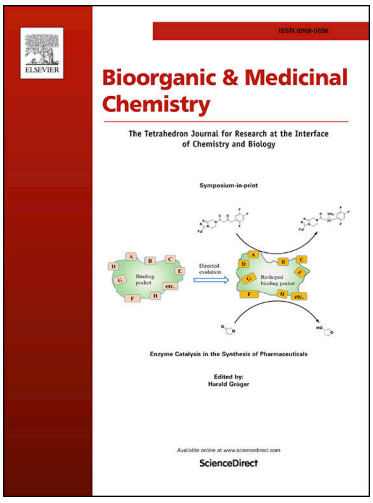

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C-2 Derivatized 8-sulfonamidoquinolines as antibacterial compounds

Emma K. Davison[a,b], John E. McGowan[a], Freda F. Li[a], Andrew D. Harper[a], Joo Young Jeong[a], Sonya Mros[c], Nichaela Harbison-Price[c], Essie M. Van Zuylen[c], Melanie K. Knottenbelt[c], Adam Heikal[c], Scott A. Ferguson[c], Michelle A. McConnell[c], Gregory M. Cook[b,c], Woravimol Krittaphol[d], Greg F. Walker[d], Margaret A. Brimble[a,b], David Rennison[a,*]

[a] *School of Chemical Sciences, University of Auckland, 23 Symonds Street, Auckland, New Zealand*
[b] *Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, Auckland 1042, New Zealand*
[c] *Department of Microbiology and Immunology, School of Biomedical Sciences, University of Otago, 720 Cumberland Street, Dunedin, New Zealand*
[d] *School of Pharmacy, University of Otago, 18 Frederick Street, Dunedin, New Zealand*

Abstract – A series of C-2 derivatized 8-sulfonamidoquinolines were evaluated for their antibacterial activity against the common mastitis causative pathogens *Streptococcus uberis*, *Staphylococcus aureus* and *Escherichia coli*, both in the presence and absence of supplementary zinc (50 μM $ZnSO_4$). The vast majority of compounds tested were demonstrated to be significantly more active against *S. uberis* when in the presence of supplementary zinc (MICs as low as 0.125 μg/mL were observed in the presence of 50 μM $ZnSO_4$). Compounds **5**, **34**-**36**, **39**, **58**, **79**, **82**, **94** and **95** were shown to display the greatest antibacterial activity against *S. aureus* (MIC ≤8 μg/mL; both in the presence and absence of supplementary zinc), while compounds **56**, **58** and **66** were demonstrated to also exhibit activity against *E. coli* (MIC ≤16 μg/mL; under all conditions). Compounds **56**, **58** and **66** were subsequently confirmed to be bactericidal against all three mastitis pathogens studied, with MBCs (≥3$\log_{10}$ CFU/mL reduction) of ≤32 μg/mL (in both the presence and absence of 50 μM $ZnSO_4$). To validate the sanitizing activity of compounds **56**, **58** and **66**, a quantitative suspension disinfection (sanitizer) test was performed. Sanitizing activity (>5$\log_{10}$ CFU/mL reduction in 5 min) was observed against both *S. uberis* and *E. coli* at compound concentrations as low as 1 mg/mL (compounds **56**, **58** and **66**), and against *S. aureus* at 1 mg/mL (compound **58**); thereby validating the potential of compounds **56**, **58** and **66** to function as topical sanitizers designed explicitly for use in non-human applications.

Keywords: 8-Hydroxyquinoline, 8-Sulfonamidoquinoline, Ionophore, Antibacterial, Bactericidal, *Streptococcus uberis*, *Staphylococcus aureus, Escherichia coli,* Bovine mastitis, Sanitizer.

* Corresponding author: Tel.: +64 9 373 7599x88527; fax: +64 9 373 7422 (school); E-mail address: d.rennison@auckland.ac.nz

1. Introduction

Bovine mastitis, an inflammatory response to a bacterial infection of the udder, is the most significant production-limiting disease for dairying worldwide,[1] and the single most common reason for antibiotic use within the dairy industry. Preventative teat sprays containing sanitizers such as chlorhexidine and iodine are important tools in the management of bovine mastitis. However, both chlorhexidine and iodine are considered equally valuable in the control of infectious diseases in humans, and as such are listed on the World Health Organization's model list of essential medicines.[2]

8-Hydroxyquinoline[3,4,5] (8-HQ, **1**, Fig. 1) has been utilized as a scaffold in the design of a number of drug candidates with wide-ranging applications such as neuroprotection,[6,7,8,9] anticancer,[10,11] antiviral,[12] antimalarial,[13] antifungal[14] and antibacterial.[15,16,17] Examples of other 8-HQs include 5-chloro-7-iodo-8-quinolinol (clioquinol, CQ, **2**, Fig. 1), 5,7-diiodo-8-quinolinol, 5,7-dibromo-8-quinolinol, 5,7-dichloro-2-methyl-8-quinolinol and 5-chloro-8-quinolinol, many of which have been developed as topical antiseptics.[18] More recently CQ, along with later-generation halogenated 8-HQs such as PBT2 (**3**, Fig. 1), have been utilized in the treatment of non-infectious pathologies such as Alzheimer's disease,[7] Huntington's disease,[8] Parkinson's disease[9] and cancer.[10,11] More pertinent to this study, PBT2 also exhibits antibacterial activity, and it has recently been shown that PBT2, in combination with zinc, can reverse antibiotic resistance.[19] Zinc homeostasis is known to play a critical role in bacterial survival, with excessive cellular zinc accumulation typically culminating in zinc intoxication.[20,21]

It is known that 8-HQ and its derivatives can act as both mono- and bi-dentate metal chelators (coordination involves the participation of both the endocyclic quinoline nitrogen and the exocyclic phenolate oxygen as donor atoms).[22,23] 8-HQs have been reported to form metal-ligand complexes with most divalent transition metal ions including Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}, with a known preference for Cu^{2+} and Zn^{2+}.[24,25] The biological mode of action of 8-HQ has been studied extensively and it has been shown that the participation of a metal is essential for effective antibacterial activity.[15,16,17] While the precise mode(s) of action of CQ and PBT2 have yet to be fully clarified, postulated mechanisms to date suggest that these molecules could be functioning as metal ionophores.[26,27,28] As lipid-soluble chemical entities with the capacity to reversibly bind and

transport metal ions across cell membranes, metal ionophores have been used as growth promotants in livestock farming for decades (e.g. monensin, an animal feed additive used to improve weight gain in beef cattle).[29,30] Though bacterial resistance can be considered a natural process, the widespread use, and in many instances misuse, of antibiotics in livestock farming has artificially driven a rise in resistant strains, heightening the likelihood of antibiotic-resistant bacteria of animal origin ultimately infecting humans.[31] Current evidence suggests that the use of metal ionophores as antibacterials would be unlikely to contribute towards the spread of antibiotic resistance in humans on the basis that they exploit a distinct mode of action not currently utilized in human medicine.[32,33] As such, zinc-based therapies could offer an attractive means of controlling bovine mastitis.

Designed as bioisosteric structural analogues of 8-hydroxyquinoline, we recently reported a series of substituted 8-sulfonamidoquinolines as zinc-dependent antibacterial compounds; with a particular emphasis on the sulfonamide group.[34] Of the >70 compounds which underwent microbiological evaluation, *N*-(4-(trifluoromethyl)benzenesulfonyl)-8-aminoquinoline analogue **4** was adjudged to have the best antibacterial/bactericidal profile against the common mastitis causative pathogens studied. Having previously probed and optimized the nature of the sulfonamide moiety, and in the continued search for antibacterial compounds designed to be used exclusively as animal therapeutics, we herein report a series of C-2 derivatized 8-sulfonamidoquinolines building on platform compound **4**.

2. Results and discussion

2.1. Synthesis

Compounds **6**-**57**, **59**-**69** and **99** were accessed through the reductive amination (using sodium triacetoxyborohydride as the hydride source) of 2-formyl-8-sulfonamidoquinoline **98** with the corresponding amine (Scheme 1). 2-Formyl-8-sulfonamidoquinoline **98** was prepared over two steps from commercially available 2-methyl-8-aminoquinoline (**96**), via sequential reaction with 4-(trifluoromethyl)benzenesulfonyl chloride (to give 8-sulfonamidoquinoline **97**) and oxidation (using selenium dioxide) (Scheme 1). Guanidine derivative **58** and carboxylic acid derivative **70** were prepared from Boc-protected guanidine **99** and *tert*-butyl ester **69**, respectively, using trifluoroacetic acid (Scheme 1). Compounds **71**-**75** were prepared (via their corresponding *N*-Boc-protected intermediates) from common *N*-hydroxysuccinimide derivative **101** (Scheme 2). *N*-Hydroxysuccinimide derivative **101** was prepared over two steps from amino acid **70** (via *N*-Boc-protected amino acid **100**) (Scheme 2). Carboxamide derivatives **76**-**78** and guanidines **79** (via Boc-protected guanidine **105**) and **80** were prepared from amine **5** using the appropriate derivatizing reagent (Scheme 3). Amine **5** was prepared from oxime **82** through catalytic hydrogenation (Scheme 3). Carboxamide derivative **81** was prepared from carboxylic acid **106**, itself accessed through the oxidation of aldehyde **98** using potassium permanganate (Scheme 4). Oximes **82**-**84** and hydrazones **85**-**87** were accessed through the reaction of aldehyde **98** and the corresponding hydroxylamine/hydrazine, respectively (Scheme 5). Acylhydrazones **88**-**91** and **107**, semicarbazone **93** and thiosemicarbazones **94** and **95** were similarly prepared from aldehyde **98** using the appropriate acylhydrazide/semicarbazide/thiosemicarbazide, respectively (Scheme 5). Acylhydrazone **92** was prepared from *N*-Boc protected acylhydrazone **107** using trifluoroacetic acid.

2.2. Antibacterial/bactericidal activity

2.2.1. Minimum inhibitory concentration (MIC)

Despite over 100 different microorganisms having been identified as potential causative agents of bovine mastitis, only a few species are considered to be of economic or epidemiological importance.[35] Of these, the most significant include contagious Gram-positive pathogens such as *Staphylococcus aureus* (*S. aureus*), and environmental Gram-positive and Gram-negative pathogens such as *Streptococcus uberis* (*S. uberis*) and *Escherichia coli* (*E. coli*), respectively.[36] Environmental pathogens can be found in soil, animal housing, feedstuffs and standing water, and as such cannot be eliminated from the herd as they form part of the natural surroundings in which dairy cattle live. These pathogens are able to survive outside the udder, and can often be found on the skin. Contagious pathogens generally live within the udder or teat skin, and can be transmitted from cow to cow, particularly at milking. Major regional variations in the prevalence and distribution of mastitis causative pathogens exist. In New Zealand, the most common mastitis causative pathogens are *S. uberis* and *S. aureus*.[37] The antibacterial activities (reported as minimum inhibitory concentrations, MIC; at which 100% growth inhibition was observed)

of compounds **4**-**95** were subsequently evaluated against *S. uberis* strain ATCC 19436, *S. aureus* strain ATCC 6538 and *E. coli* strain ATCC 10536, in both the presence and absence of supplementary zinc; a concentration of 50 μM $ZnSO_4$ (a level at which zinc will be present in significant excess to the compound) was adopted on the basis that it has no observed effect on the growth of any of the target pathogens.*

Against *S. uberis* in the presence of 50 μM $ZnSO_4$, MICs were found to be in the range of 0.125-0.5 μg/mL for mono-*N*-alkylamine substituted derivatives **5**-**18** (Table 1); little to no difference in antibacterial activity was observed between straight-, branched- and cyclic-chain analogues. Within the linear di-*N*-alkylamine series, compounds **4**[34] (R = Me), **23** (Et) and **24** (^{n}Pr) were shown to retain the activity of their mono-*N*-alkylated counterparts, as did cyclic di-*N*-alkylamine derivatives **27** (pyrrolidine), **28** (piperidine) and **29** (azepane); whereas diisopropylamine derivative **25** was found to be of lower activity (Table 2). Unsubstituted amine derivative **5** was revealed to have an MIC of 0.5 μg/mL (Table 1). To probe the effect of amino group basicity on antibacterial activity, a selection of amines bearing known 'pK_a lowering groups' were evaluated, including fluorinated alkylamines **19** (R = CH_2CH_2F) and **20** (CH_2CHF_2), allylamine **21** and propargylamine **22** (Table 1), alongside morpholine **30**, thiomorpholine **31** and *N*-methylpiperazine **32** (Table 2); no influence on activity was observed, with MICs ranging from 0.125-0.5 μg/mL. In the absence of supplementary zinc, compounds **4**-**25** and **27**-**32** were subsequently demonstrated to be significantly less active against *S. uberis* (MIC ≥16 μg/mL) (Tables 1 and 2). Of compounds **4**-**25** and **27**-**32**, only compounds **4** (MIC 4 μg/mL), **5** (MIC 4 μg/mL) and **6** (MIC 8 μg/mL) were found to have any notable antibacterial activity against *S. aureus* in the presence of 50 μM $ZnSO_4$, and only compounds **5** (MIC 8 μg/mL) and **6** (MIC 16 μg/mL) in the absence of supplementary zinc (Tables 1 and 2). Compounds **4**-**25** and **27**-**32** were all confirmed to be of lower activity against *E. coli* (MIC ≥64 μg/mL), both in the presence and absence of supplementary zinc.

A select series of *N*-substituted amines bearing either aromatic or heteroaromatic groups were next evaluated (Table 3). Against *S. uberis* in the presence of 50 μM $ZnSO_4$, unsubstituted aromatic derivatives **33** (phenyl) and **37** (benzyl) displayed levels of antibacterial activity comparable to that of their aliphatic counterparts, as did furan **43** and thiophene **44** (MIC 0.125-0.25 μg/mL). As observed previously, compounds **33**, **37**, **43** and **44** were all found to be significantly less active in the absence of supplementary zinc (MIC ≥16 μg/mL), as they were under all conditions against both *S. aureus* (MIC ≥32 μg/mL) and *E. coli* (MIC ≥64 μg/mL). To probe the effect of incorporating groups inherently capable of further chelating Zn^{2+} into the C-2 side-chain, phenol **34**, alongside phenolic analogues bearing electron-withdrawing substituents (compounds **35** and **36**), were evaluated (Table 3) (Fig. 2). While antibacterial activity against *S. uberis* in the presence of 50 μM $ZnSO_4$ largely reflected that of unsubstituted analogue **33**, activity in the absence of supplementary zinc increased, particularly for dichlorinated phenolic derivative **36** (MIC 2 μg/mL); an analogue conceptualized to increase the acidity of the phenol group, thus increasing the population of potentially chelating phenoxide present at physiological pHs.[7] This notable activity was also extended to *S. aureus* (MIC 2 μg/mL, both in the presence and absence of 50 μM $ZnSO_4$), but not *E.coli* (MIC ≥64 μg/mL, under all conditions). With a similar approach in mind, in part based on literature observations around the known zinc-chelating compound *N*,*N*,*N*′,*N*′-*tetrakis*(2-pyridylmethyl)ethylenediamine (TPEN),[38] a select series of pyridine derivatives where prepared (Table 3) (Fig. 2). Against *S. uberis* in the presence of 50 μM $ZnSO_4$, pyridines **38**, **39** and **42** (designed to probe the importance of linker length) and pyridines **40** and **41** (to probe the importance of the nitrogen atom position within the ring) displayed MICs ranging from 0.125-4 μg/mL. More noteworthy though were their MICs in the absence of supplementary zinc, where only compound **39** was demonstrated to retain its antibacterial activity (MIC 2 μg/mL), akin to compound **36**. Such activity also extended to *S. aureus* (MIC 4 μg/mL, both in the presence and absence of 50 μM $ZnSO_4$), but not *E. coli* (MIC ≥64 μg/mL, under all conditions). Di-substituted 2-pyridylmethyl-derivative **26** was also prepared to determine whether this phenomenon could be extended to the installation of two potentially chelating groups within the C-2 side chain (Table 2); however, in this particular instance, antibacterial activity was restricted to *S. uberis* in the presence of supplementary zinc only.

A select series of mono-, di- and tri-*N*-hydroxyalkylamines (C-2 aminoalcohols), including PEG derivatives, were also evaluated; in some examples alongside their corresponding methyl ethers (Table 4). Within the mono-substituted series, compounds incorporating short-chain hydroxyalkyl substituents (e.g. compounds **45** and **46**; MIC ≤0.5 μg/mL), along with methyl ether **52** (MIC 0.25 μg/mL), were shown to have marginally better antibacterial activity against *S. uberis* in the presence of 50 μM $ZnSO_4$ than their longer chain counterparts (e.g. compounds **47**, **48**, **53** and **54**; MIC ≥1 μg/mL), while

* Independently, all three mastitis-causative pathogens were previously shown to be highly tolerant to supplementary zinc salts ($ZnSO_4$), with the minimum inhibitory concentrations (MIC) ranging from 800-1600 μM for *S. uberis*, through to 6400 μM for *E. coli*.

diols **49** and **50**, and triol **51** were all confirmed to be of lower activity (MIC ≥16 μg/mL). Compounds **45-54** were subsequently demonstrated to be less active against *S. uberis* (MIC ≥16 μg/mL) in the absence of supplementary zinc, as they were against *S. aureus* (MIC ≥32 μg/mL) and *E. coli* (MIC ≥64 μg/mL) under all conditions.

Next, a focused series of *N*-aminoalkylamines (C-2 diamines) were prepared (Table 4). Against *S. uberis* in the presence of 50 μM $ZnSO_4$, compounds **55**, **56** and **62-68** displayed MICs ranging from 0.5-4 μg/mL. More pronounced though was the retention of activity of compounds **55** (R = $CH_2CH_2NMe_2$), **56** ($CH_2CH_2CH_2NMe_2$), **62** (CH_2CH_2-*N*-piperidine) and **66** ($CH_2CH_2CH_2$-*N*-piperidine) against *S. uberis* (MIC ≤8 μg/mL) in the absence of 50 μM $ZnSO_4$, and their activity against *S. aureus* (MIC 16 μg/mL), regardless of the presence or absence of supplementary zinc. Moreover, compounds **56** and **66** were also demonstrated to be active against *E. coli* (MIC 16 μg/mL; under all conditions), whereas their corresponding shorter-chain homologues, compounds **55** and **62** respectively, were revealed to be of lower activity (MIC ≥64 μg/mL). To further probe this finding, and as a further example of an analogue featuring a C-2 side-chain bearing a cationic group, guanidine **58** was prepared (Table 4). As observed previously for *N*-aminoalkylamines **56** and **66**, guanidine **58** was demonstrated to be active against *S. uberis* (MIC 8 μg/mL), *S. aureus* (MIC 8 μg/mL) and *E. coli* (MIC 16 μg/mL), regardless of the presence or absence of supplementary zinc. To investigate the importance of electrostatic charge over hydrogen-bonding, with respect to the contribution (molecular interaction) of the guanidine group, non-basic guanidine bioisostere **59** was also prepared, and was subsequently shown to be less active against all three pathogens (under all conditions). As further examples of derivatized amines, acetamide **57**, along with sulfonamides **60** and **61**, were also revealed to be of lower activity.

N-Carboxyalkylamine **70** (C-2 amino acid) was also demonstrated to be of lower activity against all three pathogens under all conditions; while its synthetic precursor, *tert*-butyl ester **69**, was found to be active against *S. uberis* (MIC 0.5 μg/mL) in the presence of 50 μM $ZnSO_4$ only (Table 5). As representative examples of carboxamides (exploiting the capacity to further functionalize the C-2 side-chain of amino acid **70** via an amide linkage), unfunctionalized amide derivatives **71** and **72**, alongside aminoalkyl-substituted amide derivatives **73** and **74** (bearing terminal dimethylamino moieties akin to those present in compounds **55** and **56**), were all revealed to be of lower activity against all three pathogens (under all conditions) (Table 5); as was hydroxamic acid derivative **75** (as an example of a compound incorporating a known zinc-chelating moiety within the C-2 side-chain)[39] (Table 5).

To investigate the importance of having an amino group at the *β*-position within the C-2 side-chain, unsubstituted acetamide **76**, alongside dimethylamino-substituted acetamides **77** and **78**, were evaluated; compounds **76** and **77** were subsequently shown only to be active against *S. uberis* (MIC 0.125 μg/mL) in the presence of 50 μM $ZnSO_4$, whereas compound **78** was revealed to be of lower activity in all instances (Table 5). Building on previous observations within the series (in terms of the better performing C-2 side-chain functional groups), guanidine **79** was prepared and demonstrated to display activity against *S. uberis* (MIC 0.5 μg/mL) in the presence of 50 μM $ZnSO_4$, and against *S. aureus* (MIC 8 μg/mL) and *E. coli* (MIC 32 μg/mL) under all conditions; thus mirroring the activity of guanidine **58**. Nitro-guanidine **80** was revealed to be of lower activity (cf. nitro-guanidine **59**) (Table 5), as was amino-substituted acetamide **81**.

Finally, as further examples of C-2 side-chain derivatives, a small series of oximes, hydrazones, acylhydrazones, semicarbazones and thiosemicarbazones were explored (Table 6). Of particular note were thiosemicarbazones **94** and **95**, which were demonstrated to be active against both *S. uberis* (MIC 4 μg/mL) and *S. aureus* (MIC 4 μg/mL), both in the presence and absence of 50 μM $ZnSO_4$, but not *E. coli* (MIC ≥64 μg/mL, under all conditions). As discussed previously for compounds **36** and **39**, thiosemicarbazone **94**[40] provides a further example of a compound inherently capable of further chelating zinc through the participation of its C-2 side chain group (Fig. 2).

2.2.2. Minimum bactericidal concentration (MBC)

Compounds **56**, **58** and **66** were next evaluated for their bactericidal activity (reported as minimum bactericidal concentrations, MBC; ≥3$\log_{10}$ CFU/mL reduction) against *S. uberis* strain ATCC 19436, *S. aureus* strain ATCC 6538 and *E. coli* strain ATCC 10536 (Table 7). Against *S. uberis* in the presence of 50 μM $ZnSO_4$, compound **56** registered the lowest MBC (0.5 μg/mL) of those compounds put forward for bactericidal evaluation; in the absence of supplementary zinc compound **56** was found to be significantly less active (MBC 8 μg/mL), whereas the bactericidal activities of compounds **58** and **66** were confirmed to be unaffected (MBC 4-8 μg/mL, with or without 50 μM $ZnSO_4$). Against *S. aureus*, compounds **56**, **58** and **66** were revealed to be equipotent, and uninfluenced by the addition of 50 μM $ZnSO_4$ (MBC 16 μg/mL, all

conditions), as they were against *E. coli* (MBC 16-32 μg/mL, all conditions). In summary, compounds **56**, **58** and **66** were demonstrated to exhibit effective broad-spectrum bactericidal activity (MBC ≤32 μg/mL) against all three mastitis-causative pathogens screened.

2.2.3. Sanitizing activity

To experimentally validate that compounds **56**, **58** and **66** meet the stern requirements of a chemical disinfectant/antiseptic, we modified a quantitative suspension test (BS EN 1656:2009, European Committee for Standardization, 2009)[41] for use in 96-well microtiter plates. The EN1656 standardized assay specifies strict conditions in which the evaluation of potential veterinary chemical disinfectants/antiseptics must be carried out under - compounds must be biologically active in the presence of a well-defined interfering agent (European Committee for Standardization, 2000); for the validation of teat spray disinfectants, the use of skim milk as an interfering agent is required. The sanitizing activity (>5$\log_{10}$ CFU/mL reduction in 5 minutes) of compounds **56**, **58** and **66** (as unoptimized formulations) against *S. uberis* ATCC 19436, *S. aureus* strain ATCC 6538 and *E. coli* strain ATCC 10536 is reported in Table 8. Our findings indicated that compounds **56**, **58** and **66** had the capacity to reduce the bacterial inoculum of both *S. uberis* and *E. coli* by >5$\log_{10}$ CFU/mL at compound concentrations as low as 1 mg/mL. Compound **58** was also able to reduce the bacterial inoculum of *S. aureus* (>5$\log_{10}$ CFU/mL) at a compound concentration of 1 mg/mL; whereas compounds **56** and **66** were revealed to be less effective/reliable. This data further validates the potential of these compounds to function as topical sanitizers against the common mastitis causative pathogens *S. uberis*, *S. aureus* and *E. coli*.

3. Conclusion

Based on the findings of this study it is evident that against *S. uberis*, 8-sulfonamidoquinolines **4**-**95** were typically reliant on the presence of supplementary zinc for effective antibacterial activity; and that of the three mastitis causative pathogens studied, *S. uberis* would appear to be significantly more sensitive to these particular 8-sulfonamidoquinoline-zinc combinations than either *S. aureus* or *E. coli*. As such it is postulated that 8-sulfonamidoquinolines,[34] as plausible bioisosteric structural equivalents of 8-hydroxyquinoline,[42] similarly share the capacity to form reversible metal-ligand complexes with divalent transition metal ions such as Zn^{2+}; potentially forming complexes akin to those described previously for PBT2 (**3**).[23] Moreover, it is suggested that these lipid-soluble complexes are endowed with the ability to transport zinc ions across bacterial cell membranes, with ensuing elevated intracellular zinc concentrations ultimately leading to zinc intoxication.[28] With regards to the antibacterial profiles of compounds **36**, **39** and **94** against *S. aureus* (and as exemplars of compounds which were revealed to be equally active both in the presence and absence of supplementary zinc against both *S. uberis* and *S. aureus*), one could postulate that these molecules could be operating via a different mechanism of action to others within this particular sub-family of compounds. In terms of offering a potential explanation for the activity of compounds **56**, **58** and **66** against *E. coli*, one postulation for such an observation could relate to the basicity of the amine/guanidine groups within the C-2 side-chain; and plausibly the participation of a charged moiety, as has been shown to be mechanistically important in the antibacterial activity of some drugs against Gram-negative pathogens.[43] For example, the presence of an additional methylene group within the C-2 side-chains of compounds **56** and **66** would reveal a terminal amine group of inherently higher pK_a than that found in corresponding homologues **55** and **62**, respectively; which were both demonstrated to be less active against *E. coli*. Further support for this hypothesis could be provided through the inactivity (against *E. coli*) of compounds **67** (morpholine) and **68** (*N*-methylpiperazine), versus compound **66** (piperidine), both of which incorporate amine groups of lower basicity. Guanidine **58** is provided as a further example of a C-2 derivatized 8-sulfonamidoquinoline bearing a cationic side-chain moiety with proven activity against *E. coli*. A more detailed mechanistic explanation sits outside the scope of this structure-activity focused manuscript. In conclusion, 8-sulfonamidoquinolines serve as potentially promising platforms in the design of preventative teat disinfectants for the preclusion of bovine mastitis, and as prospective animal-specific sanitizers *per se*.

4. Experimental

4.1. General experimental methods

All reagents were used as supplied unless otherwise stated. Solvents were purified by standard methods. Analytical thin layer chromatography (TLC) was carried out on pre-coated silica gel plates (Merck/UV254) and products were visualized by UV fluorescence. Flash chromatography was performed using silica gel (Riedel-de Haën, particle size 0.032–0.063 mm). Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker AVANCE DR × 300 (^{1}H, 300 MHz), × 400 (^{1}H, 400 MHz) or × 500 (^{1}H, 500 MHz) spectrometer at 298 K. For ^{1}H NMR data, chemical shifts are described in parts per million (ppm) relative to either $CDCl_3$ (δ 7.26), d_4-MeOH (δ 3.31), d_6-DMSO (δ 2.50) or d_6-acetone (δ 2.05), and are reported consecutively as position (δ_H), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet) and coupling constant (*J*/Hz). For ^{13}C NMR data, chemical shifts are described in parts per million (ppm) relative to either $CDCl_3$ (δ 77.16), d_4-MeOH (δ 49.00), d_6-DMSO (δ 39.52) or d_6-acetone (δ 29.84), and are reported consecutively as position (δ_C), degree of hybridization, multiplicity (d = doublet, q = quartet) and coupling constant (*J*/Hz). Mass spectra were recorded on a VG-70SE mass spectrometer using electrospray ionization (ESI) methods. The purity of all target compounds was assigned using achiral reverse-phase HPLC [Dionex P680 system using a Phenomenex Gemini C_{18}-Si column (150 mm x 4.6 mm, 5 μm)] – eluted using a gradient of 100:0% A:B to 5:95% A:B over 15 min at 1 mL/min; where solvent A was water (+0.1% v/v trifluoroacetic acid) and solvent B was acetonitrile (+0.1% v/v trifluoroacetic acid), unless stated otherwise (*denotes a gradient of 100:0% A:B to 5:95% A:B over 10 min at 1 mL/min); with detection at 254 nm.

4.2.1. *N*-(2-(((3-(Dimethylamino)propyl)amino)methyl)quinolin-8-yl)-4-(trifluoromethyl)benzenesulfonamide di-hydrochloride (56)[44]

A solution of compound **98** (5.0 g, 13.2 mmol), 3-(dimethylamino)-1-propylamine (5.0 mL, 39.7 mmol) and sodium triacetoxyborohydride (5.60 g, 26.4 mmol) in dichloromethane (250 mL) was stirred at room temperature for 18 h. The mixture was then washed with an aqueous solution of phosphate buffer (0.5 M, pH 7), the separated organic layer dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. The resulting residue was then taken up in ethyl acetate and extracted with an aqueous solution of hydrochloric acid (1 M), and the separated aqueous layer further washed with ethyl acetate. The pH of the separated (product-containing) aqueous layer was then adjusted to pH 4 through the careful addition of sodium hydrogen carbonate, and the resulting solution used directly as an HPLC loading vehicle. Purification by semi-preparative reverse phase HPLC (100:0 to 50:50 A:B over 50 minutes at a flow rate of 7 mL/min, where A = water + 0.1% v/v trifluoroacetic acid and B = acetonitrile + 0.1% v/v trifluoroacetic acid), followed by lyophilization, afforded the corresponding di-trifluoroacetate salt of compound **56**, which was then dissolved in 0.1 M hydrochloric acid and lyophilized to afford the corresponding di-hydrochloride salt. Recrystallization (EtOH) afforded compound **56** as a white solid (1.64 g, 3.04 mmol, 23%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 2.27-2.35 (2H, m), 2.78 (6H, s), 3.18 (2H, t, *J* = 7.5 Hz), 3.26 (2H, t, *J* = 7.5 Hz), 4.59 (2H, s), 7.53 (1H, t, *J* = 8.0 Hz), 7.62 (1H, d, *J* = 8.5 Hz), 7.68 (1H, dd, *J* = 8.3 and 0.9 Hz), 7.80 (1H, dd, *J* = 7.8 and 0.9 Hz), 7.92 (2H, d, *J* = 8.5 Hz), 8.22 (2H, d, *J* = 8.5 Hz), 8.40 (1H, d, *J* = 8.5 Hz), 10.29 (1H, brs), 10.81 (1H, brs); ^{13}C NMR (100 MHz, d_6-DMSO) δ 20.5 (CH_2), 42.0 (CH_3), 43.7 (CH_2), 49.5 (CH_2), 53.6 (CH_2), 117.2 (CH), 121.3 (CH), 123.1 (CH), 123.3 (C, q, *J* = 272.7 Hz), 126.6 (CH, q, *J* = 3.2 Hz), 127.0 (CH), 127.5 (C), 128.0 (CH), 132.6 (C, q, *J* = 32.4 Hz), 133.2 (C), 137.4 (C), 137.7 (CH), 143.3 (C), 151.8 (C); ESI-MS: *m/z* calcd for $C_{22}H_{25}F_3N_4O_2S$: 466.2; found $[M+H]^+$: 467.2; RP-HPLC: T_R = 9.2 min* (purity at λ_{254nm} 99%).

For analysis a small sample of di-hydrochloride salt was converted to its free base: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.79 (2H, t, *J* = 7.0 Hz), 2.27 (6H, s), 2.43 (2H, t, *J* = 7.0 Hz), 2.81 (2H, t, *J* = 7.0 Hz), 4.10 (2H, s), 7.37-7.47 (3H, m), 7.56 (2H, d, *J* = 8.2 Hz), 7.77 (1H, dd, *J* = 7.3 and 1.5 Hz), 8.00 (2H, d, *J* = 8.2 Hz), 8.05 (1H, d, *J* = 8.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.3 (CH_2), 45.3 (CH_3), 48.2 (CH_2), 55.2 (CH_2), 58.1 (CH_2), 115.9 (CH), 121.5 (CH), 122.6 (CH), 123.2 (C, q, *J* = 273 Hz), 126.1 (CH, q, *J* = 3.0 Hz), 126.5 (CH), 127.5 (C), 127.8 (CH), 133.4 (C), 134.6 (C, q, *J* = 33 Hz), 137.0 (CH), 138.1 (C), 143.3 (C), 158.8 (C).

4.2.2. *N*-(2-(((2-Guanidinoethyl)amino)methyl)quinolin-8-yl)-4-(trifluoromethyl)benzenesulfonamide di-hydrochloride (58)[44]

A solution of compound **99** (1.0 g, 1.40 mmol) in trifluoroacetic acid-dichloromethane (30 mL, 50% v/v) was stirred at room temperature for 3 h, and the solvent removed *in vacuo*. Purification by semi-preparative reverse phase HPLC (100:0 to 50:50 A:B over 50 minutes at a flow rate of 7 mL/min, where A = water + 0.1% v/v trifluoroacetic acid and B = acetonitrile + 0.1% v/v trifluoroacetic acid), followed by lyophilization, afforded the corresponding di-trifluoroacetate salt of compound **58**, which was then dissolved in 0.1 M hydrochloric acid and lyophilized to afford compound **58** as a white solid (0.92 g, 1.40 mmol, quant.). ^{1}H NMR (500 MHz, d_4-MeOH) δ 3.51 (2H, t, *J* = 6.2 Hz), 3.84 (2H, t, *J* = 6.2 Hz), 4.69 (2H, s), 7.51 (1H, t, *J* = 8.0 Hz), 7.56 (1H, d, *J* = 8.5 Hz), 7.62 (1H, dd, *J* = 7.9 and 0.8 Hz), 7.76 (2H, d, *J* = 8.4 Hz), 7.84 (1H, dd, *J* = 7.9 and 0.8 Hz), 8.25 (2H, d, *J* = 8.3 Hz), 8.34 (1H, d, *J* = 8.5 Hz); ^{13}C NMR (125 MHz, d_4-MeOH) δ 39.0 (CH_2), 47.5 (CH_2), 52.2 (CH_2), 117.8 (CH), 121.8 (CH), 124.1 (CH), 124.7 (C, q, *J* = 272.1 Hz), 127.3 (CH, q, *J* = 3.6 Hz), 128.5 (CH), 129.4 (C), 129.5 (CH), 134.7 (C), 135.5 (C, q, *J* = 32.8 Hz), 139.1 (C), 139.5 (CH), 144.7 (C), 152.3 (C), 158.9 (C); ESI-MS: *m/z* calcd for $C_{20}H_{21}F_3N_6O_2S$: 466.1; found $[M+H]^+$: 467.1; RP-HPLC: T_R = 9.3 min* (purity at λ_{254nm} 99%).

4.2.3. *N*-(2-(((3-(Piperidin-1-yl)propyl)amino)methyl)quinolin-8-yl)-4-(trifluoromethyl)benzenesulfonamide di-hydrochloride (66)[44]

A solution of compound **98** (4.45 g, 11.7 mmol), *N*-(3-aminopropyl)piperidine (5.6 mL, 35.1 mmol) and sodium triacetoxyborohydride (4.96 g, 23.4 mmol) in dichloromethane (250 mL) was stirred at room temperature for 18 h. The mixture was then washed with an aqueous solution of phosphate buffer (0.5 M, pH 7), the separated organic layer dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. The resulting residue was then taken up in ethyl acetate and extracted with an aqueous solution of hydrochloric acid (1 M), and the separated aqueous layer further washed with ethyl acetate. The pH of the separated (product-containing) aqueous layer was then adjusted to pH 4 through the careful addition of sodium hydrogen carbonate, and the resulting solution used directly as an HPLC loading vehicle. Purification by semi-preparative reverse phase HPLC (100:0 to 50:50 A:B over 50 minutes at a flow rate of 7 mL/min, where A = water + 0.1% v/v trifluoroacetic acid and B = acetonitrile + 0.1% v/v trifluoroacetic acid), followed by lyophilization, afforded the corresponding di-trifluoroacetate salt of compound **66**, which was then dissolved in 0.1 M hydrochloric acid and lyophilized to afford the corresponding di-hydrochloride salt. Recrystallization ($EtOH/Et_2O$) afforded compound **66** as a white solid (3.41 g, 5.89 mmol, 50%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 1.38-1.44 (1H, m), 1.68-1.90 (5H, m), 2.29-2.37 (2H, m), 2.82-2.91 (2H, m), 3.14-3.23 (4H, m), 3.41-3.44 (2H, m), 4.59 (2H, t, *J* = 5.3 Hz), 7.54 (1H, t, *J* = 8.0 Hz), 7.62 (1H, d, *J* = 8.5 Hz), 7.69 (1H, dd, *J* = 8.3 and 1.0 Hz), 7.79 (1H, dd, *J* = 7.8 and 1.0 Hz), 7.94 (2H, d, *J* = 8.3 Hz), 8.22 (2H, d, *J* = 8.0 Hz), 8.42 (1H, d, *J* = 8.5 Hz), 10.20 (2H, brs), 10.77 (1H, brs), 10.80 (1H, brs); ^{13}C NMR (100 MHz, d_6-DMSO) δ 19.8 (CH_2), 21.3 (CH_2), 22.1 (CH_2), 43.9 (CH_2), 49.5 (CH_2), 51.9 (CH_2), 52.8 (CH_2), 117.1 (CH), 121.3 (CH), 123.1 (CH), 123.3 (C, q, *J* = 273.0 Hz), 126.6 (CH, q, *J* = 3.4 Hz), 127.0 (CH), 127.5 (C), 128.0 (CH), 132.6 (C, q, *J* = 32.2 Hz), 133.2 (C), 137.4 (C), 137.7 (CH), 143.3 (C), 151.8 (C); ESI-MS: *m/z* calcd for $C_{25}H_{29}F_3N_4O_2S$: 506.2; found $[M+H]^+$: 507.2; RP-HPLC: T_R = 9.2 min* (purity at λ_{254nm} 99%).

For analysis a small sample of di-hydrochloride salt was converted to its free base: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40-1.50 (2H, m), 1.58-1.69 (4H, m), 1.86-1.95 (2H, m), 2.58-2.73 (6H, m), 2.86 (2H, t, *J* = 6.9 Hz), 4.12 (2H, s), 7.36-7.46 (3H, m), 7.56 (2H, d, *J* = 8.2 Hz), 7.74 (1H, dd, *J* = 7.3 and 1.5 Hz), 8.01 (2H, d, *J* = 8.2 Hz), 8.05 (1H, d, *J* = 8.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.6 (CH_2), 24.9 (CH_2), 25.0 (CH_2), 47.7 (CH_2), 54.2 (CH_2), 54.5 (CH_2), 57.0 (CH_2), 115.7 (CH), 121.3 (CH), 122.4 (CH), 123.2 (C, q, *J* = 273 Hz), 126.1 (CH, q, *J* = 3.0 Hz), 126.5 (CH), 127.5 (C), 127.8 (CH), 133.5 (C), 134.4 (C, q, *J* = 33 Hz), 137.1 (CH), 138.0 (C), 143.4 (C), 157.8 (C).

4.2.4. *N*-(2-Methylquinolin-8-yl)-4-(trifluoromethyl)benzenesulfonamide (97)[44]

A solution of 8-amino-2-methylquinoline (**96**) (10.0 g, 63.2 mmol), 4-(trifluoromethyl)benzenesulfonyl chloride (17.0 g, 69.5 mmol) and triethylamine (10.5 mL, 75.8 mmol) in dichloromethane (100 mL) was stirred at room temperature for 18 h. The mixture was then diluted with water (100 mL), the pH adjusted to pH 6-7 using aqueous phosphate buffer solution (0.5 M, pH 7) and the separated aqueous layer further extracted with dichloromethane (2 x 50 mL). The combined organic layers were washed with aqueous phosphate buffer solution (0.5 M, pH 7) (50 mL), dried over anhydrous magnesium sulfate, filtered and the filtrate concentrated *in vacuo* to afford compound **97** as a pale-grey solid (22.4 g), which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.67 (3H, s), 7.27 (1H, d, *J* = 8.4 Hz), 7.37 (1H, t, *J* = 7.8 Hz), 7.45 (1H, dd, *J* =

8.3 and 1.4 Hz), 7.60 (2H, d, *J* = 8.4 Hz), 7.79 (1H, dd, *J* = 7.4 and 1.5 Hz), 7.96 (1H, d, *J* = 8.4 Hz), 8.01 (2H, d, *J* = 8.5 Hz), 9.31 (1H, brs); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.2 (CH_3), 115.7 (CH), 122.7 (CH), 123.1 (CH), 123.2 (C, q, *J* = 273.1 Hz), 125.8 (CH), 126.1 (CH, q, *J* = 3.7 Hz), 126.5 (C), 127.8 (CH), 132.6 (C), 134.5 (C, q, *J* = 33.2 Hz), 136.5 (CH), 138.1 (C), 143.1 (C), 158.3 (C); ESI-MS: *m/z* calcd for $C_{17}H_{13}F_3N_2O_2S$: 366.1; found $[M+H]^+$: 367.1.

4.2.5. *N*-(2-Formylquinolin-8-yl)-4-(trifluoromethyl)benzenesulfonamide (98)[44]

A solution of compound **97** (22.4 g) and selenium dioxide (7.45 g, 67.2 mmol) in 1,4-dioxane (400 mL) was heated at 85 ºC for 10 h. The mixture was allowed to cool to room temperature then filtered through Celite®, and the filtrate concentrated *in vacuo*. Purification by flash chromatography (petroleum ether/ethyl acetate, 4:1→2:1→1:1) afforded compound **98** as an off-white solid (18.50 g, 48.6 mmol, 77% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57-7.63 (2H, m), 7.67 (2H, d, *J* = 8.4 Hz), 7.94 (1H, dd, *J* = 6.8 and 2.1 Hz), 8.06 (1H, d, *J* = 8.3 Hz), 8.07 (2H, d, *J* = 8.3 Hz), 8.30 (1H, dd, *J* = 8.4 and 0.7 Hz), 9.20 (1H, brs), 10.20 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 116.2 (CH), 118.5 (CH), 122.8 (CH), 123.1 (C, q, *J* = 272.5 Hz), 126.4 (CH, q, *J* = 3.7 Hz), 127.9 (CH), 129.9 (CH), 130.4 (C), 134.2 (C), 135.0 (C, q, *J* = 33.1 Hz), 138.1 (CH), 142.9 (C), 144.7 (C), 151.1 (C), 192.5 (CH); ESI-MS: *m/z* calcd for $C_{17}H_{11}F_3N_2O_3S$: 380.0; found $[M+H]^+$: 381.0.

4.2.6. *N*-(2-(((*N*,*N*'-Bis-Boc-2-Guanidinoethyl)amino)methyl)quinolin-8-yl)-4-(trifluoromethyl)benzenesulfonamide (99)[44]

A similar procedure to that described for the preparation of compound **6** was followed using compound **98** (1.0 g, 2.60 mmol), compound **109** (2.34 g, 7.80 mmol) and sodium triacetoxyborohydride (1.10 g, 5.20 mmol) in 1,2-dichloroethane (50 mL). Purification by flash chromatography (dichloromethane/methanol, 40:1→30:1→20:1) afforded compound **99** as a pale-yellow solid (1.20 g, 1.70 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (9H, s), 1.49 (9H, s), 2.88 (2H, t, *J* = 5.9 Hz), 3.54-3.60 (2H, m), 4.08 (2H, s), 7.37-7.48 (2H, m), 7.57-7.63 (3H, m), 7.80 (1H, dd, *J* = 7.4 and 1.6 Hz), 8.00 (2H, d, *J* = 8.1 Hz), 8.03 (1H, d, *J* = 8.6 Hz), 8.71-8.74 (1H, m), 11.49 (1H, brs); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2 (CH_3), 28.4 (CH_3), 40.5 (CH_2), 48.0 (CH_2), 55.0 (CH_2), 79.4 (C), 83.2 (C), 116.0 (CH), 121.6 (CH), 122.8 (CH), 123.2 (C, q, *J* = 272.7 Hz), 126.1 (CH, q, *J* = 3.0 Hz), 126.4 (CH), 127.4 (C), 127.8 (CH), 132.9 (C), 134.5 (C, q, *J* = 33.0 Hz), 136.9 (CH), 137.9 (C), 143.1 (C), 153.3 (C), 156.4 (C), 159.5 (C), 163.6 (C); ESI-MS: *m/z* calcd for $C_{30}H_{37}F_3N_6O_6S$: 666.2; found $[M+H]^+$: 667.2.

5. Acknowledgement

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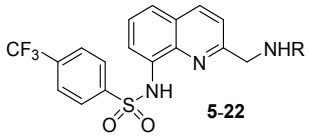
Appendix A. Supplementary data

Supplementary data to this article can be found online at

Table 1: Antibacterial activity (MIC) of compounds **5-22** against *S. uberis* ATCC 19436, *S. aureus* ATCC 6538 and *E. coli* ATCC 10536, in both the presence and absence of 50 μM $ZnSO_4$.

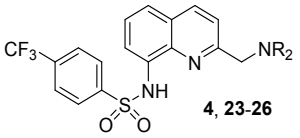


5-22

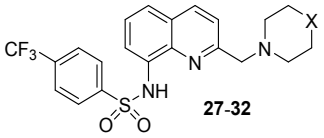
			S. uberis MIC (μg/mL)		*S. aureus* MIC (μg/mL)		*E. coli* MIC (μg/mL)	
Compound	R	% Yield[a]	+Zinc (50 μM)	No Zinc	+Zinc (50 μM)	No Zinc	+Zinc (50 μM)	No Zinc
5	H	-[b]	0.5	≥16	4	8	≥64	≥64
6	Me	62	0.25	≥16	8	16	≥64	≥64
7	Et	65	0.125	≥16	≥32	≥32	≥64	≥64
8	^{n}Pr	73	0.125	≥16	≥32	≥32	≥64	≥64
9	^{i}Pr	62	0.125	≥16	≥32	≥32	≥64	≥64
10	^{n}Bu	66	0.125	≥16	≥32	≥32	≥64	≥64
11	^{i}Bu	63	0.125	≥16	≥32	≥32	≥64	≥64
12	^{sec}Bu	28	0.125	≥16	≥32	≥32	≥64	≥64
13	^{t}Bu	65	0.25	≥16	≥32	≥32	≥64	≥64
14	^{c}Pr	61	0.25	≥16	≥32	≥32	≥64	≥64
15	^{c}Pn	67	0.125	≥16	≥32	≥32	≥64	≥64
16	^{c}Hex	12	0.125	≥16	≥32	≥32	≥64	≥64
17	$CH_2{}^{c}Pr$	73	0.25	≥16	≥32	≥32	≥64	≥64
18	$CH_2{}^{c}Hex$	45	0.125	≥16	≥32	≥32	≥64	≥64
19	CH_2CH_2F	42	0.25	≥16	≥32	≥32	≥64	≥64
20	CH_2CHF_2	42	0.25	≥16	≥32	≥32	≥64	≥64
21	$CH_2CH{=}CH_2$	53	0.125	≥16	≥32	≥32	≥64	≥64
22	$CH_2C{\equiv}CH$	57	0.125	≥16	≥32	≥32	≥64	≥64
Penicillin	-	-	0.125	0.125	0.015	0.015	6.25[c]	6.25[c]

[a] In conjunction with Scheme 1 (unless stated otherwise); [b] See Scheme 3; [c] Against *E. coli* a 1:1 mixture of penicillin/streptomycin was used as a positive control.

Table 2: Antibacterial activity (MIC) of compounds **4**, **23-32** against *S. uberis* ATCC 19436, *S. aureus* ATCC 6538 and *E. coli* ATCC 10536, in both the presence and absence of 50 μM $ZnSO_4$.



4, **23-26**

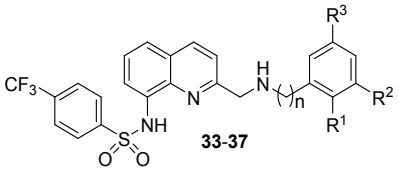


27-32

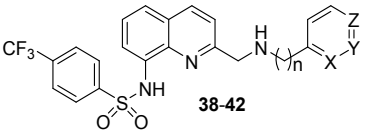
				S. uberis MIC (μg/mL)		*S. aureus* MIC (μg/mL)		*E. coli* MIC (μg/mL)	
Compound	R	X	% Yield[a]	+Zinc (50 μM)	No Zinc	+Zinc (50 μM)	No Zinc	+Zinc (50 μM)	No Zinc
4[34]	Me	-	-	0.125	≥16	4	≥32	≥64	≥64
23	Et	-	77	0.125	≥16	≥32	≥32	≥64	≥64
24	^{n}Pr	-	19	0.125	≥16	≥32	≥32	≥64	≥64
25	^{i}Pr	-	30	8	≥16	≥32	≥32	≥64	≥64
26	CH_2(2-pyridine)	-	42	4	≥16	≥32	≥32	≥64	≥64
27	-	-	33	0.125	≥16	≥32	≥32	≥64	≥64
28	-	CH_2	73	0.125	≥16	≥32	≥32	≥64	≥64
29	-	CH_2CH_2	86	0.125	≥16	≥32	≥32	≥64	≥64
30	-	O	69	0.25	≥16	≥32	≥32	≥64	≥64
31	-	S	42	0.125	≥16	≥32	≥32	≥64	≥64
32	-	NMe	61	0.5	≥16	≥32	≥32	≥64	≥64
Penicillin	-	-	-	0.125	0.125	0.015	0.015	6.25[b]	6.25[b]

[a] In conjunction with Scheme 1; [b] Against *E. coli* a 1:1 mixture of penicillin/streptomycin was used as a positive control.

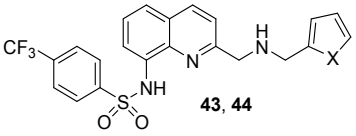
Table 3: Antibacterial activity (MIC) of compounds **33**-**44** against *S. uberis* ATCC 19436, *S. aureus* ATCC 6538 and *E. coli* ATCC 10536, in both the presence and absence of 50 μM $ZnSO_4$.



33-**37**



38-**42**

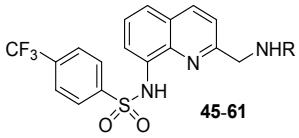


43, **44**

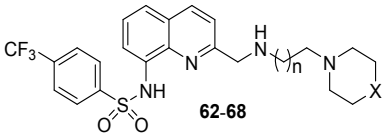
									S. uberis MIC (μg/mL)		*S. aureus* MIC (μg/mL)		*E. coli* MIC (μg/mL)	
Compound	R^1	R^2	R^3	X	Y	Z	n	% Yield[a]	+Zinc (50 μM)	No Zinc	+Zinc (50 μM)	No Zinc	+Zinc (50 μM)	No Zinc
33	H	H	H	-	-	-	0	19	0.25	≥16	≥32	≥32	≥64	≥64
34	OH	H	H	-	-	-	0	30	0.5	8	4	4	≥64	≥64
35	OH	H	NO_2	-	-	-	0	15	1	8	4	8	≥64	≥64
36	OH	Cl	Cl	-	-	-	0	38	0.5	2	2	2	≥64	≥64
37	H	H	H	-	-	-	1	46	0.125	≥16	≥32	≥32	≥64	≥64
38	-	-	-	N	CH	CH	0	15	0.5	≥16	≥32	≥32	≥64	≥64
39	-	-	-	N	CH	CH	1	69	1	2	4	4	≥64	32
40	-	-	-	CH	N	CH	1	46	4	≥16	≥32	≥32	≥64	≥64
41	-	-	-	CH	CH	N	1	34	4	≥16	≥32	≥32	≥64	≥64
42	-	-	-	N	CH	CH	2	57	0.125	≥16	≥32	≥32	≥64	≥64
43	-	-	-	O	-	-	-	50	0.25	≥16	≥32	≥32	≥64	≥64
44	-	-	-	S	-	-	-	53	0.125	≥16	≥32	≥32	≥64	≥64
Penicillin	-	-	-	-	-	-	-	-	0.125	0.125	0.015	0.015	6.25[b]	6.25[b]

[a] In conjunction with Scheme 1; [b] Against *E. coli* a 1:1 mixture of penicillin/streptomycin was used as a positive control.

Table 4: Antibacterial activity (MIC) of compounds **45-68** against *S. uberis* ATCC 19436, *S. aureus* ATCC 6538 and *E. coli* ATCC 10536, in both the presence and absence of 50 μM $ZnSO_4$.



45-61

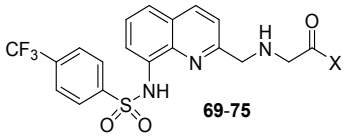


62-68

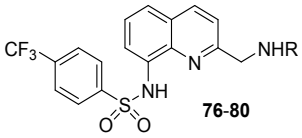
					S. uberis MIC (μg/mL)		*S. aureus* MIC (μg/mL)		*E. coli* MIC (μg/mL)	
Compound	R	X	n	% Yield[a]	+Zinc (50 μM)	No Zinc	+Zinc (50 μM)	No Zinc	+Zinc (50 μM)	No Zinc
45	CH_2CH_2OH	-	-	58	0.5	≥16	≥32	≥32	≥64	≥64
46	$CH_2CH_2CH_2OH$	-	-	50	0.125	≥16	≥32	≥32	≥64	≥64
47	$CH_2CH_2OCH_2CH_2OH$	-	-	65	1	≥16	≥32	≥32	≥64	≥64
48	$(CH_2CH_2O)_2CH_2CH_2OH$	-	-	57	1	≥16	≥32	≥32	≥64	≥64
49	$CH_2CH(OH)CH_2OH$	-	-	50	≥16	≥16	≥32	≥32	≥64	≥64
50	$CH(CH_2OH)_2$	-	-	38	≥16	≥16	≥32	≥32	≥64	≥64
51	$C(CH_2OH)_3$	-	-	26	≥16	≥16	≥32	≥32	≥64	≥64
52	CH_2CH_2OMe	-	-	57	0.25	≥16	≥32	≥32	≥64	≥64
53	$CH_2CH_2OCH_2CH_2OMe$	-	-	61	1	≥16	≥32	≥32	≥64	≥64
54	$(CH_2CH_2O)_2CH_2CH_2OMe$	-	-	53	1	≥16	≥32	≥32	≥64	≥64
55	$CH_2CH_2NMe_2$	-	-	50	1	8	16	16	≥64	≥64
56[b]	$CH_2CH_2CH_2NMe_2$	-	-	23	0.5	4	16	16	16	16
57	CH_2CH_2NHAc	-	-	65	1	≥16	≥32	≥32	≥64	≥64
58[b]	$CH_2CH_2NHC(=NH)NH_2$	-	-	-[a]	4	8	8	8	16	16
59	$CH_2CH_2NHC(=NNO_2)NH_2$	-	-	57	4	≥16	≥32	≥32	≥64	≥64
60	$CH_2CH_2NHSO_2Me$	-	-	46	0.5	≥16	≥32	≥32	≥64	≥64
61	$CH_2CH_2NHSO_2Ph$	-	-	38	0.5	≥16	≥32	≥32	≥64	≥64
62	-	CH_2	1	53	0.5	8	16	16	≥64	≥64
63	-	O	1	61	1	≥16	≥32	≥32	≥64	≥64
64	-	S	1	50	1	≥16	≥32	≥32	≥64	≥64
65[c]	-	NMe	1	23	4	≥16	≥32	≥32	≥64	≥64
66[b]	-	CH_2	2	50	2	4	16	16	16	16
67	-	O	2	69	2	≥16	≥32	≥32	≥64	≥64
68[c]	-	NMe	2	23	4	≥16	≥32	≥32	≥64	≥64
Penicillin	-	-	-	-	0.125	0.125	0.015	0.015	6.25[d]	6.25[d]

[a] In conjunction with/see Scheme 1; [b] As its di-hydrochloride salt; [c] As its di-trifluoroacetate salt; [d] Against *E. coli* a 1:1 mixture of penicillin/streptomycin was used as a positive control.

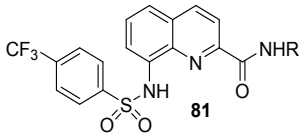
Table 5: Antibacterial activity (MIC) of compounds **69-81** against *S. uberis* ATCC 19436, *S. aureus* ATCC 6538 and *E. coli* ATCC 10536, in both the presence and absence of 50 μM $ZnSO_4$.



69-75



76-80

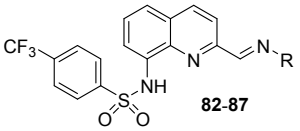


81

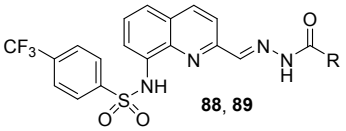
			S. uberis MIC (μg/mL)		*S. aureus* MIC (μg/mL)		*E. coli* MIC (μg/mL)	
Compound	X or R	% Yield	+Zinc (50 μM)	No Zinc	+Zinc (50 μM)	No Zinc	+Zinc (50 μM)	No Zinc
69	O^{t}Bu	-[a]	0.5	≥16	≥32	≥32	≥64	≥64
70[e]	OH	-[a]	≥16	≥16	≥32	≥32	≥64	≥64
71[e]	NH_2	-[b]	≥16	≥16	≥32	≥32	≥64	≥64
72[e]	NHMe	-[b]	≥16	≥16	≥32	≥32	≥64	≥64
73[f]	$NHCH_2CH_2NMe_2$	-[b]	≥16	≥16	≥32	≥32	≥64	≥64
74[f]	$NHCH_2CH_2CH_2NMe_2$	-[b]	≥16	≥16	≥32	≥32	≥64	≥64
75[e]	NHOH	-[b]	≥16	≥16	≥32	≥32	≥64	≥64
76	Ac	-[c]	0.125	≥16	≥32	≥32	≥64	≥64
77	$C(=O)CH_2NMe_2$	-[c]	0.125	≥16	≥32	≥32	≥64	≥64
78[e]	$C(=O)CH_2CH_2CH_2NMe_2$	-[c]	≥16	≥16	≥32	≥32	≥64	≥64
79[e]	$C(=NH)NH_2$	-[c]	0.5	≥16	8	8	16	32
80	$C(=NNO_2)NH_2$	-[c]	0.5	≥16	≥32	≥32	≥64	≥64
81[e]	$CH_2CH_2CH_2NMe_2$	-[d]	≥16	≥16	≥32	≥32	≥64	≥64
Penicillin	-	-	0.125	0.125	0.015	0.015	6.25[g]	6.25[g]

[a] See Scheme 1; [b] See Scheme 2; [c] See Scheme 3; [d] See Scheme 4; [e] As its trifluoroacetate salt; [f] As its di-trifluoroacetate salt; [g] Against *E. coli* a 1:1 mixture of penicillin/streptomycin was used as a positive control.

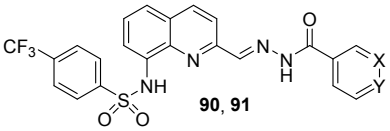
Table 6: Antibacterial activity (MIC) of compounds **82-95** against *S. uberis* ATCC 19436, *S. aureus* ATCC 6538 and *E. coli* ATCC 10536, in both the presence and absence of 50 μM $ZnSO_4$.



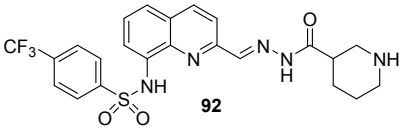
82-87



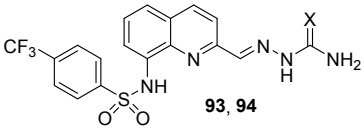
88, **89**



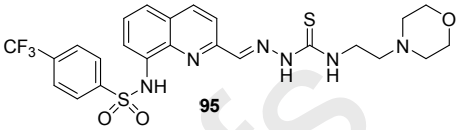
90, **91**



92



93, **94**



95

					S. uberis MIC (μg/mL)		*S. aureus* MIC (μg/mL)		*E. coli* MIC (μg/mL)	
Compound	R	X	Y	% Yield[a]	+Zinc (50 μM)	No Zinc	+Zinc (50 μM)	No Zinc	+Zinc (50 μM)	No Zinc
82	OH	-	-	76	0.125	8	4	4	≥64	≥64
83	OMe	-	-	69	0.125	≥16	≥32	≥32	≥64	≥64
84	OCH_2Ph	-	-	57	0.125	≥16	≥32	≥32	≥64	≥64
85	NH_2	-	-	61	1	≥16	16	16	≥64	≥64
86	NHMe	-	-	65	0.5	≥16	≥32	≥32	≥64	≥64
87	$NHCH_2Ph$	-	-	53	0.5	≥16	≥32	≥32	≥64	≥64
88	Me	-	-	76	0.5	≥16	16	16	≥64	≥64
89	Ph	-	-	77	0.125	≥16	≥32	≥32	≥64	≥64
90	-	N	CH	65	2	≥16	16	16	≥64	≥64
91	-	CH	N	61	2	≥16	16	16	≥64	≥64
92[b]	-	-	-	-[a]	≥16	≥16	16	16	≥64	≥64
93	-	O	-	73	2	≥16	8	16	≥64	≥64
94	-	S	-	71	1	4	4	4	≥64	≥64
95	-	-	-	61	0.125	4	4	4	≥64	≥64
Penicillin	-	-	-	-	0.125	0.125	0.015	0.015	6.25[c]	6.25[c]

[a] In conjunction with/see Scheme 5; [b] As its trifluoroacetate salt; [c] Against *E. coli* a 1:1 mixture of penicillin/streptomycin was used as a positive control.

Table 7: Bactericidal activity (MBC, ≥3log$_{10}$ CFU/mL reduction) of compounds **56**, **58** and **66** against *S. uberis* ATCC 19436, *S. aureus* ATCC 6538 and *E. coli* ATCC 10536, in both the presence and absence of 50 μM $ZnSO_4$.

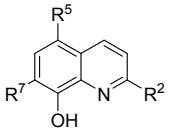
	S. uberis MBC (μg/mL)		*S. aureus* MBC (μg/mL)		*E. coli* MBC (μg/mL)	
Compound	+Zinc (50 μM)	No Zinc	+Zinc (50 μM)	No Zinc	+Zinc (50 μM)	No Zinc
56	0.5	8	16	16	16	16
58	8	8	16	16	32	32
66	4	8	16	16	16	16
Penicillin	0.06	0.03	0.015	0.015	6.25[a]	6.25[a]

[a] Against *E. coli* a 1:1 mixture of penicillin/streptomycin was used as a positive control.

Table 8: Sanitizing activity (log_{10} CFU/mL reduction in 5 minutes) of compound **56**, **58** and **66** (as unoptimized formulations) against *S. uberis* ATCC 19436, *S. aureus* ATCC 6538 and *E. coli* ATCC 10536; as determined by a modified microplate quantitative suspension test (BS EN 1656:2009) for chemical disinfectants, including EN1656 controls.

		S. uberis[b]		*S. aureus*[b]		*E. coli*[b]	
Compound[a]	Concentration (mg/mL)	Log_{10} reduction (CFU/mL)	EN1656 Pass/Fail[c]	Log_{10} reduction (CFU/mL)	EN1656 Pass/Fail[c]	Log_{10} reduction (CFU/mL)	EN1656 Pass/Fail[c]
56	10	>5	Pass	>5	Pass	>5	Pass
56	5	>5	Pass	8.76, 6.76, 4.57, 3.50, 3.10	Pass/Fail	>5	Pass
56	2.5	>5	Pass	<5	Fail	>5	Pass
56	1	>5	Pass	<5	Fail	>5	Pass
56	0.5	<5	Fail	<5	Fail	<5	Fail
56	0.25	<5	Fail	<5	Fail	<5	Fail
58	10	>5	Pass	>5	Pass	>5	Pass
58	5	>5	Pass	>5	Pass	>5	Pass
58	2.5	>5	Pass	>5	Pass	>5	Pass
58	1	>5	Pass	>5	Pass	>5	Pass
58	0.5	5.84, 3.70	Pass/Fail	5.15, 3.56	Pass/Fail	<5	Fail
58	0.25	<5	Fail	<5	Fail	<5	Fail
66	10	>5	Pass	>5	Pass	>5	Pass
66	5	>5	Pass	6.98, 6.11, 4.41, 4.29	Pass/Fail	>5	Pass
66	2.5	>5	Pass	6.28, 6.22, 3.90, 3.78	Pass/Fail	>5	Pass
66	1	>5	Pass	<5	Fail	>5	Pass
66	0.5	<5	Fail	<5	Fail	<5	Fail
66	0.25	<5	Fail	<5	Fail	<5	Fail
Growth control[d]		-	Pass	-	Pass	-	Pass
Neutralizer toxicity control[e]		-	Pass	-	Pass	-	Pass
Method validation control[f]		-	Pass	-	Pass	-	Pass

[a] Compounds were formulated in a solvent mixture composed of 35% (w/v) glycerol, 5% (w/v) nonionic surfactant mixture (Lutensol XL80, Ecoteric LA8N and Softanol 90), 0.66% (w/v) ethanol and 0.021% (w/v) Ponceau 4R dye – a formulation which was, without compound, previously demonstrated to be devoid of antibacterial activity; [b] Initial bacteria count at OD_{625} 0.3 equates to approximately 3.45×10^8 CFU/mL (data are representative of at least a biological duplicate); [c] >5log_{10} CFU/mL reduction in 5 minutes equates to a Pass according to BS EN 1656:2009; [d] Growth control: milk and bacteria only (i.e. without compound and neutralizers), to ensure that the presence of milk does not inhibit the growth of bacteria; [e] Neutralizer toxicity control: neutralizer and bacteria only (i.e. without compound and milk), to ensure neutralizer does not inhibit the growth of bacteria; [f] Method validation control: bacteria are added post compound neutralization, to ensure that the neutralizer acts against the compound, and that bacteria can grow in the presence of the compound.



1 $R^2 = R^5 = R^7 = H$
2 $R^2 = H$, $R^5 = Cl$, $R^7 = I$
3 $R^2 = CH_2NMe_2$, $R^5 = R^7 = Cl$

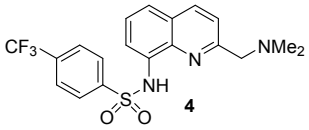


Figure 1. Known examples of 8-hydroxyquinolines exhibiting antibacterial activity (compounds **1**-**3**), and recently reported zinc-dependent antibacterial 8-sulfonamidoquinoline **4**.[34]

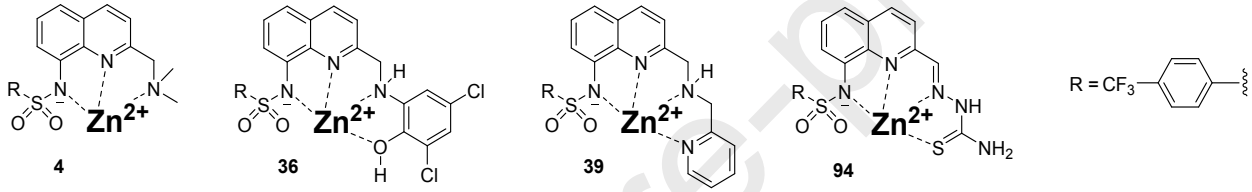
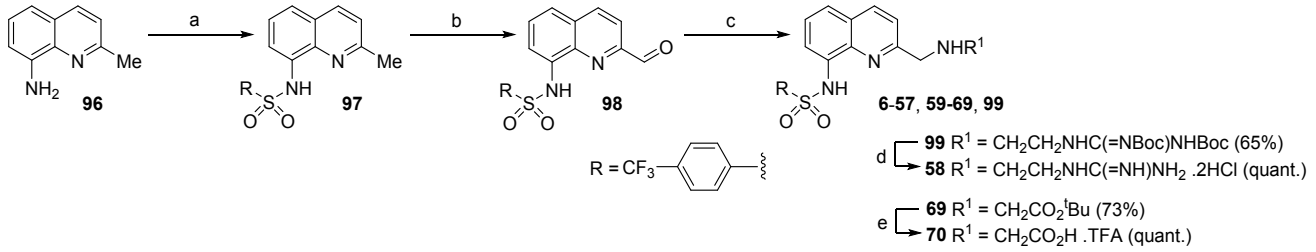
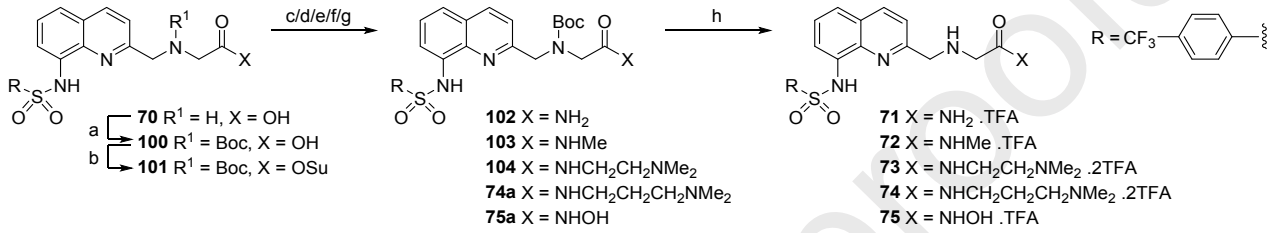


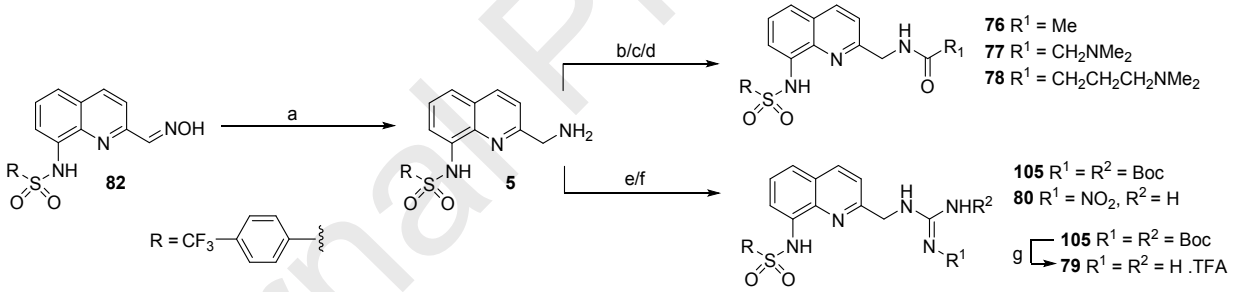
Figure 2. Postulated examples of C-2 derivatized 8-sulfonamidoquinolines (compounds **36**-Zn^{2+}, **39**-Zn^{2+} and **94**-Zn^{2+}) capable of forming tetradentate complexes with zinc (cf. compound **4**-Zn^{2+}, as an example of a compound which can only form a tridentate complex with zinc).



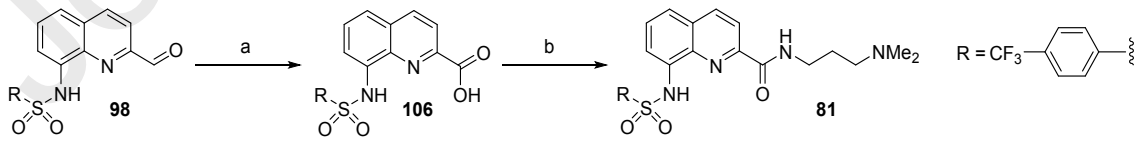
Scheme 1. Reagents and conditions: (a) 4-(trifluoromethyl)benzenesulfonyl chloride, Et_3N, DCM, RT, 18 h; (b) SeO_2, 1,4-dioxane, 85 °C, 10 h, 77% (over 2 steps); (c) Substituted amine (see experimental section for details), $NaBH(OAc)_3$, Et_3N (in some examples), 1,2-DCE or DCM, RT, 18 h (see Tables 1-4 for yields); (d) i) trifluoroacetic acid, DCM, RT, 3 h, then ii) 0.1 M HCl; (e) trifluoroacetic acid, DCM, RT, 36 h.



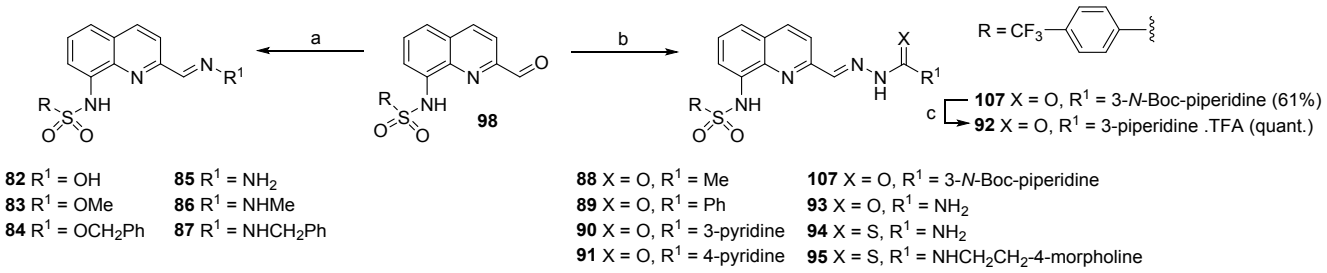
Scheme 2. Reagents and conditions: (a) di-*tert*-butyl dicarbonate, Et_3N, DMF, RT, 18 h, 79%; (b) HOSu, EDCI.HCl, DCM-DMF, RT, 18 h, 71%; (c) **101**, methanolic NH_3, DCM, RT, 18 h, 26% (to give **102**); (d) **101**, methylamine (2.0 M in THF), DCM, RT, 18 h, 66% (to give **103**); (e) **101**, *N,N*-dimethylethylenediamine, DCM, RT, 18 h, 26% (to give **104**); (f) **101**, 3-(dimethylamino)-1-propylamine hydrochloride, DCM, RT, 18 h (to give **74a**, not isolated); (g) **101**, $H_2NOH.HCl$, K_2CO_3, THF-water (1:1 v/v), RT, 18 h (to give **75a**, not isolated); (h) trifluoroacetic acid, DCM, RT, 3 h, quant. (33% over steps f and h for compound **74**, 20% over steps g and h for compound **75**).



Scheme 3. Reagents and conditions: (a) H_2, Pd-C (10 wt.%), MeOH, RT, 18 h, 64%; (b) acetyl chloride, DCM, RT, 18 h, 53% (to give **76**); (c) *N,N*-dimethylglycine hydrochloride, EDCI.HCl, Et_3N, DMF, RT, 18 h, 46% (to give **77**); (d) 4-(dimethylamino)butyric acid hydrochloride, EDCI.HCl, Et_3N, DMF, RT, 18 h, 38% (to give **78**); (e) *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamidine, THF, RT, 18 h, 85% (to give **105**); (f) *N*-nitro-*S*-methylisothiourea, EtOH, 40 °C, 3 d, 69% (to give **80**); (g) trifluoroacetic acid, DCM, RT, 3 h, quant.



Scheme 4. Reagents and conditions: (a) $KMnO_4$, acetone, RT, 4 h, 53%; (b) 3-(dimethylamino)-1-propylamine hydrochloride, EDCI.HCl, Et_3N, DCM, RT, 18 h, 15%.



Scheme 5. Reagents and conditions: (a) Substituted hydroxylamine/hydrazine (see experimental section for details), NaOAc (optional), EtOH or aq. EtOH, 70 °C, 18 h (see Table 6 for yields); (b) Substituted acylhydrazine/semicarbazide/thiosemicarbazide (see experimental section for details), EtOH or aq. EtOH, 70 °C, 18 h (see Table 6 for yields); (c) trifluoroacetic acid, DCM, RT, 3 h.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical Abstract

C-2 Derivatized 8-sulfonamidoquinolines as antibacterial compounds, Emma K. Davison, John E. McGowan, Freda F. Li, Andrew D. Harper, Joo Young Jeong, Sonya Mros, Nichaela Harbison-Price, Essie M. Van Zuylen, Melanie K. Knottenbelt, Adam Heikal, Scott A. Ferguson, Greg M. Cook, Woravimol Krittaphol, Greg F. Walker, Margaret A. Brimble, David Rennison