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New cytotoxic spongian diterpenes from the sponge *Dysidea* cf. *arenaria*

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ABSTRACT

Seven new (**1**–**7**) and three known (**8**–**10**) spongian-class diterpenes have been isolated from the sponge *Dysidea* cf. *arenaria* collected in Okinawa. Compound **6** was also isolated from the nudibranch *Chromodoris kuniei*. The structures of the new entities were elucidated by spectroscopic analyses. Three of the new spongians (**2**, **6**, and **7**) showed cytotoxicity against NBT-T2 rat bladder epithelial cells.

1. Introduction

Among sessile marine macroorganisms, sponges have been the most prolific sources of novel molecules with structural diversity and biological activity. Those belonging to the genus *Dysidea* have also been extensively studied for their chemical constituents resulting in the isolation of a number of chemically unique and biologically important molecules.[1] A representative species includes *Dysidea arenaria*, from which arenastatins were discovered as potent cytotoxins,[2] whereas a protein kinase inhibitor isoarenarol was isolated from another specimen.[3] The formers show structural similarity to cyanobacterial metabolite cryptophycins,[4] whose synthetic analog coded LY355703 is under clinical trials as an anticancer agent.[5] As Faulkner reported that a polychlorinated molecule[6] was a cyanobacterial metabolite[7] in another species *Dysidea* (currently *Lamellodysidea*) *herbacea*, arenastatins also raise a question on the origin of the molecules whether they are produced by associated cyanobacteria or by the sponge itself.[8]

On our quest for new bioactive molecules from Okinawan marine invertebrates,[9] we encountered a soft sponge similar to *D. arenaria*, but different in their chemical constituents from the one containing arenastatins or arenarol. In this manuscript we describe the isolation, structures, and biological activity of new spongian diterpenes found from the title sponge and from a nudibranch collected at the same place.

2. Results and discussion

A specimen (0.68 kg) of the sponge *D.* cf. *arenaria* was collected in Okinawa. As its lipophilic extract (4.24 g) showed cytotoxicity against cultured cells at 1 μg/mL and was found to be rich in terpenes with the ^{1}H NMR, the whole was subjected to repeated chromatographic separation to give 10 compounds (**1**–**10**). Of them, compounds **1**–**7** turned out to be new diterpenes after spectroscopic analyses, while the remaining three were identified as known spongians: dorisenone B (**8**),[10] 12-desacetylaplysillin (**9**),[11] and isoagatholactone (**10**).[12] In the following, the structures of the new molecules are described.

Compound **1** was isolated as a white solid and found to have a molecular formula as $C_{24}H_{34}O_6$ by HRESIMS. The ^{1}H NMR spectrum of **1** included two oxymethylene proton signals (δ 4.07 dd, 4.25 t) and four aliphatic methyl singlets (δ 0.81 s, 0.82 s, 0.91 s, 1.05 s) as isoagatholactone (**10**). A γ-lactone moiety was confirmed with a carbonyl (δ 169.1 s, 1770 cm^{-1}), an HMBC correlation from δ 4.25 (H-15) to C-16, and a downfield proton at δ 6.71 (H-12) assigned as β proton to the carbonyl as in **10** (δ 6.87). There are additional signals of two acetates (δ 2.04, 2.14; δ 21.2 q, 21.3 q, 169.8 s, 170.4 s) and two oxymethine signals (δ 4.77 t and 5.65 ddd) suggesting that compound **1** is an analog of **10** with two acetates. The acetates could be placed at C-7 and C-11 by COSY (H-7/H-6αβ, H-11/H-9,12) and HMBC (H-7/C-5,9, H-11/C-12,13) analyses and were α-oriented by observing NOEs (H-7/H-17, H-11/H-17,20) and coupling constants ($J_{6\alpha,7}$=3.0 Hz, $J_{6\beta,7}$=3.0 Hz, $J_{9,11}$=8.3 Hz). The configuration at C-14 was elucidated as *S** with an NOE (H-9/H-14). Thus, the structure of **1** was elucidated as 7α,11α-diacetoxyisoagatholactone or 7,11-di-*epi*-dorisenone D.[10]

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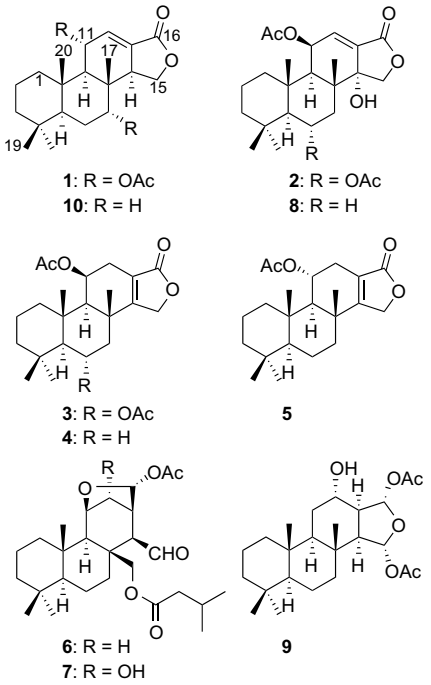

ESIMS of compound **2** established the molecular formula as $C_{24}H_{34}O_7$ indicating one additional oxygen atom to that of **1**. Two acetates (δ 2.07, 2.08; δ 21.4, 21.9, 169.7, 170.6, 1738 cm^{-1}), γ-lactone (δ 168.9 s, 1780 cm^{-1}), and its conjugated double bond (δ 6.74; δ 131.7 s, 135.3 d) were observed as in **1**. However, the presence of a tertiary hydroxyl (δ 76.7 s, 3445 cm^{-1}) and two mutually coupled (J=10.0 Hz) protons at δ 4.26 d and 4.48 d were quite reminiscent to those of dorisenone B (**8**),[10] suggesting the same lactone moiety for the ring D portion. The hydroxyl proton appearing at δ 5.89 br s in DMSO-d_6 showed an NOE with H-15α (δ 4.12), while irradiation of H-15β gave positive NOE of Me-17 confirming α orientation of the hydroxyl group. The positions of the acetates were determined by COSY (H-6/H-5,7$\alpha\beta$, H-11/H-9,12) and HMBC correlations (H-6/C-5,Ac, H-11/C-9,12,13,Ac). Both acetoxy groups at C-6 and C-11 were elucidated as α and β by observing coupling constants ($J_{6\beta,7\alpha}$=11.0 Hz, $J_{9,11}$=4.9 Hz) and positive NOEs (H-6/H-17,20, H-9/H-11).

The molecular formula of compound **3**, $C_{24}H_{34}O_6$ by HRESIMS, indicated that **3** is isomeric to compound **1**. The NMR and FTIR spectra of **3** showed the presence of two acetates (δ 2.04, 2.08; δ 169.9, 170.2, 1740 cm^{-1}), four singlet methyls (δ 0.90, 1.03, 1.18, 1.64), and α,β-unsaturated γ-lactone (δ 173.7 s, 120.7 s, 168.0 s, 1758 cm^{-1}). The position of the conjugated double bond was inferred by observing allylic proton signals at H-12 (δ 2.46 m) and mutually coupled (J=17.1 Hz) oxymethylene protons at H-15 (δ 4.69 ddd, 4.83 dt) and their HMBC correlations to C-13 and C-14. The two acetoxy groups were found to be exactly the same as **2**: 6α and 11β by observing similar oxymethine signals (δ 5.39 td, 5.78 m) to those of **2** (δ 5.33 td, 5.75 dd), coupling constants ($J_{5,6}$=11.3 Hz, $J_{6,7\alpha}$=11.3 Hz), and NOEs (H-6/H-17,20, H-9/H-11). Further analysis of 1D and 2D NMR spectra led to the gross structure of **3** and signal assignments (Table 1).

The molecular formula of compound **4**, $C_{22}H_{32}O_4$, implied that it is a diterpene with an acetate. On comparison of ^{1}H NMR data of **4** with **3**, analogous signals such as H-11 (δ 5.76 br s), H-12 (δ 2.43 m), and H-15$\alpha\beta$ (δ 4.74 dt, 4.84 dt) to those of **3** were observed, whereas there is no signal corresponding to H-6 (δ 5.39) in **3**. Further NMR studies confirmed that **4** is 11β-acetoxyspongian-13-en-16-one (Table 1).

Compound **5** had the same molecular formula as that of **4** and also contained the same functionalities: α,β-unsaturated γ-lactone (δ 135.6 s, 155.6 s, 171.3 s, 1750 sh cm^{-1}), an acetate (δ 2.08 s, δ 21.8 q, 170.2 s, 1738 cm^{-1}), and four methyl singlets (δ 0.84, 0.87, 1.00, 1.27). However, there are some differences in the signals of H-9 (δ 1.59 d), H-11 (δ 5.47 ddd), and H-12 (δ 2.30 brdd, 2.82 dd). Coupling constants ($J_{9,11}$=10.7 Hz, $J_{11,12\alpha}$=8.5 Hz) and NOEs (H-11/H-17,20) clearly concluded α-orientation of the acetoxy group at C-11. The remaining signals were assigned as in Table 1.

The molecular formula of compound **6**, $C_{27}H_{42}O_6$, exhibited seven degrees of unsaturation assignable to two ester carbonyls (δ 169.8, 172.3), an aldehyde (δ 9.79 s, δ 197.4 d), and the remaining four to rings. An isovalerate moiety was revealed by COSY connectivity from two methyl doublets (δ 0.948, 0.953, δ 22.3, 22.4) to a methine proton (δ 2.08 m), which further coupled with methylene protons at δ 2.15, and also by HMBC correlations of the ester carbonyl (δ 172.3) with δ 2.15 and oxymethylenes at δ 4.66 d and 4.85 d. An acetate (δ 2.04 s, 169.8) was found to attach to an acetal (δ 6.14 s, δ 97.5 d), and the acetal proton showed HMBC cross-peaks with C-13 and C-14. In addition to the partial structure from C-11 to C-14 disclosed by COSY, HMBC cross-peaks for H-11/C-16, H-16/C-11, H-15/C-14, and H-17/C-7,8 connected all the functionalities on the C and D rings. Since the stereochemical arrangements were revealed by positive NOEs (H-9/H-11, H-14/H-9,12α,13,15, H-16/H-15,17a, H-20/H-17b,19, Fig. 1), the whole structure of **6** can be depicted with signal assignments (Table 2).

The molecular formula of **7**, $C_{27}H_{42}O_7$, was shown to have an additional oxygen atom to **6**. Compound **7** retains the same functionalities: an acetal (δ 6.09 s; δ 96.3 d), an aldehyde (δ 9.83 s, δ 198.2 d), an acetate (δ 2.06 s, δ 21.2 q, 169.6 s), and an isovalerate (δ 172.4 s, 42.8 t, 25.5 d, 22.3 q$\times$2) with similar chemical shifts as those of **6**. As the presence of the cyclic ether was confirmed by HMBC (H-11/C-16, H-16/C-11), the remaining oxygen atom can be accounted for a secondary hydroxyl group (δ 4.73 m, δ 68.4 d, 3478 cm^{-1}) at C-12 by observing COSY (H-12/H-11,13) and HMBC (H-12/C-14, H-9,13,14/C-12) correlations. NOE study confirmed the same configurations at C-9, 11, 13, 14, and 16 (H-9/H-11,14, H-13/H-14, H-16/H-17a) with **6** except for C-12. Although no significant NOE was observed for the stereochemistry of H-12, α-orientation was elucidated for the hydroxyl group by the facts that C-9 and C-14 were upfield-shifted (C-9: $-$11.3, C-14: $-$7.6) probably due to gamma-*gauche* effect, that H-9 and H-14 were downfield-shifted (H-9: +0.65, H-14: +0.53), and that nearly the same coupling constants (6.0 Hz for **6** and 5.8 Hz for **7**) were observed for $J_{11,12\beta}$ on comparison of **6** and **7** (Table 2).

When we examined chemical content of a specimen of the nudibranch *Chromodoris kuniei* collected nearby the sponge, it was found to contain compound **6** as a major secondary metabolite as well as the sponge suggesting the dietary origin of the molecule. However, this sponge–nudibranch relation could be an exception to the feeding specificity as chromodorids prefer darwinellid sponges.[13]

Compounds **1**–**7** exhibited cytotoxicity as follows: IC_{50} >10, 1.9, >10, >10, >10, 1.8, and 4.2 μg/mL against NBT-T2 rat bladder epithelial cells.

3. Conclusion

The current study resulted in the structural elucidation of seven new spongian-class diterpenes (**1**–**7**) with their relative stereochemistry from the Dictyoceratid sponge *D.* cf. *arenaria*. Compounds **2**, **6**, and **7** showed moderate cytotoxicity against cultured cells. Although *Dysidea* sponges have been reviewed to contain rearranged spongian diterpenes,[14] we have not encountered any of them, instead compounds **6** and **7** constitute a new spongian subgroup.

Table 1
NMR data for spongians **1**–**5** in $CDCl_3$[a]

C	**1**		**2**		**3**		**4**		**5**	
	δ_C	δ_H, m, *J* in Hz	δ_C	δ_H, m, *J* in Hz	δ_C	δ_H, m, *J* in Hz	δ_C	δ_H, m, *J* in Hz	δ_C	δ_H, m, *J* in Hz
1α	39.8 t	1.23 m	39.9 t	1.17 m	39.6 t	1.02 td, 12.7, 4.0	39.8 t	0.93 td, 13.2, 4.0	41.2 t	1.09 td, 13.3, 3.5
1β		1.40 m		1.62 m		1.86 brd, 12.7		1.85 brd, 13.2		2.14 brd, 13.3
2α	18.3 t	1.40 m	17.8 t	1.45 m	17.9 t	1.52 m	18.2 t	1.50 m	18.7 t	1.39 m
2β		1.60 m		1.62 m		1.67 m		1.67 m		1.61 m
3α	41.4 t	1.21 m	43.1 t	1.21 m	42.9 t	1.23 td, 13.7, 4.4	41.7 t	1.14 td, 13.5, 4.6	41.7 t	1.17 td, 13.2, 4.0
3β		1.43 m		1.39 m		1.38 brdt, 13.7, 3.4		1.41 brd, 13.5		1.36 m
4	32.6 s		33.1 s		33.4 s		33.4 s		33.7 s	
5	47.9 d	1.45 m	58.8 d	1.34 m	59.7 d	1.25 d, 11.3	57.5 d	0.85 m	56.4 d	0.93 dd, 12.0, 2.2
6α	23.4 t	1.79 dt, 15.0, 3.0	70.5 d		69.7 d		18.5 t	1.59 m[b]	17.7 t	1.43 m[c]
6β		1.71 ddd, 15.0, 12.8, 3.0		5.33 td, 11.0, 4.4		5.39 td, 11.3, 3.7		1.73 m[b]		1.67 m[c]
7α	74.1 d		38.8 t	2.26 t, 11.0	46.1 t	1.52 brt, 11.6	40.3 t	1.48 m	36.3 t	1.35 m
7β		4.77 t, 3.0		1.63 m		2.01 dd, 11.6, 3.7		1.75 m		2.68 dt, 13.2, 3.2
8	44.1 s		39.8 s		37.5 s		37.2 s		37.6 s	
9	54.4 d	2.01 d, 8.3	48.6 d	2.05 m	56.3 d	1.35 brs	57.0 d	1.29 brs	58.8 d	1.59 d, 10.7
10	38.8 s		39.7 s		39.6 s		38.2 s		39.4 s	
11	69.5 d	5.65 ddd, 8.3, 4.0, 2.5	66.2 d	5.75 dd, 4.9, 3.9	65.7 d	5.78 m	65.8 d	5.76 brs	69.7 d	5.47 ddd, 5.8, 8.5, 10.7
12α	130.3 d	6.71 t, 4.0	135.3 d	6.74 d, 3.9	29.4 t	2.46 m (2H)	29.4 t	2.43 m (2H)	32.9 t	2.30 brdd, 18.3, 8.5
12β										2.82 dd, 18.3, 5.8
13	131.4 s		131.7 s		120.7 s		120.3 s		155.7 s	
14	42.5 d	3.34 m	76.7 s		168.0 s		169.6 s		135.6 s	
15α	66.8 t	4.25 t, 9.5	75.0 t	4.26 d, 10.0	68.3 t	4.69 ddd, 17.1, 3.1, 2.1	68.5 t	4.74 dt, 16.8, 2.7	69.8 d	4.60 dd, 16.8, 1.0[d]
15β		4.07 dd, 9.5, 8.0		4.48 d, 10.0		4.83 dt, 17.1, 2.7		4.84 dt, 16.8, 2.7		4.54 d, 16.8[d]
16	169.1 s		168.9 s		173.7 s		174.2 s		171.3 s	
17	15.6 q	0.91 s	21.1 q	1.25 s	23.8 q	1.64 s	23.0 q	1.52 s	22.0 q	1.27 s
18	33.1 q	0.81 s	36.6 q	1.07 s	36.1 q	1.03 s	33.3 q	0.86 s	33.7 q	0.87 s
19	21.2 q	0.82 s	22.2 q	0.91 s	21.8 q	0.90 s	21.2 q	0.85 s	21.5 q	0.84 s
20	16.6 q	1.05 s	18.4 q	1.32 s	18.7 q	1.18 s	17.4 q	1.07 s	16.9 q	1.00 s
6Ac			21.9 q, 170.6 s	2.07 s	21.9 q, 170.2 s	2.08 s				
7Ac	21.3 q, 170.4 s	2.04 s								
11Ac	21.2 q, 169.8 s	2.14 s	21.4 q, 169.7 s	2.08 s	21.4 q, 169.9 s	2.04 s	21.5 q, 170.1 s	2.03 s	21.8 q, 170.2 s	2.08 s

[a] Chemical shifts were referenced to $CDCl_3$ (δ_C 77.1) for ^{13}C and TMS (δ_H 0.00) for ^1H.
[b] Signals are exchangeable.
[c] Signals are exchangeable.
[d] Signals are exchangeable.

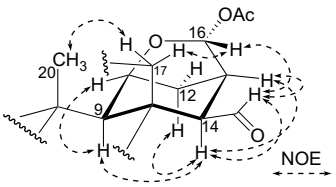

Figure 1. Selected NOEs for compound **6**.

Table 2
NMR Data for Spongians **6** and **7** in $CDCl_3$[a]

C	**6**		**7**	
	δ_C	δ_H, m, *J* in Hz	δ_C	δ_H, m, *J* in Hz
1α	39.8 t	0.83 m	39.6 t	0.77 m
1β		1.76 brd, 12.0		1.66 m
2α	18.4 t	1.50 m	18.4 t	1.47 m
2β		1.68 m		1.67 m
3α	42.0 t	1.14 td, 13.5, 4.0	41.9 t	1.16 td, 13.6, 4.0
3β		1.40 m		1.40 brd, 13.6
4	33.3 s		33.3 s	
5	56.8 d	0.88 m	56.6 d	1.00 m
6α	18.6 t	1.69 m	18.6 t	1.72 m
6β		1.50 m		1.53 m
7α	38.5 t	1.38 m	37.9 t	1.51 m
7β		2.40 brd, 13.2		2.39 dd, 9.5, 3.0
8	46.3 s		45.4 s	
9	63.9 d	1.31 s	52.6 d	1.96 s
10	37.7 s		36.9 s	
11	76.1 d	4.80 d, 6.0	75.8 d	4.48 d, 5.8
12α	38.6 t	1.52 brd, 11.2	68.4 d	
12β		2.42 dd, 11.2, 6.0		4.73 m
13	40.0 d	2.96 d, 3.9	42.7 d	2.83 brd, 4.5
14	61.6 d	2.18 m	54.0 d	2.71 d, 2.0
15	197.4 d	9.79 s	198.2 d	9.83 s
16	97.5 d	6.14 s	96.3 d	6.09 s
17a	63.6 t	4.66 d, 12.3	64.7 t	4.62 dd, 12.2, 1.7
17b		4.85 d, 12.3		4.87 d, 12.2
18	33.1 q	0.85 s	33.1 q	0.86 s
19	20.8 q	0.83 s	20.8 q	0.84 s
20	17.7 q	1.03 s	18.5 q	1.05 s
1'	172.3 s		172.4 s	
2'	42.9 t	2.15 m (2H)	42.8 t	2.13 dd, 14.9, 7.0, 2.17 dd, 14.9, 7.2
3'	25.5 d	2.08 m	25.5 d	2.08 m
4'	22.3 q	0.948 d, 6.4	22.3 q	0.94 d, 7.0
5'	22.4 q	0.953 d, 6.6	22.3 q	0.94 d, 7.0
16Ac	21.3 q, 169.8 s	2.04 s	21.2 q, 169.6 s	2.06 s

[a] Chemical shifts were referenced to $CDCl_3$ (δ_C 77.1) for ^{13}C and TMS (δ_H 0.00) for ^1H.

4. Experimental section

4.1. General experimental procedures

Precoated silica gel TLC plates (Merck) were used with detection by dipping in 1% vanillin in 20% sulfuric acid followed by heating. HPLC equipped with Hitachi 4000 pump, a Hitachi 6000 UV detector, and a Shodex RI-101 monitor was used. Optical rotation was measured on a JASCO P-1010 polarimeter. FTIR spectra were obtained on a JASCO FT/IR 300 instrument. ESIMS spectra were obtained on a PE QSTAR mass spectrometer. NMR data were measured at 500 MHz for ^1H and 125 MHz for ^{13}C on a JEOL A500 instrument.

4.2. Sponge and nudibranch

A specimen (0.68 kg, wet) of the sponge was collected at 35 m deep at Manza in Okinawa Island and brought back to the laboratory. A specimen of the same sponge was identified previously by Dr. J. N. A. Hooper, Queensland Museum and deposited at the museum with a code QMG307000. A specimen (3 g, wet) of the nudibranch *C. kuniei* was collected at the same site and dissected into two parts, mantle and viscera.

4.3. Extraction and isolation

The fresh sponge specimen was soaked in acetone (1.5 L) for three times. The combined extract was concentrated under reduced pressure and the residue was partitioned between EtOAc and H_2O. The EtOAc layer gave 4.24 g of a dark oil, which was subjected to vaccum flash chromatography on silica to give six fractions. Fraction 4 (1.68 g) showed the strongest cytotoxicity and it was separated on a silica gel column followed by HPLC purification of each subfractions to give compounds **1**–**10**. Additional amount of compounds **2**, **9**, and **10** was also obtained from fractions 1–3 and 5 by VFC. Total amounts were: **1** (5.7 mg), **2** (12.3 mg), **3** (7.3 mg), **4** (5.6 mg), **5** (9.8 mg), **6** (43.8 mg), **7** (6.5 mg), **8** (10.3 mg), **9** (6.8 mg), and **10** (10.9 mg).

Both mantle and viscera of *C. kuniei* were extracted with aqueous MeOH. The EtOAc soluble portion (6.0 mg) from the viscera was subjected for preparative TLC on silica (hexane–EtOAc, 3–1) to give 1.4 mg of compound **6** showing identical NMR data as the one from the sponge.

*4.3.1. Compound **1***

A white solid; $[\alpha]_D^{25}$ −125 (*c* 0.41, CH_2Cl_2); IR (neat) 2930, 1770, 1733, 1235 cm^{-1}; UV (MeOH) 219 nm (ε 1.1×10^4); ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 441.2240 $[M+Na]^+$ (calcd for $C_{24}H_{34}O_6Na$, 441.2252).

*4.3.2. Compound **2***

Needles (from MeOH); $[\alpha]_D^{25}$ +250 (*c* 0.28, CH_2Cl_2); IR (neat) 3445, 2930, 1780, 1738, 1224 cm^{-1}; UV (MeOH) 222 nm (ε 6.5×10^3); ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 457.2220 $[M+Na]^+$ (calcd for $C_{24}H_{34}O_7Na$, 457.2202).

*4.3.3. Compound **3***

Glass; $[\alpha]_D^{25}$ +95 (*c* 0.47, CH_2Cl_2); IR (neat) 2870, 1758, 1740, 1244 cm^{-1}; UV (MeOH) 231 nm (ε 3.1×10^3); ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 441.2258 $[M+Na]^+$ (calcd for $C_{24}H_{34}O_6Na$, 441.2252).

*4.3.4. Compound **4***

Glass; $[\alpha]_D^{25}$ +15 (*c* 0.41, CH_2Cl_2); IR (neat) 2930, 1758, 1738, 1680, 1240 cm^{-1}; UV (MeOH) 221 nm (ε 1.2×10^4); ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 383.2192 $[M+Na]^+$ (calcd for $C_{22}H_{32}O_4Na$, 383.2198).

*4.3.5. Compound **5***

Glass; $[\alpha]_D^{25}$ −28 (*c* 0.74, CH_2Cl_2); IR (neat) 2925, 1750 sh, 1738, 1240 cm^{-1}; UV (MeOH) 215 nm (ε 1.2×10^4); ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 383.2185 $[M+Na]^+$ (calcd for $C_{22}H_{32}O_4Na$, 383.2198).

*4.3.6. Compound **6***

Glass; $[\alpha]_D^{25}$ −10 (*c* 0.85, CH_2Cl_2); IR (neat) 2930, 1740, 1235 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 485.2883 $[M+Na]^+$ (calcd for $C_{27}H_{42}O_6Na$, 485.2878).

*4.3.7. Compound **7***

Glass; $[\alpha]_D^{25}$ −12 (*c* 0.44, CH_2Cl_2); IR (neat) 3478, 2930, 1740, 1236 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 501.2824 $[M+Na]^+$ (calcd for $C_{27}H_{42}O_7Na$, 501.2828).

*4.3.8. Compounds **9** and **10***

Both compounds showed the same absolute configuration ($[\alpha]_D$ +6.4, +6.5) as those reported ($[\alpha]_D$ +7.5, +6.3).[15,11]

4.4. Cytotoxicity test

NBT-T2 cells (BRC-1370) were cultured under a standard protocol using DMEM. Cultured cells were inoculated into each well (96-well plate) with 200 μL of the medium. After preincubation (24 h, 37 °C, 5% CO_2), sample solutions in MeOH were added to each well and the cells were incubated for 48 h. The medium was removed by aspiration, and MTT solution (5 mg/mL in PBS) was added to each well and incubated for 3 h. The residual formazan was dissolved in 100 μL of dimethylsulfoxide (DMSO). The absorbance was measured on a microplate reader at 570 nm.

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