Progress Toward the Synthesis of Novel Bacterial Topoisomerase Inhibitors Derived from Fluoroquinolones. N-1 Amine Derivatives.

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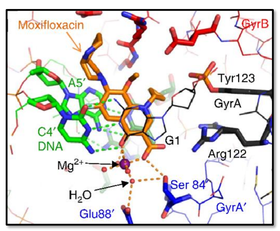
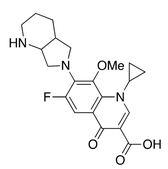
Introduction

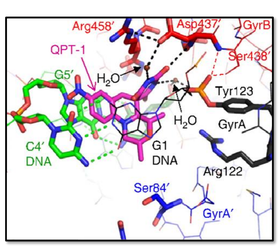
Bacterial resistance to current antibiotics is a growing problem across the globe. The CDC reported that the estimated minimum illnesses caused by antibiotic resistance in the year 2013 was over 2 million, resulting in about 23,000 deaths in the U.S.[1] In 2014, the Obama administration released an executive order mandating that research be conducted to identify solutions to the bacterial resistance problem, especially in Gram-negative bacteria. One antibiotic series that has shown some promise are the fluoroquinolones. Fluoroquinolones have been on the market for years, with a current reference example being moxifloxacin. Fluoroquinolones (FQs) work by targeting DNA gyrase and topoisomerase IV, which are clinically validated antibacterial targets. Getting antibiotics into Gram-negative bacteria is a multivariate problem. Gram-negative bacteria utilize two membranes, an outer membrane that is hydrophilic, and an inner membrane that is lipophilic. These bacteria also have antibiotic de-activating enzymes and RND efflux pumps that work to remove the antibiotics from the cell. These features make targeting of Gram-negative organisms very difficult.

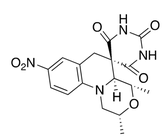
A new drug lead called QPT-1 has been shown to be active against quinolone-resistant bacteria, with low MIC values (MIC_{90} = 0.5 μg/mL) even against moxifloxacin-resistant *Staphylococcus aureus*.[2] QPT-1 works by interacting with GyrB (in red) instead of GyrA (in blue), moxifloxacin's target).[3]





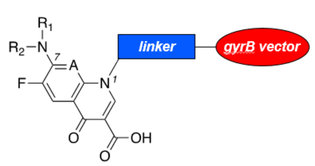
Binding site for moxifloxacin in S. aureus DNA gyrase complex





QPT-1: active against quinolone-resistant bacteria!

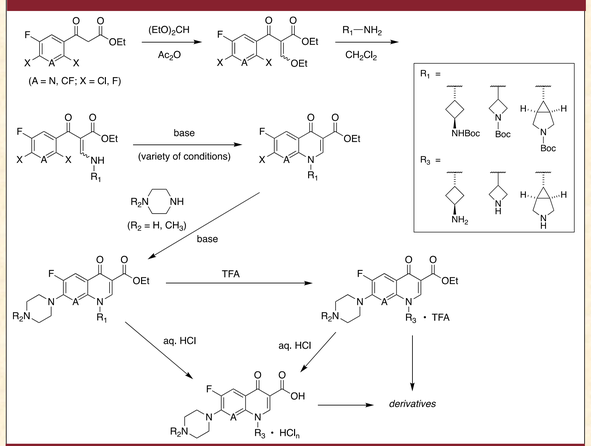
We hypothesized that adding various functional groups to different linkers attached to the *N*-1 position of a fluoroquinolone core would provide new binding interactions with the GyrB subunit and overcome pre-existing target-based fluoroquinolone resistance.



Objectives

- Design, synthesize, purify and characterize compounds for preliminary enzymatic and microbiological activity testing in Prof. Rachael Baker's lab and, eventually, at the Walter Reed Army Institute of Research (WRAIR)
- Optimize procedures for producing intermediates and final fluoroquinolone derivatives
- Identify promising residues that can be appended to the distal amino group at *N*-1 to engage the GyrB subunit of the DNA gyrase complex.

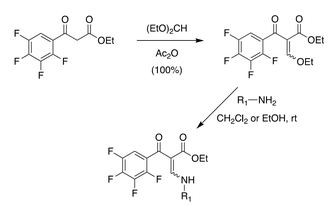
Overall Synthetic Scheme



Selected Results: Synthesis of Fluoroquinolone Ester Intermediates

Preparation of Enamine Intermediates:

- All yields 90%+

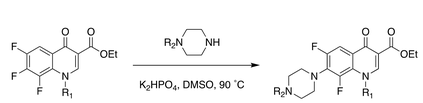


Cyclization to fluoroquinolone esters under basic conditions:

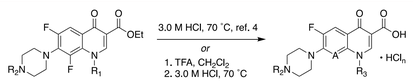
- Yields generally >75%

Addition of piperazine or *N*-methyl piperazine:

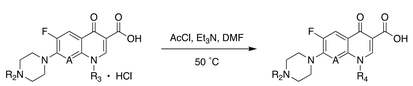
- Yields generally 80%+



Results: 7-Piperazinyl Quinolone Intermediates/Products

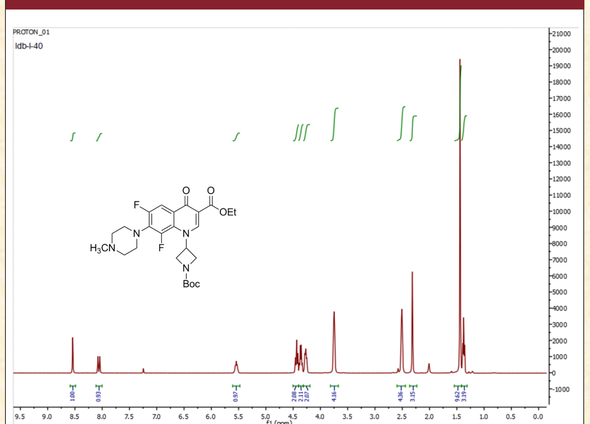


R_3	R_2	A	Yield (%)
H_2N	CH_3	CF	60
H_2N	CH_3	N	100
H_2N	H	CF	100
H_2N	H	N	100
HN	CH_3	CF	70
HN	CH_3	N	81
HN	H	CF	61
HN	H	N	100
HN	CH_3	CF	48
HN	CH_3	N	51
HN	H	CF	96
HN	H	N	58

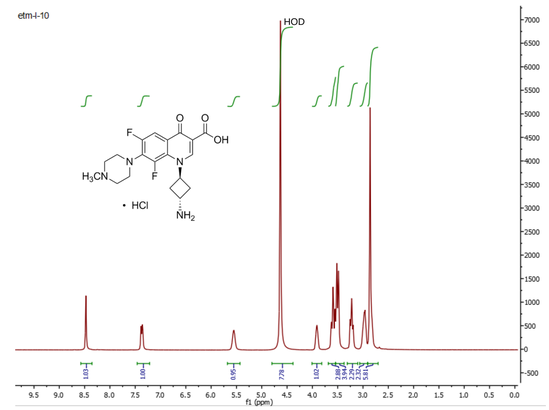


R_4	R_2	A	Yield (%)
AcHN	CH_3	CF	55
AcHN	CH_3	N	88

Results: Representative NMR Spectrum of an Ester Intermediate



Results: Representative NMR Spectrum of a Fluoroquinolone Product



Conclusions

- Advanced fluoroquinolone- and naphthyridone-based intermediates were synthesized and characterized.
- Analogues containing three different linker groups were prepared:
  - 3-aminoazetidine
  - *trans*-1,3-diaminocyclobutane
  - 6-amino-3-azabicyclo[3.1.0]hexane
- *N*-1 substituted fluoroquinolone acids are poised for further elaboration of their amino groups.
- An initial enzymatic and microbiological assessment of some synthesized products was done at Calvin College (data not shown) and will later be expanded at the Walter Reed Army Institute of Research (WRAIR).
- Structure-activity relationships (SAR) in this series will be evaluated once gyrase IC_{50} and minimum inhibitory concentration (MIC) data are 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. Miller, A. A.; *et al. Antimicrob. Agents Chemother.* **2008**, *52*, 2806-2812
3. Chan, P. F.; *et al. Nat. Commun.* **2015**, *6*, 10048.
4. Domagala, J. M.; *et al. J. Med. Chem.* **1988**, *31*, 991-1001

Acknowledgments

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