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-glu-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 Euaditus 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 bioactivities⁴⁻⁶. Among these, cyclic peptides and related congeners with unique structures and wide pharmacological profile⁴⁻⁶ have received special attention which may prove better candidates to overcome the problem of widespread 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 activity¹⁵⁻¹⁶, tyrosine inhibitory activity¹⁷⁻¹⁸ and estrogen-like activity¹⁹⁻²⁰. Recently, a natural cyclopeptide, 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 and 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 cyclopeptides²²⁻²⁴, 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-(butyl) pyrocarbonate (Boc₂O), N-p-methoxybenzyl-l-phenylalanine and N-p-methoxybenzyl-l-leucine 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), (Bruker, USA) using CDCl₃ as solvent and tetramethylsilane as internal standard (in ppm). Mass spectra were 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 HSO₄ and finally extracted with chloroform (3 x 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]D = 2.7°, Rf = 0.61; IR: 3292-2488 (m br, OH str, COOH); 3133 (m, NH str, amide); 2927, 2830 (m, CH str, asy and sym, CH₂); 1712 (s, C=O str, COOH); 1635 (s, C=O str, amide); 1531 (m, NH bend, amid); 1466 (m, CH bend (scissoring), CH₂); 1387, 1362 (m, CH bend, butyl-t); 929 (w, CH, 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 (C-β, C-β, butyl-t).

Anal. Calcd. for C₉H₁₄NO₂: 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]D = -4.0°, Rf = 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, amid); 1387, 1365 (m, CH bend, butyl-t); 933 (m, CH, 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-α, 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-α); 128.2 (2C, C-β); 126.5 (C-p); 79.5 (C-α, butyl-t); 55.1 (C-β); 57.7 (C-β, C-β, butyl-t).

Anal. Calcd. for C₁₉H₂₁NO₄: 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, [α]D -13.5 °, R -0.54; IR: 3015-2852 (sbr, NH, str, asym and sym); 2925 (m, CH str, asym, CH); 2822 (m, CH str, OCH3); 1742 (s, C-O str, ester); 1602, 1506 (sbr, NH2, bend and 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, rock, butyl-t and propyl-i). 1H NMR: 4.12 (s, 3H, OCH3), 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-CH3, J=6.15 Hz); 1.17 (br. s, NH). 13C NMR: 169.5 (C-O, ester); 53.0 (OCH3); 49.6 (C-α); 39.1 (C-β); 27.3 (C-γ); 20.9 (2C, C-δ).

Anal. Calcd. for C8H17NO3C: 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, [α]D -4.3 °, R -0.67; IR: 3372 (mbr, OH, str); 3011-2863 (sbr, NH2, str, asym and sym); 2928, 2848 (m, CH str, asym and sym, CH3); 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); 925 (s, CH def, oop, ring). 1H NMR: 7.80-7.78 (dd, 2H, H-μ, J=7.4 Hz, J=5.0 Hz); 7.56-7.54 (dd, 2H, H-μ, J=7.5 Hz, J=4.6 Hz); 5.40 (br. s, OH and NH3). 1.41-1.40 (m, 1H, H-α); 4.12 (s, 3H, OCH3); 2.20-2.18 (d, 2H, H-β, J=7.15 Hz). 13C NMR: 168.8 (C-O, ester); 156.5 (C-γ); 130.2 (2C, C-ο); 122.2 (C-δ); 117.5 (2C, C-μ); 53.1 (OCH3); 50.5 (C-α); 32.9 (C-β).

Anal. Calcd. for C8H17NO2C: 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, [α]D -88.2 °, R -0.45; IR: 3015-2853 (sbr, NH, str, asym and sym); 2925, 2847 (m, CH str, asym and sym, CH3); 1744 (s, C-O str, ester); 1599, 1502 (sbr, NH2, bend and asym and sym); 1386, 1370 (m, CH def, butyl-t); 1268 (s, C-O str, ester); 932 (w, CH, rock, butyl-t). 1H NMR: 4.75 (br. s, NH); 4.19 (s, 3H, OCH3); 3.85 (m, 2H, H-α). 13C NMR: 170.2 (C-O, ester); 52.3 (OCH3). 40.8 (C-α).


L-Proline methyl ester hydrochloride (6).

This compound was obtained according to the general procedure as viscous liquid, yield 87%, mp 177-178 °C, [α]D -40.2 °, R -0.26; IR: 3050-2840 (sbr, NH, str, asym and sym); 2995, 2987 (m, CH str, CH3, pro); 1745 (s, C-O str, ester); 1440 (sbr, NH2, bend); 1270 (s, C-O str, ester). 1H NMR: 4.73-4.70 (m, 1H, H-α); 4.05-4.01 (t, 2H, H-β); 3.86 (s, 3H, OCH3); 2.45-2.32 (m, 4H, H-β, H-γ); 2.35 (br. s, NH). 13C NMR: 171.8 (C-O, ester); 62.1 (C-α); 54.5 (OCH3); 44.7 (C-β); 30.4 (C-β); 20.2 (C-γ).

Anal. Calcd. for C7H14N2O2C: 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 Bodanszky method with certain modifications. Amino acid methyl ester hydrochloride/peptide methyl ester (10 mmol) was dissolved in CHCl3 (20 mL). To this, NMM (21 mmol) was added at 0 °C and the reaction mixture was stirred for 15 minutes. Boc-amino acid (10 mmol) in CHCl3 (20 mL) and DCC (10 mmol) were added with stirring. After 24 h, the reaction mixture was filtered and the residue was washed with CHCl3 (30 mL) and added to the filtrate. The filtrate was washed with 5% NaHCO3 and saturated NaCl solutions. The organic layer was dried over anhydrous Na2SO4, 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 amino terminal was accomplished by treatment of Boc-di/tripeptides at RT for 1 h with then acidified to pH 3.5 with 1H2SO4. The aqueous layer was extracted with Et2O (3 x 25 mL). The combined organic extracts were dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was crystallized from methanol and ether to get pure Boc-di/tripeptides.

tert-Butyloxycarbonyl-glycyl-leucine methyl ester (7). This compound was obtained according to the general procedure as semisolid mass, yield 79%, [α]D +1.8 °, R -0.36; IR: 3125 (m, NH str, amide); 2927 (m, CH str, asym, CH3); 2853 (m, CH str, sym, CH3); 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-i); 1270 (s, C-O str, ester); 930, 924 (w, CH, rock, butyl-t and propyl-i). 1H NMR: 6.52 (br. s, 1H, NH); 6.02 (br. s, 1H, NH); 4:25-4:20 (q, 1H, H-α, ester); 3.62 (s, 3H, OCH3); 3.50-3.48 (d, 2H, CH2, J=4.75 Hz); 1.54 (s, 9H, butyl-t); 1.51-1.41 (m, 3H, H-β, H-γ, H-δ); 0.96-0.94 (d, 6H, H-β, H-δ, ester); 0.61-0.58 (s, 9H, butyl-t); 1H and sym); 2.73 (C=O str, asym and sym); 2.928, 2842 (m, CH str, asym and sym, CH3); 1735 (s, C-O

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% (CH₃CN) mp 213 °C (213-214 °C, lit. [35] 213-214 °C). IR: 3372 (mbr, OH str, ester); 3129-3122 (m, NH str, amide); 3074, 3052 (w, CH str, rings); 2998-2993 (m, CH str, CH₃, pro); 2926-2923, 2852-2844 (m, CH str, asym and sym, CH); 1673, 1645-1636 (s, C=O str, 3° and 2° amide) 1585, 1582, 1472 (m, skeletal bands, rings); 1536-1530, 1255-1518 (m, NH bend, amide); 1380, 1361 (s, C=O, def, propyl-o-t₂, pro); 1233 (s, C=O, str, tyr); 922 (w, CH, rock, propyl-i); 825, 720, 688 (s, CH, def, oop, rings).


**PHARMACOLOGICAL EVALUATION**

The newly synthesized cyclohexapeptide 12 was evaluated for in vitro antibacterial, antifungal, and cytotoxic activities. The antibacterial activity was carried out against gram positive bacteria *Bacillus subtilis* (MUCM 408), *Staphylococcus aureus* (MUCM 377), gram negative bacteria *Pseudomonas aeruginosa* (MUCM 266), *Escherichia coli* (MUCM 106) and cutaneous fungi *Microsporum audouinii* (MUCM 545) and *Trichophyton mentagrophytes* (MUCM 665), damorphic fungi *Candida albicans* (MUCM 29) and pathogenic fungi *Aspergillus niger* (MUCM 188) using ciprofloxacin and griseofulvin as reference drugs. Anthelmintic activity was performed against three earthworm species *Megascoelous koninensis* (ICARB 211), *Pontoscolex corethruses* (ICARB 408) and *Eudrilus sp.* (ICARB 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 drug.

**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 method. 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 × 10⁶ spores mL⁻¹). Filter paper disks of 6 mm diameter and 2 mm thickness were sterilized at 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
Anthelmintic screening

Anthelmintic activity study was carried out against three different species of earthworms at 2 mg mL⁻¹ concentration using Garg method13. 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 Kuttan method16. 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 dulbecco's 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-O-pnp 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 1-11 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/C,H,N, 7 days, 0 °C

Scheme 1. Synthetic pathway for cherimolacyclopeptide E (12)

1672-1636 cm⁻¹ (Amide I band) and NH bending at 536-522 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 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 C₄₂H₅₂N₈O₂ 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.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>B. subtilis</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
<th>E. coli</th>
<th>C. albicans</th>
<th>M. audouinii</th>
<th>A. niger</th>
<th>T. mentagrophytes</th>
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<tbody>
<tr>
<td>12</td>
<td>–</td>
<td>–</td>
<td>28(6)*</td>
<td>24(12.5)</td>
<td>25(6)</td>
<td>9(12.5)</td>
<td>–</td>
<td>11(12.5)</td>
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<td>Control</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Ciprofloxacin</td>
<td>20(6)</td>
<td>20(12.5)</td>
<td>25(6)</td>
<td>19(12.5)</td>
<td>–</td>
<td>11(12.5)</td>
<td>18(6)</td>
<td>18(12.5)</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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*Values in parenthesis are MIC values (µg mL⁻¹).

Table 2: Anthelmintic activity data for compound 12.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>M. konkanensis</th>
<th>P. corethruses</th>
<th>Eudrilus sp</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean paralyzing time (min)*</td>
<td>Mean death time (min)*</td>
<td>Mean paralyzing time (min)</td>
</tr>
<tr>
<td>12*</td>
<td>14.20 ± 0.52</td>
<td>17.32 ± 0.47</td>
<td>27.29 ± 0.36</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mebendazole</td>
<td>10.55 ± 0.64</td>
<td>12.59 ± 0.53</td>
<td>17.58 ± 1.03</td>
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<tr>
<td>Piperazine citrate</td>
<td>12.39 ± 0.36</td>
<td>18.06 ± 0.49</td>
<td>19.06 ± 0.57</td>
</tr>
</tbody>
</table>

*Data are given as mean ± S.D. (n = 3); *c = 2 mg mL⁻¹; *0.5% Tween 80 in distilled water.

Table 3: Cytotoxic activity data for compound 12.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Conc. (µg/ml)</th>
<th>Live cells counted</th>
<th>No. of dead cells</th>
<th>% growth inhibition*</th>
<th>CTC₅₀ (µM)</th>
<th>Live cells counted</th>
<th>No. of dead cells</th>
<th>% growth inhibition*</th>
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<tbody>
<tr>
<td>12</td>
<td>62.5</td>
<td>0</td>
<td>38</td>
<td>100.0</td>
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*% growth inhibition = 100 – [(Cell_total – Cell_dead) x 100] / Cell_total; *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 µg 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 µg 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 Megacollynx 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 CTC_{50} values of 2.76 and 4.96 μM respectively (Standard drug, 5-fluorouracil (5-FU); CTC_{50} values – 37.36 and 90.55 μM), in comparison to significant cytotoxicity exhibited by natural cherimolacyclopeptide E against KB cells with IC_{50} 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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