complex side chains as well as to create further structural variation at the level of two- and three-carbon side-chain substituents.

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Pyridonecarboxylic Acids as Antibacterial Agents. 2.[1] Synthesis and Structure-Activity Relationships of 1,6,7-Trisubstituted 1,4-Dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acids, Including Enoxacin, a New Antibacterial Agent[2]

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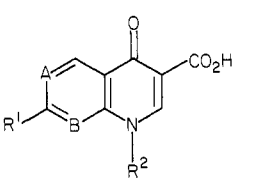
The title compounds having nitro, amino, cyano, chloro, or fluoro as the C-6 substituent were prepared. Introduction of the chloro and cyano groups at C-6 was accomplished by the Sandmeyer reaction of 6-amino-1,8-naphthyridine derivatives **9** via their 6-diazonium salts **10**. The reaction was extended to the synthesis of the 6-fluoro analogues **20**, involving the Balz–Schiemann reaction of the diazonium tetrafluoroborate **19**. Furthermore, a series of the 1-ethyl (**24** and **27–30**), 1-vinyl (**35–37**), 1-(2-fluoroethyl) (**38** and **39**), and 1-(difluoromethyl) (**40**) analogues of 7-substituted 6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acids was prepared. 1-Pyrrolidinyl and, particularly, N-substituted or unsubstituted 1-piperazinyl groups were introduced as the C-7 variants. As a result of this study, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid (**24b**; named enoxacin, originally AT-2266) was found to show the most broad and potent in vitro antibacterial activity, an excellent in vivo efficacy on systemic infections, and a weak acute toxicity. Structure–activity relationships of compounds with variations of substituents at C-1, C-6, and C-7 are also discussed.

In earlier papers, we had reported the synthesis and antibacterial activity of pyrido[2,3-*d*]pyrimidines (**1**),[3,4] 1,6- and 1,8-naphthyridines (**2** and **3**),[1] and quinolines (**4**),[5] all of which possess a 1,4-dihydro-4-oxopyridine-3-carboxylic acid moiety as a common structure. This class of compounds has increasingly attracted attention as a source of new antibacterial agents.[6] It has been reported that nalidixic acid (**3**, $R^1 = CH_3$; $R^2 = C_2H_5$)[7] and oxolinic acid [**4**, R^1 = 6,7-(-OCH_2O-), $R^2 = C_2H_5$][8] are a specific inhibitor of DNA synthesis in susceptible bacterial cells, and this inhibition is due to the prevention of DNA gyrase activity.[9] Recent findings indicate that piromidic acid (**1a**, $R^2 = C_2H_5$)[3] and pipemidic acid (**1b**, $R^2 = C_2H_5$)[4] also act by the same mechanism.[10]

Chart I[a]



1, A = B = N
2, A = N; B = CH
3, A = CH; B = N
4, A = B = CH
5, A = CR^3; B = N
6, A = CF; B = CH; $R^2 = C_2H_5$

[a] a, R^1 =  ; b, R^1 =  ; c, R^1 =  .

A continuing interest in this field led us to an investigation of 1,6,7-trisubstituted 1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acids (**5**), with the C-6 substituent being either nitro, amino, chloro, or fluoro; little information about the effect of the C-6 substituent upon antibacterial activity has been available thus far.

(1) Paper 1: Hirose, T.; Mishio, S.; Matsumoto, J.; Minami, S. *Chem. Pharm. Bull.* **1982**, *30*, 2399.
(2) This work was presented in part (a) at the 11th International Congress of Chemotherapy and 19th Interscience Conference on Antimicrobial Agents and Chemotherapy, Boston, MA, Oct 1979 (In "Current Chemotherapy and Infectious Disease"; Nelson, J. D.; Grassi, C., Eds.; American Society for Microbiology: Washington, DC, 1980; Vol. 1, p 454) and (b) at the 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, Apr 1980; Abstr, p 275. (c) Matsumoto, J.; Takase, Y.; Nishimura, Y. European Patent 9425, 1980; *Chem. Abstr.* **1980**, *93*, 168305*x*.
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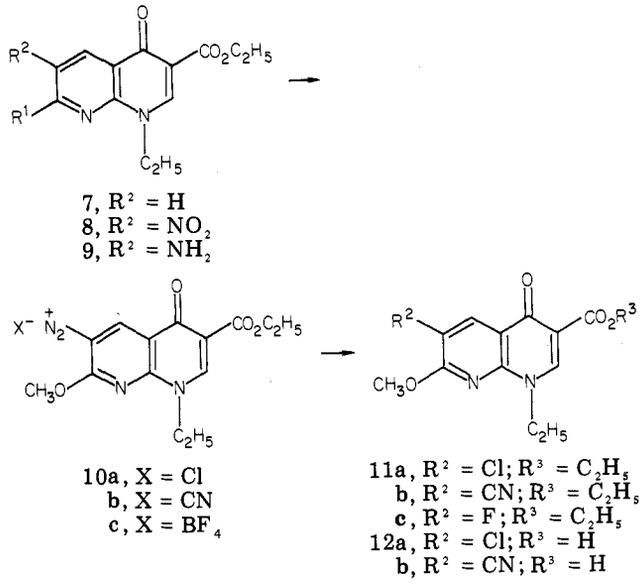
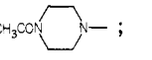
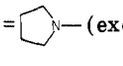
Scheme I[a]



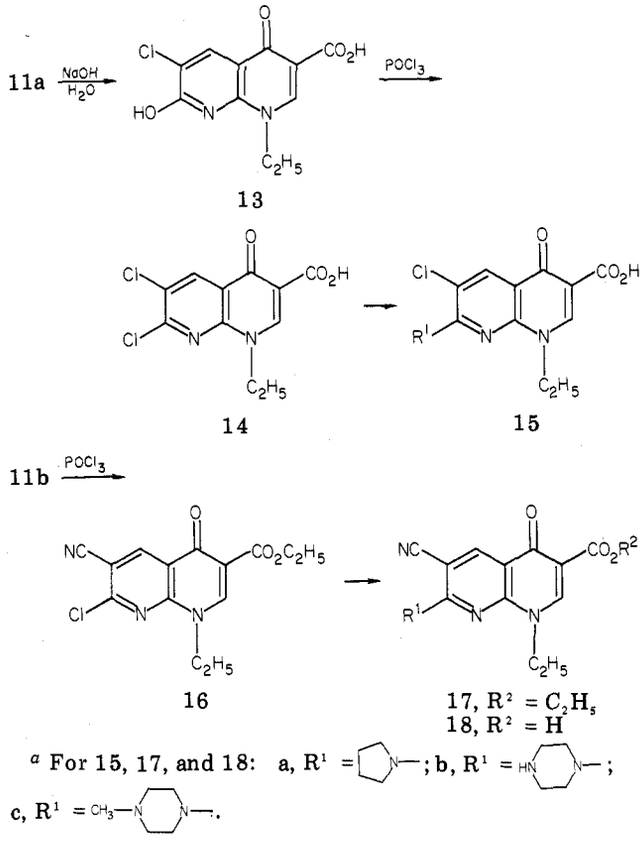
[a] For 7-9: a, $R^1 = CH_3O$; b, $R^1 =$  ; c, $R^1 =$  (except for 7).

1-Pyrrolidinyl, 1-piperazinyl, and *N*-methyl-1-piperazinyl groups were selected to be introduced at C-7 of **5** at the outset of this study on the basis of our successful development of piromidic and pipemidic acids. As a result of the present study, we found a promising candidate, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid (**24b**), as a potent new antibacterial agent. Recent papers dealing with the quinoline analogues norfloxacin (**6b**)[11] and perfloxacin (**6c**)[12] prompted us to report our results on a synthesis of the 1,8-naphthyridine derivatives **5**, as well as **24b**, and the structure–activity relationships (SAR's) associated with variations of the C-1, C-6, and C-7 substituents of **5**.

Although a number of 1,8-naphthyridine derivatives **3** without the C-6 substituent have been reported, little appears to be known about derivatives of **5** with substituents at C-6,[6,13] probably due to synthetic difficulties. An attractive intermediate for the synthesis of **5** would be a 6-diazonium salt of an appropriately functionallized naphthyridine, such as **10**, which might be converted into the 6-cyano, 6-chloro, and 6-fluoro analogues **11** under conditions of the Sandmeyer reaction or the like as depicted in Scheme I.

Chemistry. The requisite diazonium salt **10** was readily prepared by diazotization of the corresponding 6-amino compound **9** via the 6-nitro analogue **8**. Thus, ethyl 1-ethyl-1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate (**7a**) was nitrated with a mixed acid (fuming nitric acid and concentrated sulfuric acid) to give **8a** in 81% yield. Reduction of **8a** with iron powder in acetic acid gave the 6-amino derivative **9a**, which was then diazotized with sodium nitrite in concentrated hydrochloric acid. The resulting diazonium chloride **10a**, without isolation, was successively treated with cuprous chloride, giving the 6-chloro compound **11a** in 64% yield. The 6-cyano congener **11b** could analogously be prepared in good yield by diazotization of **9a** with sodium nitrite in a dilute sulfuric acid, followed by successive treatment with a mixture of cuprous cyanide and potassium cyanide. The esters **11a,b** were hydrolyzed to the acids **12a,b**, respectively.

Scheme II[a]



[a] For 15, 17, and 18: a, $R^1 =$; b, $R^1 =$; c, $R^1 =$.

Compounds **11a,b** served as the starting compounds for the preparation of the 6-chloro and 6-cyano derivatives **15** and **18**, respectively, with a cyclic amino group at C-7 (Scheme II). Alkaline hydrolysis of **11a**, followed by chlorination of **13** with phosphoryl chloride, afforded the 6,7-dichloro compound **14**. Regiospecific displacement of the 7-chloro group in **14** with the selected amine was effected by refluxing in acetonitrile to give good to excellent yields of the desired compounds **15a–c**; in this case, the possibility of an isomeric structure due to displacement of the 6-chloro group by the amine was excluded because the product **15a** was identical with a compound that was derived through an unambiguous route involving the reaction of **12a** with pyrrolidine. Treatment of the 6-cyano-7-methoxy compound **11b** with phosphoryl chloride gave directly the 6-cyano-7-chloro derivative **16**, which was then treated with the amines in acetonitrile to afford the 6-cyano-7-(cyclic amino) esters **17a–c**. These esters were converted into the corresponding carboxylic acids **18a–c** by acidic hydrolysis.

In order to introduce a fluoro group at C-6 of the 1,8-naphthyridine ring, the Balz–Schiemann reaction[14] was applied initially to the 6-amino-7-methoxy derivative **9a**. Diazotization of **9a** with sodium nitrite in 42% tetrafluoroboric acid gave an 85% yield of the diazonium tetrafluoroborate **10c**, which showed a characteristic IR absorption at 2200 cm^{-1} (ν, N≡N). However, attempts to convert the diazonium salt **10c** into the fluoro compound **11c** under a variety of conditions were unsuccessful.

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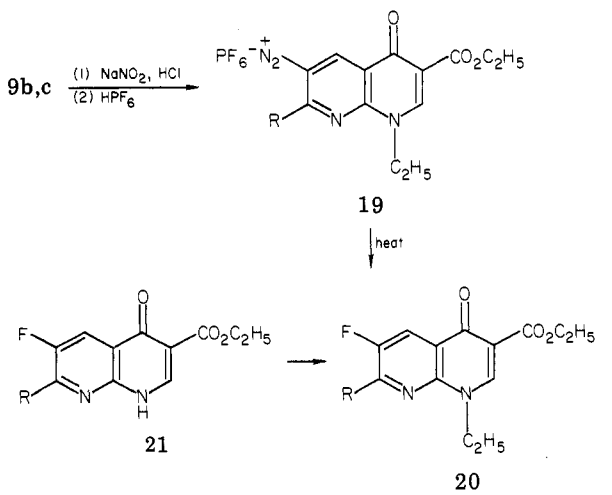
(13) (a) Suzuki, N.; Kato, M.; Dohmori, R. *Yakugaku Zasshi* **1979**, *99*, 155. (b) Suzuki, N. *Chem. Pharm. Bull.* **1980**, *28*, 761.
(14) Roe, A. *Org. React.* **1949**, *5*, 193–228.

Table I. 1-Ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid Derivatives

compd	mp, °C	recrystn solvent	yield,[a] %	synth method[b]	formula[c]
8a	224-227	EtOH	80.8	A	$C_{14}H_{15}N_3O_6$
8b	211-212	EtOH	89.5	A	$C_{19}H_{23}N_5O_6$
8c	205-207[d]	EtOH	91.6	B	$C_{17}H_{20}N_4O_5$
9a	250-252	EtOH	79.9	C	$C_{14}H_{17}N_3O_4$
9b	265-270	EtOH	78.2	C	$C_{19}H_{25}N_5O_4$
9c	270-272[e]	EtOH	37.5	C	$C_{17}H_{22}N_4O_3$
11a	156-157	EtOH-H_2O	63.7	D	$C_{14}H_{15}ClN_2O_4$
11b	221-223	EtOH	66.0	E	$C_{15}H_{15}N_3O_4$
12a	271-275	DMF	72.3	F	$C_{12}H_{11}ClN_2O_4$
12b	281-284	DMF	93.0	F	$C_{13}H_{11}N_3O_4$
13	>300	DMF	83.3	G	$C_{11}H_9ClN_2O_4$
14	262-267[f]	DMF	65.7	H	$C_{11}H_8Cl_2N_2O_3$
15a	297-300[g]	DMF	85.7	I	$C_{15}H_{16}ClN_3O_3$
			75.6	J	
15b[h]	246-248	AcOH/NH_4OH[i]	82.1	I	$C_{15}H_{17}ClN_4O_3$
15c	232-233	DMF	77.6	I	$C_{16}H_{19}ClN_4O_3$
16	252-256[j]	DMF	77.1	H	$C_{14}H_{12}ClN_3O_3$
17a	259-260	EtOH	75.3	K	$C_{18}H_{20}N_4O_3$
17b	151-154	DMF	51.7	K	$C_{18}H_{21}N_5O_3 \cdot 1.2H_2O$
17c	195-196	EtOH	74.2	K	$C_{19}H_{23}N_5O_3$
18a	296-298	DMF	91.1	F	$C_{16}H_{16}N_4O_3$
18b[k]	242-245 dec	HCl/AcONa[i]	79.4	F	$C_{16}H_{17}N_5O_3$
18c	233-235	DMF	73.6	F	$C_{17}H_{19}N_5O_3$
20a	220-222	EtOH-CH_2Cl_2	8.2	L	$C_{17}H_{20}FN_3O_3$
			84.7	M	
20b	195-197	AcOEt	36.2	L	$C_{19}H_{23}FN_4O_4$
			89.7	M	
22a	256-258	DMF	95.7	F	$C_{15}H_{16}N_4O_5$
22b	234-236	DMF	80.9	N	$C_{15}H_{17}N_5O_5 \cdot 2H_2O$
22c	219-221	EtOH-$CHCl_3$	54.9	R	$C_{16}H_{19}N_5O_5 \cdot 0.3H_2O$
23a	290-296 dec	NaOH/AcOH[i]	85.1	O	$C_{15}H_{18}N_4O_3$
23b	253-255	NaOH/AcOH[i]	29.3	N	$C_{15}H_{19}N_5O_3 \cdot 2H_2O$
23c	253-256	EtOH	26.1	P	$C_{16}H_{21}N_5O_3$
24a	299-300	AcOEt-CH_2Cl_2	73.5	F	$C_{15}H_{16}FN_3O_3$
24b[l]	220-224	EtOH-CH_2Cl_2	75.3	Q	$C_{15}H_{17}FN_4O_3$
24c	227-230	EtOH-CH_2Cl_2	75.0	R	$C_{16}H_{19}FN_4O_3$
			89.3	T	

[a] Yields are of purified products and are not maximal. [b] Capital letters refer to the method described in the Experimental Section. [c] All compounds were analyzed for C, H, N, and, where present, Cl and F; analytical results were within ±0.3% of the theoretical values. [d] Literature[13a] mp 200-201 °C. [e] Literature[13a] mp 259 °C. [f] Literature[13a] mp 270-273 °C. [g] Literature[13a] mp >300 °C. [h] HCl salt: mp 290-300 °C. Anal. ($C_{15}H_{17}ClN_4O_3 \cdot HCl$) C, H, Cl, N. [i] Purified by reprecipitation on treatment with the acid and subsequently with the base or vice versa. [j] Literature[13b] mp 243 °C. [k] HCl salt: mp 265-273 °C. Anal. ($C_{16}H_{17}N_5O_3 \cdot HCl$) C, H, Cl, N. [l] For salts, see the Experimental Section.

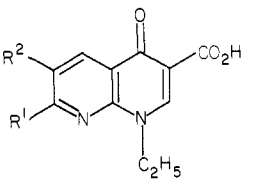
Scheme III[a]



[a] For 19-21: a, R =  ; b, R =

Compound **9b** was then employed for the Balz–Schiemann reaction. Nitration of ethyl 7-(4-acetyl-1-piperazinyl)-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (**7b**), followed by reduction of the 6-nitro compound **8b**, gave the requisite amino analogue **9b**.

Chart II[a]

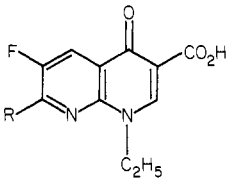


22, $R^2 = NO_2$
23, $R^2 = NH_2$
24, $R^2 = F$

[a] a, R^1 =  ; b, R^1 =  ; c, R^1 =  .

Diazotization of **9b** with sodium nitrite in 65% hexafluorophosphoric acid furnished a 94% yield of the 6-diazonium hexafluorophosphate **19b** (Scheme III). When **19b** was heated at 80–100 °C with *n*-heptane, the fluorination proceeded smoothly to give a 36% yield of the desired 6-fluoro compound **20b**, though unsatisfactory in yield. The same conditions permitted the conversion of 7-pyrrolidinyl-6-diazonium hexafluorophosphate **19a** into the 6-fluoro-7-pyrrolidinyl analogue **20a** but in a very poor yield. Compounds **20a,b** were alternatively prepared in good yields by the N-ethylation of **21a,b**, respectively. The synthesis of **21**, which is important for aspects of the large-scale preparation of **20** and its derivatives, particu-

Table II. 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acids Derivatives



compd	R	mp, °C	recrystn solvent	yield,[a] %	synth method[b]	formula[c]
27a	NH_2	>300	DMF	83.3	S	$C_{11}H_{10}FN_3O_3$
27b	$NH_2CH_2CH_2NH$	273-276	AcOH/NH_4OH[d]	83.4	T	$C_{13}H_{15}FN_4O_3$
27c	3-oxo-1-piperazinyl	>300	DMF	71.4	T	$C_{15}H_{15}FN_4O_4$
27d	$CH_2(CH_2CH_2)_2N$-	211-213	DMF	84.3	T	$C_{16}H_{18}FN_3O_3$
27e	$O(CH_2CH_2)_2N$-	261-263	DMF	79.6	T	$C_{15}H_{16}FN_3O_4$
27f	$S(CH_2CH_2)_2N$-	251-253	MeCN	83.5	T	$C_{15}H_{16}FN_3O_3S$
27g	homopiperazinyl[e]	238-240	AcOH/NH_4OH[d]	72.7	T	$C_{16}H_{19}FN_4O_3$
27h	1-azepinyl	192-193	EtOH	74.0	T	$C_{17}H_{14}FN_3O_3$
27i	$CH_2(CH_2CH_2CH_2)_2N$-	188-190	EtOH	78.1	T	$C_{18}H_{22}FN_3O_3$
27j	Ph-N(CH_2CH_2)$_2$N-	237-239	MeCN	50.4	T	$C_{21}H_{21}FN_4O_3$
27k	$PhCH_2N(CH_2CH_2)_2N$-	220-222	EtOH-CH_2Cl_2	31.7	T	$C_{22}H_{23}FN_4O_3$
27l	Et-N(CH_2CH_2)$_2$N-	193-194	EtOH-CH_2Cl_2	91.6	T	$C_{17}H_{21}FN_4O_3$
28a	*n*-Pr-N(CH_2CH_2)$_2$N-	208-209	EtOH-CH_2Cl_2	32.4	U	$C_{18}H_{23}FN_4O_3$
28b	*n*-Bu-N(CH_2CH_2)$_2$N-	184-185	EtOH-CH_2Cl_2	61.2	U	$C_{19}H_{25}FN_4O_3$
28c	*s*-Bu-N(CH_2CH_2)$_2$N-	213-214	EtOH-CH_2Cl_2	26.5	U	$C_{19}H_{25}FN_4O_3$
29	OHC-N(CH_2CH_2)$_2$N-	292-294	EtOH-CH_2Cl_2	97.5	V	$C_{16}H_{17}FN_4O_4$
30	$CH_3CON(CH_2CH_2)_2N$-	>300	EtOH-CH_2Cl_2	85.6	O	$C_{17}H_{19}FN_4O_4$

[a-c] See footnotes *a-c* in Table I. [d] Purified by reprecipitation on treatment with the acid and subsequently with the base. [e] Hexahydro-1*H*-1,4-diazepinyl.

Table III. 1,7-Disubstituted 6-Fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid Derivatives

compd	mp, °C	recrystn solvent	yield,[a] %	synth method[b]	formula[c]
31	213-215	MeCN	92.9	M	$C_{19}H_{23}FN_4O_5$
32	168-170	AcOEt	90.3	W	$C_{19}H_{22}ClFN_4O_4$
33	184-185	Me_2CO	79.8	M	$C_{19}H_{22}F_2N_4O_4$
34	282-284	AcOEt-CH_2Cl_2	19.4	X	$C_{18}H_{19}F_3N_4O_4$
35	264-266	MeCN	80.6	Y	$C_{17}H_{17}FN_4O_4$
36[d]	256-260 dec	AcOH/NH_4OH[e]	82.4	Z	$C_{15}H_{15}FN_4O_3$
37	205-207	MeCN	43.6	R	$C_{16}H_{17}FN_4O_3$
38	223-225	HCl/NH_4OH[e]	75.4	Q	$C_{15}H_{16}F_2N_4O_3$
39	224-226	MeCN	82.2	R	$C_{16}H_{18}F_2N_4O_3$
40	284-286	DMF-EtOH	97.6	Q	$C_{14}H_{13}F_3N_4O_3$

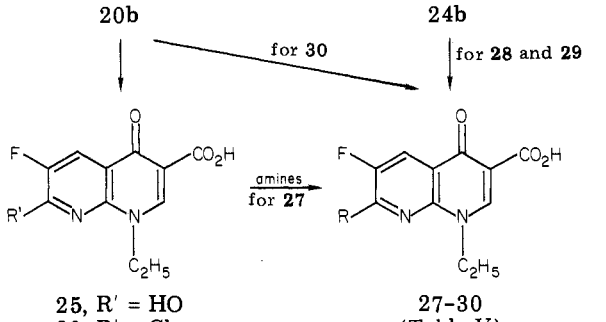
[a-c] See footnotes *a-c* in Table I. [d] HCl salt: mp ~290 °C dec (EtOH-H_2O). Anal. ($C_{15}H_{15}FN_4O_3$·HCl) C, H, Cl, F, N. Methanesulfonic acid salt: mp 292-294 °C dec (EtOH-H_2O). Anal. ($C_{15}H_{15}FN_4O_3$·CH_4SO_3·$^1/_2H_2O$) C, H, F, N, S. [e] Purified by reprecipitation on treatment with the acid and subsequently with the base.

larly **24b**, will be reported elsewhere.[15]

The 6-fluoro esters **20a,b** thus obtained were hydrolyzed under acidic conditions into the corresponding 6-fluoro carboxylic acids **24a,b**. Reductive N-methylation of **24b** upon treating with formaldehyde and formic acid afforded the *N*-methylpiperazinyl compound **24c**. Analogously, the 6-nitro carboxylic acids **22a,b** were prepared by hydrolysis of **8c,d**, respectively, and **22c** was derived from **22b** by the reductive N-methylation. The 6-amino carboxylic acids **23a-c** were obtained by reduction of the corresponding nitro compounds **22a-c**.

Since compound **24b** showed an excellent in vitro antibacterial activity as described in the following section, the 6-fluoro analogues substituted at C-7 by cyclic (and acyclic) amino groups other than the piperazinyl group of **24b** were prepared (Scheme IV). Prolonged heating of **20b** with alkali, followed by chlorination of **25** with phosphoryl chloride, afforded the 7-chloro-6-fluoro derivative **26**. Upon treatment of **26** with a variety of amines, the displacement reaction proceeded quite regioselectively

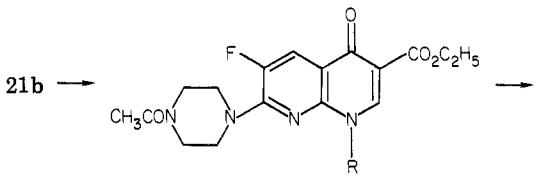
Scheme IV



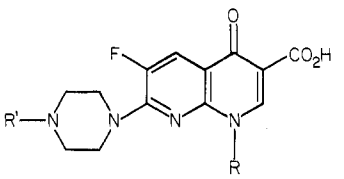
at C-7 to give the desired 7-substituted 6-fluoro derivatives **27a-l**. Reductive alkylation on the piperazinyl N-4 of **24b** was carried out by treatment with propionaldehyde, butyraldehyde, and isobutyraldehyde in formic acid to afford the corresponding N^4-alkyl derivatives **28a-c**. *N*-Formyl compound **29** was prepared by treatment of **24b** with formamide in formic acid. Partial hydrolysis of **20b** by alkali gave the N^4-acetyl carboxylic acid **30**. Among these compounds thus prepared, **28a-c**, **29**, and **30** were identified as metabolites of **24b** in animals tested.[16]

(15) Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H.; Nishimura, H. accepted for publication in *J. Heterocycl. Chem.* See also ref 2c.

Scheme V



31, R = CH_2CH_2OH
32, R = CH_2CH_2Cl
33, R = CH_2CH_2F
34, R = CHF_2



35, R = $CH{=}CH_2$; R' = CH_3CO
36, R = $CH{=}CH_2$; R' = H
37, R = $CH{=}CH_2$; R' = CH_3
38, R = CH_2CH_2F; R' = H
39, R = CH_2CH_2F; R' = CH_3
40, R = CHF_2; R' = H

Finally, the ethyl group at N-1 of **24b** and **24c** was replaced by a vinyl, fluoroethyl, or difluoromethyl group (Scheme V), because these groups seemed attractive as variants that might possibly improve the antibacterial activity of the pyridonecarboxylic acids.[17] Thus, treatment of **21b** with ethylenechlorohydrin, followed by chlorination of **31** with thionyl chloride, afforded the chloroethyl derivative **32**. On treatment of **32** with potassium hydroxide in ethanol, elimination of the hydrogen chloride proceeded smoothly to give an 81% yield of the vinyl compound **35**, along with a small amount of the hydrolyzed product, 6-fluoro-1,4-dihydro-7-hydroxy-4-oxo-1-vinyl-1,8-naphthyridine-3-carboxylic acid (**41**). The N^4-protective group in **35** was removed by alkaline hydrolysis to give the desired piperazinyl vinyl derivative **36**, which was subsequently heated with 37% formalin in formic acid to produce the N^4-methylpiperazinyl vinyl analogue **37**. Furthermore, the fluoroethyl derivative **33** was prepared from **21b** by the reaction with 2-fluoroethyl *p*-toluenesulfonate. Subsequent acid treatment of **33** provided the deprotected acid **38**, which was converted into the N^4-methylpiperazinyl analogue **39** by reductive methylation. The difluoromethyl compound **40** was prepared, though in a poor yield, by the reaction of **21b** with (difluoromethyl)carbene, formed in situ from sodium chlorodifluoroacetate, followed by deprotection of **34** with hydrochloric acid.

Biological Results and Discussion

The in vitro antibacterial activity of compounds prepared in the present study against representatives of Gram-positive (*Staphylococcus aureus* 209P JC-1) and Gram-negative bacteria (*Escherichia coli* NIHJ JC-2 and *Pseudomonas aeruginosa* Tsuchijima) is shown in Table IV. Minimal inhibitory concentrations (MIC's) of **3a–c** ($R^2 = C_2H_5$),[1] nalidixic acid, and pipemidic acid are included for comparison.

Table IV. In Vitro Antibacterial Activity[a]

	min inhibitory concn, μg/mL		
compd	*S. aureus* 209P JC-1	*E. coli* NIHJ JC-2	*P. aeruginosa* Tsuchijima
3a	12.5	25	>100
3b	25	6.25	25
3c	25	6.25	25
15a	12.5	>100	>100
15b	3.13	0.78	6.25
15c	6.25	1.56	12.5
18a	3.13	12.5	25
18b	6.25	1.56	6.25
18c	12.5	1.56	12.5
22a	25	100	>100
22b	6.25	6.25	25
22c	12.5	3.13	50
23a	>100	>100	>100
23b	>100	3.13	6.25
23c	25	1.56	12.5
24a	0.39	1.56	3.13
24b	0.78	0.2	0.78
24c	1.56	0.39	1.56
27a	>100	1.56	50
27b	25	6.25	50
27c	6.25	0.78	12.5
27d	0.78	6.25	12.5
27e	1.56	3.13	6.25
27f	1.56	3.13	12.5
27g	1.56	0.78	1.56
27h	3.13	6.25	50
27i	12.5	25	>100
27j	6.25	12.5	>100
27k	0.78	6.25	12.5
27l	0.78	0.78	3.13
28a	1.56	1.56	12.5
28b	3.13	3.13	25
28c	1.56	3.13	25
29	3.13	1.56	12.5
30	3.13	3.13	50
36	1.56	0.1	0.2
37	3.13	0.2	0.78
38	0.39	0.2	0.78
39	0.78	0.2	1.56
40	6.25	0.78	6.25
NA[b]	50	1.56	50
PPA[c]	6.25	1.56	6.25

[a] See the Experimental Section. [b] Nalidixic acid. [c] Pipemidic acid.

Firstly, we wish to discuss the SAR's associated with modification of the C-6 substituent of compounds **15**, **18**, and **22–24** compared with **3a–c** ($R^2 = C_2H_5$). Introduction of an amino, nitro, cyano, chloro, or fluoro group at C-6 of **3a–c** influenced markedly the antibacterial activity. Thus, in a series of the pyrrolidinyl compounds (**15a**, **18a**, **22a**, **23a**, and **24a**), the fluoro and cyano groups cause an increase in activity against all the bacteria tested, whereas others result in a complete loss of activity, particularly against the Gram-negative bacteria; it is noticed that a remarkable difference between the fluoro and the chloro groups in an effect upon the activity is observed in the series **a**. With respect to the piperazinyl and *N*-methylpiperazinyl derivatives (i.e., series **b** and **c** of compounds **15**, **18**, and **22–24**), introduction of the C-6 substituent tends to enhance the activity against both Gram-positive and Gram-negative organisms, with a few exceptions. In both series of compounds, the activity against *S. aureus* increases in the order $NH_2 \leq H < NO_2 = CN < Cl < F$, whereas the Gram-negative activity follows the sequence $NO_2 \leq H < NH_2 = CN = Cl < F$, with the amino, cyano, and chloro compounds being equally active. In the **b** series of compounds, the activity against *Escherichia coli* increases in the order $H = NO_2 < NH_2 < CN < Cl < F$. It is of particular interest that replacement of hydrogen by

(16) Nakamura, R.; Yamaguchi, T.; Sekine, Y.; Hashimoto, M. *J. Chromatogr.* **1983**, *278*, 321.
(17) For example, see ref 4. Albrecht, R. *Eur. J. Med. Chem.* **1977**, *12*, 231. Agui, H.; Saji, I.; Nakagome, T. Japanese Patent Kokai 139094, 1977; *Chem. Abstr.* **1978**, *88*, 105406*g*. Lee, K. T. German Offen. 2706781, 1977; *Chem. Abstr.* **1977**, *87*, 201507*r*.

Table V. Efficacy on Systemic Infections and Acute Toxicity with Oral Administration in Mice[a]

compd	*S. aureus* 50774: ED_{50}[b] (MIC)[c]	*E. coli* P-5101: ED_{50} (MIC)	*P. aeruginosa* 12: ED_{50} (MIC)	LD_{50}, mg/kg po
24b	10 (0.78)	1.8 (0.1)	9.0 (0.78)	>2000
24c	4.8 (1.56)	1.2 (3.13)	10.6 (0.2)	210
36	33.4 (3.13)	1.3 (0.1)	2.4 (0.39)	>2000
37	10.5 (3.13)	1.1 (0.1)	3.7 (1.56)	354
38	11.5 (1.56)	3.0 (0.1)	27.2 (0.78)	>2000
39	1.4 (0.39)	0.52 (0.1)	4.2 (1.56)	1866
NA[d]	>800 (50)	29.2 (3.13)	267 (100)	1800
PPA[e]	215 (25)	12.8 (3.13)	70.8 (25)	>2000

[a] See the Experimental Section. [b] In milligrams per kilogram. [c] In micrograms per milliliter. [d] Nalidixic acid. [e] Pipemidic acid.

halogen, especially fluorine, at C-6 in the 1,8-naphthyridine system results in an outstanding enhancement of the activity against both Gram-positive and Gram-negative organisms. A comparison of the activity of the piperazinyl (**15b**, **18b**, **22b**, **23b**, and **24b**) and the *N*-methyl-1-piperazinyl analogues (**15c**, **18c**, **22c**, **23c**, and **24c**) with the same C-6 substituent generally found the former to be more active than the latter, except for the activity of two sets (**22b** vs. **22c** and **24b** vs. **24c**) against *E. coli*. Compound **24a**, bearing the pyrrolidine ring at C-7, is the most potent as far as activity against *S. aureus*, whereas **24b** and **24c**, bearing the piperazine ring instead, have better activity against Gram-negative organisms. Overall, the most active is compound **24b**, which is about 30 times as active as **3b** ($R^2 = C_2H_5$) against every organism tested.

SAR's of the C-7 substituent in a series of analogues with an ethyl group at N-1 (see **24** and **27–30**) were substantially comparable to those for the corresponding 5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acids (**1**, $R^2 = C_2H_5$), which had previously been studied by us.[3,4] Thus, modification of the cyclic amino moiety at C-7 resulted in a significant decrease in activity as observed in **24a** and **27c–i** compared with **24b**. Only compound **24a** with the 1-pyrrolidinyl group, however, is more active than **24b** against the Gram-positive *Staphylococcus* strain. A comparison of the activity between **24b** and **27d**, as well as **27g** and **27h**, obviously indicates that the presence of a basic NH group in the cyclic amino function is a prerequisite to optimal activity. However, if this NH group is an amide, such as in the 3-oxo-1-piperazinyl group of **27c**, activity is decreased. Introduction of an alkyl, aralkyl, or aryl group at the piperazinyl N-4 of **24b** (i.e., **24c**, **27j–l**, and **28a–c**) causes a decrease in activity; in particular, the activity against Gram-negative bacteria is reduced markedly with an increase in the chain length and, hence, with an increase in the lipophilicity of the group introduced. Compounds **27a–c**, **29**, and **30**, identified as metabolites of **24b**,[16] are substantially less active. Of these derivatives possessing the ethyl group at N-1, no compounds superior to **24b** in activity were found.

Variation of the N-1 substituent on the naphthyridine ring significantly influenced the in vitro activity. The excellent activity of the vinyl and fluoroethyl analogues (see **36–39**) should be noted. The difluoromethyl group in **40**, however, led to a considerable decrease in activity. In each comparison between **24b** and **36**, as well as **24c** and **37**, it is revealed that replacement of the ethyl group by a vinyl group causes an increase in the Gram-negative activity, whereas it reduces appreciably the Gram-positive activity. The fluoroethyl function, in contrast to the vinyl group, enhances the Gram-positive activity, while the Gram-negative activity is essentially unchanged (compare **24b** and **24c** with **38** and **39**, respectively).

Selected compounds (**24b,c** and **36–39**) were 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 nalidixic acid and pipemidic acid. The results are listed in Table V, which includes for reference the MIC's against the organisms employed. The median effective dose (ED_{50}) values of these compounds are always much lower than those of the reference drugs.

With the exception of **38**, the in vivo efficacy on the experimental infection due to the Gram-negative bacteria was highly increased by changing the N-1 substituent from ethyl to vinyl or fluoroethyl (see **36**, **37**, and **39**). When the ethyl group of **24b** was changed to fluoroethyl (**38**), however, the efficacy on the pseudomonal infection was reduced to one-third the potency of the parent compound. On the other hand, this variation at N-1 resulted in a decreased efficacy on the infection due to *S. aureus*, a Gram-positive bacterium; for example, the most effective compound, **36**, on the pseudomonal infection is the least effective on the staphylococcal infection. Of much interest is compound **39**, which is about 7, 3.5, and 2 times as potent as **24b** on systemic infections caused by *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively. However, an acute toxicity test with a single oral administration in mice revealed the most effective compound, **39**, to possess a smaller value of the median lethal dose (LD_{50}) than that of **24b**, but it is comparable to the LD_{50} of nalidixic acid, as shown in Table V. It is noteworthy that compounds **24c** and **37**, as well as **39**, which have the N^4-methyl group on the piperazine ring in common, exhibit more potent toxicity than the corresponding N^4-unsubstituted compounds **24b**, **36**, and **38**, of which every LD_{50} is >2000 mg/kg orally in mice.

As a result, compound **24b** (named enoxacin, originally AT-2266) was found to possess broad and potent in vitro and in vivo antibacterial activity and weak oral acute toxicity. These findings indicate the possible use of **24b** as a potent, orally administrable antibacterial agent. Enoxacin (**24b**) was thus selected for further biological evaluation[18] and is now undergoing clinical trials.

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. ^1H NMR spectra were taken at 60 or 100 MHz on either a Varian EM-360A or HA-100D spectrometer with Me_4Si as an internal standard. Mass spectra were recorded on a Hitachi RMU-6L single-focusing mass spectrometer using the direct inlet system at 70-eV ionization potential. Elemental analysis are indicated only by symbols of the elements; analytical results were within ±0.3% of theoretical values. IR, ^1H NMR, and/or mass spectra

(18) Nakamura, S.; Minami, A.; Katae, H.; Inoue, S.; Yamagishi, J.; Takase, Y.; Shimizu, M. *Antimicrob. Agents Chemother.* **1983**, *23*, 641. Nakamura, S.; Nakata, K.; Katae, H.; Minami, A.; Kashimoto, S.; Yamagishi, J.; Takase, Y.; Shimizu, M. *Ibid.* **1983**, *23*, 742.

were obtained on all compounds and were consistent with assigned structures. Organic solutions were dried over anhydrous Na_2SO_4 or $MgSO_4$.

Ethyl 1-Ethyl-1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate (7a). In a similar procedure to that described in our previous paper,[1] **7a** was prepared from 2-amino-6-methoxypyridine by the Gould–Jacobs-type reaction of its malonate, followed by the N-ethylation of ethyl 1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate; the details will be reported elsewhere:[19] mp 142.5–143 °C; IR (KBr) 1680, 1640 cm^{-1}; EIMS, m/z 276 (M^+), 240 ($M^+ - C_2H_4CO_2$). Anal. ($C_{14}H_{16}N_2O_4$) C, H, N.

Ethyl 7-(4-Acetyl-1-piperazinyl)-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (7b). A mixture containing 5.0 g (17.8 mmol) of ethyl 7-chloro-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate,[1] 7.0 g (54.7 mmol) of *N*-acetylpiperazine, and 100 mL of EtOH was heated to reflux for 3 h. The reaction mixture was concentrated to dryness in vacuo. The residue was taken up in 80 mL of water, acidified with 10% HCl, and extracted with $CHCl_3$. The extract was washed with water and dried, and the solvent was evaporated in vacuo. The residue was crystallized from acetonitrile to give 6.5 g (98.1%) of **7b**: mp 195–197 °C; IR (KBr) 1720, 1680, 1610 cm^{-1}. Anal. ($C_{19}H_{24}N_4O_4 \cdot H_2O$) C, H, N.

Method A. Ethyl 1-Ethyl-1,4-dihydro-7-methoxy-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylate (8a). To a stirred mixture of concentrated HNO_3 (150 mL) and concentrated H_2SO_4 (200 mL) was added portionwise 50 g (0.181 mol) of **7a** at room temperature. After complete addition, the reaction mixture was heated at 60–70 °C for 1.5 h, poured onto 1.5 L of ice–water, and extracted with $CHCl_3$. The extract was washed with 7% $NaHCO_3$, dried, and concentrated in vacuo to give 47.0 g (80.8%) of **8a**: IR (KBr) 1720, 1610, 1540, 1360 cm^{-1}; EIMS, m/z 321 (M^+), 249 ($M^+ - C_2H_4CO_2$).

Method B. Ethyl 1-Ethyl-1,4-dihydro-6-nitro-4-oxo-7-(1-pyrrolidinyl)-1,8-naphthyridine-3-carboxylate (8c). To a stirred solution of **8a** (1.0 g, 3.11 mmol) in 30 mL of acetonitrile was added 3 mL of pyrrolidine. The mixture was heated to reflux for 3 h. The solvent was evaporated in vacuo. The residue was crystallized from AcOEt to give 1.03 g (91.6%) of **8c**: IR (KBr) 1720, 1680, 1540, 1350 cm^{-1}.

Method C. Ethyl 6-Amino-1-ethyl-1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate (9a). To a stirred solution of **8a** (20 g, 62.3 mmol) in 400 mL of AcOH was added portionwise 40 g of reduced-iron powder at such a rate that the temperature did not rise over 80 °C. After complete addition, the reaction mixture was heated at 80 °C for 1 h and cooled to room temperature. After addition of EtOH (500 mL), the mixture was filtered to remove insoluble materials, and the filtrate was concentrated to dryness in vacuo. The residue was taken up in 800 mL of water and allowed to stand under ice cooling to give 14.5 g (79.7%) of **9a**: IR (KBr) 3420, 3280, 2950, 1680, 1620 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.63 (1 H, s, H-2), 7.62 (1 H, s, H-5), 5.47 (2 H, s, NH_2), 4.45 and 4.23 (each 2 H, q, J = 7 Hz, NCH_2CH_3, OCH_2CH_3), 4.07 (3 H, s, OCH_3), 1.42 and 1.30 (each 3 H, t, J = 7 Hz, NCH_2CH_3, OCH_2CH_3).

3-(Ethoxycarbonyl)-1-ethyl-1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-6-diazonium Tetrafluoroborate (10c). To a stirred solution of **9a** (5.0 g, 17.2 mmol) in 65 mL of 42% HBF_4 was added dropwise a solution of $NaNO_2$ (1.2 g, 17.4 mmol) in 2 mL of water while the temperature was maintained at 5–10 °C. After 30 min, the precipitate was collected by filtration, washed with a mixture of MeOH–Et_2O (1:1 v/v), and crystallized from water to give 5.7 g (85.0%) of **10c**: mp 148–149 °C dec; IR (KBr) 2200, 1720, 1620 cm^{-1}. Anal. ($C_{14}H_{15}BF_4N_4O_4$) C, H, F, N.

Thermal Decomposition of 10c. A mixture containing 2.0 g (5.13 mmol) of **10c** and 2.0 g of anhydrous Na_2SO_4 was heated at 73–85 °C for 3 h. The reaction mixture was taken up in 80 mL of EtOH–$CHCl_3$ (1:1, v/v) and filtered to remove insoluble materials. The filtrate was concentrated to dryness in vacuo. The residue was chromatographed on silica gel with $CHCl_3$. The solid resulting from the main fraction was recrystallized from *n*-hexane–acetone to give 0.58 g (41.0%) of **7a**; this was identical in all respects with an authentic specimen of **7a**.

Method D. Ethyl 6-Chloro-1-ethyl-1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate (11a). To a stirred solution of **9a** (4.0 g, 13.7 mmol) in 50 mL of concentrated HCl was added dropwise a solution of $NaNO_2$ (1.5 g, 21.7 mmol) in 8 mL of water over a period of 10 min at 0–3 °C. After an additional 10 min, the reaction mixture was added to a solution of cuprous chloride (2.7 g, 13.6 mmol) in 15 mL of concentrated HCl and allowed to stir at room temperature for 1 h, after which time the temperature was maintained at 65 °C for 30 min. The mixture was neutralized with 10% NaOH and extracted with $CHCl_3$. The extract was concentrated to dryness in vacuo; the residue was crystallized from aqueous EtOH to give 2.71 g (63.7%) of **11a**: IR (KBr) 1720, 1690, 1620 cm^{-1}; EIMS, m/z 310 (M^+), 238 ($M^+ - C_2H_4CO_2$); ^1H NMR ($CDCl_3$) δ 8.70 (1 H, s, H-5), 8.60 (1 H, s, H-2), 4.45 (4 H, q, J = 7 Hz, NCH_2CH_3, $CO_2CH_2CH_3$), 4.20 (3 H, s, OCH_3), 1.55 (3 H, t, J = 7 Hz, $CO_2CH_2CH_3$), 1.44 (3 H, t, J = 7 Hz, NCH_2CH_3).

Method E. Ethyl 6-Cyano-1-ethyl-1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate (11b). To a suspension of **9a** (10 g, 34.3 mmol) in 200 mL of water was added 5.0 g of concentrated H_2SO_4. To the reaction mixture kept at 0 °C was gradually added a solution of $NaNO_2$ (2.6 g, 37.7 mmol) in 3 mL of water while the temperature was kept at 3–6 °C. The resulting mixture was added at room temperature to a solution of cuprous chloride (6.8 g, 38.0 mmol) and KCN (8.6 g, 132 mmol) in 160 mL of water. The reaction mixture was maintained at 80 °C for 1 h, basified with aqueous ammonia, and extracted with $CHCl_3$. The extract was washed with water, dried, and concentrated to dryness in vacuo. The residue was chromatographed on silical gel with $CHCl_3$ to give 6.8 g (65.8%) of **11b**: IR (KBr) 2200, 1720, 1630 cm^{-1}; EIMS, m/z 301 (M^+), 229 ($M^+ - C_2H_4CO_2$); ^1H NMR ($CDCl_3$) δ 8.94 (1 H, s, H-5), 8.58 (1 H, s, H-2), 4.42 (4 H, q, J = 7 Hz, $CO_2CH_2CH_3$, NCH_2CH_3), 1.55 (3 H, t, J = 7 Hz, $CO_2CH_2CH_3$), 1.45 (3 H, t, J = 7 Hz, NCH_2CH_3).

Method F. 6-Chloro-1-ethyl-1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylic Acid (12a). A mixture containing 3.0 g (9.65 mmol) of **11a**, 80 mL of 1 N HCl, and 20 mL of EtOH was heated at 90–95 °C for 1 h. The resulting clear solution was allowed to cool. The precipitate was filtered off, washed with EtOH, and recrystallized to give 2.3 g (72.3%) of **12a**: IR (KBr) 3000, 1700, 1600 cm^{-1}; EIMS, m/z 282 (M^+), 238 ($M^+ - CO_2$).

Method G. 6-Chloro-1-ethyl-7-hydroxy-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (13). A stirred suspension of **11a** (5.0 g, 16.1 mmol) in 50 mL of 1 N NaOH was heated at 90–95 °C for 45 min. Neutralization of the mixture with 30% AcOH afforded 4.1 g (94.7%) of **13**: IR (KBr) 1705, 1650, 1620 cm^{-1}.

Method H. 6,7-Dichloro-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (14). A mixture containing 2.7 g (10 mmol) of **13** and 15 mL of $POCl_3$ was heated to reflux for 30 min. The excess of $POCl_3$ was evaporated in vacuo, and the residue was poured onto ice–water to give precipitates, which were filtered off, washed with water, and recrystallized to give 1.9 g (65.7%) of **14**: IR (KBr) 1720 cm^{-1}; EIMS, m/z 287 (M^+), 243 ($M^+ - CO_2$).

6-Chloro-1-ethyl-1,4-dihydro-4-oxo-7-(1-pyrrolidinyl)-1,8-naphthyridine-3-carboxylic Acid (15a). (A) Method I. A mixture containing 0.50 g (1.74 mmol) of **14**, 0.75 g (10.5 mmol) of pyrrolidine, and 20 mL of acetonitrile was heated to reflux for 45 min. The solvent was evaporated in vacuo, and the residue was taken up in 10 mL of water, neutralized with 30% AcOH, and extracted with $CHCl_3$. The extract was concentrated to dryness to give 0.48 g (85.7%) of **15a**: IR (KBr) 1700, 1610 cm^{-1}; EIMS, m/z 321 (M^+), 277 ($M^+ - CO_2$), 249 ($M^+ - C_2H_4CO_2$).

According to this procedure, compounds **15b,c** were prepared from **14** with anhyrous piperazine and *N*-methylpiperazine, respectively.

(B) Method J. A mixture containing 0.50 g (1.77 mmol) of **12a**, 1.0 g (14.1 mmol) of pyrrolidine, and 30 mL of DMF was allowed to stir at 50–60 °C for 2 h. After the same workup as in method I, **15a** (0.43 g, 75.6%) was obtained.

Method K. Ethyl 6-Cyano-1-ethyl-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylate (17b). To a

(19) Matsumoto, J.; Hirose, T.; Mishio, S., manuscript in preparation.

refluxed solution of anhydrous piperazine (2.0 g, 23.2 mmol) in 30 mL of acetonitrile was added 1.0 g (3.27 mmol) of **16** over a period of about 15 min with stirring. The reaction mixture was heated to reflux for an additional 1 h and allowed to cool. The resulting precipitate was filtered off and recrystallized to give 0.60 g (51.7%) of **17b**: IR (KBr) 2200, 1710, 1620 cm^{-1}; EIMS, m/z 355 (M^+), 283 ($M^+ - C_2H_4CO_2$).

In a similar manner, compounds **17a,c** were prepared from **16** with pyrrolidine and *N*-methylpiperazine, respectively.

Ethyl 7-(4-Acetyl-1-piperazinyl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (20b). (A) Method L. To a stirred solution of **9b** (1.0 g, 2.56 mmol) in 20 mL of 1 N HCl was added dropwise a solution of $NaNO_2$ (250 mg, 3.6 mmol) in 3 mL of water, while the temperature was maintained at 0–3 °C. After the reaction mixture was stirred for 5 min, aqueous 65% HPF_6 was added to the mixture until precipitation was complete. The precipitate was filtered off and dried over P_2O_5 in vacuo to give 1.3 g (94.2%) of the diazonium salt **19b**: mp 128–131 °C dec; IR (KBr) 2180 cm^{-1}. A suspension of **19b** (1.0 g, 1.84 mmol) in 40 mL of *n*-heptane was heated to reflux for 15 min and allowed to cool. The resulting precipitate was collected by filtration, taken up in 30 mL of water, and extracted with $CHCl_3$. The extract was dried and concentrated to dryness in vacuo to afford a crude product, which was recrystallized to give 0.26 g (36.2%) of **14b**: IR (KBr) 1720, 1680, 1620 cm^{-1}; EIMS, m/z 390 (M^+), 318 ($M^+ - C_2H_4CO_2$); ^1H NMR ($CDCl_3$) δ 8.55 (1 H, s, H-2), 8.23 (1 H, d, J = 14 Hz, H-5), 4.45 (2 H, q, J = 7 Hz, $CO_2CH_2CH_3$), 4.37 (2 H, q, J = 7 Hz, NCH_2CH_3), 3.80 (8 H, s, piperazine H), 2.18 (3 H, s, $COCH_3$), 1.50 (3 H, t, J = 7 Hz, $CO_2CH_2CH_3$), 1.47 (3 H, t, J = 7 Hz, NCH_2CH_3).

Similarly prepared was compound **20a** from **9c** via **19a** using cyclohexane. Diazonium salt **19a**: mp 125–130 °C dec; IR (KBr) 2180 cm^{-1}.

(B) Method M. A mixture containing 3.62 g (10 mmol) of ethyl 7-(4-acetyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (**21b**),[15] 2.76 g (20 mmol) of K_2CO_3, and 40 mL of DMF was heated at 100 °C for 30 min with stirring. To this mixture was added 4.68 g (30 mmol) of ethyl iodide. The resulting mixture was allowed to stir at the same temperature for 3 h and filtered to remove insoluble materials. The filtrate was concentrated to dryness in vacuo. The residue was taken up in a mixture of water (30 mL) and $CHCl_3$ (50 mL). The organic phase was separated, washed with water, dried, and chromatographed on silica gel with $CHCl_3$ to give 3.5 g (89.7%) of **20b**; this compound was identical in all respects with an authentic specimen of **20b** prepared by the method L.

Also prepared according to this procedure were **20a** [from **21a**[15] with ethyl iodide (Table I)] and **31** and **33** [from **21b** with ethylene bromohydrin and 2-fluoroethyl tosylate, respectively (Table III)].

Method N. 1-Ethyl-1,4-dihydro-6-nitro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic Acid (22b). A mixture containing 4.1 g (9.82 mmol) of **8b**, 40 mL of 15% HCl, and 20 mL of EtOH was heated at 90–100 °C for 100 min with stirring and allowed to cool. The resulting precipitate (the HCl salt of **22b**) was filtered off and dissolved in a minimum volume of hot water. The aqueous solution was basified with aqueous ammonia to afford precipitates, which were filtered off and recrystallized to give 2.76 g (80.9%) of **22b**: IR (KBr) 1630, 1600, 1560, 1360 cm^{-1}; EIMS, m/z 347 (M^+), 303 ($M^+ - CO_2$).

Method O. 6-Amino-1-ethyl-1,4-dihydro-4-oxo-7-(1-pyrrolidinyl)-1,8-naphthyridine-3-carboxylic Acid (23a). A stirred suspension of **9c** (0.90 g, 2.72 mmol) in 20 mL of 1 N NaOH was heated at 80–90 °C for 15 min. The resulting solution was treated with charcoal and neutralized with AcOH to give 0.70 g (85.1%) of **23a**: IR (KBr) 3360, 3300, 1680, 1610 cm^{-1}; EIMS, m/z 302 (M^+), 258 ($M^+ - CO_2$).

Method P. 6-Amino-1-ethyl-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-1,8-naphthyridine-3-carboxylic Acid (23c). A mixture containing 0.50 g (1.36 mmol) of **22c**, 0.10 g of 5% Pd/C, and 50 mL of EtOH was hydrogenated at 50–60 °C until the required volume of H_2 had been taken up. The reaction mixture was filtered to remove the catalyst. The filtrate was concentrated to dryness in vacuo. The crystalline residue was purified by column chromatography on silica gel with $CHCl_3$ to give 0.12 g (26.1%) of **23c**: EIMS, m/z 331 (M^+), 287 ($M^+ - CO_2$), 261 [$M^+ - CH_2{=}N(CH_3)CH_2CH_3$], 217 [287 – $CH_2N(CH_3)CH_2CH_3$].

Method Q. 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic Acid (Enoxacin, 24b). A suspension of **20b** (5.0 g, 12.8 mmol) in 50 mL of 10% HCl was heated to reflux for 4 h and allowed to cool. The resulting precipitate was filtered off and washed with EtOH to give 4.15 g (91%) of **24b**·HCl. This salt was dissolved in 120 mL of hot water and treated with charcoal, and, after the addition of 20% HCl (5 mL), the solution was cooled to give the pure hydrochloride of **24b**, mp >300 °C. Anal. ($C_{15}H_{17}FN_4O_3$·HCl) C, H, Cl, F, N.

The hydrochloride was dissolved again in 20 mL of water with heating. Subsequent addition of 2.2 mL of concentrated aqueous ammonia under cooling led to precipitation of crystals, which were filtered off and washed with cold water to give 3.1 g (75.3%) of **24b**: IR (KBr) 2400–2000, 1615 cm^{-1}; EIMS, m/z 320 (M^+), 276 ($M^+ - CO_2$), 234 ($M^+ - CH_2{=}NCH_2$); ^1H NMR (Me_2SO-d_6) δ 8.92 (1 H, s, H-2), 8.00 (1 H, d, J = 14 Hz, H-5), 4.49 (2 H, q, J = 7 Hz, NCH_2CH_3), 3.76 and 2.85 (each 4 H, m, piperazine H), 1.39 (3 H, t, J = 7 Hz, NCH_2CH_3).

24b·AcOH: mp 228–229 °C (recrystallized from EtOH). Anal. ($C_{15}H_{17}FN_4O_3$·$C_2H_4O_2$) C, H, F, N. **24b·MeSO$_3$H**: mp >300 °C (recrystallized from EtOH–H_2O). Anal. ($C_{15}H_{17}FN_4O_3$·CH_4O_3S) C, H, F, N, S.

Method R. 1-Ethyl-6-fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-1,8-naphthyridine-3-carboxylic Acid (24c). A mixture containing 6.0 g (16.8 mmol) of **24b**, 12 mL of 37% formalin, and 18 mL of formic acid was heated at 120–125 °C for 17 h with stirring. The reaction mixture was concentrated to dryness in vacuo. The residue was taken up in 30 mL of water. The mixture was adjusted at first to pH 9 with 10% NaOH and subsequently to pH 7 with AcOH and then extracted with $CHCl_3$. The extract was washed with water, dried, and concentrated to dryness in vacuo. The residue was crystallized from CH_2Cl_2–EtOH to give 5.0 g (75%) of **24c**: IR (KBr) 1710, 1630 cm^{-1}; EIMS, m/z 334 (M^+), 290 ($M^+ - CO_2$), 220 [$M^+ - CH_3CH_2N(CH_3){=}CH$]; ^1H NMR ($CDCl_3$) δ 8.78 (1 H, s, H-2), 8.13 (1 H, d, J = 14 Hz, H-5), 4.48 (2 H, q, J = 7 Hz, NCH_2CH_3), 3.95 (4 H, m, CH_2NCH_2), 2.60 (4 H, m, $CH_2N^4CH_2$), 2.39 (3 H, s, NCH_3), 1.52 (3 H, t, J = 7 Hz, NCH_2CH_3), 14.2 (1 H, br s, COOH).

1-Ethyl-6-fluoro-1,4-dihydro-7-hydroxy-4-oxo-1,8-naphthyridine-3-carboxylic Acid (25). A suspension of **20b** (7.8 g, 20 mmol) in 100 mL of 20% NaOH was heated to reflux for 43 h with stirring. After ice cooling, the precipitate (the disodium salt of **25**) was filtered off and taken up in 200 mL of hot water. The aqueous solution was treated with charcoal, acidified with 20 mL of 10% HCl, while hot, and allowed to cool to give precipitates, which were filtered off and washed with water. Recrystallization from water–EtOH (1:4, v/v) gave 4.13 g (82.0%) of **25**: mp >300 °C; IR (KBr) 1710 cm^{-1}. Anal. ($C_{11}H_9FN_2O_4$) C, H, F, N.

7-Chloro-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (26). A mixture containing 9.5 g (37.7 mmol) of **25** and 50 mL of $POCl_3$ was heated to reflux for 15 min. The excess of $POCl_3$ was evaporated in vacuo, and the residue was poured into 300 mL of ice–water. After 30 min of stirring, the precipitate was filtered off and washed successively with water and acetone to give 9.3 g (91.4%) of **26**: mp 265–267 °C (recrystallized from CH_3CN); IR (KBr) 1720 cm^{-1}; EIMS, m/z 270 (M^+), 226 ($M^+ - CO_2$); ^1H NMR ($CDCl_3$) δ 8.98 (1 H, s, H-2), 8.56 (1 H, d, J = 8 Hz, H-5), 4.62 (2 H, q, J = 7 Hz, NCH_2CH_3), 1.58 (3 H, t, J = 7 Hz, NCH_2CH_3), 14.2 (1 H, s, COOH). Anal. ($C_{11}H_8ClFN_2O_3$) C, H, Cl, F, N.

Method S. 7-Amino-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (27a). To a stirred solution of **26** (1.35 g, 5 mmol) in 20 mL of DMF was added 4.8 g (50 mmol) of ammonium carbonate. The mixture was heated at 90 °C for 2 h, after which time an additional 2.4 g (25 mmol) of ammonium carbonate was added. The reaction was allowed to run for an additional 1.5 h. After the mixture was cooled, the precipitate was filtered off, washed with water, and recrystallized to give 1.1 g (87.6%) of **27a** (Table II): EIMS, m/z 251 (M^+), 207 ($M^+ - CO_2$).

Method T. 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(3-oxo-1-piperazinyl)-1,8-naphthyridine-3-carboxylic Acid (27c). A mixture containing 1.5 g (5.55 mmol) of **26**, 1.11 g (11.1 mmol) of 2-oxopiperazine, and 100 mL of acetonitrile was heated to reflux for 1 h and allowed to cool. The precipitate was filtered off,

washed with water, and recrystallized from DMF–EtOH (ca. 1:1, v/v) to give 1.35 g (71.4%) of **27c**: IR (KBr) 3400, 3200, 3050, 2400, 1710, 1670, 1620 cm^{-1}; EIMS, m/z 334 (M^+), 290 ($M^+ - CO_2$).

Method U. 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-*n*-propyl-1-piperazinyl)-1,8-naphthyridine-3-carboxylic Acid (28a). A mixture containing 1.0 g (3.12 mmol) of **24b**, 5 mL of propionaldehyde, and 5 mL of formic acid was heated at 90 °C for 17 h with stirring. The residue was taken up in 20 mL of 10% HCl and extracted with three 20-mL portions of $CHCl_3$. The combined extracts were dried and concentrated to dryness. The precipitate was recrystallized from EtOH–CH_2Cl_2 to give 0.02 g of **29** which was identical with its authentic specimen prepared by method V. The foregoing acidic aqueous layer was adjusted to pH 7 with 10% NaOH and extracted with three 20-mL portions of $CHCl_3$. The combined extracts were dried, and the solvent was evaporated. The residue was crystallized from CH_2Cl_2–EtOH (ca. 1:1, v/v) to give 0.51 g (46.5%) of **28a**.

Also prepared according to this procedure were **28b,c** (Table II) from **24b** using *n*-butyraldehyde and *sec*-butyraldehyde, respectively.

Method V. 1-Ethyl-6-fluoro-7-(4-formyl-1-piperazinyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (29). A mixture containing 5.0 g (15.6 mmol) of **24b**, 50 mL of formic acid, and 10 mL of formamide was heated to reflux for 2 h. After the mixture was cooled, the resulting precipitate was filtered off and washed with acetone to give 5.3 g (97.5%) of **29**: IR (KBr) 1720, 1670, 1625 cm^{-1}.

Method W. Ethyl 7-(4-Acetyl-1-piperazinyl)-1-(2-chloroethyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (32). To a stirred solution of **31** (5.4 g, 13.3 mmol) in 60 mL of $CHCl_3$ was added a solution of $SOCl_2$ (3.17 g, 26.6 mmol) in 10 mL of $CHCl_3$. The mixture was heated to reflux for 30 min, cooled, mixed with 30 mL of water, and neutralized with saturated $NaHCO_3$. The organic layer was separated, washed with water, dried, and concentrated to dryness. The residual solid was chromatographed on silica gel with $CHCl_3$ to give 5.1 g (90.3%) of **32**: EIMS, m/z 424 (M^+), 379 ($M^+ - C_2H_5O$), 352 ($M^+ - C_2H_4CO_2$); IR (KBr) 1720, 1620 cm^{-1}.

Method X. Ethyl 7-(4-Acetyl-1-piperazinyl)-6-fluoro-1-(difluoromethyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (34). To a refluxed solution of **21b** (10.8 g, 30 mmol) in 300 mL of DMF was added dropwise a solution of sodium chlorodifluoroacetate (11.4 g, 75 mmol) in 30 mL of DMF over a period of 5 min. The mixture was allowed to reflux for an additional 30 min and then cooled. The insoluble material was filtered off and washed with CH_2Cl_2 to give 8.5 g (78.7% recovery) of the unchanged compound **21b**. The filtrate and the washings were combined and concentrated to dryness in vacuo. The residue was triturated with hot $CHCl_3$. The $CHCl_3$ solution was washed with water and then dried over K_2CO_3, and the solvent was evaporated to leave a crude product, which was chromatographed on silica gel with $CHCl_3$; the main fraction gave 2.4 g (19.4%) of **34**: IR (KBr) 1730, 1650, 1625 cm^{-1}; EIMS, m/z 412 (M^+), 340 ($M^+ - C_2H_4CO_2$); ^1H NMR (Me_2SO-d_6) δ 8.57 (1 H, s, H-2), 7.96 (1 H, d, J = 14 Hz, H-5), 8.35 (1 H, t, J = 59 Hz, CHF_2), 4.26 (2 H, q, J = 7 Hz, NCH_2CH_3), 3.81 and 3.36 (8 H, piperazine H), 2.06 (3 H, s, $COCH_3$), 1.29 (3 H, t, J = 7 Hz, NCH_2CH_3).

Method Y. 7-(4-Acetyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-1-vinyl-1,8-naphthyridine-3-carboxylic Acid (35). To a hot solution of **32** (2.13 g, 5 mmol) in 15 mL of EtOH was added a solution of KOH (0.84 g, 15 mmol) in 15 mL of EtOH. The reaction mixture was heated to reflux for 2 h and allowed to cool. The precipitate was filtered off, washed with EtOH, and dissolved in 20 mL of boiling water. The aqueous solution was treated with charcoal and adjusted to pH 4–5 with 10% AcOH to give 1.45 g (80.6%) of **35**: IR (KBr) 1710 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.90 (1 H, s, H-2), 8.14 (1 H, d, J = 13.5 Hz, H-5), 7.83 (1 H, dd, J = 16 and 9 Hz, NC*H*=CH_2), 5.88 (1 H, dd, J = 2 and 16 Hz, NCH=C*H* trans), 5.45 (1 H, dd, J = 2 and 9 Hz, NCH=C*H* cis), 3.78 (8 H, piperazine H), 2.03 (3 H, s, $COCH_3$), 14.95 (1 H, br s, COOH).

Method Z. 6-Fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1-vinyl-1,8-naphthyridine-3-carboxylic Acid (36). A stirred suspension of **35** (4.0 g, 11.1 mmol) in 20 mL of 10% NaOH was heated to reflux for 2 h, allowed to cool, and adjusted to pH 7 with 30% AcOH. The resulting solid was filtered off, washed with water, and taken up in ca. 40 mL of 10% AcOH with heating. The solution was adjusted to pH 9 with aqueous ammonia and cooled to give precipitates, which were dissolved in 10% AcOH; the solution was neutralized with ammonia to give 2.90 g (82.4%) of **36**: IR (KBr) 1620 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.82 (1 H, s, H-2), 8.05 (1 H, d, J = 14 Hz, H-5), 7.76 (1 H, dd, J = 16 and 9 Hz, NC*H*=CH_2), 5.82 (1 H, dd, J = 16 and 2 Hz, NCH=C*H* trans), 5.40 (1 H, dd, J = 9 and 2 Hz, NCH=C*H* cis), 3.75 and 2.84 (8 H, piperazine H).

The initial filtrate and the washings were combined and concentrated to dryness. Recrystallization from an ca. 1:1 EtOH–$CHCl_3$ mixture gave 0.15 g (5.4%) of **6-fluoro-1,4-dihydro-7-hydroxy-4-oxo-1-vinyl-1,8-naphthyridine-3-carboxylic acid (41)**: mp 256–259 °C; IR (KBr) 1700 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.93 (1 H, s, H-2), 8.25 (1 H, d, J = 10 Hz, H-5), 7.73 (1 H, dd, J = 16 and 9 Hz, NC*H*=CH_2), 5.93 (1 H, dd, J = 16 and 2 Hz, NCH=C*H* trans), 5.53 (1 H, dd, J = 9 and 2 Hz, NCH=C*H* cis), ~13.0 (br, COOH and/or OH). Anal. ($C_{11}H_7FN_2O_4$) C, H, F, N.

In Vitro Antibacterial Activity. According to the method of Goto et al.,[20] 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.[21] 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 carboxymethylcellulose 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.

Acute Toxicity Test. A suspension of the test compound in 0.2% carboxymethylcellulose in different concentrations was orally given to male mice (Std-ddY, four to eight in each group) at a dose of 0.1 mL/10 g of body weight. The number of dead mice was counted after 7 days, and the LD_{50} value (milligrams per kilogram) was calculated in according to the Behrens–Kaerber method.[22]

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Registry No. **3a**, 57948-69-3; **3b**, 54132-31-9; **3c**, 54132-45-5; **7a**, 76105-70-9; **7b**, 54132-41-1; **8a**, 79286-67-2; **8b**, 87939-04-6; **8c**, 65095-80-9; **9a**, 79286-68-3; **9b**, 87938-96-3; **9c**, 70186-25-3; **10c**, 79286-70-7; **11a**, 87938-89-4; **11b**, 87938-90-7; **12a**, 87938-91-8; **12b**, 87939-18-2; **13**, 87938-92-9; **14**, 70186-28-6; **15a**, 70186-29-7; **15b**, 87938-93-0; **15c**, 87938-94-1; **16**, 75064-88-9; **17a**, 87938-95-2; **17b**, 76105-58-3; **17c**, 76105-72-1; **18a**, 76105-57-2; **18b**, 87939-19-3; **18c**, 76105-73-2; **19a**, 87939-01-3; **19b**, 87938-98-5; **20a**, 87938-99-6; **20b**, 82671-13-4; **21a**, 87939-02-4; **21b**, 84233-60-3; **22a**, 87939-20-6; **22b**, 74274-57-0; **22b**·HCl, 87939-05-7; **22c**, 87939-08-0; **23a**, 87939-06-8; **23b**, 74274-56-9; **23c**, 87939-07-9; **24a**, 74274-63-8; **24b**, 74011-58-8; **24b**·HCl, 74011-31-7; **24b**·AcOH, 75167-07-6; **24b**·$MeSO_3H$, 75167-06-5; **24c**, 74274-66-1; **25**, 87939-09-1; **25**·2Na, 87939-10-4; **26**, 79286-73-0; **27a**, 87939-11-5; **27b**, 87939-21-7; **27c**, 87939-12-6; **27d**, 74274-65-0; **27e**, 74274-64-9; **27f**, 87939-22-8; **27g**, 74274-60-5; **27h**, 87939-23-9; **27i**, 87939-24-0; **27j**, 87939-25-1; **27k**, 74274-70-7; **27l**, 74274-67-2; **28a**, 74274-68-3; **28b**, 74274-69-4; **28c**, 87939-14-8;

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29, 87939-13-7; 30, 78903-83-0; 31, 87939-03-5; 32, 87939-15-9; 33, 84209-43-8; 34, 87939-16-0; 35, 87939-17-1; 36, 74274-71-8; 36·HCl, 75167-16-7; 36·$MeSO_3H$, 75167-17-8; 37, 74274-72-9; 38, 84209-34-7; 39, 84209-33-6; 40, 87939-26-2; 41, 84424-27-1; ethyl 1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate, 87938-88-3; ethyl 7-chloro-1-ethyl-4-oxo-1,8-naphthyridine-3-carboxylate, 56654-05-8; *sec*-butyraldehyde, 78-84-2; *N*-methylpiperazine, 109-01-3; *N*-acetylpiperazine, 13889-98-0; pyrrolidine, 123-75-1; piperazine, 110-85-0; ethylene bromohydrin, 540-51-2; 2-fluorethyl tosylate, 383-50-6; 2-oxopiperazine, 5625-67-2; propionaldehyde, 123-38-6; butyraldehyde, 123-72-8; sodium chlorodifluoroacetate, 1895-39-2; ethylenediamine, 107-15-3; piperidine, 110-89-4; morpholine, 110-91-8; thiomorpholine, 123-90-0; homopiperazine, 505-66-8; azepine, 12764-48-6; azocine, 1121-92-2; 1-phenylpiperazine, 92-54-6; 1-benzylpiperazine, 2759-28-6; 1-ethylpiperazine, 5308-25-8.

Structure–Activity Relationships of Sparsomycin and Its Analogues. Octylsparsomycin: The First Analogue More Active than Sparsomycin

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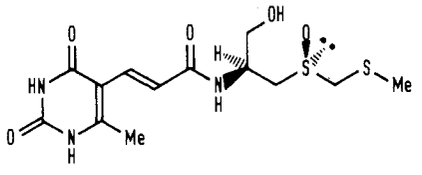
Nine analogues of sparsomycin (1) were synthesized, and their cytostatic activity was studied in an in vitro clonogenic L1210 assay by measuring the inhibition of colony formation. The activity of an analogue, expressed as an ID_{50} value, was compared to that of sparsomycin (Table I). Each analogue possesses not more than two structural modifications of the sparsomycin molecule 1. Comparison of the activity of 1 with that of the stereomers **2–4**, having R_CS_S, S_CS_S, and R_CR_S chirality, respectively, shows that the *S* configuration of the chiral carbon atom is essential for an optimal activity, whereas the *R* chirality of the sulfoxide sulfur atom of sparsomycin is of importance. Study of the ID_{50} values of the *S*-deoxo analogues **10** and **11**, as well as the compounds **14** and **15** having the β-sulfoxide function, indicate that the presence of an oxygen atom on the α-sulfur atom is essential. Isomerization of the trans double bond into the cis double bond yields isosparsomycin (**16**, Scheme II), which has a low activity. The cytostatic activity of sparsomycin seems to be related to its lipophilicity: octylsparsomycin (**19**) was shown to be three times as effective as sparsomycin.

The development of a flexible synthesis is a prerequisite for thorough studies on the biological activity and/or biochemical mechanism of the interaction of a molecule. An example, which underlines this view, is sparsomycin (1).[1]



1 : Sparsomycin (S_C -R_S)

This antibiotic has been synthesized only recently.[2-4] Consequently, the structure–activity relationship studies of 1 that have appeared so far[5-7] concern analogues in which several structural parameters have been varied simultaneously, thus allowing only a limited interpretation of the results with regard to the role of the various structural fragments.

The interpretaton and comparison of the available information on structure–activity relationships of sparsomycin encounters a second difficulty; the biological activity of the analogues has been determined in different systems (in vitro: KB cell culture[5] and cell-free ribosomal systems[6]; in vivo: P-388 system[6] and Walker 256 system[7]).

Sparsomycin (1) is a strong inhibitor of protein biosynthesis and has therefore attracted widespread attention. There is ample evidence[8] that sparsomycin has its site of interaction in the large ribosomal subunit, where it prevents peptide transfer by interfering with the peptidyltransferase center. Sparsomycin manifests its action in intact prokaryotic cells,[9] eukaryotic cells[10] (including transformed[5,11] and/or virus infected cells[12]), and various cell-free systems.[13] The behavior of sparsomycin with

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(1) Sparsomycin is a metabolite of *Streptomyces sparsogenes* (Argoudelis, A. D.; Herr, R. R. *Antimicrob. Agents Chemother.* **1962**, 780) and *Streptomyces cuspidosporus* (Higashide, E.; Hasegawa, T.; Mizuno, K.; Akaide, H. *Takeda Kenkyusho Nempo.* **1966**, *25*, 1; *Chem. Abstr.* **1967**, *66*, 54328).
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