UNIVERSITY OF CALIFORNIA SAN DIEGO

The Antibacterial Activity of Tricyclic Gyrase (GyrB/ParE) Inhibitor: A New Class of Antibacterial Agents

A thesis submitted in partial satisfaction of the
requirements for the degree Master of Science

in

Biology

by

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2019

SIGNATURE PAGE

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Co-chair

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University of California San Diego

2019

DEDICATION

I want to dedicate my thesis to my parents who have been supporting and encouraging me throughout my life. I also want to dedicate my thesis to my friends who have helped and went through the defense process together with me.

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ACKNOWLEDGEMENT

I would like to acknowledge Professor Dionicio Siegel for his support as my committee chair. Prof. Siegel has helped me with my research approach and given me insights of my research. He has also advised me on scientific paper writing and much more. Without Prof. Siegel's support, I would not be able to come this far.

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Compound synthesis scheme in Material & Methods is coauthored with Srihari Konduri. The thesis author was the primary author of this part of the thesis.

ABSTRACT OF THE THESIS

The Antibacterial Activity of Tricyclic Gyrase (GyrB/ParE) Inhibitor: A New Class of Antibacterial Agents

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Professor Dionicio Siegel, Chair

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Growing antibiotics resistance and the limited amount of effective antibiotics against Gram-negative pathogens are the most alarming problems in clinic; therefore, the search for new board-spectrum antibacterial agents becomes an imminent task in the pharmaceutical industry.

Researches have shown that Tricyclic Gyrase (GyrB/ParE) Inhibitors are a new class of board-spectrum antibacterial agents which are effective against multi-drug resistant bacteria strains. Pharmaceutical companies like Trius had synthesized such inhibitors but none of the inhibitors has made into clinical trials yet due to various safety issues and solubility problems. In this research, new tricyclic gyrase inhibitors were synthesized by modifying the functional groups. Minimum Inhibitory Concentration (MIC) Assays were used to examine their board-spectrum antibacterial potencies against nine total bacteria strains, including multi-drug resistant strains. The results showed that two of the synthesized compounds, D18 and Tri-1, have board-spectrum antibacterial activities and works against multi-drug resistant strains. Compounds Tri-2, Tri-3, and Tri-4 showed activity against Gram-positive bacteria only while compound Tri-5 showed poor antibacterial activity possible due to the 2-Methylpyrimmdin.

INTRODUCTION

As the rate of multidrug-resistance is rapidly increasing among bacteria and other micro-organisms, the search for new and effective antibiotics becomes imminent. Nowadays some widely used drugs are losing their effectiveness on common Gram-positive bacteria. Meanwhile, there is still a lack of treatment against Gram-negative bacteria infections. The purpose of this research is to look for compounds with broad-spectrum antibacterial activities that could potentially become drug candidates for diseases caused by bacteria infections.

Today, there is a growing interest on inhibitors targeting bacterial ATPases. The use of this novel mechanism of inhibition started when Novobiocin was first commercialized. Novobiocin was a GyrB single-targeting inhibitor discovered in microbial natural products in the 1950s. It was used against penicillin-resistant *S. aureus*, but the usage soon declined after the emergence of new penicillinase-stable penicillins and repeatedly reported adverse effects from patients including Rash, hematological disorders, and gastro-intestinal intolerance. In 1969, FDA officially withdrew Novobiocin for medical uses. From the 1970s, many major pharmaceutical companies started the search for new gyrase inhibitors. Roche and Bristol-Myers soon discovered coumermycin A1 which had greater antibacterial potency against Gram-positive bacteria. Coumermycin A1 made it into Phase 1 of clinical trials, but the development was halted because of its poor solubility. Soon Bristol-Myers created an analog, BL-C43, with improved solubility, but unfortunately this project was terminated at Phase 1 as BL-C43 was causing Rash and other adverse effects much like Novobiocin did. No other gyrase inhibitors have made thus far as coumermycin A1 and BL-C43 in clinical trials. As Topoisomerase IV was discovered and the structures of ATPases were revealed by X-ray crystallography in the 1990s, the concept of "DNA gyrase and topoisomerase IV dual-inhibition" emerged and it was believed to be the key

for combating drug resistance. DNA gyrase & Topoisomerase IV dual-inhibiting inhibitor has gained research attention because of its novel mechanism of competitive inhibition of the ATPase. Both DNA gyrase and Topoisomerase IV are tetramers. DNA gyrase is consisted of two GyrA & two GyrB subunits. Topoisomerase IV is consisted of two ParC and two ParE subunits. Both GyrB and ParE subunits contain ATP-binding site, which is the site of action for gyrase inhibitors. The significance of this ATP binding site is that it contains a highly conserved Asp73 residue. This amino acid provides a hydrogen bonding between Asp73 and the adenine of ATP, which is also the key interaction between Asp73 and gyrase inhibitors. The fact that mutation almost never happens at Asp73 residue makes the ATP binding sites of GyrB and ParE excellent drug targets because drug-resistance is less likely to develop; therefore, duel-inhibition compounds, targeting both GyrB and ParE, could be a solution to treat multi-drug resistant bacteria infections. However, many factors have made this search for new antibiotics challenging. Scientists found Membrane permeability and aqueous solubility are two of the most difficult problems to solve as they were looking for the suitable drug candidates. Because the targeted sites are located in cytoplasm, the inhibitors must be able to penetrate the hydrophobic membrane while maintaining their aqueous solubility. For drugs against Gram-negative bacteria, the task becomes more difficult as Gram-negative bacteria possesses both outer and inner membranes. Scientists have synthesized many small and polar compounds to counter the problem, but eventually none of the compounds made it to the clinical trials. Among many of the compounds produced by different pharmaceutical companies, Trius's tricyclic gyrase inhibitor demonstrated excellent antibacterial activities against both Gram-positive and Gram-negative pathogens. This is possibly due to the interaction between the primary amine of the compounds and another conserved residue on the ATP binding site, Asn46. The interaction between the

primary amine and Asn46 water network results in a better binding affinity. Assays also have shown that Trius's scaffold has a good level of in vivo potency and low level of protein binding. However, no other pharmacokinetic and safety data were published. In this research, the goal is to look for new tricyclic gyrase inhibitors by modifying the functional groups on the scaffold. Both Gram-positive and Gram-negative pathogens were used to examine whether the new inhibitors possess broad-spectrum antibacterial activity. Multi-drug resistant bacteria strains were also used to test and compared the performance of the new inhibitors with a commercialized drug Levofloxacin.

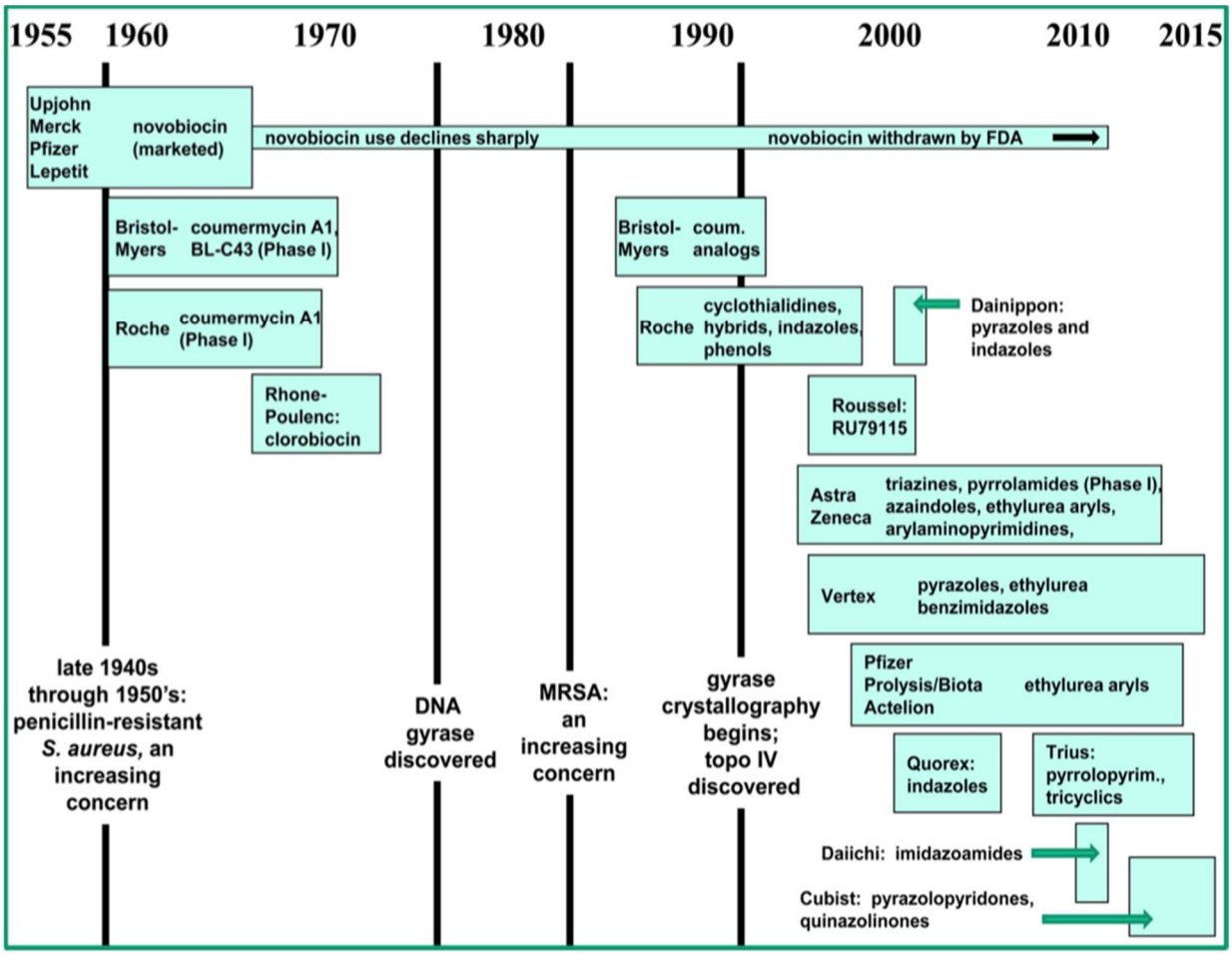
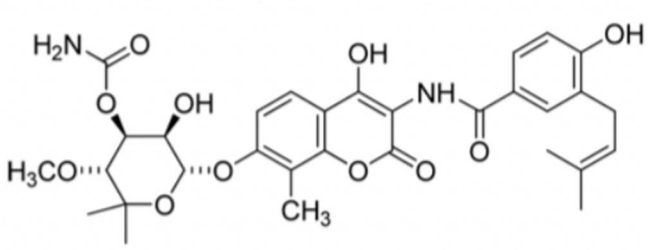
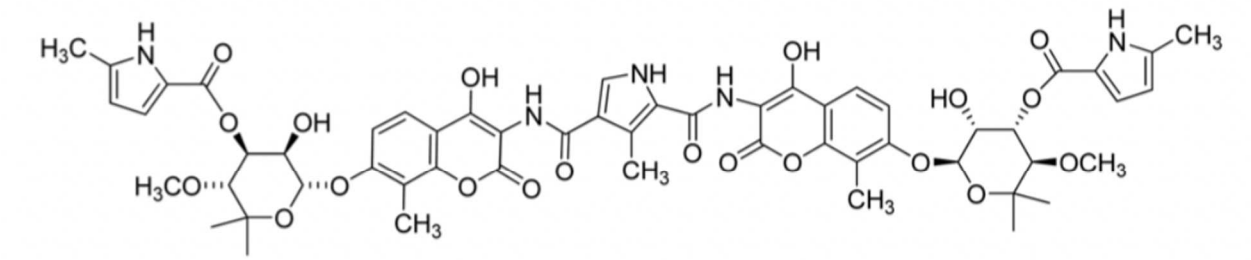


Figure 1. Timeline of GyrB/ParE Inhibitor Development and Important Research Events. Compounds proceeded to clinical trials were noted, which are coumermycin and BL-C43. Key research events relevant to GyrB/ParE inhibitor were also indicated. Figure was adapted from Bisacchi and Manchester, 2015.



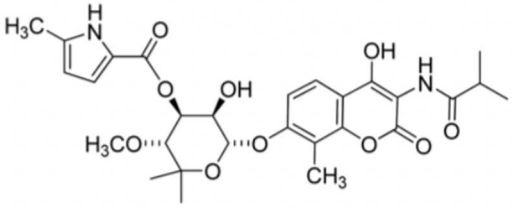
Novobiocin

Figure 2. Structure of Novobiocin. The drug was discovered by four pharmaceutical companies including Upjohn, Pfizer, Merck, and Lepetit during the 1950s. Novobiocin was commercialized under trade name Albamycin, but the usage declined sharply during the 1960s and it was officially withdrawn by FDA in 2011.



Coumermycin A1

Figure 3. Structure of Coumermycin A1 from Roche and Bristol-Meyers. The compound was an attempt for the search of new gyrase inhibitor by Roche and Bristol-Myers. Coumermycin A1 is a dimer of Novobiocin. It had greater potency against S. aureus and several Gram-negative bacteria than Novobiocin did.



BL-C43

Figure 4. Structure of BL-C43 from Bristol-Meyers. An improved version of coumermycin A1. BL-C43 showed good antibacterial potency as Novobiocin and coumermycin A1, but the project was terminated at Phase 1 because BL-C43 was causing similar adverse effects as Novobiocin did.

Table 1. MIC Values for Novobiocin and Coumermycin A1[a]. This table compares the MIC vales of Novobiocin's and coumermycin A1's. Coumermycin A1 demonstrated its potent antibacterial against Gram-positive bacteria and low protein binding in mice serum. Coumermycin A1 also has a better potency against Gram-negative bacteria than Novobiocin does, but coumermycin A1 was terminated in Phase 1 because of its low human efficacy.

	MIC (μg/mL)	
Bacterial Strain	**Novobiocin**	**coumermycin A1**
S. aureus 209P	0.05	0.0025
S. aureus 209P + 50% serum	3.2	0.16
S. aureus Smith	0.039	0.0012
S. aureus 52-34 (multi resistant)	0.78	0.0012
S. pneumoniae Type II	0.78	0.78
S. pyogenes Type 3	0.78	0.78
Neisseria sp.	12.5	12.5
E. coli ATCC 9637	50	6.25
S. flexneri	3.12	6.25
K. pneumoniae Type A	3.13	0.78
P. aeruginosa	100	12.5

[a]Data originated from Kawaguchi et al., 1965.

Table 2. MIC Values Comparison Between Novobiocin's and BL-C43's. BL-C43 had improved solubility but was not as potent as Novobiocin and coumermycin. Data was adapted from Godfrey and Price, 1972.

	MIC (μg/mL)	
Bacterial Strain	**Novobiocin**	**BL-C43**
S. aureus (Non-penicillinase producer)	<0.01	0.5
S. aureus (Penicillinase producer)	0.18	0.9
S. pneumoniae	1	3.2
S. pyogenes	0.5	2

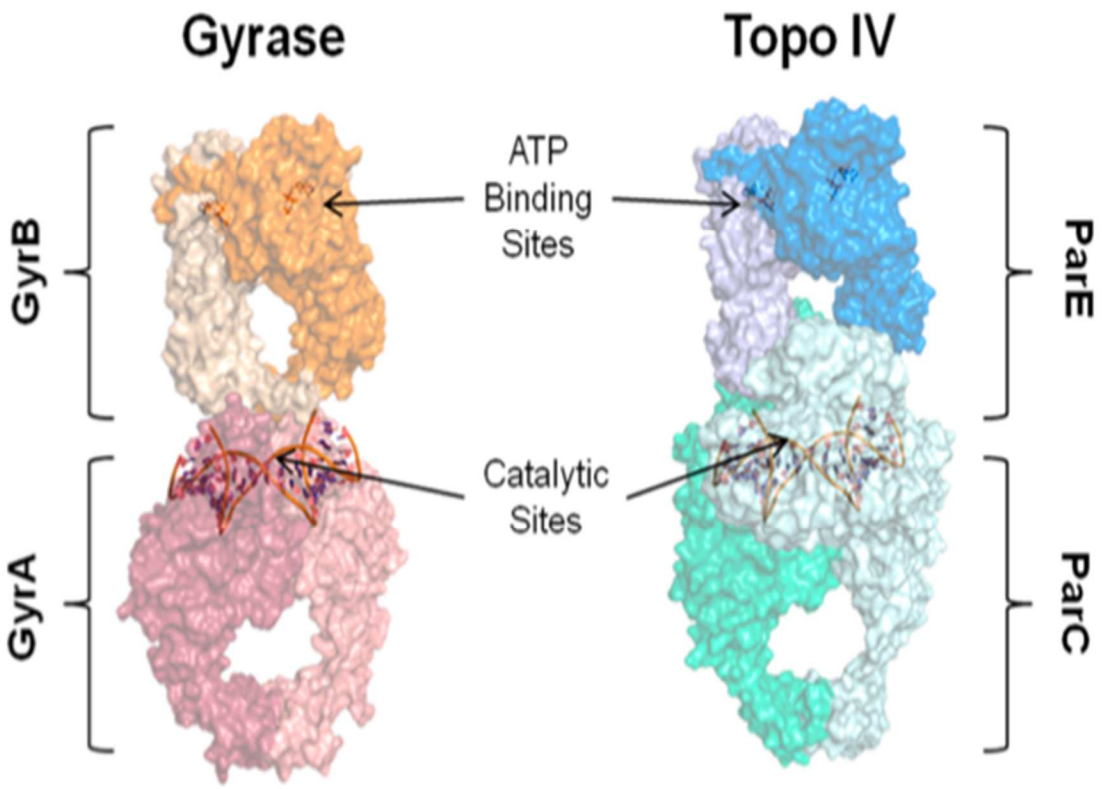


Figure 5. Structural Models of DNA Gyrase and Topoisomerase IV. The DNA gyrase structure is shown on the left and Topoisomerase IV structure is shown on the right. Different subunits are marked with different colors. GyrB and ParE subunits are marked with orange and blue respectively. GyrA and ParC are marked with red and green respectively. Arrows indicate ATP binding sites and catalytic sites within GyrB/ParE and GyrA/ParC respectively. Figure adapted from Laponogov, et al., 2009.

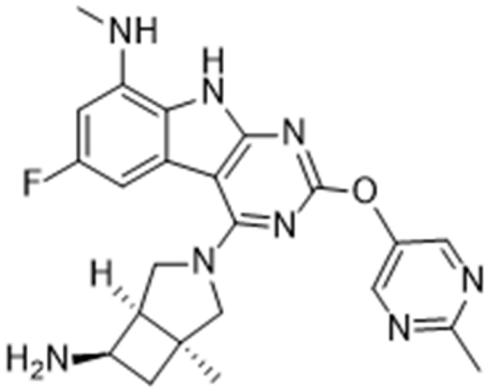


Figure 6. The Structure of Trius' Tricyclic Gyrase Inhibitor. The tricyclic gyrase inhibitor contains a tricyclic core and two functional groups attached to the core. Amines highlighted in red interact with Asp73 residue at the ATP binding site to form hydrogen bonds. The primary amine highlighted in yellow interacts with another conserved Asn46 residue at the ATP binding site.

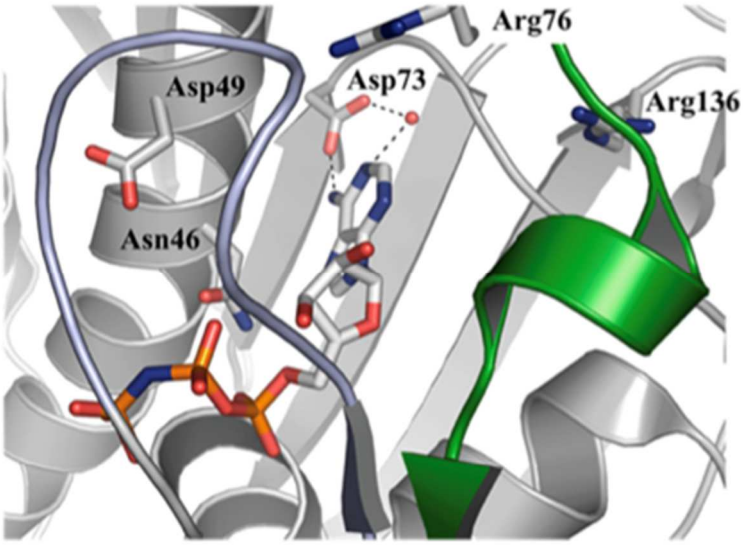


Figure 7. ATP Binding Sites of GyrB Bonding with nonhydrolyzable ATP analog, ADPNP (adenosine 5'-(β,γ-imido)triphosphate). Gyrase inhibitor functions the same as ADPNP, hydrogen bonding with the key and highly conserved Asp73 residue on the ATP binding site to inhibit the ATPase activity competitively. Another conserved residue, Asn46, interacts with the primary amine on Trius' tricyclic gyrase inhibitor to provide better selectivity. Figure adapted from Bax, et al., 2010.

RESULTS

To exam the antibacterial activities of test compounds, Minimum Inhibitory Concentration (MIC) assays were performed. The data of the assays are shown in the Table 3. Total eight compounds including two controls, Levofloxacin and Novobiocin, were tested against nine different bacterial strains. Levofloxacin and Novobiocin were selected as controls for different reasons. Levofloxacin was selected because of its extensive medical usage as a broad-spectrum antibiotic from the fluoroquinolone drug class. Direct comparison of the MIC values between the compounds and Levofloxacin demonstrates the broad-spectrum antibacterial activity of tested compounds. Novobiocin was selected because it is a GyrB single targeting inhibitor. Data compared between Novobiocin and tested compounds could show the capability of compounds at combating multi-drug resistance because of the dual-targeting mechanism. The bacteria strains used were *Group A streptococcus* (GAS 5448-M1T1), *Vancomycin-resistant Enterococcus* (VRE), *Staphylococcus aureus* (TCH 1516), *Methicillin-resistant Staphylococcus aureus* (MRSA-LAC), *Klebsiella pneumonia* (K 1100), *Acinetobacter baumannii* (AB 5075), *Acinetobacter baumannii* (AB 7978), and *Pseudomonas aeruginosa* (PA-01 & P4). Five out of nine strains were multidrug-resistant strains, which were MRSA-LAC, K 1100, AB 5075, VRE, and P4. Based on the table, Compound D18 and compound Tri-1 both demonstrated their better board-spectrum antibacterial potencies by having an overall lower MIC values against all tested strains except for *Pseudomonas aeruginosa* compared to MIC values of Levofloxacin and Novobiocin. D18 and Tri-1 also have shown excellent antimicrobial activities against multi-drug resistant strains compared to Levofloxacin and Novobiocin. For example, MIC value of D18 against Levofloxacin-resistant *Klebsiella pneumonia* (K 1100) is more than 211-fold lower than Levofloxacin's. MIC value of Tri-1 against Levofloxacin-resistant *Acinetobacter baumannii* (AB

5075) is 26-fold lower than Levofloxacin's. Compound Tri-2, Tri-3, and Tri-4 showed good antimicrobial potency against Gram-positive bacteria and their multi-drug resistant types but failed to show the potential of a board-spectrum antibacterial agent. This loss of broad-spectrum activity is possibly due to the structural change of the bicyclic amines attached on the tricyclic core. Data also suggests Tri-2 were more potent against Gram positive bacteria by having a lower MIC value compared to others. Tri-5 showed no potential of an antibacterial agent against neither Gram positive or Gram-negative bacteria possible due to the loss of 2-Methylpyrimmdin.

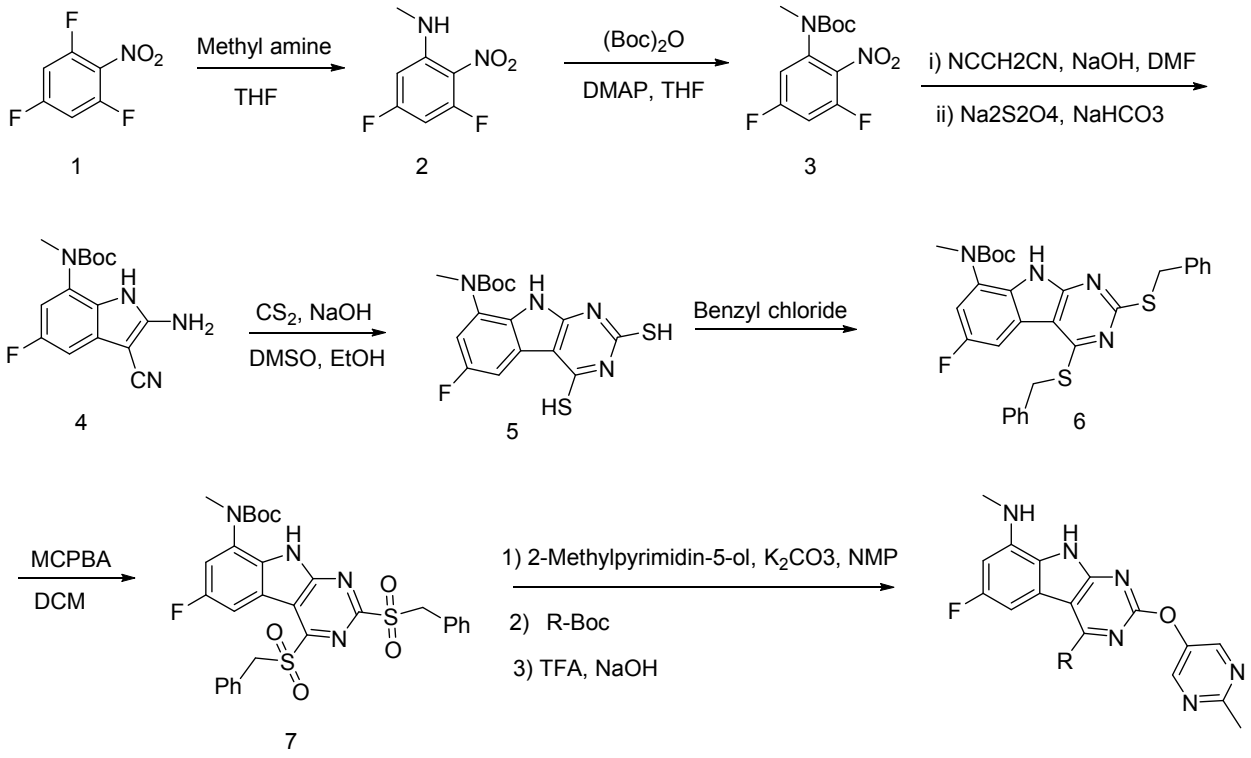
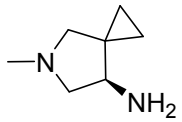
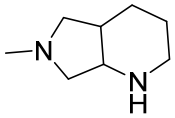
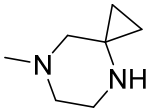
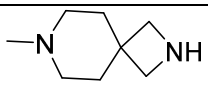


Figure 8. Chemical Synthesis Scheme for Tricyclic Gyrase Inhibitors With 2-Methylpyrimmdin-5-ol. The synthetic scheme shows the general procedure of synthesizing the tricyclic gyrase inhibitor with 2-Methylpyrimmdin-5-ol. Five out of six compounds synthesized in this research have the same general structure as shown at the last step in the scheme. This general synthetic procedure was adapted from Bensen, et al., 2012.

Table 3. Compound Code of Five Tricyclic Gyrase Inhibitors Synthesized. The table lists the compound code of synthesized tricyclic gyrase inhibitors with corresponding functional group attached to the structured shown at the last step of the above figure. Compound D18 was originated from Bensen, et al., 2012. Compounds Tri-1, Tri-2, Tri-3, and Tri-4 are new analogs.

S. No	Compound Code	R=
1	D18	
2	Tri-1	
3	Tri-2	
4	Tri-3	
5	Tri-4	

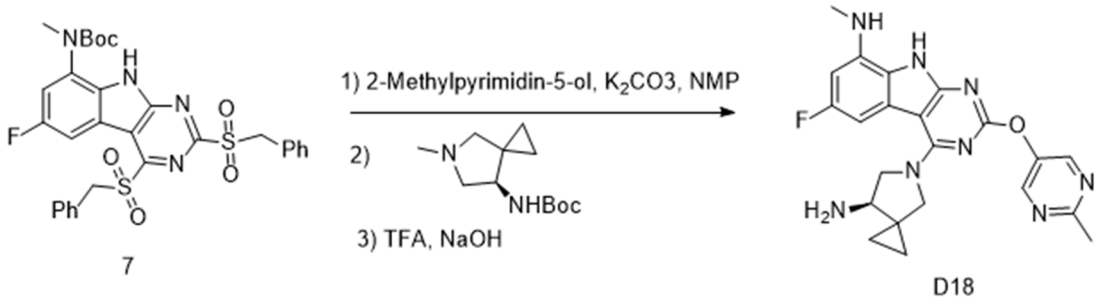


Figure 9. Synthetic Scheme For D18. Followed by the general scheme shown in Figure 8, D18 was obtained from the above reaction.

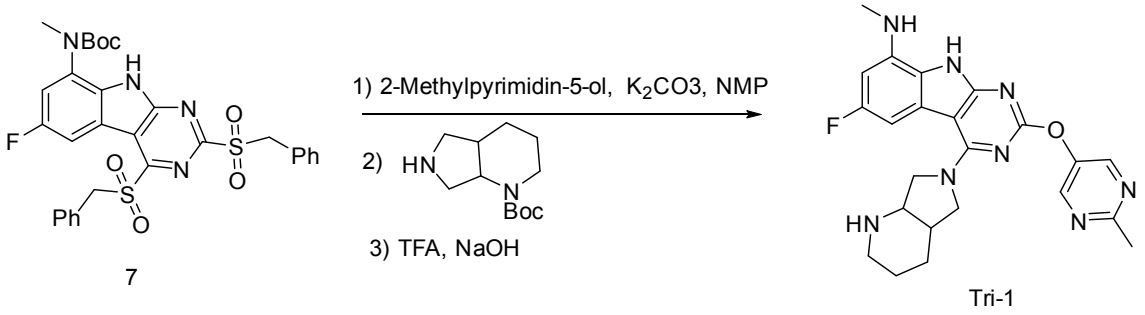


Figure 10. Synthetic Scheme For Tri-1. Followed by the general scheme shown in Figure 8, Tri-1 was obtained as a light brown solid from the above reaction.

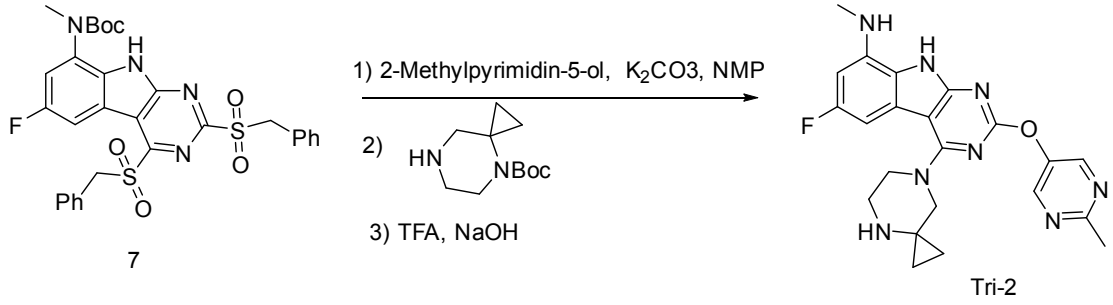


Figure 11. Synthetic Scheme For Tri-2. Followed by the general scheme shown in Figure 8, Tri-2 was obtained as a pale yellow solid from the above reaction.

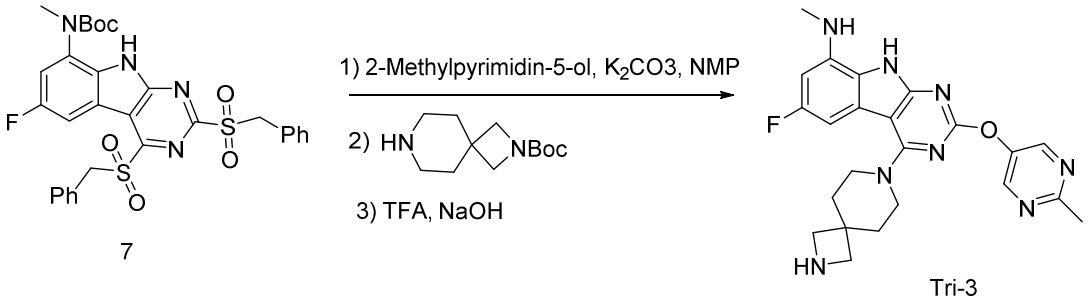


Figure 12. Synthetic Scheme For Tri-3. Followed by the general scheme shown in Figure 8, Tri-3 was obtained as a light brown solid from the above reaction.

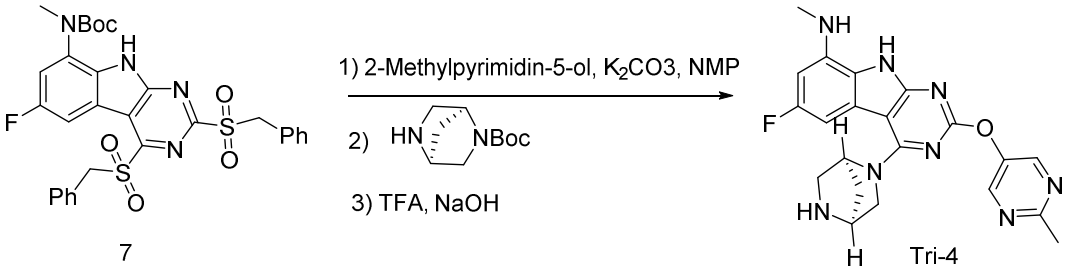


Figure 13. Synthetic Scheme For Tri-4. Followed by the general scheme shown in Figure 8, Tri-4 was obtained as a light brown solid from the above reaction.

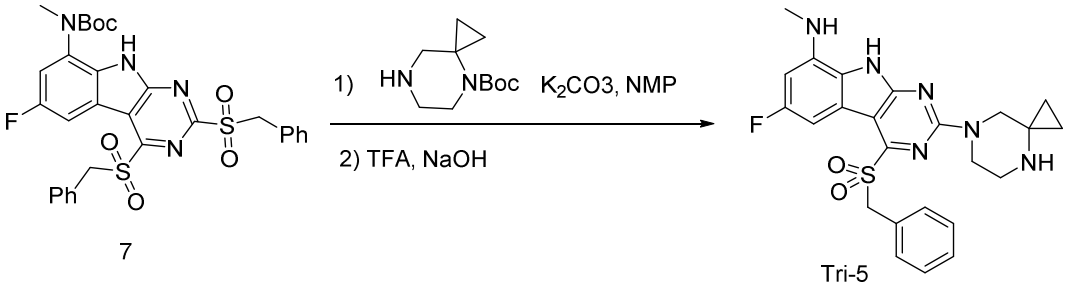


Figure 14. Synthetic Scheme For Tri-5. Followed by the general scheme shown in Figure 8, Tri-5 was obtained as a yellow solid from the above reaction.

Table 4. MIC Assay Results of Six Tested Compounds and Two Control Drugs Against Nine Bacteria Strains. MIC values were determined by using broth microdilutions according to the Clinical Laboratory Standards Institute (CLSI) guidelines. All compounds including two control compounds, Novobiocin and Levofloxacin, were tested against nine bacteria strains. Note that Levo-R indicates multidrug-resistance (including Levofloxacin) strains, and Levo-S indicates Levofloxacin-sensitive strains. Compounds Tri-5 was not tested against all strains because of its poor antibacterial activity against tested strains. (Including a drug sensitive strain)

	MIC (μg/mL)							
Bacterial Strain	**Levofloxacin**	**Novobiocin**	**D18**	**Tri-1**	**Tri-2**	**Tri-3**	**Tri-4**	**Tri-5**
Staphlococcus aureus (TCH 1516- LEVO-S)	0.141	0.239	1.36	0.089	0.34	1.4	5.25	48
Methicillin-resistant Staphylococcus aureus- LAC-LEVO R	9	0.239	0.087	0.35	0.68	1.4	5.25	-
Group A streptococcus 5448-M1T1	< 0.018	0.956	< 0.022	0.089	0.087	0.7	2.63	48
Klebsiella pneumonia K1100- LEVO-R	> 36	61.3	0.17	5.6	> 43.4	> 44.8	> 42	-
Acinetobacter baumannii 5075 LEVO-R	9	7.66	2.72	0.35	2.71	22.4	21	-
Acinetobacter baumannii 7978- LEVO-S	4.5	> 61.3	2.72	5.6	43.4	> 44.8	> 42	-
Vancomycin-resistant Enterococcus	> 36	0.956	< 0.022	0.089	0.043	0.17	0.655	> 48
Pseudomonas aeruginosa (PA-01)	0.564	> 61.3	5.44	11.2	> 43.4	> 44.8	> 42	> 48
*Pseudomonas aeruginosa (*P4- LEVO -R)	36	> 61.3	10.9	22.4	> 43.4	> 44.8	> 42	-

DISCUSSION

The fast-growing rate of antibiotics resistance and the lack of effective antibiotics against Gram-negative bacteria are the most concerning problems in the clinics. Looking at the history of antibiotics, Novobiocin was once a popular first line antibiotics used during the 1960s, but the usage declined sharply after a decade as adverse effects were reported by many patients. Although the drug itself was not successful enough, its novel mechanism of action caught much attention from many scientists. Different from quinolone antibiotics, a group of widely used antibiotics that are effective against both Gram-positive and Gram-negative bacteria, Novobiocin competitively inhibits ATPase of the GyrB subunit of DNA gyrase whereas quinolone antibiotics targets GyrA subunits of DNA Gyrase and ParC subunits of Topoisomerase IV. This new mechanism of action made Novobiocin a more potent antibacterial agent because it targets the conserved ATP binding site of GyrB. While Novobiocin was potent against Gram-positive bacteria, especially against *S. aureus*, it had poor activity against Gram-negative bacteria possibly due to its large size which prevented the molecule from entering both the inner and outer membrane. Since the usage of Novobiocin declined, companies have been searching for new ATPase inhibitors, and the idea of GyrB/ParE dual-targeting inhibitors emerged as Topoisomerase IV was discovered. Targeting two conserved ATP binding sites simultaneously results in a greater antibacterial potency and less chance of multi-drug resistance development; therefore, scientists believes that dual-targeting inhibitors have the potential of becoming the new class of board-spectrum antibacterial agents. Although most published compounds demonstrated good antibacterial potency, many of them suffered from poor membrane penetration, low aqueous solubility, and *in vivo* safety issues. Hence, none of the compounds has proceeded to clinical trials yet. Among all of the published compounds, Trius' tricyclic gyrase

inhibitor showed excellent activities across the spectrum. (Table)) The inhibitor also showed low protein binding and high *in vivo* potency in mice models. However, safety data of this inhibitor was insufficient, and no other pharmacokinetic data was released. Nevertheless, Trius' tricyclic gyrase inhibitor remains to be the one that has the most potential. In this research, the core three ring structure of Trius' compound was maintained while the functional groups attached were modified. Newly synthesized tricyclic gyrase inhibitors were then tested against both Gram-positive and Gram-negative bacteria including several of the multi-drug resistant types. Based on the results of MIC assays, those modifications yielded two drug candidates as board-spectrum antibacterial agents, Compound D-18 and Tri-1. Both compounds demonstrated their broad-spectrum antibacterial potency with D-18 having a slightly better overall activity, especially against Gram-negative bacteria. Both compounds were more effective against the multi-drug resistant types than the controls did. Compounds Tri-2, Tri-3, and Tri-4 were proved to be effective against Gram-positive bacteria, including the multi-drug resistant types. The data shows Tri-2 are more potent than Tri-3 and Tri-4 by having a much lower MIC value. Tri-5 showed Poor activity possible due to the loss of 2-Methylpyrimmdin on the tricyclic core.

MATERIALS AND METHODS

Compound Synthesis Scheme

General Procedure of the Tricyclic Gyrase Inhibitor Synthesis

2-methylpyridin-5-ol (3.6 equiv.) was added to a stirred solution of the Bi sulfone compound 7[1] (1 equiv.) in NMP (4.2 mL/mmol compound 7) in a seal tube at 23 °C. After 5 minutes of stirring, the reaction was clear. Solid K_2CO_3 (3.6 equiv.) was added in a single portion to the reaction mixture. The reaction mixture was heated to 100°C and stirred for 1 hour. Boc-protected amine (R-) (1.8 equiv.) was then added at once and maintained for 1 hour at 100°C. Water (15 mL/mmol compound7) was added and stirred for 15 mins after the mixture was cooled to room temperature. The compound was filtered and washed with water. The solid compound was purified via silica gel column chromatography using Hexanes: EtOAc (50% to 80%) to get the boc-protected compound.

The resulting compound was added in a single neck RBF and added with TFA (34 equiv.) at room temperature. After five minutes of stirring, solvent was removed under vacuum. Water and ethanol (4:1) were added with stirring. NaOH solution (1N) was slowly added up to pH 10. After stirring, the solid was filtered and washed with water twice. The final compound was obtained after drying under vacuum.

Tri-1 Synthesis Scheme

6-fluoro-N-methyl-2-((2-methylpyrimidin-5-yl)oxy)-4-(octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-9H-pyrimido[4,5-b]indol-8-amine (Tri-1): Following general procedure for compound **7** and corresponding Boc protected amine was transformed into compound Tri-1 (65%) as a light brown color solid. **^{1}H NMR** (600 MHz, DMSO) δ 8.70 (s, 2H), 7.01 (d, *J* = 10.7

Hz, 1H), 6.28 (d, *J* = 11.8 Hz, 1H), 5.54 (d, *J* = 3.8 Hz, 1H), 3.92 (m, 2H), 3.66 (m, 2H), 2.89 (d, *J* = 11.0 Hz, 1H), 2.84 (s, 3H), 2.66 (s, 3H), 2.57 (t, *J* = 10.8 Hz, 1H), 2.35 (s, 1H), 1.74 (d, *J* = 11.2 Hz, 1H), 1.70 – 1.58 (m, 2H), 1.45 (m, 1H), 1.21 (m, 1H). **^{13}C NMR-DEPTQ** (151 MHz, DMSO) δ 163.28, 161.06, 159.90, 158.38, 158.27, 157.13, 150.86, 146.27, 136.49, 136.40, 120.65, 119.16, 119.07, 94.96, 94.19, 94.16, 91.57, 50.43, 44.08, 29.89, 25.06, 22.39, 20.49. **HRMS** (ESI) calc. for $C_{23}H_{26}FN_8O$ $[M+H]^+$: 449.2208, obs. 449.2209.

Tri-2 Synthesis Scheme

6-fluoro-N-methyl-2-((2-methylpyrimidin-5-yl)oxy)-4-(4,7-diazaspiro[2.5]octan-7-yl)-9H-pyrimido[4,5-b]indol-8-amine (Tri-2): Following general procedure for compound **7** and corresponding boc protected amine was transformed into compound Tri-1 (58%) as a pale yellow color solid. **^{1}H NMR** (600 MHz, DMSO) δ 8.71 (s, 2H), 6.61 (s, 1H), 6.34 (d, *J* = 11.2 Hz, 1H), 5.58 (m, 1H), 3.67 (m, 2H), 3.51 (m, 2H), 2.86 (m, 5H), 2.66 (s, 3H), 1.23 (s, 2H), 0.36 (m, 4H). **^{13}C NMR-DEPTQ** (151 MHz, DMSO) δ 163.44, 161.36, 160.69, 159.92, 158.40, 157.83, 150.97, 146.24, 136.70, 120.75, 118.73, 95.46, 94.48, 54.13, 48.95, 44.59, 37.13, 29.88, 25.05, 11.95. **HRMS** (ESI) calc. for $C_{22}H_{24}FN_8O$ $[M+H]^+$: 435.2052, obs. 435.2051.

Tri-3 Synthesis Scheme

6-fluoro-N-methyl-2-((2-methylpyrimidin-5-yl)oxy)-4-(2,7-diazaspiro[3.5]nonan-7-yl)-9H-pyrimido[4,5-b]indol-8-amine (Tri-3): Following general procedure for compound **7** and corresponding boc protected amine was transformed into compound Tri-1 (68%) as a light brown color solid. **^{1}H NMR** (600 MHz, DMSO) δ 8.72 (s, 2H), 6.60 (d, *J* = 8.7 Hz, 1H), 6.34 (d, *J* = 11.9 Hz, 1H), 5.65 (s, 1H), 3.58 (m, 8H), 2.84 (s, 3H), 2.66 (s, 3H), 1.84 (m, 4H), 1.21 (s,

1H). **^{13}C NMR-DEPTQ** (151 MHz, DMSO) δ 163.54, 161.38, 161.13, 160.14, 158.61, 157.83, 150.93, 146.33, 136.84, 120.94, 118.91, 118.83, 96.16, 94.51, 92.48, 55.33, 44.53, 34.77, 29.98, 29.19, 25.13, 12.69. **HRMS** (ESI) calc. for $C_{23}H_{26}FN_8O$ $[M+H]^+$: 449.2208, obs. 449.2205.

Tri-4 Synthesis Scheme

4-((1S,4S)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-6-fluoro-N-methyl-2-((2-methylpyrimidin-5-yl)oxy)-9H-pyrimido[4,5-b]indol-8-amine (Tri-4): Following general procedure for compound **7** and corresponding boc protected amine was transformed into compound Tri-1 (61%) as a light brown color solid. **^{1}H NMR** (600 MHz, DMSO) δ 8.69 (s, 2H), 6.83 (d, *J* = 10.5 Hz, 1H), 6.29 (d, *J* = 11.7 Hz, 1H), 5.58 (s, 1H), 4.80 (s, 1H), 4.05 (m, 1H), 3.78 (m, 1H), 3.57 (d, *J* = 7.6 Hz, 2H), 3.04 (d, *J* = 8.1 Hz, 1H), 2.95 (m, 1H), 2.83 (s, 3H), 2.65 (s, 3H), 1.76 (dd, *J* = 69.5, 8.7 Hz, 2H), 1.21 (s, 1H). **^{13}C NMR-DEPTQ** (151 MHz, DMSO) δ 163.34, 161.08, 159.96, 158.44, 157.75, 157.42, 150.87, 146.29, 136.55, 120.71, 118.87, 94.68, 94.54, 91.88, 59.32, 55.93, 51.02, 36.01, 29.86, 25.06. **HRMS** (ESI) calc. for $C_{21}H_{22}FN_8O$ $[M+H]^+$: 421.1895, obs. 421.1890.

Tri-5 Synthesis Scheme

4-(benzylsulfonyl)-6-fluoro-N-methyl-2-(4,7-diazaspiro[2.5]octan-7-yl)-9H-pyrimido[4,5-b]indol-8-amine (Tri-5): To a stirred solution of the Bi sulfone compound **7** (1 equiv.) in NMP (4.2 mL/mmol compound 7) in a seal tube at 23 °C was added the boc protected amine (2.0 equiv.) and solid K_2CO_3 (3.6 equiv.) simultaneously in a single portion to the reaction mixture. The reaction mixture was heated to 100°C and stirred for 1 hour. After completion of 1 hr cooled to RT and add water (15 mL/mmol compound7), stirred for 15 mins. Filtered the compound and wash with water. The solid compound was purified via silica gel column chromatography using Hexanes: EtOAc (30% to 60%) to get the bocprotected compound.

Take the above compound in single neck RBF and add TFA (34 equiv.) at RT. Stirred for 5 minutes and removed the solvent under vacuum. Then added water and ethanol (4:1) and stirred. Added NaOH solution (1N) slowly up to PH> 10. Stirred and filtered the solid, washed with water twice. Dried the compound under vacuum. To get the Tri-5 compound (72 %) as a yellow color solid.

Compound synthesis scheme in Material & Methods is coauthored with Srihari Konduri. The thesis author was the primary author of this part of the thesis.

Bacterial Strains/Culturing

Nine different bacterial strains were used to exam the antibacterial activities of tested compounds, including four Gram positives and five Gram negatives. Gram positive bacteria are *Group A streptococcus* (GAS 5448-M1T1), *Vancomycin-resistant Enterococcus* (VRE), *Staphlococcus aureus* (TCH 1516), and *Methicillin-resistant Staphylococcus aureus* (MRSA-LAC), whereas TCH 1516 is Levofloxacin sensitive and MRSA-LAC is Levofloxacin resistant. Gram negative bacteria are *Klebsiella pneumonia* (KB 1100), *Acinetobacter baumannii* (AB 5075), *Acinetobacter baumannii* (AB 7978), and *Pseudomonas aeruginosa* (PA-01 & P4), whereas KB 1100, AB 5075, and P4 are Levofloxacin resistant and AB 7978 is Levofloxacin sensitive.

Minimum Inhibitory Concentration Assays

Test compounds were prepared as stock solutions by dissolving into 100% dimethylsulfoxide (DMSO). Broth microdilution were used to determine MIC values, and Ca-MHB (Mueller Hewitt broth) was used as media according to the Clinical Laboratory Standards

Institute (CLSI) guidelines. Bacteria were grown to mid-log phase (OD_{600nm} = 0.4) at 37°C with constant shaking. Then bacteria were diluted in MHB to $2x10^6$ cfu/ml, and 10 µl of it was added to each well of a 96-well assay plate containing 170 µl of MHB. Stock solutions were diluted from 1000 µM to 0.5 µM in dilution plates by two-fold dilution. 20 µl of compound solution at each concentration were added to the assay plate to give a final compound concentration ranges from 100 µM to 0.05 µM. Each well with the same concentration was repeated three times to eliminate experimental uncertainty. Assay plates were covered with parafilm and incubated at 37°C for 24 hours. After incubation, plates were read at OD_{600nm} using a VersaMax plate reader. The MIC values were determined by recording the lowest concentration of compound which inhibited bacteria growth.

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