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Synthesis and antibacterial activity of nocathiacin I analogues

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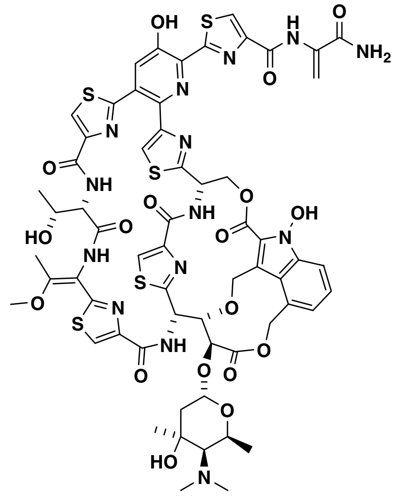
Abstract—Stereoselective reduction of dehydroalanine double bond in nocathiacin I afforded the primary amide **2**. Enzymatic hydrolysis of the amide **2** provided the carboxylic acid **3**, which upon coupling with a variety of amines furnished amides **4**–**32**. Some of these semi-synthetic derivatives have retained very good antibacterial activity and have improved aqueous solubility.

Antimicrobial resistance to current therapies is increasing at an overwhelming rate.[1] Infections caused by drug-resistant Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Enterococcus faecium* (MREF), vancomycin-resistant *Enterococci* (VRE), and penicillin-resistant *Streptococcus pneumoniae* (PRSP), once confined to hospitals, are spreading among the healthy people in larger communities. Consequently, there is an unmet medical need to develop new agents that combat these infections, including those caused by resistant bacteria. Preferably, to overcome the rapid cross-resistance development, these new agents either work by different mechanism or belong to a new class of compounds.

In an effort to develop a new antibacterial drug that would be effective against Gram-positive bacterial infections, we screened our natural product collections using multiply antibiotic-resistant strain of *Enterococcus faecium* whole cell-based assay. This screen resulted in the discovery of new class of thiazolyl peptide natural products known as nocathiacins. Nocathiacin I, a cyclic thiazolyl peptide antibiotic, was isolated from the fermentation broth of *Nocardia* sp. and fungus *Amicolaptosis* sp.[2] It displays a potent antibacterial activity against a variety of Gram-positive bacteria, including a number of multiple drug-resistant strains such as MRSA, MREF, VRE, and PRSP.[2,3a] It works by disrupting the bacterial protein biosynthesis by interacting directly with the L11 protein and 23S rRNA region of the ribosome.[3] In addition, nocathiacin I has desirable bactericidal activity against *S. aureus* and shows in vivo efficacy in a mouse systemic *S. aureus* infection model (Fig. 1).

Although nocathiacin I has an excellent spectrum of antibacterial activity, its inadequate aqueous solubility



1

Figure 1. Nocathiacin I.

Keywords: Nocathiacin I; Antibacterial activity.

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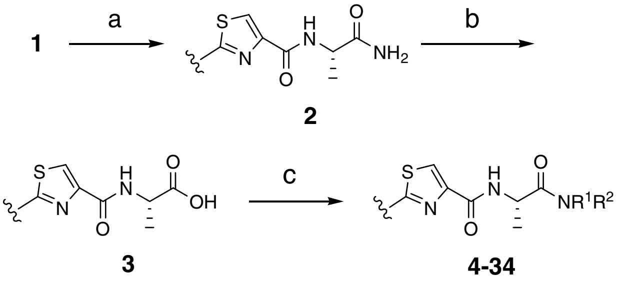
presents formidable challenge for its development as an intravenous (iv) drug. Therefore, we have undertaken an investigation to modify nocathiacin I to improve its aqueous solubility while maintaining its intrinsic biological activity. One of our approaches is to introduce polar water-solubilizing groups into the molecule through chemical transformation. In a series of publications, we have disclosed synthesis and antibacterial activity of several nocathiacin I analogues by derivatization at the indole and/or pyridine hydroxyl groups, and Michael addition of amines and thiols to the dehydroalanine side chain.[4] Most of these semi-synthetic nocathiacins have improved aqueous solubility and retained excellent in vitro and in vivo antibacterial activities of **1**. However, several hydroxyl-derived analogues suffered from poor stability, whereas Michael adducts are generated as a mixture of diastereomers thus increasing the cost of development for these adducts.

Consequently, we embarked upon developing an alternative route which could provide stable and diastereomerically pure analogues. To this end, we envisioned that stereoselective reduction of dehydroalanine double bond in **1** would provide the (*S*)-alanine amide **2** which could be hydrolyzed to carboxylic acid **3**. Then, coupling of **3** with amines would produce desired diastereomerically pure amides. Herein, we report the results of synthesis and antibacterial activities of stable and diastereomerically pure nocathiacin I analogues.

Semi-synthetic nocathiacin derivatives **4–32** were synthesized in three steps as shown in Scheme 1. We surveyed several catalysts for the asymmetric hydrogenation of dehydroalanine double bond to afford **2** and found that the rhodium-based homogeneous catalyst (*R*,*R*)-(−)-1,2-bis[(*o*-methoxypheny)(phenyl) phosphino]ethane(1,5-cyclooctadiene)rhodium(I) tetrafluoroborate ([Rh(COD)(*R*,*R*-DIPAMP)]$^+$ BF_4^-) consistently provided the primary amide **2** in >95% de and in quantitative yield.[5] We are gratified that the hydrolysis of the amide **2** to the carboxylic acid **3** took place uneventfully with an orange peel enzyme in aqueous solution.[6] This conversion was highly stereospecific and provided a single (*S*)-isomer. 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) or diphenylphosphoryl azide (DPPA)-mediated coupling of the carboxylic acid **3** with a variety of amines furnished amides **4–32** in good yields.[7]



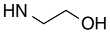
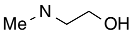
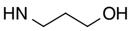
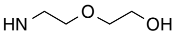
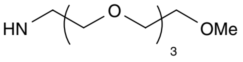
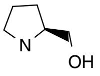
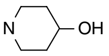
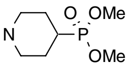
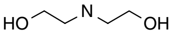
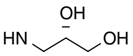
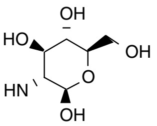
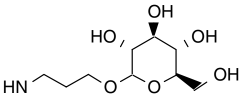
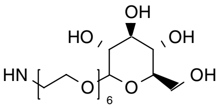
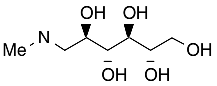
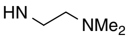
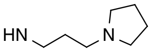
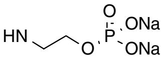
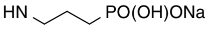
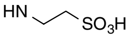
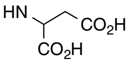
Scheme 1. Reagents and conditions: (a) catalytic [Rh(COD)(*R*,*R*-DIPAMP)]$^+$ BF_4^-, H_2 (60 psi), Pd/C; (b) orange peel enzyme/water/34 °C; (c) R^1R^2NH, EDC, PyBOP or DPPA, DMF.

The semi-synthetic nocathiacin I derivatives having diverse neutral, basic, and acidic polar functional groups were evaluated for their in vitro and in vivo antibacterial activity and results are summarized in Table 1. The semi-synthetic nocathiacins **4–11** having neutral polar groups such as hydroxyl, ether, and phosphonate have retained potent in vitro antibacterial activity of **1**. Furthermore, the in vivo potency of these compounds is comparable with that of the parent **1**. Interestingly, the amides derived from amines containing two or more hydroxyl groups have suffered from reduced in vitro and in vivo antibacterial activity. In particular, derivatives **15** and **16** derived from aminoalkyl-sugars are 100- to 1000-fold less potent than the parent against all strains tested.

Although analogues **18** and **19** with amino polar groups have reduced activity compared with **1**, still they possess good potency. As expected phosphate prodrug **20** has substantially reduced in vitro activity but maintained the in vivo potency of the parent **4**. However, introduction of non-cleavable acidic groups, derivatives **21–23**, had detrimental effect on the activity. Compound **24** derived from 4-hydroxybenzylamine has retained in vitro potency of the parent but has slightly reduced in vivo efficacy. Analogue **25** derived from 3,4-dihydroxybenzylamine has very good in vitro activity but has lost the in vivo potency compared with **1**. However, compound **26** derived from 3,5-dihydroxybenzylamine has significantly reduced activity. In addition, compounds derived from phenethylamines displayed similar trend. Analogue **27** derived from 4-hydroxy phenethylamine is as potent as **1**, whereas derivatives **28** and **29** have only moderate activity. Moreover, these derivatives have suffered from reduced in vivo potency too. 4-Aminoethylpyridine derivative **30** has moderate in vitro and in vivo activity, whereas analogues **31** and **32** with acidic groups have considerably reduced activity.

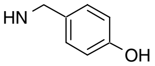
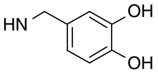
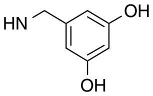
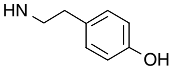
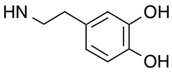
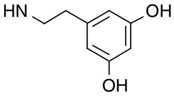
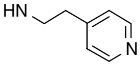
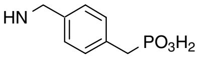
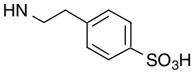
In summary, the desired diastereomerically pure semi-synthetic analogues containing polar functional groups were synthesized in three steps from the nocathiacin I by asymmetric hydrogenation, enzymatic hydrolysis, and amide bond formation. Several of these synthetic nocathiacins have very good antibacterial activity and improved water solubility. Analogues derived from amines containing single neutral polar groups have maintained very good potency, whereas those derived from amines containing two or more neutral polar groups or acidic functional groups have substantially reduced activity.

Table 1. In vitro and in vivo antibacterial activity of nocathiacin I analogues

Compound	NR^1R^2	MIC[a] (μg/mL)			PD_{50}[b] (mg/kg)	Solubility[c] mg/mL (pH)
		S. aureus A15090 (MSSA)[d]	*S. pneumo* A28272 (PRSP)	*E. faecalis* A20688 (MSEF)[d]		
1	—	0.007	0.002	0.03	0.8	0.34 (4.0)
2	NH_2	0.25	0.06	2	1.65	0.7 (3.1)
4		0.03	0.001	0.5	1.44	1.3 (3.1)
5		0.06	0.003	0.03	1.65	2.3 (3.1)
6		0.06	0.007	0.03	1.25	2.7 (2.8)
7		0.03	0.015	0.25	1	>2.9 (3.1)
8		0.06	0.003	0.5	ND	ND
9		0.03	0.015	0.25	2.18	2.2 (3.1)
10		0.007	0.003	0.06	4.35	1.1 (3.2)
11		0.015	0.015	0.03	ND	>3.0 (3.3)
12		0.25	0.25	0.5	>10	>1.2 (3.4)
13		0.25	0.03	0.25	5	>2.2 (3.1)
14		1	0.06	0.5	>10	>2.2 (3.1)
15		4	1	8	ND	>2.0 (2.8)
16		4	1	8	ND	>3.7 (2.9)
17		0.125	0.015	0.5	ND	>2.4 (3.2)
18		0.125	0.125	0.25	ND	>2.4 (3.5)
19		0.25	0.25	0.5	ND	>2.6 (3.3)
20		8	0.5	16	1.65	>4.8 (7.4)
21		8	0.25	32	ND	>1.7 (8.1)
22		0.25	0.03	0.25	>10	>2.1 (8.4)
23		16	0.25	16	ND	ND

(*continued on next page*)

Table 1 (*continued*)

Compound	NR^1R^2	MIC[a] (μg/mL)			PD_{50}[b] (mg/kg)	Solubility[c] mg/mL (pH)
		S. aureus A15090 (MSSA)[d]	*S. pneumo* A28272 (PRSP)	*E. faecalis* A20688 (MSEF)[d]		
24		0.007	0.007	0.03	4.35	0.48 (3.1)
25		0.03	0.007	0.06	>10	0.02 (2.9)
26		1	0.25	2	ND	<0.0003 (3.8)
27		0.003	0.0005	0.007	>10	0.12 (3.2)
28		0.25	0.125	0.5	>10	0.007 (3.0)
29		0.25	0.06	0.5	>10	0.02 (3.0)
30		0.125	0.015	0.06	3.3	>3.4 (3.0)
31		1	0.25	2	>10	>2.6 (7.4)
32		0.5	0.125	1	>10	>1.2 (8.7)

ND means not determined.

[a] MICs (minimum inhibitory concentration): lowest concentration of drug that inhibits visible growth of the organism.[8]

[b] PD_{50} determined by mouse systemic lethal *S. aureus* infection model.[9]

[c] Equilibrium water solubility was determined with amorphous powders.

[d] MSSA, methicillin-sensitive *S. aureus*; MSEF, methicillin-sensitive *E. faecalis*.

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7. Representative experimental procedure: To a stirred solution of carboxylic acid **3** (0.318 g, 0.214 mmol), HOBt (0.040 g, 0.3 mmol), DIEA (52 μL, 0.3 mmol), DMAP (5 mg), and ethanolamine (30 μL, 0.5 mmol) in anhydrous DMF (4 mL) at room temperature was added EDC (0.058 g, 0.3 mmol). The reaction mixture was stirred for 8 h and purified on preparative HPLC using MeOH/water containing 0.1% TFA as an eluent. The fractions containing the desired product were combined, concentrated and the aqueous solution was freeze-dried (lyophilized) to give the product (0.071 g) as a yellow fluffy solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.92 (1H, s), 10.76 (1H, s), 9.11 (1H, s), 9.09 (1H, s), 8.65 (1H, s), 8.61–8.56 (2H, m), 8.54 (1H, s), 8.50 (1H, s), 8.22 (1H, s), 8.01 (1H, s), 7.98 (1H, t, $J = 5.6$ Hz), 7.90 (1H, s), 7.86 (1H, d, $J = 11.0$ Hz), 7.75 (1H, d, $J = 8.6$ Hz), 7.38–7.35 (2H, m), 7.20 (1H, d, $J = 7.0$ Hz), 6.42–6.35 (1H, br s), 6.03 (1H, d, $J = 12.2$ Hz), 5.77–5.70 (2H, m), 5.25–5.21 (1H, m), 5.08–5.04 (2H, m), 5.01 (1H, d, $J = 7.6$ Hz), 4.81 (1H, d, $J = 10.1$ Hz), 4.57–4.52 (2H, m), 4.30 (1H, d, $J = 9.8$ Hz), 4.27–4.23 (1H, m), 4.20–3.75 (4H, br) 4.16 (1H, d, $J = 10.7$ Hz), 4.05 (1H, dd, $J = 9.3$, 1.4 Hz), 3.91 (3H, s), 3.42 (1H, t, $J = 6.2$ Hz), 3.19–3.12 (3H, m), 2.89 (3H, s), 2.87 (3H, s), 2.15–2.10 (1H, m), 2.00 (3H, s), 1.95 (1H, d, $J = 14.9$ Hz), 1.60 (3H, s), 1.39 (3H, d, $J = 7.0$ Hz), 1.16 (3H, br s), 0.81 (3H, d, $J = 7.0$ Hz). HRMS (ESI) calcd for $C_{63}H_{67}N_{14}O_{19}S_5$ (M+H): 1483.331; found: 1483.328.

8. The minimum inhibitory concentration (MIC) of a compound was obtained against a panel of bacteria using a conventional broth dilution assay in accordance with standards recommended by the National Committee for Clinical Laboratory Standards (NCCLS). The serial micro broth dilution method used Muller–Hinton medium except for the *Streptococcus pneumoniae* which was tested in 50% Muller–Hinton medium and 50% Todd–Hewitt medium. The final bacterial inoculum contained approximately 5×10^5 CFU/well and was run on microtiter plates. The volume of each well was 100 μL and the plates were inoculated at 35 °C for 18 h in ambient air.

9. PD_{50} is the amount of drug required (mg/kg) to cure 50% of infected mice subjected to a lethal systemic infection of *Staphylococcus aureus*. Adult female ICR mice were inoculated intraperitoneally with $5\text{–}6 \times 10^6$ CFU overnight culture of *S. aureus* A15090 strain suspended in 7% sterile hog gastric mucin. Drug was prepared in a 10% DMSO/5% Tween 80/85% water vehicle and administered subcutaneously, twice daily at 1 and 4 h after pathogen inoculation. The number of mice that survived in each experimental group was monitored up to 8 days after pathogen inoculation, and the 50% protective doses (PD_{50}s) of the drug-treated animals were determined by the Spearman–Karber nonparametric estimator method. Each experimental group consisted of 10 animals and a minimum of three different concentrations of drug was evaluated per compound.