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Phenylpropanosids, lignans and other constituents from *Cremanthodium ellisii*

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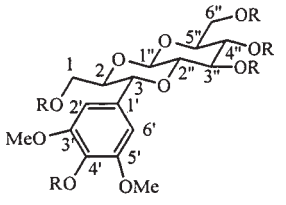
Together with twenty-six known compounds, a new phenylpropanosid, named cremanthodioside, was isolated from the whole plant of *Cremanthodium ellisii* Kitam. Their structures were elucidated by spectroscopic methods MS, IR, UV, NMR, including 2D NMR techniques, and by chemical methods. The anti-bacterial activity of compounds **1**–**6** and the anti-tumor activity of compound **1** were tested.

1. Introduction

The genus *Cremanthodium* (Compositae) is widely distributed in the mountains of the Himalayas and contiguous climatic regions. So far, 64 species of it are known throughout the world, all of which are distributed in China, especially in the northwest and southwest regions (most of the genus *Cremanthodium* grow at an elevation of 3500–5000 m) (Delectis Flora Reipublicae Popularis Sinicae Agendae Academiae Sinicae Edita 1989). Some *Cremanthodium* plants like the title species have been used as traditional Tibetan medicines for antiinflammation, detoxification, and relief of pain since ancient times (Northwestern Plateau Institute of Biology, Academia Sinica 1991).

Due to its medicinal value, *Cremanthodium ellisii* Kitam. (Chen et al. 1996, 1997) (collected in Zhang county, Gansu province) has been systematically studied by our research group. We have now also investigated *C. ellisii* collected in Huzhu county, Qinghai province. We found that their chemical constituents differed greatly because they were collected in different districts (Su et al. 2000). In this paper, we report the isolation and structural elucidation of a new phenylpropanosid (**1**) and twenty-six known compounds (**2**–**27**) from the species as a continuation of our studies. Among these, compounds **1**–**4**, **6**–**11**, **13**–**16** and **19**–**27** were isolated from this genus for the first time. In addition, six compounds **1**–**6** were assayed against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. The new compound **1** was also screened against human ovarian carcinoma (HO-8910), human leukemia (HL-60) and human hepatoma (SMMC-7721) cell lines.



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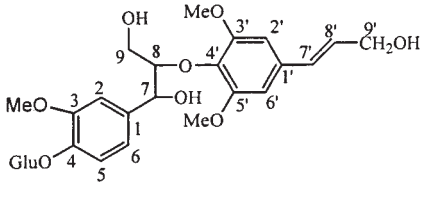

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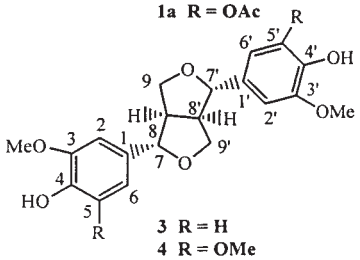

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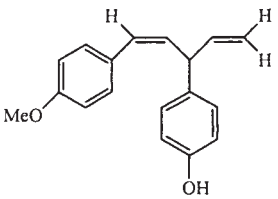

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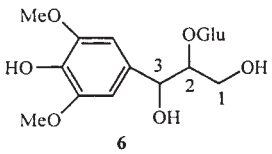

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2. Investigations, results and discussion

From the ethanol extract of the whole plant of *C. ellisii*, a new phenylpropanosid, named cremanthodioside (**1**), was isolated and elucidated, together with twenty-six known compounds: citrusin B (**2**) (Deyama et al. 1987), (+)-pinoresinol (**3**) (Deyama 1983; Fonseca et al. 1979), (+)-syringaresinol (**4**) (Abe and Yamauchi 1988; Deyama 1983), (−)-4′-*O*-methylnyasol (**5**) (Su et al. 2000), syrigoylglycerol 2-*O*-β-D-glucopyranoside (**6**) (Kijima et al. 1997), syringin (**7**) (Niwa et al. 1988), isorhamnetin (**8**) (Barbear et al. 1986), isorhamnetin-3-*O*-β-D-glactopyranoside (**9**) (Barbear et al. 1986), quercetin-3-*O*-β-D-glactopyranoside (**10**) (Barbear et al. 1986), umbelliferone (**11**) (Razdan et al. 1987), scopoletin (**12**) (Razdan et al. 1987), scopolin (**13**) (Tsukamoto et al. 1985), scoporone (**14**) (Razdan et al. 1987; Yu et al. 1998), 8-hydroxy-9,10-isobutyryloxy-thymol (**15**) (Bohlmann and Chen 1984), sitoindoside I (**16**) (Chaurasia and Wichtl 1987; Luo et al. 2001), β-sitosterol (**17**) (Greca et al. 1990), β-daucosterol (**18**) (Li et al. 1994), *trans-p*-hydroxycinnamic acid (**19**) (Sadtler Research Laboratories, Inc. 1978), *trans*-3,4-dihydroxycinnamic acid (**20**) (Flamini et al. 2001), *trans*-4-dihydroxy-3-methoxylcinnamic acid (**21**) (Sadtler Research Laboratories, Inc. 1973), 3,5-dimethoxy-4-hydroxybenzoic acid (**22**) (Sadtler Research Laboratories, Inc. 1969, Sadtler Research Laboratories, Division of Bio-Rad Laboratories, Inc. 1979), 5-hydroxymethyl furfural (**23**) (Xu et al. 1995), L-chiro-inositol (**24**) (Dorman et al. 1970), glycerol (**25**), octadecanoate glycerol (**26**), (*R*)-(*Z*, *Z*)-1-(9,12-octadecadienoate)-glycerol (**27**). The structure of the new compound **1** was identified by EIMS, FABMS, IR, UV, ^{1}H NMR, ^{13}C NMR, ^{1}H, ^{1}H COSY, HMQC, HMBC, TOCSY1D spectroscopic methods and chemical transformation. The structures of the known compounds **2**–**27** were elucidated by comparison of their spectral data (ORD, EIMS, FABMS, IR, ^{1}H NMR and ^{13}C NMR) with those published in the literature or were determined on the basis of their physical properties (m.p., R_f etc.) by comparison with those of authentic samples.

Compound **1** was obtained as white needles, m.p. 189–190 °C. Its molecular formula was deduced to be $C_{17}H_{24}O_{10}$ from the quasi-molecular ion peaks of FABMS at m/z 394.9 $[M+Li]^+$ and m/z 410.9 $[M+Na]^+$, which was supported by ^{1}H NMR, ^{13}C NMR and DEPT data (Table 1). The IR spectrum (KBr) showed the presence of alcoholic groups (3464, 3398, 3296, 1058, 1023 cm^{-1}), phenolic hydroxyl (3568, 1225 cm^{-1}), typical methoxyl absorption (2852 cm^{-1}), benzene ring (1615, 1521, 1467 cm^{-1}) and C−O−C bond (1126 cm^{-1}). Its ^{1}H NMR spectrum gave a independent aromatic proton absorption signal at δ_H 6.63 (2 H, d, J = 1.8 Hz), so that combined with the ^{13}C NMR and DEPT spectra data at δ_C 148.36 (2 × C), 136.17 (C), 128.45 (C), 106.07 (2 × CH=), compound **1** has a symmetrical four-substituted aromatic ring. Moreover, the syringyl subunit of compound **1** was deduced from signals δ_H 8.42 (1 H, brs, Ar-OH), and δ_H 3.75 (6 H, s, 2 × OMe) in the ^{1}H NMR spectrum and δ_C 56.74 (2 × OMe) in the ^{13}C NMR spectrum. In addition, the significant absorption bands at 270 and 209 nm in the UV spectrum also supported a aromatic ring with auxochromic group substitutions. Apart from the proton signals corresponding to the above mentioned groups, the ^{1}H NMR data displayed an anomeric proton signal of sugar at δ_H 4.48 (1 H, d, J = 7.5 Hz), and oxygen-bearing methylene and methine group signals, which were determined to be a glycerol moiety and a monosaccharide moiety by ^{1}H, ^{1}H COSY spectrum data. Furthermore, ^{13}C NMR and DEPT spectral data further verified this conclusion at δ_C 98.57 (anomeric carbon of sugar) and δ_C 61.12–81.34. To determine the correlations of δ_H and δ_C, the HMQC spectrum of compound **1** was obtained (Table 1). In addition, its HMBC spectrum gave long-range correlations of δ_H 6.63 (H-2′,6′) with δ_C 79.25 (C-3); and of δ_H 4.27 (H-3) with δ_C 106.07 (C-2′, 6′) showing that the skeleton of compound **1** was 3-*C*-syringy-glycerol, and the coupling constant ($J_{2,3}$) of 9.3 H_Z allowed its stereochemistry be assigned to the threo form (Herrera Braga et al. 1984). From the long-range correlations of δ_H 4.48 (H-1″) with δ_C 81.34 (C-2); of δ_H 3.68 (H-2) with δ_C 98.57 (C-1″); δ_H 2.94 (H-2″) with δ_C 79.25 (C-3); and of δ_H 4.27 (H-3) with δ_C 80.03 (C-2″), the structure of compound **1** was obtained apart from the type of the sugar. In order to confirm this point, compound **1** was acetylated to give a penta-acetate (**1a**). Besides absorption peaks of the 4-acetyl-3,5-dimethoxyl-phenyl moiety, the ^{1}H NMR spectrum of compound **1a** revealed oxygenbearing proton signals which were connected to saturated carbons. According to the ^{1}H, ^{1}H COSY spectrum of **1a**, these signals were assigned as follow: δ_H 5.33 (1 H, dd, J = 9.9, 9.6 Hz, H-3″), 5.13 (1 H, dd, J = 9.9, 9.6 Hz, H-4″), 4.68 (1 H, d, J = 8.1 Hz, H-1″), 4.47 (1 H, d, J = 9.3 Hz, H-3), 3.93 (2 H, m, H-2, 5″), 3.52 (1 H, dd, J = 9.9, 8.1 Hz, H-2″). The relatively large trans di-axial coupling constants ($J_{a,a}$) between H-1″ and H-2″ (8.1 Hz), H-2″ and H-3″ (9.9 Hz), H-3″ and H-4″ (9.6 Hz), H-4″ and H-5″ (9.9 Hz) indicated that the sugar was β-D-glucopyranose. The signals of H-2 and H-5″ were overlapped, giving the TOCSY1D spectrum of **1a** (about tri-acetate sugar), then the signals at δ_H 4.28 (1 H, dd, J = 12.3, 5.1 Hz), 4.19 (1 H, dd, J = 12.3, 1.8 Hz) were the proton of C-6″ and δ_H 4.05 (1 H, m) was the proton of C-1. Thus, the structure of compound **1** was elucidated as shown, and it was named cremanthodioside.

Compounds **1**–**6** were tested for their anti-bacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* by comparision with chloramphenicol as a control. The results indicated that they all exhibited comparatively strong activities against *S. aureus* (Table 2).

Table 1: ^{1}H (400 MHz), ^{13}C NMR (100 MHz) and HMBC data of 1 (DMSO-d_6, TMS, δ, ppm)

No.	^{1}H NMR	^{13}C NMR (DEPT)	HMBC
1	3.22 (brs)	61.12 (CH_2)	C-3
2	3.68 (brd, J = 9.3 Hz)	81.34 (CH)	C-3, 1″
3	4.27 (d, J = 9.3 Hz)	79.25 (CH)	C-1, 2, 1′, 2′, 6′, 2″
1′	–	128.45 (C)	–
2′, 6′	6.63 (d, J = 1.8 Hz)	106.07 (CH)	C-3, 1′, 3′, 4′, 6′/2′
3′, 5′	–	148.36 (C)	–
4′	–	136.17 (C)	–
1″	4.48 (d, J = 7.5 Hz)	98.57 (CH)	C-2, 2″, 5″
2″	2.94 (dd, J = 9.9, 8.1 Hz)	80.03 (CH)	C-3, 1″, 3″
3″	3.35 *	73.86 (CH)	C-2″, 4″
4″	3.15 *	71.16 (CH)	C-3″, 5″, 6″
5″	3.29 *	79.25 (CH)	C-1″, 4″, 6″
6″	3.68 *, 3.46 (dd, J = 12.3, 6.0 Hz)	61.59 (CH_2)	C-5″
OMe	3.75 (s)	56.74 (CH_3)	C-3′, 5′
Ar-OH	8.42 (brs)	–	C-3′, 4′, 5′

* Overlapped signals
** Assignments are aided by ^{1}H, ^{1}H COSY, HMQC and HMBC data
*** OH of compound **1**: 5.14-5.16 (C_1-OH, $C_{6''}$-OH), 4.64-4.68 ($C_{2''}$-OH, $C_{3''}$-OH, $C_{4''}$-OH) which were assigned by HMBC data.

Table 2: Anti-bacterial activity of compounds

Compd.	*S. aureus*	*E. coli*	*B. subtilis*
1	++	−	+
2	++	++	+
3	++	+	+
4	++	++	+
5	++	+	+
6	++	+	+
H_2O	−	−	−
Chloramphenicol	+++	+++	+++

Zone diameter of growth inhibition: < 10 mm (−), 10–12 mm (+), 13–15 mm (++), and 16–20 mm (+++)

Using the MTT method, the anti-tumor activity of compound **1** against human ovarian carcinoma (HO-8910), human leukemia (HL-60) and human hepatoma (SMMC-7721) cell lines was screened by comparision with vincristine sulphate as a control. However, they exhibited little anti-tumor activity against the cell lines (the half inhibitory concentrations (IC_{50}, ug/ml) were 286.578 ± 1.586, 333.667 ± 2.450 and > 400, respectively).

3. Experimental

3.1. Apparatus

Optical rotations: Perkin-Elmer 341 Polarimeter; UV: TU-1901 UV-VIS instrument; IR: Nicolet NEXUS 670 FT-IR instrument; EIMS: HP 5988A GC/MS instrument; FABMS data: VG-ZAB-HS mass spectrometer (at 70 eV); NMR: Varian Mercury Plus-300BB instrument; Silica gel (200–300 mesh) for column chromatography and GF254 (10-40 μ) for TLC were supplied by the Qingdao Marine Chemical factory, Qingdao, P.R. China; Spots were detected on TLC under UV lamp and by heating after spraying with 5% H_2SO_4 in C_2H_5OH or 5% $FeCl_3$ in C_2H_5OH; Melting points were determined on a Kofler melting point apparatus, and are uncorrected.

3.2. Plant material

Cremanthodium ellisii Kitam. was collected in Huzhu county, Qinghai province, P.R. China, in August 1999. It was identified by Prof. Guo-liang Zhang, School of Life Science, Lanzhou University. A voucher specimen (No. Ce-0802) was deposited in the herbarium of our institute.

3.3. Extraction and isolation

Air-dried and pulverized whole plant of *C. ellisii* Kitam. (7.5 kg) was extracted with methanol three times at room temperature (each for one week). The extract was concentrated under reduced pressure to yield a residue (340 g), from which we obtained fifteen compounds (Su et al. 2000). This was then extracted with ethanol three times under the same conditions, and the resultant extract (170 g) was suspended in hot water (60 °C, 400 ml). The suspension was extracted, successively, with petroleum ether (60–90 °C) and EtOAc, then was concentrated under reduced pressure to yield residues of 20 g (part 1) and 35 g (part 2), respectively, and the remaining residue yielded 100 g (part 3). The combined part 1 and part 2 residues (55 g) were chromatographed over a silica gel column (700 g) eluted with a gradient of petroleum ether (60–90 °C)-acetone (50 : 1 to 1 : 1 and CH_3COCH_3, 500 ml each eluent). Combination of the appropriate fractions (monitored by TLC analysis) led to eight crude fractions (A-H). Fraction A (petroleum ether-acetone 50 : 1 to 20 : 1, 15 g) was obtained as white wax and yellow oil which were apparently mainly volatile oils and fatty hydrocarbons so have not been studied in detail; fraction B (petroleum ether-acetone 15 : 1, 5 g) was mainly volatile oil and colorless needles, which were recrystallized in acetone to obtain compound **17**; fraction C (petroleum ether-acetone 10 : 1, 1 g) was repeatedly chromatographed over a silica gel column to give compound **15** (25 mg). Fraction D (petroleum ether-acetone 6 : 1, 1.5 g) was chromatographed over a silica gel column eluted with a gradient of petroleum ether (60–90 °C)-acetone (8 : 1, 5 : 1, 3 : 1, 100 ml each eluent) to obtain fractions D_1–D_3. Compound **14** (5 mg) and compound **26** (10 mg) were obtained by chromatography over a silica gel column from fraction D_1. Fraction D_2 was repeatedly chromatographed over a silica gel column to give compound **5** (60 mg). Fraction D_3 was repeatedly recrystallized in EtOAc to obtain compound **11** (12 mg); fraction E (petroleum ether-acetone 3 : 1, 5.5 g) was repeatedly recrystallized in methanol to give compound **12** (200 mg), the mother liquid was chromatographed over a silica gel column eluted with a gradient of petroleum ether (60–90 °C)-acetone (8 : 1, 6 : 1, 6 : 1, 2 : 1 and 1 : 1, 100 ml each eluent) to obtain fractions E_1–E_2. Fraction E_1 (400 mg) was rechromatographed over a silica gel column eluted with benzene-acetone (10 : 1) yielding compound **27** (30 mg). Fraction E_2 (400 mg) was repeated chromatographed over a silica gel column eluted with $CHCl_3$ to give compound **4** (15 mg). Fraction F (petroleum ether-acetone 1 : 1, 4.3 g) separated out as a white solids and was purified by prep. TLC on silica gel to obtain compound **16** (24 mg), the mother liquid was chromatographed over a silica gel column eluted with a gradient of petroleum ether (60–90 °C)-acetone (8 : 1 to 1 : 1, 100 ml each eluent) to obtain fractions F_1–F_3 after combination according to TLC analysis. Fraction F_1 (310 mg) was rechromatographed over a silica gel column eluted with benzene-acetone (8 : 1) to give compound **3** (17 mg); Compound **21** (30 mg) was obtained by prep. TLC on silica gel from fraction F_2 (50 mg). Fraction F_3 (300 mg) was rechromatographed over a polyamide column eluted with methanol-water (5 : 1), and was then purified by prep. TLC on silica gel to yield compound **20** (50 mg); fraction G (acetone, 4 g) was recrystallized in CH_3OH to give compound **18**, while the mother liquid was chromatographed over a silica gel column eluted with CH_2Cl_2–CH_3OH (10 : 1), and was then recrystallized in CH_3OH to obtain compound **8** (50 mg). Fraction H (acetone, 10 g) was recrystallized in CH_3OH to yield a mixture of compound **9** and compound **10** (50 mg), and compound **9** (25 mg) and compound **10** (5 mg) were then isolated by prep. TLC on silica gel. The mother liquid was repeatedly chromatographed over a silica gel column to yield compounds **22** (2 mg) and **19** (20 mg).

The 100 g remainder (part 3) was chromatographed over a silica gel column (700 g) eluted with a gradient of $CHCl_3$–CH_3OH (10 : 1 to 1 : 1 and CH_3OH, 500 ml each fluent). Combination of the appropriate fractions (monitored by TLC analysis) led to seven fractions (A′–G′), and compound **24** was obtained as a white powder with the chromatograph. Fraction A′ ($CHCl_3$–CH_3OH 10 : 1, 450 mg, 2000 ml) was chromatographed over a silica gel column eluted with $CHCl_3$-acetone (8 : 1), and was then purified by prep. TLC on silica gel to obtain compound **23** (5 mg). Fraction B′ ($CHCl_3$–CH_3OH 10 : 1, 1.5 g, 10000 ml) was repeatedly chromatographed over a silica gel column to give compound **13** (25 mg); fraction C′ ($CHCl_3$–CH_3OH 5 : 1, 1 g, 2500 ml) was rechromatographed over a silica gel column to obtain compound **7** (14 mg); fraction D′ ($CHCl_3$–CH_3OH 5 : 1, 1 g, 1000 ml) was chromatographed over a silica gel column eluted with EtOAc-CH_3OH (20 : 1, 10 : 1 and 5 : 1) to yield fraction D_1′ (EtOAc-CH_3OH 5 : 1, 500 mg), which was repeatedly recrystallizated in methanol to obtain compound **1** (30 mg); fraction E′ ($CHCl_3$–CH_3OH 5 : 1, 1.6 g, 4000 ml) was chromatographed over a silica gel column eluted with a gradient of EtOAc-CH_3OH (20 : 1, 10 : 1 and 5 : 1), and was then purified by prep. TLC on silica gel to yield compound **2** (30 mg); fraction F′ ($CHCl_3$–CH_3OH 5 : 1, 1.5 g, 4000 ml) was repeatedly chromatographed over a silica gel column after being chromatographed over a silica gel column eluted with EtOAc-CH_3OH (20 : 1, 10 : 1 and 5 : 1) to obtain compound **25** (100 mg). Fraction G′ ($CHCl_3$–CH_3OH 5 : 1, 1.5 g, 2500 ml) was chromatographed over a silica gel column eluted with EtOAc-CH_3OH (20 : 1, 10 : 1 and 5 : 1) to obtain crude compound **6** (50 mg), which was then repeatly recrystallized in methanol to yield compound **6** (24 mg).

3.3.1. Cremanthodioside

White needles (CH_3OH), m.p. 189–190 °C; molecular formula: $C_{17}H_{24}O_{10}$; $[\alpha]_D^{20} + 75.0$ (c, 0.2, CH_3OH); FABMS m/z (sgly): 394.9 $[M+Li]^+$, 410.9 $[M+Na]^+$ IR (ν_{max}^{KBr}, cm^{-1}): 3464, 3398, 3296, 1058, 1023 (C−O−H), 3568, 1225 (Ar-OH), 2852 (OMe); 1615, 1521, 1467 (benzene ring) and 1126 (C−O−C); UV λ_{max}^{MeOH} (nm): 270, 209; ^{1}H NMR, ^{13}C NMR, DEPT and HMBC data: see Table 1.

3.3.2. Acetylation of cremanthodiumol

Compound **1** (2 mg) was acetylated with a mixture of $(Ac)_2O$ and pyridine (500 μl each) at room temperature for 72 h and was concentrated under reduced pressure to yield the penta-acetate **1a** (3 mg). Colorless gum; molecular formula: $C_{27}H_{34}O_{15}$; $[\alpha]_D^{20} + 54.0$ (c, 0.1, $CHCl_3$); EIMS m/z (rel int): 556 $[M\text{-}Ac]^+$ (1.3), 252 (0.7), 213 (1.1), 196 (0.3), 182 (1.4), 167 (1.0), 153 (2.8), 97 (9.1), 43 (100); IR (ν_{max}^{KBr}, cm^{-1}): 2850 (OMe); 1747 (C=O), 1606, 1464 (benzene ring) and 1230, 1130, 1042 (C−O−C); UV $\lambda_{max}^{CHCl_3}$ (nm): 272, 240; ^{1}H NMR δ ppm ($CDCl_3$, 300 Hz): 6.54 (2 H, d, J = 1.8 Hz, H-2′, 6′), 5.33 (1 H, dd, J = 9.9, 9.6 Hz, H-3″), 5.13 (1 H, dd, J = 9.9, 9.6 Hz, H-4″), 4.68 (1 H, d, J = 8.1 Hz, H-1″), 4.47 (1 H, d, J = 9.3 Hz, H-3), 4.28 (1 H, dd, J = 12.3, 5.1 Hz, H-6″), 4.19 (1 H, dd, J = 12.3, 1.8 Hz, H-6″), 4.05 (2 H, m, H-1), 3.93 (2 H, m, H-2, 5″), 3.82 (3 H, s, OMe), 3.52 (1 H, dd, J = 9.9, 8.1 Hz, H-2″), 2.33, 2.02, 2.03, 2.04, 2.08 (3 H each, s, OAc).

3.3.3. Citrusin B

Colorless gum; molecular formula: $C_{27}H_{36}O_{13}$; $[\alpha]_D^{20}$ −43.6 (c, 1.7, CH_3OH); FABMS m/z (sgly): 575 $[M+Li]^+$, 591 $[M+Na]^+$ ^{1}H NMR δ ppm (CD_3OD, 300 MH_Z): 7.12 (1 H, d, J = 8.1 Hz, H-5), 7.07 (1 H, d, J = 2.0 Hz, H-2), 6.91 (1 H, dd, J = 8.1, 2.0 Hz, H-6), 6.72 (2 H, d, J = 2.0 Hz, H-2′, 6′), 6.54 (1 H, d, J = 15.9 Hz, H-7′), 6.31 (1 H, dt, J = 15.9, 5.4 Hz, H-8′), 4.94 (1 H, d, J = 5.4 Hz, H-7), 4.91 (2 H, m, H-8,

1″), 4.57 (brs, OH), 4.22 (2 H, d, J = 5.4 Hz, H-9′), 3.84 (3 H, s, C_3-OMe), 3.81 (6 H, s, $C_{3'}$, $C_{5'}$-OMe), 3.68 (2 H, dd, J = 12.3, 3.6 Hz, H-9); ^{13}C NMR δ ppm (CD_3OD, 75 MH_Z): 153.37 (C-3′, 5′), 149.28 (C-3), 146.01 (C-4), 136.32 (C-4′), 135.12 (C-1′), 133.59 (C-1), 130.20 (C-7′), 128.72 (C-8′), 119.62 (C-6), 116.45 (C-5), 111.20 (CH-2), 103.69 (C-2′, 6′), 101.78 (C-1″), 86.09 (C-8), 76.98 (C-3″), 76.61 (C-5″), 73.73 (C-2″), 72.60 (C-7), 70.16 (C-4″), 62.40 (C-9′), 61.32 (C-6″), 60.26 (C-9), 55.49 (OCH_3).

3.3.4. (+)-Pinoresinol

Colorless gum; molecular formula: $C_{20}H_{22}O_6$; $[\alpha]_D^{20}$ + 30.6 (c, 1.1, $CHCl_3$); EIMS m/z (rel int): 358 [M];$^+$ (28), 327 [M-OMe]$^+$ (4), 205 [M-ArCHO–H]$^+$ (15), 163 [ArCH=C=O–H]$^+$ (27), 151 [ArCHO–H]$^+$ (100), 137 (52), 131 (35), 124 [Ar-H]$^+$ (18), 123 [Ar]$^+$ (9); ^{1}H NMR δ ppm ($CDCl_3$, 300 MHz): 6.90 (2 H, d, J = 1.8 Hz, H-2, 2′), 6.89 (2 H, d, J = 8.4 Hz, H-5, 5′), 6.82 (2 H, dd, J = 8.4, 1.8 Hz, H-6, 6′), 5.66 (2 H, brs, Ar-OH), 4.74 (2 H, d, J = 3.6 Hz, H-7, 7′), 4.24 (2 H, dd, J = 7.2, 6.9 Hz, H-9e, 9e′), 3.90 (6 H, s, OMe), 3.86 (2 H, dd, J = 7.2, 3.3 Hz, H-9a, 9a′), 3.10 (2 H, m, H-8, 8′); ^{1}H NMR δ ppm (CD_3COCD_3, 300 MH_Z): 6.97 (2 H, d, J = 1.8 Hz, H-2, 2′), 6.80 (4 H, m, H-5, 5′, 6, 6′), 4.66 (2 H, d, J = 3.6 Hz, H-7, 7′), 4.19 (2 H, H-9e, 9e′), 3.83 (6 H, s, OMe), 3.77 (2 H, dd, J = 8.7, 6.6 Hz, H-9a, 9a′), 3.08 (2 H, m, H-8, 8′); ^{13}C NMR δ ppm ($CDCl_3$, 75 MHz): 146.93 (C-3, 3′), 145.45 (C-4, 4′), 133.10 (C-1, 1′), 119.36 (C-6, 6′), 114.68 (C-5, 5′), 108.97 (C-2, 2′), 86.29 (C-7, 7′), 71.89 (C-9, 9′), 56.34 (C–OCH_3), 54.19 (C-8, 8′); ^{13}C NMR δ ppm (CD_3COCD_3, 75 MHz): 147.68 (C-3, 3′), 146.11 (C-4, 4′), 133.53 (C-1, 1′), 118.95 (C-6, 6′), 114.80 (C-5, 5′), 109.96 (C-2, 2′), 85.97 (C-7, 7′), 71.54 (C-9, 9′), 55.59 (OCH_3), 54.57 (C-8, 8′).

3.3.5. (+)-Syringaresinol

Colorless gum; molecular formula: $C_{22}H_{26}O_8$; $[\alpha]_D^{20}$ + 14.6 (c, 0.8, $CHCl_3$); ^{1}H NMR δ ppm ($CDCl_3$, 300 MHz): 6.58 (4 H, d, J = 2.0 Hz, H-2, 2′, 6, 6′), 5.53 (2 H, brs, Ar-OH), 4.74 (2 H, d, J = 3.6 Hz, H-7, 7′), 4.28 (2 H, dd, J = 8.7, 6.6 Hz, H-9e, 9e′), 3.90 (14 H, brs, 4OMe, H-9a, 9a′), 3.10 (2 H, m, H-8, 8′); ^{13}C NMR δ ppm ($CDCl_3$, 75 MHz): 147.40 (C-3, 3′, 5, 5′), 134.55 (C-4, 4′), 132.32 (C-1, 1′), 102.96 (C-2, 2′, 6, 6′), 86.30 (C-7, 7′), 72.02 (C-9, 9′), 56.60 (OCH_3), 54.57 (C-8, 8′).

3.4. Anti-bacterial activity assays

The anti-bacterial activity assay was carried out according to the cup-plate method. Chloramphenicol was used as a positive control. Three strains of bacteria: *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*, were cultured in beef broth and incubated at 37 °C for 24 h. After dilution of the beef broth, the three bacteria were cultured individually in agar medium dishes, six cups (8 × 10 mm) were put onto the dishes, and each compound being tested (0.2 ml of 100 μg/ml) was added to the cups separately under aseptic conditions. The dishes were then cultured at 37 °C for 24 h. The zone of inhibition of the growth of bacteria, produced by diffusion of the compounds from the cup into the surrounding medium, was measured to evaluate the anti-bacterial activity. Each test was performed in duplicate.

3.5. Anti-tumor activity assays

Anti-tumor activity assays were carried out according to the sulforhodamine B (MTT) colorimetric assay. Human ovarian carcinoma (HO-8910), human leukemia (HL-60) and human hepatoma (SMMC-7721) cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, at 37 °C under a humidified atmosphere of 5% CO_2, and distributed in replicate 96-well plates with 4 × 103 cells/well for 24 h. Then, using vincristine sulfate as a positive control, compound **1** was added. After 48 h exposure to the toxins, cell viability was determined by measuring the absorbance at 515 nm with an ELISA reader. Each test was performed in 5 replicates.

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