Syntheses and antibacterial activity of novel 6-fluoro-7-(*gem*-disubstituted piperazin-1-yl)-quinolines[1]

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This paper is dedicated to Professor Zdenek (Denny) Valenta on the occasion of his 65th birthday

DANIEL T. W. CHU, AKIYO K. CLAIBORNE, JACOB J. CLEMENT, and JACOB J. PLATTNER. Can. J. Chem. **70**, 1328 (1992).

A series of quinoline and naphthyridine antibacterial agents possessing an acyclic or cyclic *gem*-disubstituted piperazine substituent at the C-7 position have been prepared and evaluated in vitro and in vivo for antibacterial activity against a variety of Gram-positive and Gram-negative organisms. They are, however, not as active as quinolones or naphthyridines with a monosubstituted piperazine substituent at C-7. The chemical synthesis of these derivatives is also described.

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On a préparé un série d'agents antibactériens dérivés de quinoléines et de naphtyridines portant comme substituant en position C-7 une pipérazine *gem*-disubstituée sur la portion aliphatique ou cyclique; on a évalué leur activité antibactérienne in vitro et in vivo contre une variété d'organismes Gram-positifs et Gram-négatifs. Ils ne sont toutefois pas aussi actifs que les quinolones ou les naphtyridines portant une pipérazine monosubstituée en position C-7. On décrit aussi la synthèse chimique de ces dérivés.

[Traduit par la rédaction]

Introduction

Since the introduction of nalidixic acid **1** (1) into clinical practice in 1963, a large number of related analogs have been synthesized. Recently, several highly potent broad-spectrum antibacterial agents of this class have been made. All of them possess a 1-substituted-1,4-dihydro-4-oxo-pyridine-3-carboxylic acid moiety and a fluorine atom at the C-6 position and are collectively known as quinolones. These agents include norfloxacin **2** (2), temafloxacin **3** (3), ciprofloxacin **4** (4), lomefloxacin **5** (5), fleroxacin **6** (6), ofloxacin **7** (7), and tosufloxacin **8** (8). They have been shown to inhibit DNA gyrase (9, 10). Chirality in the quinolone molecule may have a great impact on the biological activity. The *S* enantiomer of ofloxacin **9** (11, 12) and *S*-25930 **10** (13) were recently reported to possess greater biological activities (10- to 100-fold) than their antipodes. The enantiomers of the 3-ethylaminomethylpyrrolidin-1-yl derivative **11**, however, were reported to have similar biological activity (14).

Recently, it has been shown that coadministration of enoxacin and theophylline to rats causes an increase of elimination half-life of theophylline, leading to an increase in its plasma concentration (15). This results in increased theophylline-related adverse reactions such as headache, nausea, and tachycardia. It is suggested that enoxacin competitively inhibits the binding of theophylline to cytochrome P-448 (15). The coplanarity between the C-7 piperazinyl group and the naphthyridine nucleus of enoxacin may be a possible explanation for this interaction. To avoid this undesirable interaction with theophylline, it is highly desirable to produce a potent quinoline with a bulky substituent on the piperazine of the C-7 substituent so that the coplanarity between the C-7 substituent and the quinolone nucleus can be avoided by steric interaction.

We have synthesized both enantiomers of temafloxacin. They were found to possess identical antibacterial activities (16), suggesting that there may be a large pocket at the enzymatic site that allows a substituent at both the α and β position of the C-3 of the piperazin-1-yl group. As a continuation of our search for a potent quinolone without interaction with theophylline, we synthesized a series of 1-substituted-6-fluoro-1-(3-*gem*-disubstituted-piperazin-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acids (or naphthyridine analogs) **12** for biological evaluation. In addition to *gem*-dimethyl substitution, these derivatives also included a 3-spirocyclopropane ring since substitution at N-1 of the quinoline ring with cyclopropyl group has been shown to dramatically increase in in vitro potency (17).

Chemistry

The synthesis of the desired 1-substituted-6-fluoro-7-(3-*gem*-disubstituted-piperazin-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acids and their corresponding naphthyridine derivatives **12** (*a–j*) (Tables 1–3) is outlined in Scheme 1. They were prepared by displacement of the 7-halo of the known ethyl 1-substituted 6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate, ethyl 1-substituted-6,7-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate, or ethyl 1-substituted-6-fluoro-7-chloro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (**14**) with the appropriate 2-spiro-substituted piperazine (**13***a*,*b*) followed by acid hydrolysis. The *gem*-dimethyl derivatives were prepared by direct displacement of the 7-halo of the quinoline or naphthyridine-3-carboxylic acid derivatives (**16**) with 2,2-dimethylpiperazine (**13***c*).

The requisite 6,9-diazaspiro[4,5]decane (**13***a*) was prepared by the reaction sequence outlined in Scheme 2. Esterification of 1-amino-1-cyclopentanecarboxylic acid (**17**) with thionyl chloride in ethanol yielded the α amino acid ethyl ester hydrochloride (**18**). Condensation of **18** with carbobenzyloxyglycyl-*O*-succinimide in the presence of sodium bicarbonate gave the protected dipeptide (**19**). Removal of the protecting group by hydrogenolysis provided the free amino derivative, which cyclized to yield the diketopiper-

[1]A preliminary account of this work was presented at the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy, October, 1990, Atlanta; Abstr. No. 394.

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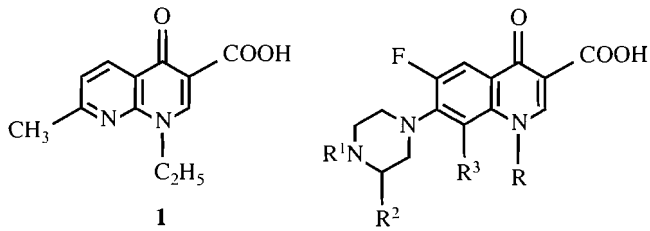
azine derivative (**20**). Borane–THF reduction of **20** gave the desired 6,9-diazaspiro[4,5]decane (**13***a*).

The above synthetic methodology was not regioselective for the synthesis of the 4,7-diazaspiro[2,5]octane (**13***b*). The key intermediate (**23**) not only provided the desired diketopiperazine **24** but also gave a substantial amount of the homoketopiperazine by-product **25** as illustrated in Scheme 3. It could be reasonable that displacement of the ethoxy group by the free amino group in **23** provided **24**. However, because of the presence of the ester function attached to the cyclopropane ring, the free amino group could also attack the carbon atom on the cyclopropane, generating a ring-opened product. To avoid this undesired ring opening, the previous reaction sequence was reversed. The desired 4,7-diazaspiro[2,5]octane (**13***b*) was prepared by a route shown in Scheme 4.



1

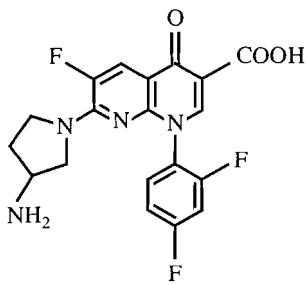
2 R = C_2H_5, $R^1 = R^2 = R^3 = H$

3 R = *o*,*p*-$C_6H_3F_2$, $R^1 = R^3 = H$, $R^2 = CH_3$

4 R = *c*-C_3H_5, $R^1 = R^2 = R^3 = H$

5 R = C_2H_5, $R^1 = H$, $R^2 = CH_3$, $R^3 = F$

6 R = C_2H_4F, $R^1 = CH_3$, $R^2 = H$, $R^3 = F$.



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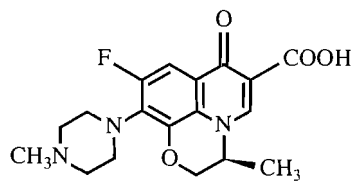

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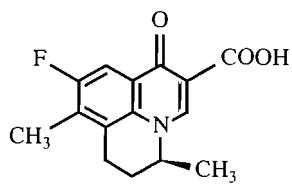

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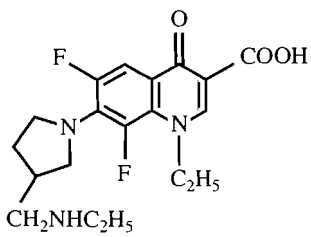

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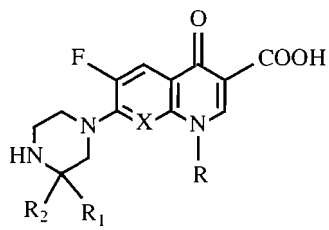

12

N-Protection of the 1-amino-1-cyclopropane carboxylic acid **21** with carbobenzyloxy-*O*-succinimide in the presence of sodium bicarbonate in THF gave the protected acid **26**. Activation of the carboxylic acid function of **26** with dicyclohexylcarbodiimide and *N*-hydroxyl-*O*-succinimide gave the *O*-succinimide derivative **27**. Treatment of **27** with ethyl glycine yielded the protected dipeptide **28**. Removal of the *N*-protecting group by hydrogenolysis yielded the free amino derivative **29**. Cyclization of **29** with DBU in refluxing toluene gave the desired diketopiperazine **24**. No ring opening by product was isolated. Borane–THF reduction of **24** provided the desired 4,7-diazaspiro[2,5]octane (**13***b*).

The 2,2-dimethylpiperazine (**13***c*) was prepared by the same reaction sequence as the preparation of 6,9-diazaspiro[4,5]decane (**13***a*) but using 2-amino-2-methylpropanoic acid instead of 1-amino-1-cyclopentanecarboxylic acid.

Results and discussion

Tables 4–6 summarize the in vitro antibacterial activity activity of the 7-*gem*-disubstituted piperazinyl quinolone derivatives (**12***a*–*j*) against ten Gram-positive bacteria (*Staphylococcus aureus* ATCC6538P, *Staphylococcus aureus* A5177, *Staphylococcus aureus* 642A, *Staphylococcus aureus* NCTC10649, *Staphylococcus aureus* CMX553, *Staphylococcus epidermidis* 3519, *Enterococcus faecium* ATCC 8043, *Streptococcus bovis* A5169, *Streptococcus agalactiae* CMX508, *Streptococcus pyogenes* EES61), and seven Gram-negative organisms (*Escherichia coli* Juhl, *Enterobacter aerogenes* ATCC13048, *Klebsiella pneumoniae* ATCC8045, *Pseudomonas aeruginosa* BMH10, *Pseudomonas aeruginosa* A5007, *Pseudomonas aeruginosa* K799/61, *Acinetobacter sp.* CMX669). The in vitro antibacterial activities are reported as minimum inhibitory concentration (MIC) in μg/mL determined by conventional agar dilution procedure. The data for ciprofloxacin (Cipro), a clinically useful antibacterial agent, are included for comparison.

The antibacterial activity for these compounds indicated that the 7-(4,7-diazaspiro[2,5]octan-7-yl)-quinolones and naphthyridines were more active than 7-(3,3-dimethylpiperazin-1-yl)-quinolones and naphthyridine derivatives, which were more active than 7-(6,9-diazaspiro[4,5]decan-9-

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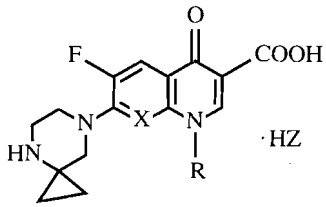
TABLE 1. Chemical structures for compound **12** (*a–d*)



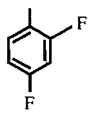
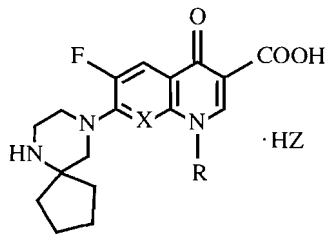
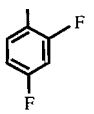
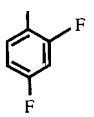
Compound	X	R	HZ
a	CH		HCl
b	CF		HCl
c	N		HCl
d	N		CF_3COOH

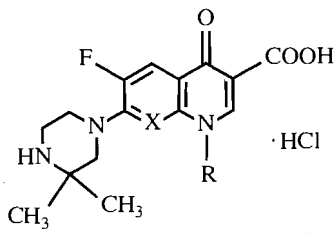
TABLE 2. Chemical structures for compound **12** (*e–g*)

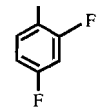
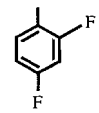


Compound	X	R	HZ
e	CH		HCl
f	CH		HCl
g	N		CF_3COOH

yl)-quinolones and naphthyridine derivatives. Although both enantiomers of temafloxacin were reported to possess identical antibacterial activity, the presence of two bulky substituents at C-3 of the piperazin-1-yl group reduced the antibacterial activity as did the 6,9-diazaspiro[4,5]decan-9-yl substituent. In general, the 1-substituted-6-fluoro-7-(4,7-diazaspiro[2,5]octan-7-yl)-1,4 - dihydro-4-oxoquinoline - 3-carboxylic acids and naphthyridine derivatives were more active against Gram-positive organisms than Gram-negative organisms. This is in contrast to traditional quinolones, which are more active against Gram-negative organisms. The 4,7-diazaspiro[2,5]octan-7-yl derivative **12***b* was the most active compound in this series and was found to be more active than ciprofloxacin against Gram-positive bacteria and slightly less active against Gram-negative bacteria.

TABLE 3. Chemical structures for compound **12** (*h–j*)



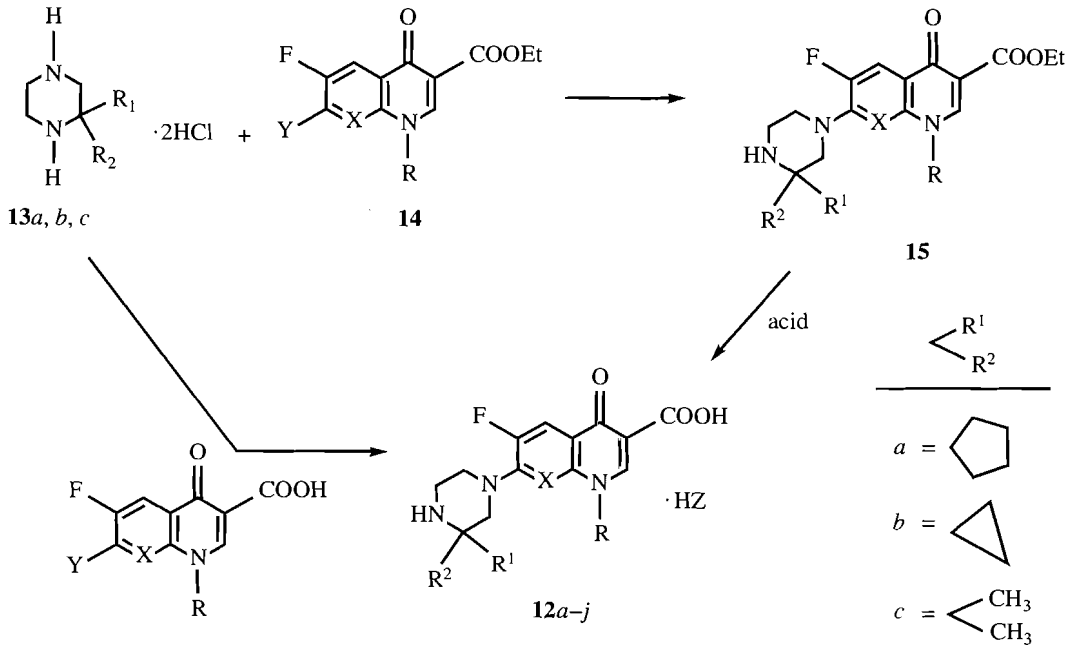
Compound	X	R
h	CH	
i	CH	
j	N	

To determine in vivo efficacy, several of these compounds were evaluated in mouse protection tests. Ciprofloxacin (Cipro) was used as a standard. The compounds were administered either orally (po) or subcutaneously (sc) with a specific amount of the test compound divided equally, to be administered 1 and 5 h after the injection. The potency is given in ED_{50} values, which are expressed as a total dose of the compound in mg/kg required to protect 50% of mice challenged intraperitoneally with the organism indicated. The results against *Staphylococcus aureus* and *E. coli* systemic infections are shown in Table 7. With the exception of **12***b*, all the compounds tested were inferior to ciprofloxacin. **12***b* possessed potency similar to ciprofloxacin against *S. aureus* infection in the mouse protection test.

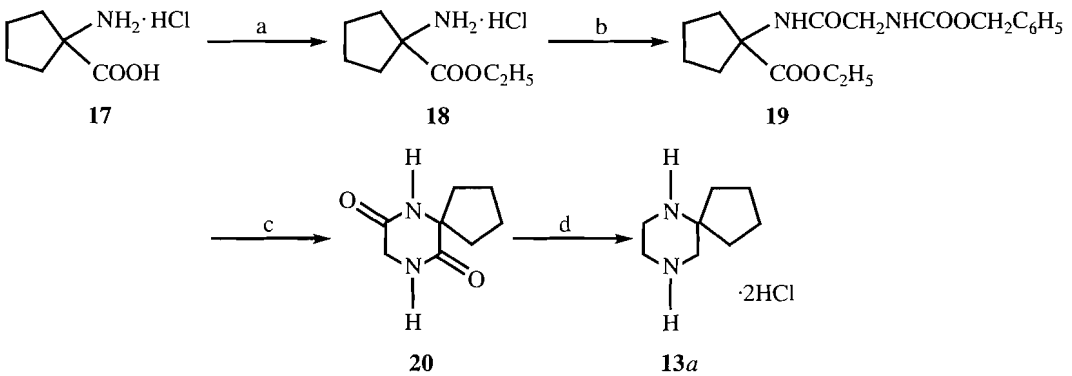
In summary, we have synthesized a series of *gem*-disubstituted piperazin-1-yl quinolone derivatives with good antibacterial activity. However, they were found to be somewhat inferior to ciprofloxacin, which is currently used clinically as a broad spectrum antibacterial agent. This new series provided better anti-Gram-positive activity than anti-Gram-negative activity, an observation contrary to classical quinolone SAR.

Experimental

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points were taken in a Thomas–Hoover capillary apparatus and were uncorrected. NMR spectra were determined on a General Electric GN-300 spectrometer operating at 300.1 MHz. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane. Significant ^1H NMR data are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, double doublet; b, broad), coupling constants(s), number of protons, and designation. The IR spectra were recorded on a Perkin–Elmer model 710A infrared spectrometer. Mass spectra were obtained with a Hewlett–Packard 5985A mass spectrometer or a Kratos MS-50 instrument with EI source (70 eV). The IR, NMR, and mass spectral data of all compounds were consistent with the assigned structures. Solutions were dried over magnesium sulfate. E. Merck silica



SCHEME 1



(a) $C_2H_5OH/SOCl_2$; (b) Cbz-glycyl-*O*-succinimide/$NaHCO_3$; (c) H_2; 10%Pd/C; (d) borane–THF/HCl

SCHEME 2

gel (230–400 mesh) obtained from VWR Scientific was used for column chromatography, and yields of the reactions were not optimized. Elemental analyses were performed by the Abbott analytical department and IR, NMR, and mass spectra were recorded by the Abbott structural chemistry department.

In vitro antibacterial activity

The in vitro antibacterial activity of the test compounds was tested in a side-by-side comparison with temafloxacin and determined by conventional agar dilution procedures. The organisms were grown overnight in brain–heart infusion (BHI) broth (Difco 0037-01-6) at 36°C. Twofold dilutions of the stock solution (2000 μg/mL) of the test compound were made in BHI agar to obtain test concentrations ranging from 200 to 0.005 μg/mL. They were then incubated at 36°C for 18 h. The minimal inhibitory concentration (MIC) was the lowest concentration of the test compund 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 water and diluting it with distilled water to the desired volume. The median lethal dose of the test organisms was determined as follows.

After 18 h of incubation, the cultures of test organism in BHI broth were serially diluted by 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 cumulative mortalities on the sixth day by using the Reed and Muench procedure (18).

After 18 h, cultures of the above were 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 (sc) or orally (po) with a specific amount of the test compound divided equally to be administered 1 and 5 h after injection. A group of 10 animals each for at least three dose levels was thus treated, and the deaths were recorded daily for 6 days. Ten mice were left untreated as infection control. ED_{50} values were calculated from the cumulative mortalities on the sixth day after infection by using the trimmed version of the logit method (19).

1-Amino-1-ethoxycarbonylcyclopentane hydrochloride (18)

To a stirred mixture of 1-amino-1-cyclopentanecarboxylic acid (50 g, 387 mmol) in absolute ethanol (600 mL) at 0°C was added,

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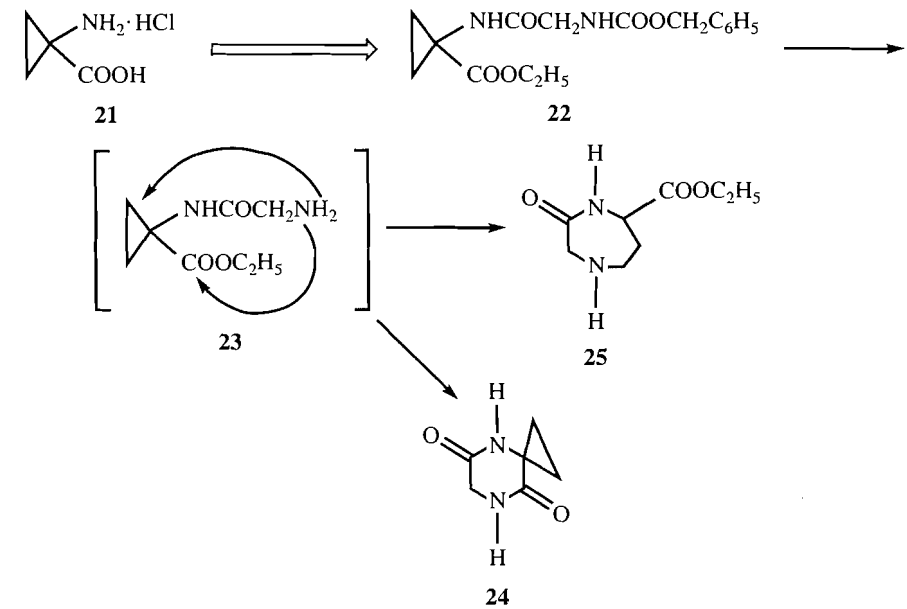
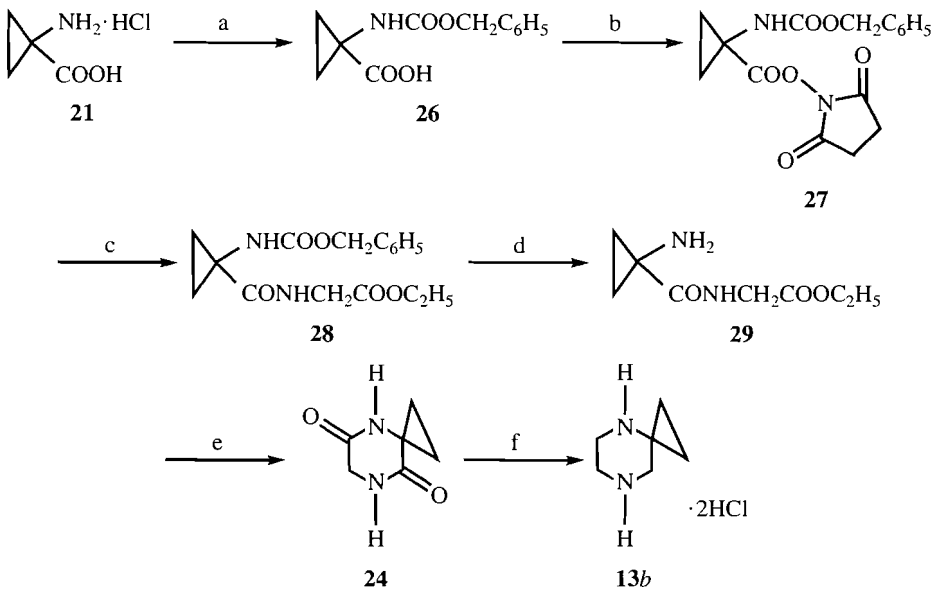


SCHEME 3



(a) Cbz-*O*-succinimide/$NaHCO_3$; THF; (b) *N*-hydroxysuccinimide/DCC; THF; (c) ethyl glycine hydrochloride/$NaHCO_3$; THF–H_2O; (d) H_2; 10%Pd/C; EtOAc; (e) DBU/toluene, heat; (f) borane–THF/HCl

SCHEME 4

dropwise, thionyl chloride (85 mL, 1.16 mol). The ice bath was removed, and the mixture was heated at reflux for 4 h and concentrated *in vacuo* to give a white solid, which was crystallized from ether–ethanol yielding **18** (62.4 g, 83%), mp 232°C. IR (KBr): 1740 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 1.24 (t, J = 7 Hz, 3H, ethyl CH_3), 1.72 (m, 2H, CH_2), 1.88 (m, 2H, CH_2), 1.97 (m, 2H, CH_2), 2.10 (m, 2H, CH_2), 4.21 (q, J = 7 Hz, 2H, ethyl CH_2), 8.80 (bs, 3H, NH_3Cl). Anal. calcd. for $C_8H_{16}ClNO_2$: C 49.61, H 8.33, N 7.23; found: C 49.75, H 8.49, N 7.22.

By use of this procedure, 2-amino-2-methylpropanoic acid gave ethyl 2-amino-2-methylpropanoate hydrochloride in 97% yield, mp 159°C. IR (KBr): 1742 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 1.24 (t, J = 7 Hz, 3H, ethyl CH_3), 1.47 (s, 6H, CH_3×2), 4.21 (q, J = 7 Hz, 2H, ethyl CH_2), 8.70 (bs, 3H, $NH_2 \cdot HCl$). Anal. calcd. for $C_6H_{14}ClNO_2$: C 42.99, H 8.36, N 8.36; found: C 42.92, H 8.61, N 8.31.

1-N-Carbobenzyloxyglycylamino-1-ethoxycarbonyylcyclopentane (19)

A solution of carbobenzyloxyglycine succinimido ester (19.4 g, 63 mmol) in THF (150 mL) was added dropwise to a solution of 1-amino-1-ethoxycarbonylcyclopentane hydrochloride (**18**) (11.6 h, 60 mmol) and sodium bicarbonate (15.1 g, 180 mmol) in water (200 mL) at 0°C. After the addition, the reaction was allowed to warm up to room temperature and was stirred for 16 h. The solvent was removed under reduced pressure and the residue was taken up in methylene chloride (350 mL) and washed twice with water. The organic portion was separated and dried. Removal of the solvent yielded 17.6 g (88%) of **19**, mp 99°C. IR

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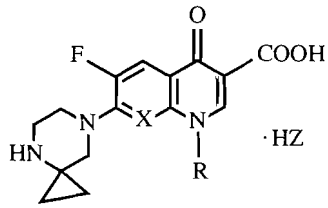
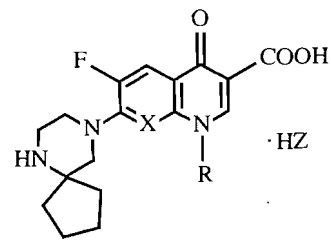
TABLE 4. In vitro activity of quinolones **12** (*a–d*)



Organism	MIC (μg/mL)				
	a	*b*	*c*	*d*	Cipro
Staph. aureus ATCC6538P	0.2	0.1	0.39	0.39	0.2
Staph. aureus A5177	0.39	0.2	0.78	0.78	0.39
Staph. aureus 642A	0.39	0.2	0.78	0.78	0.39
Staph. aureus NCTC10649	0.12	0.1	0.78	0.39	0.39
Staph. aureus CMX553	0.78	0.2	0.78	0.78	0.78
Staph. epidermidis 3519	0.39	0.2	0.78	0.78	0.39
Enter. faecium ATCC 8043	1.56	0.78	0.2	3.1	0.39
Strep. bovis A5169	6.2	3.1	2.5	6.2	1.56
Strep. agalactiae CMX508	1.56	0.78	3.1	3.1	0.78
Strep. pyogenes EES61	0.78	1.56	3.1	3.1	0.78
Escherichia coli Juhl	0.1	0.05	0.2	3.1	0.01
Enter. aerogenes ATCC13048	0.2	0.1	0.2	6.2	0.05
Kleb. pneumoniae ATCC8045	0.05	0.02	0.05	1.56	0.02
Pseudo. aeruginosa BMH10	1.56	0.78	3.1	25	0.1
Pseudo. aeruginosa A5007	1.56	1.56	3.1	50	0.1
Pseudo. aeruginosa K799/61	0.2	0.1	0.2	3.1	0.02
Acinetobacter sp. CMX669	0.39	0.1	0.39	3.1	0.39

TABLE 5. In vitro activity of quinolones **12** (*e–g*)



Organism	MIC (μg/mL)			
	e	*f*	*g*	Cipro
Staph. aureus ATCC6538P	0.39	0.39	0.39	0.2
Staph. aureus A5177	0.78	0.78	0.39	0.39
Staph. aureus 642A	0.78	0.78	0.39	0.39
Staph. aureus NCTC10649	0.39	0.39	0.39	0.39
Staph. aureus CMX553	1.56	0.78	0.78	0.78
Staph. epidermidis 3519	0.39	0.78	0.39	0.39
Enter. faecium ATCC8043	3.1	0.78	3.1	0.39
Strep. bovis A5169	6.2	3.1	12.5	1.56
Strep. agalactiae CMX508	1.56	12.5	3.1	0.78
Strep. pyogenes EES61	1.56	3.1	3.1	0.78
Escherichia coli Juhl	0.78	3.1	0.78	0.01
Enter. aerogenes ATCC13048	1.56	3.1	1.56	0.05
Kleb. pneumoniae ATCC8045	0.78	1.56	0.39	0.02
Pseudo. aeruginosa BMH10	6.2	25	12.5	0.1
Pseudo. aeruginosa A5007	12.5	25	12.5	0.1
Pseudo. aeruginosa K799/61	1.56	3.1	0.78	0.02
Acinetobacter sp. CMX669	3.1	3.1	1.59	0.39

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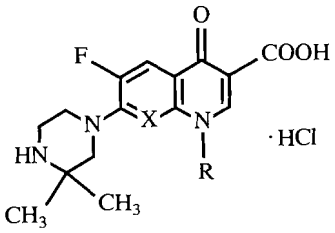
TABLE 6. In vitro activity of quinolones **12** (*h–j*)



Organism	MIC (μg/mL)			
	h	*i*	*j*	Cipro
Staph. aureus ATCC6538P	0.39	0.2	0.39	0.2
Staph. aureus A5177	0.78	0.39	0.78	0.39
Staph. aureus 642A	0.78	0.39	0.78	0.39
Staph. aureus NCTC10639	0.39	0.2	0.39	0.39
Staph. aureus CMX553	1.56	0.78	0.78	0.78
Staph. epidermidis 3519	0.78	0.78	0.78	0.39
Enter. faecium ATCC8043	1.56	1.56	3.1	0.39
Strep. bovis A5169	6.2	6.2	6.2	1.56
Strep. agalactiae CMX508	1.56	1.56	3.1	0.78
Strep. pyogenes EES61	1.56	1.56	1.56	0.78
Escherichia coli Juhl	0.2	0.78	0.78	0.01
Enter. aerogenes ATCC13048	0.39	0.78	0.78	0.05
Kleb. pneumoniae ATCC8045	0.2	0.39	0.39	0.02
Pseudo. aeruginosa BMH10	1.56	3.1	6.2	0.1
Pseudo. aeruginosa A5007	3.1	6.2	6.2	0.1
Pseudo. aeruginosa K799/61	0.39	0.39	0.78	0.02
Acinetobacter sp. CMX669	1.56	0.78	1.56	0.39

TABLE 7. Mouse protection test of selected quinolones

Test organism (Dose)	Compound	Route	ED_{50} (95% confidence limits) mg/kg
S. aureus NCTC10649 (100 × LD_{50})	**12***b*	SC	6.2 (3.7–10.3)
		PO	13.1 (9.7–17.6)
	12*c*	SC	80.7 (8.6–760.5)
		PO	>100
	12*g*	SC	16.2 (10.1–25.3)
		PO	24.0 (15.2–38.0)
	Cipro	SC	1.6 (1.0–3.5)
		PO	15.5 (9.9–24.1)
E. coli Juhl (100 × LD_{50})	**12***b*	SC	2.0 (1.3–3.2)
		PO	5.0 (3.2–7.9)
	12*c*	SC	8.0 (5.1–12.7)
		PO	19.7 (8.5–45.9)

($CDCl_3$): 1682, 1727 (CO) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.23 (t, J = 7 Hz, 3H, ethyl CH_3), 1.76 (m, 4H, CH_2×2), 1.95 (m, 2H, CH_2), 2.21 (m, 2H, CH_2), 3.86 (d, J = 5 Hz, 2H, NCH_2), 4.13 (q, J = 7 Hz, 2H, ethyl CH_2), 5.13 (s, 2H, OCH_2), 5.49 (bs, 1H, NH), 6.54 (bs, 1H, NH), 7.34 (m, 5H, aromatic H). Anal. calcd. for $C_{18}H_{24}N_2O_5$: C 62.05, H 6.94, N 8.04; found: C 62.04, H 7.05, N 8.12.

By use of this procedure, reacting ethyl 2-amino-2-methylpropionate hydrochloride instead of 1-amino-1-ethoxycarbonyl-cyclopentane hydrochloride (**18**) with carbobenzyloxyglycine succinimido ester, ethyl 2-*N*-carbobenzyloxyglycylamino-2-methylpropionate was obtained in 89% yield, mp 85°C. IR ($CDCl_3$): 1682, 1727 (CO) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.27 (t, J = 7 Hz, 3H, ethyl CH_3), 1.56 (s, 6H, CH_3×2), 3.85 (d, J = 6Hz, 2H, CH_2), 4.20 (q, J = 7 Hz, 2H, ethyl CH_2), 5.14 (s, 2H, benzyl CH_2), 5.37 (bs, 1H, NH), 6.54 (bs, 1H, NH), 7.36 (m, 5H, aromatic H). Anal. calcd. for $C_{16}H_{22}N_2O_5$: C 59.63, H 6.83, N 8.70; found: C 59.72, H 6.92, N 8.67.

By use of the above procedure, ethyl 1-amino-1-cyclopropanecarboxylate (20) yielded ethyl 1-*N*-carbobenzyloxyglycylamino-1-cyclopropanecarboxylate (**22**) in 86% yield, mp 90–91°C. IR ($CDCl_3$): 1700, 1730 (CO) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.14 (m, 2H, CH_2), 1.21 (t, J = 7 Hz, 3H, ethyl CH_3), 1.56 (m, 2H, CH_2), 3.87 (d, J = 6 Hz, 2H, NCH_2), 4.11 (q, J = 7 Hz, 2H, ethyl CH_2), 5.12

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(s, 2H, OCH_2), 5.58 (bs, 1H, NH), 6.73 (bs, 1H, NH), 7.34 (m, 5H, aromatic H). Anal. calcd. for $C_{16}H_{20}N_2O_5$: C 59.99, H 6.29, N 8.75; found: C 60.06, H 6.28, N 8.74.

*6,9-Diaza-7,10-dioxospiro[4,5]decane (**20**)*

A solution of **19** (17.6 g, 53 mmol) in ethanol (250 mL) was hydrogenated at 4 atmospheres (1 atm = 101.3 kPa) of hydrogen with 10% palladium on carbon for 18 h. The solution was then filtered and the residue was washed with ethanol. The combined solution was filtered through a 0.45 μm Nylon filter to remove the residual catalyst. Concentration of the filtrate to dryness yielded a solid, which was crystallized in acetonitrile yielding 6.35 g (71.4%) of **20**, mp >255°C. IR (KBr): 1675 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 1.66 (m, 6H, $CH_3\times2$), 2.06 (m, 2H, CH_2), 3.74 (d, J = 3 Hz, 2H, NCH_2), 7.93 (bs, 1H, NH), 8.35 (bs, 1H, NH). Anal. calcd. for $C_8H_{12}N_2O_2$: C 57.13, H 7.19, N 16.66; found C 57.33, H 7.36, H 16.78.

Using similar reaction conditions as above, ethyl 2-*N*-carbobenzyloxyglycylamino-2-methylpropionate produced 2,2-dimethyl-3,6-dioxopiperazine in 98% yield, mp 258–261°C. IR (KBr): 1680 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 1.29 (s, 6H, $CH_3\times2$), 3.78 (d, J = 2.5 Hz, 2H, CH_2), 7.91 (bs, 1H, NH), 8.23 (bs, 1H, NH). Anal. calcd. for $C_6H_{10}N_2O_2$: C 50.70, H 7.04, N 19.72; found: C 50.55, H 7.14, N 19.79.

*2,2-Dimethylpiperazine dihydrochloride (**13**c)*

2,2-Dimethyl-3,6-dioxopiperazine (15 g, 106 mmol), was suspended in THF (650 mL). A 433 mL sample of 1 M borane–tetrahydrofuran complex (433 mmol) was then added dropwise to the above stirring suspension over 1 h. Gas evolution occurred and the mixture became nearly homogeneous at the end of the addition. The mixture was heated at 60°C for 20 h. It was cooled to room temperature and 200 mL of methanol was added over a 1 h period. Gas evolution occurred and the reaction mixture was concentrated to dryness. The residue was redissolved in methanol (150 mL), excess methanolic HCl solution (500 mL) was added, and the solution was refluxed for 2 h. It was cooled and allowed to stand at room temperature overnight. The reaction was evaporated to dryness. The mixture and added acetonitrile were boiled with stirring for 0.5 h, then cooled, and the acetonitrile was decanted off. The residue was dissolved in a small amount of hot methanol. Upon cooling, crystals appeared. After addition of ether, they were filtered inside a dry-box and the residue was dried under vacuum yielding 16.5 g (84%) 2,2-dimethylpiperazine dihydrochloride (**13***c*). This solid was very hygroscopic. Upon exposure to air, it turned watery within several seconds. The IR spectrum showed no carbonyl absorption signal. ^1H NMR (D_2O) δ: 1.54 (s, 6H, $CH_3\times2$), 3.42 (s, 2H, CH_2), 3.52 (m, 2H, CH_2), 3.60 (m, 2H, CH_2). Exact Mass calcd. for $C_6H_{15}N_2$: 115.1235; measured: 115.1235.

By use of this procedure, borane–THF reduction of 6,9-diaza-7,10-dioxospiro[4,5]decane (**20**) yielded 6,9-diazaspiro[4,5]decane dihydrochloride (**13***a*) in 99% yield. This compound behaved as **13***c* and was very hygroscopic. The IR spectrum showed no carbonyl absorption signal. ^1H NMR (DMSO-d_6) δ: 1.62 (m, 2H, CH_2), 1.75 (m, 2H, CH_2), 1.90 (m, 2H, CH_2), 1.99 (m, 2H, CH_2), 3.28 (bs, 4H, $NCH_2\times2$), 3.48 (m, 2H, NCH_2), 9.98 (bs, 2H, $NH\times2$). Exact Mass calcd. for $C_8H_{17}N_2$: 141.1392; measured: 141.1392.

By use of the above procedure, borane–THF reduction of 4,7-diaza-5,8-dioxospiro[2,5]octane (**24**) yielded 4,7-diazaspiro[2,5]-octane dihydrochloride (**13***b*) in 98% yield. This compound was very hygroscopic. The IR spectrum showed no absorption signal for carbonyl group. ^1H NMR (DMSO-d_6) δ: 0.99 (m, 2H, CH_2), 1.21 (m, 2H, CH_2), 3.48 (m, 6H, $NCH_2\times3$), 9.85 (bs, 2H, NH_2Cl), 10.29 (bs, 2H, NH_2Cl). Exact Mass calcd. for $C_{16}H_{13}N_2$: 113.1079; measured: 113.1079.

Hydrogenolysis of 22

A solution of **22** (18.5 g, 58 mmol) in ethanol (250 mL) was hydrogenated at 4 atmospheres of hydrogen with 10% palladium on carbon for 18 h. The solution was then filtered and the residue was washed with ethanol. The combined solution was filtered through a 0.45 μm Nylon filter to remove the residual catalyst. Concentration of the filtrate to dryness yielded a residue, which was suspended in acetonitrile and boiled for 10 min. It was cooled and filtered, yielding 3.17 g of two products in about 1:1 ratio by TLC. ^1H NMR showed the presence of two compounds; one corresponding to **24** that had an identical spectrum to an authentic samples prepared by another route. The remaining signals in this spectrum indicated the presence of **25**. Repeated purification through several chromatographies yielded a small amount of **25** for ^1H NMR analysis. ^1H NMR ($CDCl_3$) δ: 1.22 (m, 5H, CH_2 and ethyl CH_3), 1.58 (m, 2H, NCH_2), 1.89 (bs, 1H, NH), 3.32 (bs, 2H, NCH_2CO), 3.72 (m, 1H, NCH), 4.14 (q, J = 7 Hz, 2H, ethyl CH_2), 7.70 (bs, 1H, CONH). The mass spectrum showed a m/z (M + 1) at 187 consistent with the structure assigned.

*1-N-Carbobenzyloxyamino-1-cyclopropanecarbonylic acid (**26**)*

A solution of *N*-benzyloxycarbonyloxy succinimide (60.7 g, 24 mmol) in THF (300 mL) was added to a solution of 1-amino-1-cyclopropanecarboxylic acid hydrochloride (24.5 g, 17.8 mmol) in water (45 mL). At ice temperature, a solution of sodium bicarbonate (82 g, 96 mmol) in water (500 mL) was poured into the mixture. After the addition, the reaction was allowed to warm up to room temperature and was stirred for 16 h. The solvent was removed under reduced pressure. The residue was suspended in 200 mL of methylene chloride and extracted with water (500 mL). The water solution was acidified with acetic acid and the precipitate was filtered yielding **26** (18 g, 43%), mp 157°C. IR (KBr): 1690, 1710 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 1.01 (m, 2H, CH_2), 1.31 (m, 2H, CH_2), 5.02 (s, 2H, OCH_2), 7.34 (m, 5H, aromatic H), 7.88 (s, 1H, NH), 12.42 (bs, 1H, OH). Anal. calcd. for $C_{12}H_{13}NO_4$: C 61.27, H 5.51, N 5.95; found: C 61.33, H 5.60, N 5.96.

*Preparation of active ester (**27**)*

Dicyclohexylcarbodiimide (7.74 g, 37.6 mmol) in THF (15 mL) was added dropwise to a mixture of **26** (8.83 g, 37.5 mmol) and *N*-hydroxysuccinimide (4.53 g, 39.4 mmol) in THF (60 mL). The mixture was allowed to stir at room temperature for 18 h. The precipitate was removed by filtration. The filtrate was concentrated to a residue, which was dissolved in ethyl acetate (300 mL). The organic solvent was washed with sodium bicarbonate solution. The organic layer was dried and concentrated to yield **27** (9.87 g, 79.2%), mp 119–120°C. IR (KBr): 1745, 1785, 1810 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.46 (bs, 2H, CH_2), 1.86 (bs, 2H, CH_2), 5.15 (bs, 2H, OCH_2), 5.47 (bd, 1H, NH), 7.35 (m, 5H, aromatic H). Anal. calcd. for $C_{16}H_{16}N_2O_6$: C 57.83, H 4.85, N 8.43; found: C 57.84, H 4.88, N 8.38.

*Preparation of dipeptide (**28**)*

The active ester (**27**) (2 g, 6 mmol) in THF (15 mL) was added to a chilled solution of glycine ethyl ester hydrochloride (0.8 g, 5.1 mmol) in water (5 mL). Sodium bicarbonate solution (1.4 g in 20 mL of water) was then added to the reaction mixture. After the addition, the reaction was allowed to warm up to room temperature and was stirred for 18 h. The solvent was removed under reduced pressure and the residue was washed with water and extracted with methylene chloride. The organic layer was dried and concentrated to a solid under reduced pressure, yielding a solid **28** (1.88 g, 97.5%), mp 97–98°C. IR (KBr): 1675, 1740 (CO) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.07 (bs, 2H, CH_2), 1.27 (q, J = 7 Hz, 3H, ethyl CH_3), 1.59 (m, 2H, CH_2), 3.99 (d, J = 5 Hz, 2H, NCH_2), 4.19 (q, J = 7 Hz, 2H, ethyl CH_2), 5.43 (s, 2H, OCH_2), 5.58 (s, 1H, NH), 6.98 (bs, 1H, NH), 7.34 (s, 5H, aromatic H). Anal. calcd. for $C_{16}H_{20}N_2O_5$: C 59.99, H 6.29, N 8.74; found: C 60.06, H 6.27, N 8.74.

*4,7-Diaza-5,8-dioxospiro[2,5]octane (**24**)*

A solution of the dipeptide (**28**) (8.63 g, 27 mmol) in ethyl acetate (250 mL) was hydrogenated under a hydrogen atmosphere at 4 atmospheres pressure with 10% palladium on carbon for 18 h. The

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solution was filtered and the filtrate was concentrated under reduced pressure to a residue, **29**. ^{1}H NMR (DMSO-d_6) δ: 0.77 (dd, J = 4.5 Hz, 2H, CH_2), 1.05 (dd, J = 4.5 Hz, 2H, CH_2), 1.19 (t, J = 7 Hz, 3H, ethyl CH_3), 2.31 (bs, 2H, NH×2), 3.84 (d, J = 6.5 Hz, 2H, NCH_2), 4.09 (q, J = 7 Hz, 2H, ethyl CH_2), 8.43 (t, J = 6.5 Hz, 1H, CONH). The residual liquid **29** was dissolved in toluene (200 mL). To this solution was added DBU (2 mL). After heating at 115°C for 22 h, the mixture was cooled to room temperature to yield **24** (3.2 g, 89%), mp >260°C. IR (KBr): 1685, 1695 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 0.94 (dd, J = 4.5 Hz, 2H, CH_2), 1.16 (dd, J = 4.5 Hz, 2H, CH_2), 3.85 (d, J = 2.5 Hz, 2H, NCH_2), 8.02 (bs, 1H, NH), 8.27 (bs, 1H, NH). Anal. calcd. for $C_6H_8N_2O_2$: C 51.42, H 5.75, N 20.00; found: C 51.10, H 5.66, N 19.80.

*1-Cyclopropyl-6-fluoro-7-(3,3-dimethylpiperazin-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride (**12**h)*

2,2-Dimethylpiperazine dihydrochloride (**13***c*) (423 mg, 2.3 mmol) was added to a mixture of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (21) (500 mg, 1.9 mmol) and triethylamine (10 mL) in *N*-methyl pyrrolidinone (20 mL). The reaction mixture was heated at 50°C for 18 h. The reaction was cooled to room temperature and concentrated under reduced pressure to dryness. Ethanol was added and digested under reflux for 30 min. The mixture was cooled and filtered, yielding **12***h* (525 mg, 70%), mp >260°C. IR (KBr): 1730 (CO) cm^{-1}. ^{1}H NMR (D_2O) δ: 1.22 (m, 2H, CH_2), 1.44 (m, 2H, CH_2), 1.58 (s, 6H, CH_3×2), 3.43 (s, 2H, NCH_2), 3.58 (m, 4H, NCH_2×2), 3.73 (m, 1H, CH), 7.52 (d, J_{H-F} = 6.5 Hz, 1H, C8-H), 7.54 (d, J_{H-F} = 13 Hz, 1H, C5-H), 8.67 (s, 1H, C2-H). Anal. calcd. for $C_{19}H_{23}ClFN_3O_3$: C 57.65, H 5.82, N 10.62; found: C 57.73, H 6.02, N 10.42.

By use of this procedure, 1-(2,4-difluorophenyl)-6-fluoro-7-(3,3-dimethylpiperazin-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride **12***i* was prepared by reaction of **13***c* with 1-(2,4-difluorophenyl)-6,7-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (16) in 77% yield, mp >260°C. IR (KBr): 1730 (CO) cm^{-1}. ^{1}H NMR (D_2O) δ: 1.48 (s, 6H, CH_3×2), 3.25 (s, 2H, NCH_2), 3.36 (m, 2H, NCH_2), 3.42 (m, 2H, NCH_2), 6.47 (d, J_{H-F} = 6 Hz, 1H, C8-H), 7.37 (m, 2H, aromatic H), 7.72 (m, 1H, aromatic H), 7.92 (d, J_{H-F} = 12 Hz, 1H, C5-H), 8.82 (s, 1H, C2-H). Anal. calcd. for $C_{22}H_{21}ClF_3N_3O_3 \cdot 1/4\ H_2O$: C 55.93, H 4.56, N 8.90; found: C 55.85, H 4.68, N 8.90.

By use of this procedure, 1-(2,4-difluorophenyl)-6-fluoro-7-(3,3-dimethylpiperazin-1-yl)-1,4-dihydro-4-oxo[1,8]naphthyridine-3-carboxylic acid hydrochloride (**12***j*) was prepared by reaction of **13***c* with 1-(2,4-difluorophenyl)-6-fluoro-7-chloro-1,4-dihydro-4-oxo[1,8]-naphthyridine-3-carboxylic acid (22) in 94% yield, mp >260°C. IR (KBr): 1730 (CP) cm^{-1}. ^{1}H NMR (CF_3COOH) δ: 1.43 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 3.56 (m, 2H, NCH_2), 4.03 (bs, 2H, NCH_2), 4.26 (m, 2H, NCH_2), 7.28 (m, 2H, aromatic H), 7.75 (m, 1H, aromatic H), 8.33 (d, J_{H-F} = 13 Hz, 1H, C5-H), 9.26 (s, 1H, C2-H). Anal. calcd. for $C_{21}H_{20}ClF_3N_4O_3$: C 53.79, H 4.27, N 11.95; found: C 53.58, H 4.28, N 11.73.

*Ethyl 1-cyclopropyl-6-fluoro-7-(4,7-diazaspiro[2,5]octan-7-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylate (**15**a)*

4,7-Diazaspiro[2,5]octane dihydrochloride (**13***b*) (1.5 g, 8 mmol) was added to a solution of ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**14***a*) (750 mg, 2.6 mmol) in 1-methyl-2-pyrrolidione (30 mL) at 75°C. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (1.2 g, 10.2 mmol) was then added to the reaction mixture. The mixture was heated at 100°C for 16 h. The solvent was removed by vacuum distillation. Column chromatography of the residue on silica gel using 5% methanol in methylene chloride as eluent yielded **15***a* (0.4 g, 40%), mp 223–225°C. IR ($CDCl_3$): 1680, 1720 (CO) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.68 (m, 2H, CH_2), 0.72 (m, 2H, CH_2), 1.14 (m, 2H, CH_2), 1.21 (m, 2H, CH_2), 1.49 (t, J = 7 Hz, 3H, ethyl CH_3), 3.10 (s, 2H, NCH_2), 3.21 (m, 4H, NCH_2×2), 3.43 (m, 1H, CH), 4.48 (q, J = 7 Hz, 2H, ethyl CH_2), 7.22 (d, J_{H-F} = 7 Hz, 1H, C8-H), 8.04 (d, J_{H-F} = 13 Hz, 1H, C5-H), 8.53 (s, 1H, C2-H). Anal. calcd. for $C_{21}H_{24}FN_3O_3 \cdot 1/2\ H_2O$: C 63.94, H 6.39, N 10.65; found: C 64.09, H 6.23, N 11.04.

By use of this procedure, reaction of **13***b* with ethyl 1-cyclopropyl-6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**14***b*) gave ethyl 1-cyclopropyl-6,8-difluoro-7-(4,7-diazaspiro[2,5]octan-7-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylate (**15***b*) in 42% yield. IR ($CDCl_3$): 1685, 1725 (CO) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.62 (m, 2H, CH_2), 0.69 (m, 2H, CH_2), 1.09 (m, 2H, CH_2), 1.23 (m, 2H, CH_2), 1.40 (t, J = 7 Hz, 3H, ethyl CH_3), 3.11 (m, 2H, NCH_2), 3.16 (s, 2H, NCH_2), 3.33 (m, 2H, NCH_2), 3.87 (m, 1H, CH), 4.38 (q, J = 7 Hz, 2H, ethyl CH_2), 7.90 (dd, J_{H-F} = 13 Hz, 1H, C5-H), 8.54 (s, 1H, C2-H). Anal. calcd. for $C_{21}H_{23}F_2N_3O_3 \cdot 1/4\ H_2O$: C 61.38, H 5.81, N 10.30; found: C 62.06, H 5.81, N 10.43.

Using a similar procedure, reaction of **13***b* with 1-cyclopropyl-6-fluoro-7-chloro-1,4-dihydro-4-oxo[1,8]naphthyridine-3-carboxylate (**14***c*) gave ethyl 1-cyclopropyl-6-fluoro-7-(4,7-diazaspiro-[2,5]octan-7-yl)-1,4 -dihydro- 4 -oxo[1,8]naphthyridine-3-carboxylate (**15***c*) in 46% yield, mp 174–175°C. IR ($CDCl_3$): 1685, 1725 (CO) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.62 (m, 2H, CH_2), 0.66 (m, 2H, CH_2), 1.02 (m, 2H, CH_2), 1.18 (m, 2H, CH_2), 1.41 (t, J = 7 Hz, 3H, ethyl CH_3), 3.11 (m, 2H, NCH_2), 3.47 (m, 1H, CH), 3.70 (s, 2H, NCH_2), 3.83 (m, 2H, NCH_2), 4.39 (q, J = 7 Hz, 2H, ethyl CH_2), 8.09 (d, J_{H-F} = 13 Hz, 1H, C5-H), 8.50 (s, 1H, C2-H). Anal. calcd. for $C_{20}H_{23}FN_4O_3$: C 62.16, H 6.00, N 14.50; found: C 62.31, H 5.94, N 14.93.

By use of this procedure, reacting **13***b* with ethyl 1-(2,4-difluorophenyl)-6-fluoro-7-chloro-1,4-dihydro-oxo[1,8]naphthyridine-3-carboxylate (**14***d*) gave ethyl 1-(2,4-difluorophenyl)-6-fluoro-7-(4,7-diazaspiro[2,5]octan-7-yl)-1,4-dihydro-4-oxo[1,8]naphthyridine-3-carboxylate (**15***d*) in 53% yield, mp 172–174°C. IR ($CDCl_3$): 1685, 1725 (CO) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.34 (m, 2H, CH_2), 0.54 (m, 2H, CH_2), 1.41 (t, J = 7 Hz, 3H, ethyl CH_3), 2.91 (t, J = 4 Hz, 2H, NCH_2), 3.42 (s, 2H, NCH_2), 3.56 (m, 2H, NCH_2), 3.39 (q, J = 7 Hz, 2H, ethyl CH_2), 7.06 (m, 2H, aromatic H), 7.49 (m, 1H, aromatic H), 8.11 (d, J_{H-F} = 13 Hz, 1H, C5-H), 8.39 (s, 1H, C2-H). Anal. calcd. for $C_{23}H_{21}F_3N_4O_3 \cdot 1/4\ H_2O$: C 59.67, H 4.68, N 12.10; found: C 59.57, H 4.72, N 12.06.

By a similar procedure, reaction of 6,9-diazaspiro[4,5]decane dihydrochloride (**13***a*) with quinolone ester (**14***a*) gave ethyl 1-cyclopropyl- 6-fluoro-7-(6,9 -diazaspiro[4,5]decan-9-yl)-1, 4 -dihydro-4-oxoquinoline-3-carboxylate (**15***e*) in 48% yield, mp >255°C. IR ($CDCl_3$): 1685, 1720 (CO) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.13 (m, 2H, CH_2), 1.34 (m, 2H, CH_2), 1.41 (t, J = 7 Hz, 3H, ethyl CH_3), 1.77 (m, 2H, CH_2), 2.04 (m, 2H, CH_2), 2.17 (m, 4H, CH_2×2), 3.35 (s, 2H, NCH_2), 3.43 (m, 2H, NCH_2), 3.57 (m, 2H, NCH_2), 4.40 (q, J = 7 Hz, 2H, ethyl CH_2), 7.31 (d, J_{H-F} = 6 Hz, 1H, C8-H), 8.10 (d, J_{H-F} = 13 Hz, 1H, C5-H), 8.55 (s, 1H, C2-H). Exact Mass calcd. for $C_{23}H_{28}FN_3O_3$: 413.2115; measured: 413.2113.

By use of the above procedure, reaction of **13***a* with ethyl 1-(2,4-difluorophenyl)-6,7-difluoro-1,4 -dihydro- 4 -oxoquinoline-3 -carboxylate (**14***e*) gave ethyl 1-(2,4-difluorophenyl)-6-fluoro-7-(6,9-diazaspiro[4,5]decan-9-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylate (**15***f*) in 39% yield, mp 216–218°C. IR ($CDCl_3$): 1690, 1725 (CO) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.49 (t, J = 7 Hz, 3H, ethyl CH_3), 1.59 (m, 4H, CH_2×2), 1.79 (m, 4H, CH_2×2), 2.89 (m, 4H, NCH_2×2), 3.01 (m, 2H, NCH_2), 4.39 (q, J = 7 Hz, 2H, ethyl CH_2), 6.12 (d, J_{H-F} = 6.5 Hz, 1H, C8-H), 7.15 (m, 2H, aromatic H), 7.50 (m, 1H, aromatic H), 8.06 (d, J_{H-F} = 13 Hz, 1H, C5-H), 8.31 (s, 1H, C2-H). Anal. calcd. for $C_{26}H_{26}F_3N_3O_3 \cdot 1/2\ H_2O$: C 63.15, H 5.50, N 8.50; found: C 63.25, H 5.32, N 8.53.

Using a similar procedure, reaction of **13***a* with quinoline ester (**14***d*) gave ethyl 1-(2,4-difluorophenyl)-6-fluoro-7(6,9-diazaspiro[4,5]decan-9-yl)-1,4 -dihydro-4-oxo[1,8]naphthyridine-3-carboxylate (**15***g*) in 81% yield, mp 120–122°C. IR ($CDCl_3$): 1690, 1725 (CO) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.40 (t, J = 7 Hz, 3H, ethyl CH_3), 1.46 (m, 4H, CH_2×2), 1.56 (m, 2H, CH_2), 1.68 (m, 2H, CH_2), 2.90 (t, J = 5 Hz, 2H, NCH_2), 3.42 (m, 2H, NCH_2), 3.53 (m, 2H, NCH_2), 4.49 (q, J = 7 Hz, 2H, ethyl CH_2), 7.06 (m, 2H,

aromatic H), 7.39 (m, 1H, aromatic H), 8.11 (d, J_{H-F} = 13 Hz, 1H, C5-H), 8.38 (s, 1H, C2-H). Anal. calcd. for $C_{25}H_{25}F_3N_4O_3 \cdot 1/4\ H_2O$: C 61.15, H 5.23, N 11.41; found: C 61.13, H 5.26, N 11.20.

*1-Cyclopropyl-6-fluoro-7-(4,7-diazaspiro[2,5]octan-7-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride (**12**a)*

A suspension of **15***a* (80 mg, 0.2 mmol) in 6 N HCl (3 mL) was refluxed for 48 h. The solvent was then concentrated to dryness under reduced pressure. Ethanol (5 mL) was added to digest the residue for 15 min under reflux. It was cooled, filtered, and dried to give 35 mg **12***a* (43%), mp 247–255°C (dec.). IR (KBr): 1720 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.95 (m, 2H, CH_2), 1.05 (t, J = 7 Hz, 3H, ethyl CH_3), 1.15 (m, 2H, CH_2), 1.19 (m, 2H, CH_2), 1.32 (m, 2H, CH_2), 3.42 (m, 2H, NCH_2), 3.45 (s, 2H, NCH_2), 3.62 (m, 2H, NCH_2), 3.84 (m, 1H, CH), 7.59 (d, J_{H-F} = 7 Hz, 1H, C8-H), 7.96 (d, J_{H-F} = 13 Hz, 1H, C5-H), 8.68 (s, 1H, C2-H), 9.72 (bs, 2H, NH_2Cl), 15.13 (s, 1H, OH). Anal. calcd. for $C_{19}H_{21}ClFN_3O_3 \cdot 1/2\ H_2O$: C 56.64, H 5.50, N 10.43; found: C 56.60, H 5.50, N 10.15.

By use of a similar procedure, hydrolysis of **15***b* with 6 N HCl gave 1-cyclopropyl-6,8-difluoro-7-(4,7-diazaspiro[2,5]octan-7-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride (**12***b*) in 81% yield, mp >250°C. IR (KBr): 1725 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.93 (m, 2H, CH_2), 1.14 (m, 2H, CH_2), 1.20 (m, 4H, CH_2×2), 3.35 (m, 2H, NCH_2), 3.47 (s, 2H, NCH_2), 3.65 (m, 2H, NCH_2), 4.12 (m, 1H, CH), 7.88 (dd, J_{H-F} = 12 Hz, 1H, C5-H), 8.71 (s, 1H, C2-H), 9.74 (bs, 2H, NH_2Cl), 14.66 (bs, 1H, OH). Anal. calcd. for $C_{19}H_{20}ClF_2N_3O_3 \cdot 1/2\ H_2O$: C 54.22, H 4.99, N 9.99; found: C 54.12, H 4.82, N 9.92.

Under similar conditions, hydrolysis of **15***c* with 6 N HCl gave 1-cyclopropyl-6-fluoro-7-(4,7-diazaspiro[2,5]octan-7-yl)-1,4-dihydro-4-oxo[1,8]naphthyridine-3-carboxylic acid hydrochloride (**12***c*) in 77% yield, mp >250°C. IR (KBr): 1720 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.97 (m, 2H, CH_2), 1.12 (m, 4H, CH_2×2), 1.20 (m, 2H, CH_2), 3.40 (m, 2H, NCH_2), 3.72 (m, 1H, CH), 4.02 (s, 2H, NCH_2), 4.18 (m, 2H, NCH_2), 8.17 (d, J_{H-F} = 13 Hz, 1H, C5-H), 8.64 (s, 1H, C2-H), 9.92 (bs, 2H, NH_2Cl), 15.07 (bs, 1H, OH). Anal. calcd. for $C_{18}H_{20}ClFN_4O_3 \cdot 1/2\ H_2O$: C 53.53, H 5.20, N 13.88; found: C 53.25, H 5.31, N 13.74.

Hydrolysis of **15***d* in trifluoroacetic acid and 6 N HCl mixture yielded 1-(2,4-difluorophenyl)-6-fluoro-7-(4,7-diazaspiro[2,5]octan-7-yl)-1,4-dihydro-4-oxo[1,8]naphthyridine-3-carboxylic acid trifluoroacetic acid salt **12***d*, mp 229–230°C. IR (KBr): 1675, 1730 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.53 (m, 2H, CH_2), 0.93 (m, 2H, CH_2), 3.24 (m, 2H, NCH_2), 3.66 (m, 2H, NCH_2), 3.88 (m, 2H, NCH_2), 7.37 (m, 1H, aromatic H), 7.64 (m, 1H, aromatic H), 7.83 (m, 1H, aromatic H), 8.25 (d, J_{H-F} = 13 Hz, 1H, C5-H), 8.93 (s, 1H, C2-H), 9.39 (bs, 2H, CF_3COONH_2), 14.86 (bs, 1H, OH). Anal. calcd. for $C_{23}H_{18}F_6N_4O_5$: C 50.74, H 3.33, N 10.29; found: C 50.83, H 3.53, N 10.20.

Hydrolysis of **15***e* with 6 N HCl gave 17-cyclopropyl-6-fluoro-7-(6,9-diazaspiro[4,5]decan-9-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride (**12***e*), mp >250°C. IR (KBr): 1720 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 1.18 (m, 2H, CH_2), 1.33 (m, 2H, CH_2), 1.69 (m, 2H, CH_2), 1.82 (m, 4H, CH_2×2), 1.98 (m, 2H, CH_2), 3.36 (m, 4H, NCH_2×2), 3.50 (m, 2H, CH_2), 3.83 (m, 1H, CH), 7.03 (d, J_{H-F} = 7 Hz, 1H, C8-H), 7.98 (d, J_{H-F} = 13 Hz, 1H, C5-H), 8.70 (s, 1H, C2-H), 9.30 (bs, 2H, NH_2Cl), 15.12 (bs, 1H, OH). Exact Mass calcd. for $C_{21}H_{24}FN_3O_3$: 385.1802; measured: 385.1803.

Hydrolysis of **15***f* with 6 N HCl yielded 1-(2,4-difluorophenyl)-6-fluoro-7-(6,9-diazaspiro[4,5]decan-9-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride (**12***f*), mp >250°C. IR (KBr): 1730 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 1.62 (m, 2H, CH_2), 1.82 (m, 6H, CH_2×3), 3.20 (m, 6H, NCH_2×3), 6.41 (d, J_{H-F} = 7 Hz, 1H, C8-H), 7.45 (m, 1H, aromatic H), 7.75 (m, 1H, aromatic H), 7.94 (m, 1H, aromatic H), 8.05 (d, J_{H-F} = 13 Hz, 1H, C5-H), 8.88 (s, 1H, C2-H), 9.52 (bs, 2H, NH_2Cl), 14.84 (bs, 1H, OH). Anal. calcd. for $C_{24}H_{23}ClF_3N_3O_3 \cdot 1/4\ H_2O$: C 57.83, H 4.75, N 8.43; found: C 57.66, H 4.70, N 8.41.

Hydrolysis of **15***g* in a mixture of trifluoroacetic acid and 6 N HCl yielded 1-(2,4-difluorophenyl)-6-fluoro-7-(6,9-diazaspiro[4,5]decan-9-yl)-1,4-dihydro-4-oxo[1,8]naphthyridine-3-carboxylic acid trifluoroacetic acid salt (**12***g*), mp 255°C. IR (KBr): 1680, 1735 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 1.28 (m, 2H, CH_2), 1.64 (m, 6H, CH_2×3), 3.23 (m, 2H, NCH_2), 3.59 (m, 2H, NCH_2), 3.85 (m, 2H, NCH_2), 7.38 (m, 1H, aromatic H), 7.66 (m, 1H, aromatic H), 7.84 (m, 1H, aromatic H), 8.26 (d, J_{H-F} = 13 Hz, 1H, C5-H), 8.90 (s, 1H, C2-H), 9.19 (bs, 2H, CF_3COONH_2), 14.87 (bs, 1H, OH). Anal. calcd. for $C_{25}H_{22}F_6N_4O_5 \cdot 1/4\ H_2O$: C 52.04, H 3.90, N 9.71; found: C 51.82, H 3.85, H 9.60.

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