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.

Acknowledgment. We express our appreciation to Drs. Lowell D. Hatfield and J. Alan Webber for their support of this project and to the analytical and physical chemistry sections for elemental analyses and UV, IR, and NMR spectroscopy.

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 Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285. *Received March 14, 1988*

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

(1) Kukolja, S.; Wright, W. E.; Quay, J. F.; Pfeil, J. L.; Draheim, S. E.; Eudaly, J. A.; Johnson, R. J.; Ott, J. L.; Counter, F. T.; Cooper, R. D. G.; Chauvette, R. R. *J. Med. Chem.*, preceding paper in this issue.

(2) Pfeil, J. L.; Draheim, S. E.; Counter, F. T.; Kukolja, S.; Ott, J. L., 27th Interscience Conference on Antimicrobial Agents and Chemotherapy, 4–7 October, 1987, New York, NY; Abstract 804.

(3) Kukolja, S.; Draheim, S. E.; Graves, B. J.; Hunden, D. C.; Pfeil, J. L.; Cooper, R. D. G.; Ott, J. L.; Counter, F. T. *J. Med. Chem.* **1985**, *28*, 1896.

(4) Breuer, H., US Pat. 3960849, 1976.

(5) Knott, E. B. *J. Chem. Soc.* **1945**, 455.

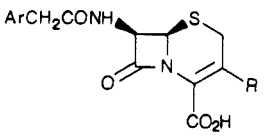
(6) Fraser, R. R. US Pat. 3296250, 1967.

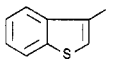
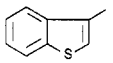
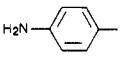
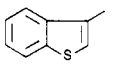
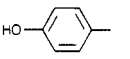
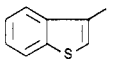
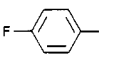
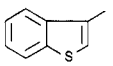
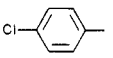
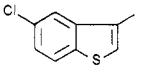
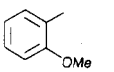
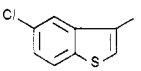
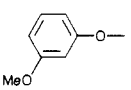
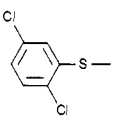
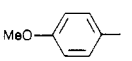
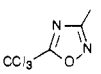
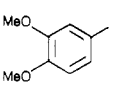
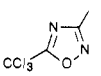
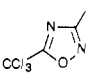
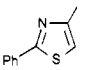
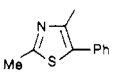
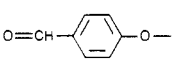
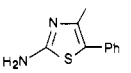
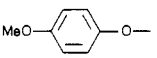
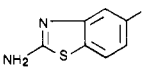
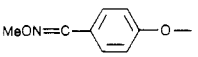
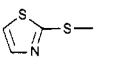
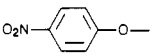
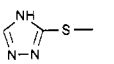
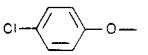
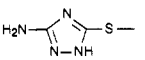
(7) Koening, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788 and 2024. Kemp, D. S.; Trangle, M.; Trangle, K. *Tetrahedron Lett.* **1974**, 2695.

(8) Stuckwisch, C. C. *J. Am. Chem. Soc.* **1949**, *71*, 3417.

0022-2623/88/1831-1993$01.50/0

Table I



no.	Ar	R	formula	anal.	no.	Ar	R	formula	anal.
1		CH_3	$C_{18}H_{16}N_2O_4S_2$	CHN	18[a]		Cl		
2[a]		Cl			19		Cl	$C_{15}H_{14}N_3O_4SCl$	CHN
3		H	$C_{17}H_{14}N_2O_4S_2$	CHN	20[a]		Cl		
4		OCH_3	$C_{18}H_{16}N_2O_5S_2$	CHN	21		Cl	$C_{15}H_{12}N_2O_4SClF$	CHN
5		$CH{=}CH_2$	$C_{19}H_{16}N_2O_4S_2$	CHN	22		Cl	$C_{15}H_{12}N_2O_4SCl_2$	CHN
6[a]		Cl			23		Cl	$C_{16}H_{15}N_2O_5SCl$	CHN
7		H	$C_{17}H_{13}N_2O_4S_2Cl$	CHN	24		Cl	$C_{16}H_{15}N_2O_5SCl$	H[b]
8		Cl	$C_{15}H_{11}N_2O_4S_2Cl_3$	CHN	25		Cl	$C_{16}H_{15}N_2O_5SCl$	CHN
9		Cl	$C_{12}H_8N_4O_5SCl_4$	CHN	26		Cl	$C_{17}H_{17}N_2O_6SCl$	CHN
10		H	$C_{12}H_9N_4O_5SCl_3$	H[b]	27[a]		Cl		
11		OCH_3	$C_{13}H_{12}N_4O_6SCl_3$	CHN	28		Cl	$C_{18}H_{14}N_3O_4S_2Cl$	CHN
12[a]		Cl			29		Cl	$C_{19}H_{16}N_3O_4S_2Cl$	CHN
13		Cl	$C_{16}H_{13}N_2O_6SCl$	CHN	30		Cl	$C_{18}H_{15}N_4S_2Cl$	CHN
14		Cl	$C_{16}H_{15}N_2O_6SCl$	CHN	31		Cl	$C_{16}H_{13}N_4O_4S_2Cl$	*c*
15		Cl	$C_{17}H_{16}N_3O_6SCl$	CHN	32		Cl	$C_{12}H_{10}N_3O_4S_3Cl$	CHN
16		Cl	$C_{15}H_{12}N_3O_7SCl$	CHN	33		Cl	$C_{11}H_{10}N_5O_4S_2Cl$	CHN
17		Cl	$C_{15}H_{12}N_2O_5SCl_2$	CHN	34		Cl	$C_{11}H_{11}N_6O_4S_2Cl$	CHN

[a] This compound was reported in the preceding paper; it is included here and in Tables III and IV for reference purposes. [b] Combustion data for this compound was not within *J. Med. Chem.* guidelines. Other spectral data support this structure. [c] Combustion data for this compound was not within *J. Med. Chem.* guidelines. Other spectral data support this structure. In addition, the analysis for the trityl-protected precursor was correct.

and elemental analyses for new cephalosporins are reported in Table I, and their pertinent NMR data in Table II.

Biological Results and Discussion

Cephalosporin Nucleus Modifications. One compound that had shown promising bioavailability in the earlier study was the 7-(benzothien-3-ylacetamido)-3-chloro cephalosporin **2**. In addition to the 3-chloro nucleus, we acylated several cephalosporin nucleii with this side chain (see Table I, compounds **1** and **3–5**). Compounds **2** and **5**, with 3-chloro and 3-vinyl substituents, produced the best Gram-positive microbiological activity; the Gram-negative activity was not as good (see Table III). A significant improvement in Gram-positive activity was seen by the addition of a chlorine atom at the 5-position of the benzothiophene ring. In fact, the MIC's displayed by **6** were among the best Gram-positive values that were obtained.

Since 7-[(2,5-dichlorothiophenoxy)acetyl]-3-acetoxymethyl cephalosporin has been shown previously to have surprisingly good Gram-positive MIC's even against methicillin resistant staphylococci and to have some oral absorbtion,[9] we prepared the (2,5-dichlorothiophenoxy)acetamido cephalosporin **8**. Although **8** displayed exceptionally good activity against Gram-positive organisms, it was found to be highly serum-bound in rats.[10]

The unique characteristics of the 7-[(2-aminothiazol-4-yl)acetyl]-3-chloro cephalosporin **27** (its broad spectrum

(9) Preston, D. A.; Huffman, G. W., personal communication.
(10) Wright, W. E., personal communication.

Table II. ^{1}H NMR Spectral Data for New Compounds[a]

compd	SCH_2 (AB q)	3'-X (s)	6-H (d)	7-H (dd)	7'-CH_2 (s)
1	3.31, 3.58 (J = 18)	2.01	5.00 (J = 4.4)	5.57 (J = 4.4, 8.1)	3.82
3	3.51 (br s)	6.40 (br s)	4.93 (J = 5.1)	5.60 (d, J = 5.1)	3.77
4	3.64 (br s)	3.75	5.03 (J = 4.4)	5.41 (J = 4.4, 7.9)	3.83
5	3.51, 3.89 (J = 17.5)	5.4, 6.8 (m, J = 11, 17.6)	5.10 (J = 4.8)	5.66	3.80
6[b]	3.48, 3.80 (J = 18.2)		5.04 (J = 4.78)	5.74 (J = 4.78, 8.18)	3.85
7	3.53 (br s)	6.45 (br s)	4.99 (J = 4.8)	5.69 (J = 4.8, 8.4)	3.81
8	3.63, 3.99 (J = 17.6)		5.16 (J = 4.8)	5.68 (J = 4.8, 7.9)	3.90
9[b]	3.47, 3.81 (J = 17)		5.04 (J = 4.8)	5.81 (J = 4.8, 8.6)	3.93
10[b]	3.55 (m)	6.68 (m)	5.01 (J = 4.7)	5.92 (J = 4.7, 8.6)	3.94
11[b]	3.44 (s)	3.91	5.06 (J = 4.1)	5.55 (J = 4.1, 7.9)	3.96
13	3.68, 4.02 (J = 18)		5.22 (J = 5)	5.74 (m)	4.75
14	3.68, 4.02 (J = 18)		5.20 (J = 5)	5.72 (J = 5, 8)	4.54
15	3.67, 4.01 (J = 18)		5.20 (J = 5)	5.71 (J = 5, 8)	4.64
16	3.34, 3.78 (J = 18)		5.02 (J = 5)	5.5 (m)	4.80
17	3.65, 4.00 (J = 18)		5.18 (J = 5)	5.70 (J = 5, 8)	4.60
19	3.64, 3.98 (J = 18)		5.14 (J = 4)	5.70 (J = 4, 7)	3.30
21	3.62, 3.98 (J = 18)		5.15 (J = 4.5)	5.66 (J = 4.5, 7)	3.48
22	3.64, 3.99 (J = 18)		5.16 (J = 4.8)	5.68 (J = 4.8, 7.9)	3.53
23	3.48, 3.80 (J = 18)		4.96 (J = 4)	5.50 (J = 4, 7)	3.29
24	3.62, 3.96 (J = 18)		5.12 (J = 4)	5.64 (J = 4, 7)	3.68
25	3.65, 3.99 (J = 18.03)		5.17 (J = 4.83)	5.68 (J = 4.83, 7.92)	3.73
26	3.65, 3.98 (J = 18)		5.16 (J = 4)	5.67 (J = 4, 7)	3.69
28	3.62, 3.96 (J = 18)		5.16 (J = 5)	5.70 (J = 5, 8)	3.76
29	3.68, 4.01 (J = 18.03)		5.21 (J = 4.8)	5.71 (J = 4.8, 8.1)	3.85
30	3.66, 4.00 (J = 18)		5.20 (J = 5)	5.70 (J = 5, 8)	3.60
31	3.64, 3.98 (J = 18)		5.16 (J = 4)	5.68 (J = 4, 7)	3.48
32	3.65, 3.99 (J = 18)		5.19 (J = 4.8)	5.71 (J = 4.8, 8)	4.06
33	3.63, 3.99 (J = 18)		5.18 (J = 4.8)	5.70 (J = 4.8, 7.9)	3.92
34	3.63, 3.99 (J = 18)		5.18 (J = 4.8)	5.69 (J = 4.8, 8.4)	3.78

[a] Chemical shifts are in δ values (ppm); coupling constants (J) in hertz; unless noted, solvent was DMSO-d_6. [b] Solvent was $CDCl_3$.

Table III. Agar Dilution Minimum Inhibitory Concentrations (μg/mL) against Representative Organisms[a]

compd	*S. aureus* (Pen G suscept)	*S. pyogenes*	*S. pneumoniae*	*H. influenzae* (β-lactamase(−))	*H. influenzae* (β-lactamase(+))	*E. coli*	*K. pneumoniae*	*E. aerogenes*	*S. typhi*
1	1	0.5	1	32	1	>	>	>	>
2	0.125	0.06	0.125	1	0.25	64	128	>	128
3	0.25	0.06	0.25	4	0.5	64	>	>	>
4	0.25	0.25	0.5	8	0.5	>	>	>	>
5	0.125	0.015	0.06	4	4	>	>	>	>
6	0.03	0.015	0.015	2	0.25	128	64	128	128
7	0.125	0.03	NT	NT	NT	128	128	>	128
8	0.03	<0.008	<0.008	1	1	32	32	>	64
9	2	0.25	0.5	4	4	64	16	>	16
10	2	2	2	32	16	32	32	128	16
11	16	4	4	32	32	>	>	>	>
12	0.25	0.125	0.125	1	2	64	128	>	64
13	0.25	0.015	0.03	2	1	>	>	>	>
14	0.25	0.06	0.125	2	2	>	>	>	>
15	0.25	0.03	0.06	4	1	>	>	>	>
16	0.25	0.015	0.03	2	1	>	>	>	>
17	0.125	0.03	0.03	1	1	>	>	>	>
18	0.5	0.125	0.125	1	1	8	4	128	4
19	0.5	0.06	0.125	1	0.5	16	2	128	2
20	2	0.125	0.25	2	2	32	8	>	8
21	0.5	0.06	0.125	4	1	32	32	>	16
22	0.25	0.06	0.125	1	0.5	64	128	>	64
23	1	1	1	2	1	>	>	>	>
24	0.5	0.125	0.25	1	0.5	64	32	>	32
25	0.5	0.125	0.125	1	1	64	32	>	32
26	0.25	0.03	0.06	1	0.5	128	128	>	128
27	2	0.125	0.25	1	1	1	0.5	8	0.25
28	0.25	0.06	0.125	4	0.5	>	>	>	>
29	0.125	0.015	0.06	32	32	>	>	>	>
30	0.03	0.03	0.03	16	16	>	>	>	>
31	0.25	0.06	0.06	0.5	0.5	64	64	>	64
32	1	0.25	0.5	NT	NT	32	16	>	16
33	4	0.5	2	4	4	16	16	>	16
34	4	1	2	4	2	16	8	>	8

[a] Determined by agar dilution method of Kirst et al.: Kirst, H. A.; Wild, G. M.; Baltz, R. H.; Hamill, R. L.; Ott, J. L.; Counter, F. T.; Ose, E. E. *J. Antibiot.* **1982**, *35*, 1675. > denotes an MIC of greater than 128 μg/mL. NT denotes not tested against that organism.

of activity and good bioavailability)[1] prompted us to pursue the synthesis of the 1,2,4-oxadiazole analogues. The oxadiazoles **9**–**11** showed moderate Gram-positive activity but again lacked the required Gram-negative activity. Compound **9** with the 3-chloro group was more active than **10** and **11** with 3-hydro and 3-methoxy substituents.

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

	S. aureus				S. pyogenes				S. pneumoniae			
compd	MIC[b]	sc	po	ratio po/sc	MIC	sc	po	ratio po/sc	MIC	sc	po	ratio po/sc
2	0.25	0.62	1.51	2.4	0.06	3.8	5.5	1.4	0.06	14.8	36.2	2.4
3	0.25	0.8	2.9	3.6	0.06	8.2	>10		0.25	>10	>10	
4	0.5	1.4	2.8	2.0	0.25	>10	>10		0.5	>10	>10	
5	0.125	0.76	3.12	4.1	0.03	3.54	14.7	4.2	0.03	8.4	>16	
8	0.03	1.3	7.7	5.9	0.125	2.8	7.2	2.6	0.015	6.3	>25	
27	1.0	2.9	13.0	4.5	0.25	2.5	1.75	0.7	0.5	21.8	31.2	1.4
31	0.25	3.54	>10		0.03	0.9	10	11	0.06	2.3	>10	
Pen V	0.04	0.04	0.46	11	0.06	0.16	0.62	3.9	0.02	2.1	2.3	1.1

[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 compounds 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 LD_{50}'s per challenge dose. The values were not obtained from a single test, but for each compound the sc and po values were run in parallel. [b] μg/mL.

Side-Chain Modifications. Because of its favorable pharmacokinetics, 7-(phenoxyacetyl)-3-chloro cephalosporin **12** was chosen for structural modification, and various para-substituted derivatives **13–17** were prepared. These modifications did not significantly improve the biological activity over that seen in the parent compound **12**. Gram-negative activity was relatively poor for all the derivatives.

Next, derivatives **19–26** of (phenylacetyl)-3-chloro cephalosporin **18** were prepared. Interestingly, **18** showed greater Gram-negative activity than its analogue **12**. As was the case with the phenoxyacetyl side chain, little improvement in activity was seen through derivatization. Compounds **22** and **26** showed the lowest Gram-positive MIC's, while **19** showed the lowest Gram-negative ones. The Gram-negative activity was not as good as that seen previously with compound **27**.[1] One observation of note: when a substituent was placed in the ortho position on the phenyl ring, as in **23**, antibiotic activity in most organisms was reduced. This might be an indication of particular spatial requirements for the enzyme receptor sites of these organisms. In the phenylthiazole series discussed below, a similar trend was seen.

The SAR of the thiazole series[1] was extended to include the 2-phenyl-, 2-methyl-4-phenyl-, 2-amino-4-phenylthiazoles **28–30** and the 2-amino-5-benzothiazole compound **31**. A comparison of the spectrum of activity of these compounds with cephalosporin **27** reveals better Gram-positive MIC's for the phenylthiazole compounds and a lack of Gram-negative activity. One interesting aspect of these data is the comparatively poor *Haemophilus influenzae* activity of compounds **29** and **30** (with *o*-phenyl substituents) when compared with those of **27**.

Finally, we prepared and tested three heterocyclic thioacetamido 3-chloro cephalosporins. As before, compounds **32–34** showed adequate Gram-positive activity, but few Gram-negative organisms were susceptible at MIC's of less than 16 μg/mL.

Efficacy in Treating Mouse Infections. Several compounds were tested in mice for their oral and subcutaneous efficacy in treating infections caused by *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae* organisms. These data are shown in Table IV. The ratio of oral (po) and subcutaneous (sc) ED_{50}'s for each compound was compared to get an idea of relative oral absorption. As Scartazzini and Bickel have pointed out,[11] comparing only the po ED_{50} values can be misleading because of differences in MIC's; a compound with a very good MIC may show a good po ED_{50} even if it is not well absorbed.

Against *S. aureus*, the compounds tested were effective in curing the infections, and all but **31** had a po/sc ratio less than 6. In the same test, penicillin V had a po/sc ratio of 11. Against *S. pyogenes*, only three of the compounds had ratios less than 5, compared to the penicillin V value of 3.9. None of the compounds was as effective orally as Pen V against the more virulent *S. pneumoniae*.

When comparing the same side chain on different nuclei (compounds **2–5**), the 3-chloro nucleus possessed the best ratios (2.4, 1.4, and 2.4) and best po ED_{50}'s. Upon comparing compounds **27** and **31**, it can seen that **27** (with the aminothiazole side chain) is more effective orally than **31**, which has the aminobenzothiazole side chain.

Summary

Modifications of the aryl group of 7-arylacetyl cephalosporins 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 **27**. 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 **2** and **27** showed the best per oral to subcutaneous ED_{50} ratios.

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 95% EtOH. 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%.

7-[(2-Amino-4-phenylthiazol-4-yl)acetamido]-3-chloro-3-cephem-4-carboxylic Acid (28). Preparation of 2-(Tritylamino)-4-phenylthiazole-5-acetic Acid. A solution of trityl chloride (25.8 g, 93 mmol) in 60 mL of chloroform was stirred at 10 °C for 15 min and added to a suspension of ethyl 2-amino-4-phenylthiazole-5-acetate (11.3 g, 43 mmol) in 100 mL of chloroform and 12.4 mL of triethylamine over a 2-min period. TLC (70% EtOAc/30% hexanes) indicated the reaction was complete in 5 min. The reaction mixture was diluted with water. The chloroform layer was separated and then washed with 5% HCl and water. After drying, the solvent was evaporated and the crude product was chromatographed on a silica gel column. Elution was done first with hexanes and then 1:1 EtOAc/hexanes. The yield of the tritylated ester was 9.9 g (46%). This ester was dissolved in 100 mL of ethanol, and 135 mL of 10% aqueous solution of KOH was added. The mixture was refluxed on a steam bath for 3 h. The EtOH was evaporated under reduced pressure; the residue was diluted with water and extracted with EtOAc. The aqueous layer was separated and acidified with 1 N HCl to pH 2.5 and then extracted twice with EtOAc. The combined

(11) Scartazzini, R.; Bickel, H. *Heterocycles* **1977**, *7*, 1167.

extracts were dried, and the solvent was evaporated to yield 3.7 g of the tritylated acid.

HBT/DCC Procedure: Preparation of the Active Ester and Coupling to the 3-Chloro Nucleus.[7] To a solution of tritylated acid (3.68 g, 7.7 mmol) in 800 mL of THF were added 1.16 g of hydroxybenzotriazole and 1.86 g of dicyclohexylcarbodiimide. The mixture was stirred for 2 h at room temperature and concentrated under reduced pressure to approximately half the original volume, and the precipitated dicyclohexylurea was filtered off. The filtrate contained the activated ester ready for coupling.

The 3-chlorocephem nucleus[12] (1.69 g) was suspended in 35 mL of water and 17.5 mL of acetone, and the pH was adjusted to 7.5 with a 45% solution of K_3PO_4. To this mixture, the previously prepared active ester was added, and the pH was maintained at 7.5. The solution was stirred for 16 h at room temperature and then concentrated to remove the acetone and THF. The residue was diluted with water and EtOAc and then acidified to pH 2.5 with 1 N HCl. The EtOAc layer was separated and washed with a 5% $NaHCO_3$ solution. The combined aqueous solutions were acidified to pH 2.5 and extracted twice with EtOAc. After drying, the solvent was evaporated to give 2.52 g of a crude product. This material was chromatographed on a silica gel column, eluting first with $CHCl_3$ and then with 5% MeOH/95% $CHCl_3$. The yield of tritylated cephalosporin was 1.09 g (20%). This material was dissolved in 98% formic acid (14 mL) and 1.5 mL of water, and the solution was stirred at room temperature. The precipitated trityl alcohol was removed by filtration. The filtrate was evaporated under reduced pressure, and then ether was added to the residue, and the mixture was stirred for 30 min before the desired compound **28** was filtered off. Yield 420 mg (62%).

By following this general procedure, cephalosporin **31** was synthesized. The following data are for the trityl protected derivative of **31**: NMR (DMSO-d_6) δ 3.46 (s, 2 H), 3.62 and 3.96 (AB q, J = 18 Hz, 2 H), 5.12 (d, J = 5 Hz, 1 H), 5.62 (dd, J = 5 and 8 Hz, 1 H), 7.0–7.5 (m, 18 H), 8.9 (br s, 1 H), 9.04 (d, J = 8 Hz, 1 H); MS, m/e (667, M^+). Anal. ($C_{35}H_{27}N_4O_4S_2Cl$) C, H, N.

General Procedure for Preparation of Heterocyclic Thioacetamido Cephalosporins 32–34: Synthesis of 7-(Bromoacetyl)-3-chlorocephalosporanic Acid. A solution of 2.5 N NaOH was added dropwise to a suspension of 1.17 g (5.0 mmol) of 7-amino-3-chlorocephalosporanic acid in 25 mL of acetone and 25 mL of H_2O until the pH was 8. At this time the nucleus went into solution. The solution was cooled to −10 °C. Bromoacetyl bromide and 1 N NaOH were slowly added to the reaction alternately, with use of the 1 N NaOH to keep the pH between 8 and 9 so that the unreacted nucleus stayed in solution. Addition was continued until TLC showed no starting nucleus remained. Total addition of $BrCH_2COBr$ was 1.6 mL (18.3 mmol, 3.7 equiv). The acetone was evaporated, and the pH was adjusted to 2.2. The water was then evaporated until the product began to precipitate. After cooling, 829 mg (47%) of product was obtained in two crops: NMR (DMSO-d_6) δ 3.51 and 3.90 (AB q, J = 17.59 Hz, 2 H), 3.93 (s, 2 H), 5.12 (d, J = 4.95 Hz, 1 H), 5.58 (dd, J = 4.95 and 7.92 Hz, 1 H), 9.31 (d, J = 7.92 Hz, 1 H); MS, m/e (355, M^+); UV (EtOH) λ_{max} 264 nm (ϵ 7000); IR (KBr) 1771 cm^{-1} (C=O).

Displacement of Bromine by Heterocyclic Thiols. The bromoacetyl cephalosporin was dissolved in CH_3CN and H_2O (1:1), and the pH was adjusted to 7.5 with NaOH. The solution was cooled in an ice water bath, and the heterocyclic thiol (1.05 equiv) was added. The pH of the reaction mixture was maintained between 7.5 and 7.8 with 0.1 N NaOH. Stirring was continued in the ice bath for 2 h and then at room temperature for 2 h until TLC showed completion of the reaction. The pH was then adjusted to 2; the solid that precipitated was filtered and dried to give the desired product.

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Oral Absorption of Cephalosporin Antibiotics. 3. Synthesis and Biological Properties of 7α-Methoxy-7β-(arylacetamido)-3-chloro-3-cephem-4-carboxylic Acids[1]

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A series of 7α-methoxy-7β-amido-3-chloro-3-cephem-4-carboxylic acids was prepared and evaluated for biological activity. When compared with the parent 7-non-methoxy analogues, these new 7α-methoxy-3-chloro cephalosporins displayed diminished antimicrobial activity.

The cephalosporin class of antibiotics has proven to be very useful in clinical medicine due to its generally broad spectrum of antibacterial activity and relative lack of toxicity. Many structural variations of the original Cephalosporin C nucleus have been synthesized chemically.[2] Others have been discovered in microbiological fermentations—among these are the cephamycins.[3] Ce-

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(2) For example, Nagata, W.; Narisada, M.; Yoshida, T. (Chapter 1), and Holden, K. G. (Chapter 2), *Chemistry and Biology of β-Lactam Antibiotics*; Morin, R. B., Gorman, M., Eds.; Academic: New York, 1982; Vol. 2.

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