Bloomington, IN, 47405. The calculations were preformed with UHF wave functions and the geometries were optimized by using a combination of the Broyden-Fletcher-Goldfarb-Shanno optimizer and Bartel's method. The mass-weighted Hessian matrix was used to calculate the vibrational frequencies. All $3n - 6$ vibrational frequencies were used to calculate the equilibrium isotope effects.[12,15]

Acknowledgment. This work was supported in part by the National Institutes of Health (Grant GM 36922).

Registry No. P-450, 9035-51-2; $^2H_2$, 7782-39-0; ω-hydroxylase, 9059-16-9; octane, 111-65-9.

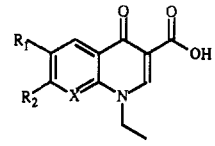
The Synthesis and Antibacterial Activities of Quinolones Containing Five- and Six-Membered Heterocyclic Substituents at the 7-Position

Curt S. Cooper,* Pamela L. Klock, Daniel T. W. Chu, and Prabhavathi B. Fernandes

Anti-Infective Research Division, Abbott Laboratories, Abbott Park, Illinois 60064. *Received August 14, 1989*

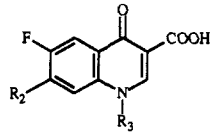
A series of 6-fluoro-7-substituted-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids were prepared. The substituents at the 7-position included five- and six-membered heterocyclic rings such as oxazoline and oxazine as well as five-membered heteroaromatic rings such as oxazoles and imidazoles. The structure-activity relationships (SAR) of these compounds indicated that oxazole substituent containing a 2-methyl group had the greatest in vitro potency. The compounds showed greater in vitro antibacterial activity against Gram-positive organisms than against Gram-negative organisms.

Oxolinic acid (**1a**) and nalidixic acid (**1b**) are members of the quinolone class of orally active antibacterial agents.[1]



1a R_1,R_2=OCH_2O X=CH
1b R_1=H R_2=CH_3 X=N
1c R_1=H R_2=4-pyridyl X=CH

Among the members of this class of compounds is rosoxacin (**1c**), which contains a heteroaromatic substituent attached to the 7-position via a carbon-carbon bond.[2] This compound has been shown to have good oral antibacterial activity in animals.[3] The antibacterial activity of the quinolones against Gram-positive organisms was increased when a fluorine atom was introduced into the 6-position, leading to compounds such as norfloxacin (**1d**)[4a]



1d R_2=1-piperazinyl R_3=CH_2CH_3
1e R_2=1-piperazinyl R_3=cyclopropyl
1f R_2=4-methyl-1-piperazinyl R_3=CH_2CH_3
1g R_2=4-methyl-1-piperazinyl R_3=4-fluorophenyl

and more recently ciprofloxacin (**1e**),[4b] perfloxacin (**1f**),[4c] and difloxacin (**1g**).[4d] Besides rosoxacin, few compounds have been reported which contain substituents attached to the 7-position of the quinolone system via a carbon-carbon linkage.[5] Most of these compounds had weak antibacterial activity. Recently, Uno et al.[6] and Culbertson et al.[7] have reported on the synthesis and biological evaluation of some 7-(azole substituted)-quinolones and Nishimura and Matsumoto[8] have reported on the preparation and evaluation of some 7-(4-pyridyl)-1,8-naphthyridine-3-carboxylic acid antibacterial agents. These papers have prompted us to report on our investigations of replacing the traditional nitrogen atom at the 7-position of the quinolone ring system with a substituent attached through a carbon-carbon bond. This series of compounds containing heterocyclic rings attached via such a carbon-carbon linkage was used to probe the importance of the nitrogen at the 7-position for antibacterial activity. In this paper we wish to report on the synthesis and biological evaluation of the antibacterial activity of a series of 6-fluoro-1-ethylquinolones which contain five- and six-membered heterocyclic or heteroaromatic substituents at the 7-position having a C-C covalent bond.

Chemistry

All of the quinolone derivatives in this study were synthesized from a common intermediate, **9**. This intermediate was prepared by the synthetic route outlined in Scheme I. The commercially available 2-fluoroacetophenone (**2**) was nitrated with fuming HNO_3 and concentrated H_2SO_4 to give **3** in 79% yield. The ketone was protected by ketalization with an appropriate diol (the choice of diol for the protecting group will be discussed later). Nitroketal **4** was reduced with Raney nickel under a hydrogen atmosphere to give amine **5**. Condensation of **5** with diethyl (ethoxymethylene)malonate (EMME) gave enamine **6**. The quinolone ring system was obtained by thermal cyclization of **6** in Dowtherm A. When ethylene glycol was used for the protection of ketone **4** (R = H), a 50:50 mixture of **7** and **7a** was obtained during the cycli-

(1) Albrecht, R. *Prog. Drug. Res.* **1977**, *17*, 9.
(2) Lesher, G. Y.; Carabates, P. M. U.S. Patent 3,907,808, 1975.
(3) O'Connor, J. R.; Dobson, R. A.; Came, P. E.; Wagner, R. B. *Curr. Chemother. Infect. Dis.* **1980**, *1*, 440.
(4) (a) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* **1980**, *23*, 1358. (b) Wise, R.; Andrews, J. M.; Edwards, L. *J. Antimicrob. Agents Chemother.* **1983**, *23*, 559. (c) Goueffon, Y.; Montay, G.; Roquet, F.; Pesson, M. *C. R. Hebd. Seances Acad. Sci.* **1971**, *292*, 37. (d) Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Pihuleac, E.; Nordeen, C. W.; Maleczka, R. E.; Pernet, A. G. *J. Med. Chem.* **1985**, *28*, 1558.
(5) (a) Artico, M.; Corelli, F.; Massa, S.; Stephancich, G.; Panico, G.; Siomonetti, N. *Farm. Ed. Sci.* **1984**, *39*, 910. (b) Uno, T.; Takamatsu, M.; Iuchi, K.; Tsukamoto, G. European Patent 115,049A, 1984.
(6) Uno, T.; Takamatsu, M.; Inoue, Y.; Kawahata, Y.; Iuchi, K.; Tsukamoto, G. *J. Med. Chem.* **1987**, *30*, 2163.
(7) Culbertson, T. P.; Domagala, J. M.; Peterson, P.; Bongers, S.; Nichols, J. B. *J. Heterocycl. Chem.* **1987**, *24*, 1509.
(8) Nishimura, Y.; Matsumoto, J. *J. Med. Chem.* **1987**, *30*, 1622.

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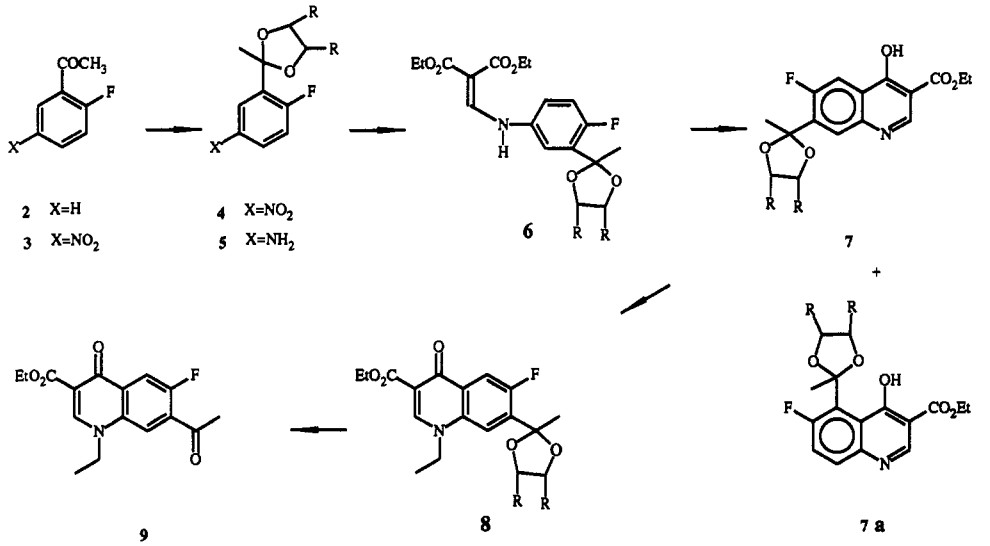
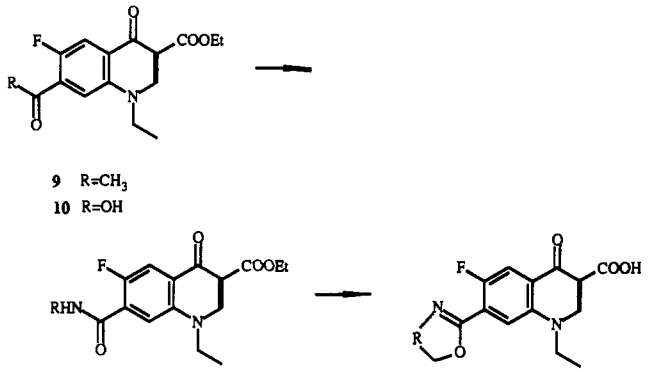
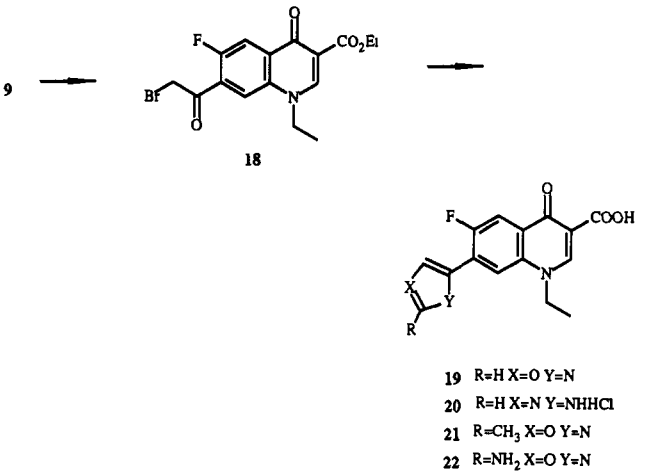
Scheme I



Scheme II



Scheme III



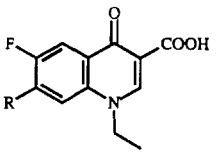
zation. The steric bulk around the ketone was increased by using 2,3-butanediol 4 (R = Me) as the protecting group. When this sterically hindered compound was cyclized thermally, a much more favorable mixture of isomers, 90:10 **7**:**7a**, was obtained. This ratio could be further enriched to 98:2 **7**:**7a** by washing the crude product with acetone. Alkylation of **7** with EtI in DMF with K_2CO_3 as the base gave **8**. The ketal and ester protecting groups were then removed in a single step by hydrolysis with HCl. The carboxylic acid was then reesterified with EtI and DBU to give the key intermediate **9**.[9] Attempts at selective removal of the ketal protecting group in the presence of the ester were not successful.

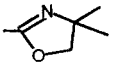
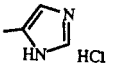
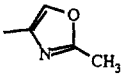
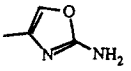
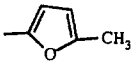
The quinolones containing an oxazoline or oxazine substituent at the 7-position were prepared from **9** by the route shown in Scheme II. In this route the methyl ketone functionality in **9** was converted to a carboxylic acid group by controlled oxidation with aqueous sodium hypochlorite, giving **10** in 71% yield.[10] Extended reaction times and higher temperatures caused hydrolysis of the ester group in addition to the desired oxidation of the methyl ketone group and lead to dicarboxylic acid **11**. The heterocyclic rings were then prepared by conversion of the carboxylic acid moiety of **10** to the corresponding amides **12**–**14** by reaction of the appropriate amino alcohols with the acid chloride, which was prepared by reaction of **10** with oxalyl chloride.[11] The amides were then cyclized with thionyl chloride to give the 7-substituted heterocyclic ring substituents.[12] The ester protecting groups were then removed by standard base hydrolysis to give oxazine **15** and oxazolines **16** and **17** after acidification.

A second series of quinolones containing heteroaromatic rings attached to the 7-position was prepared by the synthetic route shown in Scheme III. In this scheme the common intermediate **9** was functionalized to give monobromo derivative **18** by reaction with pyridinium bromide perbromide.[13,14] α-Bromoketone **18**, when heated at 110 °C in formamide, gave a mixture of the 4-oxazole analogue and the 4-imidazole analogues, which were separated by extraction of the esters.[15] Standard base hydrolysis of the esters and neutralization with acid led to oxazole **19** and imidazole **20**. The reaction of **18** with ammonium acetate

(9) Ono, N.; Yamada, T.; Saito, T.; Tanaka, K.; Kaji, A. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 2401.
(10) Neiswender, D. D.; Moniz, W. B.; Dixon, J. A. *J. Am. Chem. Soc.* **1980**, *82*, 2876.
(11) Szmuszkovicz, J. *J. Org. Chem.* **1964**, *29*, 843.
(12) Meyers, A. I.; Temple, D. L.; Haidukewych, D.; Michelich, E. D. *J. Org. Chem.* **1974**, *39*, 2787.
(13) Djerassi, C.; Scholz, C. R. *J. Am. Chem. Soc.* **1948**, *70*, 417.
(14) In addition to monobromo ketone **18**, a small amount of dibromo ketone was observed in the reaction mixture, which was removed by recrystallization.
(15) Bredereck, H.; Gompper, R. *Chem. Ber.* **1954**, *87*, 700.

Table I. 7-Substituted Quinolones



compd	R	% yield	mp,[a] °C	formula	analysis[b]
11	CO_2H	79	301–302	$C_{13}H_{10}FNO_5$	C,H,N
13	$CONHCH_2CH_2OH$	43	237–238	$C_{15}H_{15}FN_2O_5$	C,H,N
15		36	255–253	$C_{16}H_{15}FN_2O_4$	C,H,N
16		34	276–278	$C_{15}H_{13}FN_2O_4$	C,H,N
17		58	244–245	$C_{17}H_{17}FN_2O_4$	C,H,N[c]
19		58	220 dec	$C_{15}H_{11}FN_2O_4$	C,H,N
20		82	>290	$C_{15}H_{13}ClN_3O_3$	C,H,N
21		47	>290	$C_{16}H_{13}FN_2O_4$	C,H,N
22		43	>290	$C_{15}H_{12}FN_3O_4$	C,H,N[d]
24		40	257–258	$C_{17}H_{14}FNO_4$	C,H,N

[a] Melting points are uncorrected. [b] All compounds gave the analyses indicated within ±0.4% of the theoretical values unless otherwise noted. [c] C: calcd, 61.44; found, 60.69. [d] N: calcd, 12.53; found, 12.02.

Table II. In Vitro Antibacterial Activities[a]

organism	MIC,[b] μg/mL										
	11	13	15	16	17	19	20	21	22	24	1e[c]
Staphylococcus aureus ATCC 6538P	>100	>100	50	25	25	6.2	12.5	1.56	12.5	1.56	0.1
S. aureus CMX 686B	>100	>100	50	12.5	12.5	6.2	12.5	0.78	12.5	0.78	0.2
S. aureus A5177	>100	>100	>100	25	25	12.5	25	3.1	25	1.56	0.39
S. aureus 45	>100	>100	>100	50	100	25	50	3.1	50	1.56	1.56
Staphylococcus epidermidis 3519	>100	>100	>100	50	50	12.5	50	3.1	50	1.56	0.39
Streptococcus faecium ATCC 8043	>100	>100	>100	>100	>100	>100	>100	100	>100	>100	0.39
Streptococcus bovis A5169	>100	>100	>100	>100	>100	>100	>100	100	>100	>100	1.56
Streptococcus agalactiae CMS 508	>100	>100	>100	>100	>100	>100	50	50	100	50	0.39
Streptococcus pyogenes 930	>100	>100	>100	>100	>100	>100	50	100	100	50	0.39
Escherichia coli Juhl	>100	50	50	12.5	>100	12.5	25	6.2	50	12.5	0.02
Enterobacter aerogenes ATCC 13048	>100	>100	>100	12.5	>100	12.5	50	50	50	12.5	0.05
Klebsiella pneumoniae 8045	>100	50	25	6.2	100	6.2	12.5	3.1	25	6.2	0.02
Pseudomonas aeruginosa 5007	>100	>100	>100	>100	>100	>100	>100	50	>100	>100	0.2
P. aeruginosa K799/WT	>100	>100	>100	>100	>100	100	>100	50	>100	>100	0.2
Acinetobacter CMX 669	>100	>100	>100	50	>100	50	>100	12.5	>100	25	0.78

[a] Structures are shown in Table I. [b] The MIC (minimum inhibitory concentration) values were determined by the usual 2-fold agar dilution method using brain-heart infusion agar. [c] Ciprofloxacin was the reference standard.

in acetic acid at 95 °C led to the 2-methyl-4-oxazole ethyl ester, which was purified by column chromatography.[16] Base hydrolysis of the ester led to **21**. Reaction of **18** with urea in DMF at 130 °C followed by base hydrolysis and acidification led to 2-amino-4-oxazole derivative **22**.[17]

Furan derivative **24** was obtained by the reaction sequence outlined in Scheme IV. Intermediate **9** was converted to the 1,4-diketone **23** by reaction with manganese(III) acetate and isoprenyl acetate.[18] Attempts to prepare the same 1,4-diketone by other methods worked with model compounds, but they were not successful with the intact quinolone ring system. 1,4-Diketone **23** was then cyclized with *p*-toluenesulfonic acid at 110 °C to give the furan derivative **24**.[19] The ester protecting group was concomitantly removed during the ring-formation reaction.

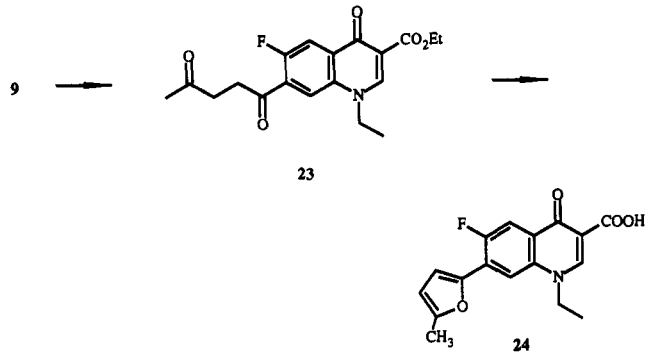
The physical properties of compounds **11**, **13**, **15–17**, **19–22**, and **24** and the structures of the 7-position substituents are shown in Table I.

Results and Discussion

The in vitro antibacterial activities of the compounds in this study were determined by conventional agar dilu-

(16) Theilig, G. *Chem. Ber.* **1953**, *86*, 96.
(17) Gompper, R.; Christmann, O. *Chem. Ber.* **1959**, *92*, 1944.
(18) Dessan, R. M.; Heiba, E. I. *J. Org. Chem.* **1974**, *39*, 3457.
(19) Scott, L. T.; Naples, J. O. *Synthesis* **1973**, 209.

Scheme IV



tion procedures. The in vitro antibacterial activity of the quinolones against several Gram-positive and Gram-negative bacteria are summarized in Table II. Activity against nine Gram-positive bacteria and six Gram-negative bacteria are some representative examples reported in the table. The data for ciprofloxacin **1e** is included for comparison.

The effect of the various heterocyclic ring substituents on the in vitro antibacterial potency is shown in Table II. Oxazine **15** was less active than either of the oxazolines **16** or **17**, with the oxazolines showing only weak activity against *Staphylococcus* organisms. The aromatic five-membered ring analogues **19–22** and **24** were more active than the oxazolines against most organisms. The antibacterial activity did not improve with the addition of a polar amino group to oxazole ring **22**. This compound was slightly less active than unsubstituted oxazole **19**. However, inclusion of the a nonpolar methyl substituent at the same position of the oxazole ring gave **21**, which was the most active compound in the series. Interestingly, furan derivative **24**, which also contained a methyl group attached to the heteroaromatic ring, was almost equipotent to **21**. Imidazole derivative **20** was about 2-fold less active than the corresponding unsubstituted oxazole **19**. Imidazole **19** is less active than the corresponding imidazoles attached to the quinolone ring at the N-1 nitrogen reported by Uno and co-workers.[6]

Culbertson et al. have reported that 7-thiazolyl-4-quinolones are active against Gram-positive and Gram-negative bacteria; they observed the greatest biological activity when the thiazole moiety contained aminomethyl substituents in contrast to the results we observed with the substituted oxazole derivatives.[7]

None of the compounds in this series possessed good broad-spectrum antibacterial activity. The most active compounds (**21** and **24**) were most effective against *Staphylococcus* organisms. The compounds in this report were generally more active against Gram-positive organisms than against Gram-negative organisms. In this series the replacement of the nitrogen atom at the 7-position of the quinolone ring system with a carbon atom did not produce quinolone derivatives with high antibacterial activity.

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 R 30-10 or Hewlett-Packard 5985 A 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 analyses (TLC) were performed on 0.25-mm silica gel G PF-254 glass plates. Column chromatography was performed on Merck 70–230 mesh silica gel.

5-Nitro-2-fluoroacetophenone (3). A flask charged with 68 mL of concentrated H_2SO_4 was cooled to −20 °C and to this was added 16.7 g (0.12 mol) of **2** dropwise with stirring. After the addition was complete a thoroughly mixed solution of 9.6 mL of fuming nitric acid and 28.4 mL of concentrated H_2SO_4 was added dropwise with stirring. The nitrating reagent was added at a rate so as to maintain a temperature of −15 °C. After the addition was complete, the reaction mixture was stirred at −15 °C for an additional 15 min and then poured into 750 g of ice. The resulting solid was isolated by suction filtration and was washed with H_2O (2 × 250 mL). The solid was dissolved in 600 mL of ether and was washed with 5% $NaHCO_3$ (2 × 500 mL) and 500 mL of H_2O. The organic layer was dried over $MgSO_4$ and filtered, and the solvent was removed on a rotary evaporator. The product **3** was isolated as a pale yellow solid weighing 17.3 g (79%): mp 49–50 °C; NMR ($CDCl_3$) δ 2.75 (d, 3 H, J = 6.9 Hz), 7.45 (dd, 1 H, J = 8.0 Hz, J = 10.0 Hz), 8.40 (m, 1 H, J = 3.0 Hz, J = 10.0 Hz, J = 2.0 Hz), 8.80 (dd, 1 H, J = 3.0 Hz, J = 6.0 Hz); mass spectrum, m/z 186 $(M)^+$, 168 $(M - CH_3)^+$, 122 $(M - CH_3 - NO_2)^+$.

5-Nitro-2-fluoro-(2,4,5-trimethyl-1,3-dioxolan-2-yl)benzene (4). Nitroketone **3** (37 g, 0.20 mol) was dissolved in 500 mL of benzene and to this was added 418 mg (2.2 mmol) of *p*-toluenesulfonic acid and 20.1 mL (0.22 mol) of 2,3-butanediol. The reaction mixture was heated under reflux and the water was removed by azeotropic distillation with a Dean–Stark trap. The reaction was stopped after 24 h, cooled to room temperature, and washed with 500 mL of 5% $NaHCO_3$ and 500 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 product was dried on a vacuum pump, giving **4** as a pale yellow oil (44.4 g, 86%): NMR ($CDCl_3$) δ 1.10 (d, 6 H, J = 6.0 Hz), 1.80 (s, 3 H), 4.40 (dq, 2 H, J = 6.0 Hz, J = 2.0 Hz), 7.30 (dd, 1 H, J = 10.0 Hz, J = 8.0 Hz), 8.20 (m, 1 H), 8.60 (dd, 1 H, J = 4.0 Hz, J = 6.0 Hz); mass spectrum, m/z 256 $(M + H)^+$, 240 $(M + H - CH_3)^+$, 226 $(M + H - Et)^+$.

5-Amino-2-fluoro-(2,4,5-trimethyl-1,3-dioxolan-2-yl)-benzene (5). A sample of protected ketone **4** (23 g, 90 mmol), dissolved in 1 L MeOH, was reduced with 2.3 g of Raney nickel #28 at room temperature under 4 atm of hydrogen for 18 h. The solvent was removed on a rotary evaporator. The residue was dissolved in 1 L of CH_2Cl_2 and was filtered through 200 g of florisil and was washed with 1 L of CH_2Cl_2. The solvent was removed on a rotary evaporator followed by drying on a vacuum pump. Product **5** (15.6 g, 77%) was isolated as a yellow oil: NMR ($CDCl_3$) δ 1.20 (d, 6 H, J = 6.0 Hz), 1.75 (s, 3 H), 4.50 (dq, 2 H, J = 6.0 Hz, J = 2.0 Hz), 7.20 (dd, 1 H, J = 10.0 Hz), 8.21 (m, 1 H, J = 4 Hz), 8.62 (dd, 1 H, J = 6.0 Hz, J = 4.0 Hz); mass spectrum, m/z 256 $(M + H)^+$, 240 $(M - Me)^+$.

Ethyl 6-Fluoro-7-(2,4,5-trimethyl-1,3-dioxolan-2-yl)-4-hydroxyquinoline-3-carboxylate (7). An oven-dried system under a positive N_2 atmosphere was charged with amine **5** (15 g, 66 mmol) suspended in 15 mL of EMME. The reaction mixture was heated at 120 °C with stirring for 3 h. The reaction mixture was dried on a vacuum pump and was used without purification. Crude product **6** was dissolved in 50 mL of diphenyl ether. This solution was added dropwise with stirring over a period of 2.5–3 h to 500 mL of Dowtherm A heated at 250 °C. The reaction mixture was heated for an additional 15 min after the addition was complete and was then cooled to 25 °C and diluted with hexane. The resulting precipitate was isolated by suction filtration and was washed with hexane followed by acetone, giving **7** as an off-white solid (13.7 g, 62%): mp 299–300 °C; NMR (DMSO-d_6) δ 1.02 (d, 6 H, J = 6.0 Hz), 1.30 (t, 3 H, J = 2.5 Hz), 1.63 (s, 3 H), 4.22 (q, 2 H, J = 7.5 Hz), 4.45 (dq, 2 H, J = 6.0 Hz, J = 2.0 Hz), 7.27 (d, 1 H, J = 11.1 Hz), 7.92 (d, 1 H, J = 6.0 Hz), 8.60 (s, 1 H), 12.50 (br s, 1 H); mass spectrum, m/z 350 $(M + H)^+$. Anal. ($C_{18}H_{20}FNO_5$): C, 61.88; H, 5.77; N, 4.01; F, 5.44. Found: C, 61.84; H, 5.69; N, 4.11; F, 5.44.

Ethyl 6-Fluoro-7-(2,4,5-trimethyl-1,3-dioxolan-2-yl)-1,4-dihydro-1-ethyl-4-oxoquinoline-3-carboxylate (8). An oven-dried system under a positive N_2 atmosphere was charged with

7 (3.5 g, 10 mmol) dissolved in 70 mL of dry DMF. The reaction mixture was heated to 75 °C with stirring. To this was added anhydrous K_2CO_3 (2.7 g, 20 mmol), and after 30 min 3.3 mL (40 mmol) of EtI was added. The reaction was stopped after 20 h and the solvent was removed on a rotary evaporator. The reaction mixture was diluted with 200 mL of CH_2Cl_2 and washed with 200 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 product was washed with hexane (2 × 20 mL) and dried under vacuum to give 8 as a white solid (3.2 g, 92%): mp 130–132 °C; NMR ($CDCl_3$) δ 1.12 (d, 6 H, J = 6.0 Hz), 1.43 (t, 3 H, J = 6.9 Hz), 1.56 (t, 3 H, J = 7.5 Hz), 1.72 (s, 3 H), 4.28 (q, 2 H, J = 7.5 Hz), 4.44 (m, 4 H), 7.80 (d, 1 H, J = 6 Hz), 8.18 (d, 1 H, J = 12 Hz), 8.53 (s, 1 H); mass spectrum, m/z 378 $(M + H)^+$.

Ethyl 6-Fluoro-7-acetyl-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylate (9). A flask was charged with 8 (3.2 g, 8.5 mmol) dissolved in 100 mL of 1:1 trifluoroacetic acid/2 N HCl. The reaction mixture was heated at 75 °C with stirring for 7 h and was then cooled to room temperature. The CF_3CO_2H was removed on a rotary evaporator and the residue was diluted with 25 mL of H_2O. The resulting precipitate was isolated by suction filtration and was washed with H_2O (2 × 50 mL). The product was dried under vacuum at 40 °C overnight. The crude product was dissolved in 40 mL of dry CH_3CN and was treated with 1.7 mL (11.3 mmol) of DBU and 1.6 mL (11.3 mmol) of EtI. The reaction mixture was stirred overnight at 25 °C under a positive N_2 atmosphere. The reaction mixture was poured into 250 mL of 1 N HCl, extracted with 250 mL of CH_2Cl_2 and washed with 250 mL of 10% Na_2CO_3 and 250 mL of H_2O. The organic layer was dried over Na_2SO_4, filtered, and then concentrated to dryness. The product was recrystallized from CH_2Cl_2, giving 9 as a white solid (2.07 g, 80%): mp 123–124 °C; NMR ($CDCl_3$) δ 1.30 (t, 3 H, J = 6.0 Hz), 1.35 (t, 3 H, J = 6.0 Hz), 2.75 (d, 3 H, J = 5.0 Hz), 4.25 (q, 2 H, J = 6.0 Hz), 4.30 (q, 2 H, J = 6.0 Hz), 7.90 (d, 1 H, J = 6.0 Hz), 8.10 (d, 1 H, J = 11.0 Hz), 8.50 (s, 1 H); mass spectrum, m/z 306 $(M + H)^+$, 260 $(M + H - EtOH)^+$, 218 $(M + H - CH_3)^+$.

Ethyl 6-Fluoro-7-carboxy-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylate (10). Ketone 9 (5.0 g, 16.3 mmol) was dissolved in 80 mL of 1:1 dioxane/water, which was added dropwise over a period of 10–15 min to a mechanically stirred solution of 75 mL of 5.25% NaOCl and 130 mL of water cooled in an ice bath. The reaction mixture was stirred at 0–5 °C for 30 min, followed by addition of 10 g of sodium bisulfite and 70 mL of 1 N HCl. The product was isolated by suction filtration and was recrystallized from EtOH, giving 10 as a white solid (3.55 g, 71%): mp 254–256 °C; NMR (CD_3CO_2D) δ 1.36 (t, 3 H, J = 6.9 Hz), 1.58 (t, 3 H, J = 7.5 Hz), 4.58 (q, 2 H, J = 6.9 Hz), 5.05 (q, 2 H, J = 7.5 Hz), 8.65 (d, 1 H, J = 9.9 Hz), 8.87 (d, 1 H, J = 6.0 Hz), 9.62 (s, 1 H); mass spectrum, m/z 308 $(M + H)^+$.

6-Fluoro-7-carboxy-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (11). A Morton flask was charged with 15 mL (9.9 mmol) of 5.25% NaOCl and 14.4 mL (14.4 mmol) of 1 N NaOH. The system was cooled in an ice bath and 9 (305 mg, 1.0 mmol) in 8 mL of dioxane was added dropwise with stirring. The reaction mixture was stirred at 0–5 °C for 4 h after the addition was complete. To this was added sodium bisulfite. The reaction mixture was washed with CH_2Cl_2 (30 mL) and was adjusted to pH 1 with 1 N HCl. The resulting precipitate was isolated by suction filtration and was washed with H_2O (2 × 20 mL). The product was dried under vacuum, giving 11 as a white solid (220 mg, 79%): mp 301–302 °C; NMR (DMSO-d_6) δ 1.45 (t, 3 H, J = 7 Hz), 3.50 (br s, 1 H), 4.68 (q, 2 H, J = 7 Hz), 8.10 (d, 1 H, J = 11 Hz), 8.38 (d, 1 H, J = 6 Hz), 9.13 (s, 1 H), 14.80 (br s, 1 H); mass spectrum, m/z 279 (M^+), 262 $(M - OH)^+$, 235 $(M - CO_2)^+$; IR (KBr) 3420 (OH), 1720 (C=O) cm^{-1}.

Ethyl 7-[(3-Hydroxypropyl)carbamoyl]-6-fluoro-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylate (12). Acid 10 (1.23 g, 4.0 mmol) was dissolved in 20 mL of dry CH_2Cl_2 to this was added 1.1 mL (8.0 mmol) of Et_3N, followed by 350 μL (4.0 mmol) of oxalyl chloride added dropwise. The reaction mixture was stirred at room temperature for 30 min. To this was added 1.5 mL (20 mmol) of 3-aminopropanol dropwise with stirring at 0 °C. After 2.5 h, the reaction mixture was poured into 100 mL of 10% $NaHCO_3$ and was washed with 100 mL of H_2O and then dried over Na_2SO_4. The organic layer was filtered and the solvent was removed on a rotary evaporator, giving amide 12 (944 mg, 65%): NMR ($CDCl_3$) δ 1.42 (t, 3 H, J = 6.9 Hz), 1.58 (t, 3 H, J = 7.5 Hz), 1.88 (qnt, 2 H, J = 6.0 Hz), 3.07 (br s, 1 H), 3.72 (q, 2 H, J = 6.0 Hz), 3.79 (t, 2 H, J = 6.0 Hz), 4.34 (q, 2 H, J = 7.5 Hz), 4.42 (q, 2 H, J = 6.9 Hz), 7.59 (m, 1 H), 8.18 (d, 1 H, J = 12.0 Hz), 8.31 (d, 1 H, J = 6.0 Hz), 8.56 (s, 1 H); mass spectrum, m/z 365 $(M + H)^+$.

Ethyl 7-[(2-Hydroxyethyl)carbamoyl]-6-fluoro-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylate (13). Acid 10 (2.01 g, 6.6 mmol) in 60 mL of CH_2Cl_2 was treated with 1.83 mL (13.2 mmol) of oxalyl chloride and 750 μL (6.6 mL) of triethylamine with protection from moisture. After 10 min, 1.98 mL (33 mmol) of 2-aminoethanol was added at 0 °C. After 3 h, the reaction mixture was poured into 100 mL of 10% $NaHCO_3$, extracted with 100 mL of CH_2Cl_2, and dried over Na_2SO_4. The organic layer was filtered and the solvent was removed on a rotary evaporator. To 460 mg (1.3 mmol) of the product suspended in 8 mL of THF was added 5.6 mL of 0.5 N NaOH. The reaction mixture was heated under reflux with removal of the THF by distillation. After 3 h, the mixture cooled to room temperature and was adjusted to pH 4 with 0.5 N HCl. The product was isolated by suction filtration and was washed with H_2O. The crude product was suspended in MeOH and was heated under reflux for 1 h. The product was isolated by suction filtration and was dried under vacuum, giving a white solid (13) (188 mg, 43%): mp 237–238 °C; NMR (CD_3CO_2D) δ 1.67 (t, 3 H, J = 7 Hz), 3.82 (t, 2 H, J = 5 Hz), 4.02 (q, 2 H, J = 5 Hz), 4.77 (q, 2 H, J = 7 Hz), 8.36 (d, 1 H, J = 11 Hz), 8.63 (d, 1 H, J = 6 Hz), 9.25 (s, 1 H); mass spectrum, m/z 323 $(M + H)^+$, 305 $(M - OH)^+$, 279 $(M - OH - C_2H_2)^+$; IR (KBr) 3420 (OH), 1720 (C=O), 1660 (C=C) cm^{-1}.

Ethyl 7-[(2-Hydroxy-1,1-dimethylethyl)carbamoyl]-6-fluoro-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylate (14). A system was charged with 307 mg (1.0 mmol) of acid 10, 131 mg (1.0 mmol) of oxalyl chloride in 10 mL CH_2Cl_2, and 280 μL (2.0 mmol) of triethylamine. The system was protected from moisture and after 30 min 448 mg (5.0 mmol) of 2-methyl-2-aminopropanol was added. After 3 h the reaction mixture was poured into 20 mL of 5% $NaHCO_3$ and extracted with 40 mL of CH_2Cl_2. The organic layer was dried over Na_2SO_4 and filtered, and the solvent was removed on a rotary evaporator. Product 14 was an off-white solid (367 mg, 97%); NMR ($CDCl_3$) δ 1.43 (t, 3 H, J = 6.9 Hz), 1.47 (s, 6 H), 1.58 (t, 3 H, J = 6.9 Hz), 3.74 (br s, 2 H), 4.01 (t, J = 7 Hz, 1 H), 4.33 (q, 2 H, J = 6.9 Hz), 4.41 (q, 2 H, J = 6.9 Hz), 7.14 (d, 1 H, J = 15 Hz), 8.24 (d, 1 H, J = 12.0 Hz), 8.28 (d, 1 H, J = 6 Hz), 8.56 (s, 1 H); mass spectrum, m/z 379 $(M + H)^+$.

7-(5,6-Dihydro-4*H*-1,3-oxazin-2-yl)-6-fluoro-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (15). An oven-dried system protected from moisture was charged with 523 mg (1.4 mmol) of amide 12. To this was cautiously added 5 mL of $SOCl_2$. After 30 min the cyclization was quenched by pouring into 2.85 mL (2.85 mmol) of 1 M NaOH; 2 mL of EtOH was used to transfer the remaining product. The reaction mixture was heated at 65 °C for 4 h. The solvent was removed and the residue was dissolved in 10 mL of water. The reaction mixture was adjusted to pH 4 with acetic acid. The product was isolated by suction filtration. The product was heated under reflux in 8 mL of EtOH for 1 h, isolated by suction filtration, washed with 2 mL of EtOH, and dried under vacuum, giving 15 as a white solid (89 mg, 36%): mp 255–256 °C; NMR (CD_3CO_2D) δ 1.65 (t, 3 H, J = 7.5 Hz), 2.07 (qnt, 2 H, J = 6.0 Hz), 3.70 (t, 2 H, J = 6.0 Hz), 4.45 (q, 2 H, J = 7.5 Hz), 4.47 (t, 2 H, J = 6.0 Hz), 8.05 (d, 1 H, J = 6.0 Hz), 8.21 (d, 1 H, J = 10.5 Hz), 8.80 (s, 1 H); mass spectrum, m/z 319 $(M + H)^+$; IR (KBr) 3420 (OH), 1720 (C=O) cm^{-1}.

7-(2-Oxazolin-2-yl)-6-fluoro-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (16). An oven-dried system protected from moisture was charged with 1.38 g (4.0 mmol) of amide 13, to this was added 3.4 mL (40 mmol) of $SOCl_2$ (exothermic), and the reaction mixture was stirred for 30 min. The reaction was quenched by pouring into 1 M NaOH. The crude product was isolated by suction filtration. The ester was then hydrolyzed by treatment of the crude product with 2.5 mL (2.5 mmol) of 1 M NaOH and 3 mL of THF and the mixture was heated at 80 °C for 10 h. The basic solution was washed with CH_2Cl_2 and was adjusted to pH 4 with acetic acid. The product was isolated by suction filtration and was washed with 15 mL of water, followed by drying under vacuum to give 16 as a white solid (148 mg, 34%):

mp 276–278 °C; NMR (CD_3CO_2D) δ 1.59 (t, 3 H, J = 7.5 Hz), 4.21 (t, 2 H, J = 9.9 Hz), 4.45 (q, 2 H, J = 7.5 Hz), 4.55 (t, 2 H, J = 9.9 Hz), 8.23 (d, 1 H, J = 6.0 Hz), 8.29 (d, 1 H, J = 10.5 Hz), 8.82 (s, 1 H); mass spectrum, m/z 305 $(M + H)^+$; IR (KBr) 3440 (OH), 1720 (C=O) cm^{-1}.

7-(4,4-Dimethyl-2-oxazolin-2-yl)-6-fluoro-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (17). An oven-dried system protected from moisture was charged with 1.6 g (4.2 mmol) of **14** dissolved in 8 mL of CH_2Cl_2. The reaction mixture was cooled in an ice bath and to this was added 3.0 g (25 mmol) of $SOCl_2$ dropwise. After stirring at room temperature for 2.5 h, the reaction mixture was cooled in an ice bath and quenched with 1 M NaOH. The reaction mixture was extracted with CH_2Cl_2 (3 × 60 mL) and was dried over Na_2SO_4. The drying agent was removed by filtration and the solvent was removed on a rotary evaporator. The crude product was purified by silica gel chromatography. The column was eluted with 2% MeOH/CH_2Cl_2. Similar fractions were then pooled and concentrated to dryness. The ester (400 mg, 1.1 mmol) in 4 mL of THF was hydrolyzed with 1.3 mL (1.3 mmol) of 1 M NaOH at 60 °C for 7 h. The reaction mixture was washed with CH_2Cl_2 (2 × 10 mL). The reaction mixture was adjusted to pH 4 with acetic acid. The product was isolated by suction filtration and was washed with 4 mL of H_2O. After drying under vacuum, product **17** was obtained as a white solid (213 mg, 58%): mp 244–246 °C; NMR (TFA) δ 1.45 (s, 6 H), 1.65 (t, 3 H, J = 6.9 Hz), 4.20 (s, 2 H), 4.45 (q, 2 H, J = 6.9 Hz), 8.22 (d, 1 H, J = 6.0 Hz), 8.27 (d, 1 H, J = 10.5 Hz), 8.82 (s, 1 H); mass spectrum, m/z 333 $(M + H)^+$; IR (KBr) 3420 (OH), 1727 (C=O) cm^{-1}.

Ethyl 7-(Bromoacetyl)-6-fluoro-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylate (18). An oven-dried system under a positive N_2 atmosphere was charged with ketone **9** (6.1 g, 20 mmol) in 100 mL of glacial acetic acid. To this was added 6.08 g (19 mmol) of pyridinium bromide perbromide. The reaction mixture was heated at 60 °C for 22 h. The cold reaction mixture was cooled and poured into 800 mL of H_2O, followed by extraction with CH_2Cl_2 (3 × 150 mL). The organic layer was dried over Na_2SO_4 and filtered, and the solvent was removed on a rotary evaporator. The product was recrystallized from EtOH to give **18** as a solid (4.27 g, 56%): mp 154–155 °C; NMR ($CDCl_3$) δ 1.43 (t, 3 H, J = 7.5 Hz), 1.59 (t, 3 H, J = 7.5 Hz), 4.32 (q, 2 H, J = 7.5 Hz), 4.42 (q, 2 H, J = 7.5 Hz), 4.60 (d, 2 H, J = 3.0 Hz), 8.07 (d, 1 H, J = 6.0 Hz), 8.31 (d, 1 H, J = 10.5 Hz), 8.56 (s, 1 H); mass spectrum, m/z 384 $(M + H)^+$, 386 $(M + H)^+$.

7-(Oxazol-4-yl)-6-fluoro-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid Hydrochloride (19). The CH_2Cl_2 extracts from the reaction of bromo ketone **18** with formamide (see **20**) were combined and dried over Na_2SO_4. The solution was filtered and the solvent was removed on a rotary evaporator. The ester (220 mg, 0.67 mmol) was suspended in 3.3 mL of 1 M NaOH. The reaction mixture was heated at 60 °C for 3 h, then cooled to 25 °C, and adjusted to pH 1 with concentrated HCl. The solvent was removed on a rotary evaporator. The product was recrystallized from 6:1 EtOH/H_2O. The product was isolated by suction filtration and was dried under vacuum to give **19** as a solid (117 mg, 58%): mp 220 °C dec; NMR (TFA) δ 1.93 (m, 3 H), 5.22 (m, 2 H), 8.60 (m, 1 H), 9.24 (m, 1 H), 9.58 (s, 1 H); mass spectrum, m/z 303 $(M + H)^+$, 285 $(M - H_2O)^+$; IR (KBr) 3440 (OH), 1720 (C=O) cm^{-1}.

7-(Imidazol-4-yl)-6-fluoro-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (20). An oven-dried system under a nitrogen atmosphere was charged with bromo ketone **18** (1.0 g, 2.6 mmol) dissolved in 10 mL (250 mmol) of formamide. The reaction mixture was heated at 110 °C for 90 h. The excess formamide was removed by distillation. The residue was poured into 100 mL of H_2O and was extracted with CH_2Cl_2 (3 × 100 mL); these extracts were used to prepare **19** (see above). The solid that did not dissolve in CH_2Cl_2 was isolated by suction filtration, washed with H_2O, and dried under vacuum. The ester (500 mg, 1.5 mmol) was suspended in 8.0 mL of 1 M NaOH and was heated at 80 °C for 5 h. The reaction mixture was cooled to 25 °C and was diluted with 25 mL of H_2O and then adjusted to pH 1 with concentrated HCl. The solvent was removed under vacuum. The residue was recrystallized from 5:1 EtOH/H_2O. The product was isolated by suction filtration and was dried under vacuum, giving **20** as a solid (421 mg, 57%): mp >290 °C; NMR (TFA) δ 1.90 (m, 3 H), 5.21 (m, 2 H), 8.37 (s, 1 H), 8.64 (d, 1 H, J = 9.6 Hz), 9.03 (s, 1 H), 9.18 (s, 1 H), 9.58 (s, 1 H); mass spectrum, m/z 302 $(M + H)^+$; IR (KBr) 3440 (OH), 1720 (C=O) cm^{-1}.

7-(2-Methyloxazol-4-yl)-6-fluoro-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (21). A mixture of bromo ketone **18** (538 mg, 1.5 mmol), NaOAc (345 mg, 4.1 mmol), and NH_4OAc (563 mg, 7.0 mmol) was heated in 5 mL of glacial acetic acid at 95 °C for 1.5 h. The HOAc was then removed under vacuum. The residue was heated in EtOH under reflux and the insoluble material was removed by filtration. The filtrate was concentrated to dryness and the product was purified by silica gel chromatography. The column was eluted with 0.5% MeOH/CH_2Cl_2 to give 102 mg (28%) of the methyloxazole ester. The ester (589 mg, 1.86 mmol) was dissolved in 6 mL of THF and 3 mL of 1 M NaOH. The reaction mixture was heated at 85 °C. The THF was removed by distillation during the reaction. The reaction was complete after 1 h. The reaction mixture was cooled to 25 °C and was diluted with 50 mL of H_2O and then neutralized with acetic acid. The product was isolated by suction filtration and washed with H_2O. The product was dried under vacuum to give **21** a white solid (256 mg, 47%): mp >290 °C; NMR (TFA) δ 1.74 (t, 3 H, J = 7.5 Hz), 2.94 (s, 3 H), 4.85 (q, 2 H, J = 7.5 Hz), 8.14 (d, 1 H, J = 3.0 Hz), 8.44 (d, 1 H, J = 6.0 Hz), 8.46 (s, 1 H); mass spectrum, m/z 317 $(M + H)^+$, 303 $(M - CH_3)^+$ (5), 273 $(M - CO_2H)^+$ (10); IR (KBr) 3440 (OH), 1720 (C=O), 1615 (C=C) cm^{-1}.

7-(2-Aminooxazol-4-yl)-6-fluoro-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (22). A system was charged with bromo ketone **18** (500 mg, 1.3 mmol) in 25 mL of dry DMF and to this was added 780 mg (13 mmol) of urea. The reaction mixture was heated at 130 °C for 18 h. After cooling, the reaction mixture was poured into 150 mL of H_2O and extracted with EtOAc (3 × 80 mL). The solvent was dried over Na_2SO_4, filtered, and removed on a rotary evaporator. The resulting solid was triturated with cyclohexane to give 295 mg (43%) of the aminooxazole ester. The ester (220 mg, 0.64 mmol) was dissolved in 3 mL of THF and was hydrolyzed with 3 mL of 1 M NaOH at 80 °C for 4 h with the THF removed by distillation during the reaction. The reaction mixture was cooled to 25 °C, diluted with 25 mL of H_2O, and then adjusted to pH 4 with HOAc. The product was isolated by suction filtration and was dried under vacuum to give **22**, a tan solid (132 mg, 66%): mp >290 °C; NMR (TFA) δ 1.68 (t, 3 H, J = 7.5 Hz), 4.73 (br s, 2 H), 4.85 (q, 2 H, J = 7.5 Hz), 8.10 (d, 1 H, J = 4.5 Hz), 8.40 (d, 1 H, J = 10.5 Hz), 8.56 (d, 1 H, J = 6.0 Hz), 9.24 (s, 1 H); mass spectrum, m/z 318 $(M + H)^+$, 274 $(M - CO_2)^+$ (80).

Ethyl 7-(1,4-Dioxopentyl)-6-fluoro-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylate (23). An oven-dried system protected from moisture was charged with 915 mg (3.0 mmol) of ketone **9** suspended in 12 mL of glacial acetic acid. The reaction mixture was heated at 70 °C, and 1.6 g (6.0 mmol) of manganese(III) acetate dihydrate and 660 μL (6.0 mmol) of isoprenyl acetate were added. After 1 h the reaction mixture was cooled to 25 °C, diluted with 100 mL of H_2O, extracted with CH_2Cl_2 (3 × 100 mL), and dried over Na_2SO_4. The solution was filtered and the solvent was removed on a rotary evaporator. The crude product was purified by column chromatography on 50 g of silica gel. The column was eluted with 1% MeOH/CH_2Cl_2. Like fractions were pooled and concentrated to give **23** (540 mg, 50%): NMR ($CDCl_3$) δ 1.48 (t, 3 H, J = 6.5 Hz), 1.55 (t, 3 H, J = 7.0 Hz), 2.27 (s, 3 H), 2.94 (t, 2 H, J = 6.0 Hz), 3.43 (m, 2 H, J = 6.0 Hz, J = 2.0 Hz), 4.29 (q, 2 H, J = 7.0 Hz), 4.43 (q, 2 H, J = 6.5 Hz), 8.05 (d, 1 H, J = 6.0 Hz), 8.29 (d, 1 H, J = 10.5 Hz), 8.67 (s, 1 H); mass spectrum, m/z 362 $(M + H)^+$.

7-(2-Methylfur-5-yl)-6-fluoro-1-ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (24). An oven-dried system under a positive N_2 atmosphere was charged with 527 mg (1.46 mmol) of **23** dissolved in 40 mL of 1:1 benzene/1,2-dichloroethane and to this was added 278 mg (1.46 mmol) of p-toluenesulfonic acid. The reaction mixture was heated at 90 °C for 20 h. After cooling to 25 °C, the reaction mixture was diluted with 50 mL of EtOAc and was washed with 5% $NaHCO_3$ (2 × 100 mL) and 100 mL of saturated NaCl. The organic layer was dried over Na_2SO_4 and filtered. The solvent was removed on a rotary evaporator. Product was recrystallized from EtOH and dried under vacuum to give **24** as a white solid (185 mg, 40%): mp 257–258 °C; NMR ($CDCl_3$) δ 1.67 (t, 3 H, J = 7.5 Hz), 2.47 (s, 3 H), 4.46 (q, 2 H, J = 7.5 Hz),

6.24 (dd, 1 H, J = 4.5 Hz, J = 1.5 Hz), 7.08 (dd, 1 H, J = 4.5 Hz, J = 4.5 Hz), 7.95 (d, 1 H, J = 6.0 Hz), 8.19 (d, 1 H, J = 12.0 Hz), 8.77 (s, 1 H); mass spectrum, m/z 316 $(M + H)^+$; IR (KBr) 3420 (OH), 1720 (C=O) cm^{-1}.

In Vitro Antibacterial Activity. The in vitro antibacterial activity of the new compounds was tested in a side-by-side comparison with ciprofloxacin (1e) 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. Twofold 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 to 0.005 μg/mL. The plate was inoculated with approximately 10^4 organisms. It was then incubated at 36 °C for 18 h. The minimum inhibitory concentrations (MIC, μg/mL) were the lowest concentrations of the test compounds that yielded no visible growth on the plate.

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Analogues of 1,5-Bis(4-amidinophenoxy)pentane (Pentamidine) in the Treatment of Experimental *Pneumocystis carinii* Pneumonia

Richard R. Tidwell,*,† Susan Kilgore Jones,† J. Dieter Geratz,† Kwasi A. Ohemeng,† Michael Cory,‡ and James Edwin Hall§

Division of Medicinal and Biological Chemistry, Department of Pathology, School of Medicine and Department of Parasitology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, and Burroughs-Wellcome Company, Cornwallis Road, Research Triangle Park, North Carolina 27709. *Received July 24, 1989*

A series of 33 analogues of the anti-*Pneumocystis carinii* drug 1,5-bis(4-amidinophenoxy)pentane (pentamidine) was synthesized for screening against a rat model of *P. carinii* pneumonia (PCP). Twenty-five of the compounds showed efficacy against PCP when compared to a saline-treated control group. Two compounds, 1,4-bis(4-amidinophenoxy)butane (butamidine, **6**) and 1,3-bis(4-amidino-2-methoxyphenoxy)propane (**DAMP**, **16**), were statistically more effective than the parent drug in treating PCP in the rat model of infection. In addition to their activity against PCP, the compounds were also evaluated for antitrypsin activity, ability to inhibit thymidylate synthetase, affinity for DNA, and toxicity. No correlation was observed between the tested molecular interactions of the diamidines and their effectiveness against PCP.

An aromatic diamidino compound, pentamidine, was discovered as early as 1957 to be an effective agent for the treatment of *P. carinii* pneumonia (PCP).[1] Since then the drug has seen continued use for the treatment of PCP despite an extensive list of adverse reactions that include nephrotoxicity, hepatotoxicity, hypotension, and sterile abscesses at the injection site.[2,3] However, pentamidine was a distant second to the relatively nontoxic diaminopyrimidinesulfonamide combinations for the treatment of PCP.[4] This preference changed drastically with the clinical upsurge of cases of PCP caused by the acquired immune deficiency syndrome (AIDS) and the observation that trimethoprim-sulfamethoxazole caused a high frequency of adverse reactions in patients with AIDS-related PCP.[5,6] This unfortunate circumstance, combined with the finding that PCP is the leading cause of morbidity and mortality in AIDS patients,[7,8] has caused an increased dependency on the use of pentamidine in treatment of AIDS-related PCP. Recent studies have shown that the toxicity of pentamidine can be greatly reduced and drug efficacy increased by aerosol administration.[9,10] Despite these findings there is still an urgent need for a safe and effective drug that can be given either by oral or by parenteral administration for treatment of PCP associated with AIDS.

There is published record of only a handful of pentamidine-related compounds as having been tested against PCP. The screening of large numbers of drugs against PCP has been limited due to the lack of a dependable in vitro assay system. Therefore, the evaluation of anti-*P. carinii* drugs has depended on a somewhat cumbersome and expensive model utilizing immunosuppressed animals. The model involves the administration of corticosteroids to rats for a period of 6-8 weeks, resulting in the spontaneous induction of PCP.[11-14] A number of drug studies have shown that the rat model of PCP is an effective predictor of drug efficacy in humans.[11,12] An early report demonstrated that a diamidine derivative, hydroxystilbamidine, showed some activity against *P. carinii* in the rat model of the disease.[11] Two recent studies demonstrated that several dicationic molecules with structures related

† Department of Pathology, University of North Carolina at Chapel Hill.
‡ Burroughs-Wellcome.
§ Department of Parasitology, University of North Carolina at Chapel Hill.

(1) Ivady, G.; Paldy, L. *Monatsschr. Kinderheilkd.* **1957**, *106*, 10.
(2) Pearson, R. D.; Hewlett, E. L. *Ann. Intern. Med.* **1985**, *103*, 782.
(3) MacFarlane, J. T.; Finch, R. G. *Thorax* **1985**, *40*, 561.
(4) Walzer, P. D.; Kim, C. K.; Cushion, M. T. In *Parasitic Infections in the Compromised Host*; Walzer, P. D., Genta, R. M., Eds.; Marcel Dekker, Inc.: New York, 1989; pp 83-178.
(5) Jaffe, H. S.; Abrams, D. I.; Ammann, A. J.; Lewis, B. J.; Golden, J. A. *Lancet* **1983**, *2*, 1109.
(6) Gordin, F. M.; Simon, G. L.; Wofsy, C. B.; Mills, J. *Ann. Intern. Med.* **1984**, *100*, 495.
(7) Niedt, G. W.; Schinella, R. A. *Arch. Pathol. Lab. Med.* **1985**, *109*, 727.
(8) Moskowitz, L.; Hensley, G. T.; Chan, J. C.; Adams, K. *Arch. Pathol. Lab. Med.* **1985**, *109*, 735.
(9) Montgomery, A. B.; Luce, J. M.; Turner, J.; Lin, E. T.; Debs, R. J.; Corkery, K. J.; Brunette, E. N.; Hopewell, P. C. *Lancet* **1987**, *ii*, 480.
(10) Debs, R. J.; Blumenfeld, W.; Brunette, E. N.; Straubinger, R. M.; Montgomery, A. B.; Lin, E.; Agabian, N.; Papahadjopoulos, D. *Antimicrob. Agents Chemother.* **1987**, *31*, 37.
(11) Frenkel, J. K.; Good, J. T.; Shultz, J. R. *Lab. Invest.* **1966**, *15*, 1559.
(12) Walzer, P. D.; Kim, C. K.; Foy, J.; Linke, M. J.; Cushion, M. T. *Antimicrob. Agents Chemother.* **1988**, *32*, 896.
(13) Hughes, W. T.; McNabb, P. C.; Makers, T. D.; Feldman, S. *Antimicrob. Agents Chemother.* **1974**, *5*, 289.
(14) Tidwell, R. R.; Kilgore, S. G.; Ohemeng, K. A.; Geratz, J. D.; Hall, J. E. *J. Protozool.* **1989**, *36*, 74S.

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