ISSN 2348-232X

Unique Research Journal of Chemistry

Available online: www.ujconline.net

Research Article

SYNTHESIS AND ANTIOXIDANT ACTIVITY OF SYNTHETIC CYCLOHEPTAPEPTIDE

Karigar Asifiqbal A*

Professor and Head, Department of Pharmaceutical Analysis, Maratha Mandal's College of Pharmacy, Belgaum 590016, Karnataka, India

Received 20-04-2016; Revised 18-05-2016; Accepted 16-06-2016

*Corresponding Author: **Dr. Asifiqbal Karigar**
Professor and Head, Department of Pharmaceutical Analysis, Maratha Mandal's College of Pharmacy, Belgaum 590016, Karnataka, India.

ABSTRACT

Most of the cyclic peptides are found to exhibit antifungal, antibacterial, antitubercular, anthelmintic, cytotoxic and anti-inflammatory activities. Glaucacyclopeptide A is a cyclic heptapeptide isolated from the seeds of Annona glauca and belongs to family Annonaceae. It Possesses various biological activities. Keeping in view of the significant biological activities exhibited by glaucacyclopeptide A, as a part of ongoing study, an attempt was made to synthesize plain, glaucacyclopeptide A, and was characterized by IR, ^{1}H NMR and FAB-Mass spectral studies. The synthesized cylopeptide showed significant antioxidant activity

Keywords: Glaucacyclopeptide A, Cyclic heptapeptide, Solution Phase Synthesis, Antioxidant activity

INTRODUCTION

Most of the cyclic peptides are found to exhibit antifungal, antibacterial, antitubercular, anthelmintic, cytotoxic and anti-inflammatory activities. Glaucacyclopeptide A is a cyclic heptapeptide isolated from the seeds of Annona glauca and belongs to family Annonaceae. It Possesses various biological activities. Keeping in view of the significant biological activities exhibited by glaucacyclopeptide A, as a part of ongoing study, an attempt was made to synthesize plain, glaucacyclopeptide A[1-8].

MATERIALS AND METHODS

All the reactions required anhydrous conditions were conducted in flame dried apparatus. Solvents and reagents were purified by standard methods. Organic extracts were dried over anhydrous sodium sulphate. All amino acids and other chemicals were obtained from spectrochem Ltd (Mumbai, India). Melting points were determined in open capillary tubes and are uncorrected. Purity of the compounds was checked by pre-coated TLC plate. IR spectra were recorded on Thermo Nicolet FTIR 330 spectrometer using a thin film supported on KBr pellets. ^{1}H NMR spectra were recorded on Bruker AC NMR spectrometer using $CDCl_3$ as solvent. FAB Mass spectra were recorded on a Joel Sx 102/DA-6000.

In order to carry out the synthesis, the cyclic heptapeptide was disconnected into one tripeptide and tetrapeptide units. Boc-Gly-Ala-Gly-OMe and Boc-Val-Val-Leu-Pro-OMe. These units were properly appropriated and coupled together to get the linear heptapeptide and was finally cyclised using p-nitrophenyl ester method (Scheme 1)

Preparation of Dipeptides: Amino acid methyl ester hydrochloride (10mmol) was dissolved in chloroform (20ml). To this, N-methyl morpholine (4ml, 10mmol) was added at 0^0C and the reaction mixture was stirred for 15 mins. Boc-amino acid (10mmol) in $CHCl_3$ (20ml) and EDC (10mmol) were added with stirring. After 12hrs, the reaction mixture was filtered and the residue was washed with $CHCl_3$ (30ml) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ (20ml) and saturated NaCl (20ml) solutions. The organic layer was filtered and evaporated in vacuum. To remove the traces of impurities the product was dissolved in minimum amount of chloroform and cooled to 0^0C. The crystallized impurities was removed by filtration. Petroleum ether was added to the filtrate at 0^0C to recrystallize the pure product. Boc-Leu-Pro-OMe (1), Boc-Val-Val-OMe (2) and Boc-Gly-Ala-OMe (3) were prepared in this manner.

Preparation of the Tetrapeptide Boc-Val-Val-Leu-Pro-OMe: The tetrapeptide was prepared from the dipeptides Boc-Leu-Pro-OMe (1) and Boc-Val-Val-OMe (2) units after appropriate deprotection at the required functional groups. The deprotected dipeptides units[9] were coupled using EDC/NMM to get the protected tetrapeptide by the procedure similar to that of the dipeptides.

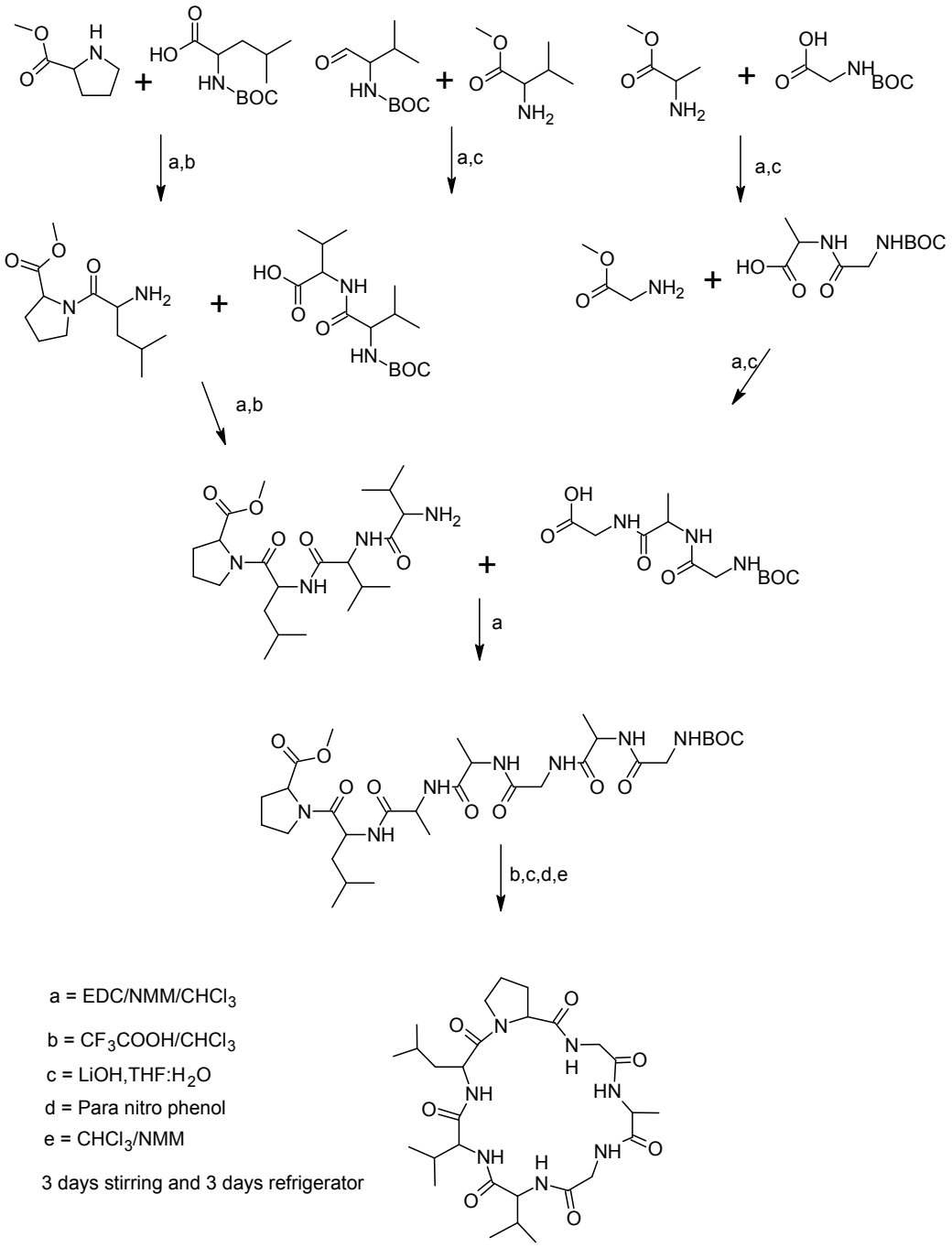
Preparation of the Tripeptide Boc-Gly-Ala-Gly-OMe: The tripeptide was prepared from the dipeptide Boc-Gly-Ala-OMe (3) and Gly-OMe (4) units after appropriate deprotection at the

required functional groups using EDC/NMM to get the protected tripeptide.

Preparation of linear heptapeptide: The ester group of the tripeptide (Boc-Gly-Ala-Gly-OMe) was removed and the Boc-group of the tetrapeptide (Boc-Val-Val-Leu-Pro-OMe) was deprotected. Both the deprotected units were coupled to get the linear heptapeptide.

Preparation of Cyclic heptapeptide: The cyclisation of the linear hexapeptide unit was carried out by the p-nitrophenyl ester method of Bodanszky[9] with certain modifications[10]. The ester group of the linear fragment was removed and the p-nitrophenyl ester group was introduced by stirring it for 12 hrs in $CHCl_3$ with p-nitrophenol at 0^0C. The reaction mixture was washed several times with saturated $NaHCO_3$ until the unreacted p-nitrophenol was removed completely and washed with 5% HCl to get Boc-peptide-pnp ester. The Boc-group also was removed, added $CHCl_3$ and NMM and the reaction mixture was kept at 0^0C for 7 days. The mixture was finally washed with 5% HCl, dried and evaporated in vacuum to get the cyclised product.

Scheme



RESULTS AND DISCUSSIONS

Chemistry

FTIR spectra of cyclic heptapeptide unit showed characteristic medium to strong bands corresponding to carbonyl stretching at 1627 cm^{-1} and NH bending at 1573 cm^{-1}, confirming the coupling reaction of the cyclic heptapeptide compound. ^{1}H NMR spectra of the synthesized compound clearly indicates the coupling of amino acids and peptides. The mass spectral data indicates stable molecular ion peak for the synthesized compound. Therefore the cyclic heptapeptide structure was confirmed by FTIR, ^{1}H NMR and MASS spectral data.

Pharmacological Studies:

Antioxidant activity:

The result of sample was compared with the standard (butyl hydroxyl toluene-BHT). With this method[11] it was possible to determine the antiradical power of an antioxidant compound by measuring the decrease in the absorbance of DPPH at 517 nm.

A color change from purple to yellow indicated that the absorbance decreased when the DPPH was scavenged by an antioxidant through donation of hydrogen to form stable DPPH molecule. **Table 1**, illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of prepared sample and standards.

Table 3: Antioxidant activity of synthesized peptide

Conc. (µg/ml)	Absorbance (Std)	%inhibition (Std)	Absorbance (Sample)	%inhibition (Sample)
10	0.1087	39.3076	0.16	20
20	0.0958	46.5103	0.13	35
50	0.0761	57.5097	0.11	45
100	0.0311	82.6353	0.09	55

CONCLUSION

In conclusion, we have synthesized a Glaucacycloptide A Cyclo [Gly-Ala-Gly-Val-Val-Leu-Pro] by using solution phase peptide synthesis. The compound was synthesized conveniently by solution phase technique and was characterized by IR, ^{1}H NMR and FAB-Mass spectral studies. The synthesized peptide exhibited significant antioxidant activity.

REFERENCES

1. Alassane We´le´, Idrissa Ndoye , Yanjun Zhang , Jean-Paul Brouard ,Jean-Louis Pousset , Bernard Bodo. Glaucacyclopeptide A from the seeds of Annona glauca.Phytochemsitry, 2005; 66: 1154-1157.
2. Chang, FR., Cheng, JL., Chui, HF., Wu, MJ., Wu, YC., 1998. Acetogenins from the seeds of Annona reticulata. Phytochemistry 47, 1057–1061.
3. Tsutomu S., Ishiyama D., Honda R., Hisato S and Tokumasu HK., J. Antibiot., 2000, 53, 597.
4. Pettit GR., Carny FG., Larry RL. and Schmidt PT., J.Med, Chem., 1994, 37, 137.
5. Morita H., Yoshida N., Takeya K., Itakowa H and Shirota O., Tetrahedron, 1996, 52, 123.
6. Sakai Y., Yoshida T., Sujita T and Agatsuma T, J. Antibiot., 1997, 50, 659.
7. Dathe M., Nikolenko H., Klose J and Bienert M., J. Biochem., 2004, 43, 9140.
8. Lee MD, Yuhong M. J., Soskis C. P., Borella M., Humanmitochondrial peptide deformylase, a new anticancer target of actinonin-based antibiotics, J. Clinical Investigation., 2004, 8, 114.
9. Beverly AT., Ara G., Roy H., Palombella V. J., Julian A., The Proteasome Inhibitor PS-341 in Cancer Therapy, Clinical Cancer Research., 1999, 5, 2638.
10. 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.
11. Ilhami G., Ekrem K., Elmastas M., Hassan YA., Determination of invitro antioxidant and Radical Scavenging Activity of Verbascum Oreophi C. Koch Var. Joannis (Fam. Scrophulariaceae). Research Journal of Biological Sciences 2007, 2, 372.

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