Asian Journal of Chemistry; Vol. 27, No. 3 (2015), 1153-1156

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.18612

Synthesis, Characterization and Anticancer Screening of Psammosilenin B: A Cyclic Octapeptide

NIRMALA V. SHINDE[1,2,*], AVINASH S. DHAKE[2] and KISHAN P. HAVAL[3]

[1]Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Kakinada-533 003, India
[2]Department of Pharmaceutical Chemistry, S.M.B.T. College of Pharmacy, Nandi Hills, Dhamangaon-422 403, India
[3]Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Sub Centre, Osmanabad-413 501, India

*Corresponding author: Tel: +91 2553 202017; E-mail: nirmalampharm@rediffmail.com

Received: 18 August 2014; *Accepted*: 7 November 2014; *Published online*: 19 January 2015; AJC-16745

Synthesis and anticancer screening of cyclic octapeptide, Psammosilenin B is described. The structure of synthesized compound was confirmed by spectral analysis including FTIR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. From the results of screening against 60 human tumour cell lines, it was concluded that the newly synthesized cyclopeptide possessed prominant anticancer activity against HOP-92 cell lines of non small cell lung cancer, PC3 cell lines of prostate cancer and significant activity against HL-60(TB) cell lines of leukemia cancer in comparision with DTP One Dose Mean Graph of vincristine.

Keywords: Cyclic peptide, Anticancer, *p*-Nitrophenyl ester method, Solution phase synthesis.

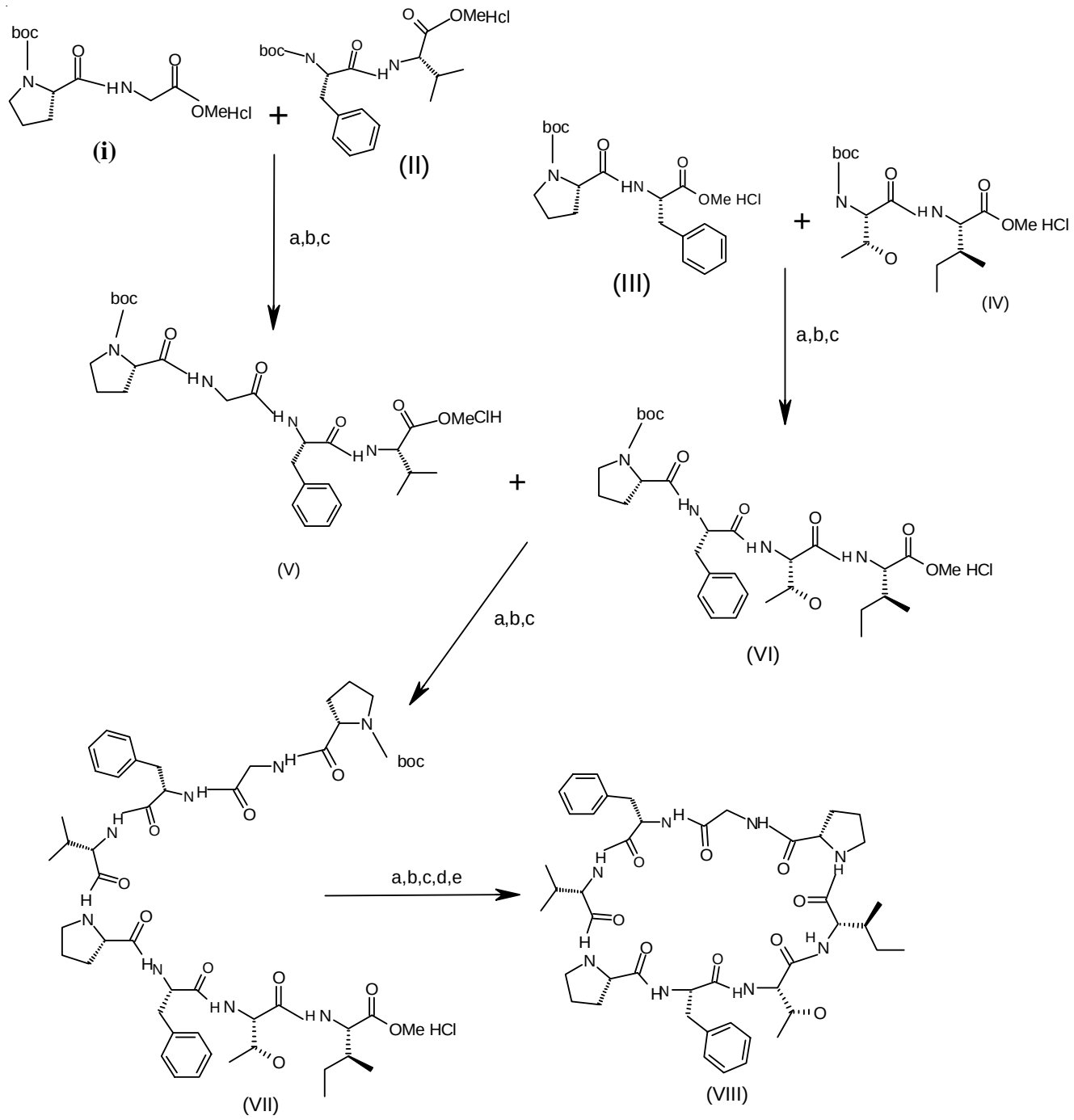
INTRODUCTION

The literature is enriched with several reports which have proved that most of the cyclic peptides are found to exhibit cytotoxic, antineoplastic, antifungal, antibacterial, insecticidal, anti-inflammatory, antitumour anthelmintic, tyrosinase inhibitory and melanin-production inhibitory activities[1-14]. Keeping in view of the significant biological activities exhibited by various cyclic peptides, as a part of ongoing study, an attempt was made towards the synthesis of a cyclic octapeptide, Psammosilenin B cyclo (-Pro-Gly-Phe-Val-Pro-Phe-Thr-Ile-), isolated from *Psammosilenin tunicoides*, a monotype genus plant belonging to the family, Caryophyllaceae[15] (**Scheme-I**). The structure of the synthesized compound was confirmed by spectral analysis. The stereochemistry of all amino acid residues was found to be in the L-configuration by GC analysis. The synthesized compound was then further subjected to anticancer screening against 60 tumour cell lines and was found to show prominent activity against some cancer cell lines and good activity against all cancer cell lines. The prominent results obtained for three cell lines were compared with results for vincristine and found to be significant.

EXPERIMENTAL

Melting point were determined by open capillary method and reported uncorrected. All required L-amino acids, di-tertbutyldicarbonate (Boc_2O), diisopropylcarbodiimide (DIPC), trifluoroacetic acid (TFA), triethylamine (TEA), pyridine and *N*-methylmorpholine (NMM) were purchased from Spectro-chem Limited (Mumbai, India). The chemical structures of all newly synthesized compounds were elucidated by means of spectral as well as elemental analysis. The IR spectra were run on JASCO 4100 FTIR spectrophotometer using a thin film supported on KBr pellets or utilizing chloroform and NaCl cells at S.M.B.T. College of Pharmacy, Dhamangaon. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC NMR spectrometer using DMSO as a solvent. The mass spectrum of the cyclopeptide was recorded on JMS-DX 303 Mass spectrometer operating at 70 eV by ESIMS/MS.

In order to carry out the first total synthesis of Psammosilenin B (VIII), disconnection strategy was employed. The cyclic octapeptide molecule was split into four dipeptide units Boc-Pro-Gly-OMe (I), Boc-Phe-Val-OMe (II), Boc-Pro-Phe-OMe (III) and Boc-Thr-Ile-OMe (IV). The required dipeptide units I, II III and IV were prepared by coupling of Boc-amino acids *viz*. Boc-Pro, Boc-Phe and Boc-Thr with corresponding amino acid methyl ester hydrochlorides such as L-Gly-OMe.HCl, L-Val-OMe.HCl, L-Phe-OMe.HCl and L-Ile-OMe.HCl employing diisopropylcarbodiimide as coupling agent and triethylamine as the base[16]. Ester group of dipeptides was removed by alkaline hydrolysis with LiOH and deprotected peptide was coupled with amino acid methyl ester hydrochlorides using DCC/diisopropylcarbodiimide and triethylamine, to get the tetrapeptide units, BOC-Pro-Gly-Phe-Val OMe HCl (V) and BOC-Pro-Phe-Thr-Isoleu OMe HCl (VI). The deprotected units were coupled to get linear octapeptide



where: a = DIPC, NMM, $CHCl_3$, RT, 24 h; b = TFA, NMM, RT, 1 h; c = LiOH, THF:H_2O (1:1), reflux, 15 min; d = pnp-, $CHCl_3$, RT, 12 h; e = NMM, $CHCl_3$, 0 °C, 7 day

Scheme-I: Synthetic route for cyclic octapeptide - Psammosilenin B

Boc-L-Pro-Gly-Phe-Val-Pro-Phe-Thr-Ile-OMe (VII). The methyl ester group of linear peptide fragment was replaced by *p*-nitrophenyl (pnp) ester group. The Boc-group of resulting compound was removed using trifluoroacetic acid and deprotected linear fragment was cyclized by keeping the whole contents at 0 °C for 7 days in presence of catalytic amount of triethylamine/*N*-methylmorpholine/pyridine to get the cyclic product (VIII).

General method for preparation of Di/Tri/Tetrapeptide intermediates: L-Amino acid methyl ester hydrochloride/dipeptide methyl ester (10 mmol) was dissolved in dichloromethane (20 mL). To this, triethylamine (2.8 mL, 20 mmol) was added at 0 °C and the reaction mixture was stirred for 15 min. Boc-L-amino acid/Boc-dipeptide (10 mmol) in dichloromethane (20 mL) and diisopropylcarbodiimide (DIPC) (2.1 g/1.26 g, 10 mmol) were added with stirring. After 24 h, the reaction mixture was filtered and the residue was washed with dichloromethane (30 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

TABLE-1
DTP ONE DOSE MEAN GRAPH FOR COMPOUND VIII (NSC: 777714/1)

Human tumor cell line	% Growth inhibition comp. VIII (NSC: 777714/1)
Leukemia	
CCRF-CEM	80.04
HL-60(TB)	94.81
K-562	79.09
MOLT-4	92.01
RPMI-8226	92.44
SR	79.95
Non-small cell lung cancer	
A549/ATCC	43.44
HOP-62	23.98
HOP-92	118.43
NCI-H226	34.64
NCI-H23	17.49
NCI-H322M	14.7
NCI-H460	44.32
NCI-H522	44.50
Colon cancer	
COLO 205	20.31
HCC-2998	11.75
HCT-116	81.24
HCT-15	22.83
HT29	23.76
KM12	40.76
SW-620	31.73
CNS cancer	
SF-268	27.20
SF-295	25.79
SF-539	16.78
SNB-19	26.42
U251	45.65
SNB-75	33
Melanoma	
LOX IMVI	11.27
MALME-3	35.98
M M14	77.78
MDA-MB-435	25.96
SK-MEL-2	11.74
SK-MEL-28	23.59
SK-MEL-5	35.16
UACC-257	39.44
UACC-62	34.42
Ovarian cancer	
IGROV1	36.36
OVCAR-3	42.42
OVCAR-4	40
OVCAR-5	10.09
OVCAR-8	31.69
NCI/ADR-RES	35.51
SK-OV-3	21.68
Renal cancer	
786-0	60.07
A498	73.93
ACHN	41.31
CAKI-1	62.38
N12C	33.50
TK-10	-15.54
UO-31	63.12
Prostate cancer	
PC-3	100.59
DU-145	16.39
Breast cancer	
MCF7	32.58
MDA-MB-231/ATCC	73.58
MDA-MB-468	40.77
HS578T	46.95
Mean	57.10
Delta	75.53
Range	133.97

and evaporated in vacuum. The crude product was recrystallized from a mixture of chloroform and petroleum ether, followed by cooling at 0 °C. By using above method, the intermediates I to VII were synthesized.

Synthesis of linear octapeptide: 7.20 g (10 mmol) of Boc-L- Pro-Gly-L-Phe-L-Val-OH was dissolved in dimethyl formamide (DMF, 25 mL) and solution was neutralized with 2.21 mL (21 mmol) of *N*-methylmorpholine at 0 °C and the resulting mixture was stirred for 15 min. 5.84 g (10 mmol) of L-Pro-L-Phe -L-Thr-L-Ile OMe was dissolved in DMF (25 mL) and the resulting solution along with 1.26 g (0.01 mol) of diisopropylcarbodiimide were added to above mixture. Stirring was first done for 1 h at 0-5 °C and then further for 35 h at room temperature. After the completion of reaction, the reaction mixture was diluted with equal amount of water. The precipitated solid was filtered, washed with water and recrystallized from a mixture of chloroform and petroleum ether (b.p. 40-60 °C) followed by cooling at 0 °C to get Boc- L- Pro-Gly-L-Phe-L-Val-L-Pro-L-Phe -L-Thr-L-Ile-OMe (VII) as pale-yellow mass.

Cyclization of linear Octapeptide fragment[16]: To synthesize (VIII), linear octapeptide unit (VII) Boc-L-Pro-Gly-L-Phe-L-Val- L-Pro-L-Phe -L-Thr-L-Ile-OMe (1.2 mmol) in $CHCl_3$ (15mL) at 0 °C. Then *p*-nitrophenol (0.27 g, 2 mmol) was added and stirred for 12 h at room temperature and washed with 10 % $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4. To the Boc-deprotected peptide-pnp-ester in $CHCl_3$ (15 mL), *N*-methyl morpholine (1.4 mL, 2 mmol.) was added and kept at 0 °C for 7 days. The reaction mixture was washed with 10 % $NaHCO_3$ until the byproduct *p*-nitrophenol was removed completely and finally washed with 5 % HCl (5 mL). The organic layer was dried over anhydrous Na_2SO_4. Chloroform and pyridine were distilled off to get the crude product of the cyclized compound, which was then recrystallized from $CHCl_3$/*n*-hexane.

Physical state: white crystals: $[\alpha]_D$ -17.7° (-18° for natural peptide; c, 0.1 in MeOH); R_f 0.76.

IR data: Intense N-H and C=O absorptions at 3300 and 1650 cm^{-1} (amide linkage): 3676.4 (OH stretch), 3293.1 (NH stretch), 3017.8 (arom-CH stretch), 2935.2 (aliph-CH stretch), 2857.8 (aliph-CH stretch), 1658.7 (C=O stretch of amide), 1530.4 (OH-bend) 1451.5 (NH bend) cm^{-1}.

FABMS showed M^+ ion peak at *m/z* 921, which corrosponds to molecular formula $C_{50}H_{80}N_8O_8$. ^{13}C NMR: showed the signals of eight amide carbonyls between δ 169.1 and 173.3. δ (ppm) 169 (C=O of Gly), 171.5 (C=O of Val and Ile), 172.3 (C=O of Pro and Thr), 173.3 (C=O of Phe), 43.6 (Cα of Gly), 55.2, 33.2,20 (Cα, Cβ, Cγ of val), 57,40,25.2 (Cα, Cβ, Cγ of Ile), 62.1, 29.4, 25.3(Cα, Cβ, Cγ of Pro), 58,41.8, 138.9 (Cα, Cβ, Cγ of Phe), 126.9-130.1 (Cδ of Phe), 48.9 (Cδ of Pro), 10.7 (Cδ of Ile).

^{1}H NMR: ^{1}H NMR spectra showed six amide protons between δ 8.58 and 9.56. δ 9.28 (H_N of Gly), δ 4.56 (dd, Hα of Gly), δ 9.08 (H_N of val), δ 5.23, 2.56 (m, Hα and Hβ of val), δ 1.39 (d Hγ of val), δ 8.70 (H_N of Ile), δ 5.23, 2.45, 1.80 (m, Hα, Hβ and Hγ of Ile), δ 8.58 (H_N of Thr), δ 5.38 m, 4.45 m, 1.57 d(Hα ,Hβ and Hγ of Thr), δ 4.19, 3.89, (m for Hα of 2 Pro), δ 2.06, 1.89 (m for Hβ of 2 Pro), δ 8.59, 9.56 H_N of 2 Phe ala, δ 3.41 d, 4 m for Hβ of 2 phe ala. Elemental analysis: C, 65.19 %, H 8.75 %, N 12.16 %.

DTP Human tumor cell line screen[17]: The synthesized compound was screened for preliminary anticancer assay by National Cancer Institute (NCI), Bethesda, USA in an *in vitro* 60 human tumor cell panel. The screening of the compound operated with the *in vitro* cell line screening project (IVCLSP), which is dedicated service, providing direct support to the DTP anticancer drug discovery program. The process utilized 60 different human tumor cell lines of the leukemia, Non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostrate and breast cancers which was aimed in showing selective growth inhibition or cell killing of particular tumor cell lines by specific compound. The screening begins with the evaluation of selected compounds against these 60 cell lines at a single dose of 10^{-5} M. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE programme.

RESULTS AND DISCUSSION

Synthesis of cyclic peptide, Psmamosilenin B was carried out with good yield. The synthesized compound had shown good activity against all cell lines of leukemia, cell lines of colon cancer (HTC 116), cell lines of Melanoma cancer (MM14), cell lines of renal cancer (A498) and cell lines of breast cancer (MDA-MB-231/ATCC). The compound had shown significant activity against cell lines of leukemia cancer (HL-60 TB) and had shown prominant activity against cell lines of non small cell lung cancer (HOP-92), cell lines of prostate cancer (PC3) and when compared with DTP one dose mean graph for vincristine (Fig. 1).

Conclusion

From the results of anticancer activity, it was concluded that, the compound has shown good anticancer activity against some cancer cell lines and development of analogs of same compound may show more activity against human tumor cell lines.

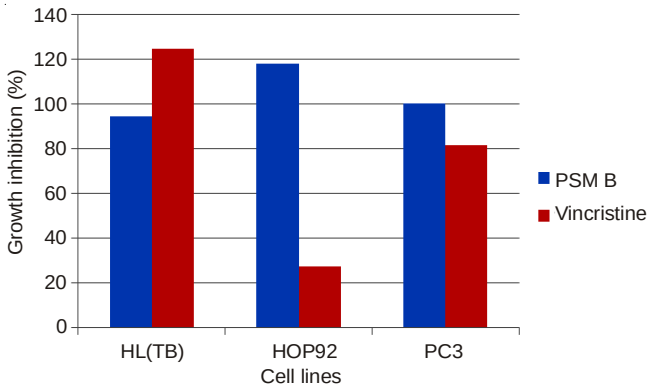


Fig. 1. Comparison of synthesized Psammosilenin B for anticancer screening with data of vincristine against human tumor cell lines

ACKNOWLEDGEMENTS

The authors are thankful to CSIR, New Delhi for financial assistance. Thanks are also due to S.M.B.T. College of Pharmacy, Dhamangaon, Nashik for providing necessary facilities and Sophisticated Analytical Instrumentation Laboratory, Chandigarh for spectral analysis. The authors grateful to NCI, USA for carrying out anticancer activity.

REFERENCES

1. H. Yoshioka, T. Aoki, H. Goko, K. Nakatsu, T. Noda, H. Sakakibara, T. Take, A. Nagata, J. Abe, T. Wakamiya, T. Shiba and T. Kaneko, *Tetrahedron Lett*.,**12**, 2043 (1971).
2. S.B.H. Kent, *Annu. Rev. Biochem*., **57**, 957 (1988).
3. T. Imaeda, Y. Hamada and T. Shioiri, *Tetrahedron*, **35**, 591 (1994).
4. H. Morita, N. Yoshida, K. Takeya, H. Itokawa and O. Shirota, *Tetrahedron*, **52**, 2795 (1996).
5. R. Fernández, S. Omar, M. Feliz, E. Quin˜oá and R. Riguera, *Tetrahedron Lett*., **30**, 6017 (1992).
6. J. Schimana, K. Gebhardt, A. Höltzel, D.G. Schmid, R. Süssmuth, J. Müller, R. Pukall and H.-P. Fiedler, *J. Antibiot*., **55**, 565 (2002).
7. L. Bourel-Bonnet, K.V. Rao, M.T. Hamann and A. Ganesan, *J. Med. Chem*., **48**, 1330 (2005).
8. M.A. Fara, J.J. Di'az-Mochon and M. Bradley, *Tetrahedron Lett*., **47**, 1011 (2006).
9. S.H. Joo, *Biomol. Ther*., **20**, 19 (2012).
10. N.V. Shinde, M. Himaja, S.K. Bhosale and R.D. Wagh, *Asian J. Chem*., **22**, 996 (2010).
11. R. Dahiya and H. Gautam, *Bull. Pharm. Res*., **1**, 1 (2011).
12. S. Chaudhary, H. Kumar, H. Verma and A. Rajpoot, *Int. J. Pharm. Tech. Res*., **4**, 194 (2012).
13. J. Zhong and Y. Chau, *Mol. Cancer Ther*., **7**, 289 (2008).
14. A.G. Shilabin, N. Kasanah, D.E. Wedge and M.T. Hamann, *J. Med. Chem*., **50**, 4340 (2007).
15. Z.T. Ding and Y.C. Wang, *Chin. Chem. Lett*., **10**, 1037 (1999).
16. M. Bodanszky and A. Bodanszky, Practice of Peptide Synthesis, Springer-Verlag, New York, p. 78 (1984).
17. NCI-60 DTP Human Tumor Cell Line Screen; http://dtp.nci.nih.gov/branches/btb/ivclsp.html.