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Four new phorbol diesters from *Croton tiglium* and their cytotoxic activities

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ABSTRACT

Phytochemical investigation of the seeds of *Croton tiglium* resulted in the isolation and structure elucidation of four new 4-deoxy-4β-phorbol diesters (**1–4**) named as 12-*O*-tiglylphorbol-4-deoxy-4β-phorbol-13-acetate (**1**), 12-*O*-tiglylphorbol-4-deoxy-4β-phorbol-13-hexadecanoate (**2**), 13-*O*-acetyl-phorbol-4-deoxy-4β-phorbol-20-oleate (**3**) and 13-*O*-acetylphorbol-4-deoxy-4β-phorbol-20-linoleate (**4**), respectively. The structures of the new compounds were established by 1D, 2D-NMR and HR-ESI–MS spectroscopic data and chemical degradation experiments. The cytotoxic activities of compounds **1–4** were evaluated against hepatic tumor cell lines (SNU387 and SNU398). Among these compounds, compound **4** exhibited the most potent activity against the SNU387 with IC_{50} value of 0.71 μM.

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1. Introduction

Croton tiglium L. belongs to the family Euphorbiaceae and is a leafy shrub with diverse biological activities. The oil prepared from the seeds of *C. tiglium* is rich in phorbol esters, which refer to tetracyclic tigliane diterpenes and generally possess tumor promoting properties. However, there are also many phorbol esters which do not cause tumorigenesis (Goel et al., 2007; El-Mekkawy et al., 2000). In the past decades, phorbol esters have been reported from several plants, such as *Sapium indicum, S. japonicum, Euphorbia frankiana, E. cocrulescence, E. ticulli, Croton spareiflorus, C. tigilium, C.ciliatoglandulifer, Jatropha curcas, Excoecaria agallocha* and *Homalanthus nutans* (Salatino et al., 2007). Phorbol esters isolated from *C. tiglium* attracted much more attention due to their exhibiting various bioactivities, including cytotoxic, anti-HIV-1 and anti-leukemia activities (Bauer et al., 1983; Brune et al., 1978; Colburn et al., 1982; El-Mekkawy et al., 1999; zur Hausen et al., 1978). Our previous phytochemical investigation of this species led to the discovery of nineteen cytotoxic phorbol esters with two kinds of backbones: 4β-hydroxy-phorbol and 4-deoxy-4α-phorbol (Zhang et al., 2013). A continuous study of this plant led to the purification of four new phorbol diesters (**1–4**) having a 4-deoxy-4β-phorbol skeleton, which had only been reported in *S. indicum* (Chumkaew et al., 2003). The present paper deals with the isolation, structural elucidation and cytotoxic activities of these compounds. (Fig. 1).

2. Results and discussion

2.1. Structure elucidation and cytotoxic activities of the new compounds

Compound **1** was assigned the molecular formula $C_{27}H_{36}O_7$ on the basis of the HRESIMS (*m/z* 495.2365 $[M+Na]^+$, calcd 495.2353) and NMR data, indicating 10 degrees of unsaturation. The IR spectrum showed the absorption bands for hydroxy (3416 cm^{-1}) and carbonyl (1713 cm^{-1}) groups. In the ^1H NMR spectrum of compound **1**, two olefinic proton singlets at δ_H 7.55 (1H, s, H-1) and 5.53 (1H, s, H-7), four methyl signals at δ_H 1.22 (3H, s, CH_3-16), 1.20 (3H, s, CH_3-17), 0.91 (3H, d, J = 6.4 Hz, CH_3-18) and 1.73 (3H, s, CH_3-19), one oxymethylene doublet at δ_H 4.03, 3.99 (2H, d, J = 13.7 Hz, CH_2-20) and one methylene quartet at δ_H 2.84, 2.15 (2H, dd, J = 18.7, 9.4 Hz, CH_2-5) were clearly observed. The ^{13}C NMR spectrum together with DEPT experiments indicated the existence of seven methyls, two methylenes, nine methines and nine quaternary

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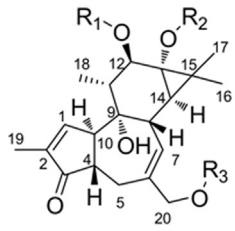

Compound	R_1	R_2	R_3
1	tiglyl	acetyl	H
2	tiglyl	hexadecanoyl	H
3	H	acetyl	oleoyl
4	H	acetyl	linoleoyl

Fig. 1. Chemical structures of compounds **1**–**4**.

carbons. The signals at δ_C 209.7 (C-3), 179.4 (C-1″) and 167.6 (C-1′) in the ^{13}C NMR spectrum were attributed to carbonyl carbons. The obvious HMBC correlations from a methyl singlet at δ_H 1.73 (3H, s, CH_3-19) to carbons at δ_C 159.7 (C-1), 136.4 (C-2) and 209.7 (C-3) suggested the presence of an α, β-unsaturated carbonyl group. The ^{1}H–^{1}H COSY correlations of H-1/H-10/H-4/H-5, of H-7/H-8/H-14 and H-12/H-11/H-18 showed the connection patterns of C-1–C-10–C-4–C-5, C-7–C-8–C-14 and C-12–C-11–C-18 (Fig. 2). These signals suggested that compound **1** possessed the 4-deoxy-4α-phorbol or 4-deoxy-4*β*-phorbol backbone (Taylor et al., 1981; Chumkaew et al., 2003). The chemical shifts of H-1 (δ_H 7.55), H-4 (δ_H 2.48), H-5 (δ_H 2.15 and 2.84) illustrated the 4-deoxy-4*β*-phorbol skeleton of **1** rather than 4-deoxy-4α-phorbol skeleton, in which the chemical shifts of these protons H-1, H-4 and H-5 were at around H-1 (δ_H 7.00), H-4 (δ_H 2.70), H-5 (δ_H 2.50 and 3.30) (Zhang et al., 2013). The chemical shift values of C-1, C-2 and C–10 at δ_C 159.7, 136.4 and 54.1 also verified the β configuration of H-4 in compound **1** (Chumkaew et al., 2003). In addition, the proposed stereochemistry of compound **1** was supported from NOE correlations between H-4/H-8/H-11 on the β face and H-1, H-7, H-14 and H-18 on the other side of the molecule (Fig. 2). The coupling constant between H-11 and H-12 was 9.7 Hz, confirming the α configuration of H-12 (Chumkaew et al., 2003). Other characteristic resonances included signals for a tiglyl group and an acetyl moiety. In the HMBC spectrum, H-12 proton showed a ^3J correlation with the carbonyl carbon of the tiglyl group (δ_C 167.6), confirming the location of the tiglyl group at C-12. Thus, the structure of **1** was determined to be 12-*O*-tiglylphorbol-4-deoxy-4*β*-phorbol-13-acetate.

Compound **2** exhibited a $[M+Na]^+$ peak at *m*/*z* 691.4548 in the HRESIMS (calcd 691.4544), consistent with a molecular formula of $C_{41}H_{64}O_7$, requiring 10 degrees of unsaturation. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **2** were similar to those of compound **1**. After hydrolysis with 0.1 M NaOMe, methyl hexadecanoate was assigned by GC–MS (t_R 17.2 min, *m*/*z* 270 $[M]^+$). In addition, NMR signals for a hexadecanoyl group in **2** were observed to replace those for an acetyl moiety in **1**. Other characteristic resonances included signals for a tiglyl group (Zhang et al., 2013; Chumkaew et al., 2003). In the HMBC spectrum, H-12 proton showed a ^3J correlation with the carbonyl carbon of the tiglyl group, confirming the location of the tiglyl group at C-12. Thus, the structure of **2** was determined to be 12-*O*-tiglylphorbol-4-deoxy-4*β*-phorbol-13-hexadecanoate.

Compound **3** was assigned the molecular formula $C_{40}H_{62}O_7$ on the basis of the HRESIMS (*m*/*z* 677.4396 $[M+Na]^+$, calcd 677.4388) and NMR data, indicating 10 degrees of unsaturation. ^{1}H NMR and ^{13}C NMR data (Tables 1 and 2) for compound **3** indicated that compounds **3** and **1** shared the same backbone. After hydrolysis with 0.1 M NaOMe, methyl oleate was identified by GC–MS (t_R 21.9 min, *m*/*z* 296 $[M]^+$). Other characteristic resonances included signals for an acetyl group and an oleyl moiety (Zhang et al., 2013). In the HMBC spectrum, H-20 showed a ^3J correlation with the carbonyl carbon of the oleyl group, confirming the location of the oleyl group at C-20. Thus, the structure of **3** was determined to be 13-*O*-acetylphorbol-4-deoxy-4*β*-phorbol-20-oleate.

Compound **4** was assigned the molecular formula $C_{40}H_{60}O_7$ on the basis of the HRESIMS (*m*/*z* 675.4220 $[M+Na]^+$, calcd 675.4231) and spectroscopic data, indicating 11 degrees of unsaturation. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **4** was quite similar to those of **3**, except that the oleoyl group in **3** was replaced by a linoleoyl group in **4**. Hydrolysis of **4** with 0.1 M NaOMe gave methyl linoleate, which was verified by GC–MS (t_R 23.0 min, *m*/*z* 294 $[M]^+$) analysis. Furthermore, In the HMBC spectrum, H-20 proton showed a ^3J correlation with the carbonyl carbon of the linoleyl group, confirming the location of the linoleyl group at C-20. Hence, the structure of **4** was determined to be 13-*O*-acetylphorbol-4-deoxy-4*β*-phorbol-20-linoleate.

The skeletons of the isolated phorbol diesters can be divided into three categories: 4*β*-hydroxy-phorbol (I), 4-deoxy-4α-phorbol (II) and 4-deoxy-4*β*-phorbol (III). Although their chemical structures are very similar, the ^{1}H NMR is still a powerful method to distinguish them. For type I skeleton, the chemical shift of H-1is usually in the range of 7.58–7.62 ppm, while the H_2-5 often appear in the region of 2.5 and 2.6 ppm, respectively (Zhang et al., 2013). For type II and III backbones, the chemical shifts of H-1, H-4 and

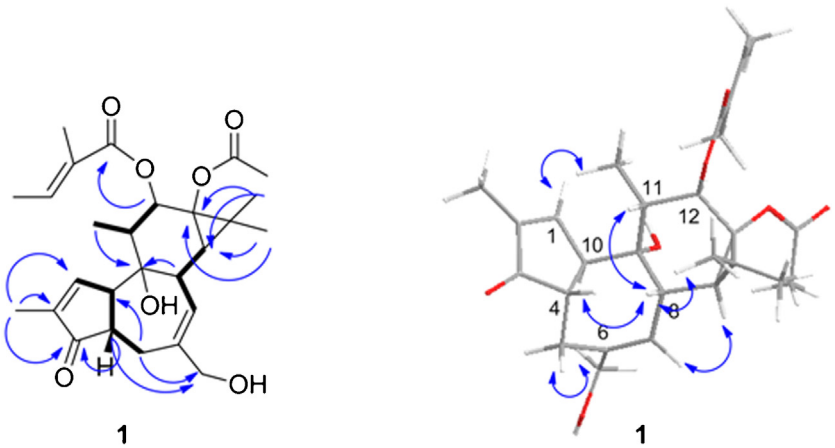

Fig. 2. Key ^{1}H–^{1}H COSY (—), HMBC (→) and NOESY (↔) correlations of **1**.

Table 1
^{1}H NMR (600 MHz) data for compounds **1**–**4** ($CDCl_3$).

No.	**1**	**2**	No.	**3**	**4**
H-1	7.55, s	7.56, s	H-1	7.54, s	7.54, s
H-4	2.48, m	2.48, m	H-4	2.47, m	2.47, m
H-5α	2.84, dd (18.7,9.4)	2.85, dd (18.2,10.2)	H-5α	2.84, dd (18.2,9.2)	2.83, dd (18.3,9.2)
H-5β	2.15, dd (18.7,9.4)	2.15, dd (18.2,10.2)	H-5β	2.13, dd (18.2,9.2)	2.13, dd (18.3,9.2)
H-7	5.53, d (5.4)	5.54, d (4.2)	H-7	5.51, d (4.1)	5.51, d (3.5)
H-8	2.38, br, m	2.39, br, m	H-8	2.35, br, m	2.35, br, m
H-10	3.24, m	3.25, m	H-10	3.14, m	3.14, m
H-11	1.60, m	1.61, m	H-11	1.42, m	1.43, m
H-12	5.47, d (9.7)	5.47, d (9.7)	H-12	4.00, d (7.6)	4.00, d (9.1)
H-14	1.08, d (5.3)	1.05, d (5.3)	H-14	1.01, d (5.7)	1.01, d (4.8)
H-16	1.22, s	1.23, s	H-16	1.23, s	1.23, s
H-17	1.20, s	1.21, s	H-17	1.21, s	1.22, s
H-18	0.91, d (6.4)	0.91, d (6.4)	H-18	1.12, d (6.4)	1.12, d (6.3)
H-19	1.73, s	1.73, s	H-19	1.75, s	1.75, s
H-20α	4.03, d (13.7)	4.03, d (13.9)	H-20α	4.49, d (12.7)	4.49, d (12.6)
H-20β	3.99, d (13.7)	4.00, d (13.9)	H-20β	4.43, d (12.7)	4.43, d (12.6)
R_1			R_2		
1′			1”		
2′			2”	2.12, s	2.12, s
3′	6.84, m	6.83, m			
4′	1.80, d (7.0)	1.80, d (7.0)	R_3		
5′	1.84, s	1.84, s	1‴		
			2‴	2.30, t (6.1)	2.30, t (7.3)
R_2			3‴	1.60, m	1.60, m
1”			4‴	1.27–1.34, m	1.29–1.36, m
2”	2.10,s	2.31, m	5‴	1.27–1.34, m	1.29–1.36, m
3”		1.60, m	6‴	1.27–1.34, m	1.29–1.36, m
4”		1.23–1.35, m	7‴	1.27–1.34, m	1.29–1.36, m
5”		1.23–1.35, m	8‴	2.01, m	2.05, m
6”		1.23–1.35, m	9‴	5.34, m	5.356, m
7”		1.23–1.35, m	10‴	5.34, m	5.36, m
8”		1.23–1.35, m	11‴	2.01, m	2.77, m
9”		1.23–1.35, m	12‴	1.27–1.34, m	5.36, m
10”		1.23–1.35, m	13‴	1.27–1.34, m	5.36, m
11”		1.23–1.35, m	14‴	1.27–1.34, m	2.05, m
12”		1.23–1.35, m	15‴	1.27–1.34, m	1.29–1.36, m
13”		1.23–1.35, m	16‴	1.27–1.34, m	1.29–1.36, m
14”		1.23–1.35, m	17‴	1.27–1.34, m	1.29–1.36, m
15”		1.23–1.35, m	18‴	0.89, t (7.2)	0.89, t (7.2)
16”		0.88, t (7.2)			

H-5 are all obviously different (Chumkaew et al., 2003; Taylor et al., 1981). The presence of two signals at around 7.04 (H-1) and 2.7 (H-4) ppm suggests the type II compounds, while the appearance of two peaks at around 7.55 (H-1) and 2.5 (H-4) ppm indicates the type III ones. Moreover, the two chemically non-equivalent protons of H_2-5 in type II individuals appear at 2.5 and 3.5 ppm, in contrast to 2.1 and 2.8 ppm in type III compounds. The substituent groups in phorbol diesters are often located at C-12, C-13 and C-20. Generally speaking, the C-13 hydroxyl is always substituted. Hence, the other substituent group is located at C-12 or C-20, which can be easily determined by HMBC experiment.

Compounds **1**–**4** were tested for their cytotoxicities against the SNU387 and SNU398 hepatic tumor cell lines using the MTT method, with paclitaxel as the positive control (Table 3). Among these phorbol diesters, compounds **3** and **4** were found to be the most potent cytotoxic against SNU387, with IC_{50} values of 1.9 and 0.71 μM, respectively.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were run on a Shimadzu UV-2550 spectrophotometer. IR data were obtained on a Perkin-Elmer 983 G spectrometer. 1D and 2D NMR spectra were recorded on a Bruker Avance 600 spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C) with TMS as the internal standard. High-resolution mass spectrometry experiments were performed on a Bruker Bio TOF-Q mass spectrometer equipped with an ESI source. GC–MS analysis was performed on Thermo Trace-PolarisQ GC–MS with an Agilent DB-1 column (60 m × 0.25 mm × 0.5 μm). Analytical HPLC was carried out on Lab Alliance Series III model 201 (SSI) using Ultimate C_{18} column (250 mm × 4.60 mm, 5 μm). Preparative HPLC was carried out on P3000 with a UV3000 detector (Beijing Chuangxintongheng Science and Technology Co., Ltd.) and Ultimate C_{18} column (250 mm × 21.2 mm, 5 μm). Normal phase column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China). TLC was detected with 254 nm UV light and visualized by spraying with a solution of 5% H_2SO_4 in C_2H_5OH followed by heating. The software ChemBioDraw was used to feature the stereochemistry of the compound in Fig. 2.

3.2. Plant material

The seeds of *C. tiglium* were collected from Silie Town, Yibin County, Sichuan Province, People's Republic of China, in November 2011. Its botanical identity as *C. tiglium* was confirmed by Prof. Feng-e Wu, Chengdu Institute of Biology, Chinese Academy of Sciences. A voucher specimen (CIB-4-304-307) has been conserved in the Center for Natural Products, Chengdu Institute of Biology, Chinese Academy of Sciences.

Table 2
^{13}C NMR (150 MHz) data for compounds **1**–**4** ($CDCl_3$).

No.	1	2	No.	3	4
1	159.7	159.8	1	160.6	160.4
2	136.4	136.4	2	132.3	132.3
3	209.7	209.7	3	207.9	207.7
4	44.2	44.2	4	44.2	44.3
5	29.6	29.6	5	28.2	29.6
6	142.1	142.0	6	137.0	138.0
7	126.4	126.5	7	127.4	127.5
8	42.1	42.1	8	40.1	40.0
9	77.8	77.8	9	78.1	78.1
10	54.1	54.1	10	56.1	55.8
11	42.6	42.3	11	45.3	45.2
12	76.0	76.0	12	72.0	72.0
13	65.4	65.1	13	65.2	65.1
14	35.6	35.8	14	35.3	35.5
15	25.6	25.7	15	26.1	26.0
16	16.8	16.8	16	16.9	16.8
17	23.7	23.8	17	23.7	23.8
18	14.4	14.4	18	14.3	14.4
19	10.2	10.2	19	10.1	10.2
20	67.4	67.4	20	77.4	77.3
R_1			R_2		
1′	167.6	167.6	1”	173.5	173.5
2′	128.5	128.6	2”	21.0	21.0
3′	137.4	137.6			
4′	14.4	14.4	R_3		
5′	12.2	11.8	1‴	174.1	174.2
			2‴	34.2	31.5
R_2			3‴	24.9	24.9
1”	179.4	176.4	4‴	29.1–29.8	29.1
2”	23.4	34.5	5‴	29.1–29.8	29.2–29.6
3”		25.7	6‴	29.1–29.8	29.2–29.6
4”		29.1–29.6	7‴	29.1–29.8	29.2–29.6
5”		29.1–29.6	8‴	27.2	27.2
6”		29.1–29.6	9‴	129.7	130.2
7”		29.1–29.6	10‴	129.8	128.1
8”		29.1–29.6	11‴	27.1	25.6
9”		29.1–29.6	12‴	29.1–29.8	127.9
10”		29.1–29.6	13‴	29.1–29.8	130.2
11”		29.1–29.6	14‴	29.1–29.8	26.3
12”		29.1–29.6	15‴	29.1–29.8	29.2–29.6
13”		29.1–29.6	16‴	31.9	29.2–29.6
14”		31.8	17‴	22.7	22.6
15”		22.6	18‴	14.1	14.0
16”		14.1			

Table 3
Cytotoxic activity evaluation of compounds **1**–**4**.[a]

compound	SNU387	SNU398
1	59.5 ± 2.1	43.7 ± 1.5
2	30.2 ± 1.4	91.2 ± 3.7
3	1.9 ± 0.2	13.5 ± 1.1
4	0.71 ± 0.08	18.2 ± 1.7
paclitaxel	0.35 ± 0.05	4.5 ± 0.5

[a] Data are expressed as IC_{50} values (μM).

3.3. Extraction, isolation and characterization of the compounds

The air-dried seeds of *C. tiglium* (7.1 kg) were powdered and extracted with MeOH three times (each 72 h) at room temperature to yield a methanolic extract, which was concentrated to yield 957.9 g of residue. The residue was suspended in water and extracted successively with petroleum ether (60–90 °C), $CHCl_3$ and *n*-BuOH. The $CHCl_3$ extract (740.4 g) was subjected to a silica gel [100–200 mesh] column eluted successively with a gradient of with EtOAc-petroleum ether (0–100%) to give nine fractions: Fr.1 (17.3 g), Fr.2 (27.1 g), Fr.3 (200.5 g), Fr.4 (156.4 g), Fr.5 (64.5 g), Fr.6 (37.1 g), Fr.7 (33.7 g), Fr.8 (7.5 g) and Fr.9 (40.5 g). Fr.7 (33.7 g) was further separated by column chromatography over silica gel (200−300 mesh) eluted successively with a gradient of $(CH_3)_2CO$—CH_2Cl_2 (0:10 to 10:0) and pooled together using HPLC to obtain 10 sub-fractions: Fr.7a (1.8 g), Fr.7b (0.9 g), Fr.7c (1.5 g), Fr.7d (2.8 g), Fr.7e (2.5 g), Fr.7f (2.9 g), Fr.7 g (3.5 g), Fr.7 h (2.7 g), Fr.7i (2.7 g), and Fr.7j (3.1 g).

Fr.7e (100 mg) was separated by semi-preparative HPLC (60% Acetonitrile-H_2O, flow rate 12 mL/min) to afford compounds **2** (13 mg) and **4** (14 mg). Fr.7 g (3.5 g) was purified by silica gel column chromatography eluted with CH_2Cl_2—CH_3OH (10:1 to 1:1) to yield five sub-fractions (Fr.7g1-Fr.7g5). Fr.7g2 (210 mg) was applied to semi-preparative HPLC (60% Acetonitrile-H_2O, flow rate 12 mL/min) to give compound **3** (14 mg). Fr.7i (2.7 g) was subject to passage over a C_{18} column (CH_3OH—H_2O, 55:45 to 80:20, stepwise) to afford four sub-fractions (Fr.7i1-Fr.7i4). Fr.7i3 (335 mg) was then purified by semi-preparative HPLC (75% Acetonitrile-H_2O, flow rate 12 mL/min) to give compound **1** (11 mg).

3.3.1. 12-O-*tiglylphorbol-4-deoxy-4*β*-phorbol-13-acetate* (***1***)

Colorless gum; $[\alpha]^{25}_D = -28.5$ (*c* 0.22, $CHCl_3$); UV (MeOH), λ_{max} (log ε): 234 (4.24) nm; IR (KBr) ν_{max}: 3416, 2930, 1713, 1658, 1464, 1386, 1259, 1164, 1037, 880 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (positive ion mode) *m/z* 495.2365 $[M+Na]^+$ (calcd 495.2353 for $C_{27}H_{36}O_7Na$).

3.3.2. 12-O-*tiglylphorbol-4-deoxy-4*β*-phorbol-13-acetate* (***2***)

Colorless gum; $[\alpha]^{25}_D = -15.1$ (*c* 0.20, $CHCl_3$); UV (MeOH), λ_{max} (log ε): 234 (3.82) nm; IR (KBr) ν_{max}: 3410, 2925, 1711, 1647, 1455, 1375, 1250, 1151, 1027, 881 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (positive ion mode) *m/z* 691.4548 $[M+Na]^+$ (calcd 691.4544 for $C_{41}H_{64}O_7Na$).

3.3.3. 13-O-*Acetylphorbol-4-deoxy-4*β*-phorbol-20-oleate* (***3***)

Colorless gum; $[\alpha]^{25}_D = +40.8$ (*c* 0.34, $CHCl_3$); UV (MeOH), λ_{max} (log ε): 231 (3.92) nm; IR (KBr) ν_{max}: 3412, 2951, 2024, 1710, 1376, 1258, 1071, 801 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (positive ion mode) *m/z* 677.4396 $[M+Na]^+$ (calcd 677.4388 for $C_{40}H_{62}O_7Na$).

3.3.4. 13-O-*acetylphorbol-4-deoxy-4*β*-phorbol-20-linoleate* (***4***)

Colorless gum; $[\alpha]^{25}_D = +30.4$ (*c* 0.15, $CHCl_3$); UV (MeOH), λ_{max} (log ε): 235 (4.02) nm; IR (KBr) ν_{max}: 3415, 3012, 2041, 1720, 1385, 1263, 1088, 803 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (positive ion mode) *m/z* 675.4220 $[M+Na]^+$ (calcd 675.4231 for $C_{40}H_{60}O_7Na$).

3.4. Cytotoxicity assays

The cytotoxicity of all isolated compounds against the SNU387 hepatic tumor cell line (from the Shanghai Cell Bank, Chinese Academy of Sciences) was measured using the microculture tetrazolium (MTT) assay (Mosmann, 1983). The cells were maintained in RPMI1640 medium with 10% FBS (fetal bovine serum), harvested and seeded in 96-well plates. Then they were treated with the test compounds at various concentrations and incubated for 48 h followed by a MTT assay. Absorbance of the solution was measured using a microplate reader spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA), at a wavelength of 570 nm. Half-maximal inhibitory concentration (IC50) values were calculated using GraphPad Prism 4 (San Diego, CA). Paclitaxel was used as the positive control. All samples were assayed in triplicate.

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