

FACILE SYNTHESIS, DOCKING STUDIES AND ANTIOXIDANT ACTIVITY OF FGVR

Himaja M^{1*}, Abdulla M¹, Karigar Asif², Ramana M.V² and Munirajasekhar¹

¹Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore 632 014, India

²Maratha Mandal's College of Pharmacy, Belgaum 590016, Karnataka, India

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*Dr (Mrs.) M. Himaja, M.Sc., PhD, Professor, Pharmaceutical Chemistry Division, School of Advanced Sciences VIT University, Vellore-632014 Tamil Nadu, India

ABSTRACT

A rational designing of linear Tetrapeptide FGVR was done and was synthesized by solution phase peptide synthesis. The docking studies of designed linear tetrapeptide FGVR was carried out by using Schrodinger Software Solutions, USA. Qikprop results show the ligand FGVR mostly act as antihypertensive and anti coagulant properties. The solution phase synthesis of FGVR is carried out by using 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as coupling agents and N-Methyl morpholine (NMM) as base. Structure of synthesized FGVR was confirmed by FTIR, ¹H NMR and Mass spectral data, and evaluated for antioxidant property by using 1,1-diphenyl-2-picryl-hydrazil (DPPH) method and the synthesized peptides FGVR possess moderate antioxidant activity.

KEYWORDS: Tetrapeptide FGVR, Solution Phase Peptide Synthesis, Schrodinger software 2009 (Docking), Antioxidant activity.

INTRODUCTION

Most of the peptides are found to exhibit antifungal, antibacterial, antitubercular, anthelmintic, cytotoxic, antioxidant, anti-inflammatory and insecticidal activities¹⁻⁹. Peptides function as hormones, enzymes, enzyme inhibitors or substrates, growth promoters or inhibitors, neurotransmitters and immuno modulators. 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 in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking. In the present work the designed ligand FGVR targeted to the cancer cell proteins, human peptide deformylase protein and HIF-1 α protein using Schrodinger software.

MATERIALS AND METHODS

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 capillary method.

Amino acids, di-tert-butylpyrocarbonate, trifluoroacetic acid, EDC, Diethyl ether, Methanol and Chloroform were obtained from and Spectrochem Ltd, Mumbai. DPPH was obtained from Spectrochem Mumbai. 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 Bruker JOEL (400MHz) NMR spectrometer. The spectra was obtained in CDCl₃ 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-Val-Arg (nitro)-OMe were properly appropriated and coupled together to get the linear tetrapeptide (Scheme 1).

Side chain protection of arginine: The guanidino group of L-arginine was protected by the introduction of nitro group (Bodanszky et al¹⁰) to prevent side chain cross reaction as it has amino group in the side chain (Scheme 1).

Preparation of Dipeptides: Amino acid methyl ester HCl (10 mmol) was dissolved in chloroform (CHCl₃) (20 mmol). To this N-methyl morpholine (NMM) (4 ml) was added at 0^oC and the reaction mixture was stirred for 15 minutes. Boc amino acid (10 mmol) in chloroform (20 ml) and EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) (10mmol) were added with stirring. After 12hrs, the reaction was filtered and the residue was washed with CHCl₃ (30ml) and added to the filtrate. The

filtrate was washed with 5% NaHCO₃ (20 ml) and plain water (20 ml). The organic layer was dried over anhydrous sodium sulphate (Na₂SO₄), filtered and evaporated in vacuum. Petroleum ether was added at 0°C to recrystallize the pure product. Boc-Phe-Gly-OMe and Boc-Val-Arg(nitro)-OMe were prepared in this manner.

Preparation of linear Tetrapeptide: The ester group of the dipeptide (Boc-Phe-Gly-OMe) was removed and the Boc-group of another dipeptide (Boc-Val-Arg(nitro)-OMe) was deprotected. Both the deprotected units were coupled to get the linear tetrapeptide (Scheme 2).

Antioxidant activity

Synthesized linear tetrapeptide FGVR screened for antioxidant activity such as free radical scavenging activity. The free radical scavenging activity of the synthesized compounds was determined by the 1,1-diphenyl-2-picryl-hydrazil (DPPH). This activity was measured by following the method described by Ilhami Gülçin et al¹¹, where in 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 FGVR, 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 tetrapeptide FGVR 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:

$$\text{DPPH Scavenging effect (\%)} = [(A_c - A_s) / A_0] \times 100$$

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 ligands FGVR and FGVR is tried to dock 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 Tabke:2. With this study it was able to Predict that the designed ligand FGVR was able to bind to the protein Human mitochondrial peptide deformylase and FGVR was able to bind to the protein HIF-1α effectively.

Synthesis: A rational designing of linear Tetrapeptide was done and were synthesized by solution phase peptide synthesis¹⁰. 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 and is shown below:

Spectral Analysis: The structure of the synthesized compound was characterized by FT-IR, ¹H NMR and FAB-MS. ¹H 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, OCH₃), 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 (ν/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. Resulting a color change from purple to yellow, 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 tetrapeptide FGVR could be conveniently prepared by EDC/NMM method. The product could be obtained in a pure form since the byproduct from EDC was water-soluble. and docking studies of tetrapeptides was designed by using Schrodinger software. Qikprop results shows the ligand FGVR mostly act as antihypertensive anticoagulant properties. Designed tetrapeptides were synthesized by solution phase technique. The synthesized compound was characterized by FTIR, ¹H NMR and FAB-Mass spectral studies. The synthesized peptide FGVR possesses moderate antioxidant activity.

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Table: 1 Docking of FGVR with Mitochondrial peptide deformylase protein

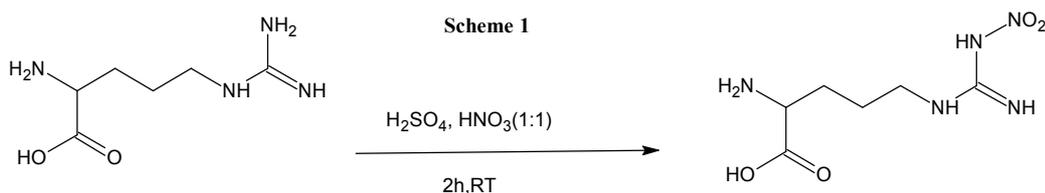
Sl.no	FGVR Isomers	Docking score
1	D-Phe-Gly-D-Val-L-Arg	-5.89
2	D-Phe-Gly-L-Val-L-Arg	-3.34
3	D-Phe-Gly-L-Val-L-Arg	-3.05

Table: 2 Physical data of synthesized peptide

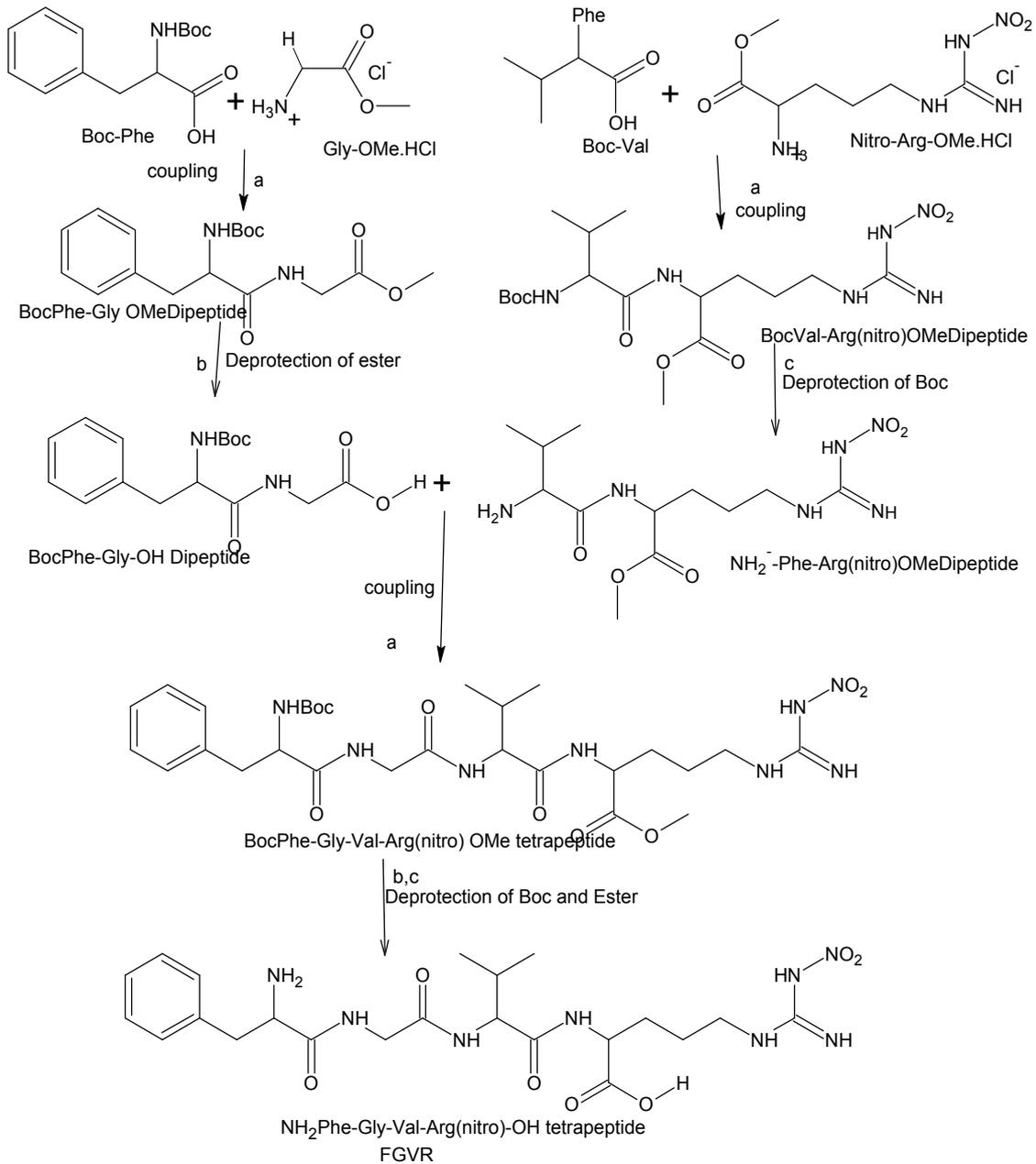
Compound	Nature	% of Yield
D-Phe-Gly-D-Val-L-Arg(nitro)	Brown Semi solid mass	78

Table: 3 Antioxidant activity of synthesized peptide

Conc. (μ g/ml)	Absorbance (Std)	% of inhibition (Std)	Absorbance (Sample FGVR)	% of inhibition (Sample FGVR)
10	0.1087	39.3076	0.15	16
20	0.0958	46.5103	0.12	33
50	0.0761	57.5097	0.10	44
100	0.0311	82.6353	0.08	66



Scheme 2



(a) EDC, Et₃N, 8h, RT, (b) LiOH, THF:H₂O, Reflux, 15 min, (c) TFA, CH₂Cl₂, 2hr, RT

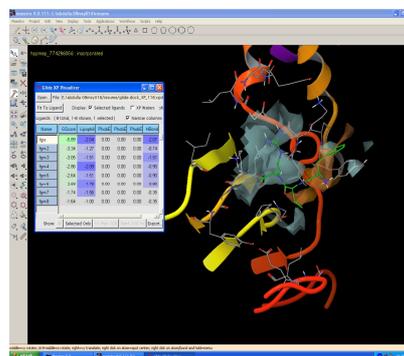


Fig-1: XP visualizer of docking of FGVR ligands Human Mitochondrial peptide deformylase
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