1642 A Novel β-lactamase Inhibitor (Durlobactam, DUR) and β-Lactams Enhance Susceptibility Against Multidrug-Resistant (MDR) *Mycobacterium abscessus (Mab)*

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Background

- Mab is a MDR nontuberculous mycobacterium that causes invasive pulmonary infections in patients with structural lung disease. Mab harbors a chromosomally encoded class A β-lactamase, Bla_{Mab}, able to hydrolyze penicillins, most cephalosporins and carbapenems.
- L,D- and D,D-transpeptidases (L,D-TP and D,D-TP, respectively) shape peptidoglycan (PG) synthesis and contribute to cell wall structure.
- Select combinations of β-lactams that inhibit L,D-TP and D,D-TPs and Bla_{Mab} are desirable as they can potentially improve treatment outcomes.
- Durlobactam (DUR) is a novel DBO β-lactamase inhibitor (BLI) with broad-spectrum activity against Ambler class A, C, and D β-lactamases (Figure 1).
- Here, we investigated the mechanism of action and efficacy of DUR alone and combined with select β-lactams in restoring susceptibility of Mab to β-lactam antibiotics.

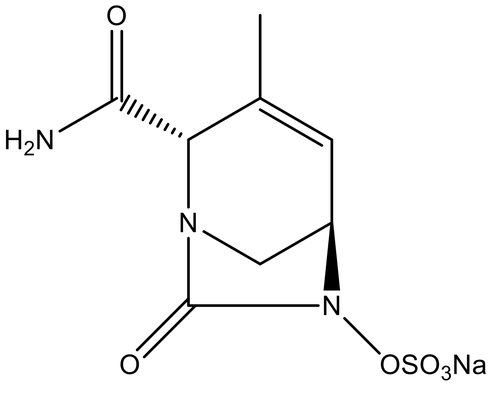


Figure 1: Chemical composition of DUR

Methods

Methods Minimum inhibitory concentrations (MICs) of cefuroxime (CEF), imipenem (IMI) and amoxicillin (Amox) with or without DUR were determined using microdilution. Approximately 5 x 10^5 colony-forming units (CFU) per milliliter were inoculated into Middlebrook 7H9 broth supplemented with 10% (vol/vol) oleic albumin dextrose catalase and 0.05% (vol/vol) Tween 80. When more than 2 drugs were combined, Amox was added at fixed concentration of 8 µg/mL to serial dilutions of CEF-DUR or IMI-DUR in a 1:1 ratio. Mab isolates were incubated with test agents at 30˚ C for 48 h, and MIC was defined as lowest antibiotic concentration that prevented visible bacterial growth. Reaction intermediates in the inactivation pathway of Bla_{Mab}, L,D-TP and D,D-TPs with DUR were captured using mass spectrometry (QTOF-MS).

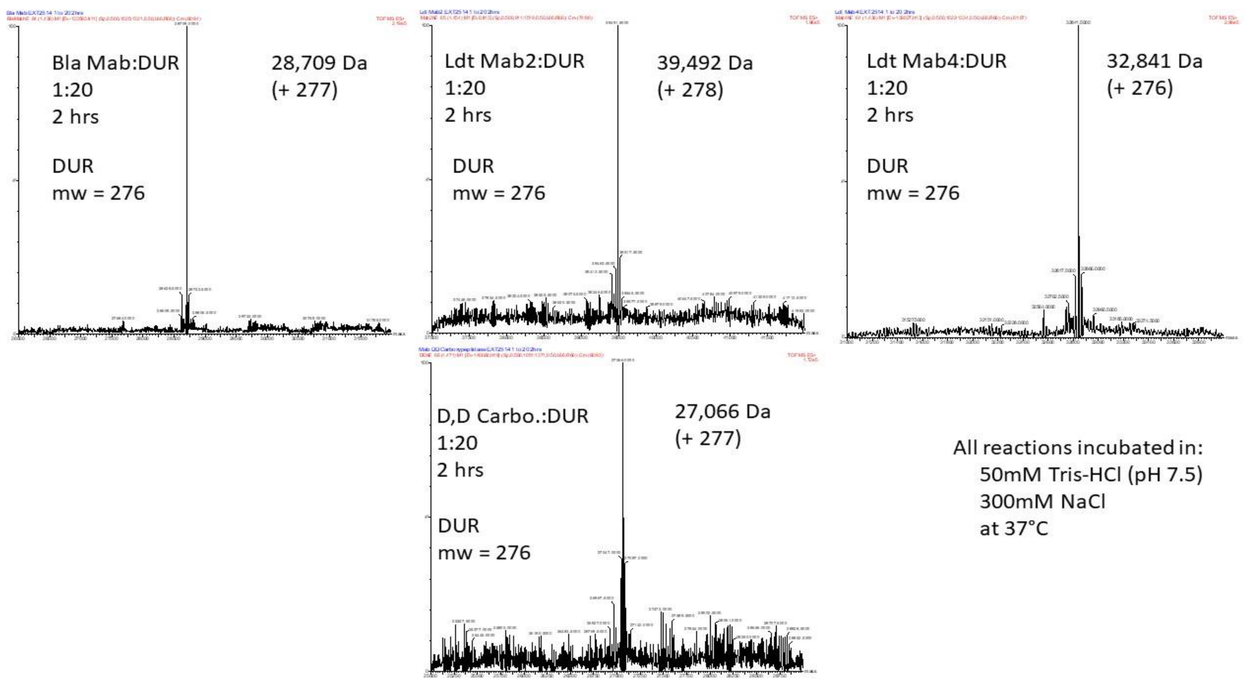
Results

Table: MIC50 and MIC90 of 100 Mab clinical strains against DUR alone and in combination with Amox, CEF and IMI

	DUR µg/mL	Amox µg/mL	Amox/DUR (1:1) µg/mL	CEF µg/mL	CEF/DUR (1:1) µg/mL	CEF/DUR + Amox 8 µg/mL	CEF/amox 8 µg/mL	IMI µg/mL	IMI/DUR (1:1) µg/mL	IMI/DUR + Amox 8 µg/mL	IMI/Amox (1:1) µg/mL
MIC50	4	≥256	2	8	1	≤0.06	4	2	2	≤0.06	1
MIC90	8	≥256	4	16	2	≤0.06	8	4	2	0.25	2

DUR, CEF (Cefuroxime), Amox (Amoxicillin), Imipenem (IMI)

Figure 2: Mass spectrometry of Bla_{Mab}, L,D-TP and D,D-TPs incubated with DUR



Mass spectrometry analyses of Bla_{Mab}, L,D-TP and D,D-TPs Mab (2,4) inactivated by DUR showed formation of stable adducts of DUR to Bla_{Mab}, L,D-TP and D,D-TPs (Figure 1)

One hundred clinically derived and previously characterized isolates were tested in these assays. MIC50 and MIC90 of DUR alone was 4 and 8 µg/mL, demonstrating intrinsic activity. Combinations of DUR-IMI or DUR-CEF plus 8 µg/mL Amox lowered MIC50 to < 0.06 µg/mL in all 100 clinical isolates (Table).

Mass spectrometry analyses of Bla_{Mab}, L,D-TP and D,D-TPs Mab (2,4) inactivated by DUR showed formation of stable adducts of DUR to Bla_{Mab}, L,D-TP and D,D-TPs (Figure 2).

Conclusion

We demonstrate that a novel DBO BLI, DUR, is an active agent with potent intrinsic activity against Bla_{Mab} and Mab L,D-TPs and D,D-TPs.

We hypothesize that DUR improves β-lactam activity by protecting against the hydrolytic activity of Bla_{Mab} and by targeting multiple steps in PG synthesis.

Acknowledgements

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