SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF C-2 CARBOXYETHENYLTHIO-CARBAPENEM DERIVATIVES†

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A number of C-2 carboxyethenylthio-carbapenem derivatives possessing either the (5*R*,6*R*,8*S*)- or the (5*R*,6*S*,8*R*)-stereochemistries have been prepared from the olivanic acids MM 22382 (**1**) and MM 22383 (**4**), respectively. Their *in vitro* antibacterial activities and stabilities to human kidney homogenate are superior to those of the parent compounds, particularly in the latter series.

The olivanic acids, together with thienamycin, the carpetimycins and PS-5 are members of a family of β-lactam antibiotics, all of which were isolated from soil microorganisms and possess the 7-oxo-1-azabicyclo[3.2.0]hept-2-ene (carbapenem) ring system.[1~3] They display potent activity against a broad range of Gram-positive and Gram-negative bacteria, including penicillin-resistant strains of *Staphylococcus aureus* and *Haemophilus influenzae* and organisms that are often resistant to other β-lactam antibiotics, such as indole-positive *Proteus, Enterobacter* spp., *Serratia marcescens* and the anaerobe *Bacteroides fragilis*.

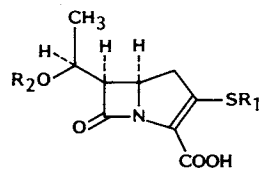
The major structural difference between the olivanic acids and the thienamycins is the absolute stereochemistry of the chiral centre at C-8, which is (*S*) for the olivanic acids and (*R*) for the thienamycins. In the olivanic acid series it is those compounds with the *cis*-orientated β-lactam (*i.e.* 5*R*,6*R*), as in MM 22382 (**1**) and MM 22380 (**2**) that are most potent. Whilst the corresponding (8*S*) hydroxyethyl compounds with the *trans*-β-lactam (*i.e.* 5*R*,6*S*), MM 22383 (**4**) and MM 22381 (**5**) also have a broad spectrum of activity, they are rather less potent than the *cis*-compounds. *N*-Acetyldehydrothienamycin (**6**) and *N*-acetylthienamycin (**7**) have the same *trans*-β-lactam orientation as MM 22383 and MM 22381 but have the (*R*) configuration at C-8. They have much improved antibacterial potency compared to MM 22383 and MM 22381, being similar to *cis*-isomers MM 22382 and MM 22380 whilst retaining the stability of the (5*R*,6*S*,8*S*) compounds towards R_{TEM} β-lactamase.[4]

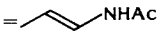
The exceptional activity of this class of compounds has prompted considerable effort in their chemical modification in order to determine the structural requirements for optimum activity and efficacy.

Earlier chemical studies in these laboratories on the isomerisation of the double bond in the acetamidoethenylthio-side chain of the olivanic acids, MM 22382 (**1**) and MM 13902 (**3**) revealed that the (*Z*)-isomers (**10**) ('*iso*'-MM 22382) and (**11**) ('*iso*'-MM 13902), respectively, were considerably more active than their natural product counterparts *in vivo*.[5] The improvement in *in vivo* activity is partly attributed to their superior intrinsic antibacterial activities *in vitro*, and partly due to enhanced mouse plasma levels as a result of improved stabilities to mouse tissue homogenates.

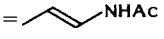
The improved biological properties of the isomeric compounds, together with the finding that the

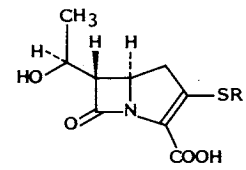
† Numbering based on 'trivial' carbapenem nomenclature. For nomenclature of β-lactam antibiotics, see ref 11.

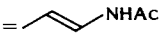


MM 22382 (**1**) R_1 =  R_2 = H

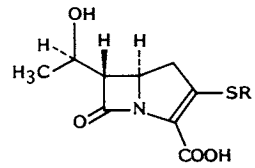
MM 22380 (**2**) R_1 =  R_2 = H

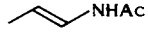
MM 13902 (**3**) R_1 =  R_2 = SO_3H

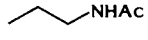


MM 22383 (**4**) R = 

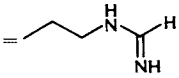
MM 22381 (**5**) R = 

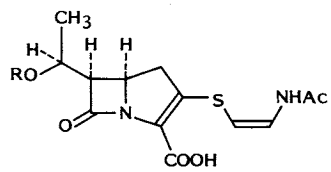


(**6**) R = 

(**7**) R = 

Thienamycin (**8**) R = 

Imipenem (**9**) R = 



'*iso*'-MM 22382 (**10**) R = H

'*iso*'-MM 13902 (**11**) R = SO_3H

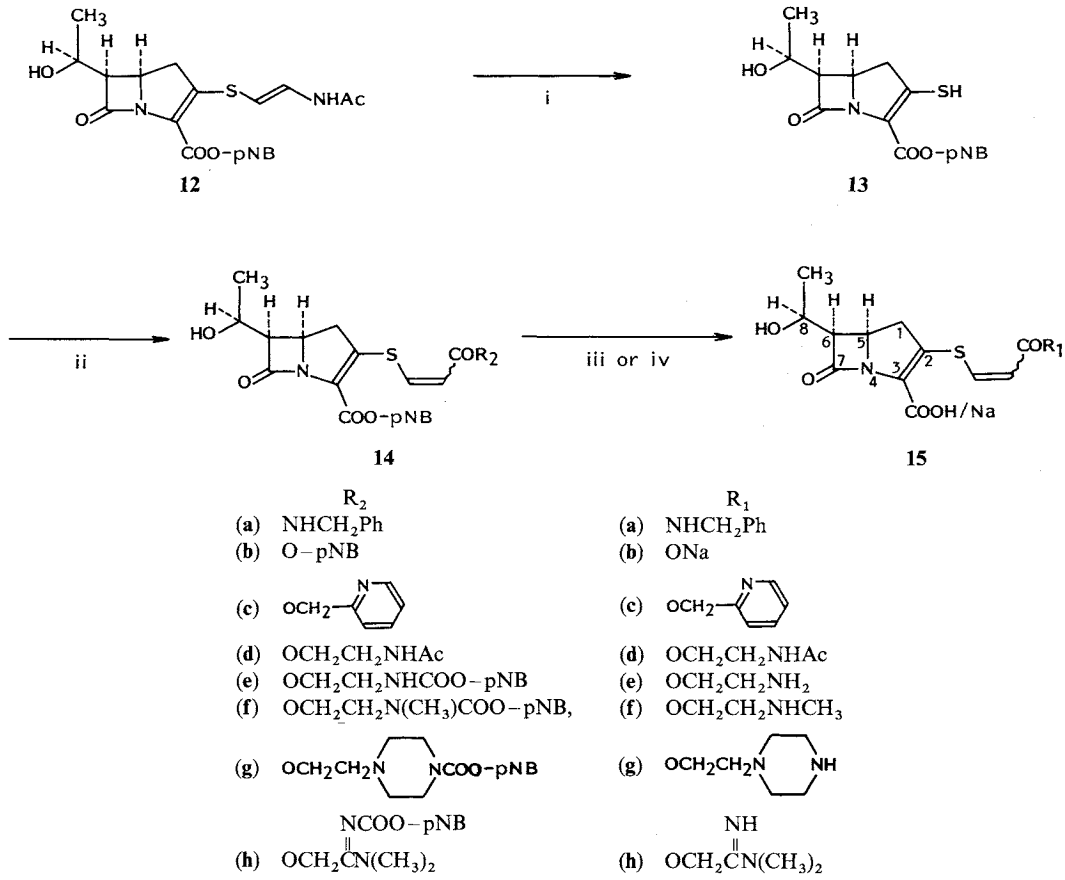
2-mercapto-carbapenem derivative (**13**) reacts readily with propiolate esters[6] prompted us to prepare a number of C-2 carboxyethenylthio-derivatives for biological evaluation.

Chemistry

The carboxyethenylthio-derivatives were prepared initially in the (5*R*,6*R*,8*S*)-series, from the readily available natural product MM 22382, by the method outlined in Scheme 1. Those which displayed interesting biological properties were then prepared with the (5*R*,6*S*,8*R*)-stereochemistry from MM 22383, after inversion of the C-8 configuration by the previously reported procedure[7] (Scheme 2). This inversion procedure involves reaction of the *p*-nitrobenzyl ester of MM 22383 with triphenylphosphine and diethylazodicarboxylate in the presence of formic acid, to provide the formate ester (**17**). Saponification of the formate ester moiety to the (8*R*)-hydroxyethyl derivative may be performed either prior to the thiol addition reaction (Method A) or, more conveniently, at the final stage of the reaction sequence by mild alkaline hydrolysis (Method B).

The C-2 mercapto-derivatives, **13**, **19**, and **22** were prepared by reaction of the appropriate acetamidoethenylthio-carbapenems, **12**, **18**, and **17**, respectively, with *N*-bromoacetamide in aqueous 1,4-dioxan.[6] Addition to the appropriate propiolate ester, or propiolic acid amide then provided the isomeric mixture of carboxyethenylthio-derivatives, **14**, **20**, and **23**. Yields for the addition to propiolate

Scheme 1.



pNB: *p*-Nitrobenzyl.

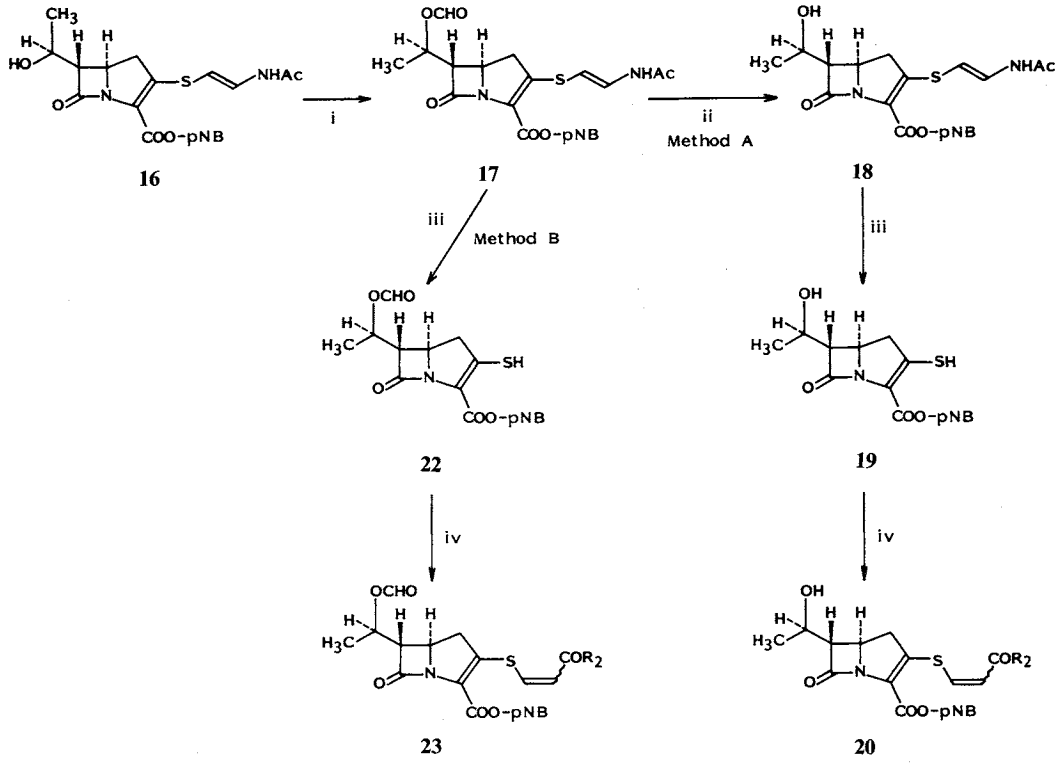
Reagents: i) *N*-Bromoacetamide, aq 1,4-dioxan, room temperature, 4.5 minutes; ii) $HC{\equiv}CCOR_2$, K_2CO_3, DMF, room temperature, 25, minutes; iii) (a) H_2, 25% aq 1,4-dioxan, 5% Pd/C, room temperature, 3.5 hours, (b) $NaHCO_3$ (for Na salts); iv) H_2, 25% aq 1,4-dioxan, pH 7.0, 0.05 M potassium phosphate buffer, 5% Pd/C, room temperature, 3.5 hours (for zwitterions).

esters ranged from 25 to 50%, whereas yields for addition to propiolic acid amides were somewhat lower (5 to 10%). The stereochemistry of the C-2 side chain double bonds was assigned on the basis of the coupling constants of the olefinic protons in the ^1H NMR spectrum;[5] $J_{CH=CH}$ was usually in the order of 10~11 Hz for the (*Z*)-isomers and 15 Hz for the (*E*)-isomers. The major product in the propiolate addition reactions was always the (*Z*)-isomer and could be obtained in pure form and free from contamination with the (*E*)-isomer by silica gel column chromatography.

Removal of the *p*-nitrobenzyl protecting groups was effected by hydrogenolysis of the esters **14**, **20**, and **23** in aqueous 1,4-dioxan over 5% palladium on carbon catalyst to yield either the zwitterion or the sodium salt of the acids **15**, **21**, and **24**. The zwitterions were obtained by hydrogenolysis in the presence of pH 7.0, 0.05 M potassium phosphate buffer while the sodium salts were obtained after the addition of 1 equivalent of sodium hydrogen carbonate.

The amidino-derivatives, **15i**~**15k** and **21i** were prepared by reaction of the parent amines **15e** and

Scheme 2.



16 17 18 22 19 23 20 24 21

R_2=(a) $NHCH_2Ph$, (b) O-pNB; (c) OEt, (d) OCH_2CH_2OH, (e) OCH_2CH_2NHAc, (f) OCH_2CH_2NHCOO-pNB, (g) $OCH_2CH_2N(CH_3)COO$-pNB. pNB: p-Nitrobenzyl

R_1=(a) $NHCH_2Ph$, (b) ONa, (c) OEt, (d) OCH_2CH_2OH, (e) OCH_2CH_2NHAc, (f) $OCH_2CH_2NH_2$, (g) $OCH_2CH_2NHCH_3$.

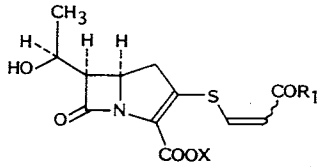
Reagents: i) PPh_3, HCOOH, EtOOC-N=N-COOEt, THF, room temperature, 1 hour; ii) NaOH (1 equiv), 30% aq 1,4-dioxan, 5°C, 5 minutes; iii), iv), v), and vi) as in Scheme 1.; vii) pH 9.0 (NaOH), H_2O, room temperature, 3.5 hours.

21f with either ethyl formimidate hydrochloride, ethyl acetimidate hydrochloride or methyl picolinimidate in concentrated aqueous solution at pH 9.0.

Results and Discussion

The comparative antibacterial activities of the various carboxyethenylthio-carbapenem derivatives are shown in Tables 1 and 2. All the compounds were highly active against a wide range of Gram-positive

Table 1. *In vitro* activity of (5*R*,6*R*,8*S*) derivatives.



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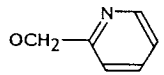
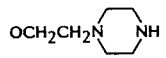
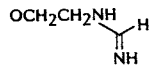
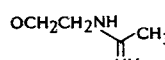
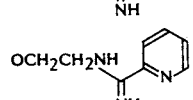
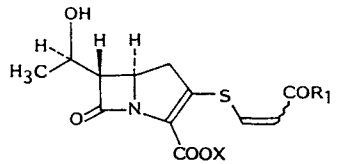
Compound	R_1	Isomer	X	Antibacterial activity (μg/ml)													Human kidney stability[a]
				E.cl. N1	*E.c.* 0111	*E.c* JT39 (R^+)	*K.p.* A	*P.m.* 977	*P.v.* W091	*P.a.* A	*S.m.* V520	*S.a.* Ox	S.a. Ru (R^+)	*S.a.* 1517	*S.f.* I	*S.p.* CN33	*S.p.* CN10	
15a	$NHCH_2Ph$	*Z*	Na	0.2	0.2	0.2	0.2	≤0.1	0.2	25	0.4	≤0.1	≤0.1	1.6	0.2	—	≤0.1	2.6
15b	ONa	*Z*	Na	0.5	0.5	12.5	2.5	1.2	2.5	>50	2.5	0.5	2.5	25	2.5	≤0.1	0.2	2.4
15c		*Z*	Na	1.6	0.4	3.1	0.8	0.8	6.2	100	6.2	0.4	0.4	3.1	0.4	—	≤0.1	0.6
15d	OCH_2CH_2NHAc	*Z*	Na	0.8	0.2	1.6	0.4	0.8	1.6	100	1.6	0.4	0.4	1.6	0.4	—	≤0.1	2.4
15e	$OCH_2CH_2NH_2$	*Z*	H	0.2	≤0.1	0.8	0.2	0.4	0.4	25	0.4	≤0.1	0.2	0.2	≤0.1	≤0.1	≤0.1	1.2
15f	$OCH_2CH_2NH(CH_3)$	*Z*	H	0.8	≤0.1	0.8	≤0.1	0.4	3.1	50	0.4	0.1	0.2	1.6	≤0.1	—	—	2.2
15g		*Z*	H	0.8	≤0.1	0.8	≤0.1	0.8	1.6	100	1.6	≤0.1	0.2	1.6	≤0.1	—	—	1.3
15h	$OCH_2C(=NH)N(CH_3)_2$	*Z*	H	0.8	≤0.1	≤0.1	0.8	0.4	12.5	100	6.2	≤0.1	0.2	3.1	0.2	—	≤0.1	1.8
15i		*Z*	H	1.6	0.2	1.6	0.8	0.4	3.1	50	1.6	≤0.1	≤0.1	1.6	—	0.8	—	2.2
15j		*Z*	H	3.1	0.2	0.2	0.2	0.4	3.1	25	1.6	≤0.1	≤0.1	0.8	≤0.1	≤0.1	≤0.1	2.5
15k		*Z*	H	1.6	0.2	6.2	1.6	0.8	6.2	100	1.6	≤0.1	≤0.1	6.2	0.2	—	≤0.1	1.2
MM 22382				3.1	0.2	25	0.4	0.2	0.8	>100	3.1	0.4	0.4	6.2	1.6	—	0.05	0.8

[a] Human kidney stability relative to MM 13902 (1.0).

Abbreviations: *E.cl.*, *Enterobacter cloacae*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *P.m.*, *Proteus mirabilis*; *P.v.*, *P. vulgaris*; *P.a.*, *Pseudomonas aeruginosa*; *S.m.*, *Serratia marcescens*; *S.a.*, *Staphylococcus aureus*; *S.f.*, *Streptococcus faecalis*; *S.p.*, *S. pneumoniae*; R^+, denotes resistance to ampicillin.

Table 2. *In vitro* activity of (5*R*,6*S*,8*R*) derivatives.



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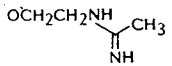
Compound	R_1	Isomer	X	Method of preparation	Antibacterial activity ($\mu g/ml^{-1}$)													Human kidney stability[a]
					E.cl. N1	*E.c.* 0111	*E.c.* JT39 (R^+)	*K.p.* A	*P.m.* 977	*P.v.* W091	*P.a.* A	*S.m.* V520	*S.a.* Ox	*S.a.* Ru (R^+)	*S.a.* 1517	*S.f.* I	*S.p.* CN33	*S.p.* CN10	
21a	$NHCH_2Ph$	*Z*	Na	B	1.6	0.2	≤0.1	≤0.1	0.8	0.4	50	0.8	≤0.1	≤0.1	6.2	0.4	≤0.1	≤0.1	1.5
21b	ONa	*Z*	Na	B	3.1	0.2	≤0.1	0.2	3.1	0.8	100	3.1	0.8	1.6	12.5	3.1	—	≤0.1	1.5
21c	OEt	*Z*	Na	A	0.4	≤0.1	≤0.1	≤0.1	0.4	0.2	50	6.2	≤0.1	≤0.1	0.8	0.2	—	—	1.7
21d	OCH_2CH_2OH	*Z*	Na	B	0.4	≤0.1	≤0.1	≤0.1	0.8	0.2	50	0.2	≤0.1	≤0.1	0.8	0.4	—	—	2.5
21e	OCH_2CH_2NHAc	*Z*	Na	B	0.4	≤0.1	≤0.1	≤0.1	0.4	0.4	50	0.4	≤0.1	≤0.1	6.2	0.8	—	≤0.1	1.7
21f	$OCH_2CH_2NH_2$	*Z*	H	A,B	0.4	≤0.1	≤0.1	≤0.1	0.8	0.4	25	0.8	≤0.1	≤0.1	0.2	0.2	≤0.1	—	2.2
21g	$OCH_2CH_2NHCH_3$	*Z*	H	B	0.4	≤0.1	≤0.1	<0.1	0.8	0.4	25	0.2	≤0.1	≤0.1	0.8	0.2	—	≤0.1	2.6
21h	$OCH_2CH_2NH_2$	*E*	H	B	3.1	0.4	0.4	0.8	1.6	1.6	50	3.1	≤0.1	0.2	1.6	0.4	—	≤0.1	1.9
21i		*Z*	H		0.8	0.2	0.2	0.2	0.8	0.8	25	0.4	≤0.1	≤0.1	1.6	0.2	—	—	2.1
Imipenem					—	<0.1	0.4	0.4	6.2	1.6	3.1	0.8	<0.1	<0.1	0.8	0.2	—	<0.1	2.2
MM 22383					25	12.5	12.5	6.2	12.5	6.2	>100	12.5	6.2	6.2	100	50	—	1.6	1.0

[a] Human kidney stability relative to MM 13902 (1.0).

Abbreviations: See Table 1.

and Gram-negative organisms, including β-lactamase producing strains. Those with the thienamycin stereochemistry (5*R*,6*S*,8*R*) were marginally better than those derivatives having the (5*R*,6*R*,8*S*) stereochemistry of MM 22382. They were clearly superior to MM 22383, confirming that the biological activity was significantly affected by the stereochemical configuration of the hydroxyethyl group at C-8.

Substituted ester derivatives, particularly aminoethyl esters **15e** and **21f** and the hydroxyethyl ester (**21d**) showed an improvement in activity over the parent carboxyethenyl compounds **15b** and **21b**. No evidence of hydrolysis to the parent acid was apparent under the test conditions. Amides **15a** and **21a** were also clearly superior to the parent acids against all organisms. The superior activity of the (*Z*)-carboxyethenylthio-isomers was demonstrated by a comparison of the antibacterial activity of the (*Z*)-isomer of aminoethylcarboxyethenyl-derivative (**21f**) with the corresponding (*E*)-isomer (**21h**). The (*Z*)-isomer (**21f**) was on average some 4-fold more active than the (*E*)-isomer (**21h**).

Unlike thienamycin and imipenem (*N*-formimidoyl thienamycin)[8,9] (Table 2), however, none of these compounds showed any appreciable activity against *Pseudomonas aeruginosa*, despite the introduction of the hydrophilic amino function.

All of the compounds in this study were examined for their stability to human kidney homogenate and their rates of degradation were measured relative to the standard compound, MM 13902. The ability of these compounds to resist degradation by the renal dehydropeptidase enzyme DHP-I showed some improvement over MM 13902. Most compounds were some 2-fold more stable than MM 13902. Indeed, some members of this new class of compounds were more stable than imipenem to human kidney homogenate.

This improved *in vitro* antibacterial activity and metabolic stability was reflected in enhanced activity *in vivo*. For example, the aminoethoxycarbonylethenylthio-compounds **15e** and **21f** were markedly more effective than MM 13902 and MM 22382 against experimental *Staphylococcus aureus* Smith and ampicillin sensitive (E8) *Escherichia coli* infections in the mouse (Table 3). Compound (**21f**) was particularly effective against the Gram-positive infection.

Table 3. *In vivo* activity of **15e** and **21f** against experimental mouse infections.

Organism	CD_{50} mg/kg (total dose)			
	15e	**21f**	MM 13902	MM 22382
S.a. Smith	4.5	<0.4	62	24
E.c. 8	3.1	8.5	56	70

Compounds dosed at 1 and 5 hours post infection.
Abbreviations: *S.a.*, *Staphylococcus aureus*; *E.c.*, *Escherichia coli*.

In conclusion, this family of C-2 modified carbapenem derivatives showed a considerable improvement in antibacterial activity and stability to the DHP-I enzyme compared to the natural products, MM 22382 and MM 22383. Whilst some members of this new class of compounds (for example **21d**, **21f** and **21g**) possessed, with the exception of *Pseudomonas* spp. improved antibacterial activity and stability to human kidney homogenate when compared with imipenem, their poor antipseudomonal activity precluded their progression.

Experimental

MM 13902, MM 22382 and MM 22383 were prepared by fermentation of *Streptomyces olivaceus* ATCC 31365 as described previously.[1,2] Imipenem was a generous gift from Merck Sharp and Dohme Laboratories, Rahway, New Jersey, U.S.A.

MIC

The compounds were serially diluted in 0.05 ml volumes of Nutrient Broth No. 2 (Oxoid) using

microtitre equipment (Dynatech). All microtitre trays were inoculated with a multipoint inoculator (Denleytech) which delivered 0.001 ml of a 1/10 dilution of an overnight broth culture of the test organism, an inoculum equivalent to 10^6 cfu/ml. The MIC was determined after incubation at 37°C for 18 hours as the lowest concentration of antibiotic preventing visible growth.

All MIC values quoted (Tables 1 and 2) were carried out on essentially pure materials.

Tissue Stability Studies

The stabilities of the compounds to human kidney homogenate were determined relative to that of MM 13902, as described previously.[10]

Mouse Protection Studies

Albino mice (OLAC MFI) weight range 18~22 g were infected intraperitoneally with 0.5 ml of a dilution of an overnight broth culture of the infecting organism suspended in 5% hog gastric mucin (American Laboratories Inc.) to yield an infective inoculum of 10 to 100 medium lethal doses. The antibiotics were administered (0.2 ml/20 g) in phosphate buffered saline (pH 7.2) subcutaneously at 1 and 5 hours post infection. Groups of 5 mice were treated with a total of four dose levels for each antibiotic used. The numbers of animals surviving 4 days after infection were recorded and the total dose required to protect 50% of the infected animals was calculated by probit transformation.

Chemistry: General

UV spectra were recorded on a Pye Unicam SP7-500 UV/VIS spectrophotometer. IR spectra were recorded on a Perkin-Elmer 197 or 457 machine. ^{1}H NMR spectra were recorded at 90 MHz on a Perkin-Elmer R32 and at 250 MHz on a Bruker WM 250 instrument with tetramethylsilane as internal standard for spectra in $CDCl_3$ and DMF-d_7, and acetonitrile as external standard for spectra in D_2O. The purity of all compounds was tested by TLC on Merck pre-coated silica gel 60 F_{254} plates. Preparative chromatography was carried out on columns of Merck silica gel 60 (1 : 1 mixture of finer than 230 mesh and 230~400 mesh ASTM) using the slightly increased pressure of a Medcalf Hy-flo pump. Sodium salts and zwitterions were purified by column chromatography over Diaion HP-20SS resin, eluting with ethanol-water mixtures and monitoring the column fractions by UV and HPLC on an Altex 110A instrument using a C_{18} μBondapak reverse phase column. The purity of the products **15** and **21** was determined by assay using the characteristic olivanic acid/thienamycin chromophore at λ_{max} nm 295~325 in the UV spectrum and was based on an estimated ε 15,000 for C-2 carboxyethenylthio-compounds. Numbering in experimental section based on 7-oxo-1-azabicyclo[3.2.0]hept-2-ene ring system according to systematic IUPAC nomenclature.[11]

General Procedure for Preparation of (5*R*,6*R*,8*S*) and (5*R*,6*S*,8*R*) Derivatives *via* **13** and **19** (Schemes 1 and 2, Method A)

p-Nitrobenzyl (5*R*,6*R*)-3-[2-*p*-Nitrobenzyloxycarbonylethenylthio]-6-[(*S*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**14b**)

A solution of the *p*-nitrobenzyl ester of MM 22382 (**12**) (0.300 g) in dioxan (6 ml) and water (0.9 ml) was stirred with *N*-bromoacetamide (0.090 g) at room temperature for 4.5 minutes. Chloroform (30 ml) was added and the organic solution was washed with 0.05 M pH 7 phosphate buffer (20 ml), followed by dilute sodium chloride solution. Evaporation of the dried ($MgSO_4$) organic solution afforded the C-2 thiol (**6**) as a foam, which was dissolved in dry DMF and stirred at room temperature for 25 minutes with *p*-nitrobenzyl propiolate (0.275 g) and potassium carbonate (0.045 g). The solution was then diluted with ethyl acetate, washed with water, saturated sodium chloride solution, dried ($MgSO_4$) and evaporated. The residue was chromatographed over silica gel, eluting with a gradient of 50~100% ethyl acetate-hexane. The first eluted component was the (*E*)-isomer of **14b** (0.028 g; 7%); UV λ_{max}^{EtOH} nm 335, 265; IR ν_{max} ($CHCl_3$) cm^{-1} 1785, 1715; ^{1}H NMR (acetone-d_6) δ 1.35 (3H, d, J=6 Hz, CH_3CH), 3.25~3.90 (3H, m, 4-CH_2+6-CH), 4.45 (3H, m, 5-CH+CH_3CH+OH), 5.35 (2H, s, CH_2Ar), 5.30 and 5.55 (each 1H, d, J=14 Hz, $ArCH_2$), 6.30 (1H, d, J=15 Hz, CH=C), 7.62~8.30 (9H, m, Ar+C=CH).

The second eluted component was the (*Z*)-isomer of **14b** (0.100 g; 26%); UV λ_{max}^{EtOH} nm (ε) 335 (21,998),

263 (21,785); IR ν_{max} (KBr) cm^{-1} 1780 and 1710; ^1H NMR (DMF-d_7) δ 1.31 (3H, d, J=6.5 Hz, CH_3CH), 3.4~3.85 (3H, m, 4-CH_2+6-CH), 4.05~4.55 (2H, m, 5-CH+$CHCH_3$), 5.11 (1H, d, J=4.5 Hz, OH), 5.42 (2H, s, CH_2Ar), 5.39 and 5.61 (each 1H, d, J=13 Hz, CH_2Ar), 6.26 (1H, d, J=10 Hz, CH=C), 7.07~8.05 (5H, m, Ar+C=CH), 8.28 (4H, d, J=9 Hz, Ar).

Disodium Salt of (5*R*,6*R*)-3-[(*Z*)-2-Carboxyethenylthio]-6-[(*S*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (**15b**)

A solution of the ester (**14b**; *Z*-isomer) (0.095 g) in 25% aq dioxan (20 ml) was shaken with hydrogen in the presence of 5% palladium on carbon catalyst (0.125 g) for 3.5 hours. Sodium hydrogen carbonate (0.024 g) was added to the mixture which was then filtered over Celite, washing well with water. The filtrate was concentrated to small volume (approx 20 ml) and washed with ethyl acetate. The solution was further concentrated and chromatographed on a column of Biogel P2. Fractions containing the product were combined and lyophilised to yield the title compound (**15b**; *Z*-isomer) as a white solid (0.028 g; 49%); UV $\lambda_{max}^{H_2O}$ nm 325 (ε 7,768); IR ν_{max} (KBr) cm^{-1} 1750 and 1590.

General Procedure for Preparation of (5*R*,6*S*,8*R*) Derivatives *via* Formate Ester (**22**; Scheme 2, Method B)

p-Nitrobenzyl (5*R*,6*S*)-3-[2-(2-*p*-Nitrobenzyloxycarbonylaminoethoxycarbonyl)ethenylthio]-6-[(*R*)-1-formyloxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**23f**)

p-Nitrobenzyl (5*R*,6*S*)-3-(*E*-2-acetamidoethenylthio)-6-[(*R*)-1-formyloxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**17**)[5] (377 mg) was dissolved in aqueous 1,4-dioxan (50 ml) and stirred at room temperature for 5 minutes with *N*-bromoacetamide (109.5 mg). Chloroform was added and the organic phase was washed with pH 7.0, 0.05 M phosphate buffer, and saturated sodium chloride solution and dried over anhydrous magnesium sulfate. Filtration and removal of the solvent at reduced pressure afforded the crude thiol (**22**) as a pale yellow oil, IR ν_{max} ($CHCl_3$) cm^{-1} 1781, 1723, 1610, 1560, 1525, 1355, 1340.

The crude thiol (**22**) was dissolved in dry dimethylformamide (10 ml) and stirred at room temperature for 15 minutes with 2-(*p*-nitrobenzyloxycarbonylamino)ethyl propiolate (350 mg) and anhydrous potassium carbonate (109.5 mg). Ethyl acetate was added and the organic solution washed with water, saturated sodium chloride solution and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed at reduced pressure to yield a yellow oil, which was chromatographed over silica gel (20 g). Elution with ethyl acetate afforded the crude product as a pale yellow foam (310 mg). This foam was rechromatographed over silica gel (15 g). Elution with chloroform afforded the product as an oil (155 mg; 29%). This consisted of *p*-nitrobenzyl (5*R*,6*S*)-3-[2-(2-*p*-nitrobenzyloxycarbonylaminoethoxycarbonyl)ethenylthio]-6-[(*R*)-1-formyloxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**23f**) as a mixture of the (*Z*)-isomer and the (*E*)-isomer in the approximate ratio of 9:1; UV λ_{max}^{EtOH} nm (ε) 334 (17,746), 265 (18,579); IR ν_{max} ($CHBr_3$) cm^{-1} 3400, 1781, 1720, 1605, 1520 and 1345; ^1H NMR ($CDCl_3$) δ 1.47 (3H, d, J=7.5 Hz, H_3CCH), 3.24 (1H, dd, J=9.5 and 17 Hz, 4-CH_a), 3.4~3.6 (4H, m, CH_2NH+6-CH+4-CH_b), 4.30 (3H, m, CO_2CH_2+5-CH), 5.20 (2H, s, CH_2Ar), 5.22~5.58 (4H, m, CH_2Ar+8-CH+NH), 6.00 (1H, d, J=10.5 Hz, *cis* CH=C), 7.26 (1H, d, J=10.5 Hz, *cis* CH=C), 7.49 (2H, d, J=8 Hz, Ar), 7.66 (2H, d, J=8 Hz, Ar), 8.07 (1H, s, CHO), 8.13~8.30 (4H, 2×d, Ar).

The (*E*)-isomer shows *inter alia* δ 6.12 (d, J=15 Hz, *trans* CH=C) and 7.77 (d, J=15 Hz, *trans* CH=C). Repeated chromatography gave the (*Z*)-isomer in pure form.

(5*R*,6*S*)-3-[(*Z*)-2-(2-Aminoethoxycarbonyl)ethenylthio]-6-[(*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (**21f**; *Z*-isomer)

The pure ester (**23f**; *Z*-isomer) (500 mg) was dissolved in 1,4-dioxan (75 ml), water (23 ml) and 0.05 M, pH 7.0 phosphate buffer and shaken with hydrogen in the presence of 5% palladium on carbon catalyst (750 mg) at ambient temperature and pressure for 3.5 hours. The solution was filtered over Celite, washing well with water, and evaporated to smaller volume at reduced pressure.

The filtrate was washed with ethyl acetate. The resulting aqueous solution containing the formyloxy-derivative (**24f**) was adjusted to pH 9.0 by the addition of 1 N NaOH solution and stirred at

this pH at ambient temperature for 3.5 hours, when HPLC indicated complete hydrolysis of the formate ester. The pH of the solution was readjusted to pH 7.0, evaporated to small volume and applied to a column of Diaion HP-20SS. Elution with a gradient of 0~5% ethanol-water afforded the title compound as a white fluffy solid (40 mg; 16%) after lyophilisation; UV $\lambda_{max}^{H_2O}$ nm (ε) 325 (10,550); IR ν_{max} (KBr) cm^{-1} 3600~3000 (br), 1750, 1705 and 1570.

General Procedure for Preparation of Amidines

(5*R*,6*R*)-3-[(*Z*)-2-(2-Acetimidoylaminoethoxycarbonyl)ethenylthio]-6-[(*S*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (**15j**; $R_1 = OCH_2CH_2NHC(CH_3)=NH$, (*Z*)-Isomer)

A solution of *p*-nitrobenzyl (5*R*,6*R*)-3-[(*Z*)-2-(2-*p*-nitrobenzyloxycarbonylaminoethoxycarbonyl)ethenylthio]-6-[(*S*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**14e**; *Z*-isomer) (210 mg) in 1,4-dioxan (30 ml), water (9 ml) and 0.05 M, pH 7.0 phosphate buffer (9.5 ml) was shaken with hydrogen for 2 hours in the presence of 5% palladium on carbon catalyst (315 mg). The solution was filtered over Celite, washing well with water (50 ml). The filtrate was concentrated to approximately 40 ml and washed with ethyl acetate (3 × 50 ml). The aqueous solution, which was estimated to contain 72 mg of the amino acid (**15e**, *Z*-isomer), based on ε 15,000 at λ_{max} nm 323 in the UV spectrum, was evaporated to small volume (10 ml) at reduced pressure. The solution was stirred at room temperature and the pH maintained at 8.5 by the addition of 1 N NaOH solution whilst ethyl acetimidate hydrochloride (183 mg) was added over a period of 15 minutes. Stirring was continued at pH 8.5 for a further 45 minutes. The pH was then readjusted to 7.0 and the solution chromatographed over Diaion HP-20. The column was eluted with a gradient of 0~25% ethanol-water and the fractions monitored by UV. Those fractions possessing an absorption at λ_{max} nm 323 in the UV spectrum were combined and the resulting solution containing (5*R*,6*R*)-3-[(*Z*)-2-(2-acetimidoylaminoethoxycarbonylethenylthio]-6-[(*S*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (**15j**; $R_1 = OCH_2CH_2NHC(CH_3)=NH$, *Z*-isomer) was lyophilised to yield a pale yellow fluffy solid (47 mg; 38%); UV $\lambda_{max}^{H_2O}$ nm (ε) 323 (10,311); IR ν_{max} (KBr) cm^{-1} 3500~3000 (br), 1750, 1685, 1638 and 1570; ^1H NMR (D_2O) δ 1.32 (3H, d, J=6 Hz, CH_3CH), 2.20 (3H, s, $CH_3C=N$), 3.2~3.8 (5H, m, 4-CH_2+6-CH+CH_2N), 4.0~4.5 (m, CO_2CH_2+8-CH+5-CH), 5.96 (1H, d, J=10 Hz, CH=C), 7.64 (1H, d, J=10 Hz, CH=C).

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