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Research Article

L-PPGL - A SYNTHETIC TETRAPEPTIDE WITH POTENT ANTIOXIDANT, ANTICANCER AND ANTITUBERCULAR ACTIVITIES

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ABSTRACT

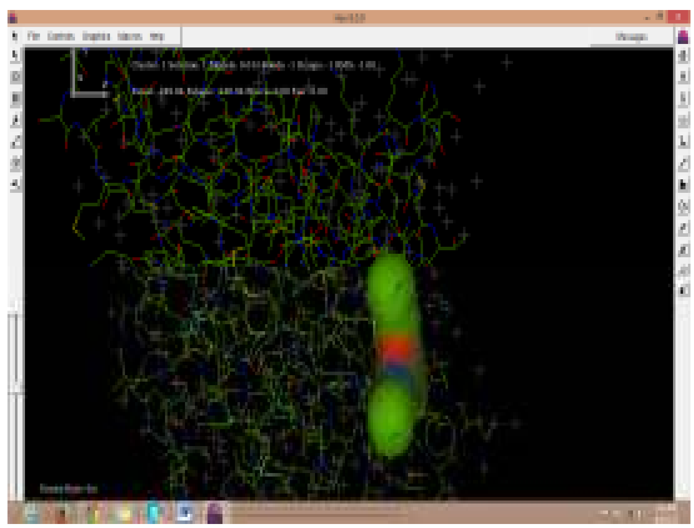
A linear tetrapeptide L-[Pro-Pro-Gly-Leu] (L-PPGL) was designed based on the structure of Wainunuamide. The tetrapeptide was synthesized by solution phase technique using TBTU/Et_3N in chloroform. The synthesized compound was subjected to antioxidant, anticancer, antitubercular and antimicrobial activities. The compound showed poor antimicrobial activity but significant antioxidant, anticancer activities and good antitubercular activities.

Keywords: Tetrapeptide, Anticancer, Antioxidant, Antitubercular, Wainunuamide.

INTRODUCTION

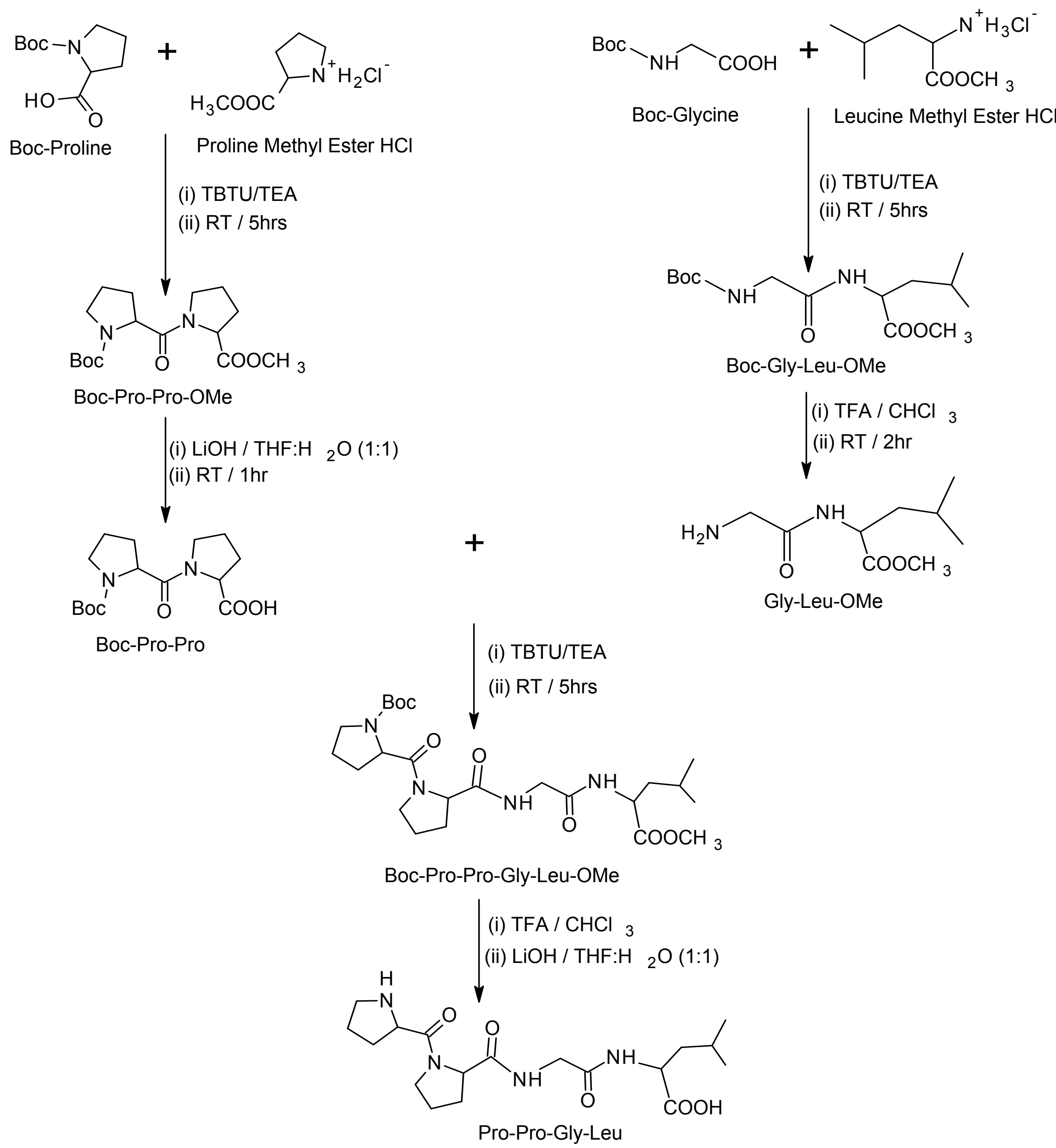
The tetrapeptide Pro-Pro-Gly-Leu (PPGL) is one of the tetrapeptides[1,2] derived from the cycloheptapeptide Wainunuamide, Cyclo-L-[Pro-Pro-Gly-Leu-Phe-Pro-His], which was isolated by Marcel et al from a *Fijian m*arine sponge of *Stylotella aurantium.* The crude extract of wainunuamide[3] exhibited strong in vitro cytotoxicity against the A2780 ovarian tumour and K562 leukaemia cancer cell line[4]. Based on the results, the derived tetrapeptide was subjected to docking using Hex software. Anticancer receptor with PDB ID: 2IOI was downloaded in pdb format from Protein Data Bank. Docking studies of the tetrapeptide with the downloaded receptor was carried out. High dock score (-249) was obtained which indicated that the tetrapeptide had a strong binding affinity towards the anticancer receptor 2IOI.

The tetrapeptide was theoretically disconnected into respective dipeptides; Boc-Pro-Pro-OMe, Boc-Gly-Leu-OMe. Synthesis of the dipeptide units was carried out by coupling the respective Boc-amino acids and Amino acid methyl ester hydrochlorides using solution phase peptide synthesis. TBTU (O-benzotriazol-1-yl)-N,N,N^1,N^1-tetramethyluroniumtetrafluoroborate) was used as the coupling reagent and triethylamine as the base. The Boc group of the dipeptide Boc-Gly-Leu-OMe was deprotected by CF_3COOH using chloroform as solvent. The carboxyl group of the dipeptide Boc-Pro-Pro-OMe was deprotected by LiOH using tetrahydrofuran: water (1:1). The tetrapeptide was prepared from the deprotected dipeptide units using the same procedure as that of dipeptide coupling. The synthesized tetrapeptide was deprotected on both the amino end and carboxylic end to get the free tetrapeptide GLFP (Scheme-1). The coupling was carried out by modifying the original procedures of Bodanzsky et al[5] and M.M.Joullie et al[6].



MATERIALS AND METHODS

All the reactions requiring anhydrous conditions were conducted in flame dried apparatus. Solvents and reagents were purified by standard methods. Organic extracts were dried over anhydrous sodium sulphate. Melting points were determined by capillary method and were uncorrected. All the chemicals were procured from Spectrochem Ltd. IR spectra were recorded on Jasco FT/IR-5300 IR spectrometer using a thin film supported on KBr pellets. The values are reported as cm^{-1}. 1H NMR spectra were recorded on Bruker JOEL(400MHz) NMR spectrometer. MASS spectra were recorded on a Joel Sx 102/DA-6000 mass spectrometer.



Scheme 1

PREPARATION OF DIPEPTIDES:

Amino acid methyl ester hydrochloride (10mmol) was dissolved in chloroform (20ml). To this, triethylamine (4ml, 28.7mmol) was added at 0^0C and the reaction mixture was stirred for 15 mins. Boc-amino acid (10mmol) in $CHCl_3$ (20ml) and TBTU (10mmol) were added with stirring. After 6hr, the reaction mixture was filtered and the residue was washed with $CHCl_3$ (30ml) and the washings were collected into the filtrate. The filtrate was washed with 5% $NaHCO_3$ (20ml) and saturated NaCl (20ml) solutions. The organic layer was washed with water three times to remove the byproducts. The organic layer was dried with anhydrous Na_2SO_4 and evaporated to dryness. The product was recrystallized from petroleum ether. Using this procedure the protected dipeptides Boc-Pro-Pro-OMe and Boc-Gly-Leu-OMe were synthesized. Physical Data is shown in Table-1.

PREPARATION OF TETRAPEPTIDE (PPGL):

The dipeptides were appropriately deprotected and the deprotected dipeptide units were coupled using TBTU/TEA to get the protected tetrapeptide by the procedure similar to that of the dipeptides. Physical data is shown in Table-1.

BIOLOGICAL ACTIVITIES:

The title compound was subjected to the following biological activity studies[7-9].

ANTICANCER ACTIVITY:
The tetrapeptide was screened for anticancer activity using MTT assay. The samples were prepared at final concentration of 100µg/ml, 75µg/ml and 50µg/ml. 5-fluorouracil was used as the positive control. All the three tetrapeptides exhibited potent anticancer activity against HeLa cell lines. The percentage of cell death is given in Table 2.

ANTIOXIDANT ACTIVITY:
Antioxidant activity was carried out for the tetrapeptide using DPPH method[5]. The result of sample was compared with that of the standard (ascorbic acid). The decrease in absorbance of DPPH at 517nm was noted. A colour change from purple to yellow indicated that absorbance decreased when DPPH was scavenged by an antioxidant through donation of hydrogen to form stable DPPH molecule. The results shown in Table 3, which indicates significant decrease of DPPH radical due to scavenging ability of prepared samples and standard. The tetrapeptides showed better antioxidant activity compared to that of the standard BHT (Butylatedhydroxytoluene).

ANTIMICROBIAL ACTIVITY:
The antimicrobial activity of the tetrapeptide was evaluated by the agar diffusion method at concentration of 50µg/0.1ml using DMSO as a solvent. The zones of inhibition were measured in mm at the end of 24 hr for bacteria and 48 hr for fungi and are reported in Tables 4 and 5 respectively. The antibacterial activity of the newly synthesized derivatives has been evaluated against both gram-positive organisms *Staphylococcus aureus*and *Bacillus subtilis*and gram negative organisms *Escherichia coli* and*Klebsiellapneumoniae.* The standard drug was Ampicillin. The antifungal screening was done by using *Aspergillusniger*and *Candida albicans.* The standard drug used was Streptomycin.

ANTITUBERCULAR ACTIVITY:
The antitubercular activity of the tetrapeptide was assessed against *M. tuberculosis* H37Rv (ATCC 27294) using microplate Alamar Blue Assay (MABA). This methodology is non toxic, uses a thermally-stable reagent and shows good correlation with BACTEC radiometric methods. The active ingredient of Alamar Blue (resazurin) is a nontoxic, cell permeable compound that is blue in color and virtually nonfluorescent. Upon entering cells, resazurin is reduced to resorufin, which produces very bright red fluorescence. The amount of fluorescence produced is proportional to the number of living cells. Streptomycin and Pyrazinamide was used as standard. The activity is expressed as theminimum inhibitory concentration (MIC) in µg/mL. The tetrapeptide showed better antitubercular activity compared to the standard drugs Streptomycin and Pyrizinamide. Minimum Inhibitory Concentration of the tetrapeptides is shown in Table 6.

RESULTS

Table 1: Physical Data of Intermediates and PPGL

Sl. No.	Compound	Physical State	M.P. (^{0}C) Lit. M.P.)	% Yield
1.	Boc-L-Pro	White Crystals	134(133-135)	84.42
2.	Boc-L-Gly	White Crystals	86 (86-89)	95.80
3.	Pro-OMe.HCl	Viscous Liquid	-	96.96
4.	Leu-OMe.HCl	White Crystals	152(151-153)	99.04
5.	Boc-Pro-Pro-OMe	Semisolid Mass	-	64.42
6.	Boc-Gly-Leu-OMe	Semisolid Mass	-	94.37
7.	Boc-Pro-Pro-Gly-Leu-OMe (PPGL)	Pale Yellow Semisolid Mass	-	88.46

Table 2: Anticancer Activity Data (MTT Assay Method)

Compound	Percentage of Viable Cells	
	Control	Treated
Pro-Pro-Gly-Leu	100	29

***Values are mean of the three experiments.**
The viable HeLa cells were calculated after 48 hrs of synthesized metallopeptides treatment and strained with trypan blue dye exclusion test.

Table 3: Antioxidant Activity Data (DPPH Method)

Compound	% Inhibition		
	25µg/ml	50µg/ml	75µg/ml
Pro-Pro-Gly-Leu	98.33	99.14	99.65
BHT (Standard)	45.96	70.16	91.93

***BHT: Butylatedhydroxytoluene**

Table 4: Antibacterial Activity Data

Compound	Zone of Inhibition (mm).*			
	S. aureus	*B. subtilis*	*E. coli*	*K. pneumoniae*
Pro-Pro-Gly-Leu	02	02	NA	NA
Ampicillin	10	12	15	11

*** NA: No Activity**

Table 5: Antifungal Activity Data

Compound	Zone of Inhibition (mm).*	
	Aspergillus niger	*Candida albicans*
Pro-Pro-Gly-Leu	02	02
Ampicillin	10	12

*** NA: No Activity**

Table 6: Antitubercular Activity Data (Microplate Alamar Blue Assay)

Compound	MIC (µg/ml)
Pro-Pro-GLy-Leu	2.1
Streptomycin (Standard)	6.25
Pyrizinamide (Standard)	3.12

***MIC: Minimum Inhibitory Concentration (lowest drug concentration required to complete inhibition of bacterial growth)**

DISCUSSION

The tetrapeptide L-PPGL resulted in a good dock score against the cancer receptor 2IOI. It was synthesized conveniently by solution phase technique using TBTU/TEA method with a high yield. The structure of the compound was confirmed by spectral analysis. The title compound showed poor antimicrobial activity, good antioxidant and antitubercular activities but potent anticancer activity against HeLa cancer cell lines, which is as good as its parent compound Wainunuamide.

CONCLUSION

L-PPGL can be a good anticancer agent comparable to the cycloheptapeptide Wainunuamide. Hence it can be a low cost anticancer drug if passes the clinical trials.

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