Synthesis, Characterization and Biological Evaluation of Cyclic Peptides: Viscumamide, Yunnanin A and Evolidine

Boja Poojary[a] and S. L. Belagali[b]

[a] Department of Post-Graduate Studies and Research in Chemistry, Mangalore University, Mangalagangotri-574 199, Mangalore, India
[b] Department of Environmental Sciences, University of Mysore, Mysore-575 006, India

Reprint requests to Dr. B. Poojary. Fax: 91 824 2287 367. E-mail: bojapoojary@yahoo.com

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Three biologically active cyclic peptides, viscumamide, yunnanin A and evolidine were synthesized and the structures were established on the basis of analytical, IR, NMR and mass spectral data. These newly synthesized cyclic peptides were evaluated for antimicrobial and pharmacological activities. Evolidine showed better growth inhibition against bacterial strains than yunnanin A and viscumamide. The anti-inflammatory activity of viscumamide is moderate and the remaining two cyclic peptides are found to be less active. But, all the three cyclic peptides showed weak anthelmintic activity.

Key words: Cyclic Peptides, Viscumamide, Yunnanin A, Evolidine, Antimicrobial Activity

Introduction

In the past two decades, a wide variety of naturally occurring bioactive cyclic peptides have been isolated from plants, marine sponges and tunicates [1]. The wide spread increase of bacterial resistance towards conventional antibiotics encouraged the exploration of novel antimicrobial molecules with unexploited mechanisms of action. Initially discovered as a defensive system in invertebrates and vertebrates, antimicrobial peptides are attracting increased interest as potential therapeutics [2 – 4]. Unlike classical antibiotics, which must penetrate the target cell, the principle mode of action of peptides involves perturbation and permeability of the cell membrane. This mechanism confers activity towards a broad spectrum of microbial cells, but is also responsible for undesired lytic activity against mammalian cells such as erythrocytes [5 – 7].

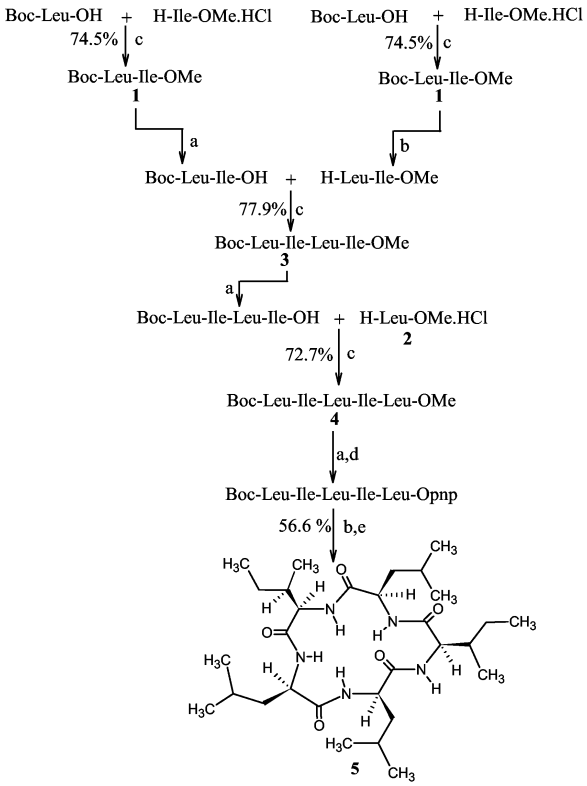
A cyclic pentapeptide, viscumamide was isolated from *Viscum album* Linn. *var. coloratum* Ohwi by Okumura and Sakurai [8] and the structure cyclo(-Leu-Ile-Leu-Ile-Leu-) was assigned to this substance on the basis of gas chromatographic studies on the products of its partial hydrolysis. They also attempted the synthesis of this cyclic peptide [9]. Itokawa *et al.* [10] isolated two novel cyclic peptides, named yunnanins A and B from *Stellaria yunnanensis franch* (Caryophyllaceae). The structures of these peptides were elucidated from spectroscopy and chemical degradations. As a part of the continuing studies in search of new bioactive cyclic peptides from higher plants, these authors have also isolated 16 novel cyclic peptides belonging to Caryophyllaceae family including Yunnanin A-C from the roots of *Stellaria yunnanensis* [11]. Some of the peptides showed tyrosinase inhibitory and cell growth inhibitory activities. The structures of these peptides were elucidated by extensive 2D NMR spectrometry, chemical degradation and ESI MS methods. To determine the importance of the specific functional groups for the overall conformation of cyclic peptides and to understand the relationships between chemical composition and three dimensional structure, conformational analysis of cyclic heptapeptides, yunnanins A and C were undertaken using X-ray crystallography, high field NMR and MD calculations. The solid and solution state conformations of isolated yunnanin A were also studied by Morita *et al*. [12] using X-ray diffraction and NMR techniques. Yunnanin A occurring in the roots of *Stellaria yunnanensis* was efficiently synthesized using a combination of solid and solution phase techniques [13]. The structural analysis on the synthesized peptide showed that the synthetic sample exhibited a configurational pattern at the proline peptide linkages identical to the natural product. The influence of different coupling reagents and metal ions in the promotion of the cyclization of linear

segment during the synthesis of yunnanin A was also studied [14 – 16]. Another cyclic heptapeptide [cyclo(-Ser-Phe-Leu-Pro-Val-Asn-Leu-)] was isolated by Eastwood *et al.* [17] from the leaves of *Evodia xanthoxyloides* and was named evolidine. The structure of this peptide was deduced from NMR studies [18]. The structure of evolidine was also confirmed by chemical degradation of the natural product and by synthesis [19]. The cyclic nature and the amino acid composition of evolidine were also investigated [20, 21]. Many authors also studied the conformation of evolidine by proton magnetic resonance and computational studies [22 – 25]. The structure assigned to the evolidine was also confirmed by its synthesis [26, 27].

As a part of our ongoing study on the synthetic and biological aspects of cyclic peptides of biological interest [28], an attempt was made towards the synthesis of viscumamide, a cyclic pentapeptide and two cyclic heptapeptides, yunnanin A and evolidine. The synthesized peptides were further subjected to antibacterial and pharmaceutical activity studies.

Results and Discussion

Retrosynthetically, we disconnected viscumamide into two dipeptide units of Boc-Leu-Ile-OMe (**1**) and a single amino acid unit, H-Leu-OMe·HCl (**2**). The required dipeptides were prepared by coupling Boc-L-amino acids with the respective L-amino acid ester hydrochlorides using DCC, HOBt and *N*-methyl morpholine according to the Bodanszky procedure [29] with suitable modifications [30]. The ester group of the dipeptide **1** was removed with LiOH and the Boc-group was removed with trifluoroacetic acid. Both the deprotected units were coupled to obtain the tetrapeptide, Boc-Leu-Ile-Leu-Ile-OMe (**3**). The resulting tetrapeptide was then condensed with H-Leu-OMe·HCl (**2**) to obtain the pentapeptide, Boc-Leu-Ile-Leu-Ile-Leu-OMe (**4**). The methyl ester group of **4** was deprotected with LiOH and *p*-nitrophenyl ester group (pnp) was introduced by treatment with *p*-nitrophenol in the presence of DCC. The Boc-group of resulting Boc-Leu-Ile-Leu-Ile-Leu-Opnp was removed by treatment with trifluoroacetic acid in chloroform. The solution of the Boc-deprotected pentapeptide *p*-nitrophenyl ester was diluted with chloroform and allowed to cyclize in the presence of pyridine [31] to obtain viscumamide (**5**) as depicted in Scheme 1.



Scheme 1. a = LiOH, THF:H_2O(1:1), RT/1 h; b = TFA, $CHCl_3$, RT/1 h; c = DCC, NMM, HOBt, DCM, RT/36 h; d = *p*-nitrophenol(pnp), DCC, $CHCl_3$, RT/12 h; e = pyridine, $CHCl_3$, 10 days/0 °C.

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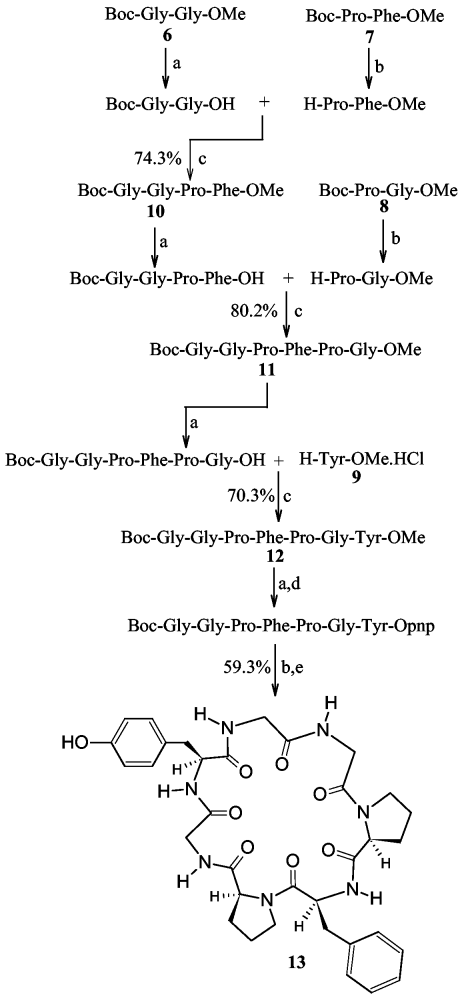
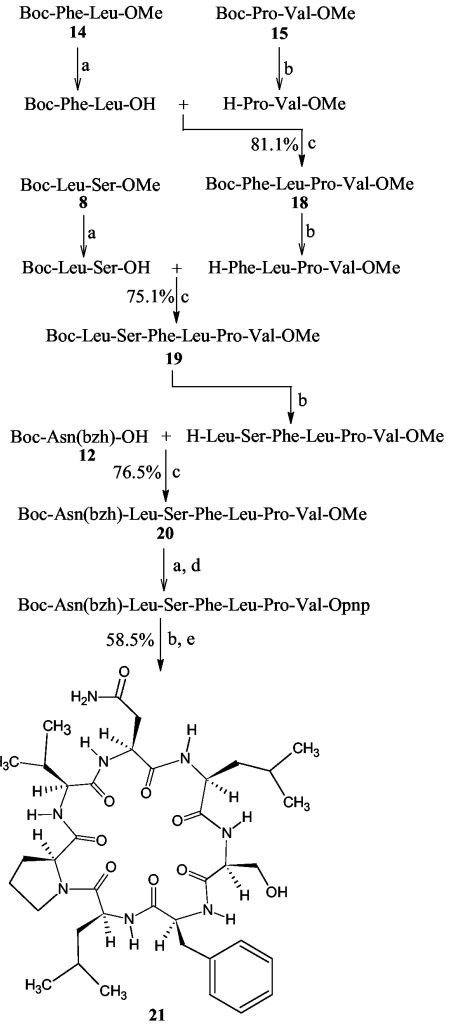
The synthesis of yunnanin A, cyclo(-Gly-Gly-Pro-Phe-Pro-Gly-Tyr-) was carried out from three dipeptide units, Boc-Gly-Gly-OMe (**6**), Boc-Pro-Phe-OMe (**7**), Boc-Pro-Gly-OMe (**8**) and a single amino acid unit H-Tyr-OMe·HCl (**9**). The dipeptides **6** and **7** were coupled after proper deprotection to get the tetrapeptide, Boc-Gly-Gly-Pro-Phe-OMe (**10**). The resulting tetrapeptide was then condensed with another dipeptide unit (**8**), after proper deprotection to yield the hexapeptide unit, Boc-Gly-Gly-Pro-Phe-Pro-Gly-OMe (**11**). It was then coupled with H-Tyr-OMe·HCl to obtain the heptapeptide, Boc-Gly-Gly-Pro-Phe-Pro-Gly-Tyr-OMe (**12**). Finally, the linear segment was cyclized to yield yunnanin A (**13**) as depicted in Scheme 2. In the same way, evolidine,[cyclo(-Leu-Ser-Phe-Leu-Pro-Val-Asn-)], was also disconnected into three dipeptide units of Boc-Phe-Leu-OMe (**14**), Boc-Pro-Val-OMe (**15**), Boc-Leu-Ser-OMe (**16**), and a single amino acid unit, Boc-Asn(bzh)-OH (**17**). The tetrapeptide unit, Boc-Phe-Leu-Pro-Val-OMe (**18**) was prepared as per the above procedure by condensing the dipeptides **14** and **15** after proper deprtection. The



Scheme 2. a = LiOH, THF:H_2O(1:1), RT/1 h; b = TFA, $CHCl_3$, RT/1 h; c = DCC, NMM, HOBt, DCM, RT/36 h; d = *p*-nitrophenol(pnp), DCC, $CHCl_3$, RT/12 h; e = pyridine, $CHCl_3$, 10 days/0 °C.



Scheme 3. a = LiOH, THF:H_2O(1:1), RT/1 h; b = TFA, $CHCl_3$, RT/1 h; c = DCC, NMM, HOBt, DCM, RT/36 h; d = *p*-nitrophenol(pnp), DCC, $CHCl_3$, RT/12 h; e = pyridine, $CHCl_3$, 10 days/0 °C.

tetrapeptide **18** was then condensed similarly with the remaining dipeptide unit **16** to yield the hexapeptide, Boc-Phe-Leu-Pro-Val-Leu-Ser-OMe (**19**). The benzhydryl(bzh) protection of the carboxamido group to yield Boc-Asn(bzh)-OH (**17**) was achieved by treating the Boc-asparagine with benzhydrol in acetic acid [29]. The Boc-group of the hexapeptide **19** was removed and then condensed with **17** to obtain Boc-Asn(bzh)-Phe-Leu-Pro-Val-Leu-Ser-OMe (**20**), the linear segment of evolidine. Finally, the cyclization of the linear segment (Scheme 3) was carried out by the *p*-nitrophenyl ester method to obtain evolidine **21**.

The intermediate and final products were purified by column chromatography using a chloroform-methanol system and recrystallized from EtOAc-petroleum ether. The newly synthesized compounds were analyzed for C,H,N and the structures were confirmed by IR, NMR and mass spectral data. The characteristic IR and NMR spectra of all the intermediate compounds were analyzed. The characteristic IR absorption bands of –CO-NH- moiety were present in the cyclized product. The NMR spectra of all cyclized products clearly indicate the presence of all respective amino acid moieties. Furthermore, the mass spectra of the cyclic peptides viscumamide, yunnanin A and evolidine showed the $[M^+ + H]$ peak at *m/z* 566, 676 and 771 which are consistent with their molecular for-

Table 1. Antimicrobial activities of viscumamide (**5**), yunnanin A (**13**), and evolidine (**21**).

Compound	Diameter of zone of inhibition (mm)					
	Antibacterial activity studies			Antifungal activity studies		
	P.aer	*E.coli*	*B.sub*	*S.aur*	*C.alb*	*A.niger*
Viscumamide (**5**)	10	08	12	08	10	10
Yunnanin A (**13**)	08	11	14	–	08	08
Evolidine (**21**)	12	–	10	18	10	14
Penicillin	12	12	18	18	–	–
Greseofulvin	–	–	–	–	20	20

mulae, $C_{30}H_{55}N_5O_5$, $C_{34}H_{41}N_7O_8$ and $C_{38}H_{58}N_8O_9$, respectively.

Biological Activity Studies

The synthesized cyclic peptides, viscumamide (**5**), yunnanin A (**13**) and evolidine (**21**) were also screened for their antibacterial, antifungal, antiinflammatory and anthelmintic activity. The antibacterial and antifungal activities were determined against four bacterial (*S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli*) and two fungal strains (*C. albicans* and *A. niger*). These activity studies were carried out according to the disc diffusion method [32]. Penicillin and griseofulvin were used as standards against bacteria and fungal strains at 10 and 25 μg/disc respectively. The results are summarized in Table 1. The antibacterial screening results indicate that viscumamide and yunnanin A are less active against all tested bacterial and fungal strains. However, evolidine is as active as the standard drug against the bacterial strains *P. aeruginosai* and *S. aureus* but only moderately active against the remaining two bacterial strains. It is also less active against fungal strains. The anti-inflammatory activity tests were carried out according to the method of Winter *et al.* [33] using Ibuprofen as the standard and the results are presented in Table 2. The anti-inflammatory data reveal that viscumamide is moderately active, but evolidine and yunnanin A are inactive as compared to the standard drug used for screening studies. The anthelmintic activity was carried out against the earthworms (*Pontoscotex corethruses*) according to Garg's method [34] (Table 3) using mebendazole as standard drug. All the three cyclic peptides are found to be less active when compared to the standard.

The synthesis of a cyclic pentapeptide and two cyclic heptapeptides was achieved in fairly good yields in a simple solution phase method. The yunnanins and evolidine were found to possess tyrosinase and growth inhibitory activities. But, the antibacterial, antifungal, anti-inflammatory and anthelmintic activity studies on these cyclic peptides are not reported in the literature. Hence the synthetic cyclic peptides are screened for these activities. The screening data of these compounds in the present study showed some promising results.

Table 2. Antiinflammatory activities of viscumamide (**5**), yunnanin A (**13**), and evolidine (**21**).

Compound	Increase in paw volume after 3 h ± S E (ml)	Percentage of inhibition of oedema after 3 h ± S E
Viscumamide (**5**)	0.68 ± 0.01	24.44
Yunnanin A (**13**)	0.86 ± 0.02	9.09
Evolidine (**21**)	0.73 ± 0.04	18.88
Ibuprofen	0.55 ± 0.03	40.50
Control	0.90 ± 0.02	–

Table 3. Anthelmintic activities of viscumamide (**5**), yunnanin A (**13**), and evolidine (**21**).

Compound	Conc of the Compd (mg)	Mean paralyzing time (min) ± S E	Mean death time (min) ± S E
Viscumamide (**5**)	100	60.00 ± 1.58	136.44 ± 2.08
	200	51.50 ± 2.02	120.04 ± 1.60
Yunnanin A (**13**)	100	82.51 ± 1.05	122.0 ± 1.02
	200	74.00 ± 1.10	102.6 ± 1.09
Evolidine (**21**)	100	99.84 ± 2.10	130.81 ± 2.04
	200	89.75 ± 1.90	115.32 ± 2.03
Mebendazole	100	18.01 ± 2.01	55.20 ± 2.00
	200	12.55 ± 1.02	32.01 ± 1.10
Control	–	–	–

Experimental Section

Melting points were taken in open capillary and are uncorrected. IR spectra (in $CHCl_3$) were recorded on a Perkin-Elmer infrared spectrophotometer. NMR spectra were recorded in $CHCl_3$/DMSO-d_6 on a 300 MHz spectrophotometer using TMS as an internal standard. The mass spectra were recorded on a FAB mass spectrometer. TLC checked the progresses of the reactions on silica gel G plates and the products were purified by silica gel column chromatography.

Procedure for the preparation of Boc-Asn(bzh)-OH (**12**)

Boc-asparagine (232 g, 10 mmol) was dissolved in acetic acid (25 ml) and benzhydrol (1.84 g, 10 mmol) was added with stirring at R.T. To the resulting mixture 0.05 ml of

concentrated sulphuric acid was added and allowed to stand overnight. The reaction mixture was poured into water (75 ml). The precipitate formed was filtered. It was dissolved in ethyl acetate, washed with water (20 ml), dried over anhydrous sodium sulphate. And ethyl acetate was removed by distillation under reduced pressure to obtain Boc-Asn(bzh)-OH. M. p. 145-146 °C (Lit. [23] 145 – 146 °C); yield 72.4%.

General procedure for the preparation of dipeptides

Amino acid methyl ester hydrochloride (10 mmol) was taken in dichloromethane (20 ml) and triethylamine (4 ml, 28.7 mmol) was added at 0 °C. The reaction mixture was stirred for 15 min and Boc-amino acid (10 mmol) in dichloromethane (20 ml) was added. DCC (2.09 g, 10 mmol) and HOBt (2.34 g, 12 mmol) were added with stirring. After 24 h, the reaction mixture was filtered and the residue was washed with dichloromethane (30 ml) and added to the filtrate. The filtrate was washed with 5% HCl (20 ml), 5% $NaHCO_3$ (20 ml), and saturated NaCl (20 ml) solutions. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum. To remove trces of the dicyclohexylurea, the product was dissolved in minimum amount of chloroform and cooled to 0 °C. The recrystallized dicyclohexylurea was removed by filtration. To the filtrate petroleum ether was added at 0 °C to recrystallize the pure product.

Boc-Leu-Ile-OMe (**1**)

IR ($CHCl_3$): $\nu = 3340$ (br.s, N-H str.), 3250 (br.s, N-H str.), 2940 (s, C-H str.), 1760 (s, C=O str. ester), 1685 (s, C=O str. amide), 1520 (s), 1450 (s), 1370 (s), 1250 (s), 1140 (s), 1070 (s), 1000 (s), 860 (s) cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.65$ (br.s, 1H, NH), 5.7 – 5.5 (br.s, 1H, -NH), 4.4 – 4.3 (m,1H, α-CH), 4.2 – 4.1 (m,1H, α-CH), 3.7 (s, 3H, O-CH_3), 2.0 – 1.7 (m, 3H, β-CH_2 and β-CH), 1.4 (s, 9H, $C(CH_3)_3$), 1.3 – 1.1 (m, 3H, γ-CH_2 and γ-CH), 0.95 (doublet overlapped with a triplet, 12H, -$C(CH_3)_2$ and –CH_3). – $C_{18}H_{34}N_2O_5$ (358): calcd. C 60.28, H 9.49, N 7.81; found C 60.38, H 9.42, N 7.93.

General procedure for the preparation of tetrapeptides

Deprotection of the carboxyl group of Boc-dipeptide-methyl ester

To a solution of the Boc-dipeptide-methyl ester (1 mmol) in tetrahydrofuran:water(1:1) (36 ml), lithium hydroxide (0.041 g, 1.5 mmol) was added at 0 °C. The mixture was stirred for 1 h at R.T. and then acidified to ph 3.5 with 1N H_2SO_4. The aqueous layer was extracted with ether (3×15 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated in vacuum to yield Boc-dipeptide.

Deprotection of the amino group of Boc-dipeptide-methyl ester

The protected dipeptide (1 mmol) was dissolved in chloroform (15 ml) and treated with trifluoroacetic acid (0.228 g, 2 mmol). The solution was stirred at R.T. for an hour, washed with saturated $NaHCO_3$ solution (5 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuum to obtain dipetide methyl ester. The product was purified by recrystallization from chloroform and petroleum ether.

Preparation of tetrapeptides

The Boc-dipeptide (1 mmol) and the dipeptide methyl ester (1 mmol) were dissolved in dichloromethane (15 ml) and triethylamine (0.4 ml) was added. The solution was cooled to 0 °C and DCC (0.216 g, 1 mmol) and HOBt (0.153 g, 1 mmol) were added and stirred for 24 h. The separated dicyclohexylurea was removed by filtration. The filtrate was washed with 10% citric acid solution (2 ml), again with 5% $NaHCO_3$ solutin (2 ml) and water (2 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness in vacuum. The residue was triturated with hexane, filtered, washed with hexane and dried. The sample was purified by silica gel column chromatography using dichloromethane-methanol system and finally recrystallized from chloroform and petroleum ether.

Boc-Leu-Ile-Leu-Ile-OMe (**3**)

IR ($CHCl_3$): $\nu = 3400$ (br.s, N-H str.), 3270 (br.s, N-H str.), 2960 (s, C-H str.), 2940 (s, C-H str.), 1750 (s, C=O str. ester), 1690 (s, C=O str. amide), 1660 (s, C=O str. amide), 1520 (m), 1430 (s), 1310 (s), 1155 (s), 1010 (s), 860 (s) cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$): $\delta = 6.4$ (br.s, 1H, NH), 5.8 (br.s, 3H, NH), 4.6 – 4.3 (m, 4H, α-CH), 3.7 (s, 3H, O-CH_3), 2.1 – 1.6 (m, 6H, β-CH_2 and β-CH), 1.45 (s, 9H, $C(CH_3)_3$), 1.3 – 1.1 (m, 6H, γ-CH_2 and γ-CH), 1.05 (doublet overlapped with a triplet, 24H, -$(CH_3)_2$ and –CH_3), – $C_{30}H_{56}N_4O_7$(568): calcd. C 63.30, H 9.85, N 9.85; found C 63.42, H 9.78, N 10.00.

Boc-Gly-Gly-Pro-Phe-OMe (**10**)

IR ($CHCl_3$): $\nu = 3310$ (br.s, N-H str.), 3010 (m, C-H str.), 2950 (s, C-H str.), 2850 (s, C-H str.), 1720 (s, C=O str. ester), 1690 (s, C=O str. amide), 1670 (s, C=O str. amide), 1650 (s, C=O str. amide), 1510 (s), 1310 (m), 1220 (s), 1140 (s), 1080 (s), 970 (s) cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.0$ (br.s, 2H, NH), 7.8 (br.s, 1H, NH), 7.4 – 7.1 (m, 5H, Ar-H), 4.5 – 4.3 (m, 2H, α-CH), 4.2 – 3.9 (m, 4H, α-CH_2), 3.7 (s, 3H, -O-CH_3), 3.6 – 3.4 (m, 2H, -N-CH_2), 3.3 – 3.1 (m, 2H, β-CH_2), 2.2 – 1.8 (m, 4H, -CH_2-CH_2-), 1.45 (s, 9H, $C(CH_3)_3$). – $C_{24}H_{34}N_4O_7$ (490): calcd. C 58.88, H 6.94, N 11.43; found C 58.88, H 6.88, N 11.53.

Boc-Phe-Leu-Pro-Val-OMe (**18**)

IR ($CHCl_3$): ν = 3327 (br.s, N-H str.), 3030 (m = C-H str.), 2970 (s, C-H str.), 2851 (s, C-H str.), 1720 (br.s, C=O str. ester), 1680 (s, C=O str. amide), 1660 (s, C=O str. amide), 1622 (s, N-H def.), 1574 (s), 1510 (s), 1430 (s), 1310 (s), 1240 (s), 1150 (s), 892 (s) cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$): δ = 8.2 (br.s, 1H, NH), 7.9 (br.s, 1H, NH), 7.5 – 7.3 (m, 5H, Ar-H), 6.9 (br.s, 1H, NH), 4.6 – 4.35 (m, 2H, α-CH), 4.3 – 4.1 (m, 2H, α-CH), 3.75 (s, 3H, O-CH_3), 3.6 – 3.3 (m, 2H, -N-CH_2), 3.1 – 2.8 (m, 2H, β-CH_2), 2.4 – 1.8 (m, 5H, -CH_2-CH_2- & β-CH), 1.45 (s, 9H, C(CH_3)$_3$), 1.3 – 1.2 (1H, m, γ-CH), 1.0 (d, J = 6.5 Hz, 12H, C(CH_3)$_2$). – $C_{31}H_{48}N_4O_7$ (588): calcd. C 63.27, H 8.16, N 9.52; found C 63.20, H 8.28, N 9.63.

Procedure for the preparation of pentapeptide, Boc-Leu-Ile-Leu-Ile-Leu-OMe (**4**)

The methyl ester group of Boc-Leu-Ile-Leu-Ile-OMe (**3**) was removed using aqueous LiOH and then condensed with H-Leu-OMe·HCl to obtain the pentapetide **4** according to the procedure employed for the preparation of tetrapeptides.

IR ($CHCl_3$): ν = 3450 (br.s, N-H str.), 3300 (br.s, N-H str.), 2980 (s, C-H str.), 2890 (s, C-H str.), 1760 (s, C=O str. ester), 1685 (s, C=O str. amide), 1670 (s, C=O str. amide), 1530 (m), 1420 (s), 1300 (s), 1160 (s), 1000 (br.s), 870 (s), 720 (s) cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$): δ = 6.6 (br.s, 1H, NH), 6.0 (br.s, 1H, NH), 5.7 (br.s, 3H, NH), 4.6 – 4.4 (m, 2H, α-CH), 4.3 – 4.1(m, 3H, α-CH), 3.65 (s, 3H, OCH_3), 2.2 – 1.8 (m, 8H, β-CH_2 and β-CH), 1.45 (s, 9H, C(CH_3)$_3$), 1.3 – 1.1 (m, 7H, γ-CH_2 and γ-CH), 0.90 (doublet overlapped with a triplet, 30H, -(CH_3)$_2$ and –CH_3). – $C_{36}H_{67}N_5O_8$ (697): calcd. C 61.90, H 9.68, N 10.10; found C 61.82, H 9.60, N 9.94.

Procedure for the preparation of hexapeptide, Boc-Gly-Gly-Pro-Phe-Pro-Gly-OMe (**11**)

The methyl ester group of Boc-Gly-Gly-Pro-Phe-OMe (**10**) and the Boc group of the dipeptide Boc-Pro-Gly-OMe (**8**) were removed using aqueous LiOH and CF_3CO_2H respectively. The resulting deprotected fragments were then condensed to get the hexapeptide **11** according to the procedure employed for the preparation of tetrapeptides.

IR ($CHCl_3$): ν = 3320 (br.s, N-H str.), 3005 (m,=C-H str.), 2960(s, C-H str.), 2840 (s, C-H str.), 1730 (s, C=O str. ester), 1690 (s, C=O str. amide), 1680 (s, C=O str. amide), 1670 (s, C=O str. amide), 1620 (s, N-H def.), 1520 (s, C-N str.), 1310 (s, C-H def.), 1220 (s, C-O str.), 1170 (s, C-O str.), 1080 (s, C-H def.), 980 (s, C-H def.) cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$): δ = 8.3 (br.s, 2H, NH), 8.0 (br.s, 2H, NH), 7.4 – 7.2 (m, 5H, Ar-H), 4.6 – 4.3 (m, 3H, α-CH), 4.2 – 4.0 (m, 6H, α-CH_2), 3.75 (s, 3H, -OCH_3), 3.4 – 3.2 (m, 6H, N-CH_2 and β-CH_2), 2.3 – 2.0 (m, 8H, -CH_2-CH_2), 1.45 (s, 9H, -C(CH_3)$_3$). – $C_{31}H_{44}N_6O_9$ (644): calcd. C 57.76, H 6.83, N 13.04; found C 57.86, H 6.95, N 12.96.

Procedure for the preparation of hexapeptide, Boc-Leu-Ser-Phe-Leu-Pro-Val- OMe (**19**)

The methyl ester group of the dipeptide, Boc-Leu-Ser-OMe (**8**) and the Boc group of the tetrapeptide, Boc-Phe-Leu-Pro-Val-OMe (**18**) were removed using aqueous LiOH and CF_3CO_2H respectively. The resulting deprotected fragments were then condensed to get the hexapeptide **19** according to the procedure employed for the preparation of tetrapeptides.

IR ($CHCl_3$): ν = 3600 (br.s, O-H str.), 3330 (br.s, N-H str.), 3060 (m,=C-H str.), 3010 (s, =C-H str.), 2940 (s, C-H str.), 2880 (s, C-H str.), 1740 (s, C=O str. ester), 1695 (s, C=O str. amide), 1680 (s, C=O str. amide), 1660 (s, C=O str. amide), 1602 (s, N-H def.), 1574 (s), 1520 (s, C-N str.), 1310 (s, C-H def.), 892 (s, C-H def.) cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$): δ = 8.3 (br.s, 2H, NH), 7.9 (br.s, 1H, NH), 7.4 – 7.1 (m, 5H, Ar-H), 6.8 (br.s, 2H, N-H), 5.1 (s, 1H, O-H), 4.7 – 4.6 (m, 1H, α-CH), 4.5 – 4.4 (m, 4H, α-CH), 4.3 – 4.0 (m, 3H, α-CH and β-CH_2), 3.75 (s, 3H, -OCH_3), 3.6 – 3.3 (m, 2H, N-CH_2), 3.2 – 2.8 (m, 2H, β-CH_2), 2.4 – 2.0 (m, 4H, -CH_2-CH_2), 1.9 – 1.6 (m, 5H, β-CH_2 and β-CH), 1.45 (s, 9H, -C(CH_3)$_3$), 1.3 – 1.15 (m, 2H, γ-CH), 0.95 (d, J = 6.5 Hz, 18H, -C(CH_3)$_2$). – $C_{40}H_{64}N_6O_{10}$ (788): calcd. C 60.91, H 8.12, N 10.66; found C 60.98, H 8.2, N 10.77.

Procedure for the preparation of heptapeptide, Boc-Gly-Gly-Pro-Phe-Pro-Gly-Tyr-OMe (**12**)

The methyl ester group of Boc-Gly-Gly-Pro-Phe-Pro-Gly-OMe (**11**) was removed using aqueous LiOH and then condensed with H-Tyr-OMe·HCl to obtain the heptapetide **12** according to the procedure employed for the preparation of tetrapeptides.

IR ($CHCl_3$): ν = 3600 (br.s, N-H str.), 3315 (br.s, N-H str.), 3080 (m,=C-H str.), 2970 (s, C-H str.), 2860 (s, C-H str.), 1725 (s, C=O str. ester), 1690 (s, C=O str. amide), 1685 (s, C=O str. amide), 1680 (s, C=O str. amide), 1675 (s, C=O str. amide), 1620 (s, N-H def.), 1520 (s, C-N str.), 1320 (s, C-H def.), 1220 (s, C-O str.), 1140 (s, C-O str.), 1080 (s, C-H def.), 970 (s, C-H def.) cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$): δ = 8.4 (br.s, 4H, NH and OH), 8.1 (br.s, 2H, NH), 7.4 – 7.1 (m, 7H, Ar-H and Ar^1-H), 6.9 (2H, d, J = 8.0 Hz, Ar^1-H), 4.5 – 4.4 (m, 4H, α-CH), 4.2 – 4.0 (m, 6H, α-CH_2), 3.7 (s, 3H, -OCH_3), 3.5 – 3.2 (m, 4H, N-CH_2), 3.1 – 2.8 (m, 4H, β-CH_2), 2.2 – 1.9 (m, 8H, -CH_2-CH_2), 1.4 (s, 9H, -C(CH_3)$_3$). – $C_{40}H_{53}N_7O_{11}$(807): calcd. C 59.48, H 6.57, N 12.14; found C 59.06, H 6.72, N 12.06.

Procedure for the preparation of heptapeptide, Boc-Asn(bzh)-Leu-Ser-Phe-Leu-Pro-Val-OMe (**20**)

The Boc-group of Boc-Leu-Ser-Phe-Leu-Pro-Val-OMe (**19**) was removed using aqueous CF_3CO_2H and then condensed with Boc-Asn(bzh)-OH (**12**) to obtain the heptapetide **20** according to the procedure employed for the preparation of tetrapeptides.

IR ($CHCl_3$): $\nu = 3610$ (br.s, O-H str.), 3320 (br.s, N-H str.), 3070 (m,=C-H str.), 3010 (s, =C-H str.), 2940 (s, C-H str.), 2860 (s, C-H str.), 1730 (s, C=O str. ester), 1695 (s, C=O str. amide), 1680 (s, C=O str. amide), 1660 (s, C=O str. amide), 1612 (s, N-H def.), 1574 (s, N-H def.), 1510 (s, C-N str.), 1310 (s, C-H def.), 992 (s, C-H def.) cm^{-1}. – ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.2$ (br.s, 3H, NH), 7.9 (br.s, 2H, NH), 7.5 – 7.1 (m, 15H, Ar-H), 6.8 (br.s, 1H, N-H), 6.0 (br.s, 1H, NH), 5.1 (s, 1H, O-H), 4.7 – 4.4 (m, 6H, α-CH), 4.3 – 4.1 (m, 3H, α-CH), 3.68 (s, 3H, OCH_3), 3.6 – 3.3 (m, 4H, N-CH_2 and β-CH_2), 3.2 – 2.8 (m, 2H, β-CH_2), 2.4 – 2.2 (m, 5H, -CH_2-CH_2 and Ar_2CH), 1.9 – 1.6 (m, 5H, β-CH_2 and β-CH), 1.45 (s, 9H, -$C(CH_3)_3$), 1.3 – 1.1 (m, 2H, γ-CH), 1.0 (d, $J = 6.5$ Hz, 6H, -$C(CH_3)_2$), 0.95 (d, $J = 6.8$ Hz, 12H, -$C(CH_3)_2$). – $C_{57}H_{80}N_8O_{12}$ (1068): calcd. C 64.04, H 7.49, N 10.49; found C 64.28, H 7.32, N 10.60.

General procedure for the preparation of cyclic peptides, viscumamide (**5**), *yunnanin A* (**13**) *and evolidine* (**21**)

The methyl ester group of the linear segment of Boc-(penta/heptapeptide)-OMe was removed using aqueous LiOH as explained earlier to yield Boc-(penta/heptapeptide)-OH. The resulting Boc-peptide-OH (1.5 mmol) was dissolved in chloroform (15 ml) at 0 °C. Then *p*-nitrophenol (0.27 g, 2 mmol) and DCC (0.31 g, 1.5 mmol) were added. The resulting mixture was stirred at R.T. for 12 h. The reaction mixture was filtered and the filtrate was washed with 10% $NaHCO_3$ solution until the excess of p-nitrophenol was removed and finally washed with 5% HCl (5 ml) to get Boc-peptide-*p*-nitrophenyl ester.

To the solution of Boc-(penta/heptapeptide)-*p*-nitrophenyl ester (1.2 mmol) in chloroform (25 ml), trifluoroacetic acid (0.274 g, 2.4 mmol) was added, stirred for 1 h at R.T. and washed with 10% sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulphate. To the resulting Boc-deprotected peptide-pnp ester in THF(25 ml), pyridine(1.4 ml, 2 mmol) was added and kept at 4 °C for seven days. The reaction mixture was washed with 10% sodium bicarbonate solution until the byproduct *p*-nitrophenol was removed completely and finally washed with 5% HCl (5 ml). The organic layer was dried over anhydrous sodium sulphate. THF and pyridine were distilled under reduced pressure to get cyclic peptide. The crude product was purified by silica gel column chromatography using the dichloromethane-methanol system and finally recrystallized from EtOAc-*n*-hexane.

Viscumamide (**5**)

M. p. 347 – 349 °C (Lit. [8] 349.5 – 351 °C). – IR ($CHCl_3$): $\nu = 3340$ (br.s, N-H str.), 3160 (br.s, N-H str.), 2960 (s, C-H str.), 2820 (s, C-H str.), 1670 (s, C=O str. amide), 1630 (s, C=O str. amide), 1490 (s, N-H def.), 1450 (s, C-H def.), 1100 (m, C-H def.) cm^{-1}. – ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 6.9$ (br.s, 3H, NH), 6.1 (br.s, 2H, NH), 4.7 – 4.4 (m,3H, α-CH), 4.3 – 4.1 (m, 2H, α-CH), 2.3 – 1.9 (m, 8H, β-CH_2 and β-CH), 1.45 – 1.15 (m, 7H, γ-CH_2 and γ-CH), 0.95 (doublet overlapped with triplet, 30H, -$C(CH_3)_2$ and CH_3). – ^{13}C NMR (DMSO-d_6): $\delta = 175.2$ (s, C=O), 174.6 (s, C=O), 174.4 (s, C=O), 60.5 (d, α-CH), 60.3 (d, α-CH), 60.2 (d, α-CH), 59.8 (d, α-CH), 59.5 (d, α-CH), 40.6 (t, β-CH_2), 40.4 (t, β-CH_2), 40.3 (t, β-CH_2), 39.7 (t, β-CH), 39.6 (d, β-CH), 25.7 (d, γ-CH), 25.4 (d, γ-CH), 25.1 (d, γ-CH), 24.9 (t, γ-CH_2), 24.7 (t, γ-CH_2), 23.3 (q, -CH_3), 23.0 (q, -CH_3), 22.7 (q, -CH_3), 22.6 (q, -CH_3), 22.4 (q, -CH_3), 19.5 (q, -CH_3), 19.2 (q, -CH_3). – FAB mass: *m/z* = 566 (m^+ + H, 40%), 523 (15%), 509 (45%), 454 (15%), 439 (90%), 410 (15%), 344 (5%), 325 (87%), 296 (34%), 232 (20%), 211 (12%), 184 (17%), 86 (10%), 57 (23%), 43 (25%). – $C_{30}H_{55}N_5O_5$ (565): calcd. C 64.29, H 9.73, N 12.39; found C 64.32, H 9.81, N 12.32.

Yunnanin A (**13**)

M. p. 235 – 237 °C. – IR($CHCl_3$): $\nu = 3600$ (br.s, O-H str.), 3400 (br.s, N-H str.), 3025 (m = C-H str.), 2945 (s, C-H str.), 2855 (s, C-H str.), 1680 (s, C=O str. amide), 1650 (s, C=O str. amide), 1630 (s, C=O str. amide), 1605 (s, N-H def.), 1520 (s, C-N str.), 1355 (s, C-H def.), 1090 (s, C-H def.), 1015 (s, C-H def.), 910 (s, C-H def.) cm^{-1}. – ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.6$ (br.s, 2H, NH), 8.4 (br.s, 2H, NH and OH), 7.9 (br.s, 2H, NH), 7.5 – 7.1 (m, 7H, Ar-H and Ar^1-H), 6.9 (d, 2H, $J = 8$ Hz), 4.6 – 4.4 (m, 4H, α-CH), 4.2 – 4.0 (m, 6H, α-CH_2), 3.6 – 3.4 (m, 4H, N-CH_2), 3.2 – 2.8 (m, 4H, β-CH_2), 2.2 – 1.8 (m, 8H, -CH_2-CH_2-). – ^{13}C NMR (DMSO-d_6): $\delta = 173.7$ (s, C=O), 173.1 (s, C=O), 172.6 (s, C=O), 171.1 (s, C=O), 170.8 (s, C=O), 170.4 (s, C=O), 168.4 (s, C=O), 155.2 (s, Ar^1-4-C), 138.6 (s, Ar-1-C), 130.4 (d, Ar^1-2,6-C), 129.3 (s, Ar^1-1-C), 128.9 (d, Ar-2,6-C), 128.2 (d, Ar-3,5-C), 126.3 (d, Ar-4-C), 114.3 (d, Ar^1-3,5-C), 61.9 (d, α-CH), 61.2 (d, α-CH), 60.5 (d, α-CH), 56.7 (d, α-CH), 55.0 (d, α-CH), 50.4 (d, α-CH), 44.2 (d, α-CH), 61.0 (t, β-CH_2), 58.4 (d, α-CH), 55.4 (d, α-CH), 53.9 (d, α-CH), 51.3 (d, α-CH), 50.4 (d, α-CH), 48.3 (t, δ(N)-CH_2), 46.8 (t, δ(N)-CH_2), 44.2 (t, α-CH_2), 42.9 (t, α-CH_2), 40.0 (t, α-CH_2), 38.0 (t, β-CH_2), 37.4 (t, β-CH_2), 29.8 (t, β-CH_2), 28.7 (t, β-CH_2), 25.7 (t, γ-CH_2), 25.3 (t, γ-CH_2). – FAB mass : *m/z* = 676 [M^+ + H].

– $C_{34}H_{41}N_7O_8$(675): calcd. C 60.45, H 6.07, N 14.52; found C 60.72, H 6.15, N 14.38.

Evolidine (**16**)

M.p. 276 – 27 °C (Lit. [11] 278 °C). – IR ($CHCl_3$): ν = 3600 (br.s, O-H str.), 3420 (br.s, N-H str.), 3050 (m,=C-H str.), 3010 (m,=C-H str.), 2950 (s, C-H str.), 2825 (s, C-H str.), 1690 (s, C=O str. amide), 1680 (s, C=O str. amide), 1650 (s, C=O str. amide), 1610 (s, N-H def.), 1605 (s, N-H def.), 1510 (s, C-N str.), 1445 (s, C-H def.), 1050 (s, C-H def.), 915 (s, C-H def.) cm^{-1}. – ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.2 (br.s, 3H, NH), 7.8 (br.s, 1H, NH), 7.4 – 7.1 (m,5H, Ar-H), 6.9 (1H, br.s, NH), 6.8 (br.s, 2H, NH_2), 6.0 (br.s, 1H, NH), 5.15 (s, 1H, -OH), 4.6 – 4.4 (m, 6H, α-CH and β-CH_2), 4.3 – 4.0 (m, 3H, α-CH), 3.6 – 3.2 (m, 4H, N-CH_2 and β-CH_2(Asn)), 3.1 – 2.9 (m, 2H, β-CH_2), 2.3 – 1.9 (m, 4H, -CH_2-CH_2-), 1.8 – 1.5 (m, 5H, β-CH_2 and β-CH), 1.4 – 1.2 (m, 2H, γ-CH), 0.95 (d, J = 6.5 Hz, 6H, -$C(CH_3)_2$), 0.90 (d, J = 6.8 Hz, 12H, -$C(CH_3)_2$). – ^{13}C NMR(DMSO-d_6): δ = 173.0 (s, C=O), 172.7 (s, C=O), 172.0 (s, C=O), 171.5 (s, C=O), 171.1 (s, C=O), 170.9 (s, C=O), 170.5 (s, C=O), 157.2 (s, C=O), 138.6 (s, Ar-1-C), 128.9 (d, Ar-2,6-C), 128.2 (d, Ar-3,5-C), 126.3 (d, Ar-4-C), 62.8 (t, β-CH_2), 60.5 (d, α-CH), 58.2 (d, α-CH), 57.1 (d, α-CH), 55.8 (d, α-CH), 54.9 (d, α-CH), 53.3 (d, α-CH), 53.1 (d, α-CH), 51.0 (t, β-CH_2), 46.9 (t, δ(N)-CH_2), 40.0 (t, β-CH_2), 39.4 (t, β-CH_2), 37.5 (t, β-CH_2), 31.6 (t, β-CH_2), 29.3 (d, β-CH), 28.8 (t, β-CH_2), 25.7 (t, γ-CH), 25.5 (t, γ-CH), 19.0 (q, -CH_3), 18.5 (q, -CH_3), 17.8 (q, -CH_3), 17.6 (q, -CH_3), 17.2 (q, -CH_3), 17.1 (q, -CH_3). – FAB mass : m/z = 771 [M^+ + H]. – $C_{38}H_{58}N_8O_9$ (770): calcd. C 59.22, H 7.53, N 14.55; found C 59.34, H 7.62, N 14.70.

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