ROOSEVELT UNIVERSITY
COLLEGE OF PHARMACY

Synthesis of truncated, methanethiosulfonate-containing analogues of ACHN-975

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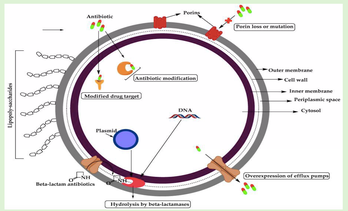
Purpose

This project aims to investigate synthetic modifications of previously reported LpxC inhibitor ACHN-975, a potential drug candidate for Gram-negative bacteria.

Introduction

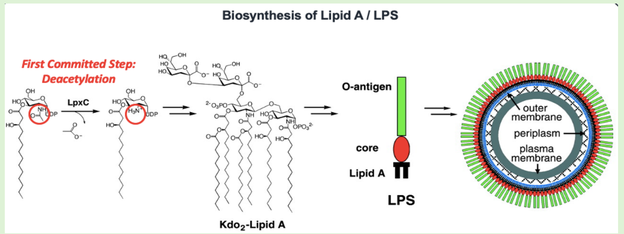
- Antibiotic resistance (AR) in Gram-negative bacteria is on the rise, causing difficult-to-treat drug resistant infections
- Per the 2019 AR threat report from the CDC, more than 2.8 million AR infections occur in U.S. each year causing 35,000 deaths
- Gram-negative bacteria are intrinsically more resistant to antibiotics than Gram-positive bacteria due to their extra outer membrane
- Most nosocomial infections are caused by Gram-negative pathogens, including hospital-acquired or ventilator-associated pneumonia, blood stream infections caused by P. aeruginosa, and urinary tract infections caused by E. coli
- There are multiple mechanisms by which gram-negative bacteria acquire resistance as shown below:



- Many pharmaceutical industries choose not to invest in new antibiotic research because the market is not lucrative

Background

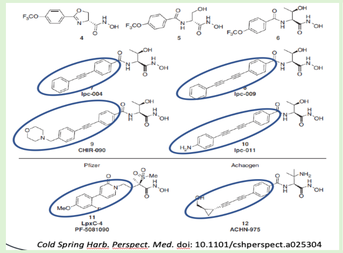
- Gram-negative bacteria contain a lipid A moiety, which is the hydrophobic anchor of lipopolysaccharide (LPS)
- LPS (endotoxin) is notorious for causing endothelial cell injury and toxic reactions by promoting proinflammatory cytokines
- Patients mostly manifest with septic shock symptoms, such as hypotension, tachycardia, fever and increased in WBCs

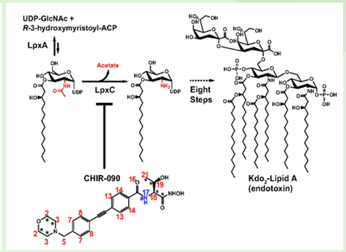


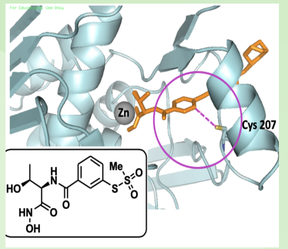
- Biosynthesis of Lipid A is a multistep process involving nine different enzymes
- The second step of this process involves deacetylation of UDP-3-O-acyl-GlcNAc and is catalyzed by LpxC
- This acetylation is irreversible and considered the committed step in the biosynthesis of Lipid A
- Gram-negative bacteria with defective Lipid A biosynthesis have shown increased permeability and sensitivity to antibiotics
- Therefore, inhibiting LpxC could be a potential strategy for the development of a new antibiotic

Hypothesis & Specific Aims

- Several LpxC inhibitors with low to sub-nanomolar activities have been discovered, but only a single agent (ACHN-975) has reached clinical trials.
- ACHN-975 failed its Phase I clinical trial due to side effects at the infusion site, theoretically because it contains a highly lipophilic side chain.
- Our research aims to re-design this known inhibitory scaffold as a bifunctional, zinc-chelating and covalent inhibitor enabling truncation of the lipophilic side chain that benefits PK

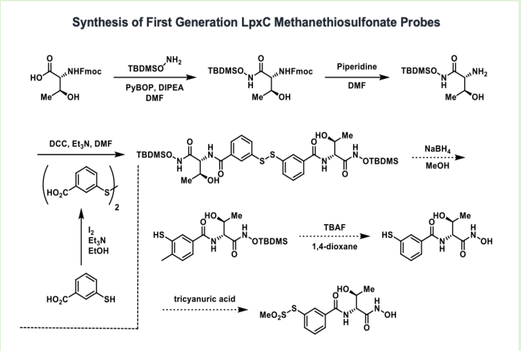






Methods

A synthetic route of truncated, electrophilic analogues is shown below in the figure:



- Step 1: FMOC-threonine was coupled with TBDMS-protected hydroxylamine with PyBOP to install the protected hydroxamic acid
- Step 2: The FMOC group was removed using piperidine in DMF to expose the free amine
- Step 3: The resulting amine was coupled with a 3-mercapobenzoic acid disulfide dimer using DCC
- Step 4: $NaBH_4$ was used to reduce the disulfide
- Step 5: TBAF was used to deprotect the TBDMS group, exposing the hydroxamate
- Step 6: The electrophilic methanethiosulfonate group was installed with tricyanuric acid in DMSO

Conclusions

- Several reversible LpxC inhibitors have been developed with only one agent reaching clinical trials but failing Phase I due to adverse effects
- To overcome the limitations of previously developed LpxC inhibitors, we hypothesize to re-design the scaffold by removing the highly lipophilic side chain
- Inhibition of LpxC is an exciting strategy in next-generation antibiotic design because it addresses LPS-associated gram-negative virulence

Future Directions

- Moving forward, we will complete the synthesis of our re-designed compound
- Once synthesized, compounds will be evaluated for dual mechanism of inhibition against EcLpxC
- Irreversible inhibition will be validated using time dependent kinetics, exhaustive dialysis, competition studies, mass spectrometry and mutant studies

References

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