H), 2.60 (m, 4 H), 3.16 (m, 4 H), 3.60 (s, 2 H), 4.41 (q, J = 7.0 Hz, 2 H), 4.84 (d, J = 6.0 Hz, 2 H), 7.17–7.40 (m, 11 H), 7.26 (t, J = 6.0 Hz, 1 H), 8.32 (s, 1 H), 8.34 (d, J = 13 Hz, 1 H). Anal. ($C_{30}H_{31}FN_6$) C, H, N.

1-Amino-5-ethyl-8-fluoro-7-(1-piperazinyl)-5*H*-pyrimido[4,5-*b*]indole (19). A solution of 18c (0.258 g, 0.52 mmol) in a mixture of EtOH (100 mL) and 1 N HCl (10 mL) was hydrogenated over 5% Pd/C (0.1 g) and PdO (0.1 g) at 78 °C for 4 h. After filtration, the pH was adjusted to 9 with 1 N NaOH and the solution concentrated in vacuo. The colorless solid was triturated with water (50 mL) and filtered, yielding 50 mg (31%) of 19: mp 219–221 °C; NMR (DMSO-d_6) δ 1.28 (t, J = 7.0 Hz, 3 H), 2.90 (m, 4 H), 3.00 (m, 4 H), 4.39 (q, J = 7.0 Hz, 2 H), 7.13 (s, 2 H), 7.20 (d, J = 7.0 Hz, 1 H), 8.21 (d, J = 13 Hz, 1 H), 8.24 (s, 1 H); MS m/e 314 (M^+).

7. Enzyme Inhibition. The compounds synthesized were evaluated in a DNA supercoiling assay.[13] Equimolar quantities of each DNA gyrase subunit[14,15] were reconstituted to the A_2B_2 complex by incubation for 30 min at 25 °C. Relaxed pUC18 DNA (7.5 nM) was incubated with DNA gyrase (3.85 nM) in 35 mM Tris-HCl (pH 7.5), 24 mM KCl, 4 mM $MgCl_2$, 1.8 mM spermidine, 9 μg/mL tRNA, 5 mM DTT, 1.4 mM ATP, 100 μg/mL BSA and the compound tested at concentrations between 0.1 and 100 μg/mL at 37 °C for 30 min. Samples were electrophoresed on a 0.8% agarose gel and the inhibiting activity of the compound tested was expressed as MNEC (maximal noneffective concentration), i.e. the highest inhibitor concentration at which all DNA is still completely supercoiled.

8. In Vitro Antibacterial Activity. The in vitro antibacterial activity of the compounds was determined in a side-by-side comparison with fleroxacin by standard agar dilution method on DST agar (Oxoid). The compounds were incorporated into a melted medium of 50 °C just prior to the pouring and use of the plates. The inoculum of approximately 10^4 colony-forming units (CFU) was prepared from appropriately diluted overnight cultures and applied to the agar surface with a multipoint inoculating device (Denley A400 multipoint inoculator). The lowest concentration of the drug that prevented the macroscopic growth of a culture after 18 h of incubation at 35 °C was recorded as minimal inhibitory concentration (MIC).

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Registry No. **5**, 106847-36-3; **6**, 137858-20-9; **7**, 578-28-9; **8a**, 137858-22-1; **8b**, 139495-69-5; **8c**, 137858-40-3; **8d**, 137858-25-4; **9a**, 139495-70-8; **9b**, 139495-71-9; **9c**, 139495-72-0; **9d**, 139495-73-1; **10**, 137858-23-2; **10a**, 139495-74-2; **11a**, 139495-75-3; **11b**, 137858-43-6; **11c**, 137858-11-8; **11d**, 139495-76-4; **11e**, 137858-28-7; **11f**, 137858-17-4; **11g**, 137858-46-9; **11h**, 137858-12-9; **11i**, 139495-77-5; **11j**, 139495-78-6; **11k**, 137882-03-2; **11l**, 139495-79-7; **11m**, 139495-80-0; **11n**, 137858-31-2; **11o**, 137858-32-3; **11p**, 137858-18-5; **11q**, 137882-07-6; **11r**, 137858-14-1; **11s**, 137858-51-6; **11t**, 137858-15-2; **11u**, 137858-54-9; **11v**, 137858-16-3; **12a**, 139495-81-1; **12b**, 137858-41-4; **12c**, 137858-26-5; **13a**, 139495-82-2; **13b**, 137858-42-5; **13c**, 137858-55-0; **13d**, 137858-45-8; **13e**, 139495-83-3; **13f**, 139495-84-4; **13g**, 137858-30-1; **13h**, 137858-49-2; **13i**, 139495-85-5; **13j**, 137858-52-7; **13k**, 137858-53-8;)14b, 139495-86-6; **14c**, 139495-87-7; **15a**, 327-92-4; **15b**, 123344-02-5; **16**, 139495-88-8; **17a**, 139495-89-9; **17b**, 139495-90-2; **17c**, 139495-91-3; **18a**, 139495-92-4; **18b**, 139495-93-5; **18c**, 139495-94-6; **19**, 139495-95-7; $CH_2(CO_2Et)_2$, 105-53-3; $HN{=}C(OEt)CH_2CO_2Et$, 27317-59-5; $CH_2(CN)CONH_2$, 107-91-5; $BzN(CH_2CH_2Cl)_2$, 55-51-6; 3′-chloro-*N*-ethyl-4′-fluoroacetanilide, 137858-19-6; *N*-methylpiperazine, 109-01-3; *N*-ethyl-4′-fluoro-5′-(4-methyl-1-piperazinyl)-2′-nitroacetanilide, 137858-21-0; cyclopropylamine, 765-30-0; 5-chloro-*N*-cyclopropyl-2-nitro-4-fluoroaniline, 135861-04-0; *N*-acetylpiperazine, 13889-98-0; 1-acetyl-4-[5-(cyclopropylamino)-2-fluoro-4-nitrophenyl]piperazine, 137858-24-3; 4-[5-(cyclopropylamino)-2-fluoro-4-nitrophenyl]piperazine, 135861-05-1; *O*-benzylhydroxylamine, 622-33-3.

Preparation and in Vitro and in Vivo Evaluation of Quinolones with Selective Activity against Gram-Positive Organisms

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A series of quinolones were prepared which contained oximes or substituted oximes as replacements for the amine substituents normally found on the pyrrolidine or piperidine fragments of quinolone antibacterial agents. These substituents led to compounds that had selective activity against Gram-positive organisms. These compounds showed in vivo activity against *Staphylococcus aureus*. Only compound **29** had in vivo activity against *Streptococcus pneumoniae*.

Introduction

The topoisomerases are a group of enzymes that control the linking number of double-stranded DNA molecules, and they are divided into three groups. The enzyme DNA gyrase is a member of the topoisomerase II group.[1] The inhibition of bacterial DNA gyrase has been the target of a worldwide research effort which began with the discovery of nalidixic acid (**1**) in the early 1960s.[2] Structure–activity relationships (SAR) of compounds based on nalidixic acid have led to a large group of synthetic antibacterial agents known collectively as the quinolones.[3] The compounds

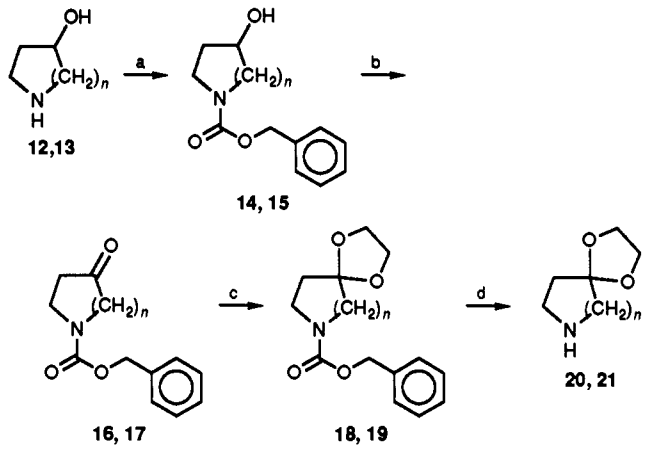
(1) (a) Wang, J. C. DNA Topoisomerases. *Annu. Rev. Biochem.* **1985**, *54*, 655–697. (b) Gellert, M.; Mizuuchi, K.; O'Dea, M. H.; Nash, H. A. DNA Gyrase: An Enzyme That Introduces Superhelical Turns into DNA. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3872–3876.

(2) Lesher, G. Y.; Froelich, E. J.; Gruett, M. D.; Bailey, J. H.; Brundage, P. R. 1,8-Naphthyridine Derivatives: A New Class of Chemotherapeutic Agents. *J. Med. Chem.* **1962**, *5*, 1063–1068.

(3) (a) Albrecht, R. Development of Antibacterial Agents of the Nalidixic Acid Type. *Prog. Drug Res.* **1977**, *21*, 9–104. (b) Chu, D. T. W.; Fernandes, P. B. Structure-Activity Relationships of the Fluoroquinolones. *Antimicrob. Agents Chemother.* **1989**, *33*, 131–135.

0022-2623/92/1835-1392$03.00/0

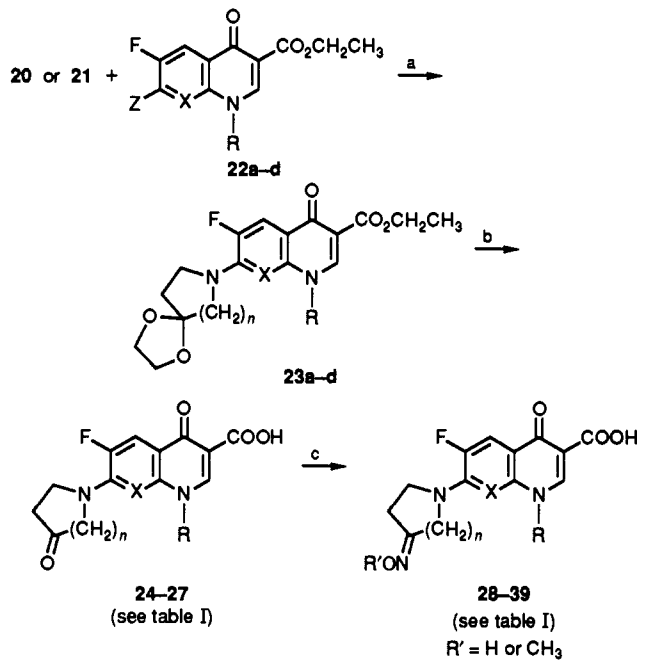
Scheme I[a]



[a] Reagents: (a) CBZ-Cl, NaOH, Et_2O, 0 °C; (b) pyridine–SO_3, DMSO, Et_3N 0 °C; (c) $(CH_2OH)_2$, toluene, p-TSA, 130 °C; (d) 20% Pd/C, CH_3OH, 4 atm H_2, 25 °C.

prepared early in this effort such as oxolinic acid (**2**),[4a] rosoxacin (**3**),[4b] and pipemidic acid (**4**)[4c] were most active against Gram-negative bacteria. Introduction of a fluorine atom at the 6-position of the quinolone ring system led to norfloxacin (**5**)[5a] which had broad-spectrum antibacterial activity. Newer members of this family include ciprofloxacin (**6**),[5b] ofloxacin (**7**),[5c] enoxacin (**8**),[5d] lomefloxacin (**9**),[5e] fleroxacin (**10**),[5f] and temafloxacin (**11**).[5g] As part of an ongoing effort to enhance the spectrum of activity of quinolone antibacterial agents we have investigated mod-

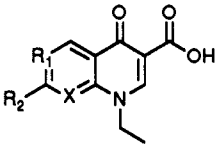
Scheme II[a]



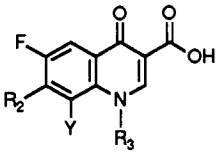
(a) X = CH, Z = F, R = 2,4-difluorophenyl
(b) X = N, Z = Cl, R = 2,4-difluorophenyl
(c) X = CF, Z = F, R = cyclopropyl
(d) X = N, Z = Cl, R = cyclopropyl

[a] Reagents: (a) pyridine; 25 °C for X = N, Z = Cl; 45 °C for X = CH, Z = F; (b) 3 M HCl, 70 °C, 24–48 h; (c) THF/EtOH, $R'ONH_2$·HCl, $NaHCO_3$.

ifications of the 7-position substituent. These investigations led to a series of compounds with selective in vitro activity against Gram-positive organisms. This series involved replacement of the amine substituent on pyrrolidine and piperidine rings with oximes and substituted oximes. We wish to report herein the preparation of these compounds along with their in vitro and in vivo antibacterial activity.[6]



1: R_1 = CH, R_2 = CH_3, X = N
2: R_1,R_2 = C—OCH_2O, X = CH
3: R_1 = CH, R_2 = 4-pyridyl, X = CH
4: R_1 = N, R_2 = 1-piperazinyl, X = N
8: R_1 = CF, R_2 = 1-piperazinyl, X = N



5: Y = H, R_2 = 1-piperazinyl, R_3 = CH_2CH_3
6: Y = H, R_2 = 1-piperazinyl, R_3 = cyclopropyl
7: Y,R_3 = OCH_2CHCH_3, R_2 = 4-methyl-1-piperazinyl
9: Y = F, R_2 = 3-methyl-1-piperazinyl, R_2 = CH_2CH_3
10: Y = F, R_2 = 4-methyl-1-piperazinyl, R_3 = CH_2CH_2F
11: Y = H, R_2 = 3-methyl-1-piperazinyl, R_3 = 2,4-difluorophenyl

Chemistry

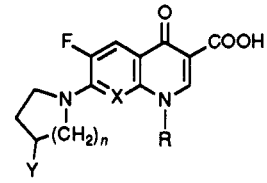
The general procedures for the preparation of the quinolone and naphthyridine ring skeletons such as **22a–d**

(4) (a) Turner, F. J.; Ringel, S. M.; Martin, J. F.; Storino, P. J.; Daly, J. M.; Schwartz, B. S. Oxolinic Acid, A New Synthetic Antibacterial Agent. *Antimicrob. Agents Chemother.* **1967**, 475–479. (b) Lesher, G. Y.; Carabates, P. M. 1,4-Dihydro-4-oxo-7-pyridyl-3-quinolinecarboxylic Acid Derivatives. U.S. Patent 3,907,809, 1975. (c) Pesson, M.; Antoine, M.; Chabassier, S.; Geiger, S.; Girard, P.; Richer, D.; deLaJudie, P.; Horvath, E.; Leriche, B.; Patte, S. Antibacterial Derivatives of 8-Alkyl-5-oxo-5,8-dihydropyrido[2,3-d]pyrimidine-6-carboxylic Acids. I. New Procedure of Preparation. *Eur. J. Med. Chem.* **1974**, *9*, 585–590.

(5) (a) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. Structure–Activity Relationships of Antibacterial 6,7- and 7,8-Disubstituted 1-Alkyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acids. *J. Med. Chem.* **1980**, *23*, 1358–1363. (b) Wise, R.; Andrews, J. M.; Edwards, L. J. In Vitro Activity of BAY 09867, A New Quinoline Derivative, Compared with Those of Other Antimicrobial Agents. *Antimicrob. Agents Chemother.* **1983**, *23*, 559–564. (c) Sato, K.; Matsuura, Y.; Inoue, M.; Une, T.; Osada, Y.; Ogawa, H.; Mitsuhaski, S. In Vitro and In Vivo Activity of DL-8280, A New Oxazine Derivative. *Antimicrob. Agents Chemother.* **1982**, *22*, 548–553. (d) Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H. Pyridonecarboxylic Acids as Antibacterial Agents. 2. Synthesis and Structure–Activity Relationships of 1,6,7-Trisubstituted 1,4-Dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acids, Including Enoxacin, A New Antibacterial Agent. *J. Med. Chem.* **1984**, *27*, 292–301. (e) Hirose, T.; Okezaki, E.; Kato, H.; Ito, Y.; Inoue, M.; Mitsuhashi, S. In Vitro and In Vivo Activity of NY-198, A New Difluorinated Quinolone. *Antimicrob. Agents Chemother.* **1987**, *31*, 854–859. (f) Hirai, K.; Aoyama, H.; Hosaka, M.; Oomori, Y.; Niwata, Y.; Suzue, S.; Irikura, T. In Vitro and In Vivo Antibacterial Activity of AM-833, A New Quinolone Derivative. *Antimicrob. Agents Chemother.* **1986**, *29*, 1059–1066. (g) Hardy, D. J.; Swanson, R. N.; Hensey, D. M.; Ramer, N. R.; Bower, R. R.; Hanson, C. W.; Chu, D. T. W.; Fernandes, P. B. Comparative Antibacterial Activities of Temafloxacin Hydrochloride (A-62254) and Two Reference Fluoroquinolones. *Antimicrob. Agents Chemother.* **1987**, *31*, 1768–1774.

(6) Some similar compounds and some hydroxylamine derivatives have been disclosed in a Japanese patent JP 0110155 (Apr 18, 1989).

Table I. 7-Substituted Quinolones



compound	n	R	X	Y	yield %	mp, °C[a]	formula[b]
24	1	o,p-difluorophenyl	CH	=O	82	245 dec	$C_{20}H_{13}F_3N_2O_4 \cdot {}^1/_2H_2O$
25	2	o,p-difluorophenyl	N	=O	89	>300	$C_{20}H_{14}F_3N_3O_4$
26	1	cyclopropyl	CH	=O	80	272–273	$C_{17}H_{15}FN_2O_4$
27	1	cyclopropyl	CF	=O	70	237–240	$C_{17}H_{14}F_2N_2O_4 \cdot {}^1/_4H_2O$
28	1	o,p-difluorophenyl	CH	=NOH	66	214–216	$C_{20}H_{14}F_3N_3O_4 \cdot {}^1/_2H_2O$
29	2	o,p-difluorophenyl	CH	=NOH	70	256–257	$C_{21}H_{16}F_3N_3O_4$
30	1	o,p-difluorophenyl	CH	$=NOCH_3$	35	>300	$C_{21}H_{16}F_3N_3O_4 \cdot 2H_2O$[c]
31	2	o,p-difluorophenyl	CH	$=NOCH_3$	62	238–240	$C_{22}H_{16}F_3N_3O_4 \cdot 1.5EtOH$
32	2	o,p-difluorophenyl	N	=NOH	86	>300	$C_{20}H_{15}F_3N_4O_4 \cdot 2H_2O$[d]
33	1	o,p-difluorophenyl	N	$=NOCH_3$	38	237–238	$C_{20}H_{15}F_3N_4O_4$
34	2	o,p-difluorophenyl	N	$=NOCH_3$	94	253–254	$C_{21}H_{17}F_3N_4O_4 \cdot {}^1/_2H_2O$
35	1	cyclopropyl	CF	=NOH	79	242–244	$C_{17}H_{15}F_2N_3O_4 \cdot CH_3OH$
36	2	cyclopropyl	CF	$=NOCH_3$	67	>300	$C_{19}H_{19}F_2N_3O_4 \cdot 3.5H_2O$[e]
37	2	cyclopropyl	CF	=NOH	70	241–243	$C_{18}H_{17}F_2N_3O_4 \cdot {}^1/_2EtOH$
38	1	cyclopropyl	N	$=NOCH_3$	35	258–260	$C_{17}H_{17}FN_4O_4 \cdot {}^1/_2H_2O$
39	1	cyclopropyl	N	=NOH	70	257–258	$C_{16}H_{15}FN_4O_4 \cdot {}^1/_4H_2O$
40	2	o,p-difluorophenyl	CH	$-NH_2$[f]			
41	1	o,p-difluorophenyl	N	$-NH_2$[f]			
42	1	cyclopropyl	N	$-NH_2$[f]			

[a] Melting points are uncorrected. [b] All compounds were analyzed for (C, H, N) and gave the analyses indicated within ±0.4% of the theoretical values unless otherwise noted. [c] N: calcd 8.99, found, 9.77; H calcd 4.31, found 3.64. [d] H: calcd 3.11, found 4.09. [e] H: calcd 5.76, found 4.03. [f] Synthesis of these compounds has been reported see ref 7.

have been described previously.[7] The pyrrolidine and piperidine substituents were prepared by the synthetic route outlined in Scheme I. This same reaction sequence was used to access the pyrrolidine and piperidine congeners. The amino group of the readily available amino alcohols **12** or **13** was protected as a benzyloxycarbonyl (Cbz) derivative, which was prepared by reaction of the amine with benzyl chloroformate in a two-phase system at 0 °C giving **14** or **15**.[8] The alcohol functionality of **14** or **15** was then oxidized to the ketone with pyridine–sulfur trioxide complex[9] giving **16** or **17**; these intermediates were purified by bulb-to-bulb distillation. The ketones were then protected by standard procedures giving the 1,3-dioxolanes **18** or **19**.[10] The Cbz protecting group was then removed by reaction with 20% Pd/C in methanol under 4 atm of hydrogen at room temperature giving **20** or **21**.[8]

The reactions depicted in Scheme II summarize the final preparation of the oxime compounds. The deprotected amine **20** or **21** was reacted with the quinolone ester (where Z = F, **22a,c**) in pyridine at 45 °C to give the displacement product **23a,c**. In the naphthyridine series **22b,d** where (Z = Cl), the displacements with **20** or **21** were carried out at room temperature to give **23b,d**. The displacement products were then recrystallized, and the ketal and ester protecting groups were simultaneously removed by hydrolysis in 3 M HCl. Reaction of the ketone groups with the appropriate hydroxylamine derivative afforded the oxime or methyl oximes **28–39** as final products.

The physical properties of compounds **24–39** and the structures of their substituents are summarized in Table I.

Results and Discussion

Compounds **24–42** were evaluated for their in vitro antibacterial activity against a variety of Gram-positive and Gram-negative bacteria. These activities were determined by conventional agar dilution procedures, and the results of these assays are summarized in Table II. Data for nine Gram-positive bacteria and six Gram-negative bacteria as representative examples are reported in the table. The data for ciprofloxacin (**6**) are included for comparison.

The ring size and functional group array of the substituent at the 7-position of the quinolone or naphthyridine ring system is known to have a strong influence on the spectrum and extent of in vitro antibacterial activity. In this study, the 7-position substituents consisted of piperidine and pyrrolidine rings which contained ketone, oxime, and methyloxime groups. When the conventional amino substituents attached to pyrrolidine or piperidine rings of quinolones were replaced with oximes or methyloximes, compounds were obtained which showed enhanced biological activity against Gram-positive organisms. This selectivity is most evident against the Staphylococcus organisms. The activity difference against the Streptococcus organisms was less pronounced. The oxime **39** was more active than the amine **42**, while the oxime **29** and the methyloxime **33** were about equipotent to the amines **40** and **41**. At the same time there was a large decrease in biological activity of all of the oximes against the Gram-negative organisms when compared to the corresponding

(7) (a) Chu, D. T. W.; Fernandes, P. B.; Claiborne, A.; Pihuleac, E.; Nordeen, C.; Maleczka, R.; Pernet, A. Synthesis and Structure–Activity Relationships of Novel Arylfluoroquinolone Antibacterial Agents. *J. Med. Chem.* **1985**, *28*, 1558–1564. (b) Chu, D. T. W.; Fernandes, P. B.; Claiborne, A.; Gracey, E.; Pernet, A. Synthesis and Structure–Activity Relationships of New Arylfluoronaphthyridine Antibacterial Agents. *J. Med. Chem.* **1986**, *29*, 2363–2369. (c) Sanchez, J. P.; Domagala, J. M.; Hagen, S. E.; Heifetz, C. L.; Hutt, M. P.; Nichols, J. B.; Trehan, A. K. Quinolone Antibacterial Agents. Synthesis and Structure–Activity Relationships of 8-Substituted Quinoline-3-carboxylic Acids and 1,8-Naphthyridine-3-carboxylic Acids. *J. Med. Chem.* **1988**, *31*, 983–991.

(8) Bergmann, M.; Zervas, L. *Chem. Ber.* **1932**, *65*, 1192–1201.

(9) Parika, J.; Doering, W. Sulfur Trioxide in the Oxidation of Alcohols by Dimethyl Sulfoxide. *J. Am. Chem. Soc.* **1967**, *89*, 5505–5507.

(10) Daignault, R.; Eliel, E. 2-Cyclohexyloxyethanol. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. 5, 303–306.

Table II. In Vitro Antibacterial Activities[a]

organism[a]	MIC (μg/mL)[c]																		
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	6[d]	40	41	42
S. aureus ATCC 6538P	0.39	0.05	1.56	0.78	<0.05	0.05	0.02	0.05	0.05	0.05	0.1	<0.005	0.05	0.01	0.02	0.02	0.1	0.1	0.02	0.1
S. aureus CMX 553	1.56	0.1	3.1	0.39	<0.05	0.1	0.05	0.2	0.2	0.1	0.39	0.02	0.1	0.05	0.05	0.05	0.2	0.39	0.05	0.1
S. aureus A5177	1.56	0.1	3.1	0.78	<0.05	0.1	0.05	0.1	0.1	0.1	0.2	0.01	0.1	0.02	0.05	0.05	0.39	0.39	0.05	0.2
S. aureus NCTC 10649	0.39	0.1	1.56	0.39	<0.05	0.1	0.05	0.05	0.1	0.05	0.2	0.01	0.05	0.02	0.02	0.05	0.39	–	–	–
S. epidermidis 3519	1.56	0.2	3.1	1.56	<0.05	0.1	0.05	0.1	0.2	0.1	0.39	0.02	0.1	0.02	0.1	0.05	0.39	0.39	0.1	0.1
Ent. faecium ATCC 8043	25	1.56	>100	6.2	0.2	0.39	0.39	3.1	1.56	0.39	3.1	0.1	1.56	0.2	0.78	0.39	0.39	0.78	0.2	0.39
St. bovis A5169	>100	3.1	>100	50	0.39	1.56	1.56	6.2	3.1	1.56	6.2	0.2	3.1	0.39	1.56	0.78	1.56	1.56	0.39	0.78
St. agalactiae CMX 508	12.5	1.56	100	12.5	0.2	0.39	0.39	1.56	0.78	0.39	1.56	0.1	1.56	0.2	0.78	0.2	0.39	0.78	0.39	0.39
St. pyogenes 930	25	1.56	>100	25	0.2	0.39	0.39	1.56	1.56	0.78	3.1	0.1	1.56	0.1	0.78	0.39	0.39	0.39	0.2	0.39
E. coli Juhl	12.5	0.39	6.2	0.39	0.2	0.39	0.78	0.2	0.78	0.78	6.2	0.02	1.56	0.2	0.39	0.02	0.02	0.1	0.02	0.01
Ent. aerogenes ATCC 13048	12.5	3.1	12.5	1.56	0.78	3.1	3.1	>100	3.1	3.1	12.5	0.39	>100	0.39	0.78	0.2	0.05	0.39	0.05	0.02
K. pneumoniae 8045	6.2	0.39	6.2	0.39	0.2	0.39	0.78	3.1	0.78	0.78	3.1	0.05	3.1	0.1	0.1	0.05	0.02	0.1	0.02	0.01
P. aeruginosa A5007	>100	12.5	>100	50	3.1	6.2	6.2	>100	12.5	6.2	50	0.78	>100	3.1	3.1	>100	0.2	0.78	0.78	0.1
P. aeruginosa K799/WT	50	3.1	>100	50	1.56	3.1	6.2	>100	6.2	6.2	25	0.78	>100	1.56	1.56	0.78	0.2	0.78	0.78	0.1
Acinetobacter CMX 669	12.5	1.56	50	3.1	0.39	1.56	0.78	6.2	3.1	1.56	12.5	0.2	3.1	0.39	0.39	0.2	0.78	0.78	0.1	0.1

[a] Microorganisms: *S. aureus*, *Staphylococcus aureus*; *S. epidermidis*, *Staphylococcus epidermidis*; *Ent. faecium*, *Enterococcus faecium*; *St. bovis*, *Streptococcus bovis*; *St. agalactiae*, *Streptococcus agalactiae*; *St. pyogenes*, *Streptococcus pyogenes*; *E. coli*, *Escherichia coli*; *Ent. aerogenes*, *Enterobacter aerogenes*; *K. Pneumoniae*, *Klebsiella pneumoniae*; *P. aeruginosa*, *Pseudomonas aeruginosa*. [b] Structures are shown in Table I. [c] The MIC (Minimum Inhibitory Concentration) values were determined by the usual 2-fold agar dilution method using brain–heart infusion agar. [d] Ciprofloxacin was the reference standard.

Table III. In Vivo Activity of Quinolones against *Staphylococcus aureus* NCTC 10649

compd	admin route	ED_{50}, mg/kg per day	95% CF lim
6	po	15.5	9.9–24.1
	sc	1.6	1.0–2.5
28	po	>12.0	–
	sc	4.1	2.4–7.0
29	po	6.0	3.8–9.5
	sc	3.2	2.0–5.0
31	po	>12.0	–
	sc	>8.0	–
32	po	>8.0	–
	sc	>2.0	–
33	po	>12.0	–
	sc	4.7	1.6–13.6
34	po	>12.0	–
	sc	>8.0	–
35	po	3.0	2.0–4.5
	sc	1.5	1.0–2.2
37	po	3.0	2.0–4.5
	sc	6.7	4.3–10.4
39	po	>12.0	–
	sc	>8.0	–

amines. Thus, the oximes and methyloxime-containing quinolones exhibit selective in vitro activity towards Gram-positive organisms.

The same selectivity was not seen with the ketones. Ketones **24–27** showed a pronounced decrease in activity against the Streptococcus organisms as well as against the Gram-negative organisms such as *Escherichia coli* and *Pseudomonas aeruginosa*. These compounds were less active than the corresponding oximes **28** and **35** against the Staphylococcus organisms. Ketones **24** and **25** were less potent than the corresponding amines **40** and **41**, especially against Gram-negative organisms.

In general, the oximes, e.g. **28**, **29**, and **32**, were more active than the methyloximes, e.g. **30**, **31**, and **33**. This difference was most pronounced against the *P. aeruginosa*. The pyrrolidine-containing oximes, e.g. **28** and **35**, were about 2-fold more active than the corresponding piperidine analogues, e.g. **29** and **37**. There was little difference in the spectrum of activity between the corresponding quinolones and naphthyridines; **32** was about equipotent to **29**, and **33** was about equipotent to **30**.

Mouse protection tests were used to evaluate the in vivo activity of the quinolones, with the compounds being administered either orally (po) or subcutaneously (sc). The activity of these compounds against *Staphylococcus aureus* is shown in Table III. The same compounds were also tested against *Streptococcus pneumoniae* in vivo. Only compound **29** had activity against this organism with an oral ED_{50} of 16.3 mg/kg per day with a 95% confidence limit of 7.3–36.3 mg/kg per day. All the other compounds had oral $ED_{50} > 70$ mg/kg per day and subcutaneous $ED_{50} > 50$ mg/kg per day against *S. pneumoniae*. Compound **35** was the most active compound in vivo against *S. aureus*. None of the compounds had any in vivo activity against *P. aeruginosa* A5007 at the dosages tested. At oral doses of up to 50 mg/kg per day and subcutaneous doses up to 25 mg/kg per day, no activity was seen in the mouse protection tests for any of the oximes.

The in vivo activity of the oximes shows the same type of selectivity as the in vitro activity, with the greatest activity seen against the Gram-positive *S. aureus* and the least activity seen against the Gram-negative *P. aeruginosa*. The oximes **29** and **32** were more active in vivo than the corresponding methyl oximes **31** and **34**. Compound **29**, which was the only compound with activity against *S. pneumoniae*, also had good in vivo activity against *S.*

aureus. The oximes were generally more active subcutaneously than orally against *S. aureus*. The most active oxime in vitro, **35**, was also the most active compound in vivo. Many of the oximes had excellent in vitro profiles which were selective against Gram-positive organisms. This selectivity was also observed in vivo, but the in vivo activity was not as great as was anticipated from the in vitro results.

Experimental Section

Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Elemental analyses were obtained for all new compounds reported. Carbon, hydrogen, and nitrogen analyses (unless otherwise specified) were within ±0.4% of the theoretical values. Microanalyses were performed by the Abbott Analytical Department. The NMR spectra were obtained on a Varian EM-360 or a General Electric QE-300 spectrometer. Resonances are reported downfield relative to tetramethylsilane as internal standard. Mass spectra were recorded on a Nermag R30-10 or Hewlett-Packard 5985A mass spectrometer in the chemical ionization mode with ammonia as the reagent gas. The IR spectra were recorded on a Nicolet 60 SX FT infrared spectrometer. Thin-layer chromatographic analysis (TLC) was performed on 0.25-mm silica gel G PF-254 glass plates. Column chromatography was performed on Merck 70–230-mesh silica gel.

***N*-(Benzyloxycarbonyl)-3-pyrrolidinol (14).** The amine **12** (13.2 g, 151.9 mmol) in 50 mL of ether and 182 mL of 1 M NaOH was cooled in an ice bath and was stirred vigorously. A solution of 21.7 mL (151.9 mmol) of benzyl chloroformate in 50 mL of ether was added dropwise with stirring. The reaction mixture was stirred in the ice bath for 3 h. The reaction mixture was then diluted with water, and the product was extracted into ether (3 × 150 mL). The organic layer was dried over Na_2SO_4 and filtered, and the solvent was removed on a rotary evaporator giving 31.6 g (94%) of **14** as a yellow liquid: NMR ($CDCl_3$) δ 1.95 (m, 2 H), 2.21 (dd, 1 H, J = 22, 3 Hz), 3.51 (m, 4 H), 4.45 (m, 1 H), 5.14 (s, 2 H), 7.37 (m, 5 H); mass spectrum, m/z 239 $(M + NH_4)^+$, 222 $(M + H)^+$.

***N*-(Benzyloxycarbonyl)-4-piperidinol (15).** The amine **13** (35.4 g, 350 mmol) was dissolved in 50 mL of ether and 420 mL of 1 M NaOH. The reaction mixture was cooled in an ice bath and 50 mL (350 mmol) of benzyl chloroformate in 100 mL of ether was added dropwise with stirring. The reaction mixture was stirred at ice-bath temperature for 3 h. The reaction mixture was diluted with 200 mL of water and was extracted with ether (3 × 200 mL). The combined organic layers were dried over Na_2SO_4 and filtered, and the solvent was removed on a rotary evaporator giving **15** as a yellow oil: NMR ($CDCl_3$) δ 1.49 (m, 2 H), 1.71 (s, 1 H), 1.87 (m, 2 H), 3.88 (m, 2 H), 3.92 (m, 1 H), 3.15 (ddd, 2 H, J = 3, 3, 9 Hz), 5.12 (s, 2 H), 7.36 (m, 5 H).

***N*-(Benzyloxycarbonyl)-3-pyrrolidinone (16).** The alcohol **14** (31.6 g, 142.8 mmol) was dissolved in 525 mL of DMSO and was cooled in an ice bath. To this was added 159.2 mL (1.14 mol) of Et_3N and 68.2 g (428.5 mmol) of pyridine–sulfur trioxide complex. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 16 h. The reaction mixture was poured into 1 L of water and was extracted with CH_2Cl_2 (3 × 300 mL). The combined organic layers were dried over Na_2SO_4 and filtered, and the solvent was removed on a rotary evaporator. The product **16** was used in the next step without purification: NMR ($CDCl_3$) δ 2.61 (2 H, J = 7.5 Hz), 3.85 (dd, 2 H, J = 7.5 Hz), 3.82 (s, 2 H), 5.18 (s, 2 H), 7.37 (m, 5 H); mass spectrum m/z 237 $(M + NH_4)^+$, 220 $(M + H)^+$.

***N*-(Benzyloxycarbonyl)-4-piperidone (17).** The alcohol **15** was oxidized in a procedure identical to that for the preparation of ketone **16**. The product was purified by Kugelrohr distillation (130 °C/4 Torr) giving 11.0 g (91%) of **17** as a clear colorless oil: NMR ($CDCl_3$) δ 2.45 (dd, 4 H, J = 6 Hz), 3.81 (dd, 4 H, J = 6 Hz), 5.18 (s, 2 H), 7.37 (m, 5 H); mass spectrum, m/z 251 $(M + NH_4)^+$, 234 $(M + H)^+$.

***N*-(Benzyloxycarbonyl)-7-aza-1,4-dioxaspiro[4.4]nonane (18).** The ketone **16** (30.6 g, 289.8 mmol) was dissolved in 600 mL of toluene. To this was added 40.4 mL (724.4 mmol) of ethylene glycol and 2.76 g (14.5 mmol) of *p*-toluenesulfonic acid. The reaction mixture was heated at 130 °C for 4 days. After cooling to room temperature, the reaction mixture was poured into 500 mL of 5% $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (2 × 300 mL). The combined organic layers were washed with water (2 × 500 mL). The organic layer was dried over Na_2SO_4 and filtered, and the solvent was removed on a rotary evaporator. The product was purified by Kugelrohr distillation (130–160 °C/1 Torr) giving 30.3 g (70%) of **18** as a clear colorless oil. NMR ($CDCl_3$) δ 2.05 (dd, 2 H, J = 7.5, J = 15 Hz), 3.46 (d, 2 H, J = 6 Hz), 3.57 (dd, 2 H, J = 6, 13.5 Hz), 3.96 (s, 4 H), 5.13 (s, 2 H), 7.35 (m, 5 H); mass spectrum, m/z 281 $(M + NH_4)^+$, 264 $(M + H)^+$.

***N*-(Benzyloxycarbonyl)-8-aza-1,4-dioxaspiro[4.5]decane (19).** The ketone **17** was converted to the ketal **19** by a similar procedure to the preparation of **18**. The product was purified by Kugelrohr distillation (125–150 °C/1 Torr) giving 21.6 g (91%) of **19** as a clear colorless oil: NMR ($CDCl_3$) δ 1.67 (m, 4 H), 3.59 (m, 4 H), 3.98 (s, 4 H), 5.13 (s, 2 H), 7.35 (m, 5 H); mass spectrum, m/z 278 $(M + H)^+$.

7-Aza-1,4-dioxaspiro[4.4]nonane (20). The protected amine **18**, 30.3 g (202 mmol) was dissolved in 200 mL of EtOAc and 50 mL of methanol and was treated with 6.5 g of 20% Pd/C under 4 atm of H_2 at room temperature for 24 h. The bulk of the catalyst was removed by gravity filtration. The filtrate was then run through a 0.45-μm nylon filter to remove the remaining traces of catalyst. The solvent was removed on a rotary evaporator. The product was purified by Kugelrohr distillation (100 °C/0.5 Torr) giving 4.4 g (31%) of **20** as a clear colorless oil: NMR ($CDCl_3$) δ 1.96 (dd, 2 H, J = 7.5 Hz), 2.12 (s, 1 H), 2.89 (s, 2 H), 3.05 (dd, 2 H, J = 7.5 Hz), 3.92 (m, 4 H); mass spectrum, m/z 130 $(M + H)^+$.

8-Aza-1,4-dioxaspiro[4.5]decane (21). The protected amine **19** was deprotected by the same procedure used for the preparation of **20** above. The product was dried on a vacuum pump giving 13.1 g (99%) of **21** as a yellow oil: NMR ($CDCl_3$) δ 1.76 (m, 4 H), 3.02 (m, 4 H), 3.98 (s, 4 H), 4.09 (s, 1 H); mass spectrum, m/z 144 $(M + H)^+$.

7-(3-Oxopyrrolidinyl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (24). The ester **22a**, 1.0 g (2.74 mmol) in 15 mL of pyridine under a positive N_2 atmosphere was heated with 1.07 g (8.22 mmol) of **20** and 2.3 mL (16.7 mmol) of Et_3N at 45 °C for 24 h. The solvent was removed on a rotary evaporator. The product was recrystallized from EtOH/hexane and was isolated by suction filtration. The product was dissolved in 3 mL of THF and was hydrolyzed with 11 mL of 3 M HCl at 70 °C for 38 h. The reaction mixture was cooled to room temperature, and the product was isolated by suction filtration. The product was washed with water (2 × 10 mL) and was dried under vacuum giving 560 mg (51%) of **24** as a white solid: NMR (CF_3CO_2D) δ 3.01 (dd, 2 H, J = 8 Hz), 4.06 (dd, 2 H, J = 8 Hz), 4.35 (s, 2 H), 6.41 (d, 1 H, J = 6 Hz), 7.35 (m, 2 H), 7.73 (m, 1 H), 8.28 (d, 1 H, J = 13 Hz), 9.13 (s, 1 H); mass spectrum, m/z 403 $(M + H)^+$.

7-(4-Oxopiperidinyl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (25). The ester **22b** was reacted with **21** instead of **20** under the same reaction conditions used in the preparation of **24** except that the reaction was run at room temperature. The hydrolysis used the same reaction conditions as for the preparation of **24**. The product was dried under vacuum giving 1.49 g (89%) of **25** as a white solid: NMR (CF_3CO_2D) δ 2.10 (m, 2 H), 2.67 (m, 2 H), 4.12 (m, 2 H), 7.25 (m, 2 H), 7.70 (m, 1 H), 8.28 (d, 1 H, J = 12 Hz), 9.19 (s, 1 H); mass spectrum, m/z 418 $(M + H)^+$; IR (KBr) 3400 (OH), 1740 (C=O), 1630 (C=O) cm^{-1}.

7-(3-Oxopyrrolidinyl)-6-fluoro-1-cyclopropyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (26). The ester **22c** (X = CH) was reacted with **20** under the same reaction conditions used in the preparation of **24** except heating at 50 °C for 6 days. The hydrolysis conditions were the same as those used for the preparation of **24**. The product was isolated by suction filtration and was dried under vacuum giving 440 mg (80%) of **26** as a white solid: NMR (CF_3CO_2D) δ 1.32 (m, 2 H), 1.48 (m, 2 H), 2.95 (m, 2 H), 4.10 (m, 1 H), 4.53 (m, 4 H), 6.90 (d, 1 H), J = 7.5 Hz), 8.92 (d, 1 H, J = 12 Hz), 8.72 (s, 1 H); mass spectrum, m/z 331 $(M + H)^+$; IR (KBr) 3420 (OH), 1770 (C=O), 1725 (C=O) cm^{-1}.

7-(3-Oxopyrrolidinyl)-6,8-difluoro-1-cyclopropyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (27). The ester **22c**

was reacted with 20 under the same reaction conditions used in the preparation of 24 except that the reaction was heated at 60 °C for 48 h. The hydrolysis conditions were the same as those used in the preparation of 24. The product was dried under vacuum to give 816 mg (70%) of 27 as a white solid: NMR (CF_3CO_2D) δ 1.41 (m, 5 H), 2.81 (t, 2 H, J = 7.5 Hz), 4.31 (s, 2 H), 4.35 (m, 2 H), 8.02 (dd, 1 H, J = 15, J = 3 Hz), 9.16 (s, 1 H); mass spectrum, m/z 349 $(M + H)^+$.

7-[3-(Hydroxyimino)pyrrolidinyl]-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (28). The quinolone 24 (887 mg, 1.90 mmol) was suspended in 18 mL of 2:1 EtOH/THF. To this was added a solution of 484 mg (6.97 mmol) of NH_2OH·HCl and 586 mg (6.97 mmol) of $NaHCO_3$ in 3 mL of H_2O. The reaction mixture was heated at 85 °C with stirring for 2 h. The reaction mixture was diluted with 20 mL of water, and the product was isolated by suction filtration. The filter cake was washed with 10 mL of MeOH and was dried under vacuum at room temperature giving 548 mg (66%) of 28 as a white solid: NMR (CF_3CO_2D) δ 3.37 (m, 2 H), 3.94 (m, 2 H), 4.92 (s, 2 H), 6.39 (m, 2 H), 7.32 (m, 2 H), 7.71 (m, 1 H), 8.28 (d, 1 H, J = 13.5 Hz), 9.00 (s, 1 H); mass spectrum, m/z 418 $(M + H)^+$; IR (KBr) 3400 (OH), 1720 (C=O), 1630 (C=O) cm^{-1}.

7-[4-(Hydroxyimino)piperidinyl]-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (29). The ester 22a (1.00 g, 2.74 mmol) in 15 mL of dry pyridine, under a positive N_2 atmosphere, was treated with 784 mg (5.48 mmol) of 21. The reaction mixture was heated at 60 °C for 48 h. After cooling to room temperature, the solvent was removed on a rotary evaporator. The product was recrystallized from i-PrOH/hexane to give 868 mg (65%) of the ester. The ester and ketal were hydrolyzed in 6 mL of 3 N HCl at 80 °C for 48 h. The product was isolated by suction filtration and was washed with water and 1:1 H_2O/EtOH. The oxime was prepared by treatment of 400 mg (0.96 mmol) of the ketone in 8 mL of 3:1 EtOH/THF with a solution of 200 mg (2.88 mmol) of hydroxylamine hydrochloride and 404 mg (4.8 mmol) of $NaHCO_3$ in 3 mL of water. The reaction mixture was heated at 65 °C for 3 h. The reaction mixture was cooled to room temperature and was diluted with 5 mL of water. The product was isolated by suction filtration and was washed with water. The product 29 (289 mg, 70%) was obtained as a white solid after drying under vacuum: NMR (CF_3CO_2D) δ 2.89 (m, 2 H), 3.05 (m, 2 H), 3.65 (m, 4 H), 6.59 (d, 1 H, J = 7 Hz), 7.34 (m, 2 H), 7.81 (m, 1 H), 8.21 (d, 1 H, J = 12 Hz), 9.08 (s, 1 H); mass spectrum, m/z 432 $(M + H)^+$; IR (KBr) 3400 (OH), 1730 (C=O), 1630 (C=N) cm^{-1}.

7-[3-(Methoxyimino)pyrrolidinyl]-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (30). The quinolone 24 was reacted with methoxylamine hydrochloride instead of NH_2OH·HCl at 50 °C for 14 h using the same procedure used for the preparation of 28. The product was isolated by suction filtration and was dried under vacuum to give 123 mg (35%) of 30 as an off-white solid: NMR ($CDCl_3/CD_3OD$) δ 2.84 (m, 2 H), 3.49 (m, 2 H), 3.88 (s, 3 H), 4.14 (m, 2 H), 5.94 (m, 1 H), 7.23 (m, 2 H), 7.61 (m, 1 H), 7.94 (m, 1 H), 8.49 (m, 1 H); mass spectrum, m/z 432 $(M + H)^+$; high resol mass spectrum, m/z 432.1177 $(M + H)^+$ (calcd for $C_{21}H_{17}F_3N_3O_4$, 432.1171); IR (KBr) 3400 (OH), 1750 (C=O), 1630 (C=N) cm^{-1}.

7-[4-(Methoxyimino)piperidinyl]-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (31). The ester 22a (1.00 g, 2.74 mmol) in 15 mL of dry pyridine was treated with 784 mg (5.48 mmol) of 21 under a positive N_2 atmosphere. The reaction mixture was heated at 60 °C with stirring for 48 h. The reaction mixture was cooled to ambient temperature, and the solvent was removed on a rotary evaporator. The product was recrystallized from i-PrOH/hexane to give 868 mg (65%) of the ester. The protecting groups were removed by hydrolysis in 6 mL of 3 N HCl at 80 °C for 48 h. The reaction mixture was cooled to room temperature, and the product was isolated by suction filtration. The oxime was prepared by reaction of 300 mg (0.72 mmol) of the ketone with 241 mg (2.89 mmol) of methoxylamine hydrochloride and 303 mg (3.61 mmol) of $NaHCO_3$ in 10 mL 1:1 EtOH/water. The reaction mixture was heated at 45 °C for 24 h. The reaction mixture was diluted with 5 mL of water and was adjusted to pH 4 with acetic acid. The product was isolated by suction filtration and was washed with 5 mL of EtOH. The product was dried under vacuum giving 198 mg (62%) of 31 as an off-white solid: NMR ($CDCl_3/CD_3OD$) δ 2.23 (dd, 2 H, J = 6 Hz), 2.49 (dd, 2 H, J = 6 Hz), 3.25 (dd, 2 H, J = 6 Hz), 3.31 (dd, 2 H, J = 6 Hz), 3.84 (s, 3 H), 6.27 (d, 1 H, J = 6 Hz), 7.27 (m, 2 H), 7.62 (m, 1 H), 8.04 (d, 1 H, J = 12 Hz), 8.62 (s, 1 H); mass spectrum, m/z 446 $(M + H)^+$; high resol mass spectrum, m/z 446.1328 $(M + H)^+$ (calcd for $C_{22}H_{19}F_3N_3O_4$, 446.1328).

7-[4-(Hydroxyimino)piperidinyl]-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (32). The naphthyridine 25 (700 mg, 1.68 mmol) was suspended in 8 mL of EtOH. To this was added a solution of 350 mg (5.04 mmol) of hydroxylamine hydrochloride and 705 mg (8.39 mmol) of $NaHCO_3$ in 3 mL of H_2O. The reaction mixture was heated at 60 °C for 6 h. The reaction mixture was cooled to room temperature and was diluted with 5 mL of water. The product was isolated by suction filtration, washed with water, and dried under vacuum giving 621 mg (86%) of 32 as a white solid: NMR (CF_3CO_2D) δ 2.91 (m, 2 H), 3.03 (m, 2 H), 4.08 (m, 4 H), 7.26 (dd, 2 H, J = 9.0 Hz), 7.70 (m, 1 H), 8.30 (d, 1 H, J = 12 Hz), 9.21 (s, 1 H); mass spectrum, m/z 433 $(M + H)^+$, 388 $(M - CO_2H)^+$, high resol mass spectrum, m/z 433.1117 $(M + H)^+$ (calcd for $C_{20}H_{16}F_3N_4O_4$, 433.1124); IR (KBr) 3400 (OH), 1630 (C=O) cm^{-1}.

7-[3-(Methoxyimino)pyrrolidinyl]-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (33). The naphthyridine 25 was reacted with methoxylamine hydrochloride instead of NH_2OH·HCl at 45 °C for 20 h using the same procedure used for the preparation of 32. The product was dried under vacuum giving 244 mg (38%) of 33 as a pale yellow solid: NMR (DMSO-d_6) δ 2.71 (m, 2 H), 3.67 (m, 2 H), 3.79 (s, 3 H), 4.20 (m, 2 H), 7.35 (m, 1 H), 7.62 (m, 1 H), 7.82 (m, 1 H), 8.12 (d, 1 H, J = 12 Hz), 8.84 (s, 1 H), 15.06 (s, 1 H); mass spectrum, m/z 433 $(M + H)^+$, 388 $(M - NOCH_3)^+$; IR (KBr) 3440 (OH), 1730 (C=O), 1630 (C=O) cm^{-1}.

7-[4-(Methoxyimino)piperidinyl]-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (34). The naphthyridine 25 was reacted with methoxylamine hydrochloride using the same procedure used for the preparation of 33. The product was dried under vacuum giving 303 mg (94%) of 34 as a white solid: NMR (DMSO-d_6) δ 2.32 (m, 2 H), 2.48 (m, 2 H), 3.65 (m, 4 H), 3.73 (s, 3 H), 7.35 (m, 1 H), 7.63 (m, 1 H), 7.82 (dt, 1 H, J = 9 Hz, J = 6 Hz), 8.17 (d, 1 H, J = 13.5 Hz), 8.87 (s, 1 H), 14.98 (s, 1 H); mass spectrum, m/z 447 $(M + H)^+$; IR (KBr) 3440 (OH), 1740 (C=O), 1630 (C=O) cm^{-1}.

7-[3-(Hydroxyimino)pyrrolidinyl]-6,8-difluoro-1-cyclopropyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (35). The ketone 27 (500 mg, 1.44 mmol) was suspended in 10 mL of 3:1 EtOH/THF, and to this was added a solution of 300 mg (4.32 mmol) hydroxylamine hydrochloride and 603 mg (7.18 mmol) of $NaHCO_3$ in 2.5 mL of water. The reaction mixture was heated at 65 °C with stirring for 2.5 h. The reaction mixture was cooled to ambient temperature and was diluted with 5 mL of water. The product was isolated by suction filtration and was washed with CH_3OH. The product was dried under vacuum giving 410 mg (79%) of 35 as a white solid: NMR (CD_2Cl_2/CD_3OD) δ 1.40 (m, 5 H), 2.92 (m, 2 H), 4.28 (m, 4 H), 6.88 (dd, 1 H, J = 3 Hz), 8.10 (dd, 1 H, J = 12 Hz), 9.15 (s, 1 H); mass spectrum, m/z 364 $(M + H)^+$, 319 $(M - CO_2H)^+$; IR (KBr) 3400 (OH), 1700 (C=O) cm^{-1}.

7-[4-(Methoxyimino)piperidinyl]-6,8-difluoro-1-cyclopropyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (36). The quinolone 22c (2.50 g, 8.03 mmol) in 30 mL of dry pyridine was treated with 1.73 g (12.03 mmol) of 21 and 4.48 mL (32.13 mmol) of Et_3N. The reaction mixture was heated at 70 °C for 24 h. The solvent was removed on a rotary evaporator. The product was dissolved in 60 mL of CH_2Cl_2 and was washed with 60 mL 1 N HCl and 60 mL 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4 and filtered, and the solvent was removed on a rotary evaporator. The product 23c was purified by silica gel chromatography. The column was eluted with a gradient of CH_2Cl_2 to 3% MeOH/CH_2Cl_2. The ester and ketal protecting groups were removed by hydrolysis with 5 mL of 3 N HCl at 70 °C for 24 h. The ketone (230 mg, 0.63 mmol) in 6 mL of EtOH was treated with a solution of 159 mg (1.90 mL) of methoxylamine hydrochloride and 267 mg (3.18 mmol) of $NaHCO_3$ in 4 mL of H_2O. The reaction mixture was heated at 42 °C for 24 h. The reaction mixture was diluted with 5 mL H_2O and the product was

isolated by suction filtration. The product was dried under vacuum giving 167 mg (67%) of **36** as a white solid: NMR (CD_2Cl_2/CD_3OD) δ 1.12 (m, 2 H), 1.22 (m, 2 H), 2.49 (m, 2 H), 2.76 (m, 2 H), 3.40 (m, 2 H), 3.48 (m, 2 H), 3.85 (s, 3 H), 3.95 (m, 1 H), 7.74 (m, 1 H), 8.78 (s, 1 H); mass spectrum, m/z 392 $(M + H)^+$; high resol mass spectrum, m/z 392.1418 $(M + H)^+$ (calcd for $C_{19}H_{20}F_2N_3O_4$, 392.1422); IR (KBr) 3440 (OH), 1620 (C=O) cm^{-1}.

7-[4-(Hydroxyimino)piperidinyl]-6,8-difluoro-1-cyclopropyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (37). The ketone derived from **23c** was reacted with NH_2OH·HCl instead of methoxylamine hydrochloride at 40 °C for 24 h using the procedure used for the preparation of **36**. The product was washed with EtOH and was dried under vacuum giving 145 mg of **37** as a white solid: NMR (CD_2Cl_2/CD_3OD) δ 1.20 (m, 4 H), 2.51 (m, 2 H), 2.81 (m, 2 H), 3.46 (m, 4 H), 3.93 (m, 1 H), 7.82 (d, 1 H, J = 12 Hz), 8.70 (s, 1 H); mass spectrum, m/z 378 $(M + H)^+$.

7-[3-(Methoxyimino)pyrrolidinyl]-6-fluoro-1-cyclopropyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (38). The ester **22d** (600 mg, 1.57 mmol) in 10 mL of dry pyridine was treated with 304 mg (2.35 mmol) of **20** and 870 μL (6.28 mmol) of Et_3N. The reaction mixture was stirred at room temperature for 6 h. The solvent was removed on a rotary evaporator. The product was dissolved in 50 mL of CH_2Cl_2 and was washed with 50 mL of 0.5 N HCl, 50 mL of 5% $NaHCO_3$, and 50 mL of H_2O. The organic layer was dried over Na_2SO_4 and filtered, and the solvent was removed on a rotary evaporator. The protecting groups were removed by hydrolysis in 4 mL of 3 N HCl at 80 °C for 48 h. The reaction mixture was diluted with 10 mL of H_2O, and the product was isolated by suction filtration. A flask was charged with 300 mg (0.90 mmol) of the ketone in 6 mL of EtOH. To this was added a solution of 303 mg (3.63 mmol) of methoxylamine hydrochloride and 380 mg (4.52 mmol) of $NaHCO_3$ in 4 mL of H_2O. The reaction mixture was heated at 42 °C for 24 h. The reaction mixture was diluted with 10 mL of H_2O, and the product was isolated by suction filtration. The product was dried under vacuum giving 113 mg (35%) of **38** as a solid: NMR ($CDCl_3/CD_3OD$) δ 1.13 (m, 2 H), 1.36 (m, 2 H), 2.96 (m, 2 H), 3.34 (s, 3 H), 3.76 (m, 1 H), 4.19 (m, 2 H), 4.61 (m, 2 H), 8.05 (d, 1 H, J = 12 Hz), 8.76 (s, 1 H); mass spectrum, m/z 361 $(M + H)^+$; IR (KBr) 3440 (OH), 1720 (C=O), 1630 (C=O) cm^{-1}.

7-[3-(Hydroxyimino)pyrrolidinyl]-6-fluoro-1-cyclopropyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (39). The ketone precursor to **38** was reacted with NH_2O-H·HCl instead of methoxylamine hydrochloride at 25 °C for 3 days using the same procedure used for the preparation of **38**. The product was dried under vacuum at room temperature giving 463 mg (70%) of **39** as a white solid: NMR (CF_3CO_2D) δ 1.30 (m, 2 H), 1.50 (m, 2 H), 2.93 (m, 2 H), 4.10 (m, 1 H), 4.20 (m, 4 H), 8.53 (d, 1 H, J = 12 Hz), 9.37 (s, 1 H); mass spectrum, m/z 375 $(M + NH_4)^+$, 347 $(M + H)^+$, 302 $(M - CO_2)^+$; IR (KBr) 3420 (OH), 1770 (C=O), 1720 (C=O), 1630 (C=N) cm^{-1}.

In Vitro Antibacterial Activity. The in vitro antibacterial activity of the synthesized compounds was tested in a side-by-side comparison with ciprofloxacin (**6**) and determined by conventional agar dilution procedures. The organisms were grown overnight in brain–heart infusion (BHI) broth (Difco 0037-01-6) at 36 °C. Two-fold dilutions of the stock solution (2000 μg/mL) of the test compound were made in BHI agar to obtain the test concentration ranging from 200–0.005 μg/mL. The plate was inoculated with approximately 10^4 organisms. It was then incubated at 36 °C for 18 h. The minimal inhibitory concentration (MIC) was the lowest concentration of the test compound that yielded no visible growth on the plate.

In Vivo Antibacterial Activity. The in vivo antibacterial activity of the test compounds was determined in CF-1 female mice weighing approximately 20 g. Aqueous solutions of the test compounds were made by dissolving the hydrochloride salt in distilled water or by dissolving the compound in dilute NaOH and diluting it with distilled water to the desired volume. The median lethal dose of the test organism was determined as follows:

After 18-h incubation, the cultures of *E. coli* Juhl in BHI broth were serially diluted by using 10-fold dilutions in 5% (w/v) hog gastric mucin. Cultures (0.5 mL), dilution from 10^{-1} to 10^{-8}, were injected intraperitoneally into mice. The LD_{50} for the test organism was calculated from the cumulative mortalities on the sixth day by using the Reed and Muench procedure.[11]

The 18-h culture of the above was diluted in 5% (w/v) hog gastric mucin to obtain 100 times the LD_{50}, and 0.5 mL was injected intraperitoneally into mice. The mice were treated subcutaneously (sc) or orally (po) with a specific amount of the test compound divided equally to be administered at 1 and 5 h after infection. A group of 10 animals each for at least three dose levels was thus treated, and the deaths were recorded daily for 6 days. Ten mice were left untreated as infection control. ED_{50} values were calculated from the cumulative mortalities on the sixth day after infection by using the trimmed version of the Logit method.[12]

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