

Abstract

Background: CBR-2092 is a novel rifamycin-quinolone hybrid antibiotic in development for the treatment of serious bacterial infections. The antimicrobial activity profile of CBR-2092 *in vitro* is described herein including assessment of its growth inhibitory and bactericidal properties.

Methods: CLSI guidelines were followed throughout with polysorbate-80 (0.002%) supplementation of broth medium as indicated. Additional studies employed standard methods; Mutant Prevention Concentrations (MPCs) were determined by plating of 10¹⁰ CFU.

Results: MIC values (MIC₅₀ and MIC range in µg/mL) determined for CBR-2092 against 51 MSSA (0.015, ≤ 0.004 to 0.03), 54 MRSA (0.015, ≤ 0.004 to 2), 35 MSSE (0.008, ≤ 0.004 to 0.015), 35 MRSE (0.5, ≤ 0.004 to 1), 35 *S. pyogenes* (0.12, 0.008 to 0.12) and 35 *S. agalactiae* (0.25, 0.06 to 2) indicate potent activity against key pathogens. CBR-2092 exhibits prolonged post-antibiotic and sub-MIC effects in *S. aureus* with values of 2.7, >4 and >4 h, respectively, determined for the PAE (3×MIC), SME (0.12×MIC) and PAE-SME (3×MIC/0.12×MIC) periods and are indicative of efficient intracellular uptake and retention. In time-kill studies, CBR-2092 exhibits bactericidal activity against both staphylococci and streptococci with rapid, quinolone-like killing of rifampin-resistant strains observed (>99.9% kill in 4 h at ≥ 2× MIC). In spontaneous resistance studies, CBR-2092 exhibits activity consistent with balanced contributions from its composite pharmacophores with an MPC of 0.12 µg/mL and a resistance frequency of <10⁻¹² determined at 1µg/mL in agar for *S. aureus* ATCC# 29213. In studies of the killing of *S. aureus* residing intracellularly in murine macrophage cells, CBR-2092 exhibits prolonged bactericidal activity (>99% kill at 24 h at ≥ 4µg/mL).

Conclusions: CBR-2092 exhibits promising activity in a range of antimicrobial assays *in vitro* that pertain to properties relevant to the effective treatment of serious bacterial infections.

Introduction

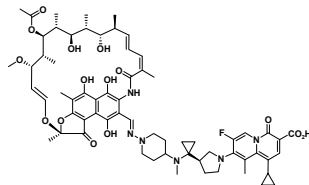
Antibiotics of the rifamycin class have proven efficacy in the treatment of persistent bacterial infections including tuberculosis and biofilm-associated infections of indwelling medical devices (6, 9, 10). However, the relative ease with which bacteria develop resistance to the rifamycins restricts 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-oxoquinolizine (7) pharmacophores via a chiral linking group. Herein we report the *in vitro* antimicrobial activity profile of CBR-2092 against key target pathogens including staphylococci, streptococci, and *M. tuberculosis*. Overall, the data suggests that CBR-2092 exhibits antimicrobial activity *in vitro* that is (i) consistent with its dual-pharmacophore character and (ii) distinct from antibiotics of either parent class. These combined features hold promise that CBR-2092 may exhibit efficacy *in vivo* that is distinct from, and superior to, parental antibiotic agents.

Methods and Materials

Determination of broth and agar microdilution MIC endpoints (2) and assessment of bactericidal activity by determination of MBC endpoints and time-kill methodology (8) was done in accordance with CLSI methodology in cation adjusted Mueller Hinton (MHII) supplemented with 0.002% (vol:vol) Polysorbate-80 (P-80), MIC and MBC analysis for *Mycobacterium tuberculosis* employed the Microplate Alamar Blue Assay (MABA) as described previously (3). Mutant Prevention Concentrations (MPCs) were determined through plating 10¹⁰ viable test organisms (5). Studies to measure Post-Antibiotic Effects (PAE) and Sub-MIC Effects (SMEs) were undertaken with *S. aureus* CB190 (ATCC # 29213) and standard methods (4). In all cases, the PAE period was determined following exposure at a 3xMIC concentration for 1 hour, the SME period determined following exposure at a 0.125xMIC concentration for 1 hour, and the PAE-SME period determined by combination of these exposures. Intracellular killing studies employed the adherent, mouse macrophage cell line J774A.1 (ATCC # TIB-67) in combination with *S. aureus* CB1406 (ATCC # 25923) or a small colony variant of *S. aureus* ATCC # 12600 (CB1927) bearing a deletion-replacement $\Delta hemB::ermC$ mutation.

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:** C₆₅ H₈₁ F N₆ O₁₅
- Molecular Weight:** 1205.38 Daltons

Panel 2: CBR-2092 *in vitro* activity - Staphylococci

Staphylococcus aureus MSSA (n=51) - broth microdilution MIC in µg/mL				
Antimicrobial agent	Resistance class	MIC range	MIC ₅₀	MIC ₉₀
CBR-2092	MSSA	≤0.004 - 0.03	0.008	0.015
Rifampin	MSSA	≤0.008 - 0.06	0.015	0.015
Ciprofloxacin	MSSA	0.06 - > 4	0.25	1

Staphylococcus aureus MRSA (n=54) - broth microdilution MIC in µg/mL				
Antimicrobial agent	Resistance class	MIC range	MIC ₅₀	MIC ₉₀
CBR-2092	MRSA	≤ 0.004 - 2	0.015	0.015
Rifampin	MRSA	≤ 0.008 - > 4	0.015	0.015
Ciprofloxacin	MRSA	0.12 - > 250	> 4	> 4

Staphylococcus epidermidis MSSE (n=35) - broth microdilution MIC in µg/mL				
Antimicrobial agent	Resistance class	MIC range	MIC ₅₀	MIC ₉₀
CBR-2092	MSSE	≤ 0.004 - 0.015	≤ 0.008	0.008
Rifampin	MSSE	≤ 0.004 - 0.06	0.015	0.015
Ciprofloxacin	MSSE	0.12 - > 16	0.25	> 4

Staphylococcus epidermidis MRSE (n=35) - broth microdilution MIC in µg/mL				
Antimicrobial agent	Resistance class	MIC range	MIC ₅₀	MIC ₉₀
CBR-2092	MRSE	≤ 0.004 - 1	0.008	0.5
Rifampin	MRSE	≤ 0.004 - > 16	0.015	> 4
Ciprofloxacin	MRSE	0.12 - > 16	> 4	> 16

CBR-2092 antimicrobial activity profiled against methicillin-susceptible or methicillin-resistant staphylococci in broth microdilution MIC assays.

- CBR-2092 exhibits potent anti-staphylococcal antimicrobial activity
- CBR-2092 potency is not impacted by methicillin resistance class
- CBR-2092 potency equivalent to rifampin and superior to ciprofloxacin

Panel 3: CBR-2092 *in vitro* activity – Streptococcus spp.

Streptococcus pyogenes (n=35) – broth microdilution MIC in µg/mL				
Antimicrobial agent	Resistance class	MIC range	MIC ₅₀	MIC ₉₀
CBR-2092	NA	0.008 - 0.12	0.03	0.12
Rifampin	NA	≤ 0.008 - 0.25	0.06	0.12
Ciprofloxacin	NA	0.12 - 2	0.5	1

Streptococcus agalactiae (n=35) - broth microdilution MIC in µg/mL				
Antimicrobial agent	Resistance class	MIC range	MIC ₅₀	MIC ₉₀
CBR-2092	NA	0.06 - 2	0.12	0.25
Rifampin	NA	0.03 - 2	0.12	0.12
Ciprofloxacin	NA	0.25 - 1	0.5	1

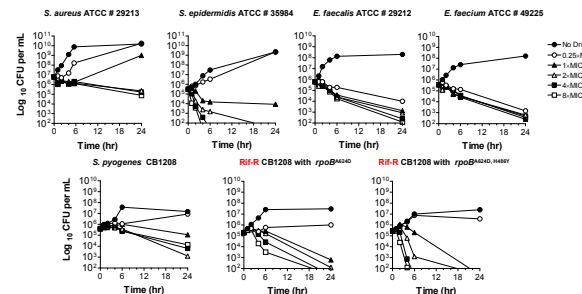
Streptococcus pneumoniae (n=55) - broth microdilution MIC in µg/mL				
Antimicrobial agent	Resistance class	MIC range	MIC ₅₀	MIC ₉₀
CBR-2092	MDS, Fq-RMDR	0.06 - 0.25	0.12	0.12
Rifampin	MDS, Fq-RMDR	0.12 - 1	0.25	0.25
Ciprofloxacin	MDS, Fq-RMDR	0.5 - > 16	2	> 16

Abbreviations: NA, not applicable; MDS, Multi-drug sensitive; Fq-RMDR, Quinolone- and/or Multi-drug resistant

CBR-2092 antimicrobial activity profiled against Streptococci in broth microdilution MIC assays.

- CBR-2092 exhibits excellent anti-streptococcal activity
- CBR-2092 potency equal to rifampin and superior to ciprofloxacin
- CBR-2092 activity not impacted by prevalent resistance classes

Panel 4: CBR-2092 time-kill studies - Gram-positive pathogens



CBR-2092 activity in time-kill assays at 0.25-8×MIC with select Gram-positive pathogens.

- Time-dependent cidal attributes typical of rifampin-sensitive gram-positive pathogens
- Concentration-dependent cidal properties versus rifampin-sensitive *S. epidermidis* 35984
- Rate of cidal increase in *S. pyogenes* engineered to rifampin resistance (*rpoB*^{MS24D-H486I})

Panel 5: CBR-2092 *in vitro* activity against *M. tuberculosis*

Antimicrobial agent	<i>M. tuberculosis</i> MIC in µg/mL by MABA for:										
	H37Rv, WT (MBC)	MOX1, FQ-R	MOX3, FQ-R	3863R, RIF-R	A406	A2584	294	IND 2039	A2341	MEX111	A2316
CBR-2092	0.06 (0.19)	0.04	0.29	0.86	0.09	0.18	0.05	0.12	0.11	0.06	0.23
Rifampin	0.04 (0.13)	0.03	0.23	> 16	0.08	0.12	0.02	0.05	0.03	0.07	0.12
Gatifloxacin	0.21	15.71	> 16	0.11	0.45	0.12	0.09	0.11	0.12	0.12	0.25

CBR-2092 antimicrobial activity profiled against *M. tuberculosis* by MABA MIC assays.

- CBR-2092 exhibits potent rifampin-like antimicrobial activity and potency
- Activity retained against rifampin-resistant or quinolone-resistant H37Rv-derivatives
- Activity retained against clinical isolates representing the major pathogenic clades

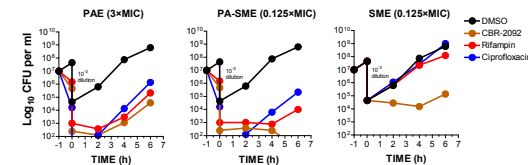
Panel 6: CBR-2092 MOA - Resistance studies

Compound	MPC (µg/mL; 10 ¹⁰ CFU)	MSW (MPC/MIC)	Resistance Freq. (1 µg/mL)
Rifampin	> 32	> 4,000	2.9×10 ⁻⁸
CBR-2092	0.12	8	< 10 ⁻¹²
Ciprofloxacin	2	8	5.0×10 ⁻⁹

CBR-2092 profiled in resistance studies versus wild-type *S. aureus*.

- CBR-2092 exhibits a Mutant Prevention Concentration (MPC) consistent with dual-pharmacophore nature
- Mutant Selection Window (MSW) (i.e., MPC/MIC) demonstrates substantial improvement over rifampin
- Spontaneous resistance frequency consistent with a low resistance potential

Panel 7: CBR-2092 Post-antibiotic and Sub-MIC effects

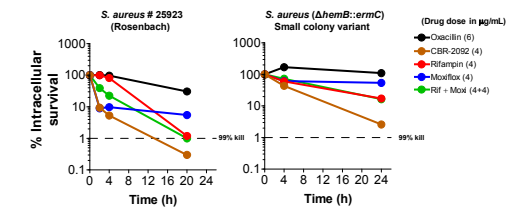


Compound	MIC (µg/mL)	Response of Staphylococcus aureus ATCC #29213 in hours		
		PAE (3×MIC)	PA-SME (0.125×MIC)	SME (0.125×MIC)
CBR-2092	0.008	2.7	> 4.6	> 4.6
Rifampin	0.008	2.7	4.6	—
Ciprofloxacin	0.24	0.6	—	—

CBR-2092 profiled in PAE, PA-SME and SME studies versus wild-type *S. aureus*.

- CBR-2092 induces a prolonged, rifampin-like Post-Antibiotic Effect (PAE)
- CBR-2092 induces a substantial SME and (PA-SME) that is distinct from either parent
- Properties consistent with efficient uptake and intracellular retention

Panel 8: CBR-2092 intracellular accumulation and efficacy



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

- Oxacillin exerts minimal overall efficacy owing to poor intracellular accumulation
- Rifampin exhibits efficient but time-dependent kill
- Moxifloxacin exhibits rapid kill but sub-population persists
- CBR-2092 exhibits rapid and effective overall kill with efficacy superior to Rifampin plus Moxifloxacin cocktail
- Activity of CBR-2092 maintained against engineered Small Colony Variant ($\Delta hemB$)

Summary and Conclusion

Summary:

- CBR-2092 is a novel rifamycin-quinolone hybrid antibiotic in development for the treatment of serious bacterial infections.
- CBR-2092 exhibits potent anti-staphylococcal activity and is unaffected by the methicillin-resistance phenotype of the test strain.
- CBR-2092 retains potent activity against select gram-positive pathogens associated with cSSSI infections.
- Killing properties of CBR-2092 as measured by time-kill assays are consistent with that of a time-dependent rifamycin-like agent. CBR-2092 also exhibits rapid quinolone-like kill versus rifampin-resistant strains, consistent with its dual-pharmacophore nature.
- Single step resistance selection studies support both the multi-targeting nature of CBR-2092 and indicate a low propensity for resistance development.
- CBR-2092 exhibits prolonged post-antibiotic and sub-MIC effects and good intracellular killing activity for MSSA or a Small Colony Variant of MSSA cultivated within J774A.1 macrophages.

Conclusion:

- CBR-2092 exhibits promising activity in a range of antimicrobial assays *in vitro* that pertain to properties relevant to the effective treatment of serious bacterial infections.

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