rification by a chromatography on silical gel and passage through a column of Dowex-50 × 8 ion-exchange resin (sodium form), compound **1b** was thus obtained in the yield of 42%: ^{1}H NMR (CD_3OD) δ 0.75 (s, 3 H, CH_3-18), 0.91 (d, 6 H, J = 6.6 Hz, 2 CH_3-26,27), 0.98 (d, 3 H, J = 6.5 Hz, CH_3-21), 1.09 (s, 3 H, CH_3-19), 3.77 (d, 1 H, J = 8.4 Hz, H-7), 3.98–4.08 (m, 4 H; 1 H-3, 1 H-4′, 2 H-5′), 4.55 (m, 1 H, H-3′), 5.31 (s, 1 H, H-6), 5.78 (d, 1 H, J = 8.1 Hz, H-5″), 6.34 (t, 1 H, J = 6.8 Hz, H-1′), 8.01 (d, 1 H, J = 8.1 Hz, H-6″); ^{13}C NMR (CD_3OD) see Table II; FAB-MS positive (matrix, 1-thioglycerol) 737 [MNa^+, 5], 715 [MH^+, 5], 383 [19], 367 [33]; FAB-MS negative (matrix, 1-thioglycerol) 691 [$(M - Na^+)^-$, 36], 481 [$(M - Na^+ - Nuc + H^+)^-$, 9], 307 [$(M - Na^+ - St + H^+)^-$, 35]. Anal. ($C_{36}H_{56}N_2O_9PNa\cdot 2H_2O$) C, H, N, P, Na.

Antiproliferative Activity in Vitro. The murine leukemia EL-4 cells were used to assess the cytotoxic profile of the compounds. Cells were maintained in 25 cm^2 tissue-culture flasks (Falcon 3042F) in RPMI-1640 medium supplemented with 10% inactivated fetal calf serum (Gibco, Bio-Cult, Glasgow, Scotland) and gentalin (20 mg/L). All assays were performed in 24-well plates (Costar). To each well were added 2.5 × 10^5 cells in 2 mL and 5 μL of ethanolic solution of tested compounds. In every case the final ethanol concentration was less than 0.25%. Cells were allowed to proliferate for 48 h at 37 °C in a humified atmosphere containing 5% CO_2. At the end of the incubation, the number of viable cells was determined by the Trypan Blue exclusion test.

Antitumor Activity in Vivo. The compounds shown in Table I were screened for in vivo antitumor activity against intraperitoneally transplanted Krebs II ascitic carcinoma in Swiss/OF1 female mice (supplied by Le Centre d'élevage Iffa Credo/France). The intraperitoneal transplantation of 1 × 10^6 ascitic Krebs II cells (in a 0.25 mL suspension of 0.9% NaCl) in OF1 mice (six mice for each group, average weight 20–25 g) was carried out with donor mice bearing 8–10-day-old tumors. Compounds **1a**, **1b**, or 5F-dUrd were dissolved in 0.9% saline. BHS-7β-OHC was dissolved in ultrapurified water. 7β-OHC was suspended in ultrapurified water and stirred with an electric minimixer to give a homogeneous suspension. A 0.2-mL solution was administered ip daily, starting 24 or 96 h after tumor transplantation, as indicated in the Table I. The mice in the control group received the same volume of 0.9% saline. Animals were observed for 80 days. Antitumor activity was evaluated by comparing the mean survival time of the treated animals (T) with that of saline-treated control animals (C): the percentage of increase in life span, % ILS = $\{(T/C) - 1\} \times 100$, and the percent of animals showing complete recovery (survival for more than 80 days) is %CR. The results presented here are derived from four independent experiments.

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Quinolone Antibacterial Agents Substituted at the 7-Position with Spiroamines. Synthesis and Structure–Activity Relationships

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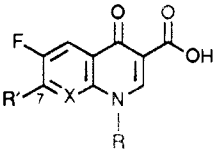
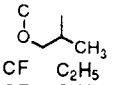
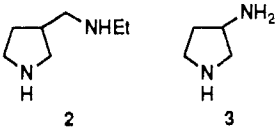
A series of fluoroquinolone antibacterials having the 7-position (10-position of pyridobenzoxazines) substituted with 2,7-diazaspiro[4.4]nonane (**4b**), 1,7-diazaspiro[4.4]nonane (**5a**), or 2,8-diazaspiro[5.5]undecane (**6b**) was prepared, and their biological activities were compared with piperazine and pyrrolidine substituted analogues. Most exhibited potent Gram-positive and Gram-negative activity, especially when side chain 4b was N-alkylated.

Quinolone antibacterial agents continue to show promise of being an important class of therapeutically useful compounds.[1] Most of these agents, which have broad spectrum activity, are substituted at the 7-position by cyclic aliphatic amines (side chains), especially diamines such as piperazine. Notable examples are norfloxacin (**1a**), ciprofloxacin (**1b**), enoxacin (**1c**), and ofloxacin (**1d**).



	R′	X	R
1a:	1-piperazinyl	CH	C_2H_5
b:	1-piperazinyl	CH	c-C_3H_5
c:	1-piperazinyl	N	C_2H_5
d:	4-methyl-1-piperazinyl		
e:	3-[(ethylamino)methyl]-1-pyrrolidinyl	CF	C_2H_5
f:	3-amino-1-pyrrolidinyl	CF	C_2H_5
g:	3-[(ethylamino)methyl]-1-pyrrolidinyl	CF	c-C_3H_5
h:	3-amino-1-pyrrolidinyl	CF	c-C_3H_5
i:	5-methyl-2,5-diabicyclo[2.2.1]hept-2-yl	CF	c-C_3H_5

Two other diamines which have been successfully employed are *N*-ethyl-3-pyrrolidinemethanamine (**2**) and 3-aminopyrrolidine (**3**), both of which have been attached to quinolone substrates by way of their ring nitrogen to give the highly active broad spectrum antibacterials **1g** and **1h**, respectively.[2]



2 **3**

Since flexible linear amines such as ethylenediamine are poor substituents for the 7-position,[1a] it would appear that some rigidity is essential for active quinolone side chains. A recent report on danofloxacin[3] (**1i**) demonstrates that quinolones with side chains less flexible than piperazine can be very active. It was therefore of interest to prepare

(1) (a) Chu, D. T. W.; Fernandes, P. B. *Antimicrob. Agents Chemother.* **1989**, 33, 131. (b) Fernandes, P. B.; Chu, D. T. W. *Annu. Rep. Med. Chem.* **1988**, *23*, 133.

(2) (a) Sanchez, J. P.; Domagala, J. M.; Hagen, S. E.; Heifetz, C. L.; Hutt, M. P.; Nichols, J. B.; Trehan, A. K. *J. Med. Chem.* **1988**, *31*, 983. (b) Egawa, H.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Okada, H.; Uno, H.; Matsumoto, J. *J. Med. Chem.* **1984**, *27*, 1543.

(3) McGuirk, P. L.; Jefson, M. R.; Mann, D. D.; Hindahl, M. S.; Cornell, C. P.; Weber, F. H. *Abstract of the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Houston, Texas, 1989, Abstr. 1186.

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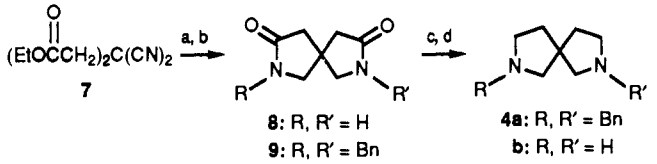
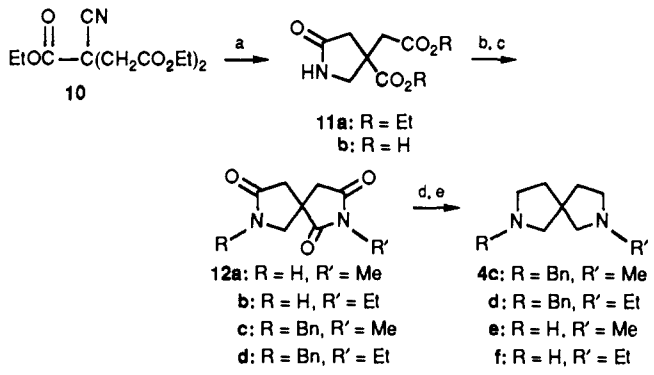
Scheme I[a]



[a] (a) H_2, Raney Co; (b) benzyl chloride, NaH; (c) $LiAlH_4$; (d) H_2, Pd/C.

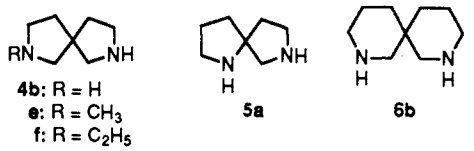
Scheme II[a]



[a] (a) H_2, Raney Co; (b) $MeNH_2$ or $EtNH_2$; (c) benzyl chloride, NaH; (d) $LiAlH_4$; (e) H_2, Pd/C.

quinolones substituted with side chains related to amines **2** and **3** but which were more conformationally restricted.

Spiroamines 2,7-diazaspiro[4.4]nonane (**4b**) and 1,7-diazaspiro[4.4]nonane (**5a**) retain the structural features of a pyrrolidine ring substituted in the 3-position by an aminomethyl or amino group corresponding to amines **2** and **3**, but are less flexible. 2,8-Diazaspiro[5.5]undecane (**6b**), although enlarged by one methylene in each ring, relates to **4b** and **5a** in that the two nitrogen atoms are separated by three carbon atoms and lie in perpendicular planes. The spiroamines were reacted with a variety of 7-haloquinolone substrates and these compounds were examined for antibacterial activity as well as inhibition of DNA gyrase, the enzyme responsible for supercoiling of DNA and the site of action for this class of compounds.[4]



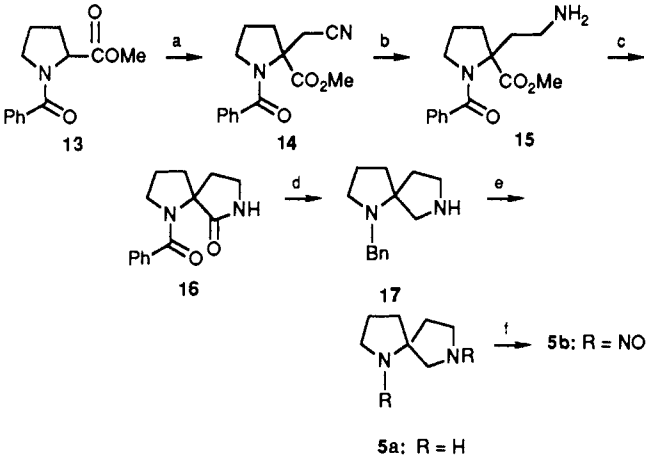
Chemistry

Spiroamine **4b** was prepared by a modification of the procedure described by Overberger,[5] Scheme I. Hydrogenation of diethyl 3,3-dicyanoglutarate (**7**) with Raney cobalt catalyst afforded 2,7-diazaspiro[4.4]nonane-3,8-dione (**8**),[6a,b] which was N-benzylated to give **9**, reduced with LAH to give **4a**,[5] and hydrogenated to afford **4b**.

Spiroamines **4e** and **4f** were prepared (Scheme II) via hydrogenation of triethyl 2-cyano-1,2,3-propanetricarboxylate (**10**) to afford the pyrrolidinone **11a**, which was further cyclized to **12a** by heating with methylamine. Subsequent N-benzylation, **12c**, LAH reduction, **4c**, and hydrogenation gave **4e**. Synthesis of the *N*-ethyl analogue **4f** differed only in that the cyclization with ethylamine was carried out on the diacid **11b**.

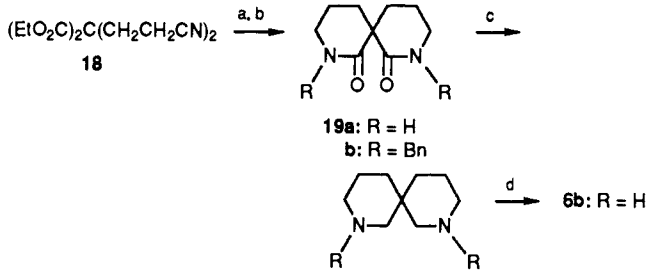
Spiroamine **5a** was prepared according to Scheme III with *N*-benzoyl-L-proline methyl ester (**13**) as the starting material. Alkylation of **13** with chloroacetonitrile afforded **14**, which was hydrogenated to **15**. Thermal cyclization afforded the spirolactam **16**, which was reduced by LAH

Scheme III[a]



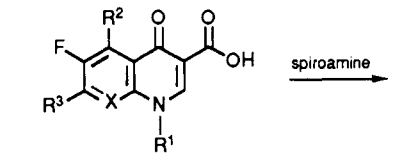
[a] (a) $ClCH_2CN$, LDA; (b) H_2, Raney Ni; (c) Δ; (d) $LiAlH_4$; (e) H_2, Pd/C; (f) $NaNO_2$, pH 2, 90 °C, 1 h.

Scheme IV[a]

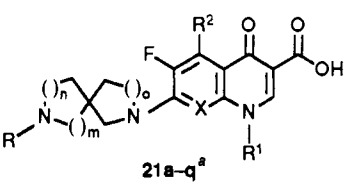


[a] (a) H_2, Raney Co; (b) benzyl chloride, NaH; (c) $LiAlH_4$; (d) H_2, Pd/C.

Scheme V



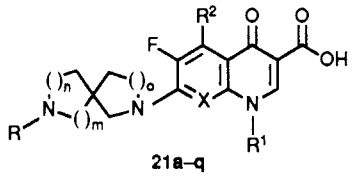
	X	R^1	R^2	R^3
20a:[16]	CH	Et	H	F
b:[12]	CF	Et	H	F
c:[17]	CF	Et	F	F
d:[18]	CN	Et	H	Cl
e:[12]	CF	c-C_3H_5	H	F
f:[19]			H	F
g:[20]			NH_2	F



[a] See Table I.

(4) Cozzarelli, N. R. *Science* **1980**, *207*, 953.
(5) Overberger, C. G.; Wang, D. W.; Hill, R. K.; Krow, G. R.; Ladner, D. W. *J. Org. Chem.* **1981**, *46*, 2757.
(6) (a) Kajtar, M.; Hollosi, M.; Kinsky, K.; Majer, Z. *Collect. Czech. Chem. Commun.* **1982**, *47*, 936. (b) Majer, Z.; Kajtar, M.; Tichy, M.; Blaha, K. *Collect. Czech. Chem. Commun.* **1982**, *47*, 950.

Table I. Spiroamine Substituted Quinolones



21a–q

compd	X	R^1	R^2	R	n, m, o	reaction time, h	yield, %	mp, °C[a]	formula	analysis
21a	CF	C_2H_5	H	H	1, 1, 1	1.5[b]	100	234–240	$C_{19}H_{21}F_2N_3O_3$	C, H, N
21b	CH	C_2H_5	H	CH_3	1, 1, 1	18[b]	64	239–241	$C_{20}H_{24}FN_3O_3$	C, H, N
21c	CF	C_2H_5	F	CH_3	1, 1, 1	18[c]	84	253–256	$C_{20}H_{22}F_3N_3O_3$	C, H, N
21d	CF	C_2H_5	H	CH_3	1, 1, 1	1.5[b]	74	229–231	$C_{20}H_{23}F_2N_3O_3$	C, H, N
21e	CF	c-C_3H_5	H	H	1, 1, 1	2[b]	94	259–260	$C_{20}H_{21}N_3F_2O_3 \cdot 0.5H_2O$	C, H, N
21f	CF	c-C_3H_5	H	CH_3	1, 1, 1	6[b]	55	216–217	$C_{21}H_{23}N_3F_2O_3$	C, H, N
21g	CF	c-C_3H_5	H	C_2H_5	1, 1, 1	3[b]	65	190–191	$C_{20}H_{25}F_2N_3O_3$	C, H, N
21h	CF	C_2H_5	H	C_2H_5	1, 1, 1	2[b]	58	199–200	$C_{21}H_{25}F_2N_3O_3$	C, H, N
21i	CF	C_2H_5	H	H	2, 0, 1	3.5[b]	45	282–283	$C_{19}H_{21}F_2N_3O_3 \cdot HCl$	C, H, N, Cl
21j	CF	C_2H_5	H	H	2, 1, 2	2[b]	63	267–268	$C_{21}H_{25}F_2N_3O_3$	C, H, N
21k	N	C_2H_5	H	H	1, 1, 1	4.5[b]	20	>300[d]	$C_{18}H_{21}FN_4O_3 \cdot HCl$	C, H, N, Cl
21l	N	C_2H_5	H	C_2H_5	1, 1, 1	24[c]	84	215–217	$C_{20}H_{25}FN_4O_3$	C, H, N
21m	N	C_2H_5	H	CH_3	1, 1, 1	2[c]	44	252–254	$C_{19}H_{23}FN_4O_3 \cdot 0.5H_2O$	C, H, N[e]
21n	N	C_2H_5	H	H	2, 1, 2	18[c]	35	288–292	$C_{20}H_{25}FN_4O_3$	C, H, N
21o	CF	c-C_3H_5	H	H	2, 0, 1	6	57	280	$C_{20}H_{21}F_2N_3O_3 \cdot 0.7H_2O$	C, H, N
21p			H	CH_3	1, 1, 1	18[b]	63	228–230[f]	$C_{21}H_{24}FN_3O_4 \cdot 0.2H_2O$	C, H, N
21q			NH_2	CH_3	1, 1, 1	4[g]	29	264–266	$C_{21}H_{25}FN_4O_4$	C, H, N

[a] Compounds were precipitated solids which melted with decomposition. [b] Refluxed. [c] Room temperature. [d] Crystallized from EtOH–H_2O. [e] N: calcd 14.61, found 14.12. HPLC: >98% pure. [f] Crystallized from MeCN. [g] Heated in DMF solution at 100 °C.

Table II. Biological Testing Results from the Drug-Induced DNA Cleavage Assay and Antibacterial Screening

	minimum inhibitor concentrations (MIC),[a] μg/mL										
	Gram-negative organisms					Gram-positive organisms					gyrase-drug induced cleavage,[a,b] μg/mL, *E. coli* H560
compd	*E. cloacae* HA 2646	*E. coli* Vogel	*K. pneumoniae* MGH-2	*P. rettgeri* H1771	*P. aeruginosa* UI-18	*S. aureus* H228	*S. aureus* UC-76	*S. faecalis* MGH-2	*S. pneumoniae* SV-1	*S. pyogenes* C203	
21a	0.2	0.1	0.2	1.6	0.8	0.1	0.05	0.2	0.2	0.1	2.5
21b	0.2	0.2	0.8	3.1	3.1	0.8	0.1	0.4	0.4	0.4	5.0
21c	0.8	0.8	1.6	6.3	6.3	0.8	0.2	1.6	0.8	1.6	5.0
21d	0.1	0.006	0.1	0.8	0.8	0.1	0.006	0.1	0.4	0.2	1.0
21e	0.2	0.2	0.4	0.8	1.6	0.2	0.025	0.4	0.1	0.025	0.1
21f	0.05	0.05	0.1	0.2	0.8	0.25	0.013	0.1	0.05	0.05	0.5
21g	0.2	0.1	0.2	0.4	1.6	0.1	0.025	0.1	0.2	0.2	0.5
21h	0.4	0.2	0.2	1.6	3.1	0.2	0.1	0.2	0.8	0.4	5.0
21i	0.2	0.2	0.4	0.8	1.6	1.6	0.8	3.1	1.6	1.6	2.5
21j	6.3	3.1	6.3	25.0	12.5	3.1	0.4	3.1	6.3	3.1	7.5
21k	0.8	0.8	3.1	12.5	0.8	3.1	0.4	1.6	0.8	0.2	5.0
21l	1.6	0.4	0.4	6.3	3.1	0.4	0.2	0.8	1.6	1.6	5.0
21m	0.4	0.1	0.4	1.6	1.6	0.1	0.025	0.2	0.8	0.4	10
21n	25	12.5	25	100	50	6.3	3.1	12.5	12.5	50	50
21o	0.025	0.025	0.1	0.8	0.2	0.05	0.4	0.4	0.4	0.4	0.5
21p	0.2	0.025	0.1	0.4	0.8	0.1	0.006	0.1	0.2	0.1	1.0
21q	0.4	0.4	0.8	6.3	12.5	0.4	0.1	0.4	0.4	0.2	1.0
1a	0.1	0.1	0.2	0.2	0.8	3.1	0.4	3.1	3.1	3.1	5.2
1b	0.013	0.025	0.025	0.025	0.1	1.6	0.1	0.4	0.4	0.2	5.2
1d	0.1	0.2	0.2	0.4	1.6	3.1	0.4	6.3	0.4	3.1	6.2
1e	0.2	0.1	0.2	0.4	1.6	0.1	0.05	0.1	0.1	0.1	2.5
1f	0.1	0.05	0.2	0.2	0.4	0.4	0.05	0.8	0.4	0.4	1.0
1g	0.1	0.05	0.1	0.2	0.4	0.025	0.006	0.025	0.025	0.025	0.25
1h	0.025	0.013	0.025	0.05	0.1	0.05	0.025	0.05	0.05	0.25	0.1

[a] Standard microdilution techniques, see ref 9. All values are accurate to ±50% and were obtained from duplicate experiments. [b] Minimum concentration of drug needed to produce linear DNA at an intensity relative to oxolinic acid at 10 mg/mL; see ref 9.

and then hydrogenated to give **5a**, a compound previously prepared by a different route and characterized by its *N,N'*-dinitroso derivative, **5b**.[6b]

The synthesis of **6b** was carried out according to literature procedures[7a,b] except that the spirolactam **19a** was N-benzylated, **19b**, prior to LAH reduction and catalytic hydrogenation (Scheme IV).

The spiroamines were coupled with quinolone substrates **20a–e** by direct displacement of the fluorine or chlorine atom at the 7-position, or at the 10-position in the case of pyridobenzoxazines **20f** and **20g** (Scheme V).

Biology

The spiroamine substituted quinolones **21** and related quinolones **1a,b,d–f** were tested against 10 organisms by using standard techniques and their MICs determined.[8]

(7) (a) Sury, E.; Hoffmann, K. *Helv. Chim. Acta* **1953**, *36*, 1815. (b) Albertson, N. F.; Fillman, J. L. *J. Am. Chem. Soc.* **1949**, *71*, 2818.

(8) Cohen, M. A.; Griffin, T. J.; Bien, P. A.; Heifetz, C. L.; Domagala, J. M. *Anticirob. Agents Chemother.* **1985**, *28*, 766.

Table III. In Vivo Mouse Protection Assay

compd		organism; protective dose (PD_{50}),[a] mg/kg				
		E. coli Vogel	*P. aeruginosa* UI-18	*S. aureus* VC76	*S. pneumoniae* SV-1	*S. pyogenes* C203
21a	sc	15				
	po	>100				
21c	sc	12.5				
	po	22				
21d	sc	3.4	70	4	11	27
	po	8.4	140	9	43	38
21e	sc	7				
	po	>100				
21f	sc	2				
	po	5				
21h	sc	6	180		43	38
	po	13	>100		68	70
21k	sc	28				
	po	>100				
21l	sc	15				
	po	10.3				
21m	sc	4.4	58	3.6	16	13
	po	11.4	200	8.5	30	27
21o	sc	1				
	po	4				
21p	sc	4.5	56	2.1	11.5	6
	po	14	200	12.5	63	31

[a] Values were obtained from 16 animals for each test and had a mean variation of ±34%.

The compounds were also tested for their inhibition of DNA gyrase isolated from *Escherichia coli* as previously described.[9,10] The concentration of drug (μg/mL) required to induce the gyrase-mediated cleavage of DNA was determined. The combined results are shown in Table II. The in vivo potency expressed as the median protective dose (PD_{50}, mg/kg) was determined in acute, lethal, systemic infections in female Charles River CD-1 mice (16 animals for each type of dose) as previously described.[10,11] Single doses of compound were administered with challenge. The results are shown in Table III.

Results and Discussion

Compounds **21a**, **21i**, and **21j** are 6,8-difluoro-1-ethyl-4-oxo-3-quinolinecarboxylic acids which are substituted at the 7-position by spiroamines **4b**, **5a**, and **6b**, each of which has a secondary amine function in the nonattached ring. Compound **21a** with a 2,7-diazaspiro[4,4]nonane (**4b**) side chain showed broad spectrum antibacterial activity; it was equivalent to norfloxacin (**1a**) against Gram-negative organisms but at least 4 times more potent against Gram-positive bacteria. Compound **21i** with a 1,7-diazaspiro-[4.4]nonane (**5a**) side chain was about equal to **21a** against Gram-negative organisms but slightly weaker against Gram-positives. Also, **21o** was more active than **21e** against Gram-negative bacteria but weaker against Gram-positives as well as gyrase (0.1 versus 0.5 μg/mL). In comparison, quinolones **1e**[12] and **1f**[13] substituted with pyrrolidines **2** and **3**, respectively, show a similar relationship in that they were about equipotent against Gram-negative bacteria, but **1e** (pyrrolidine **2**) was more active against Gram-positives.

Compound **21j** appended with the expanded spiroamine, 2,8-diazaspiro[5.5]undecane (**6b**), was the least potent, especially against Gram-negative bacteria. In addition, the gyrase inhibition values for **21a**, **21i**, and **21j** (2.5, 2.5, and 7.5 μg/mL, respectively; see Table II) reflected the same relative potencies. It has been previously noted that quinolones that inhibit gyrase at >5 μg/mL are generally poor antibacterial agents.[12] The poor antibacterial activity associated with side-chain **6b** was also observed when it was attached to a naphthyridone (**21n**), and gyrase was only inhibited at 50 μg/mL.

It is generally known that quinolones substituted at the 1-position with a cyclopropyl group are more potent than those with an ethyl substituent,[12] e.g. **1g** > **1e** and **1h** > **1f** (see Table II). A comparison of 1-ethylquinolone **21a** with 1-cyclopropyl analogue **21e** showed only minor improvement in the antibacterial activity expected from the cyclopropyl substituent, although gyrase inhibition improved by more than 10-fold. With 1,7-diazaspiro[4.4]-nonane (**5a**) as the side chain, the 1-cyclopropyl analogue **21o** did show the expected increase in potency (2–10-fold) relative to the 1-ethyl compound **21i**.

Since the 2,7-diazaspiro[4.4]nonane **4b** appeared to elicit the highest potency in general, it was of interest to compare quinolones having N-alkylated spiroamine side chains in order to assess the effect of increased lipophilicity, for example, *N*-methyl- and *N*-ethylspiroamines **4e** and **4f**. In the 1-ethyl-6,8-difluoroquinolone series substitution by the *N*-methylspiroamine **4e** to give **21d** resulted in improved activity over both **21a** (R = H) and **21h** (R = Et). The same trend in antibacterial activity also appeared in the naphthyridine series, i.e., **21m** > **21l** ≈ **21k**, although gyrase inhibition was weak and in reverse order. The conclusion that an N-methylated side chain was preferred was also supported by in vivo mouse protection data (see Table III); i.e., the 1-ethyl-6,8-difluoroquinolone **21d** (R = Me) was consistently better than **21h** (R = Et) and **21a** (R = H), and N-alkylation was especially needed for oral activity. In the 1-ethyl-6-fluoronaphthyridine series, **21m** (R = Me) was more potent than **21l** (R = Et) or **21k** (R = H) with **21k** being devoid of oral activity. This preference for N-alkylation of quinolones substituted by amino-pyrrolidines had been previously observed.[2a]

(9) Domagala, J. M.; Hanna, L. D.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Sanchez, J. P.; Solomon, M. *J. Med. Chem.* **1986**, *29*, 394.
(10) Domagala, J. M.; Heifetz, C. L.; Mich, T. F.; Nichols, J. B.; *J. Med. Chem.* **1986**, *29*, 445.
(11) Miller, L. C.; Tainter, M. L. *Proc. Soc. Exp. Biol. Med.* **1944**, *57*, 261.
(12) Domagala, J. M.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Nichols, J. B.; Solomon, M.; Worth, D. F. *J. Med. Chem.* **1988**, *31*, 991.
(13) Culbertson, T. P.; Domagala, J. M.; Mich, T. F.; Nichols, J. B.; U.S. Patent 4665079, 1987.

Since the *N*-methyl-2,7-diazaspiro[4.4]nonane (**4e**) side chain appeared to be the most favorable, it was incorporated into several other substituted quinolones and pyridobenzoxazines. Both the 6-fluoro-1-ethylquinolone **21b** and the 5,6,8-trifluoro-1-ethylquinolone **21c** were less potent than the 6,8-difluoro-1-ethylquinolone **21d**, which is consistent with quinolones with other side chains.[2a,14] The 10-substituted-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*]1,4-benzoxazine-6-carboxylic acid **21p** was equipotent with **21d** against bacteria as well as gyrase; however, the 8-amino analogue **21q** was somewhat less active.

Overall, it appeared that activity was influenced more by the structure of the spiroamine side chain than other substituents on the quinolone substrate. For example, the usual enhancement in activity associated with changing 1-ethyl to a 1-cyclopropyl substitution was not observed, i.e., 1-ethylquinolones **21a**, **21d**, and **21h** were of about the same potency as the 1-cyclopropyl analogues **21e**, **21f**, and **21g**.

Quinolones with a 3-[(ethylamino)methyl]pyrrolidine side chain (**1g**)[2a,12] and a 3-aminopyrrolidine side chain (**1h**) (CI-938)[2a] were clearly superior to analogous spiroamine substituted quinolones **21e** and **21o**. From this data it appears that quinolones with side chains derived from 2,7-diazaspiro[4.4]nonane (**4b**) and 1,7-diazaspiro[4.4]nonane (**5a**), although potent antibacterial agents, are not as active as quinolones substituted with the more flexible pyrrolidines **2** and **3**.

Experimental Section

All melting points were determined on a Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were determined on a Nicolet FTIR SX-20 spectrophotometer. Proton NMR spectra were recorded on either a Varian XL-200 or an IBM 100 WP100SY spectrometer. Shifts are reported in δ units relative to internal tetramethylsilane. Elemental analyses were performed on a Perkin-Elmer 240 elemental analyzer. All compounds had analytical results ±0.4% of theoretical values. All organic solutions were dried over magnesium sulfate. Column chromatography was performed with E. Merck silica gel 60, 70–230 mesh ASTM, and thin-layer chromatography was performed with silica gel 60 F254 plates.

2,7-Diazaspiro[4.4]nonane-3,8-dione (8). A solution of 87.55 g (0.367 mol) of diethyl 3,3-dicyanoglutarate[5] in 360 mL of DMF and 22 mL of triethylamine was treated with 22 g of Raney cobalt catalyst and hydrogenated at 2400–5400 psi for 23 h. After filtration of the catalyst and evaporation of the solvent in vacuo, the residue was stirred with 200 mL of $CHCl_3$. The solid was removed by filtration to give 18.13 g (32%) of **8** as a white solid:[15] mp 213–218 °C; NMR (DMSO-d_6) δ 2.18 (s, 4 H, CH_2), 3.13 (s, 4 H, CH_2), 7.51 (s, 2 H, NH). A sample was recrystallized for analysis from ethanol, mp 216–220 °C. Anal. ($C_7H_{10}N_2O_2$) C, H, N.

2,7-Bis(phenylmethyl)-2,7-diazaspiro[4.4]nonane-3,8-dione (9). A 60% mineral oil suspension of sodium hydride (5.76 g, 0.24 mol) was washed twice with toluene, suspended in DMF, and treated with 17.50 g (0.114 mol) of 2,7-diazaspiro[4.4]nonane-3,8-dione (**8**). The mixture was stirred 0.5 h, treated dropwise with 30.5 g (0.24 mol) of benzyl chloride, and stirred overnight. After removal of most of the solvent in vacuo, the mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with water, dried, and evaporated to afford 35.7 g (94%) of **9** as a syrup: NMR ($CDCl_3$) δ 2.43 (s, 2 H, CH_2), 3.11 (s, 2 H, CH_2), 4.33 (s, 2 H, benzyl CH_2), 7.17 (m, 5 H, phenyl). This material was used without further purification.

2,7-Bis(phenylmethyl)-2,7-diazaspiro[4.4]nonane Dihydrochloride (4a·2HCl). To a suspension of 10.00 g (0.26 mol) of lithium aluminum hydride in 200 mL of THF was added dropwise a solution of 35.0 g (0.10 mol) of **9** in 200 mL of THF. After stirring overnight at room temperature, the mixture was refluxed for 1 h, cooled, diluted with 200 mL of THF, and treated sequentially (dropwise) with 10 mL of water, 10 mL of 15% NaOH solution, and 30 mL of water. After filtration of the solids the filtrate was evaporated to a syrup, dissolved in ether, and concentrated. The residue was dissolved in 2-propanol and treated with excess 6 N HCl in 2-propanol. The product was allowed to crystallize at 0 °C to afford 31.51 g (83%) of spiroamine **4a** as its hydrochloride salt. A sample was recrystallized from 2-propanol for analysis, mp 254–256 °C. Anal. ($C_{21}H_{26}N_2 \cdot 2HCl \cdot 0.1H_2O$) C, H, N, Cl.

2,7-Diazaspiro[4.4]nonane (4b). Spiroamine **4b** was prepared in 75% yield by hydrogenation of compound **4a**·2HCl in methanol solution using 10% Pd/C catalyst: bp 70–72 °C (2 mm) [lit.[5] bp 50–52 °C (0.5 mm)]; NMR ($CDCl_3$) δ 1.73 (t, 4 H, J = 7.1 Hz, NCH_2CH_2), 1.85 (s, 2 H, NH), 2.80 (d, d, 4 H, J = 10.6, 12.9 Hz), 2.99 (t, 4 H, J = 7.1 Hz, NCH_2CH_2).

Ethyl 3-(Ethoxycarbonyl)-5-oxo-3-pyrrolidineacetate (11a). Compound **11a**, bp 176–180 °C (0.5 mm), was prepared in 80% yield by hydrogenation of triethyl 2-cyano-1,2,3-propanetricarboxylate[5] according to the procedure for compound **9** and was used without further purification.

2-Methyl-2,7-diazaspiro[4.4]nonane-1,3,8-trione (12a). A solution of 10.21 g (0.042 mol) of 11a in 20 mL of 40% aqueous methylamine was stirred at room temperature overnight. The mixture was then placed in an oil bath, and the temperature was gradually raised to 220 °C (1 h) while the volatiles were allowed to distill. The product was crystallized from ethanol to afford 5.83 g (76.2%) of **12a**, mp 202–204 °C. Anal. ($C_8H_{10}N_2O_3$) C, H, N.

2-Ethyl-2,7-diazaspiro[4.4]nonane-1,3,8-trione (12b). The diester **11a** (24.3 g, 0.1 mol) was hydrolyzed to the diacid **11b** by stirring with 165 mL of 2 N NaOH for 6 h at room temperature. The mixture was acidified with dilute HCl, evaporated to dryness, and stirred with 2-propanol. After filtration of NaCl and evaporation to dryness, the residue was dissolved in 110 mL of 70% ethylamine and gradually heated in an oil bath over 40 min to 230 °C. Cooling the reaction mixture produced a gum which crystallized from 2-propanol to give 10.1 g (52%) of **12b**: mp 168–169 °C; NMR ($CDCl_3$) δ 1.16 (t, 3 H, J = 7 Hz, CH_2CH_3), 2.29 (d, 1 H, J = 16 Hz, C_9H), 2.82 (s, 2 H, C_4H), 2.94 (d, 1 H, J = 16 Hz, C_9H), 3.30 (d, 1 H, J = 10 Hz, C_6H), 3.58 (q, 2 H, J = 7 Hz, CH_2CH_3), 3.84 (d, 1 H, J = 10 Hz, C_6H), 6.54 (br s, 1 H, NH). Anal. ($C_9H_{12}N_2O_3$) C, H, N.

2-Methyl-7-(phenylmethyl)-2,7-diazaspiro[4.4]nonane-1,3,8-trione (12c). To 0.50 g (10.4 mmol) of toluene washed 50% sodium hydride in 10 mL of DMF was added gradually a solution of 1.82 g (10 mmol) of **12a** in 20 mL DMF. The mixture was stirred 1 h, treated with 1.40 g (11 mmol) of benzyl chloride, and stirred overnight at room temperature. The solvent was removed in vacuo; the residue was diluted with water and extracted with $CHCl_3$. The organic layer was evaporated and the product was crystallized from toluene–hexanes to give 1.74 g (64%) of **12c**, mp 157–158 °C. Anal. ($C_{15}H_{16}N_2O_3$) C, H, N.

2-Ethyl-7-(phenylmethyl)-2,7-diazaspiro[4.4]nonane-1,3,8-trione (12d). Compound **12d**, mp 125–126.5 °C, was prepared in 76% yield from **12b** by the same procedure used to prepare **12c**. Anal. ($C_{16}H_{18}N_2O_3$) C, H, N.

2-Methyl-7-(phenylmethyl)-2,7-diazaspiro[4.4]nonane Dihydrochloride (4c·2HCl). Compound **4c**·2HCl, mp 233–234 °C, was prepared in 64% yield by the LAH reduction of **12c** according to the procedure used to prepare **4a**. Anal. ($C_{15}H_{22}N_2 \cdot 2HCl$) C, H, N, Cl.

2-Ethyl-7-(phenylmethyl)-2,7-diazaspiro[4.4]nonane Dihydrochloride (4d·2HCl). Compound **4d**·2HCl, mp 196–198 °C.

(14) Mich, T. F.; Domagala, J. M.; Nichols, J. B. U.S. Patent 4657913, 1987.
(15) The *R* isomer of **8**, mp 258–260 °C, was prepared by a different route, see ref 6a.
(16) Eur. Pat. App. 0000203, 1970; *Chem. Abstr.* **1979**, *90*, 163334j.
(17) Mich, T. F.; Domagala, J. M.; Nichols, J. B. U.S. Patent 4657913, 1987.
(18) Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H.; Nishimura, H. *J. Med. Chem.* **1984**, *27*, 292.
(19) Hayakawa, I.; Tunaka, Y. Hiramitsu, T. Eur. Pat. 0047005, 1982; *Chem. Abstr.* **1982**, *97*, 55821b.
(20) Domagala, J. M.; Mich, T. F.; Sanchez, J. P. Eur. Pat. Appl. EP 265230, 1988.

was prepared in 77% yield from **12d** according to the procedure used to prepare **4a**. Anal. ($C_{16}H_{24}N_2$·2HCl) C, H, N, Cl.

2-Methyl-2,7-diazaspiro[4.4]nonane Dihydrochloride (4e·2HCl). Compound **4e**·2HCl, mp 168–170 °C, was prepared in 87% yield by hydrogenation of 4c·2HCl according to the procedure used to prepare **4b** and was converted to the dihydrochloride with hydrogen chloride in 2-propanol. Anal. ($C_8H_{16}N_2$·2HCl) C, H, N, Cl.

2-Ethyl-2,7-diazaspiro[4.4]nonane Dihydrochloride (4f·2HCl). Compound 4f·2HCl, mp 168–172 °C, was prepared in 98% yield from **4d**·2HCl according to the procedure used to prepare **4b**. Anal. ($C_9H_{18}N_2$·2HCl) C, H, N, Cl.

***N*-Benzoyl-L-proline Methyl Ester (13).** A solution of 10.0 g (45.7 mmol) of *N*-benzoyl-L-proline in 100 mL of methanol was cooled in an ice bath and treated dropwise with 3.6 mL (60.4 mmol) of thionyl chloride. After standing at 0 °C for 1 h and room temperature overnight, the mixture was evaporated to dryness and the product crystallized from toluene–hexanes to give 10.2 g (96%) of **13**, mp 89–91 °C, $[\alpha]^{25}_D$ −100.1° (*c* 1.17, methanol). Anal. ($C_{13}H_{15}NO_3$) C, H, N.

1-Benzoyl-2-(cyanomethyl)proline Methyl Ester (14). To a solution of 33.8 mL (0.24 mol) of diisopropylamine in 250 mL of THF cooled to −75 °C under nitrogen was added 115 mL of 2.1 M *n*-butyllithium (hexane solution) and then 38 mL (0.25 mol) of *N,N,N′,N′*-tetramethylenediamine. After stirring for 0.5 h the mixture was treated dropwise with a solution of 55.9 g (0.23 mol) of **13** in 250 mL of THF. The mixture was stirred an additional 10 min, and treated dropwise over 0.75 h with 20 mL (0.32 mol) of chloroacetonitrile in 60 mL of THF. After stirring for 1 h at −78 °C the reaction mixture was allowed to warm to 0 °C and was concentrated under vacuum to form a dark syrup. The residue was partitioned between dichloromethane–water, and the solid was removed by filtration. The organic layer was separated, washed with 2 N HCl, dried ($MgSO_4$), and evaporated to afford 52.4 g of a dark syrup. It was chromatographed by eluting with ethyl acetate–hexanes (1:1) to afford 24.4 g (40%) of **14** as a thick syrup: IR (LF) 1635 (s), 1743 (s), 2251 (w) cm^{-1}. TLC (EtOAc–hexanes, 1:1) showed a single spot at R_f 0.3. This material was used without further purification.

1-Benzoyl-1,7-diazaspiro[4.4]nonan-6-one (16). A mixture of 19.9 g (73 mmol) of cyano ester **14** in 100 mL of methanol, 100 mL of THF, and 2 g of Raney nickel catalyst was hydrogenated at 50 psi and 50 °C for 46 h. Filtration and evaporation of solvents afforded a syrup, **15**, which was dissolved in 400 mL of toluene and refluxed 48 h. TLC ($CHCl_3$/NaOH, 20:1) showed two main spots at R_f 0.11 and 0.28. The lower spot material isolated by chromatography on silica gel ($CHCl_3$/NaOH, 30:1) afforded 6.39 g (36%) of **16**. A sample crystallized from ethanol melted 153–155 °C, solidified and remelted at 187–188 °C: NMR ($CDCl_3$) δ 1.88 (m, 1 H), 1.96–2.15 (complex m, 3 H), 2.30 (m, 1 H), 2.92 (m, 1 H), 3.35 (m, 1 H), 3.61 (m, 3 H), 5.94 (s, 1 H), 7.39 (m, 3 H), 7.54 (m, 2 H). Anal. ($C_{14}H_{16}N_2O_2$) C, H, N.

1-(Phenylmethyl)-1,7-diazaspiro[4.4]nonane Dihydrochloride (17·2HCl). A solution of 4.60 g (18.8 mmol) of **16** in 200 mL of warm THF was added dropwise to a suspension of 4.0 g LAH in 75 mL of THF. After stirring two days at room temperature, the mixture was refluxed 2 h, cooled, treated successively (dropwise) with 4 mL of water, 4 mL of 15% NaOH solution, and 12 mL of water, and the solid was removed by filtration. The filtrate was treated with excess 6 N HCl in 2-propanol, evaporated to dryness, and triturated with ether to afford 0.94 g of a very hygroscopic solid, **17**·2HCl: NMR (TFA) δ 7.48 (s, phenyl), very complex otherwise; IR (KBr) showed no amide bands. This material was used without further purification.

1,7-Diazaspiro[4.4]nonane (5a). A solution of 5.30 g (18.3 mmol) of **17**·2HCl, in 100 mL of methanol and 0.30 g of 20% Pd/C catalyst was hydrogenated at 50 psi for 1.5 h. Filtration and concentration afforded a thick syrup which did not crystallize. The free base was generated from a MeOH solution of the product by addition of a slight excess of sodium methoxide in MeOH followed by ether dilution. Filtration, evaporation of solvents, and distillation afforded 1.53 g (66%) of spiroamine **5a**: bp 92–95 °C (15 mmHg), which was very hygroscopic; NMR ($CDCl_3$) δ 1.70 (m, 6 H), 2.32 (s, 2 H), 2.50–3.30 (m, 6 H).

A sample of compound **5a** was converted into the bisnitroso derivative **5b** by literature procedure[6a] and crystallized from $CHCl_3$–ether: mp 75–76 °C (lit mp 81–82 °C); NMR ($CDCl_3$) δ 1.8–2.9 (m, 6 H), 3.6–4.3 (m, 4 H), 4.4–5.0 (m, 2 H); IR (KBr) 1409, 1319 cm^{-1}. Anal. ($C_7H_{12}N_4O_2$) C, H, N.

2,8-Bis(phenylmethyl)-2,8-diazaspiro[5.5]undecane-1,7-dione (19b). Spirolactam **19a** prepared by hydrogenation of diethyl bis(2-cyanoethyl)malonate (**18**)[7b] was benzylated by the procedure used to prepare **9** to afford **19b**, mp 122–125 °C, in 90% yield. Anal. ($C_{23}H_{26}N_2O_2$) C, H, N.

2,8-Bis(phenylmethyl)-2,8-diazaspiro[5.5]undecane Dihydrochloride (6a·2HCl). Spiroamine **6a**·2HCl was prepared from **19b** by the procedure used to prepare **4a**·2HCl and the crude solid was used without purification.

2,8-Diazaspiro[5.5]undecane Dihydrochloride (6b·2HCl). Compound **6b**·2HCl, mp >300 °C, was prepared by hydrogenation of **6a**·2HCl in 83% yield according to the procedure used to prepare **4d**. Anal. ($C_9H_{18}N_2$·2HCl) C, H, N, Cl.[7a]

General Procedure for the Preparation of Quinolone Antibacterials (Table I). All quinolone, naphthyridine, and pyridobenzoxazine substrates were prepared by literature procedures (see Scheme V). A solution of 1 mol of the 7-haloquinolone, 1 mol of the spiroamine dihydrochloride, and 3 mol of DBU in acetonitrile was stirred at room temperature or heated until the reaction was complete by TLC, MeCN–H_2O–Et_3N (18:2:1). For compounds **21e**, **21i**, **21j**, **21k**, and **21n** 2 mol of spiroamine was used without added DBU. The products precipitated during the reaction period or after cooling. Since compounds **21i** and **21k** are difficult to purify under neutral conditions, they were converted to hydrochlorides by lyophilizing aqueous solutions titrated to pH 2 with dilute hydrochloric acid.

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