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Synthesis and Biological Evaluation of Analogue of Delavayin-C: A Cyclic Heptapeptide

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The synthesis of N-methylated analogue of delavayin-C, a cyclic heptapeptide was carried out by using solution phase technique. The structure of this compound was confirmed on the basis of analytical IR, ^{1}H NMR and FAB MASS spectral data. The elemental analysis of synthesized compound was also carried out. The antibacterial activities of this peptide were carried out against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*. The antifungal activities were carried out against *C. albicans* and *C. neoformans* as test organisms. The synthesized compound had shown significant antibacterial as well as antifungal activity.

Key Words: Cyclic peptide, Antibacterial, Antifungal.

INTRODUCTION

Most of the cyclic peptides are found to exhibit antifungal, antibacterial, anthelmintic, cytotoxic, antineoplastic, insecticidal, antiinflammatory, antitumour, tyrosinase inhibitory and melanin-production inhibitory activities[1-5]. Delavayin-C[6], a naturally occurring cyclic heptapeptide, cyclo (Gly-Tyr-Tyr-Tyr-Pro-Val-Pro) was isolated from the roots of *Stellaria delavayi* and belongs to family 'Caryophyllaceae'. It possesses various biological activities. Keeping in view of the significant biological activities exhibited by delavayin-C, as a part of ongoing study, an attempt was made to synthesize a N-methylated analogue of delavayin-C cyclo[Gly-Tyr-Tyr-Tyr-Pro-(N-Me)Val-Pro], in the laboratory, as N-methylation in cyclic peptides is found to increase their activity[7]. The synthesized compound was further subjected to spectral analysis for structural confirmation. The antibacterial activity was carried out against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* and antifungal activity against *C. albicans* and *C. neoformans* by using modified Kirby Bauer method[8].

The synthesized compound has shown significant antibacterial and antifungal activity comparable with the standard drug benzyl penicillin and standard antifungal agent fluconazole, respectively.

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EXPERIMENTAL

All the chemicals used in the synthesis were of laboratory grade. Melting points were taken in open capillary tubes and are found to be uncorrected. IR spectra was recorded on JASCO FTIR 5300 IR spectrometer (in $CHCl_3$) and the chemical shift values are reported as values as ν_{max} (cm^{-1}). ^1H NMR spectra was recorded on Bruker AC NMR spectrometer (300 MHZ in $CDCl_3$) and the chemical shift values are reported as values in ppm relative to TMS ($\delta = 0$) as a internal standard. FAB MASS spectra were recorded on a Joel SX 102/DA-6000 MASS spectrometer using xenon as a carrier gas. TLC were done to check the progress of reaction by using silica gel-G plates. All the compounds gave satisfactory elemental analysis for C, H and N. The synthesis of analogue of delavayin-C was achieved by routes as in **Scheme-I.** The amino group of amino acid was protected with the *t*-butyloxycarbonyl group (Boc-) and carboxyl group was protected[9] by converting into methyl ester HCl to get respective Boc-amino acid and amino acid methyl ester HCl. The two dipeptides, Boc-Gly-Tyr-OMe (7) and Boc-Tyr-Tyr-OMe (8) were prepared by coupling of respective Boc-amino acids with amino acid methyl ester HCl by using diisopropyl carbodiimide (DIPC) as the coupling agent and N-methyl morpholine (NMM) as the base according to Bondanszky[10,11] procedure with suitable modifications. The ester group of Boc-Gly-Tyr-OMe (7) was removed by using LiOH and Boc-group of Boc-Tyr-Tyr-OMe (8) was removed by using trifluoro acetic acid. The deprotected Boc-Gly-Tyr was then coupled with Tyr-Tyr-OMe to get a tetrapeptide Boc-Gly-Tyr-Tyr-Tyr-OMe (11).

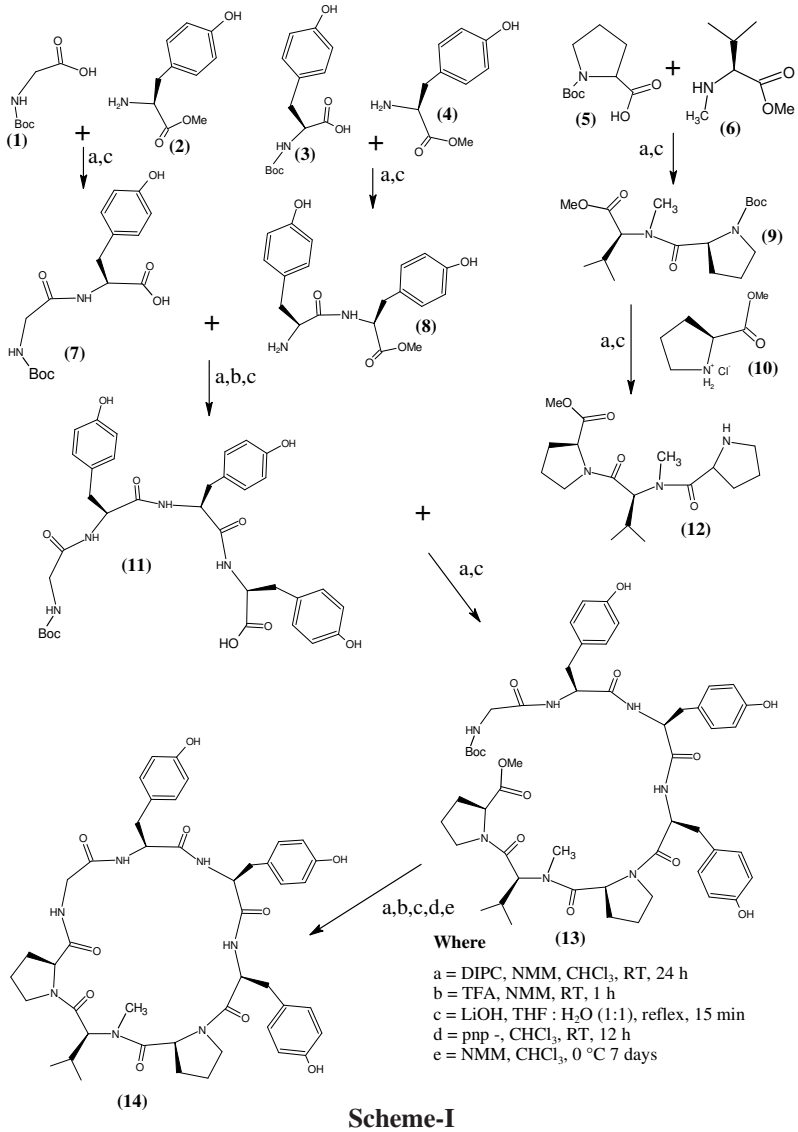
N-Methylation[12] was done for the amino acid valine by using Bentoin method[13] to get N-Me-Valine-OMe (6). Boc-Pro (5) was coupled with N-Me-Valine-OMe (6) to get a dipeptide Boc-Pro-(N-Me)Val-OMe (9). The so formed dipeptide was then coupled with a single Pro-OMe (10) unit after appropriate deprotection as above to get a tripeptide Boc-Pro-(N-Me)Val-Pro-OMe (12).

Tetrapeptide Boc-Gly-Tyr-Tyr-Tyr-OMe (11) and a tripeptide Pro-(N-Me)Val-Pro-OMe (12) were then coupled together after appropriate deprotection by using diisopropyl carbodiimide (DIPC) as the coupling agent and N-methyl morpholine (NMM) as a base to get a linear heptapeptide Boc-Gly-Tyr-Tyr-Tyr-Pro-(N-Me)Val-Pro-OMe (13). The ester group of the linear heptapeptide was removed with LiOH and *p*-nitrophenyl group (pnp-) was introduced. The Boc group was removed by trifluoro acetic acid and the linear fragment was cyclised by adding N-methyl morpholine and keeping the whole contents at 0 ºC for seven days (14).

The intermediate and the final product were purified by recrystallization from $CHCl_3$.

Preparation of cyclic heptapeptide

***p*-Nitrophenyl ester method[14]:** Cyclization of the linear fragments was attempted by *p*-nitrophenyl ester method. The ester group of the linear segment was removed with LiOH and the *p*-nitrophenyl ester group was introduced using the following procedure (**Scheme-I**):



Scheme-I

The boc-peptide carboxylic acid (1.5 mmol) was dissolved in $CHCl_3$ (15 mL) at 0 ºC. Then *p*-nitrophenol was added (0.27 g, 2 mmol) and stirred for 12 h at room temperature. The reaction mixture was filtered and the filtrate was washed with $NaHCO_3$ solution (10 %) until excess of *p*-nitrophenol was removed and finally washed with 5 % HCl (5 mL) to get Boc-peptide-pnp-ester.

To the above Boc-peptide-pnp-ester (1.2 mmol) in $CHCl_3$ (15 mL), CF_3COOH (0.274 g, 2.4 mmol) was added, stirred for 1 h at room temperature and washed with 10 % $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4. To the Boc-deprotected peptide-pnp-ester in $CHCl_3$ (15 mL), N-methyl morpholine

(1.4 mL, 2 mmol) was added and kept at 0 ºC for 7 days. The reaction mixture was washed with 10 % $NaHCO_3$ until the byproduct *p*-nitrophenol was removed completely and finally washed with 5 % HCl (5 mL). The organic layer was dried over anhydrous Na_2SO_4. Chloroform and pyridine were distilled off to get the crude product of the cyclized compound, which was then recrystallized from $CHCl_3$/*n*-hexane.

Biological activity: The synthesized compound was screened *in vitro* for its antibacterial and antifungal activity. The antibacterial activity was carried out against four bacterial species (*B. subtilis, S. aureus, E. coli* and *P. aeruginosa*). The antifungal activity was carried out against *C. albicans* and *C. neoformans* species. The activity studies were carried out by disc diffusion technique according to modified Kirby Bauer method[7].

Benzyl penicillin and fluconazole were used as standards against bacterial and fungal strains, respectively at conc. of 50 g/mL. Nutrient broth and sabouraud's agar were used as medium for antibacterial and antifungal activity. Dimethyl formamide was used as a solvent control. The synthetic peptide has shown significant activity against *B. subtilis* and *S. aureus* which are gram positive bacteria and moderate activity against *E. coli* and *P. aeruginosa* which are gram negative bacteria when compared with standard drug benzyl penicillin (Table-1). The compound has also shown significant growth of inhibition against *C. albicans* and *C. neoformans* (Table-2). Screening data of antibacterial and antifungal activity revealed that the synthetic peptide is found to be active.

TABLE-1
RESULTS OF ANTIBACTERIAL ACTIVITY BY USING DISC DIFFUSION METHOD

Sr. no.	Name of compound	Diameter of zone of inhibition (mm)			
		S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
1.	Cyclic peptide	22	15	14	10
2.	Benzyl penicillin	25	15	17	16
4.	DMF	–	–	–	–

(-) indicates no inhibition zone.

TABLE-2
RESULTS OF ANTIFUNGAL ACTIVITY BY USING DISC DIFFUSION METHOD

Sr. no.	Name of compound	Diameter of zone of inhibition (mm)
	C. albicans	*C. neoformans*
1.	Cyclic peptide	18	16
2.	Fluconazole	20	22
3.	DMF	–	–

(-) indicates no inhibition zone.

Spectral data

IR (ν_{max}, cm^{-1}): 3677 (OH stretch), 3314 (NH stretch), 3016 (arom-CH stretch), 2933 (aliph-CH stretch), 2857 (aliph-CH stretch), 1695 (N-CH_3 stretch), 1660 (C=O stretch of amide).

^{1}H NMR (300 MHZ $CDCl_3$): δ 10.4 (3H, d, NH), 8.15 (2H, d, NH) 7.8-6.0(12H, m, arom-H); 5.0 (1H, d, α-H), 4.7 (1H, d, α-H), 4.3 (1H, m, α-H), 4.5 (2H, m, α-H); 4.1 (1H, m, α-H), 3.7 (1H, m, α-H), 3.5 (10H, m, β-CH_2 of Tyr and β-CH_2 of Pro); 2.9 (3H, br.s, N-CH_3 of Val) 2.3(1H, m, β-H of Val), 0.95(6H, d, $(CH_3)_2$ of Val).

FAB Mass in m/z: Cyclo[Gly-Tyr-Tyr-Tyr-Pro-(N-Me)Val-Pro]: Molecular ion peak was observed at m/z 854 corresponds to the molecular formula $C_{45}H_{55}N_7O_{10}$. Elemental analysis: C: 63.83(63.29) %, N: 11.09(11.48)

RESULTS AND DISCUSSION

The newly synthesized compound showed significant antibacterial activity against gram positive bacteria in comparision to that of standard drug benzyl penicillin. The compounds showed potent antifungal activity in comparision to that of standard drug fluconazole.

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