Synthesis and Biological Activity of AM-112 and Related Oxapenem Analogues

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Thirty five oxapenem analogues substituted with a range of tertiary groups at C-2 have been synthesised and evaluated as broad-spectrum β-lactamase inhibitors. All analogues enhanced the activity of ceftazidime against bacterial isolates producing Class A and Class C β-lactamases. Compounds with cyclic substituents at C-1′ (attached to C-6) were associated with enhanced antibacterial activity against *Staphylococcus aureus*. (*R*) Stereochemistry at C-1′ led to synergistic activity against β-lactamase negative enterococci. (*S*) Stereochemistry at C-1′ was associated with enhanced inhibition of Class A β-lactamases and lack of synergistic activity against enterococci. AM-113 was unstable in serum and not detectable following subcutaneous or oral dosing in mice. AM-112 and AM-115 achieved good serum levels following subcutaneous dosing. AM-114 exhibited 30% bioavailability following oral dosing. AM-112 [(1′*R*,5*R*,6*R*)-2-(4-ammonio-1,1-dimethylbutyl)-6-(1′-hydroxyethyl)oxapenem-3-carboxylate] achieved the greatest protection of ceftazidime against Gram-negatives producing Class A or C β-lactamases.

β-Lactamase production, particularly Class A and Class C enzymes, is the principal mechanism of β-lactam resistance among Gram-negative bacteria.[1] Although four classes of β-lactamases are recognised, Class A and Class C β-lactamases pose the greatest threat to clinical therapy.

The β-lactamase inhibitors clavulanic acid, tazobactam and sulbactam have proven highly successful in overcoming resistance mediated by commonly encountered Class A β-lactamases, such as TEM-1 and SHV-1, and the less common mutant extended-spectrum β-lactamases (ESBLs).[2] However, many Gram-negative bacteria produce highly inducible or derepressed Class C β-lactamases. Consequently, there is a clinical requirement for β-lactamase inhibitors with a much broader spectrum of activity that includes Class C β-lactamases. Several groups have synthesised compounds which inhibit Class C β-lactamases, but none of these agents has been progressed for clinical use because of reduced activity against Class A β-lactamases, chemical instability, poor pharmacokinetics[3] or difficulty of synthesis.

Oxapenems are a class of molecules with a five-membered, oxygen-containing ring fused to a β-lactam ring with a double bond between C-2 and C-3. Early compounds were either of low[4] or moderate[5] chemical stability. However, PFAENDLER *et al.*[6] achieved enhanced stability by the introduction of a bulky tertiary-alkyl group at C-2. The lead compound from this series, AM-113 (**1a**), possessed antimicrobial activity particularly against staphylococci.[7] Further studies showed that AM-113 and other analogues also possessed broad spectrum β-lactamase inhibitory activity, including potent activity against Class C enzymes. Here we report the chemical synthesis, structure-activity relationships and biological evaluation of oxapenem analogues as broad spectrum β-lactamase inhibitors, culminating in the selection of AM-112 (**1s**) as a lead development candidate.

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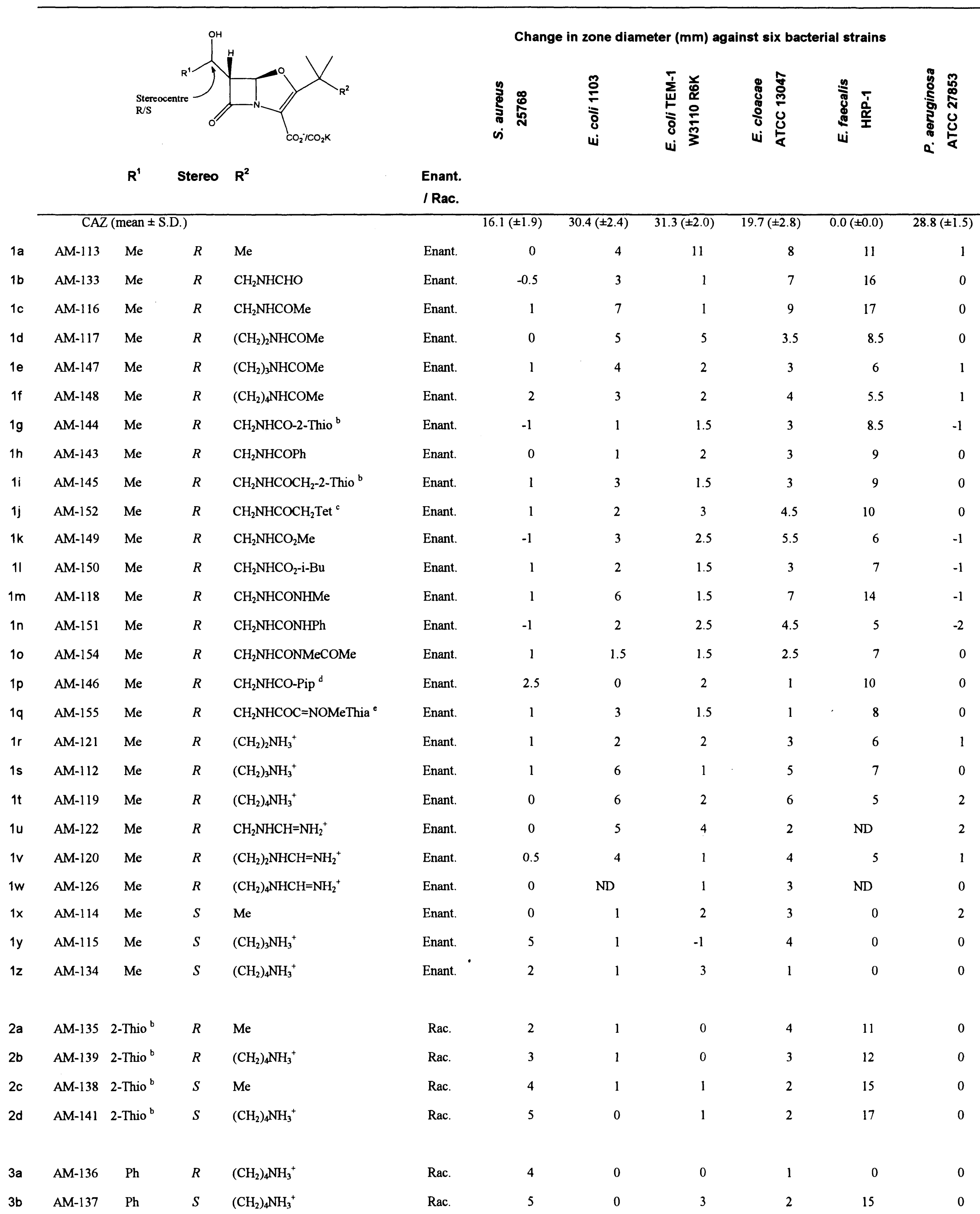
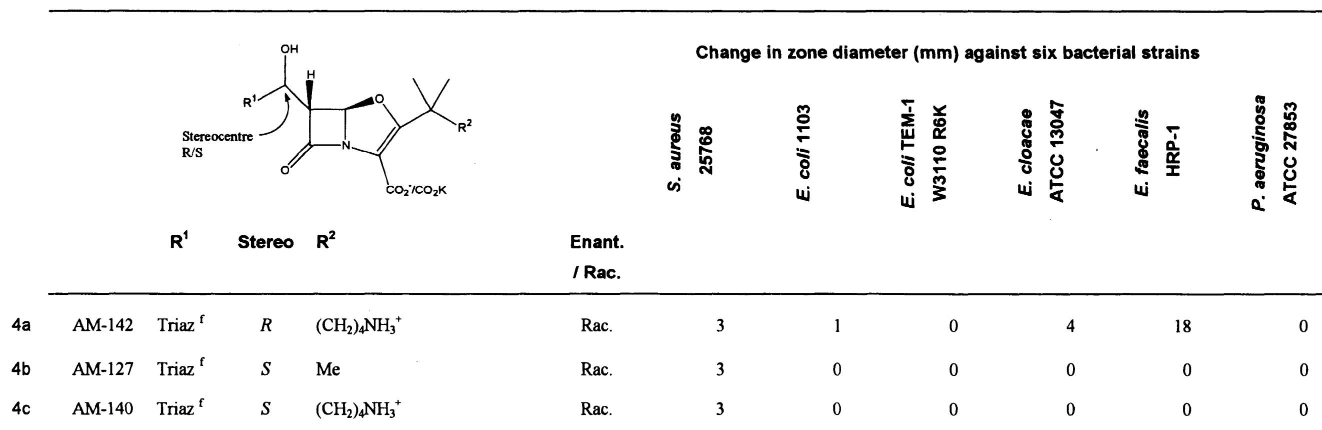
Table 1. Effect of oxapenem analogues (10 μg) upon zone diameter[a] (mm) of ceftazidime (30 μg disk) against six bacterial strains.



		R^1	Stereo	R^2	Enant. / Rac.	Change in zone diameter (mm) against six bacterial strains					
						S. aureus 25768	*E. coli* 1103	*E. coli* TEM-1 W3110 R6K	*E. cloacae* ATCC 13047	*E. faecalis* HRP-1	*P. aeruginosa* ATCC 27853
	CAZ (mean ± S.D.)					16.1 (±1.9)	30.4 (±2.4)	31.3 (±2.0)	19.7 (±2.8)	0.0 (±0.0)	28.8 (±1.5)
1a	AM-113	Me	*R*	Me	Enant.	0	4	11	8	11	1
1b	AM-133	Me	*R*	CH_2NHCHO	Enant.	-0.5	3	1	7	16	0
1c	AM-116	Me	*R*	$CH_2NHCOMe$	Enant.	1	7	1	9	17	0
1d	AM-117	Me	*R*	$(CH_2)_2NHCOMe$	Enant.	0	5	5	3.5	8.5	0
1e	AM-147	Me	*R*	$(CH_2)_3NHCOMe$	Enant.	1	4	2	3	6	1
1f	AM-148	Me	*R*	$(CH_2)_4NHCOMe$	Enant.	2	3	2	4	5.5	1
1g	AM-144	Me	*R*	CH_2NHCO-2-Thio [b]	Enant.	-1	1	1.5	3	8.5	-1
1h	AM-143	Me	*R*	$CH_2NHCOPh$	Enant.	0	1	2	3	9	0
1i	AM-145	Me	*R*	$CH_2NHCOCH_2$-2-Thio [b]	Enant.	1	3	1.5	3	9	0
1j	AM-152	Me	*R*	$CH_2NHCOCH_2Tet$ [c]	Enant.	1	2	3	4.5	10	0
1k	AM-149	Me	*R*	CH_2NHCO_2Me	Enant.	-1	3	2.5	5.5	6	-1
1l	AM-150	Me	*R*	CH_2NHCO_2-i-Bu	Enant.	1	2	1.5	3	7	-1
1m	AM-118	Me	*R*	$CH_2NHCONHMe$	Enant.	1	6	1.5	7	14	-1
1n	AM-151	Me	*R*	$CH_2NHCONHPh$	Enant.	-1	2	2.5	4.5	5	-2
1o	AM-154	Me	*R*	$CH_2NHCONMeCOMe$	Enant.	1	1.5	1.5	2.5	7	0
1p	AM-146	Me	*R*	CH_2NHCO-Pip [d]	Enant.	2.5	0	2	1	10	0
1q	AM-155	Me	*R*	CH_2NHCOC=NOMeThia [e]	Enant.	1	3	1.5	1	8	0
1r	AM-121	Me	*R*	$(CH_2)_2NH_3^+$	Enant.	1	2	2	3	6	1
1s	AM-112	Me	*R*	$(CH_2)_3NH_3^+$	Enant.	1	6	1	5	7	0
1t	AM-119	Me	*R*	$(CH_2)_4NH_3^+$	Enant.	0	6	2	6	5	2
1u	AM-122	Me	*R*	$CH_2NHCH=NH_2^+$	Enant.	0	5	4	2	ND	2
1v	AM-120	Me	*R*	$(CH_2)_2NHCH=NH_2^+$	Enant.	0.5	4	1	4	5	1
1w	AM-126	Me	*R*	$(CH_2)_4NHCH=NH_2^+$	Enant.	0	ND	1	3	ND	0
1x	AM-114	Me	*S*	Me	Enant.	0	1	2	3	0	2
1y	AM-115	Me	*S*	$(CH_2)_3NH_3^+$	Enant.	5	1	-1	4	0	0
1z	AM-134	Me	*S*	$(CH_2)_4NH_3^+$	Enant.	2	1	3	1	0	0
2a	AM-135	2-Thio [b]	*R*	Me	Rac.	2	1	0	4	11	0
2b	AM-139	2-Thio [b]	*R*	$(CH_2)_4NH_3^+$	Rac.	3	1	0	3	12	0
2c	AM-138	2-Thio [b]	*S*	Me	Rac.	4	1	1	2	15	0
2d	AM-141	2-Thio [b]	*S*	$(CH_2)_4NH_3^+$	Rac.	5	0	1	2	17	0
3a	AM-136	Ph	*R*	$(CH_2)_4NH_3^+$	Rac.	4	0	0	1	0	0
3b	AM-137	Ph	*S*	$(CH_2)_4NH_3^+$	Rac.	5	0	3	2	15	0

Table 1. (Continued)



		R^1	Stereo	R^2	Enant. / Rac.	*S. aureus* 25768	*E. coli* 1103	*E. coli* TEM-1 W3110 R6K	*E. cloacae* ATCC 13047	*E. faecalis* HRP-1	*P. aeruginosa* ATCC 27853
4a	AM-142	Triaz [f]	*R*	$(CH_2)_4NH_3^+$	Rac.	3	1	0	4	18	0
4b	AM-127	Triaz [f]	*S*	Me	Rac.	3	0	0	0	0	0
4c	AM-140	Triaz [f]	*S*	$(CH_2)_4NH_3^+$	Rac.	3	0	0	0	0	0

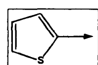
Change in zone diameter (mm) against six bacterial strains

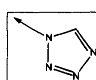
If R^2 is positively charged the compound is a zwitterion. If R^2 is neutral the compound is a potassium salt.

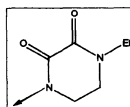
For compounds **2(a~d)**, **3(a** and **b)** and **4(a~c)** the absolute stereochemistry is the opposite of that shown in Table 1. However, for consistency within Table 1, that shown assumes that R^1 has lowest priority and is hence comparable to all other compounds in the Table.

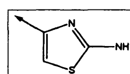
The compounds of Table 1 are either single enantiomers (Enant.) or a racemic mixture (Rac.).

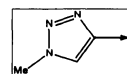
[a]Zone diameter determined using disc diffusion (IsoSensitest agar swabbed with a culture of each bacterial strain adjusted to an inoculum density corresponding to a 0.5 McFarland standard).

[b]2-Thio 

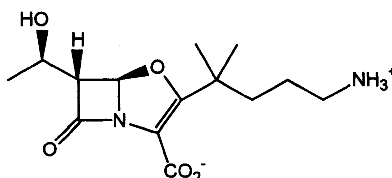
[c]Tet 

[d]Pip 

[e]Thia 

[f]Triaz 

ND not determined.



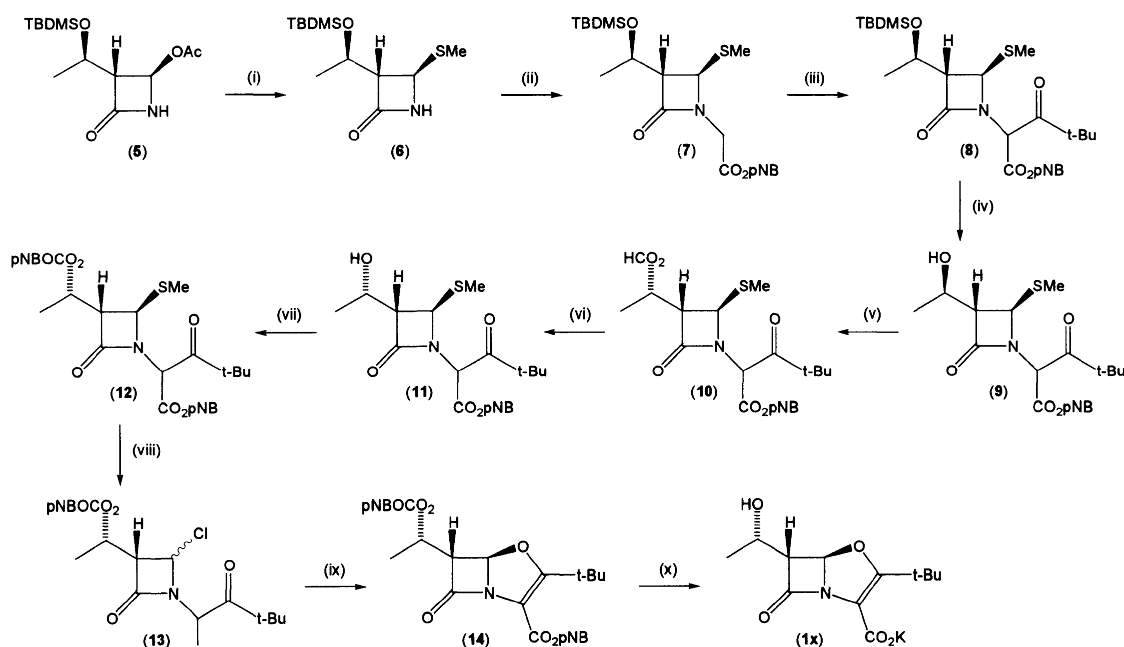
AM-112 (**1s**)

This paper contains results reported at the 41st and 42nd Interscience Conferences on Antimicrobial Agents and Chemotherapy.[8~12]

Synthesis

As an example of the preparation of the compounds in Table 1, the synthesis of AM-114 (**1x**) is shown in Scheme 1. The commercially available azetidinone (**5**) was converted to the methylthio analogue (**6**) by treatment with sodium methylmercaptide. This was alkylated by deprotonation with *n*-butyl lithium and reaction with *p*-nitrobenzyl iodoacetate to give the ester (**7**), which was in turn acylated by treatment with lithium bis(trimethylsilyl)amide and pivaloyl chloride to give the β-ketoester (**8**). The next stage was to invert the configuration of the hydroxyethyl group. This was achieved by way of a Mitsunobu reaction in which the alcohol (**9**) was converted to the formate ester (**10**), which was then selectively hydrolysed with hydrochloric acid in methanol and water to give the alcohol of the opposite stereochemistry (**11**). After protection of the alcohol as the *p*-nitrobenzyl carbonate (**12**) the azetidinone was activated for ring closure as the chloro analogue (**13**). When this compound was treated

Scheme 1. The Synthesis of AM-114 (**1x**).



Reagents: (i) NaSMe, CH_3CN, 15-20°C, 1 hour; (ii) n-BuLi, THF, -40°C, 1 hour then p-nitrobenzyl iodoacetate, THF, -10°C, 2 hours; (iii) $LiN(SiMe_3)_2$, pivaloyl chloride, THF, -70°C, 10 minutes; (iv) CH_3CO_2H, Bu_4NF, THF, 90°C, 1.5 hours; (v) diisopropyl azodicarboxylate, Ph_3P, HCO_2H, THF, 0°C, 2 hours; (vi) HCl, MeOH, H_2O, 0°C to room temperature, overnight; (vii) p-nitrobenzyl chloroformate, DMAP, CH_2Cl_2, -12°C, 3.5 hours; (viii) Cl_2, CH_2Cl_2, -40°C, 10 minutes; (ix) KOt-Bu, THF, -30°C, 1.5 hours; (x) H_2, Pd/C, EtOAc, 0°C, 40 minutes then $KHCO_3$, H_2O, 0°C, 5 minutes.TBDMS: t-butyldimethylsilyl
pNB: p-nitrobenzyl

with a base ring-closure occurred to give the oxapenem (**14**) in which the *trans* isomer shown was the major product (typically *trans*:*cis* was 2:1). Finally, hydrogenolysis led to a double deprotection of the alcohol and acid to give AM-114 (**1x**). Other compounds shown in Table 1 were made by similar methods.[6,7,13~17)]

Results and Discussion

A total of 35 oxapenems were synthesised and screened for synergy with ceftazidime (CAZ), using disc diffusion, against six strains representing a range of bacterial species (Table 1). These data allowed the following general structure activity relationships to be made. Within series 1 (R^1=Me, **1a**~**z**) when the (*R*) stereochemistry is present, all substitutions at R^2 enhanced the activity of ceftazidime to a similar extent against *Escherichia coli* and *Enterobacter cloacae* strains producing Class A and Class C β-lactamases respectively and surprisingly against *Enterococcus faecalis* HRP-1 a β-lactamase negative isolate. None of the analogues enhanced ceftazidime activity against *Pseudomonas aeruginosa* ATCC 27853. The synergistic activity against *E. faecalis* HRP-1 was lost in series 1 when the (*S*) stereochemistry was present (**1x**~**z**).

Introduction of cyclic groups at R^1 (series 2, 3 and 4) generated analogues which markedly enhanced ceftazidime zone diameter against *S. aureus* 25768, but had no beneficial effect against the Gram-negative strains. The requirement for (*R*) stereochemistry when R^1=Me (series 1) to achieve activity was not apparent in these series. When R^1 was a cyclic group, both (*R*) and (*S*) configurations exhibited synergistic interactions with ceftazidime against *E. faecalis*.

Eleven analogues from series 1, representing both salts and zwitterionic compounds, were selected for further evaluation as β-lactamase inhibitors. When tested alone, the analogues exhibited good activity against *S. aureus* (MICs 0.5~4 μg/ml) but generally poor or no activity against other bacterial species (Table 2).

The potential of the selected analogues to enhance ceftazidime activity was confirmed in MIC tests against isolates producing defined β-lactamases (Table 3). AM-112

Table 2. Antibacterial activity (MIC μg/ml[a]) of selected oxapenem analogues against a range of β-lactamase producing pathogens.

Strain	No. of isolates	1s AM-112	1a AM-113	1x AM-114	1y AM-115	1c AM-116	1d AM-117	1m AM-118	1t AM-119	1v AM-120	1r AM-121	1u AM-122
Escherichia coli	16	32	4-16	>32	>32	8->32	4->32	4->16	4->32	4-32	>16	1->4
AmpC-inducible enterics[b]	8	16->32	16->32	>32	>32	16->32	16->32	8->16	16->32	4->32	>16	≥ 4
Pseudomonads[c]	7	>32	>32	>32	>32	>32	>32	>16	>32	>32	>16	>4
Acinetobacter spp.	1	>32	>32	>32	>32	>32	>32	>16	>32	>32	>16	>4
Enterococcus spp.	8	>32	>32	>32	>32	>32	>32	>16	>32	>32	>16	>4
MSSA[d]	3	2	0.5	1	4	4	1-2	4	1	1	4	2
MRSA[e]	5	>32	16-32	>32	>32	>32	>32	>16	≥ 32	>32	>16	>4
Clostridium spp.	3	4-16	4-8	>32	1->64	2-32	1-8	8->64	16->64	4->32	8-32	>4
Bacteroides spp.	4	8-16	8	32	>8	8-16	4	ND	ND	>32	≥ 32	>4

[a]Tested in Mueller Hinton broth with an inocula of c. 10^5 cfu/ml.

[b]*Enterobacter, Citrobacter, Serratia* and *Morganella* spp.

[c]*Pseudomonas aeruginosa, Pseudomonas fluorescens, Burkolderia cepacia* and *Stenotrophomonas maltophilia.*

[d]Methicillin susceptible *Staphylococcus aureus.*

[e]Methicillin resistant *Staphylococcus aureus.*

and AM-113 were selected as the analogues exhibiting the greatest activity against isolates having Class C β-lactamases whilst also retaining excellent activity against most Class A producers. The AM-112 and AM-113 (*S*) stereochemical analogues AM-115 and AM-114 exhibited poorer activity against Class C strains but enhanced activity against Class A strains. Combinations of (*R*) and (*S*) stereoisomers (*i.e.* AM-112 and AM-115, or AM-113 and AM-114) were more potent than individual component analogues indicating the need for different stereoisomers at C-1′ to inhibit Class A and Class C β-lactamases.

The unexpected but C-1′ stereospecific synergy against *E. faecalis* was also confirmed in MIC tests (Table 4). Further tests (not shown) showed that this synergy extended to *Enterococcus casseflavus* and *Enterococcus gallinarum* but was less evident against *Enterococcus faecium.*

AM-112, AM-113, AM-114 and AM-115 exhibited potent broad spectrum inhibition of isolated Class A, C and D β-lactamases (Table 5). Contrary to results from MIC tests, the (*S*) stereoisomers AM-114 and AM-115 were the most potent analogues, suggesting that other factors such as enhanced penetration might contribute to the greater β-lactamase inhibitory activity of the zwitterionic compounds AM-112 and AM-115 against cellular enclosed enzymes.

Following subcutaneous dosing at 50 mg/kg in mice, detectable serum levels of the zwitterions AM-112 and AM-115 were achieved for 30 minutes (Figure 1). Lower levels were observed for the non zwitterionic analogue of AM-115 (*i.e.* AM-114), but the corresponding analogue of AM-112 (*i.e.* AM-113) was not detectable. In contrast, following oral dosing, only AM-114 gave detectable serum levels, indicating approximately 30% bioavailability (Figure 2). In serum stability studies, HPLC analysis showed no loss of AM-112, AM-114 or AM-115 following incubation for 60 minutes in human and marmoset sera and only *ca.* 20% in mouse sera. In contrast, AM-113 exhibited marked losses in all sera types. The pH stability of AM-112 ranged from 0% loss at pH 6.5 to 28.4% loss at pH 8.5, over 6 hours.

The results of subsequent murine infection models confirmed the ability of AM-112 to enhance the activity of ceftazidime against infections caused by *S. aureus* or Gram-negative Class A or C β-lactamase producing bacteria.

Table 3. MIC (μg/ml[a]) of ceftazidime (CAZ) alone and in the presence of oxapenem analogues (4 μg/ml) against a range of β-lactamase producing ceftazidime resistant organisms.

			1s	1a	1x	1y	1c	1d	1m	1t	1v	1r	1u	1s+1y	1a+1x
β-lactamase class	Strain	CAZ alone	AM-112	AM-113	AM-114	AM-115	AM-116	AM-117	AM-118	AM-119	AM-120	AM-121	AM-122[b]	AM-112 + AM-115[c]	AM-113 + AM-114[c]
A	*E. coli* TEM-3	32	1	1	0.5	2	0.5	0.5	2	2	4	4	0.25	1	1
	E. coli TEM-6	>64	>64	>64	8	64	32	16	>64	>64	16	>64	64	32	8
	E. coli TEM-9	>64	4	>64	16	64	>64	32	>64	>64	16	>64	>64	>64	32
	E. coli TEM-10	>64	>64	64	1	2	16	>64	32	16	4	64	2	1	2
	E. coli SHV-2	16	4	2	1	0.25	16	8	2	4	4	8	0.5	0.25	1
	E. coli SHV-5	>64	>64	>64	32	64	>64	>64	>64	>64	32	>64	>64	>64	32
B	*E.coli* IMP-1	32	32	32	32	64	64	64	32	16	64	32	32	64	16
C	*C. freundii* (C10-con)	>64	0.5	2	2	8	64	32	16	0.25	4	64	8	8	4
	E. cloacae (P99)	>64	1	4	16	8	>64	>64	64	4	4	64	8	1	8
	E. cloacae (Hennessey)	>64	16	>64	>64	64	>64	>64	>64	>64	4	>64	64	16	>64
	M. morganii (M1-con)	16	0.12	≤0.06	0.12	0.12	0.5	1	0.25	0.5	4	0.25	2	0.12	≤0.06
	B. fragilis	64	0.12	ND	1	1	1	0.12	0.5	ND	1	1	2	0.5	0.5
D	*E. coli* OXA-5	>64	>64	16	2	2	16	32	>64	32	16	>64	64	8	1

[a]Tested in Mueller Hinton broth with an inocula of c. 10^5 cfu/ml.

[b]tested at 1 μg/ml.

[c]2 μg/ml of each stereoisomer, total concentration of 4 μg/ml.

ND not determined.

CAZ ceftazidime.

Table 4. MIC (μg/ml[a]) of ceftazidime (CAZ) alone and in the presence of selected oxapenem analogues at 2 : 1 ratio against *E. faecalis*.

Organism	CAZ	CAZ + AM-112 (1s)	CAZ + AM-115 (1y)	CAZ + AM-113 (1a)	CAZ + AM-114 (1x)
E. faecalis SFZ	64	8	64	8	64
E. faecalis NCTC 10541	>64	16	64	8	>64
E. faecalis NCTC 5957	32	8	32	8	16
E. faecalis ATCC 29212	32	8	16	8	16
E. faecalis NCTC 7171	>64	32	>64	16	32
E. faecalis *VanA*	>64	32	>64	32	>64
E. faecalis *VanB*	32	8	32	8	16

[a]Tested in Mueller Hinton broth with an inocula of c. 10^5 cfu/ml.

Spectral data

AM-112 (**1s**): UV λmax nm 262 (ε = 5,000); ^{1}H NMR (400 MHz, D_2O) 5.82 (1H, br s, C*H*N), 4.26 (1H, m, C*H*OH), 3.80 (1H, dd, J = 5.2, 0.8 Hz, C*H*C=O), 2.98 (2H, br t, J = 7.2 Hz, $CH_2NH_3^+$), 1.8 – 1.6 (4H, m, $CH_2CH_2C(CH_3)_2$), 1.33 (3H, d, J = 6.4 Hz, CH_3CHOH), 1.25 (6H, s, $C(CH_3)_2$).

AM-113 (**1a**): See Reference 7.

AM-114 (**1x**): UV λmax nm 260 (ε = 5,000); ^{1}H NMR (400 MHz, D_2O) 5.74 (1H, s, C*H*N), 4.28 (1H, m, C*H*OH), 3.83 (1H, d, J = 4.4 Hz, C*H*C=O), 1.33 (3H, d, J = 6.5 Hz, CH_3CHOH), 1.25 (9H, s, $C(CH_3)_3$).

AM-115 (**1y**): UV λmax nm 262 (ε = 5,000); ^{1}H NMR (400 MHz, D_2O) 5.75 (1H, s, C*H*N), 4.28 (1H, m, C*H*OH), 3.83 (1H, d, J = 4.4 Hz, C*H*C=O), 2.98 (2H, br t, J = 7.1 Hz, $CH_2NH_3^+$), 1.8 – 1.6 (4H, m, $CH_2CH_2C(CH_3)_2$), 1.33 (3H, d, J = 6.5 Hz, CH_3CHOH), 1.25 (6H, s, $C(CH_3)_2$).

Table 5. IC_{50}[a] values (μg/ml) of clavulanic acid (CLAV) and four oxapenem compounds against β-lactamases[b] isolated from eight bacterial strains.

Strain	CLAV	AM-112 (1s)	AM-113 (1a)	AM-114 (1x)	AM-115 (1y)
Class A β-lactamases					
E. coli TEM-1	0.024	0.67	0.93	0.0005	0.05
E. coli TEM-10	0.006	0.02	0.0021	0.0015	0.0015
E. coli SHV-5	0.0016	0.048	0.029	0.003	0.017
Class C β-lactamases					
E. cloacae P99	3.36	0.0006	0.0005	0.0003	0.004
S. marcescens S2	67	0.02	0.0006	0.0014	0.027
P. aeruginosa S+A	92	0.0006	0.015	0.00004	0.006
Class D β-lactamases					
E. coli OXA-1	20.3	0.0015	0.0001	0.0007	0.019
E. coli OXA-5	41.5	0.0002	0.0002	0.0006	0.0016

[a]CLAV and oxapenems were pre-incubated with the β-lactamase for 15 minutes prior to spectrophotometric determination of the IC_{50} using nitrocefin as the substrate.[18)]
[b]β-lactamases purified and their singularity confirmed using preparative and analytical isoelectric focussing.[19, 20)]

Fig. 1. Pharmacokinetics of oxapenem analogues following subcutaneous dosing at 50 mg/kg (mean of three mice per time point).

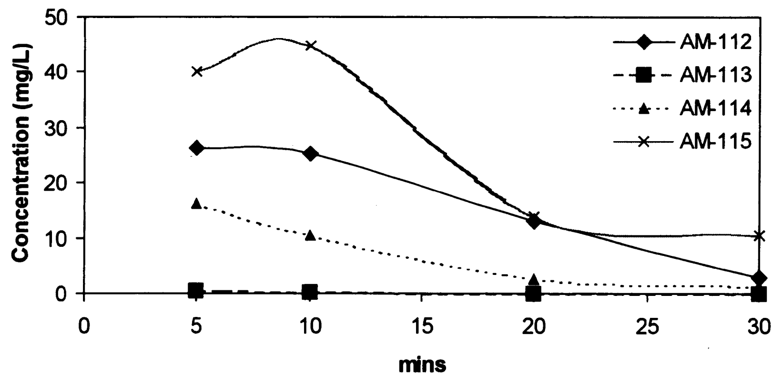


Fig. 2. Pharmacokinetics of oxapenem analogues following oral dosing at 50 mg/kg (mean of three mice per time point).

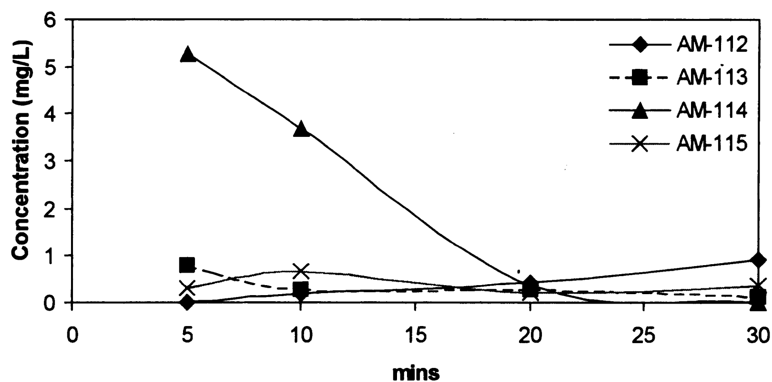


Table 6. ED_{50} values for combinations of ceftazidime (CAZ) and AM-112 (**1s**) against various pathogens in a murine intraperitoneal sepsis model.

Strain (inoculum [CFU/ml])	Compound	MIC (μg/ml)[d]	ED_{50} (mg/kg)[e]	95% CI[f]
S. aureus 3816[a]				
(8×10^6)	CAZ	16	22.6	19.4 - 26.4
(8×10^6)	AM-112	1	2.6	2.2 - 3.1
(8×10^6)	CAZ:AM-112 (4:1)	ND	4.8 + 1.2	4.0 - 5.8
(8×10^6)	CAZ:AM-112 (7:1)	ND	7 + 1	6.6 - 9.7
E. cloacae P99[b]				
(2.2×10^7)	CAZ	128	>100	ND
(2.2×10^7)	AM-112	32	19	11.6 - 33.4
(2.2×10^7)	CAZ:AM-112 (1:1)	2 + 2	2 + 2	1.0 - 5.5
(2.2×10^7)	CAZ:AM-112 (2:1)	4 + 2	3.8 + 1.9	2.3 - 5.9
(2.2×10^7)	CAZ:AM-112 (4:1)	4 + 1	11.6 + 2.9	7.2 - 17.4
K. pneumoniae SHV-5[c]				
(6×10^8)	CAZ	128	>160	ND
(6×10^8)	AM-112	16	>40	ND
(6×10^8)	CAZ:AM-112 (1:1)	16 + 16	33.6 + 33.6	25.1 - 45.1
(6×10^8)	CAZ:AM-112 (2:1)	32 + 16	23.8 + 11.9	18.8 - 30.1

Antimicrobials were administered by subcutaneous (*S. aureus* 3816 and *K. pneumoniae* SHV-5) or intravenous (*E. cloacae*) injection.

[a]0.1ml of *S. aureus* 3816 suspension was inoculated intraperitoneally into male ICR mice (20-22g Harlan Sprague Dawley, Indianapolis, ten mice per antimicrobial dose). ED_{50} was calculated from the survival rate at four days by the Spearman Kärber method.

[b]0.1ml of *E. cloacae* P99 suspension was inoculated intraperitoneally into male CD1 mice (20-22g Harlan Sprague Dawley, Indianapolis, five mice per antimicrobial dose).

[c]0.1ml of *K. pneumoniae* SHV-5 suspension was inoculated intraperitoneally into female ICR mice (20-22g Harlan Sprague Dawley, Indianapolis, five mice per antimicrobial dose).

[d]MICs quoted for CAZ:AM-112 combinations are expressed as CAZ + AM-112 MIC.

[e]ED_{50} values for CAZ:AM-112 combinations are expressed as CAZ + AM-112 ED_{50} values.

[f]95% CI, confidence intervals for combinations, only CAZ limits shown.

ND not determined.

Conclusions

Previous studies have shown that chemical instability problems associated with early oxapenem compounds[4,5] can be overcome by the introduction of a bulky tertiary-alkyl group at C-2.[6] The lead compound AM-113 from these studies not only exhibited antibacterial activity against *S. aureus* but also potent inhibitory activity against Class A and C β-lactamases in disk diffusion tests. In the

current study, we have investigated the structure activity relationships of AM-113 and an additional 34 oxapenem analogues.

All analogues enhanced the activity of ceftazidime against representative bacterial strains producing Class A or C β-lactamases. Modifications of R^2 (C-2 substituent) had no marked effect upon β-lactamase inhibitory or antibacterial activity. In contrast, inversion of stereochemistry at C-1′ not only impacted the ratio of Class A: Class C β-lactamase inhibition but also synergistic antibacterial activity with ceftazidime against *Enterococcus* spp. and pharmacokinetic properties. AM-113 was unstable in serum and gave very low blood levels in pharmacokinetic studies in mice. AM-114, the (1′*S*) epimer of AM-113, was stable in serum, exhibited 30% oral bioavailability but inferior blood levels to those of the zwitterionic compounds AM-112 and AM-115. AM-112 ((1′*R*) stereoisomer) offers advantages over its (1′*S*) epimer AM-115 as a development compound on the basis of ease of synthesis, enhanced activity against Class C β-lactamases and synergistic antibacterial activity with ceftazidime against *Enterococcus* spp.

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References

1) Livermore, D. M.: β-Lactamases in laboratory and clinical resistance. Clin. Microbiol. Rev. 8: 557~584, 1995
2) Bradford, P. A.: Extended-spectrum β-lactamases in the 21st century: characterisation, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev. 14: 933~951, 2001
3) Maiti, S. N.; O. A. Phillips, R. G. Micetich & D. M. Livermore: β-Lactamase inhibitors: agents to overcome bacterial resistance. Curr. Med. Chem. 5: 441~456, 1998
4) Cherry, P. C.; C. E. Newall & N. S. Watson: Preparation of the 7-oxo-4-oxa-1-azabicyclo[3.2.0]hept-2-ene system and the reversible cleavage of its oxazoline ring. J. Chem. Soc., Chem. Commun. 1978: 469~470, 1978
5) Murakami, M.; T. Aoki, M. Matsuura & W. Nagata: Synthesis and biological properties of 1-oxapenems. J. Antibiotics 43: 1441~1449, 1990
6) Pfaendler, H. R.; W. Hendel & U. Nagel: Stable oxapenem-3-carboxylic acids – a new class of β-lactam antibiotics. Influence of 2- and 6-alkyl substituents. Z. Naturforsch., B: Chem. Sci. 47: 1037~1049, 1992
7) Pfaendler, H. R.; F. Weisner & K. Metzger: Synthesis and antibacterial activity of (1′*R*,5*R*,6*R*)-2-*tert*-butyl-6-(1′-hydroxyethyl)oxapenem-3-carboxylic acid. Bioorg. Med. Chem. Lett. 3: 2211~2218, 1993
8) Livermore, D. M.; L. Tysall, M. Warner & I. N. Simpson: Oxapenem analogues as antibiotics and broad spectrum β-lactamase inhibitors. Abstracts of the 41st Intersci. Conf. on Antimicrob. Agents Chemother., September and December 2001, page 212
9) Jamieson, C. E.; P. A. Lambert & I. N. Simpson: Protection of ceftazidime against class A and class C β-lactamases by oxapenems. Abstracts of the 41st Intersci. Conf. on Antimicrob. Agents Chemother., September and December 2001, page 213
10) Simpson I. N.; E. Di Modugno, D. Jabes & D. Nicolau: Oxapenem AM-112: *in vivo* efficacy alone and in combination with ceftazidime in animal models of infection, involving pathogens producing class A and class C β-lactamases. Abstracts of the 41st Intersci. Conf. on Antimicrob. Agents Chemother., September and December 2001, page 213
11) White L. O.; L. Brevik, R. Owens, I. Simpson, M. Cavaleri & D. Jabes: AM-112, a novel enhanced-spectrum oxapenem β-lactamase inhibitor: oral and parental pharmacokinetics (pk), with and without ceftazidime (TAZ), in rats and mice. Abstracts of the 41st Intersci. Conf. on Antimicrob. Agents Chemother., September and December 2001, page 213
12) Simpson I. N.; C. J. Urch, D. M. Livermore, C. Brunati, M. Cavaleri & L. O. White: Broad-spectrum β-lactamase inhibitor AM-112: Synthesis and enhancement of the *in vitro* activity of ceftazidime and piperacillin. Abstracts of the 42nd Intersci. Conf. on Antimicrob. Agents Chemother., September 2002, page 177
13) Pfaendler, H. R.; T. Neumann & R. Bartsch: Synthesis and biological activity of enantiomeric oxapenemcarboxylic acids. Synthesis. 11: 1179~1184, 1992
14) Pfaendler, H. R. & W. Hendel: Preparation of stable oxapenemcarboxylates as antibacterial agents. EP 301 394, July 20, 1988
15) Pfaendler, H. R.; K. G. Metzger, R. Endermann, I. Haller, H. Wild & W. Hartwig: Preparation and formulation of oxapenemcarboxylates as β-lactamase inhibitors. EP 362 622, September 21, 1989
16) Pfaendler, H. R.: Preparation of 6-[(1*S*)-1-hydroxyalkyl]oxapenem-3-carboxylic acids and their use as β-lactamase inhibitors. EP 548 790, December 17, 1992
17) Pfaendler, H. R. & I. N. Simpson: Pharmaceutical compositions containing oxapenem-3-carboxylic acids. WO 02/32423, October 11, 2001
18) O'Callaghan, C. H.; P. W. Muggleton & G. W. Ross: Effects of β-lactamase from gram-negative organisms on cephalosporins and penicillins. Antimicrob. Agents Chemother. 8: 57~63, 1968
19) Simpson, I. N.; S. J. Plested, M. J. Budin-Jones, J. Lees, R. W. Hedges & G. A. Jacoby: Characterization of a novel plasmid-mediated β-lactamase and its contribution

to β-lactam resistance in *Pseudomonas aeruginosa.* FEMS Microbiol. Lett. 19: 23~27, 1983

20) MATTHEW, M.; A. M. HARRIS, M. J. MARSHALL & G. W. ROSS: Use of analytical isoelectric focusing for detection and identification of β-lactamases. J. Gen. Microbiol. 88: 169~178, 1975