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Synthesis, Spectroscopic and Biological Investigation of Cyclic Octapeptide: Cherimolacyclopeptide G

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A natural cyclic octapeptide cherimolacyclopeptide G (**8**) was synthesized by coupling of tetrapeptide units Boc-Gly-Ala-Val-Pro-OMe (**5**) and Boc-Ile-Tyr-Ala-Pro-OMe (**6**) after proper deprotection at carboxyl and amino terminals followed by cyclization of the linear peptide segment. The structure was elucidated by IR, ^{1}H-NMR, ^{13}C-NMR, FAB MS spectral data, and elemental analyses. The newly synthesized cyclopeptide was also evaluated for its antimicrobial, anthelmintic, and cytotoxic activities and found to exhibit potent anthelmintic and cytotoxic activity against earthworms, *Megascoplex konkanensis*, *Pontoscotex corethruses*, and *Eudrilus* species, and Dalton's lymphoma ascites (DLA) and Ehrlich's ascites carcinoma (EAC) cell lines. In addition, compound **8** possessed moderate antimicrobial activity against the pathogenic fungus *Candida albicans* and gram-negative bacterium *Pseudomonas aeruginosa*.

Key Words: Cyclic octapeptide, cherimolacyclopeptide G, antibacterial activity, antifungal activity, anthelmintic activity, cytotoxicity.

Introduction

In past decades, plants were well recognized for their ability to produce a wide spectrum of natural products with interesting biological activities.[1–6] Among these, large cyclopeptides containing 6 to 9 amino acid units have received special attention due to their unique structures and wide biological profile, which may prove better candidates to overcome the problem of the widespread increase in resistance to conventional drugs. A new potent cytotoxic cyclic peptide, cherimolacyclopeptide G, has been isolated from seeds of *Annona cherimola* and the structure was elucidated by 2D-NMR and mass spectrometry.[7]

In continuation of our research work on the synthesis of natural cyclic polypeptides of biological interest,[8–16] an attempt was made to synthesize cherimolacyclopeptide G. In view of the significant biological activities possessed by various cyclopeptides, the above synthetic peptide was further subjected to antibacterial, antifungal, anthelmintic, and cytotoxic activity studies.

Experimental

Materials

All the reactions requiring anhydrous conditions were conducted in a flame dried apparatus. Melting point was determined by open capillary method and was uncorrected. L-Amino acids, dicyclohexylcarbodiimide (DCC), trifluoroacetic acid (CF_3COOH), p-nitrophenol (PNP), N-methylmorpholine (NMM), triethylamine (TEA), di-*tert*-butylpyrocarbonate (Boc_2O), and pyridine (C_5H_5N) were obtained from Spectrochem Limited (Mumbai, India).

Instrumentation

IR spectra were recorded on a Shimadzu 8700 FTIR spectrophotometer (Shimadzu, Japan) using a thin film supported on KBr pellets for synthesized cyclic octapeptide and $CHCl_3$ as solvent for intermediate semisolids. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AC NMR spectrometer (300 MHz), (Brucker, USA) using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on a JMS-DX 303 Mass spectrometer (Jeol, Tokyo, Japan) operating at 70 eV using the fast atom bombardment technique. Elemental analyses of all compounds were performed on a Vario EL III elemental analyzer (Elementar, Germany). Optical rotation of the synthesized peptides was measured on an automatic polarimeter (Optics Tech, Ghaziabad, India) in a 2 dm tube at 25 °C using a sodium lamp and methanol as solvent. Purity of the synthesized cyclopeptide as well as intermediates was checked by TLC on precoated silica gel G plates utilizing $CHCl_3$/MeOH as developing solvent in different ratios (8:2/7:3 v/v) and brown spots were detected on exposure to iodine vapors in a tightly closed chamber.

General method for the synthesis of linear peptide fragments 1-7

Amino acid methyl ester hydrochloride/peptide methyl ester (0.01 mol) was dissolved in $CHCl_3$ (20 mL). To this was added NMM (2.23 mL, 0.021 mol) at 0 °C and the reaction mixture was stirred for 15 min. Boc-amino acid/peptide (0.01 mol) in $CHCl_3$ (20 mL) and DCC (2.1 g, 0.01 mol) were added with stirring. After 24 h, the reaction mixture was filtered and the residue was washed with $CHCl_3$ (30 mL) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ and saturated NaCl solutions. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum. The crude product was recrystallized from a mixture of chloroform and petroleum ether followed by cooling at 0 °C.

The carboxyl group of L-amino acids was protected by esterification with methanol using $SOCl_2$. Peptide units were prepared by solution phase technique[17] employing DCC as coupling agent. Furthermore, CF_3COOH was used for the removal of the Boc group, and the ester group was removed by alkaline hydrolysis with lithium hydroxide.

^{t}Butyloxycarbonyl-glycyl-alanine methyl ester (1):

Semisolid mass; Yield 84.6% (2.2 g); $[\alpha]_D$ –14.2°; R_f– 0.87; IR ($CHCl_3$): v 3122 (m, -NH str, amide), 2954, 2925 (m, -CH str, asym, CH_3 and CH_2), 2852 (m, -CH str, sym, CH_2), 1748 (s, -C=O str, ester), 1645, 1636 (s, -C=O str, 2° amide), 1534 (m, -NH bend, 2° amide), 1390, 1366 (m, -CH bend, ^{t}Butyl group), 1272 (s, C–O str, ester), 932 (w, CH_3 rocking, ^{t}Butyl group) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.50 (1H, br. s, -NH), 6.22 (1H, br. s, -NH), 4.74-4.69 (1H, m, α-H, Ala), 3.59 (3H, s, OCH_3), 3.49-3.47 (2H, d, J = 4.8 Hz,

CH_2, Gly), 1.54 (9H, s, ^{t}Butyl group), 1.29-1.27 (3H, d, J = 4.25 Hz, β-H's, Ala) ppm; Anal. Calcd. for $C_{11}H_{20}N_2O_5$: C, 50.76; H, 7.74; N, 10.76. Found: C, 50.75; H, 7.72; N, 10.80%.

^{t}Butyloxycarbonyl-valyl-proline methyl ester (2):

Dense liquid; Yield 60.4% (1.98 g); $[\alpha]_D$ –78.5°; R_f - 0.65; IR ($CHCl_3$): v 3130 (m, -NH str, amide), 2994, 2990 (m, -CH str, cyclic CH_2 and CH), 1752 (s, -C=O str, ester), 1671, 1640 (s, -C=O str, 3° & 2° amide), 1535 (m, -NH bend, 2° amide), 1393, 1370 (m, -CH bend, ^{t}Butyl group), 1384, 1359 (s, -CH bend, isopropyl group), 1270 (s, C-O str, ester), 924 (w, CH_3 rocking, isopropyl group) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.30 (1H, br. s, -NH), 4.29-4.26 (1H, t, α-H, Val), 4.03-3.98 (1H, t, α-H, Pro), 3.63 (3H, s, OCH_3), 3.50-3.47 (2H, t, δ-H's, Pro), 2.07-1.96 (4H, m, β- & γ-H's, Pro), 1.67-1.55 (1H, m, β-H, Val), 1.54 (9H, s, ^{t}Butyl group), 1.04-1.02 (6H, d, J = 5.75 Hz, γ-H's, Val) ppm; Anal. Calcd. for $C_{16}H_{28}N_2O_5$: C, 58.52; H, 8.59; N, 8.53. Found: C, 58.50; H, 8.60; N, 8.52%.

^{t}Butyloxycarbonyl-isoleucyl-tyrosine methyl ester (3):

Semisolid mass; Yield 69.4% (2.83 g); $[\alpha]_D$ +1.3°; R_f - 0.52; IR ($CHCl_3$): v 3371 (m/br, -OH str, Tyr), 3127 (m, -NH str, amide), 3062 (w, -CH str, arom. ring), 2960, 2926 (m, -CH str, asym, CH_3 and CH_2), 2872 (m, -CH str, sym, CH_3), 1750 (s, -C=O str, ester), 1642, 1635 (s, -C=O str, 2° amide), 1589, 1475 (m, skeletal bands, arom. ring), 1535, 1526 (m, -NH bend, 2° amide), 1392, 1367 (m, -CH bend, ^{t}Butyl group), 1270 (s, C–O str, ester), 1228 (s, C–O str, phenolic), 824 (s, -CH bend, oop, arom. ring) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.92-6.90 (2H, dd, J = 7.5 Hz, o-H's, Tyr), 6.79-6.77 (2H, dd, J = 7.45 Hz, m-H's, Tyr), 6.73 (1H, br. s, -NH), 5.97 (1H, s, -OH, Tyr), 5.66 (1H, br. s, -NH), 4.60-4.56 (1H, m, α-H, Tyr), 4.32-4.29 (1H, t, α-H, Ile), 3.54 (3H, s, OCH_3), 2.81-2.79 (2H, d, J = 7.15 Hz, β-H's, Tyr), 1.78-1.71 (2H, m, γ-H's, Ile), 1.54 (9H, s, ^{t}Butyl group), 1.52-1.45 (1H, m, β-H, Ile), 1.04-0.96 (6H, m, $\gamma\prime$- and δ-H's, Ile) ppm; Anal. Calcd. for $C_{21}H_{32}N_2O_6$: C, 61.75; H, 7.90; N, 6.86. Found: C, 61.73; H, 7.89; N, 6.88%.

^{t}Butyloxycarbonyl-alanyl-proline methyl ester (4):

Semisolid mass; Yield 70.3% (2.11 g); $[\alpha]_D$ –11.7°; R_f - 0.81; IR ($CHCl_3$): v 3132 (m, -NH str, amide), 2996, 2992 (m, -CH str, cyclic CH_2 and CH), 2963 (m, -CH str, asym, CH_3), 2870 (m, -CH str, sym, CH_3), 1750 (s, -C=O str, ester), 1669, 1640 (s, -C=O str, 3° & 2° amide), 1534 (m, -NH bend, 2° amide), 1395, 1372 (m, -CH bend, ^{t}Butyl group), 1272 (s, C-O str, ester) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.40 (1H, br. s, -NH), 4.60-4.55 (1H, m, α-H, Ala), 4.32-3.29 (1H, t, α-H, Pro), 3.78-3.75 (2H, t, δ-H's, Pro), 3.62 (3H, s, OCH_3), 2.06-1.97 (4H, m, β- & γ-H's, Pro), 1.59-1.57 (3H, d, J = 4.3 Hz, β-H's, Ala), 1.55 (9H, s, ^{t}Butyl group) ppm; Anal. Calcd. for $C_{14}H_{24}N_2O_5$: C, 55.99; H, 8.05; N, 9.33. Found: C, 56.02; H, 8.08; N, 9.32%.

^{t}Butyloxycarbonyl-glycyl-alanyl-valyl-proline methyl ester (5):

Semisolid mass; Yield 79.4% (3.62 g); $[\alpha]_D$ –86.4°; R_f - 0.43; IR ($CHCl_3$): v 3128, 3124 (m, -NH str, amide), 2996, 2991 (m, -CH str, cyclic CH_2 and CH), 2955, 2927 (m, -CH str, asym, CH_3 & CH_2), 2853 (m, -CH str, sym, CH_2), 1670, 1643 (s, -C=O str, 3° & 2° amide), 1752 (s, -C=O str, ester), 1532 (m, -NH bend, 2° amide), 1395, 1372 (m, -CH bend, ^{t}Butyl group), 1382, 1358 (s, -CH bend, isopropyl group), 1270 (s, C–O str, ester), 933, 921 (w, CH_3 rocking, ^{t}Butyl & isopropyl groups) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.42 (1H, br. s, -NH), 8.21 (1H, br. s, -NH), 6.99 (1H, br. s, -NH), 4.43-4.38 (1H, m, α-H, Ala), 4.19-4.16 (1H, t,

α-H, Val), 3.67-3.62 (4H, m, α-H, Pro & OCH_3), 3.54-3.52 (2H, d, $J = 4.75$ Hz, CH_2, Gly), 3.14-3.07 (2H, t, δ-H's, Pro), 2.06-1.99 (4H, m, β- & γ-H's, Pro), 1.92-1.89 (1H, m, β-H, Val), 1.55 (9H, s, ^{t}Butyl group), 1.49-1.47 (3H, d, $J = 4.3$ Hz, β-H's, Ala), 1.04-1.02 (6H, d, $J = 5.8$ Hz, γ-H's, Val) ppm; Anal. Calcd. for $C_{21}H_{36}N_4O_7$: C, 55.25; H, 7.95; N, 12.27. Found: C, 55.25; H, 7.94; N, 12.30%.

^{t}Butyloxycarbonyl-isoleucyl-tyrosinyl-alanyl-proline methyl ester (6):

Semisolid mass; Yield 76.7% (4.42 g); $[\alpha]_D$ +112.0°; R_f - 0.69; IR ($CHCl_3$): v 3371 (m/br, -OH str, Tyr), 3132, 3127 (m, -NH str, amide), 3062 (w, -CH str, arom. ring), 2996, 2992 (m, -CH str, cyclic CH_2 and CH), 2963, 2960, 2926 (m, -CH str, asym, CH_3 and CH_2), 2872, 2870 (m, -CH str, sym, CH_3), 1750 (s, -C=O str, ester), 1669, 1642, 1635 (s, -C=O str, 3° & 2° amide), 1589, 1475 (m, skeletal bands, arom. ring), 1535, 1526 (m, -NH bend, 2° amide), 1395, 1369 (m, -CH bend, ^{t}Butyl group), 1273 (s, C–O str, ester), 1228 (s, C–O str, phenolic), 824 (s, -CH bend, oop, arom. ring) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 9.39 (1H, br. s, -NH), 7.94 (1H, br. s, -NH), 6.99-6.88 (4H, m, o- & m-H's, Tyr), 5.95 (1H, s, -OH, Tyr), 5.67 (1H, br. s, -NH), 4.39-4.27 (2H, m, α-H's, Ala & Tyr), 4.23-4.20 (1H, m, α-H, Ile), 3.93-3.89 (1H, t, α-H, Pro), 3.63 (3H, s, OCH_3), 3.40-3.36 (2H, t, δ-H's, Pro), 2.80-2.78 (2H, d, $J = 7.2$ Hz, β-H's, Tyr), 2.05-1.96 (4H, m, β- & γ-H's, Pro), 1.78-1.72 (2H, m, γ-H's, Ile), 1.54 (9H, s, ^{t}Butyl group), 1.51-1.49 (3H, d, $J = 4.25$ Hz, β-H's, Ala), 1.48-1.44 (1H, m, β-H, Ile), 1.05-0.96 (6H, m, $\gamma\prime$- & δ-H's, Ile) ppm; Anal. Calcd. for $C_{29}H_{44}N_4O_8$: C, 60.40; H, 7.69; N, 9.72. Found: C, 60.39; H, 7.70; N, 9.75%.

^{t}Butyloxycarbonyl-glycyl-alanyl-valyl-prolyl-isoleucyl-tyrosinyl-alanyl-proline methyl ester (7):

Semisolid mass; Yield 80.6% (7.25 g); $[\alpha]_D$ –72.8°; R_f - 0.56; IR ($CHCl_3$): v 3369 (m/br, -OH str, Tyr), 3133, 3126, 3122 (m, -NH str, amide), 3063 (w, -CH str, arom. ring), 2996, 2994, 2990 (m, -CH str, cyclic CH_2 and CH), 2962, 2955, 2928 (m, -CH str, asym, CH_3 & CH_2), 2873, 2869 (m, -CH str, sym, CH_3), 2852 (m, -CH str, sym, CH_2), 1670, 1668, 1643 (s, -C=O str, 3° & 2° amide), 1750 (s, -C=O str, ester), 1587, 1476 (m, skeletal bands, arom. ring), 1536, 1525 (m, -NH bend, 2° amide), 1395, 1370 (m, -CH bend, ^{t}Butyl group), 1383, 1357 (s, -CH bend, isopropyl group), 1272 (s, C–O str, ester), 1228 (s, C–O str, phenolic), 932, 922 (w, CH_3 rocking, ^{t}Butyl & isopropyl groups), 825 (s, -CH bend, oop, arom. ring) cm^{-1}; ^1H-NMR (300 MHz, $CDCl_3$): δ 8.43, 8.30, 8.23 (3H, br. s, -NH, Ala^1, Ile & Val), 8.02, 7.95 (2H, br. s, -NH, Tyr & Ala^2), 6.99-6.89 (4H, m, o- & m-H's, Tyr), 6.25 (1H, br. s, -NH, Gly), 5.96 (1H, s, -OH, Tyr), 4.56-4.53 (1H, t, α-H, Ile), 4.48-4.42 (1H, m, α-H, Ala^1), 4.32-4.19 (2H, m, α-H's, Ala^2 & Tyr), 4.02-3.99 (1H, t, α-H, Val), 3.93-3.88 (1H, t, α-H, Pro^2), 3.85-3.82 (1H, t, α-H, Pro^1), 3.72-3.70 (2H, d, $J = 4.8$ Hz, CH_2, Gly^1), 3.64 (3H, s, OCH_3), 3.41-3.37 (2H, t, δ-H's, Pro^2), 3.08-3.04 (2H, t, δ-H's, Pro^1), 2.80-2.61 (4H, m, β-H's, Pro^1 & Tyr), 2.06-1.99 (4H, m, β- & γ-H's, Pro^2), 1.94-1.85 (3H, m, γ-H's, Pro^1 & β-H, Val), 1.84-1.69 (3H, m, β- & γ-H's, Ile), 1.54 (9H, s, ^{t}Butyl group), 1.51-1.49 (3H, d, $J = 4.3$ Hz, β-H's, Ala^2), 1.26-1.24 (3H, d, $J = 4.25$ Hz, β-H's, Ala^1), 1.05-0.93 (12H, m, γ-H's, Val & $\gamma\prime$- and δ-H's, Ile) ppm; Anal. Calcd. for $C_{44}H_{68}N_8O_{12}$: C, 58.65; H, 7.61; N, 12.44. Found: C, 58.68; H, 7.60; N, 12.45%.

Synthesis of cyclic octapeptide, cherimolacyclopeptide G (8)

To synthesize compound **8**, linear octapeptide unit **7** (4.51 g, 0.005 mol) was deprotected at the carboxyl end using LiOH (0.18 g, 0.0075 mol) to get Boc-Gly-Ala-Val-Pro-Ile-Tyr-Ala-Pro-OH. The deprotected octapeptide unit (4.44 g, 0.005 mol) was then dissolved in $CHCl_3$ (50 mL) at 0 °C. To the above solution was added p-nitrophenol (0.94 g, 0.0067 mol), followed by stirring at RT for 12 h. The reaction mixture was

filtered and the filtrate was washed with 10% $NaHCO_3$ solution (3 × 15 mL) until the excess of p-nitrophenol was removed, and finally washed with 5% HCl (2 × 10 mL) to get the corresponding p-nitrophenyl ester Boc-Gly-Ala-Val-Pro-Ile-Tyr-Ala-Pro-O-PNP. To this compound (4.0 g, 0.004 mol) dissolved in $CHCl_3$ (35 mL) was added CF_3COOH (0.91 g, 0.008 mol), stirred at RT for 1 h, and washed with 10% $NaHCO_3$ solution (2 × 25 mL). The organic layer was dried over anhydrous Na_2SO_4 to give Gly-Ala-Val-Pro-Ile-Tyr-Ala-Pro-O-PNP, which was dissolved in $CHCl_3$ (25 mL) and NMM/TEA/C_5H_5N (2.21 mL/2.8 mL/1.61 mL, 0.02 mol) was added. Then the entire contents were kept at 0 °C for 7 days. The reaction mixture was washed with 10% $NaHCO_3$ solution until the by-product p-nitrophenol was removed completely and finally washed with 5% HCl (3 × 15 mL). The organic layer was dried over anhydrous Na_2SO_4. Finally, chloroform was distilled off and the crude cyclized product was crystallized from $CHCl_3$ and n-hexane to obtain pure cyclo (glycyl-alanyl-valyl-prolyl-isoleucyl-tyrosinyl-alanyl-prolyl) (**8**).

White solid; mp 276-277 °C (277-278 °C); Yield 84.0% (6.46 g, NMM), 68.4% (TEA), 59.3% (C_5H_5N); $[\alpha]_D$ –52.2° (–52.0°); R_f - 0.73; IR (KBr): v 3373 (m/br, -OH str, Tyr), 3125-3121 (m, -NH str, amide), 3077 (w, -CH str, arom. ring), 2997-2992 (m, -CH str, cyclic CH_2 and CH), 2959, 2872 (m, -CH str, asym & sym, CH_3), 2926, 2850, 2847 (m, -CH str, asym and sym, CH_2), 2825 (m, -CH str, OCH_3), 1672, 1669, 1643, 1639 (s, -C=O str, 3° & 2° amide), 1588, 1472 (m, skeletal bands, arom. ring), 1537-1525 (m, -NH bend, 2° amide), 1385, 1360 (s, -CH bend, isopropyl group), 1231 (s, C–O str, phenolic), 920 (w, CH_3 rocking, isopropyl groups), 824 (s, -CH bend, oop, arom. ring) cm^{-1}; ^1H-NMR (300 MHz, $CDCl_3$): δ 9.17, 8.68, 8.36 (3H, br. s, -NH, Gly, Ile & Tyr), 8.05, 7.80, 7.56 (3H, br. s, -NH, Ala^1, Val & Ala^2), 6.99-6.89 (4H, m, o- & m-H's, Tyr), 6.63-6.60 (1H, t, α-H, Val), 6.10-5.99 (2H, m, α-H's, Ala^1 & Ala^2), 5.96 (1H, s, -OH, Tyr), 5.67-5.62 (1H, m, α-H, Tyr), 3.98-3.96 (2H, d, J = 4.75 Hz, CH_2, Gly), 3.90-3.87 (1H, t, α-H, Pro^2), 3.82-3.78 (1H, m, α-H, Ile), 3.67-3.64 (1H, t, α-H, Pro^1), 3.28-3.24 (2H, t, δ-H's, Pro^2), 3.01-2.97 (2H, t, δ-H's, Pro^1), 2.70-2.65 (4H, m, β-H's, Pro^1 & Pro^2), 2.60-2.58 (2H, d, J = 7.2 Hz, β-H's, Tyr), 1.88-1.83 (4H, m, γ-H's, Pro^1 & Pro^2), 1.76-1.73 (2H, m, γ-H's, Ile), 1.70-1.62 (1H, m, β-H, Val), 1.60-1.57 (1H, m, β-H, Ile), 1.46-1.40 (6H, m, β-H's, Ala^1 & Ala^2), 1.10-1.08 (6H, d, J = 5.8 Hz, γ-H's, Val), 1.02-0.96 (6H, m, $\gamma\prime$- & δ-H's, Ile) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ 173.7, 172.3, 171.6 (C=O, Ala^1, Pro^2 & Tyr), 171.3, 170.8, 170.3 (C=O, Ile, Val, Pro^1), 169.9, 168.4 (C=O, Ala^2 & Gly), 154.2 (p-C, Tyr), 133.4 (γ-C, Tyr), 129.9, 126.3 (o- & m-C's, Tyr), 65.2 (α-C, Pro^2), 59.3 (α-C, Pro^1), 58.8, 57.2 (α-C's, Ile & Val), 54.1, 49.5 (α-C's, Tyr & Ala^2), 48.6 (α-C, Ala^1), 47.5, 46.8 (δ-C's, Pro^2 & Pro^1), 42.2 (CH_2, Gly), 39.7, 37.4, 33.3 (β-C's, Tyr, Ile & Pro^1), 30.6, 28.3 (β-C's, Val & Pro^2), 26.3 (γ-C, Ile), 24.8, 22.7 (γ-C's, Pro^2 & Pro^1), 18.6 (γ-C's, Val), 17.0 (β-C, Ala^1), 14.2 ($\gamma\prime$-C, Ile), 11.4 (β-C, Ala^2), 10.0 (δ-C, Ile) ppm; FAB MS (m/z, relative intensity): 769 [$(M + H)^+$, 100], 741 [$(769–CO)^+$, 18.2], 698 [(Pro-Gly-Ala-Val-Pro-Ile-Tyr)$^+$, 11.8], 670 [(Pro-Ile-Tyr-Ala-Pro-Gly-Ala)$^+$, 50.8], 642 [$(670–CO)^+$, 14.3], 599 [(Pro-Ile-Tyr-Ala-Pro-Gly)$^+$, 10.1], 571 [599–CO)$^+$, 2.5], 535 [(Pro-Gly-Ala-Val-Pro-Ile)$^+$, 14.7], 507 [$(535–CO)^+$, 7.9], 445 [(Pro-Ile-Tyr-Ala)$^+$, 9.8], 422 [(Pro-Gly-Ala-Val-Pro)$^+$, 13.6], 417 [$(445–CO)^+$, 21], 394 [$(422–CO)^+$, 5.8], 374 [(Pro-Ile-Tyr)$^+$, 57.8], 325 [(Pro-Gly-Ala-Val)$^+$, 55.2], 297 [$(325–CO)^+$, 29.9], 226 [(Pro-Gly-Ala)$^+$, 16.9], 211 [(Pro-Ile)$^+$, 14.8], 183 [$(211–CO)^+$, 15.1], 155 [(Pro-Gly)$^+$, 3.5], 107 [$(C_7H_7O)^+$, 12.2], 93 [$(C_6H_5O)^+$, 7.8], 57 [$(C_4H_9)^+$, 10.6], 43 [$(C_3H_7)^+$, 8.6], 30 [$(CH_4N)^+$, 1.2], 29 [$(C_2H_5)^+$, 1.3], 15 [$(CH_3)^+$, 4.9]; Anal. Calcd. for $C_{38}H_{56}N_8O_9$: C, 59.36; H, 7.34; N, 14.57. Found: C, 59.38; H, 7.34; N, 14.56%.

Biological experimental section

Antimicrobial studies

The synthesized cyclopeptide was screened for its antimicrobial activity[18] against 4 bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) and 4 fungal strains (*Microsporum audouinii*, *Trichophyton mentagrophytes*, *Candida albicans*, and *Aspergillus niger*) at 10 μg mL^{-1} concentration. MIC values of the test compounds were determined by tube dilution technique using DMF and DMSO. A spore suspension in sterile distilled water was prepared from 5-day-old culture of the test bacteria/fungi growing on nutrient broth media/Sabouraud broth media. About 20 mL of the growth medium was transferred into sterilized petri plates and inoculated with 1.5 mL of the spore suspension (spore concentration – 6×10^4 spores mL^{-1}). Filter paper disks of 6 mm diameter and 2 mm thickness were sterilized by autoclaving at 121 °C for 15 min. Each petri plate was divided into 5 equal portions along the diameter to place each disk. Three discs of test sample were placed on 3 portions together with 1 disk with reference drug ciprofloxacin/griseofulvin and a disk impregnated with the solvent (DMF/DMSO) as a negative control. Reference drugs were also tested at the same concentration of 10 μg mL^{-1}. The petri plates inoculated with bacterial/fungal cultures were incubated at 37 °C for 18 h and 48 h, respectively. Diameters of the zones of inhibition (in mm) were measured and the average diameters for the test sample were calculated for triplicate sets. The diameters obtained for the test sample were compared with that produced by the standard drug.

Anthelmintic studies

Anthelmintic activity studies[19] were carried out against 3 different species of earthworms, *Megascoplex konkanensis*, *Pontoscotex corethruses*, and *Eudrilus* species, at 2 mg mL^{-1} concentration. Suspensions of samples were prepared by triturating synthesized compounds (100 mg) with Tween 80 (0.5%) and distilled water and the resulting mixtures were stirred using a mechanical stirrer for 30 min. The suspensions were diluted to contain 0.2% w/v of the test samples. Suspensions of the reference drugs, mebendazole and piperazine citrate, were prepared with the same concentration in a similar way. Three sets of 5 earthworms of similar sizes (5 cm in length) were placed in petri plates of 10 cm diameter containing 50 mL of suspension of test sample and reference drug at RT. Another set of 5 earthworms was kept as controls in a 50 mL suspension of distilled water and Tween 80 (0.5%). The times of paralysis and death were noted and their mean was calculated for triplicate sets. The time of death was ascertained by placing the earthworms in warm water (60 °C), which stimulated movement, if the worm was alive.

Cytotoxicity studies

Synthesized cyclopeptide **8** was subjected to short term in vitro cytotoxicity study[20] at 62.5-3.91 μg mL^{-1} using 5-fluorouracil (5-FU) as reference compound. Activity was assessed by determining the percentage inhibition of DLA and EAC cells. Both cells were cultured in the peritoneal cavity of healthy albino mice by injecting the suspension of cells (1×10^6 cells/mL) intraperitoneally. After 15-20 days, cells were withdrawn from the peritoneal cavity of the mice with the help of a sterile syringe and counted using a hemocytometer and adjusted to 1×10^6 cells/mL. Different dilutions of synthesized compound **8** ranging from 62.5 to 3.91 μg mL^{-1} were prepared in Dulbecco's minimum essential medium and 0.1 mL of each diluted test compound was added to 0.1 mL of DLA cells (1×10^6 cells/mL) and EAC cells (1×10^6 cells/mL). The resulting

suspensions were incubated at 37 °C for 3 h. After 3 h, a trypan blue dye exclusion test was performed and percentage growth inhibition was calculated. CTC_{50} values were determined by graphical extrapolation method. The controls were also tested at 62.5-3.91 μg mL^{-1} against both cell lines.

Investigation and Results

Chemistry

In the present study, a disconnection strategy was employed to carry out the first total synthesis of cherimolacyclopeptide G. The cyclic octapeptide molecule was split into 4 dipeptide units: Boc-Gly-Ala-OMe (**1**), Boc-Val-Pro-OMe (**2**), Boc-Ile-Tyr-OMe (**3**), and Boc-Ala-Pro-OMe (**4**). The required dipeptide units **1-4** were prepared by coupling of Boc-amino acids viz. Boc-Gly, Boc-Val, Boc-Ile, and Boc-Ala with corresponding amino acid methyl ester hydrochlorides such as Ala-OMe.HCl, Pro-OMe.HCl, and Tyr-OMe.HCl employing dicyclohexylcarbodiimide (DCC) as coupling agent. The ester group of dipeptide **1** was removed by alkaline hydrolysis with LiOH and the Boc-group of another dipeptide **2** was removed using CF_3COOH. Both the deprotected units were coupled with each other using DCC and N-methylmorpholine (NMM) as base, to obtain the first tetrapeptide unit Boc-Gly-Ala-Val-Pro-OMe (**5**). Similarly, dipeptide **3** after deprotection at the carboxyl terminal was coupled with dipeptide **4** after deprotection at the amino end to obtain another tetrapeptide Boc-Ile-Tyr-Ala-Pro-OMe (**6**). After removal of the ester and Boc groups of tetrapeptides **5** and **6**, deprotected units were coupled to obtain the linear octapeptide Boc-Gly-Ala-Val-Pro-Ile-Tyr-Ala-Pro-OMe (**7**). The ester group of the linear fragment was removed using LiOH and a p-nitrophenyl (PNP) ester group was introduced. The Boc-group was removed by CF_3COOH and deprotected linear fragment was then cyclized by keeping the whole contents at 0 °C for 7 days in the presence of a catalytic amount of NMM/TEA/pyridine to yield compound **8** (Figure). Structures of the newly synthesized cyclic octapeptide as well as intermediates linear di/tetra/octapeptides were confirmed by IR, ^{1}H-NMR, and elemental analysis. In addition, ^{13}C-NMR and mass spectra were recorded for the cyclopeptide.

Pharmacology

Synthesized compound **8** was screened for in vitro antimicrobial activity against the gram-positive bacteria *B. subtilis* and *S. aureus*, the gram-negative bacteria *P. aeruginosa* and *E. coli*, the cutaneous fungi *M. audouinii* and *T. mentagrophytes*, and the diamorphic fungi *C. albicans* and *A. niger* by disk diffusion method[18] and for anthelmintic activity against earthworms, *Megascoplex konkanensis, Pontoscotex corethruses*, and *Eudrilus* species, by the Garg method.[19] Synthesized cyclopeptide was also subjected to short term in vitro cytotoxicity study against DLA and EAC cell lines by the Kuttan method.[20] The results of the biological activity studies are tabulated in Tables 1-3.

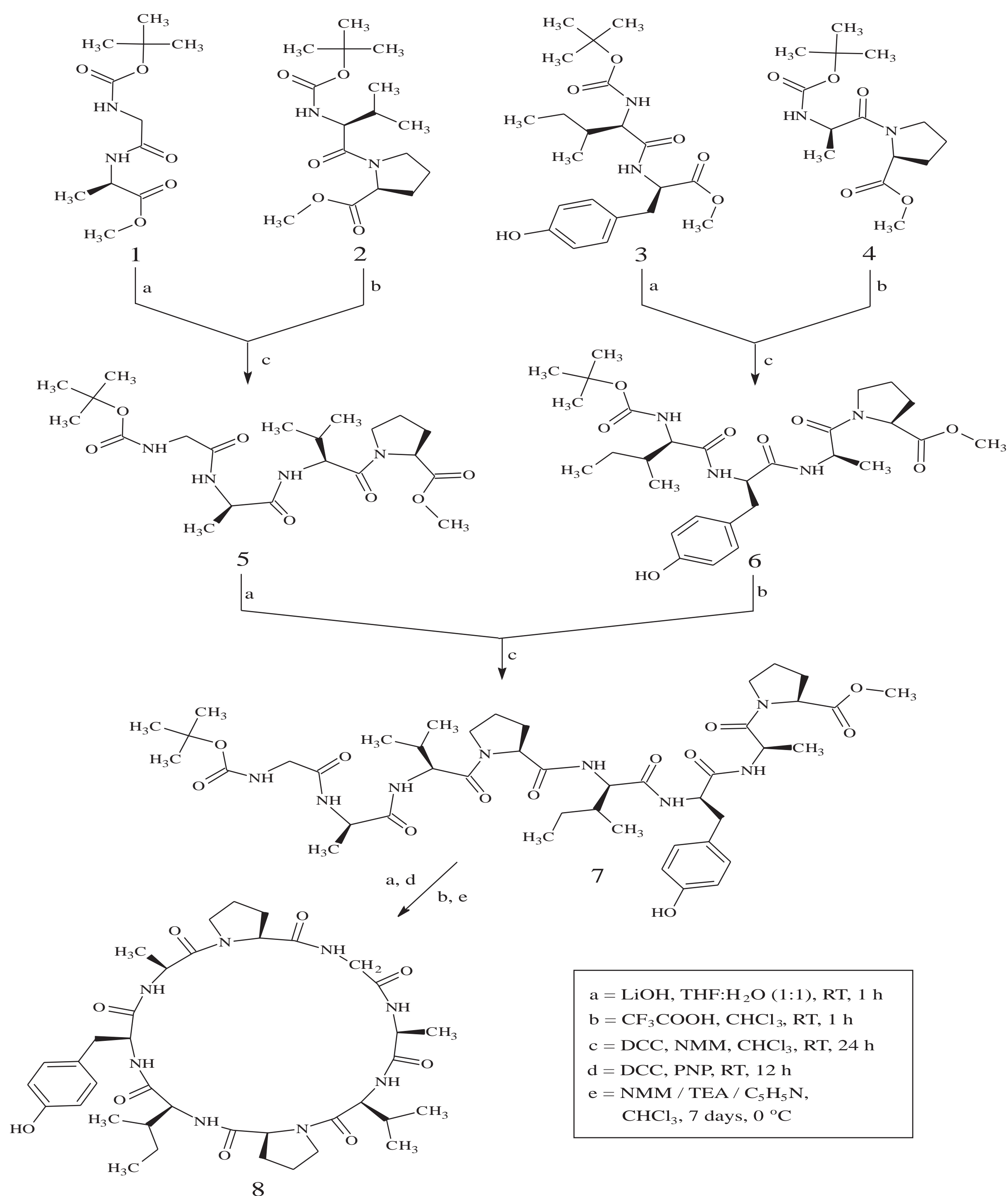

Figure. Synthetic pathway for cherimolacyclopeptide G (**8**).

Table 1. Antimicrobial activity data.

Compd.	Diameter of zone of inhibition (mm)							
	Bacterial strains				Fungal strains			
	B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*	*M. audouinii*	*A. niger*	*T. mentagrophytes*
8	–	–	21(6)[a]	8(12.5)	17(6)	9(25)	–	9(12.5)
Control	–	–	–	–	–	–	–	–
Ciprofloxacin	20(6)	20(12.5)	25(6)	19(12.5)	–	–	–	–
Griseofulvin	–	–	–	–	20(6)	17(6)	18(12.5)	20(6)

[a]Values in parentheses are MIC values (μg mL^{-1}).

Table 2. Anthelmintic activity data.

Compd.	Earthworm species					
	M. konkanensis		*P. corethruses*		*Eudrilus* sp.	
	Mean paralyzing time (min)[a]	Mean death time (min)[a]	Mean paralyzing time (min)	Mean death time (min)	Mean paralyzing time (min)	Mean death time (min)
8[b]	07.52 ± 0.59	10.36 ± 0.55	11.22 ± 0.51	13.38 ± 0.86	08.12 ± 0.48	10.08 ± 0.16
Control[c]	–	–	–	–	–	–
Mebendazole[b]	10.55 ± 0.64	12.59 ± 0.53	17.58 ± 1.03	19.42 ± 1.20	11.35 ± 0.45	13.46 ± 0.62
Piperazine citrate[b]	12.39 ± 0.36	13.52 ± 0.49	19.06 ± 0.57	22.23 ± 0.78	12.46 ± 0.15	13.58 ± 0.47

[a]Data are given as mean ± S.D. (n = 3); [b]c = 2 mg mL^{-1}; [c]0.5% Tween 80 in distilled water.

Discussion

Synthesis of cherimolacyclopeptide G was carried out successfully with good yield and NMM proved to be a yield effective base for cyclization of the linear octapeptide fragment. The structure of cyclic octapeptide was confirmed by spectral as well as elemental analysis. Cyclization of the linear peptide fragment was indicated by the disappearance of absorption bands at 1395, 1370, and 932 cm^{-1} (-CH bending and CH_3 rocking of ^{t}Butyl group) and the presence of additional Amide I and Amide II bands of the -CO-NH- moiety at 1639 cm^{-1} and 1537-1525 cm^{-1} in the IR spectra of compound **8**. Formation of cyclopeptide was further confirmed by disappearance of the singlet at 1.54 ppm corresponding to 9 protons of the ^{t}Butyl group of Boc in the ^{1}H-NMR spectrum of compound **8**. Furthermore, ^{1}H-NMR and ^{13}C-NMR spectra of synthesized cyclic octapeptide showed characteristic peaks confirming the presence all the 56 protons and 38 carbon atoms. The presence of $(M + 1)^+$ ion peak at m/z 769 corresponding to the molecular formula $C_{38}H_{56}N_8O_9$ in the mass spectra of compound **8**, along with other fragment ion peaks resulting from cleavage at Ala-Pro and Val-Pro amide bond level, showed the exact sequence of attachment of all 8 amino acid moieties in a chain. In addition, elemental analysis of compound **8** afforded values (± 0.02) strictly in accordance with the molecular composition.

Table 3. Cytotoxic activity data.

Compd.	Conc. (μg/mL)	DLA cells: Live cells counted	DLA cells: No. of dead cells	DLA cells: % growth inhibition[a]	DLA cells: CTC^b_{50} (μM)	EAC cells: Live cells counted	EAC cells: No. of dead cells	EAC cells: % growth inhibition	EAC cells: CTC_{50} (μM)
8	62.5	0	38	100.0		0	28	100.0	
	31.25	1	37	97.36		3	25	89.28	
	15.63	5	33	86.84	6.72	9	19	67.85	11.9
	7.81	17	21	55.26		15	13	46.43	
	3.91	20	18	47.36		25	3	10.71	
Control	62.5	38	0	–		28	0	–	
	31.25	38	0	–		28	0	–	
	15.63	38	0	–	–	28	0	–	–
	7.81	38	0	–		28	0	–	
	3.91	38	0	–		28	0	–	
Standard (5-FU)	62.5	0	38	100.0		0	28	100.0	
	31.25	0	38	100.0		0	28	100.0	
	15.63	10	28	73.68	37.36	11	17	60.71	90.55
	7.81	13	25	65.79		19	9	32.14	
	3.91	22	16	42.11		23	5	17.86	

[a]% growth inhibition = 100 – [{($Cell_{total}$ – $Cell_{dead}$)× 100} / $Cell_{total}$]; [b]CTC_{50} = cytotoxic conc. inhibiting 50% of percentage growth.

The synthesized cyclopeptide exhibited potent anthelmintic activity against all 3 earthworms species, in comparison to the reference compounds mebendazole/piperazine citrate, and potent cytotoxic activity against DLA and EAC cell lines with CTC_{50} values of 6.72 and 11.9 μM respectively, in comparison to the standard drug 5-fluorouracil (5-FU) (CTC_{50} values – 37.36 and 90.55 μM). Moreover, compound **8** showed a moderate level of activity against the pathogenic microbes *C. albicans* and *P. aeruginosa*. Gram-positive bacteria were found to be resistant towards compound **8** in comparison to sensitive gram-negative bacteria. On passing toxicity tests, synthesized cyclopeptide **8** may prove a good candidate for clinical studies and can be a new anthelmintic and cytotoxic drug of the future.

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References

1. H. Morita, T. Iizuka, A. Gonda, H. Itokawa and K. Takeya, **J. Nat. Prod. 69,** 839-841 (2006).
2. M. Yoshikawa, H. Matsuda, T. Morikawa, H. Xie, S, Nakamura and O. Muraoka, **Bioorg. Med. Chem. 14,** 7468-7475 (2006).
3. A. Wele, I. Ndoye, Y. Zhang, J.P. Brouard, J.L. Pousset and B. Bodo, **Phytochemistry 66,** 1154-1157 (2005).
4. H. Morita, H. Suzuki and J. Kobayashi, **J. Nat. Prod. 67,** 1628-1630 (2004).
5. S.M. Sang, A.N. Lao, Y. Leng, L. Cao, Z.L. Chen, J. Uzawa, Y. Shigeo and Y. Fujimoto, **J. Asian Nat. Prod. Res. 4,** 297-301 (2002).
6. J. Kobayashi, H. Suzuki, K. Shimbo, K. Takeya and H. Morita, **J. Org. Chem. 66,** 6626-6633 (2001).
7. A. Wele, Y. Zhang, L. Dubost, J.L. Pousset and B. Bodo, **Chem. Pharm. Bull. (Tokyo) 54,** 690-692 (2006).
8. R. Dahiya and K. Kaur, **Arzneim.-Forsch. 58,** 29-34 (2008).
9. R. Dahiya, **Acta Pol. Pharm. 64,** 509-516 (2007).
10. R. Dahiya and K. Kaur, **Arch. Pharm. Res. 30,** 1380-1386 (2007).
11. R. Dahiya, **J. Chil. Chem. Soc. 52,** 1224-1229 (2007).
12. R. Dahiya, **Pak. J. Pharm. Sci. 20,** 317-323 (2007).
13. R. Dahiya and D. Pathak, **J. Serb. Chem. Soc. 72,** 101-107 (2007).
14. R. Dahiya and D. Pathak, **Asian J. Chem. 19,** 1499-1505 (2007).
15. R. Dahiya, D. Pathak, M. Himaja and S. Bhatt, **Acta Pharm. 56,** 399-415 (2006).
16. R. Dahiya and D. Pathak, **J. Pharm. Res. 5,** 69-73 (2006).
17. M. Bodanszky and A. Bodanszky, **"The Practice of Peptide Synthesis",** pp. 78-143, Springer-Verlag, New York, 1984.
18. A.W. Bauer, W.M. Kirby, J.C. Sherris and M. Turck, **Am. J. Clin. Path. 45,** 493-496 (1966).
19. L.C. Garg and C.K. Atal, **Ind. J. Pharm. Sci. 59,** 240-245 (1963).
20. R. Kuttan, P. Bhanumathy, K. Nirmala and M.C. George, **Cancer Lett. 29,** 197-202 (1985).