MedChemComm

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: M. Stephens, N. Yodsanit and C. Melander, *Med. Chem. Commun.*, 2016, DOI: 10.1039/C6MD00365F.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/medchemcomm

View Article Online
DOI: 10.1039/C6MD00365F

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

Matthew D. Stephens[a] , Nisakorn Yodsanit[b] and Christian Melander*[a]

[a]*Department of Chemistry, North Carolina State University, 2620 Yarbrough Drive, Raleigh, NC 27695, USA*
[b]*Department of Chemistry, Mahidol University, 999 Phuttamonthon 4 Road, Salaya 73170, Thailand*

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*.

Oxazolines represent an important class of heterocylic 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]

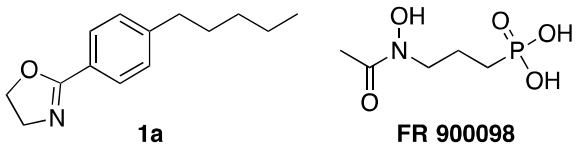
The fosmidomycin analogue FR 900098 is known to inhibit DXR (1-deoxy-d-xylulose-5-phosphate reductoisomerase), the first committed step for the biosynthesis of isoprenoids through the non-mevalonate pathway.[11,12] The non-mevalonate pathway is present in several pathogenic bacterial species, but is not present in humans making it an attractive drug target.[13-16] The production of isoprenoids through the non-mevalonate

†The authors declare no competing interests.

View Article Online
DOI: 10.1039/C6MD00365F

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 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 persistance.[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*.



1a
200 μM
4 μg mL^{-1}

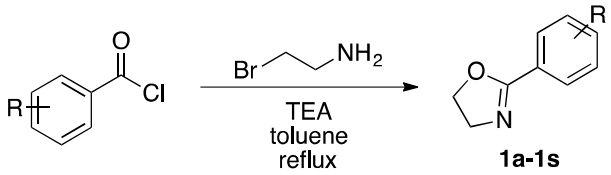
FR 900098
1,024 μg mL^{-1}

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.*

The lead compound (**1a**) was tested against *F. novicida* and reduced FR 900098's MIC 4-fold to 4 μg mL^{-1}. Oxazoline analogues were then prepared by reacting 2-

View Article Online
DOI: 10.1039/C6MD00365F

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 μg mL^{-1}. Interestingly, further extension to hexyl (**1g**) resulted in a marked decrease in MIC to 0.0156 μg 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 μg mL^{-1}.

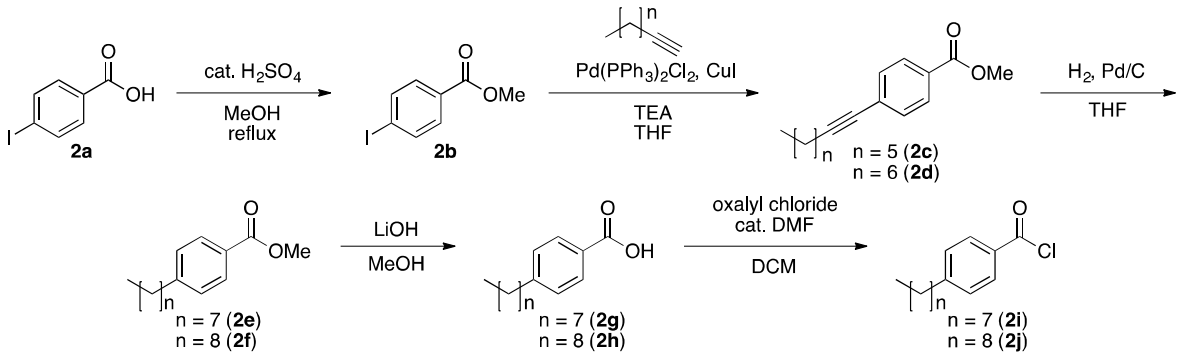


Scheme 1. General synthetic scheme for cyclization of oxazolines.

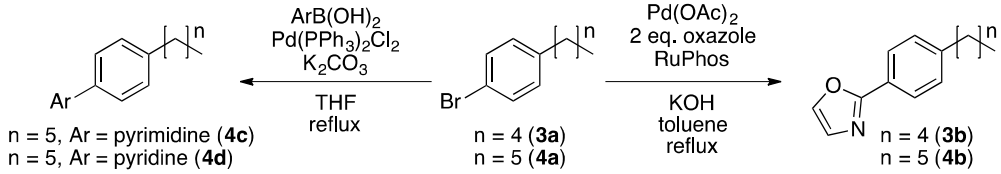
In order to synthesize the octyl and nonyl tails, 4-octyl (**2i**) and 4-nonylbenzoyl chloride (**2j**) needed to be synthesized due to lack of commercial availability. The acid chlorides were synthesized by esterification of 4-iodobenzoic acid (**2a**) in refluxing MeOH under acid catalysis (Scheme 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

View Article Online
DOI: 10.1039/C6MD00365F

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 μg mL^{-1} for **1i**, but no MIC reduction was observed for **1j** or **1k**.



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

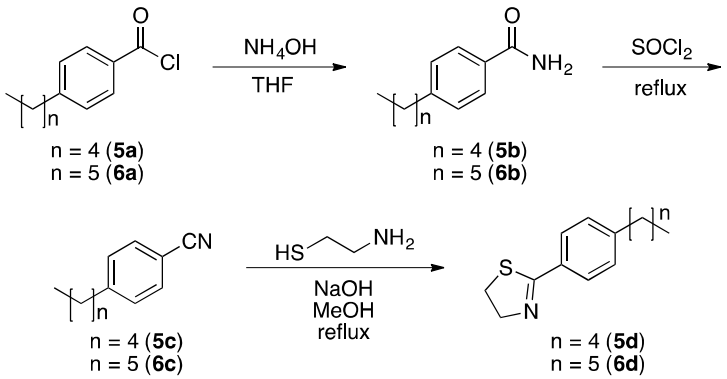


Scheme 3. Synthetic route for aryl head analogues.

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,

View Article Online
DOI: 10.1039/C6MD00365F

as was the introduction of a *meta* – bromine (**1r**). A cinnamoyl derivative (**1s**) was synthesized which actually increased the MIC to 32 μg 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 μg mL^{-1}.



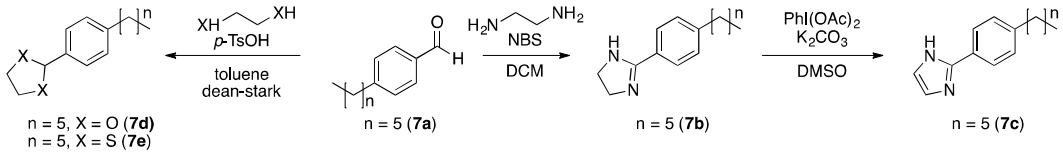
Scheme 4. Synthetic route to thiazolines.

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 heterocylic 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^{-}

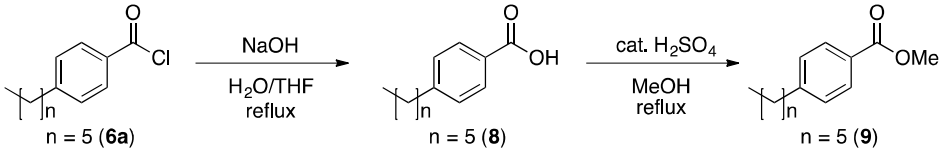
View Article Online
DOI: 10.1039/C6MD00365F

[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 NH_4OH in MeOH affording primary amides **5b** and **6b**, which were dehydrated to nitriles by refluxing $SOCl_2$ (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]

View Article Online
DOI: 10.1039/C6MD00365F



Scheme 5. Synthetic route toward imidazoles and acetals.



Scheme 6. Synthesis of the carboxylic acid (**8**) and methyl ester (**9**).

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.

View Article Online
DOI: 10.1039/C6MD00365F

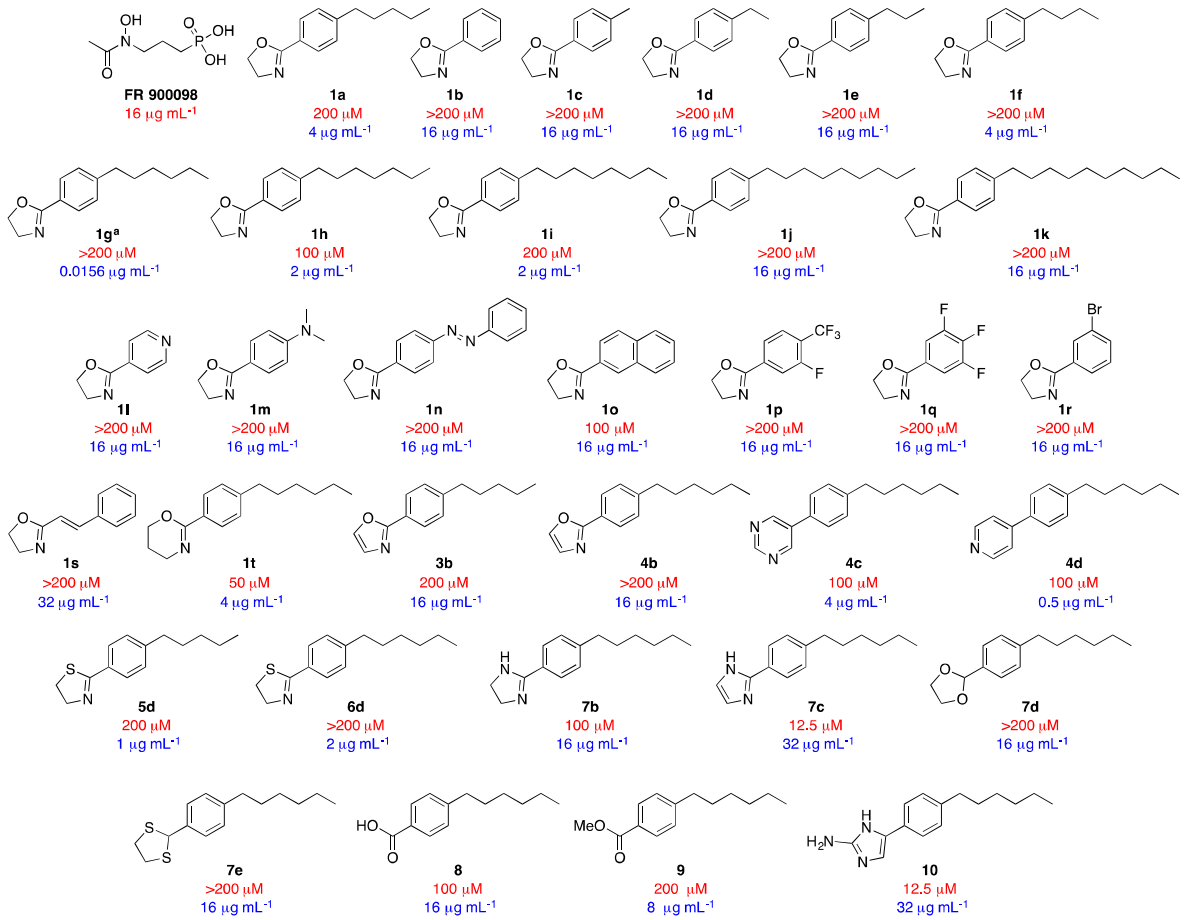


Figure 2. Analogue library. Compounds were tested at 25% of their MIC value. Values in red are MIC value of compound or antibiotic. Values in blue are MIC of antibiotic in presence of compound against *F. novicida.* [a]Compound was tested at 40 μM.

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 μg mL^{-1} to 0.0156 μg 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

View Article Online
DOI: 10.1039/C6MD00365F

compound **1g** most likely involves membrane permealization; 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.

Acknowledgements

The authors would like to thank the NIH (GM055769 and DE022350) and the Faculty of Science, Mahidol University for their generous support of this work.

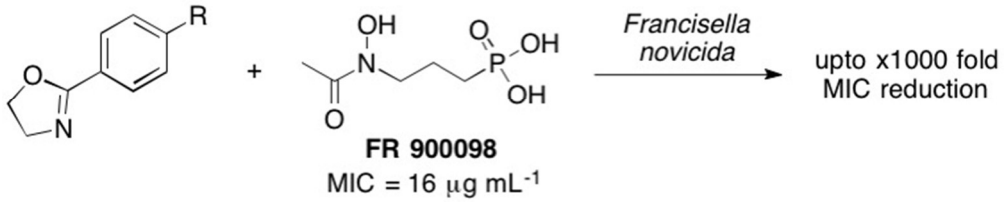
References

(1) 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.
(2) Meyers, A. I.; Temple, D. L.; Haidukewych, D.; Mihelich, E. D. *J. Org. Chem.* **1974**, *39*, 2787.
(3) Hargaden, G. C.; Guiry, P. J. *Chem. Rev.* **2009**, *109*, 2505.
(4) McManus, H. A.; Guiry, P. J. *Chem. Rev.* **2004**, *104*, 4151.
(5) Adams, N.; Schubert, U. S. *Adv. Drug Deliv. Rev.* **2007**, *59*, 1504.
(6) Hoogenboom, R. *Angew. Chem. Int. Ed.* **2009**, *48*, 7978.
(7) Kobayashi, S.; Uyama, H. *J. Polym. Sci. A Polym. Chem.* **2002**, *40*, 192.
(8) Holerca, Marian N.; Percec, V. *European J. Org. Chem.* **2000**, *2000*, 2257.
(9) Ishihara, M.; Togo, H. *Tetrahedron* **2006**, *63*, 1474.
(10) Witte, H.; Seeliger, W. *Angew. Chem. Int. Ed. Engl.* **1972**, *11*, 287.
(11) Chofor, R.; Risseeuw, M. D.; Pouyez, J.; Johny, C.; Wouters, J.; Dowd, C.S.; Couch, R. D.; Van Calenbergh, S. *Molecules (Basel, Switzerland)* **2014**, *19*, 2571.
(12) Proteau, P. J. *Bioorg. Chem.* **2004**, *32*, 483.
(13) Wiemer, A. J.; Hsiao, C. H.; Wiemer, D. F. *Curr. Top. Med. Chem.* **2010**, *10*, 1858.
(14) Jawaid, S.; Seidle, H.; Zhou, W.; Abdirahman, H.; Abadeer, M.; Hix, J. H.; van Hoek, M. L.; Couch, R. D. *PLoS ONE* **2009**, *4*, e8288.
(15) Singh, N.; Cheve, G.; Avery, M. A.; McCurdy, C. R. *Curr. Pharm. Des.* **2007**, *13*, 1161.
(16) Sangari, F. J.; Pérez-Gil, J.; Carretero-Paulet, L.; García-Lobo, J. M.; Rodríguez-Concepción, M. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 14081.
(17) Gallagher, L. A.; Ramage, E.; Jacobs, M. A.; Kaul, R.; Brittnacher, M.; Manoil, C. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 1009.
(18) Brown, A. C.; Parish, T. *BMC Microbiol.* **2008**, *8*, 78.

View Article Online
DOI: 10.1039/C6MD00365F

(19) Stephens, M. D.; Hubble, V. B.; Ernst, R. K.; van Hoek, M. L.; Melander, R. J.; Cavanagh, J.; Melander, C. *Med. Chem. Commun.* **2016**, *7*, 128.
(20) Santic, M.; Molmeret, M.; Abu Kwaik, Y. *Cell. Microbiol.* **2005**, *7*, 957.
(21) *Federal register* **2012**, *77*, 61083.
(22) Dennis, D. T.; Inglesby, T. V.; Henderson, D. A.; Bartlett, J. G.; Ascher, M. S.; Eitzen, E.; Fine, A. D.; Friedlander, A. M.; Hauer, J.; Layton, M.; Lillibridge, S. R.; McDade, J. E.; Osterholm, M. T.; O'Toole, T.; Parker, G.; Perl, T. M.; Russell, P. K.; Tonat, K. *JAMA* **2001**, *285*, 2763.
(23) Dai, S.; Mohapatra, N. P.; Schlesinger, L. S.; Gunn, J. S. *Front. Microbiol.* **2010**, *1*, 144.
(24) Berrada, Z. L.; Telford Iii, S. R. *Arch. Microbiol.* **2011**, *193*, 223.
(25) Strotman, N. A.; Chobanian, H. R.; Guo, Y.; He, J.; Wilson, J. E. *Org. Lett.* **2010**, *12*, 3578.
(26) Fujioka, H.; Murai, K.; Ohba, Y.; Hiramatsu, A.; Kita, Y. *Tetrahedron Lett.* **2005**, *46*, 2197.
(27) Ishihara, M.; Togo, H. *Synlett* **2006**, *2006*, 227.
(28) Worthington, R. J.; Bunders, C. A.; Reed, C. S.; Melander, C. *ACS Med. Chem. Lett.* **2012**, *3*, 357.

View Article Online
DOI: 10.1039/C6MD00365F



134x27mm (144 x 144 DPI)