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Article

New Azalomycin F Analogs from Mangrove *Streptomyces* sp. 211726 with Activity against Microbes and Cancer Cells

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Abstract: Seven new azalomycin F analogs (**1**–**7**) were isolated from the broth of mangrove *Streptomyces* sp. 211726, and respectively identified as 25-malonyl demalonylazalomycin F_{5a} monoester (**1**), 23-valine demalonylazalomycin F_{5a} ester (**2**), 23-(6-methyl)heptanoic acid demalonylazalomycins F_{3a} ester (**3**), F_{4a} ester (**4**) and F_{5a} ester (**5**), 23-(9-methyl)decanoic acid demalonylazalomycin F_{4a} ester (**6**) and 23-(10-methyl)undecanoic acid demalony lazalomycin F_{4a} ester (**7**). Their structures were established by their spectroscopic data and by comparing with those of azalomycins F_{3a}, F_{4a} and F_{5a}. Biological assays exhibited that **1**–**7** showed broad-spectrum antimicrobial and anti HCT-116 activities.

Keywords: azalomycin F; *Streptomyces* sp. 211726; cytotoxicity; antimicrobial activity

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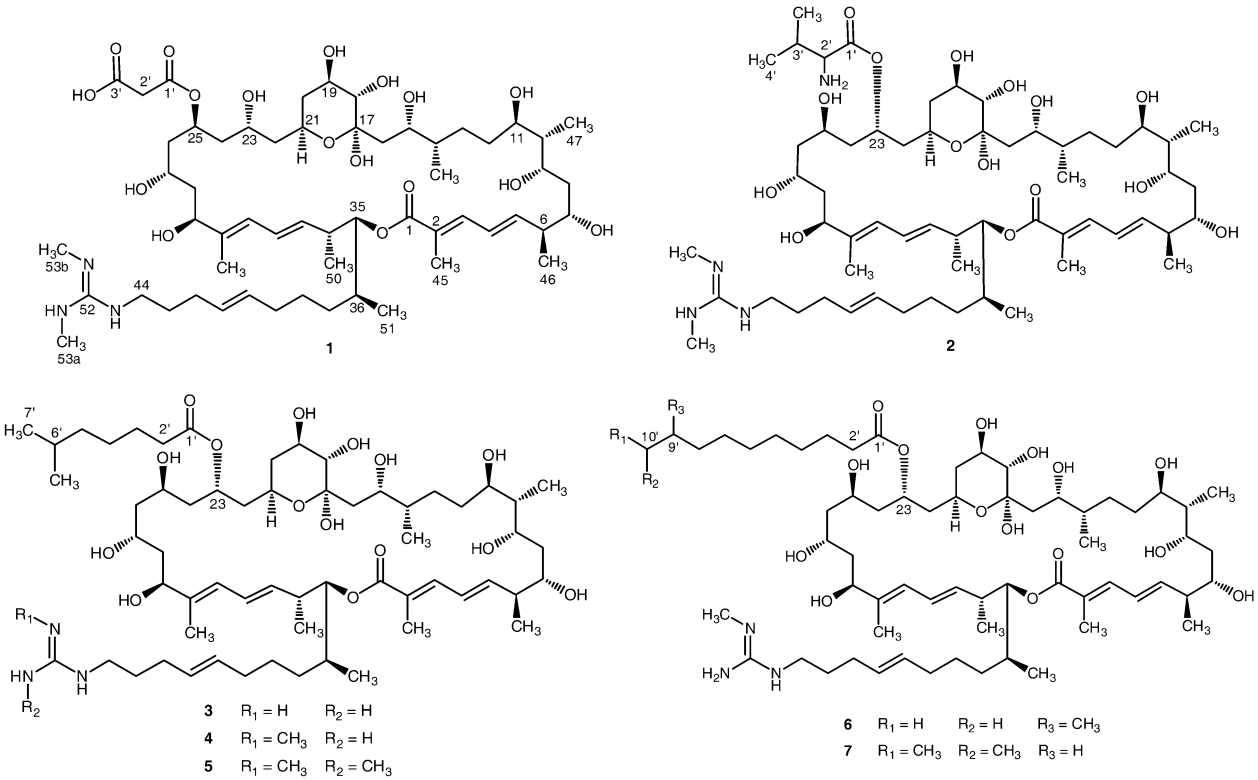
1. Introduction

Mangroves are woody plants located in tropical and subtropical intertidal coastal regions, which are high productive ecosystems [1,2]. Novel bioactive compounds have been reported from the plant materials [3–5]. Mangrove streptomycetes are also potential resources for the discovery of anti-infection, anti-tumor and hypoglycemic compounds [6–10]. *Streptomyces* sp. 211726, a remarkable producer of macrocyclic lactones, was selected from 288 strains when we carried on the chemical screening for macrolide-producing mangrove actinomycetes. Five azalomycin F analogs including azalomycins F_{3a}, F_{4a}, F_{5a}, azalomycin F_{4a} 2-ethylpentyl ester and azalomycin F_{5a} 2-ethylpentyl ester were identified from the culture broth of this strain in our previous work [11], while the HPLC profiles of the methanol extract and several macrolide constituents indicated that many azalomycin F analogs were produced by this strain. After the relative configurations of azalomycins F_{3a}, F_{4a} and F_{5a} were assigned [12], further research on minor azalomycin F analogs produced by this strain led to seven new compounds (Figure 1) which were respectively identified as 25-malonyl demalonylazalomycin F_{5a} monoester (**1**), 23-valine demalonylazalomycin F_{5a} ester (**2**), 23-(6-methyl) heptanoic acid demalonylazalomycins F_{3a} (**3**), F_{4a} (**4**) and F_{5a} (**5**) esters, 23-(9-methyl)decanoic acid demalonylazalomycin F_{4a} ester (**6**) and 23-(10-methyl)undecanoic acid demalonylazalomycin F_{4a} ester (**7**). Their structures were established by their spectroscopic data (IR, UV, NMR, MS) and by comparing with those of azalomycins F_{3a}, F_{4a} and F_{5a} which were reported in our previous paper [11], and their complete ^1H and ^{13}C assignments were achieved by using ^1H, ^{13}C, DEPT, HSQC, ^1H-^1H COSY and HMBC spectra in MeOH-d_4. Moreover, biological assays of **1**–**7** showed broad-spectrum antimicrobial activity as well as anti HCT-116 activity.

Figure 1. Structure of compounds **1**–**7** from Mangrove *Streptomyces* sp. 211726.



2. Results and Discussion

2.1. Structural Elucidation

25-Malonyl demalonylazalomycin F_{5a} monoester (**1**) was obtained as a white, amorphous powder with $[\alpha]_D^{29}$ +6.7° (*c* 0.1, MeOH). Its molecular formula $C_{57}H_{97}N_3O_{17}$ was established by the HRESIMS spectrometric data at *m*/*z* 1096.6914 $[M + H]^+$ (calcd. for $C_{57}H_{98}N_3O_{17}$, 1096.6896), which showed that its molecular formula was identical to that of azalomycin F_{5a}. Like azalomycin F_{5a}, its UV absorption maxima at 241 nm (log ε, 4.6) and 269 nm (log ε, 4.3) also indicated the presence of a conjugated diene and an α,β,γ,δ-unsaturated acid (or ester) group. The ^{13}C, DEPT and HSQC spectra of **1** (Table 1) showed one guanidino carbon signal at δ_C 157.42, three carbonyl carbon signals at δ_C 170.2, 171.9 and 173.9, ten olefinic carbon signals at δ_C 125.3, 126.8, 127.6, 128.6, 130.3, 132.6, 136.3, 140.2, 140.3 and 146.2, one quaternary hemiacetical carbon at δ_C 99.9 and one methine carbon at δ_C 70.8. So, **1** was deduced as an isomer of azalomycin F_{5a}. When we compared the ^{13}C and DEPT spectra of **1** with those of azalomycin F_{5a} [13], the signal at δ_C 46.6 (C-26) in the ^{13}C NMR spectrum of azalomycin F_{5a}, was not observed while a signal at δ_C 44.0 appeared in that of **1**. Based on the HSQC, ^1H-^1H COSY and HMBC spectra of **1**, the linking position of the malonyl group was assigned to C-25 in **1**, and the signal at δ_C 44.0 was assigned to C-26. It is interesting that **1** was found to be convertible to azalomycin F_{5a}. HPLC analysis showed that the ratio of **1** to azalomycin F_{5a} was about 15:85 after **1** stood in MeOH-d_4 at room temperature for 30 days. This phenomenon was also observed by Iwasaki S. *et al.* [14]. The compound convertible to azalomycin F_{5a} was named as azalomycin F_{5b}, although spectroscopic information and structure of azalomycin F_{5b} was not given in the paper [14]. There is not enough evidence to confirm that **1** and azalomycin F_{5b} are the same compound. So, **1** was identified as 25-malonyl demalonylazalomycin F_{5a} monoester.

Table 1. NMR spectroscopic data (400 MHz for ^1H, 100 MHz for ^{13}C) of **1**, **2**, **3** and **6** in MeOH-d_4 (δ in ppm).

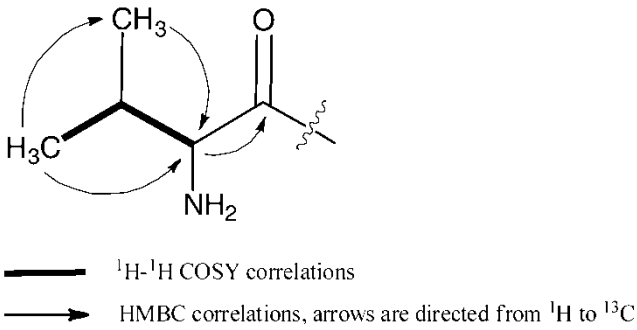
Position	1		2		3		6	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
C-1	170.2	-	170.1	-	170.1	-	170.1	-
C-2	126.8	-	126.7	-	126.8	-	126.8	-
C-3	140.3	7.09 d (11.2)	140.3	7.10 d (11.0)	140.2	7.10 d (11.2)	140.3	7.10 d (11.0)
C-4	127.6	6.43 dd (11.5, 14.9)	127.6	6.43 dd (11.5, 14.7)	127.6	6.43 dd (11.9, 14.3)	127.6	6.43 dd (11.5, 14.8)
C-5	146.2	6.07 dd (15.1, 9.0)	146.1	6.08 dd (14.8, 9.0)	146.1	6.08 dd (14.0, 9.0)	146.1	6.08 dd (14.9, 9.0)
C-6	44.8	2.43 m	44.7	2.44 m	44.6	2.43 m	44.7	2.44 m
C-7	75.9	3.80 m	75.8	3.77 m	75.8	3.77 m	75.8	3.77 m
C-8	39.3	1.50 m, 1.78 m	39.3	1.50 m, 1.78 m	39.3	1.50 m, 1.77 m	39.3	1.50 m, 1.78 m
C-9	75.4	3.80 m	75.2	3.80 m	75.2	3.80 m	75.2	3.80 m
C-10	44.7	1.54 m	44.6	1.53 m	44.5	1.54 m	44.6	1.53 m
C-11	72.2	3.91 m	72.2	3.87 m	72.2	3.91 m	72.2	3.87 m
C-12	33.5	1.62 m, 1.38 m	33.5	1.60 m, 1.38 m	33.5	1.62 m, 1.37 m	33.4	1.62 m, 1.37 m
C-13	30.7	1.30 m, 1.45 m	30.6	1.30 m, 1.42 m	30.6	1.30 m, 1.43 m	30.6	1.30 m, 1.43 m
C-14	40.6	1.60 m	40.5	1.61 m	40.7	1.61 m	40.5	1.61 m
C-15	72.7	3.86 m	72.7	3.87 m	72.4	3.86 m	72.7	3.87 m
C-16	41.9	1.80 m	41.8	1.81 m	41.9	1.82 m	41.8	1.81 m
C-17	99.9	-	100.0	-	99.8	-	99.9	-

Table 1. *Cont.*

C-18	77.5	3.34 d (9.2)	77.4	3.35 d (9.1)	77.2	3.35 d (9.2)	77.5	3.35 d (9.1)
C-19	69.9	3.87 m	69.9	3.88 m	69.7	3.87 m	69.8	3.88 m
C-20	41.4	1.89 m, 1.30 m	41.3	1.89 m, 1.30 m	41.2	1.90 m, 1.31 m	41.3	1.89 m, 1.31 m
C-21	65.7	4.17 m	66.2	4.16 m	66.3	4.16 m	66.3	4.16 m
C-22	44.5	1.52 m	41.9	1.82 m	41.8	1.88 m	41.9	1.81 m
C-23	66.3	4.03 m	70.9	5.29 m	70.7	5.27 m	70.9	5.29 m
C-24	44.6	1.69 m	44.0	1.70 m	44.0	1.72 m	44.1	1.76 m, 1.66 m
C-25	70.8	5.28 m	65.7	3.86 m	65.6	3.87 m	65.7	3.86 m
C-26	44.0	1.61 m, 1.83 m	46.3	1.51 m	46.4	1.51 m	46.3	1.51 m
C-27	65.7	3.88 m	65.8	4.04 m	65.6	4.04 m	65.8	4.04 m
C-28	44.2	1.78 m	44.1	1.54 m	44.1	1.53 m	44.1	1.63 m
C-29	74.2	4.18 m	74.2	4.18 m	74.2	4.18 m	74.2	4.18 m
C-30	140.2	-	140.1	-	140.1	-	140.1	-
C-31	125.3	5.98 d (10.4)	125.3	5.98 d (10.7)	125.2	5.98 d (10.5)	125.3	5.98 d (10.7)
C-32	128.6	6.22 dd (10.9, 14.5)	128.5	6.23 dd (10.9, 14.9)	128.6	6.22 dd (10.9, 14.9)	128.5	6.23 dd (10.9, 14.8)
C-33	136.3	5.43 m	136.3	5.44 m	136.2	5.45 m	136.3	5.44 m
C-34	41.0	2.57 m	40.7	2.57 m	41.0	2.57 m	40.9	2.57 m
C-35	80.9	4.78 dd (7.6, 4.0)	80.9	4.79 dd (7.7, 4.1)	80.8	4.78 dd (7.8, 3.9)	80.9	4.79 dd (7.6, 4.1)
C-36	35.3	1.82 m	35.3	1.82 m	35.2	1.81 m	35.3	1.82 m
C-37	34.4	1.15 m, 1.35 m	34.4	1.15 m, 1.35 m	34.5	1.15 m, 1.35 m	34.4	1.15 m, 1.35 m
C-38	27.9	1.42 m	27.8	1.42 m	27.9	1.41 m	27.9	1.42 m
C-39	33.6	1.99 m	33.6	1.99 m	33.6	1.99 m	33.6	1.99 m
C-40	132.6	5.44 m	132.5	5.44 m	132.6	5.44 m	132.6	5.44 m
C-41	130.3	5.44 m	130.3	5.44 m	130.1	5.50 m	130.2	5.44 m
C-42	30.7	2.07 m	30.4	2.07 m	30.8	2.07 m	30.6	2.07 m
C-43	29.9	1.67 m	29.8	1.64 m	29.8	1.64 m	29.8	1.64 m
C-44	42.2	3.17 t (7.3)	42.0	3.15 t (7.1)	42.0	3.15 t (7.0)	42.0	3.15 t (7.1)
C-45	12.9	1.92 s	12.9	1.92 s	12.9	1.92 s	12.9	1.92 s
C-46	17.1	1.11 d (6.8)	17.1	1.12 d (6.8)	17.1	1.11 d (6.8)	17.1	1.12 d (6.8)
C-47	10.5	0.89 d (6.9)	10.5	0.89 d (6.9)	10.5	0.89 d (6.9)	10.5	0.89 d (6.9)
C-48	15.2	0.91 d (6.7)	15.3	0.92 d (6.7)	15.3	0.91 d (6.7)	15.3	0.92 d (6.7)
C-49	13.1	1.65 s	13.1	1.65 s	13.3	1.65 s	13.1	1.65 s
C-50	17.8	1.01 d (6.7)	17.9	1.00 d (6.7)	17.9	1.00 d (6.6)	17.9	1.00 d (6.8)
C-51	14.4	0.94 d (6.7)	14.5	0.95 d (6.7)	14.4	0.94 d (6.7)	14.5	0.94 d (6.7)
C-52	157.4	-	157.4	-	158.7	-	158.3	-
C-53a	28.4	2.85 s	28.4	2.85 s			28.4	2.84 s
C-53b	28.4	2.85 s	28.4	2.85 s				
C-1′	171.9	-	174.1	-	175.5	-	175.4	-
C-2′	46.0	3.22 m	61.7	3.44 d (4.0)	35.0	2.36 t (7.4)	35.0	2.36 t (7.5)
C-3′	173.9	-	30.8	2.28 m	26.0	1.62 m	26.0	1.61 m
3′-CH_3			17.9	1.02 d (6.8)				
C-4′			19.2	1.07 d (6.8)	30.4	1.42 m	30.3	1.35 m
C-5′					40.3	1.18 m	30.4	1.31 m
C-6′					29.2	1.29 m	30.8	1.30 m
6′-CH_3					23.8	0.88 d (6.6)		
C-7′					23.7	0.88 d (6.6)	28.5	1.29 m
C-8′							40.3	1.17 m
C-9′							29.2	1.31 m
9′-CH_3							23.1	0.89 d (6.8)
C-10′							23.1	0.89 d (6.8)

23-Valine demalonylazalomycin F_{5a} ester (**2**) was obtained as a white, amorphous powder with $[\alpha]_D^{29}$ +4.4° (*c* 0.1, MeOH). Its molecular formula $C_{59}H_{104}N_4O_{15}$ was established by the HRESIMS spectrometric data at *m/z* 1109.7580 $[M + H]^+$ (calcd. for $C_{59}H_{105}N_4O_{15}$, 1109.7576). Its UV absorption maxima at 241 nm (log ε, 4.6) and 268 nm (log ε, 4.4) indicated the presence of a conjugated diene and an α,β,γ,δ-unsaturated acid (or ester) group. The ^{13}C, DEPT and HSQC spectra of **2** (Table 1) showed one guanidino carbon signal at δ_C 157.4, one carbonyl carbon signal at δ_C 170.1, ten olefinic carbon signals at δ_C 125.3, 126.7, 127.6, 128.5, 130.3, 132.5, 136.3, 140.1, 140.3 and 146.1, one quaternary hemiacetical carbon signal at δ_C 100.0, one methine carbon signal at δ_C 70.9 and two *N*-methyl carbon signals at δ_C 28.4. These spectroscopic data were very similar to azalomycin F_{5a} reported in our previous paper [11], while there was no carbonyl carbon signal at δ_C 171.6 and methylene carbon signal at δ_C 46.1. Comparing the ^{13}C, DEPT and HSQC spectra of **2** and those of azalomycin F_{5a}, two additional methyl carbon signals at δ_C 17.9 and 19.2 and two methylene carbon signals at δ_C 30.81 and 61.7 were present. Based on the correlations (Figure 2) observed in the ^1H-^1H COSY and HMBC spectra of **2**, a valyl group was established. Moreover, the correlation between H-23 (δ_H 5.29) and C-1′ (δ_C 174.1) observed in the HMBC spectrum of **2** indicated that the valyl group was linked to the lactonic ring at C-23 with an ester bond. So, **2** was identified as 23-valine demalonylazalomycin F_{5a} ester.

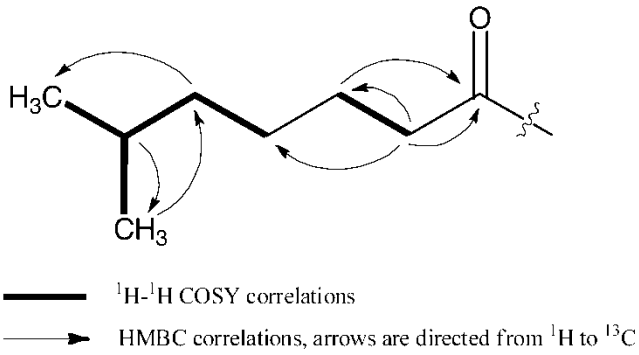
Figure 2. Key ^1H-^1H COSY and HMBC correlations of the valyl moiety in **2**.



23-(6-Methyl)heptanoic acid demalonylazalomycin F_{3a} ester (**3**) was obtained as a white, amorphous powder with $[\alpha]_D^{20}$ +6.8° (*c* 0.1, MeOH). Its molecular formula $C_{60}H_{105}N_3O_{15}$ was established by the HRESIMS spectrometric data at *m/z* 1108.7638 $[M + H]^+$ (calcd. for $C_{60}H_{106}N_3O_{15}$, 1108.7624). Its UV absorption maxima at 238 nm (log ε, 4.6) and 269 nm (log ε, 4.3) indicated the presence of a conjugated diene and an α,β,γ,δ-unsaturated acid (or ester) group. The ^{13}C, DEPT and HSQC spectra of **3** (Table 1) showed one guanidino carbon signal at δ_C 158.7, one carbonyl carbon signal at δ_C 170.1, ten olefinic carbon signals at δ_C 125.2, 126.8, 127.6, 128.6, 130.1, 132.6, 136.2, 140.1, 140.2 and 146.1, one quaternary hemiacetical carbon signal at δ_C 99.8 and one methine carbon signal at δ_C 70.7. These spectroscopic data were very similar to those of azalomycin F_{3a} [15], which were reported as supporting information in our previous paper [11], while there were no carbonyl carbon signals at δ_C 171.8 and 174.0 and methylene carbon signal at δ_C 45.8 in the ^{13}C NMR spectrum of **3**. Comparing the ^1H, ^{13}C, DEPT and HSQC spectra of **3** with those of azalomycin F_{3a}, two additional methyl carbon signals at δ_C 23.8 and 23.7, four methylene carbon signals at δ_C 40.3, 35.0, 30.4 and 26.0, one methine carbon signal at δ_C 29.2 and a carbonyl carbon signal at δ_C 175.5 were

observed in the ^{13}C NMR spectrum of **3**. Based on the correlations observed in the ^{1}H-^{1}H COSY and HMBC spectra (Figure 3), a 6-methyl heptanoyl group was deduced. Moreover, the correlations between H-23 (δ_H 5.27) and C-1′ (δ_C 175.5) observed in the HMBC spectrum of **3** indicated that the 6-methyl heptanoyl group was linked to the lactonic ring at C-23 with an ester bond. So, **3** was identified as 23-(6-methyl)heptanoic acid demalonylazalomycin F_{3a} ester.

Figure 3. Key ^{1}H-^{1}H COSY and HMBC correlations of the 6-methyl heptanoyl moiety in **3**.



23-(6-Methyl)heptanoic acid demalonylazalomycin F_{4a} ester (**4**) was obtained as a white, amorphous powder with $[\alpha]_D^{20}$ +6.4° (*c* 0.1, MeOH). Its molecular formula $C_{61}H_{107}N_3O_{15}$ was established by the HRESIMS spectrometric data at *m/z* 1122.7788 $[M + H]^+$ (calcd. for $C_{61}H_{108}N_3O_{15}$, 1122.7780). The difference of 14 mass units between **4** and **3** indicated that **4** has one methylene unit more than **3**. Similar ^{1}H, ^{13}C, DEPT spectra and UV absorption data allowed identification of these two compounds as analogs. Comparing the ^{13}C and DEPT spectra of **4** with those of azalomycin F_{4a} [16], the guanidino carbon signal at δ_C 158.3 (C-52) indicated that one methyl group was linked to a guanidino nitrogen [11], which was also corroborated by a proton signal at δ_H 2.84 (3H, s, H-53a), a carbon signal at δ_C 28.4 (C-53a) and the correlation between H-53a and C-52 observed in the HMBC spectrum of **4**. So, **4** was identified as 23-(6-methyl)heptanoic acid demalonylazalomycin F_{4a} ester.

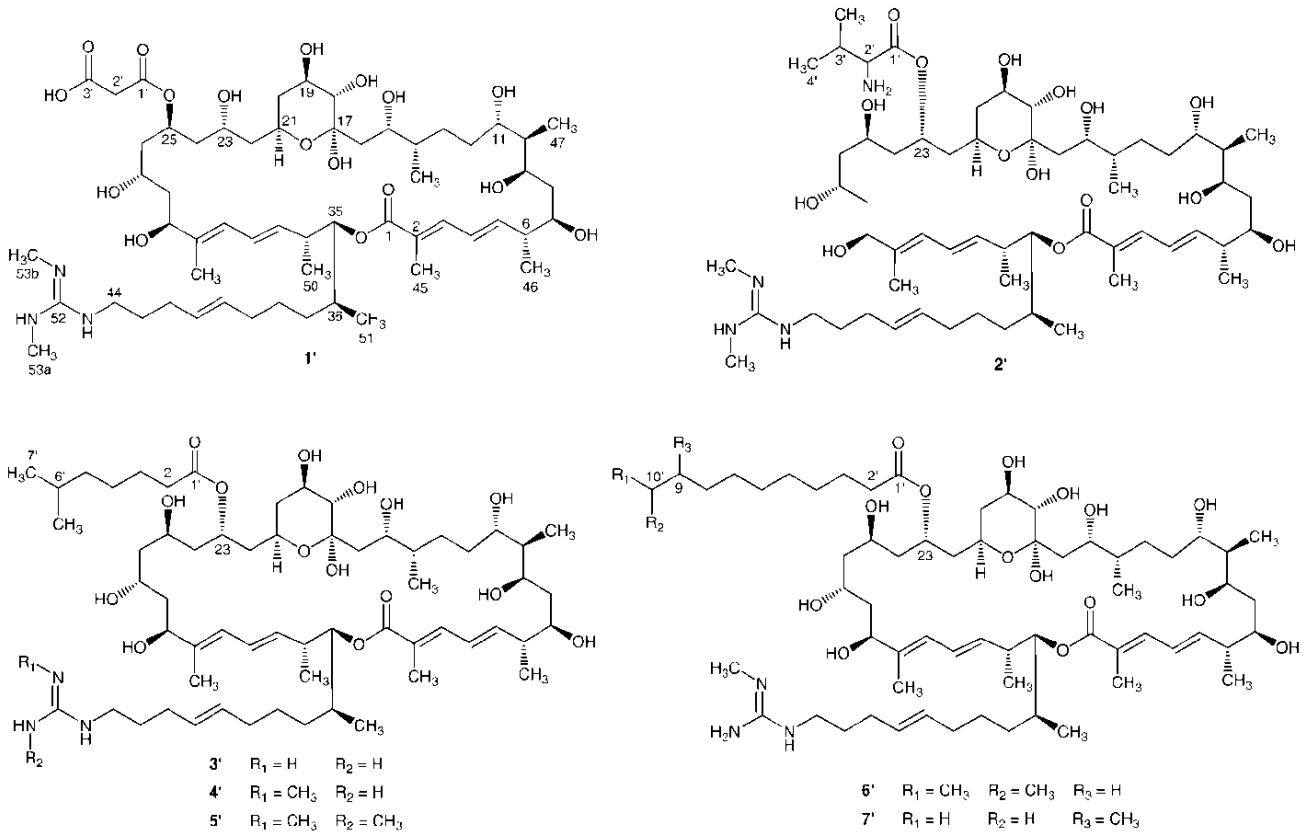
23-(6-Methyl)heptanoic acid demalonylazalomycin F_{5a} ester (**5**) was obtained as a white, amorphous powder with $[\alpha]_D^{20}$ +6.1° (*c* 0.1, MeOH). Its molecular formula $C_{62}H_{109}N_3O_{15}$ was established by the HRESIMS spectrometric data at *m/z* 1136.7956 $[M + H]^+$ (calcd. for $C_{62}H_{110}N_3O_{15}$, 1136.7937). The difference of 14 mass units between **5** and **4** indicated that **5** has one methylene unit more than **4**. Similar ^{1}H, ^{13}C, DEPT spectra and UV absorption data allowed identification of these two compounds as analogs. Comparing their ^{1}H, ^{13}C and DEPT spectra, the guanidino carbon signal at δ_C 157.4 indicated two methyl groups were linked to two guanidino nitrogens, which was also corroborated by proton signals at δ_H 2.84 (6H, s, H-53a and H-53b) and carbon signals at δ_C 28.4 (C-53a and C-53b) in the ^{1}H and ^{13}C NMR spectrum of **5**, respectively. So, **5** was identified as 23-(6-methyl)heptanoic acid demalonylazalomycin F_{5a} ester.

23-(9-Methyl)decanoic acid demalonylazalomycin F_{4a} ester (**6**) was obtained as a white, amorphous powder with $[\alpha]_D^{20}$ +6.0° (*c* 0.1, MeOH). Its molecular formula $C_{64}H_{113}N_3O_{15}$ was established by the HRESIMS spectrometric data at *m/z* 1164.8269 $[M + H]^+$ (calcd. for $C_{64}H_{114}N_3O_{15}$, 1164.8250). The difference of 42 mass units between **6** and **4** indicated that **6** has three methylene units more than **4**. Similar ^{1}H, ^{13}C, DEPT spectra (Table 1) and UV absorption data of **6** and **4** also allowed identification of these two compounds as analogs. Comparing their spectroscopic data indicated the fatty acyl side

chain of **6** has three methylenes more than **4**, which was deduced by the ^{1}H, ^{13}C, DEPT, HSQC and HMBC spectra of **6**. The ^{13}C and ^{1}H assignments of the fatty acyl side chain of **6** were achieved by its ^{1}H-^{1}H COSY, HSQC and HMBC spectra and ACD/Lab 6.0 software. **6** was identified as 23-(9-methyl)undecanoic acid demalonylazalomycin F_{4a} ester.

23-(10-Methyl)undecanoic acid demalonylazalomycin F_{4a} ester (**7**) was obtained as a white, amorphous powder with $[\alpha]_D^{20}$ +6.0° (*c* 0.1, MeOH). Its molecular formula $C_{65}H_{115}N_3O_{15}$ was established by the HRESIMS spectrometric data at *m*/*z* 1178.8426 $[M + H]^+$ (calcd. for $C_{65}H_{116}N_3O_{15}$, 1178.8406). The difference of 14 mass units between **7** and **6** indicated that **7** has one methylene unit more than **6**. Similar ^{1}H, ^{13}C, DEPT spectra and UV absorption data of them allowed identification of these two compounds as analogs. Comparing their ^{1}H, ^{13}C and DEPT spectra, there was no obvious difference between the ^{13}C NMR spectrum of **7** and **6** except that one methylene carbon signal at about δ_C 31.0 was presented in the ^{13}C NMR spectrum of **7**. So, **7** was identified as 23-(10-methyl)decanoic acid demalonylazalomycin F_{4a} ester.

Figure 4. Structures of **1′**–**7′**.



After the planar structures of **1**–**7** were established, we focused on their stereochemistries. As the core macrolide planar structures of **1**–**7** were accordingly identical to those of azalomycins F_{5a}, F_{4a} or F_{3a}, the relative configurations of the core macrolide structures of **1**–**7** except the structural fragment from C-23 to C-27 of **1** could be directly established by comparing their ^{13}C and ^{1}H NMR spectra with those of azalomycins F_{5a}, F_{4a} or F_{3a} [11–13,15,16]. Similar spectroscopic data of their core macrolide structures deduced that the relative configurations of **1**–**7** except that at C-23/C-25/C-27 of **1** were accordingly identical to those of azalomycins F_{5a}, F_{4a} or F_{3a}. Like that of azalomycin F_{5a}, the chemical

shifts for C-21 (65.7 ppm), C-23 (66.3 ppm) and C-27 (65.7 ppm) lower than 68.0 ppm in MeOH-d_4 deduced that the relavitve configuration at C-23/C-25/C-27 of **1** was also *anti*/*anti* according to the universal NMR Database **4** [12,17], which was further confirmed by two facts that **1** could be convertible to azalomycin F_{5a} in MeOH-d_4 at room temperature and that the chemical shift for C-23 was upfield by about 5.0 ppm when the malonyl group of azalomycin F_{4a} was removed [12]. Because the relative stereochemistries of C_6–C_{11} to C_{14}–C_{36} stereogenic centers of azalomycins F_{5a} remain undefined in our previous work [12], azalomycin F_{5a} was one of two possible stereoisomers which the relative configuration at C_{11}/C_{14} was *anti* or *syn*. Similarly, azalomycins F_{4a} and F_{3a} were respectively one of two possible stereoisomers like that of azalomycin F_{5a}. So, each compound of **1**–**7** was one of two possible stereoisomers numbered **1**–**7** and **1′**–**7′** presented in Figures 1 and 4, respectively.

2.2. Biological Assays

Biological assays indicated that **1**–**7** had broad-spectrum antimicrobial activity. Their minimal inhibitory concentrations (MICs) against *Candida albicans* ATCC 10231, *Staphylococcus aureus* S014, *Bacillus subtilis* S028 and *Escherichia coli* S002 were respectively 1.56–6.25, 0.39–1.56, 0.20–0.78 and 3.13–25.00 μg/mL (Table 2). Moreover, they also showed moderate cytotoxicity against human colon tumor cell HCT-116 *in vitro* with IC_{50} values of 1.81–5.00 μg/mL (Table 2).

Table 2. Minimal inhibitory concentrations (MICs) against test microbes and IC_{50} value against HCT-116 *in vitro*.

Compounds	MICs (μg/mL)				IC_{50} (μg/mL)
	***Candida albicans* ATCC 10231**	***Staphylococcus aureus* S014**	***Bacillus subtilis* S028**	***Eschzerichia coli* S002**	**HCT-116**
1	3.13	0.39	0.20	3.13	5.00
2	6.25	1.56	0.39	6.25	1.95
3	3.13	0.78	0.39	3.13	2.46
4	1.56	1.56	0.20	6.25	2.45
5	1.56	0.78	0.78	12.5	1.81
6	3.13	0.39	0.39	25.00	1.54
7	3.13	0.39	0.39	3.13	2.46
Positive controls *	2.0	0.50	0.20	2.0	0.18

* Amphotericin B for *C. albicans*, oxacillin sodium for *S. aureus* and *B. subtilis*, kalamycin for *E. coli* and doxorubicin for HCT-116 were respectively used as positive controls.

2.3. Discussion

Azalomycin F complex, including azalomycins F_{3a}, F_{4a}, F_{5a} and several minor analogs, was first isolated from the broth of *Streptomyces hygroscopicus* var. *azalomyceticus* by Arai in 1959 [18,19]. The structures of azalomycins F_{3a}, F_{4a} and F_{5a} were progressively elucidated from 1982 to 2012 [12,14,20–22], while others minor analogs were not identified. *Streptomyces* sp. 211726, isolated from mangrove rhizosphere soil, showed a remarkable productivity of macrocyclic lactones, and five main components azalomycins F_{3a}, F_{4a}, F_{5a} azalomycin F_{4a} 2-ethylpentyl ester and azalomycin F_{5a} 2-ethylpentyl ester produced by this strain were identified [11]. During our research on minor analogs

produced by this strain, seven new compounds **1**–**7** were isolated and identified in this paper. Similar to these azalomycin F analogs, many 36-membered polyhydroxyl macrolides such as RS-22, guanidylfungins, amycins, niphimycin, malonylniphimycin, dihydroniphimycin, malonyl-4,5-dihydroniphimycin, shurimycins and others were identified [23–26]. There are about thirty 36-membered polyhydroxyl macrolides identified so far, and almost all of them were produced by *Streptomyces*. These compounds possess broad-spectrum antimicrobial activity, and azalomycin F was also an inhibitor of type-I interleukin-1 receptor [27]. In our research on biological activity of azalomycin F analogs produced by *Streptomyces* sp. 211726, these twelve compounds also showed remarkable broad-spectrum antimicrobial activity. Moreover, they had moderate cytotoxicity, and the acute toxicity (LD_{50}) of a mixture of twelve azalomycin F analogs produced by this strain was 97.9 mg/kg in mice by intraperitoneal injection.

3. Experimental Section

3.1. General Experimental Procedures

Optical rotations were measured in methanol using an Autopol III digital polarimeter. UV spectra were determined by DU-800 UV/Visible spectrophotometer. IR spectra were obtained with Thermo Nicolet 380 FTIR spectrometer. All NMR experiments were recorded on a Bruker AV-400 NMR spectrometer equipped with a 5 mm PABBO BB-probe head (400 MHz for ^{1}H shifts relative to MeOH-d_4 at 3.31 ppm and 100 MHz for ^{13}C shifts relative to MeOH-d_4 at 49.05 ppm) at 30 °C. HR-ESI-MS spectra were carried on the API QSTAR Pulsar I MS System. Silica gel (Qingdao Haiyang Chemical Co. Ltd., Qingdao, China, 10–40 μm), octadecylsilyl silica gel (Silicycle, Quebec, Canada; 40–63 μm) and Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden) were used for column chromatography. Precoated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co., Ltd.) were used for thin layer chromatography. All compounds was prepared by Dionex Summit HPLC system (Dionex, Sunnyvale, CA, USA) consisting of Dionex P680 HPLC pumps (P680A HPG-4) with a UV detector (170 U), and a Shimadzu Shim-pack VP-ODS reversed-phase column (250 mm × 4.6 mm i.d., 5-μm particle size) was used.

3.2. Actinomycetes Material and Fermentation

The strain of *Streptomyces* sp. 211726 was isolated from mangrove rhizosphere soil of *Heritiera globosa* collected in Wenchang, China. Voucher specimens are stored in Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (Wuhan University), Ministry of Education, Wuhan, China. The fermentation of strain *Streptomyces* sp. 211726 was reported in our previous paper [11]. In short, the strain of *Streptomyces* sp. 211726 was cultured with liquid medium containing glucose 1.0%, soluble starch 3.5%, yeast 0.2%, casein 0.4% and NaCl 1.8% (pH 7.2 before autoclaving), and incubated at 30 °C for 10 days on a rotary shaker at 190 rpm until 100 L of broth was obtained.

3.3. Extract and Isolation

After the 100 L broth of *Streptomyces* sp. 211726 was centrifuged, the mycelia was extracted with methanol, concentrated under vacuum and freeze-dried to obtain lyophilized powder. The powder was

dissolve in $CHCl_3$:MeOH (80:20), and separated into eight fractions (1–8) on a silica gel column using gradient elution of $CHCl_3$:MeOH (3:1, 2:1 and 1:1). Fraction 2 was purified by reversed-phase C_{18} column eluted with MeOH:H_2O (60:40), semi-preparative reversed-phase C_{18} high performance liquid chromatography eluted with MeOH:H_2O (58:42) to give three pure fraction, and which were respectively concentrated under vacuum to obtain three extracts. These extracts were respectively dissolved in MeOH, purified by sephadex LH-20 column eluted with MeOH, concentrated under vacuum at 35 °C, and dried to give **1** (41 mg), **2** (22 mg) and **3** (31 mg). Similarly, fraction 3 was purified by reversed-phase C_{18} column eluted with MeOH:H_2O (65:35), semi-preparative reversed-phase C_{18} high performance liquid chromatography eluted with MeOH:H_2O (63:37) and sephadex LH-20 column eluted with MeOH to give **4** (15 mg) and **6** (27 mg); Fraction 4 was purified by reversed-phase C_{18} column eluted with MeOH:H_2O (70:30), semi-preparative reversed-phase C_{18} high performance liquid chromatography eluted with MeOH:H_2O (68:32) and sephadex LH-20 column eluted with MeOH to give **5** (10 mg) and **7** (24 mg).

25-Malonyl demalonylazalomycin F_{5a} monoester (**1**): White amorphous powder; $[\alpha]_D^{29}$ +6.7° (*c* 0.1, MeOH); UV λ_{max}^{MeOH} nm (log ε): 241(4.6), 269(4.3); IR υ_{max}^{KBr} (cm^{-1}): 3385, 2964, 2936, 1701, 1636, 1597, 1459, 1243, 1089, 1066, 969; ^{13}C NMR (MeOH-d_4, 100 MHz) and ^1H NMR (MeOH-d_4, 400 MHz) data were showed in Table 1; HRESIMS *m*/*z* 1096.6914 $[M + H]^+$ (calcd. for $C_{57}H_{98}N_3O_{17}$, 1096.6896).

23-Valine demalonylazalomycin F_{5a} ester (**2**): White amorphous powder; $[\alpha]_D^{29}$ +4.4° (*c* 0.1, MeOH); UV λ_{max}^{MeOH} nm (log ε): 241(4.6), 268(4.4); IR υ_{max}^{KBr} (cm^{-1}): 3414, 3137, 2965, 2928, 1726, 1635, 1597, 1507, 1261, 1092, 968; ^{13}C NMR (MeOH-d_4, 100 MHz) and ^1H NMR (MeOH-d_4, 400 MHz) data were showed in Table 1; HRESIMS *m*/*z* 1109.7580 $[M + H]^+$ (calcd. for $C_{59}H_{105}N_4O_{15}$, 1109.7576).

23-(6-Methyl)heptanoic acid demalonylazalomycin F_{3a} ester (**3**): White amorphous powder; $[\alpha]_D^{20}$ +6.8° (*c* 0.1, MeOH); UV λ_{max}^{MeOH} nm (log ε): 238(4.6), 269(4.3); IR υ_{max}^{KBr} (cm^{-1}): 3423, 2962, 2935, 1736, 1707, 1637, 1184, 1045, 970, 721; ^{13}C NMR (MeOH-d_4, 100 MHz) and ^1H NMR (MeOH-d_4, 400 MHz) data were showed in Table 1; HRESIMS *m*/*z* 1108.7638 $[M + H]^+$ (calcd. for $C_{60}H_{106}N_3O_{15}$, 1108.7624).

23-(6-Methyl)heptanoic acid demalonylazalomycin F_{4a} ester (**4**): White amorphous powder; $[\alpha]_D^{20}$ +6.4° (*c* 0.1, MeOH); UV λ_{max}^{MeOH} nm (log ε): 238(4.5), 269(4.4); IR υ_{max}^{KBr} (cm^{-1}): 3423, 2965, 2936, 1734, 1708, 1635, 1181, 1049, 972, 722; ^1H NMR (MeOH-d_4, 400 MHz) and ^{13}C NMR (MeOH-d_4, 100 MHz) data were showed in supplementary file; HRESIMS *m*/*z* 1122.7788 $[M + H]^+$ (calcd. for $C_{61}H_{108}N_3O_{15}$, 1122.7780).

23-(6-Methyl)heptanoic acid demalonylazalomycin F_{5a} ester (**5**): White amorphous powder; $[\alpha]_D^{20}$ +6.1° (*c* 0.1, MeOH); UV λ_{max}^{MeOH} nm (log ε): 238(4.5), 269(4.4); IR υ_{max}^{KBr} (cm^{-1}): 3425, 2963, 2935, 1734, 1708, 1636, 1185, 1047, 972, 721; ^1H NMR (MeOH-d_4, 400 MHz) and ^{13}C NMR (MeOH-d_4, 100 MHz) data were showed in supplementary file; HRESIMS *m*/*z* 1136.7956 $[M + H]^+$ (calcd. for $C_{62}H_{110}N_3O_{15}$, 1136.7937).

23-(9-Methyl)decanoic acid demalonylazalomycin F_{4a} ester (**6**): White amorphous powder; $[\alpha]_D^{20}$ +6.0° (*c* 0.1, MeOH); UV λ_{max}^{MeOH} nm (log ε): 238(4.5), 269(4.4); IR υ_{max}^{KBr} (cm^{-1}): 3425, 2968, 2935, 1734, 1708, 1636, 1185, 1047, 972, 721; ^1H NMR (MeOH-d_4, 400 MHz) and ^{13}C NMR

(MeOH-d_4, 100 MHz) data were showed in Table 1; HRESIMS *m*/*z* 1164.8269 $[M + H]^+$ (calcd. for $C_{64}H_{114}N_3O_{15}$, 1164.8250).

23-(10-Methyl)undecanoic acid demalonylazalomycin F_{4a} ester (**7**): White amorphous powder; $[\alpha]_D^{20}$ +6.0° (*c* 0.1, MeOH); UV λ_{max}^{MeOH} nm (log ε): 238(4.5), 269(4.4); IR υ_{max}^{KBr} (cm^{-1}): 3425, 2968, 2936, 1736, 1707, 1636, 1185, 1047, 972, 721; ^{13}C NMR (MeOH-d_4, 100 MHz) and ^1H NMR (MeOH-d_4, 400 MHz) data were showed in supplementary file; HRESIMS *m*/*z* 1178.8426 $[M + H]^+$ (calcd. for $C_{65}H_{116}N_3O_{15}$, 1178.8406).

3.4. Biological Assays

The MICs of all compounds against *C. albicans* ATCC 10231, *S. aureus* S014, *B. subtilis* S028 and *E. coli* S002 were determined by agar dilution method. Amphotericin B for *C. albicans*, oxacillin sodium for *S. aureus* and *B. subtilis* and kalamycin for *E. coli* were respectively used as positive controls. Yeast extract-peptone-dextrose (YPD) medium was used for *C. albicans*, beef extract-peptone medium was used for *S. aureus* and *B. subtilis*, and Luria-Bertani (LB) medium was used for *E. coli*. Their cytotoxicities were evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide) method using human colon tumor cell HCT-116, and doxorubicin was used as a positive control.

4. Conclusions

Proceed with research on minor azalomycin F analogs produced by *Streptomyces* sp. 211726, seven new compounds were isolated and identified. Biological assays of **1**–**7** showed remarkable antifungal and antibacterial activity and moderate cytotoxicity against human colon tumor cell HCT-116 *in vitro*.

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132.6 (C-40), 130.3 (C-41), 30.7 (C-42), 29.9 (C-43), 42.1 (C-44), 12.8 (C-45), 17.0 (C-46), 10.5 (C-47), 14.8 (C-48), 13.3 (C-49), 17.7 (C-50), 14.4 (C-51), 158.7 (C-52), 171.8 (C-1′), 45.8 (C-2′), 174.0 (C-3′).

16. ^{13}C NMR (MeOH-d_4, 100 MHz) data of azalomycin F_{4a}: 170.1 (C-1), 126.8 (C-2), 140.2 (C-3), 127.6 (C-4), 146.1 (C-5), 44.6 (C-6), 75.7 (C-7), 39.2 (C-8), 75.0 (C-9), 44.5 (C-10), 72.2 (C-11), 33.6 (C-12), 30.8 (C-13), 40.5 (C-14), 72.3 (C-15), 41.7 (C-16), 99.8 (C-17), 77.1 (C-18), 69.7 (C-19), 41.2 (C-20), 65.4 (C-21), 42.0 (C-22), 70.7 (C-23), 44.6 (C-24), 65.5 (C-25), 46.5 (C-26), 66.1 (C-27), 44.1 (C-28), 74.2 (C-29), 140.1 (C-30), 125.2 (C-31), 128.6 (C-32), 136.2 (C-33), 41.0 (C-34), 80.7 (C-35), 35.1 (C-36), 34.6 (C-37), 27.9 (C-38), 33.6 (C-39), 132.5 (C-40), 130.3 (C-41), 30.8 (C-42), 29.8 (C-43), 42.1 (C-44), 12.9 (C-45), 17.1 (C-46), 10.5 (C-47), 14.8 (C-48), 13.3 (C-49), 17.6 (C-50), 14.3 (C-51), 158.3 (C-52), 28.4 (C-53a), 171.6 (C-1′), 46.1 (C-2′), 173.9 (C-3′).
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