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BRIEF COMMUNICATION

Quinohemanine, a quinoxalinone-bohemamine hybrid compound from *Streptomyces* sp. CPCC 200497

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Abstract

A quinoxalinone-bohemamine hybrid compound quinohemanine (**1**), together with 1-methyl-2(*H*)-quinoxalin-2-one (**2**), was isolated from *Streptomyces* sp. CPCC 200497, a producer of quinomycins and bohemamines. Compounds **1** and **2** were purified using standard chromatographic methods, and their structures were defined through interpretation of HRMS, 1D, and 2D NMR data. Both **1** and **2** displayed moderate cytotoxicity against cancer cell line HepG2.

Streptomyces is a very important genus of bacteria for secondary metabolites with antibacterial, antifungal, and antitumor activities [1]. *Streptomyces* sp. CPCC 200497 as a soil isolate from GuangXi (China) is a talented strain for secondary metabolites. Previously, it was identified to produce the quinoxaline antibiotic quinomycins with very potent antibacterial and antitumor activities [2–4], and the pyrrolizidine alkaloid bohemamines with cytotoxicity against cancer cell lines [5]. An antiSMASH analysis of the genome of *Streptomyces* sp. CPCC 200497 revealed over thirty gene clusters, and about one-third of them showed over 30% similarity to gene clusters responsible for real secondary metabolite biosynthesis (Supplementary Table S1). Therefore, *Streptomyces* sp. CPCC 200497 may have produced other secondary metabolites besides quinomycins and bohemamines. Based on this belief, a further study of secondary metabolites of this strain was conducted by us, which led to the discovery of a new compound quinohemanine (**1**) comprising a quinoxalinone moiety attached to bohemamine A, together with a known compound 1-methyl-2(*H*)-quinoxalin-2-one (**2**) (Fig. 1). The isolation, structural identification, and cytotoxicity of **1** and **2** were described as below.

Fresh spores of *Streptomyces* sp. CPCC 200497 were spread on ISP2 plates (medium composition: yeast extract 0.4%, malt extract 1.0%, glucose 0.4%, and agar 1.5%) and were grown at 28 °C for 8 days in order to grow them into mycelia lawns. The fermentation cultures (80 L) were pooled and extracted three times (each 24 h) with equal volume of ethyl acetate (EtOAc), and the solution was evaporated to dryness under reduced pressure to yield 10 g of an EtOA extract. The extract was suspended in H_2O (100 mL), subjected to a YMC*GEL ODS-A-HG column (36 × 460 mm), and eluted with H_2O (2 L) and then 10–100% EtOH (10% up for each stepwise gradient, 2 L for each step) to give 11 fractions (F1–11). Fraction F2 was analyzed by HPLC. The chromatogram revealed two peaks at Rt 36.6 and 21.9 min, respectively, with UV–visible absorption profile different from any of the above known secondary metabolites, suggesting that they may contain un-identified secondary metabolites (Supplementary Fig. S1). Thus, fraction F2 (140 mg) was subjected to a Sephadex LH-20 column and eluted by a mixture of chloroform/methanol (1:1) to yield four fractions F2-1, F2-2, F2-3, and F2-4. Subfractions F2-1 and F2-4 were purified by semi-preparative RP-HPLC (Agilent ZorBax SB-C18, 5 µm, 9.4 × 250 mm) to afford **1** (2.1 mg) and **2** (2.0 mg), respectively (Supplementary Fig. S2).

Compound **1** was obtained as a light yellow powder. Its molecular formula was determined as $C_{23}H_{24}N_4O_4$ according to its HR-ESIMS (Supplementary Fig. S3).

The 1D and 2D NMR spectra of **1** revealed a set of signals for bohemamine unit [5, 6]. The ^{1}H NMR data of **1**

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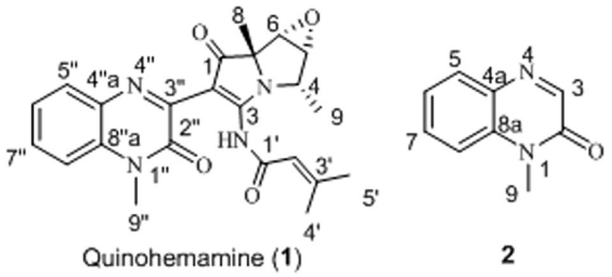

Fig. 1 The structures of **1** and **2**

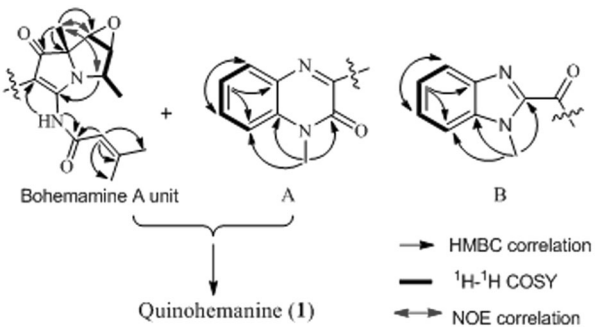

Fig. 2 ^{1}H–^{1}H COSY, key HMBC, and NOSEY correlations in quinohemamine (**1**). Two scaffolds, 4-methyl-3-oxo-3,4-dihydroquinoxalin-2-yl (A) or 1-methylbenzimidazol formyl (B), were proposed for the substructure fused to bohemamine A unit. The observed NMR chemical shifts supported only scaffold A in quinohemamine (**1**)

in DMSO-d_6 gave two methyl signals at δ 1.30 (s) and δ 1.13 (d, $J = 6.6$ Hz), two vinyl methyl signals at δ 1.80 (s) and δ 1.88 (s), an olefinic proton signal at δ 5.80 (s), an amide exchangeable proton at δ 10.47 (s), and three methine signals at δ 3.95 (q, $J = 6.6$ Hz), δ 3.76 (d, $J = 3.0$ Hz), and δ 3.81 (dd, $J = 6.6$, 3.6 Hz). The ^{13}C NMR spectrum in DMSO-d_6 showed two carbonyl signals at δ 195.0 and δ 163.9, assignable to a ketone and an amide, respectively. Other critical assignments for the bohemamine unit were based on ^{1}H–^{1}H COSY correlations of H-9/H-4/H-5/H-6, and the key HMBC correlations from H-4 to C-3/C-9, H-5 to C-4/C-6/C-7/C-9, H-8 to C-1/C-6/C-7, 3-N*H* to C-2/C-1′, H-2′ to C-1′/C-3′/C-4′/C-5′, H-4′ to C-2′/C-3′/C-5′, and H-5′ to C-2′/C-3′/C-4′ (Fig. 2, Supplementary Figs S4–9).

Compared to bohemamine A as a molecule, the bohemamine unit in **1** lost the signal for a methine group (C-2) and gained a new all-substituted olefinic carbon signal at δ_C 101.2, indicating that the bohemamine unit had a substituent at C-2 by a C–C bond (Supplementary Table S2).

In addition, the NMR spectrum of **1** displayed signals for an *ortho*-substituted benzene ring moiety (δ_H 7.70 (dd, $J =$ 8.4, 1.8 Hz), 7.32 (td, $J = 7.2$, 1.2 Hz), 7.51 (td, $J = 7.2$, 1.2 Hz), and 7.44 (dd, $J = 8.4$, 1.2)). One of the two substituents was a methylamine functionality deduced from the large methyl singlet at δ 3.49(s) with a corresponding carbon signal at δ 29.3 and the HMBC correlations from the methyl singlet to C-8″ (δ 114.6), C-8″a (δ 132.8) and C-2″ (δ 153.6), and the correlation from the methyl singlet to C-8″ is much weaker than other correlations, indicating it was an unusual 4-bond long-range correlation in HMBC. The other substituent was probably an *O*-carbon or *N*-carbon according to the HMBC correlation of protons in the benzene moiety (Fig. 2) and chemical shifts at δ_C 133.0 (C-4″a) and δ_C 132.8 (C-8″a).

Based on the molecular formula and the above NMR interpretations of **1**, a 1-methyl-2(*H*)-quinazolinone (A) or 1-methylbenzimidazol formyl (B) scaffold could be proposed for the substructure attached to the bohemamine A unit of **1** (Fig. 2). As a set of signals in good agreement with those of 1-methyl-2(*H*)-quinoxalin-2-one moiety were found in the ^{13}C NMR of **1** [7], and the signals δ_C 153.6 (C-2″) and δ_C 152.0 (C-3″) were inconsistent with those reported for 1-methylbenzimidazoles [8, 9], a 1-methyl-2(*H*)-quinazolinone moiety was confirmed to connect to the bohemamine A unit (Supplementary Table S2).

Therefore, the planar structure of **1** was established as a 1-methylquinoxalin-2(1*H*)-one unit attached to bohemamine A by two olefinic carbons (C-2 and C-3″). NOESY correlations were observed between H-8 and H-6, H-8 and H-4, suggesting the relative configuration of **1** as shown in Fig. 2, which is identical to that of bohemamine A (Supplementary Fig. S10). Compound **1** should have the same absolute configurations in the bohemamine unit as those for bohemamines, as its circular dichroism (CD) spectrum showed the same Cotton effects as bohemamines (Supplementary Fig. S13) [5, 6]. Compound **1** was named by us as quinohemanine. The NMR chemical shifts of quinohemanine were assigned completely by HSQC, ^{1}H–^{1}H COSY, and HMBC, as indicated in Table 1.

The molecular formula of **2** was determined as $C_9H_8N_2O$ according to its HR-ESIMS (Supplementary Fig. S3). A careful examination indicated that the NMR data of **2** were very similar to those of the 1-methy-2(1*H*)-quinoxalinone scaffold in **1**, except that an additional proton signal (δ_H 8.25 (s, 1*H*)) appeared in **2** (Supplementary Figs S14–15). Thus, the structure of **2** was 1-methyl-2(*H*)-quinoxalin-2-one. Compound **2** had been reported before as a synthetic molecule [7], but it was characterized here as a natural compound for the first time.

It is very interesting that **1** appeared as a secondary metabolite of *Streptomyces* sp. CPCC 200497, a producer of quinomycins and bohemamines. The 1-methylquinoxalin-2(1*H*)-one moiety in **1** may have a biosynthetic relationship with quinomycins, whose biosynthesis involves the chromophore quinoxaline-2-carboxylic acid as the starting unit. After decarboxylation, methylation, reduction, and oxidation, the chromophore is transformed to 1-methylquinoxalin-2(1*H*)-one (**2**), which is then attached to bohemamine A to yield **1** (Fig. 3).

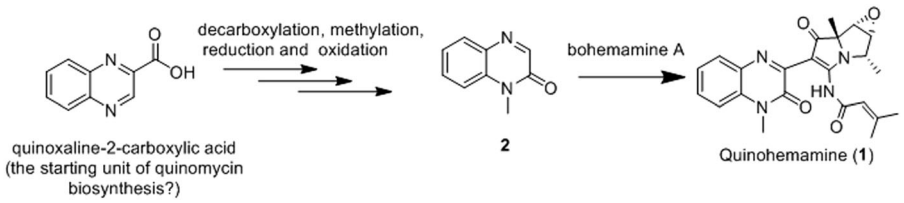

Fig. 3 The speculated biosynthesis of **1**

Table 1 ^{1}H- and ^{13}C-NMR data of quinohemanine (1) in DMSO-d_6

Position	δ_C	δ_H (J in Hz)	Position	δ_C	δ_H (J in Hz)
1	195.0		4′	27.5	1.80 s
2	101.2		5′	20.2	1.88 s
3	166.0		3-N*H*		10.47 s
4	56.5	3.95 q (6.6)	2″	153.6	
5	63.4	3.81dd (6.6, 3.6)	3″	152.0	3″
6	56.1	3.76 d (3.0)	4″a	133.0	4″a
7	74.6		5″	129.2	7.70 dd (8.4, 1.8)
8	20.1	1.30 s	6″	123.5	7.32 ddd (7.2, 7.2, 1.2)
9	14.4	1.13 d (6.6)	7″	129.3	7.51 ddd (7.2, 7.2, 1.2)
1′	163.9		8″	114.6	7.44 dd (8.4, 1.2)
2′	118.2	5.80 s	8″a	132.8	
3′	156.3		N-CH_3	29.3	3.49 s

^{1}H- and ^{13}C-NMR spectra data (δ) were obtained at 600 and 125 MHz, respectively, on VNS-600 spectrometer or Bruker 600 spectrometer, and measured in DMSO-d_6 at room temperature

Quinomycins possess a very strong cytotoxic activity against tumor cells because the quinoxaline chromophore of quinomycins can intercalate into the DNA of tumor cells, which prevents the normal transcription and replication of DNA [3, 10]. Some derivatives of bohemamines are also cytotoxic against tumor cells [5]. It is interesting to see whether **1** as a hybrid molecule with a quinoxaline scaffold and a bohemamine unit displays also cytotoxicity against tumor cell. To confirm this, an in vitro assay of **1** and **2** against liver cancer cell line HepG2 was performed using the SRB method, with doxorubicin as positive control (IC_{50}, 0.4 μM). Compounds **1** and **2** exhibited similar moderate cytotoxicity against HepG2, with IC_{50} (65.9 and 52.5 μM, respectively) comparable to that of bohemamine A reported before [5]. In addition, **1** is a weaker cytotoxic agent than quinomycins [10].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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