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Synthesis and Structure–Activity Relationships of 7-Diazabicycloalkylquinolones, Including Danofloxacin, a New Quinolone Antibacterial Agent for Veterinary Medicine[1]

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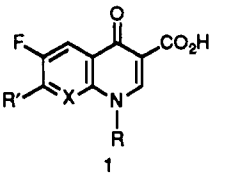
A series of novel 6-fluoro-7-diazabicycloalkylquinolonecarboxylic acids substituted with various C_8 (H, F, Cl, N) and N_1 (ethyl, cyclopropyl, vinyl, 2-fluoroethyl, 4-fluorophenyl, 2,4-difluorophenyl) substituents, as well as, 9-fluoro-10-diazabicycloalkylpyridobenzoxazinecarboxylic acids, were prepared 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-diazabicylo[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 N_1 cyclopropyl group and the C_7 (1*S*,4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]hept-2-yl appendage conferred the best overall antibacterial, physiochemical, 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**).



1

	R'	*X*	*R*
1a	1-piperazinyl	CH	C_2H_5
b	1-piperazinyl	CH	$-C_3H_7$
c	1-piperazinyl	N	C_2H_5
d	4-methyl-1-piperazinyl	$-COCH_2CH(CH_3)-$	
e	4-methyl-1-piperazinyl	CF	CH_2CH_2F
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

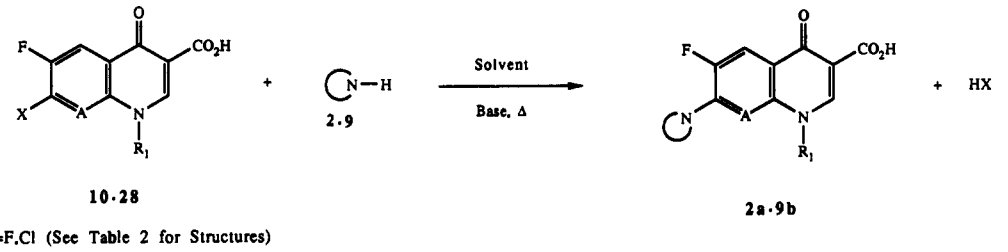
(1) This work was previously presented in part: McGuirk, P. R.; Jefson, M. R.; Mann, D. D.; Hindahl, M. S.; Cornell, C. P.; Weber, F. H. The Synthesis and Antibacterial Activity of 6-Fluoro-7-diazabicycloalkylquinolones related to CP-76,136 (Danofloxacin). *Abstracts of the 29th Interscience Conference 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.

0022-2623/92/1835-0611$03.00/0

Scheme I

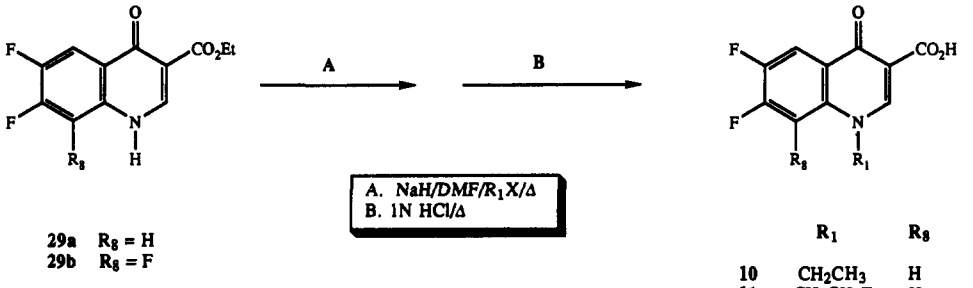


10·28

X=F,Cl (See Table 2 for Structures)

2a·9b

Scheme II

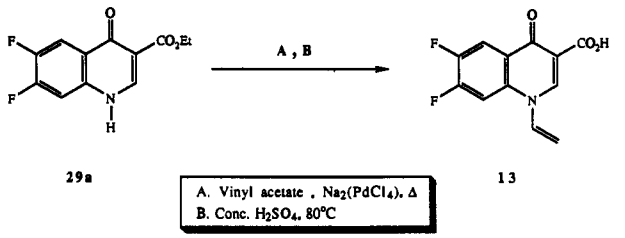


29a R_8 = H
29b R_8 = F

	R_1	R_8
10	CH_2CH_3	H
11	CH_2CH_2F	H
12	CH_3	H
14	CH_2CH_3	F
15	CH_2CH_2F	F

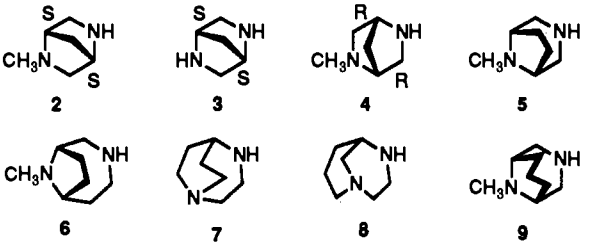
a quinolone with suitable properties would be of considerable interest in this area, where the need for new therapy, particularly against resistant strains of primarily Gram-negative bacteria, is great. As part of our effort to discover a new agent for this use we chose to investigate the properties of a group of novel[4] 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 C_7 would have a favorable effect on some of the physiochemical properties of these molecules (e.g. aqueous solubility[6] and pharmacokinetics[7]), while retaining an excellent antibacterial profile. The compounds presented were prepared by reacting the diazabicycloalkanes **2–9** with

Scheme III



29a

13



2 3 4 5

6 7 8 9

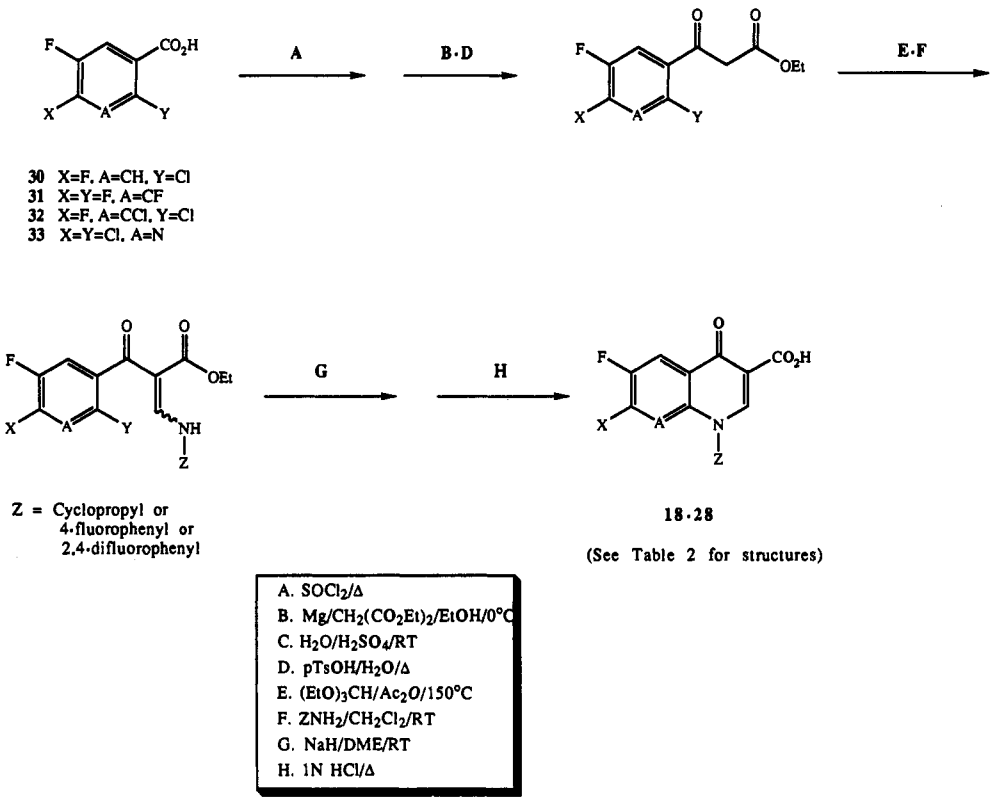
(4) During the course of this work several of the 7-diazabicycloalkylquinolone-3-carboxylic acids discussed in this paper were independently disclosed by another group. See: Hutt, M. P.; Mich, T. F.; Culbertson, T. P. U. S. Patent 4,571,396, 1986.

(5) Bulky C_7 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 (4*S*)-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 C_7-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 models[9] (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 Sturm[10] by alkylation of the esters, **29a,b** at N_1 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 $Na_2(PdCl_4)$[11] followed by ester hydrolysis in concentrated H_2SO_4 (Scheme III). Compounds **16**[12] and **17**[13] 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 **33**[14] 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 **8**[18] were each synthesized via slight

(8) (a) Sugino, A.; Peebles, C. L.; Kreuzer, K. N.; Cozzarelli, N. R. Mechanism of Action of Nalidixic Acid: Purification of *Escherichia coli NalA* Gene Product and its Relationship to DNA Gyrase and a Novel Nicking-closing Enzyme. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 4767–4771. (b) Gellert, M.; Mizuuchi, K.; O'Dea, M. H.; Itoh, T.; Tomizawa, J. Nalidixic Acid Resistance: A Second Genetic Character Involved in DNA Gyrase Activity. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 4772–4776. (c) Cozzarelli, N. R. DNA Gyrase and the Supercoiling of DNA. *Science* **1980**, *207*, 953–960. For reviews see: (d) Gellert, M. DNA Topoisomerases. *Ann. Rev. Biochem.* **1981**, *50*, 879–910. (e) Vosberg, H.-P. DNA Topoisomerases: Enzymes That Control DNA Conformation. *Curr. Top. Microbiol. Immunol.* **1985**, *114*, 19–102.

(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.

(10) Sturm, E. U.S. Pat. 4,264,604, 1981.

(11) Bayer, E.; Geckeler, K. Homogeneous Catalytic Vinylation of Cyclic Imides and Lactams for the Synthesis of N-Vinyl Monomers. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 533–534.

(12) Hayakawa, I.; Hiramitsu, T.; Tanaka, Y. U.S. Pat. 4,382,892, 1983.

(13) Wentland, M. P.; Bailey, D. M.; Cornett, R. A.; Dobson, R. A.; Powles, R. G.; Wagner, R. B. Novel Amino-Substituted 3-Quinolonecarboxylic Acid Antibacterial Agents: Synthesis and Structure-Activity Relationships. *J. Med. Chem.* **1984**, *27*, 1103–1108.

(14) Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Gracey, E. H.; Pernet, A. G. Synthesis and Structure-Activity Relationships of New Arylfluoronaphthyridine Antibacterial Agents. *J. Med. Chem.* **1986**, *29*, 2363–2369.

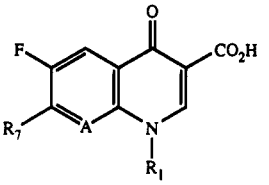
(15) Grohe, K.; Petersen, U.; Kuck, K.-H. U. S. Pat. 4,563,459, 1986.

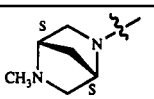
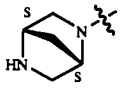
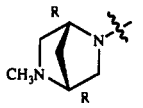
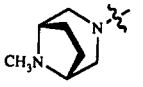
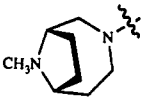
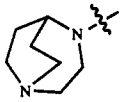
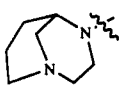
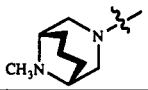
(16) Portoghese, P. S.; Mikhail, A. A. Bicyclic Bases: Synthesis of 2,5-Diazabicyclo[2.2.1]heptanes. *J. Org. Chem.* **1966**, *31*, 1059–1062.

(17) Henry, D. W.; Sturm, P. A. U. S. Pat. 3,947,445, 1976.

(18) Rubstov, M. V.; Mikhlina, 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. Mikhlina, 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.

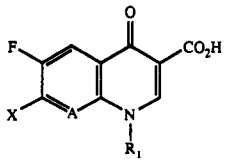
Table I. 7-Diazabicycloalkyl-Substituted Quinolones and Napthyridinones



compd	R1[a]	A	R7	method (% yield)	mp, °C	formula[b]
2a	Et	CH		A (91)	235–237	$C_{18}H_{20}FN_3O_3 \cdot 1.25H_2O$
2c	Cpr	CH		B (81)	337–339	$C_{19}H_{20}FN_3O_3 \cdot 1.5H_2O \cdot CH_3SO_3H$
2d	Cpr	CF		B(66)	295–297	$C_{19}H_{19}F_2N_3O_3 \cdot 0.5H_2O \cdot CH_3SO_3H$
2e	Cpr	CCl		A (48)	175–177	$C_{19}H_{19}ClFN_3O_3 \cdot 0.25H_2O$
2g	4-FPh	CH		B (85)	271–273	$C_{22}H_{19}F_2N_3O_3 \cdot 0.5H_2O \cdot CH_3SO_3H$
2h	4-FPh	CF		B (55)	288–289	$C_{22}H_{18}F_3N_3O_3 \cdot 0.5H_2O \cdot CH_3SO_3H$
2i	4-FPh	CCl		C (54)	200–201	$C_{22}H_{18}ClF_2N_3O_3$[c]
2j	4-FPh	N		B (66)	314–315	$C_{21}H_{18}F_2N_4O_3$[c]
2k	2,4-F_2Ph	CH		A (60)	209–212	$C_{22}H_{18}F_3N_3O_3 \cdot 2.5H_2O$
2l	2,4-F_2Ph	CF		C (45)	228–230	$C_{21}H_{17}F_4N_3O_3$[c]
2m	2,4-F_2Ph	CCl		A (51)	243–245	$C_{22}H_{17}ClF_3N_3O_3 \cdot 1.25H_2O$
3a	Et	CH		A (14)	283–285	$C_{17}H_{18}FN_3O_3 \cdot 0.25H_2O$
3c	Cpr	CH		A (63)	301–302	$C_{18}H_{18}FN_3O_3 \cdot 2.25H_2O$
3d	Cpr	CF		B (78)	258–260	$C_{20}H_{17}F_2N_3O_3 \cdot 1.5H_2O \cdot CH_3SO_3H$
3e	Cpr	CCl		C (45)	242–244	$C_{18}H_{17}ClFN_3O_3 \cdot 3.25H_2O$
3g	4-FPh	CH		B (92)	294–295	$C_{21}H_{17}F_2N_3O_3$
3h	4-FPh	CF		B (72)	290–292	$C_{21}H_{16}F_3N_3O_3 \cdot 2.25H_2O$
3l	2,4-F_2Ph	CF		C (50)	244–246	$C_{21}H_{15}F_4N_3O_3$[c]
4c	Cpr	CH		A (48)	310–312	$C_{19}H_{20}FN_3O_3 \cdot 1.5H_2O \cdot CH_3SO_3H$
5a	Et	CH		A (89)	310–312	$C_{19}H_{22}FN_3O_3 \cdot CH_3SO_3H$
5b	Et	CF		A (21)	227–228	$C_{19}H_{21}F_2N_3O_3 \cdot 0.125H_2O$
5c	Cpr	CH		A (86)	306–308	$C_{20}H_{22}FN_3O_3 \cdot 1.5H_2O \cdot CH_3SO_3H$
5d	Cpr	CF		B (63)	290–292	$C_{20}H_{21}F_2N_3O_3 \cdot 0.5H_2O \cdot CH_3SO_3H$
5e	Cpr	CCl		A[d] (13)	225–227	$C_{20}H_{21}ClFN_3O_3$[c]
5g	4-FPh	CH		A (35)	319–320	$C_{23}H_{21}F_2N_3O_3 \cdot 2.5H_2O \cdot HCl$
5n	2-FEt	CH		A (52)	270–273	$C_{19}H_{21}F_2N_3O_3 \cdot 1.5H_2O \cdot HCl$
5o	2-FEt	CF		A (56)	216–219	$C_{19}H_{20}F_3N_3O_3$[c]
5p				A[e] (21)	248–252	$C_{20}H_{22}FN_3O_4 \cdot 0.5H_2O$
5q	vinyl	CH		A (66)	230–232	$C_{19}H_{20}FN_3O_3 \cdot 0.5H_2O$
5r	Me	CH		A (68)	251–252	$C_{18}H_{20}FN_3O_3 \cdot 0.75H_2O$
6a	Et	CH		A (53)	187–188	$C_{20}H_{24}FN_3O_3 \cdot 0.25H_2O$
6b	Et	CF		A (69)	294–297	$C_{20}H_{23}F_2N_3O_3 \cdot 0.25H_2O \cdot CH_3SO_3H$
6c	Cpr	CH		A (63)	263–265	$C_{21}H_{24}FN_3O_3 \cdot H_2O \cdot CH_3SO_3H$
6d	Cpr	CF		A (54)	253–255	$C_{21}H_{23}F_2N_3O_3 \cdot 0.75H_2O \cdot CH_3SO_3H$
6f	Cpr	N		A (83)	263–264	$C_{20}H_{23}FN_4O_3 \cdot 1.125H_2O \cdot CH_3SO_3H$
6g	4-FPh	CH		B (95)	270–272	$C_{24}H_{23}F_2N_3O_3$[c]
6n	2-FEt	CH		A (13)	202–203	$C_{20}H_{23}F_2N_3O_3$[c]
6q	vinyl	CH		A (45)	214–215	$C_{20}H_{22}FN_3O_3 \cdot 0.25H_2O$
6r	Me	CH		A (8.5)	220–222	$C_{19}H_{22}FN_3O_3$[c]
7a	Et	CH		C (82)	325–327	$C_{19}H_{22}FN_3O_3 \cdot CH_3SO_3H$
7b	Et	CF		B (27)	312–313	$C_{19}H_{21}F_2N_3O_3 \cdot 3H_2O \cdot HCl$
7c	Cpr	CH		B (90)	330–332	$C_{20}H_{22}FN_3O_3 \cdot 1.5H_2O \cdot HCl$
7d	Cpr	CF		B (43)	250–252	$C_{20}H_{21}F_2N_3O_3$[e]
7g	4-FPh	CH		B (68)	320–322	$C_{23}H_{21}F_2N_3O_3 \cdot 1.5H_2O \cdot HCl$
7n	2-FEt	CH		B (50)	256–258	$C_{19}H_{21}F_2N_3O_3 \cdot 2.5H_2O \cdot HCl$
7o	2-FEt	CF		B (13)	311–313	$C_{19}H_{20}F_3N_3O_3 \cdot 1.5H_2O \cdot HCl$
7p	-CH(CH_3)CH_2OC-			A[e] (14)	230–232	$C_{20}H_{22}FN_3O_4 \cdot 2.75H_2O \cdot HCl$
7q	vinyl	CH		B (50)	255–257	$C_{19}H_{20}FN_3O_3 \cdot 2.5H_2O$
7s	$NHCH_3$	CH		B (73)	245–247	$C_{18}H_{21}FN_4O_3$[c]
8a	Et	CH		A (21)	310–312	$C_{19}H_{22}FN_3O_3 \cdot 0.25H_2O \cdot CH_3SO_3H$
8c	Cpr	CH		B (37)	332–334	$C_{20}H_{22}FN_3O_3 \cdot 0.5H_2O \cdot CH_3SO_3H$
8g	4-FPh	CH		A (17)	312–313	$C_{23}H_{21}F_2N_3O_3$[c]
9a	Et	CH		A (47)	180–182	$C_{20}H_{24}FN_3O_3 \cdot 1.25H_2O$
9c	Cpr	CH		A (66)	252–255	$C_{21}H_{24}FN_3O_3 \cdot 2.25H_2O \cdot HCl$

[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-Chloronapthyridinone 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
	$-CH(CH_3)CH_2OC-$		

[a] Me = methyl, Et = ethyl, Cpr = cyclopropyl, 4-FPh = 4-fluorophenyl, 2,4-F_2Ph = 2,4-difluorophenyl, 2-FEt = 2-fluoroethyl.

modifications of the published procedures, while **6**[19] and **9**[20] 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 N_1 nucleus a large difference in in vitro activity was not observed for any of the C_7 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 C_7 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 N_1 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 N_1 cyclopropyl derivatives **7c** and **d**, the addition of a C_8 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 N_1 ethyl series upon C_8 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 C_7 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 N_1. Within the (1*S*,4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]heptane (**2**) series the N_1 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 N_1 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 C_8 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 C_8 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-quinolonecarboxylic Acids. New Quantitative Structure-Activity Relationships at N_1 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.; Sesnie, 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 N_1-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 (μg/mL)]

compd	microorganisms (strain)[a]											
	Bb	Ec	Ph	Pm	Sc	Sa	Apl	Apy	By	Cp	Th	gyrase[b]
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 (norfloxacin)	6.25	0.10	0.05	0.05	0.10	3.13	<0.05	12.5	25	0.39	6.25	0.78

[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 (μg/mL) to induce detectable cleavage of supercoiled pBR322 substrate to the linear form.[8a,8b,28]

were the desmethyl C_8 fluoro and chloro derivatives **3d** and **3e** (0.09 μg/mL).[23]

In the 8-methyl-3,8-diazabicyclo[3.2.1]oct-3-yl (**5**) series, halogenation of N_1 cyclopropyl derivatives at the C_8 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 N_1-substituted compounds in this series were all less potent than the N_1 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-

(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.

Table IV. In Vivo Mouse Protection Assay

		organism: protective dose (PD_{50}), mg/kg		
		P. multocida	*E. coli*	*S. choleraesuis*
compd	route	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**) C_7 substituents conferred the greatest potency to the various nuclei. In addition, it was shown that the N_1-cyclopropyl-containing analogues were the most potent within a given C_7 series. Halogenation at the C_8 position in the N_1 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 (PD_{50}) 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 PD_{50}'s against this strain. Exceptions may be **2c** and **5g**, which show lower PD_{50}'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 PD_{50}'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 V[24]

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

	Compound: MED, mg/kg			
model	**2c**	**5a**	**5c**	**7a**
Pasteurella haemolytica (Cattle, SID × 2)				
IM	0.15	1.2	0.3	0.3
Actinobacillus pleuropneumoniae (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 C_7 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 PD_{50}'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. bovigenitalium* 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.

profile[7] 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 N_1 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, 3l, 5e, 5o, 6g, 6n, 6r, 7d, 7s, and 8g by high-resolution mass spectrometry.

General Procedures for the Preparation of Quinolones and Naphthyridinones (Scheme I). 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 N_2 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 Na_2SO_4, 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 $NaHCO_3$ and the product was extracted with chloroform (5 × 100 mL). The chloroform layer was dried over Na_2SO_4, 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: ^{1}H NMR (D_2O) 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 $C_{19}H_{22}FN_3O_3$ 359.1655, found 359.1705. Anal. ($C_{19}H_{22}FN_3O_3$·HCl·H_2O) 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. ($C_{19}H_{22}FN_3O_3$·CH_3SO_3H) 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 N_2 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; ^{1}H 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 $C_{19}H_{20}FN_3O_3$ 357.1489, found 357.1525. Anal. ($C_{19}H_{20}FN_3O_3$·CH_3SO_3H·1.5H_2O) 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 N_2 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; ^{1}H NMR ($CDCl_3$) 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 $C_{19}H_{22}FN_3O_3$ 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. ($C_{19}H_{22}$-FN_3O_3·CH_3SO_3H) 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-quinolinecarboxylic Acid (13). To a mixture of 1.26 g (4.98 mmol) of ester **29a** and 12.5 mL of vinyl acetate under a N_2 atmosphere was added 12.5 mg (0.037 mmol) of $Na_2(PdCl_4)$. 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 $Na_2(PdCl_4)$ 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 $MgSO_4$ and evaporated to a gold solid. The crude product was purified by SiO_2 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 H_2SO_4 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 $C_{12}H_8F_2NO_3$ $(P + H)^+$ 252.0501, found 252.0472.

DNA Gyrase Purification. DNA gyrase subunits A (1.9×10^4 U/mg) and B (0.54×10^4 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 complimentary subunit).

DNA Gyrase Cleavage Assay. The general protocol followed was modified from Gellert et al.[8b] and Sugino et al.[8a] Approximately 2 units of reconstituted DNA gyrase holoenzyme were incubated in 35 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 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 electrophosesed. 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 $N_2/CO_2/H_2$ (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 (PD_{50}). 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, 3.0×10^3 cfu/mL; *E. coli* 51A266, 3.0×10^8 cfu/mL; *S. choleraesuis* 58B010, 8.5×10^2 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 PD_{50} (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 (8000*g*) 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 5×10^8 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 alotted. 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 5×10^8 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 alloted 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% CO_2 at 37 °C. The bacteria were rinsed from the plates with saline, diluted, and standardized by OD so that 10^9 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 (^{1}H 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(1*H*)-ones as Combined Inhibitors of Thromboxane A_2 Synthase and cAMP Phosphodiesterase[1]

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A series of 1*H*-imidazol-1-yl- and 3-pyridyl-substituted 3,4-dihydroquinolin-2(1*H*)-ones was designed and synthesized as combined inhibitors of thromboxane (TXA_2) 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 TXA_2 synthase in in vitro ADP-induced aggregation experiments with human blood platelets. The TXA_2 synthase inhibitory activity was confirmed by measurement of the prostanoid metabolites derived from ^{14}C-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 TXA_2 synthase inhibitory activity. Synergistic enhancement of antiaggregatory and antithrombotic actions was expected when simultaneous stimulation of adenylate cyclase (through increased PGI_2 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 TXA_2 synthase (IC_{50} 1.2 μM) and human platelet cAMP PDE (IC_{50} 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 (TXA_2) synthase inhibition, or TXA_2 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 TXA_2 production in human platelet rich plasma, in the presence of prostacyclin (PGI_2) synthase, has been shown to redirect the balance of prostaglandin synthesis in favor of the production of PGI_2.[5] The an-

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