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 (HCO_3 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); ^{1}H NMR (D_2O) δ 2.0 (dq, 2 H, H-5′, H-5″, $J_{H5',P}$ = 19.12 Hz, $J_{H5'',P}$ = 18.14 Hz, $J_{H5',H4'}$ = 7.2 Hz, $J_{H5'',H4'}$ = 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); ^{13}C NMR (D_2O) δ 33.63 (d, $J_{C5',P}$ = 131.25 Hz), 73.8 (d, $J_{C4',P}$ = 9.75 Hz), 74.53, 80.52, 87.52, 90.49, 137.76, 142.59, 155.62, 159.33; ^{31}P NMR (D_2O) δ 19.57. Anal. ($C_{10}H_{17}N_6O_7P\cdot H_2O$) 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): (H_2O) λ_{max} 251 nm (ε 11 670), (pH 7) 251 nm (ε 11 670), (pH 1) 256 nm (ε 8040); ^{1}H NMR (H_2O) δ 1.96 (dd, H-5′ and H-5″, $J_{H5',P}$ = 17.83 Hz, $J_{H5',H4'}$ = 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′, $J_{1',2'}$ = 3.3 Hz), 7.79 (s, 1 H), 8.19 (s, 1 H); ^{13}C NMR (D_2O) δ 33.0 (d, C-5′, $J_{C5',P}$ = 132 Hz), 75.11 (d, C-4′, $J_{C4',P}$ = 10.5 Hz), 76.29, 82.16, 89.69, 92.27, 143.87, 147.44, 156.13, 157.64; ^{31}P NMR (D_2O) δ 19.242. Anal. ($C_{10}H_{16}N_5O_7P\cdot H_2O$) C, H, N, P.

In Vitro Anitumor 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) × 10^3 cells per well and 0.1–100 μM 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 μL 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 ID_{50} 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 μL each contained 4 mM ATP, 1 mM test compound, 65 mM Tris·Cl (pH 7.5), 40 mM KCl, 0.5 mM $MgCl_2$, 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 μmol 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**·NH_3, 120033-39-8; **14**·NH_3, 120033-40-1; **14**·2Na, 30784-89-5; **15**·NH_3, 120033-41-2; **16**·NH_3, 120033-42-3; **17**·NH_3, 120033-43-4; 6-chloropurine, 87-42-3; uracil, 66-22-8; N^4-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 pipemidic 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) Lesher, G. Y.; Froelich, E. J.; Gruett, M. D.; Bailey, J. H.; Brundage, P. R. *J. Med. Chem.* **1962**, *5*, 1063.
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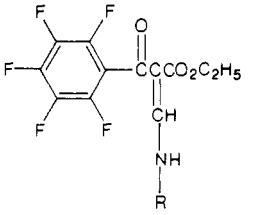
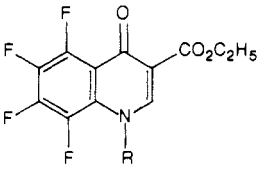
0022-2623/89/1832-1313$01.50/0

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



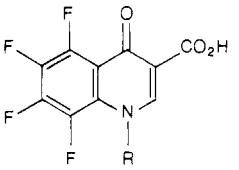
no.	R	% yield	recryst solvent	mp, °C	formula
16	cyclopropyl	65	hexanes	86–87	$C_{15}H_{12}F_5NO_3$
17	isopropyl	95	hexanes	87–89	$C_{15}H_{14}F_5NO_3$
18	4-fluorophenyl	84	CH_2Cl_2/hexanes	126–127	$C_{18}H_{11}F_6NO_3$
19	pentafluorophenyl	69	hexanes	70–71	$C_{18}H_7F_{10}NO_3$

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



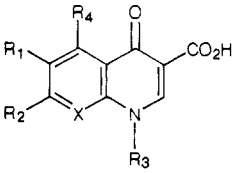
no.	R	% yield	recryst solvent	mp, °C	formula
20	cyclopropyl	96	CH_2Cl_2/hexanes	164–167	$C_{15}H_{11}F_4NO_3$
21	isopropyl	89	CH_2Cl_2/hexanes	170–172	$C_{15}H_{13}F_4NO_3$
22	4-fluorophenyl	99	CH_2Cl_2/hexanes	220–222	$C_{18}H_{10}F_5NO_3$
23	pentafluorophenyl	97	acetone/hexanes	137–139	$C_{18}H_6F_9NO_3$

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	$C_{13}H_7F_4NO_3$
24	isopropyl	100	CH_2Cl_2/hexanes	235–238	$C_{13}H_9F_4NO_3$
25	4-fluorophenyl	83	CH_2Cl_2/hexanes	268–271	$C_{16}H_6F_5NO_3$
26	pentafluorophenyl	60	acetone/hexanes	245–248	$C_{16}H_2F_9NO_3$

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: R_1 = H, R_2 = CH_3, R_3 = C_2H_5, X = N, R_4 = H
2: R_1 = N, R_2 = 1-piperazinyl, R_3 = C_2H_5, X = N, R_4 = H
3: R_1, R_2 = OCH_2O, R_3 = C_2H_5, X = CH, R_4 = H
4: R_1 = H, R_2 = 4-pyridyl, R_3 = C_2H_5, X = CH, R_4 = H
5: R_1 = F, R_2 = 1-piperazinyl, R_3 = cyclopropyl, X = CH, R_4 = H
6: R_1 = F, R_2 = 1-piperazinyl, R_3 = C_2H_5, X = CH, R_4 = H
7: R_1 = F, R_2 = 4-methyl-1-piperazinyl, X, R_3 = $COCH_2CH(CH_3)$, R_4 = H
8: R_1 = F, R_2 = 1-piperazinyl, R_3 = C_2H_5, X = N, R_4 = H
9: R_1 = F, R_2 = 4-methyl-1-piperazinyl, R_3 = C_2H_5, X = CH, R_4 = H
10: R_1 = F, R_2 = 4 methyl-1-piperazinyl, R_3 = C_2H_5, X = CF, R_4 = 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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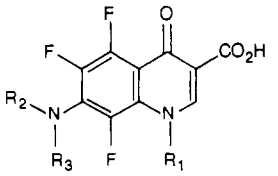
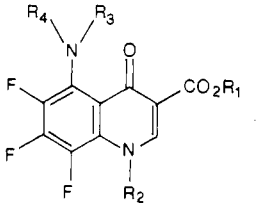
Table IV. 7-Amino-1-(substituted)-4-quinolonecarboxylic Acids



no.	R_1	R_2 R_3	% yield	recryst solvent	mp, °C	formula
27	C_2H_5	$-(CH_2)_2N[CH_3](CH_2)_2-$	53	acetone/hexanes	221–223	$C_{17}H_{18}F_3N_3O_3$[a]
28	C_2H_5	$-(CH_2)_2O(CH_2)_2-$	51	acetone	281–283	$C_{16}H_{15}F_3N_2O_4$
29	C_2H_5	$-CH_2(CH_2)_2CH_2-$	94	CH_2Cl_2/acetone	>320	$C_{16}H_{15}F_3N_2O_3$
30	C_2H_5	$-(CH_2)_2S(CH_2)_2-$	94	acetone	295–300	$C_{16}H_{15}F_3N_2O_3S$
31	isopropyl	$-(CH_2)_2N(CH_3)(CH_2)_2-$	90	CH_2Cl_2/hexanes	217–218	$C_{18}H_{20}F_3N_3O_3$
32	cyclopropyl	$-(CH_2)_2N(CH_3)(CH_2)_2-$	53	$CHCl_3$/hexanes	245	$C_{18}H_{18}F_3N_3O_3$
33	4-fluorophenyl	$-(CH_2)_2N[CH_3](CH_2)_2-$	85	acetone/hexanes	255–256	$C_{21}H_{17}F_4N_3O_3$

[a] HCl salt. Anal. C, H, F, N, Cl. Mp 271–273 °C.

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

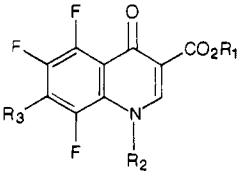


no.	R_1	R_2	R_3 R_4	% yield	recryst solvent	mp, °C	formula
34	C_2H_5	C_2H_5	$-(CH_2)_2N(CH_3)(CH_2)_2-$	51	CH_2Cl_2/hexanes	188–190	$C_{19}H_{22}F_3N_3O_3$
35	C_2H_5	C_2H_5	$-(CH_2)_2O(CH_2)_2-$	>95	CH_2Cl_2/hexanes	190–192	$C_{18}H_{19}F_3N_2O_4$
36	C_2H_5	C_2H_5	$-(CH_2)_2S(CH_2)_2-$	93	CH_2Cl_2/hexanes	185–187	$C_{18}H_{19}F_3SN_2O_3$
37	C_2H_5	C_2H_5	$-CH_2(CH_2)_2CH_2-$	5	hexanes	152–154	$C_{18}H_{19}F_3N_2O_3$
38	C_2H_5	cyclopropyl	$-(CH_2)_2N(CH_3)(CH_2)_2-$	49	CH_2Cl_2/hexanes	133–136	$C_{20}H_{22}F_3N_3O_3$
39	C_2H_5	4-fluorophenyl	$-(CH_2)_2N(CH_3)(CH_2)_2-$	74	acetone/hexanes	162–164	$C_{23}H_{21}F_4N_3O_3$
40	H	C_2H_5	$-(CH_2)_2O(CH_2)_2-$	74	H_2O	225–227	$C_{16}H_{15}F_3N_2O_4$
41[a]	H	C_2H_5	$-(CH_2)_2N(CH_3)(CH_2)_2-$	72	CH_2Cl_2/hexanes	212–214	$C_{17}H_{18}F_3N_3O_3$

[a] HCl salt. Anal. C, H, F, N, Cl, $0.3H_2O$. 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**.



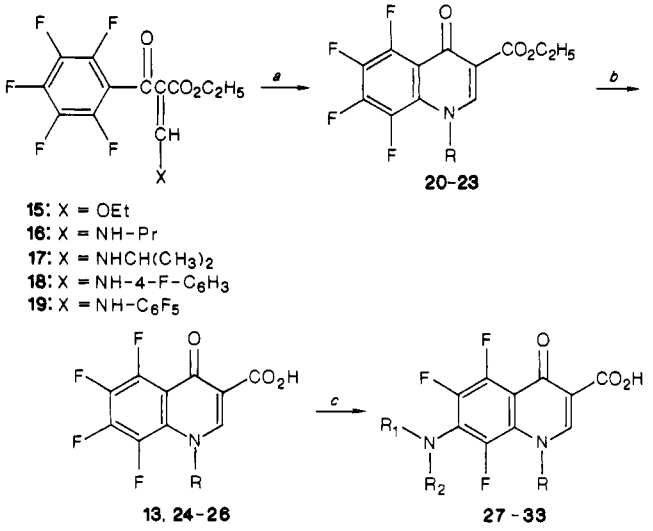
11: R_1, $R_2 = C_2H_5$, $R_3 = F$
12: $R_1 = H$, $R_2 = C_2H_5$, $R_3 = F$
13: $R_1 = H$, $R_2 = c\text{-}C_3H_5$, $R_3 = F$
14: $R_1 = H$, R_2 = alkyl and aryl, R_3 = substituted amino

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 CH_2Cl_2 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 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

Scheme I



15: X = OEt
16: X = NH-Pr
17: X = NHCH(CH$_3$)$_2$
18: X = NH-4-F-C$_6$H$_3$
19: X = NH-C$_6$F$_5$

20-23

13, 24-26

27-33

[a] K_2CO_3/DMF, 25 °C. [b] 2 N H_2SO_4. [c] NHR_1R_2/*N*-methyl-2-pyrrolidinone or -pyridine.

(12) Mich, T. F.; Domagala, J. M.; Nichols, J. B. U.S. Patent Appl. 4,657,913, April 14, 1987.

Table VI. In Vitro Antibacterial Activity[a,b] (Minimal Inhibitory Concentration (MIC), μg/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* VHG 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]

	C5-F			C6-F			C7-F			C8-F		
no.	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

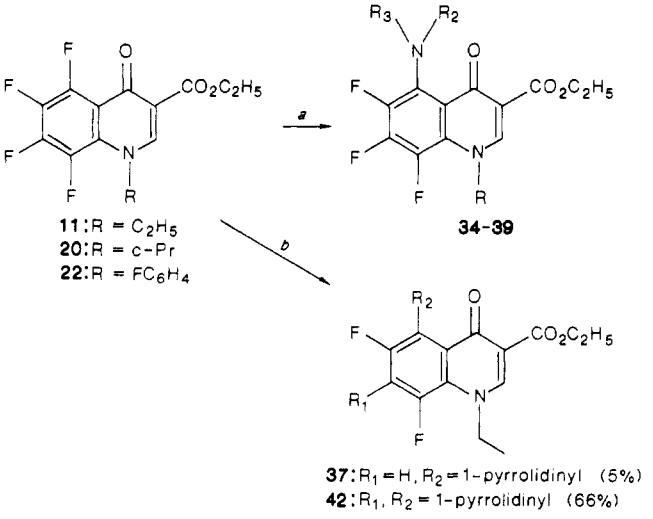
Fluorine-19 NMR was used to distinguish between 5- and 7-substituted quinolones. The chemical shifts, coupling constants, and multiplicities for selected 5- and 7-substituted quinolones are summarized in Table VII. The ^{19}F spectra of the 5-substituted quinolones were first order in all cases. The 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 ^{1}H–^{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-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

Scheme II



[a] NHR_2R_3/*N*-methyl-2-pyrrolidinone or -pyridine. [b] Excess pyrrolidine, 95 °C/pyridine.

(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 dimethylamino[15] probably will not favorably contribute to the in vitro biological activity of the quinolone.

(14) Crumplin, G. C.; Midgley, J. M.; Smith, J. T. *Topics in Antibiotic Chemistry*; Sammes, P. G., Ed.; Wiley and Sons: New York, 1980; Vol. 4, pp 1–38 and references cited therein.

(15) Domagala, J. M.; Hagen, S. E.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Sanchez, J. P.; Trehan, A. K. *J. Med. Chem.* **1988**, *31*, 506.

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

test organism	no.	administration route	ED_{50} (95% confidence limits), mg/kg	po/sc
E. coli #311	9	po[a]	1.1 (0.91–1.5)	2.75
		sc[b]	0.4 (0.3–0.5)	
	32	po	0.5 (0.4–0.7)	1.4
		sc	0.35 (0.27–0.45)	
S. aureus Smith	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 ED_{50}'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 ^{19}F 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-oxobenzenepropionate (18). A solution of **15**[12] (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 CH_2Cl_2–hexanes, mp 126–127 °C. NMR ($CDCl_3$): δ 1.17 (3 H, t, J = 7.1, CH_2CH_3), 4.13 (2 H, q, J = 7.3, CH_2CH_3), 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. ($C_{18}H_{11}F_6NO_3$) 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 H_2O and CH_2Cl_2. The organic layer was washed with cold H_2O, dried, and upon concentration yielded 12.2 g (96%) of **20**, mp 158–162 °C. A sample was crystallized from CH_2Cl_2–hexanes, mp 164–167 °C. NMR ($CDCl_3$): δ 1.17 (4 H, m, CH_2CH_2), 1.38 (3 H, t, J = 7.1, CH_2CH_3), 3.88 (1 H, m, NCH), 4.35 (2 H, q, J = 7.0, CH_2CH_3), 8.47 (1 H, s, C2-H). Anal. ($C_{15}H_{11}F_4NO_3$) C, H, F, N.

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 H_2O, 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 H_2SO_4 was stirred at 100 °C for 18 h. The cooled mixture was extracted with CH_2Cl_2 and the organic layer dried. Concentration afforded 2.12 g (100%) of **24**, mp 231–234 °C. Crystallization from CH_2Cl_2–hexanes yielded 1.5 g of white crystals, mp 235–238 °C. NMR ($CDCl_3$): δ 1.67 (6 H, dd, J_{HH} = 6.5, J_{HF} = 1.8, $CH(CH_3)_2$), 5.30 (1 H, m, J_{HH} = 6.7, J_{HF} = 3.0, $CH(CH_3)_2$), 8.86 (1 H, s, C2-H), 14.13 (1 H, s, COOH). Anal. ($C_{13}H_9F_4NO_3$) 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 **12**[12] (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 (Me_2SO-d_6): δ 1.41 (3 H, d of t, J_{HH} = 7.0, J_{HF} = 1.2, CH_2CH_3), 2.24 (3 H, s, NCH_3), 2.46 (4 H, br s, $CH_3N(CH_2)_2$), 3.39 (4 H, br s, $N(CH_2)_2$), 4.54 (2 H, m, NCH_2CH_3), 8.88 (1 H, s, C2-H). Anal. ($C_{17}H_{18}F_3N_3O_3$) 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 **11**[12] (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 CH_2Cl_2 and H_2O. Evaporation of the dried organic layer yielded 0.7 g (100%) of **35**, mp 188–191 °C. A sample was recrystallized from CH_2Cl_2–hexanes, mp 190–192 °C. NMR ($CDCl_3$): δ 1.38 (3 H, t, J = 7.1, $CO_2CH_2CH_3$), 1.48 (3 H, d of t, J_{HH} = 7.0, J_{HF} = 1.1, NCH_2CH_3), 3.27 (4 H, br s, $N(CH_2)_2$), 3.85 (4 H, br s, $O(CH_2)_2$), 4.27 (2 H, m, NCH_2CH_3), 4.36 (2 H, q, J = 7.1, $CO_2CH_2CH_3$), 8.18 (1 H, s, C2-H). Anal. ($C_{18}H_{19}F_3N_2O_4$) 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 H_2O and CH_2Cl_2. 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 ($CDCl_3$): δ 1.36 (3 H, t, J = 7.2, $CO_2CH_2CH_3$), 1.47 (3 H, d of t, J_{HH} = 7.1, J_{HF} = 1.1, NCH_2CH_3), 1.94 (4 H, m, $(CH_2)_2$), 3.48 (4 H, m, $N(CH_2)_2$), 4.23 (2 H, m, NCH_2CH_3), 4.36 (2 H, q, J = 7.2, $CO_2CH_2CH_3$), 8.11 (1 H, s, C2-H). Anal. ($C_{18}H_{19}F_3N_2O_3$) 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 ($CDCl_3$): δ 1.37 (3 H, t, J = 7.1, $CO_2CH_2CH_3$), 1.44 (3 H, d of t, J_{HH} = 7.1, J_{HF} = 1.2, NCH_2CH_3), 1.93 (8 H, m, $((CH_2)_2)_2$), 3.37 (4 H, m, $N(CH_2)_2$), 3.62 (4 H, m, $N(CH_2)_2$), 4.18 (2 H, m, NCH_2CH_3), 4.34 (2 H, q, J = 7.1, $CO_2CH_2CH_3$), 8.09 (1 H, s, C2-H). Anal. ($C_{22}H_{27}F_2N_3O_3$) 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 ($CDCl_3$): δ 1.41 (3 H, d of t, J_{HH} = 6.8, J_{HF} = 1.0, NCH_2CH_3), 3.22 (4 H, br s, $N(CH_2)_2$), 3.74 (4 H, br t, J = 3.7, $O(CH_2)_2$), 4.53 (2 H, m, NCH_2CH_3), 8.90 (1 H, s, C2-H). Anal. ($C_{16}H_{15}F_3N_2O_4$) 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 $CHCl_3$ 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 (Me_2SO-d_6): δ 1.38 (3 H, t, NCH_2CH_3), 1.91 (8 H, m, $2((CH_2)_2)$), 3.30 (4 H, m, $N(CH_2)_2$), 3.64 (4 H, m, $N(CH_2)_2$), 4.40 (2 H, m, NCH_2CH_3), 8.66 (1 H, s, C2-H). HRMS: calcd for $C_{20}H_{23}F_2N_3O_3$ 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 mm. 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) × 10^4 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 (ED_{50}) 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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