O085

1-hour Oral Session

Drug discovery

Optimizing the pyrrolocytosines for enhanced influx into Gram-negative pathogens

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Objectives The pyrrolocytosines are a novel class of ribosome inhibitors that have been rationally designed and optimized to have broad-spectrum activity, including against multidrug- (MDR) and extremely-drug (XDR) resistant Gram-negative pathogens. A highlight of the class is the lack of cross-resistance to current therapies. Previously, the molecular features that optimized avoidance of efflux for this class were disclosed. This effort was undertaken to identify areas of the molecular scaffold where influx could be impacted and optimized. Representative molecules confirmed as highly-active ribosome inhibitors, which varied in activity against MDR isolates of Enterobacteriaceae were chosen. Examples with optimized properties to avoid efflux were included to determine whether influx and efflux could be balanced on a single scaffold. Compounds were assessed for their ability to access cytoplasmic targets in *Klebsiella pneumoniae*. Isolates containing outer membrane porin defects and isolates featuring lipopolysaccharide-deficient (LPS) mutants were studied.

Methods Susceptibilities of the *K. pneumoniae* isolates to 24 pyrrolocytosines were determined by CLSI broth microdilution. To assess influx in the presence of porin defects, nine carbapenem-resistant isolates representing low-, intermediate- and high-resistance were studied. Three of these lacked a functional OmpK35 porin; three of these lacked both OmpK35 and OmpK36, one of these lacked OmpK35 and OmpK37 and two of these lacked all three. To assess influx in the presence of LPS-deficient mutants, eight *K. pneumoniae* isolates with unique LPS characteristics plus parent strains were studied. *Escherichia coli* ATCC25922 was the quality control strain.

Results Five molecules were highly active (MIC ranges 0.25 – 2 micrograms/mL) against all isolates tested. Pairs of compounds with designed modifications on the "tail" region of the pyrrolocytosine scaffold evidenced an impact on influx (Table). Molecular properties that enhance influx have been identified and incorporated into subsequent optimization cycles.

Isolate Number		ATCC 13883	ART 2008226	ART 2008136	ART 2008026	ART 2008141	ART 2008027	VA-406
Carbapenem-resistance category		WT	KPC Low	KPC Intermed	KPC High	KPC High	KPC High	KPC
Non-functional OmpK		WT SHV1	OmpK35	OmpK35	OmpK35	OmpK35, OmpK36	OmpK35, OmpK36	OmpK35, OmpK36, OmpK37
Cpd	Analog	MICs (mcg/mL)						
RX-P792	Parent	0.25	0.25	0.25	0.5	0.5	0.25	0.5
RX-P873	Tail Mod	0.25	0.25	0.5	0.25	0.25	0.25	0.5
RX-P933	Parent	0.25	2	1	2	4	2	8
RX-P934	Tail mod	0.25	1	0.5	1	1	0.5	1
P1366	Parent	0.25	4	4	32	16	4	32
P1368	Tail mod	0.5	1	0.5	4	1	1	2

Conclusions Pyrrolocytosines have been optimized to show a robustness to modifications of the outer membrane of *K. pneumoniae*. A chemical handle has been identified where influx can be modulated and optimized. This can be done preserving properties important for reducing the efflux liability, maintaining broad-spectrum activity across the Enterobacteriaceae as well as non-fermenters including *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.