solved/suspended in concentrated H_2SO_4 (30 mL) at 0 °C. After 15 min, concentrated HNO_3 (0.75 mL) was added resulting in a gradual color change from yellow to lime over 15 min. After 30 min, the reaction mixture was poured into ice/H_2O (150 mL) and the resulting suspension extracted with portions of $CHCl_3$. The extracts were washed with H_2O, dried (Na_2SO_4), and evaporated to give compound **23** as a yellow solid (509 mg, 76%). Recrystallization of the sample from MeOH–$CHCl_3$ (13:87) gave pure **23** as a yellow microcrystalline solid: mp gradual dec and blackening above 220 °C; IR (KBr) 3440 (OH), 1752 (lactone), 1655 (pyridone), 1590 (aromatic), 1532 (NO_2) cm^{-1}; ^1H NMR (TFA-d_1) δ 1.16 (t, 3, J = 7 Hz, H-18), 2.19 (q, 2, J = 7 Hz, H-19), 4.32 (s, 3, OCH_3), 5.78 (ABq, 2, J = 18 Hz, $\Delta\gamma$ = 85 Hz, H-17), 5.85 (s, 2, H-5), 8.31 (d, 1, J = 9 Hz, H-11), 8.48 (s, 1, H-14), 8.85 (d, 1, J = 9 Hz, H-12), 9.28 (s, 1, H-7); $[\alpha]^{23}_D$ 21° (c 0.10, MeOH–$CHCl_3$, 1:4); HPLC retention time 5.1 min (2.0 mL/min; $CHCl_3$–MeOH, 995:5). Anal. Calcd for $C_{21}H_{17}N_3O_7$: 423.1066. Found: 423.1065. ($C_{21}H_{17}N_3O_7$), C, H, N.

9-Amino-10-methoxy-20(*S*)-camptothecin (24). A stirred solution/suspension of $SnCl_2 \cdot 2H_2O$ (600 mg) and Sn powder (25 mg) in a mixture of absolute EtOH and concentrated HCl (3 mL each) was cooled to 0 °C and treated portionwise with solid compound **23**. The turbid mixture was permitted to warm to room temperature during which time there was a color change from lemon to orange. After 4.5 h, the solvents were removed in vacuo. The tan residue was suspended/dissolved in MeOH (50 mL) and the pH adjusted to 6–7 using concentrated NH_4OH. The sample was diluted with $CHCl_3$ and adsorbed onto Celite (3 g). Chromatography through silica gel (20 g; gradient: 500 mL MeOH–$CHCl_3$, 2:98; 500 mL MeOH–$CHCl_3$, 7:93) afforded pure **24** as an orange solid (142 mg, 75%). Compound **24** resulted as an amorphous orange solid by precipitation from MeOH–$CHCl_3$ (13:87): mp 276–280 °C dec; IR (KBr) 3465, 3378 (NH_2), 3150 (OH), 1750 (lactone), 1650 (pyridone), 1585 (aromatic) cm^{-1}; ^1H NMR (TFA-d_1) δ 1.15 (t, 3, J = 7 Hz, H-18), 2.16 (q, 1, J = 7 Hz, H-19), 4.37 (s, 3, OMe), 5.78 (ABq, 2, J = 18 Hz, $\Delta\gamma$ = 85 Hz, H-17), 5.82 (s, 2, H-5), 8.29 (d, 1, J = 9.5 Hz, H-11), 8.35 (s, 1, H-14), 8.76 (d, 1, J = 9.5 Hz, H-12), 9.61 (s, 1, H-7); $[\alpha]^{23}_D$ 27.5° (c 0.04, MeOH–$CHCl_3$, 1:4); HPLC retention time 3.3 min (2.0 mL/min; $CHCl_3$–MeOH, 98:2). Anal. Calcd for $C_{21}H_{19}N_3O_5$: 393.1324. Found: 393.1329. ($C_{21}H_{19}N_3O_5 \cdot 0.5H_2O$) C, H, N.

9-Nitro-10-hydroxy-20(*S*)-camptothecin (25). 10-Hydroxy-20(*S*)-camptothecin (**2**) (160 mg, 0.44 mmol) was dissolved/suspended in 30% aqueous HNO_3 (10 mL) at room temperature. After 1 h, concentrated HNO_3 (70%, 1 mL) was added to the stirred, turbid orange mixture, and the reaction was left for 18 h. The clear orange solution was extracted repeatedly with $CHCl_3$, and the resulting yellow solution was washed with H_2O, dried (Na_2SO_4), and evaporated in vacuo to give crude **25** as a yellow solid (193 mg). The usual chromatography of the Celite-adsorbed material on silica gel using MeOH–$CHCl_3$ (1:9) gave pure **25** (85 mg, 47%). Recrystallization (MeOH–$CHCl_3$, 13:87) gave the compound as a microcrystalline solid: mp 206–210 °C; IR (KBr) 3405 (OH), 1741 (lactone), 1657 (pyridone), 1590 (aromatic), 1528 (NO_2) cm^{-1}; ^1H NMR (TFA-d_1) δ 1.15 (t, 3, J = 7 Hz, H-18), 2.17 (q, 2, J = 7 Hz, H-19), 5.79 (ABq, 2, J = 18 Hz, $\Delta\gamma$ = 85 Hz, H-17), 5.88 (s, 2, H-5), 7.26 (s, $CHCl_3$), 8.12 (d, 1, J = 9.5 Hz, H-11), 8.33 (s, 1, H-14), 8.74 (d, 1, J = 9.5 Hz, H-12), 10.36 (s, 1, H-7); $[\alpha]^{23}_D$ 33° (c 0.1, MeOH–$CHCl_3$, 1:4); HPLC retention time 4.2 (2.0 mL/min; $CHCl_3$–MeOH, 94:6). Anal. Calcd for $C_{20}H_{15}N_3O_7$: 409.0910. Found: 409.0911. ($C_{20}H_{15}N_3O_7 \cdot 0.25CHCl_3$) C, H, N.

9-Acetamido-10-hydroxy-20(*S*)-camptothecin (26). Compound **25** (150 mg, 0.367 mmol) was combined with PtO_2 (50 mg) in absolute EtOH (50 mL) and subjected to 1 atm of H_2 for 1.5 h. The catalyst was removed by filtration (Celite), and the filter pad was washed free of adsorbed organics using $CHCl_3$–MeOH. Concentration of the gold-colored filtrate (~250 mL) containing the unstable aminophenol intermediate to half its volume gave a turbid tan-yellow solution, was further treated with Ac_2O (1 mL). After 2.5 h, the resulting clear yellow solution was adsorbed onto Celite (0.75 g) and chromatographed by silica gel column (15 g; MeOH–$CHCl_3$, 1:9) to give the pure acetamido analogue **26** as a rusty-yellow solid (69 mg, 45% overall). Precipitation from MeOH–$CHCl_3$ (13:87) afforded **26** as a pale-orange powder: mp 255–258 °C dec; IR (KBr) 3200–3450 (OH, NH), 1740 (lactone), 1655 (pyridone, amide), 1590 (aromatic) cm^{-1}; ^1H NMR (TFA-d_1) δ 1.15 (t, 3, J = 7 Hz, H-18), 2.17 (q, 2, J = 7 Hz, H-19), 2.66 (s, 3, CH_3CO), 5.78 (ABq, 2, J = 18 Hz, $\Delta\gamma$ = 85 Hz, H-17), 5.83 (s, 2, H-5), 8.11 (d, 1, J = 9.5 Hz, H-11), 8.29 (s, 1, H-14), 8.41 (d, 1, J = 9.5 Hz, H-12), 9.34 (s, 1, H-7); $[\alpha]^{23}_D$ 25.4° (c 0.067, MeOH–$CHCl_3$, 1:4); HPLC retention time 4.7 min (2.0 mL/min; $CHCl_3$–MeOH, 8:2). Anal. Calcd for $C_{22}H_{19}N_3O_6$: 421.1273. Found: 421.1277. ($C_{22}H_{19}N_3O_6 \cdot 2.0H_2O$) C, H, N.

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Registry No. **1**, 7689-03-4; **2**, 19685-09-7; **3**, 19685-10-0; **5a**, 70945-42-5; **5b**, 23126-68-3; **5c**, 56008-61-8; **5d**, 104155-87-5; **6**, 56489-01-1; **7**, 42373-30-8; (±)-**8**, 102978-40-5; (±)-**10**, 104155-88-6; (±)-**11**, 104155-89-7; (±)-**14**, 104195-61-1; (±)-**15**, 104195-62-2; (±)-**16**, 104155-91-1; (±)-**17**, 104155-92-2; (±)-**18a**, 104155-93-3; (±)-**18b**, 73466-16-7; **19**, 91421-42-0; **20**, 58546-27-3; **21**, 91421-43-1; **22**, 58546-28-4; **23**, 104155-94-4; **24**, 104155-95-5; **25**, 104267-73-4; **26**, 104155-96-6; **27**, 73427-89-1; **28**, 104155-97-7; (±)-**29**, 73466-17-8; 5-acetamido-2-nitrobenzaldehyde, 104155-86-4.

Synthesis and Structure–Activity Relationships of New Arylfluoronaphthyridine Antibacterial Agents[1]

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Novel arylfluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acids have been prepared and their antibacterial activity evaluated. These derivatives are characterized by having a fluorine atom at the 6-position, substituted amino groups at the 7-position, and substituted phenyl groups at the 1-position. The in vitro antibacterial potency is greatest when the 1-substituent is either *p*-fluorophenyl or *o,p*-difluorophenyl and the 7-substituent is a 3-amino-1-pyrrolidinyl group. 1-(2,4-Difluorophenyl)-6-fluoro-7-(3-amino-1-pyrrolidinyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**38**) was found to possess excellent in vitro potency and in vivo efficacy.

In earlier papers, we reported the syntheses and antibacterial activities of 7-(substituted amino)-6-fluoro-1-aryl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids (**1**)[2] and benzothiazolo[3,2-*a*]quinoline derivatives (**2**).[3] These

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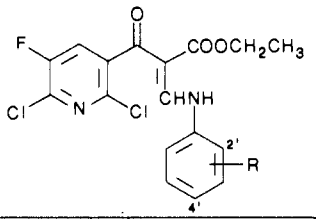
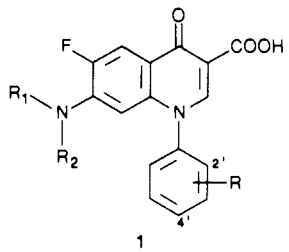
Table I. Ethyl 3-Anilino-2-(2,6-dichloro-5-fluoronicotinyl)-acrylates



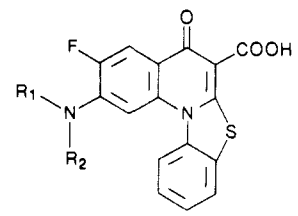
compd	R	yield,[a] %	mp, °C	formula[b]
13	H	53.5	96–97	$C_{17}H_{13}Cl_2FN_2O_3$
14	4′-F	82.7	113–115	$C_{17}H_{12}Cl_2F_2N_2O_3$
15	2′-F, 4′-F	81.4	139–140	$C_{17}H_{11}Cl_2F_3N_2O_3$

[a] Yields are not optimized. [b] C, H, and N analyses were within ±0.4% of the theoretical values, except as otherwise noted.

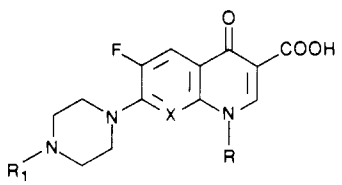
compounds possess a 1-aryl-1,4-dihydro-4-oxopyridine-3-carboxylic acid moiety. These potent antibacterials, together with pefloxacin (**3**),[4] norfloxacin (**4**),[5] ofloxacin (**5**),[6] and ciprofloxacin (**6**),[7] are 4-quinolones, a class of compounds that has attracted increasing attention as a source of new antibacterial agents.[8,9]



1



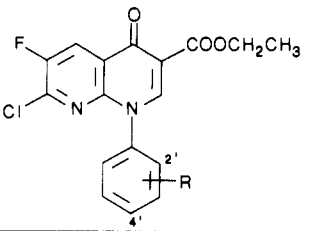
2



3, R = C_2H_5; R_1 = CH_3; X = CH
4, R = C_2H_5; R_1 = H; X = CH
5, X = R = $COCH_2CH(CH_3)$; R_1 = CH_3
6, R = C_3H_5; R_1 = H; X = CH
7, R = C_2H_5; R_1 = H; X = N

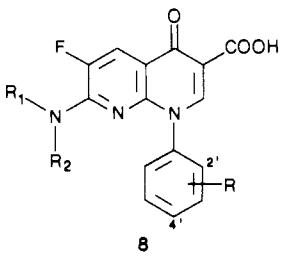
Enoxacin (**7**),[10] a naphthyridine analogue of the corresponding quinolone norfloxacin (**4**), was found to have an increased in vivo potency upon oral administration in systemic mouse protection test. As a continuation of our research for potent antibacterial agents, we have extended the introduction of the 1-aryl substituent to the naphthyridine nucleus. In this paper, we report the syntheses and antibacterial activity of 7-(substituted amino)-6-fluoro-1-aryl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid derivatives **8**. 3-Amino-1-pyrrolidinyl, 1-piperazinyl, *N*-methyl-1-piperazinyl, and 3-methyl-1-piperazinyl groups were selected to be introduced at C-7 of **8** in this study on the basis of our experience with the 1-aryl quinolone antibacterial agents.[2]

Table II. Ethyl 1-Aryl-6-fluoro-7-chloro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylates

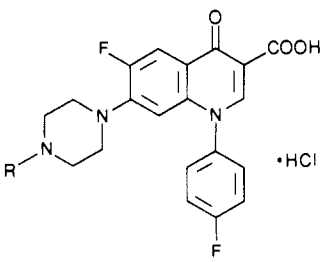


compd	R	yield,[a] %	mp, °C	formula[b]
16	H	75.8	219–220	$C_{17}H_{12}ClFN_2O_3$
17	4′-F	59.6	218–220	$C_{17}H_{11}ClF_2N_2O_3 \cdot 0.25H_2O$
18	2′-F, 4′-F	86.5	211–212	$C_{17}H_{10}ClF_3N_2O_3$

[a] See Table I, footnote a. [b] See Table I, footnote b.



8



39, R = CH_3
40, R = H

Chemistry. The general method used for the preparation of 1-alkylnaphthyridine antibacterial agents involves the alkylation of 4-hydroxy-1,8-naphthyridine-3-carboxylic acid alkyl ester with an alkyl halide to form the 1-alkylated-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid ester derivative.[8,11] This process, however, is not applicable to the introduction of a phenyl ring at the 1-position. The 1-aryl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid derivatives were synthesized as illustrated in Scheme I.

Hydrolysis of ethyl 2,6-dichloro-5-fluoronicotinate (**9**)[12] with 6 N hydrochloric acid in trifluoroacetic acid yielded the 2,6-dichloro-5-fluoronicotinic acid (**10**). Treatment of **10** with thionyl chloride gave the 2,6-dichloro-5-fluoronicotinyl chloride (**11**). Reaction of the acid chloride **11** with dilithio dianion of monoethyl malonate[13] afforded the ethyl 2,6-dichloro-5-fluoronicotinyl acetate (**12**). Treatment of this ester with triethyl orthoformate in acetic anhydride gave the one-carbon homologue enol ether intermediate, which upon evaporation of solvent to dryness was allowed to react with a slight excess of an appropriate aniline in methylene chloride at room temperature to give the ethyl 3-anilino-2-(2,6-dichloro-5-fluoro)nicotinyl-

(1) This work was presented in part at the 25th Interscience Conference on Antimicrobial Agents and Chemotherapy, Sept. 29–Oct. 2, 1985, Minneapolis, MN; Abstract 131.
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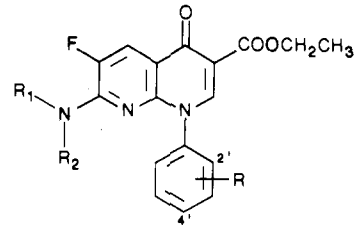
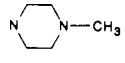
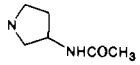
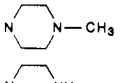
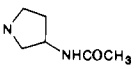
(7) Wise, R.; Andrews, J. M.; Edwards, L. J. *Antimicrob. Agents Chemother.* **1983**, *23*, 559.
(8) For a review, see: Albrecht, R. *Prog. Drug Res.* **1977**, *21*, 9.
(9) Smith, J. T. *Pharm. J.* **1984**, 299.
(10) Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H. *J. Med. Chem.* **1984**, *27*, 292.
(11) Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H.; Nishimura, H. *J. Heterocycl. Chem.* **1984**, *21*, 673.
(12) Matsumoto, H.; Miyamoto, T.; Egawa, H.; Tskasa, Y. JP Appl. 82/72981, May 7, 1982.
(13) Wierenga, W.; Skulnick, H. I. *J. Org. Chem.* **1979**, *44*, 310.

Table III. Ethyl 1-Aryl-6-fluoro-7-amino-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylates



compd	R	NR_1R_2	yield,[a] %	mp, °C	formula[b]
19	H		64.5	184–185	$C_{21}H_{21}F_4O_3 \cdot {}^1/_3CH_2Cl_2$
20	4′-F		86.7	225–226	$C_{21}H_{19}F_2N_3O_4$
21	4′-F		63.7	213–215	$C_{21}H_{20}F_2N_4O_3 \cdot 0.5H_2O$
22	4′-F		60.0	200–203	$C_{22}H_{22}F_2N_4O_3 \cdot 0.5H_2O$
23	4′-F		88.1	160–162	$C_{22}H_{22}F_2N_4O_3 \cdot 0.25H_2O$
24	4′-F		88.0	210–211	$C_{23}H_{22}F_2N_4O_4 \cdot 0.5H_2O$
25	2′-F, 4′-F		81.4	210–212	$C_{21}H_{19}F_3N_4O_3 \cdot 1.5H_2O$[c]
26	2′-F, 4′-F		85.3	173–174	$C_{22}H_{21}F_3N_4O_3$
27	2′-F, 4′-F		85.7	158–159	$C_{22}H_{21}F_3N_4O_3$
28	2′-F, 4′-F		81.2	227–229	$C_{22}H_{21}F_3N_4O_4$

[a] see Table I, footnote a. [b] See Table I, footnote b. [c] H: calcd, 4.79; found, 4.32.

acrylates **13–15** (Table I). Cyclization of compounds **13–15** with 1 M equiv of sodium hydride in tetrahydrofuran (THF) yielded ethyl 1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylates **16–18** (Table II). Displacement of the 7-chlorine atom of the carboxylates **16–18** with an appropriate amine in methylene chloride yielded the desired 7-amino derivatives **19–28** (Table III). Hydrolysis of the ethyl ester **19–28** with hydrochloric acid gave the desired 7-amino-6-fluoro-1-aryl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acids **29–38** (Table IV).

Results and Discussion

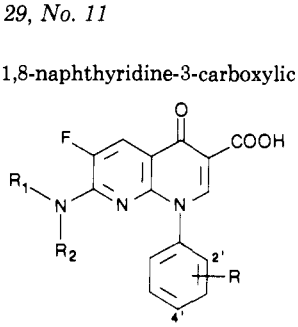
Table V summarizes the in vitro antibacterial activity of the 1-aryl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acids against five Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* CMX 686B, *Staphylococcus epidermidis* 3519, *Streptococcus faecium* ATCC 8043, and *Streptococcus pyogenes* 930) and six Gram-negative organisms (*Escherichia coli* Juhl, *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumoniae* 8045, *Pseudomonas aeruginosa* 5007, *Pseudomonas aeruginosa* K799/WT, and *Acinetobacter* CMX 669). The data for norfloxacin (**4**) and ciprofloxacin (**6**) as well as for difloxacin A-56619 (**39**) and A-56620 (**40**) of the 1-aryl-1,4-dihydro-4-oxoquinoline-3-carboxylic acid series[2] are included for comparison.

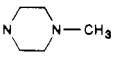
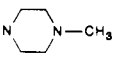
The effect of substitutions on the 1-phenyl ring of the 6-fluoro-7-piperazinyl-1,8-naphthyridines on the in vitro antibacterial potency is shown by comparing the data of compounds **29**, **31**, and **35** shown in Table V. Substitution with fluorine at the para position generally enhances overall antibacterial potency. Difluoro substitution on the phenyl group results in increased antistreptococcal activity.

The structure–activity relationships (SARs) of the C-7 substitution in a series of analogues with the *p*-fluorophenyl group at N-1 (**30–34**) or a 2′,4′-difluorophenyl group at N-1 (**35–38**) were comparable to those for the corresponding 7-(substituted amino)-6-fluoro-1-aryl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids (**1**).[2] With respect to N-1 *p*-fluorophenyl or *N*-1 2′,4′-difluorophenyl derivatives, introduction of the C-7 substituent tends to enhance the antibacterial activity. In both series of compounds, the activity against Gram-negative organisms increases in the order 3-hydroxypyrrolidinyl < 4-methylpiperazinyl ≤ 3-methylpiperazinyl < piperazinyl < 3-aminopyrrolidinyl, whereas the Gram-positive activity follows the sequence piperazinyl < 3-methylpiperazinyl ≤ 4-methylpiperazinyl < 3-hydroxypyrrolidinyl ≤ 3-aminopyrrolidinyl. Compounds **34** and **38**, both bearing a 3-aminopyrrolidinyl group at C-7, are very potent antibacterial agents with activities similar to ciprofloxacin (**6**), which is one of the leading quinolones under development.

Efficacy in systemic infections due to *S. aureus* NCTC 10649, *E. coli* Juhl, and *P. aeruginosa* 5007 in mice of several selected compounds and of ciprofloxacin (**6**) is shown in Table VI, which includes the minimal inhibitory concentrations (MICs) against the organisms employed. With the exception of **31**, the in vivo efficacy on the experimental infection due to *S. aureus* NCTC 10649 of all naphthyridines tested was greater than that of ciprofloxacin (**6**), both administered subcutaneously (sc) or orally (po). Compound **31** is as potent as ciprofloxacin when

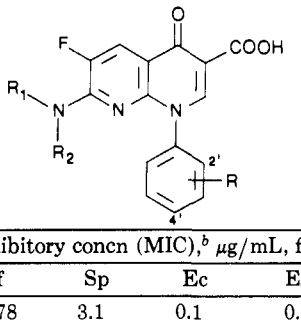
Table IV. 1-Aryl-6-fluoro-7-amino-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acids



compd	R	NR_1R_2	yield,[a] %	formula[b,c]
29	H		90	$C_{19}H_{17}FN_4O_3 \cdot HCl \cdot 0.5H_2O$[d]
30	4′-F		75.3	$C_{19}H_{15}F_2N_3O_4 \cdot 0.5H_2O$
31	4′-F		63.3	$C_{19}H_{16}F_2N_4O_3 \cdot HCl \cdot H_2O$
32	4′-F		44.9	$C_{20}H_{18}F_2N_4O_3 \cdot HCl$
33	4′-F		77.6	$C_{20}H_{18}F_2N_4O_3 \cdot HCl \cdot 0.25H_2O$
34	4′-F		61.7	$C_{19}H_{16}F_2N_4O_3 \cdot HCl \cdot 0.5H_2O$
35	2′-F, 4′-F		92.5	$C_{19}H_{15}F_3N_4O_3 \cdot HCl \cdot 0.5H_2O$
36	2′-F, 4′-F		81.2	$C_{20}H_{17}F_3N_4O_3 \cdot HCl \cdot 0.25H_2O$
37	2′-F, 4′-F		73.9	$C_{20}H_{17}F_3N_4O_3 \cdot HCl \cdot 0.25H_2O$
38	2′-F, 4′-F		80.3	$C_{19}H_{15}F_3N_4O_3 \cdot HCl \cdot 0.75H_2O$

[a] See Table I, footnote a. [b] See Table I, footnote b. [c] Melting points of all the compounds are >275 °C. [d] C: calcd, 55.14; found, 55.61.

Table V. In Vitro Antibacterial Activity of 1-Aryl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acids[a]



	minimal inhibitory concn (MIC),[b] μg/mL, for each organism given										
compd	Sa(A)	Sa	Se	Sf	Sp	Ec	Ea	Kp	Pa(5)	Pa(K)	A
4	0.78	0.78	1.56	0.78	3.1	0.1	0.2	0.1	0.39	0.39	6.2
6	0.2	0.39	0.39	0.2	0.39	0.02	0.05	0.02	0.1	0.1	1.56
29	1.56	1.56	1.56	50	6.2	0.2	0.39	0.1	1.56	1.56	1.56
30	0.05	0.05	0.2	1.56	0.78	0.39	0.39	0.1	3.1	3.1	0.39
31	0.39	0.39	0.78	3.1	1.56	0.05	0.1	0.1	0.39	0.39	0.39
32	0.2	0.2	0.39	3.1	3.1	0.2	0.2	0.1	1.56	1.56	0.2
33	0.39	0.78	0.78	6.2	3.1	0.05	12.5	0.02	1.56	1.56	0.2
34	0.5	0.1	0.1	0.39	0.2	0.05	0.1	0.05	0.1	0.2	0.1
35	0.2	0.2	0.39	0.78	0.78	0.05	0.2	0.05	0.39	0.39	0.39
36	0.2	0.2	0.39	1.56	1.56	0.39	0.39	0.39	3.1	3.1	0.39
37	0.2	0.39	0.78	3.1	1.56	0.1	3.1	0.2	1.56	1.56	0.39
38	0.05	0.05	0.1	0.2	0.2	0.02	0.1	0.02	0.2	0.39	0.1
39	0.2	0.2	0.39	1.56	1.56	0.2	0.78	0.1	0.78	1.56	0.78
40	0.2	0.2	0.39	1.56	0.78	0.05	0.2	0.02	0.39	0.39	0.2

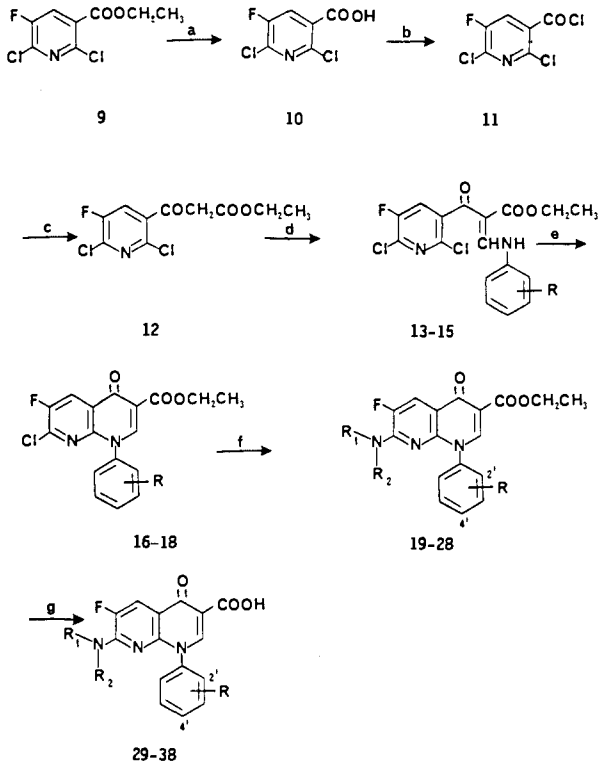
[a] Structures are shown in Table IV. [b] The MICs were determined by the 2-fold agar dilution on brain–heart infusion agar. Organisms selected for inclusion in the table are Sa(A), *Staphylococcus aureus* ATCC 6538P; Sa, *Staphylococcus aureus* CMX 686B; Se, *Staphylococcus epidermidis* 3519; Sf, *Streptococcus faecium* ATCC 8043; Sp, *Streptococcus pyogenes* 930; Ec, *Escherichia coli* Juhl; Ea, *Enterobacter aerogenes* ATCC 13048; Kp, *Klebsiella pneumoneae* 8045; Pa(5), *Pseudomonas aeruginosa* 5007; Pa(k), *Pseudomonas aeruginosa* K799/WT; A, *Acinetobacter* sp. CMX669.

Table VI. Mouse Protection Test of Selected Naphthyridines in Comparison with Ciprofloxacin (6)

test organism (dose)[a]	compd[b]	MIC, μg/mL	ED_{50}[a] (95% confidence limits), mg/kg	
			sc	po
S. aureus NCTC 10649 (100 × LD_{50})	**6**	0.25	1.6 (1.0–2.5)	15.5 (9.9–24.1)
	31	0.25	1.6 (1.0–2.5)	7.5 (4.8–11.7)
	34	0.06	0.5 (0.1–1.6)	1.6 (0.9–2.6)
	35	0.12	0.6 (0.4–1.0)	2.4 (1.5–3.7)
	38	0.03	0.2 (0.2–0.4)	1.4 (0.9–2.2)
E. coli Juhl (100 × LD_{50})	**6**	0.02	0.2 (0.1–0.2)	1.9 (1.2–3.0)
	31	0.05	1.0 (0.6–1.5)	2.8 (1.8–4.4)
	33	0.1	1.0 (0.5–2.0)	2.4 (1.6–3.7)
	37	0.1	1.4 (0.8–2.5)	3.9 (2.5–6.0)
	38	0.02	0.2 (0.2–0.3)	1.3 (0.8–2.2)
P. aeruginosa 5007 (100 × LD_{50})	**6**	0.1	2.1 (1.1–3.8)	13.3 (6.8–26.2)
	31	0.39	6.9 (5.1–9.2)	19.9 (12.8–31.3)
	34	0.1	7.4 (4.7–11.7)	18.2 (10.3–32.3)
	35	0.39	4.6 (2.6–8.0)	7.0 (5.0–12.0)
	38	0.2	3.6 (2.0–6.5)	4.9 (1.7–13.7)

[a] See Experimental Section. [b] Structures are shown in Table IV.

Scheme I[a]



[a] HCl/CF_3COOH. [b] $SOCl_2$. [c] 1, $CH_2(COOEt)COOH$/*n*-BuLi; 2, H^+. [d] 1, $CH(OC_2H_5)_3/Ac_2O$; 2, $RC_6H_4NH_2$. [e] NaH/THF. [f] NHR_1R_2/CH_2Cl_2. [g] HCl/H_2O.

tested subcutaneously and more potent when tested orally. As for Gram-negative bacteria, the in vivo efficacy of the naphthyridines is similar to or better than ciprofloxacin (6). Of much interest is compound **38**, which is 11, 1.5, and 2.5 times as potent as ciprofloxacin (6) on systemic infection caused by *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively, upon oral administration.

A comparison between **31**, a naphthyridine, and its quinolone counterpart **40** in in vivo efficacy is shown in Table VII. The naphthyridine derivative **31** shows greater oral activity against *E. coli* Juhl and *P. aeruginosa* 5007 than **40**. Even though **31** has higher MIC values than **40** against *S. aureus* NCTC 10649, its in vivo potency is about the same as **40** upon oral administration of the drugs. It appears that the naphthyridine analogue has better oral absorption than its quinolone counterpart. This is in agreement with the previous observation that enoxacin (7) has a greater increased in vivo oral efficacy in systemic infection in mice than its quinolone counterpart norfloxacin (4).

As a result of this study, it has been shown that aryl substitution on the 1-position of 6-fluoro-7-(substituted amino)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid produces potent antibacterial agents with activity comparable to that of ciprofloxacin. Compound **38**, which has a difluorophenyl substitution at the 1-position and a 3-aminopyrrolidinyl group at the 7-position, is found to possess broad and potent in vitro antibacterial activity and excellent in vivo efficacy in systemic infections in mice and is superior to ciprofloxacin, based on biological evaluations conducted during this study. Detailed accounts of its antibacterial and pharmacokinetic properties will be reported elsewhere.

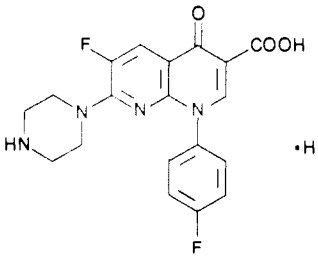
Experimental Section

Melting points were taken in a Thomas-Hoover capillary 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 Varian T-60 and HA-100 spectrometers using tetramethylsilane as an internal standard. Mass spectra were recorded on a Kratos MS-50 mass spectrometer. The IR spectra were recorded on a Perkin-Elmer Model 710 A infrared spectrometer. The IR, NMR, and MS data of all compounds were consistent with the assigned structures, and the nuclear magnetic resonance spectra of naphthyridines are given in Table VIII.

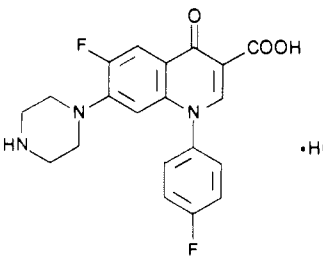
2,6-Dichloro-5-fluoronicotinic Acid (10). Ethyl 2,6-dichloro-5-fluoronicotinate (**9**)[12] (20 g, 84 mmol) was dissolved in a mixture of 40 mL of trifluoroacetic acid and 40 mL of 7.5 N HCl. The mixture was heated to reflux for 24 h. The solution was cooled, and the trifluoroacetic acid was removed by evaporation under reduced pressure. Upon cooling, 100 mL of water was added and a white precipitate formed. The precipitate was filtered, washed with hexane, and dired, yielding 12.91 g (73.2%) of 2,6-dichloro-5-fluoronicotinic acid (**10**): mp 153–154 °C. Anal. ($C_6H_2Cl_2FNO_2$) C, H, N.

Ethyl 2,6-Dichloro-5-fluoronicotinylacetate (12). 2,6-Dichloro-5-fluoronicotinic acid (**10**) (7 g, 33.3 mmol) was dissolved in thionyl chloride (35 mL). After the mixture was heated at 80 °C for 2 h, the thionyl chloride was removed by evaporation under reduced pressure yielding a yellowish 2,6-dichloro-5-fluoronicotinyl chloride. Monoethyl malonate (13.2 g, 100 mmol) and 3 mg of biquinoline was dissolved in 280 mL of dry tetrahydrofuran (THF) and cooled to −30 °C. A solution of 2.5 M *n*-butyllithium in hexane was added until a pink color remained at −5 °C (80 mL). The suspension was then cooled to −50 °C. The acid chloride obtained as described above, dissolved in 50 mL of THF, was then added to the suspension dropwise. After the addition, the dry ice bath was removed and the reaction was allowed to stir at room temperature for 1 h. The reaction was acidified with 200 mL of 1

Table VII. Comparison between Naphthyridine and Quinolone on in Vivo Efficacy



31



40

test organism (dose)[a]	compd	MIC, μg/mL	ED_{50} (95% confidence limits),[a] mg/kg sc	ED_{50} (95% confidence limits),[a] mg/kg po
S. aureus NCTC 10649	**31**	0.25	1.6 (1.0–2.5)	7.5 (4.8–11.7)
(100 × LD_{50})	**40**	0.12	1.6 (1.0–2.5)	6.5 (3.8–11.2)
E. coli Juhl	**31**	0.05	1.0 (0.6–1.5)	2.8 (1.8–4.4)
(100 × LD_{50})	**40**	0.05	0.6 (0.3–1.3)	4.3 (2.8–6.5)
P. aeruginosa 5007	**31**	0.39	6.9 (5.1–9.2)	19.9 (12.8–31.3)
(100 × LD_{50})	**40**	0.39	1.6 (1–2.5)	21.4 (13.4–34.4)

[a] See Experimental Section.

Table VIII. Nuclear Magnetic Resonance Spectra of Naphthyridines[a]

compd	solvent	aromatic	C_5-H	C_2-H	OCH_2	ethyl CH_3	others
19	$CDCl_3$	7.39 (m, 2 H), 7.52 (m, 3 H)	8.15 (d), J = 13	8.53 (s)	4.38 (q), J = 7	1.38 (t), J = 7	2.85 (dd, J = 4.5, $N(CH_2)_2$), 3.51 (dd, J = 4.5, $N(CH_2)_2$)
20	$CDCl_3$	7.31 (m, 4 H)	7.98 (d), J = 13	8.46 (s)	4.42 (q), J = 7	1.40 (t), J = 7	2.04 (m, 3, CH_2, OH), 3.62 (m, 4, $N(CH_2)_2$), 4.56 (m, 1, CH)
21	$CDCl_3$	7.61 (m, 4 H)	8.14 (d), J = 13	8.49 (s)	4.38 (q), J = 7	1.38 (t), J = 7	2.02 (sb, 1, NH), 2.86 (dd, J = 4.5, 4, $N(CH_2)_2$, 3.54 (dd, J = 4.5, 4, $N(CH_2)_2$)
22	$CDCl_3$	7.31 (m, 4 H)	8.14 (d), J = 13	8.47 (s)	4.39 (q), J = 7	1.39 (t), J = 7	2.28 (s, 3, NCH_3), 2.39 (m, 4, $N(CH_2)_2$), 3.56 (m, 4, $N(CH_2)_2$)
23	$CDCl_3$	7.31 (m, 4 H)	8.13 (d), J = 13	8.48 (s)	4.38 (q), J = 7	1.39 (t), J = 7	0.97 (d, J = 6.5, 3, CH_3), 2.53 (m, 1, CH), 2.77 (m, 2, NCH_2), 2.91 (m, 2, NCH_2), 4.07 (m, 2, NCH_2)
24	$CDCl_3$	7.32 (m, 4 H)	7.66 (d), J = 13	8.44 (s)	4.38 (q), J = 7	1.37 (t), J = 7	2.01 (m, 2, CH_2), 2.12 (s, 3, $COCH_3$), 3.54 (m, 4, $N(CH_2)_2$), 4.64 (m, 1, CH)
25	$CDCl_3$	7.04 (m, 2 H), 7.48 (m, 1 H)	8.12 (d), J = 13	8.40 (s)	4.41 (q), J = 7	1.38 (t), J = 7	2.01 (sb, 1, NH), 2.88 (dd, J = 5, 4, $N(CH_2)_2$), 3.53 (dd, J = 5, 4, $N(CH_2)_2$)
26	$CDCl_3$	7.04 (m, 2 H), 7.39 (m, 1 H)	8.12 (d), J = 13	8.39 (s)	4.37 (q), J = 7	1.39 (t), J = 7	2.27 (s, 3, NCH_3), 2.39 (m, 4, $N(CH_2)_2$), 3.54 (m, 4, $N(CH_2)_2$)
27	$CDCl_3$	7.07 (m, 2 H), 7.41 (m, 1 H)	8.12 (d), J = 13	8.41 (s)	4.37 (q), J = 7	1.39 (t), J = 7	0.98 (dd, J = 6, 3, CH_3), 2.54 (m, 1, NCH), 2.78 (m, 2, NCH_2), 2.96 (m, 2, NCH_2), 4.07 (m, 2, NCH_2)
28	$CDCl_3$	7.08 (m, 2 H), 7.38 (m, 1 H)	7.61 (d), J = 13	8.35 (s)	4.32 (q), J = 7	1.40 (t), J = 7	2.11 (m, 3, NCH_2, CH), 2.19 (s, 1, $COCH_3$), 3.49 (m, 4, $N(CH_2)_2$)
29	Me_2SO-d_6	7.63 (m, 5 H)	8.26 (d), J = 13	8.73 (s)			3.11 (m, 4, $N(CH_2)_2$), 3.73 (m, 4, $N(CH_2)_2$), 14.95 (sb, 1, COOH)
30	Me_2SO-d_6	7.58 (m, 4 H)	8.08 (d), J = 13	8.67 (s)			1.88 (m, 2, CH_2), 3.51 (m, 4, $N(CH_2)_2$), 4.35 (m, 1, *C*HOH), 5.03 (sb, 1, OH), 15.32 (sb, 1, COOH)
31	Me_2SO-d_6	7.58 (m, 4 H)	8.38 (d), J = 13	8.78 (s)			3.17 (m, 4, $N(CH_2)_2$), 3.78 (m, 4, $N(CH_2)_2$), 9.33 (sb, 2, NH_2^+)
32	Me_2SO-d_6	7.45 (m, 2 H), 7.62 (m, 2 H)	8.29 (d), J = 13	8.78 (s)			3.04 (m, 4, $N(CH_2)_2$), 3.38 (s, 1, NCH_3), 4.15 (m, 4, $N(CH_2)_2$), 10.96 (sb, 1, NH^+)
33	Me_2SO-d_6	7.46 (m, 2 H), 7.70 (m, 2 H)	8.07 (d), J = 13	8.77 (s)			1.09 (d, J = 6, 3, CH_3), 3.15 (m, 4, $N(CH_2)_2$), 3.45 (m, 1, NCH), 4.16 (m, 2, NCH_2), 9.30 (sb, 2, NH_2^+)
34	Me_2SO-d_6	7.42 (m, 2 H), 7.67 (m, 2 H)	8.11 (d), J = 13	8.67 (s)			2.13 (m, 2, CH_2), 3.82 (m, 5, $N(CH_2)_2$, CH)
35	Me_2SO-d_6	7.35 (m, 1 H), 7.62 (m, 1 H), 7.82 (m, 1 H)	8.06 (d), J = 13	8.92 (s)			3.11 (m, 4, $N(CH_2)_2$), 3.72 (m, 4, $N(CH_2)_2$), 9.18 (sb, 2, NH_2), 14.06 (sb, 1, COOH)
36	Me_2SO-d_6	7.36 (m, 1 H), 7.63 (m, 1 H), 7.82 (m, 1 H)	8.09 (d), J = 13	8.93 (s)			3.04 (m, 4, $N(CH_2)_2$), 3.41 (s, 3, NCH_3), 4.13 (m, 4, $N(CH_2)_2$), 10.89 (sb, 1, NH^+)
37	Me_2SO-d_6	7.38 (m, 1 H), 7.67 (m, 1 H), 7.84 (m, 1 H)	8.27 (d), J = 13	8.94 (d), J = 1			1.11 (dd, J = 6, 3, CH_3), 3.20 (m, 3, NCH_2, NCH), 3.98 (m, 2, NCH_2), 4.12 (m, 2, NCH_2), 9.4 (sb, 2, NH_2^+)
38	Me_2SO-d_6	7.36 (m, 1 H), 7.60 (m, 1 H), 7.81 (m, 1 H)	8.13 (d), J = 13	8.84 (s)			2.11 (m, 2, CH_2), 3.82 (m, 5, $N(CH_2)_2$, CH), 8.27 (sb, 3, NH_3^+)

[a] The chemical shifts are recorded in δ values and coupling constants in hertz. Spectra are recorded in solvent specified with tetramethylsilane as internal reference. The NMR peaks are designated as follows: s, singlet, d, doublet; t, triplet; q, quartet; m, multiplet; b, broad.

N HCl and was extracted with ether. The ether fraction was washed with saturated aqueous sodium bicarbonate solution and then water. The ether solution was dried and evaporated to dryness yielding a residue, which was washed with hexane to give 9.14 g (97.9%) of **12**: mp 64–65 °C. Anal. ($C_{10}H_8Cl_2FNO_3$) C, H, N.

Ethyl 3-*p*-Fluoroanilino-2-(2,6-dichloro-5-fluoronicotinyl)acrylate (14). A solution of ethyl 2,6-dichloro-5-fluoronicotinylacetate (**12**) (1.60 g, 5.7 mmol) in triethyl orthoformate (1.4 mL, 8.6 mmol) and acetic anhydride (10 mL, 72.3 mmol) was heated at 130 °C for 1 h with removal of ethyl acetate formed during the reaction. The solution was evaporated under reduced pressure to a mobile oil, which was then dissolved in methylene chloride (50 mL). 4-Fluoroaniline (0.82 mL, 6.3 mmol) was added to the solution. After 0.5 h, the solution was evaporated to dryness and the residue crystallized and washed with hexane, yielding 1.9 g (82.7%) of the nicotinylacrylate **14**: mp 113–115 °C. Anal. ($C_{17}H_{12}Cl_2F_2N_2O_3$) C, H, N.

By use of this procedure, compounds **13** and **15** were prepared from **12** using the appropriate anilines.

Ethyl 1-*p*-Fluorophenyl-6-fluoro-7-chloro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (17). A 60% sodium hydride in oil suspension (173 mg, 4.3 mmol) was slowly added to a cold solution of the nicotinylacrylate **14** (1.65 g, 4.1 mmol) in THF (20 mL). The mixture was heated at reflux under nitrogen atmosphere for 1 h and was cooled, washed with water, and dried, yielding 894 mg (59.6%) of **17**: mp 218–220 °C. Anal. ($C_{17}H_{11}ClF_2N_2O_3 \cdot 0.25H_2O$) C, H, N.

By use of the same methods, compounds **16** and **18** were prepared from compounds **13** and **15**.

Ethyl 1-*p*-Fluorophenyl-6-fluoro-7-(3-methyl-1-piperazinyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (23). To a solution of **17** (2 g, 5.5 mmol) in pyridine (50 mL) was added 2-methylpiperazine (1.16 g, 11 mmol), and the mixture was stirred at room temperature for 3 h. The pyridine was removed under reduced pressure, and the residue was partitioned between methylene chloride and water. The organic layer was dried and evaporated under reduced pressure to give a solid. This was suspended in water and stirred for 18 h. The solid was filtered and dried, yielding 2.07 g (88.1%) of the carboxylate **23**: mp 160–162 °C. Anal. ($C_{22}H_{22}F_2N_4O_3$) C, H, N.

By use of this procedure, compounds **19–22** and **24–28** were prepared from the appropriate carboxylates **16–18** and amines.

1-*p*-Fluorophenyl-6-fluoro-7-(3-methyl-1-piperazinyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (33). A suspension of the ethyl 1-fluorophenyl-6-fluoro-7-(3-methyl-1-piperazinyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (**23**) (1.92 g, 4.5 mmol) in 100 mL of 6 N HCl was heated at 110 °C for 24 h. The mixture was evaporated to dryness under reduced pressure. The residue was suspended in ethanol and stirred for 24 h to remove the color. An equal volume of ether was added, and the solid was filtered and dried, yielding 1.52 g (77.6%) of **33**. Anal. ($C_{20}H_{18}F_2N_4O_3 \cdot HCl \cdot 0.25H_2O$) C, H, N.

Compounds **29–32** and **34–38** were prepared in the same way.

In Vitro Antibacterial Activity. The in vivo antibacterial activity of the test compound was determined in a side-by-side comparison with ciprofloxacin (**6**) 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 a 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 minimal inhibitory concentration was the lowest concentration of the test compound that yielded no visible growth on the plate.

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

After 18 h incubation, the cultures of *E. coli* Juhl in BHI broth were serially diluted using 10-fold dilutions in 5% (w/v) hog gastric mucin. Cultures (0.5 mL), dilution from 10^{-1} to 10^{-8}, were injected intraperitoneally into mice. The LD_{50} for the test organism was calculated from the cumulative mortalities on the sixth day using the Reed and Muench procedure.[14]

The 18-h culture of the above was diluted in 5% (w/v) hog gastric mucin to obtain 100 times the LD_{50} and 0.5 mL was injected intraperitoneally into mice. The mice were treated subcutaneously or orally with a specific amount of the test compound divided equally to be administered at 1 and 5 h after infection. A group of 10 animals each for at least three dose levels were thus treated, and the deaths were recorded daily for 6 days. Ten mice were left untreated as infection control. Fifty percent effective dose values (ED_{50}) were calculated from the cumulative mortalities on the sixth day after infection using the trimmed version of the Logit method.[15]

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