The Path to New Halogenated Quinolines With Enhanced Activities Against *Staphylococcus epidermidis*

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ABSTRACT: Antibiotic-resistant bacteria and surface-attached bacterial biofilms play a significant role in human disease. Conventional antibiotics target actively replicating free-floating, planktonic cells. Unfortunately, biofilm communities are endowed with nonreplicating persister cells that are tolerant to antibiotics. Innovative approaches are necessary to identify new molecules able to eradicate resistant and tolerant bacterial cells. Our group has discovered that select halogenated quinolines (HQs) can eradicate drug-resistant, gram-positive bacterial pathogens and their corresponding biofilms. Interestingly, the HQ scaffold is synthetically tunable and we have discovered unique antibacterial profiles through extensive analogue synthesis and microbiologic studies. We recently reported the synthesis of 14 new HQs to investigate the impact of ClogP values on antibacterial and biofilm eradication activities. We conducted diverse synthetic modifications at the 2-position of the HQ scaffold in an attempt to enhance water solubility and found new compounds that display enhanced activities against *Staphylococcus epidermidis*. In particular, HQ **2** (ClogP = 3.44) demonstrated more potent antibacterial activities against methicillin-resistant *S epidermidis* (MRSE) 35984 planktonic cells (minimum inhibitory concentration = 0.59 µM) compared with methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* isolates while demonstrating potent MRSE biofilm eradication activities (minimum biofilm eradication concentration = 2.35 µM). We believe that HQ could play a critical role in the development of next-generation antibacterial therapeutics.

KEYWORDS: bacterial biofilms, biofilms-eradicating agent, drug discovery, halogenated quinoline, medicinal chemistry, persister cells, *S epidermidis*, synthetic chemistry

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Synthetically tunable antibacterial agents that operate through unique modes of action to target antibiotic-resistant and tolerant bacteria are of importance to human health.[1-4] Pathogenic bacteria present 2 distinct clinical problems: (1) acquired antibiotic resistance and (2) innate antibiotic tolerance. Bacteria acquire resistance to conventional antibiotics during therapy using one or more well-defined mechanism(s), including point mutations in antibiotic targets, enzyme-mediated antibiotic inactivation/degradation, and changes in membrane chemistry to impede drug penetration.[5] In contrast, metabolically dormant persister cells give rise to innate antibiotic tolerance. Surface-attached bacterial communities, known as "biofilms," contain enriched nonreplicating persister cell populations that demonstrate high levels of tolerance to all classes of antibiotics.[1,6] Nearly 80% of bacterial infections are biofilm associated, which are credited as the underlying cause of persistent and chronic infections.

It is important to note that all conventional antibiotic classes were initially discovered as bacterial growth–inhibiting agents in various microbiologic experiments involving planktonic bacteria and not biofilm communities.[1] To effectively identify compounds that can eradicate biofilms, new agents that operate through bacterial growth–independent mechanisms are required. Innovative discovery approaches are necessary to meet this clinical need and our group looked to nature's chemical inventory for inspiration, which has been the source of many therapeutic agents.

We are interested in the identification of natural products, and their synthetic derivatives, that operate through novel antibacterial mechanisms that enable biofilm and persister cell killing. During initial studies, we came across a report that described the pathogenesis of lung infections common among patients with cystic fibrosis (CF).[7] Many young patients with CF endure lung infections caused by the gram-positive pathogen, *Staphylococcus aureus*. As these patients age, the gram-negative bacterium *Pseudomonas aeruginosa* coinfects the lungs and eradicates the established *S aureus* population. The patients with CF are known to experience chronic lung infections that can last for more than 10 years; therefore, we hypothesized that the persistent *S aureus* infections were biofilm associated. It is believed that *P aeruginosa* uses the phenazine antibiotic pyocyanin, and other phenazines,[8] to eradicate *S aureus* during microbial warfare interactions within the lung of patients with CF.

Initial investigations focused on the synthesis of a library of chemically diverse phenazine antibiotics and phenazine derivatives for microbiologic evaluation against *S aureus*.[9] Interestingly, we found that marine-derived 2-bromo-1-hydroxyphenazine (2-Br-1-OHPhz; minimum inhibitory concentration, MIC = 6.25 µM; Figure 1) demonstrated an 8-fold increase in antibacterial potency against *S aureus* ATCC

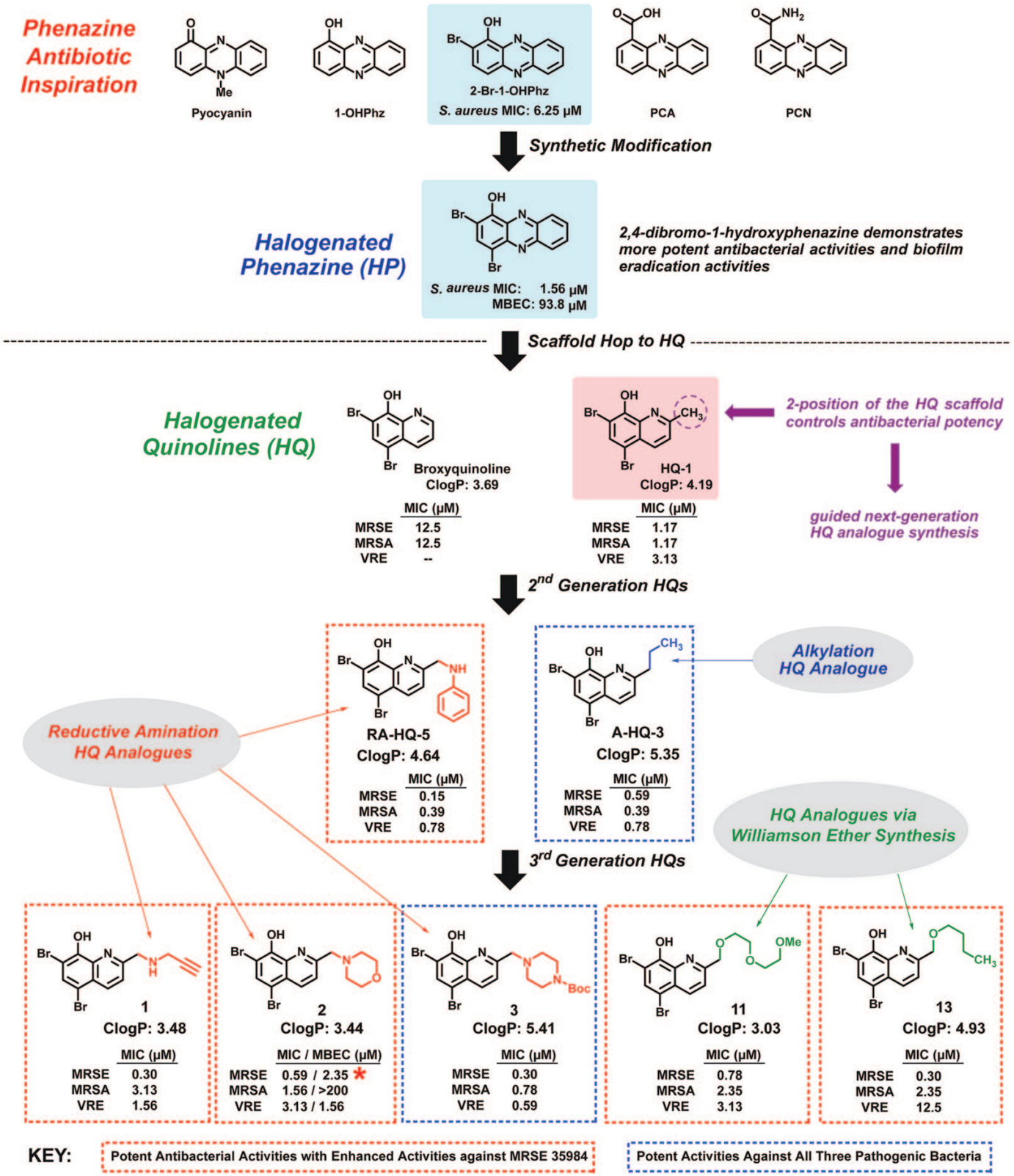

Figure 1. Phenazine antibiotic discovery of novel biofilm-eradicating agents. Halogenated quinolines were investigated following a scaffold hop from phenazine to quinoline agents. The 2-position of the halogenated quinoline scaffold can be tuned via multiple synthetic pathways, which has allowed for the discovery of potent biofilm-eradicating agents and compounds that have increased antibacterial activities against *Staphylococcus epidermidis*.

25923 compared with pyocyanin (MIC = 50 µM). Also, we found that the second bromine atom at the 4-position of the phenazine heterocycle, giving 2,4-dibromo-1-hydroxyphenazine, demonstrated further improvements in antibacterial activities against *S aureus* 25932 (MIC = 1.56 µM)[9] and eradicated surface-attached methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms in Calgary Biofilm Device (CBD) assays (minimum biofilm eradication concentration,

MBEC = 93.8 μM).[10] 2,4-Dibromo-1-hydroxyphenazine and related halogenated phenazine (HP) analogues demonstrate potent biofilm eradication activities against multiple MRSA, MRSE, and vancomycin-resistant *Enterococcus* (VRE) isolates while showing minimal cytotoxicity against HeLa cells or hemolytic activity against red blood cells.[1,3,10-12]

The discovery of HP antibacterial agents has set the stage for our group's investigations into new halogenated quinoline (HQ) analogues. We used a scaffold hop strategy (from phenazine to quinoline scaffold) to expand the synthetic analogue potential of the HP compound library (Figure 1).[13] We noticed broxyquinoline (5,7-dibromo-8-hydroxyquinoline) contained similar structural features to our HPs. We later learned that those structural similarities were critical for metal(II) chelation in addition to being the pharmacophore responsible for antibacterial and biofilm-eradicating activities.[14] When comparing broxyquinoline with an early analogue bearing a simple methyl ($-CH_3$) group at the 2-position of the HQ scaffold, we noted a significant increase in antibacterial potency against gram-positive pathogens (eg, MRSA, MRSE, VRE; see Figure 1). This simple structural modification prompted the synthesis of second-generation analogues to further probe the 2-position of the HQ scaffold. We generated a library of HQ analogues containing a diversity of functional groups at the 2-position and found that several analogues synthesized from alkylation and reductive amination pathways demonstrated potent antibacterial and biofilm eradication activities.[2,14]

Our third-generation HQ series was motivated to probe the effects that water-solubilizing moieties at the 2-position of the HQ scaffold had on antibacterial activities.[4] The most potent second-generation HQ analogues had ClogP values of ⩾4.6 and we wanted to design analogues with reduced ClogP values to improve the therapeutic potential of these compounds. With that, we designed a series of focused HQ analogues to incorporate chemical functionality to allow more favorable hydrogen-bond interactions with water. For our most recent third-generation HQ analogues, we capitalized on the reductive amination procedure we previously developed to synthesize amine-based groups at the 2-position of the HQ scaffold.[2,14] In addition, we incorporated ether- and polyethylene glycol (PEG)-based groups strategically at the 2-position of the HQ scaffold using Williamson ether syntheses.

Following the chemical synthesis of 14 new HQs bearing focused appendages at the 2-position to enhance water solubility, we evaluated these analogues against a panel of drug-resistant strains, including MRSA ATCC 1707, MRSE ATCC 35984, and VRE ATCC 700221.[4] We were interested to find 4 new HQ analogues from this collection (**1**, **2**, **11**, **13**; ClogP: 3.03-4.93) to demonstrate enhanced antibacterial activities against methicillin-resistant *S epidermidis* strain MRSE 35984. Based on MIC value assessment, MRSE 35984 demonstrated a 3- to 10-fold increase in sensitivity against these 4 HQs compared with MRSA 1707 and VRE 700221. This was the first clear demonstration of enhanced antibacterial activities of HQs (**1**, **2**, **11**, **13**; MIC = 0.30-0.78) against MRSE. In previous studies, active HQs typically demonstrated potent antibacterial activities against each of the pathogenic strains used in this most recent study, similar to **3** (ClogP: 5.41; new HQ), which reported MIC values of 0.30 to 0.78 against MRSE 35984, MRSA 1707, and VRE 700221.

Third-generation HQs **2**, **3**, **11**, and **13** demonstrated minimal cytotoxicity against HeLa cells and were advanced to biofilm eradication assays using the CBD.[4] To our delight, HQ **2** bearing a morpholine moiety at the 2-position (ClogP: 3.44) demonstrated potent eradication activity against MRSE 35984 biofilms (MBEC = 2.35 μM); however, it was unable to eradicate MRSA 1707 biofilms at 200 μM. HQ **3** bearing a Boc-protected piperazine at the 2-position of the HQ scaffold (ClogP: 5.41) reported good to excellent eradication activities against MRSE, MRSA, and VRE biofilms (MBEC = 1.56-18.8 μM) without demonstrating significant enhancements for any particular pathogen. Ether- and PEG-based HQ analogues **11** and **13** reported reduced, but equipotent biofilm eradication activities against MRSE (MBEC = 75 μM).

From these investigations, we have identified new HQ analogues with potent and enhanced antibacterial activities against methicillin-resistant *S epidermidis* 35984. These findings are indeed timely, as *S epidermidis* has emerged as an opportunistic pathogen in health care–associated infections in patients with indwelling medical devices.[15] In the clinic, *S epidermidis* infections are complicated by antibiotic-tolerant and immune-resistant biofilms that form on the surfaces of indwelling medical devices, such as catheters. The current standard of care for catheters bearing *S epidermidis* biofilms is to completely remove the biomedical device to clear the infection. Finding a viable therapeutic solution to catheter-based infections alone would be significant progress while laying the groundwork for other biofilm infections, such as prosthetic joint replacements (hip, knee replacements).

To conclude, many clinical problems associated with antibiotic resistance and antibiotic tolerance have motivated innovations in the discovery of next-generation therapeutic agents. We initially set out to identify new biofilm-eradicating agents that could target bacteria, which led to the discovery of pyocyanin-inspired HP small molecules. A major goal of this work is to develop novel synthetic analogues through medicinal chemistry campaigns; therefore, we hopped from the HP scaffold to HQs for further elaboration and development. Through the synthesis and microbiologic investigation of novel HQs, we have found the 2-position of the HQ scaffold to play a critical role in the tuning antibacterial and biofilm eradication properties. In our most recent work, we incorporated a series of diverse functional groups that enhanced water solubility compared with initial and second-generation HQ analogues. We found several new HQs, including **2**, to demonstrate enhanced antibacterial activities

against the human pathogen *S epidermidis*, which is a major clinical pathogen involved in biofilm-associated infections of indwelling medical devices. We hope to translate these provocative discoveries into next-generation human therapies that will effectively target and eradicate bacterial biofilms during infection.

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Author contributions

The author has established a research program aimed at developing novel antibacterial agents that eradicate bacterial biofilms and he wrote this manuscript.

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