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BRIEF COMMUNICATION

Semisynthesis and antibacterial activities of nidulin derivatives

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Abstract

Derivatives of the fungal depsidone, nidulin, have been synthesized in order to evaluate the potential of the chemical skeleton as antibacterial agents. Alkylation, acylation, and arylation reactions of nornidulin underwent in a regioselective manner to predominantly produce 8-*O*-substituted derivatives. Many of the semisynthetic derivatives showed more potent antibacterial activities than nidulin, In particular, 8-*O*-aryl ether derivatives displayed significant activities against Gram-positive bacteria, including Methicillin-resistant *Staphylococcus aureus*.

Nidulin (**1**), nornidulin (**2**), and related depsidones are secondary metabolites specifically produced by fermentation of the common fungi *Aspergillus unguis* and *Aspergillus nidulans* (Fig. 1). There have been several reports regarding biological activities of nidulin and closely related *Aspergillus* depsidones. Unguinol (2,4,7-trisdechloro-nornidulin) was reported to be a potential herbicide candidate due to its inhibitory activity of the C_4 plant enzyme pyruvate phosphate dikinase [1]. Unguinol and its derivatives are claimed to be animal growth permittants in a US patent [2]. Kittakoop and co-workers reported aromatase inhibitory, radical scavenging, and cytotoxic activities of nidulin and co-metabolites, aspergillusidones, from *A. unguis* CRI-282-03 [3, 4]. Recently, antibacterial, antifungal, antimalarial, and cytotoxic activities of depsidones and depsides from *A. unguis* have been reported [5, 6]. During the preparation of this paper, a report on isolation of many depsidones from *A. unguis* and evaluations of their activities against Gram-positive bacteria was published [7].

Several years ago, we isolated nidulin, nornidulin and three related depsidones from cultures of a mangrove-derived fungus *Halosarpheia kandeliae* BCC 16551. However, based on the checking of the culture collection deposit of the strain, and the failure of the reproduction by new fermentations, it was later concluded that these depsidones were most likely produced due to the contamination of *Aspergillus unguis* into a seed culture in the large-scale fermentation process. Evaluation of biological activities of the isolated depsidones revealed that nidulin exhibits activity against Gram-positive bacteria, *Bacillus cereus* (MIC 1.56 μg ml^{-1}) and *Enterococcus faecium* (MIC 3.13 μg ml^{-1}). Nornidulin showed weaker activity when compared with nidulin. As preliminary study on structure-activity relationship (SAR), we examined preparation of 3-*O*-methyl-nornidulin by alkylation of **2** with one equivalent of MeI (K_2CO_3, DMF, room temperature) expecting the production of a mixture of 3-*O*-methyl-**2** and 8-*O*-methyl-**2** (nidulin, **1**). Interestingly, the *O*-methylation underwent in a highly regioselective manner giving only nidulin (**1**). 3-*O*-Methyl-nidulin, prepared by *O*-methylation of **1** with excess MeI, was inactive against these bacteria, which suggested that 3-OH group is crucial for antibacterial activity. On the basis of these preliminary results on SAR and regioselectivity of *O*-alkylation, we had planned semisynthesis of nidulin derivatives by selective alkylation of nornidulin 8-OH to evaluate the potential of the skeleton as antibacterial agents. We report here synthesis of various nidulin derivatives and evaluation of their antibacterial activity.

As substrate for the semisynthesis, nornidulin was produced by fermentation of the commercial strain, *A. unguis* ATCC 10032, which is a known to producer of nidulin

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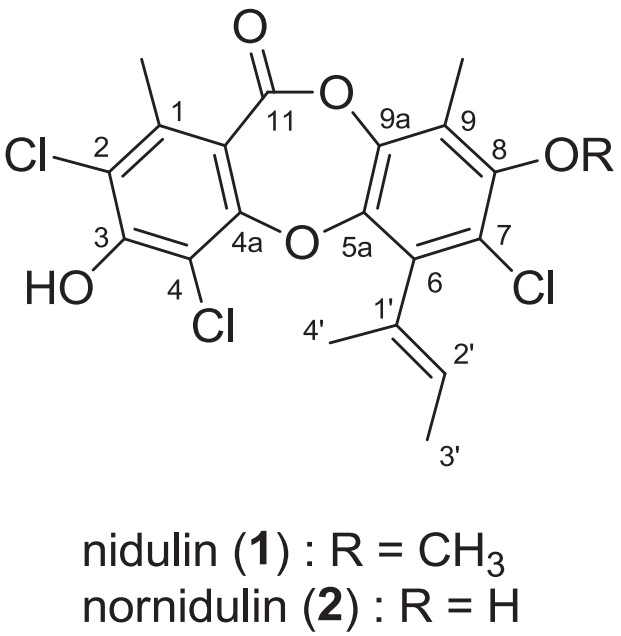

Fig. 1 Structures of nidulin and nornidulin

derivatives. Fermentation using a common media, potato dextrose broth (PDB), under static condition gave nornidulin but as a minor metabolite with unguinol as the major product. This result was probably due to the lack of chloride ion source in the liquid culture media. Similarly to the fermentation of a marine-derived *A. unguis* CRI-282-03 [3, 4], feeding NaCl worked fruitfully for ATCC 10032. Thus, addition of 2% (w/v) NaCl in PDB resulted in the enhanced production of nornidulin and nidulin as two major metabolites, while production of unguinol and other dechloro derivatives were reduced. Higher composition of NaCl (5%) resulted in serious inhibition of the mycelial growth. Gram-quantity of nornidulin was obtained using this simply optimized fermentation conditions and chromatographic separation/purification.

Reaction of nornidulin with one equivalent of alkyl, allylic, or benzylic halide (R-X; X = I, Br, Cl) and K_2CO_3 (2 equiv) in DMF at room temperature underwent in highly regioselective manner to give 8-*O*-substituted derivatives (**3**–**17**) (Fig. 2). Regioselectivity of the *O*-alkylation was confirmed by the NOESY correlation between 9-CH_3 and H_2-1″ for compound **5**, and by comparison of the ^{1}H and ^{13}C NMR spectroscopic data of the alkylated compounds with those of nidulin and nornidulin. A dimeric nidulin derivative **18** was synthesized by the reaction of **2** with a half equivalent of 1,4-diiodobutane. Nidulin derivatives with a pyridine ring (**19** and **20**) or a hydroxyl group (**21**) were also synthesized using the same procedure. A *tert*-butyl ester derivative **22** was converted into a carboxylic acid derivative **23** by deprotection with trifluoroacetic acid. Two 8-*O*-acylated derivatives, **24** and **25**, were obtained by the

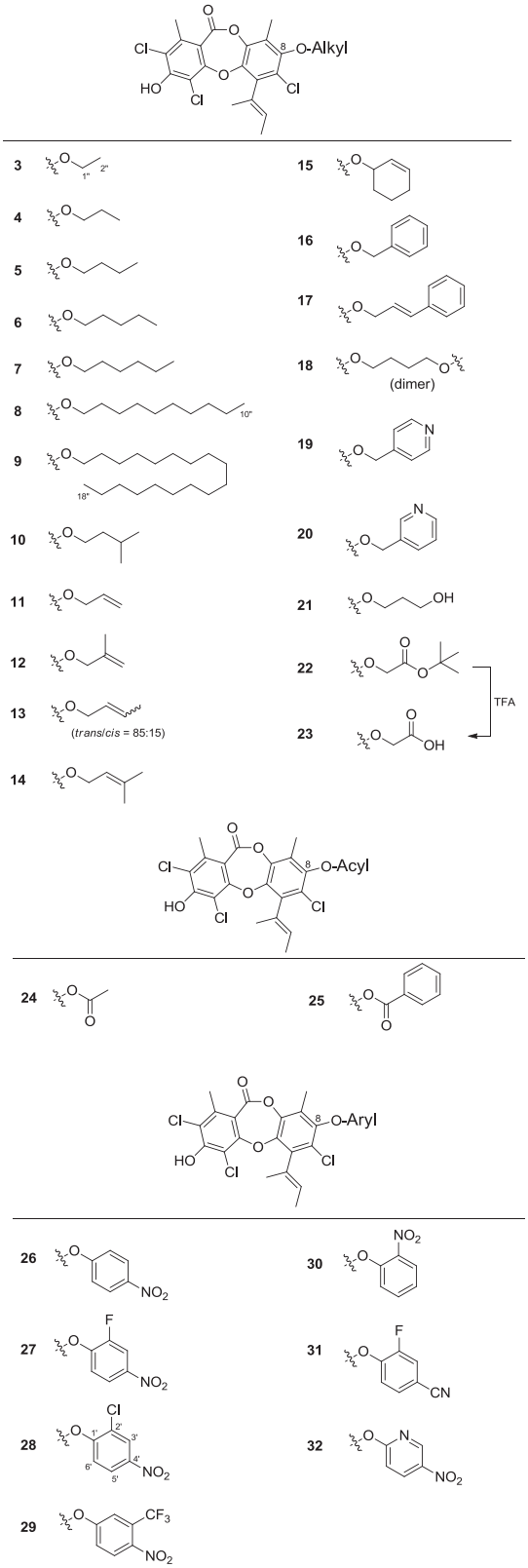

Fig. 2 Structures of the semisynthetic nidulin derivatives

reaction of **2** with acetic anhydride and benzoyl chloride, respectively. Several nornidulin 8-*O*-aryl ethers (**26**–**31**)

Table 1 Antibacterial activities and cytotoxicity of nidulin derivatives

Compound	Antibacterial activity (MIC_{90}, μg ml^{-1})				Cytotoxicity (IC_{50}, μg ml^{-1}) Vero cells[a]
	B. cereus[b]	*E. faecium*[c]	*S. aureus*[d]	MRSA[e]	
Nidulin (**1**)	1.56	3.13	6.25	4	26.5
Nornidulin (**2**)	6.25	6.25	12.5	NT	19.2
3	1.56	3.13	3.13	NT	32.8
4	0.781	1.56	3.13	NT	6.8
5	0.391	3.13	3.13	2	10.5
6	1.56	0.781	6.25	NT	3.3
7	1.56	1.56	25	NT	9.0
8	6.25	25	>50	NT	10.3
9	>25	>50	>50	NT	48.6
10	0.781	0.781	6.25	NT	3.4
11	0.781	3.13	3.13	NT	7.1
12	0.781	1.56	3.13	NT	8.1
13	1.56	1.56	3.13	NT	5.3
14	0.781	1.56	6.25	NT	5.9
15	1.56	0.781	3.13	NT	5.1
16	0.781	6.25	6.25	NT	2.2
17	0.781	1.56	25	NT	9.0
18	6.25	>50	50	NT	32.9
19	1.56	6.25	6.25	NT	7.4
20	6.25	50	50	NT	36.9
21	6.25	12.5	12.5	NT	41.6
22	0.781	3.13	6.25	NT	3.1
23	>25	>50	>50	NT	>50
24	3.13	6.25	12.5	NT	18.1
25	>25	>50	>50	NT	19.7
26	0.391	0.781	1.56	0.5	16.3
27	0.781	0.781	0.781	NT	4.0
28	0.781	0.391	0.781	1	4.5
29	0.781	0.781	1.56	NT	1.6
30	0.781	3.13	0.781	0.5	12.1
31	0.781	0.781	0.781	0.5	3.8
32	1.56	1.56	1.56	NT	6.5
Vancomycin·HCl	2.00	–	1.00	0.5	–
Rifampicin	–	1.56	0.0781		
Tetracycline·HCl	–	0.195	–		
Ellipticine					0.89

NT not tested

[a]African green monkey kidney fibroblasts

[b]*Bacillus cereus* ATCC 11778

[c]*Enterococcus faecium* ATCC 51559

[d]*Staphylococcus aureus* ATCC 29213

[e]Methicillin-resistant *Staphylococcus aureus* SK1

were synthesized by the reactions of **2** with 1 equivalent of fluorobenzenes bearing an electron-withdrawing group (–NO_2 or –CN) and K_2CO_3 (2 equiv), heating at 60 °C. Regioselectivity of the *O*-arylation was confirmed by the NOESY correlation between 9-CH_3 and H-2″/H-6″ for compound **28**, and by comparison of the ^1H and ^{13}C NMR spectroscopic data of the *O*-arylated compounds with those of nidulin and nornidulin. Similarly, a pyridyl ether

derivative **32** was synthesized from **2** and 2-chloro-5-nitropyridine.

The semisynthetic nidulin derivatives were tested for activities against Gram-positive bacteria, *B. cereus* ATCC 11778, *E. faecium* ATCC 51559, and *Staphylococcus aureus* ATCC 29213 [8], and cytotoxicity to nonmalignant Vero cells (African green monkey kidney fibroblasts) [9] (Table 1). Most of the 8-*O*-alkyl derivatives with short side-chain showed more potent antibacterial activity than nidulin. Among these derivatives, compound **5** (8-*O*-butyl) exhibited highest activity against *B. cereus* (MIC 0.391 μg ml^{-1}). Alkyl derivatives with long side chain, **8** and **9**, showed much weaker antibacterial activity. It should also be noted that certain level of steric bulkiness of the alkyl group may be accepted (**15**, **16**, and **22**). On the other hand, weak antibacterial activities of compounds **19**–**21**, and **23** suggested that polar functional group on the alkyl side-chain significantly reduces the activity. Acylated derivatives (**24** and **25**) exhibited weaker activity. All of the *O*-aryl derivatives (**26**–**32**) showed potent antibacterial activity.

To further demonstrate the antibacterial potential, nidulin (**1**, lead compound) and five high-activity derivatives were selected and tested for activity against Methicillin-resistant *S. aureus* SK1 (MRSA) [10] by one of the authors (S.P.). In particular, compounds **26**, **30**, and **31**, were significantly active also against MRSA (MIC 0.5 μg ml^{-1}).

Many of the synthetic compounds displayed weak to moderate cytotoxicity to Vero cells. Since the SAR pattern for cytotoxicity was different from that for antibacterial activities, they could potentially be distinguishable through synthetic design.

In conclusion, the present results demonstrate a possibility of the *Aspergillus* depsidone (nidulin) as a core structure for antibacterial agents. In particular, 8-*O*-aryl ether derivatives have been shown to exhibit significant activities against Gram-positive bacteria, including MRSA.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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