41011-01-2; *m*-(acetylamino)phenacyl bromide, 30095-56-8; *m*-(methylsulfonylamino)phenacyl bromide, 2065-04-5; *m*-(methoxycarbonyl)phenacyl bromide, 27475-19-0; *m*-cyanophenacyl bromide, 50916-55-7; *m*-nitrophenacyl bromide, 2227-64-7; *o*-hydroxyphenacyl bromide, 2491-36-3; *o*-acetoxyphenacyl bromide, 40231-08-1; *o*-methoxyphenacyl bromide, 31949-21-0; *o*-chlorophenacyl bromide, 5000-66-8; 3,4-dihydroxyphenacyl bromide, 40131-99-5; 3,5-dihydroxyphenacyl bromide, 62932-92-7; 3-hydroxy-4-methylphenacyl bromide, 73898-30-3; 3-hydroxy-4-methoxyphenacyl bromide, 90971-90-7; 4-chloro-3-hydroxyphenacyl bromide, 73898-34-7; 4-hydroxy-3-(methoxycarbonyl)phenacyl bromide, 36256-45-8; 4-hydroxy-3-nitrophenacyl bromide, 5029-61-8; 4-methyl-3-(methylsulfinyl)phenacyl bromide, 99583-05-8; 4-methyl-3-(methylsulfonyl)phenacyl bromide, 99583-06-9; 6-methyl-2-benzothiazolamine, 2536-91-6; 2-amino-6-benzothiazolol, 26278-79-5; 6-methoxy-2-benzothiazolamine, 1747-60-0.

New Structure–Activity Relationships of the Quinolone Antibacterials Using the Target Enzyme. The Development and Application of a DNA Gyrase Assay

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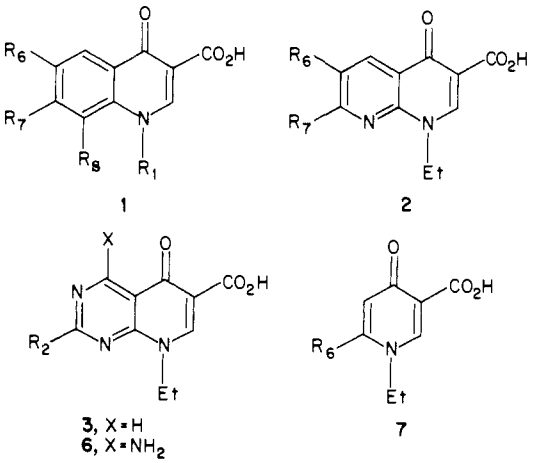
A series of 60 newly synthesized and known quinolone antibacterials, including quinoline- and 1,8-naphthyridine-3-carboxylic acids, pyrido[2,3-*d*]pyrimidine-6-carboxylic acids, and some monocyclic 4-pyridone-3-carboxylic acids, were tested and compared in a newly established, easy to perform, DNA gyrase assay. The results were correlated with minimum inhibitory concentrations (MICs) against a variety of organisms. Among the known quinolones were 14 clinically significant drugs (oxolinic acid, norfloxacin, ciprofloxacin, enoxacin, etc.) which were used as standards and compared side-by-side. The study focused on the changes in DNA gyrase inhibition brought about by certain features of the molecules, namely, the C_6-fluorine or the nature of the C_7 substituent. The intrinsic gyrase inhibition of the fused parent rings, quinoline vs. naphthyridine vs. pyrido[2,3-*d*]pyrimidine, was also explored. In all cases, loss of enzyme inhibition produced poor MICs, but some compounds with good DNA gyrase inhibition did not correspondingly inhibit bacterial growth. Possible explanations for this phenomena and the benefits of a DNA gyrase–MIC strategy for developing future structure–activity relationships are discussed.

During the last 25 years, the quinolone class of orally active antibacterials (which generically has come to include most of the 4-pyridone-3-carboxylic acid antibacterials) has been intensively studied and evaluated for use in antiinfective chemotherapy.[1] Many of the initial agents, such as oxolinic (**1a**) and nalidixic (**2a**) acids (shown in Figure 1), lacked substantial Gram-positive activity and had blood levels below their minimum inhibitory concentrations (MICs). After the discovery of norfloxacin (**1c**) and enoxacin (**2b**), which were the first members of this class of antibacterials to possess broad-spectrum activity with oral efficacy, an explosion of many new and useful agents such as ciprofloxacin (**1f**) and AM833 (**1h**) has occurred.[2] Several other significant quinolone antibacterials are shown in Figure 1.

Midway through the chemical development of this area, the mechanism of action of the quinolones was elucidated.

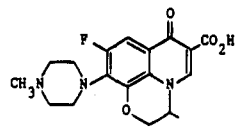


These agents were shown to be specific inhibitors of the A subunit of the bacterial topoisomerase DNA gyrase.[12]

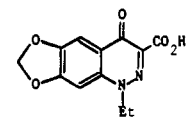
(1) Albrecht, R. *Prog. Drug. Res.* **1977**, 21, 9.
(2) Numerous studies showing the effectiveness of many of these agents have been reported at the 23rd and 24th Interscience Conference on Antimicrobial Agents and Chemotherapy. (a) Abstract: 371-382, 518-519, 647-660c, 694-708B, Oct 24–26, 1983, Las Vegas, NV. (b) Abstract: 71-82, 197, 270-279, 391-403, 455-462, 963-980, Oct 8–10, 1984, Washington, DC.
(3) Agui, H.; Mitani, T.; Izawa, A.; Komatsu, T.; Nakagome, T. *J. Med. Chem.* **1977**, *20*, 791.
(4) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* **1980**, *23*, 1358.

0022-2623/86/1829-0394$01.50/0

Name	Number	R_1	R_2	R_6	R_7	R_8	Reference
Oxolinic acid	1a	Et	-	-O-CH_2-O-		H	1
Miloxacin	1b	OCH_3	-	-O-CH_2-O-		H	3
Norfloxacin	1c	Et	-	F	HN⌒N-	H	4
Pefloxacin	1d	Et	-	F	CH_3N⌒N-	H	4
Amifloxacin	1e	$NHCH_3$	-	F	CH_3N⌒N-	H	5
Ciprofloxacin	1f		-	F	HN⌒N-	H	6
Rosoxacin	1g	Et	-	H		H	7
AM-833	1h	CH_2CH_2F	-	F	CH_3N⌒N-	F	8
Nalidixic acid	2a	Et	-	H	CH_3	-	1
Enoxacin	2b	Et	-	F	HN⌒N-	-	9
Piromidic acid	3a	Et		-	-	-	10
Pipemidic acid	3b	Et	HN⌒N-	-	-	-	10



Ofloxacin[11] 4



Cinoxacin[1] 5

Figure 1. Clinically significant quinolone type antibacterials.

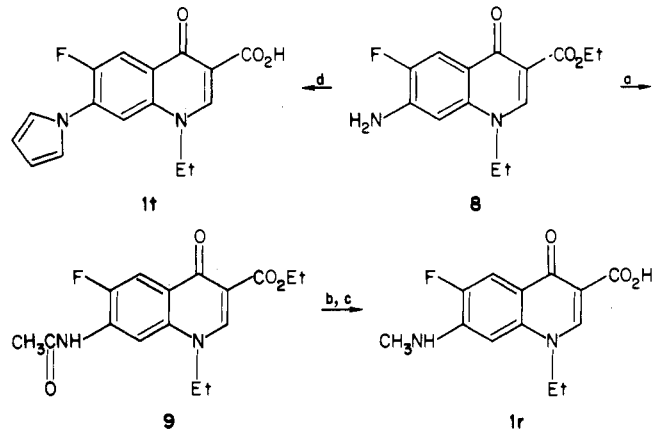
This enzyme controls the shape of bacterial DNA through its unique supercoiling and relaxing activities. Exactly how the inhibition of DNA gyrase leads to the death of the bacterial cell has recently been explored.[13] Many excellent reviews concerning DNA gyrase and supercoiling have been published.[14]

Table I. Previously Described Quinolone and Naphthyridine Antibacterials Used in This Study[a]

compd no.	R_6	R_7	R_8	lit. ref
1i	H	HN⌒N—	H	4
1j	F	H	H	1
1k	NO_2	H	H	1
1l	F	Cl	H	4
1m	F	F	F	23
1n	F	CH_3	H	4
1o	F		H	4
1p	F	HN⌒N—	F	23
1q	F	CH_3N⌒N—	F	23
2c	H	HN⌒N—		9
2d	F			9
2e	F	$H_2NCH_2CH_2NH$—		9
2f	H	HON=CH—		20
2g	H	CH_3N⌒N—N=CH—		20
2h	F			9
2i	F	EtO_2C—N⌒N—		26

[a] All compounds prepared by these referenced procedures had correct C, H, N analyses and supporting spectral data.

Scheme I[a]

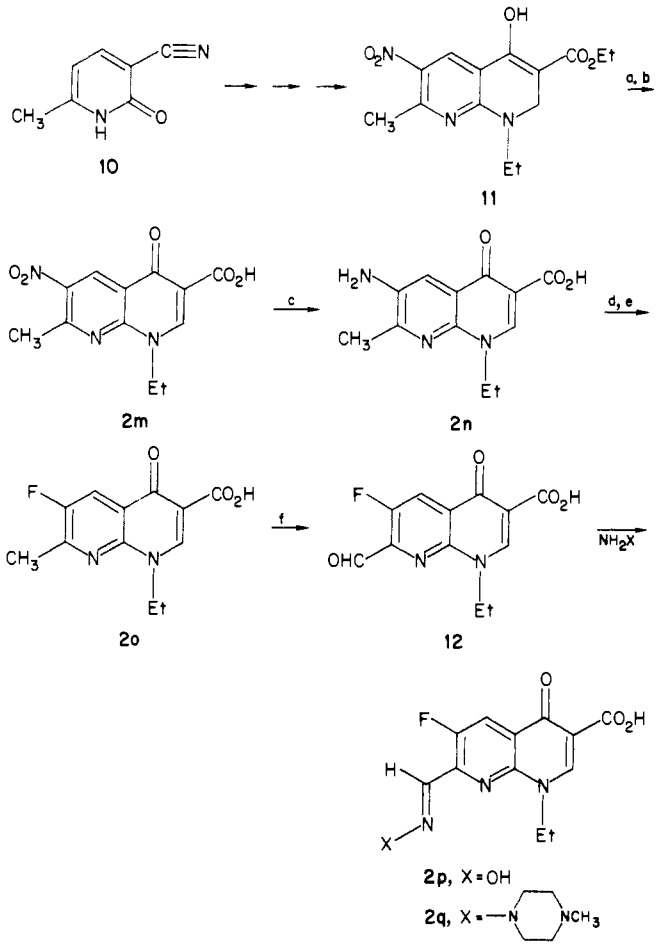


[a] (a) Ac_2O, AcOH, (b) NaH, CH_3I, (c) NaOH, (d) 1,4-dimethoxytetrahydrofuran.

While the details of the inhibition of DNA gyrase have been extensively studied with oxolinic and nalidixic acids, the supercoiling inhibition of only a few other members of the quinolone class has been reported.[12d-f,15] Virtually all of the structure–activity relationships reported to date on this group of antibacterials have utilized MICs against whole bacteria as the sole criterion for biological activity.[4,9,16,17]

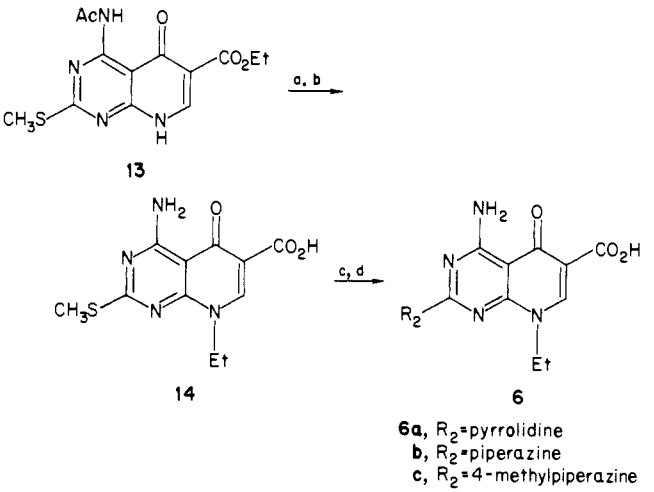
(5) Wentland, M. P.; Baily, D. M.; Cornett, J. B.; Dobson, R. A.; Powles, R. G.; Wagner, R. B. *J. Med. Chem.* **1984**, *27*, 1103.
(6) (a) Wise, R.; Andrews, J. M.; Edwards, L. J. *Antimicrob. Agents Chemother.* **1983**, *23*, 559. (b) Grohe, K.; Zeiler, H.; Metzger, K. *European Patent* 0078362, 1983; *Chem. Abstr.* **1983**, *99*, 53790h.
(7) Lesher, G. Y.; Carabateas, P. M. U.S. Patent 3907808, 1975; Chem. Abstr. **1973**, *78*, 84280n.
(8) Hirai, K.; Hosaka, M.; Oomori, Y.; Murayama, S.; Ito, A.; Takagi, K.; Irikura, T.; Mitsuhashi, S.: see ref 2a, Abstr 658.
(9) Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H.; Nishimura, H. *J. Med. Chem.* **1984**, *27*, 292.
(10) (a) Matsumoto, J.; Minami, S. *J. Med. Chem.* **1975**, *18*, 74. (b) Pesson, M.; Antoine, M.; Chabassier, S.; Geiger, S.; Girard, P.; Richer, D.; deLajudie, P.; Horvath, E.; Leriche, B.; Patte, S. *Eur. J. Med. Chem.* **1974**, *9*, 585, 591.
(11) (a) Hayakawa, I.; Hiramitsu, T.; Tanaka, Y. European Patent 0047005, 1982; Chem. Abstr. **1982**, *97*, 55821b. (b) Sato, K.; Matsuura, Y.; Inoue, M.; Une, T.; Osada, Y.; Ogawa, H.; Mitsuhashi, S. *Antimicrob. Agents Chemother.* **1982**, *22*, 548.
(12) (a) Sugino, A.; Peebles, C. L.; Kreuzer, K. N.; Cozzarelli, N. R. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 4767. (b) Crumplin, G. C.; Midgley, J. M.; Smith, J. T. "Topics in Antibiotic Chemistry"; Sammes, P. G., Ed.; Ellis Horwood: West Sussex, England, 1980; Vol. 3, pp 11–38. (c) Gellert, M.; Mizuuchi, K.; O'Dea, M. H.; Itoh, T.; Tomizawa, J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 4772. (d) Morita, J.; Kounosuke, W.; Komano, T. *Agric. Biol. Chem.* **1984**, *48*, 663. (e) Yamagishi, J.; Furutani, Y.; Inoue, S.; Ohue, T.; Nakamura, S.; Shimizu, M. *J. Bacteriol.* **1981**, *148*, 450. (f) Hogberg, T.; Khanna, I.; Drake, S. D.; Mitscher, L. A.; Shen, L. L. *J. Med. Chem.* **1984**, *27*, 306.
(13) Crumplin, G. C.; Kenwright, M.; Hirst, T. *J. Antimicrob. Chemother.* **1984**, *13*, Suppl. B, 9.
(14) (a) Cozzarelli, N. R. *Science* **1980**, *207*, 953. (b) Peebles, C. L.; Higgins, N. P.; Kreuzer, K. N.; Morrison, A.; Brown, P. O.; Sugino, A.; Cozzarelli, N. R. *Cold Spring Harbor Symp. Quant. Biol.* **1978**, *43*, 41. (c) Gellert, M.; Mizuuchi, K.; O'Dea, M. H.; Ohmori, H.; Tomizawa, J. *Ibid.* **1979**, *43*, 35.
(15) (a) Hooper, D. C.; Wolfson, J. S.; Tung, C.; Swartz, M. N.: see ref 2a, Abstr 1021. (b) Domagala, J. M.; Nichols, J.; Mich, T. F.: see ref 2b, Abstr 80.
(16) Koga, H. *Kagaku no Ryoiki, Zokan* **1982**, *136*, 177.
(17) Egawa, H.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Okada, H.; Uno, H.; Matsumoto, J. *J. Med. Chem.* **1984**, *27*, 1543.

Scheme II[a]



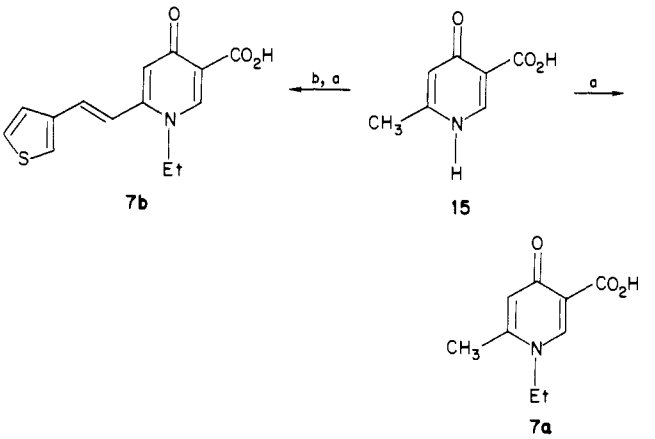
[a] (a) DDQ, (b) HCl, (c) H_2, Pd/C, AcOH, (d) $NaNO_2$, HBF_4, (e) 120 °C, (f) SeO_2, 175 °C.

Scheme III[a]



[a] (a) DMF, EtI, K_2CO_3, (b) EtOH, NaOH, (c) mCPBA, (d) R_2 nucleophile.

Scheme IV[a]



[a] (a) EtI phase transfer, (b) several steps, ref 26.

During our involvement in this area, we questioned whether the biological activity of the quinolones might not be controlled by at least two variables. The first is the inhibition of DNA gyrase and its essential ability to supercoil relaxed bacterial DNA. The second variable we considered involved the ability of these drugs to penetrate the bacterial cell wall and/or to subsequently lead to the death of the cell. Lending credence to the importance of this second variable has been the discovery of quinolone resistant factors in bacteria associated with permeability of the drug.[18] We therefore proposed that certain structural features of the quinolones are necessary for enzyme recognition and inhibition, while others may be responsible for cell penetration. In order to elucidate the existence and operation of these two variables, and to develop a more meaningful structure–activity relationship, we have studied the activity of certain quinolones utilizing both a DNA gyrase assay and traditional MICs.

In this paper we report the first complete, side-by-side comparison of the biologically significant quinolones in Figure 1 against DNA gyrase. We also report some initial structure–activity relationships for compounds selected specifically to define those features necessary for DNA gyrase activity.

Chemistry. The compounds in Figure 1 and Table I with the exception of oxolinic and nalidixic acids, and rosoxacin[19] were prepared according to literature reports. The quinolines **1w**, **1x**, and **1c′** (Table II) were prepared from norfloxacin. Likewise, **2l** was obtained from enoxacin. Many of the compounds in Table II were synthesized from the 7-chloro-6-fluoroquinoline **11** or the 7-chloro-6-fluoronaphthyridine **2** (R_6 = F, R_7 = Cl),[9] using the appropriate nucleophile to displace at C_7 (general method A described in the Experimental Section).[4,9] Compounds **1r** and **1t** were prepared from the 7-aminoquinoline **8**[4] (Scheme I). 6-Fluoronalidixic acid (**2o**) and its precursors **2m** and **2n** were obtained from the 1,2-dihydro-6-methyl-2-oxo-3-pyridinecarbonitrile (**10**) by the route in Scheme II. After nitration of **10** at C_5, the 4-pyridone-3-carboxylic acid moiety was built to form **11** by using established methodology.[1,21] The 6-fluoronalidixic acid **2o** was then oxidized to form the 7-aldehyde, which was derivatized to the oxime **2p** and hydrazone **2q**. The vinylpiperazinyl analogue of nalidixic acid **2r** was synthesized from nalidixic acid by conversion of the methyl group to a homologous enamine[22]

(18) (a) Inoue, S.; Ohue, T.; Yamagishi, J.; Nakamura, S.; Shimizu, M. *Antimicrob. Agents Chemother.* **1978**, *14*, 240. (b) Hooper, D. C.; Wolfson, J. S.; Finn, K. S.; Tung, C.; Swartz, M. N.: see ref 2b, Abstr 197.

(19) Obtained from Aldrich Chemical Co. Rosoxacin was a gift from Sterling Winthrop Co.

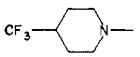
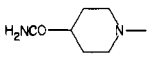
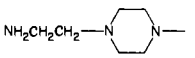
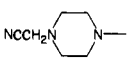
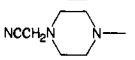
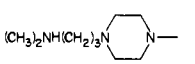
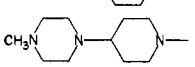
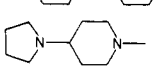
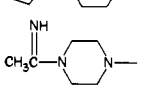
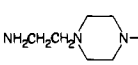
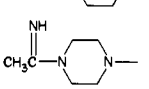
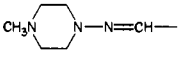
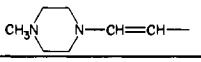
(20) Nishigaki, S.; Mizushima, N.; Senga, K. *Chem. Pharm. Bull.* **1977**, *25*, 349.

(21) Santilli, A. A.; Scotese, A. C.; Yurchenco, J. A. *J. Med. Chem.* **1975**, *18*, 1038.

(22) Rufer, C.; Kessler, H. J.; Schwarz, K. *Eur. J. Med. Chem.* **1977**, *1*, 27.

(23) Irikura, T.; Koga, H.; Murayama, S. U.S. Patent 4 398 029, 1981; Chem. Abstr. **1982**, *96*, 6607g.

Table II. Quinolone and Naphthyridine Antibacterials Prepared for This Study

compd	R_6	R_7	R_8	method of prep or prepared from[a]	mp, °C	anal.[b]	purification method[c]
1r	F	CH_3NH—	H	Scheme I	290–293	$C_{13}H_{13}FN_2O_3 \cdot 0.5H_2O$ (C, H, N)	trit MeOH
1s	F	$NH_2CH_2CH_2S$—	H	A	268–270	$C_{14}H_{15}FN_2O_3S \cdot HCl$ (C, H, N)	recryst EtOH
1t	F		H	Scheme I	215–220	$C_{16}H_{13}FN_2O_3$ (C, H, N)	recryst DMF–MeOH
1u	F		H	A	243–245	$C_{18}H_{18}F_4N_2O_3$ (C, H, N)	trit MeOH
1v	F		H	A	280–283	$C_{18}H_{20}FN_3O_4$ (C, H, N)	trit ether–MeOH
1w	F		H	norfloxacin	215–218	$C_{18}H_{23}FN_4O_3 \cdot 3H_2O$ (C, H, N, H_2O)	recryst $CHCl_3$–EtOH
1x	F		H	norfloxacin	235–237	$C_{18}H_{19}FN_4O_3 \cdot 0.25H_2O$ (C, H, N, H_2O)	recryst $CHCl_3$–EtOH
1y	H		H	1i	237–240	$C_{18}H_{20}N_4O_3 \cdot 0.15H_2O$ (C, H, N, H_2O)	recryst DMF–MeOH
1z	F		H	A	187–189	$C_{21}H_{29}FN_4O_3$ (C, H, N)	no purif
1a′	F		H	A	255–257	$C_{22}H_{29}FN_4O_3 \cdot 0.66H_2O$ (C, H, N, H_2O)	trit ether
1b′	F		H	A	259–262	$C_{21}H_{26}FN_3O_3$ (C, H, N)	trit ether
1c′	F		H	norfloxacin	306–307	$C_{18}H_{21}FN_4O_3 \cdot HCl \cdot H_2O$ (C, H, N, Cl, H_2O)	no purif
1d′	F		H	A	270–273	$C_{15}H_{15}FN_2O_3S$ (C, H, N)	recryst DMF
1e′	F		H	A	254–256	$C_{16}H_{17}FN_2O_3S$ (C, H, N)	trit H_2O–CH_3CN
2j	F			A	286–288	$C_{14}H_{14}FN_3O_3S$ (C, H, N)	recryst EtOH–H_2O
2k	F			A	267–269	$C_{17}H_{22}FN_5O_3 \cdot 3HCl \cdot H_2O$ (C, H, N, Cl)	recryst EtOH–H_2O
2l	F			enoxacin	>300	$C_{17}H_{20}FN_5O_3 \cdot HCl \cdot 1.25H_2O$ (C, H, N, Cl)	trit EtOH
2m	NO_2	CH_3		Scheme II	248–250	$C_{12}H_{11}N_3O_5$ (C, H, N)	recryst DMF–H_2O or CH_3CN
2n	NH_2	CH_3		Scheme II	298–300	$C_{12}H_{13}N_3O_3 \cdot 0.25H_2O$	recryst DMF–H_2O
2o	F	CH_3		Scheme II	248–252	$C_{12}H_{11}FN_2O_3$ (C, H, N, F)	no purif
2p	F	HON=CH—		Scheme III	258–262	$C_{12}H_{10}FN_3O_4 \cdot 0.16H_2O$ (C, H, N, H_2O)	recryst EtOH
2q	F			Scheme III	>285	$C_{17}H_{20}FN_5O_3 \cdot 1.3HCl$ (C, H, N, Cl)	recryst EtOH
2r	H			nalidixic	168–170	$C_{18}H_{22}N_4O_3$	no purif

[a] Refers to the general method or precursor used. Method A is a literature coupling method and is described in the Experimental Section. [b] Symbols refer to those elements analyzed for. Analyses were ±0.4% of theoretical values. [c] Trituration (trit) refers to grinding of the solids under solvent until a fine powder is formed. Recrystallizations (recryst) were performed by dissolving the product in the first solvent listed and using the second solvent to induce precipitation.

followed by treatment with methylpiperazine.

The 4-aminopyrido[2,3-*d*]pyrimidines (Table III) were prepared from the 2-methyl sulfide derivative **13**[24] (Scheme III) by alkylation at N_8 followed by displacement of the activated sulfide with amine nucleophiles.

The monocyclic analogues **7a** and **7b** (Scheme IV) were obtained by N-ethylation of the corresponding 4-pyridones.[25]

Biochemical Assays. Two separate assays were employed to measure the potency of the compounds against bacterial DNA gyrase obtained from *Escherichia coli* (H560). The first assay, used primarily for the standard drugs, monitors the gyrase-catalyzed conversion of relaxed plasmid DNA to its native supercoiled form. Initial inhibition and complete inhibition were readily discernible. From these two values (in micrograms/milliliter) an I_{50}

Table III. 4-Aminopyrido[2,3-*d*]pyrimidines 6 and 4-Pyridones 7

compd no.	R	mp, °C	anal.
6a		285–287	$C_{14}H_{17}N_5O_3$ (C, H, N)
6b		349–350	$C_{14}H_{18}N_6O_3$ (C, H, N)
6c		255–258	$C_{15}H_{20}N_6O_3$ (C, H, N)
7a	CH_3	188–189	$C_9H_{11}NO_3$ (C, H, N)
7b		233–235	$C_{14}H_{13}NO_3S$ (C, H, N)
7c	*p*-$CH_3OC_6H_4$—	ref 27	(C, H, N)

(24) Riskalla, B. H.; Broom, A. D. *J. Org. Chem.* **1972**, *37*, 3980.
(25) DeJohn, D.; Domagala, J. M.; Kaltenbronn, J. S.; Krolls, U. J. *J. Heterocycl. Chem.* **1983**, *20*, 1295.

Table IV. Biological Testing Results from the Gyrase Induced DNA-Cleavage Assay, Supercoiling Inhibition Assay, and Antibacterial Screening

compd no.	gyrase cleavage,[a] μg/mL	gyrase 50% inhibn,[b] μg/mL	antibacterial activity (MICs),[c,d] μg/mL					
			E. coli		*K. pneum.*	*P. aerug.*	*S. aureus*	*S. pneum.*
			H560	vogel	MGH-2	UI-18	H228	SV-1
1a (oxolinic)	10	25	0.2	0.2	0.2	6.3	1.6	100
1b (miloxacin)	10	50	0.4	0.4	0.2	12.5	6.3	100
1c (norfloxacin)	1	5.5	0.1	0.025	0.05	0.2	0.8	1.6
1d (pefloxacin)	1	5.5	0.1	0.025	0.05	0.4	0.2	0.8
1e (amifloxacin)	2.5	6.3	0.025	0.1	0.2	0.8	1.6	12.5
1f (ciprofloxacin)	0.5	5.3	0.025	0.05	0.1	0.4	3.1	1.6
1g (rosoxazin)	2.5	>10	0.2	0.2	0.4	6.3	0.4	12.5
1h (AM-833)	2.5	3.8	0.1	0.2	0.2	1.6	0.8	6.3
2a (nalidixic)	50	>100	6.3	6.3	6.3	>100	>100	>100
2b (enoxacin)	5	28	0.1	0.1	0.1	0.8	3.1	3.1
3a (piromidic)	38	>100	50	25	25	>100	12.5	>100
3b (pipemidic)	50	>100	3.1	1.6	6.3	6.3	50	>100
4 (ofloxacin)	5	6.3	0.1	0.1	0.1	0.4	0.4	0.8
5 (cinoxacin)	43	>100	50	3.1	1.6	>100	>100	>100
1i	18		6.3	6.3	6.3	25	>50	50
1j	50		25	0.8	1.6	>100	>100	>100
1k	>100		50	12.5	12.5	>100	>100	100
1l	38		1.6	0.8	1.6	50	3.1	100
1m	38		6.3	1.6	6.3	>100	50	>100
1n	25		0.4	0.2	0.2	12.5	1.6	100
1o	5	26	1.6	1.6	>100	>100	0.8	12.5
1p	1	6.3	0.1	0.2	0.1	1.6	3.1	3.1
1q	2.5		0.1	0.2	0.4	0.8	0.4	3.1
1r	18		3.1	3.1	6.3	>100	12.5	100
1s	5.0		3.1	0.8	1.6	3.1	25.0	25.0
1t	2.5	28	1.6	1.6	1.6	12.5	0.4	12.5
1u	5		6.3	6.3	>100	>100	0.1	6.3
1v	7.5		25	6.3	25	50	3.1	6.3
1w	10		25	25	50	50	12.5	50
1x	10		0.8	0.4	0.8	6.3	1.6	6.3
1y	18		50	50	>100	100	25	>100
1z	50		3.1	6.3	6.3	12.5	12.5	25
1a′	50		12.5	3.1	12.5	50	1.6	6.3
1b′	7.5		0.1	0.4	1.6	3.1	3.1	12.5
1c′	2.5		0.2	0.8	1.6	1.6	6.3	12.5
1d′	5		0.2	0.2	0.4	3.1	0.2	12.5
1e′	5		0.1	0.1	0.2	0.8	0.006	12.5
2c	75		3.1	6.3	12.5	12.5	50	50
2d	18		1.6	1.6	3.1	6.3	0.2	50
2e	50		25	12.5	25	50	>100	>100
2f	100		>100	12.5	12.5	100	50	100
2g	100		12.5	12.5	12.5	>100	12.5	100
2h	2.5	5.0	0.4	3.1	3.1	6.3	0.2	3.1
2i	18		50.0	25.0	50.0	200	3.1	100
2j	5	65	0.4	0.1	0.4	1.6	0.05	1.6
2k	6.3		12.5	12.5	25	50	50	12.5
2l	6.3		0.8	3.1	6.3	25	50	50
2m	50		50	25	50	>100	25	>100
2n	>100		100	>100	>100	>100	50	50
2o	25		3.1	3.1	6.3	50	25	>100
2p	50		>100	6.3	12.5	100	100	100
2q	100		50	25	25	>100	25	100
2r	>100		>100	>100	>100	>100	>100	>100
6a	100		100	100	>100	>100	100	25
6b	100		3.1	6.3	25	25	50	50
6c	100		3.1	1.6	6.3	100	100	100
7a	100		>100	>100	>100	>100	>100	>100
7b	100		50	25	>100	>100	>100	>100
7c	100		>100	50	50	>100	>100	>100

[a] Refers to the minimum concentration at which cleaved DNA (linear) is observed at an intensity relative to oxolinic acid at 10 μg/mL. [b] Refers to the concentration of drug that inhibits gyrase ~50% as determined by the initial inhibition + 100% inhibition/2. [c] Using standard microdilution techniques; see ref 28. [d] All results are averaged from duplicate or triplicate runs and are ± one serial dilution.

(50% inhibition in micrograms/milliliter) was estimated. The second assay, employed routinely for all compounds, utilized commercially available supercoiled plasmid DNA as substrate. This assay is specific for the quinolone family of gyrase inhibitors[12a] (as opposed to the coumarins) and it monitors the appearance of linear DNA formed from a denaturing of the drug–gyrase–DNA complex. The minimum concentration (micrograms/milliliter) of drug necessary to display this linear band (relative to oxolinic acid at 10 μg/mL) was recorded as the gyrase cleavage value listed in Table IV. The relaxed, linear, and supercoiled DNA are easily resolved and visualized by agarose gel electrophoresis with ethidium bromide stain. All the gyrase results are obtained by serial dilution initially beginning at 200 μg/mL and are accurate to ± one dilution. Repeat experiments on all active compounds begin at 20

μg/mL. results recorded in Table IV are the average of these multiple runs. The MIC values (micrograms/milliliter) were obtained by using standard microtitration techniques.[28]

Results and Discussion

All the biological results are given in Table IV. Since this is the first time that a group of compounds has been compared side-by-side using gyrase assays and MICs, the first three columns in Table IV are especially important and deserve further explanation.

The gyrase cleavage value represents a "thermodynamic" value reflecting the amount of the drug–gyrase–DNA complex present at equilibrium. Cleavage of DNA does not occur for gyrase–DNA complex where drug is not bound. Thus, the value of 0.5 μg/mL obtained for ciprofloxacin (**1f**) is interpreted to mean that at 0.5 μg/mL the same amount of drug–gyrase–DNA complex is present for ciprofloxacin as would be present for oxolinic acid at 10 μg/mL or nalidixic acid at 50 μg/mL. Whether the drug is bound to the gyrase, the DNA, or both in this complex is unknown,[29] but upon quenching with sodium dodecyl sulfate (SDS), whatever proportion of drug–complex is present is visualized as cleaved DNA. The cleavage values and I_{50} values in Table IV do correlate with the MICs vs. *E. coli* H560. This correlation was examined quantitatively. Those compounds with incomplete titrations (>100 μg/mL) were assigned the MIC value of 100 μg/mL. Considering the entire set in this manner, a modest but significant correlation was measured between the molar or log molar concentrations of the gyrase cleavage value and the *E. coli* H560 MIC ($N = 59$, $r^2 = 0.66$, $s = 0.67$, $F = 109$ for the latter). No differences were observed when quinolines and naphthyridines were treated separately.

The gyrase I_{50} values represent "kinetic" parameters and are related to how the drug actually inhibits the supercoiling process. Here the ability of the drug–gyrase–DNA complex to prevent or retard the supercoiling reaction is measured. As seen in Table IV, there is a high correlation between the gyrase cleavage values and the I_{50} values ($N = 14$, $r^2 = 0.94$, $s = 5$, $F = 103$), but with certain notable exceptions.

Enoxacin (**2b**), for example, has a cleavage value of 5 μg/mL but an I_{50} of 27.5, while ofloxacin (4), with an identical cleavage value, has an I_{50} of 6.3. Ofloxacin's I_{50} value is in line with those obtained for quinolones that have low cleavage values. Keeping in mind that the I_{50} is determined by the concentrations of drug that give initial inhibition and complete inhibition, the high I_{50} values for enoxacin and other quinolones can be understood. Occasionally a drug, as enoxacin, while showing a low concentration for initial inhibition (and thereby for the cleavage assay as well), has difficulty inhibiting the supercoiling reaction completely. This supercoiling "leakage" raises the 100% inhibition concentration and thus the I_{50}. Our assay employs relaxed Col E1 plasmid, which has multiple gyrase binding sites. It is possible that enoxacin inhibits the primary site readily, while supercoiling continues (less efficiently) at the other available sites. It is not known if this observation has biological significance in vivo. Further speculation is beyond the scope of this paper. However, the current theory is that even minor gyrase perturbation will seriously impair cell function.[13] Almost without exception (based on data not shown), the gyrase cleavage value (Table IV) for a given drug is equal (±2.5 μg/mL) to the initial inhibition concentration. Therefore the I_{50} values, which are influenced most by the higher concentrations required to stop supercoiling, really reflect the ability of the drug to inhibit supercoiling 100%. Thus ciprofloxacin, with a low cleavage value of 0.5 μg/mL, stops supercoiling completely at 10 μg/mL ($I_{50} = 5.3$), while AM833, with a cleavage value of 2.5 μg/mL, inhibits supercoiling completely at 5 μg/mL and has a lower I_{50} of 3.8 μg/mL.

Another significant point involves the relationship between gyrase inhibition and MIC. Results in Table IV clearly indicate that the gyrase inhibition and the MIC's are not directly proportional. For example, norfloxacin (**1c**) is a 10-fold more potent gyrase inhibitor than oxolinic acid (using cleavage assay), while its MIC against *E. coli* is only 2-fold improved. Ciprofloxacin is 20-fold more potent than oxolinic, while its MIC shows only an 8-fold improvement. While the gyrase results and the MICs both show ciprofloxacin > norfloxacin > oxolinic acid, the magnitudes of the differences are not close. This MIC leveling effect could be the result of different cell permeabilities or other penetration phenomena. Thus, enoxacin (**2b**), which is clearly a less potent gyrase inhibitor than norfloxacin (**1c**) by either assay, must be able to penetrate the cell with greater efficacy in order to have MICs so comparable to those of norfloxacin. As already mentioned, even quinolones with moderate gyrase inhibition, when in the cell, can inhibit cell growth, albeit, not as efficiently as the more potent inhibitors.[2b,13]

Ignoring the absolute gyrase numbers, and concentrating instead on ranges of values, an excellent correlation of gyrase inhibition and MIC can be observed. Of the 14 standard quinolones, those with drug-induced cleavage values > 10 μg/mL are weak inhibitors of the enzyme, have I_{50} values > 50 μg/mL, MICs ≥ 3.1 vs. *E. coli*, and are usually very poor against the other strains as well. These compounds are cinoxacin, pipemidic, piromidic, and nalidixic acids. Those compounds with drug-induced cleavage values from 6 to 10 μg/mL are good inhibitors. They have I_{50} values from 25 to 50 μg/mL and show good MICs (0.2–1.6 μg/mL) against a majority of strains. Oxolinic acid and miloxacin fall into this range. The best quinolones are those with cleavage values ≤ 5 μg/mL. These show (with the exception of enoxacin) I_{50} values of ≤6.3 μg/mL and excellent MIC activity with a majority of organisms inhibited at ≤0.4 μg/mL.

With use of the drug-induced cleavage values and the *E. coli* H560 MICs, the remaining quinolones can be ranked as follows: ciprofloxacin > norfloxacin, pefloxacin, amifloxacin > AM833, rosoxacin > ofloxacin and enoxacin. If the I_{50} values are used, the ordering of the drugs, except for enoxacin, is the same within experimental error. It would seem that the ideal inhibitor should have both low cleavage and I_{50} values, but it appears that ample antibacterial effect is obtained with those compounds possessing low cleavage values alone.

It is clear that the best inhibitors are without exception fluorinated quinolines or naphthyridines with a piperazine at the C_7 position. The activity of pipemidic acid proves that the piperazine moiety by itself does not make a potent drug. Three important questions therefore need to be addressed. What is the significance of the C_6-fluoro group? What is the value of the piperazine group, and its rela-

(26) Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H.; Nishimura, H. *J. Heterocycl. Chem.* **1984**, *21*, 673.
(27) Wick, A. E. UK Patent Application G.B. 2,013,190A, 1979; *Chem. Abstr.* **1979**, *91*, 211273h.
(28) Heifetz, C. L.; Chodubski, J. A.; Pearson, I. A.; Silverman, C. A.; Fisher, M. W. *Antimicrob. Agents Chemother.* **1974**, *6*, 124.
(29) Shen, L. L.; Pernet, A. G. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 307.

tionship to the C_6-fluoro, that has led so many medicinal chemists to this common endpoint? And finally, why does enoxacin, a naphthyridine, behave differently than the related quinolines? With these issues in mind and using the ranges of activity established from the analysis of the standard quinolones, the synthetic analogues can now be discussed. In most cases, the gyrase cleavage assay data will be discussed rather than I_{50} values, because of the ease of this assay, and as shown above, because of the good correlation of these data with MICs.

The Effect of the C_6-Fluorine. The effect of the C_6-fluorine is dramatically seen by comparison of the desfluoronorfoxacin **1i** and the desfluoroenoxacin **2c** with the parent drugs (Table IV). Norfloxacin is 17.5 times more potent in the gyrase cleavage assay than the desfluoro derivative **1i** and 63 times more active in the MIC vs. *E. coli* H560. This tremendous boost in activity is seen in other strains as well. Norfloxacin is 126 times more active than **1i** vs. *Klebsiella* and 125 times more active vs. *Pseudomonas*. This increase in MIC activity might be viewed as an increase in two components. A 18-fold improvement in the drug–gyrase–DNA complex binding and a 3.5-fold (63 ÷ 18) increase in cell penetration. A similar enhancement is repeated when comparing enoxacin with the desfluoro naphthyridine **2c**. In this case the gyrase activity is improved 15 times and the MIC 31-fold. Again, the C_6-fluoro group appears to impart an equally striking improvement in enzyme-complex binding, with a lesser but significant improvement in cell penetration. However, not every compound shows such an enhancement. Fluoronalidixic acid **2o** is only 2-fold more active than nalidixic in the gyrase assay and the MIC is only 2-fold improved, implying no improvement in cell penetration or growth inhibition beyond that derived from the gyrase itself. Similar results are observed with the oximes **2f** and **2p**. A comparison of the (cyanomethyl)piperazines **1y** and **1x** show that the 6-fluoro in **1x** provides only a 1.75-fold improvement in the gyrase activity but a 62.5-fold improvement in MIC, implying a 35-fold increase in penetration and growth inhibition efficiency. Thus, the 6-fluorine appears to improve both gyrase complex binding (2–17-fold) and cell penetration (1–70-fold), with each factor weighted depending on the nature of the C_7 substituent with an optimum for the combined factors when piperazine is the C_7 group. One would expect the C_6-fluoro group to be beneficial in almost any quinolone drug synthesized. Other groups at C_6 were examined such as in the 6-nitro and the 6-aminonalidixic acids **2m** and **2n**, which showed very poor gyrase and MIC activities.

Nature of the C_7 Substituent. To discuss the nature of the C_7 substituent, we have divided the compounds to be compared into three groups. The first group contains quinolines and naphthyridines with small or linear-like C_7 substituents such as H (**1j**), Cl (**1l**), CH_3 (**1n**, **2o**), $NH_2CH_2CH_2NH$ (**2e**), CH_3NH (**1r**), $NH_2CH_2CH_2S$ (**1s**), HON=CH (**2p**), the hydrazone **2q**, and vinylpiperazine **2r** (Table IV). In all of these examples, only **1s** and **1r** have gyrase complex binding values of ≤18. Compound **1s** with a cleavage value of 5 μg/mL would be expected to have MICs in the ≤0.4 range but instead has MICs ≥ 0.8 with an MIC of 3.1 μg/mL vs. *E. coli* H560. It would appear that all the small substituents lack appreciable binding to the gyrase–DNA complex and also possess correspondingly inferior MICs. It should be pointed out that compounds **1l** and **1n** have slightly better MICs than their gyrase inhibition values would have led one to predict. This could be due to increased cell penetration or possibly some other bacterial action. It is interesting to note that many of these derivatives with very simple C_7 side chains (or with none at all as **1j**) are still more active than some of the early compounds like nalidixic or pipemidic acids.

The second set of C_7 substituents constitute the medium-size groups of five- and six-membered rings with no substitution. The groups considered here are the pyrrolidines (**1o**, **2h**), pyrrole (**1t**), thiazolidines (**1d′**, **2j**), thiomorpholine (**1e′**), and the piperazines (norfloxacin and enoxacin). All of these compounds have gyrase cleavage values of ≤5 μg/mL with the piperazine in norfloxacin being an optimum at 1.0 μg/mL. A striking feature, however, is that the quinolones **1o** (pyrrolidine) and **1t** (pyrrole) show poor MICs relative to their good gyrase cleavage numbers. Their I_{50} values are equal to enoxacin's but they obviously lack the "cell permeability variable" operating for enoxacin. Meanwhile, compounds **2h**, **1d′**, **2j**, and especially **1e′** (thiomorpholine) show very good broad-spectrum activity. Note that **2j** has a high I_{50} but good MICs just as enoxacin. The gyrase–DNA complex obviously is cleaved much more effectively by derivatives with five- and six-membered rings than any of the linear or small groups previously examined. Oddly enough, the piperazine is not required for potent enzyme activity. Although outside the scope of this paper, it has been reported and observed in our laboratories that the piperazines often do show superior in vivo activity when compared to other ring systems. Yet it is still very important to realize that the most essential element of activity, enzyme inhibition, tolerates a reasonable variety of structure at C_7 with the piperazine ring being only one of the possible five- or six-membered rings acceptable. The obvious question to explore is how big can the C_7 substituent become before gyrase complex binding is negated? Any compound with a C_7 substituent larger than a five- or six-membered ring is considered in this third group of comparisons. Derivatives **1c′** and **2l** ((acetylamidino)-piperazines), **1u** ((trifluoromethyl)piperidine), and **1v** ((aminocarbonyl)piperidine) show gyrase cleavage values of 2.5–7.5 μg/mL, with correlatable MICs. These compounds represent a set of small ring substituents of two atoms or less in length. When the ring substituents grow larger (three atoms in length) as in **1w** and **2k** ((aminoethyl)piperazines), **1x** ((cyanomethyl)piperazine), and **1b′** (pyrrolidinopiperidine), the gyrase cleavage range tends to increase to 6.3–10 μg/mL. When longer substituents are attached (> three atoms) as in **1z**, **1a′**, and **2i**, the gyrase cleavage values fall off dramatically to 18–50 μg/mL.

The combined data strongly suggest that linear or small substituents, and larger groups (rings with atom chains > three), possess moderate to weak gyrase inhibition and low MICs, while five- or six-membered rings by themselves or with small substituents have very good gyrase–DNA complex binding and have good to excellent MICs as well. The kind of substituent on the ring does not profoundly influence the activity if the size requirements are met. These substituents need not be basic as one might have suspected from all the published derivatives containing piperazine. These results clearly point to a size optimum that parallels the findings of other workers.[1,4,16,17] However our gyrase results show that certain rings, such as pyrrolidine and pyrrole, **1o** and **1t**, which would not look favorable based on MIC alone, are still viable analogues if improvements in their cell permeabilities could be fashioned. Indeed, with use of gyrase activity as a guide, there appears to be much more structural flexibility at C_7 than was otherwise suspected.

Naphthyridines vs. Quinolines. One question that has never been addressed in the literature is which of the two major ring classes, quinoline or naphthyridine, provide the best activity? Unfortunately our data do not definitively answer this question because the better ring system seems to depend on the nature of the C_7 substituent. Comparison of norfloxacin and enoxacin described earlier shows the quinoline to be a 5-fold better gyrase inhibitor than the naphthyridine. The quinoline is also ~3-fold superior when comparing **1e′** and **2d** (thiomorpholine at C_7) or **1c′** and **2l** ((acetylamidino)piperazine at C_7). In all cases the MICs of the quinolines reflect the better enzyme inhibition. The trend breaks down however when **1n** and **2o** (methyl at C_7), **1o** and **2h** (pyrrolidine at C_7), and **1d′** and **2j** (thiazolidine at C_7) are compared. It would appear that, for any new C_7 substituent, both the quinoline and naphthyridine version would have to be synthesized in order to identify the preferred derivative.

Relative to the 6-fluoroquinolines or 6-fluoro-1,8-naphthyridines the pyrido[2,3-*d*]pyrimidines (pipemidic acid compared to norfloxacin or enoxacin) are substantially less active, with a gyrase cleavage value of 50 μg/mL and an I_{50} > 100 μg/mL. When pipemidic acid is compared to the nonfluorinated versions of norfloxacin and enoxacin, **1i** and **2c**, it is more active than the naphthyridine **2c** (50 vs. 75 μg/mL) but less active than the quinoline **1i** (17.5 μg/mL). In this desfluoro series with piperazine as the side chain, the quinoline **1i** is still the more active parent ring, but the pyrido[2,3-*d*]pyrimidine is slightly better than the naphthyridine **2c**. Insertion of the nitrogen in position 6 of **2c** to produce pipemidic acid is favorable by a factor of 1.5, but addition of a 6-fluoro is 10-fold better, rendering the pyridopyrimidines obsolete.

Other Factors. Two interesting derivatives with an additional C_8-fluorine were examined. These represent a difluoronorfloxacin (**1p**) and a difluoropefloxacin (**1q**). Both difluoro derivatives possess substantially less MIC activity against Gram-positive organisms. The gyrase activity of these two compounds was essentially equal to their monofluoro parents. The greater MIC, once again, could be attributable to lesser cell penetration.

Recurrent reports in the literature have suggested that a 5-amino group in oxolinic acid[1] or in ofloxacin[30] are beneficial for activity. We decided to extend this concept to the very weakly active pyrido[2,3-*d*]pyrimidines **3**. These 4-aminopyrido[2,3-*d*]pyrimidines **6a–c** were all inactive in the gyrase assay. The amino group in these derivatives has a deleterious effect, apparently on the gyrase directly.

One final issue to be determined is whether or not certain monocylic 4-pyridone-3-carboxylic acids, which were claimed as antibacterials in the patent literature,[27,31] were gyrase inhibitors. The known compound **7c**[27] and the derivatives **7a** and **7b** were synthesized and screened. Very little if any activity was observed. Several other compounds related to **7c**[27] were tested with identical results. Other researchers have claimed that analogues of **7c**, where aromatic N_1 substituents were employed instead of ethyl, possess good antibacterial activity, but the mechanism of inhibition was not disclosed.[31]

In summary, we have shown that DNA gyrase inhibition data collected for many compounds, combined with MICs, provides a much more powerful understanding of the structure–activity relationships among the quinolone antibacterials. The data strongly suggest that the activity of the quinolones is determined not only by their intrinsic inhibition of DNA gyrase but also by their ability to penetrate the bacterial cell and/or inhibit cell growth through their action on DNA gyrase. A better understanding of this latter variable is under further study. For the first time, the great enhancement in activity produced by the 6-fluorine, now common to all of the best quinolones, has been shown to be caused by a simultaneous increase in enzyme inhibition and the "cell penetration variable". Our examination of the C_7 substituents has indicated that the structural variety of groups at this position, which are tolerated and effective against DNA gyrase, may be much greater than predicted on the basis of an MIC strategy. The DNA gyrase also showed tolerance to changes at the C_8 position, but the extent of this will require further investigation. An exploration of other features of the quinolone antibacterials is in progress.

Experimental Section

Melting points were taken on a Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were determined on a Nicolet FT IR SX-20 with 2-cm^{-1} resolution. Proton magnetic resonance (NMR) spectra were recorded on either a Varian XL 200 or IBM 100 WP100SY spectrometer. Chemical shifts are reported in δ units relative to internal tetramethylsilane. Mass spectra were recorded on either a Finnigan 4500 GCMS or a VG Analytical 7070E/HF with an 11/250 Data System. Column chromatography was performed with E. Merck silica gel 60, 70–230-mesh ASTM. All the amines used were commercially available except where indicated. Solutions were dried over magnesium sulfate. Elemental analyses were performed on a Perkin-Elmer 240 elemental analyzer. All new products and intermediates had analytical results within ±0.4% of theoretical values. Spectral data has been provided only for those compounds not prepared by well-established literature conditions. The structures of all compounds were consistent with their spectral properties.

General Method A. With use of established literature conditions,[1,4,5,17] the appropriate amine was heated with the desired quinoline 1 or naphthyridine 2 (where R_7 = halogen) and base. Where noted, base was excluded if the side-chain amine was dibasic. The products were isolated by concentrating the solvent or by filtrating the reaction directly. The solids were then purified according to Table II.

7-[(2-Aminoethyl)thio]-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid Hydrochloride (1s). With use of a modified version of method A, a suspension of 0.72 g (15 mmol) of 50% sodium hydride–mineral oil in 10 mL of DMF was treated with 1.34 g (15 mmol) of thiazolidine. When gas evolution had ceased, the mixture was treated with 0.81 g (3.0 mmol) of **1l**. This mixture was stirred at room temperature for 1 h. It was concentrated and the residue dissolved in H_2O. The pH was adjusted to 7.2 and the precipitate was filtered, washed with water, redissolved in HCl at pH 2, and lyophilized to give, after purification, 0.5 g (48%) of **1s**: NMR (TFA) δ 9.3 (s, 1 H, C_2H), 8.25 (d, J = 11 Hz, 1 H, C_5H), 8.1 (d, J = 6 Hz, 1 H, C_8H), 7.2 (br s, 2 H, NH_2), 4.9 (q, J = 7 Hz, 2 H, CH_2CH_3), 3.7 (s, 4 H, $NH_2CH_2CH_2S$), 2.8 (t, J = 7 Hz, 3 H, CH_2CH_3).

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-(trifluoromethyl)-1-piperidinyl]-3-quinolinecarboxylic Acid (1u). Following the general method A, 1.00 g (3.8 mmol) of 1 (R_6 = R_7 = F, R_8 = H, R_1 = Et)[32] was reacted with 0.83 g (5.4 mmol) of 4-(trifluoromethyl)piperidine[33] with 1.0 equiv of DBU as base in acetonitrile solvent. Direct filtration and purification gave 0.67 g (44%) of **1u**.

7-[4-(Aminocarbonyl)-1-piperidinyl]-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (1v). With use of

(30) Japanese Patent 57 149 286, 1982; *Chem. Abstr.* **1983**, *98*, 72117q.

(31) Narita, H.; Konishi, Y.; Nitta, J.; Misumi, S.; Nagaki, H.; Kitayama, I.; Nagai, Y.; Watanaba, Y.; Matsabara, N.; Minami, S.; Saikawa, I. GB 2,130,580A, 1984; *Chem. Abstr.* **1984**, *101*, 171101s.

(32) European Patent Appl. 0,000,203, 1979; *Chem. Abstr.* **1979**, *90*, 163334j.

(33) Raasch, M. S. *J. Org. Chem.* **1962**, *27*, 1406.

the general method A, 5.4 g (20 mmol) of **11** was reacted with 4-piperidinecarboxamide isonipecotamide in β-picoline at reflux to yield 3.91 g (54%) of **1v**.

7-[4-[3-(Dimethylamino)propyl]-1-piperazinyl]-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (1z). With use of the general method A, 1.35 g (5.0 mmol) of **11** was reacted with 4-[3-(dimethylamino)propyl]piperazine[34] in β-picoline at reflux to yield 1.01 g (50%) of **1z**.

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-(4-methyl-1-piperazinyl)-1-piperidinyl]-4-oxo-3-quinolinecarboxylic Acid (1a′). With use of the general method A, 2.70 g (10 mmol) of **11** was reacted with 1-methyl-4-(4-piperidinyl)piperazine monoacetate in β-picoline at reflux with DBU as base to give 2.52 g (61%) of **1a′**.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-(1-pyrrolidinyl)-1-piperidinyl]-3-quinolinecarboxylic Acid (1b′). With use of the general method A, 2.70 g (10 mmol) of **11** was reacted with 4-(1-pyrrolidinyl)piperidine in refluxing β-picoline with DBU as base to give 2.33 g (60%) of **1b′**.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(3-thiazolidinyl)-3-quinolinecarboxylic Acid (1d′). With use of the general procedure A, 1.01 g (4.0 mmol) of **1** ($R_6 = R_7 = F$, $R_8 = H$, $R_1 = Et$) was reacted with thiazolidine in refluxing CH_3CN–DMF (50:50) to give, after recrystallization, 0.13 g (11%) of **1d′**.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-thiomorpholinyl)-3-quinolinecarboxylic Acid (1e′). With use of the general procedure A, 1.35 g (5.33 mmol) of **1** ($R_6 = R_7 = F$, $R_8 = H$, $R_2 = Et$) was reacted with thiomorpholine in refluxing CH_3CN–DMF (2:1) to give 1.18 g (65%) of **1e′**.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(3-thiazolidinyl)-1,8-naphthyridine-3-carboxylic Acid (2j). With use of the general procedure A, 0.5 g (1.8 mmol) of **2** ($R_6 = F$, $R_7 = Cl$) was reacted with thiazolidine in DMF at 25 °C with Et_3N as base. The mixture was concentrated after 18 h and the residue dissolved in water. The pH was adjusted to 2.0 and the solution filtered to clarify. The filtrate was lyophilized and the powder purified to give 170 mg (29%) of **2j**.

7-[4-(2-Aminoethyl)-1-piperazinyl]-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (2k). With use of the general method A, 1.0 g (3.7 mmol) of **2** ($R_6 = F$, $R_7 = Cl$) was reacted with 1-(aminoethyl)piperazine in CH_3CN at 25 °C. The solids were isolated, dissolved in water at pH 10, and filtered, and the pH was adjusted to 2.0. The solution was lyophilized and the powder purified to give 340 mg (19%) of **2k**.

1-Ethyl-6-fluoro-1,4-dihydro-7-(methylamino)-4-oxo-3-quinolinecarboxylic Acid (1r). To a solution of 3.2 g (10 mmol) of **9**[4] in 50 mL of DMF was added 0.72 g (15 mmol) of 50% sodium hydride oil dispersion. The reaction was heated to 50 °C for 1 h and treated with 1.25 mL (20 mmol) of CH_3I. After 3 h at 50 °C the mixture was concentrated and partitioned between H_2O–ether. To the water layer was added 8.0 g of KOH, and the mixture was refluxed for 2 h. The pH was adjusted to 5.0, and the solids were collected to give, after purification, 2.55 g (93%) of **1r**.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1*H*-pyrrol-1-yl)-3-quinolinecarboxylic Acid (1t). To 1.39 g (5.0 mmol) of **8**[4] was added 1.35 mL (10 mmol) of 2,5-dimethoxytetrahydrofuran and 25 mL of AcOH. The mixture was refluxed for 5 h. It was concentrated and the residue heated to 100 °C for 1 h with 25 mL of 1 N NaOH. The mixture was cooled and the pH adjusted to 4.5. The solids were collected and gave, after purification, 0.59 g (39%) of **1t**.

7-[4-(Cyanomethyl)-1-piperazinyl]-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (1x). To 3.19 g (10 mmol) of **1c** in 40 mL of DMF was added 0.83 g (11 mmol) of chloroacetonitrile and 2.8 mL (20 mmol) of Et_3N and the mixture stirred at 25 °C for 7 h. The solids were collected, dissolved in water at pH 10, and reprecipitated at pH 5.0. Purification and drying gave 2.61 g (72%) of **1x**.

7-[4-(2-Aminoethyl)-1-piperazinyl]-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (1w). A mixture of 1.8 g (5.0 mmol) of **1x**, 1.0 g (10 mmol) of Et_3N, 0.5 g of Raney cobalt, and 50 mL of *N,N*-dimethylacetamide was shaken at 100 °C in an H_2 atmosphere of 1500 psi for 5 h. The mixture was filtered, concentrated, dissolved in water at pH 9.5, and precipitated at pH 7.0 to give, after purification, 1.2 g (58%) of **1w**.

7-[4-(Cyanomethyl)-1-piperazinyl]-1-ethyl-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (1y). Following the same procedure used for **1x**, 15.05 g (50 mmol) of **1i** was converted to 12.3 g (73%) of **1y**.

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-(1-iminoethyl)-1-piperazinyl]-4-oxo-3-quinolinecarboxylic Acid (1c′). A mixture of 1.6 g (5.0 mmol) of **1c**, 0.93 g (0.75 mmol) of ethyl acetamidate hydrochloride, and 1.26 g (12.5 mmol) of Et_3N in 150 mL of EtOH was stirred at 25 °C for 72 h. The reaction was treated to a stream of anhydrous HCl gas until the mixture tested positive to moist pH paper. The precipitate was collected to give 1.8 g (87%) of **1c′**.

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-(1-iminoethyl)-1-piperazinyl]-4-oxo-1,8-naphthyridine-3-carboxylic Acid (2l). With use of the procedure for **1c′**, 0.64 g (2.0 mmol) of **2b** was converted to 0.52 g (61%) of **2l**.

1-Ethyl-1,4-dihydro-7-methyl-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (2m). To 16.0 g (52.1 mmol) of **11**[21] in 500 mL of dioxane at room temperature was added 11.8 g (52.0 mmol) of DDQ in 300 mL of dioxane. The mixture was stirred overnight and concentrated, and the residue was triturated with H_2CCl_2. The mixture was filtered and the filtrate concentrated to 18.5 g of a crude brown solid. This material was dissolved in AcOH and 60 mL of 6 N HCl was added. The reaction was refluxed for 2 h. It was concentrated, diluted with H_2CCl_2, extracted twice with H_2O, and dried. Concentration and purification gave 12.0 g (83%) of **2m** as a yellow powder: IR (KBr) 1725 cm^{-1}; NMR ($DCCl_3$ + Me_2SO-d_6) δ 9.1 (s, 1 H, Ar), 8.85 (s, 1 H, Ar), 4.5 (q, J = 7 Hz, 2 H, CH_2CH_3), 3.0 (s, 3 H, CH_3), 1.5 (t, J = 7 Hz, 3 H, CH_2CH_3).

6-Amino-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic Acid (2n). To 8.9 g (32 mmol) of **2m** was added 100 mL of AcOH and 0.4 g of 5% Pd/C. The mixture was shaken in a pressure chamber with H_2 gas at 53.5 psi for 18 h. The mixture was filtered and the filtrate concentrated and purified to give 5.0 g (65%) of **2n** as a tan powder: IR (KBr) 3353, 1708, 1615, 1480 cm^{-1}; NMR (Me_2SO-d_6) δ 15.1 (s, 1 H, OH), 8.8 (s, 1 H, C_2H), 7.6 (s, 1 H, C_5H), 5.6 (br s, 2 H, NH_2), 4.5 (q, J = 7 Hz, 2 H, CH_2CH_3), 2.45 (s, 3 H, CH_3), 1.4 (t, J = 7 Hz, 3 H, CH_2CH_3).

1-Ethyl-6-fluoro-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic Acid (2o). To 1.4 g (5.6 mmol) of **2n** in 8 mL of 50% HBF_4 was added, at −5 °C, 386 mg of $NaNO_2$ in 1 mL of H_2O. After 3 h the mixture was treated with 10 mL of ether and filtered to give 2.28 g of wet solid. This material was heated to 120 °C for 30 min under N_2. The solids were dissolved in $HCCl_3$ and extracted twice with H_2O. The organic layer was dried, concentrated, and refluxed in 20 mL of AcOH and 5.0 mL of 3 N HCl (to hydrolyze borate ester that had formed). This mixture was concentrated, dissolved in $HCCl_3$, and extracted into 1 N NaOH. The pH was adjusted to 2.5, and the solids were collected and dried to give 0.5 g (36%) of **2o** as a light yellow solid: IR (KBr) 1720, 1629, 1483 cm^{-1}; NMR (TFA) δ 9.6 (s, 1 H, C_2H), 8.6 (d, J = 9 Hz, 1 H), 5.2 (q, J = 7 Hz, 2 H, CH_2CH_3), 3.05 (d, J = 3 Hz, 3 H, CH_3), 1.85 (t, J = 7 Hz, 3 H, CH_2CH_3).

1-Ethyl-6-fluoro-7-formyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (12). To 2.0 g (8.0 mmol) of **2o**, at 175 °C, was added 1.2 g (1.5 equiv) of SeO_2 in portions. After 1 h, the mixture was cooled, treated with hot $HCCl_3$, and filtered. The filtrate was extracted with H_2O, dried, concentrated, and chromatographed (silica gel; $HCCl_3$, EtOH, AcOH, 9:0.7:0.3) to give 0.48 g (23%) of **12**: NMR (Me_2SO-d_6) δ 14.0 (s, 1 H, OH), 10.0 (s, 1 H, CHO), 9.2 (s, 1 H, C_2H), 8.6 (d, J = 12 Hz, 1 H, C_5H), 4.65 (q, J = 7 Hz, 2 H, CH_2CH_3), 1.5 (t, J = 7 Hz, 3 H, CH_2CH_3).

1-Ethyl-6-fluoro-1,4-dihydro-7-[(hydroxyimino)methyl]-4-oxo-1,8-naphthyridine-3-carboxylic Acid (2p). To a mixture of 0.2 g (0.75 mmol) of the aldehyde **12** and 0.2 g (2.8 mmol) of hydroxylamine hydrochloride in 5 mL of EtOH was added 1.0 mL of pyridine. The mixture was refluxed overnight. The solids that formed upon ice cooling were collected and purified, giving 0.18 g (86%) of **2p**: NMR (Me_2SO-d_6) δ 14.3 (s, 1 H, CO_2H), 12.4

(34) Tomcufeik, A. S.; Fabio, P. F.; Hoffman, A. M. U.S. Patent 3 331 843, 1967; *Chem. Abstr.* *67*, 90839r.

(s, 1 H, NOH), 9.2 (s, 1 H, C*H*=NOH), 8.4 (d, J = 12 Hz, 1 H, C_5H), 8.3 (s, 1 H, C_2H), 4.6 (q, J = 7 Hz, 2 H, CH_2CH_3), 1.3 (t, J = 7 Hz, 3 H, CH_2CH_3).

1-Ethyl-6-fluoro-1,4-dihydro-7-[[(4-methyl-1-piperazinyl)imino]methyl]-4-oxo-1,8-naphthyridine-3-carboxylic Acid (2q). To 0.36 g (1.36 mmol) of **12** in 10 mL of EtOH was added 0.56 g (2.73 mmol) of 4-methyl-1-piperazinamine dihydrochloride hydrate and 0.38 mL (2.73 mmol) of Et_3N. The mixture was refluxed for 1 h and stirred at room temperature overnight. The solids obtained upon cooling were collected and purified to give 0.3 g (63%) of **2q**.

1-Ethyl-1,4-dihydro-7-[2-(4-methyl-1-piperazinyl)-ethenyl]-4-oxo-1,8-naphthyridine-3-carboxylic Acid (2r). To 1.0 g (3.6 mmol) of 7-[2-(dimethylamino)ethenyl]-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid[22] was added 50 mg of Et_3N·HCl and 20 mL of *N*-methylpiperazine. The mixture was refluxed for 16 h and was concentrated. The solids were washed with 5% aqueous THF and then ether to give 1.23 g (100%) of **2r** as a slightly yellow powder: IR (KBr) 1710, 1610 cm^{-1}; NMR ($CDCl_3$) δ 8.55 (s, 1 H, C_2H), 8.55 (d, J = 12 Hz, 1 H, vinyl), 8.2 (d, J = 7 Hz, 1 H), 6.85 (d, J = 7 Hz, 1 H), 5.3 (d, J = 12 Hz, 1 H, vinyl), 4.45 (q, J = 7 Hz, 2 H, CH_2CH_3), 3.3 (m, 4 H), 2.45 (m, 4 H), 2.3 (s, 3 H, NCH_3), 1.45 (t, J = 7 Hz, 3 H, CH_2CH_3).

4-Amino-8-ethyl-5,8-dihydro-2-(methylthio)-5-oxopyrido-[2,3-*d*]pyrimidine-6-carboxylic Acid (14). To 6.4 g (20 mmol) of **13**[24] in 200 mL of DMF was added 6.9 g (2.5 equiv) of K_2CO_3 and 8.0 mL (5.0 equiv) of EtI. The mixture was stirred vigorously at 70 °C for 16 h. It was concentrated to dryness and then partitioned between H_2O and $HCCl_3$. The organic layer was separated, dried, and concentrated to give 5.6 g of ethylated product. This material was hydrolyzed directly in ethanol and 2 N NaOH to give 3.64 g (65%) of **14** as a white powder: mp 328–330 °C; IR ($HCCl_3$) 3480, 3350, 1725, 1560 cm^{-1}; NMR (TFA) δ 9.0 (s, 1 H, C_7H), 8.6 (br s, 2 H, NH_2), 4.6 (q, J = 7 Hz, 2 H, CH_2CH_3), 2.8 (s, 3 H, SCH_3), 1.6 (t, J = 7 Hz, 3 H, CH_2CH_3).

2-Substituted 4-Amino-8-ethyl-5,8-dihydro-5-oxopyrido-[2,3-*d*]pyrimidine-6-carboxylic Acids (6a–c). The General Procedure. To 300 mg (1.07 mmol) of the acid **14** in 15 mL of EtOH was added 1.1 g (5.4 mmol) of mCPBA. The mixture was stirred for 18 h at 50 °C and diluted with ether, and the solids were filtered and dried in air. The solids were then added to the amine neat or in H_2CCl_2. The reactions were diluted with ether, and the solids were collected. These were recrystallized from DMF.

4-Amino-8-ethyl-5,8-dihydro-5-oxo-2-(1-pyrrolidinyl)-pyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (6a). The general procedure was employed with pyrrolidine to yield 230 mg (71%) of **6a**: IR (KBr) 3420, 3300, 2960, 2850, 1714, 1635, 1450 cm^{-1}; NMR (TFA) δ 8.95 (s, 1 H, C_7H), 4.5 (q, J = 7 Hz, 2 H, CH_2CH_3), 3.85 (m, 4 H, pyrrolidine), 2.25 (m, 4 H, pyrrolidine), 1.65 (t, J = 7 Hz, 3 H, CH_2CH_3).

4-Amino-8-ethyl-5,8-dihydro-5-oxo-2-(1-piperazinyl)-pyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (6b). The general procedure was employed with piperazine in dichloromethane to give 300 mg (88%) of **6b**.

4-Amino-8-ethyl-5,8-dihydro-2-(4-methyl-1-piperazinyl)-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (6c). The general method was employed with 4-methylpiperazine to give 0.261 g (78%) of **6c**.

1-Ethyl-1,4-dihydro-6-methyl-4-oxo-3-pyridinecarboxylic Acid (7a). To 2.00 g (13.1 mmol) of **15**[25] suspended in 45 mL of H_2O was added 0.6 g (1.8 mmol) of tetrabutylammonium bromide and 7.07 g (3.5 equiv) of diethyl sulfate. To this mixture, at 45 °C, was added 3.37 g (4.0 equiv) of KOH. The mixture was stirred at 45 °C for 3.5 h and extracted with $HCCl_3$ and this layer was discarded. The H_2O layer was acidified to pH 2.0 and extracted with $HCCl_3$ three times. The $HCCl_3$ extract was dried and concentrated. The solids were recrystallized from hot EtOH to give 0.75 g (37%) of **7a** as pale yellow needles: IR (KBr) 1705, 1650 cm^{-1}; NMR (Me_2-d_6) δ 11.4 (s, 1 H, OH), 8.6 (s, 1 H), 6.6 (s, 1 H), 4.1 (q, J = 7 Hz, 2 H, CH_2CH_3), 2.2 (s, 3 H, CH_3), 1.3 (t, J = 7 Hz, 3 H, CH_2CH_3).

1-Ethyl-1,4-dihydro-4-oxo-6-[2-(3-thienyl)ethenyl]-3-pyridinecarboxylic Acid (7b). With use of the procedure above, 1.0 g (4.0 mmol) of 1,4-dihydro-4-oxo-6-[2-(3-thienyl)ethenyl]-3-pyridinecarboxylic acid[25] was converted to 0.76 g (69%) of **7b**: mp 233–235 °C.

DNA Gyrase Assay. Materials and Methods. The DNA gyrase was isolated from *E. coli* H560[35] and was purified according to literature procedures.[12a,36–38] The two subunits were stored separately and made to a concentration such that 1 μL of each would completely supercoil 1 μL of relaxed Col E1 plasmid DNA (at a concentration of 0.3 μg/mL) under conditions described below. Supercoiled Col E1 DNA was purchased from Sigma and purified using CsCl–ethidium bromide gradient. Relaxed Col E1 DNA was prepared from the supercoiled form with use of rat liver untwisting enzyme[39] or calf thymus relaxing enzyme[40] according to established procedures.[38] Bovine serum albumin (BSA) was denatured at 80 °C for 20 min prior to preparation of a stock solution. All biochemicals employed were commercially available from Sigma. All inorganic reagents were biochemical grade.

I_{50} Determination. To a 1.5-mL eppendorf tube, was added a reaction cocktail containing Tris-HCl pH 7.6 (40 mM), K_xPO_4 pH 7.4 (20 mM), dithiotreitol (8 mM), $MgCl_2$ (8 mM), spermidine trihydrochloride (5.8 mM), BSA (116 μg/mL), KCl (20 mM), and tRNA (100 μg/mL). To this cocktail was added the drug to be tested in 0.1 N KOH. The mixture was then treated with relaxed Col E1 DNA (~0.3 μg/mL), DNA gyrase subunit A, and DNA gyrase subunit B. The mixture was incubated for 40 min at 30 ± 1 °C and then ATP was added. After an additional 10 min the reaction was quenched by adding an aqueous solution containing 5% sodium dodecyl sulfate (SDS), 20% Ficoll, 0.025% bromophenol blue, and 25 mM Tris-HCl, pH 8.0, followed by proteinase K (1 mg/mL). This final mixture was incubated at 37 ± 1 °C. The samples were applied to a 1% agarose gel and resolved at 50 V for 16–18 h. The gel was visualized with ethidium bromide stain and fluorescence at 254 nm. The gel was photographed with use of a UV filter, and the drug concentrations corresponding to 100% supercoiling inhibition and initial inhibition (where the linear DNA first appears) were noted. These two values were added together, divided by two, and recorded as the "I_{50}".

Cleavage Assay. To the same cocktail as above was added supercoiled Col E1 DNA (0.3 μg/mL), each subunit, and finally the drug to be tested as defined above. The mixture was incubated at 30 ± 1 °C for 1 h. It was quenched and visualized as previously described. The concentration of the drug which produced a linear DNA band of equal intensity to that produced by oxolinic acid at 10 μg/mL was recorded as the gyrase cleavage value.

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Registry No. 1 (R_6 = R_7 = F_1R_8 = H_1 R^1 = Et), 70032-25-6; **1a**, 14698-29-4; **1b**, 37065-29-5; **1c**, 70458-96-7; **1d**, 70458-92-3; **1e**, 86393-37-5; **1f**, 85721-33-1; **1g**, 40034-42-2; **1h**, 74029-44-0; **1i**, 54132-24-0; **1j**, 3832-97-1; **1k**, 23790-01-4; **1l**, 68077-26-9; **1m**, 75338-42-0; **1n**, 75001-62-6; **1o**, 70459-01-7; **1p**, 99726-76-8; **1q**, 75338-41-9; **1r**, 99726-77-9; **1s**, 99726-78-0; **1t**, 91524-15-1; **1u**, 99726-79-1; **1v**, 75001-75-1; **1w**, 99726-80-4; **1x**, 99726-81-5; **1y**, 99726-82-6; **1z**, 99726-83-7; **1a′**, 99726-84-8; **1b′**, 99726-85-9; **1c′**, 99726-86-0; **1d′**, 93509-80-9; **1e′**, 93509-81-0; **2** (R_6 = F, R_7 = Cl), 79286-73-0; **2** (R_6 = N_2^+, R_7 = Me), 99727-02-3; **2a**, 389-08-2; **2b**,

(35) Strain was obtained from Dr. P. Higgins, University of Alabama, Birmingham.
(36) Otter, R.; Cozzarelli, N. R. *Methods Enzymol.* **1983**, *100*, 171.
(37) Higgins, P. N.; Peebles, C. L.; Sugino, A.; Cozzarelli, N. R. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 1773.
(38) Sugino, A.; Cozzarelli, N. R. *J. Biol. Chem.* **1980**, *255*, 6299.
(39) Sumida-Yasumoto, C.; Ikeda, J. E.; Benz, E.; Marians, K. J.; Vicuna, R.; Sugrue, S.; Zipursky, S. L.; Hurwitz, J. *Cold Spring Harbor Symp. Quant. Biol.* **1978**, *43*, 311.
(40) Commercially available from Bethesda Research Labs.

74011-58-8; **2c**, 54132-31-9; **2d**, 87939-22-8; **2e**, 87939-21-7; **2f**, 63053-03-2; **2g**, 68998-57-2; **2h**, 74274-63-8; **2i**, 92741-52-1; **2j**, 93675-10-6; **2k**, 99726-87-1; **2l**, 99726-88-2; **2m**, 24677-82-5; **2m** (ethyl ester), 99727-01-2; **2n**, 99726-89-3; **2o**, 99726-90-6; **2p**, 99726-91-7; **2q**, 99726-92-8; **2r**, 99726-93-9; **3a**, 19562-30-2; **3b**, 51940-44-4; **4**, 82419-36-1; **5**, 28657-80-9; **6a**, 99726-94-0; **6b**, 99726-95-1; **6c**, 99726-96-2; **7a**, 70934-34-8; **7b**, 99726-97-3; **8**, 91188-89-5; **9**, 99727-00-1; **11**, 57018-74-3; **12**, 99727-03-4; **13**, 36707-44-5; **14**, 99727-04-5; **15**, 33821-58-8; thiazolidine, 504-78-9; 4-(trifluoromethyl)piperidine, 657-36-3; 4-piperidinecarboxamide, 39546-32-2; 4-[3-(dimethylamino)propyl]piperazine, 877-96-3; 1-methyl-4-(4-piperidinyl)piperazine monoacetate, 99726-99-5; 4-(1-pyrrolidinyl)piperidine, 5004-07-9; thiomorpholine, 123-90-0; 1-(aminoethyl)piperazine, 140-31-8; 2,5-dimethoxytetrahydrofuran, 696-59-3; chloroacetonitrile, 107-14-2; ethyl acetamidate hydrochloride, 2208-07-3; 4-methyl-1-piperazinamine, 6928-85-4; 7-[2-(dimethylamino)ethenyl]-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, 63475-31-0; *N*-methylpiperazine, 109-01-3; pyrrolidine, 123-75-1; piperazine, 110-85-0; 1,4-dihydro-4-oxo-6-[2-(3-thienyl)]-3-pyridinecarboxylic acid, 96593-69-0.

N-Imidazolylchroman-4-ones, *N*-Imidazolyl-1-tetralones, and Their Alcohols as Hypolipemic Agents Raising High-Density Lipoproteins

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A series of 3-(1-imidazolyl)chroman-4-ones and 2-(1-imidazolyl)-1-tetralones II, some of their alcohols, and some related compounds were synthesized and tested for hypolipidemic activity. Compounds II, bearing appropriate lipophilic substituents on the phenyl ring, strongly reduced total serum cholesterol while raising high-density lipoprotein cholesterol in diet-induced hypercholesterolemic rats. 3-(1-Imidazolyl)chroman-4-ols and 2-(1-imidazolyl)-1-tetralols corresponding to II retained the hypolipidemic activity while removal of the carbonyl or hydroxy group adjacent to imidazole gave inactive compounds. Although many of the active compounds significantly increased liver weight, the one studied as a model, 6-chloro-3-(1-imidazolyl)-2,3-dihydro-4*H*-1-benzopyran-4-one (**5**), caused no peroxisome proliferation. Compound **5** and the corresponding alcohol **40**, as representatives of the ketone and alcohol series, showed significant hypolipidemic activity in normolipemic rats. Some of the compounds assayed in cholesterol biosynthesis inhibited acetate incorporation but none inhibited HMG-CoA reductase. 5-Bromo-6-hydroxy-2-(1-imidazolyl)-3,4-dihydro-1(2*H*)-naphthalenone (**38**), which showed strong activity but caused little hepatomegaly in the rat, was chosen for further pharmacological evaluation.

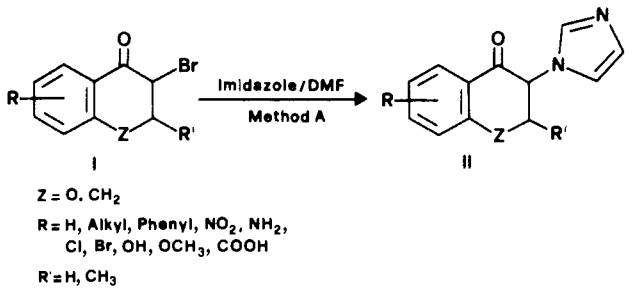
Lipid-lowering drugs have been sought for many years for the treatment of hyperlipidemia.[1] Recently new agents reducing atherogenic low-density lipoproteins (LDL) and raising high-density lipoproteins (HDL) have been sought[2] on the basis that high HDL levels are associated with a reduced risk of coronary heart disease.[3]

This paper reports a series of *N*-imidazolyl derivatives of the chroman and of the tetraline ring (Table I) and some related compounds (Table II). The compounds were tested in diet-induced hypercholesterolemic rats and some of them significantly lowered total serum cholesterol while raising HDL cholesterol. The compounds are structurally unrelated to known hypolipemic drugs although some *N*-alkylimidazole[4] and *N*-benzylimidazole[5] derivatives and more recently khellin and some related chromones and chromanones[2e] were reported to possess hypolipemic activity. The many hypolipemic chroman derivatives described by Witiak et al.[6] are more closely related to clofibrate than to the chroman derivatives described here.

Chemistry. *N*-Imidazolylchromanones and *N*-imidazolyltetralones synthesized following Schemes I and II are reported in Table I, which summarizes their physicochemical properties and biological activity in the rat made hypercholesterolemic by diet.

Compounds II where Z is CH_2, namely 2-(1-imidazolyl)-3,4-dihydro-1(2*H*)-naphthalenones, were prepared following Scheme I analogously to the procedure described by Schröder[7] et al. (method A). Compounds II where Z is oxygen, namely 3-(1-imidazolyl)-2,3-dihydro-4*H*-1-benzopyran-4-ones, were prepared in a few cases with the same method A, reported in Scheme I or, more often,

Scheme I



following Scheme II. In this case 2-bromo-2′-hydroxyacetophenones III were reacted with imidazole (method

† Department of Chemistry.
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(1) See for example: (a) Holland, G. F. "Burger's Medicinal Chemistry", Wolff, M. E., Ed.; Wiley: New York, 1979; Part 2, Chapter 35. (b) Cayen, M. N.; Kallai-Sanfacon, M. A. "Annual Reports in Medicinal Chemistry"; Hess, H. J., Ed.; Academic Press: New York, 1980; Vol. 15, p 162.
(2) See for example: (a) Day, C. E.; Schurr, P. E.; Heyd, W. E.; Lednicer, D. *Adv. Exp. Med. Biol.* **1976**, *67*, 231. (b) Sirtori, C. R.; Gomarasca, P.; d'Atri, G.; Cerutti, S.; Tronconi, G.; Scolastico, G. *Atherosclerosis* **1978**, *30*, 45. (c) Schwandt, P.; Weisweiler, P.; Neureuther, G. *Artery* **1979**, *5*, 117. (d) Sircar, I.; Hoefle, M.; Maxwell, R. E. *J. Med. Chem.* **1983**, *26*, 1020. (e) Gammill, R. B.; Day, C. E.; Schurr, P. E. *J. Med. Chem.* **1983**, *26*, 1674 and references therein.
(3) (a) Miller, N. E.; Forde, O. H.; Thelle, D. S.; Mjes, O. D. *Lancet* **1977**, *1*, 965. (b) Rossner, S.; Kjellin, K. G.; Mettinger, R. L.; Linden, A.; Soderstrom, C. E. *Lancet* **1978**, *1*, 577. (c) Goldbourt, U.; Medalie, J. H. *Am. J. Epidemiol.* **1979**, *109*, 296.
(4) Atkin, S. D.; Morgan, B.; Baggaley, K. H.; Green, J. *Biochem. J.* **1972**, *130*, 153.
(5) Baggaley, K. H.; Heald, M.; Hindley, R. M.; Morgan, B.; Tee, J. L.; Green, J. *J. Med. Chem.* **1975**, *18*, 833.

0022-2623/86/1829-0404$01.50/0