SYNTHESIS, DOCKING STUDIES AND ANTIOXIDANT ACTIVITY OF LINEAR TETRAPEPTIDE FAYV

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ABSTRACT
The linear tetrapeptide Phe-Ala-Tyr-Val (FAYV) was designed based on docking results using Schrodinger Software tool. The tetrapeptide was conveniently synthesized by solution phase peptide synthesis using Dicyclohexylcarbidomide (DCC) as coupling agent and triethyl amine as the base. Qikprop (a tool to predict drug like properties) results showed that the ligands mostly show antihypertensive and antidiabetic properties. The compounds were evaluated for antioxidant property by using 1,1-diphenyl-2-picryl-hydrazil (DPPH$^.$) method and were found to possess significant antioxidant activity.
KEY WORDS: Tetrapeptide, Solution Phase Peptide Synthesis, Schrodinger software 2009, Docking, Antioxidant activity.

INTRODUCTION

Peptides are the important class of organic compounds with potent biological activities[1-7]. Peptides function as hormones, enzymes, enzyme inhibitors or substrates, growth promoters or inhibitors, neurotransmitters and immunomodulators. Most of the peptides exhibit their biological activities through binding to corresponding acceptor molecules (receptors or enzymes).

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.

In the present work, ligand Phe-Ala-Val-Tyr (FAYV), with all amino acids having L-configuration, was designed and was targeted to the cancer cell proteins, human peptide deformylase protein and HIF 1α protein using Schrodinger software and were synthesized by solution phase peptide synthesis.

MATERIALS AND METHODS

Molecular docking: In the present work Schrodinger 2009 software was used to dock the ligand with the target protein. The designed ligand D-FAYV was docked against target protein Human Mitochondrial peptide deformylase and hypoxia-inducible factors HIF-1α. In standard virtual docking studies, ligand is docked into the binding site of a receptor where the receptor is held rigid and the ligand is free to move. Molecular docking involves the following steps (Schrodinger Software Solutions, USA), 1) Ligprep, 2) Protein preparation wizard, 3) Glide grid generation, 4) Docking.

Ligprep: Ligprep is a robust collection of tools designed to prepare high quality, all-atom 3D structures for large numbers of drug-like molecules, starting with 2D or 3D structures in SD or Maestro format. The resulting structures can be saved in either SD or Maestro format.

Protein preparation wizard: It is a tool to convert a target protein in its PDB form into a form suitable for docking with ligand and modeling calculations. The typical structure file from the PDB is not suitable for immediate use in molecular modeling calculations. A typical PDB structure file consists only of heavy atoms and may include a co crystallized ligand, water molecules, metal ions, and cofactors. Some structures are multimeric, and may need to be reduced to a single unit. Because of the limited resolution of X-ray experiments, it can be difficult to distinguish between NH and O, and the placement of these groups must be checked. PDB structures may be missing information on connectivity, which must be assigned, along with bond orders and formal charges. Schrodinger has therefore assembled a set of tools to prepare proteins in a form that is suitable for modeling calculations.

Glide grid generation: Glide searches a favorable interaction between one or more ligand molecules and a receptor molecule, usually a protein. The shape and properties of the receptor are represented on a grid by

several different sets of fields that provide more accurate scoring of the ligand poses.

Docking: Protein-Ligand docking is a molecular modeling technique is used to predict the orientation and confirmation of binding of ligands with proteins (**Figure 1**). More the value of glide score more is the interaction between the ligand and target protein (**Table 1**). The preliminary study of docking shows that the designed ligand tetrapeptides dock with the target proteins, Human Mitochondrial peptide deformylase protein and HIF-1α protein. This shows that the ligands can bind effectively to the predicted protein.

Analytical grade solvents and commercially available reagents 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 open capillary method. Amino acids, di-tert-butylpyrocarbonate, trifluoroaceticacid, DCC, 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 ν_{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 ($\delta=0$) as internal standard. FAB Mass spectra were recorded. In order to carry out the synthesis the dipeptides Boc-Phe-Gly-OMe and Boc-Tyr-Val-OMe were properly appropriated 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 DCC (Dicyclohexyl Carbodiimide) (2.2gm, 10mmol) were added with stirring. After 16hrs, 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 plain water (20 ml).The organic layer was dried over anhydrous dried with anhydrous sodium sulphate (Na_2SO_4), filtered and evaporated in vacuum. To remove the traces of dicyclohexylurea (DCU), the product was dissolved in minimum amount of $CHCl_3$ and cooled to 0^0C. The crystallized DCU was removed by filtration. Petroleum ether was added to the filtrate at 0^0C to

recrystallize the pure product. Boc-L-Phe-Ala-OMe and Boc-Tyr-Val-OMe were prepared in this manner.

Preparation of linear Tetrapeptide: The ester group of the dipepeptide (Boc-L-Phe-L-Ala-OMe) was removed and the Boc-group of another dipetide (Boc-Tyr-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 FAYV was screened for antioxidant activity such as free radical scavenging activity by 1,1-diphenyl-2-picryl-hydrazil (DPPH˙). This was measured by following the method described by Ilhami Gülçin et al[8], wherein the bleaching rate of a stable free radical, DPPH˙ 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 mM methanolic solution of DPPH was added to 3 mL of the synthesized samples FGVY, at different concentrations in methanol (10, 20, 50, 100 µg/mL). The samples were kept in the dark for 30 min after which the absorbance was measured at 517 nm in a UV spectrophotometer (Systronics 2202). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Butylated Hydroxy Toluene (BHT), which is a good antioxidant, is taken as a standard in this study. The linear tetrapeptides L-FAYV and D-FAYV showed moderate free radical scavenging activity at all different four concentrations studied. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH˙ Scavenging effect (%) = [(A_c-A_s)/A_o) 100]

Where A_c is the absorbance of the control reaction and A_s is the absorbance in the presence of sample.

RESULTS AND DISCUSSION

Docking: A Preliminary study was initially carried out with Schrodinger software where the Designed ligand FAYV was docked with Human Mitochondrial peptide deformylase and HIF 1α that were collected from PIR (Protein information resource) (listed in Table 1) and their docking score in **Table:1.** The docking score revealed that the ligand FAYV (with all L-amino acids) showed very good affinity to bind with the protein HIF-1α effectively.

Synthesis: Keeping view, the cost-effectiveness, the isomer FAYV, with all L-amino acids, was synthesized by solution phase peptide synthesis[9]. The results of all the peptides along with their physical properties have been shown in **Table 2.** The final synthesized compound was obtained in a good yield.

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): 7.0-7.2 (5H, m, Ar-H), 6.8-7.0 (4H, d, Ar-H), 6.4-6.5 (1H, S, OH), 4.2-5.2 (5H, S, αH-H), 3.6-3.7(3H, m, OCH$_3$), 3.1-3.5 (4H,S, NH), 0.8-2.3 (20H, br, Bz-H, βH, γH, of Phe, Tyr, Val and Boc), IR spectrum (v/cm^{-1}): 3433.2 cm^{-1} (OH stretch), 3287.38 cm^{-1} (NH stretch), 3017 cm^{-1} (Ar-CH stretch), 2935 cm^{-1} (Alip-CH stretch) 1652 cm^{-1} (C=O stretch. The molecular ion peak was obtained at 587 (M+2).

Antioxidant activity: The result of sample was compared with the standard (butyl hydroxyl toluene-BHT). 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

In conclusion, we have synthesized a linear tetrapeptide (L-Phe-L-Ala-L-Tyr-L-Val), and carried out docking studies of the tetrapeptides using Schrodinger software tool. Qikprop results showed that the ligand L-FAYV resembles the drug like properties related to antihypertensive and anticoagulant agents. The tetrapeptide was synthesized conveniently by solution phase technique and was characterized by IR, ^{1}H NMR and FAB-Mass spectral studies. The synthesized tetrapeptides (L-Phe-Ala-Tyr-Val) exhibited significant antioxidant activity.

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**Table 1: Docking Score of Tautomers of FAYV**

Sl.no	FAYV Isomers	Docking score
1	D-Phe-L-Ala-L-Tyr-L-Val	-5.01
2	L-Phe-L-Ala-D-Tyr-L-Val	-4.76
3	L-Phe-L-Ala-L-Tyr-L-Val	-4.43
4	L-Phe-D-Ala-L-Tyr-D-Val	-3.89

Table 2: Physical Data of FAYV

Sl.no	Compound	Nature	% of Yield
1	L-Phe-L-Ala-L-Tyr-L-Val	Light brown semisolid mass	82

Table: 3 Antioxidant activity of synthesized peptide

Conc. (µg/ml)	Absorbance (Std)	%inhibition (Std)	Absorbance (Sample FAYV)	%inhibition (Sample FAYV)
10	0.1087	39.3076	0.19	5
20	0.0958	46.5103	0.14	30
50	0.0761	57.5097	0.11	45
100	0.0311	82.6353	0.09	55

(i)DCC, Et₃N, 24h, RT
(ii)LiOH, THF:H₂0, Reflux, 15 min
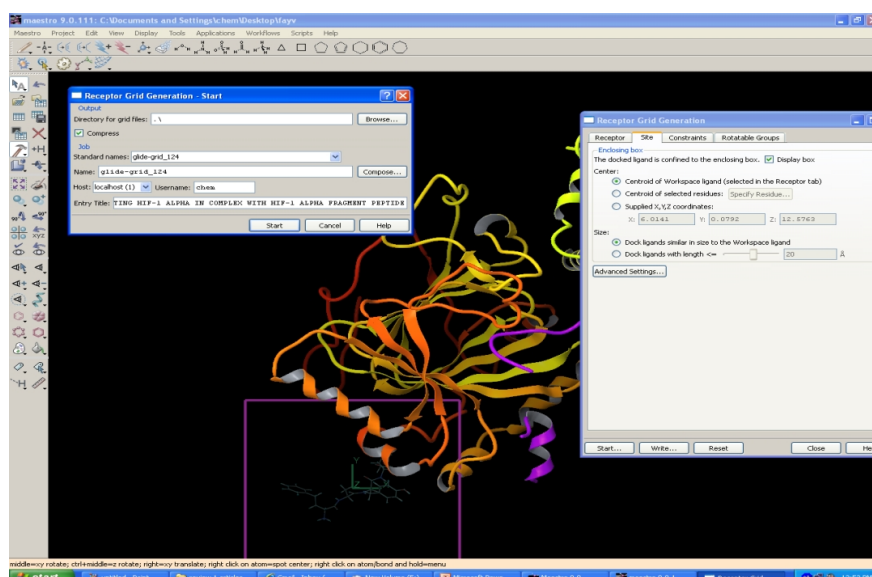(iii)TFA, CHCl₃, 2hr, RT
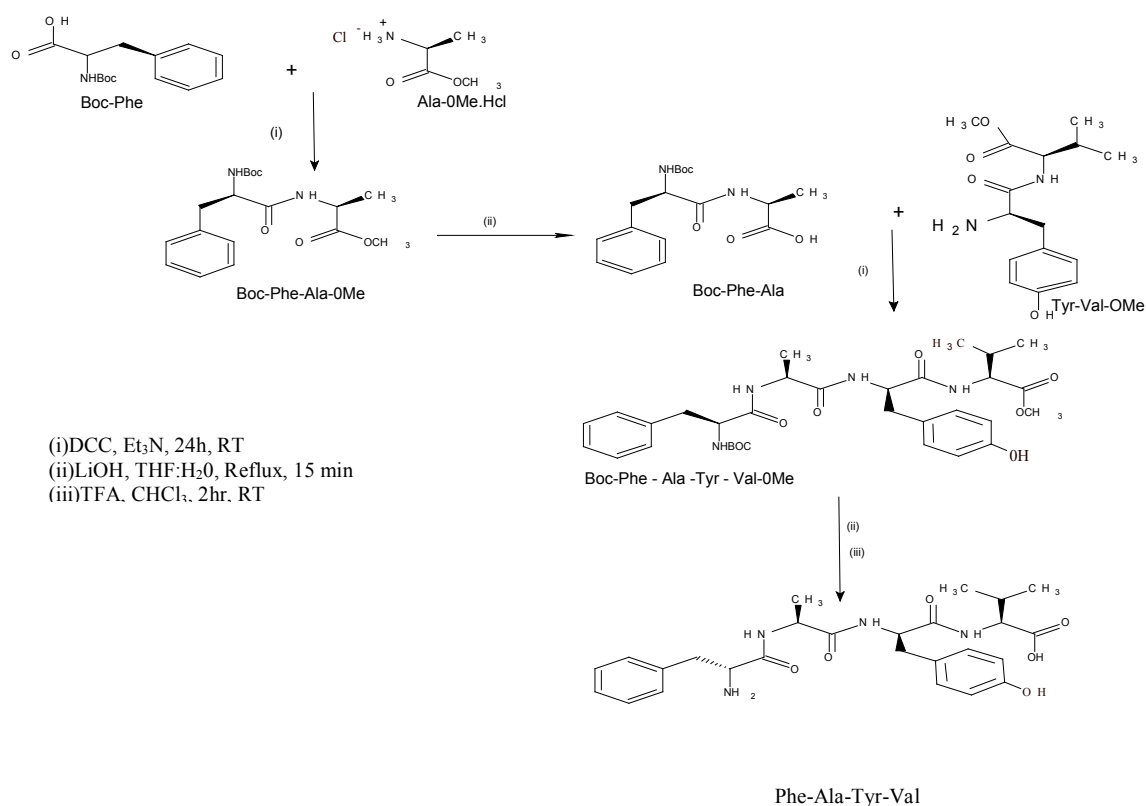
Scheme 1

Figure 1:Binding of ligand with HIF-1α protein

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