6-(SUBSTITUTED METHYLENE)PENEMS, POTENT BROAD SPECTRUM INHIBITORS OF BACTERIAL β-LACTAMASE

I. RACEMIC 6-ETHYLIDENEPENEMS

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The dehydration of various 6-(1-hydroxyethyl)penems to give *E*- and *Z*-6-ethylidenepenems is described. Both isomers have been shown to be potent broad spectrum inhibitors of bacterial β-lactamases capable of reducing the MIC values of β-lactam antibiotics such as amoxycillin and cephaloridine against a wide range of resistant organisms.

Since WOODWARD's[1] first description of the penem nucleus the chemistry and biology of these interesting members of the β-lactam family have been the subject of numerous publications.[2] Much of the effort has concentrated on the antibacterial properties of penems bearing a 1-hydroxyethyl function at the C-6 position. We wish to report the dehydration of these compounds to provide 6-ethylidenepenems,[3] a novel class of penems with interesting biological properties. Antibacterial activity is much weaker than in the 6-(1-hydroxyethyl) series, but the ethylidenepenems are potent inhibitors of a wide range of β-lactamases. Subsequent to our undertaking this study, similar properties have been reported for one related compound, 6-acetylmethylene-2-methylpenem-3-carboxylate.[4] β-Lactamase inhibitory properties have also been described for the asparenomycins, a structurally related series of carbapenems.[5]

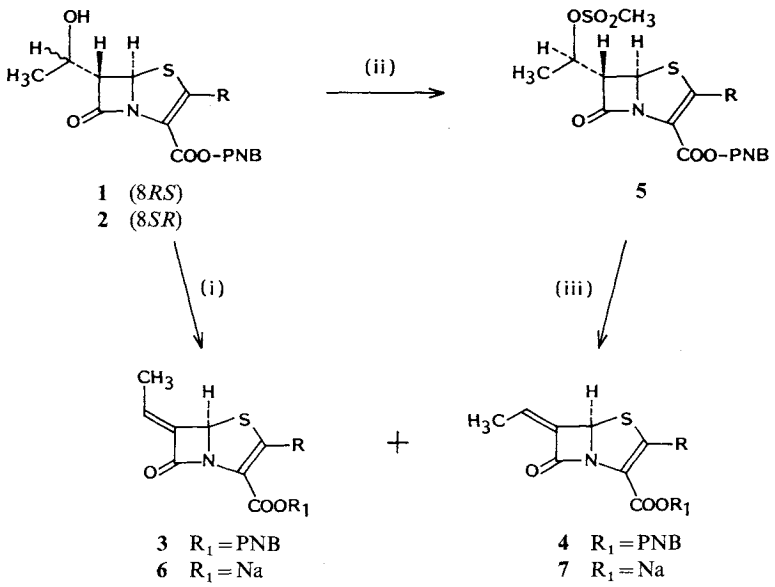
Chemistry

Dehydration of the *trans*-6-[(1*RS*)-hydroxyethyl]penem ester (**1a**)[3] under Mitsunobu conditions (triphenylphosphine/diethyl azodicarboxylate) gave the *Z*-ethylidenepenem ester (**3a**) as the major product. Similar treatment of the (1'*SR*) isomer (**2a**)[3] afforded the *E*-ethylidenepenem ester (**4a**). Both reactions exhibited good stereoselectivity the major/minor isomer ratios being approximately 10:1. Confirmation of the stereochemistry of the double bond was provided by the ^{1}H NMR data. The olefinic proton of the *Z*-isomer (**3a**) showed the expected downfield shift compared with that of the *E*-isomer (**4a**) due to the deshielding effect of the neighbouring β-lactam carbonyl group. The corresponding downfield shift of the methyl group in the *E*-isomer compared to the *Z*-isomer was also evident.

Alternatively, dehydration could be achieved by a mesylation/elimination process (Scheme 1) albeit with a loss of stereoselectivity. Thus, treatment of the mesylate (**5a**)[3] derived from the alcohol (**1a**) with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave a 2:1 mixture of the *Z*- and *E*-penems (**3a**) and (**4a**). Similar treatment of the alcohols (**1b** and **1c**)[3] gave mixtures of corresponding ethylidenepenems from which it was generally possible to isolate one or both isomers by chromatographic and/or fractional crystallisation techniques.

Deprotection of the *p*-nitrobenzyl (PNB) esters was accomplished by hydrogenolysis over palladium/carbon catalyst, providing the sodium salts (**6** and **7**) after treatment with sodium hydrogencarbonate. Chromatography on Biogel P2 provided materials which were homogeneous by NMR

Scheme 1.



1 (8*RS*)
2 (8*SR*)
5

3 R_1=PNB
6 R_1=Na
4 R_1=PNB
7 R_1=Na

a R=SEt, **b** R=$(CH_2)_2CH_3$, **c** R=H.

(i) Ph_3P-EtO_2CN=NCOOEt, (ii) CH_3SO_2Cl-Et_3N, (iii) DBU.
All compounds are racemic, only one enantiomer has been depicted.

Table 1. 6-Ethylidenepenems.

Compound No.	R	R_1	UV λ_{max} nm (ε)[a]	IR ν_{max} cm^{-1}[b]	^1H NMR[c]	
					8-CH_3 (d, J=7 Hz)	8-H (q, J=7 Hz)
3a	SEt	PNB	260 (14,520), 322 (7,980)	1785, 1700 (br)	1.82	6.44
3b	$(CH_2)_2CH_3$	PNB	267 (12,190), 296 (10,500)	1775, 1710	1.79	6.41
3c	H	PNB	265 (12,010), 296 (inflection)	1790, 1720	1.86	6.53
4a	SEt	PNB	260 (14,050), 322 (7,640)	1780, 1690	2.10	5.98
4c	H	PNB	264 (12,170), 292 (inflection)	1785, 1720	2.14	6.05
6a	SEt	Na	305 (3,280)	—	1.71	6.44
6b	$(CH_2)_2CH_3$	Na	284 (4,581)	1757, 1709, 1606	1.78	6.47
6c	H	Na	288 (3,590)	1765, 1700, 1600	1.64	6.34
7a	SEt	Na	212 (8,410), 306 (3,180)	—	1.96	6.08
7c	H	Na	290 (5,030)	—	2.03	6.17

[a] Solvent: EtOH, compounds **2** and **3**; H_2O compounds **6** and **7**.
[b] Medium: $CHCl_3$ solution, compounds **2** and **3**; KBr, compounds **6** and **7**.
[c] δ (90 or 250 MHz) centre of multiplet.

and contained up to 15% water. It was noteworthy that, in contrast to the 6-ethylidenecarbapenems,[6] no reduction of the exocyclic double bond was observed.

Spectral data of the compounds prepared are listed in Table 1.

Biology

The β-lactamase inhibitory activity of selected 6-ethylidenepenems, potassium clavulanate and sulbactam is shown in Table 2. It can be seen that both *E*- and *Z*-isomers of the penems inhibited all four β-lactamases including the Class Ia enzyme of *Enterobacter cloacae* which is only weakly inhibited by potassium clavulanate. Sulbactam showed similar broad spectrum inhibitory activity but was much less potent than the penems. The inhibitory activity of these compounds was reflected also in the synergy observed with β-lactams against β-lactamase-producing organisms. For example Table 3 shows that penems (**6a**~**6c** and **7a**) considerably reduced the MIC values of amoxycillin against *Klebsiella pneumoniae*, *Proteus vulgaris* and resistant strains of *Escherichia coli* and *Staphylococcus aureus*, and that they were as effective as potassium clavulanate against these organisms. In addition the penems improved the activity of amoxycillin against *E. cloacae* whereas potassium clavulanate had no effect. Synergism was not observed with any compound in combination with amoxycillin against *Pseudomonas aeruginosa*.

The cephalosporinase inhibitory activity of the penems (**6a** and **7a**) was seen to a greater extent when tested in combination with cephaloridine (Table 4) when both compounds considerably reduced the MICs against all the test organisms other than *P. aeruginosa*.

Table 2. β-Lactamase inhibitory activity of 6-ethylidenepenems.

Source of β-lactamase	Class[a]	I_{50} (μg/ml)[b]					
		6a	**7a**	**6c**	**7c**	Potassium clavulanate	Sulbactam
Enterobacter cloacae	Ia	0.6	0.4	1.0	0.4	>50	4.0
Escherichia coli (TEM-1)	III	0.007	0.15	0.04	0.2	0.06	1.0
Klebsiella pneumoniae	IV	0.001	0.03	0.015	0.07	0.03	6.0
Staphylococcus aureus Russell	—	0.13	2.5	0.6	3.2	0.08	1.3

[a] Classification of RICHMOND and SYKES.[8]
[b] Concentration giving 50% inhibition of the rate of hydrolysis of nitrocefin after preincubation of enzyme and inhibitor for 5 minutes.

Table 3. Activity (MIC μg/ml) of amoxycillin alone and in combination with 6-ethylidenepenems.

Organism	Class[a]	β-Lactamase inhibitor[b]					
		None	**6a**	**6b**	**6c**	**7a**	Potassium clavulanate
Enterobacter cloacae	Ia	500	25	50	250	12.5	500
Proteus vulgaris	Ic	1,000	1.0	1.0	5.0	1.0	2.5
Pseudomonas aeruginosa	Id	>1,000	>1,000	>1,000	>1,000	>1,000	>1,000
Proteus mirabilis	II	>1,000	100	100	1,000	250	10
Escherichia coli (TEM-1)	III	>1,000	5.0	10	5.0	5.0	5.0
Klebsiella pneumoniae	IV	500	2.5	5.0	2.5	2.5	5.0
Staphylococcus aureus Russell	[d]	100	0.5	<0.2[c]	1.0	0.5	0.2
E. coli (Amox^s)	—	5.0	5.0	5.0	2.5	5.0	5.0
P. mirabilis (Amox^s)	—	2.5	2.5	2.5	2.5	2.5	2.5
S. aureus Oxford	—	0.2	0.2	0.2	0.2	0.2	0.2

[a] as for Table 2.
[b] Concentration 5.0 μg/ml for Gram-negative bacteria (MIC alone 31~250 μg/ml) and 1.0 μg/ml for *S. aureus* (MIC alone 2~16 μg/ml). Amox^s indicates amoxycillin-sensitive strain.
[c] MIC of inhibitor **6b** = 1.0 μg/ml in this test.
[d] *Staphylococcus*.

Table 4. Activity (MIC μg/ml) of cephaloridine alone and in combination with 6-ethylidenepenems.

Organism	Class[a]	β-Lactamase inhibitor[b]				
		None	**6a**	**7a**	**6c**	Potassium clavulanate
Enterobacter cloacae	Ia	1,000	2.5	2.5	10	1,000
Morganella morganii	Ia	>1,000	10	25	100	>1,000
Citrobacter freundii	Ia	500	25	25	10	500
Escherichia coli	Ib	100	50	10	10	100
Proteus vulgaris	Ic	1,000	5.0	2.5	5.0	5.0
Pseudomonas aeruginosa	Id	>1,000	>1,000	>1,000	>1,000	>1,000
Proteus mirabilis	II	100	5.0	5.0	10	5.0
E. coli (Amox^s)	—	2.5	2.5	2.5	5.0	2.5
P. mirabilis (Amox^s)	—	5.0	5.0	5.0	10	5.0

[a] and [b] as for Table 3.

Conclusion

The present study has established that 6-ethylidenepenems are potent inhibitors of bacterial β-lactamase with a broader spectrum of activity than potassium clavulanate and greater potency than sulbactam. Their ability to reduce the MIC values of β-lactams against β-lactamase producing bacteria has also been demonstrated. Neither changes in the geometry of the ethylidene double bond nor of the 2-substituent resulted in any gross changes in synergistic activity.

Further studies on 6-(substituted methylene)penems and the structure-activity relationships within this novel class of β-lactamase inhibitors will form the subject of succeeding publications.

Experimental

UV spectra were recorded on a Perkin-Elmer 554 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 197 or 457 machine. ^{1}H NMR spectra were recorded at 60 MHz on a Varian EM360, at 90 MHz on a Perkin-Elmer R32, or at 250 MHz on a Bruker WM250 instrument using TMS or HOD as internal standard. Molecular ion determinations were carried out on a VG (Altringham UK) 7070F MS using PFK as standard. Preparative chromatography was carried out under slight pressure on columns of Merck Kieselgel 60 using the stated eluent. MP's were determined on a Kofler Hot Stage apparatus and are uncorrected.

β-Lactamase inhibition studies were carried out on isolated enzyme preparations by spectrophotometric monitoring of hydrolysis of nitrocefin in the presence and absence of the test compound.[7]

Synergism Studies: Serial dilutions of amoxycillin or cephaloridine were added to 18 ml volumes of Blood Agar Base (Oxoid) together with a constant concentration (1.0 μg/ml or 5.0 μg/ml) of the test inhibitor and poured into Petri-dishes. Control plates without inhibitor were also prepared. The plates were inoculated with 10^6 cfu and the MIC values recorded after 18 hours at 37°C.

General Procedures for the Dehydration of 6-(1-Hydroxyethyl)penem Esters

Diethyl Azodicarboxylate/Triphenylphosphine Method: PNB-(5*RS*,6*SR*)-2-ethylthio-6-[(1*RS*)-hydroxyethyl]penem-3-carboxylate (**1a**) (82 mg) was dissolved in dry dichloromethane (5 ml), cooled in an ice bath, and treated with triphenylphosphine (52 mg) and diethyl azodicarboxylate (35 mg). The ice bath was removed and the mixture was stirred for 15 minutes. The stirred mixture was re-cooled in an ice bath and treated with triphenylphosphine (52 mg) and diethyl azodicarboxylate (35 mg). The ice bath was again removed and after stirring for a further 15 minutes the mixture was evaporated. Chromatography (chloroform) and fractional crystallisation (ethyl acetate-petroleum ether) of crude product gave PNB-(5*RS*)-(*Z*)-6-ethylidene-2-ethylthiopenem-3-carboxylate (**3a**) (44 mg) as fine yellow needles: MP 169~171°C; UV λ_{max}^{EtOH} nm (ε) 260 (14,520), 322 (7,980); IR ν_{max} ($CHCl_3$) cm^{-1} 1785, 1700 (br); ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.36 (3H, t, J=7 Hz), 1.82 (3H, d, J=7 Hz), 2.75~3.15 (2H, m), 5.19 and 5.49 (2H,

ABq, $J=14$ Hz), 6.16 (1H, s), 6.44 (1H, q, $J=7$ Hz), 7.63 (2H, d, $J=8$ Hz), 8.19 (2H, d, $J=8$ Hz); MS m/z 392.0503 (M^+); $C_{17}H_{16}N_2O_5S_2$ requires M, 392.0500.

Similar treatment of the isomeric penem ester (**2a**) (100 mg) provided the *E*-ethylidenepenem ester (**4a**) (48 mg) as a solid.

Mesylation/Elimination Method: A solution of methanesulfonyl chloride (491 mg) in dry dichloromethane (2 ml) was added, dropwise over 2 minutes, to a stirred mixture of PNB-(5*RS*,6*SR*)-6-[(1*RS*)-hydroxyethyl]penem-3-carboxylate (**1c**) (750 mg) and triethylamine (433 mg) in dry dichloromethane (25 ml) at -10°C. After stirring at -10°C for 15 minutes the mixture was diluted with ethyl acetate (50 ml) and was washed with brine (3×10 ml). The dried ($MgSO_4$) organic layer was evaporated to give the crude mesylate (**5c**) as a gum: IR ν_{max} ($CHCl_3$) cm^{-1} 1795, 1720; ^1H NMR (60 MHz, $CDCl_3$) δ 1.61 (3H, d, $J=7$ Hz), 3.28 (3H, s), 4.12 (1H, dd, $J=2$ and 5 Hz), 5.20 (1H, dq, $J=7$ and 7 Hz), 5.28 and 5.52 (2H, ABq, $J=14$ Hz), 5.94 (1H, d, $J=2$ Hz), 7.46 (1H, s), 7.66 (2H, d, $J=8$ Hz), 8.29 (2H, d, $J=8$ Hz).

The crude mesylate (**5c**) was dissolved in dry dichloromethane (25 ml), cooled to -20°C, and treated, dropwise over 2 minutes, with a solution of DBU (488 mg) in dry dichloromethane (2 ml). After stirring at -20°C for 10 minutes the mixture was diluted with ethyl acetate (50 ml) and was washed with 5% citric acid (10 ml), brine (10 ml), saturated sodium hydrogencarbonate (10 ml) and brine (3×10 ml). The dried ($MgSO_4$) organic layer was evaporated and the residue chromatographed using dichloromethane-ethyl acetate mixtures to give two fractions. The less polar fraction was evaporated and the residue crystallised from ethyl acetate-hexane to give PNB-(5*RS*)-(*E*)-6-ethylidenepenem-3-carboxylate (**4c**) (76 mg) as small needles: MP 151~152°C; UV λ_{max}^{EtOH} nm (ε) 264 (12,170), approx 292 (inflection); IR ν_{max} ($CHCl_3$) cm^{-1} 1785, 1720; ^1H NMR (250 MHz, $CDCl_3$) δ 2.14 (3H, d, $J=7.1$ Hz), 5.28 and 5.46 (2H, ABq, $J=14$ Hz), 6.05 (1H, q, $J=7.1$ Hz), 6.30 (1H, s), 7.39 (1H, s), 7.61 (2H, d, $J=9$ Hz), 8.24 (2H, d, $J=9$ Hz); MS m/z 332.0457 (M^+); $C_{15}H_{12}N_2O_5S$ requires M, 332.0467. The more polar fraction was evaporated and the residue crystallised from ethyl acetate-hexane to give PNB-(5*RS*)-(*Z*)-6-ethylidenepenem-3-carboxylate (**3c**) (380 mg) as rods: MP 165~167°C; UV λ_{max}^{EtOH} nm (ε) 265 (12,010), 296 (inflection); IR ν_{max} ($CHCl_3$) cm^{-1} 1790, 1720; ^1H NMR (250 MHz, $CDCl_3$) δ 1.86 (3H, d, $J=7.1$ Hz), 5.28 and 5.45 (2H, ABq, $J=13$ Hz), 6.32 (1H, d, $J=1$ Hz), 6.53 (1 H, q, $J=7.1$ Hz with further fine coupling $J=$approx 1 Hz), 7.37 (1H, s), 7.61 (2H, d, $J=9$ Hz), 8.24 (2H, d, $J=9$ Hz); MS m/z 332.0474 (M^+); $C_{15}H_{12}N_2O_5S$ requires M, 332.0467. Similar treatment of compound (**1a**) gave a 2:1 ratio of the *Z*- and *E*-penem esters (**3a** and **4a**) in 62% overall yield. Treatment of compound (**1b**) gave a 5:2 ratio of *Z*- and *E*-penem esters (**3b** and **4b**) from which the pure *Z*-isomer (**3b**) (29%) was obtained by fractional crystallization, mp 101~102°C (rods ex ethyl acetate-petroleum ether).

General Procedure for the Preparation of Sodium Salts

The *Z*-ethylidenepenem ester (**3c**) (56 mg) was dissolved in a mixture of dioxan (8 ml) and water (2 ml) and was hydrogenated over 5% palladium/carbon catalyst (84 mg) for 45 minutes at 1 atmosphere. Further catalyst (56 mg) was added and the hydrogenation continued for 30 minutes. The mixture was treated with sodium hydrogencarbonate (1.42 ml of a 1% aqueous solution) and filtered through Kieselguhr, the residual catalyst being washed with a little 50% aqueous dioxan. The combined filtrates were evaporated and the residue chromatographed on Biogel P2 eluting with water. The appropriate fractions were evaporated and the residue re-evaporated from ethanol (2 ml) and dry toluene (2×2 ml) to give, after trituration with dry ether, sodium (5*RS*)-(*Z*)-6-ethylidenepenem-3-carboxylate (**6c**) (20 mg) as a buff coloured amorphous solid: UV λ_{max}^{EtOH} nm (ε) 288 (3,590); IR ν_{max} (KBr) cm^{-1} 3700~2300, 1765, 1700, 1600, 1560 (sh); ^1H NMR (250 MHz, D_2O) δ 1.64 (3H, d, $J=7$ Hz), 6.20 (1H, s), 6.34 (1H, q, $J=7$ Hz), 6.90 (1H, s).

Similar treatment of the esters (**3a**, **3b**, **4a** and **4c**) gave the corresponding sodium salts (**6a**, **6b**, **7a** and **7c**) respectively.

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