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Galiellalactone analogs and their possible precursors from *Sarcosomataceae*

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ABSTRACT

Galiellalactone analogs (**1**–**4**) (including two new compounds), together with their possible precursors (**5–9,** named pregaliellalactone B-F), were obtained from the solid cultures of an endophytic fungus *Sarcosomataceae* NO.45-1-8-1. Their chemical structures were elucidated by analyses of HR ESI-TOF MS, 1D-, 2D-NMR, CD spectra and single crystal X-ray diffraction methods. Compounds **5–9**, the possible precursors of galiellalactone analogs, were found to exist as enantiomers for the first time. The cytotoxicity of these compounds against six tumor cell lines was examined and preliminary structure–activity relationship (SAR) was also discussed.

1. Introduction

Galiellalactone, first isolated from cultures of *Galiella rufa*, was the characteristic secondary metabolite produced by the family of *Sarcosomataceae*, which was used as the chemotaxonomic marker of the *Sarcosomataceae* [1,2]. Galiellalactone was reported to be a potent inhibitor of IL-6 signaling mediated by STAT3, a signal transducer and activator of transcription that was constitutively active in several forms of cancer [3–6]. Recently, apart from the inhibitory of STAT3 signaling pathway, galiellalactone was found to inhibit other signal pathways, such as TGF-β signaling and NF-κB signaling [7,8]. Also, some of (−)-galiellalactones' precursors and structurally related compounds were reported to have nematicidal activity [9]. So, galiellalactone and its analogs showed great promising for development of new drug candidates.

In our search for bioactive components from microorganisms, four galiellalactone analogs (**1**–**4**) (including two new compounds), together with five new possible precursors (**5–9,** named pregaliellalactone B–F), were obtained from the solid cultures of *Sarcosomataceae* NO.45-1-8-1, an endophytic fungus isolated from *Cladonia gracilis* (L.) Willd. subsp. *turbinata* (Ach.) Ahti. Herein, we described the isolation and structure elucidation of new galiellalactone analogs and their possible precursors. In addition, the cytotoxicity of these compounds against six tumor cell lines was examined, and preliminary structure–activity relationship (SAR) was also discussed.

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2. Experimental

2.1. General experimental procedures

Optical rotations were obtained on a P-1020 digital polarimeter (Jasco Corporation). IR spectra were recorded on a JASCO FTIR-480 plus spectrometer. CD spectrum was recorded in $CHCl_3$ using a JASCO J-810 spectrophotometer at room temperature. NMR spectra were measured on Bruker AV 300 and 400. The chemical shifts were given in ppm relative to chemical shifts of solvent resonances (CD_3OD: δ_H 3.30/δ_C 49.0). HR-ESI-MS spectra were obtained on a Micromass Q-TOF mass spectrometer. A single-crystal X-ray diffraction was measured on Agilent SuperNova. Analytical HPLC was performed on a SHIMADZU LC-20AB Liquid Chromatograph with SPD-M20A Detector using a cosmosil C18 column (4.6 × 250 mm, 5 μm). Preparative HPLC was performed on a SHIMADZU LC-6AD Liquid Chromatograph with SPD-20A Detector using an ODS column [YMC-Pack ODS-A (10.0 × 250 mm, 5 μm, 220 and 208 nm)]. Open column chromatography (CC) was performed using silica gel (200–300 mesh, Qingdao Haiyang Chemical Group Corp., Qingdao), ODS (50 μm, YMC). TLC analysis was performed on pre-coated silica gel GF254 plates (Qingdao Haiyang Chemical Group Corp., Qingdao).

2.2. Microorganism and fermentation

The fungal strain (NO.45-1-8-1) was isolated from the host of lichen *Cladonia gracilis* (L.) Willd. subsp. *turbinata* (Ach.) Ahti collected from Changbaishan, Jilin province of China by Prof. Liang-Dong Guo and was identified as the family of *Sarcosomataceae* based on the morphological characters and analyses of the Internal Transcribed Spacer (ITS) region of the rDNA, whose sequence data have been deposited at GenBank with the accession number KP109690. The strain was preserved in the Institute of Traditional Chinese Medicine & Natural Products, Jinan University.

The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 5 days. Agar plugs were used to inoculate four Erlenmeyer flasks (250 mL), each containing 100 mL of potato dextrose broth (PDB). Four flasks of the inoculated media were incubated at 25 °C on a rotary shaker at 200 rpm for 5 days to prepare the seed culture. Fermentation was carried out in 20 Erlenmeyer flasks (500 mL), each containing 70 g of rice. Distilled H_2O (105 mL) was added to each flask, and the contents was soaked overnight before autoclaving at 120 °C for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at room temperature for 55 days.

2.3. Extraction and isolation

The fermented mycelia were extracted with EtOAc (5 × 3 L) at room temperature for three times, and the organic solvent extract was evaporated to dryness under vacuum to afford the crude extract (32.4 g). The extract was subjected to silica gel CC (ϕ 11.0 × 52 cm) using cyclohexane–ethyl acetate and ethyl acetate–CH_3OH gradient to give 14 fractions (a–n). Fraction c (1000.0 mg) was separated by ODS CC (ϕ 3.0 × 13 cm) eluted with CH_3OH–H_2O (60:40, 70:30, 80:20, 100:0, v/v) to afford 4 subfractions (c-a to c-d). Subfraction c-a (30.0 mg) was applied to RP-HPLC (50% MeOH–H_2O, a flow rate of 3.5 mL/min) to afford **5** (8.1 mg, t_R = 40.3 min). Fraction f (1500.5 mg) was subjected to ODS CC (ϕ 3.0 × 13 cm) using a CH_3OH–H_2O gradient (30:70, 40:60, 50:50, 80:20, 100:0, v/v) to give 5 subfractions (f-a to f-e). Subfraction f-b (325.3 mg) was applied to RP-HPLC (40% CH_3OH–H_2O, a flow rate of 3.2 mL/min) to afford **1** (90.6 mg, t_R = 14.3 min), **2** (16.0 mg, t_R = 15.2 min), **8** (7.5 mg, t_R = 22.2 min) and **9** (5.1 mg, t_R = 20.6 min). Fraction g (800.7 mg) was separated by ODS CC (ϕ 3 × 13 cm) eluting with CH_3OH–H_2O (30:70, 40:60, 50:50, 60:40, 100:0, v/v) to afford 5 subfractions (g-a to g-e). Subfraction g-c (23.6 mg) was applied to RP-HPLC (35% CH_3OH–H_2O, a flow rate of 3 mL/min) to afford **3** (18.6 mg, t_R = 22.5 min). Fraction h (1000.9 mg) was subjected to ODS CC (ϕ 3.0 × 13 cm) using a CH_3OH–H_2O gradient (30:70, 40:60, 50:50, 60:40, 100:0, v/v) to give 5 fractions (h-a to h-e). Subfraction h-b (153.4 mg) was applied to RP-HPLC (30% CH_3OH–H_2O, a flow rate of 3.5 mL/min) to afford **6** (16.2 mg, t_R = 13.1 min) and **7** (8.1 mg, t_R = 19.8 min). Fraction j (6000.8 mg) was separated by ODS CC (ϕ 3 × 13 cm) eluting with CH_3OH–H_2O (10:90, 20:80, 30:70, 40:60, 100:0, v/v) to afford 6 subfractions (j-a to j-f). Subfraction j-c (900.7 mg) was applied to silica gel CC (ϕ 1.5 × 19 cm) eluting with $CHCl_3$–CH_3OH (98:2, v/v) to give **4** (10.2 mg).

2.3.1. Galiellalester (**2**)

Colorless oil; $[\alpha]_D^{26}$ -23.1 (*c* 0.57, $CHCl_3$); IR (KBr) ν_{max} 3421, 2958, 2925, 2854, 1744 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESI-MS *m/z* 227 $[M + H]^+$, 249 $[M + Na]^+$; HR-ESI-MS: *m/z* 249.1089 $[M + Na]^+$ (calcd for $C_{12}H_{18}O_4Na$, 249.1103).

2.3.2. Isogaliellalactone (**3**)

White amorphous powder; $[\alpha]_D^{26}$ -52.4 (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 3430, 2957, 2929, 2857, 1697, 1567, 1247 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESI-MS *m/z* 195 $[M + H]^+$, 217 $[M + Na]^+$; HR-ESI-MS *m/z* 195.1018 $[M + H]^+$ (calcd for $C_{11}H_{15}O_3$, 195.1021).

2.3.3. Pregaliellalactone B (**5**)

Colorless oil; $[\alpha]_D^{26}$ 32.0 (*c* 0.35, $CHCl_3$); IR (KBr) ν_{max} 3438, 2928, 2855, 1589 cm^{-1}; ^1H and ^{13}C NMR data (Table 2); ESI-MS *m/z* 181 $[M + H]^+$, 203 $[M + Na]^+$; HR-ESI-MS *m/z* 181.1243 $[M + H]^+$ (calcd for $C_{11}H_{17}O_2$, 181.1229).

Table 1
Assignment of ^1H and ^{13}C NMR data for compounds **2** and **3** (δ in ppm, *J* in Hz).

Position	2[a]		3[a]	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
2		169.3 s		178.5 s
2a		132.1 s	3.11, m	49.2 d
3	6.97, m	151.1 d	5.49, m	114. 3 d
4	2.36, m	33.0 d		135.0 s
5	1.43, overlap	38.8 t	1.95, overlap	29.3 t
	1.68, overlap		2.37, m	
5a	2.04, overlap	46.8 d	2.21, m	45.7 d
6	1.69, overlap	32.6 t	1.33, m	29.4 t
	2.22, overlap		1.64, m	
7	1.51, overlap	29.5 t	1.79, overlap	32.1 t
	2.12, overlap		1.92, overlap	
7a	4.32, dd, 5.2, 1.4	80.1 d	4.70, dd, 5.8, 0.9	92.9 d
7b		83.2 s		82.5 s
8	1.06, d, 7.3	21.1 q	1.76, m	24.1 q
9	3.75, s	52.0 q		

[a] Measured at 300 MHz for ^1H and 75 MHz for ^{13}C in CD_3OD.

Table 2
Assignment of ^{1}H and ^{13}C NMR data for compounds **5–9** (δ in ppm, J in Hz).

Position	5[a]		6[b]		7[b]		8[b]		9[b]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
2		176.2 s		176.3 s		176.2 s		175.9 s		175.8, s
3		134.8 s		136.2 s		134.3 s		134.9 s		134.9, s
4	7.28, m	151.1 d	7.21, m	148.1 d	7.33, m	151.5 d	7.31, m	150.7 d	7.29, m	150.6, d
5	4.99, overlap	82.6 d	5.01,m	84.1 d	5.12, m	80.7 d	5.10,m	80.4 d	5.35, m	79.0,d
6	1.67,m	33.7 t	3.65, dd, 12.2, 4.8	62.8 t	1.73, m	37.5 t	1.88, m	33.5 t	2.83, d, 6.5	45.8, t
	1.86, m		3.84, dd, 12.2, 3.8		1.95, m		2.13, m			
7	2.18, overlap	30.3 t			3.71, dd, 6.8, 5.6	58.9 t	4.18, m	61.5 d		209.3, s
8	5.84, m	138.5 d						172.6 s	2.51, q, 7.3	37.0, t
9	5.03, m	116.1 t					2.03, s	20.8 q	1.03, t, 7.3	7.7, q
1′	2.22, overlap	28.1 t	2.24, m	28.2 t	2.22, m	28.1 t	2.23, m	28.1 t	2.22, m	28.1, t
2′	1.58, m	21.8 t	1.59, sext, 7.5	21.8 t	1.58, sext, 7.5	21.8 t	1.59, sext, 7.3	21.8 t	1.58, sext, 7.3	21.8, t
3′	0.96, t, 7.5	14.0 q	0.96, t, 7.5	14.0 q	0.96, t, 7.3	14.0 q	0.96, t, 7.3	14.0 q	0.96, t, 7.3	14.0, q

[a] Measured at 400 MHz for ^{1}H and 100 MHz for ^{13}C in CD_3OD.
[b] Measured at 300 MHz for ^{1}H and 75 MHz for ^{13}C in CD_3OD.

*2.3.4. Pregaliellalactone C (**6**)*

Colorless oil; $[\alpha]_D^{26}$ -7.6 (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 3425, 2961, 2928, 2854, 1748 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 2); ESI-MS *m/z* 157 $[M + H]^+$, 179 $[M + Na]^+$; HR-ESI-MS *m/z* 157.0861 $[M + H]^+$ (calcd for $C_8H_{13}O_3$, 157.0865).

*2.3.5. Pregaliellalactone D (**7**)*

Colorless oil; $[\alpha]_D^{26}$ 17.8 (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 3436, 2961, 2932, 2875, 1746, 1060 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 2); ESI-MS *m/z* 171 $[M + H]^+$, 193 $[M + Na]^+$; HR-ESI-MS *m/z* 171.1019 $[M + H]^+$ (calcd for $C_9H_{15}O_3$, 171.1021).

*2.3.6. Pregaliellalactone E (**8**)*

Colorless oil; $[\alpha]_D^{26}$ 14.0 (*c* 0.4, $CHCl_3$); IR (KBr) ν_{max} 3447, 2961, 2926, 2854, 1747, 1240 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 2); ESI-MS *m/z* 213 $[M + H]^+$, 235 $[M + Na]^+$; HR-ESI-MS *m/z* 213.1137 $[M + H]^+$ (calcd for $C_{11}H_{17}O_4$, 213.1127).

*2.3.7. Pregaliellalactone F (**9**)*

Colorless oil; $[\alpha]_D^{26}$ 6.4 (*c* 0.35, $CHCl_3$); IR (KBr) ν_{max} 3447, 2961, 2926, 2854, 1756; ^{1}H and ^{13}C NMR data (Table 2); ESI-MS *m/z* 197 $[M + H]^+$, 219 $[M + Na]^+$; HR-ESI-MS *m/z* 197.1183 $[M + H]^+$ (calcd for $C_{11}H_{17}O_3$, 197.1178).

*2.4. Crystal data for isogaliellalactone (**3**)*

Colorless needles, $C_{11}H_{14}O_3$ (H_2O), $M = 212.24$: orthorhombic, space group $P2_12_12_1$, $a = 7.2048(8)$ Å, $b = 9.1567(10)$ Å, $c = 16.295(2)$ Å, $\alpha = 90.00°$, $\beta = 90.00°$, $\gamma = 90.00°$, $V = 1075.0(2)$ Å3, $Z = 4$, $d_x = 1.311$ g/cm^3, $F(000) = 456.0$, μ(Cu Kα) $= 0.824$ mm^{-1}. Data collection was performed on a Sapphire CCD using graphite-monochromated Cu Kα radiation ($\lambda = 1.54184$ Å) at 293.0 K; 4838 reflections were collected to $\theta_{max} = 61.85°$; 1642 independent reflections were obtained, with 1401 reflections above the designated intensity threshold for observation [$F^2 > 2\sigma(F^2)$]. The final $R = 0.0523$, $Rw = 0.1721$, and $S = 1.138$, and Flack = 0.0 (5). Crystallographic data for compound **3** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (CCDC 1031872).

2.5. MTT assay

Cells were counted by using a hemocytometer, equally distributed in 96-well plates (5×10^3 cells per well) and treated with **1–9** for 48 h using cisplatin, paclitaxel or vorinostat as the positive controls. Cell proliferation was evaluated with an MTT assay procedure as previously described [10,11]. To determine cell viability, the medium was removed and cells were incubated with 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) at a final concentration of 0.5 mg/mL in RPMI 1640 medium containing 10% FBS for 2 h in the dark at 37 °C. Then 100 μL DMSO was added to the wells. Cultures were incubated at room temperature (RT) for 5 min and read at 492 nm.

3. Results and discussion

Compound **1** was obtained as colorless oil. The ESI-MS spectrum showed quasimolecular ions at *m/z* 195 $[M + H]^+$ and 217 $[M + Na]^+$, indicating that its molecular weight was 194. By comparison of its physical and spectroscopic data, compound **1** was identified to be (−)-galiellalactone [2].

Compound **2** was obtained as colorless oil. The HR-ESI-MS showed quasimolecular ion at *m/z* 249.1089 $[M + Na]^+$ (calcd for 249.1103), indicating the molecular formula of $C_{12}H_{18}O_4$ and accounting for 4 degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra for **2** were similar to those of (−)-galiellalactone (**1**), implying that **2** was a galiellalactone analog. The ^{1}H and ^{13}C NMR spectra for **2** showed the presence of a methoxyl signal at δ_H 3.75 (3H, s, H-9)/δ_C 52.0 (C-9), The HMBC correlation from H-9 (δ 3.75, 3H, s) to C-2 (δ 169.3) attached the methoxyl group to C-2 position, suggesting that the lactone ring in **2** was opened. The molecular formula information of **2** confirmed the conclusion mentioned above. Thus, the planar structure of **2** was determined, which was verified by ^{1}H-^{1}H COSY and HMBC spectra (Figs. 1 and 2). The stereochemistry of **2** was defined by chemical derivative method. Compound **1** was put into methanol for 2 days at room temperature. HPLC analysis revealed that compound **1** was transformed into compound **2** partially. By comparing their optical rotations, together with consideration of biogenetic pathway, compound **2** had the same stereochemistry as that of **1**. Thus, the chemical structure

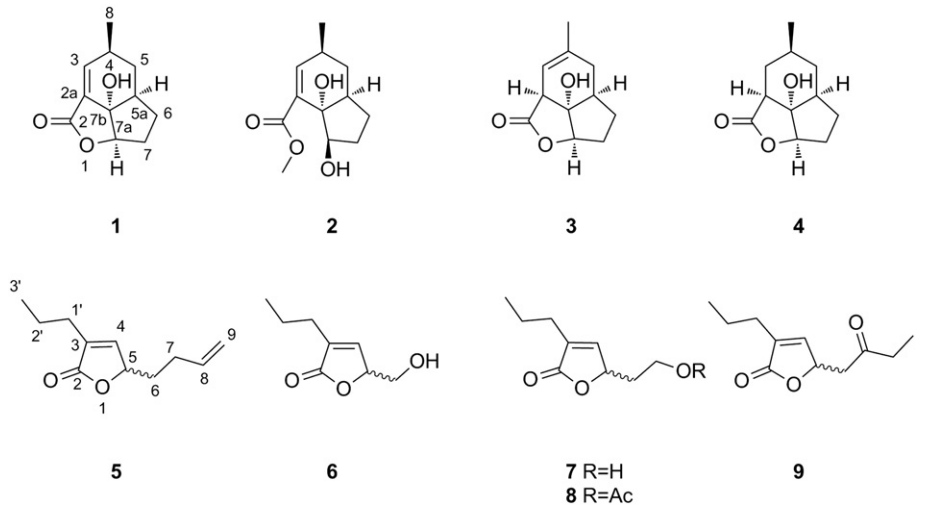

Fig. 1. Chemical structures of compounds 1-9.

of compound **2** was determined and named galiellaester. Also, the result suggested that compound **2** might be an artifact during the purification process.

Compound **3** was obtained as white amorphous powder. Its molecular formula was established to be $C_{11}H_{14}O_3$ by HR ESI-TOF MS spectrum (*m/z* 195.1018 $[M + H]^+$, calcd for 195.1021), requiring 5 degrees of unsaturation. Comparison of the ^1H and ^{13}C NMR spectra with those of (−)-galiellalactone (**1**) revealed that **3** was also a galiellalactone analog and the obvious differences between them lay in the ^{13}C chemical shift of C-2a, 3, and 4. In the ^1H and ^{13}C NMR spectra of **3**, the ^{13}C chemical shifts from δ 132.3 (C-2a), 150.4 (C-3) and 30.0 (C-4) (in **1**) to δ 49.2, 114.3 and 135.0 (in **3**), suggesting that the double bond has been shifted from Δ2a to Δ3 in **3** compared with that of **1**. The HMBC correlations from H-8 (δ 1.76, 3H, m) to C-3 (δ 114.3) and C-4 (δ 135.0) confirmed the deduction mentioned above. Therefore, the planar structure of **3** was determined and confirmed by ^1H-^1H COSY and HMBC spectra (Figs. 1 and 2). The relative configurations of **3** were assigned by the single-crystal X-ray diffraction experiment conducted with Cu Kα, which were identical with those of **1**. Considering the biogenetic pathway, together with comparison of the optical rotation with that of **1**, the absolute configurations of **3** were determined as (2a*S*, 5a*R*, 7a*R*, 7b*S*). Thus, the chemical structure of **3** was determined named isogaliellalactone.

Compound **4** was obtained as colorless oil. The ESI-MS showed quasimolecular ion at *m/z* 197 $[M + H]^+$, indicating that its molecular weight was 196, 2 Da more than that of **1**. Comparing their physical and spectroscopic data, compound **4** was assigned to be 2αβ,3-dihydrogaliellalactone [13].

Compound **5** was obtained as colorless oil. The HR-ESI-MS showed quasimolecular ion at *m/z* 181.1243 $[M + H]^+$ (calcd for 181.1229), indicating the molecular formula of $C_{11}H_{16}O_2$ and accounting for 4 degrees of unsaturation. The ^{13}C NMR signals at δ 82.6, 151.1, 134.8, and 176.2 suggested the presence of a 3,5-disubstituted-*5H*-furan-2-one moiety in **5**, which was similar to those of pregaliellalactone [2]. In the ^1H-^1H COSY spectrum of **5**, successive correlations of H-9 (δ 5.03, 2H, m), H-8 (δ 5.84, 1H, m), H-7 (δ 2.18, 2H, overlap), H-6α (δ 1.67, 1H, m)/H-6β (δ 1.86, 1H, m), H-5 (δ 4.99, 1H, m), and H-4 (δ 7.28, 1H, m) revealed the partial structure of C4-C5-C6-C7-C8-C9; correlations of H-2′ (δ 1.58, 2H, m) with H-3′ (δ 0.96, 3H, t, 7.5) and H-1′ (δ 2.22, 2H, overlap) showed partial structure of C1′-C2′-C3′. The HMBC correlations from H-6α (δ 1.67, 1H, m) and H-6β (δ 1.86, 1H, m) to C-5 (δ 82.6) and C-4 (δ 151.1) confirmed that aliphatic chain C6-C7-C8-C9 was adjacent to C-5 of the 3,5-disubstituted-furan-*5H*-2-one moiety. Meanwhile, the HMBC correlations from H-1′ (δ 2.22, 2H, overlap) to C-3 (δ 134.8) and C-4 (δ 151.1) implied the linkage of aliphatic chain C1′–C2′–C3′ with C-3 of the 3,5-disubstituted-*5H*-furan-2-one moiety. Thus, the planar structure of **5** was determined (Fig. 1). Chiral resolution of **5** was performed by Lux cellulose-2 chiral liquid chromatography (phenomenex, 5 μ, ϕ 4.6 × 250 mm) using 50% CH_3CN–H_2O at 1.0 mL min^{-1}. The HPLC chromatogram showed two enantiomers, t_R 9.94 min for **5a** and t_R 10.38 min for **5b**, and their relative content ratio was 94:6 according to their relative

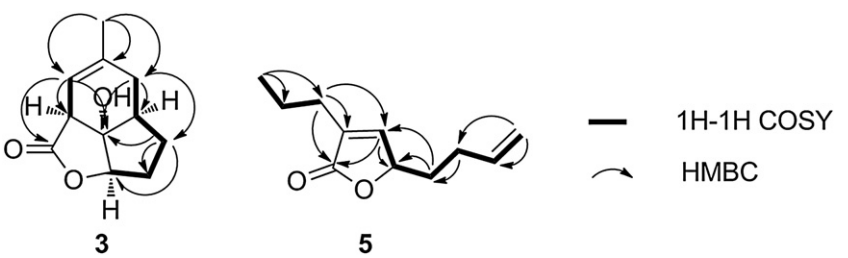

Fig. 2. Key 1H-1H COSY and HMBC correlations of compounds 3 and 5.

peak areas (94.2% for **5a** and 5.8% for **5b**). The CD spectrum of compound **5** displayed positive cotton effects at 211 nm, suggesting that the major enantiomer (**5a**) has *S* configuration [12]. Thus compound **5** was assigned to be a mixture of (5*S*)- and (5*R*)-pregaliellalactone B in the ratio of 94:6 (Fig. 3).

Compound **6** was obtained as colorless oil. Its molecular formula was established to be $C_8H_{12}O_3$ by HR ESI-TOF MS spectrum (*m/z* 157.0861 $[M + H]^+$, calcd for 157.0865), requiring 3 degrees of unsaturation. Comparison of the ^1H and ^{13}C NMR data with that of **5** suggested that compound **6** had a 3-propyl-*5H*-furan-2-one moiety in its structure and the difference between them lay in the aliphatic side chain substituent of C-5. The ^1H and ^{13}C NMR spectrum of **6** displayed a set of methylene signals at δ 3.65 (1H, dd, 12.2, 4.8, H-6a)/3.84 (1H, dd, 12.2, 3.8, H-6β) and δ 62.8 (C-6), which suggested that the side chain attached with C-5 of the 3-propyl-*5H*-furan-2-one moiety was a

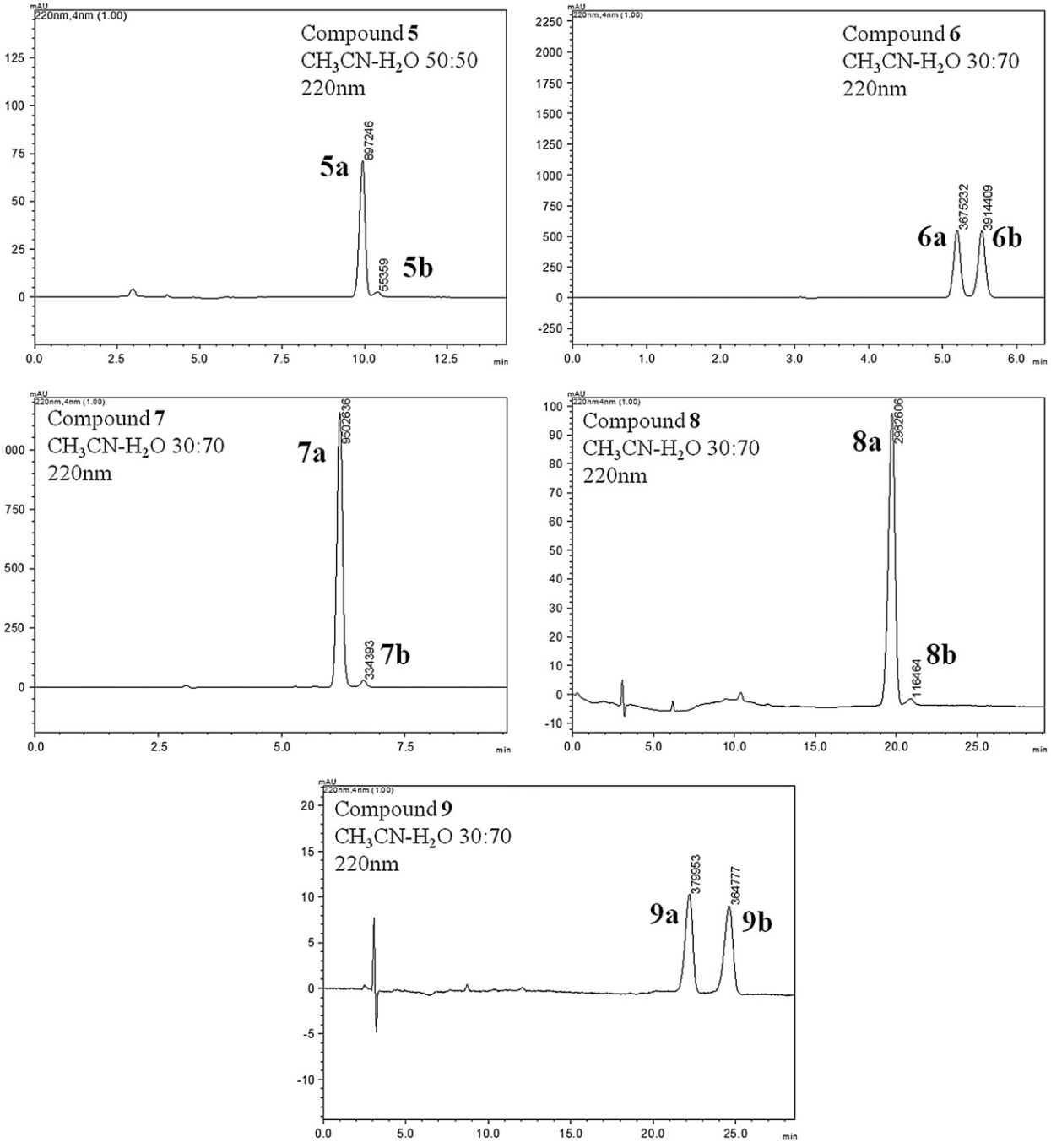

Fig. 3. Chiral HPLC analytical chromatograms of **5**–**9**.

hydroxymethyl group. Thus, the planar structure of **6** was proposed (Fig. 1) and was confirmed by ^{1}H-^{1}H COSY and HMBC spectra. Compound **6** was subject to Lux cellulose-2 chiral liquid chromatography analysis (phenomenex, 5 μ, ϕ 4.6 × 250 mm) using 30% CH_3CN–H_2O at 1.0 mL min^{-1}. The HPLC chromatogram displayed two enantiomers, t_R 5.19 min for **6a** and t_R 5.53 min for **6b**, and their relative content ratio was 48:52 according to their relative peak areas (48.4% for **6a** and 51.6% for **6b**). Enantiomer **6a** showed a negative cotton effect at 211 nm in the CD spectrum, which suggested that its absolute configuration was *S* configuration [12]. Meanwhile, enantiomer **6b** showed a positive cotton effect at 211 nm in the CD spectrum, indicating that the absolute configuration of C-5 for enantiomer **6b** was *R* configuration. Thus, compound **6** was elucidated to be a mixture of (5*S*)- and (5*R*)-3-propyl-5-(hydroxymethyl)-*5H*-furan-2-one in the ratio of 48:52 (Fig. 3 and 4).

Compound **7** was obtained as colorless oil. Its molecular formula was established to be $C_9H_{14}O_3$ by HR ESI-TOF MS spectrum (*m/z* 171.1019 [M + H]$^+$, calcd for 171.1021), requiring 3 degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra of **7** were similar to that of **6** except for an extra methylene signal at δ_H 1.73 (1H, m), 1.95 (1H, m)/δ_C 37.5 (C-6). The HMBC correlations from δ_H 1.73 (1H, m) and 1.95 (1H, m) to C-7 (δ_C 58.9), C-5 (δ_C 80.7), and C-4 (δ_C 151.5) suggested that the methylene was adjacent to the C-5 of the *5H*-furan-2-one moiety. The conclusion was confirmed by the ^{1}H-^{1}H COSY spectrum of compound **7**. Thus, the planar structure of **7** was determined as shown in Fig. 1. Chiral resolution of **7** was performed by Lux cellulose-2 chiral liquid chromatography (phenomenex, 5 μ, ϕ 4.6 × 250 mm) using 30% CH_3CN–H_2O at 1.0 mL min^{-1}. The HPLC chromatogram showed two enantiomers, t_R 6.18 min for **7a** and t_R 6.66 min for **7b**, and their relative content ratio was 97:3 according to their relative peak areas (96.6% for **7a** and 3.4% for **7b**). The CD spectrum of compound **7** displayed positive cotton effects at 211 nm, suggesting that the major enantiomer (**7a**) has *S* configuration [12]. Thus, compound **7** was identified to be a mixture of (5*S*)- and (5*R*)-3-propyl-5-(2-hydroxyethyl)-*5H*-furan-2-one in the ratio of 97:3 (Fig. 3).

Compound **8** was obtained as colorless oil. The HR-ESI-MS showed quasimolecular ion at *m/z* 213.1137 [M + H]$^+$ (calcd. for 213.1127), indicating the molecular formula of $C_{11}H_{16}O_4$ and accounting for 4 degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra for **8** showed great similarity to those of **7** except for an extra acetyl group signals at δ_H 2.03 (3H, s)/δ_C 20.8 and δ_C 172.6 (Table 2). The HMBC correlations from H-7 (δ 4.18, 2H, m) to C-8 (δ 172.6) suggested the linkage of acetyl group with C-7. Thus, the planar structure of compound **8** was determined, which was an acetylated derivative of **7**. Compound **8** was subject to Lux cellulose-2 chiral liquid chromatography analysis (phenomenex, 5 μ, ϕ 4.6 × 250 mm) using 30% CH_3CN–H_2O at 1.0 mL min^{-1}. The HPLC chromatogram displayed two enantiomers, t_R 19.73 min for **8a** and t_R 20.87 min for **8b**, and their relative content ratio was 96:4 according to their relative peak areas (96.2% for **8a** and 3.8% for **8b**). The CD spectrum of compound **8** displayed positive cotton effects at 211 nm, suggesting that the major enantiomer (**8a**) has *S* configuration [12]. Thus, compound **8** was assigned to be a mixture of (5*S*)- and (5*R*)-3-propyl-5-(2-acetyl-ethyl)-*5H*-furan-2-one in the ratio of 96:4 (Fig. 3).

Compound **9** was obtained as colorless oil. Its molecular formula was established to be $C_{11}H_{16}O_3$ by HR ESI-TOF MS spectrum (*m/z* 197.1183 [M + H]$^+$, calcd. for 197.1178), requiring 3 degrees of unsaturation. Comparison of ^{1}H and ^{13}C NMR data with those of **5** indicated that **9** also had the 3-propyl-*5H*-furan-2-one moiety and the difference between them lay in the C-5 substituent (Table 2). The HMBC correlations from H-9 (δ 1.03, 3H, t, 7.3) to C-8 (δ 37.0) and C-7 (δ 209.3) and from H-6 (δ 2.83, 2H, d, 6.5) to C-5 (δ 79.0), C-4(δ 150.6), C-7 (δ 209.3), and C-8 (δ 37.0) suggested that a 2-oxobutyl group was adjacent to C-5 in **9**. Thus, the planar structure of **9** was established as depicted (Fig. 1). Chiral resolution of **9** was performed by Lux cellulose-2 chiral liquid chromatography (phenomenex, 5 μ, ϕ 4.6 × 250 mm) using 30% CH_3CN–H_2O at 1.0 mL min^{-1}. The HPLC chromatogram displayed two enantiomers, t_R 22.20 min for **9a** and t_R 24.60 min for **9b**, and their relative content ratio was 51:49 according to their relative peak areas (51.0% for **9a** and 49.0% for **9b**). Enantiomer **9a** showed a positive cotton effect at 211 nm in the CD spectrum, which suggested that its absolute

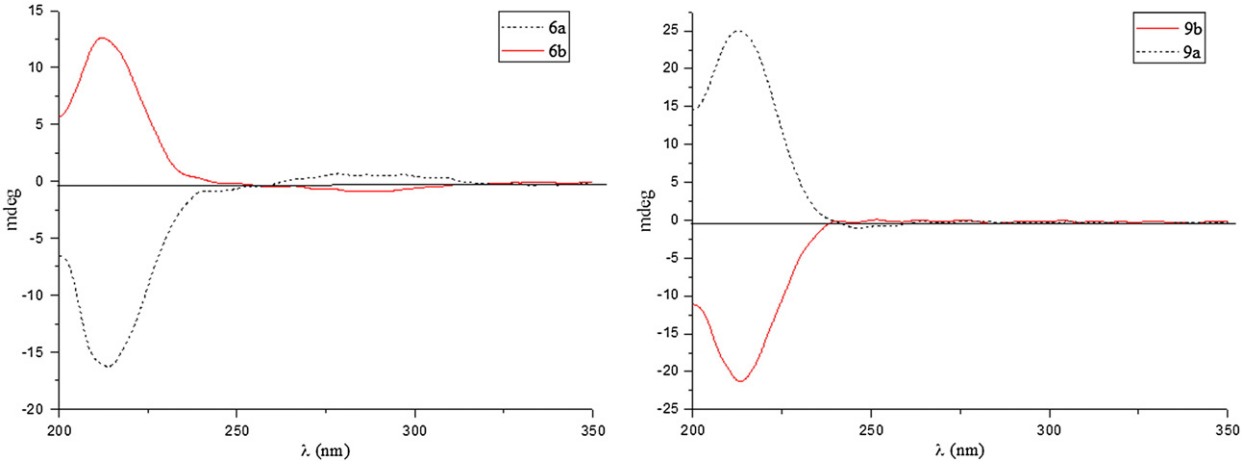

Fig. 4. CD spectra of compounds **6** and **9** in CHCl3.

Table 3
Cytotoxicity of compounds **1** and **2** (IC_{50}, μM).

Compound	HL-60	SMMC-7721	A-549	MCF-7	SW-480	RPMI-8226
1	4.43	3.60	4.76	12.69	14.09	6.67
2	8.71	3.78	5.13	16.21	12.77	7.38
Cisplatin[a]	1.23	7.10	4.50	12.41	12.42	–
Paclitaxel[a]	<0.008	<0.008	<0.008	<0.008	<0.008	–
Vorinostat[a]	–	–	–	–	–	1.73

[a] Positive control; "–" means not detected.

configuration was *S* configuration [12]. Thus, compound **9** was identified to be a mixture of (5*S*)- and (5*R*)-3-propyl-5-(2-oxobutyl)-*5H*-furan-2-one in the ratio of 51:49 (Figs. 3 and 4).

The biosynthesis of (−)-galiellalactone was presumed to be involved in an intramolecular Diels–Alder cyclization, which was very rare in nature [14,15]. Moreover, the enzyme catalyzed cyclization was highly specific since only the enantiomer (−)-pregaliellactone could be catalyzed to cyclization but not the (+)-pregaliellactone. In our study, we found that pregaliellalactone analogs (**5**–**9**), potential precursors of galiellalactone analogs, existed as enantiomers. Moreover, the content of dextro enantiomers was usually higher than that of levo enantiomers. It was a surprise that galiellalactone analogs obtained in this paper were optically pure compounds. This result revealed that enzymes in the organism preferred levo enantiomers to dextro enantiomers for the intermolecular Diels–Alder cyclization, which was consistent with previous reports [15].

Cytotoxicity of compounds **1**–**9** against tumor cell lines HL-60, SMMC-7721, A-549, MCF-7, SW-480 and RPMI-8226 was evaluated by MTT method [9,10] with cisplatin, paclitaxel or vorinostat as positive controls. As shown in Table 3, (−)-galiellalactone and galiellaester (**1** and **2**) exhibited significant cytotoxicity against the tested tumor cell lines with IC_{50} values from 3.60 to 16.21 μM, comparable to that of cisplatin or vorinostat. Other compounds showed no cytotoxicity (IC_{50} > 40 μM). Preliminary structure–activity relationship (SAR) suggested that the existence and its position of double band in the structure of galiellalactone analogs were very important for their cytotoxicity. In addition, it seemed that the lactone ring in the structure of galiellalactone analogs was not a prerequisite for their cytotoxicity.

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