

9-[5'-Deoxy-5'-(dihydroxyphosphinyl)- β -D-ribofuranosyl]guanosine (16). 9-[5'-Deoxy-2',3'-di-O-benzoyl-5'-(diethoxyphosphinyl)- β -D-ribofuranosyl]-2-bromohypoxanthine (11) (3.37 g, 5 mmol) was dealkylated with bromotrimethylsilane (2.37 g, 15 mmol) as described for 15 and debenzoylated and aminated at 140 °C for 16 h with methanolic ammonia. The reaction mixture was dried under reduced pressure and the residue was dissolved in 15 mL of water. The title compound was purified on a DEAE-cellulose column (HCO₃ form) as in the case of 13 to give 0.95 g of the monoammonium salt of 16: yield 50.2%; UV λ_{\max} (pH 7) 247 nm (ϵ 13 145), (pH 1) λ_{\max} 253 (ϵ = 15 100); ¹H NMR (D₂O) δ 2.0 (dq, 2 H, H-5', H-5'', $J_{\text{H5',P}}$ = 19.12 Hz, $J_{\text{H5'',P}}$ = 18.14 Hz, $J_{\text{H5',H4'}}$ = 7.2 Hz, $J_{\text{H5'',H4'}}$ = 4.5 Hz), 4.04 (d, 1 H), 4.17 (s, 1 H), 4.43 (br s, 1 H), 5.87 (s, 1 H, H-1'), 8.02 (s, 1 H); ¹³C NMR (D₂O) δ 33.63 (d, $J_{\text{C5',P}}$ = 131.25 Hz), 73.8 (d, $J_{\text{C4',P}}$ = 9.75 Hz), 74.53, 80.52, 87.52, 90.49, 137.76, 142.59, 155.62, 159.33; ³¹P NMR (D₂O) δ 19.57. Anal. (C₁₀H₁₇N₆O₇P·H₂O) C, H, N, P.

9-[5'-Deoxy-5'-(dihydroxyphosphinyl)- β -D-ribofuranosyl]hypoxanthine (17). 9-(5'-Deoxy-2',3'-di-O-benzoyl-5'-(diethoxyphosphinyl)- β -D-ribofuranosyl)hypoxanthine (12) (5 mmol, 2.98 g) was treated first with bromotrimethylsilane (15 mmol, 2.37 g) and then with methanolic ammonia exactly as in the case of the adenosine analogue 13. After DEAE-cellulose column chromatography the yield of 17 was 1.0 g (55% as monoammonium salt): (H₂O) λ_{\max} 251 nm (ϵ 11 670), (pH 7) 251 nm (ϵ 11 670), (pH 1) 256 nm (ϵ 8040); ¹H NMR (H₂O) δ 1.96 (dd, H-5' and H-5'', $J_{\text{H5',P}}$ = 17.83 Hz, $J_{\text{H5'',H4'}}$ = 6.4 Hz), 3.93 (t, 1 H), 4.1 (m, 1 H), 4.45 (t, 1 H), 5.86 (d, 1 H, H-1', $J_{\text{H1',2'}}$ = 3.3 Hz), 7.79 (s, 1 H), 8.19 (s, 1 H); ¹³C NMR (D₂O) δ 33.0 (d, C-5', $J_{\text{C5',P}}$ = 132 Hz), 75.11 (d, C-4', $J_{\text{C4',P}}$ = 10.5 Hz), 76.29, 82.16, 89.69, 92.27, 143.87, 147.44, 156.13, 157.64; ³¹P NMR (D₂O) δ 19.242. Anal. (C₁₀H₁₆N₅O₇P·H₂O) C, H, N, P.

In Vitro Antitumor Activity. The cell lines used were L1210 (a murine leukemia), WI-L2 (a human B-lymphoblast), CCRF-CEM (a human T-cell leukemia), and LoVo/L (a human colon carcinoma). Cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 20 mM HEPES, pH 7.4, and 2 mM glutamine. The cytotoxicity determinations were carried out in 96-well microtiter dishes containing a starting number of (5-10) \times 10³ cells per well and 0.1-100 μ M concentrations of the compounds in triplicate wells. L1210 and WI-L2 were incubated with the compounds at 37 °C for 3 days, CCRF-CEM was incubated for 4 days, and Lovo/L was incubated for 5 days. After this time period, 25 μ L of 4 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added to each well and incubation was continued for 2 h. The formazan product was dissolved in 2-propanol containing 0.04 M HCl and

the absorbance was determined with a microtiter plate reader. The absorbance was proportional to the number of cells. The absorbance values were used to calculate the ID₅₀ value for each compound, the concentration which inhibited cell growth to 50% of control cell growth.

In Vitro Antiviral Evaluation. Nucleoside 5'-phosphonates 13-17 were evaluated for their ability to inhibit virus-induced cytopathic effect (CPE) produced by six viruses. Viruses used in this evaluation included parainfluenza virus type 3, herpes simplex virus type 2, adenovirus type 2, rhinovirus type 1-A, influenza A virus, and visna virus. The assays were completed when untreated virus-infected cells showed 100% CPE, which took 5 days for visna and influenza A virus and 3 days for other viruses. Antiviral experiments were performed according to our previous procedures.²⁹

Enzymatic Reactions. The ability of certain nucleoside monophosphates and their analogues to undergo phosphorylation was assessed with commercially available enzymes. Guanylate kinase (bovine brain) and nucleoside monophosphate kinase (bovine liver) were purchased from Sigma Chemical Co., St. Louis, MO. Enzyme reaction volumes of 300 μ L each contained 4 mM ATP, 1 mM test compound, 65 mM Tris-Cl (pH 7.5), 40 mM KCl, 0.5 mM MgCl₂, and either guanylate kinase (0.1 unit/mL) or nucleoside monophosphate kinase (0.2 unit/mL). A unit of enzyme activity is defined as 1 μ mol of substrate converted per minute at 37 °C. After incubation for 2-24 h, reaction products were analyzed by high-pressure liquid chromatography (HPLC) methods as described previously.³⁰ The relative amounts of nucleoside mono-, di-, and triphosphates present at the end of the incubation periods were estimated from peak areas printed out for each HPLC run for all compounds.

Registry No. α -2, 120142-51-0; β -2, 38838-05-0; α -4, 120033-27-4; β -4, 120033-28-5; α -5, 120033-29-6; β -5, 120033-30-9; α -6, 120033-31-0; β -6, 120033-32-1; α -7, 120033-34-3; β -7, 120033-33-2; 8, 120033-35-4; 9, 120033-36-5; 10, 120058-47-1; 11, 120033-37-6; 12, 120033-38-7; 13-NH₃, 120033-39-8; 14-NH₃, 120033-40-1; 14-2Na, 30784-89-5; 15-NH₃, 120033-41-2; 16-NH₃, 120033-42-3; 17-NH₃, 120033-43-4; 6-chloropurine, 87-42-3; uracil, 66-22-8; N⁴-benzoyl cytosine, 26661-13-2; 2-bromohypoxanthine, 87781-93-9.

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Synthesis of Novel 5-Fluoro Analogues of Norfloxacin and Ciprofloxacin

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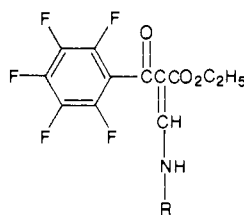
A series of polyfluoro-3-quinolonecarboxylic acids have been synthesized and their in vitro antibacterial activity evaluated. The desired 7-(substituted amino) derivatives were prepared from the 5,6,7,8-tetrafluoroquinolone acids. Conversely, amine displacement occurred primarily at the 5-position when the ester was used. Structure-activity studies indicated that the antibacterial activity was greatest when the N-1 substituent was cyclopropyl and the 7-substituent was 4-methyl-1-piperazinyl. All 5-(substituted amino) derivatives showed poor in vitro activity.

In 1963, nalidixic acid (1)¹ was introduced for the treatment of urinary infections. Since that time numerous structurally related derivatives have been synthesized. Gram-negative antibacterials such as piperimic acid (2),

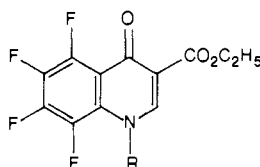
oxolinic acid (3), and rosoxacin (4) were introduced.² Recently, more highly effective broad-spectrum antibacterial agents that contain fluorine atoms have been synthesized. Compounds such as ciprofloxacin (5),³ norflox-

(1) Leshner, G. Y.; Froelich, E. J.; Gruett, M. D.; Bailey, J. H.; Brundage, P. R. *J. Med. Chem.* 1962, 5, 1063.

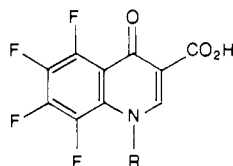
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Table I. Ethyl Perfluoro-3-oxo-2-[(substituted amino)methylene]benzenepropanoates

no.	R	% yield	recryst solvent	mp, °C	formula
16	cyclopropyl	65	hexanes	86-87	C ₁₅ H ₁₂ F ₅ NO ₃
17	isopropyl	95	hexanes	87-89	C ₁₅ H ₁₄ F ₅ NO ₃
18	4-fluorophenyl	84	CH ₂ Cl ₂ /hexanes	126-127	C ₁₆ H ₁₁ F ₆ NO ₃
19	pentafluorophenyl	69	hexanes	70-71	C ₁₈ H ₇ F ₁₀ NO ₃

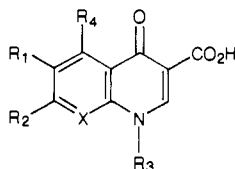
Table II. Ethyl 5,6,7,8-Tetrafluoro-1-(substituted)-1,4-dihydro-4-oxoquinoline-3-carboxylates

no.	R	% yield	recryst solvent	mp, °C	formula
20	cyclopropyl	96	CH ₂ Cl ₂ /hexanes	164-167	C ₁₅ H ₁₁ F ₄ NO ₃
21	isopropyl	89	CH ₂ Cl ₂ /hexanes	170-172	C ₁₅ H ₁₃ F ₄ NO ₃
22	4-fluorophenyl	99	CH ₂ Cl ₂ /hexanes	220-222	C ₁₈ H ₁₀ F ₅ NO ₃
23	pentafluorophenyl	97	acetone/hexanes	137-139	C ₁₈ H ₆ F ₉ NO ₃

Table III. 5,6,7,8-Tetrafluoro-1-(substituted)-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acids

no.	R	% yield	recryst solvent	mp, °C	formula
13	cyclopropyl	80	acetone/hexanes	177-180	C ₁₃ H ₇ F ₄ NO ₃
24	isopropyl	100	CH ₂ Cl ₂ /hexanes	235-238	C ₁₃ H ₉ F ₄ NO ₃
25	4-fluorophenyl	83	CH ₂ Cl ₂ /hexanes	268-271	C ₁₆ H ₆ F ₅ NO ₃
26	pentafluorophenyl	60	acetone/hexanes	245-248	C ₁₆ H ₂ F ₉ NO ₃

acin (6),⁴ ofloxacin (7),⁵ enoxacin (8),⁶ and pefloxacin (9)⁷ are included in this later group. All have fluorine at C-6.



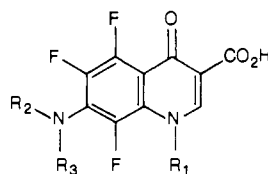
- 1: R₁ = H, R₂ = CH₃, R₃ = C₂H₅, X = N, R₄ = H
- 2: R₁ = N, R₂ = 1-piperazinyl, R₃ = C₂H₅, X = N, R₄ = H
- 3: R₁, R₂ = OCH₂O, R₃ = C₂H₅, X = CH, R₄ = H
- 4: R₁ = H, R₂ = 4-pyridyl, R₃ = C₂H₅, X = CH, R₄ = H
- 5: R₁ = F, R₂ = 1-piperazinyl, R₃ = cyclopropyl, X = CH, R₄ = H
- 6: R₁ = F, R₂ = 1-piperazinyl, R₃ = C₂H₅, X = CH, R₄ = H
- 7: R₁ = F, R₂ = 4-methyl-1-piperazinyl, X, R₃ = COCH₂CH(CH₃), R₄ = H
- 8: R₁ = F, R₂ = 1-piperazinyl, R₃ = C₂H₅, X = N, R₄ = H
- 9: R₁ = F, R₂ = 4-methyl-1-piperazinyl, R₃ = C₂H₅, X = CH, R₄ = H
- 10: R₁ = F, R₂ = 4-methyl-1-piperazinyl, R₃ = C₂H₅, X = CF, R₄ = H

Addition of fluorine at C-8, as in 10, was reported to enhance antibacterial activity.^{11a-c} However, a recent paper reports that the 6,8-difluoroarylquinolones are less active agents than the 6-fluoroarylquinolone parent.^{11d}

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- (11) (a) Domagala, J. M.; Hanna, L. D.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Sanchez, J. P.; Soloman, M. *J. Med. Chem.* 1986, 29, 394. (b) Schentag, J. J.; Domagala, J. M. *Res. Clin. Forums* 1985, 7, 9. (c) UK Patent Appl. GB 2, 057, 440A, 1979; *Chem. Abstr.* 1982, 96, 6607g. (d) Chu, D. T. W.; Fernandes, P. B.; Maleczka, R. E.; Nordeen, C. W.; Pernet, A. G. *J. Med. Chem.* 1987, 30, 504.

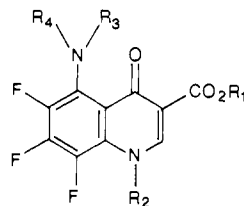
Table IV. 7-Amino-1-(substituted)-4-quinolonecarboxylic Acids



no.	R ₁	R ₂	R ₃	% yield	recryst solvent	mp, °C	formula
27	C ₂ H ₅	-(CH ₂) ₂ N(CH ₃)(CH ₂) ₂ -		53	acetone/hexanes	221-223	C ₁₇ H ₁₈ F ₃ N ₃ O ₃ ^a
28	C ₂ H ₅	-(CH ₂) ₂ O(CH ₂) ₂ -		51	acetone	281-283	C ₁₆ H ₁₅ F ₃ N ₂ O ₄
29	C ₂ H ₅	-CH ₂ (CH ₂) ₂ CH ₂ -		94	CH ₂ Cl ₂ /acetone	>320	C ₁₆ H ₁₅ F ₃ N ₂ O ₃
30	C ₂ H ₅	-(CH ₂) ₂ S(CH ₂) ₂ -		94	acetone	295-300	C ₁₆ H ₁₅ F ₃ N ₂ O ₃ S
31	isopropyl	-(CH ₂) ₂ N(CH ₃)(CH ₂) ₂ -		90	CH ₂ Cl ₂ /hexanes	217-218	C ₁₈ H ₂₀ F ₃ N ₃ O ₃
32	cyclopropyl	-(CH ₂) ₂ N(CH ₃)(CH ₂) ₂ -		53	CHCl ₃ /hexanes	245	C ₁₈ H ₁₈ F ₃ N ₃ O ₃
33	4-fluorophenyl	-(CH ₂) ₂ N(CH ₃)(CH ₂) ₂ -		85	acetone/hexanes	255-256	C ₂₁ H ₁₇ F ₄ N ₃ O ₃

^a HCl salt. Anal. C, H, F, N, Cl. Mp 271-273 °C.

Table V. 5-Amino-1-(substituted)-4-quinolonecarboxylic Acids and Carboxylates

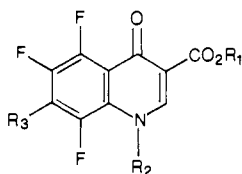


no.	R ₁	R ₂	R ₃	R ₄	% yield	recryst solvent	mp, °C	formula
34	C ₂ H ₅	C ₂ H ₅	-(CH ₂) ₂ N(CH ₃)(CH ₂) ₂ -		51	CH ₂ Cl ₂ /hexanes	188-190	C ₁₉ H ₂₂ F ₃ N ₃ O ₃
35	C ₂ H ₅	C ₂ H ₅	-(CH ₂) ₂ O(CH ₂) ₂ -		>95	CH ₂ Cl ₂ /hexanes	190-192	C ₁₈ H ₁₉ F ₃ N ₂ O ₄
36	C ₂ H ₅	C ₂ H ₅	-(CH ₂) ₂ S(CH ₂) ₂ -		93	CH ₂ Cl ₂ /hexanes	185-187	C ₁₈ H ₁₉ F ₃ SN ₂ O ₃
37	C ₂ H ₅	C ₂ H ₅	-CH ₂ (CH ₂) ₂ CH ₂ -		5	hexanes	152-154	C ₁₈ H ₁₉ F ₃ N ₂ O ₃
38	C ₂ H ₅	cyclopropyl	-(CH ₂) ₂ N(CH ₃)(CH ₂) ₂ -		49	CH ₂ Cl ₂ /hexanes	133-136	C ₂₀ H ₂₂ F ₃ N ₃ O ₃
39	C ₂ H ₅	4-fluorophenyl	-(CH ₂) ₂ N(CH ₃)(CH ₂) ₂ -		74	acetone/hexanes	162-164	C ₂₃ H ₂₁ F ₄ N ₃ O ₃
40	H	C ₂ H ₅	-(CH ₂) ₂ O(CH ₂) ₂ -		74	H ₂ O	225-227	C ₁₆ H ₁₅ F ₃ N ₂ O ₄
41 ^a	H	C ₂ H ₅	-(CH ₂) ₂ N(CH ₃)(CH ₂) ₂ -		72	CH ₂ Cl ₂ /hexanes	212-214	C ₁₇ H ₁₈ F ₃ N ₃ O ₃

^a HCl salt. Anal. C, H, F, N, Cl, 0.3H₂O. Mp 270-271 °C.

In general, optimum activity requires substitution at the N-1 position such as vinyl, ethyl, cyclopropyl, methylamino, and, more recently, fluorophenyl.^{5-10a,b} Substitution at the C-7 position with various piperazines or 3-amino-1-pyrrolidinyl enhances antibacterial activity.

Recently, the synthesis of compounds 11-13 has been disclosed.¹² This prompted us to report our work concerning the synthesis and biological data of a similar group of compounds with general structure 14.

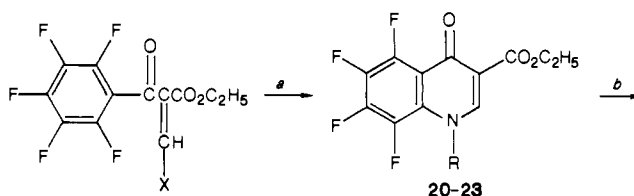


- 11: R₁, R₂ = C₂H₅, R₃ = F
 12: R₁ = H, R₂ = C₂H₅, R₃ = F
 13: R₁ = H, R₂ = *c*-C₃H₅, R₃ = F
 14: R₁ = H, R₂ = alkyl and aryl, R₃ = substituted amino

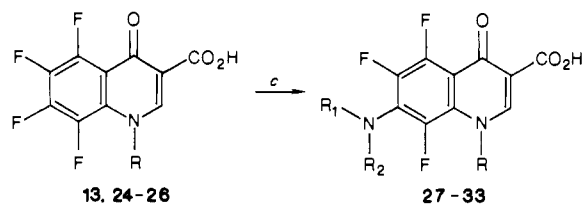
Chemistry

Scheme I shows the reaction of ethyl 2-(2,3,4,5,6-pentafluorobenzoyl)-3-ethoxyacrylate (15) with the appropriate primary amine in ethanol or CH₂Cl₂ gave the enamine esters 16-19 (Table I).¹² Cyclization was accomplished either with 1 equiv of sodium hydride or in higher yield with potassium carbonate at 22 °C in DMF. These reactions afforded the quinolone esters 20-23 (Table

Scheme I



- 15: X = OEt
 16: X = NH-Pr
 17: X = NHCH(CH₃)₂
 18: X = NH-4-F-C₆H₃
 19: X = NH-C₆F₅



^a K₂CO₃/DMF, 25 °C. ^b 2 N H₂SO₄. ^c NHR₁R₂/N-methyl-2-pyrrolidinone or -pyridine.

II). Hydrolysis of these esters with 2 N sulfuric acid yielded the 1,4-dihydro-4-oxo-3-quinolinecarboxylic acids 13, 24-26 (Table III). Acids 12, 13, 24, and 25 were then reacted with various amines which afforded the desired 7-(substituted amino) derivatives 27-33 (Table IV).

When the ethyl esters 11, 20, and 22 reacted with a sampling of the amines shown in Table IV, the 5-substituted quinolone esters 34-39 were the primary products isolated (see Scheme II and Table V). Acid hydrolysis of 34 and 35 gave the corresponding quinolone carboxylic

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Table VI. In Vitro Antibacterial Activity^{a,b} (Minimal Inhibitory Concentration (MIC), $\mu\text{g}/\text{mL}$, for Each Organism Is Given)

no.	Sa(A)	Sa(B)	Sa(C)	S(f)	Sa(D)	Ec(A)	K(P)	Et(C)	Sm	Pa	Ec(B)	Ec(C)
5	1.0	0.5	0.12	2.0	1.0	0.008	0.03	0.008	0.008	0.5	0.002	0.008
9	0.50	0.5	0.25	4.0	0.5	0.12	0.25	0.12	0.12	4.0	0.06	0.12
10	0.50	0.50	0.50	8.0	0.50	0.12	0.25	0.25	0.12	4.0	0.06	0.06
27	2.0	2.0	2.0	32.0	2.0	0.25	0.50	0.50	0.25	32.0	0.12	0.25
28	1.0	1.0	1.0	32.0	1.0	4.0	4.0	4.0	1.0	64.0	4.0	2.0
29	0.25	0.5	0.25	>128.0	0.5	4.0	>128	>128	0.5	>128	2.0	2.0
30	1.0	1.0	0.5	32.0	1.0	4.0	8.0	8.0	2.0	64.0	4.0	4.0
31	32.0	16.0	32.0	>128	64.0	4.0	8.0	8.0	4.0	>128	4.0	4.0
32	0.5	0.5	0.25	4.0	0.5	0.06	0.06	0.06	0.06	4.0	0.015	0.03
33	4.0	2.0	2.0	64.0	2.0	2.0	2.0	4.0	2.0	32.0	2.0	1.0
40	128.0	32.0	64.0	>128	128.0	>128	>128	>128	>128	>128	>128	>128
41	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
43	8.0	8.0	128.0	>128	>128	>128	>128	>128	>128	>128	>128	128.0

^a Structures shown in Tables IV and V. ^b Organisms selected for the table are as follows: Sa(A), *Staphylococcus aureus* VGH 84-47; Sa(B), *Staphylococcus aureus* K82-26; Sa(C), *Staphylococcus aureus* Smith (MP); S(f), *Streptococcus faecalis* UCI 85-30; Sa(D), *Staphylococcus aureus* ATCC 29213; Ec(A), *Escherichia coli* MOR 84-20; K(P), *Klebsiella pneumoniae* CMC 84-31; Et(C), *Enterobacter cloacae* VGH 84-39; Sm, *Serratia marcescens* MOR 84-41; Pa, *Pseudomonas aeruginosa* K84-16; Ec(B), *Escherichia coli* ATCC 25922; Ec(C), *Escherichia coli* #311 (MP).

Table VII. Fluorine-19 NMR Spectral Data of Selected 5- and 7-Substituted Quinolonecarboxylic Acids^a

no.	C5-F			C6-F			C7-F			C8-F		
	ppm	mult	J , Hz	ppm	mult	J , Hz	ppm	mult	J , Hz	ppm	mult	J , Hz
27	19.71	dd	$J_{5-6} = 19.5$ $J_{5-8} = 12.2$	15.20	dd	$J_{6-5} = 19.5$ $J_{6-8} = 6.0$				26.66	br resonance	
29	19.76	dd	$J_{5-6} = 19.5$ $J_{5-8} = 12.2$	15.10	dd	$J_{6-5} = 19.5$ $J_{6-8} = 6.0$				26.84	br resonance	
40				11.44	d	$J_{6-7} = 22.0$	13.60	t	$J_{7-8,6} = 22.0$	16.60	d	$J_{8-7} = 22.0$
41				10.52	d	$J_{6-7} = 22.0$	13.37	t	$J_{7-8,6} = 22.0$	16.52	d	$J_{8-7} = 22.0$

^a All chemical shifts are referenced to internal hexafluorobenzene at 0.0 ppm and all coupling constants refer to ^{19}F - ^{19}F couplings.

acids 41 and 40, respectively (Table V). Only in one case involving pyrrolidine as nucleophile was the 5,7-disubstitution product 42 obtained along with a small amount of the 5-substitution component 37 (Scheme II). Acid hydrolysis of 42 gave the 5,7-disubstituted quinolonecarboxylic acid 43. This preference for nucleophilic substitution at the 5-position in the case of ethyl esters over the 7-position realized in the free acid is not clear. However, fluorine spectral data clarified the positional isomer in each case.

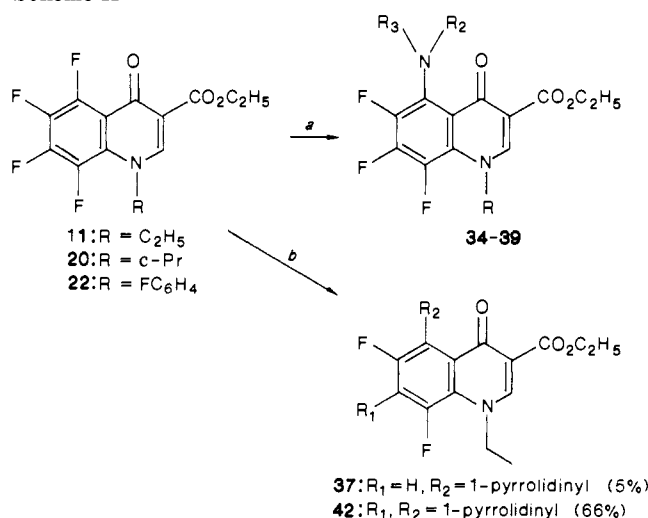
Spectral Studies

Fluorine-19 NMR was used to distinguish between 5- and 7-substituted quinolones. The chemical shifts, coupling constants, and multiplicities for selected 5- and 7-substituted quinolones are summarized in Table VII. The ^{19}F spectra of the 5-substituted quinolones were first order in all cases. The large ortho coupling constants for all three fluorine resonances ($J = 22$ Hz) and the multiplicities indicate three contiguous fluorine atoms. However, in the case of the 7-substituted quinolones the spectra were second order. For example, in the 7-morpholino-substituted quinolone 28, C5-F is a doublet of doublets and has both an ortho ($J = 19.5$ Hz) and a para coupling ($J = 12$ Hz). C6-F is also a doublet of doublets and has an ortho coupling ($J = 19.5$ Hz) and a meta coupling ($J = 6$ Hz).¹³ The C8-F is a broad resonance, and the ^{19}F - ^{19}F coupling constants could not be measured. This broadening is attributed to the long range ^1H - ^{19}F coupling from the C-7 and N-1 substituents.

Results and Discussion

Table VI summarizes the in vitro antibacterial data of the 5- and 7-substituted quinolones against five Gram-

Scheme II



^a NHR₂R₃/N-methyl-2-pyrrolidinone or -pyridine. ^b Excess pyrrolidine, 95 °C/pyridine.

positive bacteria (*Staphylococcus aureus* VGH 84-47, *Staphylococcus aureus* K-82-26, *Staphylococcus aureus* Smith (MP), *Streptococcus faecalis* UCI 85-30, and *Staphylococcus aureus* ATCC 29213) and seven Gram-negative organisms (*Escherichia coli* MOR 84-20, *Klebsiella pneumoniae* CMC-84-31, *Enterobacter cloacae* VGH 84-39, *Serratia marcescens* MOR-84-41, *Pseudomonas aeruginosa* K 84-16, *Escherichia coli* ATCC 25922, and *Escherichia coli* #311). The data for ciprofloxacin (5), pefloxacin (9), and 1-ethyl-6,8-difluoro-7-(4-methylpiperazin-1-yl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (10) are included for comparison.^{11c}

The effects of varying the substituents at the N-1 position can be seen by comparing the activity for compounds 27, 31, 32, and 33. Clearly, 32 has comparable activity to ciprofloxacin or pefloxacin. Compound 27, with an ethyl

(13) Emsely, J. W.; Feeney, J.; Sutcliffe, L. H. *High Resolution Nuclear Magnetic Resonance Spectroscopy*; Pergamon Press: Long Island City, NY, 1966; Vol. 2, Chapter 11 and references cited therein.

group at this position, exhibits slightly less activity as does 33 with a 4-fluorophenyl group. The isopropyl derivative 31 showed a marked decrease in activity.

The decreasing order of broad spectrum activity in this series of 1-(substituted)-5,6,8-trifluoro-7-(4-methylpiperazinyl)quinolones therefore is the following: cyclopropyl > ethyl > 4-fluorophenyl > isopropyl.

A comparison between 5-fluoroquinolone 27 and its parent analogue 10 indicates that the presence of this C-5 substituent slightly decreases the overall activity. This effect is even greater regarding the activity of 27 versus *Pseudomonas aeruginosa*. Substitution solely at the 5-position, as in examples 40 and 41, virtually deactivates the analogue. A vast difference in broad-spectrum behavior is seen for the regioisomers 41 vs 27. One further demonstration of the deactivation produced by a C-5 substituent other than hydrogen is seen by comparing the activity data for the 7-pyrrolidinyl derivative 29 against the 5,7-bis(1-pyrrolidinyl) analogue 43. The 5-pyrrolidinyl moiety in 43 serves only to further decrease that activity seen in 29. It is accepted that the antibacterial action of these quinolones is due to their inhibition of bacterial DNA synthesis. The chemical events can be explained, at least in part, by a complexation involving the 4-oxo 3-carboxylic moiety of the quinolone and a metal ion of the bacterial DNA gyrase enzyme.¹⁴ Electron-donating substituents such as secondary amino groups at the quinolone C-7 position favor the metal-quinolone complexation. Amine groups at the C-5 position also donate electron density to the site of complexation and thus should enhance activity while an electron-withdrawing one (fluoride) should decrease it. A recent report describes significantly enhanced antibacterial potency when an amino function is substituted at the 5-position of the 6,8-difluoro-1-cyclopropylquinolone.¹⁵ Obviously, other considerations, such as steric bulk, play an even more important role. A substantial loss of activity occurs when the C-5 hydrogen or fluoride is replaced with a larger group such as the pyrrolidinyl. The quinolone-metal complexation argument is supported when one considers the example of the slight activity loss regarding the C-5 fluoride analogue 27 vs the C-5 hydrogen in 10.

The in vitro activity of the various analogues possessing amino substituents at the 7-position with a common N-1 group (ethyl) is presented in examples 27-30. In this series, the effectiveness against Gram-positive organisms decreases in the order pyrrolidine > morpholine/thiomorpholine > 4-methylpiperazine. The Gram-negative profile of these entries, on the other hand, follows the opposite trend. The effect of these four C-7 substituents is not new and has previously been observed in similar quinolone systems.^{9a}

Significant to this work is the fact that secondary amines can be introduced at the quinolone C-5 position. Despite a similar ability to donate electron density to the 4-oxo quinolone moiety as their C-7 regioisomers, these C-5 substituted quinolones are not active. Conclusions drawn from this empirical data suggest that steric factors are the most important consideration concerning the C-5 position. Other groups much larger than amino or dimethylamino¹⁵ probably will not favorably contribute to the in vitro biological activity of the quinolone.

Table VIII. Mouse Protection Test of 32 vs Pefloxacin (9)

test organism	no.	administration route	ED ₅₀ (95% confidence limits), mg/kg	po/sc
<i>E. coli</i> #311	9	po ^a	1.1 (0.91-1.5)	2.75
		sc ^b	0.4 (0.3-0.5)	
<i>S. aureus</i> Smith	32	po	0.5 (0.4-0.7)	1.4
		sc	0.35 (0.27-0.45)	
	9	po	3.1 (2.4-4.0)	1.6
		sc	2.0 (1.6-2.3)	
32	po	3.6 (2.5-5.2)	1.7	
	sc	2.1 (1.9-2.5)		

^a Oral dosing. ^b Subcutaneous dosing.

The efficacy in systemic infection due to Gram-negative organism *E. coli* #311 and Gram-positive organism *S. aureus* Smith of 32 vs pefloxacin (9) are summarized in Table VIII. These compounds are tested in mice by oral (po) and subcutaneous (sc) routes of administration. The ratio of the po and sc ED₅₀'s are compared and serve as a measure of the relative oral absorption.

Enhanced in vivo activity of 32 vs 9 against *E. coli* #311 is realized as a much lower po/sc ratio. However, against *S. aureus* Smith a comparable ratio is witnessed.

Against *E. coli* systemic infection 32 is nearly twice as potent as pefloxacin when administered orally. Both compounds show similar activity via subcutaneous routes. The ratio po/sc for 32 (1.4) vs 9 (2.75) indicates that 32 is better absorbed. Against *S. aureus* 9 and 32 are quite similar in all aspects.

Experimental Section

Melting points were taken in a Mel-Temp melting point apparatus and are uncorrected. Elemental analyses were obtained for all new compounds reported. Carbon, hydrogen, nitrogen, and fluorine analyses (unless otherwise specified) were within 0.4% of the theoretical values. The NMR spectra were obtained with a NT-300 WB spectrometer and chemical shifts (δ) are in ppm relative to internal tetramethylsilane. The ¹⁹F spectra were obtained with a Nicolet NT-300 WB N spectrometer, referenced to internal hexafluorobenzene at 0.0 ppm. Mass spectra were recorded on a Finnigan Mat CH-7 spectrometer. High-resolution mass spectra (HRMS) were determined with a ZAB-SE mass spectrometer. The IR spectra were recorded on Perkin-Elmer Model 21 infrared spectrometer. The IR, NMR, and mass spectral data of all compounds were consistent with the assigned structures. Solutions were dried with anhydrous sodium sulfate.

Ethyl 2,3,4,5,6-Pentafluoro- α -[(4-fluorophenyl)amino]methylene]- β -oxobenzeneprionate (18). A solution of 15¹² (11.2 g, 0.033 mol) in 200 mL of absolute EtOH was cooled to 0 °C while 4-fluoroaniline (4.0 g, 0.036 mol) was added dropwise over 10 min. The stirred mixture was allowed to gradually come to room temperature over 18 h. The mixture was evaporated to half-volume and the precipitate collected to yield 11.23 g (84%) of 18, mp 124-126 °C. A sample was crystallized from CH₂Cl₂-hexanes, mp 126-127 °C. NMR (CDCl₃): δ 1.17 (3 H, t, $J = 7.1$, CH₂CH₃), 4.13 (2 H, q, $J = 7.3$, CH₂CH₃), 7.16 (2 H, dd, $J_{HH} = 8.5$, $J_{HF} = 8.4$, aromatic H), 7.27 (2 H, dd, $J_{HH} = 8.0$, $J_{HF} = 4.5$, aromatic H), 8.58 (1 H, d, $J = 13.8$, vinyl H), 12.75 (1 H, br d, $J = 13.5$, NH). Anal. (C₁₈H₁₁F₆NO₃) C, H, F, N.

Various ethyl polyfluoro-3-oxo-2-[(substituted amino)methylene]benzenepropanoates (Table I) were prepared from 15 in a similar fashion.

Ethyl 1-Cyclopropyl-5,6,7,8-tetrafluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylate (20). A mixture of 16 (13.5 g, 0.039 mol) and anhydrous potassium carbonate (16.2 g, 0.12 mol) in 100 mL of DMF was stirred at ambient temperature for 18 h. The concentrate was partitioned between cold H₂O and CH₂Cl₂. The organic layer was washed with cold H₂O, dried, and upon concentration yielded 12.2 g (96%) of 20, mp 158-162 °C. A sample was crystallized from CH₂Cl₂-hexanes, mp 164-167 °C. NMR (CDCl₃): δ 1.17 (4 H, m, CH₂CH₂), 1.38 (3 H, t, $J = 7.1$, CH₂CH₃), 3.88 (1 H, m, NCH), 4.35 (2 H, q, $J = 7.0$, CH₂CH₃), 8.47 (1 H, s, C2-H). Anal. (C₁₅H₁₁F₄NO₃) C, H, F, N.

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Various ethyl polyfluoro-1-(substituted)-3-quinoline carboxylates (Table II) were prepared in a similar fashion.

Alternatively, a solution of 16 (1.04 g, 3.0 mmol) in 5 mL of DMF was added under argon to a hexane-washed 50% sodium hydride-in-oil suspension (0.22 g, 4.5 mmol) in 5 mL of DMF and the mixture was stirred for 30 min. The mixture was poured over crushed ice. The precipitate was collected, washed with H₂O, and yielded 0.64 g (65%) of 20, mp 158–162 °C.

5,6,7,8-Tetrafluoro-1,4-dihydro-1-(1-methylethyl)-4-oxo-3-quinolinecarboxylic Acid (24). A mixture of 21 (2.32 g, 7.0 mmol) in 70 mL of 2 N H₂SO₄ was stirred at 100 °C for 18 h. The cooled mixture was extracted with CH₂Cl₂ and the organic layer dried. Concentration afforded 2.12 g (100%) of 24, mp 231–234 °C. Crystallization from CH₂Cl₂-hexanes yielded 1.5 g of white crystals, mp 235–238 °C. NMR (CDCl₃): δ 1.67 (6 H, dd, *J*_{HH} = 6.5, *J*_{HF} = 1.8, CH(CH₃)₂), 5.30 (1 H, m, *J*_{HH} = 6.7, *J*_{HF} = 3.0, CH(CH₃)₂), 8.86 (1 H, s, C2-H), 14.13 (1 H, s, COOH). Anal. (C₁₃H₉F₄NO₃) C, H, F, N.

Various polyfluoro-1-(substituted)-3-quinolinecarboxylic acids (Table III) were prepared in a similar fashion.

1-Ethyl-5,6,8-trifluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic Acid (27). A solution of 12¹² (50 mg, 0.17 mmol) in 0.3 mL of 1-methyl-2-pyrrolidone was stirred at ambient temperature while *N*-methylpiperazine (43 mg, 0.43 mmol) was added rapidly. The mixture was stirred for 2 h and ether was added. The precipitate was collected and crystallization from acetone-hexanes yielded 34 mg (53%) of 27, mp 221–223 °C. NMR (Me₂SO-*d*₆): δ 1.41 (3 H, d of t, *J*_{HH} = 7.0, *J*_{HF} = 1.2, CH₂CH₃), 2.24 (3 H, s, NCH₃), 2.46 (4 H, br s, CH₂N(CH₂)₂), 3.39 (4 H, br s, N(CH₂)₂), 4.54 (2 H, m, NCH₂CH₃), 8.88 (1 H, s, C2-H). Anal. (C₁₇H₁₈F₃N₃O₃) C, H, F, N.

Various 7-amino-1-(substituted)-4-quinolonecarboxylic acids (Table IV) were prepared in a similar fashion from 12–13 and 24–25.

Ethyl 1-Ethyl-6,7,8-trifluoro-1,4-dihydro-5-(4-morpholinyl)-4-oxo-3-quinolinecarboxylate (35). A solution of 11¹² (0.58 g, 1.8 mmol) and morpholine (0.80 g, 9.0 mmol) in 30 mL of dry pyridine was heated on a steam bath for 1.5 h. The mixture was concentrated and the residue partitioned between CH₂Cl₂ and H₂O. Evaporation of the dried organic layer yielded 0.7 g (100%) of 35, mp 188–191 °C. A sample was recrystallized from CH₂Cl₂-hexanes, mp 190–192 °C. NMR (CDCl₃): δ 1.38 (3 H, t, *J* = 7.1, CO₂CH₂CH₃), 1.48 (3 H, d of t, *J*_{HH} = 7.0, *J*_{HF} = 1.1, NCH₂CH₃), 3.27 (4 H, br s, N(CH₂)₂), 3.85 (4 H, br s, O(CH₂)₂), 4.27 (2 H, m, NCH₂CH₃), 4.36 (2 H, q, *J* = 7.1, CO₂CH₂CH₃), 8.18 (1 H, s, C2-H). Anal. (C₁₈H₁₉F₃N₂O₄) C, H, F, N.

Various 5-amino-1-(substituted)-4-quinolone esters (Table V) were prepared in a similar fashion from 11, 20, and 22.

Ethyl 1-Ethyl-6,7,8-trifluoro-1,4-dihydro-4-oxo-5-(1-pyrrolidinyl)-3-quinolinecarboxylate (37) and Ethyl 1-Ethyl-6,8-difluoro-1,4-dihydro-4-oxo-5,7-bis(1-pyrrolidinyl)-3-quinolinecarboxylate (42). A solution of 11 (3.0 g, 9.5 mmol) and pyrrolidine (1.35 g, 18.9 mmol) in 50 mL of dry pyridine was heated on a steam bath for 4 h and evaporated. The residue was partitioned between H₂O and CH₂Cl₂. The organic layer was separated, dried, and evaporated to give a solid. The solid was chromatographed on flash silica gel. Elution with 10% acetone-hexanes yielded 0.17 g (5%) of 37, mp 152–154 °C, after hexane crystallization. TLC: one spot (silica gel), acetone-hexanes (3:7), *R*_f = 0.7. NMR (CDCl₃): δ 1.36 (3 H, t, *J* = 7.2, CO₂CH₂CH₃), 1.47 (3 H, d of t, *J*_{HH} = 7.1, *J*_{HF} = 1.1, NCH₂CH₃), 1.94 (4 H, m, (CH₂)₂), 3.48 (4 H, m, N(CH₂)₂), 4.23 (2 H, m, NCH₂CH₃), 4.36 (2 H, q, *J* = 7.2, CO₂CH₂CH₃), 8.11 (1 H, s, C2-H). Anal. (C₁₈H₁₉F₃N₃O₃) C, H, F, N.

Further elution with 5% MeOH-acetone yielded 2.6 g (66%) of 42, mp 140–145 °C. A sample was recrystallized from acetone-hexanes, mp 146–148 °C. TLC: one spot (silica gel), acetone-hexanes (3:7), *R*_f = 0.5. NMR (CDCl₃): δ 1.37 (3 H, t, *J* = 7.1, CO₂CH₂CH₃), 1.44 (3 H, d of t, *J*_{HH} = 7.1, *J*_{HF} = 1.2, NCH₂CH₃), 1.93 (8 H, m, ((CH₂)₂)₂), 3.37 (4 H, m, N(CH₂)₂), 3.62 (4 H, m, N(CH₂)₂), 4.18 (2 H, m, NCH₂CH₃), 4.34 (2 H, q, *J* = 7.1, CO₂CH₂CH₃), 8.09 (1 H, s, C2-H). Anal. (C₂₂H₂₇F₂N₃O₃) C, H, F, N.

1-Ethyl-6,7,8-trifluoro-1,4-dihydro-5-(4-morpholinyl)-4-oxo-3-quinolinecarboxylic Acid (40). A mixture of 35 (0.51 g, 1.33 mmol) and 40 mL of 6 N HCl was heated on a steam bath for 1 h and then cooled. The solid was collected and dried to yield 0.35 g (74%) of 40, mp 225–227 °C. NMR (CDCl₃): δ 1.41 (3 H, d of t, *J*_{HH} = 6.8, *J*_{HF} = 1.0, NCH₂CH₃), 3.22 (4 H, br s, N(CH₂)₂), 3.74 (4 H, br t, *J* = 3.7, O(CH₂)₂), 4.53 (2 H, m, NCH₂CH₃), 8.90 (1 H, s, C2-H). Anal. (C₁₆H₁₅F₃N₂O₄) C, H, F, N.

By the above procedure, analogue 41 was prepared by hydrolysis of 34 (Table V).

1-Ethyl-6,8-difluoro-1,4-dihydro-4-oxo-5,7-bis(1-pyrrolidinyl)-3-quinolinecarboxylic Acid (43). A mixture of 42 (0.23, 5 mmol) in 50 mL of 6 N HCl was heated at 100 °C for 18 h. The mixture was evaporated to dryness under reduced pressure. The residue was dissolved in CHCl₃ and ether added until turbid. After cooling, the solid was filtered and dried, yielding 0.15 g (77%) of 43, mp 210–212 °C. NMR (Me₂SO-*d*₆): δ 1.38 (3 H, t, NCH₂CH₃), 1.91 (8 H, m, 2((CH₂)₂)), 3.30 (4 H, m, N(CH₂)₂), 3.64 (4 H, m, N(CH₂)₂), 4.40 (2 H, m, NCH₂CH₃), 8.66 (1 H, s, C2-H). HRMS: calcd for C₂₀H₂₃F₂N₃O₃ *m/z* 392.1788, found 392.1784 (M⁺).

High-pressure liquid chromatography (HPLC) was performed with Water Associates components systems and a Hewlett-Packard Model 3390A integrator. HPLC was performed with an RCM 4-μm, 5 mm × 10 cm C18 NovaPak column. The eluting solvent was 90:10 methanol-water and was monitored by UV absorption at 295 nm. A purity of 99.5% by integration was found.

Biology. The *in vitro* antibacterial effects of the compounds were determined in a side-by-side comparison with ciprofloxacin (5) and pefloxacin (9) by standard agar dilution method. Two-fold serial dilutions of the drugs were prepared in Mueller-Hinton agar. The agar surfaces in petri plates were inoculated with (1–5) × 10⁴ colony forming units (cfu) of bacteria by means of the Steers multiple inocula replicator. The lowest concentration of the drug that inhibited the macroscopic growth of a culture after 18 h incubation at 35 °C was recorded as the minimal inhibitory concentration (MIC).¹⁶

The *in vivo* antibacterial effects were determined against acute lethal infections in CD-1 female mice weighing 20 ± 2 g. The mice were infected intraperitoneally with 0.5 mL of a standardized suspension of bacteria in trypticase-soy broth (*E. coli* #311) or 5% hog gastric mucin (*S. aureus* Smith). One-half hour after infection the mice were treated with subcutaneous or oral doses at 2-fold increments of the test compounds contained in 0.5 mL of 0.2% aqueous agar. In each test five mice were treated at each dose level. Untreated control mice died in 24–48 h. The 7-day survival ratios from three separate tests were pooled for the estimation of the median effective doses (ED₅₀) by a computerized probit analysis program.¹⁷

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Registry No. 11, 106890-68-0; 12, 106890-69-1; 13, 106890-70-4; 15, 114226-95-8; 16, 107564-01-2; 17, 119877-52-0; 18, 119877-53-1; 19, 119877-54-2; 20, 107564-02-3; 21, 119877-55-3; 22, 107564-03-4; 23, 119877-56-4; 24, 119877-57-5; 25, 107564-04-5; 26, 119877-58-6; 27, 111781-36-3; 27-HCl, 111781-37-4; 28, 111760-12-4; 29, 111760-11-3; 30, 111760-13-5; 31, 119877-59-7; 32, 107564-07-8; 33, 114008-31-0; 34, 119877-60-0; 35, 119877-61-1; 36, 119877-62-2; 37, 119877-63-3; 38, 113846-23-4; 39, 113846-25-6; 40, 119877-64-4; 41, 119877-65-5; 41-HCl, 119877-51-9; 42, 119877-66-6; 43, 119877-67-7; *N*-methylpiperazine, 109-01-3; morpholine, 110-91-8; pyrrolidine, 123-75-1; thiomorpholine, 123-90-0; cyclopropylamine, 765-30-0; isopropylamine, 75-31-0; *p*-fluoroaniline, 371-40-4; perfluoroaniline, 58749-37-4.

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