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Short communication

Synthetic studies on cyclic octapeptides: Yunnanin F and Hymenistatin

Boja Poojary [a,*], Shiddappa 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*

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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

* Corresponding author.
E-mail address: bojapoojary@yahoo.com (B. Poojary).

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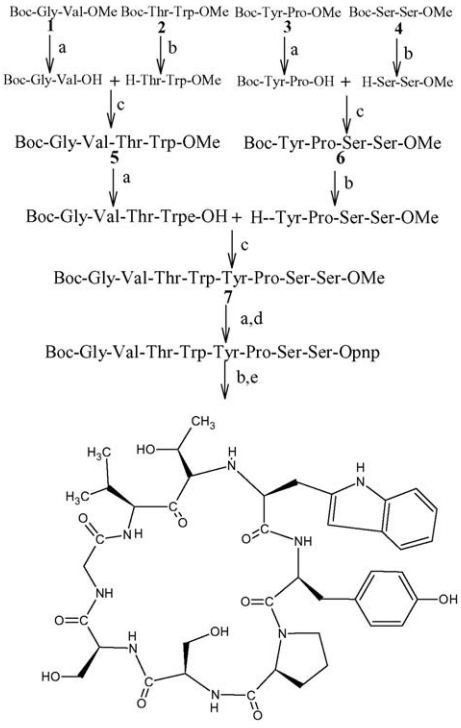
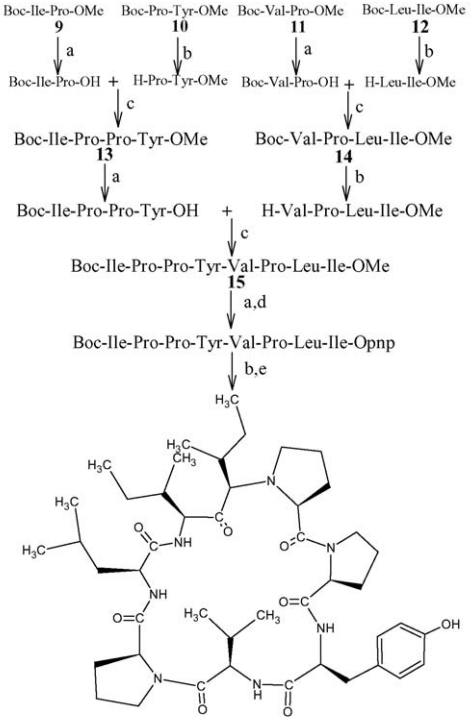
coupling Boc-amino acids with the respective amino acid ester hydrochlorides using DCC, HOBt and *N*-methyl morpholine according to Bodanszky and Bodanszky [13] procedure with suitable modifications [14]. The ester group of dipeptide **1** was removed with LiOH and the Boc-group of dipeptide **2** was 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-