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Research Article

SYNTHESIS, DOCKING STUDIES AND FREE RADICAL SCAVENGING ACTIVITY OF THE LINEAR TETRAPEPTIDE VFPF

Das M.*, Agarwal D.S., Sharma S.K., Das P., Rout P.K.
Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore - 632014, TN, India

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*Email: moonjitdas@gmail.com,

ABSTRACT
A rational designing of the linear tetrapeptide Val-Phe-Pro-Phe (VFPF) was done and was synthesized by solution phase peptide synthesis. The solution phase synthesis of VFPF was carried out by using ethyl-3-dimethylaminopropyl carbodiimide (EDC) as a coupling reagent and triethyl amine as a base. The molecular docking studies of the designed tetrapeptide VFPF was carried out by using Molegro Virtual Docker software for anticancer properties. VFPF was evaluated for antioxidant property by using 1,1-dipheny-2-picryl-hydrazil (DPPH) method and were found to possess moderate antioxidant activity.

Keywords: Tetrapeptide, Solution phase peptide synthesis, Molegro Virtual Docker software, Antioxidant activity.

INTRODUCTION

Peptides are one of the important classes of organic compounds with many biological activities. Most of the peptides are found to exhibit antifungal, antibacterial[1-2], antihelmintic, antitubercular, antioxidant and anti-inflammatory activities.[3-6] The development of rational approaches to the design of peptide ligands with specific chemical, physical and biological properties is the main goal in peptide synthesis. Peptide ligands generally act by interaction with receptor or acceptor molecules (hormones, enzymes, neurotransmitters, growth promoters and inhibitors, etc.).[7-8]

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. Most of the peptides exhibit their biological activities through binding to corresponding receptors or enzymes.[3-6] In the present work the designed ligand VFPF targeted to the cancer cell protein, Human Tumor Suppressor P53 receptor with the PDB ID: 1OLG using Molegro Virtual Docker software.

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, di-tertbutylpyrocarbonate, trifluoroacetic acid, EDC, Diethyl ether, Methanol and Chloroform were obtained from and Spectrochem Ltd, Mumbai. DPPH was 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}). ^1H NMR spectra was recorded on ^1H 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-Val-Phe- OMe and Boc-L-Pro-Phe-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 mmol). 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 with stirring. After 8hrs, 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) and distilled water (20 ml).The organic layer was dried over anhydrous sodium sulphate (Na_2SO_4), filtered and evaporated in vacuum. The crude product was recrystallized from chloroform and petroleum ether. Boc-L-Val-Phe- OMe and Boc-L-Pro-Phe-OMe were prepared in this manner.

Preparation of linear Tetrapeptide:

The ester group of the dipepeptide (Boc-L-Val-Phe-OMe) was removed and the Boc-group of another dipeptide (Boc-L-Pro-Phe-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 VFPF was screened for antioxidant activity such as free radical scavenging activity by 1, 1-diphenyl-2-picryl-hydrazil ($DPPH^{\cdot}$).[12-15] This was measured by following method wherein the bleaching rate of a stable free radical, $DPPH^{\cdot}$ is monitored at a characteristic wavelength in the presence of the sample. 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 VFPF, 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 VFPF showed moderate free

radical scavenging activity at all different three concentrations studied.

RESULTS AND DISCUSSIONS

Docking: A Preliminary study was carried out on the linear tetrapeptide VFPF using Molegro Virtual Docker software where the ligand was docked with Human Tumor Suppressor P53 receptor with the PDB ID: 1OLG (listed in Table 1). The docking score revealed that the L-(VFPF) showed highest docking score and hence a strong binding affinity towards the protein 1OLG effectively.

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

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): 7.12-7.17 (4H, d, Ar-H), 7.21-7.4 (4H, t, Ar-H), 6.8-7.08 (2H, d, Ar-H), 11-11.1 (1H, S, OH), 1.0-1.1(6H, d, Alip-CH_3), 1.9-2.2 (2H, d, -NH_2), 3.15, 2.87 (2H,d, R_2CH_2), 7.8-8.1 (2H, d, NH), IR spectrum (v/cm^{-1}): 3518.2 cm^{-1} (OH stretch), 3243.88 cm-1 (NH stretch), 3039.08 cm^{-1} (Ar-CH stretch), 2968.12 cm-1 (Alip-CH stretch), 1672 cm-1(C=O stretch). The molecular ion peak was obtained at 507 (M+1)

Antioxidant activity:

The result of the sample 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.

CONCLUSION

The linear tetrapeptide VFPF could be conveniently prepared by EDC/Et $_3$N method. The product could be obtained in a pure form since the byproduct from EDC was water-soluble. Docking studies of the tetrapeptides on the target proteins Human Tumor Suppressor P53 receptor with PDB ID: 1OLG showed that ligand VFPF resembles the drug like properties related to anticancer agents. The synthesized tetrapeptide was characterized by IR, ^{1}H NMR and FAB-Mass spectral studies. The synthesized compound VFPF exhibited moderate antioxidant activity.

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

Sl. No	Ligands	Docking Score
1	VGPF	-62.56
2	FVPF	-6.76
3	FVFP	984.53

Table 2: Physical data of VFPF

Sl.No	Compound	Nature	% of Yield
1	L-Val-Phe-Pro-Phe	Light brown semisolid mass	69

Table 3: Antioxidant activity of synthesized peptide

Conc. (µg/ml)	Absorbance (Std.)	% of inhibition (Std.)	Absorbance (Sample VFPF)	% of inhibition (Sample VFPF)
25	0.187	55.68	0.283	32.93
50	0.163	61.37	0.267	36.72
100	0.152	63.93	0.245	41.94

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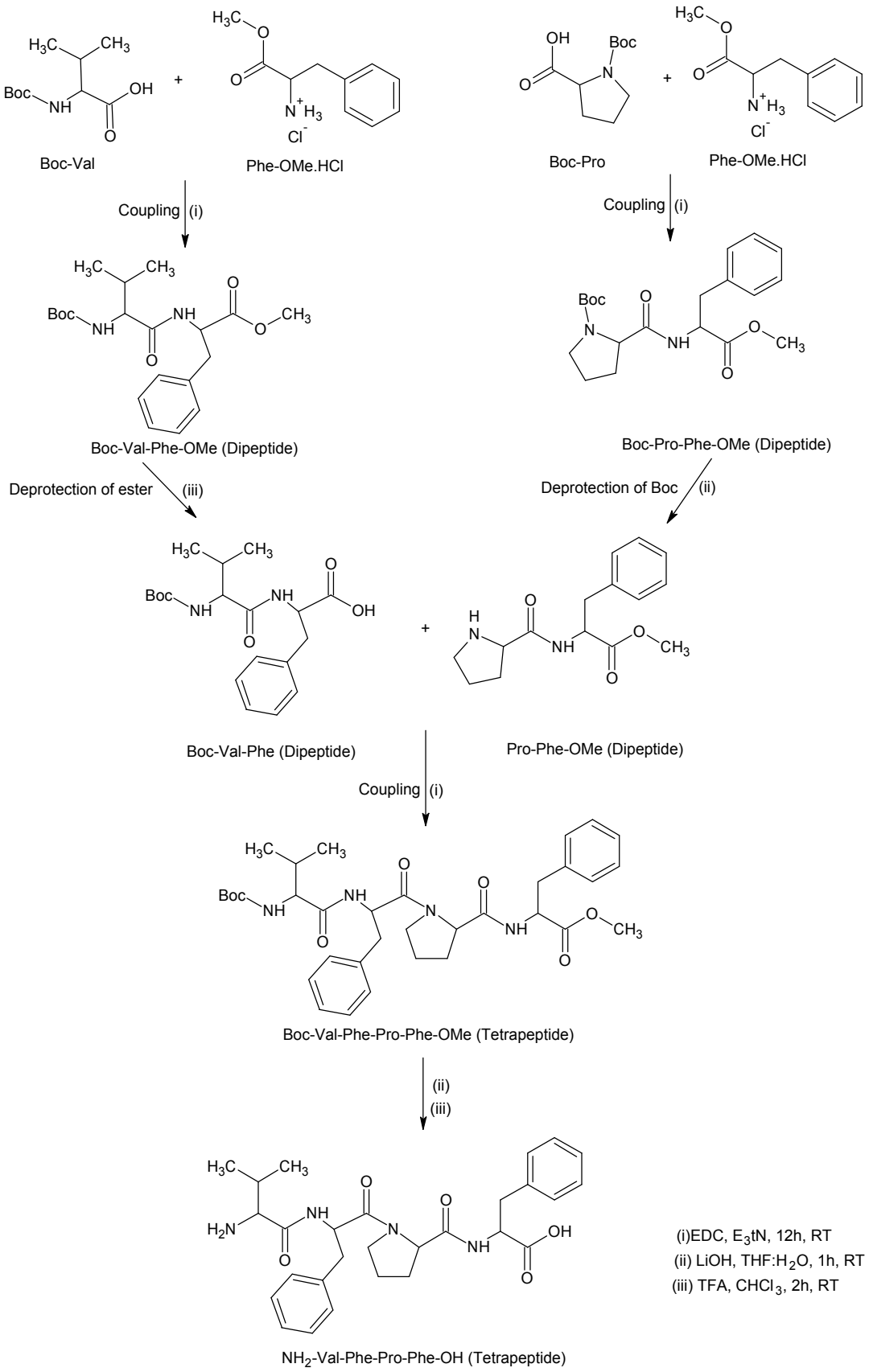
Boc-Val
Phe-OMe.HCl
Boc-Pro
Phe-OMe.HCl
Coupling (i)
Coupling (i)
Boc-Val-Phe-OMe (Dipeptide)
Boc-Pro-Phe-OMe (Dipeptide)
Deprotection of ester (iii)
Deprotection of Boc (ii)
Boc-Val-Phe (Dipeptide)
Pro-Phe-OMe (Dipeptide)
Coupling (i)
Boc-Val-Phe-Pro-Phe-OMe (Tetrapeptide)
(ii)
(iii)
NH2-Val-Phe-Pro-Phe-OH (Tetrapeptide)
VFPF
(i)EDC, E3tN, 12h, RT
(ii) LiOH, THF:H2O, 1h, RT
(iii) TFA, CHCl3, 2h, RT

Scheme 1

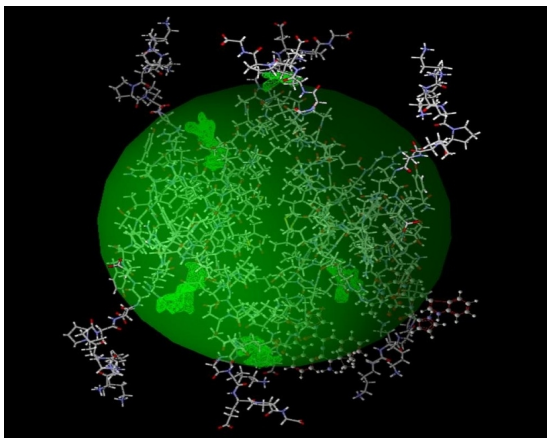

Figure 1: XP visualize of docking of the ligands (tetrapeptides) with Human Tumor Suppressor P53 receptor (PDB ID: 1OLG)

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