Synthetic and Cytotoxic and Antimicrobial Activity Studies on Annomuricatin B

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Z. Naturforsch. 2009, 64b, 237 – 244; received August 21, 2008

The first total synthesis of annomuricatin B (8) is described via coupling of the tripeptide Boc-L-asparaginyl(benzhydryl)-L-alanyl-L-tryptophan-OH and the tetrapeptide L-leucyl-glycyl-L-thryl-L-proline-OMe followed by cyclization of the linear heptapeptide fragment. On pharmacological investigation, it was observed that the cycloheptapeptide 8 displays moderate cytotoxicity against Dalton’s lymphoma ascites and Ehrlich’s ascites carcinoma cell lines with cytotoxic inhibitory concentration (50 %) values of 11.6 and 14.1 µM, in addition to potent antidermatophyte activity against Trichophyton mentagrophytes and Microsporum audouinii with a minimum inhibitory concentration of 6 µg mL⁻¹. Moreover, Gram-negative bacteria and Candida albicans were found to be moderately sensitive towards the newly synthesized peptide.

Key words: Annomuricatin B, Cycloheptapeptide, Solution-phase Synthesis, Macrocyclization, Pharmacological Activity

Introduction

During past years, a lot of work has been reported by various scientists which demonstrates the potential of higher plants to produce a wide array of natural products with interesting bioactivities [1 – 5]. Among these, cyclopolyepptide and related congeners [6] are emerging as novel organic compounds with unique structures and a wide pharmacological profile that may prove better candidates to overcome the problem of resistance towards conventional drugs. Plant-derived cyclic peptides possess a variety of biological activities including antitumor [7], vasorelaxant [8], immunosuppressive [9], tyrosinase and cyclooxygenase inhibitory [10, 11], antimalarial [12], and estrogen-like activity [13, 14]. A novel cyclic heptapeptide, annomuricatin B was isolated by column chromatography from seeds of Annona muricata (Annonaceae), and the structure was elucidated by chemical and spectral methods [15].

Prompted by the medicinal properties of plant-derived cyclopolyepptides as well as to obtain the natural peptide in good yield, the present study aimed at the synthesis of annomuricatin B employing solution-phase chemistry. The cytotoxic, antibacterial and antifungal activities of the synthesized peptide were also evaluated.

Results and Discussion

The cycloheptapeptide molecule was split into three dipeptide units Boc-L-Leu-Gly-OMe (1), Boc-L-Thr-L-Pro-OMe (2) and Boc-L-Ala-L-Trp-OMe (3) and a single amino acid unit Boc-L-Asn-OH (4). Dipeptide units 1 – 3 were prepared by coupling of Boc-amino acids such as Boc-L-Leu, Boc-L-Thr and Boc-L-Ala with the corresponding amino acid methyl ester hydrochlorides such as Gly-OMe·HCl, L-Pro-OMe·HCl and L-Trp-OMe·HCl by following the modified Bodanzsky and Bodansky method [16]. The carboxamide side chain of amino acid unit 4 was protected using benzhydrol to get Boc-L-Asn(bzh)-OH (4a). After deprotection at the carboxy terminus, dipeptide 1 was coupled with dipeptide 2 deprotected at the amino terminus, to get the tetrapeptide unit Boc-L-Leu-Gly-L-Thr-L-Pro-OMe (5). The Boc group of dipeptide 3 was removed using trifluoroacetic acid (TFA), and the deprotected peptide was coupled with the benzhydrol-protected amino acid unit 4a utilizing three different carbodiimides to
Scheme 1. Synthetic route to the cycloheptapeptide (annomuricatin B) 8. Reaction conditions: a = LiOH·H₂O (1:1), r.t., 1 h; b = TFA, CHCl₃, r.t., 1 h; c = benzhydrol, GAA, H₂SO₄, r.t., 30 min; d = EDC·HCl or DIPC, TEA or NMM, THF or DMF, r.t., 24 – 36 h; e = DIPC, pnp/pfp, r.t., 12 h; f = TEA or NMM or pyridine, 7 d, 0 °C.

get the tripeptide unit Boc-L-Asn(bzh)-L-Ala-L-Trp-OMe (6). After removal of the ester group of tripeptide 6 and the Boc group of tetrapeptide 5, the deprotected units were coupled to get the linear heptapeptide unit Boc-L-Asn(bzh)-L-Ala-L-Trp-L-Leu-Gly-L-Thr-L-Pro-OMe (7). The methyl ester group of the linear peptide fragment was replaced by p-nitrophenyl or pentafluorophenyl (Pnp or Pfp) ester groups. The Boc and Bzh groups of the resulting compound were removed using TFA, and the deprotected linear fragment was now cyclized by keeping the whole contents at 0 °C for 7 d in the presence of catalytic amounts of
Table 1. Cytotoxic activity data.

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<th>Live cells counted</th>
<th>No. of dead cells</th>
<th>% GI&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CTC&lt;sub&gt;50&lt;/sub&gt; (µM)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Live cells counted</th>
<th>No. of dead cells</th>
<th>% GI</th>
<th>CTC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
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<sup>a</sup> % Growth inhibition (GI) = 100 – \[\left(\frac{\text{Cell}_{\text{total}} - \text{Cell}_{\text{dead}}}{\text{Cell}_{\text{total}}}\right) \times 100\]; <sup>b</sup> CTC<sub>50</sub> = cytotoxic concentration inhibiting 50 % of percentage growth.

Table 2. Antifungal activity data.<sup>a</sup>

<table>
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<td>8</td>
<td>14(6)</td>
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<td>Control</td>
<td>–</td>
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<td>Griseofulvin</td>
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</table>

<sup>a</sup> Values in parentheses are MIC values (µg/mL<sup>-1</sup>).

The structure of the newly synthesized cyclic heptapeptide as well as that of the intermediate tri/tetra/heptapeptides were confirmed by FTIR and <sup>1</sup>H NMR spectroscopy, and elemental analysis. In addition, <sup>13</sup>C NMR and mass spectra were recorded for the linear as well as for the cyclic heptapeptide.

The synthesis of cyclopeptide 8 was accomplished with 85 % yield, and pyridine proved to be an effective base for cyclization of the linear heptapeptide fragment. Cyclization of the linear peptide was indicated by the disappearance of absorption bands at 1748, 1270 and 1388, 1371 cm<sup>-1</sup> (C–Ostr, ester and C–Hdef, tert-butyl groups) and the presence of additional amide I and II bands of the -CO–NH- moiety at 1637 – 1634 cm<sup>-1</sup> and 1527 – 1525 cm<sup>-1</sup> in the IR spectrum of 8. Deprotection of asparagine was confirmed by the presence of amide I and II bands (1653, 1628 cm<sup>-1</sup>) and bands at 3350, 3178 and 1405 cm<sup>-1</sup> due to N–H str and C–N str of the -CONH<sub>2</sub> moiety, and the disappearance of strong out-of-plane deformation bands at 733 – 731 cm<sup>-1</sup> and 699 – 694 cm<sup>-1</sup> due to the aromatic rings of the benzhydryl (Bzh) group, in the IR spectra and the disappearance of the multiplet at 7.23 – 7.16 and 7.09 – 7.03 ppm due to 10 protons of phenyl rings of the benzhydryl (Bzh) group in the <sup>1</sup>H NMR spectrum of 8. The formation of the cyclopeptide was further confirmed by the disappearance of singlets at 3.63 and 1.54 ppm corresponding to three protons of the methyl ester group and nine protons of the tert-butyl group of Boc in the <sup>1</sup>H NMR spectrum and the disappearance of the singlets at 154.6, 79.9 and 53.9, 28.3 ppm corresponding to carbon atoms of ester and tert-butyl groups in the <sup>13</sup>C NMR spectrum of 8. Furthermore, the <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of the synthesized cyclic heptapeptide showed characteristic peaks confirming the presence of all the 49 protons and 35 carbon atoms. The appearance of the pseudomolecular ion peak [M + 1]<sup>+</sup> at m/z = 740 corresponding to the molecular formula C<sub>35</sub>H<sub>49</sub>N<sub>9</sub>O<sub>9</sub> in the mass spectrum of 8, along with other fragment
ion peaks resulting from cleavage at ‘Gly-Thr’, ‘Thr-Pro’ and ‘Pro-Asn’ amide bonds showed the exact sequence of the attachment of all the seven amino acid moieties in a chain. In addition, the presence of the immonium ion peaks at \( m/z = 159 \) (Trp), 87 (Asn), 86 (Leu), 74 (Thr), 70 (Pro), 44 (Ala), and 30 (Gly) further confirmed all the seven amino acid moieties in the cyclopeptide structure. Furthermore, the elemental analysis of 8 afforded values with tolerances of ±0.03 in strict accordance with the molecular composition.

The synthesized cyclopeptide 8 exhibited moderate cytotoxic activity against Dalton’s lymphoma ascites (DLA) and Ehrlich’s ascites carcinoma (EAC) cell lines with CT50 values of 11.6 and 14.1 \( \mu M \), respectively, in comparison to the standard drug 5-fluorouracil (5-FU) (CT50 values = 37.4 and 90.6 \( \mu M \)) (Table 1). The possible mechanism of the cytotoxic action of 8 might be through apoptosis via induction of early cell death, nuclear fragmentation and internucleosomal DNA scission. Comparison of the antifungal activity data suggested that 8 possessed potent bioactivity against dermatophytes M. audouinii and T. mentagrophytes and moderate antifungal activity against pathogenic Candida albicans with MIC values of 6 \( \mu g/mL \) when compared to the reference drug griseofulvin (Tables 2 and 3). Moreover, a moderate level of activity was observed against Gram-negative bacteria K. pneumoniae, P. aeruginosa and E. coli for the newly synthesized cyclopeptide, in comparison to the standard drug gatifloxacin. However, 8 displayed no significant activity against neither Gram-positive bacteria nor pathogenic Ganoderma sp. and Aspergillus niger. In addition, the analysis of the pharmacological activity data revealed that cycloheptapeptide 8 displayed a higher bioactivity against pathogenic microbes and cell lines than its linear form. Thus, the inherent flexibility of linear peptides leads to different conformations which can bind to more than one receptor molecule, resulting in undesirable adverse effects.

**Conclusion**

The present study presents the successful synthesis of the natural peptide annomuricatin B (8) in good yield via coupling reactions utilizing different carboximidates. The DIPC/TEA coupling method proved to be yield-effective, in comparison to methods utilizing EDC-HCl and NMM, providing 10 – 12 % additional yield. The pentafluorophenyl ester was shown to be better for the activation of the acid functionality of the linear heptapeptide unit when compared to the p-nitrophenyl ester. Pyridine was found to be a good base for the intramolecular cyclization of the linear peptide fragment in comparison to TEA or NMM. The synthesized cycloheptapeptide displayed moderate cytotoxicity as well as potent antidermatophyte activity. In comparison, Gram-negative bacteria were found to be more sensitive than Gram-positive bacteria towards the newly synthesized peptide. On passing toxicity tests, cyclopeptide 8 may prove as a good candidate for clinical studies and can be a new antifungal and cytotoxic drug of future.

**Experimental Section**

**General methods**

Melting points were determined using a Jindal Scientific melting point apparatus (Jindal, Delhi, India) by the open capillary method and are uncorrected. IR spectra were recorded on an FTIR-8400S Fourier transform spectrophotometer (Shimadzu, Kyoto, Japan) using a thin film supported on KBr pellets for solids and CHCl3 as solvent for intermediate semisolids. \(^1\)H and \(^{13}\)C NMR spectra were recorded on a Bruker AC 300 spectrometer at 300 MHz (Bruker, IL, USA) using CDCl3 as solvent and tetramethylsilane as internal standard. Mass spectra were recorded on a Bruker 303 spectrometer at 70 eV using the fast atom bombardment technique. Elemental analyses of the cyclopeptide as well as of the intermediates were performed on a Vario EL III elemental analyzer (Elementar Vario EL III, Hanau, Germany). Optical rotation of the synthesized peptides was measured on an Optics Tech-
ology automatic polarimeter (OpticsTech, Delhi, India) in a 2 dm tube at 25 °C using a sodium lamp and methanol as solvent. Purity of the synthesized cyclopeptide and the intermediates was checked by TLC on precoated silica gel G plates (Kieselgel 0.25 mm, 60G F254, Merck, Germany) utilizing CHCl3-MeOH as developing solvent in different ratios. Brown spots were detected on exposure to iodine vapors in a tightly closed chamber.

Protection of the carboxamide side chain of Boc-L-asparagine (4)

To the solution of Boc-L-asparagine (2.32 g, 0.01 mol) in glacial acetic acid (GAA, 25 mL), benzhydrol (1.84 g, 0.01 mol) was added with stirring at room temperature (r.t.) for a time period of 30 min. Concentrated sulfuric acid in glacial acetic acid (GAA, 25 mL), benzhydrol (1.84 g, 0.01 mol) were added, the solution was warmed to 100 °C and stirred under reduced pressure. The aqueous layer was extracted with Et2O (3 × 25 mL). The combined organic extracts were dried over anhydrous Na2SO4 and concentrated under reduced pressure.

Deprotection of the tripeptide unit at the carboxyl terminal

To a solution of the tripeptide (6.7 g, 0.01 mol) in THF-H2O (1 : 1, 36 mL), LiOH (0.36 g, 0.015 mol) was added with stirring at r.t. for 1 h and then acidified to pH = 3.5 with 1 N H2SO4. The aqueous layer was extracted with Et2O (3 × 25 mL). The combined organic extracts were dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude product was finally crystallized from methanol and ether to get the pure deprotected compound 5a.
tert-Butyloxy carbonyl-L-asparaginyl(bzh)-L-alanyl-L-tryptophan (5a)

White solid. M.p. 91–93 °C. Yield 87 %. \( \alpha \) (D) = −69.7° (c = 0.25, MeOH). \( R_f = 0.77 \) (CHCl\(_3\)-MeOH = 8 : 2). – IR (KBr): \( \nu = 3489 \) (\( \delta_{\text{H}} \)-ring), 3297–2509 (O–H=ar., COOH), 3129, 3126 (N–H=ar., amide), 2975, 2955 – 2922 (C–H str, asymmetric, CH\(_3\) and CH\(_2\)), 2874, 2845 (C–H=asym, sym, CH\(_3\) and CH\(_2\)), 1713 (C=O=ar., COOH), 1645, 1639 (C=O=ar., amide), 1576–1568, 1482–1478 (skeletal bands), 1538–1533 (N=O=ar., amide). 1386, 1370 (C–H def, \( \text{MeOH} \)). R \( \text{H} \) Asn), 1.53 (s, 9H, \( \text{t} \)-butyl), 1.49 (d, \( J = 5.85 \) Hz, 3H, \( \beta \)-H\( \delta \), Ala), 1.36 (d, \( J = 5.85 \) Hz, 3H, \( \alpha \)-H\( \delta \), Thr), 1.15 (d, \( J = 6.27 \) Hz, \( \delta \)-H, Leu). 0.77 (CHCl\(_3\)-MeOH = 8 : 2). – IR (KBr): \( \nu = 3488 \) (N–H=ar., ring), 3374 (O–H=ar., sym, CH\(_3\) and CH\(_2\)), 2875, 2847, 2844 (C–H=asym, sym, CH\(_3\) and CH\(_2\)), 1748 (C=O=ar., ester), 1669, 1646 – 1639 (C=O=ar., amide), 1578–1569, 1484–1479 (skeletal bands), 1539, 1535, 1530 (N–H=def., amide), 1388, 1371 (C–H=def., tert-butyl), 1381, 1367 (C–H=def., \( \text{iso} \)-propyl), 1270, 1094 (C–O=ar., ester and C–O=H), 736–731, 699–695, 676 (C–H def, out-of-plane, rings) cm\(^{-1}\). – 1HN NMR (CDCl\(_3\)): \( \delta = 6.00 \) (br. s, 1H, \( \text{NH} \)), 7.82 (br. s, 2H, \( \text{NH} \)), 8.50 (br. s, 1H, \( \text{NH} \)), 7.55 (d, \( J = 5.85 \) Hz, 1H, \( \alpha \)-H, Ala), 5.53–5.36 (d, 2H, \( \beta \)-H\( \delta \), Ala), 4.48–4.43 (m, 1H, \( \alpha \)-H, Trp), 4.42–4.38 (m, 1H, \( \beta \)-H\( \delta \), Ala), 3.63 (s, 3H, OC\(_3\)O), 1.92 (m, 1H, \( \text{D} \)=+2.8°, N). 78 %; – \( \alpha \) (D) = −51.8° (c=0.35, MeOH). \( R_f = 0.63 \) (CHCl\(_3\)-MeOH = 9 : 1). – IR (CHCl\(_3\)): \( \nu = 3486 \) (N–H=ar., ring), 3374 (O–H=ar., sym, CH\(_3\) and CH\(_2\)), 2875, 2847, 2844 (C–H=asym, sym, CH\(_3\) and CH\(_2\)), 1748 (C=O=ar., ester), 1669, 1646–1639 (C=O=ar., amide), 1578–1569, 1484–1479 (skeletal bands), 1539, 1535, 1530 (N–H=def., amide), 1388, 1371 (C–H=def., tert-butyl), 1381, 1367 (C–H=def., \( \text{iso} \)-propyl), 1270, 1094 (C–O=ar., ester and C–O=H), 736–731, 699–694, 676 (C–H=def., out-of-plane, rings) cm\(^{-1}\). – 1HN NMR (CDCl\(_3\)): \( \delta = 8.90 \) (br. s, 1H, \( \text{NH} \)), 8.75 (br. s, 1H, \( \text{NH} \)), 8.63 (br. s, 1H, \( \text{NH} \)), 8.50 (br. s, 1H, \( \text{NH} \)), 8.19 (br. s, 1H, \( \delta \)-H, Asn), 8.16 (br. s, 1H, \( \text{NH} \)), 7.82 (br. s, 2H, \( \text{NH} \)), 7.69 (br. s, 1H, \( \text{NH} \)), 7.25 (d, \( J = 7.85 \) Hz, 1H, \( \beta \)-H, indole), 7.23–7.16 (m, 6H, \( m \)-H\( \delta \) and \( p \)-H\( \delta \), phenyl rings, Bzh), 7.15–7.10 (m, 4H, \( \delta \)-H–H\( \delta \), indole), 7.09–7.03 (m, 4H, \( \alpha \)-H–H\( \alpha \), \( \text{NH} \)), 4.5–4.1 (m, 1H, \( \alpha \)-H–H\( \alpha \), \( \text{NH} \)), 3.95–3.8 (m, 2H, \( \alpha \)-H–H\( \alpha \), \( \text{NH} \)), 3.22 (d, \( J = 5.45 \) Hz, 1H, \( \alpha \)-H–H\( \alpha \), \( \text{NH} \)), 4.75–4.68 (m, 1H, \( \alpha \)-H, Asn), 4.24–4.19 (m, 1H, \( \alpha \)-H–H\( \delta \), Trp), 4.09 (dd, \( J = 6.27 \) Hz, 1H, \( \alpha \)-H–H\( \delta \), Trp), 1.22 (d, \( J = 5.85 \) Hz, 3H, \( \beta \)-H\( \delta \), Ala), 0.99 (d, \( J = 6.15 \) Hz, 6H, \( \delta \)-H–H\( \delta \), \( \text{Me} \)).
Synthesis of the cyclic heptapeptide – annomuricatin B (8)

To synthesize compound 8, the linear heptapeptide unit 7 (5.2 g, 0.005 mol) was deprotected at the carboxyl end using LiOH (0.18 g, 0.0075 mol) to get Boc-L-Asn(bz)-L-Ala-L-Trp-L-Leu-Gly-L-Thr-L-Pro-OH. The deprotected heptapeptide unit (5.12 g, 0.005 mol) was then dissolved in CHCl3 (50 mL) at 0 °C. To this solution, 0.0067 mol of p-nitrophenol or pentafluorophenol (0.94 g or 1.23 g) and DIPC (0.63 g, 0.005 mol) were added, and stirring was done at rt. for 12 h. The reaction mixture was filtered, and the filtrate was washed with 10% NaHCO3 solution (3 × 25 mL) and finally with 5% HCl (2 × 30 mL) to get the corresponding p-nitrophenyl or pentafluoro-phenyl esters Boc-L-Asn(bz)-L-Ala-L-Trp-L-Leu-Gly-L-Thr-L-Pro-OH or Boc-L-Asn(bz)-L-Ala-L-Trp-L-Leu-Gly-L-Thr-L-Pro-OH. To this compound (4.58 g or 4.76 g, 0.004 mol) dissolved in CHCl3 (35 mL), CF3COOH (0.91 g, 0.008 mol) was added, and the mixture was 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 1-L-Asn-L-Ala-L-Trp-L-Leu-Gly-L-Thr-L-Pro-OH or 1-L-Asn-L-Ala-L-Trp-L-Leu-Gly-L-Thr-L-Pro-OH which was dissolved in CHCl3 (25 mL), and TEA/NMM/C2H5N (2.8 mL or 2.21 mL or 1.61 mL, 0.021 mol) was added. Then the mixture was kept at 0 °C for 7 d. The reaction mixture was washed with 10% NaHCO3 (3 × 25 mL) and 5% HCl (2 × 30 mL) solutions. The organic layer was dried over anhydrous Na2SO4, and the crude product was crystallized from CHCl3-η-heptane to get the pure cyclic peptide 8.

Cyclo (L-Asn-L-Ala-L-Trp-L-Leu-Gly-L-Thr-L-Pro) (8)

White crystals. M. p. 212–213 °C (213 °C for natural annomuricatin B [15]). Yield 85% (C2H5N), 78% (NMM), 69% (TEA). [α]D = −37.3° (c = 0.5, MeOH) (−37.25° for natural annomuricatin B [15]). Rf = 0.81 (CHCl3-MeOH = 9 : 1). – IR (KBr): ν = 3488 (N-H str, ring), 3372 (O-H str), 3350, 3178 (N-H str, 1° amide), 3136–3127 (N-H str, 2° amide), 2998–2993 (C=H str, CH2, Pro), 3074 (C=H str, ring), 2967, 2929, 2920 (C=H str, asym, CH3 and CH2), 2878, 2844, 2842 (C=H str, sym, CH3 and CH2), 1667, 1653, 1645–1634 (C=O str, amides), 1628 (NH2, 1° amide), 1572, 1480 (skeletal bands), 1538–1532, 1529–1525 (N-H str, amide), 1405 (C=O str, 1° amide), 1383, 1362 (C-H str, iso-propyl), 1095 (C-Oar, C-OH), 735, 677 (C-H str, out-of-plane, ring) cm−1. – 1H NMR (CDCl3): δ = 11.78 (br. s, 1 H, NH), 8.25 (br. s, 2 H, NH, indole and OH, Thr), 8.20 (br. s, 1 H, NH), 8.05 (br. s, 1 H, NH), 7.93 (br. s, 1 H, NH), 7.28 (d, J = 7.9 Hz, 1 H, β-H, Leu), 7.25 (d, J = 7.65 Hz, 1 H, β-H, indole), 7.14 (br. s, 1 H, NH), 7.13–7.10 (m, 3 H, ε-η-H’s, indole), 6.97 (br. s, 1 H, NH), 6.75 (br. s, 2 H, NH2, Asn), 6.28–6.24 (m, 1 H, α-H, Leu), 5.98–5.93 (m, 1 H, α-H, Ala), 5.78 (dd, J = 6.15 Hz, 4.9 Hz, 1 H, α-H, Thr), 5.74–5.69 (m, 1 H, α-H, Trp), 5.28 (d, J = 5.5 Hz, 2 H, α-H’s, Gly), 4.99–4.95 (m, 1 H, C=O, Asn), 3.94 (q, J = 6.9 Hz, 1 H, α-H, Pro), 3.81–3.75 (m, 1 H, β- H, Thr), 3.26 (t, J = 7.15 Hz, 2 H, β-H’s, Pro), 2.95 (d, J = 4.9 Hz, 2 H, β-H’s, Asn), 2.88 (d, J = 5.7 Hz, 2 H, β-H’s, Thr), 2.67–2.63 (m, 2 H, β-H’s, Pro), 1.88 (t, J = 5.85 Hz, 2 H, β-H’s, Leu), 1.85–1.79 (m, 2 H, γ-H’s, Pro), 1.47 (d, J = 5.75 Hz, 3 H, β-H’s, Ala), 1.42 (d, J = 5.7 Hz, 3 H, γ-H’s, Thr), 0.98 (d, J = 6.2 Hz, 6 H, δ-H’s, Leu), 0.86–0.78 (m, 1 H, γ-H, Leu). – 13C NMR (CDCl3): δ = 175.2 (C=O, Asn), 174.8 (C=O, Trp), 174.0 (C=O, Thr), 172.6 (C=O, Ala), 171.4 (C=O, Asn), 169.8 (C=O, Gly), 168.9 (C=O, Pro), 165.7 (C=O, Leu), 137.3, 129.6 (2 C, 2-C, and 3-C, indole), 125.5 (5-C, indole), 122.3 (2-C, indole), 119.2 (4-C, indole), 117.4 (6-C, indole), 112.0 (7-C, indole), 110.3 (3-C, indole), 68.4 (C-β, Thr), 59.7 (α-C, Pro), 57.2 (α-C, Thr), 56.0 (α-C, Asn), 55.7 (α-C, Trp), 50.2 (α-C, Ala), 49.5 (α-C, Leu), 48.8 (δ-C, Pro), 44.5 (α-C, Gly), 43.6 (β-C, Asn), 39.2 (β-C, Leu), 33.0 (β-C, Pro), 30.3 (γ-C, Leu), 26.8 (β-C, Trp), 25.0 (γ-C, Pro), 23.7 (2 C, δ-C, Leu), 23.1 (γ-C, Thr), 18.9 (β-C, Ala). – MS (FAB, 70 eV): m/z (%) = 740 (100) [M + 1]+, 712 (11) [740-CO]+, 683 (72) [Thr-Pro-Asn-Ala-Trp-Leu]+, 582 (39) [Pro-Asn-Ala-Trp-Leu]+, 570 (27) [Thr-Pro-Asn-Ala-Trp]+, 554 (14) [582-CO]+, 542 (18) [570-CO]+, 485 (48) [Asn-Ala-Trp-Leu]+, 384 (55) [Thr-Pro-Asn-Ala]+, 372 (29) [Asn-Ala-Trp]+, 356 (14) [384-CO]+, 344 (10) [372-CO]+, 313 (32) [Thr-Pro-Asn]+, 283 (19) [Pro-Asn-Ala]+, 199 (39) [Thr-Pro]+, 171 (10) [199-CO]+, 159 (16) [C10H11N2]+, 130 (9), 116 (11), 115 (8) [Asn]+, 102 (15) [Thr]+, 98 (13) [Pro]+, 87 (11) [C9H7N2O]+, 86 (16) [C6H12N2]+, 74 (14) [C5H7NO]+, 70 (10) [C6H9N]+, 58 (7), 57 (15), 45 (8), 44 (8) [C5H7N2O]+, 43 (16), 42 (12), 30 (9) [CH3N]+, 17 (4), 15 (6) [CH3]+, – C15H19N9O12 (3739): calcld. C 56.82, H 6.68, N 17.04; found C 56.79, H 6.70, N 17.05.
Pharmacological activity studies

Cytotoxicity screening

The synthesized linear and cyclic heptapeptides (7, 8) were subjected to a short term in vitro cytotoxicity study [17] against Dalton’s lymphoma ascites (NCRC 101) and Ehrlich’s ascites carcinoma (NCRC 69) cell lines at 62.5 – 3.91 µg mL⁻¹ using 5-fluorouracil (5-FU) as reference compound. The activity was assessed by determining the percentage inhibition of DLA and EAC cells. CTC50 values were determined by the graphical extrapolation method (Table 1).

Antimicrobial screening

The synthesized linear and cyclic heptapeptides (7, 8) were evaluated for their antimicrobial activity against six bacterial strains Corynebacterium pyogenes (MUMC 73), Staphylococcus aureus (MUMC 377), Bacillus subtilis (MUMC 408), Klebsiella pneumoniae (MUMC 95), Pseudomonas aeruginosa (MUMC 266) and Escherichia coli (MUMC 106), and five fungal strains Candida albicans (MUMC 29), Aspergillus niger (MUMC 77), Ganoderma sp. (MUMC 218), Microsporum audouinii (MUMC 545) and Trichophyton mentagrophytes (MUMC 665) at 50–60 µg mL⁻¹ using the Kirby-Bauer disk diffusion method [18]. MIC values of the test compounds were determined by the tube dilution technique. The solvents DMF and DMSO were used as negative controls, and gatifloxacin and griseofulvin were used as antibacterial and antifungal standards (Tables 2 and 3).

Experimental details of the biological activity test procedures are given in our previously published reports [19].