

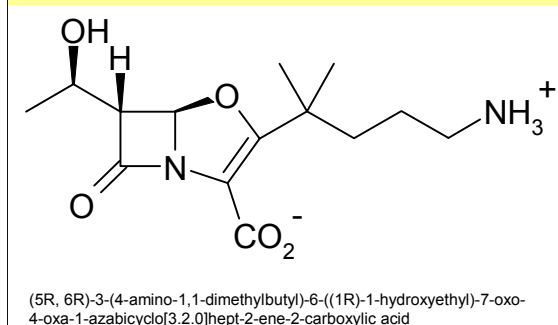
INTRODUCTION

β -Lactamase inhibitors, such as clavulanic acid, tazobactam and sulbactam, have proven to be highly successful agents in overcoming β -lactamase-mediated resistance (1). These agents are effective inhibitors of Class A β -lactamases, but lack activity against Class B, C and D enzymes (2).

AM-112 (Figure 1), under development by Amura Ltd, Cambridge, UK, is a novel oxapenem with broad-spectrum β -lactamase inhibitory activity. This class of compounds are notoriously unstable, but the presence of a tertiary substituent in the 2-position, as in AM-112, greatly increases stability. This is very important in making Amura's oxapenems viable as drug candidates. In addition, AM-112 has a (R)-1-hydroxyethyl substituent in the 6-position which, besides further stabilising the molecule, is an important molecular recognition feature.

We report on the chemical and biological properties of this compound, including its *in vivo* and *in vitro* activities against a variety of β -lactamase producing bacterial strains.

Figure 1. Chemical structure of AM-112 (PFOB)



METHODS

Preparation and inhibition of cell-free β -lactamases

β -Lactamase extracts were prepared and purified as described previously (3). AM-112 and clavulanic acid were pre-incubated with each β -lactamase for 15 minutes prior to spectrophotometric determination of IC_{50} values using Nitrocefin as substrate (4).

Renal DHP stability

AM-112, imipenem and meropenem were incubated at 37°C with porcine renal dehydropeptidase (DHP). The absorbance (at 262nm for AM-112, 296nm for imipenem and meropenem) of each solution was recorded over 3 hours.

Susceptibility tests

The activity of AM-112 and various cephalosporins alone and in combination were determined by broth microdilution (NCCLS guidelines).

Pharmacokinetics

AM-112 or ceftazidime (CAZ) and AM-112 were co-administered, in a 2:1 ratio, to mice and rats by iv injection into the caudal vein. Animals were bled pre-dose and up to two hours post dose. Blood collection from mice was performed by intra-cardiac puncture and from rats via the retro-orbital sinus into heparinised tubes under light halothane anaesthesia. At the end of the experiment, the animals were killed by asphyxiation in CO_2 . Samples were stored at -70°C until required and assayed by high performance liquid chromatography (HPLC).

In vivo infection models

Mice were infected intraperitoneally with either *S. aureus* 3816, *E. cloacae* P99 or *E. coli* SHV-5 in 6% w/v mucin, at inocula ranging from 10^6 to 10^8 cfu/ml. Post-infection, the mice were treated with different schedules of antibiotics, depending on the infecting organism. Survival of the mice was scored over the following four days and the ED_{50} was determined by the Probit method.

RESULTS

Chemical synthesis

AM-112 is made in a 7-step stereoselective sequence from a commercially available azetidinone in a current overall yield of 20%.

Patents

New patents have been filed in the last year including a composition of matter patent of selection covering AM-112. Patents covering the production, physical form and stable compositions of AM-112 have also been filed together with a patent covering its synergistic interaction with cephalosporins against enterococci.

β -Lactamase inhibition (Figure 2)

AM-112 exhibited potent inhibitory activity against Class A, C and D β -lactamases. Clavulanic acid, with IC_{50} values of 0.02 to 0.002 mg/L, was up to 100-fold more active than AM-112 against Class A enzymes. AM-112, with IC_{50} values of 0.07 to 0.007 mg/L, was 1000- to 100,000-fold more active than clavulanic acid against Class C and Class D enzymes.

Renal DHP stability (Figure 3)

AM-112 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 AM-112 exhibited little or no loss, respectively, over the same time period.

Antibacterial spectrum (Figure 4)

AM-112 exhibited potent activity (MICs ≤ 2 mg/L) against methicillin sensitive *Staphylococcus aureus*, penicillin-susceptible *Streptococcus pneumoniae*, anaerobes (Gram +ve and Gram -ve) and *Moraxella catarrhalis*. Moderate activity (MICs 4 to 16 mg/L) was observed against penicillin-intermediate and resistant *S. pneumoniae*, *Haemophilus influenzae* and *Enterobacteriaceae*. AM-112 exhibited poor activity against MRSA and *Pseudomonas aeruginosa*.

Synergy with cephalosporins against Gram-negatives with potent Class A or Class C β -lactamase activity

The MICs of 7 cephalosporins were determined alone and in the presence of AM-112 at 4 mg/L against 6 isolates producing high levels of specific β -lactamases (Table 1). All of the cephalosporins exhibited high MICs (≥ 32 mg/L) against at least 2 strains. Addition of AM-112 markedly reduced (≥ 8 -fold) the MIC of each cephalosporin against at least 3 isolates. In many cases, the reduction in MIC was >128 -fold. Cefazolin, cefuroxime, cefaclor and cefoperazone were the least β -lactamase stable compounds, exhibiting MICs of ≥ 4 mg/L against all isolates. AM-112 provided significant protection against all three isolates producing Class A β -lactamases and, with the exception of cefuroxime, protection against *Enterobacter cloacae* with Class C β -lactamases. Cefotaxime and ceftriaxone possessed good activity (MICs <1 mg/L) against isolates with Class A β -lactamases but were inactive against isolates with Class C β -lactamases. Addition of AM-112, further reduced the MIC against isolates with Class A β -lactamases and markedly reduced the MIC against *E. cloacae* isolates. Ceftazidime exhibited high MICs against isolates producing Class A and Class C β -lactamases. Addition of AM-112 reduced all ceftazidime MICs to <1 mg/L.

Synergy with cephalosporins against Enterococci

MIC and rate of kill studies have shown unexpected synergy between cephalosporins and AM-112 against some isolates of *Enterococcus* spp. (See Poster P748).

In vivo infection models

The efficacy of ceftazidime and AM-112 was assessed alone and in combination in three animal infection models (Table 2). **S. aureus 3816.** The superior *in vitro* activity of AM-112 over ceftazidime in MIC tests was reflected *in vivo* where the ED_{50} for AM-112 against *S. aureus* 3816 was ca 6-fold lower than achieved by ceftazidime. Combinations of ceftazidime and AM-112 were additive, mainly reflecting AM-112 activity. **E. cloacae P99.** The ED_{50} values for ceftazidime and AM-112 alone and in combination mirrored the marked synergistic effect noted in *in vitro* tests. The ED_{50} for ceftazidime fell from >100 mg/kg when used alone to 2 to 4 mg/kg in the presence of AM-112 at 2 mg/kg. **E. coli SHV-5.** *In vitro* tests indicated an additive rather than synergistic interaction between ceftazidime and AM-112. The MIC results were not predictive of ED_{50} values either alone or in combination. *In vivo*, combinations of ceftazidime and AM-112 were at least 8-fold more active than either single agent indicating marked synergy.

Pharmacokinetics

Ceftazidime and AM-112 were co-administered at 2:1 ratio by intravenous injection at three doses to mice and rats. AM-112 exhibited similar pharmacokinetics to ceftazidime, with a linear dose response and mean elimination half life of 8.2 mins (Figure 5). Rats. AM-112 exhibited a linear dose response. Although peak levels were similar to ceftazidime, the mean elimination half life of 8.9 mins was shorter. (Figure 6).

Figure 2. IC_{50} values (mg/L) obtained for clavulanic acid and AM-112 against Class A, C and D β -lactamases isolated from eight bacterial strains

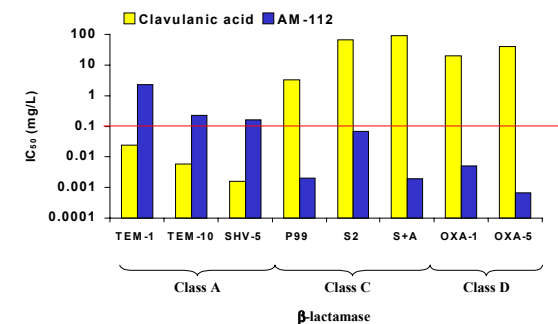


Figure 3. Stability of imipenem, meropenem and AM-112 in Tris buffer over an hour at 37°C

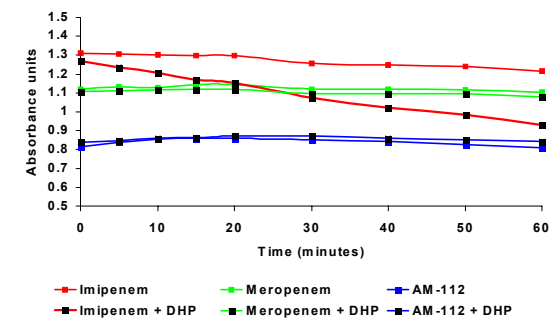


Figure 4. Antibacterial spectrum of AM-112

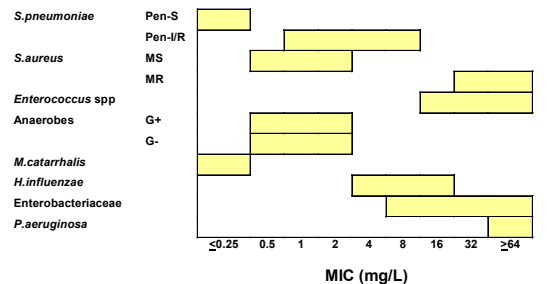


Table 1. Minimum inhibitory concentration (MIC) of seven cephalosporins alone and combined with 4mg/L of AM-112 against a panel of β -lactamase producing bacteria

Compound	MIC (mg/L)						
	Class A β -lactamases			Class C β -lactamases			
	<i>E. coli</i> TEM-1	<i>E. coli</i> TEM-10	<i>E. coli</i> SHV-5	<i>E. cloacae</i> P99	<i>E. cloacae</i> Hennessey	<i>C. diversus</i> 2046E	
Cefazolin	64	8	32	>64	>64	>64	>64
Cefazolin + AM-112	1	<0.06	<0.06	<0.06	8	>64	>64
Cefuroxime	8	16	8	>64	>64	>64	>64
Cefuroxime + AM-112	0.25	<0.06	<0.06	<0.06	>64	64	64
Cefaclor	64	32	16	>64	>64	>64	>64
Cefaclor + AM-112	0.5	<0.06	<0.06	<0.06	2	>64	>64
Ceftriaxone	0.06	0.25	2	64	>64	>64	>64
Ceftriaxone + AM-112	0.06	<0.06	<0.06	<0.06	0.5	32	32
Cefoperazone	>64	4	4	64	>64	>64	>64
Cefoperazone + AM-112	<0.06	<0.06	<0.06	<0.06	1	>64	>64
Cefotaxime	0.25	0.5	1	64	64	4	4
Cefotaxime + AM-112	<0.06	<0.06	<0.06	<0.06	0.5	0.125	0.125
Ceftazidime (CAZ)	0.5	>64	16	32	>64	1	1
Ceftazidime + AM-112	<0.06	<0.06	<0.06	<0.06	0.5	0.06	0.06

Key: ≥ 8 -fold reduction in MIC ≥ 128 -fold reduction in MIC

Figure 5. Summary of CAZ and AM-112 levels in mice following iv administration at 2:1 ratio

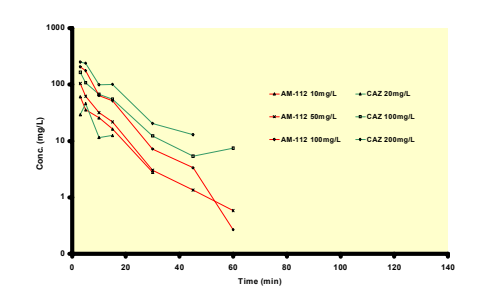


Figure 6. Summary of CAZ and AM-112 levels in rats following iv administration at 2:1 ratio

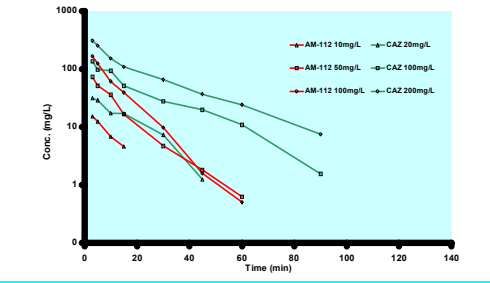


Table 2. ED_{50} values for combinations of ceftazidime (CAZ) and AM-112 against various pathogens in a murine intraperitoneal sepsis model

Organism (inoculum [CFU/ml])	Compound	MIC (mg/L)	ED_{50} (mg/kg)
<i>S. aureus</i> 3816 (3×10^7)	CAZ	16	18.3
	CAZ		22.6
	AM-112	1	2.7
<i>S. aureus</i> 3816 (8×10^6)	CAZ		2.6
	AM-112		4.8 + 1.2
	CAZ-AM-112 (4:1)	ND	7 + 1
<i>E. cloacae</i> P99 (2.2×10^7)	CAZ-AM-112 (7:1)	ND	>100
	CAZ	128	>100
	AM-112	32	19
<i>E. cloacae</i> P99 (2.2×10^7)	CAZ-AM-112 (1:1)	2 + 2	2
	CAZ-AM-112 (2:1)	4 + 2	4 + 2
	CAZ-AM-112 (4:1)	4 + 1	11 + 3
<i>E. coli</i> SHV-5 (6×10^6)	CAZ	>128	16
	AM-112		72
	CAZ-AM-112 (1:1)	8 + 8	2
<i>E. coli</i> SHV-5 (6×10^6)	CAZ-AM-112 (2:1)	16 + 8	2 + 1
	CAZ-AM-112 (2:1)		

Key: ND: Not determined Additive Synergy

CONCLUSIONS

- AM-112 is potent β -lactamase inhibitor with a broad spectrum of activity that includes Class A, C and D β -lactamases.
- AM-112 has a similar porcine DHP stability to that of meropenem, and superior to that of imipenem.
- AM-112 possesses good antibacterial activity against *S. aureus*, *S. pneumoniae*, anaerobes and *M. catarrhalis*.
- AM-112 synergises with cephalosporins, markedly enhancing their activity against *Enterobacteriaceae* isolates producing high levels of characterised β -lactamases and resulting in unexpected activity against β -lactamase negative enterococci.
- Following iv administration at 10, 50 and 100 mg/kg, AM-112 exhibits similar pharmacokinetics to ceftazidime in mice. The elimination half-life was shorter than ceftazidime in rats.
- In animal models of infection, AM-112 complements ceftazidime activity against *S. aureus* and enhances ceftazidime activity against *E. cloacae* P99 and *E. coli* SHV-5.
- AM-112 has the potential to improve the efficacy of cephalosporins, in particular ceftazidime, in treating serious infections.

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