SYNTHESIS AND BIOLOGICAL ACTIVITY OF A NEW CEPHALOSPORIN, BMY-28232 AND ITS PRODRUG-TYPE ESTERS FOR ORAL USE

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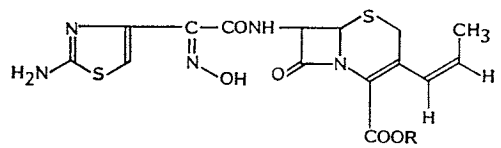
(Received for publication April 9, 1988)

The synthesis and structure-activity relationships of 7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylic acid (BMY-28232), its 3-alkenyl analogs (**6** and **7**) and *O*-substituted derivatives of the oxyimino moiety (**10**) are described, as well as the oral pharmacokinetics and *in vivo* activities of the 1-acetoxyethyl ester of BMY-28232 (BMY-28271) and its analogous esters (**11**). The 3-alkenyl groups were introduced by the Wittig reaction of the ylide (**2**) prepared from the 3-chloromethyl cephem (**1**) to afford the *Z* (main) and *E* (minor) isomers regarding the 3-side chain. The *O*-substituted derivatives (**10**) were prepared by 7-*N*-acylation of the 7-amino cephem (**4a**) with the corresponding *O*-substituted side chain acids (**8**). The prodrug esters (**11**) were prepared by esterification of BMY-28232 with an appropriate halide. BMY-28232 was the most active among the 3-alkenyl analogs tested against Gram-negative organisms and much more active than the *O*-substituted derivatives against Gram-positive bacteria. BMY-28271 showed good oral bioavailability (66%) and good *in vivo* efficacy in mice against infections of *Staphylococcus aureus* Smith (PD_{50}, 0.68 mg/kg) and *Escherichia coli* Juhl (0.54 mg/kg).

Since the discovery of cefuroxime axetil[1], a number of prodrug-type cephalosporin esters[2~8] possessing an aminothiazole moiety at the C-7 position of the cephem nucleus have been reported as orally active cephalosporins. They are mostly different from one another in the C-3 side chain.

Previously we have found 3-alkenyl cephalosporins[9] in the cephalexin-cefadroxil series showing good oral absorbability and selected a 3-(*Z*)-propenyl derivative, BMY-28100[9,10], for clinical evaluation. In the course of our research program exploring broad spectrum oral cephalosporins, we applied the C-3 alkenyl side chain to the aminothiazole cephalosporins and identified a new 3-(*Z*)-propenylcephalosporin, BMY-28232 (Fig. 1), which showed a well-balanced, broad antibacterial spectrum against Gram-positive and Gram-negative organisms including β-lactamase-producing strains. BMY-28232 was orally absorbed in mice to some extent. To improve its oral absorption, several prodrug-type esters of BMY-28232 were prepared, among which BMY-28271 (Fig. 1), the acetoxyethyl ester of BMY-28232, was selected for further evaluation in view of its excellent oral absorption and good *in vivo* activity††.

Fig. 1. BMY-28232 and BMY-28271.



BMY-28232 R=H
BMY-28271 R=CHOCOCH$_3$
|
CH$_3$

This paper describes the synthesis and the structure-activity relationships of BMY-28232

† Deceased.

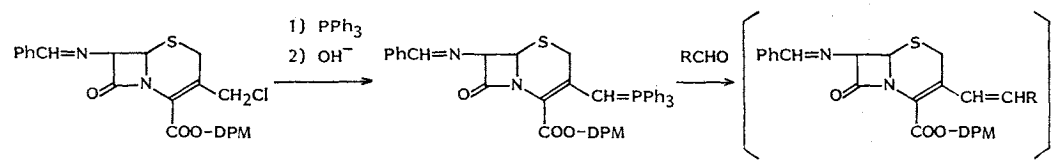
†† A part of this workes has been presented at the 27th Intersci. Conf. on Antimicrob. Agents Chemother. Meeting, Abst. No. 806, New York, 1987.

and its analogs. The oral pharmacokinetics and oral *in vivo* activities of BMY-28271 and its analogous esters are also discussed.

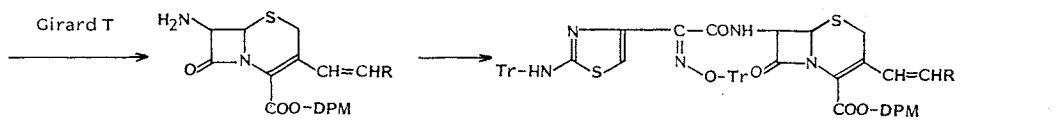
Chemistry

The 3-propenylcephalosporin acid, (BMY-28232, **6a**) was prepared by the synthetic route shown in Scheme 1. Diphenylmethyl 7-benzylideneamino-3-chloromethyl-3-cephem-4-carboxylate (**1**)[11] was treated with triphenylphosphine followed by aqueous Na_2CO_3 to give the ylide **2** in 81% yield. The Wittig reaction of **2** with acetaldehyde was carried out to afford the reaction product **3**, which was, without isolation, treated with Girard T reagent to give compound **4a** in 71% yield as colorless needles. The HPLC study (ODS, 70% MeOH - pH 7 buffer) indicated that **4a** was a mixture of two components (85 : 15) with retention time at 5.7 minutes for the major component and 7.1 minutes for the minor one. The ^1H NMR spectrum of **4a** showed a doublet at 6.23 ppm (J=12 Hz) indicating that the reaction gave predominantly the *cis* isomer in regard to the 3-side chain. Since it was difficult to separate these isomers by crystallization or column chromatography in this stage, the product was used for the next step without separation of the isomers. Compound **4a** was acylated with (*Z*)-2-(2-tritylaminothiazol-4-yl)-2-trityloxyiminoacetic acid[12] by the active ester method using 1-hydroxybenzotriazole to afford **5a** in 98% yield after column chromatography. Deblocking of **5a** with HCOOH - HCl was followed by chromatography on a column of reverse phase silica gel to separate the *Z* isomer (**6a**) in 33% yield and its *E* isomer (**7a**) in 4% yield. The 3-butenyl and 3-vinyl analogs were similarly prepared from **2**. The Wittig reaction of **2** with propionaldehyde and formaldehyde

Scheme 1. Synthesis of 3-alkenyl cephalosporins (**6** and **7**).

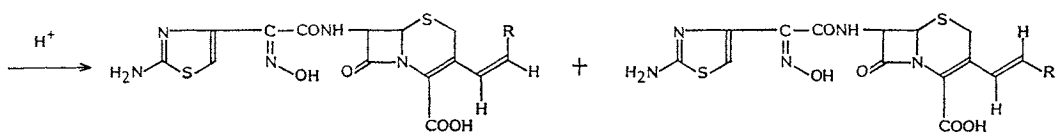


1 **2** **3**



4a R = CH_3
4b R = C_2H_5
4c R = H

5a R = CH_3
5b R = C_2H_5
5c R = H

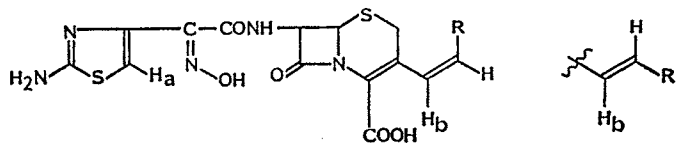


6a R = CH_3 (BMY-28232)
6b R = C_2H_5
6c R = H

7a R = CH_3
7b R = C_2H_5

Tr: $C(C_6H_5)_3$
DPM: $CH(C_6H_5)_2$

Table 1. Yields, mp, UV, ^{1}H NMR and mass data of 3-alkenyl cephalosporins (**6** and **7**).



6

7

Compound	R	Yield (%)	MP (°C, dec)	UV λ_{max} nm (ε)[a]	^{1}H NMR (80 MHz, δ in D_2O (+$NaHCO_3$))						Mass (*m/z*) $(M+1)^+$
					2-H[b]	6-H[c]	7-H[c]	H_a[d]	H_b[e]	R	
6a	CH_3	33	170	225 (18,400), 282 (15,200)	3.56	5.35	5.90	7.06	6.02	1.73 (CH_3)	410
7a	CH_3	4	200	223 (20,000), 290 (21,000)	3.69	5.25	5.83	6.97	6.55	1.85 (CH_3)	410
6b	C_2H_5	23	170	223 (17,000), 281 (15,300)	3.55	5.34	5.90	7.07	6.00	1.05 (CH_3), 2.12 (CH_2)	424
7b	C_2H_5	3	170	223 (19,400), 290 (19,200)	3.75	5.32	5.88	7.06	6.60	1.10 (CH_3), 2.25 (CH_2)	424
6c	H	27	170	223 (17,400), 286 (19,700)	3.76	5.33	5.90	7.07	6.75	5.33 (=CH), 5.50 (=CH)	396

[a] Determined in pH 7 phosphate buffer.
[b] ABq, J=18 Hz for **6a** and **6b**; s for **7a**, **7b** and **6c**.
[c] d, J=4.5 Hz.
[d] s.
[e] d, J=12 Hz for **6a** and **6b**; d, J=16 Hz for **7a** and **7b**; dd, J=12 and 18 Hz for **6c**.

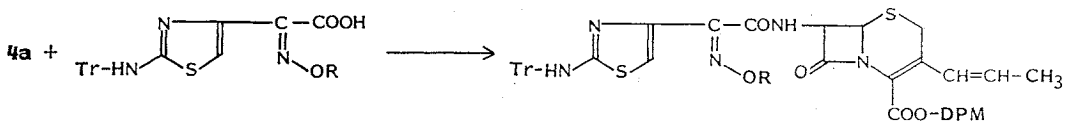
gave the 3-butenyl (**4b**) and 3-vinyl (**4c**)[13] derivatives, respectively. The HPLC study indicated that **4b** contained 11% of its *trans* isomer. The 7-*N*-acylation of **4b** followed by deblocking afforded the *cis* isomer **6b** (23%) and the *trans* isomer **7b** (3%) after chromatographic separation. The vinyl derivative **6c**[14,15] was also prepared similarly from **4c**. The chemical data of **6** and **7** are shown in Table 1.

As can be seen in the table, distinct differences between *Z* and *E* isomers on the 3-alkenyl moiety were observed in their UV and ^{1}H NMR spectra. *Z* isomers (**6a** and **6b**) had an absorption maximum at near 280 nm, whereas *E* isomers (**7a** and **7b**) showed the maximum at 290 nm with greater intensity. The vinyl derivative **6c** was similar to the *E* isomers. The ^{1}H NMR of *Z* isomers showed a doublet at *ca.* 6 ppm (J=12 Hz) assigned to a vinyl proton closer to the cephem nucleus (H_b in Table 1) and an AB quartet at *ca.* 3.6 ppm due to the 2-H protons, whereas that of *E* isomers resonated at lower field (*ca.* 6.6 ppm) with larger coupling constant (J=16 Hz) for the H_b proton and at *ca.* 3.7 ppm as a two-proton singlet for the 2-H protons. The ^{1}H NMR of **6c** was also similar to that of the *E* isomers for signals of the H_b and 2-H protons. A similar observation has been previously reported on BMY-28100 derivatives having 3-alkenyl side chains[9].

The *O*-substituted analogs of the oxyimino moiety were prepared by the *N*-acylation of **4a** with appropriate *O*-substituted aminothiazolyl acids (**8a**~**8d**[12] and **8e**[16]) followed by deblocking as shown in Scheme 2. Only the predominant *cis* isomers (**10**) were isolated by chromatographic separation from the reaction mixture of the final products. The physico-chemical data of **10** are shown in Table 2.

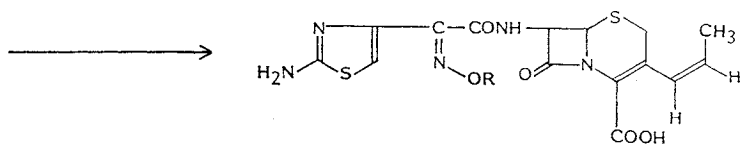
BMY-28232 (**6a**) was esterified with 1-acetoxyethylbromide[17] in *N*,*N*-dimethylformamide in the

Scheme 2. Synthesis of *O*-substituted cephalosporins (**10**).



8a R = CH_3
8b R = $CH(CH_3)_2$
8c R = $CH_2C{\equiv}CH$
8d R = $CH_2CH{=}CH_2$
8e R = $CH_2COO\,^tBu$

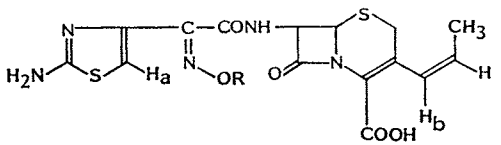
9a R = CH_3
9b R = $CH(CH_3)_2$
9c R = $CH_2C{\equiv}CH$
9d R = $CH_2CH{=}CH_2$
9e R = $CH_2COO\,^tBu$



10a R = CH_3
10b R = $CH(CH_3)_2$
10c R = $CH_2C{\equiv}CH$
10d R = $CH_2CH{=}CH_2$
10e R = CH_2COOH

Tr: $C(C_6H_5)_3$
DPM: $CH(C_6H_5)_2$

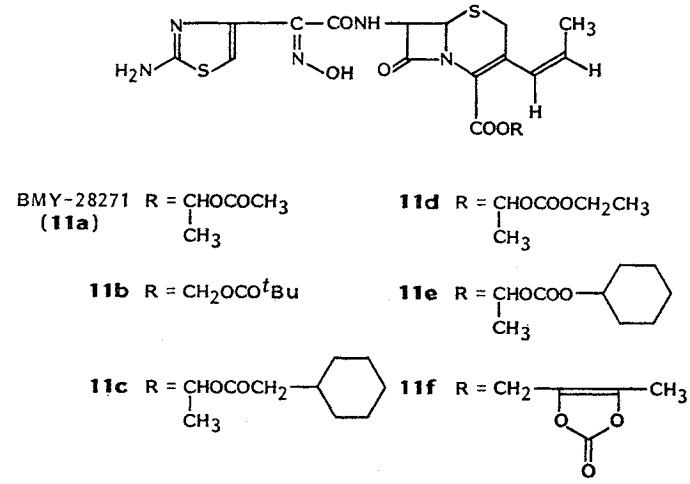
Table 2. Yields, mp, UV, ^{1}H NMR and mass data of *O*-substituted cephalosporins (**10**).



Compound	R	Yield (%)	MP (°C, dec)	UV λ_{max} nm (ε)[a]	^{1}H NMR (80 MHz, δ in D_2O (+$NaHCO_3$))							Mass (*m/z*) $(M+1)^+$
					2-H[b]	6-H[c]	7-H[c]	H_a[d]	H_b[e]	$=CCH_3$[f]	R	
10a	CH_3	35	180	232 (16,400), 283 (15,500)	3.62	5.35	5.90	7.15	6.10	1.75	4.13 (CH_3)	424
10b	$CH(CH_3)_2$	33	170~175	232 (16,700), 284 (16,200)	3.67	5.42	5.95	7.15	6.12	1.82	1.48 ($CH_3\times2$)	452
10c	$CH_2C\equiv CH$	22	155	229 (17,000), 285 (14,500)	3.62	5.38	5.92	7.21	6.08	1.75	4.98 (CH_2)	448
10d	$CH_2CH=CH_2$	50	140	232 (17,000), 285 (14,700)	3.63	5.40	5.92	7.17	6.12	1.77	[g]	450
10e	CH_2COOH	39	170	235 (14,800), 283 (12,700)	3.58	5.35	5.92	7.15	6.05	1.75	4.68 (CH_2)	468

[a] Determined in pH 7 phosphate buffer.
[b] ABq, J=18 Hz.
[c] d, J=4.5 Hz.
[d] s.
[e] d, J=12 Hz.
[f] d, J=6 Hz.
[g] The OCH_2 peak was overlapped with DOH signal.

Fig. 2. BMY-28271 and related prodrug esters.



presence of Na_2CO_3. After chromatography on a silica gel column the 1-acetoxyethyl ester (BMY-28271, **11a**) was obtained in 24% yield as an amorphous powder, which was crystallized from EtOAc. In a similar way, reaction of **6a** with pivaloyloxymethyl iodide, 1-cyclohexylacetyloxyethyl iodide[7], 1-ethoxycarbonyloxyethyl iodide, 1-cyclohexyloxycarbonyloxyethyl iodide[8] and (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl bromide[18] gave the corresponding esters (**11b**~**11f**) in 13~50% yield (Fig. 2). The physico-chemical data of **11** are shown in Table 3. As shown in the last column of Table 3, the HPLC study revealed that **11a**, **11c**, **11d** and **11e** were a mixture of diastereoisomers regarding the asymetric carbon of the ester moiety and the ratio was almost 1 : 1. This was also supported by 400 MHz ^{1}H NMR spectrum of **11a**, in which most protons appeared as a pair of signals with almost the same intensity (see Experimental part), although the presence of a pair of signals was obscure in the spectrum determined by an 80 MHz spectrometer.

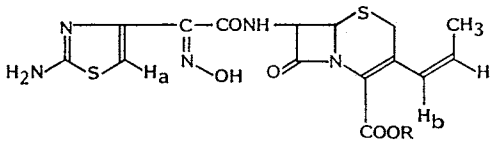
Biological Evaluation

MICs of cephalosporin acids (**6**, **7** and **10**) against 25 test organisms were determined by 2-fold serial agar dilution method in Mueller-Hinton agar. The test organisms consist of five strains each of five groups which are described in Table 4. The *in vitro* activity of the test compounds was assessed by the geometric mean of MICs for each group of the test organisms.

The *in vitro* activity of BMY-28232 (**6a**), its 3-modified analogs (**6** and **7**) and the *O*-substituted derivatives (**10**) are shown in Table 5. The 3-*Z*-propenyl derivative (BMY-28232, **6a**) was the most active among the cephalosporins tested in the present series. The 3-*Z*-butenyl (**6b**) and 3-vinyl (**6c**) derivatives were as active as **6a** against Gram-positive species (Gp-Ia and Gp-Ib), but less active than **6a** against Gram-negative groups (Gn-Ia, -Ib and -II). The *E*-propenyl (**7a**) and *E*-butenyl (**7b**) derivatives were similar to their *Z* isomers (**6a** and **6b**) in Gram-positive activity, but much less active than the latters against Gram-negative species.

Generally *O*-substitution of the hydroxyimino moiety of **6a** resulted in reducing the Gram-positive activity. Thus, the methoxyimino derivative (**10a**) was nearly as active as **6a** against Gram-negative species, but significantly less active against both Gram-positive groups (Gp-Ia and Gp-Ib). The *O*-allyl derivative (**10d**) showed fairly good Gram-positive activity, but decreased Gram-negative

Table 3. Yields, mp, UV, ^{1}H NMR, HPLC and mass data of BMY-28271 and related compounds (**11**).



Compound	R	Yield (%)	MP (°C, dec)	UV λ_{max}^{EtOH} nm (ε)	^{1}H NMR (80 MHz, δ in $CDCl_3$)					HPLC[e] room temp (minutes)	Mass (m/z) $(M+1)^+$
					2-H[a]	6-H[b]	H_a[c]	H_b[d]	Ester moiety		
11a (BMY-28271)	$CHOCOCH_3$ | CH_3	24	146~149	223 (19,000), 286 (12,000)	3.45	5.10	7.04	6.15	1.52 (3H, d), 2.10 (3H, s)	8.6, 8.9	496
11b	CH_2OCO-*tert*-Bu	50	115~120	222 (19,000), 285 (12,000)	3.45	5.10	7.00	6.15	1.25 (9H, s), 5.85 (2H, ABq)	8.0	524
11c	$-CHOCOCH_2-C_6H_5$ | CH_3	13	115~120	224 (18,000), 285 (11,300)	3.45	5.08	7.00	6.15	1.52 (3H, d), 0.90~1.90 (11H, m)	9.1, 9.6	578
11d	$CHOCOOC_2H_5$ | CH_3	17	105~110	222 (20,000), 286 (11,000)	3.47	5.10	7.03	6.17	1.36 (3H, t), 1.55 (3H, d), 4.23 (2H, q)	12.9, 14.1	526
11e	$-CHOCOO-C_6H_5$ | CH_3	28	115~120	224 (20,000), 284 (12,000)	3.44	5.07	7.00	6.15	1.55 (3H, d), 0.8~2.0 (10H, m)	9.5, 10.7	580
11f	$-CH_2-C(CH_3)=C(O)-O-C(=O)-$	39	115~120	225 (19,000), 287 (12,000)	3.45	5.11	7.00	6.13	2.17 (3H, s), 4.96 (2H, s)	4.9	522

[a] br s.

[b] d, J=5.5 Hz.

[c] s.

[d] d, J=12 Hz.

[e] Column: SSC-ODS-262, 6×100 mm (Senshu Pak), detection: UV at 254 nm, flow rate: 1 ml/minute, mobile phase: CH_3CN - pH 2.6 buffer (**11a**, 30 : 70; **11b**, 45 : 55; **11c**, 50 : 50; **11d**, 35 : 65; **11e**, 45 : 55; **11f**, 40 : 60).

Table 4. Test organisms for the primary evaluation of cephalosporins.

Group	Organism	Number of strains
Gp-Ia	Penicillinase (Pen-ase)-negative *Staphylococcus aureus*	5
Gp-Ib	Pen-ase-positive *S. aureus*	5
Gn-Ia	Cephalothin (CET)-sensitive *Escherichia coli* (2 strains), *Klebsiella pneumoniae* (1) and *Proteus mirabilis* (2)	5
Gn-Ib	CET-resistant *E. coli* (3) and *K. pneumoniae* (2)	5
Gn-II	*Morganella morganii* (1), *Enterobacter cloacae* (2) and *Serratia marcescens* (2)	5

Table 5. *In vitro* activity of BMY-28232 and analogs (Mueller-Hinton agar, 10^6 cfu/ml, 37°C, 18 hours).

Compound	Geometric mean of MIC (μg/ml)				
	Gp-Ia[a]	Gp-Ib	Gn-Ia	Gn-Ib	Gn-II
6a (BMY-28232)	0.23	0.40	0.076	0.35	5.5
6b	0.26	0.40	0.20	0.79	7.3
6c	0.30	0.53	0.17	0.91	14
7a	0.23	0.61	0.46	2.1	33
7b	0.23	0.61	0.69	3.2	22
10a	1.8	3.6	0.10	0.52	6.3
10b	1.6	2.7	0.53	1.2	8.3
10c	1.4	1.6	0.23	0.91	14
10d	0.46	0.80	0.70	2.1	29
10e	14	29	0.029	0.30	4.2
Cefuroxime	0.92	1.8	1.4	6.3	44
Cefaclor	0.92	4.7	1.1	7.2	>100

[a] See Table 4.

activity. On the contrary the *O*-carboxymethyl derivative (**10e**) was more active than **6a** and **10a** against Gram-negative groups, but showed only very weak Gram-positive activity.

In view of the above results, BMY-28232 (**6a**) was selected as a lead compound in this series and evaluated further for its *in vitro* activity, absorption and *in vivo* efficacy following intramuscular and oral administrations to mice. Table 6 shows the *in vitro* activity of **6a** compared with that of cefuroxime against standard strains of bacteria stocked in our laboratory. This confirmed the results of the primary assessment described above. As shown in Table 7, **6a** was well absorbed by intramuscular injection to mice, and was effective *in vivo* against infections produced by *Staphylococcus aureus* Smith and *Escherichia coli* Juhl. However **6a** showed limited absorption by an oral administration and its oral *in vivo* activity was less than one fifth that obtained by im route against *S. aureus* and *E. coli* infections. Modification of **6a** to its prodrug-type esters (**11a**~**11f**) resulted in a singificant improvement of its oral absorption. The pharmacokinetic parameters and *in vivo* activities against *S. aureus* Smith and *E. coli* Juhl of the prodrug esters were compared with those of cefuroxime axetil and cefaclor after an oral administration to mice. The results are summarized in Table 8. The relative oral bioavailability of the esters calculated from the oral AUC value of a prodrug and the AUC value of the parent acid (**6a**) administered intravenously at equivalent dose. This series of esters, with an exception of **11f**, showed higher oral bioavailabilities (60~66%) than those of cefuroxime axetil (46%) and cefaclor (51%).

Table 6. Antibacterial spectrum of **6a** and cefuroxime against standard strains of bacteria.

Test organism	MIC (μg/ml)	
	6a	Cefuroxime
Staphylococcus aureus FDA 209P JC-1	0.4	1.6
S. aureus Smith	0.4	1.6
S. aureus Terajima	0.4	0.8
S. aureus MS 353	0.2	0.8
Escherichia coli NIHJ JC-2	0.2	6.3
E. coli K-12 C600	0.4	6.3
Klebsiella pneumoniae PCI-602	0.025	0.05
Salmonella typhimurium IID 971	0.4	12.5
S. typhi 901	0.1	3.1
S. paratyphi 1015	0.1	0.2
S. schottmuelleri 8006	0.05	0.1
S. enteritidis G 14	0.1	3.1
Proteus mirabilis IFO 3849	0.4	3.1
P. vulgaris OX 19	0.2	3.1
P. vulgaris HX 19	0.1	0.8
Providencia rettgeri IFO 3850	<0.006	0.1
Morganella morganii IFO 3848	0.4	0.8
Enterobacter aerogenes ATCC 13048	0.8	6.3
E. cloacae 963	1.6	6.3
Serratia marcescens IAM 1184	0.8	50
Pseudomonas aeruginosa IFO 3445	>50	>50
P. aeruginosa NCTC 10490	25	50
P. aeruginosa PAO 1	>50	>50

Table 7. Blood level and PD_{50} of **6a** (BMY-28232) by im, iv, and oral administrations in mice ($n=10$).

Route	Blood level				PD_{50} (mg/kg)	
	Dose (mg/kg)	C_{max} (μg/ml)	$T_{1/2}$ (hours)	AUC (μg·hours/ml)	*S.a.*	*E.c.*
im	100	86	0.74	126	0.26	0.23
iv	100	516	0.51	117		
Oral	100	6.9	2.2	23	2.3	1.4

S.a., *Staphylococcus aureus* Smith; *E.c.*, *Escherichia coli* Juhl.

The prodrug esters in the present study (**11a**~**11f**) were all more effective than cefuroxime axetil against *S. aureus* and *E. coli* infections. BMY-28271 (**11a**) appeared to be the most effective compound against both infections and also more active than cefaclor. Other esters (**11b**~**11f**) appeared more active than cefaclor against *E. coli* Juhl, although somewhat less active against *S. aureus* Smith.

Further evaluation on BMY-28232 and selected prodrug esters will be described in a separate paper.

Experimental

MP's were determined using a Yanagimoto micro hot-stage apparatus and are uncorrected. IR spectra were recorded on a Jasco IRA-1 spectrometer and UV spectra on a Shimadzu UV-200 spectrophotometer. NMR spectra were recorded on Varian FT-80A (80 MHz) and Jeol GX-400 (400 MHz). Mass spectra were recorded on a Hitachi M-80 mass spectrometer (secondary ion (SI)-MS).

Table 8. Blood level and PD_{50} of BMY-28271 and analogs (**11**) after oral administration to mice ($n=5$).

Compound	R	Blood level (dose=100 mg/kg)			B.A.* (%)	PD_{50} (mg/kg)	
		C_{max} (μg/ml)	$T_{1/2}$ (hours)	AUC (μg·hours/ml)		*S.a.*	*E.c.*
11a (BMY-28271)	$CHOCOCH_3$ with CH_3	41	1.7	77	66	0.68	0.54
11b	CH_2OCO-*tert*-Bu	34	2.2	71	61	0.99	0.70
11c	$-CHOCOCH_2-C_6H_5$ with CH_3	33	2.3	70	60	2.7	1.4
11d	$CHOCOOCH_2CH_3$ with CH_3	32	2.9	74	63	1.4	1.1
11e	$-CHOCOO-C_6H_5$ with CH_3	48	2.0	77	66	1.0	1.2
11f	$-CH_2-$ dioxolenone $-CH_3$	16	2.3	44	38	2.0	1.2
Cefuroxime axetil		27	1.5	36	46	6.1	8.8
Cefaclor		32	1.0	41	51	0.81	1.8

* Relative bioavailability: **11a**~**11f**, [AUC(po) of the prodrugs (**11**)/AUC(iv) of (**6a**)]×100; cefuroxime axetil, [AUC(po) of cefuroxime axetil/AUC(iv) of cefuroxime]×100; cefaclor, [AUC(po) of cefaclor/AUC(iv) of cefaclor]×100.

S.a., *Staphylococcus aureus* Smith; *E.c.*, *Escherichia coli* Juhl.

Diphenylmethyl 7-Benzylideneamino-3-[(triphenylphosphoranylidene)methyl]-3-cephem-4-carboxylate (**2**)

Diphenylmethyl 7-benzylideneamino-3-chloromethyl-3-cephem-4-carboxylate (**1**, 48.4 g, 96.2 mmol) was added to a stirred solution of PPh_3 (25.2 g, 96.2 mmol) in CH_2Cl_2 (50 ml) at room temperature under nitrogen atmosphere. The resulting pale yellow solution was continued to be stirred for 4 days at room temperature. The viscous reaction mixture was diluted with CH_2Cl_2 (300 ml), and an aqueous solution of 5 N Na_2CO_3 (100 ml, 5 equiv) was added in one portion under vigorous stirring. The mixture being stirred for 50 minutes was separated and the aqueous layer was extracted with CH_2Cl_2 (3×70 ml). The combined organic extracts were dried over anhydrous $MgSO_4$ and filtered. The dark red filtrate was concentrated *in vacuo* to a volume of about 150 ml. The concentrate was poured into acetone (400 ml) with stirring and the resulting solid was collected by filtration and washed with acetone (300 ml). The solid was then dried under reduced pressure at room temperature in the dark. Yield 56.7 g (81%): UV $\lambda_{max}^{CH_2Cl_2}$ nm (ε) 255 (24,900), 388 (24,300); mp 219~220°C (dec).

Anal Calcd for $C_{46}H_{37}N_2O_3PS$: C 75.81, H 5.12, N 3.84, S 4.40.
Found: C 75.39, H 5.16, N 3.82, S 4.71.

The Wittig Reaction Products: Diphenylmethyl 7-Amino-3-(1-propenyl)-3-cephem-4-carboxylate Hydrochloride (**4a**)

To a chilled solution of lithium bromide (43.4 g, 0.5 mol) in DMF (200 ml) and CH_2Cl_2 (200 ml) was added a solution of the ylide **2** (36.5 g, 0.05 mol) in CH_2Cl_2 (200 ml). To the mixture was added acetaldehyde (50 ml) under stirring. The mixture was allowed to stand for 24 hours at room temperature and concentrated under reduced pressure. The residue was diluted with EtOAc (1.5 liters), washed with water, dried over anhydrous $MgSO_4$ and filtered. To the filtrate was added Silica gel (Wakogel C-100, 50 g). The mixture was filtered and the filtrate was concentrated to *ca.* 500 ml.

To the concentrate was added a solution of Girard T reagent (25.2 g, 0.15 mol) in MeOH (400 ml) and AcOH (20 ml) and the mixture was stirred for 20 minutes. After removal of MeOH under reduced pressure, the mixture was diluted with EtOAc (1 liter), washed with aq $NaHCO_3$ and then water. The organic solution was dried and concentrated *in vacuo*. The concentrate was diluted with ether (1 liter) and then 1.5 N HCl - MeOH (25 ml) was added to precipitate the crude product (*ca.* 20 g), which was dissolved in a mixture of MeOH (100 ml) and EtOAc (300 ml), and treated with a small amount of charcoal. The solution was filtered and the filtrate was concentrated *in vacuo* to afford a crystalline product, which was collected by filtration and dried. Yield 15.7 g (71%): MP 165~170°C; IR ν_{max}^{KBr} cm^{-1} 1782, 1722; UV λ_{max}^{EtOH} nm (ε) 285 (7,600); ^1H NMR (80 MHz, DMSO-d_6) δ 1.47 (3H, dd, J=2 and 7 Hz, CH=CHCH_3), 3.65 (2H, ABq, J=18 Hz, 2-H), 5.15 (1H, d, J=5.5 Hz, 6-H or 7-H), 5.30 (1H, d, J=5.5 Hz, 6-H or 7-H), 5.45~5.75 (1H, m, CH=C*H*CH_3), 6.23 (1H, d, J=12 Hz, C*H*=$CHCH_3$), 6.85 (1H, s, Ph_2CH), 7.1~7.5 (10H, m, phenyl). HPLC study showed that this product contained about 15% of the *E* isomer. HPLC (column Senshu Pak SSC-ODS-262, 254 nm, solvent 70% MeOH - pH 7 buffer, 2 ml/minute): *Z* isomer 5.7 minutes, *E* isomer 7.1 minutes.

Anal Calcd for $C_{23}H_{22}N_2O_3S \cdot HCl$: C 62.36, H 5.23, N 6.32, S 7.24, Cl 8.00.
Found: C 62.29, H 5.18, N 6.10, S 7.24, Cl 7.92.

Diphenylmethyl 7-Amino-3-(1-butenyl)-3-cephem-4-carboxylate Hydrochloride (**4b**)

The Wittig reaction of **2** (7.3 g, 10 mmol) using propionaldehyde instead of acetaldehyde gave 1.49 g (33%) of **4b** by a similar procedure to **4a**: MP 120~127°C; IR ν_{max}^{KBr} cm^{-1} 1780, 1710; UV λ_{max}^{EtOH} nm (ε) 286 (7,400); ^1H NMR (80 MHz, DMSO-d_6) δ 0.93 (3H, t, J=7 Hz, CH_2CH_3), 2.00 (2H, m, CH_2CH_3), 3.75 (2H, ABq, J=18 Hz, 2-H), 5.22 (1H, d, J=5.5 Hz, 6-H or 7-H), 5.40 (1H, d, J=5.5 Hz, 6-H or 7-H), 6.33 (1H, d, J=12 Hz, C*H*=$CHCH_3$), 6.97 (1H, s, Ph_2CH), 7.40 (10H, m, phenyl). HPLC study showed that this product contained 11% of the *E* isomer. HPLC (column LiChrosorb RP-18 (25 cm×4 mm i.d.), 254 nm, solvent 60% CH_3CN - pH 7 buffer, 2 ml/minute): *Z* isomer 5.1 minutes, *E* isomer 6.7 minutes.

Acylated Products (**5**): General Procedures Illustrated with the Preparation of 7-[(*Z*)-2-(2-Tritylaminothiazol-4-yl)-2-trityloxyiminoacetamido]-3-(1-propenyl)-3-cephem-4-carboxylate (**5a**)

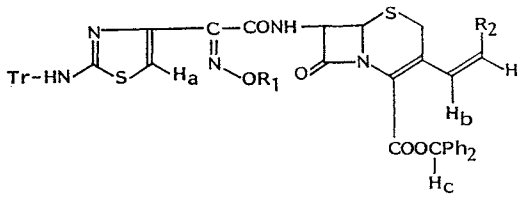
To a chilled mixture of (*Z*)-2-(2-tritylaminothiazol-4-yl)-2-trityloxyiminoacetic acid (7.27 g, 10.8 mmol) and 1-hydroxybenzotriazole hydrate (1.65 g, 10.8 mmol) in dry THF (180 ml) was added dicyclohexylcarbodiimide (DCC) (2.23 g, 10.8 mmol) and the mixture was stirred for 1 hour at 5°C and filtered to afford the active ester solution. A suspension of **4a** (3.99 g, 9 mmol) in EtOAc (50 ml) was washed with aq $NaHCO_3$ and water, successively and evaporated under reduced pressure. The residue was dissolved in THF (30 ml) and added into the above-prepared active ester solution. The mixture was stirred overnight at room temperature and concentrated under reduced pressure. The residue was dissolved in EtOAc, washed with water and concentrated under reduced pressure. The residue was chromatographed on a Silica gel column (Merck Kieselgel 60, 90 g) and the column was eluted with a mixture of toluene and EtOAc (20 : 1) to give 9.4 g (98%) of **5a** as an amorphous powder. SI-MS m/z 1,060 $(M+1)^+$.

Compounds **5b** and **5c** were prepared by a similar procedure in 87% and 95% yield, respectively. Data of **5** are summarized in Table 9.

3-*Z*- and *E*-Alkenyl Cephalosporins (**6** and **7**): General Procedure Illustrated with the Preparation of 7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)- and (*E*)-1-propenyl]-3-cephem-4-carboxylic Acids (**6a** and **7a**)

A mixture of **5a** (9.2 g, 8.7 mmol) in 85% formic acid (30 ml) was stirred for 1 hour at room temperature. Hydrochloric acid (1 ml) was added and the mixture was stirred for 2 hours. Evaporation of the mixture under reduced pressure followed by trituration of the residue with isopropyl ether gave the crude product, which was chromatographed on a column of C_{18} Silica gel (the packing of prepPAK-500/C_{18} (Waters), 300 ml). The column was eluted with water, 10% MeOH and 20% MeOH, successively and the fractions were monitored by HPLC (column Senshu Pak SSC-ODS-262, solvent CH_3CN - pH 2.6 buffer (15 : 85), 1 ml/minute, *Z* isomer 5.6 minutes, *E* isomer 7.8 minutes). The fractions contain-

Table 9. Yields and ^{1}H NMR of the acylation products (**5** and **9**).



Compound	R_1	R_2	Yield (%)	^{1}H NMR (80 MHz, δ in $CDCl_3$)						
				2-H[a]	6-H[b]	H_a[c]	H_b[d]	H_c[e]	$=CCH_3$[e]	Others
5a	$C(C_6H_5)_3$	CH_3	98	3.20	5.05	6.40	6.05	6.90	1.40	—
5b	$C(C_6H_5)_3$	C_2H_5	87	3.22	5.15	6.43	6.06	6.92	—	0.83 (CCH_3)
5c	$C(C_6H_5)_3$	H	95	3.40[c]	5.05	6.45	[f]	6.96	—	5.25 (=CH), 5.35 (=CH)
9a	CH_3	CH_3	91	3.35	5.06	6.72	6.05	6.90	1.40	4.15 (OCH_3)
9b	$CH(CH_3)_2$	CH_3	49	3.35	5.07	6.73	6.06	6.90	1.43	1.33 ($C(CH_3)_2$)
9c	$CH_2C\equiv CH$	CH_3	97	3.40	5.10	6.80	6.11	6.96	1.50	2.50 (≡CH), 4.87 (OCH_2)
9d	$CH_2CH=CH_2$	CH_3	95	3.35	5.05	6.74	6.08	6.90	1.44	4.75 (OCH_2)
9e	CH_2COO-*tert*-Bu	CH_3	70	3.32	5.07	6.80	6.10	6.88	1.42	1.40 ($C(CH_3)_3$), 5.24 (OCH_2)

[a] ABq, J=18 Hz, except **5c**.
[b] d, J=5.5 Hz.
[c] s.
[d] br d, J=11 Hz.
[e] dd, J=6 and 2 Hz.
[f] Overlapped with phenyl protons.

ing the *Z* isomer were concentrated under reduced pressure and the residue was freeze-dried to give 1.15 g (33%) of the *Z* isomer (**6a**): The fractions containing the *E* isomer were worked up similarly to give 143 mg (4%) of the *E* isomer (**7a**).

The 3-*Z*- and *E*-butenyl derivatives (**6b** and **7b**) and 3-vinyl derivative (**6c**) were prepared by a similar procedure. Physico-chemical properties are summarized in Table 1.

Acylated Products (**9**): General Procedures Illustrated with the Preparation of Diphenylmethyl 7-[(*Z*)-2-(2-Tritylaminothiazol-4-yl)-2-methoxyiminoacetamido]-3-(1-propenyl)-3-cephem-4-carboxylate (**9a**)

To a chilled mixture of (*Z*)-2-(2-tritylaminothiazol-4-yl)-2-methoxyiminoacetic acid (266 mg, 0.6 mmol) and 1-hydroxybenzotriazole hydrate (92 mg, 0.6 mmol) in THF (5 ml) was added DCC (124 mg, 0.6 mmol). The mixture was stirred for 2 hours at 5°C and filtered to afford the active ester solution. A suspension of **4a** (221 mg, 0.5 mmol) in EtOAc (5 ml) was shaken with aq $NaHCO_3$ and water, dried and evaporated under reduced pressure. The residue was dissolved in THF (1 ml) and added into the above-prepared active ester solution. The mixture was stirred overnight at room temperature and evaporated under reduced pressure. The residue was dissolved in EtOAc and the mixture was washed with aq $NaHCO_3$ and water. After concentration, the residue was chromatographed on a column of Silica gel (Merck Kieselgel 60, 10 g) and the column was eluted with toluene containing 10% of EtOAc. The fraction containing the desired product was concentrated to dryness to give 380 mg (91%) of the title compound. SI-MS *m/z* 832 $(M+1)^+$.

The *O*-substituted derivatives (**9b**~**9e**) were similarly prepared by *N*-acylation of **4a** with the corresponding *N*-tritylated side chain acids (**8b**~**8e**). Data on **9** are summarized in Table 9.

O-Substituted Cephalosporins (**10**): General Procedures Illustrated with the Preparation 7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylic Acid (**10a**)

A mixture of **9a** (350 mg, 0.42 mmol) and 85% formic acid (2 ml) was stirred for 1 hour at room temperature. Hydrochloric acid (0.1 ml) was added and the mixture was stirred for additional 2 hours. The mixture was evaporated under reduced pressure and the residue was chromatographed on a column of C_{18} Silica gel (the packing of prepPAK-500/C_{18} (Waters), 50 ml). The column was eluted with water, 10% MeOH and 20% MeOH, successively and the fractions were monitored by HPLC. The fractions containing the desired product were combined and concentrated under reduced pressure. The concentrate was freeze-dried to give 62 mg (35%) of the product as an amorphous powder. The *O*-substituted acids (**10b**~**10e**) were prepared by a similar procedure. Physico-chemical data are summarized in Table 2.

Prodrug-type Esters (**11**): General Procedure Illustrated with the Preparation of 1-Acetoxyethyl 7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylate (**11a**)

To a stirred mixture of **6a** (1.02 g, 2.5 mmol) in DMF (4 ml) was added sodium carbonate (159 mg, 1.5 mmol) and the mixture was stirred for 1 hour at room temperature. The mixture was cooled to 0°C and a solution of 1-acetoxyethyl bromide (501 mg, 3 mmol) in DMF (2 ml) was added dropwise over a period of 15 minutes. The mixture was stirred for 40 minutes at the same temperature and diluted with EtOAc (100 ml). The reaction mixture was washed with water and dried over anhydrous $MgSO_4$. The filtrate was concentrated *in vacuo* and the residue was chromatographed on a column of Silica gel (Merck Kieselgel 60, 50 g). The column was eluted with $CHCl_3$ - MeOH (20:1) and the fractions containing the desired product were combined and concentrated under reduced pressure. The residue was triturated with isopropyl ether and 296 mg (24%) of **11a** was collected by filtration. The product (100 mg) was crystallized from EtOAc to give 49.6 mg of crystalline **11a**: ^1H NMR (400 MHz, DMSO-d_6) δ 1.43 and 1.44 (3H, two d, J=5.5 Hz, $CHCH_3$), 1.59 and 1.60 (3H, two dd, J=1.8 and 7.0 Hz, $CH=CHCH_3$), 2.05 and 2.07 (3H, two s, OAc), 3.52 and 3.54 (1H, two d, J=18 Hz, 2-H), 3.59 and 3.61 (1H, two d, J=18 Hz, 2-H), 5.22 and 5.24 (1H, two d, J=4.8 Hz, 6-H), 5.62~5.74 (1H, m, $CH=CHCH_3$), 5.80 and 5.83 (1H, two dd, J=4.8 and 8.0 Hz, 7-H), 6.07 and 6.09 (1H, two dd, J=10.8 and 1.8 Hz, $CH=CHCH_3$), 6.65 and 6.66 (1H, two s, thiazole-H), 6.83 and 6.93 (1H,

two q, J=5.5 Hz, C*H*CH_3), 7.11 (2H, s, NH_2), 9.45 and 9.47 (1H, two d, J=8.0 Hz, 7-CONH), 11.291 and 11.293 (1H, two s, NOH).

Compounds **11b**~**11f** were prepared by a similar procedure using pivaloyloxymethyl iodide, 1-cyclohexylacetyloxyethyl iodide, 1-ethoxycarbonyloxyethyl iodide, 1-cyclohexyloxycarbonyloxyethyl iodide and (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl bromide, respectively, instead of 1-acetoxyethyl bromide in **11a** and compounds **11e** and **11f** were crystallized from MIBK and MEK respectively. Physico-chemical properties of **11** are summarized in Table 3.

Determination of MICs

MICs were determined on solid medium by the standard 2-fold agar dilution method in Mueller-Hinton agar (Difco) after incubation at 37°C for 18 hours with an inoculum size of 10^6 cfu/ml.

Blood Level in Mice

Male *dd*Y-mice, weighing 18 to 22 g, were given an antibiotic solution in 10% DMSO at a dose of 100 mg/kg by oral, im or iv administration. Blood samples were collected from the orbital sinuses at 0.5, 1, 2, 3, 4, 5, 6 and 7 hours after oral administration or at 10, 20, 30, 40, 50, 60, 90 and 120 minutes after im or iv administration and assayed by the paper-disc agar diffusion method using *Micrococcus luteus* PCI 1001 as an assay organism. The esters (**11**) were administered orally at a dose of 100 mg/kg equivalent to their parent acid (**6a**) and their blood levels were assayed and indicated as the concentration of **6a**. The half life ($T_{1/2}$, hours) and area under the drug concentration-time curve (AUC, μg·hours/ml) were calculated by the method of LEITNER *et al.*[19]. The relative bioavailability of **11** and cefuroxime axetil was calculated from AUC's after oral administration of these prodrug esters and those after iv administration of parent cephalosporin **6a** and cefuroxime, respectively. The relative bioavailability of cefaclor was calculated from AUC's after oral and iv administrations of cefaclor.

Protective Effect

Organisms were cultured overnight at 37°C in brain heart infusion broth and suspended in 5% hog mucin (American Laboratory, Omaha, Neb. U.S.A.). Male *dd*Y-mice were infected intraperitoneally with about 100 times of the median lethal dose of the pathogen. Mice were individually given an antibiotic solution in 10% DMSO at each dose level of 0.16, 0.63, 2.5 and 10 mg/kg orally or intramuscularly just before the bacterial challenge. The 50% protective dose (PD_{50}, mg/kg) was calculated by the method of LITCHFIELD and WILCOXON[20], from survival rate recorded on 7 days after the bacterial infection. The esters **11** were administered at doses equivalent to **6a** and their PD_{50} values were indicated as **6a**.

Acknowledgments

The authors express their appreciation to Professor M. OHASHI of the University of Electro-Communications for the mass spectral data. They also wish to thank Dr. T. TSUNO and his associates of the Analytical Chemistry Laboratory of this Institute for the microanalytical and spectral data.

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