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Potential of the Fosmidomycin analogue FR 900098 with substituted 2-oxazolines against *Francisella novicida*[†]

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Abstract

A library of 33 compounds was screened for potentiation of the antibiotic FR 900098 against the *Francisella tularensis* surrogate *Francisella novicida*. From the screen a highly potent 2-oxazoline adjuvant was discovered capable of potentiating FR 900098 with a 1000-fold reduction in MIC against the *Francisella* sub-species *F. novicida* and *F. philomiragia*.

Graphical abstract

Oxazolines represent an important class of heterocyclic compounds that are present in a wide array of natural products.¹ Additionally, oxazolines have many significant uses such as: protecting group for carboxylic acids,² ligands for asymmetric synthesis,^{3,4} and living cationic ring-opening polymerization reactions.^{5–7} Synthesis of substituted oxazolines is most commonly performed by one of three methods involving acid chlorides,⁸ aldehydes,⁹ or nitriles.¹⁰

The fosmidomycin analogue FR 900098 is known to inhibit DXR (1-deoxy-d-xylulose-5-phosphate reductoisomerase), the first committed step for the biosynthesis of isoprenoids through the non-mevalonate pathway.^{11,12} The non-mevalonate pathway is present in several pathogenic bacterial species, but is not present in humans making it an attractive drug target.^{13–16} The production of isoprenoids through the non-mevalonate pathway has been shown to be essential for the survival of *Francisella* species as mutations to the pathway are lethal.^{17,18}

Previously, our group discovered an oxazoline containing compound (**1a**) capable of potentiating FR 900098 against the Gram-negative bacteria *Francisella philomiragia* with a 256-fold reduction in MIC (Figure 1).¹⁹ Building on this discovery we opted to explore

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antibiotic potentiation against the commonly used *Francisella tularensis* surrogate *Francisella novicida*.²⁰ *F. novicida* is often used in place of *F. tularensis* since both are genetically similar but does not require special containment facilities.²¹ Antibiotic resistance and alternative treatment options for *F. tularensis* are of great interest due to its history as a biological weapon,²² extreme virulence,²³ and environmental persistence.²⁴ Herein, we report the construction of a small library of heterocyclic analogues capable of potentiating the phosphonic acid antibiotic FR 900098 against the *Francisella* sub-species *F. novicida*.

The lead compound (**1a**) was tested against *F. novicida* and reduced FR 900098's MIC 4-fold to $4\text{ }\mu\text{g mL}^{-1}$. Oxazoline analogues were then prepared by reacting 2-bromoethylamine and various acid chlorides in the presence of TEA at reflux (Scheme 1). The first series of compounds synthesized were designed in order to investigate the effect that the alkyl tail length had on antibiotic potentiation. When the tail was removed entirely ($R = H$, **1b**), no reduction in antibiotic MIC was observed (Figure 2). Modification of the tail to methyl (**1c**), ethyl (**1d**), and propyl (**1e**) substituents similarly delivered compounds that were unable to potentiate FR 900098. Increasing the tail length by a methylene unit to a butyl chain (**1f**) increased antibiotic activity 4-fold and lowered the MIC to $4\text{ }\mu\text{g mL}^{-1}$. Interestingly, further extension to hexyl (**1g**) resulted in a marked decrease in MIC to $0.0156\text{ }\mu\text{g mL}^{-1}$ a 1000-fold overall reduction. Extension of the tail to heptyl (**1h**) potentiated FR 900098 with an 8-fold reduction to an MIC of $2\text{ }\mu\text{g mL}^{-1}$.

In order to synthesize the octyl and nonyl tails, 4-octyl (**2i**) and 4-nonylbenzoyl chloride (**2j**) needed to be synthesized due to lack of commercial availability. The acid chlorides were synthesized by esterification of 4-iodobenzoic acid (**2a**) in refluxing MeOH under acid catalysis (Scheme 2). The methyl ester (**2b**) was then subjected to Sonogashira coupling with 1-octyne or 1-nonyne, giving alkynes **2c** and **2d**, which were both hydrogenated with H_2 over Pd/C in THF. The esters were then hydrolyzed with LiOH in refluxing THF/ H_2O and upon acidification gave pure carboxylic acids **2g** and **2h**. The acids were subsequently converted to the acid chlorides by treatment with oxalyl chloride in CH_2Cl_2 and a catalytic amount of DMF. Finally, the acid chlorides were cyclized to **1i** and **1j** via the approach outlined in Scheme 1. Testing of octyl (**1i**), nonyl (**1j**) and decyl (**1k**) tail lengths revealed an 8-fold reduction to an MIC of $2\text{ }\mu\text{g mL}^{-1}$ for **1i**, but no MIC reduction was observed for **1j** or **1k**.

Aside from alkyl substituents other functionalities were explored to probe the oxazolines ability to potentiate the antibiotic FR 900098. Switching the phenyl core for the heterocyclic pyridinyl core (**1l**) resulted in no change in antibiotic MIC. Incorporation of a highly electron donating *para* – dimethyl amino group (**1m**) also yielded no change in MIC, likewise for a *para* – azo motif (**1n**). Replacing the phenyl ring with a polycyclic naphthyl ring (**1o**) was non-advantageous. The results were similar for strongly electron withdrawing fluorine atoms in **1p** and **1q**, also being disadvantageous to MIC reduction, as was the introduction of a *meta* – bromine (**1r**). A cinnamoyl derivative (**1s**) was synthesized which actually increased the MIC to $32\text{ }\mu\text{g mL}^{-1}$. Interestingly, increasing the ring size of the oxazoline to a 6-membered derivative (**1t**) displayed modest activity, reducing the MIC 4-fold to $4\text{ }\mu\text{g mL}^{-1}$.

At this point our studies revealed that enhanced activity was observed with alkyl chains of pentyl, hexyl, heptyl and octyl, while other substitutions of the phenyl ring were unsuccessful at potentiating antibiotic activity. The next series of analogues were designed in order to establish the oxazolines role in activity. In order to assess whether the oxazoline was required analogues of the pentyl and hexyl tails were synthesized with oxazole head groups. Arylation of oxazole was achieved by reacting 2 equivalents of oxazole with aryl bromides (**3a** and **4a**) using Pd(OAc)₂, KOH, and RuPhos in refluxing toluene (Scheme 3).²⁵ Neither of the oxidized derivatives **3b** or **4b**, showed any activity as compared to the more saturated oxazolines. Other heterocyclic heads were heads incorporated into the oxazolines position by standard Suzuki coupling conditions. The pyrimidine (**3c**) analogue moderately potentiated FR 900098 with an MIC of 4 µg mL⁻¹, while the pyridine (**4c**) derivative was much more active lowering the MIC to 0.5 µg mL⁻¹ a 32-fold MIC reduction. We next evaluated whether substitution of the oxazoline with a thiazoline or imidazoline was tolerated. Thiazolines were synthesized by treatment of acid chlorides **5a** and **6a** with NH₄OH in MeOH affording primary amides **5b** and **6b**, which were dehydrated to nitriles by refluxing SOCl₂ (Scheme 4). Cyclization of the nitriles was performed with 2-aminoethanethiol to give thiazolines **5d** and **6d**, which reduced antibiotic MIC to 1 µg mL⁻¹ and 2 µg mL⁻¹ a 16-fold and 8-fold reduction overall, respectively. The imidazoline (**7b**) was synthesized by cyclization from **7a** with ethylenediamine and oxidation with NBS (Scheme 5).²⁶ Further oxidation of **7b** to the imidazole (**7c**) was carried out by treatment with PhI(OAc)₂.²⁷ The imidazoline, however, abrogated activity as did the more oxidized imidazole analogue. This loss in activity for the imidazoline led us to investigate the completely saturated heterocycles 1,3-dioxolane (**7d**) and 1,3-thiolane (**7e**). Acetalization and thioacetalization was carried out under standard conditions using catalytic acid and azeotropic distillation. The completely saturated analogues were inactive. Next, we sought to test the unmasked version of **1g**, the carboxylic acid (**8**). The acid was produced by hydrolysis of **6a** and was found to be unable to potentiate FR 900098 (Scheme 6). Increasing **8**'s lipophilicity by esterification with MeOH and catalytic acid failed to produce enhanced activity as the methyl ester (**9**). Surprisingly, the previously synthesized 2-aminoimidazole (**10**) analogue actually increased the MIC to 32 µg mL⁻¹, which has previously been shown to lower the MIC of β-lactam antibiotics and colistin against other gram-negative bacteria.²⁸

A time kill curve was constructed for **1g** to determine whether it was acting through a toxic or non-toxic mechanism. Analysis of the graph shows a slight delay in growth for compound containing samples as compared to strictly bacterial samples at the 4 h and 6 h time points (SI Figure 1). After 6 h samples containing **1g** began to grow at a similar rate as the control and growth became identical after 24 h. This result provides strong evidence that the oxazoline **1g** is acting through a non-bactericidal mechanism.

After discovering a highly active adjuvant for potentiation of FR 900098 against *F. novicida* we sought to establish the effectiveness of **1g** across other sub-species of the *Francisella* genus. Having previously succeeded in potentiating *F. philomiragia* with **1a** we tested **1g** against that bacterium as well. Oxazoline **1g** was slightly less active than the original lead, lowering the antibiotic MIC to 32 µg mL⁻¹ a 32-fold reduction.

In conclusion, a diverse library of heterocycles was constructed in order to investigate potentiation of the antibiotic FR 900098 against the *F. tularensis* surrogate *F. novicida*. The SAR study led to several highly active heterocycles based on the lead 2-oxazoline **1a** that were able to potentiate FR 900098. The most potent adjuvant **1g** reduced the MIC 1000-fold from 16 $\mu\text{g mL}^{-1}$ to 0.0156 $\mu\text{g mL}^{-1}$. Given the lipophilic nature of **1g**, coupled with the observation that activity falls off precipitously below 30 μM (data not shown), the mechanism of action driving FR 900098 potentiation by compound **1g** most likely involves membrane permeabilization; however further studies are necessary to validate this. Colony count enumeration provides evidence that strongly suggests that it is acting through a non-toxic mechanism. Testing of **1g** against *F. philomiragia* demonstrates **1g**'s ability to potentiate FR 900098 against multiple sub-species across the *Francisella* genus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Structures of the lead compound and antibiotic. Values in red are MIC value of compound or antibiotic. Value in blue is MIC of antibiotic in the presence of **1a** against *F. philomiragia*.

Figure 2.

Analogue library. Compounds were tested at 25% of their MIC value. Values in red are MIC value of compound or antibiotic. Values in blue are MIC of antibiotic in presence of compound against *F. novicida*. ^aCompound was tested at 40 µM.

Scheme 1.

General synthetic scheme for cyclization of oxazolines.

Scheme 2. Synthetic route to oxazolines **1i** and **1j**.

Scheme 3.

Synthetic route for aryl head analogues.

Scheme 4.
Synthetic route to thiazolines.

Scheme 5.

Synthetic route toward imidazoles and acetals.

Scheme 6.

Synthesis of the carboxylic acid (**8**) and methyl ester (**9**).