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Structure activity relationship study of burkholdine analogues toward simple antifungal agents

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ABSTRACT

Cyclic and linear lipopeptides, burkholdine analogues, were synthesized by conventional Fmoc-SPPS and cyclisation with DIPC/HOBt in the solution phase. Synthesized peptides were evaluated for antifungal activities with MIC values against *Saccharomyces cerevisiae* and *Aspergillus oryzae*. As a result, the stereochemistry of the amino acid residues and sequences of burkholdine analogues exerted a significant influence on antifungal activities. In addition, we found a linear burkholdine analogue with moderate antifungal activities.

Burkholdines (Bks) are potent antifungal cyclic lipopeptides isolated from culture of *Burkholderia* 2.2 N by Schmidt's group.[1,2] Five Bk-series compounds, Bk-1229, Bk-1079 (**1**), Bk-1215, Bk-1119 and Bk-1213, were isolated and revealed their chemical structures to date. Bk-1097 (**1**), the most simple Bk, is a cyclic octapeptide composed of L- and D-serine (Ser), L- and D-asparagine (Asp), glycine (Gly), (2*S*,3*R*)-β-hydroxyasparagine (βHYD), β-hydroxytyrosine (βHYY) and 3-amino-5,6,7-trihydroxynonadecanoic acid (ATNA). Although the absolute stereochemistry of the βHYY residue and ATNA moiety of Bk-1097 (**1**) have been reported to be 2*R*,3*R* and 3*R*,5*R*,6*S*,7*S*, respectively, there is no complete assignment for identification. In addition, potent antifungal activity of Bk-1097 (**1**) has been shown against *Saccharomyces cerevisiae* and *Aspergillus niger*. In a word, these activities are more potent than amphotericin B currently used in antifungal therapy.[3,4] Bks are promising antifungal agents that produce fungal cell membrane defects, although the inhibitory mechanism and target enzyme are still unknown. The total synthesis of Bk-1097 (**1**) has not been reported in the literature, and even synthetic study of composed unusual amino acids was not disclosed. We are interested in the chemical structure and biological activity of Bk-1097 (**1**). Generally, cyclic peptides tend to show potent activities[5–10] because of their rigid framework and preservation of sidechain trajectories. Therefore, synthesis and a structure activity relationship study of Bks were conducted. In our previous Letter, we synthesized 19 Bk-1097 analogues through the use of Fmoc-SPPS and found Bk-analogue (**2**) with an antifungal activity of 25 μg/mL as the MIC.[11] We herein report that investigation of the stereochemistry of the left sequence (***A*** and ***F*–*H*** amino acids) for the potent antifungals because those of right sequence (***A*–*D***) were mainly optimised in the previous Letter.[11] In addition, synthesis and evaluation of cyclic heptapeptides and linear octapeptides were attempted toward simple antifungal agents (Fig. 1).

The designed cyclic octapeptides were synthesized using our previous synthetic protocols.[11] The corresponding linear peptides were prepared by Fmoc-SPPS using 2-chlorotrityl chloride (2-CTC) resin.[8–11] After cleavage from the resin with 20% HFIP/CH_2Cl_2, macrolactamization between N-terminus amine and C-terminus carboxylic acid of the linear peptides in the presence of DIPC/HOBt for 14–62 h followed by global deprotection with TFA/TIPS/H_2O (95:2.5:2.5) gave the designed cyclic octapeptides (**2**)–(**17**). The stereochemistry of the ***A*** and ***F*–*H*** positions of synthetic peptides with chemical yields (*X*%, *Y*%, and *Z*%) are shown in Table 1. Synthesis of the corresponding linear peptides by ordinary Fmoc-SPPS was performed in moderate yield (*X*%) after the purification by preparative RP-HPLC. The chemical yield of Fmoc-SPPS for **16** was slightly low, because each coupling efficiency of

Abbreviations: SPPS, solid phase peptide synthesis; DMF, dimethyl formamide; DIPEA, diisopropylethylamine; DIPC, diisopropylcarbodiimide; HOBt, 1-hydroxybenzotriazole; Fmoc, 9-fluorenylmethyloxycarbonyl; HFIP, 1,1,1,3,3,3-hexafluoropropan-2-ol; TFA, trifluoroacetic acid; LAP, *N*-lauryl-3-amino-4-carbamolypropanoic acid; MIC, minimum inhibitory concentration.

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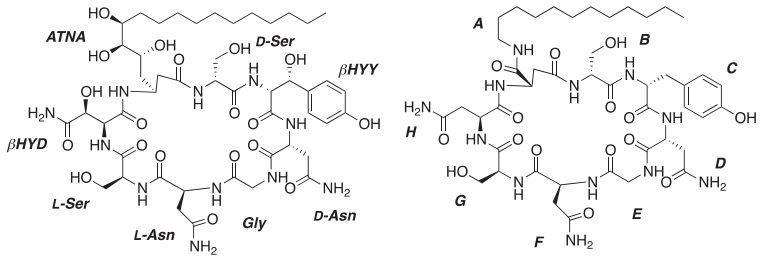
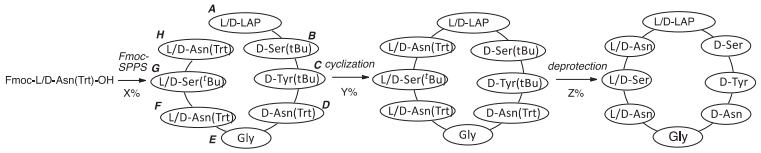


Figure 1. Chemical structure of Bk-1079 (**1**) and Bk-analogue (**2**).

Table 1
Synthesis and antifungals of cyclic octapeptide Bk-analogues



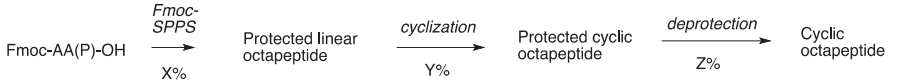
Entry	Compound[a]	*X*%	*Y*%	*Z*%	Entry	Compound[a]	*X*%	*Y*%	*Z*%
1	**2** (L-LLL)	64	55	57	9	**10** (D-LLL)	45	46	57
2	**3** (L-LLD)	62	63	38	10	**11** (D-LLD)	45	81	40
3	**4** (L-LDL)	43	53	25	11	**12** (D-LDL)	41	24	76
4	**5** (L-LDD)	42	63	15	12	**13** (D-LDD)	49	22	22
5	**6** (L-DLL)	52	46	50	13	**14** (D-DLL)	72	21	61
6	**7** (L-DLD)	45	38	36	14	**15** (D-DLD)	48	31	34
7	**8** (L-DDL)	39	60	34	15	**16** (D-DDL)	17	43	70
8	**9** (L-DDD)	42	83	41	16	**17** (D-DDD)	34	68	30

[a] *cyclo*(-L-LAP-D-Ser-D-Tyr-D-Asn-Gly-L-Asn-L-Ser-L-Asn-) (**2**) was shown as **2** (L-LLL).

Fmoc-SPPS for the sequence was not enough to give desired product with the by-products. Macrolactamization of the linear peptides successively proceeded by our optimised conditions (*Y*%). Purification of crude protected cyclic peptides was performed by silica gel column chromatography. It is noted that the cyclised efficiency of linear peptides dominated the sequences with stereochemistry, namely, conformation of the linear peptides was clearly preferred between the amine of the N-terminus and carboxylic acid of C-terminus by interaction of an intramolecular hydrogen bond. These results suggested that motif of Asn-Gly-Asn formed a β-turn structure and additionally stacked between two trityl groups of the two Asp side chains, because we understand that macrolactamization of non-protected linear peptides did not proceed in our preliminary study. In addition, some of the synthetic peptides gave low chemical yields as the final deprotection. As the reason, removal of protecting groups, especially Trityl groups, hardly proceeded to form any structures and/or there were lower solubility of the peptides with hydrophobic sidechain and hydrophilic sequence. The experimental procedure of selected cyclic peptide (**11**) with HPLC profiles and ESIMS are shown in the reference section[12] (Table 1).

To compare the hydrocarbon chain length of simplified lipo-β-amino acid, we designed *N*-hexadecyl-3-amino-4-carbamolypropanoic acid (HAP) and *N*-octyl-3-amino-4-carbamolypropanoic acid (OAP), which were coupled with linear peptides as the last component of solid support. To prepare both enantiomers of HAP and OAP residues, Fmoc-D/L-Asp(*t*Bu)-OH was condensed with hexadecylamine or octylamine using BOP/Et_3N in DMF followed by TFA-mediated deprotection to give the Fmoc-derivatives of both enantiomers containing HAP and OAP residues without any problems. Synthetic protocols with reagents and conditions, resin and purification methods were similar to Table 1. Preparations of two linear peptides containing L- or D-HAP by Fmoc-SPPS were successful in 25% and 28% overall yields, respectively (entries 1 and 2). In contrast, OAP-containing peptides were synthesized in 56% and 50% yields (entries 3 and 4). In

Table 2
Synthesis of cyclic octapeptide Bk-analogues



Entry	Lipo-β-AA	Product	*X*%	*Y*%	*Z*%
1	L-HAP	**18**	25	60	43
2	D-HAP	**19**	28	56	58
3	L-OAP	**20**	56	98	30
4	D-OAP	**21**	50	76	76