Bioorganic & Medicinal Chemistry Letters 23 (2013) 1727–1731

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel 3-*O*-carbamoyl erythromycin A derivatives (carbamolides) with activity against resistant staphylococcal and streptococcal isolates

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ARTICLE INFO

Article history:
Received 16 November 2012
Revised 7 January 2013
Accepted 16 January 2013
Available online 26 January 2013

Keywords:
Bacterial resistance
cfr
erm
MLSb
MRSA
Macrolide
Carbamolide
Clarithromycin
Oxazolidinone
Linezolid
Ribosome X-ray
Deinococcus radiodurans

ABSTRACT

A novel series of 3-*O*-carbamoyl erythromycin A derived analogs, labeled carbamolides, with activity versus resistant bacterial isolates of staphylococci (including macrolide and oxazolidinone resistant strains) and streptococci are reported. An (*R*)-2-aryl substituent on a pyrrolidine carbamate appeared to be critical for achieving potency against resistant strains. Crystal structures showed a distinct aromatic interaction between the (*R*)-2-aryl (3-pyridyl for **4d**) substituent on the pyrrolidine and G2484 (G2505, *Escherichia coli*) of the *Deinococcus radiodurans* 50S ribosome (3.2 Å resolution).

Staphylococcus aureus infections, common in both hospital and in community settings, are increasingly becoming resistant to available therapies and current standard treatment options such as the natural glycopeptide vancomycin are losing their effectiveness.[1] The newer and purely synthetic oxazolidinone class, represented by linezolid (Fig. 1), is not exempt from this trend either—particularly in light of the plasmid-carried multi-drug resistant *cfr* gene and the possibility of horizontal resistance transfer.[2] The macrolide antibiotics such as clarithromycin (Fig. 1) have been very successful for decades combating community-acquired respiratory tract infections, caused by primarily *Streptococcus pneumoniae* rather than *S. aureus*.[3]

However streptococcal susceptibilities have inevitably decreased with use of these agents as well, and multidrug-resistant pathogens to the macrolide class are a persistent and growing health concern.[4] In order to combat this problem, a number of semi-synthetic variants on the macrolide structure have been discovered.[5] The structural changes of these newer macrolides have enabled different pharmacophoric elements to interact with the bacterial ribosome—with the ketolides for example.[6] These derivatives have resulted in improved resistance profiles versus strains with the capacity to modify the target ribosome (*erm*)[7] and overcome macrolide efflux (*mef*).[8] The strategies generally rely upon

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http://dx.doi.org/10.1016/j.bmcl.2013.01.067

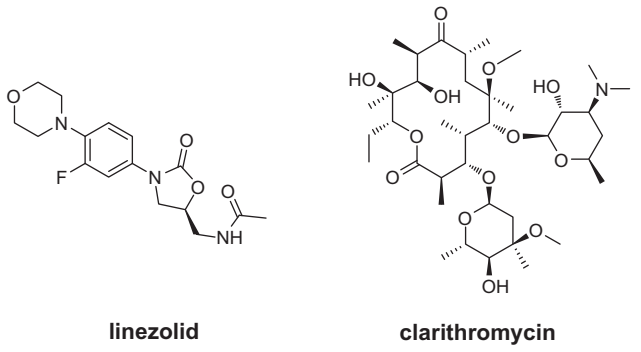


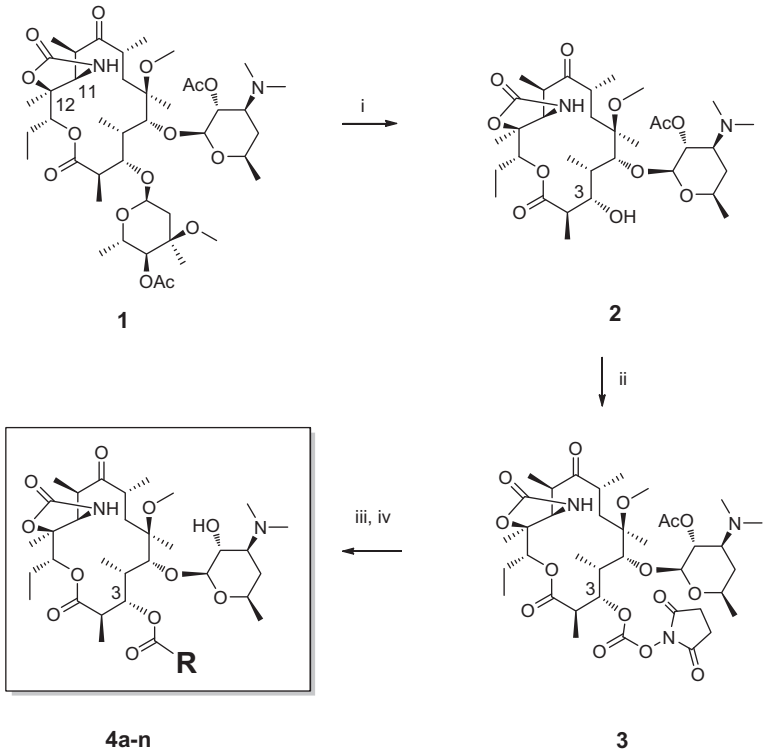
Figure 1. Structures of linezolid and clarithromycin.

modification or removal of the cladinose sugar at the 3-position and introduction of a tethered aryl moiety from different launch points of the molecule, the latter being particularly important for capturing *erm* activity.[9] The degree of resistance in different *erm* strains has been correlated with the degree of modification (methylation of A2058, *Escherichia coli*) of the bacterial ribosome.[10]

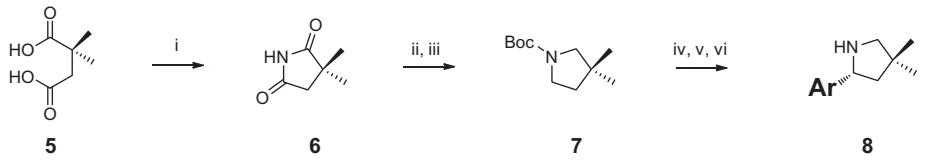
This disclosure is focused upon novel 3-*O*-carbamoyl derivatives of 6-*O*-methyl-12,11-oxycarbonylimino erythromycin A—herein labeled carbamolides—which begin to address the problem of Gram-positive resistance, with an emphasis on staphylococci and structural basis of these carbamolides binding in a unique mode to large ribosomal subunit from *Deinococcus radiodurans* (D50S). (Unless otherwise stated, in the text that follows, nucleotide numbering will refer to *D. radiodurans*, but the equivalent number in *E. coli* will be given in parentheses.)

Intermediates **1** and subsequently **2** were generated using known methods starting from clarithromycin.[11] Conversion of **2** to the versatile electrophilic carbamate precursor **3** required repeated treatment with excess di-(*N*-succinimidyl) carbonate in a solution of acetonitrile with triethylamine over several days with heating (85 °C) (Scheme 1). Succinimate **3** was purified chromatographically in 44% overall yield as a stable dry powder. Coupling of amines with **3** was affected in acetonitrile with triethylamine with heating (50 °C) overnight in generally acceptable yields of 50% or better. Amines that were not commercially available included the 4,4-dimethyl substituted pyrrolidines, the general synthesis of which is outlined in Scheme 2 starting from 2,2-dimethylsuccinic acid (**5**). Solvent-free condensation with urea yielded **6**, which was readily reduced with lithium aluminum hydride and converted to the *N*-Boc intermediate **7**. The key 2-arylation step was carried out using a variety of aryl halides and following a variation on Negishi coupling via enantio-biased anion formation.[12] Thus enantio-enriched coupling products were obtained using (−)-spartein complexed organozinc coupling to the corresponding aryl halide via palladium catalysis. The coupled products were subsequently crystallized to >98% enantiomeric excess as the (+)-phencyphos salts.[13] In this way, a series of amines were made and coupled to the succinimate template **3** to yield final test compounds (Fig. 2).

Researchers at Abbott first observed the potency advantages of fusing a carbamate across the 11,12-positions of the macrolactone ring,[14] and this structural feature is present in the ketolides telithromycin and cethromycin. We used the same 11,12-fused carbamate feature, starting from the known intermediate **1** and removing the cladinose provided the 3-hydroxy template (**2**) as a launch point for picking up novel interactions with the ribosome important for overcoming *erm* (MLSb) resistance. We found the pyrrolidinyl carbamate without any substitution (**4a**) to have weak



Scheme 1. General route for the synthesis of the carbamolide analogs with varying amines **R** (see Fig. 2). Reagents and conditions: (i) HCl/EtOH/40 °C; SiO_2 chromatography; 60–70% yld; (ii) di-(*N*-succinimidyl) carbonate (10 equiv over **5d**)/TEA/MeCN/85 °C; SiO_2 chromatography; 44% yld; (iii) coupling amine = **R** (1.2 equiv, generally as (+)-phencyphos salt form)/TEA/MeCN/50 °C; (iv) MeOH/TEA/50 °C to remove acetate protecting group; SiO_2 chromatography; 50–60% yld over two steps.



Scheme 2. General route for the synthesis of 2-aryl substituted (*R*)-4,4-dimethylpyrrolidines. Reagents and conditions: (i) urea/160 °C; 51% yld; (ii) LAH/THF/reflux; (iii) Boc anhydride/THF; 95% yld over two steps; (iv) (−)-spartein/MTBE/−78 °C/*sec*-BuLi; $ZnCl_2$/THF/−78 °C to rt; aryl-halide (**Ar**-X; X = Br or I)/$Pd(OAc)_2$/*t*-Bu_3PHBF_4; SiO_2 chromatography; (v) TFA; (vi) (+)-phencyphos/*i*-PrOH/recrystallization to enhance enantiomeric excess. 10–30% yld over three steps.

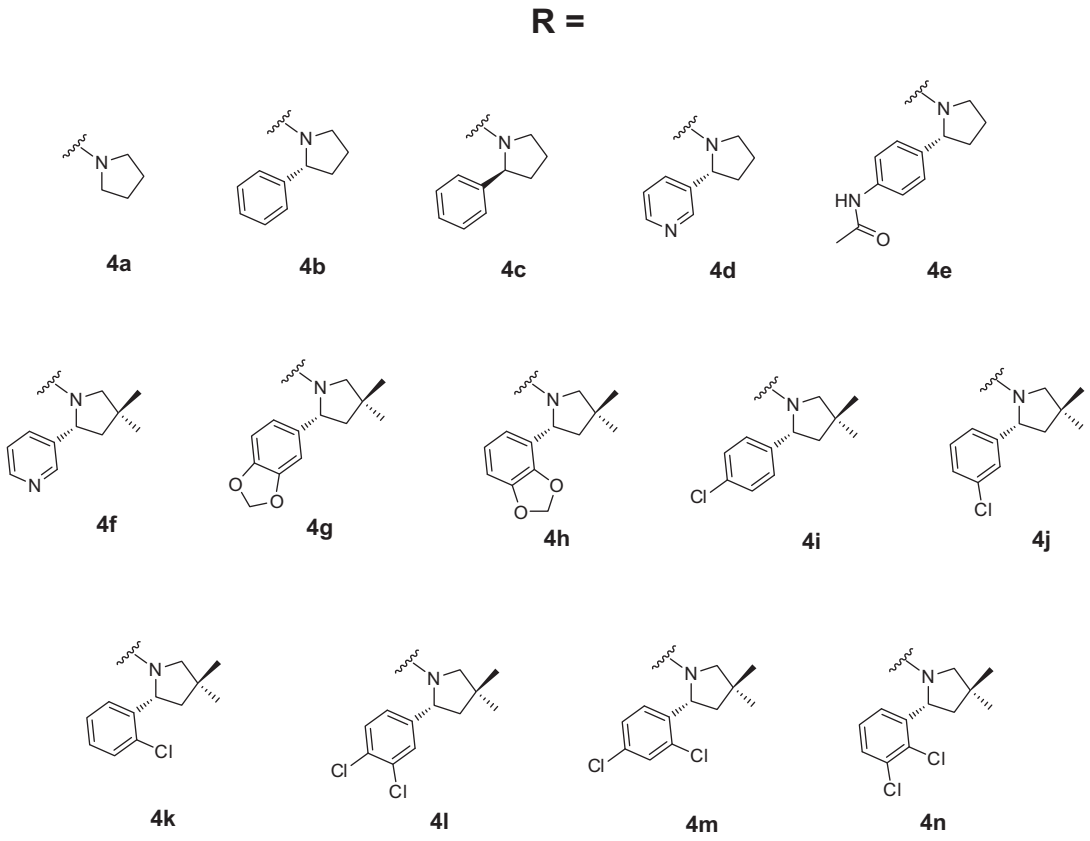


Figure 2. Pyrrolidine attachments (**R** in Scheme 1) representing key test carbamolide analogs **4a**–**n**.

Table 1
Minimum inhibitory concentrations (MICs, mg/L) of carbamolide analogs against resistant isolates

Compound ID	*Strep. pneumoniae* 1243-00 MLSb	*Strep. pyogenes* 1304-00 MLSb	*Staph. epidermidis* 1105-00 MRSE, MLSb	*Staph. aureus* 1279-07 MRSA, USA300	*Staph. aureus* 2111-10 MRSA, USA300 *cfr*	*Staph. aureus* 1281-07 MRSA, *cfr*	*Staph. aureus* 1279-07 MRSA, *cfr*	*Staph. aureus* 1609-09 MRSA, *cfr*, MLSb	*Staph. aureus* 1015-00 MRSA, MLSb	*Staph. aureus* 29213 ATCC [control strain]
Linezolid	0.5	0.5	1	2	16	32	16	16	4	4
Clarithro	>64	>64	>64	>64	>64	0.25	>64	>64	>64	0.5
4a	>64	>64	>64	>64	0.5	0.5	>64	>64	>64	2
4b	0.5	0.5	>64	16	0.25	0.125	16	>64	>64	0.25
4c	64	32	>64	>64	2	1	>64	>64	>64	4
4d	0.5	2	>64	4	0.25	0.25	0.25	>64	>64	0.25
4f	0.03	0.03	16	0.5	0.125	0.125	0.125	>64	64	0.125
4g	0.125	0.125	64	0.5	0.25	0.25	0.25	>64	>64	0.25
4h	0.03	0.03	64	0.25	0.25	0.125	0.25	>64	>64	0.25
4i	0.25	0.25	64	2	0.25	0.25	0.25	64	64	0.25
4j	0.125	0.125	64	2	0.25	0.25	0.25	64	64	0.25
4k	0.015	0.03	8	0.5	0.25	0.25	0.25	64	32	0.25
4l	1	1	16	2	0.5	0.25	0.5	64	32	0.5
4m	0.125	0.25	8	1	0.5	0.25	0.5	16	16	0.5
4n	0.125	0.125	8	1	0.5	0.5	0.5	8	8	0.5

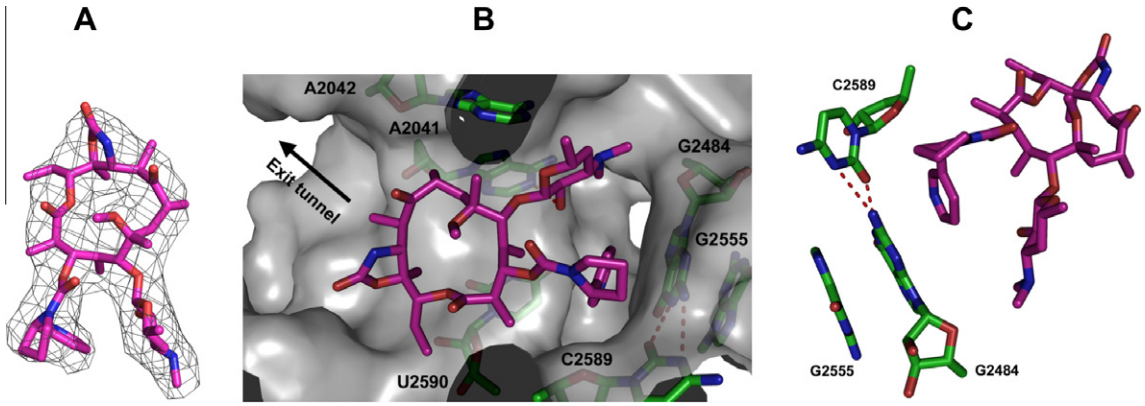


Figure 3. The X-ray crystal structure of **4d** bound to D50S at 3.2 Å resolution (PDB access code: 4IO9). (A) The (Fo–Fc) omit map at the inhibitor binding site, contoured at 3σ. (B) The pose in the nascent peptide tunnel. (C) The π-stacking interaction of the pyridyl moiety with G2484 (G2505).

minimum inhibitory concentrations (MICs, Table 1) for bacterial growth although it did show improvement versus a few of the linezolid and clarithromycin resistant strains. We were pleased however to discover that the (*R*)-2-phenyl substituent (**4b**) on the pyrrolidine ring demonstrated a significant boost in potency against the *S. pneumoniae* and *Streptococcus pyogenes* MLSb isolates. The stereochemistry at the 2-position was found to be important, as illustrated by the MIC data for the corresponding (*S*)-2-phenyl diastereomer (**4c**). Modification of the phenyl ring to heterocycles led to only modest potency changes as illustrated by the 3-pyridinyl analog **4d**. Extended substitutions such as the *N*-acetyl at the 4-position of the phenyl ring (**4e**) demonstrated no potency against resistant MLSb strains.[15] We subsequently introduced a *gem*-dimethyl function at the 4-position of the pyrrolidine with the goal of probing the effect on potency incurred by conformational locking of this ring system. The resulting (*R*)-3-(4,4-dimethylpyrrolidin-2-yl)pyridinyl analog **4f** was the first carbamolide analog to demonstrate any activity against a staphylococcal MLSb strain (*S. epidermidis* 1105-00) albeit moderate (MIC = 16 mg/L) and did not inhibit the growth of the extremely resistant MLSb *S. aureus* strains (1609-09 and 1015-00). Both analog **4d** and the *gem*-dimethyl analog **4f** gave excellent potency against the linezolid resistant (*cfr*) strains (MIC ⩽ 0.25 mg/L vs *S. aureus* 2111-10, 1281-07, and 1279-07). However, the majority of analogs showed little or no activity against the staphylococcal MLSb strains. The robust and constitutive nature of the these strains, owing to the methylation of A2041 (A2058) and the consequent steric clash with the dimethylamino function of the desosamine sugar present in the macrolide antibiotics, including clarithromycin, makes it very challenging for these templates to regain any potency against them.[16]

We were able to obtain an X-ray structure of **4d** soaked into *Deinococcus radiodurans* 50S (D50S) crystals at a resolution of 3.2 Å. This structure (Fig. 3) showed that the compound bound to the large subunit in the nascent peptide tunnel below the peptidyl transferase center. The lactone ring was oriented almost the same as the lactone ring of erythromycin[17] and inserted into the hydrophobic pocket mainly formed by residues A2041 (A2058), A2042 (A2059) and U2590 (C2611) upon binding. The 2′-OH group of the desosamine sugar formed a hydrogen bond with the N1 of A2041 (A2058). Importantly, the attachment of (*R*)-2-aryl (3-pyridyl for **4d**) substituent on the pyrrolidine placed the aryl moiety into a narrow pocket composed of G2484 (G2505), C2589 (C2610) and C2590 (C2611) and confirmed our observation of stereo-sensitivity in this region. The aryl ring formed additional interactions with G2484 (G2505), a universally conserved nucleotide in the peptidyl transferase loop, which was further stabilized by

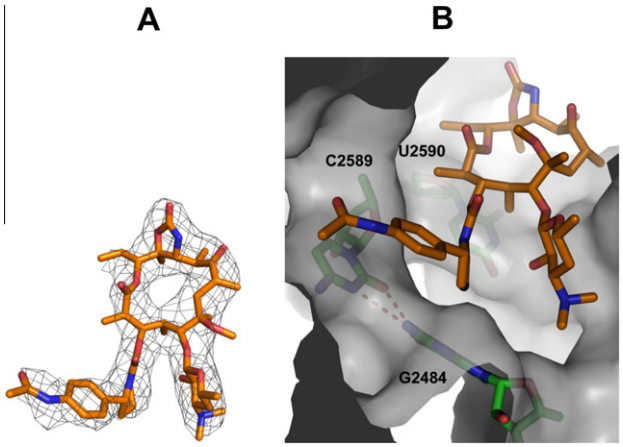


Figure 4. X-ray crystal structure of **4e** bound to D50S at 3.2 Å resolution (PDB access code: 4IOA). (A) The (Fo–Fc) omit map at the inhibitor binding site, contoured at 3σ. (B) Interaction of **4e** in the nascent peptide tunnel showing the rotated pyrrolidinyl side chain (cf. Figs. 3 and 5).

forming a H-bond with C2589 (C2610) and interaction with G2555 (G2576) (Fig. 3). Interestingly, the less active *N*-acetyl analog (**4e**, Fig. 4) had the pyrrolidine rotated by almost 180° relative to **4d** such that the whole pyrrolidinyl sidechain rotated out to interact with domain V of the 23S rRNA—presumably due to the limited pocket size defined by G2484 (G2505), C2589 (C2610) and C2590 (C2611) and its inability to accommodate the *N*-acetyl function. As a result, the pyrrolidine made a van der Waals contact with G2484 (G2505) and the *N*-acetyl formed additional stacking interactions with C2589 (C2610). As noted, this rotation was not beneficial for antibacterial activity. Looking to improve the potency of our analogs, we speculated that the addition of the 4,4-dimethyl substitution pattern on the pyrrolidine would limit the conformational flexibility on this ring system, thereby enhancing the aryl-ribosome interaction and consequently the potency. Consistent with this hypothesis, the crystal structure of the 4,4-dimethyl analog **4f** at 3.6 Å resolution showed the same aromatic stacking interaction with G2484 (G2505) with the *gem*-dimethyl function solvent exposed rather than making a direct interaction with the ribosome (Fig. 5). The two benzo-[1,3]-dioxole analogs **4g** and **4h** however failed to show any improvement in potency. Chloro substitution in the 4- (**4i**) and 3- (**4j**) positions also did not improve antibacterial activity, however the 2-chloro (**4k**) had reasonable potency against the *S. epidermidis* 1105-00 (MIC = 8 mg/L). The 3,4-dichloro (**4l**) analog was less potent than the 2,4- (**4m**) and in particular the 2,3-dichloro substitution (**4n**)—the latter being

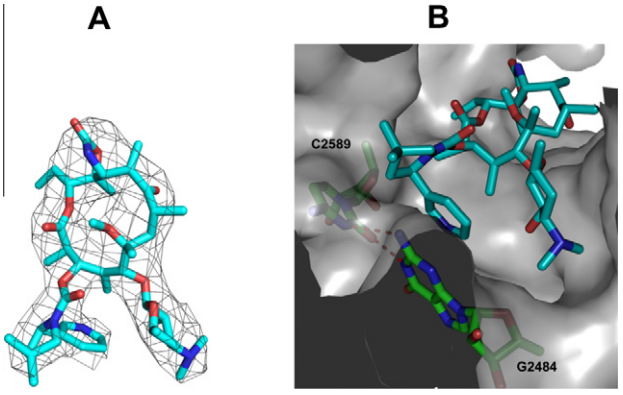


Figure 5. X-ray crystal structure of **4f** in complex with D50S at 3.6 Å resolution (PDB access code: 4IOC). (A) the (Fo–Fc) omit map at the inhibitor binding site, contoured at 3σ. (B) Details of interaction showing the same π-stacking interaction with G2484 (G2504).

the only analog to achieve MICs ⩽ 8 mg/L versus all of the strains tested, including the most recalcitrant staphylococcal MLSb and *cfr* isolates (*S. aureus* 1609-09 and 1015-00). While the aromatic stacking interaction is clearly important for potency, the precise nature of why this particular substitution pattern has a potency advantage is not clear and may also be a function of bacterial cell penetration.

We have disclosed a novel series of 3-*O*-carbamate erythronolide A analogs, herein dubbed carbamolides, which demonstrate antibacterial activity against macrolide and oxazolidinone resistant strains of staphylococci and streptococci, including some degree of activity versus MLSb *S. aureus* isolates. We have further provided crystallographic evidence for a unique aromatic stacking interaction within a tight pocket of the bacterial ribosome that appears to be critical to the in vitro potency of these molecules in inhibiting bacterial growth.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.01.067.

References and notes

1. (a) Nannini, E.; Murray, B. E.; Arias, C. A. *Curr. Opin. Pharmacol.* **2010**, *10*, 516; (b) Rossolini, G. M.; Mantengoli, E.; Montagnani, F.; Pollini, S. *Curr. Opin. Microbiol.* **2010**, *13*, 582.
2. Toh, S.-M.; Xiong, L.; Arias, C. A.; Villegas, M. V.; Lolans, K.; Quinn, J.; Mankin, A. S. *Mol. Microbiol.* **2007**, *64*, 1506.
3. Kaneko, T.; Dougherty, T. J.; Magee, T. V. In *Macrolide Antibiotics. Comprehensive Medicinal Chemistry II*; Taylor, J. B., Triggle, D. J., Eds.; Therapeutic Areas II: Cancer, Infectious Diseases, Inflammation and Immunology and Dermatology; Elsevier Ltd: Oxford, UK, 2007; Vol. 7, p 519.
4. (a) Jenkins, S. G.; Farrell, D. J. *Emerg. Infect. Dis.* **2009**, *15*, 1260; (b) Metlay, J. P.; Fishman, N. O.; Joffe, M. M.; Kallan, M. J.; Chittams, J. L.; Edelstein, P. H. *Emerg. Infect. Dis.* **2006**, *12*, 1223; (c) Gould, I. M. *Int. J. Antimicrob. Agents* **2009**, *34*, S8.
5. (a) Pereira, D.; Fernandes, P. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 510; (b) Kirst, H. A. *Expert Opin. Ther. Patents* **2010**, *20*, 1343; (c) Zhang, L.; Song, L.; Liu, Z.; Lu, H.; Lu, Y.; Li, Z.; Ma, S. *Eur. J. Med. Chem.* **2010**, *45*, 915; (d) Pandya, M.; Chakrabarti, A.; Rathy, S.; Katoch, R.; Venkataraman, R.; Bhateja, P.; Mathur, T.; Kumar, G. R.; Malhotra, S.; Rao, M.; Bhadauria, T.; Barman, T. K.; Das, B.; Upadhyay, D.; Bhatnagar, P. K. *Int. J. Antimicrob. Agents* **2010**, *36*, 169; (e) Wu, Z.; Lu, Y.; Luo, M.; He, X.; Xiao, Y.; Yang, J.; Pan, Y.; Qiu, G.; Guo, H.; Hu, H.; Zhou, D.; Hu, X. *Eur. J. Med. Chem.* **2010**, *45*, 3636; (f) Xu, P.; Liu, L.; Chen, X.-Z.; Li, Y.; Liu, J.; Jin, Z.-P.; Wang, G.-Q.; Lei, P.-S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4079; (g) Tang, D.; Gai, Y.; Polemeropoulos, A.; Chen, Z.; Wang, Z.; Or, Y. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5078; (h) Akritopoulou-Zanze, I.; Phelan, K. M.; Marron, T. G.; Yong, H.; Ma, Z.; Stone, G. G.; Daly, M. M.; Hensey, D. M.; Nilius, A. M.; Djuric, S. W. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3809; (i) Xu, P.; Liu, L.; Jin, Z.; Lei, P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3330; (j) Zhu, B.; Marinelli, B. A.; Abbanat, D.; Foleno, B. D.; Henninger, T. C.; Bush, K.; Macielag, M. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1054; (k) Zuckerman, J. M. *Infect. Dis. Clin. North Am.* **2004**, *18*, 621.
6. (a) Berisio, R.; Harms, J.; Schluenzen, F.; Zarivach, R.; Hansen, H. A. S.; Fucini, P.; Yonath, A. *J. Bacteriol.* **2003**, *185*, 4276; See also: (b) Poehlsgaard, J.; Douthwaite, S. *Nat. Rev. Microbiol.* **2005**, *3*, 870.
7. Weisblum, B. *Antimicrob. Agents Chemother.* **1995**, *39*, 577.
8. Wierzbowski, A. K.; Boyd, D.; Mulvey, M.; Hoban, D. J.; Zhanel, G. G. *Antimicrob. Agents Chemother.* **2005**, *49*, 4635.
9. Ma, Z.; Nemoto, P. A. *Curr. Med. Chem.* **2002**, *1*, 15.
10. Wolter, N.; Smith, A. M.; Farrell, D. J.; Northwood, J. B.; Douthwaite, S. D.; Klugman, K. P. *Antimicrob. Agents Chemother.* **2008**, *52*, 435.
11. Tanikawa, T.; Asaka, T.; Kashimura, M.; Suzuki, K.; Sugiyama, H.; Sato, M.; Kameo, K.; Morimoto, S.; Nishida, A. *J. Med. Chem.* **2003**, *46*, 2706.
12. Campos, K. R.; Klapars, A.; Waldman, J. H.; Dormer, P. G.; Chen, C.-Y. *J. Am. Chem. Soc.* **2006**, *128*, 3538.
13. (a) ten Hoeve, W.; Wynberg, H. *J. Org. Chem.* **1985**, *50*, 4508; (b) Wijnberg, H.; ten Hoeve, W. European Patent Appl. 1986, EP 180276.; See also: (c) Leeman, M.; Querniard, F.; Vries, T. R.; Kaptein, B.; Kellogg, R. M. *Org. Process Res. Dev.* **2009**, *13*, 1379.
14. Baker, W. R.; Clark, J. D.; Stephens, R. L.; Kim, K. H. *J. Org. Chem.* **1988**, *53*, 2340.
15. MIC vs *S. pyogenes* 1068 > 64 mg/L.
16. Schluenzen, F.; Zarivach, R.; Harms, J.; Bashan, A.; Tocilj, A.; Albrecht, R.; Yonath, A.; Franceschi, F. *Nature* **2001**, *413*, 814.
17. Dunkle, J. A.; Xiong, L.; Mankin, A. S.; Cate, J. H. D. *Proc. Natl. Acad. Sci.* **2010**, *107*, 17152.