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Research Article

SYNTHESIS, DOCKING AND BIOLOGICAL STUDIES OF THE LINEAR TETRAPEPTIDE PWPV-A POTENT INSECTICIDAL AGENT

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ABSTRACT

Linear Tetrapeptide L-PWPV was designed and synthesized by solution phase peptide synthesis based on dock score. The molecular docking studies of the designed tetrapeptide L-PWFV 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 compound was analyzed by FTIR, ^{1}HNMR and MASS data and was subjected to antioxidant activity using 1,1-dipheny-2-picryl-hydrazil (DPPH) method and insecticidal activity by 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 $_P$53 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 the 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 condition for all the reactions was maintained 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 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 (d=0) as internal standard. FAB Mass spectra were recorded. In order to carry out the synthesis the dipeptides Boc-L-Pro-Trp-OMe and Boc-L-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^{0}C and the reaction mixture was stirred for 15 minutes. Boc-amino acid (10 mmol) in chloroform (20 ml) and EDC (10mmol) were added and the reaction mixture was 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 from chloroform and petroleum ether. Boc-L-Pro-Trp- OMe and Boc-L-Phe-Val-OMe were prepared in this manner [11].

Preparation of linear Tetrapeptide:

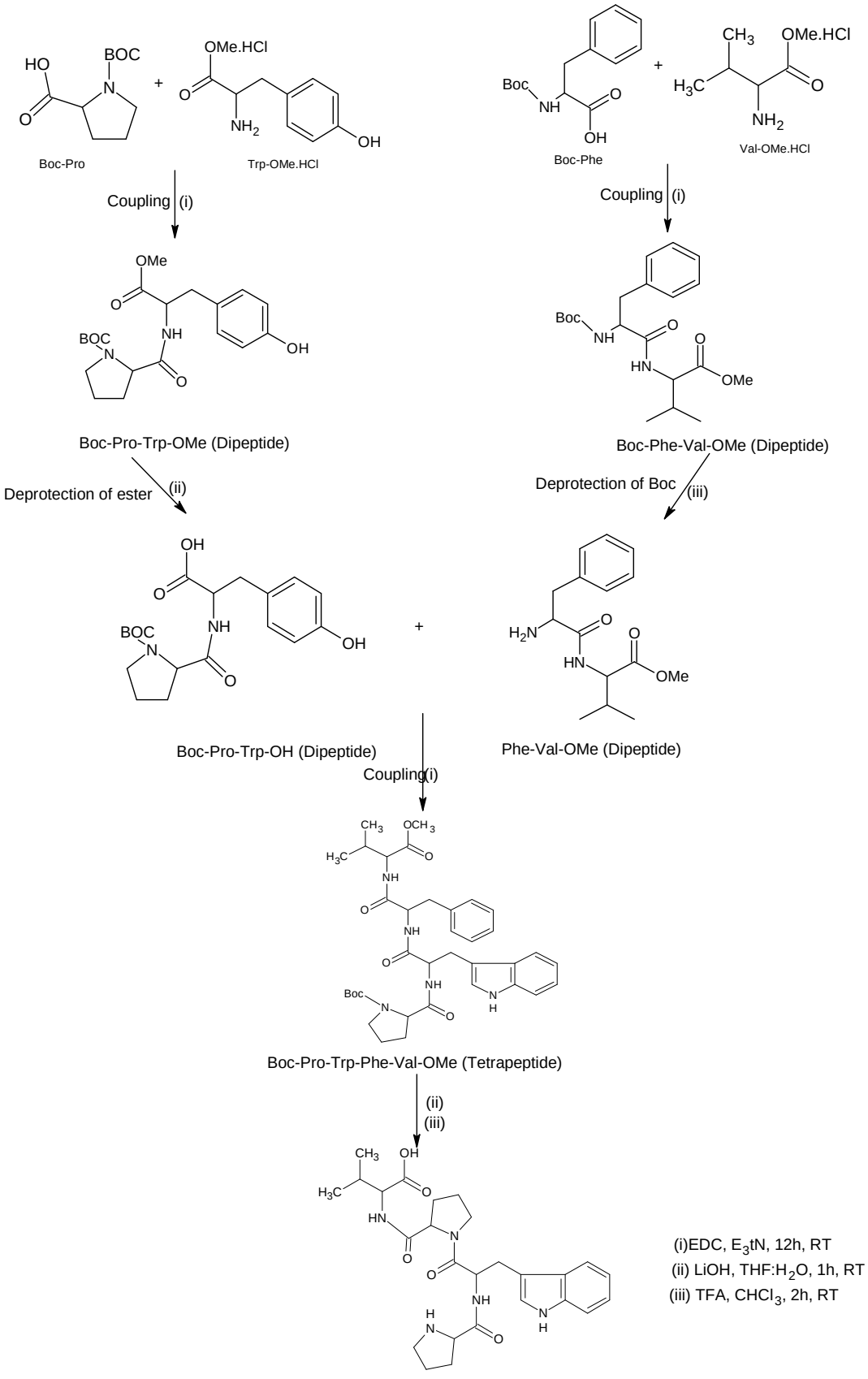
The ester group of the dipepeptide (Boc-L-Pro-Trp- OMe) was removed and the Boc-group of another dipeptide (Boc-L-Phe-Val-OMe) was deprotected by standard methods. Both the deprotected units were coupled to get the protected linear tetrapeptide which was deprotected at both the ends to get the targeted compound.

Antioxidant Activity

The synthesized linear tetrapeptide PWFV 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*, wherein 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 PWFV, 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 PWFV showed moderate free radical scavenging activity at all the 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]. Whatmann filter paper was cut according to the inner diameter of the Petri plate (9.2cm). 25mg of the each test compounds was dissolved in 1ml of chloroform. The solution was uniformly spread onto the filter paper and was allowed to dry. The concentration of each test compound was 0.75mg/cm2 area. Control (without sample) and a Standard drug were maintained in a similar way. The termites were introduced onto the filter paper placed in the petriplate and the lid was closed which contained a thin layer of wet cotton bed. The death time of the insects was noted down.


Boc-Pro
Trp-OMe.HCl
Boc-Phe
Val-OMe.HCl
Coupling (i)
Coupling (i)
Boc-Pro-Trp-OMe (Dipeptide)
Boc-Phe-Val-OMe (Dipeptide)
Deprotection of ester (ii)
Deprotection of Boc (iii)
Boc-Pro-Trp-OH (Dipeptide)
Phe-Val-OMe (Dipeptide)
Coupling (i)
Boc-Pro-Trp-Phe-Val-OMe (Tetrapeptide)
(ii)
(iii)
Pro-Trp-Phe-Trp (Tetrapeptide)
PWFV
(i)EDC, E3tN, 12h, RT
(ii) LiOH, THF:H2O, 1h, RT
(iii) TFA, CHCl3, 2h, RT

Scheme 1

RESULTS AND DISCUSSION

Docking

A Preliminary study was carried out on the linear tetrapeptide PWFV 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-(PWFV) 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

S. No.	Ligands	Docking Score
1	*L-(PWFV)	-171.072
2	L-(FVPV)	-140.843
3	L-(FWPV)	-137.182
4	L-(PWFV)	-154.943

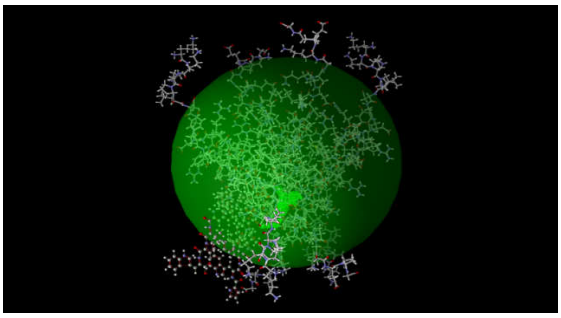


Fig. 1: Docking of the ligands (tetrapeptides) with Human Tumor Suppressor P53 receptor (PDB ID: 1OLG)

Synthesis

The isomer PWFV 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 PWFV

S. No.	Compound	Nature	% of Yield.
1	L-Pro-Trp-Phe-Val	Light brown, semisolid mass	74.8

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): 7.1-7.2(1H,t,Ar-H), 7.3-7.6(1H,d,Ar-H), 7.2-7.3(1H,d,Ar-H), 7.4--1.6(9H,s,O(CH_3)$_3$, 3.6-3.7(3H,s,OCH_3), IR spectrum (v/cm^{-1}): 3320-3325 cm^{-1}(N-H stretch), 3050-3060 cm^{-1}(Ar-C-H stretch), 2920-2970cm^{-1}(Alip.C-H stretch), 1670-1680cm^{-1}(C=O stretch), 1380-1400 cm^{-1}(C-N stretch)., The molecular ion peak was obtained at 662.

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 PWFV)	% of inhibition (Sample PWFV)
25	0.187	55.68	0.361	14.45
50	0.163	61.37	0.303	28.19
100	0.152	63.93	0.211	47.63

Insecticidal activity

The sample result was compared with the standard (Chloropyrifos). With this method it was possible to determine the insecticidal activity for L-(PWFV) 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-(PWFV)	25mg/66.4424cm^2	011
Chloropyrifos.	25mg/66.4424cm^2	2.45

CONCLUSION

The linear tetrapeptide PWFV 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 tetrapeptide L-PWFV was synthesized based on dock score and was characterized by IR, ^{1}H NMR and MASS spectral studies.

The compound showed moderate antioxidant activity in comparison with ascorbic acid but potent insecticidal activity as compared to the standard chloropyrifos.

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