

# Oxapenem analogues as antibiotics and broad-spectrum $\beta$ -lactamase inhibitors

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## BACKGROUND

- $\beta$ -Lactamases are the main source of resistance to  $\beta$ -lactam antibiotics in Gram-negative bacteria whereas target modification is more important in Gram-positive species.
- No  $\beta$ -lactam escapes all  $\beta$ -lactamases, and existing  $\beta$ -lactamase inhibitors are strongly active only against Class A enzymes.
- Many experimental penem-related  $\beta$ -lactams have broad spectra of activity against bacteria and act as inhibitors of Class C as well as Class A  $\beta$ -lactamases.
- We examined several oxapenem  $\beta$ -lactams as antibiotics, and as potentiators of ceftazidime against  $\beta$ -lactamase producers.

## MATERIALS & METHODS

### Agents tested

- Eleven 6-(1'-hydroxyethyl) oxapenem analogues were tested (Figure 1).
- These had alkyl, amino-alkyl, amidinoalkyl, acetamidoalkyl or methyl ureidoalkyl C-2 side chains.
- Analogues AM-113 and AM-114 were C1' stereoisomers of each other, as were AM-112 and AM-115.

### Bacteria tested

- Panels of 56 organisms were tested. These included:
  - E. coli* transconjugants with known  $\beta$ -lactamases.
  - Enterobacteriaceae mutants hyperproducing AmpC  $\beta$ -lactamases.
  - Recent clinical isolates.
- Most major pathogens groups, including Enterobacteriaceae, *P. aeruginosa*, *Acinetobacter* spp., *S. aureus*, MRSA, enterococci, and anaerobes.

### Susceptibility test methods

- NCCLS agar dilution: Mueller-Hinton agar with inocula of  $c.10^5$  cfu.
- Oxapenems were tested alone as antibiotics.
- In synergy experiments, the oxapenems at 4, 1 or 0.25 mg/L were combined with doubling dilutions of ceftazidime.
- The stereoisomeric pairs (AM-113 and AM-114; AM-112 and AM-115) were tested as 50:50 mixtures as well as individually.

Figure 1. Chemical structures of oxapenem analogues

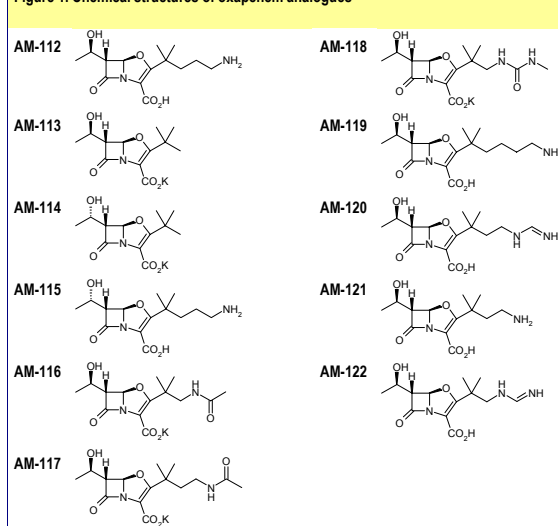


Table 1. Antibacterial activity of oxapenem analogues

Strains	MIC (mg/L)											
	CLAV	AM-112	AM-113	AM-114	AM-115	AM-116	AM-117	AM-118	AM-119	AM-120	AM-121	AM-122
<i>E. coli</i> <sup>a</sup>	32	32	4-16	>32	>32	8->32	4->32	4->16	4->32	4-32	>16	1->4
AmpC-inducible enterics <sup>b</sup>	32->64	16->32	16->32	>32	>32	16->32	16->32	8->16	16->32	4->32	>16	≥4
<i>Pseudomonads</i> <sup>c</sup>	>64	>32	>32	>32	>32	>32	>32	>16	>32	>32	>16	>4
<i>Acinetobacter</i>	16	>32	>32	>32	>32	>32	>32	>16	>32	>32	>16	>4
Enterococci	>64	>32	>32	>32	>32	>32	>32	>16	>32	>32	>16	>4
MSSA	16	2	0.5	1	4	4	1-2	4	1	1	4	2
MRSA	>64	>32	16-32	>32	>32	>32	>32	>16	≥32	>32	>16	>4
Clostridia	8->64	4-16	4-8	>32	1->64	2-32	1-8	8->64	16->64	4->32	8-32	>4
<i>Bacteroides</i>	4-16	8-16	8	32	>8	8-16	4	ND	ND	>32	≥32	>4

Key: All MICs <4 mg/L MICs range from < 4 ->16 mg/L All MICs 8 - 16 mg/L MICs range from < 8 ->16 mg/L MICs range from < 4 ->16 mg/L

<sup>a</sup>. Anomolously low MIC results are omitted. <sup>b</sup>. *Enterobacter*, *Citrobacter*, *Serratia* and *Morganella* spp. <sup>c</sup>. *P. aeruginosa*, *P. fluorescens*, *B. cepacia*, and *S. maltophilia*

Table 2. Ability of oxapenems (at 4 mg/L unless otherwise stated) to potentiate ceftazidime against selected resistant strains

Strains	MIC (mg/L) of ceftazidime in the presence of $\beta$ -lactamase inhibitor at 4 mg/L														
	None	CLAV	AM-112	AM-113	AM-114	AM-115	AM-116	AM-117	AM-118	AM-119	AM-120	AM-121	AM-122*	AM-112 + AM-115**	AM-113 + AM-114**
<b>Class A, B and D <math>\beta</math>-lactamases</b>															
<i>E. coli</i> TEM-3	32	0.25	1	1	0.5	2	0.5	0.5	2	2	4	4	0.25	1	1
<i>E. coli</i> TEM-6	>64	0.5	>64	>64	8	64	32	16	>64	>64	16	>64	64	32	8
<i>E. coli</i> TEM-9	>64	1	4	>64	16	64	>64	32	>64	>64	16	>64	>64	>64	32
<i>E. coli</i> TEM-10	>64	1	>64	64	1	2	16	>64	32	16	4	64	2	1	2
<i>E. coli</i> SHV-2	16	0.5	4	2	1	0.25	16	8	2	4	4	8	0.5	0.25	1
<i>E. coli</i> SHV-5	>64	1	>64	>64	32	64	>64	>64	>64	32	>64	>64	>64	>64	32
<i>E. coli</i> OXA-5	>64	0.5	>64	16	2	2	16	32	>64	32	16	>64	64	8	1
<i>E. coli</i> IMP-1	32	16	32	32	32	64	64	64	32	16	64	32	32	64	16
<b>Class C <math>\beta</math>-lactamases</b>															
<i>C. freundii</i> (C10-con)	>64	64	0.5	2	2	8	64	32	16	0.25	4	64	8	8	4
<i>E. cloacae</i> (P99)	>64	>64	1	4	16	8	>64	>64	64	4	4	64	8	1	8
<i>E. cloacae</i> (Hennessey)	>64	>64	16	>64	>64	64	>64	>64	>64	4	>64	64	16	>64	>64
<i>M. organii</i> (M1-con)	16	32	0.12	≤0.06	0.12	0.125	0.5	1	0.25	0.5	4	0.25	2	0.12	≤0.06
<i>B. fragilis</i>	64	0.5	0.12	-	1	1	1	0.12	0.5	-	1	1	2	0.5	0.5

Key: Ceftazidime MIC reduced to <8 mg/L (Susceptible) Ceftazidime MIC reduced to 16 mg/L (Intermediate) \* 1 mg/L; \*\* Both at 2 mg/L

Table 3. Susceptibility of key phenotypes to ceftazidime and oxapenems

Strains	No tested	No isolates susceptible to ceftazidime (16 mg/L) plus inhibitor (4mg/L)												
		CAZ	CLAV	AM-112	AM-113	AM-114	AM-115	AM-116	AM-117	AM-119	AM-120	AM-121	AM-122	AM-112 + AM-113 + AM-115 + AM-114
ESBL +ve <i>E. coli</i>	8	2	8	5	5	7	5	4	4	5	7	4	5	6
AmpC derepressed enterobacteria	7	3	3	6	6	6	6	3	3	6	3	6	7	6
Strains with OXA-5, IMP-1 and PER-1 enzymes	3	0	3	1	1	1	1	1	1	0	2	1	0	2
Non-fermenters without characterised mechanisms	7	5	5	5	3	4	2	6	6	1	1	2	1	2
Enterococci	8	0	0	3	5	0	0	0	0	5	5	0	0	3
Methicillin-susceptible staphylococci	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Methicillin-resistant staphylococci	4	0	0	0	0	0	0	0	0	1	0	0	0	0
Anaerobes	8	7	2	7	Void	6	6	7	7	Void	6	6	6	6

Key: Majority of isolates susceptible

Figure 2a. Dose/response for ceftazidime + oxapenem combinations vs. *E. coli* TEM-3

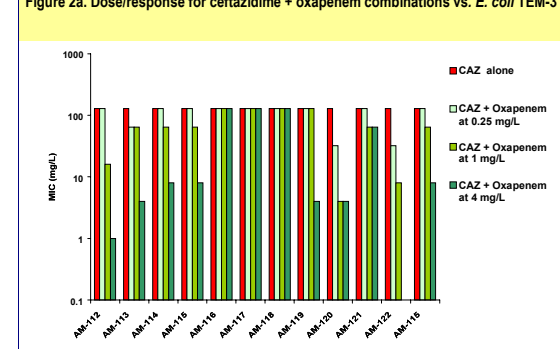


Figure 2b. Dose/response for ceftazidime + oxapenem combinations vs. *E. cloacae* P99 (AmpC hyperproducing)

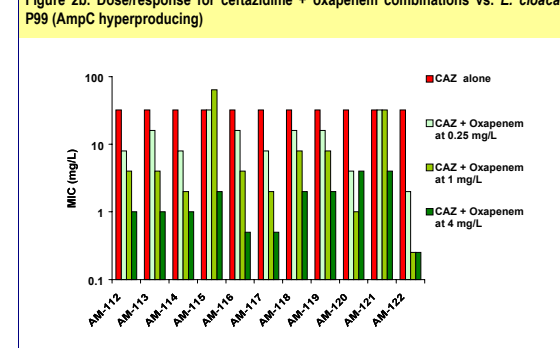
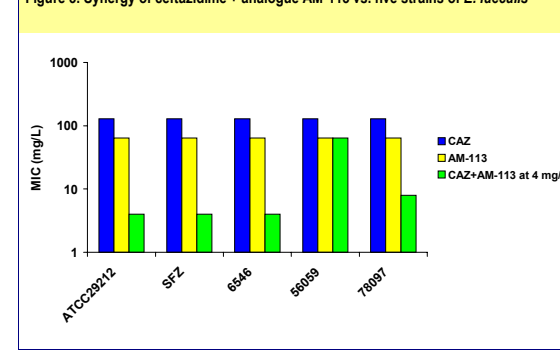


Figure 3. Synergy of ceftazidime + analogue AM-113 vs. five strains of *E. faecalis*



## RESULTS

### Antibacterial activity (Table 1)

- In general, the analogues had:
  - MICs of 1-4 mg/L vs. MSSA.
  - MICs of 4-16 mg/L vs. many *E. coli* and anaerobes.
  - MICs of 16->32 mg/L vs. *Citrobacter*, *Enterobacter*, *Morganella* and *Serratia* spp.
  - MICs >32 mg/L for *Pseudomonas*, *Acinetobacter*, *Burkholderia* and *Stenotrophomonas* spp. enterococci and MRSA.

### Specifically:

- Compound AM-122 had the best anti-*E. coli* activity, with MICs of 4 mg/L.
- AM-113 had superior antibacterial activity to its C1' stereoisomer, AM-114.
- AM-112 had superior activity to its stereoisomer, AM-115.

### Activity as synergists

#### Synergy related to $\beta$ -lactamase inhibition

- The analogues showed dose-dependent synergy with ceftazidime. MICs of the cephalosporin were progressively reduced as the oxapenem concentrations were raised. This is illustrated for *E. coli* TEM-3 in Figure 2a and for *E. cloacae* P99 (AmpC-hyperproducing) in Figure 2b.

#### Table 2 shows the MICs when ceftazidime was combined with the oxapenems at the highest concentrations tested (generally 4 mg/L).

- All the analogues strongly potentiated ceftazidime vs. a *B. fragilis* strain; vs. *M. organii* with a derepressed AmpC enzyme and vs. *E. coli* with TEM-3.
- Synergy was consistently poor *E. coli* with TEM-9, SHV-5 or PER-1.
- Compounds AM-112, AM-120 and AM-122 had their best synergistic activity against strains with AmpC enzymes.
- Compounds AM-114 and AM-115 had the best activity against isolates with TEM or SHV ESBLs.

- AM-112 and AM-115 were tested together on the logic that their activities against AmpC enzymes and ESBLs should be complementary. However, the combination was not significantly better than ceftazidime plus either component alone. AM-113 plus AM-114 was marginally better than ceftazidime plus either component alone.

#### Synergy unrelated to $\beta$ -lactamases

- AM-113 4 mg/L, reduced ceftazidime MICs for *E. faecalis* to 4-8 mg/L, compared with ≥32 mg/L for AM-113 or ceftazidime alone (Figure 3). Weaker synergy was seen between ceftazidime and AM-120 or AM-112 against *E. faecalis*. The mechanism is unknown.
- Synergy between ceftazidime and oxapenems was also seen with *E. faecium*, but MICs of ceftazidime for this species were rarely brought to below 16 mg/L.

#### Antagonisms

- Many analogues antagonised ceftazidime vs. the sole *P. fluorescens* strain tested with the MIC of ceftazidime raised up to 32-fold by oxapenems at 4 mg/L. Weaker antagonism was also seen vs. *B. cepacia*, but not against any commoner pathogen.

#### Combined activity of ceftazidime + oxapenem combinations

- Table 3 summarises the activity of ceftazidime + oxapenem combinations, disregarding whether activity arose from  $\beta$ -lactamase-dependent or -independent synergy and/or from the inherent antibacterial activity of either component. It assumes a breakpoint of 16 mg/L for ceftazidime, and an inhibitor concentration of 4 mg/L, except with analogue AM-122, (1 mg/L).
- On this assessment, the most effective oxapenem partners were (in no particular order) AM-112, AM-113, AM-114, AM-115 and AM-122.

## CONCLUSIONS

- The oxapenems, were strongly active vs. MSSA, with MICs of 1-4 mg/L, and had MICs of 4-16 mg/L for *E. coli* and many anaerobes. Other bacteria were more resistant.
- Oxapenem/ceftazidime combinations achieved a broad-spectrum activity, including many strains that were ceftazidime-resistant via ESBL production or derepression of AmpC enzymes.
- Although *E. faecalis* isolates were consistently resistant to both oxapenems and ceftazidime, they were susceptible to several oxapenem/ceftazidime combinations.
- The most promising analogues were AM-112, AM-113, AM-114, AM-115 and AM-122.