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Non-glycosidic iridoids from *Cymbaria mongolica*

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Abstract

Six non-glycosidic iridoids, i.e. (1*R*,4*S*,4a*S*,7*S*,7a*S*)-7-hydroxyl-4-hydroxy- methyl-7-methyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopenta[*e*]pyran-3-one (**1**), (1*S*,4*R*,4a*S*,7*S*,7a*S*)-7-hydroxyl-4-hydroxymethyl-7-methyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopenta[*e*]-pyran-3-one (**2**), (1*R*,4*R*,4a*S*,7*S*,7a*S*)-7-hydroxyl-4-hydroxy-methyl-7-methyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopenta[*e*]pyran-3-one (**3**), (1*R*, 4*R*, 4a*S*, 7a*S*)-4,7-dihydroxymethyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopenta-6-ene[*e*]pyran-3-one (**4**), (1*R*, 4*R*, 4a*S*, 7a*S*)-4,7-dihydroxymethyl-1-hydroxyl-1,4,4a, 7a-tetrahydrocyclopenta-6-ene[*e*]pyran-3-one (**5**), (1*R*, 4*S*, 4a*S*, 7a*S*)-4,7-dihydroxy-methyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopenta-6-ene[*e*]pyran-3-one (**6**), as well as five known non-glycosidic iridoids mussaenin A (**7**), gardendiol (**8**), isoboonein (**9**), 4-epi-alyxialactone (**10**) and rehmaglutin D (**11**) have been isolated from the Chinese medicinal plant *Cymbaria mongolica*. Their structures were elucidated by spectroscopic methods. These compounds exhibit significant antitumor and antibacterial activity.

Keywords: *Cymbaria mongolica*; Scrophulariaceae; Non-glycosidic iridoids; Cytotoxicity; Antibiosis

1. Introduction

Cymbaria mongolica (Scrophulariaceae) is a perennial herbaceous plant growing mainly in north China. Its rhizome has been used as a traditional Chinese medicine for treatment of rheumatism and dermatosis since ancient times (Ma et al., 1982). Its phytochemical or pharmacological properties have not been reported to date. In our effort to find active components from Chinese medicinal plants (Dai et al., 2001) we found from the acetone extract of the whole plant of *Cymbaria mongolica* six new non-glycosidic iridoids **1**–**6** described below as well as five known non-glycosidic iridoids mussaenin A (**7**), gardendiol (**8**), isoboonein (**9**), 4-epi-alyxialactone (**10**) and rehmaglutin D (**11**). We report herein the isolation, structural elucidation and antitumor and antibacterial activities of these compounds.

2. Results and discussion

From the acetone extract of the whole plant of *Cymbaria mongolica* eleven non-glycosidic iridoids (**1**–**11**) were obtained and purified by repeated chromatography on silica gel and Sephadex LH-20. Each of the isolates was subjected to detailed spectroscopic analyses to establish their chemical structures.

Compound **1** was obtained as a white amorphous powder. HR–EIMS gave a molecular ion peak at m/z 230.1058, corresponding to the molecular formula $C_{11}H_{18}O_5$ (calc. 230.1057). Its IR spectrum showed characteristic bands of hydroxyl (3368 cm^{-1}) and δ-lactone (1735 cm^{-1}) functionalities. The presence of the δ-lactone moiety was also supported by the signal at δ 173.0 in the ^{13}C NMR spectrum. The ^1H NMR spectrum showed signals of two methyls and three protons on oxygenated carbons (Table 1). The ^{13}C NMR and DEPT spectra exhibited signals of 11 carbons (2×C, 4×CH, 3×CH_2 and 2×CH_3) (Table 2). Taking into account the three double bond equivalents, compound **1** was deduced to be a iridolactone bicyclic monoterpene. The structure was confirmed by 2D NMR spectroscopic analysis. The ^1H–^1H COSY and HMQC spectra of **1** suggested the presence of a –O–CH–CH–CH(–CH–CH_2–O)–CH_2–CH_2– moiety. In the HMBC spectrum clear correlations were observed between the quaternary carbon at δ 80.1 (C-8) with the signals at δ 1.23 (CH_3-10), δ 1.97 (H-7β) and δ 2.69 (H-9), between the carbonyl carbon at δ 173.0 (C-3) with the H-4 (δ 2.00) and H-1 (δ 5.07), and between the methine carbon at δ 92.7

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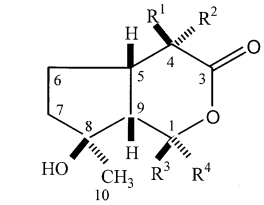
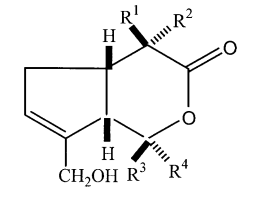
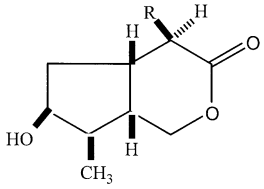


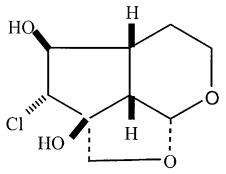
	R^1	R^2	R^3	R^4
1	CH_2OH	H	OCH_3	H
2	H	CH_2OH	H	OCH_3
3	H	CH2OH	OCH3	H
7	CH2OH	H	H	H



	R^1	R^2	R^3	R^4
4	H	CH_2OH	OCH_3	H
5	H	CH_2OH	OH	H
6	CH2OH	H	OCH3	H
8	CH2OH	H	H	H



9 R = H; **10** R = CH_2OH



11

(C-1) and 3.70 (OCH_3-1) (Table 3). The coupling constant of 8.8 Hz between H-1 and H-9 suggested that **1** should take a boat-lactone conformation of the iso-iridomyrmecin-type (Fig. 1) as discussed previously (Sisido et al., 1968; Uesato et al., 1987). The coupling constant of 11.4 Hz between H-4 and H-5 indicated a trans-diaxial relationship, hence H-4 must be α-oriented. The clear NOE correlations between 10-CH_3 and H-1α, H-4α, H-7α indicated that the 10-CH_3 group must also be α-oriented. Comparison of the ^{13}C NMR spectral data of **1** with those of mussaenin A (**7**) (Zhao et al, 1996) showed that H-1β in **7** is replaced by a methoxy group in **1**. Therefore, **1** was assigned to be a new non-glycosidic iridoid (1*R*,4*S*,4a*S*,7*S*,7a*S*)-7-hydroxyl-4-hydroxymethyl-7-methyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopenta-[*e*]pyran-3-one (1β-methoxyl-mussaenin A). The NMR spectral data of mussaenin A (**7**) (Zhao et al., 1996) were also listed in Tables 1 and 2 for comparison.

Compound **2** was obtained as a colorless gum. HR–EIMS showed a molecular ion peak at *m*/*z* 230.1062, corresponding to the molecular formula $C_{11}H_{18}O_5$ (calc. 230.1057). Its IR spectrum showed absorption bands for hydroxyl (3399 cm^{-1}) and lactone (1733 cm^{-1}) moieties. The ^1H, ^{13}C and 2D NMR spectral data of **2** were quite similar to those of **1,** except for significant differences in some coupling constants (Tables 1 and 2). The coupling constant of 3.0 Hz for H-9 and H-1β, and that of 3.0 Hz for H-4 and H-5 suggested that **2** had an iridomyrmecin type conformation with both the methoxyl and hydroxymethyl groups α-oriented (Fig. 1) as described previously (Sisido et al., 1968; Uesato et al., 1987). Thus, **2** was assigned as an diastereomer of **1**, i.e. (1*S*,4*R*,4a*S*,7*S*,7a*S*)-7-hydroxyl-4-hydroxymethyl-7-methyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopenta[*e*]pyran-3-one (1α-methoxyl-4-epi-mussaenin A).

Compound **3** was obtained as a colorless gum, whose HR–EIMS gave a molecular ion peak at *m*/*z* 230.1062 corresponding to the molecular formula $C_{11}H_{18}O_5$ (calc. 230.1057). Its IR spectrum showed absorption bands for hydroxyl (3399 cm^{-1}) and lactone moieties (1732 cm^{-1}).

Table 1
^1H NMR spectral data of compounds **1**–**8** (400 MHz, $CDCl_3$)[a]

H	**1**	**2**	**3**	**4**	**5**	**6**	**7**[b]	**8**[c]
1α	5.07 *d* (8.8)		5.00 *d* (7.2)	4.81 *d* (8.3)	4.19 *d* (8.7)	4.46 *d* (8.3)	4.06 *dd* (12.4, 11.0)	4.0 *t* (11.6)
1β		5.16 *d* (3.0)					4.34 *dd* (11.1, 5.9)	4.62 *dd* (11.6, 6.0)
4	2.00 *ddd* (11.4, 8.5, 5.7)	2.10 *ddd* (7.5,5.7, 3.0)	2.06 *ddd* (7.2, 5.8, 3.2)	2.33 *ddd* (4.0, 5.2, 5.8)	2.37 *ddd* (5.6,5.9, 5.2)	2.99 *ddd* (11.6, 4.7, 4.8)	2.71 *ddd* (9.0, 5.1, 3.7)	2.52 *m*
5	3.10 *m*	3.09 *m*	2.61 *m*	2.59 *m*	2.80 *m*	2.91 *m*	2.89 *m*	2.71 *m*
6α	1.75 *m*	1.60 *m*	1.61 *m*	2.01 *m*	2.18 *m*	2.19 *m*	1.71 *m*	2.25 *brd* (16. 4)
6β	1.97 *m*	2.14 *m*	1.98 *m*	2.70 *m*	2.22 *m*	2.27 *m*	2.50 *m*	2.80 *brdd* (16.4, 9.7)
7α	1.70 *m*	1.79 *m*	1.75 *m*	5.70 *s*	5.73 *s*	5.81 *s*	1.81 *m*	5.17 *brs*
7β	1.97 *m*	1.83 *m*	1.76 *m*				1.87 *m*	
9	2.69 *dd* (8.8, 10.8)	2.63 *dd* (3.0, 10.1)	1.80 *m*	2.36 *t* (8.3)	2.38 *dd* (8.3, 8.7)	2.41 *dd* (8.3, 8.4)	2.63 *ddd* (12.4,9.4, 5.9)	3.20 *m*
10	1.23 *s*	1.28 *s*	2.39 *dd* (7.2, 8.0)	4.20 *s*	4.25 *s*	4.23 *s*	1.75 *s*	4.15 *m*
11a	3.24 *dd* (11.4, 8.5)	3.48 *dd* (12.0, 7.5)	1.35 *s*	3.49 *dd* (12.0, 5.2)	3.46 *dd* (12.0, 5.9)	3.71 *dd* (12.0, 4.7)	4.15 *dd* (11.2, 5.1)	3.75 *dd* (11.8, 6.4)
11b	3.85 *dd* (11.4, 5.7)	4.06 *dd* (12.0, 5.7)	3.73 *dd* (12.1, 7.2)	3.95 *dd* (12.0, 5.8)	4.00 *dd* (12.0, 5.2)	4.10 *dd* (12.0, 4.8)	4.22 *dd* (11.2, 3.7)	3.85 *dd* (11.8, 3.2)
OCH_3	3.70 *s*	3.75 *s*	3.95 *dd* (12.1, 5.8) 3.72 *s*	3.71 *s*		3.68 *s*		

[a] Chemical shifts (ppm), multiplicity, and coupling constants (Hz in parentheses).
[b] From Zhao et al., 1996.
[c] From Zhao et al., 1994.

Table 2
^{13}C NMR spectral data of compounds **1**–**8** (100.58 MHz, $CDCl_3$)

C	1	2	3	4	5	6	7[a]	8[b]
1	92.7	91.7	94.6	95.2	92.9	98.7	67.2	69.0
3	173.0	172.9	173.6	173.9	173.7	172.4	175.3	175.4
4	49.3	46.5	47.7	43.6	39.5	40.9	45.5	35.0
5	38.3	34.8	38.3	39.6	38.3	40.5	35.4	46.5
6	23.2	27.1	27.3	35.9	29.7	30.9	29.7	38.5
7	37.9	38.9	40.2	128.4	127.2	128.7	39.1	128.2
8	80.1	80.1	79.7	143.1	143.2	144.8	80.1	140.8
9	48.2	46.8	49.9	50.9	50.9	50.7	49.2	45.7
10	24.1	25.0	25.5	60.1	60.3	61.4	23.4	60.6
11	63.7	61.4	61.0	63.6	63.7	62.3	60.9	60.2
OCH_3	51.7	51.9	51.0	51.9		51.6		

[a] From Zhao et al., 1996.
[b] From Zhao et al., 1994.

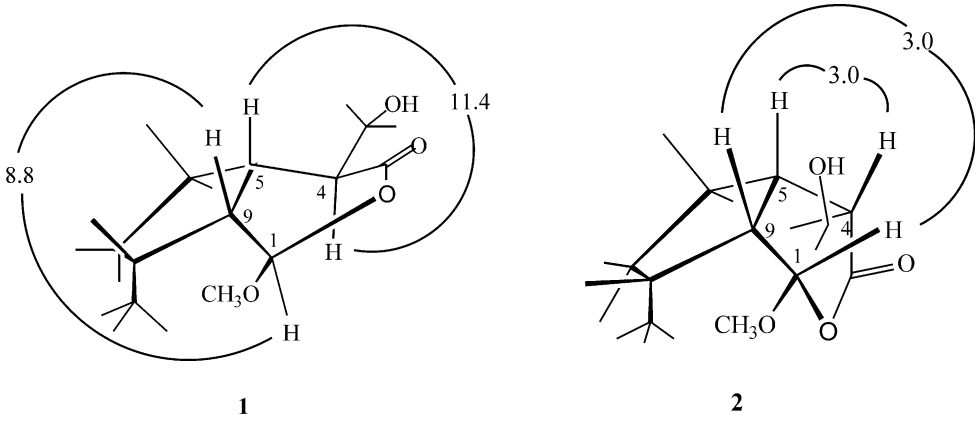
Table 3
HMBC correlations of compounds **1** and **6**

1		6	
H-1	C-3, C-9, OCH_3	H-1	C-9, C-8, OCH_3
H-4	C-3, C-5, C-6, C-9, C-11	H-4	C-3, C-5, C-6, C-11
H-7	C-5, C-6, C-8	H-7	C-5, C-6, C-8, C-9, C-10
H-9	C-1, C-3, C-5, C-6, C-8	H-9	C-1, C-5, C-6, C-7, C-8
H-10	C-7, C-8, C-9	H-10	C-7, C-8, C-9

The ^1H and ^{13}C NMR spectral data and 2D NMR spectrum of **3** were quite similar to those of **1**, except for a significant difference in the coupling constant of H-4 and H-5 (Table 1). The coupling constant of 3.2 Hz indicated an equatorial-axial relationship between H-4 and H-5, suggesting that the 4-hydoxymethyl group must be α-oriented as was next confirmed by clear NOE correlations between 10-CH_3 and H-1α, H-7α and H-11. Therefore, **3** was assigned to be (1*R*,4*R*,4a*S*,7*S*,7a*S*)-7-hydroxyl-4-hydroxymethyl-7-methyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopenta[*e*]pyran-3-one (1β-methoxyl-4-epi-mussaenin A).

Compound **4** was obtained as a colorless gum, and was assigned a molecular formula of $C_{11}H_{16}O_5$ by HR–EIMS (228.0896; calc. 228.0890). Its IR spectrum showed absorptions at 3390 cm^{-1} (OH), 1732 cm^{-1} (C=O) and 1087 cm^{-1} (C–O–C). The ^{13}C NMR spectrum of **4** showed signals for one trisubstituted double bond, two methylenes bearing oxygen functions, one methylene and five methines, of which one connected two oxygen functionalities (Table 2). Comparison of these data with those of compound **1** suggested that **4** was a non-glycosidic iridoid with two hydroxyls and a trisubstituted double bond. The presence of an allylic moietiey was confirmed by the cross-peaks between H-6α/H-7, H-10/H-7 and H-9/H-7 in the ^1H–^1H COSY spectrum. The location of the trisubstituted double bond and the hydroxyl groups were assigned at C-7 (δ 128.4), C-10 (δ 60.1) and C-11 (δ 63.6), respectively, based on the HMBC spectrum which showed correlations of C-7/H-6, H-10; C-8/H-7, H-9, H-10; C-4/H-5, H-11 and C-10/H-7. The coupling constant of 8.3 Hz for H-9/H-1 demonstrated that **4** possessed an iso-iridomyrmecin type structure, i. e. the C-1 methoxyl was β-oriented. The coupling constant of 4.0 Hz between H-4 and H-5 indicated an equatorial-axial relationship between these protons, hence the C-4 hydroxymethyl group must be α-oriented. Therefore, **4** was assigned as (1*R*,4*R*,4a*S*,7a*S*)-4,7-dihydroxymethyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopent-6-ene[*e*]pyran- 3-one (1β-methoxyl-4-epigardendiol). The NMR spectral data of gardendiol (**8**) (Zhao et al., 1994) were also listed in Tables 1 and 2 for comparison.

The ^1H, ^{13}C NMR and DEPT spectra of compound **5** were similar to those of **4** except for the absence of the OMe group and the upfield shift of C-1 and H-1 (δ 92.9 and 4.19 respectively) than those of **4** (δ 95.2 and 4.81 respectively). It indicated that the presence of a hydroxyl group on C-1. Hence **5** was assigned as (1*R*, 4*R*, 4a*S*, 7a*S*)- 4,7-dihydroxymethyl-1-hydroxyl-1,4,4a,7a- tetrahydrocyclopenta-6-ene[*e*]pyran-3-one (1β-hydroxyl-4-epigardendiol).



1 **2**

Fig. 1. The possible conformation of compounds **1** and **2**.

The ^1H, ^{13}C NMR and DEPT spectra of compound **6** were similar to those of **4** except for the significantly larger coupling constant of 11.6 Hz for H-4 / H-5 that demonstrated the β-orientation of the C-4 hydroxymethyl group (Tables 1–3). Thus, **6** is assigned as (1*R*, 4*S*, 4a*S*, 7a*S*)-4,7-dihydroxymethyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopenta-6-ene[*e*]pyran-3-one (1β-methoxyl-gardendiol).

Compounds **7**–**11** were identified as mussaenin A, gardendiol, isoboonein, 4-epi-alyxialactone and rehmaglutin D respectively, by comparing their NMR spectral data with those reported in the literatures (Zhao et al., 1994, 1996; Bianco et al., 1994; Topcu et al., 1990; Morota et al., 1989, respectively).

The cytotoxicity and antibacterial activity of compounds **1**–**11** have not been reported previously. Therefore, the antitumour activity of **1**–**11** was determined using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay (Price and McMillan, 1990) with three tumor cell lines, i.e. human hepatoma cells, (SMMC-7721), human uterine cervix carcinoma cells (Hela) and mouse melanotic carcinoma cells (B16) with vincristine as a positive control (Table 4). It is seen from the table that all of these compounds exhibit moderate in vitro cytotoxic activity against these tumor cells. The 50% inhibitory concentration (IC_{50}) values of some compounds are comparable to vincristine whereas others are much smaller than vincristine.

The antibacterial activity of **1**–**11** was determined against *Bacillus subtilis, Escherichia coli* and *Staphylococcus aureus* and compared with that of chloramphenicol (Table 5). It is noted that these non-glycosidic iridoids, especially **1**, possess antibacterial activity close to that of chloramphenicol.

Table 4
Antitumor activity of compounds **1**–**11**[a]

Compound	SMMC-7721	B16	Hela
1	97.6±2.4	88.3±5.6	86.5±5.0
2	111.6±4.5	96.5±4.8	87.9±5.9
3	98.6±2.0	100.2±3.3	83.2±4.6
4	85.1±3.5	56.4±4.7	71.8±4.1
5	88.8±1.9	40.2±2.6	73.2±2.2
6	78.3±1.6	75.2±4.2	75.4±2.5
7	102.3±3.2	78.2±3.9	94.2±4.3
8	100.1±4.3	88.4±3.5	96.3±3.3
9	135.2±5.6	120.3±1.8	101.2±2.1
10	122.2±5.3	90.1±2.2	92.3±1.7
11	45.4±3.8	60.7±1.9	71.2±2.9
Vincristine	63.2±1.8	70.7±2.8	67.2±2.2

[a] Activities are expressed as IC_{50} (50% inhibitory concentration) in μg ml^{-1}. Data are expressed as mean ± standard deviations of triplicate determinations.

Table 5
Antibacterial activity of compounds **1**–**11**[a]

Compound	*B. subtilis*	*E. coli*	*S. aureus*
1	14.5±1.0	13.9±1.5	14.5±1.5
2	12.8±0.9	14.9±1.4	13.7±2.0
3	13.9±0.9	14.1±2.9	12.5±1.9
4	11.7±0.8	13.5±1.2	9.5±1.7
5	14.0±1.1	12.4±0.8	13.2±0.5
6	11.1±1.2	12.8±1.3	13.3±0.4
7	14.2±0.7	13.6±1.1	12.6±1.5
8	10.9±0.8	11.8±1.0	13.1±0.6
9	13.5±0.6	12.0±1.5	10.1±1.4
10	12.2±1.3	9.9±1.2	9.3±1.7
11	13.4±1.8	14.5±0.9	13.2±0.8
Chloramphenicol	14.5±1.1	14.9±1.3	15.1±1.2

[a] Activities are expressed as the diameter of the inhibitory zone in mm. Data are expressed as mean ± standard deviations of triplicate determinations.

3. Experimental

3.1. General

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. The IR spectra were taken on a Nicolet 170SX FT–IR spectrometer. ^1H, ^{13}C NMR and 2D NMR spectra were recorded using a Brüker AM 400 NMR spectrometer at 400 and 100.58 MHz respectively with TMS as internal standard. HR–EIMS and EIMS data were obtained using a Brüker APEX II FT–MS and HP-5988 MS spectrometers respectively. Silica gel (200–300 and 300–400 mesh) was used for CC and silica GF_{254} for TLC. Spots were detected on TLC under UV or by heating after spraying with 5% H_2SO_4 in C_2H_5OH.

3.2. Plant material

The whole plants of *Cymbaria mongolica* were collected in the suburb of Lanzhou city, Gansu province, China, in August 1999. A voucher specimen (No. 9902) was deposited at the National Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou, China, and identified by Professor Yong-Hong Zhang at the Department of Chemistry, Lanzhou University.

3.3. Extraction and isolation

The chopped whole dry plant material (2.5 kg) was extracted repeatedly (3 times, 7 days each time) with acetone at room temperature to give a residue (68 g) after evaporation. This residue was separated by CC using 700 g silica gel (200–300 mesh) with a gradient (2000 ml each eluant) of petroleum ether–acetone (20:1, 15:1, 10:1, 7:1, 5:1, 3:1, 1:1, 0:1). The gummy crude extract containing **4**–**8** was obtained from the fraction eluted with petroleum ether–acetone (7:1), and **1**–**3** and

9–**11** from the fraction eluted with petroleum ether–acetone (5:1) and subjected to gel filtration (Sephadex, LH-20) followed by silica gel (300–400 mesh) column chromatography eluted with petroleum ether–AcOEt (6:1 and 4:1 respectively) to give **1** (15 mg), **2** (20 mg), **3** (17 mg), **4** (20 mg), **5** (35 mg), **6** (17 mg), **7** (18 mg), **8** (15 mg), **9** (28 mg), **10** (25 mg) and **11** (25 mg).

*3.4. (1R,4S,4aS,7S,7aS)-7-hydroxyl-4-hydroxymethyl-7-methyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopenta[e]-pyran-3-one (**1**)*

White amorphous powder. $[\alpha]_D^{25}$ +33.5 (*c* 0.35, $CHCl_3$); HR–EIMS: *m/z* = 230.1058 [$C_{11}H_{18}O_5$, requires: 230.1057]; IR (KBr): ν_{max} = 3368 (OH), 1735 (C=O) cm^{-1}. EIMS: *m/z* (rel. int.) = 230 (3) $[M]^+$, 215 (6), 212 (24), 198(43), 166 (80), 134 (49), 79 (44), 43 (100). For ^1H and ^{13}C NMR spectral data see Tables 1 and 2. For HMBC correlations see Table 3.

*3.5. (1S,4R,4aS,7S,7aS)-7-hydroxyl-4-hydroxymethyl-7-methyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopenta[e]-pyran-3-one (**2**)*

Colorless gum. $[\alpha]_D^{25}$ +23.7 (*c* 0.25, $CHCl_3$); HR–EIMS: *m/z* = 230.1062 [$C_{11}H_{18}O_5$, requires: 230.1057]; IR (KBr): ν_{max} = 3399 (OH), 1733 (C=O) cm^{-1}. For ^1H and ^{13}C NMR spectral data see Tables 1 and 2.

*3.6. (1R,4R,4aS,7S,7aS)-7-hydroxyl-4-hydroxymethyl-7-methyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopenta[e]-pyran-3-one (**3**)*

Colorless gum: $[\alpha]_D^{25}$ +23.9 (*c* 0.45, $CHCl_3$); HR–EIMS: *m/z* = 230.1062 [$C_{11}H_{18}O_5$, requires: 230.1057]; IR (KBr): ν_{max} = 3399 (OH), 1733 (C=O) cm^{-1}. EIMS: *m/z* (rel. int.) 230 (5) $[M]^+$, 191 (11), 71 (37), 43 (100). For ^1H and ^{13}C NMR spectral data see Tables 1 and 2.

*3.7. (1R,4R,4aS,7aS)-4,7-dihydroxymethyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopenta-6-ene[e]pyran-3-one (**4**)*

Colorless gum: $[\alpha]_D^{25}$ 27.8 (*c* 0.20, $CHCl_3$); HR–EIMS: *m/z* = 228.0896 [$C_{11}H_{16}O_5$, requires: 228.0890]; IR (KBr): ν_{max} = 3390 (OH), 1732 (C=O) cm^{-1}. EIMS: *m/z* (rel. int.) 228 (5) $[M]^+$, 210 (23), 177 (40), 154 (40), 121 (37), 96 (100), 43 (35). For ^1H and ^{13}C NMR spectral data see Tables 1 and 2.

*3.8. (1R,4R,4aS,7aS)-4,7-dihydroxymethyl-1-hydroxyl-1,4,4a,7a-tetrahydrocyclopenta-6-ene[e]pyran-3-one (**5**)*

Colorless gum: $[\alpha]_D^{25}$ +32.0 (*c* 0.30, $CHCl_3$); IR (KBr): ν_{max} = 3390 (OH), 1733 (C=O) cm^{-1}. EIMS: *m/z* (rel. Int) 216 (5)$[M]^+$. For ^1H and ^{13}C NMR spectral data see Tables 1 and 2.

*3.9. (1R,4S,4aS,7aS)-4,7-dihydroxymethyl-1-methoxyl-1,4,4a,7a-tetrahydrocyclopenta-6-ene[e]pyran-3-one (**6**)*

Colorless gum: $[\alpha]_D^{25}$ −8.5 (*c* 0.10, $CHCl_3$); HR–EIMS: *m/z* = 228.0903 [$C_{11}H_{16}O_5$, requires: 228.0890]; IR (KBr): ν_{max} = 3393 (OH), 1730 (C=O), 1060 (C–O–C) cm^{-1}. EIMS: *m/z* (rel. int.) 228 (8) $[M]^+$, 211 (7), 132 (37), 105 (37), 91 (26), 77 (22), 67 (13). For ^1H and ^{13}C NMR spectral data see Tables 1 and 2. For HMBC correlations see Table 3.

3.10. Cytotoxicity assay

The cytotoxicity of compounds **1**–**11** was tested in three cell lines: human hepatoma (SMMC-7721), mouse melanoma (B16) and human carcinoma of uterine cervix (HeLa). Cells were cultured at 37 °C under a humidified atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal calf serum and dispersed in replicate 96-well plates with 1×10^4 cells/well for 24 h. Compounds **1**–**11** (10–400 μM) or vincristine (positive control) were then added. After 48 h exposure to the toxins, cell viability was determined by the [3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide] (MTT) colorimetric assay (Price and McMillan, 1990) by measuring the absorbance at 595 nm with an ESILA reader. Each test was performed in triplicate.

3.11. Antibacterial assay

The paper-disk method (Xu and Bian, 1982) was used for antibacterial tests. A 10 μg portion of compounds **1**–**11** or chloramphenicol (used as a positive control) was applied onto a paper disk, and the paper disk was air-dried. Then the disks were placed on agar plates that had been seeded with *B. subtilis*, *E. coli* or *S. aureus*, respectively, and incubated at 37 °C for 24 h. The antibacterial activity was determined by measuring the diameter of the inhibitory circles. Each test was performed in triplicate.

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