7-Azetidinylquinolones as Antibacterial Agents. 3.[1] Synthesis, Properties and Structure–Activity Relationships of the Stereoisomers Containing a 7-(3-Amino-2-methyl-1-azetidinyl) Moiety[2]

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A series of stereochemically pure 7-(3-amino-2-methyl-1-azetidinyl)-1,4-dihydro-6-fluoro-4-oxoquinoline- and -1,8-naphthyridine-3-carboxylic acids, with varied substituents at the 1-, 5-, and 8-positions, was prepared to determine the effects of chirality on potency and *in vivo* efficacy relative to the racemic mixtures (for part 2, see: *J. Med. Chem.* **1994**, *37*, 4195–4210). A series of chiral 9-fluoro-2,3-dihydro-3-methyl-7-oxo-10-(substituted-1-azetidinyl)-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acids was synthesized to study the effect of the azetidine moiety on tricyclic quinolone antibacterial agents. A series of amino acid prodrugs of chiral naphthyridines **24a** and **24b** and quinolone **33a** (cetefloxacin) was prepared and evaluated for antibacterial activity, solubility, and pharmacokinetic behavior. The absolute conflguration of the new azetidinylquinolones was established by X-ray analysis of one of the diastereomeric salts of the resolved azetidinols (**15**) and of compound **25a** (E-4767), which showed the best *in vitro* and *in vivo* overall profile. Structure–activity relationship studies indicated that the absolute stereochemistry at the asymmetric centers of both the azetidine and the oxazine rings was critical to increase *in vitro* activity and oral efficacy. The 3*S* configuration in the pyridobenzoxazine series and the (2*S*,3*R*) configuration of the 3-amino-2-methylazetidine moiety for all new compounds conferred the best antibacterial activity.

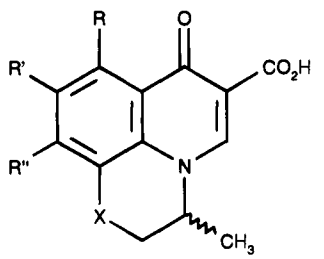
Quinolone antibacterial agents continue to represent an important new class of therapeutically useful compounds and have been the subject of many recent reviews.[3]

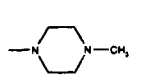
The importance of chiral chemical compounds in biological, pharmaceutical, and pharmacokinetic phenomena is well-documented.[4] In favorable cases the enantiomers of drugs have been shown to result in enhanced selectivity, greater potency, and reduced side effects.

Nearly all clinically useful quinolone antibacterial agents developed to date are either achiral or racemic mixtures. Recently,[5,6] however, optically active centers have increasingly been introduced into the structures of synthetic quinolones. In some case the racemic mixtures have been resolved as individual enantiomers or they have been synthesized in a chiral manner. A substantial difference in potency has then been observed between the chiral forms. Most of the tricyclic quinolones possess an asymmetric center in the quinolizine or benzoxazine rings (Chart 1), but among them only a few are capable of resolution into antipodes.[5] The (*S*)-enantiomers of the tricyclic quinolones have been reported to exhibit greater biological activities (10–100-fold) than their antipodes.[5] Recently, optically active enantiomers of flumequine (**1**),[5a] methylflumequine (**2**),[5c] and **3**[5b] have been obtained by asymmetric synthesis and the more outstanding, the (*S*)-(−)-enantiomer of ofloxacin **4**, known as levofloxacin, has been prepared using its optically resolved synthetic intermediate[5d,e] or by an efficient asymmetric synthesis.[5f]

With regard to quinolones and naphthyridinones, some examples have been reported in the literature on

Chart 1



	X	R	R'	R"
1	CH_2	H	F	H
2	CH_2	H	F	CH_3
3	O	CH_3	H	Cl
4	O	H	F	

the influence of side-chain asymmetry on antibacterial activity.[6] The (*S*)-enantiomer of **5** is about 4 times more potent *in vitro* than (*R*)-**5**.[6a] Although (*S*)-(−)-**6** does show a consistent trend toward increased potency against Gram-positive organisms, there is no significant potency difference with its enantiomer (*R*)-(+)-**6**.[6b] The (*S*)-(+) enantiomer of tosufloxacin (**7**) is 2-4 times more active than its (*R*)-(−) enantiomer.[6c] (*R*)-**8** shows 10-60-fold greater potency than its antipode.[6d] Although pairs of enantiomers have not been synthesized, the enantiomerically homogeneous series (4*S*)-**9** shows that the absolute stereochemistry at the 2-position of the pyrrolidine ring is critical in exhibiting potent antibacterial activity.[6d] (3*R*)-3-(1-Amino-1-methylethyl)-1-pyr-

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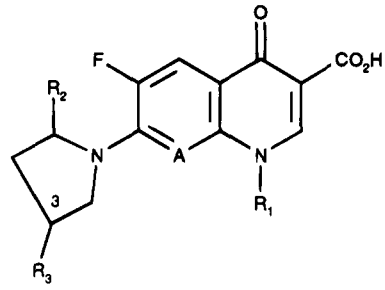
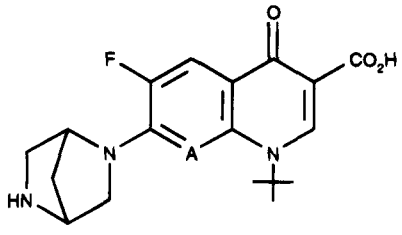
Chart 2



	5	6	7	8	9	10
R_2	H	H	H	CH_2OH	CH_3	H
R_3	OH	CH_2NHEt	NH_2	H	NH_2	$CR_4R_5(NH_2)$



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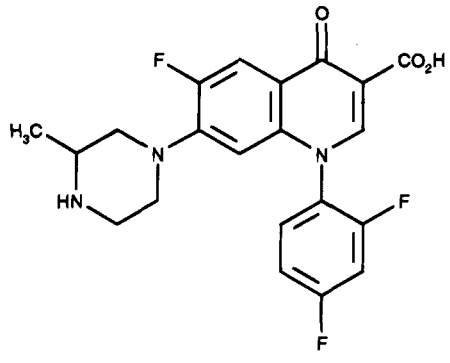

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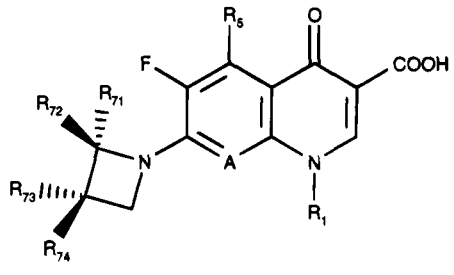
Chart 3

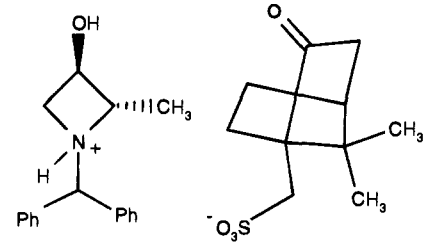


13: A = CH, CF, CCl, N
R_1 = c-C_3H_5, Et, 4-FPh, 2,4-F_2Ph

14: A-R_1 = C-O-CH_2-CH(CH_3)

rolidinyl and (3*R*)-3-[(1*S*)-1-aminoethyl]-1-pyrrolidinyl derivatives **10** were identified as the most potent stereoisomers in this series.[6e,f] (1*R*,4*R*)-**11** stereoisomers are 2-8-fold more potent than its (1*S*,4*S*) counterparts.[6g] Although no difference in *in vitro* antibacterial activities was observed between the enantiomers of temafloxacin (**12**), a slightly better pharmacokinetic profile was observed for (*S*)-(−)-**12** in mice.[6h]

On the other hand, we have shown that replacing the 1-piperazinyl or 1-aminopyrrolidinyl moiety of quinolones and naphthyridinones with 3-amino- or 3-amino-3-methyl-1-azetidinyl rings (**13**; R_{71} = R_{72} = H; R_{73} = H, CH_3; R_{74} = NH_2) greatly enhanced *in vivo* efficacy.[7] Recently, racemic 2,3-disubstituted 1-azetidinyl derivatives (**13**; R_{71}, R_{72}, R_{73} = H, CH_3; R_{74} = NH_2) have been reported as particularly potent members of this class of antibacterial agents.[1] Among them the *trans*-3-amino-2-methyl-1-azetidinyl group conferred the best overall antibacterial, pharmacokinetic, and physicochemical properties to the 7-azetidinylquinolones. It seemed of interest to us to know which of the stereoisomers was more potent or even if one of the enantiomers was the active component of the racemic mixtures. In this paper, we report the efficient synthesis and *in vitro* antibacterial activities of stereoisomers of quinolones and naphthyridinones **13**, and pyridobenzoxazines **14**, as well as the *in vivo* activity comparison between these compounds and their corresponding racemic mixtures in mouse protection tests. We have also developed the reactions of amino acids or peptides with stereoisomers **13** in order to improve their very low solubility in water and at physiological pH. We have also carried out the single-crystal X-ray analysis of compounds **15** and **25a** in order to establish their absolute configuration and to compare **25a** with the unsubstituted and 3-monosubstituted azetidinylquinolones previously analyzed.[1]



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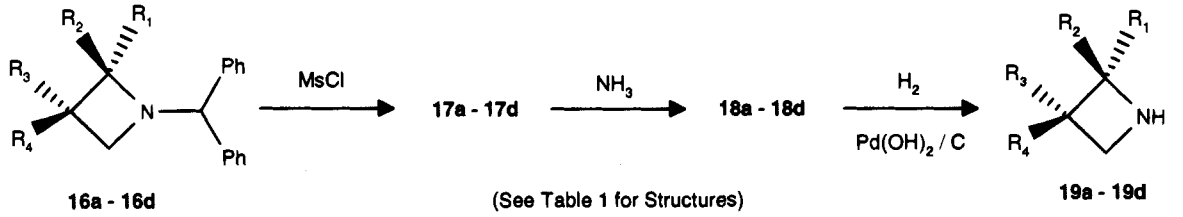
Chemistry

The chiral 3-amino-2-methylazetidines used in this study are new compounds that we have prepared in our

Scheme 1



Scheme 2

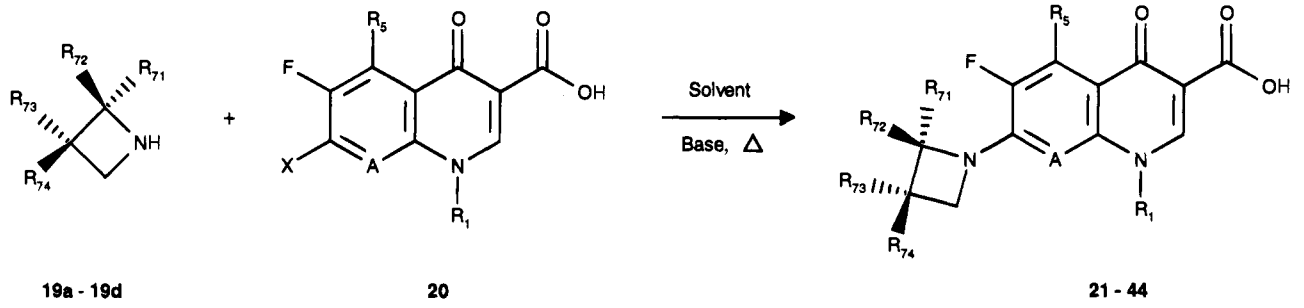
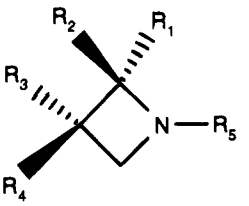


Table 1. Azetidine Nucleus[a]



compd	stereo	R_1	R_2	R_3	R_4	R_5
16a	2*S*,3*R*	CH_3	H	H	OH	Ph_2CH
16b	2*R*,3*S*	H	CH_3	OH	H	Ph_2CH
16c	2*S*,3*S*	CH_3	H	OH	H	Ph_2CH
16d	2*R*,3*R*	H	CH_3	H	OH	Ph_2CH
17a	2*S*,3*R*	CH_3	H	H	MsO	Ph_2CH
17b	2*R*,3*S*	H	CH_3	MsO	H	Ph_2CH
17c	2*S*,3*S*	CH_3	H	MsO	H	Ph_2CH
17d	2*R*,3*R*	H	CH_3	H	MsO	Ph_2CH
18a	2*S*,3*R*	CH_3	H	H	NH_2	Ph_2CH
18b	2*R*,3*S*	H	CH_3	NH_2	H	Ph_2CH
18c	2*S*,3*S*	CH_3	H	NH_2	H	Ph_2CH
18d	2*R*,3*R*	H	CH_3	H	NH_2	Ph_2CH
19a	2*S*,3*R*	CH_3	H	H	NH_2	H
19b	2*R*,3*S*	H	CH_3	NH_2	H	H
19c	2*S*,3*S*	CH_3	H	NH_2	H	H
19d	2*R*,3*R*	H	CH_3	H	NH_2	H

[a] Abbreviation: Ms = methylsulfonyl.

laboratories.[1,8] 3-Azetidinols are key compounds in the synthesis of 3-aminoazetidines. The *N*-(diphenylmethyl)azetidinols **16** have been obtained in a stereospecific fashion by treatment of the monomesylate derived from *N*-(diphenylmethyl)-3-amino-1,2-butanediols with triethylamine,[9] or by resolution of the racemic mixture, which in turn was synthesized from 1-hydroxy-2-butene.[1] Resolution of (±)-*trans*-1-(diphenylmethyl)-3-hydroxy-2-methylazetidine was achieved by fractional recrystallization of the (+)-(1*S*)-camphorsulfonic salt from water. The less soluble diastereomeric salt provided (+)-(2*R*,3*S*)-1-(diphenylmethyl)-3-hydroxy-2-methylazetidinyl (1*S*)-camphorsulfonate with 97% optical purity, as determined by HPLC. From basified mother liquor and treatment with (−)-(1*R*)-camphorsulfonic acid, **15** was obtained with 96% optical purity, as determined by HPLC.

An amino group was introduced at the 3-position of 1-benzhydrylazetidine (Scheme 1) by sequential methanesulfonate ester formation (**17**) and displacement with ammonia to obtain **18** with stereospecific retention of configuration.[1] Removal of the (diphenylmethyl) group yields **19**, which could be condensed with the quinolone nuclei **20** (Scheme 2) to yield compounds **21**−**44** following synthetic routes previously reported.[7,10] Physical properties of chiral compounds **21**−**44** and their structures are summarized in Tables 2 (quinolone and naphthyridinone) and 3 (pyridobenzoxazine).

Amino acid derivatives **46** were obtained (Scheme 3) by reaction of naphthyridinones **24a** and **24b**, and quinolone **33a** with the amino acid active esters of *N*-hydroxysuccinimide **45**, whose amino functional group was protected with a suitable group such as those used in peptide synthesis.[11] Removal of the protective groups was carried out by means of a catalytic hydrogenation (method a), or by acidolysis employing trifluoroacetic acid (method B). The corresponding salts **47** and **48** were obtained by treatment with hydrochloric acid or *p*-toluenesulfonic acid in ethanol. Physical properties of compounds **47** and **48** and their structures are displayed in Table 4.

X-ray Crystallographic Study

The absolute configurations of the isolated 3-hydroxy-2-methylazetidine stereoisomers were confirmed by X-ray crystallography of (−)-(1*R*)-camphorsulfonate of **16a** (compound **15**). Cell parameters and characteristics are described in Table 5. Compound **16a** has 2*S*,3*R* configuration (Figure 1). According to previously reported work,[12] the four-membered azetidine ring was found to be buckled ranging from 0 to 11°, but larger buckling (from 14 to 27°) was observed in 3-hydroxyazetidine derivatives. The azetidine ring of **15** is present in a buckled form to an extent of 22.2° (angle of puckering, θ = 157.8°). The endocyclic N−C bonds [1.518(3) and 1.531(3) Å] are longer than the exoxyclic

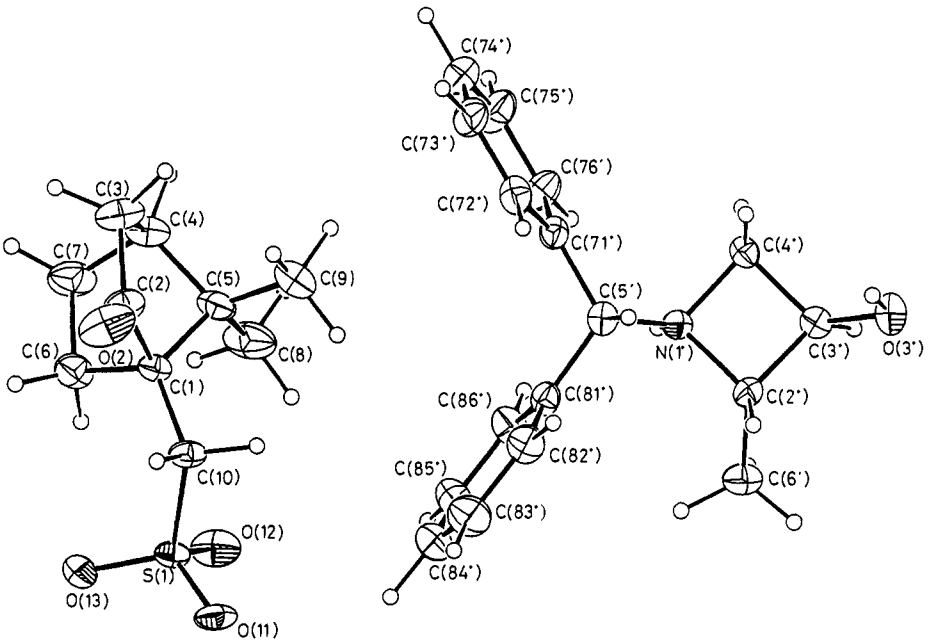


Figure 1. Single-crystal X-ray structure of **15**.

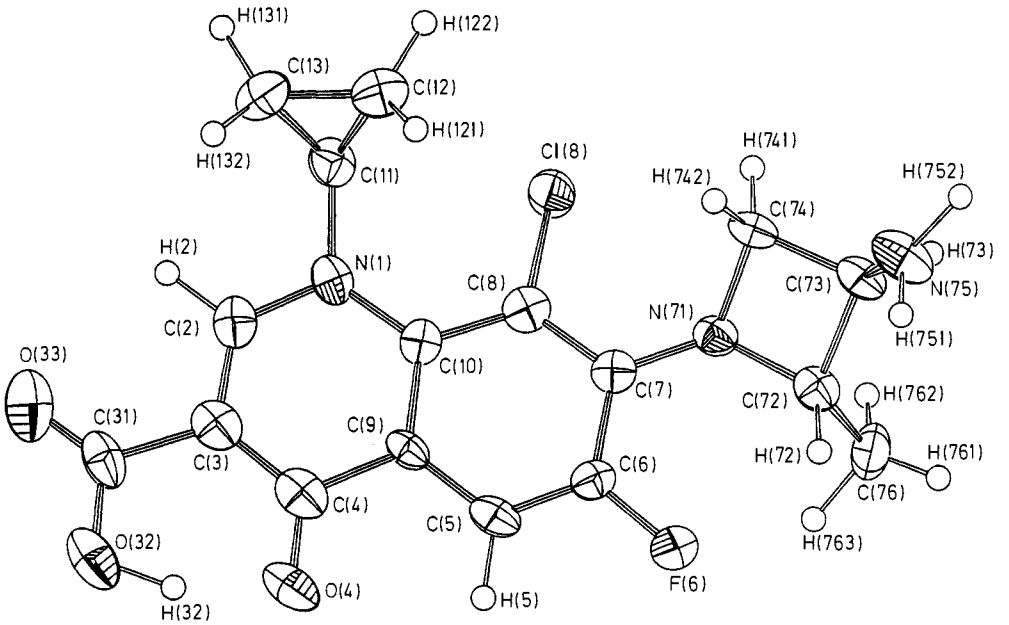
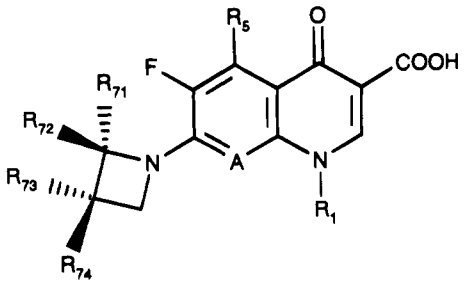


Figure 2. Single-crystal X-ray structure of **25a**.

one [1.501(3) Å]. This lengthening may be atributed to the strain in the four-membered ring.

Among the thousands of synthesized quinolones, only a few structures have been reported using X-ray crystallography.[13] Recently,[1] we described the single-crystal X-ray analysis of two 7-(unsubstituted-azetidinyl)-quinolones (UAQ) and a 7-(3-(ethylamino)azetidinyl)-quinolone (3AQ). Concerning 7-(3-amino-2-methylazetidinyl)quinolones, compound **25a** (E-4767) afforded suitable crystals for X-ray analysis and the 2*S*,3*R* absolute configuration was confirmed (Figure 2). The 2*S*,3*R* absolute configuration of **25a** also shows conclusively that the introduction of an amino group at the 3-position of the azetidinol, by activation of the hydroxyl as the mesylate and subsequent displacement with the nucleophile, proceeds with retention of configuration, since the starting material was (2*S*,3*R*)-1-(diphenylmethyl)-3-hydroxy-2-methylazetidine. Cell parameters and characteristics are described in Table 5. The angle of puckering for azetidine ring is 170.1°, and the endocyclic N-C bonds [1.490(8) and 1.492(8) Å] are substantially longer than the exocyclic one [1.370(8) Å]. The azetidine ring deviates from the plane determined by the quinoline [33.5(3)°] to a greater extent than for UAQ and 3AQ [9.2(3) and 16.0(1)°, respectively],[1] probably due to the presence of a chlorine atom at the 8-position of **25a**. We may argue the same reason for the measured angle between the cyclopropyl ring and the quinoline least-square plane [115.0(4)°]. The amino acid 3AQ showed a zwitterionic character,[1] but the carboxylic group has a nonionic character in amino acid **25a**. An intramolecular hydrogen bond between the carboxylic acid and the carbonyl group forms a quasi-planar pseudo-six-membered ring, which does not deviate significantly from the planarity determined by the quinoline ring [4.3(3)°]. These hydrogen bonds and

Table 2. Physical Data of the Quinolones and Naphthyridinones Prepared for This Study[a]



compd	A	R_1	R_5	R_{71}	R_{72}	R_{73}	R_{74}	stereo[b]	mp, °C	$[\alpha]^{20}_D$, deg (c, 0.5 N NaOH)	analyses[c]	% yield[d]
21	CH	c-C_3H_5	H	H	CH_3	NH_2	H	racemic				
21a	CH	c-C_3H_5	H	CH_3	H	H	NH_2	2*S*,3*R*	252–254	−12.1 (0.30)	$C_{17}H_{18}FN_3O_3 \cdot 0.3H_2O$	87
21b	CH	c-C_3H_5	H	H	CH_3	NH_2	H	2*R*,3*S*	242–244	+13.7 (0.38)	$C_{17}H_{18}FN_3O_3 \cdot 0.1H_2O$	81
22	CF	c-C_3H_5	H	H	CH_3	NH_2	H	racemic				
22a	CF	c-C_3H_5	H	CH_3	H	H	NH_2	2*S*,3*R*	231–233	−10.6 (0.27)	$C_{17}H_{17}F_2N_3O_3 \cdot 0.2H_2O$	82
22b	CF	c-C_3H_5	H	H	CH_3	NH_2	H	2*R*,3*S*	229–231	+9.4 (0.26)	$C_{17}H_{17}F_2N_3O_3 \cdot 0.3H_2O$	89
23	CF	c-C_3H_5	H	H	CH_3	H	NH_2	racemic				
23c	CF	c-C_3H_5	H	CH_3	H	NH_2	H	2*S*,3*S*	193–197	−32.3 (0.69)	$C_{17}H_{17}F_2N_3O_3 \cdot 0.7H_2O$	71
23d	CF	c-C_3H_5	H	H	CH_3	H	NH_2	2*R*,3*R*	196–200	+32.0 (0,50)	$C_{17}H_{17}F_2N_3O_3 \cdot 0.5H_2O$	64
24	N	c-C_3H_5	H	H	CH_3	NH_2	H	racemic				
24a	N	c-C_3H_5	H	CH_3	H	H	NH_2	2*S*,3*R*	236–239	−12.1 (0.94)	$C_{16}H_{17}FN_4O_3 \cdot 0.4H_2O$	85
24b	N	c-C_3H_5	H	H	CH_3	NH_2	H	2*R*,3*S*	231–236	+10.5 (1.0)	$C_{16}H_{17}FN_4O_3 \cdot 0.9H_2O$	76
25	CCl	c-C_3H_5	H	CH_3	H	H	NH_2	racemic				
25a	CCl	c-C_3H_5	H	CH_3	H	H	NH_2	2*S*,3*R*	221–225	−156.0 (0.30)	$C_{17}H_{17}ClFN_3O_3$	76
25b	CCl	c-C_3H_5	H	H	CH_3	NH_2	H	2*R*,3*S*	249–252	+155.2 (0.88)	$C_{17}H_{17}ClFN_3O_3$	88
26	CF	c-C_3H_5	NH_2	CH_3	H	H	NH_2	racemic				
26a	CF	c-C_3H_5	NH_2	CH_3	H	H	NH_2	2*S*,3*R*	210–218	−45.4 (0.35)	$C_{17}H_{18}F_2N_4O_3 \cdot 0.2H_2O$	75
27a	N	c-C_3H_5	CH_3	CH_3	H	H	NH_2	2*S*,3*R*	199–201	−7.1 (1.0)	$C_{17}H_{19}FN_4O_3 \cdot 0.7H_2O$	80
28	CF	C_2H_5	H	CH_3	H	H	NH_2	racemic				
28a	CF	C_2H_5	H	CH_3	H	H	NH_2	2*S*,3*R*	206–211	−5.0 (0.92)	$C_{16}H_{17}F_2N_3O_3$	78
29a	CF	C_2H_5	NH_2	CH_3	H	H	NH_2	2*S*,3*R*	263–267	−22.0 (1.0)	$C_{16}H_{18}F_2N_4O_3 \cdot 0.4H_2O$	73
30	CF	4-FPh	H	CH_3	H	H	NH_2	racemic				
30a	CF	4-FPh	H	CH_3	H	H	NH_2	2*S*,3*R*	250–254	−12.5 (1.0)	$C_{20}H_{16}F_3N_3O_3 \cdot 0.6H_2O$	93
31	CF	2,4-F_2Ph	H	CH_3	H	H	NH_2	racemic				
31a	CF	2,4-F_2Ph	H	CH_3	H	H	NH_2	2*S*,3*R*	197–200	−14.0 (0.30)	$C_{20}H_{15}F_4N_3O_3 \cdot 0.5H_2O$	82
32	N	2,4-F_2Ph	H	CH_3	H	H	NH_2	racemic				
32a	N	2,4-F_2Ph	H	CH_3	H	H	NH_2	2*S*,3*R*	191–196	−33.5 (0.65)	$C_{19}H_{15}F_3N_4O_3 \cdot 0.8H_2O$	59
32b	N	2,4-F_2Ph	H	H	CH_3	NH_2	H	2*R*,3*S*	183–187	+38.4 (1.0)	$C_{19}H_{15}F_3N_4O_3$	68
33	CH	2,4-F_2Ph	H	CH_3	H	H	NH_2	racemic				
33a	CH	2,4-F_2Ph	H	CH_3	H	H	NH_2	2*S*,3*R*	227–230	−40.8 (1.1)	$C_{20}H_{16}F_3N_3O_3 \cdot 1.1H_2O$	71
33b	CH	2,4F_2Ph	H	H	CH_3	NH_2	H	2*R*,3*S*	207–212	+38.6 (1.0)	$C_{20}H_{16}F_3N_3O_3 \cdot 0.5H_2O$	52
34	CCl	2,4F_2Ph	H	CH_3	H	H	NH_2	racemic				
34a	CCl	2,4F_2Ph	H	CH_3	H	H	NH_2	2*S*,3*R*	180–181	−70.8 (0.75)	$C_{20}H_{15}ClF_3N_3O_3 \cdot 0.2H_2O$	34
35a	CF	2,4F_2Ph	NH_2	CH_3	H	H	NH_2	2*S*,3*R*	246–248	−49.3 (0.5)	$C_{20}H_{16}F_4N_4O_3 \cdot 0.7H_2O$	65
36a	N	2,4F_2Ph	CH_3	CH_3	H	H	NH_2	2*S*,3*R*	126–128	−16.0 (0.5)	$C_{20}H_{17}F_3N_4O_3 \cdot 0.8H_2O$	53

[a] Abbreviations: c-C_3H_5 = cyclopropyl, 4-FPh = 4-fluorophenyl, 2,4-F_2Ph = 2,4-difluorophenyl. [b] Racemic compounds were previously described.[1] [c] C, H, and N analyses where within ±0.4% of the theoretical values for the formula shown. [d] Yields are those obtained from the coupling step to final product.

angle are practically the same as for 8-fluoro-7-(unsubstituted-azetidinyl)quinolone[1] analogs of **25a** [O(32)–H(32) 1.07(9) Å, O(4)···H(32) 1.49 (9) Å, <O···H–O 160.0(8)°].

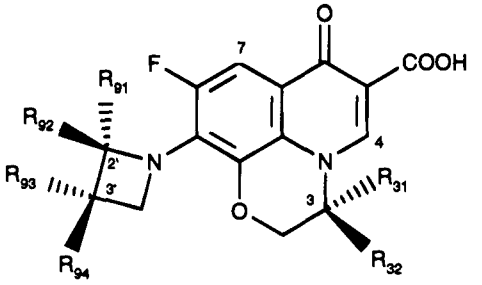
Biological Assays

Compounds **21**–**44** and **47**–**48** were evaluated for *in vitro* antibacterial activity versus a variety of Gram-positive and Gram-negative bacteria. These activities are reported as minimum inhibitory concentration (MIC, μg/mL). Representative data for the stereoisomers are displayed in Table 6. Data for racemic mixtures as well as for ciprofloxacin and levofloxacin are provided for comparison. The *in vivo* efficacy of several stereoisomers determined by the mouse protection test is shown in Table 7. The potency is given in ED_{50} values which are expressed as the total dose of compound in mg/kg required to protect 50% of the mice challenged intraperitoneally with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, or *Escherichia coli*. Data for racemic mixtures are provided for comparison, and ciprofloxacin and levofloxacin were used as standards. The compounds were administered orally (po). Blood levels of selected quinolones after oral administration (50 mg/kg) in mice are displayed in Table 8.

Results and Discussion

We have shown previously[1] that the introduction of a methyl group at C-2 of a 3-aminoazetidinyl group attached at C-7 of **13** markedly influenced the antibacterial activity with respect to the mono-substituted and 3,3-disubstituted azetidine.[7] We also found[1] that the *trans*-3-amino-2-methyl-1-azetidinyl moiety (compound **22**) produces 2–8 times better activity than the *cis*-3-amino-2-methyl-1-azetidinyl substituent, **23**. In this study, we have focused on the four stereoisomers of 2-methyl-3-amino-1-azetidinyl derivatives. The enantiomer (2*R*,3*S*)-**22b** and its epimer (2*R*,3*R*)-**23d** were

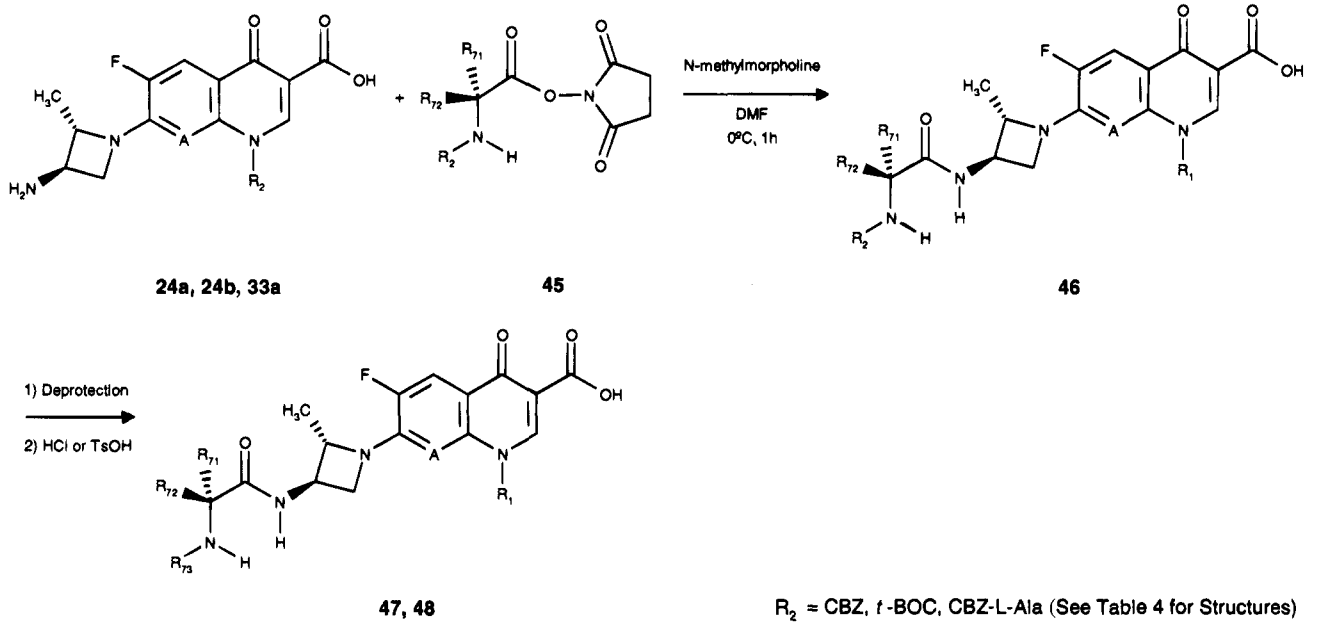
Table 3. Physical Data of the Pyridobenzoxazines Prepared for This Study[a]



compd	R_{31}	R_{32}	R_{91}	R_{92}	R_{93}	R_{94}	stereo	mp, °C	$[\alpha]^{20}_D$, deg (c, 0.5 N NaOH)	analyses[b]	% yield[c]	NMR, δ[d] C_4H[e]	C_7H[f]
37	H	CH_3	H	H	NH_2	H	3*S*	236–240	−78.8 (0.41)	$C_{16}H_{16}FN_3O_4 \cdot 0.5H_2O$	65	8.88	7.52
38	CH_3	H	H	H	NH_2	CH_3	3*R*	>300	+82.2 (0.43)	$C_{17}H_{18}FN_3O_4$	57	8.62	7.50
39	H	CH_3	H	H	NH_2	CH_3	3*S*	>300	−83.1 (0.41)	$C_{17}H_{18}FN_3O_4 \cdot 0.4H_2O$	57	8.66	7.47
40	H	CH_3	H	H	NHMe	CH_3	3*S*	>300	−77.4 (0.50)	$C_{18}H_{20}FN_3O_4 \cdot 0.5H_2O$	83	8.91	7.55
41	H	CH_3	H	H	NMe_2	H	3*S*	>300	−79.6 (0.41)	$C_{18}H_{20}FN_3O_4 \cdot 1.2H_2O$	64	8.57	7.52
42	H	CH_3	H	H	NMe_2	CH_3	3*S*	298–299	−74.6 (0.40)	$C_{19}H_{22}FN_3O_4 \cdot 0.1H_2O$	56	8.76	7.50
43	H	CH_3	H	H	CH_2NHEt	CH_3	3*S*	242–245	−56.1 (0.48)	$C_{20}H_{24}FN_3O_4 \cdot 0.1H_2O$	37	8.86	7.48
44a	H	CH_3	CH_3	H	H	NH_2	3*S*,2'*S*,3'*R*	217–221	−30.2 (0.36)	$C_{17}H_{18}FN_3O_4 \cdot 0.2H_2O$	72	8.92	7.57
44b	H	CH_3	H	CH_3	NH_2	H	3*S*,2'*R*,3'*S*	217–219	−106.8 (0.31)	$C_{17}H_{18}FN_3O_4 \cdot 1.0H_2O$	54	8.92	7.58

[a] Abbreviations: Me = methyl, Et = ethyl. [b] See Table 2. [c] Yields are those obtained from the coupling step to final product, including deprotection when appropriate.[1] [d] Solvent: DMSO-d_6, TFA. [e] Singlet. [f] Doublet.

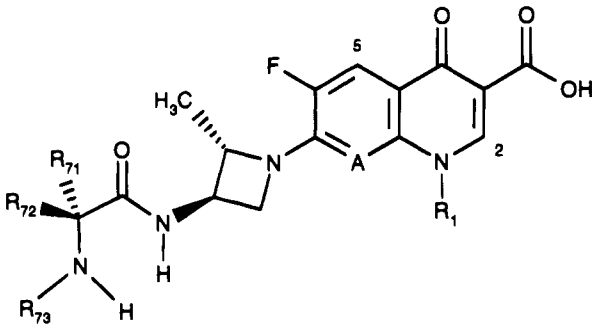
Scheme 3



slightly less potent than or as potent as ciprofloxacin against Gram-positive bacteria, but they were appreciably less active against Gram-negative microorganisms. Conversely, the enantiomer (2*S*,3*R*)-**22a** was not only more potent but also resulted in a general increase in Gram-positive *in vitro* potency over ciprofloxacin by a factor of 2–16. Moreover, the excellent Gram-negative activity was retained or improved 4 times, except for *P. aeruginosa*, against which it was one dilution less active. In summary, the *in vitro* activity for the stereoisomers of 3-amino-2-methyl-1-azetidinylquinolones **22** and **23** shows the following decreasing trend: 2*S*,3*R* > 2*S*,3*S* > 2*R*,3*S* ≅ 2*R*,3*R*. The important feature of these results is the difference in activity between each of the pairs of enantiomers (**22a/22b**, **23c/23d**) as well as the preferred absolute stereochemistry *S* at C-2 of the azetidine group.

After examination of the MIC values against Gram-positive and Gram-negative organisms, it is found that the *in vitro* activity associated with an 8-unsubstituted-7-azetidinylquinolone is comparable with that of the corresponding naphthyridine (**21a/24a**; **21b/24b**; **33a/32a**; **33b/32b**). This conclusion was also achieved in a related study[14] concerning piperazinyl and substituted pyrrolidinyl side chains at the 7-position of 8-unsubstituted quinolones and naphthyridines. Conversely to what is described in the literature for 7-pyrrolidinyl-substituted *N*-cyclopropylquinolones[14] and *N*-(4-fluorophenylquinolones),[15] the *in vitro* activity of 7-azetidinylquinolones bearing an 8-F, fluctuates in a narrow range relative to 8-H (**22a/21a**; **22b/21b**; **31a/33a**). The presence of a chlorine at C-8 resulted in a general increase in *in vitro* potency for the *N*-cyclopropyl compounds (**25a/21a**), while the presence of chlorine at

Table 4. Physical Data of the *N*-Amino Acid-Substituted Azetidinylquinolones and -naphthyridinones Prepared for This Study[a]



compd	A	R_1	azetidine stereo	R_{71}	R_{72}	R_{73}	salt	mp, °C	$[\alpha]^{20}_D$, (*c*, 0.5 N NaOH)	analyses[b]	method (% yield)[c]	NMR, δ[d] C_2H[e]	NMR, δ[d] C_5H[f]
47aA	N	c-C_3H_5	2*S*,3*R*	H	CH_3	H	HCl	190–192	+16.2 (0.88)	$C_{19}H_{23}ClFN_5O_4{\cdot}0.7H_2O$	A (38)	8.58	8.00
47aD	N	c-C_3H_5	2*S*,3*R*	CH_3	H	H	HCl	238–240	+3.4 (0.73)	$C_{19}H_{23}ClFN_5O_4{\cdot}1.1H_2O$	A (39)	8.54	7.91
47bD	N	c-C_3H_5	2*R*,3*S*[g]	CH_3	H	H	HCl	193–195	−16.1 (0.67)	$C_{19}H_{23}ClFN_5O_4{\cdot}0.4H_2O$	A (77)	8.59	8.01
47aL	N	c-C_3H_5	2*S*,3*R*	H	^{i}Bu	H	HCl	181–184	+23.8 (0.75)	$C_{22}H_{19}ClFN_5O_4{\cdot}0.7H_2O$	B (34)	8.98	8.01
47aAA	N	c-C_3H_5	2*S*,3*R*	H	CH_3	A	HCl	188–191	+16.6 (0.70)	$C_{22}H_{28}ClFN_6O_5{\cdot}0.9H_2O$	A (37)	8.52	7.88
48aA	CH	2,4-F_2Ph	2*S*,3*R*	H	CH_3	H	TsOH	172–175	−21.0 (0.90)	$C_{30}H_{29}F_3N_4O_7S{\cdot}1.6H_2O$	A (68)	8.60	7.85
48aD	CH	2,4-F_2Ph	2*S*,3*R*	CH_3	H	H	HCl	207–211	-24.5 (0.83)	$C_{23}H_{22}ClF_3N_4O_4{\cdot}0.7\ H_2O$	A (61)	8.65	7.87
48aN	CH	2,4-F_2Ph	2*S*,3*R*	H	^{n}Pr	H	TsOH	164–167	-12.3 (0.79)	$C_{32}H_{33}F_3N_4O_7S{\cdot}1.1\ H_2O$	B (56)	8.80	7.95

[a] Abbreviations: c-C_3H_5 = cyclopropyl, 2,4-F_2Ph = 2,4-difluorophenyl, A = L-alanine, D = D-alanine, L = L-leucine, AA = L-alanine-L-alanine, N = L-norvaline, ^{i}Bu = isobutyl, ^{n}Pr = *n*-propyl, TsOH = *p*-toluensulfonic acid. [b] See Table 3. [c] Overall yield (see Scheme 3). [d] Solvent: DMSO-d_6, TFA. [e,f] See Table 3. [g] The stereochemistry is the oposite to that showed in the picture.

Table 5. Crystal and Refinement Parameters for Compounds **15** and **25a**

	15[a]	**25a**[b]
formula	$C_{27}H_{35}NO_5S$	$C_{17}H_{17}ClFN_3O_3$
crystal color	colorless	colorless
crystal size/mm	0.23 × 0.20 × 0.17	0.30 × 0.20 × 0.10
symmetry	monoclinic, $P2_1$	orthorhombic, $P2_12_12_1$
unit-cell determination	least-squares fit from 25 reflections	
	(15° < θ < 20°)	(10° < θ < 16°)
unit cell dimension		
a/Å	10.190(6)	6.989(2)
b/Å	11.966(5)	10.469(3)
c/Å	10.793(4)	21.458(6)
β/deg	102.03(5)	
packing: *V*/Å, *Z*	1287(1), 2	1570.2(8), 4
d_c/g cm^{-3}, *M*, *F*(000)	1.25, 485.6, 520	1.55, 365.8, 760
μ/cm^{-1}, *T*/K	1.54, 293	2.74, 200
λ/Å	0.71073	0.71073
technique	diffractometer: Enraf-Nonius CAD-4 single-crystal graphite crystal monochromator: Mo Kα, ω–2θ scans	
scan time	1 min per reflection	2 min per reflection
number of reflections		
measured	6948	3411
independent	4484	2761
observed	3245[3σ(*I*) criterion]	1597[3σ(*I*) criterion]
R_{int}	0.018	0.038
standard reflections	three reflections every 60 min	
range *h*,*k*,*l*	−9,−11,−10 to 12,14,12	−8,−12,−25 to 8,12,25
drift correction	0.98–1.02	0.98–1.01
absorption corr; ψ-scans	0.89–1.03	0.61–1.46
solution and refinement	direct methods; full matrix least-squares on F_o	
parameters: no. of var	313	239
final shift/error	0.002	0.0042
weighting scheme	$\Sigma w(F_o - Fc)^2$, $w = 1/[\sigma^2(F_o) + gF_o^2]$ with $\sigma(F_o)$ from counting statistics	
g	0.0001	0.001
max. thermal value/Å^2	U_{33}[O(2)] = 0.126(2)	U_{33}[N(75)] = 0.077(5)
final ΔF peaks/e Å^{-3}	0.23, −0.34	0.72, −0.59
final *R* and R_w	0.034, 0.033	0.059, 0.059

[a] Solvent of recrystallization = water. [b] Solvent of recrystallization = dimethylformamide–water (95:5).

C-8 for the *N*-(2,4-difluorophenyl) analogs resulted in a 2-fold decrease in activity (**34a/33 a**).

As previously reported[16] in the context of a QSAR study for the 1-position of 7-pyrrolidinyl-substituted quinolones, the cyclopropyl derivative of 7-azetidinylquinolone **22a** was more active *in vitro* than the corresponding ethyl **28a**, 4-fluorophenyl **30a**, and 2,4-difluorophenyl **31a** (**22a** > **30a** ≅ **31a** > **28a**). Recently,[13h,17] comparison of 8-F quinolones with their 5-amino derivatives showed this latter being more potent *in vitro*. In our series, the influence brought about by adding a 5-amino group to **22a** to yield **26a**

Table 6. *In vitro* Antibacterial Activity of 7-Azetidinyl-Substituted Quinolones (MIC, μg/mL)[a,b]

compd	Bs	Bc	Sf	Sa	Se	Pa	Mm	Pv	Kp	Ec	Ecl
21	0.03	0.06	0.25	0.12	0.12	0.25	0.03	0.06	0.03	0.015	0.03
21a	0.015	0.015	0.12	0.06	0.06	0.12	0.015	0.06	0.015	0.015	0.015
21b	0.25	1	2	1	1	4	0.5	1	0.25	0.25	0.25
22	0.03	0.06	0.25	0.06	0.06	0.25	0.03	0.06	0.03	0.015	0.03
22a	0.015	0.06	0.25	0.015	0.015	0.25	0.015	0.06	0.015	0.015	0.015
22b	0.12	0.5	2	0.25	0.25	4	0.25	0.5	0.12	0.25	0.25
23	0.06	0.12	1	0.12	0.12	1	0.12	0.25	0.12	0.06	0.06
23c	0.03	0.06	0.5	0.06	0.12	0.5	0.06	0.12	0.03	0.03	0.03
23d	0.25	0.25	2	0.25	1	2	0.25	1	0.25	0.25	0.25
24	0.03	0.06	0.25	0.12	0.12	0.5	0.03	0.06	0.03	0.03	0.03
24a	0.015	0.015	0.12	0.06	0.06	0.12	0.015	0.06	0.015	0.015	0.015
24b	0.06	0.25	2	0.5	0.25	2	0.25	0.5	0.06	0.12	0.12
25	0.03	0.03	0.06	0.03	0.03	0.12	0.03	0.03	0.015	0.015	0.03
25a	0.015	0.015	0.015	0.015	0.015	0.12	0.015	0.015	0.015	0.015	0.015
25b	0.12	0.5	0.25	0.5	0.5	4	0.25	0.25	2	0.25	0.25
26	0.015	0.015	0.06	0.015	0.015	0.25	0.015	0.06	0.015	0.015	0.015
26a	0.015	0.015	0.12	0.015	0.015	0.12	0.015	0.06	0.015	0.015	0.015
27a	0.25	0.25	0.12	0.12	0.12	0.5	0.12	0.25	0.12	0.12	0.12
28	0.06	0.25	1	0.25	0.12	1	0.06	0.25	0.015	0.06	0.06
28a	0.12	0.25	2	0.25	0.25	2	0.12	0.5	0.06	0.12	0.12
29a	0.06	0.25	0.25	0.12	0.25	0.5	0.06	0.25	0.25	0.25	0.06
30	0.12	0.25	2	0.25	0.12	2	0.25	0.5	0.12	0.12	0.12
30a	0.06	0.12	1	0.12	0.25	0.5	0.12	0.25	0.015	0.06	0.015
31	0.06	0.12	1	0.12	0.12	1	0.25	0.5	0.06	0.06	0.06
31a	0.06	0.12	1	0.12	0.12	0.5	0.25	0.5	0.015	0.06	0.06
32	0.06	0.25	1	0.12	0.25	1	0.25	1	0.06	0.12	0.12
32a	0.015	0.06	0.5	0.06	0.12	0.5	0.25	0.5	0.015	0.06	0.06
32b	0.25	0.5	4	0.5	0.5	8	1	2	0.25	0.5	1
33	0.03	0.25	1	0.12	0.12	2	0.25	0.5	0.015	0.06	0.12
33a	0.06	0.25	1	0.12	0.12	1	0.25	0.5	0.06	0.06	0.12
33b	0.25	0.5	4	0.25	0.25	8	1	2	0.25	0.5	1
34	0.03	0.03	0.25	0.12	0.06	0.5	0.25	0.25	0.12	0.03	0.12
34a	0.12	1	2	0.25	0.5	2	0.5	1	0.5	0.25	0.25
35a	0.03	0.12	0.12	0.06	0.12	0.5	0.12	0.5	0.25	0.03	0.12
36a	0.015	0.06	0.25	0.06	0.06	0.5	0.25	1	0.12	0.06	0.12
37	0.06	0.12	0.12	0.12	0.06	0.25	0.06	0.25	0.015	0.015	0.015
38	2	4	4	4	4	32	8	8	8	8	4
39	0.015	0.25	0.5	0.25	0.12	0.5	0.015	0.25	0.015	0.015	0.015
40	0.06	0.12	0.5	0.12	0.12	1	0.06	0.5	0.015	0.015	0.06
41	0.015	0.06	2	0.12	0.12	4	0.12	0.25	0.015	0.06	0.06
42	0.06	0.5	2	0.25	0.12	4	0.25	0.5	0.12	0.12	0.5
43	0.06	0.5	2	0.5	0.25	8	1	1	1	0.25	0.5
44a	0.015	0.06	0.12	0.015	0.015	0.25	0.015	0.06	0.015	0.015	0.015
44b	0.12	0.5	1	0.25	0.25	4	0.12	0.5	0.06	0.25	0.25
47aA	0.12	1	8	0.5	0.5	8	0.5	1	0.06	0.12	0.12
47aD	0.12	0.5	1	0.25	1	8	2	2	0.5	0.25	0.25
47bD	0.5	1	1	1	0.25	16	8	8	8	1	4
47aL	0.12	0.12	2	0.25	0.25	8	0.25	0.25	0.06	0.06	0.12
47aAA	1	16	16	4	8	16	4	8	8	2	2
48aA	0.5	1	8	1	16	8	4	16	2	1	2
48aN	0.25	0.5	4	0.5	0.5	16	4	8	0.25	0.5	1
CIP[c]	0.06	0.25	0.5	0.25	0.5	0.12	0.06	0.06	0.03	0.03	0.03
LEV[d]	0.03	0.25	0.25	0.25	0.12	0.5	0.03	0.5	0.03	0.03	0.03

[a] Structures are shown in Tables 2–4. [b] Organisms selected for the table are as follows: Bs, *Bacillus subtilis* ATCC 6633; Bc, *Bacillus cereus* ATCC 11778; Sf, *Streptococcus faecalis* ATCC 10541; Sa, *Staphylococcus aureus* ATCC 25178; Se, *Staphylococcus epidermidis* ATCC 155–1; Pa, *Pseudomonas aeruginosa* ATCC 10145; Mm, *Morganella morganii* ATCC 8019; Pv, *Proteus vulgaris* ATCC 8427; Kp, *Klebsellia pneumoniae* ATCC 10031; Ec, *Escherichia coli* ATCC 23559; Ecl, *Enterobacter cloacae* ATCC 23355. [c] CIP: ciprofloxacin. [d] LEV: levofloxacin.

appeared to slightly improve the *in vitro* activity, particularly against Gram-positive and *P. aeruginosa*. 5-Methyl-7-aminopyrrolidinyl-substituted naphthyridones were reported[18] to have better *in vitro* activity than the 5-hydrogen analogs. Concerning 7-azetidinyl-naphthyridinones, the 5-methyl group maintained the *in vitro* activity with the 1-(2,4-difluorophenyl) moiety (**36a/32a**), but it gave poorer activity with the 1-cyclopropyl substitution (**27a/24a**).

The 2*S* stereoisomers **21a**, **23c**, and **25a**–**33a** are at least as potent in *in vivo* tests as the racemic mixtures **21**, **23**, and **25**–**33**, respectively, and **22a** and **24a** display a 3-fold improvement in *in vivo* efficacy versus the corresponding racemic mixtures **22** and **24**. Among 7-[(2*S*,3*R*)-3-Amino-2-methyl-1-azetidinyl]-1-cyclopropylquinolones **21a**–**27a**, the most potent members of this series *in vitro* (**22a**, **24a**, and **25a**) also show the best activities *in vivo*. It has been widely published[1,13h,14,17a] that 8-H and 8-F and 5-NH_2-substituted quinolones decrease their *in vivo* potency with respect to 8-F and 8-Cl quinolones and naphthyridines bearing a cyclopropyl group at the 1-position. Our results in Table 7 corroborate the diminished *in vivo* efficacy related to 7-azetidinyl-8-unsubstituted- (**21a**) and 5-amino-7-azetidinyl-8-fluoroquinolone (**26a**).

As shown in Table 7, the compounds evaluated (including 2*S*,3*R* enantiomers and their antipodes 2*R*,3*S*) resulted in an increased potency po against *S. aureus* over ciprofloxacin by a factor of 2–12 (**22a**, **25a**). The data showed that *in vivo* efficacy of 2*S*,3*R* stereoisomers

Table 7. Efficacy on Systemic Infections after Oral Administration in Mice of Selected Quinolones (ED_{50}, mg/kg)

compd	*S. aureus* HS-93	*E. coli* HM-42	*P. aeruginosa* HS-116
21	22.3	3.5	108
21a	17.0	2.9	100
22	9.0	2.0	22
22a	3.5	1.0	18
22b	12.2	10.2	65
23	18.6	6.2	123
23c	5.4	6.1	117
24	13.8	3.5	95
24a	4.6	1.0	34
25	9.6	2.8	38
25a	3.6	2.1	36
25b	19.1	18.4	147
26	8.7	4.2	102
26a	11.4	4.1	96
28	5.1	4.0	56
28a	5.9	3.9	60
30	5.7	2.5	55
30a	4.8	2.1	53
31	10.0	2.3	61
31a	11.2	3.0	98
32	8.9	3.3	140
32a	6.6	4.2	156
32b	8.6	10.3	400
33	8.1	6.0	78
33a	7.0	4.0	64
33b	16.1	10.0	90
39	19.1	6.7	121
44a	6.9	5.9	26
44b	46.1	50.0	53
47aL	9.4	2.0	63
CIP[a]	45.1	3.0	70
LEV[b]	14.0	5.0	109

[a] CIP: ciprofloxacin. [b] LEV: levofloxacin.

Table 8. Blood Level of Selected Quinolones after Oral Administration in Mice[a] (50 mg/kg)

compd	AUC[b]	compd	AUC
21a	4.0	**32b**	28.3
21b	2.2	**33a**	28.4
22a	21.8	**33b**	35.9
22b	13.6	**39**	8.5
24a	19.2	**44a**	2.3
24b	22.3	**47aA**	28.8[c]
25a	5.2	**47aD**	0.4[c]
26a	11.4	**47bD**	0.0[d]
28a	21.0	**47aL**	24.9[c]
29a	12.5	**47aAA**	19.2[c]
30a	40.9	CIP[e]	2.3
32a	38.2	LEV[f]	10.2

[a] These data were determined by a bioassay procedure and represent total activity present in the serum. [b] Area under the concentration−time curve recorded at 0.5, 1, 2, and 4 h after dosing (AUC, 0−4 h), μg/mL per hour. [c] AUC of the parent compound **24a**. [d] AUC of the parent compound **24b**. [e] CIP: ciprofloxacin. [f] LEV: levofloxacin.

(ED_{50} = 1−4 mg/kg) and ciprofloxacin (ED_{50} = 3 mg/kg) displayed a comparable potency against *E. coli*. Concerning *P. aeruginosa*, we have to point out a greater dispersion of results than for other strains. Although most of the 2*S*,3*R* enantiomers showed a similar *in vivo* efficacy to ciprofloxacin, some compounds (**22a**, **24a**, **25a**) displayed 2−4 times more activity than ciprofloxacin. The 2*S*,3*R* stereoisomers 1-(4-fluorophenyl)- (**30a**) and 1-(2,4-difluorophenyl)quinolone (**33a**) (cetefloxacin) showed an analogous *in vivo* profile to that of ciprofloxacin against Gram-negative strains, but displayed over 6-fold improvement against *S. aureus*.

In the pyridobenzoxazine series, our findings led to results (Table 6) similar to those of ofloxacin and its derivatives.[5] The 3*S*-(10-aminoazetidinyl) derivative **37** resulted in an increase in Gram-positive *in vitro* potency over levofloxacin by a factor of 2, and the Gram-negative activity was retained. The (*S*)-(−) enantiomer **39** was 16−512 times better than its antipode **38**, and overall, it has an excellent activity with a broad spectrum comparable to levofloxacin. It is interesting to observe, from a molecular biological standpoint, that the same enantiopreference is seen in the aminoazetidinyl and piperazinyl series. The importance of stereochemistry at the azetidine ring on the antibacterial activity of (3*S*)-10-azetidinyl-3-methylpyridobenzoxazines can be seen by the antibacterial activity comparison of the 2′*S*,3′*R* and 2′*R*,3′*S* diastereomers **44a** and **44b**, respectively, as shown in Table 6. The diastereomer **44b** shows 2−8 times weaker activity than levofloxacin, while the 2′*S*,3′*R* isomer **44a** was 2−8 times more active than levofloxacin. Concerning *in vivo* efficacy, the comparison of 3-amino-3-methylazetidinyl derivative **39** and levofloxacin shows that they have similar potency (Table 7). Once again **44a** was the most potent member of the pyridobenzoxazine series *in vivo*, exhibiting twice the efficacy versus its diastereomer **44b** against *P. aeruginosa* and 7-fold improvement against *E. coli* and *S. aureus*. The 2′*S*,3′*R* diastereomer **44a** compares very favorably with levofloxacin.

Table 9. Aqueous Solubility of Selected Compounds

compd	solubility (μg/mL)[a] H_2O	solubility (μg/mL)[a] pH 7.4	compd	solubility (μg/mL)[a] H_2O	solubility (μg/mL)[a] pH 7.4
24a	23.0	16.5	**33a**	8.0	5.9
47aA	>500	>500	**48aA**	>500	>500
47aD	495	386	**48aN**	>500	>500
47bD	>500	>500			
47aL	>500	>500			
47aAA	>500	>500			

[a] Solubility determined at 25 °C in water and in a pH 7.4 buffer. See the Experimental Section.

Results of preliminary pharmacokinetic studies of selected compounds in mice are displayed in Table 8. As described for the racemic *trans*-3-amino-2-methyl series,[1] several selected stereoisomers showed areas under the plasma level curves 10−17 times greater than ciprofloxacin. 1-(4-Fluorophenyl) derivative **30a** and 1-(2,4-difluorophenyl) derivative **33a** (cetefloxacin) displayed promising pharmacokinetic properties.

Concerning amino acid derivatives **47** and **48**, *in vitro* activity resulted in a decrease as compared with the parent drug (Table 6). The amino acid analogs showed equal or less *in vivo* efficacy. The L-amino acid derivatives were enzymatically cleaved after oral administration in mice to release parent drugs (Table 8), but no blood levels of parent drug were detected when D-amino acid derivatives **47aD** and **47bD** were administered in mice. On the other hand, the amino acid prodrugs showed over 20 times improved solubility in water with regard to parent drugs (Table 9).

In summary, 1-cyclopropyl-8-haloquinolones **22a** and **25a** and naphthyridine **24a** bearing a (2*S*,3*R*)-3-amino-2-methylazetidine ring at C-7 exhibited very good *in vivo* efficacy against Gram-negative and especially against Gram-positive organisms. 1-Cyclopropyl-8-chloroquinolone **25a** (E-4767) showed the best *in vitro* overall profile, and the L-alanyl derivative of **24a** (**47aA**)

and the 1-(2,4-difluorophenyl) derivative **33a** (cetefloxacin) displayed promising pharmacokinetic properties.

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from commercial sources and used without further purification. All melting points were determined on a Bausch & Lomb apparatus and are uncorrected. Infrared (IR) spectra were determined in KBr with a Nicolet FT-IR 5DXC spectrophotometer. Proton magnetic resonance spectra were recorded with either a Bruker AM-100 spectrometer operating at 100 MHz or a Varian Unity 300 spectrometer operating at 300 MHz. Chemical shifts are expressed in ppm (δ) relative to internal tetramethylsilane. Mass spectra were obtained with a Finnigan Mat TSQ-70 mass spectrometer. The IR and NMR spectral data of all compounds were consistent with the assigned structures. Elemental analyses were obtained for all new quinolones reported. Carbon, hydrogen, and nitrogen analyses were within 0.4% of theoretical values. All organic phases were dried over anhydrous $MgSO_4$ and removed *in vacuo* with a Büchi rotatory evaporator at aspiratory pressure. Chromatography was done using the medium-pressure flash method and Merck silica gel 60 (230–400 mesh ASTM).

Optical Resolution of (±)-*trans*-1-(diphenylmethyl)-3-hydroxy-2-methylazetidine (16a + 16b). From a solution of (±)-*trans*-1-(diphenylmethyl)-3-hydroxy-2-methylazetidine (**16**, 60.5 g, 0.239 mmol) and (+)-(1*S*)-camphorsulfonic acid (55.54 g, 0.239 mmol) in ethanol (200 mL) was obtained after evaporation and washing twice with diethyl ether the salt mixture (110 g). A 40 g (82.4 mmol) sample of the diastereomeric mixture was recrystallized from water (800 mL) to afford (+)-(2*R*,3*S*)-1-(diphenylmethyl)-3-hydroxy-2-methylazetidinyl (1*S*)-camphorsulfonate (13.87 g, 69%), $[\alpha]^{24}_D$ +45.6° (*c* 1.0, CH_3OH), optical purity (97:3) determined by HPLC: ENANTIOPAC (α-glicoprotein on silica gel), 4 × 100 mm column (LKB-Pharmacia); solvent, 5 mM (+)-camphorsulfonic acid in 10 mM phosphate buffer (pH 6); flow rate, 0.5 mL/min; t_R 10.6 min.

From the mother liquor, azetidinol free base (13.62 g, 53.83 mmol) was obtained after treatment with 0.5 N NaOH (HPLC **16a**:**16b** 75:25). The azetidinol mixture (13.62 g) and (−)-(1*R*)-camphorsulfonic acid (13.75 g, 53–81 mmol) in water (300 mL) gave (−)-(2*S*,3*R*)-1-(diphenylmethyl)-3-hydroxy-2-methylazetidinyl (1*R*)-camphorsulfonate (**15**, 15.17 g, 76%), $[\alpha]_D$ −47.2° (α 1.0, CH_3OH), optical purity (96:4) determined by HPLC as described before: t_R 18.8 min. The base **16a** was liberated from **15** to afford an optically pure compound (HPLC 99.5:0.5), $[\alpha]_D$ −103.1° (*c* 1.0, CH_3OH).

Preparation of Aminoazetidines (Scheme 1). (2*S*,3*R*)-1-(Diphenylmethyl)-2-methyl-3-(methylsulfonyloxy)-azetidine (17a). To a stirred solution of **16a** (7.9 g, 31.2 mmol) and triethylamine (5 g, 49.5 mmol) in CH_2Cl_2 (50 mL) was added dropwise a solution of methanesulfonyl chloride (5.3 g, 46.8 mmol) in CH_2Cl_2 (20 mL), and the mixture was stirred for 24 h at room temperature. The organic solution was washed several times with water (30 mL), and the solvent was removed *in vacuo* to obtain an oil, which was crystallized with petroleum ether to afford **17a** (10.8 g, 97%): $[\alpha]^{20}_D$ −98.0° (*c* 0.25, $CHCl_3$); mp 72–76 °C; IR(KBr) 1361, 1178, 1152, 708 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.87 (d, J = 6 Hz, 3H), 2.80 (s, 3H), 2.82 (m, 1H), 3.43 (t, J = 7 Hz, 1H), 3.74 (t, J = 7 Hz, 1H), 4.46 (s, 1H), 4.60 (m, 1H), 7.32 (m, 10H).

(2*S*,3*R*)-3-Amino-1-(diphenylmethyl)-2-methylazetidine (18a). A mixture of **17a** (7.2 g, 21.7 mmol), 2-propanol (40 mL), and ammonium hydroxide (30%, 25 mL) was heated at 70 °C for 3 h. 2-Propanol was removed *in vacuo*, and the resulting solution was alkalinized with Na_2CO_3 and extracted with CH_2Cl_2 to give **18a** (4.7 g, 86%). **18a·2HCl**: mp 152–153 °C; IR (KBr) 3400–2300, 1453, 704 cm^{-1}; **18a**: $[\alpha]^{20}_D$ −110.3° (*c* 0.3, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.64 (d, J = 7 Hz, 3H), 2.20 (q, J = 7 Hz, 1H), 2.63 (t, J = 7 Hz, 1H), 2.90 (quint, J = 7 Hz, 1H), 3.50 (t, J = 7 Hz, 1H), 4.20 (s, 1H), 7.20 (m, 10H).

(2*R*,3*S*)-3-Amino-1-(diphenylmethyl)-2-methylazetidine (18b): $[\alpha]^{20}_D$ −112.3° (*c* 0.3,$CHCl_3$).

(2*S*,3*S*)-3-Amino-1-(diphenylmethyl)-2-methylazetidine (18c). 18c·2HCl: mp 130–132 °C; IR (KBr) 3348, 1492, 1450, 703 cm^{-1}. **18c**: $[\alpha]^{20}_D$ −73.3° (*c* 0.3, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.63 (d, J = 6 Hz, 3H), 1.64 (br, 2H), 2.09 (d, J = 4 Hz, 2H), 3.35 (m, 2H), 4.34 (s, 1H), 7,29 (m, 10H).

(2*R*,3*R*)-3-Amino-1-(diphenylmethyl)-2-methylazetidine (18d): $[\alpha]^{20}_D$ +74.0° (*c* 0.3, $CHCl_3$).

(2*S*,3*R*)-3-Amino-2-methylazetidine Dihydrochloride (19a). A mixture of **18a** (4.5 g, 13.8 mmol) and 10% $Pd(OH)_2$/C (0.5 g) in ethanol (90 mL) was treated with H_2 at room temperature and 60 psi for 2 h. The mixture was filtered, the solvent was evaporated, and the residue was washed with benzene to give **19a** (1.8 g, 82%): $[\alpha]^{20}_D$ −21.0° (*c* 1.0, CH_3OH); mp 163–165 °C; IR (KBr) 3500–2100, 1561, 1451, 1365, 1403 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 1.51 (d, J = 7 Hz, 3H), 3.92 (m, 3H), 4.60 (m, 1H), 9.2 (br, 5H).

General Procedure for the Preparation of Quinolones, Naphthyridines, and Pyridobenzoxazines (Scheme 2). Preparation of 7-[(2*S*,3*R*)-3-Amino-2-methyl-1-azetidinyl]-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxo-3-quinolinecarboxylic Acid (Cetefloxacin, 33a). A mixture containing 8.0 g (23.7 mmol) of 6,7-difluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid,[10] 5.7 g (35.8 mmol) of (2*S*,3*R*)-3-amino-2-methylazetidine dihydrochloride (**19a**), and 25 mL (245 mmol) of triethylamine in 80 mL of pyridine was heated to reflux for 3 h and then cooled to room temperature. After concentration of the reaction mixture under reduced pressure, the residue was diluted with water. The precipitated solid was collected by filtration and washed with water to give the crude product. This solid was dissolved in water, made basic with concentrated ammonium hydroxide, and filtered, and the pH was adjusted to 7.2 by elimination of NH_3. The precipitated solid was collected and washed successively with water and ethanol to give **33a** (7.7 g, 81%): mp 227–230 °C; $[\alpha]^{20}_D$ −40.8° (*c* 1.1, NaOH 0.5 N); IR (KBr): 1630, 1611, 1509 cm^{-1}; ^{1}H-NMR (DMSO-d_6/TFA) δ 1.28 (d, 3H), 3,-62 (m, 1H), 3.92 (m, 1H), 4.31 (m, 2H), 5.76 (d, J = 6.8 Hz, 1H), 7.41 (m, 1H), 7.67 (m, 1H), 7.91 (m, 1H), 7.94 (d, J = 12.7 Hz, 1H), 8.23 (br, 3H), 8.79 (s, 1H). Optical purity (>99%) was determined by HPLC: Lichrospher RP18, 4 × 125 mm column (Merck); solvent, 6 mM L-phenylalanine and 3 mM $CuSO_4$ (pH 3.5)/CH_3OH (60:40); flow rate, 0.8 mL/min; temperature 0 °C; t_R 15.5 and 17.5 min for **33a**; 12.4 and 26.5 min for **33b**.

7-[(2*S*,3*R*)-3-Amino-2-methyl-1-azetidinyl]-1-cyclopropyl-1,4-dihydro-6-fluoro-4-oxo-3-naphthyridinecarboxylic Acid (24a). Optical purity (98.3%) was determined by HPLC: Suplex PKB 100, 4 × 150 mm column (Supelco); solvent, 6 mM L-phenylalanine and 3 mM Cu SO_4 (pH 3.5)/CH_3OH (85:15); flow rate 1 mL/min; temperature 25 °C; t_R 50.9 min for **24a**; t_R 60.5 min for **24b**.

General Procedures for the Preparation of *N*-Amino Acid-Substituted Azetidinylquinolones and -naphthyridines (Scheme 3). Method A. Preparation of the Hydrochloride of 7-[(2*S*,3*R*)-3-(Alanylamino)-2-methyl-1-azetidinyl]-1-cyclopropyl-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (47aA·HCl). *N*-CBZ-Ala-*N*-hydroxysuccinimide (**N-CBZ-45A**) (0.74 g, 2.31 mmol) was added to a solution of 7-[(2*S*,3*R*)-3-amino-2-methyl-1-azetidinyl]-1-cyclopropyl-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**24a**) (0.70 g, 2.11 mmol) and *N*-methylmorpholine (0.21 g, 2.11 mmol) in dry dimethylformamide (30 mL) cooled to 0 °C. The solution was kept at this temperature for 1 h and then at room temperature for 8 h. The resulting solution was added to a solution of hydrochloric acid (200 mL, 0.5 N). The obtained precipitate was filtered and washed with water, and the solid was dried over P_2O_5 to give 7-[(2*S*,3*R*)-3-N-CBZ-Ala-amino-2-methyl-1-azetidinyl]-1-cyclopropyl-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**46aA**) (1.1 g, 97%): mp 211–213 °C; $[\alpha]^{20}_D$ +27.7° (*c* 0.78, DMSO); IR (KBr) 3325, 1720, 1680, 1632, 1509, 1449, 1328 cm^{-1}; ^{1}H-NMR (DMSO-d_6/TFA) δ 1.11 (m, 4H), 1.21 (d, J = 7.0 Hz, 3H), 1.59 (d, J = 6.1 Hz, 3H), 3.60 (m, 1H), 3.85–

4.70 (br, 5H), 5.01 (s, 2H), 7.30 (s, 5H), 7.97 (d, J = 11.5 Hz, 1H), 8.56 (s, 1H).

Pd/C (10%) (0.08 g) was added to a solution of **46aA** (0.96 g, 1.78 mmol) in 80 mL of dimethylformamide, and the mixture was kept under hydrogen atmosphere for 24 h. The catalyst was filtered off and washed with dimethylformamide. The solvent was evaporated at reduced pressure, and the resulting solid was crystallized from an ethanol–water mixture to give 7-[(2*S*,3*R*)-3-Ala-amino-2-methyl-1-azetidinyl]-1-cyclopropyl-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**47aA**) (0.50 g, 69%): mp 220–222 °C; $[\alpha]^{20}_D$ +16.9° (*c* 0.75, DMSO); IR (KBr) 3630–2420, 1630, 1510, 1500, 1450, 1362, 1320 cm^{-1}; ^{1}H-NMR (DMSO-d_6/TFA) δ 1.16 (m, 4H), 1.39 (d, J = 7.00 Hz, 3H), 1.65 (d, J = 6.2 Hz, 3H), 3.55–4.00 (m, 2H), 4.00–4.80 (br, 4H), 8.02 (d, J = 11.6, 1H), 8.15 (br, 3H), 8.60 (s, 1H), 8.95 (m, 1H).

47aA (0.35 g, 0.86 mmol) was treated with a solution of EtOH–HCl. The solvent was evaporated at reduced pressure to give the hydrochloride of 7-[(2*S*,3*R*)-3-Ala-amino-2-methyl-1-azetidinyl]-1-cyclopropyl-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**47aA·HCl**, 0.37 g, 98%): mp 190–192 °C; $[\alpha]^{20}_D$ +16.2° (*c* 0.88, DMSO); IR (KBr) 3620–2400, 1718, 1686, 1631, 1561, 1490, 1449, 1328 cm^{-1}; ^{1}H-NMR (DMSO-d_6/TFA) δ 1.10 (m, 4H), 1.38 (d, J = 7.0 Hz, 3H), 1.63 (d, J = 6.2 Hz, 3H), 3.50–4.00 (m, 2H), 4.00–4.80 (br, 4H), 8.00 (d, J = 11.5 Hz, 1H), 8.16 (br, 3H), 8.58 (s, 1H), 9.13 (m, 1H).

Optical purity (>98%) was determined by HPLC: Suplex PKB 100, 4 × 150 mm column (Supelco); solvent, 6 mM L-phenylalanine and 3 mM $CuSO_4$ (pH 3.5)/CH_3OH (80:20); flow rate 1 mL/min; temperature 25 °C; t_R 62.3 min.

Method B. Preparation of the Hydrochloride of 1-Cyclopropyl-1,4-dihydro-6-fluoro-7-[(2*S*,3*R*)-3-Leu-amino-2-methyl-1-azetidinyl]-4-oxo-1,8-naphthyridine-3-carboxylic Acid (47aL·HCl). *N-t*-BOC-Leu-*N*-hydroxysuccinimide ester (**N-BOC-45L**) (0.43 g, 1.32 mmol) was added to a solution of 7-[(2*S*,3*R*)-3-amino-2-methyl-1-azetidinyl]-1-cyclopropyl-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**24a**, 0.40 g, 1.20 mmol) and *N*-methylmorpholine (0.12 g, 1.20 mmol) in dry dimethylformamide (20 mL) cooled to 0 °C. The temperature was maintained for 1 h, and the solution was stirred at room temperature overnight. The resulting solution was poured into a solution of hydrochloric acid (200 mL, 0.5 N). The precipitate was filtered off and washed with water, and the solid was dried over P_2O_5 to give 7-[(2*S*,3*R*)-3-*N*-*t*-BOC-Leu-amino-2-methyl-1-azetidinyl]-1-cyclopropyl-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**46aL**, 0.60 g, 92%): mp 117–120 °C; $[\alpha]^{20}_D$ +17.0° (*c* 0.71, DMSO); IR (KBr) 3318, 2962, 1719, 1631, 1509, 1447, 1368, 1331 cm^{-1}; ^{1}H-NMR (DMSO-d_6/TFA) δ 0.85 (d, J = 5.9 Hz, 6H), 1.14 (m, 4H), 1.35 (s, 12H), 1.59 (d, J = 5.9 Hz, 3H), 3.55–4.70 (br, 6H), 7.97 (d, J = 11.5 Hz, 1H), 8.57 (s, 1H).

46aL (0.54 g, 0.99 mmol) and trifluoroacetic acid (15 mL) were kept at room temperature for an hour. Diethyl ether was added, the precipitate was filtered off, and the solid was washed with diethyl ether. The salt formed was dissolved in water and adjusted to a pH of approximately 7.6 with NH_3. The precipitate was filtered, washed with water, and dried over P_2O_5 to give 7-[(2*S*,3*R*)-3-Leu-amino-2-methyl-1-azetidinyl]-1-cyclopropyl-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**47aL**, 0.25 g, 57%): mp 216–218 °C; $[\alpha]^{20}_D$ +9.7° (*c* 0.76 DMSO); IR (KBr) 3331, 2962, 1724, 1636, 1571, 1509, 1449 cm^{-1}; ^{1}H-NMR (DMSO-d_6/TFA) δ 0.92 (d, J = 4.8 Hz, 6H), 1.13 (m, 4H), 1.65 (d, J = 5.9 Hz, 6H), 3.68 (m, 2H), 4.05–4.80 (m, 4H), 8.03 (d, J = 11.7 Hz, 1H), 8.15 (br, 3H), 8.60 (s, 1H), 9.09 (m, 1H).

47aL (0.20 g, 45 mmol) was treated with a solution of EtOH–HCl. The solvent was evaporated at reduced pressure to give the hydrochloride of 7-[(2*S*,3*R*)-3-Leu-amino-2-methyl-1-azetidinyl]-1-cyclopropyl-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**47aL·HCl**, 0.21 g, 98%): mp 181–184 °C; $[\alpha]^{20}_D$ +23.8° (*c* 0.75, DMSO); IR (KBr) 3600–2400 (br) 1718, 1687, 1630, 1562, 1512, 1449, 1325 cm^{-1}; ^{1}H-NMR (DMSO-d_6/TFA) δ 0.89(d, J = 5.2 Hz, 6H), 1.11 (m, 4H), 1.63 (d, J = 5.5 Hz, 6H), 3.43 (m, 2H), 4.05–4.80 (br, 4H), 8.01 (d, J = 11.4 Hz, 1H), 8.15 (br, 3H), 8.58 (s, 1H), 9.19 (m, 1H).

Single-Crystal X-ray Analysis of 15 and 25a. Crystallographic data were collected on an Enraf-Nonius CAD4 single crystal diffractometer with Mo Kα radiation and a graphite crystal monochromator. Unit cell dimensions were determined from the angular settings of 25 reflections within the θ ranges shown in Table 5. Space groups were determined from systematic absences or structure determination. The reflections were measured using the $\omega-2\theta$ scan technique with a variable scan rate and a maximum scan time of 60 s (**15**) or 120 s (**25a**) per reflection. The intensity was checked throughout data collection by monitoring three standard reflections every 60 min. Final drift corrections are shown in Table 5. A profile analysis was performed on all reflections.[19a,b] A semiempirical absorption correction, ψ-scan based, was applied. Symmetry equivalent and double-measured reflections were averaged, $R_{int} = \Sigma(|I| - \langle I\rangle)/\Sigma I$. Lorentz and polarization corrections were applied and the data were reduced to $|F_c|$ values. The structure was solved by Direct Methods using the program SHELX86[19c] and expanded by DIRDIF.[19d] Isotropic least-squares refinement, using SHLX76,[19e] was performed until convergence. An empirical absorption correction was applied.[19f] Maximum and minimum correction factors are shown in Table 5. Further refinements included anisotropic thermal parameters for all non-hydrogen atoms. All hydrogen atoms were isotropically refined with a common thermal parameter. The minimized function was $\Sigma w(F_o - F_c)^2$, $w = 1.0/(\sigma^2(F_o) + gF_o^2)$ with $\sigma(F_o)$ from counting statistics. Atomic scattering factors were taken from ref 19g. The plots were made using the EUCLID package.[19h] Geometrical calculations were made with PARST.[19i] All crystallographic calculations were carried out on a MicroVax-3400. Fractional coordinates, bond distances, bond angles, structure amplitudes, anisotropic thermal parameters, H-atom parameters, distances and angles involving H atoms, distances, angles, least-squares-planes data, and torsion angles are available as supplementary material.

Solubility Studies. A known excess weight of the compound was added to water or to 0.05 M phosphate buffer (pH 7.4) into a suitable container. The solution was shaken for 24 h in a Heto shaking water bath, at 25 °C. The suspension was filtered (0.22-μm filter) and the first portion discarded to ensure saturation of the filter. An aliquot of the filtrate was diluted with either 0.1 N HCl or 0.1 N NaOH and analyzed spectrophotometrically at the wavelength corresponding to the maximum absorbance of the compound.

Microbiology. General Procedures for *in Vitro* Studies. The *in vitro* antibacterial activity was studied by side-by-side comparison with Ciprofloxacin and levofloxacin and determined by a serial 2-fold agar dilution technique using Mueller Hinton medium. The inoculum size was adjusted to 10^5 cfu/mL, and concentrations of the compounds ranged from 0.007 to 16 μg/mL. Minimum inhibitory concentrations (MICs) were defined as the lowest concentration of the compound that prevented visible growth of bacteria after incubation at 37 °C for 18 h.

***In Vivo* Studies (Mouse Protection Tests).** The screening *in vivo* was carried out with 4 groups of 10 mice each. The mice were infected intraperitoneally with a suspension containing an amount of the indicated organism slightly greater than its lethal dose 100 (LD_{100}). Each group was treated orally with the test compound administered as a single dose immediately after infection. Four different doses, one per group, were selected depending on the *in vitro* activity of the test compound. ED_{50} values were calculated by interpolation among survival rates in each group after a week. They express the total dose of compound (mg/kg) required to protect 50% of the mice from an experimentally induced lethal systemic infection of the indicated organism.

Pharmacokinetic Studies. General Procedure. Mice were given a single 50 mg/kg oral dose. At the specified time intervals (0.5, 1, 2, and 4 h after dosing), blood was collected from groups of six mice. All samples were assayed by a disk agar diffusion bioassay procedure. *Bacillus subtilis* ATCC 6633 was used as the assay organism and Seed Agar as the growth medium. The plates were incubated at 37 °C for 18 h.

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Supplementary Material Available: Tables of atomic coordinates, thermal parameters, bond lengths, bond angles, anisotropic temperature factors, torsion angles, and angles between planes for compounds **15** (JF 911) and **25a** (E-4767) (19 pages). Ordering information is given on any current masthead page.

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(2) A preliminary account of this work was presented (a) at the 30th ICAAC (Interscience Conference Antimicrobial Agents Chemotherapy), Atlanta, October 1990, Abstr. No. 396. (b) at the XIIth International Symposium on Medicinal Chemistry, Basel (Switzerland), September 1992, Abstr. No. P-076.B; and (c) at the XVI Congress of the International Union of Crystallography, Beijing (China), August 1993, Abstr. No. PS-06.02.13.

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