



DOCKING, SYNTHESIS AND ANTITUBERCULAR ACTIVITY OF SOME HEPTAPEPTIDE DERIVATIVES

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ABSTRACT

Docking was done by using Hex software. Docking helps to determine which compounds can be synthesized. Glauca cyclopeptide A is a cyclic heptapeptide isolated from the seeds of *Annona glauca*, was synthesized by solution-phase peptide technique. Glauca cyclopeptide A and its N-methylated analogue were synthesized using Dicyclohexylcarbodiimide (DCC) and triethyl amine (Et₃N) as base. Structures of all the newly synthesized compounds were confirmed by FTIR, ¹H NMR and Mass spectral data. Glauca cyclopeptide analogs were screened for their antitubercular activity and was assessed against *M. tuberculosis* H₃₇Rv (ATCC 2729411). The compounds exhibit significant antitubercular activity.

KEY WORDS: Glauca cyclopeptide A, heptapeptide, Solution Phase Synthesis, Docking, Hex 5.1 software, Antitubercular activity.

INTRODUCTION

Glauca cyclopeptide A is a cyclic heptapeptide isolated from the seeds of *Annona glauca* and belongs to family Annonaceae¹. Plants of the Annonaceae family are very important sources of edible fruits and material for perfumery, and are used in folk medicine in various capacities, such as antitumoral, paracitidial and antidiarrhoeal agents²⁻⁵. Keeping in view the significant biological activities, as a part of ongoing study, an attempt was made to synthesize some plain and N-methylated heptapeptides in the laboratory, as the N-methylation in cyclic peptide is found to increase their activity^{6,7}. The antitubercular activity of compounds was assessed against *M. tuberculosis* H₃₇Rv (ATCC 2729411). Docking studies also carried out for all synthesized heptapeptides by using Hex 5.1 software and 2 YES as target protein. The synthesized Glauca cyclopeptide A and its N-methylated analogue have shown significant antitubercular activity comparable with the standard drugs.

EXPERIMENTAL

Materials & Methods

All the reactions required anhydrous conditions were conducted in flame dried apparatus. Solvents and reagents were purified by standard methods. Organic extracts were dried over anhydrous sodium sulphate. All amino acids and other chemicals were obtained from spectrochem Ltd (Mumbai, India). Melting points were determined in open capillary tubes and are uncorrected. Purity of the compounds was checked by pre-coated TLC plate. IR spectra were recorded on Thermo Nicolet FTIR 330 spectrometer using a thin film supported on KBr pellets. ¹H NMR spectra were recorded on Bruker AC NMR spectrometer using CDCl₃ as solvent. FAB Mass spectra were recorded on a Joel Sx 102/DA-6000.

Docking

Docking was done by using Hex software. Docking helps to determine which compounds can be synthesized.

Molecular docking involves the following steps using Hex 5.1 software:

- Identify a target protein 2 YES from the Protein data Bank.
- Download PDB FILE (text) and save in Example Folder of Hex 5.1
- Draw all the ligands using Chem Sketch.
- Generate 3-D view (SDF format), convert it into MOL file.
- Convert into PDB format by using Swiss PDB viewer and save it.
- Open Hex 5.1 software, select appropriate protein and ligand and perform Docking.
- Tabulation the all ligands docking score.

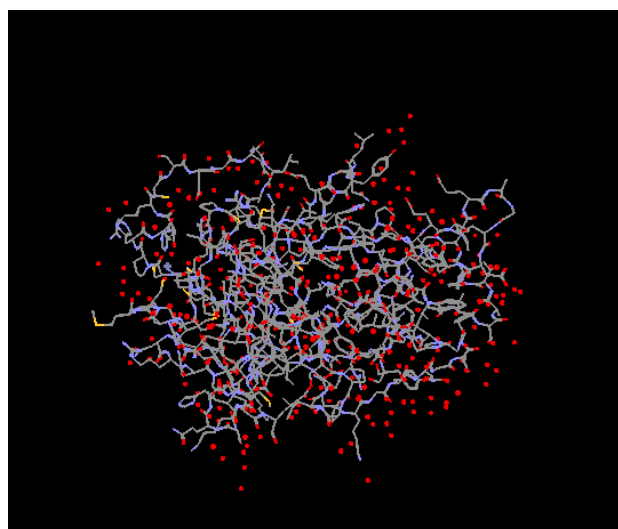


Fig. 1: 3D- view of 2YES protein

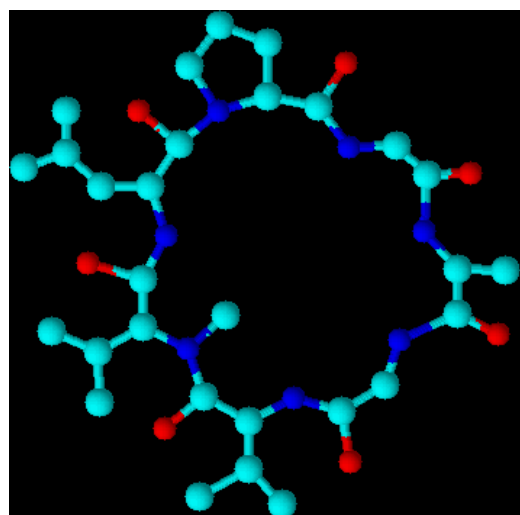
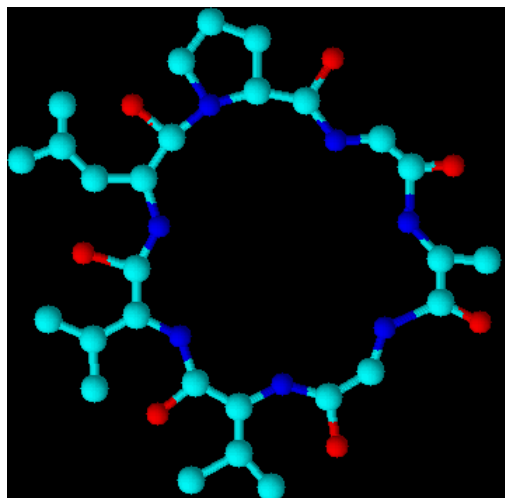


Fig. 2: **Ligands:** 1. glaucacyclopeptide A
2. N-methylated glaucacyclopeptide A

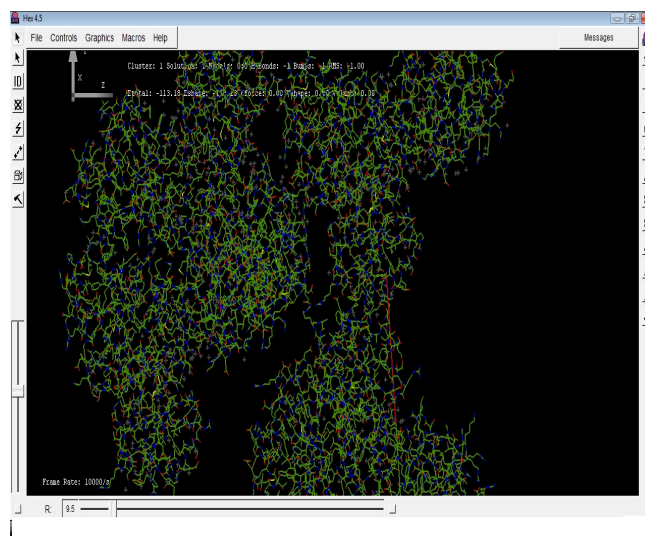
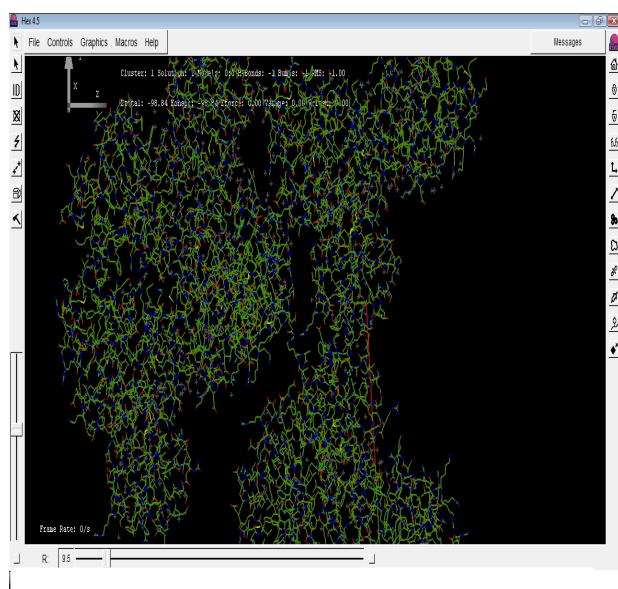
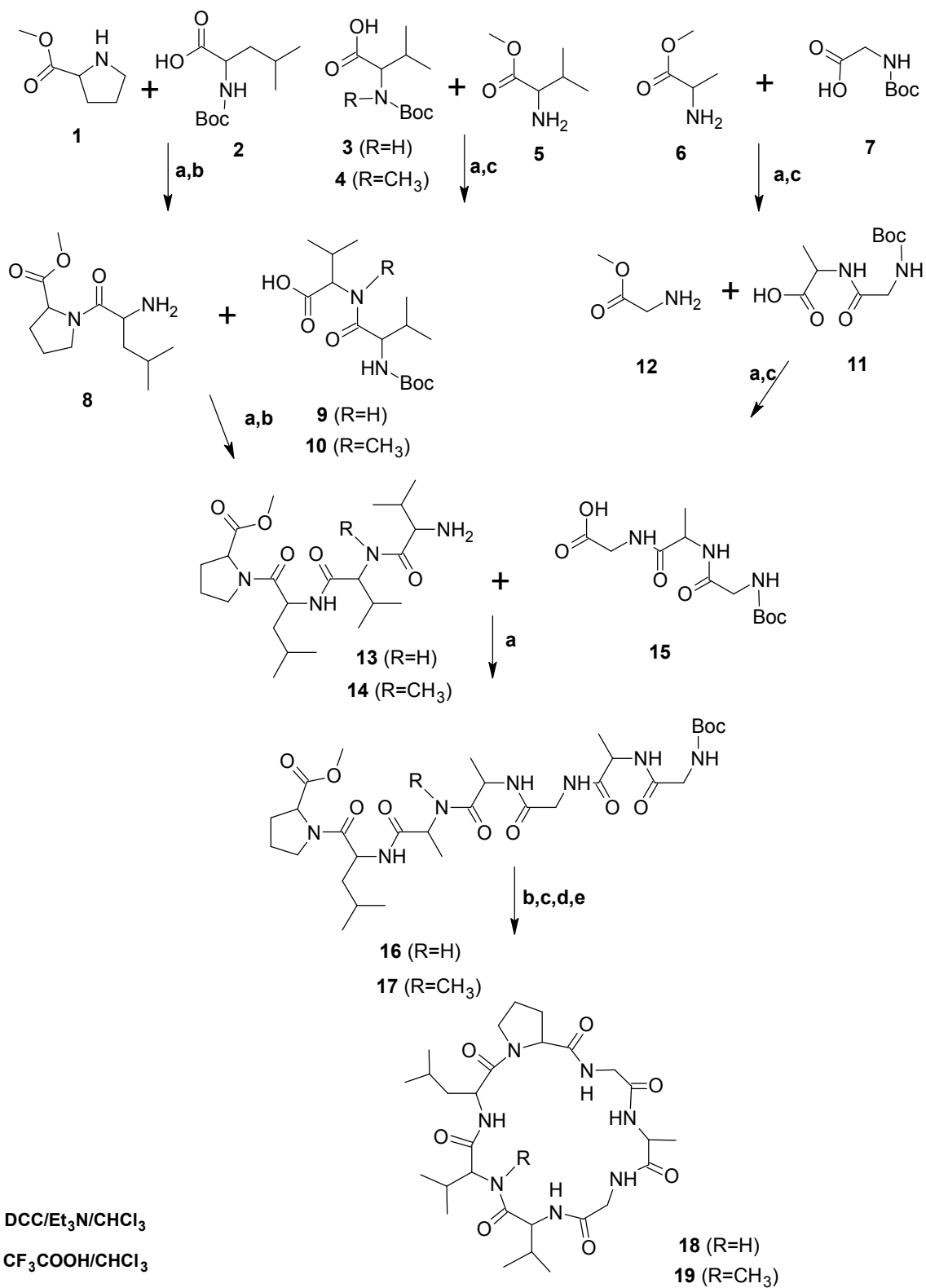


Fig. 3: binding interaction of 2 YES with
glaucacyclopeptide A(18) and N-methylated
glaucacyclopeptide A (19)

In order to carry out the synthesis, the cyclic heptapeptide was disconnected into one tripeptide and tetrapeptide units. Boc-Gly-Ala-Gly-OMe and Boc-Val-Val-Leu-Pro-OMe. These units were properly appropriated and coupled together to get the linear heptapeptide and were finally cyclised using *p*-nitrophenyl ester method⁸⁻¹⁰ (Scheme 1).

Preparation of Dipeptides: Amino acid methyl ester hydrochloride (10mmol) was dissolved in chloroform (20ml). To this, triethyl amine (4ml, 10mmol) was added at 0°C and the reaction mixture was stirred for 15 mins. Boc-amino acid (10mmol) in CHCl₃ (20ml) and DCC (10mmol) were added with stirring. After 12hrs, the reaction mixture was filtered and the residue was washed with CHCl₃ (30ml) and added to the filtrate. The filtrate was washed with 5% NaHCO₃ (20ml) and saturated NaCl (20ml) solutions. The organic layer was filtered and evaporated in vacuum. To remove the traces of impurities the product was dissolved in minimum amount of chloroform and cooled to 0°C. The crystallized impurity was removed by filtration. Petroleum ether was added to the filtrate at 0°C to recrystallize the pure product. Boc-Leu-Pro-OMe (1), Boc-Val-Val-OMe (2) and Boc-Gly-Ala-OMe (3) were prepared in this manner.

Scheme 1



a = DCC/Et₃N/CHCl₃

b = CF₃COOH/CHCl₃

c = LiOH, THF: H₂O

d = Para nitro phenol

e = CHCl₃/NMM

7 days refrigerator

Preparation of the Tetrapeptide Boc-Val-Val-Leu-Pro-OMe: The tetrapeptide was prepared from the dipeptides Boc-Leu-Pro-OMe (1) and Boc-Val-Val-OMe (2) units after appropriate deprotection at the required functional groups. The deprotected dipeptides units were coupled using DCC/ Et_3N to get the protected tetrapeptide by the procedure similar to that of the dipeptides.

Preparation of the Tripeptide Boc-Gly-Ala-Gly-OMe: The tripeptide was prepared from the dipeptide Boc-Gly-Ala-OMe (3) and Gly-OMe (4) units after appropriate deprotection at the required functional groups using DCC/ Et_3N to get the protected tripeptide.

Preparation of linear heptapeptide: The ester group of the tripeptide (Boc-Gly-Ala-Gly-OMe) was removed and the Boc-group of the tetrapeptide (Boc-Val-Val-Leu-Pro-OMe) was deprotected. Both the deprotected units were coupled to get the linear heptapeptide.

Preparation of Cyclic heptapeptide (18&19): The cyclisation of the linear heptapeptide unit was carried out by the p-nitrophenyl ester method of Bodanszky⁸ with certain modifications. The ester group of the linear fragment was removed and the p-nitrophenyl ester group was introduced by stirring it for 12 hrs in CHCl_3 with p-nitrophenol at 0°C . The reaction mixture was washed several times with saturated NaHCO_3 until the unreacted p-nitrophenol was removed completely and washed with 5% HCl to get Boc-peptide-pnp ester. The Boc-group also was removed, added CHCl_3 and Et_3N and the reaction mixture was kept at 0°C for 7 days. The mixture was finally washed with 5% HCl, dried and evaporated in vacuum to get the cyclised product¹⁰.

Antitubercular activity

The antimycobacterial activity for all synthesized heptapeptides was assessed against *M. tuberculosis* H₃₇Rv (ATCC 27294) using microplate Alamar Blue assays (MABA)¹¹⁻¹². This methodology is nontoxic, uses a thermally-stable reagent and shows good correlation with proportional and BACTEC radiometric methods¹³, and the activity expressed as the minimum inhibitory concentration (MIC) in $\mu\text{g/mL}$. The MICs of the compounds were depicted in Table 1. Streptomycin and Pyrazinamide was used as standards. N-methylated analogue showed better in vitro antitubercular activity at minimum concentrations compared with synthesized Glaucacyclopeptide A and standard drugs.

RESULTS AND DISCUSSION

FTIR spectra of cyclic heptapeptide unit showed characteristic medium to strong bands corresponding to carbonyl stretching at 1627 cm^{-1} and NH bending at 1573 cm^{-1} , confirming the coupling reaction of the cyclic heptapeptide compound. ^1H NMR spectra of the synthesized compound clearly indicates the coupling of amino acids and peptides. The mass spectral data indicates stable molecular ion peak for the synthesized compound. Therefore the cyclic heptapeptide structure was confirmed by FTIR, ^1H NMR and MASS spectral data.

SPECTRAL DATA

Tetrapeptide (Boc-Val-Val-Leu-Pro-OMe):

Brown sticky semisolid mass, Molecular Formula: $\text{C}_{27}\text{H}_{48}\text{N}_4\text{O}_7$, FTIR (KBr cm^{-1}): 3327(-NH Stretch), 2982 & 2927(-CH Stretch), 1749 (CO of ester group), 1681(CO Stretch), 1570(-NH Bend), 1461 (-CH bend).

Tripeptide (Boc-Gly-Ala-Gly-OMe):

Yellowish sticky semisolid mass, Molecular Formula: $\text{C}_{13}\text{H}_{23}\text{N}_3\text{O}_6$, FTIR (KBr cm^{-1}): 3329(-NH Stretch), 2927 & 2850(-CH Stretch), 1700 (CO of ester group), 1650(CO Stretch), 1527(-NH Bend), 1466 (-CH bend).

Cyclic heptapeptide (Cyclo-[Gly-Ala-Gly-Val-Val-Leu-Pro]):

yield 69%, m.p. $167-168^\circ\text{C}$, yellowish crystals, Molecular weight: 593, Molecular Formula: $\text{C}_{28}\text{H}_{47}\text{N}_7\text{O}_7$, FTIR (KBr): 3327(-NH Stretch), 2929 & 2850(-CH Stretch), 1720 (CO Stretch), 1535(-NH Bend), 1436 (-CH bend); ^1H NMR (CDCl_3): 6.87-5.5 (s, 6H, -NH), 4.3-4.1 (m, 3H), 3.5-3.4 (m, 2H, -CH₂), 3.2-3.1 (m, 2H, -CH₂), 1.5 (m, 2H, -CH₂), 1.6 (m, 3H, -CH), 1.3-1.0 (m, 21H, 7(-CH₃)). FABMASS: m/z: 594 (M+1).

Cyclic heptapeptide (Cyclo-[Gly-Ala-Gly-Val-(N-Me)Val-Leu-Pro]):

yield 71%, m.p. $182-183^\circ\text{C}$, yellowish brown crystals, Molecular weight: 607, Molecular Formula: $\text{C}_{29}\text{H}_{49}\text{N}_7\text{O}_7$, FTIR (KBr): 3329(-NH Stretch), 2941 & 2852(-CH Stretch), 1718 (CO Stretch), 1575(-NH Bend), 1448 (-CH bend); ^1H NMR (CDCl_3): 5.28-5.0 (s, 5H, -NH), 4.5-4.4 (m, 4H), 3.7-3.6 (m, 4H, -CH₂), 2.8 (s, 3H, N-CH₃), 2.19-2.17 (m, 2H, -CH₂), 2.15-2.10 (m, 2H, -CH₂), 1.9 (m, 3H, -CH), 1.8-1.4 (m, 21H, 7(-CH₃)). FABMASS: m/z: 607 (M+1).

The MICs of the compounds were depicted in Table 1. Streptomycin and Pyrazinamide was used as standards. N-methylated analogue showed better in vitro antitubercular activity at minimum concentrations compared with synthesized linear heptapeptide, glaucacyclopeptide A and standard drugs. All the synthesized heptapeptides showed good docking score and N-methylated showed better docking score compared with non methylated ones.

Table 1: Antitubercular activity of synthesized heptapeptides

| Sl No | Compound | Composition | MIC ($\mu\text{g/mL}$) | PDB CODE | E SCORE (KJ/ Mol) |
|-------|--------------|--|--------------------------|----------|-------------------|
| 1 | 16 | Gly-Ala-Gly-Val-Val-Leu-Pro | 12.5 | 2YES | -144.60 |
| 2 | 17 | Gly-Ala-Gly-Val-(N-Me)Val-Leu-Pro | 6.25 | 2YES | -145.74 |
| 3 | 18 | [Cyclo(Gly-Ala-Gly-Val-Val-Leu-Pro)] | 6.25 | 2YES | -150.23 |
| 4 | 19 | [Cyclo(Gly-Ala-Gly-Val-(N-Me)Val-Leu-Pro)] | 1.6 | 2YES | -151.93 |
| 5 | Streptomycin | - | 6.25 | 2YES | -125.43 |
| 6 | Pyrazinamide | - | 3.12 | 2YES | -123.07 |

The MIC values were evaluated at concentration range, 0.1-100 $\mu\text{g/mL}$. The figure in the table showed the value in $\mu\text{g/mL}$.

CONCLUSION

All the four heptapeptides were synthesized by solution phase technique of peptide synthesis using Dicyclohexylcarbodiimide (DCC) and triethyl amine (Et_3N) as base. All the synthesized compounds showed potent antitubercular activity. N-methylated analogue of linear heptapeptide (17) and cyclic heptapeptide (19) showed better in vitro antitubercular activity at minimum concentrations as compared to linear (16) and cyclic (18) non methylated analogs and further the N-methylated cyclic peptide (19) showed better antitubercular activity compared to standard drugs streptomycin and Pyrazinamide. All the compounds showed good docking score. The increased activity of N-methylated analogue may be assumed due to the change in the hydrogen bond formation and increased lipophilic

character of the molecule which enhances the permeability of the molecule into the Mtb cell wall.

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