JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Antimalarial β-Carboline and Indolactam Alkaloids from *Marinactinospora thermotolerans*, a Deep Sea Isolate

Hongbo Huang,[†,∥] Yueliang Yao,[†,∥] Zhengxiang He,[‡] Tingting Yang,[§] Junying Ma,[†] Xinpeng Tian,[†] Yayong Li,[‡] Caiguo Huang,[§] Xiaoping Chen,[‡] Wenjun Li,[⊥] Si Zhang,[†] Changsheng Zhang,[†] and Jianhua Ju[†,*]

[†]CAS Key Laboratory of Marine Bio-resources Sustainable Utilization, Guangdong Key Laboratory of Marine Materia Medica, RNAM Center for Marine Microbiology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Rd., Guangzhou 510301, People's Republic of China

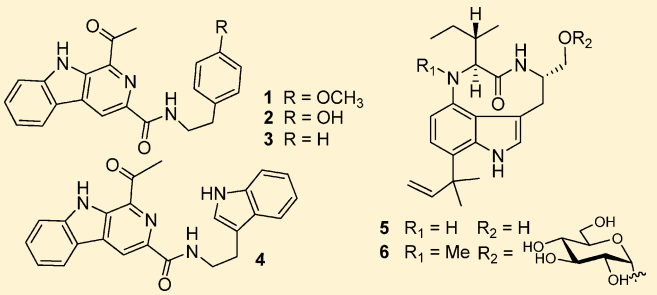
[‡]State Key Laboratory of Respiratory Disease, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, 190 Kaiyuan Ave., Guangzhou 510530, People's Republic of China

[§]Department of Biochemistry and Molecular Biology, The Second Military Medical University, 800 Xiangyin Road, Shanghai 200433, People's Republic of China

[⊥]Yunnan Institute of Microbiology, Yunnan University, 2 North Cuihu Road, Kunming 650091, People's Republic of China

Supporting Information

ABSTRACT: Four new β-carboline alkaloids, designated marinacarbolines A–D (**1**–**4**), two new indolactam alkaloids, 13-*N*-demethyl-methylpendolmycin (**5**) and methylpendolmycin-14-*O*-α-glucoside (**6**), and the three known compounds 1-acetyl-β-carboline (**7**), methylpendolmycin (**8**), and pendolmycin (**9**) were obtained from the fermentation broth of *Marinactinospora thermotolerans* SCSIO 00652, a new actinomycete belonging to the family *Nocardiopsaceae*. Their structures were elucidated by extensive MS and 1D and 2D NMR spectroscopic data analyses. The structure of compound **1** was further confirmed by single-crystal X-ray crystallography. The new compounds **1**–**6** were inactive against a panel of eight tumor cell lines (IC_{50} > 50 μM) but exhibited antiplasmodial activities against *Plasmodium falciparum* lines 3D7 and Dd2, with IC_{50} values ranging from 1.92 to 36.03 μM.



Actinomycetes are important economical microorganisms and play a leading role in the production of bioactive natural products.[1] In the past 10 years, new compounds originating from marine actinomycetes have surpassed the production by their terrestrial counterparts, and marine actinomycetes are considered an exciting new resource for drug discovery.[2,3] It has been demonstrated that new genera or species of marine actinomycetes are capable of producing novel chemotypes. For example, the antitumor antibiotic salinosporamides from the new genus *Salinispora*,[4] antibacterial abyssomicins from the new species *Verrucosispora* sp. AB-18-032,[5,6] and the antitumor and antibacterial marinomycins from the new genus *Marinispora*[7] are several structure classes with novel scaffolds. These discoveries inspired us to search for new actinomycetes from the South China Sea and to explore their novel, biologically active secondary metabolites. Recently, we reported two new genera and one new species of marine actinomycetes from the South China Sea marine sediments.[8−10] Among them, the actinomycete strain SCSIO 00652 was indentified to be a novel genus, designated *Marinactinospora thermotolerans*, belonging to the family *Nocardiopsaceae*.[8] This strain was isolated from a sediment sample collected at a depth of 3865 m. A chemical investigation of this strain resulted in the isolation of nine alkaloids, including four new β-carboline alkaloids, designated marinacarbolines A–D (**1**–**4**), two new indolactam alkaloids, 13-*N*-demethyl-methylpendolmycin (**5**) and methylpendolmycin-14-*O*-α-glucoside (**6**), and the three known analogues 1-acetyl-β-carboline (**7**), methylpendolmycin (**8**), and pendolmycin (**9**). In inhibition assays of in vitro growth of *Plasmodium falciparum*, compounds **1**–**6** exhibited antiplasmodial activity against drug-sensitive line 3D7 and drug-resistant line Dd2 of *P. falciparum*. In this paper, we report the

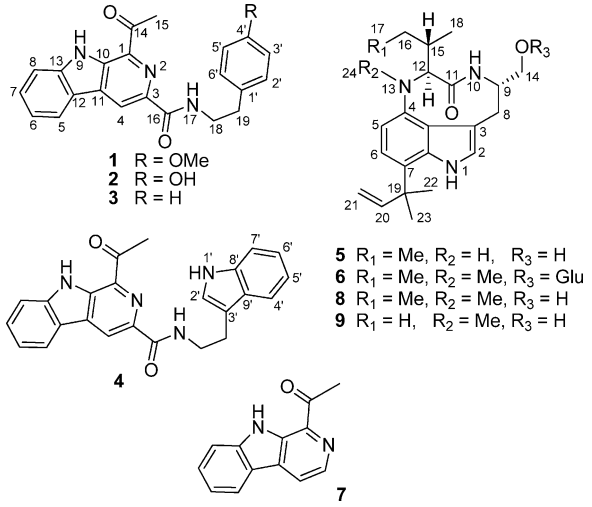
Received: May 11, 2011
Published: October 6, 2011

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Spectroscopic Data for Compounds 1−4

	1[a]		2[b]		3[a]		4[a]	
position	δ_C, type	δ_H, mult (J/Hz)	δ_C, type	δ_H, mult (J/Hz)	δ_C, type	δ_H, mult (J/Hz)	δ_C, type	δ_H, mult (J/Hz)
1	133.5, C		133.9, C		133.5, C		133.4, C	
3	138.8, C		138.8, C		139.2, C		138.7, C	
4	118.3, CH	9.00, s	117.8, CH	9.08, s	118.4, CH	9.09, s	118.6, CH	8.98, s
5	122.2, CH	8.17, d (8.0)	122.2, CH	8.45, d (8.0)	122.3, CH	8.22, d (7.5)	122.0, CH	8.16, d (8.0)
6	121.5, CH	7.33, dt (8.0, 3.0)	120.7, CH	7.34, t (8.0)	121.5, CH	7.38, t (7.5)	121.3, CH	7.31, dt (8.0, 2.5)
7	129.7, CH	7.57, m	129.2, CH	7.62, t (8.0)	129.7, CH	7.63, t (7.5)	129.6, CH	7.56, m
8	112.2, CH	7.57, m	113.2, CH	7.83, d (8.0)	112.2, CH	7.59, d (7.5)	112.2, CH	7.56, m
10	136.0, C		134.7, C		136.3, C		136.5, C	
11	132.6, C		131.8, C		132.7, C		132.6, C	
12	120.9, C		120.2, C		121.1, C		120.8, C	
13	141.6, C		142.3, C		141.6, C		141.6, C	
14	202.2, C		202.2, C		202.3, C		202.3, C	
15	25.6, CH_3	2.71, s	25.9, CH_3	2.87, s	25.7, CH_3	2.75, s	25.4, CH_3	2.75, s
16	164.9, C		163.7, C		164.6, C		164.9, C	
17		8.08, t (5.5)		8.31, s		8.07, t (5.5)		8.18, t (5.5)
18	40.7, CH_2	3.76, q (7.0)	40.7, CH_2	3.57, t (7.0)	40.7, CH_2	3.84, q (7.0)	39.7, CH_2	3.84, q (6.5)
19	34.8, CH_2	2.91, t (7.0)	34.5, CH_2	2.80, t (7.0)	34.8, CH_2	2.91, t (7.0)	25.2, CH_2	3.11, t (6.5)
1′	130.9, C		129.4, C		130.9, C			
2′	129.9, CH	6.86, d (8.5)	129.5, CH	6.71, d (8.0)	129.0, CH	7.34, m	122.0, CH	7.11, s
3′	114.2, CH	7.21, d (8.5)	115.2, CH	7.11, d (8.0)	128.8, CH	7.34, m	112.2, C	
4′	158.7, C		155.6, C		126.7, CH	7.28, t (7.0)	118.1, CH	7.60, d (8.0)
5′	114.2, CH	7.21, d (8.5)	115.2, CH	7.11, d (8.0)	128.8, CH	7.34, m	119.1, CH	7.01, t (8.0)
6′	129.9, CH	6.86, d (8.5)	129.5, CH	6.71, d (8.0)	129.0, CH	7.34, m	121.8, CH	7.11, t (8.5)
7′							111.3, CH	7.35, d (8.5)
8′							136.2, C	
9′							127.2, C	
4′-OMe	55.3, CH_3	3.76, s						
4′−OH				9.21, br s				

[a]Recorded in $CDCl_3$. [b]Recorded in DMSO-d_6.

fermentation, isolation, structure elucidation, cytotoxicities, and antiplasmodial activities of these compounds.



RESULTS AND DISCUSSION

Since *M. thermotolerans* SCSIO 00652 is a novel genus isolated from a deep sea sample, chemical screening was adopted to explore its metabolite profile. The strain was initially fermented using five media,[11] and the fermentation broth from each was extracted with butanone and analyzed by HPLC-(DAD)-UV. On the basis of the initial results, we further optimized the fermentation conditions to using modified-ISP4 medium and analyzed its butanone extract by HPLC-UV. Comparisons of the HPLC metabolite profiles of the organic extracts from the different fermentation conditions revealed that using modified-ISP4 medium yielded the most abundant secondary metabolites. Two sets of metabolites with characteristic UV absorption bands were observed upon HPLC-(DAD)-UV analysis. Large-scale fermentation (8 L) and subsequent HPLC-UV-guided fractionation led to the purification of six new (**1**−**6**) and three known compounds (**7**−**9**), belonging to *β*-carboline and indolactam alkaloids, respectively. The three known compounds 1-acetyl-*β*-carboline (**7**),[12] methylpendolmycin (**8**),[13] and pendolmycin (**9**)[14] were identified by interpreting their MS and ^{1}H and ^{13}C NMR spectroscopic data and comparing them with those reported in the literature.

Marinacarboline A (**1**) was isolated as pale yellow crystals. Its molecular formula was determined as $C_{23}H_{21}N_3O_3$ by HRESIMS, requiring 15 degrees of unsaturation. The UV spectrum of **1** exhibited characteristic absorption bands at 216, 298, and 375 nm, diagnostic of a *β*-carboline chromophore.[15,16] The ^{1}H, ^{13}C, and DEPT NMR spectroscopic data of **1** (Table 1) displayed signals assignable to one methyl (C-15), one methoxy, two methylenes (C-18, C-19), nine aromatic methines, eight unsaturated quaternary carbons, and two carbonyls (C-14, C-16). The $^{1}H-^{1}H$ COSY and HSQC NMR experiments suggested the presence of 1,2-disubstituted and 1,4-disubstituted benzenes (C-5−C-8, C-2′(6′)−C-3′(5′)) and an aliphatic fragment (C-18−C-19) (Figure 1). The methyl singlet at δ_H 2.71 (H_3-15) showed HMBC correlation to the carbonyl at δ_C 202.2 (C-14), suggesting the presence of an

dx.doi.org/10.1021/np200399t|J. Nat. Prod. 2011, 74, 2122−2127

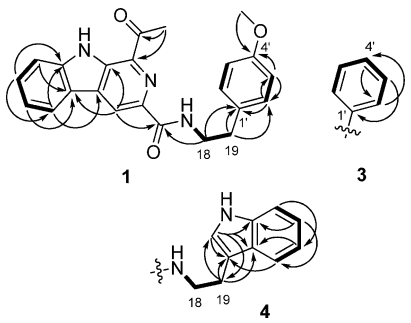


Figure 1. ^{1}H–^{1}H COSY (bold) and selected HMBC correlations (arrow) of **1**, **3**, and **4**.

acetyl group. Additional HMBC correlation of H_3-15/C-1 located the acetyl group at C-1 to form the 1-acetyl-β-carboline moiety in **1**. In the ^{1}H NMR spectrum of **1**, a downfield singlet was readily discernible at δ 9.0, indicating the proton was localized at H-4, corresponding to the peri position of the β-carboline skeleton. The HMBC correlations from H-4 to C-10 and C-11 supported this assumption. The singlet H-4 also showed HMBC correlation to a carbonyl at δ 164.9 (C-16), suggesting C-16 was connected to C-3. Therefore, a 1,3-disubsituted β-carboline core structure was established. One para-substituted benzene moiety was inferred upon considering the presence of two pairs of ortho-coupled aromatic protons at δ 7.21 (2H, d, J = 8.5 Hz, H-3′, H-5′) and δ 6.86 (2H, d, J = 8.5 Hz, H-2′, H-6′). The methoxy group at δ 3.76 was placed at the para-substituted aromatic ring, as judged by its HMBC correlation with C-4′. The C-18–C-19 fragment was deduced to be linked at C-1′ according to the HMBC correlations of H-18/C-1′,H-19/C-1′,2′(6′), and H-2′(6′)/C-19. Considering the molecular formula of **1**, as well as the chemical shifts of the C-16 (δ 164.9) and C-18 (δ 40.7) in **1**, we postulated that an amide bond was present between C-16 and C-18. The HMBC correlation from H-18 to C-16 confirmed the amide linkage. Hence, the structure of marinacarboline A was constructed. An analysis of the single-crystal X-ray diffraction data of **1** unambiguously confirmed this structure (Figure 2).

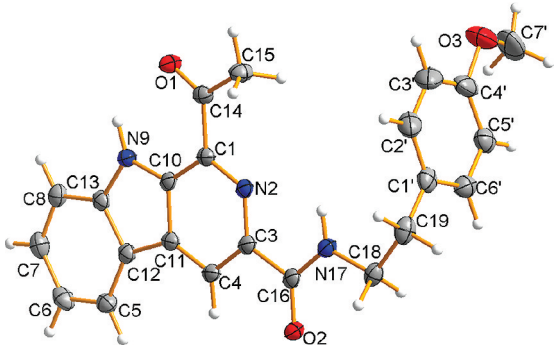


Figure 2. ORTEP structure of **1**.

Marinacarboline B (**2**) was obtained as a yellowish powder. It exhibited a prominent quasimolecular peak at m/z 372.1350 $[M - H]^-$ in HRESIMS, indicating the molecular formula $C_{22}H_{19}N_3O_3$, which is 14 mass units smaller than that of **1** and corresponds to the loss of a methyl group. The UV, ^{1}H, and ^{13}C NMR spectra of **2** strongly resembled those of **1** (Table 1), except that the signal for the methoxy group in **1** was missing and, in turn, a hydroxyl proton signal was observed at δ 9.21 in **2**. These changes suggested a hydroxyl group instead of a methoxy group was attached at C-4′ in **2**. Therefore, compound **2** was identified to be 4′-*O*-desmethyl marinacarboline A.

Marinacarboline C (**3**) had a molecular formula of $C_{22}H_{19}N_3O_2$, as measured by HRESIMS, which was 16 mass units less than that of **2**. The UV, ^{1}H, and ^{13}C NMR spectra of **3** and **2** were also very similar. Careful examinations of the NMR data of **3** and **2** revealed the signals for C-4′ OH as well as the oxygen-bearing aromatic quaternary carbon at C-4′ (δ 155.6) in **2** were missing; instead, an additional aromatic methine signal at C-4′ was present(δ_H 7.28 (t, J = 7.8 Hz), δ_C 126.7). The ^{1}H–^{1}H COSY spectrum of **3** revealed a spin system of five aromatic protons (H-2′ to H-6′, Figure 1), indicating that the 4′-OH-substituted benzene moiety in **2** was replaced by a phenyl moiety in **3**. The HMBC correlations of H-2′(6′)/C-4′, H-3′(5′)/C-1′, and H-4′/C-2′(6′) substantiated the above conclusion. Thus, compound **3** was established as 4′-deshydroxy-marinacarboline B.

Marinacarboline D (**4**) was also obtained as a yellowish powder. Its molecular formula was established by HRESIMS analysis as $C_{24}H_{20}N_4O_2$, suggesting 17 degrees of unsaturation. Compound **4** had the same core structure of 1-acetyl β-carboline moiety as found in compounds **1**–**3**, which was inferred by comparing the ^{1}H and ^{13}C NMR spectroscopic data of **4** (Table 1) with those of **1**–**3**. The presence of this moiety was further supported by analyses of the ^{1}H–^{1}H COSY, HSQC, and HMBC NMR data of **4**. Detailed analyses of the remaining ^{1}H, ^{13}C, and 2D NMR spectroscopic data of **4** disclosed that **4** possess another tryptamine moiety in the molecule. The ^{1}H–^{1}H COSY correlations of H-4′/H-5′/H-6′/H-7′ confirmed a 1,2-disubstituted benzene in the tryptamine substructure. The H-2′ at δ 7.11 showed HMBC correlations to C-3′, C-8′, and C-9′. The important HMBC correlations of H-18/C-3′, H-19/C-3′,C-2′,C-9′, H-2′/C-19, and H-18/C-16 not only confirmed the presence of a tryptamine moiety but also connected C-18 with C-16 through an amide linkage (Figure 1). Hence, the structure of **4** was determined.

Compound **5** was isolated as a gray-brown solid. Its molecular formula was determined as $C_{22}H_{31}N_3O_2$ by the HRESIMS showing a quasimolecular peak at m/z 392.2303 $[M + Na]^+$, indicating nine degrees of unsaturation. Compound **5** had an indolactam skeleton, as deduced by the close similarities of its ^{1}H and ^{13}C NMR spectroscopic data with those of methylpendolmycin.[13] 1D and 2D (COSY, HSQC, and HMBC) NMR spectra of **5** were acquired, which allowed the full assignments of its ^{1}H and ^{13}C signals (Table 2). The ^{1}H NMR spectrum of **5** showed two ortho-coupled protons at δ_H 6.70 (H-5) and 6.99 (H-6), which was supported by a ^{1}H–^{1}H COSY correlation, and one isolated aromatic proton at δ_H 6.96 assignable to H-2 in the indole moiety. The HMBC correlations from H-2 to C-3 and C-7a, from H-5 to C-7, and from H-6 to C-4 and C-7a led to the assignment of the remaining ^{13}C NMR signals in the indole moiety. The two terminal olefinic protons at δ_H 5.12 (d, J = 17.5 Hz, H-21$_a$) and 5.11 (d, J = 10.5 Hz, H-21$_b$) were found coupled with a third olefinic proton at δ_H 6.11 (dd, J = 17.5, 10.5 Hz, H-20). This end olefin was deduced to be linked to a propane unit to form 3-substituted 3-methylbutenyl on the basis of the HMBC correlations from H_3-22 and H-23 to C-20 and from H-21 and H-20 to C-19 (Figure 3). Further HMBC correlations from H-20, H_3-22, and H_3-23 to C-7 and from H-6 to C-19 localized the 3-substituted 3-methylbutenyl at C-7 of the indole moiety. Analysis of DEPT and ^{1}H–^{1}H COSY spectra of **5** revealed an

dx.doi.org/10.1021/np200399t|*J. Nat. Prod.* 2011, 74, 2122–2127

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Spectroscopic Data for Compounds 5 and 6

	5[a]		6[b]	
position	δ_C, type	δ_H, mult (J/Hz)	δ_C, type	δ_H, mult (J/Hz)
2	124.6, CH	6.96, s	121.4, CH	6.75, s
3	111.7, C		113.5, C	
3a	114.1, C		119.2, C	
4	142.3, C		146.3, C	
5	115.8, CH	6.70, d (8.0)	106.3, CH	6.42, d (8.0)
6	120.1, CH	6.99, d (8.0)	118.6, CH	6.96, d (8.0)
7	128.0, C		122.9, C	
7a	137.7, C		137.4, C	
8	30.7, CH_2	3.18, dd (15.0, 7.0)	34.3, CH_2	3.13, br d (15.0)
		2.97, dd (15.0, 4.5)		2.96, br d (15.0)
9	54.6, CH	5.20, m	53.3, CH	4.31, m
11	177.7, C		174.5, C	
12	74.0, CH	3.61, d (10.0)	69.5, CH	4.40, d (9.5)
14	66.2, CH_2	3.72, dd (11.0, 4,5)	69.8, CH_2	3.55, dd (11.0, 4,5)
		3.64, dd (11.0, 7.0)		3.45, br d (11.0)
15	38.2, CH	2.14, m	34.3, CH	2.34, m
16	26.5, CH_2	1.95, m	24.5, CH_2	1.34, m
				0.59, m
17	10.6, CH_3	1.00, t (7.4)	10.3, CH_3	0.55, t (6.0)
18	16.4, CH_3	1.04, d (6.7)	17.3, CH_3	0.83, d (6.5)
19	41.4, C		40.1, C	
20	148.9, CH	6.11, dd (17.5, 10.5)	149.4, CH	6.14, dd (17.5, 10.5)
21	112.2, CH_2	5.12, d (17.5)	111.4, CH_2	5.27, d (17.5)
		5.11, d (10.5)		5.17, d (10.5)
22	28.0, CH_3	1.44, s	26.9, CH_3	1.45, s
23	28.0, CH_3	1.49, s	27.3, CH_3	1.48, s
24			33.2, CH_3	2.84, s
1′			100.4, CH	4.82, br s
2′			70.5, CH	3.45, br s
3′			71.0, CH	3.95, br s
4′			73.0, CH	3.54, br s
5′			75.4, CH	3.05, m
6′			61.0, CH_2	3.69, m; 3.92, m

[a]Recorded in CD_3OD. [b]Recorded in $CDCl_3$.

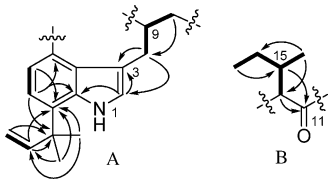


Figure 3. ^{1}H–^{1}H COSY (bold) and selected HMBC correlations (arrow) of **5**.

aliphatic chain (C-8/C-9/C-14) consisting of three methines, two of which were inferred to be nitrogen- and oxygen-bearing methines in light of their ^{13}C NMR chemical shifts at δ_C 54.6 (C-9) and δ_H 66.2 (C-14). This aliphatic chain was placed at C-3 of the indole moiety according to the HMBC correlations from H-8 to C-3 and C-2. Thus, compound **5** was deduced to have a fragment A substructure as shown in Figure 3. Two ^{1}H–^{1}H spin systems of H-12/H-15 and H-18/H-15/H-16/H-17 in the COSY spectrum of **5** suggested the presence of a substituted isobutyl group. A carbonyl (C-11) was connected to the isobutyl at C-12, as supported by the HMBC correlations from H-15 to C-11 and from H-12 to C-11. Thus, fragment B in **5** was constructed as shown in Figure 3. Taking the molecular formula and degrees of unsaturation into consideration, fragments A and B were connected through two imino groups, establishing the planar structure of **5**. The absolute configuration of **5** was determined to be 9*S*,12*S*,15*S* by comparing its CD spectrum (Figure S27, Supporting Information) with those of the known compounds methylpendolmycin,[13] lyngbyatoxin A,[17,18] and (−)-indolactam I, whose structures were confirmed by chemical synthesis and biosynthesis.[19] Therefore, compound **5** was determined to be 13-*N*-demethyl-methylpendolmycin.

Compound **6** had a molecular formula of $C_{29}H_{43}N_3O_7$, as determined by HRESIMS showing a quasimolecular peak at m/z 568.3029 $[M + Na]^+$. The ^{1}H and ^{13}C NMR spectroscopic data of **6** implied that **6** was a glycoside of 16-methylpendolmycin.[13] A set of 2D NMR (COSY, HMQC and HMBC) spectra of **6** was acquired, resulting in the full assignments of its ^{1}H and ^{13}C signals. Detailed analyses of the 1D and 2D NMR data of **6** demonstrated that **6** had signals attributable to the methylpendolmycin aglycon moiety. In addition, compound **6** displayed ^{1}H and ^{13}C NMR signals for a sugar moiety. This sugar was deduced as an α-linked glucose on the basis of the anomeric methine at δ_H 4.82 (br s) and δ_C 100.4 (C-1′), four oxygen-bearing methines at δ_C 75.4 (C-3′), 73.0 (C-5′), 71.0 (C-2′), and 70.5 (C-4′), and an oxygen-bearing methylene at δ_C 61.0 (C-6′). When the ^{13}C NMR spectroscopic data were compared with those for methylpendolmycin (**8**, Table S4, Supporting Information), the glycosylation shifts were observed at C-14 ($\Delta\delta_C$ +4.8) and C-9 ($\Delta\delta_C$ −2.6) in the aglycone moiety, which confirmed that the α-glucose was linked at C-14 of the aglycone. Therefore, compound **6** was determined to be methylpendolmycin-14-*O*-α-glucoside.

The β-carboline alkaloids have been found in plants, marine invertebrates, and animals but extremely rarely in bacteria and display a large variety of physiological and pharmacological properties, such as interaction with DNA and with 5-hydroxytryptamine and dopamine receptors and antitumor, antimicrobial, antiviral, and antiparasitic activities.[20] Notably, the antimalarial activity of such alkaloids has attracted attention. For example, manzamine A had been reported to exhibit in vivo antimalarial activity in mice.[21] Of particular interest is that a structurally closely related synthetic β-carboline analogue, spiroindolone, has recently been reported to exhibit antimalarial activity at nanomolar concentrations.[22] The nine-membered indolactam alkaloids belong to a small group of alkaloids found in cyanobacteria[17,23] and actinobacteria,[24,25] which displayed toxicity to fish,[23] protein kinase C activating property, and tumor-promoting activity.[26,27]

We evaluated the cytotoxicities of compounds **1**–**9** against a panel of human tumor cell lines using a published method,[28] revealing that these compounds were not significantly cytotoxic (IC_{50} > 50 μM; for details see Table S1 in the Supporting Information). Compounds **1**–**6** were tested for their antiplasmodial activities against *Plasmodium falciparum* line 3D7, a drug-sensitive strain, and Dd2, a multi-drug-resistant strain, using a previously described method.[29] The results of the antiplasmodial assays are given in Table 3. Notably, marinacarboline A (**1**) and compound **6** inhibited *P. falciparum* line Dd2 with IC_{50} values of 1.92 and 5.03 μM, respectively, and marinacarbolines C (**3**) and D (**4**) inhibited *P. falciparum* lines 3D7 and Dd2 with IC_{50} values between 3.09 and 5.39 μM.

dx.doi.org/10.1021/np200399t|*J. Nat. Prod.* 2011, 74, 2122–2127

Table 3. Antiplasmodial Activities of Compounds 1−6 against *P. falciparum* Lines 3D7 and Dd2 (μM)

	3D7[a]	Dd2[b]
1	36.03 ± 15.68	1.92 ± 1.03
2	16.65 ± 7.47	15.59 ± 6.41
3	3.09 ± 2.86	3.38 ± 2.85
4	5.39 ± 1.68	3.59 ± 1.20
5	20.75 ± 2.92	18.67 ± 1.58
6	10.43 ± 1.32	5.03 ± 1.57
chloroquine[c]	0.0128 ± 0.0027	0.0974 ± 0.0059

[a]Drug-sensitive line. [b]Multi-drug-resistant line. [c]Positive control.

These results further support that these β-carboline and indolactam alkaloids could serve as leads in the development of new antimalarial drugs.

EXPERIMENTAL SECTION

General Experimental Procedures. NMR spectra were recorded on an Avance 500 spectrometer (Bruker) at 500 MHz for ^{1}H nuclei and 125 MHz for ^{13}C nuclei. Chemical shifts (δ) in MeOH-d_4 are referenced to residual solvent signals (δ_H 3.35 and δ_C 49.23), and other chemical shifts (δ) in $CDCl_3$ and DMSO-d_6 are given with reference to TMS. Coupling constants (J) are given in Hz. ESIMS spectra were detected on an Esquire 3000^{plus} spectrometer (Bruker). HRESIMS spectra were recorded on a Q-TOF-Micromass spectrometer (Waters) and a microTOF-QII mass spectrometer (Bruker). Column chromatography (CC) was performed on silica gel (200−300 mesh, Qingdao Marine Chemicals) and Sephadex LH-20 (Amersham Pharmacia). Semipreparative HPLC was operated on a 210 solvent delivery module with a 335 PDA detector (Varian) using an YMC-Pack ODS-A column (250 × 10 mm, 5 μm). Single-crystal data were measured on a Smart-1000 CCD diffractometer (Bruker) using Mo Kα radiation.

Bacterial Material. The actinomycete strain SCSIO 00652 was isolated from a marine sediment sample collected at a depth of 3865 m in the northern South China Sea, People's Republic of China. It was identified as a new genus and species, *Marinactinospora thermotolerans*, of the family *Nocardiopsaceae*.[8] A voucher strain of this actinomycete has been preserved at the RNAM Center for Marine Microbiology, South China Sea Institute of Oceanology, Chinese Academy of Sciences.

Fermentation and Extraction. *M. thermotolerans* SCSIO 00652 was grown on modified ISP4 medium agar plates consisting of 1.5% agar, 1% starch, 0.1% K_2HPO_4, 0.1% $MgSO_4{\cdot}7H_2O$, 0.1% peptone, 0.05% yeast extract, 0.2% $(NH_4)_2SO_4$, 0.2% $CaCO_3$, 3% marine salt, and trace element solution (pH 7.0). A spore suspension and mycelium was inoculated into each of the 250 mL Erlenmeyer flasks containing 50 mL of liquid modified ISP-4 medium. The flasks were incubated at 28 °C on a rotary shaker (200 rpm) for 2 days. Each seed culture (50 mL) was transferred into a 1000 mL Erlenmeyer flask containing 200 mL of modified ISP-4 medium. The flasks were incubated on a rotary shaker (200 rpm) at 28 °C for 9 days. After fermentation, the culture (8 L) was centrifuged to yield supernatant and a mycelium cake. The supernatant was extracted with an equal volume of butanone three times and evaporated to dryness. The mycelium cake was extracted three times with 2 L of acetone and the organic phase evaporated to dryness. The two organic extracts were combined to give 3.1 g of residue.

Isolation. The crude extract was subjected to silica gel CC using gradient elution with a petroleum ether and EtOAc mixture from 90/10 to 0/100 (v/v) to give nine fractions (Fr.1−Fr.9). Fr.2, which was eluted by petroleum ether/EtOAc (50/50), was purified repeatedly by silica gel CC and eluted with $CHCl_3$/MeOH (98/2 → 85/15) to obtain **8** (52 mg), **9** (6 mg), and Fr.2-3. Fr.2-3 was purified by semipreparative HPLC with an ODS column (YMC, 250 × 10 mm, 5 μm) to give **3** (8 mg), **4** (12 mg), and **1** (10 mg). The solvent system consisted of solvent A (0.1% HOAc/15% CH_3CN in H_2O) and solvent B (0.1% HOAc/80% CH_3CN in H_2O). Compounds were eluted at 2.5 mL/min with a linear gradient from 20% to 60% solvent B over the course of 12 min, followed by holding at 60% solvent B for 12 min, and then eluted with 100% solvent B for 10 min; UV detection was at 280 nm. Fr.3, eluted with petroleum ether/EtOAc (10/90), was subjected to silica gel CC with $CHCl_3$/MeOH (95/5 → 85/15) as eluent to give **2** (6 mg), **6** (7 mg), and Fr.3-3. Fr.3-3 was further purified by semipreparative HPLC to give **5** (4 mg) under the same chromatography conditions as described above. Fr.4, eluted with petroleum ether/EtOAc (80/20), was chromatographed on silica gel with petroleum ether/EtOAc (80/20 → 30/70) as eluent to give **7** (15 mg).

Compound ***1****:* yellowish needles; UV (PDA) λ_{max} 216, 298, 375 nm; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; (−)-ESIMS m/z 386.0 $[M - H]^-$; (−)-HRESIMS m/z 386.1519 $[M - H]^-$ (calcd for $C_{23}H_{20}N_3O_3$ 386.1510).

Compound ***2****:* yellowish solid; UV (PDA) λ_{max} 221, 285, 374 nm; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; (−)-ESIMS m/z 372.1 $[M - H]^-$; (−)-HRESIMS m/z 372.1350 $[M - H]^-$ (calcd for $C_{22}H_{18}N_3O_3$ 372.1354).

Compound ***3****:* yellowish solid; UV (PDA) λ_{max} 218, 282, 373 nm; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; (−)-ESIMS m/z 356.2 $[M - H]^-$; (−)-HRESIMS m/z 356.1421 $[M - H]^-$ (calcd for $C_{22}H_{18}N_3O_2$ 356.1405).

Compound ***4****:* yellowish solid; UV (PDA) λ_{max} 219, 282, 375 nm; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; (−)-ESIMS m/z 395.1 $[M - H]^-$; (−)-HRESIMS m/z 395.1524 $[M - H]^-$ (calcd for $C_{24}H_{19}N_4O_2$ 395.1513).

Compound ***5****:* gray brown solid; $[\alpha]_D^{25} = -45°$ (c 0.33, MeOH); UV (PDA) λ_{max} 228, 285 nm; ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; (+)-ESIMS m/z 369.7 $[M + H]^+$, 391.9 $[M + Na]^+$, 407.7 $[M + K]^+$; (+)-HRESIMS m/z 392.2303 $[M + Na]^+$ (calcd for $C_{22}H_{31}N_3O_2Na$ 392.2308)

Compound ***6****:* brown solid; $[\alpha]_D^{25} = -67°$ (c 0.12, $CHCl_3$); UV (PDA) λ_{max} 224, 302 nm; ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; (+)-ESIMS m/z 383.5 $[M - 162 + H]^+$, 545.8 $[M + H]^+$, 567.9 $[M + Na]^+$; (+)-HRESIMS m/z 568.3029 $[M + Na]^+$ (calcd for $C_{29}H_{43}N_3O_7Na$ 568.2993).

X-ray Crystallographic Analysis of 1. A yellowish crystal of **1** was obtained in $CHCl_3$/MeOH (3/1). The crystal data of **1** were recorded on a Bruker Smart 1000 CCD single-crystal diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å). The structure was solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques.[30] Crystallographic data for **1** have been deposited in the Cambridge Crystallographic Data Center with the deposition number CCDC 823830. A copy of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax, +44(0)-1233-336033; e-mail, deposit@ccdc.cam.ac.uk).

Crystal data: monoclinic, $C_{23}H_{21}N_3O_3$, space group $P2_1/c$, a = 16.0472(10) Å, b = 8.5891(5) Å, c = 15.6785(9) Å, α = 90°, β = 116.5630(10)°, γ = 90°, V = 1932.9(2) Å^3, Z = 4, D_{calcd} = 1.331 Mg/m^3, μ = 0.090 mm^{-1}, $F(000)$ = 816, crystal size 0.45 × 0.43 × 0.28 mm^3, 4188 independent reflections (R_{int} = 0.0243). The final indices were R1 = 0.0431, wR2 = 0.1061 ($I > 2\sigma(I)$).

Cytotoxicity Assay. This experiment was performed using a published protocol.[28] The cell growth inhibitory activities of compounds **1**−**9** were determined against MCF-7 (breast cancer), SW1990 (pancreatic cancer), SMMC-7721 (liver cancer), NCI-H460 (lung cancer), A549 (lung cancer), HeLa (cervical cancer), DU 145 (prostate cancer), and MDA-MB-231 (breast cancer) cell lines using the MTT method. The cancer cell lines were cultured in RPMI-1640 supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 at 37 °C. In brief, 100 μL portions of cell suspensions were plated in 96-well plates to a final concentration of 2 × 10^3 cells per well and incubated for 12 h. A 50 μL amount of the test compound solution (in DMSO) at various concentrations was added to each well. After exposure to ophiobolin O for 48 h, 50 μL of MTT solution (1 mg/mL in PBS) was added to each well, and the plates were incubated for 4 h at 37 °C. Then, 200 μL of DMSO was added to each well. The absorbance caused by formazan crystallization

was read at 550 nm using a microplate reader (Model 550, Bio-Rad). The calculation of cell viability used the following formula: cell viability (%) = (average $A_{550\ nm}$ of the treated group)/(average $A_{550\ nm}$ of the untreated group) × 100.

Antiplasmodial Activity Assay. This experiment was conducted according to a recently described protocol.[29] Briefly, compound solutions were serially diluted and administered in quadruplicate to parasite cultures in 96-well plates to achieve 0.2% parasitemia with a 2% hematocrit. The plates were then incubated for 72 h at 37 °C. Following incubation, 100 μL of lysis buffer containing 0.2 μL/mL SYBR Green I was added to each well. The plates were incubated for 1 h in the dark, and a 96-well fluorescence plate reader (Multilabel HTS Counter, PerkinElmer) was used to measure relative fluorescence. The 50% inhibitory concentration (IC_{50}) was determined using a nonlinear regression analysis of the logistic dose response curves using the software GraphPad Prism (GraphPad Software Inc., La Jolla, CA).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures giving 1D and 2D NMR spectra of compounds **1**–**6**, a CIF file giving crystallographic data for **1**, and figures giving CD spectra of **5** and **6** and ^{1}H and ^{13}C NMR spectroscopic data of compounds **7**–**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel/fax: +86-20-89023028. E-mail: jju@scsio.ac.cn.

Author Contributions

‖These authors contributed equally to this work.

■ ACKNOWLEDGMENTS

We thank the analytical facility center of the South China Sea Institute of Oceanology for recording NMR data. This work was supported in part by grants from the Knowledge Innovation Programs of the Chinese Academy of Sciences (KSCX2-YW-G-065, KZCX2-YW-JC202, LYQY200805, SQ201015, and KZCX2-EW-G-12), the National Natural Science Foundation of China for Young Scholars (31000051), the Science and Technology Planning Project of Guangdong Province (2010B030600010), the National Basic Research Program of China (2010CB833805), and the Scientific Research Foundation for the Returned Overseas Chinese Scholars of the State Education Ministry. J.J. is a scholar of the "100 Talents Project" of the Chinese Academy of Sciences (08SL111001).

■ REFERENCES

(1) Berdy, J. *J. Antibiot.* **2005**, *58*, 1–26.
(2) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666–673.
(3) Lam, K. S. *Curr. Opin. Microbiol.* **2006**, *9*, 245–251.
(4) Williams, P. G.; Buchanan, G. O.; Feling, R. H.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *J. Org. Chem.* **2005**, *70*, 6196–6203.
(5) Riedlinger, J.; Reicke, A.; Zähner, H.; Krismer, B.; Bull, A. T.; Maldonado, L. A.; Ward, A. C.; Goodfellow, M.; Bister, B.; Bischoff, D.; Süssmuth, R. D.; Fiedler, H. P. *J. Antibiot.* **2004**, *57*, 271–279.
(6) Bister, B.; Bischoff, D.; Ströbele, M.; Riedlinger, J.; Reicke, A.; Wolter, F.; Bull, A. T.; Zähner, H.; Fiedler, H.-P.; Süssmuth, R. D. *Angew. Chem., Int. Ed.* **2004**, *43*, 2574–2576.
(7) Kwon, H. C.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *J. Am. Chem. Soc.* **2006**, *128*, 1622–1632.
(8) Tian, X. P.; Tang, S. K.; Dong, J. D.; Zhang, Y. Q.; Xu, L. H.; Zhang, S.; Li, W. J. *Int. J. Syst. Evol. Microbiol.* **2009**, *59*, 948–952.
(9) Tian, X. P.; Zhi, X. Y.; Qiu, Y. Q.; Zhang, Y. Q.; Tang, S. K.; Xu, L. H.; Zhang, S.; Li, W. J. *Int. J. Syst. Evol. Microbiol.* **2009**, *59*, 222–228.
(10) Tian, X. P.; Zhang, Y. Q.; Li, Q. X.; Zhi, X. Y.; Tang, S. K.; Zhang, S.; L, W. J. *Int. J. Syst. Evol. Microbiol.* **2009**, *59*, 745–749.
(11) Duan, C.; Yao, Y.; Wang, Z.; Tian, X.; Zhang, S.; Zhang, C.; Ju, J. *Chin. J. Mar. Drugs* **2010**, *29*, 12–20.
(12) Shin, H. J.; Lee, H.-S.; Lee, D.-S. *J. Microbiol. Biotechnol.* **2010**, *20*, 501–505.
(13) Sun, H. H.; White, C. B.; Dedinas, J.; Cooper, R.; Sedlock, D. M. *J. Nat. Prod.* **1991**, *54*, 1440–1443.
(14) Yamashita, T.; Imoto, M.; Isshiki, K.; Sawa, T.; Naganawa, H.; Kurasawa, S.; Zhu, B.-Q.; Umezawa, K. *J. Nat. Prod.* **1988**, *51*, 1184–1187.
(15) Sun, B.; Morikawa, T.; Matsuda, H.; Tewtrakul, S.; Wu, L. J.; Harima, S.; Yoshikawa, M. *J. Nat. Prod.* **2004**, *67*, 1464–1469.
(16) Wang, W.; Nam, S.-J.; Lee, B.-C.; Kang, H. *J. Nat. Prod.* **2008**, *71*, 163–166.
(17) Aimi, N.; Odaka, H.; Sakai, S.; Fujiki, H.; Suganuma, M.; Moore, R. E.; Patterson, G. M. L. *J. Nat. Prod.* **1990**, *53*, 1593–1596.
(18) Edwards, D. J.; Gerwick, W. H. *J. Am. Chem. Soc.* **2004**, *126*, 11432–11433.
(19) Endo, Y.; Hasegawa, M.; Itai, A.; Shudo, K. *Tetrahedron* **1987**, *43*, 3695–3704.
(20) Cao, R.; Peng, W.; Wang, Z.; Xu, A. *Curr. Med. Chem.* **2007**, *14*, 479–500.
(21) Ang, K. K.; Holmes, M. J.; Higa, T.; Hamann, M. T.; Kara, U. A. *Antimicrob. Agents Chemother.* **2000**, *44*, 1645–1649.
(22) Rottmann, M.; McNamara, C.; Yeung, B. K. S.; Le, M. C. S.; Zou, B.; Russell, B.; Seitz, P.; Plouffe, D. M.; Dharia, N. V.; Tan, J.; Cohen, S. B.; Spencer, K. R.; González-Páez, G. E.; Lakshminarayana, S. B.; Goh, A.; Suwanarusk, R.; Jegla, T.; Schmitt, E. K.; Beck, H. P.; Brun, R.; Nosten, F.; Renia, L.; Dartois, V.; Keller, T. H.; Fidock, D. A.; Winzeler, E. A.; Diagana, T. T. *Science* **2010**, *329*, 1175–1180.
(23) Cardellina, J. H. II; Marner, F.-J.; Moore, R. E. *Science* **1979**, *204*, 193–195.
(24) Sakai, S.; Aimi, N.; Yamaguchi, K.; Hitotsuyanagi, Y.; Watanabe, C.; Yokose, K.; Koyama, Y.; Shudo, K.; Itai, A. *Chem. Pharm. Bull.* **1984**, *32*, 354–357.
(25) Izumikawa, M.; Khan, S. T.; Komaki, H.; Takagi, M.; Shin-ya, K. *J. Antibiot.* **2010**, *63*, 33–36.
(26) Fujiki, H.; Mori, M.; Nakayasu, M.; Terada, M.; Sugimura, T.; Moore, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3872–3876.
(27) Nakagawa, Y.; Irie, K.; Yanagita, R. C.; Ohigashi, H.; Tsuda, K. *J. Am. Chem. Soc.* **2005**, *127*, 5746–5747.
(28) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
(29) He, Z.; Chen, L.; You, J.; Qin, L.; Chen, X. *Int. J. Antimicrob. Agents* **2010**, *35*, 191–193.
(30) Sheldrick, G. M. *SHELXTL-97, Program for Crystal Structure Solution and Refinement*; University of Göttingen, Göttingen, Germany, 1997.