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

II. RACEMIC FURYL AND THIENYL DERIVATIVES

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A series of racemic 6-(substituted methylene)penems have been prepared. These compounds contain a 5-membered monoheteroaromatic ring at C-8. The antibacterial/synergistic and β-lactamase inhibitory activities of both *E*- and *Z*-isomers and 2-substituted derivatives are compared.

In our previous paper we described the synthesis and biological activity of 6-ethylidenepenems (**1**)[1]. These novel penem derivatives are potent broad-spectrum β-lactamase inhibitors and also act as synergists with penicillins and cephalosporins against β-lactamase producing bacteria. We have investigated the replacement of the C-8 methyl group with other functions and in this paper we wish to report on the extension of this work to 6-(substituted methylene)penems (**2**) containing a 5-membered monoheteroaromatic moiety at C-8[2].

Chemistry

Reaction of the 2-unsubstituted penem acetates (**3a**～**3d**)[2] with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at low temperature resulted in the smooth loss of acetic acid and excellent yields of the *Z*-isomers (**4a**～**4d**) were obtained together with small amounts of the *E*-isomers (**5a**～**5d**). The isomers were readily separable by column chromatography.

Substituents linked to the 2 position *via* carbon or sulfur were also prepared. For example in the 2-furyl series, the 2-ethylthio penem (**3e**)[2] proved to be a versatile intermediate as it could be transformed into other 2-thio derivatives by way of the "sulfoxide displacement process"[3,4]. Thus, oxidation to the sulfoxide (**3f**) followed by reaction with a thiol provided the 2-alkylthio penems (**3g** and **3h**). The *Z*-isomers (**4e, 4g** and **4h**) were obtained from the corresponding acetates on treatment with DBU.

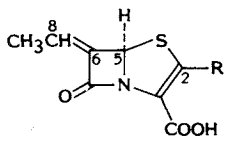
In the 3-furyl series, the 2-hydroxymethyl derivative (**3j**)[2] was converted to the *Z* alcohol (**4j**) on base treatment. Further chemical modification of hydroxyl group led to the synthesis of the penems (**4k**～**4m**).

The *p*-nitrobenzyl (PNB) protecting group of the *Z*- and *E*-isomers (**4** and **5**) was removed by hydrogenolysis over palladium on carbon followed by treatment with sodium hydrogencarbonate. The resulting sodium salts (**6** and **7**) were obtained as homogeneous freeze-dried solids after chromatography on Biogel P2.

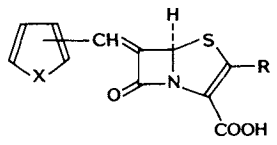
Biology

The β-lactamase inhibitory activity against cell-free enzyme preparations and the synergistic effects with amoxycillin or cephaloridine against whole cells are given in Table 1.

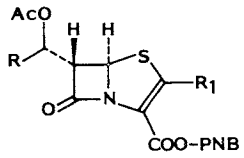
The *E*-isomers (**7a**～**7d**) had poorer inhibitory activity than the equivalent *Z*-isomers (**6a**～**6d**) against



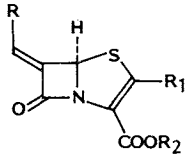
1



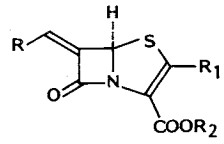
2 X=O, S



3



4 R_2=PNB
6 R_2=Na



5 R_2=PNB
7 R_2=Na

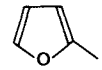
a R=  R_1=H

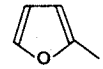
b R=  R_1=H

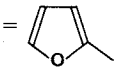
c R=  R_1=H

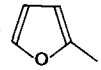
d R=  R_1=H

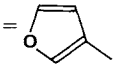
e R=  R_1=SEt

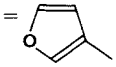
f R=  R_1=S(O)Et

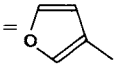
g R=  R_1=SCH_2CH_2OH

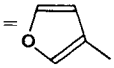
h R=  R_1=$SCH_2CH_2COOPNB$

i R=  R_1=SCH_2CH_2COONa

j R=  R_1=CH_2OH

k R=  R_1=CH_2OCONH_2

l R=  R_1=CH_2OAc

m R=  R_1=$CH_2OCH_2OCH_3$

All compounds are racemic, only one enantiomer is depicted.

all five enzymes and were also inferior synergists with amoxycillin. The reduction in synergistic activity seen with most of the *E*-isomers was pronounced, particularly against *Proteus mirabilis*, *Klebsiella pneumoniae* and *Enterobacter cloacae*. An exception was the 2-furyl derivative *E*-isomer (**7c**) which was as potent as the corresponding *Z*-isomer against several of the organisms despite a 100-fold difference in inhibitory activity. These results could reflect different rates of penetration into the bacterial periplasm.

The introduction of either alkylthio or alkyl substituents into the 2-position of the more active *Z*-isomer (*i.e.* **6e**, **6g** and **6i**~**6m** generally reduced inhibitory activity particularly against TEM-1 and *K. pneumoniae* β-lactamases. The synergistic activity of these 2-substituted compounds, relative to that of the unsubstituted *Z*-isomers, were reduced most against *E. cloacae* and *Escherichia coli* and least against *P. mirabilis* and *Staphylococcus aureus*.

The most active derivatives *in vitro* were the 2-unsubstituted *Z*-isomers (**6a**~**6d**). These four compounds had synergistic activity with amoxycillin similar to that seen with the corresponding 6-ethylidenepenem

Table 1. Synergistic and β-lactamase inhibitory activity.

Class	I_{50}[b] (μg/ml)					Antibiotic MICs (μg/ml) in presence of 1 μg/ml inhibitor				
						Cephaloridine	Amoxycillin			
	E.cl. I	*P.m.* II	*E.co.* III	*K.p.* IV	*S.a.* —	*E.cl.* I	*P.m.* II	*E.co.* III	*K.p.* IV	*S.a.* —
1[a]	1.000	NT	0.040	0.015	0.600	256	>256	4	2	1.0
6a	0.020	0.400	0.008	0.028	0.400	8	8	4	32	0.5
7a	3.000	In	3.500	2.200	In	256	>256	128	>128	>8.0
6b	0.003	0.090	0.002	0.003	0.021	16	8	2	4	0.3
7b	0.320	5.500	0.350	0.300	1.000	256	256	16	128	8
6c	0.005	0.025	0.003	0.002	0.013	16	4	2	8	0.3
7c	0.400	5.500	0.350	0.150	5.500	32	128	8	4	2
6d	0.005	0.012	0.001	0.003	0.045	8	16	2	16	0.5
7d	NT	NT	NT	NT	NT	>256	NT	32	>128	>8.0
6e	0.003	0.020	0.130	0.040	0.012	128	64	16	64	0.3
6g	0.150	0.006	0.025	NT	0.030	>256	8	16	64	0.3
6i	NT	NT	NT	NT	NT	128	16	256	>128	2.0
6j	0.005	0.070	0.030	0.035	0.060	32	4	8	64	1.0
6k	0.025	0.025	0.100	0.025	0.400	256	8	64	128	4.0
6l	0.025	0.012	0.095	0.050	0.900	256	8	16	128	4.0
6m	0.013	0.024	0.080	0.045	0.030	256	32	128	128	0.5
Clavulanic acid	In	0.030	0.050	0.019	0.060	>256	4	2	2	0.5
Sulbactam	2.8	0.080	1.90	10.0	1.50	256	32	128	32	4.0
Tazobactam	0.02	0.02	0.02	0.10	0.35	128	1	16	16	1.0
No inhibitor	—	—	—	—	—	>256	>256	>256	256	128.0

Abbreviations: *E.cl.*, *Enterobacter cloacae*; *P.m.*, *Proteus mirabilis*; *E. co.*, *Escherichia coli* (TEM-1); *K.p.*, *Klebsiella pneumoniae*; *S.a.*, *Staphylococcus aureus*.

[a] Structure **1**: R=H, *Z*-isomer.

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

NT: Not tested. In: Inactive at 10 μg/ml. Class: Enzyme classification based on RICHMOND and SYKES[7].

(**1**: R=H, *Z*-isomer); but were better synergists against *E. cloacae* and *P. mirabilis*. The synergistic activity of these compounds with amoxycillin and cefazolin *in vivo* is given in Table 2. Whereas all four compounds had similar activity *in vitro* (**6c**) proved to be the most active compound *in vivo*. However against *E. coli* TEM-1 the *in vivo* activity was still inferior to that shown by clavulanic acid.

Conclusion

Replacement of the methyl substituent at C-8 in 6-ethylidenepenems (**1**) with either a thiophene or furan ring has resulted in a considerable increase in synergistic activity and has also indicated a preferred double bond geometry and 2-substituent. Thus the *Z*-isomers (**6**) bearing a hydrogen at the 2-position were found to be the most potent derivatives. Further studies on structure-activity relationships involving the extension of this work to include other 6-(heterocyclylmethylene)penems will be reported.

Experimental

β-Lactamase inhibition studies were carried out on isolated enzyme preparations[5] by spectrophotometric monitoring of hydrolysis of nitrocefin in the presence and absence of test compound.

MICs were determined in Microtiter plates by serial dilution of amoxycillin or cephaloridine in broth,

Table 2. *In vivo* synergistic activity (antibiotic CD_{50}[a] as mg/kg $\times 3$).

	Escherichia coli (TEM-1)	*Enterobacter cloacae* (class 1)
Antibiotic	Amoxycillin	Cefazolin
Inhibitor dose (mg/kg)[b]	5	2
Antibiotic alone	1,000	200
Antibiotic + **6a**	200	100
Antibiotic + **6b**	200	200
Antibiotic + **6c**	63	60
Antibiotic + **6d**	200	175
Antibiotic + potassium clavulanate	2	NT

[a] CD_{50}: Curative dose for 50% of mice following dosing subcutaneously.
[b] All β-lactamase inhibitors were inactive when dosed alone at the dose level used in the combination.
NT: Not tested.

followed by addition of inhibitor (1 μg/ml) and organism (approx 2×10^6 cfu/ml), as previously described[6]. MIC values were recorded after incubation at 37°C for 18 hours.

Synergistic effects *in vivo* were carried out by infecting mice intraperitoneally with a lethal dose of the organism and treating groups with a range of doses of the antibiotic together with a fixed dose of inhibitor (either 5 or 2 mg/kg). Controls received the inhibitor alone or a range of doses of antibiotic alone. The dose of antibiotic required to protect 50% of the mice was calculated 4 days after infection.

For chromatographic and spectral details, see Part I[1]. The preparation of the penem derivatives (**3**) has been described in a patent application[2].

PNB-(5*RS*,6*SR*)-6-[acetoxy(2-furyl)methyl]-2-ethylsulfinylpenem-3-carboxylate (**3f**)

A solution of *m*-chloroperbenzoic acid (207 mg, 3.78 mmol) in dichloromethane (2 ml) was added in one portion to a stirred, ice bath cooled, mixture of the penem (**3e**) (465 mg, 0.92 mmol) in dichloromethane (20 ml) and saturated $NaHCO_3$ solution (20 ml). After 10 minutes the mixture was diluted with dichloromethane (10 ml). The organic layer was separated, washed with brine, then dried and evaporated. Chromatography eluting with EtOAc-hexane mixtures gave the title compound (**3f**) (266 mg, 55%) as a 1:1 mixture of sulfoxide diastereoisomers: IR ν_{max} ($CHCl_3$) cm^{-1} 1800, 1745, 1705; ^1H NMR (250 MHz, $CDCl_3$) δ 1.40 (3H, t, $J=7$ Hz), 2.05 (3H, s), 2.90~3.25 (2H, m), 4.43 (1H, dd, $J=2$ and 4 Hz), 5.22 and 5.45 (2H, ABq, $J=14$ Hz), 6.00 ($\frac{1}{2}$H, d, $J=2$ Hz), 6.10 ($\frac{1}{2}$H, d, $J=2$Hz), 6.24 (1H, d, $J=4$ Hz), 6.31~6.49 (2H, m), 7.39 (1H, br s), 7.57 and 7.59 (2H, each d, $J=8$ Hz), 8.22 (2H, d, $J=8$ Hz).

PNB-(5*RS*,6*SR*)-6-[acetoxy(2-furyl)methyl]-2-(2-hydroxyethylthio)penem-3-carboxylate (**3g**)

A solution of diisopropylethylamine (25 mg, 0.19 mmol) in acetonitrile (2.5 ml) was added dropwise to a solution of the sulfoxide (**3f**) (100 mg, 0.19 mmol) and 2-mercaptoethanol (30 mg, 0.38 mmol) in acetonitrile (5 ml) at -40°C. After 10 minutes the mixture was diluted with EtOAc and washed successively with 5% (w/v) citric acid solution, saturated $NaHCO_3$ and brine, then dried and evaporated. Chromatography eluting with EtOAc-hexane mixtures gave the title compound (**3g**) (61 mg, 61%): IR ν_{max} ($CHCl_3$) cm^{-1} 3600~3100, 1790, 1740, 1690; ^1H NMR (250 MHz, $CDCl_3$) δ 2.02 (1H, t, $J=5.9$ Hz), 2.09 (3H, s), 3.12 (1H, dt, $J=6.2$ and 13.9 Hz), 3.22 (1H, dt, $J=6.0$ and 13.9 Hz), 3.85~3.95 (2H, m), 4.30 (1H, dd, $J=1.8$ and 4.5 Hz), 5.26 and 5.46 (2H, ABq, $J=13.8$ Hz), 5.96 (1H, d, $J=1.8$ Hz), 6.28 (1H, d, $J=4.5$ Hz), 6.39 (1H, dd, $J=1.9$ and 3.4 Hz), 6.48 (1H, d, $J=3.4$ Hz), 7.41 (1H, d, $J=1.9$ Hz), 7.62 (2H, d, $J=8.7$ Hz), 8.22 (2H, d, $J=8.7$ Hz).

Compound **3h** was prepared as described above.

Penem Esters (**4** and **5**): General Elimination Procedure

A solution of the penem **3** in dichloromethane at -40°C under argon was treated dropwise with a solution of DBU (2 equivalents) in dichloromethane. After 15 minutes the reaction mixture was diluted with dichloromethane, washed with 5% citric acid solution and then brine. The dried ($MgSO_4$) solution

Table 3. Spectral data of penem esters (**4** and **5**).

Compound No.	IR ν_{max} ($CHCl_3$) cm^{-1} (β-lactam)	UV λ_{max}^{EtOH} nm (ε_m)	^1H NMR ($CDCl_3$)		
			5-CH[a]	8-CH[b]	2-Substituent
4a	1780	271 (14,350), 316 (25,440)	6.50	7.40	7.36 (1H, s)
5a	1770	267 (15,240), 319 (19,580)	6.45	6.77	7.42 (1H, s)
4b	1785	295 (24,240)	6.57	7.22	7.36 (1H, s)
5b	1770	306 (24,220)	6.44	6.69	7.43 (1H, s)
4c	1780	265 (11,710), 315 (31,250)	6.59	6.96	7.38 (1H, s)
5c	1770	262 (14,775), 325 (23,140)	6.41	6.53	7.39 (1H, s)
4d	1780	295 (23,000)	6.53	7.11	7.35 (1H, s)
5d	1770	262 (16,500), 300 (17,250)	6.42	6.57	7.42 (1H, s)
4e	1775	260 (16,370), 319 (36,060)	6.44	6.90	1.36 (3H, t, J=7 Hz), 2.75~3.20 (2H, m)
4g	1770	262 (15,900), 320 (35,020)	6.46	6.94	1.93 (1H, t, J=6.2 Hz), 3.08~3.26 (2H, m), 3.84~3.92 (2H, m)
4h	1770	262 (13,900), 329 (17,480)	6.48	6.94	2.85 (2H, t, J=7.2 Hz), 3.14~3.26 (2H, m), 5.24 (2H, s)
4j	1790	293 (25,700)	6.37	7.10	3.15 (1H, t, J=6.9 Hz), 4.62~4.68 (2H, m)
4k	1780	267 (20,130), 294 (25,390)	6.38	7.10	4.68 (2H, br s), 5.10, 5.53 (2H, ABq, J=15.5 Hz)
4l	1780	264 (20,440), 295 (26,354)	6.38	7.11	2.10 (3H, s), 5.10, 5.52 (2H, ABq, J=15.4 Hz)
4m	1775	266 (20,450), 294 (26,225)	6.35	7.09	3.37 (3H, s), 4.65 (2H, s), 4.68, 4.92 (2H, ABq, J=15.7 Hz)

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

was evaporated and chromatographed eluting with dichloromethane - EtOAc mixtures to give the *Z*-isomer (**4**). In some cases a small amount of the less polar *E*-isomer (**5**) was obtained. (With the alcohols (**3g** and **3j**), 3 equivalents of DBU were necessary). Spectral data for these compounds are shown in Table 3.

Penems (**6** and **7**): General Deprotection Procedure

The penem ester **4** (or **5**) was dissolved in a 4:1 mixture of dioxan and water and hydrogenated at 1 atmosphere over 5% palladium on carbon (1.5 weight equivalents) until starting material could no longer be detected on TLC (30~40 minutes). $NaHCO_3$ solution (1%, 1 equivalent) was added and the mixture filtered through Kieselguhr washing with dioxan-water mixtures. The filtrate was evaporated and chromatographed on Biogel P2 eluting with water to give, after freeze-drying, the sodium salt **6** (or **7**) as a yellow amorphous solid. (With the diester **4h** twice the amount of catalyst and $NaHCO_3$ were used). Spectral data for these compounds (Table 4) showed that the salts were homogeneous and contained up to 15% water.

PNB-(5*RS*)-2-Carbamoylmethyl-(*Z*)-6-(3-furyl)methylenepenem-3-carboxylate (**4k**)

A suspension of the alcohol (**4j**) (80 mg, 0.19 mmol) in dichloromethane (8 ml) at 5°C was treated with a solution of trichloroacetylisocyanate (72 mg, 0.38 mmol) in dichloromethane (4 ml). The reaction mixture was stirred at room temperature for 0.5 hour then diluted with EtOAc and successively washed with

Table 4. Spectral data of penem salts (**6** and **7**).

Compound No.	UV $\lambda_{max}^{H_2O}$ nm (ε_m)	^{1}H NMR (D_2O)		
		5-CH[a]	8-CH[b]	2-Substituent
6a	308 (17,600)	6.60	7.50	7.06 (1H, s)
7a	317 (11,570)	6.47	7.04	7.15 (1H, s)
6b	296 (22,510)	6.69	7.26	7.04 (1H, s)
7b	298 (14,980)	6.47	6.90	7.13 (1H, s)
6c	308 (21,020)	6.64	7.08	7.06 (1H, s)
7c	318 (13,335)	6.45	6.73	7.12 (1H, s)
6d	291 (18,000)	6.68	7.22	7.09 (1H, s)
7d	296 (26,020)	6.39	6.75	7.16 (1H, s)
6e	238 (7,420), 315 (24,770)	—	—	—
6g	236 (7,420), 314 (23,600)	6.52	7.08	2.94~3.20 (2H, m), 3.88~3.95 (2H, m)
6i	312	6.56	7.09	2.56~2.65 (2H, m), 2.98~3.25 (2H, m)
6j	291 (18,275)	6.51	7.21	4.54, 4.76 (2H, ABq, J=14.9 Hz)
6k	271 (16,940)	6.51	7.25	5.00, 5.40 (2H, ABq, J=15 Hz)
6l	290 (16,950)	6.51	7.21	2.10 (3H, s), 5.03, 5.04 (2H, ABq, J=14.0 Hz)
6m	289 (14,450)	6.51	7.21	3.40 (3H, s), 4.57, 4.89 (2H, ABq, J=13.9 Hz), 4.71 (2H, s)

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

saturated $NaHCO_3$ solution and brine. The dried organic phase was evaporated and then dissolved in MeOH (2 ml) containing 2,6-lutidine (4 mg). After 18 hour dichloromethane was added and the solution was washed successively with 5% (w/v) citric acid solution, saturated $NaHCO_3$ solution and brine. The dried organic phase was evaporated and then chromatographed eluting with dichloromethane - EtOAc mixtures to give the title compound **4k** (53 mg, 60%). Spectral data are shown in Table 3.

PNB-(5*RS*)-2-Acetoxymethyl-(*Z*)-6-(3-furyl)methylenepenem-3-carboxylate (**41**)

A suspension of the alcohol (**4j**) (50 mg, 0.12 mmol) in dichloromethane (2 ml) at 5°C was treated with 4-dimethylaminopyridine (1.5 mg, 0.01 mmol), triethylamine (15.8 mg, 0.16 mmol) and acetic anhydride (16 mg, 0.16 mmol). After 0.5 hour at room temperature, the mixture was diluted with EtOAc and washed successively with 5% (w/v) citric acid solution, saturated $NaHCO_3$ solution and brine. The dried organic phase was evaporated and then chromatographed eluting with dichloromethane - EtOAc mixtures to give the title compound **41** (53 mg, 96%). Spectral data are shown in Table 3.

PNB-(5*RS*)-(*Z*)-6-[(3-Furyl)methylene]-2-(methoxymethyloxymethyl)penem-3-carboxylate (**4m**)

A mixture of the alcohol (**4j**) (50 mg, 0.12 mmol), 2,6-lutidine (39 mg, 0.36 mmol) and chloromethyl methyl ether (29 mg, 0.36 mmol) in dichloromethane (4 ml) was stirred at 40°C for 24 hours. The mixture was diluted with EtOAc and washed successively with 5% (w/v) citric acid, saturated $NaHCO_3$ and brine. The dried organic phase was chromatographed eluting with dichloromethane - EtOAc mixtures to give the title compound **4m** (46 mg, 84%). Spectral data are shown in Table 3.

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