

Oxapenem AM-114 (XOB): In vitro activity alone and in combination with amoxicillin or cefaclor against community-acquired respiratory tract pathogens

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INTRODUCTION

The oxapenems are a series of novel β -lactams under development, by Amura Ltd, Cambridge, UK, as broad-spectrum β -lactamase inhibitors.

The lead compound, AM-112, also possesses potent activity as an antibacterial agent against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, many anaerobe species and, surprisingly, also interacts synergistically with cephalosporins against β -lactamase negative enterococci. AM-112 is being progressed as an injectable agent in combination with ceftazidime for the treatment of moderate to severe hospital infections.

XOB (AM-114, Figure 1) also possesses broad-spectrum β -lactamase inhibitory activity. In contrast to AM-112, XOB possesses little antibacterial activity but is orally absorbed. Hence XOB is being considered for use in combination with orally administered β -lactams to treat community and outpatient infections, eg respiratory and urinary tract infections.

Streptococcus pneumoniae, *Haemophilus influenzae* and *Moraxella catarrhalis* are the principal pathogens responsible for community-acquired respiratory tract infections. Routine therapy for such infections is β -lactams, macrolides or fluoroquinolones. However, the empirical use of many antimicrobial agents has been compromised by rapidly emerging resistance, particularly to β -lactams and macrolides.

In common with most bacterial species, *H.influenzae* and *M.catarrhalis* owe their β -lactam resistance to the production of specific β -lactamases. Rare isolates of β -lactamase negative ampicillin resistant (BLNAR) *H.influenzae* with altered penicillin binding proteins (PBPs) also occur. *S.pneumoniae* do not produce detectable β -lactamases, but have developed an elaborate mosaic system of altered PBPs. *S.pneumoniae* are generally categorised as penicillin-susceptible (Pen-S, MIC ≤ 0.06 mg/L), -intermediate resistant (Pen-I, MIC 0.12-1 mg/L) or resistant (MIC ≥ 2 mg/L).

The present study reports on the in vitro activity of XOB alone and in combination with amoxicillin (AMX) or cefaclor (CCL) against isolates of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* with characterised resistance phenotypes.

METHODS

Susceptibility tests

- The minimum inhibitory concentrations (MIC) of amoxicillin (AMX), cefaclor (CCL) and XOB alone, and in combination (at a ratio of 2:1 AMX or CCL to XOB or using fixed sub-MIC concentrations of XOB) were determined using a standard microdilution technique according to NCCLS guidelines.
- Appropriate ATCC quality control strains, where possible, were included on each test occasion.
- Amoxicillin/clavulanate (AMC) was included as a control antimicrobial agent.

Assay strains

- The 35 assay strains comprised *S.pneumoniae* (15), *H.influenzae* (14) and *M.catarrhalis* (6).
- Isolates of *S.pneumoniae* were selected to exhibit susceptibility (Pen-S), intermediate resistance (Pen-I) or full resistance (Pen-R) to penicillin.
- Isolates of *H.influenzae* were selected to exhibit high (β la+) or low (β la-) levels of β -lactamase production. In addition, 4 isolates of β -lactamase negative, ampicillin-resistant (BLNAR) organisms were included.
- All isolates of *M.catarrhalis* produced easily detectable amounts of β -lactamase.

RESULTS

S.pneumoniae (Table 1)

- XOB was moderately active against Pen-S and Pen-I isolates (MICs 1 to 2 mg/L) but inactive (MICs >64 mg/L) against Pen-R isolates.
- CCL was 2 to 4-fold more active than XOB.
- AMX was the most active single agent with MICs of ≤ 0.06 mg/L against Pen-S and Pen-I isolates and gave MICs of 0.5 to 2 mg/L against Pen-R isolates.
- Addition of XOB or clavulanic acid did not enhance the activity of AMX against this β -lactamase negative species.
- Addition of XOB, at half the concentration of CCL, resulted in a 2 to 4-fold decrease in CCL MIC against 7/11 Pen-S and Pen-I isolates. For one strain, #18, the ≥ 4 -fold decrease in MIC of both CCL and XOB was indicative of synergy.
- Similarly, addition of XOB at 0.5 mg/L resulted in a 2 to 4-fold decrease in CCL MIC against 9/11 Pen-S and Pen-I isolates. For 3/6 strains, (#4, #13 and #16) the ≥ 4 -fold decreases in MIC of both CCL and XOB were indicative of synergy.

H.influenzae (Table 2)

- XOB was moderately active (MICs 8 – 16 mg/L) against *H.influenzae*, irrespective of resistance phenotype.
- CCL exhibited MICs of 0.5 to 2 mg/L against β -lactamase negative isolates and, generally, MICs of 4 to 16 mg/L against β -lactamase positive or BLNAR isolates.
- AMX exhibited highly variable activity with MICs of ≤ 0.25 mg/L against β -lactamase negative isolates, 1 to 16 mg/L against BLNAR isolates and >64 mg/L against β -lactamase positive isolates.
- Addition of clavulanic acid, at 2:1 ratio, enhanced AMX activity against β -lactamase positive isolates resulting in MICs of 0.5 to 1 mg/L, as observed with β -lactamase negative isolates, but had no effect against BLNAR isolates.
- Addition of XOB, at 2:1, also enhanced AMX activity against β -lactamase positive isolates resulting in MICs of 2 to 4 mg/L. XOB at a fixed concentration of 0.5 mg/L was less effective in protecting AMX from β -lactamase attack.
- Addition of XOB, at 2:1, generally enhanced CCL activity at least 4-fold against β -lactamase positive isolates. Fixed XOB concentrations of 0.06 and 0.5 mg/L were slightly less effective.
- Interestingly, the addition of XOB achieved a small reduction in CCL MIC against several BLNAR.

M.catarrhalis (Table 3)

- XOB was very active (MICs 0.125 - 1 mg/L) against *M.catarrhalis*.
- CCL and AMX were less active than XOB with MIC ranges of 1 to 2 mg/L and 4 to 16 mg/L respectively.
- XOB, at either 2:1 ratio or at a fixed concentration of 0.06 mg/L, enhanced the activity of both AMX and CCL resulting in MICs of ≤ 0.25 mg/L, similar to those obtained with amoxicillin/clavulanate.

Table 1. MIC (mg/L) of AMX, XOB, CCL alone and combinations of AMX+XOB or CCL+XOB against *S. pneumoniae* with different penicillin susceptibility

Strain	Phenotype	XOB	AMX	AMX+XOB (2:1)	AMX+XOB @ 0.06 mg/L	AMX+XOB @ 0.5 mg/L	AMC	CCL	CCL+XOB (2:1)	CCL+XOB @ 0.06 mg/L	CCL+XOB @ 0.5 mg/L
#1	Pen-S	1	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	0.5	0.25	1	0.25
#3	Pen-S	1	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	0.25	0.125	1	0.25
#5	Pen-S	1	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	0.25	0.25	0.5	0.125
#9	Pen-S	1	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	0.25	0.25	1	0.125
#11	Pen-S	2	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	0.5	0.25	0.5	0.25
#4	Pen-I	1	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	0.5	0.25	0.25	0.125
#6	Pen-I	2	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	1	1	1	0.5
#13	Pen-I	1	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	0.5	0.25	0.5	0.125
#14	Pen-I	1	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	0.25	0.25	0.5	0.125
#16	Pen-I	2	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	1	0.5	1	≤ 0.06
#18	Pen-I	2	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06	1	0.25	1	1
#7	Pen-R	>64	2	2	2	1	2	>64	64	>64	>64
#8	Pen-R	>64	1	1	1	1	1	>64	>64	>64	>64
#24	Pen-R	64	0.5	0.5	0.5	0.25	0.25	64	32	64	64
#33	Pen-R	>64	2	1	2	2	2	>64	64	>64	>64

Key: Synergy (≥ 4 fold decrease in MIC of both components) Additive effect

Table 2. MIC (mg/L) of AMX, XOB, CCL alone and combinations of AMX+XOB or CCL+XOB against *H. influenzae* with different β -lactam resistance phenotypes

Strain	Phenotype	XOB	AMX	AMX+XOB (2:1)	AMX+XOB @ 0.06 mg/L	AMX+XOB @ 0.5 mg/L	AMX + Clavulanate (2:1)	CCL	CCL+XOB (2:1)	CCL+XOB @ 0.06 mg/L	CCL+XOB @ 0.5 mg/L
#3	β la-	16	0.125	0.25	0.25	0.25	0.25	2	2	2	2
#18	β la-	8	0.25	0.25	0.25	0.25	0.25	1	1	1	1
#24	β la-	16	0.25	0.25	0.5	0.5	0.25	2	2	2	2
#36	β la-	8	0.25	0.25	0.25	0.125	0.25	2	2	2	1
#42	β la-	16	0.125	0.125	0.125	0.125	0.125	0.5	1	1	1
#12	β la+	16	>64	2	>64	4	1	16	2	8	2
#28	β la+	8	>64	4	>64	8	1	8	2	2	1
#30	β la+	8	>64	2	>64	4	0.5	4	2	2	2
#32	β la+	16	>64	4	64	4	0.5	4	1	2	2
#40	β la+	16	>64	4	>64	8	1	4	1	2	1
#49	BLNAR	8	1	1	1	0.25	1	4	4	4	2
#50	BLNAR	8	1	1	1	0.5	1	4	4	4	4
#51	BLNAR	16	4	4	4	4	4	8	4	8	4
#52	BLNAR	64	16	8	16	16	16	64	16	64	32

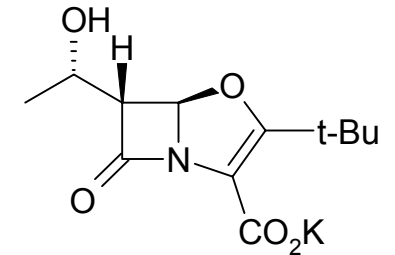
Key: Synergy (>4 fold decrease in MIC of both components) Additive effect

Table 3. MIC (mg/L) of AMX, XOB, CCL alone and combinations of AMX+XOB or CCL+XOB against *M. catarrhalis*

Strain	XOB	AMX	AMX+XOB (2:1)	AMX+XOB @ 0.06 mg/L	AMX+XOB @ 0.5 mg/L	AMX + Clavulanate (2:1)	CCL	CCL+XOB (2:1)	CCL+XOB @ 0.06 mg/L	CCL+XOB @ 0.5 mg/L
#1	0.5	16	0.25	0.25	$\leq 0.06^*$	0.125	2	0.25	0.25	$\leq 0.06^*$
#2	1	8	0.125	0.125	$\leq 0.06^*$	0.125	2	0.25	0.5	$\leq 0.06^*$
#9	1	8	0.25	0.25	$\leq 0.06^*$	0.125	2	0.25	0.5	$\leq 0.06^*$
#10	1	4	0.25	0.25	$\leq 0.06^*$	0.125	4	0.25	0.5	$\leq 0.06^*$
#11	0.25	8	≤ 0.06	≤ 0.06	$\leq 0.06^*$	≤ 0.06	2	0.125	0.125	$\leq 0.06^*$
#12	0.25	4	≤ 0.06	≤ 0.06	$\leq 0.06^*$	1	1	0.25	0.125	$\leq 0.06^*$

Key: Synergy (>4 fold decrease in MIC of both components) Additive effect * XOB concentration exceeds the MIC

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

CONCLUSIONS

- XOB (AM-114) is a novel oxapenem, broad-spectrum β -lactamase inhibitor but also possesses good antibacterial activity against *S.pneumoniae* and *M. catarrhalis* and moderate activity against *H.influenzae*.
- XOB exhibits synergy with both amoxicillin and cefaclor against β -lactamase positive strains of *M. catarrhalis* and *H. influenzae*.
- As with clavulanic acid, XOB does not enhance the activity of amoxicillin against *S.pneumoniae*.
- XOB acted additively in combination with cefaclor against most isolates of *S.pneumoniae*, with some instances of synergy. This synergy is likely to reflect complementation at the PBP level as previously noted with AM-112 and cephalosporins against β -lactamase negative enterococci.
- XOB exhibits similar synergistic β -lactamase inhibitory activity to clavulanic acid against the principal respiratory tract pathogens where Class A β -lactamases predominate.
- Further studies are warranted to investigate:
 - the interaction of XOB with other oral β -lactams against respiratory tract pathogens
 - the interaction of XOB with other oral β -lactams against urinary tract pathogens where organisms with Class A and Class C β -lactamases occur.