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4-Aminothiazolyl analogs of GE2270 A: Design, synthesis and evaluation of imidazole analogs

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ABSTRACT

Imidazole analogs of the antibiotic natural product GE2270 A (**1**) were designed, synthesized, and evaluated for Gram positive bacteria growth inhibition. A recently reported, copper-mediated synthesis was exploited to prepare 4-thiazolyl imidazole analogs of **1**. The synthesis described represents a structurally complex, natural product-based application of this recently reported synthetic methodology. In addition, the biological evaluation of the imidazole-based analogs further define the SAR of the 4-aminothiazolyl-based antibacterial template.

Introduction: In 1991, Selva et al. from Lepetit Research Institute reported the structure and antibiotic activity of GE2270 A (**1**). This thiopeptide-based natural product was isolated from a fermentation broth of *Planobispora rosea*[1] and displayed exquisite in vitro antibacterial activity against methicillin resistant staphylococci (MRSA) and vancomycin resistant enterococci (VRE), with minimum inhibitory concentrations <1 μg/mL. Thiopeptide-based actinomycetes metabolites are characterized by their highly modified, sulfur containing macrocyclic peptide structures, which possess a tri- or tetra-substituted nitrogen-containing heterocycle core (blue color, Fig. 1). Nearly all of the members of the thiopeptide class inhibit bacterial growth by inhibiting protein synthesis, however their cellular targets are distinct. For example, the structurally complex polycycles of the Thiostreptons[2] (**2**, Fig. 1) and Nocathiacins[2] (**3**) bind to the 23S rRNA component of the bacterial 50S ribosomal subunit at the same site as the L11 ribosomal subunit, while **1** and the Thiomuracin[3] (**4**) monocycles target the prokaryotic chaperone, elongation factor Tu (EF-Tu).[4]

Recently there has been increased interest in **1** and related thiopeptides. In 2003, Vicuron Inc. reported semisynthetic derivatives of **1** intended for parenteral administration.[5] A previously described acid-mediated decomposition of the oxazoline sidechain allowed for SAR investigation of carbon-based sidechain analogs, which included terminal carboxylic acids.[6] One compound was reportedly selected for advancement to the clinic as a topical treatment for acne.[7] In addition, partial and total syntheses of **1** have been achieved by several academic groups.[8] Structural papers[9] and solution conformation studies[10] of related natural products have also recently been reported. Our own drug discovery efforts have included the fermentation, isolation, and characterization of the Thiomuracins.[3]

More recently, we described the identification, decomposition, and subsequent stabilization of 4-aminothiazolyl analogs of **1**.[11] Due to promising initial in vitro antibacterial results, the 4-aminothiazolyl template was selected for further medicinal chemistry optimization. In order to further extend the SAR and mimic the oxazoline ring structure of GE2270 A while retaining exquisite antibacterial activity, 4-thiazolyl N-linked imidazole analogs were envisioned. Thus, the acid sensitive oxazoline sidechain[1] of **1** would be replaced by a 4-thiazolyl, N-linked imidazole. This aromatic heterocyclic replacement offered the opportunity to stabilize the sidechain structure of **1** while also acting as a linchpin for further diversification and SAR

Abbreviations: MIC, minimum inhibitory concentration; G+, gram positive; MRSA, methicillin resistant staphylococci; VRE, vancomycin resistant enterococci; *S. aureus*, *Staphylococcus aureus*; *E. faecalis*, *Enterrococcus faecalis*; *E. faecium*, *Enterococcus faecium*; *S. pyogenes*, *Streptococcus pyogenes*; EF-Tu, elongation factor Tu.

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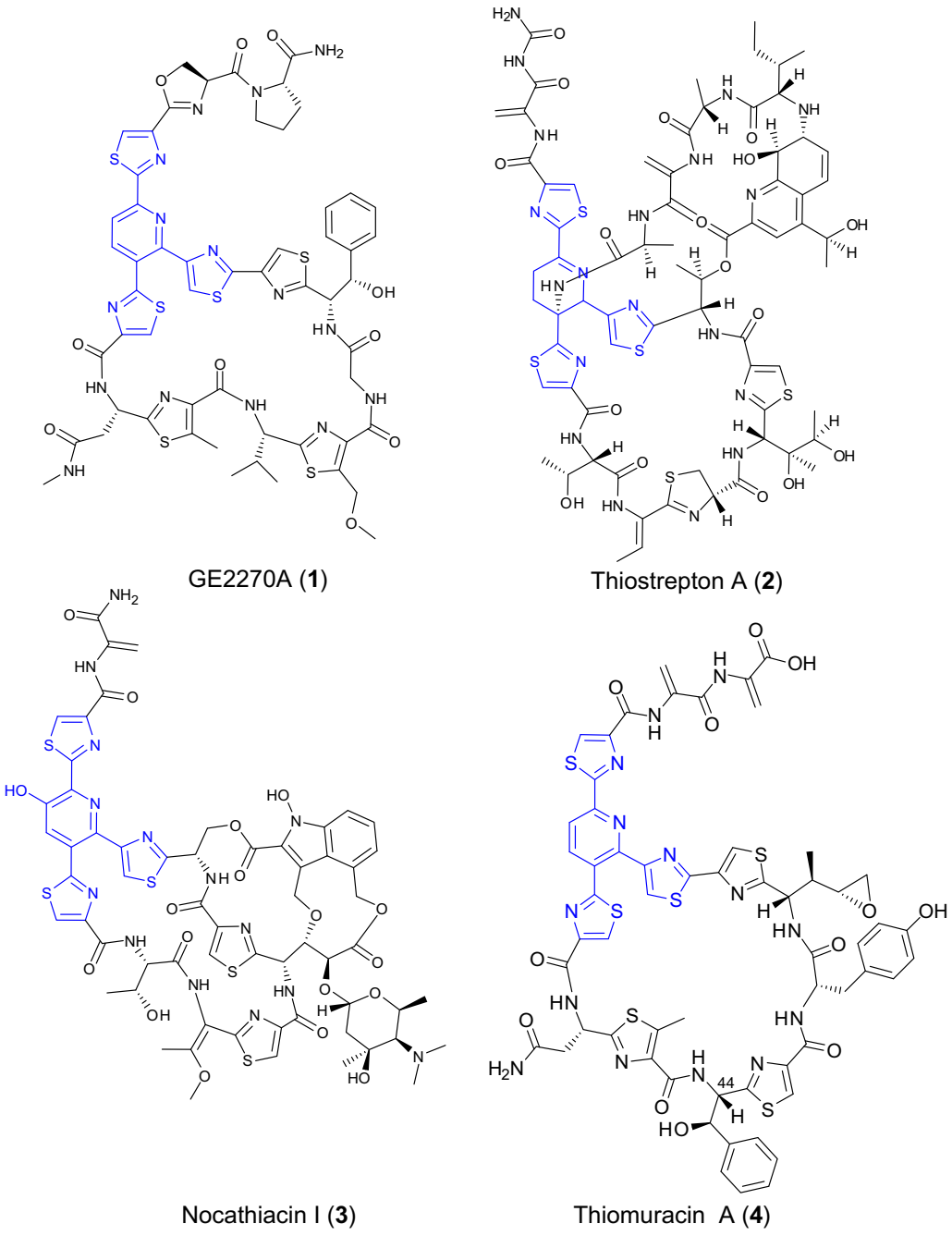
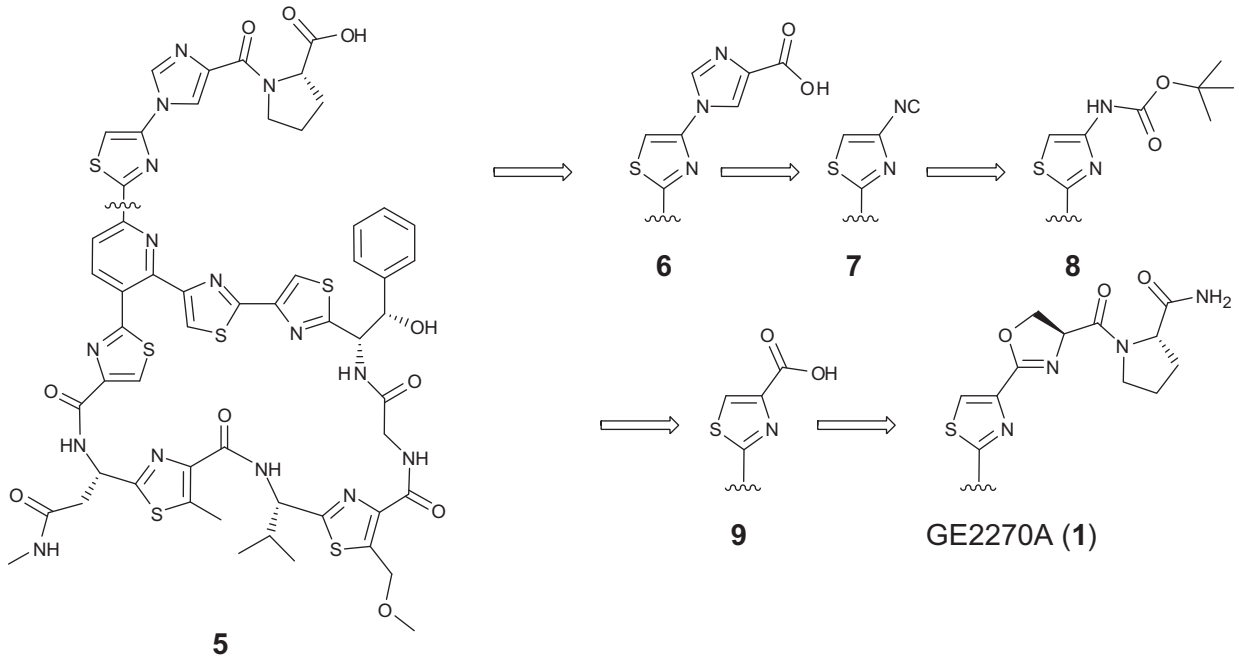


Figure 1. Thiopeptide-based natural products.

characterization. Towards this end, N-linked imidazole analogs were designed, synthesized, and evaluated for Gram positive bacterial growth inhibition.

In 2006, Kanazawa et al. reported the efficient, direct synthesis of imidazoles through a copper-catalyzed cross-cycloaddition between two different isocyanides.[12] Therefore, a 4-thiazolyl isocyanide would be required to form the N-linked imidazole analogs of **1** (Scheme 1). In the retrosynthetic sense, imidazolyl-proline **5** was disconnected at the proline amide bond revealing imidazole-acid **6**. Disconnection of the imidazole ring revealed isocyanate **7**, which was envisioned to arrive from the previously described Boc-protected 4-aminothiazole (**8**).[11] This strategic intermediate results from the Curtius rearrangement of known acid **9**,[1] a derivative of **1**.

Results and discussions: In the forward synthetic sense (Scheme 2), selective removal of the Boc protecting group under acidic conditions (TFA or HCl)[11] provided the stable amine salt which was used directly in a formylation reaction, furnishing formamide **10**. Treatment with phosphorous oxychloride then afforded isonitrile **7**, the imidazole precursor. In the cyclization event, commercially available isocyanoacetate, copper oxide, and phenanthroline were stirred in the presence of the isonitrile at elevated temperature (80 °C, THF). This process furnished the imidazole-4-carboxylic acid ethyl ester in good overall yield (**11**). Base-mediated hydrolysis then afforded the imidazole-4-carboxylic acid analog (**6**). Subsequent amide coupling utilizing polymer supported DCC[13] afforded proline analog **5**, and by the same methods the acyclic analog (**12**).



Scheme 1. Retrosynthesis of imidazole-based analogs.

Analogs **1**, **6**, **5**, and **12** were then evaluated in MIC (minimum inhibitory concentration) assays for Gram positive bacteria growth inhibition (Table 1).[14] Four organisms comprised our antibacterial screen and included *Enterrococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. All three imidazole-based analogs showed similar bacterial growth inhibition results. Unfortunately, overall the imidazole congeners all proved significantly less potent (MICs 4 to >32 μg/mL) than the natural product (MICs <1 μg/mL). Moderate activity (MICs 4–8 μg/mL) was observed against *E. faecalis* and *S. aureus* assays, while no activity was detected against *E. faecium* and *S. pyogenes*. However, using an in vitro bacterial cell extract assay measuring inhibition of protein synthesis in the absence of the cell membrane,[15] imidazoles **5** and **12** both showed activities commensurate to the natural product (50–64% inhibition @ 2 μM). We speculate that the moderate MIC of compounds **6**, **5**, and **12** results from weaker cell penetration or permeability rather than insufficient protein synthesis inhibition. Furthermore, the ionizable nature of the imidazolyl-carboxylic acid derivatives may contribute to this effect.

In summary, 4-aminothiazolyl imidazole analogs of the antibiotic natural product GE2270 A were designed, synthesized, and evaluated for Gram positive bacterial growth and protein synthesis inhibition. A recently reported, copper-mediated isonitrile cyclization provided efficient synthetic access to the imidazole derivatives. The in vitro activities of these novel congeners were moderate in MIC assays in three of four target Gram positive organisms and equipotent to the natural product in a cell extract assay measuring protein synthesis inhibition. The imidazole analogs described provide further understanding of the aminothiazole sidechain SAR, and demonstrate a complex application of a useful and efficient method of constructing imidazole heterocycles. Finally, the 4-aminothiazolyl-imidazole template described herein represents a unique, semi-synthetically derived scaffold for further antibacterial drug discovery. Certainly, the discovery of innovative chemical scaffolds that address underexploited antibacterial mechanisms of action remains a pressing need in infectious disease care in order to combat increasing clinical resistance. Further lead optimization efforts regarding 4-aminothiazole derivatives of **1** will be reported in due course.

Materials and methods: NMR: proton NMR spectra were recorded on a Bruker 400 MHz ultrashield spectrometer. Chemical shifts are reported relative to methanol (δ 3.31), dimethyl sulfoxide (δ 2.50), or chloroform (δ 7.26).

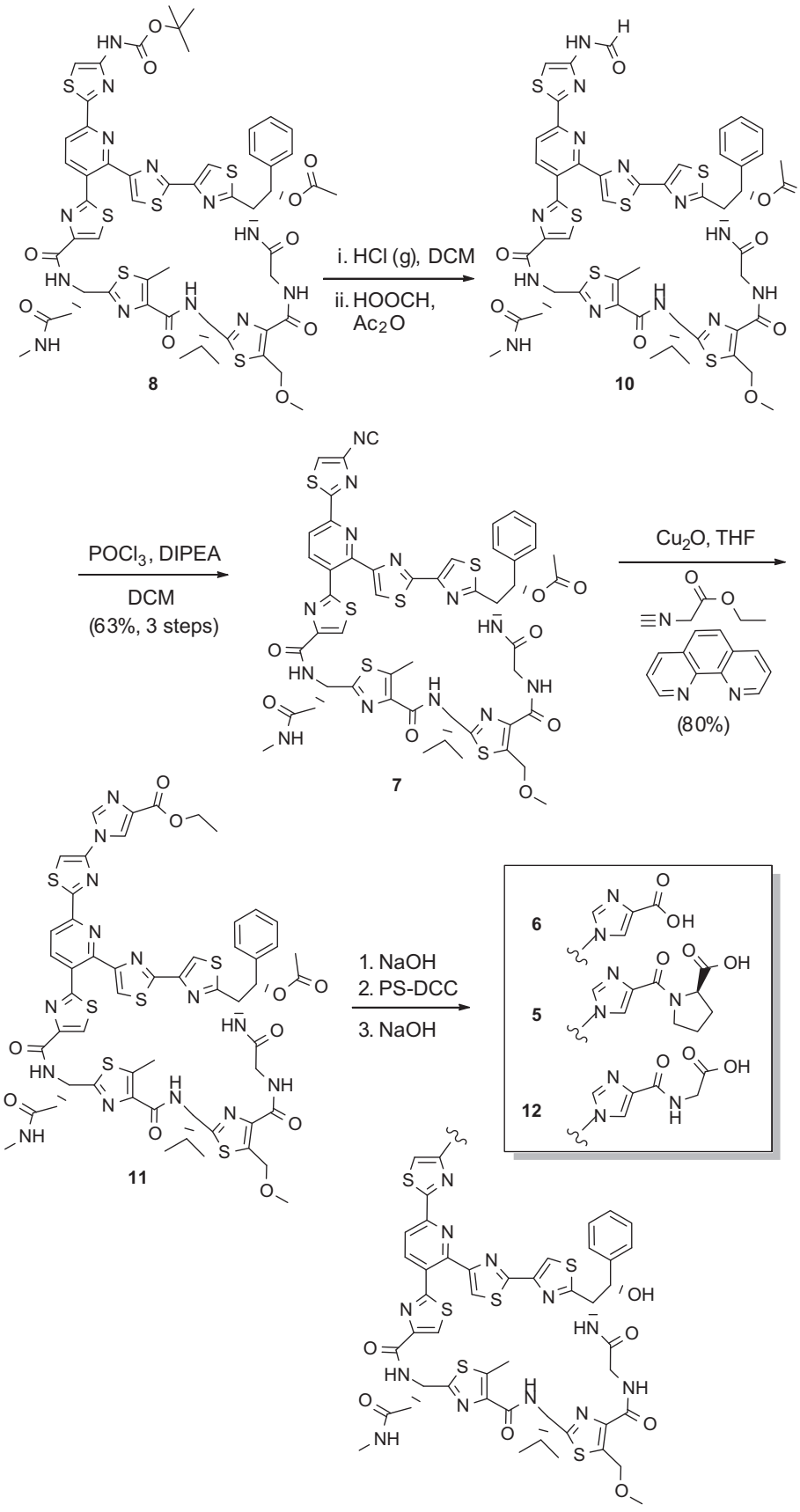
LCMS: compounds were analyzed on an Inertsil ODS-3 column (C18, 50 × 4.6 mm, 3 μM) with a 2 min gradient elution (25% acetonitrile/H_2O/5 mM ammonium formate) and a flow rate of 4 mL/min.

HPLC: purification utilizes a C8 or C18 column (30 × 100 mm, 5 μM, brand: Sunfire or XTerra) and is performed according to two methods. Method 1 consists of 0.1% TFA in 10–95% ACN in H_2O. Method 2 consists of 10 mM NH_4OH in 40–95% ACN in H_2O.

LCUV: analysis utilizes an Atlantis brand C18 column (150 mm) with a 20 min gradient elution (0–95% acetonitrile in water +0.1% TFA). All compounds tested in MIC assays were >95% purity by LCUV analysis, LCMS, and ^1H NMR.

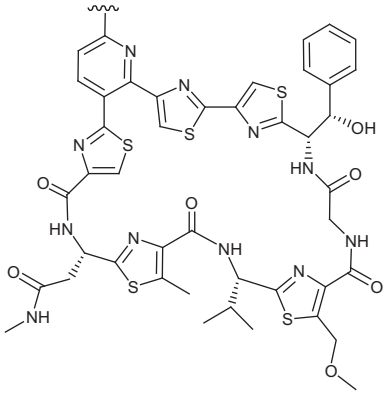
Preparation of Imidazole (**6**)*:* Steps 1 and 2: To a suspension of Boc–amine **8** (480 mg, 0.647 mmol) in DCM (150 mL) was added HCl (g, stream) for 20 min. The reaction was capped and stirred for 30 min. The reaction was concentrated to dryness and taken to the next step with no further purification. LCMS: R_t = 1.45 min, $[M+H]^+$ 1138. The residue was suspended in DCM (150 mL) and a solution of formic acid in acetic anhydride (1 mL, 3:2) is added followed by stirring at rt for 2 h. The crude formamide (**10**, 500 mg crude) is concentrated to dryness and stored in vacuo for 12 h. LCMS: R_t 1.46 min, $[M+H]^+$ 1166.

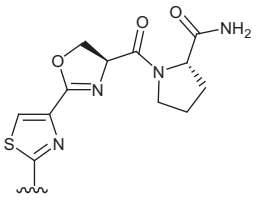
Step 3: To a suspension of the intermediate crude formamide (**10**, 90 mg, 0.771 mmol) in DCM (100 mL) was added DIPEA (1 mL, 5.74 mmol) and $POCl_3$ (100 μL, 1.27 mmol). The reaction was stirred at rt for 30 min and a second addition of DIPEA (1 mL, 5.74 mmol) and $POCl_3$ (100 μL, 1.27 mmol) was added. The reaction was stirred an additional 30 min and then concentrated onto silica gel. Purification by flash chromatography (gradient elution: 60–100% EtOAc in heptane) affords 55 mg (63%, 3 steps) of isonitrile (**7**). LCMS: R_t 1.72 min, [M] 1148.



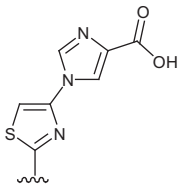
Scheme 2. Synthesis of 4-thiazolyl-imidazole analogs.

Table 1
Minimum inhibitory concentrations (MIC) and inhibition of prokaryotic protein synthesis

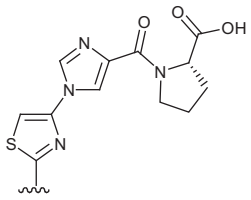




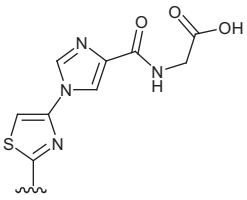
1



6



5



12

E. faecaelis	0.5	4	8	8
E. faecium	0.25	>32	>32	32
S. aureus	0.25	4	8	4
S. pyogenes	1	>32	>32	>32
Inhibition of protein expression	61%	0%	53%	64%

Step 4: To a solution of isonitrile (**7**, 155 mg, 0.135 mmol) in THF (8 mL) was added Cu_2O (cat.), ethyl isocyanoacetate (13.8 mg, 0.122 mmol) and phenanthroline (3.1 mg, 0.017 mmol). The reaction was placed in a sealed tube and heated to 80 °C for 2 h, then cooled to rt. The reaction was concentrated and purified by flash chromatography (gradient elution: 50–100% EtOAc in heptane; second column: gradient elution: 0–10% MeOH in DCM) to furnish 136 mg (80%) imidazole-ester **11**. LCMS: R_t 1.62 min, $[M+2H]^+$ 1262.

Step 5: To a solution of imidazole **11** in MeOH (8 mL) and H_2O (2 mL) was added NaOH (*s*, 10 mg, 0.25 mmol) and reaction was heated to 40 °C for 12 h. The reaction was cooled to rt and concentrated to dryness. Final purification by HPLC (gradient elution: 45–55% acetonitrile in H_2O with 0.1% TFA) generates 3 mg of **6** as a TFA salt. LCMS: R_t 1.13 min, $[M+H]^+$ 1191. HRMS: 1191.1949; calculated: 1191.1975. ^{1}H NMR (DMSO-d_6) δ ppm 9.02 (d, 1H), 8.68 (d, 2H), 8.60 (s, 1H), 8.55–8.58 (m, 2H), 8.41–8.47 (m, 2H), 8.38 (d, 1H), 8.25–8.30 (m, 2H), 7.20–7.39 (m, 8H), 5.99–6.06 (m, 1H), 5.17–5.32 (m, 3H), 4.95–5.03 (m, 3H), 4.22–4.32 (m, 1H), 3.74–3.83 (m, 1H), 3.38 (s, 3H), 2.67–2.75 (m, 1H), 2.58 (s, 3H), 2.45–2.48 (m, 3H), 2.12–2.21 (m, 1H), 1.25–1.35 (m, 1H), 0.81–0.91 (m, 6H).

Preparation of Imidazole (**12**)*:* Step 1: To a suspension of crude imidazole **6** (180 mg, 0.151 mmol) in DCM (10 mL) and pyridine (500 μL) is added PS-DCC (0.453 mmol) and amino-acetic acid methyl ester (20 mg, 0.227 mmol). The reaction is stirred at rt for 48 h and then heated to 40 °C for 2 h. The residue is concentrated onto silica gel and purified by flash chromatography (gradient elution: 50–100% EtOAc in heptanes) to generate 77 mg of methyl ester intermediate. LCMS: R_t 1.35 min, $[M+H]^+$ 1262.

Step 2: The methyl ester (77 mg, 0.061 mmol) was suspended in MeOH (5 mL) and H_2O (1 mL) and NaOH (*s*, 40 mg, 1.00 mmol) was added. The reaction was stirred at rt for 12 h and then concentrated onto silica gel for purification by flash chromatography (gradient elution: 0–10% MeOH in DCM + 0.1% acetic acid) afforded 4.2 mg of imidazole acid **12**. LCMS: R_t 1.18 min, $[M+2H]^+$ 1249. HRMS: (M/2) 624.6121, calculated: 624.6129. ^{1}H NMR (DMSO-d_6) δ ppm 9.12 (d, 1H), 8.68 (d, 2H), 8.55–8.63 (m, 2H), 8.36–8.48 (m, 4H), 8.27 (d, 2H), 8.02 (s, 1H), 7.19–7.41 (m, 8H), 6.17 (s, 1H), 5.16–5.32 (m, 3H), 4.95–5.03 (m, 3H), 4.23–4.33 (m, 1H), 3.74–3.84 (m, 1H), 3.61–3.69 (m, 2H), 3.38 (s, 3H), 2.67–2.76 (m, 1H), 2.58 (s, 3H), 2.45–2.48 (m, 3H), 2.12–2.21 (m, 1H), 1.27–1.38 (m, 1H), 0.81–0.90 (m, 6H).

Preparation of Imidazole (**5**)*:* Step 1: To a suspension of crude imidazole acid **6** (276 mg, 0.232 mmol) in DCM (20 mL) and pyridine (1.0 mL) was added PS-DCC (0.695 mmol) and (*R*)-pyrrolidine-2-carboxylic acid methyl ester (60 mg, 0.463 mmol). The reaction was stirred and heated to 40 °C for 24 h. The reaction was concentrated onto silica gel and purified (twice) by flash chromatography (gradient elution: 0–5% MeOH in DCM) to generate 170 mg of methyl ester intermediate. LCMS: R_t 1.45 min, $[M+2H]^+$ 1303.

Step 2: The methyl ester (170 mg, 1.305 mmol) was suspended in MeOH (10 mL) and H_2O (2 mL) and NaOH (*s*, 40 mg 1.00 mmol) were added. The reaction was stirred at rt for 18 h then heated to 40 °C for 12 h. The reaction was mounted onto silica gel for purification by flash chromatography (gradient elution: 0–10% MeOH in DCM) followed by HPLC purification (gradient elution: 40–60% acetonitrile in H_2O with 0.1% TFA) which afforded 5 mg of imidazole acid **5**. LCMS: R_t 1.11 min, $[M+H]^+$ 1288. HRMS: (M/2) 644.6292; calculated: 644.6285. ^{1}H NMR (DMSO-d_6) δ ppm 9.07 (d, 1H), 8.68 (d, 2H), 8.61 (s, 1H) 8.49–8.55 (m, 1H) 8.45 (d, 3H), 8.37 (d, 1H), 8.25–8.32 (m, 2H), 7.21–7.41 (m, 8H), 6.05 (s, 1H), 5.17–5.33 (m, 3H), 4.95–5.04 (m, 3H), 4.24–4.33 (m, 1H), 4.06–4.18 (m, 1H), 3.75–3.85 (m, 1H), 3.38 (s, 3H), 2.67–2.76 (m, 1H), 2.58 (s, 3H), 2.44–2.48 (m, 3H), 2.07–2.34 (m, 4H), 1.83–2.03 (m, 3H), 1.25–1.36 (m, 1H), 0.81–0.91 (m, 6H).

MIC assays: MIC assays were conducted according to the Clinical and Laboratory Standards Institute (CLSI). Wikler, Cockerill, Bush et

al. (2009); Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard-Eighth Edition. CLSI M07-A8, Vol. 29, No. 2. Bacterial strains included *E. faecalis* (ATCC 29212), *E. faecium* (Professor Chopra, Univ. Leeds UK, strain 7130724), *S. aureus* (MRSA from Professor Willinger, isolated from a pharyngeal smear, AKH Vienna A4.018), *S. pyogenes* (ATCC BAA-595). Wikler, Cockerill, Bush et al. (2009); Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth informational supplement. CLSI M100-S19, Vol. 29, No. 3.

In vitro transcription/translation assays: The *E. coli* S30 extract system for circular DNA (Promega Cat # L1020) was used per recommendation of the manufacturer with slight modifications. Briefly, 3.5 μL of 286 ng/μl template DNA (pBEST*luc*™) was mixed with 1.0 μL each of 1 mM 'methionine minus' and 'cysteine minus' amino acid mixes, 8 μL of S30 premix, 6 μL of S30 extract and 0.5 μL of the test agents at 40× final concentration, in a total volume of 20 μL. The reaction mixtures were incubated for 2 h at 37 °C in 384 well flat bottom white plate (Corning, catalog # 3704). The formation of luciferase was measured by adding equal volume (20 μL) of Steady-Glo luciferase reagent (Promega Cat # 27104) and emitted light was detected with a luminometer (Molecular Devices, LMaxll plate reader). Kirromycin and puromycin, which are known to be protein synthesis inhibitors, were used as positive controls. Ampicillin was used as a negative control. The Rabbit Reticulocyte TnT® Quick coupled Transcription/Translation system (Promega catalog # L1170) was used as recommended by the manufacturer except that the assay volumes were scaled down from 50 to 20 μL. Briefly the assay components consisted of 16 μL of TnT® Quick master mix, 3 μL of (pT7*luc*) at 167 ng/μl, 0.5 μL of methionine and 0.5 μL of the test compound at 40× final concentration in a final volume of 20 μL. The reacation mixtures were incubated at 37 °C for 75 min. Luminescence was detected as described above.

Acknowledgments

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References and notes

1. Selva, E.; Beretta, G.; Montanini, N.; Saddler, G. S.; Gastaldo, L.; Ferrari, P.; Lorenzetti, R.; Landini, P.; Ripamonti, F.; Goldstein, B. P.; Berti, M.; Montanaro, I.; Denaro, M. *J. Antibiot.* **1991**, *7*, 693; The structure was later corrected and verified: Tavecchia, P.; Gentili, P.; Kurz, M.; Sottani, C.; Bonfichi, R.; Selva, E.; Lociuro, S.; Restelli, E.; Ciabatti, R. *Tetrahedron* **1995**, *16*, 4867. For acidic decomposition see above.
2. For a review of thiopeptide natural products see: (a) Bagley, M. C.; Dale, J. W.; Merritt, E. A.; Xiong, X. *Chem. Rev.* **2005**, *105*, 685; (b) Rezanka, T.; Sobotka, M.; Spizek, J.; Sigler, K. *Anti-Infect. Agents Med. Chem.* **2006**, *5*, 187; (c) Hughs, R. A.; Moody, C. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 2; Thiostrepton: (d) Mocek, U.; Zeng, Z.; O'Hagan, D.; Zhou, P.; Fan, L. D. G.; Beale, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1993**, *115*, 7992; Nocathiocins: (e) Pucci, M. A.; Bronson, J. J.; Barret, J. F.; DenBleyker, K. L.; Driscotto, L. F.; Fung-Tomc, J. C.; Ueda, Y. *Antimicrob. Agents Chemother.* **2004**, *48*, 3697; (f) Naidu, B. N.; Sorensen, M. E.; Bronson, J. J.; Pucci, M. J.; Clark, J. M.; Ueda, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2069. and references cited therein; Amythiomicins (g) Shiminaka, K.; Kinoshita, N.; Iinuba, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1994**, *6*, 668.
3. Thiomuracins: Morris, R. P.; Leeds, J. A.; Naegeli, H. U.; Oberer, L.; Memmert, K.; Weber, E.; LaMarche, M. J.; Parker, C. N.; Burrer, N.; Esterow, S.; Hein, A. E.; Schmitt, E. K.; Krastel, P. *J. Am. Chem. Soc.* **2009**, *131*, 5946.
4. For review of the elongation factors see: Lim, V. I.; Curran, J. F.; Garber, M. B. *J. Mol. Biol.* **2005**, *351*, 470; Nilsson, J.; Nissen, P. *Curr. Opin. Struct. Biol.* **2005**, *15*, 349.
5. Clough, J.; Chen, S.; Gordon, E. M.; Hackbarth, C.; Lam, S.; Trias, J.; White, R. J.; Candiani, G.; Donadio, S.; Romano, G.; Ciabatti, R.; Jacobs, J. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3409; Malabarba, M.; Cavaleri, M.; Mosconi, G.; Jabes, D.; Romano, G.; WO03105881.
6. Tavecchia, P.; Kurz, M.; Colombo, L.; Bonofichi, R.; Selva, E.; Lociuro, S.; Marzorati, E.; Ciabatti, R. *Tetrahedron* **1996**, *26*, 8763.
7. Butler, M. S. *Nat. Prod. Rep.* **2005**, *22*, 162.
8. (a) Heckmann, G.; Bach, T. *Angew. Chem., Int. Ed.* **2005**, *44*, 1199; (b) Nicolaou, K. C.; Zou, B.; Dethe, D. H.; Li, D. B.; Chen, D. Y. K. *Angew. Chem., Int. Ed.* **2006**, *45*, 7786.
9. (a) Heffron, S. E.; Jurnak, F. *Biochemistry* **2000**, *39*, 37; (b) Heffron, S. E.; Moeller, R.; Jurnak, F. *Acta Cryst.* **2006**, *D62*, 433; (c) Parmeggiani, A.; Nissen, P. *FEBS Lett.* **2006**, *580*, 4576.
10. Lewis, R. J.; Hughs, R. A.; Alcaraz, L.; Thompson, S. P.; Moody, C. J. *Chem. Commun.* **2006**, 4215.
11. (a) LaMarche, M.J.; Leeds, J.A.; Dzink-Fox, J.; Gunderson, K.; Krastel, P.; Memmert, K.; Patane, M.A.; Rann, E.M.; Schmitt, E.; Tiamfook, S.; Wang, B. *J. Med. Chem*. ASAP Article March 15, 2011.; (b) LaMarche, Matthew J.; Bushell, Simon,; Patane, Michael, Whitehead, Lewis; Aminothiazoles and Their Uses. WO2007142986.
12. Kanazawa, C.; Kamijo, S.; Yamamoto, Y. *J. Am. Chem. Soc.* **2006**, *128*, 10662.
13. Weinshenker, G. M. et al *Org. Synth., Coll.* **1988**, *6*, 951; Guan, Y. et al *J. Comb. Chem.* **2000**, *2*, 297; Senton, K. et al *J. Comb. Chem.* **2000**, *5*, 336; Cano, M. et al *J. Org. Chem.* **2002**, *67*, 129.
14. MIC assays were conducted according to the Clinical and Laboratory Standards Institute (CLSI). Wikler, M.A., Cockerill, F.R., Bush, et al (**2009**); Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard- Eighth Edition. CLSI M07-A8, Vol. 29, No. 2. Bacterial strains included *E. faecalis* (ATCC 29212), *E. faecium* (Professor Chopra, Univ. Leeds UK, strain 7130724), *S. aureus* (MRSA from Professor Willinger, isolated from a pharyngeal smear, AKH Vienna A4.018), *S. pyogenes* (ATCC BAA-595). Wikler, M.A., Cockerill, F.R., Bush, et al (2009); Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth informational supplement. CLSI M100-S19, Vol. 29, No. 3.
15. (a) Zubay, G. *Annu. Rev. Genet.* **1973**, *7*, 267; (b) Zubay, G. *Methods Enzymol.* **1980**, *65*, 856.