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ORIGINAL ARTICLE

Two piperazic acid-containing cyclic hexapeptides from *Streptomyces alboflavus* 313

Shaopeng Wei · Lixia Fan · Wenjun Wu · Zhiqin Ji

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Abstract Two novel cyclic hexapeptides, designated NW-G10 (**1**) and NW-G11 (**2**), were isolated from the fermentation broth of *Streptomyces alboflavus* 313. Their relative structures were elucidated on the basis of extensive spectroscopic analysis, and the absolute configurations of several constituent amino acids were determined by Marfey's method. NW-G10 (**1**) and NW-G11 (**2**) exhibited significant activity against Gram-positive bacteria, such as *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus*, including methicillin-resistant *Staphylococcus aureus* (MRSA), but they are not active against gram negatives.

Keywords Structure elucidation · Cyclic hexapeptide · Antibacterial activity

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S. Wei · Z. Ji
College of Plant Protection, Northwest A&F University, Yangling 712100, Shaanxi, People's Republic of China

S. Wei · L. Fan · W. Wu
Shaanxi Province Key Laboratory of Research and Development on Botanical Pesticide, Northwest A&F University, Yangling 712100, Shaanxi, People's Republic of China

W. Wu · Z. Ji
State Key Laboratory of Crop Stress Biology in Arid Areas, Northwest A&F University, Yangling 712100, Shaanxi, People's Republic of China

Z. Ji (✉)
Institute of Pesticide Science, Northwest A&F University, Xinong 61#, Yangling 712100, Shaanxi, People's Republic of China
e-mail: jizhiqin@nwsuaf.edu.cn

Introduction

Since monamycins were first isolated from *Streptomyces jamaicensis* in 1959 (Hassall and Magnus 1959), a series of natural peptides which contain the moiety of piperazine-3-carboxylic acid have been found among the metabolites of microorganisms, such as antrimycin (Shimada et al. 1981), azinothricin (Maehr et al. 1986; Smitka et al. 1988; Sakai et al. 1997; Maskey et al. 2006), himastatin (Lam et al. 1990; Leet et al. 1990, 1996), chloptosin (Umezawa et al. 2000), luzopeptins (Konishi et al. 1981), lydiamycin (Huang et al. 2006), and piperazimycin (Miller et al. 2007). These natural products exhibit impressive antimicrobial, antitumour, and anti-HIV activities. Attracted by their intriguing chemical structures and a medicinally relevant biological activity profile, many piperazic acid-containing natural products have been successfully synthesized in recent years (Kamenecka and Danishefsky 1998a, b; Yu et al. 2010; Oelke et al. 2010, 2011; Setsuya et al. 2011).

In our previous investigation of natural products from microorganisms for drug discovery, two piperazic acid-containing cyclic hexapeptides, NW-G01 and NW-G03, were discovered in the fermentation broth of *Streptomyces alboflavus* 313 (Guo et al. 2009, 2010, 2011). These natural cyclic hexapeptides could selectively inhibit Gram-positive bacteria, such as *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus*, including methicillin-resistant *Staphylococcus aureus* (MRSA), whereas they were not active against Gram-negative bacteria. In our continuous investigation, two novel analogs of NW-G01, NW-G10 (**1**) and NW-G11 (**2**) were isolated as trace constituents from the same microorganism by large-scale fermentation. Herein, we report the structure elucidation and antibacterial activity of these two novel cyclic hexapeptides.

Experimental procedures

Materials

Melting points were measured on a WPR apparatus and are uncorrected (Shanghai Jingke Co.). IR spectra were recorded on a Nicolet FT-IR 750 spectrometer. UV spectra were obtained using a Shimadzu UV-2401A spectrometer. Optical rotations were measured with a Horiba SEPA300 polarimeter. ESI–MS/MS and HR-ESI–MS spectra were obtained on a Finnigan LCQ Advantage ion-trap mass spectrometer (Thermo Fisher Co.) and an APEX II FT-ICR mass spectrometer (Bruker Daltonics Inc.). ^{1}H, ^{13}C NMR, DEPT, HSQC, HMBC and NOESY spectra were obtained on Bruker Avance III-500 NMR spectrometer (Bruker Daltonics Inc.), with TMS as internal standard (^{1}H at 500 MHz, ^{13}C at 125 MHz, respectively). The LC–MS experiments were performed on the Finnigan LCQ Advantage Max ion-trap mass spectrometer (Thermo Fisher Co.) coupled with a surveyor HPLC.

Methods

Microorganism and fermentation

The producing strain *S. alboflavus* 313 was isolated from a soil sample collected in Qinling Mountain, Shaanxi Province, China, and identified by its morphology, physiology, biochemistry and 16S rRNA gene sequence. The voucher specimen of this streptomycete was deposited at the Institute of Pesticide Science, Northwest A&F University, China.

The spores of *S. alboflavus* 313 grown on Gause's No. 1 agar were used to inoculate into a 250-mL flask containing 50 mL of a sterile seed medium consisting of glucose 0.8 %, soluble starch 0.8 %, beef extract 0.6 %, peptone 1.0 %, and NaCl 0.5 %, pH 7.2. The flask was shaken on a shaker at 180 rpm for 24 h at 28 °C. Ten milliliters of the seed culture was transferred to 250 mL flasks containing 50 mL of a sterile producing medium consisting of glucose 3.0 %, millet steep liquor 1.0 %, peptone 1.5 %, NaCl 0.5 %, and $CaCO_3$ 0.5 %, pH 7.2. Fermentation was carried out at 180 rpm for 5 days at 28 °C on a rotary shaker.

Extraction and isolation

The culture of 200 L was filtered with cheesecloth to separate the medium and culture liquid. The filtrate was absorbed onto HPD400 macroporous resin (Baoen Co. Ltd., Hebei, China), and then eluted with water and methanol in sequence. The methanol fraction was evaporated in vacuum. The concentrate was subjected to a silica gel column (600 g, 200–300 mesh, Qingdao Marine Chemical Co. Ltd., Shandong, China) and eluted with the mixture of EtOAc–MeOH at the ratio of 75:25 (v/v). The antimicrobial fraction was concentrated in vacuum, and then subjected to an ODS-AP flash chromatography (50 μm, Daiso Co. Ltd., Osaka, Japan), eluted with the mixture of MeOH–water at the ratio of 60:40 (v/v). The active fractions were concentrated in vacuum and further purified on a Shimadzu 6AD HPLC apparatus (Shimadzu Co. Ltd., Tokyo, Japan) equipped with a column of Hypersil ODS-BP (20 × 250 mm, 10 μm, flow rate 8.0 mL/min) to afford two novel cyclic hexapeptides, NW-G10 (**1**, 5.8 mg) and NW-G11 (**2**, 5.2 mg).

Acid hydrolysis and Marfey's analysis

Approximately 1.0 mg of **1** or **2** was hydrolyzed with 100 μL of 6 N HCl at 110 °C for 24 h. The acid hydrolysate was evaporated to dryness and dissolved in 100 μL of distilled water. To 50 μL of the solution, 80 μL of 1 N $NaHCO_3$ and 100 μL of 1 % acetone solution of L-FDAA (J&K chemical Co. Beijing, China) were added, the mixture was heated at 50 °C for 1 h. After cooling to room temperature, the reaction mixture was neutralized with 40 μL 1 N HCl, and evaporated to dryness (Fujii et al. 1997). The residue was then dissolved in 0.2 mL acetonitrile and injected for HPLC.

Chromatography

The HPLC instrument used for the analysis was a Finnigan Surveyor equipped with a photodiode array detector. Separations were carried out on a Hypersil Gold column (150 × 2.1 mm) (Thermo, San Jose, USA). Acetonitrile–0.1 % acetic acid was used as the mobile phase under a linear gradient elution mode (acetonitrile, 15–70 %, 40 min). The flow rate was 0.2 mL/min with UV detection at 340 and 200–600 nm by photodiode array detection.

LC–MS

The LC–MS experiments were performed on the Finnigan LCQ Advantage Max ion-trap mass spectrometer (Thermo Fisher Co.) coupled with a surveyor HPLC. The sample solution from the outlet of the DAD detector was injected to the ESI interface directly. The ESI voltage was 5.4 kV with the sheath and auxiliary gas set at 60 and 5 arbitrary units, respectively, and the capillary was heated to 250 °C. A mass range of *m/z* 200–850 was covered, and data were collected in positive mode.

Antibacterial assay

The standard bacterial strains *Bacillus cereus* (1.1846), *Bacillus subtilis* (1.88), *Staphylococcus aureus* (1.89), *Escherichia coil* (1.1574), and *Pseudomonas aeruginosa* (1.2031) were obtained from the China General

Microbiological Culture Collection Center. A clinical isolate of MRSA was obtained from Nanjing Medical University, China. Ampicillin sodium (Sigma, Shanghai, China) was used as positive control. The antibacterial activities of compounds against six strains of bacteria were evaluated by the micro-broth dilution method in 96-well plates. The inoculum was prepared by suspending several colonies from an overnight culture of tested bacteria from 0.5 % sheep blood agar media in Müller-Hinton broth (Hangzhou Microbial Reagent Co. Ltd., Zhejiang, China) and adjusting to a 0.5 McFarland standard (approximately 1.5×10^8 colony-forming units per mL). A further dilution of 1:200 was made by placing 0.25 mL of the adjusted suspension into 49.75 mL of Müller-Hinton broth. The tested compounds were firstly dissolved in DMSO at the concentration of 10 mg/mL, and it was diluted ten folds with sterile water to give the stock solution. Twofold serial dilutions of the tested compounds were prepared in Müller-Hinton broth. Then the dilutions and inoculated suspension of the bacteria were delivered to wells of a 96-well plate at the ratio of 1:1. The final concentration of inoculum in each well was 3.7×10^5 colony-forming units per mL. After incubation for 24 h at 30 °C, the MICs were examined. Experiments were repeated triplicate and standard ampicillin sodium was used as the positive control.

Chemistry

*NW-G10 (**1**)*

Amorphous white powder; m.p. 206 °C(dec.); $[\alpha]_D$ −95.8 (*c* 0.25, MeOH); UV (MeOH): λ_{max} 210, 240, 300 nm; IR (KBr) ν_{max} 3,426, 2,931, 1,750, 1,657, 1,414, 1,339, 1,234, 1,162 and 648 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; positive HR-ESI–MS *m/z* 769.3182 $[M + H]^+$ (cacld for $C_{35}H_{46}ClN_{10}O_8$, 769.3189).

*NW-G11 (**2**)*

Amorphous white powder; m.p. 195 °C(dec.); $[\alpha]_D$ −49.8 (*c* 0.25, MeOH); UV (MeOH): λ_{max} 220, 230, 305 nm; IR (KBr) ν_{max} 3,425, 3,298, 2,935, 1,639, 1,441, 1,409, 1,255, 1,102 and 924 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; positive HR-ESI–MS *m/z* 781.3193 $[M + H]^+$ (cacld for $C_{36}H_{46}ClN_{10}O_8$, 781.3189).

Results and discussion

Extraction and isolation

Two hundred liters of filtered fermentation broth of *S. alboflavus* 313 was passed through a column of macroporous resin (5 kg), and then eluted with water and methanol in sequence. The concentrated methanol fraction was subjected to a column of silica gel and eluted with the mixture of EtOAc–MeOH, the active fraction was subsequently purified by reverse phase (RP) flash chromatography and RP-HPLC to afford two novel cyclic hexapeptides, NW-G10 (**1**) and NW-G11 (**2**).

Structure elucidation

NW-G10 (**1**) was obtained as a white powder. Its molecular formula was established as $C_{35}H_{45}ClN_{10}O_8$ by HR-ESI–MS (*m/z* 769.3182, $[M + H]^+$; cacld for $C_{35}H_{46}ClN_{10}O_8$, 769.3189). The presence of chlorine was suggested by the isotope abundance peaks in the MS spectrum. Like the IR spectrum with typical absorption bands of amides carbonyls at 1,658 cm^{-1}, the NMR data showed typical characteristics of peptides, for example resonances for amide carbonyls in the ^{13}C NMR spectrum and for α proton in the ^1H NMR spectrum (Table 1). The ^{13}C NMR and DEPT spectra (Table 1) of **1** showed the presence of 35 carbon signals, which were recognized as three methyls, one oxygenated methyl, seven methylenes, eight sp^3 methines, one oxygenated sp^3 quaternary C-atom, five sp^2 methines, four sp^2 quaternary C-atoms, and six amide carbonyl carbons. ^1H and ^{13}C chemical shift assignments were made by standard 1D and 2D NMR techniques, such as DEPT, HSQC, and HMBC.

The ^1H NMR spectrum of **1** showed the presence of two sp^3 methines at δ 5.34(d, $J = 5.5$) and δ 2.25(m), two secondary methyl groups at δ 0.99(d, $J = 7.0$) and δ 1.02(d, $J = 7.0$), they were assigned to the α, β, λ and λ′ protons of valine based on the HSQC and HMBC spectra of **1**, and by comparison with the corresponding chemical shifts and coupling constants of NW-G01 and NW-G03. At the same time, the cross peak between H-α at δ 5.34 and the carbonyl carbon at δ 173.4 was observed on the HMBC spectrum. Thus, the valine (Val) moiety was elucidated. Alanine (Ala), and two molecules of piperazic acid (PA) could also be determined based on the 1D, 2D NMR spectra, and by comparison to the data of NW-G01.

The signals observed at δ 4.83(t, $J = 8.0$), δ 2.36(m) and δ 2.51(m), δ 7.23(d, $J = 8.0$), δ 6.83(dd, $J = 8.0, 2.0$), δ 6.73(d, $J = 2.0$), δ 5.68(s) were assigned to the H-2, H-3, H-4, H-5, H-7, and H-8a protons of the chlorinated pyrroloindoline moiety (Trp derivative) based on the HSQC and HMBC spectra of **1**, and by comparison with the corresponding chemical shifts and coupling constants of NW-G01 and NW-G03. The long-range coupling between H-α at δ 4.83 and the carbonyl carbon at δ 172.8 was observed on the HMBC spectrum. Thus, the 6-chloro-3a-hydroxy-1,2,3,3a,8,8a-hexhydropyrrolo-[2,3-*b*]indole-2-carboxylic acid moiety, a chlorinated pyrroloindoline

Table 1 NMR data of NW-G10 (**1**) and NW-G11(**2**) (^{1}H measured at 500 MHz, ^{13}C measured at 125 MHz, in CD_3OD)

Position	NW-G10 (**1**)		NW-G11 (**2**)	
	δC (ppm)	δH (ppm, J = Hz)	δC (ppm)	δH (ppm, J = Hz)
Valine (Val)				
CO	173.4		172.6	
C_α	55.6	5.34(d, J = 5.5)	54.6	5.22(d, J = 5.5)
C_β	29.7	2.25(m)	29.4	2.07, 1.38(m)
C_γ	16.3	0.99(d, J = 7.0)	17.5	0.92(d, J = 7.0)
$C_{\gamma'}$	18.4	1.02(d, J = 7.0)	18.5	1.02(d, J = 7.0)
Alanine or *N*-methylalanine (Ala)				
CO	175.4		171.8	
C_α	44.8	5.39(q, J = 7.0)	48.1	5.72(q, J = 7.0)
C_β	16.5	1.35(d, J = 7.0)	13.1	1.40(d, J = 7.0)
N-CH_3	–	–	29.1	3.10(s)
Piperazic acid (PA_1)				
CO	173.1		171.9	
C_α	52.4	5.00(m)	54.9	5.50(brs)
C_β	24.3	2.25(m), 2.06(m)	24.9	2.32(m), 1.75(m)
C_γ	20.1	1.93(m), 1.62(m)	19.0	2.34(m), 2.24(m)
C_δ	46.4	3.28(m), 2.96(m)	46.2	3.17(dd, J = 12.5, 3.0), 2.91(m)
5-Methoxy-2,3-dihydropyridazine-3-carboxylic acid (PA_2)				
CO	171.8		170.0	
C_α	52.7	6.30(d, J = 6.0)	53.1	6.36(d, J = 6.0)
C_β	92.9	5.30(dd, J = 6.0, 2.5)	92.7	5.40(dd, J = 6.0, 2.5)
C_γ	146.4		146.4	
C_δ	136.0	6.86(d, J = 2.5)	135.6	6.79(d, J = 2.5)
OCH_3	54.2	3.67(s)	54.1	3.63(s)
Piperazic acid or 2,3,4,5-tetrahydropyridazine-3-carboxylic acid (PA_3)				
CO	171.0		172.1	
C_α	51.5	4.71(m)	50.6	5.18(m)
C_β	24.2	2.25(m), 2.06(m)	20.1	2.01(m), 1.60(m)
C_γ	19.3	1.93(m), 1.62(m)	17.0	2.11(m)
C_δ	45.9	3.16(m), 3.00(m)	144.4	7.13(brs)
6-Chloro-3a-hydroxy-1,2,3,3a,8,8a-hexhydropyrrolo-[2,3-*b*]indole-2-carboxylic acid (Trp)				
CO	172.8		171.0	
C_2	61.2	4.83(t, J = 8.0)	61.7	4.31(t, J = 8.0)
C_3	40.7	2.51(m), 2.36(m)	38.0	2.72(m), 2.58(m)
C_{3a}	88.7		86.3	
C_{3b}	130.0		128.6	
C_4	123.6	7.23(d, J = 8.0)	124.3	7.25(d, J = 8.0)
C_5	119.4	6.83(dd, J = 8.0, 2.0)	119.2	6.81(dd, J = 8.0, 2.0)
C_6	135.0		135.5	
C_7	111.0	6.73(d, J = 2.0)	111.4	6.75(d, J = 2.0)
C_{7a}	149.4		149.9	
C_{8a}	85.9	5.68(s)	83.0	5.30(s)

derivative considered to be derived from tryptophan (Trp), was elucidated.

In the ^{13}C NMR spectrum of **1**, three unsaturated carbon signals were observed at δ 92.9, δ 136.0, and δ 146.4, they were assigned to methine, methine, and quaternary carbon, respectively, based on the DEPT spectrum. It indicated the presence of C=C and C=N in the amino acid residue, and in which one hydrogen atom of the unsaturated carbon was

replaced. In ^{1}H-^{1}H-COSY spectrum, there was strong coupling signals between sp^3 methine (δ 6.30) and sp^2 methine (δ 5.30), the long-range coupling from the sp^3 methine proton (δ 6.30) to carbonyl (δ 171.8) and unsaturated carbons (δ 92.9, δ 146.4) were also observed in the HMBC spectrum, it diagnosed the presence of a 2,3-dihydropyridazine-3-carboxylic acid moiety. The signal observed at $\delta_{H/C}$ 3.67(s)/54.2 was assigned to the oxygenated methyl group based on the spectra of DEPT and HSQC, and a cross peak between the protons of methyl and the quaternary carbon at δ 146.4 was observed in the HMBC spectrum. Thus, the last amino acid was determined as 5-methoxy-2,3-dihydropyridazine-3-carboxylic acid.

The sequence linkage of the amino acids in **1** could be readily determined by MS/MS experiment (Figs. 2, 3). The product ions observed in the MS/MS spectrum were derived mostly from the cleavage of peptide bonds, and the amino acid content of characteristic fragments could be deduced based on the mass values of amino acid residues. Fragment ions observed at *m/z* 251, 349, 448, 519, 631 were deduced as the moieties of PA_2-PA_3 (P_2P_3), PA_1-Trp (P_1T), PA_1-Trp-Val (P_1TV), Ala-PA_1-Trp-Val (AP_1TV) and Ala-PA_1-Trp-Val-PA_3 (AP_1TVP_3), it revealed most information of the sequence linkage of the amino acids in **1**, except for the position of the unsaturated piperazic acid. The ion observed at *m/z* 560 was determined as PA_1-Trp-Val-PA_3 (P_1TVP_3), it indicated that 5-methoxy-2,3-dihydropyridazine-3-carboxylic acid (PA_2) was connected to Ala. The conclusion could also be testified by the results of HMBC experiment, which showed correlation from the α-methine protons of amino acid residue to carbonyl carbon of the neighboring residues.

For the determination of the absolute configurations, an acidic hydrolysate was generated according to the Marfey's protocol (Fujii et al. 1997). Subsequent HPLC analysis indicated the presence of L-alanine and D-valine. Because of the standard *R*- and *S*-piperazic acids were not obtained commercially, the 1-fluoro-2,4-dinitrophenyl-5-L-alanineamide (FDAA) derivatives of acidic hydrolysate of NW-G01 were prepared and analyzed using LC/DAD/MS, the absolute structure of this peptide has been established determinately by single-crystal X-ray diffraction and Marfey's method. Two PA-L-FDAA peaks ($[M + H]^+$, *m/z* 383) were observed in the selected ion chromatogram (SIC), and the ratio of their areas was 1:2, it was in accord with the structure of NW-G01, into which two molecules of *R*-PA and one *S*-PA were incorporated. Because only one peak was observed in the mass chromatogram of NW-G10 (**1**), piperazic acids in this compound could be determined as *R* configuration by comparison of its retention time with that of *R*-PA-L-FDAA. Unfortunately, FDAA derivatives of the unsaturated piperazic acid (PA_2) and the chlorinated Trp were not observed in Marfey's experiment, it may due to they were unstable in strong acidic conditions. The stereochemical configuration of these two rare amino acids will be assigned in further investigation by synthesis route (Fig. 1).

NW-G11 (**2**) was obtained as a white powder. Its molecular formula was established as $C_{36}H_{45}ClN_{10}O_8$ by HR-ESI–MS (*m/z* 781.3197, $[M + H]^+$; cacld for $C_{36}H_{46}ClN_{10}O8$, 781.3189). The presence of chlorine was suggested by the isotope abundance peaks in the MS spectrum. Like the IR spectrum with typical absorption bands of amides carbonyls at 1,639 cm^{-1}, the NMR data showed typical characteristics of peptides, for example resonances for amide carbonyls in the ^{13}C NMR spectrum and for α proton in the ^{1}H NMR spectrum (Table 1). The ^{13}C NMR and DEPT spectra (Table 1) of **2** showed the presence of 36 carbon signals, which were recognized as four methyls, one oxygenated methyl, six methylenes, eight sp^3 methines, one oxygenated sp^3 quaternary C-atom, six sp^2 methines, four sp^2 quaternary C-atoms, and six amide carbonyl carbons. ^{1}H and ^{13}C chemical shift assignments were made by standard 1D and 2D NMR techniques, such as DEPT, HSQC, and HMBC. The moieties of valine, N-methyl-alanine, piperazic acid, and chlorinated pyrroloindoline derivative were elucidated based on the HSQC and HMBC spectra of **2**, and by comparison with the corresponding chemical shifts and

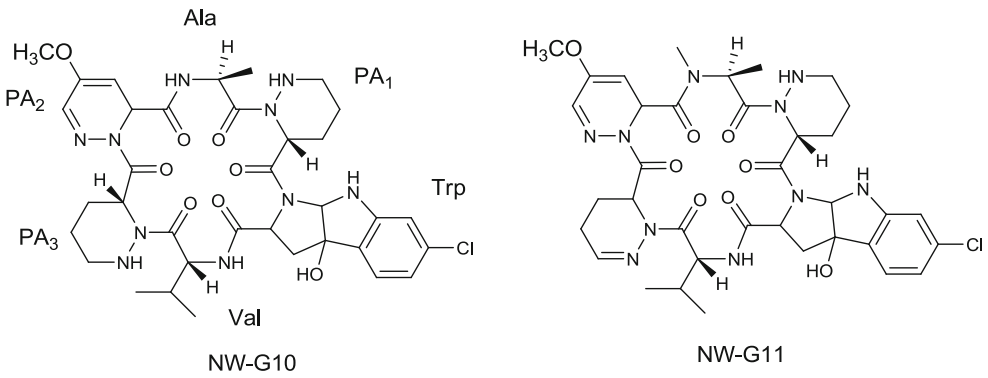

Fig. 1 Structures of NW-G10 (**1**) and NW-G11 (**2**)

coupling constants of NW-G01. 5-Methoxy-2,3-dihydropyridazine-3-carboxylic acid could also be determined based on the NMR data, and by comparison with the corresponding data of **1**.

Careful comparison of the DEPT spectra of **2** with that of **1**, an aromatic methine was new appeared at $\delta_{H/C}$ 7.13/144.4, and this proton was correlated to the β and γ carbons (δ 17.0 and δ 20.1) in HMBC spectrum. As only one remaining methenyl carbon was present, a 2,3,4,5-tetrahydropyridazine-3-carboxylic acid was diagnosed easily. This could also be testified by the correlation between H-α and carbonyl carbon (δ 172.1), as well as the results of MS/MS experiment.

The sequence linkage of the amino acids in **2** could be readily determined by the characteristic MS/MS fragments as described above (Figs. 2, 3). The conclusion could also be testified by the results of HMBC experiment, which showed correlation from the α-methine protons of particulars amino acid residues to the carbonyl carbon of the neighboring residues (Fig. 1).

N-Methyl-L-alanine, D-valine and *R*-piperazic acids could be recognized by application of the Marfey's method as described above, whereas the absolute configurations of other amino acids were not assigned in this investigation, because their FDAA derivatives were not be obtained by acid hydrolysis and Marfey's analysis.

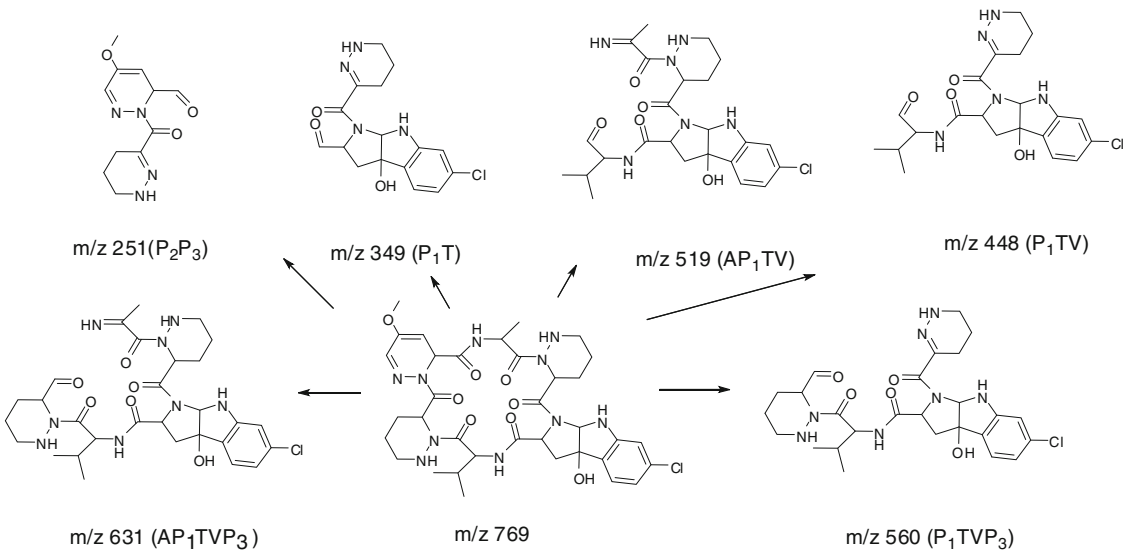

Fig. 2 Fragment pathway of NW-G10 (**a**) and NW-G11 (**b**)

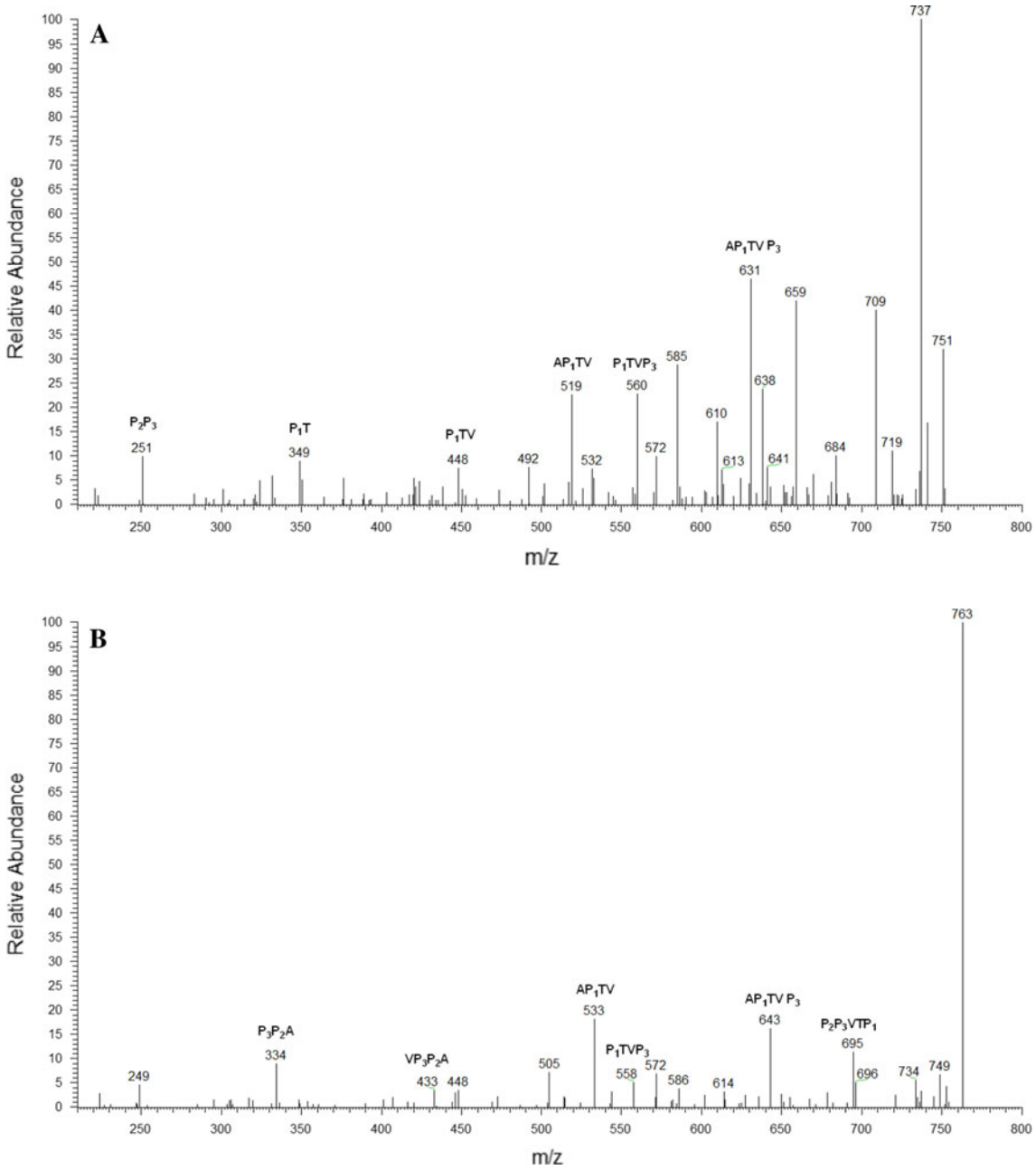

Fig. 3 MS/MS spectra of NW-G10 (**a**) and NW-G11 (**b**)

Antibacterial activity

The antibacterial activities of **1** and **2** against several strains of bacteria have been evaluated by micro-broth dilution method as we described previously (Ji et al. 2007). The results indicated that **1** and **2** could effectively inhibit Gram-positive bacteria, such as *Bacillus cereus*, *B. subtilis Staphylococcus aureus*, and MRSA, whereas they were insensitive to Gram-negative bacteria (Table 2). The minimum inhibition concentrations (MICs) of **1**, **2** and Ampicillin against *B. cereus* were 6.25, 25.0 and 6.25 μg/ml, their MICs against *B. subtilis* were 3.13, 25.0 and 1.56 μg/ml, their MICs against *S. aureus* were 6.25, 25.0 and 6.25 μg/ml, respectively, the antibacterial activity of **1** was higher than **2**. Because two amino acids are different in their structures, the results could not give any conclusion on the relationship of structure–activity. Compared to ampicillin, the MICs of **1** and **2** against MRSA were equal to their MICs against *S. aureus*, it revealed that this type of cyclic hexapeptides has a different mechanism of action from commercial β-lactam antibiotics.

Table 2 MICs of NW-G10 (**1**) and NW-G11(**2**) against several strains of bacteria

Tested bacteria	MIC (μg/ml)		
	1	**2**	Ampicillin sodium
Bacillus cereus (CGMCC 1.1846)	6.25	25.0	6.25
Bacillus subtilis (CGMCC 1.88)	3.13	25.0	1.56
Staphylococcus aureus (CGMCC 1.89)	6.25	25.0	6.25
Escherichia coil (CGMCC 1.1574)	>100	>100	3.13
Pseudomonas aeruginosa (CGMCC 1.2031)	>100	>100	>100
MRSA	6.25	25.0	>100

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