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γ-Pyranone derivatives and other constituents from *Erigeron annuus*

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Two new γ-pyranone derivatives and a new triterpene, together with eight known compounds, were isolated from the aerial parts of *Erigeron annuus*. Their structures were elucidated by spectroscopic methods. Besides, the anti-tumor activities of the γ-pyranone derivatives to human hepatoma (SMMC-7721), human embryo liver (L-02) and human leukemia (HL-60) cells were evaluated.

1. Introduction

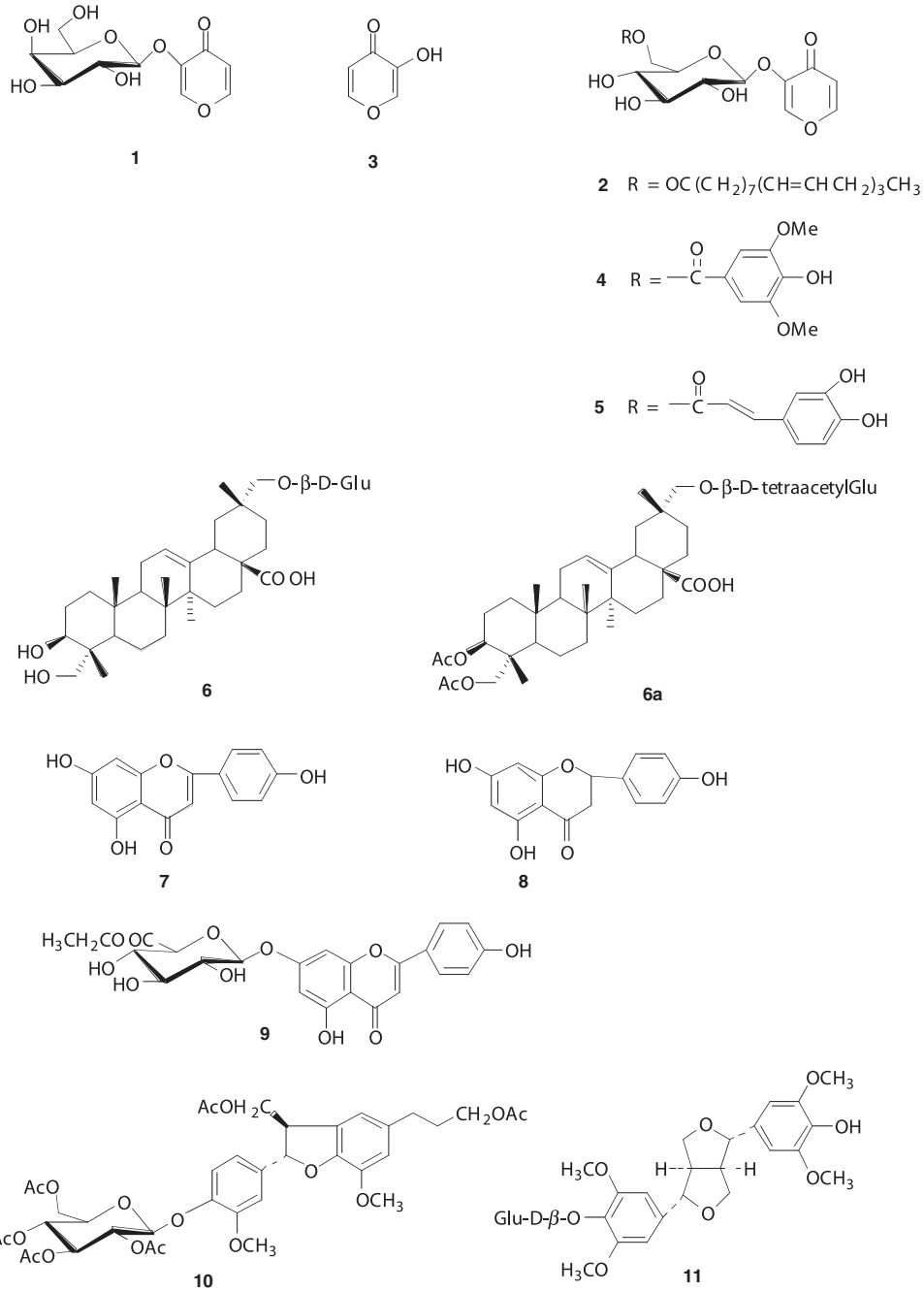
3-Hydroxyl-γ-pyranone and its derivatives have been reported to be abundantly contained in the *Erigeron* family (Mathela et al. 1984; Yue et al. 1994; Zhang et al. 1998; Oh et al. 2002) and to show strong activities of treating apoplexy of the brain (Zhang et al. 2000) and regulating blood pressure (Zhang et al. 1981). They also possess protective effects against injury due to ischemic reperfusion (Zhang et al. 2001). In our continuous study (Li et al. 2005) for bioactive constituents from *Erigeron annuus* (L.) Pers. (Compositae), which is widely distributed in China (Delectis Florae Reipublicae Popularis Sinicae Agendae Academiae Sinicae Edita, 1985) and also a useful traditional medicine for the treatment of indigestion, enteritis, epidemic hepatitis and hematuria (Jiangsu College of New Medicine, 1977), we got a series of γ-pyranone derivatives and phenypropanoids along with a new triterpene. This paper reports the isolation and structure elucidation of two new γ-pyranone derivatives 3-*O*-β-D-galactopyranosyl-γ-pyranone (**1**), 3-*O*-β-D-(6′-*O*-linolenic) glucopyranosyl-γ-pyranone (**2**) and a new triterpene glycoside 29-*O*-β-D-glucopyranosy-3β, 23-dihydroxyolean-12-en-28-oic (**6**), as well as eight known compounds 3-hydroxyl-γ-pyranone (**3**), 3-*O*-β-D-[6′-(4″-hydroxy-3″,5″-dimethyoxybenzoyl)] glucopyranosyl-γ-pyranone (**4**), erigeside (**5**), apigenin (**7**), 5,7,4′-trihydroxyflavanone (**8**), apigenin-7-*O*-β-D-glucuronideethylester (**9**), dehydrodiconiferyl alcohol-4-β-D-glucoside hexaacetate (**10**) and (+)-syringaresinol *O*-β-D-glucopyranoside (**11**). In addition, the anti-tumor activities of compounds **1**–**4** were tested against human hepatoma (SMMC-7721), human embryo liver (L-02) and human leukemia (HL-60) cells with 10-hydroxycamptothecine as a standard. The result showed that compound **2** had weak activity against HL-60 with an IC50 value of 63.50 μg/ml.

2. Investigations, results and discussion

Compound **1** was obtained as white amorphous powder. It is easily soluble in H_2O, but only slightly soluble in MeOH. Its molecular formula was established as $C_{11}H_{14}O_8$ on the basis of HR-ESI-MS (m/z = 275.0767, $[M+H]^+$ requires 275.0761). The IR spectrum showed absorptions for hydroxyl groups (3303, 1080, 1038 cm^{-1}). Besides, diagnostic bands of γ-pyranone were at 1656 and 1510 cm^{-1} (Nakanishi et al. 1977). Its UV absorptions at 223.7, 261.5 nm also indicated an α, β-unsaturated keto group in the pyranone ring. In its ^1H and ^{13}C NMR spectra (Table 1) the typical signals for a β-D-galactoside were readily recognized, which was further confirmed by comparing with an authentic compound by PC after an acid hydrolysis of **1**. The remaining signals of the aglycone were similar to those of 3-hydroxyl-γ-pyranone (Zhang et al. 1998). Thus the structure of compound **1** was elucidated to be 3-*O*-β-D-galactopyranosyl-γ-pyranone.

The molecular of compound **2** was established to be $C_{29}H_{42}O_9$ on the basis of HR-ESI-MS (m/z = 535.2903, $[M+H]^+$ requires 535.2902). Its IR spectrum showed absorptions for hydroxyl groups (3393, 1073 cm^{-1}) and an α, β-unsaturated keto group (1640 cm^{-1}). The latter was further confirmed by the absorptions at 221.5, 260.3 nm in the UV spectrum. Its ^1H and ^{13}C NMR spectra indicated the typical signals of a β-D-glucopyranoside, the remaining signals of the aglycone showed that this compound was both a derivative of 3-hydroxyl-γ-pyranone and an ester of linolenic acid (Shen et al. 1986). The upfield shift (about 3 ppm) of C-5′ (δ 74.5) of the sugar and downfield shift (about 1 ppm) of C-6′ (δ 63.8) indicated that there must be a group attached to C-6′ of the glucopyranosyl moiety by an oxygen atom. This was further confirmed by the correlations of the H-6′ (δ 4.08, dd, J = 11.7, 6.3 Hz, H-6a; δ 4.32, d, J = 11.7 Hz, H-6b) of the sugar moiety with the carbonyl group (δ 173.0) in ester (i.e. C-1″ of the linolenic acyloxy group) in HMBC spectrum. Besides, the cross peak between C-3 (δ 144.6) of the pyranone moiety and H-1′ (δ 4.87, d, J = 7.8 Hz) of the sugar moiety indicated that 3-hydroxyl-γ-pyranone was attached to C-1′ of β-D-glucopyranosyl by an oxygen atom. Thus the structure of compound **2** was established as 3-*O*-β-D-(6′-*O*-linolenic)glucopyranosyl-γ-pyranone. All the proton and carbon signals were assigned by $^1H-^1H$ COSY and HMQC spectra.

Scheme



The glycoside **6** was obtained through its hexaacetate **6a** (see Experimental). Compound **6a** exhibited ion peaks at m/z 903 $[M+H]^+$ in the FAB-MS and m/z 920.5020 ($[M+NH_4]^+$, requires 920.5002) in the HR-ESI-MS spectrum, compatible with the molecular formula $C_{48}H_{70}O_{16}$. The IR spectrum showed bands for −COOH (3343, 2750, 1752 cm^{-1}), C−O (1226 cm^{-1}) and double bond (1694 cm^{-1}) functional groups. The ^1H NMR spectrum showed six methyls due to the acetyl groups (δ 2.09, s, 3 H; δ 2.07, s, 3 H; δ 2.03, s, 9 H; δ 2.02, s, 3 H). It further showed another five methyl signals at δ 0.75, δ 0.82, δ 0.93, δ 0.96, δ 1.12, each appearing as a singlet. The broad-band ^{13}C NMR spectrum of **6a** showed the presence of 48 carbon atoms in the molecule, whose multiplicities were determined by DEPT experiments. Out of these, 12 carbon signals were due to the 6 acetyl groups and 6 carbon signals were in the aglycosidic region corresponding to the a D-glucose moiety. The remaining 30 carbon signals were attributable to the aglycone. The ^1H NMR spectrum of **6a** also exhibited a characteristic signal for an anomeric proton at δ 4.45. The coupling constant of H-1′ (J = 7.8 Hz) implied β-configuration of the sugar residue. The DEPT spectrum of **6a** also showed that the aglycone had two secondary carbon atoms bearing hydroxyl groups (δ 81.1 and δ 65.3), which was further confirmed by the signals at δ 3.03 (1 H, d, J = 11.7 Hz, H-29a), δ 3.72 (1 H, d, J = 11.7 Hz, H-29b), δ 3.80 (1 H, d, J = 11.7 Hz, H-23a) and δ 3.89 (1 H, d, J = 11.7 Hz, H-23b) in the ^1H NMR spectrum. All this suggested that the aglycone was consistent with an olean triterpene skeleton. This was further confirmed by the cross peaks between H-24 (δ 0.83) and C-3 (δ 74.5), C-4 (δ 40.5), C-5 (δ 47.7),

Table 1: The ^{1}H and ^{13}C NMR of compounds 1–4 (δ, ppm, ^{1}H NMR at 300 MHz and ^{13}C NMR at 75 MHz)

	1[a]		2[b]		3[c]		4[d]	
	H	C	H	C	H	C	H	C
2	8.06 (s)	146.2	8.13 (s)	146.4	7.87 (s)	146.8	8.16 (s)	144.3
3		145.5		144.6		139.2		146.5
4		175.5		173.4		173.8		173.0
5	6.35 (d, 6.0)	115.7	6.42 (d, 5.7)	116.9	6.49 (d, 5.1)	113.8	6.37 (d, 5.1)	116.8
6	7.90 (d, 6.0)	157.7	8.14 (d, 5.7)	156.4	7.79 (d, 5.1)	155.4	8.05 (d, 5.1)	156.4
1′	4.66 (d, 6.3)	101.2	4.87 (d, 7.8)	100.5			4.94 (d, 7.2)	100.5
2′	3.37 (t, 6.0)	72.8	3.22 (t, 7.8)	73.7			3.27 (t, 7.2)	73.7
3′	3.28 (d, 5.4)	75.2	3.20 (t, 6.3)	76.9			3.25 (t, 6.0)	77.0
4′	3.39 (brs)	69.2	3.16 (t, 9.3)	70.3			3.22 (t, 6.6)	70.7
5′	3.34 (m)	76.3	3.55 (t, 7.5)	74.5			3.75 (t, 6.6)	74.7
6′a	3.58 (dd, 12.3, 5.4)	60.5	4.08 (dd, 11.7, 6.3)	63.8			4.23 (dd, 10.5, 7.5)	64.7
6′b	3.74 (d, 12.3)		4.32 (d, 11.7)				4.63 (d, 11.7)	

Solvents: a (D_2O); b (d-DMSO); c ($CDCl_3$); d (d-DMSO)
b: the linolenic group: ^{1}H NMR: δ 2.27 (H-2″, 2 H, t, 6.9 Hz), δ 1.49 (H-3″, 2 H, m), δ 1.23 (H-4″–7″, 8 H, m), δ 2.03 (H-8″ 17″, 4 H, q, 8.4 Hz), δ 5.32 (H-9″ 10″ 12″ 13″ 15″ 16″, 6 H, m), δ 2.77 (H-11″ 14″, 4 H, t, 6.0 Hz), δ 0.92 (H-18″, 3 H, t, 6.9 Hz); ^{13}C NMR: (C-1″–18″): δ 173.0, 34.1, 25.0, 29.7, 29.4, 29.1, 29.7, 27.3, 130.6, 128.6, 25.9, 128.6, 128.2, 25.9, 128.2, 127.7, 20.7, 14.6
d: the 4″-hydroxy-3″, 5″-dimethyoxybenzoyl group: ^{1}H NMR: δ 7.22 (H-2″ 6″, 2 H, s), δ 9.33 (−OH, 1 H, brs), δ 3.81 (−MeO, 6 H, s); ^{13}C NMR: 119.9, 107.6, 148.3, 141.5, 148.3, 107.6, 166.2, 56.8 (−MeO)

Table 2: Half inhibitory concentrations (IC50) of compounds 1–4 (μg/ml)

Compound	SMMC-7721	L-02	HL-60
1	>200	>200	>200
2	121.87	>200	63.50
3	>200	>200	>200
4	>200	>200	>200
10-Hydroxycamptothecine	0.0157	0.0006	0.0084

C-23 (δ 65.3), and H-30 (δ 0.93) and C-29 (δ 81.1), C-19 (δ 40.0), C-20 (δ 35.1), C-21 (δ 28.2) in the HMBC spectrum. These cross peaks also suggested that there were two hydroxyl groups attached to C-3 and C-23, respectively. The coupling constant of H-3 (δ 4.81, 1 H, dd, J = 11.1, 4.8 Hz) suggested that the hydroxyl group at C-3 should be in β configuration. The downfield shift of C-29 to δ 81.1 suggested that the β-D-glucose was attached to C-29, which was confirmed by the cross peak between H-1′ (1 H, d, J = 7.8 Hz) and C-29. The broad singlet at δ 5.28 in the ^{1}H NMR spectrum of **6a** was characteristic for H-12 of oleane triterpene with a double bond laying at C_{12} (C_{13}). The NOE difference spectra showed gains for Me-24 (102%) when irradiating Me-26 and for H-30 (77%) when irradiating Me-25. But no methyl groups showed gains when Me-27 was irradiated. Thus the structure of compound **6a** should be elucidated as 3β, 23-dihydroxyl-29-*O*-β-D-glucopyranosylolean-12-en-28-oic hexaacetate. The ^{13}C NMR data of the aglycone part were similar to those of the reported compound 3β,23,29-trihydroxyolean-12-en-28 oic acid (Masazumi et al. 1999). So the structure of compound **6** should be deduced to be 3β,23-dihydroxyl-29-*O*-β-D- glucopyranosylolean-12-en-28-oic acid.

Compound **3**–**5**, **7**–**11** were identified as 3-hydroxyl-γ-pyranone (**3**) (Zhang et al. 1998), 3-*O*-β-D-[6′-(4″-hydroxy-3″,5″-dimethyoxybenzoyl)]glucopyranosyl-γ-pyranone (**4**) (Chen et al. 2002), erigeside (**5**) (Zhang et al. 1998), apigenin (**7**) (Li et al. 1999), 5,7,4′-trihydroxyflavanone (**8**) (Hu et al. 1999), apigenin-7-*O*-β-D-glucuronideethylester (**9**) (Ahmed et al. 1989), dehydrodiconiferyl alcohol-4-*O*-β-D-glucoside-hexaacetate (**10**) (Salama et al. 1981) and (+)-syringaresinol *O*-β-D-glucopyranoside (**11**) (Chen et al. 2002) respectively by comparison of their spectra data (^{1}H NMR and ^{13}C NMR) with those reported in the literature.

Using SRB method (Lee et al. 2003), the anti-tumor activities of compound **1**–**4** against human hepatoma (SMMC-7721), human embryo liver (L-02) and human leukemia (HL-60) cells were studied with the standard 10-hydroxycamptothecine. The half inhibitory concentrations (IC50) against the three cells were listed in Table 2. Among the four compounds tested, **2** exhibited weak anti-tumor activity especially against human leukemia (HL-60) cells.

3. Experimental

3.1. Equipment

Silica gel (200–300 mesh) used for column chromatography (CC) and silica GF_{254} (10–40 μm) for TLC were supplied by the Qingdao Marine Chemical factory, Qingdao, P.R. China. TLC were detected at 254 nm or by heating after being sprayed with 5% H_2SO_4 in C_2H_5OH (v/v). macroreticular resin (D101) was supplied by Tianjin Youchang Industrial Trade Co., LTD. Optical rotations were measured using a Perkin Elmer Model 341. IR spectra were obtained on a Nicolet NEXUS 670 FT-IR spectrometer. NMR spectra were recorded on a Varian Mercury-300BB NMR instrument (^{1}H NMR spectra at 300 MHz and ^{13}C NMR at 75 MHz) with TMS as the internal standard and d-$CHCl_3$, D_2O, d_6-DMSO or d_6-$(Me)_2CO$ as solvents. FAB-MS were measured on ZAB-HS MS instrument and the positive HR-ESIMS (secondary ion mass spectrometry) were carried out on a Bruker APEX II with glycerol as the matrix. Melting points were uncorrected.

3.2. Plant material

The aerial parts of *Erigeron annuus* were collected from Taibai Mountain (altitude: 800 m) in Shaanxi Province, P.R. China, in August 2000, and authenticated by Prof. Zhenhai Wu of Northwest Sci-Tech University of Agriculture and Forestry. A voucher specimen (No. 2000815) was deposited in the College of Chemistry and Chemical Engineering, Lanzhou University.

3.3. Extraction and isolation

The air-dried powered aerial parts of *Erigeron annuus* (7.4 kg) was extracted (×3) with MeOH (31.3 l) at room temperature. The extract was evaporated to dryness under reduced pressure and the residue was dissolved in H_2O. The aqueous solution was partitioned with EtOAc and *n*-BuOH successively to give fractions E and B, respectively. Fr.E (120.0 g) was chromatographed on a column of silica gel column (200–300 mesh, 1.0 kg), eluted with petroleum ether containing gradually increasing amounts of EtOAc and finally with MeOH. Combination of the appropriate fractions (monitored by TLC analysis) led to ten fractions. Fr.E.7 (5 : 1 and 3 : 1, 20 g) yielded a colorless crystal which was further purified by recrystallization from MeOH to afford compound **3** (3.0 g). The remaining solution was combined and evaporated to dryness (16.0 g) under reduced pressure, then it was chromatographed on a silica gel column (200–300 mesh, 160 g), eluted with a gradient of acetone in $CHCl_3$ to give compound **8** (2 mg). Fr.E.9 (0 : 1, 18.3 g) was chromatographed on a silica gel column (200–300 mesh, 200 g), eluted with $CHCl_3$/acetone (2 : 1) to fur-

nish a crude fraction (100 mg) containing **2**, which was further purified by PTLC over a silica gel plate (silica GF254, 10–40 μm, 25 × 25 cm × 1.0 mm) using $CHCl_3$/MeOH (8 : 1) as a developing system to give **2** (20 mg). Fr.E.10 (MeOH, 41.3 g) was chromatographed on a silica gel column (200–300 mesh, 290 g), eluted with a gradient of MeOH in $CHCl_3$ (20 : 1–0 : 1) to yield a white amorphous compound and a yellow one. The white powder was further purified by recrystallization from MeOH to afford compound **1**, while the yellow one was purified by PTLC over a silica gel plate (silica GF254, 10–40 μm, 25 × 25 cm × 1.0 mm) using EtOAc/MeOH/H_2O (6 : 1 : 0.5) as a developing system to give **5** (5 mg). A crude fraction (400 mg) containing compounds **10** and **6** was also got from this part. Various developing systems had been tried to separate the two compounds on silica gel columns (200–300 mesh). But all resulted in failure. Isolation of **6** and **10** was managed through acetylation (pyridine-Ac_2O 1 : 1) of the crude fraction followed by column chromatography using petroleum ether/acetone (4 : 1) as eluent. Fr.B was first subjected to a macroreticular resin column (D101, 500 ml), eluted first with distilled H_2O, then with EtOH. The EtOH part was evaporated to dryness under reduced pressure and then was subjected to column chromatography over silica gel (200–300 mesh, 350 g) eluted with $CHCl_3$ containing gradually increasing amounts of MeOH to give ten crude fractions Fr.B.1 – Fr.B.10. Recrystallization of Fr.B.4, Fr.B.10 and Fr.B.11 from MeOH gave compound **7** (20 mg), **4** (30 mg) and **9** (30 mg), respectively. Compound **11** (20 mg) was got from Fr.B.9 by column chromatography on silica gel (200–300 mesh, 4 g) with $CHCl_3$/MeOH (9 : 1) as the eluent.

3.4. 3-O-β-D-Galactopyranosyl-γ-pyranone (**1**)

White amorphous powder; m.p. 181–183 °C (MeOH); $[\alpha]_D^{22}$: −66.4° (c 6.8, H_2O); IR (ν_{max}, cm^{-1}, KBr): 3303, 1642, 1081, 1038, 1114; HR-ESIMS m/z: 275.0767 $[M+H]^+$ (calcd. for 275.0761 $[C_{15}H_{26}O_2 + H]^+$); ^1H and ^{13}C NMR data see Table 1.

3.5. 3-O-β-D-(6′-O-Linolenic)glucopyrannosyl-γ-pyranone (**2**)

White amorphous powder; m.p. 143–145 °C (Me_2CO); $[\alpha]_D^{19}$: −51° (c 0.125, CH_3OH); IR (ν_{max}, cm^{-1}, KBr): 3393, 2921, 2853, 1640, 1073; HR-ESIMS m/z: 535.2903 $[M+H]^+$ (calcd. for 535.2902 $[C_{29}H_{42}O_9 + H]^+$); ^1H and ^{13}C NMR data see Table 1.

3.6. 3-Hydroxyl-γ-pyranone (**3**)

Colorless columns, m.p. 117–120 °C (MeOH); ^1H and ^{13}C NMR data see Table 1.

3.7. 3-O-β-D-[6′-(4″-Hydroxy-3″,5″-dimethyoxybenzoyl)]glucopyranosyl-γ-pyranone (**4**)

White amorphous powder; m.p. 139–140 °C (MeOH); ^1H and ^{13}C NMR data see Table 1.

3.8. 29-O-β-D-Glucopyranosy-3β,23-dihydroxyolean-12-en-28-oic (**6a**)

Colorless oil; $[\alpha]_D^{25}$: +5° (c 0.6, $CHCl_3$); IR (ν_{max}, neat): 3343, 1752, 1226, 1694 cm^{-1}; FAB-MS: $[C_{48}H_{70}O_{16} + H]^+$ m/z = 903.2; HR-ESIMS m/z = 920.5020 (calcd. for 920.5002 $[C_{48}H_{70}O_{16} + NH_4]^+$); ^1H NMR ($CDCl_3$, δ, ppm, 300 MHz) 5.29 (1 H, brs, H-12), 3.03 (1 H, d, J = 11.7 Hz, H-29a), 3.72 (1 H, d, J = 11.7 Hz, H-29b), 4.81 (1 H, dd, J = 11.1, 4.8 Hz, H-3α), 3.80 (1 H, d, J = 11.7 Hz, H-23a), 3.89 (1 H, d, J = 11.7 Hz, H-23b), 0.83 (3 H, s, H-24), 0.97 (3 H, s, H-25), 0.75 (3 H, s, H-26), 1.12 (3 H, s, H-27), 0.93 (3 H, s, H-30), 4.45 (1 H, d, J = 7.8 Hz, H-1′), 5.05 (1 H, t, J = 9.3 Hz, H-2′), 5.24 (1 H, t, J = 9.3 Hz, H-3′), 5.12 (1 H, t, J = 9.3 Hz, H-4′), 3.66 (1 H, m, H-5′), 4.29 (1 H, dd, J = 12.3, 4.8 Hz, H-6′a), 4.15 (1 H, d, J = 12.3 Hz, H-6′b), 2.01–2.10 (6 CH_3CO-); ^{13}C NMR ($CDCl_3$, δ, ppm, 75 MHz, C_1-C_{30}) 36.8, 23.3, 74.5, 40.5, 47.7, 17.9, 32.2, 41.5, 47.7, 37.7, 22.8, 123.0, 143.3, 39.3, 27.4, 23.4, 46.6, 40.0, 40.0, 35.1, 28.2, 31.4, 65.3, 13.1, 15.8, 16.9, 25.7, 182.3, 81.1, 19.3, $C_{1'}-C_{6'}$: 101.7, 71.5, 71.7, 68.5, 72.7, 62.0; CH_3CO-: 20.6, 170.0.

3.9. SRB assay

Using SRB (sulforhodamine B) method (Lee et al. 2003), the anti-tumor activities of compounds **1**–**4** against human hepatoma (SMMC-7721), human embryo liver (L-02) and human leukemia (HL-60) cells were studied with the standard 10-hydroxycamptothecine. The test plates were incubated for 3 days at 37 °C in a 5% CO_2 incubator and after the incubation periods, the cells were fixed by the addition of aqueous TCA solution (4 °C for 30 min) and the fixed cells were stained with SRB (0.4% w/v in 1% aqueous acetic acid) for 30 min, the bound dye was solubilized with 200 μl of 10 mM tris-base (pH 10.0) and the absorbance was determined at 515 nm. The half inhibitory concentration (IC50) against the three cell lines are listed in Table 2. Among the four compounds tested, **2** exhibited weak anti-tumor activity especially against human leukemia (HL-60) cells.

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