In Vivo Activity and Structural Characterization of a New Generation γ-Lactam Siderophore Antibiotic Against Multidrug-Resistant Gram-Negative Bacteria and *Acinetobacter* spp.

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REVISED ABSTRACT

Background: Multidrug-resistant (MDR) *A. baumannii* presents a critical need for innovative antibacterial development. We have identified a new series of γ-lactam antibiotics that target penicillin binding proteins (PBPs) and incorporate a siderophore moiety to facilitate periplasmic uptake.[1] YU253911, an advanced iteration of this class showed potent *in vitro* activity against clinically relevant Gram-negative organisms including *Acinetobacter* spp.

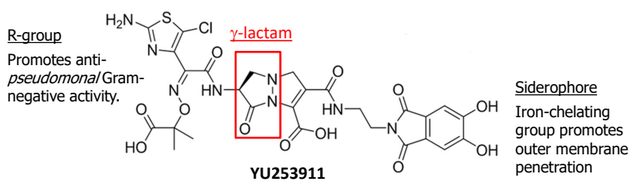
Methods: Minimum inhibitory concentrations (MICs) for YU253911 were determined using broth microdilution against MDR *K. pneumoniae* and *P. aeruginosa* strains and a 198-member panel of clinical isolates of *Acinetobacter* spp. The antibiotic's target protein was evaluated by binding studies with Bocillin, a fluorescent penicillin analog, and the structure of the active site-acylation product complex was determined for *P. aeruginosa* PBP3 by X-ray crystallography. Murine pharmacokinetics were determined for YU253911, and the compound was evaluated in vivo against *P. aeruginosa* in a mouse thigh infection model at 50 and 100 mg/kg q6h using subcutaneous dosing.

Results: MIC testing for YU253911 afforded an MIC_{50} of 0.5 and 1 μg/mL against 23 samples each of MDR *P. aeruginosa* and *K. pneumoniae*, similar potency as described with previous γ-lactam agents. Testing against a 198-member *Acinetobacter* spp. panel revealed an MIC_{50} of 0.5 μg/mL and an MIC_{90} of 16 μg/mL, which compared favorably to all tested β-lactams including penicillins, cephalosporins, monobactams and carbapenems (MIC_{50} = 8 to >16 μg/mL). Competitive binding studies with Bocillin against the antibiotic's putative target protein, PBP3, revealed an IC_{50} of 5 μM. The generation of a high-resolution crystal structure of the *P. aeruginosa* PBP3-YU253911 acylation product revealed key protein-ligand interactions. YU253911 showed promising preclinical pharmacokinetics in mice with a 15 h half-life and demonstrated a dose-dependent reduction in colony forming units from 50 and 100 mg/kg q6h dosing in a mouse thigh infection model using *P. aeruginosa*.

Conclusions: YU253911, a new generation γ-lactam antibiotic effective against MDR *A. baumannii* and *P. aeruginosa* demonstrated promising in in vitro potency and favorable pharmacokinetics which correlated with *in vivo* efficacy.

BACKGROUND

Figure 1. Structure and design features of the γ-lactam antibiotic YU253911.[1]



METHODS

YU253911 minimum inhibitory concentrations (MICs) were determined by broth microdilution against representative γ-lactam-susceptible/carbapenem–resistant *K. pneumoniae* and *P. aeruginosa* strains (23 each),[1] as well 198 *Acinetobacter spp. clinical* isolates, including 98 carbapenem-resistant *A. baumannii* strains.[2] All studies were performed according to modified Clinical and Laboratory Standards Institute (CLSI) guidelines using iron-depleted media. YU253911's putative target protein was evaluated by competitive binding studies with Bocillin, a fluorescently-labeled penicillin analog according to an established procedure.[3] The crystal structure of the *P. aeruginosa* PBP3-YU253911 acylation product was solved using a 2.0 Å-resolution synchrotron radiation diffraction dataset of apo-enzyme crystals soaked with YU253911 as previously described.[1] Pharmacokinetics were determined in CD-1 mice after single administration of a 50 mg/kg i.v. bolus by HPLC-MS/MS analysis of blood aliquots at 0.05, 0.167, 0.5, 1, 2, 4, 6, and 24 h. The compound's in vivo efficacy was evaluated in a *P. aeruginosa* thigh infection model in neutropenic mice following a standard method.[4] Briefly animals were inoculated intramuscularly with 1×10^6 colony forming units (CFU) of strain AR-0229. YU253911 was dosed subcutaneously every 6 h at 50 and 100 mg/kg for 24 h; colistin (30 mg/kg, q 12h) was used as a positive control. The bacterial burden of harvested tissue was determined at 24 h.

RESULTS

Figure 2. YU253911 MICs against representative γ-lactam-susceptible/carbapenem–resistant *K. pneumoniae* and *P. aeruginosa* strains (23 each). YU253911 maintains microbiologic potency against MDR Gram-negative rods previously described for this γ-lactam-siderophore class.[1]

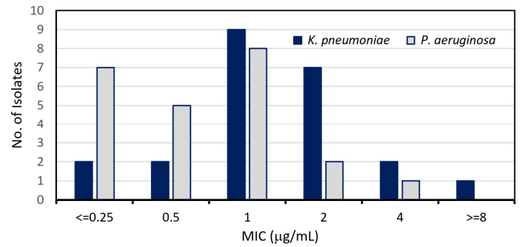
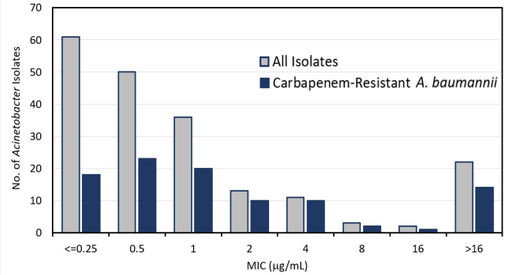
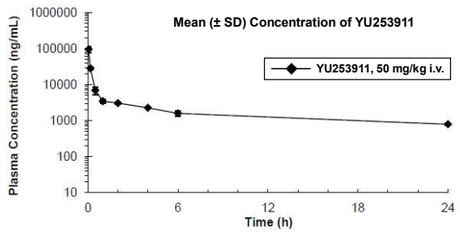


Figure 3. YU253911 MICs (μg/mL) against 198 *Acinetobacter* spp. clinical isolates, including 98 carbapenem-resistant *A. baumannii* strains.



Compound	MIC_{50}	MIC_{90}
YU253911	0.5	>16
Aztreonam	>32	>32
Ceftazidime	64	>64
Meropenem	8	>64
Ceftazidime/ avibactam	16	>64
Ceftolozane/ tazobactam	8	>64

Figure 4. Murine pharmacokinetic parameters from i.v. administration of YU253911.



$t_{1/2}$ (h)	15
C_0 (ng/mL)	159069
AUC_{last} (h·ng/mL)	55972
AUC_{inf} (h·ng/mL)	72839
AUC Extrapolated	0.23
MRT (h)	15
Vss (L/kg)	10
Cl (mL/min/kg)	11

Figure 5. Preliminary in vivo efficacy of YU253911 in a murine soft tissue infection model.

- Mouse thigh infection model using *P. aeruginosa* AR-0229 (a carbapenem-resistant strain; YU253911 MIC = 0.5 μg/mL)
- A statistically significant and dose-dependent reduction in cfu/g of tissue versus vehicle control was determined after 24 h of qid subcutaneous (SC) dosing .

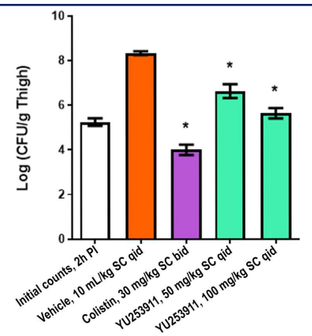


Figure 6. The YU253911 IC_{50} for *P. aeruginosa* PBP3 was determined using a competitive assay with Bocillin, a fluorescent PBP3 substrate. The calculated IC_{50} is the concentration of YU253811 which reduced the Bocillin-labeled fluorescence by 50%.

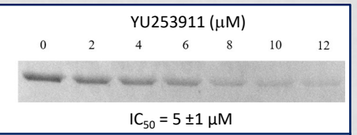
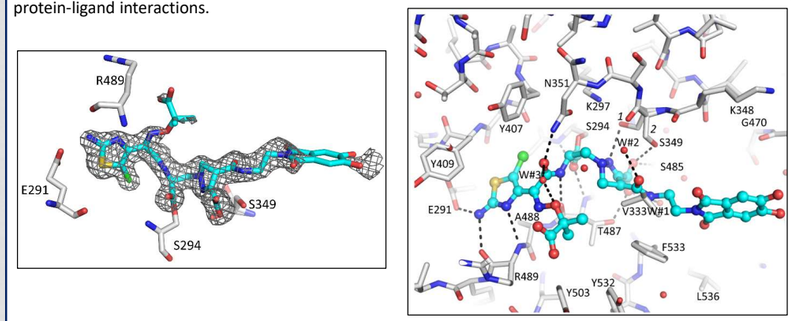


Figure 7. 2 Å resolution crystal structure of the YU253911 *P. aeruginosa* PBP3 acylation product showing key protein-ligand interactions.



Left, unbiased omit electron density for YU253911 ligand contoured at 3 σ is well defined except for the 2-carboxypropan-dimethyl moiety and catechol moiety. The latter moiety serves to promote bacterial cell uptake of the compound via its iron uptake pathway.

Right, YU253911 ligand interactions observed in *P. aeruginosa* PBP3 active site:

- Covalent bond with catalytic S294 thus inactivating the PBP.
- The carbonyl oxygen is situated in the oxyanion hole formed by backbone nitrogens of S294 and T487.
- The chlorine substituent makes a unique "C–Cl···O" interaction with the carbonyl oxygen of Y407, a favorable halogen–protein interaction that is uncommonly described.[5]
- The aminothiazole ring hydrogen bonds with E291 and backbone atoms of R489.
- The adjacent amide moiety hydrogen bonds with residues on both side of the active site (N351 and T487).
- The carboxyl moiety interacts with residues S485, T487, and S349; the nitrogen of the attached 5-membered ring interacts with one of the conformations of S349.
- PBP3 hydrophobic aromatic wall–ligand interactions (residues F533, Y532, and Y503) are also observed.

CONCLUSIONS

- YU253911 demonstrated promising in vitro activity against carbapenem resistant clinical isolates of *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*.
- A high-resolution crystal structure of YU253911 complexed with PBP3 validates its cellular target and allows for further optimization of the chemical structure and new analogs.
- Preliminary in vivo experiments show favorable pharmacokinetic properties and efficacy in a soft tissue murine infection model.
- Ongoing studies on the mechanism of resistance will be reported in due course, as well as experiments which demonstrate the utility of partner agents to overcome inherent resistance.

ACKNOWLEDGMENTS

- This study was supported in part by funds and facilities provided by the Cleveland Department of Veterans Affairs, Veterans Affairs Merit Review Program award number 1I01BX001974, from the Biomedical Laboratory Research & Development Service of the VA Office of Research and Development (to RAB) and the Geriatric Research Education and Clinical Center VISN 10.
- Additional funding was made available from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award numbers R01AI063517, R01AI072219, and R01AI100560 to RAB. We also thank SSRL beamline support for help with data collection.
- This work was also supported by PITCH—Program in Innovative Therapeutics for Connecticut's Health, funded through the Connecticut Bioscience Innovation Fund.

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