Original Article

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Total Synthesis of Bacicyclin Analog, cyclo-(D-Phe-Lys-Ile-Val-MeLeu-Gly), using a Combination of Solid- and Solution-Phase Method

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Abstract

Bacicyclin [cyclo-(D-Phe-D-Ala-Ile-Val-Leu-Gly)] is a cyclic hexapeptide with antimicrobial activity against *Enterococcus faecalis* and *Staphylococcus aureus* with MIC values of 8 and 12 μM, respectively. Studies on bacicyclin analog were conducted to investigate how the substitution of bacicyclin sequences affects its antibacterial activity. In the studies, two residues, D-Ala and Leu, were replaced by respective Lys and MeLeu residues. Bacicyclin analog [cyclo-(D-Phe-Lys-Ile-Val-MeLeu-Gly)], was successfully synthesized by constructing the linear precursor on 2-chlorotrityl chloride resin using Fmoc-based strategy. The HATU/HOAt reagent was applied in all peptide bond formation. The desired linear peptide was released from the resin using trifluoroethanol:acetic acid:dichloromethane (2:2:6) giving the product in 92% yield. Cyclization of the linear peptide was carried out using HATU as a cyclization agent and DIPEA as base in 1mM solution. Deprotection of Boc group was then carried out under 95% trifluoroacetic acid in dichloromethane. The synthesized product was then purified using semi-preparative RP-HPLC to obtain bacicyclin analog (10.3% yield). The purity of cyclic product was analyzed by analytical RP-HPLC (t_R = 35.2). The purified product was characterized by HR-TOF-MS ES$^+$ giving molecular ion peak at *m/z* $[M+H]^+$= 672.4456 and confirmed with ^{1}H-NMR (500 MHz CD_3OD-d_4) and ^{13}C-NMR (125 MHz CD_3OD-d_4). The antibacterial activity of bacicyclin analog was tested and showing a lower antibacterial activity than bacicyclin. The result indicated that the amino acid sequence dictates the antibacterial activity. This finding gives additional information on the relationship between peptide sequence and antibacterial activity.

Keywords: Bacicyclin analog, solid-phase peptide synthesis, cyclization, cyclic hexapeptide.

INTRODUCTION

The increasing number of resistant bacteria strain has become a global health issue that can cause 10 million death by 2050 if there is no new antimicrobial approaches implemented [1]. AMP has become one of approaches to overcome this issue, due to its broad antimicrobial activity [2] [3]. AMP in cyclic form exhibits unique advantages over linear peptide because of their lower hidrophobicity, lower toxicity, higher stability and higher affinity [4]. These advantages have made cyclic AMP a promising source of new drugs candidate [5].

Bacicyclin (**1**) (Fig. 1) [cyclo-(D-Phe-D-Ala-Ile-Val-Leu-Gly) is a cyclic hexapeptide first isolated by Wiese et al. [5] from marine *Bacillus sp.* Strain (BC028) that have antibacterial actiity againts *Enterococcus faecalis* and *Staphylococcus aureus* with MIC values of 8 and 12 μM, respectively. Bacicylin and its analogs were successfully synthesized by Chen et al. [6] using a combination of solid- and solution-phase methods showing a moderate antibacterial activity against *S.aureus* with IC_{50} >75 μg/mL. The difference in the antibacterial properties between the synthetic bacicylin and its natural product was described due to the difference in the conformation and purities between the natural product and the synthetic product [6].Maharani et al. [7] also synthesized a reversed-bacicyclin using a a combination of solid- and solution-phase methods giving 43.7% yield of the product. The synthetic reversed-bacicylin showed a lower antibacterial activity, indicating the peptide sequence influences the bioactivity.

According to Tores et al. [1], increasing the cationic charge and hydrophobicity of peptide will increase their antibacterial activity. In order to increase the antibacterial activity of bacicyclin, an analog was designed by replacing Ala residue with Lys to provide a cationic charge [8] with decreased hidrophobicity [9] and Leu with MeLeu to increase the hidrophobicity [10,11]. The presence of unnatural amino acid residues, D-Phe and MeLeu, in the structure could make the analog more resistant to proteases [12] and facilitate the cyclisation step because of their β-turn inducer properties [13]. Herein, we report the total synthesis of a bacicyclin analog, cyclo-(D-Phe-Lys-Ile-Val-MeLeu-Gly) (**2**) and linear bacicyclin analog, H_2N-D-Phe-Lys-Ile-Val-MeLeu-Gly-OH (Fig. 1).

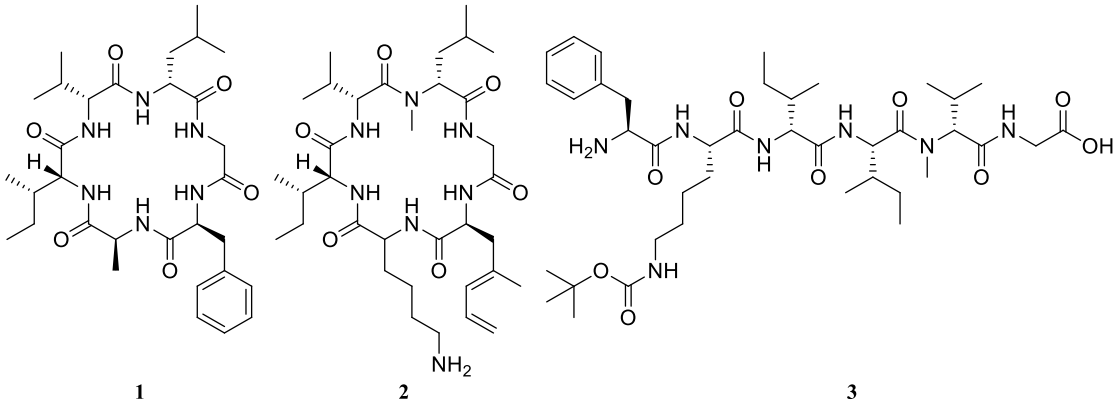

Fig 1. Structure of bacicyclin (**1**), bacicylin analog (**2**), and linear bacicyclin analog (**3**)

Most cyclic peptides were synthesized via a combination of solid- and solution-phase method, where the linear precursors were constructed in solid-phase and cyclisation was carried out in solution phase. Several peptides synthesized using this method are wollamide, desotamide A & B [14], exumolide A & B [11], c-PLAI and its analog [8,15]. This method was also used in the current synthesis. Initially, the cyclisation site was determined and the site between Gly at the C-terminus and Phe at the N-terminus was chosen. The selection was due to Gly at C-terminus can suppress the epimerization potential [16]. The solid-phase linear peptide synthesis was carried out on 2-chlorotrityl resin with Fmoc strategy. Lys side chain was protected by Boc group that is acid labile that made it safe during peptide elongation. The antibacterial properties of the synthesized product was evaluated againts *S.aureus* and *E.faecalis*

EXPERIMENTAL SECTION

Materials

The chemicals used were 2-chlorotrityl chloride resin, dimethylformamide (DMF), dichloromethane (DCM), 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium-3-oxide hexafluorophosphate (HATU), *n*-hexane *N,N*-diisopropylethylamine (DIPEA), 1-hydroxy-7-azabenzotriazole (HOAt), piperidine, ethyl acetate, and trifluoroacetic acid. All amino acid residues, Fmoc-D-phenylalanine, Fmoc-L-lysine(Boc),

Fmoc-L-isoleucine, Fmoc-L-valine, Fmoc-L-N-Me-leucine, Fmoc-L-glycine, and 2-chlorotrityl chloride (0.972 mmol/g) were purchased from GL-biochem Ltd., Shanghai, China.

Instrumentation

Purity analysis of the linear and cyclic hexapeptides was performed on a Waters RP-HPLC with Waters 2998 Photodiode Array Detector (PDA) with a wavelength of 210, 240, and 254 nm and LiChrospher 100 C-18 column (5 μm). Deionized water (A) and acetonitrile (B) were used as the mobile phase with gradient elution with a flow rate of 1.0 mL/min and column temperature 25 °C for 40 min. The purification of peptides was peformed on Waters RP-HPLC with Waters 2998 Photo Diode Array Detector (PDA) with a wavelenght of 210, 240, and 254 nm and Phenomenex 300 C-18 column (5 μm). Acetonitrile (A) and deionized water (B) were used as the mobile phase with addition of 0.1% trifluoroacetic acid (TFA) (vol/vol) and gradient elution with a flow rate of 2.0 mL/min and column temperature 25 °C for 40 min. The peptides were characterized by ^{1}H- and ^{13}C-NMR spectra using an Agilent ^{1}H-NMR 500 MHz and ^{13}C-NMR 125 MHz with deuterated solvent. The mass spectra were obtained from Waters HR-ToF-MS Lockspray. The absorbance of the loaded resin was measured on a UV-Vis Spectrophotometer (TECAN Infinite Pro 200).

Methods

Synthesis of linear hexapeptides bacicyclin analog precursor

The synthesis was performed on 2-chlorotrityl chloride resin (250 mg, 0.375 mmol), which was swollen in dichloromethane (10 mL) for 15 min at room temperature. Fmoc-Gly-OH (111,5 mg, 0.375 mmol) was loaded onto the resin in a mixture of dichloromethane (5 mL) and DIPEA (127 μL, 0.7 mmol). To measure the loading resin value, 20% piperidine in DMF (3 mL) was added to 0.7 mg Fmoc-D-Phe-resin in an eppendorf tube and the mixture was left for 30 min, followed by sonification for 5 min before the absorbance was measured at 290 nm. Then, the resin was capped by adding 10 mL of MeOH:DCM:DIPEA (15:80:5) twice before the addition of 20% piperidine in DMF (5 mL) for 2 × 5 min to eliminate the Fmoc group, yielding a free amino group on the resin. NH_2-Gly-Resin was coupled with the second residue, Fmoc-N-Me-Leu-OH, using a combination of HATU (144.0 mg, 0.378 mmol) and HOAt (51.5 mg, 0.378 mmol) as a coupling agent and DIPEA 128.8 μL, 0,99 mmol) as a base in DMF (5 mL) for 4 h at room temperature. The Fmoc group was removed from Fmoc-N-Me-Leu-Gly-Resin using 20% piperidine in DMF (5 mL) for 2 × 5 min to afford the Fmoc-MeLeu-Gly-Resin. This cycle of coupling and Fmoc deprotection was repeated with subsequent Fmoc-protected amino acids to obtain the D-Phe-Lys(Boc)-Ile-Val-MeLeu-Gly-Resin. Finally, the peptide was cleaved from the resin using a mixture of TFE:AcOH:dichloromethane (2:2:6) (10 mL) for 2 × 90 min. After the collection of filtrate and subsequent TFE and AcOH evaporation, the crude peptide was repeatedly washed with dichloromethane and dried under vacuum. The protected linear peptide was injected into an analytical RP-HPLC (5–40% acetonitrile in water for 40 min, flow rate 1 mL/min, λ 240 nm) and characterized using HR-ToF-MS.

Synthesis of a cyclic hexapeptides

The protected linear hexapeptide (34 mg, 0.04 mmol) was dissolved in DMSO (500 μL, 6.4 mmol), then dichloromethane (50 mL) before the addition of HATU (6 equiv. 98.17 mg, 0.25 mmol) and DIPEA (12 equiv. 87.8 μL, 0.51 mmol). The reaction mixture was stirred for 48 h at room temperature (monitored by TLC), deprotected by 95% TFA in DCM for 90 min, then evaporated under vacuum to yield the crude cyclic product as a dark-yellow oil which was extracted between ethyl acetate (50 mL) and brine solution (3 × 30 mL). The organic fractions were combined and evaporated to give crude peptides as a bright-yellow solid, which were purified by RP-HPLC to obtain the desired product (3,5 mg; yield 10,3%).

Bacicyclin analog (**2**): Bright-yellow solid; 10.3% yield. ^{1}H-NMR (500 MHz, CD_3OD, δ, ppm) and ^{13}C-NMR (125 MHz, CD_3OD, δ, ppm) data can be seen in Table 1. HR-TOF-MS *m/z* $[M+H]^+$ 672.4456 (calcd. $C_{35}H_{58}N_7O_6$ *m/z* 672.4443).

Deprotection of protected linear hexapeptides of precursor of bacicyclin analog

The protected linear hexapeptide (15 mg) was deprotected by 95% TFA in DCM for 90 min, then evaporated under vacuum to yield the crude cyclic product as a dark-yellow oil, which was extracted between ethyl acetate (50 mL) and brine solution (3 × 30 mL). The organic fractions were combined and evaporated to give crude peptides as a bright-yellow solid, which were purified by RP-HPLC to obtain the desired product (11 mg; yield 73,3%).

Linear bacicyclin analog (**3**): Bright-yellow solid; 73.3% yield. ^{1}H-NMR (500 MHz, CD_3OD, δ, ppm) and ^{13}C-NMR (125 MHz, CD_3OD, δ, ppm) data can be seen in Table 1. HR-TOF-MS *m/z* $[M+2H]^+$ 691.4407 (calcd. $C_{35}H_{61}N_7O_7$ *m/z* 691.4554).

Antibacterial assay

The microdilution method described by Mustafa et al. [17] with some modifications was also performed to evaluate the antibacterial activity of the test peptides against *S. aureus* and *E. faecalis*. Briefly, the peptides were dissolved in 2% DMSO at a concentration of 1 g/mL to prepare a series of serial dilutions (1000; 500; 250; 125; 62.5; 31.25; 15.62; 7.81; 3.90; 1.95; 0.97 and 0.48 ppm). The sample solutions, ciprofloxacin, daptomycin, and 2% DMSO were placed in a 96-well microplate and incubated at 37 °C for 18-24 h before the absorbance was measured at 600 nm to calculate the MIC values.

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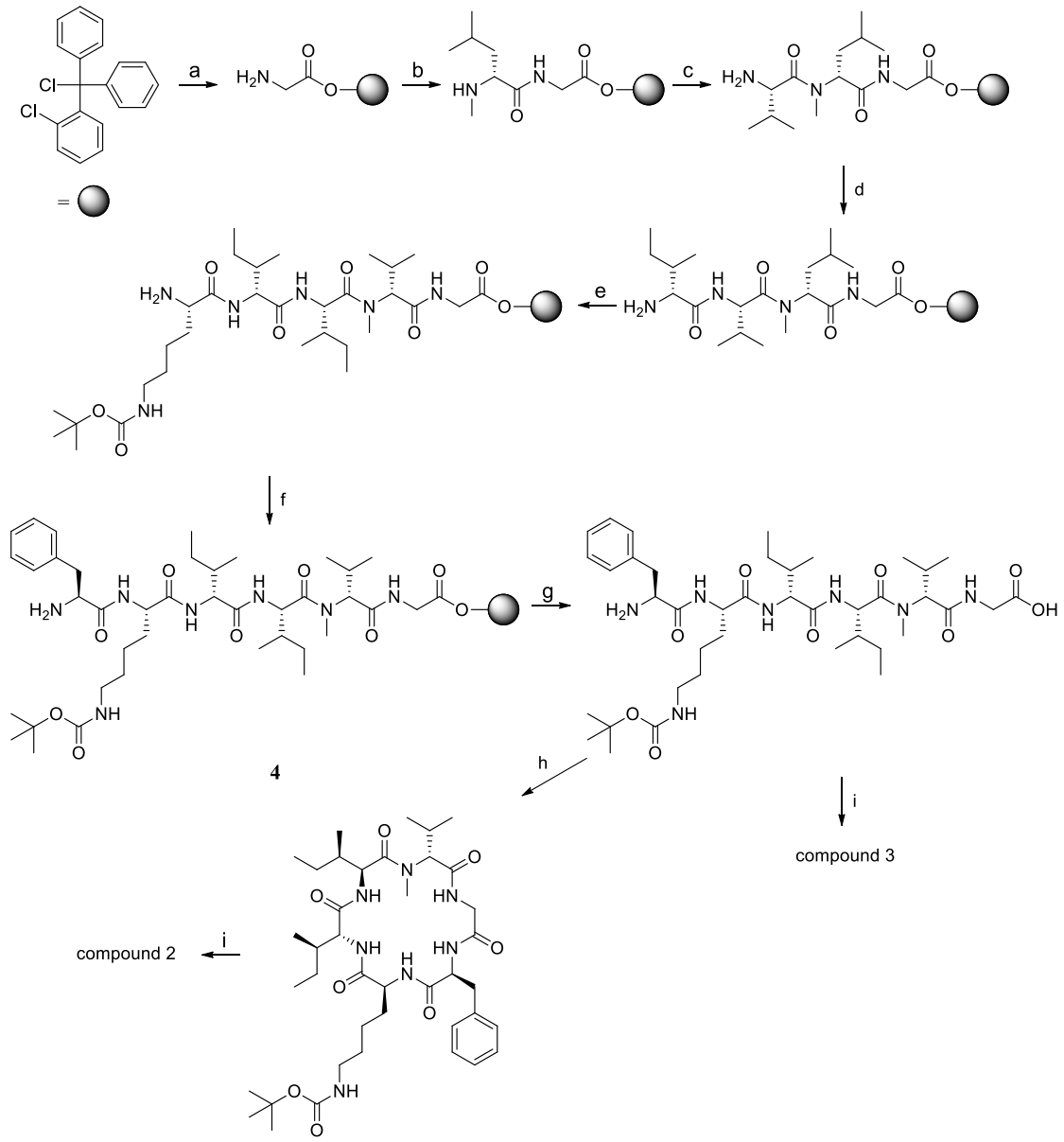
RESULTS AND DISCUSSION

The synthesis procedure of bacicyclin analog is shown in Scheme 1. The linear hexapeptide was prepared via an SPPS method using 2-chlorotrityl chloride (2-CTC) resin because the resin can suppress diketopiperazine formation due to bulky size of the chlorotrityl group and provide a mild acidic cleavage condition. The synthesis was initiated by the loading of first residue, Fmoc-Gly-OH, onto resin to obtain 0,50 mmol/g, which was categorized as good (0.2-0.8). The unreacted sites were then capped using MeOH:DIPEA:DCM (15:5:85), and the Fmoc group was deprotected with 20% piperidine in DMF to obtain a free amino group. The product was then coupled with Fmoc-N-Me-Val-OH using HATU/HOAt as the coupling reagent, and DIPEA as the base before the peptide was elongated by the attachment of subsequent L-leucine, L-valine, L-isoleucine, and D-phenylalanine residues and final Fmoc deprotection to give the linear hexapeptidyl NH_2-D-Phe-Lys(Boc)-Ile-Val-N-Me-Leu-Gly-Resin (**4**).



(a) (1) Fmoc-Gly-OH, DIPEA, in DCM, 4 h, rt, 20% piperidine in DMF, (2) MeOH:DIPEA:DCM (3:1:16), 2x20 min, rt,
(b) (1) Fmoc-MeLeu-OH, HATU/HOAt, in DMF, 4 h, rt, (2) 20% piperidine in DMF, 2x5 min,
(c) (1) Fmoc-Val-OH, HATU/HOAt, in DMF, 4 h, rt, (2) 20% piperidine in DMF, 2x5 min,
(d) (1) Fmoc-Ile-OH, HATU/HOAt, in DMF, 4 h, rt, (2) 20% piperidine in DMF, 2x5 min,
(e) (1) Fmoc-Lys(Boc)-OH, HATU/HOAt, in DMF, 4 h, rt, (2) 20% piperidine in DMF, 2x5 min,
(f) (1) Fmoc-D-Phe-OH, HATU/HOAt, in DMF, 4 h, rt, (2) 20% piperidine in DMF, 2x5 min,
(g) TFE:AcOH:DCM (2:2:6) 2x90 min, rt, (h) HATU, DIPEA, in DCM, 10-3 M, 48 h, rt.
(i) 95% TFA in DCM, 90 min, rt.

Scheme 1. Fmoc-based SPPS, solution-phase macrocyclization and Boc deprotection of bacicyclin analog.

The protected linear hexapeptide was cleaved from the resin using a mixture of TFE:AcOH:DCM (2:2:6) to give crude linear peptide, consisting two peaks on the HPLC chromatogram at a retention time of 27,5 and 33,0 minutes (Fig. 2). The two peaks were analysed by HR-TOF-MS showing molecular ion peak at *m/z* 790,5081 (*m/z* calcd. 790,5079) for the desired product (t_R = 33.032 min) and at *m/z* 643,4448 (*m/z* calcd. 643,4442) $[M]^+$ for pentapeptide product (H_2N-Lys(Boc)-Ile-Val-MeLeu-Gly-OH (t_R = 27.529 min). The pentapeptide product is a deletion product that was formed due to the failure of coupling reaction of Phe to Lys(Boc)-peptide-resin. Thus due to the occurrence of aggregation at fifth position NH_2-Lys(Boc)-peptide-resin sequence made the coupling of the sixth residue (Phe) had failed [18, 19].

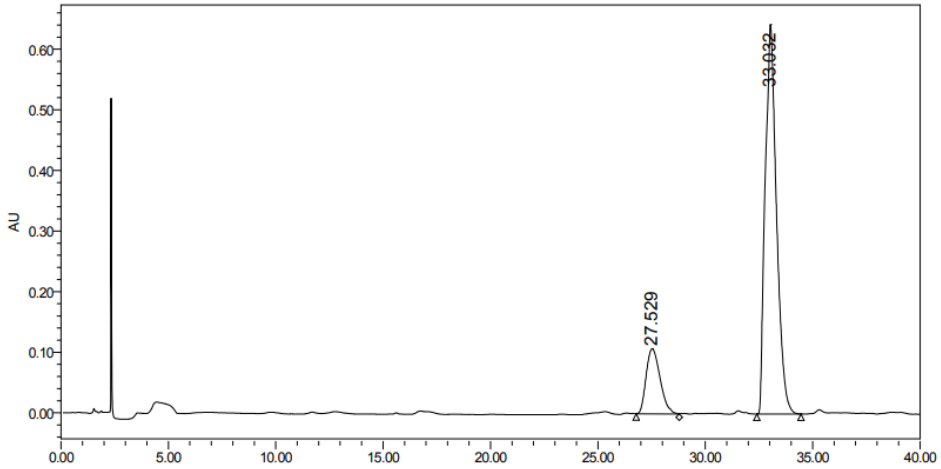


Fig 2. Analytical RP-HPLC chromatogram of protected linear bacicyclin analog **4** in acetonitrile:water (5–40% linear gradient) at a flow rate of 1 mL/min and λ 240 nm

Fig 3. MS spectra of crude of the linear protected hexapeptide

The protected linear hexapeptide was divided into two batches. The first batch is used for cyclization and another batch is used for the direct deprotection to give the unprotected linear precursor. The first linear hexapeptide was less soluble in DCM, DMF, acetonitrile, and other organic solvents but dissolved well in DMSO. Therefore, to avoid difficulties with DMSO removal after the cyclization, a minimal amount of DMSO was used to dissolve the peptide. Macrocyclization was firstly trialled in a dilute solution of 0.001 M in

dichloromethane following the protocol of Ma and colleagues [20] and then was optimized by Maharani et al. [21]. The reaction was monitored by TLC (n-hexane:isopropanol (7:3) and peptide dimerization was avoided by conducting the macrocyclization reaction at a very dilute peptide concentration (less than 1 mM). The reaction was complete in 48 h, then, without prior purification, the crude cyclic product was Boc-deprotected by 95% TFA in DCM for 90 min. The second batch of the protected linear hexapeptide was Boc-deprotected using 95% TFA in DCM for 90 min to give linear hexapeptide unprotected. The linear peptide was used for bioassay comparison with the cyclic analog.

Crude of bacicyclin (**2** and **3**) analogs was then purified by semi-preparative RP-HPLC with deionized water (A) and acetonitrile (B) as the mobile phase and the addition of 0,1% trifluoroacetic acid (TFA) (vol/vol). The purification was used a gradient elution from 20% to 80% of B with a flow rate of 2.0 mL/min and column temperature 25°C for 40 min, yielding 3,5 mg of compound **2** (10,3%) and 11 mg of compound **3** (73,3%) both as bright-yellow solid from each crude. The purity of peptide **2** and **3** was checked by analytical RP-HPLC (Fig. 4; retention time = 35.2 min, Fig 5; retention time = 35,2) and it was characterized by HR-TOF-MS, ^{1}H-NMR, and ^{13}C-NMR. The mass spectra of HR-TOF-MS showed a correct molecular ion peak of the compound **2** with *m/z* 672.4456 $[M+H]^+$ (calcd. *m/z* 672.4443) (Fig. 6) and compound **3** with *m/z* 691.4407 $[M+2H]^+$ (calcd. *m/z* 691.4554) (Fig. 7).

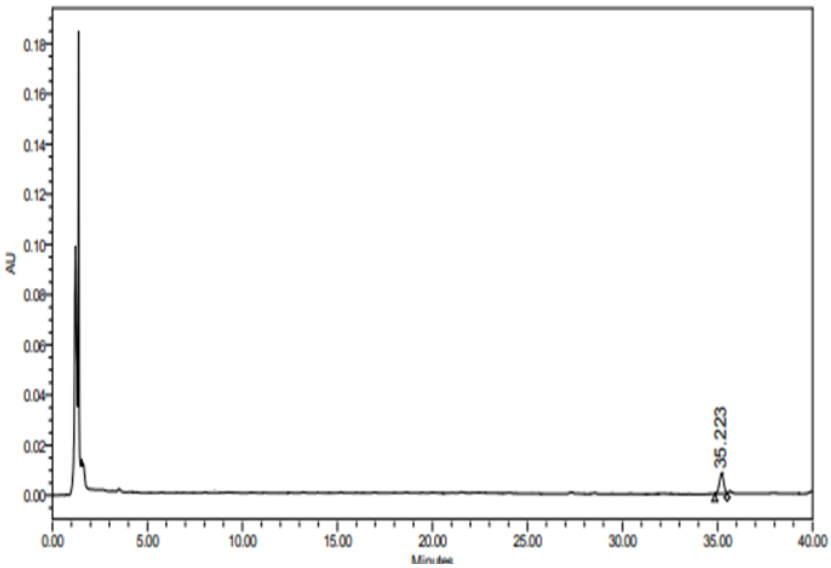


Fig 4. Analytical RP-HPLC chromatogram of bacicyclin analog (**2)** in acetonitrile:water (5–40% linear gradient) at a flow rate of 1 mL/min and λ 240 nm (purity 100%)

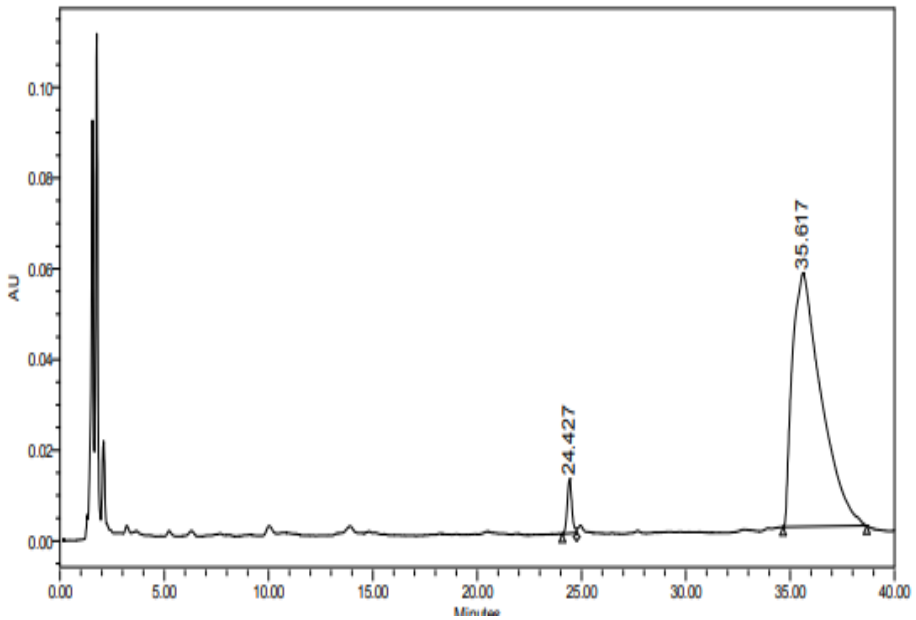


Fig 5. Analytical RP-HPLC chromatogram of linear bacicyclin analog (**3)** in acetonitrile:water (5–40% linear gradient) at a flow rate of 1 mL/min and λ 240 nm (purity 96%)

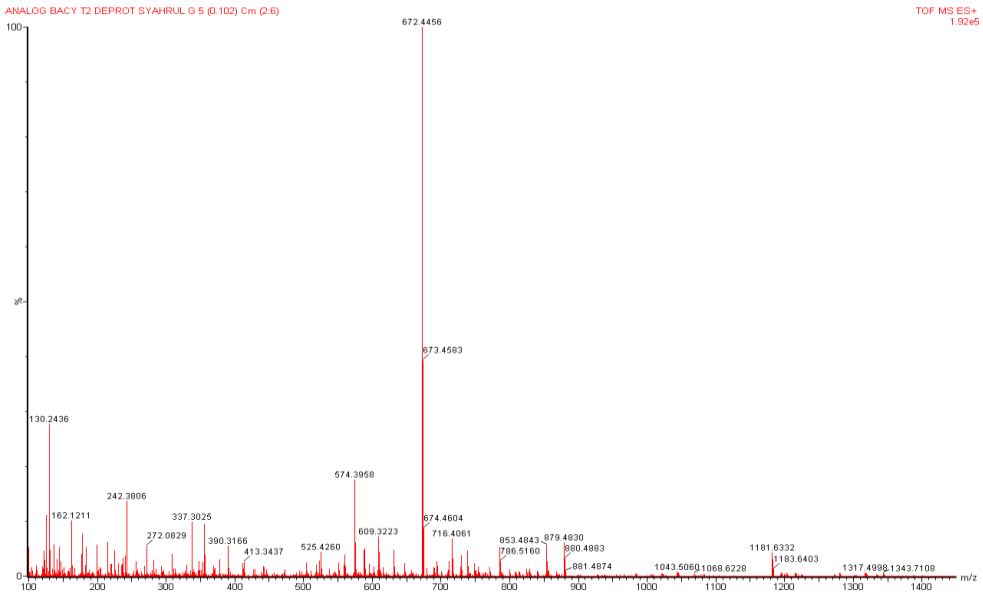


Fig 6. MS spectra of the compound **2** [cyclo-(D-Phe-Lys-Ile-Val-MeLeu-Gly)]

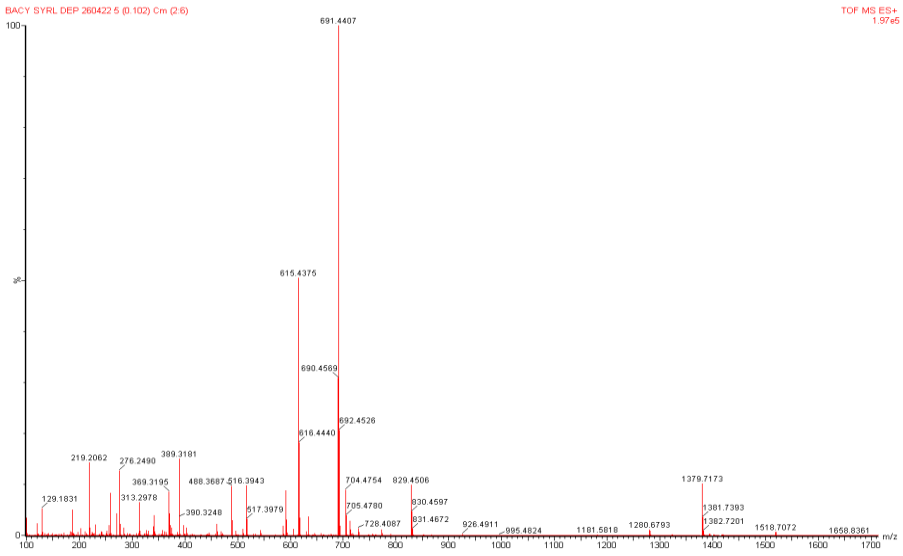


Fig 7. MS spectra of the compound **3** (D-Phe-Lys-Ile-Val-MeLeu-Gly)

Table 1. ^{1}H-NMR and ^{13}C-NMR spectra data of bacicyclin analog,linear bacicyclin analog and comparison with bacicyclin spectral data by Wiese and colleagues

Asam Amnio	Bacicyclin [5]		Bacicyclin analog (2)		Linear Bacicyclin analog (3)	
	600 MHz CD_3OD-d_4	150 MHz CD_3OD-d_4	500 MHz CD_3OD-d_4	125 MHz CD_3OD-d_4	500 MHz CD_3OD-d_4	125 MHz CD_3OD-d_4
	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
			Gly			
CO		179.1		175.07		178.29
α	3.36 (t)	41.4	4.02 (t)	44.37	4.22 (m)	40.36
			Leu			
CO		171.5		169.68		168.07
α	3.8 (t)	52.7	4.51 (t)	53.44	4.12 (t)	53.2
N	-	-	3.27 (m)	38.94	3.12 (m)	37.15
β	1.58 (m)	41.8	1.59 (m)	38.52	1.59 (m)	36.73
γ	1.62(m)	26.2	1.67 (m)	25.32	1.62 (m)	24.61
δ	0.91 (d)	22.5	0.97 (d)	22.58	0.97 (d)	22.26
δ'	0.96 (d)	21.6	0.97 (d)	22.42	0.97 (d)	22.17
			Val			
CO		175.5		172.98		172.99
α	4.21 (dd)	60.6	4.51 (dd)	58.38	4.25 (dd)	57.75
β	1.98 (m)	31.8	1.96 (m)	30.3	2.12 (m)	31.6
γ	0.89 (d)	19.5	0.89 (d)	19.04	0.89 (d)	18.24
γ '	0.88 (d)	18.9	0.89 (d)	18.31	0.89 (d)	17.52

			Ile			
CO		173.2		172.49		171.91
α	4.19 (d)	58.9	4.41 (dd)	56.31	4.62 (d)	54.7
β	2.03 (m)	36.1	2.31 (m)	36.5	2.09 (m)	36.6
γ '	0.84	12.1	0.97	14.92	1.02	14.9
γ	1.28	27.5	1.35	26.35	1.77	26.67
δ	0.86	14.6	0.86	10.62	0.95	9.86
	Ala				**Lys**	
CO		173.4		172.57		172.13
α	4.3 (q)	49.9	4.98 (d)	55.84	4.63 (d)	54.3
β	1.17 (d)	18.1	1.83 (m)	29.72	1.56 (m)	30.55
γ			1.28 (d)	19.04	1.47 (d)	20.71
δ			1.76 (t)	23.38	1.64 (dd)	24.61
ε			2.99 (dd)	43.41	2.86 (q)	38.95
	N rantai samping		5.08 (dd)		5.23 (dd)	
			Phe			
CO		173.9		172.57	5.23 (dd)	172.33
α	4.37 (dd)	56.8	4,98 (d)	56.06	4.90 (d)	54.55
β/ β’	3.31/3.05 (m)	29.1	3.36;3.26 (m)	29.43	3.87 (m)	30.48
Bzl-i		128.9		136.46		134.41
Bzl-o	7.22 (m)	112.3	7.30 (m)	126.71	7.33 (m)	127.38
Bzl-m	6.98 (m)	119.4	7.23 (m)	128.38	7.28 (m)	128.68
Bzl-p	7.18 (m)	126.4	7.26 (m)	129.27	7.37 (m)	129.13

The ^{1}H-NMR and ^{13}C-NMR spectra of **2** and **3** where quite different, with the presence of the more deshielding carbonyl chemical shift at 178.29 ppm at the linear peptide **3** indicating carboxyl group from Gly as C terminus. The absence of amine proton were caused by the use of deuterated methanol (polar protic) that facilitate proton exchange [22] between compound and solvent [23]. The successful formation of an amide bond between the amine group of Phe with carboxyl group of Gly at compound **2** was proven by the presence of deshielded carbonyl signal of Gly at 175,07 ppm. Overall, at NMR sectra of compound **2** displayed six amide-type carbonyls (δ 175.07, 169.68, 172.98, 172.49, 172.57, 172,57 ppm), four sp^2 carbons (δ 136.44, 126.71, 128.38, 129.27), six α carbons (δ 44.37, 53.44, 58.38, 56.31, 55.84, 56.06 ppm), three methine carbons (δ 25.32, 30.30, 36.50 ppm), seven methylene carbons (δ 25.32, 26.35, 29.43, 29.72, 19.04, 23,38. 43,41 ppm), and seven methyl carbons (δ 38.94, 22.58, 22.42, 19.04, 18.31, 14.92, 10.62 ppm). NMR spectra of compound **3** displayed six amide-type carbonyls (δ 178.29, 168.07, 172.99, 171.91, 172.23, 172.33), four sp^2 carbons (δ 134.41, 127.38, 128.68, 129.13), six α carbon (δ 40.43, 53.2, 57.75, 54.7, 54.3, 54.55), three methine carbons (δ 24.61, 31.60, 36.6), seven methylene carbons (δ 36.73, 26.67, 30.55, 20.71, 24.61, 38.95, 30.48), and seven methyl carbons (δ 31.2, 22.26, 22.17, 18.24, 17.52, 14.39, 9.89). The NMR spectral data of **2** and **3**, particularly at the chemical shifts of Gly, Leu, Val, Ile, and Phe residues were similar to the NMR data obtained by Wiese et al. for bacicyclin.

The antimicrobial activities of **2** and **3** were assessed, showing that the linear and cyclic peptides of the bacicyclin analog exhibited weak antibacterial activity against *S. aureus* and *E. faecalis* but were not as effective as the natural product [5], synthetic bacicylin [6], or reversed analog [7], indicating that the replacement of one residue with another residue affected the biological properties of the peptide.

Table 2. MIC value of bacicyclin analogs **2** and **3** with positive control ciprofloxacin and vancomycin againts *S.aureus* and *E.faecalis*

Compound	MIC value (µg/mL)	
	***Staphylococcus aureus* (ATCC 6538)**	***Enterococcus faecalis* (ATCC 29212)**
ciprofloxacin	0.48	3.9
vancomycin	6.25	12.5
bacicyclin analog **2**	>500	500
linear bacicyclin analog **3**	>500	>500

Biological activity of cyclic peptide is slightly better than in linear form, this is because cyclic peptide can adopt a favourable orientation towards the membrane and acquire an ordered structure that allows a high charge density and amphipathic arrangement. Cyclic peptide also locate itself deeper in the membrane as well than its linear counterpart [24]. The difference in biological activity between the naturally occurring compounds and the synthetic compounds may be attributed to the conformational difference, or the presence of biologically active impurities in the naturally isolated material, and/or changes in the configuration during synthesis [6], as some groups reported [25]. The substitution of D-Ala with Lys also contributed to decrease the activity of bacicyclin analog, maybe the hydrophilicity of Lys reduces the cell permeability of the peptide. The different bioassay protocol that we use in this research with Wiese and Chen may also give different result.

CONCLUSION

Bacicyclin analog [cyclo-(D-Phe-Lys-Ile-Val-MeLeu-Gly) and its linear peptide, H_2N-D-Phe-Lys-Ile-Val-MeLeu-Gly-OH, has been successfully synthesized in a two-step process, achieving an overall yield of 10,3% and 73,3%, respectively. Biological assay showed that cyclic peptide had slightly better activity than its linear form. However, the biological properties of the synthetic peptides were inactive compared to the natural products. This finding gives information on the relationship between peptide sequence and biological properties.

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