4-17-2013

Identification of a compound that disrupts the primosome function in Neisseria gonorrhoeae DNA replication restart

Identification of a compound that blocks the primosome function in *Neisseria gonorrhoeae* DNA Replication Restart

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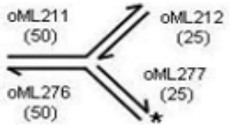
Abstract

The effects of two lead compounds, Penicillin G potassium salt and Paroxetine Hydrochloride hemihydrate, on *Neisseria gonorrhoeae* DNA replication restart pathway were investigated. DNAunwinding assays and steady state kinetics wereused to evaluate the extent of inhibition of each compound. DNA unwinding assays and steady state kinetics suggest that paroxetine inhibits PriA catalyzed DNA replication restart.

Fork 2 DNA

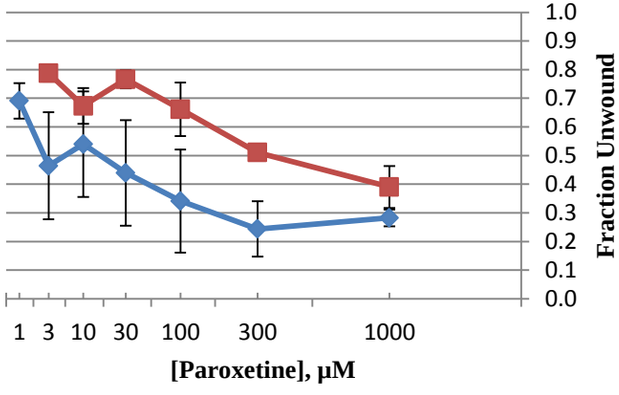


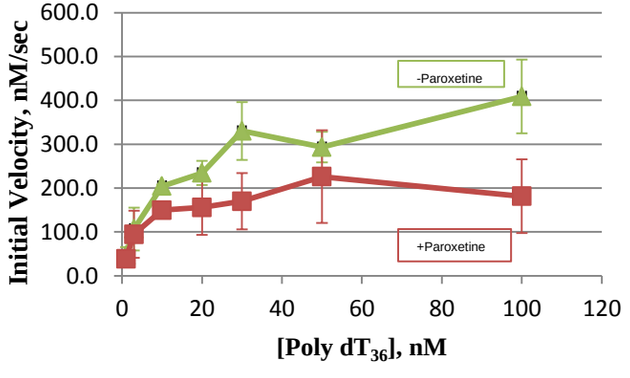
Introduction

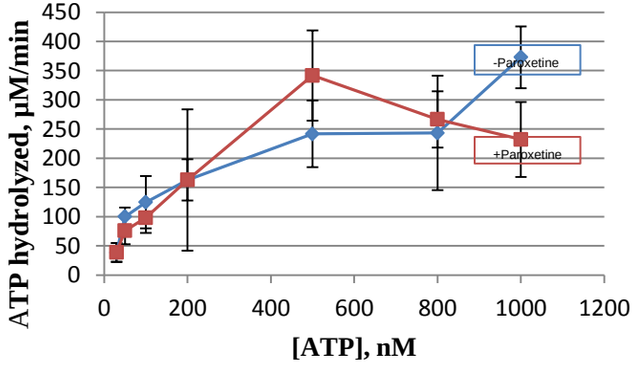
Evidence that PriA plays an important role in DNA repair and how it provides resistance to oxidative agents emphasize the importance of DNA replication restart for the survival of this bacteria. Developing antibacterial compounds that target this pathway could lead to vital discoveries in the field of medicinal research. This study under taken to understand the mechanistic features of inhibition these lead compounds

Materials and Methods

- Helicase Assay Buffer (stored at 4°C)
- Fork 2DNA(stored at -80°C)
- ATP, PriA and PriB proteins
- Unwinding assays were performed using protocol 1.4 and 3.7
- Degree of unwinding was quantified using fluorescence anisotropy.
- IC_{50} was determined for paroxetine
- Steady state trends were investigated using a coupled spectrophotometric assay







Results

DNA Unwinding Assays

- Paroxetine disrupts PriA's DNA
- Unwinding ability
  - Degree of unwinding
  - decreases with concentration
- IC_{50} was determined

ATP Hydrolysis Assays

- Kinetic parameters were determined for two profiles
- Paroxetine exhibits a competitive mode of inhibition on the PriA:DNA complex

	-Paroxetine	+ Paroxetine
$V_{max, DNA}$, nM/sec	426 ±145	222 ±111
$K_{m, DNA}$, nM	11 ±10	6.5 ±5.8
k_{cat}, s^{-1}	42±14	22±11

	- Paroxetine	+ Paroxetine
$V_{max, DNA}$, nM/sec	415 ± 128	270 ±9
$K_{m, DNA}$, nM	161±117	52±5
k_{cat}, s^{-1}	41 ±12	27 ±1

Future work

- Reevaluate the inhibitory properties of the first lead compound
- Identification of a more effective antibiotic
- Evaluate the inhibitory properties of these compounds in other bacteria