ORGANIC LETTERS

2000
Vol. 2, No. 7
973−976

Synthesis and Antifungal Activity of Rhodopeptin Analogues. 1. Modification of the East and South Amino Acid Moieties

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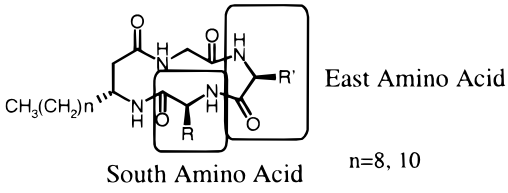
Received February 7, 2000

ABSTRACT



Synthetic Rhodopeptin Analogs

Structure–activity relationships of the east and south amino acid modified analogues of rhodopeptins, novel antifungal cyclic tetrapeptides isolated from *Rhodococcus* species Mer-N1033, have been investigated. It was observed that a basic amino acid moiety (lysine or ornithine) as the east amino acid and a hydrophobic and bulky neutral amino acid (i.e., γ-methylleucine) as the south amino acid were indispensable structure motifs for antifungal activity of rhodopeptin analogues.

The occurrence of opportunistic fungal infections, caused by *Candida* sp., *Cryptococcs neoformans*, and *Aspergillus* sp., has risen sharply in recent years among patients immunocompromised by cancer chemotherapy, organ transplant, hematological malignancy, and HIV infection. There are only a few drugs available for treating systemic antifungal infection, e.g., amphotericin B (AMPH) and the azoles such as fluconazole (FLCZ) and itraconazole (ITCZ), and thus the need for other effective antifungal agents has intensified.[1]

Rhodopeptins comprise a family of novel antifungal cyclic tetrapeptides, isolated from *Rhodococcus* species Mer-N1033.[2] The rhodopeptins, which are composed of three α-amino acids and one β-amino acid with a lipophilic side chain (Figure 1), exhibit fungicidal activity against *Candida* sp. and *Cryptococcus neoformans* and fungistatic activity against *Aspergillus fumigatus*.

Prompted by the unique structure and antifungal activity, we executed a structure−activity relationship (SAR) study

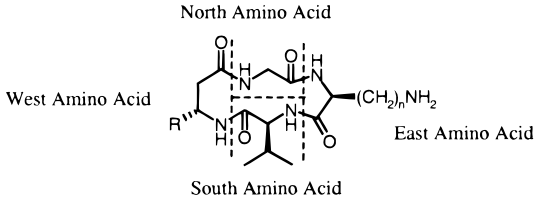
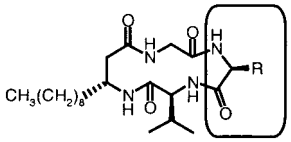


Figure 1. **1**: $n = 3$, R = $(CH_2)_6CH(CH_3)CH_2CH_3$ (rhodopeptin C1). **2**: $n = 4$, R = $(CH_2)_8CH(CH_3)_2$ (rhodopeptin B5). **3**: $n = 4$, R = $(CH_2)_8CH_3$.

(1) (a) De Lucca, A. J.; Walsh, T. J. *Antimicrob. Agents Chemother.* **1999**, *43*, 1. (b) Barrett, J. F.; Klanbert, D. H. *Ann. Rep. Med. Chem.* **1992**, *27*, 149. (c) Barrett, J. F.; Hey, R. J.; Peterson, P. K. *New strategies in Fungal Disease*; Churchill Livingstone: Edinburgh, 1992. (d) Yamaguchi, H.; Kobayashi, G. S.; Takahashi, H. *Recent Progress in Antifungal Chemotherapy*; Marcell Dekker Inc.: New York, 1991. (e) Balkovec, J. M. *Exp. Opin. Invest. Drugs* **1994**, *3*, 65.

10.1021/ol005631c CCC: $19.00
Published on Web 03/15/2000

Table 1. Antifungal Activity of the East Amino Acid Analogues of Rhodopeptin[a]



East Amino Acid

		MIC (mg/mL)[b]				
compound	east amino acid	*C. albicans* ATCC24433	*C. tropicalis* TIMM0313	*C. krusei* TIMM0269	*C. glabrata* ATCC2001	*A. fumigatus* TIMM0063
3	Lys	4	4	2	4	>128
7	Orn	4	1	2	2	>32
14	Arg	2	1	2	2	64
15	His	>32	>32	>32	>32	>32
16	Glu	>64	>64	>64	>64	>64
17	N^6-Me-Lys	4	2	8	4	>128
18	N^6-^{i}Pr-Lys	8	8	8	8	>128
19	N^6,N^6-di-Me-Lys	8	4	8	4	>128
20	N^6-Me-N^6-Bn-Lys	>128	>128	>128	>128	>128
21	N^6-Ac-Lys	>16	>16	>16	>16	>16
AMPH		4	4	4	2	4
FLCZ		>128	>128	128	128	>128

[a] Boc-Lys(Me, Bn) was synthesized from Boc-Lys(Cbz).[8] Reductive amination of Cbz-Lys-OMe with formaldehyde, followed by deprotection, furnished Cbz-Lys(Me_2). The other protected amino acids were commercially available. [b] Minimum inhibitory concentration. Medium: synthetic amino acid medium fungal (SAAMF).

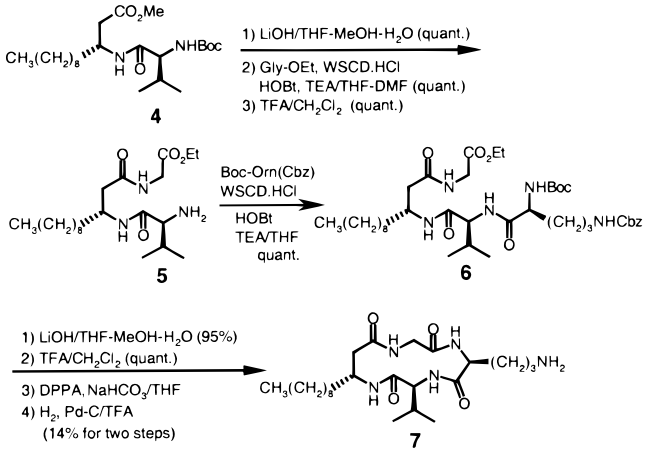
of rhodopeptin derivatives. For convenience, the cyclic tetrapeptide structure was divided into four parts, the north, west, south, and east amino acids as illustrated in Figure 1. Herein we report the synthesis and antifungal activity of east and south amino acid modified analogues of the rhodopeptins.

The east amino acid analogues were constructed on the basis of the previously reported protocol for the synthesis of rhodopeptin analogue **3**.[3] The synthesis of ornithine analogue **7** is depicted as an example (Scheme 1). Starting with dipeptide **4**,[3] deprotection and successive condensations with glycine and ornithine residues provided tetrapeptide **6**. Deprotection and cyclization using diphenylphosphoryl azide (DPPA),[4] followed by removal of the Cbz group, furnished compound **7**. Analogous chemistry was utilized to prepare the remainder of the analogues listed in Table 1.

The synthesis of γ-methylleucine (γ-MeLeu) derivative **13** exemplifies the preparation of the compounds listed in Table 2 (Scheme 2). Anticipated steric problems dictated that tetrapeptide cyclization for this series of compounds would rely upon ring closure between the C-terminus of the west amino acid and the north amino acid N-terminus. From the results of our preliminary study which determined that antifungal activity required lipophilic side chains ranging from 9 to 11 carbons, we prepared both nonyl and undecyl derivatives of these compounds.

Wittig reaction of *n*-dodecanal **8** with (carbomethoxymethylene)triphenylphosphorane provided the α,β-unsaturated ester.[3] Michael addition of (*R*)-1-phenylethylamine to the ester furnished a mixture of diastereomers.[5] After chromatographic separation, hydrogenolysis of the desired isomer **9** provided (*R*)-3-undecyl-β-alanine methyl ester **10**. Condensation of **10** with Boc-γ-methylleucine, followed by removal of the Boc group and another condensation with Boc-Gly-Lys(Cbz),[3] provided tetrapeptide **12**. Deprotection with trifluoroacetic acid and cyclization with DPPA, followed by hydrogenolysis, furnished the desired compound **13**.

Scheme 1



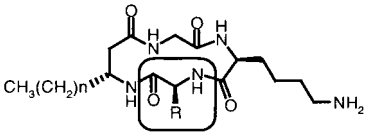
(2) (a) Chiba, H.; Agematu, H.; Kaneto, R.; Terasawa, T.; Sakai, K.; Dobashi, K.; Yoshioka, T. *J. Antibiot.* **1999**, *52*, 695. (b) Chiba, H.; Agematu, H.; Dobashi, K.; Yoshioka, T. *J. Antibiot.* **1999**, *52*, 700.

(3) Chiba, H.; Agematu, H.; Sakai, K.; Dobashi, K.; Yoshioka, T. *J. Antibiot.* **1999**, *52*, 710.

(4) Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc*. **1972**, *94*, 6203.

(5) Furukawa, M.; Okawara, T.; Terawaki, Y. *Chem. Pharm. Bull.* **1977**, *25*, 1319.

Table 2. Antifungal Activity of the South Amino Acid Analogues of Rhodopeptin



South Amino Acid

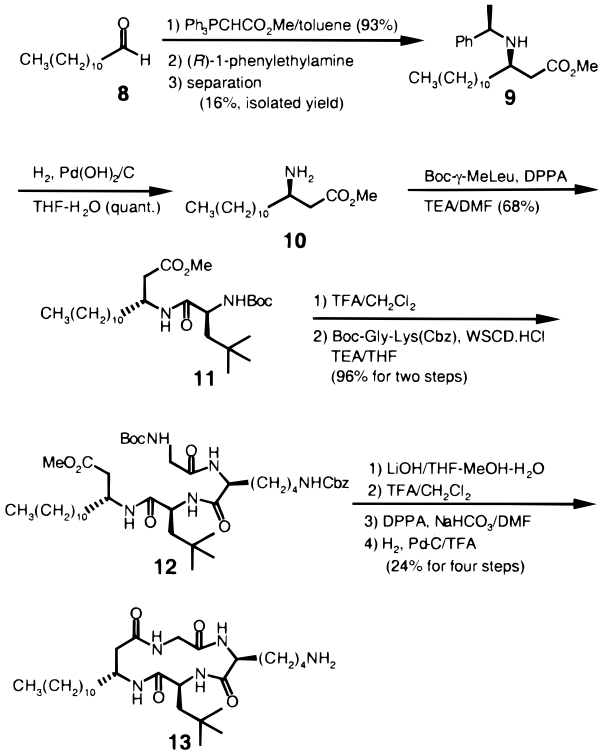
			MIC (mg/mL)[a]				
compound	*n*	south amino acid	*C. albicans* ATCC24433	*C. tropicalis* TIMM0313	*C. krusei* TIMM0269	*C. glabrata* ATCC2001	*A. fumigatus* TIMM0063
3	8	Val	4	4	2	4	>128
22	8	Lys	64	128	>128	128	>128
23	8	Glu	64	>128	>128	32	>128
24	10	Val	128	8	128	8	128
25	10	Ile	16	8	64	8	16
26	10	Leu	8	4	128	8	>128
27	10	Cha	64	32	16	8	64
28	10	*tert*-Leu	16	8	16	8	32
13	10	γ-MeLeu	4	4	8	4	8
AMPH			4	4	4	2	4
FLCZ			>128	>128	128	128	>128

[a] Minimum inhibitory concentration. Medium: synthetic amino acid medium fungal (SAAMF).

The minimum inhibitory concentration values (MIC, μg/mL) of the east amino acid analogues against four strains of *Candida* sp. and *Aspergillus fumigatus* TIMM0063 are presented in Table 1.[6] The activities of AMPH[7] and FLCZ[7] are shown as a reference.

The ornithine analogue **7** displayed increased potency in comparison to the lysine analogue **3**. The arginine analogue **14** possessed higher activity against *Candida* sp. and was also slightly active against *Aspergillus fumigatus*. In contrast, the less basic histidine derivative **15** and the glutamic acid derivative **16** containing an acidic functional group were inactive at the maximum soluble concentration. A glycine analogue was completely insoluble in the medium, precluding measurement of its activity. The results with N^6-substituted lysine derivatives (**17**−**20**) demonstrated that mono- and bis-substitution with a small alkyl group could be possible, whereas substitution with a larger group, e.g., benzyl, resulted in loss of activity. The acylated analogue **21** showed decreased solubility in the medium and was inactive at the maximum soluble concentration.

Scheme 2



(6) The in vitro susceptibility testings were done by a microdilution method, using 96-well microplates (flat bottom). Synthetic amino acid medium fungal (SAAMF; pH 7.0 at 30 °C, Nippon Bio-Supp. Center, Tokyo, Japan) was used throughout in this study. Freshly grown yeasts on slopes of sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, MI) were suspended with physiological saline and counted in a hemacytometer and cell concentration was adjusted to 1×10^6 cells per mL. When *A. fumigatus* was studied, subcultured organisms were suspended with saline containing 0.1% Tween 80 (monooleate polyoxyethylenesorbitan, Sigma Chemicals, St. Louis, MO), and then conidia were collected by passing the culture through a glass filter. Test compounds were initially dissolved in dimethyl sulfoxide (DMSO; Nakarai Chemicals, Kyoto, Japan) and further diluted with distilled water. Serial 2-fold dilution (256 to 0.125 μg/mL, 100 μL per well) were dispensed with the aid of an automatic dispenser (Model, Dinatech, Kyoto, Japan) and 2× concentrated medium was added to each well (100 μL per well). The final concentration of DMSO contained in culture in wells was 2% at maximum. After inoculation (5 μL per well) was done by use of an automatic inoculator (Model, Dinatech), plates were gently but thoroughly shaken and were incubated at 30 °C for 48 h (*Candida* sp.) or 72 h (*A. fumigatus*). The inoculum size was 5×10^3 cells per mL. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of a compound which gave no visibly detectable fungal growth.

(7) Drug used: amphotericin B (AMPH; Fungizone, Bristol Myers Squibb, New Brunswick, NJ) and fluconazole (FLCZ; Diflucan, Pfizer, New York,) were obtained commercially.

The antifungal activity of the south amino acid analogues is presented in Table 2.[6] The activity of lysine derivative **22** containing a basic functional group was significantly decreased. The glutamic acid derivative **23** with an acidic functional group was also less active. Both isoleucine analogue **25** and leucine analogue **26** displayed moderate activity against *Candida* species, with **25** showing activity against *Aspergillus* species as well. The more bulky lipophilic amino acid derivatives, cyclohexylalanine (Cha, **27**) and *tert*-leucine **28**, did not display significantly improved antifungal activities. However, γ-methyllecine (γ-MeLeu) analogue **13** exhibited activity comparable to that of AMPH against both *Candida* and *Aspergillus* species. Furthermore **13** showed lower toxicity[9] than AMPH, with GI_{50} values of 13.6 and 1.0 μg/mL, respectively.

(8) Andruszkiewicz, R. *Pol. J. Chem*. **1988**, *62*, 257.
(9) MTT assay against P388.

In conclusion, we have found that a basic amino acid moiety (lysine or ornithine) as the east amino acid and a hydrophobic and bulky neutral amino acid (i.e. γ-methylleucine) as the south amino acid were indispensable structural motifs for antifungal activity of rhodopeptin analogues. Studies on the mechanism of action of these compounds are currently in progress in our laboratories.

Acknowledgment. We thank Mr. Chiba (Mercian Co. Ltd.) for useful discussions. We also thank Prof. William Moser of IUPUI for critiquing the manuscript.

Supporting Information Available: Spectroscopic and analytical data for compounds **5**−**7** and **11**−**28**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL005631C