Bioorganic & Medicinal Chemistry 20 (2012) 5290–5295

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Indole alkaloids from two cultured cyanobacteria, *Westiellopsis* sp. and *Fischerella muscicola*

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ARTICLE INFO

Article history:
Received 10 April 2012
Revised 13 June 2012
Accepted 18 June 2012
Available online 5 July 2012

Keywords:
Cyanobacteria
Stigonematales
Westiellopsis sp.
Fischerella muscicola
Indole alkaloids
Cytotoxicity
20S Proteasome
Antimicrobial

ABSTRACT

Chemical investigation of two cultured cyanobacteria, *Westiellopsis* sp. (SAG strain number 20.93) and *Fischerella muscicola* (UTEX strain number LB1829) led to the isolation of three hapalindole-type alkaloids, namely hapalindole X (**1**), deschloro hapalindole I (**2**), and 13-hydroxy dechlorofontonamide (**3**), along with ten known indole alkaloids (hapalindoles A, C, G, H, I, J, and U, hapalonamide H, anhydrohapaloxindole A, and fischerindole L) and fischerellins A and B. The structures were determined by a combination of spectroscopic analyses mainly based on 1D and 2D NMR and HRESIMS data. Selected compounds were evaluated for cytotoxicity and exhibited weak to moderate cytotoxicity against HT-29, MCF-7, NCI-H460, SF268, and IMR90 cells. All compounds, except hapalindole C, were evaluated for 20S proteasome inhibition and displayed either weak or no inhibition at 25 μg/mL. Selected compounds were also evaluated for antimicrobial activity, and hapalindoles X (**1**) and A, and hapalonamide H showed potent activity against both *Mycobacterium tuberculosis* and *Candida albicans* with MIC values ranging from 0.6 to 2.5 μM.

1. Introduction

More than 4000 strains of cyanobacteria (blue-green algae) have been studied to date, with more than 1000 secondary metabolites described.[1–4] This includes over 70 indole alkaloids from branched filamentous cyanobacteria belonging to the order Stigonematales.[5–21] These alkaloids include hapalindoles,[5–9] fischerindoles,[10–12] welwitindolinones,[11] ambiguines,[13–17] hapalindolinones,[18] hapaloxindoles,[19,20] and fontonamides.[19,20] All have polycyclic carbon skeletons derived from L-tryptophan and geraniol pyrophosphate and possess diverse biological activities, such as antibacterial, antifungal, and antialgal activities.[5,10,21]

As part of our ongoing collaborative natural product drug discovery project,[22] we evaluated organic extracts of cultured cyanobacteria for cytotoxicity using a set of human cancer cell lines designated HT-29 (colon), MCF-7 (breast), NCI-H460 (lung), and SF268 (CNS). The organic extract of *Westiellopsis* sp. (SAG 20.93) displayed cytotoxicity against HT-29 cells. Bioassay-guided fractionation led to isolation of hapalindole X (**1**) and deschloro hapalindole I (**2**) along with previously known hapalindoles A, C, G, H, I, J, and U, and hapalonamide H. The organic extract of *Fischerella muscicola* (UTEX LB1829) inhibited the growth of MCF-7, NCI-H460, and SF268 cells, and 13-hydroxy dechlorofontonamide (**3**) was isolated along with previously known hapalindoles A, H, I, and J, as well as fischerellins A and B. The three new hapalindole-type alkaloids were named hapalindole X (**1**), deschloro hapalindole I (**2**), and 13-hydroxy dechlorofontonamide (**3**) in line with the tradition used for previously reported hapalindole-type alkaloids from cyanobacteria (Fig. 1). We herein present the isolation, structure elucidation, and bioactivity evaluation of these compounds.

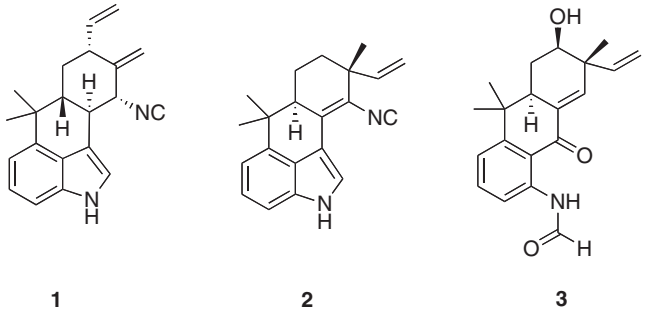

Figure 1. Structures of hapalindole X (**1**), deschloro hapalindole I (**2**), and 13-hydroxy dechlorofontonamide (**3**) isolated from *Westiellopsis* sp. (SAG 20.93) and *Fischerella muscicola* (UTEX LB1829). *Absolute configurations shown are arbitrary as only relative configurations were determined in all three compounds.

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http://dx.doi.org/10.1016/j.bmc.2012.06.030

2. Results and discussion

2.1. Structure elucidation

Hapalindole X (**1**) was obtained as a yellow amorphous powder. The HRESIMS pseudo-molecular $[M+H]^+$ ion at *m/z* 303.1853 indicated a molecular formula of $C_{21}H_{22}N_2$. The UV absorptions [λ_{max} (log ε) 223 (6.48) 275 (7.32) nm] and the ^{1}H NMR signals [δ_H 8.07 (H-1), 7.67 (H-2), 7.18 (H-6 and 7), and 7.02 (H-5)] indicated the presence of an indole moiety. Resonances at δ_H 5.94 (H-20), 5.19 (H-21*E*), and 5.14 (H-21*Z*) and at δ_H 1.48 (H_3-17) and 1.08 (H_3-18) showed the presence of a vinyl and two methyl groups, respectively. The two resonances at δ_H 5.45 (H-19a) and 5.06 (H-19b) were assigned to an exomethylene moiety. In addition, one methylene [δ_H 2.11 (H-14_{eq}) and 1.37 (H-14_{ax})] and four methines [δ_H 4.24 (H-11), 3.08 (H-10), 2.64 (H-13), and 1.77 (H-15)] were observed. Correlations observed in the COSY spectrum connected these latter resonances and the vinyl group to form a 7-carbon spin system (Fig. 2 and Table 1). The ^{13}C NMR spectrum of **1** displayed all 21 carbon resonances required by the molecular formula. Of the thirteen resonances observed between 100 and 160 ppm, eight (δ_C 140.7, 133.7, 125.2, 123.3, 118.8, 113.4, 113.2, and 108.54) were assigned to the indole moiety and two (δ_C 139.1 and 116.3) were attributed to a vinyl group. The carbon resonances at δ_C 144.7 and 108.53 were assigned to an exomethylene moiety, and the final carbon resonance at δ_C 159.5 was assigned to an isonitrile moiety. These partial structures were connected by correlations observed in the HMBC spectrum (Fig. 2 and Table 1). A correlation from H-5 to C-16 connected the indole moiety to the *gem*-dimethyl group. Correlations from both H_3-17 and H_3-18 to C-15 further connected this group to the 7-carbon H-11 to H_2-21 fragment established in the COSY spectrum. The exomethylene moiety was placed at C-12 by a correlation from H-19a to C-12, and connected to C-11 and C-13 by correlations from both H-19a and H-19b to C-11 and C-13. The final connectivity from C-10 to C-3 was determined by a correlation from H-10 to C-3, and completed the planar structure of **1**. Although a direct correlation from H-11 to C-22 was not observed, the ^{13}C NMR chemical shift at δ_C 159.5 and a characteristic IR peak at 2142 cm^{-1} indicated the presence of an isonitrile. The position of the isonitrile at C-11 was determined based on the ^{13}C NMR chemical shift of C-11 (δ_C 63.0). The relative configuration of **1** was determined by correlations observed from 2D NOESY spectrum (Table 1). Correlations observed between H-13 and H-15, H-11, and H-14_{eq} indicated H-11, H-13, and H-15 all to be in

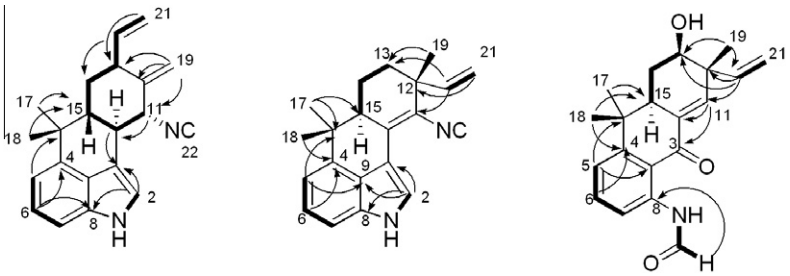

Figure 2. Key COSY (▬) and HMBC (—) correlations of compounds **1**–**3**.

Table 1
NMR data of hapalindole X (**1**) in $CDCl_3$

Position	δ_C, mult.[a]	δ_H	mult. (*J* in Hz)[b]	COSY[b]	HMBC[b]	NOESY[b]
1		8.07	br s	2		2
2	118.8, CH	7.67	t (1.7)	1, 10[c]	3, 8, 9	1, 10, 11
3	113.4, C					
4	140.7, C					
5	113.2, CH	7.02	d (6.8)	6	7, 9, 16	17, 18
6	123.3, CH	7.18	t (6.8)	5, 7	4, 8	
7	108.54, CH	7.18	d (6.8)		5, 9	
8	133.7, C					
9	125.2, C					
10	42.7, CH	3.08	t (11.1)	11, 15	2, 3, 11, 15	11, 14_{ax}, 15, 18
11	63.0, CH	4.24	d (11.1)	10, 19a, 19b	10, 12	10, 13, 15
12	144.7, C					
13	45.9, CH	2.64	br t	14_{eq}, 14_{ax}, 20		11, 14_{eq}, 15, 20
14_{eq}	33.1, CH_2	2.11	dt (12.6, 3.0)	13, 14_{ax}, 15	10, 12	13, 14_{ax}, 15
14_{ax}		1.38	ddd (12.6)	13, 14_{eq}, 15	10, 12, 13, 15, 20	10, 14_{eq}, 18
15	48.7, CH	1.77	ddd (12.6, 11.1, 3.0)	10, 14_{eq}, 14_{ax}		11, 13, 14_{eq}
16	37.9, C					
17	25.08, CH_3	1.48	s	18	4, 15, 16, 18	5
18	25.05, CH_3	1.08	s	17	4, 15, 16, 17	5, 10
19a	108.53, CH_2	5.45	t (1.7)	11	11, 12, 13	11, 19b
19b		5.06	t (1.7)	11	11, 13	19a, 20
20	139.1, CH	5.94	ddd (17.6, 10.4, 7.8)	13, 21*E*, 21*Z*	13, 14	14_{ax}, 21*E*, 21*Z*
21*E*	116.3, CH_2	5.19	dd (10.4, 1.7)	20	13	
21*Z*		5.14	dt (17.6, 1.7)	20	13, 20	13, 20
22	159.5, C					

[a] Recorded at 225 MHz.
[b] Recorded at 600 MHz.
[c] A weak four-bond correlation resulting from 'W' configuration.

the same plane. Correlations observed between H-14$_{ax}$ and H-10 indicated these to be in the opposite plane. Thus, the relative configuration of **1** was established as 10*S**, 11*R**, 13*R**, and 15*R**.

Deschloro hapalindole I (**2**) was obtained as a white amorphous powder. The HRESIMS pseudo-molecular $[M+H]^+$ ion at *m/z* 303.1863 indicated a molecular formula of $C_{21}H_{22}N_2$. The ^{1}H NMR signals of an indole moiety [δ_H 8.32 (H-1), 7.89 (H-2), 7.206 (H-7), 7.202 (H-6), and 7.04 (H-5)], a vinyl group [δ_H 5.94 (H-20), 5.21 (H-21*E*), and 5.18 (H-21*Z*)], and three methyl singlets [δ_H 1.52 (H$_3$-17), 1.36 (H$_3$-19), and 1.04 (H$_3$-18)] indicated **2** to be a hapalindole-type alkaloid. The UV absorptions [λ_{max} (log ε) 223 (8.31) 247 (7.63) 280 (7.32) 324 (7.67)] were markedly similar as those reported for hapalindole I, structure of which contained a chlorine atom on position 13.[6] Taken together, **2** was suggested to be the deschloro derivative of hapalindole I and this was explained by a comparison of the ^{1}H NMR chemical shifts. The major differences between hapalindole I and **2** were observed at H-13 [δ_H 4.14 vs δ_H 1.76 (H-13$_{eq}$) and 1.72 (H-13$_{ax}$) in **2**] and H-14 [H-14$_{eq}$

Table 2
NMR data of deschloro hapalindole I (**2**) in $CDCl_3$

Position	δ_C, mult.[a]	δ_H	mult. (*J* in Hz)[b]	COSY[b]	HMBC[b]	NOESY[b]
1		8.32	br s	2		
2	123.3, CH	7.89	d (2.4)	1	3, 8, 9	
3	109.9, C					
4	140.7, C					
5	114.5, CH	7.04	m	6	4, 7, 9, 10, 16	17, 18
6	124.4, CH	7.202	m	7	4, 5, 8	
7	109.2, CH	7.206	m	6	5, 9	
8	133.1, C					
9	124.9, C					
10	132.4, C					
11	121.6, C					
12	40.6, C					
13$_{eq}$	35.8, CH_2	1.76	m	14$_{ax}$	12, 19	19
13$_{ax}$		1.72	m	13$_{eq}$		15
14$_{eq}$	20.1, CH_2	1.97	m	14$_{ax}$, 15	13	15
14$_{ax}$		1.83	m	13$_{eq}$, 14$_{eq}$, 15	13	18, 19
15	47.4, CH	2.66	dd (9.6, 6.6)	14$_{eq}$, 14$_{ax}$	10, 11, 16, 17	13$_{ax}$, 14$_{eq}$, 14$_{ax}$, 17
16	38.9, C					
17	24.6, CH_3	1.52	s	18	4, 5, 16, 18	
18	26.1, CH_3	1.04	s	17	4, 5, 16, 17	14$_{ax}$
19	23.6, CH_3	1.36	s		11, 12, 13, 20	
20	145.2, CH	5.94	dd (17.4, 10.8)	21*E*, 21*Z*	11, 12, 13, 20	13$_{ax}$, 19
21*E*	114.7, CH_2	5.21	d (10.8)	20	12	19
21*Z*		5.18	d (17.4)	20	12, 20	19
22	[c]					

[a] Chemical shifts determined from gHSQC and gHMBC experiments recorded at 600 MHz.
[b] Recorded at 600 MHz.
[c] Signal not observed.

Table 3
NMR data of 13-hydroxy dechlorofontonamide (**3**) in $CDCl_3$

Position	δ_C, mult.[a]	δ_H	mult. (*J* in Hz)[b]	COSY[b]	HMBC[b]	NOESY[b]
1		11.67	s	2		2
2	159.9, CH	8.47	s	1	8	1
3	188.9, C					
4	153.7, C					
5	118.8, CH	7.19	d (8.2)	6	7, 9, 16	17
6	135.3, CH	7.51	t (8.2)	5, 7	4, 8	5
7	119.3, CH	8.59	d (8.2)	6	5	
8	140.8, C					
9	117.7, C					
10	133.6, C					
11	144.5, CH	7.10	d (2.4)	15	3, 10, 13, 15, 20	19
12	44.0, C					
13	64.8, CH	4.05	dd (12.8, 3.4)	14$_{eq}$, 14$_{ax}$	14, 15, 19, 20	14$_{eq}$, 15, 20
13-OH		1.54	s			
14$_{eq}$	30.8, CH_2	2.32	ddd (12.8, 6.0, 3.4)	13, 14$_{ax}$, 15	10, 12, 13	13, 15, 17
14$_{ax}$		2.09	q (12.8)	13, 14$_{eq}$, 15	12, 13, 16	18, 19
15	44.2, CH	2.82	ddd (12.8, 6.0, 2.4)	14$_{eq}$, 14$_{ax}$	11, 14, 16, 18	13, 17
16	37.8, C					
17	24.2, CH_3	1.44	s		4, 15, 16, 18	5
18	25.4, CH_3	1.04	s		4, 15, 16, 17	
19	19.9, CH_3	1.35	s		11, 12, 13, 20	11, 20, 21*Z*
20	142.9, CH	5.92	dd (17.4, 10.7)	21*E*, 21*Z*	11, 12, 13, 19	19
21*E*	115.1, CH_2	5.20	d (10.7)	20	12, 13[c]	
21*Z*		5.18	d (17.4)	20	12, 20	19

[a] Chemical shifts determined from gHSQC and gHMBC experiments recorded at 600 MHz.
[b] Recorded at 600 MHz.
[c] A four-bond correlation resulting from 'W' configuration.

(δ_H 2.41 vs δ_H 1.97 in **2**) and H-14$_{ax}$ (δ_H 2.23 vs δ_H 1.83 in **2**)]. In addition, minor differences in the ^{1}H NMR chemical shifts were observed around H-13 [H-21*E* (δ_H 5.46 vs δ_H 5.21 in **2**), H-21*Z* (δ_H 5.36 vs δ_H 5.18 in **2**), H-20 (δ_H 5.85 vs δ_H 5.94 in **2**), and H$_3$-19 (δ_H 1.47 vs δ_H 1.36 in **2**)]. The planar structure was confirmed by correlations observed in the COSY and HMBC spectra (Fig. 2 and Table 2). The correlation to an isonitrile moiety was not observed in the HMBC spectrum and amount of the compound was not enough to obtain a carbon spectrum. However, the presence of the isonitrile moiety was confirmed by a characteristic IR peak at 2135 cm^{-1}. The 2D NOESY spectrum was analyzed to determine the relative configuration of **2**. Correlations observed between H-14$_{ax}$ and H$_3$-18 and H$_3$-19, and between H-15 and H-13$_{ax}$, H-14$_{eq}$, and H$_3$-17 indicated H-15 and H$_3$-19 to be in the opposite plane. Thus, the relative configuration of **2** was established as 12*R** and 15*S**, which is identical to that observed for hapalindole I.

13-Hydroxy dechlorofontonamide (**3**) was obtained as a pale yellow amorphous powder. The HRESIMS pseudo-molecular [M−H]$^-$ ion at *m/z* 324.1630 indicated a molecular formula of $C_{20}H_{23}NO_3$. The ^{1}H NMR chemical shifts of **3** (Table 3) closely resembled those reported for dechlorofontonamide,[20] with the major differences being observed at H-13 [δ_H 1.73 (H-13$_{eq}$) and 1.64 (H-13$_{ax}$) vs δ_H 4.05 in **3**] and H-14 [H-14$_{eq}$ (δ_H 1.96 vs δ_H 2.32 in **3**) and H-14$_{ax}$ (δ_H 1.69 vs δ_H 2.09 in **3**)]. Some additional minor differences in the ^{1}H NMR chemical shifts between dechlorofontonamide and **3** were observed at H-21*E* (δ_H 5.05 vs δ_H 5.20 in **3**), H-21*Z* (δ_H 5.01 vs δ_H 5.18 in **3**), H-20 (δ_H 5.85 vs δ_H 5.92 in **3**), and H$_3$-19 (δ_H 1.19 vs δ_H 1.35 in **3**). Together, these findings indicated **3** to be the hydroxyl derivative of dechlorofontonamide, consistent with the molecular formula of $C_{20}H_{23}NO_3$. The hydroxyl group was placed at C-13 by analysis of the COSY spectrum, which indicated a CH–CH$_2$–CH spin system from C-15 to C-13, combined with the ^{13}C NMR chemical shift of C-13 (δ_C 64.8) (Table 3). The relative configuration of **3** was determined by analysis of 2D NOESY spectrum. Correlations observed between H-13 and H-15 and H-14$_{eq}$ indicated H-13 and H-15 to be in the same plane. Correlations observed between H-14$_{ax}$ and H$_3$-18 and H$_3$-19 indicated H-15 and H$_3$-17 to be in the opposite plane. Thus, the relative configuration of **3** was established as 12*R**, 13*S**, and 15*S**, which is identical to those reported for both fontonamide and dechlorofontonamide.

Table 4
Cytotoxic activity of selected compounds isolated from *Westiellopsis* sp. (SAG 20.93) and *Fischerella muscicola* (UTEX LB1829)

	IC$_{50}$ (μM)				
	HT-29 [a]	MCF-7 [b]	NCI-H460 [c]	SF268 [d]	IMR90[e]
Hapalindole X (**1**)	24.8	35.4 ± 2.8	23.0 ± 4.6	23.5 ± 9.5	113.2 ± 13.2
13-hydroxy dechlorofontonamide (**3**)	NA	>100	>100	>100	>100
Hapalindole I	NA	>100	68.5 ± 11.0	93.1 ± 14.0	>100
Hapalindole J	28.6	43.7 ± 10.0	12.0 ± 1.9	16.9 ± 3.4	39.1 ± 6.6
Hapalindole A	31.3	30.7 ± 7.1	17.0 ± 4.8	16.3 ± 7.1	39.1 ± 10.2
Hapalindole U	52.6	>100	>100	>100	>100
Hapalindole C	52.6	>100	53.7 ± 15.5	88.6 ± 17.6	>100
Hapalindole H	10.8	16.3 ± 3.3	8.5 ± 3.7	10.6 ± 4.5	31.9 ± 11.6
Anhydrohapaloxindole A	NT	56.7 ± 10.5	18.7 ± 6.2	26.0 ± 9.0	80.1 ± 24.5
Fischerindole L	48.2	28.3 ± 8.1	15.1 ± 2.6	17.4 ± 8.6	46.3 ± 13.7
Camptothecin	NT	0.08 ± 0.1	0.002 ± 0.0	0.07 ± 0.1	0.43 ± 0.5

[a] Human colon adenocarcinoma.
[b] Human breast carcinoma.
[c] Human large cell lung carcinoma.
[d] Human glioblastoma cancer cell.
[e] Human lung fibroblast normal cell. IC$_{50}$ values were obtained by averaging the results from three independent experiments. NT represents not tested. NA represents not active.

Table 5
MIC and IC$_{50}$ values for selected compounds isolated from and *Westiellopsis* sp. (SAG 20.93) and *Fischerella muscicola* (UTEX LB1829)

	MIC (μM)						IC$_{50}$ (μM)
	M. tuberculosis [a]	*M. smegmatis* [b]	*C. albicans* [c]	*S. aureus* [d]	*E. coli* [e]	*A. baumannii* [f]	Vero
Hapalindole X (**1**)	2.5	78.8	2.5	9.1	>100	>100	35.2
Deschloro hapalindole I (**2**)	>100	>100	>100	>100	>100	>100	>100
13-Hydroxy dechlorofontonamide (**3**)	>100	>100	>100	>100	>100	>100	>100
Hapalindole I	2.0	>100	>100	>100	>100	>100	>100
Hapalindole J	4.3	39.0	0.7	8.4	>100	>100	31.9
Hapalindole A	< 0.6	18.2	1.2	3.9	8.0	>100	25.6
Anhydrohapaloxindole A	16.2	>100	1.9	27.3	>100	>100	79.9
Hapalonamide H	1.2	34.3	< 0.6	>100	>100	>100	13.6
Fischerindole L	22.0	63.0	1.2	6.4	>100	>100	< 9.2
Fischerellin A	43.1	>100	>100	>100	>100	>100	94.8
Fischerellin B	23.0	>100	>100	>100	>100	>100	>100
Rifampin	0.24						
Streptomycin		0.41					
Amphotericin B			0.12				
Ampicillin				1.54			
Gentamycin					2.3		
Doxycycline						0.35	

[a] *Mycobacterium tuberculosis.*
[b] *Mycobacterium smegmatis.*
[c] *Candida albicans.*
[d] *Staphylococcus aureus.*
[e] *Escherichia coli.*
[f] *Acinetobacter baumannii.*

2.2. Biological evaluation

Selected compounds were evaluated for cytotoxicity against HT-29 (colon), MCF-7 (breast), NCI-H460 (lung), and SF268 (CNS) cancer cells, and IMR90 (lung) cells (Table 4). Hapalindole X (**1**) showed moderate cytotoxicity against all four cancer cells and relatively low cytotoxicity against IMR90 cells. The most cytotoxic compound was hapalindole H with IC_{50} values ranging from 8.5 to 31.9 μM, while 13-hydroxy dechlorofontonamide (**3**) was inactive in all cell lines tested. Mo et al. previously reported antimicrobial activities of hapalindole-related alkaloids,[16,17] and selected compounds were further evaluated for antimicrobial activity against a panel of microorganisms including *Mycobacterium tuberculosis*, *Mycobacterium smegmatis*, *Candida albicans*, *Escherichia coli*, and *Acinetobacter baumannii* as well as for Vero cell toxicity (Table 5). Hapalindole X (**1**) displayed antimicrobial activities against both *M. tuberculosis* and *C. albicans* with MIC values of 2.5 μM and moderate cytotoxicity in the Vero cell assay with an IC_{50} value of 35.2 μM. Hapalindole I showed inhibitory activity against *M. tuberculosis* with a MIC value of 2.0 μM and no detectable cytotoxicity against Vero cells. Hapalindole A was the most active compound against *M. tuberculosis* with a MIC value of less than 0.6 μM. Both deschloro hapalindole I (**2**) and 13-hydroxy dechlorofontonamide (**3**) showed no antimicrobial activity. None of the evaluated compounds inhibited the growth of *A. baumannii*. Only hapalindole A inhibited the growth of the gram-negative bacteria *E. coli* (MIC value of 8.0 μM).

3. Conclusions

The bioassay-guided investigation of two cultured cyanobacteria, *Westiellopsis* sp. and *Fischerella muscicola*, both belonging to the order Stigonematales, led to the isolation of three new hapalindole-type alkaloids. Hapalindole X (**1**) represents the first report of a tetracyclic hapalindole with an exocyclic methylene moiety. This moiety was found at C-12, a position substituted with a vinyl group and a methyl group in all previously reported tetracyclic hapalindoles. In hapalindole X (**1**), the vinyl group was located at C-13, suggesting the presence of a new biosynthetic pathway in this organism. Most hapalindoles have been reported in both chlorinated and deschlorinated forms, and we herein report the isolation of deschlorinated hapalindole I. Hapalonamides are considered oxidation products of the hapalindoles,[19] and it can be assumed that 13-hydroxy dechlorofontonamide (**3**) is the oxidation product of hapalindole A.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Varian Cary 50 Bio spectrophotometer. IR spectra were obtained on a FTIR-410 Fourier transform infrared spectrometer. 1D and 2D NMR spectra were obtained at room temperature on a Bruker Avance DRX 600 MHz spectrometer with a 5 mm CPTXI Z-gradient probe. ^{13}C NMR spectrum was obtained on a Bruker AV 900 MHz NMR spectrometer with a 5 mm ATM CPTCI Z-gradient probe. ^{1}H and ^{13}C chemical shifts were referenced to a residual proton, 7.24 and 77.0 ppm in $CDCl_3$. HRESIMS were obtained using a Shimadzu IT-TOF spectrometer.

4.2. Biological material

Westiellopsis sp. and *Fischerella muscicola* were initially acquired from the culture collections of Algae at the University of Göttingen (SAG strain number 20.93) and at the University of Texas at Austin (UTEX strain number LB1829), respectively. Each cyanobacterium was grown in a 2.8 L Fernbach flask containing 2 L of inorganic media (Z media for SAG 20.93 and BG-12 media for UTEX LB1829) under aeration.[23] Cultures were illuminated with fluorescent lamps at 1.93 klx with an 18/6 h light/dark cycle at 22 °C. After eight weeks, the biomass of each cyanobacterium was harvested by centrifugation, and lyophilized.

4.3. Extraction and isolation

The lyophilized biomass (1.00 g) of *Westiellopsis* sp., from total of 4 L culture, was extracted by maceration with CH_2Cl_2/MeOH (1:1 v/v) at room temperature and the solvent was evaporated in vacuo. The extract (330.7 mg) was redissolved in CH_2Cl_2/MeOH (1:1 v/v) and mixed with Diaion® HP20SS resin. The mixture was dried in vacuo and the dried mixture was fractionated on a Diaion® column using a step gradient with increasing amount of 2-propanol in water to afford eight fractions. Fractions 5–7, eluting with 70%, 80%, and 90% 2-propanol, respectively, displayed cytotoxicity against HT-29 cells. Fractions 5 and 6 were subjected to HPLC-ESIMS analysis (Varian Microsob C_8, 2.0 × 250 mm, flow rate 0.2 mL/min) with 30–100% MeOH gradient over 35 min containing 0.1% formic acid. The analysis of MS spectra indicated the presence of potentially new indole alkaloids. Fractions 5 and 6 were combined and further subjected to reversed-phase HPLC (column Alltima C_8 Dynamax, 10 × 250 mm, flow rate 3 mL/min) with 70–85% MeOH/H_2O gradient over 40 min and yielded **1** (1.05 mg, t_R 40.0 min) and **2** (0.62 mg, t_R 42.8 min) along with hapalonamide H (1.02 mg, t_R 16.0 min), hapalindoles U (0.66 mg, t_R 26.2 min), C (0.41 mg, t_R 28.5 min), G (0.29 mg, t_R 29.3 min), J (2.92 mg, t_R 30.5 min), A (5.15 mg, t_R 32.4 min), H (4.18 mg, t_R 37.8 min), and I (0.64 mg, t_R 43.5 min). The extract (327.0 mg) of *Fischerella muscicola* was obtained from the lyophilized biomass (6.26 g from 13 L culture) as described above. Eight fractions were obtained using the Diaion fractionation protocol described above. The combined fractions 5–7 (70%, 80%, and 90% 2-propanol) were subjected to reversed-phase HPLC (column Alltima C_8, 10 × 250 mm, flow rate 3.0 mL/min) with 80% MeOH isocratic to yield **3** (0.94 mg, t_R 15.2 min) along with fischerindole L (1.09 mg, t_R 13.3 min), hapalindoles J (1.14 mg, t_R 14.1 min), A (3.86 mg, t_R 15.9 min), and I (0.49 mg, t_R 17.6 min), fischerellins A (1.06 mg, t_R 18.8 min) and B (1.27 mg, t_R 21.7 min). Fraction 6 was subjected to reversed-phase HPLC (Alltima C_8, 10 × 250 mm, flow rate 3.0 mL/min) with 70–85% MeOH/H_2O gradient over 50 min and yielded anhydrohapaloxindole A (1.13 mg, t_R 26.8 min).

4.3.1. Hapalindole X (1)

Yellow amorphous powder; $[\alpha]_{D^{25}}$ +138.0° ($CHCl_3$, *c* 0.14); UV (MeOH) λ_{max} (log ε) 223 (6.48) 275 (7.32); IR (neat) ν_{max} 3410, 2969, 2142, 1630, 1437, 1374, 1093, 920, 825, 776, 749 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 303.1853 $[M+H]^+$ (calcd for $C_{21}H_{23}N_2$, 303.1861).

4.3.2. Deschloro hapalindole I (2)

White amorphous powder; $[\alpha]_{D^{25}}$ +11.0° ($CHCl_3$, *c* 0.05); UV (MeOH) λ_{max} (log ε) 223 (8.31) 247 (7.63) 280 (7.32) 324 (7.67); IR (neat) ν_{max} 3478, 2924, 2135, 1683, 1601, 1457, 1377, 1207, 1138, 1056, 843, 749, 724 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; HRESIMS *m/z* 303.1863 $[M+H]^+$ (calcd for $C_{21}H_{23}N_2$, 303.1861).

4.3.3. 13-hydroxy dechlorofontonamide (3)

Pale yellow amorphous powder; $[\alpha]_{D^{25}}$ −45.6° ($CHCl_3$, *c* 0.05); UV (MeOH) λ_{max} (log ε) 207 (4.99) 238 (5.40) 287 (5.27) 343

(4.83); IR (neat) ν_{max} 3750, 3200, 1698, 1654, 1599, 1507, 1252, 902, 813 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; HRESIMS *m/z* 324.1630 $[M-H]^-$ (calcd for $C_{20}H_{22}NO_3$, 324.1600).

4.4. Cytotoxicity assay

Cytotoxicity against NCI-H460, MCF-7, SF268, and IMR90 cells was measured as described previously.[24] Cytotoxicity against HT-29 cells was measured using a commercially available kit according to the manufacturer's instructions (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega Corp, Madison, WI, USA).

4.5. 20S proteasome assay

Evaluation of inhibition of the 20S proteasome was performed as previously described.[25]

4.6. *Mycobacterium tuberculosis*, other organisms and vero cell cytotoxicity

Experiments were performed as previously described.[16,17]

Acknowledgement

This research was supported by the National Cancer Institute (PO1CA125066). We thank the Research Resources Center (RRC) at UIC for high-resolution mass spectrometry.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.06.030.

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