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Letters

Nanomolar Inhibitors of *Staphylococcus aureus* Methionyl tRNA Synthetase with Potent Antibacterial Activity against Gram-Positive Pathogens

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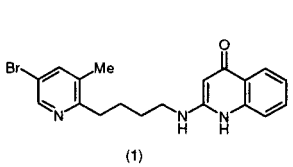
Abstract: Potent nanomolar inhibitors of *Staphylococcus aureus* methionyl tRNA synthetase have been derived from a file compound high throughput screening hit. Optimized compounds show excellent antibacterial activity against staphylococcal and enterococcal pathogens, including strains resistant to clinical antibiotics. Compound **11** demonstrated in vivo efficacy in an *S. aureus* rat abscess infection model.

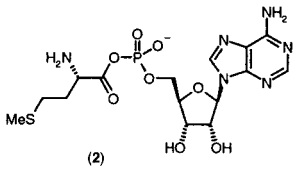
Introduction. The inexorable rise in the incidence of bacterial resistance and the attendant healthcare issues have been widely noted. Of particular concern has been the rise of methicillin resistant *Staphylococcus aureus* (MRSA), with MRSA strains now accounting for over 40% of cases of *S. aureus* bacteraemia.[1] An enormous increase has also occurred in the incidence of infections with vancomycin resistant enterococci (VRE), many strains of which have become resistant to all established classes of antibiotics.[2] This scenario has driven the search for new classes of antimicrobial agents which act on novel bacterial targets and thus should not be susceptible to established mechanisms of bacterial resistance.

The aminoacyl tRNA synthetase family of enzymes are responsible for charging tRNA molecules with their cognate amino acid in an essential step of protein synthesis.[3,4] The clinical antibiotic mupirocin (pseudomonic acid), a recommended topical agent for the prevention and eradication of MRSA outbreaks, is an inhibitor of bacterial isoleucyl tRNA synthetase. The other 18 aminoacyl tRNA synthetases present in Gram-positive bacteria are unexploited as antibacterial targets, and selective inhibition of one of these enzymes would provide an antibiotic acting by a novel mode of action. We have undertaken high throughput screening of all aminoacyl tRNA synthetases from *S. aureus*.[5−7] Here we describe potent Gram-positive antibacterial agents derived from a file compound screening hit against methionyl tRNA synthetase (MRS).

Screening Lead. High throughput screening of *S. aureus* MRS[8,9] afforded the hit **1** which had an IC_{50} value of 350 nM. The MRS inhibition was found to be competitive with respect to methionine with a K_i of 100 nM, despite little formal similarity to the tightly bound intermediate methionyl adenylate (**2**). In a standard antibacterial assay against a range of pathogens, compound **1** had no Gram-positive antibacterial activity up to the highest concentration tested (64 μg/mL).[10]



(1)



(2)

Synthesis. The inhibitors reported here were synthesized as shown in Scheme 1. The key intermediate is the amine (**5**), prepared in two steps from the quinolone (**3**). Reductive alkylation of **5** with 3,4-dichlorobenzaldehyde afforded **9** (SB-299683). A similar reaction under more forcing conditions with the ketones **7** and **8** gave the tetrahydroquinoline derivatives **10** (SB-362916) and **11** (SB-425076). Where necessary, the

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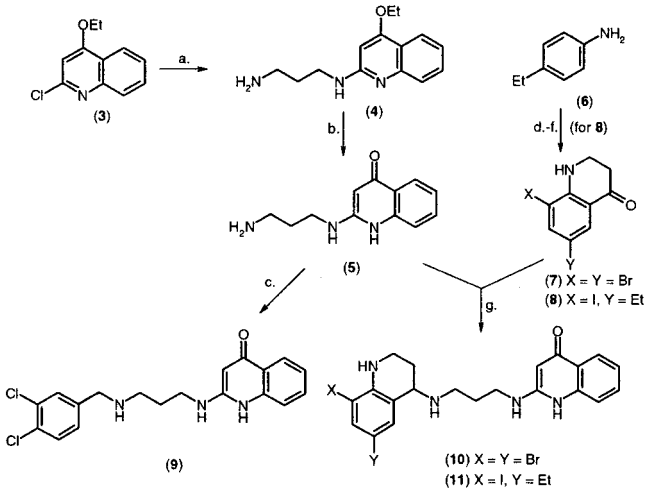
Scheme 1[a]



[a] Reagents: (a) $H_2N(CH_2)_3NH_2$/60 °C/48 h; (b) conc. HCl/reflux/24 h; (c) 3,4-diCl-PhCHO/$NaBH_3CN$/AcOH/MeOH; (d) propiolactone/MeCN/reflux/3 h; (e) P_2O_5/polyphosphoric acid/100 °C/2 h; (f) ICl/2 M HCl; (g) **7** or **8**/$NaBH_3CN$/AcOH/MeOH/reflux.

cyclic ketone was prepared from a suitably substituted aniline, for example **8** from **6**.

Enzyme Inhibition. Initial structure−activity analysis of the MRS inhibition of close analogues of **1** indicated the quinolone moiety was important for MRS inhibition but that there was significant potential for variation at the left-hand side and linker parts of the molecule. Substantially improved inhibition was obtained by introducing a secondary amine in the linker between the left-hand aryl ring and the quinolone heterocycle; for example the dichlorobenzyl-amine compound (**9**) had an IC_{50} of 16 nM against *S. aureus* MRS. Compound **9** maintained competitive kinetics with respect to methionine, with a K_i value of 5 nM.

Cyclization around the left-hand aryl ring led to the tetrahydroquinolines **10** and **11**. These compounds were potent inhibitors of *S. aureus* MRS with IC_{50} values of 8 nM and 12 nM, respectively.[9] The concentration of enzyme in the assay is 3 nM, and as the IC_{50} values are approaching the tight binding limit of the assay, the true potency of the compounds is likely to be somewhat greater than these figures suggest. To substantiate this, the inhibitors were tested in a pyrophosphate exchange assay using a large excess of substrates. This type of exchange assay has successfully differentiated tight binding inhibitors of other tRNA synthetases.[11] In the exchange assay, compounds **9**, **10**, and **11** had IC_{50} values of 500 nM, 1.0 nM, and 1.4 nM, respectively, indicating that the tetrahydroquinolines are considerably more potent than **9**.

The inhibitors were all highly selective for the bacterial MRS, showing no inhibition of rat liver MRS at concentrations up to 1000 nM. Further details of the structure−activity studies of the series will be published elsewhere.

In vitro antibacterial activity. Whole-cell antibacterial activity of compounds **9**−**11** was determined by broth microdilution.[12] In line with its increased enzyme inhibition over **1**, compound **9** shows significant antibacterial activity against *S. aureus* and *Enterococcus faecalis* (Table 1). The further enhancement in MRS potency achieved by compounds **10** and **11** results in excellent antibacterial activity against these organisms. Against the major respiratory tract pathogens, the compounds showed good activity against some strains of *Streptococcus pneumoniae* but no activity against the Gram-negative organism *Haemophilus influenzae*.

Table 1. In Vitro Minimum Inhibitory Concentration (MIC) of MRS Inhibitors against Strains of *S. aureus* and *E. faecalis*

	MIC (μg/mL)				
	amoxicillin	mupirocin	**9**	**10**	**11**[a]
S. aureus Oxford	0.125	0.125	4	<0.06	0.25
S. aureus WCUH29	>64	0.25	8	0.125	0.25
E. faecalis 1	0.5	64	2	<0.06	<0.06
E. faecalis 7	0.5	64	2	<0.06	0.125

[a] SB-425076 was tested as the dihydrochloride salt.

Table 2. In Vitro Antibacterial Activity of Compounds **10** and **11** against Panels of Staphylococci and Enterococci, Where MIC90 Is the Concentration Required To Inhibit 90% of the Strains Tested[13]

	MIC90 (μg/mL)			
	S. aureus ($n = 31$)	*S. epidermidis* ($n = 10$)	*E. faecalis* ($n = 10$)	*E. faecium* ($n = 10$)
amoxicillin	>16	8−16	>16	>16
10	0.5	0.5	0.03	<0.016
11	1	1	0.25	0.06

Compounds **10** and **11** were tested against larger collections of staphylococcal and enterococcal isolates using the NCCLS recommended procedure for broth microdilution.[13] The organisms tested consisted of reference as well as clinical isolates. As shown in Table 2, the MIC90 values against *S. aureus*, *Staphylococcus epidermidis*, *E. faecalis*, and *Enterococcus faecium* were all ≤ 1 μg/mL. Included in this evaluation were strains resistant to many clinical antibiotics, including 18 MRSA, 9 mupirocin resistant *S. aureus*, 3 mupirocin resistant *S. epidermidis*, and 2 vancomycin resistant *S. epidermidis* isolates. The *E. faecalis* and *E. faecium* collections each contained two VRE strains.

Time-kill viability studies were performed with compound **10** at concentrations up to 100 μg/mL against *S. aureus* Oxford and *E. faecalis* 1.[14] Although growth was inhibited, there was no significant reduction in bacterial counts from the test inoculum (1×10^6 cfu/mL) for either organism, up to 24 h. These results suggest that the inhibitory effect of this compound is bacteriostatic, consistent with what would be expected for a tRNA synthetase inhibitor.

Mechanism of Antibacterial Action. An expected response to the treatment of *S. aureus* with a tRNA synthetase inhibitor is an increase in the intracellular pool of guanosine 3′-diphosphate, 5′-triphosphate (ppGpp) due to the induction of the stringent response.[15,16] Cultures of *S. aureus* were treated with varying concentrations of compound **9** for 30 min, and nucleotides were extracted and then separated by HPLC.[17,18] Compound **9** at concentrations equal to MIC and 2 times MIC led to an increase in intracellular pppGpp pools between 9-fold and 10-fold relative to an untreated control (Figure 1). Identical cultures treated with 4 times MIC of mupirocin exhibited an approximately 30-fold increase in the intracellular pppGpp pool. Control compounds chloramphenicol and ciprofloxacin failed to elicit an increase in pppGpp pools leading to the

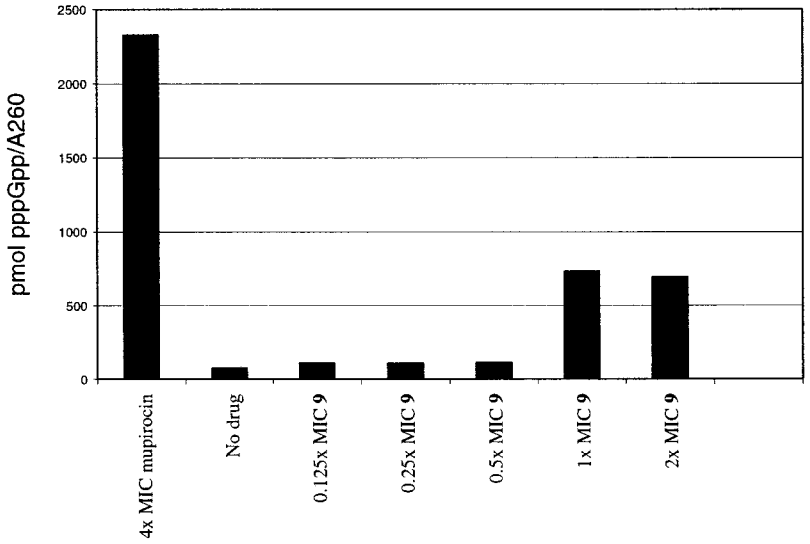


Figure 1. Quantitation of intracellular pppGpp pools in *S. aureus* following 30 min treatment with compound **9** (MIC = 4 μg/mL) and mupirocin (MIC = 0.125 μg/mL). Levels are presented as pmol/A260 unit of extract. Levels were determined from extracts run in triplicate which varied by less than 5%.

conclusion that **9** causes an amino acid starvation likely due to inhibition of MRS activity.

The mode of action of compound **9** was further confirmed by analysis of resistant mutants. *S. aureus* RN4220 mutants resistant to **9** were obtained by both chemical mutagenesis and serial passaging in the presence of drug. Nitrosoguanidine-derived mutants exhibited >4-fold increase in MIC to **9** following two passages in the presence of drug. Nucleotide sequence analysis of the MRS gene of these mutants revealed a change at nucleotide position 699, leading to an isoleucine to phenylalanine amino acid alteration. Spontaneous mutants isolated following one or four passages in the presence of drug both revealed a nucleotide alteration distinct from the chemically induced mutants, a G to A conversion at nucleotide 148, resulting in a glycine to serine amino acid alteration. Both classes of mutants were subsequently shown to be resistant to compound **10**. The mutant *metS* genes were cloned into a *S. aureus*/*Escherichia coli* shuttle vector, and the resulting plasmids conferred >4-fold resistance to **9** confirming that the resistance was due to the alterations contained in the mutant alleles.

Efficacy in Vivo. Compound **11** was tested for efficacy against *S. aureus* in a groin abscess infection in rats. Animals (specific pathogen free Sprague−Dawley rats from Charles River, Raleigh, NC) were inoculated with approximately 6.0 $\log_{10}$ cfu *S. aureus* WCUH29 in 0.5 mL of semisolid agar via subcutaneous injection into the groin. The compound, dosed as the mesylate salt, was administered as an intravenous bolus injection (21 mg/kg body weight) at 1, 5, and 24 h post-infection. After 48 h, viable bacterial numbers in the abscess were determined. Compound **11** showed good efficacy, reducing bacterial counts significantly compared with untreated animals. At a dose of 21 mg/kg, **11** produced similar efficacy to erythromycin dosed at 50 mg/kg (Table 3).

Conclusion. Compounds **10** and **11** are potent and selective inhibitors of *S. aureus* MRS. They show very good antibacterial activity against staphylococci and enterococci, including strains with resistance to known classes of antibiotics. Compound **11** was also efficacious against a *S. aureus* rat abscess infection in vivo. These compounds represent a mechanistically novel class of potential antibiotics against Gram-positive organisms.

Table 3. In Vivo Efficacy of Compound **11** against *S. aureus* WCUH29 in a Groin Abscess Infection in Rats

compound	dose (mg/kg)	bacterial counts ($\log_{10}$ cfu)
nontreated controls		7.0 ± 0.6
erythromycin	50	3.7 ± 1.2[a]
11	21	3.9 ± 0.8[a]

[a] Significant difference compared to nontreated controls, $p < 0.01$.

Supporting Information Available: Synthetic procedures and characterization data for compounds shown in Scheme 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Recombinant *S. aureus* MRS[19] was overexpressed in *E. coli* and purifed using standard procedures.
(9) Inhibitors were tested in a full aminoacylation assay essentially as described for other aminoacyl tRNA synthetases.[20,21] SAR screening was carried at a methionine concentration equal to K_m and with ATP in excess (2.5 mM).
(10) The Gram-positive organisms tested in this assay included strains of *E. faecalis*, *S. aureus*, *S. pneumoniae*, and *Streptococcus pyogenes*.
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(13) National Committee for Clinical Laboratory Standards. 2000. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard-Fifth Edition. NCCLS document M7-A5, NCCLS, Wayne PA. Number of isolates in each profile were *S. aureus*, $n = 31$; *S. epidermidis*, $n = 10$; *E. faecalis*, $n = 10$; *E. faecium*, $n = 10$.
(14) An inoculum equivalent in turbidity to a 0.5 MacFarland standard ($\sim 1 \times 10^8$ cfu/mL) was prepared in saline from a 24 h culture of each organism. A 1:2 dilution of this inoculum was made in cation adjusted Mueller Hinton broth. A final 1:100 dilution was made into flasks containing compound **10** (at 0.1, 1, 10, and 100 × MIC) to produce a final 20 mL volume. After the organism and compound were added, the flasks were placed on a shaker in a 35 °C incubator containing 5% CO_2. A 10 μL aliquot was taken from each flask at 0, 2, 4, 8, and 24 h. Ten-fold serial dilutions of the sample were prepared in a microtitre plate. Three 20 μL aliquots from each well were plated onto Mueller Hinton agar and incubated overnight at 35 °C. Colony counts were determined at the dilution that provided distinguishable colonies. An average of the three samples was determined and used to estimate the number of colony forming units per milliliter (cfu/mL) in the original sample.
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