

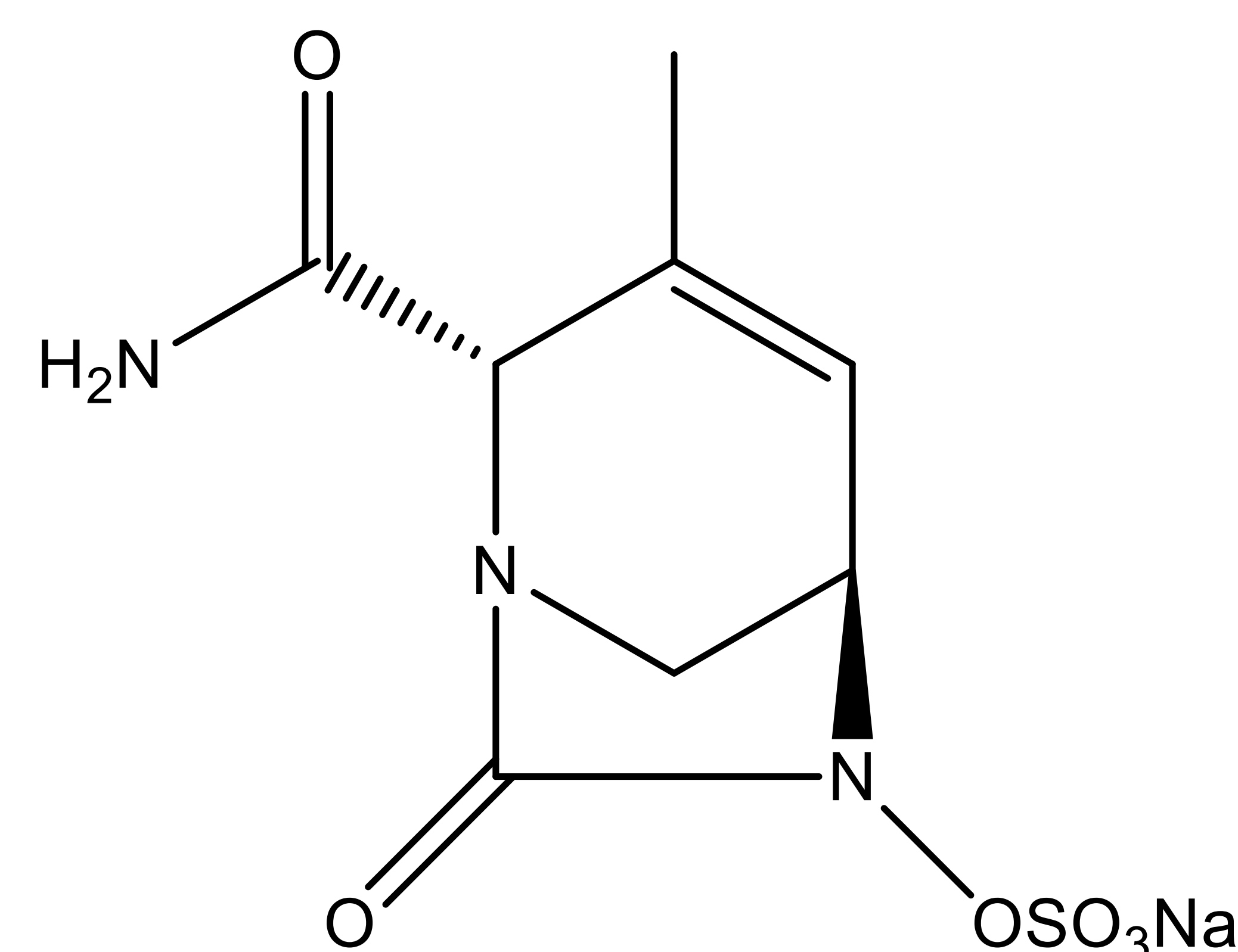
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## Background

- Mab is a MDR nontuberculous mycobacterium that causes invasive pulmonary infections in patients with structural lung disease. Mab harbors a chromosomally encoded class A  $\beta$ -lactamase, Bla<sub>Mab</sub>, 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  $\beta$ -lactams that inhibit L,D-TP and D,D-TPs and Bla<sub>Mab</sub> are desirable as they can potentially improve treatment outcomes.
- Durlobactam (DUR) is a novel DBO  $\beta$ -lactamase inhibitor (BLI) with broad-spectrum activity against Ambler class A, C, and D  $\beta$ -lactamases (Figure 1).
- Here, we investigated the mechanism of action and efficacy of DUR alone and combined with select  $\beta$ -lactams in restoring susceptibility of Mab to  $\beta$ -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  $5 \times 10^5$  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  $\mu\text{g/mL}$  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<sub>Mab</sub>, L,D-TP and D,D-TPs with DUR were captured using mass spectrometry (QTOF-MS).

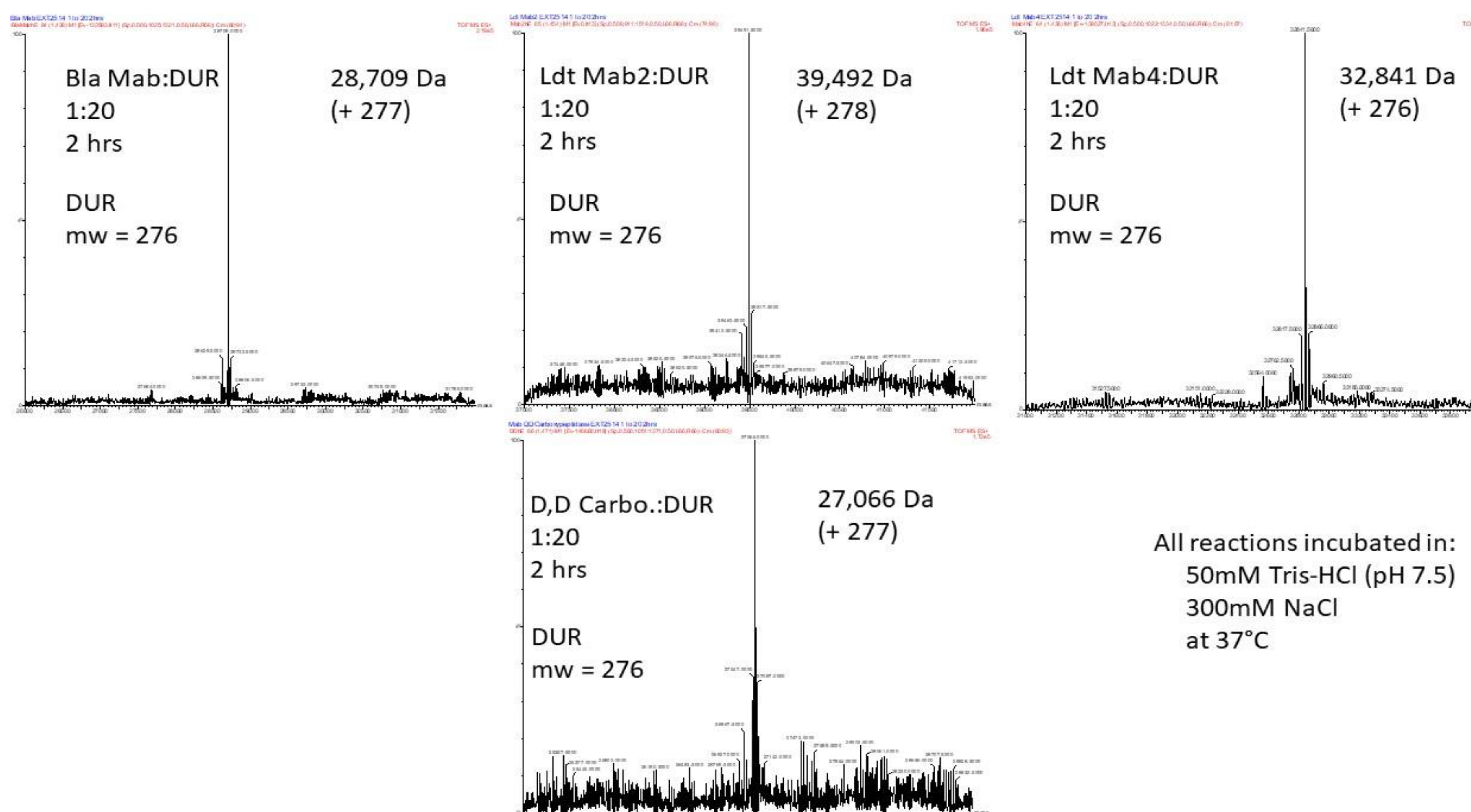
## Results

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

	DUR $\mu\text{g/mL}$	Amox $\mu\text{g/mL}$	Amox/DUR (1:1) $\mu\text{g/mL}$	CEF $\mu\text{g/mL}$	CEF/DUR (1:1) $\mu\text{g/mL}$	CEF/DUR + Amox 8 $\mu\text{g/mL}$	CEF/amox 8 $\mu\text{g/mL}$	IMI $\mu\text{g/mL}$	IMI/DUR (1:1) $\mu\text{g/mL}$	IMI/DUR + Amox 8 $\mu\text{g/mL}$	IMI/Amox (1:1) $\mu\text{g/mL}$
<b>MIC50</b>	4	$\geq 256$	2	8	1	$\leq 0.06$	4	2	2	$\leq 0.06$	1
<b>MIC90</b>	8	$\geq 256$	4	16	2	$\leq 0.06$	8	4	2	0.25	2

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

**Figure 2:** Mass spectrometry of Bla<sub>Mab</sub>, L,D-TP and D,D-TPs incubated with DUR



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

Mass spectrometry analyses of Bla<sub>Mab</sub>, L,D-TP and D,D-TPs Mab (2,4) inactivated by DUR showed formation of stable adducts of DUR to Bla<sub>Mab</sub>, 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<sub>Mab</sub> and Mab L,D-TPs and D,D-TPs.

We hypothesize that DUR improves  $\beta$ -lactam activity by protecting against the hydrolytic activity of Bla<sub>Mab</sub> and by targeting multiple steps in PG synthesis.

## Acknowledgements

This work was supported by Entasis Therapeutics

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