Determining the Effectiveness of Drugs using EC50 and MIC Assays

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Summary

Background

- Antibiotic resistance comes from bacterial mutations in the antibiotic binding site (which prevents antibiotic binding)
- These new antibiotics target both DNA Gyrase *and* DNA Topoisomerase IV (fluoroquinolones)
  - This improves efficacy because if one binding site is mutated, the other is still available
    - This makes it harder for bacteria to form resistance against the drug

Methods

1) Gyrase Assay
   - Tests what the drug does to the DNA Gyrase inside the bacteria
     - Gyrase supercoils DNA – without which the DNA can't form chromosomes -> can't replicate
2) Minimum Inhibitory Concentration (MIC) Assay
   - Tests whether the drug can penetrate the bacteria's thick cell wall to disable DNA Gyrase

Results/Data

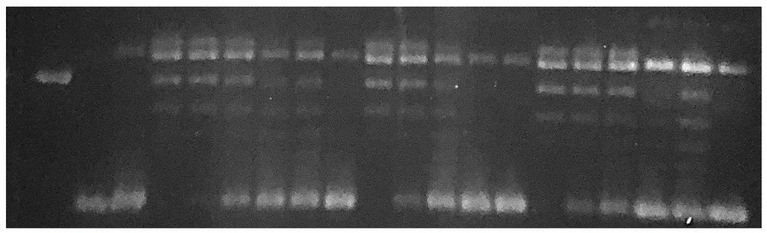
- Standard deviations of EC50s and MICs are used to quantify the effectiveness of the compounds

Conclusions/Outcomes/Future

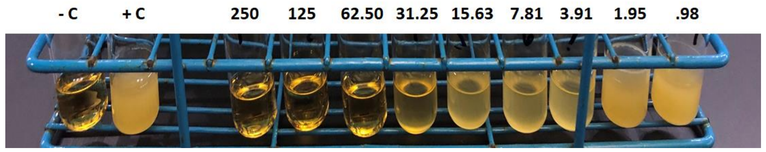
- Conclusion: effect of structure on binding affinity and ability to get into the bacteria (see Results)
- Complete a new set of assays with the best compounds:
  - TopoIV Assay: tests that the drugs are dual-targeting
  - Human Topo II Assay: tests that the drugs won't affect human Topoisomerase II (only bacterial)
  - Resistance Testing: tests the efficacy of the compounds against bacteria that have resistance to fluoroquinolones

A methodology for determining potency of modified antibiotics

Results

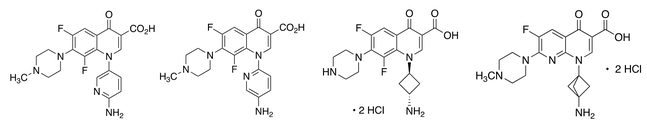


Gyrase Assay with AKE-I-39, AKE-I-51, & ETM-I-15 ([]s = 32-1or2 µg/mL). Yields the Effective Concentration needed to kill 50% of the bacteria (EC50). (LM = Linear Marker; SCM = Supercoiled Marker)



MIC Assay with RLB-XIV-15 in K. pneumoniae ([]s = serial dilutions starting at 250 ug/mL). Yields the Minimum Inhibitory Concentration needed to prevent bacterial growth.

Example Structures:



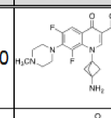
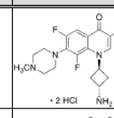
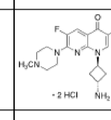
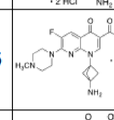
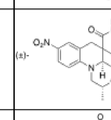
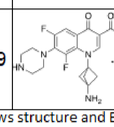
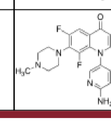
AKE-I-39 AKE-I-51 ETM-I-15 RLB-XIV-15

Results

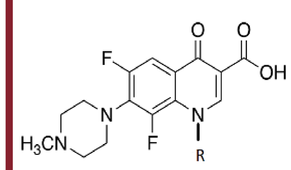
Compound Identifier	Avg # of SD's from Mean	Ring Structure	R Group
RLB-XIII-140	-0.92		
ETM-I-11	-0.64		
MRB-I-3	-0.56		
AKE-I-39	-0.55		
ETM-I-10	-0.03		
RLB-XIV-15	0.08		
RLB-XIII-149	0.62		

This table shows an example of how to compare structural changes between compounds. Using this table, ring structure and R group can be easily compared, using standard deviation (as explained more in the table note below).

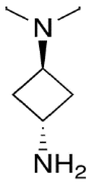
Top Compounds with Structure, EC50, and MIC

	Compound Identifier	Chemical Structure	EC50 or MIC	Compound Identifier	Chemical Structure	EC50 or MIC
EC50	RLB-XIII-140		2.79	ETM-I-10		10.6
MIC (KP)			18. ug/mL			29. ug/mL
MIC (SA)			78. ug/mL			88. ug/mL
EC50	ETM-I-11		9.20	RLB-XIV-15		12.0
MIC (KP)			8. ug/mL			42. ug/mL
MIC (SA)			21. ug/mL			26. ug/mL
EC50	MRB-I-3		5.41	RLB-XIII-149		2.05
MIC (KP)			42. ug/mL			75. ug/mL
MIC (SA)			17. ug/mL			350. ug/mL
EC50	AKE-I-39		9.98	This table shows structure and EC50/MIC results of the above, best-performing compounds, in order of how many standard deviations away from the mean in the negative direction the compound is (how effective it is).		
MIC (KP)			5. ug/mL			
MIC (SA)			42. ug/mL			

Most Effective Ring Modification, Based on Table Above



Most Effective R Group, Based on Table Above



These results can tell us which ring structure modifications and R groups should be used as a template, and which should be avoided, when synthesizing new antibiotics.

*note: Some ring structures/ R groups were never able to permeate the bacteria, even if they bound to the protein.

References and Acknowledgments

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