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Research Article

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Designing and synthesis of biologically active natural octapeptide, polycarponin B and its analog

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ABSTRACT

The present study describes designing of Polycarponin B and its N-methylated analog by retrosynthetic analysis. Synthesis of the titled compounds was then carried out by solution phase technique. The structure of the synthesized compounds was confirmed by IR, ^{1}H NMR, ^{13}C NMR, Mass and elemental analysis. The compounds were evaluated for antimicrobial, antifungal and antioxidant activity. From the results of biological activity it was concluded that newly synthesized compound possessed very good activity against Gram positive bacteria and moderate activity against Gram negative bacteria and pathogenic fungi. The N-methylated analog of Polycarponin B showed increase in the antimicrobial and antioxidant activity in comparison with Polycarponin B.

Keywords: cyclopeptide, antimicrobial, antioxidant, *p*-nitro phenyl ester method

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INTRODUCTION

Owing to the different pharmacological activities possessed by various cyclic peptides, synthesis of naturally active cyclic peptides has attracted much attention of the researchers. Keeping in view of significant biological activities exhibited byvarious cyclic peptides[1-5], as a part of ongoing study, an attempt was made towards the synthesis of Polycarponin B a cyclic octapeptide, cyclo (-Gly-Ile-Val-Leu-Val-Gly-Leu-Pro) isolated from the whole plants of *polycarpon prostratum*[6]. As N-methylation of cyclic peptides was found to show increase in the biological activity, N-methylated analog of Polycarponin B was also synthesized[9,10]. The synthesized compounds were tested for antimicrobial and antioxidant activity by following standard protocol. The compounds had also shown very good activity against gram positive bacteria's and moderate activity against pathogenic fungi in comparison with benzyl penicillin and fluconazole, as standard. The synthesized compounds had also shown good antioxidant activity and N-methylated analog had shown potent activity in comparison with Polycarponin B.

EXPERIMENTAL SECTION

All the amino acids and other chemicals required to carry out the synthesis were purchased from Spectrochem Limited (Mumbai, India). Melting points were determined by using SYSTRONIC digital melting point apparatus. The chemical structures of newly synthesized compounds were confirmed by means of spectral as well as elemental analysis. The IR spectra were run on JASCO 4100 FTIR spectrophotometer using a thin film supported on KBr

pellets or utilizing chloroform and NaCl cells. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC NMR spectrometer using DMSOas a solvent. The mass spectrum of the cyclopeptide was recorded on JMS-DX 303 Mass spectrometer operating at 70 eV by ESIMS/MS.

In order to carry out the total synthesis of cyclopeptide, Polycarponin B, cyclo (-Gly-Ile-Val-Leu-Val-Gly-Leu-Pro), it wasdisconnected into four dipeptide units through retrosynthetic analysis. The four dipeptide units (1, 2,3,4) were then prepared by coupling Boc- amino acids with respective amino acid methyl ester hydrochlorides by using DIPC as coupling agent. The dipeptide units were then again coupled together to get tetrapeptides (5, 6). The tetrapeptides were coupled to get linear octapeptide (7), which was then cyclized by using p-nitro phenyl ester method[7] to get titled compound(8). The ester group was removed by using LiOH, before cyclization. The analog of titled compound was prepared by N-methylation at valine by following standard protocol[10].

Antimicrobial activity

The synthesized compounds were screened *in vitro* for its antibacterial and antifungal activity by using disc diffusion method and tube dilution technique. The bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeuroginosa*) and fungal strains (*Candida albicans* and *Aspergillus niger)* were obtained from the national collection of industrial micro-organisms (NCIM), branch of national chemical laboratory (NCL) Pune, India.

For the disc diffusion method, the activity studies were carried out according to modified Kirby-Bauer method[11]. Benzyl penicillin and fluconazole were used as standards against bacterial and fungal strains, respectively at a concentration of50 µg/ml. Nutrient broth and Sabourds agar were used as a medium and dimethylformamide (DMF) was used as a solvent control for carrying out the activity. After preparation of the disc, allowed to stand for 24 h at37^{0}. The zone of inhibition, observed around the disks after incubation, was measured.

Compound inhibiting growth of microorganisms was further tested for minimum inhibitory concentration (MIC). A solution of the compound was prepared in DMF and a series of doubling dilutions prepared with sterile pipettes so as to get the conc. from 500-62.5µg/ml. To each of a series of sterile stoppered test tubes, a standard volume of nutrient broth medium was added. A control tube containing no antimicrobial agent was included. The inoculum consisting of an overnight broth culture of microorganisms was added to separate tubes. The tubes were incubated at 37^{0}for 24 h and examined for turbidity. The tube with highest dilution showing no turbidity was the one containing compound with MIC. Screening data of antibacterial and antifungal activity revealed that the synthetic peptide and its analogue are found to be active. The results are shown in Tables 1 and 2.

From the results of biological activity it was concluded that newly synthesized compounds possessed very good activity against *B. subtilis* and *S.aureus*(Gram positive bacteria) and moderate activity against *E. coli* and *P. aeruginosa (*Gram negative bacteria) and pathogenic fungi. The N-methylated analog of Polycarponin B showed more antimicrobial and antioxidant activity in comparison with Polycarponin B.

Antioxidant activity[12]:

DPPH radical scavenging activity: DPPH is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole and the delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substrate that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color.

In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals was monitored.

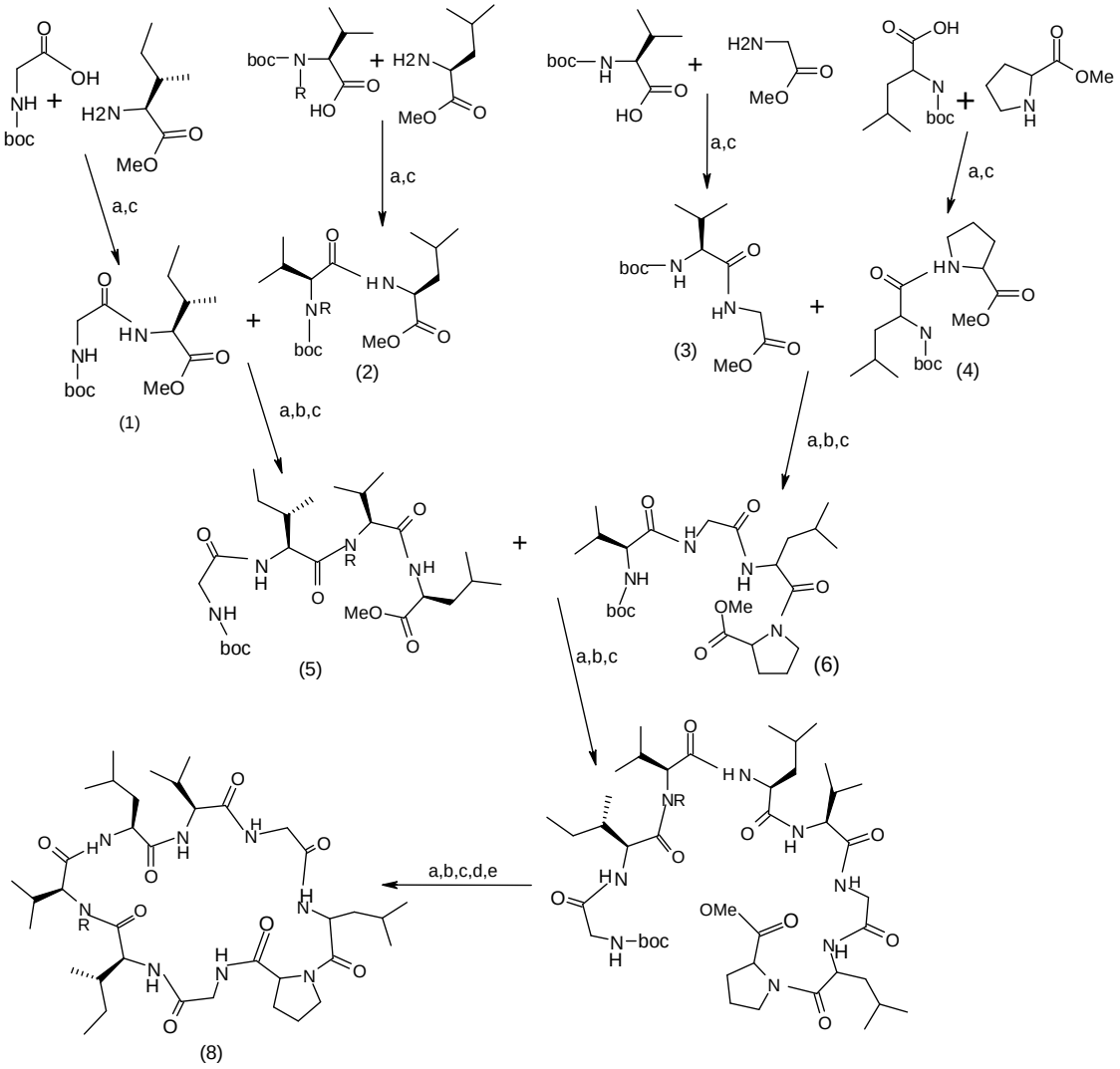
The effect of synthesized compound on DPPH radical was assayed using the method of Mensor et al (2001)17. A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different conc. of synthesized compound and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without test compound served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$\textbf{\% scavenging activity} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Where A control = absorbance of DPPH in methanol
A sample= absorbance of Sample/ standard+ DPPH+ methanol

The results for antioxidant activity are shown in Tables 3

Scheme 1: Synthetic route for Polycarponin B and its analog



For Poly B, R= H and ***Where:*** *a= DIPC, NMM, $CHCl_3$, RT, 24h,*
For analog, R= CH_3 b= TFA, NMM, RT, 1h,
c= LiOH, THF: H_2O(1:1), reflux, 15 mins
d= pnp-, $CHCl_3$, RT, 12h,
e= NMM, $CHCl_3$, 0°C, 7days

RESULTS AND DISCUSSION

Spectral Data:

1) Polycarponin B:

Physical state: Colorless solid needles

IR Data: shows peak at 290 and 1640 cm^{-1} which is characteristic peak of amino and amide carbonyl group

FABMS showed M^+ ion peak at *m/z* 749, which matches to the molecular formula $C_{37}H_{64}O_8N_8$

^{13}C NMR: showed presence of eight amide carbonyl signals
δ170.4(C=O of Gly), δ44.9 (Cα of Gly), δ169.3 (C=O of Gly_2), 44.9(Cα of Gly_2), δ173.4 (C=O of Pro), δ59.7 (Cα of Pro), δ 29.7 (Cβ of Pro), δ 26.5(Cγ of Pro), δ172.5(C=O of Ile),δ60.2(Cα of Ile), δ 36.9 (Cβ of Ile), δ 25.6, 16.4 (Cγ of Ile),δ171.1(C=O of Val),δ61.9(Cα of Val), δ 30 (Cβ of Val), δ 19.1, 19.7 (Cγ of Val),δ172.7(C=O of Val_2),δ62.7(Cα of Val_2), δ 30.9 (Cβ of Val_2), δ 19.3, 19.6 (Cγ of Val_2),δ171.4(C=O of Leu),δ50.1(Cα of Leu), δ 41.1 (Cβ of Leu), δ 25.2 (Cγ of Leu),δ173.4(C=O of Leu_2),δ53.7(Cα of Leu_2), δ 42.4(Cβ of Leu_2), δ25.6(Cγ of Leu_2).

^{1}H NMR: showed seven amide N-H signals
δ10.20, (HN of Gly), δ 4.46 (dd, Hα of Gly), δ 3.97 **(**dd, Hα of Gly**),** δ 9.53 (dd, HN of Gly_2), δ 4.79 **(**dd, Hα of Gly_2**),** δ 4.74 and 2.08, 1.92 (Hα, Hβ and Hγof Pro), δ 8.87 (HN of Ile), δ 4.6 and 2.38**(**Hα, Hβof Ile), δ 8.42, 4.41, 1.04(H**N,** Hα, and Hγ of Val), δ 9.4, 4.6, 2.04(H**N,** Hα, and Hβ of Val_2), δ 7.69 d, 5.3, 2 (H**N,** Hα, and Hβ of Leu),δ 8.69 d, 7.1, 3.1(H**N,** Hα, and Hβ of Leu_2)

Elemental Analysis: C: 63.12, H: 12.19, N: 12.69, O: 14.59

2) Polycarponin B analog:
Physical state: Colorless solid needles
IR Data: shows peak at 290 and 1640 cm^{-1} which is characteristic peak of amino and amide carbonyl group. It also showed strong peak at 1695which is characteristic peak for N-CH_3 stretch.

FABMS showed M^+ ion peak at *m/z* 764, which matches to the molecular formula $C_{38}H_{66}O_8N_8$

^{13}C NMR: showed peak atδ38.8 (3H, N-CH_3 of Val) which characterizes substituted methyl group
δ170.1(C=O of Gly), δ 44.6 (Cα of Gly), δ169 (C=O of Gly_2), 45.1(Cα of Gly_2), δ173.6(C=O of Pro), δ59.9 (Cα of Pro), δ 29.9 (Cβ of Pro), δ 26.6(Cγ of Pro), δ172.8(C=O of Ile),δ60(Cα of Ile), δ 37(Cβ of Ile), δ 25.8, 16.6 (Cγ of Ile),δ170.9(C=O of Val),δ61.9(Cα of Val), δ 30 (Cβ of Val), δ 19.3, 19.6 (Cγ of Val),δ172.6(C=O of Val_2),δ62.6(Cα of Val_2), δ 30.7 (Cβ of Val_2), δ 19.3, 19.7 (Cγ of Val_2),δ171.1(C=O of Leu),δ50.4(Cα of Leu), δ 41 (Cβ of Leu), δ 25.3 (Cγ of Leu),δ173.6(C=O of Leu_2),δ53.5(Cα of Leu_2), δ 42.4(Cβ of Leu_2),

^{1}H NMR: showed peak at δ 2.9(3H, N-CH_3 of Val) which characterizes substituted methyl group
δ 10.21, (HN of Gly), δ 4.44 (dd, Hα of Gly), δ 3.96 **(**dd, Hα of Gly**),** δ 9.55 (dd, HN of Gly_2),δ 4.78 **(**dd, Hα of Gly_2**),** δ 4.74 and 2.06, 1.92 (Hα, Hβ and Hγ of Pro),δ 8.88 (HN of Ile)

δ 4.6 and 2.34**(**Hα, Hβof Ile), δ 8.44, 4.42, 1.03(H**N,** Hα, and Hγ of Val), δ 9.2, 4.4, 2.02(H**N,** Hα, and Hβ of Val_2), δ 7.66 d, 5.1, 2 (H**N,** Hα, and Hβ of Leu),δ 8.66 d, 7.2, 3.3(H**N,** Hα, and Hβ of Leu_2)

Results of Biological Activity:

Table No.1: Results of Antimicrobial activity by using Disc diffusion method

Compound	Diameter of zone of inhibition					
	S. aureus,	***B. subtilis,***	***E. coli,***	***P. aeuroginosa***	***C. Albicans***	***A. Niger***
Poly B	20	13	10	11	14	12
analog of Poly B	22	15	12	12	15	14
Benzyl Penicillin	25	15	16	17	-	-
Fluconazole	-	-	-	-	20	18
DMF	-	-	-	-	-	-

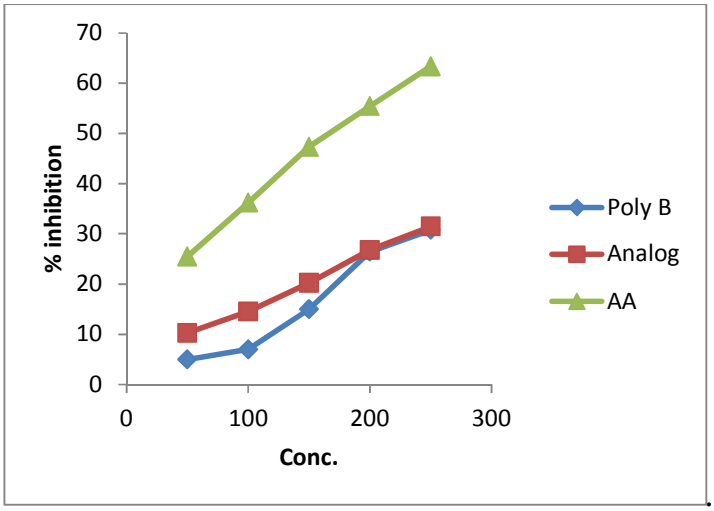
Table No.2: Results of MIC for the test compounds by using Tube dilution technique

Compound	Minimum inhibitory Concentration					
	S. aureus,	***B. subtilis,***	***E. coli,***	***P. aeuroginosa***	***C. Albicans***	***A. Niger***
Poly B	62.5	62.5	125	250	500	500
analog of Poly B	31.25	62.5	62.5	250	250	250
Benzyl Penicillin	0.95	1.9	3.9	62.5	-	-
Fluconazole	-	-	-	-	15.6	15.6

Table No.3: Results of Antioxidant activity by using DPPH method

Compound	% inhibition					
Conc (µg/ml)	**50**	**100**	**150**	**200**	**250**	**IC 50**
Poly B	11.28	16.25	20.12	26.45	30.78	446.77
analog of Poly B	10.28	14.54	20.25	26.78	31.47	419.46
Ascorbic acid	25.48	36.2	47.33	55.41	63.31	182.88

Fig. 1: DPPH radical scavenging activity of synthesized compounds in comparison with standard



CONCLUSION

The designed compounds have been synthesized with good yield by using solution phase synthesis. The synthesized compounds had shown very good activity against gram positive bacteria's and moderate activity. The compounds had also shown good antioxidant activity and N-methylated analog had shown potent activity in comparison with Polycarponin B. Synthesis of N- methylated analogs of the parent molecule may lead to the development of more active analog.

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