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Isolation, structure, and antibacterial activity of thiazomycin A, a potent thiazolyl peptide antibiotic from *Amycolatopsis fastidiosa*

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ABSTRACT

Thiazolyl peptides are a class of thiazole-rich macrocyclic potent antibacterial agents. Recently, we described thiazomycin, a new member of thiazolyl peptides, discovered by a thiazolyl peptide specific chemical screening. This method also allowed for the discovery of a new thiazolyl peptide, thiazomycin A, which carries modification in the oxazolidine ring of the amino sugar moiety. Thiazomycin A is a specific inhibitor of protein synthesis (IC_{50} 0.7 μg/mL) and a potent Gram-positive antibacterial agent with minimum inhibitory concentration (MIC) ranging 0.002–0.25 μg/mL. The isolation and structure elucidation and biological activities of thiazomycin A are described.

1. Introduction

Emergence of multidrug resistant bacteria is no longer a purview of hospital borne-infections. It is now also observed in the community. In addition, it is growing rapidly in both settings. The dire consequences of this phenomenon can be seen from the results of a recent study, which show that methicillin resistant *Staphylococcus aureus* caused over 18,000 deaths in United States.[1] It is clear that there is a medical need for new antibiotics. Most of the antibacterial leads, that have led to clinically used antibiotics, were discovered more than 45 years ago.[2,3] Incremental improvements of these leads continue to deliver antibiotics that provide effective treatment options for bacterial infections. However, additional improvements are becoming significantly challenging. Fortunately the strains that are resistant to one antibiotic are susceptible to many others and thus many of these infections can still be treated. However, the emergence of multidrug resistant pathogens mentioned above and poor tissue distribution of otherwise effective antibiotic at the site of infection have led to treatment failures. In order to avoid epidemic of untreatable bacterial infections, new treatment options must be made available by discovery of new structural chemotypes that inhibit growth of bacteria by new modes of action (e.g., platensimycin[4,5] and platencin[6,7]).

Thiazolyl peptides are a class of naturally occurring antibiotics produced by soil dwelling filamentous bacteria and exemplified by thiostrepton, micrococcin,[8] glycothiohexide-α[9,10], S54832A-I,[8] MJ347-81F4A and B,[11] and nocathiacins I–IV (**1**–**4**).[12,13] These compounds are some of the most potent in vitro antibacterial agents known but were not developed as clinical agents due to poor physicochemical properties, most notably low aqueous solubility. With the development of newer chemistry and newer formulations, we recently undertook efforts to discover new thiazolyl peptides and reported the discovery of thiazomycin (**5**) by congener mining using chemical screening methods using LCHRMS.[14,15] Continued mining led to the discovery of a new congener thiazomycin A (**6**), an equally potent antibacterial agent, from *Amycolatopsis fastidiosa*. The isolation, structure elucidation, and full biological characterization of thiazomycin A are described herein.

2. Results and discussions

The *A. fastidiosa* strain MA7332 (ATCC 202099) was grown in 23 L fermentation tanks for 10 days on a production medium different from the medium used for the production of thiazomycin (**5**), and which apparently did not produce any thiazomycin A (**6**). The medium used for this study consisted of acid hydrolyzed casein, soybean meal, and beef extract compared to peptone and allophosite used for the production of thiazomycin (**5**). The harvested batch was extracted with EtOAc and acetone. The solid from the combined extract was washed with hexane and 1:1 MeOH/CH_2Cl_2. The latter wash was chromatographed on silica gel and eluted with a step gradient of 1–7.5% MeOH–$CHCl_3$ with 1% AcOH followed by 7.5–15% MeOH–$CHCl_3$ + 1% NH_4OH. The fractions

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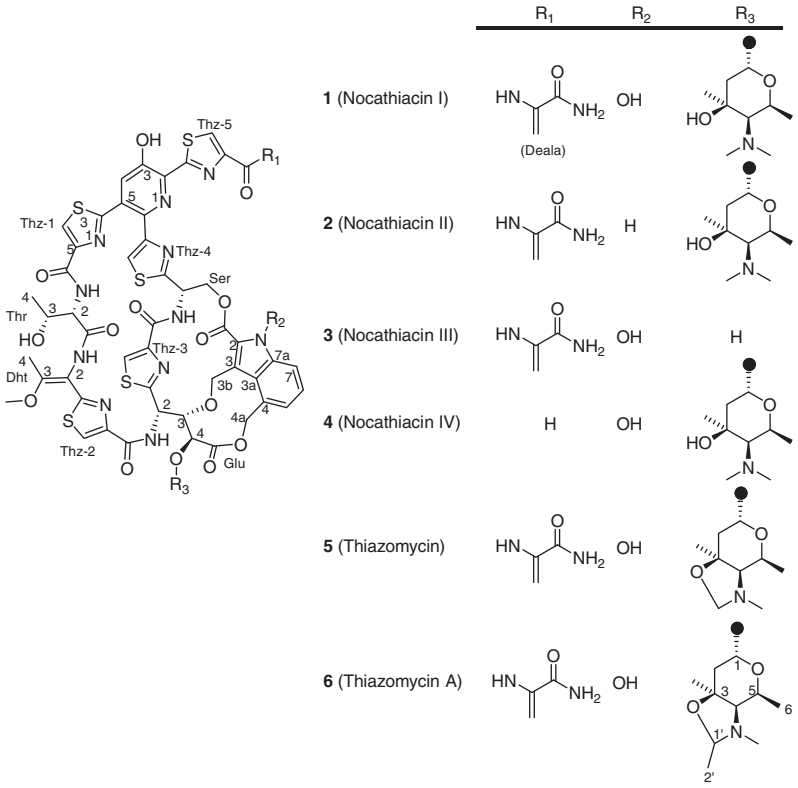

eluting with 5% MeOH contained nocathiacin III (**3**) and fractions eluting late with 7.5–15% MeOH contained nocathiacin I (**1**). The middle fractions eluting with 7.5% MeOH with NH_4OH were further chromatographed by reversed-phase HPLC to afford 7 mg (0.07 mg/L) of compound **6** as an amorphous powder. LC–MS analysis of the crude extract indicated the presence of nocathiacins I, II, III, and IV, thiazomycin, and thiazomycin A in an approximate ratio of 100:25:8:4:0.1:0.1.

3. Structure elucidation

ESI mass spectral analysis of thiazomycin A (**6**) produced a molecular weight of 1448 which was analyzed for a molecular formula of $C_{62}H_{60}N_{14}O_{18}S_5$ by HRESIFTMS. Comparison of the formula with the thiazomycin formula indicated the presence of an extra CH_2. The ESIMS of **6** produced a fragment ion at

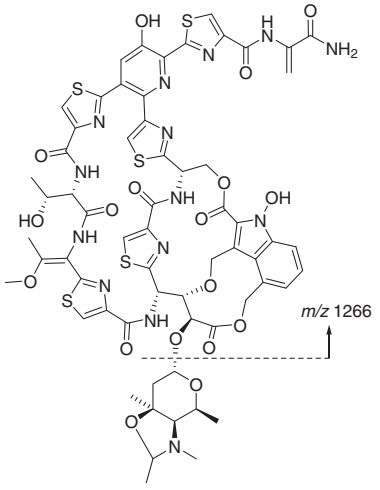

Figure 1. Key ESIMS fragmentation of thiazomycin A.

m/z 1266, the common ion produced by nocathiacin I (**1**) and thiazomycin (**5**), arising from the cleavage of the glycosidic bond suggesting the presence of a common thiazolyl peptide core structure in all three compounds (Fig. 1). These data indicated that the structural differences were in the sugar moiety. These observations were confirmed by comparison of the ^1H and ^{13}C NMR spectra of thiazomycin and thiazomycin A in two independent solvents, that is, DMSO-d_6 and 5:1 mixture of $CDCl_3 + CD_3OD$. The latter solvent afforded better resolution and sharper lines compared to the former solvent. In fact, in the former solvent a number of ^1H and ^{13}C NMR signals were missing due to signal broadening and could not be detected even in the gHSQC experiment. This phenomenon was particularly pronounced with resonances of the sugar moiety. The ^1H and ^{13}C NMR resonances of the thiazolyl peptide portion of the molecule of thiazomycin A were identical to corresponding resonances of thiazomycin. Comparison of the resonances of the sugar moiety indicated the absence of the methylene group (δ_H 4.25, d, J = 5.5 Hz; δ_H 5.13, br d, J = 5.5 Hz; δ_C 83.4) and showed the presence of a methine (δ_H 4.46, br m; δ_C 91.9) and a methyl group (δ_H 1.45, br s; δ_C 14.5), suggesting the presence of a methyl substituted oxazolidine ring. This finding was confirmed by HMBC correlations of C-1′ with the H_3-2′ methyl and the C4′-NMe group protons. The reciprocal HMBC from H-2′ was not observed due to signal broadening even in $CDCl_3$–CD_3OD. Complete set of ^1H, ^{13}C, COSY, TOCSY, gHSQC, and gHMBC data was recorded in both solvents and assignments are listed in Table 1. Like thiazomycin, the NMR spectra in the mixed solvents were highly dependent on the concentration of solute and the ratio of the two solvents. In the mixed solvents, NH protons exhibited slow deuterium exchange and were easily detected but exchanged over time and were not useful for HMBC correlations. Based on these data structure **6** was assigned for thiazomycin A. The absolute configuration of thiazomycin A is inferred to the absolute configuration of nocathiacin I and thiazomycin which are co-produced in the fermentation broth.

Table 1
^{1}H and ^{13}C NMR assignments of 25 mM thiazomycin A TFA salt (**6**)

Position	δ_C A	δ_H, J (Hz) A	δ_C B	δ_H, J (Hz) B	HMBC H → C
Thz-1(N)					
Thz-1 (C_2)	167.4	—	163.8	—	
Thz-1 (C_4)	125.4	8.33, s	126.3	8.63, s	Thz-1 (C-2, 5, C=O)
Thz-1(C_5)	149.5	—	149.5	—	
Thz-1(CO)	161.7	—	158.0	—	
Thr (CO)	169.3		ND		
Thr (NH)		7.94, d, 4.0			
Thr (C_2)	56.0	4.22, br d, 3.5	55.4	4.15, m	
Thr (C_3)	64.1	2.87, m	65.0	2.87, m	
Thr (C_4)	17.5	1.41, d, 6.0	17.4	1.12, d, 6.0	Thr (C-2, 3)
Dht (NH)					
Dht (C_2)	110.1		109.5		
Dht (C_3)	158.4		161.3		
Dht (C_4)	13.3	1.89, s	12.8	1.99, s	Dht (C-2, 3)
Dht (OMe)	55.3	3.80, s	55.8	3.86, s	Dht (C-3)
Thz-2 (N)					
Thz-2 (C_2)	162.1	—	162.8	—	
Thz-2 (C_4)	124.0	7.93, s	125.3	8.20, s	Thz-2 (C-2, 5, C=O)
Thz-2 (C_5)	145.8		146.0		
Thz-2 (CO)	161.3		160.0		
Glu (NH)		8.38, d, 10		8.39, d, 8.6	
Glu (C_2)	48.4	5.98, dd, 10, 1.5	49.2	5.85, m	Glu (C-3), Thz-2 (C=O), Thz-3 (C-2)
Glu (C_3)	81.0	3.81, dd, 10, 1.5	79.2	3.87, m	Glu (C-4, C=O)
Glu (C_4)	69.2	4.43, d, 9.5	69.2	4.39, d, 9.5	Glu (C-2, 3, C=O), Sug-C-1
Glu (CO)	171.4		171.6		
Thz-3 (N)					
Thz-3(C_2)	166.3		167.1		
Thz-3 (C_4)	126.3	8.24, s	125.5	8.51, s	Thz-3 (C-5, 2, C=O)
Thz-3 (C_5)	148.9		148.7		
Thz-3 (CO)	160.3		160.1		
Ser (NH)		8.09, d, 11		7.89, d, 11	
Ser (C_2)	48.7	5.67, dd, 11, 6.5	49.4	5.76, br m	Thz-3 (C=O), Thz-4 (C-2)
Ser (C_3)	64.1	4.41, br d, 11 5.28, dd, 11, 6.0	63.1	4.51, br d, 12 5.22, br m	Thz-3 (C=O), Thz-4 (C-2)
Thz-4 (N)					
Thz-4 (C_2)	169.1		ND		
Thz-4 (C_4)	120.1	7.69, s	120.1	7.85, s	Thz-4 (C-5, 2)
Thz-4 (C_5)	155.0		ND		
Pyr (C_2)	134.4		134.9		
Pyr (C_3)	151.3		151.0		
Pyr (C_4)	126.6	7.62, br s	127.0	7.96, s	Pyr (C-2, 6, 3), Thz-1 (C-2)
Pyr (C_5)	129.9		ND		
Pyr (C_6)	143.8		142.8		
Thz-5 (N)					
Thz-5 (C_2)	165.9		166.9		
Thz-5 (C_4)	124.3	8.21, s	127.4	8.56, s	Thz-5 (C-2, 5, C=O)
Thz-5 (C_5)	149.6		149.7		
Thz-5 (CO)	159.2		158.5		
Deala (NH)					
Deala (C_2)	132.8		134.4		
Deala (C_3)	103.7	5.58, s 6.57, s	103.8	5.74, s 6.34, s	Deala (C-2, C=O)
Deala (CO)	165.1		164.9		
Deala (NH_2)					
Indole (CO)	161.4		ND		
Indole (OH)					
Indole (C_2)	126.3		126.2		
Indole (C_3)	109.7		110.6		
Indole (C_{3a})	119.3		119.3		
Indole (C_{3b})	65.9	4.17, d, 10 4.84, d, 10	64.4	4.14, d, 10.3 4.79, d, 10.3	
Indole (C_4)	127.5		128.2		
Indole (C_{4a})	67.1	4.93, d, 12 5.96, d, 12	67.5	5.06, d, 12 5.95, d, 12	Indole (C-3a, 5, 4), Glu (C=O)
Indole (C_5)	123.7	7.13, d, 7.0	123.0	7.17, d, 8.6	Indole (C-3a, 4a, 7)
Indole (C_6)	124.9	7.37, t, 7.0	124.1	7.34, t, 8.6	Indole (C-4, 7a)
Indole (C_7)	111.9	7.76, d, 7.0	112.6	7.70, d, 8.6	Indole (C-3a, 5)
Indole (C_{7a})	135.2		135.0		
Sug (C_1)	93.1	5.07, dd, 8, 6	93.6	5.86, br m	Sug (C-5); Glu (C-4)
Sug (C_2)	35.4	1.80, dd, 16, 8 2.41, dd, 18, 6	ND	1.93, br m 2.24, br m	Sug (C-4, 3)
Sug (C_3)	79.3		ND		
Sug (C_3-Me)	24.8	1.39, s	24.3	1.40, br s	Sug (C-2, 4, 3)
Sug (C_4)	72.6	3.00, br s	ND	Broadened	Sug (C-3-Me, NMe)
Sug (C_4-NMe)	39.6	2.73, s	ND	Broadened	Sug (C-4, C-1′)
Sug (C_5)	63.5	3.93, q, 6.5	ND	Broadened	Sug (C-6, 4, 1)
Sug (C_6)	15.1	0.75, d, 6.5	15.0	0.82, br s	Sug (C-5, 4)
Sug (C-1′)	91.9	4.46, br m	ND	Broadened	
Sug (C-2′)	14.5	1.45, br s	ND	Broadened	Sug (C-1′)

A, $CDCl_3/CD_3OD$, 5:1; B, DMSO-d_6.

Table 2
In vitro antibacterial activity of thiazomycin A (MIC in μg/mL)

Organism	Strain #	Thiazomycin A	Thiazomycin	Linezolid
Streptococcus pneumoniae (penicillin^{S})*	CL2883	0.002	0.004	1
Enterococcus faecalis (vancomycin^{S})	CL8516	0.25	0.064	1
S. aureus (MSSA)	MB2865	0.06	0.016	2
S. aureus (MRSA, macrolide^{R}) COL	MB5393	0.06	0.032	2
S. aureus	EP167	0.004	NT	NT
Haemophilus influenzae	MSD 2261	>32	>32	>32
Escherichia coli	MB2884	>32	>32	>32
Candida albicans	MY1055	>32	>32	>32

All strains were tested in cation adjusted Mueller Hinton Broth (CAMHB) medium unless mentioned otherwise. *Medium: CAMHB + 2.5% lysed horse blood.

The aqueous solubility of thiazomycin A (<0.1 mg/mL) was similar to thiazomycin. Although this compound was produced as a minor congener of nocathiacin I just like thiazomycin, it is reasonable to expect that the production titer and ratio of the three compounds can be favorably improved by mutation and fermentation medium manipulations.

Thiazomycin A (**6**) inhibited growth of Gram-positive bacteria with potency similar to thiazomycin (Table 2). It showed MIC of 0.002 μg/mL against *Streptococcus pneumoniae*, 0.06 μg/mL against *S. aureus* Smith strain (MB2865), 0.004 μg/mL against *S. aureus* (EP167), 0.06 μg/mL against methicillin resistant *S. aureus* COL strain (MRSA, MB5393), and 0.25 μg/mL against *Enterococcus faecalis*. Like thiazomycin and many other thiazolyl peptides **6** was most sensitive against *S. pneumoniae* and least sensitive against *Enterococcus* sp. The in vitro activity of thiazomycin A is generally slightly lower than thiazomycin and nocathiacin I except for *S. pneumoniae* suggesting a detrimental role of the methyl substitution in the sugar moiety. It did not exhibit any activity against Gram-negative bacteria consistent with thiazomycin and other thiazolyl peptides due to lack of entry. It also did not inhibit growth of *Candia albicans* indicating selectivity for inhibition of growth of bacteria and not eukaryotic cells. It inhibited protein synthesis with IC_{50} value of 0.7 μg/mL and did not inhibit any other macromolecules (RNA, DNA, peptidoglycan, and phospholipid) of *S. aureus* (EP167) thus exhibiting strong selectivity for inhibition of protein synthesis (Fig. 2). The compound showed significant difference in the IC_{50} value of the protein synthesis inhibition and MIC value. There are a number of differences in the two assays that could account for this discrepancy. For example, the assays are carried out in different media, the concentration of bacteria differs by a factor of 1000 in the two assays and the incubation time is only 30 min in the labeling assay whereas the MIC determination requires an 18–20 h incubation.

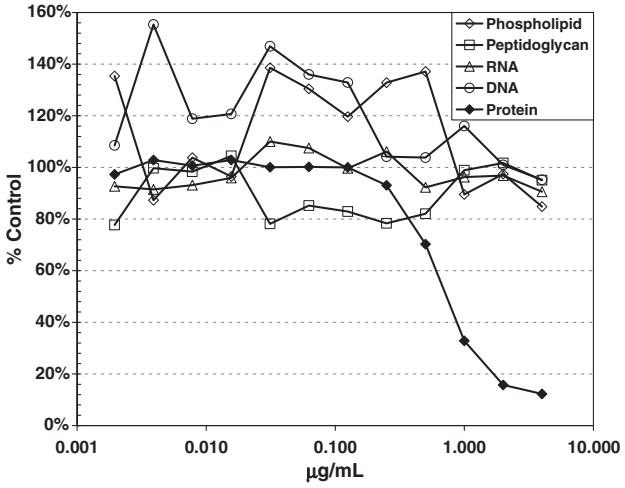

Figure 2. Inhibition of macromolecular synthesis by thiazomycin A.

In conclusion, this paper describes the isolation and structure elucidation of a new thiazolyl peptide congener of thiazomycin and nocathiacins. It is a highly potent antibiotic like its brethrens thiazomycin and nocathiacins. The congener mining is a powerful method for the discovery of related secondary metabolites. It is particularly useful for the discovery of analogs that can augment and perhaps solve some of key in the structure–activity relationship questions not accessible by standard chemical modifications.

3. Experimental

3.1. General procedure

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 spectral data were obtained on a Perkin-Elmer Spectrum One spectrometer. UV spectrum was recorded on a Perkin-Elmer Lambda 35 UV/Vis spectrometer. The NMR spectra were obtained on a Varian Inova 500 MHz spectrometers operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C nuclei. The chemical shifts were referenced to residual $CDCl_3$ (δ_H 7.27 and δ_C 77.0 ppm) and DMSO-d_6 (δ_H 2.49 and δ_C 39.5 ppm). Data were collected uniformly at 25 °C in 3 mm NMR tubes. A Nalorac 3 mm H{CN} indirect Z-gradient probe was used for all samples. Varian standard pulse sequences were used for all data collection. The 2D TOCSY data were collected with a 4900 Hz spin-lock field held for 80 ms, using the flopsy16 mixing scheme. Proton homonuclear correlation data were obtained with the Varian GCOSY or DQF-COSY pulse sequences. Single and multiple bond heteronuclear connectivity data were observed using the GHSQC and GHMBC pulse sequences, respectively. The GHMBC data were collected using a mixing time optimized for a 7 Hz heteronuclear coupling constant. High-resolution mass spectra were obtained on a Thermo Finnigan LTQ-FT using electrospray ionization using a Finnigan Ion Max source with source fragmentation on and equal to 18 V.

3.2. Fermentation conditions of ATCC 202099 (MA7332)

A 1 mL frozen vegetative stock culture of *A. fastidiosa* ATCC 202099 (MA7332) was used to inoculate 50 mL of seed medium, in a 250 mL flask, containing the following components per liter of water: soluble starch (Sigma #S-9765), 20 g; dextrose, 5 g; N-Z amine TypeEKC (Kerry Bio-Science, Hoffman Estates, IL), 3 g; Bacto yeast extract (Becton–Dickinson), 2 g; Pharmamedia (Traders Protein, Memphis, TN), 5 g; calcium carbonate, 1 g. The culture was incubated at 32 °C on a rotary shaker operating at 220 rpm for 2 days. Twenty milliliters of the resulting culture was used to inoculate 500 mL of the seed medium, in a 2 L flask. The culture was incubated at 32 °C on a rotary shaker operating at 180 rpm for 2 days. The resulting 500 mL culture was used to inoculate 15 L of production medium, in a 23 L fermenter, containing the following components per liter of water: soluble starch, 25 g; glucose, 15 g; acid hydrolyzed casein 7.5 g; yeast extract, 12 g; soybean

meal, 3.5 g; beef extract, 3.5 g; anti-foam P2000 (polymeric material made by Dow Chemical, Midland, MI, that prevents foaming), 1 mL. The production fermentation tanks were operated at 32 °C, a back-pressure of 5 psi, and an agitation rate of 300 rpm. Air was sparged through the fermenter at five standard L/min (slpm) and pH was controlled at 7.0 by addition of NaOH and H_2SO_4. The fermenter was operated for 10 days at which time the culture was harvested for isolation of metabolites.

3.3. Isolation of thiazomycin A

The 100 L broth from six tanks was pooled and was extracted with 50 L EtOAc by shaking overnight. The top EtOAc layer was removed, and the aqueous layer was filtered through Celite. The EtOAc extract was concentrated to dryness and triturated with hexane to obtain 10 g of solid material. The filtered cake consisting of cells was extracted with 2× 20 L acetone. Removal of acetone under reduced pressure afforded a precipitate which was filtered through a sintered glass funnel. Solids from EtOAc and acetone extracts were pooled and washed with hexane (500 mL) and dissolved in a 1:1 mixture of MeOH/CH_2Cl_2 (500 mL) and filtered through a sintered glass funnel. The filtrate was concentrated to dryness under reduced pressure to give 42 g of a solid which was dissolved in a 1:1 mixture of CH_2Cl_2/MeOH (200 mL), and pre-adsorbed onto 50 g silica gel and applied to a 12 × 17 cm sintered glass funnel packed with 550 g silica gel and eluted sequentially with 1% MeOH/$CHCl_3$ + 1% AcOH (2 L), 2% MeOH/$CHCl_3$ + 1% AcOH (1 L), 3% MeOH/$CHCl_3$ + 1% AcOH (1 L), 4% MeOH/$CHCl_3$ + 1% AcOH (1 L), 5% MeOH/$CHCl_3$ + 1% AcOH (4 L), 6% MeOH/$CHCl_3$ + 1% AcOH (1 L), 7.5% MeOH/$CHCl_3$ + 1% AcOH (3 L), 7.5% MeOH/$CHCl_3$ + 1% NH_4OH (5 L), 15% MeOH/$CHCl_3$ + 1% NH_4OH (2 L), 30% MeOH/$CHCl_3$ + 1% NH_4OH (2 L), 60% MeOH/$CHCl_3$ + 1% NH_4OH (2 L), 100% MeOH with 1% NH_4OH (3 L). Five hundred milliliters of each fraction was collected giving a total of 54 fractions. Fractions 11–18 eluting with 5% MeOH mostly contained nocathiacin III (**3**) and was not purified further. Fractions 29–30 eluting with 7.5% MeOH–$CHCl_3$ + 1% NH_4OH was concentrated under reduced pressure to give 333.7 mg of a powder containing compound **6**. The fractions eluting with 7.5–15% MeOH–$CHCl_3$ + 1% NH_4OH contained nocathiacins I, II, IV (**1**, **2**, and **4**) and was not purified further. The fractions eluting just before compound **6** contained thiazomycin (**5**). The fractions containing compound **6** were chromatographed by reversed-phase HPLC (Zorbax SB-phenyl, 21.2 × 250 mm). Elution with a 45 min gradient of 40–50% aqueous CH_3CN + 0.1% TFA at a flow rate of 12 mL/min and lyophilization of fractions eluting at 39–40 min afforded 7 mg (0.07 mg/L) of thiazomycin A (**6**) as a beige amorphous powder. α_D^{23} +78 (*c* 0.5, MeOH/THF, 1:1); UV (MeOH/THF, 1:1) λ_{max} 222 (ε 52,736), 288 (23,110), 362 (9,383); IR (ZnSe) ν_{max} 3387, 2978, 1742, 1667 (br, strong), 1531, 1475, 1422, 1320, 1252, 1200, 1128, 1097, 1070, 1025, 832, 801, 721 cm^{-1}; ESIMS (*m/z*) 1471 [M+Na], 1449 [M+H], 1266; HRESIMS (*m/z*) 1449.2930 (calcd for $C_{62}H_{60}N_{14}O_{18}S_5$ + H: 1449.2892); 1266.1610 (calcd for $C_{52}H_{44}N_{13}O_{16}S_5$, 1266.1633); For ^1H and ^{13}C NMR see Table 1.

3.4. 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, protocol, which involved twofold serial broth dilution method as previously described.[16] The culture was incubated at 37 °C for 18–20 h before activity was read. MIC is defined as the lowest concentration of antibiotic which inhibited visible growth.

3.5. Macromolecular synthesis inhibition in *S. aureus*

Staphylococcus aureus Ep167 was incubated to log phase at 37 °C at 220 rpm in NB medium (1% NaCl, 34 μg/mL chloramphenicol). A 0.05 mL aliquot of this culture was mixed with 0.05 mL aliquots of NB (1% NaCl) that contained various concentrations of the test compounds and one of the following (erythromycin was included in the incubation to negate the effects of the stringent response): peptidoglycan and phospholipid syntheses: 20 μg/mL of erythromycin, 0.5 μCi/mL of [^{14}C-(U)]glycine (Perkin-Elmer), and 0.5 μCi/mL of [2-^{3}H]glycerol (Perkin-Elmer); DNA and RNA syntheses: 20 μg/mL of erythromycin, 0.5 μCi/mL of [2-^{14}C]thymidine (Perkin-Elmer), and 3 μCi/mL of [5,6-3H]uracil (Perkin-Elmer); protein synthesis: 5 μCi/mL of L-[4,5-3H]leucine (Perkin-Elmer). The samples were incubated with aeration at 37 °C. After 30 min, incorporation was terminated by the addition of 0.025 mL of 25% TCA. The TCA-insoluble fraction was collected on a glass microfiber filter mat (Perkin-Elmer 1405-421) with a Molecular Devices Micro96 Cell Harvester. The filter mat was dried under a stream of hot air for 5 min. It was then placed in sample bag (Perkin-Elmer 1450-432); 4 mL of Betaplate Scint scintillation fluid was added. The bag was heat sealed and placed in a cassette (Perkin-Elmer 1450-104). Radioactivity was measured in a Perkin-Elmer MicroBeta Plate 1450 scintillation counter and % inhibition was plotted against concentration (Fig. 2).

Acknowledgments

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Supplementary data

^1H and ^{13}C NMR spectra of thiazomycin A. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.08.079.

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