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ORIGINAL ARTICLE

New isofuranonaphthoquinones and isoindolequinones from *Streptomyces* sp. CB01883

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Isofuranonaphthoquinones (IFQs) and Isoindolequinones (IIQs) comprise a small family of natural products, with the latter ones are especially uncommon in nature. Here we report the discovery of seven new IFQs, IFQ A-G (1–7), and three new IIQs, IIQ A-C (8–10), along with the known anthraquinone desoxyerythrolaccin (11), from *Streptomyces* sp. CB01883, expanding the chemical diversity of this family of natural products. The structures of these natural products were established on the basis of their HR-ESI-MS and nuclear magnetic resonance (NMR) spectroscopic data. All compounds were assessed for antibacterial activity, with 11 and 1, 5–7 exhibiting moderate and weak activities, respectively, against several Gram-positive bacteria tested. Bioinformatics analysis of the *Streptomyces* sp. CB01883 genome revealed the *ifq* gene cluster that showed identical genetic organization, with high-sequence identity, to the *ifn* gene cluster recently cloned from *Streptomyces* sp. RI-77 and confirmed to encode the biosynthesis of two IFQs, JBIR-76 and JBIR-77. Co-isolation of IFQs with IIQs from *Streptomyces* sp. CB01883 and facile chemical transformation of selected IFQs to IIQs, as exemplified by 1 to 9, together with the finding of the *ifq* cluster that most likely only encodes IFQ biosynthesis, support the proposal that IIQs may be derived nonenzymatically from IFQs in the presence of an amine.
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INTRODUCTION

Isofuranonaphthoquinones (IFQs) and isoindolequinones (IIQs) comprise a small family of natural products featuring a characteristic tricyclic naphtho[2,3-*c*]furan(or pyrrole)-4,9-dione ring scaffold, with varying substitutions at rings A, B or C (Figure 1a).[1] The first reported IFQ natural product was isolated from the fungus *Nectria haematococca* in 1983.[2] To date, the majority of this family of natural products are produced by fungi and plants.[1] Actinobacteria, especially the genus *Streptomyces*, have also been shown to be sources of IFQs and IIQs.[3,4] The first IIQs, featuring the 2*H*-benzo[*f*]isoindole-4, 9-dione scaffold, were bhimamycin C and D, isolated from *Streptomyces* sp. GW32/698 in 2003.[3] The IFQs have been reported to have antibacterial, antioxidant, antiplasmodial, cytotoxic and Fe(III) chelation activities.[3–6]

Nothing was known about IFQ biosynthesis until the recent cloning and characterization of the *ifn* gene cluster from *Streptomyces* sp. RI-77, which was confirmed experimentally to encode the production of two IFQs JBIR-76 and JBIR-77 (Figure 2a).[5,6] JBIR-76 and JBIR-77 biosynthesis featured a type II polyketide synthase that assembles an octaketide intermediate from malonyl CoA precursors and a Baeyer-Villiger monooxygenase that catalyzes a key C–C bond cleavage, affording the characteristic IFQ scaffold of JBIR-76 and JBIR-77 (Figure 2b).[6] The biosynthetic origin of IIQs and their relationship to IFQs have not been addressed. It is worth noting that IIQs are always co-isolated with related IFQs and have been prepared upon heating the related IFQs with an amine (albeit in low yields).[3] These observations have raised the question if IIQs are artifacts of isolation that could be derived spontaneously from IFQs in the presence of an amine.

The Natural Products Library Initiative (NPLI) at the Scripps Research Institute (TSRI) aims at constructing a natural products library with unique chemical and structural diversity that complements the small-molecule collection at TSRI. The NPLI biases natural products from Actinomycetales that are isolated from unexplored or underexplored ecological niches and unavailable in public strain collections.[7–17] The current library at TSRI consists of (i) purified natural products with fully assigned structures, (ii) C-18 medium-pressure liquid chromatography fractions, and (iii) crude extracts of microbial fermentation. Typically, strains were fermented in multiple media and subjected to HPLC and LC-MS analysis. Those with HPLC profiles showing rich chemical diversity are given high priority for further natural products isolation and structural elucidation.

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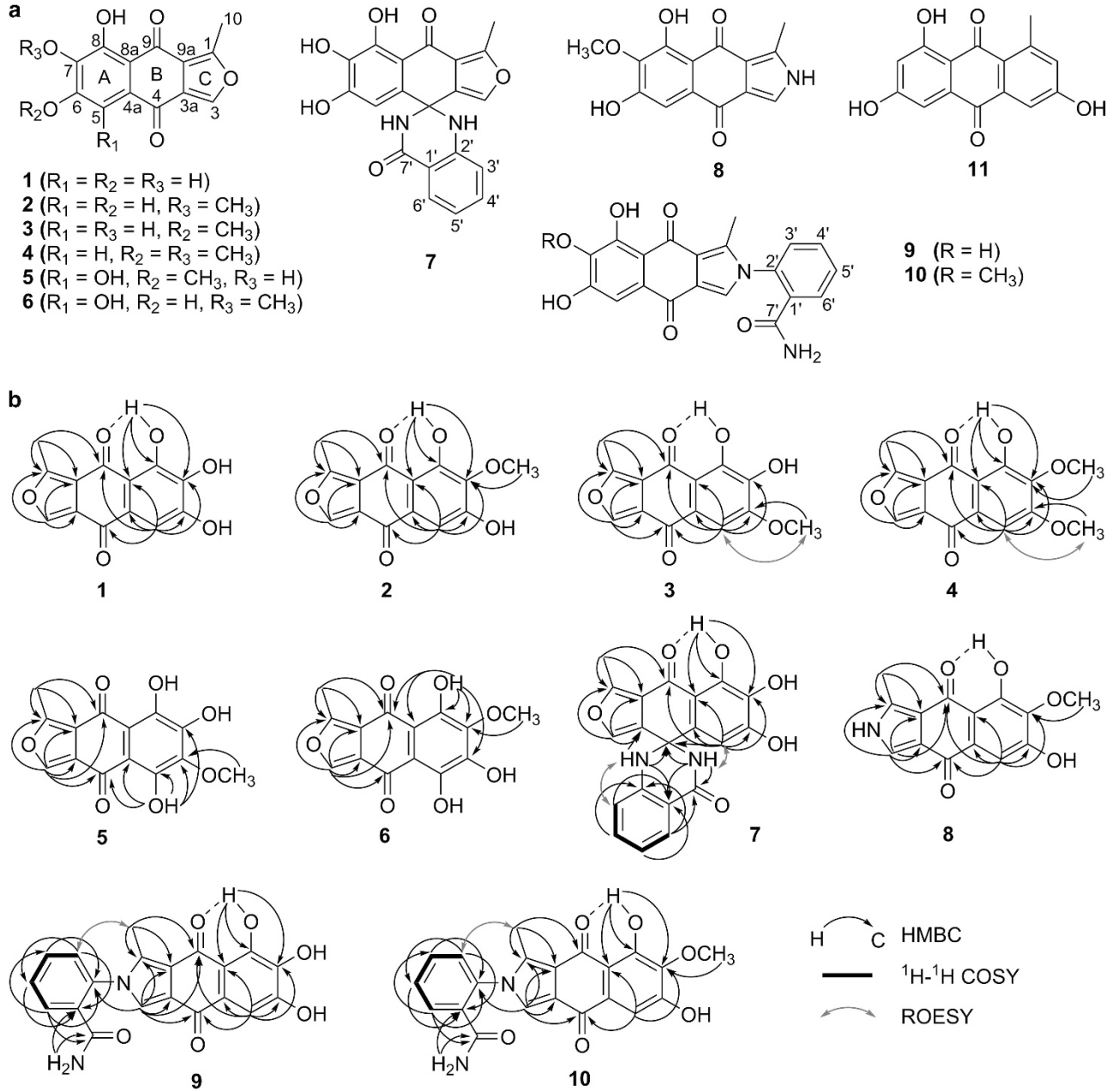

Figure 1 The seven isofuranonaphthoquinones (IFQs; **1**–**7**), three isoindolequinones (IIQs; **8**–**10**) and the known anthraquinone (**11**) from *Streptomyces* sp. SB3501. (**a**) Structures of IFQ A-G (**1**–**7**), IIQ A-C (**8**–**10**) and desoxyerythrolaccin (**11**). (**b**) The key ^{1}H–^{1}H COSY, ROESY and HMBC correlations supporting the structures of IFQ A-G (**1**–**7**) and IIQ A-C (**8**–**10**). A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Here we report the discovery of seven new IFQs, IFQ A-G (**1**–**7**) and three new IIQs, IIQ A-C (**8**–**10**), along with the known anthraquinone desoxyerythrolaccin (**11**),[18] from *Streptomyces* sp. SB3501, a mutant strain of *Streptomyces* sp. CB01883. The new IFQs and IIQs feature varying substitutions at the A-ring (**1**–**6**), B-ring (**7**) and C-ring (**8**–**10**). All compounds (**1**–**11**) were assayed for antibacterial activities, with **11** and **1**, **5**–**7** exhibiting moderate and weak activities, respectively, against several Gram-positive bacteria tested. The *ifq* (isofuranonaphthoquinone) gene cluster encoding **1**–**6** biosynthesis was identified by mining the *Streptomyces* sp. CB01883 genome, facilitated by the *ifn* gene cluster recently cloned for JBIR-76 and JBIR-77 biosynthesis in *Streptomyces* sp. RI-77.[6] We also showed that simply mixing IFQ A (**1**) with anthranilamide in refluxing ethanol afforded IIQ B (**9**) and the reaction could be accelerated upon acid catalysis, supporting the proposal that IIQs may be derived nonenzymatically from IFQs in the presence of an amine.

RESULTS AND DISCUSSION

Strain selection, taxonomy and fermentation optimization

Streptomyces sp. CB01883 was isolated from a forest soil sample collected in Guangnan County, Yunnan Province, China. It grows and sporulates well on ISP4 agar medium, and was classified as a *Streptomyces* species on the basis of a phylogenetic analysis using the concatenated partial sequences of three housekeeping genes 16 S rRNA, *rpoB* and *trpB* (Genebank accession number KT722854, KT736417 and KT793843, respectively).[14–16] In our continued effort

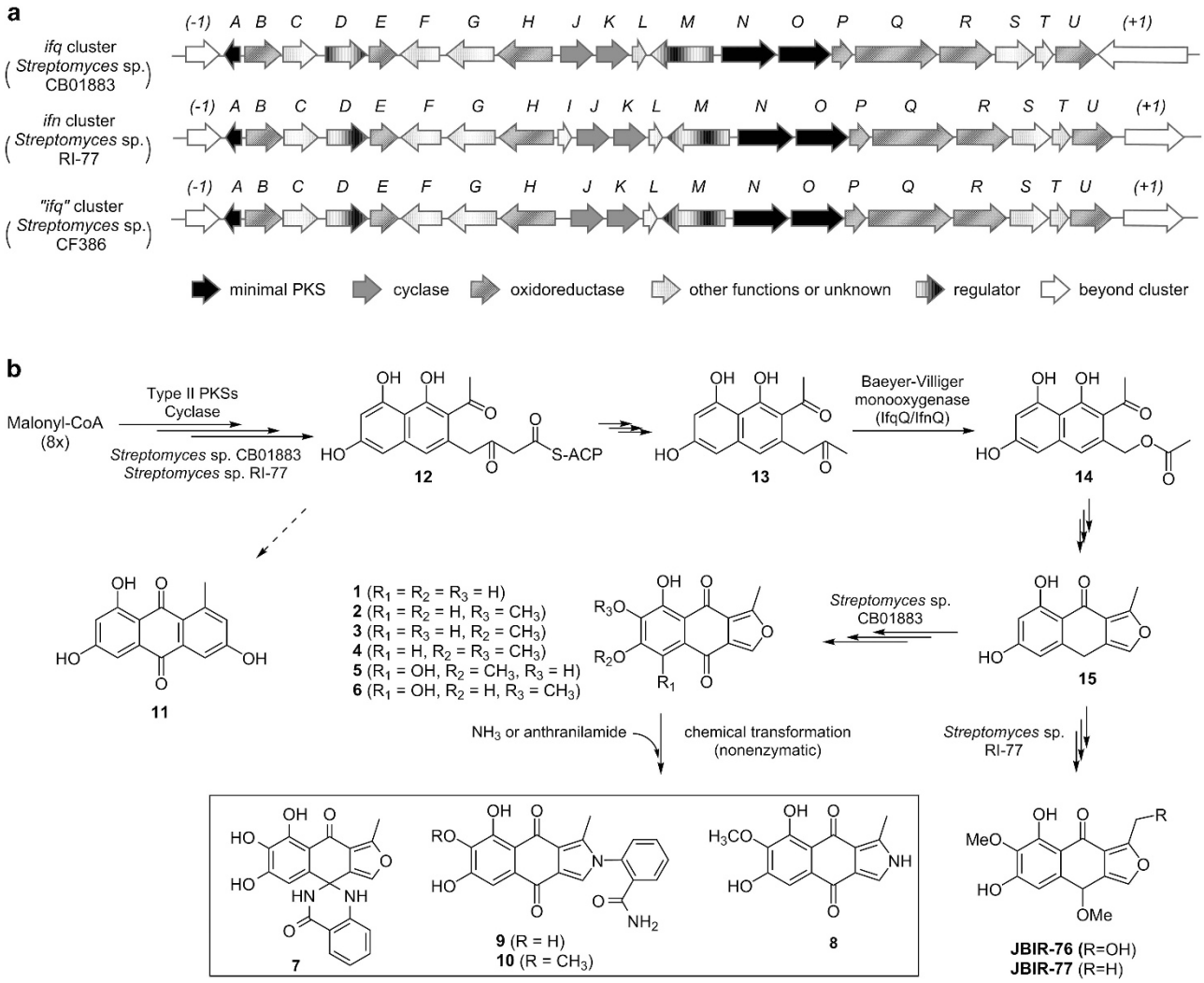

Figure 2 Biosynthesis of isofuranonaphthoquinones (IFQs) and the origin and biosynthetic relationship of isoindolequinones (IIQs) to IFQs. (**a**) Genetic organization and comparison of confirmed (*ifn* from *Streptomyces* sp. RI-77), proposed (*ifq* from *Streptomyces* sp. CB01883) and putative ('*ifq*' from *Streptomyces* sp. CF386) gene clusters for IFQ biosynthesis. (**b**) A unified pathway for IFQ biosynthesis, featuring common intermediates **12**, **13**, **14** and **15** and divergence from **15** to JBIR-76 and JBIR-77 in *Streptomyces* sp. RI77 and **1**–**6** in *Streptomyces* sp. CB01883, as well as chemical transformation of IFQs in the presence of amines to account for IIQ formation. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

to study the biosynthesis of hybrid peptide-polyketide natural products,[19–21] we sequenced the genome of *Streptomyces* sp. CB01883 and deleted a gene from a hybrid peptide-polyketide natural product biosynthetic gene cluster to generate the *Streptomyces* sp. SB3501 mutant strain. *Streptomyces* sp. SB3501 was fermented in four media (Supplementary Table S1), and their crude extracts were subjected to HPLC and LC-MS analysis, revealing rich metabolite profiles (Supplementary Figure S1). *Streptomyces* sp. SB3501 therefore was given high priority for natural products isolation, aiming at discovering new natural products.

The fermentation of *Streptomyces* sp. SB3501 in medium M1 yielded the richest metabolite profile that included a major metabolite with $[M+H]^+$ ion at *m/z* 261, which did not match the natural products in our current library at the time, indicative of a new natural product. To isolate and identify this major metabolite, we first made a crude extract from 1-L fermentation of *Streptomyces* sp. SB3501 in medium M1 and subjected it to a combination of silica gel, Sephadex LH-20 and C-18 chromatography, resulting in the isolation and identification of this metabolite, named as IFQ A (**1**), a new member of the IFQ family of natural products. We also compared the metabolite profiles of *Streptomyces* sp. CB01883 wild-type and SB3501 mutant strains in medium M1. Although **1** was produced by both strains, its titer in *Streptomyces* sp. SB3501 was significantly higher than that from the *Streptomyces* sp. CB01883 wild type (Supplementary Figure S2), hence the choice of *Streptomyces* sp. SB3501 as the preferred strain for larger scale fermentation and natural product production. Thus, from 20-L fermentation of *Streptomyces* sp. SB3501 in medium M1, we subsequently isolated six additional IFQs, named IFQ B-G (**2**–**7**), three IIQs, named IIQ A-C (**8**–**10**) and a known anthraquinone desoxyerythrolaccin (**11**),[18] together with **1** as the major metabolite.

Structural elucidation

IFQ A (**1**) was isolated as a yellow solid. The molecular formula of **1** was determined as $C_{13}H_8O_6$ on the basis of HR-ESI-MS analysis, affording an $[M+H]^+$ ion at *m/z* 261.0397 (calculated for $[M+H]^+$ ion at *m/z* 261.0394), and ^1H and ^{13}C NMR data (Tables 1 and 2). Initial interpretation of the MS, ^1H NMR, ^{13}C NMR, HSQC and HMBC spectra (Figure 1b) suggested that the structure of **1** was similar to a

Table 1 ^{1}H (700 MHz) NMR data (δ_H, *J* in Hz) for 1–6 in DMSO-d_6[a]

Position	***1***	***2***	***3***	***4*** [b]	***5***	***6***
3	8.47, s	8.58, s	8.50, s	8.04, s	8.57, s	8.61, s
5	7.20, s	7.25, s	7.30, s	7.46, s		
10	2.69, s	2.73, s	2.69, s	2.78, s	2.71, s	2.69, s
5-OH					13.62, s	13.21, br s
6-OH	9.82[c], br s	11.07, br s				10.89, br s
6-OCH_3			3.95, s	4.05, s	3.88, s	
7-OH	10.70[c], br s		10.50, brs		11.08, br s	
7-OCH_3		3.87, s		4.03, s		3.89, s
8-OH	12.98, s	13.18, s	12.33, s	13.08, s	13.33, br s	13.60, s

Abbreviations: DMSO, dimethyl sulfoxide; NMR, nuclear magnetic resonance.
[a]Assignments were based on HSQC, HMBC, ^{1}H–^{1}H COSY and ROESY correlations.
[b]NMR data were recorded in $CDCl_3$.
[c]Interchangeable.

Table 2 ^{13}C (175 MHz) NMR data (δ_C) for 1–6 in DMSO-d_6[a]

Position	***1***	***2***	***3***	***4***[b]	***5***	***6***
1	159.9	160.1	160.2	159.9	160.4	159.9
3	145.1	145.7	145.2	143.6	145.2	145.9
3a	123.2	122.9	123.0	122.9	123.0	122.8
4	178.1	178.3	177.8	178.6	182.5	183.9
4a	127.5	131.8	126.9	131.5	107.2	111.0
5	108.8	108.9	104.2	103.8	154.8	150.0
6	151.9	157.3	151.7	158.1	141.1	148.0
7	139.1	139.9	140.7	141.4	149.4	141.5
8	152.7	158.0	152.7	157.3	150.3	154.4
8a	112.4	112.5	113.7	113.9	111.0	107.6
9	185.8	185.6	186.1	185.3	185.1	184.2
9a	116.7	116.7	116.6	116.8	116.4	116.3
10	14.1	14.1	14.1	14.0	14.1	14.0
6-OCH_3			56.6	56.5	60.7	
7-OCH_3		60.5		61.0		60.8

Abbreviations: DMSO, dimethyl sulfoxide; NMR, nuclear magnetic resonance.
[a]Assignments were based on HSQC, HMBC, ^{1}H-^{1}H COSY, and ROESY correlations.
[b]NMR data were recorded in $CDCl_3$.

known IFQ from an *Actinoplanes* isolate,[4] with the exception of an additional phenolic group on the A-ring of **1**. Further observation of HMBC correlations from H-5 (δ_H 7.20) to C-4 (δ_C 178.1), C-4a (δ_C 127.5), C-6 (δ_C 151.9), C-7 (δ_C 139.1) and C-8a (δ_C 112.4) established the phenolic group at C-6. Taken together, the combination of HR-ESI-MS and 1D and 2D NMR analysis unambiguously established the structure of **1** (Figure 1a).

IFQ B (**2**) was obtained as a yellow solid. HR-ESI-MS analysis yielded an $[M+H]^+$ ion at *m/z* 275.0551, giving the molecular formula of **2** as $C_{14}H_{10}O_6$ (calculated for $[M+H]^+$ ion at *m/z* 275.0550). The ultraviolet (UV) and infrared spectroscopy (IR) spectra of **2** resembled those of **1**, and most of the ^{1}H and ^{13}C NMR data of **2** were highly similar to those of **1** (Tables 1 and 2). In the ^{1}H NMR, ^{13}C NMR and HSQC spectra of **2**, additional signals attributed to a methoxyl group (δ_H 3.87 and δ_C 60.5) were observed, indicating one hydroxyl group in the A-ring was replaced by a methoxyl group. This was confirmed by the HMBC correlations from 7-OCH_3 to C-7 (δ_C 139.9) and from 8-OH (δ_H 13.18) to C-7 (δ_C 139.9), C-8 (δ_C 158.0) and C-8a (δ_C 112.5; Figure 1b), hence the unambiguous assignment of the **2** structure (Figure 1a).

IFQ C (**3**) was purified as a yellow solid. HR-ESI-MS gave an $[M+H]^+$ ion at *m/z* 275.0555, consistent with the molecular formula $C_{14}H_{10}O_6$ (calculated for $[M+H]^+$ ion at *m/z* 275.0550), which was the same as **2**. The ^{1}H and ^{13}C NMR spectra of **3** resembled those of **2**, and the major differences observed in the NMR spectra could be attributed to the different position of the methoxyl group (Tables 1 and 2). HMBC and ROESY correlations of **3** provided evidence for the methoxyl group (δ_H 3.95, δ_C 56.6) to be at C-6 of the A-ring. Specifically, HMBC correlations were observed from H-5 (δ_H 7.30) to C-4 (δ_C 177.8), C-4a (δ_C 126.9), C-6 (δ_C 151.7), C-7 (δ_C 140.7) and C-8a (δ_C 113.7), and from 6-OCH_3 (δ_H 3.95) to C-6 (δ_C 151.7). The ROESY spectrum showed a key correlation of H-5 (δ_H 7.30) with 6-OCH_3 (δ_H 3.95; Figure 1b). Taken together, the structure of **3** was unambiguously assigned (Figure 1a).

IFQ D (**4**) was obtained as a yellow solid. The molecular formula $C_{15}H_{12}O_6$ was derived from HR-ESI-MS data, affording an $[M+H]^+$ ion at *m/z* 289.0712 (calculated for $[M+H]^+$ ion at *m/z* 289.0707). The ^{1}H NMR spectrum of **4** was comparable to those of **2** and **3**, except for the absence of a proton signal corresponding to the hydroxyl group at the A-ring. One new methoxyl group signal was shown in the NMR spectra, hence suggesting **4** would be a 6,7-dimethoxyl congener of **1** (Tables 1 and 2). The HMBC correlations from 6-OCH_3 (δ_H 4.05) to C-6 (δ_C 158.1), from 7-OCH_3 (δ_H 4.03) to C-7 (δ_C 141.4) and from 8-OH (δ_H 13.08) to C-7 (δ_C 141.4), C-8 (δ_C 157.3), and C-8a (δ_C 113.9), as well as the key ROESY correlation between 6-OCH_3 (δ_H 4.05) and H-5 (δ_H 7.46) confirmed the positions of these two methoxyl groups (Figure 1b), hence the assignment of the structure of **4** (Figure 1a).

IFQ E (**5**) and IFQ F (**6**) were both isolated as yellow solids. HR-ESI-MS yielded $[M+H]^+$ ions at *m/z* 291.0499 and 291.0501 respectively, consistent with the same molecular formula of $C_{14}H_{10}O_7$ (calculated for $[M+H]^+$ ion at *m/z* 291.0499) for both **5** and **6**. The ^{1}H and ^{13}C NMR spectra of **5** and **6** were almost identical to those of ventilone F, a known IFQ isolated from the plant *Ventilago goughii*[22] (Tables 1 and 2). Detailed analysis of the HSQC and HMBC spectra of **5** and **6** showed that they could be the tautomeric forms of ventilone F, which were confirmed by (i) the correlations from H_3-10 (δ_H 2.71) to C-1 (δ_C 160.4), C-9 (δ_C 185.1) and C-9a (δ_C 116.4), from OH-5 (δ_H 13.62) to C-4 (δ_C 182.5), C-4a (δ_C 107.2), C-5 (δ_C 154.8), C-6 (δ_C 141.1) and C-7 (δ_C 149.4), and from 6-OCH_3 (δ_H 3.88) to C-6 (δ_C 141.1) in the HMBC spectrum of **5** and (ii) the correlations from H_3-10 (δ_H 2.69) to C-1 (δ_C 159.9), C-9 (δ_C 184.2) and C-9a (δ_C 116.3), from OH-8 (δ_H 13.60) to C-6 (δ_C 148.0), C-7 (δ_C 141.5), C-8 (δ_C 154.4), C-8a (δ_C 107.6) and C-9 (δ_C 184.2), and from 7-OCH_3 (δ_H 3.89) to C-7 (δ_C 141.5) in the HMBC spectrum of **6** (Figure 1b). Thus, the structures of **5** and **6** were ambiguously assigned (Figure 1a).

Table 3 ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR data (δ_H, δ_C, *J* in Hz) for 7–10 in DMSO-d_6[a]

	7		***8***		***9***		***10***	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	157.6		137.6		139.2		136.6	
3	137.7	7.55, s	122.5	7.53, s	126.7	7.52, s	126.0	7.47, s
3a	130.2		122.0		121.4		121.6	
4	65.7		178.9		178.7		180.5	
4a	138.2		132.1		127.3		132.1	
5	108.1	6.92, s	108.8	7.10, s	108.3	7.16, s	114.2	6.73, s
6	152.6		157.7		151.2		157.9	
7	133.1		139.3		138.5		140.0	
8	152.7		157.7		152.6		157.9	
8a	109.6		111.6		112.1		106.5	
9	185.7		186.3		186.8		183.3	
9a	114.9		116.7		117.1		118.0	
10	14.1	2.64, s	13.0	2.55, s	12.2	2.36, s	11.9	2.32, s
6-OH		10.37, br s				10.43, br s		
7-OH		8.83, br s				9.58, br s		
7-OCH_3			60.2	3.80, s			59.1	3.70, s
8-OH		13.22, s		13.67, br s		13.43, s		13.71, s
1′	113.7				136.0		136.2	
2′	146.0				134.5		134.7	
3′	114.7	6.57, d (7.7)			128.8	7.54, m	128.8	7.50, m
4′	134.2	7.27, td (7.7, 1.5)			131.1	7.65, m	131.0	7.63, m
5′	117.8	6.73, t (7.7)			130.3	7.64, m	130.0	7.60, m
6′	127.7	7.71, dd (7.7, 1.5)			128.7	7.67, m	128.6	7.62, m
7′	162.9				168.2		168.3	
2′-NH		7.36, s						
7′-$NH_{(2)}$		8.61, s				7.88, s		7.81, s
						7.49, s		7.41, s

Abbreviations: DMSO, dimethyl sulfoxide; NMR, nuclear magnetic resonance.
[a]Assignments were based on HSQC, HMBC, ^{1}H-^{1}H COSY and ROESY correlations.

IFQ G (**7**) was isolated as a golden solid in its racemic form {$[\alpha]_D^{25}$ 0.0 (*c* 0.10, MeOH)}. The molecular formula of **7** as $C_{20}H_{14}N_2O_6$ was determined by HR-ESI-MS, affording an $[M+H]^+$ ion at *m/z* 379.0924 (calculated for $[M+H]^+$ ion at *m/z* 379.0925). The ^{1}H and ^{13}C NMR data of **7** (Table 3) differed substantially from **1**-**6**, and close analysis of the splitting patterns for the coupled aromatic proton signals in the ^{1}H NMR and ^{1}H-^{1}H COSY spectra suggested the presence of an additional 1,2-disubstituted benzene moiety in **7**. HMBC correlations from 7′-NH (δ_H 8.61) to C-7′ (δ_C 162.9) and C-1' (δ_C 113.7), and from H-6′ (δ_H 7.71) to C-7′ (δ_C 162.9) indicated the connection of C-1′ with C-7′. The HMBC correlations from 2′-NH (δ_H 7.36) to C-1′ (δ_C 113.7), and from H-4′ (δ_H 7.27) and H-6′ (δ_H 7.71) to C-2′ (δ_C 146.0) confirmed the position of a second NH at C-2′ (Figure 1b). The other signals observed in the ^{1}H and ^{13}C NMR of **7** were very close to those of **1**, indicating a similar structure except for the absence of a carbonyl carbon and the presence of a quaternary carbon C-4 (δ_C 65.7) in **7**, which could be deduced as an aminal function (Tables 1 and 2). In the HMBC spectrum, correlations from 2′-NH (δ_H 7.36), 7′-NH (δ_H 8.61) and H-5 (δ_H 6.92) to C-4 (δ_C 65.7), and from 2′-NH (δ_H 7.36) to C-3a (δ_C 130.2) identified the formation of a spiro ring system (Figure 1b). This assignment was supported by the ROESY experiment of **7**, in which the correlations of 2′-NH (δ_H 7.36) with H-3′ (δ_H 6.57), and 7′-NH (δ_H 8.61) with H-5 (δ_H 6.92) were indeed observed. Taken together, the extensive NMR analysis finally allowed the unambiguous assignment of the **7** structure (Figure 1a).

IIQ A (**8**) was obtained as a golden solid. HR-ESI-MS analysis of **8** yielded an $[M+H]^+$ ion at *m/z* 274.0710, consistent with the molecular formula $C_{14}H_{11}NO_5$ (calculated for $[M+H]^+$ ion at *m/z* 274.0710). The ^{1}H NMR data were similar to those of **2**, and comparison of partial ^{13}C NMR data with those of **2** confirmed their close similarity except for the signals of C-1 and C-3, which were up-field shifted from δ_C 160.1 and 145.7 in **2** to δ_C 137.6 and 122.5 in **8**, respectively (Tables 1,2 and 3). Considering the ^{1}H NMR data and molecular formula of **8**, we suggested these two carbons might be connected to a secondary amine, which was further confirmed by the correlations from H-3 (δ_H 7.53) to C-1 (δ_C 137.6), C-3a (δ_C 122.0), C-4 (δ_C 178.9) and C-9a (δ_C 116.7), and from H_3-10 (δ_H 2.55) to C-1 (δ_C 137.6), C-9 (δ_C 186.3) and C-9a (δ_C 116.7) in the HMBC spectrum. The position of the methoxyl group (δ_H 3.80) at C-7 (δ_C 139.3) was established by the HMBC correlations from H-5 (δ_H 7.10) and 7-OCH_3 (δ_H 3.80) to C-7 (δ_C 139.3) and the absence of ROESY correlation between H-5 (δ_H 7.10) and 7-OCH_3 (δ_H 3.80) (Figure 1b), hence the final assignment of the **8** structure (Figure 1a).

IIQ B (**9**) was isolated as a dark yellow solid. The HR-ESI-MS analysis gave an $[M+H]^+$ ion at *m/z* 379.0928, establishing a molecular formula of $C_{20}H_{14}N_2O_6$ (calculated for $[M+H]^+$ ion at *m/z* 379.0925). The ^{13}C NMR spectrum had similar resonances with those of **1**, except for the signals of C-1 and C-3, which were up-field shifted from δ_C 159.9 and 145.1 in **1** to δ_C 139.2 and 126.7 in **9**, respectively, and an additional aromatic moiety (Table 3). These data suggested the two carbons to be connected to a nitrogen atom, thus resembling **8**.

Analysis of the signals in the ^{1}H NMR, ^{13}C NMR and ^{1}H-^{1}H COSY spectra revealed the presence of a 1,2-disubstituted benzene moiety in **9**, which was supported by HMBC correlations from 7′-NH_2 (δ_H 7.88, 7.49) to C-7′ (δ_C 168.2) and C-1′ (δ_C 136.0), from H-6′ (δ_H 7.67) to C-2′ (δ_C 134.5) and C-7′ (δ_C 168.2), from H-3′ (δ_H 7.54) and H-5′ (δ_H 7.64) to C-1′ (δ_C 136.0), and from H-4′ (δ_H 7.65) to C-2′ (δ_C 134.5). ROESY correlation of H_3-10 (δ_H 2.36) with H-3′ (δ_H 7.54) and HMBC correlation from H-3 (δ_H 7.52) to C-2′ (δ_C 134.5) confirmed the connection of isoindolenaphthoquinone fragment with the 1,2-disubstituted benzene moiety through a C–N bond (Figure 1b). Thus, the structure of **9** was unambiguously assigned (Figure 1a).

IIQ C (**10**) was isolated as a dark yellow solid. Its molecular formula was determined as $C_{21}H_{16}N_2O_6$ based on HR-ESI-MS analysis, which afforded an $[M+H]^+$ ion at *m/z* 393.1081 (calculated for $[M+H]^+$ ion at *m/z* 393.1081). ^{1}H and ^{13}C NMR data of **10** were highly similar to those of **9** (Table 3). Further comparison of 1D and 2D NMR data revealed that **10** was a 7-OCH_3 congener of **9**, which was confirmed by HMBC correlations from 7-OCH_3 (δ_H 3.70) and H-5 (δ_H 6.73) to C-7 (δ_C 140.0), and from 8-OH (δ_H 13.71) to C-7 (δ_C 140.0), C-8 (δ_C 157.9) and C-8a (δ_C 106.5). Complete analysis of the ^{1}H NMR,^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, HMBC and ROESY spectra (Figure 1b) provided further evidences supporting for the structural assignment of **10** (Figure 1a).

Desoxyerythrolaccin (**11**) was isolated as an orange solid, whose structure was confirmed by comparison of its MS and NMR data with those published previously.[18]

Antibacterial activities

IFQs have been reported to inhibit the growth of different bacteria.[3] We first subjected the 11 compounds to antibacterial assay, using the agar diffusion method with 7 mm paper discs containing 100 μg of compounds or tetracycline as a positive control, against selected Gram-positive bacteria, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 23857 and *Mycobacterium smegmatis* ATCC 607, and the Gram-negative bacterium *Escherichia coli* ATCC 25922. For the active compounds, their minimum inhibitory concentrations (MICs) were then determined by the broth dilution method.[23] The assays were performed in the 96-well plates in duplicate with Müller-Hinton broth. As summarized in Table 4, **11** exhibited moderate antibacterial activity against *S. aureus* ATCC 25923, *B. subtilis* ATCC 23857 and *M. smegmatis* ATCC 607, with the MIC values of 3.4, 3.4 and 1.7 μg ml^{-1}, respectively, which were similar to the reported MIC values for the R1128 substances, structurally similar to **11**, against other *S. aureus* and *B. subtilis* species.[24] Although none of the IIQs (**8**-**10**) was active, several IFQs (**1** and **5**–**7**) showed weak activity against the Gram-positive bacteria tested with MIC values higher than 50 μg ml^{-1}. All compounds showed no antibacterial activity against the Gram-negative *E. coli* ATCC 25922, which was consistent with the results observed for similar natural products.[4,24]

Table 4 Antibacterial activity of compounds 1–11 (MIC, μg ml^{-1})[a]

Compounds	*S. aureus*	*B. subtilis*	*M. smegmatis*
1	86.7	>100	>100
5	58	58	72.5
6	58	58	72.5
7	75.6	>100	94.5
11	3.4	3.4	1.7
Tetracycline	5	10	7.5

Abbreviations: MIC, minimum inhibitory concentration.
[a]No antibacterial activity was observed for compounds 2–4, and 8–10 at the concentration of 100 μg ml^{-1}.

Identification of the *ifq* Cluster and a proposed biosynthetic pathway for IFQs

Inspired by the recently reported *ifn* gene cluster from *Streptomyces* sp. RI-77, which was confirmed to encode the biosynthesis of JBIR-76 and JBIR-77,[6] two IFQs structurally similar to **1**–**6** (Figure 2), we decided to identify the *ifq* gene cluster in *Streptomyces* sp. CB01883 to shed light on IFQ and IIQ biosynthesis. Bioinformatics analysis[25] of the *Streptomyces* sp. CB01883 genome revealed the *ifq* gene cluster that showed identical genetic organization, with high sequence identity, to the *ifn* gene cluster[6] (Figure 2a). With the exception of *ifnI*, which was annotated to encode a 161-amino acid hypothetical protein[6] and is missing from the *ifq* cluster, pairwise comparison of the annotated proteins between the two clusters revealed high amino acid identity (Supplementary Table S2), strongly supporting that the *ifq* gene cluster most likely encodes the biosynthesis of IFQs, such as **1**–**6**, in *Streptomyces* sp. CB01883.

Thus, we now propose a unified pathway for IFQ biosynthesis, featuring the same acyl carrier protein-tethered octaketide intermediate **12**, intermediates **13** and **14**, substrate and product of the key Baeyer-Villiger monooxygenase (IfnQ or IfqQ) to furnish the characteristic IFQ scaffold, and the pre-IFQ intermediate **15**. At this point, the biosynthetic pathway diverges with **15** being further oxidized and methylated to JBIR-76 and JBIR-77 in *Streptomyces* sp. RI77 or to IFQ A-F (**1**–**6**) in *Streptomyces* sp. CB01883, respectively (Figure 2b). As the *ifn* and *ifq* gene clusters are nearly identical, the divergence is unlikely resulted from the enzymes encoded within the two clusters. Rather, variations in fermentation conditions, hence discrepancy in relative enzyme activities within the gene clusters, as well as other adventitious enzyme activities beyond the gene clusters, might be the likely causes that resulted in production and isolation of JBIR-76 and JBIR-77 in *Streptomyces* sp. RI-77[6] and IFQ A-F (**1**–**6**) in *Streptomyces* sp. SB3501, respectively, as the major metabolites (Figure 2b). Interestingly, in additional to be isolated from both plants and microorganisms,[26,27] **11** has also been produced by a recombinant *Streptomyces* strain expressing the *actI*/*actVII*/*actIV* genes that encoded the type II polyketide synthase and associated cyclases for actinorhodin biosynthesis.[18] Since similar acyl carrier protein-tethered octaketide intermediates have been well established for actinorhodin biosynthesis,[18] **11** could be viewed as a shunt product of **12** (Figure 2b), the isolation of which provided further support for the proposed pathway for **1**-**6** biosynthesis in *Streptomyces* sp. SB3501.

Intrigued by the fact that *Streptomyces* sp. RI-77 and *Streptomyces* sp. CB01883 contain nearly identical gene clusters (that is, *ifn* and *ifq*, respectively) yet produced varying IFQs (that is, JBIR-76 and JBIR-77, and IFQ A-F, respectively) under the conditions studied, we carried out virtual survey[17,25] of all bacterial genomes available in public databases, in an attempt to search for additional *ifn*- or *ifq*-like gene clusters. We identified six additional clusters, all of which are from actinomycetes (Supplementary Figure S79). In particular, the '*ifq*' gene cluster from *Streptomyces* sp. CF386 is highly homologous to both *ifn* and *ifq* gene clusters (Figure 2a and Supplementary Table S2), indicative of *Streptomyces* sp. CF386 as a potential producer of JBIR-76, JBIR-77 or IFQ A-G. The other five gene clusters displayed many differences, in both genetic organization and the genes within the clusters, to the *ifq*, *ifn* and '*ifq*' gene clusters (Supplementary Figure S79), indicative of them as potential sources to discover, thereby further expanding the IFQ family of natural products.

Chemical transformation of IFQs to IIQs

The proposed unified pathway for IFQ biosynthesis, as exemplified by JBIR-76 and JBIR-77 in *Streptomyces* sp. RI-77[6] and **1–6** in *Streptomyces* sp. SB3501 (Figure 2b), together with the previous observation that IIQs were always co-isolated with related IFQs,[3,28] raised an intriguing question about the origin of IIQs and their biosynthetic relation to IFQs. To date, three other IIQ congeners have been isolated from microorganisms, including azamonosporascone from the pathogenic fungus *Monosporascus cannonballus*,[28] and bhimamycin C and D from *Streptomyces* sp. GW32/698.[3] It is tempting to speculate that IIQs could simply be derived nonenzymatically from the corresponding IFQs by an amine directly attacking the isofurano moiety to afford the characteristic isoindole moiety of IIQs (Supplementary Figure S80). Thus, chemical reactions between **2** and NH_3, and between **1** or **2** and anthranilamide could account for the formation of **8** and **9** or **10**, respectively (Figure 2b). Alternatively, condensation between **1** and anthranilamide, by first regioselectively attacking C-4 of **1** followed by an imine-mediated ring closure (Supplementary Figure S80), could account for the formation of **7** (Figure 2b), although IFQ with a spiro-fused aminal structure like **7** is not known previously.

The idea of IIQs deriving nonenzymatically from IFQs in the presence of an amine in fact could be traced back to the early observation that IIQ, such as bhimamycin C or D, could be obtained from the corresponding IFQ, bhimamycin A or B, by direct heating in the presence of an amine, such as ethanolamine or anthranilic acid, respectively, albeit in low yields.[3] We now show that simply heating a solution of **1** and anthranilamide in ethanol can readily afford **9** directly, and this reaction can be significantly accelerated upon acid catalysis (Supplementary Figure S81). On the other hand, all attempts to prepare **7** by mixing **1** with anthranilamide were not successful, in spite of the fact that **7** was isolated in a racemic form, indicative of its non-enzymatic origin.

In summary, we have isolated seven new IFQs (**1–7**), three new IIQs (**8–10**) and a known anthraquinone desoxyerythrolaccin (**11**),[18] from a mutant strain of *Streptomyces* sp. CB01883 selected based on the chemical profiling during fermentation optimization. The new compounds feature varying modifications at the A-ring (**1–6**), B-ring (**7**) and C-ring (**8-10**), expanding the structural diversity of the IFQ and IIQ family of natural products. Antibacterial assays have showed that **11** and **1**, **5-7** exerted moderate and weak activity, respectively, against several Gram-positive bacteria tested. Bioinformatics analysis of the *Streptomyces* sp. CB01883 genome resulted in the identification of the *ifq* cluster, enabling us to (i) propose a unified pathway for the biosynthesis of the IFQ family of natural products and (ii) speculate the origin of IIQs and its biosynthetic relation to IFQs. The proposal that IIQs may be derived nonenzymatically from IFQs was supported by chemical transformations of selected IFQs into IIQs in the presence of an amine. The presence and diversity of *ifq*-like gene clusters in sequenced microbial genomes highlights once again that *Nature* is the ultimate combinatorial biosynthetic chemist and presents us with a great opportunity to discover novel natural products from underexplored microorganisms.

MATERIALS AND METHODS

General experimental procedures

Optical rotations were recorded on an AUTOPOL IV automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). UV spectra were obtained on a NanoDrop 2000C spectrophotometer (Thermo Scientific, Wilmington, DE, USA). IR spectra were collected with a Spectrum One FT-IR spectrometer (PerkinElmer, Shelton, CT, USA). HR-ESI-MS was conducted on an Agilent 1260 Infinity LC coupled to a 6230 TOF equipped with an Agilent Poroshell 120 EC-C18 column (50 mm × 4.6 mm, 2.7 μm). NMR data were acquired using a Bruker Avance III Ultrashield 700 MHz spectrometer at 700 MHz for ^{1}H NMR and 175 MHz for ^{13}C NMR. Chemical shifts were given in δ (p.p.m.) and referenced to the solvent signal (dimethyl sulfoxide (DMSO)-d_6, δ_H 2.50, δ_C 39.5; $CDCl_3$ and δ_H 7.26, δ_C 77.1) as the internal standard, and coupling constants (J) were reported in Hz. HPLC was conducted on a Varian semipreparative HPLC system (Woburn, MA, USA) with a Prostar 330 detector, using a GRACE Apollo C_{18} column (250 × 4.6 mm, 5 μm) for analysis, and an Alltima C_{18} column (250 × 10.0 mm, 5 μm) for purification.

Cultivation, fermentation and isolation

Streptomyces sp. SB3501 was grown on ISP4 agar medium (Difco, Becton, Dickinson and Company, Sparks, MD, USA) for 7 days, and then cultured in 50 ml tryptic soy broth medium (Bacto, Becton, Dickinson and Company) in a 250-ml Erlenmeyer flask on a rotary shaker (New Brunswick Scientific Innova 44 incubator, Hauppauge, NY, USA) at 250 r.p.m. and 28 °C. After 2 days, each seed culture broth (50 ml) was inoculated into a 2-l Erlenmeyer baffled flasks, each containing 500 ml of production medium M1 (20 l in total; pH 7.2 before sterilization) consisting of 1.5% malt extract (Bacto), 0.5% soluble starch (Sigma-Aldrich, St Louis, MO, USA), 1% glucose (Sigma-Aldrich), 0.4% tryptone (Fisher Scientific, Fair Lawn, NJ, USA), 0.05% K_2HPO_4 (Fisher Scientific), 0.02% $MgSO_4{\cdot}7H_2O$ (Fisher Scientific), 0.005% methionine (Alfa Aesar, A Johnson Matthey Company, Heysham, Lancs, UK), 0.00001% vitamin B12 (Sigma-Aldrich) in 1 l of deionized water. The cultivation was continued on shaking incubators for 6 days, with 3% Diaion HP-20 resin (Supelco, Bellefonte, PA, USA) added after 1 day growth in the 2-l flask. *Streptomyces* sp. CB01883 wild-type was cultivated under the same condition as a control.

After fermentation, the resin and the biomass were collected and centrifuged, and extracted with acetone three times. The acetone extract was evaporated to dryness under reduced pressure, which was then partitioned between ethyl acetate and water. The ethyl acetate phase was dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a dark brown residue (3.26 g). The residue was fractionated by silica gel (33 g, 230–400 mesh) chromatography (3 × 30 cm) eluted with a gradient of CH_2Cl_2-MeOH solvent system (v/v 100:0, 100:1, 100:2, 100:4, 100:8, 100:16, 100:32 and 0:100, each 450 ml) to give eight fractions. Fraction 3 (CH_2Cl_2-MeOH, 100:2) was separated by Sephadex LH-20 (GE Healthcare) chromatography (2 × 30 cm) eluted with MeOH (500 ml) to yield 5 subfractions. All of these fractions and subfractions were further purified by semipreparative HPLC eluted with a linear gradient of MeOH-H_2O (containing 0.1% CH_3COOH) solvent system (0–35 min, 0–100% MeOH in H_2O; 35–50 min, 100% MeOH; 50–51 min, 100-0% MeOH in H_2O; 51–56 min, 100% H_2O) at a flow rate of 3 ml min^{-1} and with UV detection at 254 nm. Subfraction 2 of fraction 3 was purified by semipreparative HPLC to afford **11** (5.1 mg, retention time (Rt) = 34.2 min).[18] Fraction 4 (CH_2Cl_2-MeOH, 100:4) was purified by semipreparative HPLC to give **1** (53.5 mg, Rt = 29.0 min), **2** (8.6 mg, Rt = 32.7 min), **3** (5.5 mg, Rt = 30.7 min), **4** (0.4 mg, Rt = 39.8 min), **5** (1.6 mg, Rt = 33.0 min), **6** (1.3 mg, Rt = 33.4 min), **8** (0.8 mg, Rt = 28.6 min), and **10** (3.5 mg, Rt = 29.5 min). Fraction 5 (CH_2Cl_2-MeOH, 100:8) was purified by semipreparative HPLC to yield **7** (2.8 mg, Rt = 27.0 min) and **9** (9.6 mg, Rt = 26.4 min).

*IFQ A (**1**).* yellow solid; UV (DMSO) λ_{max} (log ε) 289 (4.51), 388 (4.19) nm; IR (neat) ν_{max} 3389, 2988, 1655, 1591, 1559, 1452, 1342, 1314, 1287, 1214, 1180, 1123, 1079, 932, 798, 751 cm^{-1}; ^{1}H NMR (700 MHz) data, Table 1; ^{13}C NMR (175 MHz) data, Table 2; HR-ESI-MS *m/z* 261.0397 $[M+H]^+$ (calculated for $C_{13}H_9O_6$, 261.0394).

*IFQ B (**2**).* yellow solid; UV (DMSO) λ_{max} (log ε) 282 (4.59), 387 (4.36) nm; IR (neat) ν_{max} 3149, 1663, 1568, 1417, 1384, 1340, 1291, 1263, 1207, 1171, 1125, 974, 926, 783, 746 cm^{-1}; ^{1}H NMR (700 MHz) data, Table 1; ^{13}C NMR (175 MHz) data, Table 2; HR-ESI-MS *m/z* 275.0551 $[M+H]^+$ (calculated for $C_{14}H_{11}O_6$, 275.0550).

*IFQ C (**3**).* yellow solid; UV (DMSO) λ_{max} (log ε) 287 (4.52), 383 (4.21) nm; IR (neat) ν_{max} 3344, 1570, 1451, 1363, 1325, 1275, 1213, 1122, 1039, 926, 797, 752 cm^{-1}; ^{1}H NMR (700 MHz) data, Table 1; ^{13}C NMR (175 MHz) data,

Table 2; HR-ESI-MS *m/z* 275.0555 $[M+H]^+$ (calculated for $C_{14}H_{11}O_6$, 275.0550).

*IFQ D (**4**).* yellow solid; UV (DMSO) λ_{max} (log ε) 276 (4.07), 345 (3.77), 388 (3.68) nm; IR (neat) ν_{max} 3379, 2926, 1627, 1589, 1563, 1450, 1412, 1361, 1311, 1286, 1125, 916, 774, 742 cm^{-1}; ^1H NMR (700 MHz) data, Table 1; ^{13}C NMR (175 MHz) data, Table 2; HR-ESI-MS *m/z* 289.0712 $[M+H]^+$ (calculated for $C_{15}H_{13}O_6$, 289.0707).

*IFQ E (**5**).* yellow solid; UV (DMSO) λ_{max} (log ε) 279 (4.48), 378 (4.15), 445 (4.24) nm; IR (neat) ν_{max} 1598, 1567, 1451, 1367, 1302, 1175, 1118, 999, 924, 774 cm^{-1}; ^1H NMR (700 MHz) data, Table 1; ^{13}C NMR (175 MHz) data, Table 2; HR-ESI-MS *m/z* 291.0499 $[M+H]^+$ (calculated for $C_{14}H_{11}O_7$, 291.0499).

*IFQ F (**6**).* yellow solid; UV (DMSO) λ_{max} (log ε) 272 (4.42), 445 (4.25) nm; IR (neat) ν_{max} 1567, 1431, 1366, 1299, 1169, 1125, 1018, 997, 925, 771 cm^{-1}; ^1H NMR (700 MHz) data, Table 1; ^{13}C NMR (175 MHz) data, Table 2; HR-ESI-MS *m/z* 291.0501 $[M+H]^+$ (calculated for $C_{14}H_{11}O_7$, 291.0499).

*IFQ G (**7**).* golden solid; $[\alpha]_D^{25}$ 0.0 (*c* 0.10, MeOH); UV (DMSO) λ_{max} (log ε) 259 (4.33), 336 (4.25) nm; IR (neat) ν_{max} 3316, 1611, 1484, 1356, 1319, 1200, 1160, 1106, 1025, 759 cm^{-1}; ^1H (700 MHz) and ^{13}C (175 MHz) NMR data, Table 3; HR-ESI-MS *m/z* 379.0924 $[M+H]^+$ (calculated for $C_{20}H_{15}N_2O_6$, 379.0925).

*IFQ A (**8**).* golden solid; UV (DMSO) λ_{max} (log ε) 267 (4.53), 326 (4.34), 398 (4.19) nm; IR (neat) ν_{max} 3253, 2988, 1614, 1570, 1538, 1449, 1392, 1353, 1268, 1087, 747 cm^{-1}; ^1H (700 MHz) and ^{13}C (175 MHz) NMR data, Table 3; HR-ESI-MS *m/z* 274.0710 $[M+H]^+$ (calculated for $C_{14}H_{12}NO_5$, 274.0710).

*IFQ B (**9**).* dark yellow solid; UV (DMSO) λ_{max} (log ε) 281 (4.53), 328 (4.20), 400 (4.08) nm; IR (neat) v_{max} 3342, 2931, 1665, 1618, 1541, 1359, 1315, 1243, 1025, 759 cm^{-1}; ^1H (700 MHz) and ^{13}C (175 MHz) NMR data, Table 3; HR-ESI-MS *m/z* 379.0928 $[M+H]^+$ (calculated for $C_{20}H_{15}N_2O_6$, 379.0925).

*IFQ C (**10**).* dark yellow solid; UV (DMSO) λ_{max} (log ε) 272 (4.22), 331 (4.21), 400 (3.80) nm; IR (neat) ν_{max} 3397, 2972, 1667, 1608, 1540, 1394, 1287, 1244, 1055, 742 cm^{-1}; ^1H (700 MHz) and ^{13}C (175 MHz) NMR data, Table 3; HR-ESI-MS *m/z* 393.1081 $[M+H]^+$ (calculated for $C_{21}H_{17}N_2O_6$, 393.1081).

*Desoxyerythrolaccin (**11**).* orange solid; NMR data were almost identical with those reported previously;[18] HR-ESI-MS *m/z* 271.0604 $[M+H]^+$ (calculated for $C_{15}H_{10}O_5$, 271.0601).

Antibacterial assays

The antibacterial activity of compounds **1–11** were evaluated against the Gram-positive *S. aureus* ATCC 25923, *B. subtilis* ATCC 23857 and *M. smegmatis* ATCC 607, and the Gram-negative *E. coli* ATCC 25922 in accordance with the previously reported methods.[16,23] In the assays, the antibacterial activity was first tested using the agar diffusion method with 7 mm paper discs containing 100 μg of compounds and tetracycline as the positive control.[16] MIC values for compounds that displayed inhibition zones were then determined in the 96-well plates duplicate with Müller-Hinton broth.[16,23] The effect of these compounds on the bacterial growth was assessed after incubation at 37 °C for 18 h, and the MIC was determined as the lowest concentration exhibiting no growth compared with the broth control.

Genome sequencing of *Streptomyces* sp. CB01883 and annotation of the *ifq* gene cluster

Genome sequencing was performed using an Illumina MiSeq sequencer (2 × 300 paired end sequencing) at the Next Generation Sequencing and Microarray Core Facility, The Scripps Research Institute. Read quality filtering was performed using an in-house developed tool. Adapter trimming and *de novo* assembly was done with CLC Genomics Workbench version 7.5.1 (CLC Bio., Aarhus, Denmark) using default settings. The resulting contigs were further extended and joined into a final scaffold by SSPACE version 2.0 using all quality filtered reads.[29] The remaining gaps inside the final scaffold were partially or completely filled using the quality filtered reads by GapFiller version 1.10.[30] Genome sequence of *Streptomyces* sp. CB01883 has been deposited into GenBank with the accession number LIWA00000000. The *ifq* gene cluster were annotated and deposited into GenBank with the accession number KX358898. The boundaries of *ifq* gene cluster were estimated by bioinformatics analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Piggott, M. J. Naphtho[23-*c*]furan-4,9-diones and related compounds: theoretically interesting and bioactive natural and synthetic products. *Tetrahedron* **61**, 9929–9954 (2005).
2 Parisot, D., Devys, M., Férézou, J. P. & Barbier, M. Pigments from *Nectria haematococca*: anhydrofusarubin lactone and nectriafurone. *Phytochemistry* **22**, 1301–1303 (1983).
3 Fotso, S. *et al.* Bhimamycin A-E and bhimanone: isolation, structure elucidation and biological activity of novel quinine antibiotics from a terrestrial Streptomycete. *J. Antibiot.* **56**, 931–941 (2003).
4 Zhang, Q. B. *et al.* Isofuranonaphthoquinone produced by an *Actinoplanes* isolate. *J. Nat. Prod.* **72**, 1213–1215 (2009).
5 Motohashi, K., Izumikawa, M., Kagaya, N., Takagi, M. & Shin-ya, K. JBIR-76 and JBIR-77, modified naphthoquinones from *Streptomyces* sp. RI-77. *J. Antibiot.* **69**, 707–708 (2016).
6 Katsuyama, Y. *et al.* Involvement of the Baeyer-Villiger monooxygenase IfnQ in the biosynthesis of isofuranonaphthoquinone scaffold of JBIR-76 and -77. *ChemBioChem* **17**, 1021–1028 (2016).
7 Huang, S.-X. *et al.* Erythronolides H and I, new erythromycin congeners from a halophilic actinomycete *Actinopolyspora* sp. YIM90600. *Org. Lett.* **11**, 1353–1356 (2009).
8 Huang, S.-X. *et al.* Discovery and total Synthesis of a new estrogen receptor heterodimering actinopolymorphol A from *Actinopolymorpha rutilus*. *Org. Lett.* **12**, 3525–3527 (2010).
9 Huang, S.-X. *et al.* Cycloheximide and congeners as inhibitors of eukaryotic protein synthesis from endophytic actinomycetes *Streptomyces* sps. YIM56132 and YIM56141. *J. Antibiot.* **64**, 164–166 (2011).
10 Yu, Z. *et al.* Tirandamycins from *Streptomyces* sp. 17944 inhibiting the parasite *Brugia malayi* asparagine tRNA synthetase. *Org. Lett.* **13**, 2034–2037 (2011).
11 Zhao, L.-X. *et al.* Actinopolysporins A-C and tubercidin as a Pdcd4 stabilizer from the halophilic actinomycete *Actinopolyspora erythraea* YIM 90600. *J. Nat. Prod.* **74**, 1990–1995 (2011).
12 Huang, S.-X. *et al.* Neaumycin - a new macrolide from *Streptomyces* sp. NEAU-x211. *Org. Lett.* **14**, 1254–1257 (2012).
13 Yu, Z., Vodanovic-Jankovic, S., Kron, M. & Shen, B. New WS9326A congeners from *Streptomyces* sp. 9078 inhibiting *Brugia malayi* asparaginyl-tRNA synthetase. *Org. Lett.* **14**, 4946–4949 (2012).
14 Xie, P. *et al.* Biosynthetic potential-based strain prioritization for natural product discovery - a showcase for diterpenoid producing actinomycetes. *J. Nat. Prod.* **77**, 377–387 (2014).
15 Hindra *et al.* Strain prioritization for natural product discovery by a high-throughput real-time PCR method. *J. Nat. Prod.* **77**, 2296–2303 (2014).
16 Ma, M. *et al.* Angucyclines and angucyclinones featuring c-ring cleavage and expansion from *Streptomyces* sp. CB01913. *J. Nat. Prod.* **78**, 2471–2480 (2015).
17 Rudolf, J. D., Yan, X. & Shen, B. Genome neighborhood network reveals insights into enediyne biosynthesis and facilitates prediction and prioritization for discovery. *J. Ind. Microbiol. Biotechnol.* **43**, 261–276 (2016).
18 Bartel, P. L. *et al.* Biosynthesis of anthraquinones by interspecies cloning of actinorhodin biosynthesis genes in Streptomycetes: clarification of actinorhodin gene functions. *J. Bacteriol.* **172**, 4816–4826 (1990).
19 Du, L., Sanchez, C. & Shen, B. Hybrid peptide-polyketide natural products: biosynthesis and prospects toward engineering novel molecules. *Metab. Eng.* **3**, 78–95 (2001).

20 Du, L., Sanchez, C., Chen, M., Edwards, D. J. & Shen, B. The biosynthetic gene cluster for the antitumor drug bleomycin from *Streptomyces verticillus* ATCC15003 supporting functional interactions between nonribosomal peptide synthetases and a polyketide synthase. *Chem. Biol.* **7**, 623–642 (2000).
21 Tang, G.-L., Cheng, Y.-Q. & Shen, B. Leinamycin biosynthesis revealing unprecedented architectural complexity for a hybrid polyketide synthase and nonribosomal peptide synthetase. *Chem. Biol.* **11**, 33–45 (2004).
22 Jammula, S. R., Pepalla, S. B., Telikepalli, H., Rao, K. V. J. & Thomson, R. H. An acetonaphthone and an isofuranonaphthoquinone from *Ventilago goughii*. *Phytochemistry* **30**, 2427–2429 (1991).
23 Wiegand, I., Hilpert, K. & Hancock, R. E. W. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat. Protoc.* **3**, 163–175 (2008).
24 Hori, Y. *et al.* R1128 substances, novel non-steroidal estrogen-receptor antagonists produced by a *Streptomyces*. I. Taxonomy, fermentation, isolation and biological properties. *J. Antibiot.* **46**, 1055–1062 (1993).
25 Weber, T. *et al.* AntiSMASH 3.0 — a comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic Acids Res.* **43**, W237–W243 (2015).
26 Mehandale, A. R., Rama Rao, A. V., Shaikh, I. N. & Venkataraman, K. Desoxyerythrolaccin and laccaic acid D. *Tetrahedron Lett.* **18**, 2231–2234 (1968).
27 Yagi, A., Makino, K. & Nishioka, I. Studies on the constituents of *Aloe sapnaria* HAW. I. The structures of tetrahydroanthracene derivatives and the related anthraquinones. *Chem. Pharm. Bull.* **22**, 1159–1166 (1974).
28 Stipanovic, R. D., Zhang, J. X., Bruton, B. D. & Wheeler, M. H. Isolation and identification of hexaketides from a pigmented *Monosporascus cannonballus* isolate. *J. Agric. Food Chem.* **52**, 4109–4112 (2004).
29 Boetzer, M., Henkel, C. V., Jansen, H. J., Butler, D. & Pirovano, W. Scaffolding preassembled contigs using SSPACE. *Bioinformatics* **27**, 578–579 (2011).
30 Boetzer, M. & Pirovano, W. Toward almost closed genomes with GapFiller. *Genome Biol.* **13**, R56 (2012).

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