In Vitro Antibacterial Susceptibility Testing of Sulopenem Against Category A and B Bio-Threat Bacterial Pathogens

Michael W. Dunne, MD[1], Steven I. Aronin, MD[1], Stephanie A. Halasohoris[2], Lisa M. Pysz, BS[2], Sanae Lembirik, BS[2], and James M. Meinig, PhD[2]
[1]Iterum Therapeutics, Old Saybrook, CT 06475; [2]US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702.

ABSTRACT

Background: Sulopenem is a thiopenem β-lactam antibiotic being developed for the treatment of infections caused by multi-drug resistant bacteria. Sulopenem possesses potent activity against species of the Enterobacterales that encode ESBLs or AmpC-type β-lactamases that confer resistance to third generation cephalosporins. It has also demonstrated good *in vitro* microbiological activity against a range of bacterial pathogens including penicillin resistant *S. pneumoniae,* β-lactamase-producing *H. influenzae* and *M. catarrhalis*. Sulopenem is available as intravenous and oral pro-drug formulations, and its activity aligns with the most urgent drug-resistant antimicrobial threats defined by the CDC.

Materials/methods: Bacterial inoculums were prepared by suspending colonies into cation adjusted Mueller Hinton broth (CAMHB) from 18-24 h (*B. anthracis, B. pseudomallei* and *B. mallei* plates incubated at 35°C); or 36-48 h (*F. tularensis* and *Y. pestis* plates incubated at 35°C and 28°C, respectively). Sheep blood agar plates were used for *B. anthracis* and *Y. pestis*. Chocolate agar plates were used for *F. tularensis, B. pseudomallei* and *B. mallei*. Suspended cultures were diluted with CAMHB to achieve a turbidity equivalent to a 0.5 McFarland standard. MICs were determined by the microdilution method in 96-well microplates according to CLSI guidelines (Clinical and Laboratory Standards Institute, 2020). Antibiotic ranges used for sulopenem were 0.03 - 64 µg/mL and 0.004 - 8 µg/mL for the diversity strains of *B. anthracis, F. tularensis, Y. pesis, B. mallei,* and *B. pseudomallei*, based on a final well volume of 100 µl after inoculation.

Results: A summary of sulopenem MIC_{90} results versus bio-threat bacterial pathogens in presented in the table. Criteria for down selection into mice was met for all pathogens except *F. tularensis*.

	B. anthracis	*F. tularensis*	*Y. pestis*	*B. mallei*	*B. pseudomallei*
Criteria	MIC_{90} (µg/ml) ≤1	MIC_{90} (µg/ml) ≤1	MIC_{90} (µg/ml) ≤1	MIC_{90} (µg/ml) ≤4	MIC_{90} (µg/ml) ≤4
Sulopenem	0.03	32	0.12	0.25	1

Sulopenem MIC_{90} Summary for Down-Selection Criteria

Conclusions: Sulopenem is active *in vitro* against a number of bio-threat pathogens at concentrations likely to be achieved after oral dosing in humans and meets criteria to be tested in the murine model of *B. anthracis, Y. pestis, B. mallei,* and *B. pseudomallei*.

INTRODUCTION

- The threat of bioterrorism has increased in the past 20-25 years.
- The Centers for Disease Control and Prevention (CDC) has classified bioterrorism agents into three categories, based on ease of dissemination, potential to cause severe disease, and predicted mortality rate:

Category	Characteristics
A	Highest risk to national security and public health: • Easily disseminated or transmitted from person to person • High mortality rates and potential for major public health impact • Might cause public panic and social disruption • Require special action for public health preparedness
B	Second highest priority: • Moderately easy to disseminate • Moderate morbidity rates and low mortality rates • Require specific enhancements for diagnostic capacity and disease surveillance
C	Third highest priority, includes emerging pathogens that could be engineered for mass dissemination in the future because of: • Availability • Ease of production and dissemination • Potential for high morbidity and mortality rates and major health impact

- Current antibiotic treatment options against Category A and B biothreat pathogens are limited.
- Current agents are at risk for engineered antibiotic resistance.
- New therapeutic options are therefore needed for prophylaxis and treatment of the diseases caused by these pathogens.

*In general, a 4 µg/ml *in vitro* MIC_{90} value for *B. mallei,* and *B. pseudomallei* and a 1 µg/ml *in vitro* MIC_{90} value for *B. anthracis, F. tularensis, and Y. pestis* supports the progression of an antibiotic into *in vivo* studies.

  - These values are reasonably achievable in serum, without regard for tissue accumulation or individual drug performance.

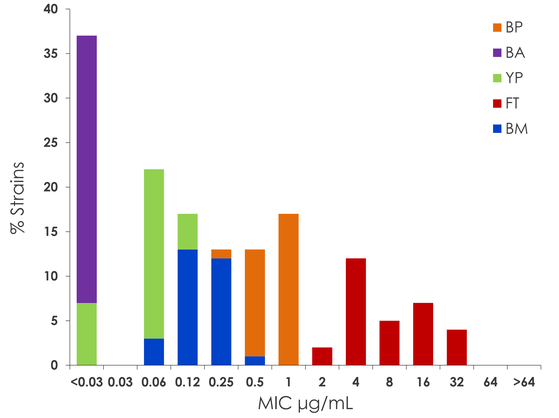
- Sulopenem is a thiopenem β-lactam antibiotic being developed for the treatment of infections caused by multi-drug resistant bacteria.
- Sulopenem's activity aligns with the most urgent drug-resistant antimicrobial threats defined by the CDC, including potent activity against species of the Enterobacterales that encode ESBLs or AmpC-type β-lactamases that confer resistance to third generation cephalosporins.
- Sulopenem has also demonstrated good *in vitro* microbiological activity against a range of bacterial pathogens including penicillin resistant *S. pneumoniae,* β-lactamase-producing *H. influenzae* and *M. catarrhalis*.
- Sulopenem is available as intravenous and oral pro-drug formulations.

METHODS

- *In vitro* antibacterial activity of sulopenem was evaluated against the geographically bio-diverse sets of 30 strains each of *Bacillus anthracis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Francisella tularensis*, and *Yersinia pestis*.
- Bacterial inoculums were prepared by suspending colonies into cation adjusted Mueller Hinton broth (CAMHB):
  - *B. anthracis, B. pseudomallei* and *B. mallei* plates were incubated at 35°C for 18-24 h
  - *F. tularensis* and *Y. pestis* plates were incubated at 35°C and 28°C, respectively, for 36-48h
- Sheep blood agar plates were used for *B. anthracis* and *Y. pestis.*
- Chocolate agar plates were used for *F. tularensis, B. pseudomallei* and *B. mallei.*
- Suspended cultures were diluted with CAMHB to achieve a turbidity equivalent to a 0.5 McFarland standard.
- MICs were determined by the microdilution method in 96-well microplates according to CLSI guidelines (Clinical and Laboratory Standards Institute, 2020).
- Antibiotic ranges used for sulopenem were 0.03 - 64 µg/mL and 0.004 - 8 µg/mL for the diversity strains of *B. anthracis, F. tularensis, Y. pestis, B. mallei,* and *B. pseudomallei*, based on a final well volume of 100 µl after inoculation.

RESULTS

Figure 1: MIC Distribution for Sulopenem Starting Concentration at 64 µg/mL (N=30)



RESULTS

Figure 2: MIC Distribution for Sulopenem Starting Concentration at 8 µg/mL (N=30)

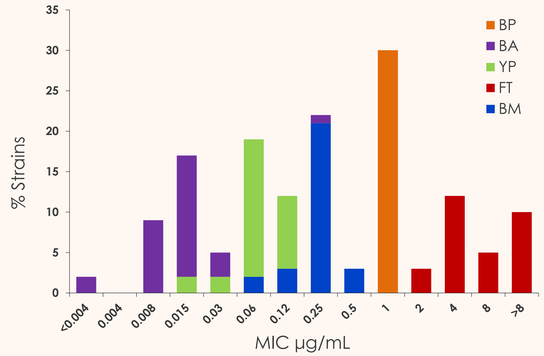


Table 1: MIC Summary for Sulopenem Against Category A Pathogens

B. anthracis (n=30)			*F. tularensis* (n=30)			*Y. pestis* (n=30)		
MIC Range	MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}
<0.004 – 0.25	0.015	0.03	2 - 32	8	32	0.015 – 0.125	0.063	0.12

All values listed are in µg/mL

Table 2: MIC Summary for Sulopenem Against Category B Pathogens

B. mallei (n=30)			*B. pseudomallei* (n=30)		
MIC Range	MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}
0.06 – 0.50	0.25	0.50	1	1	1

All values listed are in µg/mL

Table 3: MIC_{90} Summary for Down-Selection Criteria

	B. anthracis	*F. tularensis*	*Y. pestis*	*B. mallei*	*B. pseudomallei*
Criteria	MIC_{90} (µg/ml) ≤1	MIC_{90} (µg/ml) ≤1	MIC_{90} (µg/ml) ≤1	MIC_{90} (µg/ml) ≤4	MIC_{90} (µg/ml) ≤4
Sulopenem	0.03	32	0.12	0.25	1

All values listed are in µg/mL

CONCLUSIONS

- Sulopenem is active *in vitro* against a number of Category A and Category B bio-threat pathogens at concentrations likely to be achieved after oral dosing in humans.
- Based on the results of our *in vitro* testing (summarized in Table 3), sulopenem meets criteria* to be tested in the murine model of *Bacillus anthracis, Yersinia pestis, Burkholderia mallei,* and *Burkholderia pseudomallei*.

REFERENCES

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