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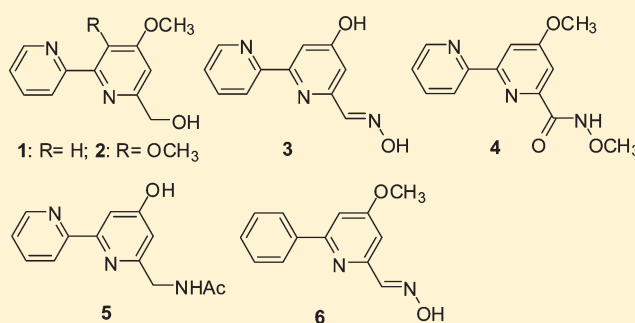
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Cytotoxic Bipyridines from the Marine-Derived Actinomycete *Actinoalloteichus cyanogriseus* WH1-2216-6Peng Fu,<sup>†,‡</sup> Shuxia Wang,<sup>†,‡</sup> Kui Hong,<sup>§</sup> Xia Li,<sup>⊥</sup> Peipei Liu,<sup>†</sup> Yi Wang,<sup>†</sup> and Weiming Zhu<sup>\*,†</sup><sup>†</sup>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<sup>§</sup>College of Pharmacy, Wuhan University, Wuhan 430071, People's Republic of China<sup>⊥</sup>Marine College, Shandong University at Weihai, Weihai 264209, People's Republic of China

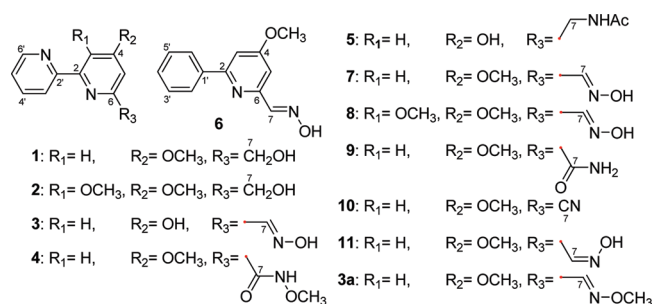
## Supporting Information

**ABSTRACT:** Five new bipyridine alkaloids (1–5) and a new phenylpyridine alkaloid (6), which we name caerulomycins F–K, along with five known analogues (7–11), were isolated from the marine-derived actinomycete *Actinoalloteichus cyanogriseus* WH1-2216-6. The structures of 1–6 were established on the basis of spectroscopic analyses and chemical methods. Compounds 1–10 showed cytotoxicity against the HL-60, K562, KB, and A549 cell lines, with IC<sub>50</sub> values of 0.26 to 15.7 μM. Compounds 7 and 8 also showed antimicrobial activities against *Escherichia coli*, *Aerobacter aerogenes*, *Pseudomonas aeruginosa*, and *Candida albicans*, with MIC values of 9.7 to 38.6 μM.



The actinomycetes are a prolific source of structurally new and biologically active metabolites and are responsible for producing over 45% of all known microbial natural products.<sup>1</sup> Most research on actinomycetes has been carried out with terrestrial species.<sup>2,3</sup> However, the discovery of new lead compounds from common soil-derived actinomycetes has declined in the past two decades.<sup>4</sup> Examining the natural product profiles of the under-exploited marine-derived actinomycetes has become a research hot spot in drug discovery. To date, nearly 400 new compounds with cytotoxicity and antimicrobial activity have been isolated from marine actinomycetes.<sup>5–8</sup> As part of our ongoing research on new antitumor compounds from marine-derived actinomycetes, strain WH1-2216-6, identified as *Actinoalloteichus cyanogriseus*, was isolated from marine sediments and was found to exhibit significant cytotoxic effects on the K562 cell line. A series of metabolites contained in the extract of strain WH1-2216-6 showed UV absorptions similar to those of bipyridine alkaloids such as caerulomycins A–E, caerulomycinonitrile, and caerulomycinamide in HPLC–UV analysis at 230 and 270 nm.<sup>9–13</sup> Bipyridine alkaloids are known to have antibiotic,<sup>10,14</sup> phytotoxic,<sup>12</sup> and immunosuppressant activity.<sup>15</sup> A chemical investigation resulted in the isolation of five new bipyridine alkaloids and a new phenylpyridine alkaloid, which we have named caerulomycins F–K (1–6). Five known analogues, caerulomycin A (7),<sup>16,17</sup> caerulomycin C (8),<sup>18,19</sup> caerulomycinamide (9),<sup>11</sup> caerulomycinonitrile (10),<sup>11</sup> and (Z)-4-methoxy-2,2'-bipyridine-6-carbaldehyde oxime (11), were also isolated.<sup>17</sup> Potent cytotoxicities were observed for compound 3 against HL-60, compound 4 against K562, compound 8 against K562, and compound 7 against HL-60 and A549 cells, with IC<sub>50</sub> values of 1.6, 0.37, 1.8, 0.71, and 0.26 μM, respectively. Compounds 7 and

8 also showed antimicrobial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* with MIC values of 10.9/9.7, 21.8/38.6, and 21.8/19.3 μM, respectively.

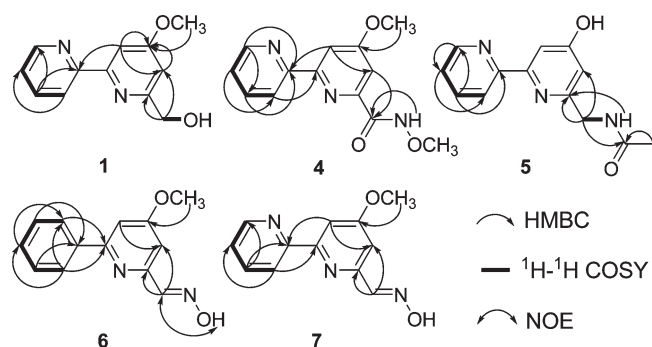


## RESULTS AND DISCUSSION

The bioactive EtOAc extract of *A. cyanogriseus* WH1-2216-6 was chromatographed on a silica gel column, and extensive reversed-phase preparative HPLC was carried out to give compounds 1–11. Among them, 7 was the major compound isolated (4 g from a 50 L culture, compared to all other compounds, which were isolated in quantities less than 50 mg). Its molecular formula was tentatively assigned as C<sub>12</sub>H<sub>11</sub>N<sub>3</sub>O<sub>2</sub> on the basis of a molecular ion peak at *m/z* 230 [M + H]<sup>+</sup> observed by ESIMS. Analysis of the 1D <sup>13</sup>C NMR data for 7 revealed four quaternary carbons, seven methylene carbons, and one methoxyl carbon. The <sup>1</sup>H NMR spectrum (Table S1) showed four signals at 8.40

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**Figure 1.** Key HMBC,  $^1\text{H}$ – $^1\text{H}$  COSY, and NOE correlations for **1** and **4**–**7**.

(d,  $J = 7.7$  Hz), 7.96 (td,  $J = 7.7$ , 1.9 Hz), 7.48 (ddd,  $J = 7.7$ , 4.5, 1.3 Hz), and 8.70 (d,  $J = 3.9$  Hz), which were assigned to a 2-disubstituted pyridine ring system, and two signals at 7.92 (d,  $J = 2.6$  Hz) and 7.34 (d,  $J = 2.6$  Hz) assigned to a 2,4,6-trisubstituted pyridine ring system. HMBC correlations (Figure 1) from H-3' to C-2 and from H-3 to C-2' identified a bipyridine structure. Further comparison of NMR spectra (Table S1) with reported data<sup>16,17</sup> indicated that compound **7** was caerulomycin A.

Caerulomycin F (**1**) was obtained as a white, amorphous powder. Its molecular formula was assigned as  $\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_2$  from the HRESIMS peak at  $m/z$  217.0976  $[\text{M} + \text{H}]^+$ , which requires eight degrees of unsaturation. The IR spectrum showed the presence of a hydroxy group ( $3450\text{ cm}^{-1}$ ) and an aromatic system ( $1652\text{ cm}^{-1}$ ). With the exception that an oxygenated methylene signal ( $\delta_{\text{C/H}}$  64.2/4.62) in **1** replaced an oxime methine signal ( $\delta_{\text{C/H}}$  148.8/8.13) in **7**, the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **1** (Table 1) were very similar to those of **7** (Table S1).<sup>16,17</sup> This suggested that the same 4-methoxy-2,2'-bipyridine skeleton found in **7** was present in **1**. Upfield shifts for C-2 to C-6 indicated that **1** was the hydroxymethyl-substituted derivative of **7**. 2D NMR correlations (Figure 1) also support the structure of **1**. Thus, the structure of compound **1** was determined to be 4-methoxy-2,2'-bipyridine-6-methanol.

Caerulomycin G (**2**) was obtained as colorless needles. The molecular formula was determined to be  $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}_3$  from the  $[\text{M} + \text{H}]^+$  peak at  $m/z$  247.1079 in the HRESIMS spectrum. The IR spectrum established the presence of hydroxy groups ( $3622\text{ cm}^{-1}$ ) and an aromatic system ( $1553\text{ cm}^{-1}$ ). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **2** (Table 1) were almost identical to those of **1** except that the aromatic methine signal at  $\delta_{\text{C/H}}$  106.4/7.08 in **1** was replaced by a methoxy signal at  $\delta_{\text{C/H}}$  61.5/3.70 and an aromatic quaternary carbon signal at  $\delta_{\text{C}}$  142.6. In addition, upfield shifts were observed for C-2 to C-4, supporting the postulation that **2** was the 3-methoxy-substituted derivative of **1**. Therefore, compound **2** was identified as 3,4-dimethoxy-2,2'-bipyridine-6-methanol.

Caerulomycin H (**3**) was obtained as a white, amorphous powder, and the molecular formula  $\text{C}_{11}\text{H}_9\text{N}_3\text{O}_2$  was assigned on the basis of the HRESIMS peak at  $m/z$  216.0781  $[\text{M} + \text{H}]^+$ . Careful comparison of its  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (Table 1) with those of **7** (Table S1) revealed that **3** contained the same 2,2'-bipyridine-6-carbaldehyde oxime skeleton, but that the  $\text{CH}_3\text{O}$ -4 of **7** was replaced by  $\text{HO}$ -4 in **3**. To confirm the structure of compound **3**, both  $\text{HO}$ -8 and  $\text{HO}$ -4 of **3** and  $\text{HO}$ -8 of **7** were methylated, which resulted in the production of a

common product as expected (**3a**). 1D NMR data of the oxime moiety in **3** were very similar to those of **7**, which indicated that the oxime moiety of **3** was also of the *E* configuration. Thus, the structure of compound **3** was determined to be (*E*)-4-hydroxy-2,2'-bipyridine-6-carbaldehyde oxime.

Caerulomycin I (**4**) was obtained as colorless needles, and the molecular formula  $\text{C}_{13}\text{H}_{13}\text{N}_3\text{O}_3$  was assigned on the basis of the HRESIMS peak at  $m/z$  282.0855  $[\text{M} + \text{Na}]^+$ . The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (Table 1) of **4** were very similar to those of **9** (Table S1),<sup>11</sup> with the exception that an amide proton signal at  $\delta_{\text{H}}$  8.45 (1H, brs) was replaced by a methoxy signal at  $\delta_{\text{C/H}}$  63.9/3.78. Furthermore, another amide proton signal was shifted downfield to  $\delta$  12.15, suggesting that **4** was the methoxy-substituted derivative of **9** at the amide nitrogen. The nearly identical NMR data of the methoxy to those of *O*-methylbenzohydroxamic acid ( $\delta_{\text{C/H}}$  63.9/3.78 vs 63.1/3.71)<sup>20,21</sup> established the placement of the methoxy group on the amido nitrogen atom, and the assignment was further supported by key cross-peaks from H-3 ( $\delta_{\text{H}}$  8.04) and the amide group proton ( $\delta_{\text{H}}$  12.15) to C-7 ( $\delta_{\text{C}}$  161.3) in the HMBC spectrum (Figure 1). Therefore, the structure of compound **4** was identified as *N*,4-dimethoxy-2,2'-bipyridine-6-carboxamide.

The molecular formula of caerulomycin J (**5**, isolated as a yellow oil) was determined as  $\text{C}_{13}\text{H}_{13}\text{N}_3\text{O}_2$  on the basis of the molecular ion peak at  $m/z$  266.0905  $[\text{M} + \text{Na}]^+$  in the HRESIMS spectrum. With the exception of the oxime moiety, the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **5** were similar to those of **3** (Table 1), indicating the same molecular skeleton. Compared with **3**, the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (Table 1) of **5** showed additional signals for a methylene ( $\delta_{\text{C/H}}$  44.6/4.30) and an acetyl group ( $\delta_{\text{C/H}}$  170.1 and 23.2/1.92), while the oxime methine signals ( $\delta_{\text{C/H}}$  149.6/8.09) in **3** were not observed. These observations suggested that the oxime group of **3** was reduced to the corresponding aminomethyl in **5**. This postulation was confirmed by a key  $^1\text{H}$ – $^1\text{H}$  COSY correlation between the NH ( $\delta_{\text{H}}$  8.44) and the  $\text{CH}_2$  ( $\delta_{\text{H}}$  4.30) and key HMBC correlations between the NH and C-6 ( $\delta_{\text{C}}$  159.9) and between the  $\text{CH}_2$  and C-5 ( $\delta_{\text{C}}$  109.3) and C-6 (Figure 1). HMBC correlations observed between the  $\text{CH}_2$  and the acetyl carbon indicated that the acetyl was connected to the NH. Thus, the structure of **5** was elucidated as *N*-[(4-hydroxy-2,2'-bipyridine-6-yl)methyl]-acetamide.

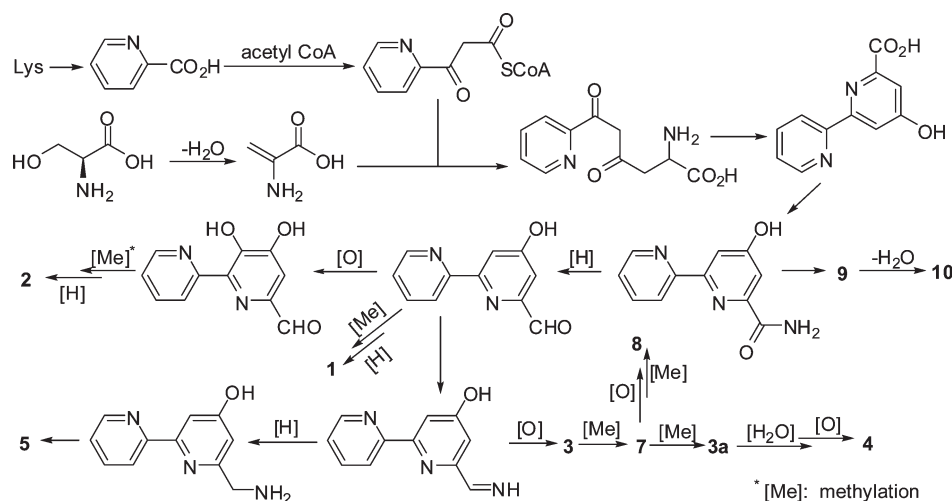
Caerulomycin K (**6**) was isolated as colorless needles. Its molecular formula was assigned as  $\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}_2$  on the basis of a HRESIMS peak at  $m/z$  229.0968  $[\text{M} + \text{H}]^+$ . The IR spectrum showed the presence of hydroxy groups and aromatic rings. Two sets of coupled  $^1\text{H}$  NMR signals at  $\delta_{\text{H}}$  8.10 (2H, d,  $J = 8.3$  Hz), 7.49 (2H, t,  $J = 7.8$  Hz), 7.44 (t,  $J = 7.3$  Hz) and 7.48 (br s), 7.26 (d,  $J = 2.3$  Hz) revealed the presence of a monosubstituted benzene ring and a 2,4,6-trisubstituted pyridine ring, and these assignments were also supported by the  $^{13}\text{C}$  NMR spectrum (Table 1). A methoxy signal at  $\delta_{\text{C/H}}$  55.6/3.93 and an oxime signal at  $\delta_{\text{C/H}}$  149.0/8.13 and  $\delta_{\text{H}}$  11.72 were also observed. These data were very similar to those of **7** (Table S1). Further comparison of their 1D NMR spectra indicated that one pyridine ring of **7** was replaced by a benzene ring in **6**. This deduction was confirmed by cross-peaks in the  $^1\text{H}$ – $^1\text{H}$  COSY spectrum from H-2'/6' ( $\delta_{\text{H}}$  8.10) to H-4' ( $\delta_{\text{H}}$  7.44) through H-3'/5' ( $\delta_{\text{H}}$  7.49) and key HMBC correlations from H-2'/6' to C-2 ( $\delta_{\text{C}}$  157.6), from H-7 ( $\delta_{\text{H}}$  8.13) to C-5 ( $\delta_{\text{C}}$  103.8) and C-6 ( $\delta_{\text{C}}$  153.5), and from H-3 ( $\delta_{\text{H}}$  7.48) to C-5 (Figure 1). Meanwhile, an NOE correlation between H-7 and HO-8 indicated that the oxime

Table 1.  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data for 1–6 (600 MHz, 150 MHz, DMSO- $d_6$ , TMS,  $\delta$  ppm)

position	1		2		3		4		5 <sup>a</sup>		6	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)
2	155.0, C		150.8, C		157.3, C		157.0, C		149.7, C		157.6, C	
3	106.4, CH	7.80, d (2.3)	142.6, C		107.5, CH	7.79, d (2.2)	108.7, CH	8.04, d (2.3)	107.2, CH	7.61, brs	106.9, CH	7.48, s
3-OCH <sub>3</sub>			61.5, CH <sub>3</sub>	3.70, s								
4	167.0, C		160.2, C		165.7, C		167.9, C		166.5, C		166.6, C	
4-OH (4-OCH <sub>3</sub> )	55.3, CH <sub>3</sub>	3.92, s	56.4, CH <sub>3</sub>	3.94, s			56.3, CH <sub>3</sub>	3.96, s		11.09, brs	55.6, CH <sub>3</sub>	3.93, s
5	104.1, CH	7.08, d (2.3)	105.2, CH	7.22, s	108.9, CH	7.21, d (2.2)	108.9, CH	7.54, d (2.3)	109.3, CH	6.65, brs	103.8, CH	7.26, d (2.3)
6	163.7, C		157.0, C		153.9, C		151.5, C		159.9, C		153.5, C	
7	64.2, CH <sub>2</sub>	4.62, s	64.7, CH <sub>2</sub>	4.51, d (6.7)	149.6, CH	8.09, s	161.3, C		44.6, CH <sub>2</sub>	4.30, d (5.9)	149.0, CH	8.13, s
7-OH (NH)		5.52, brs		5.47, d (6.6)				12.15, s		8.44, t (5.9)		11.72, s
N-OH (N-OCH <sub>3</sub> )												
1'						11.67, s	63.9, CH <sub>3</sub>	3.78, s			138.2, C	
2'	156.2, C		158.7, C		155.3, C		154.5, C		155.7, C		128.6, CH	8.10, d (8.3)
3'	120.7, CH	8.37, d (7.7)	124.5, CH	7.64, d (7.5)	121.2, CH	8.36, d (7.7)	122.4, CH	8.86, d (8.2)	121.2, CH	8.34, d (8.3)	126.8, CH	7.49, t (7.8)
4'	137.2, CH	7.93, td (7.8, 1.9)	136.9, CH	7.87, t (7.5)	137.8, CH	7.93, t (6.6)	137.7, CH	7.96, td (7.8, 1.8)	137.7, CH	7.91, td (7.8, 1.8)	129.3, CH	7.44, t (7.3)
5'	124.3, CH	7.45, ddd (7.5, 4.9, 1.3)	123.5, CH	7.40, dd (7.5, 4.4)	124.9, CH	7.45, t (6.6)	125.3, CH	7.47, ddd (7.3, 4.6, 0.9)	124.6, CH	7.42, dd (6.4, 4.6)	126.8, CH	7.49, t (7.8)
6'	148.1, CH	8.68, d (4.1)	149.2, CH	8.64, d (5.5)	149.8, CH	8.67, d (4.6)	149.6, CH	8.67, d (4.1)	149.7, CH	8.64, d (4.1)	128.6, CH	8.10, d (8.3)

<sup>a</sup> The NMR data for the acetyl are  $\delta_{\text{H}}$  1.92 (s, 3H) and  $\delta_{\text{C}}$  23.2 (CH<sub>3</sub>) and 170.1 (C).

Scheme 1. Plausible Biogenetic Pathways of 1–5 and 7–10

Table 2. Cytotoxicities against Tumor Cells for 1–10 and 3a (IC<sub>50</sub>,  $\mu$ M)

	1	2	3	4	5	6	7	8	9	10	3a
HL-60	>50	>50	1.6	>50	>50	>50	0.71	>50	>50	>50	>50
K562	15.7	NT <sup>a</sup>	NT	0.37	15.0	>50	>50	1.8	NT	15.0	31.8
A549	>50	>50	8.4	>50	>50	>50	0.26	>50	>50	>50	>50
KB	>50	NT	NT	5.2	25.7	>50	>50	3.1	NT	>50	>50

<sup>a</sup> NT = not tested.

moiety had the *E* configuration (Figure 1). Thus, the structure of compound 6 was elucidated as (*E*)-4-methoxy-2-phenylpyridine-6-carbaldehyde oxime.

It was also established that 11 could be formed from 7 by direct photoisomerization, indicating that *E*-aldoximes can be transformed into *Z*-aldoximes using light (Figure S32). Even though 11 was present in the extract before chromatography (Figure S33), it is still possible that 11 could be formed non-enzymatically during the extraction process. The HPLC profile of the EtOAc extract (Figure S33) also showed 4 is a natural product.

Compounds 1–5 and 7–10 are probably biosynthesized from amino acid and polyketide pathways (Scheme 1).<sup>13,22</sup> Intramolecular reactions of lysine can produce picolinic acid. Picolinic acid (likely as its CoA ester) may condense with acetyl CoA to form picolinoylacyl CoA, which undergoes condensation with 2-aminoacrylic acid derived from serine to produce 4-hydroxybipyridine-6-carboxylic acid. The bipyridine carboxylic acid can be converted to the corresponding bipyridine amide intermediate that would yield 9 upon methylation. Compound 10 can be produced by the dehydration of 9. The bipyridine amide or acid can undergo reduction to yield the bipyridine carbaldehyde, which through methylation, reduction, and oxidation steps can yield 1 and 2. The bipyridine carbaldehyde is also postulated to form the oxime by incorporation of ammonia and oxidation. The oxime can be further processed to yield compounds 3, 3a, 4, and 7 (Scheme 1). Compound 5 is probably formed from the carbaldehyde by ammonia incorporation, reduction, and acylation.<sup>13</sup> Compound 6 may be formed by the same biogenetic pathway as 7 except starting with benzoylacyl CoA in place of picolinoylacyl CoA.

Compounds 1–10 were evaluated for their cytotoxicity against the K562, HL-60, and KB cell lines using the MTT method,<sup>23</sup> and the A549 cell line using the SRB method (Table 2).<sup>24</sup> The new compounds 1 and 3–5 were found to be active against K562, HL-60, A549, and KB cells with IC<sub>50</sub> values ranging from 0.37 to 31.8  $\mu$ M. Compound 4 had the most potent activity, with an IC<sub>50</sub> value of 0.37  $\mu$ M against K562 cells. Caerulomycin A (7) showed potent cytotoxicity against the HL-60 and A549 cell lines, with IC<sub>50</sub> values of 0.71 and 0.26  $\mu$ M, respectively. Caerulomycin C (8) showed moderate cytotoxicity against K562 and KB cells, with IC<sub>50</sub> values of 1.8 and 3.1  $\mu$ M, respectively. Weak cytotoxicities for caerulomycinonitrile (10) against A549 cells, and 3a against K562 cells, were also observed, with IC<sub>50</sub> values of 15.0 and 31.8  $\mu$ M, respectively. The other compounds isolated were found to be inactive (IC<sub>50</sub> > 50  $\mu$ M) against the cell lines tested. Antimicrobial activities against *Escherichia coli*, *Aerobacter aerogenes*, *Pseudomonas aeruginosa*, and *Candida albicans* were also evaluated for compounds 1, 4–8, 10, and 3a using an agar dilution method.<sup>25</sup> Compound 7 showed weak antimicrobial activities against *E. coli*, *P. aeruginosa*, and *C. albicans* with MIC values of 10.9, 21.8, and 21.8  $\mu$ M, respectively. Compound 8 showed weak antimicrobial activities against *E. coli*, *A. aerogenes*, *P. aeruginosa*, and *C. albicans* with MIC values of 9.7, 19.3, 38.6, and 19.3  $\mu$ M, respectively. Compound 3a selectively inhibited the growth of *E. coli* with a MIC value of 41.1  $\mu$ M. The other compounds did not show antimicrobial activity (MIC  $\geq$  100  $\mu$ M). The results showed that the antimicrobial activity of bipyridines is not due to general metal chelation, but more importantly might be related to the oxime moiety.



## ■ EXPERIMENTAL SECTION

**General Experimental Procedures.** UV spectra were recorded on a Beckman DU 640 spectrophotometer. IR spectra were taken on a Nicolet Nexus 470 spectrophotometer as KBr discs.  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and DEPT spectra of compounds **1**–**6** and 2D NMR spectra of compounds **4** and **5** were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as an internal standard, and chemical shifts were recorded as  $\delta$  values. 2D NMR spectra of compound **6** were recorded on a Bruker Avance 500 spectrometer. 1D NOE spectra were obtained on a Varian INOVA 400 spectrometer. ESIMS utilized a Q-TOF Ultima Global GAA076 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column [YMC-pak ODS-A,  $10 \times 250$  mm,  $5 \mu\text{m}$ ,  $4 \text{ mL/min}$ ]. TLC and column chromatography (CC) were performed on plates precoated with silica gel GF<sub>254</sub> ( $10$ – $40 \mu\text{m}$ ) 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). Marimum salt used is made from the evaporation of seawater collected in Laizhou Bay (Weifang Haisheng Chemical Factory).

**Actinomycete Material.** The actinomycete *Actinoalloteichus cyanogriseus* WH1-2216-6 was isolated from marine sediments collected from the seashore of Weihai, China. The marine sediments (2 g) were air dried for 15 days in a 45 mL sterile centrifuge tube. The dried sediments were diluted into  $10^{-3}$  g/mL, 100  $\mu\text{L}$  of which was dispersed across a solid-phase agar plate (2216 media) and incubated at  $28^\circ\text{C}$  for 10 days. Then a single colony was transferred on Gause's synthetic agar media. It was identified according to its morphological characteristics and 16S rRNA gene sequences (see Supporting Information; GenBank EU596461) and has been preserved in the China Center for Type Culture Collection (patent depository number CCTCC M 209277). Working stocks were prepared on Gause's synthetic agar slants and stored at  $4^\circ\text{C}$ .

**Fermentation and Extraction.** Spores were directly inoculated into 500 mL Erlenmeyer flasks containing 150 mL fermentation media (glucose 20 g, beef extract 3 g, yeast extract 10 g, soluble starch 10 g, peptone 10 g,  $\text{K}_2\text{HPO}_4$  0.5 g,  $\text{MgSO}_4$  0.5 g,  $\text{CaCO}_3$  2 g, and marimum salt 33 g, dissolved in 1 L of tap water, pH 7.0). The flasks were incubated on a rotary shaker at 140 rpm and  $28^\circ\text{C}$ . After 11 days of cultivation, 50 L of whole broth was extracted three times with EtOAc (50 L each). The EtOAc extract was concentrated under reduced pressure to give a dark brown gum (28.0 g).

**Purification.** The gum (28.0 g) was separated into 10 fractions on a silica gel VLC column using stepwise gradient elution, with  $\text{CH}_2\text{Cl}_2$ /petroleum ether (50–100%) followed by  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  (0–50%). Fraction 4 (5.1 g,  $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 50:1) was recrystallized from  $\text{MeOH}$  to give compound **7** (4.0 g), and the mother liquor of the recrystallization was then purified by semipreparative HPLC (45%  $\text{MeOH}/\text{H}_2\text{O}$ ) to afford compound **11** (3.3 mg,  $t_{\text{R}}$  22 min). Fraction 3 (4.2 g) was separated into four subfractions by VLC on RP-18 silica using stepwise gradient elution with 5–90%  $\text{MeOH}/\text{H}_2\text{O}$ . Subfraction 3-2 (650 mg) was further purified by semipreparative HPLC (70%  $\text{MeOH}/\text{H}_2\text{O}$ ) to yield **10** (6.0 mg,  $t_{\text{R}}$  7.2 min). Fraction 5 (4.5 g) was separated into nine subfractions by VLC on RP-18 using stepwise gradient elution with 5–90%  $\text{MeOH}/\text{H}_2\text{O}$ . Subfraction 5-1 (1.3 g) was separated into six subfractions on Sephadex LH-20, eluting with  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  (1:1). Subfractions 5-1-2 (96 mg) and 5-1-3 (260 mg) were further purified by semipreparative HPLC (45%  $\text{MeOH}/\text{H}_2\text{O}$ ) to yield **2** (3.1 mg,  $t_{\text{R}}$  11.5 min) and **8** (13.6 mg,  $t_{\text{R}}$  13.1 min), respectively. Subfraction 5-3 (1.1 g) was separated into five further subfractions by Sephadex LH-20, eluting with  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  (1:1). Subfractions 5-3-3 (330 mg) and 5-3-4 (235 mg) were further separated by semipreparative HPLC to yield **4** (53.0 mg,  $t_{\text{R}}$  14.2 min) and **1** (12.0 mg,  $t_{\text{R}}$  9.7 min), eluting with 47% and 60%

$\text{MeOH}/\text{H}_2\text{O}$ , respectively. Subfraction 5-4 (940 mg) was further separated into seven subfractions by Sephadex LH-20, eluting with  $\text{MeOH}$ . Subfraction 5-4-5 (107 mg) was further purified by semipreparative HPLC (60%  $\text{MeOH}/\text{H}_2\text{O}$ ) to yield **6** (3.5 mg,  $t_{\text{R}}$  13.3 min). Fraction 6 (1.1 g) was separated into three subfractions by Sephadex LH-20, eluting with  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  (1:1). Subfraction 6-2 (370 mg) and 6-3 (240 mg) were further purified by semipreparative HPLC to yield **9** (10.0 mg,  $t_{\text{R}}$  10.8 min) and **3** (3.0 mg,  $t_{\text{R}}$  13.7 min), eluting with 40% and 30%  $\text{MeOH}/\text{H}_2\text{O}$ , respectively. Fraction 7 (1.5 g) was subjected to CC using a stepwise gradient elution of EtOAc/petroleum ether (25–100%) to afford four subfractions. Subfraction 7-5 (250 mg) was further purified by semipreparative HPLC (35%  $\text{MeOH}/\text{H}_2\text{O}$ ) to yield **5** (3.2 mg,  $t_{\text{R}}$  8.9 min).

**Caerulomycin F (1):** white, amorphous powder; UV ( $\text{MeOH}$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 203 (2.41), 253 (1.16) nm; IR (KBr)  $\nu_{\text{max}}$  3449, 2924, 1652, 1464, 1381, 1113, 1099,  $1056 \text{ cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR, see Table 1; HRESIMS  $m/z$  217.0976 [ $\text{M} + \text{H}$ ] $^+$  (calcd for  $\text{C}_{12}\text{H}_{13}\text{N}_2\text{O}_2$ , 217.0977).

**Caerulomycin G (2):** colorless needles ( $\text{MeOH}$ ); mp  $127^\circ\text{C}$ ; UV ( $\text{MeOH}$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 214 (3.16), 277 (2.62) nm; IR (KBr)  $\nu_{\text{max}}$  3749, 3622, 2906, 2846, 1779, 1692, 1553, 1454, 1069,  $670 \text{ cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR, see Table 1; HRESIMS  $m/z$  247.1079 [ $\text{M} + \text{H}$ ] $^+$  (calcd for  $\text{C}_{13}\text{H}_{15}\text{N}_2\text{O}_3$ , 247.1083).

**Caerulomycin H (3):** white, amorphous powder; UV ( $\text{MeOH}$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 240 (2.91), 280 (2.75) nm; IR (KBr)  $\nu_{\text{max}}$  3171, 2953, 1651, 1502, 1378, 1302, 1016, 980, 797,  $738 \text{ cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR, see Table 1; HRESIMS  $m/z$  216.0781 [ $\text{M} + \text{H}$ ] $^+$  (calcd for  $\text{C}_{11}\text{H}_{10}\text{N}_3\text{O}_2$ , 216.0773).

**Caerulomycin I (4):** colorless needles ( $\text{MeOH}$ ); mp  $101^\circ\text{C}$ ; UV ( $\text{MeOH}$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 230 (3.66), 278 (3.23) nm; IR (KBr)  $\nu_{\text{max}}$  3257, 3098, 3065, 2939, 1676, 1584, 1480, 1426, 1356, 1219, 1034, 875, 780,  $671 \text{ cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR, see Table 1; HRESIMS  $m/z$  282.0855 [ $\text{M} + \text{Na}$ ] $^+$  (calcd for  $\text{C}_{13}\text{H}_{13}\text{N}_3\text{O}_3\text{Na}$ , 282.0855).

**Caerulomycin J (5):** yellow oil; UV ( $\text{MeOH}$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 230 (3.83), 273 (3.63) nm; IR (KBr)  $\nu_{\text{max}}$  3288, 2926, 1633, 1537, 1442, 1372, 1027, 794,  $621 \text{ cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR, see Table 1; HRESIMS  $m/z$  266.0905 [ $\text{M} + \text{Na}$ ] $^+$  (calcd for  $\text{C}_{13}\text{H}_{13}\text{N}_3\text{O}_2\text{Na}$ , 266.0905).

**Caerulomycin K (6):** colorless needles ( $\text{MeOH}$ ); mp  $153^\circ\text{C}$ ; UV ( $\text{MeOH}$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 210 (2.24), 242 (3.34) nm; IR (KBr)  $\nu_{\text{max}}$  3748, 3167, 3062, 2925, 2852, 1593, 1562, 1463, 1359, 1052,  $987 \text{ cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR, see Table 1; HRESIMS  $m/z$  229.0968 [ $\text{M} + \text{H}$ ] $^+$  (calcd for  $\text{C}_{13}\text{H}_{13}\text{N}_2\text{O}_2$ , 229.0977).

**Chemical Transformations of 3 and 7.** A suspension of NaH (1.0 mg) in 1.0 mL of anhydrous DMF was treated with a solution of compound **3** (1.0 mg) in 1.0 mL of DMF at  $0^\circ\text{C}$ . The reaction mixture was stirred at  $0^\circ\text{C}$  for 30 min, and then  $\text{CH}_3\text{I}$  (10  $\mu\text{L}$ ) was added. After 30 min, a saturated solution of  $\text{NH}_4\text{Cl}$  (5.0 mL) was added to quench the reaction. The product was extracted with EtOAc ( $4 \times 10 \text{ mL}$ ) and purified by VLC on RP-18 silica to give **3a** (1.0 mg, 94%). Using the same procedure, compound **7** also afforded **3a** (0.9 mg, 85%). Product **3a** was identified as *O*-methyl-(*E*)-4-methoxy-2,2'-bipyridine-6-carbaldehyde oxime by comparison of its  $^1\text{H}$  NMR spectrum with that reported in the literature.<sup>11</sup> Compound **3a**: white, amorphous powder; UV ( $\text{MeOH}$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 231 (3.63), 257 (3.41) nm;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz)  $\delta$  3.98 (s, 3H,  $\text{OCH}_3$ -8), 4.04 (s, 3H,  $\text{OCH}_3$ -4), 7.32 (ddd, 1H,  $J = 7.1, 4.9, 1.1 \text{ Hz}$ , H-5'), 7.38 (d, 1H,  $J = 2.8 \text{ Hz}$ , H-5), 7.82 (td, 1H,  $J = 7.7, 1.7 \text{ Hz}$ , H-4'), 7.95 (d, 1H,  $J = 2.8 \text{ Hz}$ , H-3), 8.21 (s, 1H, H-7), 8.43 (d, 1H,  $J = 7.7, \text{H-3'}$ ), 8.67 (d, 1H,  $J = 4.1 \text{ Hz}$ , H-6'); ESIMS  $m/z$  244 [ $\text{M} + \text{H}$ ] $^+$ .

**Photoisomerization.** The isomerization of **7** (1 mg) was carried out under the following experimental conditions: reaction times, 1 h, 4 h, 40 h; solvent (2 mL),  $\text{Me}_2\text{CO}$ ; temperature,  $25^\circ\text{C}$ ; light source, mercury vapor lamp or no light (control). The reaction mixtures obtained from each set of reaction conditions were analyzed by HPLC (50%  $\text{MeOH}$ ) (Figure S32). Compound **7** was found to isomerize to **11** when light was

applied, while it was stable in the absence of light. The proportion of 7 that had isomerized with the application of light after 1, 4, and 40 h was 8.4%, 14%, and 33%, respectively.

## ■ ASSOCIATED CONTENT

**S Supporting Information.** NMR spectra for compounds 1–6, NMR data for compounds 7–11, HPLC analysis of the product of photoisomerization of 7, a description of the bioassay protocols used, and 16S rRNA sequences of *A. cyanogriseus* WH1-2216-6 are available free of charge via the Internet at <http://pubs.acs.org>.

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