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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF DICHOTOMIN A ANALOGS

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Received: 25-07-2010; Revised: 24-08-2010; Accepted: 16-09-2010

ABSTRACT
A solution phase peptide synthesis was employed to synthesize Dichotomin A analogs. The N, O-dimethylation on Tyrosine and configurational change of L- to D- on Valine was made on Dichotomin A to derive the compounds 1) Cyclo-L-[Gly-Thr-Phe-Leu-(N-CH_3, O-CH_3) Tyr-Val] and 2) Cyclo-L-[Gly-Thr-Phe-Leu-(N-CH_3,O-CH_3)Tyr-D-Val]. The structure of these compounds was confirmed by IR, ^{1}H NMR, and FABMASS. The synthesized compounds were tested for their biological activities against bacterial and fungal organisms and were found to be active. Compound (2) carrying D-valine unit have shown more antimicrobial activity than the compound carrying L-valine unit.

KEYWORDS: Dichotomin A, Cyclic hexapeptide, Solution Phase Synthesis, Antimicrobial activity

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INTRODUCTION

A cyclic hexapeptide dichotomin-A, Cyclo-L-[Gly-Thr-Phe-Leu-Tyr-Val] isolated from the roots of *Stellaria dichotoma*, showed cell growth inhibitory activity[1] and used as folk medicine for antifebrile. Several peptide antibiotics such as geodiamolides, arylomycin, clavariosporin were found to contain N-methylated amino acids in their ring structures[2-4]. A review of the structures of cyclic peptides exhibiting antimicrobial activity showed presence of D- amino acid and / or N- methylated amino acid units in the molecule. Hence two cyclic hexapeptides N-methylated analogs of dichotomin-A have been designed. Both the cyclic hexapeptides comprise of N, O- dimethylated Tyr units but the second one in addition to that contains one D-Val instead of L-Val. The two molecule (1) Cyclo-L-[Gly-Thr-Phe-Leu-(N-CH_3,O-CH_3) Tyr-Val] (2) Cyclo-L-[Gly-Thr-Phe-Leu-(N-CH_3, O-CH_3) Tyr-D-Val] were synthesized by solution phase technique of peptide synthesis using dicyclohexylcarbodimide (DCC) as the coupling agent and triethylamine (TEA) as a base. The enhanced biological activity by the N-methylation and configurational changes[5,6] on the cyclic peptides directed us to synthesize these compounds with intention to increase the antimicrobial activity.

EXPERIMENTAL

MATERIALS AND METHODS

All the reactions requiring anhydrous conditions were conducted in flame dried apparatus. The amino acids used are L- and D-amino acid, purchased from Spectrochem Private Limited, Mumbai, India. Solvents and reagents were purified by standard methods. Boc-amino acids, amino acid methyl ester hydrochlorides were prepared by standard procedures[7]. N-methylated amino acids were prepared using NaH/CH_3I by Benoiton method[8]. Organic extracts were dried over anhydrous sodium sulphate. Melting points were determined by an open capillary method and are uncorrected. The completion of the reaction and purity of the compounds were checked by thin layer chromatography. IR spectra were recorded on Nicolet impact 400 FT/IR spectrometer using KBr pressed pellet technique. ^{1}H NMR spectra were recorded on GEOL-JMS D–300 (MHz) NMR spectrometer. MASS spectra were recorded on Shimandzu GC-MS (at 70 eV) Mass Spectrometer using xenon as the carrier gas.

Preparation of Dipeptides: Amino acid methyl ester hydrochloride (10mmol) was dissolved in chloroform (20ml). To this, triethylamine (4ml, 28.7mmol) was added at 0^0C and the reaction mixture was stirred for 15 mins. Boc-amino acid (10mmol) in $CHCl_3$ (20ml) and DCC (10mmol) were added with stirring. After 12hrs, the reaction mixture was filtered and the residue was washed with $CHCl_3$ (30ml) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ (20ml) and saturated NaCl (20ml) solutions. The organic layer was filtered and evaporated in vacuum. To remove the traces of the dicyclohexylurea (DCU), the product was dissolved in minimum amount of chloroform and cooled to 0^0C. The crystallized DCU was removed by filtration. Petroleum ether was added to the filtrate at 0^0C to recrystallize the pure product. Boc-Gly-Thr-OMe, Boc-Phe-Leu-OMe and Boc-L-(N-CH_3, O-CH_3)-Tyr-L-Val-OMe, Boc-L-(N-CH_3, O-CH_3)-Tyr-D-Val-OMe were prepared in this manner.

Preparation of the Tetrapeptide Boc-Gly-Thr-Phe-Leu-OMe: The tetrapeptide was prepared from the dipeptide The tetrapeptide were prepared from the dipeptide Boc-Gly-Thr-OMe (1) and Boc-Phe-Leu-OMe (2) units after appropriate deprotection at the required functional groups using DCC/Et_3N to get the protected tripeptide.

Preparation of linear hexapeptide: The Boc-group of the dipeptide Boc-L-(N-CH_3,O-CH_3)-Tyr-L-Val-OMe and Boc-L-(N-CH_3,O-CH_3)-Tyr-D-Val-OMe was removed and the ester group of the tetrapeptide (3) was deprotected. Both the deprotected units were coupled to get the two linear hexapeptide.

Preparation of Cyclic hexapeptide (1) and (2): The cyclisation of the linear hexapeptide unit was carried out by the p-nitrophenyl ester method of Bodanszky[9] with certain modifications. The ester group of the linear fragment was removed and the p-nitrophenyl ester group was introduced by stirring it for 12 hrs in $CHCl_3$ with p-nitrophenol at 0^0C. The reaction mixture was washed several times with saturated $NaHCO_3$ until the unreacted p-nitrophenol was removed completely and washed with 5% HCl to get Boc-peptide-pnp ester. The Boc-group also was removed, added $CHCl_3$ and pyridine and the reaction mixture was kept at 0^0C for 10 days. The mixture was finally washed with 5% HCl, dried and evaporated in vacuum to get the cyclised product.

Cyclic hexapeptide (1): Cyclo-L-[Gly-Thr-Phe-Leu-(N-CH_3,O-CH_3) Tyr-Val]: IR (KBr): 3676, 3328, 3034.2, 1721.2, 1627.8, 1575; ^{1}H NMR ($CDCl_3$): δ 7.4-7.0 (m, 9H, Ar-H), 7.6 (s, 1H, -NH), 7.0-6.9 (s, 3H, -NH), 5.5 (s, 1H, -NH), 4.6 (m, 2H,) 4.3-4.1 (m, 3H,) 3.7 (s, 3H, OCH_3), 3.5-3.4 (m, 2H, -CH_2), 3.2-3.1 (m, 2H, - CH_2), 2.2 (s, 3H, N-CH_3), 2.0 (m, 2H, -CH_2), 1.6 (m, 3H, -CH), 1.3-1.0 (m, 15H, 5(-CH_3). FABMASS: m/z: 708 (M+1).

Cyclic hexapeptide 2: Cyclo-L-[Gly-Thr-Phe-Leu-(N-CH_3, O-CH_3) Tyr-D-Val]: IR (KBr): 3640, 3255.4, 3097.5, 1722.1, 1658.4, 1612.9; ^{1}H NMR ($CDCl_3$): 7.6 (s, 1H, -NH), 7.4-7.1 (m, 9H, Ar-H), 7.1 (s, 3H, -NH), 6.85 (s, 1H, -NH), 4.7(s, 1H, -NH), 4.6-4.4 (m, 2H), 4.4-4.1 (m, 3H), 3.75 (s, 3H, -OCH_3), 3.6-3.4 (m, 2H, -CH_2), 3.45-3.2 (m, 2H, -CH_2), 2.0 - 2.5 (s, 3H, N-CH_3), 2.0 -1.0 (m, 2H, -CH_2). FABMASS: m/z 708 (M+1)

EVALUATION OF ANTIMICROBIAL ACTIVITY

Both the compounds were screened for antibacterial and antifungal activities using the cup-plate agar diffusion method[10] by measuring the zone of inhibiton. A 24 h culture of bacterial strains of S. aureus, B. subtilis, p. aeruginosa and E. coli were cultivated in nutrient broth medium and the fungal strains of A. flavus, A. fumigates and C. albicans were cultivated in Fluid Sabraud's medium respectively. Both the compounds were tested at a concentration level of 25 µg/ml. Dimethyl formamide was used as a solvent and as control. Ciprofloxacin and Fluconazole were used as a standard for comparison of the results. The diameter of zone of inhibition was measured after 24h incubation at 37^0C.

RESULTS AND DISCUSSION

The cyclic hexapeptides were obtained in moderate yield (Table 1) and their structure was confirmed by IR, ^{1}H NMR and FABMASS spectroscopy. The compounds were screened for antibacterial and antifungal activities using the bacterial strains of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and the fungal strains of *Asperigillus flavus, Asperigillus fumigates and Candida albicans* respectively. Both the cyclic hexapeptides have shown good antimicrobial activities compared to their standard drugs but the antifungal activity is more prominent. Compound (2) showed enhanced activity than Compound (1) in both the cases. The increased activity of the compounds may be assumed due to the change in the hydrogen bond formation and increased liphophilic character of the molecule which enhances the permeability of the molecule into the bacteria and fungi. In addition to N, O-dimethylation the configurational changes also play an important role to increase the antimicrobial activity. Further studies on other amino acid units are necessary to know the effect of N-methylation and configurational changes to conclude some structure activity relationship of Dichotomin A.

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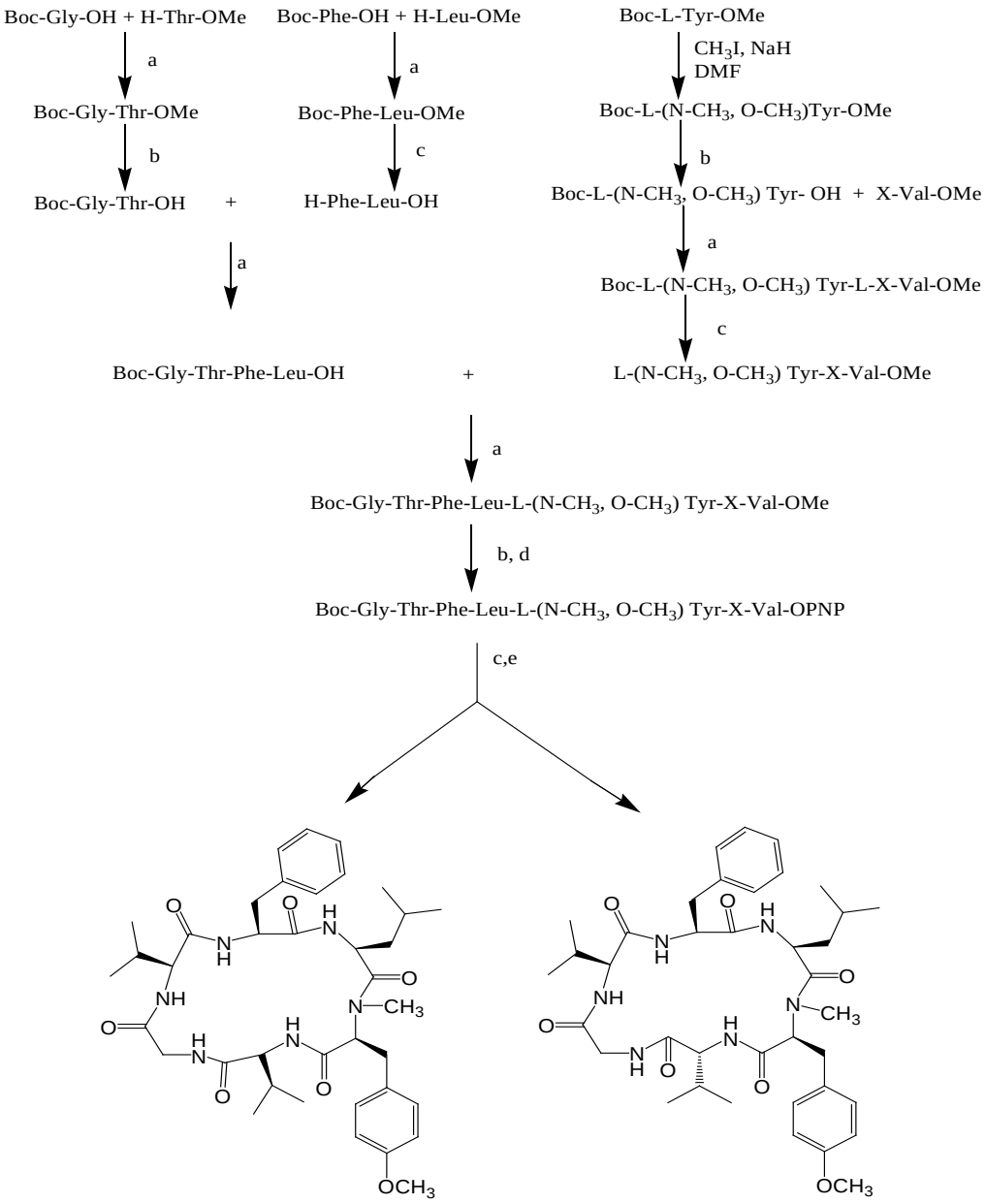
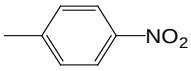


Where

X= L and D

PNP = 

a = $CHCl_3$, DCC, Et_3N, 24h, RT, b = THF: H_2O (1:1), LiOH, 1h, RT, c = $CF_3COOH/CHCl_3$, 1h, RT
d = p-nitrophenol, e = $CHCl_3$, pyridine, 10 days, 0^0C

Scheme-I

Table 1: Physical data of synthesized Cyclic hexapeptides

Compounds	**Composition**	**Physical state**	**% yield**
CP-1	Cyclo–L-[Gly-Thr-Phe-Leu-(N-CH_3,O-CH3)Tyr-L-Val]	brown solid	56.6
CP-2	Cyclo–L-[Gly-Thr-Phe-Leu-(N-CH_3,O-CH3)Tyr -D-Val]	brown solid	61.8

Table 2: Antibacterial and antifungal activities of the compounds

Comp. no↓ Organism→	**Diameter of zone of inhibition(mm)**						
	S. aureus	***B. subtilis***	***E. coli***	**P. aer**	***C. albicans***	***A. flavus***	***A. fumigatus***
CP-1	17	17	19	18	15	13	13
CP-2	19	18	21	18	16	13	14
Ciprofloxacin	23	22	23	23	–	–	–
Fluconazole	–	–	–	–	16	17	17

(–) indicates no inhibition zone (no activity)

Source of support: Nil, Conflict of interest: None Declared