

Protection of ceftazidime against Class A and Class C β -lactamases by oxapenemsC E Jamieson¹, P A Lambert¹ & I N Simpson²¹Aston University, Birmingham, B4 7ET; ²Micron Research, Cambridge CB3 7ES, UKP A Lambert
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INTRODUCTION

Mutations in Class A TEM and SHV β -lactamases have led to the emergence of plasmid mediated, extended spectrum β -lactamase enzymes (ESBLs), which destroy 3rd and 4th generation cephalosporins. Some Class C enzymes are now plasmid mediated, potentially leading to their rapid spread among bacterial species.

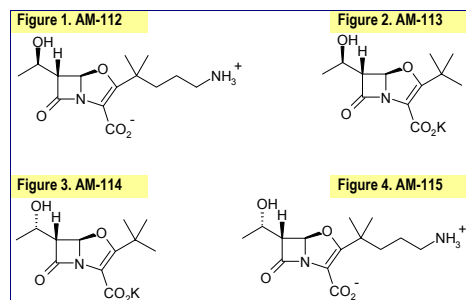
Ceftazidime is a broad-spectrum β -lactam cephalosporin, with good activity against Gram-negative species. It is stable to many β -lactamases, but susceptible to ESBLs and Class C enzymes. Resistance to this agent is increasing due to bacterial β -lactamase production and overuse (Zhang and Li 2001). One strategy to protect the activity of ceftazidime is co-administration with a β -lactamase inhibitor. Existing β -lactamase inhibitors such as clavulanic acid and tazobactam are effective inhibitors of Class A enzymes (Payne et al 1994), but lack efficacy against the Class C cephalosporinases (Bush et al 1995).

Oxapenems are a novel class of compounds that have potent, broad-spectrum β -lactamase inhibitory properties. We have investigated both the inhibitory profile of four closely related oxapenem compounds in a cell free assay and the ability of these oxapenems to protect ceftazidime against a panel of bacterial strains which produce enzymes from Class A (including ESBLs), Class C and Class D β -lactamases.

METHODS

Oxapenems

Four oxapenems (AM-112, AM-113, AM-114 and AM-115) were obtained from Amura Ltd, Cambridge. The four analogues are closely related; AM-112 and AM-115, possessing the same substituent at C2 and differing only in the stereochemistry at C1' (attached to C6). Similarly, AM-113 and AM-114 possess the same substituent at C2 (a t-butyl group), but again differ in stereochemistry at C1'. Structures are shown in Figures 1-4.

Preparation and inhibition of cell-free β -lactamases

β -Lactamase extracts were prepared and purified as described previously (Simpson et al., 1983). Oxapenem compounds and clavulanic acid were pre-incubated with the β -lactamase for 15 minutes prior to spectrophotometric determination of the IC₅₀ using nitrocefins as substrate (O'Callaghan et al. 1969).

Susceptibility tests

The MICs of ceftazidime alone, the oxapenems alone and ceftazidime in combination with each of the oxapenems in a 1:1 and 2:1 ratio were determined by agar dilution against 35 bacterial strains, according to NCCLS guidelines.

Table 1. In vitro activity (MIC) of ceftazidime alone and in the presence of oxapenems against *Escherichia coli* producing plasmid-mediated Class A β -lactamases

Organism:	MIC (mg/L) of CAZ and oxapenems alone					MIC (mg/L) of CAZ in the presence of oxapenems										
	CAZ	AM-112	AM-113	AM-115	AM-114	CAZ + oxapenems at 2:1 ratio				CAZ + oxapenems at 1:1 ratio						
						CAZ+ AM-112	CAZ+ AM-113	CAZ+ AM-115	CAZ+ AM-114	CAZ+ AM-112	CAZ+ AM-113	CAZ+ AM-115	CAZ+ AM-114			
<i>E. coli</i> ATCC 25922	0.25	8	4	>64	32	0.25	0.03	0.125	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
<i>E. coli</i> ATCC 35218	0.125	8	4	>64	16	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
<i>E. coli</i> J53-1	0.125	8	8	>64	32	0.125	0.125	0.125	0.25	0.25	0.25	0.25	0.125	0.125	0.125	0.125
<i>E. coli</i> TEM-1	0.25	16	4	>64	16	0.5	0.06	0.25	0.25	0.25	0.5	0.5	0.25	0.25	0.25	0.25
<i>E. coli</i> TEM-3	16	8	16	>64	64	2	2	2	2	4	4	2	2	2	2	2
<i>E. coli</i> TEM-6	>64	16	8	>64	32	4	2	8	2	8	4	8	2	2	2	2
<i>E. coli</i> TEM-9	>64	8	8	>64	32	8	2	8	4	8	4	4	2	2	2	2
<i>E. coli</i> TEM-10	>64	8	16	>64	64	16	2	8	4	8	4	4	2	2	2	2
<i>E. coli</i> SHV-1	2	8	8	>64	32	0.125	0.125	0.125	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
<i>E. coli</i> SHV-2	0.25	2	4	>64	16	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
<i>E. coli</i> SHV-3	0.125	32	2	>64	8	0.125	0.03	0.03	0.03	0.03	0.03	0.03	0.125	0.03	0.03	0.03
<i>E. coli</i> SHV-4	>64	16	8	>64	32	8	0.03	2	4	4	8	2	1	1	1	1
<i>E. coli</i> SHV-5	16	16	16	>64	64	16	2	4	2	8	8	8	1	1	1	1
<i>E. coli</i> OXA-1	0.25	8	8	>64	32	0.25	0.25	0.25	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0.25
<i>E. coli</i> OXA-2	0.25	16	4	>64	32	0.25	0.125	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
<i>E. coli</i> OXA-3	0.5	16	16	>64	64	0.5	0.25	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
<i>E. coli</i> OXA-5	0.5	8	16	>64	32	0.25	0.03	0.25	0.25	0.25	0.25	0.5	0.5	0.25	0.25	0.25
<i>E. coli</i> PSE-4	0.125	16	16	>64	32	0.5	0.125	0.125	0.5	0.25	0.25	8	0.5	0.5	0.5	0.5
<i>M. Morganii</i> M1-con	8	64	4	>64	16	1	1	0.25	0.25	1	1	1	1	1	1	0.25

Key: 4 fold reduction in CAZ MIC \geq 8 fold reduction in CAZ MIC Most potent oxapenem in reducing CAZ MIC

RESULTS

Inhibition of cell-free β -lactamases

- The IC₅₀ value for each inhibitor against each β -lactamase was calculated from a plot of the inhibitor concentration against the percentage inhibition of the enzyme, compared to a control enzyme. IC₅₀ values (mg/L) calculated for each inhibitor are summarised in Figure 5.
- Clavulanic acid exhibited good activity against each of the Class A enzymes with IC₅₀ values of 0.0016 – 0.024 mg/L, but was at least 100 – 50,000 fold less effective against the Class C and Class D enzymes with IC₅₀ values of 3.34 – 92 mg/L.
- Each of the four oxapenem compounds was a highly active β -lactamase inhibitor against Class A, C and D β -lactamases.
- AM-112 and AM-113 were 30 – 40 fold less active than clavulanic acid against the Class A TEM-1 and SHV-5 enzymes but at least 1000-fold more active than clavulanic acid against Class C and D β -lactamases.
- AM-114 and AM-115 exhibited clavulanic acid levels of activity against the Class A enzymes and were also highly active against the Class C and D β -lactamases.

Susceptibility tests

Ceftazidime alone

- Ceftazidime was particularly active (MICs <1 mg/L) against *Escherichia coli* without plasmid-mediated β -lactamases; however, possession of TEM- or SHV- derived ESBLs generally conferred resistance (MIC \geq 16 mg/L) (Table 1).
- Ceftazidime was generally inactive against Enterobacteriaceae and *P. aeruginosa* with high levels of Class C β -lactamases (Table 2), *Enterococcus faecalis* and methicillin-resistant *Staphylococcus aureus* (MRSA) (Table 3).
- Activity was moderate (MIC 4-8 mg/L) against methicillin-susceptible *Staphylococcus aureus* (MRSA).

Table 2. In vitro activity (MIC) of ceftazidime alone and in the presence of oxapenems against Gram-negative bacteria with inducible or derepressed Class C β -lactamases

Organism:	MIC (mg/L) of CAZ and oxapenems alone					MIC (mg/L) of CAZ in the presence of oxapenems										
	CAZ	AM-112	AM-113	AM-115	AM-114	CAZ + oxapenems at 2:1 ratio				CAZ + oxapenems at 1:1 ratio						
						CAZ+ AM-112	CAZ+ AM-113	CAZ+ AM-115	CAZ+ AM-114	CAZ+ AM-112	CAZ+ AM-113	CAZ+ AM-115	CAZ+ AM-114			
<i>E. cloacae</i> P99	32	2	8	>64	64	4	8	4	8	4	4	2	4	4	4	4
<i>E. cloacae</i> Hennessy	>64	32	64	>64	>64	4	8	8	8	4	16	4	4	4	4	4
<i>E. cloacae</i> B4-con	>64	16	32	>64	>64	4	16	16	16	4	8	8	8	8	8	8
<i>S. marcescens</i> S2-con	1	16	32	>64	64	0.5	1	0.5	0.5	0.03	0.03	0.25	0.5	0.5	0.5	0.5
<i>C. freundii</i> C2-con	64	16	4	>64	32	2	2	4	4	0.03	4	2	2	2	2	2
<i>M. Morganii</i> M1-con	8	64	4	>64	16	1	1	0.25	0.25	1	1	1	0.25	0.25	0.25	0.25
<i>P. aeruginosa</i> ATCC 27853	2	>64	>64	>64	>64	2	2	4	2	4	2	2	2	2	2	2
<i>P. aeruginosa</i> 1405-con	>64	>64	>64	>64	>64	64	32	32	32	64	64	16	32	32	32	32
<i>P. aeruginosa</i> 2297-con	>64	>64	>64	>64	>64	32	32	32	32	32	32	16	16	16	16	16

Key: 4 fold reduction in CAZ MIC \geq 8 fold reduction in CAZ MIC Most potent oxapenem in reducing CAZ MICTable 3. In vitro activity (MIC) of ceftazidime alone and in the presence of oxapenems against *Staphylococcus aureus* and *Enterococcus faecalis*

Organism:	MIC (mg/L) of CAZ and oxapenems alone					MIC (mg/L) of CAZ in the presence of oxapenems										
	CAZ	AM-112	AM-113	AM-115	AM-114	CAZ + oxapenems at 2:1 ratio				CAZ + oxapenems at 1:1 ratio						
						CAZ+ AM-112	CAZ+ AM-113	CAZ+ AM-115	CAZ+ AM-114	CAZ+ AM-112	CAZ+ AM-113	CAZ+ AM-115	CAZ+ AM-114			
<i>S. aureus</i> ATCC 29213	8	1	0.5	2	1	2	4	1	2	4	4	0.5	2	2	2	2
<i>S. aureus</i> NCTC 6571	4	1	0.5	2	16	1	4	1	2	2	4	0.5	1	1	1	1
<i>S. aureus</i> Innsbruck (MRSA)	>64	2	1	4	16	2	0.03	0.125	0.06	0.03	0.03	0.03	0.03	0.03	0.03	0.03
<i>E. faecalis</i> ATCC 29212	32	32	8	>64	>64	8	16	8	16	8	16	4	16	16	16	16
<i>E. faecalis</i> NCTC 10541	>64	8	2	>64	>64	16	64	8	>64	16	>64	2	>64	>64	>64	>64
<i>E. faecalis</i> NCTC 5957	32	32	8	>64	>64	8	32	8	16	0.03	0.03	0.25	0.03	0.03	0.03	0.03
<i>E. faecalis</i> van A	>64	>64	2	>64	64	32	>64	32	>64	64	>64	0.25	>64	>64	>64	>64
<i>E. faecalis</i> van B	32	>64	16	>64	>64	8	32	8	16	16	32	8	16	16	16	16

Key: 4 fold reduction in CAZ MIC \geq 8 fold reduction in CAZ MIC Most potent oxapenem in reducing CAZ MIC

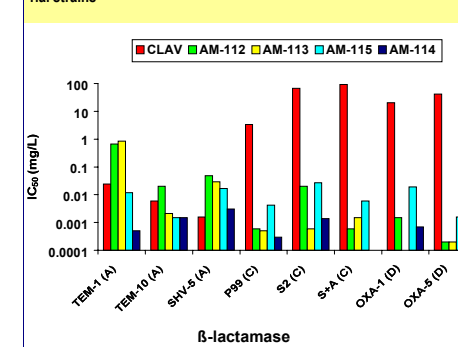
Activity directly attributable to oxapenem

Oxapenems alone

- AM-115 was inactive (MIC >64 mg/L) against all strains tested except *S. aureus* (MIC 2 mg/L).
- AM-114 exhibited MICs of 16-64 mg/L against most Enterobacteriaceae, 1-16 mg/L vs MSSA and was inactive against *E. faecalis*, *P. aeruginosa* and MRSA.
- AM-112 and AM-113 were active against MSSA (MIC 0.5-1 mg/L), exhibited moderate activity (MICs 8-32 mg/L) against Enterobacteriaceae and were inactive against *P. aeruginosa*. AM-113 also exhibited variable activity against *E. faecalis*.

Combinations of ceftazidime and oxapenems

- There was no obvious difference in the potency of 1:1 or 2:1 CAZ: oxapenem combinations. However, there were differences in the inhibition profiles and potencies of the four oxapenems.
- All four oxapenems reduced the CAZ MIC against the majority of β -lactamase-producing isolates, except *P. aeruginosa*.
- AM-113 and AM-114 were 2 – 4 more potent than AM-112 and AM-115 as inhibitors of Class A β -lactamases (Table 1).
- AM-112 was the most potent inhibitor of Class C β -lactamases (Table 2), although there was an indication of inhibition of *P. aeruginosa* β -lactamase by both AM-114 and AM-115.
- Although the CAZ MIC was reduced against isolates of *S. aureus*, this reflected the intrinsic activity of the oxapenems rather than synergy (Table 3).
- AM-112 and AM-115 acted synergistically with CAZ against β -lactamase-negative *E. faecalis* (Table 3).

Figure 5. IC₅₀ values (mg/L) obtained for clavulanic acid and four oxapenem compounds against β -lactamase isolated from eight bacterial strains

CONCLUSIONS

- Oxapenems are a novel class of β -lactamase inhibitors with a broad spectrum of activity against Class A (including ESBLs), Class C and Class D enzymes, in both cell free and whole cell tests.
- The inhibition profile of the oxapenems is determined by the substituent at C2 and the stereochemistry of the side-chain attached to C6 (at C1').
- Possession of the AM-112/AM-115 substituent at C2 appears to confer enhanced activity against Class C β -lactamases whereas the AM-113/AM-114 substituent is associated with enhanced activity vs Class A β -lactamases.
- Changes in stereochemistry at C1' (attached to C6), appears to confer little effect upon β -lactamase inhibitory activity, but the AM-112/AM-113 configuration is associated with enhanced antibacterial activity.
- The oxapenems warrant further investigation as agents to protect and enhance the antibacterial spectrum and activity of β -lactam antibiotics.

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