Bulletin of Pharmaceutical Research 2011;1(1):1-10
An Official Publication of Association of Pharmacy Professionals
ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

RESEARCH ARTICLE

SOLUTION PHASE SYNTHESIS AND BIOEVALUATION OF CORDYHEPTAPEPTIDE B

Rajiv Dahiya[1]* and Hemendra Gautam[2]

[1]*Department of Pharmaceutical Chemistry, Globus College of Pharmacy, Bhopal - 462 045, Madhya Pradesh, India*
[2]*Department of Pharmaceutical Chemistry, NRI Institute of Pharmacy, Bhopal - 462 021, Madhya Pradesh, India.*

**E-mails*: drrajivdahiya@rediffmail.com, hemendra_gautam2002@yahoo.com
Tel.: +91-9630229885, +91-755-4285308.

Received: January 1, 2011 / Revised: January 20, 2011 / Accepted: January 27, 2011

A natural phenylalanine-rich cyclic peptide - cordyheptapeptide B was synthesized by coupling of *N*-methylated tetrapeptide and tripeptide units after proper deprotection at carboxyl and amino terminals followed by cyclization of linear heptapeptide fragment. Required tetrapeptide and tripeptide units were prepared by coupling of Boc-protected dipeptides *viz.* Boc-Phe-*N*(Me)Gly-OH and Boc-Leu-Ile-OH with respective dipeptide methyl ester Pro-*N*(Me)Phe-OMe and amino acid methyl ester hydrochloride *N*(Me)Phe-OMe·HCl. Cyclization of linear polypeptide unit was done by pentafluorophenyl ester method. The structure of synthesized cyclopeptide was elucidated by spectral as well as elemental analysis. The newly synthesized cyclopeptide was evaluated for its antimicrobial and cytotoxic potential, and found to exhibit potent cytotoxicity against *Dalton's lymphoma ascites* (DLA) and *Ehrlich's ascites carcinoma* (EAC) cell lines, in addition to good antidermatophyte activity against *Trichophyton mentagrophytes* and *Microsporum audouinii*. Moreover, cyclopeptide displayed moderate antimicrobial activity against gram negative bacteria *Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

Key words: *Cordyceps* sp, Cyclic heptapeptide, Peptide synthesis, Cytotoxicity, Pharmacological activity, Antibacterial activity.

INTRODUCTION

Past literature has proved the ability of fungi, bacteria, higher plants and marine sponges to produce a wide spectrum of natural products with diverse bioactivities (Haritakun *et al* 2007; Kornsakulkarn *et al* 2009; Jia *et al* 2007; Ebaba *et al* 2010; Daly *et al* 2009). Among these, cyclic peptides with unique structures and wide biological profile, are emerged as vital organic structures which may overcome the problem of resistance towards conventional agents. A novel natural cyclopeptide, cordyheptapeptide B has been earlier, isolated from a fungal strain *Cordyceps* sp and the absolute configuration of the cordyheptapeptide B was indicated by chromatographic analysis of acid hydrolyzate (Isaka *et al* 2007). Keeping in view the biological potential of natural cyclopolypeptides (Dahiya and Pathak, 2006; Pathak and Dahiya, 2003) and in continuation of our efforts toward synthesizing natural cyclic peptides (Dahiya 2007a; 2007b; 2007c; 2008a; 2008b; 2008c; 2008d; Dahiya and Gautam, 2010a; 2010b; 2010c; 2011; Dahiya and Kaur, 2007; 2008; Dahiya and Kumar, 2007; 2008; Dahiya and Pathak, 2006; 2007a; 2007b; Dahiya and Sharma, 2008; Dahiya *et al* 2006; 2009a; 2009b; 2009c) this study was directed toward the synthesis of a novel *N*-methylated cyclic peptide cordyheptapeptide B. The synthesized cyclic heptapeptide was also evaluated for its antibacterial, antifungal and cytotoxic potential.

MATERIALS AND METHODS

General experimental part

Melting point was determined by open capillary method and is uncorrected. L-amino acids,

diisopropylcarbodiimide (DIPC), trifluoroacetic acid (TFA), pentafluorophenol (pfp), pyridine (C_5H_5N), *N*-methylmorpholine (NMM), triethylamine (TEA) and di-*tert*-butyl-pyrocarbonate (Boc_2O) were purchased from Spectrochem Limited (Mumbai, India). IR spectra were recorded on a Shimadzu 8700 FTIR spectrophotometer (Shimadzu, Japan) using a thin film supported on KBr pellets for synthesized cyclic heptapeptide and $CHCl_3$ as solvent for intermediate semisolids. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AC NMR spectrometer (300 MHz) using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. 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 synthesized 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.

General method for the synthesis of dipeptide fragments (1-3)

N-methyl amino acid methyl ester hydrochloride (0.01 mol) was dissolved in $CHCl_3$ (20 ml). To this, NMM (0.021 mol, 2.23 ml) was added at 0°C and the reaction mixture was stirred for 15 min. Boc-amino acid (0.01 mol) in 20 ml of $CHCl_3$ and DIPC (0.01 mol) were added with stirring. After 24 h, the reaction mixture was filtered and the residue was washed with $CHCl_3$ (25 ml) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ and saturated NaCl solutions. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum. The crude product was recrystallized from a mixture of chloroform and petroleum ether (b.p. 60-80°C) followed by cooling at 0°C (**Table 1**).

General method for the synthesis of tri/ tetra/heptapeptide fragments (4-6)

N-methyl amino acid methyl ester hydrochloride or dipeptide/tripeptide methyl ester (0.01 mol) was dissolved in DMF (25 ml). To this, NMM (0.021 mol) was added at 0°C and the reaction mixture was stirred for 15 min. Boc-dipeptide (0.01 mol) in DMF (25 ml) and DIPC (0.01 mol) were added with stirring. Stirring was first done for 1 h at 0-5°C and then further for 24 h at room temperature (RT). After the completion of the reaction, the reaction mixture was diluted with an equal amount of water. The precipitated solid was then, filtered and washed with water, and finally recrystallized from mixture of chloroform and petroleum ether (b.p. 60-80°C) followed by cooling at 0°C to get title compounds (**Table 1**).

Table 1. Physical characterization of the synthesized compounds **1-8**

Compd.	Physical state	Mol. formula (Mol. wt.)	M.p. (°C)	Yield (%)	R_f[a]	$\alpha_{[D]}$[b]	Elemental analysis Calcd. / found (%)		
							C	H	N
1	Semisolid mass	$C_{18}H_{26}N_2O_5$	–	73.3	0.63	–37.1	61.70	7.48	7.99
		(350.41)					61.69	7.46	8.02
2	Semisolid mass	$C_{21}H_{30}N_2O_5$	–	69.2	0.73	–103.2	64.60	7.74	7.17
		(390.48)					64.62	7.75	7.19
3	Semisolid mass	$C_{18}H_{34}N_2O_5$	–	69.9	0.58	–19.6	60.31	9.56	7.81
		(358.48)					60.34	9.55	7.83
4	Semisolid mass	$C_{11}H_{16}NO_2$	–	77.5	0.81	+61.8	68.02	8.30	7.21
		(194.25)					67.99	8.29	7.23
5	Semisolid mass	$C_{33}H_{44}N_4O_7$	–	79.2	0.48*	–52.3	65.11	7.29	9.20
		(608.73)					65.10	7.32	9.22
6	Semisolid mass	$C_{28}H_{45}N_3O_6$	–	73.0	0.77	+98.1	64.71	8.73	8.09
		(519.68)					64.69	8.75	8.10
7	Semisolid mass	$C_{55}H_{77}N_7O_{10}$	–	82.7	0.86*	–72.4†	66.31	7.79	9.84
		(996.25)					66.34	7.80	9.85
8	White solid	$C_{49}H_{65}N_7O_7$ (864.09)	92-93	86.0	0.69*	–81.9‡ (–82.0)	68.11	7.58	11.35
							68.09	7.59	11.38

[a] ($CHCl_3$:MeOH / 9:1); * ($CHCl_3$:MeOH / 8:2); [b] *c*, 0.25 in $CHCl_3$; † *c*, 0.1 in $CHCl_3$; ‡ *c*, 0.05 in $CHCl_3$

Cyclization of the linear heptapeptide fragment (7)

To synthesize cycloheptapeptide **8**, linear peptide unit **7** (0.005 mol, 4.98 g) was deprotected at carboxyl end using lithium hydroxide (0.0075 mol, 0.18 g) to get Boc-Phe-*N*(Me)Gly-Pro-*N*-(Me)Phe-Leu-Ile-*N*-Me Phe-OH. The deprotected heptapeptide unit (0.005 mol, 4.9 g) was now dissolved in 50 ml of chloroform at 0°C. To the above solution, pentafluorophenol (pfp, 0.0067 mol) was added and stirred at room temperature for 12 h. The reaction mixture was filtered and the filtrate was washed with 10% sodium bicarbonate solution (3 × 15 ml) until excess of pentafluorophenol was removed and finally washed with 5% HCl (2 × 10 ml) to get the corresponding pentafluorophenyl ester Boc-Phe-*N*(Me)Gly-Pro-*N*-(Me)Phe-Leu-Ile-*N*-(Me)Phe-Opfp. To this compound (0.004 mol, 4.6 g) dissolved in 35 ml of chloroform, trifluoroacetic acid (0.008 mol, 0.91 g) was added, stirred at room temperature for 1 h and washed with 10% sodium bicarbonate solution (2 × 25 ml). The organic layer was dried over anhydrous sodium sulphate to get the Phe-*N*-(Me) Gly-Pro-*N*-(Me) Phe-Leu-Ile-*N*-(Me) Phe-Opfp which was dissolved in 25 ml of chloroform and triethylamine/*N*-methyl morpholine/pyridine (0.021 mol, 2.21 ml / 2.8 ml /1.61 ml) was added. Then, whole contents were kept in a refrigerator at 0°C for 7 days. The reaction mixture was washed with 10% sodium carbonate solution until the byproduct pentafluorophenol (pfp) was removed completely and finally washing was done with 5% HCl (3 × 15 ml). The organic layer was dried over anhydrous sodium sulphate. Finally, chloroform was distilled off and the crude cyclized product was crystallized using a mixture of chloroform and *n*-hexane to get the pure cyclo(-Phe-*N*(Me)Gly-Pro-*N*-(Me)Phe-Leu-Ile -*N*-(Me) Phe-) **8**.

*tert-Butyloxycarbonyl-phenylalanyl-N-methyl-glycine methyl ester (**1**)*

IR ($CHCl_3$): *v* 3138 (m, -NH str, amide), 2929, 2624 (m, -CH str, asym, CH_2), 2854 (m, -CH str, sym, CH_2), 2869, 2865 (m, -CH str, sym, CH_3), 1746 (s, -C=O str, ester), 1644 (s, -C=O str, 2° amide), 1589, 1478 (m, skeletal bands, arom. ring), 1533 (m, -NH bend, 2° amide), 1465 (m, -CH bend(scissoring), CH_2), 1393, 1377 (s, -CH bend, *tert*-Butyl group), 1272 (s, C-O str, ester), 932 (w, CH_3 rocking, *tert*-Butyl group), 725, 689 (s, -CH bend, out-of-plane, arom. ring) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.53-7.49 (2H, tt, *J* = 6.75, 4.45 Hz, *m*-H's, Phe), 6.96 (1H, br. s, -NH, Phe), 6.92-6.89 (1H, t, *J* = 6.25 Hz, *p*-H, Phe), 6.85-6.83 (2H, dd, *J* = 8.8, 4.15 Hz, *o*-H's, Phe), 5.05-5.01 (1H, q, *J* = 5.5 Hz, α-H, Phe), 4.24 (2H, s, α-H, Gly), 3.72 (3H, s, OCH_3), 3.14-3.12 (2H, d, *J* = 5.6 Hz, β-H's, Phe), 3.05 (3H, s, NCH_3, Gly), 1.53 (9H, s, *tert*-Butyl group) ppm.

*tert-Butyloxycarbonyl-prolyl-N-methyl-phenyl alanine methyl ester (**2**)*

IR ($CHCl_3$): *v* 2998, 2995 (m, -CH str, cyclic CH_2, Pro), 2925 (m, -CH str, asym, CH_2), 2872 (m, -CH str, sym, CH_3), 1744 (s, -C=O str, ester), 1662, 1659 (s, -C=O str, 3° amide), 1588, 1475 (m, skeletal bands, arom. ring), 1392, 1379 (s, -CH bend, *tert*-Butyl group), 1269 (s, C-O str, ester), 930 (w, CH_3 rocking, *tert*-Butyl group), 722, 686 (s, -CH bend, oop, arom. ring) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.15-7.12 (1H, t, *J* = 6.2 Hz, *p*-H, Phe), 7.06-7.02 (2H, tt, *J* = 6.7, 4.5 Hz, *m*-H's, Phe), 6.75-6.73 (2H, dd, *J* = 8.75, 4.2 Hz, *o*-H's, Phe), 5.15-5.12 (1H, t, *J* = 5.15 Hz, α-H, Phe), 4.13-4.10 (1H, t, *J* = 6.85 Hz, α-H, Pro), 3.52 (3H, s, OCH_3), 3.23-3.20 (2H, t, *J* = 7.2 Hz, δ-H's, Pro), 3.07-3.05 (2H, d, *J* = 5.55 Hz, β-H's, Phe), 3.02 (3H, s, NCH_3, Phe), 2.58-2.53 (2H, q, β-H's, Pro), 1.93-1.87 (2H, m, γ-H's, Pro), 1.48 (9H, s, *tert*-Butyl group) ppm.

*tert-Butyloxycarbonyl-leucyl-isoleucine methyl ester (**3**)*

IR ($CHCl_3$): *v* 3139, 3136 (m, -NH str, amide), 2926, 2851 (m, -CH str, asym and sym, CH_2), 2965, 2961, 2868-2865 (m, -CH str, asym and sym, CH_3), 1743 (s, -C=O str, ester), 1645, 1641 (s, -C=O str, 2° amide), 1536, 1532 (m, -NH bend, 2° amide), 1462 (m, -CH bend(scissoring), CH_2), 1391, 1374 (s, -CH bend, *tert*-Butyl group), 1380, 1362 (s, -CH bend, *iso*-propyl group), 1269 (s, C-O str, ester), 934, 921 (w, CH_3 rocking, *tert*-Butyl and *iso*-propyl groups) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 6.45 (1H, br. s, -NH, Ile), 6.02 (1H, br. s, -NH, Leu), 4.30-4.26 (1H, q, *J* = 6.75 Hz, α-H, Leu), 4.22-4.19 (1H, t, *J* = 8.6 Hz, α-H, Ile), 3.52 (3H, s, OCH_3), 1.98-1.85 (3H, m, β-H's, Leu and Ile), 1.71-1.66 (2H, m, γ-H's, Ile), 1.61-1.55 (1H, m, γ-H's, Leu), 1.54 (9H, s, *tert*-Butyl group), 1.03-1.01 (6H, d, *J* = 6.25 Hz, δ-H's, Leu), 0.95-0.93 (3H, d, *J* = 7.75 Hz, δ-H's, Ile), 0.95-0.93 (3H, d, *J* = 5.9 Hz, γ'-H's, Ile) ppm.

*N-methyl-phenylalanine methyl ester hydrochloride (**4**)*

IR ($CHCl_3$): *v* 2625, 2851 (m, -CH str, asym and sym, CH_2), 2869, 2866 (m, -CH str, sym, CH_3),

1742 (s, -C=O str, ester), 1586, 1473 (m, skeletal bands, arom. ring), 1271 (s, C-O str, ester), 728, 687 (s, -CH bend, oop, arom. ring) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.49-9.43 (2H, m, NH_2^+), 7.51-7.48 (1H, t, *J* = 6.3 Hz, *p*-H, Phe), 7.12-7.08 (2H, dd, *J* = 8.75, 4.15 Hz, *o*-H's, Phe), 7.06-7.02 (2H, tt, *J* = 6.7, 4.45 Hz, *m*-H's, Phe), 3.85 (3H, s, OCH_3), 3.65-3.61 (1H, m, α-H, Phe), 3.28-3.26 (2H, d, *J* = 5.55 Hz, β-H's, Phe), 2.52 (3H, s, *N*CH_3) ppm.

*tert-Butyloxycarbonyl-phenylalanyl-N-methyl-glycyl-prolyl-N-methyl-phenylalanine methyl ester (**5**)*

IR ($CHCl_3$): *v* 3136 (m, -NH str, amide), 2999, 2996 (m, -CH str, cyclic CH_2, Pro), 2927, 2622, 2854-2851 (m, -CH str, asym and sym, CH_2), 2870, 2866 (m, -CH str, sym, CH_3), 1742 (s, -C=O str, ester), 1664-1659, 1642 (s, -C=O str, 3° and 2° amide), 1587, 1479 (m, skeletal bands, arom. rings), 1532 (m, -NH bend, 2° amide), 1391, 1376 (s, -CH bend, *tert*-Butyl group), 1270 (s, C-O str, ester), 933 (w, CH_3 rocking, *tert*-Butyl group), 726-722, 690, 687 (s, -CH bend, out-of-plane, arom. ring) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.52-7.48 (2H, tt, *J* = 6.8, 4.45 Hz, *m*-H's, Phe-1), 7.15-7.12 (1H, t, *J* = 6.2 Hz, *p*-H, Phe-2), 7.05-7.01 (2H, tt, *J* = 6.75, 4.5 Hz, *m*-H's, Phe-2), 6.93-6.90 (1H, t, *J* = 6.25 Hz, *p*-H, Phe-1), 6.86-6.84 (2H, dd, *J* = 8.8, 4.15 Hz, *o*-H's, Phe-1), 6.75-6.73 (2H, dd, *J* = 8.75, 4.2 Hz, *o*-H's, Phe-2), 6.51 (1H, br. s, -NH, Phe-1), 5.39-5.36 (1H, t, *J* = 5.2 Hz, α-H, Phe-2), 4.87-4.83 (1H, q, *J* = 5.55 Hz, α-H, Phe-1), 4.45-4.42 (1H, t, *J* = 6.9 Hz, α-H, Pro), 3.89 (2H, s, α-H, Gly), 3.59-3.56 (2H, t, *J* = 7.15 Hz, δ-H's, Pro), 3.54 (3H, s, OCH_3), 3.17-3.11 (4H, m, β-H's, Phe-1 and Phe-2), 2.99 (3H, s, *N*CH_3, Phe), 2.94 (3H, s, *N*CH_3, Gly), 2.69-2.64 (2H, q, β-H's, Pro), 1.96-1.89 (2H, m, γ-H's, Pro), 1.52 (9H, s, *tert*-Butyl group) ppm.

*tert-Butyloxycarbonyl-leucyl-isoleucyl-N-methyl-phenylalanine methyl ester (**6**)*

IR ($CHCl_3$): *v* 3136, 3132 (m, -NH str, amide), 2929, 2854 (m, -CH str, asym and sym, CH_2), 2967-2963, 2869, 2863 (m, -CH str, asym and sym, CH_3), 1745 (s, -C=O str, ester), 1667, 1644-1641 (s, -C=O str, 3° and 2° amide), 1588, 1471 (m, skeletal bands, arom. ring), 1539, 1535 (m, -NH bend, 2° amide), 1466 (m, -CH bend (scissoring), CH_2), 1393, 1375 (s, -CH bend, *tert*-Butyl group), 1379, 1364 (s, -CH bend, *iso*-propyl group), 1274 (s, C-O str, ester), 933, 920 (w, CH_3 rocking, *tert*-Butyl and *iso*-propyl groups), 729, 685 (s, -CH bend, oop, arom. ring) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.15-7.12 (1H, t, *J* = 6.25 Hz, *p*-H, Phe), 7.10 (1H, br. s, -NH, Ile), 7.05-7.01 (2H, tt, *J* = 6.75, 4.45 Hz, *m*-H's, Phe), 6.75-6.72 (2H, dd, *J* = 8.75, 4.2 Hz, *o*-H's, Phe), 6.04 (1H, br. s, -NH, Leu), 4.45-4.42 (1H, t, *J* = 5.15 Hz, α-H, Phe), 4.37-4.34 (1H, t, *J* = 8.55 Hz, α-H, Ile), 4.20-4.16 (1H, q, *J* = 6.8 Hz, α-H, Leu), 3.55 (3H, s, OCH_3), 3.08-3.06 (2H, d, *J* = 5.6 Hz, β-H's, Phe), 3.02 (3H, s, *N*CH_3), 2.05-1.96 (3H, m, β-H's, Leu and Ile), 1.66-1.59 (2H, m, γ-H's, Ile), 1.58-1.53 (1H, m, γ-H's, Leu), 1.51 (9H, s, *tert*-Butyl group), 1.03-1.01 (3H, d, *J* = 5.85 Hz, γ'-H's, Ile), 1.01-0.99 (6H, d, *J* = 6.3 Hz, δ-H's, Leu), 0.96-0.94 (3H, d, *J* = 7.8 Hz, δ-H's, Ile) ppm.

*tert-Butyloxycarbonyl-phenylalanyl-N-methyl - glycyl-prolyl-N-methyl-phenylalanyl-leucyl-isoleucyl-N-methyl-phenylalanine methyl ester (**7**)*

IR ($CHCl_3$): *v* 3138-3133 (m, -NH str, amide), 2998, 2995 (m, -CH str, cyclic CH_2, Pro), 2928-2623, 2856-2852 (m, -CH str, asym and sym, CH_2), 2966, 2869, 2865 (m, -CH str, asym and sym, CH_3), 1747 (s, -C=O str, ester), 1668-1663, 1645, 1641 (s, -C=O str, 3° and 2° amide), 1589-1586, 1479-1475 (m, skeletal bands, arom. rings), 1536, 1533-1530 (m, -NH bend, 2° amide), 1468 (m, -CH bend(scissoring), CH_2), 1395, 1374 (s, -CH bend, *tert*-Butyl group), 1378, 1365 (s, -CH bend, *iso*-propyl group), 1268 (s, C-O str, ester), 935, 919 (w, CH_3 rocking, *tert*-Butyl and *iso*-propyl groups), 729-725, 689-686 (s, -CH bend, oop, arom. ring) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.78 (1H, br. s, -NH, Leu), 7.59 (1H, br. s, -NH, Ile), 7.51-7.47 (2H, tt, *J* = 6.75, 4.5 Hz, *m*-H's, Phe-1), 7.24-7.19 (2H, tt, *J* = 6.8, 4.45 Hz, *m*-H's, Phe-2), 7.16-7.13 (1H, t, *J* = 6.3 Hz, *p*-H, Phe-3), 7.06-7.01 (3H, m, *p*-H, Phe-2 and *m*-H's, Phe-3), 6.92-6.89 (1H, t, *J* = 6.25 Hz, *p*-H, Phe-1), 6.87-6.85 (2H, dd, *J* = 8.75, 4.2 Hz, *o*-H's, Phe-1), 6.76-6.69 (4H, m, *o*-H's, Phe-2 and Phe-3), 6.45 (1H, br. s, -NH, Phe-1), 5.12-5.09 (1H, t, *J* = 5.15 Hz, α-H, Phe-2), 4.65-4.61 (1H, q, *J* = 5.6 Hz, α-H, Phe-1), 4.48-4.45 (1H, t, *J* = 6.85 Hz, α-H, Pro), 4.42-4.39 (1H, t, *J* = 5.2 Hz, α-H, Phe-3), 4.38-4.34 (1H, q, *J* = 6.75 Hz, α-H, Leu), 4.23-4.20 (1H, t, *J* = 8.6 Hz, α-H, Ile), 3.87 (2H, s, α-H, Gly), 3.65-3.62 (2H, t, *J* = 7.2 Hz, δ-H's, Pro), 3.53 (3H, s, OCH_3), 3.20 (3H, s, *N*CH_3, Gly), 3.13-3.07 (4H, m, β-H's, Phe-1 and Phe-3), 3.05 (3H, s, *N*CH_3, Phe-3), 3.02 (3H, s, *N*CH_3, Phe-2), 2.99-2.97 (2H, d, *J* = 5.6 Hz, β-H's, Phe-2), 2.71-2.67 (2H, q, β-H's, Pro), 2.06-2.01 (1H, m, β-H, Ile), 1.96-1.89 (2H, m, γ-H's, Pro), 1.83-1.80 (2H, t, β-H's, Leu), 1.64-1.59 (2H, m, γ-H's, Ile), 1.54 (9H, s, *tert*-Butyl group), 1.49-1.43 (1H, m, γ-H's, Leu), 1.04-1.02 (3H, d, *J* = 5.9 Hz, γ'-

H's, Ile), 1.00-0.98 (6H, d, *J* = 6.25 Hz, *δ*-H's, Leu), 0.95-0.93 (3H, d, *J* = 7.75 Hz, *δ*-H's, Ile) ppm.

*Cyclo(-phenylalanyl-N-methyl-glycyl-prolyl-N-methyl-phenylalanyl-leucyl-isoleucyl-N-methyl-phenylalanyl-) (**8**)*

Yield 87.3% (3.77 g, pyridine), 74.1% (3.2 g, NMM), 66.0% (2.85 g, TEA); IR (KBr): *ν* 3139, 3137-3132 (m, -NH str, amide), 2999-2995 (m, -CH str, cyclic CH_2, Pro), 2926, 2622, 2855-2852 (m, -CH str, asym and sym, CH_2), 2969, 2868-2864 (m, -CH str, asym and sym, CH_3), 1669, 1665, 1646-1640 (s, -C=O str, 3° and 2° amide), 1588, 1585, 1477, 1473 (m, skeletal bands, arom. rings), 1538, 1533-1529 (m, -NH bend, 2° amide), 1381, 1366 (s, -CH bend, *iso*-propyl group), 921 (w, CH_3 rocking, *iso*-propyl groups), 728, 722, 688-685 (s, -CH bend, oop, arom. ring) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.58 (1H, br. s, -NH, Phe-1), 9.12 (1H, br. s, -NH, Leu), 9.09 (1H, br. s, -NH, Ile), 7.25-7.20 (4H, m, *m*-H's, Phe-2 and Phe-3), 7.19-7.15 (2H, tt, *J* = 6.85, 4.5 Hz, *m*-H's, Phe-1), 7.06-7.01 (2H, m, *p*-H, Phe-2 and Phe-3), 6.99-6.96 (1H, t, *J* = 6.25 Hz, *p*-H, Phe-1), 6.84-6.82 (2H, dd, *J* = 8.75, 4.15 Hz, *o*-H's, Phe-1), 6.75-6.69 (4H, m, *o*-H's, Phe-2 and Phe-3), 5.70-5.67 (1H, t, *J* = 5.15 Hz, *α*-H, Phe-3), 5.31-5.28 (1H, t, *J* = 8.55 Hz, *α*-H, Ile), 5.28 (2H, s, *α*-H, Gly), 5.10-5.06 (1H, q, *J* = 6.8 Hz, *α*-H, Leu), 4.41-4.38 (1H, t, *J* = 6.9 Hz, *α*-H, Pro), 4.36-4.31 (2H, m, *α*-H's, Phe-1 & Phe-2), 3.63-3.60 (2H, t, *J* = 7.15 Hz, *δ*-H's, Pro), 3.05 (3H, s, *N*CH_3, Gly), 2.90 (3H, s, *N*CH_3, Phe-3), 2.86 (3H, s, *N*CH_3, Phe-2), 2.70-2.66 (2H, q, *β*-H's, Pro), 2.63-2.58 (4H, m, *β*-H's, Phe-2 and Phe-3), 2.57-2.53 (2H, d, *J* = 5.55 Hz, *β*-H's, Phe-1), 1.88-1.85 (2H, t, *β*-H's, Leu), 1.83-1.78 (2H, m, *γ*-H's, Pro), 1.66-1.61 (2H, m, *γ*-H's, Ile), 1.47-1.42 (1H, m, *β*-H, Ile), 0.99-0.97 (6H, d, *J* = 6.3 Hz, *δ*-H's, Leu), 0.96-0.94 (3H, d, *J* = 5.85 Hz, *γ'*-H's, Ile), 0.93-0.91 (3H, d, *J* = 7.75 Hz, *δ*-H's, Ile), 0.86-0.81 (1H, m, *γ*-H's, Leu) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ 174.1, 172.8, 171.6 (3C, C=O, Ile, Phe-1 and Phe-2), 171.0, 170.7, 170.3 (3C, C=O, Phe-3, Leu and Pro), 169.2 (C=O, Gly), 139.3 (*γ*-C, Phe-2), 139.0, 131.9 (2C, *γ*-C's, Phe-1 & Phe-3), 130.5 (2C, *m*-C's, Phe-2), 129.8 (2C, *m*-C's, Phe-3), 129.2 (2C, *o*-C's, Phe-1), 128.8 (2C, *o*-C's, Phe-3), 128.1 (2C, *o*-C's, Phe-2), 127.7 (2C, *m*-C's, Phe-1), 127.3, 127.0 (2C, *p*-C's, Phe-2 and Phe-3), 126.6 (*p*-C, Phe-1), 61.2, 59.8 (2C, *α*-C's, Phe-3 and Phe-2), 54.9, 54.4 (2C, *α*-C's, Leu and Ile), 52.7 (*α*-C, Pro), 47.7, 47.3 (2C, *α*-C's, Phe-1 and Gly), 45.6 (*δ*-C, Pro), 44.0 (*β*-C, Leu), 41.5 (*β*-C, Phe-1), 38.8, 38.4, 35.6 (3C, *β*-C's, Phe-2, Phe-3 and Ile), 35.1, 33.9, 33.4 (3C, *N*CH_3, Phe-2, Gly and Phe-3), 30.7 (*β*-C, Pro), 26.9 (*γ*-C, Leu), 24.4, 23.6 (2C, *γ*-C's, Ile and Pro), 22.7 (2C, *δ*-C's, Leu), 16.4 (*γ'*-C, Ile), 10.1 (*δ*-C, Ile) ppm; FAB MS (*m/z*, relative intensity): 865 [(M + H)$^+$, 100], 837 [(865–CO)$^+$, 12.7], 794 [(Pro-*N*(Me)Phe-Leu-Ile-*N*(Me)Phe-Phe)$^+$, 38.5], 766 [(794–CO)$^+$, 15.3], 751 [(Ile-*N*(Me)Phe-Phe-*N*(Me)Gly-Pro-*N*(Me)Phe)$^+$, 33.2], 723 [(751–CO)$^+$, 11.6], 703 [(Phe-*N*(Me)Gly-Pro-*N*(Me)Phe-Leu-Ile)$^+$, 22.9], 675 [(703–CO)$^+$, 13.7], 646 [(Pro-*N*(Me)Phe-Leu-Ile-*N*(Me)Phe)$^+$, 76.8], 618 [(646–CO)$^+$, 21.5], 590 [(Ile-*N*(Me)Phe-Phe-*N*(Me)Gly-Pro)$^+$, 48.6], 562 [(590–CO)$^+$, 16.5], 493 [(Ile-*N*(Me)Phe-Phe-*N*(Me)Gly)$^+$, 27.4], 485 [(Pro-*N*(Me)Phe-Leu-Ile)$^+$, 52.4], 477 [(Phe-*N*(Me)Gly-Pro-*N*(Me)Phe)$^+$, 37.2], 465 [(493–CO)$^+$, 10.2], 457 [(485–CO)$^+$, 11.9], 449 [(477–CO)$^+$, 11.2], 422 [(Ile-*N*(Me)Phe-Phe)$^+$, 22.5], 394 [(422–CO)$^+$, 14.8], 372 [(Pro-*N*(Me)Phe-Leu)$^+$, 60.2], 344 [(372–CO)$^+$, 14.8], 316 [(Phe-*N*(Me)Gly-Pro)$^+$, 19.9], 288 [(316–CO)$^+$, 9.6], 275 [(Ile-*N*(Me)Phe)$^+$, 40.3], 259 [(Pro-*N*(Me)Phe)$^+$, 29.6], 247 [(275–CO)$^+$, 15.1], 231 [(259–CO)$^+$, 10.2], 219 [(Phe-*N*(Me)Gly)$^+$, 31.3], 191 [(219–CO)$^+$, 10.9], 148 [(Phe)$^+$, 14.7], 120 [($C_8H_{10}N$)$^+$, 8.9], 114 [(Ile)$^+$, 19.6], 98 [(Pro)$^+$, 13.3], 91 [(C_7H_7)$^+$, 17.3], 86 [($C_5H_{12}N$)$^+$, 11.4], 77 [(C_6H_5)$^+$, 12.7], 57 [(C_4H_9)$^+$, 14.2], 43 [(C_3H_7)$^+$, 9.8], 42 [(C_3H_6)$^+$, 8.7], 29 [(C_2H_5)$^+$, 7.9], 15 [(CH_3)$^+$, 13.9].

Biological activity studies

Antibacterial screening

The synthesized peptide derivatives were screened for their antibacterial activity against two gram positive bacterial strains *B. subtilis*, *S. aureus* and two gram negative bacterial strains *P. aeruginosa*, *K. pneumonia* (Bauer *et al* 1966). MIC values of test compounds were determined by Tube Dilution Technique. All the synthesized compounds were dissolved separately to prepare a stock solution of 1 mg/ml using DMF. Stock solution was aseptically transferred and suitably diluted with sterile broth medium to contain seven different concentrations of each test compound in different test tubes. All the tubes were inoculated with one loopful of one of the test bacterium. The process was repeated with different test bacteria and different samples. Tubes inoculated with bacterial cultures were incubated at 37°C for 18 h and the presence/absence of growth of the bacteria was observed. From these results, MIC of each test compound was determined against each test bacterium. A spore suspension in sterile distilled water was prepared from 5 days old culture of the test bacteria growing on nutrient broth

media. About 20 ml of the growth medium was transferred into sterilized petri plates and inoculated with 1.5 ml of the spore suspension (spore concentration – 6×10^4 spores/ml). 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 were placed on three portions together with one disc with reference drug ciprofloxacin and a disk impregnated with the solvent (DMF) as negative control. Test sample and reference drugs were tested at the concentration of 12.5-6 *µ*g/ml. The petri plates inoculated with bacterial cultures were incubated at 37°C for 18 h. Diameters of the zones of inhibition (in mm) were measured and the average diameters for test sample were calculated of triplicate sets. The diameters obtained for the test sample were compared with that produced by the standard drug – gatifloxacin (**Table 2**).

Antifungal screening

Serial plate dilution method was used for the evaluation of antifungal activity against diamorphic fungal strain *C. albicans* and three other fungal strains, including *A. niger* and two dermatophytes *M. audouinii* and *T. mentagrophytes* (Khan, 1997). MIC values were determined by employing the same technique as used for antibacterial studies using DMSO instead of DMF and tubes inoculated with fungal cultures were incubated at 37°C for 48 h. After incubation, the presence/absence of growth of the fungi was observed and MIC of test compounds was determined against each test fungus. A spore suspension in normal saline was prepared from culture of the test fungi on sabouraud's broth media. After transferring growth medium, petri plates were inoculated with spore suspension. After drying, wells were made using an agar punch and test samples, reference drug and negative control (DMSO) were placed in labeled wells in each petri plate. Test samples and griseofulvin were tested at 12.5-6 *µ*g/ml concentration. Petri plates inoculated with fungal cultures were incubated at 37°C for 48 h. Antifungal activity was determined by measuring diameter of inhibition zone for triplicate sets. Activity of compounds was compared with griseofulvin (**Table 2**).

Cytotoxicity screening

Synthesized cyclopeptide was subjected to short term *in vitro* cytotoxicity study at 62.5-3.91 *µ*g/ml using 5-fluorouracil (5-FU) as reference compound (Kuttan *et al* 1985). Activity was assessed by determining the percentage inhibition of DLA and EAC cells. Both cells were cultured in the peritoneal cavity of healthy albino mice by injecting the suspension of cells (1×10^6 cells/ml) intraperitoneally. After 15-20 days, cells were withdrawn from the peritoneal cavity of the mice with help of sterile syringe and counted using haemocytometer and adjusted to 1×10^6 cells/ml. Different dilution of all selected compounds ranging from 62.5–31.25 *µ*g/ml 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 (1×10^6 cells/ml) and EAC cells (1×10^6 cells/ml). Resulted suspensions were incubated at 37°C for 3 h. After 3 h, tryphan blue dye exclusion test was performed and percentage growth inhibition was calculated. CTC_{50} values were determined by graphical extrapolation method. Controls were also tested at 62.5-3.91 *µ*g/ml against both cell lines. The results of cytotoxicity studies are listed in the **Table 3**.

RESULTS

Chemistry

In the present study, disconnection strategy was employed to carry out the first total synthesis of cyclic heptapeptide **8**. The cyclopeptide molecule was split into three dipeptide units Boc-Phe-*N*(Me)Gly-OMe **1**, Boc-Pro-*N*(Me)Phe-OMe **2**, Boc-Leu-Ile-OMe **3** and *N*(Me)Phe-OMe·HCl **4**. *N*-methylation of boc-protected phenylalanine methyl ester was done by treatment with methyliodide and sodium hydride as per the literature method (Das and Himaja, 2010). Required dipeptide units **1-3** were prepared by coupling of Boc-amino acids viz. Boc-L-Phe, Boc-L-Pro and Boc-L-Leu with corresponding amino acid methyl ester hydrochlorides such as L-*N*(Me)Gly-OMe·HCl, L-*N*(Me)Phe-OMe·HCl and L-Ile-OMe·HCl employing DIPC as coupling agent (Bodanszky and Bodanszky, 1984). Ester group of **1** was removed by alkaline hydrolysis with LiOH and Boc-group of **3** was removed using TFA. Both the deprotected units were coupled using DIPC and NMM as base, to get tetrapeptide unit Boc-Phe-*N*(Me)Gly-Pro-*N*(Me)Phe-OMe **5**. Similarly, **3** after deprotection at carboxyl terminal, was coupled with **4** to get tripeptide Boc-Leu-Ile-*N*(Me)Phe-OMe **6**. After removal of ester and Boc groups of **5** and **6**, deprotected units were coupled to get linear heptapeptide Boc-Phe-*N*(Me)Gly-Pro-*N*(Me)Phe-Leu-Ile-*N*(Me) Phe-OMe **7**. The ester group of linear fragment

was removed using LiOH and pentafluorophenyl (pfp) ester group was introduced. The Boc-group was removed using TFA and deprotected linear fragment was now cyclized by keeping the whole contents at 0°C for 7 days in presence of catalytic amount of base to get the cyclic product **8** (**Figure A**). Structure of the newly synthesized cyclic heptapeptide and intermediates linear di/tri/ tetra/hepta peptides were confirmed by IR, ^{1}H NMR as well as elemental analysis. In addition, ^{13}C NMR and mass spectra were recorded for the cyclopeptide.

Pharmacology

Antimicrobial activity results for cyclopeptide **8** against Gram-positive bacteria *B. subtilis* and *S. aureus,* Gram-negative bacteria *P. aeruginosa* and *K. pneumoniae,* cutaneous fungi *M. audouinii* and *T. mentagrophytes,* diamorphic fungi *C. albicans* and *A. niger* by disk diffusion method, are tabulated in **Table 2**. The *in vitro* cytotoxicity study results against DLA and EAC cell lines are tabulated in **Table 3**.

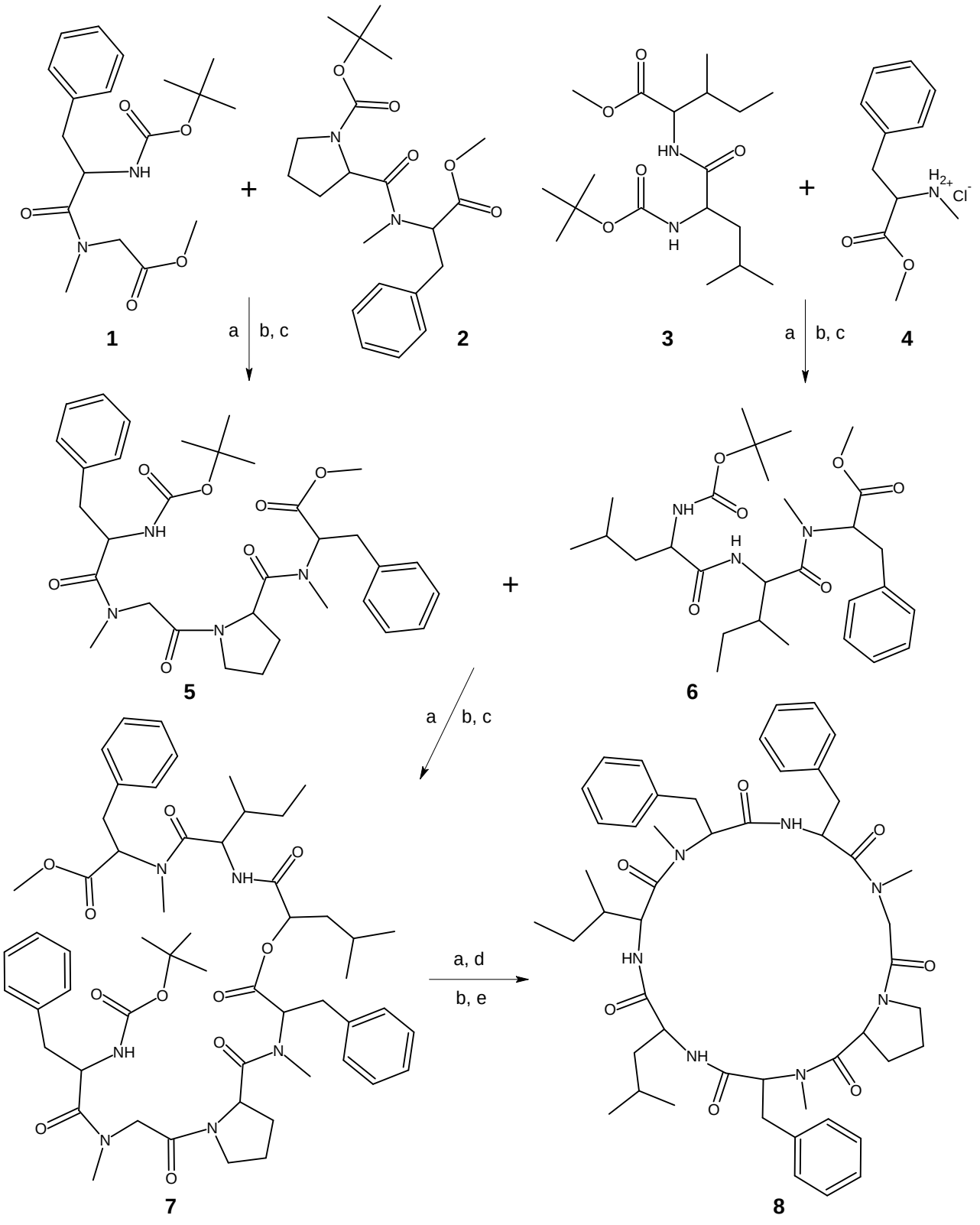
DISCUSSION

Synthesis of cycloheptapeptide was carried out successfully with good yield and pyridine was proved to be a yield effective base for cyclization of linear heptapeptide fragment. Structure of cyclic heptapeptide was confirmed by spectral as well as elemental analysis. Cyclization of linear peptide fragment was indicated by disappearance of absorption bands at 1747, 1268 and 1395, 1374 cm^{-1} (-C=O str of ester and -CH deformation of *tert*-Butyl group) in IR spectra of the compound **8**. Formation of cyclopeptide was further confirmed by disappearance of singlet at 3.53 and 1.54 ppm corresponding to three protons of methyl ester group and nine protons of *tert*-Butyl group of Boc, in ^{1}H NMR spectrum of compound **8**. Furthermore, ^{1}H NMR and ^{13}C NMR spectra of synthesized cyclic heptapeptide showed characteristic peaks confirming presence all the 65 protons and 49 carbon atoms. Presence of $(M + 1)^{+}$ ion peak at *m/z* 865 corresponding to the molecular formula $C_{49}H_{65}N_7O_7$ in mass spectra of compound **8**, along with other fragment ion peaks resulting from cleavage at 'Leucyl–Isoleucyl', 'Glycyl–Prolyl', and '*N*(Me)-Phenylalanyl–Phenylalanyl' amide bond levels, showed exact sequence of attachment of all the seven amino acid moieties in a chain. In addition, elemental analysis of compound **8** afforded values (±0.03) strictly in accordance to the molecular composition. Synthesized cyclic peptide exhibited potent cytotoxic activity against DLA and EAC cell lines with CTC_{50} values of 7.4 and 13.56 M respectively, in comparison to standard drug 5-fluorouracil (5-FU) (CTC_{50} values - 37.36 and 90.55 μM). Compound **8** also showed good activity against pathogenic dermatophytes *T. mentagrophytes* and *M. audouinii*, in comparison to standard drug - griseofulvin. Moreover, synthesized cyclopeptide displayed moderate level of activity against Gram-negative bacteria. Gram-positive bacteria were found to be resistant towards the compound **8** in comparison to the sensitive Gram-negative bacteria. On passing toxicity tests, the synthesized *N*-methylated cyclohepta peptide **8** may prove good candidate for clinical studies and can be the new cytotoxic and antidermatophyte drug of future.

Table 2. Antimicrobial activity data for synthesized cyclopeptide **8**

Compd.	Diameter of zone of inhibition (mm)							
	Bacterial strains				Fungal strains			
	B. subtilis	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*	*M. audouinii*	*A. niger*	*T. mentagrophytes*
8	–	–	17(6)[a]	19(6)	9(12.5)	20(6)	–	22(6)
Control	–	–	–	–	–	–	–	–
Gatifloxacin	19(12.5)	28(6)	24(6)	25(6)	–	–	–	–
Griseofulvin	–	–	–	–	20(6)	17(6)	18(12.5)	20(6)

[a] Values in bracket are MIC values (μg/ml)



a = LiOH, THF:H_2O (1:1), RT, 1 h; b = TFA, $CHCl_3$, RT, 1 h; c = DIPC, NMM, $CHCl_3$/DMF, RT, 24 h; d = DIPC, pfp, RT, 12 h; e = TEA/NMM/pyridine, $CHCl_3$, 7 days, 0 ^oC.

Figure A. Synthetic pathway for cordyheptapeptide B (**8**)

Table 3. Cytotoxic activity data for synthesized cyclopeptide **8**

Compd.	Conc. (μg/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)
8	62.5	0	38	100.0		0	28	100.0	
	31.25	4	34	89.47		3	25	89.29	
	15.63	7	31	81.57	7.4	11	17	60.71	13.56
	7.81	15	23	60.53		17	11	39.29	
	3.91	26	12	31.58		25	3	10.71	
Control	62.5	38	0	–		28	0	–	
	31.25	38	0	–		28	0	–	
	15.63	38	0	–	–	28	0	–	–
	7.81	38	0	–		28	0	–	
	3.91	38	0	–		28	0	–	
Standard (5-FU)	62.5	0	38	100.0		0	28	100.0	
	31.25	0	38	100.0		0	28	100.0	
	15.63	10	28	73.68	37.36	11	17	60.71	90.55
	7.81	13	25	65.79		19	9	32.14	
	3.91	22	16	42.11		23	5	17.86	

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

ACKNOWLEDGEMENT

The authors are thankful to Sophisticated Analytical Instrumentation Laboratory, School of Pharmaceutical Sciences, Rajiv Gandhi Proudyogiki Vishwavidyalaya (RGPV), Bhopal, Madhya Pradesh, India for the spectral analysis.

REFERENCES

Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Path*. 1966;45(4):493-6.

Bodanszky M, Bodanszky A. The Practice of Peptide Synthesis, Springer-Verlag: New York, 1984; 78-143.

Dahiya R. Synthesis, characterization and biological evaluation of a glycine-rich peptide - cherimola-cyclopeptide E. *J. Chil. Chem. Soc.* 2007a;52(3):1224-9. [DOI: 10.4067/S0717-97072007000300000]

Dahiya R. Synthesis of a phenylalanine-rich peptide as potential anthelmintic and cytotoxic agent. *Acta Pol. Pharm.* 2007b;64(6):509-16.

Dahiya R. Synthetic and pharmacological studies on longicalycinin A. *Pak. J. Pharm. Sci.* 2007c;20(4):317-23.

Dahiya R. Synthesis, spectroscopic and biological investigation of cyclic octapeptide: cherimolacyclo-peptide G. *Turk. J. Chem.* 2008a;32(2):205-15.

Dahiya R. Total synthesis and biological potential of psammosilenin A', *Arch. Pharm. Chem. Life Sci.* 2008b;341(8):502-9. [DOI: 10.1002/ardp.200800006]

Dahiya R. Synthetic studies on a cyclic hexapeptide from *Dianthus superbus*. *Chem. Pap.* 2008c;62(5):527-35. [DOI: 10.2478/s11696-008-0052-9]

Dahiya R. Synthesis and *in vitro* cytotoxic activity of a natural peptide of plant origin. *J. Iran. Chem. Soc.* 2008d;5(3):445-52.

Dahiya R, Gautam H. Total synthesis and antimicrobial activity of a natural cycloheptapeptide of marine-origin. *Mar. Drugs* 2010a;8(8):2384-94. [DOI: 10.3390/md8082384]

Dahiya R, Gautam H. Synthetic and pharmacological studies on a natural cyclopeptide from *Gypsophila arabica*. *J. Med. Plant. Res.* 2010b;4(19):1960-6.

Dahiya R, Gautam H. Toward the first total synthesis of gypsin D: A natural cyclopolypeptide from *Gypsophila arabica*. *Am. J. Sci. Res.* 2010c;11:150-8.

Dahiya R, Gautam H. Toward the synthesis and biological screening of a cyclotetrapeptide from marine bacteria. *Mar. Drugs* 2011;9(1):71-81. [DOI: 10.3390/md9010071]

Dahiya R, Kaur K. Synthetic and biological studies on natural cyclic heptapeptide: segetalin E. *Arch. Pharm. Res.* 2007;30(11):1380-6. [DOI: 10.1007/BF02977360]

Dahiya R, Kaur K. Synthetic and pharmacological investigation of segetalin C as a novel antifungal and cytotoxic agent. *Arzneim. Forsch.* 2008;58(1):29-34.

Dahiya R, Kumar A. Synthesis and biological activity of a potent analog of natural cyclopeptide. *Int. J. Nat. Appl. Sci.* 2007;3(4):433-40.

Dahiya R, Kumar A. Synthetic and biological studies on a cyclopolypeptide of plant origin. *J. Zhejiang Univ. Sci. B* 2008;9(5):391-400. [DOI: 10.1631/jzus.B0720001]

Dahiya R, Pathak D. Synthetic studies on a natural cyclic tetrapeptide - halolitoralin C. *J. Pharm. Res.* 2006; 5(3):69-73.

Dahiya R, Pathak D. Synthesis, characterization and biological evaluation of halolitoralin B - A natural cyclic peptide. *Asian J. Chem.* 2007a;19(2):1499-505.

Dahiya R, Pathak D. First total synthesis and biological evaluation of halolitoralin A. *J. Serb. Chem. Soc.* 2007b; 72(2):101-7. [DOI: 10.2298/JSC0702101D]

Dahiya R, Sharma RD. Synthesis and bioactivity of a novel cyclic hexapeptide from *Stellaria delavayi*. *Eur. J. Sci. Res.* 2008;21(2):277-87.

Dahiya R, Pathak D. Himaja M, Bhatt S. First total synthesis and biological screening of hymenamide E. *Acta Pharm.* 2006;56(4):399-415.

Dahiya R, Maheshwari M, Kumar A. Toward the synthesis and biological evaluation of hirsutide. *Monatsh. Chem.* 2009a;140(1):121-7. [DOI: 10.1007/s00706-008-005c2-z]

Dahiya R, Kumar A, Gupta R. Synthesis, cytotoxic and antimicrobial screening of a proline-rich cyclopoly-peptide. *Chem. Pharm. Bull.* 2009b;57(2):214-7. [DOI: 10.1248/cpb.57.214]

Dahiya R, Maheshwari M, Yadav R. Synthetic, cytotoxic and antimicrobial activity studies on annomuricatin B. *Z. Naturforsch.* 2009c;64b(2):237-44.

Dahiya R, Pathak D. Cyclic peptides: new hope for antifungal therapy. *Egypt. Pharm. J. (NRC)* 2006;5(2):189-99.

Daly NL, Rosengren KJ, Craik DJ. Discovery, structure and biological activities of cyclotides. *Adv. Drug Deliv. Rev.* 2009;61(11):918-30. [DOI:10.1016/j.addr.2009.05.003]

Das P, Himaja M. Design and synthesis of 4-[2′-(5′-nitro) imidazolyl] benzoyl (*N*-methyl) amino acids and peptides. *Int. J. Drug Dev. Res.* 2010;2(2):364-70.

Ebada SS, Lin W, Proksch P. Bioactive sesterterpenes and triterpenes from marine sponges: occurrence and pharmacological significance. *Mar. Drugs* 2010;8(2): 313-46. [DOI: 10.3390/md8020313]

Haritakun R, Srikitikulchai P, Khoyaiklang P, Isaka M. Isariotins A-D, alkaloids from the insect pathogenic fungus *Isaria tenuipes* BCC 7831. *J. Nat. Prod.* 2007;70 (9):1478-80. [DOI: 10.1021/np070291q]

Isaka M, Srisanoh U, Lartpornmatulee N, Boonruangprapa T. ES-242 Derivatives and Cycloheptapeptides from *Cordyceps* sp. Strains BCC 16173 and BCC 16176. *J. Nat. Prod.* 2007;70(10):1601-4. [DOI: 10.1021/np070 357h]

Jia A-Q, Tan N-H, Zhou J. Cyclopeptides from *Sagina japonica* (Caryophyllaceae). *Chem. Biodivers.* 2007;4(2): 241-7. [DOI: 10.1002/cbdv.200790029]

Khan ZK. *In vitro* and *vivo* screening techniques for bioactivity screening and evaluation. Proceeding International workshop UNIDO-CDRI, 1997;210-1.

Kornsakulkarn J, Thongpanchang C, Lapanun S, Srichomthong K. Isocoumarin glucosides from the scale insect fungus *Torrubiella tenuis* BCC 12732. *J. Nat. Prod.* 2009;72(7):1341-3. [DOI: 10.1021/np900 082h]

Kuttan R, Bhanumathy P, Nirmala K, George MC. Potential anticancer activity of turmeric (*Curcuma longa*). *Cancer Lett*. 1985;29(2):197-202. [DOI: 10.1016/0304-3835(85)90159-4]

Pathak D, Dahiya R. Cyclic peptides as novel antineoplastic agents: a review. *J. Sci. Pharm.* 2003;4(4):125-31.

*****