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ARTICLE TYPE

Genome Scanning Inspired Isolation of Reedsmycins A-F, Polyene-polyol Macrolides from *Streptomyces* sp. CHQ-64

Qian Che,[§a] Tong Li,[§a] Xiaofang Liu,[a] Tingting Yao,[a] Jing Li,[b] Qianqun Gu,[a] Dehai Li,[*a] Wenli Li,[*a] and Tianjiao Zhu[*a]

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Genome scanning of the reeds rhizosphere soil-derived *Streptomyces* sp. CHQ-64 revealed a partial gene cluster, putatively encoding a polyene-polyol compound. Inspired by this finding, six new polyene-polyol macrolides, reedsmycins A-F (**1-6**), were isolated guided by the characteristic NMR signals. Their structures were elucidated using mass spectrometry and extensive NMR spectroscopy. Among them, reedsmycin F (**6**) possessed a rare 31-membered macroring containing a tetrahydrofuran motif, and reedsmycin A (**1**) exhibited promising activity against *Candida albicans* with MIC of 25-50 μM, in comparable level as that of the positive control nystatin.

Introduction

The genome-guided strategy for isolation of new natural products was prompted by the development of the high throughput sequencing technique. [1, 2] Polyketide (PK) is one of the major family of natural products with structural and biological diversities. The backbones of PKs are assembled by polyketide synthetases (PKSs), and hence PKS genes are important molecular indicators for discovery PKs compounds. During our genome scanning program for the gene clusters encoding PKs, a 28-kb DNA fragment containing two partial type I PKSs genes was identified from the genome of the marine-derived *Streptomyces* sp. CHQ-64, and their functional domain composition indicated the potential producibility of polyene-polyol macrolides by this strain.

Previously, two classes of cytotoxic hybrid isoprenoid alkaloids merging the amino acid and mavalonate pathways were obtained from this strain guided by the bioassay results.[3,4] Inspired by genome scanning information, we checked the noncytotoxic fractions of the *Streptomyces* sp. CHQ-64 fermentation products by ^{1}H NMR analysis. Among them, the characteristic signals related to polyene-polyol structures were observed in the fraction eluted by CH_2Cl_2/MeOH (5:1-1:1) on the silica column. By further NMR-guided fractionation, six new polyene-polyol macrolides, named reedsmycins A-F (**1-6**), were isolated. Herein, we report the genome-guided discovery, isolation, structural elucidation, and antifungal activities of these new compounds.

Results and Discussion

A 28-kb DNA fragment, processing an overall G+C content of 68.5%, was obtained by genome scanning of *Streptomyces* sp. CHQ-64. Two partial open reading frames (*orfs*) were identified within the DNA fragment, which putatively encoded type I PKS. The module and domain organizations of ORF1-2 were deduced (Fig. 1), and their conserved motifs were analyzed. The KS domains contain the Cys-His-His catalytic triad required for the decarboxylative condensation.[5] The ACP domains display the conserved L/IG(x)DS motif harboring the serine residue that was essential for 4′-phopspho-panthehenylation.[6] The 13 active site residues in all the ATs are QQGHSIGRFHTHV, indicating that they are specific for malonyl-CoA.[7] The KR domains contain the conserved consensus sequence G*X*G*XX*G*XX*A for NAD(P)H binding; KR1-3 harbor the conserved Trp141 and belong to A-type, catalyzing the formation of an *S*-configured alcohol; KR4-6 are B-type with the conserved LDD-motif being replaced with IDD in KR4 and LED in KR5-6, hence generating a *R*-configured alcohol (Table S1).[8] Functional DH domains featuring the conserved consensus sequence H*XXX*G*XXX*P are found for modules 4-6,[9] which catalyze elimination of water from *R*-configured hydroxyl group to form *E/Z*-configured double bond [10]. The above findings indicated that this DNA fragment was probably involved in the gene cluster biosynthesizing polyene-polyol compounds as predicted in Fig. 1.

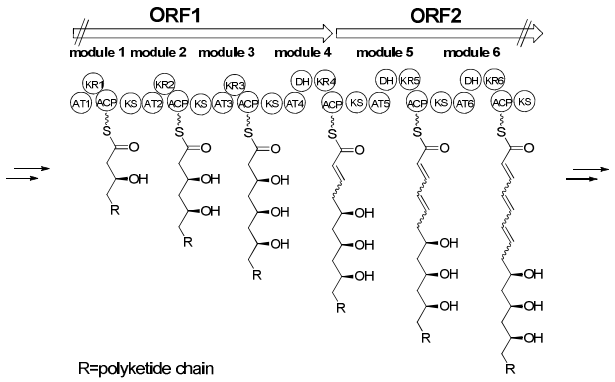


Fig. 1. Domain organization of the partial ORF1-2 and their proposed biosynthetic product.

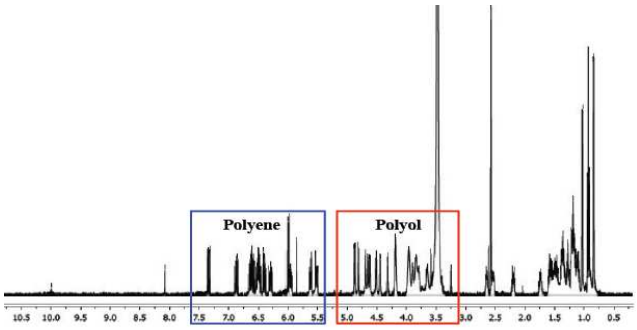
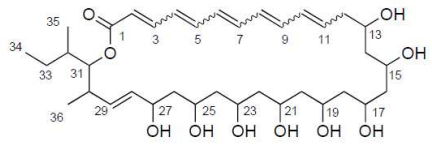


Fig. 2. The ^{1}H NMR Spectrum (600 MHz, in DMSO-d_6) of the fraction eluted by CH_2Cl_2/MeOH (5:1-1:1) on silica column.

With the noncytotoxic subfractions of the fermentation product in hand, the ^{1}H NMR was employed to detect whether the polyene-polyol-like compounds existed in the *Streptomyces* sp. CHQ-64 products. Delightedly, the fraction eluted by CH_2Cl_2/MeOH (5:1-1:1) on silica column, indeed showed the characteristic signals related to polyene-polyol structures (Fig. 2). This fraction was further investigated by further fractionation, leading to the isolation of six new polyene-polyol macrolides, named reedsmycins A-F (**1-6**) (Fig. 3).



1 $\Delta^{2,3} = E$; $\Delta^{4,5} = E$; $\Delta^{6,7} = E$; $\Delta^{8,9} = E$; $\Delta^{10,11} = E$
2 $\Delta^{2,3} = E$; $\Delta^{4,5} = E$; $\Delta^{6,7} = Z$; $\Delta^{8,9} = E$; $\Delta^{10,11} = E$
3 $\Delta^{2,3} = Z$; $\Delta^{4,5} = E$; $\Delta^{6,7} = E$; $\Delta^{8,9} = E$; $\Delta^{10,11} = E$
4 $\Delta^{2,3} = E$; $\Delta^{4,5} = E$; $\Delta^{6,7} = E$; $\Delta^{8,9} = E$; $\Delta^{10,11} = Z$
5 $\Delta^{2,3} = E$; $\Delta^{4,5} = Z$; $\Delta^{6,7} = E$; $\Delta^{8,9} = Z$; $\Delta^{10,11} = E$

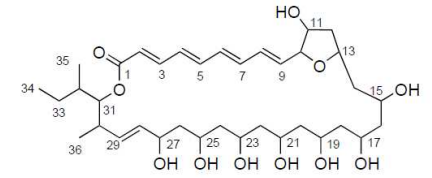


Fig. 3 The chemical structures of compounds **1-6**.

Reedsmycin A (**1**) was purified as a yellow powder and was determined to have the molecular formula $C_{36}H_{58}O_{10}$ by HRESIMS. The IR absorption at 1696 cm^{-1}, as well as the carbon signal observed at δ_C 166.1 in ^{13}C NMR spectrum, revealed the presence of an ester group in **1** (Table 1). The ^{1}H NMR spectrum of **1** clearly showed the feature of polyunsaturated and polyhydroxylated compounds, with 12 olefinic protons from 7.19 ppm and 5.41 ppm, and 9 protons of oxygenated methines between 4.77 and 3.55 ppm (Table 2). Further analysis of the ^{1}H NMR spectrum of **1** identified 20 aliphatic protons in the region of δ_H 2.58−1.02, three methyl groups at δ_H 0.98, 0.89, and 0.80 and eight exchangeable protons in the region of δ_H 4.71−4.08, which illustrated that reedsmycin A (**1**) possessed at least 8 hydroxyl groups.

The combination of COSY (Fig. 4) and HMQC experimental data indicated a conjugated pentaene moiety from H-2 (δ_H 5.87) to H-11 (δ_H 6.47), which was connected to a repeating 1,3-hydroxy group moiety from H-12 (δ_H 2.47, 2.13) to H-27 (δ_H 4.08), and further extended the spin system to H-34 (δ_H 0.89). The two methyls CH_3-35 (δ_H 0.80) and CH_3-36 (δ_H 0.98) were located on C-32 (δ_C 34.7) and C-30 (δ_C 35.6) based on the COSY correlations (H-35/H-32, H-36/H-30) and HMBC correlations from H-35 to C-31 and from H-36 to C-29, C-30 and C-31. Finally, the planar structure of **1** was determined as a macrolide by the HMBC correlations from H-2, H-3 (δ_H 7.19) and H-31 (δ_H 4.77) to the carbonyl carbon C-1 (δ_C 166.1) and named as reedsmycin A (**1**).

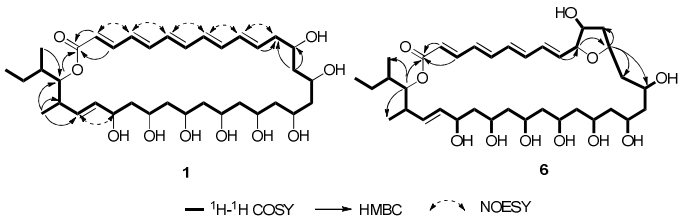


Fig. 4. Selected 2D NMR correlations for compounds **1** and **6**.

The geometries of the six double bonds (Δ^2 to Δ^{10} and Δ^{28}) were determined as *E* on the basis of their characteristically large coupling constants ($J \geq 14.0$ Hz) (Table 1) together with the NOESY correlations for H-3/H-5, H-5/H-7, H-7/H-9, H-2/H-4, H-4/H-6, H-6/H-8 and H-27/H-29.

The complex polyol segments and overlap of the ^{1}H NMR signals make the elucidation for the stereochemistry of the polyene-polyol macrolides a serious challenge. A literature survey showed that the attempts to assign the relative configuration of the polyol system were carried out including X-ray crystallography[11,12], application of Kishi's Universal NMR Database[13,14] or ^{13}C NMR acetonide analysis[15,16]. Unfortunately, none of the above methods worked properly for **1**, resulting in its configurations unclear.

Reedsmycin B (**2**) was obtained as a yellow powder. Its molecular formula was determined as $C_{36}H_{58}O_{10}$ on the basis of HRESIMS, requiring 8 degrees of unsaturation. Compound **2** showed the same features of the NMR, UV, and mass spectra with those of **1**, indicating a structurally related isomer of **1**. Detailed analysis of 1D- and 2D-NMR spectroscopy revealed that **2** had a smaller coupling constant ($^3J_{H\text{-}6,H\text{-}7}$ = 11.0 Hz) for $\Delta^{6,7}$, indicated the *Z* configuration of this double bond in **2** (Table 2). Similarly, reedsmycins C-E (**3-5**) were also structurally related isomers of **1** on the basis of IR, UV, HRESIMS, UV, ^{1}D and ^{2}D NMR spectroscopy. Comprehensive NMR analysis (Table 2) showed that the differences between these isomers (**3-5**) were the positions of *Z* geometric double bonds ($\Delta^{2,3} = Z$ (**3**), $\Delta^{10,11} = Z$ (**4**), and $\Delta^{4,5} = Z$ and $\Delta^{8,9} = Z$ (**5**), respectively). Compounds **3-5** were named as reedsmycins C-E, respectively.

Reedsmycin F (6) was obtained as a pale yellow powder. Its molecular formula was determined as $C_{36}H_{58}O_{11}$ according to the HRESIMS at *m/z* 689.3860 $[M + Na]^+$, indicating eight degrees of unsaturations and possessing one more oxygen than that of **1**. The general features of the NMR spectra of **6** were very similar to those of **1**, indicating that they are structurally related compounds. The main differences were observed at the positions of C-10 and C-11, where the two olefinic methines of **1** were replaced by two oxygenated methines in **6**. The UV absorption

maximum at 327 nm also revealed the smaller conjugated system in **6**. Since seven unsaturations were accounted for the five double bonds and the macrolide feature, **6** was inferred to contain an additional ring. The tetrahydrofuran (THF) ring was indicated by the ^{13}C chemical shifts at C-10 (δ_C 86.3) and C-13 (δ_C 75.8), the COSY correlations (H-10/H-11(OH-11)/H-12/H-13) and the HMBC correlations from H-10 (δ_H 4.17) to C-13. The NOESY correlations of H-10/OH-11 (δ_H 5.09) and H-13 (δ_H 4.34) /H-15 (δ_H 4.34) while no correlation for H-11 (δ_H 3.95)/H-13 implied the H-10/OH-11-*cis,* H-13/H-15-*cis* and the C-11/C-13-*trans* configurations in the THF ring.

Reedsmycin A (**1**) possessed a similar polyene-polyol feature to those antibiotics such as bahamaolides[17], marinisporolides[14], mycoticins[18], dermostatins[19], roflamycoin[20], and roxaticin[11], which were all isolated from *Streptomyces* species. Previous biosynthetic studies conducted with these oxo polyenes showed that they share similar biosynthetic pathway.[11] Based on the structure of reedsmycin A, it is proposed to be biosynthesized by 16 rounds of condensation probably using propionate as the start unit, 2 methylmalonyl CoA and 14 malonyl CoA as the extender units successively. As shown in Fig 1, the 28-kb DNA fragment is possibly responsible for the biosynthesis of the C5-C18 fragment of reedsmycin A, generating 3 *S*-configured hydroxyl groups (at C13, C15 and C17) and 3 *E*/*Z*-configured double bonds ($\Delta^{6,7}$, $\Delta^{8,9}$, and $\Delta^{10,11}$).

Polyene macrolides are potent antifungal agents that also exhibit a range of promising biological activities against parasites, enveloped viruses, tumor cells, prion diseases.[20,21] In our current research, all the compounds showed no cytotoxicities on K562, A549 and HL-60 tumor cell lines (IC_{50} > 50 μM). The antifungal activities of reedsmycins A-F (**1-6**) were also evaluated against *Candida albican*s using nystatin as the positive control[22] (Table 3). Compound **1** showed pronounced activity with MIC of 25-50 μM, which was stronger than those of the compounds containing *Z* double bond. Additionally, reedsmycin F (**6**), lack of one double bond but containing THF ring, exhibited no inhibitory activity. The results indicated that the number and geometry of the double bonds are essential to the antifungal activity.

Experimental Section

General

Specific rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on Beckman DU 640 spectrophotometer. CD spectra were measured on JASCO J-715 spectropolarimeter. IR spectra were taken on a Nicolet Nexus 470 spectrophotometer in KBr discs. NMR spectra were recorded on a JEOL JNM-ECP 600 and Bruker-400 spectrometers using TMS as internal standard, and chemical shifts were recorded as δ values. ESIMS utilized on a Thermo Scientific LTQ Orbitrap XL mass spectrometer. Semipreparative HPLC was performed using an ODS column [HPLC (YMC-Pack ODS-A, 10 × 250 mm, 5 μm, 4 mL/min)]. TLC and column chromatography (CC) were performed on plates precoated with silica gel GF254 (10–40 μm) and over silica gel (200–300 mesh, Qingdao Marine Chemical Factory), and Sephadex LH-20 (Amersham Biosciences), respectively. Vacuum-liquid chromatography (VLC) was carried out over silica gel H (Qingdao Marine Chemical Factory). Marinum salt used is made from the evaporation of sea water collected in Laizhou Bay (Weifang Haisheng Chemical Factory).

Strains and culture conditions

Streptomyces sp. CHQ-64（Former name: AH1-5) was isolated from reeds rhizosphere soil collected from the mangrove conservation area of Guangdong province, China. The voucher specimen is deposited in our laboratory at –80°C. The producing strain was prepared on ISP-2 agar slants at 3.3% salt concentration and stored at 4°C. For biological assays, *Candida albicans* was cultured overnight at 28 °C in MH broth.

Bioinformatic analysis

The sequence was analyzed for putative *orfs* with the FramePlot 2.3.2 program.[23] The proposed function of the *orfs* was accomplished by using the Blast programs.[24]. The module and domain organizations of ORF1-2 were deduced by SBSPKS analysis.[25]

Nucleotide Sequence Accession Number

The nucleotide sequence reported in this paper has been deposited in the GenBank database under accession number KM411608.

Purification

The crude extract (35.5 g) was subjected to vacuum liquid chromatography over a silica gel (200–300 mesh) column using stepwise gradient elution with the mixtures of petroleum ether-$CHCl_3$-MeOH to give eight fractions. Fraction 7 (0.5 g) was purified by repeated ODS CC to afford six subfractions (fractions 7.1-7.6). Fraction 7.3 was further purified on Sephadex LH-20 and semipreparative HPLC (75% MeOH) to give compound **6** (2 mg, t_R 12 min), compound **5** (10 mg, t_R 21 min), compound **2** (3 mg, t_R 22 min), compound **3** (2 mg, t_R 24 min), compound **4** (2 mg, t_R 25 min) and compound **1** (20 mg, t_R 29 min), respectively.

Table 1. ^{13}C NMR Data for Compounds **1-6** (recorded in DMSO-d_6)

	1[a]	**2**[b]	**3**[b]	**4**[b]	**5**[a]	**6**[b]
position	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type
1	166.1, qC	166.4, qC	166.0, qC	166.8, qC	166.0, qC	166.7, qC
2	120.4, CH	120.9, CH	117.7, CH	120.9, CH	121.2, CH	121.3, CH
3	144.5, CH	137.5, CH	139.5, CH	145.3, CH	138.9, CH	144.9, CH
4	129.8, CH	130.5, CH	124.3, CH	131.3, CH	125.8, CH	130.6, CH
5	140.9, CH	145.3, CH	138.2, CH	141.6, CH	137.3, CH	141.3, CH
6	135.7, CH	128.4, CH	126.9, CH	132.6, CH	126.8, CH	132.1, CH

7	130.6, CH	134.7, CH	138.6, CH	138.2, CH	137.7, CH	137.4, CH
8	137.6, CH	127.1, CH	131.1, CH	133.1, CH	130.9, CH	129.6, CH
9	131.4, CH	136.4, CH	136.5, CH	131.4, CH	135.8, CH	137.2, CH
10	132.0, CH	132.8, CH	132.6, CH	130.5, CH	132.6, CH	86.3, CH
11	133.7, CH	133.9, CH	134.3, CH	130.8, CH	133.1, CH	75.7, CH
12	42.2, CH_2	39.7, CH_2	42.7, CH_2	44.3, CH_2	38.7, CH_2	39.6, CH_2
13	67.3, CH	66.7, CH	68.6, CH	66.4, CH	(66.8-63.5)[d], CH	75.8, CH
14	45.7, CH_2	46.7, CH_2	46.8, CH_2	(47.8-44.5)[d], CH_2	(45.8-40.3)[d], CH_2	41.9, CH_2
15	65.6, CH	67.9, CH	66.9, CH	65.5, CH	(66.8-63.5)[d], CH	65.7, CH
16	47.5, CH_2	46.2[c], CH_2	46.2, CH_2	(47.8-44.5)[d], CH_2	(45.8-40.3)[d], CH_2	(47.2-46.0)[d], CH_2
17	64.3, CH	63.6, CH	65.5, CH	67.0, CH	(66.8-63.5)[d], CH	65.9, CH
18	45.9, CH_2	46.0[c], CH_2	45.6, CH_2	(47.8-44.5)[d], CH_2	(45.8-40.3)[d], CH_2	(47.2-46.0)[d], CH_2
19	66.1, CH	67.5, CH	67.0, CH	63.2, CH	(66.8-63.5)[d], CH	64.3, CH
20	46.5, CH_2	46.2[c], CH_2	45.1, CH_2	(47.8-44.5)[d], CH_2	(45.8-40.3)[d], CH_2	(47.2-46.0)[d], CH_2
21	66.6, CH	66.5, CH	63.6, CH	66.4, CH	(66.8-63.5)[d], CH	66.5, CH
22	42.3, CH_2	44.2, CH_2	46.5, CH_2	(47.8-44.5)[d], CH_2	(45.8-40.3)[d], CH_2	(47.2-46.0)[d], CH_2
23	65.4, CH	68.1, CH	66.6, CH	65.2, CH	(66.8-63.5)[d], CH	66.8, CH
24	46.1, CH_2	45.2, CH_2	47.8, CH_2	(47.8-44.5)[d], CH_2	(45.8-40.3)[d], CH_2	(47.2-46.0)[d], CH_2
25	62.9, CH	65.7, CH	64.9, CH	66.1, CH	(66.8-63.5)[d], CH	65.2, CH
26	45.6, CH_2	45.6, CH_2	45.9, CH_2	(47.8-44.5)[d], CH_2	(45.8-40.3)[d], CH_2	(47.2-46.0)[d], CH_2
27	69.2, CH	69.7, CH	69.6, CH	69.4, CH	69.3, CH	69.1, CH
28	133.3, CH	134.7, CH	134.0, CH	134.1, CH	134.1, CH	134.2, CH
29	129.6, CH	132.2, CH	130.7, CH	130.8, CH	131.5, CH	130.5, CH
30	35.6, CH	38.7, CH	36.1, CH	36.2, CH	37.6, CH	36.5, CH
31	77.8, CH	78.7, CH	78.5, CH	79.0, CH	78.6, CH	78.3, CH
32	34.7, CH	36.3, CH	35.4, CH	35.5, CH	35.8, CH	35.3, CH
33	24.9, CH_2	26.4, CH_2	25.3, CH_2	25.1, CH_2	25.5, CH_2	25.7, CH_2
34	10.7, CH_3	11.8, CH_3	11.1, CH_3	11.1, CH_3	11.1, CH_3	11.3, CH_3
35	15.5, CH_3	16.4, CH_3	16.0, CH_3	15.9, CH_3	15.1, CH_3	16.0, CH_3
36	11.6, CH_3	14.3, CH_3	12.2, CH_3	12.3, CH_3	14.0, CH_3	12.7, CH_3

[a] Spectra were recorded at 100 MHz for ^{13}C NMR using TMS as internal standard.
[b] Spectra were recorded at 150 MHz for ^{13}C NMR using TMS as internal standard.
[c] Interchangeable within column.
[d] Signals could not be individually assigned.

Table 2. ^{1}H NMR Data for Compounds **1-6** (recorded in DMSO-d_6)

	1[b]	2[a]	3[a]	4[a]	5[b]	6[a]
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
2	5.87, d (14.8)	5.93, d (15.4)	5.65, d (11.5)	5.94, d (15.4)	5.98, d (15.0)	5.95, d (15.4)
3	7.19, dd (11.5, 15.4)	7.36, dd (11.0, 14.9)	7.24, t (11.5, 12.1)	7.24, dd (11.6, 15.4)	7.71, dd (12.3, 15.3)	7.21, dd (11.5, 15.4)
4	6.44, dd (11.6, 14.8)	6.46, dd (11.0, 14.3)	7.16, t (10.4, 12.7)	6.46, dd (11.0, 15.4)	6.20, t (11.4, 11.5)	6.47, m
5	6.73, dd (11.0, 14.3)	7.35, dd (11.5, 15.4)	6.44, t (11.0, 13.2)	6.77, dd (11.6, 14.6)	6.47, dd (11.5, 11.9)	6.72, dd (11.0, 14.9)
6	6.37, dd (10.4, 14.8)	6.12, t (11.0, 11.0)	6.89, t (13.7, 13.1)	6.38, t (12.1, 14.3)	6.84, t (12.8, 13.3)	6.38, dd (11.0, 14.9)
7	6.25, dd (11.5, 14.8)	6.28, t (11.0, 11.6)	6.50, t (12.1, 13.2)	6.56, dd (11.0, 14.8)	6.54, dd (9.9, 14.7)	6.47, m
8	6.49, dd (11.5, 14.8)	6.89, dd (13.7, 12.7)	6.30, t (12.1, 13.7)	6.36, t (11.6, 14.3)	6.40, t (10.0, 10.7)	6.32, dd (11.5, 14.9)
9	6.34, dd (11.0, 14.3)	6.40, dd (10.4, 14.9)	6.39, t (9.9, 16.5)	6.64, dd (12.1, 14.3)	6.40, t (10.0, 10.7)	5.94, m
10	6.16, dd (10.4, 14.8)	6.20, dd (11.0, 15.4)	6.17, t (11.6, 14.3)	6.20, t (11.0, 11.5)	6.16, m	4.17, m
11	6.47, m	5.92, m	5.82, m	5.68, m	5.90, m	3.95, m
12	2.47, m ; 2.13, m	2.31, m ; 2.23, m	2.44, m ; 2.12, m	2.66, m ; 2.20, m	2.36, m	1.78, dd (6.6, 11.5) ; 1.62, m
13	3.81, m	3.86, m	3.78, m	3.79, m	(3.45-3.95)[c], m	4.34, d (5.0)
14	1.54, m; 1.38, m	(1.51-1.20)[c], m	1.58, m; 1.33, m	(1.60-1.10)[c], m	(1.60-1.10)[c], m	1.66, m; 1.54, m

15	3.55, m	3.70, m	(3.82-3.90)[c], m	3.67, m	(3.45-3.95)[c], m	3.70, m
16	1.54, m; 1.23, m	(1.51-1.20)[c], m	1.10, m	(1.60-1.10)[c], m	(1.60-1.10)[c], m	(1.70-1.10)[c], m
17	3.71, m	3.90, m	(3.82-3.90)[c], m	3.93, m	(3.45-3.95)[c], m	3.78, m
18	1.14, m; 1.06, m	(1.51-1.20)[c], m	1.45, m; 1.16, m	(1.60-1.10)[c], m	(1.60-1.10)[c], m	(1.70-1.10)[c], m
19	3.88, m	3.88, m	(3.82-3.90)[c], m	3.91, m	(3.45-3.95)[c], m	3.91, m
20	1.14, m	(1.51-1.20)[c], m	1.19, m	(1.60-1.10)[c], m	(1.60-1.10)[c], m	(1.70-1.10)[c], m
21	3.74, m	3.87, m	(3.82-3.90)[c], m	3.87, m	(3.45-3.95)[c], m	3.83, m
22	1.31, m; 1.10, m	1.43, m; 1.40, m	1.29, m	(1.60-1.10)[c], m	(1.60-1.10)[c], m	(1.70-1.10)[c], m
23	3.75, m	3.82, m	3.62, m	3.84, m	(3.45-3.95)[c], m	3.89, m
24	1.28, m; 1.12, m	1.49, m; 1.42, m	1.52, m; 1.23, m	(1.60-1.10)[c], m	(1.60-1.10)[c], m	(1.70-1.10)[c], m
25	3.89, m	3.57, m	3.68, m	3.41, m	(3.45-3.95)[c], m	3.72, m
26	1.17, m	1.51, m; 1.35, m	1.34, m; 1.15, m	(1.60-1.10)[c], m	(1.60-1.10)[c], m	(1.70-1.10)[c], m
27	4.08, m	4.05, m	4.02, m	4.01, m	4.07, m	4.07, m
28	5.41, dd (4.4, 16.0)	5.39, m	5.45, m	5.43, m	5.42, m	5.41, dd (4.9, 16.0)
29	5.51, dd (5.0, 16.0)	5.40, m	5.45, m	5.43, m	5.42, m	5.49, dd (6.0, 15.4)
30	2.58, m	2.49, m	2.57, m	2.56, m	2.55, m	2.57, m
31	4.77, dd (2.8, 9.4)	4.76, t (5.5, 6.6)	4.76, d (9.3)	4.76, dd (2.0, 12.1)	4.77, t (5.8, 6.3)	4.77, dd (2.8, 8.8)
32	1.68, m	1.65, m	1.68, m	1.67, m	1.66, m	1.63, m
33	1.44, m; 1.15, m	1.26, m; 1.10, m	1.47, m; 1.15, m	1.49, m; 1.15, m	1.32, m; 1.10, m	1.44, m; 1.52, m
34	0.89, t (7.1, 7.7)	0.86, t (7.1, 7.7)	0.89, t (6.5, 7.7)	0.89, t (7.1, 7.7)	0.87, t (7.4, 8.8)	0.88, t (7.2, 7.7)
35	0.80, d (6.6)	0.86, d (4.4)	0.80, d (6.0)	0.77, d (6.6)	0.85, d (5.8)	0.79, d (6.6)
36	0.98, d (6.6)	0.92, d (7.1)	0.97, d (6.1)	0.97, d (6.6)	0.93, d (6.7)	0.99, d (6.6)

[a] Spectra were recorded at 600 MHz for ^{1}H NMR using TMS as internal standard.
[b] Spectra were recorded at 400 MHz for ^{1}H NMR using TMS as internal standard.
[c] Signals could not be individually assigned.

Table 3. Antifungal Activities of the Tested Compounds

	Compounds						
Candida albicans	**1**	**2**	**3**	**4**	**5**	**6**	nystatin
MIC (μM)	25-50	100-200	50-100	50-100	50-100	> 200	25-50

Reedsmycin A (1): yellow powder, $[\alpha]^{24}_D$ - 35.4 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3380, 2938, 1696, 1575, 1127, 1012 cm^{-1}; UV (MeOH) λ_{max} (log ε) 369 (1.09); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 673.3917 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_{10}Na$, 673.3922).

Reedsmycin B (2): yellow powder, $[\alpha]^{24}_D$ - 89.8 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3419, 2935, 1628, 1429, 1383, 1260, 1126 cm^{-1}; UV (MeOH) λ_{max} (log ε) 364 (1.11); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 673.3911 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_{10}Na$, 673.3922).

Reedsmycin C (3): yellow powder, $[\alpha]^{24}_D$ - 83.4 (*c* 0.08, MeOH); IR (KBr) ν_{max} 3382, 2937, 1689, 1578, 1427, 1381, 1299, 1126 cm^{-1}; UV (MeOH) λ_{max} (log ε) 363 (1.04); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 673.3920 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_{10}Na$, 673.3922).

Reedsmycin D (4): yellow powder, $[\alpha]^{24}_D$ - 75.8 (*c* 0.09, MeOH); IR (KBr) ν_{max} 3381, 2937, 1617, 1577, 1127, 1012 cm^{-1}; UV (MeOH) λ_{max} (log ε) 366 (1.04); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 673.3912 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_{10}Na$, 673.3922).

Reedsmycin E (5): yellow powder, $[\alpha]^{24}_D$ -157.0 (*c* 0.07, MeOH); IR (KBr) ν_{max} 3381, 2937, 1700, 1616, 1577, 1427, 1379, 1297, 1128 cm^{-1}; UV (MeOH) λ_{max} (log ε) 366 (1.09); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 673.3911 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_{10}Na$, 673.3922).

Reedsmycin F (6): pale yellow powder, $[\alpha]^{24}_D$ + 58.9 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3383, 2936, 1699, 1595, 1427, 1123, 1092, 1011 cm^{-1}; UV (MeOH) λ_{max} (log ε) 327 (1.05); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 689.3860 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_{11}Na$, 689.3817).

Bioassays

The antifungal activity of the compounds were tested against *Candida albicans.* Solutions of the compounds were made up in

methanol and dispensed into 96-well plates using the 2 × microdilution method to give seven concentrations in the range of 200–3.125 μmol for each compound. MH broth was used as a blank control, and methanol was used as a negative control, while nystatin was used as a positive control. The bioassay was replicated three times for each compound. The plates were incubated for 72 h at 28 °C in the dark. Fungal growth in the wells was visually examined and the lowest concentration that inhibited hyphal growth in all the replicates was recorded as the MIC. [22]

Conclusions

In conclusion, inspired by the genome scanning result, six new skipped-polyol polyene macrolides were isolated from the noncytotoxic fractions of the fermentation products of *Streptomyces* sp. CHQ-64. Reedsmycin F (**6**) possessed a rare tetrahydrofuran ring in the structure compared with other known polyene-polyol macrolides. Reedsmycin A (**1**) showed promising antifungal activity. This genome-guided strategy was more efficient than the traditional methods, and would be applicable for other compounds with characteristic structures.

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Notes and references

[a] *Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, People's Republic of China.*

[b] *College of Marine Life Sciences, Ocean University of China, Qingdao 266003, People's Republic of China*

[§] *These authors have contributed equally to this work.*

**To whom correspondence should be addressed. Tel: 0086-532-82031632 and Fax: 0086-532-82033054. E-mail: dehaili@ouc.edu.cn (D. Li), liwenli@ouc.edu.cn (W. Li) and zhutj@ouc.edu.cn (T. Zhu).*

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