(2S,3aS,7aS)-2-[(Benzyloxy)carbonyl]perhydroindole, Tosylate (12). This compound was prepared by using the method described previously[21] for a mixture of isomers. A mixture of 11 (17 g, 0.1 mol), benzyl alcohol (40 g, 0.37 mol), *p*-toluenesulfonic acid (PTSH; 30 g, 0.16 mol), and toluene (300 mL) was stirred under reflux for 4 h. Water was collected in a Dean-Stark trap. Then toluene was removed by evaporation. The residue was triturated with diisopropyl oxide and the resulting solid was collected by filtration to yield 40 g (93%) of title compound melting at 162 °C. Anal. ($C_{23}H_{29}NO_5S$) C, H, N, S.

(2S,3aS,7aS)-1-[N-[1(S)-Carbethoxybutyl]glycinyl]-2-[(benzyloxy)carbonyl]perhydroindole (13). To a solution of 12 (4.31 g, 10 mmol), triethylamine (1.4 mL, 10 mmol), and 10 (2.03 g, 10 mmol) in DMF (50 mL) was added a solution of HOBT (1.35 g, 10 mmol) and DCC (2.06 g, 10 mmol) in DMF (10 mL). The reaction mixture was stirred for 20 h at room temperature. Dicyclohexylurea (DCU) was removed by filtration and DMF was evaporated in vacuo. The residue was dissolved in ethyl acetate and washed with a 5% solution of $NaHCO_3$ in water then with brine. The organic layer was dried on $CaSO_4$, filtered, and concentrated. The product was purified by silica gel chromatography with ethyl acetate as eluent to afford 2.5 g (56%) of title compound. Anal. ($C_{25}H_{36}N_2O_5$) C, H, N.

(2S,3aS,7aS)-1-[N-[1(S)-Carbethoxybutyl]glycinyl]-2-carboxyperhydroindole, *tert*-Butylamine Salt (14). A mixture of 13 (2.3 g, 52 mmol) and Pd/C (10%, 0.5 g) in absolute ethanol (50 mL) was shaken in a pressure bottle on a Parr hydrogenator at 3 kg/cm^2 for 18 h at room temperature. The catalyst was removed by filtration and the filtrate was evaporated to afford 1.7 g (92%) of the crude acid form of 14. Crystallization of *tert*-butylamine salt from diisopropyl oxide gave pure 14 as colorless crystals melting at 122 °C. Anal. ($C_{22}H_{41}N_3O_5$) C, H, N.

(2S,3aS,7aS)-1-[N-[1(S)-Carboxybutyl]glycinyl]-2-carboxyperhydroindole (2b). A solution of 14 (0.83 g, 2.5 mmol) in 0.2 N NaOH (37.5 mL, 7.5 mmol) was allowed to stand for 3 days. Hydrochloric acid (1 N, 7.5 mL, 7.5 mmol) was added and the reaction mixture evaporated to dryness. The residue was dissolved in absolute ethanol (50 mL) and sodium chloride was removed by filtration. After evaporation of ethanol the product was dissolved in water; the solution was filtered and lyophilized to afford 0.58 g (72%) of title compound. Anal. ($C_{16}H_{26}N_2O_5$) C, H, N.

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(2S,3aS,7aS)-1-[N-[1(S)-Carbethoxybutyl]-(R)-alanyl]-2-carboxyperhydroindole Hydrochloride (15). To a mixture of *N*-[1(*S*)-carbethoxybutyl]-(*R*)-alanine hydrochloride[1] (9.9 g, 39.1 mmol), triethylamine (5.4 mL, 39.1 mmol), and (2*S*,3a*S*,7a*S*)-2-(*tert*-butoxycarbonyl)perhydroindole[1] (8.8 g, 39.1 mmol) in DMF (250 mL) was added a solution of HOBT (4.65 g, 39.1 mmol) in DMF (100 mL) then DCC (8.1 g, 39.1 mmol). The reaction mixture was stirred for 20 h at room temperature. The DCU was removed by filtration and the DMF was evaporated in vacuo. The residue was dissolved in ethyl acetate and washed with a 5% solution of $NaHCO_3$ in water then brine. The organic layer was dried on $CaSO_4$, filtered, and concentrated to afford 16 g of crude (2*S*,3a*S*,7a*S*)-1-[*N*-[1(*S*)-carbethoxybutyl]-(*R*)-alanyl]-2-(*tert*-butoxycarbonyl)perhydroindole as an oil. This oil was dissolved in a solution of hydrochloric acid in 4 N ethyl acetate (250 mL) and the solution stirred for 20 h at room temperature. The product was collected by filtration and washed three times with ethyl acetate (50 mL) to afford 8.2 g (51.8%) of title compound melting at 180 °C with decomposition. Anal. ($C_{19}H_{33}ClN_2O_5$) C, H, N, Cl.

(2S,3aS,7aS)-1-[N-[1(S)-Carboxybutyl]-(R)-alanyl]-2-carboxyperhydroindole (2a). A solution of 15 (5 g, 12.3 mmol) in 0.5 N NaOH (100 mL, 50 mmol) was allowed to stand at room temperature for 48 h. The solution was acidified (pH = 2) by addition of 4 N HCl and was added to a column of Dowex-50W × 8 (H^+ form). The ion-exchange resin was washed with water then eluted with water-pyridine (9:1). The eluates were evaporated to dryness. The residue was triturated with acetone and the solid was collected by filtration to yield 1.9 g (45%) of title compound. Anal. ($C_{17}H_{28}N_2O_5$) C, H, N.

Registry No. 2, 95153-31-4; **2a**, 130982-51-3; **2b**, 130933-17-4; 10, 130933-18-5; 11, 80875-98-5; 12, 94062-52-9; 13, 130933-19-6; 14, 130933-21-0; 15, 130982-52-4; perindopril, 82834-16-0; (*S*)-norvaline ethyl ester hydrochloride, 40918-51-2; bromoacetic acid, 79-08-3; (*S*)-2-carboxyindoline, 79815-20-6; benzyl alcohol, 100-51-6; angiotensin converting enzyme, 9015-82-1.

Supplementary Material Available: Tables of atomic coordinates for H atoms and isotropic thermal parameter, anisotropic thermal coefficients for non-hydrogen atoms, bond length, and valency angles for 2, IR, ^{1}H NMR, and TLC studies for all new compounds, optical rotation values for 2 and 11, ^{13}C NMR studies for 2, 2a, and 2b, HPLC studies for 2, 2a, 2b, and 14, MS studies for 2, 2b, and 14 (15 pages); a list of observed and calculated structure factors for 2 (11 pages). Ordering information is given on any current masthead page.

Dual-Action Cephalosporins: Cephalosporin 3′-Quaternary Ammonium Quinolones[1]

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When cephalosporins exert their biological activity by reacting with bacterial enzymes, opening of the β-lactam ring can lead to expulsion of the 3′-substituent. A series of cephalosporins was prepared in which antibacterial quinolones were linked to the 3′-position through a quaternary nitrogen. Like the 3′-ester-linked dual-action cephalosporins reported earlier, these compounds demonstrated a broad spectrum of antibacterial activity derived from cephalosporin-like and quinolone-like components, suggesting a dual mode of action.

When cephalosporins react with bacterial enzymes, opening of the β-lactam ring leads to liberation of the 3′-substituent, if that substituent can function as a leaving group.[2-8] When the leaving group possesses antibacterial activity of its own, the cephalosporin should exhibit a dual mode of action.[9-11] As a rationale for drug design, this

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(2) Hamilton-Miller, J. M. T.; Newton, G. G. F.; Abraham, E. P. *Biochem. J.* **1970**, *116*, 371.
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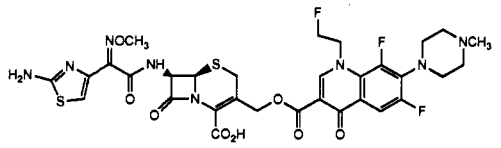
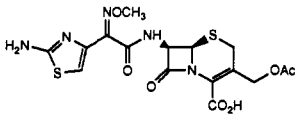
0022-2623/91/1834-0669$02.50/0

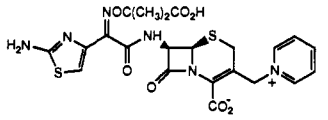
Chart I. Structures of Reference Compounds



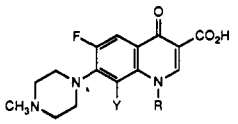
Ro 23-9424 (1)



Cefotaxime (CTX)

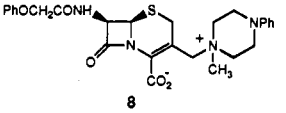


Ceftazidime (CAZ)



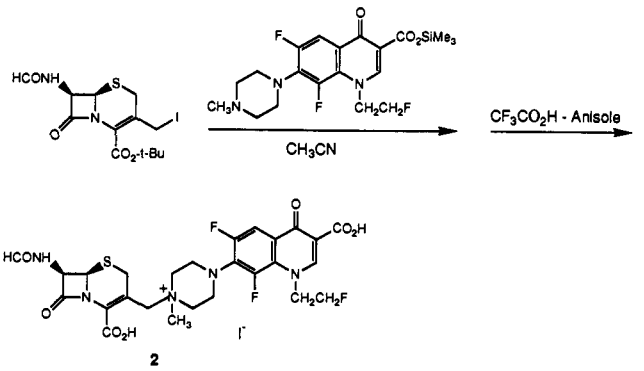
Fleroxacin (FLX); Y = F, R = FCH_2CH_2-

Pefloxacin; Y = H, R = Et



8

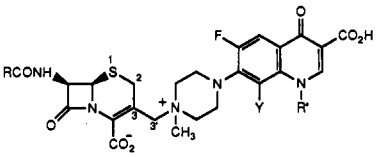
Scheme I



2

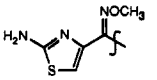
mechanism has been discussed in some detail, and the synthesis and biological activity of a class of dual-action cephalosporins in which antibacterial quinolones are ester-linked to the cephalosporin 3′-position have been described.[12-23] These compounds showed excellent broad-

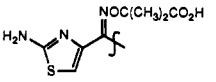
Table I. Cephalosporin 3′-Quaternary Ammonium Quinolones



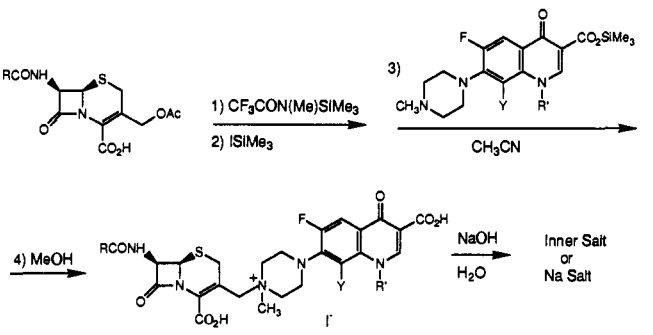
no.	R	R′	Y	salt[a]
2	H	CH_2CH_2F	F	HI
3	ATM[b]	CH_2CH_2F	F	HI
4	$PhOCH_2$	CH_2CH_2F	F	HI
5	ATIBA[c]	CH_2CH_2F	F	Na
6	ATM	Et	H	HI

[a] Salt used for biological testing.

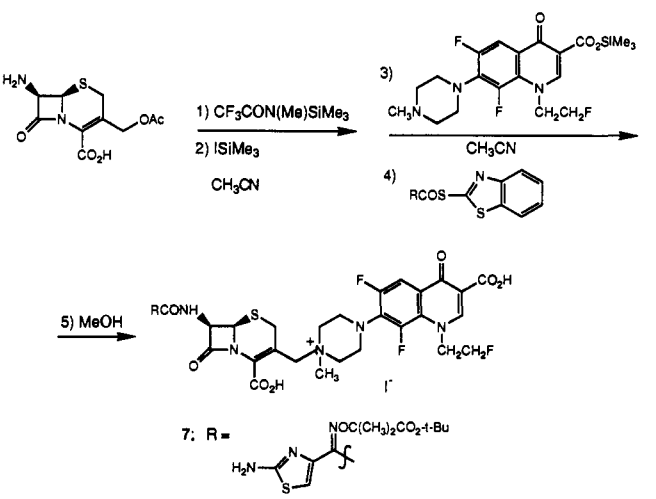
[b] ATM = 

[c] ATIBA = 

Scheme II



Scheme III



spectrum antibacterial activity reflecting both cephalosporin and quinolone-like contributions. A substantial

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Table II. In Vitro Activity

organisms	MIC, μg/mL								
	1	2	3	4	5	6	8	CTX	FLX
Escherichia coli 257	0.063	2	0.125	0.5	0.25	0.5	32	0.031	0.031
Klebsiella pneumoniae A	0.125	2	0.5	0.5	0.25	1	32	0.031	0.031
Enterobacter cloacae 5699	0.25	4	0.5	0.5	0.25	1	64	0.125	0.063
Enterobacter cloacae P99	0.125	0.5	0.5	0.5	1	1	>128	64	0.031
Citrobacter freundii BS #16	0.25	16	2	2	2	4	>128	128	0.25
Serratia marcescens SM	0.125	8	1	2	1	1	>128	0.25	0.25
Pseudomonas aeruginosa 5712	16	128	32	32	16	64	>128	64	4
Staphylococcus aureus Smith (MS)	1	4	4	0.5	16	2	0.063	1	0.25
Staphylococcus aureus 95 (MR)	1	1	4	1	8	4	128	128	0.25
Streptococcus pneumoniae 6301	≤0.008	16	0.063	0.25	0.5	0.063	0.125	0.016	8
Streptococcus pyogenes 4	≤0.008	8	0.125	0.063	0.5	0.125	0.031	≤0.008	4

body of evidence suggests that, in addition to providing β-lactam activity, these bifunctional cephalosporins can act as targeted prodrugs for delivery of quinolones at or near the site of action. This earlier work led to the development of Ro 23-9424 (**1**) as a candidate for clinical trial (Chart I).

We now report on a new class of bifunctional cephalosporins in which the 3'-position is bonded to an antibacterial quinolone through a quaternary nitrogen. This functionality can act as a leaving group and seemed appropriate, since the beneficial influence of a quaternary nitrogen substituent on the biological activity of β-lactam antibacterials has been noted.[24,25] The purpose of this research was to prepare parenteral cephalosporins with an expanded spectrum in which the better features of two major classes of antibacterials were combined. These features include the solubility, superior pharmacokinetics, low toxicity, and potent activity against streptococci associated with cephalosporins, as well as the broad spectrum of activity of the quinolones, which includes β-lactam-resistant strains.

Chemistry

Cephalosporins 2–6 (Table I) were prepared by the methodologies outlined in Schemes I–III. The 3'-iodocephalosporin *tert*-butyl esters used earlier to prepare cephalosporin 3'-quinolone esters were also suitable intermediates for preparing 3'-quaternary quinolones.[12,26,27] Displacement with a tertiary amine and deprotection of the intermediate ester obtained from the quaternization step led to the desired product. Compound **2** was prepared by this method (Scheme I), which is quite general but requires the preparation of the starting iodide. Usually it was more convenient to utilize the multistep, one-pot synthesis shown in Scheme II, which was adapted from procedures used by others to introduce substituted pyridinium and *N*-methylpyrrolidinium groups at the 3'-position.[28,29] The starting cephalosporins were readily prepared by methods in the literature or were commercially available, as in the case of cefotaxime. Initial treatment with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) provided the trimethylsilyl (TMS) ester in which any sensitive functionality on the acyl side chain was also TMS protected. Reaction with iodotrimethylsilane then gave the 3'-iodo derivative, which was used in situ. Addition of the TMS ester of an antibacterial quinolone containing a tertiary amine function led to quaternization. Upon solvolysis with methanol the product precipitated as a quaternary ammonium iodide. The quaternary iodide could then be converted to a zwitterion or sodium salt by appropriate treatment with sodium phosphate buffer, sodium bicarbonate, or sodium hydroxide. The two compounds prepared in greatest amount, **3** and **5**, were purified as sodium salts By C_{18} reverse-phase chromatography.

Methylene chloride was generally used for the preparation of the TMS-protected iodo intermediate, while the preferred solvent for the quaternization step was acetonitrile. In one example, the synthesis of **5**, acetonitrile was used with satisfactory results for the entire sequence, in order to avoid the inconvenience of changing solvents. Some isomerization of the cephem double bond was occasionally noted. Since this rearrangement is base-catalyzed, the basic conditions of the quaternization reaction were probably primarily responsible. Reducing the amount of quinolone TMS ester used seemed to help. Conditions were optimized for each reaction at mole ratios of from 0.3 to 0.75. Under these conditions products were relatively free of both the Δ^2 isomer and unchanged quinolone. However, subtle factors can influence the double-bond migration. In one case, the choice of solvent proved critical. When cefotaxime was treated with MSTFA in methylene chloride and reprecipitated after 5 min by addition of methanol, no significant isomerization occurred. However, when acetonitrile was used as the solvent in a similar experiment, up to 30% of Δ^2 isomer appeared in the recovered cefotaxime. Fortunately, this behavior was not general. The starting material for the synthesis of **5** showed no tendency toward isomerization in similar experiments.

The multistep, one-pot synthetic sequence could be extended one step further. Compound **7**, a potential intermediate for the synthesis of **5**, was prepared from 7-aminocephalosporanic acid (7-ACA) as shown in Scheme III. In this alternate approach, 7-ACA was subjected to the reaction sequence leading to introduction of the 3'-quaternary substituent, and the resulting intermediate was acylated in situ with a thioester.[30] This methodology

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Table III. Binding of Dual-Action Cephalosporins to Essential PBPs of *E. coli* DC-O

compound	concentration, μg/mL, required for 90% inhibition of [^{14}C]Pen G binding: PBP 1a 90 kDa	PBP 1b 90 kDa	PBP 2 66 kDa	PBP 3 60 kDa	morphology	MIC, μg/mL
1	0.1	2	100	0.1	F[a]	0.12
3	>100	>100	100	0.1	F	0.2
4	10	100	>100	2	F	8
5	2	30	100	0.1	F	0.2
CTX	0.1	0.5	100	0.1	F/L[b]	0.06

[a] F, filaments. [b] L, lysis.

should be general in its applicability, but was not further explored.

Results and Conclusions

The five cephalosporin 3′-quaternary quinolones were screened for in vitro antibacterial activity, along with the reference compounds cefotaxime (CTX), fleroxacin (FLX), Ro 23-9424 (1), and 8 (Table II). Compounds **2–5** contained fleroxacin as the quinolone component, while **6** was derived from pefloxacin, a slightly less potent quinolone. Compound **2** was significantly less active than the other bifunctional compounds, especially against the streptococci, probably because the 7-formylamino substituent did not confer good β-lactam activity. Cephalosporins with a 7-(phenoxyacetyl)amino substituent usually exhibit significant activity only against Gram-positive bacteria.[12] The model compound **8**, containing a 3′-quaternary ammonium substituent which, when released, would not show activity of its own, was no exception. However, in addition to its Gram-positive activity, the bifunctional compound **4** also demonstrated excellent activity against Gram-negative strains, except for *Pseudomonas aeruginosa*. Relative to the reference cephalosporins and quinolone, compounds **3–6** showed a broadened spectrum of activity. Compared to cefotaxime, they exhibited increased potency against *Enterobacter cloacae* P99, *Citrobacter freundii* BS #16, and *Staphylococcus aureus* 95, which is a methicillin-resistant β-lactamase producer. Compared to fleroxacin, they were significantly more potent against the streptococci. Compound **1**, in which fleroxacin is ester-linked to the 3′-position, showed better in vitro activity than its quaternary-linked isomer **3**, which was the most active of the quaternary bifunctional cephalosporins.

The pattern of both cephalosporin-like and quinolone-like activities, which was also noted earlier for the cephalosporin 3′-quinolone esters,[12] suggests a dual mode of action. Although the mechanism by which this dual-action is manifested has not been as extensively investigated for the cephalosporin 3′-quaternaries as for the 3′-esters, several possibilities should be considered.

(1) Both β-lactam and quinolone activities could be intrinsic to the intact molecule. Certainly, the bifunctional compounds can exert β-lactam activity. Compounds **1** and **3–5** behaved toward penicillin-binding proteins (PBPs) like typical cephalosporins (Table III). The major determinant of binding to PBP 3 appeared to be the 7-acylamino function. Whether the quinolone was ester-linked (**1**) or quaternary-linked (**3**), the affinity for PBP 3 remained the same as that of cefotaxime. The possibility that the intact molecule possesses quinolone activity seems less likely, but must be considered, since unlike the ester-linked compounds, the quaternary-linked compounds contain a free carboxylic acid function, at the 3-position of the quinolone nucleus, which is essential for quinolone activity. Quinolones act by inhibiting bacterial DNA gyrase. Studies of replicative DNA biosynthesis, which is a measure of DNA gyrase activity,[31,32] have shown that the cephalosporin 3′-quaternaries are far less inhibitory than the free quinolone.[33] For example, in this assay **3** showed an IC_{50} of 50 μg/mL, compared to 1 μg/mL for fleroxacin. Whether this represents the actual activity of the intact molecule or of a minor amount of fleroxacin, either present as a contaminant or generated in situ during the assay, is not clear. What is clear is that this result places an upper limit on the quinolone-like activity to be expected from the intact molecule. Thus, it appears that this component of the antibacterial spectrum must come from quinolone liberated by some process from the bifunctional molecule.

Table IV. Efficacy of 3 in Systemic Murine Infections

infection	ED_{50}, mg/kg, sc: 3	CTX	FLX
E. coli 257	<0.5	0.37	0.25
S. marcescens SM	10	>50	<2
P. aeruginosa 5712	>250	>250	14
S. pneumoniae 6301	12	90	>250
S. pyogenes 4	<5	7	>250

(2) Significant degradation of the original molecule to yield bioactive products, i.e. in situ hydrolysis to produce a 3′-hydroxycephalosporin and a quinolone, could account for the observed activity. The in vitro antibacterial assay requires an 18-h incubation. If the degradation half-life of the bifunctional compound is relatively short on this time scale, so as to permit a buildup of active hydrolysis products during the critical early hours of the incubation period, this could significantly influence MICs. Therefore, in vitro data should be interpreted with caution, and correlation with in vivo models of infection is important for predicting clinical efficacy. The quaternary-linked compounds were much more stable than the ester-linked compounds. For example, the degradation half-lives of **1** and **5** at 37 °C in pH 6.5 phosphate buffer were 6.9 and 68 h, respectively, as determined by HPLC analysis. In studies conducted with a 2-h incubation to minimize possible degradation, comparisons of the effects on growth of *Escherichia coli* JF568 and a porin-deficient mutant JF703 led to the conclusion that **1** and **5** can penetrate the bacterial outer membrane as intact molecules, through porins, like typical cephalosporins,[34] and that they penetrate more slowly than cefotaxime or desacetylcefotaxime.[15,33]

(3) Bacterial–enzyme-mediated ring opening of the B-lactam followed by elimination of the quinolone may occur, according to the putative mechanism which provided the rationale for design of dual-action cephalosporins.[12] While this mechanism is not established with any certainty, is difficult to prove unequivocally, and may not be the exclusive mechanism by which these compounds act, circumstantial evidence suggests that it may be operative for both the cephalosporin 3′-quinolone esters and for the 3′-quaternary quinolones.

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Table V. Efficacy of 5 in Systemic Murine Infections

infection	ED_{50}, mg/kg, sc		
	5	CAZ	FLX
E. coli 257	0.28	<0.5	0.26
K. pneumoniae A	3	<2	0.3
E. cloacae 5699	<5	25	<2
C. freundii BS#16	9	22	2
S. marcescens SM	8	67	<2
P. aeruginosa 5712	>250	217	31
S. aureus Smith (MS)	2.6	<2	0.65
S. aureus 753 (MR)	44	62	2.8
S. pneumoniae 6301	8	3	>250
S. pyogenes 4	54	12	>250

Limited in vivo testing in murine infections was conducted with 3 (Table IV). Against *E. coli*, *Serratia marcescens*, and two Gram-positive infections, 3 showed excellent activity. However, it was not active against the *P. aeruginosa* infection. Because of limited aqueous solubility (0.5%) and toxicity (LD_{50} = 55 mg/kg, iv, in mice), no further studies were carried out with this compound.

On the basis of favorable in vitro activity and aqueous solubility (≥10%) under neutral conditions, 5 was selected for a more thorough evaluation. Compound 5 proved comparatively nontoxic, with an LD_{50} in mice of 770 mg/kg iv, compared to an LD_{50} of 245 mg/kg for the quinolone component, fleroxacin. In the mouse protection test, 5 showed excellent activity against 7 of 10 infections, and moderate activity against two other infections, but was inactive against *P. aeruginosa* (Table V). Results for ceftazidime (CAZ) and fleroxacin (FLX) are included for comparison. In results reported elsewhere,[14,21] Ro 23-9424 (1) demonstrated activity against all of the infections of the in vivo screen used in this study. Thus it appears that none of the new quaternary dual-action cephalosporins can match the excellent in vitro and in vivo activity of Ro 23-9424, which is currently under development as a clinical candidate.

Experimental Section

Physical Chemistry. Infrared spectra (IR) were recorded on a Digilab FTS 15-E spectrophotometer. Mass spectra (MS) were obtained on a VG7070E-HF mass spectrometer in the positive-ion fast-atom-bombardment mode with glycerol or thioglycerol as the solvent. Proton nuclear magnetic resonance spectra (NMR) were obtained on a Varian XL-400 instrument. Chemical shifts (δ) are expressed in parts per million (ppm) downfield from tetramethylsilane, with coupling constants (J) in hertz (Hz).

HPLC Analyses. Conditions for HPLC analyses of products for purity and for monitoring stability studies were typically as follows: A Hamilton PRP-1 (250 mm × 4.1 mm) column was used, with UV detection at 280 nm, and a mobile phase consisting of a 0.01 M solution of tetradecyltrimethylammonium bromide in a mixture of 70% 0.072 M, pH 8.2 phosphate buffer, and 30% acetonitrile.

Biological Assays. In vitro antibacterial testing was performed by using serial 2-fold dilutions of the agents. Results are expressed as minimum inhibitory concentrations (MICs). MICs for aerobic nonfastidious organisms were determined by the broth microdilution method, as recommended by the NCCLS.[35] *Streptococcus pneumoniae* and *Streptococcus pyogenes* were tested with use of a broth macrodilution assay in cation-supplemented Mueller-Hinton broth containing 10% goat serum. The standard agar dilution procedure[35,36] was employed for testing staphylococci.

In vivo antibacterial activity was assessed in the mouse protection test by using procedures described previously.[37] CD1 Swiss albino mice (18–20 g, Charles River Breeding Laboratories, Kingston, NY) were infected intraperitoneally with 0.5 mL of an appropriately diluted suspension of the bacterial culture. Serial dilutions of the antibacterial agents were prepared to yield concentrations ranging from 0.05 to 250 mg/kg. The infected mice were immediately treated (within 5 min) with 1.0 mL of the test solution subcutaneously at the dorsal base of the neck. Six mice were used for each treatment and for control groups. Only one treatment was given, except for experiments with *P. aeruginosa* where a second treatment was administered 3 h after infection. The method of Reed and Muench[38] was used to determine the 50% effective (i.e. protective) dose (ED50).

The PBP binding assay was carried out with solubilized membranes from sonicated *E. coli* DC-0, as previously described.[39] PBP binding was measured as inhibition of [^{14}C]penicillin G binding. Cell morphology was determined by microscopic examination after a 3-h incubation with the test compound.

Replicative DNA biosynthesis was measured as the ATP-dependent incorporation of [^{3}H]thymidine into trichloroacetic acid insoluble material by toluene-treated *E. coli* H560 cells.[31] Results are expressed as IC_{50}s, the concentrations required for 50% inhibition.

Membrane permeation studies to determine the extent and pathway of entry of intact dual-action cephalosporins in *E. coli* were conducted as previously described.[15]

Nomenclature. For purposes of discussion, the familiar and traditional cephem numbering system has been used in the text. Note that in the experimental section compounds are named according to the conventions of the Chemical Abstracts Service.

(6*R-trans*)-4-[3-Carboxy-6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-4-oxoquinolin-7-yl]-1-[[2-carboxy-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpiperazinium Iodide (4). Under an argon atmosphere, a mixture of 1.22 g (3 mmol) of (6*R-trans*)-3-[(acetyloxy)methyl-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid, 8 mL of dry methylene chloride, and 1.67 mL (9 mmol) of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) was stirred for 1 h; 0.85 mL (6 mmol) of iodotrimethylsilane was added, and the mixture stirred for 2 h. The solution was then concentrated to dryness under reduced pressure, and the residual oil was dissolved in 8 mL of acetonitrile; 0.34 mL (4.2 mmol) of anhydrous THF was added, and the mixture was stirred for 5 min. A solution prepared from 0.37 g (1 mmol) of 6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid, 0.37 mL (2 mmol) of MSTFA, and 6 mL of acetonitrile was added, and stirring was continued for 2 h. The mixture was chilled in ice, and 0.5 mL of methanol was added. The solid which precipitated was filtered, washed with acetonitrile, and dried under reduced pressure to obtain 0.38 g (45%) of product: NMR (Me_2SO-d_6) δ 3.15 (s, 3 H, NCH_3), 3.45–3.85 (m, 9 H, 4 × NCH_2 and CH of SCH_2), 3.95 (d, 1 H, J_{gem} = 16.5 Hz, CH of SCH_2), 4.39 and 4.77 (AB, 2 H, J_{gem} = 13 Hz, NCH_2), 4.61 and 4.64 (AB, 2 H, J_{gem} = 15 Hz, OCH_2CO), 4.83–5.07 (m, 4 H, NCH_2CH_2F), 5.24 (d, 1 H, J = 5 Hz, CH), 5.82 (dd, 1 H, J = 5 and 7 Hz, CH), 6.95 (d, 2 H, J = 8 Hz, Ar), 6.97 (t, 1 H, J = 8 Hz, Ar), 7.29 (t, 2 H, J = 8 Hz), 7.96 (d, 1 H, J = 12 Hz, Ar), 8.91 (s, 1 H, =CH–), 9.18 (d, 1 H, J = 7 Hz, NH); IR (KBr) 3400, 1788, 1728, 1700, 1612, cm^{-1}; MS m/z 716 (cation).

[6*R*-[6α,7β(*Z*)]]-1-[[7-[[2-Amino-4-thiazolyl][(1-carboxy-1-methylethoxy)imino]acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-4-[3-carboxy-6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-4-oxoquinolin-7-yl]-1-methylpiperazinium Hydroxide Inner Salt Sodium Salt (5). Under an argon atmosphere, a mixture of 5.12 g (8 mmol) of [6*R*-[6α,7β(Z)]]-3-[(acetyloxy)methyl]-7-[[(2-amino-4-thiazolyl)[1-(1-carboxy-1-methylethoxy)imino]acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trifluoroacetic

(35) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A. National Committee for Clinical Laboratory Standards, Villanova, PA, 1985.
(36) Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A3. National Committee for Clinical Laboratory Standards, Villanova, PA, 1984.
(37) Beskid, G.; Christenson, J. G.; Cleeland, R.; DeLorenzo, W.; Trown, P. W. *Antimicrob. Agents Chemother.* **1981**, *20*, 159.
(38) Reed, L. J.; Muench, H. *Am. J. Hyg.* **1938**, *27*, 493.
(39) Georgopapadakou, N. H.; Liu, F. Y. *Antimicrob. Agent Chemother.* **1980**, *18*, 148.

acid salt,[40] 48 mL of dry acetonitrile, and 12 mL (64 mmol) of MSTFA was stirred for 30 min; 2.0 mL (14 mmol) of iodotrimethylsilane was added dropwise, and the mixture stirred for 30 min. With momentary cooling in ice, 1.12 mL (14 mmol) of dry THF was added. After 10 min, a solution prepared from 2.27 g (6 mmol) of 6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid, 24 mL of acetonitrile, and 1.28 mL (7.2 mmol) of MSTFA was added, and the mixture was stirred for 1.5 h. The mixture was concentrated under reduced pressure, and the residual oil was dissolved in 40 mL of acetonitrile. With ice-cooling, 4 mL of methanol was added, resulting in a thick precipitate. After settling for a few minutes, the precipitate was filtered and washed with four 10-mL portions of acetonitrile. After drying, the solid was triturated with 60 mL of methanol, filtered, and washed with four 10-mL portions of methanol. The solid thus obtained was suspended in water, and aqueous sodium bicarbonate was added to pH 7. The solution of crude product was purified by C_{18} reverse-phase HPLC in three steps. First a column of 50 g of Waters C_{18} silica was used, with water followed by 30% acetonitrile in water as eluants. Then, with use of a 0.025 molar pH 7 sodium phosphate buffer–acetonitrile gradient, the product was further purified by HPLC on a Waters Prep 500A with C_{18} columns. Finally, the product was desalted on a flash column of 60 g of C_{18} silica with water and 20% acetonitrile in water as eluants. After the product was concentrated under reduced pressure to eliminate the organic solvent and freeze-dried, 1.0 g (20%) of the title compound was obtained: NMR (Me_2SO-d_6-D_2O) δ 1.37 (s, 3 H, CH_3), 1.44 (s, 3 H, CH_3), 3.10 (s, 3 H, NCH_3), 3.39 and 3.88 (AB, 2 H, J_{gem} = 16.5 Hz, SCH_2), 3.40–3.70 (m, 8 H, 4 × NCH_2), 4.12 and 5.17 (AB, 2 H, J_{gem} = 12.5 Hz, NCH_2), 4.62–4.94 (m, 4 H, NCH_2CH_2F), 5.15 (d, 1 H, J = 5 Hz, CH), 5.73 (d, 1 H, J = 5 Hz, CH) 6.74 (s, 1 H, Ar), 7.83 (d, 1 H, J = 12 Hz, Ar), 8.47 (br s, 1 H, =CH–); IR (KBr) 3400, 1772, 1618, 1595 cm^{-1}; MS m/z = 859 $(M + H)^+$.

[6*R*-[6α,7β(*Z*)]]-3-[(Acetyloxy)methyl]-7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (Cefotaxime). The free anhydrous form of cefotaxime used as a starting material for preparation of compounds **3** and **6** was obtained from the commercially available sodium salt as follows. A solution of 10 g (0.021 mol) of cefotaxime sodium salt (Claforan from Hoechst-Roussel Pharmaceuticals Inc.) in 180 mL of water was cooled in ice and 21 mL of 1 N HCl was added. The resulting mixture was stirred for 20 min and freeze dried. The residue was triturated with six 150-mL portions of acetone. The combined extracts were dried with 4A molecular sieves, filtered, and concentrated to dryness under reduced pressure to recover 7.70 g (81%) of cefotaxime.

[6*R*-[6α,7β(*Z*)]]-1-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-en-3-yl]methyl]-4-[3-carboxy-6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-4-oxo-7-quinolinyl]-1-methylpiperazinium Iodide (3). With cefotaxime as the starting cephalosporin and fleroxacin in a mole ratio of 0.54 as the starting quinolone, **3** was prepared according to procedures similar to those for the preparation of **4**. After trituration with acetonitrile, **3** was obtained in 95% yield: NMR (Me_2SO-d_6) δ 3.15 (s, 3 H, NCH_3), 3.50–3.90 (m, 9 H, 4 × NCH_2 and CH of SCH_2), 3.86 (s, 3 H, OCH_3), 3.96 (d, 1 H, J_{gem} = 17 Hz, CH of SCH_2), 4.42 and 4.73 (AB, 2 H, J_{gem} = 14 Hz, NCH_2), 4.85–5.10 (m, 4 H, NCH_2CH_2F), 5.29 (d, 1 H, J = 5 Hz, CH), 5.93 (dd, 1 H, J = 5 and 7 Hz, CH), 6.86 (s, 1 H Ar), 7.24 (s, 2 H, NH_2), 7.98 (d, 1 H, J = 12 Hz, Ar), 8.94 (s, 1 H, =CH–), 9.67 (d, 1 H, J = 7 Hz, NH); IR (KBr) 3420, 1775, 1720, 1675, 1618 cm^{-1}.

Sodium Salt of 3. A suspension of 900 mg (1.01 mmol) of the above iodide in water was neutralized with 0.1 N sodium hydroxide, and the resulting solution was freeze-dried. The residue was purified by C_{18} reverse-phase HPLC on a Waters Prep 500A, eluting with a water–acetonitrile gradient (0–40%). Evaporation and lyophilization of the appropriate fractions afforded 344 mg (43%) of product: NMR (Me_2SO-d_6) δ 3.10 (s, 3 H, NCH_3), 3.40–3.76 (m, 9 H, 4 × NCH_2 and CH of SCH_2), 3.84 (d, 1 H, J = 16 Hz, CH of SCH_2), 3.84 (s, 3 H, OCH_3), 4.10 and 5.19 (AB, 2 H, J_{gem} = 14 Hz, NCH_2), 4.70–4.94 (m, 4 H, NCH_2CH_2F), 5.13 (d, 1 H, J = 5 Hz, CH), 5.65 (dd, 1 H, J = 5 and 7 Hz, CH), 6.74 (s, 1 H, Ar), 7.23 (s, 2 H, NH_2), 7.82 (d, 1 H, J = 12 Hz, Ar), 8.55 (s, 1 H, =CH–), 9.58 (d, 1 H, J = 7 Hz, NH); IR (KBr) 3410, 1772, 1665, 1618 cm^{-1}; MS m/z 787 $(M + H)^+$.

[6*R*-[6α,7β(*Z*)]]-1-[[7-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-4-(3-carboxy-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-methylpiperazinium Iodide (6). With cefotaxime as the starting cephalosporin and pefloxacin in a mole ratio of 0.3 as the starting quinolone, **6** was prepared by procedures similar to those for the preparation of **4**. After trituration first with acetonitrile and then with methanol, **6** was obtained in 93% yield: NMR (Me_2SO-d_6) δ 1.45 (br t, 3 H, CH_3 of NEt), 3.15 (s, 3 H, NCH_3), 3.50–3.95 (m, 9 H, 4 × NCH_2 and CH of SCH_2), 3.85 (s, 3 H, OCH_3), 3.99 (d, 1 H, J_{gem} = 16.5 Hz, CH of SCH_2), 4.43 and 4.70 (AB, 2 H, J_{gem} = 14 Hz, NCH_2), 4.61 (br q, 2 H, CH_2 of NEt), 5.31 (d, 1 H, J = 5 Hz, CH), 5.94 (dd, 1 H, J = 5 and 8 Hz, CH), 6.76 (s, 1 H, Ar), 7.30 (br s, 2 H, NH_2), 7.31 (d, 1 H, J = 6.5 Hz, Ar), 8.03 (d, 1 H, J = 12.5 Hz, Ar), 9.03 (s, 1 H, =CH–), 9.68 (d, 1 H, J = 8 Hz, NH); IR (KBr) 1785, 1720, 1680, 1628 cm^{-1}; MS m/z = 729 (cation).

(6*R*-*trans*)-4-[3-Carboxy-6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-quinolinyl]-1-[[2-[(1,1-dimethylethoxy)carbonyl]-7-(formylamino)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpiperazinium Iodide. A mixture of 0.87 g (2.35 mmol) of 6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid, 0.51 mL (2.6 mmol) of MSTFA, and 5 mL of dry acetonitrile was stirred for 30 min; 1.00 g (2.36 mmol) (6*R*-*trans*)-7-(formylamino)-3-(iodomethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 1,1-dimethylethyl ester was added, and stirring was continued for 24 h. The resulting precipitate was filtered and discarded. The mother liquor was absorbed onto a column of 5 g of C_{18} silica. After elution with water, 10%, and 20% aqueous methanol, the appropriate fractions were combined and concentrated somewhat under reduced pressure to obtain a precipitate. After filtration and drying, 380 mg (20%) of the title compound was obtained: IR (KBr) 3440, 1785, 1720, 1610 cm^{-1}; MS m/z = 666 (cation).

(6*R*-*trans*)-4-[3-Carboxy-6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-quinolinyl]-1-[[2-carboxy-7-(formylamino)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpiperazinium Iodide (2). A mixture of 200 mg (0.252 mmol) of the above ester, 0.2 mL anisole, and 2.5 mL of trifluoroacetic acid was stirred for 3 h at room temperature. After filtration of the insoluble portion, the solution was concentrated under reduced pressure. The residue was dissolved in 10 mL of acetonitrile, and 200 mL of ether was added to precipitate a solid. After filtration and drying, 135 mg (73%) of the product was obtained: NMR (Me_2SO-d_6) δ 3.14 (s, 3 H, NCH_3), 3.50–3.85 (m, 9 H, 4 × NCH_2 and CH of SCH_2), 3.95 (d, 1 H, J_{gem} = 16.5 Hz, CH of SCH_2), 4.35 (d, 1 H, J_{gem} = 13 Hz, CH of NCH_2), 4.82–5.05 (m, 5 H, NCH_2CH_2F and CH of NCH_2), 5.23 (d, 1 H, J = 5 Hz, CH), 5.84 (dd, 1 H, J = 5 and 7 Hz, CH), 7.96 (d, 1 H, J = 13 Hz, Ar), 8.17 (s, 1 H, NCHO), 8.92 (s, 1 H, =CH–), 9.11 (d, 1 H, J = 7 Hz, NH); IR (KBr) 3400, 1780, 1720, 1685 cm^{-1}; MS m/z = 610 (cation).

[6*R*-[6α,7β(*Z*)]]-1-[[7-[[(2-Amino-4-thiazolyl)[[2-(1,1-dimethylethoxy)-1,1-dimethyl-2-oxoethoxy]imino]acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-4-[3-carboxy-6,8-difluoro-(2-fluoroethyl)-1,4-dihydro-4-oxoquinolin-7-yl]-1-methylpiperazinium Iodide (7). Under an argon atmosphere, a mixture of 272 mg (1 mmol) of 7-aminocephalosporanic acid, 0.67 mL (3.6 mmol) of MSTFA, and 3 mL of dry acetonitrile was stirred for 30 min; 0.25 mL (1.75 mmol) of iodotrimethylsilane was then added and stirring continued for another 30 min. The mixture was cooled momentarily, and 0.14 mL (1.75 mmol) of anhydrous THF was added. After 10 min, a solution prepared from 277 mg (0.75 mmol) of 6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid, 0.17 mL (0.9 mmol) of MSTFA, and 3 mL of dry acetonitrile was added. Stirring at room temperature was continued for 2.5 h; 478 mg (1 mmol) of 2-[[[1-(2-amino-4-thiazolyl)-2-[(2-benzothiazolyl)thio]-2-oxoethylidene]amino]oxy]-2-methylpropanoic acid 1,1-dimethylethyl ester and

(40) Heymes, R. U. S. Patent 4,288,434, Sept. 8, 1981.

4 mL of dry acetonitrile were added, and the mixture was stirred overnight. After filtration to remove a small amount of insoluble solid, the mixture was concentrated to dryness under reduced pressure. The residual oil was redissolved in 4 mL of acetonitrile, and with ice cooling, 0.16 mL of methanol was added. The mixture was stirred for 1 min and allowed to stand for 3 min, before filtering. After the mixture was washed with three 3-mL portions of acetonitrile and dried under reduced pressure, 530 mg of product was obtained: NMR (Me_2SO-d_6) δ 1.36 (s, 12 H, *t*-Bu and CH_3), 1.40 (s, 3 H, CH_3), 3.12 (s, 3 H, NCH_3), 3.40–3.86 (m, 9 H, 4 × NCH_2 and CH of SCH_2), 3.96 (d, 1 H, J_{gem} = 16 Hz, CH of SCH_2), 4.40, 4.66 (AB, 2 H, J_{gem} = 13 Hz, NCH_2), 4.62–5.06 (m, 4 H, NCH_2CH_2F), 5.26 (d, 1 H, J = 5 Hz, CH), 5.93 (dd, 1 H, J = 5 and 7 Hz, CH), 6.68 (s, 1 H, Ar), 7.25 (s, 2 H, NH_2), 7.92 (d, 1 H, J = 12 Hz, Ar), 8.88 (s, 1 H, =CH–), 9.44 (d, 1 H, J = 7 Hz, NH).

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Synthesis of Halogen-Substituted 1,5-Benzothiazepine Derivatives and Their Vasodilating and Hypotensive Activities

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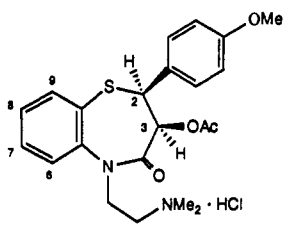
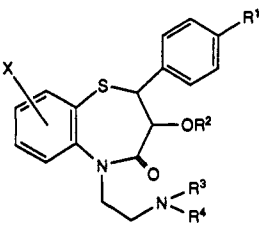
In an attempt to improve the effectiveness and duration of the action of diltiazem (1), a 1,5-benzothiazepine calcium channel blocker, its derivatives (2) with halogen substituents on the fused benzene ring were synthesized. These compounds were evaluated for their effects on vertebral and coronary blood flows and antihypertensive activity. The structure–activity relationships are discussed. The 8-chloro derivative ((+)-**2b**), the most potent compound in this series, was selected for clinical evaluation as a cerebral vasodilating and antihypertensive agent.

Diltiazem (1)[1] is a potent calcium channel blocker and has been widely used as an effective antianginal and antihypertensive agent.[2] Our previous study[3] on the structure–activity relationships (SAR) of some 40 derivatives of 1 made clear the effect of substituents at the positions 2, 3, and 5 and their stereochemical requirements for activity. The effect of substitution on the fused benzene ring of 1, however, remained uncertain, since only the 7-chloro derivative (2, X = 7-Cl, R^1 = OMe, R^2 = Ac, R^3 = R^4 = Me) has been synthesized.[1a] In an attempt to



Diltiazem (2*S*, 3*S*) (1)



(2)

improve the effectiveness and duration of the action of diltiazem (1) and to gain further insight into the SAR, we introduced halogen substituents at the positions 6–9 of 1 in the present study. Described herein are the synthesis as well as the vasodilating and antihypertensive activities of this new series of derivatives (2). The SAR are also discussed.

Chemistry

The synthesis of *cis*-2-aryl-2,3-dihydro-3-hydroxy-1,5-benzothiazepin-4(5*H*)-one (5), a requisite intermediate for 2, is shown in Scheme I. Fusion of the halogen-substituted 2-aminothiophenol 3 with the *trans*-3-arylglycidic ester 4 at about 160 °C gave the cis lactam 5. This reaction involves cis opening of the oxiran ring of 4 by the thiol group of 3 followed by intramolecular cyclization to give the cis lactam 5 predominantly. Although the yield was rather poor, this simplest method was mainly employed for the preliminary synthesis of 5 (Table I, method A). The unwanted trans isomer 6 was isolated as a minor product in some cases (Table I, **6a,b,h,j**).

The stereochemistry of these lactams (5 and 6) was deduced from the vicinal coupling constant between the methine protons at C_2 and C_3 (about 6 Hz and 11 Hz for cis and trans isomers, respectively)[4] (Table II). The reaction of 2-amino-3-chlorothiophenol (**3a**), bearing a substituent ortho to the amino group, with the glycidic ester **4a** gave the intermediate amino ester **7e** predominantly together with the lactams (**5a** and **6a**, Table I). More practically, the cis lactam 5 was prepared via the amino ester (7) (Scheme I). Heating of 2-amino-5-chlorothiophenol (**3c**) with the glycidic esters **4a** and **4b** in a nonpolar solvent at lower temperature (65–130 °C) gave the threo amino esters **7a** and **7b** in moderate yield (Table III, method F). Alkaline hydrolysis of 7 gave the amino acid **10** (Table IV, method I).

Alternatively, the amino acids **10** and **11** were also obtained via the nitro esters **8** and **9**. Recently, we reported that some Lewis acids, such as halides or carboxylates of tin or zinc, catalytically effect ready and highly stereose-

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