Synthesis of Novel Antibacterial Agents Targeting Bacterial Topoisomerases: 6,8-Difluoro Fluoroquinolones

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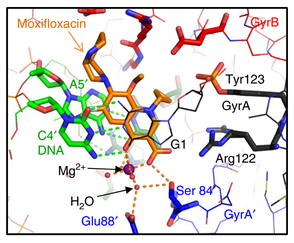
Introduction

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.



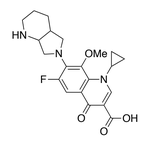
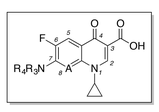


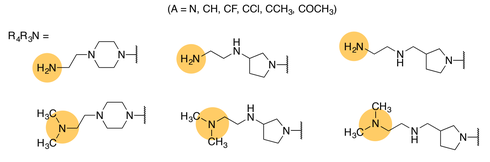
Figure 1. Binding site for moxifloxacin in *S. aureus* DNA gyrase complex.[4]

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, $COCH_3$)

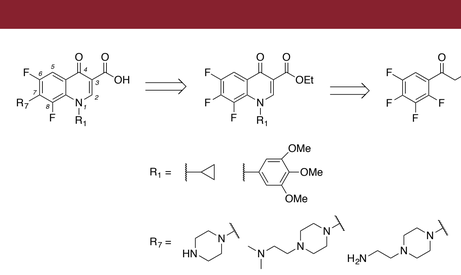
R_4R_3N =



Principal Objectives

- Generate adequate supplies of key N-1 substituted 6,7,8-trifluoroquinolone ester intermediates to explore the subsequent nucleophilic aromatic substitution reaction
- Identify the best method for introducing the desired triamino substituents to the C-7 position
  - Direct amination with a triamine fragment
  - Aminoalkylation of a diamine-substituted quinolone
- Begin exploring compounds bearing a 3,4,5-trimethoxyphenyl moiety at N-1
- Purify and characterize compounds for testing at Walter Reed Army Institute of Research

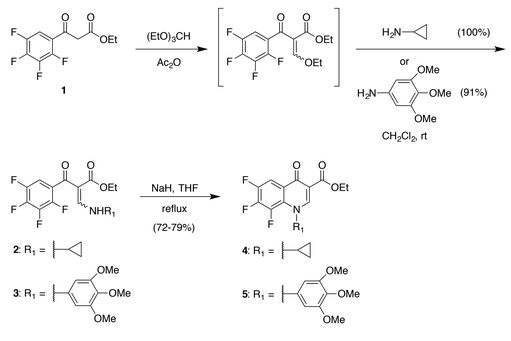
Retrosynthetic Analysis



Results: Synthesis of Quinolone Ester Intermediates

Ethyl (2,3,4,5-tetrafluorobenzoyl)acetate **(1)** was used as the starting material. Conversion to the ethoxymethylene derivative with triethylorthoformate and acetic anhydride, followed by the addition of cyclopropyl amine or 3,4,5-trimethoxyaniline, afforded the corresponding enamine products **2** and **3**, respectively, in good yields (Scheme1). Base-induced cyclization then provided the key 6,7,8-trifluoroquinolone ester intermediates **4** and **5**.

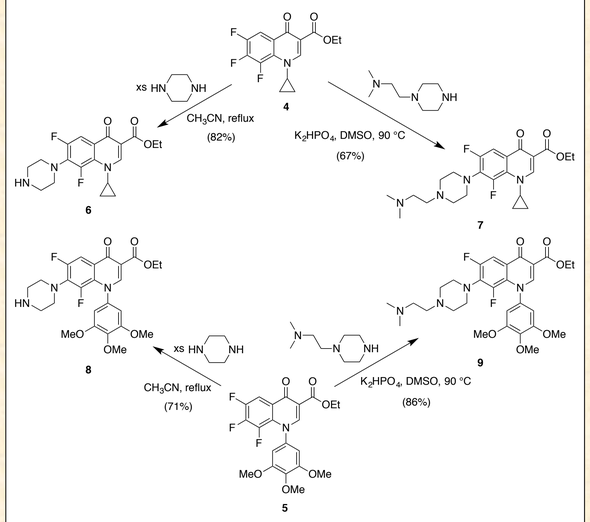
Scheme 1



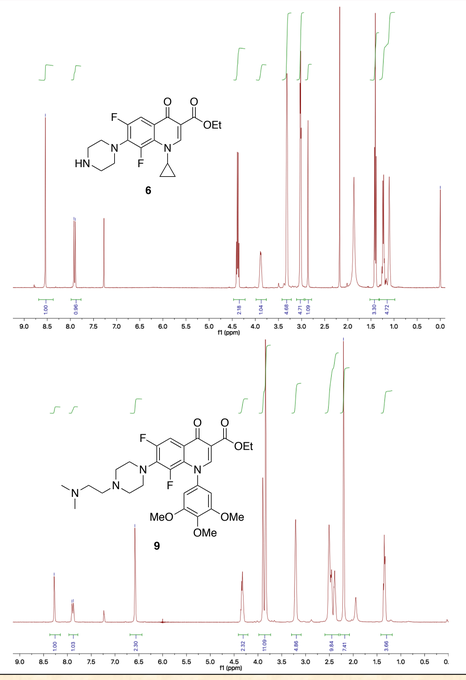
Results: C-7 Nucleophilic Aromatic Substitution

With quinolone esters **4** and **5** in hand, the stage was set for exploring the nucleophilic aromatic substitution reaction at C-7, with a wide range of amine nucleophiles within scope. We initially examined piperazine, 1-[2-(dimethylamino)ethyl]piperazine and Boc-pyrrolidine (not shown) as nucleophilic partners in this reaction. The indicated C-7 substituted quinolone esters **6**, **7**, **8** and **9** were obtained in good isolated yields (Scheme 2).

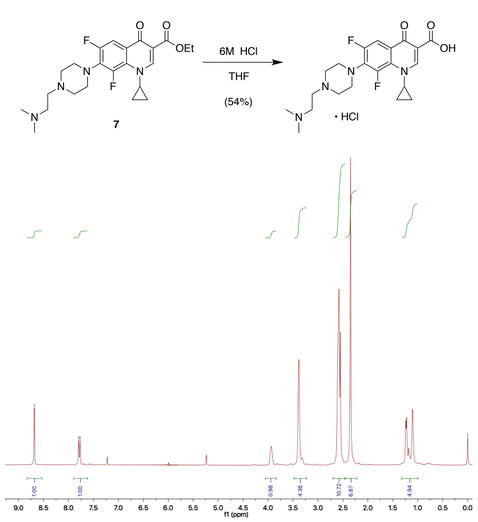
Scheme 2



Representative NMR Spectra of Quinolone Esters



Results: Ester Hydrolysis and NMR Spectrum of Fluoroquinolone Final Product



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

1. Centers for Disease Control and Prevention (CDC). Antibiotic resistance threats in the Unitied States, 2013. Atlanta:CDC;2013. Available from http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf
2. Pendleton, J.N.; Gorman, S.P.; Gilmore, B.F. *Expert Rev. Anti Infect. Ther.* **2013**,*11*, 297-308.
3. Van Bambeke, F.; Michot, J.-M.; Van Eldere, J.; Tulkens, P.M. *Clin Microbiol Infect.* **2005**, *11*, 256-80.
4. Chan, P. F.; *et al. Nat. Commun.* **2015**, *6*,10048.

Acknowledgments

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