Synthesis of Novel Antibacterial Agents Targeting Bacterial Topoisomerases: 8-Methoxy & 8-Chloro Fluoroquinolones

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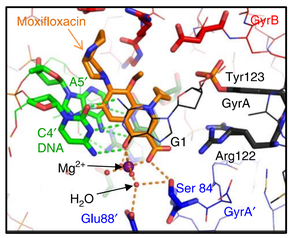
Introduction

Bacterial resistance to currently available antimicrobial agents continues to be a growing threat to public health. The CDC recently reported that each year in the United States at least 2 million people acquire serious infections caused by bacteria resistant to one or more antibacterial agents, with 23,000 of them dying as a direct result.[1] Many more die from underlying medical conditions that are exacerbated by these difficult-to-treat infections. Multidrug-resistant (MDR) strains of the so-called 'ESKAPE' pathogens are of particular concern because of their association with considerable morbidity and mortality in the hospital setting.[2] The Gram-negative 'KAPE' organisms – *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp. – are especially problematic because of the dearth of new and effective agents found in the existing clinical development pipeline. The recent emergence of infections caused by Gram-negative pathogens such as carbapenem-resistant Enterobacteriaceae (CRE) has further increased the magnitude of the problem.[1]

The fluoroquinolones (FQs), exemplified by moxifloxacin *(vide infra),* are generally broad-spectrum antibacterial agents that have been on the market for many years and have been useful, at least in part, in treating Gram-negative infections.[3] The FQs target bacterial DNA gyrase (A subunit)[4] and topoisomerase IV (C subunit), tetrameric enzymes that can now be considered as clinically validated.



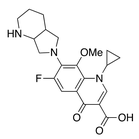
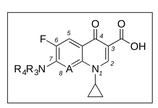


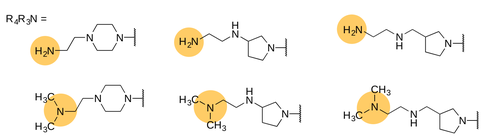
Figure 1. Binding site for moxifloxacin in *S. aureus* DNA gyrase complex.[4]

One way to potentiate the activity of antibiotics against Gram-negative bacteria is to incorporate additional basic amino groups. At physiologic pH, such groups are generally protonated. The resulting quaternary ammonium salts enhance penetration of the Gram–negative outer membrane. Polymyxin B is an exemplar in this area. We hypothesized that the Gram-negative activity of fluoroquinolones would be further enhanced by appending an additional basic amino group, highlighted below, to the usual C-7 diamine substituent.



(A = N, CH, CF, CCl, CCH_3, $COCH_3$)

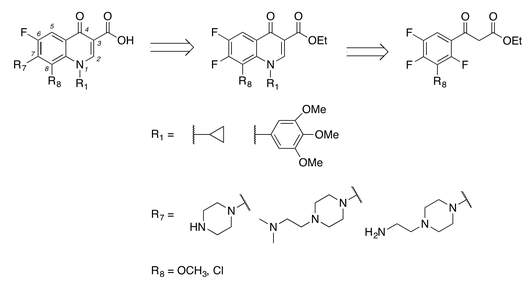
R_4R_3N =



Objectives

- Synthesize adequate supplies of N-1 substituted 8-methoxy and 8-chloro fluoroquinolone ester intermediates
- Probe most efficient pathway for amine substitution at the C-7 position
  - Direct amination through a boronate ester
  - Direct amination with triamine fragment
  - Aminoalkylation with a piperazine-substituted fluoroquinolone
- Purify and characterize compounds for testing at Walter Reed Army Institute of Research

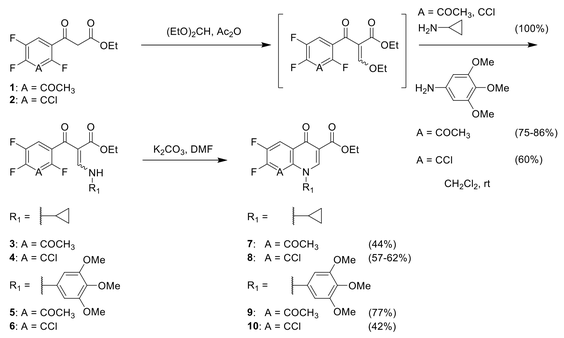
Retrosynthetic Analysis



Results: Synthesis of Fluoroquinolone Ester Intermediates

Ethyl 3-oxo-3-(2,4,5-trifluoro-3-methoxyphenyl)propanoate (**1**) and Ethyl 3-(3-chloro-2,4,5-trifluorophenyl)-3-oxopropanoate (**2**) were the starting materials. Conversion to the ethoxymethylene derivative with triethylorthoformate and acetic anhydride, followed by the addition of the cyclopropyl amine or 3,4,5-trimethoxyaniline afforded the corresponding enamine products **3**, **4**, **5**, and **6** in good yields (Scheme 1). Base induced cyclization provided the 8-methoxy and 8-chloro fluoroquinolone ester intermediates **7**, **8**, **9**, and **10**.

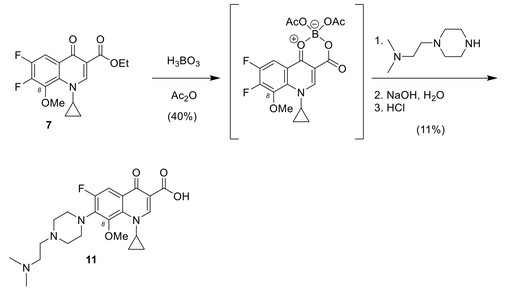
Scheme 1



Results: C-7 Nucleophilic Aromatic Substitution Pathways

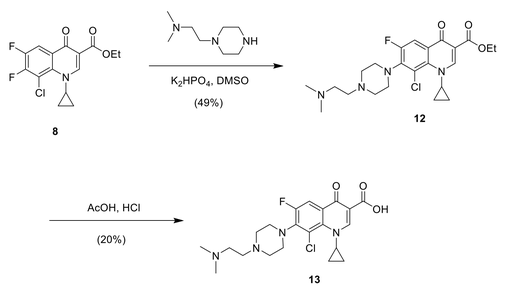
After repeated attempts at a direct amination, we quickly realized that activation of the quinolone core by conversion to the corresponding boronate ester complex would be necessary in order to predispose the 8-methoxy fluoroquinolone ester **7** towards the desired C-7 nucleophilic aromatic substitution reaction. Subsequent saponification of the boronate complex with aqueous hydroxide, followed by a pH adjustment to the neutral range with aqueous hydrochloric acid, then afforded the novel fluoroquinolone **11** in a modest, un-optimized yield of 11% (Scheme 2).

Scheme 2



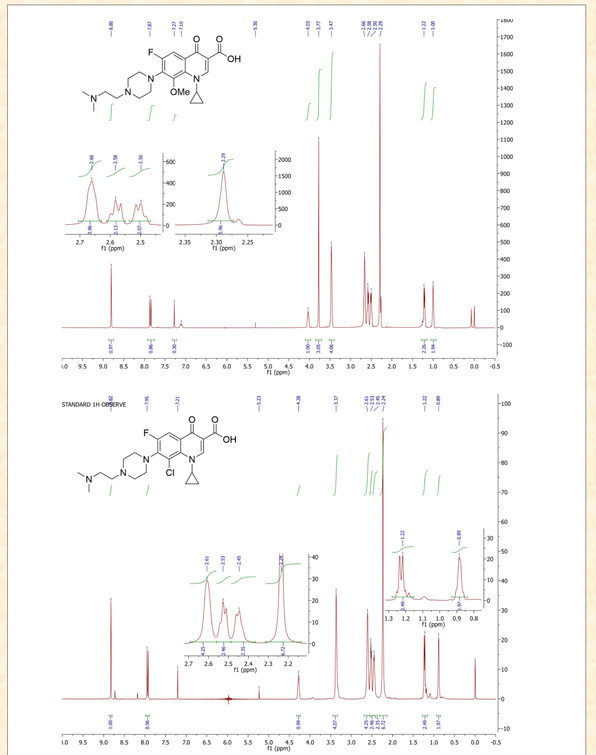
The 8-chloro fluoroquinolone series was accessed by directly reacting intermediate fluoroquinolone ester **8** with 1-[2-(dimethylamino)ethyl]piperazine to yield the penultimate C-7 substituted fluoroquinolone ester **12**. No prior activation of the quinolone beta-keto ester moiety with boronic acid was required. Straightforward acidic hydrolysis of the ester then provided the novel fluoroquinolone **13** in modest yield (Scheme 3).

Scheme 3



Further substitution reactions were also explored with fluoroquinolone esters **9** and **10**, bearing a 3,4,5-trimethoxyphenyl moiety at the N-1 position (not shown).

Results: NMR Spectra of Fluoroquinolone Final Products



Conclusions

- N-1 cyclopropyl and 3,4,5-trimethoxyphenyl analogs were synthesized and characterized.
- Initial microbiological assessment of the final products will be done at the Walter Reed Army Institute of Research (WRAIR).
- Structure-activity relationships (SAR) in this series will be evaluated once minimum inhibitory concentration (MIC) data is available.

References

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WRAIR Walter Reed Army Institute of Research Soldier Health • World Health