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A new furobenzopyranone and other constituents from *Anaphalis lactea*

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Together with twenty-one known compounds, a new furobenzopyranone was isolated from the whole plant of *Anaphalis lactea*. Their structures were elucidated by spectroscopic methods MS, IR, UV, NMR, including 2D-NMR techniques. The anti-bacterial activity of compounds **1**, **4–6**, **14**, **15** and the anti-tumor activity of compounds **4–6** were tested.

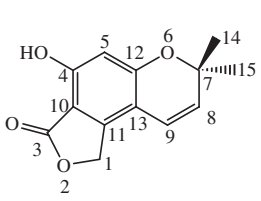
1. Introduction

There are about 80 species of *Anaphalis* (Compositae) all over the world and more than 50 species distributed in China (Editorial Committee of Zhongguozhiwuzhi, Chinese Academy of Sciences 1979). But only a few have been studied chemically. The whole plant of *Anaphalis lactea* Maxim. has long been used as a Tibetan medicine for invigorating the circulation of blood, relieving phlegm and hemostasia (Jiangsu College of New Medicine 1977), however, its chemical constituents have not been previously investigated. In this paper, we report the isolation and structural elucidation of a new furobenzopyranone (**1**) and twenty-one known compounds (**2**–**22**). Compound **9** was a new one which was isolated and identified from *Ligularia dolichobotrys* by our research group (Li et al. in press). Almost the the same time, we obtained it from *A. lactea*. In addition, six of the compounds isolated (**1**, **4–6**, **14**, **15**) were assayed against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*, three kaempferol flavonoids (**4–6**) were screened against human hepatoma (Bel-7402) and human ovaria carcinoma (HO-8910) cell lines.

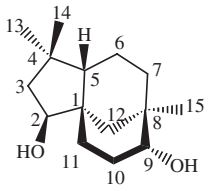
2. Investigations, results and discussion

From the acetone extract of the whole plant of *A. lactea,* a new furobenzopyranone anaphalisol (**1**) was isolated and elucidated, together with twenty-one known compounds: clovane-2β,9α-diol (**2**) (Heymann et al. 1994), flavone (**3**) (Iinuma et al. 1980), kaempferol-3-*O*-β-D-glucopyranoside (**4**) (Markham et al. 1978), kaempferol-3-*O*-[6″-*O*-(*trans*-*p*-coumaroyl)]-β-D-glucopyranoside (**5**) (Zhang et al. 1997; Zhou et al. 2001), kaempferol-3-*O*-[6″-*O*-(*trans*-*p*-coumaroyl)-4″-*O*-acetyl]-β-D-glucopyranoside (**6**) (Romussi et al. 1988), 1-hydroxyl-2,3,4,5-tetramethoxyxanthone (**7**) (Ghosal et al. 1975; Li et al. 1998), 3β-oleanolic acid (**8**) (Liu et al. 1999), 3β,7α,22-trihydroxy-5-en-stigmast (**9**) (Li et al. in press), 3β,7α-dihydroxy-5-en-stigmast (**10**) (Greca et al. 1990), β-sitosterol (**11**), daucosterol (**12**), 3α-spinasterol (**13**) (Itoh et al. 1981; Xu et al. 1998), 6-(4′-hydroxystyryl)-4-methoxy-2-pyrone (**14**) (Talapatra et al. 1976), 6-(4′-*O*-β-D-glucopyranose-styryl)-4-methoxy-2-pyrone (**15**) (Romo et al. 1972), (*E*)-3-(3,4-dihydroxyphenyl)-propenoic acid (**16**) (Asahi Research Center 1986), (*E*)-3-(3,4-dihydroxy phenyl)-propenoic acid methyl ester (**17**), methyl 3,4-dihydroxy benzoate (**18**) (Luo et al. 2001), 4-hydroxy benzoic acid (**19**) (Asahi Research Center 1985), inositol (**20**), 1-methyl- inositol (**21**) and stearic acid (**22**). The structure of the new compound **1** was identified by EI-MS, FAB-MS, IR, UV, ^{1}H NMR, ^{13}C NMR, HMBC spectroscopic methods and comparing with the spectral data of similar compounds. The structures of the known compounds **2**–**10** and **13**–**19** were elucidated by comparison with their spectral data (EI-MS, FAB-MS, IR, ^{1}H NMR and ^{13}C NMR) with those published in the literature. In addition, compounds **11**, **12** and **20**-**22** were determined on the basis of their physical properties by comparison with those of authentic samples, respectively.

Compound **1** was obtained as colorless gum, its FAB-MS gave quasi-molecular ion peaks at *m/z* 239.2 $[M + Li]^+$ and *m/z* 255.1 $[M + Na]^+$, combined with the peak of EI-MS ($[M]^+$ at *m/z* 232), the molecular formula of **1** was deduced to be $C_{13}H_{12}O_4$, which was supported by ^{1}H NMR, ^{13}C NMR and DEPT data (Table 1). The IR spectrum (KBr) showed the presence of hydroxyl (3528 cm^{-1}), α,β-unsaturated γ-lactone (1723 cm^{-1}), benzene ring (1635, 1600, 1464 cm^{-1}) and C–O–C bond (1335, 1152, 1046 cm^{-1}). Its ^{1}H NMR spectrum gave the typical signals of 2,2-dimethyl-chromene derivative at δ 6.15 (1 H, d, 9.9 Hz), 5.63 (1 H, d, 9.9 Hz), 1.46 (6 H, s), two independent signals at δ 6.35 (1 H, s), 5.25 (2 H, s) and an aromatic hydroxyl signal at δ 7.65 (1 H, brs) which disappeared on addition of D_2O. ^{13}C NMR and DEPT spectra revealed 13 carbons (2 × CH_3, 1 × CH_2, 3 × CH, 7 × C) (Table 1). The signals at δ 161.1 (C), 157.6 (C), 143.0 (C), 108.6 (C), 104.0 (C) and 103.9 (CH) (benzene ring), at δ 129.8, 116.6 (2 × CH, the sp^2 double bond), 78.1 (C) and 28.5 (2 × CH_3), further confirmed the skeleton of 2,2-dimenthyl-chromene. Apart from the carbon signals corresponding to the above mentioned groups,



1



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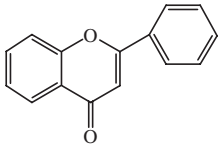

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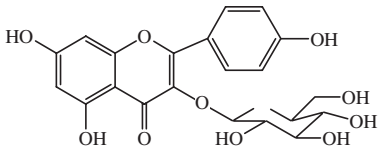

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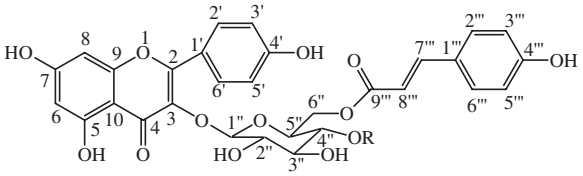
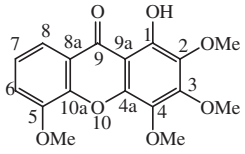


5 R=H

6 R=Ac



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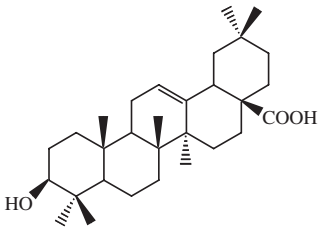

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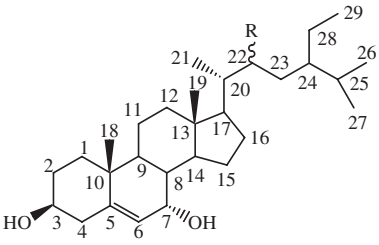
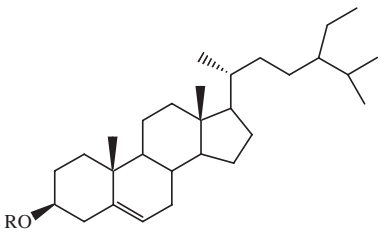


9 R=OH

10 R=H



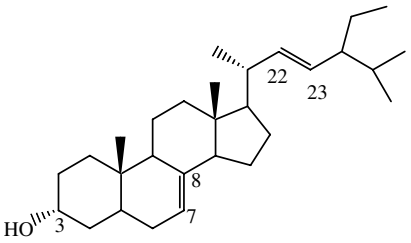
11 R=H

12 R=GLC

the ^{13}C NMR and DEPT spectra also displayed a carbonyl carbon at δ 172.5 and a oxygen-bearing carbon methylene at δ 69.4, which could be due to a γ-lactone moiety, as followed by the molecular formula and the IR spectrum. Moreover, the significant absorption band at 245 nm in the UV spectrum also supported a γ-lactone benzofurane ring. Considered a single signal of aromatic proton in ^{1}H NMR spectrum and a signal of hydroxy in ^{1}H NMR and IR spectra, the benzene ring had five substitutions including a hydroxy. From the above data, the structure of compound **1** was deduced as furobenzopyranone. In addition, its HMBC spectrum gave the long-range correlations between δ 6.35 (H-5) with δ 161.1 (C-12), 157.6 (C-4), 108.6 (C-13), 104.0 (C-10); δ 6.15 (H-9) with δ 161.1 (C-12), 143.0 (C-11), 108.6 (C-13); δ 5.25 (H-1) with δ 172.5 (C-3), 143.0 (C-11), 108.6 (C-13), 104.0 (C-10) (Table 1). Therefore, compound **1** was established and named anaphalisol. Moreover, compared the ^{1}H NMR

Table 1: ^{1}H NMR, ^{13}C NMR, DEPT data and HMBC correlations of 1 (δ, ppm, TMS, $CDCl_3$)

No.	^{1}H NMR	^{13}C NMR (DEPT)	HMBC
1	5.25 (s)	69.4 (CH_2)	C-3, 10, 11, 12, 13
3	–	172.5 (C)	–
4	–	157.6 (C)	–
5	6.35 (s)	103.9 (CH)	C-4, 10, 12, 13
7	–	78.1 (C)	–
8	5.63 (d, J = 9.9 Hz)	129.8 (CH)	C-7, 9, 13, 14, 15
9	6.15 (d, J = 9.9 Hz)	116.6 (CH)	C-7, 8, 11, 12, 13
10	–	104.0 (C)	–
11	–	143.0 (C)	–
12	–	161.1 (C)	–
13	–	108.6 (C)	–
14	1.46 (s)	28.5 (CH_3)	C-7, 8, 15
15	1.46 (s)	28.5 (CH_3)	C-7, 8, 14
OH	7.65 (brs)	–	–



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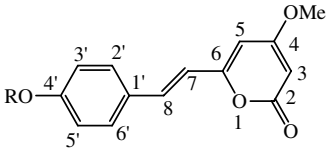
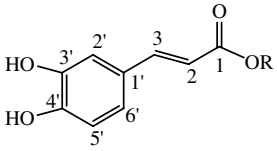


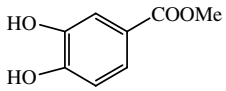
14 R=H

15 R=GLC



16 R=H

17 R=Me



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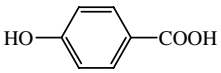

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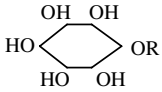

20 R=H

21 R=OMe

$CH_3(CH_2)_{14}COOH$

22

spectrum of compound **1** with that of the known compound phthalidochromene (Jakupovic et al. 1987), the only difference between them was that phthalidochromene had a signal at δ 3.93 (OMe), but compound **1** had a signal at δ 7.65 (Ar−OH). So their structure's difference was the group at C-4, the phthalidochromene was OMe (δ 3.93), however, anaphalisol was OH (δ 7.65). As a result, the structure of anaphalisol was further elucidated.

The compounds **1**, **4**–**6**, **14**, **15** were tested for their anti-bacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* by comparision with standard chloramphenicol. The results indicated that compounds **1** and **15** exhibited strong activities against *B. subtilis* (Table 2).

Using SRB method, the anti-tumor activities of compounds **4**–**6** against human hepatoma (Bel-7402) and human ovaria carcinoma (HO-8910) cell lines were screened by comparision with the standard vincristin sulphate. But they showed no effect on the growth of the cell lines at a concentration of 50 ug/ml, so they exhibited little anti-tumor activities against the cell lines.

Table 2: Anti-bacterial activity of compounds insolated

Compd.	*B. subtilis*	*S. aureus*	*E. coli*
1	+++	++	+
4	++	++	−
5	++	+	+
6	+	+	++
14	+	+	−
15	+++	++	−
H_2O	−	−	−
Chloramphenicol	+++	+++	+++

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

3. Experimental

3.1. Apparatus

Optical rotations: Perkin-Elmer 341 Polarimeter; UV: TU-1901 UV-VIS instrument; IR: Nicolet NEXUS 670 FT-IR instrument; EI-MS: HP 5988A GC/MS instrument; FAB-MS data: VG-ZAB-HS mass spectrometer (at 70 eV); NMR: Bruker AM-400 FT-NMR and Varian Mercury Plus-300 FT-NMR 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; Melting points were determined on a Kefler melting point apparatus, and are uncorrected.

3.2. Plant material

Anaphalis lactea Maxim. was collected in Yuzhong city, Gansu province, P.R. China, in August 2000. It was identified by Prof. Xun Pu, College of Biological Science, Lanzhou University. A voucher specimen (No. 20000801) was deposited in the herbarium of our institute.

3.3. Extraction and isolation

The air-dried whole plant of *A. lactea* Maxim. (2.0 kg) was pulverized and extracted with acetone three times at room temperature. The extract was concentrated under reduced pressure to yield a residue (52 g) which was chromatographed over a silica gel column eluted with a gradient of petroleum ether (60–90 °C)-EtOAc (30 : 1 to 1 : 1 and EtOAc, 500 ml each eluent). Combination of the appropriate fractions (monitored by TLC analysis) led to five fractions (A-E). Sfr. A (petroleum ether-EtOAc 30 : 1 to 20 : 1, 8 g) was obtained as white wax which was mainly volatile oil and fatty hydrocarbon according to first inference, so had not been studied carefully; Sfr. B (petroleum ether-EtOAc 15 : 1–10 : 1, 10 g) was chromatographed over a silica gel column

eluted with a gradient of petroleum ether (60–90 °C)-acetone (50 : 1 to 20 : 1, 100 ml each eluent) to obtain sfr. B_1–B_4 after combination according to TLC analysis. Compound **11** (200 mg) was obtained by recrystallization in acetone and EtOAc from sfr. B_1 and compound **22** (20 mg) was also obtained from sfr. B_1. Sfr. B_2 was rechromatographed over a silica gel column eluted with petroleum ether (60–90 °C)-EtOAc (10 : 1) to obtain compounds **1** (5 mg) and **13** (10 mg). Compound **2** (15 mg) was obtained by rechromatograph over a silica gel column eluted with petroleum ether (60–90 °C)-acetone (8 : 1) from sfr. B_3. Sfr. B_4 was repeated chromatographed over a silica gel column yield compounds **3** (3 mg), **7** (8 mg) and **10** (2 mg); Sfr. C (petroleum ether-EtOAc 7 : 1, 2 g) was subjected to a silica gel column eluted with petroleum ether (60–90 °C)-acetone (30 : 1 and 10 : 1) to afford sfr. C_1 (obtained from sfr. petroleum ether-acetone 10 : 1), which was rechromatographed over a silica gel column eluted with petroleum ether (60–90 °C)-acetone (8 : 1), then repeated recrystallized in acetone and $CHCl_3$ to afford compound **8** (21 mg); Sfr. D (petroleum ether-EtOAc 3 : 1, 4 g) was chromatographed over a silica gel column eluted with petroleum ether (60–90 °C)-acetone (10 : 1, 100 ml each eluate), Combination of the appropriate eluates according to TLC analysis to afforded sfr. D_1 and sfr. D_2. Sfr. D_1 was repeated rechromatographed over silica gel column to yield compound **14** (25 mg). Sfr. D_2 was subjected to silica gel column three times, eluted with $CHCl_3$, petroleum ether (60–90 °C)-acetone (10 : 1) and $CHCl_3$–CH_3OH (80 : 1), respectively, to yield compound **9** (12 mg); Sfr. E (EtOAc 22 g) was subjected to a silica gel column eluted with a gradient of $CHCl_3$–CH_3OH (30 : 1 to 1 : 1, 200 ml each eluate) to obtain five parts (E_1–E_5) according to TLC analysis. Sfr. E_1 was repeated recrystallized in acetone to afford compound **5** (200 mg), the mother liquor after concentration was rechromatographed over silica gel column eluted with $CHCl_3$-methanol (10 : 1) to obtain compound **6** (25 mg). Compound **12** (100 mg) was obtained by repeated recrystallization in methanol from sfr. E_2. Sfr. E_3 was repeated recrystallized in acetone, then rechromatographed over polyamide column eluted with methanol to obtain compound **4** (20 mg). Compound **21** (22 mg) was obtained by repeated recrystallization in acetone from sfr. E_4. Sfr. E_5 was chromatographed over silica gel column eluted with $CHCl_3$–CH_3OH (10 : 1) after repeated recrystallization in acetone, then rechromatographed over polyamide column eluted with methanol-H_2O (2 : 1) to yield compound **4** (20 mg).

After acetone extracted, we extracted with CH_3OH three times at room temperature and concentrated under reduced pressure, to give a residue (100 g), which was chromatographed on a silica gel column eluted with a gradient of $CHCl_3$–CH_3OH (10 : 1 to 1 : 1, 500 ml each fluent), then repeated chromatographed over silica gel column or polyamide column, and repeated recrystallizated to obtain **9** (50 mg), **16** (5 mg), **17** (8 mg), **18** (10 mg), **19** (15 mg), **20** (20 mg), and **4**, **5**, **6**, **12**, **21** (as same as Sfr. E).

3.3.1. Anaphalisol (**1**)

Colorless gum; molecular formula: $C_{13}H_{12}O_4$; FAB-MS *m/z* (3 NBA): 239.2 $[M + Li]^+$, 255.1 $[M + Na]^+$; EI-MS *m/z* (rel int): 232 $[M]^+$ (7.6), 217 $[M\text{-}Me]^+$ (100), 189 $[M-Me-CO]^+$ (12); IR (ν_{max}^{KBr}, cm^{-1}): 3528 (OH), 1723 (α,β-unsaturated γ-lactone), 1635, 1600, 1464 (benzene ring) and 1335, 1152, 1046 (C–O–C); UV λ_{max}^{CHCl3} (nm): 245; ^{1}H NMR, ^{13}C NMR, DEPT and H MBC data: see Table 1.

3.3.2. Clovane-2β,9α-diol (**2**)

Colorless needles ($CHCl_3$); molecular formula: $C_{15}H_{26}O_2$; m.p. 153–154 °C; $[\alpha_D]^{21}$+2.6 (*c*, 1.15, CH_3COCH_3); EI-MS m/z (rel int): 238 $[M]^+$ (5.4), 220 $[M\text{-}H_2O]^+$ (14), 205 $[M\text{-}H_2O\text{-}Me]^+$ (9.3), 202 $[M\text{-}2 \times H_2O]^+$ (4.4), 187 $[M\text{-}2 \times H_2O\text{-}Me]^+$ (6.0), 179 (28), 164 $[179\text{-}Me]^+$ (94); ^{1}H NMR δ ppm ($CDCl_3$, 300 MHz): 3.79 (1 H, dd, 10.2, 5.7 Hz, H-2), 3.33 (1 H, brs, H-9), 1.98 (1 H, t, 14.7 Hz, H-10), 1.70 (1 H, dd, 11.7, 5.7 Hz, H-3), 1.64 (1 H, m, H-10), 1.51 (1 H, dd, 11.7, 10.2 Hz, H-3), 1.42 (1 H, m, H-5), 1.03 (3 H, s, H-14), 0.95 (3 H, s, H-15), 0.85 (3 H, s, H-13); ^{13}C NMR δ ppm ($CDCl_3$, 75 MHz): 81.1 (C-2), 75.4 (C-9), 50.7 (C-5), 47.6 (C-3), 44.3 (C-1), 37.3 (C-4), 35.7 (C-12), 34.9 (C-8), 33.3 (C-7), 31.6 (C-14), 28.5 (C-15), 26.6 (C-11), 26.1 (C-10), 25.6 (C-13), 20.8 (C-6).

3.3.3. Flavone (**3**)

Yellow amorphous powder; molecular formula: $C_{15}H_{10}O_2$; EI-MS *m/z* (rel int): 222 $[M]^+$ (68), 194 $[M\text{-}CO]^+$ (28), 120 [A ring]$^+$ (100), 102 [B ring]$^+$ (14), 92 [a ring-CO]$^+$ (55); ^{1}H NMR δ ppm ($CDCl_3$, 300 MHz): 8.24 (1 H, dd, 8.4, 1.8 Hz, H-5), 7.94 (2 H, dd, 8.4, 1.8 Hz, H-2′, 6′), 7.71 (1 H, td, 8.4, 1.8 Hz, H-7), 7.58 (1 H, dd, 8.4, 1.8 Hz, H-8), 7.53 (3 H, m, H-3′, 4′, 5′), 7.44 (1 H, td, 8.4, 1.8 Hz, H-6), 6.85 (1 H, s, H-3); ^{13}C NMR δ ppm ($CDCl_3$, 75 MHz): 178.7 (C-4), 163.7 (C-2), 157.0 (C-9), 134.0 (C-7), 131.9 (C-1′, 4′), 129.3 (C-3′, 5′), 126.6 (C-2′, 6′), 126.0 (C-5), 125.5 (C-6), 123.8 (C-10), 118.3 (C-8), 107.8 (C-3).

*3.3.4. Kaempferol-3-O-β-*D*-glucopyranoside* (**4**)

Yellow amorphous powder; molecular formula: $C_{21}H_{20}O_{11}$; $[\alpha_D]^{21}$–16.0 (c, 0.40, CH_3OH); FAB-MS m/z (GLY): $[M + Li]^+$: 455.2, $[M + Na]^+$: 471.1; ^{1}H NMR δ ppm (DMSO-d_6, 400 MHz): 12.51 (1 H, brs, C_5-OH) 8.00 (2 H, d, 8.8 Hz, H-2′, H-6′), 6.86 (2 H, d, 8.8 Hz, H-3′, H-5′), 6.26 (1 H, d, 2 Hz, H-8), 6.06 (1 H, d, 2 Hz, H-6), 5.39 (1 H, d, 7.2 Hz, H-1″), 3.08-3.56 (6 H, m, H-2″, H-3″, H-4″, H-5″, H-6″); ^{13}C NMR δ ppm (DMSO-d_6, 100 MHz): 176.8 (C-4), 168.3 (C-7), 161.0 (C-5), 160.1 (C-4′), 156.7 (C-9), 155.5 (C-2), 133.0 (C-3), 130.6 (C-2′, 6′), 120.8 (C-1′), 115.1 (C-3′, 5′), 102.4 (C-10), 101.3 (C-1″), 99.7 (C-6), 94.1 (C-8), 77.3 (C-5″), 76.5 (C-3″), 74.2 (C-2″), 69.8 (C-4″), 60.8 (C-6″).

*3.3.5. Kaempferol-3-O-[6″-O-(trans-p-coumaroyl)]-β-*D*-glucopyranoside* (**5**)

Yellow amorphous powder; molecular formula: $C_{30}H_{26}O_{13}$; $[\alpha_D]^{21}$–31.6 (c, 0.78, CH_3OH); IR (ν_{max}^{KBr}, cm^{-1}): 3460, 3252, 3163, 1684, 1608, 1502, 1358, 1296, 1183, 1067, 826; ^{1}H NMR δ ppm (DMSO-d_6, 400 MHz): 12.57 (1 H, s, C_5-OH), 7.98 (2 H, d, 8.5 Hz, H-2′, H-6′), 7.36 (2 H, d, 8.4 Hz, H-2‴, H-6‴), 7.33 (1 H, d, 15 Hz, H-7‴), 6.85 (2 H, d, 8.5 Hz, H-3′, H-5′), 6.78 (2 H, d, 8.4 Hz, H-3‴, H-5‴), 6.38 (1 H, s, H-8), 6.14 (1 H, s, H-6), 6.10 (1 H, d, 15 Hz, H-8‴), 5.44 (1 H, d, 6.7 Hz, H-1″), 4.27 (1 H, d, 12 Hz, H-6″), 4.02 (1 H, dd, 12, 6.2 Hz, H-6″), 3.17–3.37 (4 H, m, H-2″, H-3″, H-4″, H-5″); ^{13}C NMR δ ppm (DMSO-d_6, 100 MHz): 177.4 (C-4), 166.2 (C- 9‴), 164.2 (C-7), 161.2 (C-5), 160.0 (C-4′), 159.8 (C-4‴), 156.5 (C-9), 156.4 (C-2), 144.6 (C-7‴), 133.1 (C-3), 130.8 (C-2′, 6′), 130.1 (C-2‴, 6‴), 124.9 (C-1‴), 120.8 (C-1′), 115.8 (C-3‴, 5‴), 115.1 (C-3′, 5′), 113.7 (C-8‴), 103.9 (C-10), 101.0 (C-1″), 98.8 (C-6), 93.7 (C-8), 76.2 (C-3″), 74.2 (C-2″), 74.1 (C-5″), 70.0 (C-4″), 63.0 (C-6″).

*3.3.6. Kaempferol-3-O-[6″-O-(trans-p-coumaroyl)-4″-O-acetyl]-β-*D*-glucopyranoside* (**6**)

Yellow amorphous powder; molecular formula: $C_{32}H_{28}O_{14}$; $[\alpha_D]^{21}$–56.0 (c, 0.55, CH_3OH); ^{1}H NMR δ ppm (DMSO-d_6, 400 MHz): 12.51 (1 H, s, C_5-OH), 7.99 (2 H, d, 8.8 Hz, H-2′, H-6′), 7.38 (2 H, d, 8.8 Hz, H-2‴, H-6‴), 7.35 (1 H, d, 15.6 Hz, H-7‴), 6.87 (2 H, d, 8.8 Hz, H-3′, H-5′), 6.79 (2 H, d, 8.8 Hz, H-3‴, H-5‴), 6.38 (1 H, d, 2 Hz, H-8), 6.15 (1 H, d, 2 Hz, H-6), 6.10 (1 H, d, 15.6 Hz, H-8‴), 5.50 (1 H, d, 7.9 Hz, H-1″), 5.46 (1 H, t, 9.2 Hz , H-4″), 2.02 (3 H, s, acetyl); ^{13}C NMR δ ppm (DMSO-d_6, 100 MHz): 177.2 (C-4), 169.7 (C=O, acetyl), 165.9 (C-9‴), 164.4 (C-7), 161.2 (C-5), 160.1 (C-4′), 159.9 (C-4‴), 156.7 (C-9), 156.4 (C-2), 144.8 (C-7‴), 132.9 (C-3), 130.8 (C-2′, 6′), 130.2 (C-2‴, 6‴), 124.9 (C-1‴), 120.7 (C-1′), 115.8 (C-3‴, 5‴), 115.1 (C-3′, 5′), 113.4 (C-8‴), 103.8 (C-10), 100.9 (C-1″), 98.8 (C-6), 93.7 (C-8), 74.1 (C-2″), 73.4 (C-3″), 71.4 (C-5″), 70.8 (C-4″), 61.9 (C-6″), 20.8 (CH_3, acetyl).

3.3.7. 1-Hydroxyl-2,3,4,5-tetramethoxyxanthone (**7**)

Yellow amorphous powder; molecular formula: $C_{17}H_{16}O_7$; FAB-MS m/z (3 NBA): 333.1 $[M + H]^+$, 332.1 $[M]^+$; EI-MS m/z (rel int): 332 $[M]^+$ (54), 317 $[M\text{-}Me]^+$ (100), 302 $[M\text{-}2 \times Me]^+$ (16), 289 $[M\text{-}ME\text{-}CO]^+$ (6.7), 287 $[M\text{-}3Me]^+$ (14), 274 $[M\text{-}2 \times Me\text{-}CO]^+$ (8.8), 259 $[M\text{-}3 \times Me\text{-}CO]^+$ (16); ^{1}H NMR δ ppm ($CDCl_3$, 300 MHz): 12.58 (1 H, s, C_1-OH), 7.81 (1 H, dd, 7.5, 1.2 Hz, H-8), 7.31 (1 H, t, 7.5 Hz, H-7), 7.25 (1 H, dd, 7.5, 1.2 Hz, H-6), 4.15 (3 H, s, 4-OMe), 4.03 (3 H, s, 3- OMe), 4.02 (3 H, s, 2- OMe), 3.95 (3 H, s, 5-OMe); ^{13}C NMR δ ppm ($CDCl_3$, 75 MHz): 181.9 (C-9), 154.4 (C-3), 150.8 (C-1, 10a), 149.0 (C-5), 146.6 (C-4a), 135.7 (C-2), 133.0 (C-4), 123.9 (C-7), 121.1 (C-9a), 116.8 (C-8), 116.2 (C-6), 105.3 (C-8a), 62.2 (C_4-OMe), 62.0 (C_3-OMe), 61.4 (C_2-OMe), 56.7 (C_5-OMe).

3.3.8. 3β-Oleanolic acid (**8**)

Colorless needles (CH_3OH); molecular formula $C_{30}H_{48}O_3$; m.p. 307–308 °C; $[\alpha_D]^{21}$+11.6 (c, 0.43, DMSO); ^{1}H NMR δ ppm (DMSO-d_6, 300 MHz): 11.98 (1 H, s, COOH-28), 5.13 (1 H, brs, H-12), 4.30 (1 H, OH-3), 3.42 (1 H, dd, 13.8, 9.0 Hz, H-3), 1.06 (3 H, s, Me), 0.90 (3 H, s, Me), 0.83 (3 H, s, Me), 0.79 (3 H, s, Me), 0.77 (3 H, s, Me), 0.72 (3 H, s, Me), 0.68 (3 H, s, Me); ^{13}C NMR δ ppm (DMSO-d_6, 75 MHz): 179.3 (C-28), 144.5 (C-13), 122.2 (C-12), 77.5 (C-3), 55.4 (C-5), 47.7 (C-9), 46.3 (C-19), 46.1 (C-17), 42.0 (C-14), 41.4 (C-18), 40.0 (C-8), 39.0 (C-4), 38.9 (C-1), 37.2 (C-10), 34.0 (C-21), 33.5 (C-29), 33.1 (C-7), 32.7 (C-22), 31.1 (C-20), 28.9 (C-23), 28.2 (C-2), 27.9 (C-15), 26.3 (C-27), 24.0 (C-30), 23.5 (C-11), 23.3 (C-16), 18.7 (C-6), 17.5 (C-26), 16.8 (C-25), 15.8 (C-24).

3.3.9. 3β,7α,22-Trihydroxy-5-en-stigmast (**9**)

Colorless needles (acetone); molecular formula: $C_{29}H_{50}O_3$; m.p. 123–124 °C; $[\alpha_D]^{21}$–31.4 (c, 0.35, $CHCl_3$); EI-MS m/z (rel int): 446 $[M]^+$ (0.4), 428 $[M\text{-}H_2O]^+$ (0.8), 413 $[M\text{-}H_2O\text{-}Me]^+$ (6.6), 412 $[M\text{-}H_2O\text{-}Me\text{-}H]^+$ (22), 410 $[M\text{-}2H_2O]^+$ (11), 398 $[M\text{-}H_2O\text{-}2 \times Me]^+$ (3.8), 111 (24), 97 (49), 85 (36), 83 (58), 71 (47), 69 (68), 57 (77), 55 (88), 43 (100); ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 5.61 (1 H, 4.8 Hz, H-6), 3.86 (1 H, m, H-7), 3.60 (1 H, m, H-3), 1.00 (3 H, s, H-18), 0.94 (3 H, d, 6.6 Hz, H-26), 0.88 (6 H, m, H-27, H-29), 0.79 (3 H, d, 6.6 Hz, H-21), 0.72 (3 H, s, H-19); ^{13}C NMR δ ppm ($CDCl_3$, 100 MHz): 146.3 (C-5), 123.8 (C-6), 71.3 (C-3, 22), 65.3 (C-7), 52.8 (C-17), 49.1 (C-14), 42.5 (C-13, 24), 42.3 (C-9), 42.0 (C-4), 41.4 (C-

20), 39.2 (C-12), 37.4 (C-8, 10), 37.0 (C-1), 31.4 (C-2), 29.9 (C-23), 28.7 (C-25), 27.5 (C-16), 24.4 (C-15), 23.6 (C-28), 20.7 (C-11), 20.5 (C-26), 18.2 (C-19), 17.5 (C-27), 12.3 (C-21), 11.9 (C-29), 11.6 (C-18).

3.3.10. 3β,7α-Dihydroxy-5-en-stigmast (**10**)

Colorless needles (acetone); molecular formula: $C_{29}H_{50}O_2$; $[\alpha_D]^{21}-25.0$ (c, 0.08, acetone); EI-MS m/z (rel int): 430 $[M]^+$ (1.1), 412 $[M\text{-}H_2O]^+$ (18), 397 $[M\text{-}H_2O\text{-}Me]^+$ (2.7), 379 $[M\text{-}2H_2O\text{-}Me]$ (6.2), 111 (20), 97 (33), 85 (27), 83 (47), 71 (40), 69 (61), 57 (69), 55 (84), 43 (100); ^{1}H NMR δ ppm ($CDCl_3$, 300 MHz): 5.58 (1 H, 4.8 Hz, H-6), 3.86 (1 H, m, H-7), 3.60 (1 H, m, H-3), 1.00 (3 H, s, H-19), 0.92 (3 H, d, 6.6 Hz, H-21), 0.86 (3 H, t, 7.5 Hz, H-29), 0.83 (3 H, d, 6.9 Hz, H-26), 0.80 (3 H, d, 7.2 Hz, H-27), 0.68 (3 H, s, H-18).

3.3.11. 3α-Spinasterol (**13**)

Colorless needles (acetone); molecular formula: $C_{29}H_{48}O$; m.p. 151–152 °C; $[\alpha_D]^{21}-11.1$ (c, 0.18, $CHCl_3$); ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 5.17 (2 H, m, H-22, 23), 5.03 (1 H, dd, 14.4, 8.8 Hz, H-7), 3.61 (1 H, m, H-3), 1.03 (3 H, d, 6.6 Hz, H-21), 0.86 (3 H, d, 6.2 Hz, H-26), 0.83 (3 H, t, 7.2 Hz, H-29), 0.82 (3 H, d, 6.2 Hz, H-27), 0.81 (3 H, s, H-19), 0.55 (3 H, s, H-18), 1.04–2.03 (25 H, m, CH and CH_2); ^{13}C NMR δ ppm ($CDCl_3$, 100 MHz): 139.6 (C-8), 138.2 (C-22), 129.4 (C-23), 117.4 (C-7), 71.1 (C-3), 55.9 (C-17), 55.1 (C-14), 51.2 (C-24), 49.4 (C-9), 43.3 (C-13), 40.8 (C-20), 40.3 (C-5), 39.5 (C-12), 38.0 (C-2), 37.1 (C-1), 34.2 (C-10), 31.8 (C-25), 31.5 (C-4), 29.6 (C-6), 28.5 (C-16), 25.4 (C-28), 23.0 (C-15), 21.6 (C-26), 21.4 (C-11), 21.1 (C-21), 19.0 (C-27), 13.0 (C-19), 12.2 (C-29), 12.0 (C-18).

3.3.12. 6-(4′-Hydroxystyryl)-4-methoxy-2-pyrone (**14**)

Yellow amorphous powder; molecular formula: $C_{14}H_{12}O_4$; FAB-MS m/z (3 NBA): $[M + H]^+$ 245.1; EI-MS m/z (rel int): 244 $[M]^+$ (100), 216 $[M\text{-}CO]^+$ (35), 201 $[M\text{-}Me\text{-}CO]^+$ (8.8), 173 $[M\text{-}Me\text{-}2 \times CO]^+$ (59), 125 (15), 119 (12), 69 (42); IR (ν_{max}^{KBr}, cm^{-1}): 3260, 1700, 1608, 1549, 1443, 1406, 1259, 1153, 958, 821; ^{1}H NMR δ ppm (DMSO-d_6, 400 MHz): 7.48 (1 H, d, 8.4 Hz, H-2′, H-6′), 7.23 (1 H, d, 16.0 Hz, H-8), 6.78 (2 H, d, 8.4 Hz, H-3′, 5′), 6.76 (1 H, d, 16.0 Hz, H-7), 6.21 (1 H, d, 2.0 Hz, H-5), 5.58 (1 H, d, 2.0 Hz, H-3); ^{13}C NMR δ ppm (DMSO-d_6, 100 MHz): 170.9 (C-2), 162.7 (C-4), 158.9 (C-4′), 134.4 (C-6, 8), 129.2 (C-2′, 6′), 126.2 (C-1′), 116.1 (C-7), 115.8 (C-3′, 5′), 100.0 (C-5), 88.0 (C-3), 56.3 (C-OMe).

3.3.13. 6-(4′-O-β-D-Glucopyranose-styryl)-4-methoxy-2-pyrone (**15**)

Yellow amorphous powder; molecular formula: $C_{20}H_{22}O_9$; $[\alpha_D]^{21}-40.0$ (c, 0.2., CH_3OH); ^{1}H NMR δ ppm (DMSO-d_6, 300 MHz): 7.58 (1 H, d, 8.4 Hz, H-2′, H-6′), 7.28 (1 H, d, 16.0 Hz, H-8), 7.03 (2 H, d, 8.4 Hz, H-3′, 5′), 6.88 (1 H, d, 16.0 Hz, H-7), 6.26 (1 H, d, 1.8 Hz, H-5), 5.61 (1 H, d, 1.8 Hz, H-3), 4.91 (1 H, d, 7.2 Hz, H-1″), 3.67 (2 H, m, H-6″), 3.15-3.45 (4 H, m, H-2″, H-3″, H-4″, H-5″); ^{13}C NMR δ ppm (DMSO-d_6, 75 MHz): 171.6 (C-2), 163.4 (C-4), 159.3 (C-4′), 134.5 (C-6, 8), 129.6 (C-2′, 6′), 129.5 (C-1′), 118.4 (C-7), 117.2 (C-3′, 5′), 101.4 (C-1″), 100.7 (C-5), 89.0 (C-3), 77.7 (C-5″), 77.2 (C-3″), 73.9 (C-2″), 70.3 (C-4″), 61.3 (C-6″), 57.1 (C-OMe).

3.3.14. (E)-3-(3,4-Dihydroxyphenyl)-propenoic acid (**16**)

Yellow amorphous powder; molecular formula: $C_9H_8O_4$; ^{1}H NMR δ ppm (CD_3OD, 300 MHz): 7.46 (1 H, d, J = 15.9 Hz, H-3), 7.03 (1 H, d, J = 1.8 Hz, H-2′), 6.91 (1 H, d, J = 8.4 Hz, H-5′), 6.77 (1 H, dd, J = 8.4, 1.8 Hz, H-6′), 6.24 (1 H, d, J = 15.9 Hz, H-2); ^{13}C NMR δ ppm (CD_3OD, 75 MHz): 171.5 (C-1), 147.8 (C-3′), 145.5 (C-4′), 144.2 (C-3), 127.1 (C-1′), 121.4 (C-6′), 116.6 (C-5′), 115.3 (C-2), 113.8 (C-2′).

3.3.15. (E)-3-(3,4-Dihydroxyphenyl)-propenoic acid methyl ester (**17**)

Yellow amorphous powder; molecular formula: $C_{10}H_{10}O_4$; EI-MS m/z (rel int): 194 $[M]^+$ (76), 163 $[M\text{-}OMe]^+$ (100), 145 $[M\text{-}OMe\text{-}H_2O]^+$ (24), 117 $[M\text{-}OMe\text{-}H_2O\text{-}CO]$ (20), 89 $[M\text{-}OMe\text{-}H_2O\text{-}2 \times CO]^+$ (30); ^{1}H NMR δ ppm (acetone -d_6, 300 MHz): 7.53 (1 H, d, 15.6 Hz, H-3), 7.17 (1 H, d, 1.8 Hz, H-2′), 7.04 (1 H, dd, 8.4, 1.8 Hz, H-6′), 6.88 (1 H, d, 8.4 Hz, H-5′), 6.28 (1 H, d, 15.6 Hz, H-2), 3.72 (3 H, s, OMe).

3.3.16. Methyl 3,4- dihydroxy benzoate (**18**)

Yellow amorphous powder; molecular formula: $C_8H_8O_4$; EIMS m/z (rel int): 168 $[M]^+$ (38), 137 $[M\text{-}OMe]^+$ (100), 109 $[M\text{-}OMe\text{-}CO]^+$ (25), 81 $[M\text{-}OMe\text{-}2CO]^+$ (14), 53 $[M\text{-}OMe\text{-}3 \times CO]^+$ (14); In addition, it was identified by comparison of its physical properties with that of authentic sample.

3.3.17. 4-Hydroxy benzoic acid (**19**)

Yellow amorphous powder; molecular formula: $C_7H_6O_3$; EI-MS m/z (rel int): 138 $[M]^+$ (66), 121 $[M\text{-}OH]^+$ (100), 93 $[M\text{-}COOH]$ (29), 65 $[M\text{-}COOH\text{-}CO]^+$ (26); ^{1}H NMR δ ppm (acetone-d_6, 300 MHz): 7.91 (2 H, dd, 8.1, 1.8 Hz, H-2, 6), 6.91 (2 H, dd, 8.1, 1.8 Hz, H-3, 5).

3.3.18. β-Sitosterol (**11**)*, β-daucosterol* (**12**)*, inositol* (**20**)*, 1-methyl- inositol* (**21**) *and stearic acid* (**22**)

β-Sitosterol (**11**), β-daucosterol (**12**), inositol (**20**), 1-methyl- inositol (**21**) and stearic acid (**22**) were identified on the basis of by comparison of their physical properties (m.p., R_f, ORD et al.) with those of authentic samples, respectively.

3.4. Anti-bacterial assays

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

3.5. Anti-tumor activity assays

Anti-tumor activity assays were carried out according to sulforhodamine B (SRB) colorime-tric assay. Human ovarian neoplasm cell HO-8910 and human hepatoma cell Bel-7402 were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, at 37 °C under a humidified atmosphere of 5% CO_2, and dispersed in replicate 96-well plates with 4 × 103 cells/well for 24 h. Then, used vincristine sulfate as a positive control, compounds **4**–**6** were 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 replicate.

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