SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF A GLYCINE-RICH PEPTIDE – CHERIMOLACYCLOPEPTIDE E

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ABSTRACT

The synthesis of a natural cyclic peptide cherimolacyclopeptide E (**12**) by coupling of tripeptide units Boc-gly-leu-gly-OCH₃ (**9**) and Boc-phe-tyr-pro-OCH₃ (**10**) after proper deprotection at carboxyl and amino terminals followed by cyclization of linear hexapeptide segment, is described. Structure elucidation of the cyclopeptide **12** is based on detailed spectral analysis such as FTIR, ¹H NMR, ¹³C NMR, FAB MS and elemental analysis. After biological screening, the newly synthesized peptide exhibited high cytotoxicity against *Dalton's lymphoma ascites* (DLA) and *Ehrlich's ascites carcinoma* (EAC) cell lines with CTC₅₀ values of 2.76 and 4.96 µM, and potent antimicrobial activity against pathogenic microbes *P. aeruginosa*, *E. coli* and *C. albicans* with MICs between 12.5-6 µg/mL. Furthermore, compound **12** possessed moderate anthelmintic activity against earthworms *M. konkanensis*, *P. corethruses* and *Eudrilus* sp. at 2 mg/mL dose level.

Keywords: cherimolacyclopeptide E, cyclic hexapeptide, cytotoxicity, antimicrobial evaluation, anthelmintic activity

INTRODUCTION

During past decades, plants have well proved their potential to produce a wide spectrum of natural products with interesting bioactivities¹⁻⁶. Among these, cyclic peptides and related congeners with unique structures and wide pharmacological profile⁷⁻¹⁰ have received special attention which may prove better candidates to overcome the problem of wide spread increase of resistance towards conventional drugs. Different pharmacological activities possessed by cyclic peptides obtained from latex, seeds and roots of plants, include antitumour activity¹¹⁻¹³, immunosuppressive and antimalarial activity¹⁴, vasorelaxant activity^{15,16}, tyrosinase inhibitory activity^{17,18} and estrogen-like activity^{19,20}. Recently, a natural cyclopolypeptide, cherimolacyclopeptide E, has been isolated from seeds of small tree *Annona cherimola* and the structure was elucidated by MS/MS fragmentation experiments, extensive 2D-NMR analysis/chemical degradation and showed significant cytotoxic activity against KB (human nasopharyngeal carcinoma) cells²¹.

As part of our ongoing efforts on synthetic aspects of bioactive cyclic peptides²²⁻²⁶, the present study was aimed at synthesis of novel cyclic hexapeptide - cherimolacyclopeptide E **12**. Keeping in view of significant bioactivities possessed by various cyclopeptides^{27,28}, the above synthetic peptide was further subjected to antibacterial, antifungal, anthelmintic and cytotoxic activity studies.

EXPERIMENTAL

GENERAL

All the reactions requiring anhydrous conditions were conducted in flame dried apparatus. Melting point was determined by open capillary method and is uncorrected. L-amino acids, dicyclohexylcarbodiimide (DCC), trifluoroacetic acid (TFA), p-nitrophenol (pnp), N-methylmorpholine (NMM), triethylamine (TEA), di-*tert*-butylpyrocarbonate (Boc₂O) and pyridine (C₅H₅N) were obtained from Spectrochem Limited (Mumbai, India). IR spectra were recorded on Shimadzu 8700 FTIR spectrophotometer (Shimadzu, Japan) using a thin film supported on KBr pellets for solids and CHCl₃ as solvent for intermediate semisolids (in cm⁻¹). ¹H NMR and ¹³C NMR spectra were recorded on Bruker AC NMR spectrometer (300 MHz), (Brucker, USA) using CDCl₃ as solvent and tetramethylsilane as internal standard (in ppm). Mass spectra was recorded on JMS-DX 303 Mass spectrometer (Jeol, Tokyo, Japan) operating at 70 eV using fast atom bombardment technique. Elemental analyses of all compounds were performed on Vario EL III elemental analyzer (Elementar, Germany). Optical rotation of the peptides was measured on automatic polarimeter (Optics Tech, Ghaziabad, India) in a 2 dm tube at 25 °C using sodium lamp and methanol as solvent. Purity of synthesized cyclopeptide as well as intermediates was checked by TLC on precoated silica gel G plates utilizing CHCl₃/CH₃OH as developing solvent in different ratios (9:1/7:3 v/v) and brown spots were detected on exposure to iodine vapours in a tightly closed chamber.

General procedure for synthesis of Boc-amino acids (1,2)

L-amino acid (20 mmol) was dissolved in 1N NaOH (20 mL) and isopropanol (20 mL). Di-*tert*-butylpyrocarbonate (6 mL, 26 mmol) in isopropanol (10 mL) was added followed by 1N NaOH (20 mL) to the resulting solution. The solution was stirred at RT for 2 h, washed with light petroleum ether (bp 40-60 °C) (20 mL), acidified to pH 3.0 with 2N H₂SO₄ and finally extracted with chloroform (3 × 20 mL). The organic layer was dried over anhydrous Na₂SO₄ and evaporated under reduced pressure to give the crude product which was finally crystallized from chloroform and petroleum ether (bp 40-60 °C).

tert-Butyloxycarbonyl-glycine (1).

This compound was obtained according to the general procedure as white crystals, mp 93 °C, yield 83%, [α]_D²⁰ +2.7°, R_f - 0.61; IR: 3292-2488 (m/br, OH str, COOH); 3133 (m, NH str, amide); 2927, 2850 (m, CH str, asym and sym, CH₂); 1712 (s, C=O str, COOH); 1635 (s, C=O str, amide); 1531 (m, NH bend, amide); 1466 (m, CH bend (scissoring), CH₂); 1387, 1362 (m, CH bend, butyl-*t*); 929 (w, CH₃ rocking, butyl-*t*). ¹H NMR: 10.02 (br. s, 1H, OH); 6.04 (br. s, 1H, NH); 3.96-3.94 (d, 2H, CH₂, J=4.8 Hz); 1.54 (s, 9H, butyl-*t*). ¹³C NMR: 172.7 (COOH); 157.4 (C=O, boc); 79.6 (C-α, butyl-*t*); 44.2 (C-α, gly); 25.3 (3C, C-β, butyl-*t*).

Anal. Calcd. for C₇H₁₃NO₄: C, 47.99; H, 7.48; N, 8.00. Found: C, 50.03; H, 7.46; N, 7.99.

tert-Butyloxycarbonyl-phenylalanine (2).

This compound was obtained according to the general procedure as white crystals, yield 88%, mp 85-86 °C, [α]_D²⁰ -4.0°, R_f - 0.78; IR: 3298-2485 (m/br, OH str, COOH); 3089, 3034 (w, CH str, ring); 2928 (m, CH str, asym, CH₂); 1715 (s, C=O str, COOH); 1632 (s, C=O str, amide); 1582, 1482 (m, skeletal bands, ring); 1542 (m, NH bend, amide); 1387, 1365 (m, CH bend, butyl-*t*); 933 (w, CH₃ rock, butyl-*t*); 730, 693 (s, CH bend, oop, ring). ¹H NMR: 10.4 (br. s, 1H, OH); 7.30-7.26 (tt, 2H, H-*m*); 7.10-7.08 (dd, 2H, H-*o*, J=6.4 Hz, J=4.2 Hz); 7.00-6.96 (t, 1H, H-*p*); 5.89 (br. s, 1H, NH); 5.13-5.08 (q, 1H, H-α); 3.25-3.23 (d, 2H, J=5.8 Hz, H-β); 1.55 (s, 9H, butyl-*t*). ¹³C NMR: 173.8 (COOH); 155.2 (C=O, boc); 138.0 (C-γ); 129.3 (2C, C-*o*); 128.2 (2C, C-*m*); 126.5 (C-*p*); 79.5 (C-α, butyl-*t*); 55.1 (C-α); 37.7 (C-β); 27.8 (3C, C-β, butyl-*t*).

Anal. Calcd. for C₁₄H₁₉NO₄: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.35; H, 7.23; N, 5.32.

General procedure for synthesis of L-amino acid methyl ester hydrochlorides (3-6)

Thionyl chloride (1.4 mL, 20 mmol) was added to methanol (100 mL) slowly at 0 °C and L-amino acid (20 mmol) was added to above solution. The resulting mixture was refluxed for 8-10 h at ambient temperature. Solvent was evaporated and the residue was triturated with ether at 0 °C until excess dimethyl sulphite was removed. The crude product was crystallized from methanol and

ether at 0 °C to get pure amino acid methyl ester hydrochloride.

L-Leucine methyl ester hydrochloride (3).

This compound was obtained according to the general procedure as white crystals, yield 79%, mp 118 °C, $[\alpha]_D -13.5^\circ$, R_f - 0.54; IR: 3015-2852 (s/br, NH_3^+ str, asym and sym); 2925 (m, CH str, asym, CH_2); 2822 (m, CH str, OCH_3); 1742 (s, C=O str, ester); 1602, 1506 (s/br, NH_3^+ bend, asym and sym); 1390, 1369 (m, CH def, butyl-*t*); 1378, 1362 (s, CH def, propyl-*t*); 1269 (s, C-O str, ester); 931, 920 (w, CH_3 rock, butyl-*t* and propyl-*t*). ^1H NMR: 4.12 (s, 3H, OCH_3); 4.03-3.99 (t, 2H, H- β); 3.55-3.52 (m, 1H, H- α); 1.85-1.73 (m, 1H, H- γ); 1.20-1.18 (d, 6H, H- δ , $J=6.15$ Hz); 1.17 (br. s, NH_3^+). ^{13}C NMR: 169.5 (C=O, ester); 53.0 (OCH_3); 49.6 (C- α); 39.1 (C- β); 27.3 (C- γ); 20.9 (2C, C- δ).

Anal. Calcd. for $\text{C}_7\text{H}_{16}\text{ClNO}_2$: C, 46.28; H, 8.88; N, 7.71. Found: C, 46.25; H, 8.90; N, 7.69.

L-Tyrosine methyl ester hydrochloride (4).

This compound was obtained according to the general procedure as white crystals, yield 84%, mp 190 °C, $[\alpha]_D -4.3^\circ$, R_f - 0.67; IR: 3372 (m/br, OH str); 3011-2863 (s/br, NH_3^+ str, asym and sym); 2928, 2848 (m, CH str, asym and sym, CH_2); 1750 (s, C=O str, ester); 1588, 1475 (m, skeletal bands, ring); 1388, 1372 (m, CH def, butyl-*t*); 1227 (s, C-O str, phenolic); 1272 (s, C-O str, ester); 825 (s, CH def, oop, ring). ^1H NMR: 7.80-7.78 (dd, 2H, H-*m*, $J=7.4$ Hz, $J=5.0$ Hz); 7.56-7.54 (dd, 2H, H-*o*, $J=7.5$ Hz, $J=4.6$ Hz); 5.40 (br. s, OH and NH_3^+); 4.13-4.09 (m, 1H, H- α); 4.12 (s, 3H, OCH_3); 2.20-2.18 (d, 2H, H- β , $J=7.15$ Hz). ^{13}C NMR: 168.8 (C=O, ester); 156.5 (C-*p*); 130.2 (2C, C-*o*); 122.2 (C- γ); 117.5 (2C, C-*m*); 53.1 (OCH_3); 50.5 (C- α); 32.9 (C- β).

Anal. Calcd. for $\text{C}_{10}\text{H}_{14}\text{ClNO}_3$: C, 51.84; H, 6.09; N, 6.05. Found: C, 51.82; H, 6.10; N, 6.06.

Glycine methyl ester hydrochloride (5).

This compound was obtained according to the general procedure as white solid, yield 90%, mp 174-175 °C, $[\alpha]_D -88.2^\circ$, R_f - 0.45; IR: 3015-2853 (s/br, NH_3^+ str, asym and sym); 2925, 2847 (m, CH str, asym and sym, CH_2); 1744 (s, C=O str, ester); 1599, 1502 (s/br, NH_3^+ bend, asym and sym); 1386, 1370 (m, CH def, butyl-*t*); 1268 (s, C-O str, ester); 932 (w, CH_3 rock, butyl-*t*). ^1H NMR: 4.75 (br. s, NH_3^+); 4.19 (s, 3H, OCH_3); 3.85 (m, 2H, H- α); ^{13}C NMR: 170.2 (C=O, ester); 52.3 (OCH_3); 40.8 (C- α).

Anal. Calcd. for $\text{C}_3\text{H}_8\text{ClNO}_2$: C, 28.70; H, 6.42; N, 11.16. Found: C, 28.69; H, 6.42; N, 11.20.

L-Proline methyl ester hydrochloride (6).

This compound was obtained according to the general procedure as viscous liquid, yield 87%, $[\alpha]_D -40.2^\circ$, R_f - 0.26; IR: 3050-2840 (s/br, NH_2^+ str, asym and sym); 2995, 2987 (m, CH str, CH_2 , pro); 1745 (s, C=O str, ester); 1440 (s/br, NH_2^+ bend); 1270 (s, C-O str, ester). ^1H NMR: 4.73-4.70 (m, 1H, H- α); 4.05-4.01 (t, 2H, H- δ); 3.86 (s, 3H, OCH_3); 2.45-2.32 (m, 4H, H- β , H- γ); 2.35 (br. s, NH_2^+). ^{13}C NMR: 171.8 (C=O, ester); 62.1 (C- α); 54.5 (OCH_3); 44.7 (C- δ); 30.4 (C- β); 20.2 (C- γ).

Anal. Calcd. for $\text{C}_6\text{H}_{12}\text{ClNO}_2$: C, 43.51; H, 7.30; N, 8.46. Found: C, 43.49; H, 7.33; N, 8.45.

General procedure for the synthesis of linear peptide fragments (7-11)

Peptide units were prepared according to Bodanzsky method with certain modifications²⁹. Amino acid methyl ester hydrochloride/peptide methyl ester (10 mmol) was dissolved in CHCl_3 (20 mL). To this, NMM (21 mmol) was added at 0 °C and the reaction mixture was stirred for 15 minutes. Boc-amino acid/peptide (10 mmol) in CHCl_3 (20 mL) and DCC (10 mmol) 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 crystallized from a mixture of chloroform and petroleum ether followed by cooling at 0 °C.

Deprotection at carboxyl terminal was done by adding lithium hydroxide (0.36 g, 15 mmol) to a solution of Boc-di/tripeptide methyl ester (10 mmol) in THF : H_2O (1 : 1, 36 mL) at 0 °C. Resulting mixture was stirred at RT for 1 h and then acidified to pH 3.5 with 1N H_2SO_4 . The aqueous layer was extracted with

Et_2O (3×25 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was crystallized from methanol and ether to get pure Boc-di/tripeptides.

Deprotection at amino terminal was accomplished by treatment of Boc-di/tripeptides (10 mmol) dissolved in CHCl_3 (15 mL) with trifluoroacetic acid (2.28 g, 20 mmol). The resulting solution was stirred at RT for 1 h, washed with saturated NaHCO_3 solution (25 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by crystallization from CHCl_3 and petroleum ether (bp 40-60 °C) to get pure di/tripeptide methyl esters.

tert-Butyloxycarbonyl-glycyl-leucine methyl ester (7).

This compound was obtained according to the general procedure as semisolid mass, yield 79%, $[\alpha]_D +1.8^\circ$, R_f - 0.36; IR: 3125 (m, -NH str, amide); 2927 (m, -CH str, asym, CH_2); 2853 (m, -CH str, sym, CH_2); 1742 (s, C=O str, ester); 1642, 1639 (s, C=O str, 2° amide); 1535, 1529 (m, -NH bend, 2° amide); 1392, 1368 (m, CH bend, butyl-*t*); 1380, 1363 (s, CH def, propyl-*t*); 1270 (s, C-O str, ester); 930, 924 (w, CH_3 rock, butyl-*t* and propyl-*t*). ^1H NMR: 6.52 (br. s, 1H, NH); 6.02 (br. s, 1H, NH); 4.25-4.20 (q, 1H, H- α , leu); 3.62 (s, 3H, OCH_3); 3.50-3.48 (d, 2H, CH_2 , gly, $J=4.75$ Hz); 1.54 (s, 9H, butyl-*t*); 1.51-1.41 (m, 3H, H- β , H- γ , leu); 0.96-0.94 (d, 6H, H- δ , leu, $J=6.1$ Hz). ^{13}C NMR: 179.4 (C=O, ester); 174.6 (C=O, amide); 155.8 (C=O, boc); 79.4 (C- α , butyl-*t*); 52.5 (OCH_3); 49.2 (C- α , leu); 46.8 (C- α , gly); 43.8 (C- β , leu); 29.3 (3C, C- β , butyl-*t*); 25.0 (C- γ , leu); 22.6 (2C, C- δ , leu).

Anal. Calcd. for $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_5$: C, 55.61; H, 8.67; N, 9.26. Found: C, 55.62; H, 8.65; N, 9.25.

tert-Butyloxycarbonyl-phenylalanyl-tyrosine methyl ester (8).

This compound was obtained according to the general procedure as white crystals, yield 77%, mp 76 °C, $[\alpha]_D -9.6^\circ$, R_f - 0.74; IR: 3373 (m/br, OH str, tyr); 3125 (m, NH str, amide); 3065, 3052 (w, CH str, rings); 2925, 2846 (m, CH str, asym and sym, CH_2); 1749 (s, C=O str, ester); 1645, 1638 (s, C=O str, amide); 1587, 1479, 1476 (m, skeletal bands, rings); 1536, 1526 (m, NH bend, amide); 1390, 1365 (m, CH bend, butyl-*t*); 1272 (s, C-O str, ester); 825, 710, 695 (s, CH def, oop, rings). ^1H NMR: 7.51-7.47 (tt, 2H, H-*m*, phe); 6.92-6.88 (m, 3H, H-*p*, phe and H-*o*, tyr), 6.86-6.84 (dd, 2H, H-*o*, phe, $J=6.45$ Hz, $J=4.15$ Hz); 6.80-6.78 (dd, 2H, H-*m*, tyr, $J=7.45$ Hz, $J=5.1$ Hz); 6.66 (br. s, 1H, NH); 6.58 (br. s, 1H, NH); 5.95 (s, 1H, OH, tyr); 4.72-4.69 (q, 1H, H- α , phe); 4.60-4.56 (m, 1H, H- α , tyr); 3.54 (s, 3H, OCH_3); 3.02-2.76 (m, 4H, H- β , phe and tyr); 1.55 (s, 9H, butyl-*t*). ^{13}C NMR: 172.3 (C=O, phe); 169.6 (C=O, ester); 153.8 (C-*p*, tyr); 152.0 (C=O, boc); 133.3 (C- γ , phe); 130.8 (2C, C-*m*, phe); 129.8 (2C, C-*o*, tyr); 129.1 (2C, C-*o*, phe); 128.9 (2C, C-*p*, phe); 127.8 (C- γ , tyr); 126.0 (2C, C-*m*, tyr); 79.8 (C- α , butyl-*t*); 57.3 (C- α , phe); 49.3 (C- α , tyr); 53.9 (OCH_3); 38.5, 37.5 (2C, C- β , phe and tyr); 27.5 (3C, C- β , butyl-*t*).

Anal. Calcd. for $\text{C}_{24}\text{H}_{30}\text{N}_2\text{O}_6$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.15; H, 6.85; N, 6.32.

tert-Butyloxycarbonyl-glycyl-leucyl-glycine methyl ester (9).

This compound was obtained according to the general procedure as semisolid mass, yield 79%, $[\alpha]_D +19.6^\circ$, R_f - 0.28; IR: 3126 (m, NH str, amide); 2924, 2850, 2845 (m, CH str, asym and sym, CH_2); 1752 (s, C=O str, ester); 1644, 1638 (s, C=O str, amide); 1532, 1525 (m, NH bend, amide); 1389, 1366 (m, CH bend, butyl-*t*); 1382, 1360 (s, CH def, propyl-*t*); 1268 (s, C-O str, ester); 932, 920 (w, CH_3 rock, butyl-*t* and propyl-*t*). ^1H NMR: 7.56, 7.15, 7.10 (br. s, 3H, NH); 4.57-4.53 (q, 1H, H- α , leu); 4.03-4.01 (d, 2H, CH_2 , gly-2, $J=4.75$ Hz); 3.74-3.72 (d, 2H, CH_2 , gly-1, $J=4.8$ Hz); 3.65 (s, 3H, OCH_3); 1.86-1.82 (t, 2H, H- β , leu); 1.54 (s, 9H, butyl-*t*); 1.50-1.42 (m, 1H, H- γ , leu); 0.99-0.97 (d, 6H, H- δ , leu, $J=6.1$ Hz). ^{13}C NMR: 171.2 (C=O, leu); 169.3 (C=O, ester); 166.1 (C=O, gly-1); 158.0 (C=O, boc); 79.4 (C- α , butyl-*t*); 53.2 (OCH_3); 47.8 (C- α , leu); 44.1 (C- α , gly-1); 40.1 (C- β , leu); 41.5 (C- α , gly-2); 30.2 (3C, C- β , butyl-*t*); 25.9 (C- γ , leu); 22.8 (2C, C- δ , leu).

Anal. Calcd. for $\text{C}_{16}\text{H}_{29}\text{N}_3\text{O}_6$: C, 53.47; H, 8.13; N, 11.69. Found: C, 53.45; H, 8.15; N, 11.70.

tert-Butyloxycarbonyl-phenylalanyl-tyrosinyl-proline methyl ester (10).

This compound was obtained according to the general procedure as semisolid mass, yield 76%, $[\alpha]_D -65.0^\circ$, R_f - 0.82; IR: 3369 (m/br, OH str, tyr); 3128, 3122 (m, NH str, amide); 3066, 3041 (w, CH str, rings); 2996, 2982 (m, CH str, CH_2 , pro); 2928, 2842 (m, CH str, asym and sym, CH_2); 1753 (s, C=O

str, ester); 1669, 1640, 1636 (s, C=O str, 3° and 2° amide); 1589, 1581, 1478, 1473 (m, skeletal bands, rings); 1535, 1522 (m, NH bend, amide); 1392, 1366 (m, CH bend, butyl-*t*); 1270 (s, C–O str, ester); 822, 713, 696 (s, CH def, oop, rings). ¹H NMR: 8.65 (br. s, 1H, NH); 7.51–7.48 (tt, 2H, *H-m*, phe); 6.99–6.97 (dd, 2H, *H-m*, tyr, *J*=7.5 Hz, *J*=5.15 Hz); 6.92–6.89 (m, 3H, *H-p*, phe and *H-o*, tyr); 6.85–6.83 (dd, 2H, *H-o*, phe, *J*=6.5 Hz, *J*=4.1 Hz); 6.56 (br. s, 1H, NH); 5.94 (s, 1H, OH, tyr); 4.61–4.52 (m, 2H, *H-α*, phe and tyr); 3.94–3.89 (t, 1H, *H-α*, pro); 3.62 (s, 3H, OCH₃); 3.40–3.36 (t, 2H, *H-δ*, pro); 2.97–2.82 (m, 4H, *H-β*, phe and tyr); 2.06–1.96 (m, 4H, *H-β*, *H-γ*, pro); 1.55 (s, 9H, butyl-*t*). ¹³C NMR: 176.1 (C=O, phe); 169.2 (C=O, ester); 167.8 (C=O, tyr); 153.9 (C=O, boc); 153.4 (C-*p*, tyr); 133.5 (C-*γ*, phe); 130.9 (2C, C-*m*, phe); 129.7, 129.2 (4C, C-*o*, tyr and phe); 128.9 (C-*γ*, tyr); 128.4 (C-*p*, phe); 125.2 (2C, C-*m*, tyr); 79.9 (C-*α*, butyl-*t*); 58.2 (C-*α*, pro); 54.1 (OCH₃); 51.2, 48.8 (2C, C-*α*, phe and tyr); 45.2 (C-*δ*, pro); 38.0, 37.6 (2C, C-*β*, phe and tyr); 28.7 (C-*β*, pro); 27.3 (3C, C-*β*, butyl-*t*); 24.3 (C-*γ*, pro).

Anal. Calcd. for C₂₉H₃₇N₃O₇: C, 64.55; H, 6.91; N, 7.79. Found: C, 64.55; H, 6.90; N, 7.82.

tert-Butyloxycarbonyl-glycyl-leucyl-glycyl-phenylalanyl-tyrosinyl-proline methyl ester (11).

This compound was obtained according to the general procedure as semisolid mass, yield 88%, [α]_D²⁰ –11.2°, R_f 0.43; IR: 3365 (m/br, OH str, tyr); 3132, 3129, 3123 (m, NH str, amide); 3062 (w, CH str, rings); 2995, 2980 (m, CH str, CH₂, pro); 2929, 2852–2849 (m, CH str, asym and sym, CH₂); 1750 (s, C=O str, ester); 1672, 1639, 1636 (s, C=O str, 3° and 2° amide); 1586, 1580, 1476, 1469 (m, skeletal bands, rings); 1535, 1532–1524 (m, NH bend, amide); 1390, 1365 (m, CH bend, butyl-*t*); 1382, 1359 (s, CH def, propyl-*i*); 1272 (s, C–O str, ester); 929, 922 (w, CH₃ rock, butyl-*t* and propyl-*i*); 824, 712, 696 (s, CH def, oop, rings); 1.55 (s, 9H, butyl-*t*). ¹H NMR: 9.49, 8.68, 8.02, 7.51 (br. s, 4H, NH); 7.21–7.16 (tt, 2H, *H-m*, phe); 7.05–7.02 (t, 1H, *H-p*, phe); 6.98–6.88 (m, 4H, *H-o*, *H-m*, tyr); 6.83–6.81 (dd, 2H, *H-o*, phe, *J*=6.5 Hz, *J*=4.15 Hz); 7.52 (br. s, 1H, NH); 5.98 (s, 1H, OH, tyr); 4.41–4.34 (q, 1H, *H-α*, tyr); 4.26–4.21 (q, 1H, *H-α*, phe); 4.20–4.15 (q, 1H, *H-α*, leu); 4.03–4.01 (d, 2H, CH₂, gly-2, *J*=4.75 Hz); 3.94–3.91 (t, 1H, *H-α*, pro); 3.62 (s, 3H, OCH₃); 3.53–3.51 (d, 2H, CH₂, gly-1, *J*=4.8 Hz); 3.41–3.38 (t, 2H, *H-δ*, pro); 2.81–2.72 (m, 4H, *H-β*, phe and tyr); 2.09–1.95 (m, 4H, *H-β*, *H-γ*, pro); 1.86–1.83 (t, 2H, *H-β*, leu); 1.54 (s, 9H, butyl-*t*); 1.51–1.40 (m, 1H, *H-γ*, leu); 1.03–1.01 (d, 6H, *H-δ*, leu, *J*=5.95 Hz). ¹³C NMR: 175.3 (C=O, gly-2); 168.9 (C=O, tyr); 167.9 (C=O, ester); 165.7 (C=O, gly-1); 166.3 (C=O, leu); 165.7 (C=O, phe); 157.5 (C=O, boc); 153.9 (C-*p*, tyr); 139.4 (C-*γ*, phe); 135.6 (C-*γ*, tyr); 130.4, 129.1 (4C, C-*o*, C-*m*, phe); 128.3, 127.8 (4C, C-*o*, C-*m*, tyr); 127.2 (C-*p*, phe); 79.5 (C-*α*, butyl-*t*); 57.3, 55.5 (2C, C-*α*, pro and tyr); 53.5 (OCH₃); 52.6, 47.6 (2C, C-*α*, phe and leu); 46.0 (C-*α*, gly-2); 45.3 (C-*δ*, pro); 44.1 (C-*α*, gly-1); 39.6 (C-*β*, leu); 37.2, 36.8 (2C, C-*β*, phe and tyr); 30.7 (C-*β*, pro); 29.2 (3C, C-*β*, butyl-*t*); 27.5 (C-*γ*, leu); 24.5 (C-*γ*, pro); 22.2 (2C, C-*δ*, leu).

Anal. Calcd. for C₃₉H₅₄N₆O₁₀: C, 61.08; H, 7.10; N, 10.96. Found: C, 61.05; H, 7.10; N, 11.00.

Procedure for synthesis of cyclohexapeptide, cherimolacyclopeptide E (12)

To synthesize compound **12**, linear hexapeptide unit **11** (5 mmol) was deprotected at carboxyl end using LiOH (0.18 g, 7.5 mmol) to get Boc-gly-leu-gly-phe-tyr-pro-OH. The deprotected hexapeptide unit (5 mmol) was now dissolved in CHCl₃ (50 mL) at 0 °C. To the above solution, p-nitrophenol (pnp) (0.94 g, 6.7 mmol) was added and stirred at RT for 12 h. The reaction mixture was filtered and the filtrate was washed with 10% NaHCO₃ solution (3 × 15 mL) until excess of p-nitrophenol was removed and finally washed with 5% HCl (2 × 10 mL) to get the corresponding p-nitrophenyl ester Boc-gly-leu-gly-phe-tyr-pro-O-pnp. To this compound (4 mmol) dissolved in CHCl₃ (35 mL), CF₃COOH (0.91 g, 8 mmol) was added, stirred at RT for 1 h and washed with 10% NaHCO₃ solution (2 × 25 mL). The organic layer was dried over anhydrous Na₂SO₄ to get Gly-leu-gly-phe-tyr-pro-O-pnp which was dissolved in CHCl₃ (25 mL) and TEA/MMM/C₂H₅N (2.8 mL/2.21 mL/1.61 mL, 20 mmol) was added. Then, whole contents were kept at 0 °C for 7 days. The reaction mixture was washed with 10% NaHCO₃ solution until the byproduct p-nitrophenol was removed completely and finally washed with 5% HCl (3 × 15 mL). The organic layer was dried over anhydrous Na₂SO₄. Finally, chloroform was distilled off and crude cyclized product was crystallized from CHCl₃ and n-hexane.

Cyclo (gly-leu-gly-phe-tyr-pro) (12).

This compound was obtained according to the above mentioned procedure as white crystals, yield 75% (NMM), 61% (TEA), 56% (C₂H₅N), mp 213 °C (213–214 °C), [α]_D²⁰ –55.7° (–56.0°), R_f 0.65; IR: 3372 (m/br, OH str, tyr); 3129–3122 (m, NH str, amide); 3074, 3052 (w, CH str, rings); 2998–2993 (m, CH str, CH₂, pro); 2926–2923, 2852–2844 (m, CH str, asym and sym, CH₂); 1673, 1645–1636 (s, C=O str, 3° and 2° amide); 1585, 1582, 1472 (m, skeletal bands, rings); 1536–1530, 1525–1518 (m, NH bend, amide); 1380, 1361 (s, CH def, propyl-*i*); 1233 (s, C–O str, tyr); 922 (w, CH₃ rock, propyl-*i*); 825, 720, 688 (s, CH def, oop, rings). ¹H NMR: 9.18, 8.10, 7.61, 7.54 (br. s, 4H, NH); 7.20–7.16 (tt, 2H, *H-m*, phe); 7.10 (br. s, 1H, NH); 7.02–6.99 (t, 1H, *H-p*, phe); 6.98–6.89 (m, 4H, *H-o*, *H-m*, tyr); 6.84–6.82 (dd, 2H, *H-o*, phe, *J*=6.45 Hz, *J*=4.1 Hz); 6.26–6.22 (q, 1H, *H-α*, leu); 5.96 (s, 1H, OH, tyr); 5.75–5.71 (m, 1H, *H-α*, tyr); 5.65–5.61 (q, 1H, *H-α*, phe); 5.24–5.22 (d, 2H, CH₂, gly-2, *J*=4.8 Hz); 3.96–3.94 (d, 2H, CH₂, gly-1, *J*=4.75 Hz); 3.90–3.87 (t, 1H, *H-α*, pro); 3.28–3.24 (t, 2H, *H-δ*, pro); 2.69–2.65 (m, 2H, *H-β*, pro); 2.62–2.58 (m, 4H, *H-β*, phe and tyr); 1.89–1.78 (m, 4H, *H-γ*, pro and leu); 0.99–0.97 (d, 6H, *H-δ*, leu, *J*=6.0 Hz); 0.86–0.75 (m, 1H, *H-γ*, leu). ¹³C NMR: 172.9 (C=O, pro); 171.5, 170.6 (C=O, leu and phe); 169.9 (C=O, tyr); 168.5, 167.3 (C=O, gly-1 and gly-2); 154.2 (C-*p*, tyr); 137.9 (C-*p*, phe); 132.8 (C-*γ*, tyr); 129.4, 128.5 (2C, C-*o*, C-*m*, phe); 126.7 (2C, C-*o*, tyr); 124.2 (C-*γ*, phe); 122.3 (2C, C-*m*, tyr); 62.2, 56.9 (2C, C-*α*, pro and tyr); 52.3, 51.6 (2C, C-*α*, leu and phe); 48.0 (C-*δ*, pro); 43.9 (C-*β*, leu); 42.9 (C-*α*, gly-2); 40.6 (C-*α*, gly-1); 38.6, 37.5 (2C, C-*β*, phe and tyr); 30.9 (C-*β*, pro); 27.2 (C-*γ*, leu); 24.7 (C-*γ*, pro); 22.5 (2C, C-*δ*, leu). MS (FAB): *m/z* [%] = 635.7 [(M⁺ + 1)⁺, 100], 607.7 [(635.7–CO)⁺, 32], 488.5 [(tyr-pro-gly-leu-gly)⁺, 28], 460.5 [(488.5–CO)⁺, 12], 431.5 [(tyr-pro-gly-leu)⁺, 22], 403.5 [(431.5–CO)⁺, 16], 318.4 [(tyr-pro-gly)⁺, 69], 290.3 [(318.4–CO)⁺, 20], 261.3 [(tyr-pro)⁺, 29], 233.3 [(261.3–CO)⁺, 11], 164.2 [(tyr)⁺, 15], 136.1 [(164.2–CO)⁺, 10], 107.1 [(C₇H₇O)⁺, 6], 93.1 [(C₆H₅O)⁺, 4], 91.0 [(C₆H₇)⁺, 5], 57.1 [(C₃H₉)⁺, 9], 43.1 [(C₃H₇)⁺, 9], 30.0 [(CH₄N)⁺, 4], 29.0 [(C₂H₅)⁺, 4], 15.0 [(CH₃)⁺, 7]

Anal. Calcd. for C₃₃H₄₂N₆O₇: C, 62.45; H, 6.67; N, 13.24. Found: C, 62.42; H, 6.70; N, 13.25.

PHARMACOLOGICAL EVALUATION

The newly synthesized cyclohexapeptide **12** was evaluated for *in vitro* antibacterial, antifungal, anthelmintic and cytotoxic activities. The antimicrobial activity was carried out against gram positive bacteria *Bacillus subtilis* (MUMC 408), *Staphylococcus aureus* (MUMC 377), gram negative bacteria *Pseudomonas aeruginosa* (MUMC 266), *Escherichia coli* (MUMC 106) and cutaneous fungi *Microsporium audouinii* (MUMC 545) and *Trichophyton mentagrophytes* (MUMC 665), diamorphic fungi *Candida albicans* (MUMC 29) and pathogenic fungi *Aspergillus niger* (MUMC 188) using ciprofloxacin and griseofulvin as reference drugs. Anthelmintic activity was performed against three earthworm species *Megascoplex konkanensis* (ICARBC 211), *Pontoscotex corethruses* (ICARBC 408) and *Eudrilus* sp. (ICARBC 042) using mebendazole and piperazine citrate as standard drugs. The cytotoxic activity was tested against Dalton's Lymphoma Ascites (DLA) cells and Ehrlich's Ascites Carcinoma (EAC) cells using 5-fluorouracil (5-FU) as reference compound. Bacterial and fungal cultures were obtained from the Manipal University Mycological Center (MUMC, Manipal, India) and earthworm species were obtained from Indian Council of Agricultural Research Breeding Center (ICARBC, Kasaragod, India).

Antimicrobial screening

Antimicrobial activity study was carried out against eight pathogenic microorganisms at 12.5–6 µg mL^{–1} concentration using Kirby-Bauer disk diffusion method³⁰. MIC values of test compound were determined by Tube Dilution Technique using DMF and DMSO. A spore suspension in sterile distilled water was prepared from 5 days old culture of the test bacteria/fungi growing on nutrient broth media/sabouraud's 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 × 10⁴ 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 five equal portions along the diameter to place one disc. Three discs of test sample **12** were placed on three portions together with one disc with reference drug ciprofloxacin/griseofulvin and a disk impregnated with the solvent (DMF/DMSO) as negative control. Reference drugs were also tested at the same concentration of 12.5–6 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 test sample were calculated for triplicate sets triplicate sets. The diameters obtained for the test sample **12** were compared with that produced by the standard drugs.

Anthelmintic screening

Anthelmintic activity study was carried out against three different species of earthworms at 2 mg mL⁻¹ concentration using Garg method³¹. Suspension of sample was prepared by triturating synthesized compound **12** (0.1 g) with tween 80 (0.5%) and distilled water and the resulting mixture was stirred using a mechanical stirrer for 30 minutes. The suspension was diluted to contain 0.2% w/v of the test sample. Suspension of reference drugs, mebendazole and piperazine citrate were prepared with the same concentration in a similar way. Three sets of five earthworms of almost similar sizes (2 inch in length) were placed in petri plates of 4 inch diameter containing 50 mL of suspension of test sample and reference drugs at RT. Another set of five earthworms was kept as control in 50 mL suspension of distilled water and tween 80 (0.5%). The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50 °C) which stimulated the movement, if the worm was alive.

Cytotoxicity screening

Synthesized cyclopeptide **12** was subjected to short term *in vitro* cytotoxicity study at 62.5–3.91 µg mL⁻¹ using Kutan method³². 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 × 10⁶ cells/mL) intraperitoneally. After 15–20 days, cells were withdrawn from the peritoneal cavity of the mice with help of sterile syringe and counted using haemocytometer and adjusted to 1 × 10⁶ cells/mL. Different dilution of test compound **12** ranging from 62.5–3.91 µg mL⁻¹ were prepared in dulbecoccs minimum essential medium and 0.1 mL of each diluted test compound was added to 0.1 mL of DLA cells (1 × 10⁶ cells/mL) and EAC cells (1 × 10⁶ cells/mL). Resulting suspensions were incubated at 37 °C for 3 h. After 3 h, trypan blue dye exclusion test was performed and percentage growth inhibition was calculated. CTC₅₀ values were determined by graphical extrapolation method. Controls were also tested at 62.5–3.91 µg mL⁻¹ against both cell lines.

RESULTS AND DISCUSSION

CHEMISTRY

In present work, disconnection strategy was employed to carry out the first total synthesis of cherimolacyclopeptide E (**12**). The cyclic hexapeptide molecule was split into two amino acid units Gly-OCH₃·HCl (**5**), Pro-OCH₃·HCl (**6**) and two dipeptide units Boc-gly-leu-OCH₃ (**7**), Boc-phe-tyr-OCH₃ (**8**). The required dipeptide units **7**, **8** were prepared by coupling of Boc-amino acids viz. Boc-gly (**1**) and Boc-phe (**2**) with corresponding amino acid methyl ester hydrochlorides such as Leu-OCH₃·HCl (**3**), Tyr-OCH₃·HCl (**4**) employing dicyclohexylcarbodiimide (DCC) as coupling agent. Ester group of dipeptide **7** was removed by alkaline hydrolysis with LiOH to get Boc-gly-leu-OH which was coupled with amino acid methyl ester hydrochloride **5** using DCC and N-methylmorpholine (NMM), to get the first tripeptide unit Boc-gly-leu-gly-OCH₃ (**9**). Similarly, dipeptide **8** was deprotected at carboxyl end to get Boc-phe-tyr-OH and coupled with amino acid methyl ester hydrochloride **6** to get another tripeptide unit Boc-phe-tyr-pro-OCH₃ (**10**). After removal of ester group of tripeptide **9** and Boc group of tripeptide **10**, deprotected units were coupled with each other to get linear hexapeptide unit Boc-gly-leu-gly-phe-tyr-pro-OCH₃ (**11**). The ester group of linear fragment was removed using LiOH and p-nitrophenyl (pnp) ester group was introduced. After removing Boc-group of resulting unit Boc-gly-leu-gly-phe-tyr-pro-Opnp with CF₃COOH, deprotected linear fragment was cyclized by keeping the whole contents at 0 °C for 7 days in presence of catalytic amount of TEA/NMM/pyridine to get cyclic compound **12** (Scheme 1).

Synthesis of cyclohexapeptide **12** and intermediate linear peptide segments **1-11** was carried out successfully with good yield (>75%) and NMM was proved to be a yield effective base for cyclization of linear hexapeptide unit in comparison to TEA and pyridine. IR spectra of all peptide units **7-11** showed characteristic medium to strong bands corresponding to carbonyl stretching at

i) DCC, NMM, CHCl₃, RT, 24 h; ii) LiOH, THF:H₂O (1:1), RT, 1 h; iii) TFA, CHCl₃, RT, 1 h; iv) pnp, CHCl₃, RT, 12 h; v) TEA/NMM/C₃H₅N, 7 days, 0 °C

Scheme 1. Synthetic pathway for cherimolacyclopeptide E (**12**)

1672–1636 cm⁻¹ (Amide I band) and NH bending at 1536–1522 cm⁻¹ (Amide II band), confirming positivity of coupling reaction. Cyclization of linear peptide fragment **11** was supported by disappearance of absorption bands at 1750 cm⁻¹ and 1390, 1365 cm⁻¹ (C=O stretching of ester and CH bending of *tert*-Butyl group) and presence of additional Amide I and Amide II bands of the -CO-NH- moiety at 1645–1642 cm⁻¹ and 1521–1518 cm⁻¹ in IR spectra of the compound **12**. Formation of cyclopeptide was further confirmed by disappearance of signals at 157.5, 79.5, 53.3, 29.2 ppm corresponding to Boc and ester group in ¹³C NMR spectrum and disappearance of singlets at 3.62 and 1.54 ppm corresponding to three protons of methyl ester and nine protons of *tert*-Butyl group of Boc, in ¹H NMR spectrum of compound **12**. Furthermore, ¹H NMR and ¹³C NMR spectra of synthesized cyclic hexapeptide showed characteristic peaks confirming presence of all the 42 protons and 33 carbon atoms. Presence of (M⁺ + 1)⁺ ion peak (base peak) at m/z 635.7 corresponding to the molecular formula C₃₃H₄₂N₆O₇ in mass spectra of compound **12**, along with other fragment ion peaks resulting from cleavage at gly-phe amide bond level, showed exact sequence of attachment of all the six amino acid moieties in a chain. In addition, elemental analysis of compound **12** afforded values (± 0.03) strictly in accordance to the molecular composition.

PHARMACOLOGY

Synthesized compound **12** was tested for *in vitro* antimicrobial, anthelmintic and cytotoxicity activities and results of biological activity studies are tabulated in Table 1, Table 2 and Table 3.

Table 1: Antimicrobial activity data for compound **12**.

Compd.	Diameter of zone of inhibition (mm)							
	Bacterial strains				Fungal strains			
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>M. audouinii</i>	<i>A. niger</i>	<i>T. mentagrophytes</i>
12	–	–	28(6) ^a	24(12.5)	25(6)	9(12.5)	–	11(12.5)
Control	–	–	–	–	–	–	–	–
Ciprofloxacin	20(6)	20(12.5)	25(6)	19(12.5)	–	–	–	–
Griseofulvin	–	–	–	–	20(6)	18(6)	18(12.5)	20(6)

^a Values in parenthesis are MIC values ($\mu\text{g mL}^{-1}$).**Table 2:** Anthelmintic activity data for compound **12**.

Compd.	Earthworm species					
	<i>M. konkanensis</i>		<i>P. corethruses</i>		<i>Eudrilus sp</i>	
	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)
12^b	14.20 $\pm$ 0.52	17.32 $\pm$ 0.47	27.29 $\pm$ 0.36	29.32 $\pm$ 0.57	14.56 $\pm$ 0.21	17.05 $\pm$ 0.11
Control ^c	–	–	–	–	–	–
Mebendazole ^b	10.55 $\pm$ 0.64	12.59 $\pm$ 0.53	17.58 $\pm$ 1.03	19.42 $\pm$ 1.20	11.35 $\pm$ 0.45	13.46 $\pm$ 0.62
Piperazine citrate ^b	12.39 $\pm$ 0.36	13.52 $\pm$ 0.49	19.06 $\pm$ 0.57	22.23 $\pm$ 0.78	12.46 $\pm$ 0.15	13.58 $\pm$ 0.47

^a Data are given as mean $\pm$ S.D. (n = 3); ^b c = 2 mg mL⁻¹; ^c 0.5% Tween 80 in distilled water**Table 3:** Cytotoxic activity data for compound **12**.

Compd.	Conc. ($\mu\text{g/mL}$)	DLA cells				EAC cells			
		Live cells counted	No. of dead cells	% growth inhibition ^a	CTC ₅₀ ^b (μM)	Live cells counted	No. of dead cells	% growth inhibition	CTC ₅₀ (μM)
12	62.5	0	38	100.0		0	28	100.0	
	31.25	2	36	94.74		3	25	89.29	
	15.63	7	31	81.58	2.76	9	19	67.86	4.96
	7.81	11	27	71.05		14	14	50.00	
	3.91	20	18	47.37		24	4	14.29	
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	62.5	0	38	100.0		0	28	100.0	
(5-FU)	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 = $100 - [\{ (\text{Cell}_{\text{total}} - \text{Cell}_{\text{dead}}) \times 100 \} / \text{Cell}_{\text{total}}]$; ^b CTC₅₀ = cytotoxic conc. inhibiting 50% of percentage growth

Investigation of antimicrobial activity revealed that synthesized cyclohexapeptide **12** possessed potent antifungal activity against pathogenic fungi *Candida albicans* and good antibacterial activity against gram negative bacteria. Compound **12** was almost 25% more active against pathogenic yeast *C. albicans* with MIC value of 6 $\mu\text{g mL}^{-1}$, in comparison to reference drug griseofulvin and exhibited 12-26% more antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli* with MIC value of 6 and 12.5 $\mu\text{g mL}^{-1}$, in comparison to standard drug ciprofloxacin. Gram positive bacteria and fungus *Aspergillus niger* were found to be resistant to synthesized peptide.

However, compound **12** exhibited only slight antifungal activity against dermatophytes *Microsporum audouinii* and *Trichophyton mentagrophytes*.

Comparison of anthelmintic data indicated that compound **12** showed moderate anthelmintic activity against all three earthworms *Megascopex konkanensis*, *Pontoscotex corethruses* and *Eudrilus sp.* in comparison to reference drugs mebendazole and piperazine citrate at concentration of 2 mg mL⁻¹. *Pontoscotex corethruses* was found to be the least sensitive earthworm species.

Moreover, compound **12** possessed high cytotoxic activity against DLA and

EAC cell lines with CTC₅₀ values of 2.76 and 4.96 µM respectively (Standard drug, 5-fluorouracil (5-FU): CTC₅₀ values – 37.36 and 90.55 µM), in comparison to significant cytotoxicity exhibited by natural chermolacyclopeptide E against KB cells with IC₅₀ value of 0.017 µM. On passing toxicity tests, synthesized cyclohexapeptide **12** may prove good candidate for clinical studies and can be new antimicrobial and cytotoxic drug of future.

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