Abstract #: 4136
AAR-Saturday-508
ASM Microbe 2024
Atlanta, Georgia

VNRX-14079, a cyclic boronate penicillin-binding-protein inhibitor, retains potent antibacterial and bactericidal activity against ceftriaxone-resistant *Neisseria gonorrhoeae*

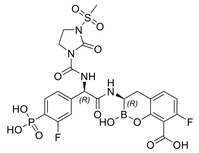
Brittany Miller, Tsuyoshi Uehara, Allison L. Zulli, Stephen M. Condon, Denis M. Daigle

Venatorx Pharmaceuticals, Inc.
Malvern, PA
miller@venatorx.com
610-644-8935

Introduction

Drug-resistant *Neisseria gonorrhoeae* is classified as an "urgent threat" pathogen according to the United States CDC. New treatment options for gonorrhea infections are needed as resistance to the standard of care agent (intramuscular-administered ceftriaxone) is emerging. To address penicillin-binding protein 2 (PBP2)-mediated ceftriaxone resistance in *N. gonorrhoeae*, Venatorx initiated a discovery effort to identify non-beta-lactam boronate-based PBP inhibitors. This initiative led to the discovery of VNRX-14079, a novel cyclic boronate-based inhibitor of PBP2 that shows potent activity against both wild-type and drug-resistant *N. gonorrhoeae.*

VNRX 14079 Structure



Materials/Methods

- Agar and broth microdilution MIC assays were conducted and interpreted according to CLSI[1,2] using GC agar supplemented with 1% IsoVitaleX and an optimized supplementation of 12 µM $Fe(NO_3)_3$ for robust growth and fastidious broth (FB) prepared according to Cartwright *et. al*[4] with an adjustment in final pH to 7.2 +/- 0.2 respectively.
- Resistance frequency experiments were conducted using the modified GC agar except for WHO Q. Growth of WHO Q was minimal on GC agar and was instead tested on chocolate agar for optimal growth.
- Time kill assays were conducted using FB in a 96-well microtiter plate based on methods described by Forester *et. al.*[3] with modifications. Inoculum consisted of 90 µL of a 10^5 CFU/mL bacterial suspension. The plate was incubated at 37°C in a 5% CO_2 humidified environment shaking at 200 RPM for 4 hours to allow the bacteria to reach log phase of growth. After the initial growth phase, 10 µL of appropriate 10× drug dilutions (4× and 16× modal broth microdilution MICs as well as one dilution above the ceftriaxone susceptibility breakpoint of ≤0.25 µg/mL (0.5 µg/mL) were tested when appropriate) were added to each desired well for a total volume of 100 µL. Also, 10 µL of FB was added to the wells serving as the positive controls. At time -4 hour (time of inoculation), 0-hour (time of drug addition), 2-hour, 4-hour, 6-hour, and 24-hour, a limiting dilution method was used to monitor bacterial growth. At each time point, CFU/mL was determined using 16 serial dilutions with mixing 63 µL from each condition/dilution with 137 µL of FB by pipetting. Growth in the first dilution represents 23 CFU/mL (7.3 × 3.17) and growth in each consecutive dilution represents 7.3 × $3.17^{(n)}$ CFU/mL (n means n^{th} dilution). Bacterial growth at 28-hour was validated prior to time kill assays to ensure appropriate growth could be maintained throughout the duration of the experiment.

Table 1: *Neisseria gonorrhoeae* Agar Dilution MICs in µg/mL in 6 wild-type and 5 mosaic PBP2 producers

Species	Strain ID	PBP2 allele	VNRX-14079	Ceftriaxone	Zoliflodacin	Azithromycin	Penicillin G	Ciprofloxacin
N. gonorrhoeae	ATCC 49226	*penA* -22	0.03	0.008	0.12	0.25	0.5	0.004
N. gonorrhoeae	FA1090	*penA* -1	0.016	≤0.002	0.03	0.06	0.06	0.004
N. gonorrhoeae	MS11	*penA* -22	0.06	0.016	0.12	0.25	1	0.004
N. gonorrhoeae	WHO G	*penA* -2	0.03	0.008	0.12	0.12	0.5	0.12
N. gonorrhoeae	WHO K	Mosaic *penA* -10	0.12	0.12	0.12	0.25	2	>16
N. gonorrhoeae	WHO L	*penA* -7	0.5	0.25	0.12	0.25	2	16
N. gonorrhoeae	WHO M	*penA* -2	0.06	0.008	0.06	0.25	>64	2
N. gonorrhoeae	CDC-0914	Mosaic *penA* -64	0.06	0.5	0.12	1	2	>16
N. gonorrhoeae	CDC-0197	Mosaic *penA* -34	0.06	0.06	0.06	>4	1	16
N. gonorrhoeae	WHO X (H041)	Mosaic *penA* -37	0.25	2	0.12	0.25	2	>16
N. gonorrhoeae	WHO Q (G7944)	Mosaic *penA* -60	0.06	0.5	0.06	>4	1	16

Table 2 and Figure 1: Correlation Between Broth and Agar Microdilution Assays for Compound Optimization

Species	Strain ID	PBP2 allele	VNRX-6752		VNRX-6884		VNRX-6958		VNRX-14079		Penicillin G	
			Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar
N. gonorrhoeae	ATCC 49226	*penA* -22	0.5	0.5	0.06	0.06	0.06	0.06	0.06	0.03	0.5	0.5
N. gonorrhoeae	FA1090	*penA* -1	0.03	0.06	0.016	0.016	0.016	0.016	0.03	0.016	0.06	0.06
N. gonorrhoeae	MS11	*penA* -22	0.5	0.5	0.06	0.06	0.06	0.06	0.06	0.06	0.5	1
N. gonorrhoeae	WHO G	*penA* -2	0.5	0.25	0.06	0.03	0.06	0.06	0.06	0.03	0.25	0.5
N. gonorrhoeae	WHO K	Mosaic *penA* -10	4	4	0.25	0.25	0.25	0.5	0.12	0.12	2	2
N. gonorrhoeae	WHO L	*penA* -7	8	8	0.5	1	0.5	1	0.5	0.5	2	2
N. gonorrhoeae	WHO M	*penA* -2	0.25	0.5	0.06	0.06	0.06	0.06	0.06	0.06	>32	>64
N. gonorrhoeae	WHO Z	Mosaic *penA* -64	0.5	2	0.12	0.25	0.12	0.25	0.03	0.06	1	2
N. gonorrhoeae	CDC-0197	Mosaic *penA* -34	0.5	2	0.12	0.25	0.12	0.25	0.06	0.06	0.25	1
N. gonorrhoeae	WHO X (H041)	Mosaic *penA* -37	4	16	0.5	1	1	1	0.12	0.25	2	2
N. gonorrhoeae	WHO Q (G7944)	Mosaic *penA* -60	1	4	0.12	0.5	0.12	0.25	0.06	0.06	1	1

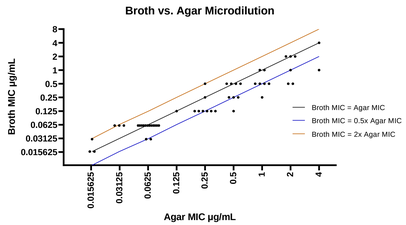
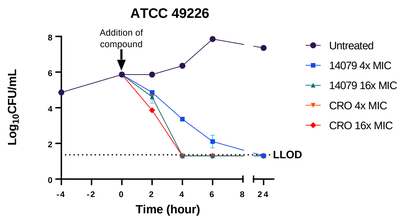
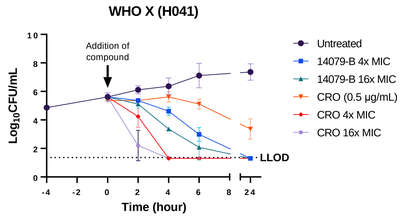


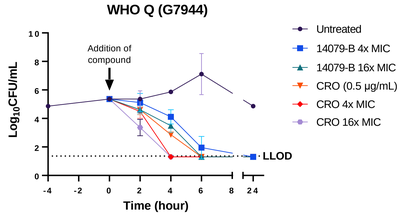
Figure 2: Time Kill Data for VNRX-14079 vs. ceftriaxone in wild-type and mosaic PBP2 producing *N. gonorrhoeae*



49226 Modal Broth MIC µg/mL	
VNRX-14079	0.06
Ceftriaxone	0.004



WHO X Modal Broth MIC µg/mL	
VNRX-14079	0.12
Ceftriaxone	1



WHO Q Modal Broth MIC µg/mL	
VNRX-14079	0.06
Ceftriaxone	0.25

Table 3: Resistance Frequencies

Compound	Concentration	ATCC 49226	WHO X	WHO Q
VNRX-14079	4× MIC	$<1.56 \times 10^{-9}$	$<1.79 \times 10^{-9}$	$<4.17 \times 10^{-9}$
	16× MIC	$<1.56 \times 10^{-9}$	$<1.79 \times 10^{-9}$	$<4.17 \times 10^{-9}$
Ceftriaxone	4× MIC	$<1.56 \times 10^{-9}$	$<1.79 \times 10^{-9}$	$<4.17 \times 10^{-9}$
	16× MIC	$<1.56 \times 10^{-9}$	$<1.79 \times 10^{-9}$	$<4.17 \times 10^{-9}$

Summary of Results

- VNRX-14079 had an MIC_{90} of 0.25 µg/mL in the panel of 11 *N. gonorrhoeae* isolates tested, while ceftriaxone, zoliflodacin, azithromycin, penicillin G, and ciprofloxacin had MIC_{90s} of 0.5, 0.12, >4, 2, and >16 µg/mL, respectively.
- Broth microdilution MICs were within 2-4 doublings of their respective agar MIC ensuring that the fastidious broth microdilution is a quick and reproducible assay for compound screening.
- Both VNRX-14079 and ceftriaxone exhibited undetectable resistance frequencies of $<10^{-9}$ in the CLSI QC strain ATCC 49226 and ceftriaxone-resistant strain WHO Q, and $<4 \times 10^{-9}$ in WHO X.
- VNRX-14079 and ceftriaxone both exhibited rapid bactericidal activity (3-$\log_{10}$ CFU reduction) and sustained activity through 24 hours in ATCC 49226, WHO Q, and WHO X when tested at 4× and 16× the MIC.
- Ceftriaxone at one doubling above the susceptibility breakpoint of ≤0.25 µg/mL (0.5 µg/mL) showed sustained bactericidal activity through 24 hours in WHO Q (2× MIC). In WHO X, bacteriostasis was observed through 6 hours and a 2-$\log_{10}$ CFU reduction was observed at 24 hour (not bactericidal).

Conclusions

VNRX-14079 showed potent antibacterial activity against representative susceptible and resistant isolates (within a doubling dilution of zoliflodacin and more potent than ceftriaxone), a low frequency of resistance, and was bactericidal in time-kill studies against both wild-type and mosaic PBP2 producing *N. gonorrhoeae*. These results support further development of VNRX-14079 as a promising anti-gonorrhea agent.

References

1. CLSI. 2018. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-Eleventh Edition. M07Ed11E, 11th Edition.
2. CLSI. 2023. Performance standards for antimicrobial susceptibility testing. M100, 33rd Edition.
3. Foerster, S., Unemo, M., Hathaway, L.J. *et al.* Time-kill curve analysis and pharmacodynamic modelling for in vitro evaluation of antimicrobials against *Neisseria gonorrhoeae* . *BMC Microbiol* **16**, 216 (2016)
4. Cartwright CP, Stock F, Gill VJ. 1994. Improved enrichment broth for cultivation of fastidious organisms. J Clin Microbiol 32:

This project has been funded in whole or in part by NIH R01 grant (5R01AI41239)