

AM-114: Chemistry and biological activity of a novel orally absorbed broad-spectrum oxapenem β -lactamase inhibitor

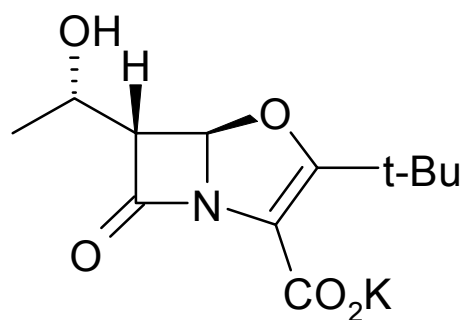
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INTRODUCTION

AM-114 (Amura Ltd, Cambridge, UK) is a novel oxapenem (Figure 1) with broad-spectrum β -lactamase inhibitory activity, against both class A and class C enzymes. Unlike AM-112, AM-114 exhibits significant oral absorption.

In this report, we describe the chemical synthesis of AM-114 and its activity alone and in combination with ceftazidime (CAZ) or cefaclor (CCL), against CAZ-resistant isolates of *Klebsiella pneumoniae* SHV-5 and *Enterobacter cloacae* P99 in MIC tests and animal models of infection.

Figure 1. Chemical structure of 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

SYNTHESIS OF AM-114

Stage 1 (Figure 2)

- The starting material (Compound I) was made as described in Poster F 345.
- Compound I and pivaloyl chloride were treated with LHMDs in THF at -78°C.
- The product was purified by chromatography.
- The yield of Compound II was 86%.
- The product was isolated as a mixture of diastereoisomers.

Stage 2

- Compound II was desilylated with acetic acid and tertbutylammonium fluoride in THF.
- The product was purified by chromatography.
- The yield of Compound III was 82%.
- The product was isolated as a mixture of diastereoisomers.
- The product is a crystalline solid.

Stage 3

- The key inversion step was carried out by way of a Mitsunobu Reaction.
- Compound III was treated with formic acid, diisopropyl azodicarboxylate and triphenylphosphine.
- The product was purified by chromatography.
- The yield of Compound IV was 44%.
- A by-product was the product of elimination of water to give a mixture of E and Z exocyclic alkenes.

Stage 4

- The formate ester of Compound IV was selectively hydrolysed with HCl in water / methanol.
- The crude product was used in the next step.
- The yield of Compound V was ~100%

Stage 5

- The hydroxy group of Compound V was protected by treatment with p-nitrobenzyl chloroformate and DMAP.
- The product was purified by chromatography.
- The yield of Compound VI was 81%.

Stage 6

- Compound VI was treated with chlorine at -40°C.
- The product was isolated by chromatography at 0°C.
- The yield of Compound VII was 85%.
- The product was isolated as a mixture of diastereoisomers.

Stage 7

- Compound VII was treated with potassium t-butoxide at -30°C to form the oxapenem ring system.
- Cold chromatography at -12°C was used to separate the cis and trans isomers.
- Fractional crystallisation from ethyl acetate / hexane at -20°C was also used to separate the isomers by precipitating the cis isomer (with a little trans) and leave the pure trans isomer in the mother liquor.
- The combined yield of cis and trans isomers of Compound VIII was 84%.
- The typical cis:trans ratio was 1:2
- The cis isomer could be equilibrated to cis:trans ratio of 31:69 in ethyl acetate at room temperature.

Stage 8

- Compound VIII was hydrogenated over palladium on carbon in ethyl acetate to deprotect both the carboxylic acid and the alcohol.
- The yield of AM-114 was 59%.
- AM-114 was obtained as a voluminous white solid.

Overall yield: 10.6%

Figure 2. Synthesis of AM-114

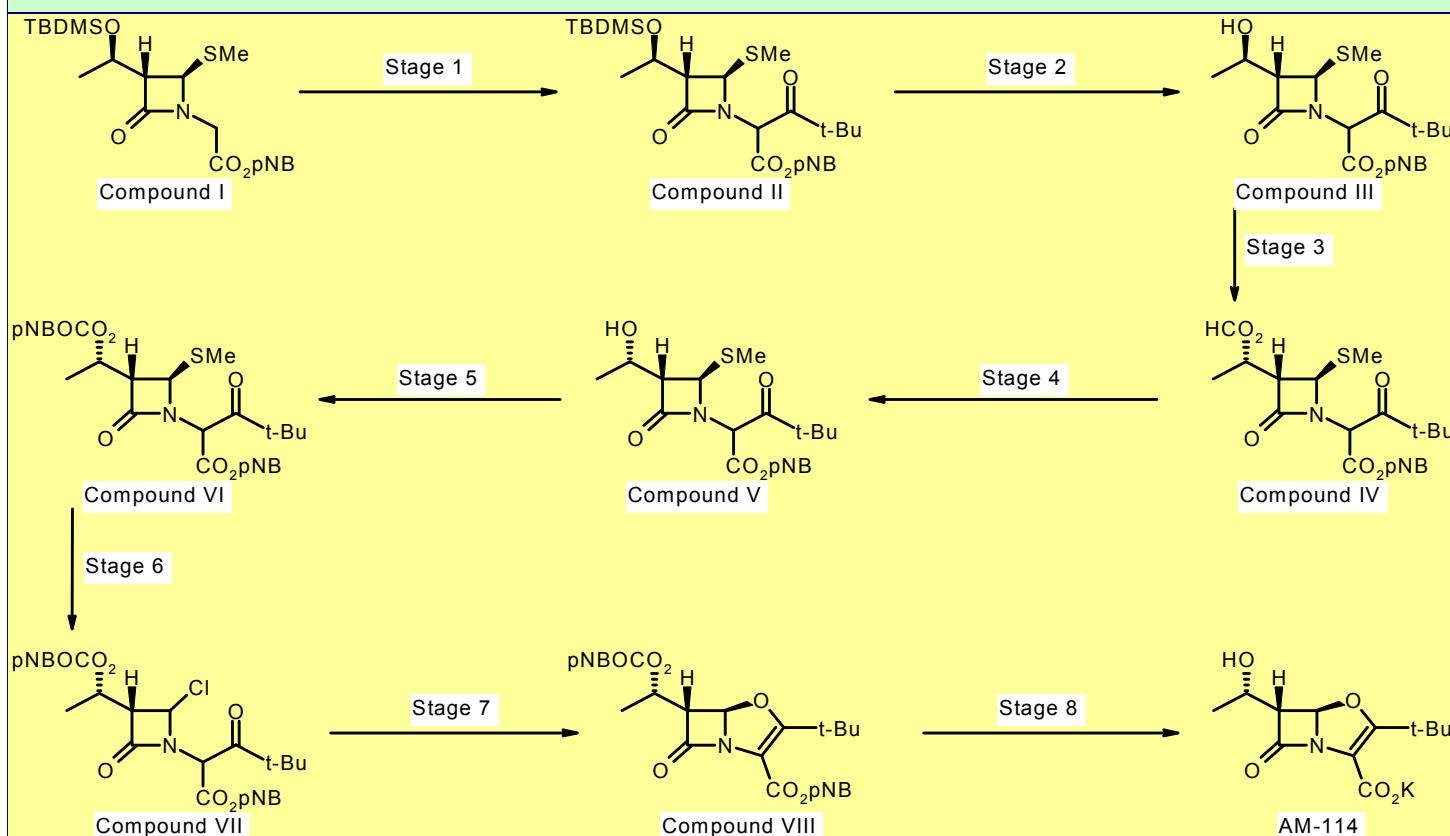


Table 1. Summary of MIC and ED₅₀ results

	Drug	Ratio	MIC (mg/L)	ED ₅₀ (mg/kg)		Route
				(1)	(2)	
<i>E. cloacae</i> P99	CAZ		32	>100		sc
	AM-114		64	>100		sc
	CAZ + AM-114	1:1	4 + 4	38 + 38		sc
<i>K. pneumoniae</i> SHV-5	CAZ		128	24	>160	sc
	AM-114		>128	>160	>160	sc
	CAZ + AM-114	1:1	4 + 4	14 + 14	>40 + >40	sc
<i>K. pneumoniae</i> SHV-5	CCL		128	113		po
	AM-114		>128	80		po
	CCL + AM-114	1:1	2 + 2	17 + 17		po
<i>K. pneumoniae</i> SHV-5	CCL		8 + 4	57 + 28		po
	CCL + AM-114	2:1	8 + 4	57 + 28		po

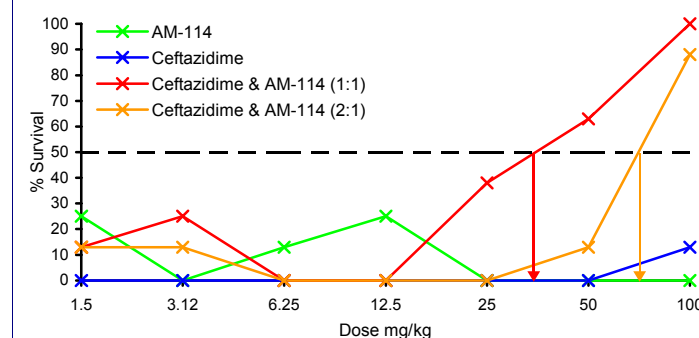
Key:

4 - fold reduction in the CAZ or CCL MIC
≥8 - fold reduction in the CAZ or CCL MIC

Figure 3. Percentage survival of infected and treated mice

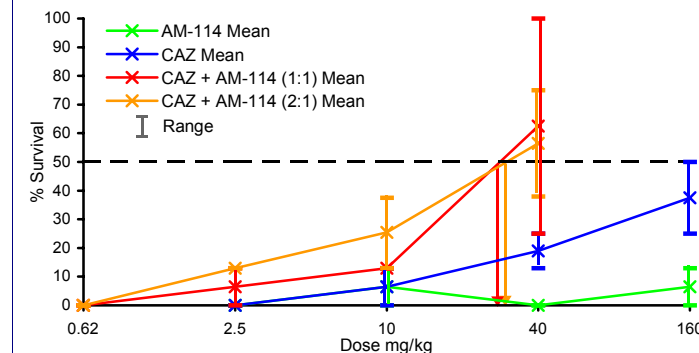
a) Infection: *E. cloacae* P99

Treatment: CAZ +/- AM-114 by subcutaneous route



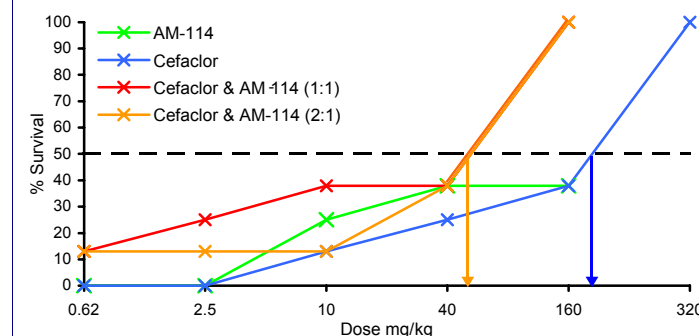
b) Infection: *K. pneumoniae* SHV-5

Treatment: CAZ +/- AM-114 by subcutaneous route



c) Infection: *K. pneumoniae* SHV-5

Treatment: CCL +/- AM-114 by oral route



BIOLOGICAL METHODS

Susceptibility tests

Minimum inhibitory concentrations (MICs) were determined by broth microdilution carried out in accordance with NCCLS guidelines (NCCLS 2000).

In vivo efficacy

Mice were infected with *E. cloacae* P99 (class C β -lactamase) or *K. pneumoniae* SHV-5 (class A β -lactamase [ESBL-type]) and treated with AM-114, CAZ, CCL alone or in combination. The ED₅₀ was defined as the cephalosporin dose which achieved 50% survival of infected mice.

1. *E. cloacae*

Mice were infected by an intraperitoneal injection (5×10^7 CFU/ml) of *E. cloacae*. A series of two-fold dilutions of the test agents were prepared in sterile water for injection such that administration of the dilutions in 0.2 ml volumes (0.4 ml for all 100 mg/kg AM-114 doses) would yield doses of 100, 50, 25, 12.5, 6.25, 3.12, and 1.5 mg/kg. Four regimens were tested: CAZ alone, AM-114 alone, CAZ + AM-114 (1:1) and CAZ + AM-114 (2:1).

Groups of 8 mice/dose were tested for each of the four treatments. All CAZ doses and immediate subsequent AM-114 doses were administered as a single subcutaneous dose at the dorsal base of the neck. Antibiotic administration occurred approximately one hour after infection, and again four hours later (five hours after infection). A control group of eight mice received 0.2 ml of saline instead of antibiotic.

2. *K. pneumoniae*

Mice were infected intraperitoneally with 10^5 CFU/mouse of *K. pneumoniae* SHV-5.

a) CAZ +/- AM-114 administered subcutaneously

10 minutes after infection, 4 groups of 8 mice were treated by sc route with two-fold dilutions of CAZ alone, AM-114 alone or of the two drugs combined in a 1:1 and 2:1 ratio (CAZ:AM-114). This experiment was conducted on two occasions.

b) CCL +/- AM-114 administered po

10 minutes after infection, another 4 groups of 8 mice were treated orally with two fold dilutions of CCL alone, AM-114 alone, or with a combination of the two drugs in 1:1 or 2:1 ratio.

RESULTS

E. cloacae P99

CAZ +/- AM-114; subcutaneous administration

- Alone, CAZ and AM-114 exhibited little activity against *E. cloacae* P99 either *in vitro* (MICs of 32 and 64 mg/L, respectively) (Table 1) or *in vivo* (both ED₅₀s >100 mg/kg) (Figure 3a).
- Combinations of CAZ and AM-114 at 1:1 or 2:1 ratio interacted synergistically both *in vitro* and *in vivo*.
 - At 1:1 ratio, the CAZ MIC was reduced 8-fold to 4 mg/L; the corresponding ED₅₀ was reduced at least 3-fold to 38 mg/kg.
 - At 2:1 ratio, the synergistic effect was less marked.

K. pneumoniae SHV-5

a) CAZ +/- AM-114; subcutaneous administration

- Alone, CAZ and AM-114 showed little or poor activity against *K. pneumoniae* SHV-5 either *in vitro* (MICs of >128 mg/L) (Table 1) or *in vivo* (ED₅₀s >160 mg/kg) (Figure 3b).
- Combinations of CAZ and AM-114 at 1:1 or 2:1 ratio interacted synergistically both *in vitro* and *in vivo*.
 - At 1:1 ratio, the CAZ MIC was reduced 32-fold to 4 mg/L. In the efficacy test, the ED₅₀ was reduced to 14 mg/kg on occasion 1. On occasion 2, the survival was 25% at 40 mg/kg.

b) CCL +/- AM-114; oral administration

- Alone, CCL and AM-114 showed little or poor activity against *K. pneumoniae* SHV-5 either *in vitro* (MICs of >128 mg/L) (Table 1) or *in vivo* (ED₅₀s 113 and 80 mg/kg respectively) (Figure 3c).
- Combinations of CCL and AM-114 at 1:1 or 2:1 ratio interacted synergistically both *in vitro* and *in vivo*.
 - At 1:1 ratio, the CCL MIC was reduced 64-fold to 2 mg/L; the corresponding ED₅₀ was reduced to 17 mg/kg.
 - At 2:1 ratio, the synergistic effect was less marked.

CONCLUSIONS

- AM-114 (under development by Amura Ltd) is a novel oxapenem, which can be synthesised in 8 stages with an overall yield of 10.6%.
- AM-114 possesses little antibacterial activity against ceftazidime resistant *E. cloacae* P99 and *K. pneumoniae* SHV-5.
- AM-114 is a potent inhibitor of class A and class C β -lactamases and can enhance ceftazidime and cefaclor against isolates producing these enzymes.
- Administered subcutaneously, AM-114 enhances the activity of ceftazidime against ceftazidime-resistant isolates of *K. pneumoniae* SHV-5 and *E. cloacae* P99.
- Administered orally, AM-114 enhances the activity of cefaclor against *K. pneumoniae* SHV-5.
- AM-114 is the first orally absorbed broad-spectrum β -lactamase inhibitor.