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Short communication

Synthetic studies on cyclic octapeptides: Yunnanin F and Hymenistatin

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Abstract

Two biologically active cyclic peptides, Yunnanin F **8** and Hymenistatin **16** were synthesized and the structures were established on the basis of analytical, IR, NMR and mass spectral data. The newly synthesized compounds were screened for their antimicrobial and pharmacological activities. These cyclic octapeptides have shown moderate to good growth inhibition against bacterial strains and weak activity against fungal strains more than that of the standard drug against only *Pseudomonas aeruginosa* but weak to moderate activity against remaining three bacterial strains. They have shown very weak activity against fungal strains. Yunnanin F possessed good anthelmintic activity while Hymenistatin possessed very low activity, but both showed moderate anti-inflammatory activity.

Keywords: Cyclic peptides; Yunnanin F; Hymenistatin; Antimicrobial activity; Pharmacological activity; *p*-Nitrophenyl ester method

1. 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]. Recently, a large number of these cyclic peptides are emerging as an important class of organic compounds due to their unique structure and biological activities. The wide spread increase of bacterial resistance towards conventional antibiotics encourages 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].

In the continued investigation of the roots of *Stellaria yunnanensis*, Morita et al. [8] isolated a new biologically active cyclic octapeptide, Yunanin F. The structure of this peptide was elucidated by extensive spectroscopic evidences and chemical degradations. Yunnanin F exhibited cytotoxic activity. Another cyclic octapeptide, Hymenistatin active against the P388 leukemia cell line was isolated by Pettit et al. [9] from the Western Pacific ocean sponge *Hymeniacidon* sp. The structural determination of this peptide was accomplished utilizing NMR FABMS/MS techniques followed by chromatographic analysis. The structure of Hymenistatin was also confirmed by the solid phase synthesis [10].

In continuation of our research work of synthesizing natural cyclic peptides of biological interest [11,12], an attempt was made towards the synthesis of Yunnanin F and Hymenistatin. Keeping in view of significant biological activities exhibited by various cyclic peptides, the above synthetic peptides were further subjected to antibacterial and pharmaceutical activity studies.

2. Chemistry

For the synthesis of these cyclic octapeptides, disconnection approach was used. In order to carry out the total synthesis of Yunnanin F, cyclo(Gly-Val-Thr-Trp-Tyr-Pro-Ser-Ser-), it was disconnected into four dipeptide units, Boc-Gly-Val-OMe **1**, Boc-Thr-Trp-OMe **2**, Boc-Tyr-Pro-OMe **3** and Boc-Ser-Ser-OMe **4**. The required dipeptides were prepared by

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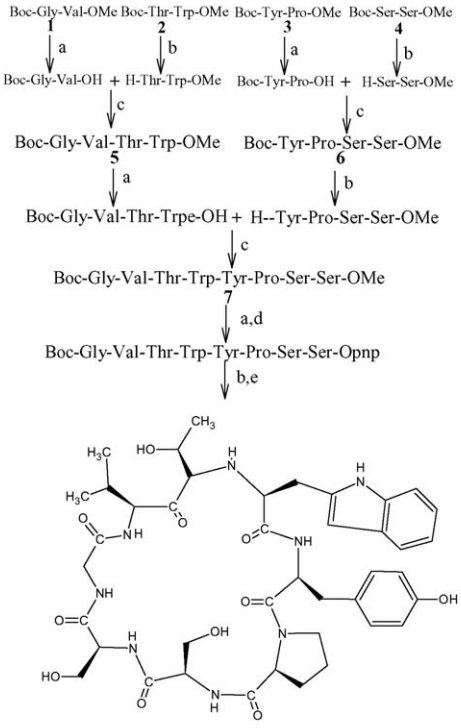
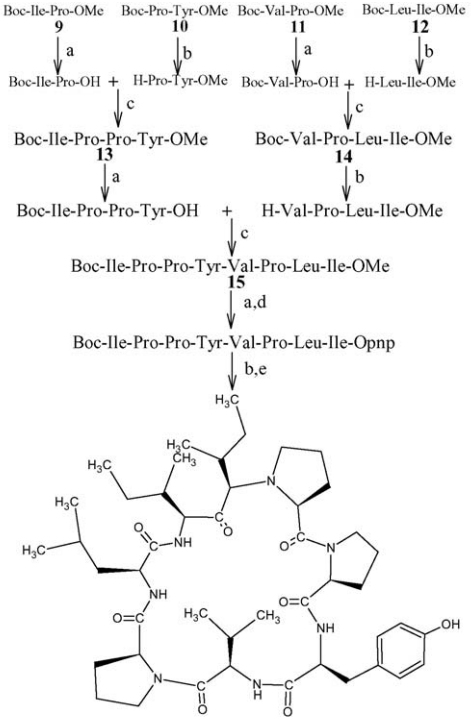
coupling Boc-amino acids with the respective amino acid ester hydrochlorides using DCC, HOBt and *N*-methyl morpholine according to Bodanszky and Bodanszky [13] procedure with suitable modifications [14]. The ester group of dipeptide **1** was removed with LiOH and the Boc-group of dipeptide **2** was removed with trifluoroacetic acid. Both the deprotected units were coupled to get the tetrapeptide, Boc-Gly-Val-Thr-Trp-OMe **5**. The remaining two dipeptides (**3** and **4**) were also coupled similarly to obtain the another tetrapeptide, Boc-Tyr-Pro-Ser-Ser-OMe **6**. These tetrapeptides were then coupled after proper deprotection using DCC, HOBt and NMM to get the octapeptide, Boc-Gly-Val-Thr-Trp-Tyr-Pro-Ser-Ser-OMe **7**. The methyl ester group of **7** was deprotected with LiOH and *p*-nitrophenyl (pnp) ester group was introduced by treating it with *p*-nitrophenol in presence of DCC. The Boc-group of resulting Boc-Gly-Val-Thr-Trp-Tyr-Pro-Ser-Ser-Opnp was removed by treating it with trifluoroacetic acid in chloroform. The solution of Boc-deprotected octapeptide pnp ester was diluted with chloroform and allowed to cyclize [15] in presence of pyridine to obtain Yunnanin F **8** as depicted in Fig. 1. In the same way, Hymenistatin [cyclo(-Ile-Pro-Pro-Tyr-Val-Pro-Leu-Ile-)] was also disconnected into four dipeptide units of Boc-Ile-Pro-OMe **9**, Boc-Pro-Tyr-OMe **10**, Boc-Val-Pro-OMe **11** and Boc-Leu-Ile-OMe **12**. The tetrapeptide units, Boc-Ile-Pro-Pro-Tyr-OMe **13** and Boc-Val-Pro-Leu-Ile-OMe **14** of were prepared as per the above pro-



Yunnanin F **(8)**

Where pnp = p-nitrophenyl
a = LiOH, THF:H_2O(1:1),RT/1h b = TFA,$CHCl_3$, RT/1h
c = DCC, NMM, HOBt, DCM, RT/36 h d = *p*-nitrophenol,DCC,$CHCl_3$,RT/12 h
e = Pyridine, $CHCl_3$,10days/0^0C

Fig. 1. Synthesis of Yunnanin F.



Hymenistatin **(16)**

Where pnp = p-nitrophenyl
a = LiOH, THF:H_2O(1:1),RT/1h b = TFA,$CHCl_3$, RT/1h
c = DCC, NMM, HOBt, DCM, RT/36 h d = *p*-nitrophenol,DCC,$CHCl_3$,RT/12 h
e = Pyridine, $CHCl_3$,10days/0^0C

Fig. 2. Synthesis of Hymenistatin.

cedure by condensing the required dipeptides (**9**–**12**) after proper deprotection. The resulting tetrapetides (**13** and **14**) were then coupled after proper deprotection using DCC, HOBt and NMM to get the octapeptide, Boc-Ile-Pro-Pro-Tyr-Val-Pro-Leu-Ile-OMe **15**, the linear segment of Hymenistatin. Finally, the cyclization of the linear segment (Fig. 2) was carried out by the pnp ester method to obtain Hymenestatin **16**.

3. Biological activity studies

The synthesized cyclic peptides, Yunnanin F and Hymenistatin were also screened for its antibacterial, antifungal, anti-inflammatory and anthelmintic activity. The antibacterial and antifungal activity are carried out against four bacterial (*Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*) and two fungal strains (*Candida albicans* and *Aspergillus niger*). These activity studies were carried out according to disc diffusion method [16]. Penicillin and griseofulvin were used as standards against bacteria and fungal strains at 10 and 25 μg per disc, respectively. The results are summarized in Table 1. The anti-inflammatory activity was carried out according to the method of Winter et al. [17] using ibuprofen as the standard and the results are presented in Table 2. The anthelmintic activity was carried out against the earthworms (pontoscotex corethruses) accord-

Table 1
Antimicrobial activity data

Compound	Diameter of zone of inhibition					
	Antibacterial activity studies			Antifungal activity studies		
	P. aeruginosa	*E. coli*	*B. subtilis*	*S. aureus*	*C. albican*	*A. niger*
Yunnanin F **8**	14	08	10	16	10	08
Hymenistatin **16**	14	10	16	08	08	09
Penicillin	12	12	18	18	–	–
Greseofulvin	–	–	–	–	20	20

Table 2
Anti-inflammatory activity data

Compound	Increase in paw volume after 3 h ± S.E. (ml)	Percentage inhibition of oedema after 3 h ± S.E.
Yunnanin F **8**	0.74 ± 0.03	17.78
Hymenistatin **16**	0.77 ± 0.03	14.44
Ibuprofen	0.55 ± 0.03	40.50
Control	0.90 ± 0.02	–

Table 3
Anthelmintic activity data

Compound	Concentration of the compound (mg)	Mean paralyzing time (min) ± S.E.	Mean death time (min) ± S.E.
Yunnanin F **8**	100	28.50 ± 2.20	55.30 ± 1.10
	200	14.64 ± 1.90	29.05 ± 1.60
Hymenistatin **16**	100	98.24 ± 1.20	118.3 ± 1.10
	200	85.18 ± 1.03	106.6 ± 2.12
Mebendazole	100	18.01 ± 2.01	35.20 ± 2.00
	200	12.55 ± 1.02	32.01 ± 1.10
Control	–	–	–

ing to Garg and Atal [18] method (Table 3) using mebendazole as standard drug.

4. Results and discussion

The intermediate and final products were purified by column chromatography using 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 the cyclized products clearly indicated the presence of all respective amino acid moieties. Further more, the mass spectra these cyclic octapeptides Yunnanin F and Hymenistatin showed the $[M^+ + H]$ peak at *m/z* 878 and 893, which are consistent with their molecular formulae, $C_{42}H_{55}N_9O_{12}$ and $C_{47}H_{72}N_8O_9$, respectively.

The antibacterial activity data presented in Table 1 indicates that both synthesized cyclic octapeptides have shown the growth inhibition more than that of the standard drug against only *P. aeruginosa* but weak to moderate activity against remaining three bacterial strains. They have shown very weak activity against fungal strains. The anti-inflammatory data reveals that both of them are less active as compared with the standard drug, ibuprofen (Table 2). Yunnanin F possessed good anthelmintic activity while Hymenistatin possessed very low activity as compared to the standard.

5. Conclusions

We have successfully synthesized two cyclic octapeptides, Yunnanin F and Hymenistatin by solution phase method in good yield. Also, we have studied their antimicrobial and pharmacological activities. The antimicrobial results indicated the moderate to good antibacterial activity of synthetic cyclic peptides when compared to the reference drug penicillin. Evaluation of anti-inflammatory activity revealed very weak activity of these cyclic octapeptides as compared to the standard drug, ibuprofen. Among the two synthesized cyclic peptides, only Yunnanin F showed a good anthelmintic activity.

6. Experimental protocols

6.1. General chemistry

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 $CDCl_3$/DMSO-d_6 on a 300 MHz spectrophotometer using TMS as an internal standard. The mass spectra were recorded on a FAB mass spectrometer. The progresses of the reactions were checked by TLC on silica gel G plates and the products were purified by silica gel column chromatography.

Eight dipeptides, Boc-Gly-Val-OMe **1**, Boc-Thr-Trp-OMe **2**, Boc-Tyr-Pro-OMe **3**, Boc-Ser-Ser-OMe **4**, Boc-Ile-Pro-OMe **9**, Boc-Pro-Tyr-OMe **10**, Boc-Val-Pro-OMe **11** and Boc-Leu-Ile-OMe **12** were prepared and these dipeptides were used for the preparation of four tetrapeptides (Fig. 1), Boc-Gly-Val-Thr-Trp-OMe **5**, Boc-Tyr-Pro-Ser-Ser-OMe **6**, Boc-Ile-Pro-Pro-Tyr-OMe **13** and Boc-Val-Pro-Leu-Ile-OMe **14** after proper deprotection using LiOH and trifluoroacetic acid according to Bodanszky procedure with suitable modifications [12]. The tetrapetides (**5**) and (**6**), were then condensed as per the procedure used for the preparation of tetrapeptides to obtain octapetide, Boc-Gly-Val-Thr-Trp-Tyr-Pro-Ser-Ser-OMe **7**, the linear segment of Yunnanin F. The linear segment

of Hymenistatin, Boc-Ile-Pro-Pro-Tyr-Val-Pro-Leu-Ile-OMe **15** was obtained by coupling tetrapeptides (**13**) and (**14**) according to above procedure.

*6.1.1. Boc-Gly-Val-Thr-Trp-OMe **5***

Yield: 79%. IR($CHCl_3$): *ν* 3580 (O–H str.), 3295 (N–H str.), 3010 (=C–H str.), 2955 (C–H str.), 2860 (C–H str.), 1730 (C=O str. ester), 1690 (C=O str. amide), 1655 (C=O str. amide), 1650 (C=O str. amide), 1530, 1450, 1390, 1265, 1160, 1090, 890 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.0 (s, 1H, NH), 8.5 (br.s, 2H, NH), 8.1 (br.s, 2H, NH), 7.8–7.2 (m, 5H, Ar-H), 5.2 (m, 1H, OH), 4.9–4.7 (m, 2H, α-CH), 4.6–4.5 (m, 1H, α-CH), 4.3–4.2 (m, 1H, α-CH), 4.1–4.0 (m, 2H, α-CH_2), 3.7(s, 3H, O–CH_3), 3.5–3.3 (m, 2H, β-CH_2), 1.6–1.5 (m, 1H, β-CH), 1.4 (s, 9H, $C(CH_3)_3$), 1.2 (d, 3H, J = 6.5 Hz, CH_3), 0.95 (d, 6H, J = 6.5 Hz); $C_{28}H_{41}N_5O_8$(575): Calc. C 58.43, H 7.13, N 12.17; Found: C 58.58, H 7.25, N 12.30.

*6.1.2. Boc-Tyr-Pro-Ser-Ser-OMe **6***

Yield: 84.5%. IR($CHCl_3$): *ν* 3490 (O–H str.), 3320 (N–H str.), 3090 (=C–H str.), 2940 (C–H str.), 2851 (C–H str.), 1730 (C=O str. ester), 1690 (C=O str. amide), 1680 (C=O str. amide), 1650 (C=O str. amide), 1622 (N–H def.), 1570, 1500, 1430, 1370, 1240, 1160, 895 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.3 (br.s, 1H, OH), 8.7 (br.s, 2H, NH), 8.3 (br.s, 1H, NH), 7.8 (br.s, 1H, NH), 7.0 (d, 2H, J = 8.0 Hz, 2,6-Ar-H), 6.85 (d, 2H, J = 8.0 Hz, 3,5-Ar-H), 5.2–5.1 (m, 2H, OH), 4.9–4.7 (m, 4H, α-CH), 4.5–4.2 (m, 4H, α-CH_2), 3.7 (s, 3H, O–CH_3), 3.6–3.4 (m, 2H, –N–CH_2), 3.2–3.0 (m, 2H, β-CH_2), 2.2–1.7 (m, 4H, –CH_2–CH_2–), 1.4 (s, 9H, $C(CH_3)_3$); $C_{26}H_{38}N_4O_{10}$(566): Calc. C 55.12, H 6.71, N 9.89; Found: C 55.24, H 6.58, N 9.73.

*6.1.3. Boc-Ile-Pro-Pro-Tyr-OMe **13***

Yield: 86.6%. IR($CHCl_3$): *ν* 3510 (O–H str.), 3480 (N–H str.), 3015 (m, =C–H str.), 2980(C–H str.), 2840 (C–H str.), 1720 (C=O str. ester), 1670(C=O str. amide), 1650 (C=O str. amide), 1610 (N–H def.), 1500 (C–N str.), 1300 (C–H def.), 1260 (C–O str.), 1015 (C–H def.), 970 (C–H def.) cm^{-1}; ^1H NMR (300 MHz, *CDCl*$_3$): δ 9.25 (br.s, 1H, OH), 7.0 (d, 2H, J = 8.0 Hz, 2,6-Ar-H), 6.85 (d, 2H, J = 8.0 Hz, 3,5-Ar-H), 6.8 (br.s, 1H, NH), 6.2 (br.s, 1H, NH), 4.7–4.5 (m, 2H, α-CH), 4.4–4.1 (m, 2H, α-CH), 3.75 (s, 3H, OCH_3), 3.5–3.3 (m, 4H, N–CH_2), 3.2–3.1 (m, 2H, β-CH_2), 2.3–1.7 (m, 9H, –CH_2–CH_2 and β-CH), 1.45 (s, 9H, $C(CH_3)_3$), 1.3–1.1(m, 2H, γ-CH_2), 1.0 (doublet overlapped with triplet, 6H, CH_3). –$C_{31}H_{46}N_4O_8$ (602): Calc. C 61.79, H 7.64, N 9.30; Found: C 61.76, H 7.89, N 9.90.

*6.1.4. Boc-Val-Pro-Leu-Ile-OMe **14***

Yield: 79.9%. IR($CHCl_3$): *ν* 3520 (N–H str.), 3450 (N–H str.), 2960 (C–H str.), 2920 (C–H str.), 2880 (C–H str.), 1740 (C=O str. ester), 1690 (C=O str. amide), 1630 (C=O str. amide), 1500, 1437 (C–N str.), 1311 (C–H def.), 1245, 1159, 1080, 892 cm^{-1}; ^1H NMR (300 MHz (*CDCl*$_3$)): δ 6.6 (br.s, 2H, NH), 5.9 (br.s, 1H, NH), 4.6–4.5 (m, 1H, α-CH), 4.3–4.0 (m, 3H, α-CH), 3.75 (s, 3H, OCH_3), 3.6–3.4 (m, 2H, N–CH_2), 2.4–2.0 (m, 4H, –CH_2–CH_2), 1.9–1.6 (m, 4H, β-CH_2 and β-CH), 1.45 (s, 9H, $C(CH_3)_3$), 1.3–1.1 (m, 3H, γ-CH_2 and γ-CH), 1.0 (doublet overlapped with triplet, 18H, CH_3 and $C(CH_3)_2$); $C_{28}H_{50}N_4O_7$ (554): Calc. C 60.65, H 9.03, N 10.11; Found: C 60.78, H 9.12, N 10.21.

*6.1.5. Boc-Gly-Val-Thr-Trp-Tyr-Pro-Ser-Ser-OMe **7***

Yield: 68%. IR ($CHCl_3$): *ν* 3590 (O–H str.), 3320 (N–H str.), 3040 (=C–H str.), 2985 (C–H str.), 2850 (C–H str.), 1730 (C=O str. ester), 1680 (C=O str. amide), 1675 (C=O str. amide), 1665 (C=O str. amide), 1600, 1530, 1470, 1320, 1240, 1160, 1080, 840 cm^{-1}; ^1H NMR (300 MHz (*CDCl*$_3$)): δ 9.0 (br.s, 1H), 6.9 (m, 9H, Ar-H), 5.2 (s, 1H, –OH), 5.18 (s, 1H, –OH), 4.8–4.7 (m, 4H, α-CH), 4.6–4.5 (m, 5H, α-CH), 4.4–4.2 (m, 5H, α-CH_2 and α-CH), 3.7 (s, 3H, OCH_3), 3.6–3.4 (m, 2H, N–CH_2), 3.2–3.0 (m, 4H, β-CH_2), 2.2–1.7 (m, 4H, –CH_2–CH_2–), 1.6–1.5 (m, 1H, β-CH), 1.4 (s, 9H, $C(CH_3)_3$), 1.3 (d, 3H, J = 6.5 Hz, CH_3), 0.95 (d, 6H, J = 6.5 Hz, –$C(CH_3)_2$); $C_{48}H_{67}N_9O_{15}$(1009): Calc. C 57.09, H 6.64, N 12.49; Found: C 57.16, H 6.62, N 12.61.

*6.1.6. Boc-Ile-Pro-Pro-Tyr-Val-Pro-Leu-Ile-OMe **15***

Yield: 69.3%. IR($CHCl_3$): *ν* 3550 (O–H str.), 3460 (N–H str.), 3010 (=C–H str.), 2980 (C–H str.), 2920 (C–H str.), 1730 (C=O str. ester), 1690(C=O str. amide), 1670 (C=O str. amide), 1630 (C=O str. amide), 1610 (N–H def.), 1540 (C–N str.), 1510, 1450 (C–H def.), 1380 (C–H def.), 1260 (C–O str.), 1100 (C–O str.), 970 (C–H def.) cm^{-1}; ^1H NMR (300 MHz (*CDCl*$_3$)): δ 9.2 (br.s, 1H, OH), 8.4 (br.s, 1H, NH), 7.5 (br.s, 1H, NH), 7.0 (d, 2H, 2,6-Ar-H), 6.9 (2H, d, J = 8.0 Hz, 3,5-Ar-H), 6.8 (br.s, 2H, NH), 6.3 (br.s, 2H, NH), 4.7–4.4 (m, 4H, α-CH), 4.3–4.0 (m, 4H, α-CH), 3.75 (s, 3H, OCH_3), 3.6–3.4 (m, 6H, N–CH_2), 3.2–3.0 (m, 2H, β-CH_2), 2.4–2.0 (m, 12H, –CH_2–CH_2), 1.9–1.6 (m, 5H, β-CH_2 and β-CH), 1.4 (s, 9H, $C(CH_3)_3$), 1.3–1.1 (m, 5H, γ-CH_2 and γ-CH), 0.95 (doublet overlapped with triplet, 24H, J = 6.5 Hz, CH_3 and $C(CH_3)_2$); $C_{53}H_{84}N_8O_{12}$ (1024): Calc. C 62.11, H 8.20, N 10.94; Found: C 62.16, H 8.12, N 10.88.

*6.1.7. General procedure for the preparation of cyclic octapeptides, Yunnanin F **8** and Hymenistatin **16***

To the solution of Boc-heptapeptide pnp ester (1.2 mmol) in chloroform (15 ml), trifluoroacetic acid (0.274 g, 2.4 mmol) was added, stirred for 1 h at room temperature and washed with 10% sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulfate. To the resulting Boc-deprotected peptide-pnp ester in THF (15 ml), pyridine (1.4 ml, 2 mmol) was added and kept at 4 °C for 7 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 sulfate. 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.

6.1.8. Yunnanin F ***8***

Yield: 56%; m.p.: 245–248 °C. IR($CHCl_3$): *ν* 3600 (O–H str.), 3320 (N–H str.), 3030 (=C–H str.), 2940 (C–H str.), 2850 (C–H str.), 1680 (C=O str. amide), 1650 (C=O str. amide), 1630 (C=O str. amide), 1605(N–H def.), 1530 (C–N str.), 1430 (C–H def.), 1150 (C–H def.), 1075 (C–H def.), 975 (C–H def.) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): *δ* 9.2 (1H, br.s, OH), 9.0 (1H, br.s, NH), 8.8 (br.s, 2H, NH), 8.5 (br.s, 2H, NH), 8.2 (br.s, 3H, NH), 7.9 (br.s, 1H, NH), 7.8–7.2 (m, 7H, Ar-H and Ar(Trp)–H), 6.95 (2H, d, *J* = 8.0 Hz, 3,5-Ar-H), 5.2 (s, 1H, OH), 5.1 (s, 1H, OH), 4.8–4.7 (m, 4H, β-CH_2), 4.6–4.5 (m, 5H, α-CH and β-CH), 4.4–4.0 (m, 5H, α-CH_2 and α-CH), 3.6–3.4 (m, 2H, N–CH_2), 3.2–3.0 (m, 4H, β-CH_2), 2.2–1.8 (m, 4H, –CH_2–CH_2–), 1.5–1.4 (m, 1H, β-CH), 1.3 (d, 3H, *J* = 6.5 Hz, –CH), 0.9 (d, 6H, *J* = 6.5 Hz, –$C(CH_3)_2$); ^{13}C NMR (DMSO-d_6): *δ* 174.8 (s, C=O), 173.5 (s, C=O), 173.0 (s, C=O), 172.4 (s, C=O), 170.9 (s, C=O), 170.8 (s, C=O), 170.1(s, C=O), 169.4 (s, C=O), 154.8 (s, Ar-4-C), 137.4 (s, Trp-8-C), 130.1 (d, Ar-2,6-C), 129.0 (s, Ar-1-C), 128.8 (s, Trp-9-C), 125.5 (d, Trp-2-C), 122.2 (d, Trp-6-C), 119.9 (d, Trp-5-C), 119.3 (d, Trp-4-C), 113.9 (d, Ar-3,5-C), 112.6 (s, Trp-3-C), 110.1 (d, Trp-7-C), 62.9 (t, β-CH_2) 62.2 (t, β-CH_2), 61.0 (d, α-CH), 60.1 (t, β-CH_2), 59.5 (d, α-CH), 56.7 (d, α-CH), 46.4 (t, α-CH), 57.2 (d, α-CH), 57.0 (d, α-CH), 55.4(d, α-CH), 53.2(d, α-CH), 48.3 (t, *δ*(N)–CH_2), 36.9 (t, β-CH_2), 27.4 (t, β-CH_2), 30.1 (d, β-CH), 28.5 (t, β-CH_2), 25.3(t, γ-CH_2), 21.5 (q, CH_3), 19.4 (q, CH_3), 19.0 (q, CH_3); FAB mass: *m/z* 878 [M^+ + H]; $C_{42}H_{55}N_9O_{12}$ (877): Calc. C 57.47, H 6.27, N 14.37; Found: C 57.60, H 6.15, N 14.42.

6.1.9. Hymenistatin ***16***

Yield: 58.9%; m.p.: 180–181 °C (Ref. [9] 180–182 °C)). IR($CHCl_3$): *ν* 3580 (O–H str.), 3270 (N–H str.), 3005 (=C–H str.), 2985 (C–H str.), 2930 (C–H str.), 1685 (C=O str. amide), 1660 (C=O str. amide), 1610 (N–H def.), 1530 (C–N str.), 1500 (C–H def.), 1450 (C–H def.), 1170 (C–H def.), 970 (C–H def.) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): *δ* 9.25 (br.s, 1H, OH), 7.6 (br.s, 1H, NH), 7.0 (d, 2H, 2,6-Ar-H), 6.9 (2H, d, *J* = 8.0 Hz, 3,5-Ar-H), 6.6 (br.s, 2H, NH), 6.2 (br.s, 2H, NH), 4.8–4.5 (m, 4H, α-CH), 4.4–4.1 (m, 4H, α-CH), 3.6–3.2 (m, 4H, N–CH_2), 3.2–3.0 (m, 2H, β-CH_2), 2.4–2.1 (m, 12H, –CH_2–CH_2–), 1.8–1.5 (m, 5H, β-CH_2 and β-CH), 1.3–1.2 (m, 5H, γ-CH_2 and β-CH), 1.3–1.2 (m, 5H, γ-CH_2 and γ-CH), 1.0 (doublet overlapped with triplet, 24H, CH_3 and $C(CH_3)_2$); ^{13}C NMR (DMSO-d_6): *δ* 173.5 (s, C=O), 173.2 (s, C=O), 172.9 (s, C=O), 172.8 (s, C=O), 172.5 (s, C=O), 172.0 (s, C=O), 171.6 (s, C=O), 171.5 (s, C=O), 157.6 (s, Ar-1-C), 130.7 (d, Ar-2,6-C), 127.1 (s, Ar-4-C), 115.9 (d, Ar-3,5-C), 62.8 (t, β-CH_2), 61.8 (d, α-CH), 60.7 (d, α-CH), 60.5 (d, α-CH), 59.9 (d, α-CH), 58.4 (d, α-CH), 57.7 (d, α-CH), 57.6 (d, α-CH), 56.7 (d, α-CH), 47.2 (t, *δ*(N)–CH_2), 47.0 (t, *δ*(N)–CH_2), 46.9 (t, *δ*(N)–CH_2), 40.0 (t, β-CH_2), 39.3 (t, β-CH), 39.2 (t, β-CH), 36.8 (t, β-CH_2), 31.6(t, β-CH_2), 29.3 (d, β-CH), 28.8 (t, β-CH_2), 28.5 (t, β-CH_2), 28.2 (t, β-CH_2), 25.7 (t, γ-CH), 25.2 (t, γ-CH_2), 25.1 (t, γ-CH_2), 24.9 (t, γ-CH_2), 24.6 (t, γ-CH), 24.5 (t, γ-CH), 20.3 (q, –CH_3), 19.8 (q, –CH_3), 19.5 (q, –CH_3), 19.4 (q, –CH_3), 18.5 (q, –CH_3), 17.8 (q, –CH_3); FAB mass: *m/z* 893 [M^+ + H]; $C_{47}H_{72}N_8O_9$(71): Calc. C 63.23, H 8.07, N 12.56; Found: C 63.15, H 8.21, N 12.50.

6.2. Biological experimental section

6.2.1. Antibacterial and antifungal activity

The synthesized cyclic octapeptides were screened for their antibacterial activity against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli* and antifungal activity against *C. albicans* and *A. niger* by disc diffusion method. The discs measuring 6 mm in diameter were punched from Whatman No. 1 filter paper. Batches of 100 discs were dispensed to each screw capped bottles and sterilized by dry heal at 140 °C for 1 h. The solutions of test compounds were prepared in dimethyl sulfoxide. The samples of test compounds were tested for antibacterial and antifungal activity at 10 and 25 μg level, respectively. One milliliter containing 100 times the amount of chemical in each disc was added to each bottle, which contains 100 discs. The discs of each concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria and fungi separately. The incubation was carried out at 37 °C for 24 h. Penicillin, streptomycin and griseofulvin were used as standard drugs for antibacterial and antifungal activity study. The solvent and growth controls were kept and zones of inhibition were noted. The results of such studies are given in Table 1.

6.2.2. Anti-inflammatory activity

Winter's hind paw method was used in the present study for the evaluation of the anti-inflammatory activity on albino rats. Carragenin at a concentration of 1 mg ml^{-1} was injected subcutaneously into the hind paw of the rat, to produce the oedema. Forty healthy rats of both sexes (body weight 100–200 g), pregnant females excluded, were selected and made in to four groups of 10 animals each. One group of animals was kept as control that received 2% w/v acacia mucilage, which was used to suspend the sample. Another group received the standard drug ibuprofen, 20 mg kg^{-1} body weight intraperitonially. Remaining two groups of animals were given a dose of different test compounds (20 mg kg^{-1} body weight). After 30 min, 0.1 ml of w/v carragenin was injected subcutaneously in to the right hind paw and the paw volume was measured by a mercury plethysmometer and then measured again after 3 h. The mean increase of paw volume was compared with that of the control group and percent inhibition values were calculated. The experimental results are fisted in Table 2.

6.2.3. Anthelmintic activity

Anthelmintic activity studies were carried out against earthworms, Pomoscolex corethrusus by Garg's method. Suspensions of the samples were prepared by triturating the samples with 0.5% tween 80 and distilled water. The suspensions were diluted to contain 0.1% and 0.2% w/v of the test samples. 0.1% and 0.2% w/v suspensions of the standard drug,

mebendazole were also prepared in a similar way. Ten earthworms of similar size were placed in a Petri plate of 4 in. diameter containing 50 ml of suspension of the test standard drug at room temperature. Another set of 10 earth worms were kept as control in 50 ml suspension of distilled water and 0.5% tween 80. Fifty milliliters each of the suspensions of the test compounds were added in to separate Petri plates containing 10 earthworms in each. The time required for the paralysis and deaths of the earthworms were noted. The death time was ascertained by placing the earthworms in warm water at 50 °C, which stimulated the movement if the worm was alive. The results are summarized in Table 3.

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