Unique Research Journal of Chemistry
Available online: www.ujconline.net
Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF A NOVEL CYCLIC HEXAPEPTIDE DERIVED FROM DESTRUXIN-B

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Received: 09-08-2013; Revised: 07-09-2013; Accepted: 05-10-2013

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ABSTRACT

A novel cyclic hexapeptide was synthesized from Destruxin family. The compound was characterized by IR, ^{1}H NMR and FABMASS spectral analysis. The synthesized compound was evaluated for antibacterial, antifungal and insecticidal activities. The compounds have showed moderate to significant activity in comparison to the standard drug.
Keywords: Cyclic Hexapeptide, Destruxin, Antibacterial, Antifungal and Insecticidal.

INTRODUCTION

Peptides, in particular cyclic peptides, are among the various organic compounds that have received the most attention during last three decades due to their unique strctures and biological activities. Destruxin-B[1], a cyclic hexa depsipeptide contains two N-Methyl groups and one ester link in the structure. In continuation of our work, synthesis of a novel cyclic hexapeptide Cyclo[Leu-Pro-Phe-Val-Val-β-Ala], has been designed based on the structure of Destruxin-B with absence of both the N-Methyl groups and ester link. The synthesis was carried out by solution phase technique of peptide synthesis. The synthesized product was characterized by PMR, IR and Mass spectra data and was evaluated for antibacterial, antifungal and insecticidal activities.

Antibacterial activity was carried out against *E. coli, P. aeruginosa, B.subtilis* and *S. aureus*. Antifungal activity by *C.albicans* and insecticidal activity studied using earthworms *C.formosanus*.

MATERIALS AND METHODS

General method of Peptide synthesis:

Formation of a selective peptide bond between two L-amino acids requires the protection of amino group of one amino acid and the carboxylic group of other. The Brodanszky method[2], the protection of amino group by di-tertiary butyl pyrocarbonate (Boc-)$_2$O and carboxylic group was protected by esterification.

Synthesis of dipeptides:

Amino acid methyl ester hydrochloride (10 mmol) was dissolved in chloroform (20 ml). To this, triethylamine (4 ml, 28.7 mmol) was added at 0°C and the reaction mixture was stirred for 15 mins. Boc-amino acid (10 mmol) in $CHCl_3$ (20 ml) and DIPC (10 mmol) were added with stirring. After 36 hrs, the reaction mixture was filtered and the residue was washed with $CHCl_3$ (30 ml) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ (20 ml) and saturated NaCl (20 ml) solutions. The organic layer was dried over anhydrous Na_2SO_4.

Using the above method the following Boc-dipeptide methyl esters were prepared.

Deprotection of the Amino and Carboxyl Groups: For the synthesis of the tri, penta and hexapeptides, deprotection of the amino and the carboxyl groups were done at the appropriate sites according to the following general procedure.

Deprotection of the Carboxyl Group: To a solution of the protected peptide (1.0 mmol) in THF: H_2O (1:1) (36 ml), LiOH (1.5mmol) was added at 0^0C. The mixture was stirred for 1 hour at room temperature and then acidified to pH 3.5 with 1N H_2SO_4. The aqueous layer was extracted with Et_2O (3 x 15ml). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure.

Deprotection of the amino group: The protected peptide (1mmol) was dissolved in $CHCl_3$ (15ml) and treated with CF_3COOH (2 mmol, 0.228 gr). The solution was stirred at room temperature for 1hr. washed with saturated $NaHCO_3$ (5

ml). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The product was purified by recrystallization from $CHCl_3$ and petroleum ether.

The deprotected single dipeptide units were coupled with one amino acid methyl ester hydrochloride unit using DIPC/NMM to get the protected tripeptide by the procedure similar to that of the dipeptides, in similar way penta and hexa peptide was prepared by deprotection of amino acid methyl ester and Boc-respetively to get protected hexapeptide.

Preparation of the Cyclic Peptides:

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 :

The Boc-peptide carboxylic acid (1.5 mmol) was dissolved in $CHCl_3$ (15 ml) at 0^0C. Then *p*-nitrophenol was added (0.27 g, 2 mmol) and stirred for 12 hours 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 hour 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), pyridine (1.4 ml, 2mmol) was added and kept at 0^0C for 10 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.

Physical data of cyclic hexapeptide given in Table-1

Evaluation of antimicrobial activity:

Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli were cultivated in nutrient broth medium. Candida albicans by Fluid Sabraud's medium.

Evaluation of the antimicrobial activity of all compounds was initially performed employing the disc diffusion technique[3]. Each compound was tested at a level of 50 µg/disc using dimethyl formamide (DMF) as the solvent. Ampicillin and Grisiofulvin were used as standard antibiotics against bacterial and fungal species at 10 and 25µg/disc, respectively. The diameter of zone of inhibition was measured after 24h and 48h at 37^0 C. Results of antimicrobial activity showed in table-2

Evaluation of insecticidal activity:

The insecticidal activity test of cyclic hexapeptide on *Coptotermes formasanus* was investigated according to the method of Inamori et al[4].

100mg. of the test sample was dissolved in 8ml of acetone. 4ml of the test sample was transferred volumetrically into a second container and diluted with another 4ml of acetone. Again 4ml of the second solution was transferred volumetrically into a third container & was diluted with 4ml of acetone. Thus the test solutions were prepared at 5 different concentrations. In similar way solutions with standard drug were prepared at 5 different concentrations[5-10].

Results of antimicrobial activity showed in Table-3.

RESULTS AND DISCUSSION

IR, ^{1}H NMR and FABMASS spectral data confirmed the molecular structure of synthesized compound. The spectral data given below.

^{1}H NMR (300MH$_Z$, CDCl$_3$):

δ 8.15 (1H, br. s, NH), δ7.8 (1H, br, s, NH), δ6.25 (1H, br, s, NH)δ6.1(1H, br, s, NH), δ5.3 (1H, m, NH), δ 5.15 (1H, d, α-H), δ4.7 (1H, t, α-H), δ4.6(1H, d, m, α-H), δ 4.3-4.0(2H, M, CH_2,of β-Ala), δ3.8-3.5(2H, m, α -CH_2 of β-Ala), δ3.5-3.2(6H , β-CH_2 of Phe, N-CH_2 OF Pro, β-CH-of Val), δ 2.2-1.8(7H, m, β -CH_2 & γ-CH of Pro , β-CH of Val), δ 1.1-0.8(18H, d, -CH_3 protans).

IR ($CHCl_3$): 3361.5(NH-stretch.), 3064.0(Ar-CH stretch.), 2932.2(aliph.-CH stretch.), 1720(C=O of ester), 1648.5(C=O of amide), 1534.7(-NH bend), 1453.7 (-CH bend) Cm^{-1}.

FABMASS in m/z: Cyclo[Leua-Pro-Phe-Val-Val-β-Ala]

Molecular ion peak was observed at m/z 626 corresponds to the molecular formula $C_{34}H_{51}N_5O_7$.

Table 1: Physical data of cyclic peptide

Sl No.	Cyclic peptide	Physical state	Rf value	% Yield
1.	Cylco[Leu-Pro-Phe-Val-Val-β-Ala]	Semisolid mass	0.59	73.17

Solvent system: Methanol: Chloroform: Water = 3:5:2

Table 2: Results of Antimicrobial activity

Sl. No.	Compound	Diameter of Zone of inhibition(mm)				
		B. sub	***S. aur***	***E. coli***	***P. aer***	***C. alb***
1.	CP1	12	16	15	14	19
2.	Ampicillin	14	17	16	18	-
3.	Griseofulvin	-	-	-	-	16
4.	DMF	-	-	-	-	-

(-) indicates no inhibition zone (no activity)

Table 3: Results of Insecticidal activity

Sample→ Conc.mg/ml↓	% of Mortality			
	Compound 1		Standard	
	12 h	24h	12h	24h
0.2	100	100	100	100
0.1	100	100	91	100
0.05	100	100	60	72
0.025	76	89	54.5	58
0.0125	45	54.5	25	50

Note: Experimental size 25 insects / group, Temperature 28^0 C.
No death of insect was observed in the control even after 72 h.

CONCLUSION

From the results given above, the structure of cyclic hexapeptide confirmed by IR, ^{1}H NMR and the m/z value by FABMASS.

Biological results of the synthesized compound showed moderate antibacterial activity in comparison to the standard drug Ampicillin. Antifungal activity of newly synthesized cyclic hexapeptide showed significant activity at concentration of 50µg/disk in comparison to standard drug Griseofulvin. The cyclic peptide showed potent insecticidal activity against *C. formosanus* even at the conc. of 0.05mg/cm^2 as compared to the standard Chloropyrifus.

Finally the cyclic hexapeptide proves that presence of N-methyl groups & heterodetic linkages are not essential in destruxin B to exhibit antifungal and insecticidal activity.

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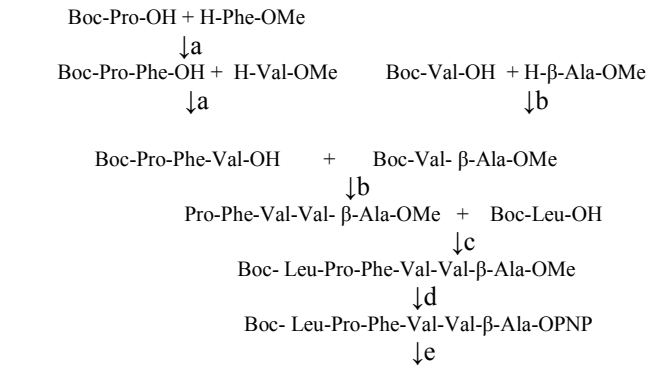
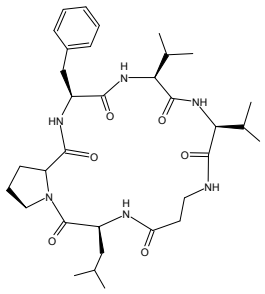


a → i) DIPC, Et_3N, 24h
ii)LiOH, THF:H_2 O (1:1) reflux, 15 mins.
b → i) DIPC, Et_3N, 24h
ii) TFA, $CHCl_3$, 1h
c → DIPC, Et_3N, 24h
d → i)LiOH, THF:H_2 O (1:1) reflux, 15 mins.
ii)p-nitrophenol, DIPC, Et_3N, 24h
iii)TFA, $CHCl_3$, 1h
e → NMM, $CHCl_3$, 0^0, 5days



Source of support: Nil, Conflict of interest: None Declared