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Research Article

SYNTHESIS, DOCKING AND BIOLOGICAL STUDIES OF THE LINEAR TETRAPEPTIDE PWPV

M. Himaja[1]*, Sanjay S[1], Poppy Das[1],Munirajasekhar D[1], Karigar A.A[2]
[1]Department of Pharmaceutical Chemistry, School of Advanced Sciences, VIT University, Vellore-632014, India
[2]Department of Pharmaceutical Analysis, Maratha Mandal's College of Pharmacy, Belgaum 590016, Karnataka, India

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

ABSTRACT
Linear Tetrapeptides L-PWPV (Pro-Trp-Pro-Val) was designed and synthesized by solution phase peptide synthesis based on dock score. The molecular docking studies of the designed tetrapeptide L-PWPV was carried out using Molegro Virtual Docker software for tumor cancer protein(1OLG). The linear tetrapeptide was synthesized by coupling protected amino acids (dipeptides) using EDC (ethyl-3-(N,N-dimethylamino)propyl carbodiimide) as coupling reagent. The compounds were analyzed by FTIR, ^{1}H NMR and MASS data and subjected to antioxidant activity using 1,1-dipheny-2-picryl-hydrazil (DPPH) method and insecticidal activity using Morita et al method.
Keywords: Tetrapeptide, Solution phase peptide synthesis, Molegro Virtual Docker software, L-PWPV, DPPH, Antioxidant and Insecticidal activities.

INTRODUCTION

Peptides are one of the important classes of organic compounds with many biological activities[1]. Most of the peptides are found to exhibit antifungal, antibacterial, anthelmintic, antitubercular, antioxidant and anti-inflammatory activities[2-6]. Peptide ligands generally act by interaction with receptor or acceptor molecules (hormones, enzymes, neurotransmitters, growth promoters and inhibitors, etc.). Docking is frequently used to predict the binding orientation of small drug candidates to their protein targets in order to predict the affinity and activity of the small molecule[7-9]. Most of the peptides exhibit their biological activities through binding to corresponding receptors or enzymes[10]. In the present work the designed ligand PWPV targeted to the cancer cell protein, Human Tumor Suppressor P53 receptor with the PDB ID: 1OLG using Molegro Virtual Docker software. The synthesis was carried out using EDC as a coupling reagent and triethyl amine as base. The structure of the tetrapeptide was confirmed by spectral analysis (^{1}H NMR, MASS, FTIR).

MATERIALS AND METHODS

Commercially available reagents and analytical grade solvents were used without further purification. Anhydrous conditions for all the reactions were conducted in dried apparatus. All the reactions were magnetically stirred unless otherwise stated. Organic extracts were dried over anhydrous sodium sulphate. Melting points were determined by capillary method. Amino acids, Diethyl ether, Methanol and Chloroform were obtained from and Spectrochem Ltd, Mumbai. DPPH, di-tertbutylpyrocarbonate, trifluoroacetic acid, EDC were obtained from AVRA. IR spectra were recorded on FTIR spectrometer using a thin film support on KBr pellets. The values are reported as υ_{max} (cm^{-1}). ^{1}H NMR spectra was recorded on ^{1}H NMR Brucker JOEL (400MHz) NMR spectrometer. The spectra was obtained in $CDCl_3$ and the chemical shift values are reported as values in ppm relative to TMS (δ=0) as internal standard. FAB Mass spectra were recorded. In order to carry out the synthesis the dipeptides Boc-Pro-Trp-OMe and Boc-Pro-Val-OMe were appropriately deprotected and coupled together to get the linear tetrapeptide (Scheme 1).

Preparation of Dipeptides:

Amino acid methyl ester HCl (10 mmol) was dissolved in chloroform ($CHCl_3$) (20 ml). To this triethylamine (Et_3N) (4 ml, 28.7 mmol) was added at 0^0C and the reaction mixture was stirred for 15 minutes. Boc-amino acid (10 mmol) in chloroform (20 ml) and EDC (10mmol) were added and kept for stirring. After 12hrs, the reaction mixture was filtered and the residue was washed with $CHCl_3$ (30ml) and the washings were added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ (20 ml), 5% HCl (20 ml) and distilled water (20 ml).The organic layer was dried over anhydrous sodium sulphate (Na_2SO_4), filtered and evaporated. The crude product was recrystallized with chloroform and petroleum ether. Boc-Pro-Trp-OMe and Boc-Pro-Val-OMe were prepared in this manner[11].

Preparation of linear Tetrapeptide:

The ester group of the dipeptide Boc-Pro-Trp-OMe was removed and the Boc-group of another dipeptide Boc-Pro-Val-OMe was deprotected. Both the deprotected units were coupled and to get the protected linear tetrapeptide which was deprotected at both the ends to get the title compound.

ANTIOXIDANT ACTIVITY

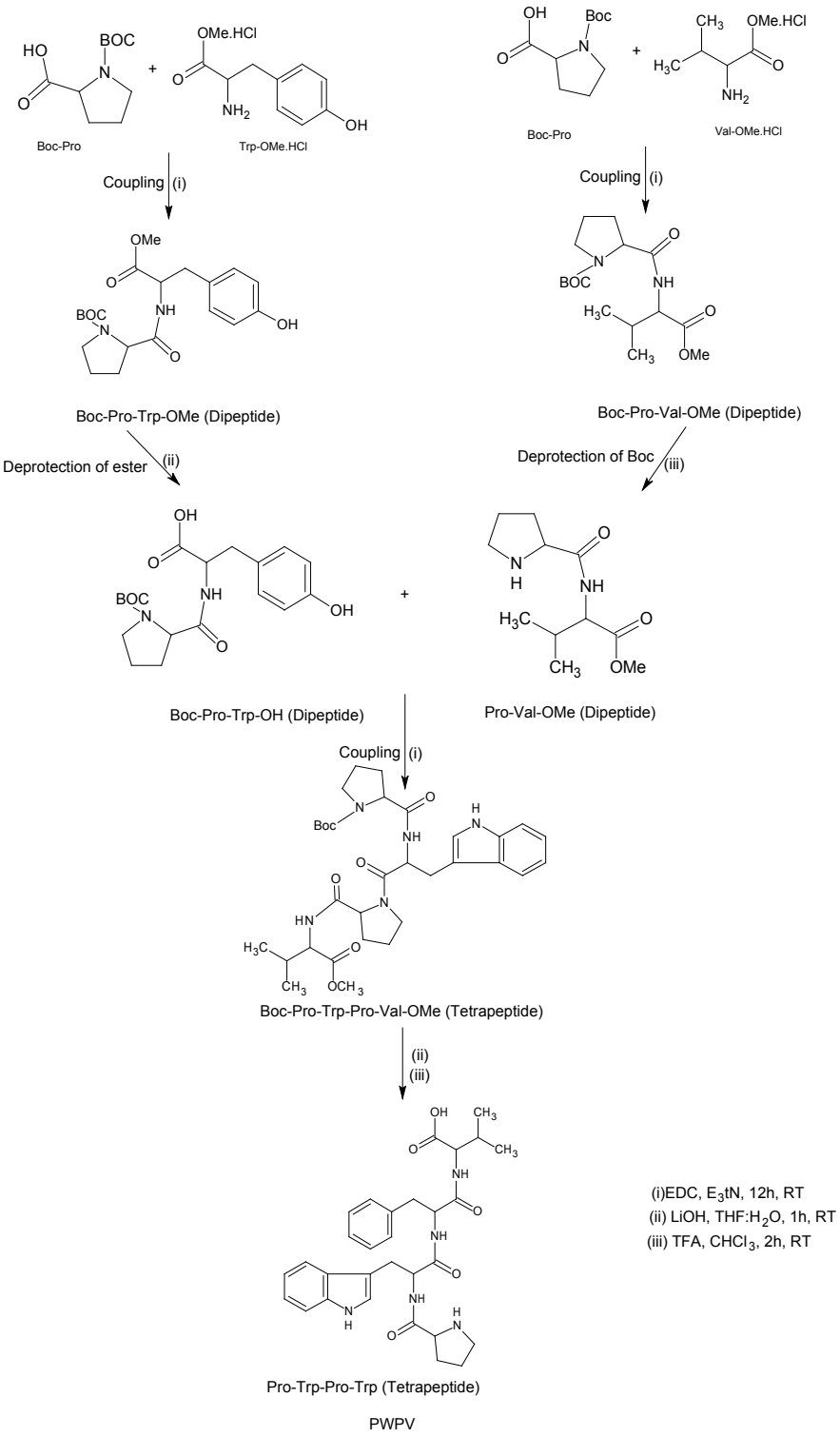
The synthesized linear tetrapeptide PWPV was screened for antioxidant activity i.e free radical scavenging activity by 1, 1-diphenyl-2-picryl-hydrazil (DPPH)[12]. This was measured by following method described by Ilhami Gulcin *et al*, where in the bleaching rate of a stable free radical, DPPH is monitored at a characteristic wavelength in the presence of the sample[13]. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 1 mL of 0.1 M methanolic solution of DPPH was added to 3ml of the synthesized sample PWPV, at different concentrations in methanol (25, 50, 100µg/mL). The samples were kept in the dark for 30 min after which the absorbance was measured at 517 nm in a UV spectrophotometer (Jasco V-670 spectrophotometer). Methanol was used as the blank. The measurements were

done in triplicate. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid was taken as a standard in this study. The tetrapeptide PWPV showed moderate free radical scavenging activity at all different three concentrations studied.

INSECTICIDAL ACTIVITY

Insecticidal activity of the synthesized compounds was carried out against the termites (Coptotermes formosanus) using Morita et al method[14]. Firstly the Whatmann filter paper was cut according to the inner diameter of the Petri plate (9.2cm), later 25mg of the each test compounds was dissolved in 1ml of chloroform and then the solution was uniformly spread onto the filter paper and allowed it to dry. The concentration of the each test compound is 0.75mg/cm2 area. A control (without sample) and a Standard drug were maintained in a similar way. Later on the termites were spreaded onto the filter paper placed in the petriplate and then close the lid which contains a thin layer of wet cotton bed. The death time of the insects was noted down.

Scheme 1



RESULTS AND DISCUSSIONS

Docking: A Preliminary study was carried out on the linear tetrapeptide PWPV using Molegro Virtual Docker software where the ligand was docked with Human Tumor Suppressor $_P$53 receptor with the PDB ID: **1OLG** (listed in Table 1). The docking score revealed that the L-(PWPV) showed highest docking score and hence a strong binding affinity towards the protein 1OLG effectively.

Table: 1 Docking of the ligands (tetrapeptides) with Human Tumor Suppressor P53 receptor

Sl. No	Ligands	Docking Score
1	*L(PWPV)	-151.367
2	L(WPPV)	-132.325
3	L(WPVP)	-137.761
4	L(PWVP)	-145.361

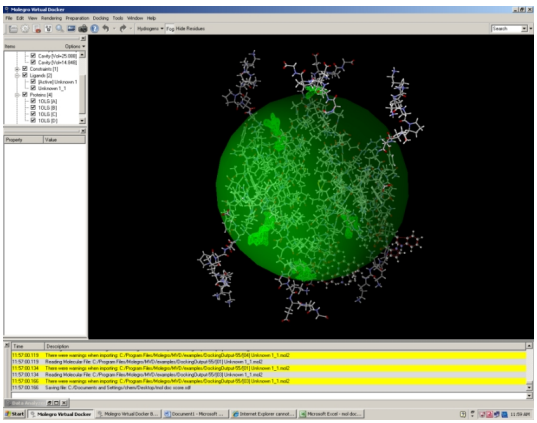


Figure 1 XP visualize of docking of the ligands (tetrapeptides) with Human Tumor Suppressor P53 receptor (PDB ID: 1OLG)

Synthesis: The isomer PWPV was synthesized by solution phase peptide synthesis. The results of the peptide along with its physical properties are shown in Table 2.

Table: 2 Physical data of PWPV

Sl.No	Compound	Nature	% of Yield
1	L-Pro-Trp-Pro-Val	Dark brown semisolid mass	71.9

Spectral Analysis: The structure of the synthesized compound was characterized by FT-IR, ^{1}H NMR and FAB-MS. ^{1}H NMR spectrum (δ, ppm): 7-7.2(1H,t,Ar-H), 7.3-7.6(1H,d,Ar-H), 10.1-10.2(1H,d,indole N-H), 8 8.2(1H,d,sec.amide N-H), 1.3-1.4(9H,s,O(CH_3)$_3$, 3.6-3.7(3H,s,OCH_3), IR spectrum (v/cm^{-1}): 3310-3320 cm^{-1} (N-H stretch), 3050-3060 cm^{-1}(Ar-C-H stretch), 2820-2970cm^{-1} (Aliph-C-H stretch), 1670-1675 cm^{-1} (C=O stretch), 1380-1390 cm^{-1}(C-N stretch), The molecular ion peak was obtained at 612.

Antioxidant activity:

The sample result was compared with the standard (ascorbic acid). With this method 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 3 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.)	% of inhibition (Std.)	Absorbance (Sample PWPV)	% of inhibition (Sample PWPV)
25	0.187	55.68	0.402	4.73
50	0.163	61.37	0.311	26.30
100	0.152	63.93	0.287	31.99

Insecticidal activity:

The sample result was compared with the standard (Chloropyrifos). With this method it was possible to determine the insecticidal activity for L-(PWPV) by comparing dead time with standard drug chloropyrifos. Table 4 illustrates a significant insecticidal activity of prepared sample and standards.

Table 4: Result of Insecticidal activity

Compound	Concentration of the compound (mg/66.4424cm^2)	Dead time(Hrs.mins)
L-(PWPV)	25mg/66.4424cm^2	0.21
Chloropyrifos.	25mg/66.4424cm^2	2.45

CONCLUSION

The linear tetrapeptide PWPV could be conveniently prepared by EDC/Et 3N method. The product could be obtained in a pure form since the byproduct from EDC was water-soluble. Linear tetrapeptides L-PWPV was synthesized based on dock score and was characterized by FTIR, ^{1}H NMR and MASS spectral studies. The compounds showed moderate antioxidant activity in comparison with ascorbic acid but potent insecticidal activity as compared to the standard chloropyrifos.

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