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Synthesis, in vitro, and in vivo antibacterial activity of nocathiacin I thiol-Michael adducts

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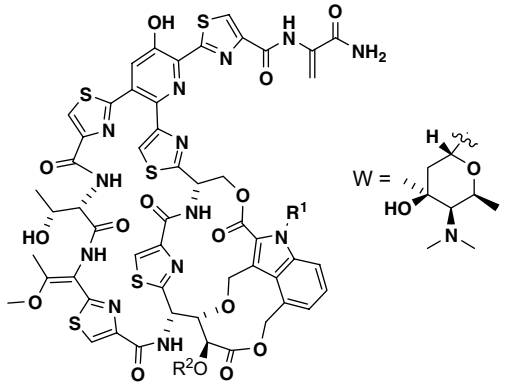
Abstract—The synthesis and antibacterial activity of a series of nocathiacin I derivatives (**4**–**20**) containing polar water solubilizing groups is described. Thiol-Michael adducts containing acidic polar groups have reduced antibacterial activity whereas those with basic polar groups have retained very good antibacterial activity.

Antibacterial resistance to hospital-acquired Gram-positive bacterial pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), has been increasing at an alarming rate.[1] Furthermore, these drug-resistant bacteria commonly infect healthy people in larger communities, thus creating a serious health problem around the globe.



1: Nocathiacin I: R^1 = OH; R^2 = W
2: Nocathiacin II: R^1 = H; R^2 = W
3: Nocathiacin III: R^1 = OH; R^2 = H

Figure 1. Nocathiacins I–III.

Consequently, the need to develop new agents that combat bacterial infections, including those resistant to existing therapies, are continuing to grow.

Nocathiacin I (**1**), a powerful antibiotic that belongs to a new class of tricyclic thiazolyl natural products, was independently discovered by two research groups (Fig. 1).[2] This antibiotic is closely related to nosiheptide and possesses very potent antibacterial activity against Gram-positive bacteria including several multiple drug-resistant strains such as MRSA, methicillin-resistant *Enterococcus faecium* (MREF), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and VRE.[2,3a] In addition, it exhibits an excellent in vivo efficacy and has bactericidal activity against *S. aureus*. The antibacterial activity of **1** is due to its disruption of bacterial protein synthesis by interacting directly with the 23S rRNA region of the ribosomal protein L11.[3]

Although nocathiacin I exhibits an excellent antibacterial profile, it has very poor aqueous solubility thus preventing its development as an intravenous (iv) drug. In an effort to enhance the aqueous solubility, we decided to introduce polar water solubilizing groups into the molecule through derivitization.[4] In this article; we describe the synthesis and antibacterial activity of thiol-Michael adducts of **1**.

Our attempts to prepare conjugate thiol adducts of **1** using known conditions were unsuccessful.[5] Under these conditions several unidentified products were formed, presumably due to the presence of very labile functional

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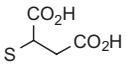
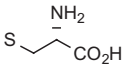
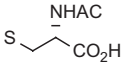
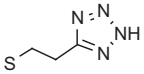
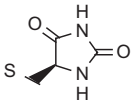
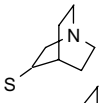
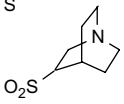
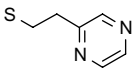
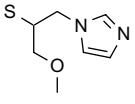
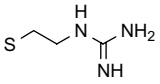
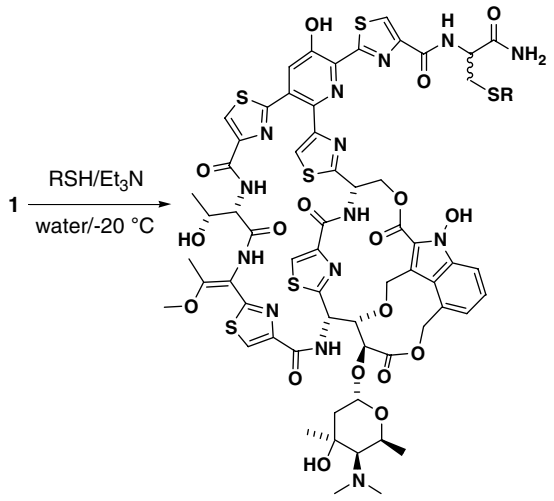
groups in **1**. Consequently, we investigated alternative conditions and found that Michael addition worked well in the presence of Et_3N in water. Furthermore, the Michael addition of thiols to **1** was more efficient and consistently provided higher isolated yields when carried out in frozen-water (Scheme 1).[5] In a typical procedure, a homogeneous reaction mixture obtained by stirring **1**, Et_3N and an appropriate thiol was frozen in a freezer maintained at −20 °C (Scheme 1).[6] Once the reaction is completed, the reaction mixture is acidified with aqueous HCl, warmed to room temperature, and purified. Under these conditions, thiols containing both acidic and basic functional groups have undergone conjugate additions and have provided Michael adducts in very good yields (Table 1). Thiol-Michael adducts were generated as an inseparable 1:1 mixture of diastereomers.

Table 1. In vitro and in vivo antibacterial activity of nocathiacin I thiol-Michael adducts

Compound	RS/RSO$_2$	MIC (μg/mL)[a]			PD_{50}[b] (mg/kg)	Solubility,[c] mg/mL (pH)
		S. aureus A15090 (MSSA)[d]	*S. pneumoniae* A28272 (PRSP)	*E. faecalis* A20688 (MSEF)[d]		
1	Nocathiacin I	0.007	0.002	0.03	0.8	0.34 (4.0)
Thiols adducts with acidic polar group						
4	SCH_2CO_2H	2	0.125	1	>10	0.22 (8.0)
5	$SCH_2CH_2CO_2H$	1	0.01	?	>10	0.014 (7.3)
6		4	0.25	4	>10	2.0 (6.7)
7		1	2	4	ND[e]	>3.1 (2.9)
8		2	0.125	8	>10	0.4 (7.4)
9		2	0.25	32	>10	0.001 (3.8)
10		4	0.03	128	>10	0.284 (3.4)
11	$SCH_2CH_2SO_3Na$	4	0.06	128	>10	>3.6 (8.6)
12	$SCH_2CH_2PO(ONa)OH$	2	0.25	32	ND[e]	>3.1 (7.1)
Thiols adduct with basic polar group						
13	$SCH_2CH_2NMe_2$	0.125	0.015	0.25	1	>3.7 (5.7)
14	$SCH_2CH_2NEt_2$	0.125	0.015	0.25	1.1	>10 (3.9)
15	$SO_2CH_2CH_2NEt_2$	0.06	0.007	0.03	1.4	11.5 (3.3)
16		2	0.25	2	2.5	>2.6 (4.1)
17		0.5	0.06	1	ND[e]	2.8 (4.3)
18		0.125	0.015	0.25	4.4	0.23 (4.1)
19		0.25	0.03	0.125	1.4	>12.8 (4.3)
20		4	2	>128	ND[e]	>2.3 (4.0)

[a] MICs (minimum inhibitory concentration): lowest concentration of the drug that inhibits visible growth of the organism.[7]
[b] PD_{50} determined by mouse systemic lethal *S. aureus* infection model.[8]
[c] Equilibrium water solubility was determined with amorphous powders.
[d] MSSA: methicillin-sensitive *S. aureus*; MSEF: methicillin-sensitive *E. faecalis*.
[e] ND: not determined.



Scheme 1.

Oxidations of sulfide adducts **14** and **16** with sodium tungstate dihydrate/30% aqueous hydrogen peroxide at room temperature provided the sulfones **15** and **17** in high yield.

Thiol-Michael adducts of **1** possessing polar functional groups were screened for their in vitro activity (MIC) against a panel of Gram-positive bacteria.[7] In addition, these compounds were evaluated for their in vivo efficacy (PD_{50}) against a lethal *S. aureus* systemic infection model in mouse.[8] The in vitro and in vivo antibacterial activity, and solubility data of these adducts **4**–**20** are summarized in Table 1.

Although thiol-Michael adducts **4**–**12** carrying polar acidic groups have good in vitro activity against *S. pneumoniae*, these derivatives have significantly reduced in vitro activity against all strains evaluated when compared to **1**. In addition, the in vivo potency of these adducts are found to be substantially less than that of **1**. This unexpected loss of in vivo activity of these derivatives may partly be due to the poor cell penetration of these analogues because they may exist as zwitterions at the physiological pH.

In general Michael adducts derived from thiols containing basic groups are more potent than those with acidic groups. For example, adducts **13**–**15** containing dialkylamino groups have good in vitro antibacterial activity and are as potent as **1** against a lethal *S. aureus* systemic infection model in mouse. Similarly, analogues **18** and **19** have good in vitro activity and in vivo efficacy. However, adducts **16**, **17**, and **20** have substantially reduced in vitro antibacterial activity when compared to **1**.

In summary, we have prepared several thiol-Michael adducts of nocathiacin I containing both acidic and basic polar groups and evaluated them against several bacterial strains. Adducts with acidic functional groups have reduced in vitro and in vivo antibacterial activity when compared with the parent. In contrast, several analogues with basic polar groups have retained good in vitro activity and in vivo potency of the parent. Furthermore, most of these analogues have improved aqueous solubility when compared with the parent and displayed good solution stability up to 24 h at ambient temperature.

Acknowledgements

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6. Representative experimental procedure: To a stirred suspension of Nocathiacin I (0.2875 g, 0.20 mmol) and 2-(*N*,*N*-dimethylamino)ethanethiol·HCl (0.284 g, 2.0 mmol) in deionized water (10 mL) at room temperature was added Et_3N (0.35 mL, 2.5 mmol). The reaction mixture was stirred until it turns into a clear homogeneous solution (~5 min). Then, the reaction mixture was left in the freezer maintained at −20 °C until the reaction was completed (24 h) as judged by HPLC analysis. Then, aqueous HCl (1 N, 4 mL) was added to the frozen solid reaction mixture, warmed to room temperature and purified using MPLC on preparative C-18 column using 10–50% acetonitrile/water containing trace HCl as eluent. The fractions containing the desired

product were combined, concentrated, and the aqueous solution was freeze dried (lyophilized) to give the product as a yellow fluffy solid (0.277 g, 87%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.77 (1H, s), 9.21 (1H, dd, J = 8.55, 2.44 Hz), 9.12 (1H, s), 8.66 (1H, s), 8.60 (1H, d, J = 8.55 Hz), 8.53 (2H, s), 8.25 (1H, s), 8.02 (1H, d, J = 2.14 Hz), 7.91 (1H, d, J = 4.58 Hz), 7.87 (1H, d, J = 10.99 Hz), 7.75 (1H, d, J = 8.55 Hz), 7.73 (1H, br s), 7.38–7.32 (3H, m), 7.19 (1H, d, J = 7.02 Hz), 6.01 (1H, d, J = 12.20 Hz), 5.75 (1H, dd, J = 11.15, 4.14 Hz), 5.70 (1H, d, J = 8.54 Hz), 5.25–5.20 (1H, m), 5.09–5.04 (2H, m), 5.00 (1H, br s), 4.78 (1H, d, J = 10.38 Hz), 4.71–4.66 (1H, m), 4.53 (1H, d, J = 11.29 Hz), 4.31 (1H, d, J = 9.77 Hz), 4.26 (1H, t, J = 5.95 Hz), 4.14 (1H, d, J = 10.37 Hz), 4.04 (1H, d, J = 9.15 Hz), 3.91 (3H, s), 3.82 (1H, br s), 3.34 (6H, s), 3.31–3.20 (2H, m), 3.16 (1H, dd, J = 14.04, 3.36 Hz), 2.98–2.87 (3H, m), 2.74 (3H, s), 2.72 (3H, s), 2.72–2.64 (2H, m), 2.54 (1H, s), 2.44 (1H, br s), 2.07–2.03 (1H, m), 2.00 (3H, s), 1.87 (1H, d, J = 13.43 Hz), 1.51 (3H, s), 1.17 (3H, s), 0.68 (3H, s). HRMS (ESI) calcd for $C_{65}H_{72}N_{15}O_{18}S_6$ (M+H): 1542.3504; found 1542.354. Anal. Calcd for $C_{65}H_{71}N_{15}O_{18}S_6 \cdot 1.7HCl \cdot 4H_2O$: C, 46.56; H, 8.45; N, 12.53; Cl, 3.59; found: C, 46.26; H, 8.46; N, 12.41; Cl, 3.16.

7. 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 *S. 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.
8. PD_{50} is the amount of drug required (mg/kg) to cure 50% of infected mice subjected to a lethal systemic infection of *S. aureus*. Adult female ICR mice were inoculated intraperitoneally with $5–6 \times 10^6$ 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 administrated 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 the drug was evaluated per compound.