4 H), 3.32 (m, 1 H), 3.60 (s, 3 H), 4.28 (m, 1 H), 4.45 (m, 1 H), 4.68 (m, 1 H), 5.00 (m, 1 H), 5.49 (m, 1 H), 5.70 (m, 1 H), 6.01 (br d, 1 H), 7.14–7.24 (m, 15 H), 7.40 (br d, 1 H), 7.70 (br s, 1 H), 8.13 (d, 1 H); positive ion MS (calculated for $C_{48}H_{62}F_2N_4O_8$ + 1) 861.46141, obsd 861.46141.

Renin-Inhibition Studies. The inhibitory potency of the compounds was measured by their ability to inhibit the cleavage of human angiotensinogen by human kidney renin. Human kidney renin (0.02 ng, purified as described[22]) was incubated with angiotensinogen (500 ng, partially purified from outdated human plasma up through the DE-52 chromatography step as described[23]) for 60 min at 37 °C in citrate phosphate buffer (0.1 M, pH 7.20, 0.10 mL) containing inhibitor. The inhibitors were delivered to the assay from stock solutions in methanol. The reactions were stopped by immersion in a boiling water bath for 1 min. The angiotensin I produced was quantitated by radioimmunoassay by competition with ^{125}I-labeled angiotensin I by using a Travenol Labs Angiotensin I radioimmunoassay kit. A plot of renin activity vs. inhibitor concentration was made and the concentration of inhibitor required to inhibit the enzymes by 50% (IC^5_0) was estimated graphically. Since the angiotensinogen concentration in this assay is much lower than its Michaelis constant,[22] the IC_{50} is equal to the inhibition constant (K_i), assuming competitive inhibition. The K_i values determined in this manner are reliable to about ±30% on the basis of multiple repitition of the measurement.

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Registry No. 4, 96056-65-4; (3*S*)-4, 97920-08-6; **5**, 108385-36-0; **6**, 109064-95-1; (3*S*,4*R*)-**6**, 109121-33-7; **7**, 109064-96-2; **7**·TFA, 109064-97-3; **8**, 109064-98-4; **9**, 108385-34-8; **10**, 98858-47-0; 11, 109064-99-5; **12**, 109065-00-1; **13**, 109065-01-2; 14, 109065-02-3; **15**, 109065-03-4; (2*S*)-**15**, 109065-09-0; **16**, 109065-04-5; **17**, 109065-06-7; **18**, 109065-07-8; 5(*S*)-[(*tert*-butyloxycarbonyl)-amino]-6-phenyl-2(*R*)-(phenylmethyl)-3-*trans*-hexenoic acid, 108385-61-1; ethyl bromodifluoroacetate, 667-27-6; *N*-(*tert*-butyloxycarbonyl)leucinal, 58521-45-2; L-leucyl-L-phenylalaninamide hydrochloride, 74214-38-3; *N*-(*tert*-butyloxycarbonyl)-L-leucyl-L-phenylalaninamide, 33900-15-1; *N*-(*tert*-butyloxycarbonyl)-L-phenylalanyl-L-phenylalanine, 13122-90-2; renin, 9015-94-5.

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Pyridonecarboxylic Acids as Antibacterial Agents. 9.[1] Synthesis and Antibacterial Activity of 1-Substituted 6-Fluoro-1,4-dihydro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylic Acids

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The title compounds (**7a–e**) with ethyl, 2-fluoroethyl, 2-hydroxyethyl, vinyl, or cyclopropyl groups, respectively, at C-1 were prepared by the method involving the Balz–Schiemann reaction of 2-(4-pyridyl)pyridine- and 7-(4-pyridyl)-1,8-naphthyridinediazonium tetrafluoroborates (**15** and **27**). The 1-ethyl, 1-(2-fluoroethyl), and 1-vinyl derivatives showed in vitro activities as potent as the corresponding 7-(1-piperazinyl) analogues against *Staphylococcus aureus* 209P JC-1 and *Escherichia coli* NIHJ JC-2 but were less active against *Pseudomonas aeruginosa* 12. Among the 7-(4-pyridyl) derivatives having the different C-1 substituent, 1-cyclopropyl derivative **7e** was found to be the most active. In vivo efficacy of **7e** was superior to that of enoxacin against experimental infections due to *S. aureus* 50774. Some aspects of structure–activity relationships associated with the C-1, C-6, and C-7 substituents were discussed.

During the last decade, a new class of pyridonecarboxylic acid antibacterials with much improved potency and broad antibacterial spectrum has been developed. Included in this class are pefloxacin,[2] norfloxacin,[3] enoxacin (**1**),[4] ofloxacin,[5] and ciprofloxacin,[6] which are chemically characterized by having both fluorine and piperazine substituents in each molecule. A combination of the piperazine at C-7 with the fluorine at C-6 on the quinolone or azaquinolone ring system is now well known to be a reliable means for obtaining a potent analogue of this class. Further efforts have been devoted thus far to a search of C-7 substituents that might cause a greater increase in antibacterial activity. We found previously that a 3-aminopyrrolidine substituent, instead of the C-7 piperazine, was efficient for enhancing antibacterial activity in combination with the C-6 fluorine.[7] Lesher et al.[8] reported the synthesis of rosoxacin (**2**), in which a 4-pyridyl group

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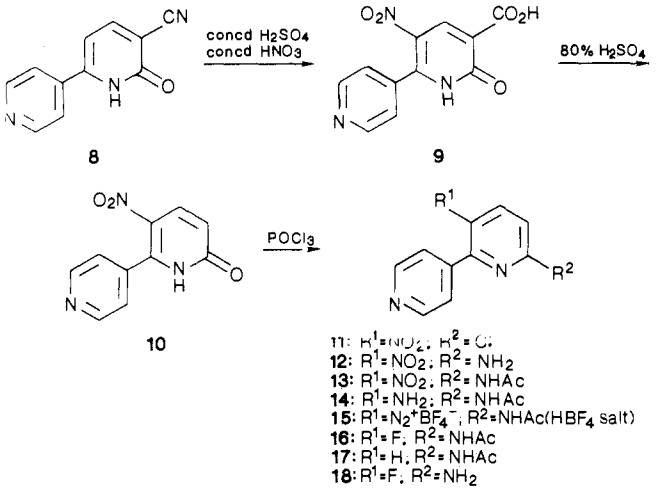
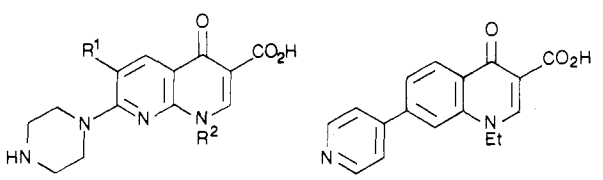
Scheme I

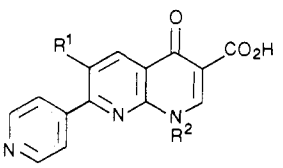


was comprised as the C-7 substituent on the quinolone ring, with fairly potent activity against a wide range of bacteria. Therefore, it was expected that an introduction of the C-6 fluorine to rosoxacin analogues having the C-7 pyridine would cause an enhancement of activity. The present study was undertaken to see if this structure–activity relationship expectation was true or not and to find new promising candidates for antibacterial agents. Thus a series of 1-substituted 6-fluoro-1, 4-dihydro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylic acids (**7a–e**) was synthesized and evaluated for their antibacterial activity, compared with those of the corresponding 6-fluoro-7-(1-piperazinyl) (**1**, **4**, and **5**),[4] 6-defluoro-7-(1-piperazinyl) (**3**)[9] and 6-defluoro-7-(4-pyridyl) analogues (**2** and **6**).[8]



1 (enoxacin): R^1=F; R^2=Et
3: R^1=H; R^2=Et
4: R^1=F; R^2=CH_2CH_2F
5: R^1=F; R^2=$CH{=}CH_2$

2 (rosoxacin)

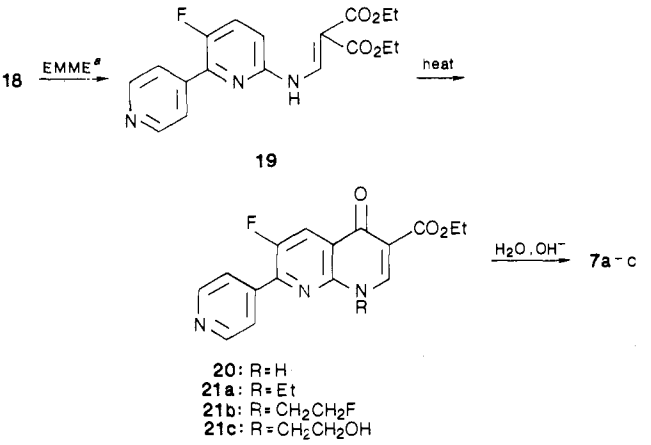


6: R^1=H; R^2=Et
7a: R^1=F; R^2=Et
7b: R^1=F; R^2=CH_2CH_2F
7c: R^1=F; R^2=CH_2CH_2OH
7d: R^1=F; R^2=$CH{=}CH_2$
7e: R^1=F; R^2=c-C_3H_5

Chemistry

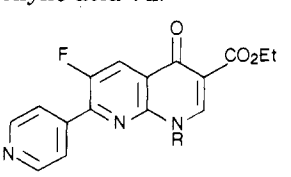
An intermediate, 6-(acetylamino)-3-fluoro-2-(4-pyridyl)pyridine (**16**), was prepared by the route depicted in Scheme I. 1,2-Dihydro-2-oxo-6-(4-pyridyl)-nicotinonitrile (**8**), derived by the Lesher's method,[8b] was treated with a mixed acid (concentrated sulfuric acid and concentrated nitric acid) to give the nitro compound **9**, though the yield was unsatisfactory. Treatment of **9** with 80% sulfuric acid afforded 1,6-dihydro-3-nitro-6-oxo-2-(4-pyridyl)pyridine (**10**). Chlorination of **10** with phosphoryl chloride, followed by amination of the product **11** with ethanolic ammonia in a sealed tube and successive acetylation of the resulting product **12** with acetic anhydride, gave 6-(acetylamino)-3-nitro-2-(4-pyridyl)pyridine (**13**) in good yield. Hydrogenation of **13** with Raney Ni afforded the amino analogue **14**, which, without isolation, was diazotized with sodium nitrite in 42% tetrafluoroboric acid. Heating the resulting diazonium salt **15** in xylene led to the Balz–Schiemann reaction to give the requisite fluoropyridine **16**. In connection with this, it is of interest to note that when **14** was diazotized with isoamyl nitrite in ethanol, a loss of the diazonium group occurred with ease to give the C-3 hydrogen analogue **17**, which served as an intermediate for the synthesis of the defluoro compound **6**. Although it was expected that **17** could be derived from **8** via the corresponding denitro analogues of **10–12** by the foregoing method (Scheme I), an attempted amination of the denitro compound of **11** was unsuccessful, probably for lack of activation caused by the nitro group. The deacetyl derivative of **17** had already been described by Lesher et al.[8b] Their method, however, involves more drastic reaction conditions. Hence the present procedure for the synthesis of **17** via **14** seems much more convenient.

Scheme II



[a] EMME = diethyl (ethoxymethylene)malonate.

The desired 1-substituted 6-fluoro-1, 4-dihydro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylic acids (**7a–c**) were prepared by the route shown in Scheme II. 6-Amino-3-fluoro-2-(4-pyridyl)pyridine (**18**) derived from **16** was treated with diethyl (ethoxymethylene)malonate (EMME). Thermal cyclization of the product **19** gave ethyl 6-fluoro-1,4-dihydro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylate (**20**). Treatment of the sodium salt of **20** with ethyl iodide, 2-fluoroethyl tosylate, and ethylenebromohydrin gave the 1-substituted analogues **21a–c**, respectively. These were hydrolyzed with sodium hydroxide to afford the corresponding carboxylic acids **7a–c**. Chlorination of **21c** with thionyl chloride followed by treatment of the resulting 1-(2-chloroethyl) derivative **22** with an alkali resulted in the elimination of hydrochloric acid and concomitant hydrolysis of the ester group to give the 1-vinyl carboxylic acid **7d**.



22: R=CH_2CH_2Cl

Since the 1-cyclopropyl derivative **7e** is not accessible by the method involving the N-alkylation of **20** (Scheme

(9) Hirose, T.; Mishio, S.; Matsumoto, J.; Minami, S. *Chem. Pharm. Bull.* **1982**, *30*, 2399.

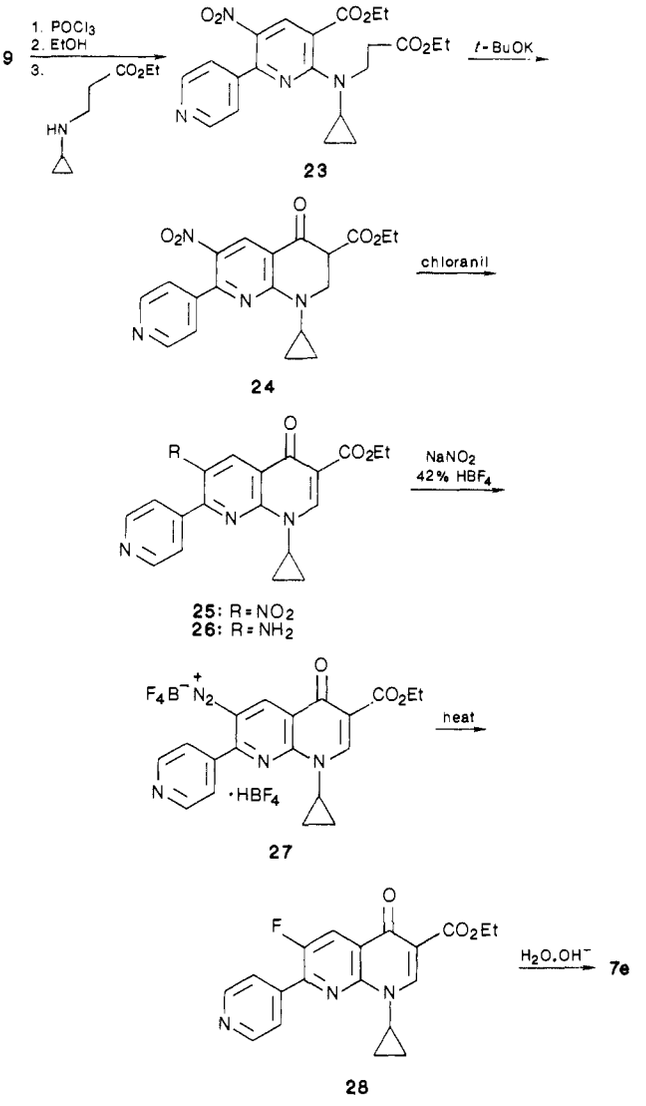
Table I. In Vitro Antibacterial Activity[a]

compd	min inhibitory concn, μg/mL		
	S. aureus 209P JC-1	*E. coli* NIHJ JC-2	*P. aeruginosa* 12
7a	0.39	0.2	3.13
7b	0.39	0.2	1.56
7c	50	1.56	50
7d	1.56	0.78	6.25
7e	0.2	0.1	0.78
3	12.5	6.25	12.5
4	0.39	0.2	0.78
5	1.56	0.1	0.39
6	1.56	0.78	12.5
RSX[b]	0.78	0.39	3.13
ENX[c]	0.78	0.2	0.78

[a] See the Experimental Section. [b] Rosoxacin. [c] Enoxacin.

II), it was prepared by another method involving the Dieckmann cyclization as shown in Scheme III. Thus **9** was chlorinated with phosphoryl chloride and treated successively with ethanol and ethyl 3-(cyclopropylamino)-propionate to give the diester **23**. Treatment of **23** with potassium *tert*-butoxide resulted in the Dieckmann cyclization to give the keto ester **24**. Oxidation of **24** with chloranil gave the 1,4-dihydro-6-nitro-4-oxo-1, 8-naphthyridine derivative **25** in good yield. Hydrogenation of **25** followed by diazotization of the resulting amine **26** with sodium nitrite in 42% tetrafluoroboric acid afforded the borofluorodiazoate **27**, which was subjected to the thermal decomposition at 100 °C to give ethyl 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylate (**28**). This ester **28** was hydrolyzed with an alkali to the desired compound **7e**.

Scheme III



Biological Results and Discussion

The in vitro antibacterial activity of compounds **7a–e** against representatives of Gram-positive (*Staphylococcus aureus* 209P JC-1) and Gram-negative (*Escherichia coli* NIHJ JC-2 and *Pseudomonas aeruginosa* 12) bacteria were tested and the results are summarized in Table I. Included for comparison are the activity of enoxacin (**1**), rosoxacin (**2**), defluoroenoxacin [1-ethyl-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid] (**3**),[9] 6-fluoro-1-(2-fluoroethyl)-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid (**4**),[4] 6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1-vinyl-1,8-naphthyridine-3-carboxylic acid (**5**),[4] and defluoro-**3a** [1-ethyl-1,4-dihydro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylic acid] (**6**).[8b]

In comparison of enoxacin with defluoroenoxacin (**3**), the remarkable influence of a combination of the C-7 piperazine with the C-6 fluorine on activities was observed as reported previously.[4] Thus enoxacin is more than 16 times as active as **3** against all the bacteria tested. The combination of the C-7 pyridine with the C-6 fluorine (compare **7a** with **6**), however, was not so highly effective as we expected; **7a** is only 4 times as active as **6** against all the bacteria. Replacement of the piperazinyl group of enoxacin (**1**), **4**, and **5** by the 4-pyridyl group (giving **7a**, **7b**, and **7d**, respectively) resulted in little change in activity against *S. aureus* 209P JC-1 and *E. coli* NIHJ JC-2 whereas, against *P. aeruginosa* 12, it caused a considerable decrease in activity.

Variation of the N-1 alkyl group influenced antibacterial activity with varied results. The 2-hydroxyethyl derivative **7c** is much less active than the ethyl counterpart **7a**, and the vinyl derivative **7d** is 2–4 times less active than **7a** against the bacteria tested. There is essentially no difference in activity between the 2-fluoroethyl derivative **7b** and the ethyl analogue **7a**.

Among compounds **7a–e**, which possess the 4-pyridyl group at C-7, the cyclopropyl derivative **7e** was found the most active in vitro. Compound **7e**, along with **7b**, was then tested on systemic infections due to *S. aureus* 50774, *E. coli* P-5101, and *P. aeruginosa* 12, with oral administration in mice and compared with rosoxacin and enoxacin. The results are given in Table II, which includes for reference MICs against organisms employed. Compounds **7b**

Table II. Oral Efficacy on Systemic Infections in Mice[a]

compd	*S. aureus* 50774		*E. coli* P-5101		*P. aeruginosa* 12	
	ED_{50}[b]	MIC[c]	ED_{50}	MIC	ED_{50}	MIC
7b	3.37	0.39	4.91	0.1	14.9	1.56
7e	5.43	0.1	4.48	0.05	15.0	0.78
RSX[d]	6.8	0.39	7.3	0.2	29.2	3.13
ENX[e]	10.0	0.78	1.9	0.1	9.0	0.78

[a] See the Experimental Section. [b] In milligrams/kilogram. [c] In micrograms/milliliter. [d] Rosoxacin. [e] Enoxacin.

and **7e** are more potent than rosoxacin against all the experimental infections tested but less active than enoxacin against infections due to Gram-negative bacteria (*E. coli* and *P. aeruginosa*).

As a result of the present study, the combination of the C-6 fluorine with the 7-(4-pyridyl) substituent is not so effective for enhancing antibacterial activity as expected. However, 6-fluoro-7-(4-pyridyl)-1,8-naphthyridine derivatives were found to have potent antibacterial activity in vitro and in vivo against Gram-positive bacteria. In particular, **7b** and **7e** are worth further evaluation as possible potent antibacterials.

Experimental Section

Chemistry. All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Hitachi Model 215 spectrophotometer. ^{1}H NMR spectra were taken at 60 or 100 MHz on either a Varian EM-360A or a HA-100D spectrometer. Mass spectra was recorded on a Hitachi RMU-6L spectrometer. The spectral data were obtained on all compounds and were consistent with the assigned structures. Elemental analysis are indicated only by symbols of the elements; analytical results were within ±0.4% of theoretical values.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylic Acid (7a). A mixture containing 500 mg (1.5 mmol) of **21a** and 10 mL of 2 N NaOH was heated at 90–100 °C for 20 min with stirring. The mixture was cooled and adjusted to pH 7.0–7.5 with AcOH. The precipitate was filtered off and recrystallized from EtOH to give 200 mg (44%) of **7a**, mp 286–288 °C. Anal. ($C_{16}H_{12}FN_3O_3$) C, H, F, N.

According to this procedure, compounds **7b**, **7c**, and **7e** were prepared from **21b**, **21c**, and **28**, respectively. **7b**: mp 270–272 °C (recrystallized from EtOH; 36%). Anal. ($C_{16}H_{11}F_2N_3O_3$) C, H, F, N. **7c**: mp 285–289 °C (recrystallized from DMF; 91%). Anal. ($C_{16}H_{12}FN_3O_4$) C, H, F, N. **7e**: mp >300 °C (recrystallized from $CHCl_3$–EtOH; 54%). Anal. ($C_{17}H_{12}FN_3O_3$) C, H, F, N.

6-Fluoro-1,4-dihydro-4-oxo-7-(4-pyridyl)-1-vinyl-1,8-naphthyridine-3-carboxylic Acid (7d). A mixture containing 200 mg (0.5 mmol) of **22** and 10 mL of 1 N NaOH was heated at 90–100 °C for 4 h with stirring and then cooled. The resulting solid was collected and dissolved in water, and the solution was adjusted to pH 6.0 with AcOH. The precipitate was filtered off to give 85 mg (51.3%) of **7d**, which was recrystallized from EtOH; mp 254–257 °C. Anal. ($C_{16}H_{10}FN_3O_3$) C, H, F, N.

1,2-Dihyro-5-nitro-2-oxo-6-(4-pyridyl)nicotinic Acid (9). To a stirred mixture of concentrated H_2SO_4 (180 mL) and concentrated HNO_3 (120 mL) was added portionwise 1,2-dihydro-2-oxo-6-(4-pyridyl)nicotinonitrile (**8**)[8b] (47 g, 194 mmol) at 90–95 °C. The reaction mixture was stirred at the same temperature for 1.5 h, poured onto ice, and adjusted to pH 3.0–3.5 with 40% NaOH. The resulting solid was collected and dissolved in water and the solution was adjusted to pH 6.5–7.0 with 10% NaOH. The precipitate was filtered off and washed successively with water and EtOH to give 35 g (56%) of **9**, which was recrystallized from DMF; mp >300 °C. Anal. ($C_{11}H_7N_3O_5$) C, H, N.

1,6-Dihydro-3-nitro-6-oxo-2-(4-pyridyl)pyridine (10). A mixture containing 35 g (134 mmol) of **9** and 100 mL of 80% H_2SO_4 was heated to reflux for 5 h and poured onto ice. The resulting solution was neutralized with 40% NaOH. The resulting solid was collected, washed with water, and recrystallized from EtOH to give 13.8 g (47%) of **10**, mp >300 °C. Anal. ($C_{10}H_7N_3O_3$) C, H, N.

6-Chloro-3-nitro-2-(4-pyridyl)pyridine (11). A suspension of **10** (13 g, 60 mmol) in 40 mL of $POCl_3$ was heated at 80 °C for 17 h with stirring, poured onto ice, and adjusted to pH 9 with 10% NaOH. The resulting solid was collected, washed with water, and dried to give 18.8 g (96%) of **11**, which was recrystallized from *i*-Pr_2O; mp 137–138 °C. Anal. ($C_{10}H_6ClN_3O_2$) C, H, Cl, N.

6-Amino-3-nitro-2-(4-pyridyl)pyridine (12). In a 100-mL sealed tube were placed 5.5 g (29 mmol) of **11** and 50 mL of 12% ethanolic ammonia. The mixture was heated at 135 °C for 4 h and then cooled. The water was added and the mixture was concentrated in vacuo. The resulting solid was collected and recrystallized from EtOH to give 4.82 g (95%) of **12**, mp >300 °C. Anal. ($C_{10}H_8N_4O_2$) C, H, N.

6-(Acetylamino)-3-nitro-2-(4-pyridyl)pyridine (13). A mixture containing 4.3 g (20 mmol) of **12**, 3.0 g (30 mmol) of Ac_2O, and 30 mL of AcOH was heated to reflux for 4 h. The mixture was concentrated to dryness in vacuo. The residue was crystallized from EtOH to give 4.6 g (90%) of **13**, mp 285–289 °C. Anal. ($C_{12}H_{10}N_4O_3$) C, H, N.

6-(Acetylamino)-3-fluoro-2-(4-pyridyl)pyridine (16). A mixture containing 7.7 g (33.3 mmol) of **13**, 5 g of Raney Ni, and 200 mL of EtOH was shaken under H_2 gas until the required volume of hydrogen was absorbed. The mixture was filtered to remove the catalyst and the filtrate was concentrated to dryness in vacuo to give 3-amino analogue **14**, which was dissolved in 100 mL of 42% HBF_4. To the mixture kept at 7–10 °C was gradually added a solution of $NaNO_2$ (2.5 g, 36.2 mmol) in 10 mL of water. After 10 min of stirring with ice-cooling, 400 mL of cold EtOH was added to the mixture and the reaction mixture was stirred for an additional 20 min. The precipitate was collected, washed with EtOH, and dried in vacuo to give 12.7 g of **15**, which was suspended in 150 mL xylene. The suspension was heated to reflux for 3 h and then cooled. The resulting solid was collected and suspended in water and the suspension was made alkaline with 10% NaOH. The precipitate was filtered off and washed with water to give 5.35 g (78%) of **16**, which was recrystallized from EtOH; mp 254–256 °C. Anal. ($C_{12}H_{10}FN_3O$) C, H, F, N.

2-(Acetylamino)-6-(4-pyridyl)pyridine (17). A mixture containing 16.5 g (64 mmol) of **13**, 8 g of Raney Ni, and 500 mL of EtOH was shaken under H_2 gas until the required volume of hydrogen was absorbed. The mixture was filtered to remove the catalyst. To the filtrate was added 20 g (171 mmol) of isoamyl nitrite. The mixture was heated to reflux for 3 h and concentrated to dryness in vacuo. The residue was triturated with Et_2O to give 9.0 g (66.1%) of **17**. The structure of **17** was confirmed by spectral data and by conversion to 2-amino-6-(4-pyridyl)pyridine, mp 193–196 °C (lit.[8b] mp 195–197 °C), on treating with 10% HCl at 90°C for 1 h.

Diethyl [[*N*-[3-Fluoro-2-(4-pyridyl)-6-pyridyl]amino]-methylene]malonate (19). A mixture containing 5.3 g (22.9 mmol) of **16**, 70 mL of MeOH, and 35 mL of 15% HCl was heated to reflux for 1.5 h. The resulting mixture was concentrated to dryness in vacuo, and the residue was dissolved in water. The solution was made alkaline with 20% NaOH. The precipitate, 6-amino-3-fluoro-2-(4-pyridyl)pyridine (**18**), was filtered off, dried, and then added to a solution of 5.9 g (27.3 mmol) of diethyl (ethoxymethylene)malonate in 20 mL of EtOH. The mixture was heated to reflux for 2 h and concentrated to dryness in vacuo, and the residue was triturated with Et_2O to give 6.7 g (82%) of **19**, which was recrystallized from AcOEt; mp 140–141 °C. Anal. ($C_{18}H_{18}FN_3O_4$) C, H, F, N.

Ethyl 6-Fluoro-1,4-dihydro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylate (20). A mixture containing 600 mg (1.7 mmol) of **19** and 6 mL of Dowtherm A was heated at 250–252 °C for 10 min and allowed to cool to room temperature. The resulting solid was collected. The mother liquor was additionally heated at 250–252 °C for 10 min and worked up again with the above procedure. The combined solid was washed with EtOH to give 390 mg (75%) of **20**, which was recrystallized from DMF; mp 280–285 °C. Anal. ($C_{16}H_{12}FN_3O_3$) C, H, F, N.

Ethyl 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylate (21a). A stirred suspension of 2.63 g (8.4 mmol) of **20** and 403 mg (8.4 mmol) of 50% NaH (in a mineral oil) in 30 mL of DMF was heated at 60 °C for 30 min. To this mixture was added 1.31 g (8.4 mmol) of ethyl iodide. The mixture was kept at the same temperature with stirring for 1.5 h and then filtered to remove the insoluble materials. The filtrate was concentrated to dryness in vacuo. The residue was taken up in a mixture of water and $CHCl_3$. The organic layer was separated and concentrated to dryness in vacuo. The residue was chromatographed on silica gel with $CHCl_3$ to give 2.06 g (72%) of **21a**, which was recrystallized from AcOEt; mp 185–188 °C. Anal. ($C_{18}H_{16}FN_3O_3$) C, H, F, N.

According to this procedure, compounds **21b** and **21c** were prepared from **20**. **21b**: mp 200–201 °C (recrystallized from AcOEt; 50%). Anal. ($C_{18}H_{15}F_2N_3O_3$) C, H, F, N. **21c**: mp 223–225 °C (recrystallized from EtOH; 44%). Anal. ($C_{18}H_{16}FN_3O_4$) C, H, F, N.

Ethyl 1-(2-Chloroethyl)-6-fluoro-1,4-dihydro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylate (22). A mixture containing 1.5 g (4.2 mmol) of **21c**, 1.0 g (8.4 mmol) of $SOCl_2$, and 30 mL of $CHCl_3$ was heated to reflux for 30 min. After the mixture was cooled, ice and 10% NaOH were added. The organic layer was separated and concentrated to dryness in vacuo. The residue was crystallized from CH_3CN to give 1.25 g (79%) of **22**, mp 192-194 °C. Anal. ($C_{18}H_{15}ClFN_3O_3 \cdot 0.5H_2O$) C, H, Cl, F, N.

Ethyl 2-[*N*-Cyclopropyl-*N*-[2-(ethoxycarbonyl)ethyl]-amino]-5-nitro-6-(4-pyridyl)nicotinate (23). A mixture containing 2.0 g (7.7 mmol) of **9** and 20 mL of $POCl_3$ was heated to reflux for 2 h and then concentrated to dryness in vacuo. The residue was taken up in a mixture of $CHCl_3$ and EtOH. The solution was heated to reflux for 1 h and concentrated to dryness in vacuo, and the residue was taken up in a mixture of ice, 10% NaOH, and $CHCl_3$. The organic layer was separated and concentrated to dryness in vacuo. To the residue was added 2.4 g (15.4 mmol) of ethyl 3-(cyclopropylamino)propionate in 30 mL of EtOH. The mixture was heated to reflux for 1.5 h and then concentrated to dryness in vacuo. The residue was taken up in a mixture of water and $CHCl_3$. The organic layer was separated and concentrated to dryness in vacuo. The residue was chromatographed on silica gel with $CHCl_3$ to give 1.5 g (61%) of **23**, which was recrystallized from *i*-Pr_2O; mp 106-107 °C. Anal. ($C_{21}H_{24}N_4O_6$) C, H, N.

Ethyl 1-Cyclopropyl-1,2,3,4-tetrahydro-6-nitro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylate (24). To a solution of **23**, (2.25 g, 5.3 mmol) in 230 mL of *t*-BuOH was added portionwise 920 mg of *t*-BuOK (8.2 mmol) at room temperature and the mixture was stirred at the same temperature for 7 h, and then 200 mL of water was added. The reaction mixture was adjusted to pH 7.0 with AcOH. The precipitate was filtered off, washed with water, and dried to give 1.6 g (83%) of **24**, which was recrystallized from EtOH; mp 195-198 °C. Anal. ($C_{16}H_{18}N_4O_5$) C, H, N.

Ethyl 1-Cyclopropyl-1,4-dihydro-6-nitro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylate (25). A mixture containing 550 mg (1.4 mmol) of **24**, 650 mg (2.6 mmol) of chloranil, and 30 mL of dioxane was heated at 60 °C for 1 h with stirring and then concentrated to dryness in vacuo, and the residue was taken up in a mixture of 1 N NaOH and $CHCl_3$. The organic layer was separated and concentrated to dryness in vacuo and the residue was crystallized from AcOEt to give 500 mg (92%) of **25**, mp 200-201 °C. Anal. ($C_{19}H_{16}N_4O_5$) C, H, N.

Ethyl 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylate (28). A mixture containing 1.85 g (4.9 mmol) of **25**, 100 mg of 5% Pd-C, and 40 mL of AcOH was shaken under H_2 gas until the required volume of hydrogen was absorbed. The reaction mixture was filtered to remove the catalyst and the filtrate was concentrated to dryness in vacuo. The residue was dissolved in 25 mL of 42% HBF_4. To this solution kept at 1-10 °C was gradually added a solution of $NaNO_2$ (500 mg, 7.2 mmol) in 3 mL of water. The mixture was stirred at the same temperature for an additional 20 min and diluted with 90 mL of cold EtOH. The resulting solid was collected, dried in vacuo, and suspended in 70 mL of toluene. The suspension was heated at 100 °C for 1 h with stirring. The solvent was removed by decantation and the residue was taken up in a mixture of 1 N NaOH and $CHCl_3$. The organic layer was separated, concentrated to dryness in vacuo and the residue was crystallized from AcOEt to give 700 mg (42%) of **28**, mp 209-211 °C. Anal. ($C_{19}H_{16}FN_3O_3$) C, H, F, N.

In Vitro Antibacterial Activity. According to the method of Goto et al.,[10] the MIC (in micrograms per milliliter) was determined by the twofold agar dilution method using Mueller-Hinton agar (pH 7.4, Difco); bacterial inocula contained approximately 10^6 colony-forming units and the bacterial growth was observed after 20-h incubation at 37 °C.

In Vivo Efficacy on Systemic Infections. In vivo activity assay was carried out according to the method of Shimizu et al.[11] Groups of 10 or more male mice (Std-ddY, 20 ± 2 g) were infected with *Staphylococcus aureus* 50774 (iv, 5×10^8 cells), *Escherichia coli* P-5101 (ip, 9×10^6 cells), and *Pseudomonas aeruginosa* 12 (ip, 4×10^3 cells). The test compounds were suspended in 0.2% sodium (carboxymethyl)cellulose and administered orally at 0 and 6 h postinfection. Survival rates were evaluated after 2 weeks for the staphylococcal infection and after 1 week for others.

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Synthesis and Biological Properties of Actinomycin D Chromophoric Analogues Substituted at the 7-Carbon with Aziridine and Aminopropoxy Functions

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The growing importance of functionalized aziridines in numreous organic biomolecules led us to develop syntheses of novel actinomycin D (AMD) analogues substituted with an aziridine. Reaction of 7-hydroxyactinomycin D with 2-(iodomethyl)aziridine produced the desired 7-(2-aziridinylmethoxy)actinomycin analogue. In an attempt to develop an alternate route to this analogue, 7-(2-azido-3-iodopropoxy)actinomycin was subjected to reduction with dimethylamine-borane complex; the reaction did not produce the three-membered aziridine; instead the reaction product was found to be linear 7-(2-aminopropoxy)actinomycin D. Calf-thymus-DNA binding of these analogues was comparable to that of AMD as examined by UV-visible difference spectral measurements, thermal denaturation of DNA, and CD techniques. The analogues were found to be about $^1/_4$ to $^1/_{30}$ as cytotoxic to human lymphoblastic CCRF-CEM leukemia and B_{16} melanoma cells in vitro as AMD.

Actinomycin D (AMD, **1b**) has been used clinically as a chemotherapeutic agent in the treatment of Wilms' tumor[1] and gestational choriocarcinoma[2] for some time. It is known to bind to double-helical DNA by intercalation

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