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Research Article

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF LINEAR TETRAPEPTIDE D-Ala-L-(Gly-Val-Val) (AGVV)

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ABSTRACT

A rational designing of the linear tetrapeptide D-Ala-L-(Gly-Val-Val) (AGVV) was done and was synthesized by solution phase peptide synthesis. The method includes synthesis of the tetrapeptide AGVV by using ethyl-3-dimethylaminopropylcarbodiimide (EDC) as a coupling reagent and triethylamine (TEA) as the base. The molecular docking studies of the designed tetrapeptide AGVV was carried out by using Molegro Virtual Docker software for anticancer properties. The synthesized compound was characterized by FT-IR, ^{1}H-NMR and Mass spectral data and was evaluated for antioxidant and anthelmintic activities. The linear tetrapeptide exhibited moderate antioxidant activity and good anthelmintic activity.

Keywords: Tetrapeptide, Solution phase peptide synthesis, EDC, Antioxidant activity, Anthelmintic activity.

INTRODUCTION

Peptides belongs to one of the most important classes of organic compounds with many biological activities like antifungal, antibacterial[1,2], antioxidant, anthelmintic, antitubercular, and anti-inflammatory activities[3-5]. The main goal in peptide synthesis is the development of approaches to design peptide ligands with specific chemical, physical and biological activities. Peptides ligands generally act by interaction with acceptor or receptor molecules like enzymes, hormones, neurotransmitters, etc[6,7].

Docking studies is frequently used to predict the binding orientation of drug candidates to their protein targets in order to predict their affinity and activities. In the present work the designed ligand AGVV was targeted to the cervical cancer cell protein, Fibroblast growth factor receptor 2 with PDB ID: 4J96 using Molegro Virtual Docker software.

In order to synthesize, the molecule was disconnected into two dipeptide units, which were synthesized and coupled using solution phase technique. The method includes the introduction of tertiary butyloxycarbonyl group (Boc) into amino acids to protect the amino group forming Boc-amino acid. The protection of the carbonyl group was done by converting into corresponding methyl ester. The protected amino acids were coupled using EDC to get the protected dipeptides. The Boc-group was removed by trifluoroacetic acid (TFA) and the ester group was removed by lithium hydroxide to get the desired tetrapeptide, AGVV. The structure of the tetrapeptide was confirmed by FT-IR, ^{1}H-NMR and Mass spectral data. The synthesized tetrapeptide was evaluated for antioxidant and anthelmintic activities. The compound showed moderate antioxidant and good anthelmintic activities.

MATERIALS AND METHODS

Commercially available reagents and analytical grade solvents were used without further purification. Anhydrous conditions for all the reactions were carried out 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, ditertbutylpyrocarbonate and trifluoroacetic acid were obtained from Spectrochem Ltd., Mumbai. EDC, DPPH, Diethyl ether, Methanol and Chloroform was obtained from AVRA. IR spectra were recorded on FT-IR spectrometer using a thin film support on KBr pellets. The values are reported as ν_{max} (cm^{-1}). ^{1}H NMR spectra was recorded on 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. The protection of amino and carboxyl group and their deprotection were done by standard procedures[8,9].

Preparation of Dipeptides:
Amino acid methyl ester hydrochloride (10 mmol) was dissolved in $CHCl_3$ (20 ml). To this, TEA (4 ml) was added and the reaction mixture was stirred for 15 minutes. Boc-amino acid (10 mmol) in $CHCl_3$ (20 ml) and EDC (10 mmol) were added with stirring. After 12 hour, the reaction mixture was transferred to a separating funnel and was washed with 5% $NaHCO_3$ (20 ml), 5% HCl (20 ml) and distilled H_2O (20 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in a vacuum. The residue was purified by recrystallization from $CHCl_3$[9].

Preparation of linear Tetrapeptide:
The ester group of the dipeptide Boc-D-Ala-L-Gly-OMe was removed and the Boc-group of another dipeptide Boc-L-Val-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 (Scheme 1).

Antioxidant Activity:
The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging effect was carried out according to the Gulcin *et al.* method.[10] Compounds of different concentrations were prepared in methanol. 1 ml of the sample solution having different concentrations (25, 50 and 100 µg/ml) were taken in different test tubes and to that 4 ml of 0.1 mM methanolic solution of DPPH was added and shaken vigorously. The tubes were then incubated in a dark place at room temperature for 30 minutes. A DPPH blank was prepared without compound and methanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-Visible spectrophotometer (Jasco V-670). The free radical scavenging activities were expressed as the inhibition percentage and were calculated using this formula,

Radical Scavenging activity (%) = $[(A_c-A_s)/A_c)\ 100]$

Where A_c is absorbance of the control (blank, without compound) and A_s is absorbance of the compound.

Anthelmintic Activity:
Anthelmintic activity study was carried out against earthworms (*Eudrilus eugeniae*) by Garg *et al.* method.[11] Suspension of the sample was prepared by triturating the sample with 15% Tween 80 and distilled water and the mixture was stirred using a magnetic stirrer for 30 minutes. The resulting suspension was used for the activity studies. The suspension was diluted to contain 100 mg in 20 ml of the test sample. The standard drug, mebendazole was also prepared with the same concentration in a similar way. Earthworms were placed in three petridishes containing 20 ml of each sample, standard drug and control (20 ml suspension of distilled water and 15% Tween 80) respectively at room temperature. The time required for the paralysis and death of the earthworms were noted. The death time was ascertained by placing the earthworms in warm water at 50^0C, which stimulated the movement if the earthworms were alive.

RESULTS AND DISCUSSION

Docking Studies:
A preliminary study was carried out on the linear tetrapeptide AGVV using Molegro Virtual Docker software where the ligand was docked with the cervical cancer cell protein, Fibroblast growth factor receptor 2 with PDB ID: 4J96 (Figure 1) along with two other ligands, GVVA and VAGV (listed in Table 1). The docking score revealed that the ligand AGVV showed highest docking score and hence a strong binding affinity towards the protein 4J96 effectively.

Physical Data and Spectral Analysis:
Yield 76.4%; light brown semisolid; IR spectrum (ν/cm^{-1}): 3435.2 cm^{-1} (OH stretch), 3289.48 cm^{-1} (NH stretch), 2936 cm^{-1} (Alip-CH stretch), 1651 cm^{-1} (C=O stretch); ^{1}H NMR spectrum (δ, ppm): 11.1 (1H, s, OH), 7.9-8.0 (2H, s, NH), 1.04 (12H, d, CH_3), 1.28 (3H, d, CH_3) 2.68-2.8 (1H, m, CH), 4.1 (2H, d, CH_2), 2.05 (2H, s, NH_2); The molecular ion peak was obtained at 342 (M+2).

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 change of color from purple to yellow indicated that the absorbance decreased when the DPPH was scavenged by an antioxidant through donation of hydrogen atom to form stable DPPH molecule. Table 2 illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of prepared sample and standard.

Anthelmintic activity:
The synthesized compound has shown good anthelmintic activity. The result of the sample was compared with the standard drug, mebendazole. Table 3 illustrates the results of the anthelmintic activity of the sample and standard drug.

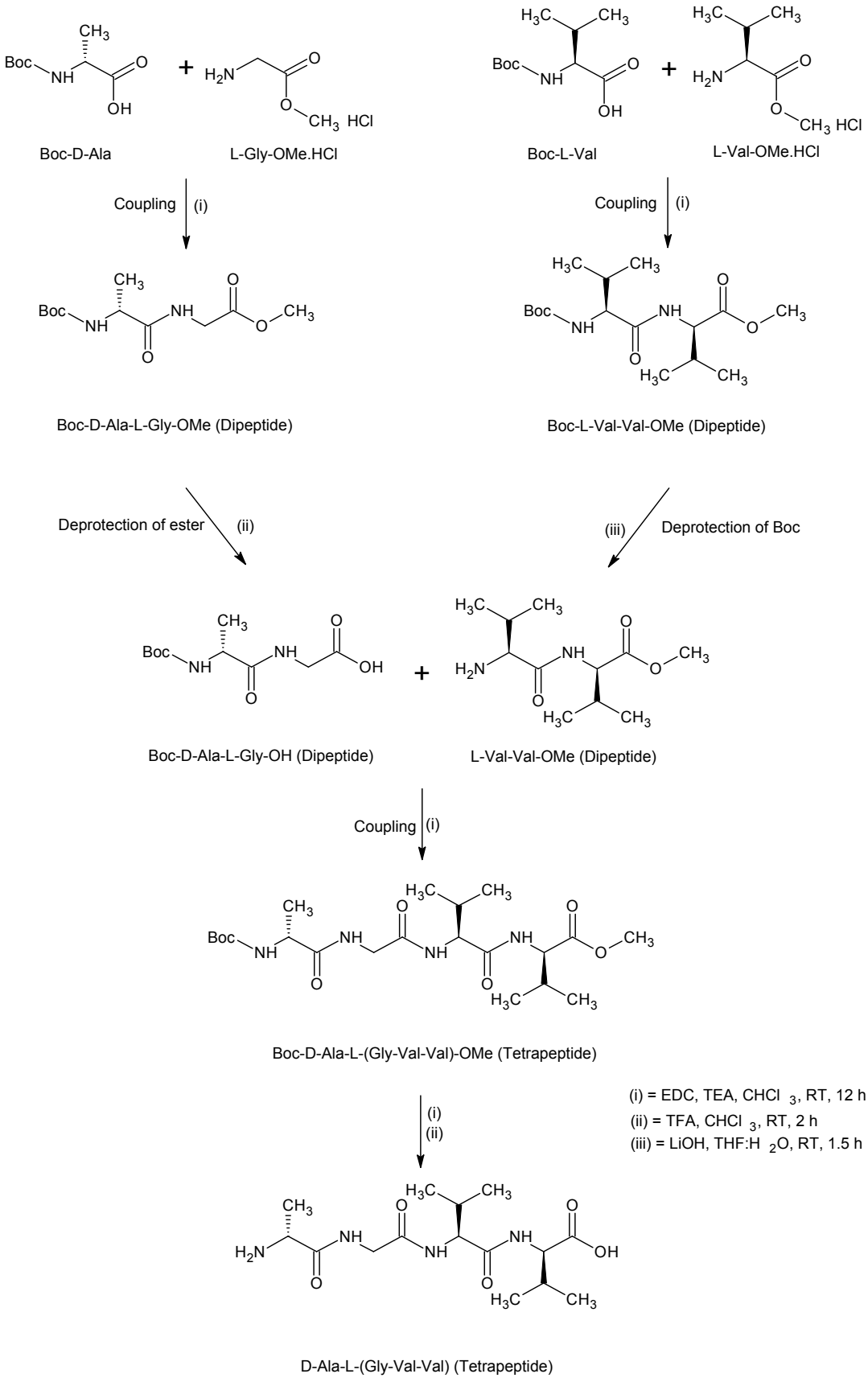
Table 1: Docking scores of the ligands (tetrapeptides)

Sl. No.	Ligands	Docking Score
1	AGVV	-75.6354
2	GVVA	-70.9385
3	VAGV	-68.9439

Table 2: Antioxidant activity of AGVV

Conc. (µg/ml)	Absorbance (Std.)	% of inhibition (std.)	Absorbance (AGVV)	% of inhibition (AGVV)
25	0.117	37.09	0.129	30.64
50	0.083	55.37	0.098	47.31
100	0.052	72.04	0.067	63.97

Scheme 1


Boc-D-Ala
L-Gly-OMe.HCl
Boc-L-Val
L-Val-OMe.HCl
Coupling (i)
Boc-D-Ala-L-Gly-OMe (Dipeptide)
Boc-L-Val-Val-OMe (Dipeptide)
Deprotection of ester (ii)
(iii) Deprotection of Boc
Boc-D-Ala-L-Gly-OH (Dipeptide)
L-Val-Val-OMe (Dipeptide)
Coupling (i)
Boc-D-Ala-L-(Gly-Val-Val)-OMe (Tetrapeptide)
(i) = EDC, TEA, CHCl 3, RT, 12 h
(ii) = TFA, CHCl 3, RT, 2 h
(iii) = LiOH, THF:H 2O, RT, 1.5 h
(i)
(ii)
D-Ala-L-(Gly-Val-Val) (Tetrapeptide)

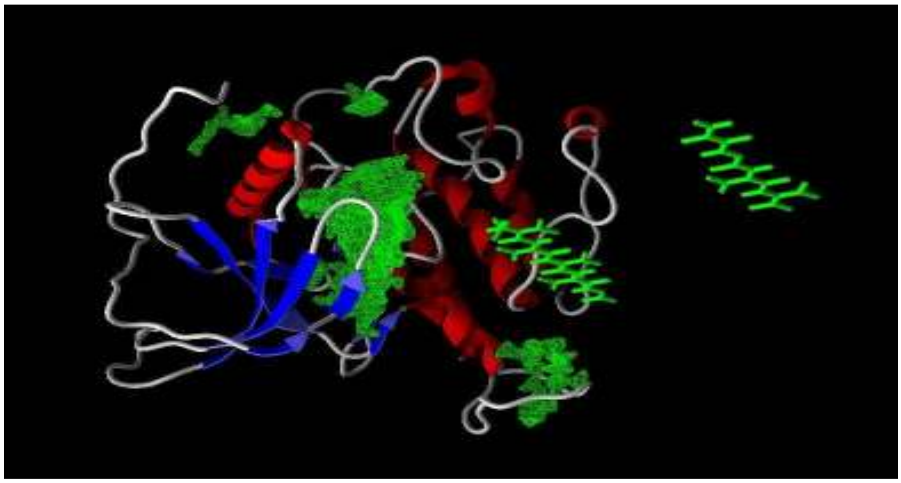


Figure 1: Screenshot of the docking of the ligands with the receptor (4J96)

Table 3: Anthelmintic activity of AGVV

Sl. No.	Compound	Conc. (mg)	Paralyzing time (Mins. Secs.)	Death time (Mins. Secs.)
1	AGVV	100	63.55	67.6
2	Mebendazole (Std.)	100	55.25	58.9
3	Control	-	-	-

CONCLUSION

The tetrapeptide could be conveniently prepared by EDC/TEA method in less time (12 hours), with a high yield (76%) and in a pure form since the byproduct from EDC was water soluble. Docking study of the tetrapeptide on the target protein, Fibroblast growth factor receptor 2 with PDB ID: 4J96 showed that the ligand AGVV resembles the drug like properties of anticancer agents. The synthesized tetrapeptide was characterized by FT-IR, ^{1}H- NMR and Mass spectral data. The compound AGVV exhibited moderate antioxidant activity due to the presence of labile hydrogen atoms and good anthelmintic activity probably due to the presence of D-Alanine.

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