



King Saud University
Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com



ORIGINAL ARTICLE

Total synthesis and anticancer activity of a cyclic heptapeptide from marine sponge using water soluble peptide coupling agent EDC

Mohanam Anand ^a, Vaithialingam Selvaraj ^{b,*}, Muthukaruppan Alagar ^c

^a Department of Chemistry, Kingston Engineering College, Vellore 59, India

^b Nanotech Research Lab, Department of Chemistry, University College of Engineering Villupuram (A Constituent College of Anna University, Chennai 600 025), Kakuppam, Villupuram 605103, Tamil Nadu, India

^c Department of Chemical Engineering, A.C.Tech Campus, Anna University, Chennai 600 025, Tamil Nadu, India

Received 30 October 2012; accepted 20 May 2014

KEYWORDS

Marine natural product;
Euryjanicin A – cyclic heptapeptide;
Solution phase synthesis;
Anticancer activity

Abstract Present investigation comprises of synthesis of a novel proline containing cyclic heptapeptide – *Euryjanicin A*, which was previously isolated from the marine sponge – *Prosuberites laughlini*. Naturally isolated cyclic heptapeptide was synthesized using solution phase peptide technique. All the coupling reactions were performed at room temperature via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) – coupling reagent and triethylamine (Et₃N) – base. The chemical structure of the finally synthesized cyclic heptapeptide was elucidated using FTIR, ¹H & ¹³C NMR and mass spectral data, as well as elemental analyses. The newly synthesized peptide was subjected to anticancer activity against HeLa (Human Colon) cancer cell line and showed potent anticancer activity.

© 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Naturally isolated cyclic peptides have become increasingly important because of their wide range of pharmacological activities and interesting chemical structures (He et al., 1993;

Wipf, 1995). A variety of active anticancer agents are derived from marine sponges, plants and terrestrial microorganisms (Blunt et al., 2009; Zheng et al., 2011).

Extensive literature survey has been done on anticancer cyclic peptides isolated from marine sponges. Examples of such biologically active peptides are Axinellins (Keyzers and Davies-Coleman, 2005), Kapakahines (Fusetani and Matsunaga, 1993), Microsclerodermins (Randazzo et al., 1998), Polytheonamides (Nakao et al., 1995), Papuamides (Yeung et al., 1996), Stylistins (Qureshi et al., 2000), Hymenamides (Hamada et al., 2005), Wainunuamide (Ford et al., 1999), dominican (Mohammed et al., 2006), etc.

A new proline containing cyclic heptapeptide, *Euryjanicin A* (8) has been isolated from the marine sponge *Prosuberites*

* Corresponding author. Tel./fax: +91 1462 224500.

E-mail addresses: vselva@aucev.edu.in, rajselva_77@yahoo.co.in (V. Selvaraj).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

<http://dx.doi.org/10.1016/j.arabjc.2014.05.037>

1878-5352 © 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Please cite this article in press as: Anand, M. et al., Total synthesis and anticancer activity of a cyclic heptapeptide from marine sponge using water soluble peptide coupling agent EDC. Arabian Journal of Chemistry (2015), <http://dx.doi.org/10.1016/j.arabjc.2014.05.037>

laughlini indigenous to *Puerto Rico*, which showed potent cytotoxic activity against different cancer cell lines (Kobayashi et al., 1993; Tsuda et al., 1993, 1994). Indeed there is no systematic evidence on the naturally isolated cyclic peptide – *Euryjanicin A*. In this context, an attempt has been made to synthesize *Euryjanicin A*-cyclic heptapeptide. The molecule 1) *Cyclo-L-[Phe-Ser-Ile-Pro-Try-Pro-Leu]* was synthesized via solution phase technique of peptide synthesis using *1-ethyl-3-(3-dimethylaminopropyl) carbodiimide* (EDC) as the coupling agent and triethylamine (Et_3N) as the base. The synthesized cyclic heptapeptide was subjected to their anticancer activity against HeLa cancer cell lines using MTT assay.

2. Experimental

2.1. Materials

All the reactions requiring anhydrous conditions were conducted in flame dried apparatus. Melting point was determined using open capillary method and was uncorrected. Solvents and reagents were purified by standard methods. Organic extracts were dried over anhydrous sodium sulphate.

L Amino acids, *1-ethyl-3-(3-dimethylaminopropyl) carbodiimide* (EDC), trifluoroacetic acid (TFA), *p*-nitrophenol (PNP), triethylamine (TEA), di-*tert*-butylpyrocarbonate (Boc_2O) and pyridine ($\text{C}_5\text{H}_5\text{N}$) were obtained from Spectrochem Limited (Mumbai, India). IR spectra were recorded on Thermo Nicolet 330 FTIR spectrophotometer (Thermo Nicolet, USA) using a thin film supported on KBr pellets for the synthesized cyclic heptapeptide – *Euryjanicin A* and Chloroform is used as a solvent for intermediate peptides.

^1H and ^{13}C NMR spectra were recorded on Bruker AC NMR spectrometer (500 and 125 MHz), (Bruker, USA) using CDCl_3 as solvent and tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on Joel Sx 102/DA-6000 mass spectrometer (Jeol, Tokyo, Japan) operating at 70 eV using fast atom bombardment technique. Elemental analyses for all the synthesized peptides were performed on Vario EL III elemental analyzer (Elementar, Germany). Purity of the synthesized cyclic peptide as well as their intermediates was checked by TLC on pre-coated silica gel G plates using $\text{CHCl}_3/\text{MeOH}$ as developing solvent system in different ratios (9:1/8:2 v/v) and brown spots were detected on exposure to iodine vapors in a tightly closed chamber (or) bluish spots were detected in UV chamber.

2.2. General procedure for the preparation of linear, tetra and tripeptide segments (Bodanszky et al., 1984)

Amino acid methyl ester hydrochloride (or) dipeptide methyl ester (10 mmol) was dissolved in chloroform (20 mL). To this, triethylamine (4 mL, 28.7 mmol) was added at 0°C and the reaction mixture was stirred for about 15 min. Boc-amino acid (or) Boc-dipeptide (10 mmol) in CHCl_3 (20 mL) and EDC (10 mmol) was added, while stirring the reaction mixture. After 8 h, the reaction mixture was filtered and the residue was washed with CHCl_3 (30 mL) and added to the filtrate. The reaction filtrate was washed with 5% NaHCO_3 (20 mL) and saturated NaCl (20 mL) solutions. The organic layer was filtered and evaporated in vacuum. The crude product was

recrystallized using the solvent system – chloroform and petroleum ether (1:2 ratio) followed by cooling at 0°C .

2.2.1. *Tert-Butyloxycarbonyl-L-Tryptophanyl-L-Prolyl-L-Leucine-OMe* (5)

Yield 87%, $\text{C}_{28}\text{H}_{40}\text{N}_4\text{O}_6$, 528.29. FTIR (KBr ν cm^{-1}): 3212 (N–H stretch), 2997–2989 (C–H stretch), 2965, 1752 (C=O stretch, ester), 1536 (N–H bend, 2° amide), 1388, 1375 (C–H bend, *tert*-butyl), 1269 (C–O stretch, ester) cm^{-1} . ^1H NMR (500 MHz, CDCl_3): δ 10.1 (1H, br.s, NH of Trp. Ring), 8.63 (1H, br.s, NH of Trp), 8.22 (1H, br.s, NH of Leu), 7.18–7.02 (4H, m, Phenyl ring of Trp), 4.40 (t, 1H, N–CH of Pro), 3.5–3.4 (m, 2H, – CH_2 of Pro), 3.2–3.0 (t, 2H, N– CH_2 of Pro), 2.9 (d, 2H, – CH_2 of Trp) 2.8–2.5 (m, 2H, – CH_2 of Pro), 4.42 (t, 1H, –CH of Leu), 3.63 (s, 3H, – OCH_3 of Leu), 1.8 (d, 2H, – CH_2 of Leu), 1.7 (m, 1H, –CH of Leu), 1.0 (s, 6H, – CH_3 of Leu), 0.9 (s, 9H, – CH_3 of Boc-group). Calcd. $\text{C}_{28}\text{H}_{40}\text{N}_4\text{O}_6$: C, 63.62; H, 7.63; N, 10.60; found: C, 63.65; H, 7.65; N, 10.63%.

2.2.2. *Tert-Butyloxycarbonyl-L-Phenylalanyl-L-Seryl-L-Isoleucyl-L-Proline-OMe* (6)

Yield 85%, $\text{C}_{29}\text{H}_{44}\text{N}_4\text{O}_8$, 576.36. FTIR (KBr ν cm^{-1}): ν 3319 (N–H stretch), 3072, 3058 (Aromatic C–H stretch), 2998, 2987 (Aliphatic C–H stretch), 1751 (C=O stretch, ester), 1541, (N–H bend) cm^{-1} . ^1H NMR (CDCl_3): δ 8.63 (1H, s, –NH of Phe), 8.52 (1H, s, –NH of Ser), 8.43 (1H, s, –NH of Ile), 8.20 (1H, s, –NH of Pro), 7.20–7.02 (5H, m, Ar–H of Phe), 6.70 (1H, s, –OH of Ser), 4.92 (1H, t, –CH of Phe), 4.61 (1H, t, –CH of Ser), 4.53 (1H, d, –CH of Ile), 4.40 (1H, t, N–CH of Pro), 4.15–3.92 (2H, d, – CH_2 of Ser), 3.64 (s, 3H, – OCH_3 of Pro), 3.51–3.40 (2H, m, N– CH_2 of Pro), 2.92 (2H, d, – CH_2 of Phe), 2.50 (1H, m, –CH of Ile), 2.34–2.09 (2H, m, – CH_2 of Pro), 2.02–1.92 (2H, m, – CH_2 of Pro), 1.29 (2H, m, – CH_2 of Ile), 1.06 (3H, d, – CH_3 of Ile), 0.96 (3H, t, – CH_3 of Ile), 0.89 (s, 9H, – CH_3 of Boc group). Calcd. $\text{C}_{29}\text{H}_{44}\text{N}_4\text{O}_8$: C, 60.40; H, 7.69; N, 9.72; found C, 60.41; H, 7.70; N, 9.75%.

2.3. Deprotection of tripeptide unit at the carboxyl terminal

To the solution of tripeptide **5** (4.4 g, 0.01 mol) in $\text{THF-H}_2\text{O}$ (1:1, 36 mL), 0.36 g (0.015 mol) of LiOH was added at 0°C . The reaction mixture was stirred at room temperature for about one hour and then acidified to pH 3.5 using 1 N H_2SO_4 . The aqueous layer was extracted with diethyl ether (3×25 mL). The organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was recrystallized using methanol and petroleum ether (1:2) to afford pure deprotected tripeptide unit.

2.4. Deprotection of tetrapeptide unit at amino terminal

The tetrapeptide **6** (6.36 g, 0.01 mol) was dissolved in CHCl_3 (15 mL) and treated with CF_3COOH (2.28 g, 0.02 mol). The resulting solution was stirred at room temperature for about one hour and 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 through crystallization using CHCl_3 and petroleum ether (1:2) to afford pure deprotected tetrapeptide unit.

2.5. Procedure for the synthesis of the linear heptapeptide unit

The deprotected tetrapeptide (5.36 g, 0.01 mol) was dissolved in tetrahydrofuran (35 mL). To this reaction mixture, triethyl amine (2.8 mL, 0.021 mol) was added at 0 °C and the resulting solution was stirred for about 15 min. Furthermore, the deprotected tripeptide (4.25 g, 0.01 mol) was dissolved in tetrahydrofuran (35 mL) and then finally the coupling agent; EDC (1.26 g, 0.01 mol) was added at 0 °C. After 8 h, the reaction mixture was filtered and the filtrate was washed with 5% NaHCO₃ and saturated NaCl solutions (30 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered, and evaporated in vacuum. The crude product was recrystallized using CHCl₃ and petroleum ether (1:2) by cooling at 0 °C to afford the pure linear heptapeptide.

2.5.1. Tert-Butyloxycarbonyl-L-Phenylalanyl-L-Seryl-L-Isoleucyl-L-Prolyl-L-Tryptophanyl-L-Prolyl-L-Leucine-OMe (7)

Yield 81%, C₅₁H₇₂N₈O₁₁, 972.53. FTIR (KBr ν cm⁻¹): ν 3375 (OH stretch, Tyr), 3132 (N—H stretch), 3069 (C—H stretch, ring), 2992–2984 (C—H stretch), 1758 (C=O stretch, ester), 1541 (N—H bend) cm⁻¹. ¹H NMR (CDCl₃): δ 10.4 (1H, br.s, NH of Trp. Ring), 8.65 (1H, br.s, NH of Trp), 8.62 (1H, s, —NH of Phe), 8.52 (1H, s, —NH of Ser), 8.43 (1H, s, —NH of Ile), 8.24 (1H, br.s, NH of Leu), 8.22 (1H, s, —NH of Pro), 7.20–7.04 (5H, m, Ar—H of Phe), 7.18–7.01 (4H, m, Phenyl ring of Trp.), 6.72 (1H, s, —OH of Ser), 4.93 (1H, t, —CH of Phe), 4.61 (1H, t, —CH of Ser), 4.53 (1H, d, —CH of Ile), 4.42 (2H, t, N—CH of Pro), 4.17–3.91 (2H, d, —CH₂ of Ser), 4.41 (t, 1H, —CH of Leu), 3.59 (s, 3H, —OCH₃ of Leu), 3.50–3.41 (m, 4H, N—CH₂ of Pro), 3.38–2.96 (t, 4H, N—CH₂ of Pro), 2.94 (2H, d, —CH₂ of Phe), 2.91–2.52 (m, 4H, —CH₂ of Pro), 2.53 (1H, m, —CH of Ile), 2.34–2.09 (2H, m, —CH₂ of Pro), 2.02–1.93 (2H, m, —CH₂ of Pro), 1.24 (2H, m, —CH₂ of Ile), 1.07 (3H, d, —CH₃ of Ile), 0.97 (3H, t, —CH₃ of Ile), 1.82 (d, 2H, —CH₂ of Leu), 1.71 (m, 1H, —CH of Leu), 1.01 (s, 6H, —CH₃ of Leu), 0.92 (s, 9H, —CH₃ of Boc-group). Calcd. C₅₁H₇₂N₈O₁₁: C, 62.94; H, 7.46; N, 11.51; found C, 62.95; H, 7.48; N, 11.53%.

2.6. Synthesis of the cyclic heptapeptide 8

The cyclization of linear heptapeptide unit was carried out via p-nitro phenyl ester method of Bodanszky (Dahiya, 2007a,b) with certain modifications. The ester group of the linear segment was removed using Lithium hydroxide and then the p-nitro phenyl ester group was introduced using the subsequent standard method (Dahiya, 2007a,b).

To synthesize *Euryjanicin A* (8), linear heptapeptide unit 7 (4.72 g, 0.005 mol) was deprotected at carboxyl end using LiOH (0.18 g, 0.0075 mol) to get *Boc-L-Phenylalanyl-L-Seryl-L-Isoleucyl-L-Prolyl-L-Tryptophanyl-L-Prolyl-L-Leucine-OMe*. The deprotected heptapeptide unit (4.65 g, 0.005 mol) was dissolved in CHCl₃ (50 mL) at 0 °C. To this solution, p-nitrophenol (0.94 g or 1.23 g, 0.0067 mol) and EDC (0.63 g, 0.005 mol) were added and then the reaction mixture was stirred at room temperature for about 12 h. The reaction mixture was filtered and the filtrate was washed with 10% NaHCO₃ solution (3 × 25 mL) and finally washed with 5% HCl (2 × 30 mL) to get the corresponding p-nitro-phenyl

ester *Boc-L-Phenylalanyl-L-Seryl-L-Isoleucyl-L-Prolyl-L-Tryptophanyl-L-Prolyl-L-Leucine-Opnp*.

The p-nitro phenyl ester peptides (4.2 g 0.004 mol) were dissolved in CHCl₃ (35 mL), and CF₃COOH (0.91 g, 0.008 mol) was added and stirred at room temperature for about one hour. The resulting solution was washed with 10% NaHCO₃ solution (2 × 25 mL). The organic layer was dried over anhydrous Na₂SO₄ to get *L-Phenylalanyl-L-Seryl-L-Isoleucyl-L-Prolyl-L-Tryptophanyl-L-Prolyl-L-Leucine Opnp*, which was dissolved in CHCl₃ (25 mL) and pyridine (2.8 mL, 0.021 mol) was added. The final alkaline solution was kept at 0 °C for about 3 days. Once again, 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 crude cyclized peptide product was crystallized using CHCl₃ and n-hexane (1:2 ratios) to acquire pure cyclic peptide – *Euryjanicin A*.

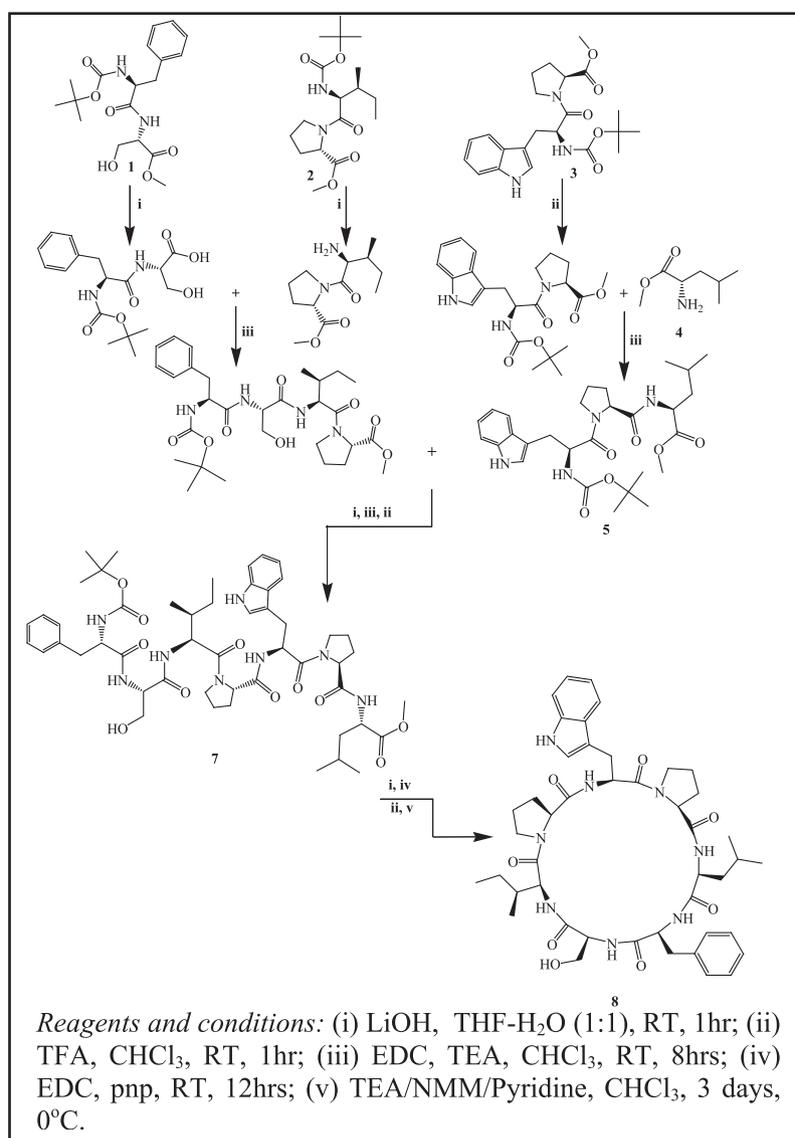
2.6.1. Cyclo (L-Phenylalanyl-L-Seryl-L-Isoleucyl-L-Prolyl-L-Tryptophanyl-L-Prolyl-L-Leucine) (8)

Yield 87%, C₄₅H₆₀N₈O₈, 841.05. FTIR (KBr ν cm⁻¹): ν 3134 (N—H stretch), 3070 (C—H stretch), 2927–2922 (C—H stretch, asym), 2876–2872, 1759 (C=O stretch, ester), 1539 (N—H bend) cm⁻¹. ¹H NMR (CDCl₃): δ 10.3 (1H, br.s, NH of Trp. Ring), 8.63 (1H, br.s, NH of Trp), 8.61 (1H, s, —NH of Phe), 8.53 (1H, s, —NH of Ser), 8.41 (1H, s, —NH of Ile), 8.23 (1H, br.s, NH of Leu), 8.21 (1H, s, —NH of Pro), 7.22–7.05 (5H, m, Ar—H of Phe), 7.19–7.02 (4H, m, Phenyl ring of Trp.), 6.73 (1H, s, —OH of Ser), 4.92 (1H, t, —CH of Phe), 4.62 (1H, t, —CH of Ser), 4.52 (1H, d, —CH of Ile), 4.43 (2H, t, N—CH of Pro), 4.16–3.92 (2H, d, —CH₂ of Ser), 4.42 (t, 1H, —CH of Leu), 3.52–3.40 (m, 4H, N—CH₂ of Pro), 3.39–2.95 (t, 4H, N—CH₂ of Pro), 2.92 (2H, d, —CH₂ of Phe), 2.90–2.53 (m, 4H, —CH₂ of Pro), 2.54 (1H, m, —CH of Ile), 2.31–2.01 (2H, m, —CH₂ of Pro), 2.02–1.93 (2H, m, —CH₂ of Pro), 1.24 (2H, m, —CH₂ of Ile), 1.07 (3H, d, —CH₃ of Ile), 0.97 (3H, t, —CH₃ of Ile), 1.83 (d, 2H, —CH₂ of Leu), 1.72 (m, 1H, —CH of Leu), 1.03 (s, 6H, —CH₃ of Leu). ¹³C NMR (CDCl₃): δ 173.5 (C=O, Ile), 172.6 (C=O, Ser), 172.7, 171.5 (2C, C=O, Pro), 171.9 (C=O, Leu), 170.9 (C=O, Trp), 169.9 (C=O, Phe), 139.5–127.3 (6C, Aromatic carbon of Phe), 126.5–111.1 (7C, Carbons of Trp), 61.8 (1C, —CH₂ OH of Ser), 60.4, 60.7 (2C, α -CH₂ of Pro-1 & Pro-2), 56.9 (1C, —CH of Ile), 56.5 (1C, —CH of Leu), 55.4 (—CH of Trp), 54.9 (—CH of Ser), 52.7 (—CH of Leu), 46.6, 45.8 (2C, δ -CH₂, Pro-1 & Pro-2), 41.2 (—CH₂ of Leu), 31.2, 30.5 (2C, β -CH₂, Pro-1 & Pro-2), 22.6, 21.7 (2C, δ -CH₂, Pro-1 & Pro-2), 37.6 (—CH₂ of Leu), 37.1 (—CH of Ile), 24.7 (—CH₂ of Ile), 22.3, 22.7 (2C, —CH₃ of Leu), 14.6 (1C, —CH₃ of Ile), 10.9 (1C, —CH₃ of Ile); EI/MS *m/z* [EI]: 827 [(M)⁺, 100], Calcd. For C₄₅H₆₀N₈O₈: C, 65.09; H, 7.08; N, 12.08; found: C, 65.11; H, 7.05; N, 12.10%.

2.7. Anticancer activity

2.7.1. MTT [3-(4, 5-dimethylthiazole-2-yl)-2,5-diphenyltetrazoliumbromide] Assay – (Carnichael et al., 1987; Mosmann et al., 1983)

The Human colon cancer cell lines were obtained from ATCC and maintained in DMEM (Hi-Media Laboratories Pvt. Ltd.,



Scheme 1 The synthetic route in the preparation of the cyclic heptapeptide *Euryjanicin A* (8).

Mumbai, India) supplemented with 10% heat inactivated FBS (v/v), streptomycin (100 µg/ml) and penicillin (100 µg/ml). The cell line was maintained at 37 °C with 5% carbon dioxide in CO₂ incubator. The MTT cell proliferation assay was used to evaluate the anticancer activity of cyclic heptapeptide using the Cell Quantification MTT cell viability assay kit (Bio assay Systems). The optical density was measured at 570 nm for each well on the absorbance plate reader. Trypan blue dye exclusion assay was also used to count the number of viable and non-viable HeLa cancer cells in the culture medium after drug treatment. Treatment with Cisplatin at the same concentration served as positive control.

3. Results and discussion

In order to carry out the first total synthesis of *Euryjanicin A*, disconnection strategy was employed. The cyclic heptapeptide molecule was detached into three dipeptide units Boc-L-Phe-L-Ser-OMe (1), Boc-L-Ile-L-Pro-OMe (2), Boc-L-Trp-L-Pro-OMe (3), and single amino acid unit L-Leu-OMe-HCl (4).

The necessary dipeptides (1–3) were prepared by coupling of Boc-amino acids, *viz.* Boc-L-Phe, Boc-L-Ile and Boc-L-Trp with the corresponding amino acid methyl ester hydrochlorides *viz.* L-Ser-OMe-HCl, L-Pro-OMe-HCl and L-Leu-OMe-HCl employing EDC as coupling agent (Williams and Ibrahim, 1981). Ester group of the dipeptide (3) was removed by alkaline hydrolysis with Lithium hydroxide and the deprotected peptide was coupled with amino acid methyl ester hydrochloride (4) using EDC and triethylamine (TEA), to get the tripeptide unit Boc-L-Trp-L-Pro-Leu-OMe (5). Correspondingly, dipeptide (1) after deprotection at the carboxyl end was coupled with dipeptide (2) deprotected at amino terminal, to get the tetrapeptide unit Boc-L-Pro-L-Ser-L-Ile-L-Pro-OMe (6). Subsequent removal of the ester group of tetrapeptide (6) and the Boc group of tripeptide (5), these deprotected units were coupled to get the linear heptapeptide unit Boc-L-Phe-L-Ser-L-Ile-L-Pro-L-Trp-L-Pro-Leu-OMe (7). The methyl ester group of the linear peptide fragment was replaced by *p*-nitro phenyl (PnP) ester group. The Boc-group of the resulting compound was removed using

trifluoroacetic acid and then the deprotected linear fragment was cyclized by the addition of chloroform and pyridine at 0 °C and the reaction mixture was kept for about 3 days in refrigerator to get cyclic heptapeptide (**8**) as described in the Scheme 1.

Infrared spectrum of *Euryjanicin A* showed characteristic medium to strong bands corresponding to the carbonyl stretching at 1652.41 cm^{-1} and NH bending at 1528.35 cm^{-1} , confirmed the coupling reaction of the cyclic heptapeptide. In the Proton NMR spectra of **8**, formation of cyclic heptapeptide was confirmed by the disappearance of singlet at δ 3.58 and δ 1.52 ppm corresponding to the three protons of methyl ester group and nine protons of the Boc *tert*-butyl group. Furthermore, ^1H & ^{13}C NMR spectra of the synthesized cyclic heptapeptide showed characteristic peaks confirming the presence of all sixty methyl, methylene and methine protons and forty five carbon atoms. The mass spectral data clearly confirmed the presence of molecular ion peak (M^+) at m/z 827 for the synthesized compound. All these spectral data of *Euryjanicin A* clearly confirmed the coupling reaction of amino acids and peptides.

The effect of *Euryjanicin A* on the growth of HeLa cancer cells was tested under *invitro* conditions determined using five different concentrations (10, 20, 30, 40 and 50 $\mu\text{g}/\text{mL}$) and the cell survival after 48 h was illustrated in Fig. 1. Cyclic

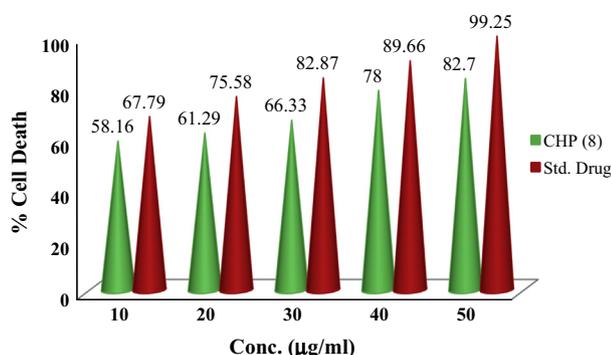


Figure 1 Anticancer activity of *Euryjanicin A* (**8**) cyclic heptapeptide.

Table 1 Effect of cyclic heptapeptide [CHP] (**8**) on HeLa cancer cell lines.

Compd.	Conc. ($\mu\text{g}/\text{mL}$)	Hela cancer cell lines	
		Absorbance (OD)	% Cell death
Cyclic heptapeptide (CHP) 8	50	1.985	82.70
	40	1.872	78.00
	30	1.592	66.33
	20	1.471	61.29
	10	1.396	58.16
Standard (Cisplatin)	50	2.382	99.25
	40	2.152	89.66
	30	1.989	82.87
	20	1.814	75.58
	10	1.627	67.79

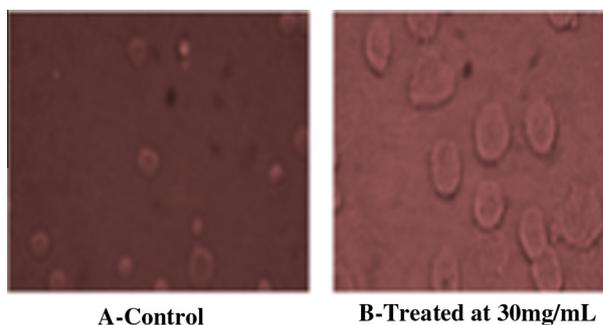


Figure 2 Anticancer activity of *Euryjanicin A* (**8**). (i) A-control and (ii) B-treated at 30 mg/mL .

heptapeptide significantly reduced the growth of HeLa cancer cells and the results are shown in Table 1.

Screening concentrations of 50 and 40 $\mu\text{g}/\text{mL}$ result to a reduction in viable cell of 17.3% and 22% respectively. At 30 $\mu\text{g}/\text{mL}$ screening concentration of *Euryjanicin A* reduced viable cells of 33.67%. Cyclic heptapeptide at 10 and 20 $\mu\text{g}/\text{mL}$ produced potent inhibitory effects [38.71% and 41.29% viable cells] against HeLa cancer cell line. The result of the study clearly indicates the positive control cisplatin to have the significant inhibition of tumor cell growth for the cell line in comparison with the cyclic heptapeptide. Cisplatin at 50 μM produced the considerable inhibitory result on the HeLa cell line resulting in a 99.25% cell death (see Fig. 2).

4. Conclusion

In the present work, we have used an effective coupling reagent, EDC, for the coupling of peptide and amino acids in good to high yields. *Euryjanicin A* was naturally isolated from Caribbean marine peptide and the marine peptide was synthetically prepared in high yield using a new water soluble coupling agent EDC. The synthesized cyclic heptapeptide possessed high cytotoxic activity against HeLa cell lines with respect to standard drug cisplatin. The synthesized cyclic heptapeptide compound was used as effective anticancer drug in future after clinical trials.

Acknowledgments

The authors wish to thank Anna University, University College of Engineering Villupuram and Kingston Engineering Management for providing the research facilities. The authors are thankful to SAIF, IIT Chennai for spectral analysis.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.arabjc.2014.05.037>.

References

Blunt, J.W., Copp, B.R., Hu, W.P., Munro, M.H.G., Northcote, P.T., Prinsep, M.R., 2009. Nat. Prod. Rep. 26, 170–244.

- Bodanszky, M., Bodanszky, A., 1984, first ed.. In: Practice of Peptide Synthesis, vol. 91 Springer-Verlag, New York, p. 131.
- Carnichael, J., DeGraff, W.G., Gazdar, A.F., Minna, J.D., Mitchell, J.B., 1987. *Cancer Res.* 47, 936–942.
- Dahiya, R., 2007a. *Acta Pol. Pharm.* 64, 509–516.
- Dahiya, R., 2007b. *Pak. J. Pharm. Sci.* 20, 317–323.
- Ford, P.W., Gustafson, K.R., McKee, T.C., Shigematsu, N., Maurizi, L.K., Pannell, L.K., Williams, D.E., De Silva, E.D., Lassota, P., Allen, T.M., Van Soest, R., Andersen, R.J., Boyd, M.R., 1999. *J. Am. Chem. Soc.* 121, 5899–5909.
- Fusetani, N., Matsunaga, S., 1993. *Chem. Rev.* 93, 1793–1806.
- Hamada, T., Matsunaga, S., Yano, G., Fusetani, N., 2005. *J. Am. Chem. Soc.* 127, 110–118.
- He, Y.B., Huang, Z., Raynor, K., Reisine, T., Goodman, M., 1993. *J. Am. Chem. Soc.* 115, 8066–8072.
- Keyzers, R.A., Davies-Coleman, M.T., 2005. *Chem. Soc. Rev.* 34, 355–365.
- Kobayashi, J., Tsuda, M., Nakamura, T., Mikami, Y., Shigemori, H., 1993. *Tetrahedron* 49, 2391–2402.
- Mohammed, R., Peng, J., Kelly, M., Hamann, M.T., 2006. *Nat. Prod.* 69, 1739–1744.
- Mosmann, T., 1983. *J. Immunol. Meth.* 65, 55–63.
- Nakao, Y., Yeung, B.K.S., Yoshida, W.Y., Scheuer, P.J., 1995. *J. Am. Chem. Soc.* 117, 8271–8272.
- Qureshi, A., Colin, P.L., Faulkner, D.J., 2000. *Tetrahedron* 56, 3679–3685.
- Randazzo, A., Dal Piaz, F., Orru, S., Debitus, C., Roussakis, C., Pucci, P., Gomez-Paloma, L., 1998. *Eur. J. Org. Chem.*, 2659–2665.
- Tsuda, M., Shigemori, H., Mikami, Y., Kobayashi, J., 1993. *Tetrahedron* 49, 6785–6796.
- Tsuda, M., Sasaki, T., Kobayashi, J., 1994. *Tetrahedron* 50, 4667–4680.
- Williams, Andrew, Ibrahim, T., 1981. *J. Am. Chem. Soc.* 103, 7090–7095.
- Wipf, P., 1995. *Chem. Rev.* 95, 2115–2134.
- Yeung, B.K.S., Nakao, Y., Kinnel, R.B., Carney, J.R., Yoshida, W.Y., Scheuer, P.J., Kelly-Borges, M., 1996. *J. Org. Chem.* 61, 7168–7173.
- Zheng, Lan-Hong, Wang, Yue-Jun, Sheng, Jun, Wang, Fang, Zheng, Yuan, Lin, Xiu-Kun, Sun, Mi, 2011. *Mar. Drugs* 9, 1840–1859.