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Original article

Synthesis and cytotoxicity of some new eriocalyxin B derivatives

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Abstract

Eriocalyxin B (**1**) was regarded as the promising candidate for new anticancer agent because of its potent activity and novel mechanism of action. Systematic modifications of **1** were done, and nineteen derivatives were synthesized and their cytotoxicities against five tumor cell lines were evaluated. The structure–activity relationship (SAR) of **1** confirmed that the α,β-unsaturated ketone moieties in ring A and D are the leading active sites; the 7,20-epoxy moiety, OH-6 and OH-7 play an important role in keeping the cytotoxicity. The 6,7-seco derivative **19** had remarkable activity while derivative **20** oxidized from **19** was completely inactive, which suggested that the carboxyl group could destroy the cytoxicity of **20** despite the presence of α,β-unsaturated ketone moiety.

Keywords: Eriocalyxin B; Synthesis; Cytotoxicity; SAR

1. Introduction

Plants of genus *Isodon* (*Rabdosia*), widely distributed in southwestern China, have been used as an anti-inflammatory, antibacterial and antitumor agent in local folk medicine for a long time [1]. Up to now, more than 400 new diterpenoids (mainly *ent*-kauranoids) with a diversity of highly oxygenated structures have been isolated from plants of this genus, and a number of these diterpenoids were found to have potent bioactivities [2]. Oridonin and ponicidin, *ent*-kauranoids isolated from *Isodon rubescens*, were reported to have antiangiogenic activity [3] and inhibition activity of NF-κB DNA-binding [4]. Eriocalyxin B (**1**) (Fig. 1) was firstly isolated from the leaves of *Isodon eriocalyx var. laxiflora* in 1982 [5], and had the highest content of 0.84% in the dried leaves of the plant [6]. Compound **1** showed significant inhibitory effect on K562 and T24 cells with IC_{50} values of 0.373 and 0.087 μg/mL, respectively [7]. Subsequently, it was found to possess the antiangiogenic activity and inhibitory activity toward telomerase of K562 tumor cell [2]. Recently, it was reported to have the activity of inducing apoptosis of t(8;21) leukemia cells through NF-κB and MAPK signaling pathways and triggers degradation of AML1-ETO oncoprotein in a caspase-3-dependent manner [8], and inhibit NF-κB activation by interfering with the binding of both p65 and p50 to the response element in a non-competitive manner [9]. However, no report about structure–activity relationship (SAR) of **1** based on modification could be traced, although some chemistry and SAR of other *ent*-kaurane diterpenoids have been reported [10–12]. Therefore, systematic modification and SAR studies on **1** are important for understanding this interesting compound and searching for drug candidate. In the present paper, we described the modification of **1** and discussion of SAR. Nineteen derivatives were synthesized, and their cytotoxicities were screened against five tumor cell lines.

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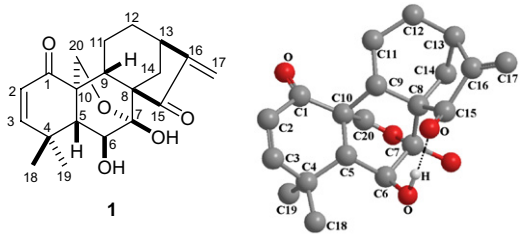


Fig. 1. The structure and the view of eriocalyxin B.

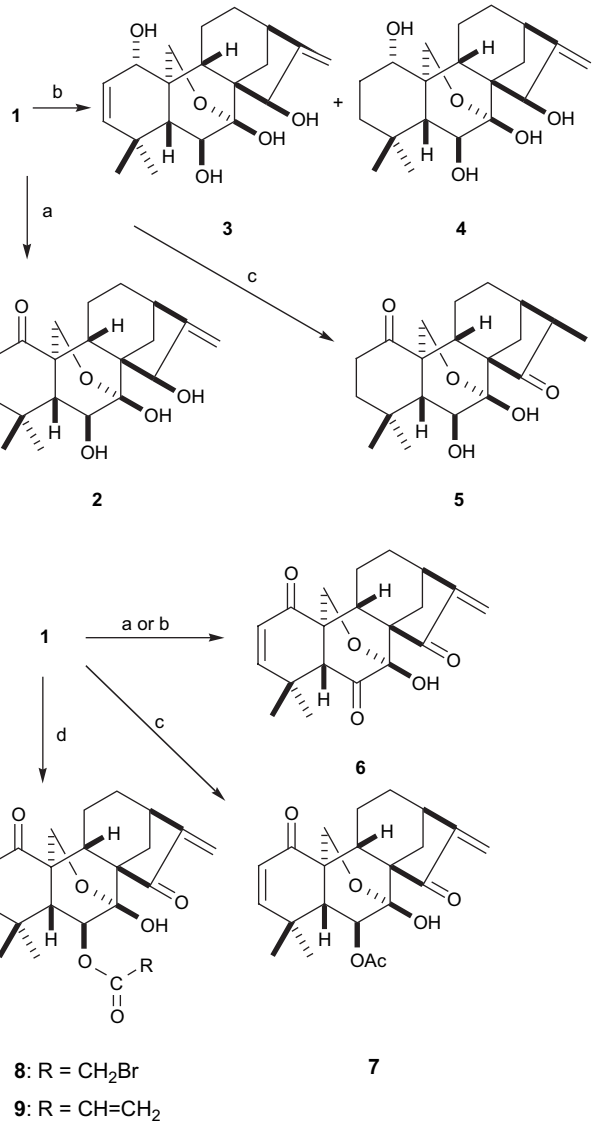
2. Chemistry

Compound **1**, belonging to 7,20-epoxy-*ent*-kaurane-type diterpenoid, contains the α,β-unsaturated ketone moieties in ring A and D and a 6-hydroxyl-7-hemiacetal group. Conformational study and the NMR spectra of **1** [5,13] suggested the presence of the hydrogen bonding between the OH-6 and the carbonyl group at C-15 (Fig. 1). To explore the active center and the factors enhancing the activity of **1**, the study was designed as reduction of α,β-unsaturated ketones, modification on 6-hydroxyl-7-hemiacetal unit and preparation of its 3,20-epoxy or 6,7-seco derivatives.

The α,β-unsaturated ketone at ring D could be reduced selectively with 1 equiv of sodium borohydride to produce **2**, previously reported as the natural product [14]. When 3 equiv of sodium borohydride was employed, the α,β-unsaturated ketones at both ring A and ring D were reduced, giving compounds **3** and **4**. Reduction of the double bond between C-2 and C-3 and the double bond between C-16 and C-17 were accomplished by catalytic hydrogenation on 10% Pd/C to yield compound **5** (Scheme 1).

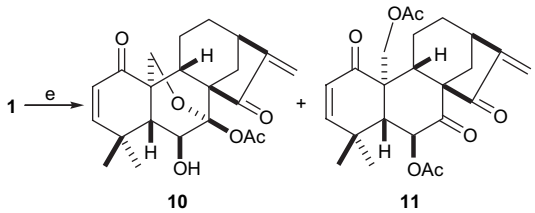
The preparation of 6-oxo derivative **6** was carried out by refluxing **1** with pyridinium chlorochromate in dichloromethane or by reaction with Jones' reagent in acetone at room temperature. Acetylation of **1** at OH-6 produced **7** with acetic anhydride in tetrahydrofuran in the presence of boron trifluoride etherate. Furthermore, treatment of **1** with bromoacetyl bromide and 3-chloropropionyl chloride, respectively, in the presence of a catalytic sodium hydride in pyridine and tetrahydrofuran, gave corresponding 6-substituted derivatives **8** and **9**, while treatment of **1** with 4-chlorobutyryl chloride gave very complex products. To incorporate acetyl in OH-7, compound **1** was reacted with acetic anhydride catalyzed by 4-dimethylaminopyridine in pyridine, affording the desired compound **10** in low yield and byproduct **11** in high yield (Scheme 2).

Treatment of **1** with *p*-toluenesulfonic acid in tetrahydrofuran could produce 3,20-epoxy-*ent*-kaurane diterpenoid **12**. In dichloromethane, compound **1** was treated with acetyl chloride, bromoacetyl bromide, 3-chloropropionyl chloride and 4-chlorobutyryl chloride, respectively, the corresponding 3,20-epoxy-6-substituted derivatives **13**–**16** were obtained (Scheme 3). When **1** was treated with sodium hydroxide in water, a retro-Dieckmann reaction occurred and gave *ent*-abietane derivatives **17** and **18**. Conversion of **1** to 6,7-seco-*ent*-kaurane-type derivative **19** was accomplished

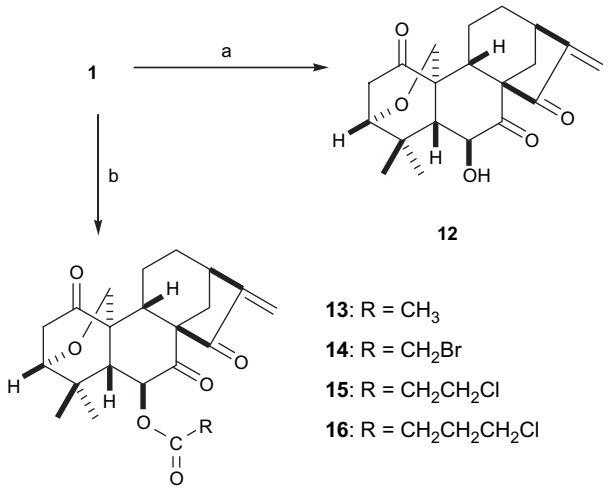


Scheme 1. (a) $NaBH_4$, MeOH, 1 equiv; (b) $NaBH_4$, MeOH, 3 equiv; (c) H_2, Pd/C.

using sodium periodate as reagent as reported before [5,15]. In addition, this conversion could also be carried out through refluxing **1** with Dess-Martin periodinane in dichloromethane, which was found accidentally in our experiments. Further oxidation of **19** with silver oxide produced **20** (Scheme 4).



Scheme 2. (a) PCC, CH_2Cl_2, reflux; (b) Jones' reagent, acetone; (c) Ac_2O, $BF_3{\cdot}Et_2O$, THF; (d) acyl chloride, NaH, Pyridine, THF; (e) Ac_2O, DMAP, pyridine.



Scheme 3. (a) TsOH, THF, reflux; (b) acyl chloride, CH_2Cl_2.

3. Pharmacology

The cytotoxicity of **1** and its derivatives (**2**–**20**) was assessed against five tumor cell lines, K562, OVCA2780, A549, DU145 and MCF-7. Cisplatin (DDP) was used as positive control in this experiment. The IC_{50} values of these compounds are summarized in Table 1. The detailed procedures were described in Section 6.

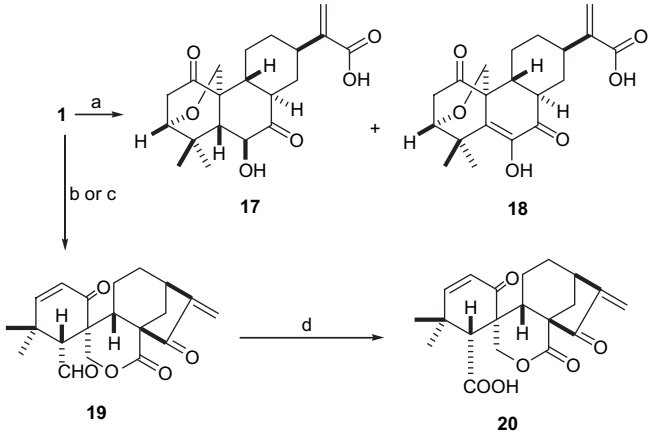
4. Results and discussion

Reduction of the carbonyl group at C-15 to the hydroxyl of **1** giving **2** resulted in distinctly decreased activity of **2** against tested cell lines, which hinted the importance of the α,β-unsaturated ketone moiety in ring D. Further reduction of the carbonyl at C-1 and the double bond at C-2 and C-3 yielded **3** and **4**, both **3** and **4** entirely lost cytotoxicity. Moreover, the double bond between C-2 and C-3 and the double bond between C-16 and C-17 were saturated to produce **5** that didn't exhibit any activity for tested cell lines. Above results strongly suggested that the α,β-unsaturated ketone moieties in ring A and ring D are the active sites of **1**.

Compared to compound **1**, 6-oxo derivative **6**, 6-acetyl derivative **7** and 7-acetyl derivative **10** exhibited weaker activity against K562, MCF-7, OVCA2780, A549 and DU145, which directly suggested the importance of the hydroxyls at C-6 and C-7 of **1**. This could be ascribed to the formation of the internal hydrogen bonds of OH-6 and OH-7 with carbonyl group at C-15. In order to further confirm the role of the hydroxyl group at C-6 for activity of **1**, other 6-substituted derivatives **8** and **9** were prepared. Interestingly, compound **8** showed good activity against five tumor cell lines, however, there was still a little decrease in the activity of **8** against five tumor cells compared to **1**. Similar result could also be found in derivative **9**. Judged by the activity of **6**, **7**, **8**, **9** and **10**, OH-6 and OH-7 are necessary for the good activity of **1**.

Another structural feature of **1** is the 7,20-epoxy moiety in the molecule. When **1** was transformed to its 3,20-epoxy derivative **12** whose cytotoxicity was reduced against five tumor cell lines indicated that the 7,20-epoxy moiety was optimal for activity. This conclusion was further confirmed by comparing the 6-substititued-3,20-epoxy derivatives **13**, **14** and **15** with corresponding 6-substituted-7,20 epoxy compounds **7**, **8** and **9**.

Opening ring D gave **17** and **18**. Both **17** and **18** had no activity, which confirmed that the α,β-unsaturated ketones at ring A and ring D are important. Oxidation of **1** gave 6, 7-seco derivative **19** with good activity against K562, A549 and MCF-7. Interestingly, oxidation of the CHO of **19** to COOH gave the inactive form of **20**. This result revealed that carboxyl group could destroy activity of **20** despite the presence of α,β-unsaturated ketone moiety.



Scheme 4. (a) NaOH, H_2O; (b) $NaIO_4$, MeOH/H_2O; (c) Dess-Martin periodinate, CH_2Cl_2, reflux; (d) Ag_2O, KOH, MeOH.

Table 1
In vitro cytotoxicity of eriocalyxin B and its analogues

Compound	IC_{50} (μg/mL)				
	K562	OVCA2780	A549	DU145	MCF-7
DDP	0.28	0.34	0.63	0.37	0.42
1	1.32	1.80	2.49	1.34	0.15
2	33.25	52.75	15.27	>100	83.61
3	>100	>200	>100	>100	>100
4	>100	>200	>100	>100	>100
5	>100	>200	>100	>200	>200
6	4.94	7.28	33.18	11.98	59.60
7	25.00	11.93	31.00	80.63	20.06
8	1.5	2.06	4.86	4.52	0.85
9	5.05	4.33	33.00	41.22	6.56
10	7.23	13.12	38.94	74.73	>100
11	28.09	9.71	61.80	17.90	24.61
12	3.73	16.94	27.22	33.16	8.87
13	19.3	11.3	10.08	31.87	36.69
14	4.54	14.94	10.9	26.58	67.08
15	8.88	10.48	20.28	51.00	22.68
16	>100	42.64	68.12	>100	>100
17	>100	>200	>200	>200	>200
18	>200	>200	>200	>200	>200
19	0.27	20.66	2.26	47.42	0.257
20	>100	>200	>100	>100	>100

5. Conclusions

In conclusion, systemic modification was performed on the lead compound **1**. The chemical behavior of **1** was described. Nineteen derivatives have been synthesized and evaluated for their cytotoxicity against K562, OVCA2780, A549, DU145 and MCF-7 cell lines. Some of these derivatives (**6**–**15**, **19**) showed different activity against the tumor cells with IC_{50} values varied from 0.26 to 11.9 μg/mL, although no one was better than **1**. From the SAR study, the following conclusions can be drawn as (1) α,β-unsaturated ketone units in ring A and D are the most important active sites, (2) the hydroxyl groups at C-6 and C-7 play important role in enhancing the activity, (3) 7,20-epoxy moiety is more optimal for the cytotoxicity than corresponding 3,20-epoxy one, and (4) The 6,7-seco type derivative **19** possessed good activity against K562, A549 and MCF-7 while its derivative **20** showed no activity against five cell lines. This result revealed that carboxyl group could destroy activity of **20** despite the presence of α,β-unsaturated ketone moiety.

On the basis of the above results, the research on synthesizing more derivatives with good activity is ongoing in our laboratory. Moreover, the results described herein might offer some useful information of structural modification and biological activity for **1**.

6. Experimental

6.1. Chemistry

^1H and ^{13}C NMR experiments were performed on a Bruker AM-400 spectrometer at ambient temperature. 2D NMR spectra were recorded on Bruker DRX-500 NMR instrument. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were obtained on a UV 2401 PC spectrometer. ESIMS and HRESIMS were taken on a VG Auto Spec-3000 or on a Finnigan MAT 90 instrument. Melting points were determined (uncorrected) on an XRC-1 micro melting point apparatus. Optical rotations were measured with a Horiba SEPA-300 polarimeter. Column chromatography was performed on silica gel (Qingdao Marine Chemical Inc. China). The starting material compound **1** was prepared by Xue-Mei Niu from the leaves of *Isodon eriocalyx var. laxiflora* according to previous method [7].

6.1.1. Synthesis of compound 2

Compound **1** (250 mg, 0.726 mmol) was dissolved in methyl alcohol (10 mL). To this solution at 0 °C was added sodium borohydride (31.5 mg, 0.799 mmol) in three portion. The mixture was stirred at room temperature until all starting material was consumed. The mixture was diluted with ethyl acetate and washed with brine and water. The organic layer was then dried (sodium sulfate) and concentrated. The residue was purified by column chromatography (chloroform–acetone, 8.5:1.5) to give **2** (102 mg, 40.6%) as white powder; mp 218–220 °C; $[\alpha]_D^{25}$ −127.2° (*c* 0.48, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 206 (4.04) nm; IR (KBr) ν_{max} 3389, 3184, 2934, 1713, 1663, 1494, 1379, 1278, 1077, 1050 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 5.79 (1H, d, $J = 10.1$ Hz, H-2), 6.83 (1H, d, $J = 10.1$ Hz, H-3), 3.84 (1H, dd, $J = 9.4$, 1.7 Hz, H-6α), 2.53 (1H, m, H-13α), 4.35 (1H, t, $J = 2.6$ Hz, H-15α), 4.98 (2H, m, H-17), 1.26 (3H, s, Me-18), 1.18 (3H, s, Me-19), 4.17 (1H, d, $J = 10.1$ Hz, H-20a), 3.88 (1H, d, $J = 10.1$ Hz, H-20b); ^{13}C NMR (acetone-d_6, 100 MHz) δ 198.9 (C, C-1), 128.1 (CH, C-2), 161.0 (CH, C-3), 36.4 (C, C-4), 55.5 (CH, C-5), 73.5 (CH, C-6), 97.6 (C, C-7), 53.2 (C, C-8), 41.5 (CH, C-9), 46.6 (C, C-10), 18.4 (CH_2, C-11), 33.3 (CH_2, C-12), 36.5 (CH, C-13), 26.1 (CH_2, C-14), 75.1 (CH, C-15), 161.3 (C, C-16), 108.0 (CH_2, C-17), 30.0 (CH_3, C-18), 24.7 (CH_3, C-19), 65.4 (CH_2, C-20); HRESIMS *m/z* 369.1678 (calcd for $C_{20}H_{26}O_5Na$: 369.1677). The β-orientation of 15-OH was determined by the ROESY correlation of H-15α with H-14α.

6.1.2. Synthesis of compounds 3 and 4

Compound **1**(400 mg, 1.162 mmol) was dissolved in methyl alcohol (10 mL). To this solution at 0 °C was added sodium borohydride (131.97 mg, 3.487 mmol) in three portions. The mixture was stirred at room temperature until all starting material was consumed. The mixture was diluted with ethyl acetate and washed with brine and water. The organic layer was then dried (sodium sulfate) and concentrated. The residue was purified by column chromatography (chloroform–methanol, 9.8:0.2) to give **3** (239 mg, 59.09%) and **4** (26 mg, 6.39%).

6.1.2.1. Compound 3. White powder; mp 200–202 °C; $[\alpha]_D^{25}$ −3.29° (*c* 0.09, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 205.4 (3.52) nm; IR (KBr) ν_{max} 3414, 2958, 2926, 1630, 1462, 1381, 1280, 1127, 1072, 1047 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 4.09 (1H, d, $J = 2.0$ Hz, H-1β), 5.32 (1H, dd, $J = 2.3$, 10.3 Hz, H-2), 5.53 (1H, dd, $J = 2.3$, 10.3 Hz, H-3), 3.86 (1H, overlap, H-6α), 2.59 (1H, dd, $J = 4.4$, 9.3 Hz, H-13α), 4.44 (1H, d, $J = 2.0$ Hz, H-15α), 5.03 (1H, br s, H-17a), 4.98 (2H, br s, H-17b), 1.17 (3H, s, Me-18), 1.09 (3H, s, Me-19), 4.20 (1H, d, $J = 9.8$ Hz, H-20a), 3.87 (1H, overlap, H-20b); ^{13}C NMR (CD_3OD, 100 MHz) δ 71.1 (CH, C-1), 127.3 (CH, C-2), 139.4 (CH, C-3), 35.5 (C, C-4), 56.6 (C, C-5), 74.9 (CH, C-6), 97.9 (C, C-7), 53.0 (C, C-8), 42.8 (CH, C-9), 40.2 (C, C-10), 18.3 (CH_2, C-11), 33.0 (CH_2, C-12), 38.1 (CH, C-13), 27.0 (CH_2, C-14), 75.4 (CH, C-15), 161.8 (C, C-16), 107.5 (CH_2, C-17), 32.4 (CH_3, C-18), 26.4 (CH_3, C-19), 63.8 (CH_2, C-20); HRESIMS *m/z* 371.1843 (calcd for $C_{20}H_{28}O_5Na$, 371.1834). The relative configuration of OH-1α and OH-15β was revealed by the ROESY correlation of H-1β with both H-5β and H-9β, and of H-15α with H-14α.

6.1.2.2. Compound 4. White powder; mp 215–217 °C; $[\alpha]_D^{25}$ −29.4° (*c* 0.29, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 205 (3.80) nm; IR (KBr) ν_{max} 2928, 2863, 1631, 1454, 1392, 1260, 1164, 1128, 1077, 1065 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 3.49 (1H, dd, $J = 10.5$, 6.4 Hz, H-1β), 3.66 (1H, d, $J = 6.0$ Hz, H-6α), 2.52 (1H, m, H-13α), 4.40 (1H, t, $J = 2.4$ Hz, H-15α), 5.02 (1H, d, $J = 1.0$ Hz, H-17a), 4.97

(1H, d, $J = 1.1$ Hz, H-17b), 1.09 (3H, s, Me-18), 0.99 (3H, s, Me-19), 4.20 (1H, dd, $J = 1.0$, 8.9 Hz, H-20a), 3.93 (1H, dd, $J = 1.5$, 9.6 Hz, H-20b); ^{13}C NMR (acetone-d_6, 100 MHz) δ 74.9 (CH, C-1), 33.4 (CH_2, C-2), 40.0 (CH_3, C-3), 34.5 (C, C-4), 59.2 (CH, C-5), 75.0 (CH, C-6), 97.6 (C, C-7), 53.0 (C, C-8), 44.6 (CH, C-9), 42.0 (C, C-10), 19.4 (CH_2, C-11), 30.6 (CH_2, C-12), 37.9 (CH, C-13), 26.6 (CH_2, C-14), 75.6 (CH, C-15), 162.0 (C, C-16), 107.5 (CH_2, C-17), 33.1 (CH_3, C-18), 22.2 (CH_3, C-19), 64.0 (CH_2, C-20); HRESIMS *m/z* 373.1980 (calcd for $C_{20}H_{30}O_5Na$, 373.1990). The α-orientation of the 1-OH and the β-orientation of the15-OH groups were apparent from the ROESY correlation of H-1β with both H-5β and H-9β, and of H-15α with H-14α.

6.1.3. Synthesis of compound ***5***

Compound **1** (500 mg, 1.453 mmol) was dissolved in ethyl alcohol (10 mL). To this solution was added 10% Pd/C (75 mg). The mixture was stirred under hydrogen atmosphere at room temperature for 2 h. The reaction mixture was filtered and the solvent was evaporated under reduced pressure. Flash chromatography of the residue, eluting with chloroform–ethyl acetate (9:1), gave compound **5** (436 mg, 86.2%) as white powder; mp 215–217 °C; $[\alpha]_D^{25} + 71.7°$ (*c* 0.51, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 3.21 nm; IR (KBr) ν_{max} 3356, 3257, 2959, 2913, 1718, 1697, 1468, 1374, 1289, 1200, 1150, 1080, 1060 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 3.49 (1H, dd, $J = 7.8$, 11.6 Hz, H-6α), 2.40 (1H, m, H-13α), 2.30 (1H, t, $J = 7.0$ Hz, H-16α), 0.95 (3H, d, $J = 7.0$ Hz, H-17), 0.99 (1H, s, Me-18), 0.85 (1H, s, Me-19), 4.03 (1H, dd, $J = 1.6$, 10.3 Hz, H-20a), 3.73 (1H, dd, $J = 1.6$, 10.3 Hz, H-20b); ^{13}C NMR (CD_3OD, 100 MHz) δ 214.0 (C, C-1), 36.8 (CH_2, C-2), 39.9 (CH_3, C-3), 33.8 (C, C-4), 63.0 (CH, C-5), 74.4 (CH, C-6), 96.7 (C, C-7), 60.8 (C, C-8), 48.6 (CH, C-9), 48.4 (C, C-10), 19.2 (CH_2, C-11), 19.8 (CH_2, C-12), 32.4 (CH, C-13), 28.3 (CH_2, C-14), 225.8 (C, C-15), 51.3 (CH, C-16), 11.1 (CH_3, C-17), 31.1 (CH_3, C-18), 23.7 (CH_3, C-19), 65.8 (CH_2, C-20); HRESIMS *m/z* 371.1837 (calcd for $C_{20}H_{28}O_5Na$, 371.1834). The β-orientation of 17-CH_3 was determined by the ROESY correlation of H-16α with H-14α.

6.1.4. Synthesis of compound ***6***

6.1.4.1. Method A. To a solution of pyridinium chlorochromate (1.50 g, 6.973 mmol) in dichloromethane (10 mL) was added **1** (300 mg, 0.872 mmol) in dichloromethane (3 mL). The mixture was heated at 45 °C for 12 h. The reaction mixture was filtered and purified through the column chromatography (petroleum ether–ethyl acetate, 8:2) and concentrated to afford **6** (46 mg, 15.4%).

6.1.4.2. Method B. Compound **1** (250 mg, 0.726 mmol) was dissolved in acetone. To this solution was added Jones' reagent dropwise until a red color persists. The mixture was stirred at room temperature until all starting material was consumed. Afterwards, the mixture was diluted with water and extracted with ethyl acetate. The extract was washed with saturated sodium bicarbonate solution and water, dried over sodium sulfate and then concentrated in vacuo. The residue was purified by column chromatography (chloroform–ethyl acetate, 9.5:0.5) to give **6** (58 mg, 24.3%).

6.1.4.3. Compound ***6***. White powder; mp 170–172 °C; $[\alpha]_D^{25}$ −231.1° (*c* 0.33, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 230 (4.27) nm; IR (KBr) ν_{max} 3438, 2982, 2863, 1737, 1663, 1466, 1374, 1264, 1056 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 5.79 (1H, d, J = 10.2 Hz, H-2), 6.74 (1H, d, $J = 10.3$ Hz, H-3), 5.39 (2H, d, $J = 2.2$ Hz, H-17), 1.60 (3H, s, Me-18), 1.23 (3H, s, Me-19), 4.33 (1H, d, $J = 10.0$ Hz, H-20a), 3.97 (1H, dd, $J = 1.8$, 10.1, Hz, H-20b); ^{13}C NMR (acetone-d_6, 100 MHz) δ 195.4 (C, C-1), 126.6 (CH, C-2), 160.9 (CH, C-3), 36.6 (C, C-4), 60.4 (CH, C-5), 202.2 (C, C-6), 93.4 (C, C-7), 55.9 (C, C-8), 47.3 (CH, C-9), 49.2 (C, C-10), 20.1 (CH_2, C-11), 29.8 (CH_2, C-12), 35.1 (CH, C-13), 24.5 (CH_2, C-14), 204.5 (C, C-15), 153.7 (C, C-16), 115.8 (CH_2, C-17), 31.4 (CH_3, C-18), 23.4 (CH_3, C-19), 65.9 (CH_2, C-20); HRESIMS *m/z* 365.1359 (calcd for $C_{20}H_{22}O_5Na$, 365.1364).

6.1.5. Synthesis of compound ***7***

A solution of boron trifluoride etherate (0.027 mL, 0.218 mmol) in acetic anhydride (0.962 mL, 8.717 mmol) was added dropwise to the solution of compound **1** (150 mg, 0.436 mmol) in tetrahydrofuran with stirring. After being stirred for 2 h the mixture was quenched by addition of 5% sodium bicarbonate aqueous solution and extracted with ethyl acetate. The organic layer was then washed with water and dried over sodium sulfate. The solution was then evaporated. The residue was purified by column chromatography (petroleum ether–acetone, 9.5:0.5) to give compound **7** (134 mg, 79.6%) as white powder; mp 182–184 °C; $[\alpha]_D^{25}$ −187.1° (*c* 0.56, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 230.6 (4.16) nm; IR (KBr) ν_{max} 3443, 2952, 1734, 1706, 1666, 1643, 1379, 1260, 1053 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 5.81 (1H, d, $J = 10.1$ Hz, H-2), 6.81 (1H, d, $J = 10.1$ Hz, H-3), 5.29 (1H, d, $J = 8.6$ Hz, H-6α), 2.98 (1H, m, H-13), 5.69 (1H, s, H-17a), 5.26 (1H, s, H-17b), 1.22 (3H, s, Me-18), 1.14 (3H, s, Me-19), 4.25 (1H, dd, $J = 10.1$, 1.3 Hz, H-20a), 3.95 (1H, dd, $J = 10.1$, 1.7 Hz, H-20b), 2.12 (3H, s, OAc); ^{13}C NMR (acetone-d_6, 100 MHz) δ 196.8 (C, C-1), 128.1 (CH, C-2), 160.4 (CH, C-3), 36.3 (C, C-4), 54.0 (CH, C-5), 74.3 (CH, C-6), 97.4 (C, C-7), 58.6 (C, C-8), 48.2 (CH, C-9), 47.3 (C, C-10), 19.7 (CH_2, C-11), 30.4 (CH_2, C-12), 34.4 (CH, C-13), 26.6 (CH_2, C-14), 202.1 (C, C-15), 155.0 (C C-16), 114.4 (CH_2, C-17), 29.8 (CH_3, C-18), 21.5 (CH_3, C-19), 65.8 (CH_2, C-20), 173.4 (s, OAc), 24.9 (q, OAc); HRESIMS *m/z* 409.1636 (calcd for $C_{22}H_{26}O_6Na$, 409.1627).

6.1.6. General procedures for the synthesis of compounds ***8*** *and* ***9***

Sodium hydride (290.561 mg, 60%, 7.264 mmol) washed with dry ethyl ether was firstly dissolved in dry tetrahydrofuran (10 mL). To this solution was added **1** (250 mg, 0.726 mmol) followed by corresponding acyl chloride (0.872 mmol) and

pyridine 0.5 mL. The mixture was stirred at room temperature and monitored by TLC until all starting material was consumed. The reaction was then quenched by water and extracted with ethyl acetate, washed with water and dried over sodium sulfate. The organic layer was evaporated and purified by column chromatography using appropriate eluent.

*6.1.6.1. Compound **8***. Chromatography: petroleum ether–ethyl acetate, 9:1; yellow powder, yield: 16.5%; mp 164–166 °C; $[\alpha]_D^{25}$ −106.3° (*c* 0.75, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 223.8 (4.29) nm; IR (KBr) ν_{max} 3386, 2931, 2869, 1729, 1660, 1377, 1299, 1057 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 6.05 (1H, d, $J = 10.1$ Hz, H-2), 7.07 (1H, d, $J = 10.1$ Hz, H-3), 5.57 (1H, d, $J = 8.3$ Hz, H-6α), 3.20 (1H, m, H-13α), 5.93 (1H, s, H-17a), 5.33 (1H, s, H-17b), 1.37 (3H, s, Me-18), 1.24 (3H, s, Me-19), 4.50 (1H, d, $J = 10.2$ Hz, H-20a), 4.21 (1H, dd, $J = 10.2$, 1.2 Hz, H-20b), 4.43 (1H, d, $J = 12.6$ Hz, H-2′a), 4.30 (1H, d, $J = 12.6$ Hz, H-2′b); ^{13}C NMR (acetone-d_6, 100 MHz) δ 196.7 (C, C-1), 128.1 (CH, C-2), 160.3 (CH, C-3), 36.2 (C, C-4), 54.0 (CH, C-5), 75.3 (CH, C-6), 96.9 (C, C-7), 58.5 (C, C-8), 48.0 (CH, C-9), 47.1 (C, C-10), 19.6 (CH_2, C-11), 30.4 (CH_2, C-12), 34.3 (CH, C-13), 26.6 (CH_2, C-14), 202.2 (C, C-15), 154.8 (C, C-16), 114.6 (CH_2, C-17), 30.0 (CH_3, C-18), 24.8 (CH_3, C-19), 65.8 (CH_2, C-20), 168.6 (C, C-1′), 27.4 (CH_2, C-2′); HRESIMS *m/z* 487.0728 (calcd for $C_{22}H_{25}O_6NaBr$, 487.0723).

*6.1.6.2. Compound **9***. Chromatography: petroleum ether–ethyl acetate, 8:2; yellow powder, yield: 33.8%; mp 188–190 °C; $[\alpha]_D^{25}$ −122.8° (*c* 0.47, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 229.2 (4.14) nm; IR (KBr) ν_{max} 3414, 2956, 2931, 1727, 1669, 1462, 1405, 1377, 1281, 1127, 1055 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 5.92 (1H, d, $J = 9.0$ Hz, H-2), 6.87 (1H, d, $J = 10.0$ Hz, H-3), 5.55 (1H, d, $J = 8.9$ Hz, H-6α), 3.03 (1H, m, H-13α), 6.02 (1H, s, H-17a), 5.40 (1H, s, H-17b), 1.30 (3H, s, Me-18), 1.22 (3H, s, Me-19), 4.57 (1H, d, $J = 9.8$ Hz, H-20a), 4.24 (1H, d, $J = 9.9$ Hz, H-20b), 6.51 (1H, dd, $J = 10.5$, 17.2, Hz, H-2′), 7.07 (1H, d, $J = 17.2$ Hz, H-3′a), 6.08 (1H, d, $J = 9.7$ Hz, H-3b′); ^{13}C NMR (CD_3OD, 100 MHz) δ 197.7 (C, C-1), 128.2 (CH, C-2), 161.4 (CH, C-3), 36.5 (C, C-4), 54.0 (CH, C-5), 73.9 (CH, C-6), 97.4 (C, C-7), 59.2 (C, C-8), 48.6 (CH, C-9), 47.6 (C, C-10), 20.1 (CH_2, C-11), 30.5 (CH_2, C-12), 34.6 (CH, C-13), 27.0 (CH_2, C-14), 203.2 (C, C-15), 155.2 (C, C-16), 115.4 (CH_2, C-17), 29.6 (CH_3, C-18), 25.2 (CH_3, C-19), 66.2 (CH_2, C-20), 167.5 (C, C-1′), 130.2 (CH, C-2′), 132.5 (CH_2, C-3′); HRESIMS *m/z* 421.1621 (calcd for $C_{23}H_{26}O_6Na$, 421.1627).

*6.1.7. Synthesis of compounds **10** and **11***

Compound **1** (400 mg, 1.162 mmol) was dissolved in pyridine (6 mL). To this solution was added 4-dimethylaminopyridine as catalyst, followed by acetic anhydride (0.877 mL 9.298 mmol). The mixture was stirred at room temperature and monitored by TLC until all starting material was consumed. The reaction mixture was then diluted with ethyl acetate and washed with diluted hydrochloric acid and water. The organic layer was then dried (sodium sulfate) and evaporated. Flash chromatography of the residue, eluting with petroleum ether–ethyl acetate (8.5:1.5), gave compounds **10** (52 mg, 12.9%) and **11** (385.1 mg, 85.8%).

*6.1.7.1. Compound **10***. White solid; mp 134–136 °C; $[\alpha]_D^{25}$ −61.3° (*c* 0.56, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 230.8 (4.15) nm; IR (KBr) ν_{max} 3364, 2960, 1759, 1706, 1661, 1640, 1220, 1085, 1059 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 5.85 (1H, d, $J = 10.1$ Hz, H-2), 6.89 (1H, d, $J = 10.1$ Hz, H-3), 5.13 (1H, dd, $J = 9.1$, 12.0 Hz, H-6α), 5.55 (1H, d, $J = 12.0$ Hz, OH-6β), 3.12 (1H, dd, $J = 4.7$, 9.3 Hz, H-13α), 6.00 (1H, s, H-17a), 5.62 (1H, s, H-17b), 1.37 (3H, s, Me-18), 1.26 (3H, s, Me-19), 4.23 (1H, d, $J = 9.8$ Hz, H-20a), 4.06 (1H, d, $J = 9.9$ Hz, H-20b), 2.00 (3H, s, OAc); ^{13}C NMR (acetone-d_6, 100 MHz) δ 196.7 (C, C-1), 127.6 (CH, C-2), 161.7 (CH, C-3), 36.5 (C, C-4), 57.8 (CH, C-5), 68.9 (CH, C-6), 101.6 (C, C-7), 60.7 (C, C-8), 49.1 (CH, C-9), 47.1 (C, C-10), 19.8 (CH_2, C-11), 29.8 (CH_2, C-12), 34.8 (CH, C-13), 25.7 (CH_2, C-14), 207.8 (C, C-15), 153.6 (C, C-16), 119.4 (CH_2, C-17), 29.6 (CH_3, C-18), 24.9 (CH_3, C-19), 66.2 (CH_2, C-20), 167.9 (s, OAc), 21.7 (q, OAc); HRESIMS *m/z* 409.1633 (calcd for $C_{22}H_{26}O_6Na$, 409.1627).

*6.1.7.2. Compound **11***. White solid; mp 208–210 °C; $[\alpha]_D^{25}$ −197.6° (*c* 0.30, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 203.8 (4.45) nm; IR (KBr) ν_{max} 3030, 1746, 1689, 1643, 1375, 1226, 1082, 1032 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 5.71 (1H, d, $J = 10.2$ Hz, H-2), 6.47 (1H, d, $J = 10.2$ Hz, H-3), 5.69 (1H, d, $J = 13.4$ Hz, H-6α), 3.03 (1H, br s, H-13α), 5.78 (1H, s, H-17a), 5.34 (1H, s, H-17b), 4.68 (1H, d, $J = 12.3$ Hz, H-20a), 4.39 (1H, d, $J = 12.3$ Hz, H-20b), 1.21 (3H, s, Me-18), 1.12 (3H, s, Me-19), 2.03 (3H, s, OAc), 1.91 (3H, s, OAc); ^{13}C NMR (acetone-d_6, 100 MHz) δ 199.2 (C, C-1), 124.0 (CH, C-2), 158.0 (CH, C-3), 37.4 (C, C-4), 49.8 (CH, C-5), 74.1 (CH, C-6), 203.1 (C, C-7), 63.3 (C, C-8), 43.3 (CH, C-9), 52.7 (C, C-10), 21.6 (CH_2, C-11), 34.2 (CH_2, C-12), 37.1 (CH, C-13), 31.6 (CH_2, C-14), 202.3 (C, C-15), 151.2 (C, C-16), 116.7 (CH_2, C-17), 33.6 (CH_3, C-18), 22.0 (CH_3, C-19), 64.8 (CH_2, C-20), 170.0 (C, OAc), 170.0 (C, OAc), 21.1 (CH_3, OAc), 20.8 (CH_3, OAc); HRESIMS *m/z* 451.1723 (calcd for $C_{20}H_{26}O_6Na$, 451.1732).

*6.1.8. Synthesis of compound **12***

Compound **1** (250 mg, 0.726 mmol) was dissolved in tetrahydrofuran (10 mL). To this solution was added *p*-toluenesulfonic acid (276.352 mg, 1.453 mmol). The mixture was refluxed until all starting material was consumed. The reaction mixture was then diluted with ethyl acetate and washed with water, dried over sodium sulfate and evaporated. Flash chromatography of the residue, eluting with petroleum ether–ethyl acetate (8.5:1.5), gave compound **12** (95 mg, 38%) as white powder; mp 180–182 °C; $[\alpha]_D^{25}$ −194.3° (*c* 1.10, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 231.2 (3.84) nm; IR (KBr) ν_{max} 3436, 2940, 2875, 1746, 1704, 1645, 1455, 1387, 1105, 1064 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.74 (1H, br s,

H-3β), 2.07 (1H, d, $J = 11.7$ Hz, H-5β), 4.55 (1H, d, $J = 11.3$ Hz, H-6α), 6.11 (1H, s, H-17a), 5.40 (1H, s, H-17b), 1.25 (3H, s, Me-18), 1.10 (3H, s, Me-19), 4.69 (1H, d, $J = 9.4$ Hz, H-20a), 4.11 (1H, d, $J = 9.4$ Hz, H-20b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 207.7 (C, C-1), 41.8 (CH_2, C-2), 77.2 (CH, C-3), 37.7 (C, C-4), 50.6 (CH, C-5), 71.3 (CH, C-6), 206.9 (C, C-7), 59.2 (C, C-8), 38.7 (CH, C-9), 51.9 (C, C-10), 20.5 (CH_2, C-11), 31.3 (CH_2, C-12), 36.7 (CH, C-13), 36.9 (CH_2, C-14), 200.2 (C, C-15), 146.9 (C, C-16), 117.6 (CH_2, C-17), 29.3 (CH_3, C-18), 23.0 (CH_3, C-19), 61.5 (CH_2, C-20); HRESIMS *m/z* 367.1531 (calcd for $C_{20}H_{24}O_5Na$,: 367.1521).

6.1.9. General procedures for the synthesis of compounds **13–16**

Compound **1** (250 mg, 0.726 mmol) was dissolved in dry dichloromethane. To this solution was added the corresponding acyl chloride (1.453 mmol). The mixture was stirred and monitored by TLC until all starting material was consumed. The reaction mixture was then diluted with ethyl acetate and washed with water. The organic layer was dried (sodium sulfate) and evaporated. The residue was purified by column chromatography using the appropriate eluent.

6.1.9.1. Compound ***13***. Chromatography: chloroform–acetone, 19.5:0.5; white powder, yield: 82.5%; mp 232–234 °C; $[\alpha]_D^{25}$ −215.0° (*c* 0.54, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 235.4 (3.73) nm; IR (KBr) ν_{max} 2931, 2883, 1759, 1745, 1725, 1643, 1454, 1387, 1261, 1229, 1111, 1063 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.72 (1H, t, $J = 1.5$ Hz, H-3β), 5.57 (1H, d, $J = 12.6$ Hz, H-6α), 6.09 (1H, s, H-17a), 5.38 (1H, s, H-17b), 1.27 (3H, s, Me-18), 1.09 (3H, s, Me-19), 4.74 (1H, dd, $J = 3.4$, 9.7 Hz, H-20a), 4.15 (1H, d, $J = 9.2$ Hz, H-20b), 2.21 (3H, s, OAc); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 207.2 (C, C-1), 41.7 (CH_2, C-2), 77.0 (CH, C-3), 37.4 (C, C-4), 46.9 (CH, C-5), 73.2 (CH, C-6), 200.0 (C, C-7), 59.6 (C, C-8), 38.1 (CH, C-9), 51.7 (C, C-10), 20.3 (CH_2, C-11), 31.3 (CH_2, C-12), 36.6 (CH, C-13), 36.4 (CH_2, C-14), 198.7 (C, C-15), 146.9 (C, C-16), 1117.3 (CH_2, C-17), 29.1 (CH_3, C-18), 23.0 (CH_3, C-19), 61.3 (CH_2, C-20), 169.6 (C, OAc), 20.7 (CH_3, OAc); HRESIMS *m/z* 409.1624 (calcd for $C_{22}H_{26}O_6Na$, 409.1627).

6.1.9.2. Compound ***14***. Chromatography: chloroform–ethyl acetate, 19.5:0.5; white powder, yield: 63.8%; mp 226–228 °C; $[\alpha]_D^{25}$ −196.4° (*c* 0.76, CH_3OH); UV (CH_3OH): λ_{max} (log ε) 236.6 (3.87) nm; IR (KBr) ν_{max} 2958, 1744, 1724, 1642, 1458, 1277, 1206, 1115, 1063 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 3.72 (1H, d, $J = 3.6$ Hz, H-3β), 5.66 (1H, d, $J = 12.6$ Hz, H-6α), 5.92 (1H, s, H-17a), 5.41 (1H, s, H-17b), 1.28 (3H, s, Me-18), 1.14 (3H, s, Me-19), 4.93 (1H, d, $J = 9.8$ Hz, H-20a), 4.16 (1H, dd, $J = 1.6$, 9.8 Hz, H-20b), 4.26 (1H, d, $J = 12.6$ Hz, H-2′a), 4.14 (1H, d, $J = 12.6$ Hz, H-2′b); ^{13}C NMR (acetone-d_6, 100 MHz) δ 207.9 (C, C-1), 42.3 (CH_2, C-2), 77.7 (CH, C-3), 38.3 (C, C-4), 47.5 (CH, C-5), 76.0 (CH, C-6), 200.7 (C, C-7), 60.7 (C, C-8), 39.4 (CH, C-9), 52.4 (C, C-10), 21.1 (CH_2, C-11), 31.9 (CH_2, C-12), 37.6 (CH, C-13), 36.9 (CH_2, C-14), 199.0 (C, C-15), 149.2 (C, C-16), 116.3 (CH_2, C-17), 29.0 (CH_3, C-18), 23.1 (CH_3, C-19), 61.6 (CH_2, C-20), 166.7 (C, C-1′), 26.2 (CH_2, C-2′); HRESIMs *m/z* 487.0728 (calcd for $C_{22}H_{25}O_6NaBr$, 487.0732).

6.1.9.3. Compound ***15***. Chromatography: chloroform–ethyl acetate, 19.5:0.5; white powder, yield: 28.9%; mp 127–129 °C; $[\alpha]_D^{25}$ −99.7° (*c* 0.21, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 231.2 (3.77) nm; IR (KBr) ν_{max} 2958, 2930, 1741, 1643, 1459, 1382, 1261, 1201, 1149, 1064 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 3.71 (1H, d, $J = 3.7$ Hz, H-3β), 5.67 (1H, d, $J = 12.6$ Hz, H-6α), 5.91 (1H, s, H-17a), 5.41 (1H, s, H-17b), 1.29 (3H, s, Me-18), 1.12 (3H, s, Me-19), 4.93 (1H, d, $J = 9.9$ Hz, H-20a), 4.15 (1H, d, $J = 9.8$ Hz, H-20b), 3.88 (2H, m, H-3′); ^{13}C NMR (acetone-d_6, 100 MHz) δ 208.1 (C, C-1), 42.3 (CH_2, C-2), 77.7 (CH, C-3), 38.0 (C, C-4), 47.4 (CH, C-5), 74.8 (CH, C-6), 200.9 (C, C-7), 60.6 (C, C-8), 39.4 (CH, C-9), 52.4 (C, C-10), 21.2 (CH_2, C-11), 31.9 (CH_2, C-12), 37.6 (CH, C-13), 36.9 (CH_2, C-14), 199.4 (C, C-15), 149.2 (C, C-16), 116.2 (CH_2, C-17), 29.1 (CH_3, C-18), 23.1 (CH_3, C-19), 61.6 (CH_2, C-20), 169.7 (C, C-1′), 38.1 (CH_2, C-2′), 40.0 (CH_2, C-3′); HRESIMS *m/z* 457.1404 (calcd for $C_{23}H_{27}O_6NaCl$, 457.1393).

6.1.9.4. Compound ***16***. Chromatography: chloroform–ethyl acetate, 19.5:0.5; white powder, yield: 83.2%; mp 74–76 °C; $[\alpha]_D^{25}$ −134.3° (*c* 0.61, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 233.4 (3.75) nm; IR (KBr) ν_{max} 2925, 2872, 2855, 1740, 1644, 1457, 1260, 1203, 1143, 1063 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 3.71 (1H, overlap, H-3β), 5.63 (1H, d, $J = 12.7$ Hz, H-6α), 5.91 (1H, s, H-17a), 5.40 (1H, s, H-17b), 1.25 (3H, s, Me-18), 1.11 (3H, s, Me-19), 4.91 (1H, d, $J = 9.9$ Hz, H-20a), 4.15 (1H, dd, $J = 1.6$, 9.9 Hz, H-20b), 3.72 (2H, overlap, H-4′); ^{13}C NMR (acetone-d_6, 100 MHz) δ 208.0 (C, C-1), 42.3 (CH_2, C-2), 77.6 (CH, C-3), 38.0 (C, C-4), 47.4 (CH, C-5), 74.4 (CH, C-6), 200.8 (C, C-7), 60.6 (C, C-8), 39.4 (CH, C-9), 52.4 (C, C-10), 21.2 (CH_2, C-11), 31.9 (CH_2, C-12), 37.6 (CH, C-13), 36.9 (CH_2, C-14), 199.6 (C, C-15), 149.2 (C, C-16), 116.2 (CH_2, C-17), 29.2 (CH_3, C-18), 23.2 (CH_3, C-19), 61.7 (CH_2, C-20), 171.7 (C, C-1′), 31.7 (CH_2, C-2′), 28.7 (CH_2, C-3′), 44.7 (CH_2, C-4′); HRESIMS *m/z* 471.1547 (calcd for $C_{24}H_{29}O_6NaCl$, 471.1550).

6.1.10. Synthesis of compounds **17** and **18**

To a solution of sodium hydroxide (87.168 mg, 2.179 mmol) in water (15 mL) was added **1** (250 mg, 0.726 mmol). The solution was stirred at room temperature until all starting material was consumed. The mixture was diluted with ethyl acetate and washed with diluted hydrochloric acid and water. The organic layer was dried over sodium sulfate and evaporated. Flash chromatography of the residue, eluting with chloroform–ethyl acetate/formic acid

(9:0.8:0.2), gave compounds **17** (120 mg, 45.4%) and **18** (48.12 mg, 18.4%).

*6.1.10.1. Compound **17***. Yellow powder; mp 212–214 °C; $[\alpha]_D^{25}$ −59.2° (*c* 0.36, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 210.0 (3.80) nm; IR (KBr) ν_{max} 3372, 2946, 2926, 1713, 1689, 1625, 1400, 1258, 1219, 1182, 1060 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 3.68 (1H, dd, $J = 2.0$, 3.4 Hz, H-3β), 4.47 (1H, d, $J = 7.9$ Hz, H-6α), 6.19 (1H, s, H-17a), 5.65 (1H, s, H-17b), 4.64 (1H, d, $J = 9.1$ Hz, H-20a), 4.03 (1H, dd, $J = 1.6$, 9.1 Hz, H-20b), 1.43 (3H, s, Me-18), 1.01 (3H, s, Me-19); ^{13}C NMR (acetone-d_6, 100 MHz) δ209.8 (C, C-1), 42.1 (CH_2, C-2), 78.2 (CH, C-3), 38.4 (C, C-4), 55.7 (CH, C-5), 74.1 (CH, C-6), 211.2 (C, C-7), 46.1 (CH, C-8), 43.7 (CH, C-9), 50.4 (C, C-10), 29.2 (CH_2, C-11), 32.3 (CH_2, C-12), 38.1 (CH, C-13), 32.2 (CH_2, C-14), 168.1 (C, C-15), 146.2 (C, C-16), 123.2 (CH_2, C-17), 29.4 (CH_3, C-18), 23.5 (CH_3, C-19), 60.3 (CH_2, C-20); HRESIMS *m/z* 385.1622 (calcd for $C_{20}H_{26}O_6Na$, 385.1627).

*6.1.10.2. Compound **18***. Yellow solid; mp 210–212 °C; $[\alpha]_D^{25}$ + 3.2° (*c* 0.52, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 278.0 (4.01) nm; IR (KBr) ν_{max} 3266, 2947, 2926, 1735, 1702, 1663, 1639, 1628, 1401, 1236, 1103, 1053 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 3.79 (1H, t, $J = 2.6$ Hz, H-3β), 6.19 (1H, s, H-17a), 5.64 (1H, s, H-17b), 1.51 (3H, s, Me-18), 1.14 (3H, s, Me-19), 4.28 (1H, d, $J = 9.1$ Hz, H-20a), 4.12 (1H, d, $J = 9.1$ Hz, H-20b); ^{13}C NMR (acetone-d_6, 100 MHz) δ 207.0 (C, C-1), 41.5 (CH_2, C-2), 78.3 (CH, C-3), 40.9 (C, C-4), 144.9 (C, C-5), 133.0 (C, C-6), 194.9 (C, C-7), 42.9 (CH, C-8), 34.8 (CH, C-9), 52.7 (C, C-10), 27.6 (CH_2, C-11), 32.4 (CH_2, C-12), 38.2 (CH, C-13), 31.9 (CH_2, C-14), 168.0 (C, C-15), 146.1 (C, C-16), 123.0 (CH_2, C-17), 23.1 (CH_3, C-18), 21.5 (CH_3, C-19), 65.0 (CH_2, C-20); HRESIMS *m/z* 383.1461 (calcd for $C_{20}H_{24}O_6Na$, 383.1470).

6.1.11. Synthesis of compound **19**

6.1.11.1. Method A. Compound **1** (240 mg, 0.697 mmol) was dissolved in methyl alcohol (10 mL). To this solution was added sodium periodate (2.24 g, 10.460 mmol) in water (5 mL). The mixture was stirred at room temperature for 24 h and then diluted with ethyl acetate. The solvent was filtered and evaporated to give **19** (238 mg, 99.7%) as white powder.

6.1.11.2. Method B. A solution of **1** (200 mg, 0.581 mmol) in dichloromethane (8 mL) was added to a solution of Dess–Martin periodinate (2.4 mL, 1.162 mmol) in dichloromethane (8 mL) with stirring. After 60 min the mixture was diluted with ethyl acetate and 10 mL of 1.3 M sodium hydroxide. After the mixture was stirred for 10 min, the organic layer was washed with 1.3 M sodium hydroxide and water, dried over sodium sulfate to give **19** (199 mg, 100%) as white powder.

*6.1.11.3. Compound **19***. White solid; mp 148–150 °C; $[\alpha]_D^{25}$ + 90.9° (*c* 0.32, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 226.6 (4.20) nm; IR (KBr) ν_{max} 2961, 2932, 2873, 2751, 1747, 1720, 1676, 1643, 1466, 1378, 1263, 1232, 1129, 1047 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 5.88 (1H, d, $J = 8.2$ Hz, H-2), 6.80 (1H, d, $J = 8.2$ Hz, H-3), 3.31 (1H, d, $J = 2.3$ Hz, H-5β), 9.99 (1H, d, $J = 2.3$ Hz, H-6), 5.91 (1H, s, H-17a), 5.56 (1H, s, H-17b), 1.41 (3H, s, Me-18), 1.34 (3H, s, Me-19), 4.99 (1H, d, $J = 9$ Hz, H-20a), 4.78 (1H, d, $J = 9.0$ Hz, H-20b); ^{13}C NMR (acetone-d_6, 100 MHz) δ 198.5 (C, C-1), 125.2 (CH, C-2), 158.4 (CH, C-3), 36.8 (C, C-4), 57.6 (CH, C-5), 202.4 (CH, C-6), 169.3 (C, C-7), 59.1 (C, C-8), 42.9 (CH, C-9), 51.2 (C, C-10), 18.3 (CH_2, C-11), 30.5 (CH_2, C-12), 35.7 (CH, C-13), 30.8 (CH_2, C-14), 202.6 (C, C-15), 152.1 (C, C-16), 118.7 (CH_2, C-17), 31.4 (CH_3, C-18), 24.4 (CH_3, C-19), 69.3 (CH_2, C-20); HRESIMS *m/z* 365.1365 (calcd for $C_{20}H_{22}O_5Na$, 365.1364).

6.1.12. Synthesis of compound **20**

To a solution of **19** (25 mg, 0.0726 mmol) in methyl alcohol (3 mL) was added a solution of silver oxide (84.168 mg, 0.363 mmol) in water (0.5 mL). To this solution was added dropwise with stirring 3 mL of potassium hydroxide (219 mg potassium hydroxide dissolved in 30 mL water). The mixture was stirred until all starting material was consumed. The reaction mixture was filtered and then diluted with ethyl acetate. The solvent was washed with diluted hydrochloric acid and water, dried over sodium sulfate and evaporated. The residue was recrystallized from acetone to give compound **20** (19.2 mg, 73.3%) as white crystals; mp 218–220 °C; $[\alpha]_D^{25}$ −114.6° (*c* 0.30, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 206.4 (4.02) nm; IR (KBr) ν_{max} 3436, 2958, 2929, 2860, 1729, 1687, 1626, 1376, 1272, 1151, 1045 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.77 (d, 1H, $J = 10.0$ Hz, H-2), 6.66 (1H, d, $J = 10.1$ Hz, H-3), 6.18 (s, 1H, H-17a), 5.56 (s, 1H, H-17b), 1.23 (s, 3H, Me-18), 1.20 (s, 3H, Me-19), 4.40 (d, 1H, $J = 11.6$ Hz, H-20a), 4.14 (d, 1H, $J = 11.8$ Hz, H-20b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 199.9 (C, C-1), 125.6 (CH, C-2), 160.1 (CH, C-3), 36.2 (C, C-4), 61.1 (CH, C-5), 174.1 (C, C-6), 169.2 (C, C-7), 57.0 (C, C-8), 44.3 (CH, C-9), 52.4 (C, C-10), 24.8 (CH_2, C-11), 29.6 (CH_2, C-12), 35.9 (CH, C-13), 31.7 (CH_2, C-14), 200.8 (C, C-15), 144.7 (C, C-16), 123.8 (CH_2, C-17), 31.5 (CH_3, C-18), 20.8 (CH_3, C-19), 68.4 (CH_2, C-20); HRESIMS *m/z* 381.1045 (calcd for $C_{20}H_{24}O_6Na$, 381.1314).

6.2. Cells and in vitro cytotoxicity assay

6.2.1. Cell lines

The cell lines used were human leukemia cell line (K562); ovarian cancer cell line (OVCA2780); human lung cancer cell line (A549); human prostate cancer cell line (DU145) and human breast cancer cell line (MCF-7).

An MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay was performed in 96-well plates. K562 cells at the log phase of their growth cycle (1.25×10^5 cell/mL) were added to each well (90 μL/well), then treated in four replicates at various concentrations of the samples (1–100 μg/mL), and incubated for 48 h at 37 °C in a humidified atmosphere of 5% CO_2. After 48 h, 10 μL of MTT solution (5 mg/mL) per well was added to each cultured medium, which were incubated for further 4 h. Then, a three-system solution of 10% SDS–5% isobutanol–0.012 mol/L hydrochloric acid was added to each well (100 μL/well). After 12 h at room temperature, the OD of each well was measured on a Microplate Reader (BIO-TEK instruments Inc EL311S) at a wavelength of 570 nm. In these experiments, the negative reference agents was 0.1% DMSO, and cisplatin (DDP) was used as the positive reference substance with concentration of 1–80 μg/mL. The same method was used in cytotoxic testing against OVCA2780, A549, DU145 and MCF-7 cell lines.

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