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Synthesis and antimicrobial activities of structurally novel *S,S′*-bis(heterosubstituted) disulfides

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ABSTRACT

The central focus of this study is on the antibacterial and antifungal properties of synthetically produced *S,S′*-bis(heterosubstituted) disulfides as a means to control the growth of various infection-causing pathogens. *Staphylococcus aureus*, *Francisella tularensis* and *Candida albicans* were each found to be highly susceptible to several of these compounds by agar or broth dilution and Kirby-Bauer diffusion assays. These structurally simple, low molecular weight disulfides have shown promising bioactivities and may serve as leads to the development of effective new antibacterials for pathogenic bacteria such as methicillin-resistant *S. aureus* and *F. tularensis*.

The concern over multidrug-resistant bacteria and the need for more effective antibacterials has truly reached a critical level, given the high incidence of life-threatening, life-altering drug-resistant infections. *Staphylococcus aureus* (Staph), a Gram-positive facultative anaerobe, resides in the nostrils and on the skin of human beings, and starts to cause infection once it enters the blood stream. Antibiotic-resistant variants of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA), the single most important bacterial pathogen causing deadly infections, are of the most immediate concern. MRSA causes infections of skin and soft tissue,[1] as well as pulmonary,[2] osteoarticular,[3] hemorrhagic adrenal gland (Waterhouse-Friderichsen syndrome[4]) and ophthalmic infections.[5] According to the United States Centers for Disease Control and Prevention (CDC), MRSA accounted for more than 94,000 life-threatening infections and nearly 19,000 deaths in the United States alone in 2005.[6] The CDC also emphasizes that the prevalence of multi-drug-resistant (MDR) bacteria including MRSAs is rising at an alarming rate. MRSA mutants that thrive in the community, referred to as community-acquired MRSA (CA-MRSA), are more lethal and complex in nature than those originating in hospitals, which are referred to as hospital-acquired MRSA (HA-MRSA).

A second bacterial pathogen, *Francisella tularensis* live vaccine strain (*F. tularensis* LVS), is a Gram-negative intracellular bacterium that is the causative agent of tularemia known as rabbit fever. Symptoms of tularemia include fever, lethargy, anorexia, signs of septicemia, and in extreme cases can cause death. As few as 10–50 cfu (colony-forming units) are sufficient to cause an infection.[7] Due to its high pathogenicity and ease of spread by aerosol, *F. tularensis* is considered for usage in biological warfare, and as such, is classified as a Class A agent by the U.S. government.[8] *F. tularensis* is susceptible to carbapenems, ceftriazone, ceftazidime, rifampin and certain macrolides, but there remains a lack of clinical data to recommend any of these compounds for clinical use.[9] Moreover, there is broad resistance to erythromycin among strains of *F. tularensis* subspecies *holarctica*.[10] Ciprofloxacin is the only drug that is considered ideal for the treatment of tularemia, although with concerns over its continued effectiveness.

Our laboratory has been investigating new types of synthetic antibacterial agents for use against pathogenic microbes. Previously, we have reported on N-thiolated β-lactams,[11–17] N-thiolated 2-oxazolidinones[18] and most recently, aryl–alkyl disulfides[19] that are all effective in vitro growth inhibitors of MRSA and *Bacillus* bacteria (Fig. 1). The mode of action and structure–activity profiles of these three chemical classes differ dramatically from those of the traditional β-lactam, oxazolidinone, or disulfide-containing antibiotics. Investigations in our laboratory have illustrated that these highly lipophilic compounds can carry a wide range of substituents, and are reactive towards thiophilic agents that under certain conditions (such as in the bacterial cytoplasm) can block metabolic

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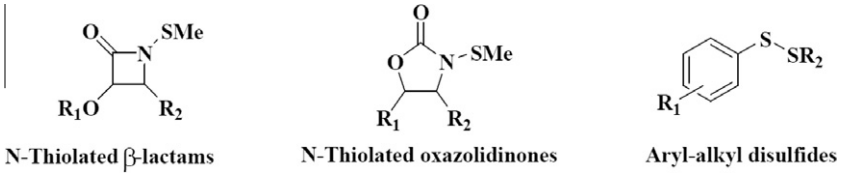


Figure 1. Thiophilic antibacterials previously developed in our laboratories.

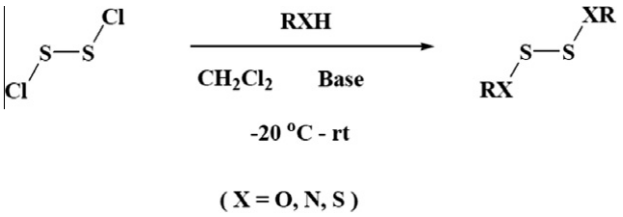
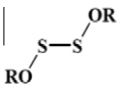


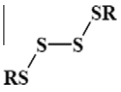
Figure 2. Synthesis of *S*,*S*′-bis(heterosubstituted) disulfides **1**–**6**.

Table 1
MIC's of *S*,*S*′-bis(alkoxy) disulfides **1a**–**e** against *S. aureus*



Compound	*R*	MSSA (μg/mL)	MRSA (μg/mL)
1a	Propyl	32	32
1b	Isopropyl	1	0.5
1c	Butyl	0.25	8
1d	*sec*-Butyl	16	8
1e	Phenyl	2	2
Penicillin G (control)		0.25	64

Table 2
MIC's of *S*,*S*′-bis(organothio) disulfides **2a–e** against *S. aureus*

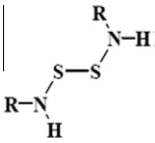


Compound	*R*	MSSA (μg/mL)	MRSA (μg/mL)
2a	Propyl	32	32
2b	Isopropyl	32	32
2c	Butyl	64	32
2d	*sec*-Butyl	32	64
2e	Phenyl	8	8
Penicillin G (control)		0.25	64

pathways used by bacteria for membrane formation and possibly other vital cellular processes.

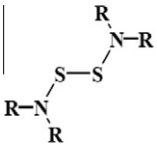
This work describes experiments on a fourth series of sulfur-containing antibacterials, *S*,*S*′-bis(heterosubstituted) disulfides. In this study, we investigated the synthesis and antimicrobial properties of these intriguing low molecular weight compounds, which at least structurally are related to naturally-occurring trisulfide and polysulfide antibiotics.[20] These investigations were initiated by the synthesis of a small library of *S*,*S*′-bis(heterosubstituted) disulfides to assess the electronic effects of different heteroatoms on microbiological activity. These derivatives were prepared by treating an alcohol, amine or thiol with sulfur monochloride at −20 °C in the presence of triethylamine as a Lewis base (Fig. 2). The desired disulfide products were obtained in 70–90% yields after column chromatography, and characterized by ^{1}H NMR spectroscopy, prior to antimicrobial testing.

Table 3
MIC's of *S*,*S*′-bis(alkylamino) disulfides **3a**–**e** against *S. aureus*



Compound	*R*	MSSA (μg/mL)	MRSA (μg/mL)
3a	Propyl	8	4
3b	Isopropyl	32	32
3c	Butyl	8	32
3d	*sec*-Butyl	16	16
3e	Phenyl	4	4
Penicillin G (control)		0.25	64

Table 4
MIC's of *S*,*S*′-bis(dialkylamino) disulfides **4a**–**e** against *S. aureus*



Compound	*R*	MSS (μg/mL)	MRSA (μg/mL)
4a	Methyl	0.25	0.5
4b	Ethyl	2	2
4c	Isopropyl	2	1
4d	Allyl	1	0.5
4e	Isobutyl	16	16
Penicillin G (control)		0.25	64

The antimicrobial activity of these *S*,*S*′-bis(heterosubstituted) disulfide compounds was evaluated against bacterial targets of interest to us, including methicillin-susceptible *S. aureus* (MSSA, ATCC 25923), methicillin-resistant *S. aureus* (MRSA, ATCC 43300, β-lactamase-producing), a live vaccine strain (LVS) of *F. tularensis* subspecies *holarctica*, nine subspecies of *Bartonella*, and *Escherichia coli* K12. The testing included the determination of the minimum inhibitory concentration (MIC), Kirby-Bauer diffusion assay and growth viability assay. Minimum fungicidal concentration (MFC) and viability assays were also performed against the fungus, *Candida albicans*. To assess antibacterial activity, we conducting susceptibility screening by determining the MIC values for each analogue by agar dilution using a 24-well plate, according to the National Committee for Clinical Laboratory Standards (NCCLS).[21] After preparing the agar, the bacteria were allowed to grow for 24 h in the presence of varying concentrations of antibiotic within the agar. All the antimicrobial assays were performed in triplicate, using either penicillin G or ciprofloxacin as positive controls and DMSO as a negative control. Among the bacteria initially examined, antimicrobial activity was not observed for any of the compounds against *Bartonella* ssp. or *E. coli* K12. However, the disulfides