was dissolved in THF (9 mL) and treated with 1 M TBAF in THF (6 mL) with stirring at ambient temperature. After stirring for 20 h the THF was removed and the residue treated with H_2O to precipitate 0.5 g (98%) of 12 as a white solid: mp 233-235 °C (after chromatography) (SiO_2, 3% MeOH-$CHCl_3$ saturated NH_3); ^1H NMR (DMSO-d_6) δ 8.34 (t, J = 6 Hz, 1 H, NH), 7.83 (d, J = 9 Hz, 1 H, NH), 7.54-7.44 (m, 2 H, NH), 7.26-7.03 (m, 13 H, ArH), 6.41 (d, J = 9 Hz, 1 H, ArH), 4.54 (d, J = 6 Hz, 1 H, OH), 4.43 (qd, J = 6, 15 Hz, 2 H, CH_2), 4.20 (t, J = 8 Hz, 1 H, CH), 3.58-3.53 (m, 1 H, CH), 3.44-3.40 (m, 1 H, CH), 2.91-2.43 (m, 5 H, CH_2), 1.75-1.61 (m, 2 H, CH_2), 1.45-1.02 (m, 3 H, CH_2), 1.29 (s, 9 H, CH_3), 0.84-0.73 (m, 6 H, CH_3). Anal. ($C_{38}H_{49}N_5O_5$) C, H, N.

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Dual-Action Cephalosporins: Cephalosporin 3'-Quinolone Carbamates

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A series of cephalosporins has been prepared in which the 3'-position was linked to the nitrogen of the antibacterial quinolone ciprofloxacin through a carbamate function. Like the ester-linked and quaternary-linked dual-action cephalosporins reported earlier, these carbamate-linked compounds exhibited a broad antibacterial spectrum derived from both cephalosporin-like and quinolone-like activities, suggesting a dual mode of action. Studies to elucidate details of the mechanism of action have been inconclusive. Ciprofloxacin liberated as a consequence of bacterial enzyme-mediated reactions may contribute to the second mode of action, although some evidence indicates that the intact carbamate-linked bifunctional molecules may possess intrinsically both β-lactam and quinolone activities.

Introduction

When cephalosporins exert their biological activity by covalently binding to bacterial enzymes, opening of the β-lactam ring is accompanied by liberation of the 3'-substituent, if that substituent can function as a leaving group.[1-7] When the eliminated substance possesses antibacterial activity of its own, the cephalosporin should exhibit a dual mode of action.[8-10] As a rationale for drug design, this mechanism has been discussed in some detail, and we have reported the synthesis and biological activity of two classes of dual-action cephalosporins.[10-21] In these

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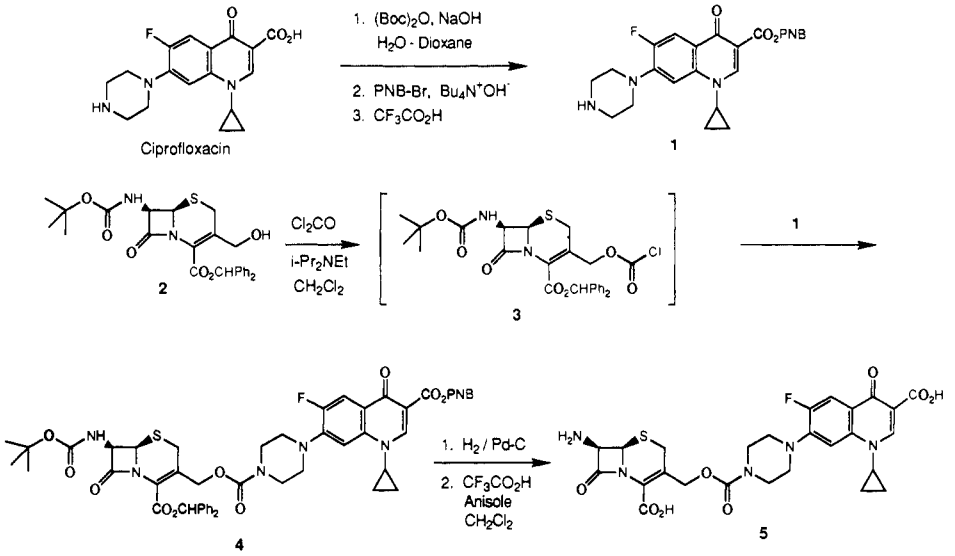
Scheme I[a]



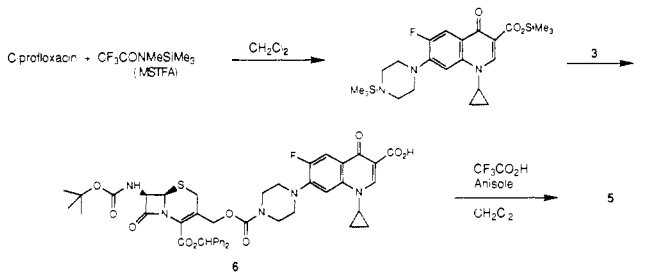
[a] PNB = 4-$O_2NC_6H_4CH_2^-$.

previously described cephalosporins, antibacterial quinolones were linked to the 3′-position either by an ester bond[10-19] or by a bond through a quaternary nitrogen.[20-21] These compounds demonstrated a broad spectrum of antibacterial activity derived from both cephalosporin and quinolone components. The antibacterial activities of quinolones and cephalosporins are complementary; quinolones are active against β-lactam-resistant strains, while cephalosporins are more potent against streptococci. The cephalosporins often exhibit better solubility under physiological conditions and superior pharmacokinetic profiles. Now, in our continuing effort to prepare bifunctional cephalosporins that combine the better features of two major classes of antibacterials, we have synthesized new examples in which quinolones are linked to the cephalosporin 3′-position through a carbamate function.[22-24] Our goal in this program is to find compounds that are significantly superior in terms of activity, solubility, stability, ease of synthesis, and/or pharmacokinetic profile to the ester-linked bifunctional cephalosporin **17** (Ro 23-9424),[10] which is currently undergoing clinical evaluation.

Chemistry

The dual-action cephalosporins of the carbamate type were synthesized according to Schemes I and II and Chart I. All compounds in this report incorporate ciprofloxacin as the quinolone component, although the methodology is quite general and can be applied to other quinolones that have in their structure a primary or secondary amino group. Initially, the 4-nitrobenzyl ester of ciprofloxacin (**1**) was used for the critical step in which the carbamate linkage was established (Scheme I). The protected cephem alcohol **2** reacted with phosgene and diisopropylethylamine to give intermediate chloroformate ester **3**. Further reaction with **1** provided the protected carbamate **4**. Removal of the 4-nitrobenzyl ester was accomplished by hydrogenolysis over 10% palladium on carbon catalyst, a procedure that did not affect the diphenylmethyl ester. Subsequent deprotection with trifluoroacetic acid–anisole then gave the key intermediate **5**. A more efficient alternate synthesis of this intermediate is shown in Scheme II. Here the chloroformate ester **3** was formed as before. Reaction with the bis-trimethylsilyl derivative of ciprofloxacin to give **6**, followed by one-step deprotection, gave **5** while avoiding the juggling of protecting groups required in Scheme I. Intermediate **5** was then acylated by a variety of standard methods to obtain the target compounds (Chart I). The bifunctional carbamates were generally purified as sodium salts by reverse-phase chromatography on C_{18}-silica and were precipitated from solution as zwitterions or free acids by acidification. Products were analyzed for purity by HPLC as described in the Experimental Section.

Scheme II



Results and Conclusions

In vitro antibacterial activities of the bifunctional cephalosporins and comparison compounds are shown in Table I. In general, all of the cephalosporin quinolone carbamates showed excellent broad-spectrum activity. The reference cephalosporin having a 7-(phenoxyacetyl)amino substituent (**16**) (Chart II) demonstrated potent activity against selected Gram-positive bacteria but no significant

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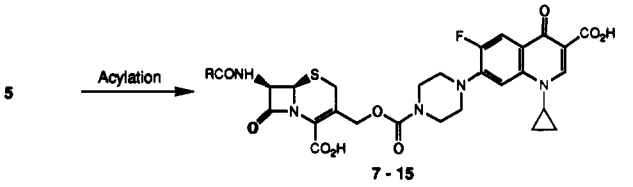
Table I. In Vitro Activity of Bifunctional Cephalosporins and Reference Compounds: MIC (μg/mL)

organisms	CTX[a]	CIP[b]	16	7	8	9	10	11	12	13	14	15
Escherichia coli 257	0.031	0.008	64	0.031	0.063	0.063	0.031	0.063	0.063	0.063	0.063	0.031
E. coli ATCC 25922	0.063	0.008	128	0.031	0.063	0.063	0.031	0.063	0.063	0.063	0.063	0.031
E. coli TEM-1[c]	0.063	0.016	128	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.031
Citrobacter freundii BS-16[c]	32	0.031	>128	0.125	0.063	0.125	0.125			0.063	0.063	0.031
Klebsiella pneumoniae A	0.031	0.25	128	0.125	0.25	0.25	0.125	0.125	0.25	0.125	0.125	0.063
Enterobacter cloacae P99[c]	64	0.008	>128	≤0.016	0.031	0.63	0.063	0.063	0.031	≤0.016	0.031	≤0.016
Serratia marcescens 1071[c]	32	0.063	>128	0.25	0.25	0.5	0.25	0.125	0.125	0.125	0.125	0.125
Proteus vulgaris 1028 BC[c]	>128	0.016	>128	0.063	0.125	0.125	0.125	0.063	0.063	0.031	0.063	0.063
P. mirabilis 90	≤0.016	0.063	32	0.063	0.125	0.25	0.125	0.5	1	0.5	1	0.25
Pseudomonas aeruginosa 8780	16	0.25	>128	1	1	1	1	2	2	1	2	1
P. aeruginosa 18S/H[c]	128	0.25	>128	1	0.5	1	1	1	1	0.5	1	0.5
Staphylococcus aureus Smith	2	0.125	0.063	0.5	0.25	1	0.5	1	0.5	0.25	0.5	0.25
S. aureus 67-0[d]	>128	0.5	128	2	2	4	4	4	4	2	4	2
S. aureus 753[d]	>128	0.25	128	2	1	4	4	2	1	1	1	0.5
Micrococcus luteus ATTC 9341	0.031	2	0.125	0.031	0.031	1	0.5	0.063	0.5	0.125	0.125	0.031
Streptococcus pneumoniae 6301	≤0.016	1	0.125	≤0.016	0.031	0.25	0.125	0.031	0.063	0.031	0.031	0.031
S. pyrogenes 4	≤0.016	0.5	0.125	≤0.016	0.031	0.25	0.063	0.031	0.125	0.031	0.063	≤0.016
Enterococcus faecalis ATCC 29212	0.25	1	32	0.25	4	1	1	0.5	4	4	4	4

[a] Cefotaxime. [b] Ciprofloxacin. [c] Constitutive β-lactamase producer. [d] Methicillin resistant.

Chart I. Carbamate-Linked Bifunctional Cephalosporins



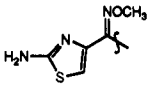
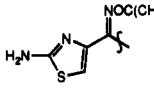
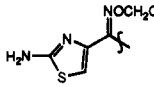
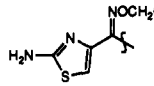
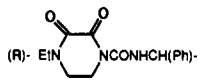
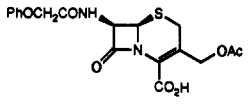
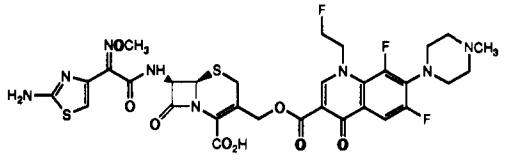
no.	Acylation Method	R
7	Thioester	
8	Acid Chloride	$PhOCH_2$-
9	Thioester (t-Bu Ester)	
10	Thioester (t-Bu Ester)	
11	Thioester	
12	Mixed Anhydride	H-
13	Acid Chloride	$NCCH_2$-
14	DCC - NHBT	
15	DCC - NHBT	(R)- PhCH(OH)-

Chart II. Reference Structures


16


17

activity against the Gram-negative strains. The bifunctional carbamate **8**, having the same acylamino group, showed potent activity against both Gram-positive and Gram-negative organisms. Compared to ciprofloxacin, the dual-action cephalosporins were more active against the streptococci and against *Micrococcus luteus*, with potency being highly dependent upon the 7-acylamino substituent. Compared to the reference cephalosporins, the bifunctional

Table II. In Vivo Activity of **7**, Cefotaxime (CTX), and Ciprofloxacin (CIP)

		mouse protection test: ED_{50}, mg/kg, sc		
infecting organism	LD_{50}s	7	CTX	CIP
S. aureus 753 (MR)	6	28	>100	2
S. pneumonaie 6301	5623	10	15	>50
P. aeruginosa 8780	240	33	>100	3

compounds exhibited improved activity against β-lactam resistant strains. Especially notable was the activity against methicillin-resistant *Staphylococcus aureus* and the β-lactamase overproducer *Enterobacter cloacae* P99, strains that are resistant to the third-generation cephalosporins. Thus, the antibacterial spectra of the bifunctional carbamates seem to be derived from both cephalosporin- and quinolone-like activities. This pattern of activity, which was also noted earlier for the ester-linked[10] and quaternary-linked[20,21] bifunctional cephaloporins, suggests a dual mode of action.

In limited in vivo testing of **7** (Ro 24-4383) this pattern persisted (Table II). In the mouse protection test, **7**

Table III. Binding of Dual-Action Cephalosporins to Essential PBPs of *E. coli* UB1005 (DC-0). Concentration (μg/mL) Required for 90% Inhibition of [^{14}C]Pen G Binding

compd	PBP 1a 90 kDa	PBP 1b 90 kDa	PBP 2 66 kDa	PBP 3 60 kDa	morphology	MIC (μg/mL)
cefotaxime	≤0.1	0.5	100	≤0.1	F/L[a]	0.06 (0.02)[b]
ceftazidime	10	10	>100	0.5	F	0.1 (0.06)
cefoperazone	10	10	2	≤0.1	F	1 (0.02)
16	0.5	100	>100	10	F	>128 (8)
7	100	30	>100	0.5	F	0.5 (0.03)
8	>100	>100	>100	>100	F	2 (1)
9	100	10	>100	≤0.1	F	1 (0.25)
10	100	10	>100	≤0.1	F	1 (0.12)
11	2	10	100	≤0.1	F	1 (0.06)
12	>100	>100	>100	100	F	2 (1)
13	>100	>100	>100	100	F	1 (0.5)
14	10	10	>100	0.5	F	1 (0.12)

[a] F, filaments; L, lysis. [b] Numbers in parentheses refer to MICs for DC2, a permeability mutant.[37]

proved more effective than cefotaxime in treating infection due to *Pseudomonas aeruginosa* or methicillin-resistant *Staphylococcus aureus* and more effective than ciprofloxacin in treating infection due to *Streptococcus pneumoniae*.

The mechanisms by which these compounds exert their observed dual mode of action involve two biological targets. Cephalosporins, like all β-lactam antibacterials, act by acylating active-site serine residues of peptidoglycan transpeptidases.[25,26] Quinolones act by inhibiting bacterial DNA gyrase.[27] In their behavior toward penicillin-binding proteins (PBPs), the bifunctional carbamates acted like typical cephalosporins; the affinity for PBP 3 of *E. coli* was strongly dependent upon the nature of the 7-acylamino substituent (Table III). The quinolone activity may originate with elimination of ciprofloxacin following the enzyme-mediated opening of the β-lactam, according to the putative mechanism discussed earlier as a rationale for drug design.[10] However, the possibility that the intact bifunctional molecule itself might have intrinsic quinolone activity as well as β-lactam activity has to be considered, since structural features known to be essential for DNA gyrase inhibitory activity are incorporated into the dual-action cephalosporins. Studies of replicative DNA biosynthesis in *E. coli*, which is a conveniently measured index of DNA gyrase activity,[28,29] indicated that all of the carbamate-linked bifunctional compounds had significant activity in this assay (Table IV).[30] Had the compounds not been active, the results would have been definitive. The observed activity requires a more equivocal interpretation. It is possible that this activity is manifested by the intact molecule. The *E. coli* cells are permeabilized by treatment with toluene,[28] which facilitates access to the quinolone target. Whether the intact bifunctional compounds could penetrate both the outer and inner membranes and reach this target in normal intact bacteria is open to question. Although the *E. coli* H560 used in these experiments is not considered to be a β-lactamase producer, the possibility of low level β-lactamase production cannot be discounted, nor can the possibility that other enzyme-mediated processes may release, or facilitate the hydrolytic release of, ciprofloxacin with sufficient efficiency to influence the assay. Thus, the question of the relative importance of quinolone activity due to the intact bifunctional cephalosporins versus quinolone activity due to in situ liberation of ciprofloxacin is unresolved.

Table IV. Effects of Carbamate-Linked Cephalosporin Quinolones and Ciprofloxacin on Replicative DNA Biosynthesis in *E. coli* H560 (Permeabilized Cells)

compd	repl. DNA bios. IC_{50} (μg/mL)	MIC (μg/mL) *E. coli* UB1005[a]	MIC (μg/mL) *E. coli* ATCC25922
7	0.9	0.5	0.03
8	4.2	2	0.06
9	2.0	1	0.06
10	1.7	1	0.03
11	1.2	1	0.06
12	0.5	2	0.06
13	1.0	1	0.06
14	9.0	1	0.06
15	4.0	1	0.03
ciprofloxacin	0.3	0.25	0.01

[a] A nalidixic acid resistant strain.[37]

In a study designed to demonstrate the extent and pathway of entry through the bacterial outer membrane, the effects of adding 10 μg/mL of cefotaxime, ciprofloxacin, or 7 upon the growth of wild-type *E. coli* JF568, porin-deficient *E. coli* JF703 (which lacks the porin OmpF), and RTEM-β-lactamase producing *E. coli* RC709 were investigated. In the wild-type *E. coli*, growth was inhibited by all of the antibacterials. In the case of *E. coli* JF703, when cefotaxime or 7 was added, growth continued, albeit at a slightly reduced rate compared to the control. When ciprofloxacin was added, growth ceased. Thus, 7 behaved like cefotaxime rather than like ciprofloxacin. This suggests that 7 is acting initially as the intact cephalosporin and penetrates the outer membrane via the porin pathway. In the case of *E. coli* RC709, ciprofloxacin completely inhibited growth; 7 proved more effective than cefotaxime, indicating that the presence of TEM-1 β-lactamase potentiated the growth-inhibitory effect of 7. Thus, enzyme-mediated release of quinolone may occur in the periplasm.

On the basis of the evidence cited above, including in vitro and in vivo activity, PBP binding characteristics,

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studies related to DNA gyrase inhibition, and bacterial penetration behavior toward wild-type and porin-deficient *E. coli*, the mechanism of action cannot be precisely defined. It appears possible that the intact bifunctional cephalosporin may be capable of directly attacking either of two biological targets. Alternatively, it may act initially as a β-lactam and then, upon opening of the β-lactam, exert a second mode of action, as a targeted prodrug for the quinolone component, by releasing ciprofloxacin in situ. Moreover, hydrolytic instability of the carbamate linkage is an additional complicating factor to be considered. Structure–activity relationships should be interpreted with caution, since the in vitro assay is conducted at 36 °C for 16 h. Although the cephalosporin quinolone carbamates are significantly more stable than the ester-linked compounds reported earlier,[10] the carbamates can still undergo gradual hydrolysis to bioactive products. In pH 7.4 phosphate buffer at 37 °C, **7** had a degradation half-life of 10.5 h, as determined by HPLC analysis. The major products of hydrolysis seemed to be the 3′-hydroxycephalosporin and the quinolone, on the basis of HPLC retention times and UV spectra. If significant concentrations of quinolone liberated by other than enzyme-mediated processes (i.e., through chemical hydrolysis) accumulate in the critical early hours of the incubation period, MICs may be influenced to the extent that hydrolysis competes with enzyme-mediated release of quinolone. On the time scale imposed by pharmacokinetics[31] upon rodent models of infection, and the relatively brief time scale of the PBP-binding, DNA gyrase related, and membrane-penetration studies, chemical degradation is unlikely to be a significant factor. The correlation of in vivo with in vitro results adds support to the thesis that **7** is acting as a dual-action cephalosporin, regardless of the precise mechanism.[32] The nature of the mechanisms by which the dual mode of action occurs is under continuing investigation. Further biological studies with **7** (Ro 24-4383) are ongoing, and results will be published in due course.

Experimental Section

Physical Chemistry. Infrared spectra were recorded on a Digilab FTS 15-E spectrometer. Mass spectra were obtained on a VG7070E-HF mass spectrometer in the positive-ion fast atom bombardment mode, using glycerol or thioglycerol as the solvent. Proton nuclear magnetic resonance spectra 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.

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. In some experiments minor adjustments in pH and in concentration of acetonitrile were made to improve resolution. In stability studies the decrease in integration of the product peak was followed at least until it reached 50% of the original value (i.e., through 1 half-life). In all cases semilogarithmic plots of product-peak integrations against time were essentially linear. In one experiment to identify the products of hydrolysis of compound **7** at 37 °C in pH 7.4 phosphate buffer, the reaction was monitored by HPLC with UV detection at 254 nm, with the standard analytical conditions as described above. The two major peaks appearing due to hydrolysis had retention times and UV spectra corresponding to those of authentic samples of ciprofloxacin (t_R 2.85 min) and desacetylcefotaxime (t_R 3.38 min). The retention time for **7** was 9.40 min.

Biological Assays. Minimum inhibitory concentrations (MICs) were determined by the agar dilution method.[33] Serial 2-fold dilutions of the compounds were prepared in water to give concentrations that, when diluted 10-fold in agar, ranged from 128 to 0.0156 μg/mL. For very active compounds further dilutions were carried out to 0.000 977 μg/mL. Three agar media were employed: brain heart infusion agar (Difco Laboratories, Detroit, MI) supplemented with 20 units/mL bovine liver catalase (Sigma Chemical Company, St. Louis, MO) for the streptococci; Mueller-Hinton agar (Difco) containing 3% agar for *Proteus*; and Mueller-Hinton agar for all other microorganisms. Petri dishes (10 cm) containing 20-mL final volume were inoculated with the aid of a Steers replicator (Craft Machine, Chester, PA). Overnight broth cultures diluted 100-fold served as inocula. This procedure results in an inoculum of approximately 3×10^4 colony-forming units per spot. MICs were determined after overnight incubation at 36 °C.

In vivo antibacterial activity (Table II) was assessed in the mouse protection test, using procedures described previously.[34] 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. The bacterial challenge for each infection is expressed as the multiple of LD_{50}s injected. Serial dilutions of the antibacterial agents were prepared to yield concentrations ranging from 250 to 0.05 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[35] was used to determine the 50% effective (i.e., protective) dose (ED_{50}).

Replicative DNA biosynthesis inhibition was determined by measuring the ATP-dependent incorporation of [^{3}H]thymidine into trichloroacetic acid insoluble material by toluene-permeabilized *E. coli* H560 cells, as previously described.[28] Results are expressed as IC_{50}s, the concentrations for 50% inhibition.

The PBP binding assay was carried out with solubilized membranes from sonicated *E. coli* UB1005, as previously described.[36] PBP binding was measured as inhibition of [^{14}C]-penicillin G binding. Cell morphology was determined by microscopic examination after a 3-h incubation at 37 °C with the test compound in antibiotic medium 3 (Difco).

Studies to determine the extent and pathway of entry of intact dual-action cephalosporins in *E. coli* were conducted with *E. coli* JF568 and the permeability mutant JF703, as previously described.[13]

Organisms used in Tables III and IV and for permeability studies: *E. coli* UB 1005 and its permeability mutant DC2 (D.

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Clark, So. Ill. Univ.);[37] *E. coli* H560 (B. Bachmann, Yale Univ.); *E. coli* JF568 and JF703 (J. Foulds, NIH).

Reference antibiotics: ciprofloxacin (Bayer); cefoperazone (Pfizer); cefotaxime (Hoechst); ceftazidime (Glaxo). Compound **16** was prepared according to the literature procedure.[38]

(6*R-trans*)-7-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[[[[4-[1-cyclopropyl-6-fluoro-1,4-dihydro-3-[[(4-nitrophenyl)methoxy]carbonyl]-4-oxo-7-quinolinyl]-1-piperazinyl]carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Diphenylmethyl Ester (4). Under an argon atmosphere, a solution of 0.58 mL (1.12 mmol) of 20% phosgene in toluene and 20 mL of methylene chloride was cooled at 0–5 °C. A solution of 0.497 g (1 mmol) of (6*R-trans*)-3-(hydroxymethyl)-7-[[(1,1-dimethylethoxy)-carbonyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid diphenylmethyl ester (**2**) in 8 mL of methylene chloride was added along with 0.195 mL (1.14 mmol) of diisopropylethylamine. The mixture was stirred for 15 min at 0–5 °C, before removing the ice bath. Stirring was continued for 1 h and 40 min at ambient temperature. The resulting solution was added to a solution of 0.550 g (1.18 mmol) of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (4-nitrophenyl)methyl ester (**1**) and 0.205 mL (1.20 mmol) of diisopropylethylamine in 17 mL of methylene chloride. The mixture was stirred at room temperature under argon for 3 h. The mixture was then concentrated under reduced pressure, and the residue was purified by flash chromatography, eluting with ethyl acetate–hexane followed by ethyl acetate. The appropriate fractions were combined and concentrated to dryness under reduced pressure to provide 0.425 g (42.9%) of **4**: NMR ($CDCl_3$) δ 1.15 and 1.35 (2 m, 4 H, CH_2CH_2), 1.47 (s, 9 H, *t*-Bu), 3.20 and 3.60 (2 m, 8 H, $N[CH_2CH_2]_2N$), 3.45 (m, 1 H, CH), 3.47 and 3.62 (2 d, 2 H, J = 18 Hz, CH_2S), 4.88 and 5.15 (2 d, 2 H, J = 14 Hz, CH_2O), 4.99 (d, 1 H, J = 5 Hz, CH), 5.19 (d, 1 H, J = 9 Hz, NH), 5.48 (s, 2 H, OCH_2Ar), 5.67 (dd, 1 H, J = 5 and 9 Hz, CH), 6.97 (s, 1 H, CH), 7.25–7.48 (m, 11 H, Ar), 7.73 and 8.26 (2 d, 4 H, J = 8 Hz, nitrophenyl), 8.12 (d, 1 H, J = 13 Hz, Ar), 8.58 (s, 1 H, =CH); IR (KBr) 1789, 1720, 1622, 1520 cm^{-1}; MS *m/z* 989 $(M + H)^+$.

(6*R-trans*)-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]-oxy]methyl]-7-[[(1,1-dimethylethoxy)carbonyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Diphenylmethyl Ester (6). A mixture of 0.414 g (0.418 mmol) of **4**, 0.602 g of 10% Pd on carbon catalyst, and 40 mL of THF (distilled from sodium benzophenone ketyl) was hydrogenated at atmospheric pressure over a period of about 3.5 h. After filtration of the catalyst, the solvent was evaporated under reduced pressure. The residue was chromatographically purified on a Chromatron Model 7924 preparative, centrifugally accelerated, radial TLC apparatus, using ethyl acetate followed by ethyl acetate–acetone–methanol–water (70:10:5:5) as eluant, to obtain 251.6 mg (70.4%) of **6**: NMR ($CDCl_3$) δ 1.20 and 1.40 (2 m, 4 H, CH_2CH_2), 1.47 (s, 9 H, *t*-Bu), 3.24 and 3.60 (2 m, 8 H, $N[CH_2CH_2]_2N$), 3.46 and 3.61 (2 d, 2 H, J = 18 Hz, CH_2S), 3.53 (m, 1 H, CH), 4.88 and 5.13 (2 d, 2 H, J = 14 Hz, CH_2O), 4.98 (d, 1 H, J = 5 Hz, CH), 5.21 (d, 1 H, J = 9 Hz, NH), 5.67 (dd, 1 H, J = 5 and 9 Hz, CH), 6.96 (s, 1 H, CH), 7.27–7.48 (m, 11 H, Ar), 8.06 (d, 1 H, J = 15 Hz, Ar), 8.79 (s, 1 H, =CH); MS *m/z* 854 $(M + H)^+$.

Alternate Synthesis of 6. Under an atmosphere of argon, a solution of 9.92 g (0.02 mol) of **2** in 200 mL of methylene chloride was stirred for 45 min with 4-Å molecular sieves, chilled in ice, and transferred to a dropping funnel. This solution and 3.84 mL (0.0224 mol) of diisopropylethylamine were added at approximately equivalent rates over a 5–10-min period to a stirred solution of 13.6 mL (0.0262 mol) of 20% phosgene in toluene and 140 mL of methylene chloride, with cooling in an ice bath. Stirring was continued with cooling for 30 min, and for another 45 min at ambient temperature. Approximately 150 mL of methylene chloride was added, and then the reaction mixture was concentrated under reduced pressure to its original volume. The mixture was chilled in ice and added over a 10-min period with stirring and ice-cooling to a solution prepared from 5.28 g (0.016 mol) of ciprofloxacin,[39] 7.36 mL (0.04 mol) of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), and 160 mL of methylene chloride. Stirring was continued for 20 min before removing the ice bath and then for another 1.5 h. The mixture was concentrated to dryness under reduced pressure. The residue was taken up in 350 mL of ethyl acetate and filtered to remove the insoluble portion. The ethyl acetate solution was washed with two 100-mL portions of pH 4.0 phosphate buffer, adding small amounts of brine to facilitate separation of the phases. The organic phase was finally washed with two 100-mL portions of brine, dried (Na_2SO_4), and concentrated to dryness under reduced pressure. The residue was triturated with 80 mL of ethanol to obtain a solid. After filtering, washing with cold ethanol, and drying under reduced pressure, 9.00 g (66%) of **6** was obtained.

(6*R-trans*)-7-Amino-3-[[[[4-(3-carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]-carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Trifluoroacetic Acid Salt (5). A solution of 20 g (0.0235 mol) of **6** and 40 mL of anisole in 350 mL of dry methylene chloride was cooled to 0 °C. Cold trifluoroacetic acid (280 mL) was added, and the mixture was stirred at 0 °C under argon for 3 h and 50 min. The mixture was concentrated to dryness under reduced pressure. The residue was treated with 225 mL of methylene chloride followed by 700 mL of ethyl acetate. The resultant gummy precipitate gradually solidified on stirring and intermittant trituration. The solid was filtered, washed with ether, and dried under reduced pressure for 5 h, to obtain 14.5 g (88%) of **5**: NMR (Me_2SO-d_6) δ 1.17 and 1.32 (2 m, 4 H, CH_2CH_2), 3.36 and 3.62 (2 m, 8 H, $N[CH_2CH_2]_2N$), 3.68 and 3.76 (2 d, 2 H, J = 18 Hz, CH_2S), 3.81 (m, 1 H, CH), 4.79 and 5.11 (2 d, 2 H, J = 13 Hz, CH_2O), 5.17 and 5.22 (2 d, 2 H, J = 5 Hz, CHCH), 7.59 (d, 1 H, J = 8 Hz, Ar), 7.93 (d, 1 H, J = 15 Hz, Ar), 8.67 (s, 1 H, =CH).

[6*R*-[6α,7β(*Z*)]]-7-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[[[4-(3-carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]-carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (7). A suspension of 9.00 g (0.0126 mol) of **5** in 175 mL of THF was stirred and cooled at 10 °C. A solution of 5.04 g (0.0503 mol) of potassium bicarbonate in 175 mL of water was added. The mixture was stirred at about 10 °C for 25 min to obtain complete solution. With continued stirring and cooling at 10 °C, a solution of 4.50 g (0.0128 mol) of (*Z*)-2-amino-α-(methoxyimino)-4-thiazoleethanethioic acid *S*-2-benzothiazolyl ester in 40 mL of THF was added. After 25 min the cooling bath was removed, and the mixture was stirred overnight at room temperature. The mixture was extracted with two 500-mL portions of ethyl acetate. The organic extracts were back-washed with water, the water-wash then being combined with the original aqueous phase. The aqueous solution was concentrated under reduced pressure to remove residual organic solvents, and 6 N HCl was added to adjust the pH to 7.55. The solution was applied to a column of approximately 220 g of C_{18}-silica from Waters and chromatographed under low pressure, using a stepwise gradient of 0.1 M pH 7.5 sodium phosphate buffer–acetonitrile, from 0 to 20% acetonitrile. The appropriate fractions were combined, cooled in ice, and acidified to pH 3.0 by addition of 6 N HCl. The solid precipitate was filtered and then suspended in cold water and stirred for 5–10 min before filtering again. After repeated washing with water, the solid was dried under reduced pressure to obtain 6.09 g (59.8%) of **7**: NMR (Me_2SO-d_6) δ 1.19 and 1.33 (2 m, 4 H, CH_2CH_2), 3.33 and 3.62 (2 m, 8 H, $N[CH_2CH_2]_2N$), 3.57 and 3.67 (2 d, 2 H, J = 18 Hz, CH_2S), 3.83 (m, 1 H, CH), 3.84

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(39) The ciprofloxacin used in these experiments was dried under reduced pressure at 100 °C and contained less than 0.5% H_2O by Karl Fischer analysis.

(s, 3 H, MeO), 4.74 and 5.06 (2 d, 2 H, J = 13 Hz, CH_2O), 5.16 (d, 1 H, J = 5 Hz, CH), 5.80 (dd, 1 H, J = 5 and 8 Hz, CH), 6.74 (s, 1 H, thiazole), 7.23 (s, 2 H, NH_2), 7.59 (d, 1 H, J = 7 Hz, Ar), 7.94 (d, 1 H, J = 13 Hz, Ar), 8.68 (s, 1 H, =CH), 9.62 (d, 1 H, J = 8 Hz, NH); IR (KBr) 1782, 1700, 1628 cm^{-1}; MS m/z 771 (M + H)$^+$. Anal. ($C_{32}H_{31}FN_8O_{10}S_2 \cdot 1.5H_2O$) C, H, N, S, H_2O.

(6*R*-*trans*)-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-8-oxo-7-[(phenoxyacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (8). A suspension of 184 mg (0.262 mmol) of **5** in 5 mL of THF was stirred at 0–5 °C, and a solution of 101 mg (1.2 mmol) of sodium bicarbonate in 4.5 mL of water was added. A solution of 54 mg (0.314 mmol) of phenoxyacetyl chloride in 1.5 mL of THF was added dropwise. Stirring was continued at 0–5 °C for 20 min and then for 3 h at ambient temperature. The mixture was concentrated under reduced pressure to remove THF. The aqueous residue was diluted with water, washed with ethyl acetate, cooled in ice, and acidified to pH 2.5 to precipitate the product. After filtration, the solid was washed on the filter with water and ethyl acetate, to obtain, after drying, 130 mg (68%) of **8**. Further purification was accomplished by reverse-phase HPLC on C_{18}-silica, using a pH 7.5 sodium phosphate buffer–acetonitrile gradient. The residue obtained after evaporation and freeze-drying of the appropriate fractions was dissolved in water. The solution was filtered and then acidified to pH 2.5 to precipitate the product: NMR (Me_2SO-d_6) δ 1.19 and 1.33 (2 m, 4 H, CH_2CH_2), 3.3 and 3.62 (2 m, 8 H, $N[CH_2CH_2]_2N$), 3.56 and 3.68 (2 d, 2 H, J = 18 Hz, CH_2S), 3.82 (m, 1 H, CH), 4.60 and 4.66 (2 d, 2 H, J = 15 Hz, CH_2O), 4.75 and 5.08 (2 d, 2 H, J = 13 Hz, CH_2O), 5.14 (d, 1 H, J = 5 Hz, CH), 5.74 (dd, 1 H, J = 5 and 8 Hz, CH), 6.95 and 7.30 (2 m, 5 H, Ph), 7.60 (d, 1 H, J = 7 Hz, Ar), 7.96 (d, 1 H, J = 13 Hz, Ar), 8.68 (s, 1 H, =CH), 9.12 (d, 1 H, J = 8 Hz, NH); MS m/z 722 (M + H)$^+$.

[6*R*-[6α,7β(*Z*)]]-7-[[(2-Amino-4-thiazolyl)[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-3-[[[[4-(3-carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (9). A suspension of 552 mg (0.786 mmol) of **5** in 14 mL of THF was cooled in ice. A solution of 240 mg (2.85 mmol) of sodium bicarbonate in 15 mL of water was added, and the mixture stirred for 20 min. A solution of 384 mg (0.804 mmol) of 2-[[[1-(2-amino-4-thiazolyl)-2-[(2-benzothiazolyl)thio]-2-oxoethylidene]amino]oxy]-2-methylpropanoic acid 1,1-dimethylethyl ester in 6 mL of THF was then added. Stirring was continued for 15 min with ice cooling and then overnight at room temperature. Under reduced pressure, the mixture was concentrated to remove THF. The remaining aqueous solution was washed with ethyl acetate and acidified to pH 2.7. The solid precipitate was filtered, washed with water, and dried under reduced pressure. The intermediate thus obtained (540 mg, 76%) was dissolved with cooling in 2.4 mL of anisole and 6 mL of trifluoroacetic acid and kept overnight at 0 °C. The mixture was concentrated under reduced pressure. Methylene chloride (10 mL) was added, and the mixture was again evaporated under reduced pressure. On addition of 4 mL of methylene chloride and 16 mL of ethyl acetate, the residue solidified. After filtering, washing with ethyl acetate, and air drying, 520 mg (90%) of product was obtained, in the form of a trifluoroacetic acid salt. This was dissolved along with 4 equiv of sodium bicarbonate in 0.025 M pH 7.5 sodium phosphate buffer and purified chromatographically on C_{18}-silica, using pH 7.5 buffer–acetonitrile as eluant. The appropriate fractions were combined and acidified to pH 3 to precipitate a solid. This was filtered, washed with water, and dried under reduced pressure to give **9**: NMR (Me_2SO-d_6) δ 1.19 and 1.33 (2 m, 4 H, CH_2CH_2), 1.44 and 1.46 (2 s, 6 H, 2 Me), 3.32 and 3.62 (2 m, 8 H, $N[CH_2CH_2]_2N$), 3.56 and 3.68 (2 d, 2 H, J = 20 Hz, CH_2S), 3.82 (m, 1 H, CH), 4.74 and 5.09 (2 d, 2 H, J = 14 Hz, CH_2O), 5.18 (d, 1 H, J = 5 Hz, CH), 5.86 (dd, 1 H, J = 5 and 8 Hz, CH), 6.73 (s, 1 H, thiazole), 7.30 (s, 2 H, NH_2), 7.60 (d, 1 H, J = 7 Hz, Ar), 7.95 (d, 1 H, J = 13 hz, Ar), 8.68 (s, 1 H, =CH), 9.45 (d, 1 H, J = 8 Hz, NH); IR (KBr) 1782, 1703, 1628 cm^{-1}; MS m/z 843 (M + H)$^+$.

[6*R*-[6α,7β(*Z*)]]-7-[[(2-Amino-4-thiazolyl)[(carboxymethoxy)imino]acetyl]amino]-3-[[[[4-(3-carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (10). Using procedures similar to those for the synthesis of **9**, but substituting the appropriate thioester, **10** was prepared: NMR (Me_2SO-d_6) δ 1.19 and 1.33 (2 m, 4 H, CH_2CH_2), 3.3 and 3.62 (2 m, 8 H, $N[CH_2CH_2]_2N$), 3.55 and 3.67 (2 d, 2 H, J = 20 Hz, CH_2S), 3.83 (m, 1 H, CH), 4.59 (s, 2 H, CH_2), 4.76 and 5.07 (2 d, 2 H, J = 14 Hz, CH_2O), 5.18 (d, 1 H, J = 5 Hz, CH), 5.83 (dd, 1 H, J = 5 and 8 Hz, CH), 6.80 (s, 1 H, thiazole), 7.27 (s, 2 H, NH_2), 7.59 (d, 1 H, J = 7 Hz, Ar), 7.95 (d, 1 H, J = 13 Hz, Ar), 8.68 (s, 1 H, =CH), 9.65 (d, 1 H, J = 8 Hz, NH); IR (KBr) 1780, 1698, 1628 cm^{-1}; MS m/z 815 (M + H)$^+$.

[6*R*-[6α,7β(*Z*)]]-7-[[[(2-Amino-2-oxoethoxy)imino](2-amino-4-thiazolyl)acetyl]amino]-3-[[[[4-(3-carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (11). Using procedures similar to those for the synthesis of **7**, but substituting the appropriate thioester, **11** was prepared: NMR (Me_2SO-d_6) δ 1.19 and 1.33 (2 m, 4 H, CH_2CH_2), 3.3 and 3.61 (2 m, 8 H, $N[CH_2CH_2]_2N$), 3.57 and 3.68 (2 d, 2 H, J = 18 Hz, CH_2S), 3.82 (m, 1 H, CH), 4.42 (s, 2 H, CH_2O), 4.74 and 5.09 (2 d, 2 H, J = 13 Hz, CH_2O), 5.20 (d, 1 H, J = 5 Hz, CH), 5.87 (dd, 1 H, J = 5 and 8 Hz, CH), 6.86 (s, 1 H, thiazole), 7.10 and 7.50 (2 s, 2 H, $CONH_2$), 7.30 (s, 2 H, NH_2), 7.60 (d, 1 H, J = 7 Hz, Ar), 7.95 (d, 1 H, J = 13 Hz, Ar), 8.68 (s, 1 H, =CH), 9.83 (d, 1 H, J = 8 Hz, NH); IR (KBr) 1780, 1685, 1628 cm^{-1}; MS m/z 814 (M + H)$^+$.

(6*R*-*trans*)-3-[4-[[[(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-7-(formylamino)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (12). A solution of 104 mg (1.18 mmol) of acetic formic anhydride in 1.5 mL of THF was added with ice-cooling to a solution of 276 mg (0.393 mmol) of **5**, 7 mL of water, 4 mL of THF, and 99 mg (1.18 mmol) of sodium bicarbonate. After 10 min, the ice bath was removed, and the mixture was stirred for 3 h at room temperature. The mixture was concentrated under reduced pressure to remove THF. The residual aqueous mixture was adjusted to pH 7.2 by addition of aqueous sodium bicarbonate. The mixture was washed with ethyl acetate and the aqueous solution cleared by filtration. The pH was adjusted to 3 by addition of 1 N HCl, to precipitate a solid. After filtering, washing with water, and drying under reduced pressure, 130 mg (54%) of **12** was obtained: NMR (Me_2SO-d_6) δ 1.19 and 1.33 (2 m, 4 H, CH_2CH_2), 3.3 and 3.62 (2 m, 8 H, $N[CH_2CH_2]_2N$), 3.57 and 3.67 (2 d, 2 H, J = 18 Hz, CH_2S), 3.82 (m, 1 H, CH), 4.76 and 5.08 (2 d, 2 H, J = 13 Hz, CH_2O), 5.13 (d, 1 H, J = 5 Hz, CH), 5.79 (dd, 1 H, J = 5 and 9 Hz, CH), 7.60 (d, 1 H, J = 7 Hz, Ar), 7.95 (d, 1 H, J = 13 Hz, Ar), 8.15 (s, 1 H, HCON), 8.68 (s, 1 H, =CH), 9.07 (d, 1 H, J = 9 Hz, NH); IR (KBr) 3420, 1782, 1700, 1625 cm^{-1}; MS m/z 616 (M + H)$^+$.

(6*R*-*trans*)-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-7-[(cyanoacetyl)amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (13). A mixture of 510 mg (6 mmol) of cyanoacetic acid, 15 mL of methylene chloride, and 0.03 mL of DMF was stirred and cooled to 0 °C, and 764 mg (6 mmol) of oxalyl chloride was added. After 5 min, the cooling bath was removed, and the mixture was stirred for 2 h at room temperature. Methylene chloride (15 mL) was added, and the solution was concentrated under reduced pressure to an approximate volume of 8 mL. This solution was diluted with methylene chloride to a volume of 24 mL.

An 8-mL (2 mmol) portion of the above-prepared solution of cyanoacetyl chloride was added at 0 °C to a solution of 701 mg (1 mmol) of **5**, 15 mL of methylene chloride, and 404 mg (4 mmol) of triethylamine. The mixture was stirred for 5 min at 0 °C and for 3 h at room temperature. A small amount of precipitate was removed by filtration. With cooling in ice, 20 mL of 0.025 M pH 7.5 sodium phosphate buffer was added, along with aqueous sodium bicarbonate solution to maintain the pH at 7.5. The aqueous solution was washed with ethyl acetate, cooled in ice, and adjusted to pH 2.9 by addition of 2 N HCl, to precipitate a solid. After filtering, washing with water, and drying under reduced pressure, 430 mg (65%) of product was obtained.

A portion of this product was further purified by chromatography on C_{18}-silica, eluting with a stepwise gradient of 0–40%

acetonitrile in 0.025 M pH 7.5 sodium phosphate buffer. The appropriate fractions were acidified to pH 2.9 to precipitate product 13, which was filtered, washed with water, and dried under reduced pressure: NMR (Me_2SO-d_6) δ 1.20 and 1.33 (2 m, 4 H, CH_2CH_2), 3.3 and 3.67 (2 m, 8 H, $N[CH_2CH_2]_2N$), 3.58 and 3.70 (2 d, 2 H, J = 18 Hz, CH_2S), 3.77 and 3.83 (2 d, 2 H, J = 20 Hz, CH_2CN), 3.82 (m, 1 H, CH), 4.75 and 5.10 (2 d, 2 H, J = 13 Hz, CH_2O), 5.14 (d, 1 H, J = 5 Hz, CH), 5.72 (dd, 1 H, J = 5 and 8 Hz, CH), 7.60 (d, 1 H, J = 8 Hz, Ar), 7.95 (d, 1 H, J = 13 Hz, Ar), 8.68 (s, 1 H, =CH), 9.31 (d, 1 H, J = 8 Hz, NH); IR (KBr) 1782, 1722, 1702, 1665 cm^{-1}; MS m/z 655 $(M + H)^+$.

[6*R*-[6α,7β(*R*)]]-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-7-[[[[(4-ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino]phenylacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (14). Using procedures similar to those used to prepare 15, 5 was acylated with (*R*)-α-[[(4-ethyl-2,3-dioxopiperazinyl)carbonyl]amino]benzeneacetic acid by the DCC–NHBT method to obtain 14: NMR (Me_2SO-d_6) δ 1.07 (t, 3 H, J = 7 Hz, Me), 1.19 and 1.33 (2 m, 4 H, CH_2CH_2), 3.25 and 3.65 (2 m, $N[CH_2CH_2]_2N$, CH_2S, and HOD), 3.82 (m, 1 H, CH), 3.91 (q, 2 H, CH_2, J = 7 Hz), 4.72 and 5.04 (2 d, 2 H, J = 13 Hz, CH_2O), 5.02 (d, 1 H, J = 5 Hz, CH), 5.65 (d, 1 H, J = 7 Hz, CH), 5.76 (dd, 1 H, J = 5 and 8 Hz, CH), 7.30–7.47 (2 m, 5 H, Ph), 7.59 (d, 1 H, J = 7 Hz, Ar), 7.95 (d, 1 H, J = 13 Hz, Ar), 8.68 (s, 1 H, =CH), 9.47 (d, 1 H, J = 8 Hz, NH) 9.86 (d, 1 H, J = 8 Hz, NH); IR (KBr) 1783, 1712, 1690, 1628 cm^{-1}; MS m/z 889 $(M + H)^+$.

[6*R*-[6α,7β(R)]]-3-[[[4-[(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-7-[(hydroxyphenylacetyl)amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (15). A mixture of 310 mg (2.04 mmol) of (*R*)-(−)-mandelic acid, 20 mL of THF, 276 mg (2.04 mmol) of 1-hydroxybenzotriazole (NHBT), and 462 mg (2.24 mmol) of 1,3-dicyclohexylcarbodiimide (DCC) was stirred for 2 h and filtered. The filtrate was added at 0 °C to a solution prepared from 1.10 g (1.57 mmol) of 5, 4 mL of THF, 14 mL of water, and 396 mg (4.71 mmol) of sodium bicarbonate. The mixture was stirred cold for 20 min and at room temperature for 4 h. Under reduced pressure, the THF was evaporated. The remaining aqueous mixture was adjusted to pH 7.5 with aqueous sodium bicarbonate and washed with ethyl acetate. The aqueous solution was concentrated slightly under reduced pressure to eliminate traces of organic solvents, treated with activated charcoal, and filtered. On acidification to pH 2.9 with 1 N HCl, a precipitate formed. A portion of the crude product obtained by filtration was redissolved at pH 7.5 and purified by chromatography on C_{18}-silica, eluting with a stepwise gradient of 0–50% acetonitrile in 0.025 M pH 7.5 sodium phosphate buffer. The appropriate fractions were combined and acidified to pH 2.90 to precipitate the product. After filtering, washing with water, and drying under reduced pressure, 15 was obtained in 18% yield: NMR (Me_2SO-d_6) δ 1.19 and 1.33 (2 m, 4 H, CH_2CH_2), 3.3 and 3.60 (2 m, $N[CH_2CH_2]_2N$, half of CH_2S, and HOD), 3.53 (d, 1 H, J = 18 Hz, half of CH_2S), 3.82 (m, 1 H, CH), 4.73 and 5.06 (2 d, 2 H, J = 14 Hz, CH_2O), 5.08 (d, 1 H, J = 5 Hz, CH), 5.12 (d, 1 H, J = 5 Hz, CH), 5.72 (dd, 1 H, J = 5 and 9 Hz, CH), 6.16 (d, 1 H, J = 5 Hz, OH), 7.25–7.48 (2 m, 5 H, Ph), 7.60 (d, 1 H, J = 7 Hz, Ar), 7.95 (d, 1 H, J = 13 Hz, Ar), 8.68 (s, 1 H, =CH), 8.73 (d, 1 H, J = 9 Hz, NH); IR (KBr) 1782, 1700, 1685, cm^{-1}; MS m/z 722 $(M + H)^+$.

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Potential Antitumor Agents. 63. Structure–Activity Relationships for Side-Chain Analogues of the Colon 38 Active Agent 9-Oxo-9*H*-xanthene-4-acetic Acid

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A series of 16 analogues of the solid tumor active compound 9-oxo-9*H*-xanthene-4-acetic acid (XAA), with variations in the acetic acid side chain, have been prepared and evaluated for their ability to cause early haemorrhagic necrosis of colon 38 tumors in mice. The results extend the previous SAR for this class and confirm the necessity for a carboxylic acid group in a fixed disposition with respect to the xanthenone chromophore. None of the compounds showed superior potency to XAA itself, with virtually all alterations in the nature of the anionic center or its geometry with respect to the chromophore greatly reducing or abolishing activity. However, α-methylation of the side chain was permissible, and the two enantiomers of 5-methyl-α-methyl-XAA were separated and tested. Both were active, but the *S*-(+) enantiomer was much more dose-potent than the *R*-(−) enantiomer, in both the in vivo tumor necrosis assay and an in vitro assay measuring the stimulation of nitric oxide production by macrophages. This suggests that the enantiomers have different intrinsic activities, rather than differing in their vivo metabolism.

The drug flavone-8-acetic acid (1) (FAA, NSC 347512) has been reported to have solid-tumor selective activity in experimental animal models,[1,2] involving the induction of selective tumor necrosis,[3] induction of cytokines,[4] vascular collapse leading to blood flow shut-down,[5,6] and

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