Articles

Synthesis and Antibacterial Activity of New Quinolones Containing a 7-[3-(1-Amino-1-methylethyl)-1-pyrrolidinyl] Moiety. Gram-Positive Agents with Excellent Oral Activity and Low Side-Effect Potential

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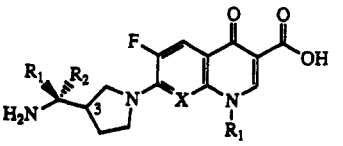
A series of the *R* and *S* isomers of 7-[3-(1-amino-1-methylethyl)-1-pyrrolidinyl]-1,4-dihydro-4-oxoquinoline- and 1,8-naphthyridine-3-carboxylic acids was prepared to determine the effect on potency of the two methyl groups adjacent to the distal nitrogen in the pyrrolidinyl moiety. The antibacterial efficacy of these dimethylated derivatives was compared to the relevant 7-[3-(aminomethyl)-1-pyrrolidinyl] parent compounds and, to a lesser extent, the 7-[3-(1-aminoethyl)-1-pyrrolidinyl] analogues. The activity of the title and reference compounds was assayed in vitro using an array of Gram-negative and Gram-positive organisms and in vivo using a mouse infection model. Selected derivatives were then screened for potential side effects in a phototoxicity mouse model and an in vitro mammalian cell cytotoxicity protocol. The results showed that the *R* isomer displayed a 2–20-fold advantage in activity in vitro and a 2–15-fold advantage in vivo over the *S* isomer. Although equipotent to the 7-[3-(aminomethyl)-1-pyrrolidinyl] parent compounds in vitro, the *R* isomers of the 7-[3-(1-amino-1-methylethyl)-1-pyrrolidinyl] analogues showed a dramatic increase in in vivo potency, especially via the oral route of administration. These same *R* isomers also appeared to possess a reduced risk of phototoxicity and cytotoxicity. This combination of superior in vivo performance with a low degree of phototoxicity and mammalian cell cytotoxicity recommends these agents for further study. Of these agents, naphthyridine **16-R** represents the optimal blend of potency and safety.

Introduction

The broad-spectrum potency and in vivo efficacy of the quinolone antibacterials have generated much enthusiasm in the medical community and have prompted extensive research in the pharmaceutical industry as well. As a byproduct of this scrutiny, a plethora of reviews and structure–activity relationship studies of the quinolones has appeared in the scientific literature.[1-7,11] Much of the focus of these SAR studies has centered on the C-8[8] and C-7[9-11] substituents, since these positions significantly influence the antibacterial spectrum as well as the overall activity, both in vitro and in vivo. Replacing the "standard" piperazine moiety at C-7 (found in the marketed compounds ciprofloxacin and lomefloxacin) with a 3-aminopyrrolidine or a 3-(aminomethyl)pyrrolidine produces compounds with enhanced in vitro activity (especially against Gram-positive organisms) but with a disappointing loss of in vivo efficacy. The in vivo activity can be regained and even improved by the addition of a halogen atom at C-8, but this substitution also exacerbates such unwanted side effects as phototoxicity[12] and cytotoxicity.[13,14] Obviously, it would be advantageous if the pyrrolidine side chain itself could be modified to impart an intrinsic in vivo boost.

Toward this end, we have studied the effects on potency of alkylation of the C-7 pyrrolidine. Alkylation of the pyrrolidine moiety at positions 2[15] and 4[16] of the (3-aminopyrrolidinyl)quinolones has been reported to increase oral activity. In the 3-(aminomethyl)pyrrolidinyl series, methylation of either the exocyclic amine[9,10] or the 3-position[17] of the pyrrolidine ring also increases in vivo efficacy (Figure 1, **1a**). Recently we reported that addition of a methyl group to the methylene spacer itself (Figure 1, **1b**) elicits a profound enhancement in activity both in vitro and in vivo with an improved safety profile for some analogues.[18] Although exciting, this particular discovery comes with a daunting synthetic challenge—that is, the construction of a complex pyrrolidine containing two chiral centers. We reasoned that incorporation of yet another methyl group at the methylene spacer (to give **1c**, Figure 1) would reduce somewhat the synthetic complexity and could improve efficacy or safety even further. We report herein the synthesis of the *R* and *S* isomers of **1c** and the excellent in vitro and in vivo potency of the *R* isomer.[19]



1a: $R_1 = R_2 = H$
1b: $R_1 = CH_3$, $R_2 = H$
1c: $R_1 = R_2 = CH_3$

Figure 1.

Chemistry

The quinolone nuclei necessary for the preparation of final products were synthesized according to literature

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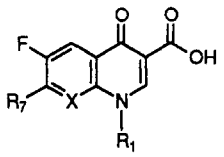
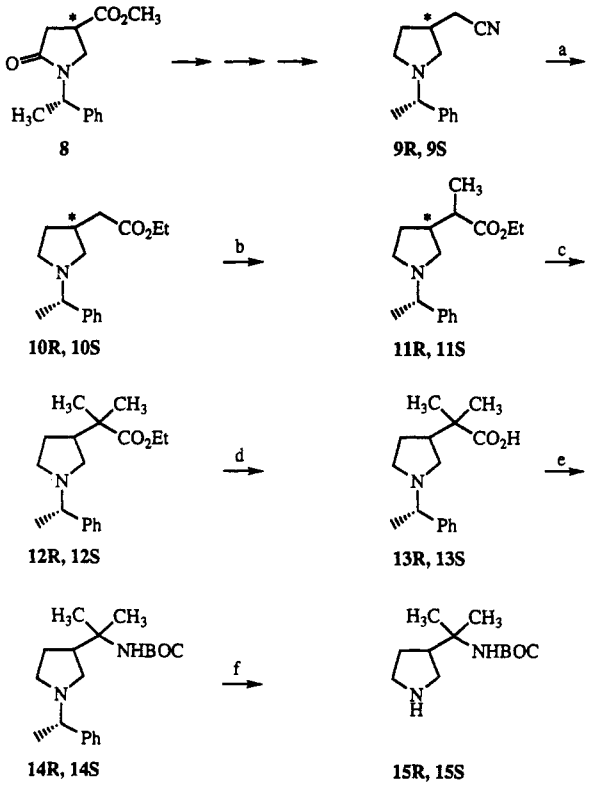
Table 1. Previously Described Quinolone and Naphthyridine Intermediates



2

compd no.	substituents in 2			ref
	R_1	R_7	X	
3	c-C_3H_5	Cl	N	18
4	c-C_3H_5	F	CH	8
5	c-C_3H_5	F	C-OCH_3	20
6	c-C_3H_5	F	C-Cl	8
7	2,4-F_2Ph	Cl	N	21

Scheme I[a]



[a] (a) H_2SO_4, EtOH; (b) LDA, −78 °C, then CH_3I; (c) LDA, 0 °C, then CH_3I; (d) NaOH; (e) DPPA, t-BuOH; (f) H_2, Pd/C.

procedures (see Table 1). The synthesis of the requisite pyrrolidines is outlined in Scheme 1. Pyrrolidinone 8 was prepared as a mixture of *R* and *S* isomers at the 3-position of the pyrrolidine ring. Chromatographic separation of this mixture into the component diastereomers and subsequent elaboration into the corresponding nitriles (**9-R** and **9-S**, where letters "R" and "S" refer to the stereochemistry at C-3) was effected following literature procedures.[22] Despite repeated attempts, dialkylation of the nitriles was not successful. Therefore, pyrrolidines **9-R** and **9-S** were hydrolyzed to ethyl esters **10-R** and **10-S**, which were then dialkylated in a two-step procedure to give dimethyl esters **12-R** and **12-S**. This conversion occurred in a straightforward manner but was fairly sensitive to the reaction temperature and the amount of base; analysis by NMR and HPLC verified the incorporation of two methyl groups. Base hydrolysis of the ester and Curtius rearrangement of the resulting acid gave penultimate intermediates **14-R, 14-S**. The 1-phenylethyl group was removed by catalytic hydrogenation to afford target pyrrolidines **15-R** and **15-S** as waxy solids. All intermediates as well as the final side chains were analyzed by standard spectroscopic techniques and supported by elemental analyses. Optical rotations for the intermediates were internally consistent and agreed with known literature values where applicable.

The coupling reactions of the appropriate pyrrolidine side chain (**15-R** or **15-S**) and the quinolone substrates followed well-established literature precedents.[8] Physical data and methods of preparation for the new and reference quinolones prepared for this study are listed in Table 2. As is the case for the side chains **15-R** and **15-S**, the letter "R" or "S" appearing in the compound number refers to the stereochemistry at the 3-position of the pyrrolidine ring.

Biological Assays

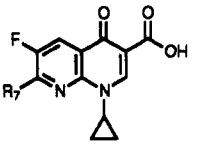
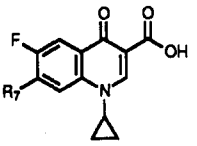
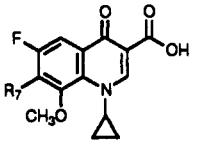
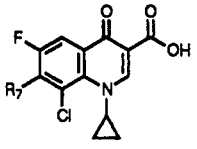
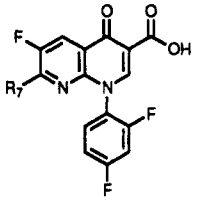
The quinolones prepared for this study were tested against 10 representative Gram-positive and Gram-negative organisms using standard microtitration techniques.[23] Their minimum inhibitory concentrations (MICs, μg/mL) are presented in Table 3. Also included are the activities of two reference compounds: ciprofloxacin (which is currently marketed) and CI-938 (chosen for its excellent Gram-positive potency). Structures are included in Figure 2. In vivo efficacy was also measured, using previously reported methods.[24] The potency was determined in acute, lethal systemic infections in female Charles River CD-1 mice and is expressed as the median protective dose (PD_{50}, mg/kg, Table 3).

Those compounds which exhibited interesting in vitro and in vivo activity were then screened for possible side effects. Oral phototoxicity was assayed in the depilated female CD-1 mice using a protocol reported elsewhere.[12] In summary, five animals/dose (and control) were exposed to UVA radiation for a 3-h duration following oral administration; the process was repeated each day for 4 days, and any redness or erythema relative to control was designated as positive. Results are given in Table 4 and are reported as the highest dose producing no effect. The other side-effect screen estimates clastogenic potential via a mammalian cell cytotoxicity assay.[14] Cytotoxicity was measured in Chinese hamster V-79 cells, which were grown overnight and treated with drug at 37 °C for 3 h. At this time, the compound-containing media was replaced with fresh media, and the cells were incubated for 5 days before examination for colony formation. The concentration of drug which inhibited colony formation by 50% (IC_{50}, μg/mL) relative to control is recorded in Table 4.

Results and Discussion

Previous researchers have attempted to delineate the influence on antibacterial activity of the stereochemistry at the pyrrolidinyl 3-position.[18,25] A study of representative quinolones containing a 3-(aminomethyl)pyrrolidinyl moiety at C-7 [Figure 1, **1a**] revealed no significant difference in in vitro and in vivo activity between the *R* and *S* enantiomers. This observation led some investigators to conclude that chirality remote from the quinolone nucleus has little effect on overall antibacterial potency.[11] However, for those [(aminomethyl)pyrrolidinyl]quinolones with a single methyl group on the methylene spacer (and therefore a new chiral center, see Figure 1, **1b**), the compounds with an "*R*" configuration at C-3 were 3–15

Table 2. Physical Properties for the Quinolone Antibacterials Tested in This Study

	R_7 =	compd no.[a]	method of prep[b] or ref	method of purification of final product[c]	yield[d] (%)	mp (°C)	analysis formula (elements analyzed)
	$R_1 = R_2 = H$	**16A**	ref 8				
	$R_1 = H, R_2 = CH_3$	**16-RR**	ref 18				
	$R_1 = CH_3, R_2 = H$	**16-RS**	ref 18				
	$R_1 = R_2 = CH_3$	**16-R**	A	trit. 2-PrOH	69	>300	$C_{19}H_{23}FN_4O_3 \cdot 1.2HCl \cdot 1.5H_2O$ (C, H, N, Cl)
	$R_1 = R_2 = CH_3$	**16-S**	A	trit. 2-PrOH	76	280–282	$C_{19}H_{23}FN_4O_3 \cdot 1.5HCl \cdot 2H_2O$ (C, H, N, Cl)
	$R_1 = R_2 = H$	**17A**	ref 8				
	$R_1 = R_2 = CH_3$	**17-R**	A	recryst. $EtOH/H_2O$	85	256–257	$C_{20}H_{24}FN_3O_3 \cdot HCl \cdot 1.2H_2O$ (C, H, N, Cl)
	$R_1 = R_2 = CH_3$	**17-S**	A	trit. EtOAc	78	246–248	$C_{20}H_{24}FN_3O_3 \cdot 1.15HCl \cdot 3.7H_2O$ (C, H, N, Cl, H_2O)
	$R_1 = R_2 = H$	**18A**	ref 18				
	$R_1 = R_2 = CH_3$	**18-R**	B	trit. 2-PrOH	47	225–227	$C_{21}H_{26}FN_3O_4 \cdot 1.15HCl \cdot 2H_2O$ (C, H, N, Cl)
	$R_1 = R_2 = CH_3$	**18-S**	B	trit. 2-PrOH	58	228–330	$C_{21}H_{26}FN_3O_4 \cdot 1.3HCl \cdot 1.7H_2O$ (C, H, N, Cl)
	$R_1 = R_2 = H$	**19A**	ref 14				
	$R_1 = R_2 = CH_3$	**19-R**	A	trit. 2-PrOH	70	225–227	$C_{20}H_{23}ClFN_3O_3 \cdot 1.8HCl \cdot 0.8H_2O$ (C, H, N, Cl)
	$R_1 = R_2 = CH_3$	**19-S**	A	trit. 2-PrOH	75	221–223	$C_{20}H_{23}ClFN_3O_3 \cdot HCl \cdot 2H_2O$ (C, H, N)
	$R_1 = R_2 = H$	**20A**	ref 18				
	$R_1 = R_2 = CH_3$	**20-R**	A	recryst. $EtOH/H_2O$	51	242–243	$C_{22}H_{21}F_3N_4O_3 \cdot 0.9HCl \cdot 0.5H_2O$ (C, H, N, Cl)
	$R_1 = R_2 = CH_3$	**20-S**	A	recryst. 2-PrOH	43	216–218	$C_{22}H_{21}F_3N_4O_3 \cdot HCl \cdot 3H_2O$ (C, H, N)

[a] The *R* or *S* designation refers to the stereochemistry at C-3 of the pyrrolidinyl side chain. [b] See the Experimental Section for general methods. [c] Trituration (trit.) refers to grinding the solids under solvent to produce a fine solid. [d] Yields are those from coupling to the final products, including deprotections and hydrolyses.

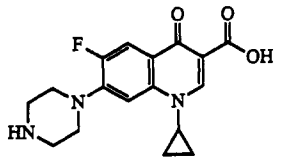
Table 3. In Vitro and in Vivo Activity of the Quinolones from Table 2

	in vitro antibacterial activity (MICs),[a] μg/mL										in vivo antibacterial activity (PD 50, mg/kg)[b]			
	Gram-negative organisms					Gram-positive organisms					E. coli		S. pyog.	
compd. no.	E. cloac MA 2646	E. coli Vogel	K. pneum MGH-2	P. rettg M1771	P. aerug U1-18	S. aureus H 228	S. aureus UC-76	S. faecalis MGH-2	S. pneum SV-1	S. pyog C 203	po[c]	sc[d]	po[c]	sc[d]
16A	0.1	0.05	0.1	0.4	0.2	0.1	0.003	0.025	0.1	0.025	17	1	39	4
16-RR	0.05	0.05	0.1	0.4	0.8	0.05	0.006	0.05	0.006	0.013	6	1	14	2
16-RS	0.05	0.025	0.05	0.1	0.4	0.025	0.003	0.013	0.003	0.013	2	0.4	3	1
16-R	0.1	0.05	0.2	0.4	1.6	0.1	0.003	0.05	0.006	0.013	5	1	4	1
16-S	0.2	0.2	0.4	0.8	3.1	0.1	0.025	0.10	0.05	0.10	10	3	32	6
17A	0.1	0.05	0.1	0.2	0.8	0.05	0.006	0.05	0.013	0.013	>100	2	>100	4
17-R	0.05	0.05	0.1	0.2	0.8	0.05	0.006	0.025	0.003	0.003	18	2	20	1
17-S	0.4	0.4	0.8	1.6	3.1	0.4	0.1	0.4	0.2	0.2			>100	13
18A	0.05	0.05	0.1	0.1	0.8	0.025	0.003	0.013	0.003	0.003			11	0.6
18-R	0.1	0.1	0.2	0.4	1.6	0.013	0.003	0.013	0.003	0.003	26	3	2	0.6
18-S	0.2	0.2	0.4	0.8	3.1	0.05	0.013	0.05	0.025	0.025			29	3
19A	0.025	0.025	0.05	0.05	0.4	0.003	0.003	0.006	0.003	0.003	33	1	5	0.2
19-R	0.05	0.05	0.1	0.2	0.8	0.006	0.003	0.013	0.003	0.003	8	1	2	0.1
19-S	0.1	0.1	0.2	0.4	1.6	0.025	0.013	0.05	0.013	0.025	27	4	13	4
20A	0.05	0.05	0.1	0.2	0.4	0.05	0.013	0.025	0.025	0.025				
20-R	0.2	0.2	0.4	0.8	3.1	0.05	0.025	0.1	0.05	0.05				
20-S	0.4	0.4	0.8	1.6	3.1	0.4	0.1	0.4	0.4	0.4				
21	0.05	0.05	0.05	0.1	0.4	3.1	0.2	0.8	1.6	0.8	1	0.3	180	19
22	0.025	0.013	0.025	0.05	0.1	0.05	0.025	0.05	0.05	0.05	1	0.2	8	2

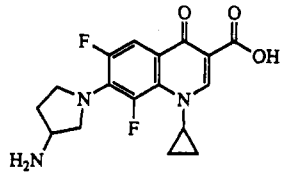
[a] Minimum inhibitory concentrations. [b] Dose required to prevent death in 50% of animals. [c] Oral administration. [d] Subcutaneous administration.

times more potent than their "*S*" counterparts. For these reasons, the effect of chirality on the efficacy of the dimethyl analogues could not be predicted a priori.

The data listed in Table 3 clearly demonstrate that the [α,α-dimethyl(aminomethyl)pyrrolidinyl]quinolones show a marked difference in potency between enantiomers.



ciprofloxacin (21)



22 (CI-938)

Figure 2. Reference Quinolones.

Table 4. Results of Selected Quinolones in the Mouse Phototoxicity and Hamster V-79 Cell Cytotoxicity Assays

compd no.	phototoxicity no-effect dose (mg/kg)	clonogenic cytotoxicity 50% inhibition (μg/mL)
16A		42
16-RR	67	100
16-RS	30	33
16-R	73	130
16-S	55	
17A		81
17-R		190
18A		10
18-R		18
18-S		>500
19-R		<8
21	>100	>200
22	3	30

Against Gram-negative organisms, the *R* isomers (**16-R**, **18-R**, **19-R**, and **20-R**) display a 2-fold improvement in in vitro potency over the *S* isomers. This enhancement is more pronounced for the Gram-positive pathogens; in this case, the (*R*)-pyrrolidinylquinolones are 4–6 times more active than their *S* counterparts in vitro. Most striking is the difference in efficacy for the *R* and *S* isomers of the 1-cyclopropyl-8-unsubstituted-quinolones **17-R** and **17-S**, where the (*R*)-pyrrolidine confers a 7-fold boost in activity against Gram-negative strains and 25-fold improvement against Gram-positive strains. The in vivo activity accentuates this trend: without exception, the *R* enantiomer displays a 2–15-fold advantage over the less potent *S* enantiomer, particularly in the *Streptococcus pyogenes* infection model (see **19-R** vs **19-S**). As before, the Gram-positive potency appears to be more highly influenced by the pyrrolidinyl C-3 stereochemistry than does the Gram-negative efficacy.[18]

The more active *R* isomers were then compared to those analogues which did not contain methyl groups at the α-position of the (aminomethyl)pyrrolidinyl moiety. If a "significant" change in activity is said to be 2-fold or better, then the dimethyl derivatives are roughly equipotent to the unsubstituted parent compound in vitro (see **16A** vs **16-R**, **17A** vs **17-R**, **18A** vs **18-R**, and **19A** vs **19-R**). The sole exception is the 5-fold loss in Gram-negative activity seen in dimethylating **20A** to give **20-R**. However, the dimethyl derivatives do show an appreciable improvement in oral efficacy in both the *S. pyogenes* and *Escherichia coli* infection models. The *R* isomers consistently display a 3–10-fold superiority over the reference quinolones. Since the *R* isomers do not confer any boost in efficacy in vitro, this enhancement in oral potency is even more impressive. Indeed, the improvement of in vivo activity should be interpreted as a true enhancement in *oral* activity since alkylation does not affect the SC values reported in Table 3 (see, for example, **16A** vs **16-R**, **17A** vs **17-R**).

Of course, a comparison between chiral dimethyl derivatives and their racemic unsubstituted parents may not be a valid analogy. It has been shown, however, that chiral pyrrolidinemethanamines impart only a 2-fold superiority in oral efficacy vs Gram-positive infections over their racemic counterparts.[25] If this is true, then the *R* stereoisomers still possess a 2–5-fold advantage in oral activity over the parent quinolones. Again, this phenomenon can probably be attributed to an enhanced absorption conferred by the *R* isomers.

Apparently, then, two methyl groups on the methylene spacer are better than none—but are two better than one? To answer this question we compared naphthyridine **16-R** with the analogous monomethyl derivatives, **16-RR** and **16-RS**, which contain the same "*R*" stereochemistry at the C-3 position of the pyrrolidine ring. In this limited data set, the dimethyl analogue is roughly equipotent with the less active monomethyl enantiomer (**16-RR**) in vitro, but slightly more active in vivo against *S. pyogenes*.

Several of the chiral [α,α-dimethyl(aminomethyl)-pyrrolidinyl]quinolones were then screened for clastogenicity (in an in vitro cell assay) and phototoxicity (in an animal model). The results of these tests are summarized in Table 4. Since currently marketed quinolones possess an IC_{50} of >100 μg/mL in the mammalian cell cytotoxicity screen, we used this value as the benchmark for the dimethyl analogues. Of those *R* isomers displaying good in vitro potency, two agents (**16-R** and **17-R**) also meet the cytotoxicity criteria, while one agent (**18-R**) gives an unacceptable response. That **18-R** fails to meet the cytotoxicity criteria is not unexpected, since prior studies have shown that clastogenicity is affected greatly by substitution patterns at C-8 and N-1[13]-alkylation of the pyrrolidine ring is not sufficient to overcome the detrimental influence of the methoxy group at C-8.[14]

In most cases, however, addition of the two methyl groups elicits a decrease in the clastogenic potential over the parent compounds with a concomitant increase in in vivo efficacy (see, for example, **16A** vs **16-R** and **17A** vs **17-R**). When compared to the monomethyl analogues **16-RR** and **16-RS**, the dimethyl derivative **16-R** shows similar clastogenic potential to the less potent enantiomer **16-RR** (IC_{50} = 130 μg/mL for **16-R** and IC_{50} = 100 μg/mL for **16-RR**), both of which are markedly less cytotoxic than the more active **16-RS** (IC_{50} = 33 μg/mL). On the basis of this evidence, the risk of clastogenicity appears to be the least for the dimethylated derivatives, followed by the monomethylated compound and then the unsubstituted parent. This trend corroborates the previous assertion that increasing bulk around the distal nitrogen results in less cytotoxic agents.[14]

Compound **16-R**, equally efficacious to **17-R** in vitro but superior in vivo, was next evaluated in the phototoxicity assay. Conceivably, the enhanced oral absorption of **16-R** could translate into increased skin tissue penetration and therefore greater phototoxicity. Fortunately this is not the case: the oral no-effect dose (NED) of 73 mg/kg obtained is significantly higher than the 30 mg/kg value associated with the current clinically useful quinolones. Thus, the potential for cytotoxic or phototoxic side effects appears minimal for naphthyridine **16-R**.

In summary, we have shown that the incorporation of two methyl groups at the methylene spacer of [(amino-

methyl)pyrrolidinyl]quinolones (Figure 1) yields antibacterial agents with excellent Gram-positive potency in vivo with a low potential for phototoxicity and cytotoxicity. In all cases, the *R* stereoisomer displayed a 2–20-fold improvement in vitro and a 2–15-fold improvement in vivo over the less potent *S* isomer. Although the unsubstituted parent compounds were roughly equipotent to these *R* derivaties in vitro, the superior in vivo performance of the [3-(1-amino-1-methylethyl)pyrrolidinyl]quinolones recommend these agents as candidates for future development. Compound **16-R** (PD 138312), in particular, possesses exceptional oral efficacy against gram positive pathogens, as well as an impressive side-effect profile. For these reasons, this naphthyridine has been targeted for further preclinical examination.

Experimental Section

Melting points were taken on a Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were determined in KBr or as a liquid film on a Nicolet FT IR SX-20 spectrophotometer. Proton magnetic resonance (NMR) were recorded on a Bruker AM 250 spectrometer, and chemical shifts are reported in δ units relative to internal tetramethylsilane. Column chromatography was effected with E. Merck silica gel, 230–400 mesh ASTM. Elemental analyses were performed on a Perkin-Elmer Model 240 elemental analyzer, and all compounds had analytical results of ±0.4% of theoretical values. Final compounds were assayed for purity by using a Waters high-performance liquid chromatography (HPLC) system equipped with a 5-μm Alltech CN column and a mobile phase consisting of 20% THF/80% 0.5 M $NH_4H_2PO_4$ (adjusted to pH 3 with H_3PO_4). In all cases, purity exceeded 97%.

Ethyl [*S*-(*R,*S**)]-1-(1-Phenylethyl)-3-pyrrolidineacetate (10-R).** Concentrated H_2SO_4 (100 mL) was added slowly to an ice-cold solution of EtOH (200 mL), and the mixture was stirred at 0 °C for 10 min. To this mixture was added a solution of 25.6 g (0.119 mol) of **9-R**[22] in 50 mL of EtOH, and the mixture was refluxed for 20 h. The solution was then cooled to 0 °C, diluted with H_2O (700 mL), and neutralized to pH 7.0 by slow addition of 2 N NaOH with stirring. The mixture was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and brine and was dried. Concentration gave 24.7 g (79%) of **10-R** as a clear oil: NMR ($CDCl_3$) δ 1.22 (t, 3H), 1.36 (d, 3H), 1.46 (m, 1H), 2.13 (m, 2H), 2.37 (m, 4H), 2.68 (t, 2H), 3.18 (q, 1H), 4.09 (q, 2H), 7.29 (m, 5H); $[\alpha]^{25}_D$ −37.2° (*c* 1.0, CH_3OH) Anal. ($C_{16}H_{23}NO_2$) C, H, N.

(3*S*)-Ethyl [*S*-(*R,*R**)]-1-(1-Phenylethyl)-3-pyrrolidineacetate (10-S).** The title compound was prepared from **9-S** in 83% yield as described previously for **10-R**: NMR ($CDCl_3$) δ 1.22 (t, 3H), 1.34 (d, 3H), 1.42 (m, 1H), 2.05 (m, 2H), 2.36 (m, 2H), 2.55 (m, 3H), 2.85 (t, 1H), 3.18 (q, 1H), 4.09 (q, 2H), 7.30 (m, 5H).

Ethyl [*S*-(*R,*S**)]-α-Methyl-1-(1-phenylethyl)-3-pyrrolidineacetate (11-R).** A solution of 4.9 mL (32 mmol) of diisopropylamine in 100 mL of dry THF was cooled to −78 °C under argon, treated dropwise with 20 mL of 1.6 M *n*-butyllithium (32 mmol), and stirred for 20 min. To this LDA solution was added a solution of 6.53 g (25 mmol) of **10-R** in 20 mL of dry THF, and the solution was stirred at −78 °C for 1 h. Iodomethane (4.7 mL, 75 mmol) was added all at once. The reaction mixture was stirred for 30 min at −78 °C and diluted with water. The solution was warmed to room temperature. Concentration gave an oil which was partitioned between $CHCl_3$ and saturated $NaHCO_3$. The organic phase was washed with brine, dried, and concentrated. The residued was chromatographed on silica gel, eluting with 98:2 $CHCl_3$/MeOH, to give 5.62 g (82%) of title compound **11-R** as an oil: NMR ($CDCl_3$) δ 1.19 (m, 6H), 1.45 (m, 3H), 1.58 (m, 1H), 2.00 (m, 1H), 2.41 (m, 4H), 2.79 (m, 2H), 3.37 (m, 1H), 4.08 (q, 2H), 7.27 (m, 5H); $[\alpha]^{25}_D$ −24.8° (*c* 1.0, CH_3OH) Anal. ($C_{17}H_{25}NO_2 \cdot 0.1CHCl_3$) C, H, N, Cl.

Ethyl [*S*-(*R,*R*)]-α-Methyl-1-(1-phenylethyl)-3-pyrrolidineacetate (11-S).** The title compound was prepared from **10-S** in 82% yield as described previously for **11-R**: NMR ($CDCl_3$) δ 1.18 (m, 6H), 1.45 (m, 3H), 1.50 (m, 1H), 1.97 (m, 1H), 2.37 (m, 5H), 2.95 (m, 1H), 3.33 (m, 1H), 4.10 (m, 2H), 7.30 (m, 5H).

Ethyl [*S*-(*R,*S**)]-α,α-Dimethyl-1-(1-phenylethyl)-3-pyrrolidineacetate (12-R).** A solution of 8.9 mL (63 mmol) of diisopropylamine in 75 mL of dry THF was cooled to −78 °C under argon, treated dropwise with 37 mL of 1.6 M *n*-butyllithium (59 mmol), and stirred for 20 min. To this LDA solution was added a solution of 5.45 g (20 mmol) of **11-R** in 20 mL of dry THF, and the mixture was warmed to 0 °C. The solution was stirred at 0 °C for 30 min, quenched with 6.2 mL (99 mmol) of iodomethane, and stirred for 30 min. Water (2 mL) was added and the mixture stirred for 30 min. Concentrations gave an oil which was partitioned between $CHCl_3$ and saturated $NaHCO_3$. The organic layer was washed with brine, dried, and concentrated to an oil which was chromatographed on silica gel, eluting with $CHCl_3$/MeOH, 99:1. The title compound was obtained as a clear oil (5.33 g, 93%): NMR ($CDCl_3$) δ 1.11 (s, 6H), 1.21 (t, 3H), 1.35 (d, 3H), 1.61 (m, 1H), 1.86 (m, 1H), 2.21 (m, 2H), 2.48 (m, 2H), 2.75 (dd, 1H), 3.14 (q, 1H), 4.09 (q, 2H), 7.31 (m, 5H); $[\alpha]^{25}_D$ −48.2° (*c* 1.0, CH_3OH). Anal. ($C_{18}H_{27}NO_2 \cdot 0.25CHCl_3$) C, H, N.

Ethyl [*S*-(*R,*R**)]-α,α-Dimethyl-1-(1-phenylethyl)-3-pyrrolidineacetate (12-S).** The title compound was prepared from **11-S** in 75% yield as described previously in **12-R**, except that the final product was purified via distillation (bulb-to-bulb, 150 °C): NMR ($CDCl_3$) δ 1.12 (d, 6H), 1.21 (t, 3H), 1.35 (d, 3H), 1.55 (m, 1H), 1.78 (m, 1H), 2.19 (t, 1H), 2.42 (t, 2H), 2.50 (m, 1H), 2.73 (t, 1H), 3.15 (q, 1H), 4.05 (m, 2H), 7.30 (m, 5H); $[\alpha]^{25}_D$ −38.0° (*c* = 1.0, CH_3OH). Anal. ($C_{18}H_{27}NO_2$) C, H, N.

[*S*-(*R,*S**)]-α,α-Dimethyl-1-(1-phenylethyl)-3-pyrrolidineacetic Acid (13-R).** A solution of 5.25 g (18.0 mmol) of **12-R** in MeOH (120 mL) was treated with 27.2 mL (54.0 mmol) of 2 N NaOH, and the mixture was refluxed for 18 h. The solution was cooled, concentrated, and diluted with H_2O. The aqueous solution was washed with ether and then neutralized to pH 7.0 with 6 N HCl. The mixture was extracted with $CHCl_3$. The organic layer was washed with water, dried, and concentrated to give 3.39 g (72%) of **13-R** as a white foam: NMR ($CDCl_3$) δ 1.20 (s, 3H), 1.27 (s, 3H), 1.60 (d, 3H), 1.83 (m, 1H), 1.98 (m, 1H), 2.17 (m, 1H), 2.42 (q, 1H), 2.64 (t, 1H), 2.99 (t, 1H), 3.54 (m, 1H), 3.73 (q, 1H), 7.37 (m, 5H), 11.60 (bs, 1H); $[\alpha]^{25}_D$ −4.4° (*c* 1.0, CH_3OH). Anal. ($C_{16}H_{23}NO_2 \cdot 0.3CHCl_3$) C, H, N.

[*S*-(*R,*R**)]-α,α-Dimethyl-1-(1-phenylethyl)-3-pyrrolidineacetic Acid (13-S).** A solution of 11.2 g (38.7 mmol) of **12-S** in 6 N HCl (75 mL) was refluxed for 7 h, cooled to room temperature, and concentrated. The residue was triturated with 2-PrOH/ether, 1:5. The solids that formed were filtered, washed with ether, and dried in vacuo to give 8.35 g (73%) of **13-S** as the hydrochloride salt: NMR (DMSO-d_6) δ 1.10 (s, 6H), 1.62 (d, 3H), 1.75 (m, 1H), 1.90 (m, 1H), 2.67–3.03 (m, 4H), 3.73 (m, 1H), 4.42 (m, 1H), 7.43 (m, 3H), 7.69 (m, 2H); $[\alpha]^{25}_D$ −19.0° (*c* 1.0, CH_3OH). Anal. ($C_{16}H_{23}NO_2 \cdot HCl$) C, H, N, Cl.

1,1-Dimethylethyl [*S*-(*R,*S**)]-[1-Methyl-1-[1-(1-phenylethyl)-3-pyrrolidinyl]ethyl]carbamate (14-R).** A mixture of freshly distilled t-BuOH (150 mL) and 3-Å molecular sieves was stirred for 30 min and then treated with 5.46 g (21.0 mmol) of **13-R** and 3.2 mL (23.0 mmol) of Et_3N. After being stirred for 1 h, the solution was treated with 4.7 mL (21.0 mmol) of diphenyl phosphorazidate and refluxed for 30 h. The reaction mixture was cooled and concentrated. The residue was dissolved in CH_2Cl_2, and the organic phase was washed with saturated $NaHCO_3$ and brine, dried, and concentrated. The crude product was chromatographed on silica gel, eluting with $CHCl_3$, to give 2.2 g (33%) of **14-R** as a solid: NMR ($CDCl_3$) δ 1.23–1.47 (m, 18H), 1.80 (m, 2H), 2.14 (m, 2H), 2.25 (m, 1H), 2.59 (m, 1H), 3.00 (d, 1H), 3.15 (q, 1H), 6.38 (bs, 1H), 7.32 (m, 5H); $[\alpha]^{25}_D$ +3.6° (*c* 1.0, CH_3OH). Anal. ($C_{20}H_{32}N_2O_2 \cdot 0.2H_2O$) C, H, N.

1,1-Dimethylethyl [*S*-(*R,*R**)]-[1-Methyl-1-[1-(1-phenylethyl)-3-pyrrolidinyl]ethyl]carbamate (14-S).** The title compound was prepared from **13-S** in 45% yield as described previously for **14-R**: NMR ($CDCl_3$) δ 1.29 (d, 6H), 1.37 (t, 3H), 1.48 (s, 9H), 1.85 (m, 2H), 2.04–2.40 (m, 3H), 2.45–2.58 (m, 1H), 3.15 (m, 2H), 6.9 (bs, 1H), 7.30 (m, 5H); $[\alpha]^{25}_D$ +1.2° (*c* 1.0, CH_3OH).

1,1-Dimethylethyl(*R*)-[1-Methyl-1-(3-pyrrolidinyl)ethyl]carbamate (15-R). A mixture of 2.2 g (6.7 mmol) of **14-R**, 0.5 g of 20% palladium on carbon, and 100 mL of MeOH was shaken

under hydrogen gas (50 psi) in a Parr shaker for 14 h. The catalyst was filtered, and the filtrate was concentrated to give 1.55 g (90%) of the title compond as a white foam: NMR ($CDCl_3$) δ 1.32 (d, 6H), 1.45 (bs, 9H), 1.85 (m, 1H), 2.03 (m, 1H), 3.15 (m, 3H), 3.30 (m, 2H), 4.54 (bs, 1H), 6.60 (bs, 2H); $[\alpha]^{24}_D$ +22.0° (c = 1.0, CH_3OH). Anal. ($C_{12}H_{24}N_2O_2 \cdot 0.9HCl \cdot 0.2H_2O$) C, H, N, Cl.

1,1-Dimethylethyl (*S*)-[1-Methyl-1-(3-pyrrolidinyl)ethyl]-carbamate (15-S). The title compound was prepared from **14-S** in 85% yield as described previously for **15-R**: NMR ($CDCl_3$) δ 1.14 (d, 3H), 1.30 (d, 3H), 1.42 (bs, 9H), 1.75 (m, 1H), 1.94 (m, 1H), 2.82–3.35 (m, 4H), 3.6 (m, 1H), 4.0 (bs, 2H); $[\alpha]^{25}_D$ −26.1° (c 1.0, CH_3OH).

General Method A. Preparation of (*S*)-7-[3-(1-Amino-1-methylethyl)-1-pyrrolidinyl]-8-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (19-S). A solution of 0.66 g (2.2 mmol) of **3**, 0.7 g (3.1 mmol) of **15-S**, 0.72 g (7.1 mmol) of triethylamine, and 30 mL of CH_3CN was refluxed for 18 h. The solution was concentrated, and the residue was chromatographed on silica gel, eluting with $CHCl_3$/MeOH, 9:1. The solid obtained was dissolved in CH_2Cl_2 (20 mL), cooled to 5 °C, and treated with a steady stream of gaseous HCl for 10 min. The mixture was allowed to warm to room temperature overnight. The solvent was evaporated and the residue triturated with 2-PrOH/ether. The solids were filtered, washed with ether, and dried to give 0.73 g (75% from **3**) of the title compound: mp 220–223 °C; NMR (DMSO-d_6) δ 0.90–1.23 (m, 4H), 1.32 (s, 6H, 2 CH_3 groups), 1.95 (m, 1H), 2.10 (m, 1H), 2.55 (m, 1H), 3.53 (m, 2H), 3.73 (m, 2H), 4.38 (m, 1H), 7.87 (d, J = 14 Hz, 1H, C-5 H), 8.23 (m, 2H, NH_2), 8.82 (s, 1H, C-2 H). Anal. ($C_{20}H_{23}ClFN_3O_3 \cdot HCl \cdot 2H_2O$) C, H, N, Cl.

Compounds **16-S**, **17-S**, and **19-R** were prepared in identical fashion to **19-S**. Compounds **16-R**, **17-R**, **20-R**, and **20-S** were prepared similarly except that the intermediate *tert*-butoxycarbonyl compound was not chromatographed; the final deprotected quinolone was recrystallized as indicated in Table 2.

General Method B. Preparation of (*R*)-7-[3-(1-Amino-1-methylethyl)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic Acid (18-R). A solution of 0.60 g (1.7 mmol) of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic acid–boron difluoride complex (**5**),[20] 0.52 g (2.3 mmol) of pyrrolidine **15-R**, 0.68 g (5.3 mmol) of diisopropylethylamine, and 25 mL of CH_3CN was stirred at room temperature for 18 h. The mixture was concentrated, and the residue was dissolved in EtOH (50 mL) and Et_3N (5 mL). The reaction mixture was refluxed for 18 h, cooled, and concentrated. The product was chromatographed on silica gel, eluting with $CHCl_3$/MeOH, 9:1, to give a yellow foam, mp 70–72 °C.

The penultimate intermediate was dissolved in 20 mL of CH_2Cl_2, cooled to 5 °C, and treated with a steady stream of gaseous HCl for 10 min. The mixture was allowed to warm to room temperature and concentrated. This paste was triturated with 2-PrOH, and the solids were filtered, washed with ether, and dried to give 0.36 g (47% overall) of the title compound as the hydrochloride salt: mp 225–227 °C; NMR (DMSO-d_6) δ 0.91–1.48 (m, 10H), 1.79 (m, 1H), 2.07 (m, 1H), 2.55 (m, 1H), 3.55–3.75 (m, 7H), 4.15 (m, 1H), 7.70 (d, J = 17.7 Hz, 1H, C-5 H), 8.67 (s, 1H). Anal. ($C_{21}H_{26}FN_3O_4 \cdot 1.15HCl \cdot 2H_2O$) C, H, N, Cl. Compound **18-S** was prepared in identical fashion.

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