UNIQUE JOURNAL OF PHARMACEUTICAL AND BIOLOGICAL SCIENCES
Research Article

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME CYCLIC PENTAPEPTIDES

M. Himaja[1*], Archana[2], Karigar Asif[3]

[1]Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore 632014, India
[2]Dept of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, Mangalore, KTK, India
[3]Dept of Pharmaceutical Analysis, Maratha Mandal College of Pharmacy, Belgaum, Karnataka, India

*Corresponding Author: **Dr. (Mrs). M. Himaja**,
Professor, Pharmaceutical Chemistry Division. School of Advanced Sciences, VIT University, Vellore – 632014 Tamil Nadu, India.
Email: dr_himaja@yahoo.com Mobile: +91-9944796228

Received 30-05-2013; Revised 24-06-2013; Accepted 03-07-2013

ABSTRACT

Cyclic peptides exhibiting antimicrobial activity showed that almost all cyclic peptides contain proline, valine, phenylalanine in their structures. Three cyclic pentapeptides have been designed containing nitro-arginine, two proline units, phenylalanine and a valine unit, with varied sequences in order to carry out a study on variation of activity with variation in the sequence of amino acids. Structures of all the newly synthesized compounds were confirmed by FTIR, ^{1}H NMR and Mass spectral data. All the synthesized analogs were screened for their bactericidal activity and fungicidal activity. Bactericidal activity against two Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and two Gram-negative bacteria *Escheridhia coli*, *Pseudomonas aeruginosa* and fungicidal activity against two fungi *Aspergillus niger* and *Candida albicans* and found to be active against these microorganisms.

Keywords: Cyclic Pentapeptides, Amino acids, Dicyclohexylcarbodiimide, Antibacterial activity, Antifungal activity.

INTRODUCTION

Peptides are the important class of organic compounds with potent biological activities[1-7]. A review of the structures of cyclic peptides exhibiting antimicrobial activity showed that almost all cyclic peptides contain proline, valine, phenylalanine in their structures. Moreover, compounds containing proline units showed good antimicrobial activity.

Peptide antibiotics are catagorised as homomeric (built up of only amino acids) and heteromeric (containing amino acids along with other moieties). Further division leads to homodetic and heterodetic peptides (rings containing other linkages) such as depsipeptides, which contain hydroxy and amino acid residues joined by amide and ester bonds.

Antibiotics effective against all microorganisms are not likely to be found, and a reasonable effort should be concentrated on the more specialised antibiotics. The following have been clinically used effectively: bacitracins, capreomycin, cycloserine, actinomycin D, gramicidin S, polymixin, tyrocidine and viomycin.

The inherent medicinal properties of cyclic peptides prompted scientists to isolate these compounds from natural sources. Since minute quantities are obtained from natural sources, many of these compounds are attempted to synthesise in the laboratories of many scientists. Antibacterial activity studies performed on these synthetic peptides proved to give good results.

MATERIALS AND METHODS

All the three cyclic pentapeptides contain two proline units, one phenylalanine unit, one valine unit and a nitro-arginine unit with varied sequence. Synthesis of the cyclic pentapeptides was carried out by the solution phase peptide synthesis.

In order to carry out the synthesis, all the three cyclic pentapeptides were disconnected into two dipeptide units and a single amino acid Boc-(nitro)Arginine. The dipeptides were prepared from the respective protected amino acids. The amino group was protected with tert. Butyloxycarbonyl (Boc-) group and the carboxyl group was protected by converting into the methyl ester. The Boc-amino acids were coupled with the amino acid methyl ester hydrochlorides by dicyclohexylcarbodiimide (DCC) as the coupling agent and N-methylmorpholine (NMM) as the base to get the protected dipeptides. These are coupled, after appropriate deprotection, with Boc-(nitro)arginine to get the pentapeptides, which were finally cyclised by p-nitrophenyl ester method using high-dilution technique to get the cyclic pentapeptides.

Synthesis of Cyclic Pentapeptide 1:

Cyclic pentapeptide: cyclo[nitroArg-Phe-Pro-Pro-Val] (1) was disconnected into Boc-Phe-Pro-OMe (2), Boc-Pro-Val-OMe (3) and Boc-(nitro)Arginine (4).

The synthesis of the pentapeptide 1 (1) was carried out as follows.

The ester group of Boc-Phe-Pro-OMe (2) was removed with LiOH and the Boc-group of Boc-Pro-Val-OMe (3) was removed with trifluoroacetic acid (TFA).

The deprotected dipeptides were coupled using DCC and NMM to get the tetrapeptide Boc-Phe-Pro-Pro-Val-OMe (5).

The Boc-group of the tetrapeptide was removed using TFA. The deprotected unit was coupled with Boc-(nitro)Arg (4) to obtain the pentapeptide Boc-(nitro)Arg-Phe-Pro-Pro-Val-OMe (6).

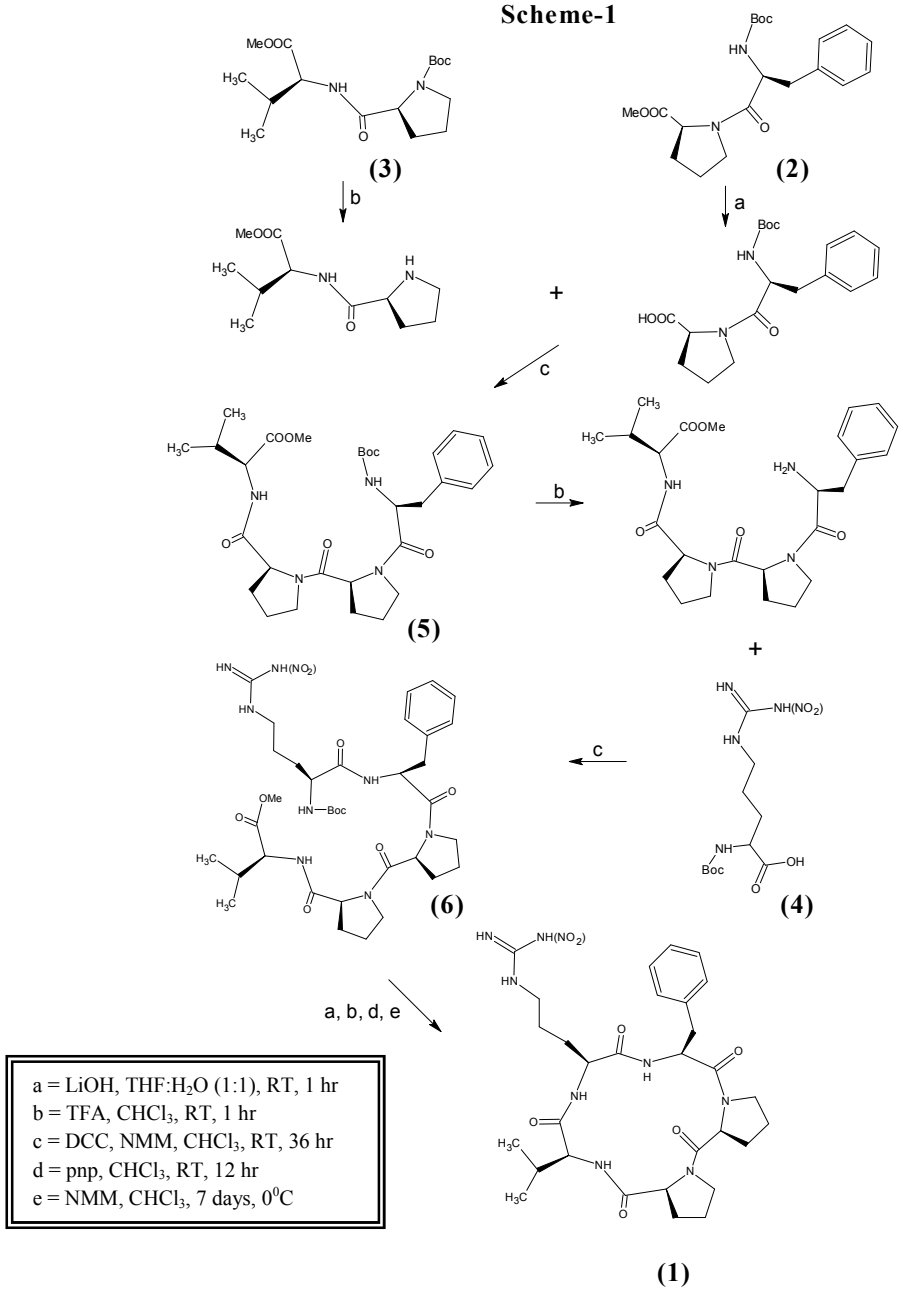
The ester group of the pentapeptide was removed by LiOH and p-nitrophenyl group (pnp-) was introduced.

The Boc- group was removed by TFA and the linear fragment was cyclised by adding NMM and keeping the whole contents at 0^0C for seven days to get the cyclic pentapeptide-1 (1).

The structure of the molecule was confirmed by IR, ^{1}H NMR, FABMS and elemental analysis.

The above synthetic steps are shown in Scheme-1.

Scheme-1



Synthesis of Cyclic Pentapeptide 2:

Cyclic pentapeptide: cyclo[nitroArg-Pro-Phe-Pro-Val] (7) was disconnected into Boc-Pro-Phe-OMe (8), Boc-Pro-Val-OMe (3) and Boc-(nitro)Arginine (4).

The synthesis of the pentapeptide 2 (7) was carried out as follows.

The ester group of Boc-Pro-Phe-OMe (8) was removed with LiOH and the Boc-group of Boc-Pro-Val-OMe (3) was removed with trifluoroacetic acid (TFA).

The deprotected dipeptides were coupled using DCC and NMM to get the tetrapeptide Boc-Pro-Phe-Pro-Val-OMe (9).

The Boc-group of the tetrapeptide was removed using TFA. The deprotected unit was coupled with Boc-(nitro)Arg (4) to obtain the pentapeptide Boc-(nitro)Arg-Pro-Phe-Pro-Val-OMe (10).

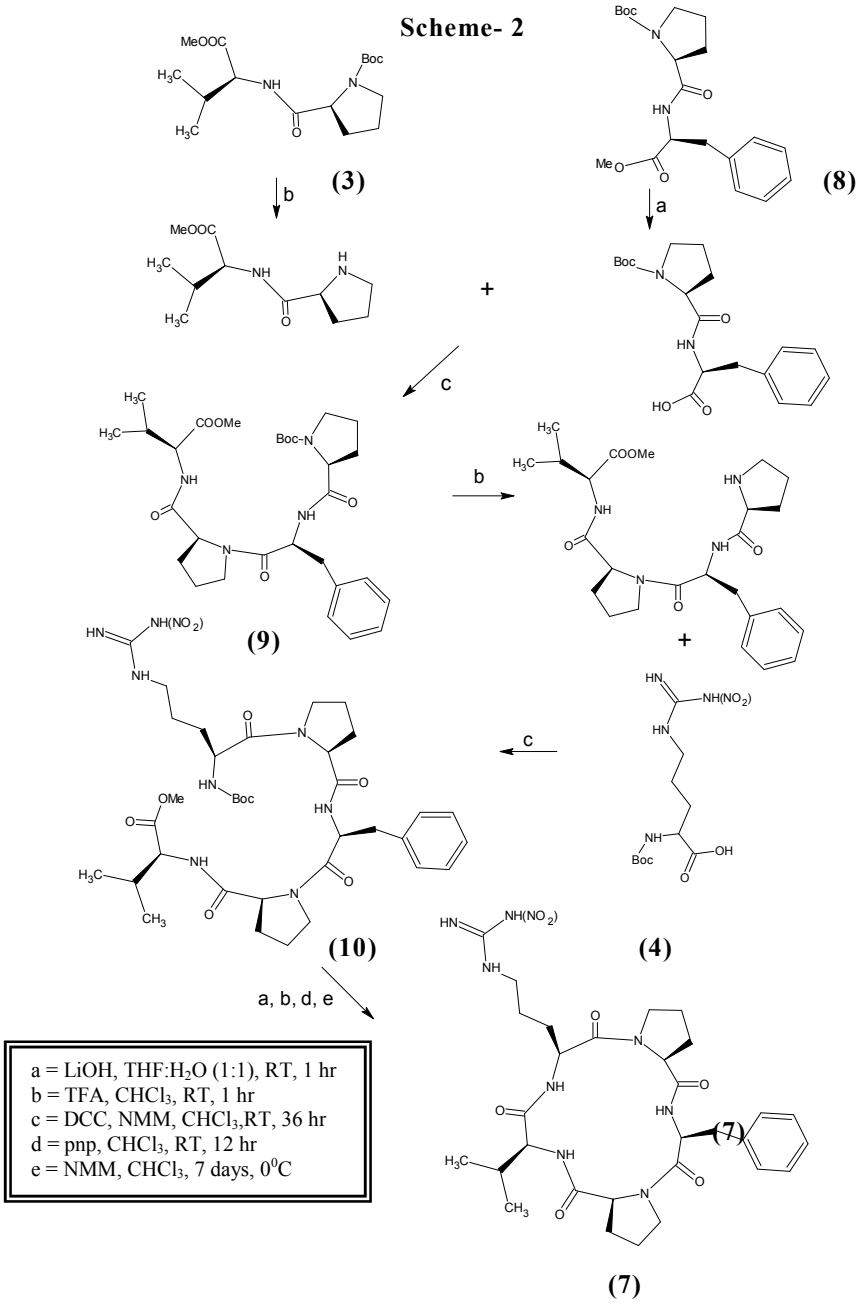
The ester group of the pentapeptide was removed by LiOH and p-nitrophenyl group (pnp-) was introduced.

The Boc-group was removed by TFA and the linear fragment was cyclised by adding NMM and keeping the whole contents at 0^0C for seven days to get the cyclic pentapeptide-2 (7).

The structure of the molecule was confirmed by IR, ^{1}H NMR, FABMS and elemental analysis.

The above synthetic steps are shown in Scheme-2.

Scheme- 2



Synthesis of Cyclic Pentapeptide 3:

Cyclic pentapeptide: cyclo[nitroArg-Pro-Val-Pro-Phe] (11) was disconnected into Boc-Pro-Val-OMe (3), Boc-Pro-Phe-OMe (8) and Boc-(nitro)Arginine (4).

The synthesis of the pentapeptide 3 (11) was carried out as follows.

The ester group of Boc-Pro-Val-OMe (3) was removed with LiOH and the Boc-group of Boc-Pro-Phe-OMe (8) was removed with trifluoroacetic acid (TFA).

The deprotected dipeptides were coupled using DCC and NMM to get the tetrapeptide Boc-Pro-Val-Pro-Phe-OMe (12).

The Boc-group of the tetrapeptide was removed using TFA. The deprotected unit was coupled with Boc-(nitro)Arg (4) to obtain the pentapeptide Boc-(nitro)Arg-Pro-Val-Pro-Phe-OMe (13).

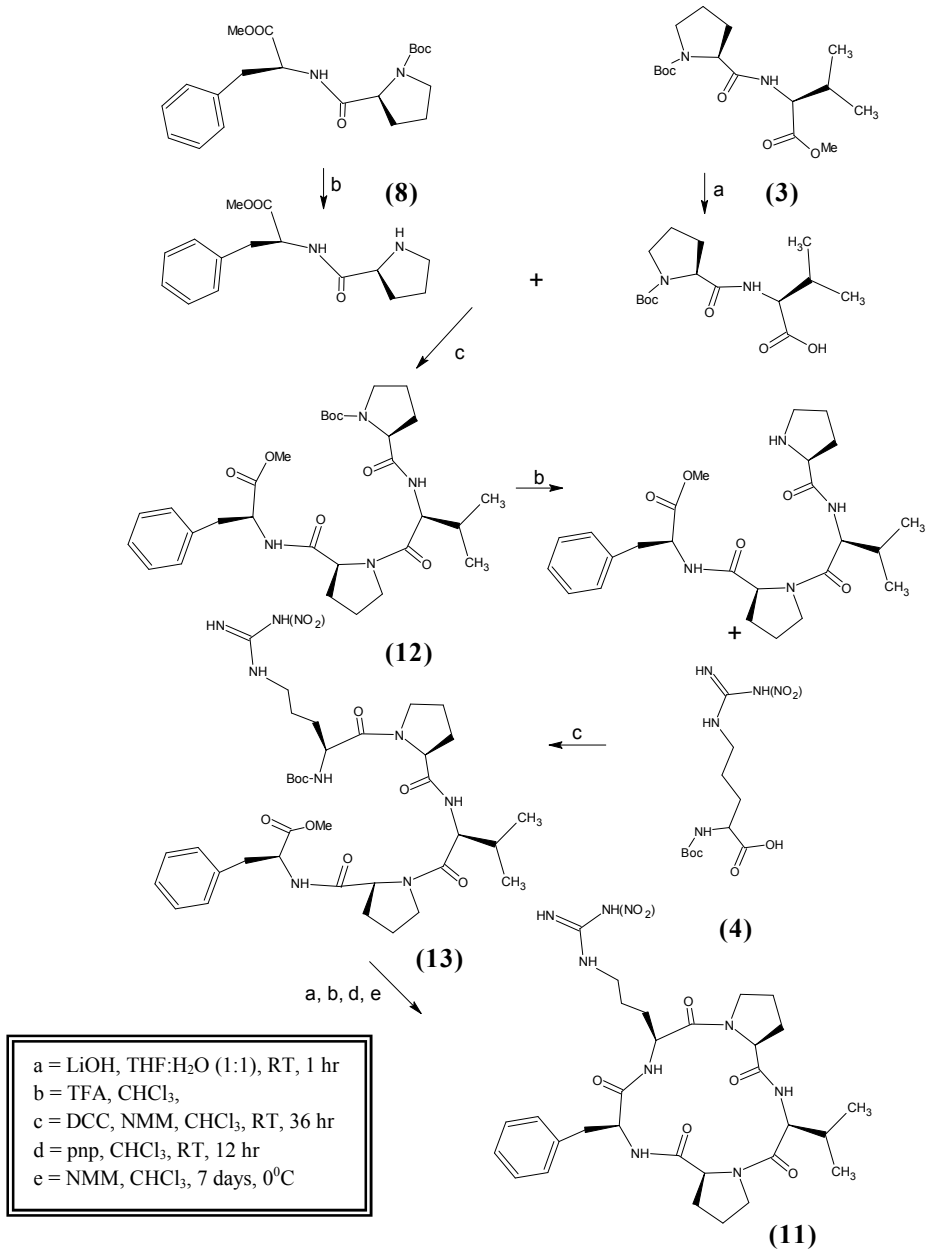
The ester group of the pentapeptide was removed by LiOH and p-nitrophenyl group (pnp-) was introduced.

The Boc- group was removed by TFA and the linear fragment was cyclised by adding NMM and keeping the whole contents at 0^0C for seven days to get the cyclic pentapeptide-3 (11).

The structure of the molecule was confirmed by IR, ^{1}H NMR, FABMS and elemental analysis.

The above synthetic steps are shown in Scheme-3.

Scheme- 3

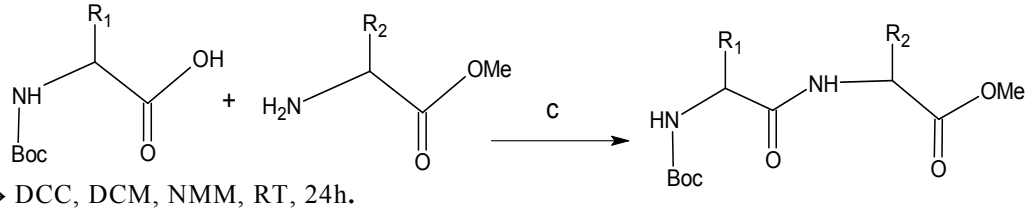


General Method of Peptide Synthesis:

Preparation of the Dipeptides:

Out of the various reported methods for peptide coupling, we adopted the methods proposed by Bodanszky[8] method, which was convenient and useful. From these procedures we derived our own more convenient method for the peptide coupling reactions. The dipeptides were prepared by using Boc-amino acid and amino acid methyl ester hydrochloride, TEA and DCC (Scheme 4).

Scheme 4



C → DCC, DCM, NMM, RT, 24h.

The structures of all the dipeptides were confirmed by spectral analysis.

Preparation of the tetrapeptides:

The tetrapeptides were prepared from the dipeptide units after appropriate deprotection at the desired functional groups. The Boc- group was deprotected by trifluoroacetic acid (TFA) and the ester group was removed by hydrolysing with LiOH. The following tetrapeptides were prepared by coupling the deprotected dipeptide units using the procedure similar to that of the dipeptide coupling (Table-2).

Preparation of the Pentapeptides (Linear Fragments)

The Boc-group of the tetrapeptides was removed by TFA/$CHCl_3$ and the deprotected units were coupled with Boc-(nitro)Arginine the procedure similar to that of the dipeptide coupling to get the pentapeptide 1,2 and 3. Structure of compounds was confirmed by spectral analysis (Table 3).

Cyclisation of the Linear Pentapeptide 1, 2 & 3:

Cyclisation of the linear pentapeptide unit was carried out by the p-nitrophenyl ester method of Bodanszky[8] with certain modifications.

The ester group of the linear fragment was removed with LiOH and the p-nitrophenyl eater group was introduced by stirring the deprotected pentapeptide in $CHCl_3$ with p-nitrophenol. The reaction mixture was washed several times with saturated $NaHCO_3$ until the unreacted p-nitrophenol was removed completely. The Boc- group was removed by TFA using the standard procedure. To the deprotected linear fragment a catalytic amount of N-methylmorpholine was added and the reaction mixture was kept at 0^0C for 10 days. Washed several times with saturated $NaHCO_3$ until the byproduct, p-nitrophenol was completely removed, finally washed with 5% HCl and water dried over anhydrous Na_2SO_4 and evaporated in vacuum to get the cyclised product which was crystallised from $CHCl_3$.

The structures cyclic pentapeptide 1, 2 and 3 were confirmed by IR, ^{1}H NMR, FABMS and elemental analysis.

Evaluation of Antimicrobial Activity

All the plates were kept in the refrigerator for 30 minutes to allow the diffusion of the sample into the surrounding agar medium. Then the plates inoculated with bacterial cultures were incubated at 37 ^{0}C for 18 hours and those with fungi were incubated at 25^0C for 48 hours. Diameter of the zones of inhibition wherever produced were measured and the average diameter for each sample was calculated. The diameters obtained for the test samples were compared with that produced by the standard antibiotics, benzylpenicillin for antibacterial activity and griseofulvin for antifungal activity[9]. The results are given in Table 5.

RESULTS AND DISCUSSION

Evaluation of antibacterial and antifungal activity was carried out for all the synthesised cyclic pentapeptides. All the compounds showed moderate antibacterial and antifungal activities.

Cyclic Peptide 1: cyclo[nitro-Arginyl-Phenylalanyl-Prolyl-Prolyl-Valine] (1)

^{1}H NMR (300MHz, $CDCl_3$) : δ7.4-7.0(6H, m), 6.9(2H, d, J = 7.2Hz), 6.4-6.2(1H, br. S), 5.2-5.1(2H, br. S), 4.9-4.6(2H, m), 4.6-4.4(1H, m), 4.4-4.1(2H, m), 3.85-3.6(4H, m), 3.6-3.0(4H, m), 2.4-1.6(8H, m), 1.2-1.0(1H, m), 0.9(6H, d, J = 7.0Hz).

IR ($CHCl_3$): 3290(br. s), 2910 (s), 2885(s), 1740(s), 1690(s), 1675(s), 1670(s), 1660(s), 1495(s), 1410(m), 1380(s), 1330(s), 1135(m), 1050 (br. s), 910(s)cm^{-1}.

FABMS: m/z $(M + 1)^+$: 643

Cyclic Peptide-2: cyclo[nitro-Arginyl-Prolyl-Phenylalanyl-Prolyl-Valine](7)

^{1}H NMR (300MHz, $CDCl_3$) : δ7.8-7.6(4H, m), 7.6-7.2(2H, m), 7.0(2H, d, J = 7.2Hz), 6.5-6.3(1H, br. S), 5.2-5.0(2H, br. S), 4.9-4.6(2H, m), 4.6-4.4(1H, m), 4.4-4.2(2H, m), 3.9-3.6(4H, m), 3.6-3.0(4H, m), 2.4-1.6(8H, m), 1.2-1.0(1H, m), 0.9(6H, d, J = 7.0Hz).

IR ($CHCl_3$): 3300(br. s), 2950(s), 2880(s), 1745(s), 1700(s), 1685(s), 1670(s), 1660(s), 1495(s), 1380(s), 1330(s), 1135(m), 1050 (br. s), 910(s)cm^{-1}.

FABMS: m/z $(M + 1)^+$: 643

Cyclic Peptide-3: cyclo[nitro-Arginyl-Prolyl-Valyl-Prolyl-Phenylalanine] (11)

^{1}H NMR (300MHz, $CDCl_3$) : δ7.6-7.2(6H, m), 6.9(2H, d, J = 7.2Hz), 6.4-6.2(1H, br. s), 5.2-5.0(2H, br. s), 4.85-4.6(2H, m), 4.7-4.4(1H, m), 4.5-4.1(2H, m), 3.85-3.6(4H, m), 3.6-3.2(4H, m), 2.5-1.6(8H, m), 1.2-1.0(1H, m), 0.9(6H, d, J = 7.0Hz).

IR ($CHCl_3$): 3320(br. s), 2990 (s), 1735(s), 1695(s), 1670(s), 1655(s), 1640(s), 1495(s), 1415(m), 1390(s), 1330(s), 1135(m), 1050 (br. s), 915(s)cm^{-1}.

FABMS: m/z $(M + 1)^+$: 643

Table 1: Physical data of the synthesised dipeptides

Sl. No	Boc-dipeptide-OMe	Physical state	% Yield
1	Boc-Pro-Val-OMe	Viscous liquid	90.58
2	Boc-Pro-Phe-OMe	Semisolid mass	90.94
3	Boc-Phe-Pro-OMe	Semisolid mass	85.04

Table 2: Physical Data of the Tetrapeptides

Sl. No	Boc-tetrapeptide-OMe	Physical state	% Yield
1	Boc-Phe-Pro-Pro-Val-OMe	Semisolid mass	94.79
2	Boc-Pro-Phe-Pro-Val-OMe	Semisolid mass	96.25
3	Boc-Pro-Val-Pro-Phe-OMe	Semisolid mass	93.66

Table 3: Physical Data of the Pentapeptides

Sl. no	Boc-tetrapeptide-OMe	Physical state	% Yield
1	Boc-(nitro)Arg-Phe-Pro-Pro-Val-OMe	Semisolid mass	89.41
2	Boc-(nitro)Arg-Pro-Phe-Pro-Val-OMe	Semisolid mass	98.93
3	Boc-(nitro)Arg-Pro-Val-Pro-Phe-OMe	Semisolid mass	97.88

Table 4: Physical Data of the Cyclic Pentapeptides

Sl no	Cyclised Product	Physical state	% Yield
1	Cyclo-(nitro)Arg-Phe-Pro-Pro-Val-OMe	Semisolid mass	87.2
2	Cyclo-(nitro)Arg-Pro-Phe-Pro-Val-OMe	Semisolid mass	87.2
3	Cyclo-(nitro)Arg-Pro-Val-Pro-Phe-OMe	Semisolid mass	89.3

Table 5: Data of Antimicrobial Activity

Sl. No.	Compound No.	Diameter of Zone of Inhibition (in mm)					
		B. sub.	*S. aur.*	*E. coli*	*P. aer.*	*C. alb.*	*A. nigar*
1	1	11	–	9	–	9	8
2	7	10	11	10	11	9	10
3	11	12	10	11	10	8	8
4	Benzyl penicillin	15	16	14	16	–	–
5	Griseofulvin	–	–	–	–	15	16

CONCLUSION

Three cyclic pentapeptides have been synthesized by prescribed Scheme with good yields by solution phase peptide synthesis. Structures of all the newly synthesized compounds were confirmed by FTIR, 1H NMR and Mass spectral data. All the synthesized analogs were screened for their bactericidal activity and fungicidal activity. Bactericidal activity against two Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and two Gram-negative bacteria *Escheridhia coli*, *Pseudomonas aeruginosa* and fungicidal activity against two fungi *Aspergillus niger* and *Candida albicans*, All the synthesized compounds found to be active against test microorganisms.

REFERENCES

1. Beverly AT, Ara G, Roy H, Palombella VJ, Julian A, The Proteasome Inhibitor PS-341 in Cancer Therapy, Clinical Cancer Research., 1999; 5: 2638.
2. Boger DL, Jiacheng Zhou, Brian Writer and Kitos PA. Key analogs of the Tetrapeptide Subunit of RA-VII and Deoxybouvardin, Bioorganic and Medicinal Chemistry, 1995; 3: 1579.
3. Katsuhirokudo SO, Yasuyuki T, Benkeblia N, Shiomi N, Antioxidative activities of some peptides isolated from hydrolyzed potato protein extract", Journal of functional foods., 2009; 1: 170.
4. Luke Simmons KL. McPhail B, Eduardo OB, Susan L, William HG, Belamide A, A new antimitotic tetrapeptide from a Panamanian marine cyanobacterium", Tetrahedron Letters., 2006; 47: 3387.
5. Gunnur Dikmen Z, Gellert GC., Pakize Dogan, Heejeong Yoon, Lee YB, Chang HA, Shay JW. In vivo and Invitro Effects of a HIF-1α Inhibitor, RX-0047, Journal of Cellular Biochemistry, 2008; 104: 985.
6. Lee MD, Yuhong MJ, Soskis CP, Borella M. Humanmitochondrial peptide deformylase, a new

anticancer target of actinonin-based antibiotics, J. Clinical Investigation., 2004; 114: 8.
7. Alejandro MS. Mayera, Kirk R. Gustafson. Marine pharmacology in 2005–2006: Antitumor and cytotoxic compounds, European journal of Cancer, 2008; 44: 2357.
8. Bodanszky M, and Bodanszky A. Practice of Peptide Synthesis. (Springer-Verlag, New York); 1984. p. 78.
9. Clinical microbiology procedures handbook. Henry D Isenberg. Volume 1. American society for microbiology/ Washington, D.C, 1992.

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