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Lyngbyaureidamides A and B, two anabaenopeptins from the cultured freshwater cyanobacterium *Lyngbya* sp. (SAG 36.91)

Jiachen Zi, Daniel D. Lantvit, Steven M. Swanson, Jimmy Orjala *

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood St., (M/C 781), Chicago, IL 60612, United States

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ABSTRACT

Two anabaenopeptin-type peptides, lyngbyaureidamides A and B, together with two previously reported peptides lyngbyazothrins C and D, were isolated from the cultured freshwater cyanobacterium *Lyngbya* sp. (SAG 36.91). Their structures were determined by spectroscopic and chemical methods. Lyngbyazothrins C and D were also able to inhibit the 20S proteasome with IC_{50} values of 7.1 μM and 19.2 μM, respectively, while lyngbyaureidamides A and B were not active at 50 μM.

1. Introduction

Cyanobacteria are known to be a rich source of secondary metabolites with diverse chemical structures and biological activities (Wagoner et al., 2007; Tan, 2007; Müeller et al., 2006; Luesch et al., 2001; Singh et al., 2005; Jaiswal et al., 2008). Many of these metabolites have been found to inhibit various proteases and we have initiated a screening program to discover cyanobacterial inhibitors of the 20S proteasome, the catalytic core of the major proteolytic system in eukaryotic cells and an established anticancer target. One of the active extracts was *Lyngbya* sp. (SAG 36.91), which showed inhibitory activity against the 20S proteasome at a concentration of 50 μg/mL. Bioassay-guided fractionation led to the isolation of four cyclic peptides, including two new anabaenopeptin-type peptides lyngbyaureidamides A (**1**) and B (**2**) and the previously reported peptides lyngbyazothrins C (**3**) and D (**4**). Lyngbyazothrins C (**3**) and D (**4**) were originally reported as an inseparable mixture with antibacterial activity from this strain (Zainuddin et al., 2009). Lyngbyazothrins C (**3**) and D (**4**) were recently reported individually as portoamides A and B (Leão et al., 2010).

2. Results and discussion

Lyngbya sp. (SAG 36.91) was mass cultured in Z medium (Mian et al., 2003). The organic extract was active in our 20S proteasome assay and was fractionated using a diaion HP20SS resin column. The activity was recovered in fraction 3, eluting with 40% isopropanol in water and further purified by HPLC, which led to the isolation of four peptides: lyngbyaureidamides A (**1**) and B (**2**) and the previously reported peptides lyngbyazothrins C (**3**) and D (**4**) (Fig. 1).

Lyngbyaureidamide A (**1**) was obtained as a yellowish gum. The molecular formula was determined as $C_{46}H_{61}N_7O_9$ by HRMS (*m*/*z* 878.4426 $[M+Na]^+$). The proton spectrum of **1** contained signals for six amide protons (δ_H 8.92, 8.75, 7.13, 7.03, 6.61 and 5.93), a *N*-methyl group (δ_H 2.64) and six α-protons (δ_H 4.95, 4.78, 4.16, 3.95, 3.87 and 3.77). The latter set correlated with six carbon resonances (δ_C 54.9, 49.0, 53.4, 55.2, 57.3 and 56.1) in the HSQC spectrum. Detailed 2D NMR spectroscopic analysis (Table 1 and Fig. 2) indicated the presence of six amino acid residues, three of which were the common amino acids Lys, Ile and Phe. The structures of three remaining non-standard amino acid residues were determined by analysis of COSY, HSQC and HMBC data. The structure of *N*-MeAla was determined by the HMBC correlation from the *N*-methyl singlet to the *N*-MeAla C-1 (δ_C 170.6), in combination with a COSY correlation between *N*-MeAla H-α and *N*-MeAla H_3-β (δ_H 1.25). The presence of a homophenylalanine (Hph) residue was deduced by the HMBC correlation from Hph H_2-γ (δ_H 2.39) to C-2′/C-6′ (δ_C 128.6) connecting the two partial structures established by COSY correlations (Hph NH to H_2-γ and a mono substituted aromatic ring C-1′–C-6′). Similarly, the presence of a homotyrosine (Hty) residue was deduced by the HMBC correlations from Hty H_2-γ (δ_H 2.48 and 2.67) to C-2′/C-6′ (δ_C 129.5), connecting Hty NH to H_2-γ partial structure to the *para*-substituted aromatic ring (Hty C-1′–C6′).

The structure of **1** was established by interpretation of the HMBC spectrum (Table 1 and Fig. 2). The correlations from the

* Corresponding author. Tel.: +1 312 996 5583; fax: +1 312 996 7107.
E-mail address: orjala@uic.edu (J. Orjala).

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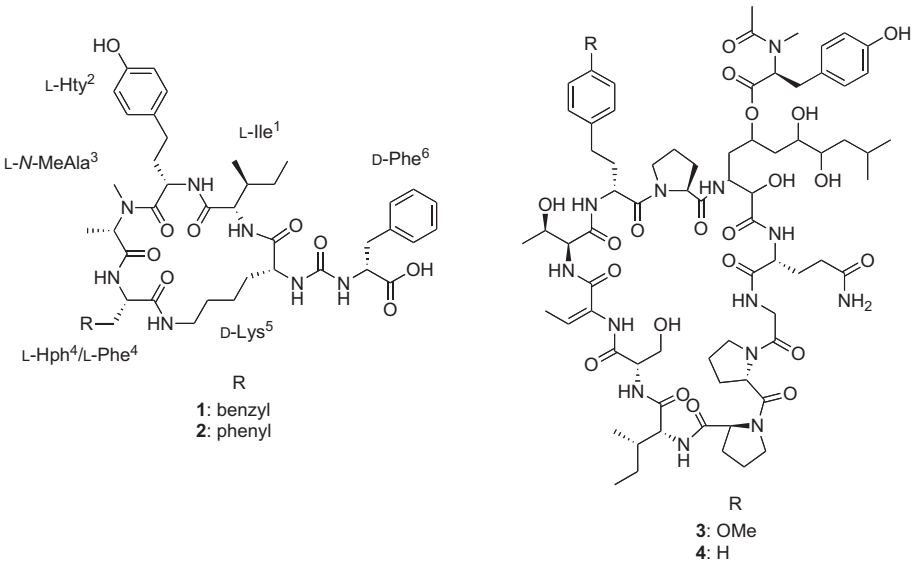

Fig. 1. Structure of lyngbyaureidamide A (**1**) and B (**2**), lyngbyazothrins C (**3**) and D (**4**).

NH of Hph to C-1 of *N*-MeAla (δ_C 170.6), from H_{NMe}- of *N*-MeAla to C-1 of Hty (δ_C 171.8), from the NH of Hty to C-1 of Ile (δ_C 173.2), from the NH of Ile to C-1 of Lys (δ_C 172.9) and from ε-NH of Lys to C-1 of Hph (δ_C 171.5), indicated that these five residues formed a 19-membered peptide ring, characteristic of the anabaenopeptin-type peptides (Welker and von Dohren, 2006). Finally, analysis of the correlations from H-α of Lys and H-α of Phe to the same sp^2 carbon at δ_C 157.1 suggested that the remaining Phe residue was attached to the 19-membered ring through an ureido group. This ureido group also accounted for the remaining carbonyl moiety implied by the molecular formula. The absolute configurations of amino acid residues of **1** were determined by Marfey's method (Marfey, 1984). The result of the HPLC analysis showed that *N*-MeAla, Hty, Ile and Hph all were of the L configuration, while Lys and Phe both were D.

Lyngbyaureidamide B (**2**) was obtained as a yellow gum. The HRMS quasimolecular peak at *m*/*z* 864.4255 $[M+Na]^+$ suggested a molecular formula of $C_{45}H_{59}N_7O_9$. The 1D and 2D NMR spectroscopic data of **2** were very similar to those of **1**. The only difference was the loss of the Hph moiety and the appearance of an additional Phe moiety in **2**. The placement of the second Phe moiety was determined by analysis of the ROESY spectrum of **2**. The correlations between NH of Phe^4 (δ_H 8.75) and H-α of *N*-$MeAla^3$ (δ_H 4.78) and ε-NH of Lys^5 (δ_H 7.16) indicated that Hph had been replaced by Phe. This was confirmed by the HMBC correlations from the NH of Phe^4 to C-1 of *N*-$MeAla^3$ (δ_C 170.6), and from ε-NH of Lys^5 to C-1 of Phe^4 (δ_C 171.5). The amino acid residues of **2** were defined as L-Ile, L-Hty, L-*N*-MeAla, L-Phe, D-Phe and D-Lys by Marfey's method), indicating the replacement of L-Hph^4 by L-Phe^4. This assignment was further supported by comparison of the NMR spectroscopic data of lyngbyaureidamide B with those published for other anabaenopeptins containing a L-Hty-L-*N*-MeAla-L-Phe-D-Lys sequence as part of their 19-membered peptide ring (Williams et al., 1996; Zafrir-Ilan and Carmeli, 2010). No significant deviation (<0.8 ppm) was found for these ^{13}C chemical shifts. In addition, the NMR chemical shifts for Phe^6 of lyngbyaureidamide B closely matched those of Phe^6 of **1**. Together, this indicated L-Phe to be in position 4.

Anabaenopeptins, a group of cyclic hexapeptides, are characterized by a 19-membered peptide ring that is formed by cyclization between the C-terminal amino acid and the ε-amine of a lysine residue. The α-amine of the lysine is further linked through an ureido group to a side-chain amino acid. Anabaenopeptin-type peptides have been obtained from several cyanobacterial species, including *Anabaena* (Harada et al., 1995; Grach-Pogrebinsky and Carmeli, 2008), *Aphanizomenon* (Murakami et al., 2000), *Lyngbya* (Matthew et al., 2008), *Microcystis* (Williams et al., 1996; Beresovsky et al., 2006; Gesner-Apter and Carmeli, 2009; Zafrir-Ilan and Carmeli, 2010), *Nodularia* (Fujii et al., 1997), *Oscillatoria* (Sano and Kaya, 1995; Shin et al., 1997; Itou et al., 1999), *Planktothrix* (Sano et al., 2001; Okumura et al., 2009), *Schizothrix* (Reshef and Carmeli, 2002), and *Tychonema* (Müeller et al., 2006), as well as from sponges (Kobayashi et al., 1991a,b; Schmidt et al., 1997; Uemoto et al., 1998; Robinson et al., 2007; Plaza et al., 2010). The exocylic amino acid for all anabaenopeptin–type peptides reported to date has L configuration and lyngbyaureidamide A (**1**) and B (**2**) are the first examples of a D-configuration amino acid residue at this position (D-Phe).

All isolates were evaluated for their 20S proteasome inhibitory activity (chymotrypsin-like activity). Lyngbyazothrins C (**3**) and D (**4**) were found to be active with IC_{50} values of 7.1 μM and 19.2 μM, respectively, while lyngbyaureidamide A (**1**) and B (**2**) were found to be inactive at 50 μM. None of the four compounds showed cytotoxic activities against the human colon cancer cell line designated HT-29 at 50 μM. Lyngbyazothrins C (**3**) and D (**4**) were originally obtained as an inseparable mixture from this cyanobacterial strain and shown to inhibit the growth of *Escherichia coli* (Zainuddin et al., 2009). The same two structures were recently also reported as pure compounds, named portoamides A and B, obtained from an *Oscillatoria* sp. (Leão et al., 2010). The portoamides were found to act synergistically to inhibit the growth of the green microalga *Chlorella vulgaris*. We here were also able to separate lyngbyazothrins C (**3**) and D (**4**), the two major components of *Lyngbya* sp. (SAG 36.91) by HPLC. Comparison of the spectroscopic data confirmed that lyngbyazothrins C and D were identical to portoamides A and B, respectively.

3. Conclusions

Bioassay-guided investigation (proteasome inhibition) of the cyanobacterium *Lyngbya* sp. (SAG 36.91) led to the isolation of four peptides. The previously reported lyngbyazothrins C (**3**) and D (**4**) accounted for the 20S proteasome inhibitory activity. In addition, two new anabaenopeptin-type peptides, lyngbyaureidamide A (**1**) and B (**2**) were obtained.

Table 1
NMR spectroscopic data (δ) of compounds **1** and **2** in DMSO-d_6.

Unit	C/H No.	**1**				**2**			
		δ_C[a]	δ_H[b]	COSY	HMBC	δ_C[c]	δ_H[b]	COSY	HMBC
Ile[1]	NH		7.03	H_α	C-1$_{Lys}$		7.14	H_α	C-1$_{Lys}$
	C-1	173.2				173.0			
	C_α	57.3	3.87	NH, H_β		57.1	3.97	NH, H_β	
	C_β	35.9	1.54	H_α, H_γ, H'_γ	C-1$_{Ile}$	36.1	1.77	H_α, H_γ, H'_γ	C-1$_{Ile}$
	C_γ	25.4	1.45	H_β, H_δ		25.3	1.56	H_β, H_δ	
			1.04	H_β, H_δ			1.17	H_β, H_δ	
	C'_γ	15.5	0.89	H_β		15.4	1.01	H_β	
	C_δ	10.8	0.73	H_γ		10.7	0.82	H_γ	
Hty[2]	NH		8.92	H_α	C-1$_{Ile}$		8.94	H_α	C-1$_{Ile}$
	C-1	171.8				171.4			
	C_α	49.0	4.78	NH, H_β		49.2	4.71	NH, H_β	
	C_β	33.8	1.93	H_α, H_γ	C-1$_{Hty}$	33.7	1.87	H_α, H_γ	C-1$_{Hty}$
			1.80	H_α, H_γ			1.72	H_α, H_γ	
	C_γ	30.9	2.67	H_β	C-1′, 2′, 6′	31.0	2.64	H_β	C-1′, 2′, 6′
			2.48	H_β			2.43	H_β	
	C-1′	131.2				131.7			
	C-2′,6′	129.5	7.04	H-3′,5′		129.5	7.01	H-3′,5′	
	C-3′,5′	115.5	6.69	H-2′,6′		115.6	6.67	H-2′,6′	
	C-4′	156.0				156.0			
	4′-OH		9.26				9.26		
N-MeAla[3]	*N*-Me	28.8	2.64		C-1$_{NMe-Ala}$, C_α, C-1$_{Hty}$	27.4	1.77		C-1$_{NMe-Ala}$, C_α, C-1$_{Hty}$
	C-1	170.6				170.6			
	C_α	54.9	4.95	H_β		54.7	4.78	H_β	
	C_β	14.5	1.25	H_α	C-1$_{NMe-Ala}$	14.3	1.06	H_α	C-1$_{NMe-Ala}$
Hph[4]/Phe[4]	NH		8.75	H_α	C-1$_{NMe-Ala}$		8.75	H_α	C-1$_{NMe-Ala}$
	C-1	171.5				171.5			
	C_α	53.4	4.16	NH, H_β		55.4	4.35	NH, H_β	
	C_β	34.5	2.08	H_α, H_γ	C-1$_{Hph}$	37.9	3.29	H_α	C-1$_{Phe4}$ and C-1′, 2′, 6′
			1.90	H_α, H_γ			2.78	H_α	
	C_γ	32.3	2.39	H_β	C-1′, 2′, 6′				
	C-1′	141.9				139.1			
	C-2′,6′	128.6	7.07			129.3	7.05		
	C-3′,5′	128.5	7.23			128.7	7.20		
	C-4′	126.2	7.15			126.4	7.15		
Lys[5]	NH		6.61	H_α			6.62	H_α	
	C-1	172.9				172.9			
	C_α	55.2	3.95	NH, H_β	C$_{Ureido}$	55.4	3.96	NH, H_β	C$_{Ureido}$
	C_β	32.7	1.54	H_α, H_γ	C-1$_{Lys}$	32.7	1.59	H_α, H_γ	C-1$_{Lys}$
	C_γ	20.7	1.26	H_β		20.8	1.29	H_β	
			1.09				1.13		
	C_δ	28.5	1.45	H_ε		28.5	1.48	H_ε	
			1.38				1.43		
	C_ε	38.7	3.50	ε-NH, H_δ		38.8	3.56	ε-NH, H_δ	
			2.79	ε-NH, H_δ			2.81	ε-NH, H_δ	
	ε-NH		7.13	H_ε	C-1$_{Hph}$		7.16	H_ε	C-1$_{Phe4}$
Phe[6]	NH		5.93	H_α			5.96	H_α	
	C-1	173.0				173.3			
	C_α	56.1	3.77	NH, H_β	C$_{Ureido}$	55.4	3.96	NH, H_β	C$_{Ureido}$
	C_β	38.2	3.00	H_α	C-1$_{Phe}$, and C-1′, 2′, 6′	38.2	3.01	H_α	C-1$_{Phe6}$ and C-1′, 2′, 6′
			2.94	H_α			2.96	H_α	
	C-1′	140.1				139.8			
	C-2′,6′	130.1	7.06			130.0	7.11		
	C-3′,5′	127.6	7.12			127.9	7.19		
	C-4′	125.6	7.07			126.0	7.14		
Ureido	C$_{Ureido}$	157.1				157.3			

[a] Chemical shifts were determined by HSQC and HMBC experiments recorded at 600 MHz.
[b] Recorded at 600 MHz.
[c] DEPTQ experiment recorded at 226 MHz.

4. Experimental

4.1. General experimental procedures

The optical rotations were determined on a Perkin–Elmer 241 polarimeter. UV spectra were obtained on a Varian Cary 50 Bio spectrophotometer. IR spectra were obtained on a Jasco FTIR-410 Fourier transform infrared spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance DRX600 MHz NMR spectrometer with a 5 mm CPTXI Z-gradient probe and a Bruker Avance II900 MHz NMR spectrometer with a 5 mm ATM CPTCI Z-gradient probe, referenced to the corresponding solvent peaks. HRMS spectra were obtained on a Shimadzu IT-TOF spectrometer. HPLC separations were performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual wavelength absorbance detector, with an Alltima (250 × 10 mm i.d.) preparative column packed with C_{18} (5 μm) or a Varian (250 × 10 mm i.d.) semipreparative column packed with C_8 (5 μm).

4.2. Biological material

Lyngbya sp. (SAG 36.91) from Culture Collection of Algae, Göttingen, Germany) was grown in 21 L of aerated inorganic Z media (Mian et al., 2003). Cultures were illuminated with fluorescent

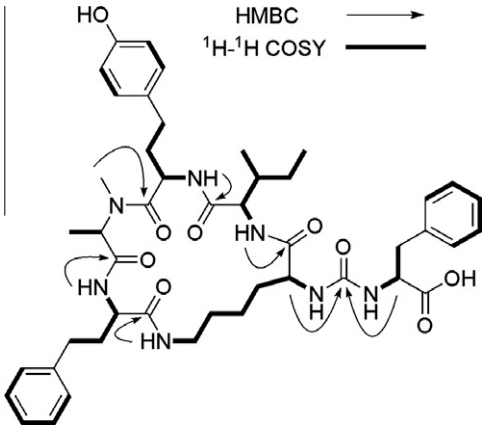

Fig. 2. ^{1}H–^{1}H COSY and key HMBC correlations of **1**.

lamps at 1.93 klx with an 18/6 h light/dark cycle. The temperature of the culture room was maintained at 22 °C. After 7 weeks, the biomass of cyanobacteria was harvested by centrifugation and freeze-dried (yield 11.24 g).

4.3. Extraction and isolation

Freeze-dried *Lyngbya* biomass (11.24 g) was extracted, each for two hrs with CH_2Cl_2/MeOH (200 mL × 5, 1:1 v/v) at room temperature to yield a crude extract (984.5 mg). A portion of the crude extract (849.8 mg) was fractionated using a Diaion HP-20 column (10 g, 130 × 25 mm) with increasing amounts of *i*-PrOH:H_2O (0:100; 20:80; 40:60; 70:30; 80:20; 90:10; 100:0, fraction size 100 mL) to generate eight sub-fractions. Fraction 3 (eluted with *i*-PrOH 40:60, v/v) was the most active fraction and showed 78.4% inhibition against 20S proteasome at 50 μg/mL. This fraction (103.5 mg) was subjected to reversed-phase HPLC (Alltima C_{18}, 5 μm, 250 × 10 mm) with a solvent gradient of MeOH:H_2O (35:65 → 90:10) over 50 min to afford two subfractions (3a at 32–33 min, and 3b at 35–36 min). Subfraction 3a (8.9 mg) was further separated by reversed-phase HPLC (Varian C_8, 5 μm, 250 × 10 mm), using MeOH–H_2O (55:45) as mobile phase, to yield **1** (1.6 mg, eluting after 112 min) and **2** (1.1 mg eluting after 110 min). Subfraction 3b (67.3 mg) was subjected to reversed-phase HPLC (Varian C_8, 5 μm, 250 × 10 mm), eluted by MeOH–H_2O (65:35), to give **3** (20.5 mg, eluting after 183 min) and **4** (10.3 mg, eluting after 188 min).

4.3.1. Lyngbyaureidamide A (*1*)

Yellowish gum; $[\alpha]_D$ −27.3 (*c* 0.09 MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.21) nm; IR (neat) ν_{max} 3295, 3250, 2932, 2863, 1643, 1548, 1503, 1452, 1250, 1007 cm^{-1}; For ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; HRMS *m*/*z* 878.4426 [M+Na]$^+$ (calcd for $C_{46}H_{61}N_7O_9Na$, 878.4428).

4.3.2. Lyngbyaureidamide B (*2*)

Yellow gum; $[\alpha]_D$ −41.1 (*c* 0.06 MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.27) nm; IR (neat) ν_{max} 3286, 2928, 1643, 1548, 1524, 1504, 1250, 1024 cm^{-1}; For ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; HRMS *m*/*z* 864.4255 [M+Na]$^+$ (calcd for $C_{45}H_{59}N_7O_9Na$, 864.4272).

4.3.3. Lyngbyazothrin C (*3*)

Yellow gum; $[\alpha]_D$ −14.6 (*c* 0.1 MeOH); IR (neat) ν_{max} 3321, 2957, 1652, 1601, 1506, 1250, 1214 cm^{-1}; ^{1}H and ^{13}C NMR data as described (Zainuddin et al., 2009; Leão et al., 2010); HRMS *m*/*z* 1554.7771 [M+Na]$^+$ (calcd for $C_{74}H_{109}N_{13}O_{22}Na$, 1554.7708).

4.3.4. Lyngbyazothrin D (*4*)

Yellow gum; $[\alpha]_D$ −17.2 (*c* 0.1 MeOH); IR (neat) ν_{max} 3307, 2971, 1664, 1605, 1470, 1207, 1128 cm^{-1}; ^{1}H and ^{13}C NMR data as described (Zainuddin et al., 2009; Leão et al., 2010). HRMS *m*/*z* 1524.7673 [M+Na]$^+$ (calcd for $C_{45}H_{59}N_7O_9Na$, 1524.7602).

4.4. Racemization of D-Homotyrosine

LD-Hty was obtained from D-Hty via an acetylation–deacetylation process (Sealock, 1946). D-homotyrosine hydrobromide (100 mg, 0.00036 mol; Chem-Impex International Inc., Wood Dale, USA) and NaOH (46.6 mg, 0,00115 mol) were dissolved in H_2O (1.12 mL), and Ac_2O (600 μL, Sigma–Aldrich Inc., Louis, USA) was added in ten portions at 3 min intervals with continuous stirring. The reaction mixture was stirred at 65 °C for 6 h, and then evaporated *in vacuo* to give a thick syrup (132 mg), which was extracted by acetone and concentrated *in vacuo* to afford crude diacetylated LD-homotyrosine (99 mg) as yellow oil, confirmed by IT–TOF MS: HRMS *m*/*z* 302.1004 [M+Na]$^+$ (calcd for $C_{14}H_{17}NO_5Na$, 302.1004). The crude diacetylated LD-homotyrosine was dissolved in 2 N NaOH (400 μL), and stirred at room temperature for 30 min. The resulting solution was neutralized to pH 7 by 6 N HCl and evaporated *in vacuo* to give a thick syrup which was extracted by acetone and concentrated *in vacuo* to afford crude *N*-acetylated LD-homotyrosine (78 mg) as yellow oil, confirmed by IT–TOF MS: HRMS *m*/*z* 260.0898 [M+Na]$^+$ (calcd for $C_{12}H_{15}NO_4Na$, 260.0899). The residue was hydrolyzed with 6 N HCl (2 mL) in a sealed thick glass tube at 102 °C for 3.5 h. The solvent was removed under reduced pressure to give crude LD-homotyrosine (70.1 mg) as a red solid, $[\alpha]_D$ ≈0, confirmed by IT–TOF MS. LD-Homotyrosine: HRMS *m*/*z* 196.0968 [M+H]$^+$ (calcd for $C_{10}H_{14}NO_3$, 196.0895).

4.5. Acid hydrolysis of 1 and 2, and Marfey's analyses of the hydrolyzates

In separate reactions, compounds **1** (0.2 mg) or **2** (0.2 mg) were dissolved in 6 N HCl (0.4 ml), and heated at 110 °C in a sealed vial for 13 h. The cooled reaction mixture was evaporated to dryness under reduced pressure. The amino acid mixture was resuspended in H_2O (40 μL). A solution of 1% (1-fluoro-2,4-dinitrophenyl)-5-L-alanine amide (FDAA) in acetone (100 μL) and 1 M $NaHCO_3$ (100 μL) was added to each reaction vessel. The reaction mixture was stirred at 40 °C for 2 h and the resulting solution was neutralized using 2 M HCl. The FDAA-amino acid derivatives, from hydrolyzates, were compared with similarly derivatized standard amino acids by HPLC analysis at 340 nm. The column (Alltima RP-C_8, 5 μm, 250 × 4.6 mm) was eluted with a linear gradient of (A) CH_3CN containing 0.65% AcOH and (B) H_2O containing 0.65% AcOH from 20% to 42% (A) over 70 min followed by another gradient elution from 42% to 45% (A) over 20 min at flow rate: 0.5 mL/min. The standards gave the following retention times in min: 48.2 for FDAA; 67.5 for L-Ile, 76.4 for D-Ile; 66.8 for L-*allo*-Ile, 75.9 for D-*allo*-Ile; 69.5 for L-Phe, 76.1 for D-Phe; 71.1 for L-Lys, 73.4 for D-Lys; 77.8 for L-Hph, 85.8 for D-Hph; 58.0 for L-Hty, 63.2 for D-Hty; 42.1 for L-*N*-MeAla, 47.2 for D-*N*-MeAla.

4.6. 20S Proteasome assay

The proteasome assay was performed according to the protocol provided with the BIOMOL 20S Proteasome Assay Kit for Drug Discovery (BIOMOL International LP, Plymouth Meeting, PA, USA Catalog Number AK740-0001). The protocol was modified such that the 10 min incubation period was performed at 37 °C. Enzyme was acquired from BostonBiochem (20S proteasome, human, BostonBiochem, USA) and substrate from BIOMOL (Suc-LLVY-AMC). This substrate is specific for the chymotrypsin-like activity of the 20S

proteasome. Fluorescence was measured using either a Tecan Genios Pro microplate reader or a Hewlett Packard model AF10000 fluorimeter with an excitation wavelength of 360 nm and an emission wavelength of 460 nm. The positive control was bortezomib (IC_{50} 2.5 nM).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2011.09.017.

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