Determining the Effectiveness of Drugs using EC50 and MIC Assays

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A methodology for determining potency of modified antibiotics

**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 (must 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.

<table>
<thead>
<tr>
<th>Compound Identifier</th>
<th>Avg # of SD’s from Mean</th>
<th>Ring Structure</th>
<th>R Group</th>
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<tbody>
<tr>
<td>RLB-XII-140</td>
<td>0.32</td>
<td>Yellow</td>
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<tr>
<td>ETM-I-11</td>
<td>0.64</td>
<td>Red</td>
<td></td>
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<tr>
<td>MRB-I-3</td>
<td>0.56</td>
<td>Orange</td>
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<tr>
<td>AKE-I-39</td>
<td>0.55</td>
<td>Green</td>
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<tr>
<td>ETM-I-10</td>
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<tr>
<td>RLB-XIV-15</td>
<td>0.08</td>
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<tr>
<td>RLB-XII-149</td>
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<td>Yellow</td>
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</table>

**Examples**
- **Gyrase Assay with AKE-I-39, AKE-I-51, & ETM-I-15**: 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**: Yields the Minimum Inhibitory Concentration needed to prevent bacterial growth.

**References and Acknowledgments**

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