

Broad-spectrum β -lactamase inhibitor AM-112: Synthesis and enhancement of the in vitro activity of ceftazidime and piperacillin

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INTRODUCTION

AM-112 (Amura Ltd, Cambridge, UK) is a novel oxapenem inhibitor of Class A, C and D β -lactamases.

We describe the chemical synthesis of AM-112, synergy with ceftazidime (CAZ) and piperacillin (PIP) against isolates with specific β -lactamases; also pharmacokinetic / pharmacodynamic studies in mice.

SYNTHESIS OF AM-112

Stage 1 (Figure 1)

- The starting azetidinone was treated with sodium methyl mercaptan in acetonitrile at -15 to -20°C.
- The product was isolated by crystallisation from hexane.
- The *trans* isomer was isolated exclusively.
- The yield of Compound III was 78%.
- The product was crystalline (white needles), m.p. 93°C.

Stage 2

- Compound III was deprotonated with butyllithium at -45 to -35°C.
- The anion produced was condensed with the iodoacetate (Compound IX (Figure 2)) at <-5°C.
- The product was isolated crude and stored as a ~40% THF solution.
- The approximate yield of crude product was 75%.

Stage 3

- Compound IV and the acid chloride (Compound X (Figure 2)) were treated with LHMDS at <-65°C.
- The product was purified by chromatography.
- The product was stored as a ~14% solution.
- The product was isolated as a mixture of diastereoisomers.
- The yield of Compound V was 68%.

Stage 4

- Compound V was desilylated with tertbutylammonium fluoride and acetic acid in THF.
- The product was purified by chromatography.
- The product was isolated as a mixture of diastereoisomers.
- The yield of Compound VI was 77%.

Stage 5

- Compound VI can be chlorinated with either chlorine or methyl sulphenyl chloride.
- A radical inhibitor was used to suppress possible side reactions.
- The product was used crude in the next stage.
- The chloroazetidinone was produced as a mixture of α and β stereoisomers.
- The approximate yield of the crude product was 94%.

Stage 6

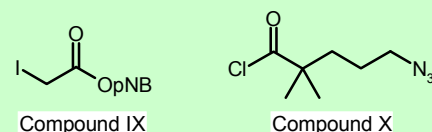
- Compound VII was treated with triethylamine in THF at -50°C to 20°C to form the oxapenem ring system.
- Cold chromatography at -15°C was used to separate the *trans* isomer.
- The compound was stored at -20°C to avoid epimerisation.
- The combined yield of the *cis* and *trans* isomers of compound VIII was 70%.
- The typical *cis:trans* ratio was 1:2.
- The *cis* isomer can be equilibrated to a 1:3 *cis:trans* mixture in a non-nucleophilic solvent at room temperature.

Stage 7

- Compound VIII was hydrogenated to deprotect both the amine and the carboxylic acid in a two-phase ethyl acetate and aqueous reaction.
- To avoid epimerisation the temperature was kept at 0°C.
- AM-112 was isolated from the aqueous phase by lyophilisation.
- Most by-products from the reaction were removed in the organic phase.
- The yield of AM-112 was 80%.
- AM-112 was produced as a voluminous off-white solid.

Overall yield: 16.1%

Figure 2: Structures of Compounds IX and X



BIOLOGICAL METHODS

Susceptibility tests

The MICs of CAZ and PIP alone, and with AM-112, at a fixed concentration of 4 mg/L, against bacterial isolates with specific Class A, C or D β -lactamases were determined using agar dilution, according to NCCLS guidelines (NCCLS 2000).

Figure 1: Synthesis of AM-112

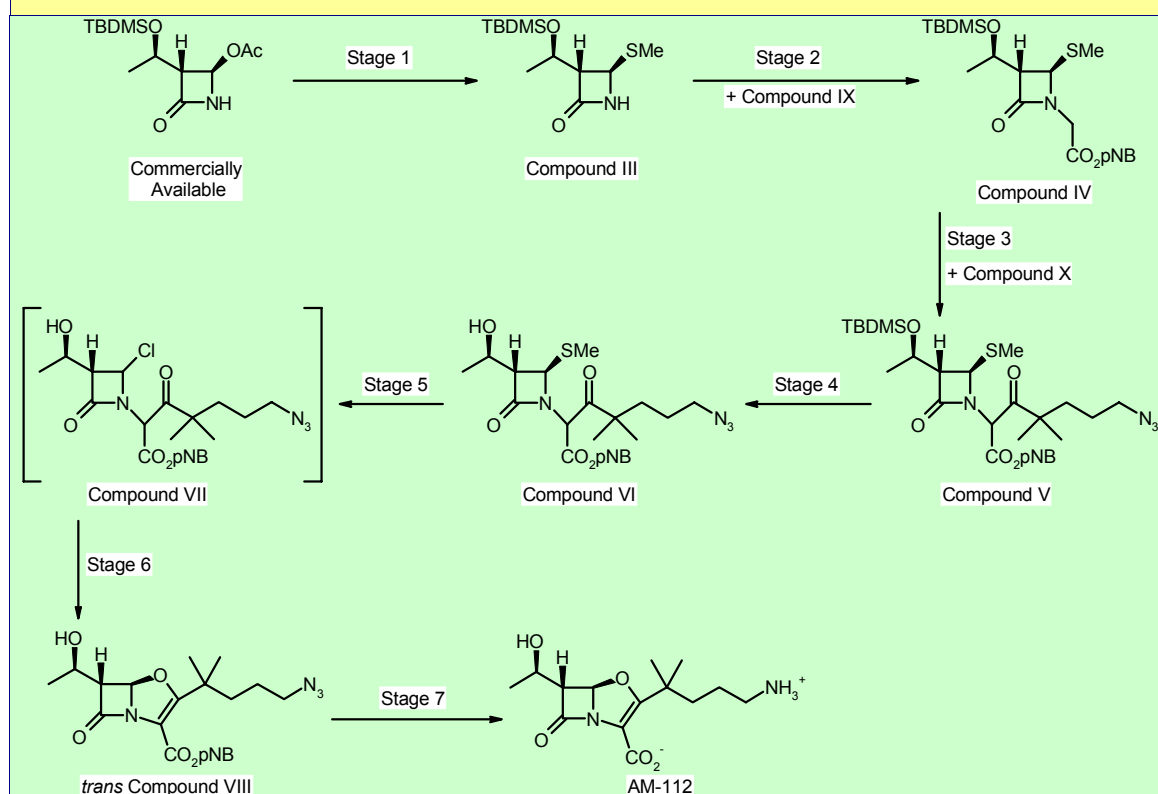


Table 1. MICs of CAZ, PIP alone and in the presence of AM-112 at 4 mg/L against isolates with Class A β -lactamases

Species	Strain	Expression	AM-112	MIC (mg/L)			
				CAZ	CAZ + AM-112	PIP	PIP + AM-112
<i>E. coli</i>	53-1	-	16	0.125	0.125	4	4
<i>E. coli</i>	62-1	-	16	0.25	0.125	4	4
<i>E. coli</i>	62/TEM-1	A	32	0.25	0.125	>128	>128
<i>E. coli</i>	53/TEM-2	A	16	0.25	0.06	>128	>128
<i>E. coli</i>	62/TEM-3	A(ESBL)	16	32	2	>128	>128
<i>E. coli</i>	53/TEM-6	A(ESBL)	32	>128	32	>128	>128
<i>E. coli</i>	53/SHV-1	A	32	0.5	0.125	>128	>128
<i>E. coli</i>	53/SHV-3	A(ESBL)	8	1	0.03	64	4
<i>E. coli</i>	53/SHV-5	A(ESBL)	32	>128	>128	>128	>128
<i>P. vulgaris</i>	VA1-CON	A+	32	1	0.25	>128	64
<i>P. vulgaris</i>	VA1-DEF	A-	32	0.125	0.06	1	1
<i>P. vulgaris</i>	V2	A	32	0.125	0.06	8	8
<i>P. vulgaris</i>	V2-CON	A+	32	1	0.06	>128	32
<i>P. vulgaris</i>	V2-DEF	A-	32	0.125	0.06	4	1
<i>P. vulgaris</i>	V3	A	32	0.125	0.125	8	16
<i>P. vulgaris</i>	V3-CON	A+	32	1	0.25	>128	128
<i>P. vulgaris</i>	V3-DEF	A-	32	0.125	0.06	8	0.5

Table 2. MICs of CAZ, PIP alone and in the presence of AM-112 at 4 mg/L against isolates with Class C β -lactamases

Species	Strain	Expression	AM-112	MIC (mg/L)			
				CAZ	CAZ + AM-112	PIP	PIP + AM-112
<i>C. freundii</i>	C2	C	128	1	1	16	8
<i>C. freundii</i>	C2-CON	C+	32	64	8	>128	64
<i>C. freundii</i>	C2-DEF	C-	16	0.5	0.125	8	1
<i>C. freundii</i>	C4	C	64	64	1	128	8
<i>C. freundii</i>	C4-CON	C+	64	128	8	>128	8
<i>C. freundii</i>	C4-DEF	C-	32	32	0.125	64	0.5
<i>C. freundii</i>	C10	C	32	8	0.25	8	2
<i>C. freundii</i>	C10-CON	C+	32	128	8	128	8
<i>C. freundii</i>	C10-DEF	C-	16	0.25	0.03	2	0.5
<i>C. freundii</i>	C12	C	64	0.25	0.25	4	4
<i>C. freundii</i>	C12-CON	C+	32	>128	16	>128	8
<i>C. freundii</i>	C12-DEF	C-	8	0.5	0.03	1	0.25
<i>E. cloacae</i>	84-CON	C+	32	>128	32	>128	128
<i>E. cloacae</i>	84-DEF	C-	32	4	0.5	32	4
<i>E. cloacae</i>	100-CON	C+	16	32	0.25	128	4
<i>E. cloacae</i>	100-DEF	C-	16	0.5	0.125	8	0.25
<i>E. cloacae</i>	684	C	64	1	4	16	16
<i>E. cloacae</i>	684-CON	C+	64	128	32	>128	128
<i>E. cloacae</i>	684-DEF	C-	16	0.5	0.125	4	2
<i>S. marcescens</i>	S2	C	128	0.25	0.25	8	4
<i>S. marcescens</i>	S2-CON	C+	64	2	0.5	128	16
<i>S. marcescens</i>	S2-DEF	C-	128	0.25	0.25	8	2
<i>S. marcescens</i>	S7	C	64	0.25	0.25	4	2
<i>S. marcescens</i>	S7-CON	C+	64	4	0.25	128	4
<i>S. marcescens</i>	S7-DEF	C-	128	0.25	0.25	8	4
<i>M. morgani</i>	M1	C	64	1	0.25	16	1
<i>M. morgani</i>	M1-CON	C+	64	32	0.5	>128	2
<i>M. morgani</i>	M1-DEF	C-	32	0.125	0.06	1	0.25
<i>M. morgani</i>	M3-CON	C+	64	64	1	>128	
<i>M. morgani</i>	M3-DEF	C-	64	0.25	0.25	1	0.5
<i>M. morgani</i>	M6	C	64	8	0.125	16	1
<i>M. morgani</i>	M6-CON	C+	64	64	0.125	128	1
<i>M. morgani</i>	M6-DEF	C-	32	0.125	0.03	1	0.25

Table 3. MICs of CAZ, PIP alone and in the presence of AM-112 at 4 mg/L against isolates with Class D β -lactamases

Species	Strain	Expression	AM-112	MIC (mg/L)			
				CAZ	CAZ + AM-112	PIP	PIP + AM-112
<i>E. coli</i>	53-1	-	16	0.125	0.125	4	4
<i>E. coli</i>	53/OXA-1	D	16	0.5	0.125	>128	8
<i>E. coli</i>	53/OXA-2	D	16	0.25	0.25	>128	>128
<i>E. coli</i>	53/OXA-3	D	32	1	0.25	32	8
<i>E. coli</i>	53/OXA-4	D	32	0.25	0.125	64	4
<i>E. coli</i>	53/OXA-5	D	32	128	4	>128	32
<i>E. coli</i>	53/OXA-7	D	32	1	0.5	>128	32

Bacterial isolates

- Isogenic strains of *Escherichia coli* producing plasmid mediated Class A β -lactamases, including extended spectrum β -lactamases (ESBLs) (Table 1).
- Clinical isolates of *Proteus vulgaris* with normal inducible (A) and mutants with depressed (A+) or reduced (A-) levels of Class A β -lactamase production (Table 1).
- Clinical isolates of *Citrobacter freundii*, *Enterobacter cloacae*, *Serratia marcescens*, *Morganella morganii* with normal inducible (C) and mutants with derepressed (C+) or reduced (C-) levels of Class C β -lactamase production (Table 2).
- Isogenic *Escherichia coli* strains producing plasmid mediated Class D β -lactamases (Table 3).
- Clinical isolates of *Pseudomonas aeruginosa* with normal inducible levels (C) or mutants with elevated (C+) or reduced (C-) of Class C β -lactamase production (Table 4).

Figure 3a. AM-112 & CAZ: Mouse pharmacokinetics 100 & 200 mg/kg

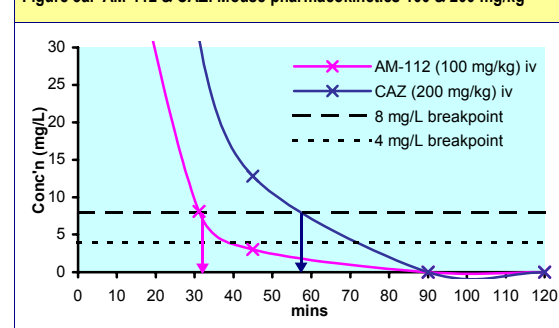


Figure 3b. AM-112 & CAZ: Mouse pharmacokinetics 50 & 100 mg/kg

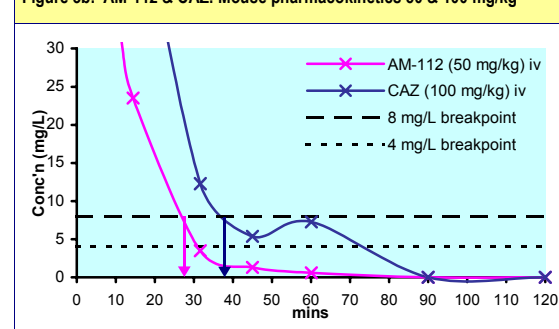


Figure 3c. AM-112 & CAZ: Mouse pharmacokinetics 10 & 20 mg/kg

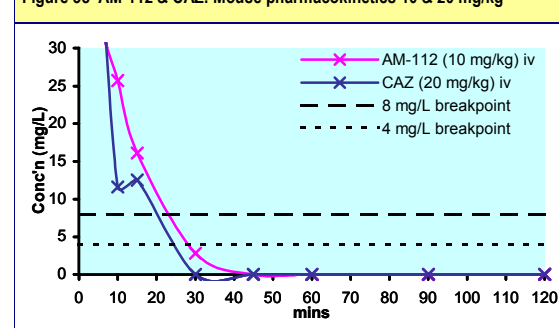


Table 4. MICs of CAZ, PIP alone and in the presence of AM-112 at 4 mg/L against isolates of *P. aeruginosa*

Species	Strain	Expression	AM-112	MIC (mg/L)			
				CAZ	CAZ + AM-112	PIP	PIP + AM-112
<i>P. aeruginosa</i>	1405-con	C+	>128	128	128	>128	>128
<i>P. aeruginosa</i>	1405-def	C-	>128	8	8	64	16
<i>P. aeruginosa</i>	2297	C	>128	2	4	8	8
<i>P. aeruginosa</i>	2297-con	C+	>128	64	64	>128	>128
<i>P. aeruginosa</i>	2297-def	C-	>128	4	4	8	8

Key: 4-fold reduction in CAZ or PIP MIC \geq 8-fold reduction in CAZ or PIP MIC

Pharmacokinetic and Pharmacodynamic studies

The pharmacokinetics of CAZ and AM-112 in mice were determined following co-administration at 2:1 ratio by iv injection at 3 doses (200:100 mg/kg, 100:50 mg/kg and 20:10 mg/kg). Samples were removed at intervals up to 120 mins and assayed using HPLC.

The time that AM-112 exceeded 4 mg/L was compared to the time that CAZ exceeded the NCCLS breakpoint of 8 mg/L (ie T_{mic}).

RESULTS

AM-112 enhancement of CAZ and PIP in vitro activity

- AM-112 possessed weak antibacterial activity against Enterobacteriaceae (MICs 16-64 mg) and was inactive against *P. aeruginosa* (Tables 1-4).
- Isolates producing Class A, C and D β -lactamases were generally more resistant to CAZ and PIP (Tables 1-4).
- AM-112, at 4 mg/L, reduced PIP and CAZ MICs at least 4-fold and generally at least 8-fold against many Enterobacteriaceae with Class A, C or D β -lactamases (Tables 1-3).
- 16 isolates of Enterobacteriaceae were fully resistant (MIC \geq 32 mg/L) to CAZ. Following addition of AM-112, only 3 isolates displayed full resistance and one isolate was intermediate resistant (MIC 16 mg/L).
- 26 isolates of Enterobacteriaceae were fully resistant (MIC \geq 128 mg/L) to PIP and a further 5 isolates were intermediate resistant (MIC 32-64 mg/L). Following addition of AM-112, 16 isolates displayed full resistance and 5 isolates were intermediate resistant.

Pharmacokinetic and Pharmacodynamic studies

- AM-112 displayed similar pharmacokinetics to CAZ (Figures 3a-c).
- Following iv administration of CAZ and AM-112 in 2:1 combination, AM-112 levels were approximately half CAZ levels over 60 mins.
- CAZ levels exceeded the NCCLS breakpoint of 8 mg/L for 57, 36, and 20 mins following dosing at 200, 100 and 20 mg/kg respectively.
- AM-112 levels exceeded the target dose of 4 mg/L for 38, 22 and 24 mins following dosing at 100, 50 and 10 mg/kg respectively.

CONCLUSIONS

- AM-112 can be synthesised in 7 steps with an overall yield of 16%.
- AM-112 enhances the in vitro activity of both ceftazidime and piperacillin against Enterobacteriaceae producing class A, C and D β -lactamases.
- At 2:1 ratio, combinations of ceftazidime and AM-112 are more effective than piperacillin and AM-112.
- AM-112 and ceftazidime possess similar pharmacokinetics in mice, following iv administration.
- Following dosing at a 2:1 ratio, ceftazidime efficacy is likely to be protected by AM-112 levels of at least 4 mg/L for the whole of the normal dosage period. These results indicate the potential for bid dosing of AM-112 in humans.

* This poster is dedicated to Dr Les White who died this year