blood. The wells were incubated at 37 °C for 2 h and examined visually for red blood cell lysis. The prodrugs that were expected to be anionic at pH 7 were not lytic up to concentrations of 400 μg/mL. However, the amine-bearing, cationic derivatives were substantially more lytic than the other prodrugs or 4.

The minimum fungicidal concentrations (MFC's) were determined against a panel of *Candida* sp. pathogens.[3b] Wells containing 150 μL of yeast nitrogen base with 1% dextrose were inoculated with approximately 5×10^3 CFU of yeast. These cultures were clinical isolates that are maintained in the Merck collection. Growth was allowed for 24 h at 35 °C in wells containing a range, by 2-fold serial dilution, from 128 to 0.06 μg/mL of drug to determine minimum inhibitory concentrations (MIC's). Subsequently, 1.5-μL aliquots were used to inoculate Sabouraud dextrose agar (SDA) plates, and these were incubated at 35 °C for 24 h. The MFC was defined as the minimum concentration of drug showing less than five colonies per spot. Control plates showed a substantial amount of growth. The hydrolytically stable derivatives were notably less active against *Candida* sp. than the parent 4. This result was also reflected in the glucan synthesis inhibition assay.[9b] These results suggested that the phenolic hydroxyl is important for antifungal activity.

Compounds **2**, **4**, and **8a–i** were evaluated in vivo in the target organ kidney assay (TOKA).[25] DBA/2 mice (5 per dosage level) were challenged, by tail vein injection, with 5×10^4 cells of *C. albicans* (MY 1055) suspended in sterile saline. Mice were dosed intraperitoneally with a vehicle sham or drug at 1.5, 3.0, and 6.0 mg/kg (MPK) twice daily for 4 days. Seven days postinfection, the animals were sacrificed and their kidneys were removed, prepared, and cultured on SDA plates at 35 °C for 24 h. The number of colony forming units per gram of kidney (CFU/g) was determined. The minimum effective dose (MED_{99}) was the dose required to reduce the CFU/g by at least 99% over the vehicle sham control. Control groups typically gave 10^6–10^7 CFU of *C. albicans* per gram of kidney. The hydrolytically stable carbamate prodrugs were ineffective while the more easily hydrolyzed compounds **8d–h** produced MED_{99}'s of 3 or 6 MPK. The phosphate **8i**, although it was very resistant to hydrolysis, was as effective as **4**, giving an MED_{99} of 3 MPK. When dosed intravenously via the tail vein, **8i** produced an MED_{99} of 2.5 MPK.

The compounds were evaluated in a rat PCP model.[8a] Immunosuppressed Sprague–Dawley rats (5 per dosage level) with a confirmed *P. carinii* infection were dosed subcutaneously at 0.15 and 0.60 mg/kg (MPK) twice daily for 4 days. Compound **2** was dosed at several levels up to 2.5 MPK. On the fifth day, the rats were sacrificed and the lungs were removed and prepared. The number of cysts per lung was determined by microscopic examination of a known amount of lung homogenizate. The minimum effective dose (MED_{90}) was the dose required to reduce the number of cysts by at least 90% over the vehicle sham controls. Control groups typically ranged from 10^7 to 10^8 cysts per lung. The relative activity of compounds **8a–i** correlated roughly with the ease of solution hydrolysis as was seen in the TOKA except for the phosphate ester. Cilofungin **2** was at least 15 times less potent than **4** or the phosphate ester **8i** in this PCP model.

Although several compounds showed acceptable efficacy in vivo in both PCP and TOKA assays, only the phosphate prodrug **8i** (L-693,989) showed high activity in both assays and additionally possessed superior solution stability. The observation that **8i** was hydrolytically stable and yet had in vivo activity comparable to the parent **4** suggested that the phosphate ester underwent rapid enzymatic hydrolysis. Preliminary pharmacokinetic data in primates (rhesus and chimpanzee) showed that prodrug **8i** was efficiently converted to parent drug **4** and produced a more sustained therapeutic level of **4** than direct iv administration of **4** itself.[26] Because of the favorable overall profile, the phosphate ester was chosen for further evaluation as a potential clinical agent for the treatment of PCP and candidiasis.

Acknowledgment. We thank Dr. Lawrence Colwell, Ms. Amy Hale, and Dr. Jerrold Liesch for performing mass spectral measurements.

Supplementary Material Available: NMR spectra for **6**, **7a,b,f–i**, and **8a–i** (16 pages). Ordering information is given on any current masthead page.

(25) Bartizal, K.; Abruzzo, G.; Trainor, C.; Puckett, J.; Fromtling, R. A New Target Organ Kidney Assay (TOKA) of Systemic Candidiasis in Congenitally Immune Deficient Mice for Discovery and Evaluation of Fungistatic/cidal Agents. *Proc. 91st General Meeting ASM*, Dallas, TX, May 5–9, 1991, Abstr. No. A-81.

(26) Hajdu, R.; Sundelof, J. G.; Bartizal, K.; Abruzzo, G.; Trainor, C.; Thompson, R.; Kropp, H. Comparative Pharmacokinetics in Four Animal Species of L-688,786 and Its Water-Soluble Prodrug, L-693,989. *Proc. 31st ICAAC*, Chicago, IL, Sept. 29–Oct. 2, 1991, Abstr. No. 209.

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Potent Non-6-Fluoro-Substituted Quinolone Antibacterials: Synthesis and Biological Activity

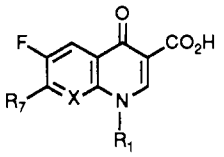
The fluoroquinolone antibacterials[1] represented generically by **1** (Table I) have generated much excitement after the discovery that a fluorine atom at C-6 enhances antibacterial activity. Norfloxacin is generally considered to be the first derivative noted for a significant increase in activity. However, flumequine was the first to demonstrate the advantage of a C-6 fluorine atom. The next entries into this class of antibacterials were ofloxacin, ciprofloxacin, and more recently tosufloxacin, all of which contain a piperazinyl or aminopyrrolidinyl moiety for R_7 and diverse groups for R_1.

Structure–activity studies have demonstrated that the optimum substituent at the C-6 position was a fluorine atom[2] in both the quinolone and naphthyridone series, and

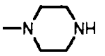
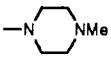
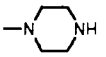
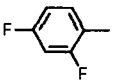
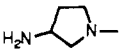
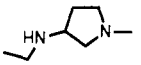
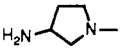
(1) For a recent review on the new generation of quinolones see: Wentland, M. P. Structure–activity relationships of fluoroquinolones. In *New generation of quinolones*; Siporin, C., Heifetz, C. L., Domagala, J. M., Eds.; Marcel Dekker, Inc.: New York, 1990; pp 1–43.

0022-2623/92/1835-0198$03.00/0

Table I



1

name	X	R_1	R_7
flumequin			H
norfloxacin	CH	Et	
ofloxacin			
ciprofloxacin	CH		
tosufloxacin	N		
1a (CI 934)	CF	Et	
1b (PD 117596)	CF		

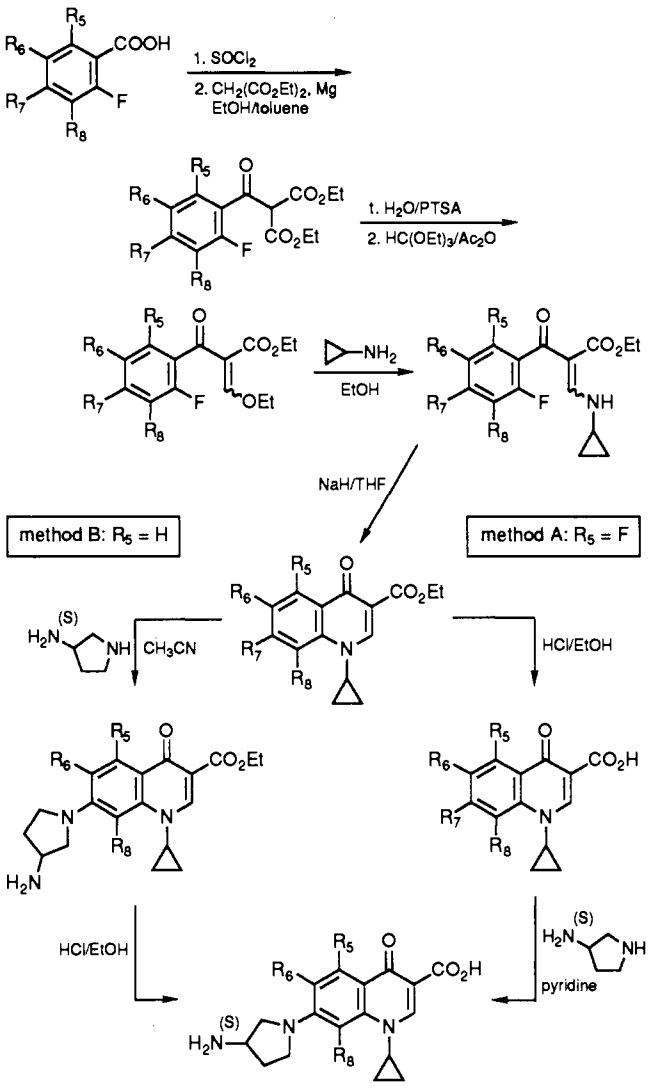
more recently, a second fluorine atom was found desirable at the C-8 position for optimal in vivo efficacy (e.g. **1a** and **1b**). During the last few years almost all structural modifications of the classical structures that have been reported in the scientific literature leading to new potent compounds have been made to structures containing a C-6 fluorine atom. Still the exact role of the C-6 fluorine substituent has never been clarified. As part of our continuing efforts in structure–activity studies[3] in the quinolone and naphthyridone series, we have discovered that derivatives without fluorine atom at the C-6 position could retain overall antimicrobial activity comparable to that of ciprofloxacin.

In this communication we describe the synthesis and biological activity of a series of 7-(aminopyrrolidinyl)-1-cyclopropyl-1,4-dihydro-4-oxo-3-quinolinecarboxylic acids in which the fluorine atom is appended at C-5 or C-8 and then compare of these compounds to known references and C-6 fluoro analogues.

Chemistry

The compounds used in this investigation were synthesized as shown in Scheme I following the standard chemistry of the quinolone series.[4] Two methods of introducing the pyrrolidine moiety were used.[5a,b] In method A, the ester function of the C-5 fluoroquinolones was hydrolyzed to avoid substitution of the C-5 fluorine by the pyrrolidine, and in method B the ester hydrolysis was unnecessary until after substitution. Yields were generally higher using method B.

Scheme I



The in vitro antibacterial activity was studied in a side-by-side comparison with ciprofloxacin and determined by the serial 2-fold dilution technique using nutrient broth. The inoculum size was adjusted to 10^6 CFU/mL, and the concentration of the compounds ranged from 0.0005 to 250 μg/mL. Minimum inhibitory concentrations were defined as the lowest concentration that prevented visible growth of bacteria after incubation at 37 °C for 18 h.

Results and Discussion

Table II summarizes the in vitro antibacterial data of the quinolones synthesized for this study against three Gram-positive and six Gram-negative organisms. The data

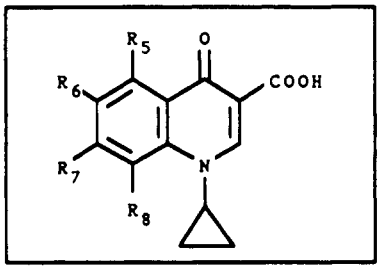
(2) Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H.; Nishimura, H. Pyridonecarboxylic Acids as Antibacterial Agents. 2. Synthesis and Structure-Activity Relationships of 1,6,7-Trisubstituted 1,4-Dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acids, Including Enoxacin, a new Antibacterial agent. *J. Med. Chem.* **1984**, *27*, 292–301.

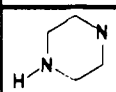
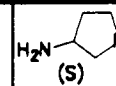
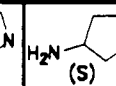
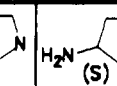
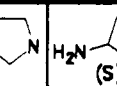
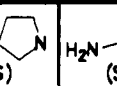
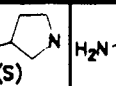
(3) Remuzon, P.; Bouzard, D.; Di Cesare, P.; Essiz, M.; Jacquet, J. P.; Kiechel, J. R.; Ledoussal, B.; Kessler, R. E.; Fung-Tomc, J. Fluoronaphthyridines and Quinolones as antibacterial Agents. 3. Synthesis and Structure-Activity Relationships of New 1-(1,1-Dimethyl-2-fluoroethyl), 1-[1-Methyl-1-(fluoromethyl)-2-fluoroethyl], and 1-[1,1-Difluoromethyl)-2-fluoroethyl] Substituted Derivatives. *J. Med. Chem.* **1991**, *34*, 29–37 and references cited therein.

(4) For a recent review on the chemistry of quinolones, see: Bouzard, D. Recent Advances in the Chemistry of Quinolones. In *Recent Progress in the Chemical Synthesis of antibiotics*; Lukacs, G., Ohno, M., Eds.; Springer-Verlag: New York, 1990; pp 247–283.

(5) (a) Moran, D. B.; Ziegler, C. B.; Dunne, T. S.; Kuck, N. A.; Lin, Y.-i. Synthesis of novel 5-fluoro analogues of norfloxacin and ciprofloxacin. *J. Med. Chem.* **1989**, *32*, 1313–1318. (b) Schibamori, K.; Egawa, H.; Miyamoto, T.; Nishimura, Y.; Itokawa, A.; Nakano, J.; Matsumoto, J. Regioselective displacement reactions of 1-cyclopropyl-5,6,7,8-tetrafluoro-4(1H)-oxoquinoline-3-carboxylic acid with amine nucleophiles. *Chem. Pharm. Bull.* **1990**, *38*, 2390–2396.

Table II. In Vitro Antimicrobial Activity (MIC, μg/mL)



		2[a]	3	4	5	6	7	8
	R_5	H	H	H	H	H	F	F
	R_6	F	F	F	H	H	H	H
	R_7							
	R_8	H	H	F	H	F	H	F
STRAINS	S. Pneumoniae 9585	0.25	0.13	0.03	2	0.5	4	4
	E. faecalis 9809	0.5	0.25	2	4	0.25	16	4
	S. aureus 9537	0.06	0.03	0.008	0.25	0.03	1	0.13
	E. coli 15119	0.008	0.004	0.008	0.008	0.008	0.13	0.06
	K. pneumoniae 9664	0.06	0.03	0.03	0.06	0.016	0.5	0.13
	E. cloacae 9656	0.03	0.004	0.016	0.13	0.03	0.13	0.06
	P. mirabilis 9900	0.008	0.03	0.016	0.13	0.03	0.5	0.13
	M. morganii 15153	0.08	0.016	0.016	0.13	0.016	0.25	0.06
	P. aeruginosa 9843	0.25	0.25	0.13	0.5	0.25	4	2

a) Ciprofloxacin

for ciprofloxacin (**2**) are included for comparison. The effectiveness was **3** = **4** = **6** > **5** > **8** > **7** against Gram-positive as well as Gram-negative organisms.

The permutation of the fluorine atom from the C-6 to the C-8 position (**3** vs **6**) result in an equivalent in vitro activity. Koga[6] did the same experiment in another series (1-ethyl-7-piperazinylquinolones) and found the C-8 fluoro compound to be 10-fold less active. It seems that the efficacy of the fluorine atom is a function of the choice of the N-1 and/or C-7 substituents.

The move of the fluorine from the C-6 to the C-5 position (**3** vs **7**) led to a large loss of activity. Moran[6] described the same tendency by comparing 5,6,8-trifluoroquinolones to 6-fluoroquinolones, but in that case, the loss of activity was less dramatic, due possibly to the influence of the other fluorine atoms. In the same way, compound **5** without fluorine displayed an intermediate activity between C-5 and C-6 fluoroquinolones, pointing out the detrimental influence of the C-5 fluorine atom.

Not surprisingly, the addition of a second fluorine atom at the C-8 position on a C-6 fluoroquinolone (**3** vs **4**) did not modify dramatically the in vitro antibacterial activity. The addition of a fluorine atom at the C-8 position on the poorly active C-5 fluoroquinolone (**6** vs **8**) led to a very weak increase of potency: the detrimental influence of the C-5 fluorine atom was not counterbalanced by the C-8 fluorine atom.

This work has clearly shown that the C-6 fluoro atom can be moved to the C-8 position while incurring only a minor loss of potency. This fact has increased the avenues for further modifications of the quinolone nucleus at the C-5 and C-6 positions. The detrimental effect of the C-5 fluorine atom was clearly established, and it is not possible to counterbalance this effect by other substitutions. As in the case of the C-6 position, the efficacy of the C-8 fluorine is dependant on the N-1 and/or C-7 substitution.

Supplementary Material Available: General experimental procedures, ^1H NMR data for **3**–**8**, and ED_{50} values (protective dose on mice) for **2**, **3**, and **6** (3 pages). Ordering information is given on any current masthead page.

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(6) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. Structure-activity Relationships of antibacterial 6,7 and 7,8-disubstituted 1-alkyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids. *J. Med. Chem.* **1980**, *23*, 1358–1363.