Unique Journal of Engineering and Advanced Sciences
Available online: www.ujconline.net
Research Article

SYNTHESIS OF FUNGISPORIN ANALOGS AS POTENT ANTIMICROBIAL AGENTS

Himaja M[1*], Elizabeth Luiz[2], Karigar Asif[3]

[1]Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore-632014, TN, India
[2]NGSM Institute of Pharmaceutical Sciences, Mangalore, Karnataka, India
[3]Dept of Pharmaceutical analysis, Maratha Mandal College of Pharmacy, Belgaum, Karnataka, India

Received: 12-08-2013; Revised: 10-09-2013; Accepted: 09-10-2013

*Corresponding Author: **Dr. (Mrs). M. Himaja**,
Professor and Program Chair Pharmaceutical Chemistry Division School of Advanced Sciences VIT University, Vellore – 632014
Tamil Nadu, India. Email: drmhimaja@gmail.com, Mobile: +91-9944796228

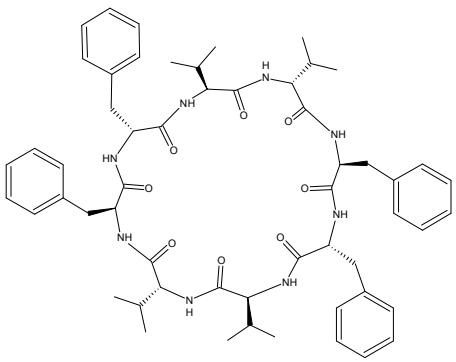
ABSTRACT

Two analogs of Fungisporin were synthesised by solution phase peptide synthesis with satisfactory yields and were characterised by FTIR, ^{1}H NMR, ^{13}C NMR, FABMASS and elemental analysis. The synthesized cyclic peptides were evaluated for antibacterial and antifungal. Cyclic peptide-1 cyclo-(VVFFVVFF) was moderately active, however, its analogcyclo-(D-Val-VFFVVFF) showed enhanced activity as compared to the first one aganist both Gram positive and Gram negative bacteria.

Keywords: Cyclic Octapeptide, Fungisporin, Peptide synthesis, Antibacterial activity, antifungal activity.

INTRODUCTION

Peptides are important class of organic compounds with potent pharmacological activities[1-7]. Cyclic peptides are among the various organic compounds that have received the most attention during last three decades due to their unique structures and biological activities. Fungisporin is one among the few cyclic peptide antibiotics possessing relatively simple chemical structure. It is a cyclic octapeptide, produced by various *Penicillium* and *Aspergillus* species. It was first isolated by Sumiki et al[8]. Its structural elucidation was carried out by Miyao et al[9] and synthesis was carried out by Studer et al[10]. It is found to be active against various Gram positive organisms.



Fungisporin

The structure of Fungisporin analog consists of two D-valine, two D-Phenylalanine, two L-valine and two L-Phenylalanine units: Cyclo[D-Val-L-Val-D-Phe-L-Phe-D-Val-L-Val-D-Phe-L-Phe].

MATERIALS AND METHODS

Synthesis of Cyclic Octapeptide-I (Cyclo [VVFFVVFF]):

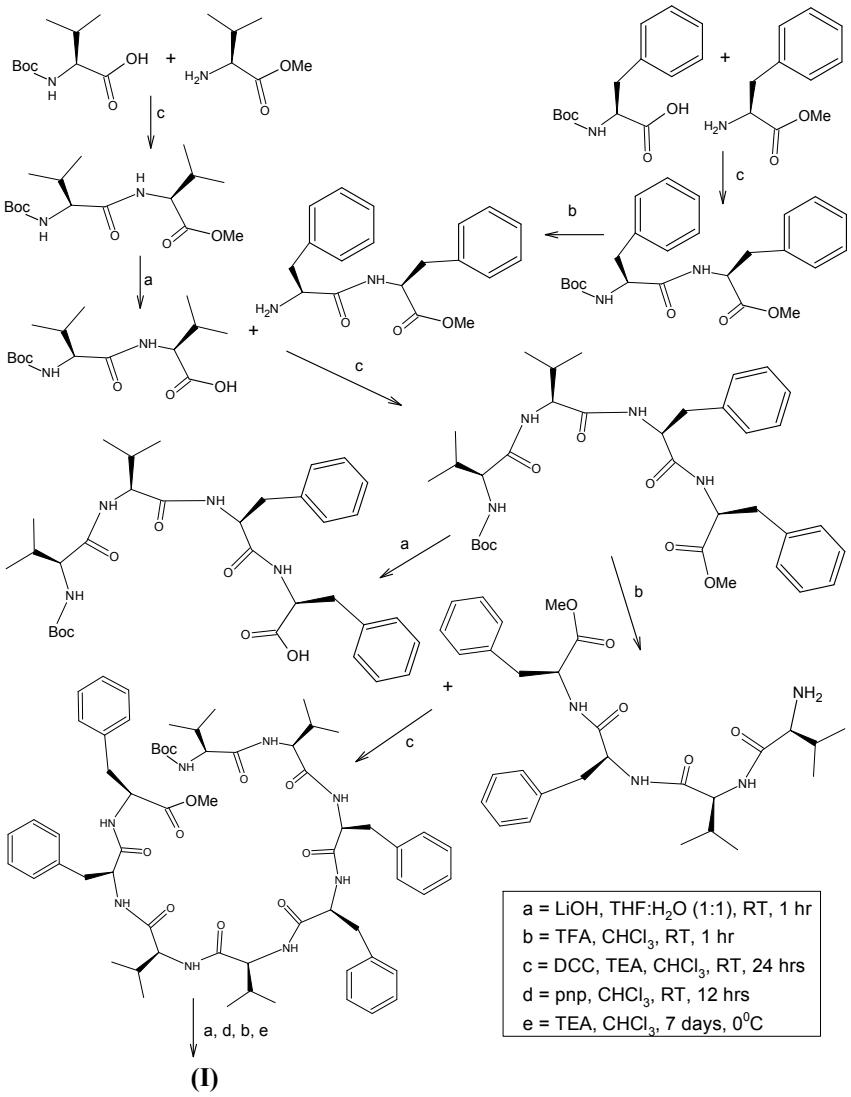
The Cyclic octapeptide I, Cyclo-L-[Val-Val-Phe-Phe-Val-Val-Phe-Phe] was disconnected into four dipeptide units i.e. two units of Boc-L-Val-L-Val-OMe and two units of Boc-Phe-Phe-OMe.

The synthesis of the Cyclo-octapeptide I was carried out as follows.

- Boc-Val and Val-OMe were coupled using DCC/TEA to get the dipeptide BOC-Val-Val-OMe. Boc-Phe-Phe-OMe was prepared in a similar way[11.12].
- The ester group of Boc-Val-Val-OMe was removed with LiOH and the Boc-group of a second portion of Boc-Phe-Phe-OMe was removed with trifluoroacetic acid (TFA).
- The deprotected dipeptides were coupled using DCC and TEA to get the tetrapeptide Boc-Val-Val-Phe-Phe-OMe, which was again divided into two portions.
- The Boc-group of one portion was removed using TFA and the ester group of the second portion of the tetrapeptide was removed by LiOH.

- The deprotected tetrapeptides were coupled using DCC and TEA to get the octapeptide Boc-Val-Val-Phe-Phe-Val-Val-Phe-Phe-OMe.
- The ester group of the linear peptide was removed and p-nitrophenyl group was introduced into the carboxyl end.
- The Boc- group was removed by TFA and the linear fragment was cyclised by adding TEA and keeping the whole contents at 0^0C for seven days to get the cyclic octapeptide-I.

The above synthetic steps are shown in Scheme-1



Scheme 1

Synthesis of Cyclic Octapeptide-II:

The Cyclic octapeptide Cyclo-[D-Val-VFFVVFF] was carried out in similar way as compared to Cyclic octapeptide I but just instead of L-Valine, D-Valine was used[11,12]. The structure of the synthesized molecules was confirmed by IR, ^{1}H NMR, ^{13}C NMR, FABMS and elemental analysis.

Evaluation of Antimicrobial Activity:

Determination of MIC by tube dilution method:

Graded concentrations of the test compound were prepared by serial dilution in DMF and into an Fluid Saboroud medium and inoculated with a test organism. The tubes were inoculated with one loopful of 24 hour culture of one of the test organisms. A positive control was prepared in a similar way except that the test compound was not added. A negative control was prepared in a similar way except that the test compound was not added and the tube was not inoculated with test organism. All tubes were incubated at appropriate conditions. Tubes inoculated with bacterial cultures were incubated aerobically at 37 ^{0}C for 24 hours and tubes inoculated with fungal cultures were incubated aerobically at 25 ^{0}C for 48 hours. After incubation, presence/absence of the growth of the organism was observed and from the results, the MIC of the test compound was calculated[13].The tubes were observed for the presence/absence of growth and the results are given in Table-2 and 3.

Sensitivity Testing:

Sterile paper disks of 6mm diameter and appox. 1 mm thickness were used. Nutrient agar medium and Sabouraud's

agar medium were used for testing the sensitivity of the bacterial and fungal cultures respectively. The compounds were tested at a concentration of 50µg. Same test organisms which were used for MIC were used for sensitivity testing also. Ciprofloxacin (10µg) and fluconazole (25µg) were used as standard antibiotics to compare the antibacterial and antifungal activity respectively.

Nutrient agar medium/Sabouraud's agar medium were prepared from the corresponding dehydrated media and sterilised by autoclaving at 121^0C for 15 minutes. 15ml of the sterile agar medium was poured aseptically into sterile petri dishes and allowed to solidify. 24 hour culture of the test organisms were suitably diluted with sterile water. 0.1 ml of one of the test organisms is added into each petri dish and spread on the surface of the medium using a sterile bent glass rod. Six Sterile paper disks were aseptically placed on the surface of the agar medium in each plate at equal distance. 10 µl (50µg) of test solution (5 mg/ml) was applied in triplicate. 10 µl (10µg) of ciprofloxacin solution (1 mg/ml), 10 µl (25µg) of fluconazole solution (2.5 mg/ml) and 10 µl of DMF were applied on each of the remaining disks in each plate. The plates were placed in a refrigerator for 1 hour and then incubated at 37^0C for bacteria and 25^0C for fungal cultures. In a similar way the remaining compounds were tested against each of the test organisms. Diameter of the zones of inhibition were measured, average values are calculated and the results were shown in Table 4.

RESULTS AND DISCUSSION

Cyclic Peptide I: Cyclo-[VVFFVVFF):

^{1}H NMR(300MHz, $CDCl_3$):δ 7.8-7.7 (1H, br. s, -NH), 7.4-7.1(20H, m. Ar. protons), 6.6(2H, br.s, -NH), 6.3(1H, br. s, -NH), 6.0(1H, br.s, –NH)5.1(3H, br. s, -NH) 4.8(2H, m, α-H), 4.4 (1H, m, α-H), 4.3 (3H, m, α-H), 4.2(2H, m, α-H), 3.3-3.0(8H, m, $-CH_2$), 1.5-1.1(4H, m, β-H), 1.0-0.9(24H, m, $-CH_3$).

IR ($CHCl_3$): 3300.2(br. s, -NH Stretch), 3080(s), 2932.8(s, -CH stretch), 1655.1(s, C=O stretch), 1533.0 (s, -NH bend), 1453.2 (s, NH bend) cm^{-1}.

^{13}C NMR:(75.467MHz, $CDCl_3$): 170.8(C=O of Val), 153.5(C=O of Phe) 133.3(C,Phe) 129.3 (C_2 & C_6, Phe) 129.1 (C_3 & C_5, Phe) 127.4 (C_4, Phe) 59.04(α-C), 54.6(α-C), 53.5(α-C) 51.7.0 (α-C), 50.1(α-C), 49.7(β-C), 48.1(β-C), 37.9(β-C), 33.9(β-C), 33.2 (β-C), 32.4(β-C)31.5 (β-C), 25.9 (CH_3), 25.6 (CH_3), 25.5 (CH_3), 25.3 (CH_3), 24.9 (CH_3), 24.7 (CH_3), 24.6 (CH_3), 19.2 (CH_3).

FABMASS: m/z : $(M + 1)^+ = 982$

Elemental Analysis:

Found (Calcd) %C: 64.9 (65.43), %N: 9.76(10.91)

Cyclic Peptide II: Cyclo[D-Val-VFFVVFF]:

^{1}H NMR(300MHz, $CDCl_3$): δ 8.05 (1H, br. s, -NH), 7.4-7.1(20H, m. Ar. protons), 7.3(1H, s, -NH), 5.4(3H, br. s, -NH), 5.5(1H, br. s, -NH), 4.7(1H, m, α-H), 4.5(2H, m, α-H), 4.1(4H, m, α-H), 3.5-3.0(8H, m, $-CH_2$), 1.5-1.0(4H, m, β-H), 1.0-0.9(24H, m, $-CH_3$).

IR ($CHCl_3$): 3300.1(br. s, -NH Stretch), 3091.5 (s, -CH stretch), 2934.4(s, -CH stretch), 1648.5(s, C=O stretch), 1533(s, -NH bend), 1452(s, NH bend) cm^{-1}.

^{13}C NMR:(75.467MHz, $CDCl_3$): 136.2(C_1 of Phe), 129.3 (C_2 & C_6, Phe) 129.1 (C_3 & C_5, Phe) 127.7 (C_4, Phe) 61.1 (α-C), 58.98(α-C), 56.85(α-C), 54.5(α-C) 53.16 (α-C), 51.04(α-C), 50.12(α-C), 49.91(β-C), 48.65(β-C), 47.83(β-C), 40.99(β-C), 33.9(β-C), 33.84 (β-C), 32.98(β-C), 31.44 (β-C), 29.56 (β-C), 25.83 (CH_3), 25.56 (CH_3), 25.39 (CH_3), 25.25 (CH_3), 24.91 (CH_3), 24.71 (CH_3), 24.48(CH_3), 19.2 (CH_3).

FABMASS: m/z : $(M + 1)^+ = 982$

Elemental Analysis: Found (Calcd) : %C: 66.11 (65.43), %N: 9.89 (10.91)

Table 1: Physical data of cyclic peptide

Compd. No.	Cyclised Product	Physical state	% Yield
(I)	Cyclo-L-[Val-Val-Phe-Phe-Val-Val-Phe-Phe]	Semisolid mass	34.7
(II)	Cyclo-[D-Val-L-(Val-Phe-Phe -Val-Val-Phe-Phe)]	***Semisolid mass***	49.01

Solvent system: Methanol: Chloroform: Water = 3:5:2

Table 2: Minimum Inhibitory Concentration for Antibacterial Activity

Comp. No↓	**PRESENCE/ABSENCE OF GROWTH**																							
Organism→	**S. aureus**						**B. subtilis**						**E. coli**						**P. aeruginosa**					
Dilution→	I	II	III	IV	V	VI	I	II	III	IV	V	VI	I	II	III	IV	V	VI	I	II	III	IV	V	VI
I	–	–	+	+	+	+	–	+	+	+	+	+	–	–	–	+	+	+	–	–	+	+	+	+
II	–	–	–	+	+	+	–	–	–	+	+	+	–	–	–	+	+	+	–	–	–	+	+	+

'+' indicates presence of growth , ' –' indicates absence of growth

Table 3: Minimum inhibitory concentration for antifungal activity

Compd. No. ↓	PRESENCE/ABSENCE OF GROWTH																	
Organism →	*C. albicans*						*A. flavus*						*A. fumigatus*					
Dilution →	I	II	III	IV	V	VI	I	II	III	IV	V	VI	I	II	III	IV	V	VI
I	–	–	+	+	+	+	–	–	+	+	+	+	–	–	+	+	+	+
II	–	–	+	+	+	+	–	–	+	+	+	+	–	–	+	+	+	+

Table 4: Antimicrobial activity- Sensitivity Testing

Compound No ↓	Diameter of zone of inhibition						
Organism →	*S. aureus*	*B.subtilis*	*E. coli*	*P. aeruginosa*	*C albicans*	*A. flavus*	*A. fumigatus*
I	13	11	12	13	11	9	9
II	14	14	16	16	12	10	8
Ciprofloxacin	25	24	25	25	–	–	–
Fluconazole	–	–	–	–	16	17	17

CONCLUSION

The synthesized fungisporin analogs could be conveniently synthesized by solution phase technique with satisfactory yields. Both the cyclic peptides were characterized by IR, ^{1}H NMR, ^{13}C NMR, FABMASS and elemental analysis.

The synthesized cyclic peptides were evaluated for antibacterial and antifungal activities (MIC) from 200µg to 6.25µg. All compounds were found to be considerably active against bacteria from 50-100µg and moderately active against fungi from 100-200µg. Sensitivity testing was carried out by agar disk diffusion method at 50µg level.

Fungisporin analog cyclo[VVFFVVFF] was moderately active, cyclic octapeptide II, cyclo[D-Val-VFFVVFF] showed enhanced activity as compared to the first one against both Gram positive and Gram negative bacteria.

Enhanced activity of cyclic octapeptide II may be due to the introduction of D-valine unit into the ring structure.

Further studies of the biological activity on cyclic octapeptide I and II may reveal interesting activities like cytotoxic,

immunosuppressive activity and tyrosinase inhibitory activity.

REFERENCES

1. Beverly AT, Ara G, Roy H, Palombella VJ, Julian A, "The Proteasome Inhibitor PS-341 in Cancer Therapy", *Clinical Cancer Research.*, 1999; 5: 2638.
2. Kim SH, Jung MH, Ahn S, Lee CW, Kim SN, Journal of Antibiotics, 2002; 55: 598-601.
3. Hashizuke, S. Hirosawa, R. Sawa, Y. Muraoka, D. Ikeda, Journal of Antibiotics, 2004; 57: 52-58.
4. Dale L. Boger, Jiacheng Zhou, Brian Writer and Paul A. Kitos" Key analogs of the Tetrapeptide Subunit of RA-VII and Deoxybouvardin", *Bioorganic and Medicinal Chemistry,* 1995; 3: 1579.
5. Katsuhirokudo, Shuichi O, Yasuyuki T, Benkeblia N, Shiomi N, " Antioxidative activities of some peptides isolated from hydrolyzed potato protein extract", *Journal of functional foods.,* 2009; 1: 170.
6. Dathe M, Nikolenko H, Kolse J, Bienert M, Biochemistry, 2004; 43: 9140-50.
7. Luke Simmons KL. McPhail B, Eduardo OB, Susan L, William HG, Belamide A, A new antimitotic tetrapeptide from a Panamanian marine cyanobacterium", *Tetrahedron Letters.,* 2006; 47: 3387.
8. Gunnur Dikmen Z, Ginelle C. Gellert, Pakize Dogan, Heejeong Yoon, Young Bok Lee, Chang Ho Ahn and Jerry W. Shay" In vivo and Invitro Effects of a HIF-1α Inhibitor, RX-0047'', *Journal of Cellular Biochemistry*, 2008; 104: 985.
9. Lee MD, Yuhong MJ, Soskis CP, Borella M. Humanmitochondrial peptide deformylase, a new anticancer target of actinonin-based antibiotics, J. Clinical Investigation., 2004; 114: 8.
10. Alejandro M. S. Mayera, Kirk R. Gustafson. Marine pharmacology in 2005–2006: Antitumor and cytotoxic compounds. European journal of Cancer, 2008; 44: 2357.
11. Belagali S. L., Mathew T., Himaja M. and Kociensky P., Ind. J. Chem., 1995, 34B, 45.
12. Bodanszky. M., and Bodanszky. A., Practice of Peptide Synthesis. (Springer-Verlag, New York); 1984. p. 78.
13. Clinical microbiology procedures handbook. Henry D Isenberg.Volume 1. American society for microbiology/ Washington, D.C, 1992.

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