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Three new polymeric isopropenyl benzofurans from *Ligularia stenocephala*

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Three new polymeric isopropenyl benzofurans, 4-methyl-2,4-bis(5,6-dimethoxy-2-benzofuranyl)-1-pentene, stenocephalin A (**1**), 4,6-dimethyl-2,4,6-tri(5,6-dimethoxy-2-benzofuranyl)-1-heptene, stenocephalin B (**2**) and 4,6,8-trimethyl-2,4,6,8-tetra(5,6-dimethoxy-2-benzofuranyl)-1-nonene, stenocephalin C (**3**), together with seven known compounds (**4**–**10**) were isolated from the roots of *Ligularia stenocephala*. The structures of the new compounds were elucidated on the basis of spectral evidence, especially on 2D NMR. In addition, the cytotoxic activity and the anti-bacterial activity of compounds **2**, **3**, **5** and **6** were tested.

1. Introduction

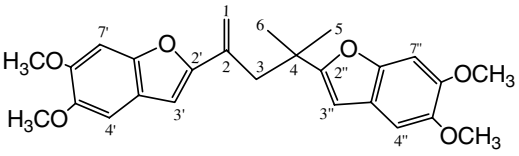
Ligularia stenocephala (Compositae) has long been used as a Chinese folk medicine in the treatment of edema and scrofula (Jiang 1985). Previous study of this plant by a Japanese research group yielded a benzofuran derivative, 5,6-dimethoxy-2-isopropenylbenzofuran (Murae et al. 1968). We re-examined the roots of *L. stenocephala* collected in Henan province, China and obtained three new polymeric isopropenyl benzofurans (**1**–**3**) and seven known compounds (**4**–**10**). The anti-bacterial activity of compounds **2**, **3**, **5** and **6** against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli,* as well as the cytotoxic activity of compounds **2**, **3**, **5** and **6** against human hepatoma (Bel-7402) and human ovarian neoplasm (HO-8910) cell lines were assayed.

2. Investigations, results and discussion

The extract from the roots of *Ligularia stenocephala* was rechromatographed over silica gel to yield three new compounds (**1**–**3**) and seven known compounds. The known compounds were determined to be 5,6-dimethoxy-2-isopropenylbenzofuran (**4**) (Murae et al. 1968), euparin (**5**) (Steelink et al. 1979), β-dictyopterol (**6**) (Hu et al. 1997), 3,5-bis(3,3-dimethylallyl)-4-hydroxyacetophenone (**7**) (Bohlmann et al. 1973), 2-acetyl-5,6-dimethoxybenzofuran (**8**) (Jia et al. 1994), (24R)-stigmast-7,22(E)-dien-3α-ol (**9**) (Jahan et al. 1995) and β-sitosterol (**10**) (Chen et al. 1998) by comparison of their spectral data (^{1}H, ^{13}C NMR, DEPT and EI-MS) with those reported in the literature respectively.

Compound **1** was obtained as a white powder, its HR-ESIMS showing $[M+NH_4]^+$ at m/z 454.2216 (calc. for $C_{26}H_{32}O_6N$ 454.2224) which suggested the molecular formula $C_{26}H_{28}O_6$, which was further confirmed by the ^{13}C and DEPT NMR spectra. The IR spectrum showed absorptions at 3155, 1622, 1486, 1122, 840 cm^{-1} and the UV (λ_{max}) absorption at 329 nm, combined with ^{13}C NMR spectral data (Table 1) and ^{1}H NMR spectral feature, are typical of benzofuran rings (Murae et al. 1968). Moreover, the ^{1}H NMR spectrum displayed four downfield singlet signals (δ 6.80, 6.85, 6.89 and 7.03, each 1 H) and IR spectrum gave absorption at 840 cm^{-1} suggesting the presence of 1,2,4,5-tetrasubstituted benzene rings. Four downfield methoxyl groups (δ 3.87 s, 3.88 s, 3.89 s and 3.93 s, each 3 H) may be located on benzene rings from the ^{1}H, ^{13}C and DEPT NMR spectra. The positions of the four OCH_3 groups were further confirmed at C-5′ (δ 146.2), C-6′ (δ 147.2), C-5″ (δ 146.3) and C-6″ (δ 148.1) from the long-range correlations of $4\times OCH_3$ with C-5′, C-6′, C-5″ and C-6″ in the HMBC spectrum. The EI-MS gave a strong peak at m/z 219 (100), which also showed that compound **1** had a isopropyl dimethoxybenzofuran moiety (Murae et al. 1968). Furthermore, the ^{1}H NMR spectrum revealed the presence of two identical tertiary methyl groups (δ 1.39 s, 2×3 H), one terminal double bond (δ 4.76, 5.70, s, each 1 H) and one methylene group (δ 2.82 s, 2 H), and combined with one quaternary carbon signal (δ 37.0) of the ^{13}C NMR spectrum, a partial structure of $-C(=CH_2)CH_2C(CH_3)_2-$ could be deduced from the HMBC correlations of H-1 (δ 4.76, 5.70) with C-2 (δ 134.3), C-3 (δ 44.3) and C-2′ (δ 156.5); H-3 (δ 2.82) with C-1 (δ 114.8), C-2 (δ 134.3), C-4 (δ 37.0), C-5 (δ 26.8), C-6 (δ 26.8) and C-2″ (δ 164.0). Thus, the above moiety was attached to C-2′ and C-2″ of the benzofuran rings, respectively. On the basis of the above evidence, the structure of compound **1** was identified as 4-methyl-2,4-bis(5,6-dimethoxy-2-benzofuranyl)-1-pentene and named as stenocephalin A.

Compound **2** was also obtained as a white powder, and the molecular formula was deduced as $C_{39}H_{42}O_9$ from its HR-ESIMS $[M+H]^+$at m/z 655.2910 (calc. for $C_{39}H_{43}O_9$ 655.2902). The IR spectrum of **2** at 3118, 1621, 1544,



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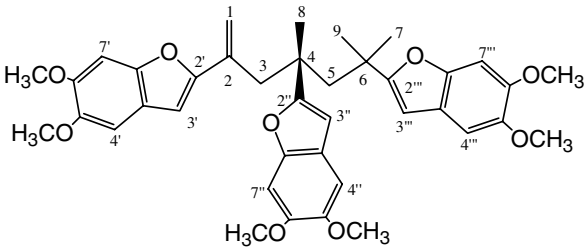

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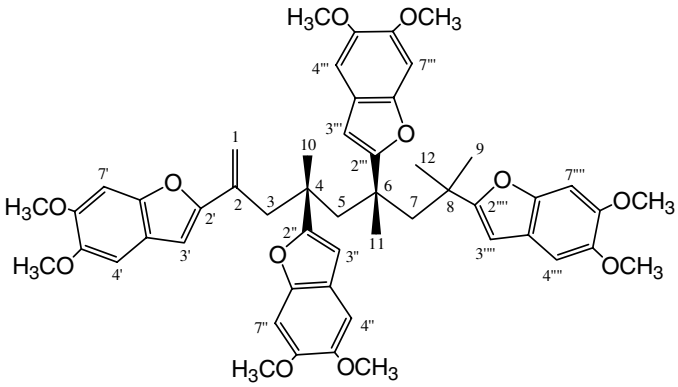

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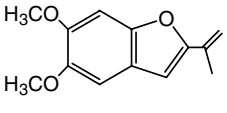

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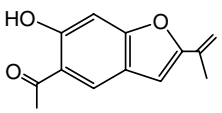

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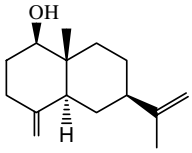

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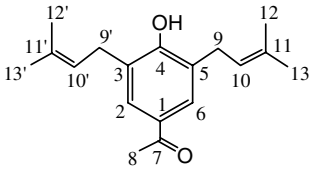

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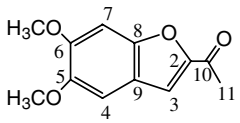

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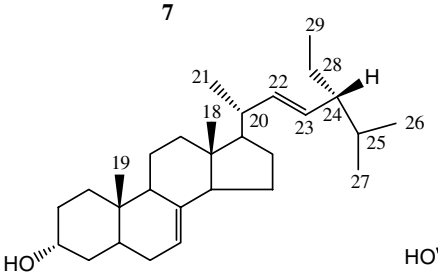

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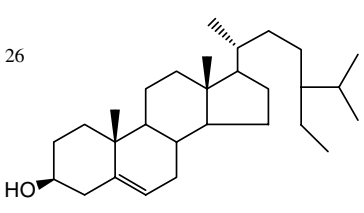

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1489, 1212, 1125, 850 cm^{-1} and UV at 307.0 nm, combined with ^{1}H and ^{13}C NMR spectral data (Table 1), suggested the presence of benzofuran rings (Murae et al. 1968). Analysis of the ^{1}H NMR of **2** indicated the presence of six methoxyl groups (δ 3.82–3.89) at aromatic rings; two kinds of singlet Ar-H (δ 6.64–6.74 and 6.80–6.88) in a 1,4-relationship of benzene rings. These relationships can be observed in its HMBC and HMQC spectra. The EI-MS also gave a strong peak at m/z 219 (82), suggesting that compound **2** had an isopropyl dimethoxybenzofuran fragment like that of **1**. The ^{13}C NMR spectrum of **2** showed the presence of one terminal double bond (δ 115.1 and 133.4), three tertiary methyl groups (δ 21.1, 26.8 and 30.4), two methylene groups (δ 46.1 and

Table 1: ^{13}C NMR data of 1, 2 and 3 (100 MHz, δ, ppm, $CDCl_3$, TMS)

	1	2	3		1	2	3
C(1)	114.8 (t)	115.1 (t)	115.3 (t)	C(5‴)	– – –	145.9[b] (s)	146.1[c] (s)
C(2)	134.3 (s)	133.4 (s)	133.3 (s)	C(5⁗)	– – –	– – –	146.2[c] (s)
C(3)	44.3 (t)	46.1 (t)	46.9 (t)	C(6′)	147.2[b] (s)	146.2[b] (s)	146.8[c] (s)
C(4)	37.0 (s)	40.3 (s)	40.1 (s)	C(6″)	148.1[b] (s)	146.9[b] (s)	146.9[c] (s)
C(5)	26.8 (q)	51.2 (t)	52.0 (t)	C(6‴)	– – –	146.9[b] (s)	147.0[c] (s)
C(6)	26.8 (q)	36.1 (s)	39.6 (s)	C(6⁗)	– – –	– – –	147.9[c] (s)
C(7)	– – –	30.4 (q)	53.7 (t)	C(7′)	95.0[c] (d)	94.8 (d)	94.8[d] (d)
C(8)	– – –	21.1 (q)	36.0 (s)	C(7″)	95.3[c] (d)	94.8 (d)	95.0[d] (d)
C(9)	– – –	26.8 (q)	29.7 (q)	C(7‴)	– – –	94.8 (d)	95.0[d] (d)
C(10)	– – –	– – –	20.2 (q)	C(7⁗)	– – –	– – –	95.2[d] (d)
C(11)	– – –	– – –	20.5 (q)	C(8′)	149.0[d] (s)	148.5[c] (s)	149.1[e] (s)
C(12)	– – –	– – –	27.9 (q)	C(8″)	149.4[d] (s)	148.7[c] (s)	148.4[e] (s)
C(2′)	156.5 (s)	156.5 (s)	156.4 (s)	C(8‴)	– – –	149.2[c] (s)	148.3[e] (s)
C(2″)	164.0 (s)	161.3 (s)	161.7 (s)	C(8⁗)	– – –	– – –	148.6[e] (s)
C(2‴)	– – –	164.3 (s)	162.1 (s)	C(9′)	120.6[e] (s)	120.5[d] (s)	120.6[f] (s)
C(2⁗)	– – –	– – –	163.9 (s)	C(9″)	120.8[e] (s)	120.6[d] (s)	120.6[f] (s)
C(3′)	101.1 (d)	100.4 (d)	102.5[a] (d)	C(9‴)	– – –	120.6[d] (s)	120.7[f] (s)
C(3″)	102.8 (d)	102.7 (d)	103.0[a] (d)	C(9⁗)	– – –	– – –	120.7[f] (s)
C(3‴)	– – –	103.2 (d)	102.7[a] (d)	C(10′)	56.2[f] (q)	56.0[e] (q)	56.0[g] (q)
C(3⁗)	– – –	– – –	100.3 (d)	C(10″)	56.2[f] (q)	56.1[e] (q)	56.0[g] (q)
C(4′)	102.1[a] (d)	101.7[a] (d)	101.7[b] (d)	C(10‴)	– – –	56.1[e] (q)	56.1[g] (q)
C(4″)	102.3[a] (d)	101.8[a] (d)	101.8[b] (d)	C(10⁗)	– – –	– – –	56.1[g] (q)
C(4‴)	– – –	101.9[a] (d)	102.0[b] (d)	C(11′)	56.4[f] (q)	56.2[e] (q)	56.2[g] (q)
C(4⁗)	– – –	– – –	102.1[b] (d)	C(11″)	56.4[f] (q)	56.3[e] (q)	56.2[g] (q)
C(5′)	146.2[b] (s)	145.8[b] (s)	145.9[c] (s)	C(11‴)	– – –	56.3[e] (q)	56.3[g] (q)
C(5″)	146.3[b] (s)	145.9[b] (s)	145.9[c] (s)	C(11⁗)	– – –	– – –	56.3[g] (q)

[a, b, c, d, e, f, g] Assignments in the same compound with the same sign may be alternatives although those given here are preferred

51.2) and two quaternary carbon atoms (δ 40.3 and 36.1), and was supported by the ^1H NMR spectrum. Therefore, a partial structure of $-C(=CH_2)CH_2C(CH_3)CH_2C(CH_3)_2-$ was deduced from the HMBC correlations of H-1 with C-2, C-3 and C-2′; H-3 with C-1, C-2, C-4, C-5, C-8 and C-2″; H-5 with C-3, C-4, C-6, C-7, C-8, C-9, C-2″ and C-2‴; in which the C-2, C-4 and C-6 were attached to C-2′, C-2″ and C-2‴ of benzofuran rings, respectively. In addition, the relative stereochemistry of **2** was deduced from a clear NOE between 8-CH_3 and H-3″ (9%) as well as H-3‴ (5%). Thus, compound **2** was established as being 4,6-dimethyl-2,4,6-tri(5,6-dimethoxy-2-benzofuranyl)-1-heptene and named as stenocephalin B.

Compound **3**, a white powder, had almost the same IR and UV absorptions as those of **2**. It had the composition $C_{52}H_{56}O_{12}$, as determined by FAB-MS $[M+H]^+$ at m/z 873 and HR-ESIMS $[M+Na]^+$ at m/z 895.3631 (calc. for $C_{52}H_{56}O_{12}Na$ 895.3664). The spectral features of compound **3** were very similar to those of **1** and **2** (Table 1). The ^{13}C NMR spectra indicated the presence of eight methoxyl groups (δ 56.0–56.3), four quaternary carbon atoms (δ 120.6–120.7), sixteen oxygenated quaternary carbon atoms (δ 145.9–163.9), and twelve methine (δ 94.8–103.0) in the downfield. In addition, the EI-MS gave a strong peak at m/z 219 (100), implying the presence of a fragment of isopropyl dimethoxybenzofuran. Based on the above evidence, compound **3** was shown to possess a moiety with four of the isopropyl dimethoxybenzofurans. Moreover, the signals of four tertiary methyl groups (δ 20.2, 20.5, 27.9 and 29.7), three methylene groups (δ 46.9, 52.0 and 53.7), one terminal double bond (δ 115.3 and 133.3) and three quaternary carbons (δ 40.1, 39.6 and 36.0) were shown in the ^{13}C and DEPT NMR spectra. It was supported by the ^1H NMR spectrum. In the HMBC study of **3**, the long-range correlations of H-1 with C-2, C-3 and C-2′; H-3 with C-1, C-2, C-4, C-5, C-10 and C-2″; H-5 with C-3, C-4, C-6, C-7, C-10, C-11, C-2″ and C-2‴; H-7 with C-5, C-6, C-8, C-9, C-11, C-12, C-2‴ and C-2⁗ suggested the presence of a partial structure of $-C(=CH_2)CH_2C(CH_3)CH_2C(CH_3)CH_2C(CH_3)_2-$, in which the C-2, C-4, C-6 and C-8 were attached to C-2′, C-2″, C-2‴ and C-2⁗ of the benzofuran rings, respectively. Also, the relative stereochemistry of **3** was deduced from a clear NOE between 10-CH_3 and H-3″ (8%) as well as H-3‴ (4%); between 11-CH_3 and H-3‴ (7%) as well as H-3″ (3%). Therefore, the structure of **3** was determined to be 4,6,8-trimethyl-2,4,6,8-tetra(5,6-dimethoxy-2-benzofuranyl)-1-nonene, and named as stenocephalin C.

Using the SRB method (Skehan et al. 1990), the cytotoxic activities of compounds **2**, **3**, **5** and **6** against human hepatoma (Bel-7402) and human ovaria carcinoma (HO-8910) cell lines were assayed by comparison with vincristine sulphate as standard. From the cytotoxic activity data (Table 2), it was found that euparin (**5**) has a significant inhibitory effect on cells HO-8910 (IC_{50} value 10.2 μg/ml) and Bel-7402 (IC_{50} value 27.3 μg/ml), which was as great as the positive control vincristine sulfate, while β-dictyopterol (**6**) exhibited appreciable cytotoxic activity against HO-8910 (IC_{50} value 59.8 μg/ml) and Bel-7402 (IC_{50} value 58.6 μg/ml), but although stenocephalin B **(2)** and stenocephalin C **(3)** had no effect because of their poor water-solubility.

Table 2: Cytotoxic activity of compounds 2, 3, 5 and 6

Compound	IC_{50} (μg/ml)	
	Bel-7402	HO-8910
Stenocephalin B (**2**)	– – –	– – –
Stenocephalin C (**3**)	– – –	– – –
Euparin (**5**)	27.3 ± 3.7	10.2 ± 3.8
β-Dictyopterol (**6**)	58.6 ± 8.8	59.8 ± 10.8
Vincristine sulfate	25.9 ± 3.4	20.7 ± 1.9

Table 3: Anti-bacterial activity* of compounds 2, 3, 5 and 6

Compound	*B. subtilis*	*S. aureus*	*E. coli*
Stenocephalin B (**2**)	+	+	+
Stenocephalin C (**3**)	++	+	+
Euparin (**5**)	++	++	+++
β-Dictyopterol (**6**)	++	++	++
H_2O	−	−	−
Chloramphenicol	+++	+++	+++

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

Compounds **2**, **3**, **5** and **6** were tested for their anti-bacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* by comparison with the standard, chloramphenicol. The results indicated that euparin (**5**) exhibited strong activity against *E. coli* (Table 3).

3. Experimental

3.1. Apparatus

Melting points were determined with a Kofler melting point apparatus and are uncorrected. Optical rotations were taken on a Perkin-Elmer 341 polarimeter. IR spectra were measured on a Nicolet 170 SX FT-IR instrument. UV spectra were recorded on a Shimadzu UV-260 instrument. ^{1}H NMR, ^{13}C NMR and 2D NMR spectra were recorded on a Bruker AM-400 FT-NMR spectrometer using TMS as the internal standard. HR-ESIMS data were recorded on a Bruker APEX II mass spectrometer. EI-MS data were obtained on an HP-5988A GC/MS instrument and FAB-MS data were taken on a VG-ZABHS mass spectrometer. Silica gel (200–300 mesh) used for column chromatography and silica gel GF_{254} for TLC were made by the Qingdao Marine Chemical Factory of China. Vincristine sulfate used as a positive control was supplied by Shanghai Hualian Pharmaceutical Company. Spots were detected on TLC under UV or by heating after spraying with 5% H_2SO_4 in C_2H_5OH (v/v).

3.2. Plant material

The roots of *L. stenocephala* (Maxim.) Matsum. et Koidz. were collected in Henan province, P. R. China, in July 2001. It was identified by Prof. C. S. Zhu of the Henan Agricultural University. A voucher specimen (No. 200171) was deposited in College of Chemistry and Chemical Engineering, Lanzhou University.

3.3. Extraction and isolation

The air-dried roots of *L. stenocephala* (3.1 kg) were pulverized and extracted four times at room temperature with petroleum ether (60–90 °C)-Et_2O-MeOH (1 : 1 : 1) (7 days each time). The solvent was evaporated under reduced pressure to yield a residue (165 g), which was subjected to column chromatography over silica gel (1000 g) and eluted with a gradient of petroleum ether/EtOAc (30 : 1, 20 : 1, 10 : 1, 5 : 1, 3 : 1, 2 : 1, 1 : 1, 0 : 1) to give eight fractions (Fr. 1–8) according to their TLC (silica gel GF_{254}) analysis. Fr. 1 (700 mg) was recrystallized with petroleum ether/Me_2CO (5 : 1) to afford **5** (270 mg). Fr. 2 (982 mg) was separated by column chromatography on silica gel (80 g) eluted with petroleum ether/Me_2CO (25 : 1, 20 : 1, 15 : 1) to afford three fractions (Fr. 2a–2c) according to their TLC (silica gel GF_{254}) analysis. Fr. 2a (210 mg) was further separated by column chromatography on silica gel (30 g) eluted with petroleum ether/Me_2CO (25 : 1) and purified by preparative TLC (silica gel GF_{254}) with petroleum ether/Me_2CO (5 : 2, R_f 0.46) to obtain **4** (7 mg). Fr. 2b (323 mg) was crystallized from petroleum ether/EtOAc (5 : 1) to give **7** (208 mg). Fr. 2c (232 mg) was separated by column chromatography on silica gel (30 g) eluted with petroleum ether/Me_2CO (20 : 1) to yield **6** (39 mg). Fr. 3 (154 mg) was separated by column chromatography on silica gel (15 g) eluted with petroleum ether/EtOAc (10 : 1) to afford **10** (27 mg) and crude **9**, then the crude **9** was further isolated by column chromatography on silica gel (8 g) eluted with petroleum ether/EtOAc (15 : 1) to obtain **9** (8 mg). Fr. 4 (391 mg) was separated by column chromatography on silica gel (35 g) eluted with $CHCl_3$/Me_2CO (50 : 1) to yield **8** (7 mg). Fr. 5 (212 mg) was separated by column chromatography on silica gel (20 g) eluted with $CHCl_3$/Me_2CO (40 : 1) and further purified by preparative TLC (silica gel GF_{254}) with $CHCl_3$/Me_2CO (20 : 1, R_f 0.66) as developer to obtain **1** (7 mg). Compound **1** exhibited a purple colour under UV and blue on TLC (silica gel GF_{254}) by heating after spraying with 5% H_2SO_4 in C_2H_5OH. Fr. 6 (610 mg) was separated by column chromatography on silica gel (65 g) eluted with $CHCl_3$/Me_2CO (40 : 1) to give **3** (35 mg) and crude **2**, the crude **2** being further purified by preparative TLC (silica gel GF_{254}) with $CHCl_3$/Me_2CO (20 : 1, R_f 0.57) as developer to obtain **2** (26 mg). Compounds **3** and **2** exhibited purple coloration under UV and blue on TLC (silica gel GF_{254}) by heating after spraying with 5% H_2SO_4 in C_2H_5OH.

3.3.1. Stenocephalin A (**1**)

White powder, molecular formula: $C_{26}H_{28}O_6$; m.p. 80–82 °C ($CHCl_3$); UV ($CHCl_3$): 329.0 nm (2.51); IR (ν_{max}^{KBr}, cm^{-1}): 3155, 2859, 1622, 1546, 1486, 1320, 1212, 1122, 1028, 928, 839 cm^{-1}; HR-ESIMS m/z 454.2216 $[M+NH_4]^+$ (calcd. for $[C_{26}H_{28}O_6+NH_4]^+$: 454.2224); EI-MS m/z (rel int): 436 ($[M]^+$ 6), 421 ($[M\text{-}15^+$ 1), 348 (2), 219 (100), 203 (4), 181 (7), 175 (6), 149 (10), 91 (45); ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 4.76 (1 H, s, 1a-H), 5.70 (1 H, s, 1b-H), 2.82 (2 H, s, 3-H), 1.39 (2 × 3 H, s, 5,6-H), 6.20 (1 H, s, 3′-H), 6.42 (1 H, s, 3″-H), 6.80 (1 H, s, 4′-H), 6.85 (1 H, s, 4″-H), 6.89 (1 H, s, 7′-H), 7.03 (1 H, s, 7″-H), 3.87–3.93 (4 × 3 H, s, 4 × OCH_3), ^{13}C NMR (DEPT): Table 1.

3.3.2. Stenocephalin B (**2**)

White powder, molecular formula: $C_{39}H_{42}O_9$; m.p. 184–186 °C ($CHCl_3$); $[\alpha]_D^{23}$: +20.0° (c 0.6, $CHCl_3$); UV ($CHCl_3$): 307.0 nm (1.69); IR (ν_{max}^{KBr}, cm^{-1}): 3118, 3082, 2993, 2831, 1621, 1544, 1489, 1466, 1321, 1212, 1125, 936, 850 cm^{-1}; EI-MS m/z (rel int): 655 ($[M+1]^+$ 1), 536 (2), 435 (2), 219 (82), 203 (5), 175 (5), 160 (3); HR-ESIMS m/z 655.2910 $[M+H]^+$ (calcd. for $[C_{39}H_{42}O_9+H]^+$: 655.2902); ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 4.53 (1 H, s, 1a-H), 5.59 (1 H, s, 1b-H), 2.60 (1 H, d, J = 14.7 Hz, 3a-H), 2.97 (1 H, d, J = 14.7 Hz, 3b-H), 2.19 (1 H, d, J = 14.1 Hz, 5a-H), 2.56 (1 H, d, J = 14.1 Hz, 5b-H), 1.35 (3H, s, 7-H), 1.07 (3 H, s, 8-H), 1.11 (3 H, s, 9-H), 5.94–6.31 (3 × 1 H, s, 3′, 3″, 3‴-H), 6.64–6.74 (3 × 1 H, s, 4′, 4″, 4‴-H), 6.80–6.88 (3 × 1 H, s, 7′, 7″, 7‴-H), 3.82–3.89 (6 × 3 H, s, 6 × OCH_3), ^{13}C NMR (DEPT): Table 1.

3.3.3. Stenocephalin C (**3**)

White powder, molecular formula: $C_{52}H_{56}O_{12}$; m.p. 174–175 °C ($CHCl_3$); $[\alpha]_D^{23}$: −7.0° (c 1.0, $CHCl_3$); UV ($CHCl_3$): 303.8 nm (0.59); IR (ν_{max}^{KBr}, cm^{-1}): 3116, 3081, 2993, 2831, 1622, 1548, 1487, 1439, 1323, 1246, 1123, 935, 843; FAB-MS m/z (rel int): 873 ($[M+1]^+$ 38), 857 ($[M\text{-}CH_3]^+$ 4), 655 (6), 437 (32), 435 (60); EI-MS m/z (rel int): 435 (1), 275 (3), 219 (100), 203 (20), 175 (9), 160 (9); HR-ESIMS m/z 895.3631 $[M+Na]^+$ (calcd. for $[C_{52}H_{56}O_{12}+Na]^+$: 895.3664); ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 4.52 (1 H, s, 1a-H), 5.57 (1 H, s, 1b-H), 2.49 (1 H, d, J = 13.6 Hz, 3a-H), 2.90 (1 H, d, J = 13.6 Hz, 3b-H), 2.29 (1 H, d, J = 14.0 Hz, 5a-H), 2.42 (1 H, d, J = 14.0 Hz, 5b-H), 2.03 (1 H, d, J = 14.4 Hz, 7a-H), 2.42 (1 H, d, J = 14.4 Hz, 7b-H), 1.27 (3 H, s, 9-H), 0.75 (3 H, s, 10-H), 0.82 (3 H, s, 11-H), 1.04 (3 H, s, 12-H), 5.82–6.20 (4 × 1 H, s, 3′, 3″, 3‴, 3⁗-H), 6.64–6.70 (4 × 1 H, s, 4′, 4″, 4‴, 4⁗-H), 6.78–6.96 (4 × 1 H, s, 7′, 7″, 7‴, 7⁗-H), 3.80–3.91 (8 × 3 H, s, 8 × OCH_3); ^{13}C NMR (DEPT): Table 1.

3.3.4. 5,6-Dimethoxy-2-isopropenylbenzofuran (**4**)

White needles, molecular formula: $C_{13}H_{14}O_3$; m.p. 72.5–73.5 °C (Me_2CO); EI-MS m/z (rel int): 218 ($[M]^+$ 100), 203 ($[M\text{-}15]^+$ 68), 175 (17), 160 (20), 147 (36), 132 (51), 119 (38), 91 (24), 69 (35), 41 (12); ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 7.01 (1 H, s, 4-H), 6.95 (1 H, s, 7-H), 6.52 (1 H, s, 3-H), 5.68 (1 H, s, 11a-H), 5.08 (1 H, s, 11b-H), 3.90 (3 H, s, OCH_3), 3.89 (3 H, s, OCH_3), 2.09 (3 H, s, 12-H); ^{13}C NMR δ ppm ($CDCl_3$, 100 MHz): 156.1 (s, C-2), 102.9 (d, C-3), 102.2 (d, C-4), 146.3 (s, C-5), 148.2 (s, C-6), 95.0 (d, C-7), 149.5 (s, C-8), 120.7 (s, C-9), 132.8 (s, C-10), 111.5 (t, C-11), 19.3 (q, C-12), 56.1 (q, OMe), 56.3 (q, OMe). The ^{13}C NMR data of **4** have not been reported in the literature previously.

3.3.5. Euparin (**5**)

Yellow needles, molecular formula: $C_{13}H_{12}O_3$; m.p. 116–117 °C (Me_2CO); IR ν_{max}^{KBr}, cm^{-1}): 3434 (OH), 3096, 1678 (C=O), 1631, 1560, 1465, 1262, 1140, 890, 831, 794; EI-MS m/z (rel int): 216 ($[M]^+$ 57), 201 ($[M\text{-}15]^+$ 100), 173 (27), 115 (19), 91 (11), 77 (8), 69 (19), 43 (30); ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 12.53 (1 H, s, OH), 7.88 (1 H, s, 4-H), 6.96 (1H, s, 7-H), 6.53 (1 H, s, 3-H), 5.75 (1 H, s, 11a-H), 5.19 (1 H, s, 11b-H), 2.67 (3 H, s, 14-H), 2.10 (3 H, s, 12-H);

3.3.6. β-Dictyopterol (**6**)

Yellow gum, molecular formula: $C_{15}H_{24}O$; $[\alpha]_{23}^{D}$: +16.9° (c 0.88, Me_2CO); IR (ν_{max}^{KBr}, cm^{-1}): 3380 (OH), 3080, 2918, 1714, 1646, 1441, 1378, 1017, 887; EI-MS m/z (rel int): 220 ($[M]^+$ 1), 205 ($[M\text{-}15]^+$ 3), 202 (2), 187 (4), 159 (8), 179 (2), 107 (27), 93 (35), 85 (100), 41 (84); ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 4.75 (1 H, brs, 12a-H), 4.72 (1 H, brs, 12b-H), 4.70 (1 H, brs, 15a-H), 4.50 (1H, brs, 15b-H), 3.40 (1 H, dd, J = 4.8, 11.5 Hz, 1α-H), 2.29 (1 H, dddd, J = 13.4, 5.1, 2.3 Hz, 3β-H), 1.94 (2H, m, 9β, 3α-H), 1.82 (1 H, tt, J = 11.0, 4.2 Hz, 7α-H), 1.74 (3 H, s, 13-H), 1.61 (2 H, m, 2β, 8β-H), 1.55 (2 H, m, 5α,6β-H), 1.41 (1 H, m, 2α-H), 1.32 (2 H, m,

6α,8α-H), 0.95 (1 H, m, 9α-H), 0.69 (3 H, s, 14-H); ^{13}C NMR δ ppm ($CDCl_3$, 100 MHz): 79.2 (d, C-1), 31.4 (t, C-2), 34.1 (t, C-3), 148.7 (s, C-4), 47.5 (d, C-5), 26.4 (t, C-6), 45.2 (d, C-7), 28.8 (t, C-8), 36.9 (t, C-9), 40.2 (s, C-10), 150.4 (s, C-11), 108.3 (t, C-12), 20.9 (q, C-13), 10.2 (q, C-14), 106.8 (t, C-15).

3.3.7. 3,5-Bis(3,3-dimethylallyl)-4-hydroxyacetophenone (**7**)

Colorless needles, molecular formula: $C_{18}H_{24}O_2$, m.p. 92–93 °C (Me_2CO); IR (ν_{max}^{KBr}, cm^{-1}): 3389 (OH), 3098, 2967, 1650 (C=O), 1584, 1472, 1434, 1275, 1188, 876, 843; EI-MS m/z (rel int): 272 ($[M]^+$ 30), 257 ($[M\text{-}15]^+$ 15), 229 (21), 217 (49), 201 (72), 173 (100), 161 (33), 115 (15), 91 (16), 77 (13), 69 (15), 43 (78); ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 7.64 (2 H, s, 2,6-H), 6.00 (1 H, s, OH), 5.32 (2 H, t, J = 7.2 Hz 10, 10′-H), 3.39 (4 H, d, J = 7.2 Hz 9,9′-H), 2.54 (3 H, s, 8-H), 1.79 (12 H, s, 12, 12′, 13, 13′-H); ^{13}C NMR δ ppm ($CDCl_3$, 100 MHz): 129.6 (s, C-1), 128.6 (d, C-2,6), 127.0 (s, C-3,5), 157.3 (s, C-4), 197.4 (s, C-7), 26.1 (q, C-8), 29.3 (t, C-9,9′), 121.3 (d, C-10,10′), 134.7 (s, C-11,11′), 25.6 (q, C-12,12′), 17.7 (q, C-13,13′). The ^{13}C NMR data for **7** were not reported in the literature previously.

3.3.8. 2-Acetyl-5,6-dimethoxybenzofuran (**8**)

Yellowish needles, molecular formula: $C_{12}H_{12}O_4$; m.p. 114–115 °C ($CHCl_3$); IR (ν_{max}^{KBr}, cm^{-1}): 3116, 3087, 1670 (C=O), 1620, 1546, 1489, 1440, 1134, 850; EI-MS m/z (rel int): 220 ($[M]^+$ 13), 205 ($[M\text{-}15]^+$ 16), 177 (4), 135 (7), 119 (9), 77 (10), 63 (8), 43 (100); ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 7.44 (1 H, s, 3-H), 7.07 (1 H, s, 4-H), 7.06 (1 H, s, 7-H), 3.97 (3 H, s, OCH_3), 3.94 (3 H, s, OCH_3), 2.57 (3 H, s, 11-H).

3.3.9. (24R)-Stigmast-7,22(E)-dien-3α-ol (**9**)

Colorless needles, molecular formula: $C_{29}H_{48}O$; m.p. 149–150 °C (Me_2CO); $[\alpha]_D^{23}$: +16.2° (c 0.3, $CHCl_3$); EI-MS m/z (rel int): 412 ($[M]^+$ 16), 397 (9), 394 (1), 369 (8), 273 (17), 271 (72), 255 (34), 253 (9), 83 (70), 81 (100), 55 (99), 43 (65); ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 5.18 (1 H, dd, J = 8.7, 15.0 Hz 22-H), 5.10 (1 H, dd, J = 15.0, 7.5 Hz 23-H), 5.02 (1 H, m, 7-H), 3.59 (1 H, m, 3-H), 1.07 (3H, d, J = 7.5 Hz 21-H), 0.86 (3 H, s, 19-H), 0.83 (3 H, d, J = 7.5 Hz 27-H), 0.81 (3 H, t, J = 7.8 Hz 29-H), 0.80 (3 H, d, J = 7.5 Hz 26-H), 0.54 (3 H, s, 18-H); ^{13}C NMR δ ppm ($CDCl_3$, 100 MHz): 37.9 (t, C-1), 28.5 (t, C-2), 71.0 (d, C-3), 34.2 (t, C-4), 40.8 (d, C-5), 31.4 (t, C-6), 117.4 (d, C-7), 139.5 (s, C-8), 49.4 (d, C-9), 37.1 (s, C-10), 21.5 (t, C-11), 39.4 (t, C-12), 43.2 (s, C-13), 55.1 (d, C-14), 23.0 (t, C-15), 29.6 (t, C-16), 55.8 (d, C-17), 12.0 (q, C-18), 13.0 (q, C-19), 40.2 (d, C-20), 21.1 (q, C-21), 138.2 (d, C-22), 129.4 (d, C-23), 51.2 (d, C-24), 31.9 (d, C-25), 19.0 (q, C-26), 21.3 (q, C-27), 25.4 (t, C-28), 12.2 (q, C-29).

3.3.10. β-Sitosterol (**10**)

Colorless needles, molecular formula: $C_{29}H_{50}O$; m.p. 141–142 °C (Me_2CO); $[\alpha]_D^{23}$: −36.2° (c 0.74, $CHCl_3$). It was identified by direct comparison with authentic samples by TLC and m.p.

3.4. Cytotoxic activity assays

The cytotoxic activity assay was carried out according to the SRB method (Skehan et al. 1990). Human ovarian neoplasm cells (HO-8910) and human hepatoma cells (Bel-7402) 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 4 × 103 cells/well for 24 h. Then, using vincristine sulfate as a positive control, compounds **2**, **3**, **5** and **6** were added. After 48 h exposure to the toxins, cell viability was determined by the sulforhodamine B (SRB) colorimetric assay by measuring the absorbance at 515 nm with an ELISA reader. Each test was performed in 5 replicates.

3.5. Anti-bacterial assays

The anti-bacterial activity assay was carried out according to the cup-plate method, using chloramphenicol as a positive control. Three strains of bacteria: *Bacillus subtilis*, *Echerichia coli* and *Staphylococcus aureus*, were cultured in beef broth and incubated at 37 °C for 24 h. After dilution of the beef broth, the three bacteria were cultured in separate agar medium dishes, six cups (8 × 10 mm) were put onto the dishes, and each compound tested (0.2 ml of 100 ug/ml) was added to the respective cup under aseptic conditions. Then the dishes were 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.

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