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# Development of Quinoline-Based Disruptors of Biofilm Formation Against *Vibrio cholerae*

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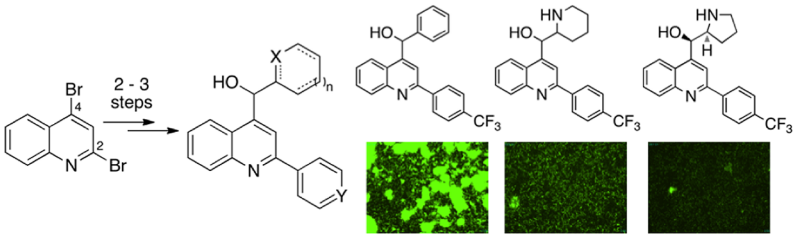
## ABSTRACT




**Biofilm formation is a major cause of bacterial persistence in nosocomial infections, leading to extended treatment times and increased rates of morbidity and mortality. Despite this, there are currently no biofilm inhibitors approved for clinical use. The synthesis and biological evaluation of a library of amino alcohol quinolines as lead compounds for the disruption of biofilm formation in *Vibrio cholerae* is now reported. Application of selective metal−halogen exchange chemistry installed both stereocenters in one step, to afford a simpler scaffold than the initial lead molecule, with an $EC_{50}$ < 10 $\mu$M.**

Bacterial biofilms are surface-associated bacterial assemblages containing microbial cells encased in an extracellular matrix of exopolysaccharides, proteins and DNA. Biofilm formation is increasingly being recognized as a major component of bacterial pathogenesis, with current estimates suggesting that up to 75% of pathogenic microbial infections are biofilm mediated.[1] This is considered to be a significant contributor to the development and persistence of nosocomial (hospital acquired) infections. Biofilm-associated infections are particularly difficult to clear because cells within the matrix can enter a latent state that reduces their susceptibility to traditional antibiotics. This results in a significant increase in bacterial persistence, leading to a concomitant increase in antibiotic treatment times, and a subsequent acceleration in the development of drug-resistance.[2] Despite detailed investigation of several compound series in recent years,[3] there are still no commercially available biofilm inhibitors that are approved for clinical use.

To discover new therapeutic options for biofilm-associated infections, we recently developed an image-based high content screening platform for the discovery of biofilm inhibitors in the model organism *Vibrio cholerae*.[4] Previous screening of a 3080-member small molecule library from the National Cancer Institute (NCI) revealed 29 compounds with the ability to selectively disrupt biofilm formation. Of these, two (NSC 13480, **3**, $EC_{50}$ = 11.1 $\mu$M; NSC 305787, **4**, $EC_{50}$ = 10.6 $\mu$M) were closely related, possessing a 2,4-disubstituted quinoline core and a $\beta$-amino alcohol motif (Figure 1). Many FDA approved drugs contain this privileged quinoline ring structure,[5]

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suggesting that this scaffold could be a valuable starting point for the development of new biofilm inhibitors.[6]

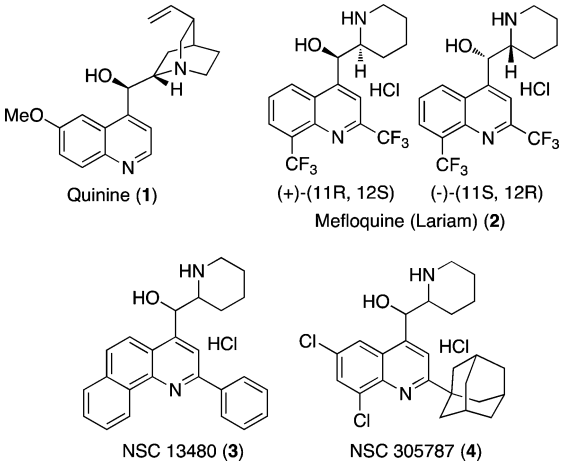
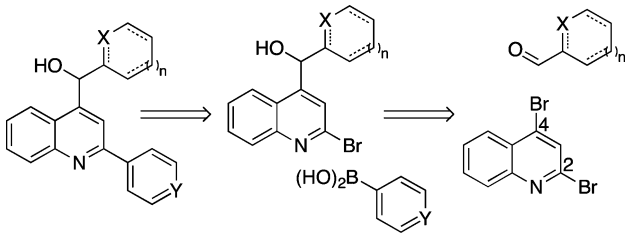



**Figure 1.** Initial lead compounds and related compounds.

The $\beta$-amino alcohol quinoline series are a well-studied class of compounds originally developed for antimalarial therapy by the Walter Reed Army Institute of Research as synthetic analogues of the natural product quinine (**1**).[7] This work ultimately lead to the development of mefloquine (**2**) for both prophylactic and therapeutic use against malaria. Recent studies have shown that mefloquine is effective against a number of other targets, including Gram-positive bacteria. However, previous work has reported that mefloquine and its derivatives are inactive against Gram-negative bacteria.[8]

The original racemic synthesis of mefloquine in 1971 afforded the drug in five steps.[9] More recently, attention has shifted to the effects of individual enantiomers on both antimalarial and neurotoxic activities,[10] culminating in two reports presenting enantioselective syntheses of the mefloquine scaffold in 2011.[11] Each of these studies employed a series of oxidation state manipulations and rearrangements to control the installation of the contiguous stereocenters of the $\beta$-amino alcohol motif. Given that recent studies indicate that the (−) form of the drug causes pronounced neurological side effects,[12] while the (+) form is responsible for the antimalarial activity, we were motivated to design a synthetic approach that could provide concise access to either racemic or enantiomerically pure forms of the $\beta$-amino alcohol scaffold using the same set of building blocks and chemical transformations, to allow for future development of either racemic or chiral approaches using the same synthetic methodology.

**Scheme 1.** Retrosynthetic Analysis



Retrosynthetically, we envisioned a C−C bond disconnection at the 2-position of the quinoline[13] (derived from Suzuki cross coupling chemistry) and a subsequent C−C bond disconnection at the 4-position, which could be installed via the regiospecific Grignard chemistry recently developed by Knochel and co-workers[14] (Scheme 1). It was proposed that this methodology could be employed to install the key $\beta$-amino alcohol motif in one step from 2,4-dibromoquinoline and an appropriately protected aryl/alkyl aldehyde. Because the predicted approach of the two substrates in the Grignard step follows the Felkin Ahn model, we hypothesized that the resulting products would be created with full stereochemical control at these two new stereogenic centers.

In this initial study, we elected to explore the effects of perturbation of the $\beta$-amino alcohol (ring size, aromaticity, N excision) and/or modification of the aryl group at the 2-position (H-donors/acceptors and stereoelectronic effects) on biofilm formation. To this end, we selected four aldehyde substrates and four boronic acids from which to create a 16-member screening library that examined the effects of systematic variation of these factors at the two key positions on the scaffold.

All library members were synthesized using the same general synthetic scheme. As a representative example, compound **5** was treated with *i*-PrMgCl•LiCl at −78 °C and underwent metal−halogen exchange exclusively at the 4-position (Figure 2). Nucleophilic addition to protected aldehyde **6** proceeded in 67% yield, to afford alcohol **10** as a single pair of enantiomers.[15] Subsequent Suzuki cross-coupling with boronic acid **14** using the $Pd(PPh_3)_4$ catalyst, followed by trityl deprotection with HCl, gave the desired amino alcohol **26** in good overall yield. Determination of the relative and absolute configurations of final compounds was accomplished via a combination of X-ray crystallographic analysis for compound **33** and comparisons

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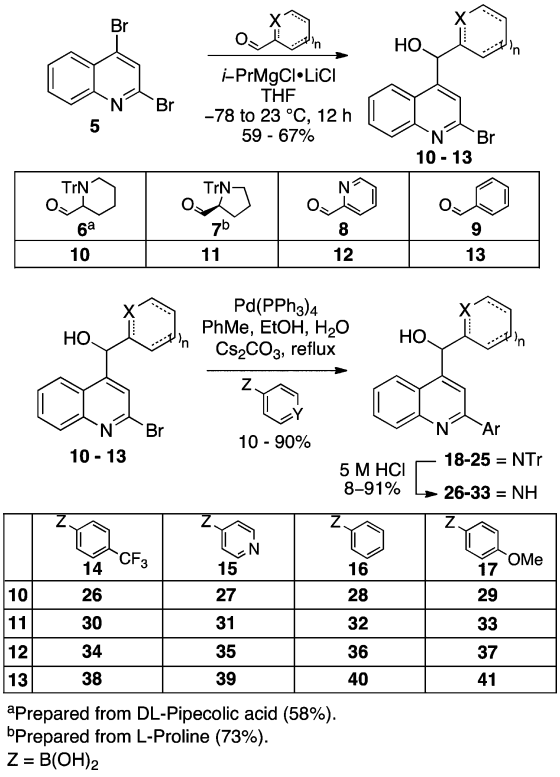
of key $^3J_{HH}$ coupling constants between the hydroxy- and amino-methine protons for compounds **26**–**33**.




| | 14 | 15 | 16 | 17 |
|---|---|---|---|---|
| 10 | 26 | 27 | 28 | 29 |
| 11 | 30 | 31 | 32 | 33 |
| 12 | 34 | 35 | 36 | 37 |
| 13 | 38 | 39 | 40 | 41 |

[a]Prepared from DL-Pipecolic acid (58%).
[b]Prepared from L-Proline (73%).
Z = $B(OH)_2$

**Figure 2.** Synthetic approach to compounds **26**–**41**.

Screening of both this 16-member library and 11 structurally related analogues from the NCI and commercial sources was performed using our standard in-house image-based biofilm screen. Briefly, this screen examines the ability of test compounds to inhibit the formation of biofilms on solid surfaces by treatment of test wells with compounds at the time of innoculation, followed by incubation for 4.5 h, washing, and imaging of biofilms formed on the surface of screening wells. The results of this analysis revealed three main SAR conclusions:

(1) The $\beta$-amino alcohol moiety is essential for reducing biofilm formation. This is evidenced by the lack of activity for compounds **34**–**37** and **38**–**41**, which contain respectively the pyridinyl and phenyl rings in place of the piperidinyl ring system, and the lack of the secondary amine from the original lead compounds.

(2) The quinoline motif is required for activity but is not in and of itself sufficient to impact biofilm formation. This conclusion is supported by the lack of activity for ephedrine (**42**), which contains the $\beta$-amino alcohol but lacks the quinoline motif, and compounds **45**, **49**, and **50**, which contain the quinoline but lack the $\beta$-amino alcohol (Figure 3).

Chelation of free iron has been shown to directly impact biofilm formation in *Pseudomonas aeruginosa*.[16]

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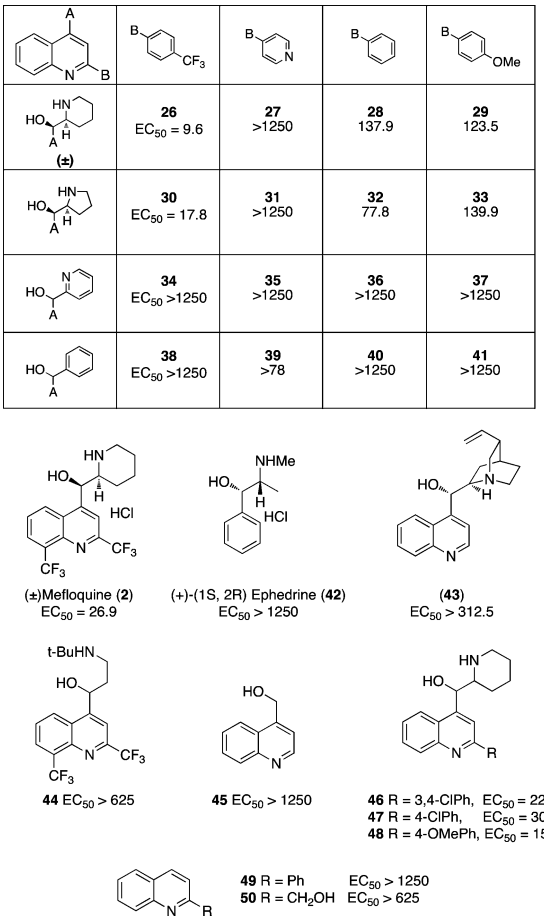


**Figure 3.** Biofilm formation $EC_{50}$ values for library members. Values in $\mu$M.

These SAR results indicate that, in *Vibrio cholerae*, bidentate chelators such as compounds **42** and **50** have no direct impact on biofilm formation.

(3) Substitution on the aryl ring at the 2-position of the quinoline impacts biofilm formation. In particular, inclusion of a pyridine ring eliminated activity in all cases, while inclusion of either phenyl or 4-(methoxy)phenyl substituents afforded compounds with weaker $EC_{50}$ values than those containing 4-(trifluoromethyl)phenyl substituents.

To be valuable in a clinical setting, lead compounds should be capable of disrupting existing preformed biofilms, in addition to inhibiting initial bacterial attachment and biofilm formation on surfaces. To examine the effectiveness of compound **26** at eliminating preformed biofilms, we treated static cultures of *V. cholerae* with **26** at both $t = 0$ (initial bacterial inoculation) and $t = 5$ h (preformed biofilms). Figure 4 demonstrates that compound **26** is capable of both preventing initial bacterial attachment (Figure 4A) and inducing detachment of preformed biofilms (Figure 4B).

There are few current examples of compounds capable of inducing bacterial detachment once biofilm formation is established,[3] making this result particularly valuable

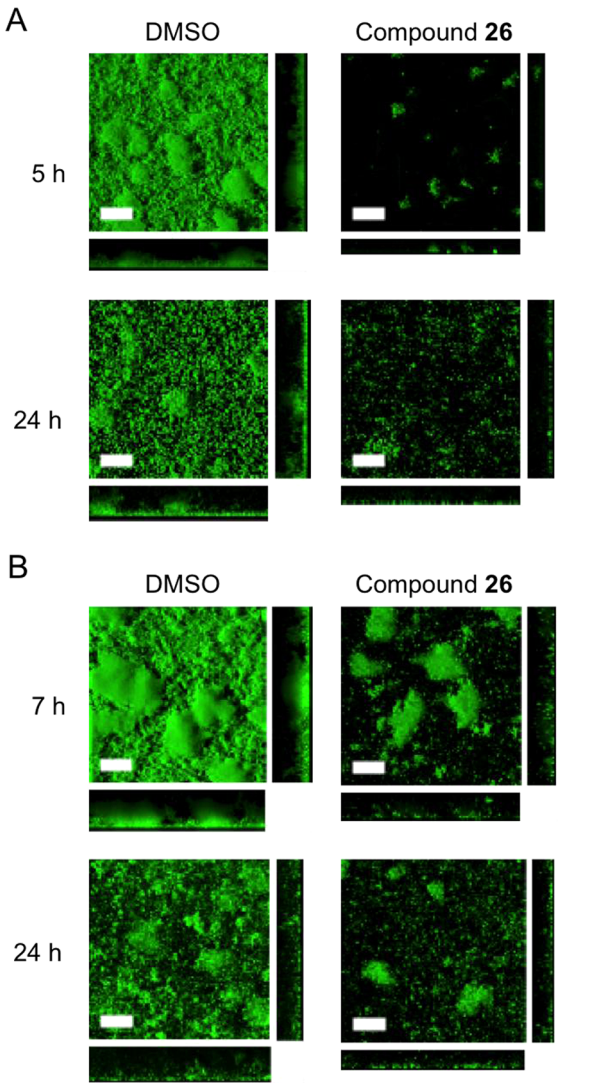


**Figure 4.** Confocal laser scanning microscopy images of top-down (large panels) and side (small panels) projections of biofilm structures formed by *V. cholerae* wild type at 5 or 7 and 24 h postinoculation. Compound **26** (50 $\mu$M) and DMSO control (0.4%) were added either during inoculation (A) or 5 h postinoculation (B). White bars represent 40 $\mu$m. Assays were repeated with two biological replicates.

from an applications perspective. In particular, few current FDA-approved antibiotics are effective against the biofilm forms of bacterial pathogens, meaning that clearance of established biofilm-mediated infections is particularly challenging.[17] In many cases, the only therapeutic options are irrigation (for open wounds) and repeated courses of antibiotic treatments, which are only marginally effective and provide a fertile environment for the development of antimicrobial resistance. The discovery that **26** is effective at reducing bacterial cell densities for both initial and preformed biofilms is therefore significant, as it offers the promise for the development of new therapeutic options for biofilm-mediated bacterial infections based on this compound scaffold.

In addition to evaluation of these compounds on biofilm formation and detachment, compounds **26**–**41** were screened for antibiotic activity against a diverse panel of clinically relevant bacterial pathogens using our recently reported BioMAP screening platform.[18] These results were in line with previously published studies of quinoline amino alcohols as antimicrobial agents, with activities in the low micromolar range against Gram-positive strains and little activity against Gram-negative organisms (Table S1). In addition, growth curves were obtained for **26** at 50 $\mu$M, using both $OD_{595}$ and colony forming unit (cfu) counts as independent readouts for cell survival (Figure S2). Under these experimental conditions, compound **26** was shown to inhibit growth of *V. cholerae*, suggesting that this compound has the ability to kill *V. cholerae* cells in both the biofilm and planktonic states.[19]

In conclusion, we report the synthetic development of a new class of disruptors of biofilm formation in *V. cholerae*. Application of recently established regioselective metal–halogen exchange methodology led to the creation of a library of synthetic analogues, one of which possesses a simpler structure and improved potency over the original lead compounds. This compound (**26**) is able to disrupt biofilm formation at low micromolar concentrations and to eliminate preformed biofilms under static conditions. This quinoline-based scaffold therefore represents a new avenue in the search for therapeutics capable of eliminating biofilm-mediated infections.

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**Supporting Information Available.** Experimental details for all synthetic procedures and biological screening, $^{1}H$ and $^{13}C$ spectra for all compounds, ORTEP and cif files for the X-ray crystal structure for compound **33**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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