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Microwave-enhanced Friedländer synthesis for the rapid assembly of halogenated quinolines with antibacterial and biofilm eradication activities against drug resistant and tolerant bacteria†‡

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Herein, we disclose the development of a catalyst- and protecting-group-free microwave-enhanced Friedländer synthesis which permits the single-step, convergent assembly of diverse 8-hydroxyquinolines with greatly improved reaction yields over traditional oil bath heating (increased from 34% to 72%). This rapid synthesis permitted the discovery of novel biofilm-eradicating halogenated quinolines (MBECs = 1.0–23.5 μ M) active against MRSA, MRSE, and VRE. These small molecules exhibit activity through mechanisms independent of membrane lysis, further demonstrating their potential as a clinically useful treatment option against persistent biofilm-associated infections.

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The synthesis of quinoline-based scaffolds receives much attention due to the ubiquity of these heterocycles among bioactive molecules.¹ In particular, 8-hydroxyquinolines have seen widespread use in therapeutics, demonstrating an array of medicinal applications (*e.g.*, antibacterial, anti-cancer, anti-neurodegenerative, anti-viral, *etc.*).² However, many syntheses of novel 8-hydroxyquinoline scaffolds utilize harsh acidic conditions (Fig. 1a) and/or require *O*-methylated aniline starting materials.^{3,4} These synthetic routes typically suffer from limited substrate scope, involve arduous workups and require deprotection to obtain 8-hydroxyquinolines.

Of particular interest to our group are the biofilm-eradicating properties of halogenated 8-hydroxyquinolines (HQs). Biofilms (*i.e.* surface-adhered bacterial communities encased within self-produced extracellular polymeric substances) are implicated in 80% of all bacterial infections (Fig. 1c).^{5,6} Worse still, bacterial infections lead to the death of more than 500 000 people annually in the U.S. alone.^{7,8} The prognosis of biofilm-related infections is exacerbated by the presence of persister cells, a distinct subset of bacterial populations which demonstrate non-dividing, dormant phenotypes.⁹ These bacterial populations are endowed with a

heightened degree of antibiotic tolerance which has proven notoriously difficult to overcome using growth-dependent conventional antibiotics.¹⁰

There are currently no clinically available agents that eradicate biofilm-associated infections; that is, agents which effectively kill biofilm-encased persister cells *via* growth-independent mechanisms. Few small molecules are known to eradicate bacterial biofilms. Lewis recently reported the eradication of *Staphylococcus aureus* persister cells and biofilms using a mouse model with ADEP4 (an acyldepsipeptide ClpP protease inhibitor) and Rifampicin co-treatment.¹¹ Others have employed antimicrobial peptides (AMPs) or mimics thereof.^{12,13} Although these AMP-like agents show promise, their mechanism of action involves the lysis of cellular membranes, which may not lend itself to bacterial selectivity. Alas, our current antimicrobial arsenal is completely incapable of addressing persistent bacterial phenotypes and the advancement of therapeutic agents able to combat them remains under-pursued. Innovative approaches by which therapeutics can be rapidly generated must be developed to counter these pathogens and remedy our inability to treat biofilm-associated infections.

Our group has recently identified a series of halogenated quinolines capable of eradicating bacterial biofilms while demonstrating minimal mammalian cytotoxicity and haemolytic activities (HQ 1, Table 1).^{14–16} Our first efforts toward the synthesis of HQs employed a Döbner–Miller reaction (Fig. 1a). However, due to harsh conditions, the necessity for demethylation, and the limitation of viable commercially available α,β -unsaturated ketones or aldehydes, we surmised

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Fig. 1 a) Common approaches for 8-hydroxyquinoline synthesis. b) Microwave-enhanced Friedländer reaction from readily available starting materials. c) Illustration of both free-floating planktonic cells and surface-attached bacterial biofilms.

this approach was not amenable to library synthesis. Alternatively, the Combes synthesis was explored, but attempts to synthesize HQs were met with failure as significant quantities of side products were observed and only trace amounts of the desired 8-hydroxyquinoline were obtained. Still motivated to further investigate HQs with greater structural diversity, we turned to the Friedländer quinoline synthesis,¹⁷ which proved to be a superior alternative. This synthesis proceeds under mild conditions without the need for catalysts or hydroxyl protecting groups. Additionally, the Friedländer reaction permits the use of readily available starting materials (aldehydes, β -keto esters, ketones and nitriles) to afford quinolines with diverse substitution patterns at the 2-, 3-, and 4-positions in a single step.

Thus, our synthesis began with the generation of 2-amino-3-hydroxybenzaldehyde **20** on gram scale, which was first condensed with commercially available ketones to afford 8-hydroxyquinolines with substitution at the 2- or 2- and 3-po-

sitions (**2a**, **3a**, **7a–10a**) in low to moderate yield (Fig. 2). Access to exclusively 3-substituted quinolines from aldehyde starting materials, however, proved troublesome under both basic and acidic conditions with traditional thermal heating (*i.e.* oil bath). To our surprise, the utilization of microwave irradiation afforded the previously unattainable 3-substituted 8-hydroxyquinolines (**4a**, **5a**, and **6a**) in 52% yield.

Further investigation into this microwave-assisted Friedländer reaction of 12 diverse substrates revealed marked improvements in yields for the majority of the quinoline library, from an overall average of 34% for traditional oil bath heating compared to 72% under microwave irradiation (Fig. 2b). Increased yields were observed in the condensation of **20** with methyl ketones (8-hydroxyquinolines **1a**, **2a**, **3a**), 1,3-dicarbonyls (**7a**, **8a**, **9a**), 3-pentanone (**10a**), and malononitrile (**12a**). It is worth noting, however, that attempts to improve the yield of 8-hydroxyquinoline **11a**, which requires a condensation with an acetophenone rather than a benzaldehyde (see ESI†), were unsuccessful. Following assembly of the core scaffold, 8-hydroxyquinolines were brominated at the 5- and 7-positions using 2.2 equivalents of *N*-bromosuccinimide to afford the final halogenated quinoline analogues in 22–94% yield (HQs **1–11**, Fig. 2b, ESI†). It should be noted that difficulty with brominating **12a** in addition to the poor solubility prompted the discontinuation of its advancement to the corresponding HQ.

Our HQ library was screened for antibacterial activity against a panel of human pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA-2 clinical isolate), methicillin-resistant *Staphylococcus epidermidis* (MRSE ATCC 35984), and vancomycin-resistant *Enterococcus faecium* (VRE ATCC 700221). In preliminary MIC assays, several HQs exhibited increased antibacterial activity compared to previously reported HQ **1** across our panel of pathogens (Table 1). HQs **8** and **9** demonstrated improved activity against MRSA-2, reporting MICs of 0.39 μM and 0.59 μM , respectively (see ESI†). Against MRSE 35984, HQs **8**, **10**, and **15** proved to be more potent than HQ **1** with MICs of 0.10 μM , 0.15 μM , and 0.10 μM . Four HQ analogues (**8–10**, **15**) reported improved MIC activity compared to HQ **1** against VRE with **15** demonstrating the highest potency against this strain (MIC = 0.30 μM). The MIC of these HQs permitted the identification of a subset of the most potent compounds to be evaluated for biofilm eradication activity.

Minimum bactericidal concentration (MBC), an evaluation of activity against planktonic cells, and minimum biofilm eradication concentration (MBEC) values were evaluated in tandem by use of the Calgary biofilm device (CBD).^{18–20} This apparatus allows the establishment of biofilms onto pegs mounted to the lid of a 96-well plate. The pegs are submerged in inoculated media and, following an incubation and concomitant biofilm establishment, are rinsed and transferred to a second 96-well plate containing serially-diluted test compound, allowing biofilm-eradicating agents to kill bacterial biofilms. Following a second incubation, the pegs are again transferred to a new 96-well plate containing only

a) Microwave Friedländer Affording Diverse 8-Hydroxyquinolines

b) Comparison of Oil Bath vs. Microwave Friedländer Yields

Entry	Product	Friedländer Method	Oil Bath Conversion (%)	Microwave Conversion (%)
1a. X = H 1. X = Br		A	37	84
2a. X = H 2. X = Br		A	28	76
3a. X = H 3. X = Br		A	60	83
4a. X = H 4. X = Br		A	No Yield	52
5a. X = H 5. X = Br		A	No Yield	48
6a. X = H 6. X = Br		A	No Yield	52
7a. X = H 7. X = Br		B	58	90
8a. X = H 8. X = Br		C	65	78
9a. X = H 9. X = Br		B	26	77
10a. X = H 10. X = Br		A	24	95
11a. X = H 11. X = Br		C	93	38
12a. X = H		B	70	92
Average Friedländer Yield			38%	72%

Fig. 2 a) Microwave-enhanced Friedländer synthesis of 8-hydroxyquinolines from a diverse array of starting materials. b) Yield comparison of oil bath heating *versus* microwave irradiation for the synthesis of 8-hydroxyquinolines.

Fig. 3 a) HQ 8 esters and amides. b) HQs with improved activities compared to HQ 1 against MRSA-2, MRSE, and VRE. c) Calgary biofilm device assay with HQs 1, 9, and front-running antibiotic vancomycin against MRSA BAA 1707.

fresh media, wherein viable biofilms, after compound treatment, are able to recover and disperse planktonic cells contained within, resulting in turbid wells. The absence of turbidity observed in this final 96-well plate represents eradicated biofilms. Thus, the lowest concentration of test compound that yields a non-turbid well is considered its MBEC. The 96-well plate from which the pegged lid is last removed allows the determination of MBC (see ESI†).

HQ 9 proved to be one of the most potent biofilm eradicators ever reported against both MRSA (MBEC = 3.9–23.5, Fig. 3c, Table 1 and Table S1†) and MRSE (MBEC = 1.0 μM). The promising biofilm eradication activities of 8 against MRSA-2 (MBEC = 31.3 μM) prompted the synthesis of a small library of ester (HQs 14–16) and amide (HQs 17–19) analogues of this HQ (Fig. 3a). However, these analogues, in addition to the carboxylic acid intermediate (HQ 13), exhibited

Table 1 Summary of biological investigations of HQ analogues (MIC, MBC, MBEC, and haemolysis)^a

Compound	MRSA-2 MIC	MRSA-2 MBC/MBEC	MRSE 35984 MIC	MRSE 35984 MBC/MBEC	VRE 700221 MIC	VRE 700221 MBC/MBEC	% haemolysis (200 μ M)
1	0.78	23.5 ^b /46.9 ^b	0.20	7.8/3.0 ^b	2.35 ^b	3.0 ^b /2.0	$\leq$ 1
2	25	—	0.39	—	>100	—	1.9
3	37.5 ^b	—	4.69 ^b	—	25	—	$\leq$ 1
4	3.13	15.6/46.9 ^b	1.17 ^b	23.5 ^b /31.3	4.69 ^b	7.8/5.9 ^b	$\leq$ 1
5	6.25	46.9 ^b /188 ^b	0.59 ^b	62.5/46.9 ^b	12.5	31.3/23.5 ^b	2.4
6	50	—	3.13	—	18.8 ^b	—	4.6
7	1.56	250/>1000	6.25	—	25	—	2.3
8	0.39	31.3/31.3	0.10 ^d	93.8 ^a /23.5 ^b	0.78	3.0 ^b /2.0	2.2
9	0.59 ^b	15.6 ^c /23.5 ^b	3.13	5.9 ^b /1.0 ^d	0.78	46.9 ^b /23.5 ^b	2.5
10	6.25	>1000/>1000	0.15 ^b	—	0.78	—	$\leq$ 1
11	6.25	—	1.17 ^b	—	4.69 ^b	—	$\leq$ 1
13	50	—	25	—	>100	—	2.5
14	1.56	—	0.30 ^b	—	2.35 ^b	—	1.5
15	6.25	375 ^b />1000	0.10 ^d	46.9 ^b /15.6 ^c	0.30 ^b	3.9 ^c /1.5 ^b	7.9
16	12.5	—	1.56	—	25	—	$\leq$ 1
17	37.5 ^b	—	12.5	—	50	—	7.0
18	6.25	>2000/>2000	6.25	—	25	—	13.0
19	100	—	50	—	>100	—	$\leq$ 1
QAC-10	3.13	31.3/125	2.35 ^a	31.3/31.3	2.35 ^b	3.0 ^b /3.0 ^b	>99
Vancomycin	0.59 ^b	3.0 ^b />2000	0.78	7.8/>2000	>100	>200/200 ^b	$\leq$ 1
Daptomycin	4.69 ^b	62.5 ^b />2000	12.5	—	—	—	1.7
Linezolid	3.13	15.6/>2000	3.13	—	3.13	4.69 ^c /1.56	$\leq$ 1

^a All MIC, MBC, and MBEC values reported in μ M. ^b Midpoint value of a two-fold range. ^c Midpoint value of a four-fold range. ^d Lowest concentration tested. Each data point is the result of three independent experiments.

no improvement in biofilm eradication activity against any pathogen with respect to 8. The most promising analogue against VRE was HQ 15 (MBEC = 1.5 μ M), exhibiting activity which correlates with its potent MIC. Although we observed improved MBEC activity for HQ 1 against MRSA-2 compared to previous studies,^{15,16} the potency was similar to biofilm eradication activities observed against other MRSA strains during these investigations (Table S1†).

The reported activities show promise, especially when in comparison to known biofilm eradicator QAC-10 (a quaternary ammonium cation)¹³ and front-running MRSA treatments vancomycin, linezolid, and daptomycin, which were used as positive controls in our biofilm eradication assays (Table 1). Despite demonstrating potent activity against planktonic bacteria (MBC), the clinically-available antibiotics exhibit no biofilm eradication activities (MBEC > 2000 μ M) against MRSA-2. Furthermore, active HQs report MBEC:MBC ratios of $\sim$ 1, demonstrative of efficacious biofilm-eradicating agents (Fig. 3c). Haemolysis assays were also conducted, wherein we observed negligible haemolytic activity at 200 μ M, with HQ 18 being the only analogue to report >10% haemolysis.

It is understood that many 8-hydroxyquinolines with medicinal utility demonstrate activity through mechanisms involving metal chelation.² Specifically, the phenolic oxygen and adjacent nitrogen atoms have been shown to participate in coordination to metal(II) cations. Although further investigation into the mode of action for our HQs is underway, we believe these agents exhibit activity *via* a metal(II)-dependent mechanism. This hypothesis is corroborated by our previous

reports of the modulation of antibacterial activities of HQs following co-treatment with metal(II) cations.^{15,16}

Improved syntheses of 8-hydroxyquinolines are invaluable to the discovery of therapeutic agents in multiple disease areas important to human health. The incorporation of readily available materials to assemble libraries in short, convergent syntheses is paramount to drug discovery. This microwave-mediated synthesis can incorporate a multitude of available starting materials, including aldehydes, β -keto esters, ketones and nitriles to directly generate 8-hydroxyquinolines with 2-, 3-, or 4-position substitutions that may otherwise be unattainable *via* strongly basic or acidic conditions and traditional oil bath heating. These advances permitted the rapid, facile synthesis of a series of novel halogenated quinolines that potently eradicate drug-resistant *S. aureus*, *S. epidermidis*, and *E. faecium* biofilms without demonstrating significant haemolytic toxicity. These data suggest that halogenated quinolines could prove to be a promising class of compounds capable of treating biofilm-associated infections.

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