

Comparative In Vitro Activity of CBR-2092, a Novel Rifamycin-Quinolone Hybrid Antibiotic, and Rifampin+Quinolone Combinations.

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Abstract

Background: CBR-2092 is a novel rifamycin-quinolone hybrid antibiotic in development for the treatment of serious bacterial infections. The comparative activity of CBR-2092 and rifampin+quinolone cocktail combinations is compared herein in a series of *in vitro* studies.

Methods: Checkerboard assays and FIC indices were determined by the broth microdilution method. High density time-kill studies employed starting cultures at ~10⁹ CFU/mL. Mutant Prevention Concentrations (MPCs) were determined by plating of 10¹⁰ CFU. Studies of biofilm efficacy employed colony biofilm assays or a drip-flow reactor system.

Results: Antagonism of the rapid killing of *S. aureus* by quinolones in the presence of rifampin is apparent in checkerboard assays via MBC endpoints and in high density time-kill assays. In the latter, a rifampin+ciprofloxacin combination (1+0.25 µg/mL) is bacteriostatic (< 99.9% kill at 24 h) against *S. aureus* ATCC# 29213 and results in the outgrowth of rifampin-resistant isolates. In contrast, CBR-2092 is observed to be bactericidal (≥ 99.9% kill at 24 h at 0.25 µg/mL) without the emergence of rifampin resistance. Also in contrast to tested rifampin+quinolone cocktails, CBR-2092 promotes eradication (≥ 7-Log₁₀ CFU reduction) of colony biofilms formed by *S. aureus* MSSA or MRSA isolates and exhibits bactericidal activity (>99% kill at 24 h at 4 µg/mL) that is greater than that of rifampin+moxifloxacin (≤ 99% kill at 4+4 µg/mL) combination in killing *S. aureus* cells within murine macrophages. Finally, combined data from a series of studies indicate that CBR-2092, unlike multiple quinolones, is not a substrate for the NorA or MepA quinolone efflux pumps in *S. aureus*.

Conclusions: CBR-2092 exhibits activity in a number of settings *in vitro* that is improved over that observed with rifampin+quinolone combinations possibly reflecting the non-susceptibility of CBR-2092 to efflux pathways and the apparent lack of self-antagonism.

Introduction

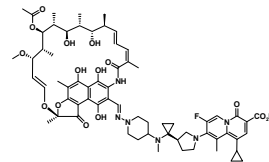
Antibiotics of the rifamycin class have proven efficacy in the treatment of persistent bacterial infections including tuberculosis and biofilm-associated infections of indwelling medical devices (4, 8, 10). However, the relative ease with which bacteria develop resistance to the rifamycins restricts their clinical use to antibiotic combination regimens (1). In a program directed toward the synthesis and evaluation of rifamycin-based multi-functional antibiotics, a series of compounds were prepared that covalently combine rifamycin and quinolone pharmacophores to form stable hybrid antibiotic agents.

CBR-2092 combines the rifamycin SV and 4H-4-oxoquinolizone (5) pharmacophores via a chiral linking group. Herein we describe a series of *in vitro* studies that compare the relative antimicrobial activity and properties of CBR-2092 with those of rifampin+fluoroquinolone cocktail combinations that could be dosed clinically. Overall, the combined data is indicative of properties of CBR-2092 that are distinct from, and superior to, rifampin+fluoroquinolone cocktail combinations including a non-susceptibility to fluoroquinolone efflux pathways. These data hold promise that CBR-2092 may exhibit efficacy and resistance-development properties *in vivo* that are superior to rifampin+fluoroquinolone cocktails.

Methods and Materials

Determination of broth and agar microdilution MIC endpoints (2) and assessment of bactericidal activity by the time-kill methodology (7) was done in accordance with CLSI methodology in cation adjusted Mueller Hinton (MHII) supplemented with 0.002% (vol/vol) Polysorbate-80 (P-80). Mutant Prevention Concentrations (MPCs) were determined through plating 10¹⁰ viable test organisms (3). Fractional Inhibition Concentrations (FICs) were determined by broth microdilution methods. Studies of biofilm efficacy employed an adapted Colony Biofilm assay (9) and an enhanced Drip-Flow Reactor system (6), wherein rifampin and quinolones were dosed at current CLSI breakpoints. Intracellular killing studies employed the adherent, mouse macrophage cell line J774A.1 (ATCC # TIB-67) in combination with *S. aureus* CB1406 (ATCC # 25923).

Panel 1: CBR-2092 Structure



- Chemical Name:** R-3-[[4-[1-[[3-Carboxy-1-cyclopropyl-7-fluoro-9-methyl-4-oxo-4H-quinolizine-8-yl]-pyrrolidin-3-yl-cyclopropyl]-methylamino]-piperidin-1-ylimino]-methylene]-rifamycin SV
- Chemical Formula:** C₆₅H₈₁F N₆O₁₅
- Molecular Weight:** 1205.38 Daltons

Panel 2: CBR-2092 efflux avoidance – MICs

Antimicrobial Tested	MIC in µg/mL or fold change in MIC for:					
	WT (#29213)	<i>norA</i> ^{ΔP}	Fold change (<i>norA</i> ^{ΔP})	WT (SA-9325-4)	<i>mepA</i> ^{ΔP} (SA-K2068)	Fold change (<i>mepA</i> ^{ΔP})
CBR-2092	0.015	0.015	1	0.004	0.004	1
Rifampin	0.008	0.008	1	0.004	0.004	1
Ciprofloxacin	0.24	2	8	0.24	2	8
Levofloxacin	0.24	0.5	2	0.24	1	4
Gatifloxacin	0.12	0.24	2	0.12	0.5	4
Moxifloxacin	0.06	0.06	1	0.06	0.24	4

CBR-2092 evaluated in MIC assays versus *S. aureus* with basal (WT) or elevated *NorA*^{ΔP} or *MepA*^{ΔP} efflux activity.

- Tested quinolones show varying susceptibility to *NorA* and/or *MepA* efflux
- CBR-2092 and rifampin MICs are unaffected by elevated *NorA* or *MepA* efflux

Panel 3: CBR-2092 efflux avoidance – MPCs

Antimicrobial or cocktail (Rifampin fixed at 125×MIC)	Ratio of MPC values for:	
	<i>norA</i> ^{ΔP} / <i>norA</i> ^{WT}	<i>mepA</i> ^{ΔP} / <i>mepA</i> ^{WT}
CBR-2092	1	1
Ciprofloxacin	4	nd
Rifampin (1) + Ciprofloxacin	4	8
Rifampin (1) + Levofloxacin	2	2
Rifampin (1) + Moxifloxacin	1	4

CBR-2092 profiled in resistance studies versus *S. aureus* with basal (WT) or elevated *NorA*^{ΔP} or *MepA*^{ΔP} efflux expression.

- CBR-2092 MPC unaffected by elevated *NorA* or *MepA* expression
- MPC for ciprofloxacin - a *NorA* substrate - impacted by elevated *NorA* expression
- Rifampin+fluoroquinolone cocktails differentially impacted by elevated *NorA* or *MepA* expression

Panel 4: CBR-2092 lack of antagonism – FIC/FBC data for cocktails

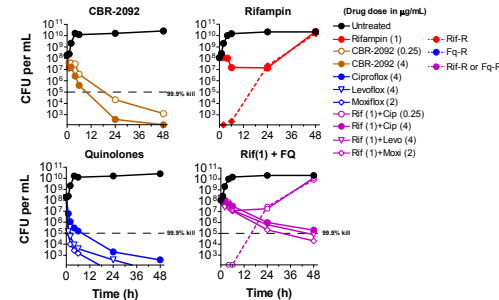
Strain	Result type (in µg/mL)	Alone		Combination		FIC or FBC	Conclusion
		Rifampin	Ciprofloxacin	Rif	Cipro		
ATCC 29213	MIC	0.008	0.5	0.004	0.25	1	Indifferent
ATCC 29213	MBC _{99.9}	1	0.5	0.25	8	16.3	Antagonism

Strain	Result type (in µg/mL)	Alone		Combination		FIC or FBC	Conclusion
		Rifampin	Levofloxacin	Rif	Levo		
ATCC 29213	MIC	0.008	0.25	0.008	0.25	2	Indifferent
ATCC 29213	MBC _{99.9}	1	0.25	0.25	4	16.3	Antagonism

Rifampin plus quinolone cocktails evaluated through FIC/FBC assays with *S. aureus*.

- In MIC assays, combined effects of rifampin plus quinolone indifferent (FIC = 1-2)
- In MBC assays, rifampin strongly antagonizes quinolone kill (FBC = 16.3)

Panel 5: CBR-2092 lack of antagonism – high density time-kills



CBR-2092 activity in high density (10⁹ CFU/mL) time-kill assays versus *S. aureus*.

- Rifampin ineffective due to failure to suppress outgrowth of rifamycin-resistant mutants
- Rapid quinolone cidal activity antagonized by co-administration of rifampin at 1 µg/mL
- CBR-2092 does not appear to self antagonize and fully suppresses rifampin-resistance

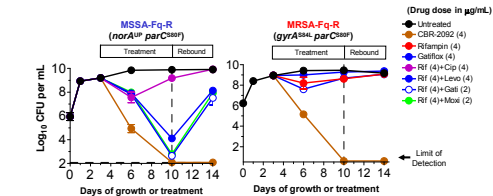
Panel 6: CBR-2092 lack of resistance – high density time-kills

Drug or cocktail tested	CBR-2092 or FQ dose (in µg/mL) required to prevent Rif-R development in:			
	WT Parent (#29213)	<i>norA</i> ^{ΔP}	<i>parC</i> ^{ΔP}	<i>norA</i> ^{ΔP} <i>parC</i> ^{ΔP}
CBR-2092	None observed	None observed	None observed	None observed
Rif (1) + Cipro	1	2	2	> 4
Rif (1) + Levo	None observed	1	1	4

CBR-2092 superior resistance properties in high density (10⁹ CFU/mL) time-kills.

- CBR-2092 fully suppresses rifampin resistance development and is unaffected by typical quinolone-resistance mutations (*parC*^{ΔP} and/or *norA*^{ΔP})
- Rifampin plus quinolone cocktails are differentially impacted by typical quinolone-resistance mutations (*parC*^{ΔP} and/or *norA*^{ΔP}) and require co-administration of higher doses of quinolone to suppress rifamycin-resistance development

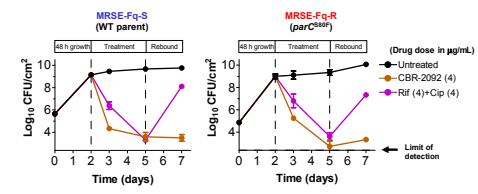
Panel 7: CBR-2092 biofilm efficacy – colony biofilm studies



CBR-2092 profiled in the colony biofilm model versus Fq-R MSSA and MRSA.

- Rifampin loses efficacy due to resistance development
- Rif + Cipro cocktail ineffective and fails to suppress emergence of Rif-R (not shown)
- Rif + Levo (or Gati, or Moxi) cocktails effective early, but fail to eradicate biofilm 'persisters'
- CBR-2092 exhibits superior efficacy & fully suppresses resistance-development – Apparent eradication of 'persister' population in biofilms

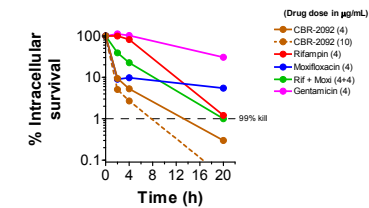
Panel 8: CBR-2092 biofilm efficacy – drip-flow studies



CBR-2092 profiled in drip flow biofilm model with WT or Fq-R MRSE.

- Rif + Cipro cocktail effective early, but fail to eradicate biofilm 'persisters'
- CBR-2092 exhibits superior efficacy & suppresses resistance-development

Panel 9: CBR-2092 intracellular accumulation and efficacy



CBR-2092 profiled in intracellular killing assay with *S. aureus* infected J774A.1 cells

- Gentamicin has minimal overall efficacy owing to poor intracellular accumulation
- Rifampin exhibits efficient but time-dependent kill
- Moxifloxacin exhibits rapid initial kill but persistent sub-population apparent
- CBR-2092 exhibits rapid and effective overall kill with efficacy superior to Rifampin plus Moxifloxacin cocktail

Summary and Conclusion

Summary:

- CBR-2092 is a novel rifamycin-quinolone hybrid antibiotic in development for the treatment of serious bacterial infections.
- CBR-2092 antimicrobial activity and resistance prevention properties are unaffected by gain of function mutations in *norA* or *mepA* which show differential effects on parent class comparators including rifampin + fluoroquinolone cocktails.
- Rapid quinolone cidal activity is antagonized by co-administration of rifampin, whereas, CBR-2092 exhibits superior killing (and resistance prevention) properties possibly owing to a lack of self-antagonism or avoidance of basal efflux mechanisms.
- CBR-2092 – optimized for biofilm activity – exhibits superior *in vitro* biofilm efficacy and exhibits intracellular bactericidal activity that is greater than that of rifampin plus moxifloxacin and other Rif + Fq cocktails.

Conclusion:

- CBR-2092 exhibits *in vitro* activity that is improved over that observed with rifampin+quinolone combinations possibly reflecting the non-susceptibility of CBR-2092 to efflux pathways and the apparent lack of self-antagonism.

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