AAI101, a novel β-lactamase inhibitor: microbiological and enzymatic profiling

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Revised Abstract

Background: AAI101 is a novel β-lactamase inhibitor, active against ESBLs and other β-lactamases. AAI101 combined with cefepime is in Phase 2 clinical trials. The objective of this study was to determine differences between AAI101 and tazobactam in their inhibition of selected β-lactamases of clinical relevance.

Methods: Isogenic *E. coli* strains expressing single clinically relevant β-lactamases were tested for susceptibility. Periplasmic β-lactamase extracts from selected strains were used to determine IC_{50}s for AAI101 and for tazobactam. β-Lactamases with low IC_{50}s for AAI101 (SHV-1, CTX-M-15, KPC-2) were purified and steady-state inactivation kinetics determined for AAI101, tazobactam, and avibactam (inhibitor structures, **Figure 1 (top)**). Mass spectrometry (MS) was used to assess the formation of intermediates (intermediate structures, **Figure 1 (bottom)**) during inactivation of SHV-1, CTX-M-15, and KPC-2 with tazobactam and AAI101. To evaluate the interactions in the active site of the β-lactamase upon acylation by AAI101 or tazobactam, molecular models were generated.

Results: AAI101 restored activity of cefepime against isogenic *E. coli* strains producing defined β-lactamases; cefepime/AAI101 was more potent than cefepime, and piperacillin/AAI101 was more potent than piperacillin/tazobactam (**Table 1**). Inactivation kinetics of SHV-1 by AAI101, tazobactam, and avibactam, revealed that AAI101 acylated SHV-1 faster than avibactam, but similar to tazobactam (**Figure 2 (left); Table 2**). AAI101, avibactam, and tazobactam displayed similar kinetics towards CTX-M-15 (**Figure 2 (center); Table 2**). AAI101 acylated KPC-2 faster than tazobactam, at a rate comparable to that of avibactam (**Figure 2 (right); Table 2**). Avibactam had a slightly slower off rate (k_{off}) than tazobactam or AAI101 (**Table 3**). Partition ratios revealed that more tazobactam was turned over by all three β-lactamases at 15 min (**Table 4**). Inactivation of SHV-1 by AAI101 and tazobactam monitored at different time points by MS revealed that SHV-1 is completely modified (-Δ14 ± 5 Da) upon reaction with AAI101, but not with tazobactam (**Figure 3A**). This modified SHV-1 is inactive towards nitrocefin (**Figure 3B**). A modified CTX-M-15 (-Δ14 ± 5 Da) also was identified upon incubation with AAI101 and tazobactam (**Figure 3A**); this modification inactivated CTX-M-15 (**Figure 3B**). However, the -Δ14 ± 5 Da modification was not observed with KPC-2 upon reaction with AAI101 or tazobactam (**Figure 3A**). However, apo-KPC-2 was evident within 15 min of incubation with tazobactam, and only the acyl-enzyme form was present following incubation with AAI101 (**Figure 3A**). After 24 h KPC-2 was fully active following incubation with AAI101 or tazobactam (**Figure 3B**). AAI101 and tazobactam were docked into the CTX-M-15 active site and molecular dynamics simulations (MDS) were conducted. The hydrogen bonding interactions of AAI101 and tazobactam with the active site pocket of CTX-M-15 are different (**Figure 4**).

Conclusion: Addition of AAI101 restores the activity of cefepime *vs.* a selected array of β-lactamases expressed in *E. coli* in an isogenic background. The inhibitory kinetics of β-lactamases by AAI101 and tazobactam and MS/MDS studies indicate different mechanisms of β-lactamase inhibition by these two β-lactamase inhibitors.

Background

- **Extended-spectrum β-lactamases (ESBLs) are the principal β-lactam resistance mechanism amongst Enterobacteriaceae,[1] and their global prevalence is increasing around the world.[2]**
- **Class A ESBLs are typically susceptible to inactivation by clavulanic acid, sulbactam, tazobactam, and avibactam.**
- **However, erosion of the efficacy of BLIs due to emergence and spread of new β-lactamases has created a need for new BLIs.**
- **The aim of this study was to determine the activity of a novel penicillanic acid sulfone β-lactamase inhibitor, AAI101, and to compare its activity with those of other β-lactamase inhibitors.**

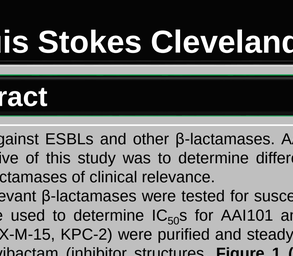

Figure 1. Chemical structures of the β-lactamase inhibitors and the intermediates (tazobactam)[3] formed during the reaction course.

Materials and Methods

Susceptibility testing. Minimum inhibitory concentrations (MICs) were determined by broth microdilution according to methods described by the Clinical and Laboratory Standards Institute.

Inhibitor kinetics. Determination of kinetic parameters k_2/K, k_{off}, and k_{cat}/k_{inact} has been described previously, and full methodology and equations used are defined in Papp-Wallace *et al.*[3]

Mass spectrometry. To discern the nature of the intermediates of inactivation in the reaction pathway with β-lactamases, electrospray ionization mass spectrometry (ESI-MS) was performed.[3,4]

Molecular modeling. Discovery Studio 3.1 molecular modeling software (Accelrys, Inc. San Diego, CA) was used to dock AAI101 and tazobactam into the active site of CTX-M-15 (PDB 4HBU). Molecular dynamic simulations were conducted for 80 ps, as described previously.[3,4]

Results

Susceptibility Testing

Results: AAI101 restored activity of cefepime and piperacillin against isogenic *E. coli* strains producing defined β-lactamases. Cefepime/AAI101 was more potent than cefepime, and piperacillin/AAI101 was more potent than piperacillin/tazobactam (**Table 1**).

Table 1. Susceptibility testing results in mg/L. The concentration of β-lactam was varied while the β-lactamase inhibitors, AAI101 and tazobactam were held at the fixed concentrations listed.

β-lactamase (classification) (amino acid substitutions present)	CEFEPIME	CEFEPIME/ AAI101 (4 mg/L)	CEFEPIME/ AAI101 (8 mg/L)	PIPERACILLIN/ TAZOBACTAM (4 mg/L)	PIPERACILLIN/ AAI101 (4 mg/L)	PIPERACILLIN/ AAI101 (8 mg/L)	IMIPENEM	MEROPENEM
E. coli DH10B*	<= 0.06	<= 0.06	<= 0.06	2	2	2	0.25	<= 0.06
Class A								
SHV-1 (OSBL)	2	0.25	<= 0.06	> 256	16	8	0.25	<= 0.06
SHV-2 (ESBL) (G238S)	4	<= 0.06	0.12	32	4	4	0.25	<= 0.06
SHV-5 (ESBL) (G238S, E240K)	8	<= 0.06	<= 0.06	256	4	4	0.25	<= 0.06
SHV-7 (ESBL) (I8F, R43S, G238S, E240K)	8	<= 0.06	<= 0.06	32	4	4	0.25	<= 0.06
SHV-8 (ESBL) (D179N)	2	<= 0.06	<= 0.06	2	2	2	0.25	<= 0.06
SHV-10 (IR) (S130G)	<= 0.06	<= 0.06	<= 0.06	> 256	> 256	256	0.25	<= 0.06
SHV-14 (OSBL) (I8F, R43S)	0.25	<= 0.06	<= 0.06	> 256	4	4	0.25	<= 0.06
SHV-26 (OSBL) (A187T)	0.25	<= 0.06	<= 0.06	> 256	4	4	0.25	<= 0.06
SHV-30 (ESBL) (I8F, R43S, G238S)	2	0.12	0.12	32	2	2	0.25	<= 0.06
SHV-49 (IR) (M69I)	<= 0.06	<= 0.06	<= 0.06	> 256	> 256	128	0.25	<= 0.06
SHV-84 (IR) (K234R)	0.12	<= 0.06	<= 0.06	8	8	8	0.25	<= 0.06
SHV-102 (ESBL) (G238A)	16	0.12	<= 0.06	> 256	8	2	0.25	<= 0.06
SHV-106 (ESBL) (I8F, G238S)	4	<= 0.06	<= 0.06	16	2	2	0.25	<= 0.06
SHV-120 (ESBL) (E240K)	0.25	<= 0.06	<= 0.06	> 256	16	8	0.25	<= 0.06
SHV-129 (ESBL) (G238S, E240K, R275L, N276D)	16	<= 0.06	<= 0.06	128	4	2	0.25	<= 0.06
SHV-141 (ESBL) (R43S, G238S)	0.25	<= 0.06	<= 0.06	2	2	2	0.12	<= 0.06
SHV-154 (ESBL) (R43S, G238S, E240K)	8	<= 0.06	<= 0.06	4	4	4	0.25	<= 0.06
SHV-161 (OSBL) (R43S)	0.5	<= 0.06	<= 0.06	> 256	8	4	0.25	<= 0.06
TEM-10 (ESBL) (R164S, E240K)	4	<= 0.06	<= 0.06	4	4	4	0.25	<= 0.06
TEM-26 (ESBL) (E104K, R164S)	0.5	<= 0.06	<= 0.06	2	2	2	0.12	<= 0.06
TEM-30 (IR) (R244S)	<= 0.06	<= 0.06	<= 0.06	256	64	16	0.25	<= 0.06
CTX-M-14 (ESBL)	8	<= 0.06	0.12	2	2	2	0.12	<= 0.06
CTX-M-15 (ESBL)	32	<= 0.06	<= 0.06	2	2	2	0.25	<= 0.06
KPC-2 (carbapenemase)	4	0.12	0.12	256	16	8	4	2
KPC-3 (carbapenemase)	4	0.25	<= 0.06	256	32	8	2	0.5
Class C								
CMY-2 (AmpC)	0.25	<= 0.06	<= 0.06	4	8	8	0.25	<= 0.06
PDC-3 (AmpC)	<= 0.06	<= 0.06	<= 0.06	2	8	4	0.25	<= 0.06
ADC-7 (AmpC)	0.12	0.12	<= 0.06	8	8	8	0.25	<= 0.06
Class D								
RGN238 OXA-1 (penicillinase)	2	0.25	0.12	256	64	32	0.25	<= 0.06
OXA-24/40 (carbapenemase)	<= 0.06	<= 0.06	<= 0.06	128	128	64	1	0.5
OXA-48 (carbapenemase)	0.12	0.12	<= 0.06	256	256	128	2	1
OXA-51 (carbapenemase)	<= 0.06	<= 0.06	<= 0.06	8	8	8	0.25	<= 0.06
OXA-58 (carbapenemase)	0.12	0.12	0.12	> 256	> 256	> 256	2	0.25

*All *bla* genes were expressed from pBC SK(+) in *E. coli* DH10B, except *bla*OXA-1 which was expressed from plasmid RGN238.
OSBL, original-spectrum β-lactamase; ESBL; extended-spectrum β-lactamase; IR, inhibitor-resistant (clavulanic acid).
Susceptible (green), Intermediate or Susceptible-Dose Dependent (gold), Resistant (red) – based on 2017 β-lactam breakpoints (CLSI M100-S27).

Inhibition kinetics

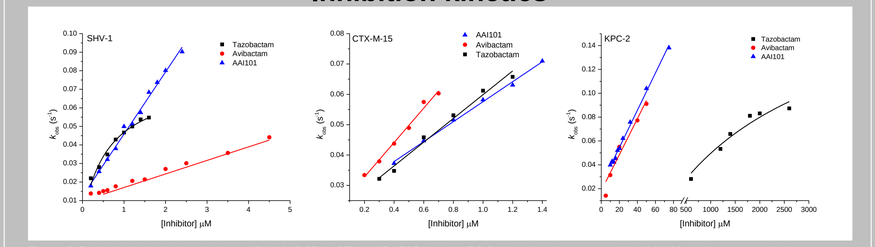

Figure 2. Plots displaying the inactivation of SHV-1 (OSBL), CTX-M-15, (ESBL), and KPC-2 (class A carbapenemase) by AAI101, tazobactam and avibactam.

Table 2. k_2/K or k_{inact}/K_I ($M^{-1}s^{-1}$) (time-dependent inhibition)

β-lactamase	AAI101	Tazobactam	Avibactam
SHV-1	233,100 ± 23,300	390,000 ± 39,000	60,300 ± 6,000[5]
CTX-M-15	187,200 ± 18,720	223,200 ± 22,320	333,000 ± 33,300
KPC-2	16,500 ± 2,200	2,500 ± 200[6]	21,580 ± 2,200[4]

Table 3. k_{off} ($M^{-1}s^{-1}$) (dissociation of inhibitor from β-lactamase)

β-lactamase	AAI101	Tazobactam	Avibactam
SHV-1	0.0021 ± 0.0002	Not measurable	0.0008 ± 0.0001
CTX-M-15	0.0010 ± 0.0001	0.0011 ± 0.0001	0.0008 ± 0.0001
KPC-2	0.0011 ± 0.0001	0.0008 ± 0.0001	0.00014 ± 0.00001[5]

Table 4. k_{cat}/k_{inact} (partition ratio or turnover number) at 15 min

β-lactamase	AAI101	Tazobactam	Avibactam
SHV-1	5	15	1
CTX-M-15	1	2	1
KPC-2	100	500	5

Results: Inactivation kinetics of SHV-1 by AAI101, tazobactam, and avibactam revealed that AAI101 acylated SHV-1 faster than avibactam, but at a similar rate as tazobactam (**Figure 2 (left); Table 2**). AAI101, avibactam, and tazobactam were equally effective kinetically against CTX-M-15 (**Figure 2 (center); Table 2**). AAI101 acylated KPC-2 faster than tazobactam, at a rate comparable to that of avibactam (**Figure 2 (right); Table 2**). Avibactam possessed a slightly slower off rate (k_{off}) compared to tazobactam and AAI101 (**Table 3**). Partition ratios revealed that more tazobactam than AAI101 or avibactam was turned over by all three β-lactamases at 15 min (**Table 4**).

Mass spectrometry

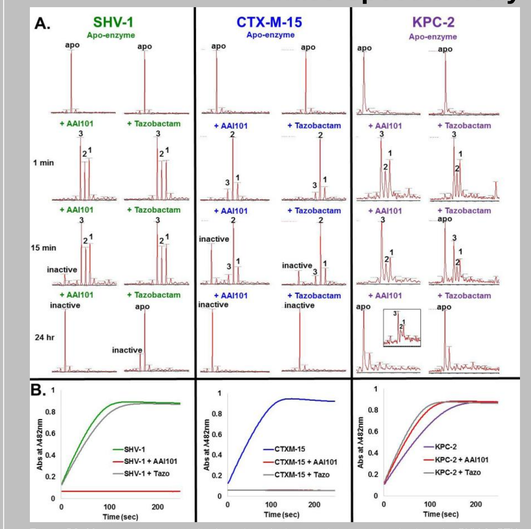

Figure 3A. Mass spectra showing the formation of intermediates during inactivation of SHV-1, CTX-M-15, and KPC-2 by AAI101 or tazobactam at different time points. Peaks labeled 1, 2, and 3 correspond to intermediates found in **Figure 1, bottom**.
Figure 3B. Nitrocefin hydrolysis by SHV-1, CTX-M-15, and KPC-2 after 24 h incubation with AAI101 (red) or tazobactam (gray).

Results: The reaction course of penicillanic acid sulfones with β-lactamases involves various intermediates depicted in **Figure 1, bottom**[3] with eventual deacylation. Inactivation of SHV-1 by AAI101 and tazobactam monitored at different time points by mass spectrometry revealed that SHV-1 is modified completely [a loss of 14 ± 5 Da was observed (-Δ14 ± 5 Da)] upon reaction with AAI101, but not with tazobactam (**Figure 3A**). This modified SHV-1 is inactive upon reaction with nitrocefin (**Figure 3B**). Similarly, a modified CTX-M-15 (-Δ14 ± 5 Da) was identified upon incubation with AAI101 and tazobactam (**Figure 3A**); this modification also inactivated CTX-M-15 (**Figure 3B**). Conversely, the -Δ14 ± 5 Da modification was not observed with KPC-2 upon reaction with AAI101 or tazobactam (**Figure 3A**). However, apo-KPC-2 was evident within 15 min of incubation with tazobactam, whereas only acyl-enzyme was present with AAI101 (**Figure 3A**). After 24 h KPC-2 was fully active after incubation with AAI101 or tazobactam (**Figure 3B**).

Molecular Modeling

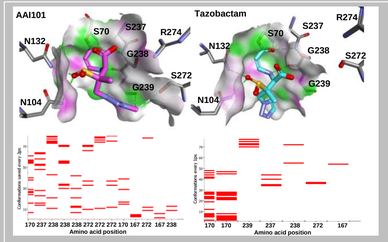

Figure 4. Molecular models of CTX-M-15 with AAI101 (pink) and tazobactam (cyan) (Top). All hydrogen bonding interactions (red line) between active site residues and the triazole ring of AAI101 (left) or tazobactam (right) during molecular dynamics simulations are graphed (bottom).

Results: AAI101 (imine) and tazobactam (imine) were docked into the CTX-M-15 active site, and molecular dynamics simulations (MDS) were conducted for 80 ps (**Figure 4**). Analysis of the complete MDS trajectory revealed that the methyl group on the triazole ring of AAI101 allows for many hydrogen bonding interactions (classical and non-classical) that are not observed with tazobactam. Specifically, AAI101 is able to form hydrogen bonds with G238 and S272, while tazobactam's triazole ring is flipped toward 102-108 loop. Upon acylating the active site of CTX-M-15, the additional methyl group on AAI101 allows for a different set of interactions to occur compared to tazobactam. The impact of these differences remains to be assessed.

Conclusions and Future Directions

AAI101 is a potent extended-spectrum β-lactamase inhibitor which differs mechanistically from tazobactam:

- **Towards a panel of isogenic *E. coli* each expressing a single, defined β-lactamase, AAI101 restored the potency of cefepime, and AAI101 was a better partner for piperacillin than tazobactam.**
- **Kinetic and mass spectrometric measurements revealed increased potency of AAI101 over tazobactam:**
  - **Rate of inactivation of SHV-1 followed the order AAI101 > tazobactam >> avibactam (Figure 2); moreover, mass spectrometric data revealed that only AAI101 modified this β-lactamase to an inactive form (Figure 3B).**
  - **Inhibition of KPC-2 followed the order avibactam > AAI101 >> tazobactam (Figure 2, Table 2).**
  - **AAI101, tazobactam, and avibactam were similarly inhibitory towards CTX-M-15 (Figure 2, Table 4).**
- **Molecular dynamic simulation revealed that AAI101 forms more hydrogen bonds than tazobactam in the active site of CTX-M-15 (Figure 4), implying that AAI101 may bind more tightly than tazobactam to the active site of CTX-M-15.**
- **Additional studies are intended to refine the mechanistic details of AAI101 interactions with β-lactamases.**

References

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