Published in final edited form as:

Bioorg Med Chem Lett. 2019 June 01; 29(11): 1386–1389. doi:10.1016/j.bmcl.2019.03.038.

Synthesis and Biological Evaluation of Solithromycin Analogs Against Multidrug Resistant Pathogens

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Abstract

Novel antibacterial drugs that treat multidrug resistant pathogens are in high demand. We have synthesized analogs of solithromycin using Cu(I)-mediated click chemistry. Evaluation of the analogs using Minimum Inhibitory Concentration (MIC) assays against resistant *Staphylococcus aureus*, *Escherichia coli*, and multidrug resistant pathogens *Enterococcus faecium* and *Acinetobacter baumannii* showed they possess potencies similar to those of solithromycin, thus demonstrating their potential as future therapeutics to combat the existential threat of multidrug resistant pathogens.

Graphical Abstract

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Supplementary Material

General methods, minimum inhibitory concentration (MIC) method, Vero cell cytotoxicity, synthetic methods for the synthesis of alkynes **11–12**, Cu(I)-catalyzed click synthesis and characterization of triazoles **16–17**, **19**, **20–21**, and **23–24**. Spectral data (¹H and ¹³C NMR) for **11–12**, **16–17**, **19**, **20–21**, and **23–24**.

Keywords

multidrug resistance; antibiotic; solithromycin; click chemistry

The onset of multidrug resistant (MDR) bacterial infections in both nosocomial and community-acquired settings is a significant public health threat.¹ The Infectious Diseases Society of America has reported that most infections are caused by so-called “ESKAPE” pathogens consisting of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*² Macrolide antibiotics have proven to be one of the safest and most effective drug classes in medicine but resistance has undermined efficacy. Therefore, the need for novel antibiotics to combat MDR bacteria is a matter of utmost significance.^{3,4}

Macrolide antibiotics are potent against Gram-positive bacteria, such as *Staphylococci* and *Streptococci*, in which the mode of action involves protein synthesis inhibition via binding reversibly to the bacterial ribosome.^{5,6} The susceptibility of macrolides toward Gram-negative bacteria, however, is limited and includes *Neisseria gonorrhoeae* and *Haemophilus influenzae*.⁷

A timeline of macrolide antibiotic development spanning four generations is shown in Figure 1. Resistance to 1st generation erythromycin (ERY, **1**)⁸ and 2nd generation macrolide antibiotic clarithromycin (CLA, **2**)⁹ and azithromycin (AZY) prompted the discovery of the 3rd generation ketolides telithromycin (KET, **3**)¹⁰ and cethromycin (CET).^{7,11} Key innovation elements of the ketolide class included (1) the replacement of the cladinose deoxysugar at C3 on the erythromycin framework with a keto (hence the term ‘ketolide’) and (2) the introduction of a pendant biaryl side-chain from the N11 oxazolidinone moiety, which makes additional π – π contacts with the ribosome. Notably, other approaches relying on Pd-catalyzed glycosylation have been reported for installing modified sugars on the methymycin aglycone in the quest for enhancing antibacterial properties.¹² The discovery of the 4th generation (i.e., fluoroketolides), represented by solithromycin (SOL, **1**),^{13–15} was enabled by Cu(I)-catalyzed combinatorial click chemistry.^{16–18} However, the development of SOL has been hampered by issues related to liver toxicity, as reported by the FDA in Fall 2016.¹⁹ This finding underscores the urgent need for novel potent and bioactive analogs that display satisfactory safety profiles.

In 2016, we demonstrated that ribosomes and 50S ribosomal subunits from *E. coli* were able to template the Huisgen [3+2] dipolar cycloaddition reaction of macrolidetethered azide **5** and 3-ethynylaniline (**6**) to synthesize solithromycin (**4**) and several triazole congener (i.e., *in situ* click chemistry).²⁰ A library of aliphatic, aromatic and heteroaromatic terminal alkynes was employed to probe the extent to which the ketolide side-chain engaged in π -stacking with the ribosomes or the 50S subunit. In 2018, we reported this method could be translated *in cellulo*, which circumvents the need to isolate bacterial ribosomes.²¹ Herein, we report the Cu(I)-catalyzed synthesis of solithromycin analogs **16–24** (Scheme 1A), several of which were not prepared during the *in situ* or *in cellulo* campaigns.

The library of terminal alkynes featured both aromatic **6–12** (Scheme 1B) and heteroaromatic **13–15** (Scheme 1C) members. The rationale for the above nine side-chain analogs of SOL (**4**) was to probe the role of H-bonding and π -stacking of the aromatic nucleus within the ribosome binding site and attendant antibacterial activity as measured by MIC. For example, acetamide **7** is expected to enhance H-bonding donation of analog **16** while also decreasing the electron-density of the benzene ring. Analog **17** is a regioisomer of SOL (**4**) wherein the amino group is in the 4- as opposed to 3-position. The 4-methoxy group in analog **18** possesses diminished π -density vis-à-vis **4** and can only serve as an H-bond acceptor. Analog **19**, derived from *O*-propargyl phenol (**10**), extends the aromatic nucleus with an ethereal oxygen. Nitroarene analogs **20** and **21** were chosen to test the consequences of markedly decreased electron density in the π -stacking event. As thiophene is a bioisostere for benzene,²² we employed two regioisomers of this heterocycle in analogs **22** and **23** (Scheme 1C). Finally, the use of 2-aminothiazole analog **24** was inspired by the aniline residue in SOL (**4**).

Solithromycin (**4**) and analogs **16–24** were synthesized using the “aqueous ascorbate” procedure of Fokin and Sharpless, which uses catalytic CuSO₄ and sodium ascorbate as stoichiometric reductant in *tert*-BuOH/water (1:1) at room temperature for 24 h (Scheme 1A).¹⁷ Isolated yields of 1,4-triazoles **4** and **16–24** ranged from 70–90% (see supporting information).

To evaluate the potency and scope of **4** (SOL) and analogs **16–24**, minimum inhibitory concentration (MIC) assays were performed against *Staphylococcus aureus* (4 strains) and *Escherichia coli* (5 strains).²³ The results are shown in Table 1.

Results of the MIC evaluation with wild type and resistant *S. aureus* strains revealed that acetamide analog **16** was four- to eight-fold more potent than SOL against wild type; however, it was not effective against resistant UCN strains (Table 1).²⁴ Regioisomeric SOL analog **17**, with its aromatic amino group in the 4- as opposed to 3-position, was equipotent with solithromycin (**4**) but like **16** did not inhibit the resistant strains. The remaining analogs **18–22** were all equipotent or more potent than SOL against the wild type strain; moreover, analog **20** was equipotent with SOL against the UCN17 resistant strain. On balance, the modifications to the benzene ring were not particularly effective in overcoming resistant *S. aureus* strains, indicating a need for alternative tactics.

Results of the MIC evaluation with *E. coli* strains were more promising (Table 1). The 4-amino regioisomer of SOL, analog **17**, was two-fold more potent than SOL against DK, DK3535, and SQ171 strains. Nitrophenol analog **20** was four-fold more potent than SOL against wild type DK strain and equipotent with SOL against resistant DK2058G strain. Analog **21** was two-fold more potent than SOL against the SQ171 strain and, significantly, four-fold more potent than SOL against the resistant SQ171(2058G) strain. Heterocyclic analogs **22–24** were largely either more potent than or equipotent with SOL against the tested strains, demonstrating this particular motif is promising and should be investigated further.

In addition to screening our library of SOL analogs for susceptibility against *S. aureus* and *E. coli*, Gram-positive *Enterococcus faecium* and Gram-negative *Acinetobacter baumannii* were also targeted as these are ESKAPE pathogens (*vide supra*). *E. faecium* accounts for enterococcal infections such as urinary tract infections, endocarditis, intra-abdominal, and bacteremia.^{25,26} There is a strong demand for novel antibacterial agents against *E. faecium*,²⁶ particularly as MDR strains have emerged that thwart treatment by vancomycin, penicillin, and aminoglycoside antibiotics.^{27,28}

Despite the fact that macrolides in general are less active against Gram negatives, screening against *Acinetobacter baumannii* revealed some promising results (Table 2). Curiously, both thiophene analogs **22** and **23** were four-fold and two-fold more potent, respectively, than SOL (**4**). The remaining triazoles were either equipotent or two-fold less potent than **4**. *A. baumannii* is a fast-evolving, opportunistic pathogen that thrives in a hospital setting leading to urinary tract infections, meningitis, and wound infections.²⁵ Developing antibacterials that treat *A. baumannii* is challenging due to the diverse repertoire of resistance mechanisms it has acquired, which include drug-modifying enzymes against β -lactams and aminoglycosides, efflux mechanisms and modifications to outer membrane proteins.²⁹

The results of MIC evaluation of SOL and analogs **16–24** against *E. faecium* are shown in Table 2. While analogs **16**, **17**, and **24** were equipotent with SOL (**4**), we found that analog **19** was two-fold more potent, analogs **18** and **21** were sixteen-fold more potent, analog **23** was 256-fold more potent, and significantly, analog **20** was 4096-fold more potent SOL (**4**).

The extremely low MICs for analogs **20** and **23** against *E. faecium* encouraged us to investigate the biological activity of other known macrolides such as ERY (**1**) and AZY. Results are shown in Table 3. MIC values for both ERY (**1**) and AZY were 12 and 25 $\mu\text{g/mL}$, respectively, which are in fact high compared to analogs **20**, **23**, and even SOL (**4**). This emphasized the need for investigating analogs **20** and **23** in downstream assays to better understand their value as drug discovery entities. We, thus, began by assessing the mammalian cell cytotoxicity with Vero cells (African green monkey kidney epithelial cell line). The CC_{50} was quantified as the minimum concentration of compound resulting in 50% growth inhibition of this model cell line.³⁰ Accordingly, the CC_{50} values of **20** and **23** were 12 and 25 $\mu\text{g/mL}$, respectively. The selectivity index ($\text{SI} = \text{CC}_{50}/\text{MIC}$) for **20** and **23** was then calculated for both to be in significant excess of 10, which is a minimum value for demonstrating a reasonable toxicity window in early drug discovery compounds.³¹

In summary, we have synthesized solithromycin (**4**) and nine analogs thereof **16–24** using Cu(I)-catalyzed click chemistry and evaluated all ten compounds against wild type and resistant isolates of Gram-positive *S. aureus*, *E. coli*, and *E. faecium*, in addition to Gram-negative *A. baumannii*. While many compounds were equipotent with **4**, we found several analogs that displayed enhanced activity against *E. coli*, *E. faecium*, and *A. baumannii* strains that justify *in vivo* studies to develop novel antibacterials that can address the existential threat of multidrug resistant pathogens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The NIH (GM126221) and Temple University supported this research. Support for the NMR facility at Temple University by a CURE grant from the Pennsylvania Department of Health is gratefully acknowledged. The Rutgers University research was supported by the NIH (U19AI109713 and T32AI125185). We thank Prof. Alexander Mankin for kindly providing us with *S. aureus* (UCN14, UCN17, UCN18) and *E. coli* bacterial strains (DK, DK3535, DK2058G, SQ171, SQ1712058G). We thank Dr. Christiana N. Tejjaro for assistance with MIC evaluation. Finally, we thank Dr. Charles DeBrosse, Director of the Temple University NMR Facility in the Department of Chemistry, for kind assistance with NMR experiments.

Abbreviations

MDR	multidrug resistance
MIC	Minimum Inhibitory Concentration
ERY	erythromycin
CLA	clarithromycin
AZY	azithromycin
TEL	telithromycin
CET	cethromycin
SOL	solithromycin
CC₅₀	Cytotoxicity Concentration 50%
SI	Selectivity Index

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Figure 1.
Structures of Four Generations of Macrolide Antibiotics and Timeline of Discovery.

Scheme 1.

(A) Cu-catalyzed click coupling of ketolide azide **5** and terminal alkynes **6-15** to form 1,4-triazoles **4 (SOL)** and **16-24**. Structures of (B) aromatic and (C) heteroaromatic terminal alkynes in the library with corresponding cycloadducts.

Table 1.

MIC ($\mu\text{g/mL}$) values for SOL (**4**) and analogs **16–24** against *S. aureus* (wt), resistant *S. aureus* (UCN14, UCN17, UCN18), and resistant *E. coli* bacterial strains (DK, DK3535, DK2058G, SQ171, SQ1712058G).

Compounds	MIC ($\mu\text{g/mL}$)								
	<i>S. aureus</i>	<i>S. aureus</i>	<i>S. aureus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>
	wt	UCN14	UCN17	UCN18	DK	DK3535	DK2058G	SQ171	SQ1712058G
SOL (4)	0.06–0.12	8	4	4	2	2	4	>32	>32
16	0.016	>128	64	64	4	4	8	64	>64
17	≤ 0.031	16	8	16	1	1	4	16	>32
18	≤ 0.0625	>128	32	32	2	2	2	64	>64
19	≤ 0.0625	64	8	4	2	4	4	>32	>32
20	≤ 0.0625	16	4	8	0.5	8	4	32	>32
21	≤ 0.0625	64	32	32	4	2	2	16	8
22	≤ 0.0625	32	8	8	2	1	1	32	>64
23	0.25	128	128	128	4	4	4	8	>64
24	0.25	64	64	64	2	1	1	>32	>32
	Equipotent with SOL (4)			More potent than SOL (4)					

Table 2.

MIC ($\mu\text{g/mL}$) values for SOL (**4**) and analogs **16–24** against multidrug resistant *E. faecium* (NCTC 7171) and *A. baumannii* (ATCC 19606) bacterial strains.

Compound	<i>A. baumannii</i> (ATCC 19606)	<i>E. faecium</i> (NCTC 7171)
SOL (4)	25	0.024
16	50	0.024
17	25	0.024
18	25	0.0015
19	25	0.012
20	25	0.000059
21	50	0.0015
22	6.5	0.049
23	12	0.000094
24	>50	0.024

Table 3.

MIC ($\mu\text{g/mL}$), CC_{50} ($\mu\text{g/mL}$), and SI values for analogs **20** and **23** against multidrug resistant *E. faecium* (NCTC 7171) in comparison to ERY (**1**) and AZY.

Compound	<i>E. faecium</i> (NCTC 7171)	Vero cell CC_{50}	$\text{SI} = \text{CC}_{50}/\text{MIC}$
ERY (1)	12	ND	ND
AZY	25	ND	ND
20	0.0000059	12	2.0×10^6
23	0.000094	25	2.7×10^5

ND = Not Determined