Combating Antibiotic Resistance with Novel Fluoroquinolone-Based Compounds

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Abstract

- Every year, approximately 23,000 people in the United States die from infections caused by Antibiotic Resistant (ABR) bacteria (1).
- Many ABR bacteria have developed resistance to the most commonly prescribed class of antibiotic drugs, the fluoroquinolones (FQs).
- FQs show high penetration into Gram-positive and Gram-negative bacteria, strong activity against DNA gyrase and Topoisomerase IV and specificity for bacterial enzymes over human enzymes.

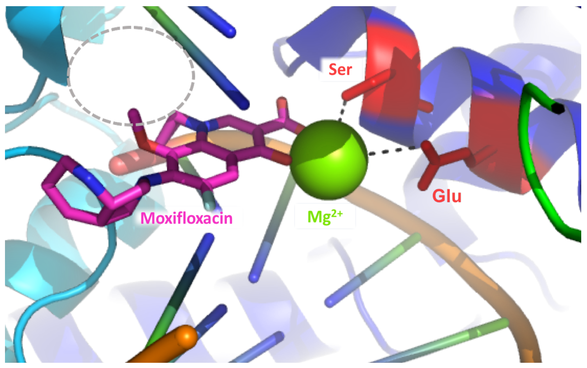


Figure 1: FQs bind to DNA Gyrase and Topoisomerase IV through a water mediated Mg^{2+} interaction. The Mg^{2+} is held in the enzyme active site through interactions with a serine and glutamate residue. Most FQ resistant strains of bacteria contain mutations in these two amino acids.

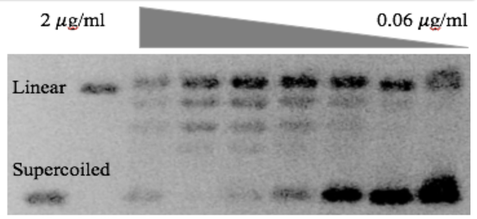
- Due to growing resistance to currently available FQs, there is a need to develop novel FQ based compounds. Ideally these compounds will form additional interactions in the enzyme active site to compensate for a possible loss of the Mg^{2+} interaction.
- The principal objectives of this project were to screen these novel FQ- based compounds to assess their efficacy via enzyme activity assays and MIC studies, and to develop modelling strategies for predicting the activity of additional novel compounds.

References

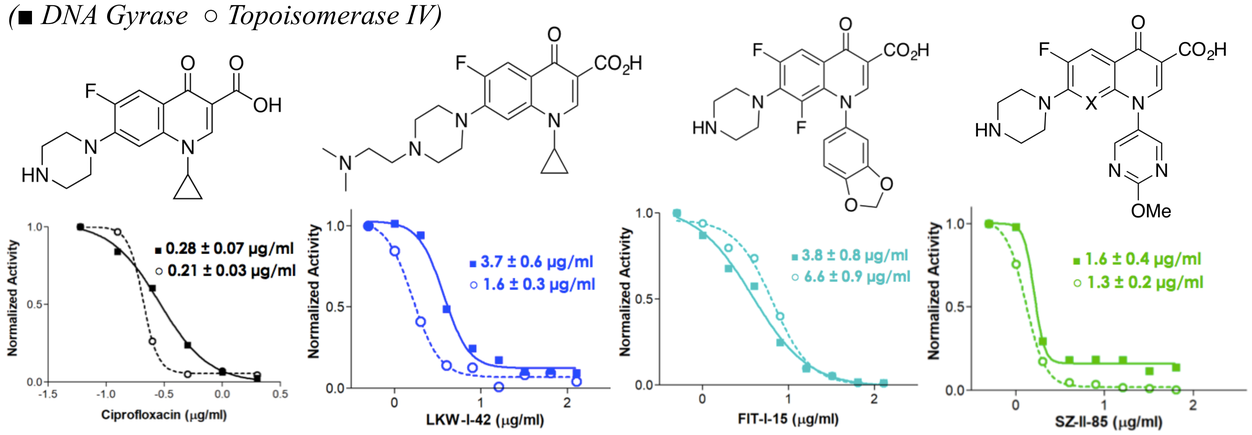
(1) Antibiotic/Antimicrobial Resistance, Centers for Disease Control and Prevention . Accessed 1 Aug. 2017.

Methods and Results

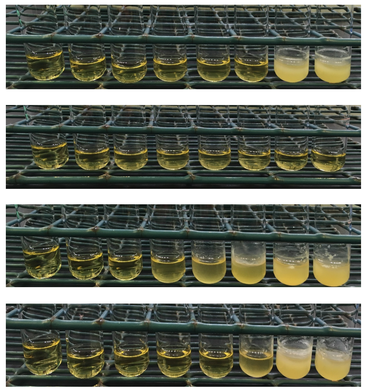
Enzyme Assays: DNA gyrase (assay on right in the presence of Ciprofloxacin) and Topoisomerase IV were sensitive to inhibition by 3 of 13 preliminary compounds. DNA gyrase generates supercoiled DNA while Topoisomerase IV decatenates DNA. The amount of final DNA was quantified to determine an EC_{50} value for each of the compounds.



(■ DNA Gyrase ○ Topoisomerase IV)

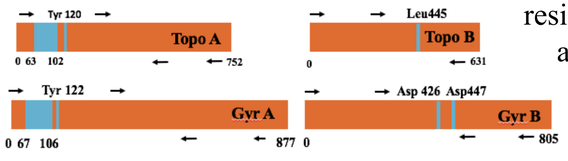


Minimum Inhibitory Concentration (MIC) Assays: Only two of the three compounds that showed activity against DNA gyrase and Topoisomerase IV had an effect on the growth of gram-negative and gram-positive bacteria. SZ-II-85 did not appear to be taken up by the bacteria, possibly due to changes in polarity or size from the particular functional groups added.

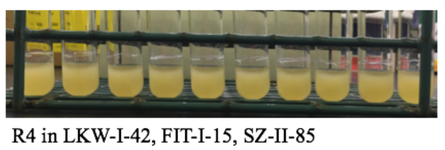


Strain	Moxifloxacin (μg/ml)	LKW-I-42 (μg/ml)	FIT-I-15 (μg/ml)	SZ-II-85 (μg/ml)
S. aureus	0.03	2	8	>32
K. pneumoniae	0.33	16	4	>32
E. coli	<0.08	2	2	>32
P. aeruginosa	2.6	32	4	>32
E. aerogenes	0.67	8	2	>32

Fluoroquinolone Resistant Bacteria: A FQ-resistant strain of *K. pneumoniae* was developed to test the effectiveness of the compounds against FQ-resistant bacteria. The sequencing strategy for the resistant bacteria is shown below. The arrows indicate the location of the sequencing primers and common resistance mutations are highlighted in blue.



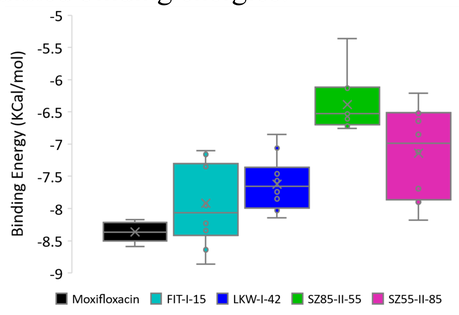
Strain	Moxifloxacin (μg/ml)	LKW-I-42, FIT-I-15, SZ-II-85 (μg/ml)
R4	16	<0.06

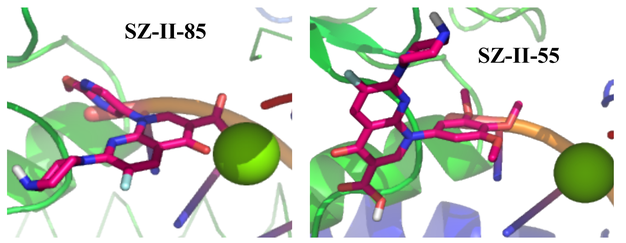


R4 in LKW-I-42, FIT-I-15, SZ-II-85

Molecular Modeling

AutoDock was used to model the possible binding modes for the novel compounds. The modeling was done in the presence of the Mg^{2+} ion. Structures of compounds known to bind experimentally docked in the same orientation as Moxifloxacin, while a compound known to not bind (SZ-II-55) docked in a different orientation and exhibited deviations in calculated binding energies.





Conclusions

- We identified 3 novel FQ-based compounds that bound to the DNA-enzyme complex, two of which were active against gram-negative and gram-positive bacterial cultures
- Molecular modeling provides a tool to predict whether future novel compounds will be active against gyrase and Topo IV
- Future tests will include Human Topoisomerase II assay to determine if the compounds are selective for the bacterial enzymes over the human ones.

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