

## Activity of oxapenem AM-112 as an antibiotic and as an inhibitor of Class A and C $\beta$ -lactamases

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### BACKGROUND

- The clinical efficacy of  $\beta$ -lactams is increasingly constrained by resistance, chiefly mediated by  $\beta$ -lactamases in Gram-negative bacteria and by target modification in Gram-positive organisms.
- AM-112 (Figure 1) is a novel 6-(1'-hydroxyethyl) oxapenem, under investigation as an antibiotic and as a  $\beta$ -lactamase inhibitor. It was chosen as one of the most promising among a series of oxapenem analogues (see adjacent Poster, 379).
- AM-112's antibiotic and  $\beta$ -lactamase inhibitory properties were investigated for bacteria with known resistance mechanisms. Ceftazidime and aztreonam were examined as partner drugs.

### MATERIALS AND METHODS

#### Bacteria tested

A panel of 56 organisms was tested. These included:

- E. coli* transconjugants with known  $\beta$ -lactamases.
- Enterobacteriaceae mutants hyperproducing AmpC  $\beta$ -lactamases.
- Recent clinical isolates.
- Representatives of most major nosocomial pathogen groups.

#### Susceptibility test methods

- NCCLS agar dilution: Mueller-Hinton agar with inocula of c. 10<sup>5</sup> cfu.
- AM-112 was tested alone as an antibiotic.
- In synergy experiments, AM-112 (4, 1 or 0.25 mg/L) was combined with doubling dilutions of ceftazidime or aztreonam; alternatively AM-112 was used as the minor component in 1:1, 1:2, 1:3 and 1:4 mixtures with ceftazidime or aztreonam.

### RESULTS

#### Antibacterial activity of AM-112

- AM-112 had low MICs (2-4 mg/L) for methicillin-susceptible staphylococci and a very low MIC for the sole *Fusobacterium* sp. tested. MICs for most other bacteria were from 8-32 mg/L, but non-fermenters, MRSA and enterococci generally were more resistant (Table 1).

Table 1. MICs of AM-112 for test pathogens

Pathogen	MIC range mg/L
<i>E. coli</i> , mostly with ESBLs (16)	8->32
AmpC-hyperproducing <i>E. cloacae</i> (2), <i>C. freundii</i> (2), <i>M. morgani</i> (1), <i>S. marcescens</i> (2), also <i>P. vulgaris</i> hyperproducing $\beta$ -lactamase	16->32
<i>P. aeruginosa</i> (3), <i>P. fluorescens</i> (1), <i>Acinetobacter</i> sp. (1), <i>B. cepacia</i> (2) & <i>S. maltophilia</i> (1)	32->32
<i>E. faecalis</i> (5), <i>E. faecium</i> (3)	>32
Methicillin-susceptible <i>S. aureus</i> (3) & <i>S. epidermidis</i> (1)	2-4
MRSA (3)	32->32
Methicillin-resistant <i>S. epidermidis</i> (1)	8
<i>B. fragilis</i> (4)	8-16
<i>C. difficile</i> (2), <i>C. perfringens</i> (1)	4-16
<i>Fusobacterium</i> sp. (1)	0.5

#### Interactions of AM-112 with ceftazidime and aztreonam

- AM-112, at 4 mg/L, achieved good potentiation of ceftazidime or aztreonam against most Enterobacteriaceae strains with derepressed AmpC  $\beta$ -lactamases (Figure 2). Except in the case of *E. cloacae* Hennessey, which remained resistant in the presence of AM-112, the MICs of ceftazidime and aztreonam were reduced from 16-128 mg/L to 0.5-2 mg/L.
- Good potentiation of ceftazidime was achieved vs. *B. fragilis* isolates, with MICs reduced from 32-64 mg/L to 0.5-2 mg/L (Figure 2).
- Potentiation against the *E. coli* transconjugants with Class A and D ceftazidimases was variable, being good against those with e.g. TEM-3 and SHV-2 enzymes and weak against those with e.g. TEM-9 and SHV-5. This may reflect relative affinity for the particular enzymes, or their quantity in the particular strains tested. Potentiation of aztreonam was better than that of ceftazidime against transconjugants with TEM-10 and OXA-5 enzymes (Figure 3).
- Potentiation of ceftazidime was not achieved against an *E. coli* transconjugant with IMP-1 metallo- $\beta$ -lactamase. Aztreonam remained active against this organism, being stable to metallo- $\beta$ -lactamases (Figure 3).

Figure 1. Chemical structure of AM-112

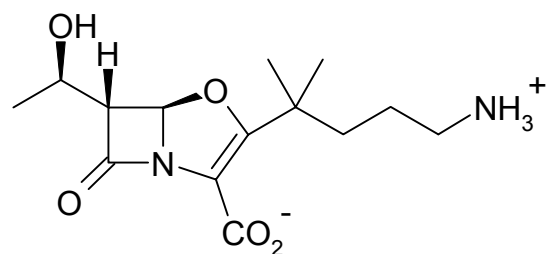


Figure 2. MICs of (a) ceftazidime  $\pm$  AM-112, 4 mg/L or (b) aztreonam  $\pm$  AM-112, 4 mg/L vs. bacteria with chromosomal  $\beta$ -lactamases

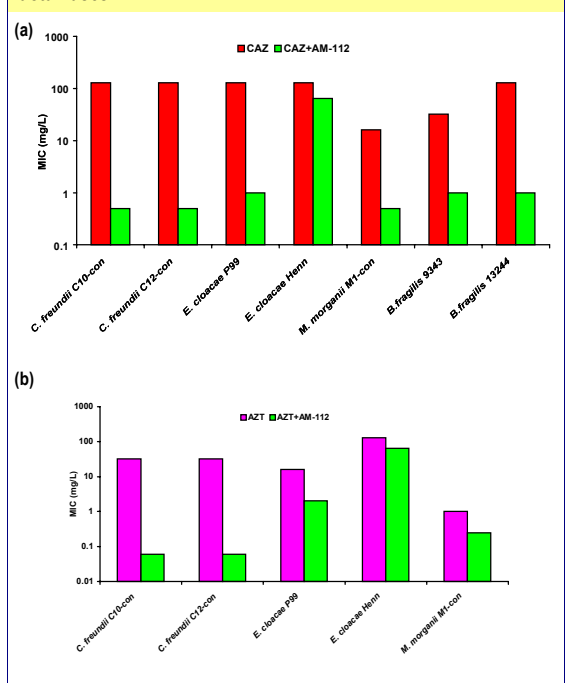


Figure 3. MICs of (a) ceftazidime  $\pm$  AM-112, 4 mg/L or (b) aztreonam  $\pm$  AM-112, 4 mg/L vs. *E. coli* transconjugants with ceftazidimases

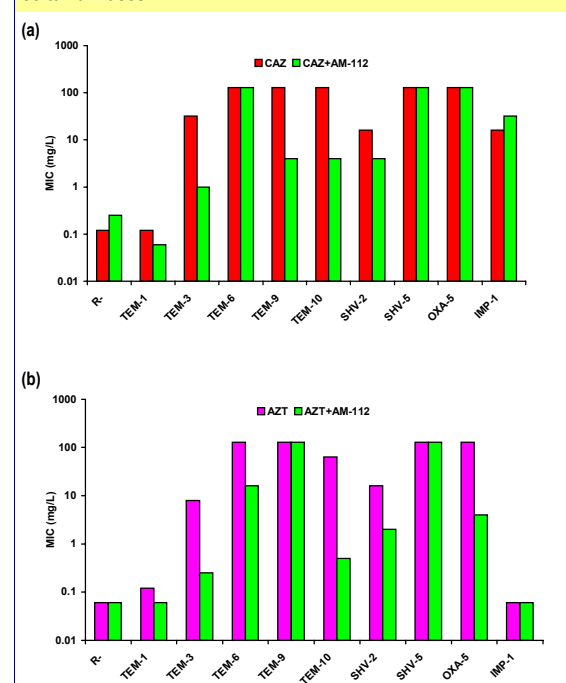


Figure 5. Effects of fixed ratios of ceftazidime : AM-112 vs. *E. coli* transconjugants with ceftazidimases

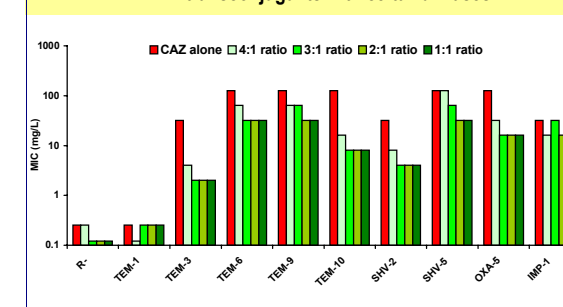


Figure 6. Synergy of AM-112 + ceftazidime vs. enterococci

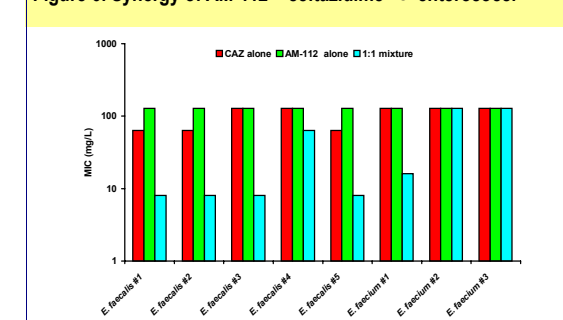
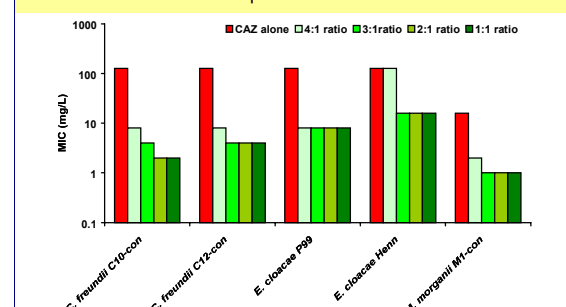


Figure 4. MICs of ceftazidime + fixed ratios of AM-112 vs. strains with chromosomal Class C  $\beta$ -lactamases



- Whether tested with fixed concentration or as fixed ratios, AM-112 achieved a dose-dependent effect against strains with derepressed AmpC enzymes and ESBLs. Nevertheless, a 1:1 ratio was not materially inferior to a 1:2 (AM-112: partner) ratio. This is illustrated for fixed ratios (Figures 4 and 5).
- Synergy between ceftazidime and AM-112 was seen against most isolates of *E. faecalis* and *E. faecium*, although the mechanism is unclear. Although MICs of ceftazidime and AM-112 were  $\geq 64$  mg/L for all isolates, 4/5 *E. faecalis* and 1/3 *E. faecium* were susceptible to 8-16 mg/L ceftazidime in the presence of AM-112, 4 mg/L (Figure 6). Synergy against enterococci was not seen between AM-112 and aztreonam.

### CONCLUSIONS

- AM-112 has moderate antibacterial activity against MSSA and anaerobes, activity against enterococci and Gram-negative aerobes was weak.
- Combination of AM-112 with ceftazidime would extend the cephalosporin's spectrum to include anaerobes and *S. aureus*, also many AmpC-derepressed strains and some with ESBLs.
- Synergy with ceftazidime against enterococci is surprising and its mechanism deserves further investigation.
- Aztreonam/AM-112 was slightly superior to ceftazidime + AM-112 vs. many strains with ESBLs; equivalent vs. those with hyperproduced AmpC enzymes, but (unsurprisingly) inferior against Gram-positive cocci, which are inherently resistant to monobactams.