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Dibohemamines I–O from *Streptomyces* sp. GZWMJZ-662, an endophytic actinomycete from the medicinal and edible plant *Houttuynia cordata* Thunb.

Dong-Yang Wang^{1,2†}, Ming-Xing Li^{1,2†}, Yan-Chao Xu^{1,3}, Peng Fu⁴, Wei-Ming Zhu^{1,4*} and Li-Ping Wang^{1,2*} 

Abstract

A chemical investigation of *Streptomyces* sp. GZWMJZ-662, an endophytic actinomycete isolated from *Houttuynia cordata* Thunb., has yielded eleven bohemamine dimers (**1–11**). Notably, the newly identified dibohemamines I–O (**1–7**) have not been previously reported. Their structures were elucidated through detailed spectroscopic analysis, encompassing high-resolution electrospray ionization mass, nuclear magnetic resonance, infrared radiation, ultraviolet–visible, and electronic circular dichroism spectroscopy. Dibohemamine I (**1**) exhibited selective cytotoxic effects against the cancer cell lines 786-O and GBC-SD among the 18 cell lines evaluated, with the half-inhibitory concentration values of 3.24 ± 0.20 and 7.36 ± 0.41 μ M, respectively.

Keywords Endophytic actinomycete, Secondary metabolite, Dibohemamine, Cytotoxicity

[†]Dong-Yang Wang and Ming-Xing Li Contributed equally to this work.

*Correspondence:

Wei-Ming Zhu
weimingzhu@ouc.edu.cn

Li-Ping Wang
wangliping2022@gmc.edu.cn

Full list of author information is available at the end of the article

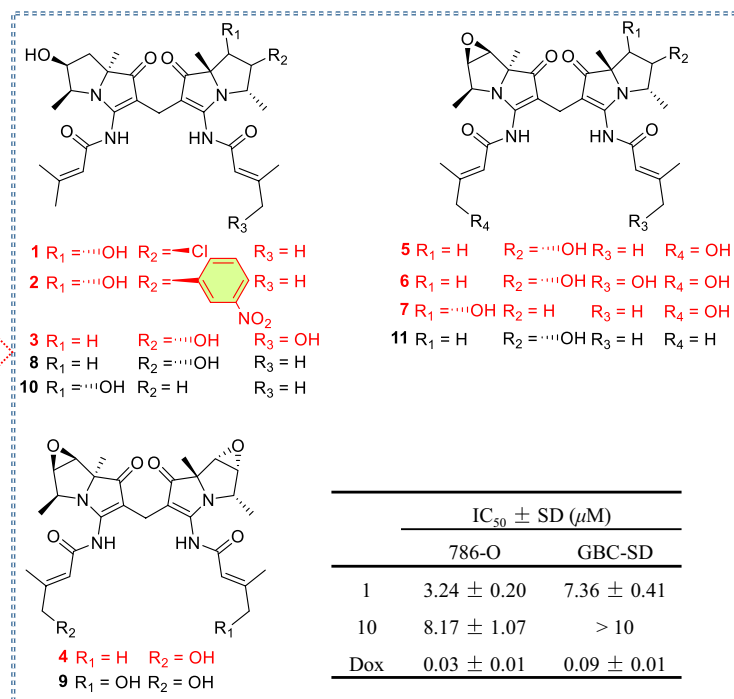
Graphical Abstract



Streptomyces sp. GZWMJZ-662,
Endophytic Actinobacteria
from *Houttuynia cordata* Thunb.



Rice-soybean medium



1 Introduction

Actinomycetes, a group of bacteria thriving in the diverse environments of natural ecosystems, produced many biologically active metabolites [1–3]. The bacterial density of microbial communities that inhabit the soil and rhizosphere around plant roots, can reach up to 10^6 cells per cubic millimeter, and actinomycetes account for a notable 4% of the total population [4]. The capacity of endophytic actinomycetes to synthesize compounds with innovative structures and remarkable bioactivity has been well documented, which is a testament to their potential research area in the field of natural product chemistry [5, 6]. Despite their proven capabilities, endophytic actinomycetes remain a relatively unexplored group within the microbial world, with much of their potential still shrouded in mystery [7]. Intriguingly, studies suggest that these endophytic bacteria are capable of producing metabolites with pharmacological activities that closely resemble, or even exactly match those of their host plants [8]. *Houttuynia cordata* Thunb. is a food and medicine homology plant with diverse phytochemical constituents, which contribute to its wide range of biological activities [9]. The antitumor effects of its extract have been extensively studied, highlighting its potential as a source of therapeutic agents [10–13]. However, metabolites

produced by endophytic actinomycetes within *H. cordata* have not yet been fully studied.

During our research, furanopyrones A and B with potent cytotoxic activity have been identified from an endophytic fungus in *H. cordata* [14]. Our ongoing study on antitumor compounds focuses on the metabolites of endophytic actinomycetes within *H. cordata*. Further exploration of *Streptomyces* sp. GZWMJZ-662 yielded seven novel bohemamine dimers, dibohemamines I–O (1–7) (Fig. 1). Four known dibohemamines were also identified, including dibohemamine B (8) [15], dibohemamine G (9) [16], dibohemamine C (10) [15], and dibohemamine F (11) [17].

2 Results and discussions

The molecular formula of **1** was determined as $\text{C}_{29}\text{H}_{39}\text{O}_6\text{N}_4\text{Cl}$ via HR-ESIMS (Fig. S2). The IR spectrum (Fig. S1) indicate that **1** contains methylene group (2930 , 1451 cm^{-1}), α,β -unsaturated ketone (1692 cm^{-1}), and amide group (1646 , 1554 cm^{-1}) [15]. The ultraviolet–visible (UV–Vis) spectrum of **1** showed peaks at 248 , 285 , and 346 nm , similar to those of bohemamines and dibohemamines [15, 18]. The comparison of the NMR data of **1** (Table 1, Figs. S3 and S4) with that of bohemamines and dibohemamines indicates that **1**

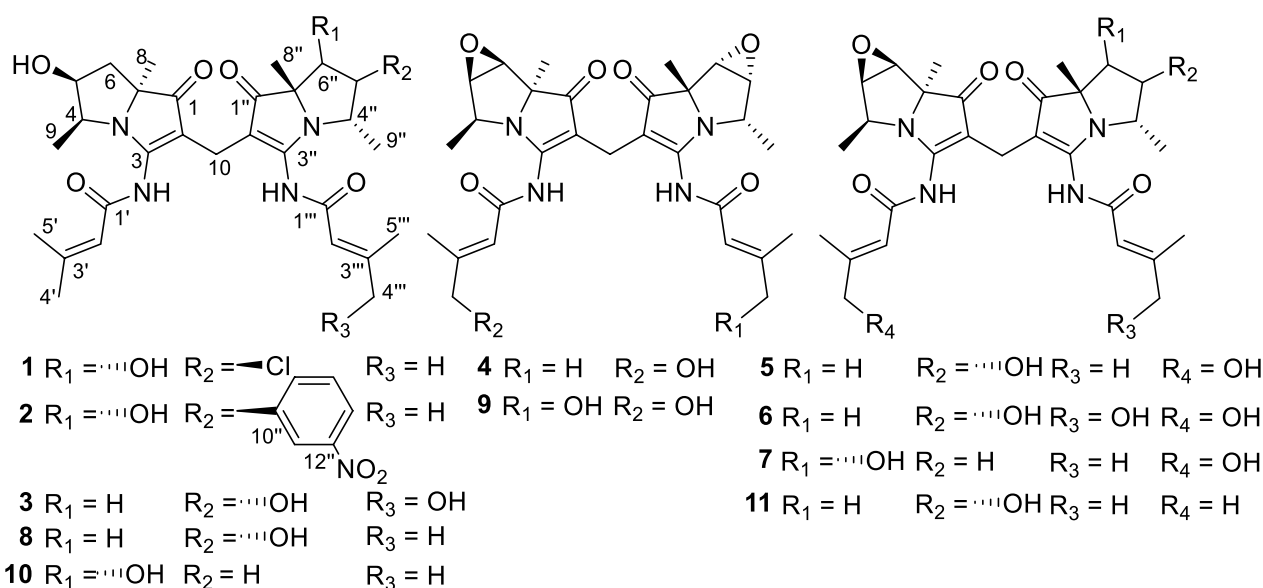


Fig. 1 The chemical structures of compounds 1–11

should be an analog of dibohemamine containing one bohemamine B moiety and one 5-Cl-bohemamine C moiety [18]. The bohemamine B moiety in **1** was confirmed via the spin system of $H_2\text{-}6/H\text{-}5/H\text{-}4/H_3\text{-}9$, and the key HMBC from $H_3\text{-}8$ to $C\text{-}1/C\text{-}6/C\text{-}7$, from $H_3\text{-}9$ to $C\text{-}4/C\text{-}5$, and from $H\text{-}2'$ to $C\text{-}1'/C\text{-}3'/C\text{-}4'/C\text{-}5'$, as well as the NOESY correlation of $H\text{-}2'$ to $H_3\text{-}9$ (Fig. 2). The 5-Cl-bohemamine C moiety of **1** was confirmed via the spin system of $H_2\text{-}6''/H\text{-}5''/H\text{-}4''/H_3\text{-}9''$, HMBC from $H_3\text{-}8''$ to $C\text{-}1''/C\text{-}6''/C\text{-}7''$, from $H_3\text{-}9''$ to $C\text{-}4''/C\text{-}5''$, and from $H\text{-}2''$ to $C\text{-}1''/C\text{-}3''/C\text{-}4''/C\text{-}5''$, and the key NOESY correlation of $H\text{-}2''$ to $H_3\text{-}9''$. These two bohemamines connected through a methylene carbon between $C\text{-}2$ and $C\text{-}2''$, which was confirmed by the HMBC from $H_2\text{-}10$ to $C\text{-}1/C\text{-}2/C\text{-}3/C\text{-}1'/C\text{-}2'/C\text{-}3'$ [17]. Thus, the skeleton of compound **1** was confirmed and was designated as dibohemamine I.

The NOESY correlations (Fig. 2) of $H_3\text{-}9$ to $H\text{-}6a$, $H_3\text{-}8$ to $H\text{-}5/H\text{-}6b$, $H\text{-}5''$ to $H_3\text{-}9''$, and $H\text{-}6''$ to $H\text{-}4''/H_3\text{-}8''$ suggest that the relative configurations of the two moieties are respectively identical to that of bohemamine B and 5-Cl-bohemamine C. Additionally, the absolute configuration of dibohemamine I was ascertained by comparing its ECD spectrum with those of reported dibohemamines (Fig. 3) [15–17].

The molecular formula of **2** was determined as $C_{35}H_{43}O_8N_5$ via the HR-ESIMS peak at m/z 662.3174 $[M+H]^+$ (calcd for $C_{35}H_{44}O_8N_5$, 662.31844). The analysis of **2** via NMR data (Table 1; Fig. 2) reveals it as an analog of dibohemamine with one bohemamine B unit and one 5''-substituted bohemamine C unit. These

two bohemamines are connected through a methylene carbon between $C\text{-}2$ and $C\text{-}2''$ indicated by the HMBC from $H_2\text{-}10$ to $C\text{-}1/C\text{-}2/C\text{-}3/C\text{-}1'/C\text{-}2'/C\text{-}3'$ [17]. Furthermore, the COSY correlations of $H\text{-}13''/H\text{-}14''/H\text{-}15''$ and the HMBC from $H\text{-}11''$ to $C\text{-}12''/C\text{-}13''/C\text{-}15''$, and from $H\text{-}14''$ to $C\text{-}10''/C\text{-}12''$ indicate the presence of a *meta*-disubstituted benzene. The connection between $C\text{-}5''$ in bohemamine C and $C\text{-}10''$ in disubstituted benzene was built via the HMBC correlations from $H\text{-}11''/H\text{-}15''$ to $C\text{-}5''$. The remaining one nitrogen and two oxygen atoms, along with the characteristic strong absorption bands at 1567 and 1383 cm^{-1} in IR spectrum [19], indicates that the $C\text{-}12''$ position is substituted by a nitro group. Consequently, the planar structure of **2** has been elucidated and is named dibohemamine J.

The NOESY of $H_3\text{-}8$ to $H\text{-}6b/H\text{-}5$, $H\text{-}6a$ to $H_3\text{-}9$, $H\text{-}15''$ to $H_3\text{-}8''/H\text{-}6''$, and $H\text{-}4''$ to $H\text{-}11''$ suggest that the nitrobenzene group exhibits the β -configuration, and the relative configurations of other chiral centers in dibohemamine I are identical to that of dibohemamine C (10). Subsequently, the configuration of **2** was confirmed according to its ECD spectrum (Fig. 3).

The molecular formula of dibohemamine K (**3**) is confirmed as $C_{29}H_{40}O_7N_4$ via HR-ESIMS, indicating that dibohemamine K contains one additional oxygen atom compared to dibohemamine B (**8**). Its similar NMR data (Table 1) with those of **8** (Table S1) reveals that a methyl group in **8** is replaced by a hydroxymethyl group, proved via the key HMBC from $H_2\text{-}4'''$ to $C\text{-}5'''/C\text{-}3'''/C\text{-}2'''$. The *E*-configuration of $\Delta^{2'''}$ was proved via the NOE correlation between $H\text{-}2'''$ and $H_2\text{-}4'''$ (Fig. 2).

Table 1 The ^1H (600 MHz) and ^{13}C (150 MHz) NMR Data of **1–3** in CD_3OD

No.	1^a		2^a		3^a	
	δ_{C} , type	δ_{H} , mult. (J in Hz)	δ_{C} , type	δ_{H} , mult. (J in Hz)	δ_{C} , type	δ_{H} , mult. (J in Hz)
1	204.8, C		204.3, C		204.4, C	
2	106.3, C		109.4, C		104.9, C	
3	166.9, C		166.7, C		166.7, C	
4	60.2, CH	4.04, dq (6.9, 6.7)	60.3, CH	4.06, qd (6.7, 6.0)	60.3, CH	4.06, qd (6.7, 5.9)
5	73.9, CH	4.72, ddd (11.6, 6.9, 5.7)	70.1, CH	4.73, ddd (11.4, 6.0, 5.9)	74.2, CH	4.71, ddd (11.3, 5.9, 5.7)
6	36.7, CH ₂	1.56, t (11.6) 1.99, dd (11.6, 5.7)	37.0, CH ₂	1.61, t (11.4) 2.00, dd (11.4, 5.9)	37.0, CH ₂	1.60, t (11.3) 2.00, dd (11.3, 5.7)
7	74.2, C		74.2, C		74.2, C	
8	25.9, CH ₃	1.38, s	25.9, CH ₃	1.39, s	25.8, CH ₃	1.38, s
9	10.3, CH ₃	0.81, d (6.7)	10.4, CH ₃	0.89, d (6.7)	10.3, CH ₃	0.88, d (6.7)
10	13.4, CH ₂	2.87, d (15.7) 2.82, d (15.7)	13.8, CH ₂	2.96, d (15.6) 2.91, d (15.6)	13.7, CH ₂	2.89, d (10.0) 2.87, d (10.0)
1'	165.0, C		164.9, C		164.9, C	
2'	118.3, CH	6.14–6.15, m	118.3, CH	6.13–6.14, m	118.1, CH	6.11–6.12, m
3'	160.9, C		160.9, C		161.1, C	
4'	27.8, CH ₃	2.04, d (1.1)	27.8, CH ₃	2.02, d (1.1)	27.8, CH ₃	2.03, d (0.9)
5'	20.6, CH ₃	2.27, d (1.1)	20.6, CH ₃	2.26, d (1.1)	20.6, CH ₃	2.26, d (0.9)
1''	200.9, C		203.2, C		204.6, C	
2''	105.2, C		104.3, C		105.1, C	
3''	166.4, C		166.6, C		166.7, C	
4''	67.1, CH	4.23, q (6.9)	60.6, CH	4.77, qd (6.7, 2.5)	60.2, CH	4.06, qd (6.7, 5.9)
5''	72.5, CH	4.21, br s	86.3, CH	5.21, dd (2.5, 2.4)	74.3, CH	4.71, ddd (11.3, 5.9, 5.7)
6''	80.2, CH	4.20, br s	80.6, CH	4.46, d (2.4)	37.1, CH ₂	1.62, t (11.3) 2.00, dd (11.3, 5.7)
7''	83.2, C		80.1, C		74.3, C	
8''	23.9, CH ₃	1.66, s	26.0, CH ₃	1.19, s	25.9, CH ₃	1.39, s
9''	19.1, CH ₃	1.26, d (6.9)	16.1, CH ₃	1.43, d (6.7)	10.3, CH ₃	0.89, d (6.7)
10''			133.5, C			
11''			147.1, CH	9.62, s		
12''			146.8, C			
13''			148.1, CH	9.14, d (8.0)		
14''			130.0, CH	8.35, dd (8.0, 6.3)		
15''			147.0, CH	9.32, d (6.3)		
1'''	164.8, C		165.3, C		165.1, C	
2'''	118.0, CH	6.11–6.12, m	118.3, CH	6.15–6.16, m	115.3, CH	6.39–6.40, m
3'''	161.3, C		161.4, C		162.7, C	
4'''	27.7, CH ₃	2.04, d (1.1)	27.7, CH ₃	2.04, d (1.1)	67.4, CH ₂	4.14, br s
5'''	20.7, CH ₃	2.26, d (1.1)	20.7, CH ₃	2.29, d (1.1)	16.1, CH ₃	2.16, br s

^a The chemical shifts of carbons were obtained via DEPTQ experiments

Furthermore, compounds **3** and **8** share identical absolute configurations at the chiral centers. This was concluded based on the similarity observed in their NOESY and ECD spectra. (Figs. 2 and 3).

The molecular formula of **4** was $\text{C}_{29}\text{H}_{36}\text{O}_7\text{N}_4$ (HR-ESIMS m/z 575.2468, calcd for $\text{C}_{29}\text{H}_{36}\text{O}_7\text{N}_4\text{Na}$, 575.24762). The NMR spectra (Figs. S27–S32) were similar to those of dibohemamine G (**9**), a semisynthetic

dibohemamine [13]. The NMR data (Table 2) reveal that a hydroxymethyl group at C-4' or C-4''' in **9** is replaced by a methyl group ($\delta_{\text{C/H}}$ 67.3/4.12). The *E*-configuration of Δ^2 was proved via the NOESY of H-2' to H₂-4' (Fig. 4). Therefore, the molecular framework of **4** is established as a heterodimer comprising bohemia A and bohemia K, which are linked through a methylene bridge between C-2 and C-2'' positions. The

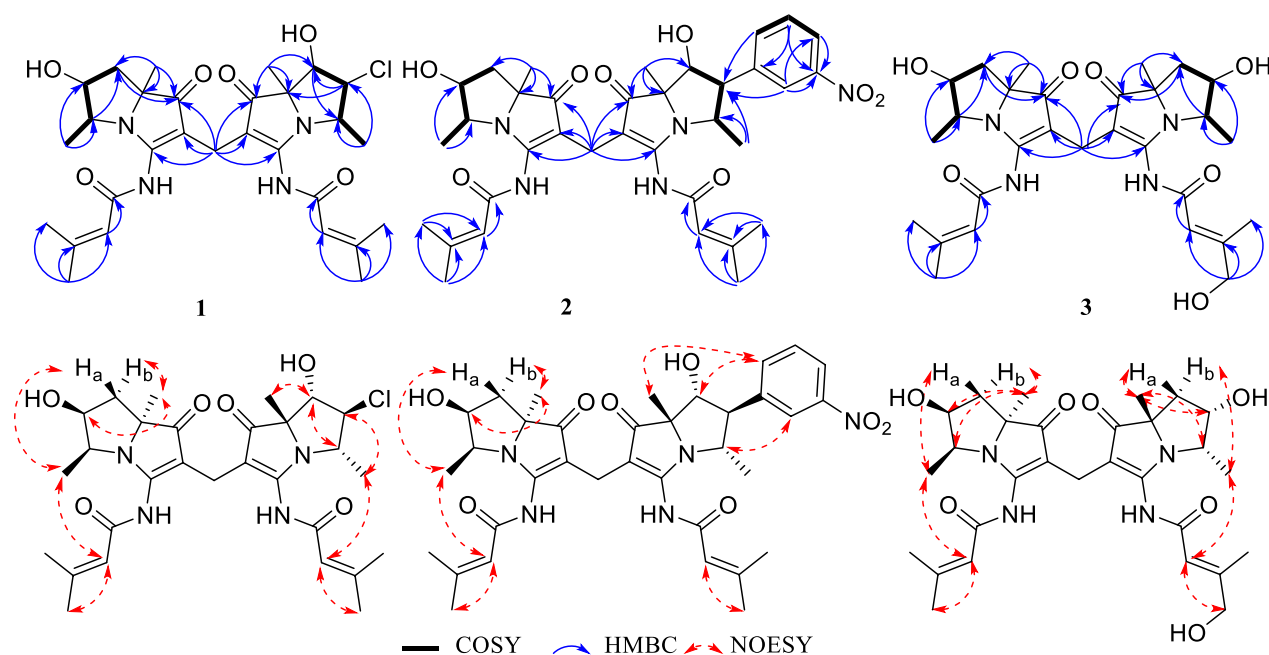


Fig. 2 Key 2D NMR correlations of dibohemamines I–K (**1–3**)

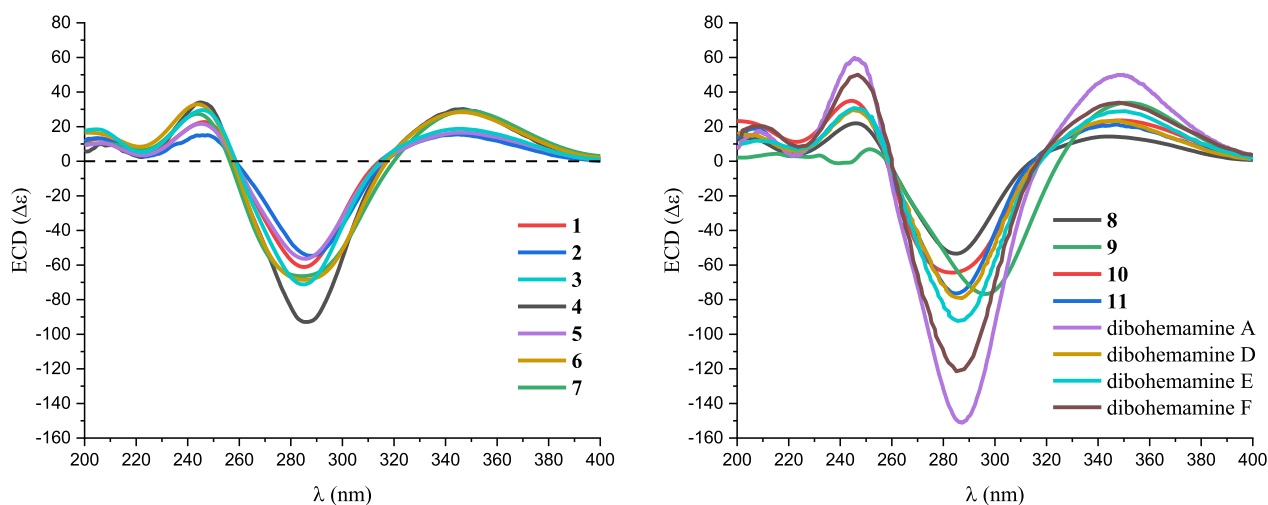


Fig. 3 Experimental ECD spectra of compounds **1–11** and reported ECD spectra of dibohemamines A [15] and D–F [17]

configuration of the two pyrrolizidine cores within **4** are congruent with those of **9**, based on the similarity of their NOE correlations and ECD curves (Figs. 3 and 4).

The molecular formula of bohemiaamine M (**5**) was confirmed as $C_{29}H_{38}O_7N_4$ by HR-ESIMS spectrum. This formula indicates an additional oxygen atom in its structure compared to that of dibohemiaamine F (**11**). The similar NMR data with those of **11** (Tables 3 and S2) reveals that a methyl group in **11** is replaced by a hydroxymethyl group. The HMBC from $H_{2-4'}$ to $C_{2-2'}/C_{3-3'}/C_{5-5'}$, and

the NOESY of $H_{2-2'}$ to $H_{2-4'}/H_{3-9}$ suggest a hydroxyl group substitution at the $C_{4-4'}$ position. The absolute configuration of the pyrrolizidine cores of **5** was confirmed to be the same as **11** via the ECD spectra (Fig. 3).

Compound **6** has a molecular formula of $C_{29}H_{38}O_8N_4$, which has one additional oxygen atom than **5**. The NMR data (Table 2) of **5** and **6** are strikingly similar except a single methyl signal that is replaced by a hydroxymethyl signal in **6**. The HMBC confirms that $C_{4-4'}$ and $C_{4-4''}$ positions are substituted by hydroxyl groups. The

Table 2 The ^1H (600 MHz) and ^{13}C (150 MHz) NMR data of **4–7** in CD_3OD

No.	4		5		6 ^a		7	
	δ_{C} , type	δ_{H} , mult. (J in Hz)	δ_{C} , type	δ_{H} , mult. (J in Hz)	δ_{C} , type	δ_{H} , mult. (J in Hz)	δ_{C} , type	δ_{H} , mult. (J in Hz)
1	200.2, C		201.0, C		201.0, C		201.3, C	
2	104.0, C		104.3, C		105.1, C		105.2, C	
3	168.3, C		168.6, C		168.4, C		168.9, C	
4	57.7, CH	3.92, qd (6.6, 3.5)	57.7, CH	3.92, q (6.5)	57.7, CH	3.92, q (6.5)	57.7, CH	3.90, q (6.6)
5	65.4, CH	3.70, dd (3.5, 3.0)	65.4, CH	3.68, d (2.9)	65.4, CH	3.68, d (3.0)	65.3, CH	3.66, d (2.9)
6	56.8, CH	3.63, d (3.0)	56.7, CH	3.62, d (2.9)	56.7, CH	3.62, d (3.0)	56.7, CH	3.60, d (2.9)
7	77.2, C		77.1, C		77.2, C		77.0, C	
8	20.0, CH_3	1.40, s	19.5, CH_3	1.42, s	19.7, CH_3	1.40, s	19.6, CH_3	1.37, s
9	14.2, CH_3	1.31, d (6.6)	14.3, CH_3	1.32, d (6.5)	14.2, CH_3	1.31, d (6.5)	14.3, CH_3	1.32, d (6.6)
10	14.4, CH_2	2.83, s	14.1, CH_2	2.95, d (15.8) 2.83, d (15.8)	14.1, CH_2	2.82, d (15.8) 2.91, d (15.8)	14.5, CH_2	2.87, d (15.6) 2.97, d (15.6)
1'	166.3, C		166.4, C		166.3, C		166.6, C	
2'	115.2, CH	6.33–6.34, m	114.9, CH	6.33–6.34, m	115.0, CH	6.33–6.34, m	115.1, CH	6.33, br s
3'	162.5, C		162.9, C		162.8, C		162.6, C	
4'	67.3, CH_2	4.12, br s	67.3, CH_2	4.14, d (1.2)	67.3, CH_2	4.13, br s	67.3, CH_2	4.14, br s
5'	16.1, CH_3	2.14, d (0.7)	16.1, CH_3	2.16, br s	16.1, CH_3	2.16, br s	16.1, CH_3	2.16, br s
1''	200.0, C		201.0, C		202.9, C		201.3, C	
2''	104.3, C		105.1, C		104.3, C		105.2, C	
3''	168.3, C		166.5, C		166.5, C		165.6, C	
4''	57.8, CH	3.93, qd (6.6, 3.5)	60.4, CH	4.12, q (6.7)	60.3, CH	4.10, q (6.8)	56.9, CH	4.14, overlap
5''	65.4, CH	3.70, dd (3.5, 3.0)	74.2, CH	4.79, ddd (11.8, 6.7, 5.8)	74.2, CH	4.76, ddd (11.8, 6.8, 5.8)	44.8, CH_2	1.97, overlap 2.87, overlap
6''	56.8, CH	3.64, d (3.0)	37.0, CH_2	1.67, t (11.8) 2.06, dd (11.8, 5.8)	36.9, CH_2	1.66, t (11.8) 2.02, dd (11.8, 5.8)	74.6, CH	4.19, d (3.6)
7''	77.1, C		74.3, C		74.1, C		84.5, C	
8''	20.1, CH_3	1.40, s	25.7, CH_3	1.40, s	25.8, CH_3	1.39, s	23.1, CH_3	1.41, s
9''	14.2, CH_3	1.30, d (6.6)	10.4, CH_3	0.92, d (6.7)	10.4, CH_3	0.92, d (6.8)	20.6, CH_3	1.24, d (6.5)
1'''	165.9, C		164.7, C		162.5, C		164.7, C	
2'''	118.3, CH	6.01–6.02, m	118.2, CH	6.09–6.10, m	115.1, C	6.40–6.41, m	118.2, C	6.07, br s
3'''	160.6, C		161.1, C		165.1, C		161.1, C	
4'''	27.8, CH_3	1.99, d (1.1)	27.8, CH_3	2.00, d (1.0)	67.3, CH_2	4.13, br s	27.7, CH_3	1.99, br s
5'''	20.7, CH_3	2.24, d (1.1)	20.7, CH_3	2.24, d (1.0)	16.1, CH_3	2.15, br s	20.7, CH_3	2.23, br s

^a The chemical shifts of carbons were obtained via DEPTQ experiments

E-configurations of $\Delta^{2'}$ and $\Delta^{2''}$ were confirmed via the NOESY of $\text{H}_2\text{-4}'$ to $\text{H-2}'$, and $\text{H}_2\text{-4}'''$ to $\text{H-2}'''$, respectively. Furthermore, the absolute configuration of **6** is identical to that of **5**, indicated via similar NOESY correlations (Figs. 4, S40, and S48) and ECD (Fig. 3) spectra.

Compound **7** is also a dibohemamine, with a molecular formula of $\text{C}_{29}\text{H}_{38}\text{O}_7\text{N}_4$. The comparison of its NMR data (Table 2) with the known dibohemamine reveals that it is a hydroxyl substituted dibohemamine D [17]. The hydroxyl substitution at the C-4' position is suggested via the NOESY correlations of $\text{H-2}'$ to $\text{H}_2\text{-4}'$ / $\text{H}_3\text{-9}$, and the HMBC from $\text{H-4}'$ to C-2' / C-3' / C-5'. The absolute configuration of **7** was confirmed to be

identical to that of dibohemamine D by the NOESY correlations (Fig. 4) and ECD spectra (Fig. 3).

Compounds **1–11** were evaluated for the cytotoxic activity against eighteen cell lines [14, 20]. In which, dibohemamine I (**1**) showed potential cytotoxic activities against human renal clear cell adenocarcinoma (786-O, $\text{IC}_{50} = 3.24 \pm 0.20 \mu\text{M}$) and the gallbladder cancer (GBC-SD, $\text{IC}_{50} = 7.36 \pm 0.41 \mu\text{M}$) cell lines. Dibohemamine C (**10**) showed specific cytotoxic activities against 786-O with an IC_{50} value of $8.17 \pm 1.07 \mu\text{M}$.

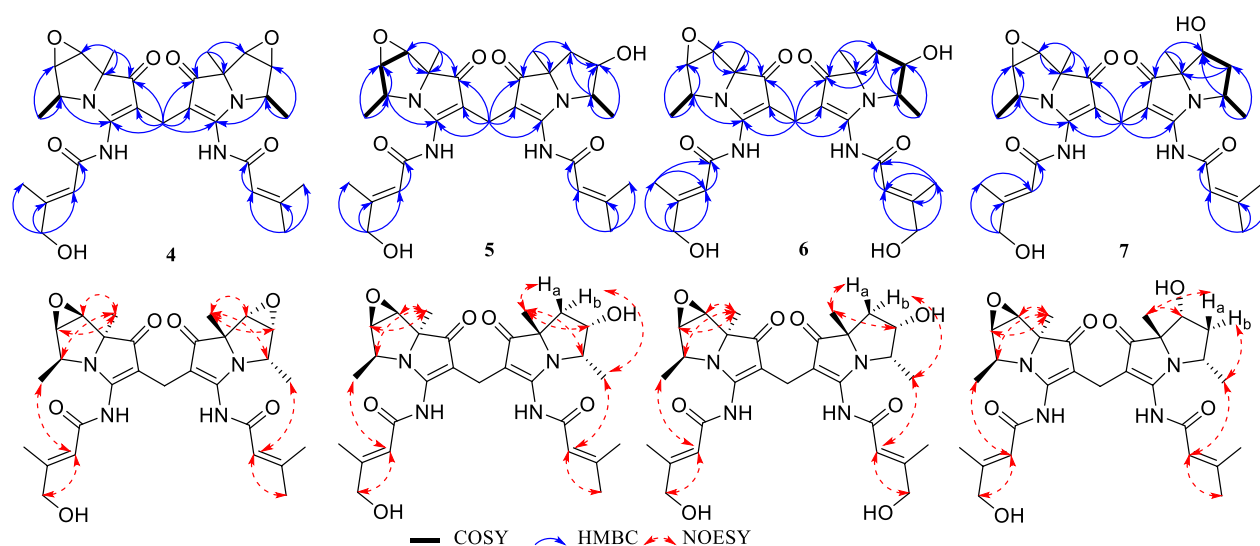


Fig. 4 Key 2D NMR correlations of dibohemamines L–O (4–7)

Table 3 Cytotoxic activities of compounds **1** and **10**

Compounds	IC ₅₀ ± SD (μM)	
	786-O	GBC-SD
1	3.24 ± 0.20	7.36 ± 0.41
10	8.17 ± 1.07	> 10
Dox	0.03 ± 0.01	0.09 ± 0.01

3 Conclusions

Although bohemiaamine dimers have been successfully semisynthesized through a chemical reaction involving bohemiaamines and formaldehyde, the production of bohemiaamines remains dependent on scarce natural sources [15, 16]. The pursuit of bioactive dimers from natural origins remains a paramount strategy for the discovery of novel bioactive dibohemiaamines. In this study, seven new dibohemiaamines (**1**–**7**) were obtained from an endophytic *Streptomyces* species in *H. Cordata*. Dibohemiaamine **J** (**2**) represents a new skeleton with a nitrobenzene-substituted bohemiaamine moiety. To date, only six natural and twelve semisynthetic bohemiaamine dimers have been reported [15–17]. Dibohemiaamines B–F and four semisynthetic dimers exhibited cytotoxic activity towards non-small cell lung or liver cancer cells. [15, 17] This study reveals that dibohemiaamines **I** (**1**) and **C** (**10**) exhibit potent cytotoxic effects on human renal clear cell adenocarcinoma and gallbladder cancer cell lines, thereby broadening the horizons for the therapeutic applications of these compounds within the realm of oncology.

4 Experimental

4.1 General experimental procedures

The equipment employed for acquiring HRESIMS, NMR, UV–Vis, IR, ECD, and ORD data, as well as the HPLC systems utilized for both analysis and separation, are consistent with those detailed in previous studies [14, 20]. UV–Vis, ECD, and ORD were measured in the solutions of MeOH. For semi-preparative HPLC separations, either ODS-A columns (YMC, 5 μm, 1 × 25 cm, 4 mL/min) or πNAP columns (COSMOSIL, 5 μm, 1 × 25 cm, 4 mL/min) were deployed.

4.2 Strain material, fermentation and isolation

Strain GZWMJZ-662 was obtained from the roots of *H. cordata*, [14] and was determined as *Streptomyces* species (16S rRNA, GenBank No. OR083423). The strain was grown on ISP2 liquid cultured medium in 500 mL Erlenmeyer flasks for 3 days (28 °C and 180 rpm) to yield seed liquid. Two hundreds of sterile cultural bags each containing 50 g rice, 10 g soybean powder, 0.25 g sodium chloride and 50 mL water were static culture for one month after seed liquid (9 mL) was added. The culture media were extracted with ethyl acetate (EtOAc) and methanol (MeOH) (10:1, v/v). After evaporated, the residual was redispersed into MeOH (5 L) and washed petroleum ether (PE) (5 L × 3 times). Then, the methanol was evaporated to yield duck extract (270 g).

The extract was loaded to a normal-phase silica gel column, and eluted by PE, DCM, and DCM:MeOH (100:1 to 1:1, v/v). After thin-layer chromatography detection and sample combination, 37 fractions (Fr1 – Fr37) were obtained.

Fr.20 (8.9 g) was separated by Sephadex LH-20 (MeOH:DCM=1:1, v/v) into Fr.20.1 to Fr.20.10. Fr.20.5 (510 mg) was initially separated by PTLC using EtOAc, yielding Fr.20.5.1 to Fr.20.5.3. Fr.20.5.1 (115 mg) was further separated using HPLC (ODS-A column) to obtain **11** (t_R =25.5 min, 8.8 mg) using 60% MeOH-H₂O containing 0.05% TFA. Fr.20.5.2 (64 mg) was separated by an intelligent flash purification system (C18 column) using a gradient elution of 5% to 100% MeOH-H₂O (containing 0.1% TFA) to obtain **4** (10.1 mg). Fr.20.5.3 (39 mg) was separated by HPLC using ODS-A column to obtain **1** (t_R =66 min, 2.1 mg) using 65% MeOH-H₂O (containing 0.05% TFA) at 10 mL/min. Fr.20.4 (741 mg) was separated by a flash purification system (C18 column) using a gradient elution of 5% to 100% MeOH-H₂O (0.1% TFA) to obtain 8 fractions Fr.20.4.1 to Fr.20.4.8). Fr.20.4.8 (27 mg) was separated by HPLC (ODS-A column) to obtain **2** (t_R =20.3 min, 2.2 mg) using 60% MeOH-H₂O (0.05% TFA).

Fr.25 (7.5 g) was separated by Sephadex LH-20 into 9 fractions (Fr.25.1–Fr.25.9) using MeOH and DCM (1:1, v/v). Fr.25.7 (564 mg) was separated by a C18 flash chromatography column using a gradient elution of 5% to 100% MeOH-H₂O (0.1% TFA) to obtain 8 sub-fractions (Fr.25.7.1–Fr.25.7.8). Fr.25.7.1 (41 mg) was washed with MeOH and DCM separately, resulting in a white insoluble substance **9** (7.8 mg). Fr.25.7.6 (49 mg) was separated by a semi-preparative μ NAP column to obtain **10** (t_R =18.0 min, 5.5 mg) using 80% MeOH-H₂O (containing 0.05% TFA). Fr.25.7.4 (44 mg) was separated by a semi-preparative μ NAP column to obtain **7** (t_R =25.5 min, 3.5 mg) using 65% MeOH-H₂O (0.05% TFA).

Fr.28 (4.714 g) was separated by Sephadex LH-20 into 9 fractions (Fr.28.1–Fr.28.9) using MeOH and DCM (1:1, v/v) as the eluent. Fr.28.6 (226.7 mg) was further separated by Toyopearl HW-40F resin into 2 fractions (Fr.28.6.1 and Fr.28.6.2) using methanol as the eluent. Fr.28.6.1 (93 mg) was separated by an ODS-A chromatography column to obtain **8** (t_R =14.3 min, 8.7 mg) using 75% MeOH-H₂O (containing 0.05% TFA). Similarly, Fr.28.6.2 (103 mg) was separated by a semi-preparative ODS-A chromatography column to obtain **5** (t_R =18 min, 20.9 mg) using 55% MeOH-H₂O (0.05% TFA).

Fr.30 (8.791 g) was separated by Sephadex LH-20 into 9 fractions (Fr.30.1–Fr.30.9) using equal volume of MeOH and DCM as eluent. Fr.30.6 (1.9 g) was further subjected to a C18 flash chromatography column (5% to 100% MeOH-H₂O, containing 0.1% TFA) to obtain 15 fractions (Fr.30.6.1–Fr.30.6.15). Fr.30.6.4 (98 mg) was then purified by semi-preparative HPLC (ODS-A) to obtain **6** (t_R =12.0 min, 5.1 mg) using 50% MeOH-H₂O (containing 0.05% TFA).

Fr.31 (2.7 g) was separated by Toyopearl HW-40F gel resin into 11 fractions (Fr.31.1–Fr.31.11) using equal volume of MeOH and DCM the eluent. Fr.31.4 (408 mg) underwent initial separation by a Flash chromatography column (C18) using a gradient elution of 5% to 100% MeOH-H₂O (containing 0.1% TFA) to obtain 4 fractions (Fr.31.4.1–Fr.31.4.4). Fr.31.4.1 (120 mg) was further purified by a semi-preparative ODS-A chromatography column to obtain **3** (t_R =12.4 min, 28.4 mg) using 65% MeOH-H₂O (containing 0.05% TFA).

4.3 Spectroscopic data of compounds

4.3.1 Diboheamine I (1)

White powder; $[\alpha]_D^{25} = -79.0$ (c 0.1); UV-Vis $\lambda_{\max}(\log \epsilon)$ 248 (4.45), 285 (4.18), 346 (4.11) nm; IR (KBr) $\nu_{\max}$: 3272, 3151, 2979, 2930, 1712, 1692, 1646, 1554, 1451, 1217, 1132, 1073, 1013, 925, 845, 800, 724, 664, 582 cm⁻¹; ECD (0.87 mM) $\lambda_{\max}(\Delta \epsilon)$ 246 (+22.6), 285 (−61.1), 345 (+15.9) nm; chemical shifts of ¹H and ¹³C (Table 1); HR-ESIMS m/z 597.2433 [M+Na]⁺ (calcd for C₂₉H₃₉O₆N₄ClNa 597.24503).

4.3.2 Diboheamine J (2)

White powder; $[\alpha]_D^{25} = -167.0$ (c 0.1); UV-Vis $\lambda_{\max}(\log \epsilon)$ 247 (4.16), 288 (3.84), 344 (3.81) nm; IR (KBr) $\nu_{\max}$: 3273, 2979, 2933, 1682, 1646, 1567, 1495, 1446, 1383, 1206, 1183, 1133, 1064, 1011, 842, 801, 722, 664, 587, 544 cm⁻¹; ECD (0.76 mM) $\lambda_{\max}(\Delta \epsilon)$ 248 (+15.0), 288 (−54.8), 345 (+15.5) nm; chemical shifts of ¹H and ¹³C (Table 1); HR-ESIMS m/z 662.3174 [M+H]⁺ (calcd for C₃₅H₄₄O₈N₅, 662.31844).

4.3.3 Diboheamine K (3)

White powder; $[\alpha]_D^{25} = -78.0$ (c 0.1); UV-Vis $\lambda_{\max}(\log \epsilon)$ $\lambda_{\max}(\log \epsilon)$ 249 (4.50), 284 (4.23), 346 (4.20) nm; IR (KBr) $\nu_{\max}$: 3275, 3217, 2980, 2934, 1712, 1692, 1643, 1554, 1449, 1375, 1331, 1214, 1183, 1135, 1074, 1017, 926, 898, 843, 801, 760, 724, 661, 587 cm⁻¹; ECD (0.22 mM) $\lambda_{\max}(\Delta \epsilon)$ 248 (+28.7), 285 (−71.2), 343 (+18.7) nm; chemical shifts of ¹H and ¹³C (Table 1); HR-ESIMS m/z 557.2946 [M+H]⁺ (calcd for C₂₉H₄₁O₇N₄, 557.29698).

4.3.4 Diboheamine L (4)

White powder; $[\alpha]_D^{25} = -110.0$ (c 0.1); UV-Vis $\lambda_{\max}(\log \epsilon)$ 246 (4.51), 285 (4.22), 347 (4.19) nm; IR (KBr) $\nu_{\max}$: 3210, 3031, 2977, 2916, 1708, 1646, 1567, 1492, 1455, 1370, 1324, 1214, 1123, 1053, 1015, 902, 843, 700.1, 674, 587, 530 cm⁻¹; ECD (0.45 mM) $\lambda_{\max}(\Delta \epsilon)$ 245 (+34.0), 285 (−92.8), 347 (+30.2) nm; chemical shifts of ¹H and ¹³C (Table 2); HR-ESIMS m/z 575.2468 [M+Na]⁺ (calcd for C₂₉H₃₆O₇N₄Na, 575.24762).

4.3.5 Diboheamine M (5)

White powder; $[\alpha]_D^{25} = -87.0$ (c 0.1); UV–Vis $\lambda_{\max}(\log \epsilon)$ 247 (4.47), 285 (4.20), 346 (4.19) nm; IR (KBr) $\nu_{\max}$: 3273, 3148, 2980, 2936, 1708, 1683, 1647, 1554, 1452, 1372, 1324, 1211, 1185, 1135, 1074, 1015, 929, 906, 843, 801, 724, 662, 590 cm^{-1} ; ECD (0.23 mM) $\lambda_{\max}(\Delta \epsilon)$ 245 (+21.6), 286 (−56.4), 346 (+16.5) nm; chemical shifts of ^1H and ^{13}C (Table 2); HR-ESIMS m/z 555.2809 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{29}\text{H}_{39}\text{O}_7\text{N}_4$, 555.28133).

4.3.6 Diboheamine N (6)

White powder; $[\alpha]_D^{25} = -113.3$ (c 0.1); UV–Vis $\lambda_{\max}(\log \epsilon)$ 249 (4.50), 284 (4.23), 346 (4.20) nm; IR (KBr) $\nu_{\max}$: 3273, 3210, 2982, 2933, 2834, 1712, 1693, 1674, 1550, 1492, 1455, 1399, 1372, 1326, 1214, 1176, 1123, 1079, 1054, 1017, 977, 928, 902, 845, 774, 699, 668, 586 cm^{-1} ; ECD (0.22 mM) $\lambda_{\max}(\Delta \epsilon)$ 244 (+32.9), 285 (−68.6), 346 (+28.4) nm; chemical shifts of ^1H and ^{13}C (Table 2); HR-ESIMS m/z 571.2740 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{29}\text{H}_{39}\text{O}_8\text{N}_4$, 571.27624).

4.3.7 Diboheamine O (7)

White powder; $[\alpha]_D^{25} = -175.3$ (c 0.1); UV–Vis $\lambda_{\max}(\log \epsilon)$ 248 (4.46), 285 (4.17), 347 (4.13) nm; IR (KBr) $\nu_{\max}$: 3273, 3210, 2982, 2939, 1683, 1647, 1545, 1512, 1446, 1377, 1326, 1208, 1135, 1067, 1011, 929, 905, 843, 801, 724, 668, 579, 536 cm^{-1} ; ECD (0.90 mM) $\lambda_{\max}(\Delta \epsilon)$ 244 (+27.4), 284 (−66.4), 348 (+29.2) nm; chemical shifts of ^1H and ^{13}C (Table 2); HR-ESIMS m/z 555.2791 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{29}\text{H}_{39}\text{O}_7\text{N}_4$, 555.28133).

4.4 Cytotoxicity assay

The cytotoxic activity of **1–11** against eighteen cell lines were assayed as our described previously [14, 20]. The tested cell lines see Table S3.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s13659-024-00494-4>.

Supplementary Material 1.

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Author contributions

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Data availability

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare no conflict of interest.

Author details

¹State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang 550014, China. ²Natural Product Research Center of Guizhou Province, Guiyang 550014, China. ³School of Pharmaceutical Sciences, Guizhou Medical University, Guiyang 561113, China. ⁴School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, China.

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