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Inhibition of Neisseria gonorrhoeae Replication Restart Pathways
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Abstract
DNA replication is essential for the survival of all living organisms. DNA damage that occurs in bacterial cells can lead to cell death by inhibiting the replication of genetic material. Bacteria, such as Neisseria gonorrhoeae, have developed a method to avoid cell death due to premature termination of DNA replication called a DNA replication restart pathway. Previous research has determined that there are two proteins, PriA and PriB, which play an important role in this pathway. Collectively these proteins bind to the DNA and act as enzymes, facilitating the reloading of the replication machinery and initiating replication without an origin sequence. This study explored possible inhibitors to the function of PriA and PriB and explored the method through which these inhibitors function. The inhibitors studied could potentially be developed into novel antibiotics against N. gonorrhoeae.

Introduction
DNA replication is essential to the survival of all living organisms. DNA replication is catalyzed by a number of enzymes. A class of enzymes called helicases function to unwind and separate the double helices into two individual strands of genetic material. The replicative helicase binds to the double stranded DNA, unwinding it to create a “replication fork”. DNA polymerases catalyze the addition of nucleotides to the individual strands of the DNA (1). This process will continue until the full chromosome is replicated, after which the DNA polymerase dissociates from the DNA leaving two full chromosomes. However, DNA damage can lead to termination of DNA synthesis before the whole chromosome is replicated (2). The termination of DNA synthesis often leads to the collapse of the replication fork, which then calls on repair pathways to perform their function to ensure that the chromosome is replicated (3). If a cell is not able to replicate its genetic material, the cell will die.

Discovery of Lead Compounds and Validation

Helicase Assay and High Throughput Screening
This functional assay was performed to demonstrate the ability of PriA and PriB to catalyze the hydrolysis of ATP. This assay used an ATP regeneration system that converts ADP to ATP in a reaction that is coupled to the conversion of NADH to NAD+. The coupled reaction was monitored spectrophotometrically by measuring the decrease in absorbance at 340 nm due to NADH oxidation. This spectrophotometric assay was used to see if the inhibitors affect PriA-catalyzed ATP hydrolysis.

Characterization of Lead Compounds

Equilibrium Binding Assay
This functional assay was performed to demonstrate the ability of PriA to bind to Fork 1 DNA and the ability of PriB to bind to ssDNA oligo (ssDNA oM6228) using fluorescence polarization spectroscopy to test protein-DNA interactions. This assay measured the rates of rotational diffusion, or tumbling, as a read-out of complex formation, where small molecules have low polarization because they tumble slower than larger molecules, which have higher polarization.

Equilibrium Binding Assay – PriB with ssDNA oM6228
Average Kd = 47.1 ± 6.9 nM

Results
Helicase assays were performed in the presence of inhibitors to determine the IC50 concentration of the PriB inhibition. This data was used to determine the IC50 concentration of the PriA inhibition, which was then used in the additional functional assays to determine the mode of inhibition.

Conclusions
This study has shown that PriA functions as an inhibitor to the helicase activity of PriA and PriB which was shown in the Helicase assays and IC50 determination. CGS 15943 affects PriA’s ability to bind to DNA while having little to no effect on PriB’s DNA binding which was shown in the Equilibrium binding assay. CGS 15943 is not a competitive inhibitor based on the ATPase results. CGS 15943 is either a mixed or uncompetitive inhibitor – it either binds to the enzyme on a site distinct from the active site, binds to the enzyme – substrate complex, or both.

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References