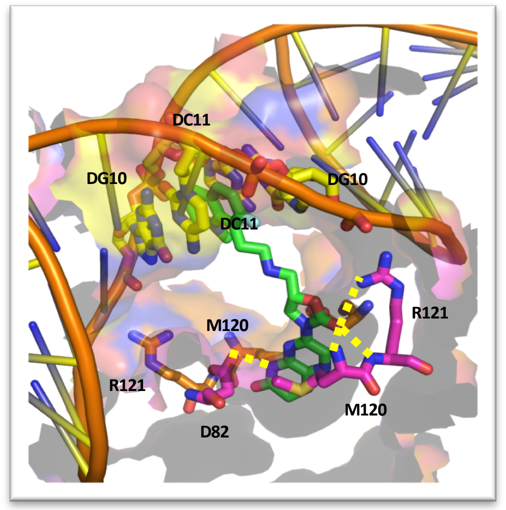
BWC0977, a novel dual target topoisomerase inhibitor: antimicrobial potency, spectrum and mechanism of action.

S. Hameed, S. Sharma, **R. Nandishaiah**, N. Katagihallimath, N. Bharatham, A. Shanbhag, S. Rajagopal, P. Bhowmik, P. Amar, R. Narjari, V. Ramachandran, S. Datta & V. Balasubramanian
Bugworks Research India Pvt. Ltd. Bengaluru 560065; **email: radha@bugworksresearch.com**

Introduction

- We have identified an antibacterial preclinical candidate BWC0977 that is equipotent against targets - Gyrase and Topoisomerase IV. It exhibits broad spectrum antibacterial activity against the ESKAPE pathogens.
- BWC0977 inhibits gyrase by stabilizing single strand breaks in the DNA unlike Ciprofloxacin that stabilizes double strand breaks.
- Computational studies predicted that the residues M120, D82 and R121 of GyrA are important for interacting with BWC0977. The upward shift in inhibition of supercoiling activity of the corresponding mutant enzymes confirms these interactions.
- BWC0977 triggers an SOS response in bacterial cells similar to Ciprofloxacin through the induction of recA, recN, sulA and lexA promoters[1,2].
- BWC0977 is bactericidal, shows time dependent kill and a post antibiotic effect of 1.5 to 2 hrs.
- Efforts to raise resistant mutants against BWC0977 in *Escherichia coli* and other Gram-negative organisms by single step methods yielded no mutants indicating very low resistance frequency. Though mutants obtained by long serial passage experiments showed a shift in MIC, sequencing revealed no target mutations.

Interaction Residues of Gyrase



- Side chains of the M120 residues at the GyrA dimer interface forms strong hydrophobic interactions with the pyrazino-oxazinone ring of BWC0977.
- -NH of pyrazino-oxazinone ring forms H-bond with D82.
- R121 interacts with Oxazolidinone ring of BWC0977.

Major Interaction residues
Ciprofloxacin : S83L
BWC0977 : M120K & D82N

Compound	*E. coli* gyrase SC, IC_{50} in µM			
	WT	S83L	M120K	D82N
BWC0977	0.004	0.013	92	3.6
Ciprofloxacin	0.183	11.6	0.91	3.1

The shift in IC_{50} of BWC0977 with M120K and D82N mutant gyrase enzymes confirms their interactions invitro.

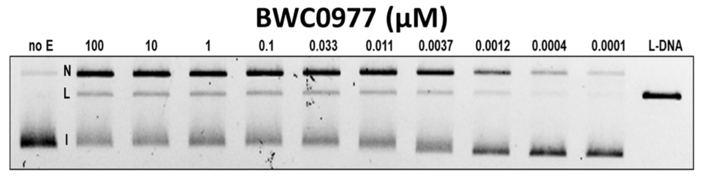
Target Inhibition across Species

Compound	IC_{50} (µM)					
	E. coli		*P. aeruginosa*		*S. aureus*	
	Gyrase	Topo IV	Gyrase	Topo IV	Gyrase	Topo IV
BWC0977	0.004	0.01	0.009	0.071	0.01	0.095
Ciprofloxacin	0.189	16.3	0.244	22.9	25.5	8.5

BWC0977 inhibits gyrase and topo IV with nanomolar potency across species. Ciprofloxacin shows higher micromolar inhibition on S. aureus gyrase activity.

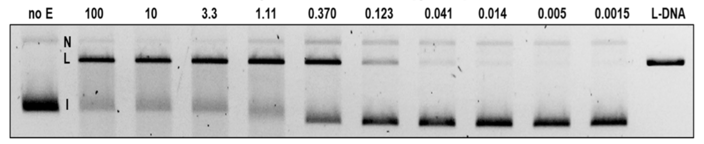
Mode of Inhibition

BWC0977 (µM)



Inhibition of DNA gyrase through formation of single-strand breaks in the plasmid DNA

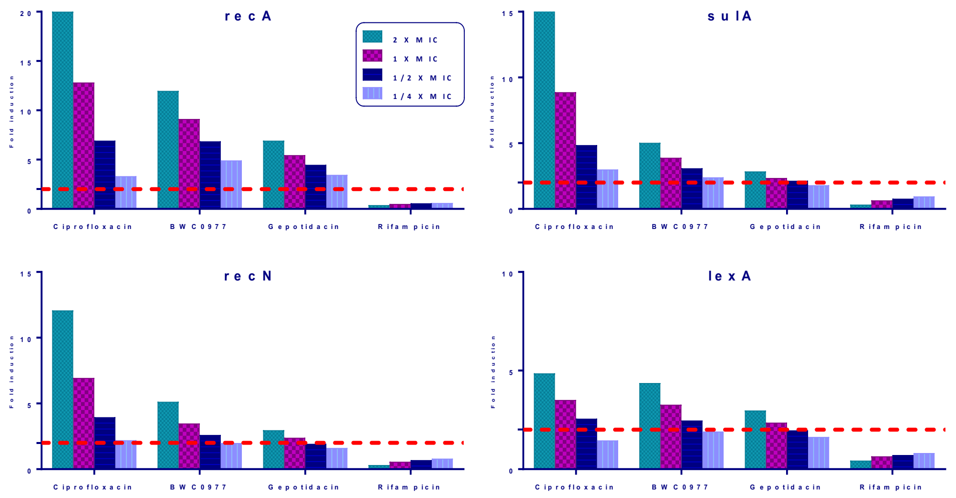
Ciprofloxacin (µM)



Inhibition of DNA gyrase through stabilization of double-strand breaks in the plasmid DNA

Cleavage-complex assays were performed with *E. coli* gyrase using supercoiled plasmid in the absence of ATP to study the mode of inhibition of compounds.

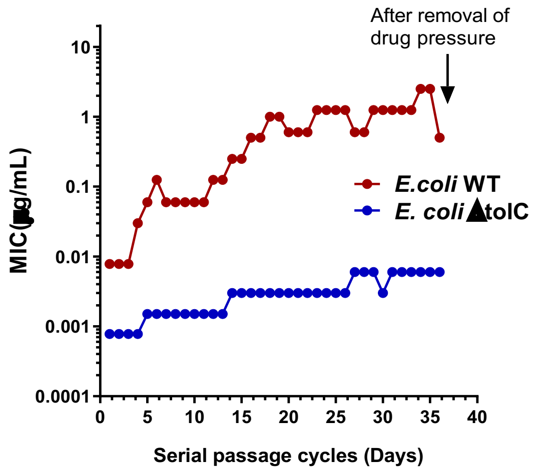
SOS Response to DNA Damage



- *E. coli* SOS-inducible promoters fused to fast-folding green fluorescent protein (GFP) on a low-copy plasmid in *E. coli* MG1655 were used as reporter strains[2].
- The selected reporter strains were treated with varying concentrations of compounds during early growth phase (A_{600} = 0.2, growing at 37°C) and the GFP fluorescence was measured at the end of 6 hours of induction.
- RFUs were normalized to the cell growth and fold induction was measured as a ratio of the treated vs. untreated cultures.

BWC0977 induces SOS response similar to Ciprofloxacin and Gepotidacin.

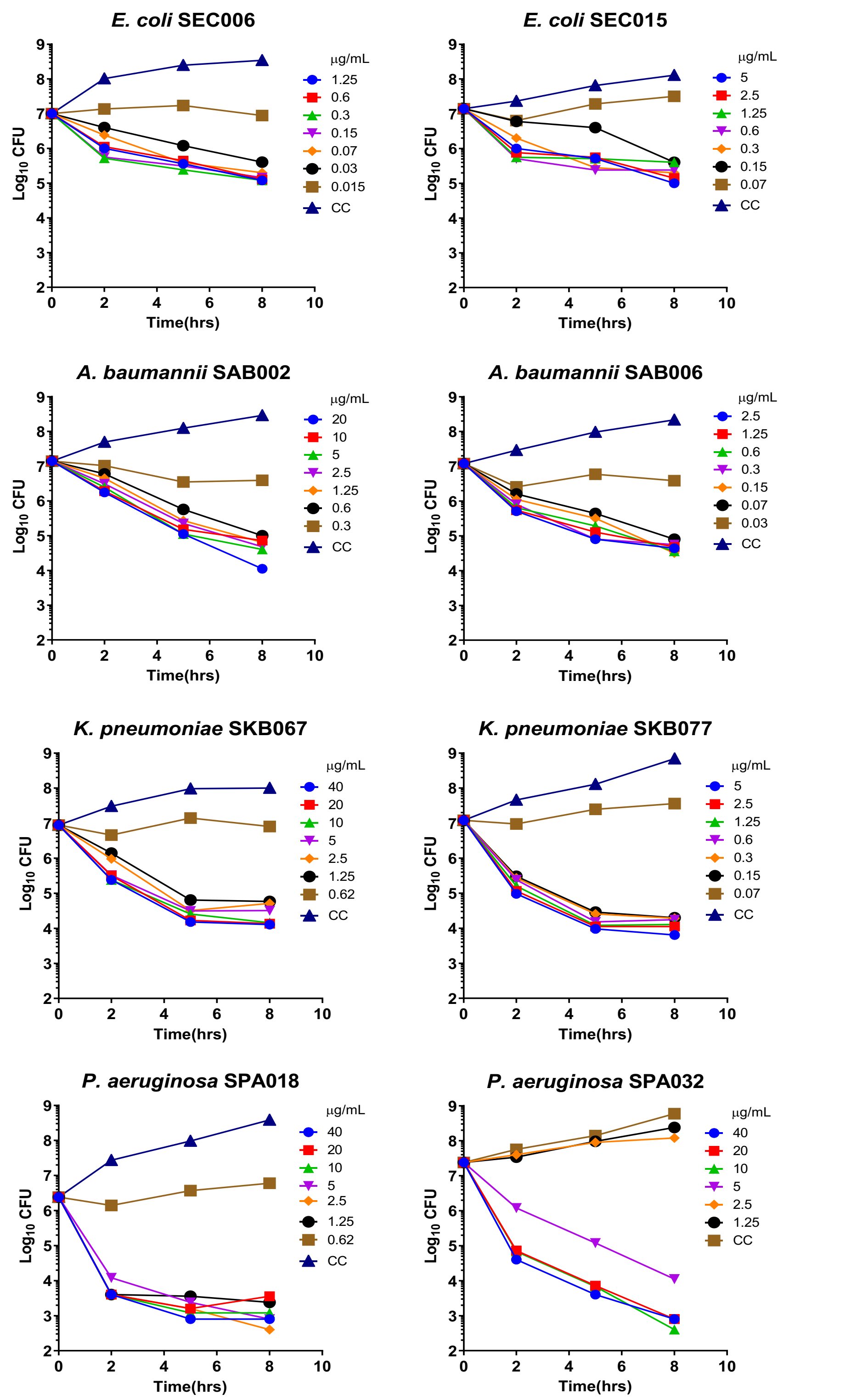
Resistant Mutant Generation



- Single step selection methods to raise resistant mutants yielded no mutants.
- A serial passage MIC protocol was done to obtain mutants in both wildtype and ΔtolC *E. coli.*

A 100-fold shift in MIC was observed in the wildtype *E. coli* at the end of 35 days while 10-fold shift was observed in ΔtolC. Individual colonies isolated from the wildtype culture were grown in the absence of drug pressure. The MIC of these stable mutants were 0.5µg/ml. The gyrA and parC genes of these mutants were sequenced. No mutations were identified in the target genes.

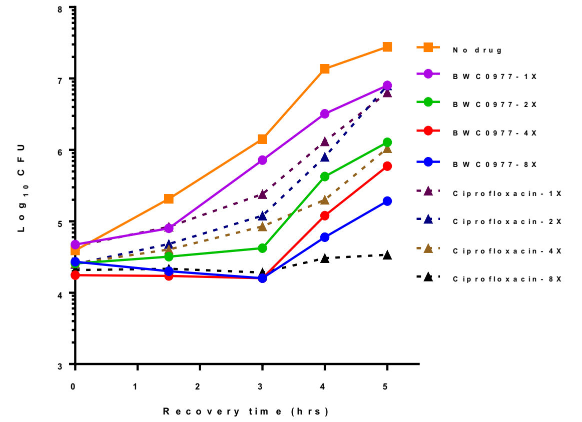
Killing Kinetics



Clinical isolate	MIC (µg/mL)	E_{max} (log_{10} CFU at 4X MIC)
SEC006	0.015	2
SEC015	0.15	1.9
SKB067	1.25	2.8
SKB077	0.15	3.1
SAC002	1.25	2.3
SAC006	0.15	2.4
SPS018	1.25	3
SPS032	5	4.2

BWC0977 was bactericidal in quinolone resistant clinical strains in a time dependent pattern.

Post Antibiotic Effect



PAE in hours		
Concentration	BWC0977	Ciprofloxacin
1X MIC	0.28	0.82
2X MIC	1.35	1.1
4X MIC	1.6	1.6
8X MIC	2.5	>2.5

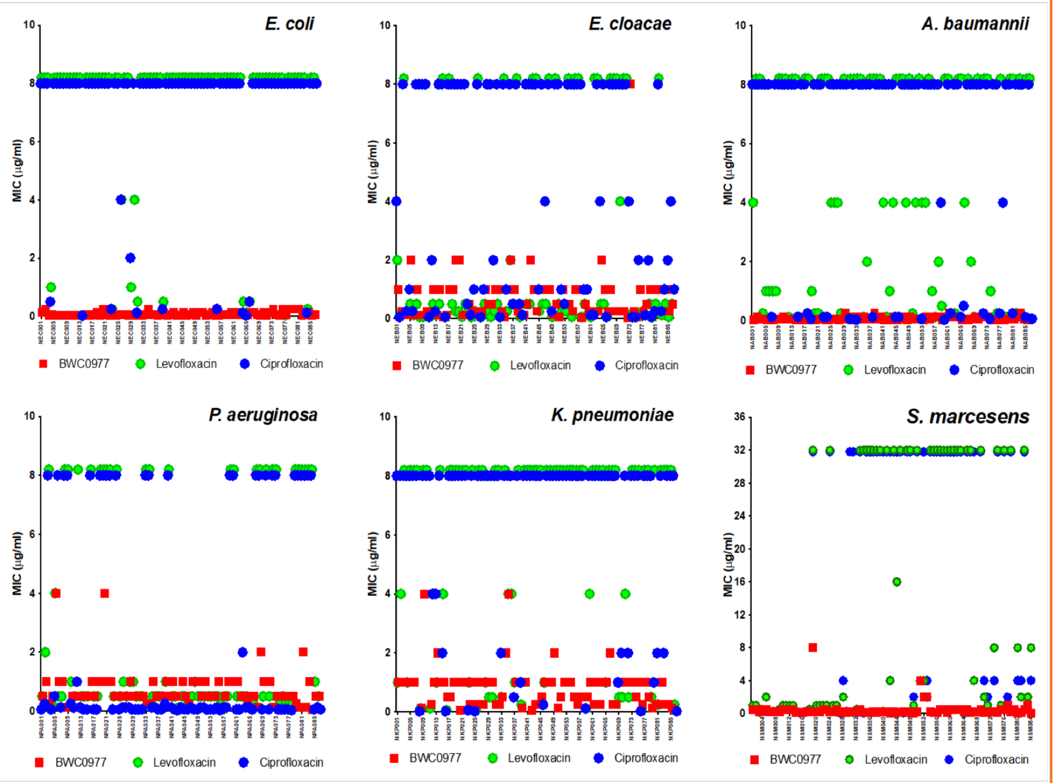
BWC0977 post antibiotic effect is similar to Ciprofloxacin.

Combination Studies

Antibiotic	FIC (A) antibiotic	FIC (B) BWC0977	FIC index (A+B)	Interaction
Amoxicillin	0.75	0.5	1.25	Indifference
Ampicillin	0.5	0.5	1	Indifference
Azithromycin	0.5	0.5	1	Indifference
Aztreonam	0.5	0.5	1	Indifference
Cefotaxime	0.25	0.5	0.75	Additive
Cefepime	0.125	0.5	0.625	Additive
Chloramphenicol	0.5	0.25	0.75	Additive
Ciprofloxacin	0.125	0.5	0.625	Additive
Clarithromycin	0.5	0.25	0.75	Additive
Clindamycin	0.5	0.25	0.75	Additive
Erythromycin	0.75	0.5	1.25	Indifference
Kanamycin	0.25	0.5	0.75	Additive
Levofloxacin	0.25	0.5	0.75	Additive
Meropenem	0.5	0.5	1	Indifference
Metronidazole	0.75	0.5	1.25	Indifference
Moxifloxacin	0.5	0.5	1	Indifference
Nitrofurantoin	0.75	0.5	1.25	Indifference
Polymyxin	0.5	0.25	0.75	Additive
Streptomycin	0.25	0.5	0.75	Additive
Vancomycin	0.5	0.25	0.75	Additive

BWC0977 exhibited additive and indifferent interactions. No antagonism was observed with any class of antibiotics.

Effective against Ciprofloxacin Resistant Clinical Isolates



No cross-resistance observed between fluroquinolones and BWC0977.

References

1. Zaslaver A. et al, 2006. A comprehensive library of fluorescent transcriptional reporters for Escherichia coli.
2. Scott D. mills et al, 2014. A Novel High-Throughput Cell-Based Assay Aimed at Identifying Inhibitors of DNA Metabolism in Bacteria.
3. PCT WO/2018/225097

Acknowledgement

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