Molecular interactions of BWC0977; a dual inhibitor of DNA gyrase and Topoisomerase IV

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Background

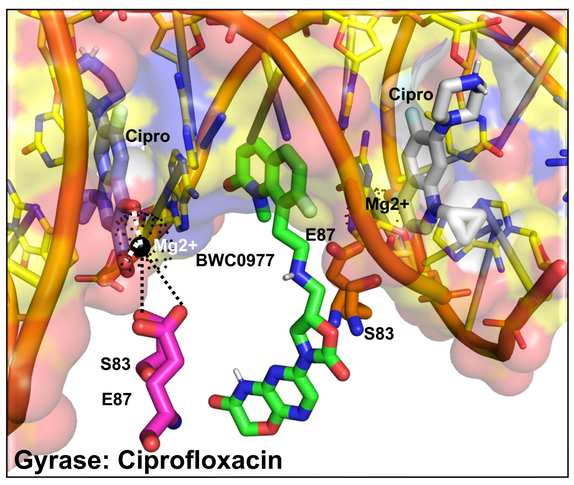
Antibiotic resistance due to target mutations has made treating bacterial infections challenging. BWC0977 is an oxazolidinone class of novel bacterial topoisomerase inhibitor (NBTI) targeting the essential enzymes; DNA gyrase and topoisomerase IV. The dual target inhibition strategy can potentially delay the development of resistance in the clinic.

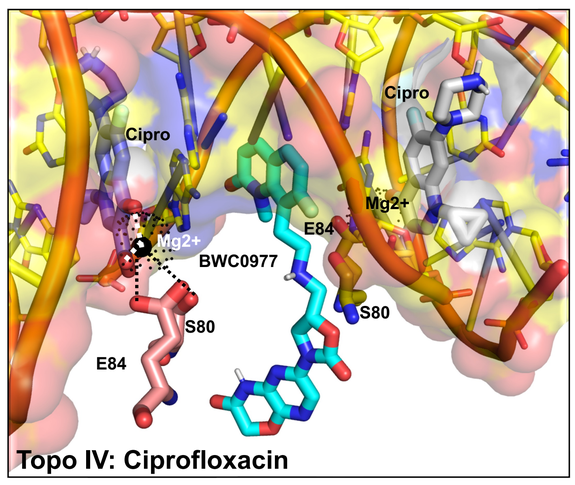
Summary

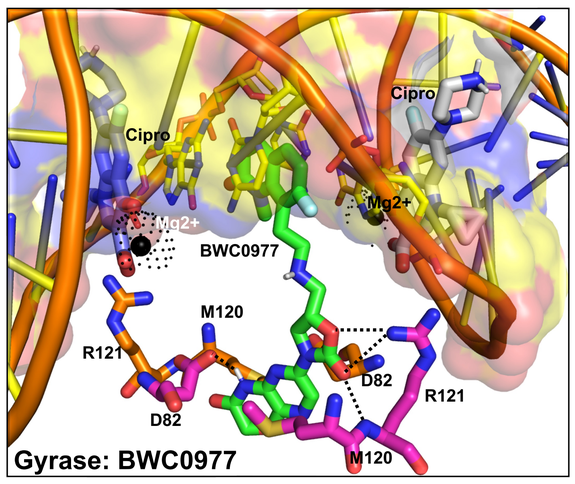
NBTIs have been reported to interact with GyrA:D82 and ParC:D79 residues in Gram-positive bacteria like *S. aureus*. *E. coli* with mutations in GyrA:D82N or ParC:D79N were engineered using a ssDNA recombineering technique, and these strains with a single mutation had marginal effect on the minimum inhibitory concentration (MIC) values whereas GyrA:D82N + ParC:D79N double mutation conferred high resistance to BWC0977. Computational modelling studies suggest that ciprofloxacin and BWC0977 access different binding pockets of the target, with no common interacting residues, thereby reducing the likelihood of developing cross-resistance.

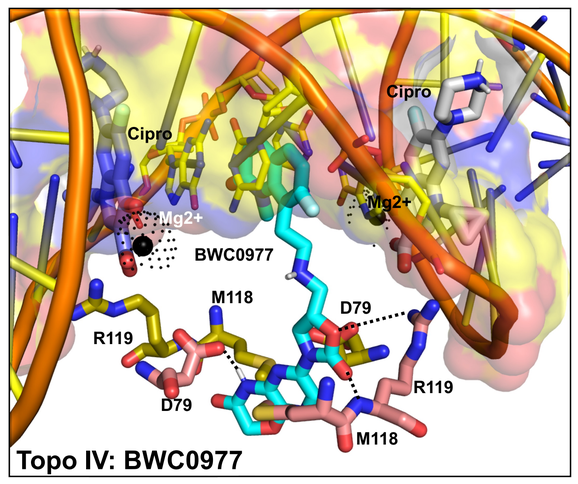
Binding mode prediction

Two ciprofloxacin molecules- one per GyrA subunit bind to the gyrase - DNA cleavage complex, whereas a single BWC0977 molecule is predicted to bind at the GyrA dimer interface in the *E. coli* gyrase - DNA complex.









- A key binding interaction of Ciprofloxacin is mediated by Mg^{2+} with the side chain of S83:Gyrase (S80:Topo IV). A mutation of this residue is the most common cause of fluoroquinolone resistance.
- The N-methyl quinolone of BWC0977 stacks with the DNA base pairs that are different from ciprofloxacin-DNA interactions, and the -NH of pyrazino-oxazinone forms stable H-bond interactions with the carboxylate side chain of D82 at the dimer interface.
- The side chain of M120 in gyrase (M118 in Topo IV) forms Van der Waals interactions with the pyrazino-oxazinone ring of BWC0977 at the dimer interface. And R121 side chain of the gyrase (R119 of Topo IV) interacts with the oxazolidinone ring of BWC0977.
- The important BWC0977 interaction residues are at lease 4Å away from the interaction residues of ciprofloxacin.

The collective evidence from the computational modelling studies suggests that none of the BWC0977 interacting residues are directly involved in ciprofloxacin binding.

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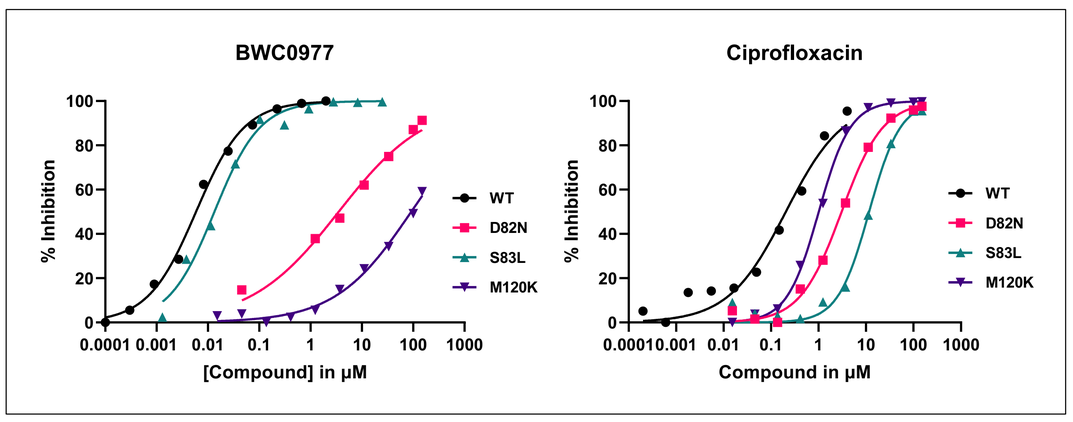
Mutant gyrase enzyme activity



Compound	*E. coli* gyrase SC, IC_{50} in µM			
	WT GyrA	S83L GyrA	D82N GyrA	M120K GyrA
BWC0977	0.004	0.013	3.6	92
Ciprofloxacin	0.183	11.6	3.1	0.9

The increase in IC_{50} of BWC0977 with D82**N** and M120**K** mutant gyrase enzymes confirms their interaction residues in the target.

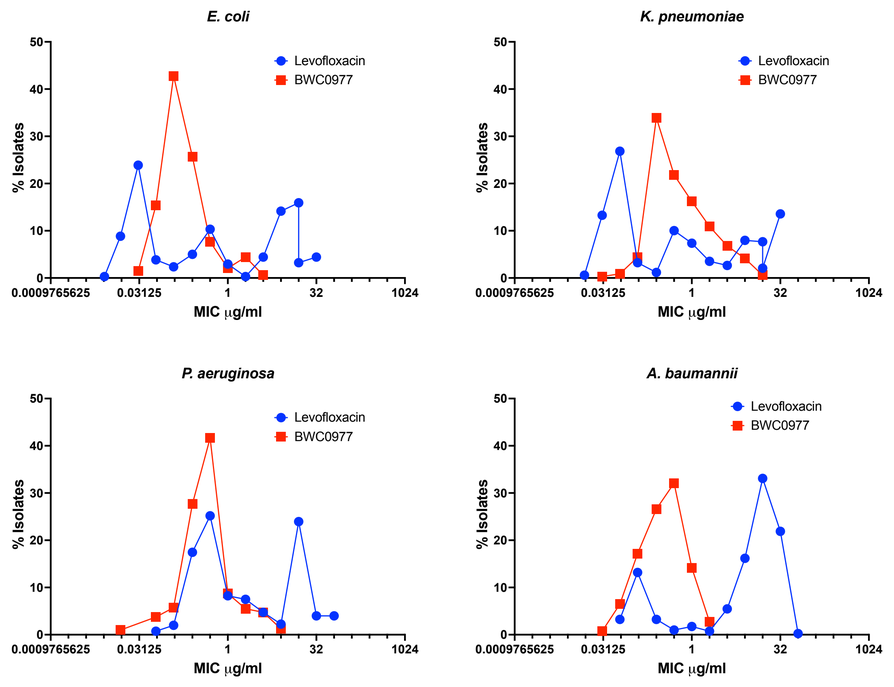
MIC of *E. coli* strain with engineered target mutations

Mutation		MIC (µg/mL)				
Gyrase	Topo IV	Ciprofloxacin	Levofloxacin	BWC0977	Gepotidacin	Ampicillin
WT	WT	0.01	0.03	0.03	0.47	5.0
D82N	WT	0.08	0.13	0.08	0.42	5.0
WT	D79N	0.01	0.02	0.03	0.83	2.5
D82N	D79N	**0.21**	**0.25**	**7.5**	**> 40**	**3.3**
M120A	M118A	0.01	0.02	0.0625	0.63	4.2
R121K	WT	0.083	0.08	0.010	0.13	4.2
WT	R119K	0.021	0.04	0.026	0.42	5.0

E. coli, K-12 MG1655 strain was used to generate target mutants for BWC0977. The MIC values are the mean of 3 experiments.

- GyrA:R121K + ParC:R119K double mutation led to poor growth implicating a fitness cost. Since this mutation is deleterious for the strain, the chances of selecting such a mutant against BWC0977 in the clinic could be low.
- The upward shift in MIC of BWC0977 in the GyrA:D82N + ParC:D79N double mutant strain and in the IC50 with the D82N and M120K gyrase mutant proteins suggests that these are the major interaction residues.

Multi-modal distribution of MIC of Levofloxacin indicates resistance



The unimodal distribution of BWC0977 MIC_{90} compared to the multimodal distribution for Levofloxacin indicate the probable lack of pre-existing resistance to BWC0977.

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