Thiazomycins, Thiazolyl Peptide Antibiotics from *Amycolatopsis fastidiosa*

Chaowei Zhang, Kithsiri Herath, Hiranthi Jayasuriya, John G. Ondeyka, Deborah L. Zink, James Occi, Gwyneth Birdsall, Jayashree Venugopal, Misti Ushio, Bruce Burgess, Prakash Masurekar, John F. Barrett,[†] and Sheo B. Singh*

Natural Products Chemistry, Merck Research Laboratories, 126 E. Lincoln Avenue, Rahway, New Jersey 07065

Received December 9, 2008

Thiazolyl peptides are a class of highly rigid trimacrocyclic compounds consisting of varying but large numbers of thiazole rings. The need for new antibacterial agents to treat infections caused by resistant bacteria prompted a reinvestigation of this class, leading to the previous isolation of thiazolyl peptides, namely, thiazomycin (**5**) and thiazomycin A (**6**), congeners of nocathiacins (**1**−**4**). Continued chemical screening led to the isolation of six new thiazolyl peptide congeners (**8**−**13**), of which three had truncated structures lacking an indole residue. From these, compound **8** showed activity similar to thiazomycin. Two compounds (**9** and **10**) showed intermediate activities, and the three truncated compounds (**11**−**13**) were essentially inactive. The discovery of the truncated compounds revealed the minimal structural requirements for activity and suggested probable biosynthetic pathways for more advanced compounds. The isolation, structure elucidation, antibacterial activity, and proposed biogenesis of thiazomycins are herein described.

The discovery and development of antibiotics to treat life-threatening infections by bacteria is perhaps one of the greatest accomplishments of the mid twentieth century.[1,2] However, bacteria continue to evolve, becoming resistant to existing antibiotics. One recent report suggested that infection by methicillin-resistant *Staphylococcus aureus* (MRSA) was responsible for over 18 000 deaths in the United States based on 6 months surveillance followed by extrapolation to a whole year.[3] While MRSA infections are prevalent and the most known, infection by the drug-resistant *Pseudomonas aeriginosa* and other pathogens perhaps is more serious. Chemical modifications of the leads discovered in the mid twentieth century led to incremental improvements, which in turn resulted in the development of new antibiotics with improved properties. These compounds have provided new treatment options and served us well. However new modifications leading to improved activity are becoming more challenging, and the discovery of new lead classes with new mechanisms of action or new lead classes for even known mechanisms of action is more critical now than ever before. Platensimycin[4,5] and platencin[6,7] are some of such discoveries that were recently reported.

Thiazolyl peptides are a class of naturally occurring antibiotics produced by soil bacteria. Micrococcin, the first member of this class, was reported in 1948 followed by the report of thiostrepton in 1954.[8] These polycyclic peptides are represented by over 90 members, and all possess a large number of thiazole residues and are some of the most potent *in vitro* growth inhibitors of Gram-positive bacteria.[8] The developments of this class of compounds as clinical agents have been hampered due to extremely poor physicochemical properties, most notably low aqueous solubility. The increased drug resistance has created a serious unmet medical need and has changed the landscape of antibacterial development. This, combined with the potent activities and novel mode of action of these compounds, prompted us to reexamine them. This effort was also aided by the significant advances in chemistry and biology in the past decade. Close examination of the literature on the members of this class, glycothiohexide α,[9,10] S54832A-I,[8] MJ347-81F4A and B,[11] and nocathiacins,[12,13] appeared more attractive because of the presence of the amino-glycosidic residue as one of the structural components of these compounds, which provided better potential for improvement of aqueous solubility. This was exemplified by chemical modifications of nocathiacin I, including a recent method developed for efficient hydrolysis of the side chain.[14−23] These successes bolstered our thought process, and we initiated an investigation to discover new thiazolyl peptides with chemical functionalities that can provide additional opportunities for chemical modifications, which could lead to compounds with better physical and pharmaceutical properties. Such compounds could be amenable to development as new antibiotics without cross resistance to existing antibiotics. To accomplish this goal, we undertook two approaches. In the first approach, we acquired known producers of thiazolyl peptides and related cultures from internal and external sources. The fermentation extracts prepared from them were subjected to both biological and chemical screening. The second approach consisted of designing thiazolyl peptide-sensitive and -resistant paired strains of an *S. aureus* assay for random screening of natural product extracts. Both of these approaches have been successful in discovering new thiazolyl peptides.[24−27]

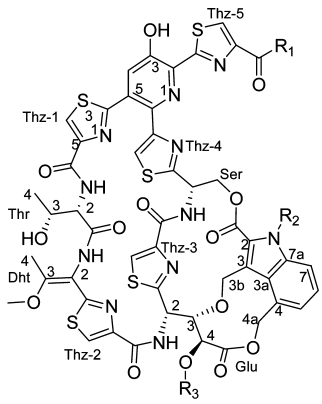
LCMS screening of the extracts of *Amycolatopsis fastidiosa* (ATCC 202099), which is the original producer of nocathiacins, showed the presence of a number of novel thiazolyl peptides, albeit in very low amounts, in addition to a larger abundance of nocathiacins I−IV (**1**−**4**). We recently reported discoveries of thiazomycin (**5**)[25,26] and thiazomycin A (**6**)[27] from the extracts of *A. fastidiosa.* This extract was found to be a treasure trove for the isolation of at least 13 thiazolyl peptides. These include seven previously known compounds, nocathiacins I−IV (**1**−**4**), thiazomycin (**5**), thiazomycin A (**6**), and **7**, and six new thiazolyl peptides, thiazomycins B−D (**8**−**10**) and truncated thiazomycins E_1−E_3 (**11**−**13**), reported herein. The discovery of truncated thiazomycins provided the opportunity, for the first time, to shed some light on the plausible biosynthetic relatedness of many of these compounds. The isolation, structure elucidation, antibacterial activities, and biogenetic proposal of the new thiazomycins and several known thiazomycins and nocathiacins has been described.

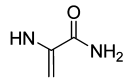
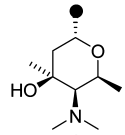
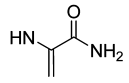
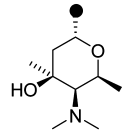
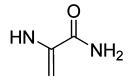
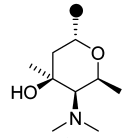
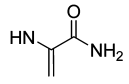
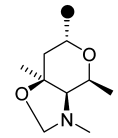
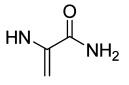
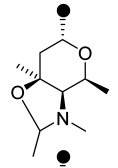
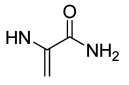
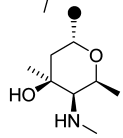
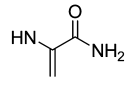
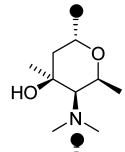
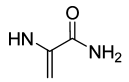
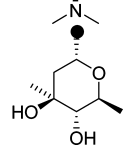
Results and Discussion

The producing strain, *Amycolatopsis fastidiosa* MA7332 (ATCC 202099), was grown in a 30 L fermentation tank using dextrose, peptone, primary yeast, and allophosite as production medium. The fermentation tank was maintained at pH 7.0 and harvested after 13 days. The broth was extracted with EtOAc and filtered. The mycelia were extracted with acetone. The acetone extract contained most of the thiazolyl peptides, as determined by reversed-phase HPLC analysis with diode array detection. The extract showed a HPLC profile, represented in Figure 1. The HPLC chromatogram showed

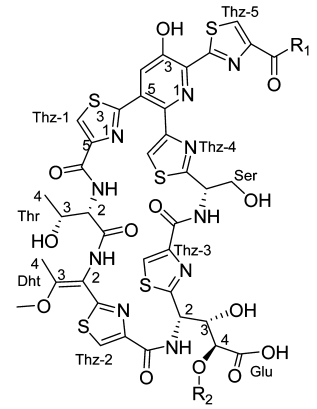
* Corresponding author. E-mail: sheo_singh@merck.com.
[†] Deceased.

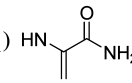
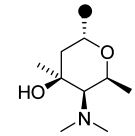
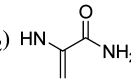
10.1021/np800783b CCC: $40.75
Published on Web 03/31/2009



	R_1	R_2	R_3
1 (nocathiacin I)		OH	
2 (nocathiacin II)		H	
3 (nocathiacin III)		OH	H
4 (nocathiacin IV)	NH_2	OH	
5 (thiazomycin)		OH	
6 (thiazomycin A)		OH	
7		OH	
8 (thiazomycin B)		H	H
9 (thiazomycin C)	NH_2	H	
10 (thiazomycin D)		OH	

that nocathiacin I (**1**) (~100 mg/L) was the major metabolite produced by this organism. Nocathiacins III (**3**), II (**2**), and IV (**4**) were produced in relatively lower amounts, and many of the new metabolites were produced in very low amounts. The acetone extract was concentrated to dryness and triturated/washed with hexanes to remove fatty material. The remaining solid was dissolved in a 1:1 mixture of CH_2Cl_2−MeOH and chromatographed on Sephadex LH20 using a step gradient of 1:4 hexanes−CH_2Cl_2 followed by



	R_1	R_2
11 (thiazomycin E_1)		
12 (thiazomycin E_2)		H
13 (thiazomycin E_3)	NH_2	H

2.5−100% MeOH−CH_2Cl_2 (Scheme 1). The early eluting fractions from the LH20 column (F03−F06) were further chromatographed on silica gel followed by reversed-phase HPLC chromatography to concentrate thiazomycin (**5**), which was subsequently isolated using a preferential protonation approach.[26] Analogous chromatography of the middle fraction (F07) on silica gel and RP HPLC afforded thiazomycins B (**8**) and D (**10**). Silica gel followed by another Sephadex LH20 chromatography of later eluting fractions (F09, F10) provided nocathiacin III (**3**). Silica gel chromatography of the subsequent fraction (F12) gave the major component, nocathiacin I (**1**). Silica gel chromatography of fraction 16 followed by RP HPLC using a polar AQ column afforded the truncated thiazomycin E_1 (**11**). Direct RPHPLC of the fractions F19−F21 again on the polar column furnished thiazomycins E_2 (**12**) and E_3 (**13**) in only trace amounts. Thiazomycin C (**9**) was isolated from another fermentation batch, which was harvested after 7 days by sequential silica gel, Diol, and silica gel chromatographies. Nocathiacins II (**2**) and IV (**4**) were similarly isolated from different batches and are not reported here. The identities of nocathiacins I−IV (**1**−**4**) and **7** were confirmed by comparison of spectroscopic data.[11−13] The structure elucidation of thiazomycin (**5**) and thiazomycin A (**6**) was reported earlier.[26,27]

MS analysis of **8** (thiazomycin B) gave a molecular weight of 1249 and a molecular formula of $C_{52}H_{43}N_{13}O_{15}S_5$. The UV spectrum showed three absorption maxima (λ_{max} 218, 294, 375) characteristic of thiazolyl peptides of the nocathiacin/thiazomycin class. The ^{1}H NMR analysis showed six olefinic downfield singlets (δ_H 7.72−8.32) consistent with the presence of five thiazoles and a pyridine unit. The ^{1}H NMR spectrum also showed the presence of the olefinic methylene group (δ_H 5.57 and 6.52) of a Deala residue and ABC coupled aromatic protons along with two pairs of oxy-methylene protons, suggesting the presence of an intact bicyclic thiazolyl peptide core as in thiazomycin. The ^{1}H NMR spectrum showed the absence of resonances for sugar residues and the presence of the C-4 methine resonance of a Glu residue. These assignments were confirmed by comparison of the ^{13}C NMR resonances with the corresponding resonances of thiazomycin and nocathiacin III and confirmed by 2D NMR (COSY, TOCSY, HSQC, and HMBC) data. On the basis of the NMR data along with the molecular

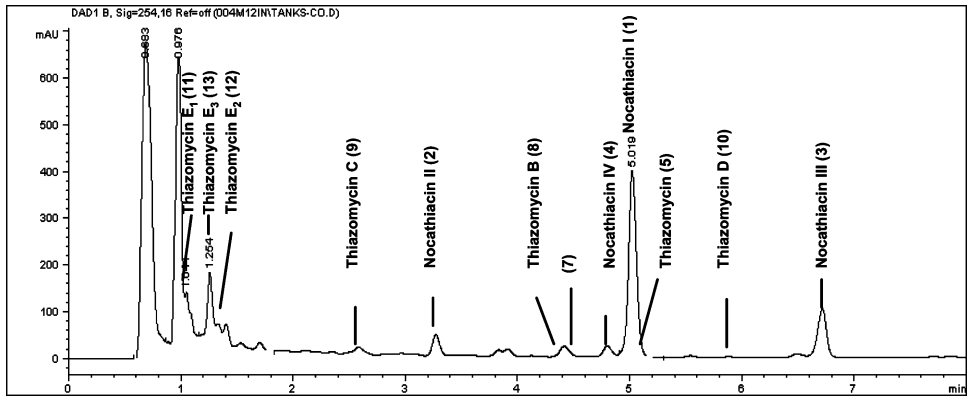
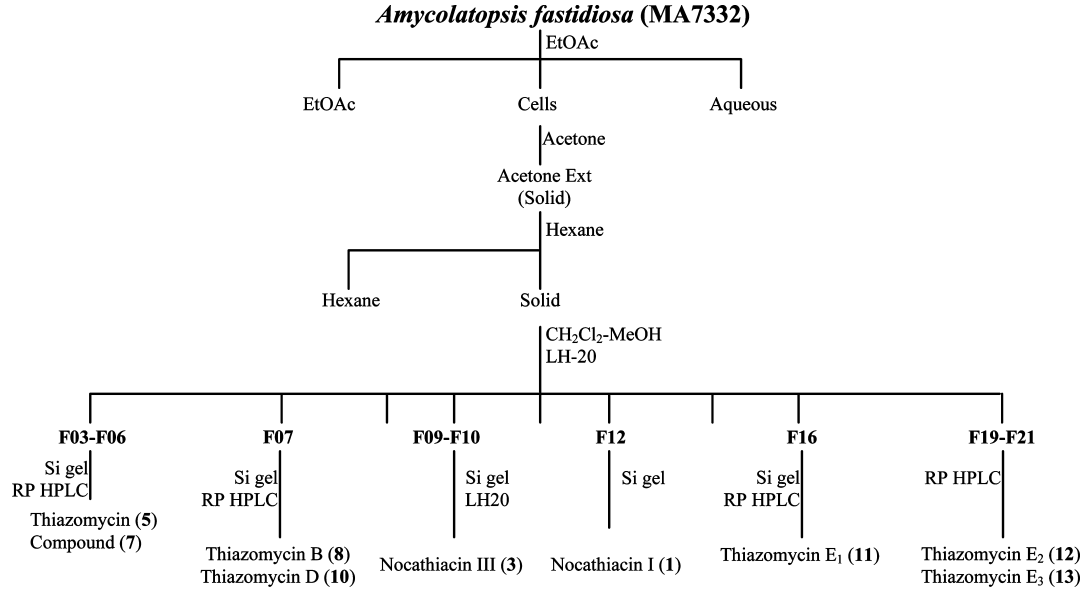


Figure 1. HPLC chromatogram of the acetone extract of *Amycolatopsis fastidiosa* (MA7332) detected at 254 nm. [Conditions: Zorbax RX C_8 (4.6 × 150 mm), 10 min gradient of 10−90% aqueous CH_3CN + 0.1% TFA, 1 mL/min.]

Scheme 1. Isolation of Thiazolyl Peptides



formula of an *N*-deoxy nocathiacin III, structure **8** was assigned to thiazomycin B.

Thiazomycin C (**9**) showed a molecular weight of 1351 and molecular formula of $C_{58}H_{57}N_{13}O_{16}S_5$ which was reduced by $C_3H_3NO_2$ from nocathiacin I and by C_3HNO_2 from thiazomycin. The UV spectrum was consistent with thiazolyl peptides. The ^1H NMR spectrum of **9** had no olefinic methylene signals of a Deala residue. No other difference in the spectrum compared to the nocathiacins was observed. Loss of the Deala residue would account for the loss of C_3H_3NO. The ESIMS spectrum gave a fragment ion at *m*/*z* 1181 indicating loss of the sugar residue; therefore, an *N*-deoxy nocathiacin IV structure was assigned for **9**.

ESIMS analysis of **10** (thiazomycin D) revealed a molecular weight of 1409 and a formula of $C_{59}H_{55}N_{13}O_{19}S_5$. The UV spectrum showed absorption maxima consistent with the thiazolyl peptides. The formula of **10** consists of an extra oxygen and loss of C_2H_5N from the formula of nocathiacin I (**1**). ESIMS produced a fragment ion at *m*/*z* 1266 that is a common fragment ion observed for **1** and **5**, which could arise from **3** by the loss of a sugar residue. The ^1H NMR spectrum of **10** did not show resonances for the dimethyl amino group, but showed a doublet for H-4 with a large coupling (J = 9.5 Hz), suggesting replacement of the dimethyl amino group with an OH group. The large coupling (J = 9.5 Hz) between H-4 and H-5 suggested a diaxial interaction, thus reversal of the configuration at C-4 of the sugar. The remainder of the NMR spectrum was identical to **1** and **5**; thus a C-4 α-hydroxy sugar structure (**10**) was assigned for thiazomycin D.

A molecular weight of 1253 and molecular formula of $C_{50}H_{55}N_{13}O_{16}S_5$ were obtained from HRESIFTMS analysis of thiazomycin E_1 (**11**). ESIMS produced a fragment ion at *m*/*z* 1083, suggesting a loss of 170 amu, consistent with the loss of the sugar residue and structurally similar to **1**. The NMR spectra of **11** had all of the signals of **1**, except for the proton and carbon resonances of the indole unit. This finding was supported by >1 ppm upfield shifts of both the β-proton resonances (appearing at δ_H 3.70 and 3.50) of the serine residue and ∼0.3 ppm upfield shifts of H-3 and H-4 of the Glu residue, consistent with loss of the indole unit. These data and 2D NMR data helped in the assignment of structure **11** for thiazomycin E_1, the first of the truncated thiazomycins.

Thiazomycin E_2 (**12**) had a molecular weight of 1082 and a molecular formula of $C_{41}H_{38}N_{12}O_{14}S_5$, which differs by 171 amu and $C_9H_{17}NO_2$ from **11**, consistent with the loss of the sugar residue. The ^1H and ^{13}C NMR spectra of **12** were essentially identical to the spectra of **11** except for the absence of the ^1H and ^{13}C resonances associated with the sugar residue, thus establishing structure **12** for thiazomycin E_2.

Thiazomycin E_3 (**13**) was the smallest of the thiazomycins, with a molecular weight of 1013 ($C_{38}H_{35}N_{11}O_{13}S_5$), suggesting the loss of a Deala residue from **12**. This conclusion was confirmed by the analysis of the ^1H NMR spectrum. Thus, structure **13** was assigned for thiazomycin E_3.

Compounds **8**−**13** were evaluated for their ability to inhibit the growth of Gram-positive bacterial strains and were compared with thiazomycin (**5**), linezolid, and erythromycin (Table 1). Thiazo-

Table 1. Antibacterial Activities (MIC, μg/mL) of Thiazomycins **8**−**10**[a]

microorganism	strain no.	phenotype	**8**	**9**	**10**	**5**	linezolid	erythromycin
S. aureus	MB5393	MRSA, Mac^R, Imp^R	0.02	0.05	0.031	0.003	4	>32
S. aureus	MB2865	MSSA	0.02	0.05	0.03	0.006	4	0.1
S. aureus +50% human serum	MB2865	MSSA	0.25	0.1	1	0.05	2	0.1
S. aureus	CL5814	MRSA, Mac^S, Lin^R	0.02	0.03	0.06	0.01	>32	1
S. aureus	CL5705	MRSA, Mac^R	0.03	0.1	0.1	0.01	2	>32
S. aureus	CL5706	MRSA, Mac^R, VISA	0.03	0.1	0.1	0.01	2	>32
S. hemolyticus	CL8544	Amp^S	0.03	0.1	0.06	0.02	2	1
S. epidermidis	CL8040		0.03	0.1	0.03	0.03	1	0.25
Enterococcus faecalis	CL8516	Van^S	0.1	>0.1	0.1	0.02	2	1
E. faecalis	CL5246	Van^R, Lin^S, Mac^R	0.06	0.1	0.1	0.01	8	32
E. faecium	CL5791	Van^R, Lin^R	0.004	0.1	0.1	0.002	32	>32
E. faecium + 50% human serum	CL5791	Van^R, Lin^R	0.1	0.1	1	0.01	>32	>32
Streptococcus pneumoniae[b]	CL8002	Pen^S	0.008	0.01	0.1	0.003	0.5	2
S. pneumoniae[b]	CL5771	Pen^R	0.004	0.006	0.03	0.003	0.5	2
S. pyogenes	CL10440		0.004	0.006	0.02	0.002	0.5	</=0.03
Candida albicans	MY1055		>32	>32	>32	>32	>32	>32

[a] Pen^R (penicillin resistant), Pen^S (penicillin sensitive), Van^S (pancomycin sensitive), Van^R (vancomycin resistant), Mac^R (macrolide resistant), Lin^R (linezolid resistant), Amp^R (ampicillin resistant), VISA (vancomycin intermediate *S. aureus*), MRSA (methicillin-resistant *S. aureus*), MSSA (methicillin-sensitive *S. aureus*), Imp^R (imipenem resistant). All strains were tested in cation adjusted Mueller Hinton broth (CAMHB) medium unless mentioned otherwise. [b] Medium: CAMHB + 2.5% lysed horse blood.

Table 2. Antibacterial Activities (MIC, μg/mL) of Thiazomycins **11**−**13**

microorganism	strain no.	phenotype	**11**	**12**	**13**	linezolid
S. aureus	MB2865	MSSA	100	>200	>200	4
E. faecalis	CL8516	Van^S	>200	>200	200	2
S. pneumoniae	CL8002	Pen^S	200	>200	200	0.5

mycins **8**−**10** showed more potent activity than linezolid and erythromycin against all strains tested and were generally less active than thiazomycin. All three compounds generally showed better MIC values against *Streptococcus* spp. (MIC values 0.004−0.03 μg/mL) than *S. aureus* (MIC values 0.02−0.1 μg/mL), similar to that of thiazomycin and nocathiacins. All these compounds were less active against *Enterococcus* spp. (MIC values 0.004−0.1 μg/mL). It also appears that the Deala residue is as important for potency as the dimethyl amino group (**9** vs **10**) except for Streptococci, in which a dimethyl amino group appears to contribute more to activity (MIC values 0.006 vs 0.02/0.03 μg/mL). These compounds showed only modest activity when tested in the presence of 50% human serum. Since these compounds inhibit growth of bacteria by inhibition of protein synthesis by a novel mode, one not common to clinical antibacterial agents, it is therefore not surprising that the activity of these compounds was not affected by various resistant phenotypes. The resistance phenotypes included *S. aureus* with methicillin resistance (MRSA), vancomycin intermediate resistance (VISA), linezolid resistance, macrolide resistance, and imipenem resistance, and *S. pneumoniae* with penicillin resistance, Enterococci with vancomycin resistance (VRE), macrolide resistance, and multidrug-resistance including vancomycin, linezolid, and macrolide. Both macrolide and linezolid are protein synthesis inhibitors; however the strains that are resistant to these antibiotics are sensitive to compounds **8**−**10**, like thiazomycin, confirming a different site of binding of these protein synthesis inhibitors from those for macrolides and linezolid. The truncated compounds **11**−**13** were also tested in a smaller bacterial panel (Table 2). These compounds were significantly less active than their larger brethren (**1**−**10**), confirming the criticality of the indole unit and requirement of the tricyclic system.

The isolation of truncated thiazomycins E_1−E_3 (**11**−**13**) as congeners could shed some light on the overall biogenesis of the more advanced nocathiacins (**1**−**4**) and thiazomycins (**5**−**10**). On the basis of the isolation of these congeners, a logical biosynthetic sequence may be postulated that involves certain of the newly isolated compounds (Figure 2). It appears that the smallest thiazolyl peptide, **13**, is biosynthesized from eight molecules of serine/cysteine, two molecules of threonine, and one molecule of glutamic acid (Figure 2).[28] These amino acids go through coupling and condensation to form the first macrocycle. It has been shown that the tetrasubstituted pyridine ring is formed from condensation of three molecules of serine/cysteine.[29] The biosynthetic route potentially diversifies after the synthesis of **13** (or its carboxylic acid intermediate), which includes both sequential and parallel coupling of a serine/cysteine followed by dehydration to attach a dehydroalanine unit at the terminal end (**12**), formation of a glycosidic linkage (**11**), and the coupling and cyclization with a substituted indole residue (**3** and **9**). It appears that once the indole residue is attached and the tricyclic system is formed, the generation of the simplest compounds, **3**/**4**/**9**, could potentially take a different route and go through addition of either sugar or Deala moieties in various combinations to produce compounds, e.g., nocathiacin I (**1**). The simplest route for thiazomycin (**5**) and thiazomycin A (**6**) could be envisioned via intermediacy of **7**. The amino sugars could be derived from the hydroxy sugar, e.g., thiazomycin D (**10**). Alternatively, compounds **5**, **6**, and **7** could all be formed by metabolism of **1**. The reductive conversion of nocathiacin I (**1**) to thiazomycin (**5**) has been reported.

In summary, repeated fractionation of an extract of *A. fastidiosa* led to the identification of 13 compounds including six new ones. These compounds included three truncated congeners lacking an indole residue, which provided significant information concerning the antibacterial SAR relationships and clues relating to their biogenesis.

Experimental Section

General Experimental Procedures. All reagents were obtained from Sigma-Aldrich and were used without further purification. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter, and IR spectra were obtained on a Perkin-Elmer Spectrum One spectrometer.

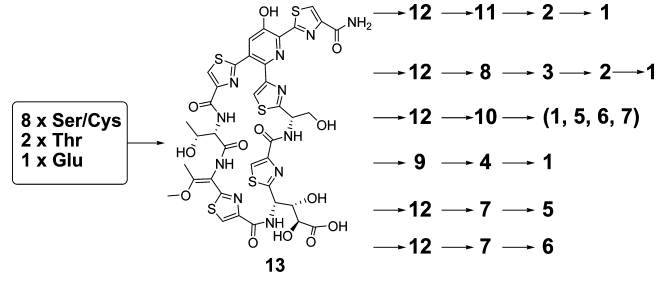


Figure 2. Biogenesis of nocathiacins and thiazomycins.

The NMR spectra were obtained on Varian Inova 500 or 600 MHz spectrometers operating at 500 or 600 MHz for ^{1}H and 125 or 150 MHz for ^{13}C nuclei. The residual solvent signal was used as a reference. High-resolution mass spectra were obtained on a Thermo Finnigan LTQ-FT with the standard Ion Max API source (without the sweep cone) and ESI probe.

Fermentation for Production of Thiazomycins 5 and 9−13. A 1 mL frozen vegetative stock culture of *Amycolatopsis fastidiosa* ATCC 202099 (MA7332) was used to inoculate 50 mL of seed medium, in a 250 mL flask, containing the following components per L of water: starch, 20 g; dextrose, 5 g; N-Z amine, 3 g; yeast extract, 2 g; pharmamedia, 5 g; calcium carbonate, 1 g. The culture was incubated at 32 °C on a rotary shaker operating at 220 rpm for 3 days. Then 20 mL of the resulting culture was used to inoculate 500 mL of seed medium, in a 2 L flask, containing the same components as for the 50 mL culture listed above. The culture was incubated at 32 °C on a rotary shaker operating at 180 rpm for 1 day. The resulting 500 mL culture was used to inoculate 20 L of media, in a 30 L fermenter, containing the following components per L of H_2O: dextrose, 20 g; peptone, 5 g; primary yeast, 10 g; Allophosite, 5 g; antifoam (P2000), 1 mL. The production fermentation was operated at a temperature of 32 °C, a back-pressure of 5 psi, and an agitation rate of 300 rpm. Air was sparged through the fermenter at 10 slpm (standard liters per minute), and pH was controlled at 7.0 by addition of aqueous solutions of NaOH and H_2SO_4. The fermenter was operated for 13 days, at which time the culture was harvested and compounds were extracted and isolated as described below.

Isolation of Thiazomycins 5 and 9−13. A 18 L fermentation broth (pH 5.0) was extracted twice with 18 and 16 L each of EtOAc by shaking overnight at room temperature. The EtOAc layers were separated, pooled, and concentrated under reduced pressure to give 7.1 g of solid. The aqueous layer was filtered through Celite, and the cake was extracted twice with 8 L of acetone each. Pooled acetone extracts were concentrated to dryness to give 15.6 g of solid, which was placed on a sintered funnel and washed with hexane (4 × 150 mL), providing 12.8 g of solid, which was suspended in 250 mL of 1:1 DCM−MeOH and filtered. An aliquot of 35 g of Sephadex LH20 was poured onto the filtrate, and the solvent was removed under reduced pressure. The LH20 powder coated with the compounds was charged on top of a 2.0 L Sephadex LH20 column packed in 1:4 hexane−DCM. The column was fitted with screw caps on both ends with the attached filter. Solvent was pumped from the top using a FMI pump. The column was eluted with two column volumes each of 1:4 hexane−DCM (fraction 3) and 2.5% MeOH−DCM (fractions 4−6), 1.5 column volume of 5% MeOH−DCM (fractions 7−11), one column volume of 10% MeOH−DCM (fractions 12−15), and 1.5 column volumes each of 20% (fraction 16−18), 50% (19−21), and finally 100% MeOH. Fractions were pooled based on TLC analysis and analytical HPLC, affording fractions 1−24 as listed in parentheses with the elution solvents.

Isolation of Thiazomycin (5). LH20 fraction 3 (1 g) was dissolved in MeOH−DCM (1:1) and adsorbed onto 5 g of silica gel. Solvent was removed under vacuum and the residue subjected to vacuum liquid chromatography (VLC) on 100 g of silica gel in a 20 cm i.d. sintered funnel. This was eluted with 0.5 L of $CHCl_3$, followed by 1 L of 10% MeOH−$CHCl_3$, 0.5 L of 20% MeOH−$CHCl_3$, and 1 L of 50% MeOH−$CHCl_3$, and finally washed with 5% NH_4OH in 50% MeOH−$CHCl_3$. Fractions from the 10% MeOH−$CHCl_3$ elution were concentrated under reduced pressure and lyophilized to yield 650 mg of a solid material, which was purified by preparative HPLC in 13 identical runs using Zorbax SB Phenyl (21 × 250 mm) eluting with a 36 min gradient of 40 to 50% acetonitrile−water containing 0.1% TFA, at a flow rate of 12 mL/min. Compound **5** eluted at 32−33 min. It was lyophilized to yield 42 mg of light yellow powder, which was further purified by two preparative HPLCs using a shallower gradient on Zorbax SB Phenyl (21 × 250 mm eluting with a 42 min gradient of 40 to 50% acetonitrile−H_2O containing 0.1% TFA, at 12 mL/min). Compound **5** eluted in 33−37 min. The fractions were pooled and lyophilized to yield 17.5 mg of **5** as a pale amorphous powder.

Isolation of Thiazomycin D (10). LH-20 fraction 7, which eluted in 5% MeOH in DCM, was concentrated to dryness (400 mg) and was subjected to silica gel chromatography in a prepacked sintered glass funnel (40 g silica gel), eluted with AcOH−MeOH−$CHCl_3$, 1:0:99, 1:1:98, 1:2:97, 1:3:96, 1:4:95, 100 mL for each, 1:5:94 (5 × 100 mL), followed by NH_4OH−MeOH−$CHCl_3$, 1:7.5:91.5 (100 mL), 1:10:89 (3 × 100 mL), 1:25:74 (2 × 100 mL), 1:99:0 (100 mL). Fractions eluted with the last 300 mL of 1:5:94 AcOH−MeOH−$CHCl_3$ elution solvent were pooled and adjusted to pH 7.0 with NH_4OH, concentrated to dryness, triturated with H_2O, and filtered, and the filtrate was concentrated to dryness to afford 33.5 mg of powder, which was then chromatographed on preparative-HPLC (YMC ODS-AQ, 20 × 250 mm, 5 mL/min, 40−50% aqueous acetonitrile with 0.05% TFA over 60 min, 215 nm). The fractions eluted at 31 and 46 min were lyophilized to furnish **8** (1.5 mg, 0.083 mg/L) and **10** (0.5 mg, 0.027 mg/L), respectively, as a pale, amorphous powder.

Isolation of Thiazomycin E_1 (11). LH20 fraction 16 (432 mg) was dissolved in $CHCl_3$−MeOH (1:1), preadsorbed onto 2 g of silica gel, solvents were removed under reduced pressure, and the powder was applied onto a silica gel-packed sintered glass funnel (25 g) under low vacuum and eluted with MeOH−$CHCl_3$, 2.5%, 5.0%, 7.5%, 10%, 15%, 20%, 30%, 50%, 75%, 100 mL each followed by 400 mL wash with 100% MeOH. The MeOH fraction was concentrated to give 84.4 mg of powder, which was subjected to preparative-HPLC fractionation (YMC ODS-AQ, 20 × 250 mm, 10 mL/min, 25−35% acetonitrile without TFA over 40 min). The fraction that eluted at 25 min was lyophilized to afford **11** (4.4 mg, 0.24 mg/L) as a pale, amorphous powder.

Isolation of Thiazomycins E_2 (12) and E_3 (13). LH-20 fraction 21 (175.0 mg) was chromatographed by RPHPLC (YMC ODS-AQ, 20 × 250 mm) with a 40 min gradient of 25−35% aqueous CH_3CN + 0.05% TFA at 12 mL/min. Fractions eluted at 22 and 31 min were lyophilized to afford 0.4 mg (0.022 mg/L) and 0.8 mg (0.044 mg/L) of **13** and **12** as pale, amorphous powders.

Isolation of Thiazomycin C (9). A 15 L fermentation of *A. fastidiosa* grown in a 23 L fermentation tank for 7 days as described earlier was extracted with 15 L of EtOAc and filtered. The filtrate was concentrated under reduced pressure using a rotary evaporator and lyophilized to produce 15 g of oily material. The extract (15 g) was dissolved in 50 mL of 90% MeOH−DCM, charged onto a 2 L Sephadex LH20 column, and eluted with MeOH−DCM (3:1) at a flow rate of 5 mL/min. Fractions of 40 mL were collected. Compounds of interest eluted in fractions 21−36 (0.8−1.4 CV). These fractions were pooled and concentrated to dryness to give 4.5 g of oily material. This material was dissolved in 50% MeOH−DCM and adsorbed onto 12 g of silica gel. It was dried under vacuum and purified using VLC on 100 g of silica gel in an 8.5 cm i.d. sintered funnel. This was washed with 2 L of MeOH−H_2O−DCM (3:0.025:97). Compound **9** eluted with 2 L of MeOH−H_2O−DCM (35:0.025:65). It was concentrated under reduced pressure and lyophilized to yield 1.8 g of solid material, which was dissolved in 10% MeOH−DCM and adsorbed onto 3 g of Diol (DL12S50, 120A, s-50 μm YMC Co. Ltd. Japan). It was concentrated under vacuum and purified on a 150 g Diol column (1.75 × 8 in.) at a flow rate of 5 mL/min. This column was eluted with DCM, followed by MeOH−H_2O−DCM (1:0.5:99), with increasing percentages of MeOH ending with MeOH−H_2O−DCM (15:1:84), and finally washed with (10:1:89) MeOH−NH_4OH−DCM. Fractions of 50 mL were collected. The fractions that eluted with the last solvent were pooled to provide 48 mg of semipurified material, which was dissolved in 10% MeOH−DCM, preadsorbed onto 0.5 g of silica gel, and purified on 10 g of silica gel (column 0.5 × 8.5 in.) using a flow rate of 1.5 mL/min. This was eluted with 250 mL of $CHCl_3$, followed by $CHCl_3$−NH_4OH with an increasing percentage of MeOH [i.e., MeOH−NH_4OH−$CHCl_3$, 2.5:0.125:97.5 (600 mL), 3:0.125:97 (800 mL), 5:0.125:95 (800 mL), 10:0.125:90 (1.4 L), 15:0.125:85 (600 mL), 25:0.125:75 (200 mL), 40:0.125:60 (200 mL)]. The fractions containing **9** eluted with 10:0.125:90 (MeOH−NH_4OH−$CHCl_3$). They were pooled, concentrated under reduced pressure, and lyophilized to afford 4 mg (0.27 mg/L) of **9**.

Thiazomycin B (8): UV (CH_3CN−H_2O) λ_{max} 218, 294, 375 nm; ^{1}H NMR ($CDCl_3$:CD_3OD, 5:1, 500 MHz) Thz-1: 8.32 (1H, s, H-4); Thr: 7.30 (1H, d, J = 7.5 Hz, NH), 4.21 (1H, dd, J = 7.5, 1.5 Hz, H-2), 1.85 (1H, m, H-3), 1.25 (3H, d, J = 6.3 Hz, H-4); Dht: 9.7 (1H, s, NH), 1.85 (3H, s, H-4), 3.74 (3H, s, OMe); Thz-2: 8.18 (1H, s, H-4); Glu: 8.31 (1H, d, J = 10 Hz, NH), 5.95 (1H, dd, J = 10, 2 Hz, H-2), 3.81 (1H, dd, J = 10, 2 Hz, H-3), 4.20 (1H, d, J = 10 Hz, H-4); Thz-3: 7.92 (1H, s, H-4); Ser: 7.81 (1H, d, J = 11 Hz, NH), 5.70 (1H, dd, J = 11, 4 Hz, H-2), 4.59 (1H, brd, J = 11 Hz, H-3), 5.31 (1H, dd, J = 11, 4 Hz, H-3); Thz-4: 7.57 (1H, s, H-4); Pyr: 7.72 (1H, brs, H-4); Thz-5: 8.23 (1H, s, H-4); Deala: 5.57 (1H, d, J = 1.8 Hz, H-3), 6.52 (1H, d, J = 1.8 Hz, H-3), 10.7 and 8.09 (1H each, brs, NH_2); indole: 4.08 (1H, d, J = 10 Hz, H-3b), 5.07 (1H, d, J = 10 Hz, H-3b), 4.93

(1H, d, J = 12 Hz, H-4a), 5.99 (1H, d, J = 12 Hz, H-4a), 7.07 (1H, d, J = 8.0 Hz, H-5), 7.30 (1H, t, J = 8.0 Hz, H-6), 7.61 (1H, d, J = 8.0 Hz, H-7); ^{13}C NMR ($CDCl_3$−CD_3OD, 5:1, 125 MHz) δ Thz-1: 164.3 (C-2), 124.3 (C-4), 150.1 (C-5), 159.2 (C═O); Thr: 168.2 (C═O), 54.6 (C-2), 66.2 (C-3), 17.6 (C-4), 168.3 (C═O); Dht: 110.0 (C-2), 159.2 (C-3), 12.9 (C-4), 55.3 (OMe), Thz-2: 166.3 (C-2); 124.1 (C-4), 149.1 (C-5), 161.7 (C═O); Glu: 49.2 (C-2), 81.4 (C-3), 67.3 (C-4), 174.4 (C═O); Thz-3: 166.4 (C-2), 123.6 (C-4), 146.0 (C-5), 161.8 (C═O); Ser: 50.7 (C-2), 64.1 (C-3); Thz-4: 168.2 (C-2), 119.3 (C-4), 154.9 (C-5); Pyr: 134.2 (C-2), 151.6 (C-3), 125.9 (C-4), 129.5 (C-5), 142.6 (C-6); Thz-5: 169.3 (C-2), 125.9 (C-4), 149.4 (C-5), 158.5 (C═O); Deala: 132.8 (C-2), 103.7 (C-3), 165.7 (C═O); indole: 161.4 (C═O), 126.3 (C-2), 115.8 C-3), 123.4 (C-3a), 64.4 (C-3b), 127.0 (C-4), 68.0 (C-4a), 122.8 (C-5), 124.1 (C-6), 115.8 (C-7), 136.4 (C-7a); ESIMS (*m*/*z*) 1272 [M + Na], 1250 [M + H]; HRESIFTMS (*m*/*z*) 1250.1670 (calcd for $C_{52}H_{43}N_{13}O_{15}S_5$ + H, 1250.1683).

Thiazomycin C (9): UV (CH_3CN−H_2O) λ_{max} 218, 298, 375 nm; ^{1}H NMR ($CDCl_3$ + CD_3OD, 500 MHz) δ Thz-1: 8.38 (1H, s, H-4); Thr: 7.38 (1H, d, J = 8 Hz, NH), 4.28 (1H, dd, J = 8, 4.5 Hz, H-2), 1.98 (1H, m, H-3), 1.29 (3H, d, J = 6.0 Hz, H-4); Dht: 7.78 (1H, brs, NH), 3.85 (3H, s, OMe), 1.94 (3H, s, H-4); Thz-2: 8.15 (1H, s, H-4); Glu: 8.43 (1H, d, J = 10 Hz, NH), 5.81 (1H, dd, J = 10, 1.5 Hz, H-2), 3.91 (1H, dd, J = 10, 1.5 Hz, H-3), 4.37 (1H, d, J = 10 Hz, H-4); Thz-3: 8.28 (1H, s, H-4); Ser: 7.81 (1H, d, J = 11.5 Hz, NH), 5.75 (1H, dd, J = 11, 4 Hz, H-2), 5.38 (1H, dd, J = 11.5, 4.5 Hz, H-3), 4.66 (1H, d, J = 11.5 Hz, H-3); Thz-4: 7.99 (1H, s, H-4); Pyr: 7.62 (1H, s, H-4); Thz-5: 8.26 (1H, s, H-4); indole: 7.66 (1H, d, J = 8.5 Hz, H-7), 7.37 (1H, dd, J = 8.5, 7 Hz, H-6), 7.14 (1H, d, J = 7 Hz, H-5), 6.13 (1H, d, J = 12.5 Hz, H-4a), 5.16 (1H, d, J = 10 Hz, H-3b), 4.96 (1H, d, J = 12.5 Hz, H-4a), 4.19 (1H, d, J = 10 Hz, H-3b); Sug: 5.13 (1H, d, J = 4 Hz, H-1), 4.06 (1H, dq, J = 1.2, 7 Hz, H-5), 3.01 (1H, brs, H-4), 2.96 (6H, s, NMe_2), 2.13 (1H, d, J = 15 Hz, H-2), 2.06 (1H, m, H-2), 1.70 (3H, s, C-3 Me), 0.88 (1H, d, J = 7 Hz, H-6); ESIMS (*m*/*z*) 1352 [M + H], 1181 [M − Sug], 1153. HRESIFTMS (*m*/*z*) 1352.2756 (calcd for $C_{58}H_{57}N_{13}O_{16}S_5$ + H, 1352.2728).

Thiazomycin D (10): UV (CH_3CN−H_2O) λ_{max} 222, 290, 375 nm; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ Thz-1: 8.31 (1H, s, H-4); Thr: 4.28 (1H, d, J = 4.5 Hz, H-2), 2.93 (1H, m, H-3), 1.39 (3H, d, J = 6.4 Hz, H-4); Dht: 3.83 (3H, s, OMe), 1.91 (3H, s, H-4); Thz-2: 8.00 (1H, s, H-4); Glu: 8.51 (1H, d, J = 9 Hz, NH), 5.81 (1H, dd, J = 9, 1.5 Hz, H-2), 3.91 (1H, dd, J = 10, 1.5 Hz, H-3), 4.42 (1H, d, J = 10 Hz, H-4); Thz-3: 8.29 (1H, s, H-4); Ser: 8.11 (1H, d, J = 11 Hz, NH), 5.69 (1H, dd, J = 11, 5.5 Hz, H-2), 5.30 (1H, dd, J = 11, 5.5 Hz, H-3), 4.45 (1H, d, J = 11 Hz); Thz-4: 7.69 (1H, s, H-4); Pyr: 7.66 (1H, s, H-4), Thz-5: 8.24 (1H, s, H-4); Deala: 6.62 (1H, d, J = 1.5 Hz, H-3), 5.54 (1H, d, J = 1.5 Hz, H-3); indole: 7.83 (1H, d, J = 8.0 Hz, H-7), 7.42 (1H, dd, J = 8.0, 7.5 Hz, H-6), 7.16 (1H, d, J = 7.5 Hz, H-5), 6.07 (1H, d, J = 12.5 Hz, H-4a), 4.95 (1H, d, J = 12.5 Hz, H-4a), 4.90 (1H, d, J = 11 Hz, H-3b), 4.23 (1H, d, J = 11 Hz, H-3b), Sug: 4.89 (1H, d, J = 4.5 Hz, H-1), 3.35 (1H, m, H-5), 3.05 (1H, d, J = 9.5 Hz, H-4), 2.17 (1H, d, J = 13.5 Hz, H-2), 1.89 (1H, dd, J = 13.5, 4.5 Hz, H-2), 1.49 (3H, s, C-3Me), 0.67 (3H, d, J = 6.5 Hz, H-6); ESIMS (*m*/*z*) 1432 [M + Na], 1266 [M − Sug]; HRESIMS (*m*/*z*) 1410.2429 (calcd for $C_{59}H_{55}N_{13}O_{19}S_5$ + H, 1410.2419).

Thiazomycin E_1 (11): $[\alpha]^{23}_D$ 60 (*c* 0.5, MeOH−THF, 1:1); UV (MeOH + THF, 1:1) λ_{max} 224 (ϵ 60 244), 364 (11 502); IR (ZnSe) ν_{max} 3368, 1657 (br, strong), 1536, 1479, 1423, 1321, 1251, 1124, 1025, 887, 761 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ Thz-1: 8.43 (1H, s, H-4); Thr: 7.38 (1H, brs, NH), 4.66 (1H, brd, J = 8 Hz, H-2), 4.78 (1H, m, H-3), 1.30 (3H, d, J = 6 Hz, H_3-4); Dht: 9.55 (1H, brs, Dht-NH), 2.02 (3H, s, H_3-4), 3.88 (3H, s, OMe); Thz-2: 8.19 (1H, s, H-4); Glu: 8.51 (1H, d, J = 8.8 Hz, NH), 5.66 (1H, dd, J = 9.3, 3 Hz, H-2), 4.00 (1H, m, H-3), 3.72 (1H, m, H-4); Thz-3: 8.35 (1H, s, H-4); Ser: 8.61(1H, brs, Ser-NH), 5.27 (1H, dt, J = 4.5, 10 Hz, H-2), 3.70 (1H, m, H-3), 3.50 (1H, t, J = 10 Hz, H-3); Thz-4: 7.81 (1H, s, H-4); Pyr: 7.87 (1H, s, H-4); Thz-5: 8.55 (1H, s, H-4); Deala: 10.08 (1H, s, NH), 8.05 and 7.59 (1H each, brs, Deala-NH_2), 6.35 and 5.72 (1H each, brs, Deala-H_2-3); Sug: 5.12 (1H, m, H-1), 1.88 (1H, dd, J = 14, 3.5 Hz, H-2), 1.74 (1H, brd, J = 14 Hz, H-2), 1.46 (3H, s, C-3-Me), 2.36 (1H, brs, H-4), 4.02 (1H, m, H-5), 0.95 (3H, d, J = 6 Hz, H_3-6), 2.60 (6H, brs, N-Me_2); ^{13}C NMR (DMSO-d_6,125 MHz) δ Thz-1: 164.2 (C-2), 125.4 (C-4), 149.9 (C-5), 160.1 (C═O), Thr: 56.0 (C-2), 69.4 (C-3), 20.9 (C-4), 169.0 (C═O), Dht: 110.2 (C-2), 160.8 (C-3), 12.7 (C-4), 55.7 (OMe), Thz-2: 162.2 (C-2), 123.0 (C-4), 146.6 (C-5), 160.0 (C═O); Glu: 49.6 (C-2), 72.7 (C-3), 72.4 (C-4), 171.7 (C═O), Thz-3: 169.3 (C-2), 124.8 (C-4), 149.2 (C-5), 160.5 (C═O), Ser: 52.9 (C-2), 62.1 (C-3), Thz-4: 168.8 (C-2), 118.4 (C-4), 153.6 (C-5), Pyr: 135.1 (C-2), 151.5 (C-3), 127.2 (C-4), 129.5 (C-5), 141.6 (C-6), Thz-5: 166.7 (C-2), 127.1 (C-4), 149.4 (C-5), 158.5 (C═O); Deala: 165.1 (C═O), 134.2 (C-2), 103.5 (C-3), Sug: 94.4 (C-1), 40.1 (C-2), 67.2 (C-3), 30.1 (C-3-Me), 68.4 (C-4), 44.1 (N-Me_2), 65.1 (C-5), 18.2 (C-6); ESIMS (*m*/*z*) 1276 [M + Na], 1083 [M − Sug], 1065 (M − Sug − H_2O), 1037 [M − Sug − $CONH_2$]; HRESIMS (*m*/*z*) 1254.2551 (calcd for $C_{50}H_{55}N_{13}O_{16}S_5$ + H, 1254.2571).

Thiazomycin E_2 (12): UV (CH_3CN−H_2O) λ_{max} 220, 295 (sh), 375 nm; ^{1}H NMR (DMSO-d_6, 500 MHz) δ Thz-1: 8.45 (1H, s, H-4); Thr: 7.60 (1H, br, NH), 4.66 (1H, bd, J = 9 Hz, H-2), 3.68 (1H, br, H-3), 0.90 (3H, d, 6.5 Hz, H-4); Dht: 3.89 (3H, s, OMe), 2.05 (3H, s, H-4), Thz-2: 8.20 (1H, s, H-4), Glu: 5.88 (1H, dd, J = 10, 2.5 Hz, H-2), 4.05 (1H, br), 3.60 (1H, br), Thz-3: 8.40 (1H, s, H-4); Ser: 5.32 (1H, dt, J = 11, 5 Hz, H-2), 3.73 (1H, dd, J = 11, 5 Hz, H-3), 3.51 (1H, t, J = 11 Hz, H-3); Thz-4: 7.86 (1H, s, H-4); Pyr: 7.92 (1H, s, H-4),Thz-5: 8.59 (1H, s, H-4); Deala: 6.34 (1H, s, H-3), 5.74 (1H, s, H-3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ Thz-1: 164.0 (C-1), 125.5 (C-4), 149.8 (C-5), 160.4 (C═O); Thr: 56.3 (C-2), 71.5 (C-3), 16.9 (C-4),169.4 (C═O); Dht: 110.0 (C-2), 160.5 (C-3), 12.7 (C-4), 55.8 (OMe); Thz-2: 162.2 (C-2), 123.5 (C-4), 146.6 (C-5), 160.4 (C═O); Glu: 49.7 (C-2), 73.6 (C-3), 66.1 (C-4), 173.6 (C═O),; Thz-3: 169.1 (C-2), 125.7 (C-4), 149.2 (C-5), 160.5 (C═O); Ser: 53.1 (C-2), 62.2 (C-3); Thz-4: 169.4 (C-2), 119.0 (C-4), 153.4 (C-5); Pyr: 135.1 (C-2), 150.8 (C-3), 127.3 (C-4), 129.6 (C-5), 142.6 (C-6); Thz-5: 167.1 (C-2), 127.1 (C-4), 149.8 (C-5), 158.4 (C═O); Deala: 165.1 (C═O), 134.3 (C-2), 103.8 (C-3); ESIMS (*m*/*z*) 1105 [M + Na], 1083 [M + H], 1081 [M − H]; HRESIFTMS (*m*/*z*) 1083.1277 (calcd for $C_{41}H_{38}N_{12}O_{14}S_5$ + H, 1083.1312).

Thiazomycin E_3 (13): UV (CH_3CN−H_2O) λ_{max} 222, 295 (sh), 370 nm; ^{1}H NMR (DMSO-d_6, 500 MHz) δ Thz-1: 8.45 (1H, s, H-4); Thr: 7.40 (1H, br, NH), 4.65 (1H, bd, J = 7.3 Hz, H-2), 3.73 (1H, m, H-3), 0.88 (3H, d, J = 6.5 Hz, H-4); Dht: 3.88 (3H, s, OMe), 2.03 (3H, s, H-4); Thz-2: 8.19 (1H, s, H-4),Glu: 8.6 (1H, br, NH), 5.86 (1H, bd, J = 9.5 Hz, H-2), 4.03 (1H, m, H-3), 3.59 (1H, m, H-4); Thz-3: 8.39 (1H, s, H-4), Ser: 5.31 (1H, m, H-2), 3.77 (1H, m, H-3), 3.49 (1H, m, H-3); Thz-4: 7.84 (1H, s, H-4), Pyr: 7.94 (1H, s, H-4); Thz-5: 8.56 (1H, s, H-4); ESIMS (*m*/*z*) 1036 [M + Na], 1014 [M + H], 1012 [M − H]; HRESIFTMS (*m*/*z*) 1014.1073 (calcd for $C_{38}H_{35}N_{11}O_{13}S_5$ + H, 1014.1097).

Minimum Inhibitory Concentration (MIC). The MIC against each of the strains was determined by National Committee for Clinical Laboratory Standards (NCCLS), now called the Clinical Laboratory Standards Institute, by the 2-fold serial broth dilution method as previously described.[30] The culture was incubated at 37 °C for 20 h before activity was read. MIC is defined as the lowest concentration of antibiotic that inhibited visible growth.

References and Notes

(1) Walsh, C. T. *Antibiotics: Actions, Origin, Resistance*; ASM Press: Washington, DC, 2003.
(2) Singh, S. B.; Barrett, J. F. *Biochem. Pharmacol.* **2006**, *71*, 1006–1015.
(3) Klevens, R. M.; Morrison, M. A.; Nadle, J.; Petit, S.; Gershman, K.; Ray, S.; Harrison, L. H.; Lynfield, R.; Dumyati, G.; Townes, J. M.; Craig, A. S.; Zell, E. R.; Fosheim, G. E.; McDougal, L. K.; Carey, R. B.; Fridkin, S. K. *J. Am. Med. Assoc.* **2007**, *298*, 1763–1771.
(4) Singh, S. B.; Jayasuriya, H.; Ondeyka, J. G.; Herath, K. B.; Zhang, C.; Zink, D. L.; Tsou, N. N.; Ball, R. G.; Basilio, A.; Genilloud, O.; Diez, M. T.; Vicente, F.; Pelaez, F.; Young, K.; Wang, J. *J. Am. Chem. Soc.* **2006**, *128*, 11916–11920, and 15547.
(5) Wang, J.; Soisson, S. M.; Young, K.; Shoop, W.; Kodali, S.; Galgoci, A.; Painter, R.; Parthasarathy, G.; Tang, Y. S.; Cummings, R.; Ha, S.; Dorso, K.; Motyl, M.; Jayasuriya, H.; Ondeyka, J.; Herath, K.; Zhang, C.; Hernandez, L.; Allocco, J.; Basilio, A.; Tormo, J. R.; Genilloud, O.; Vicente, F.; Pelaez, F.; Colwell, L.; Lee, S. H.; Michael, B.; Felcetto, T.; Gill, C.; Silver, L. L.; Hermes, J. D.; Bartizal, K.; Barrett, J.; Schmatz, D.; Becker, J. W.; Cully, D.; Singh, S. B. *Nature* **2006**, *441*, 358–361.
(6) Wang, J.; Kodali, S.; Lee, S. H.; Galgoci, A.; Painter, R.; Dorso, K.; Racine, F.; Motyl, M.; Hernandez, L.; Tinney, E.; Colletti, S.; Herath, K.; Cummings, R.; Salazar, O.; Gonzalez, I.; Basilio, A.; Vicente, F.; Genilloud, O.; Pelaez, F.; Jayasuriya, H.; Young, K.; Cully, D.; Singh, S. B. *Proc. Natl. Acad. Sci. (USA)* **2007**, *104*, 7612–7616.
(7) Jayasuriya, H.; Herath, K. B.; Zhang, C.; Zink, D. L.; Basilio, A.; Genilloud, O.; Diez, M. T.; Vicente, F.; Gonzalez, I.; Salazar, O.;

Pelaez, F.; Cummings, R.; Ha, S.; Wang, J.; Singh, S. B. *Angew. Chem., Int. Ed. Engl.* **2007**, *46*, 4684–4688.
(8) Bagley, M. C.; Dale, J. W.; Merritt, E. A.; Xiong, X. *Chem. Rev.* **2005**, *105*, 685–714.
(9) Northcote, P. T.; Siegel, M.; Borders, D. B.; Lee, M. D. *J. Antibiot. (Tokyo)* **1994**, *47*, 901–908.
(10) Northcote, P. T.; Williams, D.; Manning, J. K.; Borders, D. B.; Maiese, W. M.; Lee, M. D. *J. Antibiot. (Tokyo)* **1994**, *47*, 894–900.
(11) Sasaki, T.; Otani, T.; Matsumoto, H.; Unemi, N.; Hamada, M.; Takeuchi, T.; Hori, M. *J. Antibiot. (Tokyo)* **1998**, *51*, 715–721.
(12) 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. (Tokyo)* **2003**, *56*, 226–231.
(13) Constantine, K. L.; Mueller, L.; Huang, S.; Abid, S.; Lam, K. S.; Li, W.; Leet, J. E. *J. Am. Chem. Soc.* **2002**, *124*, 7284–7285.
(14) Connolly, T. P.; Regueiro-Ren, A.; Leet, J. E.; Springer, D. M.; Goodrich, J.; Huang, X. S.; Pucci, M. J.; Clark, J. M.; Bronson, J. J.; Ueda, Y. *J. Nat. Prod.* **2005**, *68*, 550–553.
(15) Hrnciar, P.; Ueda, Y.; Huang, S.; Leet, J. E.; Bronson, J. J. *J. Org. Chem.* **2002**, *67*, 8789–8793.
(16) 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*, 5573–5577.
(17) Naidu, B. N.; Sorenson, M. E.; Hudyma, T.; Zheng, X.; Zhang, Y.; Bronson, J. J.; Pucci, M. J.; Clark, J. M.; Ueda, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3743–3746.
(18) Naidu, B. N.; Sorenson, M. E.; Bronson, J. J.; Pucci, M. J.; Clark, J. M.; Ueda, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2069–2072.
(19) Naidu, B. N.; Sorenson, M. E.; Matiskella, J. D.; Li, W.; Sausker, 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*, 3545–3549.
(20) Pucci, M. J.; Bronson, J. J.; Barrett, J. F.; DenBleyker, K. L.; Discotto, L. F.; Fung-Tomc, J. C.; Ueda, Y. *Antimicrob. Agents Chemother.* **2004**, *48*, 3697–3701.
(21) Regueiro-Ren, A.; Ueda, Y. *J. Org. Chem.* **2002**, *67*, 8699–8702.
(22) 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.
(23) Xu, L.; Farthing, A. K.; Shi, Y. J.; Meinke, P. T.; Liu, K. *J. Org. Chem.* **2007**, *72*, 7447–7450.
(24) Zhang, C.; Occi, J.; Masurekar, P.; Barrett, J. F.; Zink, D. L.; Smith, S.; Onishi, R.; Ha, S.; Salazar, O.; Genilloud, O.; Basilio, A.; Vicente, F.; Gill, C.; Hickey, E. J.; Dorso, K.; Motyl, M.; Singh, S. B. *J. Am. Chem. Soc.* **2008**, *130*, 12102–12110.
(25) Singh, S. B.; Occi, J.; Jayasuriya, H.; Herath, K.; Motyl, M.; Dorso, K.; Gill, C.; Hickey, E.; Overbye, K. M.; Barrett, J. F.; Masurekar, P. *J. Antibiot. (Tokyo)* **2007**, *60*, 565–571.
(26) Jayasuriya, H.; Herath, K.; Ondeyka, J. G.; Zhang, C.; Zink, D. L.; Brower, M.; Gailliot, F. P.; Greene, J.; Birdsall, G.; Venugopal, J.; Ushio, M.; Burgess, B.; Russotti, G.; Walker, A.; Hesse, M.; Seeley, A.; Junker, B.; Connors, N.; Salazar, O.; Genilloud, O.; Liu, K.; Masurekar, P.; Barrett, J. F.; Singh, S. B. *J. Antibiot. (Tokyo)* **2007**, *60*, 554–564.
(27) Zhang, C.; Zink, D. L.; Ushio, M.; Burgess, B.; Onishi, R.; Masurekar, P.; Barrett, J. F.; Singh, S. B. *Bioorg. Med. Chem.* **2008**, *16*, 8818–8823.
(28) Singh, S. B.; Herath, K. B.; Yu, N. X.; Walker, A. A.; Connors, N. *Tetrahedron Lett.* **2008**, *49*, 6265–6268.
(29) Mocek, U.; Knaggs, A. R.; Tsuchiya, R.; Nguyen, T.; Beale, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1993**, *115*, 7557–7568.
(30) Onishi, H. R.; Pelak, B. A.; Gerckens, L. S.; Silver, L. L.; Kahan, F. M.; Chen, M. H.; Patchett, A. A.; Galloway, S. M.; Hyland, S. A.; Anderson, M. S.; Raetz, C. R. *Science* **1996**, *274*, 980–982.

NP800783B