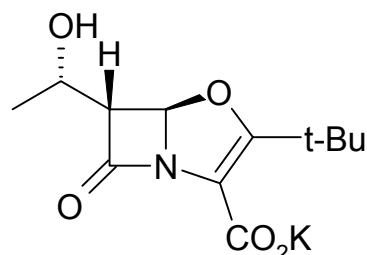


INTRODUCTION

β -Lactamase production is the principal mechanism of resistance to penicillins and cephalosporins. Co-administration of a β -lactamase inhibitor, such as clavulanic acid, tazobactam or sulbactam, has proven highly successful in protecting penicillins against some of these enzymes. However, existing β -lactamase inhibitors are generally ineffective against Class C β -lactamases. In addition, tazobactam is not available for oral administration.

XOB (AM-114) is a novel oxapenam with broad-spectrum β -lactamase inhibitory activity, under development by Amura Ltd, Cambridge, UK (Figure 1). This report details preliminary in vitro and in vivo studies upon XOB.

Figure 1. Chemical structure of XOB (AM-114)



Potassium (5R, 6R)-3-(t-butyl)-6-((1S)-1-hydroxyethyl)-7-oxo-4-oxa-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

METHODS

Preparation and inhibition of cell-free β -lactamases

β -Lactamase extracts were prepared and purified as described previously (1). IC₅₀ values were determined spectrophotometrically (2).

Renal DHP stability

Solutions of XOB, imipenem and meropenem were prepared at 0.1 mg/ml in 0.01M Tris-HCl buffer (pH 7.4) and incubated at 37°C. Porcine renal dehydropeptidase (DHP), obtained from Sigma (L2783) was added to the β -lactam solutions to give a final concentration of 0.01 units/ml. The absorbance (at 262 nm for XOB, 296 nm for imipenem and meropenem) of each solution was recorded over one hour.

Morphology studies

E. coli DC0 was incubated with XOB at 1 mg/L and 8 mg/L for 45 minutes at 37°C. Cells were stained with acridine orange and examined by fluorescence microscopy ($\times 1000$).

Susceptibility tests

The MICs of XOB and ceftazidime, alone and in combination, were determined for 36 bacterial strains (NCCLS guidelines).

Serum levels in mice

Serum levels of XOB in mice, following oral and sub-cutaneous administration at 50 mg/kg, were determined using a HPLC assay.

RESULTS

β -Lactamase inhibition (Figure 2)

- Clavulanic acid was an effective inhibitor of the Class A enzymes, with IC₅₀ values below 0.1 mg/L but lacked activity against Class C and Class D enzymes, with IC₅₀ values between 3 and 92 mg/L.
- XOB possessed similar activity to clavulanic acid against Class A β -lactamases but was markedly more effective against Class C and Class D enzymes, with all IC₅₀ values below 0.0015 mg/L.

Morphology studies

- Figure 3a shows the normal cell morphology of *E. coli* DC0.
- There was little change in the cell morphology after incubation with XOB at 1 mg/L (Figure 3b).
- When incubated with XOB at 8 mg/L, there was marked elongation of the cells, consistent with inhibition of PBP3 (Figure 3c).

Renal DHP stability (Figure 4)

- XOB and meropenem exhibited little degradation over one hour when incubated in Tris-HCl buffer (0.01M, pH 7.4). Imipenem exhibited a 7% loss over the same time period.
- In the presence of DHP, imipenem concentration was reduced by 27% (includes 7% chemical degradation). Meropenem and XOB were more stable, losing 2.5% and 0.6% respectively over the same time period.

Susceptibility tests

- XOB exhibited moderate activity (MICs 1-16 mg/L) against some isolates of *E. coli*, *Morganella* and *S. aureus*, but was inactive (MICs >32 mg/L) against the majority of isolates tested (Tables 1-3).
- Ceftazidime (CAZ) alone lacked activity (MIC > 64 mg/L) against a number of *E. coli* strains in possession of the TEM- or SHV- derived ESBL (Table 1). Combination with XOB (1:1 or 2:1) resulted in synergy, with up to a 32-fold reduction in CAZ MIC.
- Activity of CAZ against the Gram-negative strains with Class C β -lactamases was variable (Table 2). Addition of XOB at 1:1 or 2:1 ratio resulted in marked synergy against all CAZ-resistant Enterobacteriaceae.
- Addition of XOB had little beneficial effect upon CAZ activity against *S. aureus* or *Enterococcus* spp. (Table 3).

Serum Levels in Mice (Figure 5)

- Following subcutaneous administration, XOB achieved a peak serum level of 16.2 mg/L at 5 mins, falling to 2.6 mg/L at 20 mins.
- Following oral administration, serum levels were 32 – 35% of those achieved by subcutaneous dosing.

Figure 2. IC₅₀ values for clavulanic acid and XOB against Class A, C and D β -lactamases

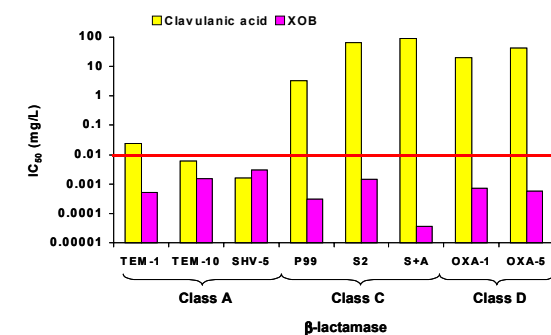


Figure 3. *E. coli* DC0 after incubation with XOB for 45 minutes at 37°C

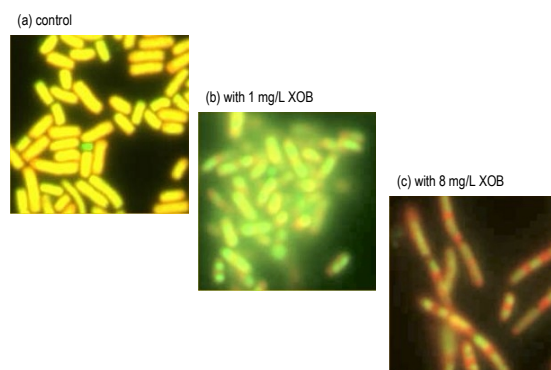


Figure 4. Stability of imipenem, meropenem and XOB (0.1mg/ml) in Tris buffer (pH 7.4) in the presence of DHP, over one hour at 37°C

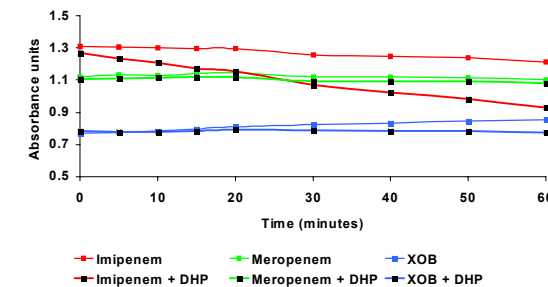


Figure 5. XOB serum levels in mice following oral or subcutaneous dosing at 50 mg/kg

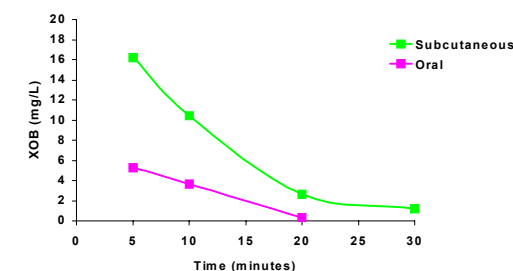


Table 1. In vitro activity of ceftazidime (CAZ) alone and in the presence of XOB against *E. coli* producing plasmid mediated Class A β -lactamases

Organism	MIC (mg/L)			
	CAZ	XOB	CAZ+XOB (1:1)	CAZ+XOB (2:1)
<i>E. coli</i> J53-1	0.125	32	0.125	0.25
<i>E. coli</i> OXA-1	0.25	32	0.25	0.25
<i>E. coli</i> OXA-2	0.25	>64	0.25	0.25
<i>E. coli</i> OXA-3	0.5	>64	0.5	0.5
<i>E. coli</i> OXA-5	0.5	32	0.25	0.25
<i>E. coli</i> SHV-1	2	32	0.25	0.25
<i>E. coli</i> SHV-2	0.25	16	0.125	0.125
<i>E. coli</i> SHV-3	0.125	8	0.03	0.03
<i>E. coli</i> SHV-4	>64	32	2	4
<i>E. coli</i> SHV-5	16	64	1	2
<i>E. coli</i> TEM-1	0.25	16	0.25	0.25
<i>E. coli</i> TEM-3	16	64	2	2
<i>E. coli</i> TEM-6	>64	32	2	2
<i>E. coli</i> TEM-9	>64	32	2	4
<i>E. coli</i> TEM-10	>64	64	2	4
<i>E. coli</i> PSE-4	0.125	32	0.5	0.25
<i>E. coli</i> ATCC 35218	0.125	16	0.125	0.125
<i>E. coli</i> ATCC 25922	0.25	32	0.25	0.25

Key: 4 fold reduction in CAZ MIC ≥ 8 fold reduction in CAZ MIC

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

Organism	MIC (mg/L)			
	CAZ	XOB	CAZ+XOB (1:1)	CAZ+XOB (2:1)
<i>P. aeruginosa</i> 2297-con	>64	>64	16	32
<i>P. aeruginosa</i> 1407-con	>64	>64	32	32
<i>P. aeruginosa</i> ATCC 27853	2	>64	2	2
<i>E. cloacae</i> P99+	32	64	4	8
<i>E. cloacae</i> Hennessy	>64	>64	4	8
<i>E. cloacae</i> 84-con	>64	>64	8	16
<i>C. freundii</i> C2-con	64	32	2	4
<i>S. marcescens</i> S2-con	1	>64	0.5	0.5
<i>M. organii</i> M1-con	8	16	0.25	0.25

Key: 4 fold reduction in CAZ MIC ≥ 8 fold reduction in CAZ MIC

Table 3. In vitro activity of ceftazidime (CAZ) alone and in the presence of XOB against *S. aureus* and *E. faecalis*

Organism	MIC (mg/L)			
	CAZ	XOB	CAZ+XOB (1:1)	CAZ+XOB (2:1)
<i>S. aureus</i> NCTC 6571	4	16	1	2
<i>S. aureus</i> ATCC 29213	8	1	2	2
<i>E. faecalis</i> NCTC 07171	>64	>64	32	32
<i>E. faecalis</i> NCTC 10547	>64	>64	>64	>64
<i>E. faecalis</i> NCTC 5957	32	>64	0.03	16
<i>E. faecalis</i> ATCC 29212	32	>64	16	16
<i>E. faecalis</i> 56059 vanA	>64	64	>64	>64
<i>E. faecalis</i> 78097 vanB	32	>64	16	16

Key: 4 fold reduction in CAZ MIC ≥ 8 fold reduction in CAZ MIC

CONCLUSIONS

- XOB is a novel oxapenam with potent broad-spectrum β -lactamase inhibitory activity against Class A (including ESBLs), Class C and Class D enzymes, in both cell-free and whole cell tests.
- XOB lacks significant antibacterial activity alone, but a concentration of 8 mg/L causes filamentation in *E. coli* DC0, suggesting inhibition of penicillin binding protein three (PBP 3).
- The stability of XOB to porcine renal dehydropeptidase was similar to that of meropenem, and superior to imipenem.
- Combination of CAZ and XOB enhances the activity of CAZ against most β -lactamase producing strains, except some *P. aeruginosa* strains.
- XOB exhibited significant oral absorption (~35%) in mice.
- Further investigation of XOB as an orally and/or parenterally administered β -lactamase inhibitor is warranted.

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- O'Callaghan C, Morris A, Kirby SM, Shingler AH. Novel method for detection of β -lactamases by using a chromogenic cephalosporin substrate. *Antimicrobial Agents and Chemotherapy* 1972;1:283-8.