Targeting Drug Resistant Bacteria: Deoxynybomycin and its Derivatives

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Introduction

Antibiotic resistant bacteria are becoming an increasing threat in medicine as the heavy selective pressure from clinical use continues. The discovery and synthesis of new classes of antibiotics is vital to solving this worldwide problem.

Fluoroquinolones were first introduced to treat gram-positive and gram-negative infections. The mechanism of action for fluoroquinolones is to target bacterial type IIA topoisomerases. More specifically, DNA gyrase is typically targeted in Gram-negative bacteria, while topoisomerase IV is more commonly targeted in Gram-positive bacteria. Both DNA gyrase and topoisiomerase IV modulate levels of DNA supercoiling in bacteria. Inhibition of these enzymes prevents bacterial DNA replication and causes bacterial death. The over prescription and overuse of fluoroquinolones has led to widespread resistance to the antibiotic. Much of this resistance originates from point mutations in the GyrA subunit of DNA gyrase and the ParC subunit of topoisomerase IV. These mutations in the GyrA subunit and the ParC subunit result in a decreased binding affinity of fluoroquinolone to its target.

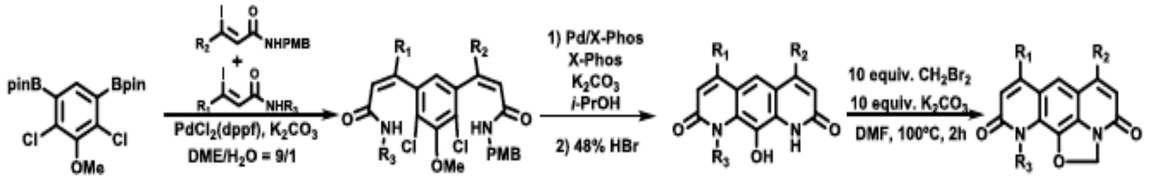
A compound, deoxynybomycin (DNM), was first synthesized when trying to determine the structure of nybomycin. DNM was found to have antibacterial activity against a range of bacteria, including fluoroquinolone-resistant MRSA that contains a S84L mutation in the GyrA subunit of DNA gyrase. This activity led to further studies to explore the possibility of a new class of antibiotics that are able to target resistance mechanisms, like those present in fluoroquinolone resistant bacteria.

Objectives

- Synthesize derivatives of DNM to optimize the potency and solubility of the compound
- Perform antibiotic susceptibility tests to determine if the derivatives of DNM are effective against fluoroquinolone resistant bacteria by targeting the resistance mechanisms of these bacteria
- Determine if wild type gram-positive bacteria that lack the S84L mutation in DNA gyrase and ParC S80F mutation in topoisomerase IV develop resistance to DNM derivatives

Method

A modular synthetic route was first used by Dr. Elizabeth Parkinson to access several DNM derivatives. The synthesis begins with a Suzuki cross coupling of two different iodoamides that have modifications depending on the DNM derivative being synthesized. This cross coupling is performed with a symmetrical aryl boronate, and results in both symmetrical and asymmetrical products. The asymmetrical product is identified and separated, and a Buchwald-Hartwig ring closing mechanism catalyzed by palladium is performed. The resulting diazanthracene is then deprotected by adding HBr, revealing a dianthracenol. A methylene bridge is then inserted using a compound that will result in the desired derivative. The synthesis of these derivatives takes 7 steps and results in an ~11% total yield.



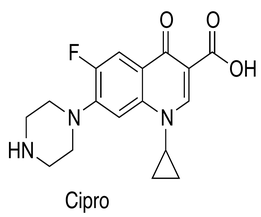
Antibiotic susceptibility for all strains of bacteria used was performed in triplicate using the microdilution broth method as outlined by the Clinical and Laboratory Standards Institute. Each well had 2 µL of 50x stock solution containing the compounds being tested, except the live and dead control cells which had vehicle instead. MH Broth was added to each well and bacteria with a concentration of $5x10^5$ cfu/mL was added to each well except for the dead control cells. Each well of the well plate had a volume of 100 µL and a concentration of $5x10^5$ cfu/mL. After incubation, the results were read on a Molecular Devices SpectraMax Plus 384 Microplate reader at λ=600nm. MIC values were defined by the lowest concentration to result in >90% growth inhibition.

Results

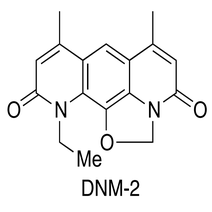
For the antibiotic susceptibility tests, a control strain of bacteria, ATCC 29213 (WT), was used and had no known resistance to any of the compounds tested. NRS3 (MRSA), was used and has GyrA S84L and ParC S80F mutations, which confer resistance to a variety of fluoroquinolones, including Ciprofloxacin. Four compounds were tested against these two strains of bacteria. Ciprofloxacin was used as a control to test the effectiveness of our synthesized compounds in treating resistant bacterial strains. DNM-2, APR-II-231, and OP were experimental DNM derivatives tested. The structures of all 4 compounds are shown below.

Minimum Inhibitory Concentration of Compounds (µg/mL)

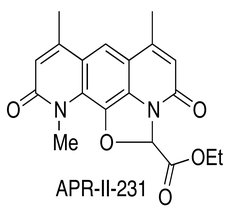
	Compound			
Bacterial Strain	Ciprofloxacin	DNM-2	APR-II-231	OP
ATCC 29213	0.12	4.0	>16.0	>32.0
NRS3 (MRSA)	16.0	0.03	0.5	0.5



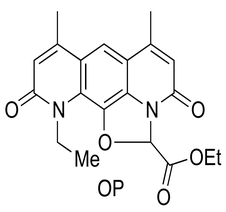
Cipro



DNM-2



APR-II-231



OP

Conclusions

When the wild type ATCC 29213 bacterial strain was treated with Ciprofloxacin, the MIC value showed that a relatively low amount of the compound was sufficient to inhibit the bacterial growth, while the MICs for DNM-2, APR-II-231, and OP were significantly higher. The opposite was found when the resistant NRS3 (MRSA) strain was treated with Ciprofloxacin, as the MIC was comparable to APR-II-231 in the wild type strain. DNM-2, APR-II-231, and OP, on the other hand, were found to have low MIC values, indicating that low amounts of these compounds were effective and sufficient in inhibiting bacterial growth. These results also support the idea that bacteria lacking the S84L mutation in DNA gyrase are able to develop resistance to DNM derivatives. We can conclude that the mutation of the serine to leucine or isoleucine is vital in bacteria's sensitivity to DNM and its derivatives. With this knowledge, a resistance/sensitization cycle can be applied to DNM and fluoroquinolones to optimize the sensitivity of the bacteria being treated with the two compounds and to help ensure that the bacteria will still be killed even if resistance emerges.

References

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