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ORIGINAL ARTICLE

Thiazomycin, nocathiacin and analogs show strong activity against clinical strains of drug-resistant *Mycobacterium tuberculosis*

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Thiazolyl peptides are a class of natural products with potent Gram-positive antibacterial activities. Lack of aqueous solubility precluded this class of compounds from advancing to clinical evaluations. Nocathiacins and thiazomycins are sub-classes of thiazolyl peptides that are endowed with structural features amenable for chemical modifications. Semi-synthetic modifications of nocathiacin led to a series of analogs with improved water solubility, while retaining potency and antibacterial spectrum. We studied the activities of a selection of two natural products (nocathiacin and thiazomycin) as well as seven polar semi-synthetic analogs against twenty clinical strains of *Mycobacterium tuberculosis* with MDR phenotypes. Two compounds show useful activity against H37Rv strain with MIC values ⩽1 μM, two (⩽0.5 μM) and three (⩽10 μM). These two derivatives showed MIC values ⩽2.5 μM against most of the 20 MDR strains regardless their resistance profile. Specifically, these lack cross-resistance to rifampicin, isoniazid and moxifloxacin.
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Nocathiacins and thiazomycins are members of the thiazolyl peptide class of natural product antibiotics produced by *Amycolatopsis fastidiosa*.[1–4] These compounds are highly potent broad-spectrum Gram-positive agents.[5] Importantly, they exhibit comparable activity against drug-resistant strains of Gram-positive pathogens including MRSA and show potent *in vivo* activity when dosed by parenteral administration.[5] The natural thiazolyl peptides are highly lipophilic and poorly soluble in aqueous media severely limiting their potential as therapeutic agents. Syntheses of a series of polar analogs have been reported by targeted semi-synthetic efforts with retention of potency and Gram-positive antibacterial spectrum.[6,7] Nocathiacin I was reported to show potent activity against the laboratory strain of *Mycobacterium tuberculosis* H37Rv.[8] Tuberculosis caused by drug-resistant *M. tuberculosis* strains continues to spread unabated in many regions of the world, which lack effective treatment options.[9] The exceptional potency against Gram-positive bacteria, lack of cross-resistance to known antibiotics, coupled with the ready availability of the natural and semi-synthetic compounds in our sample collection, provided the impetus to investigate a series of these compounds against a panel of genetically defined clinical strains of *M. tuberculosis* with a variety of drug-resistance profiles.

We selected nine compounds for this study. They include the two parent natural products plus seven semi-synthetic water-soluble derivatives of nocathiacin I. All compounds (**1**–**9**) were tested against three strains (Table 1) of *M. tuberculosis* including the laboratory H37Rv strain. Two compounds (**1** and **2**) were available in larger amounts and were evaluated extensively against a panel of 20 clinical *M. tuberculosis* strains with an array of drug-resistance profiles (Table 2). Although it is well appreciated that nocathiacin I compounds are unlikely to yield drug candidates with oral efficacy, with advanced delivery technology it is feasible to administer derivatives of this class directly to the lung by inhaled routes. The activity profile and structure activity relationship of these compounds are discussed herein.

MATERIALS AND METHODS

Reagents and test compounds

All reagents, including the antibiotic controls—isoniazid (Inh), rifampicin (Rif) and moxifloxacin hydrochloride (moxi)—were obtained from Sigma-Aldrich (St Louis, MO, USA) unless otherwise indicated. Nocathiacin I (**1**) and thiazomycin (**4**) (Figure 1) were obtained from the MRL sample repository and were originally isolated from extracts of *Amycolatopsis fastidiosa*.[3,10] The semi-synthetic derivatives (**2**, **3** and **5**–**9**) (Figure 1) also were obtained from the MRL sample repository and were prepared as described.[6,11] The solubility of

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hydrochloride salts of the parent natural products **1** and **4** in 5% dextrose-water was ~0.34 mg ml^{-1}, whereas the solubility of the semi-synthetic derivatives **2**, **3**, and **5–9** was >10 mg ml^{-1}.

Sources of strains

Strains of *M. tuberculosis* were selected from the PHRI TB Center collection containing more than 33 000 clinical isolates and represent all nine clusters that define the *M. tuberculosis* phylogenetic tree (Figure 2).

MIC or growth inhibition determination

Twenty strains were sub-cultured on the 7H11 Middlebrook agar media (VWR) enriched with BBL Middlebrook OADC (Fisher), collected using cotton swab (VWR) and resuspended in phophate-buffered saline to 1.0 McFarlands standard (corresponds to 1×10^7 CFU per ml). Ten microlitre of three dilutions (1:10 in phophate-buffered saline) were plated (corresponding to 10^2, 10^3 and 10^4 CFU) onto one quadrant of X-plates (VWR) containing 2.5, 25 or 250 (or 1, 10 or 100) μM of a given compound (dissolved in DMSO). Rif, Inh and moxi were used as control anti-tuberculosis drugs at concentration 0.1, 1.0, 10; 0.2, 1.0, 10 and 0.2, 2.0 and 10 μM. Viability of clinical isolates was determined on the control plates with no drug. Plates were incubated at 37 °C for 14–21 days.

Table 1 MIC (μM) of nocathiacin-I (1) and nocathiacin analogs against selected clinical strains of *Mycobacterium tuberculosis* (Mtb)

Compounds	*H37Rv*	*C, 913*	*W, 565*
1	⩽2.5	⩽2.5	>2.5 to <25
2	⩽2.5	⩽2.5	>2.5 to <25
3	⩽1	⩽1	⩽1
4	⩽10	>10 to ⩽100	>10 to ⩽100
5	⩽10	⩽10	⩽10
6	⩽1	⩽10	⩽10
7	>10 to ⩽100	>10 to ⩽100	>10 to ⩽100
8	⩽10	>10 to ⩽100	>10 to ⩽100
9	>100	>10 to ⩽100	>100
Inh	⩽0.2	⩽0.2	>10
Rif	⩽0.1	⩽0.1	>10

RESULT AND DISCUSSION

Thiazolyl peptide classes of compounds were discovered as early as the 1950s and are well studied. They are highly potent antibacterial agents.[12] However, none of the compounds from this class could be developed as a clinical agent due to extremely poor water solubility. Nocathiacins and thiazomycins are recent entries in the thiazolyl peptide class with potent activity.[10,13] They are endowed with structural features that are reasonably amenable to chemical modifications; therefore, we undertook semi-synthetic modification of the most abundant of the natural products, nocathiacin I (**1**) and thiazomycin (**4**) (Figure 1), leading to the synthesis of a series of highly potent, broad-spectrum Gram-positive agents with improved water solubility and *in vivo* activity.[6,7] Seven structurally diverse analogs (**2–3**, **5–9**, Figure 1), with modifications on the pyridyl hydroxyl group and/or replacement of the dehydroalanine amide with polar substituents, were selected for this study. For initial biological evaluations, three representative *M. tuberculosis* strains were selected to assess potency, spectrum and structure activity relationship (SAR). The test strains included the laboratory strain H37Rv, and the two most successful *M. tuberculosis* clones from New York City patients since the re-emergence in the early 1990s; the highly multidrug-resistant 'W' strain that was a nosocomial pathogen across numerous hospitals and the pan-susceptible community acquired 'C' strain that spread among the homeless.[14,15] The three strains were challenged against compounds **1** and **2** at concentrations of 2.5, 25 and 250 μM, whereas compounds **3–9,** for which limited quantities were available, were tested at 1, 10 and 100 μM. The inhibitory activities against *M. tuberculosis* strains are presented in Table 1.

The natural product nocathiacin I showed potent activity against all three strains with MIC of ⩽2.5 μM. Thiazomycin was 4-fold less

Table 2 MIC (μM) of nocathiacin-I (1) and a polar analog (2) against 20 drug susceptible and resistance clinical strains of *Mycobacterium tuberculosis* (Mtb)

Strain	*Strain #*	*RFLP*	*Cluster #*	*Resistance*	*Reference*	***1***[a]	***2***[a]	*Rif*	*Inh*	*Moxi*
M. tuberculosis	11677	BE	I	SUSC	Rif^S, Inh^S, Mox^S	⩽25	⩽25	⩽0.1	⩽0.2	<0.2
M. tuberculosis	565	W	II	MDR	Rif^R, Inh^R, Mox^S	⩽25	⩽25	⩾10	⩾10	⩽0.2
M. tuberculosis	18460	BE	I	MDR	Rif^R, Inh^R, Mox^S	⩽2.5	⩽2.5	⩽10	⩽10	⩽0.2
M. tuberculosis	18343	MC	I	POLY	Rif^S, Inh^R, Mox^R	⩽2.5	⩽25	⩽0.1	>10	>10
M. tuberculosis	13923	HD17	II	SUSC	Rif^S, Inh^S, Mox^S	⩽25	⩽25	⩽0.1	⩽0.2	⩽0.2
M. tuberculosis	8600	KY	II	MDR	Rif^R, Inh^R, Mox^S	⩽2.5	⩽2.5	⩽10	>10	⩽0.2
M. tuberculosis	10525	LL	IIA	MDR	Rif^R, Inh^R, Mox^S	⩽2.5	⩽25	>10	⩽10	⩽0.2
M. tuberculosis	16116	CN1	IIA	SUSC	Rif^S, Inh^S, Mox^S	⩽2.5	⩽2.5	⩽0.1	⩽0.2	⩽0.2
M. tuberculosis	1868	AU	III	MDR	Rif^R, Inh^R, Mox^S	⩽25	⩽2.5	⩽10	>10	⩽0.2
M. tuberculosis	10367	C	IV	SUSC	Rif^S, Inh^S, Mox^S	⩽2.5	⩽2.5	⩽0.1	⩽0.2	⩽0.2
M. tuberculosis	12850	AH	V	SUSC	Rif^S, Inh^S, Mox^S	⩽2.5	⩽2.5	⩽0.1	⩽0.2	⩽0.2
M. tuberculosis	15552	AH13	V	MDR	Rif^R, Inh^R, Mox^S	⩽2.5	⩽2.5	>10	>10	⩽0.2
M. tuberculosis	6134	CS	VI	MDR-+	Rif^R, Inh^R, Mox^R	⩽2.5	⩽2.5	>10	>10	>10
M. tuberculosis	12556	AI36	VI	SUSC	Rif^S, Inh^S, Mox^S	⩽2.5	⩽2.5	⩽0.1	⩽0.2	⩽0.2
M. tuberculosis	9139	AF	VIII	MONO-R	Rif^S, Inh^R, Mox^S	<2.5	⩽2.5	⩽0.1	⩽10	⩽0.2
M. tuberculosis	10975	P	VI	MDR	Rif^R, Inh^R, Mox^S	⩽25	⩽250	>10	>10	⩽0.2
M. tuberculosis	7791	001	VII	MONO-R	Rif^S, Inh^R, Mox^S	⩽2.5	⩽2.5	⩽1	⩽10	⩽0.2
M. tuberculosis	30034	BJ59	VII	SUSC	Rif^S, Inh^S, Mox^S	⩽2.5	⩽2.5	⩽1	⩽1	⩽0.2
M. tuberculosis	30425	AE21	III	SUSC	Rif^S, Inh^S, Mox^S	⩽2.5	⩽2.5	⩽1	⩽1	⩽0.2
M. tuberculosis	H37Rv	control	VIII	SUSC	Rif^S, Inh^S, Mox^S	⩽2.5	⩽25	⩽0.1	⩽0.2	⩽0.2

Abbreviation: RFLP, restriction fragmentation length polymorphism.
[a]The lowest tested concentration of compounds **1** and **2** was 2.5 μM and next tested concentration was 25 μM. When MIC data for a particular strain fell between the two test concentrations (2.5 and 25 μM), ⩽25 μM have been listed as the MIC value for **1** and **2** in the respective columns.

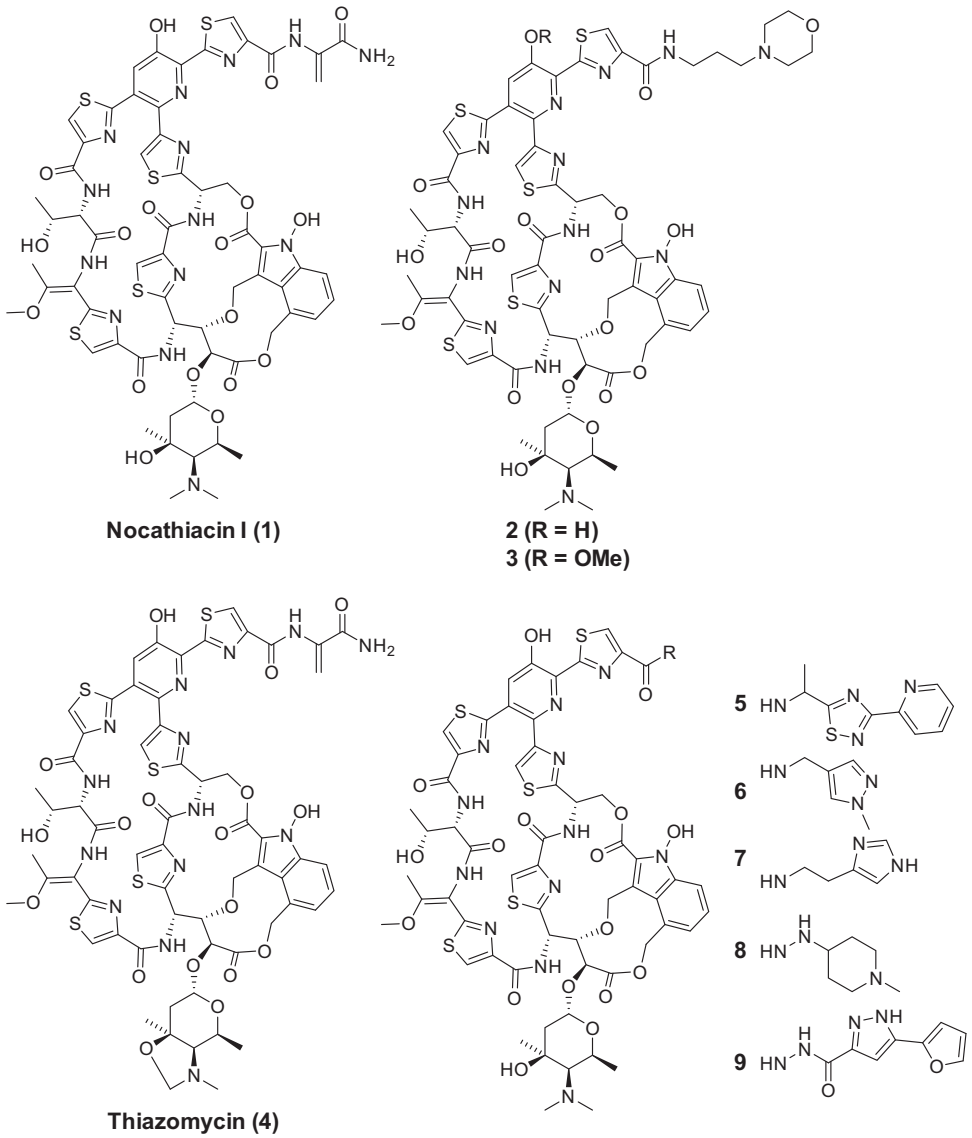

Figure 1 Structures of nocathiacin (**1**), thiazomycin (**4**) and analogs (**2**–**3**, **5**–**9**).

potent (MIC $\leqslant 10\,\mu M$) against the H37Rv strain and ~40-fold less active against the other two strains. Substitution of nocathiacin's dehydroalanine with a morpholine propyl amide produced analog **2** with fully retained *in vitro* activity and significantly improved water solubility (0.34 vs $>10\,mg\,ml^{-1}$).[6] Substitution of the pyridyl OH of **2** with a methyl ether produced compound **3** with further improved potency. Substitutions of dehydroalanine with other heterocycles (**5**–**9**) diminished potency by more than fourfold with exception of 1-methyl-pyrazolyl-methyl amide (**6**), which showed an improved MIC (~1 μM) against H37Rv but diminished activity against the other two strains.

Of the three best derivatives (**1**–**3**), **1** and **2** were immediately available in larger amounts for testing and were further evaluated at 2.5, 25 and 250 μM against a series of eighteen additional clinical *M. tuberculosis* strains with varied drug susceptible and resistant profiles. The strains were selected on the basis of both their resistance profile and their genetic diversity. The phylogenetic relationships of the test strains are mapped onto the tree in Figure 2. On the basis of comparative SNP analysis, the species are divided into three principal genetic groups and further distinguished into nine genetic clusters[16,17] The MIC data for these two compounds are presented in Table 2. In general, both analogs showed potent activity with an MIC of $\leqslant 2.5\,\mu M$ against most strains, whereas only for three strains (11 677, 13 923 and 10 975) noted reduced potency (MIC $>2.5\,\mu M$). It is important to note that these strains are genetically distinct: two are pan-susceptible and one is multidrug-resistant. This finding, along with the fact that MDR strains from different genetic clusters had an MIC of $\leqslant 2.5\,\mu M$, provides good evidence that compounds **1** and **2** do not show cross-resistance to isoniazid, rifampin and moxifloxacin and its activity has no obvious genetic restrictions.

These thiazolyl peptides show potent activity against *M. tuberculosis* regardless of resistance profile, which makes them interesting candidates for potential development. The solubility characteristics of the compounds does not have a critical role in their potency, which provides avenues for two different approaches for potential development (for example, soluble vs less-soluble compound) of inhaled products. Both of these analogs have shown potent systemic *in vivo* activity with sub $mg\,kg^{-1}$ ED_{99} against murine *Staphylococcus aureus*

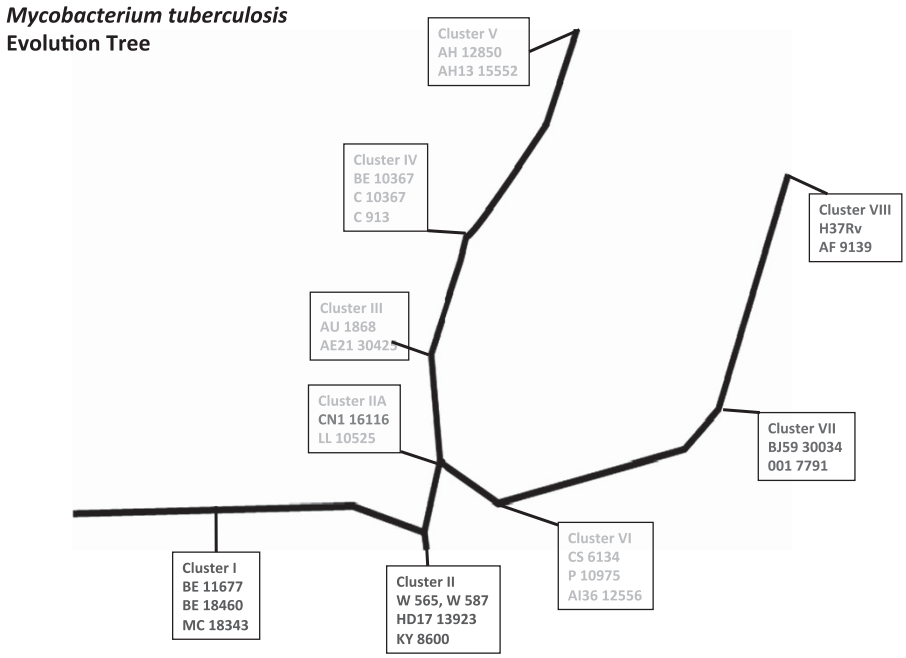

Figure 2 Phylogenetic tree of *M. tuberculosis* strains used for this study. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

infection models.[5,6] Further studies are needed to validate an *in vivo* effect of these compounds against *M. tuberculosis* and to assess whether they are worthy of further development by alternative inhaled dosing paradigms for serious life threatening tuberculosis.

CONFLICT OF INTEREST

The work described in this paper was conducted and supported by Merck & Co., Inc., a for-profit public company dedicated to discovery, development, manufacturing and sale of antibiotics and other drugs. The remaining authors declare no conflict of interest.

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