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

III. STRUCTURE-ACTIVITY RELATIONSHIPS OF THE 5-MEMBERED HETEROCYCLIC DERIVATIVES

I. BENNETT, N. J. P. BROOM*, G. BRUTON, S. CALVERT, B. P. CLARKE, K. COLEMAN*, R. EDMONDSON, P. EDWARDS, D. JONES, N. F. OSBORNE and G. WALKER

SmithKline Beecham Pharmaceuticals, Chemotherapeutic Research Centre, Brockham Park, Betchworth, Surrey, RH3 7AJ, UK

(Received for publication September 7, 1990)

Sodium (5*RS*)-*Z*-6-(heterocyclylmethylene)penem-3-carboxylates (**2**) are a series of extremely potent inhibitors of bacterial β-lactamases. A variety of 5-membered heteroaromatic derivatives have been prepared and structure-activity studies reveal a preferred substituent orientation.

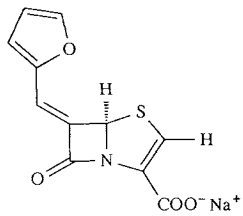
One of these derivatives, the 1-methyl-1,2,3-triazolyl compound (**5m**) is a more potent synergist of amoxycillin than clavulanic acid, sulbactam or tazobactam.

Earlier papers[1,2] have described the synthesis and biological properties of a series of racemic 6-(substituted methylene)penems. Of particular interest was the 2-furyl derivative (**1**) which had the *Z* configuration about the C-6–C-8 double bond and was unsubstituted at the 2-position[2]. This paper outlines some of the further work on a series of penems (**2**) in which we sought to investigate the effect of varying aromatic heterocyclic substituents on the biological properties of the series. A number of 5-membered heterocycles have been synthesised and some conclusions on structure-activity relationships within this group of derivatives are presented.

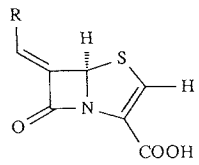
Chemistry

The *Z*-isomers (**4a**～**4q**) were prepared in excellent yield by treating the 2-unsubstituted penem acetates (**3a**～**3q**)[3] with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at low temperature. Removal of the *p*-nitrobenzyl (PNB) protecting group was readily achieved by hydrogenolysis over palladium on carbon followed by treatment with sodium hydrogen carbonate. The resulting sodium salts (**5a**～**5q**) were obtained as homogeneous freeze-dried solids after chromatography on Biogel P2.

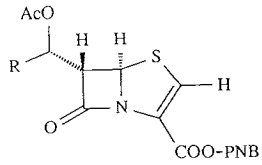
The UV spectra of the penems (**5a**～**5q**) implied a degree of π orbital overlap between the aromatic heterocyclic ring and the exocyclic double bond consistent with a coplanar conformation. Molecular models suggested that in compounds bearing either a substituent or a proton at position 4′ (see Fig. 1) conformation A could show fewer non-bonded interactions between the heterocycle and the penem nucleus than conformation B. Support for this view was also obtained from ^{1}H NMR data on some of these compounds (**4g**, **4j** and **5m**). NOE's were observed between the heterocyclic ring proton (4′-H) and both 8-H and 5-H. The results of the experiments are shown in Table 1 and may be interpreted as indicating a fast equilibrium between conformers **A** and **B**. Furthermore the differences in internuclear distances and the ratio of **A**:**B** from the NOE studies imply that **A** is the major conformer[4].



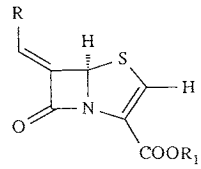
1



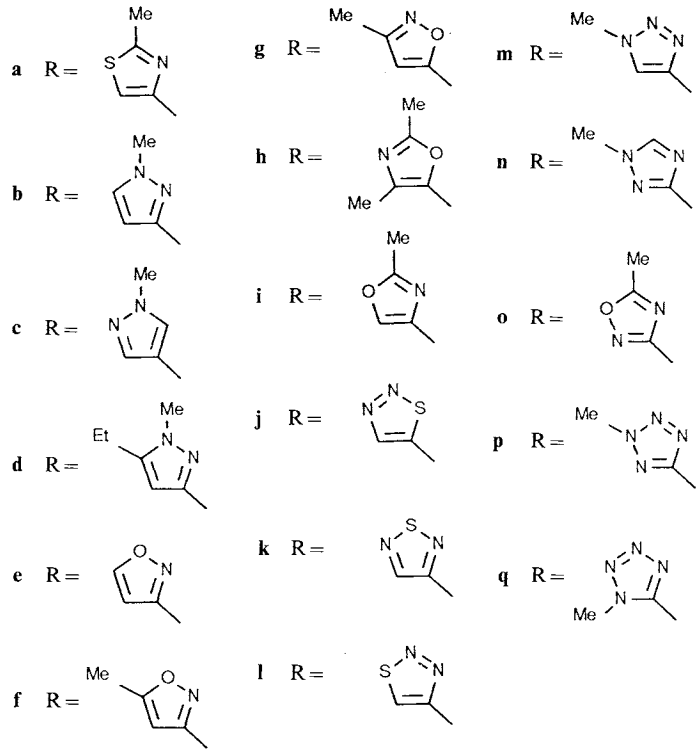
2 R = 5-ring heterocycle



3

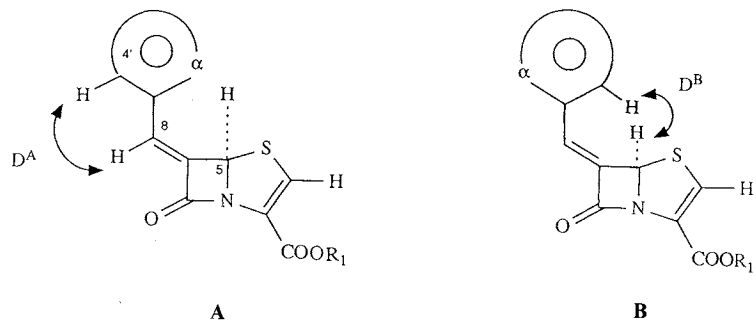


4 R_1 = PNB
5 R_1 = Na



All compounds are racemic, only one enantiomer is depicted.

Fig. 1. Planar conformations of the penem molecule (**2**).
α = N, O or S.



A **B**

Biological Properties and Structure-activity Relationships

The inhibitory activity seen against cell-free β-lactamases is shown in Table 2. The heterocyclic derivative (**5a**～**5m**, **5o**～**5q**) showed the same high level of inhibitory activity observed with previously described penems[1,2], such as the 2-furyl derivative (**1**), and were generally more active than clavulanic acid, sulbactam or tazobactam.

The synergy (of antibacterial activity) with amoxycillin is shown in Table 3, where the differences in the potency of these compounds were more pronounced. For example, **5h** and **5i** had similar inhibitory activity against the Class IV enzyme (Table 2), but were very different in the degree of which they protected amoxycillin against a strain of *Klebsiella pneumoniae* producing the same enzyme (Table 3). In such cases, the difference may be attributable to differing rates of penetration into the bacterial periplasm, and the compounds showing the best activity in Table 3 (**5b**, **5d**, **5e**, **5k**, **5l** and **5m**) are considered those which penetrate the bacterial outer membrane and have good β-lactamase inhibitory activity. These six compounds showed a broader spectrum of activity than either clavulanic acid, sulbactam or tazobactam, with the most notable improvements in activity seen against the Class Ia and V β-lactamase producing organisms.

Table 1. Results of NOE studies and internuclear distances[a].

	D^A (Å)	D^B (Å)	A:B
4g	2.93	2.68	93:7
4j	2.71	2.54	55:45
5m	2.85	2.51	70:30

[a] See Fig. 1.

Table 2. β-Lactamase inhibitory activity.

Compound	I_{50}[a] (μg/ml)					
	E.cl. Ia[b]	*P.m.* II	*E.co.* (TEM-1) III	*K.p.* IV	*E.co.* (OXA-1) V	*S.a.*
1	0.005	0.025	0.003	0.002	0.002	0.013
5a	0.001	0.005	0.002	0.001	0.002	0.015
5b	0.003	0.016	0.003	0.002	0.004	0.026
5c	0.004	0.32	0.003	0.009	0.002	0.6
5d	0.5	0.011	0.004	0.002	0.003	0.033
5e	0.002	0.03	0.005	0.004	0.03	0.02
5f	NT	0.001	0.003	0.004	0.001	0.014
5g	0.01	0.009	0.014	0.01	0.065	0.059
5h	0.002	1.0	0.06	0.003	0.001	0.3
5i	0.007	0.015	0.001	0.001	0.003	0.035
5j	0.12	0.16	0.013	0.065	0.005	0.1
5k	0.013	0,017	0.003	0.002	0.002	0.04
5l	0.053	0.013	0.002	0.002	0.003	0.03
5m	0.001	0.005	0.003	0.001	0.001	0.005
5n	NT	NT	NT	NT	NT	NT
5o	0.017	0.007	0.02	0.008	0.013	0.024
5p	0.007	0.008	0.05	0.025	0.009	0.07
5q	0.001	0.006	0.005	0.047	0.002	0.025
Clavulanic acid	>50:0	0.03	0.05	0.019	0.6	0.06
Sulbactam	2.8	0.08	1.9	10.0	3.2	1.5
Tazobactam	0.02	0.02	0.02	0.1	1.15	0.35

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

[b] Enzyme classification based on RICHMOND and SYKES[6].

Abbreviations: *E.cl.*, *Enterobacter cloacae*; *P.m.*, *Proteus mirabilis*; *E.co.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *S.a.*, *Staphylococcus aureus*.

NT: Not tested.

Table 3. Antibacterial activity of amoxycillin in the presence of 1 μg/ml inhibitor.

Compound	Amoxycillin MIC (μg/ml)					
	E.cl. Ia[a]	*P.m.* II	*E.co.* (TEM-1) III	*K.p.* IV	*E.co.* (OXA-1) V	*S.a.*
None	512	>512	>512	256	>512	128
1	16	4	2	4	32	0.3
5a	NT	8	8	4	NT	0.3
5b	2	4	1	2	16	0.5
5c	4	64	4	32	32	>8
5d	2	4	8	8	16	1.0
5e	2	2	1	4	16	0.1
5f	64	2	8	8	64	0.1
5g	8	4	8	16	16	0.3
5h	NT	256	128	64	NT	>8
5i	128	8	4	4	64	0.3
5j	128	>512	64	>64	64	0.5
5k	4	2	8	8	4	0.1
5l	2	16	2	2	8	0.1
5m	2	4	4	2	4	Inhib
5n	32	4	16	1	8	0.5
5o	64	8	128	32	>512	0.3
5p	256	4	4	8	>512	0.3
5q	256	64	32	32	>512	0.5
Clavulanic acid	>512	16	8	4	>512	0.5
Sulbactam	256	64	128	64	>512	4.0
Tazobactam	256	16	8	16	>512	1.0

The antibacterial activity of the inhibitors was 1~16 μg/ml against *S.a.* and 32~256 μg/ml against the other five organisms.

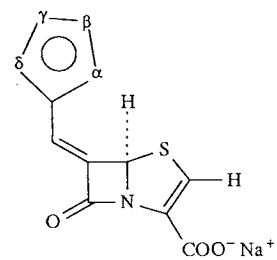
[a] Enzyme classification based on RICHMOND and SYKES[6].

Abbreviations: See Table 2.

NT: Not tested.

Inhib: Inhibitor showed antibacterial activity (MIC 1 μg/ml).

Using the differing degrees of synergy seen with amoxycillin in Table 3, it was possible to draw certain conclusions on the effects of various heterocyclic structural elements on biological activity. Using the general structure (**6**) as a template for the penems (**5a**~**5q**), the following structure-activity requirements were obtained.



6

The most active derivatives had oxygen or preferably nitrogen at the α-position and a C-H function at the δ-position. For example, the pyrazole (**5b**) and the triazole (**5m**) were more effective than the pyrazole (**5c**) which contained C-H as the α and δ substituents. In the thiadiazole series, **5j** (α=S) was less active than the regioisomers (**5k** and **5l**) (α=N).

The addition of a further hetero atom to the δ position did not result in an increase but a decrease in activity; compare the tetrazole (**5p**) with the 1,2,3-triazole (**5m**) or the oxadiazole (**5o**) with the oxazole (**5i**).

Whilst the presence of small alkyl groups at either the β or γ positions was well tolerated, the introducion of even a methyl substituent at the δ position on either carbon or nitrogen resulted in a less active compound.

Thus the oxazole (**5h**) and the tetrazole (**5q**) showed weak synergistic activity. As these derivatives had good β-lactamase inhibitory activity, these differences may be indicative of a penetration effect as previously described.

In a previous paper[2] we noted the poor *in vivo* activity of the 2-furyl derivative (**1**) relative to clavulanic acid against an *Escherichia coli* TEM-1 infection in mice. A number of these penems had *in vitro* activity similar to that of **1** against a TEM-1 producing *E. coli* and eight derivatives were tested *in vivo* against an *E. coli* TEM-1 infection (Table 4). Five of these compounds (**5a**, **5b**, **5i**, **5l** and **5m**) were more potent synergists of amoxycillin than **1** but only the 1,2,3-triazole (**5m**) proved more potent than clavulanic acid or tazobactam.

The differences observed *in vivo* presumably reflect the differing distribution characteristics and/or metabolic stability of the penems. The results of further studies in these areas form the subject of a succeeding publication.

Table 4. CD_{50} of amoxycillin in the presence of 2 mg/kg inhibitor against a TEM-1 producing *Escherichia coli* infection in mice.

Compound	CD_{50} (mg/kg × 2)
None	>1,000
1	240
5a	91
5b	85
5c	>250
5e	>250
5i	143
5l	80
5m	13
5n	>250
Clavulanic acid	70
Sulbactam	>250
Tazobactam	70

Table 5. Spectral data of penem esters (**4**).

Compound	IR ν_{max} ($CHCl_3$) cm^{-1} (β-lactam)	UV λ_{max}^{EtOH} nm (ε_m)	^1H NMR ($CDCl_3$)			
			2-CH[a]	5-CH[b]	8-CH[b]	Heterocycle
4a	1775	299 (23,100)	7.37	6.64	7.00	2.73 (3H, s), 7.40 (1H, s)
4b	1775	297 (30,430)	7.35	6.50	7.04	3.96 (3H, s), 6.40 (1H, d, J=2.3 Hz)
4c	1780	298 (34,025)	7.34	6.48	7.10	3.96 (3H, s), 7.50 (1H, s), 7.51 (1H, s)
4d	1780	301 (32,440)	7.34	6.48	6.99	1.28 (3H, t, J=7.5 Hz), 2.61 (2H, q, J=7.5 Hz), 3.82 (3H, s), 6.17 (1H, s)
4e	1790	256 (24,740), 305 (10,620)	7.41	6.49	7.05	6.50 (1H, d, J=1.2 Hz), 8.53 (1H, d, J=1.9 Hz)
4f	1785	256 (28,800), 304 (12,010)	7.40	6.47	6.98	2.49 (3H, s), 6.10 (1H, d, J=0.6 Hz)
4g	1775	290 (24,000)	7.40	6.55	6.98	2.34 (3H, s), 6.35 (1H, s)
4h	1780	311 (34,430)	7.37	6.55	6.96	2.27 (3H, s), 2.51 (3H, s)
4i	1785	287 (27,260)	7.37	6.63	6.93	2.48 (3H, s), 7.78 (1H, s)
4j	1790	267 (19,200), 296 (19,640)	8.00	6.89	7.92	9.92 (1H, s)
4k	1760	267 (18,180), 301 (25,990)	7.94	6.75	7.67	9.11 (1H, s)
4l	1785	272 (31,210)	7.94	6.82	7.69	9.50 (1H, s)
4m	1780	282 (27,180)	7.39	6.68	7.06	4.15 (3H, s), 7.72 (1H, s)
4n	1780	272 (21,425)	7.38	6.58	7.12	3.97 (3H, s), 8.06 (1H, s)
4o	1780	243 (22,955), 303 (10,915)	7.40	6.58	7.10	2.64 (3H, s)
4p	1785	—	7.41	6.60	7.26	4.41 (3H, s)
4q	1785	—	7.45	6.63	7.04	4.17 (3H, s)

[a] s.

[b] Either br s or d, J=0.5~1.0 Hz.

Table 6. Spectral data of penem salts (**5a**~**5q**).

Compound	UV $\lambda_{max}^{H_2O}$ nm (ε_m)	^{1}H NMR (D_2O)			
		2-CH[a]	5-CH[b]	8-CH[b]	Heterocycle
5a	295 (23,300)	7.02	6.65	7.13	2.71 (3H, s), 7.69 (1H, s)
5b	291 (23,230), 360 (2,040)	7.00	6.52	7.09	3.90 (3H, s), 6.42 (1H, d, J=2.3 Hz) 7.62 (1H, d, J=2.3 Hz)
5c	293 (23,150)	7.05	6.59	7.18	3.92 (1H, s), 7.69 (1H, s), 7.86 (1H, s)
5d	289 (23,180)	6.98	6.45	6.97	1.19 (3H, t, J=7.5 Hz), 2.59 (2H, q, J=7.5 Hz), 3.75 (3H, s), 6.17 (1H, s)
5e	248 (14,470), 290 (infl), 375 (1,310)	7.10	6.59	7.22	6.59 (1H, s), 8.71 (1H, s)
5f	287 (16,000), 366 (1,400)	7.10	6.64	7.14	2.30 (3H, s), 6.61 (1H, s)
5g	287 (15,970), 366 (1,420)	7.10	6.64	7.14	6.41 (1H, s)
5h	305 (22,330)	7.06	6.61	7.11	2.22 (3H, s), 2.28 (3H, s)
5i	286 (20,000)	7.05	6.64	7.07	2.47 (3H, s), 8.06 (1H, s)
5j	295 (15,450)	7.06	6.56	7.59	8.94 (1H, s)
5k	244 (9,520), 301 (19,790)	7.10	6.68	7.48	8.88 (1H, s)
5l	273 (21,580)	7.05	6.67	7.46	9.15 (1H, s)
5m	282 (19,880)	7.07	6.62	7.21	4.13 (1H, s), 8.16 (1H, s)
5n	274 (11,150), 375 (1,030)	7.04	6.59	7.11	3.95 (3H, s), 8.39 (1H, s)
5o	240 (14,400), 290 (5,300)	7.11	6.62	7.14	2.63 (3H, s)
5p	254 (9,495)	7.10	6.62	7.32	4.41 (3H, s)
5q	253 (13,910), 370 (940)	7.10	6.61	7.32	4.16 (3H, s)

[a] s.

[b] Either br s or d, J=0.5~1.0 Hz.

Experimental

β-Lactamase inhibition studies were carried out on isolated enzyme preparations as previously described[5].

MIC determinations were carried out in microtitre plates as previously described[5].

The 50% curative dose (CD_{50}) determinations were performed in mice. The organism (*E. coli* E96) was suspended in 3% hog gastric mucin +1% carboxymethylcellulose at $100 \times LD_{50}$, and 0.5 ml of suspension was injected ip into groups of five mice. Compounds were administered subcutaneously at 2 mg/kg with varying doses of amoxycillin at 1 and 5 hours post-infection. Survivors were recorded over a 4-day period. The CD_{50} of amoxycillin in the presence of inhibitor was calculated using log probit analysis.

For chromatographic and spectral details see Part I[1]. The preparation of the penem derivatives (**3**) has been described in a patent application[3]. The penem esters (**4**) were prepared from the acetates (**3**)[3] using the general elimination procedure described in Part II[2]. Spectral data for these compounds are shown in Table 5. The sodium salts (**5**) were obtained from the esters (**4**) using the general deprotection procedure described in Part II[2]. The spectral data for these compounds (Table 6) showed that the salts were homogeneous and contained up to 15% water.

The interatomic distances D^A (8-H to 4'-H) and D^B (5-H to 4'-H) were measured from structures generated using the following method. The penem nucleus was obtained from an X-ray of **5m** and the heterocycles were added on using CHEM-X. The geometries of these structures were then optimised by

AMPAC (AM1).

Acknowledgements

The authors would like to thank Dr. J. H. C. NAYLER and Dr. I. FRANCOIS for their advice and encouragement. They are also indebted to Mr. J. W. TYLER and Dr. J. EVERETT for the ^{1}H NMR data and to Mrs. P. A. HUNTER and Mr. D. GRIFFIN for some of the biological data.

References

1) BASKER, M. J. & N. F. OSBORNE: 6-(Substituted methylene)penems, potent broad spectrum inhibitors of bacterial β-lactamase. I. Racemic 6-ethylidinepenems. J. Antibiotics 43: 70~75, 1990
2) BROOM, N. J. P.; K. COLEMAN, P. A. HUNTER & N. F. OSBORNE: 6-(Substituted methylene)penems, potent broad spectrum inhibitors of bacterial β-lactamase. II. Racemic furyl and thienyl derivatives. J. Antibiotics 43: 76~82, 1990
3) OSBORNE, N. F. (Beecham): Novel compounds. Eur. Pat. Appl. 0 154 132 A, Sept. 11, 1985
4) NOGGLE, J. H. & R. E. SCHIRMER (*Ed.*): The Nuclear Overhauser Effect. Academic Press, 1971
5) BROOKS, G.; K. COLEMAN, J. S. DAVIES & P. A. HUNTER: Synthesis and β-lactamase inhibitory activity of 9-(2-amidoethenylthio)-9-deoxy derivatives of clavulanic acid. J. Antibiotics 41: 892~898, 1988
6) RICHMOND, M. H. & R. B. SYKES: The β-lactamases of Gram negative bacteria and their possible physiological role. Adv. Microb. Physiol. 9: 31~88, 1973