Bioorganic & Medicinal Chemistry Letters 19 (2009) 3531–3535

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Nocathiacin analogs: Synthesis and antibacterial activity of novel water-soluble amides

Libo Xu *, Amy K. Farthing †, James F. Dropinski, Peter T. Meinke, Christine McCallum, Penny S. Leavitt, Emily J. Hickey, Lawrence Colwell, John Barrett, Kun Liu

Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA

ARTICLE INFO

Article history:
Received 2 April 2009
Revised 27 April 2009
Accepted 30 April 2009
Available online 5 May 2009

Keywords:
Nocathiacin
Water-soluble
Thiazolyl peptide
Antibacterial

ABSTRACT

Novel water-soluble amide analogs were synthesized from nocathiacin I (**1**) through the formation of the carboxylic acid intermediate followed by coupling to primary or secondary amines. Several compounds with potent antibacterial activity and adequate water solubility were identified. Of these, compound **19** was selected for more extensive evaluation because of its excellent in vitro antibacterial activity and in vivo efficacy, as well as clean off-target screening.

Nocathiacins are a novel class of antibiotics that belong to the thiazolyl peptide family.[1,2] Nocathiacin I (**1**) is structurally identical to MJ347-81F4 component A,[3] and displays potent in vitro antibacterial activity against a variety of Gram-positive bacteria, including many drug-resistant strains, and exhibits in vivo efficacy in a systemic *Staphylococcus aureus* infection mouse model.[4,5] However, the poor aqueous solubility of nocathiacin I limits its potential for further development as a hospital i.v. drug for serious infections. Nonetheless, with its novel structure and high intrinsic potencies, nocathiacin I provides a promising lead for the development of new antibacterial agents with broad spectrum efficacy against resistant Gram-positive pathogens with minimal risk of cross-resistance to currently marketed agents. We have undertaken an investigation to chemically modify nocathiacin I (**1**) through mild and selective chemistry to improve its aqueous solubility while maintaining its biological activity.

Several approaches have been applied to nocathiacin I and analogs to obtain compounds with increased water solubility.[6–10] As part of our own efforts in this area, we recently discovered[11] a facile conversion of nocathiacin I to an important and versatile intermediate nocathiacin acid (**2**) via selective degradation of the dehydroalanine side chain. In this Letter, we describe the synthesis and SAR of a variety of novel water-soluble amide analogs utilizing the pivotal carboxylic acid intermediate (Fig. 1).

Amides with a diverse water-solubilizing functionality attached were conveniently prepared in high yields by coupling nocathiacin acid (**2**) with primary or secondary amines via standard peptide chemistry (PyBop or EDC/HOBt) (Scheme 1). Alternatively, coupling of **2** with *N*-Boc-ethylenediamine followed by deprotection with TFA gave compound **12**, which was used as an intermediate to generate structurally diverse compounds through acylation,

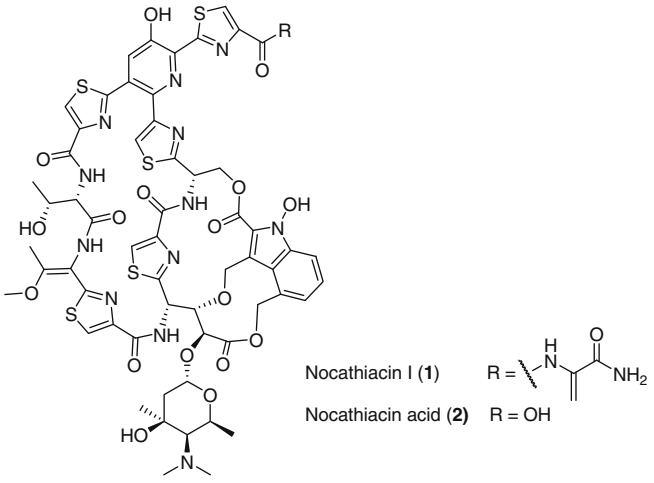
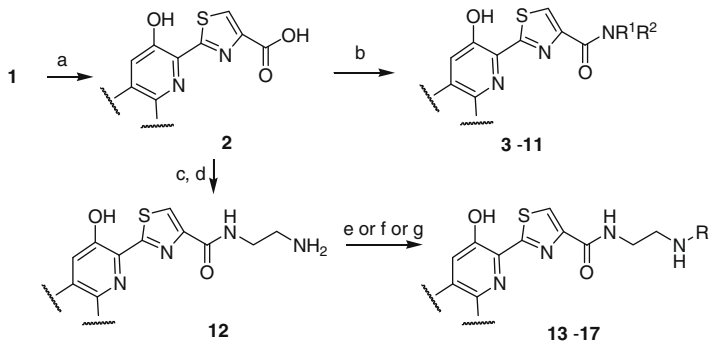


Figure 1. Structures of nocathiacins I and nocathiacin acid **2**.

* Corresponding author. Tel.: +1 732 5944963; fax: +1 732 5949556.
E-mail address: libo_xu@merck.com (L. Xu).
† Present address: CryoLife, Kennesaw, GA 30144, USA.

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.04.144

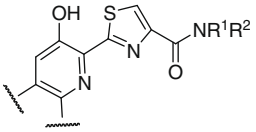


Scheme 1. Reagents: (a) TFAA, Py, THF, then aq $NaHCO_3$; (b) R^1R^2NH, EDC, HOBt, DMF; (c) $NH_2(CH_2)_2NHBoc$, EDC, HOBt, DMF; (d) TFA/DCM; (e) RCOOH, EDC, HOBt, DMF; (f) RCHO, $NaCNBH_3$, MeOH, AcOH; (g) $(CH_3)_3SiNCO$, TEA, DMF.

reductive amination or urea formation under mild and selective conditions.

The acid (**2**) itself exhibited little antibacterial activity, but using it as an intermediate, we have generated novel amides bearing different water-solubilizing groups. Previous SAR study in the dehydroalanine region[9] suggests acidic functional groups or polyhydroxyls are poorly tolerated, therefore we primarily focused on substituents with various basic groups. Table 1 lists some repre-

Table 1
Antibacterial activity of nocathiacin analogs **3–17**



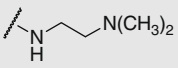
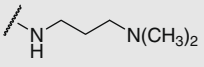
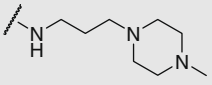
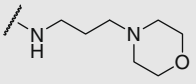
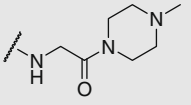
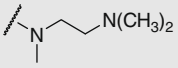
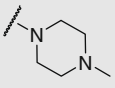
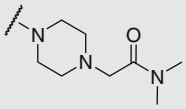
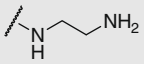
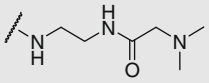
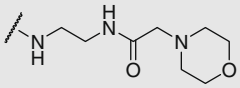
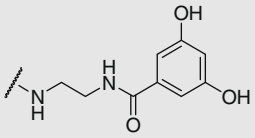
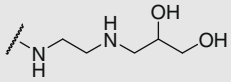
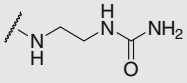
Compound	NR1R2	MIC[a] (μg/mL)			ED99[b] (mg/kg)	Solubility[c] (mg/mL)	AT2 IC50[d] (μM)
		S. aureus MB2865	*S. pneumo* CL2883	*E. faecalis* C21560			
1	Nocathiacin I	0.007	0.002	0.03		0.34	ND[e]
2	Nocathiacin acid	8	2	>8	ND	ND	1.12
3		0.015	0.00046	0.06	0.17	>10	0.190
4		0.06	0.00095	0.12	> 0.5	>10	ND
5		0.015	0.00095	0.12	0.13	>10	<0.010
6		0.011	0.007	0.016	0.24	>10	0.260
7		0.0038	0.00048	0.03	0.13	>10	ND
8		0.06	0.0038	0.12	>0.5	>10	ND
9		0.06	0.015	0.06	0.24	>10	0.102

Table 1 (*continued*)

Compound	NR1R2	MIC[a] (μg/mL)			ED99[b] (mg/kg)	Solubility[c] (mg/mL)	AT2 IC50[d] (μM)
		S. aureus MB2865	*S. pneumo* CL2883	*E. faecalis* C21560			
11		0.12	0.0038	0.25	ND	>10	ND
12		0.5	0.06	2	ND	>10	ND
13		0.019	0.00059	0.09	0.20	>10	0.078
14		0.0038	0.00048	0.06	0.05	>10	0.250
15		>1	ND	>1	ND	ND	ND
16		0.5	0.125	2	ND	10	ND
17		0.06	0.0075	0.5	ND	<10	ND

[a] MIC: minimum inhibitory concentration; the minimum concentration at which 100% inhibition of bacterial growth was observed in liquid culture by turbidity method.
[b] ED_{99}: Dosage that induces ⩾ 2 log10 cfu kidney burden reduction in a systemic *S. aureus* infection mouse model (SATOA). Vancomycin is included for comparison and its ED_{99} is normally 0.17–0.27 mg/kg.
[c] Aqueous solubility data were derived from amorphous powder of lyophilizing materials as their TFA salts and measured by nephelometry.
[d] AT_2 IC_{50} values determined with four dose levels of each compound according to the literature.[18]
[e] ND: not determined.

sentative compounds and their antibacterial activities against three selected bacterial strains.

A number of secondary amides bearing tertiary nitrogen as the solubility-enhancing element, as exemplified by **3**-**7**, exhibited potent in vitro antibacterial activity with MIC ranges comparable to nocathiacin I. Compounds **3**, **5**, **6** and **7** also demonstrated excellent in vivo efficacy. In the systemic *S. aureus* infection mouse model (SATOA),[12] **5** at 0.5 mg/kg iv produced 5.8 log reduction in bacterial load relative to control, and the calculated ED_{99} is 0.13 mg/kg. Similarly, compound **6** reduced the bacterial load by 4.68 log units with an ED_{99} of 0.24 mg/kg.

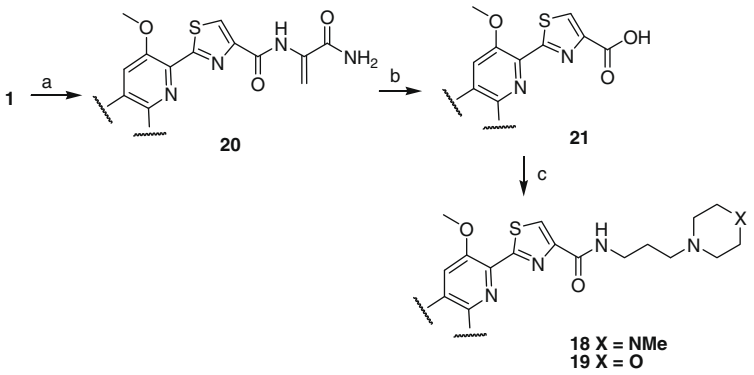
Open-chain tertiary amides, exemplified by **8**, demonstrated similar in vitro antibacterial activity as compounds in the primary amide series, but they were less efficacious when tested in the SATOA model. However, some cyclic secondary amides derived from piperazine (**9**–**10**) displayed potent antibacterial activity, both in vitro and in vivo. Compound **11** was less potent than **9** and **10**, especially against *S. aureaus* and *Enterococcus faecalis.*

It is interesting to note that compound **12**, with a terminal primary amino group, showed significantly reduced antibacterial activity relative to compound **3**. Two amides prepared from **12**, namely **13** and **14**, displayed excellent activity both in vitro and in vivo. Incorporation of non-basic polar moieties, such as bis-phenol in **15**, attenuated activity. Compounds obtained from reductive amination, such as **16**, showed weak antibacterial activity. Although compound **17** displayed modest in vitro antibacterial activity, it has limited aqueous solubility.

The potent compounds in Table 1, especially those which demonstrated good in vivo efficacy, were screened for off-target liabilities. These efforts revealed that a majority of the compounds tested displayed potent binding to the angiotension II receptor type 2 (AT_2) with IC_{50} values ranging from below 10 nM (**5**) to 459 nM (**10**). Although AT_2 receptor is expressed at low levels in the cardiovascular system in adults, it is up-regulated during certain pathological conditions, and recent investigations have implicated a role for the AT_2 receptor in cardiovascular, brain and renal function as well as in the modulation of various biological processes involved in development, cell differentiation, tissue repair and apoptosis.[13–15] Significant efforts were directed at addressing this off-target activity, which led to the discovery that the observed AT_2 binding can be significantly attenuated by introducing substitutions at the pyridyl hydroxyl and/or indole hydroxyl group. More specifically, methylation of pyridyl hydroxyl in compound **6** afforded **19**, which exhibited a 20-fold reduction in AT_2 activity (IC_{50} 5.07 μM, Table 2), while maintaining potent antibacterial activity.

Table 2
Effect of substitution on AT_2 binding and antibacterial activity

Compounds	MIC (μg/mL) (*S. aureus* 2865)	AT2 IC50 (μM)
5	0.015	<0.01
6	0.011	0.26
18	0.09	0.85
19	0.010	5.07



Scheme 2. Reagents: (a) $TMSCH_2N_2$, THF/MeOH; (b) TFAA, Py, THF, then aq $NaHCO_3$; (c) RNH_2, EDC, HOBt, DMF.

Although modification of indole hydroxyl also mitigated AT_2 binding, it often resulted in deleterious effects to antibacterial activity (data not shown).

The synthesis of compounds **18** and **19** is summarized in Scheme 2. Methylation with TMS diazomethane in THF and methanol gave almost exclusively pyridyl OMe nocathiacin I (**20**).[16] Treatment with TFAA/pyridine in THF converted **20** to the corresponding acid **21**, which was coupled to the requisite amine components to form **18** and **19**, respectively. Direct methylation of **5** and **6** with TMS diazomethane can also yield the desired products, but it was difficult to separate them from bismethylated products, especially on a large scale.

The HCl salt of compound **19** has a solubility of 68 mg/mL in water at a native pH of 3.4. It is among the most potent nocathiacin analogs synthesized so far. It exhibits excellent in vitro antibacterial activity against a variety of Gram-positive pathogens, including methicillin-resistant *S. aureus* (MRSA) and Vancomycin-resistant *enterococci* (VRE) (MICs 0.00095 and 0.06 μg/mL, respectively). It is comparable to nocathiacin I in vitro against a panel of *E. faecalis* (MICs 0.015–0.06 μg/mL), which is the limiting strain for this class of compounds and becoming more prevalent in the clinic. In a systemic *S. aureus* mouse infection model (SATOA, i.v., bid), the dose required to reduce kidney colony forming units by 99% (ED_{99}) was 0.27 mg/kg for **19**, versus 12 mg/kg for linezolid. In a more stringent mouse MRSA COL thigh model,[19] **19** (i.v., bid) @ 1 mg/kg dosage produced 1.22 log reduction in bacterial load relative to control over a 24 h period, comparable to the efficacy of vancomycin at 100 mg/kg.

The pharmacokinetics of **19** in preclinical species is summarized in Table 3. It has a terminal half life of ~5–7 h in mice, rats, dogs and monkeys. The clearance ranges from low in dogs and monkeys to high in rats. In bile duct-cannulated male rats dosed i.v. with **19** at 2 mg/kg, biliary and urinary excretion of intact parent constituted one of the elimination routes, while metabolism accounted for another clearance pathway (data not shown).

Compound **19** in vitro exhibited IC_{50} values $\geqslant 100\,\mu M$ for reversible inhibition of CYP1A2, 2C9, 2C19, 2D6 and 3A4, and an IC_{50} value of 71 μM for CYP2C8. It was neither a time-dependent inhibitor of CYP3A4, nor an activator of human PXR.

Table 3
Mean pharmacokinetic parameters of **19** in preclinical species

Parameters[a]	Mouse[b]	Rat[b]	Dog[b]	Monkey[b]
Clp (mL/min/kg)	32.1	51.3	15.6	15.0
V_{dss} (L/kg)	18.7	15.5	3.3	1.2
$T_{1/2}$ (h)	5.0	6.4	5.4	6.5

[a] Cl_p, plasma clearance (blood clearance for mice); V_{dss}, steady-state volume of distribution; $T_{1/2}$, terminal half-life.
[b] The iv dose was 1 mg/kg in EtOH/PEG400/H_2O (10:15:75) for all species.

In summary, we have synthesized a variety of amide analogs of nocathiacin I through a versatile carboxylic acid intermediate. Many of the newly synthesized analogs display comparable potency to nocathiacin I against a wide spectrum of bacteria, but with dramatically improved aqueous solubility at acidic pH, and are not accessible through previously established chemistry. Compound **19** was chosen to be further evaluated because of its excellent in vitro antibacterial activity and in vivo efficacy.

Acknowledgements

We wish to acknowledge the skills and hard work of the many scientists who provided biological support including Gowri Bhat, Nichelle Newsome, Rosalind Jenkins, Suzy Kwon, Andrew S. Misura and Charles J. Gill. The authours would also like to thank members of Fermentation Development & Operations and Biopurification Development for the supply of nocathiacin I.

References and notes

1. 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.
2. Leet, J. E.; Li, W.; Ax, H. A.; Matson, J. A.; Huang, S.; Huang, R.; Cantone, J. L.; Drexler, D.; Dalterio, R.; Lam, K. S. *J. Antibiot.* **2003**, *56*, 232.
3. Sasaki, T.; Otani, T.; Matsumoto, H.; Hamada, M.; Takeuchi, T.; Hori, M. *J. Antibiot.* **1998**, *51*, 715.
4. Pucci, M. J.; Bronson, J. J.; Barrett, J. F.; DenBleyker, K. L.; Discotto, L. F.; Fung-Tomc, J.; Ueda, Y. *Antimicrob. Agents Chemother.* **2004**, *48*, 3697.
5. 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.
6. 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.
7. Naidu, B. N.; Sorenson, M. E.; Zhang, Y.; Kim, O. K.; Matiskella, J. D.; Wichtowski, J. A.; Connolly, T. P.; Li, W.; Lam, K. S.; Bronson, J. J.; Pucci, M. J.; Clark, J. M.; Ueda, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5573.
8. 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.
9. Naidu, B. N.; Sorenson, M. E.; Matiskella, J. D.; Wichtowski, J. A.; Li, W.; Sauaker, J. B.; Zhang, Y.; Connolly, T. P.; Lam, K. S.; Bronson, J. J.; Pucci, M. J.; Yang, H.; Ueda, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3545.
10. Hrnciar, P.; Ueda, Y.; Huang, S.; Leet, J. E.; Bronson, J. J. *J. Org. Chem.* **2002**, *67*, 8789.
11. Xu, L.; Farthing, A. K.; Shi, Y. J.; Meinke, P. T.; Liu, K. *J. Org. Chem.* **2007**, *72*, 7447.
12. Mice were challenged IP with *S. aureus* MB 2865 at 1.8 × 103 cfu/mouse. Mice (3/group) were treated with test compounds (i.v., *b.i.d.*). Kidneys were aseptically collected 24 h after challenge.
13. Matsubara, H. *Circ. Res.* **1998**, *83*, 1182–1191.
14. Wolf, G. *Nephrol. Dial. Transplant.* **2002**, *17*, 195.
15. Kaschina, E.; Unger, T. *Blood Press* **2003**, *12*, 70.

16. The reaction product of **1** with TMS diazomethane was previously assigned to be indole-*N*-OMe nocathiacin I.[6] However, several lines of evidence from our NMR and chemical derivatization studies support the assignment of pyridyl-OH methylated **20** as the reaction product. The NMR peak of the methyl protons appears as a singlet at 4.3 ppm in CD_3OD. In 2D NMR, NOE between methyl protons and H-4 of pyridine moiety was observed. We also observed coupling of methyl protons to C-3 of pyridine in HMBC. Nocathiacin I can be converted to its deoxy indole analog nocathiacin II[17] when treated with ethyl bromopyruvate and BTPP in DMF. **20** should not undergo similar indole-OH cleavage if the indole-OH was methylated. However, we found that **20** was converted cleanly to the deoxy indole product, identical in all respects to that of methylation product of nocathiacin II by treatment with $TMSCH_2N_2$.
17. Connolly, T. P.; Regueiro-Ren, A.; Leet, J. E.; Springer, D. M.; Goodrich, J.; Huang, X.; Pucci, M. J.; Clark, J. M.; Bronson, J. J.; Ueda, Y. *J. Nat. Prod.* **2005**, *68*, 550.
18. Whitebread, S. E.; Taylor, V.; Bottari, S. P.; Kamber, B.; de Gasparo, M. *Biochem. Biophys. Res. Commun.* **1991**, *181*, 1365.
19. Mice were challenged IM with *Staphylococcus aureus* 'COL' at 2.3×10^7 cfu/mouse. Mice (4/group) were treated with test compounds intravenously (0.2 ml, iv, bid.). Thighs were collected aseptically 24 h after challenge.