C NMR data for compound **3** revealed that its structure bore a close resemblance to that of the C-20 non-oxygenated-*ent*-kaurane

Table 1 ^1H NMR spectroscopic data of silvaticusins A–C (**1–3**) (δ in ppm, J in Hz)

No	1 ^a	2 ^a	3 ^b
1a	0.89, m	0.82, m	1.93, overlap
1b	1.27, m	1.15 d, (13.5)	1.65, overlap
2a	1.08, m	1.26, m	1.92, overlap
2b	1.31, m	1.26, m	1.92, overlap
3a	1.23, m	1.09, m	4.04, m
3b	1.23, m	1.34, m	–
5	1.56, d, (5.3)	1.41, dd, (11.7, 6.9)	1.59, br d, (12.4)
6a	4.21, d, (5.3)	2.04, m	2.16, q, (12.4)
6b	–	3.57, m	1.74, m
7	–	–	4.80, dt, (12.4, 4.7)
9	2.53, dd, (15.3, 6.3)	1.56, m	1.42, overlap
11a	1.79, m	1.99, m	1.43, overlap
11b	1.95, m	1.99, m	1.52, m
12a	4.32, t, (8.2)	4.26, t, (8.2)	1.66, overlap
12b	–	–	0.83, m
13	3.18, br s	3.52, br s	3.23, br s
14	5.34, br s	5.59, br s	5.09, br s
15	5.72, br s	–	–
17a	5.36, d, (1.9)	5.49, s	6.30, s
17b	5.74, d, (1.9)	6.38, s	5.39, s
18	1.15, s	1.04, s	9.5, s
19	1.05, s	0.77, s	1.39, s
20a	4.07, d, (9.6)	4.09, d, (10.0)	1.07, s
20b	4.12, d, (9.6)	4.31, d, (10.0)	–

^a Recorded on a 500 MHz NMR spectrometer in pyridine- d_5 . ^b Recorded on an 800 MHz NMR spectrometer in pyridine- d_5 .

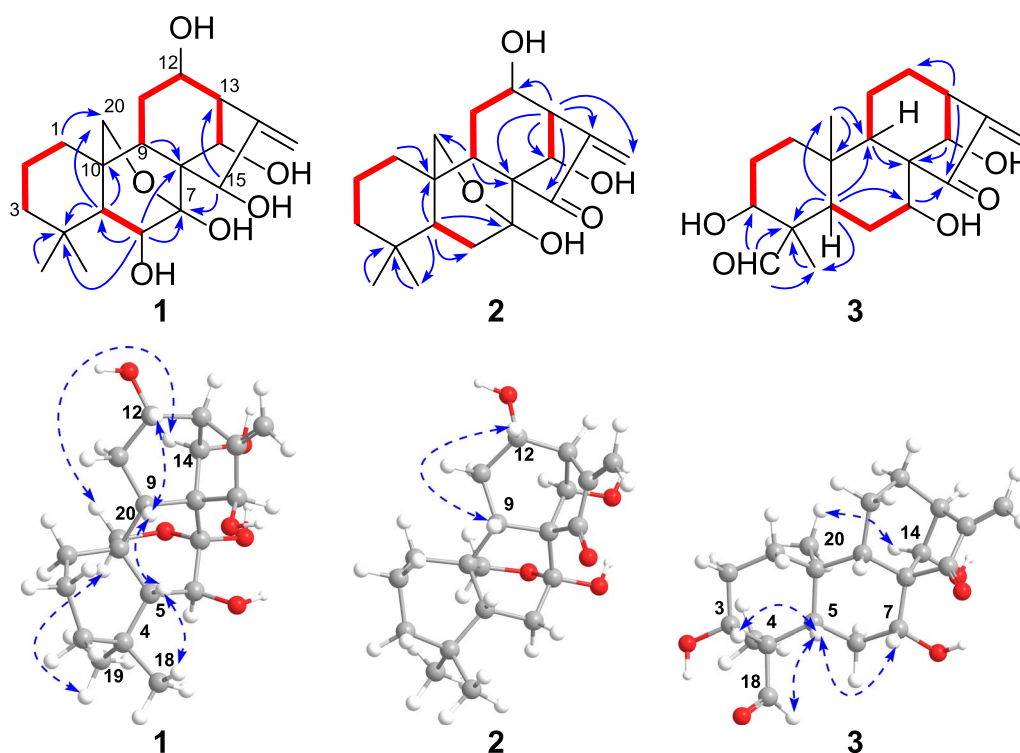
Table 2 ^{13}C NMR spectroscopic data of silvaticusins A–C (1–3) (δ in ppm)

No	1 ^a	2 ^a	3 ^b
1	31.0, CH ₂	30.9, CH ₂	30.3, CH ₂
2	41.5, CH ₂	19.0, CH ₂	26.4, CH ₂
3	19.1, CH ₂	40.9, CH ₂	70.6, CH
4	34.0, C	34.0, C	55.1, C
5	57.8, CH	48.9, CH	43.6, CH
6	73.3, CH	32.3, CH ₂	30.9, CH ₂
7	99.9, C	98.1, C	72.6, CH
8	53.5, C	59.8, C	60.8, C
9	40.0, CH	48.9, CH	53.3, CH
10	36.1, C	36.1, C	37.6, C
11	27.1, CH ₂	28.2, CH ₂	17.0, CH ₂
12	76.5, CH	74.7, CH	36.8, CH ₂
13	57.9, CH	55.3, CH	45.8, CH
14	74.3, CH	71.9, CH	74.5, CH
15	73.3, CH	203.3, C	206.6, C
16	156.3, C	149.7, C	148.5, C
17	111.1, CH ₂	119.1, CH ₂	115.6, CH ₂
18	33.6, CH ₃	20.8, CH ₃	205.8, CH
19	22.5, CH ₃	32.4, CH ₃	8.6, CH ₃
20	65.7, CH ₂	66.4, CH ₂	17.3, CH ₃

^a Recorded on a 500 MHz NMR spectrometer in pyridine-*d*₅. ^b Recorded on a 600 MHz NMR spectrometer in pyridine-*d*₅.

diterpenoid macroclynin C [16], sharing an identical molecular weight of 348 Daltons. The ^1H – ^1H COSY spectrum, with key correlations of H₂-1/H₂-2/H-3, H-5/H₂-6/H-7, and H-9/H₂-11/H₂-12/H-13/H-14, and the HMBC spectrum (Fig. 2), showing correlations from CHO-18 to C-3, C-4, and C-19, and from H-13 to C-12 and C-15, have pinpointed a hydroxyl group at C-12 in silvaticusin C (3). This differs from macroclynin C's hydroxyl group at C-3, with the rest of their structures remains congruent. As for the stereochemistry of 3, appointing H-5 as β -oriented, the H-3/H-5 β , H-7/H-5 β , H-9/H-5 β , CHO-18/H-5 β , H₃-20/H₃-19, H-14/H₃-20 correlations in the ROESY spectrum indicated the β orientation of H-3, H-7, CHO-18, and HO-14. Therefore, the structure of silvaticusin C (3) was assigned as 3 α ,7 α ,14 β -trihydroxy-*ent*-kaur-16-en-18-al-15-one.

Silvaticusin D (4) was isolated as a white amorphous powder. The molecular formula of C₄₂H₅₅O₁₄ was inferred from the (+)-HRESIMS ion at *m/z* 789.3458 [M + Na]⁺ (calcd 789.3457) and ^{13}C NMR data. Detailed analysis of the ^1H and ^{13}C NMR data of 4 suggested that it is a diterpene dimer composed of two 6,7-seco-6,20-epoxy-*ent*-kauranoids analogous to the structures of epinodosin [17, 18] and enmein-3-acetate [19], respectively. This deduction was further supported by H-1/H₂-2/H₂-3, and H-9/H-11/H₂-12/H-13/H-14, H-1'/


Fig. 2 ^1H – ^1H COSY (bold), selected HMBC (arrow), and key ROESY (dashed arrow) correlations of silvaticusins A–C (1–3)

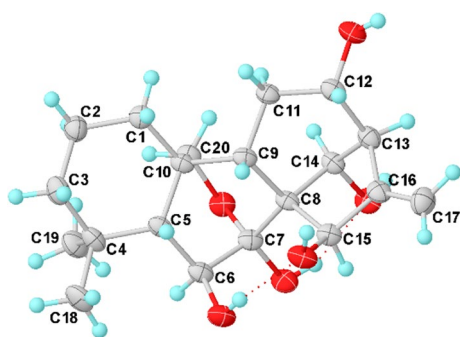


Fig. 3 Crystallographic structure of silvaticusin A (1)

$H_2-2'/H-3'$, and $H-9'/H_2-11'/H_2-12'/H-13'/H-14'$ correlations in the $^1H-^1H$ COSY spectrum, as well as HMBC correlations from H-6 to C-4, C-5, C-10, C-20, from H-11 to C-8, C-10, C-13, from H-6' to C-4', C-10', C-20', from H-3' to C-1', C-2', C-4', C-5', -OAc. The lack of NMR signals for the typical terminal double bonds at C-16/C-17, a feature usually presents in *ent*-kauranoids, in both monomers of compound **4**, implies that these double bonds are involved in the dimerization. This, combined with the one remaining degree of unsaturation and key $^1H-^1H$ COSY correlation between H-17 and H-17', along with HMBC correlations of H-13/C-16, $H_2-17/C-13$, H-13'/C-16', and $H_2-17'/C-13'$, supported a head-to-head [2+2] cycloaddition mechanism for dimerization. Accordingly, the planar structure of compound **4** is established as illustrated in Fig. 4.

The stereochemistry of most chiral centers was determined using ROESY correlations and coupling constants, except for C-3', where overlapping NMR signals obscured its configuration determination. As for the two new chiral centers (C-16 and C-16') formed during dimerization, correlations of H-13/H-17a, H-13/H-17'a, and H-13'/H-17'b in the ROESY spectrum suggested the α orientation of C-17 methylene, and the β orientation of C-17'. Then, the two possible epimers, (1*S*, 5*R*, 6*R*, 8*S*, 9*S*, 10*S*, 11*S*, 13*S*,

16*R*, 1'*S*, 3'*S*, 5'*R*, 6'*R*, 8'*S*, 9'*S*, 10'*S*, 13'*R*, 16'*R*)-**4** (**4a**) and (1*S*, 5*R*, 6*R*, 8*S*, 9*S*, 10*S*, 11*S*, 13*S*, 16*R*, 1'*S*, 3'*R*, 5'*R*, 6'*R*, 8'*S*, 9'*S*, 10'*S*, 13'*R*, 16'*R*)-**4** (**4b**) were subjected to NMR computation at the mPW1PW91-SCRF/6-31+G(d,p)//B3LYP-D3BJ/6-31G(d) level of theory. In the subsequent DP4+ analysis [20] comparing isomers **4a** and **4b**, **4a** exhibited a conclusive probability of 100% (Figure S46), thereby substantiating its designation as the correct structure. Then, TDDFT ECD calculation of (1*S*, 5*R*, 6*R*, 8*S*, 9*S*, 10*S*, 11*S*, 13*S*, 16*R*, 1'*S*, 3'*S*, 5'*R*, 6'*R*, 8'*S*, 9'*S*, 10'*S*, 13'*R*, 16'*R*)-**4** (**4a**) were carried out at the PBE0-SCRF/6-311+G(2d,p) and CAM-B3LYP-SCRF/6-311+G(2d,p) level of theory, respectively, and the calculated curves matched the experimental curve (Fig. 5), thereby determining the absolute configuration of compound **4** as 1*S*, 5*R*, 6*R*, 8*S*, 9*S*, 10*S*, 11*S*, 13*S*, 16*R*, 1'*S*, 3'*S*, 5'*R*, 6'*R*, 8'*S*, 9'*S*, 10'*S*, 13'*R*, and 16'*R* (Table 3).

Previous research on diterpenoids from the *Isodon* genus has shown that the *exo*-methylene cyclopentanone D-ring system is crucial for their antitumor and anti-inflammatory effects, with C-14 hydroxylation usually enhancing these effects [21]. In this research, compounds **2** and **3**, which possess this D-ring system, were evaluated for cytotoxicity against HL-60, A-549, SMMC-7721, MDA-MB-231, and SW-480 cell lines using the MTS assay [22], with cisplatin and paclitaxel as positive controls. Compounds **2** and **3** exhibited significant cytotoxic activity, with IC_{50} values ranging from 1.27 ± 0.08 to 7.52 ± 0.33 μ M for all five cell lines (Table 4).

3 Experimental

3.1 General experimental procedures

General experimental procedures can be found in the Supplementary Material (Part 1).

3.2 Plant material

The source of the plant material can be found in the Supplementary Material (Part 2).

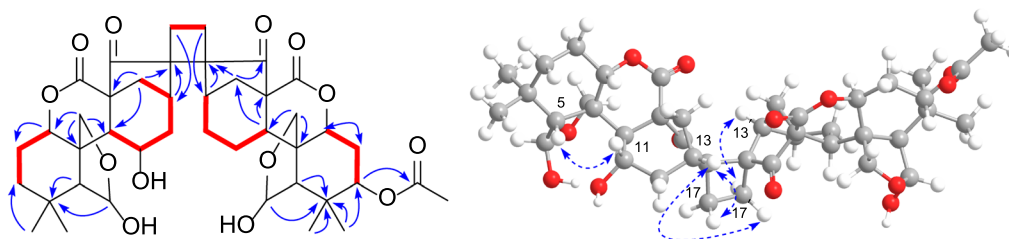


Fig. 4 $^1H-^1H$ COSY (bold), selected HMBC (arrow), and key ROESY (dashed arrow) correlations of silvaticusin D (4)

3.3 Extraction and isolation

The detailed procedures for extraction and isolation can be found in in the Supplementary Material (Part 3).

3.4 Characteristic data of compounds 1–4

Silvaticusin A (**1**): colorless needles; mp 195~197 °C; $[\alpha]_D^{20}$ –16.04 (*c* 0.106, MeOH); IR (KBr) $\nu_{\max}$ 3391, 2948, 2929 cm^{–1}; ¹H and ¹³C NMR data, see Tables 1 and 2; positive-ion HREIMS [M+Na]⁺ *m/z* 389.1936 (calcd for C₂₀H₃₀O₆Na, 389.1935).

Crystallographic data for silvaticusin A (**1**): C₂₀H₃₀O₆·2(H₂O), *M*=402.47, *a*=6.4305(2) Å, *b*=16.4111(5) Å, *c*=18.9447(5) Å, α =90°, β =90°, γ =90°, *V*=1999.26(10) Å³, *T*=150.(2) K, space group *P*212121, *Z*=4, μ (Cu K α)=0.851 mm^{–1}, 14,239 reflections measured, 3632 independent reflections (*R*_{int}=0.1309). The final *R*_i values were 0.0415 (*I*>2 σ (*I*)). The final *wR*(*F*²) values were 0.1046 (*I*>2 σ (*I*)). The final *R*_i values were 0.0639 (all data). The final *wR*(*F*²) values were 0.1146 (all data). The goodness of fit on *F*² was 1.042. Flack parameter=–0.13(15).

Silvaticusin B (**2**): white, amorphous powder; $[\alpha]_D^{20}$ –48.66 (*c* 0.06, MeOH); IR (KBr) $\nu_{\max}$ 3427, 2948, 2928, 1724 cm^{–1}; ¹H and ¹³C NMR data, see Tables 1 and 2; positive-ion HREIMS [M+H]⁺ *m/z* 349.2010 (calcd for C₂₀H₂₉O₅, 349.2010).

Silvaticusin C (**3**): white, amorphous powder; $[\alpha]_D^{25}$ –73.33 (*c* 0.09, MeOH); ¹H and ¹³C NMR data,

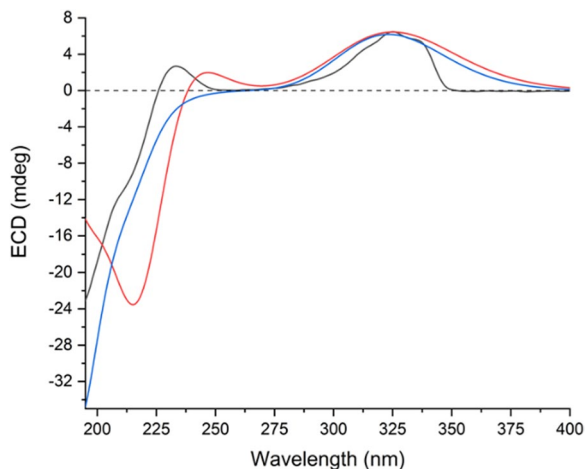


Fig. 5 Experimental ECD spectrum of silvaticusin D (**4**) (black). Calculated ECD spectra of (1*S*, 5*R*, 6*R*, 8*S*, 9*S*, 10*S*, 11*S*, 13*S*, 16*R*, 1'*S*, 3'*S*, 5'*R*, 6'*R*, 8'*S*, 9'*S*, 10'*S*, 13'*R*, 16'*R*)-**4** (**4A**) at PBE0-SCRF/6–311 + G(2d,p) (red) and CAM-B3LYP-SCRF/6–311 + G(2d,p) (blue, shift = + 15 nm) level of theory

Table 3 ¹H and ¹³C NMR spectroscopic data of silvaticusin D (**4**) (δ in ppm)

No	δ_H (mult., <i>J</i> in Hz)	δ_C , type	No	δ_H (mult., <i>J</i> in Hz)	δ_C , type
1	5.65, m	78.5, CH	2'a	2.13, m	27.2, CH ₂
2a	1.83, m	23.7, CH ₂	2'b	2.27, (12.9)	–
2b	2.04, s	–	3'	5.01, overlap	77.3, CH
3a	1.30, m	37.1, CH ₂	4'	–	34.5, C
3b	1.46, m	–	5'	2.42, d, (3.3)	51.2, CH
4	–	31.3, C	6'	5.78, d, (3.3)	101.8, CH
5	2.71, d, (3.1)	55.6, CH	7'	–	172.0, C
6	5.77, d, (3.1)	101.8, CH	8'	–	58.0, C
7	–	172.4, C	9'	2.84, m	47.6, CH
8	–	57.8, C	10'	–	49.7, C
9	2.80, d, 3.7	49.7, CH	11'a	2.11, m	19.1, CH ₂
10	–	49.6, C	11'b	1.56, m	–
11	5.00, m	65.1, CH ₂	12'a	1.36, m	21.6, CH ₂
12a	1.75, m	33.3, CH ₂	12'b	1.37, m	–
12b	2.09, m	–	13'	3.12, d, (11.1)	33.9, CH
13	3.32, m	34.2, CH	14'a	2.47, d, (13.3)	32.2, CH ₂
14a	2.55, m	33.4, C	14'b	2.50, d, (13.3)	–
14b	3.80, m	–	15'	–	216.5, C
15	–	216.8, C	16'	–	63.2, C
16	–	63.3, C	17'a	1.91, m	23.9, CH ₂
17a	2.20, m	24.3, CH ₂	17'b	2.21, s	–
17b	1.89, m	–	18'	1.02, s	27.3, CH ₃
18	0.98, s	23.1, CH ₃	19'	0.99, s	22.5, CH ₃
19	0.96, s	32.9, CH ₃	20'a	4.46, d, (9.0)	74.3, CH ₂
20a	4.27, d, (9.0)	73.9, CH ₂	20'b	4.25, d, (9.0)	–
20b	4.52, d, (9.0)	–	3'-OAc	–	169.8, C
1'	4.88, m	72.9, CH		2.23, s	20.6, CH ₃

Recorded on an 800 MHz NMR spectrometer in pyridine-*d*₅

see Tables 1 and 2; positive-ion HREIMS [M+H]⁺ *m/z* 349.2008 (calcd for C₂₀H₂₉O₅, 349.2010).

Silvaticusin D (**4**): white, amorphous powder; $[\alpha]_D^{25}$ –22.61 (*c* 0.092, MeOH); ¹H and ¹³C NMR data, see Table 3; positive-ion HREIMS [M+Na]⁺ *m/z* 789.3458 (calcd for C₄₂H₅₅O₁₄Na, 789.3457).

3.5 X-ray crystal structure analysis

The X-ray crystal structure analysis of silvaticusin A (**1**) can be found in in the Supplementary Material (Part 4).

3.6 The cytotoxicity assay

The cytotoxicity assay has been described previously [23].

Table 4 Cytotoxic activities of compounds **2** and **3** against five human tumor cell lines

Compound	HL-60	A-549	SMMC-7721	MDA-MB-231	SW-480
2	1.65 ± 0.02	3.23 ± 0.14	1.27 ± 0.08	1.99 ± 0.05	2.51 ± 0.14
3	2.76 ± 0.18	7.52 ± 0.33	4.13 ± 0.09	5.43 ± 0.11	4.48 ± 0.11
Paclitaxel	< 0.008	< 0.008	0.095 ± 0.007	< 0.008	< 0.008
Cisplatin	2.92 ± 0.15	11.95 ± 0.25	20.52 ± 0.63	19.28 ± 0.30	21.32 ± 1.05

Results are expressed as IC₅₀ values in μM. Cisplatin and paclitaxel were used as positive controls

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s13659-024-00465-9>.

Supplementary Material 1. Supplementary data associated with this article (¹H, ¹³C NMR, DEPT, HSQC, HMBC, ¹H – ¹H COSY, NOESY, HREIMS, UV, ECD and IR spectra of silvaticusins A–D (**1–4**); Computational data of silvaticusin D (**4**)).

Acknowledgements

We thank Service Center for Bioactivity Screening, Key Laboratory of Phytochemistry and Natural Medicines, Kunming Institute of Botany for bioactivity screening.

Author contributions

PTP conceived and designed the research; QXH, KH carried out the experiment and wrote the manuscript; PTP, HPH, HDS and KH supervised the whole study and critically reviewed the manuscript. All authors read and approved the final manuscript.

Funding

This work was financially supported by the National Science Fund for Distinguished Young Scholars (82325047), Second Tibetan Plateau Scientific Expedition and Research (STEP) program (2019QZKK0502), NSFC-Joint Foundation of Yunnan Province (U2002221), and Youth Innovation Promotion Association CAS (2023409).

Data availability

The data that support the findings of this study are openly available in the Science Data Bank at <https://doi.org/https://doi.org/10.57760/sciencedb.09413>.

Declarations

Competing interests

Pema-Tenzin Puno is the journal's editor but was not involved in the peer review or decisionmaking process in this article. The other authors have no conflicts of interest to disclose.

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Received: 3 June 2024 Accepted: 18 July 2024

Published online: 15 August 2024

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