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Synthesis and Structure-Activity Relationships of 7-Diazabicycloalkylquinolones, Including Danofloxacin, a New Quinolone Antibacterial Agent for Veterinary Medicine¹

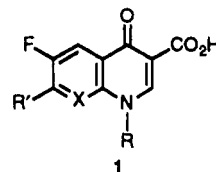
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A series of novel 6-fluoro-7-diazabicycloalkylquinolonecarboxylic acids substituted with various C₈ (H, F, Cl, N) and N₁ (ethyl, cyclopropyl, vinyl, 2-fluoroethyl, 4-fluorophenyl, 2,4-difluorophenyl) substituents, as well as, 9-fluoro-10-diazabicycloalkylpyridobenzoxazinecarboxylic acids, were prepared and evaluated for antibacterial activity against a range of important veterinary pathogenic bacteria. The diazabicycloalkyl side chains investigated at the 7-position (benzoxazine 10-position) include (1*S*,4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]heptane (2), (1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptane (3), (1*R*,4*R*)-5-methyl-2,5-diazabicyclo[2.2.1]heptane (4), 8-methyl-3,8-diazabicyclo[3.2.1]octane (5), 9-methyl-3,9-diazabicyclo[4.2.1]nonane (6), 1,4-diazabicyclo[3.2.2]nonane (7), 1,4-diazabicyclo[3.3.1]nonane (8), and 9-methyl-3,9-diazabicyclo[3.3.1]nonane (9). Among these side chains, in vitro potency was not highly variable; other properties therefore proved more critical to the selection of possible development candidates. However, the relative potencies observed for several of these compounds in mouse, swine, and cattle infection models correlated well with those seen in vitro. A combination of the N₁ cyclopropyl group and the C₇ (1*S*,4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]hept-2-yl appendage conferred the best overall antibacterial, physicochemical, and pharmacodynamic properties. Hence, danofloxacin (Advocin, 2c) (originally CP-76,136, 1-cyclopropyl-6-fluoro-7-[(1*S*,4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]hept-2-yl]-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid) was selected as a candidate for development as a therapeutic antibacterial agent for veterinary medicine.

Quinolone antibacterial agents continue to represent an important new class of therapeutically useful compounds,² in particular those analogues substituted at the 6-position with fluorine and at the 7-position with cyclic aliphatic diamines, such as piperazine, methylpiperazine, and 3-

aminopyrrolidine. Noteworthy examples are norfloxacin (1a), ciprofloxacin (1b), enoxacin (1c), ofloxacin (1d), fleroxacin (1e), temafloxacin (1f), and tosufloxacin (1g).



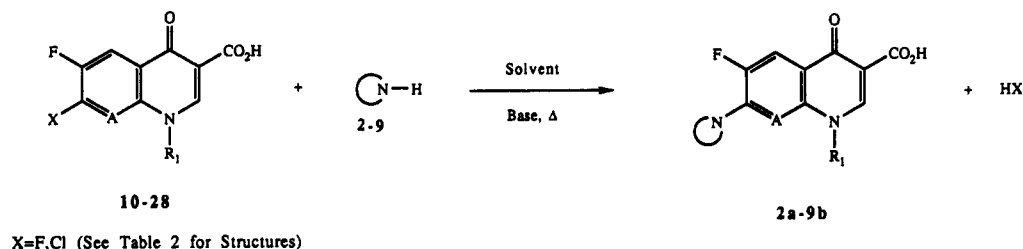
R'	X	R
1a: 1-piperazinyl	CH	C ₂ H ₅
b: 1-piperazinyl	CH	-C ₃ H ₇
c: 1-piperazinyl	N	C ₂ H ₅
d: 4-methyl-1-piperazinyl	-COCH ₂ CH(CH ₃)-	
e: 4-methyl-1-piperazinyl	CF	CH ₂ CH ₂ F
f: 3-methyl-1-piperazinyl	CH	2,4-difluorophenyl
g: 3-amino-1-pyrrolidinyl	N	2,4-difluorophenyl

In recent years, several of these agents have been applied successfully in the treatment of a wide range of human bacterial infections. Less attention has been paid to their application in veterinary medicine.³ It seemed to us that

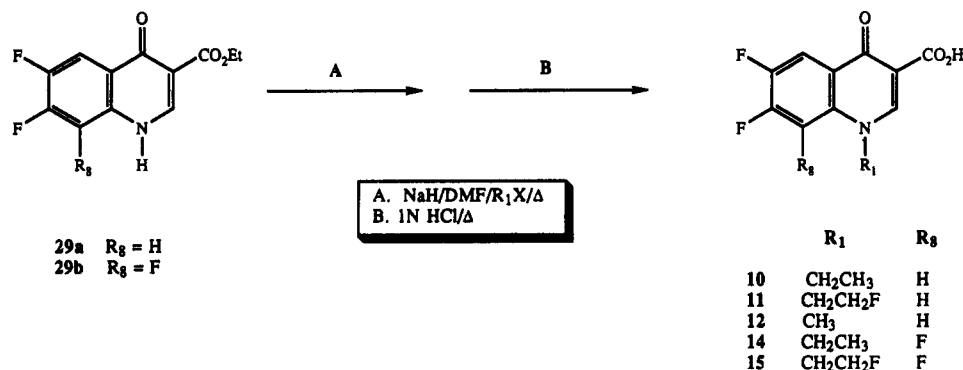
- (1) This work was previously presented in part: McGuirk, P. R.; Jefson, M. R.; Mann, D. D.; Hindahl, M. S.; Cornell, C. P.; Weber, F. H. The Synthesis and Antibacterial Activity of 6-Fluoro-7-diazabicycloalkylquinolones related to CP-76,136 (Danofloxacin). *Abstracts of the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Houston, TX, 1989, Abstract 1186 and The Synthesis and Antibacterial Activity of Danofloxacin (CP-76,136): A New Quinolone for Veterinary Medicine. *Abstracts of the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Houston, TX, 1989, Abstract 1187.
- (2) (a) Chu, D. T. W.; Fernandes, P. B. Structure-Activity Relationships of the Fluoroquinolones. *Antimicrob. Agents Chemother.* 1989, 33, 131-135. (b) Fernandes, P. B.; Chu, D. T. W. Quinolone Antibacterial Agents. In *Annu. Rep. Med. Chem.*; Academic Press, Inc.: San Diego, CA, 1988; Vol. 23, pp 133-140. (c) Fernandes, P. B.; Chu, D. T. W. Quinolones. In *Annu. Rep. Med. Chem.*; Academic Press, Inc.: Orlando, FL, 1987, Vol. 22, pp 117-126. (d) Cornett, J. B.; Wentland, M. P. Quinolone Antibacterial Agents. In *Annu. Rep. Med. Chem.*; Academic Press, Inc.: Orlando, FL, 1986; Vol. 21, pp 139-148. (e) Wentland, M. P.; Cornett, J. B. Quinolone Antibacterial Agents. In *Annu. Rep. Med. Chem.*; Academic Press, Inc.: Orlando, FL, 1985; Vol. 20, pp 145-154.

- (3) Vancutsem, P. M.; Babish, J. G.; Schwark, W. S. The Fluoroquinolone Antimicrobials: Structure, Antimicrobial Activity, Pharmacokinetics, Clinical Use in Domestic Animals and Toxicity. *Cornell Vet.* 1990, 80, 173-186.

Scheme I

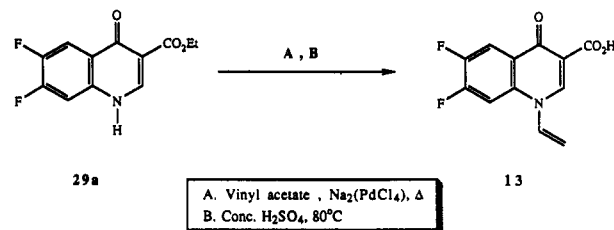


Scheme II

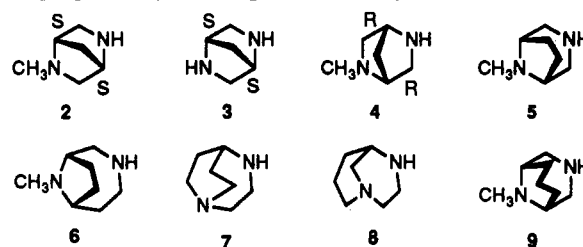


a quinolone with suitable properties would be of considerable interest in this area, where the need for new therapy, particularly against resistant strains of primarily Gram-negative bacteria, is great. As part of our effort to discover a new agent for this use we chose to investigate the properties of a group of novel⁴ 6-fluoro-7-diazabicycloalkylquinolone-3-carboxylic acids (10-diazabicycloalkylpyridobenzoxazinones) and 6-fluoro-7-diazabicycloalkyl-1,8-naphthyridinone-3-carboxylic acids on the premise that the bulky,⁵ more lipophilic diazabicycloalkyl substituent at C₇ would have a favorable effect on some of the physicochemical properties of these molecules (e.g. aqueous solubility⁶ and pharmacokinetics⁷), while retaining an ex-

Scheme III



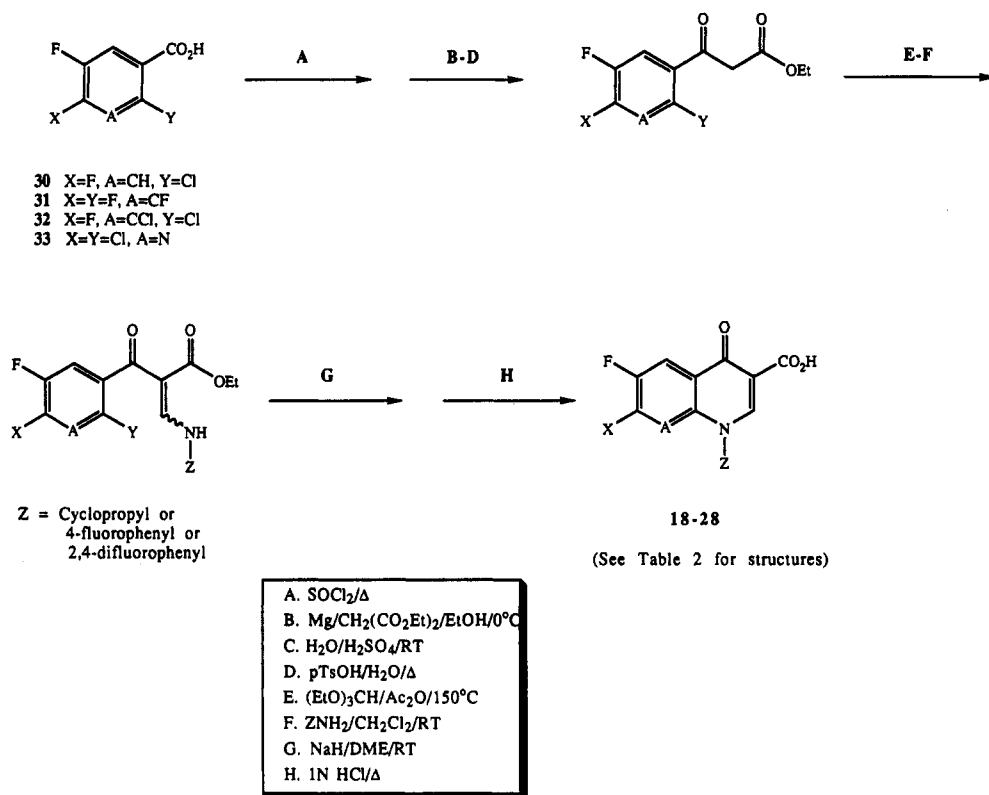
cellent antibacterial profile. The compounds presented were prepared by reacting the diazabicycloalkanes 2-9 with



- (4) During the course of this work several of the 7-diazabicycloalkylquinolone-3-carboxylic acids discussed in this paper were independently disclosed by another group. See: Hutt, M. P.; Mich, T. F.; Culbertson, T. P. U. S. Patent 4,571,396, 1986.
- (5) Bulky C₇ substituents are known to confer other favorable properties on quinolones such as sparfloxacin (AT-4140): Miyamoto, T.; Matsumoto, J.; Chiba, K.; Egawa, H.; Shibamori, K.; Minamida, A.; Nishimura, Y.; Okada, H.; Kataoka, M.; Fujita, M.; Hirose, T.; Nakano, J. *Synthesis and Structure-Activity Relationships of 5-Substituted 6,8-Difluoroquinolones, Including Sparfloxacin, a New Quinolone Antibacterial Agent with Improved Potency.* *J. Med. Chem.* 1990, 33, 1645-1656. Other reports on bridged piperazine-containing quinolones include: (a) Bouzard, P.; Di Cesare, P.; Essiz, M.; Jacquet, J. P.; Kiechel, J. R.; Remuzon, P.; Weber, T. Oki; Masuyoshi, M.; Kessler, R. E.; Fung-Tomc, J.; Desiderio, J. Fluoronaphthyridines and Quinolones as Antibacterial Agents. 2. Synthesis and Structure-Activity Relationships of New 1-tert-Butyl 7-Substituted Derivatives. *J. Med. Chem.* 1990, 33, 1344-1352. (b) Rosen, T.; Chu, D. T. W.; Lico, I. M.; Fernandes, P. B.; Marsh, K.; Shen, L.; Cepa, V. G.; Pernet, A. G. Design, Synthesis and Properties of (4S)-7-(4-Amino-2-substituted-pyrrolidin-1-yl)quinolone-3-carboxylic Acids. *J. Med. Chem.* 1988, 31, 1598-1611. (c) Kiely, J. S.; Hutt, M. P.; Culbertson, T. P.; Bucsh, R. A.; Worth, D. F.; Lesheski, L. E.; Gogliotti, R. D.; Sesnie, J. A.; Solomon, M.; Mich, T. F. Quinolone Antibacterials: Preparation and Activity of Bridged Bicyclic Analogues of the C₇-Piperazine. *J. Med. Chem.* 1991, 34, 656-663.

- (6) (a) Rosen, T.; Chu, D. T. W.; Cooper, C. S.; Fernandes, P. B.; Maleczka, R. E.; Pernet, A. The Synthesis and Antibacterial Activity of Enantiomerically Homogeneous Substituted 7-(4-Aminopyrrolidinyl)-1-aryl-6-fluoroquinolones. *Abstracts of the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy*, New Orleans, Louisiana, 1986, Abstr. 427. (b) Chu, D. T. W.; Lico, I.; Fernandes, P. B.; Shen, L.; Pernet, A. The Design, Synthesis and Properties of A-65485 and Related Quinolone Antibacterial Agents. *Abstracts of the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy*, New York, New York, 1987, Abstr. 253. (c) Hagen, S. E.; Domagala, J. M.; Heifetz, C. L.; Sanchez, J. P.; Solomon, M. New Quinolone Antibacterial Agents. Synthesis and Biological Activity of 7-(3,3- or 3,4-Disubstituted-1-pyrrolidinyl)-quinolone-3-carboxylic Acids. *J. Med. Chem.* 1990, 33, 849-854.
- (7) The pharmacokinetic properties of danofloxacin were presented previously: Frame, G. M.; Mann, D. D.; Lynch, M. J. Pharmacokinetics of the Quinolone Antibiotic Danofloxacin (CP-76,136) in Cattle, Swine and Poultry. *Abstracts of the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Houston, Texas, 1989, Abstr. 1188.

Scheme IV



a variety of 7-haloquinolone (10-position for pyridobenzoxazinones) and 7-halonaphthyridinone substrates and were examined for in vitro and in vivo antibacterial activity as well as inhibition of DNA gyrase, the enzyme responsible for DNA supercoiling and the site of action for this class of compounds.⁸ Selected analogues which exhibited favorable properties were further evaluated in use—animal respiratory infection models⁹ (vide infra). The effect of these structural variations on antimicrobial activity, efficacy, aqueous solubility, and activity against DNA gyrase is discussed herein.

Chemistry

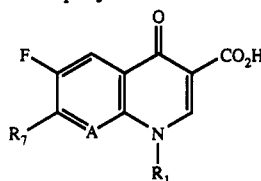
The title compounds 2a–9c (Table I) were prepared by reacting the appropriately substituted quinolone or naphthyridinone nucleus (10–28) with the requisite diazabicycloalkane (2–9) according to the general procedures A–C (Scheme I). The nuclei 10–28 (Table II) were prepared either directly according to or by slight modification of previously published chemistry. Thus 10–12, 14, and 15 were prepared according to Sturm¹⁰ by alkylation of the

esters, 29a,b at N₁ followed by hydrolysis of the ester in refluxing 1 N HCl (Scheme II). Nucleus 13 was prepared by treatment of 29a with vinyl acetate in the presence of Na₂(PdCl₄)¹¹ followed by ester hydrolysis in concentrated H₂SO₄ (Scheme III). Compounds 16¹² and 17¹³ were each prepared directly according to the literature, and nuclei 18–28 were synthesized by merging the appropriate commercially available benzoic acid 30–32 or the nicotinic acid 33¹⁴ and either cyclopropylamine, 4-fluoroaniline, or 2,4-difluoroaniline according to the procedure of Grohe et al.¹⁵ (Scheme IV). In similar fashion the diazabicycloalkanes 2–4,¹⁶ 5,¹⁷ and 7 and 8¹⁸ were each synthesized via slight

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- (9) Mann, D. Efficacy of the Quinolone Antibiotic Danofloxacin (CP-76,136) in Food Producing Animal Disease Models. *Abstracts of the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Houston, Texas, 1989, Abstract 1189.

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- (15) Grohe, K.; Petersen, U.; Kuck, K.-H. U.S. Pat. 4,563,459, 1986.
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- (18) Rubstov, M. V.; Mikhлина, E. E.; Vorob'eva, V. Ya.; Yanina, A. D. Beckmann Rearrangement in the Heterocyclic Series. Rearrangement of Oximes of 3-Quinuclidone and 1-Azabicyclo[3.2.1]-6-octanone. *Zh. Obshch. Khim.* 1964, 34, 2222–2226; *Chem. Abstr.* 1964, 61, 9481. Mikhлина, E. E.; Vorob'eva, V. Ya.; Shedchenko, V. I.; Rubstov, M. V. Structure of Products of Rearrangement of 3-Quinuclidone by the Reactions of Schmidt and Beckmann. *Zh. Org. Khim.* 1965, 1, 1352–1353.

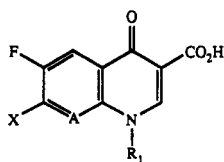
Table I. 7-Diazabicycloalkyl-Substituted Quinolones and Naphthyridinones



compd	R1 ^a	A	R7	method (% yield)	mp, °C	formula ^b
2a	Et	CH		A (91)	235–237	C ₁₈ H ₂₀ FN ₃ O ₃ ·1.25H ₂ O
2c	Cpr	CH		B (81)	337–339	C ₁₉ H ₂₀ FN ₃ O ₃ ·1.5H ₂ O·CH ₃ SO ₃ H
2d	Cpr	CF		B(66)	295–297	C ₁₉ H ₁₈ F ₂ N ₃ O ₃ ·0.5H ₂ O·CH ₃ SO ₃ H
2e	Cpr	CCl		A (48)	175–177	C ₁₉ H ₁₉ ClFN ₃ O ₃ ·0.25H ₂ O
2g	4-FPh	CH		B (85)	271–273	C ₂₂ H ₁₉ F ₂ N ₃ O ₃ ·0.5H ₂ O·CH ₃ SO ₃ H
2h	4-FPh	CF		B (55)	288–289	C ₂₂ H ₁₈ F ₃ N ₃ O ₃ ·0.5H ₂ O·CH ₃ SO ₃ H
2i	4-FPh	CCl		C (54)	200–201	C ₂₂ H ₁₈ ClF ₂ N ₃ O ₃ ^c
2j	4-FPh	N		B (66)	314–315	C ₂₁ H ₁₈ F ₂ N ₄ O ₃ ^c
2k	2,4-F ₂ Ph	CH		A (60)	209–212	C ₂₂ H ₁₈ F ₃ N ₃ O ₃ ·2.5H ₂ O
2l	2,4-F ₂ Ph	CF		C (45)	228–230	C ₂₁ H ₁₇ F ₄ N ₃ O ₃ ^c
2m	2,4-F ₂ Ph	CCl		A (51)	243–245	C ₂₂ H ₁₇ ClF ₃ N ₃ O ₃ ·1.25H ₂ O
3a	Et	CH		A (14)	283–285	C ₁₇ H ₁₈ FN ₃ O ₃ ·0.25H ₂ O
3c	Cpr	CH		A (63)	301–302	C ₁₈ H ₁₈ FN ₃ O ₃ ·2.25H ₂ O
3d	Cpr	CF		B (78)	258–260	C ₂₀ H ₁₇ F ₂ N ₃ O ₃ ·1.5H ₂ O·CH ₃ SO ₃ H
3e	Cpr	CCl		C (45)	242–244	C ₁₈ H ₁₇ ClFN ₃ O ₃ ·3.25H ₂ O
3g	4-FPh	CH		B (92)	294–295	C ₂₁ H ₁₇ F ₂ N ₃ O ₃
3h	4-FPh	CF		B (72)	290–292	C ₂₁ H ₁₆ F ₃ N ₃ O ₃ ·2.25H ₂ O
3l	2,4-F ₂ Ph	CF		C (50)	244–246	C ₂₁ H ₁₅ F ₄ N ₃ O ₃ ^c
4c	Cpr	CH		A (48)	310–312	C ₁₉ H ₂₀ FN ₃ O ₃ ·1.5H ₂ O·CH ₃ SO ₃ H
5a	Et	CH		A (89)	310–312	C ₁₉ H ₂₂ FN ₃ O ₃ ·CH ₃ SO ₃ H
5b	Et	CF		A (21)	227–228	C ₁₉ H ₂₁ F ₂ N ₃ O ₃ ·0.125H ₂ O
5c	Cpr	CH		A (86)	306–308	C ₂₀ H ₂₂ FN ₃ O ₃ ·1.5H ₂ O·CH ₃ SO ₃ H
5d	Cpr	CF		B (63)	290–292	C ₂₀ H ₂₁ F ₂ N ₃ O ₃ ·0.5H ₂ O·CH ₃ SO ₃ H
5e	Cpr	CCl		A ^d (13)	225–227	C ₂₀ H ₂₁ ClFN ₃ O ₃ ^c
5g	4-FPh	CH		A (35)	319–320	C ₂₃ H ₂₁ F ₂ N ₃ O ₃ ·2.5H ₂ O·HCl
5n	2-FEt	CH		A (52)	270–273	C ₁₉ H ₂₁ F ₂ N ₃ O ₃ ·1.5H ₂ O·HCl
5o	2-FEt	CF		A (56)	216–219	C ₁₉ H ₂₀ F ₃ N ₃ O ₃ ^c
5p				A ^e (21)	248–252	C ₂₀ H ₂₂ FN ₃ O ₄ ·0.5H ₂ O
5q	vinyl	CH		A (66)	230–232	C ₁₈ H ₂₀ FN ₃ O ₃ ·0.5H ₂ O
5r	Me	CH		A (68)	251–252	C ₁₈ H ₂₀ FN ₃ O ₃ ·0.75H ₂ O
6a	Et	CH		A (53)	187–188	C ₂₀ H ₂₄ FN ₃ O ₃ ·0.25H ₂ O
6b	Et	CF		A (69)	294–297	C ₂₀ H ₂₃ F ₂ N ₃ O ₃ ·0.25H ₂ O·CH ₃ SO ₃ H
6c	Cpr	CH		A (63)	263–265	C ₂₁ H ₂₄ FN ₃ O ₃ ·H ₂ O·CH ₃ SO ₃ H
6d	Cpr	CF		A (54)	253–255	C ₂₁ H ₂₃ F ₂ N ₃ O ₃ ·0.75H ₂ O·CH ₃ SO ₃ H
6f	Cpr	N		A (83)	263–264	C ₂₀ H ₂₃ FN ₄ O ₃ ·1.125H ₂ O·CH ₃ SO ₃ H
6g	4-FPh	CH		B (95)	270–272	C ₂₄ H ₂₃ F ₂ N ₃ O ₃ ^c
6n	2-FEt	CH		A (13)	202–203	C ₂₀ H ₂₃ F ₂ N ₃ O ₃ ^c
6q	vinyl	CH		A (45)	214–215	C ₂₀ H ₂₂ FN ₃ O ₃ ·0.25H ₂ O
6r	Me	CH		A (8.5)	220–222	C ₁₉ H ₂₂ FN ₃ O ₃ ^c
7a	Et	CH		C (82)	325–327	C ₁₉ H ₂₂ FN ₃ O ₃ ·CH ₃ SO ₃ H
7b	Et	CF		B (27)	312–313	C ₁₉ H ₂₁ F ₂ N ₃ O ₃ ·3H ₂ O·HCl
7c	Cpr	CH		B (90)	330–332	C ₂₀ H ₂₂ FN ₃ O ₃ ·1.5H ₂ O·HCl
7d	Cpr	CF		B (43)	250–252	C ₂₀ H ₂₁ F ₂ N ₃ O ₃ ^c
7g	4-FPh	CH		B (68)	320–322	C ₂₃ H ₂₁ F ₂ N ₃ O ₃ ·1.5H ₂ O·HCl
7n	2-FEt	CH		B (50)	256–258	C ₁₉ H ₂₁ F ₂ N ₃ O ₃ ·2.5H ₂ O·HCl
7o	2-FEt	CF		B (13)	311–313	C ₁₉ H ₂₀ F ₃ N ₃ O ₃ ·1.5H ₂ O·HCl
7p	-CH(CH ₃)CH ₂ OC-			A ^e (14)	230–232	C ₂₀ H ₂₂ FN ₃ O ₄ ·2.75H ₂ O·HCl
7q	vinyl	CH		B (50)	255–257	C ₁₉ H ₂₀ FN ₃ O ₃ ·2.5H ₂ O
7s	NHCH ₃	CH		B (73)	245–247	C ₁₈ H ₂₁ FN ₄ O ₃ ^c
8a	Et	CH		A (21)	310–312	C ₁₉ H ₂₂ FN ₃ O ₃ ·0.25H ₂ O·CH ₃ SO ₃ H
8c	Cpr	CH		B (37)	332–334	C ₂₀ H ₂₂ FN ₃ O ₃ ·0.5H ₂ O·CH ₃ SO ₃ H
8g	4-FPh	CH		A (17)	312–313	C ₂₃ H ₂₁ F ₂ N ₃ O ₃ ^c
9a	Et	CH		A (47)	180–182	C ₂₀ H ₂₄ FN ₃ O ₃ ·1.25H ₂ O
9c	Cpr	CH		A (66)	252–255	C ₂₁ H ₂₄ FN ₃ O ₃ ·2.25H ₂ O·HCl

^a Me = methyl, Et = ethyl, Cpr = cyclopropyl, 4-FPh = 4-fluorophenyl, 2,4-FPh = 2,4-difluorophenyl, 2-FEt = 2-fluoroethyl. ^b C, H, and N analyses were within ±0.4% of the theoretical values, except as otherwise noted. ^c Formula determined by high-resolution mass spectral (HRMS) analyses. ^d *N,N*-Dimethylformamide (DMF) was used as reaction solvent instead of pyridine. ^e Dimethyl sulfoxide (DMSO) was used as reaction solvent instead of pyridine.

Table II. 7-Fluoroquinolone and 7-Chloronaphthyridinone Nuclei



compd	R1 ^a	A	X
10	Et	CH	F
11	2-FEt	CH	F
12	Me	CH	F
13	vinyl	CH	F
14	Et	CF	F
15	2-FEt	CF	F
16			F
17	NHCH ₃	CH	F
18	Cpr	CH	F
19	4-FPh	CH	F
20	2,4-FPh	CH	F
21	Cpr	CF	F
22	4-FPh	CF	F
23	2,4-FPh	CF	F
24	Cpr	CCl	F
25	4-FPh	CCl	F
26	2,4-FPh	CCl	F
27	Cp	N	Cl
28	4-FPh	N	Cl

-CH(CH₃)CH₂OC-

^aMe = methyl, Et = ethyl, Cpr = cyclopropyl, 4-FPh = 4-fluorophenyl, 2,4-F₂Ph = 2,4-difluorophenyl, 2-FEt = 2-fluoroethyl.

modifications of the published procedures, while 6¹⁹ and 9²⁰ were prepared according to the literature.

Results and Discussion

Table III summarizes the in vitro antibacterial activity of the 7-diazabicycloalkyl-4-quinolones against five aerobically grown Gram-negative bacteria (*Bordetella bronchiseptica* 73A009, *Escherichia coli* 51A538, *Pasteurella haemolytica* 59B018, *Pasteurella multocida* 59A006, and *Salmonella choleraesuis* 58B015), one aerobically grown Gram-positive bacterium (*Staphylococcus aureus* 54B004), three anaerobically grown Gram-negative bacteria (*Actinobacillus pleuropneumoniae* 54B004, *Bacteroides vulgatus* 73E029, and *Treponema hyodysenteriae* 94A007), and two anaerobically grown Gram-positive bacteria (*Actinomyces pyogenes* 14D002 and *Clostridium perfringens* 10A009).

In general, for a given N₁ nucleus a large difference in in vitro activity was not observed for any of the C₇ diazabicycloalkyl substituents. The relative bulk or total number of carbon atoms of the substituent was not the only determinant of activity. For instance, tying the alkyl group of the distal nitrogen back into the ring, as in 7 and 8, provided compounds more potent than their isomeric counterparts bearing free methyl groups at the distal site. It can be safely said, however, that side chains 2-4, 7, and 8 showed generally better antibacterial activity than side chains 5, 6, and 9.

As mentioned above, incorporation of a 1,4-diazabicyclo[3.2.2]non-4-yl substituent (7), a 1,4-bridged homopiperazine, or the isomeric 1,4-diazabicyclo[3.3.1]non-4-yl substituent (8), a 1,3-bridged piperazine, at the quinolone C₇ position imparted the most potent antibacterial activity

in vitro when compared with the other diazabicycloalkane substituents studied. As shown by the data in Table III, placing a cyclopropyl group at N₁ as in 7c,d and 8c conferred the best activity within the two series containing 7 and 8. This observation is consistent with that found for numerous other quinolone series.²¹ In comparing N₁ cyclopropyl derivatives 7c and d, the addition of a C₈ fluorine generally had the effect of improving the in vitro activity against anaerobically grown bacteria by approximately 4-fold with no effect on the activity against aerobically grown pathogens. In contrast no improvement in potency was observed in the N₁ ethyl series upon C₈ halogenation as shown by comparing 7a with 7b.

As with 7 and 8, the (1*S*,4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]hept-2-yl C₇ substituent 2, which can be viewed as either a 2,5-bridged piperazine or a 2,4-bridged pyrrolidine, provided the most potency when combined with a cyclopropyl group at N₁. Within the (1*S*,4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]heptane (2) series the N₁ ethyl-, 2-fluorophenyl-, and 2,4-difluorophenyl-containing analogues were consistently less potent than the cyclopropyl analogue against the key Gram-negative pathogens and were at best equipotent against *Staphylococcus aureus*. In this bridged diazabicycloalkyl series, absolute stereochemistry had very little effect on antibacterial activity.²² The *R,R* isomer in the N₁ cyclopropyl series 4c was 4 times more potent against *E. coli* DNA gyrase but was either equipotent or 2-4 times less active in vitro than 2c, as shown in Table III. Introduction of a halogen at C₈ produced increased potency against both bacteria and DNA gyrase for analogues 2d,e. This is similar to what is observed when aminopyrrolidine is placed at the quinolone 7-position and is consistent with the notion that 2,5-diazabicyclo[2.2.1]heptane behaves more like a pyrrolidine than piperazine.²¹

Removal of the methyl group from the distal nitrogen, as in 3c, led to a substantial decrease in activity against bacteria. As with the 2 series, an increase in potency at both the enzyme level and in whole bacteria was seen upon introduction of a C₈ halogen. Of all the compounds tested, the most potent compounds against *E. coli* DNA gyrase

(19) Michaels, R. J.; Zaugg, H. E. Synthesis of 9-Methyl-3,9-diazabicyclo[4.2.1]nonane. *J. Org. Chem.* 1960, 25, 637.

(20) Barnes, R. A.; Fales, H. M. The Synthesis of the 3,9-Diazabicyclo[3.3.1]nonane Ring System. *J. Am. Chem. Soc.* 1953, 75, 975-977.

(21) Similar results were obtained in a quinolone series containing piperazine at the 7 position: (a) Domagala, J. M.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Nichols, J. B.; Solomon, M.; Worth, D. F. 1-Substituted 7-[3-[(Ethylamino)methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolone-carboxylic Acids. New Quantitative Structure-Activity Relationships at N₁ for the Quinolone Antibacterials. *J. Med. Chem.* 1988, 31, 991-1001. (b) Domagala, J. M.; Bridges, A. J.; Culbertson, T. P.; Gambino, L.; Hagen, S. E.; Harrick, G.; Porter, K.; Sanchez, J. P.; Sessie, J. A.; Spense, G.; Szotek, D. D.; Wemple, J. Synthesis and Biological Activity of 5-Amino and 5-Hydroxyquinolones, and the Overwhelming Influence of the Remote N₁-Substituent in Determining the Structure-Activity Relationship. *J. Med. Chem.* 1991, 34, 1142-1154. (c) Hagen, S. E.; Domagala, J. M.; Heifetz, C. L.; Johnson, J. Synthesis and Biological Activity of 5-Alkyl-1,7,8-Trisubstituted-6-fluoroquinolone-3-carboxylic Acids. *J. Med. Chem.* 1991, 34, 1155-1161.

(22) The effect of chirality at C7 on antibacterial activity is variable and is dependent on the structure of both the N1 and C7 substituents: (a) Remuzon, P.; Bouzard, D.; DiCesare, P.; Essiz, M.; Jacquet, J. P.; Kiechel, J. R.; Ledoussal, B.; Kessler, R. E.; Fung-Tomc, J. Fluoronaphthyridines and Quinolones as Antibacterial Agents. 3. Synthesis and Structure-Activity Relationships of New 1-(1,1-Dimethyl-2-fluoroethyl)-1-[1-Methyl-1-(fluoromethyl)-2-fluoroethyl], and 1-[1,1-(Difluoromethyl)-2-fluoroethyl] Substituted Derivatives. *J. Med. Chem.* 1991, 34, 29-37. (b) Chu, D. T. W.; Nordeen, C. W.; Hardy, D. J.; Swanson, R. N.; Guardina, W. J.; Pernet, A. G.; Plattner, J. J. Synthesis, Antibacterial Activities, and Pharmacological Properties of Enantiomers of Temafloxacin Hydrochloride. *J. Med. Chem.* 1991, 34, 168-174.

Table III. In Vitro Antibacterial Activity against Selected Laboratory Strains of Veterinary Pathogens [MIC's ($\mu\text{g}/\text{mL}$)]

compd	microorganisms (strain) ^a											gyrase ^b
	Bb	Ec	Ph	Pm	Sc	Sa	Apl	Apy	By	Cp	Th	
2a	1.56	0.20	0.39	0.10	0.39	0.39	0.78	>3.13	>3.13	0.20	1.56	3.12
2c	0.78	0.05	0.05	0.01	0.05	0.39	0.05	>3.13	3.13	0.20	0.39	0.78
2d	0.39	<0.05	<0.05	<0.05		0.10	<0.05	0.78	3.12	3.12	1.56	0.39
2e	0.39	<0.05		<0.05	<0.05	0.10	<0.05	0.39	0.78	0.20	6.25	0.19
2g	1.56	0.20	0.10	0.02	0.20	0.20	0.10	3.13	1.56	0.10	0.78	1.56
2h	>1.56	0.20	0.20	0.02	0.39	0.39	0.05	>3.13	3.13	0.39	1.56	0.39
2i	>1.56	0.78	0.78	0.05	1.56	0.78	0.78	>3.13	>3.13	0.78	>3.13	1.56
2j	>1.56	0.20	0.05	0.02	0.20	0.20	0.10	>3.13	3.13	0.39	3.13	1.56
2k	1.56	0.10	0.05	0.01	0.20	0.10	0.005	0.78	0.78	0.20	>3.13	0.78
2l	>1.56	0.20	0.10	0.02	0.39	0.20	0.10	1.56	1.56	0.39	>3.13	0.78
2m	>1.56	0.20	0.10	0.01	0.39	0.20	0.10	3.13	0.78	0.39	>3.13	3.12
3a	1.56	0.78	0.39	0.20	0.39	>1.56	0.39	3.13	>3.13	0.39	1.56	3.12
3c	>1.56	0.10	0.20	0.20	0.05	1.56	0.10	3.13	>3.13	0.10	1.56	0.39
3d	0.39	0.01	0.005	0.005	0.01	0.39	<0.005	0.39	1.56	0.05	1.56	0.09
3e	0.39	0.02	0.005	0.005	0.02	0.20	0.005	0.39	0.10	0.10	1.56	0.09
3g	>1.56	0.10	0.10	0.02	0.20	0.78	0.10	3.13	3.13	0.20	1.56	1.56
3h	>1.56	0.10	0.10	0.10	0.20	0.78	0.10	>3.13	>3.13	0.20	0.78	0.78
3l	1.56	0.05	0.05	0.01	0.10	0.39	0.02	0.78	1.56	0.20	3.13	0.78
4c	1.56	0.05	0.05	0.02	0.10	0.39	0.05	3.13	3.13	0.20	1.56	0.19
5a	>1.56	0.78	0.39	0.20	0.78	0.20	0.78	>3.13	>3.13	0.39	3.13	3.12
5b	12.5	0.78	0.39	0.20	1.56	0.39	0.20	6.25	>25	1.56	6.25	3.12
5c	1.56	0.10	0.10	0.05	0.20	0.10	0.39	3.13	1.56	0.10	0.78	0.78
5d	3.13	0.20	0.10	0.10	0.39	0.39	0.39	1.56	25	0.78	0.78	0.78
5e	>1.56	0.39	0.20	0.05	0.78	0.39	0.20	3.13	0.78	0.78	>3.13	1.56
5g	>1.56	1.56	0.78	0.20	>1.56	0.78	0.78	>3.13	>3.13	0.78	3.13	1.56
5n	3.13	0.20	0.20	0.20	0.39	0.20	0.10		6.25	0.39	3.13	3.12
5o	6.25	0.78	0.78	0.39	1.56	1.56	0.39	>3.13	>3.13	0.78	>3.13	0.39
5p	3.13	0.39	0.10	0.10	0.78	1.56	0.10	0.78	6.25	0.39	3.13	6.25
5q	12.5	0.78	0.39	0.10	0.78	0.20	0.20	3.13	6.25	0.78	1.56	0.39
5r	>25	3.13	0.20	0.78	3.13	0.20	0.78	3.13	12.5	1.56	0.78	3.12
6a	>1.56	>1.56	1.56	0.39	>1.56	1.56	0.78	>3.13	>3.13	1.56	>3.13	1.56
6b	1.56	0.39	0.78	0.20	1.56	0.78	0.78	>3.13	>3.13	3.13	>3.13	3.12
6c	>1.56	0.39	0.20	0.05	0.39	0.39	0.20	3.13	3.13	0.20	1.56	3.12
6d	>1.56	0.10	0.10	0.02	0.10	0.20	0.10	1.56	0.78	0.39	>3.13	0.39
6f	0.78	0.39	0.39	0.10	0.78	0.78	0.20	>3.13	>3.13	0.78	3.13	3.12
6g	>1.56	1.56	1.56	0.20	>1.56	0.78	0.78	>3.13	>3.13	0.39	3.13	0.19
6n	1.56	1.56	1.56	0.39	>1.56	0.78	0.39	>3.13	>3.13	1.56	>3.13	3.12
6q	1.56	0.78	1.56	0.20	1.56	1.56	0.78	>3.13	>3.13	0.78	3.13	6.25
6r	>25	6.25	6.25	6.25	6.25	1.56						6.25
7a	>1.56	0.20	0.10	0.05	0.20	0.39	0.05	3.13	>3.13	0.10	1.56	1.56
7b	0.78	0.20	0.20	0.10	0.20	0.39	0.05	>3.13	1.56	0.78	1.56	1.56
7c	0.39	0.02	0.01	<0.002	0.02	0.05	0.05	1.56	0.39	0.05	0.39	0.39
7d	0.39	0.01	0.01	0.002	0.01	0.05	0.01	0.39	0.10	0.10	0.78	0.39
7g	1.56	0.20	0.10	0.02	0.20	0.20	0.05	3.13	0.78	0.10	0.78	0.78
7n	1.56	0.20	0.02	0.05	0.10	0.78	0.02	>3.13	>3.13	0.20	1.56	0.39
7o	6.25	0.39	<0.05	0.20	0.20	0.78	<0.05	3.13	6.25	0.78	6.25	0.19
7p	3.13	0.20	<0.05	<0.05	0.39	0.78	<0.05	1.56	6.25	0.78	3.13	1.56
7q	1.56	0.10	0.05	0.05	0.10	0.20	0.02	3.13	3.13	0.20	0.39	0.39
7s	12.5	0.39	0.10	0.10	1.56	1.56	<0.05	6.25	>25	0.78	3.13	1.56
8a	>1.56	0.20	0.10	0.02	0.20	0.39	0.05	>3.13	3.13	0.39	0.39	3.12
8c	0.78	0.05	0.02	0.002	0.05	0.10	0.02	3.13	0.78	0.20	0.20	0.78
8g	1.56	0.10	0.05	0.01	0.20	0.10	0.05	3.13	0.39	0.20	0.20	0.78
9a	6.25	1.56	0.10	0.78	1.56	0.10	0.20	6.25	1.56	0.39	6.25	3.12
9c	1.56	0.39	0.20	0.05	0.39	0.20	0.10	3.13	1.56	0.39	>3.13	1.56
1a	6.25	0.10	0.05	0.05	0.10	3.13	<0.05	12.5	25	0.39	6.25	0.78

(norfloxacin)

^a Aerobically grown: Bb = *Bordetella bronchiseptica* (73A009); Ec = *Escherichia coli* (51A538); Ph = *Pasteurella haemolytica* (59B018); Pm = *Pasteurella multocida* (59A006); Sc = *Salmonella choleraesuis* (58B015); Sa = *Staphylococcus aureus* (54B004). Anaerobically grown: Apl = *Actinobacillus pleuropneumoniae* (54B004); Apy = *Actinomyces pyogenes* (14D002); Bv = *Bacteroides vulgatus* (78E029); Cp = *Clostridium perfringens* (10A009); Th = *Treponema hyodysenteriae* (94A007). ^b Gyrase = The *E. coli* DNA cleavage endpoint value which is the minimum amount of drug required ($\mu\text{g}/\text{mL}$) to induce detectable cleavage of supercoiled pBR322 substrate to the linear form.^{8a,8b,28}

were the desmethyl C₈ fluoro and chloro derivatives **3d** and **3e** (0.09 $\mu\text{g}/\text{mL}$).²³

(23) Although gyrase is considered the target enzyme for the fluoroquinolones there is often a poor correlation between gyrase inhibitory concentrations and MIC's. It was recently found that inhibition of DNA synthesis correlates more closely with antibacterial activity: Piddock, L. J. V.; Walters, R. N.; Diver, J. M. Correlation of Quinolone MIC and Inhibition of DNA, RNA, and Protein Synthesis and Induction of the SOS Response in *Escherichia coli*. *Antimicrob. Agents Chemother.* 1990, 34, 2331-2336.

In the 8-methyl-3,8-diazabicyclo[3.2.1]oct-3-yl (5) series, halogenation of N₁ cyclopropyl derivatives at the C₈ position to give **5d,e** had the effect of either decreasing in vitro activity or simply maintaining it. In no case was there an increase in potency upon halogenation as was observed with the 2, 3, and 7 bearing series. The other N₁-substituted compounds in this series were all less potent than the N₁ cyclopropyl analogue **5c**.

Compounds containing 9-methyl-3,9-diazabicyclo-[4.2.1]non-3-yl (**6**) or 9-methyl-3,9-diazabicyclo[3.3.1]non-3-yl (**9**) as 7-position substituents showed decreased an-

Table IV. In Vivo Mouse Protection Assay

compd	route	organism: protective dose (PD ₅₀), mg/kg		
		P. <i>multocida</i>		S. <i>choleraesuis</i>
		59A006	51A266	58B010
2a	sc	1.35	1.45	
	po			
2c	sc	0.31	0.40	1.12
	po	0.38	0.80	2.42
2g	sc	0.29	0.94	
	po			
3c	sc	0.20	0.92	
	po			
4c	sc	0.22		
	po			
5a	sc	1.31	2.23	
	po	2.21		
5c	sc	0.34	0.15	
	po	0.98	3.80	
5g	sc	0.37		
	po			
6a	sc	1.03		
	po			
6c	sc	0.43		
	po			
7a	sc	0.63	1.10	
	po	1.90	10.23	
7c	sc		0.09	
	po			
8a	sc	0.71	1.36	
	po			
8c	sc	0.15	0.31	
	po			
norflox (1a)	sc	0.63	0.46	
	po	5.54	8.84	

tibacterial activity in vitro (Table III) as well as decreased solubility properties (Table V) and were not further evaluated.

In summary, from the in vitro antibacterial data contained in Table III we determined that the 1,4-diazabicyclo[3.2.2]non-4-yl (7) and the 1,4-diazabicyclo[3.3.1]non-4-yl (8) C₇ substituents conferred the greatest potency to the various nuclei. In addition, it was shown that the N₁-cyclopropyl-containing analogues were the most potent within a given C₇ series. Halogenation at the C₈ position in the N₁ cyclopropyl series can afford compounds with very potent activity against whole bacteria and *E. coli* DNA gyrase, but this is not always the case, as seen in the 8-methyl-3,8-diazabicyclo[3.2.1]oct-3-yl (5) series, where activity is diminished.

Protective dose (PD₅₀) data is shown in Table IV. Overall, the in vitro trends exemplified in Table III were also evident in vivo. Compounds 2c, 2g, 4c, 5c, 6c, 7a, 8a, and 8c, which display the most potent activity in vitro against *Pasteurella multocida*, also show the lowest subcutaneous PD₅₀'s against this strain. Exceptions may be 2c and 5g, which show lower PD₅₀'s than suggested by their MIC's. Worthy of note is that while MIC's against *P. multocida* vary by 200-fold for this group of compounds, subcutaneous PD₅₀'s only differ by approximately 10-fold. Of particular interest is the observation that while norfloxacin 1a is 10- and 20-fold less potent orally than subcutaneously against *P. multocida* and *E. coli*, respectively, compound 2c differs by, at most, only 2-fold.

In addition to in vitro and in vivo potency, a useful therapeutic veterinary agent must optimally exhibit good aqueous solubility properties at physiological pH, in order to allow development of a preconstituted dosage form. With this in mind, the solubilities of a selected group of compounds were measured. The data shown in Table V²⁴

Table V. Aqueous Solubility of Selected Compounds at pH 7.2

compound	solubility, mg/mL	compound	solubility, mg/mL
ciprofloxacin (1b)	0.090	2k	0.019
N-methylciprofloxacin	0.082	3c	0.077
norfloxacin (1a)	0.350	3g	0.027
pefloxacin (N-Me 1a)	0.197	5a	0.530
2a	0.453	5c	0.343
2c	0.444	7a	0.049
2g	0.021	7c	0.079
2h	0.011	9c	0.054

Table VI. Minimum Effective Doses (mg/kg) of Selected Analogues in Cattle and Swine Respiratory Disease Models

model	Compound: MED, mg/kg			
	2c	5a	5c	7a
<i>Pasteurella haemolytica</i> (Cattle, SID × 2)				
IM	0.15	1.2	0.3	0.3
<i>Actinobacillus pleuropneumoniae</i> (Swine, SID × 1)				
IM	0.15	0.6	0.3	0.3
PO	0.6	5.0	2.5	

^a MED's of positive controls: *Pasteurella haemolytica* model—sulbactam/ampicillin 1.6/3.3 mg/kg (one-half the recommended label dose), gentamycin 1.0 mg/kg (small animal label dose 2–4 mg/kg), erythromycin 30 mg/kg (7–15 times the label dose). All positive controls were administered intramuscularly (SID × 2). *Actinobacillus pleuropneumoniae* model—penicillin 13 200 U/kg SID × 1 intramuscularly (two times the normal label dose).

indicate that acceptable solubility properties were shown by compounds 2a,c and 5a,c. The more potent series bearing C₇ substituent 7 (1,4-diazabicyclo[3.2.2]non-4-yl) had less favorable intrinsic aqueous solubility and was not pursued. Soluble compounds from the 8-methyl-3,8-diazabicyclo[3.2.1]oct-3-yl (5) series were considered less potent overall in vitro than compounds from the 5-methyl-2,5-diazabicyclo[2.2.1]hept-2-yl (2) series.²⁵ As compound 2c (danofloxacin)²⁶ had a potent and balanced in vitro spectrum, excellent aqueous solubility and comparable PD₅₀'s in mice following subcutaneous and oral administration, it was chosen for closer study as a potential drug candidate.

Danofloxacin was evaluated against a range of field-isolated veterinary pathogens and was found to exhibit excellent potency.¹ It was also potent against *Mycoplasma* spp: MIC's (μg/mL) *Mycoplasma hyorhinis* ATCC 17981 (0.20), *M. hyopneumoniae* ATCC 25934 (0.20), *M. bovis genitalium* ATCC 19852 (0.20), *M. gallisepticum* ATCC 19610 (≤0.05), and *M. bovis* ATCC 25523 (0.20).²⁷

As exemplified by the data presented in Table VI, extension of the attractive in vitro and pharmacokinetic

(24) Inspection of the solubility data for the standard drugs shows that N-methylation of the piperazine ring of ciprofloxacin does not lead to a significant change in aqueous solubility whereas N-methylation of norfloxacin (1a) to give pefloxacin serves to decrease solubility approximately 1.75-fold from 0.350 mg/mL to 0.197 mg/mL. Interestingly, in the case of desmethyl danofloxacin 3c (aqueous solubility, 0.077 mg/mL) N-methylation had a dramatic influence on aqueous solubility at pH 7.2, leading to a 6-fold improvement (aqueous solubility of danofloxacin, 2c, 0.444 mg/mL) and thus providing the required properties for an injectable formulation.

(25) In addition, 5c was poorly tolerated on intramuscular injection.

(26) Jefson, M. R.; McGuirk, P. R. U. S. Patent No. 4,861,799, 1989.

(27) The MIC's against the *Mycoplasma* strains obtained from the America Type Culture Collection (ATCC) were performed by 2-fold dilution in microtiter trays containing ATCC 243 medium.

profile⁷ observed with danofloxacin (**2c**) to food-producing animal disease models was explored in comparison with analogues **5a**, **5c**, and **7a**. In cattle, activity after im injection (SID × 2) was determined in an acute fibrinonecrotic pneumonia induced by endotracheal challenge with *Pasteurella haemolytica*. In this model, danofloxacin (**2c**) exhibited a minimum effective dose (the lowest dose that showed significant reduction of lung pathology, 65% reduction) of 0.15 mg/kg and was 2–8 times more potent than the other analogues tested. In swine, danofloxacin (**2c**) was compared with **5a**, **5c**, and **7a** after im injection or oral gavage (po) (SID × 1) in a periacute necrohemorrhagic pneumonia produced by intranasal instillation of *Actinobacillus pleuropneumonia*. In this model, danofloxacin (**2c**) was found to be 2–4 times more potent than the other analogues tested when administered im (SID × 1) with a minimum effective dose of 0.15 mg/kg. When administered po (SID × 1) danofloxacin (**2c**) was 4–8 times more effective than the two N₁ ethyl analogues **5a** and **7a**, with a minimum effective dose of 0.6 mg/kg.

Overall, of the bridged diazabicycloalkyl quinolones investigated in this study, danofloxacin (**2c**) demonstrates the best composite of properties. Its excellent aqueous solubility properties, broad spectrum, and potent efficacy in use animal infection models, by two routes of administration, make it an attractive candidate for clinical evaluation.

Experimental Section

General Methods. All materials were obtained from commercial suppliers and used without further purification. Melting points are uncorrected. NMR spectra were recorded on either a Bruker WM-250 (250 MHz) or a Bruker AM-300 (300 MHz) or a Varian XL-300 (300 MHz) spectrometer. High-resolution mass spectra were obtained on either an AEI MS-30 or a Kratos Concept 1S instrument. Elemental analyses were performed by the Analytical Department of the Central Research Division, Pfizer, Inc., Groton, CT and agree within 0.4% of the calculated values. Molecular formulas were confirmed for compounds **2i**, **2j**, **3i**, **5e**, **5o**, **6g**, **6n**, **6r**, **7d**, **7s**, and **8g** by high-resolution mass spectrometry.

General Procedures for the Preparation of Quinolones and Naphthyridinones (Scheme 1). Method A. Preparation of 1-Ethyl-6-fluoro-7-(8-methyl-3,8-diazabicyclo[3.2.1]oct-3-yl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (5a**).** A stirred suspension of 1-ethyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (**10**) (3.0 g, 11.9 mmol) and 8-methyl-3,8-diazabicyclo[3.2.1]octane dihydrochloride (**5**) (4.5 g, 22.7 mmol) in 15 mL of dry pyridine under N₂ was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (0.7 g, 4.6 mmol). The mixture was heated to 80 °C for 3 h. A solution resulted which was cooled to room temperature and poured into 50 mL of water. The aqueous solution was washed five times with 100 mL of chloroform. The combined organic layer was dried over Na₂SO₄, filtered and concentrated in vacuo. The resulting solid was washed with diethyl ether, dissolved in water adjusted to pH = 1 with 1 N HCl and washed with chloroform. The aqueous layer was then neutralized by the addition of saturated NaHCO₃ and the product was extracted with chloroform (5 × 100 mL). The chloroform layer was dried over Na₂SO₄, filtered, and concentrated to 25 mL in volume. The product was then precipitated as a white solid by the addition of 75 mL of diethyl ether to the concentrated solution. The solid was collected by suction filtration, washed with diethyl ether and dried to afford 2.77 g (65%) of **5a**, mp 244–245 °C.

5a was converted to its hydrochloride salt according to the following general procedure: To a suspension of **5a** (1.3 g, 3.6 mmol) in 50 mL of sterile water was added 3.25 mL (3.25 mmol, 0.9 equiv) of 1 N HCl. The mixture was allowed to stir at room temperature for 30 min and was then filtered of undissolved material, and the filtrate was concentrated in vacuo. The resulting solid was taken up in 20 mL of cold methanol, filtered, washed once with 20 mL of cold methanol, and dried to yield 1.0 g (65%)

of the hydrochloride salt of **5a** as a white solid, mp 299–300 °C; ¹H NMR (D₂O) δ 8.35 (1 H, s), 7.27 (1 H, d, *J* = 15.4 Hz), 6.84 (1 H, d, *J* = 7.7 Hz), 4.30 (2 H, q, *J* = 7.7 Hz), 4.15 (2 H, br s), 3.73–3.88 (2 H, m), 3.40–3.55 (2 H, m), 2.92 (3 H, s), 2.28–2.50 (4 H, m), 1.44 (3 H, t, *J* = 7.7 Hz); HRMS calcd for C₁₉H₂₂FN₃O₃ 359.1655, found 359.1705. Anal. (C₁₉H₂₂FN₃O₃·HCl·H₂O) C, H, N.

5a was also converted to its mesylate salt according to the following general procedure: To a suspension of **5a** (132 mg, 0.37 mmol) in 4 mL of dry ethanol was added 24 mL (0.37 mmol, 1.0 equiv) of methanesulfonic acid. The resulting creamy white suspension was heated to reflux for 30 min, cooled to room temperature, filtered, washed with diethyl ether, and dried to give 150 mg (89%) of the mesylate salt of **5a** as a white solid, mp 310–312 °C. Anal. (C₁₉H₂₂FN₃O₃·CH₃SO₃H) C, H, N.

Compounds **2a**, **2e**, **2k**, **2m**, **3a**, **3c**, **4c**, **5b**, **5c**, **5e**, **5g**, **5n–r**, **6a–d**, **6f**, **6n**, **6q**, **6r**, **7p**, **8a**, **8g**, **9a**, and **9c** were also prepared according to method A.

Method B. Preparation of 1-Cyclopropyl-6-fluoro-7-((1*S*,4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]hept-2-yl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (2c**).** A stirred suspension of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (**18**) (2.20 g, 8.30 mmol) and (1*S*,4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]heptane dihydrochloride (**2**) (1.83 g, 9.96 mmol, 1.2 equiv) in 25 mL of dry pyridine under N₂ was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (3.02 g, 19.92 mmol, 2.4 equiv). The reaction mixture was heated to 80 °C for 2 h, during which time a copious precipitate fell. After 2 h of heating the reaction mixture was cooled to room temperature, and the precipitate was collected by suction filtration, washed with cold ethanol, and dried to give 2.39 g (81%) of **2c** as a white solid, mp 275–276 °C.

The mesylate salt of **2c** was prepared by suspending **2c** (2.39 g, 6.69 mmol) in 20 mL of distilled water and adding methanesulfonic acid (642 mg, 6.69 mmol, 1.0 equiv). The resulting solution was stirred at room temperature for 15 min and then poured into 300 mL of absolute ethanol and cooled to 0 °C for 2 h. The resulting crystals were collected by suction filtration and dried to yield a pure white solid (2.79 g, 92%); mp 337–339 °C; ¹H NMR (DMSO-*d*₆) δ 8.56 (1 H, s), 7.82 (1 H, d, *J* = 16.6 Hz), 7.10 (1 H, d, *J* = 8.3 Hz), 4.42 (1 H, br s), 3.58–3.80 (2 H, m), 3.41–3.54 (2 H, m), 3.32 (3 H, s), 2.76 (2 H, br d, *J* = 8.3 Hz), 1.90 (2 H, br d, *J* = 8.3 Hz), 1.01–1.36 (4 H, m); HRMS calcd for C₁₉H₂₀FN₃O₃ 357.1489, found 357.1525. Anal. (C₁₉H₂₀FN₃O₃·CH₃SO₃H·1.5H₂O) C, H, N.

Compounds **2d**, **2g**, **2h**, **2j**, **3d**, **3g**, **3h**, **5d**, **6g**, **7b–d**, **7g**, **7n**, **7o**, **7q**, **7s**, and **8c** were also prepared according to method B.

Method C. Preparation of 1-Ethyl-6-fluoro-7-(1,4-diazabicyclo[3.2.2]non-4-yl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (7a**).** A stirred suspension of 1-ethyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (**10**) (18.4 g, 72.8 mmol) and 1,4-diazabicyclo[3.2.2]nonane (**7**) (13.8 g, 109.2 mmol) in 500 mL of dry pyridine under N₂ was heated to 100 °C for 18 h. The resulting solution was then cooled to room temperature resulting in the formation of a thick slurry. This slurry was then poured into 1.5 L of stirring cold water. The resulting mixture was a clear solution immediately following the addition of the cooled reaction mixture to the cold water, and then gradually a precipitate formed with continued stirring. After 30 min of stirring the precipitate was filtered, washed three times with 100 mL of cold ethanol, and dried to afford 21.5 g (82%) of **7a**: mp 246–247 °C; ¹H NMR (CDCl₃) δ 8.60 (1 H, s), 8.00 (1 H, d, *J* = 13.5 Hz), 6.75 (1 H, d, *J* = 7.7 Hz), 4.30 (2 H, q, *J* = 6.9 Hz), 4.02 (1 H, br s), 3.5–3.58 (2 H, m), 3.20–3.30 (2 H, m), 3.00–3.20 (4 H, m), 2.05–2.25 (2 H, m), 1.74–1.95 (2 H, m), 1.59 (3 H, t, *J* = 6.9 Hz); HRMS calcd for C₁₉H₂₂FN₃O₃ 359.1646, found 359.1648.

The mesylate salt of **7a** was prepared by suspending **7a** (48.8 g, 136 mmol) in 150 mL of distilled water and adding methanesulfonic acid (13.4 g, 140 mmol, 1.03 equiv). The resulting solution was stirred at room temperature for 15 min and then poured into 1.0 L of absolute ethanol and cooled to 0 °C for 2 h. The resulting crystals were collected by suction filtration and dried to yield a pure white solid (43.3 g, 79%); mp 325–327 °C. Anal. (C₁₉H₂₂FN₃O₃·CH₃SO₃H) C, H, N.

Compounds **2i**, **2l**, **3e**, and **3l** were also prepared according to method C.

6,7-Difluoro-1-vinyl-1,4-dihydro-4-oxo-3-quinoline-carboxylic Acid (13). To a mixture of 1.26 g (4.98 mmol) of ester 29a and 12.5 mL of vinyl acetate under a N₂ atmosphere was added 12.5 mg (0.037 mmol) of Na₂(PdCl₄). The resulting mixture was heated to reflux for 40 h. At 20 h, 10 mL of DMF (for solubility) and an additional 30 mg (0.089 mmol) of Na₂(PdCl₄) was added. The reaction was then cooled and the vinyl acetate was removed under reduced pressure. The residue was taken up in 150 mL of chloroform, filtered of starting material (0.54 g recovered), and washed with three 200-mL portions of water. The washed organic layer was dried over anhydrous MgSO₄ and evaporated to a gold solid. The crude product was purified by SiO₂ column chromatography eluting with 7:3 ethyl acetate/hexanes to give 0.82 g (59%) of pure ethyl 6,7-difluoro-1-vinyl-1,4-dihydro-4-oxoquinoline-3-carboxylate as pale yellow needles: mp 150–151.5 °C; ¹H NMR (DMSO-*d*₆) δ 8.60 (1 H, s), 8.09 (1 H, dd, *J* = 10.7, 8.9 Hz), 7.99 (1 H, dd, *J* = 12.2, 6.6 Hz), 7.47 (1 H, dd, *J* = 14.9, 8.1 Hz), 5.83 (1 H, dd, *J* = 14.9, 1.7 Hz), 5.51 (1 H, dd, *J* = 8.1, 1.7 Hz), 4.24 (2 H, q, *J* = 7.1 Hz), 1.29 (3 H, t, *J* = 7.1 Hz). The intermediate ester (0.80 g, 2.87 mmol) was taken up in 20 mL concentrated H₂SO₄ and heated to 80 °C for 5 h. After cooling, the dark brown solution was poured over ice resulting in a precipitate which was filtered, washed several times with cold methanol, and dried to give 492 mg (68%) of 13 as a pale gray powder: mp 207–210 °C; ¹H NMR (DMSO-*d*₆) δ 8.83 (1 H, s), 8.29 (1 H, dd, *J* = 10.3, 8.7 Hz), 8.18 (1 H, dd, *J* = 12.0, 6.6 Hz), 7.57 (1 H, dd, *J* = 14.8, 8.0 Hz), 6.00 (1 H, dd, *J* = 14.8, 2.0 Hz), 5.67 (1 H, dd, *J* = 8.0, 2.0 Hz); FAB-HRMS calcd for C₁₂H₈F₂NO₃ (P + H)⁺ 252.0501, found 252.0472.

DNA Gyrase Purification. DNA gyrase subunits A (1.9 × 10⁴ U/mg) and B (0.54 × 10⁴ U/mg) were purified from *Escherichia coli* overproducer strains by the method of Mizuuchi et al.²⁸ One unit of gyrase activity is defined as the amount of enzyme necessary to completely supercoil 0.3 mg of pBR322 in 30 min at 37 °C; one unit of holoenzyme used in the gyrase assays is composed of one unit activity of subunit A and one unit of subunit B (assayed independently in the excess of the complementary subunit).

DNA Gyrase Cleavage Assay. The general protocol followed was modified from Gellert et al.²⁸ and Sugino et al.²⁹ Approximately 2 units of reconstituted DNA gyrase holoenzyme were incubated in 35 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 5 mM spermidine hydrochloride, 5 mM DDT, 0.2 mM ATP, 50 mg/mL bovine serum albumin, 100 mg/mL *E. coli* t-RNA, 0.3 mg of pBR322 DNA in 30-mL reactions containing the appropriate concentration of drug being tested (usually 2-fold serial dilutions from 500 mg/mL down to 0.39 mg/mL) at 25 °C. After 60 min, the reaction is stopped by the addition of sodium dodecyl sulfate to a final concentration of 0.2% SDS, followed by deproteination by the addition of proteinase K at 90 mg/mL, and incubation for 30 min at 37 °C. After deproteination, the reaction mixture is combined with 1 mL of tracking dye (50% glycerol (w/v) and 0.125% bromophenol blue), loaded onto a 0.7% TBE-agarose gel (89 mM tris(hydroxymethyl)aminomethane, 89 mM boric acid, and 2 mM EDTA, pH 8.3), and electrophoresed. The denaturing of the drug-gyrase-DNA complex leads to the appearance of linear DNA detected as a single band between relaxed and supercoiled DNA after separation of the products by electrophoresis, staining with ethidium bromide, and photographed on a UV-light transilluminator. The DNA cleavage endpoint value is the minimum amount of drug required to induce detectable cleavage of the supercoiled pBR322 substrate to the linear form.

Aqueous Solubility Measurements. An excess amount of each compound (~5 mg) was equilibrated at room temperature (23 ± 1 °C) using a wrist-action shaker in two separate vials containing 3 mL of 0.05 M phosphate buffer at pH 7.2. At various time points up to three days, duplicate 200-mL samples of each suspension were removed and forced through a microfiltration device equipped with a 0.45-mm nylon filter. Two aliquots of each filtrate were then diluted with mobile phase and assayed by HPLC. After each sampling dilute NaOH or phosphoric acid

was utilized to adjust the pH of the remaining suspension to 7.2. Preliminary studies suggested that 3 days is a sufficient time for equilibration to occur in most cases for these compounds. No effort was made to characterize the solid material remaining in the vials at the end of the study period.

Antibacterial Susceptibility Test. MIC's were determined as described by Dirlam et al.²⁹ as modified by Jefson et al.³⁰ All anaerobically grown bacteria were tested on Tryptose agar (Difco) supplemented with 5% bovine blood (TBA) and incubated 48 h at 39 °C in a Coy (Ann Arbor, MI) anaerobe chamber containing an N₂/CO₂/H₂ (80:10:10) atmosphere. MIC's for aerobically grown bacteria were determined in an identical manner except Brain heart infusion (BHI) agar (Difco) was used and plates were incubated aerobically at 37 °C for 18–20 h.

Mouse Protection Studies (PD₅₀). Mice in groups of 10 (female CF-1 mice (Charles River Labs) weighing 15 grams each) were infected with an intraperitoneal challenge consisting of 0.5 mL of the appropriate bacterial suspension (*P. multocida* 59A006, 3.0 × 10⁸ cfu/mL; *E. coli* 51A266, 3.0 × 10⁸ cfu/mL; *S. choleraesuis* 58B010, 8.5 × 10² cfu/mL). Mice were treated with drug administered either subcutaneously (sc) or orally (po) at 30 min, 4 h, and 24 h after challenge. The percentage of surviving mice was recorded at 96 h post challenge and the in vivo potency of each drug was determined by calculating the PD₅₀ (dose which protects 50% of the infected animals) by means of probit analysis.

Bovine Pasteurella haemolytica Respiratory Model. Animals. All calves were inspected at the source for good respiratory health. If calves were from a new source, a nasal swab was taken to establish absence of *P. haemolytica*, and at least two calves were moved on station and challenged with *P. haemolytica* to insure susceptibility.

Inoculum. *P. haemolytica* strain B280PA was grown overnight at 37 °C in TP (tryptose phosphate broth enriched with 10% heat-inactivated equine serum). An aliquot of this overnight culture was standardized by optical density measurements (OD) and used to inoculate 200-mL shake cultures which were incubated for 4.5–5.5 h at 37 °C. The cultures were centrifuged (8000g) at 4 °C and the pellets washed once in tryptose phosphate broth. The bacteria were resuspended in cold broth and standardized by OD so that the inoculum was approximately 5 × 10⁸ cfu/mL.

Challenge Procedure. The day prior to challenge, body temperatures were recorded, calves were acid treated intratracheally with 0.6 mL of 8% acetic acid (24 h) and the treatment groups allotted. On day 0, calves were acid treated as before (4 h), body temperature and clinical parameters recorded (dyspnea, coughing and depression), and the calves challenged with a 50-mL inoculum (approximately 5 × 10⁸ cfu/mL) endotracheally.

Treatment. All medication was administered according to experimental design after challenge. Calves were dosed by im injection with either a 2.5% or a 5% solution of the test compound in sterile water into the area of semimembranosus or semitendinosus muscles on day 0 and day 1 (SID × 2).

Evaluation Parameters. Body temperature and clinical parameters were recorded daily (day 0–5). At trial termination, all surviving calves were killed, the lungs and heart removed and the pleural cavity inspected. The lungs were then examined grossly and the lesions scored. *In this model, lung pathology as reflected in lung lesion scores and mortality were considered key indicators of disease severity.* Sections of the lung or lesion were also removed and cultured. Any calf dying prior to day 5 was necropsied and the lungs scored and cultured.

Swine Actinobacillus pleuropneumoniae Respiratory Model. Animals. Cross-bred swine weighing 8.5–12.5 kg were purchased from local sources 10–14 days prior to the planned initiation of the experiment. All pigs received ration PG-5 Banminth/Mecadox for 5 days and then nonmedicated PG-5 for the remaining preconditioning period and during the experimental

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period. Pigs were allotted to treatment groups one day prior to the challenge with *A. pleuropneumoniae* inoculum.

Inoculum. *A. pleuropneumoniae* strain B316HM was incubated on enriched Brain heart infusion (BHI) agar plates overnight in 5–10% CO₂ at 37 °C. The bacteria were rinsed from the plates with saline, diluted, and standardized by OD so that 10⁹ cfu/mL of bacteria were present in the inoculum.

Challenge Procedure. Pigs were challenged by intranasal administration of the inoculum. Three 1-mL increments were administered per nostril using a 50-mL Roux Automatic syringe fitted with a small teat cannula.

Treatment. All im-dosed pigs were treated according to the experimental design within 1 h of challenge and in the order of challenge. Intramuscular (im) administration of either a 1% or a 2.5% solution of the test compound in sterile water was made in the ham with a 20-gauge needle. Oral gavage dosing of 0.5% solutions in sterile water was via a gastric tube and all medication was rinsed from the tube with water before it was removed. All medication was administered only once (SID × 1).

Evaluation Parameters. All pigs were evaluated daily during the experiment for body temperature, depression, and dyspnea. At necropsy, the lungs of all pigs were scored for lung pathology.

As with the *P. haemolytica* model, lung pathology as reflected in the lung lesion scores and mortality are considered the key indicators of disease severity. A sample of lung was also cultured.

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Supplementary Material Available: Proton magnetic resonance (¹H NMR) and high-resolution mass spectral (HRMS) data for all compounds included in Table I (2a–9c) (15 pages). Ordering information is given on any current masthead page.

3,4-Dihydroquinolin-2(1H)-ones as Combined Inhibitors of Thromboxane A₂ Synthase and cAMP Phosphodiesterase¹

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A series of 1H-imidazol-1-yl- and 3-pyridyl-substituted 3,4-dihydroquinolin-2(1H)-ones was designed and synthesized as combined inhibitors of thromboxane (TXA₂) synthase and cAMP phosphodiesterase (PDE) in human blood platelets. A number of structures, e.g. 4b, 7a, 7e, 13a, and 21–25, were superior to dazoxiben 26 as inhibitors of TXA₂ synthase in in vitro ADP-induced aggregation experiments with human blood platelets. The TXA₂ synthase inhibitory activity was confirmed by measurement of the prostanoid metabolites derived from ¹⁴C-labeled arachidonic acid. Three compounds (7a, 7e, and 25) demonstrated in vitro inhibition of human platelet cAMP PDE at micromolar concentrations in conjunction with their TXA₂ synthase inhibitory activity. Synergistic enhancement of antiaggregatory and antithrombotic actions was expected when simultaneous stimulation of adenylate cyclase (through increased PGI₂ production) and inhibition of platelet cAMP PDE were possible from the same compound. Ex vivo and in vivo experiments were conducted in rats and mice, respectively, to evaluate the effects of compounds 7e and 23 on platelet aggregation and thrombotic events within these animals. Compound 7e, which has a comparable level of TXA₂ synthase (IC₅₀ 1.2 μM) and human platelet cAMP PDE (IC₅₀ 6.4 μM) inhibitory activities, was found to be orally bioavailable with a long duration of action and offered effective protection against mortality in a collagen-epinephrine-induced pulmonary thromboembolism model in mice. Significant blood pressure and heart rate effects were observed for several compounds, e.g. 7e, 9e, 13a, 13d, 18, 20, 21, and 23, when dosed orally in conscious spontaneously hypertensive rats.

Introduction

During the past several years, investigations directed toward the inhibition of blood platelet aggregation have involved a number of approaches which include adenylate cyclase stimulation, cAMP phosphodiesterase inhibition, thromboxane (TXA₂) synthase inhibition, or TXA₂ receptor antagonism.^{2–4} As evidenced by the number of pathways considered, the problem of effective in vivo control of thrombosis and hemostasis is a multidimensional challenge. An approach which would address more than one of these pathways simultaneously may prove more effective than a unilateral approach in the inhibition of platelet aggregation.

In the arachidonic acid metabolic cascade, in vitro inhibition of TXA₂ production in human platelet rich

plasma, in the presence of prostacyclin (PGI₂) synthase, has been shown to redirect the balance of prostaglandin synthesis in favor of the production of PGI₂.⁵ The an-

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