Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 5573–5577
Bioorganic & Medicinal Chemistry Letters

Nocathiacin I analogues: synthesis, in vitro and in vivo biological activity of novel semi-synthetic thiazolyl peptide antibiotics

B. Narasimhulu Naidu,* Margaret E. Sorenson, Yunhui Zhang, Oak K. Kim, John D. Matiskella, John A. Wichtowski, Timothy P. Connolly, Wenying Li, Kin S. Lam, Joanne J. Bronson, Michael J. Pucci, Junius M. Clark and Yasutsugu Ueda

The Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA

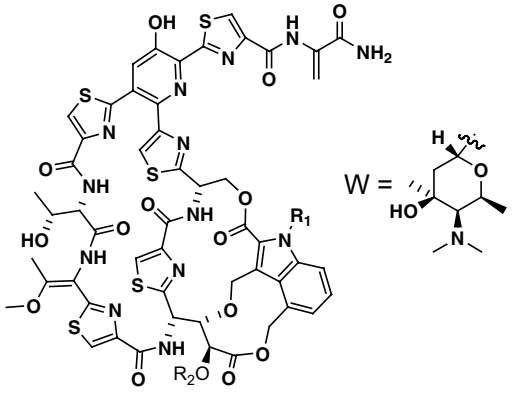
Received 2 August 2004; revised 20 August 2004; accepted 26 August 2004
Available online 18 September 2004

Abstract—Several nocathiacin I analogues (**4**–**35**) were synthesized and evaluated for their antibacterial activity. Most of these semi-synthetic analogues retained very good in vitro and in vivo antibacterial activity of **1**.

1. Introduction

Thiazolyl peptide antibiotics are highly modified cysteine-containing macrocyclic peptides with several distinctive common features: the presence of thiazole rings, unusual amino acids, dehydro amino acids, and a highly substituted pyridine centerpiece. Even though these antibiotics have been known for nearly half-a-century, so far no thiazolyl peptide has entered clinical study for human use, presumably due to poor in vivo activity. The most prominent members of this class of antibiotics are thiostrepton,[1] which is used for the treatment of bovine mastisis, and nosiheptide.[2]

Recently, two groups independently discovered a new class of thiazolyl peptide antibiotics known as nocathiacins (Fig. 1).[3–5] These compounds are structurally related to glycothiohexide α,[6] a nosiheptide-type thiazolyl peptide. Nocathiacins display potent antibacterial activity against a variety of Gram-positive bacteria, including a number of multiple drug resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Enterococcus faecium* (MREF), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *Enterococcuus faecium* (VREF).[3,5,7a] In addition, nocathiacin I (**1**) shows in vivo efficacy in a mouse systemic *S. aureus* infection model and possesses desirable bactericidal activity.[3,7] Like other thiazolyl peptide antibiotics, nocathiacins disrupt bacterial protein biosynthesis by interacting directly with the 23S rRNA region of the ribosomal protein L11.[7a,8]



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.

Keywords: Nocathiacins; Conjugate addition; Frozen water; Antibacterial activity.
* Corresponding author. Tel.: +1 203 677 6574; fax: +1 203 677 7702; e-mail: narasimhulu.naidu@bms.com

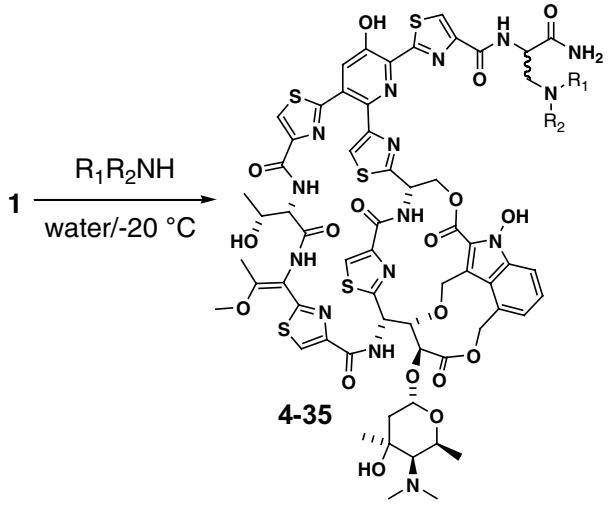
0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.08.058

As a part of our efforts to develop new antibacterial agents active against resistant bacteria and suitable for intravenous (iv) administration, we embarked on an investigation to modify the nocathiacins to improve their aqueous solubility while maintaining their intrinsic biological activity. To this end, we decided to introduce polar water-solubilizing groups into the molecule through chemical transformation.[9] Examination of the nocathiacin structure suggests several sites for possible chemical modification such as the hydroxypyridine, N-hydroxyindole, and dehydroalanine side chain. In this paper, we describe the synthesis and antibacterial activity of novel derivatives of **1** obtained by the conjugate addition of amines to the dehydroalanine side chain.

2. Chemistry and biology results

Initial efforts to carry out conjugate additions between **1** and amines in organic solvents were unsuccessful and provided several unidentified products, presumably due to the presence of very labile functional groups in **1**. Consequently, we investigated other conditions and found that conjugate additions worked well in water. Most interestingly, we found that conjugate additions were more efficient and consistently provided higher isolated yields when carried out in frozen water (Scheme 1).[10,11] In a typical procedure,[12] a homogeneous reaction mixture obtained by stirring **1** and an appropriate amine was frozen in a freezer maintained at −20 °C. Once the reaction is completed, the reaction mixture is acidified with aqueous HCl, warmed to room temperature, and purified. Under these conditions primary, secondary, and cyclic amines with diverse functional groups have undergone conjugate additions and provided moderate to good isolated yields of nocathiacin analogues **4**–**35** (Table 1).

The analogues of **1** possessing polar functional groups were screened for in vitro activity (MIC) against a panel of Gram-positive bacteria.[13] In addition, these compounds were evaluated for their in vivo efficacy (PD_{50}) against a lethal *S. aureus* systemic infection model in mouse.[14] The in vitro and in vivo antibacterial activity of compounds **4**–**35** are summarized in Table 1.



Scheme 1.

The methylamine analogue **4** has significantly reduced in vitro activity against *S. aureus* and *E. faecalis* but retained excellent in vivo efficacy. Furthermore, ethylenediamine analogues **5**–**7** also have substantially decreased antibacterial activity when compared to the parent nocathiacin. Interestingly, PEG-analogue **8** has moderate in vitro and in vivo activity whereas amino-PEG derivative **9** has significantly reduced antibacterial activity compared with **1**. Analogues **10**–**12**, with an additional heterocycle, exhibited moderate to poor in vitro antibacterial activity. Even though compounds **10** and **11** have lost significant in vivo activity, the in vivo efficacy of analogue **12** is comparable with **1**.

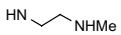
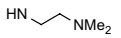
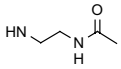
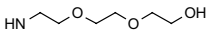
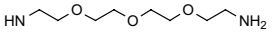
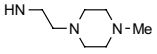
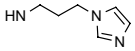
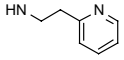
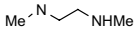
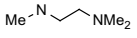
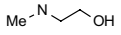
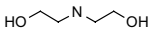
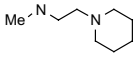
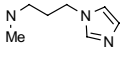
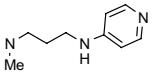
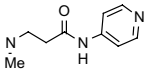
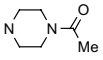
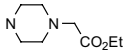
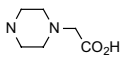
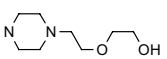
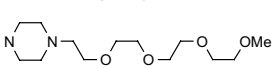
In general the secondary amine derived nocathiacin analogues are more potent than primary amine derived analogues. For example, the dimethylamine analogue **13** is much more potent than methylamine adduct **4** and has an excellent in vitro antibacterial activity and in vivo efficacy. Similarly, N,N′-dimethylethylenediamine and N,N,N′-trimethylethylenediamine derivatives, **14** and **15**, respectively, are more potent than the corresponding primary amine derived analogues **5** and **6**. The 2-(methylamino)ethanol analogue **16** exhibited antibacterial profiles comparable to **1** whereas diethanolamine analogue **17** has moderate in vitro activity and in vivo efficacy. Analogues **18**–**21** with an additional heterocycle have decreased antibacterial activity when compared with the parent nocathiacin.

Introduction of cyclic amines into the side chain also has no significant detrimental effect on the antibacterial activity of **1**. For instance, pyrrolidine and 4-hydroxypiperidine analogues **22** and **23** have very potent antibacterial activity and also have excellent in vivo efficacy. Furthermore, the in vitro and in vivo antibacterial activities of morpholine analogue **24** are comparable to those of the parent nocathiacin.

Introduction of piperazines into the side chain decreased in vitro activity for some analogues up to 100-fold relative to **1**. On the other hand, most of these piperazine analogues have retained good in vivo activity of the parent. Furthermore, from the data in Table 1, it is clear that a variety of substituents on the distal piperazine nitrogen are well tolerated. Replacing the methyl group on the piperazine distal nitrogen with the electron withdrawing acetyl group has resulted in modest loss of activity (cf. **25** vs **26**) whereas replacing it with the carboethoxymethyl group improved in vitro activity (cf. **25** vs **27**). Interestingly, compound **28** with 2-(piperazin-1-yl)acetic acid has significantly reduced antibacterial activity as compared to ester analogue **27**. This adverse effect may partially be due to poor bacterial cell penetration because of the zwitterionic nature of **28**.

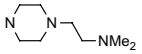
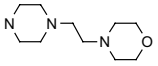
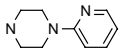
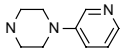
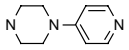
Analogues **29** and **30** with two or more glycol units on the distal piperazine nitrogen have retained moderate antibacterial activity against *S. aureus* and *S. pneumoniae* but have significantly reduced activity against

Table 1. In vitro and in vivo antibacterial activity of semi-synthetic nocathiacin I analogues (**4**–**35**)

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)[d]	*E. faecalis* A20688 (MSEF)[d]		
1	Nocathiacin I	0.007	0.001	0.03	0.8	0.34 (4.0)
Primary amine analogues						
4	NHMe	2	0.001	8	1.6	>3.5 (2.9)
5		0.25	0.03	0.5	ND[e]	>2.5 (2.6)
6		2	0.03	4	4.2	>3.8 (3.1)
7		1	0.125	4	>10	>3.0 (3.1)
8		0.25	0.06	0.5	4.6	>3.1 (3.3)
9		1	0.25	1	>10	>2.8 (3.2)
10		0.25	0.015	4	>10	>2.9 (2.8)
11		0.5	0.06	1	10	>10.0 (3.2)
12		1	0.25	1	2.8	>10.0 (3.4)
Secondary amine analogues						
13	NMe_2	0.06	0.03	0.125	<0.62	>10.0 (3.3)
14		0.06	0.001	0.5	>10	>2.8 (3.0)
15		0.06	0.007	0.5	3.3	>10.0 (2.9)
16		0.015	0.015	0.06	0.8	>10.0 (3.4)
17		0.125	0.015	0.25	5	>1.7 (2.9)
18		0.125	0.03	1	3.2	>2.7 (3.0)
19		0.125	0.015	0.125	5	>10.6 (3.9)
20		1.0	0.25	0.5	>10	>3.0 (3.0)
21		0.5	0.03	0.5	8.7	>3.0 (2.8)
Cyclic amine analogues						
22		0.06	0.015	0.5	2.5	>3.0 (2.9)
23		0.06	0.007	0.25	2.1	>2.7 (3.8)
24		0.007	0.015	0.015	0.8	>2.1 (3.1)
25		0.25	0.125	0.5	1.4	>10.0 (3.4)
26		0.5	0.03	2.0	5.0	>2.4 (2.7)
27		0.06	0.007	0.25	ND[e]	ND[e]
28		1.0	0.03	1.0	>10	>2.6 (3.3)
29		0.5	0.125	1.0	1.34	>3.0 (3.1)
30		0.5	0.25	4.0	1.5	>1.5 (3.5)

(*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)[d]	*E. faecalis* A20688 (MSEF)[d]		
31		0.5	0.25	0.125	5.3	>3.8 (3.4)
32		0.25	0.06	0.5	1.4	>12.3 (3.0)
33		0.125	0.03	0.25	<0.62	>2.4 (3.0)
34		0.06	0.007	0.25	1.43	>2.3 (3.0)
35		0.25	0.06	0.5	4.5	12.5 (3.0)

[a] MICs (minimum inhibitory concentration): lowest concentration of drug that inhibits visible growth of the organism.[13]
[b] PD_{50} determined by mouse systemic lethal *S. aureus* infection model.[14]
[c] Equilibrium water solubility was determined with amorphous powders.
[d] MSSA: methicillin-sensitive *S. aureus*; MSEF: methicillin-sensitive *E. faecalis*.
[e] ND means not determined.

E. faecalis. The 1-(2-dimethylaminoethyl) piperazine analogue **31** has modest antibacterial activity whereas analogue **32**, with 1-(2-morphilinylethyl) piperazine, has good in vitro activity and the in vivo activity is comparable to that of the parent nocathiacin **1**. An interesting trend in the antibacterial activity was observed with 1-pyridylpiperazine analogues **33**–**35**. The in vitro activity of the 1-(2-pyridyl) piperazine analogue **33** is about 10-fold weaker but the in vivo efficacy is comparable to **1**. On the other hand, the antibacterial profiles of the 1-(3-pyridyl) piperazine analogue **34** are comparable to those of **1** whereas the 1-(4-pyridyl) piperazine analogue **35** has significantly reduced antibacterial activity in comparison to that of the parent nocathiacin **1**.

In summary, in order to improve the aqueous solubility, we prepared several amine containing derivatives via conjugate addition of amines with diverse polar groups to the side chain of nocathiacin I. In general, analogues derived from primary amines have reduced antibacterial activity, whereas several analogues derived from secondary amines and cyclic amines have comparable antibacterial activity profiles to the parent nocathiacin. In addition, most of these compounds have excellent aqueous solubility at pH 3–5. Based on the overall in vitro and in vivo antibacterial activity, aqueous solubility, pharmacokinetics, stability, and ease of synthesis, analogue **16** (BMS-411886) was selected as a lead candidate for further safety evaluation.

Acknowledgements

We thank members of the Process Research Group at New Brunswick for the supply of nocathiacin I, and Microbiology, Pharmaceutics, and Discovery Analytical groups at Wallingford for their support in obtaining the data.

References and notes

1. (a) Vandeputte, J.; Dutcher, J. D. *Antibiot. Annu.* **1956**, *1955/1956*, 560–561; (b) Anderson, B.; Hodgkin, D. C.; Viswamitra, M. A. *Nature* **1970**, *225*, 233–235.
2. Pascard, C.; Ducruix, A.; Lunel, J.; Prange, T. *J. Am. Chem. Soc.* **1977**, *99*, 6418–6423.
3. Li, W.; Leet, J. E.; Ax, H. A.; Gustavson, D. R.; Brown, D. M.; Turner, L.; Brown, K.; Clark, J.; Yang, H.; Fung-Tomc, J.; Lam, K. S. *J. Antibiot.* **2003**, *56*, 226–231.
4. Leet, J. E.; Li, W.; Ax, H. A.; Matson, J. A.; Huang, S.; Huang, R.; Cantone, J. L.; Drexler, D.; Dalterio, R. A.; Lam, K. S. *J. Antibiot.* **2003**, *56*, 232–242.
5. Sasaki, T.; Otani, T.; Matsumoto, H.; Unemi, N.; Hamada, M.; Takeuchi, T.; Hori, M. *J. Antibiot.* **1998**, *8*, 715–721.
6. Northcote, P. T.; Siegel, M.; Borders, D. B.; Lee, M. D. *J. Antibiot.* **1994**, *47*, 901–908.
7. (a) Ueda, Y.; Barrett, J.; DenBleyker, K.; Fung-Tomc, J.; Discotto, L.; Stickle, T.; Kolek, B.; Pucci, M. *Abstracts of Papers*, 43rd Annual ICAAC Meeting, Chicago, IL, Sept 14–17; American Society for Microbiology: Washington, DC, 2003; F-1842; (b) Yang, H.; Chaniewski, S.; Clark, J.; Ferraro, C.; Gali, V.; Lamb, L.; Medina, I. *Abstracts of Papers*, 43rd Annual ICAAC Meeting, Chicago, IL, Sept 14–17; American Society for Microbiology: Washington, DC, 2003; B-319.
8. (a) Xing, Y. Y.; Draper, D. E. *Biochemistry* **1996**, *35*, 1581–1588; (b) Draper, D. E. In *Protein-RNA Recognition in a Highly Conserved Ribosomal Domain Targeted by Thiazole Antibiotics*; Draper, D. E., Ed.; Academic: San Diego, 1998; pp 113–125; (c) Heffron, S. E.; Jurnak, F. *Biochemistry* **2000**, *39*, 37–45.
9. (a) Hrnciar, P.; Ueda, Y.; Huang, S.; Leet, J. E.; Bronson, J. J. *J. Org. Chem.* **2002**, *67*, 8789–8793; (b) Regueiro-Ren, A.; Naidu, B. N.; Zheng, X.; Hudyma, T. W.; Connolly, T. P.; Matiskella, J. D.; Zhang, Y.; Kim, O. K.; Sorenson, M. E.; Pucci, M.; Clark, J.; Bronson, J. J.; Ueda, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 171–175; (c) Naidu, B. N.; Sorenson, M. E.; Hudyma, T. W.; Zheng, X.; Zhang, Y.; Bronson, J. J.; Pucci, M. J.; Clark, J. M.; Ueda, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3743–3746.

10. Naidu, B. N.; Li, W.; Sorenson, M. E.; Connolly, T. P.; Wichtowski, J. A.; Zhang, Y.; Kim, O. K.; Matiskella, J. D.; Lam, K. S.; Bronson, J. J.; Ueda, Y. *Tetrahedron Lett.* **2004**, *45*, 1059–1063.
11. Conjugate adducts were formed as 1:1 diastereomeric mixture and the antibacterial activity of the individual isomers is comparable to that of the mixture.
12. Representative experimental procedure: To a stirred solution of 2-(methylamino)ethanol (0.28 mL, 3.50 mmol, 10 equiv) in de-ionized water, (17.5 mL) at room temperature was added nocathiacin I (0.5032 g, 0.35 mmol, 1 equiv). The reaction mixture was stirred until it turns a clear homogeneous solution (~1–2 min). Then, the reaction mixture was left in the freezer maintained at −20 °C until the reaction is 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 (0.30 g, 57%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.95 (1H, dd, J = 17.5, 8.33 Hz), 10.78 (1H, s), 9.60–9.35 (2H, m), 9.12 (1H, s), 8.75 (1H, br s), 8.66 (1H, s), 8.59 (2H, s), 8.54 (1H, s), 8.23 (1H, s), 8.05 (1H, s), 7.93 (1H, d, J = 18.2 Hz), 7.85 (2H, m), 7.81 (1H, d, J = 9.9 Hz), 7.54 (1H, d, J = 18.0 Hz), 7.35 (2H, m), 7.19 (1H, d, J = 9.8 Hz), 6.40 (1H, s), 6.03 (1H, d, J = 15.0 Hz), 5.75 (1H, m), 5.71 (1H, d, J = 14.9 Hz), 5.33 (1H, br s), 5.21 (1H, d, J = 20.0 Hz), 5.05 (3H, m), 5.00 (1H, m), 4.72 (1H, d, J = 14.7 Hz), 4.52 (1H, d, J = 14.8 Hz), 4.30 (1H, d, J = 10.1 Hz), 4.25 (1H, m), 4.15 (1H, d, J = 9.8 Hz), 4.05 (1H, d, J = 10.0 Hz), 3.94–3.85 (4H, m), 3.77 (2H, m), 3.72–3.61 (2H, m), 3.39 (4H, m), 3.17, (4H, s), 3.12 (1H, s), 2.89 (1H, m), 2.73 (2H, s), 2.54 (1H, m), 2.13, (1H, m), 2.00 (3H, s), 1.93 (1H, d, J = 9.9 Hz), 1.61 (3H, s), 1.15 (3H, s), 0.80 (3H, d, J = 6.9 Hz). HRMS (ESI) calcd for $C_{64}H_{70}N_{15}O_{19}S_5$ (M+H): 1512.358; found: 1512.358. Anal. Calcd for $C_{64}H_{69}N_{15}O_{19}S_5 \cdot 2HCl \cdot 4H_2O$: C, 46.37; H, 4.80; N, 12.67; S, 9.67; Cl, 4.28. Found: C, 46.45; H, 4.76; N, 12.77; S, 9.84; Cl, 4.69.
13. 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.
14. 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 × 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 3 different concentrations of drug was evaluated per compound.