Synthesis of Tetracyclic Indolines as Resistance Modifying Agents for the Treatment of MRSA

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Synthesis of Tetracyclic Indolines as Resistance Modifying Agents for the Treatment of MRSA

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Abstract

The issue of antibiotic resistance is not being solved by current efforts to discover new classes of antibiotics. In an attempt to combat this issue our lab has created a series of resistance-modifying agents (RMAs) based on natural indole alkaloids, which have been used to treat many different diseases. Through structure-activity relationship (SAR) studies, we have been optimizing tetracyclic indoline compounds that potentiate β-lactam antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA), but do not exhibit antibacterial activity on their own. This is important as it reduces selective pressures for resistance while allowing us to utilize previously obsolete antibiotic classes in the fight against resistant bacteria. In previous SAR studies done by the Wang Lab, a potent compound, 6a, was discovered as a potent lead compound. Additionally, the absolute stereochemistry of the tetracyclic ring structure was found to be essential to this class of RMAs. The research in this study focuses on synthesizing novel analogs from commercially available building blocks. It was determined that the addition of an ester group to the D-ring could not be tolerated; however a primary alcohol group can. This research also supports previous studies that have optimized the carbamate functional group on the D-ring of this tetracyclic scaffold.
Introduction

1.1 Current status of antibiotic resistance and need for alternatives

Antibiotic resistance is a pressing issue that we cannot ignore. The Centers for Disease Control estimates that at a minimum 23,000 people will die each year from antibiotic-resistant infections.\textsuperscript{1} Methicillin resistant \textit{Staphylococcus aureus} is expected to be responsible for nearly half of those deaths and is listed as a “serious threat” by the CDC. In addition to being deadly, antibiotic-resistant diseases are also costly with an estimated $20 billion a year being spent on healthcare directly related to this condition and $35 billion in societal costs.\textsuperscript{2}

Between 1930 and 1962 there were 20 new classes of antibiotics developed, including \( \beta \)-lactams, like methicillin. These antibiotics were medically useful for 50 years before major resistance issues occurred, limiting their effectiveness against infections.\textsuperscript{3} Within the past 40 years only two new classes of antibiotics have been developed, and currently very little effort is being put into developing antibiotics by the pharmaceutical industry. Out of all the drugs that were allocated for research and development at the top 15 pharmaceutical companies in 2009, only 5 were antibiotics.\textsuperscript{4} What little research is being done on antibiotic development has mostly has been focused on creating new antibiotics based on known chemical scaffolds.\textsuperscript{5} The issue with this method of discovery, however, is that traditional antibiotics are prone to resistance being developed against them.\textsuperscript{6}

Bacterial resistance is observed towards most antibiotics within a couple years of being introduced to market.\textsuperscript{3} While resistance can occur more slowly, it is an issue that will continually arise due to the selective pressures of evolution. Every time an antibiotic is used, it kills all the organisms that have not evolved resistance mechanisms against it,
leaving behind the organisms that have. These resistant bacteria can reproduce and pass resistance to the next generation or transfer their resistance genes to non-resistant organisms through horizontal gene transfer. There are no antibiotics that resistance has not been discovered for, and the antibiotic pipeline is drying up, so it is clear that in order to combat the issue of resistance we will need to develop novel ways of targeting infections.⁷

1.2 Mechanisms of resistance

Antibiotics have been developed to target many aspects of microbial biochemical machinery, mainly through the inhibition of the synthesis of the cell wall, nucleic acids, or necessary enzymes (Figure 1.2). Bacteria have evolved several different mechanisms of resistance to these various classes of antibiotics. These mechanisms can be acquired through random mutations or the acquisition of genetic information that codes for the resistance mechanisms. The most common mechanisms of resistance present in *S. aureus* are modifications to the penicillin binding protein, inactivation of β-lactam antibiotics by β-lactamases, and export of antibiotics by efflux pumps.⁸ Discovery of these mechanisms is important in order to develop treatments for resistance.
1.2.1 Penicillin Binding Proteins

β-lactam antibiotics function by targeting the penicillin binding proteins (PBPs) that cross-link the peptidoglycan strands of the bacterial cell wall. β-lactams are structurally analogous to the D-ala-D-ala residue that is the target of the active site of the enzyme. By binding and forming an acyl-enzyme complex in the active site, the β-lactam effectively inhibits the PBP. In the case of MRSA, however, a modified PBP exists, which has a lowered affinity for β-lactams. PBP2a has an altered active site in which the catalytic serine residue to which β-lactams bind is inaccessible. PBP2a is encoded by the mecA gene, which is expressed by the binding of β-lactams to an extracellular penicillin-binding domain, MecR1, which causes the cleavage of the transcriptional repressor of mecA, MecI. While the wild-type PBPs are still present in organisms that exhibit PBP2a, the presence of the altered transpeptidase allows for enough cross-linking of peptidoglycan for the cell to be able to survive therapeutic doses of methicillin.
1.2.2 β-lactamases

β-lactamases also function to prevent β-lactam antibiotics from inhibition of cell wall synthesis, however instead of an altered binding site, the β-lactamase simply degrades the antibiotic through hydrolysis of the amide bond in the four-membered β-lactam ring. There are two categories of β-lactamases; the serine β-lactamases and metallo β-lactamases, which use zinc ions to facilitate hydrolysis.\textsuperscript{12} β-lactamase is encoded by the \textit{blaZ} gene and its expression is regulated by a mechanism similar to that of \textit{mecA} expression. Binding of β-lactams to BlaR1 causes it to cleave itself, which in turn causes the cleavage of BlaI, the transcriptional repressor of \textit{blaZ}.\textsuperscript{13} There is evidence to suggest that BlaI and BlaR1 may also be able to regulate PBP2a production as well.\textsuperscript{14}

![Diagram of β-lactamase production](image)

\textbf{Figure 1.2.2:} The mechanism by which \textit{blaZ} produces β-lactamase through binding of β-lactams to the intracellular BlaR1 protein. (adapted from Lowy (13))

1.2.3 Efflux Pumps

While β-lactamase and altered PBP activity are the most common mechanisms of resistance in drug-resistant \textit{S. aureus} infections, the actions of efflux pumps on resistance have become of interest in recent studies. They are often the first mechanism of antibiotic
resistance for bacteria and may account for up to 10% of resistance in MRSA strains.\textsuperscript{15} Efflux pumps function to transport toxic materials outside of the bacterial cell, including antibiotics. Some efflux pumps are specific to a single substrate whereas others can transport several different molecules leading to multi-drug resistance, which is why the study of efflux pumps is increasingly relevant.\textsuperscript{16} In \textit{S. aureus}, efflux pumps mainly contribute to resistance against quinolone antibiotics.\textsuperscript{17}

\subsection*{1.3 Resistance-modifying agents}

An important factor in the newest developments of antibiotic resistance treatments is the utilization of synergistic effects. Synergy occurs when the administration of a resistance-modifying agent (RMA) along with an existing antibiotic improves the efficacy of that antibiotic. Synergistic effects that occur between an RMA and an antibiotic function by the suppression of a resistance mechanism by the RMA so that the antibiotic is able to function again.\textsuperscript{18} One example that is currently on the market is the amoxicillin/clavulanic acid combination for the treatment of bacterial infections. Amoxicillin functions as a β-lactam antibiotic and clavulanic acid acts as a β-lactamase inhibitor. Resistance, while still a threat, has so far been observed to be rare towards these inhibitors, possibly due to the structural similarities between them and β-lactams themselves.\textsuperscript{19} Many other RMAs function as efflux pump inhibitors\textsuperscript{20, 21}, or modulate the expression of efflux pumps.\textsuperscript{22} Additionally RMAs can function as membrane permeabilizers, or as inhibitors of enzymatic targets that have been modified by resistance, such as PBP2a.\textsuperscript{14}
1.4 Indoles as biologically active compounds

Many plant alkaloids have been found to have promising biological activity. Of those compounds, indoles have shown to be biologically active in several areas. Indoles are naturally occurring in many sources, such as the amino acid tryptophan, which is the precursor for the synthesis of many other biological molecules. Studies have found indole structures to exhibit anti-cancer, anti-hypertensive, and antidepressant activities, among many others. The heterocyclic nature of the indole is thought to impart its diverse biological activities, as many biological molecules contain heterocycles, particularly nitrogen containing ones. With 80% of top pharmaceuticals produced in 2010 containing the moiety, it has clearly proven to be effective.

Indoles have also been shown to exhibit antimicrobial activity alone, as well as in combination with known antibiotics for synergistic activities against bacterial infections. In a study involving drug-resistant E. coli, naturally occurring indole alkaloids were shown to enhance the antimicrobial effects of nalidixic acid and tetracycline through inhibition of efflux pumps. Structure-activity relationship studies on various indole and 2-aryl indole analogues have potentiated antibiotics by means of NorA efflux pump inhibition. These findings make indole and indoline molecules a good target for resensitization activity studies.
**Previous Research in the Wang Lab**

Previous studies done in the Wang lab have served to optimize the structures of the indoline molecules that are tested for resistance modifying activity. By screening a variety of bio-inspired indole alkaloids for re-sensitization activity we have identified several hit compounds to make further modifications to. Structure-activity relationship studies have served as the basis for the rationale of the structural modifications made to each compound. Compounds with the most activity are chosen for further modification in order to determine which positions should be modified. Activity is measured using minimum inhibitory concentrations (MIC) and minimum re-sensitizing concentration (MRC) values. MIC values are used to determine if the compound has antibacterial activity on its own, whereas the MRC value measures the ability of the compound to function as a RMA. Each activity test gives information about which functional groups on the molecule can or cannot be further optimized.

Screening of one of the initial libraries of 120 indolines using the standard Clinical Laboratory Standards Institute (CLSI) broth microdilution method yielded nine compounds that decreased the MIC of methicillin against MRSA by 32μg/mL or more, the most potent of which was a compound named Of1. Of1, termed such because of its open ring fused structure, had a MIC of 8μg/mL when combined with methicillin. This is in comparison to a MIC of 128μg/mL for methicillin used alone against this strain of MRSA. Of1 was also tested with other β-lactam antibiotics and was found to decrease MIC in all of them. The work of Dr. Jessica Podoll suggests that Of1 stabilizes the binding of BlaI to DNA, which reduces transcription of the bla operon, which codes for
β-lactamase production. It is theorized that it may do the same for the mec operator promoter region. 

In our lab’s most recent structure-activity relationship study, a library of compounds based on the parent structure Kf18 were tested for resistance-modifying activity using the CLSI microdilution assay. Kf18 was tested against several classes of antibiotics and was found to selectively re-potentiate β-lactam antibiotics (Table 1). From this SAR study, compound 6a (Figure 2) was found to have the lowest MRC of the analogues tested. This was discovered through testing of various substitutions to the group on the D-ring nitrogen of the compound, with an ethyl carbamate exhibiting the lowest MRC of the compounds tested (Table 2). Modifications were also made by various aromatic substitutions to the compound and changing the C-ring to a 6 membered ring. Compound 6a potentiated β-lactam antibiotics in two strains of MRSA, yet showed no antibacterial activity of its own, or as a β-lactamase inhibitor.

Figure 2: Compound 6a, the parent structure for my compounds. (adapted from Zhu et al. (33))

My project focuses on making further modifications to the structure of 6a beginning with functionalizations at carbon 5 of the D ring to see if changes to this position can be tolerated and will still confer biological activity. We hypothesized that any compounds made from this parent structure will have similar biological activity to it. A racemic ethyl ester was added at this position with the rationale that its similar size to
the ethyl carbamate may be easily tolerated. The racemic starting material was chosen so that if the compounds made were found to have activity, they could be re-synthesized as enantiomerically pure stereoisomers and tested individually. Stereochemistry can have an effect on the activity of a compound due to the fact that two different enantiomers will have different 3-dimensional conformations and thus may interact with biological substrates differently, so it is necessary to figure out which enantiomer imparts the most activity. This was the beginning compound in my SAR studies to which I made other modifications including reductions of the carbamate and ester groups.

**Table 1**: Kf18, the lead compound in the SAR that produced 6a, is found to selectively re-potentiate β-lactam antibiotics in MRSA. Kf18 is found to have no resistance-modifying activity when used with other classes of antibiotics. (adapted from Zhu et al. (33))

<table>
<thead>
<tr>
<th>Entry</th>
<th>Antibiotic</th>
<th>MIC&lt;sub&gt;a&lt;/sub&gt;</th>
<th>MIC (+Kf18)&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Fold of potentiation</th>
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<tbody>
<tr>
<td>1</td>
<td>Methicillin</td>
<td>128</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>Amox/clav</td>
<td>32</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Cefazolin</td>
<td>128</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>Meropenem</td>
<td>32</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>Oxacillin</td>
<td>64</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Tetracycline</td>
<td>32</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>Ciprofloxacin</td>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>Clindamycin</td>
<td>256</td>
<td>256</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>Erythromycin</td>
<td>128</td>
<td>128</td>
<td>1</td>
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<tr>
<td>10</td>
<td>Gentamycin</td>
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<td>256</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>Streptomycin</td>
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</tr>
<tr>
<td>12</td>
<td>Daptomycin</td>
<td>256</td>
<td>256</td>
<td>1</td>
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</tbody>
</table>

<sup>a</sup> Minimum inhibitory concentration in μg/mL in MRSA strain ATCC BAA-44.
<sup>b</sup> MIC value in the presence of 20 μM of Kf18.
Table 2: Results of previous SAR studies performed in the Wang lab. The most potent of these compounds, 6a, was chosen as the lead compound for my SAR studies. (adapted from Zhu et al. (33))

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cmpd</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>Y</th>
<th>MRC (cefazolin)ᵃᵇ</th>
<th>MRC (amox/clav)ᵃᶜ</th>
<th>MICᵇ⁻¹</th>
<th>GI₅₀ᵃᵈ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kf18</td>
<td>4,6-diMe</td>
<td>Me</td>
<td>Me</td>
<td>CH₂CH₂</td>
<td>16</td>
<td>16</td>
<td>&gt;64</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>13a</td>
<td>4,6-diMe</td>
<td>Me</td>
<td>Et</td>
<td>CH₂CH₂</td>
<td>2</td>
<td>4</td>
<td>&gt;64</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>13b</td>
<td>4,6-diMe</td>
<td>Me</td>
<td>CH₂CCH</td>
<td>CH₂CH₂</td>
<td>64</td>
<td>64</td>
<td>&gt;64</td>
<td>17</td>
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<td>4</td>
<td>11</td>
<td>4,6-diMe</td>
<td>Me</td>
<td>H</td>
<td>CH₂CH₂</td>
<td>4</td>
<td>8</td>
<td>&gt;64</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>13c</td>
<td>H</td>
<td>Me</td>
<td>Me</td>
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<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>13d</td>
<td>5-Me</td>
<td>Me</td>
<td>Me</td>
<td>CH₂CH₂</td>
<td>8</td>
<td>4</td>
<td>&gt;64</td>
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<tr>
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<td>8</td>
<td>4</td>
<td>&gt;64</td>
<td>53</td>
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<tr>
<td>8</td>
<td>6a</td>
<td>4,6-diMe</td>
<td>Et</td>
<td>Me</td>
<td>CH₂CH₂</td>
<td>2</td>
<td>2</td>
<td>&gt;64</td>
<td>61</td>
</tr>
<tr>
<td>9</td>
<td>13f</td>
<td>5-F</td>
<td>Et</td>
<td>Me</td>
<td>CH₂CH₂</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>10</td>
<td>13g</td>
<td>5-F-6-MeO</td>
<td>Et</td>
<td>Me</td>
<td>CH₂CH₂</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
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<tr>
<td>12</td>
<td>14b</td>
<td>5-Me</td>
<td>Me</td>
<td>Me</td>
<td>CH₂</td>
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<td>16</td>
<td>&gt;64</td>
<td>15</td>
</tr>
<tr>
<td>13</td>
<td>14c</td>
<td>5-Cl</td>
<td>Me</td>
<td>Me</td>
<td>CH₂</td>
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<td>16</td>
<td>&gt;64</td>
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<tr>
<td>14</td>
<td>14d</td>
<td>5-Me</td>
<td>Et</td>
<td>Me</td>
<td>CH₂</td>
<td>64</td>
<td>32</td>
<td>&gt;64</td>
<td>43</td>
</tr>
<tr>
<td>15</td>
<td>14e</td>
<td>5-Cl</td>
<td>Et</td>
<td>Me</td>
<td>CH₂</td>
<td>16</td>
<td>8</td>
<td>&gt;64</td>
<td>41</td>
</tr>
</tbody>
</table>

a. Numbers are in μg/mL.
b. MRSA NRS-100.
c. MRSA ATCC BAA-44.
d. HeLa cells.
Results

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>MRC</th>
<th>MRC</th>
<th>MIC&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>GI&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(cefa)</td>
</tr>
<tr>
<td>8</td>
<td>COOEt</td>
<td>COOEt</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Me</td>
<td>COOEt</td>
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<td>&gt;64</td>
<td>&gt;64</td>
<td>117.2</td>
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<td>10</td>
<td>COOEt</td>
<td>CH₂OH</td>
<td>32</td>
<td>16</td>
<td>&gt;64</td>
<td>13.7</td>
</tr>
</tbody>
</table>

Table 3: The results of the biological testing of compounds 8, 9, and 10. a= μg/mL; b=ATCC BAA-44

All compounds were tested with cefazolin to measure β-lactam potentiation and amoxicillin/clavulanic acid to measure its synergistic action with that therapeutic combination. Mammalian toxicity (GI<sub>50</sub>) values were obtained using HeLa cells. The MRC value is obtained from the concentration of RMA at which no observable overnight growth has occurred in the presence of antibiotics. The MIC value is obtained from concentrations of RMA at which no observable overnight growth has occurred in the absence of antibiotics. No GI<sub>50</sub> value was obtained for compound 8, as it exhibited no activity.
Discussion

We hypothesized that the compounds made would follow the trends of activity shown in the previous SAR studies due to their highly similar structures. For compound 7, however, this was not the case, as it exhibited no resistance modifying activity. The presence of the carbamate, however, is important to the activity of the compound, due to the lack of activity exhibited by compound 9. This is also supported by our previous studies, which had found optimal activity for compounds with that functional group. Although the carbamate and ester are of similar size, and are located in similar positions on the molecule, the carbamate must be specifically imparting activity to the molecule.

Due to the fact that previous compounds tested containing a methyl group on the D-ring nitrogen, as is present in compound 9, showed activity as well, the lack of activity for this compound can be attributed to the presence of the ester, not the methyl group. So the negative results from both compounds 8 and 9 show us that not only is the carbamate an essential part of the molecule, but an ester on carbon 5 cannot be tolerated.

We did find that compound 10 exhibited resistance-modifying activity. Compared to the lead compound, 6a, however, its activity is decreased. While 6a had a MRC of 2μg/mL, compound 10 exhibited a MRC of 32 μg/mL. This would then follow a trend that the larger a functional group at this position, the less biological activity it has, given that the ester confers no activity and no functionalization, as in the lead compound, 6a, confers the most activity. The testing of more functional groups at this position would be needed to support this assertion, however.

A racemic starting material (1) was used for all compounds made, with the rationale that if the compound was found to be biologically active then each stereoisomer
could be isolated and tested separately to see if one enantiomer had more activity than the other. During cyclization there also exist two possible isomers, and endo and an exo product, however this stereochemistry is determined by the stereochemistry of the ester group. Therefore there are only two stereoisomers present in each of the compounds tested. Separation of these stereoisomers can be done for active compounds, such as compound 10, and could be the focus of future work.

All the compounds tested exhibited no growth inhibiting activity alone. This means that none of our compounds act as antibacterial agents by themselves, rather they selectively potentiate β-lactams in drug-resistant S. aureus strains. This is theorized to be an important characteristic for RMAs as it causes less direct selective pressure for resistance. This can be seen in the decreased rates of resistance found in β-lactamase inhibitor use. Another important aspect of our studies is the determination of mammalian toxicity. Too low of a GI50 concentration would be worrisome for any compound that is to be used as a therapeutic. The GI50 of compound 10 is not an improvement over that of the lead compound, 6a, and coupled with the decrease in potency it makes for a less promising scaffold to move forward with. With the overall goal of this study being to determine whether or not modifications to 6a could be tolerated, however, we now have important information for future SAR studies.

**Future Directions**

Future research on modifications to compound 6a could focus on different modifications to the D-ring of the tetracyclic compound. Specific modifications at carbon 5 could incorporate either smaller moieties or larger, alkyl groups to establish a clearer
trend of activity for modifications at this position. The separation of the two stereoisomers could be done for compound 10 in order to determine if one confers additional activity over the other. Determination of an exact mechanism of action could also aid in the discovery and modification of new RMAs.

**Conclusion**

This study aimed to discover if making additional modifications to a potent lead compound, 6a, could be tolerated to confer biological activity as a resistance-modifying agent in the treatment of MRSA. This study found that the addition of a primary alcohol (10) can be tolerated on the D-ring of compound, 6a, but did not confer additional biological activity beyond that observed in 6a. The testing of compound 9 also confirmed that the carbamate group is essential to the resistance-modifying activity of this compound. Further studies should focus on the addition of functional groups of different sizes to the D-ring in order to establish any trends in modification of biological activity.
Experimental

Instruments and Materials

Unless otherwise noted, reagents were obtained commercially and used without further purification. CH$_2$Cl$_2$ was distilled from CaH$_2$ under a nitrogen atmosphere. THF was distilled from sodium–benzophenone under a nitrogen atmosphere. Toluene was distilled from sodium under a nitrogen atmosphere. All chemicals were purchased from Sigma-Aldrich, TCI America, or Alfa Aesar (United States) unless otherwise noted. Thin-layer chromatography (TLC) analysis of reaction mixtures was performed on Dynamic adsorbents silica gel F-254 TLC plates. Flash chromatography was carried out on Zeoprep 60 ECO silica gel. All $^1$H and $^{13}$C NMR data were obtained using a Bruker 300 Ultrashield spectrometer. Chemical shifts are reported in ppm. Compound purity ($\geq 95\%$) was confirmed on the basis of the integration of the area under the UV absorption curve at $\lambda = 254$ or 210 nm signals using an Agilent 1260 series HPLC system coupled with a 6120 Quadrupole mass spectrometer.

Procedures
Figure 3: The synthesis scheme for compound 7. Reagents and conditions: a) SOCl₂, EtOH; b) DMAP, DIPEA, ethyl chloroformate, CH₃CN; c) THF, -40°C; d) TBAF, THF, 0°C; e) TCT, EtOH, 80°C; f) XPhos AuNTF₂, toluene, 80°C

5-Ethylcarboxyl-2-pyrrolidinone (1)

To a solution of (+/-)-2-Pyrrolidinone-5-carboxylic acid in anhydrous EtOH at 0°C was added SOCl₂ (1.2 equivalents). The solution was stirred overnight at room temperature. The mixture was concentrated in vacuo and re-dissolved in ethyl acetate, then washed with potassium carbonate and brine and dried over anhydrous Na₂SO₄. The product was filtered through a silica column and concentrated in vacuo to produce a white crystalline product (86.6%, 5.2373g). TLC (100% ethyl acetate) Rₚ=0.2; ¹H NMR (300 MHz, CDCl₃) δ: 6.28 (s, 1H), 6.06 (s, 1H), 4.31-4.08 (m, 5H), 2.61-2.15 (m, 6H), 1.67 (s, 1H), 1.31 (td, J = 7.1, 1.8 Hz, 4H).

1,2-Pyrrolidinedicarboxylic acid, 5-oxo-, 1,2-diethyl ester (2)

5-Ethylcarboxyl-2-pyrrolidinone (1) was dissolved in acetonitrile, then DMAP (0.5 equivalents) and DIPEA (1.5 equivalents) were added and the solution was cooled to 0°C. Ethyl chloroformate (1.5 equivalents) was added and the reaction was stirred overnight at room temperature. The solution was washed with brine and dried over anhydrous Na₂SO₄ and concentrated in vacuo. The crude product was purified by column chromatography on silica gel with 1:1 hexanes:ethyl acetate as the eluting
solvent. The eluent was concentrated to produce yellow crystals. (90%, 2.5493g).

TLC (1:1 hexanes: ethyl acetate) Rf=0.25; ¹H NMR (300 MHz, CDCl₃) δ: 4.73-4.63 (m, 1H), 4.44-4.22 (m, 3H), 4.28-4.07 (m, 1H), 2.77-2.58 (m, 1H), 2.61-2.45 (m, 1H), 2.37 (ddt, J = 13.3, 10.5, 9.2 Hz, 1H), 2.18-2.01 (m, 1H), 2.06 (s, 1H), 1.40-1.21 (m, 6H).

**Figure 4**: Synthesis of compound 3. Reagents and conditions: a) n-BuLi, TMSCl, THF; b) TPP, NBS, DCM; c) Mg, I₂, Et₂O, 30°C

**6-(Trimethylsilyl)-5-Hexyne-1-ol (3a)**

5-Hexyne-1-ol was dissolved in THF under inert argon atmosphere. The reaction flask was cooled to -78°C and n-butyllithium (2 equivalents) was added. The reaction was stirred for 60 min. then TMSCl (2 equivalents) was added and the reaction was left to stir overnight at room temperature. The reaction flask was cooled to 0°C and an excess of 1M HCl was added and stirred for 30 min. The reaction was diluted in ethyl acetate and washed with sodium bicarbonate and brine, then dried over anhydrous Na₂SO₄. The crude product was purified by column chromatography on silica gel with 3:1 (hexanes: ethyl acetate) as the eluting solvent to yield a colorless oil. (64.6%, 5.6004g). TLC (1:1
hexanes: ethyl acetate) R_f=0.75; ^1H NMR (300 MHz, CDCl_3) δ: 3.70 (td, J = 6.2, 5.3 Hz, 2H), 2.35-2.18 (m, 2H), 1.80-1.51 (m, 5H), 1.28 (t, J = 7.2 Hz, 1H), 0.16 (s, 9H)

(6-bromohex-1-yn-1-yl)trimethylsilane (3b)

6-(Trimethylsilyl)-5-Hexyne-1-ol (3a) was dissolved in DCM. Triphenylphosphine (1.2 equivalents) was then added to the flask, and the mixture was cooled to -30°C. N-bromosuccinimide (1.1 equivalents) was added, then the reaction was stirred overnight at room temperature under argon atmosphere. The reaction was quenched with diethyl ether, then washed with sodium bicarbonate and brine. Hexanes were added to the remaining organic layer and stirred for 15 minutes, then the solution was dried over anhydrous Na_2SO_4. The product was concentrated in vacuo, then re-dissolved in hexanes and filtered through a silica plug to remove triphenylphosphine, yielding a colorless oil (78.34%, 6.0071g) TLC (1:1 hexanes: ethyl acetate) R_f=0.8; ^1H NMR (300 MHz, CDCl_3) δ: 3.46 (t, J = 6.7 Hz, 2H), 2.29 (t, J = 7.0 Hz, 2H), 2.10-1.89 (m, 2H), 1.79-1.61 (m, 2H), 0.17 (s, 9H).

(6-(trimethylsilyl)hex-5-yn-1-yl)magnesium bromide (3)

A stir bar and magnesium strips (1.3 equivalents) were added to a flask and flame-dried to remove water. The flask was placed under argon atmosphere and iodine and diethyl ether were added. Bromide (3b) was added until the solution changed color from yellow to clear. The solution was heated at 30°C to reflux, then the remaining bromide was
added as the reflux calmed down. The reaction was stirred for 2 hours and immediately used in the following synthesis. TLC (1:1 hexanes: ethyl acetate) Rf=0.75.

![Chemical Structure]

**Ethyl 2-((ethoxycarbonyl)amino)-5-oxo-11-(trimethylsilyl)undec-10-ynoate (4)**

1,2-Pyrrolidinedicarboxylic acid, 5-oxo-, 1,2-diethyl ester (2) was dissolved in THF, then cooled to -40°C. (6-(trimethylsilyl)hex-5-yn-1-yl)magnesium bromide (3) (1.2 equivalents) was added and the reaction was stirred for 1.5 hours. The reaction was then quenched with 1:1 Methanol:Acetic acid solution. The mixture was then diluted in ethyl acetate and washed with sodium bicarbonate and brine, then dried over anhydrous Na₂SO₄. The crude product was purified by column chromatography on silica gel with 4:1 (hexanes: ethyl acetate) as the eluting solvent to yield a colorless oil. (0.9006g, 36.17%) TLC (4:1 hexanes: ethyl acetate) Rf=0.25; ¹H NMR (300 MHz, CDCl₃) δ: 4.39 – 4.18 (m, 1H), 4.24 – 4.07 (m, 2H), 3.82 – 3.65 (m, 1H), 2.67 – 2.39 (m, 2H), 2.35 – 2.19 (m, 1H), 2.06 (s, 1H), 2.02 – 1.83 (m, 0H), 1.77 – 1.62 (m, 1H), 1.63 – 1.44 (m, 1H), 1.38 – 1.20 (m, 4H), 0.16 (d, J = 1.7 Hz, 6H). ¹³C NMR (75 MHz, CDCl₃) δ 106.83, 84.78, 77.42, 77.20, 77.00, 76.58, 61.57, 53.31, 42.27, 28.04, 26.51, 22.86, 19.67, 14.54, 14.15, 0.13.

![Chemical Structure]

**Ethyl 2-((ethoxycarbonyl)amino)-5-oxoundec-10-ynoate (5)**

Ethyl 2-((ethoxycarbonyl)amino)-5-oxo-11-(trimethylsilyl)undec-10-ynoate (4)
was dissolved in THF and cooled to 0°C. TBAF (1.2 equivalents) was added and the reaction was stirred for 1 hour, then quenched with water. The product was extracted with ethyl acetate and washed with brine, dried over anhydrous Na$_2$SO$_4$. The crude product was purified on a column with 3:1 hexanes:ethyl acetate as the eluting solvent to yield a colorless oil. (74.8%, 0.5474g). TLC (2:1 hexanes: ethyl acetate) R$_f$=0.2; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 5.27 (d, $J= 8.1$ Hz, 0H), 4.38 – 4.06 (m, 4H), 2.61 – 2.46 (m, 1H), 2.45 (t, $J= 7.3$ Hz, 1H), 2.30 – 2.10 (m, 1H), 2.06 (s, 2H), 2.02 – 1.86 (m, 1H), 1.84 – 1.59 (m, 1H), 1.62 – 1.45 (m, 1H), 1.39 – 1.20 (m, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 83.98, 77.21, 68.58, 61.58, 61.20, 53.31, 42.21, 38.36, 27.84, 26.51, 22.76, 18.22, 14.53, 14.15.

![Figure 5: Methylation of 3,5-dimethylhydrazine.](image)

**1-(3,5-dimethylphenyl)-1-methylhydrazine (6)**

Free base hydrazine is generated first from 3,5-dimethylhydrazine. The starting material is added to ethyl acetate, then washed with 2M NaOH. Ethyl acetate is removed in vacuo and the compound is left on hi vac overnight. The compound was then dissolved in THF. Sodium bis(trimethylsilyl)amide (1 equivalent) was then added and allowed to stir for one hour. Methyl iodide (1 equivalent) was added and the reaction was stirred overnight at room temperature. The reaction was quenched with methanol, then washed with sodium bicarbonate and brine, and dried over anhydrous Na$_2$SO$_4$. The crude product was
purified on by column chromatography on silica gel using 4:1 (hexanes: ethyl acetate) as the eluting solvent. The fractions of the product were collected and concentrated in vacuo. A 1M HCl/ethyl acetate solution was added until the product crystallized, then was concentrated in vacuo to yield an off-white powder. (27.8%, 0.3002g). TLC (1:1 hexanes: ethyl acetate) Rf=0.4; \(^1\)H NMR (300 MHz, CDCl\(_3\)) δ: 7.04 (s, 1H), 6.87 (s, 1H), 3.29 (s, 3H), 2.32 (d, \(J = 0.7\) Hz, 6H), 1.27 (s, 1H).

**Ethyl 2-((ethoxycarbonyl)amino)-3-(2-(hex-5-yn-1-yl)-1,4,6-trimethyl-1H-indol-3-yl)propanoate (7)**

Ketone, TCT (1.5 equivalents), and 1-(3,5-dimethylphenyl)-1-methylhydrazine (5) (1.1 equivalents) were combined in sealed tube and dissolved in ethanol. The reaction was stirred overnight at 80°C. The reaction was concentrated in vacuo and re-dissolved in ethyl acetate, then washed with DI water and brine and dried over anhydrous Na\(_2\)SO\(_4\). The crude product was purified via column chromatography with 4:1 (hexanes: ethyl acetate) as the eluting solvent to yield a yellow oil. (0.087, 23.9%) TLC (4:1 hexanes: ethyl acetate) Rf=0.3; \(^1\)H NMR (300 MHz, CDCl\(_3\)) δ: 6.91 (s, 1H), 6.70 (s, 1H), 5.11 (s, 1H), 4.47 (d, \(J = 7.6\) Hz, 1H), 4.29 – 4.03 (m, 4H), 3.64 (s, 3H), 3.38 (dd, \(J = 14.8, 7.1\) Hz, 1H), 2.75 (t, \(J = 7.6\) Hz, 1H), 2.69 (s, 2H), 2.44 (s, 3H), 2.26 (td, \(J = 6.7, 2.8\) Hz, 3H), 1.98 (t, \(J = 2.6\) Hz, 1H), 1.56 (d, \(J = 1.4\) Hz, 2H), 1.40 – 1.04 (m, 8H). \(^{13}\)C NMR (300 MHz, CDCl\(_3\)) δ: 172.76, 155.99, 137.70, 137.55, 130.64, 129.28, 123.82, 123.31, 122.78,
Diethyl 1,3,5-trimethyl-10-methylene-7,8,9,10-tetrahydro-5H,6H-5a,10a-(epiminoethano)cyclohepta[b]indole-12,13-dicarboxylate (8)

Ethyl 2-((ethoxycarbonyl)amino)-3-(2-(hex-5-yn-1-yl)-1,4,6-trimethyl-1H-indol-3-yl)propanoate (7) was diluted in toluene. 10% gold xphos catalyst was diluted in toluene, then stirred for 20 min. This catalyst was added dropwise to the indole, then heated to 80°C for 24 hours. The crude product was purified by column chromatography on silica gel with 10:1 (hexanes: ethyl acetate) as the eluting solvent. (63.3%, 0.0313g). TLC (4:1 hexanes: ethyl acetate) R_f=0.6; ^1H NMR (300 MHz, CDCl3) δ: 6.29 (s, 1H), 6.13 (t, J = 14.0 Hz, 1H), 5.00 (d, J = 6.1 Hz, 1H), 4.91 (s, 1H), 4.16 (s, 1H), 4.08 – 3.93 (m, 2H), 3.05 (s, 2H), 2.95 (d, J = 12.0 Hz, 1H), 2.45 – 2.31 (m, 1H), 2.29 (s, 2H), 2.19 (s, 2H), 2.10 (d, J= 14.8 Hz, 2H), 1.76 (s, 2H), 1.58 (s, 4H), 1.47 – 1.36 (m, 2H), 1.36 – 1.22 (m, 8H), 1.21 – 1.07 (m, 3H), 1.07 – 0.82 (m, 4H). ^13C NMR (300 MHz, CDCl3) δ: 138.63, 122.79, 121.69, 121.50, 104.58, 104.25, 60.86, 60.10, 35.55, 32.94, 32.39, 30.93, 30.41, 29.70, 25.71, 24.36, 23.96, 21.58, 17.14, 14.55, 14.13. ESI m/z calculated for C_{25}H_{34}N_{2}O_{4} [M+H]^+ 427.25, found 427.2.

Selective Reduction Reactions
**Ethyl 1,3,5,13-tetramethyl-10-methylene-7,8,9,10-tetrahydro-5H,6H-5a,10a-(epiminoethano)cyclohepta[b]indole-12-carboxylate (9)**

Diethyl 1,3,5-trimethyl-10-methylene-7,8,9,10-tetrahydro-5H,6H-5a,10a-(epiminoethano)cyclohepta[b]indole-12,13-dicarboxylate (8) was diluted in THF and cooled to -78°C. LiAlH₄ (3 equivalents) was added dropwise under inert atmosphere. After 1.5 hours at room temperature the reaction was cooled to -78°C again and quenched with ethyl acetate. Rochelle’s salt was added at room temperature and the reaction was stirred for 2 hours then washed with brine and dried over anhydrous Na₂SO₄. The product was purified on a column with 4:1 hexanes: ethyl acetate as the eluting solvent and yielded a white crystalline compound. (24.0%, 6.9 mg). TLC (4:1 hexanes: ethyl acetate) Rᵣ=0.3; 'H NMR (300 MHz, CDCl₃) δ: 6.20 (s, 1H), 6.02 (d, J = 19.7 Hz, 1H), 5.16-5.03 (m, 2H), 4.21-3.99 (m, 3H), 2.93 (s, 2H), 2.91-2.71 (m, 2H), 2.30 (d, J = 12.4 Hz, 5H), 2.27-2.13 (m, 4H), 2.13-1.95 (m, 9H), 1.35-1.02 (m, 11H), 1.02-0.82 (m, 3H).

ESI m/z calculated for C₂₃H₃₂N₂O₂ [M+H]^+ 369.25, found 369.2.
13-(ethoxycarbonyl)-1,3,5-trimethyl-10-methylene-7,8,9,10-tetrahydro-5H,6H-5a,10a-(epiminoethano)cyclohepta[b]indole-12-carboxylic acid (10)

Diethyl 1,3,5-trimethyl-10-methylene-7,8,9,10-tetrahydro-5H,6H-5a,10a-(epiminoethano)cyclohepta[b]indole-12,13-dicarboxylate (8) was dissolved in THF:MeOH 1:1 solution and cooled to -78°C. LiBH$_4$ (15 equivalents) was added and let stir for 24 hours. The reaction was quenched with NH$_4$Cl, then diluted in water and extracted with ethyl acetate. The crude product was purified using preparative TLC and yielded a yellow oil. (21.9%, 3.2mg) TLC (2:1 hexanes: ethyl acetate) R$_f$=0.5; $^1$H NMR (300 MHz, CDCl$_3$) δ: 6.27 (s, 1H), 6.15 (s, 1H), 5.05 (s, 2H), 4.29 (s, 1H), 4.16-3.96 (m, 1H), 4.01-3.79 (m, 1H), 2.84-2.62 (m, 4H), 2.42 (d, J = 14.4 Hz, 1H), 2.30 (s, 3H), 2.26 (d, J = 14.4 Hz, 0H), 2.24-2.11 (m, 1H), 2.11 (s, 3H), 2.06 (s, 1H), 1.93-1.76 (m, 1H), 1.68 (s, 2H), 1.58 (s, 1H), 1.19 (t, J = 7.1 Hz, 2H). ESI m/z calculated for C$_{23}$H$_{32}$N$_2$O$_3$ [M+H]$^+$ 385.24, found 385.2.
References


