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RESEARCH PAPER

TOTAL SYNTHESIS OF A NATURAL CYCLOOLIGOPEPTIDE FROM FRUITS OF SUGAR-APPLES

Rajiv Dahiya[1]*, Sunil Singh[2], Komalpreet Kaur[3] and Ramninder Kaur[3]

[1]*Laboratory of Peptide Research and Development, School of Pharmacy, Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Trinidad & Tobago, West Indies*
[2]*Research Scholar, Department of Pharmacy, Mewar University, Gangrar, Chittorgarh-312901, Rajasthan, India*
[3]*Department of Pharmaceutical Chemistry, G.H.G. Khalsa College of Pharmacy, Gurusar Sadhar-141104, Ludhiana, Punjab, India*

**E-mail*: rajiv.dahiya@sta.uwi.edu
Tel.: +1868 4935655.

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A cyclopentapeptide, fanlizhicyclopeptide B, previously isolated from the fruits of *Annona squamosa*, was synthesized via coupling of dipeptide l-Isoleucyl-l-Tyrosyl methyl ester with tripeptide Boc-l-Alanyl-Glycyl-l-Proline followed by cyclization of the linear pentapeptide fragment. Structure of the synthesized cyclooligopeptide was confirmed using quantitative elemental analysis, FTIR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. Results of pharmacological activity studies indicated that the newly synthesized cyclopentapeptide displayed remarkable anthelmintic potential against *Megascoplex konkanensis*, *Pontoscotex corethruses* and *Eudrilus eugeniea* at 2 mg/ml and potent anti-dermatophytic activities against *Trichophyton mentagrophytes* and *Microsporum audouinii* at concentration of 6 mcg/ml.

Key words: Fanlizhicyclopeptide B, *Annona squamosa*, Solution-phase synthesis, Biological activity.

INTRODUCTION

Discovery and development of new therapeutic agents has been a continuing process. In-spite of the fact that large numbers of therapeutic molecules are available for human health care programs, the thrust for safer and effective medicines is increasing. There is an utmost need to understand the principles of traditional systems of medicine more precisely in the light of modern science and there is increasing awareness and general acceptability of the use of herbal drugs in today's medical practice. Therefore, the world is witnessing an unprecedented growth in the usage of herbal products (Kunle *et al* 2012; Kumari *et al* 2016). Among natural products, cyclooligopeptides are a special group of bioactive compounds with interesting pharmacological and biochemical properties which occur in higher plant species. Recently, plants fruit-derived cyclopolypeptides have received attention of researchers and scientists (Tan and Zhou, 2006) which exhibit a wide range of biological activities *viz.* immunosuppressive activity (Morita *et al* 1997; Picur *et al* 1998), vasorelaxant activity (Morita *et al* 2006), cell growth inhibitory activity (Morita *et al* 1996) and anti-inflammatory activity (Noh *et al* 2015). A natural cyclic pentapeptide, fanlizhicyclopeptide B was isolated from the fruits of *Annona squamosa* (sugar-apples) and its structure was elucidated by ESI MS/MS, 1D and 2D NMR data and chemical degradation (Wu *et al* 2014). Keeping in view broad range of pharmacological activities possessed by natural cyclopeptides (Fang *et al* 2016; Dahiya, 2013; Dahiya and Pathak, 2006a; Pathak and Dahiya,

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2003) and in continuation of synthetic studies of our research group toward natural peptides and their analogs (Dahiya 2007a; 2007b; 2007c; 2008a; 2008b; 2008c; 2008d; Dahiya and Gautam, 2010a; 2010b; 2010c; 2011a; 2011b; 2011c; 2011d; Dahiya and Kaur, 2007a; 2008a; Dahiya and Kumar, 2007; 2008; Dahiya and Pathak, 2006b; 2007a; 2007b; Dahiya and Sharma, 2008; Dahiya and Singh, 2017a; 2017b; 2017c; 2016; Dahiya *et al* 2006; 2009a; 2009b; 2009c; 2016; Kumar *et al* 2017), the present investigation directed toward the synthesis, structure elucidation and the biological evaluation of a sugar-apple derived cyclopenta-peptide, fanlizhicyclopeptide B for the antibacterial, antifungal and anthelmintic potential.

EXPERIMENTAL

Chemistry

Melting point was determined by the open capillary method and is uncorrected. IR spectra were recorded using an FTIR-8400S fourier transform spectrophotometer (Shimadzu, Kyoto, Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 300 spectrometer at 300 MHz (Brucker, IL, USA). Mass spectra was recorded on a JMS-DX 303 spectrometer (Jeol, Tokyo, Japan). Elemental analysis was performed on a Vario EL III elemental analyzer (Elementar Vario EL III, Hanau, Germany) and optical rotation of the synthesized peptides was measured on an Optics Technology automatic polarimeter (OpticsTech, Delhi, India). Purity of the synthesized peptides was checked by TLC on precoated silica gel G plates (Kieselgel 0.25 mm, 60G F_{254}, Merck, Germany).

General procedure for the synthesis of linear di/tripeptide segments (I, II, IV)

To the solution of the amino acid methyl ester hydrochloride (0.01 mol) in tetrahydrofuran (THF, 25 ml), N-methylmorpholine (NMM) / triethylamine (TEA) (2.23 ml/2.8 ml, 0.021 mol) was added at 0 °C, and the reaction mixture was stirred for 15 min. The Boc-protected amino acid/dipeptide (0.01 mol) in THF (25 ml), *N,N′*-diisopropylcarbodiimide / *N*-(3-dimethylamino-propyl)-*N′*-ethylcarbodiimide hydrochloride (DIPC/EDC.HCl, 1.26 g/1.92 g, 0.01 mol) and 1-hydroxybenzotriazole (HOBt, 1.34 g, 0.01 mol) were added with stirring to the above reaction mixture. Stirring of the resulting mixture was continued for 24 h at RT. The reaction mixture was filtered and the residue was washed with THF (25 ml) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ and saturated NaCl solutions. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum. The crude product was recrystallized from a mixture of chloroform and petroleum ether (b.p. 40-60°C) followed by cooling at 0°C to get the title compounds.

Deprotection of the dipeptide unit (I) at the amino terminal

Boc-protected dipeptide (I, 4.08 g, 0.01 mol) was dissolved in $CHCl_3$ (15 ml) and treated with CF_3COOH (2.28 g, 0.02 mol). The resulting solution was stirred at room temperature for 1 h, washed with a saturated $NaHCO_3$ solution (25 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by crystallization from the $CHCl_3$ and petroleum ether (b.p. 40-60°C) to get the pure deprotected compound Ia.

Deprotection of the tripeptide unit (IV) at the carboxyl terminal

To a solution of the tripeptide (IV, 3.57 g, 0.01 mol) in THF·H_2O (1:1, 36 ml), LiOH (0.36 g, 0.015 mol) was added at 0°C. The mixture was stirred at room temperature for 1 h and then acidified to *p*H = 3.5 with 1 N H_2SO_4. The aqueous layer was extracted with Et_2O (3 × 25 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was finally crystallized from methanol and ether to get the pure deprotected compound IVa.

Procedure for the synthesis of linear pentapeptide unit and its cyclized form (V, VI)

Dipeptide methyl ester, l-Ile-l-Tyr-OMe (Ia, 3.08 g, 0.01 mol) was dissolved in 30 ml of dichloromethane (DCM) and 2.23 ml/2.8 ml (0.021 mol) of TEA/NMM was added at 0°C and the resulting mixture was stirred for 15 min. Boc-protected tripeptide, Boc-l-Ala-Gly-l-Pro-OH (IVa, 3.43 g, 0.01 mol) was dissolved in 30 ml of DCM and DIPC/EDC.HCl (1.26 g/1.92 g, 0.01 mol) and HOBt (1.34 g, 0.01 mol) were added to above mixture with stirring. Stirring was continued for 24 h, after which the reaction mixture was filtered and the filtrate was washed with 25 ml each of 5% $NaHCO_3$ and saturated NaCl solutions. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum. The crude product was recrystallized

from a mixture of chloroform and petroleum ether (b.p. 40-60°C) followed by cooling at 0°C to get the Boc-l-Ala-Gly-l-Pro-l-Ile-l-Tyr-OMe (V) as yellowish semisolid mass. Linear pentapeptide unit (V, 3.17 g, 0.005 mol) was then, deprotected at the carboxyl terminal using lithium hydroxide (LiOH, 0.18 g, 0.0075 mol) to obtain the Boc-l-Ala-Gly-l-Pro-l-Ile-l-Tyr-OH (Va). To a solution of the deprotected heptapeptide (3.1 g, 0.005 mol) in $CHCl_3$ (50 ml), pentafluorophenol (*pfp*, 1.23 g, 0.0067 mol) and DCC (1.06 g, 0.005 mol) were added followed by stirring at RT for 12 h. Filtrate of the above reaction mixture was washed with 10% $NaHCO_3$ (3 × 20 ml) and 5% HCl (2 × 20 ml) solutions to obtain the corresponding pentafluorophenyl ester Boc-l-Ala-Gly-l-Pro-l-Ile-l-Tyr-O*pfp* (Vb). Boc-group of the resulting unit (3.14 g, 0.004 mol) was removed using CF_3COOH (0.91 g, 0.008 mol) to get the deprotected product l-Ala-Gly-l-Pro-l-Ile-l-Tyr-O*pfp* (Vc) which was dissolved in $CHCl_3$ (25 ml) and TEA/NMM/pyridine (2.8 ml/2.21 ml/1.61 ml, 0.021 mol) was added. Then, the whole contents were kept at 0°C for 7 days. The reaction mixture was washed with 10% $NaHCO_3$ (3 × 25 ml) and 5% HCl (2 × 25 ml) solutions. The organic layer was dried over anhydrous Na_2SO_4 and crude cyclized compound was recrystallized from CH_2Cl_2/*n*-hexane to obtain the pure cyclic product (VI).

Cyclo(l-Alanyl-Glycyl-l-Prolyl-l-Isoleucyl-l-Tyrosyl) (VI)

Pale yellow solid; m.p. 121-123 °C (d); Yield 87 % (C_5H_5N), 79 % (NMM), 73 % (TEA); $[\alpha]_D$ = –113.5° (*c* = 0.41, MeOH) (–113.6° for natural fanlizhicyclopeptide B (Wu *et al* 2014); R_f = 0.68 ($CHCl_3$·MeOH - 9:1); IR (KBr): *v* = 3375 (O–H_{str}, aromatic ring), 3128-3124, 3121 (N–H_{str}, amide), 3068-3063 (Ar–H_{str}, aromatic ring), 2998-2992 (C–H_{str}, cyclic CH_2), 2969, 2925, 2918 (C–H_{str}, asym, CH_3 and CH_2), 2842, 2837 (C–H_{str}, sym, CH_2), 1668, 1645-1639 (C=O_{str}, 3° and 2° amide), 1567, 1435 (skeletal bands), 1535, 1531-1527 (N–H_{def}, amide), 714, 685 (C–H_{def}, oop, aromatic ring) cm^{-1}; ^1H NMR ($CDCl_3$): δ = 8.68 (br. s, 1 H, N*H*, Ile), 8.35 (br. s, 1 H, N*H*, Tyr), 7.72 (br. s, 1 H, N*H*, Ala), 7.25 (br. s, 1 H, N*H*, Gly), 6.99, 6.96 (dd, *J* = 8.6, 4.9 Hz, 2 H, *o*-H's, Tyr), 6.88, 6.85 (dd, *J* = 8.6, 5.3 Hz, 2 H, *m*-H's, Tyr), 5.97 (br. s, 1 H, O*H*, Tyr), 5.94-5.89 (m, 1 H, α-H, Ala), 5.68-5.64 (q, *J* = 7.85 Hz, 1 H, α-H, Tyr), 5.29 (d, *J* = 5.5 Hz, 2 H, α-H's, Gly), 3.89 (t, 1 H, *J* = 6.9 Hz, α-H, Pro), 3.81 (t, *J* = 8.6 Hz, 1 H, α-H, Ile), 3.25 (t, 2 H, *J* = 7.15 Hz, δ-H, Pro), 2.68-2.64 (m, 2 H, β-H's, Pro), 2.37 (d, *J* = 5.5 Hz, 2 H, β-H's, Tyr), 1.85-1.79 (m, 2 H, γ-H's, Pro), 1.63-1.58 (m, 2 H, γ-H's, Ile), 1.53-1.48 (m, 1 H, β-H's, Ile), 1.44 (d, 3 H, *J* = 5.85 Hz, β-H's, Ala), 1.01 (d, *J* = 5.9 Hz, 3 H, γ'-H's, Ile), 0.96 (t, 3 H, *J* = 7.8 Hz, δ-H's, Ile); ^{13}C NMR ($CDCl_3$): δ = 173.3 (C=O, Ala), 172.1 (C=O, Tyr), 170.7 (C=O, Ile), 170.2 (C=O, Pro), 163.5 (C=O, Gly), 152.6 (*p*-C, Tyr), 133.7 (γ-C, Tyr), 129.2 (2 C, *o*-C's, Tyr), 127.9 (2 C, *m*-C's, Tyr), 59.0, 56.2, 53.7 (3 C, α-C's, Ile, Pro and Tyr), 49.2, 48.7 (2 C, α-C's, Gly and Ala), 48.0 (δ-C, Pro), 39.9, 36.4, 32.7 (3 C, β-C's, Tyr, Ile and Pro), 24.4, 22.8 (2 C, γ-C's, Ile and Pro), 17.8 (β-C, Ala), 16.9 (γ'-C, Ile), 10.6 (δ-C, Ile); MS (FAB, 70 eV): *m/z* (%) = 502 (100) $[M + 1]^+$, 474 (14) $[502\text{-}CO]^+$, 431 (64) [Gly-Pro-Ile-Tyr]$^+$, 403 (15) $[431\text{-}CO]^+$, 389 (38) [Tyr-Ala-Gly-Pro]$^+$, 377 (11) $[405\text{-}CO]^+$, 374 (76) [Pro-Ile-Tyr]$^+$, 361 (18) $[389\text{-}CO]^+$, 348 (59) [Ile-Tyr-Ala]$^+$, 346 (15) $[374\text{-}CO]^+$, 339 (49) [Ala-Gly-Pro-Ile]$^+$, 320 (16) $[348\text{-}CO]^+$, 311 (14) $[339\text{-}CO]^+$, 292 (61) [Tyr-Ala-Gly]$^+$, 277 (28) [Ile-Tyr]$^+$, 268 (46) [Gly-Pro-Ile]$^+$, 240 (13) $[268\text{-}CO]^+$, 235 (45) [Tyr-Ala]$^+$, 211 (39) [Pro-Ile]$^+$, 207 (11) $[235\text{-}CO]^+$, 198 (10) $[226\text{-}CO]^+$, 183 (10) $[211\text{-}CO]^+$, 155 (29) [Gly-Pro]$^+$, 136 (28) [Tyr immonium ion, $C_8H_{10}NO]^+$, 129 (19) [Ala-Gly]$^+$, 127 (10) $[155\text{-}CO]^+$, 107 (10) $[C_7H_7O]^+$, 93 (13) $[C_6H_5O]^+$, 86 (21) [Ile immonium ion, $C_5H_{12}N]^+$, 70 (38) [Pro immonium ion, $C_4H_8N]^+$, 57 (18) $[C_4H_9]^+$, 44 (18) [Ala immonium ion, $C_2H_6N]^+$, 30 (16) [Gly immonium ion, $CH_4N]^+$, 29 (12) $[C_2H_5]^+$, 17 (11) $[OH]^+$, 15 (24) $[CH_3]^+$; $C_{25}H_{35}N_5O_6$ (501): calcd. C 59.87, H 7.03, N 13.96; found C 59.88, H 7.05, N 13.95.

Pharmacology

Anthelmintic evaluation

Newly synthesized linear pentapeptide and pentacyclopeptide (V, VI) were subjected to anthelmintic activity studies against three earthworm species *Megascoplex konkanensis*, *Pontoscotex corethruses* and *Eudrilus eugeniea* at 2 mg/ml concentration (Garg and Atal, 1963). Tween 80 (0.5%) in distilled water was used as control and mebendazole was used as standard drug.

Antibacterial and antifungal evaluation

The synthesized linear pentapeptide and pentacyclopeptide (V, VI) were evaluated for their antimicrobial activity against against Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, the Gram-negative bacteria *Pseudomonas*

aeruginosa, *Klebsiella pneumonia*, dermatophytes *Microsporum audouinii*, *Trichophyton mentagrophytes*, diamorphic fungi *Candida albicans* and other fungal strains, including *Aspergillus niger* at 25-6 μg/ml concentration (Bauer *et al* 1966). MIC values of test compounds were determined by tube dilution technique. The Petri plates inoculated with bacterial cultures were incubated at 37°C for 18 h and those inoculated with fungal cultures were incubated at 37°C for 48 h. Gatifloxacin and griseofulvin were used as reference drugs and DMF/DMSO were used as control.

Experimental details of the biological activity studies are described in our previously published reports (Dahiya and Pathak, 2007c; Dahiya and Kaur, 2007b; 2008b; Dahiya, 2008e; Dahiya *et al* 2008; Dahiya and Mourya, 2012; 2013).

RESULTS AND DISCUSSION

The cyclopentapeptide molecule was split into two dipeptide units Boc-l-Ile-l-Tyr-OMe (I), Boc-l-Ala-Gly-OMe (II) and a single amino acid unit l-Pro-OMe·HCl (III). Dipeptide units (I, II) were prepared by coupling of Boc-amino acids like Boc-l-Ile-OH and Boc-l-Ala-OH with corresponding amino acid methyl ester hydrochlorides like l-Tyr-OMe·HCl and Gly-OMe·HCl (Bodanzsky and Bodanzsky, 1966).

After deprotection at the carboxy terminal, dipeptide (II) was coupled with amino acid unit (III), to get the tripeptide unit Boc-l-Ala-Gly-l-Pro-OMe (IV). The carboxyl group of tripeptide (IV) was removed by alkaline hydrolysis using lithium hydroxide (LiOH) and the deprotected peptide (IVa) was coupled with dipeptide unit (Ia), deprotected at amino terminal using trifluoroacetic acid (CF_3COOH), utilizing two different carbodiimides to get the linear pentapeptide unit Boc-l-Ala-Gly-l-Pro-l-Ile-l-Tyr-OMe (V). The methyl ester group of the linear peptide fragment was replaced by pentafluorophenyl (*pfp*) ester group. The Boc-group of resulting compound was removed using CF_3COOH and the deprotected linear fragment was now cyclized by keeping the whole contents at 0°C for 7 days in the presence of catalytic amounts of TEA or NMM or pyridine to get the cyclic product (VI) (**Figure 1**). The structure of the newly synthesized cyclooligopeptide as well as that of the intermediate di/tri/pentapeptides were confirmed by FT-IR, ^1H NMR spectroscopy and elemental analysis. In addition, mass spectra and ^{13}C NMR spectroscopy were recorded for the linear and cyclic pentapeptides.

The synthesis of cyclooligopeptide VI was accomplished with 87% yield, and pyridine proved to be an effective base for cyclization of the linear pentapeptide unit. Cyclization of the linear peptide fragment was supported by the disappearance of absorption bands at 1745, 1271 and 1389, 1367 cm^{-1} ($C{=}O_{str}$, $C{-}O_{str}$, ester and $C{-}H_{def}$, *tert*-butyl groups) in IR spectra of compound VI. The formation of the cyclopeptide was further confirmed by the disappearance of singlets at 3.54 and 1.54 ppm corresponding to three protons of the methyl ester group and nine protons of the *tert*-butyl group of Boc in the ^1H NMR spectrum and the disappearance of the singlets at 156.2, 79.8 and 52.5, 28.7 ppm corresponding to carbon atoms of ester and *tert*-butyl groups in the ^{13}C NMR spectrum of compound VI. Furthermore, the ^1H NMR and ^{13}C NMR spectra of the synthesized cyclic pentapeptide showed characteristic peaks confirming the presence of all the 35 protons and 25 carbon atoms. The appearance of the pseudomolecular ion peak $(M + 1)^+$ at m/z = 502 corresponding to the molecular formula $C_{25}H_{35}N_5O_6$ in the mass spectrum of VI, along with other fragment ion peaks resulting from cleavage at 'Pro-Gly', 'Gly-Ala', 'Tyr-Ile', 'Ala-Tyr' and 'Ile-Pro' amide bonds showed the exact sequence of the attachment of all the five amino acid moieties in a chain. In addition, the presence of the immonium ion peaks at m/z = 136 (Tyr), 86 (Ile), 70 (Pro), 44 (Ala) and 30 (Gly) further confirmed all the amino acid moieties in the cyclopeptide structure. Furthermore, the elemental analysis of cyclopeptide VI afforded values with tolerance of ±0.02 strictly in accordance with the molecular composition.

Biological activity

The anthelmintic activity results for cyclopeptide VI against three earthworm species *M. konkanensis*, *P. corethruses* and *E. eugeniea* at 2 mg/ml concentration using Garg's method are compiled in **Table I**. Moreover, antimicrobial activity results for compound VI against four bacteria *B. subtilis* and *S. aureus*, *P. aeruginosa* and *K. pneumonia*, cutaneous fungi *M. audouinii* and *T. mentagrophytes*, diamorphic fungi *C. albicans* and *A. niger* by disk diffusion method, are tabulated in **Table II**.

Comparison of antifungal activity data suggested that cyclooligopeptide VI possessed potent

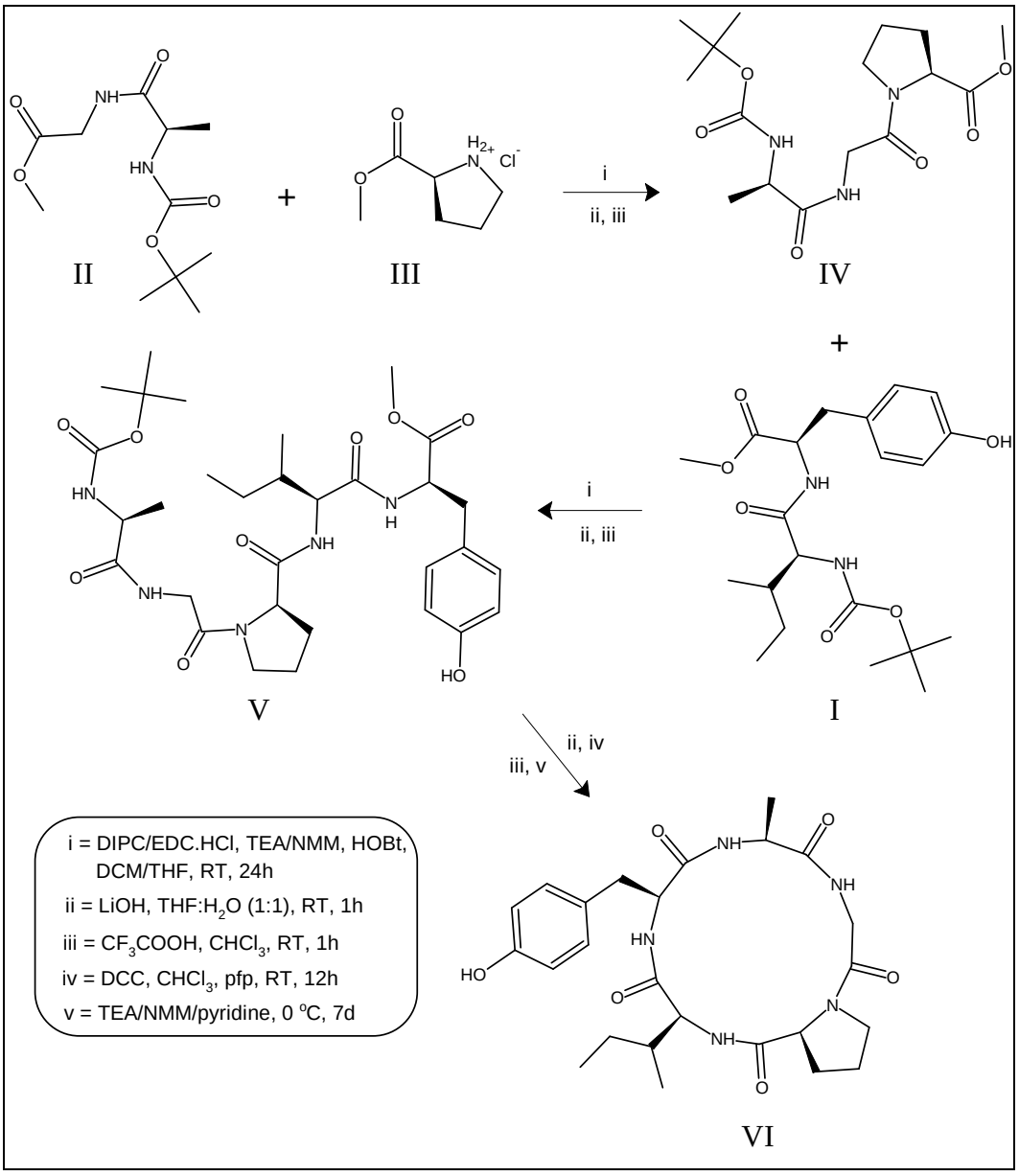

Figure 1. Synthetic route to the pentacyclopeptide (fanlizhicyclopeptide B) (VI)

Table I. Anthelmintic evaluation data for the linear and cyclopentapeptide (V, VI)

Compound	Earthworm species					
	M. konk.		***P. core.***		***E. euge.***	
	Mean paralyzing time (min)‡	Mean death time (min)‡	Mean paralyzing time (min)	Mean death time (min)	Mean paralyzing time (min)	Mean death time (min)
V	14.54 ± 0.20	22.58 ± 0.44	18.26 ± 0.42	28.55 ± 0.11	13.08 ± 0.34	24.38 ± 0.49
VI	10.16 ± 0.33	16.49 ± 0.52	13.46 ± 0.26	22.27 ± 0.17	10.49 ± 0.26	19.39 ± 0.41
Control#	–	–	–	–	–	–
Mebendazole	13.63 ± 0.33	22.43 ± 0.27	17.56 ± 0.49	29.49 ± 0.15	13.50 ± 0.39	24.07 ± 0.44

M. konk.: Megascoplex konkanensis, P. core.: Pontoscotex corethruses, E. euge.: Eudrilus eugeniea
‡ Data are given as mean ± S.D. (n = 3); # Tween 80 (0.5%) in distilled water

bioactivity against dermatophytes *M. audouinii* and *T. mentagrophytes* with MIC values of 6 μg/ml when compared to the reference drug griseofulvin. From the analysis of anthelmintic activity data, it is observed that the cyclooligopeptide VI displayed remarkable activity against all three earthworm species *M. konkanensis*, *P. corethruses* and *E. eugeniea*, in

comparison to standard drug mebendazole. Moreover, a moderate level of activity was observed against the Gram-negative bacteria *P. aeruginosa* and *Klebsiella pneumonia* for the newly synthesized cyclopeptide, in comparison to the standard drug gatifloxacin. However, compound VI displayed no significant activity against neither Gram-positive bacteria nor pathogenic *C. albicans* and *A. niger*. In addition, the analysis of the pharmacological activity data revealed that pentacyclopeptide VI displayed a higher bioactivity against pathogenic microbes and earthworms than its linear form V, which is due to fact that cyclization of peptides reduces the degree of freedom for each constituent within the ring and thus substantially leads to reduced flexibility, increased potency and selectivity of cyclic peptide.

Table II. Antimicrobial evaluation data for linear and cyclopentapeptide (V, VI)

Compound	Diameter of zone of inhibition (mm)							
	Bacterial strains				*Fungal strains*			
	B. sub.	*S. aur.*	*P. aeru.*	*K. pneu.*	*C. alb.*	*M. audo.*	*A. niger*	*T. menta.*
V	–	–	15(6)	18(6)	10(25)	16(6)	–	19(6)
VI	–	11(25)	19(6)	21(6)	14(25)	22(6)	–	23(6)
Control*	–	–	–	–	–	–	–	–
Gatifloxacin	18(12.5)†	27(6)	23(6)	25(6)	–	–	–	–
Griseofulvin	–	–	–	–	20(6)	18(6)	20(12.5)	19(6)

B. sub.: Bacillus subtilus, S. aur.: Staphylococcus aureus, P. aeru.: Pseudomonas aeruginosa, K. pneu.: Klebsiella pneumonia, C. alb.: Candida albicans, M. audo.: Microsporum audouinii, A. niger: Aspergillus niger, T. menta.: Trichophyon mentagrophytes
† Values in bracket are MIC values (μg/ml) * DMF / DMSO

CONCLUSION

The first total successful synthesis of the natural peptide fanlizhicyclopeptide B (VI) was accomplished with good yield *via* coupling reactions utilizing different carbodiimides. The DIPC / NMM coupling method proved to be yield-effectice, in comparison to methods utilizing EDC·HCl / DIPC and TEA, providing 8% additional yield. The pentafluorophenyl ester was shown to be better for the activation of the acid functionality of the linear pentapeptide unit. Pyridine was found to be a good base for the intramolecular cyclization of the linear peptide fragment in comparison to TEA or NMM. The synthesized pentacyclopeptide displayed potent anthelmintic activity and effectiveness against pathogenic dermatophtytes. In addition, Gram-negative bacteria were found to be more sensitive than Gram-positive bacteria towards the newly synthesized peptide. On passing toxicity tests, pentacyclopeptide VI may prove as a good candidate for clinical studies and can be a new anti-dermatophyte and anthelmintic drug of future.

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