Novel Fluoroquinolone Antibacterial Agents Containing Oxime-Substituted (Aminomethyl)pyrrolidines: Synthesis and Antibacterial Activity of 7-(4-(Aminomethyl)-3-(methoxyimino)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro[1,8]naphthyridine-3-carboxylic Acid (LB20304)[†,1]

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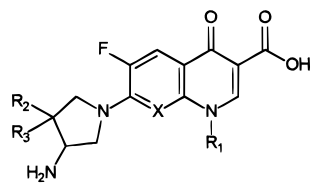
New pyrrolidine derivatives, which bear an alkyloxime substituent in the 4-position and an aminomethyl substituent in the 3-position of the pyrrolidine ring, have been synthesized and coupled with various quinolinecarboxylic acids to produce a series of new fluoroquinolone antibacterials. These fluoroquinolones were found to possess potent antimicrobial activity against both Gram-negative and Gram-positive organisms, including methicillin resistant *Staphylococcus aureus* (MRSA). Variations at the C-8 position of the quinolone nucleus included fluorine, chlorine, nitrogen, methoxy, and hydrogen atom substitution. The activity imparted to the substituted quinolone nucleus by the C-8 substituent was in the order F (C_5-NH_2) > F (C_5-H) > naphthyridine > Cl = OMe = H against Gram-positive organisms. In the case of Gram-negative strains, activity was in the order F (C_5-NH_2) > naphthyridine = F (C_5-H) > H > Cl > OMe. The advantages provided by the newly introduced oxime group of the quinolones were clearly demonstrated by their comparison to a desoximino compound **30**. In addition, the oxime moiety greatly improved the pharmacokinetic parameters of the novel quinolones. Among these compounds, compound **20** (LB20304) showed the best *in vivo* efficacy and pharmacokinetic profile in animals, as well as good physical properties. The MICs (μg/mL) of LB20304, compound **30**, and ciprofloxacin against several test organisms are as follows: *S. aureus* 6538p (0.008, 0.031, and 0.13), methicillin resistant *S. aureus* 241 (4, 16, and 128), *Streptococcus epidermidis* 887E (0.008, 0.016, and 0.13), methicillin resistant *S. epidermidis* 178 (4, 32, and 128), *Enterococcus faecalis* 29212 (0.063, 0.13, and 1), *Pseudomonas aeruginosa* 1912E (0.25, 0.5, and 0.13), *Escherichia coli* 3190Y (0.008, 0.016, and 0.008), *Enterobacter cloacae* P99 (0.008, 0.031, and 0.008), *Actinobacter calcoaceticus* 15473 (0.063, 0.13, and 0.25). On the basis of these promising results, LB20304 was selected as a candidate for further evaluation.

Introduction

Since the discovery of norfloxacin, most of the quinolone antibacterials research has been focused on the basic group at the C-7 position to produce new potent quinolones, namely, ciprofloxacin, ofloxacin, lomefloxacin, fleroxacin, and sparfloxacin, all of which contain a piperazine derivative at the C-7 position.[2−4] Recently, Warner-Lambert reported that this piperazine structure has been successfully replaced with two appropriate mimics, 3-aminopyrrolidine[5] and 3-(aminomethyl)pyrrolidine,[6] Tosufloxacin[7] and CI-960 (clinafloxacin)[6] are two representative quinolones containing an aminopyrrolidine residue, and they are currently undergoing clinical trials in the USA. Du6859a, another quinolone substituted by a modified aminopyrrolidine function, is in clinical trial phase III.[8] So far no quinolone antimicrobial agent possessing pyrrolidine substitution has been approved on a worldwide basis.[9]

The introduction of the noted pyrrolidine derivatives to the quinolones resulted in a dramatic improvement of *in vitro* Gram-positive activity compared to piperazinyl analogs. In particular, when a halogen atom was placed at the C-8 position of the quinolone ring, the pyrrolidinyl compounds showed good *in vivo* efficacy in animals.[6] This change, however, also gave rise to undesirable side effects such as phototoxicity[2,10] and cytotoxicity.[2,11] In order to circumvent these problems, additional attempts have been made to modify the pyrrolidinyl moiety. Alkylation at the 4-position of 3-amino- or 3-(aminomethyl)pyrrolidine produced novel quinolones with increased oral activity,[12] and methylation of either the exocyclic amine or the 3-position of 3-aminopyrrolidine also improved *in vivo* potency.[13] Very recently, Warner-Lambert reported that the addition of a methyl or dimethyl group to the methylene spacer of the (aminomethyl)pyrrolidine elicited a profound enhancement in both *in vitro* and *in vivo* activities.[14]



1. R_1= 2,4-difluorophenyl, R_2, R_3= H, X= N (tosufloxacin)

2. R_1= cyclopropyl, R_2, R_3= H, X= CCl (CI-960, clinafloxacin)

3. R_1=2'-fluorocyclopropyl, R_2, R_3= -CH_2CH_2-, X=CCl (Du6859a)

However, most of the quinolones currently on the market or under development have only moderate activity against many Gram-positive cocci including *staphylococci* and *streptococci.* This insufficient activity

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† This paper is dedicated to Professor Yoshito Kishi at Harvard University on the occasion of his 60th birthday.

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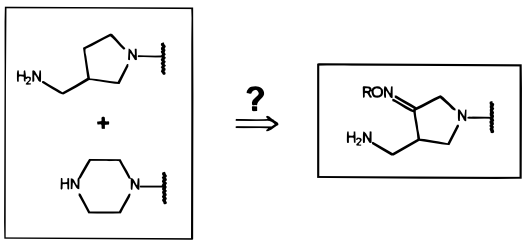
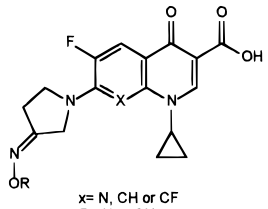
has not only limited their use in infections caused by these organisms, such as respiratory tract infections,[15] but has also been believed to be one of the reasons for the rapidly developing quinolone resistance.[16] Therefore, recent efforts have been directed toward the synthesis of new quinolones that can provide improved Gram-positive antibacterial activity, while retaining the good Gram-negative activity of ciprofloxacin.[17,18]

As part of an ongoing program to find potent, orally-active, broad-spectrum antibacterial agents, who display strong Gram-positive activity, we have focused our attention on the modification of the C-7 basic group of the quinolone. From our own research in C-7 amine modifications of the quinolones, we realized that an aminomethyl group on the pyrrolidine ring was essential for good *in vitro* (especially Gram-positive) activity. Since our goal was the discovery of new quinolone antibacterial agents with both strong Gram-positive activity and improved physical property, we designed novel pyrrolidines, which possessed both an alkyloxime substituent and an aminomethyl substituent. This structural modification of the pyrrolidine ring was expected to allow modulation of the physical properties of the corresponding quinolones while retaining their strong biological activity, thereby possibly improving their pharmacokinetic properties and *in vivo* potency.[5]

We believed for several reasons that the oximino group would be an excellent choice for our purpose. First of all, this functional group can be readily obtained from the corresponding ketone in a single operation. Secondly, it is a quite common and stable functional group frequently employed in current drugs (for example, see the most popular antibiotics, third and fourth generation cephalosporins). Thirdly, the oxime has a lone pair of electrons on the nitrogen that can participate in hydrogen bonding with the drug target, which in the case of quinolone is DNA gyrase. Finally, the oximino group has an oxygen atom that can accommodate a variety of alkyl (R) or acyl groups. By changing the R group, we anticipated that we could tune the liphophilicity of the novel compounds in order to attain the highest potency and most favorable physical properties.

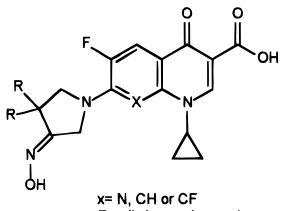


There have been two reports where the oxime functionality was introduced into the C-7 amine group of quinolone antibacterials. Cooper *et al.* (Abbott laboratories) introduced the oxime function into pyrrolidine or piperidine rings as an amino group surrogate.[20] Although these quinolones showed enhanced *in vitro* antibacterial activity against Gram-positive strains, they displayed rather weak potency against Gram-negative organisms. In addition, their *in vivo* efficacy was not as good as was anticipated from the *in vitro* results. Kaken Pharmaceutical has recently disclosed another series of oxime-containing quinolones that have antimicrobial activity profiles similar to those of the Abbott compounds.[21] Kaken compounds contained pyrrolidine rings substituted by an additional lipophilic substituent, such as a dimethyl or cyclopropyl group next to the oxime moiety. In this case the oxime group was also used as an amino group mimic.



x= N, CH or CF
R= H or CH_3

Abbott compound



x= N, CH or CF
R= alkyl or cyclopropyl

Kaken compound

In this paper, we describe the design and synthesis of oxime-functionalized pyrrolidine derivatives and a series of novel fluoroquinolone compounds containing these amines at the 7-position. The novel pyrrolidines are structurally unique, having an alkyloxime group at the 4-position and an aminomethyl substituent at the 3-position of the ring. We also report herein the excellent antibacterial activity data of the new quinolones and structure−activity relationship (SAR) of the pyrrolidinyl group. Finally, we have included the pharmacokinetic profile in animals of the development candidate LB20304 and its *in vivo* efficacy data.

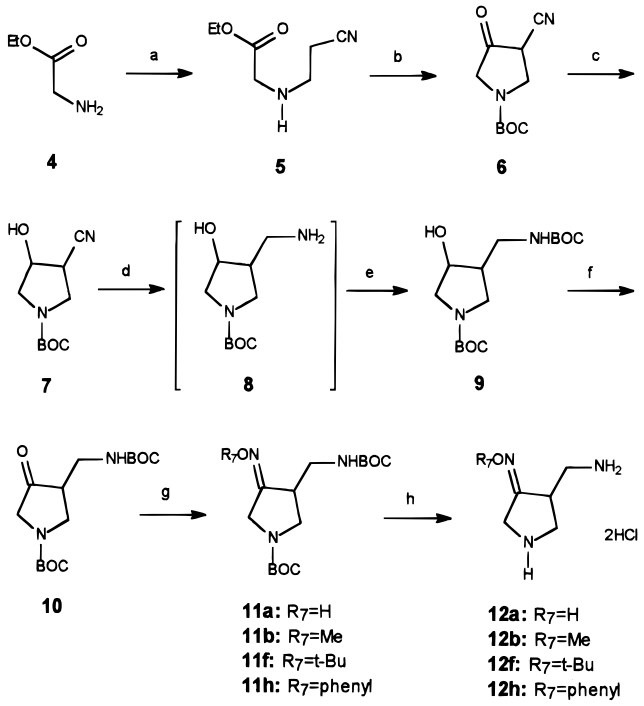
Chemistry

The synthesis of the new pyrrolidine derivatives is outlined in Scheme 1. Ethylglycine hydrochloride **4** was reacted with acrylonitrile in aqueous KOH at 60 °C, and the resulting Michael adduct was subsequently treated with di-*tert*-butyl dicarbonate to produce BOC-protected cyano ester **5**. The cyano ester **5** was smoothly cyclized to the cyano ketone **6** by NaOEt in 92% yield. Selective reduction of the cyano group to the primary amine, in the presence of the ketone moiety of the cyano ketone **6**, turned out to be rather sluggish and difficult to scale up. Even though various metals (for example Pd, Pt, or Raney Ni) were used for selective catalytic hydrogenation, we were not able to obtain the desired product in acceptable yield. Protection of the ketone and subsequent reduction of the cyano group by typical reducing agents met with no success. Also, direct reduction of both functional groups by lithium aluminum hydride yielded the amino alcohol **8** in poor yield (35−45%). Therefore the cyano ketone **6** had to be first converted to the cyano alcohol **7** by sodium borohydride in quantitative yield, and the cyano group of the corresponding alcohol **7** was subsequently reduced by lithium aluminum hydride to form **8**, in an overall yield of 83% for the two steps.

The alcohol **8** was isolated as a *tert*-butyloxycarbonyl (BOC) protected form **9** by treatment with di-*tert*-butyl dicarbonate in dioxane−H_2O at pH 8.5. When the reaction medium was more basic than pH 8.5, or typical BOC protecting conditions were applied to this compound, the undesirable bis-BOC-protected compound was obtained.

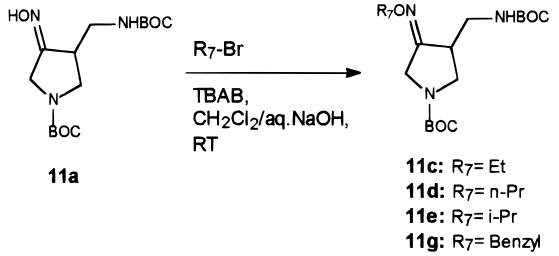
Parikh−Doering oxidation (sulfur trioxide−pyridine complex in DMSO)[22] of the alcohol **9** afforded the ketone **10** in 85% yield. The ketone **10** was smoothly converted to the oxime **11** by reaction with various *O*-alkylhydroxylamines in EtOH−THF at 40 °C. When a par-

Scheme 1. Synthesis of 3-(Alkoxyloximino)-4-(aminomethyl)pyrrolidine Derivatives[a]



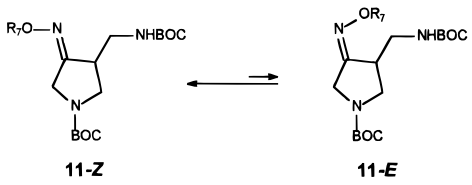
[a] Reagents: (a) CH_2=CHCN, NaOH, 60 °C; (b) $(t\text{-BOC})_2O$, $CHCl_3$, then NaOEt, EtOH, reflux; (c) $NaBH_4$, EtOH, 0 °C; (d) LAH, THF, −5 °C; (e) $(t\text{-BOC})_2O$, $NaHCO_3$, dioxane−H_2O; (f) pyridine−SO_3, Et_3N, DMSO, 5 °C; (g) R_7ONH_2·HCl, $NaHCO_3$, EtOH−THF, 40 °C; (h) acetyl chloride, MeOH, 0 °C.

Scheme 2. Alternative Synthesis of 3-(Alkyloximino)-4-(aminomethyl)pyrrolidine Derivatives

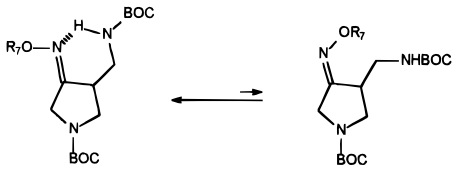


ticular *O*-alkylhydroxylamine was not commercially available, an alternative procedure was applied (Scheme 2). The ketone **10** was first converted to the free oxime **11a** by treatment with hydroxylamine, and **11a** was alkylated with the corresponding alkyl halide, affording the alkyloximes **11c,d,e,g** in good yield.

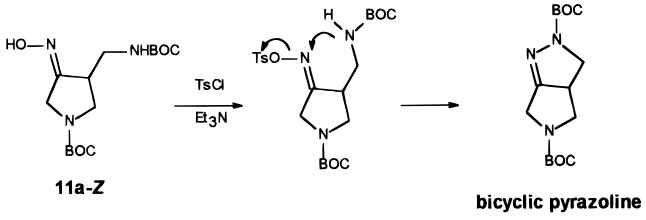
Since the oximino group can exist in the *E* or *Z* configuration, it was necessary to determine the geometry of each oxime intermediate.[23] The oximes **11** bearing various alkyl groups (R_7) were found to exist in a single configuration by TLC in several solvent systems and NMR as well as GC data. Since X-ray crystallography or NOE experiments were not applicable to **11**,[24] we have collected indirect but conclusive evidences and were able to determine the geometry of **11** as the *Z* configuration based on the following facts. First of all, the oxime **11** should be the *Z* isomer for thermodynamic reasons. It is well-known from the literature that the geometry of the oxime is very sensitive to its steric environment. For example, when a cyclic oxime has an α-substituent, the OR group of the oxime tends to orient itself in the opposite direction to avoid unfavorable steric interaction.[25] Since **11** has a quite bulky BOC-aminomethyl substituent next to the oxime moiety, we believe **11** adopts the more stable *Z* configuration.



Consideration should be given to hydrogen bonding stabilization of the *Z* form. In this configuration, the lone pair electrons of the oxime nitrogen atom are well placed to participate in the hydrogen bonding with the adjacent amino group, whereas with the *E* configuration this is not the case. As a result of stabilization by hydrogen bonding, in addition to the consequences of steric crowding, the *Z* form would be expected to be more energetically favored than its *E* isomer.



The *Z* configuration of **11** was also confirmed by means of a chemical transformation. When compound **11a** was treated with tosyl chloride (TsCl) and triethylamine in methylene chloride, clean formation of the bicyclic pyrazoline was observed. Since only the *Z*-tosylate can produce the bicyclic pyrazoline, the stereochemistry of **11a** can be surmised to be *Z*.[26]



The bis-BOC protective groups of the oxime (**11**) were removed by hydrochloric acid in MeOH in quantitative yield to afford the novel pyrrolidine salts **12**. Here again, the compounds **12** were found to exist in a single configuration by NMR and HPLC. The geometry of **12b** was determined to be *Z* by its transformation to **11b-*Z*** under virtually neutral conditions (pH 7.0, BOC_2O in aqueous dioxane). In this reaction medium we could eliminate any possibility of acid- or base-catalyzed *E*−*Z* isomerization. In addition, the *Z* geometry of **12b** was supported by its conversion to the final product, which also has a *Z* configuration (*vide infra*).

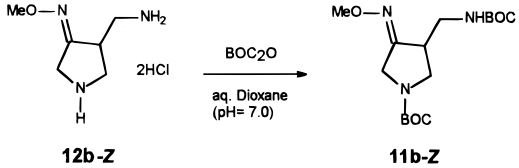
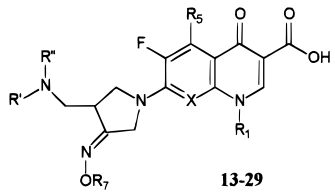


Table 1. Physical Data of the New Quinolone Compounds



13-29

compd	X	R_1	R_5	R_7	R′	R″	yield (%)	mp (°C)[a]	formula[b]	MS (FAB, M + H)
13	CF	C_2H_5	H	CH_3	H	H	67	242−245	$C_{18}H_{20}F_2N_4O_4{\cdot}2H_2O$	395
14	CF	cyclopropyl	H	CH_3	H	H	55	256−258	$C_{19}H_{20}F_2N_4O_4{\cdot}H_2O$	405
15	CCl	cyclopropyl	H	CH_3	H	H	42	241−245 (dec.)	$C_{19}H_{20}F_1N_4O_4Cl$	423
16	CH	cyclopropyl	H	CH_3	H	H	37	274−276	$C_{19}H_{21}F_1N_4O_4{\cdot}{}^1/_2H_2O$	389
17	CF	cyclopropyl	NH_2	CH_3	H	H	40	238−240	$C_{19}H_{21}F_2N_5O_4{\cdot}H_2O$	422
18	COMe	cyclopropyl	H	CH_3	H	H	43	244−246	$C_{20}H_{23}F_1N_4O_5{\cdot}{}^1/_2H_2O$	419
19	N	2,4-difluorophenyl	H	CH_3	H	H	25	248−250 (dec.)	$C_{21}H_{18}F_3N_5O_4{\cdot}H_2O$	462
20	N	cyclopropyl	H	CH_3	H	H	85	235−237	$C_{18}H_{20}F_1N_5O_4{\cdot}H_2O$	390
21	N	cyclopropyl	H	C_2H_5	H	H	57	251−253	$C_{19}H_{22}F_1N_5O_4{\cdot}2H_2O$	404
22	N	cyclopropyl	H	*n*-propyl	H	H	66	228−230	$C_{20}H_{24}F_1N_5O_4{\cdot}H_2O$	418
23	N	cyclopropyl	H	isopropyl	H	H	70	261−263	$C_{20}H_{24}F_1N_5O_4{\cdot}H_2O$	418
24	N	cyclopropyl	H	*tert*-butyl	H	H	61	254−256	$C_{21}H_{26}F_1N_5O_4{\cdot}H_2O$	432
25	N	cyclopropyl	H	benzyl	H	H	57	243−244	$C_{24}H_{24}F_1N_5O_4{\cdot}2H_2O$	466
26	N	cyclopropyl	H	phenyl	H	H	72	222−224	$C_{23}H_{22}F_1N_5O_4{\cdot}2H_2O$	452
27	N	cyclopropyl	H	H	H	H	61	232−233	$C_{17}H_{18}F_1N_5O_4{\cdot}2H_2O$	376
28	N	cyclopropyl	H	CH_3	H	CH_3	48	235−237	$C_{19}H_{22}F_1N_5O_4{\cdot}{}^1/_2H_2O$	404
29	N	cyclopropyl	H	CH_3	CH_3	CH_3	59	266−268	$C_{20}H_{24}F_1N_5O_4{\cdot}{}^1/_2H_2O$	418

[a] Melting points are uncorrected. [b] Analyses for C, H, and N were within ±0.4% of the theoretical values.

Scheme 3. Synthesis of New Quinolones

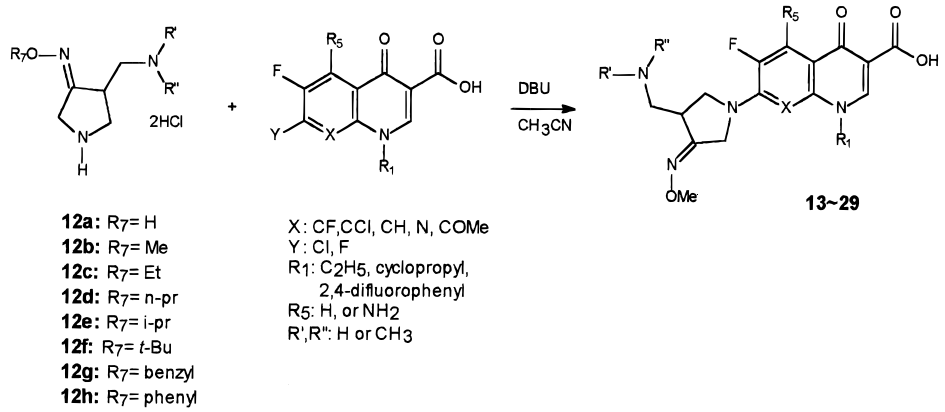
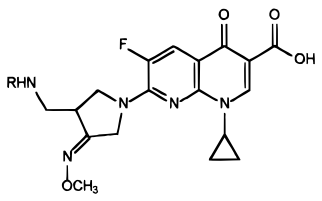


Figure 1. X-ray structure of *t*-BOC analog of LB20304.

The coupling reactions of the pyrrolidine salt **12** and various quinolone or naphthyridone nuclei followed well-established literature procedures (Scheme 3).[6,27,28] The structure and physical data of the new quinolone compounds prepared for this study are listed in Table 1.

The oxime geometry of the final compound was determined to have the *Z* configuration, by application of the same chemical transformation method. We further confirmed the structure of the final compound unambiguously by X-ray crystallographic analysis. Although we were not successful in preparing X-ray quality single crystals of compound **20** (LB20304) or the other final products, we were able to obtain X-ray data for the *t*-BOC derivative of LB20304. As expected, the geometry of the oxime group on the pyrrolidine ring was *Z* with hydrogen bonding observed between the oxime nitrogen atom (N20) and the nitrogen atom of the aminomethyl group (N24). (See Figure 1. *Please note that the numbering of the X-ray structure is different from that of general quinolone.*[29])



Results and Discussion

The data listed in Tables 2 and 3 supports the validity of our strategy. Novel quinolones containing new pyrrolidines were found to have strong antibacterial activities against not only Gram-negative strains, but also

Gram-positive organisms, including methicillin resistant *Staphylococcus aureus* (MRSA). They showed very strong activity against Gram-positive bacteria such as *S. aureus*, *Streptococcus epidermidis*, *and Bacillus subtilis*, with MIC values being mostly less than 0.008 μg/mL. The novel quinolones were especially potent against methicillin resistant *S. aureus* (MRSA, 16−256-fold enhancement compared to ciprofloxacin) and methicillin resistant *S. epidermidis* (MRSE, 32−512-fold enhancement compared to ciprofloxacin), against which ciprofloxacin was virtually ineffective (MIC 64 and 128 μg/mL, respectively).

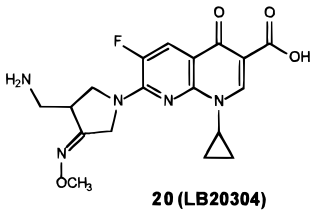
Unlike Abbott or Kaken's oxime-containing quinolones, the novel compounds in this study also displayed excellent activity against Gram-negative strains (MIC values mostly lower than 0.008 μg/mL). Their potency was comparable to ciprofloxacin against *Escherichia coli*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*. They showed even better antibacterial activity than ciprofloxacin against some Gram-negative strains, for example *Actinobacter calcoaceticus*. These results show that the novel quinolones are truly strong and broad-spectrum antibacterial agents.

Variations at the C-8 position of the quinolone nucleus in this study included fluorine, chlorine, nitrogen, methoxy, and hydrogen substitution (Table 2). The activity imparted to the substituted quinolone ring by the C-8 substituent was in the order F (C_5-NH_2) > F (C_5-H) > naphthyridine > Cl = OMe = H against Gram-positive organisms. In the case of Gram-negative strains, the activity was in the order F (C_5-NH_2) > naphthyridine = F (C_5-H) > H > Cl > OMe. It is also interesting to note that the naphthyridone-type compounds showed comparable potency to its C_8-F quinolone counterparts. They were also more potent than either their C_8-Cl or C_8-H analogs against all organisms. These results are somewhat inconsistent with structure−activity relationships for other quinolones as *in vitro* activities of naphthyridone compounds are often intrinsically lower than their C_8-F and C_8-Cl quinolone counterparts, although naphthyridones have been reported to have toxicological advantages compared to their quinolone analogs.[2]

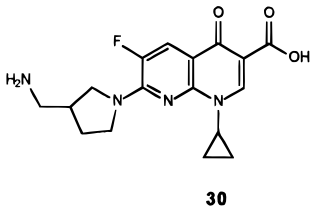
The size and lipophilicity of the alkyl group of the oxime moiety were considered to be key factors in determining biological activity. We have briefly investigated the SAR of the alkyloxime group (Table 3). Compound **27**, which possesses an unsubstituted oxime group, showed relatively low potency, while alkyloxime-containing compounds (compounds **20**−**25**) exhibited excellent activities against all organisms. These results run contrary to the activity profiles of Abbott or Kaken's oxime-substituted quinolones, in which case, methyloxime-containing compounds were reported to be less potent than free oxime-substituted quinolones. In addition, their compounds showed poor Gram-negative activity, while the activity of our quinolones was comparable to ciprofloxacin against the same strains. We believe the oxime functional group in our quinolones plays a different role to that in Abbott or Kaken's quinolones. In the latter cases the free oxime moiety was introduced instead of a hydrophilic amino moiety, whereas the alkyloxime in our compounds was used as a lipophilic alkyl group mimic. This explanation can be further supported by the fact that the biological activity increased as the size of the alkyl chain of the alkyloxime was enlarged (compounds **20**−**25**).

At this point it is necessary to mention the importance of the oxime functional group with respect to biological activity. In order to define more clearly the role of this special functional group for the activity profile, we have prepared the desoximino aminomethyl pyrrolidine derivative (compound **30**). When directly compared to compound **20** (LB20304) and other oxime-containing quinolones in this study, the desoximino compound **30** exhibited decreased antibacterial activity against all organisms. For example, the oxime-containing compound **20** was 4−8 times more active than its desmethyl analog compound **30** against *S. aureus*, MRSA, and MRSE. It was also 2−4 times more potent against *E. coli*, including TEM series. Comparable activity was observed against *P. aeruginosa* and other Gram negative organisms between compounds **20** and **30**. It is also worth noting the remarkable effect of the oxime moiety on *in vivo* efficacy in rats (*vide infra*).

When the primary amine group of the (aminomethyl)-pyrrolidine fragment was methylated or dimethylated (compounds **28**, **29**), significant loss in biological activity was observed against all organisms.



20 (LB20304)



30

Among those novel quinolones with excellent *in vitro* activity, compound **20** exhibited the most favorable pharmacokinetic profile in animals after oral administration (Table 4). In rats, this compound was well absorbed (AUC = 8.5 mg·h/mL) and showed good bioavailablity (F = 95%) and a long serum half-life (t = 2.62 h). Furthermore, in dogs LB20304 was found to have a much longer serum half-life (t = 5.12 h) than that of ciprofloxacin (t = 1.7 h).

When directly compared to the desoximino compound **30** in rats, compound **20** was superior to **30** in every respect. For example, the bioavailability of **30** (10%) was nine times lower than that of LB20304 (95%) and the C_{max} of **30** (0.30 μg/mL) was at least 8 times lower than that of LB20304 (2.44 μg/mL). From these results, it is clear that the oxime functional group changes the physicochemical property of novel quinolones, thus significantly improving the pharmacokinetic parameters.

Mouse protection tests were used to evaluate the *in vivo* efficacy of compound **20**, with the compound being administered orally (Table 5). The efficacy of this compound was tested against four representative strains: *S. aureus* and *S. pneumonia* were selected for Gram-positive bacteria, and *P. aeruginosa* and *E. coli* were chosen for Gram-negative bacteria.

In vivo efficacy is well reflected in *in vitro* inhibitory activity. The ED_{50} of compound **20** against *S. aureus* was 1.17 mg/kg with a 95% confidence limit of 0.52−2.10 mg/kg per day, which was about 6 times stronger than ciprofloxacin (ED_{50} of 6.05 mg/kg) and 7 times more potent than the desoximino compound **30** (ED_{50} of 8.03 mg/kg). Compound **20** showed at least a 20-fold en-

Table 2. *In Vitro* Antimicrobial Activities of New Quinolone Compounds

	minimum inhibitory concentrations (MIC), μg/mL													
	Gram-positive organisms							Gram-negative organisms						
compd	*S. aureus 6538p*	*S. aureus giorgio*	*S. aureus 241*	*S. epidermidis 887E*	*S. epidermidis 178*	*B. subtilis ATCC 6633*	*S. faecalis 29212*	*E. coli 10536*	*E. coli 3190Y*	*E. coli TEM9 2639E*	*P. aeruginosa 1912E*	*E. cloacae P99*	*K. aerogenes 1082E*	*A. calcoaceticus 15473*
13	0.031	0.063	4	0.063	4	0.031	0.25	0.063	0.063	0.5	2	0.13	0.5	1
14	≤0.008	≤0.008	0.5	≤0.008	0.5	≤0.008	0.031	≤0.008	≤0.008	0.031	0.25	0.016	0.031	0.031
15	0.031	0.016	1	0.031	1	0.016	0.13	0.031	0.016	0.25	1	0.063	0.25	0.25
16	0.008	0.016	4	0.016	4	≤0.008	0.031	≤0.008	≤0.008	0.031	0.25	0.008	0.031	0.13
17	≤0.008	≤0.008	0.25	≤0.008	0.25	≤0.008	0.016	≤0.008	≤0.008	0.016	0.25	≤0.008	0.016	0.031
18	0.031	0.016	1	0.031	1	0.031	0.063	0.063	0.031	0.25	2	0.063	0.25	0.25
19	0.031	0.031	8	0.016	8	0.016	0.13	0.063	0.008	0.25	0.5	0.063	0.25	0.13
20	≤0.008	≤0.008	4	≤0.008	4	≤0.008	0.063	≤0.008	≤0.008	0.031	0.25	≤0.008	0.031	0.063
30	0.031	0.031	16	0.016	32	0.016	0.13	0.031	≤0.008	0.063	0.5	0.031	0.063	0.13
CFLX	0.13	0.13	64	0.13	128	0.031	0.5	0.016	≤0.008	0.016	0.13	≤0.008	0.016	0.25

Table 3. *In Vitro* Antimicrobial Activities of New Quinolone Compounds

	minimum inhibitory concentrations (MIC), μg/mL													
	Gram-positive organisms							Gram-negative organisms						
compd	*S. aureus 6538p*	*S. aureu giorgio*	*S. aureus 241*	*S. epidermidis 887E*	*S. epidermidis 178*	*B. subtilis ATCC 6633*	*S. faecalis 29212*	*E. coli 10536*	*E. coli 3190Y*	*E. coli TEM9 2639E*	*P. aeruginosa 1912E*	*E. cloacae P99*	*K. aerogenes 1082E*	*A. calcoaceticus 15473*
20 (LB20304)	≤0.008	≤0.008	4	≤0.008	4	≤0.008	0.063	≤0.008	≤0.008	0.031	0.25	≤0.008	0.031	0.063
21	≤0.008	≤0.008	2	≤0.008	2	≤0.008	0.063	≤0.008	≤0.008	0.031	0.5	≤0.008	0.031	0.016
22	0.008	≤0.008	2	≤0.008	1	≤0.008	0.063	≤0.008	≤0.008	0.063	1	≤0.008	0.13	0.031
23	≤0.008	≤0.008	2	≤0.008	1	≤0.008	0.063	≤0.008	≤0.008	0.063	1	≤0.008	0.13	0.031
24	0.008	≤0.008	1	≤0.008	1	≤0.008	0.063	0.016	0.016	0.063	2	0.016	0.25	0.031
25	≤0.008	≤0.008	2	≤0.008	2	≤0.008	0.031	≤0.008	≤0.008	0.063	1	≤0.008	0.063	≤0.008
26	0.016	0.016	≤0.008	≤0.008	16	≤0.008	0.13	0.031	0.016	0.13	2	0.031	0.13	0.016
27	0.031	0.031	8	0.031	8	0.016	0.25	0.031	0.016	0.13	2	0.031	0.13	0.13
28	0.063	0.13	128	0.13	128	0.25	0.5	0.063	0.063	0.5	2	0.13	0.5	1
29	0.13	0.13	32	0.13	16	0.016	1	0.13	0.063	1	16	0.13	0.016	0.5
30	0.031	0.031	16	0.016	32	0.016	0.13	0.031	≤0.008	0.063	0.5	0.031	0.063	0.13
CFLX	0.13	0.13	64	0.13	128	0.031	0.5	0.016	≤0.008	0.016	0.13	≤0.008	0.016	0.25

Table 4. Pharmacokinetic Data of Compound **20**, Compound **30**, and Ciprofloxacin in Rat[a] and Dog[b]

animal	compd	route	AUC (μg h/mL)	half-life (h)	C_{max} (μg/mL)	T_{max} (h)	F (%)
rat	**20**	po	8.50	2.33	2.44	0.33	95.3
	30	po	0.38	2.20	0.30	0.17	10.1
	ciprofloxacin	po	2.59	1.87	0.90	0.11	45.4
dog	**20**	po	7.55	5.12	1.34	1.13	71
	30	ND[c]	ND	ND	ND	ND	ND
	ciprofloxacin	po	4.54	1.70	0.91	1.12	75

[a] SD rat, dose: 20 mg/kg. [b] Dose: 4 mg/kg. [c] ND: not determined.

Table 5. *In Vivo* Efficacy of Compound **20**, Compound **30**, and Ciprofloxacin against Systemic Infection in Mouse

infected bacteria	compd[a]	MIC (μg/mL)	ED_{50} (mg/kg)[b]	95% confidence limit (mg/kg)
S. aureus giorgio	**20**	≤0.008	1.17	0.52−2.10
	30	0.031	8.03	−[c]
	ciprofloxacin	0.13	6.05	1.60−14.2
Str. pneumonia 77A	**20**	0.016	7.64	0.78−17.20
	30	0.063	ND[d]	ND[d]
	ciprofloxacin	0.5	>200	
P. aeruginosa 1912E	**20**	0.25	2.08	0.76−4.94
	30	0.5	29.75	−[c]
	ciprofloxacin	0.13	2.34	0.51−11.98
E. coli 851E	**20**	≤0.008	0.47	0.00−1.54
	30	0.031	>13.5	
	ciprofloxacin	≤0.008	0.20	0.01−0.54

[a] Antimicrobial agents were orally administrated twice at 1 and 4 h after infection. [b] ED_{50}, 50% effective dose. [c] −, confidence limits could not be calculated. [d] ND: not determined.

hancement (ED_{50} of 7.64 mg/kg) in activity compared to ciprofloxacin (ED_{50} of >200 mg/kg) against *S. pneumonia*. The ED_{50} value of compound **20** (2.08 mg/kg) was somewhat better than that of ciprofloxacin (2.34 mg/kg) against the Gram negative strain *P. aeruginosa*, while it was 15 times better than the compound **30** (ED_{50} of 29.75 mg/kg). Although compound **20** exhibited a little lower *in vivo* efficacy than ciprofloxacin (ED_{50} of 0.47 *vs* 0.20 mg/kg, respectively), it was far more effective against *E. coli* than **30** (ED_{50} of >13.5 mg/kg). Finally, the methanesulfonate form of compound **20** was found to be highly soluble in water (162, 19, 0.5 mg/mL at pH 2, 6, 7, respectively, at 25 °C).

In conclusion, we designed and synthesized novel quinolone agents derivatized by oxime-substituted (aminomethyl)pyrrolidines. They were found to possess very potent antibacterial activities against both Gram-negative and Gram-positive organisms, including methicillin resistant *S. aureus* (MRSA). Among these compounds, compound **20** showed excellent *in vivo* efficacy, good bioavailability, and long half-life as well as high water solubility (methanesulfonate form). The remarkable effects of the newly introduced oxime functional group on biological activity and pharmacokinetic profiles in animals were clearly demonstrated by direct comparison of compound **20** to its desoximino compound **30**. On the basis of these promising results, compound **20** was advanced to preclinical and clinical studies.[30]

Experimental Procedures

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 spectra (NMR) were recorded on JEOL 500 or Bruker DMX 600 spectrometers, and chemical shifts are reported in δ units relative to internal tetramethylsilane. GC−MS spectra were taken from a JEOL JMS DX300 mass spectrometer with a MP2080 data system. Column chromatography was performed using silica gel (Merck, 230−400 mesh ASTM). Final compounds were purified using preparative HPLC (a Waters Associates system, UV detection at 289 or 272 nm), equipped with a PLRP-S, 25 × 300 mm, 5 μm, 100 A pore size column and a mobile phase consisting of a H_2O-CH_3CN gradient which contained 0.1% trifluoroacetic acid. In all cases, purities were higher than 95%. Elemental analyses were performed on a Perkin-Elmer 2400 CHN elemental analyzer, and all compounds had analytical results within ±0.4% of their theoretical values.

Synthesis of ((2-Cyanoethyl)amino)acetic Acid Ethyl Ester (5). Glycine ethyl ester hydrochloride **4** (139.6 g, 1 mol) was dissolved in 80 mL of distilled water, and to this solution was added 230 mL of an aqueous solution of KOH (67.3 g, 1.3 molar equiv). CH_3CN (106.2 g, 2 molar equiv) was added to the reaction mixture while stirring at 50−60 °C. The reaction mixture was stirred for 5 h at the same temperature and then cooled to room temperature. The aqueous layer was extracted twice with Et_2O, and the combined organic layer was dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated and the residue was distilled under reduced pressure (140−150 °C/10.25 Torr) to afford 65.6 g (yield, 48%) of the title compound **5**: ^{1}H NMR ($CDCl_3$, ppm) δ 4.20 (2H, q, J = 7.1 Hz), 3.48 (2H, s), 2.96 (2H, t, J = 6.7 Hz), 2.54 (2H, t, J = 6.7 Hz), 1.30 (3H,t, J = 7.1 Hz); MS (FAB, *m/e*) 157 (M + H). Anal. ($C_7H_{12}N_2O_2$) C, H, N.

Synthesis of 4-Cyano-1-*N*-(*tert*-butoxycarbonyl)pyrrolidin-3-one (6) To a solution of the ester **5** (29 g, 0.186 mol) in 200 mL of $CHCl_3$ was added di-*tert*-butoxycarbonyl dicarbonate (45 g, 0.205 mol), and the reaction mixture was stirred for 17 h at room temperature. The reaction mixture was concentrated *in vacuo* to give the BOC-protected compound, which was pure enough to be used for the next step without further purification. The crude BOC-protected compound was dissolved in absolute EtOH (200 mL), and this solution was slowly added to a NaOEt solution, which was prepared by adding 6 g of sodium (Na) turnings to 220 mL of refluxing absolute EtOH. The reaction mixture was stirred for an additional hour under reflux and concentrated under reduced pressure. The residue was diluted with water, and the aqueous layer was washed twice with CH_2Cl_2. The aqueous layer was adjusted to pH 4 with 1 N HCl and extracted three times with EtOAc (300 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* to give the ketone compound **6** (35.9 g, two-step overall yield: 92%) as a slightly yellow solid: ^{1}H

NMR ($CDCl_3$, ppm) δ 4.5 (1H, m), 4.05 (1H, m), 3.9−3.5 (3H, m), 1.5 (9H, s); MS (FAB, *m/e*) 211 (M + H). Anal. ($C_{10}H_{14}N_2O_3$) C, H, N.

Synthesis of 4-Cyano-1-*N*-(*tert*-butoxycarbonyl)pyrrolidin-3-ol (7). The ketone compound **6** (10 g, 0.047 mol) in 500 mL of EtOH was cooled to 0 °C (ice bath), and to this solution was added portionwise 3.6 g (0.094 mol) of $NaBH_4$ over a period of 20 min. The reaction mixture was stirred for 30 min at the same temperature and concentrated under reduced pressure. The residue was diluted with EtOAc (400 mL), washed with water, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure to give a residue which was purified by column chromatography (silica gel, hexane/EtOAc = 4:1), affording the alcohol **7** (10.1g, 0.047 mol) as a colorless oil in quantitative yield: ^{1}H NMR ($CDCl_3$, ppm) δ 4.5 (1H, m), 3.8 (3H, m), 3.3 (1H, m), 3.0 (1H, m), 1.4 (9H, s); MS (FAB, *m/e*) 213 (M + H). Anal. ($C_{10}H_{16}N_2O_3$) C, H, N.

Synthesis of 4-((*N*-(*tert*-Butoxycarbonyl)amino)methyl)-1-*N*-(*tert*-butoxycarbonyl)pyrrolidin-3-ol (9). The alcohol **7** was dissolved in 200 mL of dry THF, and this solution was cooled to −5 °C using an ice−salt bath. $LiAlH_4$ (2.6 g, 0.066 mol) was then added over a period of 20 min. The reaction mixture was stirred for 30 min at the same temperature and quenched with 2.6 mL of water, 2.6 mL of 15% NaOH solution, and finally with 7.8 mL of water. This mixture was stirred for 1 h at room temperature. After 6 g of anhydrous $MgSO_4$ was added the mixture was stirred for a further 30 min and filtered. The filtrate was concentrated to yield the crude amine product **8**. The latter was diluted with 200 mL of dioxane−water (2:1 by volume) followed by portionwise addition of di-*tert*-butoxycarbonyl dicarbonate (12.3 g, 0.056 mol). The mixture was stirred for 30 min and concentrated *in vacuo*, and the residue was diluted with EtOAc (400 mL). This solution was washed with water and saturated saline, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*, and the residue was purified by column chromatography (silica gel, hexane/EtOAc = 3:1) to give 12.3 g (yield, 83%) of the title compound **9** as a colorless oil: ^{1}H NMR ($CDCl_3$, ppm) δ 4.95 (1H, m), 4.1 (1H, m), 3.5 (2H, m), 3.3−3.0 (4H, m), 2.1 (1H, m), 1.45 (18H, s); MS (FAB, *m/e*) 317 (M + H). Anal. ($C_{15}H_{28}N_2O_5$) C, H, N.

Synthesis of 4-((*N*-(*tert*-butoxycarbonyl)amino)methyl)-1-*N*-(*tert*-butoxycarbonyl)pyrrolidin-3-one (10). To a solution of the alcohol **9** (14 g, 0.044 mol) in DMSO (64 mL) was added Et_3N (18.5 mL, 3 molar equiv), and the mixture was cooled to 5 °C (ice bath). When the mixture by the wall of the reaction flask begins to freeze, pyridine−sulfur trioxide ($Py·SO_3$, 12.7 g, 1.8 molar equiv) oxidant was added portionwise thereto. After the addition was completed, the ice bath was removed and the reaction solution was stirred for 3 h at room temperature. The reaction mixture was diluted with water and extracted three times with EtOAc (300 mL). The combined extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue thus obtained was purified by column chromatography (silica gel, hexane/EtOAc = 3:1) to give 11.8 g (yield, 85%) of the ketone **10** as a colorless oil: ^{1}H NMR ($CDCl_3$, ppm) δ 4.95 (1H, bs), 4.11 (1H, m), 3.90 (1H, m), 3.69 (1H, m), 3.40 (3H, m), 2.78 (1H, m), 1.45 (9H, s), 1.40 (9H, s); MS (FAB, *m/e*) 315 (M + H). Anal. ($C_{15}H_{26}N_2O_5$) C, H, N.

Synthesis of 4-((*N*-(*tert*-butoxycarbonyl)amino)methyl)-1-*N*-(*tert*-butoxycarbonyl)pyrrolidin-3-one *O*-Methyloxime (11b). To a solution of the ketone **10** (260 mg, 0.83 mmol) in a mixture of 5 mL of 95% EtOH and 2.5 mL of THF were added methoxylamine hydrochloride (256 mg, 3.7 molar equiv) and $NaHCO_3$ (257 mg, 3.7 molar equiv) dissolved in 2.5 mL of distilled water. The reaction mixture was stirred for 1 h at 40 °C and concentrated under reduced pressure. The residue was diluted with EtOAc (50 mL), washed successively with water and saturated saline solution, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*, and the residue was purified by column chromatography (silica gel, hexane/EtOAc = 3:1), affording the oxime compound **11b** (250 mg, yield 88%) as a colorless oil: ^{1}H NMR ($CDCl_3$, ppm) δ 4.98 (1H, bs), 4.08 (2H, bs), 3.81 (3H, s), 3.75 (1H, m), 3.50 (1H, bs), 3.41 (1H, bs), 3.28 (1H, m), 3.00 (1H, m), 1.40 (18H, s); MS (FAB, *m/e*) 344 (M + H). Anal. ($C_{16}H_{29}N_3O_5$) C, H, N.

Synthesis of 4-((*N*-(*tert*-butoxycarbonyl)amino)methyl)-1-*N*-(*tert*-butoxycarbonyl)pyrrolidin-3-one Oxime (11a). To a solution of the ketone **10** (300 mg, 0.95 mmol) in a mixture of 5 mL of 95% EtOH and 2.5 mL of THF was added hydroxylamine hydrochloride (232 mg, 3.5 molar equiv) and $NaHCO_3$ (281 mg, 3.5 molar equiv) dissolved in 2.5 mL of distilled water. The reaction mixture was stirred for 1 h at 40 °C, and concentrated under reduced pressure. The residue was diluted with EtOAc (50 mL), washed successively with water and saturated saline solution, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*, and the residue was purified by column chromatography (silica gel, hexane/EtOAc = 2:1) to give the oxime **11a** (292 mg, yield 93%) as a colorless oil: ^{1}H NMR ($CDCl_3$, ppm) δ 9.70 (1H, bs), 5.05 (1H, bs), 3.83−2.79 (7H, m), 1.42 (18H, s). MS (FAB, *m/e*) 330 (M + H). Anal. ($C_{15}H_{27}N_3O_5$) C, H, N.

Synthesis of 4-((*N*-(*tert*-butoxycarbonyl)amino)methyl)-1-*N*-(*tert*-butoxycarbonyl)pyrrolidin-3-one *O*-Isopropyloxime (11e). To a solution of the free oxime **11a** (292 mg, 0.88 mmol) and *n*-Bu_4NBr (85 mg, 0.26 mmol) in 15 mL of CH_2Cl_2 were added isopropyl bromide (216 mg, 1.76 mmol) and 5 mL of a 15% solution of NaOH. The reaction mixture was stirred for 5 h at room temperature and diluted with CH_2Cl_2 (20 mL). The organic layer was washed successively with water and saturated saline solution, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*, and the residue was purified with column chromatography (silica gel, hexane/EtOAc = 3:1) to give the desired compound **11d** (265 mg, yield 81%) as a colorless oil: ^{1}H NMR ($CDCl_3$, ppm) δ 5.05 (1H, bs), 4,11 (2H, s), 4.01 (1H, m), 3.55−3.25 (3H, m), 3.40 (1H, m), 3.02 (1H, m), 1.55 (18H, s), 1.05 (1H, d); MS (FAB, *m/e*) 372 (M + H). Anal. ($C_{18}H_{33}N_3O_5$) C, H, N.

Synthesis of 3,3a,4,6-Tetrahydropyrro[3,4-*c*]pyrazole-2,5-dicarboxylic Acid Di-*tert*-butyl Ester. A solution of the free oxime **11a** (30 mg, 0.09 mmol) in 3 mL of CH_2Cl_2 was cooled to 0 °C. To this solution were added Et_3N (25 μL, 0.18 mmol) and *p*-TsCl (20 mg, 0.108 mmol), and the reaction mixture was stirred for 10 min at the same temperature. After 3 h at room temperature, the reaction mixture was concentrated and the residue was purified by column chromatography (silica gel, hexane/EtOAc = 3:1) to give the cyclized compound (29.8 mg, yield 95%) as a colorless oil: ^{1}H NMR ($CDCl_3$, ppm) δ 4.10 (4H, m), 3.65 (1H, m), 3.51 (1H, m), 3.05 (1H, m), 1.53 (9H, s), 1.50 (9H, s); MS (FAB, *m/e*) 312 (M + H). Anal. ($C_{15}H_{24}N_4O_3$) C, H, N.

Synthesis of 4-(Aminomethyl)pyrrolidin-3-one-*O*-Methyloxime Dihydrochloride (12b). Acetyl chloride (10 mL) was added slowly to 20 mL of MeOH at 0 °C. The reaction mixture was stirred for 30 min at the same temperature, followed by addition of the oxime **11b** (990 mg, 2.97 mmol) in 10 mL of methanol. The reaction mixture was stirred for 50 min at room temperature and concentrated under reduced pressure. The residue was suspended with EtOAc and filtered. The solid obtained was washed twice with EtOAc and dried *in vacuo* to give 648 mg (yield 94%) of the dihydrochloride **12b** as a white solid: ^{1}H NMR (DMSO-d_6, ppm) δ 10.0 (3H, bs), 8.35 (2H, bs), 3.90 (2H, m), 3.80 (3H, s), 3.69 (1H, m), 3.40 (2H, m), 3.12 (2H, m); MS (FAB, *m/e*) 144 (M + H). Anal. ($C_6H_{15}N_3OCl_2$) C, H, N.

Synthesis of 7-(4-(Aminomethyl)-3-(methoxyimino)-pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic Acid (20). 7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (141 mg, 0.5 mmol) and 4-(aminomethyl)-pyrrolidin-3-one *O*-methyloxime dihydrochloride (110 mg, 0.5 mmol) were added to 15 mL of dry CH_3CN. Then, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 4.6 g, 30 mmol) was added at 0 °C, and the reaction mixture was stirred for 30 min at room temperature. Distilled water (15 mL) was added to the reaction mixture. The precipitated solids were filtered, washed with CH_3CN and Et_2O, and dried *in vacuo* to yield 167 mg (yield 85%) of coupling product **20**. The product was recrystallized from $CHCl_3$−EtOH to give an off-white amorphous solid: ^{1}H NMR (DMSO-d_6, ppm) δ 8.61 (1H, s), 8.05

(1H, d, J = 13.0 Hz), 4.55 (2H, s), 3.85 (3H, s), 3.84 (1H, s), 3.70(1H, m), 3.05 (1H, m), 2.85−2.75 (2H, m), 1.20−1.10 (4H, m); MS (FAB, *m/e*) 390 (M + H). Anal. ($C_{18}H_{20}F_1N_5O_4 \cdot H_2O$) C, H, N.

***In Vitro* Activity.** Compounds **13**−**29** were evaluated for their *in vitro* antibacterial activity against representative Gram-positive and Gram-negative organisms using standard agar dilution method. Mueller−Hinton medium (Difco) was used for testing aerobic and facultative organisms. For *Streptococcus pneumoniae* and *Streptococcus pyogenes*, Mueller−Hinton broth (MHB) was supplemented with 5% lysed horse serum. For *Haemophillus influenzae*, MHB was supplemented with 5% Fildes enrichment (Difco). Test strains were grown for 18 h in MHB. These cultures were diluted with the same fresh medium to a density of approximately 10^7 CFU/mL and applied to Mueller−Hinton agar (MHA) plates which have serially diluted antimicrobial agent by use of an automatic MIC-2000 multipin inoculator (Dynatech Laboratories) to yield 10^4 CFU per spot for most organisms. MICs were determined after 18 h of incubation at 35 °C. The concentrations of the bacterial suspensions were determined by measuring the optical density or the turbidity and were verified by determining standard colony counts on antibiotic-free agar plates. The MIC was considered to be the lowest concentration that completely inhibited growth on agar plates, disregarding a single colony or a faint haze caused by the inoculum. Their minimum inhibitory concentrations (MICs) are presented in Tables 2 and 3. Also included is the activity of reference compound, ciprofloxacin.

***In Vivo* Activity.** The *in vivo* activities of compound **20**, desoximino compound **30**, and ciprofloxacin were determined against systemic infection model in mice. Test organisms for infection were cultured on Tryptic Soy Agar Medium (Difco) at 35 °C for 18 h and were suspended in gastric mucin (Difco). Male ICR mice (weight 19−21 g) in four groups of six mice each were infected intraperitoneally with 0.5 mL of a bacterial suspension corresponding to an inoculum range of 5−10 times the MLD (minimal lethal dose) of bacteria. Four dose levels were used for each antibiotic, depending on the *in vitro* antimicrobial activities of the compounds. Mice were orally administered twice at 1 and 4 h postinfection with various dose regimens of antibiotics. Mortality was recorded for 7 days, and the median effective dose needed to protect 50% of mice (ED_{50}) was calculated by the method of Bliss (Table 5). All untreated mice died within 2 days after infection.

Pharmacokinetic Studies. General Procedure. A 20 mg/kg dose of compound **20**, desoximino compound **30,** or ciprofloxacin was either intravenously injected into the femoral vein of SD rats (n = 5) or orally administered using an intubation tube. Plasma samples (100 μL) were collected up to 4 h for intravenous injection, and for up to 8 h for oral administration. The plasma concentrations of compounds were determined using agar−well diffusion method. *S. aureus* giorgio was used as assay organism. The plates were incubated at 35 °C for 18 h. For dogs, 4 mg/kg of compounds were given.

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Supporting Information Available: Tables of experimental details of the X-ray crystallographic studies, positional parameters, general displacement parameter expressions, bond distances, and bond angles (12 page). Ordering information is given on any current masthead page.

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(24) We have done some NOE experiments with **11b** and final compound **20**, but we did not observe any positive correlation between the crucial protons.

(25) For a structurally similar example, see; Tsukamoto, S.; Fujii, M.; Yasunaga, T.; Matsuda, K.; Wanibuchi, F.; Hidaka, K.; Furuya, T.; Tamura, T. Synthesis and Structure-Activity Studies of a Series of 1-Oxa-8-azaspiro[4,5]decanes as M1 Muscarinic Agonists. *Chem. Pharm. Bull.* **1995**, *43*, 842.

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(30) Compound **20** (LB20304**)** has successfully completed phase I clinical trial as a racemic mixture in Great Britain in 1996 and is currently awaiting phase II clinical trial. The asymmetric synthesis of each enantiomer for further evaluation is actively in progress.

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