AK072019: A Molecular Inhibitor of *MRSA* Acetate Kinase Exhibiting Cellular-Level Efficacy against *Staphylococcus aureus*

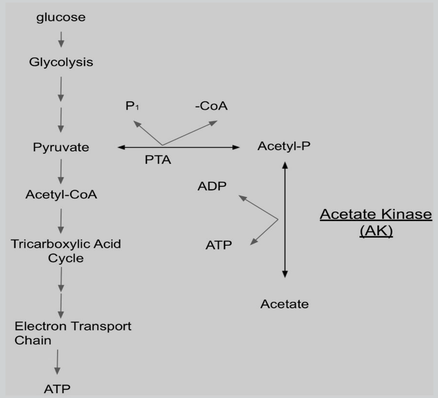
Madison Kovar, Mitchell Lonneman, Chun Wu (Ph.D.)
Mount Marty University, 1105 W 8th St. Yankton SD

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections pose a significant therapeutic challenge, driving the need for novel antibiotics. Our lab targeted the bacterial central metabolism, specifically MRSA acetate kinase (ACK), as a potential drug target for MRSA infection due to its in silico essentiality to MRSA and absence in humans. MRSA ACK was successfully cloned, expressed, purified, and characterized. High-throughput inhibitor screening identified 38 potential hits, with compound AK072019 ranking high in our priority list. This poster displayed the inhibition kinetics, antibacterial activity, and selectivity of AK072019 at the molecular and cellular levels. Kinetic assays revealed AK072019 to be a competitive inhibitor of *MRSA* ACK, exhibiting a Ki value of 448.0 µM. Comparative analysis of antibacterial activity of inhibitor AK072019 against 17 species of representative Gram positive and Gram-negative bacteria was conducted by Kirby-Bauer disk diffusion method. The results demonstrated that AK072019 selectively inhibit the growth of Gram-positive bacteria *Staphylococcus aureus, Staphylococcus epidermidis and Bacillus cereus* without inhibiting the rest of Gram-positive and Gram-negative bacteria tested. The observed efficacy and selectivity of AK072019 underscore its potential as a candidate of narrow-spectrum antibiotic. Despite AK072019 displaying micromolar-level inhibition, it is remarkable that this molecular inhibitor of a newly identified drug target exhibits selective efficacy at the cellular level. These findings provide the foundation for further investigations aiming to develop secondary inhibitors of MRSA ACK with improved inhibitory activity and cellular efficacy, that holds significant promise for combatting MRSA infections effectively.

Introduction

- Methicillin/Multidrug resistant *Staphylococcus aureus* (MRSA) are strains of *S. aureus* that are resistant to one or more antibiotics.
- An increase in MRSA infections is pushing for new antibiotics.
- Currently existing antibiotics only target four cellular functions, *i.e.* cell wall synthesis, folic acid production, DNA replication, protein synthesis.
- For novel antibiotic development, central metabolism was selected as a new drug target.
- Our previous research has identified *MRSA* Acetate Kinase (ACK) as a potential drug target for central metabolism antibiotics. This enzyme was cloned, expressed, purified an d characterized. Compound AK072019 was identified as one of the hits through High throughput inhibitor screening



Scheme 1. Metabolic Role of *MRSA* acetate kinase

Purpose

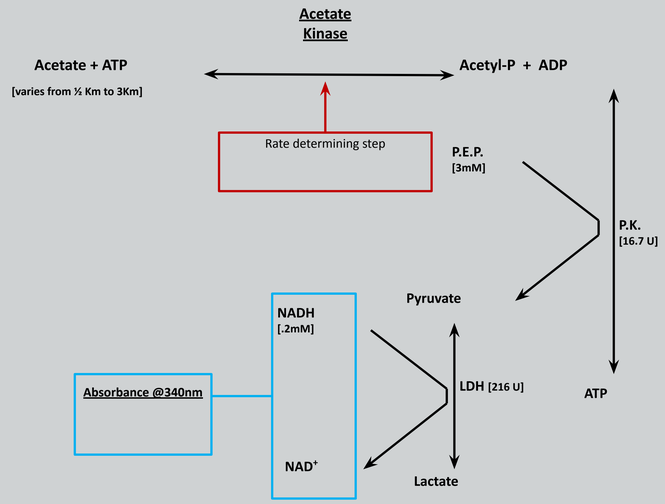
Investigate the molecular and cellular efficacy of AK072019

Methods

- Kinetic Assay as the molecular levels
- Kirby-Bauer disk diffusion susceptibility test at the cellular level

Results

Molecular Level Analysis



Scheme 2: ACK/PK/LDH kinetic assay coupling system

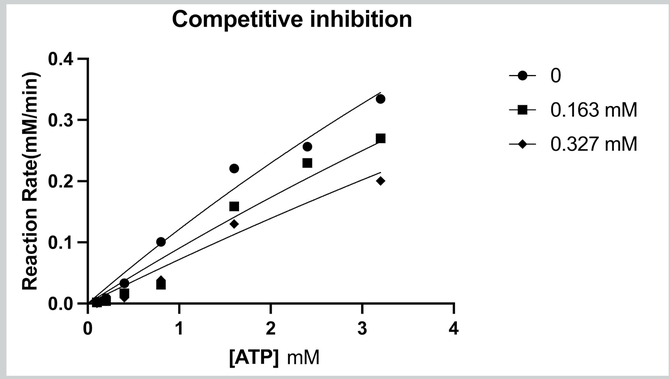


Figure 1 Non-linear regression of Competitive Inhibition of ACK by AK 072019. Concentrations of AK 072019 are 0(•), 0.163 mM ( ) and 0.327 mM(·), respectively. The assay were performed in 1 mL assay solutions containing 0.1 to 3.2 mM ATP, 3 mM PEP, 100 mM NaOAc, 5 mM $MgCl_2$, 0.2 mM NADH, 10 units/mL LDH, 5 unite/mL PK in 60 mM K^+HEPES (pH 7.0, 25 ˚C). The reaction was initiated by the addition of AK. The initial velocity data were analyzed using the following two equations $K_{m(Obs)}$=Km*(1+[I]/Ki), v_0=v_{max}*[ATP]/($K_{m(Obs)}$+[ATP]), and the computer program Prism GraphPad.

Cellular Level Analysis

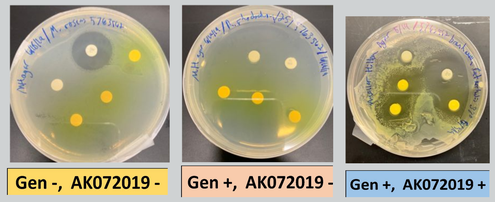


Fig. 2. Kirby-Bauer disk diffusion susceptibility test. Bacteria grown on Mueller-Hinton agar with disk containing 10 µg gentamicin (top middle, positive control) and 10 µL DMSO (top right, negative control) and three replicates of 500 µg inhibitor AK072019 (bottom three). Note: a) Gentamicin positive, AK072019 positive; b) Gentamicin positive, AK072019 negative; c) Gentamicin negative, AK072019 positive;

Gram Positive	Kirby Bauer AK072109	Gram Negative	Kirby Bauer AK072019
Staphylococcus aureus	Gen +/AK072019 + (14 ± 2 mm, n=3)	*Branhamella Catarrhalis*	Gen +/AK072019 – n= 3
Staphylococcus epidermidis	Gen +/AK072019 + (16 mm ± 4mm, n=2)	*Citrobacter freundii*	Gen +/AK072019 – n= 2
Bacillus cereus	Gen +/AK072019 + (16 mm ± 4mm, n=2)	*Enterobacter aerogenes*	Gen +/AK072019 – n= 2
Corynebacterium xerosis	Gen +/AK072019 – n=3	*Enterobacter cloacae*	Gen +/ AK072019 – n= 2
Micrococcus roseus	Gen +/AK072019 – n= 3	*Escherichia coli*	Gen +/ AK072019 – n= 2
Micrococcus luteus	Gen +/AK072019 – n=3	*Pseudomonas fluorescens*	Gen +/AK072019 – n= 2
Streptomyces griseus	Gen +/AK072019 – n=2	*Rhodospirillum rubrum*	Gen +/AK072019 – n= 2
Bacillus coagulans	Gen -/AK072019 – n=2	*Serratia liquefaciens*	Gen +/AK072019 – n= 2
Rhodococcus rhodochrous	Gen –/AK072019 – n=3		

Table 1: Zone of Inhibition for Kirby-Bauer antibiotic susceptibility test. Gen —Gentamicin, + — positive, - —negative
n —number of consistent trails

Discussions

- AK072019 inhibits *MRSA* Acetate Kinase at the molecular level with K*i* value of 448.0 µM
- AK072019 inhibits the growth of *Staphylococcus aureus* at the cellular level
- AK072019 exhibits micromolar-level inhibition, highlighting its potential as a molecular inhibitor for ACK, a newly identified drug target. Remarkably, it demonstrates selective efficacy at the cellular level. These findings serve as a basis for future studies seeking to develop secondary inhibitors targeting MRSA ACK, with enhanced inhibitory activity and cellular effectiveness. This research holds significant promise in effectively combating MRSA infections.

Conclusions

AK072019 demonstrates micromolar-level inhibition and exhibits selective efficacy at the cellular level as a molecular inhibitor for a newly identified drug target. These findings establish a basis for future investigations to develop secondary inhibitors of MRSA ACK, with enhanced inhibitory activity and cellular efficacy, presenting promising opportunities for effectively combating MRSA infections.

Acknowledgements

This presentation was made possible by NIH Grant Number 2 P20 RR016479 from the INBRE Program of the National Center for Research Resources.

References

•Haag, NL , Velk, KK, Wu, C (2012). “Potential Antibacterial Targets in Bacterial Central Metabolism”. International Journal on Advances in Life Sciences. 4 (1&2,):21-32.
•Bauer, AW; Kirby, WM; Sherris, JC; Turck, M (1966). "Antibiotic susceptibility testing by a standardized single disk method". American journal of clinical pathology. 4 (4): 493–6
•“Non-linear regression of Competitive Inhibition was performed using GraphPad Prism version 9.5.1 (528) for Mac, GraphPad Software, San Diego, California USA, www.graphpad.com”
•Equation 3.1 in: RA Copeland, Evaluation of Enzyme Inhibitors in Drug Discovery, Wiley 2005. IBSN:0471686964.RA Copeland, Evaluation of Enzyme Inhibitors in Drug Discovery, Wiley 2005. IBSN:0471686964.