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Cangumycins A−F, six new angucyclinone analogues with immunosuppressive activity from *Streptomyces*

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[ABSTRACT] Cangumycins A−F (**1**–**6**), six new angucyclinone analogues, together with two known ones (**7** and **8**), were isolated from the fermentation broth of a soil-derived *Streptomyces* sp. KIB-M10. Structures of these compounds were elucidated *via* a joint use of spectroscopic analyses and single-crystal X-ray diffractions. Among them, cangumycins E (**5**) and F (**6**) share a C-ring cleaved backbone, and cangumycins B (**2**) and E (**5**) exhibit potent immunosuppressive activity (IC_{50} 8.1 and 2.7 μmol·L^{-1}, respectively) against human T cell proliferation at a non-cytotoxic concentration.

[KEY WORDS] *Streptomyces*; Angucyclinones; Benz[*a*]anthracenes; Natural products; Immunosuppressive activity

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Introduction

Angucyclines/angucyclinones, harbouring a tetracyclic benz[*a*]anthracene core, are the largest family of aromatic polyketide natural products biosynthesized by type II polyketide synthases (PKSs) [1-5]. Generally speaking, angucyclines refer to the ones with a glycoside moiety, whereas angucyclinones are limited to a subtype without any sugar linkage [6]. Since the first angucyclinone, tetrangomycin, was discovered in 1965 [2], hundreds of angucyclines/angucyclinones analogues have been reported up to now, employing *Streptomyces* as the main producers [1-3]. With many recorded modifications, like poly-oxidizable sites, amino acid incorporations, and multifarious ring cleavages, angucyclines/angucyclinones possess abundantly diverse structures [5], which may exhibit different biological properties, such as antibacterial, antitumor, and enzyme inhibitory activities [1-3, 5].

In the continuous efforts on the chemical screening of extract libraries from some actinomycetes (mainly *Streptomyces*) by DAD-HPLC in our lab [7-8], an extract from *Streptomyces* sp. KIB-M10 with strongly long ultraviolet absorptions captured our attention. As a result, six new angucyclinone analogues, cangumycins A−F (**1**–**6**) (Fig. 1), together with two known ones (**7** and **8**), were isolated from the fermentation broth of this selected strain. Herein, the isolation, structure elucidation, and immunosuppressive screening of these angucyclinones are described.

Results and Discussion

Ten days of liquid fermentation of *Streptomyces* sp. KIB-M10 led to the isolation of six new angucyclinone analogues, cangumycins A−F (**1**–**6**) (Fig. 1), together with two known ones (**7** and **8**). The known ones were identified as

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Dedicated to Professor SUN Han-Dong on the Occasion of His 80th Birthday

elmycin A (**7**) [9] and elmycin B (**8**) [10], respectively, based on the literature reports. As shown in Fig. 2, the absolute configurations of elmycins A (**7**) and B (**8**) were directly assigned by single-crystal X-ray diffractions here for the first time.

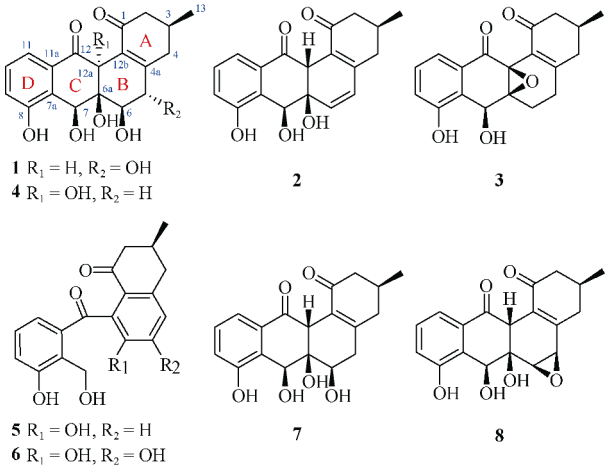

Fig. 1 Structures of compounds 1–8

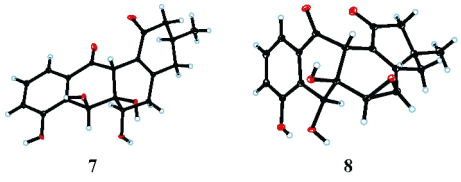

Fig. 2 X-ray crystal structures of compounds 7 and 8

Cangumycin A (**1**) was obtained as white powder. HRESI-MS analysis (*m/z* 359.1136 [M – H]$^-$, Calcd. for 359.1136) of **1** revealed its molecular formula as $C_{19}H_{20}O_7$, containing ten degrees of unsaturation. According to previous reports [5, 11-15], a comprehensive analysis of the ^{1}H and ^{13}C NMR of **1** (Tables 1 and 2) indicated that cangumycin A (**1**) was presumably an angucyclinone analogue.

A series of correlated signals [H_2-2/C-1; H-3/C-1; H_2-4/(C-4a, C-12b)] in the HMBC spectrum of **1**, as well as the ^{1}H-^{1}H COSY coupling fragment H_2-2/H-3/H_2-4(/H_3-13), corporately demonstrated the presence of six-membered carbon ring A (C-1/C-2/C-3/C-4/C-4a/C-12a) in **1** (Fig 3, left). Further HMBC correlations of **1** [H-5/(C-4, C-12b); H-6/C-6a; H-12a/(C-1, C-4a, C-6, C-6a, C-12b)], along with the COSY cross peaks of H-5/H-6 verified that another six-membered carbon ring B (C-4a/C-5/C-6/C-6a/C-12a/C-12b) was fused with ring A through two shared carbons (C-4a and C-12b) (Fig. 3, left). In addition, based on several HMBC connections of H-7/(C-6, C-6a, C-7a, C-8, C-11a, C-12a); H-9/C-8; H-10/(C-8, C-11a); H-11/C-12; and H-12a/(C-6, C-6a, C-12), a subunit comprised by ring C (C-6a/C-7/C-7a/C-11a/C-12/C-12a) and ring D (C-7a/C-8/C-9/C-10/C-11/C-11a) was found to be coupled with ring B through a carbon-carbon single bond (C-6a–C-12a). In the end, in view of the chemical formula and the unsaturated number of **1**, every undefined oxidation locus (C-5, C-6, C-6a, C-7 and C-8) in **1** was all deduced to be decorated with a hydroxy group. Thus, the chemical structure of **1** in plane was established as shown in Fig. 3.

Table 1 ^{1}H NMR data for compounds 1–4, 7 and 8 in DMSO-d_6 (*J* in Hz)

Position	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**7**[b]	**8**[a]
2-i	2.47 m;	2.48 m	2.43 m	2.43 m	2.61 dd (16.4, 3.9)	2.28[c]
2-ii	2.04[c]	2.10[c]	2.35 m	2.24 m	2.16[c]	2.12 m
3	2.08[c]	2.10[c]	2.25 m	2.22 m	2.29 m	2.06 m
4-i	2.81 dd (17.9, 3.6)	2.36 m	1.99[c]	2.04[c]	2.34[c]	2.60 dd (18.4, 4.0)
4-ii	1.82 m	2.12[c]	1.54 dd (13.8, 11.7)	1.52 m	2.12[c]	2.28[c]
5-i	3.97 d (7.8)	5.95 d (9.3)	1.80 m	2.06[c]	2.49 d (8.0)	3.46 br d (3.9)
5-ii			1.69 m	1.93 dd (14.1, 4.2)	2.34[c]	
6-i	3.02 d (7.8)	5.78 d (9.3)	1.94 m	4.10 br d (4.2)	3.51 t (8.0)	3.38 br s
6-ii			2.00[c]			
7	5.27 s	4.95 s	5.06 s	5.61 s	5.55 s	5.04 s
9	7.11 dd (6.9, 2.3)	7.09 d (7.2)	7.03 m	7.03 d (7.8)	7.12 d (7.8)	7.06 m
10	7.25[c]	7.16[c]	7.27[c]	7.26 t (7.8)	7.28 t (7.8)	7.24[c]
11	7.23[c]	7.18[c]	7.27[c]	7.32 d (7.8)	7.39 d (7.8)	7.24[c]
12a	4.12 s	4.21 s			4.32 s	3.63 s
13	1.03 d (5.7)	1.01 d (6.6)	1.01 d (6.3)	1.01 d (6.6)	1.09 d (6.1)	0.99 d (6.0)

[a] Recorded at 600 MHz; [b] Recorded at 400 MHz; [c] Overlapped signals

Next, the assignment of the relative configuration of **1** was discussed. As reported [3, 5-6, 11-15], the methyl modification at C-3 is usually *β*-oriented. In view of the shared biosynthetic origin with elmycins A (**7**) and B (**8**), as well as their coexistence after the fermentation, the orientation of OH-7, OH-6a, OH-6 and 3-Me in **1** were all suggested to be *β* like in elmycins A (**7**) and B (**8**). Further ROESY correlations of H_3-13/H-4ii, H-4ii/H-5, H-12a/H-7, H-12a/H-6, and H-6/H-7 indicated that OH-5 and H-12a were all *α*-configured (Fig. 3, right).

Table 2 ^{13}C NMR data for compounds 1–4, 7 and 8 in DMSO-d_6

Position	1[a]	2[a]	3[a]	4[a]	7[b]	8[a]
1	197.5, C	196.7, C	204.2, C	204.3, C	200.7, C	196.4, C
2	45.0, CH_2	45.2, CH_2	50.8, CH_2	50.9, CH_2	46.4, CH_2	45.0, CH_2
3	28.7, CH	29.3, CH	28.0, CH	27.9, CH	30.5, CH	29.1, CH
4	33.1, CH_2	36.6, CH_2	47.7, CH_2	47.6, CH_2	39.5, CH_2	36.8, CH_2
4a	156.0, C	147.1, C	151.0, C	153.6, C	158.5, C	151.8, C
5	73.2, CH	129.4, CH	33.0, CH_2	37.5, CH_2	39.8, CH_2	52.4, CH
6	73.2, CH	137.7, CH	29.2, CH_2	67.6, CH	67.7, CH	57.2, CH
6a	75.2, C	70.4, C	72.4, C	73.3, C	75.6, C	3.6, C
7	62.2, CH	65.9, CH	75.0, CH	68.8, CH	64.2, CH	66.9, CH
7a	128.6, C	129.0, C	127.0, C	127.2, C	128.9, C	127.0, C
8	156.5, C	155.8, C	157.9, C	158.1, C	158.0, C	156.4, C
9	120.7, CH	120.5, CH	121.7, CH	121.9, CH	122.0, CH	120.4, CH
10	128.8, CH	128.3, CH	128.4, CH	128.5, CH	130.6, CH	129.0, CH
11	116.4, CH	116.6, CH	116.6, CH	117.3, CH	118.6, CH	117.2, CH
11a	131.1, C	135.4, C	132.1, C	131.6, C	132.6, C	133.1, C
12	196.0, C	196.6, C	187.7, C	186.5, C	198.0, C	196.7, C
12a	47.4, CH	45.0, CH	74.2, C	76.1, C	49.2, CH	48.2, CH
12b	128.4, C	129.6, C	136.9, C	132.3, C	129.6, C	131.8, C
13	20.7, CH_3	20.8, CH_3	21.3, CH_3	21.4, CH_3	21.2, CH_3	20.7, CH_3

[a] Recorded at 150 MHz; [b] Recorded at 100 MHz

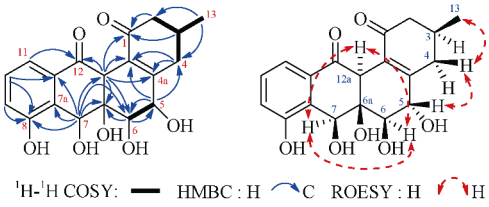

Fig. 3 Key 2D NMR correlations of 1

$C_{19}H_{18}O_5$, a chemical formula with an unsaturation index of 11, was assigned to cangumycin B (**2**) from its HRESI-MS data (*m/z* 349.1043 $[M + Na]^+$, Calcd. for 349.1046). The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **2** were closely resembling to those of elmycin A (**7**), except that a methylene (δ_C 39.8, C-5) and an oxygen-bearing methine (δ_C 67.7, C-6) in **7** were replaced by two newly formed olefinic methines (δ_C 129.4, 137.7) in **2**. Additionally, the chemical shifts of C-4a, C-6a, and C-12a in **2** were all upshifted when compared with those of **7**. Consequently, **2** was inferred to be different from **7** in ring B. ^{1}H-^{1}H COSY correlations between H-5 and H-6, and HMBC correlations of H-5/(C-4a, C-6a, C-12b) and H-6/(C-4a, C-6a, C-7, C-12a) verified the existence of a double bond at C-5 and C-6 in **2** (Fig. 4, left). The relative configurations at C-3, C-6a, C-7, and C-12a in **2** were inferred to be the same as in **7**, on the basis of the identical biosynthesis pathway, coexistence during the isolation, as well as the absence of ROESY interaction between H-7 and H-12a.

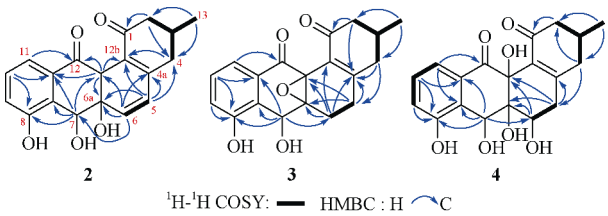

Fig. 4 Key 2D NMR correlations of 2–4

Cangumycin C (**3**) was also isolated as white amorphous powder. Its molecular formula was deduced to be $C_{19}H_{18}O_5$ based on the HRESI-MS data (*m/z* 325.1081 $[M - H]^-$, Calcd. for 325.1081), consistent with 11 degrees of unsaturation. The NMR data of **3** (Tables 1 and 2) and panglimycin F [11] were similar, except that two oxygenated sp^3 carbons (δ_C 77.3, C-12b and 77.1, C-4a) in panglimycin F were replaced by two quaternary olefinic carbons (δ_C 151.0 and 136.9) in **3**. HMBC correlations of (H-2i, H-4i, H-5ii, H-6ii)/C-4a and H-6i/C-12b in **3** clearly presented that a double bond was located at C-4a and C-12b (Fig. 4, middle). Likewise, from a biosynthetic view, the stereochemistry of C-3, C-7, and the epoxide moiety at C-6a and C-12a in **3** was all supposed to be *β*-oriented based on the stereochemistry of elmycin A (**7**).

The molecular formula of cangumycin D (**4**) was indicated as $C_{19}H_{20}O_7$ from its HRESI-MS data (*m/z* 383.1100 $[M + Na]^+$, Calcd. for 383.1101), with two hydrogen atoms and two oxygen atoms more than that of cangumycin C (**3**). A comparison of the ^{1}H and ^{13}C NMR data of **3** and **4** (Tables 1

and 2) found that a methylene (δ_C 29.2, C-6) in **3** was replaced by an oxygen-bearing sp^3 carbon (δ_C 67.6). Further detailed HMBC spectrum analysis restricted all the oxidation locus in **4** at C-1, C-6, C-6a, C-7, C-8, C-12, and C-12a (Fig. 4, right). And based on its chemical composition, the degrees of unsaturation, as well as the corresponding carbon chemical shifts, the ambiguous oxidation locus at C-6, C-6a, C-7, C-8 and C-12a in **4** should all connect with hydroxy groups, while C-1 and C-12 should be two carbonyls. The stereochemistry of C-3, C-6, C-6a and C-7 in **4** was suggested to be the same with elmycins A (**7**) and B (**8**) based on the same biosynthetic route, while the orientation of OH-12a was deduced as *α*, for a possibility that the vicinal hydroxy substituents at C-6a and C-12a may formed *via* a nucleophilic ring opening of a *β*-oriented epoxide moiety (like in compound **3**) caused by an attack of water to C-12a from the *α*-side.

The HRESI-MS analysis (*m/z* 325.1080 $[M - H]^-$, Calcd. for 325.1081) of cangumycin E (**5**) showed a molecular formula of $C_{19}H_{18}O_5$. According to the literature report [5], the ^1H and ^{13}C NMR data of **5** (Table 3) indicated that it has an angucyclinone backbone featuring C-ring cleavage. Detailed analyses of ^1H-^1H COSY and HMBC spectra confirmed the planar structure of **5** as shown in Fig. 5. The elemental composition of cangumycin F (**6**) was determined to be $C_{19}H_{18}O_6$ from its HRESI-MS data (*m/z* 365.0994 $[M + Na]^+$, Calcd. for 365.0996), with one more oxygen atom than that of **5**. The ^1H and ^{13}C NMR spectra of **6** (Table 3) closely resembled to those of **5**, except that the proton signal for H-6 in **6** was missing, and the carbon signal for C-6 in **6** was transformed into a quaternary one and severely downshifted. Therefore, a hydroxylation was supposed to take place at C-6 in **6**, on an occasion that the molecular weight variation (Δ 16) between **5** and **6** could be balanced. The relative configuration of C-3 in compounds **5** and **6** were assumed to be *β* due to a common biosynthetic origin of angucyclinones as reported [3, 5-6, 11-15].

All the isolates were submitted to an immunosuppressive screening using the method reported before [16]. As a result, only cangumycins B (**2**) and E (**5**) significantly inhibited human T cell proliferation activated by anti-CD3/anti-CD28 antibodies with IC_{50} values of 8.1 and 2.7 μmol·L^{-1}, respectively. In addition, the CC_{50} value of cangumycin B (**2**) to human resting naïve T cells was 34.2 μmol·L^{-1}, and when the same assay was treated with cangumycin E (**5**), the CC_{50} value turned out to be higher than 80 μmol·L^{-1}, suggesting cangumycins B (**2**) and E (**5**) inhibited T cell proliferation by immunosuppressive activity instead of cytotoxicity.

Experimental

General experimental procedures

X-ray data were collected using a Bruker APEX DUO diffractometer (Bruker Corpration, Germany). A Shimadzu UV-2700 spectrophotometer (Shimadzu Corpration, Japan) was used to acquire all the UV spectra. Optical rotations were recorded in MeOH using a Autopol IVS2&Plus polarimeter (Rudolf Group, Germany). IR spectra were measured on a

Table 3 ^1H (600 MHz) and ^{13}C NMR (150 MHz) data for compounds 5 and 6 in DMSO-d_6

Position	5		6	
	δ_H	δ_C	δ_H	δ_C
1		199.1, C		199.0, C
2-i	2.46 m	46.2, CH_2	2.34 m	45.9, CH_2
2-ii	2.24 m		2.11 [a]	
3	2.18 m	30.0, CH	2.11 [a]	30.2, CH
4-i	2.91 d (15.5)	36.5, CH_2	2.79 d (15.7)	37.0, CH_2
4-ii	2.61 dd (15.5, 10.3)		2.54 [a]	
4a		135.2, C		138.1, C
5	7.27 d (8.3)	130.5, CH	6.68 br s	114.3, CH
6	7.15 d (8.3)	121.8, CH		153.1, C
6a		153.0, C		141.9, C
7	4.98 s	56.8, CH_2	4.97 s	56.7, CH_2
7a		126.8, C		126.8, C
8		156.6, C		156.4, C
9	6.96 d (8.0)	119.4, CH	6.94 d (8.0)	119.2, CH
10	7.04 t (7.9)	127.5, CH	7.03 t (7.9)	127.4, CH
11	6.67 d (7.8)	121.5, CH	6.69 d (7.8)	121.6, CH
11a		138.4, C		138.6, C
12		197.8, C		195.6, C
12a		128.0, C		128.6, C
12b		130.3, C		120.8, C
13	1.02 d (6.3)	20.8, CH_3	0.99 d (5.5)	20.9, CH_3

[a] Overlapped signals

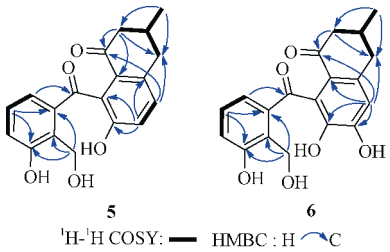

Fig. 5 Key 2D NMR correlations of 5 and 6

Nicolet™ iS™ 10 FT-IR spectrometer with KBr disks (Thermo Fisher Scientific Inc., United States). Two different Bruker spectrometers (AVANCE III-400 and AVANCE III-600, Bruker Corpration, Germany) were used for the NMR data collection, and all the NMR data were recorded in DMSO-d_6, using TMS as the internal standard. A Waters Xevo TQ-S Mass Spectrometer (Waters Corporation, United Kingdom) was used to acquire ESI-MS spectra, while HRESI-MS data collection was conducted on an Agilent G6230 Q-TOF Mass Spectrometer (Agilent Technologies Inc., United States). Silica gel (200–300 mesh) and Sephadex LH-20 (18−111 μm) were used in column chromatography (CC), while precoated silica gel GF_{254} plates (0.20−0.25 mm in thickness) were used for thin-layer chromatography (TLC) analyses. A HITACHI

Chromaster equipment (Hitachi Corpration, Japan) furnished with a DAD detector and an YMC-Triart C_{18} column (250 mm × 10 mm i.d., 5 μm) was used for semipreparative HPLC. All the HPLC analyses were performed at a flow rate of 3.0 mL·min^{-1} and a column temperature at 28 °C, and 0.1% (*V/V*) acetic acid was added to each HPLC mobile phase.

Strain material

Strain *Streptomyces* sp. KIB-M10 was isolated from a soil sample collected in Cangshan Mountain in Dali City, Yunnan Province, China. This strain was identified by Dr. YAN Yi-Jun, associate professor in State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences. Its *16S rRNA* gene sequence (Gen Bank No. MN173850) shows 99.86% identity to *Streptomyces narbonesis* strain F31 (Gen Bank No. KU324447.1).

Fermentation, extraction and isolation

Strain *Streptomyces* sp. KIB-M10 was grown on MS agar plates (soybean meal 20 g, yeast extract 4 g, and agar 20 g in 1 L water, pH 7.2) for 5 days at 30 °C. Then, it was inoculated into 250 mL baffle Erlenmeyer flasks containing 50 mL of sterile seed medium (Tryptone soy broth, 30g·L^{-1}) and cultivated for 36 hours at 30 °C on a rotary shaker (250 r·min^{-1}). After that, aliquots (12.5 mL) of the culture were transferred into 1000 mL baffled Erlenmeyer flasks filled with 250 mL of a production medium consisting of 4% dextrin, 4% lactose, 0.5% yeast extract, and 0.5% $CaCO_3$ (pH 7.2−7.3) and cultured on a rotary shaker (200 r·min^{-1}) at 30 °C for 10 days.

The supernatant of the fermentation broth (20 L) was extracted with EtOAc (10 L× 3 times). The mycelium was extracted with acetone and the extract was concentrated in vacuo, then the residual aqueous concentrate was extracted with EtOAc. Both organic phase were evaporated to remove the solvent and combined as a crude extract, which was then subjected on silica gel CC [petroleum ether/EtOAc (10 : 0, 10 : 1, 5 : 1, 2 : 1, 1 : 1, 0 : 1) and EtOAc /CH_3OH (2 : 1, 0 : 1)], and yielded eight fractions (A−H). Compound **1** (4.0 mg, 28.0% MeCN in H_2O, t_R = 10.4 min) and **6** (7.8 mg, 28.0% MeCN in H_2O, t_R = 26.2 min) were obtained from the fraction G (EtOAc/CH_3OH, 2 : 1) by semipreparative HPLC. Fraction E (petroleum ether/EtOAc, 1 : 1) was sequentially subjected to Sephadex LH-20 CC ($CHCl_3$/CH_3OH, 1 : 1) and semipreparative HPLC in sequence to afford compounds **3** (6.2 mg, 34.5% MeOH in H_2O, t_R = 26.5 min), **5** (4.0 mg, 27.0% MeOH in H_2O, t_R = 53.2 min) and **8** (67.5 mg, 34.5% MeOH in H_2O, t_R = 28.9 min). Fraction F (petroleum ether/EtOAc 0 : 1) was subjected to Sephadex LH-20 CC (CH_3OH) to generate seven subtractions, which were further purified by semipreparative HPLC to yield compounds **2** (14.4 mg, 14.0% MeCN in H_2O, t_R = 27.3 min), **4** (2.2 mg, 17.0% MeCN in H_2O, t_R = 24.6 min) and **7** (23.0 mg, 24.0% MeCN in H_2O, t_R = 11.2 min).

*Cangumycin A (**1**)*

Yellow powder; $[\alpha]_D^{21.9}$ +69.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 250 (5.02) nm; IR (KBr) ν_{max} 3416, 2956, 2926, 2872, 1663, 1590, 1466, 1384, 1292, 1092, 1025, 971, 800, 752 cm^{-1}; ^{1}H NMR (600 MHz) data see Table 1; ^{13}C NMR (150 MHz) data see Table 2; HRESI-MS *m/z* 359.1136 [M – H]$^-$ (Calcd. for $C_{19}H_{19}O_7$, 359.1136).

*Cangumycin B (**2**)*

Yellow powder; $[\alpha]_D^{21.0}$ +547.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 256 (5.32) nm; IR (KBr) ν_{max} 3388, 2955, 2925, 2872, 1682, 1639, 1591, 1466, 1383, 1291, 1076, 1023, 943, 862, 793, 751 cm^{-1}; ^{1}H NMR (600 MHz) data see Table 1; ^{13}C NMR (150 MHz) data see Table 2; HRESI-MS *m/z* 349.1043 [M + Na]$^+$ (Calcd. for $C_{19}H_{18}O_5Na$, 349.1046).

*Cangumycin C (**3**)*

Yellow powder; $[\alpha]_D^{21.9}$ +37.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 264 (4.89) nm; IR (KBr) ν_{max} 3419, 2955, 2926, 2871, 2853, 1694, 1667, 1602, 1581, 1462, 1297, 1024, 998, 782, 719 cm^{-1}; ^{1}H NMR (600 MHz) data see Table 1; ^{13}C NMR (150 MHz) data see Table 2; HRESI-MS *m/z* 325.1081 [M – H]$^-$ (Calcd. for $C_{19}H_{17}O_5$, 325.1081).

*Cangumycin D (**4**)*

Yellow powder; $[\alpha]_D^{20.8}$ +44.0 (*c* 0.1, MeOH); UV (MeOH) λmax (log ε) 261 (4.88) nm; IR (KBr) ν_{max} 3424, 2957, 2927, 2871, 1693, 1669, 1624, 1583, 1464, 1384, 1299, 1277, 1100, 1063, 1044, 936, 853, 756, 574 cm^{-1}; ^{1}H NMR (600 MHz) data see Table 1; ^{13}C NMR (150 MHz) data see Table 2; HRESI-MS *m/z* 383.1100 [M + Na]$^+$ (Calcd. for $C_{19}H_{20}O_7Na$, 383.1101).

*Cangumycin E (**5**)*

Light yellow gum; $[\alpha]_D^{21.4}$ –42.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 313 (3.48) nm; IR (KBr) ν_{max} 3430, 2955, 2926, 2871, 1664, 1585, 1460, 1382, 1340, 1299, 1217, 1002, 842, 743 cm^{-1}; ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data see Table 3; HRESI-MS *m/z* 325.1080 [M – H]$^-$ (Calcd. for $C_{19}H_{17}O_5$, 325.1081).

*Cangumycin F (**6**)*

Light yellow gum; $[\alpha]_D^{20.9}$ –29.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 320 (4.75) nm; IR (KBr) ν_{max} 3424, 2955, 2925, 2871, 1659, 1586, 1459, 1383, 1307, 1103, 997, 875, 797, 738 cm^{-1}; ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data see Table 3; HRESI-MS *m/z* 365.0994 [M + Na]$^+$ (Calcd. for $C_{19}H_{18}O_6Na$, 365.0996).

*Elmycin A (**7**)*

Yellow crystals (70% MeOH in H_2O); $[\alpha]_D^{21.4}$ +61.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 223 (5.29) nm; IR (KBr) ν_{max} 3416, 2954, 2924, 2872, 1682, 1651, 1639, 1590, 1467, 1385, 1291, 1091, 1075, 1004, 949, 861, 794, 760 cm^{-1}; ^{1}H NMR (400 MHz) data see Table 1; ^{13}C NMR (100 MHz) data see Table 2; HRESI-MS *m/z* 343.1186 [M – H]$^-$ (Calcd. for $C_{19}H_{19}O_6$, 343.1187).

Crystal data for 7

$C_{19}H_{20}O_6{\cdot}C_2H_6OS$, M = 422.48, *a* = 7.9318(4) Å, *b* = 9.3973(5) Å, *c* = 27.3222(14) Å, α = 90°, β = 90°, γ = 90°, *V* = 2036.53(18) Å^3, *T* = 100.(2) K, space group *P*212121, *Z* = 4, μ(Cu Kα) = 1.768 mm^{-1}, 17461 reflections measured, 3942

independent reflections (R_{int} = 0.0221). The final R_1 values were 0.0274 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0772 ($I > 2\sigma(I)$). The final R_1 values were 0.0275 (all data). The final $wR(F^2)$ values were 0.0773 (all data). The goodness of fit on F^2 was 1.076. Flack parameter = 0.033(3). The crystallographic data for **7** has been deposited with the Cambridge Crystallographic Data Centre (deposition No. CCDC 1940296).

*Elmycin B (**8**)*

Yellow crystals (90% MeOH in H_2O); $[\alpha]_D^{20.9}$ +17.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 221 (5.13) nm; IR (KBr) ν_{max} 3427, 2956, 2922, 2873, 2854, 1678, 1590, 1466, 1384, 1293, 1089, 1027, 928, 794 cm^{-1}; ^1H NMR (600 MHz) data see Table 1; ^{13}C NMR (150 MHz) data see Table 2.

*Crystal data for **8***

$C_{19}H_{18}O_6$, M = 342.33, a = 7.2068(7) Å, b = 10.9211(11) Å, c = 19.927(2) Å, α = 90°, β = 90°, γ = 90°, V = 1568.4(3) $Å^3$, T = 100.(2) K, space group $P212121$, Z = 4, μ(Cu Kα) = 0.904 mm^{-1}, 11081 reflections measured, 3080 independent reflections (R_{int} = 0.0341). The final R_1 values were 0.0276 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0707 ($I > 2\sigma(I)$). The final R_1 values were 0.0278 (all data). The final $wR(F^2)$ values were 0.0710 (all data). The goodness of fit on F^2 was 1.041. Flack parameter = 0.03(4). The crystallographic data for **8** has been deposited with the Cambridge Crystallographic Data Centre (deposition No. CCDC 1940302).

Immunosuppressive bioassay

The immunosuppressive bioassay was conducted using the method reported before [16].

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