Oral Absorption of Cephalosporin Antibiotics. 1. Synthesis, Biological Properties, and Oral Bioavailability of 7-(Arylacetamido)-3-chloro Cephalosporins in Animals[1]

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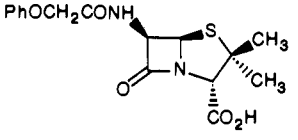
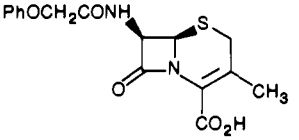
A number of 7-(arylacetamido)-3-substituted cephalosporins were prepared and tested in animals for oral absorbability. Bioavailability in mice, rats, dogs, and monkeys was determined after oral or parenteral administration. Oral bioavailability of five compounds selected for more intensive study was generally higher than that of penicillin V in all species tested. The results of ED_{50} testing against experimental infections in mice generally supported the bioavailability studies. Antibiotic activities were evaluated against Gram-positive and Gram-negative organisms with some derivatives expressing in vitro activity similar to cefaclor. The plasma half-life in rats was relatively short and the plasma curves were strongly influenced by probenecid, indicating rapid renal secretion. Some 7-(arylacetamido)-3-chloro cephalosporins are orally absorbed in animals to a greater extent than penicillin V, an antibacterial agent of proven clinical utility.

Inspection of the structural formulas of orally administered cephalosporins currently used in clinical medicine reveals one common feature: they all contain a 7-arylglycine side chain. However, if one examines clinically useful penicillins, it can be seen that, in addition to some with the phenylglycine side chain, there are several orally absorbed penicillins with non-arylglycine side chains, e.g., penicillin V, penicillin G, cloxacillin, and dicloxacillin.[2] Thus, it was questioned whether the amino group of the arylglycine side chain is indeed essential for the oral absorption of cephalosporin antibiotics or whether some arylacetamido cephalosporins might also be orally absorbed. A literature search revealed that cephalosporin 8 (with the phenylacetyl side chain and no substituent at the 3-position) was reported to be well absorbed after oral administration.[3] In addition, Huffman and Preston discovered in 1969 that (phenylthio)acetyl derivatives of 7-ACA were orally absorbed.[4]

In order to examine if cephalosporins having the same side chains as penicillin V and G might be orally active, we selected a group of 7-arylacetamido cephalosporins for oral absorbability testing. Because of the structural similarity between penicillin V and compound 1, the latter was chosen as the starting candidate for our studies. However,



penicillin V



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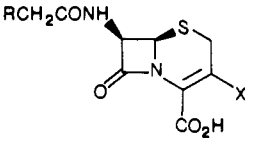
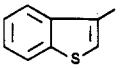
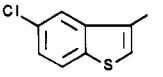
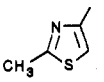
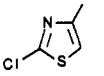
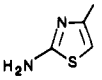
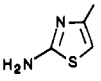
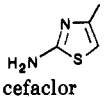
since 1 displays only moderate antibacterial activity, other more potent cephalosporins (2–8) were also selected for preliminary in vitro and in vivo testing (see Tables I and II). Most of these compounds are structurally similar to 1 with changes only at the 3-position. From the results shown in Table I, it appeared that 2 (with a 3-chloro substituent) was orally absorbed in mice to the greatest extent. Therefore, we prepared a series of 3-chloro ceph-

Table I. Structures and Oral Bioavailability Results in Mice[a]



compd no.	R	X	% bioavailability
1	PhO	CH_3	37
2	PhO	Cl	56–74
3	PhO	H	22
4	PhO	OCH_3	14
5	PhO	$CH{=}CH_2$	36–64
6	PhO	CH_2OCH_3	19
7	Ph	Cl	37
8	Ph	H	12–20
9	*p*-$(HO)C_6H_4O$	Cl	21
10	*p*-$(HO)C_6H_4$	Cl	14
11		Cl	44–60
12		Cl	47–54
13		Cl	47
14		Cl	ND
15		Cl	66
16		Cl	56
17		Cl	64
18		OCH_3	<5
19		$CH{=}CH_2$	25–35
20	cefaclor		76
21	penicillin V		29

[a] Compounds 3 and 4 were prepared by J. Fisher, 5 by D. O. Spry, 6 by R. T. Vasileff, and 12 by K. Kuhler. We are thankful for generous supplies of these compounds. ND = not done.

(1) Wright, W. E.; Quay, J. F.; Kukolja, S.; Eudaly, J. A.; Stucky, J. R.; Johnson, R. J.; Pfeil, J. L.; Draheim, S. E.; Ott, J. L.; Counter, F. T.; Cooper, R. D. G.; Shoufler, J. R.; Johnson, J. A. 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, 28 September–1 October, 1986, New Orleans, LA; Abstract 594.

(2) (a) *Chemistry and Biology of β-Lactam Antibiotics*; Gorman, M., Morin, R. B., Eds.; Academic: New York, 1982; Vol. 3, pp 382 and 390. (b) Price, K. *Structure-Activity Relationship among the Semisynthetic Antibiotics*; Perlman, D., Ed.; Academic: New York, 1977; p 29.

(3) (a) Scartazzini, R.; Bickel, H. *Heterocycles* **1977**, *7*, 1172. (b) Jung, F. A.; Pilgrim, W. R.; Poyser, J. Ph.; Siret, P. J. *Topics in Antibiotic Chemistry*; Sammes, P. G., Ed.; Wiley: New York, 1980; Vol. 4, p 42.

(4) Preston, D. A.; Huffman, G. W., personal communication.

0022-2623/88/1831-1987$01.50/0

Table II. Minimum Inhibitory Concentration Values (μg/mL) of 7-Arylacetamido Cephalosporins[a]

compd no.	S. aureus			S. epidermidis		S. pyogenes	S. pneumo.	H. influenzae		E. coli	Salmonella	Shigella sonnei	K. Pneumo.	E. aerogenes
	X1.1	V41	S13E	Epil	222	C203	Park	sens CL	res 76	EC14	X514	N9	X68	EB17
1	2	32		16	4	2	2	16	8					
2	0.25	8	16	4	0.25	0.125	0.125	1	2	64			128	
3	0.5	16	16	2	0.5	0.5	1	32	8	32				
4	2	32	16	8	2	1	1	4	4					
5	0.25	16	16	8	0.5	0.125	0.125	4	1					
6	0.25	0.5	32	0.5	0.25	0.06	0.125	2	2					
7	0.5	16	32	2	1	0.125	0.125	1	1	8	4	64	4	
8	1	16	32	4	1	0.25	1	4	1	8	8	64	4	64
9	0.5	128	128	16	1	0.06	0.125	2	1	64	64	64		
10	2	16	128	8	2	0.125	0.25	2	2	32	8	128	8	
11	0.5	16	16	2	1	0.125	0.125	1	1	4	2	32	2	64
12	0.5	8	16	4	1	0.125	0.125	1	1	8	1	32	2	
13	0.125	8	8	1	0.25	0.06	0.125	1	0.25	64	128	128	128	
14	0.03	2	2	0.5	0.125	0.015	0.015	1	0.25	64	128	128	128	128
15	1	128	128	8	2	0.25	1	2	1	16	4	64	8	
16	1	64	64	8	2	0.25	0.5	2	2	16	8	64	8	
17	2	32	32	8	2	0.125	0.25	1	1	1	0.25	4	0.5	8
18	8	32		128	32	0.5	2	8	8	8	2	32	8	32
19	1	8	16	8	1	0.125	0.5	4	2	4	2		2	128
cefaclor	1	16	64	8	4	0.25	0.5	1	2	1	0.5	4	1	16
Pen V	0.06	32	16	0.125	0.06	0.06	16	64						

[a] Determined by agar dilution method of Kirst et al. *J. Antibiot.* 1982, *35*, 1675. Blank spaces indicate an MIC >128 μg/mL.

alosporins (**9**–**17**) for further testing in mice and higher species.

Chemistry

Several of the compounds desired for testing had been previously described.[5] New compounds were prepared by N-acylation of the corresponding cephem nuclei. Acylation for compounds **9**–**16** was carried out in aqueous medium under Schotten–Bauman conditions. Compounds **17**–**19** were made by acylation of the silyl esters of the corresponding cephem nuclei with [2-(tritylamino)thiazol-4-yl]acetyl chloride and subsequent hydrolysis of the esters and removal of the trityl protective groups with 98% formic acid.

Biological Results and Discussion

Microbiological Data. Antibiotic activities were evaluated against Gram-positive and Gram-negative organisms; the minimum inhibitory concentrations (MIC's) are shown in Table II. For comparison, the activities of cefaclor and penicillin V are also included. The in vitro susceptibility of microorganisms in agar dilution tests indicates that compound **2** displays good antibacterial activity against Gram-positive organisms and *Haemophilus influenzae* but poor activity against other Gram-negative bacteria. However, the 3-chloro cephalosporins **7**, **8**, **11**, **12**, and **17** show much better Gram-negative activity. It is noteworthy that cephalosporin **17** has an antibacterial spectrum similar to that of cefaclor. Since compound **17** showed both an excellent spectrum of microbiological activity and oral bioavailability, we decided to determine if the amino group on the thiazole ring in **17** was essential for both of these properties. Therefore, we synthesized the methyl and chloro analogues **15** and **16**. The bioavailabilities of **15** and **16** are comparable to **17**, showing that the amino group is not essential for oral absorption. However, the microbiological spectra for these two analogues were not as good as that of **17**. Neither of these analogues possesses any significant activity against *Escherichia coli* and *Klebsiella* strains, while the aminothiazolyl compound **17** shows good activity against these organisms. The potency of all 3-chloro derivatives against *H. influenzae* is good and similar to that of cefaclor.

(5) (a) Chauvette, R. B.; Pennington, P. A. *J. Med. Chem.* **1975**, *18*, 403. (b) Scartazzini, R.; Bickel, H. *Helv. Chim. Acta* **1972**, *55*, 429.

Oral Absorption and Efficacy in Mice. Oral absorption was determined by comparing the percentage of the dose excreted in the urine after oral or parenteral dosage to groups of mice. Calculation of bioavailability (BA) was made with the following formula:

$$\frac{\text{percent of dose in urine after po}}{\text{percent of dose in urine after sc}} \times 100 = \% \text{ of BA (bioavailability)}$$

The results of this evaluation (shown in Table I) indicate that several of these compounds appear to be absorbed to a greater extent than one would have expected from historical data on the oral absorption of cephalosporins having non-arylglycine side chains. Particularly, the 3-chloro compounds **2**, **7**, **11**–**13**, and **15**–**17** and 3-vinyl compounds **5** and **19** display good bioavailability in the mouse model. For comparison, the bioavailabilities for cefaclor (76%) and penicillin V (29%) were also determined. To estimate in vivo efficacy after oral administration, ED_{50} testing against experimental infections in mice was also undertaken. The results shown in Table III demonstrate both parenteral and oral activity in mice infected with *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*. The ED_{50} values for cephalosporins **2**, **7**, **11**, **13**, **14**, and **17** indicate relatively efficient absorption after oral administration and are generally consistent with the bioavailability results by urinary excretion. These bioavailability and ED_{50} results of the test compounds, penicillin V, cefaclor, and cephalexin, shown in Table I and III, demonstrate the well-known complex therapeutic interaction of absolute bioavailability, compound potency, infection characteristics, and pharmacokinetic behavior. Since the initial experiments in mice were encouraging for certain of the (arylacetamido)-3-chloro cephalosporins, it was decided to expand studies to other animal species.

Oral Absorption in Dogs and Monkeys. On the basis of mouse data and microbiological screening, five 7-(arylacetamido)-3-chloro cephalosporins, **2**, **7**, **11**, **13**, and **17**, were selected for further studies in higher animal species. Figures 1 and 2 show mean antibacterial activity in the plasma of six female mongrel dogs after intravenous or oral administration of the selected cephalosporins. Penicillin V is also included for comparison. The curves in Figures 1 and 2 were obtained by measuring the antibacterial activity in dog plasma at intervals following intravenous or oral administration of the test compounds. The bioa-

Table III. Efficacy of Selected Cephalosporins against Lethal Mouse Infections: ED_{50} Values (mg/kg × 2)[a]

	S. pyogenes C203			*S. aureus* 3055			*S. pneumonia* Park		
compd no.	po	sc	MIC[b]	po	sc	MIC	po	sc	MIC
2	7.07	5.69	0.06	2.04	1.25	0.25	20	13.4	0.06
3	>10	>10	0.25	7.7	1.36	0.5	>50	25.8	0.25
5	8.3	1.67	0.125	0.89	0.25	0.25	>10	>10	0.25
7	6.41	3.68	0.125	5.4	1.38	0.5	15.1	12.3	0.25
8	19.8	12.5	0.25	5.4	1.06	1	25	35	1
11	4.35	1.46	0.125	5.96	1.25	0.5	25.1	14.4	0.25
13	5.5	3.8	0.06	1.51	0.62	0.25	36.2	14.8	0.06
14	1.68	0.83	0.03	2.2	3.5	0.06	12.5	3.1	0.02
17	1.75	2.5	0.25	13.0	2.9	1.0	31.2	21.8	0.5
Pen V	0.62	0.16	0.06	0.5	0.04	0.04	2.8	2.1	0.02
cefaclor	1.25	1.25	0.25	1.36	1.32	1	10.7	11.5	2
cephalexin	3.3	2.9	0.5	0.34	0.2	1	51	23	4

[a] Compounds were administered either subcutaneously (sc) or orally (po) to 19–21-g random sex ICR mice at 1- and 5-h intervals after intraperitoneal bacterial challenge. Each compound was tested at five 2-fold dilution levels; eight mice were used per dilution. In order to standardize these values, the challenge was taken from a frozen pool of organisms and contained 50–500 LC_{50}'s per challenge dose. The values were not obtained from a single test, but for each compounds the sc and po values were run in parallel. [b] μg/mL.

Table IV. Oral Bioavailability of Selected 7-(Arylacetamido)-3-chlorocephems and Penicillin V in Female Mongrel Dogs[a]

	AUC				24-h urinary excretion: % of dose	
compd no.	po	iv	% bioavailable	$T_{1/2}$, min	po	iv
2	1000 ± 110	3450 ± 360	30	37	18 ± 2.1	48 ± 3.9
7	970 ± 220	5840 ± 450	17	60	15 ± 2.7	67 ± 2.1
11	930 ± 60	4520 ± 480	21	57	17 ± 1.7	75 ± 2.1
13	<48	1760 ± 60	<3	26	1.5 ± 0.5	20 ± 2.8
17	560 ± 100	1990 ± 380	28	70	10 ± 1.6	36 ± 6.2
Pen V	180 ± 30	1520 ± 350	11	18	4.9 ± 1.0	32 ± 8.3

[a] Data listed with error estimates represent the mean ± SEM for six dogs. AUC = area under the plasma concentration vs time curve in μg min/mL. $T_{1/2}$ calculated from the β-phase (terminal phase) of the mean iv plasma curve. Dose: 15 mg/kg.

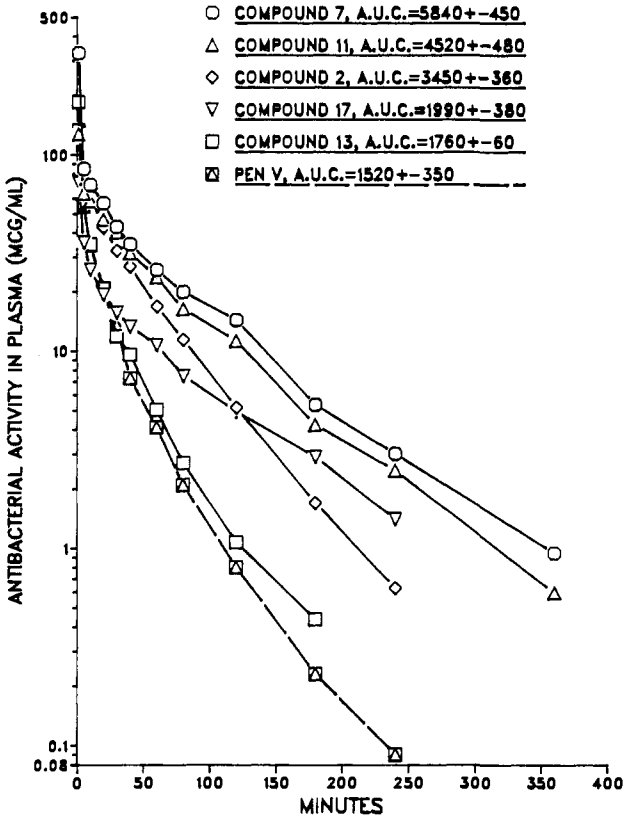


Figure 1. Antibacterial activity in plasma of female mongrel dogs after intravenous administration of single 15 mg/kg doses in solution.

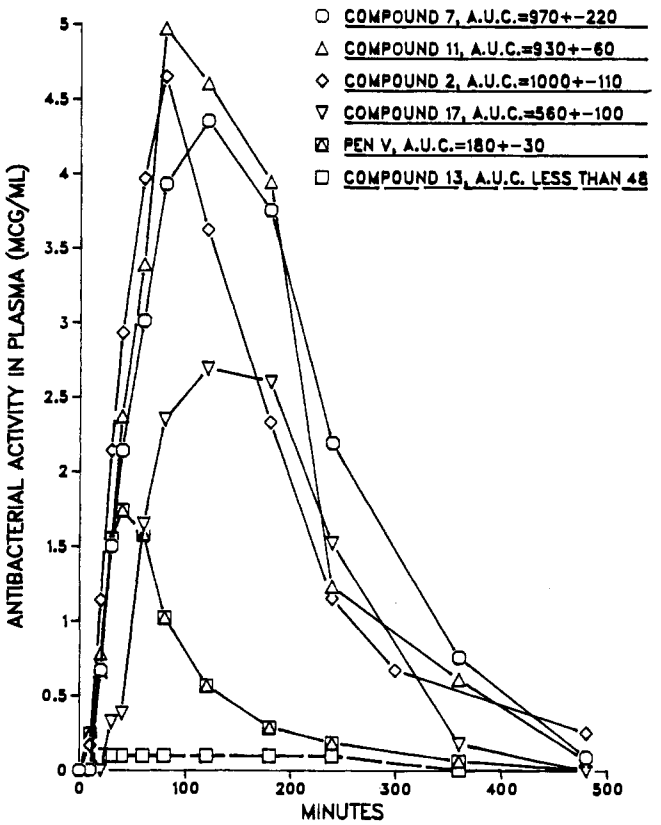


Figure 2. Antibacterial activity in plasma of female mongrel dogs after oral administration of single 15 mg/kg doses in solution.

vailability (BA) was calculated by using the area under the curve (AUC) obtained in Figures 1 and 2 (iv and po plasma activity) and also by comparison of the percent of dose in the urine. The numerical results of oral bioavailability for the selected cephalosporins and penicillin V obtained in female mongrel dogs, as well as half-lives from the β-phase of the intravenous plasma curve, are shown in Table IV.

Studies in dogs showed that the BA of the derivatives was relatively low, but still generally higher than penicillin

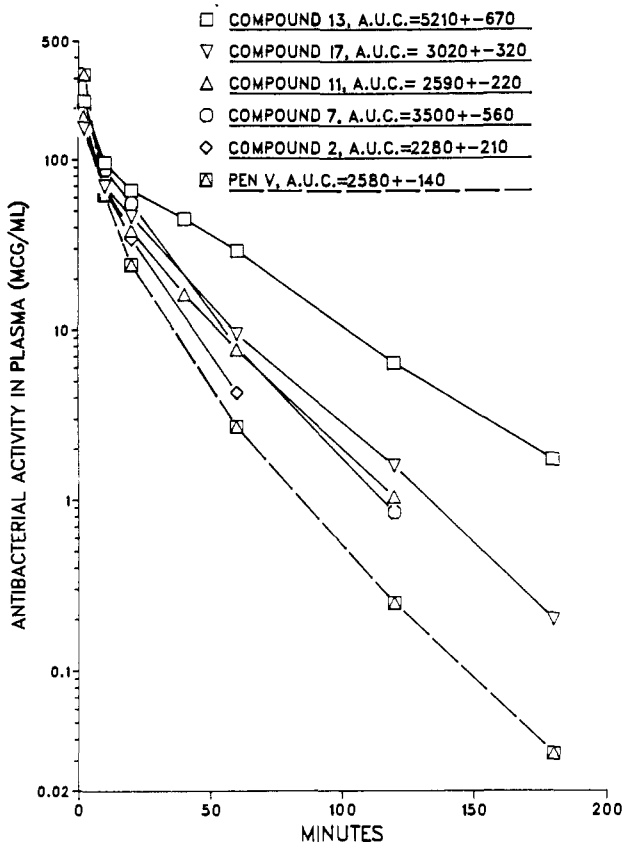


Figure 3. Antibacterial activity in plasma of six rhesus monkeys after intravenous administration of single 30 mg/kg doses in solution.

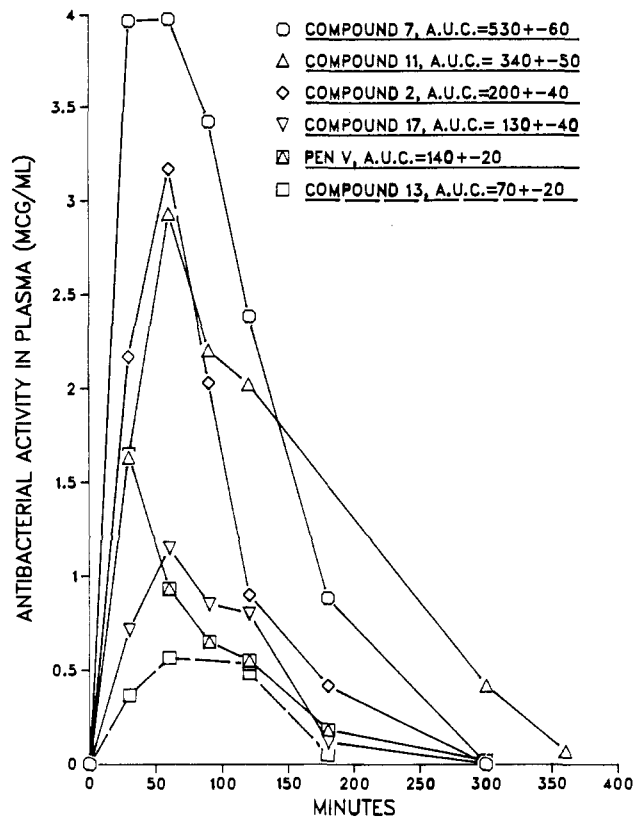


Figure 4. Antibacterial activity in plasma of six rhesus monkeys after oral administration of single 30 mg/kg doses in solution.

Table V. Oral Bioavailability of Selected 7-(Arylacetamido)-3-chlorocephems and Penicillin V in Rhesus Monkeys[a]

compd no.	AUC po	AUC iv	% bioavailable	$T_{1/2}$, min
2	300 ± 40	2280 ± 210	13.1	14
7	530 ± 60	3500 ± 560	15.1	17
11	340 ± 50	2590 ± 220	13.1	17
13	70 ± 20	5210 ± 670	1.3	30
17	130 ± 40	3020 ± 320	4.2	21
Pen V	140 ± 20	2580 ± 140	5.5	12

[a] Data listed with error estimates represent the mean ± SEM for six monkeys. AUC = area under the plasma concentration vs time curve in μg min/mL. $T_{1/2}$ calculated from the β-phase of the mean iv plasma curve. Dose: 30 mg/kg.

V. Two derivatives (2 and 17) had bioavailabilities by AUC of 30% and 28%. The BA calculated from urine excretion after oral or intravenous dosage was consistent with the AUC results.

Similar oral bioavailability testing for the five selected cephalosporins was performed in rhesus monkeys; the results are shown in Figures 3 and 4 and Table V. Oral bioavailability of the tested compounds in six rhesus monkeys was generally low and did not follow the bioavailability rank order of the same five derivatives in dogs and mice. However, three of the five cephalosporins showed BA greater than penicillin V, an oral antibiotic of proven clinical utility.

Oral Absorption and Plasma Levels in Rats. In an attempt to better define the bioavailability of this type of cephalosporin, urinary excretion after subcutaneous or oral dosage to rats was determined for 2 and 17 in a manner similar to that for mice. These results are summarized in Table VI. These results in rats were lower in apparent bioavailability and more variable than in mice—only 23.1% and 19.2% bioavailability for 2 and 17, respectively. However, when compared in this type of test with the oral bioavailability of penicillin V, the low bioavailability for 2 and 17 seemed less discouraging. Mean bioavailability for penicillin V in this rat model was 11.6% and highly variable. When nine animals were dosed orally with penicillin V, and their individual percent urinary excretion was compared with the mean values of nine animals dosed subcutaneously, individual oral bioavailabilities ranged from 4% to 34%. Compound 17 was similarly variable—individual oral bioavailabilities for the five animals varied from 2.8% to 51%.

The rapid urinary excretion of 2 and 17 in rats suggested active renal secretion, an excretion mechanism known to occur with penicillin V in animals and humans. Because

Table VI. Urinary Recovery and Oral Bioavailability of Compounds 2, 17 and Penicillin V in Rats

compd no.	% urinary recovery,[a] mean ± SD (N, range) po	% urinary recovery,[a] mean ± SD (N, range) sc	mean % bioavailable	range of bioavailability individual rats,[b] %
2	10.1 ± 8.3 (9, 3–24)	43.8 ± 6.3 (9, 38–58)	23.1	6.8–54
17	16.2 ± 11 (12, 2–43)	84.3 ± 8.9 (5, 68–91)	19.2	2.8–51
Pen V	2.6 ± 2.5 (9, 1–8)	22.5 ± 5.5 (9, 13–31)	11.6	4–34

[a] Values are mean recovery ± 1 standard deviation; (N) = number of animals; (range) = range of values. [b] Calculated by dividing the lowest and highest individual oral urine recoveries by the mean subcutaneous recovery. Dose = 20 mg/kg.

Table VII. Effect of Probenecid on the Plasma Levels and Half-Lives of Compounds 2, 17, and Penicillin V in Rats

compd no.		mean plasma levels, μg/mL[a] time after dose						AUC,[b] 0–3 h	$T_{1/2}$, min
		5 min	20 min	40 min	1 h	2 h	3 h		
2	control	45.7	19.4	8.2	3.2	0.5	0.5	1245	14.5
2	probenicid[c]	66.0	39.1	25.2	18.4	4.6	1.1	3083	30.0
17	control	40.0	12.5	1.6	<1.3	<1.3	<1.3	804	7.5
17	probenicid	57.5	32.0	15.6	9.8	1.8	<1.3	2126	23.5
Pen V	control	30.4	7.0	1.6	0.4	<0.3	<0.3	595	8.9
Pen V	probenicid	59.0	30.4	14.1	6.8	0.8	<0.3	1918	19.4

[a] Values are the mean of two jugular vein cannulated animals. [b] AUC units are μg min/mL. [c] Probenicid administered iv at 100 mg/kg 5 min prior to test compounds. Test compounds dosed iv at 20 mg/kg.

of the interest in a longer acting drug, this was viewed as a negative pharmacokinetic property. The half-life of penicillin V after iv dosage is even shorter than that of 2, although both compounds were similarly bound by rat plasma (about 70%). Compounds 2 and 17, as well as penicillin V, were similarly affected by coadministered probenecid, a compound known to decrease rate of renal secretion of several organic compounds including penicillin V. The half-life of each compound was approximately doubled (see Table VII). Similar to penicillin V, 7-(arylacetamido)-3-chlorocephems have vigorous renal secretion and short plasma half-lives in animal models.

Summary

A selected number of 7-(arylacetamido)-3-chloro cephalosporins were prepared and tested in animals for oral bioavailability. Bioavailability in mice after oral administration was unexpectedly high for certain of these nonarylglycine derivatives. Oral absorption of several derivatives selected for further study was generally higher than that of penicillin V in mice, rats, dogs, and monkeys. The half-lives in rats were relatively short, and the plasma curves were strongly influenced by probenecid, indicating rapid renal secretion. From these studies, it is concluded that the α-amino group on the 7-arylacetamido side chain functionality is not essential for oral bioavailability, at least in certain animal species. In fact, a number of 7-(arylacetamido)-3-chloro cephalosporins are orally absorbed in animals to a greater extent than penicillin V.

Experimental Section

IR spectra were recorded on a Nicolet FT-IR Model 10-DX instrument. UV spectra were recorded on a Cary Model 219 spectrometer in the solvent indicated. NMR spectra were determined on JEOL FX-90Q and General Electric QE-300 instruments. TLC was done with Merck silica gel plates. Elemental analyses were performed by the microanalytical group of the Lilly Research Laboratories. Analytical results indicated by symbols of the elements were within ±0.4%.

General Procedure for Preparation of 7-Arylacetamido Cephalosporins in Aqueous Acetone. The acid chloride was prepared from an aromatic acetic acid and oxalyl chloride in benzene, in the presence of a catalytic amount of DMF. Next, the cephem nucleus was dissolved in 50% aqueous acetone in the presence of $NaHCO_3$ and acylated by dropwise addition of the previously prepared acid chloride. A typical procedure is illustrated in the following example.

7-[(5-Chlorobenzothiophene-3-yl)acetamido]-3-chloro-3-cephem-4-carboxylic Acid (14). 5-Chlorobenzothiophene-3-acetic acid (2.27 g, 10 mmol) was suspended in 50 mL of benzene, and 3.0 mL of oxalyl chloride was added, followed by 1–2 drops of DMF. Foaming and bubbling occurred immediately. The mixture was stirred at room temperature for 90 min until everything was in solution. The solvent was evaporated, and the residue was dissolved again in 10 mL of benzene and evaporated. The 3-chloro-3-cephem nucleus[5a] (2.8 g, 12.0 mmol) was suspended in 50 mL of water and 50 mL of acetone. By careful addition of $NaHCO_3$ (2.52 g, 30 mmol), the nucleus was dissolved and the solution was cooled in an ice bath. The acid chloride was dissolved in 50 mL of acetone and added dropwise to the cold solution of nucleus. The mixture was stirred at ice-bath temperature for 1 h and for 3 h at room temperature. The progress of reaction was followed by TLC (EtOAc/AcOH, 4:1). At the end of reaction, the acetone was evaporated, the aqueous part was washed with 25 mL of EtOAc, and the EtOAc layer was discarded. Again to the aqueous solution was added 25 mL of EtOAc, and the pH of the aqueous solution was adjusted to 2.5 with 1 N HCl. The EtOAc extract was dried ($MgSO_4$), and the solvent was partially evaporated. After the mixture was cooled and kept in a refrigerator, 1.22 g of colorless crystals were collected: NMR (Me_2SO-d_6) δ 3.66 and 3.94 (AB q, J = 18.0 Hz, 2 H), 3.79 (s, 2 H), 5.14 (d, J = 4.8 Hz, 1 H), 5.66 (dd, J = 4.8 and 8.2 Hz, 1 H), 7.35 (dd, J = 2 and 8.5 Hz, 1 H), 7.6 (s, 1 H), 7.9 (d, J = 2 Hz, 1 H), 7.97 (d, J = 8.5 Hz, 1 H), 9.26 (d, J = 8.2 Hz, 1 H); MS, m/e (444, M + H). Anal. ($C_{17}H_{12}N_2O_4S_2Cl_2$) C, H, N, Cl.

By following the general procedure, the following compounds were prepared:

7-[(4-Hydroxyphenoxy)acetamido]-3-chloro-3-cephem-4-carboxylic acid (9): NMR (Me_2SO-d_6) δ 3.35 and 3.75 (AB q, J = 17.2 Hz, 2 H), 4.47 (s, 2 H), 5.01 (d, J = 4.8 Hz, 1 H), 5.49 (dd, J = 4.8 and 8.4 Hz, 1 H), 7.4–7.8 (m, 4 H), 8.85 (d, J = 8.4 Hz, 1 H), 9.10 (br s, 1 H); UV (EtOH) λ_{max} 223 (ε 10544) and 266 nm (ε 7927); MS, m/e (340, M + −44).

7-[(4-Hydroxyphenyl)acetamido]-3-chloro-3-cephem-4-carboxylic acid (10): NMR (Me_2SO-d_6) δ 3.37 (s, 2 H), 3.66 and 3.93 (AB q, J = 18.0 Hz, 2 H), 5.15 (d, J = 4.8 Hz, 1 H), 5.66 (dd, J = 4.8 and 8 Hz, 1 H), 6.63 and 7.00 (AB q, J = 8.6 Hz, 4 H), 9.02 (d, J = 8 Hz, 1 H), 9.18 (s, 1 H); UV (EtOH) λ_{max} 225 (ε 10042) and 267 nm (ε 8874); MS, m/e (369, M + H). Anal. ($C_{15}H_{13}N_2O_5SCl$) C, H, N.

7-(Benzothiophene-3-ylacetamido)-3-chloro-3-cephem-4-carboxylic acid (13): NMR (Me_2SO-d_6) δ 3.66 and 3.93 (AB q, J = 18 Hz, 2 H), 3.82 (s, 2 H), 5.14 (d, J = 4.8 Hz, 1 H), 5.67 (dd, J = 4.8 and 8.4, 1 H), 7.1–8.0 (m, 5 H), 9.23 (d, J = 8.4 Hz, 1 H); MS, m/e (408, M^+). Anal. ($C_{17}H_{13}N_2O_4S_2Cl$) C, H, N.

7-[(2-Methylthiazol-4-yl)acetamido-]-3-chloro-3-cephem-4-carboxylic acid (15): NMR (Me_2SO-d_6) δ 2.60 (s, 3 H), 3.65 and 3.95 (AB q, J = 18 Hz, 2 H), 5.15 (d, J = 5 Hz, 1 H), 5.70 (dd, J = 5 and 9 Hz, 1 H), 7.15 (s, 1 H), 9.00 (d, J = 9 Hz, 1 H); MS, m/e (374, M^+); UV (EtOH) λ_{max} 247 nm (ε 8446); IR (KBr) 1797 cm^{-1} (C=O). Anal. ($C_{13}H_{12}N_3O_4S_2Cl$) C, H, N.

7-[(2-Chlorothiazol-4-yl)acetamido]-3-chloro-3-cephem-4-carboxylic acid (16):[7] NMR (Me_2SO-d_6) δ 3.60 (s, 2 H), 3.65 and 3.95 (AB q, J = 18 Hz, 2 H), 5.15 (d, J = 5 Hz, 1 H), 5.7 (dd, J = 5 and 9 Hz, 1 H), 7.40 (s, 1 H), 9.10 (d, J = 9 Hz, 1 H); MS, m/e (394, M^+); UV (EtOH) λ_{max} 257 nm (ε 9880); IR (KBr) 1775 cm^{-1} (C=O). Anal. ($C_{12}H_9N_3O_4S_2Cl_2$) C, H, N, Cl.

General Procedure for Preparation of 7-(2-Aminothiazol-4-yl)acetamido Cephalosporins. Acylation of the silyl ester of a corresponding cephem nucleus with [2-(tritylamino)thiazol-4-yl]acetyl chloride was first achieved in an aprotic solvent and then the ester group hydrolyzed and the trityl protective function were removed. A typical procedure is illustrated in the following example:

7-[(2-Aminothiazol-4-yl)acetamido]-3-chloro-3-cephem-4-carboxylic Acid (17).[7] **(A) Preparation of the Acid Chloride.** To a mixture of ether (10 mL) and DMF (0.145 mL, 1.87 mmol),

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(7) Kukolja, S.; Wright, W. E. US Pat. 4 683 227, 1987.

cooled in an ice bath, was added oxalyl chloride (0.16 mL, 1.87 mmol) with stirring. Gas evolution ceased in ca. 5 min, and a colorless precipitate formed. The ether layer was discarded, and to the solid salt was added 6.5 mL of CH_2Cl_2. The suspension was cooled to −10 °C, and [2-(tritylamino)thiazol-4-yl]acetic acid (625 mg, 1.56 mmol) was added at once. In less than 5 min, all was in solution, indicating the acid chloride had formed. This cold solution was added to the previously prepared silyl ester of 3-chloro cephem nucleus.

(B) Preparation of the Silyl Ester and Subsequent Acylation. To a suspension of 3-chloro-3-cephem nucleus (369 mg, 1.56 mmol) in DMF (4.5 mL) was added *N,N'*-bis(trimethylsilyl)urea[6] (352 mg, 1.72 mmol), and the mixture was stirred at room temperature for 45 min. The formed solution was cooled to −10 °C, and pyridine (0.28 mL, 3.43 mmol) was added. To this cold solution was added the earlier prepared solution of the acid chloride dropwise in 5 min, and the mixture was stirred between −5 and −10 °C for 30 min. In order to hydrolyze the silyl ester, CH_2Cl_2 (2 mL) and water (20 mL) were added, and the mixture was stirred for 15 min without cooling. The layers were separated, and the aqueous one was extracted with CH_2Cl_2 (3.0 mL). The combined CH_2Cl_2 solutions were filtered from the small amount of insoluble material, and to the filtrate was added EtOAc (2.0 mL). By cooling and scratching, crystallization was induced. The next morning, 480 mg of crystalline 7-[2-[[(triphenylmethyl)-amino]thiazol-4-yl]acetamido]-3-chloro-3-cephem-4-carboxylic acid was collected: NMR ($CDCl_3$) δ 3.40 and 3.75 (AB q, J = 18 Hz, 2 H), 3.50 (s, 2 H), 4.95 (d, J = 5 Hz, 1 H), 5.60 (dd, J = 5 and 9 Hz, 1 H), 6.15 (s, 1 H), 7.25 (s, 15 H), 7.95 (s, 1 H), 8.60 (d, J = 9 Hz, 1 H).

This product was suspended in 2.0 mL of 98% of formic acid. The suspension was cooled in an ice bath, and the mixture was stirred until a solution was obtained. The solution was then warmed to about room temperature, and stirring was continued for 2 h. The insoluble triphenylcarbinol was filtered and washed with formic acid. The wash and filtrate were combined and stirred with charcoal for 30 min. The charcoal was filtered, and the filtrate was poured slowly into acetone with stirring at room temperature. The solution was kept in a refrigerator overnight, and the colorless crystalline product was filtered off and dried: NMR (Me_2SO-d_6) δ 3.37 (s, 2 H), 3.65 and 3.93 (AB q, J = 18 Hz, 2 H), 5.17 (d, J = 4.7 Hz, 1 H), 5.70 (dd, J = 4.7 and 8.4 Hz, 1 H), 6.25 (s, 1 H), 7.00 (br s, 1 H), 8.98 (d, J = 8.4 Hz, 1 H); MS, m/e (375, M + H); UV (EtOH) λ_{max} 260 (ε 9700); IR (KBr) 1771 cm^{-1} (C=O). Anal. ($C_{12}H_{11}N_4O_4S_2Cl$) C, H, N, O, S, Cl.

The compounds described below were prepared by following the general procedure:

7-[(2-Aminothiazol-4-yl)acetamido]-3-methoxy-3-cephem-4-carboxylic acid (18): NMR (Me_2SO-d_6) δ 3.33 (s, 2 H), 3.56 and 3.66 (AB q, J = 16.7 Hz, 2 H), 3.73 (s, 3 H), 5.04 (d, J = 4.2 Hz, 1 H), 5.41 (dd, J = 4.2 and 8.1 Hz, 1 H), 6.24 (s, 1 H), 6.87 (br s, 2 H), 8.83 (d, J = 8.1 Hz, 1 H); MS, m/e (371, M + H); UV (EtOH) λ_{max} 259 (partially soluble); IR (KBr) 1753 cm^{-1} (C=O). Anal. ($C_{13}H_{14}N_4O_6S_2$) C, H, N.

7-[(2-Aminothiazol-4-yl)acetamido]-3-vinyl-3-cephem-4-carboxylic Acid (19). After removal of the trityl group, the crude product was purified by chromatography over silica gel and eluted with a gradient system of $CHCl_3$ → 10% MeOH/$CHCl_3$: NMR (Me_2SO-d_6) δ 3.4 (s, 2 H), 3.55 and 3.85 (AB q, J = 18 Hz, 2 H), 5.1 (d, J = 4.5 Hz, 1 H), 5.2 (d, J = 11 Hz, 1 H), 5.5 (d, J = 18 Hz, 1 H), 5.6 (dd, J = 4.5 and 9 Hz, 1 H), 6.20 (s, 1 H), 6.85 (dd, J = 11 and 18 Hz, 1 H), 8.8 (d, J = 9 Hz, 1 H); MS, m/e (367, M + H); UV (EtOH) λ_{max} 285 nm (ε 8700).

Absorption Studies in Mice. Procedures used were modifications of a procedure reported earlier.[8] Cox male standard mice, [Lai:COX (standard) BR] were housed overnight in a wire-bottom cage with free access to water and liquid diet. The result was a gastrointestinal tract free of solids, yet without the nutritional shock and coprophagy that often accompanies overnight starvation of rodents. Dose solutions were prepared in saline at 2 mg/mL for subcutaneous or oral administration. In all cases the compounds were administered to the mice at 20 mg/kg on equal weight basis. After dosing, the mice were housed in groups in wire bottom cages designed for the collection of urine. After 2 h, the animals were anesthetized with ether and the urinary bladder and contents were combined with the urine collected from the cages to represent the total urinary excretion in that time period.

The biological activity of the samples was determined by a conventional agar well procedure using *Bacillus subtilis* ATCC 6633 as the test organism. Standard curves were prepared in the same vehicle as that being assayed.

Absorption Studies in Rats. Male Wistar rats [Hap(WI)BR] weighing between 200 and 300 g were cannulated in the jugular vein by using conventional procedures. The cannula was directed under the skin to emerge behind the neck and was kept free from clotting with a 1000 units/mL sodium heparin solution. Iv dosing and blood sampling were later carried out through the cannula and accompanied by appropriate flushing procedures using saline or heparin solution. Animals were allowed to recover overnight with free access to food and water. At midnight, lights were turned on to simulate daytime to suppress further eating. This resulted in an empty upper GI tract when oral dosing was anticipated. Animals were dosed parenterally or orally at 20 mg/kg with the test compound dissolved in normal saline at 6 mg/mL. Urine was collected through wire-bottom cages and blood was sampled through the cannula. Urine and heparinized plasma samples were refrigerated until assayed either the same day or following day with the same microbiological assay procedure used for mice.

Absorption Studies in Dogs. Six female mongrel dogs (14–23 kg) were fasted overnight in their home cages with free access to water. On the day of the study they were transferred to metabolism cages for the duration of the test. Thirty minutes prior to the dose a Foley retention catheter (French No. 14) was placed in the urinary bladder and secured by inflation of the balloon with water. Dogs to be given intravenous doses of antibiotic were given 500 mL of tap water via a temporary stomach tube. Dogs to be given oral solution doses were given 100 mL of water in the same way.

After 30 min the urinary bladder was emptied, and urine volume and pH were recorded. A sample of the urine was placed in a 15-mL screw-cap vial. At the same time a blood sample was withdrawn from a front leg vein into a heparinized Vacutainer. This sample was centrifuged to sediment cellular elements, and plasma was collected. Samples of plasma and urine were immediately placed in an ice bath and then frozen until the day of assay. Immediately following, single doses of antibiotics in solution were given through a stomach tube into the stomach or by a syringe into a cephalic vein. Oral solution doses were flushed through the tube with a volume of tap water sufficient to bring the total administered volume to 500 mL. Thereafter, samples of plasma and urine were collected at intervals and treated as described above.

On the day of the experiment, standard solutions of the antibiotics were prepared in commercial dog serum and in buffer containing 150 mM NaCl, 25 mM Na_2HPO_4, and 5 mM acetic acid, pH 6.0. The standard solutions were stored frozen alongside the biological samples until the day of assay. On the day of assay, biological samples and standard solutions were thawed, and their relative antibacterial activity was measured in a disk-plate agar diffusion test against *Escherichia coli* MB 3804.

Necessary dilutions of the biological samples were prepared by addition of aliquots of the sample to the same media used to prepare the standard solutions.

For intravenous studies, samples of the antibiotics were dissolved in isotonic saline or in buffer containing 150 mM NaCl, 25 mM Na_2HPO_4, and 5 mM acetic acid; all oral solution studies samples were dissolved in water.

Absorption Studies in Monkeys. Rhesus monkeys (*Macaca mulatta*) were obtained from the Charles River Breeding Laboratories (Wilmington, MA) breeding colony on Key Lois. Monkeys were individually caged upon arrival and isolated for at least 2 months. Twelve adult males weighing 4.73 ± 0.38 kg were assigned to the study. Each compound was administered orally and intravenously to the same group of six animals.

Monkeys were fasted overnight prior to the day of study. During the study monkeys were housed in individual cages with suspended floors under controlled temperature and humidity. At

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the beginning of the test period the animals were removed from their cages and immobilized. A blood sample was removed from one femoral vein by venipuncture and treated as described in the preceding section. Single doses of antibiotic in solution were then administered into the stomach through a temporary nasogastric tube (oral) or by syringe into one saphenous vein (iv). Thereafter, samples of plasma were collected at intervals. Following the dose, the monkeys received six biscuits of Purina certified primate chow No. 5048 and water was available ad lib. Plasma samples were immediately chilled and then frozen and stored at -70 °C until they could be assayed by microbiological assay procedures.

Intravenous studies were performed with isotonic lactated Ringer solution as a solvent; samples for all oral solution studies were dissolved in water.

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Registry No. 1, 10209-11-7; 2, 73426-29-6; 3, 36923-27-0; 4, 51803-42-0; 5, 34712-49-7; 6, 24670-45-9; 7, 68506-27-4; 8, 33477-97-3; 9, 115384-96-8; 10, 115339-15-6; 11, 53483-71-9; 12, 115384-97-9; 13, 115338-99-3; 14, 115339-03-2; 15, 110425-17-7; 16, 110425-18-8; 17, 110425-20-2; 17 (2-(tritylamino)thiazolyl deriv), 115385-03-0; 18, 115384-98-0; 19, 79350-10-0; *p*-(HO)$C_6H_4OCH_2CO_2H$, 1878-84-8; *p*-(HO)$C_6H_4OCH_2COCl$, 115384-99-1; *p*-(HO)$C_6H_4CH_2CO_2H$, 156-38-7; *p*-(HO)$C_6H_4CH_2COCl$, 37859-23-7; 7-amino-3-chloro-3-cephem-4-carboxylic acid, 53994-69-7; 5-chlorobenzo[*b*]thiophene-3-acetic acid, 17266-30-7; 5-chlorbenzo[*b*]thiophene-3-acetyl chloride, 100068-26-6; benzo[*b*]thiophene-3-acetic acid, 1131-09-5; benzo[*b*]thiophene-3-acetyl chloride, 100068-20-0; 2-methyl-4-thiazoleacetic acid, 13797-62-1; 2-methyl-4-thiazoleacetyl chloride, 115385-00-7; 2-chloro-4-thiazoleacetic acid, 29676-72-0; 2-chloro-4-thiazoleacetyl chloride, 115385-01-8; 2-(tritylamino)-4-thiazoleacetic acid, 64220-26-4; 2-(tritylamino)-4-thiazoleacetyl chloride, 115385-02-9; 7-amino-3-methoxy-3-cephem-4-carboxylic acid, 51803-38-4; 7-amino-3-vinyl-3-cephem-4-carboxylic acid, 79349-82-9.

Oral Absorption of Cephalosporin Antibiotics. 2. Expanded Structure–Activity Relationships of 7-(Arylacetamido)-3-substituted Cephalosporins[1]

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The structure–activity relationship for 7-arylacetamido cephalosporins has been extended. Modifications of the 7-aryl group led to improvements in microbiological activity against Gram-positive organisms. However, Gram-negative activity was generally much poorer than that of the lead compound 7-[(2-aminothiazol-4-yl)acetamido]-3-chlorocephalosporanic acid (A). Modifications of the 3-position did not significantly change the microbiological activity or spectrum. Of the compounds selected for mouse protection studies (ED_{50}'s), 7-[(benzothien-3-yl)acetamido]-3-chloro cephalosporin and A showed the best per oral to subcutaneous ED_{50} ratios.

In paper one of this series, we discussed the oral bioavailability of various 7-(arylacetyl)-3-substituted cephalosporins.[1] Here we will describe continuing efforts to determine which structural elements are necessary to improve the microbiological spectrum of this class of compounds while retaining oral efficacy. A number of new cephalosporins were synthesized and tested. Our goal was to expand the microbiological spectrum, particularly activity against Gram-negative organisms. Pharmacologically we wanted to have useful oral bioavailability, which we defined as greater than or equal to that of penicillin V.

Two general approaches to extending this structure–activity relationship (SAR) were taken.[2] In the first, cephalosporin nuclei were acylated with the side chains that had shown promising bioavailability in the earlier study.[1] Cephalosporin nuclei used were 3-chloro, -hydro, -methoxy, -vinyl, and -methyl. The second approach involved acylation of the 3-chlorocephem nucleus with different side chains. On the basis of microbiological spectra and/or unique structures, some compounds were then selected for oral efficacy studies (ED_{50}'s) in mice.

Chemistry

Most of the aromatic acetic acids used for preparation of new compounds were commercially available. The following substituted acetic acids or their esters were prepared according to described methods: ethyl benzothien-5-ylacetate,[3] 5-(trichloromethyl)-1,2,4-oxadiazole-3-acetic acid,[4] 2-phenylthiazole-4-acetic acid,[5] and 2-methyl-4-phenylthiazole-5-acetic acid.[6] Most of these substituted acetic acids were converted to acid chlorides for N-acylation of the corresponding cephem nuclei; acylations were performed in aqueous medium under Schotten–Bauman conditions as described in paper one. In the other cases, couplings were carried out according to the HBT/DCC procedure[7] (see the Experimental Section).

The amino groups in 2-amino-4-phenylthiazole-5-acetic acid[6] and ethyl 2-aminobenzothiazole-5-acetate[8] were protected by tritylation with trityl chloride, the esters were hydrolyzed to the acids, which in turn were converted to the activated HBT esters and coupled to the 3-chlorocephem nucleus. The trityl functions were then removed by treatment with formic acid to give cephalosporins **30** and **31**.

The heterocyclic thioacetamido cephalosporins **32–34** were prepared by displacement of the bromo group in 7-(bromoacetamido)-3-chloro-3-cephem-4-carboxylic acid with the corresponding heterocyclic thiols. The structures

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