with each concentration of CPE-C in liquid cell culture medium. The plates were returned to the CO_2 incubator at 37 °C. After four days incubation, the cytotoxicity was measured by performing viable cell counts (utilizing the trypan blue dye exclusion method) on the drug-related and untreated control cell cultures.

U.S. Army antiviral tests were carried out at Southern Research Institute under standard protocols developed by the Department of Antiviral Studies, U.S. Army Medical Research Institute of Infectious Disease.[33]

Acknowledgment. We gratefully acknowledge Dr. James A. Kelley, Laboratory of Medicinal Chemistry (LMC), for obtaining and interpreting the mass spectral data, Dr. Karl Flora, Pharmaceutical Resources Branch, NCI, for technical assistance, and Yetta Buckberg, LMC, for secretarial support.

(33) Sidwell, R. W.; Huffman, J. H. *Appl. Microbiol.* **1971**, *22*, 797.

Synthesis and Bacterial DNA Gyrase Inhibitory Properties of a Spirocyclopropylquinolone Derivative

Mark P. Wentland,*,† Robert B. Perni,† Peter H. Dorff,† and James B. Rake‡

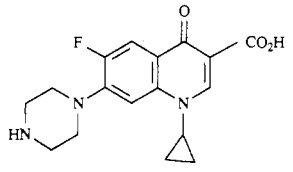
Departments of Medicinal Chemistry and Microbiology, Sterling-Winthrop Research Institute, Rensselaer, New York 12144.
Received January 11, 1988

A novel conformationally restricted 1-cyclopropylquinolone (**1**) that incorporates structural features of both ofloxacin and ciprofloxacin has been prepared. Compound **1** was found to be a DNA gyrase inhibitor having potency similar to ofloxacin but less than ciprofloxacin. The cellular inhibitory and in vivo antibacterial potencies of **1** were found to be less than those of the two reference agents.

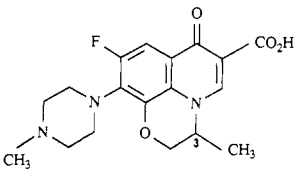
The exceptional in vitro antibacterial properties of ciprofloxacin (CIP) relative to many other quinolones can be attributed to its 1-cyclopropyl substitution.[1-4] Those quinolones that have ethyl (e.g. norfloxacin),[5] aryl (e.g. difloxacin),[6] or methylamino (e.g. amifloxacin)[7] appendages at position 1 are generally less potent in vitro, especially vs Gram-negative bacteria.

Quinolones exert their antibacterial effect via inhibition of bacterial DNA gyrase, an enzyme essential for procaryotic DNA replication.[2-4] While the molecular interaction of quinolones with the gyrase enzyme is not fully understood, quinolones have been reported to bind to single-stranded DNA but not to gyrase or double-stranded DNA.[8,9]

As part of a study into the conformational requirements of 1-cyclopropylquinolones with respect to their role as inhibitors of gyrase function, we have prepared a novel, conformationally restricted analogue (**1**) of CIP.[10] For **1**, the quaternary carbon of the cyclopropyl ring is incorporated into an additional ring, which ultimately links positions 1 and 8 of the quinolone. The resulting benzoxazine structure can also be considered a spirocyclopropyl variant of the clinically useful quinolone, ofloxacin (OFL).[2]



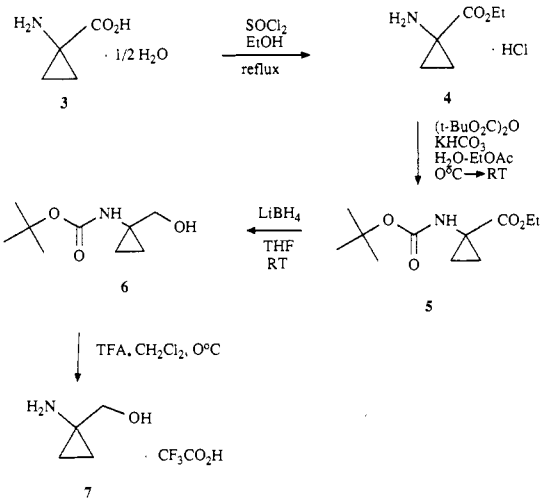
Ciprofloxacin(CIP)



Ofloxacin(OFL)

An additional analogue, **2**,[11] was prepared to assess the biological effect upon disubstitution at position 3 of OFL. Compound **2** lacks the pseudo-π interactions[12] the cyclopropyl group of **1** might have with the quinoline ring or biochemical target but maintains a spatial requirement at position 3 more similar to **1** than OFL. Recent studies have shown that the *S*-(−) enantiomer of OFL (the methyl group orientated β as normally drawn) is a 30-fold more potent *E. coli* gyrase inhibitor than its mirror image.[13]

Scheme I



† Department of Medicinal Chemistry.
‡ Department of Microbiology.

(1) Burnie, J.; Burnie, R. *Drugs Future* **1984**, *9*, 179.
(2) Wentland, M. P.; Cornett, J. B. *Annu. Rep. Med. Chem.* **1985**, *20*, 145.
(3) Cornett, J. B.; Wentland, M. P. *Annu. Rep. Med. Chem.* **1986**, *21*, 139.
(4) Fernandes, P. B.; Chu, D. T. W. *Annu. Rep. Med. Chem.* **1987**, *22*, 117.
(5) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* **1980**, *23*, 1358.
(6) Chu, D. T. W.; Fernandes, P. B.; Maleczka, R. E., Jr.; Nordeen, C. W.; Pernet, A. G. *J. Med. Chem.* **1987**, *30*, 504.
(7) Wentland, M. P.; Bailey, D. M.; Cornett, J. B.; Dobson, R. A.; Powles, R. G.; Wagner, R. B. *J. Med. Chem.* **1984**, *27*, 1103.
(8) Shen, L. L.; Pernet, A. G. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 307.
(9) Franco, R. J.; Drlica, K. *Biochem. Soc. Trans.* **1986**, *14*, 499.
(10) After submission of this manuscript, a synthesis of **1** was published utilizing similar methodology: Jpn. Pat. 62-198685. No quantification of biological activity was given.
(11) Subsequent to the completion of this work, a report appeared describing the synthesis of **2** by a similar procedure: Grohe, K.; Schriewer, M.; Zeiler, H.-J.; Metzger, K. G. EP 206,076. Grohe, K.; Schriewer, M.; EP 206,101. No quantification of biological activity was provided.

0022-2623/88/1831-1694$01.50/0 © 1988 American Chemical Society

Table I. Comparative Gyrase Inhibitory and Antibacterial Properties of Quinolones

compd	IC_{50}, μg/mL *E. coli* gyrase	MIC,[a] μg/mL, vs						in vivo, mice vs *E. coli* Vogel: PD_{50}, mg/kg[b]	
		E. coli	*P. aerug.*	*S. aureus*	*S. faecalis*	*S. pneum.*	*B. fragilis*	sc	po
1	1	0.125	4	2	8	8	8	1.9 (1.5–2.6)	6.3 (4.7–8.3)
2	1	0.5	32	2	16	8	16	3.8 (3.2–4.2)	10.1 (7.5–13.8)
OFL	1	0.008	0.5	0.25	2	0.25	0.5	1.2 (0.9–1.5)	2.2 (1.7–3.0)
CIP	0.3	0.004	0.125	0.25	0.5	0.25	2	0.20 (0.14–0.29)	0.86 (0.62–1.21)

[a] *Escherichia coli* ATCC 1-25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Streptococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 6301, and *Bacteroides fragilis* ATCC 25285. [b] 95% confidence limits are in parentheses.

Chemistry

The syntheses of compounds **1** and **2** were accomplished by using a procedure (Schemes II and III) similar to that recently utilized for preparing optically active OFL.[13] For compound **1**, the requisite (1-aminocyclopropyl)methanol (**7**) was readily prepared (Scheme I) in four steps from 1-amino-1-cyclopropanecarboxylic acid (**3**). Esterification of **3** under standard conditions (thionyl chloride, EtOH, reflux)[14] followed by protection of the amine moiety as its *tert*-butyl carbamate (di-*tert*-butyl dicarbonate, $KHCO_3$, H_2O, EtOAc) gave the protected amino acid **5**. Reduction of **5** with lithium borohydride[14] (THF, 23 °C) gave alcohol **6** in high overall yield from **3**. Cleavage of the *tert*-butyl carbamate was accomplished with trifluoroacetic acid (25% in CH_2Cl_2, 0 °C). The resulting amino alcohol was subsequently isolated as the trifluoroacetate salt (**7**) since the free amine appears to be unstable.[15]

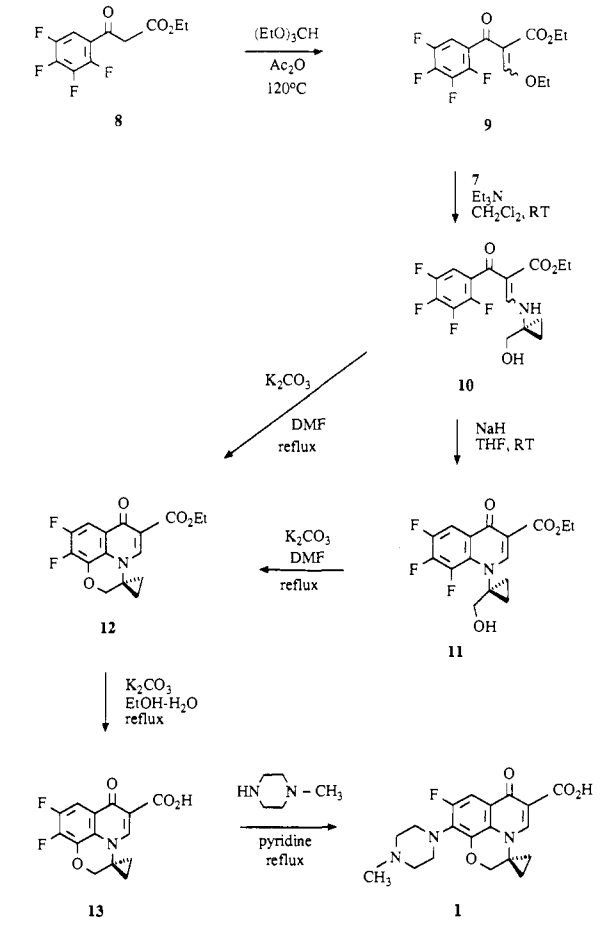
Enol ether **9**, serving as the common starting point for the synthesis of both **1** and **2**, was prepared from ethyl 3-oxo-3-(2,3,4,5-tetrafluorophenyl)propanoate (**8**) by treatment with triethyl orthoformate and acetic anhydride (Scheme II).[13] Condensation of **9** with amine salt **7** in the presence of triethylamine resulted in the smooth formation of **10** as a mixture of *E* and *Z* isomers. Base-induced cyclization of **10** with sodium hydride (THF, 23 °C) gave **11**. The final ring closure to the oxazine **12** was accomplished with more vigorous conditions (K_2CO_3, DMF, 130 °C). Alternatively, **10** was converted directly to **12** under these same conditions. Saponification of **12** (K_2CO_3, aqueous EtOH, reflux)[16] gave acid **13**, which was converted to the target compound **1** by treatment with 4-methylpiperazine in refluxing pyridine.

The dimethyl analogue **2**,[11] was prepared in a similar but slightly more efficient sequence (Scheme III). Condensation of **9** with 2-amino-2-methyl-1-propanol in dichloromethane gave **14** in good yield.[11] When **14** was heated at reflux in THF with potassium hydroxide followed by the addition of water, oxazine **15**[11] was obtained cleanly following acidification with acetic acid. Treatment of **15** with 4-methylpiperazine (vide supra) afforded **2**. Unfortunately, direct ring closure/hydrolysis of **10** failed to give appreciable yields of **13**, possibly due to competing attack of hydroxide on the cyclopropyl ring.

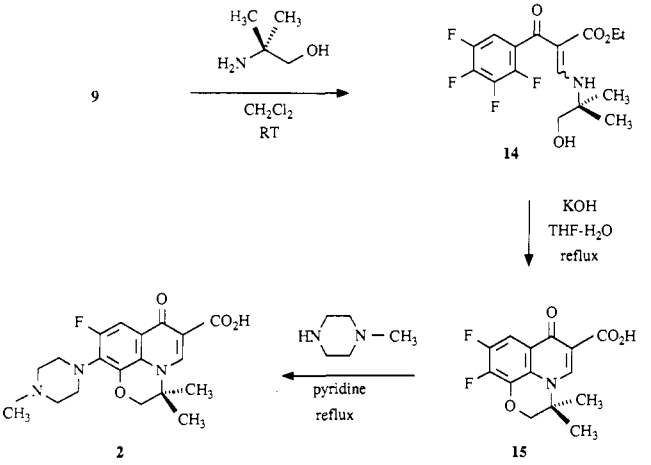
Results and Discussion

The gyrase inhibitory and antibacterial properties of compound **1** compared to **2**, CIP, and OFL are found in Table I. The *E. coli* gyrase assay used was a standard, published procedure using relaxed PBR322 plasmid DNA as substrate for *E. coli* gyrase from separately purified A and B subunits from Gellert overproducing strains.[17] The

Scheme II



Scheme III



(12) Legon, A. C.; Millen, D. J. *Chem. Commun.* **1987**, 986.
(13) Mitscher, L. A.; Sharma, P. N.; Chu, D. T. W.; Shen, L. L.; Pernet, A. G. *J. Med. Chem.* **1987**, *30*, 2283.
(14) Silverman, R. B.; Invergo, B. J.; Mathew, J. *J. Med. Chem.* **1986**, *29*, 1840.
(15) When **7** was isolated as the free base, subsequent reaction with **9** gave no new products. While **9** was recovered unchanged, **7** was not detected, indicating the free base is inherently unstable.
(16) Hydrolysis of **12** utilizing KOH(aq) failed. NMR of the crude reaction mixture suggests that the cyclopropyl ring is being opened by hydroxide ion.

IC_{50} values in micrograms/milliliter correspond to the inhibitor concentration that reduces supercoiled DNA product formation to half that observed under standard conditions. Quantification was made visually by comparing the lanes in an electrophoretic gel of DNA–gyrase–drug mixtures at various drug concentrations with lanes having DNA and either 0, 0.3, 0.5, 0.7, or 1 unit of gyrase. The in vitro cellular inhibitory (MIC, micrograms/milliliter) potencies vs *Escherichia coli* (ATCC 1-25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Streptococcus faecalis* (ATC 29212), *Streptococcus pneumoniae* (ATCC 6301), and *Bacteroides fragilis* (ATCC 25285) and the in vivo (PD_{50}, milligrams/kilogram, sc and po in mice) antibacterial properties of the test compounds against *E. coli* Vogel were determined by conventional procedures.[7]

Compounds **1** and **2** were found to be potent inhibitors of *E. coli* gyrase function with CIP being slightly more potent than the new quinolones and OFL. The cellular inhibitory potencies, however, of **1** and **2** vs *E. coli* were significantly decreased relative to the two standards. This may have been a consequence of relatively low intracellular concentrations of **1** and **2**. Similar comparative MIC data were also noted against the Gram-positive organisms, and the anaerobe *B. fragilis*.

In the mouse protection model vs *E. coli*, derivatives **1** and **2** when administered sc displayed surprisingly good protection in light of the poor MIC values. The PD_{50} value of **1**, for example, was approximately 1.5-fold greater than that of OFL, representing a much narrower potency difference than the 16-fold differential in their MIC's. Upon comparison of **1** and CIP, the former was 10- and 32-fold less potent in vivo and in vitro (MIC), respectively. The correlation between cellular and in vivo potency of **2** vs *E. coli* was similar to that noted for **1**; i.e., greater in vivo potency than would be predicted by its MIC relative to OFL and CIP. Upon oral administration of compounds **1** and **2** in the mouse model, an approximate 3-fold increase in the PD_{50} values was observed relative to subcutaneous administration. This difference in protection between the two routes of administration for **1** and **2** is qualitatively similar to that observed with CIP and OFL.

One interpretation of the gyrase inhibition data might be that the conformation of CIP at the active site differs from that of **1**. The β-cyclopropyl CH_2 of **1** might simply be filling the space normally occupied by the methyl of (*S*)-(−)-OFL or the β-methyl of **2**. The conformation of **1** might preclude the pseudo-π electronic interactions that the cyclopropyl moiety of CIP might have the quinoline ring or biochemical target. It is probably not just the presence of a cyclopropyl appendage that is an important determinant to activity but the orientation of the group as well.

From the OFL enantiomer data,[13] it seems that a methyl orientated α at position 3 is detrimental to gyrase inhibitory activity. However, the data generated from this study shows that α-substituted quinolones (e.g. **1** and **2**) that have β-substituents as well are potent gyrase inhibitors. Within the limits of these studies, binding of quinolones to their biochemical target appears to be facilitated by β-substitution and is not appreciably affected by α-substitution.

Experimental Section

General Methods. Melting points were determined on a Thomas-Hoover melting point apparatus in open capillaries and are uncorrected. Proton NMR spectra were obtained on an IBM AM-200 FT-NMR spectrometer at 200 MHz and are reported in parts per million downfield from tetramethylsilane. Chemical-ionization mass spectra were determined with a Hewlett-Packard 5980A mass spectrometer. Infrared spectra were obtained with a Nicolet 10DX FTIR spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and were within ±0.4% of theoretical values. Flash chromatography on silica gel (200–400 mesh) refers to the method developed by Still.[18] Tetrahydrofuran (THF) was distilled from sodium benzophenone. Organic extracts were dried over anhydrous sodium sulfate. Reactions were run under a nitrogen atmosphere.

Ethyl 1-[[(1,1-Dimethylethoxy)carbonyl]amino]cyclopropanecarboxylate (5). To a stirred suspension of 1-amino-1-cyclopropanecarboxylic acid hemihydrate (Aldrich) (2.00 g, 18.2 mmol) in absolute ethanol (50 mL) at 0 °C was added dropwise, over 10 min, thionyl chloride (4.0 mL, 55 mmol). The ice bath was removed, and the mixture was heated at reflux for 2 h and concentrated in vacuo to give **4** as a white solid.[14] This material was dissolved in ethyl acetate (50 mL) and cooled to 0 °C. A solution of potassium bicarbonate (3.0 g) in water (15 mL) was added dropwise over 10 min with vigorous stirring. A solution of di-*tert*-butyl dicarbonate (5.0 g, 23 mmol) in ethyl acetate (15 mL) was added. The reaction mixture was allowed to warm to ambient temperature and stirred 30 h. An additional portion of di-*tert*-butyl dicarbonate (1.0 g, 4 mmol) in ethyl acetate (5 mL) was added, and the mixture was stirred for 17 h. The layers were separated, and the organic phase was dried and concentrated in vacuo to give a colorless oil, which was used directly without further purification. An analytical sample was prepared by removal of excess di-*tert*-butyl dicarbonate and *tert*-butyl alcohol via distillation (high vacuum, steam bath). The residue was crystallized from hexane (−78 °C) and recrystallized from pentane (−78 °C, two times) to give a white powder, **5**: mp 44–46 °C; ^1H NMR ($CDCl_3$) 4.15 (q, 2 H), 1.58–1.40 (m, 2 H), 1.47 (s, 9 H), 1.25 (t, 3 H), 1.20–1.07 (m, 2 H) ppm. Anal. ($C_{11}H_{19}NO_4$) C, H, N.

Dimethylethyl [1-(Hydroxymethyl)cyclopropyl]carbamate (6). To a stirred solution of crude **5** (from 18.2 mmol of **3**) in THF (10 mL) at room temperature was added via syringe over 15 min a solution of lithium borohydride[14] in THF (15.0 mL of 2.0 M solution, 30 mmol). The resulting solution was stirred at room temperature overnight. The reaction vessel was cooled in an ice bath and treated with 50% aqueous acetic acid dropwise until gas evolution ceased. The mixture was diluted with water (25 mL) and ether (50 mL), and the layers were separated. The ethereal solution was washed with 5% aqueous $NaHCO_3$ (25 mL) and brine (25 mL) and dried. Concentration in vacuo gave white crystalline material, which was subjected to flash chromatography (1:1 EtOAc/hexane) to afford **6** as white crystals (3.36 g, 98%). An analytically pure sample was recrystallized from EtOAc/hexane: mp 83.5–85.5 °C; ^1H NMR ($CDCl_3$) 3.60 (s, 2 H), 1.46 (s, 9 H), 0.84 (s, 4 H), ppm. Anal. ($C_9H_{17}NO_3$) C, H, N.

(1-Aminocyclopropyl)methanol Trifluoroacetate (7). A solution of **6** (1.84 g, 9.8 mmol) in dichloromethane (20 mL) was cooled to 0 °C and treated with trifluoroacetic acid (5 mL). The resulting solution was stirred at 0 °C for 30 min and concentrated in vacuo to give a colorless gum, which was stored under high vacuum (<2 Torr) for 24 h before subsequent use: ^1H NMR (TFA-*d*) 4.08 (s, 2 H), 1.40–1.05 (m, 4 H) ppm.

Ethyl 2,3,4,5-Tetrafluoro-α-[[[1-(hydroxymethyl)cyclopropyl]amino]methylene]-β-oxobenzenepropanoate (10). A mixture of β-keto ester **8** (1.20 g, 4.5 mmol) in distilled acetic anhydride (1.5 mL, 15.9 mmol) and distilled triethyl orthoformate (0.80 mL, 4.8 mmol) was heated at 130 °C for 2 h and concentrated in vacuo. The residue was azeotroped with toluene (50 mL) to remove traces of acetic anhydride leaving **9** as an orange oil. Enol ether **9** was dissolved in dichloromethane (5 mL) and added to a premixed solution of **7** (0.96 g, 4.8 mmol) and triethylamine (1.5 mL) in dichloromethane (10 mL) at room temperature. The resulting solution was stirred at room temperature for 1 h and concentrated in vacuo. The gummy residue was purified by flash chromatography (20% EtOAc/hexane) to give **10** (1.27 g, 77%) as a viscous yellow oil, which slowly crystallized on standing. This

(17) Mizuuchi, K.; Mizuuchi, M.; O'Dea, M.; Gellert, M. *J. Biol. Chem.* **1984**, *259*, 9199.

(18) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

material was homogeneous by TLC (EtOAc), although obtained as a mixture of *E* and *Z* isomers.[19] Analytically pure material was obtained by recrystallization from EtOAc/hexane as an off-white powder (mp 93–97 °C): ^{1}H NMR ($CDCl_3$) for **10Z** 8.27 (d, 1 H), 7.05–6.90 (m, 1 H), 4.05 (q, 2 H), 3.70 (d, 2 H), 1.15–0.90 (m, 7 H). **10E**: 8.24 (d, 1 H), 7.23–7.05 (m, 1 H), 4.02 (q, 2 H), 3.67 (d, 2 H), 1.15–0.90 (m, 7 H) ppm; *Z*:*E*, ca. 2:1, IR (KBr) 3480 (m), 1690 (s), 1642 (s), 1262 (s), 1055 (s) cm^{-1}; CIMS, *m*/*e* 362 (MH^+). Anal. ($C_{16}H_{15}F_4NO_4$) C, H, N.

Ethyl 6,7,8-Trifluoro-1,4-dihydro-1-[(1-hydroxymethyl)-cyclopropyl]-4-oxo-3-quinolinecarboxylate (11). A solution of **10** (3.29 g, 9.1 mmol) in dry THF (50 mL) was treated with sodium hydride (60% suspension in oil, 0.42 g, 10.5 mmol) and stirred at ambient temperature for 45 min. The mixture was concentrated to dryness in vacuo and the residue was partitioned between water (50 mL) and dichloromethane (100 mL). The organic layer was separated, dried, and concentrated in vacuo to give 11 as a yellow solid (2.75 g, 88%). The product was purified by flash chromatography (EtOAc) and recrystallized (EtOAc) to give analytically pure material: mp 127–129 °C; ^{1}H NMR ($CDCl_3$) 8.34 (s, 1 H), 7.45 (m, 1 H), 4.83–4.65 (m, 1 H), 4.45–4.12 (m, 2 H) 3.31 (d, 1 H), 1.50–1.10 (m, 7 H) ppm; CIMS, *m*/*e* 342 (MH^+). Anal. ($C_{16}H_{14}F_3NO_4$) C, H, N.

Ethyl 9′,10′-Difluoro-7′-oxospiro[cyclopropane-1,3′-(2′*H*)-[7*H*]pyrido[1,2,3-*de*][1,4]benzoxazine]-6′-carboxylate (12). **Method A.** A solution of **10** (2.52 g, 7.4 mmol) in dry DMF (30 mL) was treated with powdered, anhydrous potassium carbonate (3.0 g, 22 mmol) and stirred at 130 °C for 4 h. The DMF was removed in vacuo, and the residue was partitioned between water (50 mL) and dichloromethane (100 mL). The organic layer was separated, dried, and concentrated in vacuo to afford **12** (1.36 g, 66%).[20] Flash chromatography (EtOAc) afforded clean material suitable for subsequent use. An analytical sample was prepared by recrystallization from EtOAc: mp 269–271 °C; ^{1}H NMR (TFA-*d*) 9.00 (s, 1 H), 8.10 (dd, 1 H), 4.70 (q, 2 H), 5.64 (s, 2 H), 1.85 (m, 2 H), 1.70 (m, 2 H), 1.54 (t, 3 H) ppm; IR (KBr) 2290 (w), 1680 (s), 1485 (s), 1250 (s), 795 (s) cm^{-1}; CIMS, *m*/*e* 322 (MH^+). Anal. ($C_{16}H_{13}F_2NO_4$) C, H, N.

Method B. The same procedure as for the conversion of **10** to **12** with quinolone **11** as the starting material afforded oxazine **12** in 78% yield prior to chromatography. This material was identical by TLC and NMR with that obtained from **10**.

9′,10′-Difluoro-7′-oxospiro[cyclopropane-1,3′(2′*H*)-[7*H*]-pyrido[1,2,3-*de*][1,4]benzoxazine]-6′-carboxylic Acid (13). A solution of **12** (0.41 g, 1.3 mmol) in ethanol (10 mL) was treated with a solution of K_2CO_3 (0.43 g, 3.1 mmol) in water (5 mL), and the resulting mixture was heated at reflux for 2 h. The reaction mixture was concentrated to dryness in vacuo. The residue was dissolved in water (20 mL), the insoluble material was filtered off, and the filtrate was treated with glacial acetic acid. The resulting mixture was cooled in an ice bath for 5 min, and the precipitate was collected by suction filtration to afford, after drying under high vacuum, 0.38 g (100%) of **13** as a yellow powder suitable for use without further purification. An analytical sample was prepared by recrystallization from glacial acetic acid (mp >300 °C): ^{1}H NMR (TFA-*d*) 9.06 (s, 1 H), 8.25 (dd, 1 H), 5.68 (s, 2 H), 1.98 (m, 2 H), 1.70 (m, 2 H) ppm; IR (KBr), 3050 (m), 2630 (br), 1710 (s), 1487 (s), 1072 (s) cm^{-1}; CIMS, *m*/*e* 294 (MH^+). Anal. ($C_{14}H_9F_2NO_4$) C, H, N.

9′-Fluoro-10′-(4-methyl-1-piperazinyl)-7′-oxospiro[cyclopropane-1,3′(2′*H*)-[7*H*]pyrido[1,2,3-*de*][1,4]benzoxazine]-6′-carboxylic Acid Monohydrochloride Hemihydrate (1). A solution of **13** (375 mg, 1.28 mmol) in dry pyridine (7 mL) with *N*-methylpiperazine (0.40 mL, 3.6 mmol) was heated at reflux for 4 h. *N*-Methylpiperazine (0.10 mL) was added, and the reaction mixture was heated at reflux an additional 1.5 h. Following concentration to dryness in vacuo, the residue was dissolved in boiling water (30 mL) and a small amount of undissolved material was filtered off. The solution was concentrated in vacuo to ca. 15 mL, heated on a steam bath, and treated with 1 N aqueous HCl (10 mL). The resulting solution was allowed to stand at room temperature for 2 h. The precipitate was collected and dried under high vacuum (80 °C) to afford **1** as a tan powder (255 mg, 48%): mp 250 °C dec; ^{1}H NMR (TFA-*d*) 8.95 (s, 1 H), 8.03 (d, 1 H), 4.65 (s, 2 H), 4.13–3.75 (m, 6 H), 3.58–3.30 (m, 2 H), 3.17 (s, 3 H), 1.94 (m, 2 H), 1.67 (m, 2 H) ppm; IR (KBr) 1712 (s), 1621 (s), 1468 (s), 1072 (s), 800 (m) cm^{-1}; CIMS, *m*/*e* 374 (MH^+). Anal. ($C_{19}H_{20}FN_3O_4 \cdot HCl \cdot {}^1/_2H_2O$) C, H, N.

Ethyl 2,3,4,5-Tetrafluoro-α-[[(2-hydroxy-1,1-dimethylethyl)amino]methylene]-β-oxobenzenepropanoate (14).[11] A solution of enol ether **9** (vide supra) (5.07 g, 19.2 mmol) in dichloromethane (40 mL) was treated with a solution of 2-amino-2-methylpropanol (1.78 g, 20.0 mmol) in dichloromethane (10 mL) and stirred at room temperature for 35 min. The reaction mixture was concentrated in vacuo, and the orange, gummy residue was crystallized from ether/hexane to give **14** as bright yellow crystals (5.53 g, 80%).[19] An analytical sample was prepared by recrystallization from EtOAc/hexane (two times): mp 110–112 °C (lit.[11] mp 93–94 °C); ^{1}H NMR ($CDCl_3$) for **14Z** 8.25 (d, 1 H), 7.05–6.92 (m, 1 H), 4.07 (q, 2 H), 3.60 (s, 2 H), 1.41 (s, 6 H), 1.07 (t, 3 H); for **14E** 8.27 (d, 1 H), 7.17–7.05 (m, 1 H), 4.02 (q, 2 H), 3.60 (s, 2 H), 1.39 (s, 6 H), 0.96 (t, 3 H), ppm; *Z*:*E*, ca. 3:1; IR (KBr) 3480 (s), 2980 (m), 1685 (s), 1637 (s), 1058 (s), 848 (s) cm^{-1}; CIMS, *m*/*e* 364 (MH^+). Anal. ($C_{16}H_{17}F_4NO_4$) C, H, N.

9,10-Difluoro-2,3-dihydro-3,3-dimethyl-7-oxo-7*H*-pyrido-[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic Acid (15).[11] A solution of **14** (3.22 g, 8.9 mmol) in THF (25 mL) was treated with solid potassium hydroxide (1.70 g, 30 mmol) and stirred vigorously at reflux for 2 h. Water (10 mL) was added, and the resulting red solution was heated at reflux an additional 1 h. The reaction mixture was allowed to cool to room temperature, and a small amount of insoluble material was filtered off. The solvents were removed in vacuo, and the residue was dissolved in water (25 mL). Acidification with glacial acetic acid (5 mL) gave a precipitate, which was collected. Recrystallization from glacial acetic acid afforded **15** as a white powder (1.68 g, 64%): mp >300 °C (lit.[11] mp >300 °C); ^{1}H NMR (TFA-*d*) 9.48 (s, 1 H), 8.06 (d, 1 H), 4.5 (s, 2 H), 1.95 (s, 6 H) ppm; IR (KBr) 1730 (s), 1475 (s), 1115 (s), 805 (s) cm^{-1}; CIMS, *m*/*e* 296 (MH^+). Anal. ($C_{14}H_{11}F_2NO_4$) C, H, N.

9-Fluoro-2,3-dihydro-3,3-dimethyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic Acid (2).[11] The reaction was carried out as for the preparation of **1** with **15** as the substrate, but the workup was modified. The reaction mixture was concentrated to dryness in vacuo, and the residue was suspended in water. The solids were collected, dried under high vacuum, and recrystallized from ethyl acetate to give **2** as a light tan solid (70%): mp 272 °C dec (lit.[11] mp 272–274 °C); ^{1}H NMR (TFA-*d*) 9.37 (s, 1 H), 8.06 (d, 1 H), 4.55 (s, 2 H), 4.10–3.70 (m, 6 H), 3.60–3.35 (m, 2 H), 3.21 (s, 3 H), 1.90 (s, 6 H) ppm; IR (KBr) 2950 (w), 2800 (w), 1715 (s), 1618 (s), 1445 (s), 805 (s) cm^{-1}; CIMS, *m*/*e* 376 (MH^+). Anal. ($C_{19}H_{22}FN_3O_4$) C, H, N.

Acknowledgment. We gratefully acknowledge the fine technical assistance of Dianne Deuel, Richard Dobson, and Roland Wagner in the microbiological evaluation of the test compounds. We thank the Analytical Chemical Department of this Institute for spectral measurements. The many helpful discussions with Drs. George Lesher, David Sedlock, and Susan Coughlin of this Institute and Dr. Padam Sharma of the University of Kansas are also gratefully acknowledged.

Registry No. **1**, 114636-45-2; **1** (free base), 113211-53-3; **2**, 107359-24-0; **3**, 22059-21-8; **4**, 42303-42-4; **5**, 107259-05-2; **6**, 107017-73-2; **7**, 114636-46-3; **8**, 94695-50-8; **9**, 94714-58-6; (*Z*)-**10**, 114636-47-4; (*E*)-**10**, 114636-48-5; **11**, 113211-50-0; **12**, 113211-51-1; **13**, 113211-52-2; (*E*)-**14**, 114636-49-6; (*Z*)-**14**, 114636-50-9; **15**, 107358-79-2; ClP, 85721-33-1; OFL, 100986-85-4; *N*-methylpiperazine, 109-01-3; 2-amino-2-methylpropanol, 124-68-5.

(19) The major isomer was assigned the *Z* stereochemistry on the basis of steric arguements.

(20) Although the NMR of crude **12** is quite clean, tricyclic ester **12** must be chromatographed prior to hydrolysis. If crude material was used, the yield of **13** was only ca. 40%.