Eur J Med Chem (1992) 27, 131–140

Synthesis and antibacterial activity of C4 substituted monobactams

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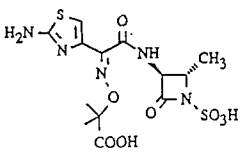
(Received 28 May 1991; accepted 19 September 1991)

Summary — The synthesis and antibacterial activity of a series of monobactams having various substituents at C4 position is described. An efficient route to versatile intermediates **4a–c** from 6-aminopenicillanic acid (6-APA) has been developed. Derivatives **10e**, **11**, **21** showed good to moderate activity against Gram-negative bacteria with the exception of *Pseudomonas aeruginosa*. Introduction of a catechol moiety on the C4 side chain only slightly improved the activity against *P aeruginosa*.

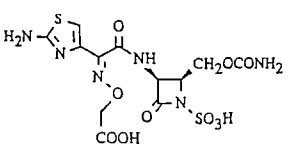
monobactam / catechol / antibacterial activity / azetidinone / beta-lactam

Introduction

Since the discovery of monobactams by Takeda and Squibb in 1981 [1, 2], a considerable number of chemical modifications have been reported. The substitution of the C4 position by alkyl groups has been studied and has led to compounds with improved antibacterial activity against Gram-negative bacteria and better stability against β-lactamases [3–7]. Aztreonam [8] and carumonam [7], which display excellent antimicrobial activity, have been developed for clinical use. However, both show weak activity against Gram-positive bacteria.



AZTREONAM



CARUMONAM

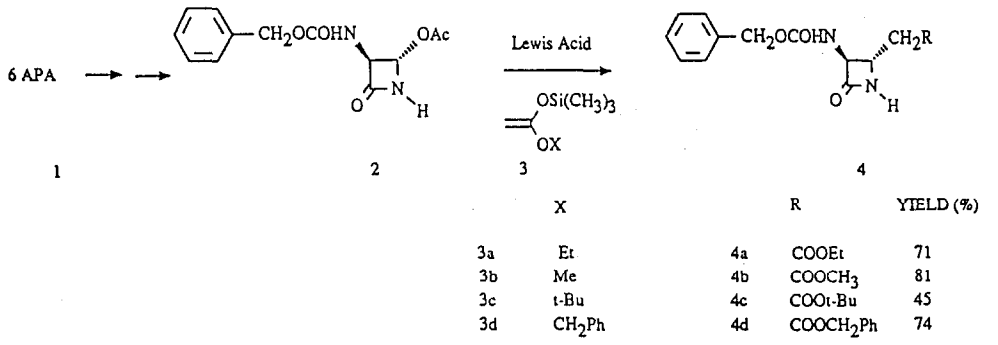
In an attempt to investigate structural factors governing these deficiencies, and also to improve the overall Gram-negative activity, monobactams bearing new C4 functionalised alkyl substituents were synthesised. It was anticipated that by introduction of various chemical functions, the physico-chemical properties of the molecule could be altered and that specific interactions with target enzymes could be achieved. In this paper we describe the synthesis of a series of *trans* C4 alkyl substituted monobactams from the readily available 4-acetoxy azetidinone **2**. Their antibacterial activity is compared with that of aztreonam.

Chemistry

The strategy for the preparation of the title compounds involved the initial introduction of an acetic acid residue at the C4 position followed by further conversion of this acid into various functions. A synthesis of the key intermediates **4a–d** was achieved as outlined in scheme 1.

A crucial step in the synthesis of **4** is the formation of the C-C bond at C4. Among the various methods of C4 alkylation of the readily available 4-acetoxyazetidinone **2**, organosilicon chemistry seemed to be the most attractive: Lewis acid catalysed addition of silyl enol ethers or *O*-silyl ketene acetals to 4-acetoxyazetidinone results in efficient carbon–carbon bond formation and has been successfully applied to the synthesis of carbapenems [9–12]. *O*-Silyl ketene acetals **3** [13–15] were therefore prepared using standard procedures and reacted with **2** (obtained from 6-APA) to give stereoselectively *trans* β-lactam azetidinones **4** in yields ranging from 45 to 81% (scheme 1).

Among various Lewis acids (ZnI_2, TMSTf, $TiCl_4$, $ZnCl_2$, $Zn(OAc)_2$), zinc acetate in toluene was found to be the optimum choice and also the most convenient to handle. Under these conditions, the reaction was readily carried out on a 30 g scale.



	X		R	YIELD (%)
3a	Et	4a	COOEt	71
3b	Me	4b	$COOCH_3$	81
3c	t-Bu	4c	COOt-Bu	45
3d	CH_2Ph	4d	$COOCH_2Ph$	74

Scheme 1.

Synthesis of ***10a, 10e, 11, 12a***

The preparation of ester and acid derivatives **10a**, **10e**, **12a**, from key intermediates **4a–c** (scheme 2) involved the deprotection of the C3 amino group (by catalytic hydrogenation), introduction of the oximino aminothiazole side-chain (in the presence of EEDQ), sulfonation of the N1 nitrogen (with the pyridine–SO_3 complex) and final acidic deprotection.

The amide derivative **11** was prepared from methyl ester **4b** which, after saponification with K_2CO_3, reacted with *t*-butylglycine ester to give azetidinone **4f** which was taken through to **11** by the sequence of reactions previously described for the preparation of **10a**.

Synthesis of the amide derivatives ***16–25***

A more convergent route was investigated. Sulfonation at an early stage was considered to be more practical; furthermore, protection of the C4 acid function by a group readily cleaved by hydrogenolysis (the conditions used for the deprotection of the C3 amino group) was also investigated, as outlined in scheme 3.

Benzyl protected azetidinone **4d** was therefore prepared and sulfonated with the sulfur trioxide–pyridine complex, followed by treatment with potassium phosphate buffer to give **13** in good yield.

Hydrogenolysis of the protecting groups followed by acylation with the appropriate acid chloride afforded **15**.

Functionalisation of the C4 acid was achieved by reaction with the appropriate amine in the presence of isobutylchloroformate as the coupling agent. After acid treatment, the final compounds were purified by preparative HPLC.

Pharmacological results

Effect of the C4 substituent in the C3 neutral oximino series (table I)

The results of *in vitro* antibacterial evaluation are summarised in table I. The MIC values of 4-alkyl substituted monobactams against *Staphylococcus aureus* and a variety of Gram-negative bacteria were determined by the agar dilution method; aztreonam was used as the reference compound.

Most of the compounds showed moderate to good antibacterial activity against Gram-negative bacteria with the exception of *Pseudomonas aeruginosa*. Among them **10e**, **11**, **21** were found to be the most active although they were not as potent as aztreonam. They were ineffective against Gram-positive bacteria (results not reported) which is consistent with previous findings for monobactam derivatives [3].

The introduction of a positive charge at C4 was anticipated to increase the Gram-negative activity, an effect which is frequently observed in the cephalosporins [16, 17]. However the potency of **18**, **19**, **20** (charged at physiological pH) is comparable with that of the uncharged analogues.

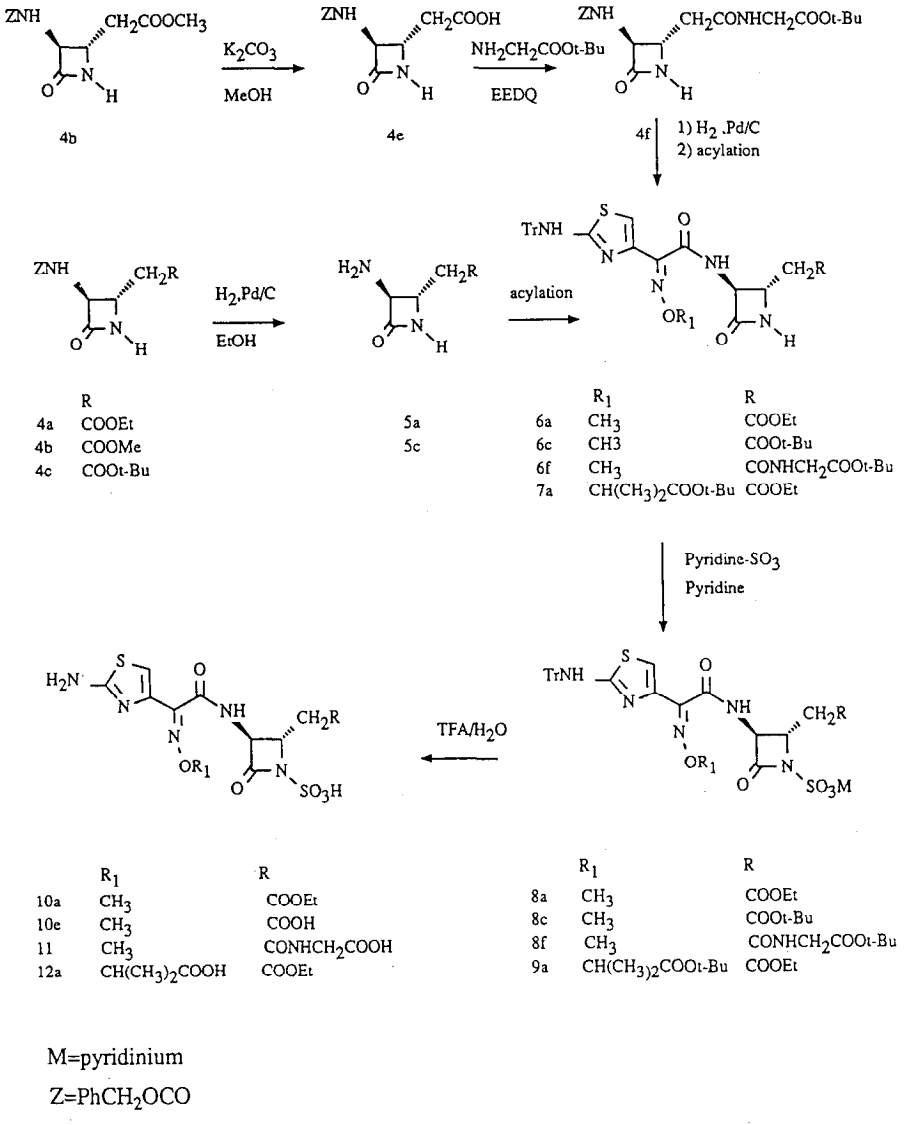
Effect of the C3 side chain (table II)

Increased activity against *P aeruginosa*, in addition to other Gram-negative organisms is usually observed in the monobactam series, when the methyl group on the oximino C3 side chain is replaced by an isobutyric acid residue. In our series, the activity against *P aeruginosa* was not affected by such a replacement but the β-lactamase stability was increased especially with regard to β-lactamase producers *E cloacae* $P99^+$ and *K oxytoca* $K1^+$.

Effect of the incorporation of a catechol residue

β-Lactam antibiotics variously substituted with catechol or related isosteres show exceptionally good activity against *P aeruginosa* and other Gram-negative bacteria. It has been found that a *tonB*- dependent illicit transport route was responsible for the enhanced antibacterial activity [18].

In the monobactam series, introduction of a catechol or hydroxy pyridone moiety has led to compounds with improved *in vitro* activity [19–21]. In order to test the properties of C4 catechol substituted monobactams, synthesis of compounds **24** and **25**



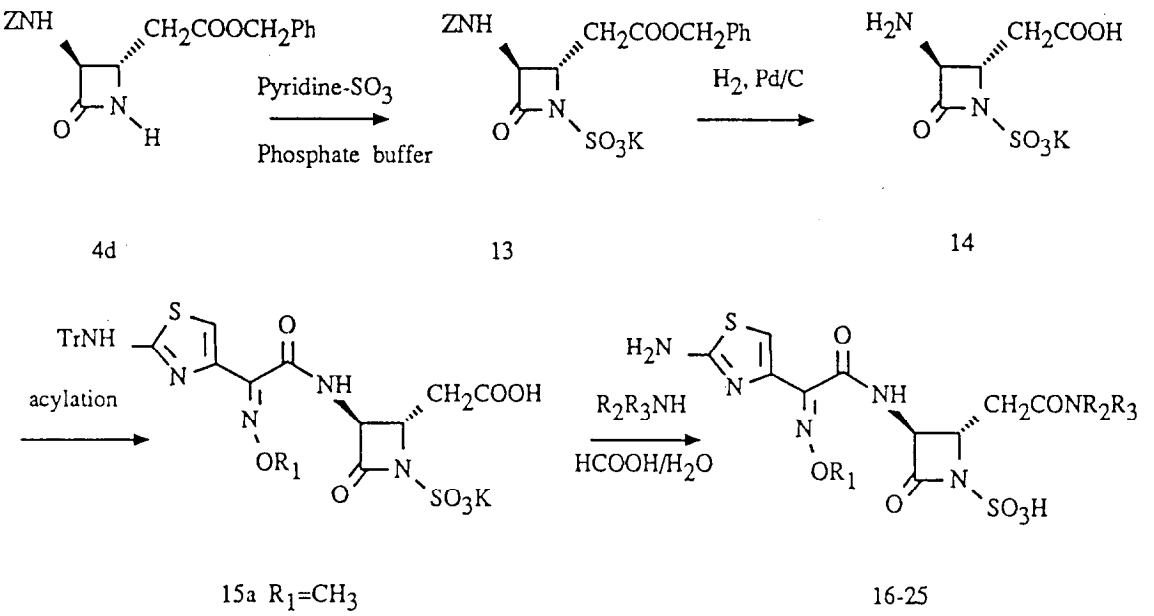
Scheme 2.

incorporating this function on the C4 side-chain was undertaken. The antimicrobial properties of **24** and **25** are compared with **12a** in table III.

In contrast with reported results, the activity against Gram-negative bacteria including *P aeruginosa* was only slightly improved. This improvement can be explained by a better penetration of the molecule through the outer membrane (compare *P aeruginosa* 799 WT with the permeability mutant 799/61 lacking the outer membrane).

Conclusion

An efficient route to versatile and chiral intermediates **4a–4e** from 6-APA has been developed. These compounds allowed the synthesis of various C4 substituted monobactams. In addition, they have potential as precursors of functionalised chiral α, β diaminodiacids by opening of the β-lactam ring. The structure activity relationship was investigated: among the series of compounds which have been tested for antibacterial activity, **10e**, **11** and **21** have moderate to good activity against Gram-negative bacteria with the exception of *P aeruginosa*. All were ineffective against Gram-positive bacteria which is consistant with previous findings concerning monobactam derivatives. Compounds incorporating a catechol function at C4 have improved activity against *P aeruginosa*. The improvement in anti-



Scheme 3.

bacterial activity although moderate compared with that which is reported for catechol monobactams [19–21] was achieved by improved penetration through the outer membrane.

Experimental protocols

IR spectra were recorded as KBr pellets on a Perkin–Elmer 781 spectrophotometer. ^{1}H NMR spectra were recorded on a 90 MHz JEOL FX 90Q spectrometer using tetramethylsilane as internal standard. Chemical shifts (δ) are reported in parts per million (ppm) relative to TMS. The following solvents were used: DMSO–d_6, DMSO–d_6–CD_3COOD (90:10), DMSO–d_6–CD_3COOD–CF_3COOD (75:15:10), $CDCl_3$, $CDCl_3$–CD_3COOD (90:10).

Melting points were measured on a Kofler melting point apparatus and are uncorrected.

Mass spectra were obtained on a Jeol D 300 mass spectrometer. The final acids described in tables I, II and III were usually hygroscopic solids, which on analysis proved to be a mixture of free acid and salts. Meaningful microanalyses were therefore difficult to obtain. IR, ^{1}H NMR and mass spectra have been used to confirm the proposed structures.

Trans-4-acetoxy-3-benzyloxycarbonylamino-2-azetidinone **2** *[22]*
To a suspension of methyl (3S, 5R, 6R)-6-benzyloxycarbonylamino-2,2-dimethyl penam-3-carboxylate [23] (86.8 g, 0.23 mol) in acetic acid (500 ml) was added mercuric acetate (152 g, 0.47 mol). The reaction mixture was heated at 70°C for 15 min. After filtration of the precipitate and evaporation under vacuum, the residue was taken up in water and the pH adjusted to pH 5 by addition of 6 N NaOH. After extraction with CH_2Cl_2 the organic layer was washed with water, dried over $MgSO_4$ and evaporated. The residue was then evaporated to dryness to give a syrup (88 g, 95%).

^{1}H NMR ($CDCl_3$) δ: 1.95(3H, s), 2.06(3H, s), 2.22(3H, s), 3.76(3H, s), 4.85(1H, d, J = 7.7 Hz), 5.14(2H, s), 5.84(1H, d, J = 7.7 Hz), 6.13(1H, d, J = 1.3 Hz). To a solution of the above compound (82.6 g, 0.21 mol) in acetone (1 l) cooled at 10°C was added a mixture of acetic acid (120 ml) and water (600 ml). The mixture was cooled to 0°C and $KMnO_4$ (50.2 g, 0.31 mol) was added portionwise. The reaction mixture was stirred for 30 min at 10°C. After addition of AcOEt (750 ml), SO_2 was bubbled for 10 min. The mixture was extracted with AcOEt (2 x 500 ml), the organic layer was washed with a saturated solution of $NaHCO_3$ (1 l), washed with H_2O (2 x 500 ml) and dried over $MgSO_4$. The solvent was evaporated and the residue chromatographed on a silica gel column; the fractions eluted with CH_2Cl_2–AcOEt (1:1) gave **2** as colourless crystals (35.85 g, 61%); mp 113–115°C.

^{1}H NMR ($CDCl_3$ + CD_3COOD) δ: 2.1(3H, s), 4.68(1H, s), 5.16(2H, s), 5.84(1H, s), 7.35(5H, s).

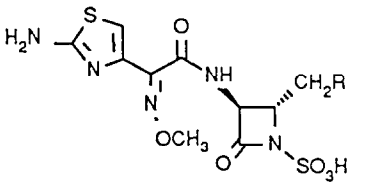
Trans-3-benzyloxycarbonylamino-4-ethoxycarbonylmethyl-2-azetidinone ***4a***
A suspension of **2** (9.8 g, 0.035 mol), anhydrous zinc acetate (9.8 g, 0.053 mol) and *O*-silyl ketene acetal **3a** [13] (14 ml, 0.07 mol) in toluene (175 ml) was heated at 70°C for 2.5 h under argon atmosphere. After filtration and evaporation, the residue was purified by filtration on silica gel and elution with CH_2Cl_2–Et_2O (1:1) gave **4a** as a colourless solid: 7.7 g (71%); mp 101–103°C.

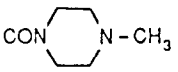
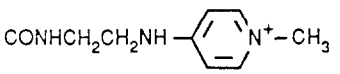
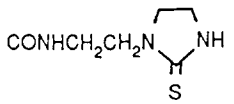
^{1}H NMR ($CDCl_3$ + CD_3COOD) δ: 1.25(3H, t, J = 7.7 Hz), 2.36–3.12(2H, m), 3.2–4(1H, m), 4.15(2H, q, J = 7.7 Hz), 4.34–4.6(1H, m), 5.12(2H, s), 7.33(5H, s).

Trans-3-benzyloxycarbonylamino-4-methoxycarbonylmethyl-2-azetidinone ***4b***
The title compound was prepared in 81% yield using *O*-silylketene acetal **3b** [13] in an analogous way.

^{1}H NMR ($CDCl_3$ + CD_3COOD) δ: 2.32–3.12(2H, m), 3.72(3H, s), 3.8–4.07(1H, m), 4.28–4.56(1H, m), 5.15(2H, s), 7.37(5H, s).

Table I. Antibacterial activity. Effect of the C4 substitution.



Entry	R	MIC (μg/ml)[a]							
		E cloacae		K oxytoca		E coli		P vulgaris	P aeruginosa
		P99+ [b]	P99- [c]	K1+ [b]	K1- [c]	J53.2	DCO		PU21
10a	COOEt	32	4	32	0.5	-	0.5	0.5	128
10e	COOH	8	2	0.25	0.25	0.5	0.5	0.25	128
11	$CONHCH_2COOH$	32	0.5	8	0.25	0.5	0.5	0.5	128
16	$CONHCH_2COOCH_3$	128	32	128	2	8	8	8	128
17	$CONHCH_2CN$	64	4	4	1	4	4	1	128
18		64	16	32	2	4	4	2	128
19	$CONHCH_2CH_2NH_2$	64	64	64	8	16	16	8	128
20		64	8	16	1	2	2	1	128
21		32	2	16	0.12	0.5	0.25	0.12	128
	Aztreonam	8	0.06	16	0.06	0.06	0.12	0.015	4

[a]IST growth medium, inoculum 10^4 cfu per spot; [b]constitutive derepressed type I β-lactamase producer; [c]inducible type IV β-lactamase producer

Trans-3-benzyloxycarbonylamino-4-t-butyloxycarbonylmethyl-2-azetidinone ***4c***

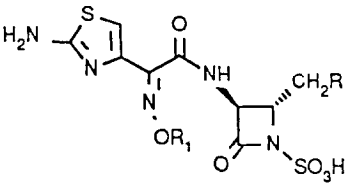
The title compound was prepared in 45% yield using *O*-silylketene acetal **3c** [14] in an analogous way.

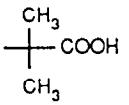
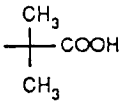
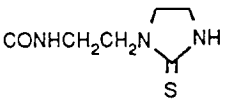
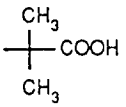
^{1}H NMR ($CDCl_3$ + CD_3COOD) δ: 1.44(9H, s), 2.35–3.1(2H, m), 3.8–4.1(1H, m), 4.32–4.6(1H, m), 5.13(2H, s), 7.34(5H, s).

Trans-3-benzyloxycarbonylamino-4-carboxymethyl-2-azetidinone ***4e***

To a cooled solution of **4b** (1.2 g, 4.1 mmol) in methanol (12 ml) was added a solution of K_2CO_3 (0.57 g, 4.1 mmol) in water (8 ml). After 4 h at 20°C, the methanol was evaporated under vacuum and the aqueous layer washed with AcOEt,

Table II. Antibacterial activity. Effect of the C3 side chain.



Entry	R	R_1	MIC (μg/ml) [a] E cloacae P99+ [b]	E cloacae P99- [c]	K oxytoca K1+ [b]	K oxytoca K- [c]	E coli J53.2	E coli DCO	P vulgaris	P aeruginosa PU21
10a	COOEt	CH_3	32	4	32	0.5	-	0.5	0.5	128
12a	"		16	8	8	1	-	2	0.25	128
19	$CONHCH_2CH_2NH_2$	CH_3	64	64	64	8	16	16	8	128
22	"		64	16	16	4	2	2	2	128
21		CH_3	32	2	16	0.12	0.5	0.25	0.12	128
23	"		4	4	2	1	1	1	0.06	128

[a]IST growth medium, inoculum 10^4 cfu per spot; [b]constitutive derepressed type I β-lactamase producer; [c]inducible type IV β-lactamase producer.

acidified to pH 2 and extracted with AcOEt to give after evaporation **4e** which solidified in an AcOEt–ether mixture (0.6 g, 53%).

^{1}H NMR (DMSO–d_6 + CD_3COOD) δ: 2.3–2.9(2H, m), 3.5–3.92(1H, m), 4.34(1H, d, J = 2 Hz), 5.08(2H, s), 7.36(5H, s).

Trans-3-benzyloxycarbonylamino-4-t-butyloxycarbonylmethyl-carbamoylmethyl-2-azetidinone ***4f***

A solution of **4e** (1 g, 3.6 mmol), *t*-butyl glycinate ester (0.5 ml, 3.6 mmol) and EEDQ (0.89 g, 3.6 mmol) in CH_2Cl_2 (20 ml) was stirred at 20°C for 1.5 h. After evaporation the residue was dissolved in AcOEt and washed successively with 1 N HCl and saturated $NaHCO_3$. After evaporation **4f** was obtained as a foam (1.2 g, 86%).

^{1}H NMR (DMSO–d_6 + CD_3COOD) δ: 1.42(9H, s), 2.1–2.9(2H, m), 3.5–3.9(3H, m), 4.2–4.4(1H, m), 5.08(2H, s), 7.38(5H, s).

Trans-3-amino-4-ethoxycarbonylmethyl-2-azetidinone ***5a***

Hydrogen was bubbled through a suspension of **4a** (0.929, 3 mmol) and 10% palladium on carbon (0.6 g) in EtOH (30 ml). After 15 min at room temperature, the catalyst was filtered off and the filtrate evaporated under vacuum to give an oil (0.52 g, 100%) which was used for the synthesis of **6a** without further purification.

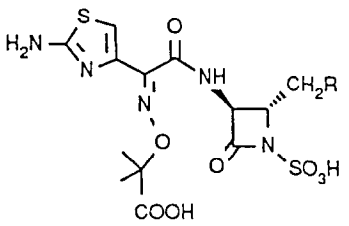
Trans-3-amino-4-t-butyloxycarbonylmethyl-2-azetidinone ***5c***

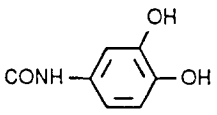
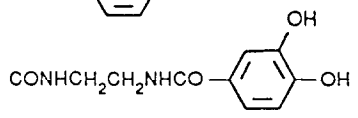
The title compound was prepared from **4c** in 96% yield as above.

Trans-3-[2-(2-tritylaminothiazol-4-yl)-2-((Z)-methoxyimino)acetamido]-4-ethoxycarbonylmethyl-2-azetidinone ***6a***

A solution of **5a** (0.51 g, 3 mmol), 2-(2-tritylaminothiazol-4-yl)-2-((*Z*)-methoxyimino)acetic acid (1.5 g, 3.5 mmol) and

Table III. Antibacterial activity. Effect of the incorporation of a catechol residue.



Entry	R	MIC (μg/ml) [a]								
		E cloacae		K oxytoca		E coli		P aeruginosa		
		P99+ [b]	P99- [c]	K1+ [b]	K- [c]	J53.2	DC0	PU21	799WT [d]	799/61 [e]
12a	COOEt	16	8	8	1	-	2	>32	>32	0.12
24		32	4	4	1	0.5	0.5	16	1	0.25
25		16	8	1	0.25	0.12	0.25	16	0.5	0.12
	Aztreonam	8	0.06	16	0.06	0.06	0.12	4	1	<0.015

[a]IST growth medium, inoculum 10^4 cfu per spot; [b]constitutive derepressed type I β-lactamase producer; [c]inducible type IV β-lactamase producer; [d]parent organism; [e]permeability mutant

EEDQ (0.86 g, 3.5 mmol) in CH_2Cl_2 (50 ml) was stirred at room temperature for 1.5 h. The solvent was evaporated and the residue redissolved in AcOEt; the organic layer was washed successively with diluted HCl, $NaHCO_3$ and H_2O, dried and concentrated under reduced pressure to give a foam (1.35 g, 75%).

^{1}H NMR (DMSO-d_6 + CD_3COOD) δ: 1.19(3H, t, J = 7 Hz), 2.5–2.9(2H, m), 3.7–4.1(1H, m), 3.84(3H, s), 4.1(2H, q, J = 7 Hz), 4.55(1H, d, J = 2 Hz), 6.75(1H, s), 7.31(15H, s).

Trans-3-[2-(2-tritylaminothiazol-4-yl)-2-((Z)-methoxyimino)acetamido]-4-t-butyloxycarbonylmethyl-2-azetidinone ***6c***

A solution of **5c** (0.53 g, 2.7 mmol), 2-(2-tritylaminothiazol-4-yl)-2-((Z)-methoxyimino)acetic acid (1.3 g, 3 mmol) and EEDQ (0.74 g, 3 mmol) in CH_2Cl_2 (50 ml) was stirred at room temperature for 1.5 h. The solvent was evaporated and the residue was dissolved in AcOEt; the organic layer was washed successively with diluted HCl, $NaHCO_3$ and H_2O, dried and concentrated under reduced pressure to give **6c** as a foam (1.07 g, 64%).

^{1}H NMR ($CDCl_3$ + CD_3COOD) δ: 1.45(9H, s), 2.3–3.2(2H, m), 3.8–4.2(1H, m), 4.04(3H, s), 4.62(1H, d, J = 2.2 Hz), 6.72(1H, s), 7.31(15H, s).

Trans-3-[2-(2-tritylaminothiazol-4-yl)-2-((Z)-methoxyimino)acetamido]-4-(t-butyloxycarbonylmethylcarbamoylmethyl)-2-azetidinone ***6f***

A suspension of **4f** (1.1 g, 2.8 mmol) and 10% palladium on carbon (0.75 g) in EtOH (40 ml) was stirred under an H_2 atmosphere for 1 h 30. The catalyst was filtered off and the filtrate was evaporated under vacuum to give an oil (0.7 g, 97%).

A solution of the above oil (0.46 g, 1.8 mmol), 2-(2-tritylaminothiazol-4-yl)-2-((Z)-methoxyimino)acetic acid (0.93 g, 2 mmol) and EDDQ (0.5 g, 2 mmol) in CH_2Cl_2 was stirred at 20°C for 1 h 30. After concentration under vaccum, the residue was dissolved in AcOEt and washed successively with 0.1 N HCl and saturated $NaHCO_3$. **6f** was obtained as an oil which solidified in ether (0.76 g, 63%).

^{1}H NMR (DMSO-d_6 + CD_3COOD) δ: 1.4(9H, s), 2.0–2.9(2H, m), 3.56–3.96(3H, m), 3.82(3H, s), 4.6(1H, d, J = 2 Hz), 6.81(1H, s), 7.32(15H, s).

Trans-3-[2-(2-tritylaminothiazol-4-yl)-2-((Z)-1-t-butyloxycarbonyl-1-methylethoxyimino)acetamido]-4-ethoxycarbonylmethyl-2-azetidinone ***7a***

A solution of **5a** (0.51 g, 1.5 mmol), 2-(2-tritylaminothiazol-4-yl)-2-((Z)-1-*t*-butyloxycarbonyl-1-methylethoxyimino)acetic acid (0.85 g, 1.5 mmol), EEDQ (0.37 g, 1.5 mmol) and *N*-

ethyldiisopropylamine (0.26 ml, 1.5 mmol) in CH_2Cl_2 (10 ml) was stirred at room temperature. After evaporation, the residue was dissolved in AcOEt; the organic layer was washed successively with diluted HCl, $NaHCO_3$ and H_2O, dried and concentrated under reduced pressure to give **7a** as a foam which was purified on a silica gel column. The fractions eluted with CH_2Cl_2–ether (1:1) gave **7a** (0.5 g, 50%).

^1H NMR (DMSO–d_6 + CD_3COOD) δ: 1.0–1.7(18H, m), 2.5–2.9(2H, m), 3.9–4.2(1H, m), 4.1(2H, d, J = 7 Hz), 4.6(1H, d, J = 2 Hz), 6.73(1H, s), 7.3(15H, s).

Pyridinium trans-3-[2-(2-tritylaminothiazol-4-yl)-2-((Z)-methoxyimino)acetamido]-4-ethoxycarbonylmethyl-2-azetidinone-1-sulfonate ***8a***

General procedure. To a stirred solution of **6a** (0.45 g, 0.75 mmol) in pyridine (5 ml) was added the complex pyridine–SO_3 (0.48 g, 3 mmol); the mixture was heated at 80°C for 2 h under an argon atmosphere. After evaporation to dryness, the residue was triturated with ether to give a solid which was further washed with water and ether to give **8a** (0.4 g, 70%).

^1H NMR ($CDCl_3$ + CD_3COOD) δ: 1.2(3H, t, J = 7 Hz), 2.5–3.5(2H, m), 3.8–4.5(3H, m), 4.12(3H, s), 4.8–5.0(1H, d, J = 2.1 Hz), 6.88(1H, s), 7.32(15H, s), 7.7–8.1(2H, m), 8.15–8.55(1H, m).

8c was prepared from **6c** by the above method in a 71% yield. ^1H NMR (DMSO–d_6 + CD_3COOD) δ: 1.36(9H, s), 2.–3.25(2H, m), 3.7–4.1(1H, m), 3.9(3H, s), 4.75(1H, d, J = 2.1 Hz), 6.86(1H, s), 7.38(15H, s), 7.95–8.3(2H, m), 8.48–8.76 (1H, m), 8.84–9.08(2H, m).

Trans-3-[2-(2-aminothiazol-4-yl)-2-((Z)-methoxyimino)acetamido]-4-ethoxycarbonylmethyl-2-azetidinone-1-sulfonic acid ***10a***

General procedure for ***10a, 10e, 12a.*** **8a** (0.335 g, 0.44 mmol) was added to a cooled solution (5°C) of TFA–H_2O (90:10) (7.5 ml) and the mixture was stirred at room temperature for 30 min. After evaporation to dryness the residue was purified by preparative HPLC on a Whatman ODS3 Magnum column; elution with MeOH–$(NH_4)_2CO_3$ 2 g/l pH 6 (25:75) and evaporation gave **10a** (45 mg, 22%). IR (KBr) cm^{-1}: 1765. ^1H NMR (DMSO–d_6 + CD_3COOD) δ:1.2(3H, t, J = 7 Hz), 2.5–3.4(2H, m), 3.9(3H, s), 3.8–4.3(3H, m), 4.7(1H, d, J = 2.6 Hz), 6.8(1H, s).

10e was prepared by a procedure similar to that described for **10a**. **10e**: (55 mg, 32%). IR (KBr) cm^{-1}: 1760. ^1H NMR (DMSO–d_6 + CD_3COOD + CF_3COOD) δ: 2.3–3.3 (2H, m), 4.0(3H, s), 3.8–4.15(1H, m), 4.84(1H, d, J = 2.5 Hz), 7.05(1H, s).

9a prepared by a procedure similar to that described for **8a** was treated as above to give **12a** (10 mg, 7%). IR (KBr) cm^{-1}: 1770. ^1H NMR (DMSO–d_6 + CD_3COOD) δ: 1.19(3H, t, J = 7 Hz), 1.43(6H, s), 2.0–3.4(2H, m), 3.9–4.3(3H, m), 4.86(1H, d, J = 2.1 Hz), 6.76(1H, s).

Trans-3-[2-(2-aminothiazol-4-yl)-2-((Z)-methoxyimino)acetamido]-4-(carboxymethylcarbamoylmethyl)-2-azetidinone-1-sulfonic acid ***11***

A solution of **6f** (0.68 g, 1 mmol) and pyridine–SO_3 complex (0.64 g, 4 mmol) in pyridine (10 ml) was heated at 80°C for 3 h under argon atmosphere. After evaporation to dryness the residue was triturated with H_2O to give a solid which was washed with ether, dried under vacuum, redissolved in a mixture of TFA/H_2O (90:10) (5 ml) at 0°C. After 30 min the mixture was concentrated under reduced pressure. The residue was triturated with ether to give a solid which was purified by preparative HPLC on a Whatman ODS3 Magnum column. Elution with $(NH_4)_2CO_3$ 2 g/l pH 6 followed by evaporation and treatment with acetone afforded **11** as a powder (45 mg, 11%). IR (KBr) cm^{-1}: 1775.

^1H NMR (D_2O + CD_3COOD + CF_3COOD) δ: 2.6–3.4(2H, m), 3.07(3H, s), 4.01(2H, s), 4.36–4.64(1H, m), 4.88(1H, d, J = 2 Hz), 7.16(1H, s). MS (–FAB): 463 $(M-H)^-$.

Synthesis of potassium trans-3-[2-(2-tritylaminothiazol-4-yl)-2-((Z)methoxyimino)acetamido]-4-carboxymethyl-2-azetidinone-1-sulfonate ***15a***

A suspension of **2** (30 g, 0.108 mol), *O*-silyl ketene acetal **3d** [15] (40 ml, 0.122 mol) and anhydrous zinc acetate (10 g, 0.5 mol) was heated at 65°C in toluene (350 ml) for 2 h under an argon atmosphere. After filtration and evaporation the residue was purified by filtration on silica gel and elution with a mixture of CH_2Cl_2–ether (1:1) to give **4d** as an oil which later solidified (29.5 g, 74%).

^1H NMR (DMSO–d_6 – CD_3COOD) δ: 2.64–2.96(2H, m), 3.72–4.0(1H, m), 4.28–4.6(1H, m), 5.08(2H, s), 5.13(2H, s), 7.4(10H, s).

A mixture of **4d** (45 g, 0.122 mol) and pyridine–SO_3 complex (80 g, 0.49 mol) in pyridine (500 ml) was heated at 80°C under an argon atmosphere. After evaporation to dryness, the residue was washed with water, dissolved in chloroform and washed with potassium phosphate buffer. The solvent was evaporated and the residue triturated with ether to give **13** as a foam (44 g, 75%).

Hydrogen was bubbled through a stirred suspension of **13** (6 g, 0.12 mol) and 10% palladium on carbon (4 g) in DMF (80 ml) at room temperature for 1.5 h. The catalyst was removed by filtration and the filtrate evaporated under vacuum to give after trituration with ether a solid which was further washed with acetonitrile and ether to give **14** (2.07 g, 64%).

To a suspension of **14** in CH_2Cl_2 (20 ml) was added BSA (3.5 ml, 14 mmol). The mixture was stirred at room temperature for 1 h and the resulting solution was added to a solution of 2-(2-tritylaminothiazol-4-yl)-2-((Z)-methoxyimino)acetic acid chloride (3.24 g, 7 mmol) in CH_2Cl_2 (10 ml) at –45°C. After 15 min at –45°C the mixture was further stirred at room temperature for 1 h, washed with water, dried and evaporated to dryness. The residue was then triturated with ether to give **15a** as a foam (2.7 g, 52%).

^1H NMR (DMSO–d_6 + CD_3COOD + CF_3COOD) δ: 2.2–3.3(2H, m), 3.8–4.1(1H, m), 3.98(3H, s), 4.7–4.9(1H, m), 6.96(1H, s), 7.1–7.7(15H, m).

Compound **15b** was prepared by a similar method to that described above from **14**.

^1H NMR (DMSO–d_6 + CD_3COOD + CF_3COOD) δ: 1.53(6H, s), 2.3–3.4(2H, m), 3.9–4.2(1H, m), 4.78–5.0(1H, m), 7.07(1H, s).

Trans-3-[2-(2-aminothiazol-4-yl)-2-((Z)-methoxyimino)acetamido]-4-substituted-2-azedidinone-1-sulfonic acid ***16***

General procedure. To a solution of **15a** (200 mg, 0.29 mmol) and triethylamine (81.6 µl, 0.58 mmol) in CH_2Cl_2 (5 ml) was added at –80°C isobutylchloroformate (79.4 mg, 0.58 mmol). After 20 min a solution of glycine methyl ester hydrochloride (35.1 mg, 0.58 mmol) and triethylamine (40.6 µl, 0.58 mmol) in CH_2Cl_2 (1 ml) was run in. The mixture was stirred at room temperature for 1 h and extracted with CH_2Cl_2; the organic layer washed with a solution of KH_2PO_4 (pH 5.5). After evaporation to dryness the residue was triturated with ether to give a solid which was deprotected using a mixture of HCOOH/H_2O (7:3, 2 ml) at 40°C for 1 h. The solvent was evaporated and the residue was triturated with ether and then purified by preparative HPLC (ODS column) and eluted

with MeOH/$(NH_4)_2CO_3$ 2 g/l pH 6 to give **16** (18 mg, 12%) (table IV).

Compounds **17–21** were prepared by a similar method to that described above. Results are shown in table IV.

*Trans-3-[2-(2-aminothiazol-4-yl)-2-((Z)-1-carboxy-1-methyl-ethoxyimino)acetamido]-4-substituted-2-azetidinone-1-sulfonic acids **22–25***

Compounds **22–25** were prepared by a similar method to that described for compound **16** starting from **15b**. Results are shown in table IV.

In the synthesis of **25**, after treatment with TFA, the mixture was stirred in MeOH–H_2O (1:3) (15 ml), adjusted to pH 8 with NH_4OH for 2 h and further purified on HP20SS resin.

The required starting amines (**26–30**) were prepared as below:

*N-(t-butyloxycarbonyl)ethylenediamine **26** was prepared as reported in [24]*

*N-[4-(1-methylpyridinium)]ethylenediamine **27***

To a solution of **26** (320 mg, 2 mmol) and triethylamine (202 mg, 2 mmol) in CH_2Cl_2 (10 ml) was added 4-chloro-1-methylpyridinium iodide [25–27] (460 mg, 1.8 mmol). After 1 h at room temperature, the mixture was evaporated to dryness and treated with TFA/CH_2Cl_2 (1:1), (5 ml) for 1 h. After evaporation to dryness, the residue was triturated with AcOEt to give **27** as a solid (150 mg, 26%).

^1H NMR (DMSO-d_6 – CD_3COOD) δ: 3.4–3.8(4H, m), 4(3H, s), 6.8–7.2(2H, m), 8–8.5(2H, m).

*1-(2-Aminoethyl)-2-imidazolidinethione **28** was prepared as reported in [28]*

*3,4-di-t-butoxyaniline **29***

A suspension of 3,4-dihydroxynitrobenzene (6 g, 0.038 mol) and *N,N'*-diisopropyl-*O-t*-butylisourea (50 g, 0.25 mol) [29] was stirred at room temperature overnight. After filtration of the precipitate the residue was chromatographed on silica gel; elution with petroleum ether–ether (95:5) afforded 3,4-di-*t*-butoxy-nitrobenzene (6.2 g, 60%).

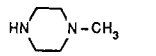
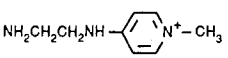
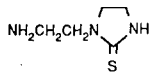
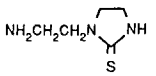
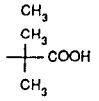
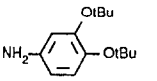
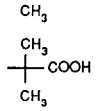
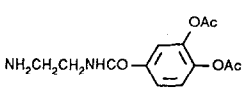
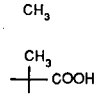
A suspension of the above compound (1 g, 3.7 mmol) and 10% palladium on carbon in EtOH (30 ml) was hydrogenated. After 30 min the catalyst was filtered off and the filtrate evaporated to dryness to give **29** as an oil (0.85 g, 95%).

NMR ($CDCl_3$): 1.4(18H, s), 7.1(1H, d, J = 10 Hz), 7.9(2H, m).

*N-(2,3-diacetoxybenzoyl)ethylenediamine **30***

To a solution of *N*-(*t*-butoxycarbonyl)ethylenediamine **26** (2.9 g, 18 ml) and triethylamine (2.5 ml, 1.8 mmol) in CH_2Cl_2 (30 ml) at 0°C was added 2,3-di-acetoxybenzoyl chloride (4.6 g, 18 mmol). The mixture was stirred for 1 h at room

Table IV. Spectral properties of compounds **16–25**. A = DMSO, B = DMSO-d_6 + CD_3COOD + CF_3COOD.

ENTRY	STARTING AMINE R_2R_3NH		OXIME R_1	YIELD % from **15a** or **15b**	IR (KBr) ν C=O (cm^{-1})	^1H NMR (SOLVENT) δ(ppm), J(Hz) MS
16	$NH_2CH_2COOCH_3$		CH_3	12	1765	(B) 2.6-3.3(2H,m), 3.6(3H,s), 3.8-4.2(6H,m), 4.8(1H,d,J=2.5Hz), 7.15(1H,s) (+FAB) : 479 $(M+H)^+$
17	NH_2CH_2CN		CH_3	16	1765	(B) 2.6-3.4(2H,m), 3.8-4.2(3H,m), 4(3H,s), 4.7-4.9(1H,m), 4.8-5.0(1H,m), 7.14(1H,s) (-FAB) : 444 $(M-H)^-$
18			CH_3	13	1765	(B) 2.83(3H,s), 2.3-3.6(10H,m), 3.95(3H,s), 7.1(1H,s)
19	$NH_2CH_2CH_2NH$-Boc	**26**	CH_3	12	1770	(B) 2.6-3.4(6H,m), 3.8-4.1(4H,m), 4.9(1H,d,J=2.5Hz), 7.1(1H,s)
20		**27**	CH_3	13	1770	(B) 3.2-3.5(4H,m), 2.6-3.2(2H,m), 3.8-4.1(1H,m), 3.9(3H,s), 4(3H,s), 4.8-5(1H,m) 6.8-7.1(2H,m), 7.15(1H,s), 7.9-8.3(2H,m)
21		**28**	CH_3	10	1770	(B) 2.7-3.2(2H,m), 3.2-3.8(8H,m), 3.9-4.2(1H,m), 4(3H,s), 5.2(1H,d,J=2.5Hz), 7.4(1H,s)
22	$NH_2CH_2CH_2NH$-Boc	**26**		29	1770	(A) 1.52(6H,s), 1.9-2.05(1H,m), 2.5-3.5(6H,m), 3.9-4.2(1H,m), 7.09(1H,s)
23		**28**		14	1770	(B) 1.53(6H,s), 2.5-3.88(10H,m), 3.8-4.16(1H,m), 4.8(1H,d,J=2Hz), 7.26(1H,s)
24		**29**		23	1770	(B) 2.6-3.4(2H,m), 4-4.3(1H,m), 4.95(1H,d,J=2.5Hz), 6.5-7(2H,m), 7.05(1H,s), 7-7.3(1H,m) (-FAB) : 585 $(M-H)^-$
25		**30**		6	1765	(B) 1.55(6H,s), 2.6-3.4(2H,m), 3.1-3.5(4H,m), 3.9-4.2(1H,m), 4.8-5(1H,m) 6.8(1H,d,J=7.6Hz), 7.1-7.5(3H,m) (-FAB) : 656 $(M-H)^-$

temperature. After evaporation to dryness the residue was extracted with AcOEt, the organic layer was then dried and evaporated to give a solid which was purified on silica gel and eluted with CH_2Cl_2–AcOEt (6:4) (4.9 g, 71%).

^1H NMR ($CDCl_3$ – D_2O): 1.4(9H, s), 2.25(6H, s), 3.2–3.6(4H, m), 7.2(1H, d, J = 8.4 Hz), 7.6–7.8(2H, m).

A solution of the above compound (2 g, 5.2 mmol) in a mixture of TFA–H_2O (9:1) (40 ml) was stirred at room temperature for 30 min. After evaporation to dryness, the residue was triturated with toluene to give **30** as a solid (2 g, 96%).

NMR (DMSO–d_6): 2(6H, s), 2.5–3(2H, m), 3–3.5(2H, m), 7.1(1H, d, J = 7.6 Hz), 7.3–7.8(2H, m).

Acknowledgments

We thank Drs TD Hennessey, NAC Curtis and their colleagues at ICI Pharmaceuticals (Macclesfield, UK) for performing the biological evaluation.

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