A Novel Antibacterial 8-Chloroquinolone with a Distorted Orientation of the N1-(5-Amino-2,4-difluorophenyl) Group

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Fluoroquinolones represent a major class of antibacterial agents with great therapeutic potential. In this study, we designed *m*-aminophenyl groups as novel N-1 substituents of naphthyridones and quinolones. Among newly synthesized compounds, 7-(3-aminoazetidin-1-yl)-1-(5-amino-2,4-difluorophenyl)-8-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4**) has extremely potent antibacterial activities against Gram (+) as well as Gram (−) bacteria. This compound is significantly more potent than trovafloxacin against clinical isolates: 30 times against *Streptococcus pneumoniae* and 128 times against methicillin resistant *Staphylococcus aureus*. The structure−activity relationship (SAR) study revealed that a limited combination of 1-(5-amino-2,4-difluorophenyl) group, 7-(azetidin-1-yl) group, and 8-Cl atom (or Br atom or Me group) gave potent antibacterial activity. An X-ray crystallographic study of a 7-(3-ethylaminoazetidin-1-yl)-8-chloro derivative demonstrated that the N-1 aromatic group was remarkably distorted out of the core quinolone plane by steric repulsion between the C-8 Cl atom and the N-1 substituent. Furthermore, a molecular modeling study of **4** and its analogues demonstrated that a highly distorted orientation was induced by a steric hindrance of the C-8 substituent, such as Cl, Br, or a methyl group. Thus, their highly strained conformation should be a key factor for the potent antibacterial activity.

Introduction

Norfloxacin, the first "new quinolone", opened a new era in the treatment of infectious diseases in 1980.[1] A number of new compounds with successful chemical modifications, such as ciprofloxacin (**1**),[2] tosufloxacin (**2**),[3] and ofloxacin,[4] had been brought into clinical use. These compounds offered novel N-1 substituents, such as cyclopropyl, 2,4-difluorophenyl, and 1,8-bridged groups. For further improvement, the cyclopropyl group was widely used as an N-1 substituent because 1-cyclopropyl compounds generally gave superior antibacterial activity.[5,6] Various structural modifications have been conducted, and a number of structure−activity relationships (SARs) have been accumulated as follows: a combination of the C-7 pyrrolidinyl group and the C-8 chlorine atom remarkably enhances the activity,[5,7] a methoxy group or a trifluoromethyl group at the C-8 position reduces the phototoxicity,[8,9] and a replacement of the 1-cyclopropylquinolone core structure with a novel scaffold, 4*H*-4-oxoquinolidine, gives excellent activity.[10] These SARs provided a great deal of useful information to obtain improved compounds with enhanced activity and good safety profiles. Several novel 1-cyclopropylquinolones have been developed to overcome the critical problems related to resistant strains,[11] phototoxicity,[12] and cardiac dysrhythmia.[13] However, these problems have not been satisfactorily resolved. Recently, a fluorine atom was introduced to the N1-cyclopropyl group to enhance the activity.[14]

On the other hand, the structural modifications of N-1 aryl compounds have not been fully studied. Chu et al. reported the SAR of N-1 aryl quinolones carrying the fluorophenyl, hydroxyphenyl, or methoxyphenyl groups.[15] This study indicated that a 2,4-difluorophenyl group was the most preferable N-1 aryl substituent; therefore, many 1-(2,4-difluorophenyl)naphthyridones and quinolones were synthesized. By contrast, little is known about N-1 phenyl groups with other substituents, such as an amino group. Thus, we focused our effort on the structural modification of the N-1 phenyl group and synthesized novel N-1 phenyl groups that have an amino group and fluorine atoms. We found that the 1-(5-amino-2,4-difluorophenyl) analogue **3** gave slightly superior activity to **2**. Although compound **3** had a closely related structure to **2**, there was an unexpected discrepancy in phototoxicity between the two compounds. Compound **2**, a typical N-1 aryl naphthylidone, demonstrated moderate phototoxicity in a mouse model at an intravenous dose of 40 mg/kg. On the contrary, compound **3** showed no phototoxicity in the same mouse model.[16] This fact enabled us to continue to synthesize the 1-(5-amino-2,4-difluorophenyl) derivatives.

In this study, we synthesized a novel antibacterial quinolone, 7-(3-aminoazetidin-1-yl)-1-(5-amino-2,4-difluorophenyl)-8-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4**), and its derivatives, which contain a 1,8-naphthyridone or quinolone core structure. A SAR study on the novel compounds was carried out to determine the effects of (i) the substitution pattern

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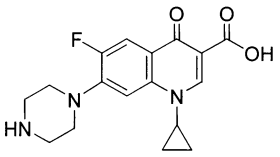
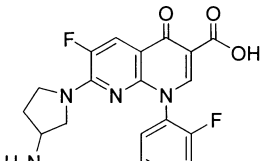
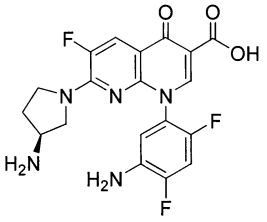


1 *Ciprofloxacin*



2 *Tosufloxacin*



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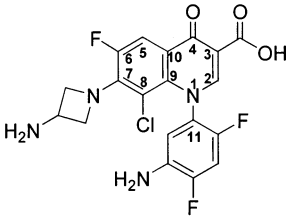


4

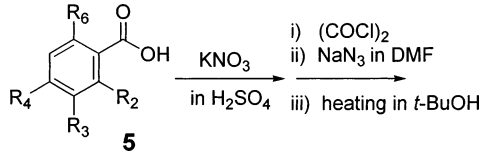
on the N-1 phenyl ring and (ii) the combination of C-7 and C-8 substituents. Furthermore, we were able to describe a novel correlation between the activity and the distorted orientation of the N-1 aromatic group based on X-ray crystallographic and molecular modeling studies.

Chemistry

Novel 1,8-naphthyridone and quinolone derivatives were synthesized according to Schemes 1−5. Scheme 1 shows the preparation of N-1 substituents, *tert*-butyl aminophenylcarbamates **7a**−**e**. The intermediates, *m*-nitrobenzoic acids, were prepared by nitration of the corresponding benzoic acids **5a**−**e** with KNO_3 in concentrated H_2SO_4 and then converted to the nitrophenylcarbamate derivatives **6a**−**e** by Curtius rearrangement. Catalytic hydrogenation of the nitro group gave **7a**−**e**. The reaction of **8** or **11a**−**e** with triethyl orthoformate, following substitution with **7a**−**e** and cyclization, gave the corresponding naphthyridone-3-carboxylates **9a**−**e** or quinolone-3-carboxylates **12a**−**e** (see Schemes 2 and 3). Deprotection was achieved in a mixture of an aqueous solution of HCl and acetic acid to obtain **10a**−**e** and **13a**−**e**.

Aromatic nucleophilic substitution of the 7-halogeno group (on **10** and **13**) with selected cyclic amines (i.e.,

Scheme 1



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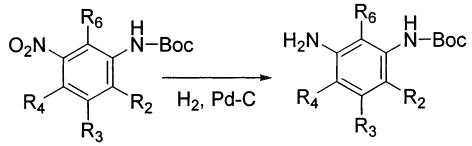
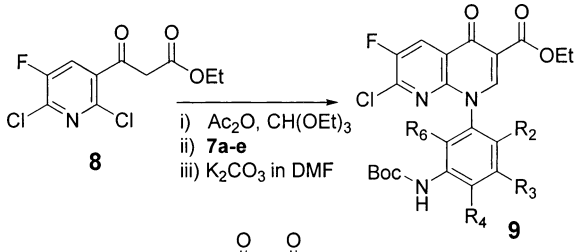


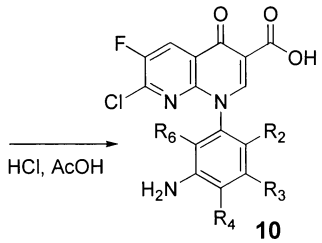
6 **7**

a: $R_2 = R_4 = F$, $R_3 = R_6 = H$
b: $R_2 = F$, $R_3 = R_4 = R_6 = H$
c: $R_4 = F$, $R_2 = R_3 = R_6 = H$
d: $R_2 = R_3 = R_4 = F$, $R_6 = H$
e: $R_2 = R_6 = F$, $R_3 = R_4 = H$

Scheme 2



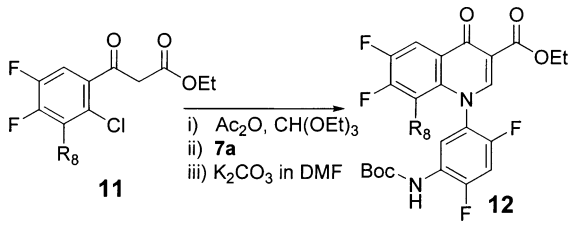
8 **9**



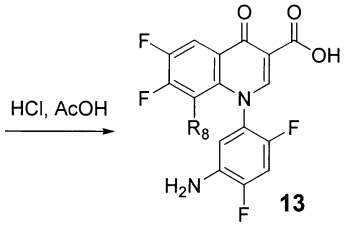
10

a: $R_2 = R_4 = F$, $R_3 = R_6 = H$
b: $R_2 = F$, $R_3 = R_4 = R_6 = H$
c: $R_4 = F$, $R_2 = R_3 = R_6 = H$
d: $R_2 = R_3 = R_4 = F$, $R_6 = H$
e: $R_4 = R_6 = F$, $R_2 = R_3 = H$

Scheme 3



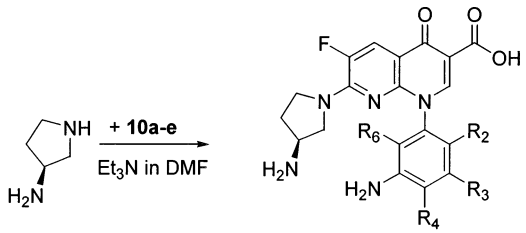
11 **12**



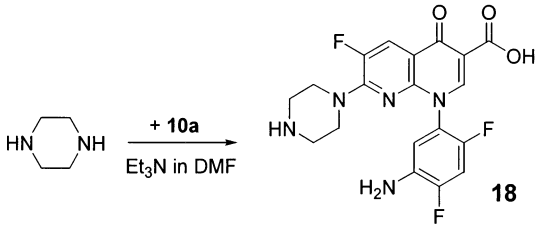
13

a: $R_8 = H$
b: $R_8 = F$
c: $R_8 = Cl$
d: $R_8 = Br$
e: $R_8 = CH_3$

Scheme 4



3: $R_2 = R_4 = F$, $R_3 = R_6 = H$
14: $R_2 = F$, $R_3 = R_4 = R_6 = H$
15: $R_4 = F$, $R_2 = R_3 = R_6 = H$
16: $R_2 = R_3 = R_4 = F$, $R_6 = H$
17: $R_4 = R_6 = F$, $R_2 = R_3 = H$



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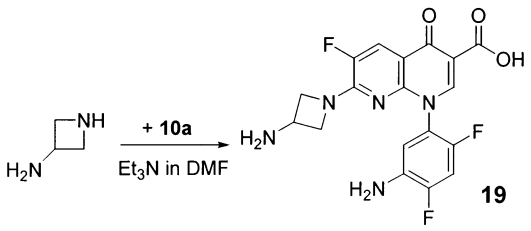

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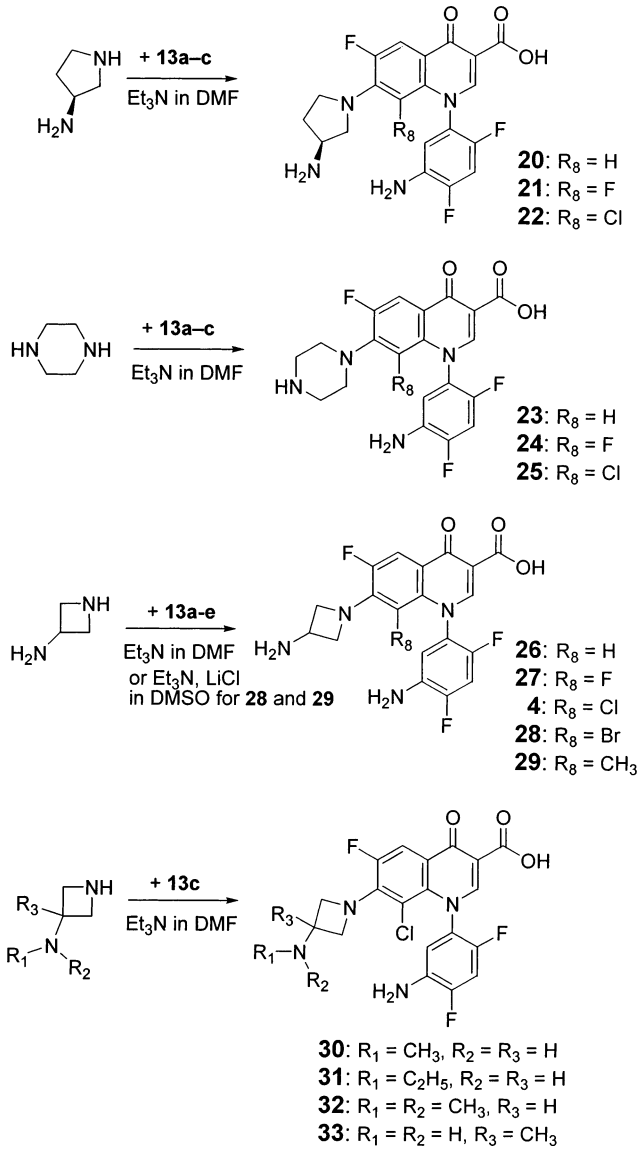
Scheme 5



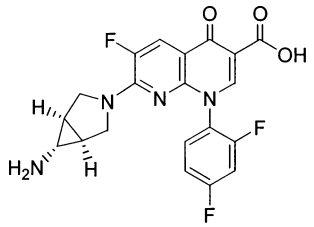
pyrrolidine, piperazine, and azetidine) gave compounds **3**, **4**, and **14**−**33** (see Scheme 4 for 1,8-naphthyridone derivatives and Scheme 5 for quinolone derivatives). The requisite 3-aminoazetidine and derivatives were synthesized according to a method similar to established procedures.[17,18] The substitution reaction of **10a**−**e** and **13a**−**c** was completed within a few hours in the presence of Et_3N at 80 °C in dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), or MeCN. On the other hand, a longer reaction time (ca. 1 day) and a higher temperature (>100 °C) were required to complete the reaction of the less reactive 8-Br (**13d**) and 8-Me (**13e**) derivatives. Such conditions resulted in low yields (<20%) because of the competitive decarboxylation at the 3-position. Previously, an analogous nucleophilic substitution of an 8-Br quinolone derivative without decarboxylation by derivatizing to its boron difluoride intermediate was accomplished.[19] Applying this method to the reaction of **13d**,**e** with 3-aminoazetidine did not improve the yield. After the examination of many additives (organic bases and inorganic salts) and reaction conditions (temperature and solvent), Li^+ ion was identified as a novel accelerator for the C-7 substitution reaction. In the presence of 4.5 equiv of LiCl, a moderate reaction condition at 60 °C for 2 h in DMSO gave ca. 80% yield (see Experimental Section). The catalytic effect of the Li^+ ion is possibly due to its chelation with 3-carboxylic acid and 4-carbonyl groups.

Table 1. In Vitro Antibacterial Activities

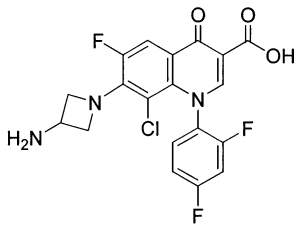
compd no.	GM-MICs (μg/mL) Gram (+)	Gram (−)
7-(3-aminopyrrolidinyl)		
14 (naphthyridone)	1.99	0.86
15 (naphthyridone)	0.45	0.14
16 (naphthyridone)	3.22	1.81
17 (naphthyridone)	1.61	0.32
3 (naphthyridone)	0.26	0.10
20 (8-H)	0.22	0.14
21 (8-F)	0.34	0.22
22 (8-Cl)	1.16	1.21
7-(piperazin-1-yl)		
18 (naphthyridone)	5.81	1.72
23 (8-H)	1.05	0.15
24 (8-F)	2.60	0.39
25 (8-Cl)	1.61	0.52
7-(3-aminoazetidinyl)		
19 (naphthyridone)	0.65	0.15
26 (8-H)	0.49	0.12
27 (8-F)	0.42	0.08
4 (8-Cl)	0.07	0.03
28 (8-Br)	0.07	0.04
29 (8-CH_3)	0.09	0.03
30 (8-Cl)	0.08	0.04
31 (8-Cl)	0.12	0.08
32 (8-Cl)	0.12	0.22
33 (8-Cl)	0.10	0.06
reference compds		
1	0.99	0.04
2	0.44	0.12
34	0.29	0.30
35 (8-Cl)	0.13	0.06

Results and Discussion

SAR. Compounds **3**, **4**, and **14**−**33** were evaluated for their in vitro antibacterial activities against a variety of Gram (+) bacteria (13 strains) and Gram (−) bacteria (14 strains). The geometric means of minimum inhibitory concentrations (GM-MICs) for the Gram (+) and Gram (−) bacteria were calculated to facilitate a comparison of their activities (Table 1). The activities of four reference compounds, **1** (a standard compound for Gram (−) bacteria), **2**, trovafloxacin (**34**)[20] (a standard compound for Gram (+) bacteria), and **35**[17] (the desamino analogue of **4**), against those bacteria were also examined. The MICs of **1**, **4**, **34**, and **35** against four clinical isolates (*Streptococcus pneumoniae*, methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and *Escherichia coli*) are summarized in Table 2.



34 *Trovafloxacin*



35

To elucidate the effect of the substitution pattern of the N-1 phenyl ring on the activity, the activities of

Table 2. In Vitro Antibacterial Activities against Clinical Isolates[a]

compd no.	MICs (μg/mL)			
	sp	MRSA	pa	ec
4	0.008	1	1	1
1	1	128	8	128
34	0.25	128	16	>128
35	0.03	8	16	32

[a] sp, *S. pneumoniae* W1466; MRSA, MRSA W9501; pa, *P. aeruginosa* W348; ec, *E. coli* W9523.

compounds **3** and **14**−**17**, which varied in the N-1 substituent for a same 7-[(3*S*)-3-aminopyrrolidin-1-yl]-1,8-naphthyridone skeleton, were evaluated. The result clearly shows that the antibacterial activity of these analogues is very sensitive to the precise substitution pattern on the N-1 phenyl ring. Among the compounds, the 5-amino-2,4-difluorophenyl analogue **3** has the strongest activity. Removal of a fluorine atom from the N-1 phenyl group of **3** reduced the activity against Gram (+) and Gram (−) bacteria. The activities of **14** (5-amino-2-fluorophenyl) and **15** (3-amino-4-fluorophenyl) were reduced to one-eighth and ca. one-half, respectively, as compared with that of **3**. Regarding the position of the *m*-amino group, the 5-amino-2,4-difluorophenyl derivative **3** is more active than its regio isomer **17** (3-amino-2,4-difluorophenyl analogue): six times against Gram (+) bacteria and three times against Gram (−) bacteria. The addition of a *m*-fluorine atom to **3** significantly reduced the activity to 1/12 against Gram (+) bacteria and 1/18 against Gram (−) bacteria (see the 5-amino-2,3,4-trifluorophenyl analogue **16**). Because the activity of 5-amino-2,4-difluorophenyl derivative **3** was markedly higher than those of the other N-1 derivatives, we further investigated the effects of the substituents at C-7 and C-8 positions of 1-(5-amino-2,4-difluorophenyl) derivatives in the next step.

Several quinolones (H, F, or Cl atom at the C-8 position) and naphthyridones carrying a 7-(3-aminopyrrolidin-1-yl), 7-(piperazin-1-yl), or 7-(3-aminoazetidin-1-yl) group were synthesized (**3**, **4**, and **18**−**33**). The activities of these 1-(5-amino-2,4-difluorophenyl) derivatives strongly depend on a combination of C-7 and C-8 substituents (see Table 1). All piperazine analogues (**18** and **23**−**25**) were less active than the aminopyrrolidinyl derivative **3**. Among them, 8-H derivative **23** was the most active against Gram (+) and Gram (−) bacteria. The naphthyridone analogue **18** was significantly less active than the quinolone derivatives **23**−**25**, especially against Gram (−) bacteria.

Among the 7-(3-aminopyrrolidin-1-yl) derivatives (**3** and **20**−**22**), the naphthyridone analogue **3** and the 8-H quinolone derivative **20** showed comparable activity against Gram (+) and Gram (−) bacteria. The activity of the 8-F quinolone derivative **21** was reduced to ca. one-half for that of **3** against Gram (+) and Gram (−) bacteria. Notably, there was a remarkable difference between the activities of 8-F and 8-Cl derivatives. The introduction of a chlorine atom to the C-8 position (**22**) significantly reduced the activity as compared with **3**: ca. one-fifth against Gram (+) bacteria and 1/12 against Gram (−) bacteria.

By contrast, in the series of the 7-(3-aminoazetidin-1-yl) derivatives, the C-8 chlorine atom gave greatly enhanced antibacterial activity against Gram (+) and Gram (−) bacteria (see compound **4**). The chlorine derivative **4** was more active than the 8-F derivative **27**: ca. six times against Gram (+) and three times against Gram (−) bacteria. The naphthyridone analogue **19** and the 8-H analogue **26** were slightly less active than the fluorine derivative **27**. In the 7-(3-aminoazetidin-1-yl) analogues, the order of activity imparted by the C-8 substituent was Cl ≫ F ≥ H ≥ naphthyridone. This order of activity is opposite to that of 7-(3-aminopyrrolidin-1-yl) analogues (naphthyridone ≥ H ≥ F ≫ Cl) as shown above. It is worth noting that the C-8 chlorine atom makes a dramatic difference in the antibacterial activity of the pyrrolidine and azetidine series. One possible cause for such a discrepancy in the activity is the bulkiness of the chlorine atom. To test this assumption, two 7-(3-aminoazetidin-1-yl) derivatives with a large C-8 substituent, the 8-bromo analogue **28** and the 8-methyl analogue **29**, were synthesized. Both compounds were almost as active as **4** and exhibited remarkably potent activity superior to that of the 8-F analogue **27**. These results demonstrate that a larger C-8 substituent is required for the potent activity in 7-(3-aminoazetidin-1-yl) derivatives.

Significantly enhanced activity was obtained only when an azetidinyl group was introduced to the C-7 position of 8-chloroquinolone containing the 1-(5-amino-2,4-difluorophenyl) group. The azetidine analogue **4** was at least 15 times more potent than the piperazine analogue **25** and the pyrrolidine analogue **22**. Such a dramatic enhancement of the activity, however, was not observed in the other C-8 series of compounds (8-H, -F quinolone, or naphthyridone). A limited combination, the 7-azetidinyl group and the 8-Cl (or Br) atom, enhances the activity synergistically. Thus, the best combination of the 7-(3-aminoazetidin-1-yl) group and the 8-Cl atom (see compound **4**) gave extremely low GM-MICs of 0.07 μg/mL against Gram (+) bacteria and 0.03 μg/mL against Gram (−) bacteria.

Frigola et al. already reported the desamino compound **35**[17] with the same C-7 and C-8 substituents as compound **4**. Compound **35** gave almost half the activity of compound **4**. However, against clinical isolates, compound **4** was remarkably more potent than **35** (Table 2): four times more active against *S. pneumoniae* and 8−32 times more active against ciprofloxacin resistant strains (MRSA, *P. aeruginosa*, and *E. coli*). The *m*-amino group on the N-1 phenyl ring enhanced the activity, especially against quinolone resistant strains. The mechanism for enhancing the activity is unclear; however, there might be two possible reasons for it: (i) the electrostatic effect of the amino group and (ii) the hydrogen bond formation at the binding site.

For further improvement of the activity, various alkyl groups were introduced to the 3-aminoazetidine moiety of **4** (compounds **30**−**33**). Unfortunately, this attempt resulted in reduced activity against Gram (−) bacteria. The methylamino derivative **30** was as active as **4**, and the 3-amino-3-methyl derivative **33** exhibited slightly reduced activity. The introduction of the ethyl group to the 3-amino group (**31**) reduced the activity to ca. one-half of that of **4** against Gram (+) and Gram (−) bacteria. The activity of the dimethylamino analogue **32** against Gram (−) bacteria was reduced to one-seventh of that of **4**. The reason for the decreased

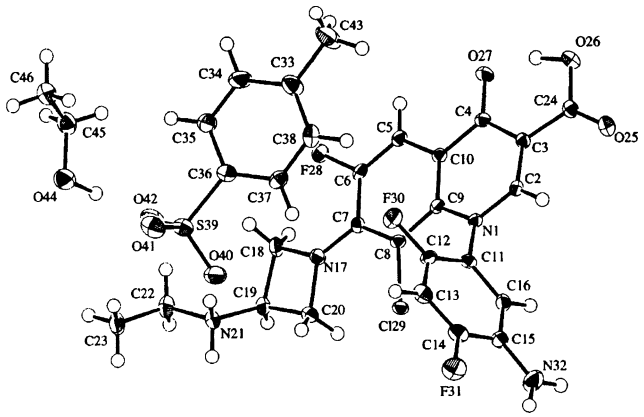


Figure 1. X-ray crystal structure of **31**·TsOH·EtOH. Atoms are drawn with 50% probability ellipsoids.

activity against Gram (−) bacteria might be an increased lipophilicity.

The activity of **4** was compared with those of **1** and **34**. The GM-MIC of **4** against Gram (+) bacteria was ca. four times superior to that of **34** and that against Gram (−) bacteria was almost equal to that of **1**. Moreover, compound **4** was significantly more potent than **1** and **34** against clinical isolates. Typical MIC values (μg/mL) of compounds **4**, **1**, and **34** against clinical isolates are listed in Table 2. Compound **4** exhibited 8−128 times superior activity to those of **1** and **34** against ciprofloxacin resistant MRSA, *P. aeruginosa*, and *E. coli*. Furthermore, compound **4** gave an excellent activity against *S. pneumoniae*, i.e., 30 times more potent than **34**. Compound **4** had the extremely potent activity against Gram (+) bacteria as well as Gram (−) bacteria, including quinolone resistant clinical isolates.

Phototoxicity is one of the most noticeable side effects induced by "new quinolones". Especially, quinolone compounds containing a fluorine or chlorine atom at the C-8 position have been reported to have a critical problem in phototoxicity.[5,21] The 1-(5-amino-2,4-difluorophenyl) derivatives with a halogen atom (F, Cl, or Br) at C-8, however, showed none or very low phototoxicity, which is similar to that of ofloxacin in a mouse model (data not shown).[16]

Followingly, to determine a more detailed role of a C-8 substituent and an *m*-aminophenyl group of **4** and its derivatives in the biological activities, an X-ray crystallographic study, molecular modeling, and measurements of pK_a values and dissociation constants with Mg^{2+} ions were carried out.

X-ray Crystal Structure of 31·TsOH·EtOH. Among all of the attempts to isolate an appropriate crystal of the quinolones for X-ray crystal analysis, a *p*-TsOH salt of 3-ethylaminoazetidinyl derivative **31** was crystallized as colorless prisms from a DMSO/EtOH solution. The ^{1}H NMR data were compatible with a structural formula of **31**·TsOH·EtOH. The X-ray crystal structure is shown in Figure 1. The carbonyl oxygen, carboxylic acid, and C-6 F atom are almost in a quinolone core ring plane. On the other hand, the N-1 aromatic group is remarkably distorted out of the plane. The strain of the N_1-C_{11} bond from the plane is evaluated as a torsion angle of 155.2(2)° for $C_{10}-C_9-N_1-C_{11}$ (cf. 180° for strain free). Furthermore, the angle of 114.6(2)° for $C_2-N_1-C_{11}$ is even smaller than that of 124.0(2)° for $C_9-N_1-C_{11}$, indicating a shift of the N-1 aromatic group toward the C-2 hydrogen atom. This strained conformation is possibly due to the steric repulsion of the N-1 aromatic substituent and the C-8 Cl atom. As was reported for X-ray crystal structures of nalidixic acid and its derivatives,[22,23] a $\pi-\pi$ ring stacking interaction in a pair of **31** molecules was observed. The intermolecular distance of two quinolone planes is ca. 3.6 Å, where each N-1 aromatic group is directed toward the opposite sides. The protonated ethylamino nitrogen $H^+\cdot N_{21}$ makes an ion pair with a tosylate O_{40} anion with a distance of 2.863(2) Å. The *m*-amino nitrogen atom on the N-1 aromatic group is positioned to interact by hydrogen bonds with the EtOH oxygen atom ($N_{32}H\cdots O$, N−O distance of 3.032(3) Å) and the carbonyl oxygen atom ($N_{32}H\cdots O_{27}$, N−O distance of 3.013(2) Å). The observation of these hydrogen bonds suggests that the *m*-amino group might be an effective electron acceptor in the quinolone binding site.

Molecular Modeling Study. To rationalize the correlation between the antibacterial activity and the strained conformation, we attempted to obtain the X-ray crystal structures of other C-8 derivatives. However, appropriate crystals for X-ray analysis have not been obtained because this series of compounds had a marked tendency to give very small crystals. Therefore, we calculated the minimum energy conformations for C-8 derivatives **4** and **26**−**29**. The calculations of these compounds were performed as their zwitterionic species, since the deprotonation constants (pK_a) of 3-carboxylic acid and the 3-amino group in the azetidine moiety for **4** were 5.4 and >9, respectively (see Experimental Section). Typical calculated structures are shown as side and top views in Figure 2. The obtained conformation for **4** (see Figure 2a) is evidently distorted, being almost the same as the X-ray crystal structure of its ethyl derivative **31**. The N-1 aromatic group is located above the quinolone core ring plane. Here again, we evaluate the structural strain of the N-1 aromatic group as a torsion angle for $C_{10}-C_9-N_1-C_{11}$ and the angles around the N_1 atom. The torsion angle of 159.7° for $C_{10}-C_9-N_1-C_{11}$, the angle of 115.4° for $C_2-N_1-C_{11}$, and the angle of 123.8° for $C_9-N_1-C_{11}$ in **4** are similar to the crystal data of **31** (155.2, 114.6, and 124.0°, respectively). Larger substituents, C-8 Br atom of **28** and the methyl group of **29**, induced slightly more strained conformations, as shown by the smaller torsion angles of 155.3 and 149.5°, respectively. By contrast, the smaller substituents, C-8 F and H groups, gave less strain to the N-1 aromatic group (see Figure 2b for **27** and c for **26**): torsion angles of −178.6 and −178.0° for $C_{10}-C_9-N_1-C_{11}$ and angles of 114.9 and 118.2° for $C_2-N_1-C_{11}$. These torsion angles demonstrate that the N_1-C_{11} bonds of **27** and **26** are not distorted from the quinolone core ring plane.

The three-dimensional orientation of the N-1 aromatic group of **4** was also confirmed by an upfield shift of ^{1}H NMR for the C-2 hydrogen atom, which would be caused by the shielding effect of the N-1 phenyl group: δ 8.42 for **4** and δ 8.64 for **26** in a DMSO solution. These results obviously demonstrate that steric repulsion of the C-8 substituent causes the distoted orientation of the N-1 phenyl group. The highly distorted compounds

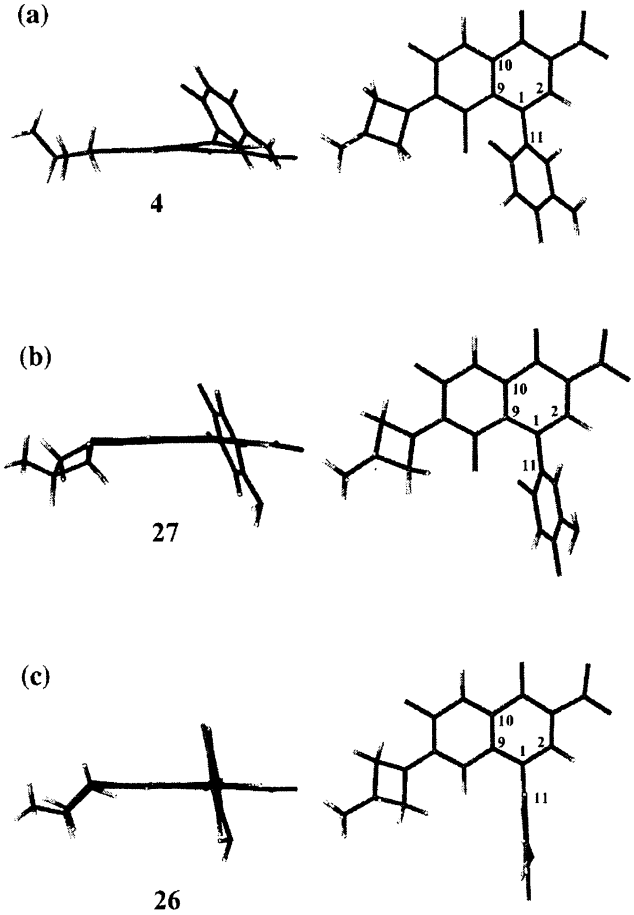


Figure 2. Minimum energy structures of twitterionic species: side and top views for (a) **4**, (b) **27**, and (c) **26**.

4, **28**, and **29** have significantly stronger antibacterial activity than the less-strained analogues **26** and **27**, as mentioned above. Therefore, the distorted orientation of the N-1 pheny group is a key factor for the stronger activity. The other combination with 7-(3-aminopyrrolidin-1-yl) group and the same N-1 aromatic group, however, shows an inverse relationship between the antibacterial activity and the degree of distorted orientation (see the activity-order of **20** (C-8 H) > **21** (F) ≫ **22** (Cl) in Table 1).

Recently, an X-ray crystal structure of the 1:1 Mg^{2+} complex with norfloxacin was reported, in which the oxygen atoms of quinolone carbonyl and carboxylate anion coordinate to a Mg^{2+} ion bidentately.[24] The antibacterial activity of quinolone is proposed to be achieved via binding to the gyrase−DNA complex in the presence of Mg^{2+} ion.[25] Because these reports suggest that the Mg^{2+}−quinolone complex is important for biological activity, we determined the pK_a values and dissociation constants with Mg^{2+} ions for **4** and **27**. Compounds **4** and **27** have almost the same pK_a values of 5.4 ± 0.1 and Mg^{2+} dissociation constants of 2.0 ± 0.2 mM (see Experimental Section). Although the steric repulsion of the C-8 Cl atom and the N-1 phenyl group induces the distorted conformation, it does not affect the physicochemical properties of quinolone carboxylic acid. Therefore, we conclude that the shape of 1-(5-amino-2,4-difluorophenyl)-8-chloroquinolone should be very important for an interaction at the target site.

Conclusions

We have synthesized a series of antibacterial naphthyridones and quinolones appended with a novel 5-amino-2,4-difluorophenyl group at the N-1 position. The antibacterial activities of these compounds strongly depend on the combination of C-7 and C-8 substituents. The SAR study showed that a limited combination, 3-aminoazetidin-1-yl group at the C-7 and Cl (or Br) atom at the C-8 on the quinolone ring (i.e., **4** or **28**), gave potent activity. Compound **4** had extremely stronger activity than clinically used compounds, including **1**, **2**, and **34**. The amino group on the N-1 phenyl ring enhanced the activity, especially against quinolone resistant strains. An X-ray crystallographic analysis and a molecular modeling study revealed that the distorted orientation of the N-1 phenyl group was a key factor for the potent activity. These findings should be useful for the design of newer quinolones. We conclude that compound **4** may be promising as a new class of quinolone antibacterial compounds. Further modification of the N-1 aromatic group in **4** is now under consideration.

Experimental Section

General Methods. All chemicals used were of reagent grade. Column chromatography was performed using silica gel 60 (60−200 mesh). Reactions were monitored by thin-layer chromatography on silica gel (Merck 60F_{254}) plates using UV light (254 nm) for detection. Melting points were determined with a Yanagimoto melting point apparatus and were not corrected. Infrared (IR) spectra were determined in KBr on a JASCO FT/IR-5300. NMR spectra were recorded with a JEOL JNM-ECP 500 at 500 MHz (^{1}H NMR) and at 125 MHz (proton decoupling ^{13}C NMR). Chemical shifts are expressed in ppm (δ) relative to internal tetramethylsilane, and coupling constants (J values) are in hertz. Mass spectra were obtained with a JEOL JMS-GC mate in fast-atom bombardment (FAB) mode (with 1:1 glycerol/DMSO matrix). Elemental analytical data (C, H, N) within 0.4% were obtained on a Perkin-Elmer 2400 CHN analyzer. UV spectra were recorded on a Hitachi U-3500 spectrophotometer equipped with a thermoelectric cell temperature controller at 35.0 ± 0.1 °C. Before pH measurement at 35.0 ± 0.1 °C with I = 0.10 (NaCl), the pH electrode system (DKK Corporation Multi Channel Ion Meter IOL-40 with a Ross Combination pH Electrode 8102BN) was calibrated with standard pH solutions (pH 4.02 and 6.84) under the nitrogen atmosphere (>99.999% purity) at 35.0 ± 0.1 °C.

***tert*-Butyl 2,4-Difluoro-5-nitrophenylcarbamate (6a).** To a solution of 2,4-difluorobenzoic acid (**5a**) (158.0 g, 1.0 mol) in concentrated sulfuric acid (500 mL) was added portionwise potassium nitrate (111.0 g, 1.1 mol) with ice-cooling and stirring. The reaction mixture was stirred at room temperature for 24 h. The solution was poured into 2 L of ice water and allowed to stand overnight. The precipitated solid was collected by filtration, washed with water, and dried over P_2O_5 to give 2,4-difluoro-5-nitrobenzoic acid (141.0 g, 70%). ^{1}H NMR (DMSO-d_6): δ 8.60 (1H, dd, J = 8.7, 7.3 Hz), 7.86 (1H, t, J = 11.0 Hz). To a solution of 2,4-difluoro-5-nitrobenzoic acid (122.0 g, 0.60 mol) in CH_2Cl_2 (500 mL) and DMF (1.0 mL) at room temperature was added dropwise oxalyl chloride (105 mL, 1.2 mol). The reaction mixture was stirred at room temperature for 18 h, and the solvent was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (120 mL) and DMF (150 mL). To the solution was added portionwise sodium azide (47.8 g, 0.66 mol) with ice cooling. After the reaction mixture was stirred at room temperature for 20 min, 2-methyl-2-propanol (300 mL) was added. After the reaction mixture was heated to reflux for 2 h, the solvent was evaporated. The residue was extracted with ethyl acetate (600 mL), and the obtained extract was washed with aqueous 2% (w/v) sodium bicarbonate. After

the organic layer was dried over $MgSO_4$, the solvent was evaporated. Purification by silica gel column chromatography (CH_2Cl_2) gave **6a** as a yellowish powder (149 g, 93% yield); mp 82−83 °C. ^{1}H NMR ($CDCl_3$): δ 8.95 (1H, s), 7.04 (1H, t, J = 10.1 Hz), 6.69 (1H, s), 1.53 (9H, s). ^{13}C NMR ($CDCl_3$): δ 154.0 (dd, J = 257, 11 Hz), 151.6 (s), 150.7 (dd, J = 264, 13 Hz), 133.7 (s), 124.2 (dd, J = 12, 4 Hz), 117.0 (s), 105.8 (dt, J = 25, 4 Hz), 82.4 (s), 28.1 (s). IR: 1705, 1547, 1344, 1151, 891, 858 cm^{-1}. MS m/z: 275 (M + H)$^+$.

***tert*-Butyl 2-Fluoro-5-nitrophenylcarbamate (6b).** 2-Fluoro-5-nitrobenzoic acid (yellowish solid, 85% yield). ^{1}H NMR (DMSO-d_6): δ 8.61 (1H, dd, J = 6.4, 3.2 Hz), 8.49 (1H, dt, J = 9.2, 3.2 Hz), 7.63 (1H, t, J = 9.2 Hz). The nitrophenylcarbamate derivative **6b** (yellow solid 75% yield); mp 98−99 °C. ^{1}H NMR ($CDCl_3$): δ 9.09 (1H, s), 7.95−7.88 (1H, m), 7.20 (1H, dd, J = 9.6, 6,9 Hz), 6.83 (1H, s), 1.56 (9H, s). ^{13}C NMR (DMSO-d_6): δ 152.6 (s), 149.6 (d, J = 257 Hz), 136.4 (s), 136.3 (d, J = 11 Hz), 125.2 (d, J = 8 Hz), 118.7 (d, J = 22 Hz), 113.9 (s), 80.0 (s), 27.9 (s). IR: 1707, 1535, 1346, 1157, 877, 828 cm^{-1}. MS m/z: 257 (M + H)$^+$.

***tert*-Butyl 4-Fluoro-3-nitrophenylcarbamate (6c).** 4-Fluoro-3-nitrobenzoic acid (yellowish solid, 88% yield). ^{1}H NMR (DMSO-d_6): δ 8.57 (1H, dd, J = 7.3, 1.9 Hz), 8.31 (1H, ddd, J = 8.7, 4.2, 1.9 Hz), 7.72 (1H, dd, J = 11.4, 8.7 Hz). The nitrophenylcarbamate derivative **6c** (yellow solid, 70% yield); mp 117−118 °C. ^{1}H NMR ($CDCl_3$): δ 8.14 (1H, dd, J = 6.4, 2.7 Hz), 7.60 (1H, brs), 7.21 (1H, t, J = 9.7 Hz), 6.64 (1H, brs), 1.53 (9H, s). ^{13}C NMR (DMSO-d_6): δ 156.4 (d, J = 247 Hz), 152.6 (s), 143.7 (s), 127.9 (d, J = 14 Hz), 119.4 (d, J = 10 Hz), 117.4 (s), 116.5 (d, J = 23 Hz), 80.4 (s), 27.9 (s). IR: 1709, 1547, 1348, 1159, 873 cm^{-1}. MS m/z: 257 (M + H)$^+$.

***tert*-Butyl 5-Nitro-2,3,4-trifluorophenylcarbamate (6d).** 5-Nitro-2,3,4-trifluorobenzoic acid (yellowish solid, 85% yield). ^{1}H NMR (DMSO-d_6): δ 8.43 (1H, dt, J = 6.4, 2.1 Hz). The nitrophenylcarbamate derivative **6d** (yellowish solid, 61% yield); mp 100−102 °C. ^{1}H NMR ($CDCl_3$): δ 8.78 (1H, brs), 6.74 (1H, s), 1.55 (9H, s). ^{13}C NMR (DMSO-d_6): δ 152.5 (s), 145.8 (dd, J = 260, 12 Hz), 141.4 (dd, J = 261, 12 Hz), 140.2 (dd, J = 250, 5 Hz), 132.9 (s), 124.3 (d, J = 7 Hz), 112.9 (s), 80.8 (s), 27.8 (s). IR: 1720, 1547, 1342, 1165, 879, 808 cm^{-1}. MS m/z: 293 (M + H)$^+$.

***tert*-Butyl 2,6-Difluoro-3-nitrotrifluorophenylcarbamate (6e).** 2,6-Difluoro-3-nitrobenzoic acid (yellowish solid, 87% yield). ^{1}H NMR (DMSO-d_6): δ 8.39 (1H, dt, J = 9.2, 5.5 Hz), 7.48 (1H, dt, J = 9.2, 1.4 Hz). The nitrophenylcarbamate derivative **6e** (yellowish solid, 73% yield); mp 113−114 °C. ^{1}H NMR ($CDCl_3$): δ 8.00 (1H, ddd, J = 9.7, 8.3, 5.5 Hz), 7.08 (1H, dt, J = 8.3, 2.3 Hz), 6.07 (1H, s), 1.52 (9H, s). ^{13}C NMR (DMSO-d_6): δ 160.7 (dd, J = 259, 4 Hz), 152.6 (s), 151.5 (dd, J = 261, 6 Hz), 134.2 (s), 124.3 (d, J = 11 Hz), 117.3 (dd, J = 19, 17 Hz), 112.1 (dd, J = 23, 3 Hz), 80.2 (s), 27.8 (s). IR: 1705, 1545, 1346, 1157, 866, 821 cm^{-1}. MS m/z: 275 (M + H)$^+$.

***tert*-Butyl 5-Amino-2,4-difluorophenylcarbamate (7a).** Under hydrogen atmosphere, a solution of **6a** (48.0 g, 176 mmol) and palladium black (0.5 g) in MeOH (400 mL) was stirred at room temperature for 3 days. After the catalyst was filtered, the filtrate was concentrated under reduced pressure. Purification by silica gel column chromatography (20:1 CH_2Cl_2/ethyl acetate) gave the phenylcarbamate **7a** as a colorless powder (32 g, 75% yield); mp 120−121 °C. ^{1}H NMR ($CDCl_3$): δ 7.57 (1H, brs), 6.77 (1H, t, J = 10.1 Hz), 6.52 (1H, s), 3.57 (2H, s), 1.50 (9H, s). ^{13}C NMR (DMSO-d_6): δ 153.1 (s), 145.9 (dd, J = 227, 11 Hz), 145.1 (dd, J = 235, 10 Hz), 132.3 (dd, J = 14, 2 Hz), 122.0 (dd, J = 13, 3 Hz), 111.5 (s), 103.3 (dt, J = 24, 3 Hz), 78.9 (s), 28.0 (s). IR: 3364, 1728, 1319, 1163 cm^{-1}. MS m/z: 245 (M + H)$^+$. Anal. ($C_{11}H_{14}F_2N_2O_2$) C, H, N.

***tert*-Butyl 5-Amino-2-fluorophenylcarbamate (7b).** Colorless needles from hexane in 85% yield; mp 95−96 °C. ^{1}H NMR ($CDCl_3$): δ 7.49 (1H, brs), 6.82 (1H, dd, J = 11.0, 8.7 Hz), 6.66 (1H, s), 6.23 (1H, ddd, J = 8.7, 3.7, 2.8 Hz), 3.55 (2H, s), 1.53 (9H, s). ^{13}C NMR (DMSO-d_6): δ 152.9 (s), 146.2 (d, J = 231 Hz), 144.9 (s), 126.1 (d, J = 13 Hz), 115.0 (d, J = 20 Hz), 109.3 (s), 109.2 (s), 78.9 (s), 28.0 (s). IR: 3362, 1720, 1248, 1165 cm^{-1}. MS m/z: 227 (M + H)$^+$. Anal. ($C_{11}H_{15}FN_2O_2$) C, H, N.

***tert*-Butyl 3-Amino-4-fluorophenylcarbamate (7c).** Yellowish solid (47% yield) purified by silica gel column chromatography (20:1 CH_2Cl_2/ethyl acetate); mp 125−126 °C. ^{1}H NMR ($CDCl_3$): δ 7.04 (1H, brs), 6.86 (1H, dd, J = 10.6, 8.7 Hz), 6.44 (1H, ddd, J = 8.7, 6.4, 2.8 Hz), 6.34 (1H, brs), 3.72 (2H, brs), 1.50 (9H, s). ^{13}C NMR (DMSO-d_6): δ 152.7 (s), 146.3 (d, J = 232 Hz), 136.1 (d, J = 14 Hz), 135.8 (s), 114.3 (d, J = 19 Hz), 106.2 (s), 105.8 (s), 78.5 (s), 28.1 (s). IR: 3312, 1697, 1305, 1163 cm^{-1}. MS m/z: 227 (M + H)$^+$. Anal. ($C_{11}H_{15}FN_2O_2$) C, H, N.

***tert*-Butyl 5-Amino-2,3,4-trifluorophenylcarbamate (7d).** Pale brown solid (26% yield) purified by silica gel column chromatography (40:1 CH_2Cl_2/ethyl acetate); mp 123−124 °C. ^{1}H NMR ($CDCl_3$): δ 7.35 (1H, brs), 6.53 (1H, s), 3.67 (2H, brs), 1.52 (9H, s). ^{13}C NMR (DMSO-d_6): δ 152.9 (s), 139.9 (ddd, J = 242, 15, 13 Hz), 135.4 (dd, J = 227, 7 Hz), 134.6 (dd, J = 236, 12 Hz), 133.1 (d, J = 21 Hz), 123.0 (d, J = 9 Hz), 104.5 (s), 79.3 (s), 27.9 (s). IR: 3364, 1734, 1267, 1155 cm^{-1}. MS m/z: 263 (M + H)$^+$. Anal. ($C_{11}H_{13}F_3N_2O_2$) C, H, N.

***tert*-Butyl 3-Amino-2,6-difluorophenylcarbamate (7e).** Pale brown solid (38% yield) purified by silica gel column chromatography (20:1 CH_2Cl_2/ethyl acetate); mp 103−104 °C. ^{1}H NMR ($CDCl_3$): δ 6.72 (1H, dt, J = 9.2, 1.8 Hz), 6.56 (1H, dt, J = 9.2, 5.1 Hz), 5.90 (1H, brs), 3.58 (2H, brs), 1.49 (9H, s). ^{13}C NMR (DMSO-d_6): δ 153.2 (s), 149.0 (dd, J = 235, 2 Hz), 145.9 (dd, J = 242, 3 Hz), 133.2 (dd, J = 13, 2 Hz), 114.7 (dd, J = 17, 15 Hz), 112.3 (s), 110.1 (d, J = 21 Hz), 78.9 (s), 27.9 (s). IR: 3213, 1705, 1282, 1167 cm^{-1}. MS m/z: 245 (M + H)$^+$. Anal. ($C_{11}H_{14}F_2N_2O_2$) C, H, N.

Ethyl 1-(5-*tert*-Butoxycarbonylamino-2,4-difluorophenyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (9a). To a solution of acetic anhydride (2.8 mL, 30 mmol) and triethyl orthoformate (2.6 mL, 15 mmol) was added ethyl 2,6-dichloro-5-fluoronicotinoyl acetate (**8**, 2.8 g, 10 mmol). The mixture was heated at 140 °C for 6 h. After the solvent was evaporated, the residue was dissolved in CH_2Cl_2 (10 mL). To a resulting solution was added dropwise a solution of **7a** (2.43 g, 10 mmol) in CH_2Cl_2 (10 mL), followed by stirring at room temperature for 1 h. After the solvent was evaporated, DMF (8 mL) and K_2CO_3 (1.39 g, 10 mmol) were added to the residue followed by stirring at 80 °C for 30 min. The solution was poured into water (400 mL), and the precipitate was collected by filtration. The solid was purified by silica gel column chromatography (20:1 CH_2Cl_2/ethyl acetate) to give **9a** as a yellowish solid (4.6 g, 93% yield); mp 191−192 °C. ^{1}H NMR ($CDCl_3$): δ 8.56 (1H, s), 8.48 (1H, d, J = 7.3 Hz), 8.35 (1H, brs), 7.12 (1H, dd, J = 10.6, 8.9 Hz), 6.81 (1H,s), 4.41 (2H, q, J = 7.1 Hz), 1.52 (9H, s), 1.41 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$): δ 173.4 (s), 164.1 (s), 152.7 (d, J = 251 Hz), 152.2 (dd, J = 252, 13 Hz), 152.0 (s), 151.7 (dd, J = 250, 11 Hz), 149.3 (s), 144.7 (s), 142.8 (d, J = 23 Hz), 124.6 (dd, J = 11, 3 Hz), 123.3 (d, J = 10 Hz), 123.2 (d, J = 2 Hz), 123.1 (d, J = 21 Hz), 119.2 (s), 113.3 (s), 104.7 (dt, J = 25, 3 Hz), 82.0 (s), 61.4 (s), 28.1 (s), 14.3 (s). IR: 3315, 1738, 1718, 1631, 1543, 1161 cm^{-1}. MS m/z: 498 and 500 for Cl isotopic peaks of (M + H)$^+$.

Ethyl 1-(5-*tert*-Butoxycarbonylamino-2-fluorophenyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (9b). Colorless solid (90% yield); mp 237−238 °C. ^{1}H NMR (DMSO-d_6): δ 9.74 (1H, brs), 8.74 (1H, s), 8.53 (1H, d, J = 7.7 Hz), 7.88 (1H, brs), 7.55−7.51 (1H, m), 7.43 (1H, t, J = 9.1 Hz), 4.24 (2H, q, J = 7.4 Hz), 1.47 (9H, s), 1.27 (3H, t, J = 7.4 Hz). ^{13}C NMR (DMSO-d_6): δ 172.5 (s), 163.2 (s), 152.7 (s), 152.1 (d, J = 246 Hz), 151.9 (d, J = 258 Hz), 149.2 (s), 144.8 (s), 140.9 (d, J = 22 Hz), 136.7 (s), 126.6 (d, J = 15 Hz), 123.4 (d, J = 21 Hz), 122.8 (d, J = 2 Hz), 120.9 (d, J = 6 Hz), 118.4 (s), 116.5 (d, J = 20 Hz), 112.7 (s), 79.6 (s), 60.4 (s), 28.0 (s), 14.1 (s). IR: 3281, 1736, 1705, 1608, 1406, 1182 cm^{-1}. MS m/z: 480 and 482 for Cl isotopic peaks of (M + H)$^+$.

Ethyl 1-(3-*tert*-Butoxycarbonylamino-4-fluorophenyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (9c). Colorless powder (71% yield); mp 214−215 °C. ^{1}H NMR ($CDCl_3$): δ 8.63 (1H, s), 8.48 (1H, d, J = 7.3 Hz), 8.28 (1H, brs), 7.26 (1H, dd, J = 11.1, 8.7 Hz), 7.00 (1H, ddd, J = 8.7, 4.5, 2.8 Hz), 6.90 (1H, s), 4.40 (2H, q, J = 7.3 Hz), 1.52 (9H, s), 1.41 (3H, t, J = 7.3 Hz). ^{13}C NMR ($CDCl_3$): δ 173.3 (s), 164.3 (s), 152.6 (d, J = 262 Hz), 151.9 (s), 151.5 (d, J = 246 Hz), 149.4 (s), 145.2 (s), 142.5 (d, J = 22 Hz), 135.9 (d, J = 3 Hz), 128.5 (d, J = 12 Hz), 123.4 (d, J = 2 Hz), 123.1 (dd, J = 21, 2 Hz), 121.4 (d, J = 8 Hz), 118.5 (s), 115.6 (d, J = 21 Hz), 112.7 (s), 81.9 (s), 61.3 (s), 28.2 (s), 14.3 (s). IR: 3254, 1728, 1612, 1539, 1406, 1159 cm^{-1}. MS m/z: 480 and 482 for Cl isotopic peaks of $(M + H)^+$.

Ethyl 1-(5-*tert*-Butoxycarbonylamino-2,3,4-trifluorophenyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (9d). Colorless needles (92% yield); mp 208−209 °C. ^{1}H NMR ($CDCl_3$): δ 8.54 (1H, s), 8.48 (1H, d, J = 6.8 Hz), 8.16 (1H, brs), 6.82 (1H, s), 4.41 (2H, q, J = 7.4 Hz), 1.52 (9H, s), 1.41 (3H, t, J = 7.4 Hz). ^{13}C NMR (DMSO-d_6): δ 172.6 (s), 163.2 (s), 152.6 (s), 152.0 (d, J = 258 Hz), 149.4 (s), 144.7 (s), 143.6 (dd, J = 253, 10 Hz), 142.8 (dd, J = 251, 12 Hz), 140.9 (d, J = 22 Hz), 139.4 (dt, J = 259, 13 Hz), 124.4 (d, J = 9 Hz), 123.5 (d, J = 21 Hz), 123.2 (d, J = 11 Hz), 122.8 (d, J = 3 Hz), 118.1 (s), 112.8 (s), 80.3 (s), 60.4 (s), 27.8 (s), 14.1 (s). IR: 3304, 1738, 1726, 1608, 1548, 1471, 1163 cm^{-1}. MS m/z: 516 and 518 for Cl isotopic peaks of $(M + H)^+$.

Ethyl 1-(3-*tert*-Butoxycarbonylamino-2,4-difluorophenyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (9e). Colorless solid (67% yield); mp 128−131 °C. ^{1}H NMR (DMSO-d_6): δ 9.16 (1H, brs), 8.75 (1H, s), 8.56 (1H, d, J = 7.8 Hz), 7.68 (1H, ddd, J = 8.2, 5.5, 2.7 Hz), 7.40 (1H, t, J = 9.2 Hz), 4.24 (2H, q, J = 7.4 Hz), 1.43 (9H, s), 1.28 (3H, t, J = 7.4 Hz). ^{13}C NMR (DMSO-d_6): δ 172.6 (s), 163.1 (s), 158.0 (d, J = 249 Hz), 153.3 (dd, J = 249, 6 Hz), 152.7 (s), 151.9 (d, J = 258 Hz), 149.4 (s), 144.9 (s), 141.0 (d, J = 22 Hz), 127.3 (d, J = 10 Hz), 123.7 (d, J = 13 Hz), 123.4 (d, J = 20 Hz), 122.8 (d, J = 3 Hz), 116.1 (t, J = 17 Hz), 112.8 (s), 112.0 (d, J = 22 Hz), 79.8 (s), 60.4 (s), 27.8 (s), 14.1 (s). IR: 3304, 1730, 1612, 1527, 1406, 1178 cm^{-1}. MS m/z: 498 and 500 for Cl isotopic peaks of $(M + H)^+$.

Ethyl 1-(5-*tert*-Butoxycarbonylamino-2,4-difluorophenyl)-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (12a). Colorless solid (76% yield); mp 235−236 °C. ^{1}H NMR (DMSO-d_6): δ 9.42 (1H, brs), 8.59 (1H, s), 8.14 (1H, dd, J = 10.1, 8.7 Hz), 8.10 (1H, t, J = 8.0 Hz), 7.71 (1H, t, J = 10.1 Hz), 7.22 (1H, dd, J = 11.2, 6.2 Hz), 4.22 (2H, q, J = 7.3 Hz), 1.45 (9H, s), 1.27 (3H, t, J = 7.3 Hz). ^{13}C NMR (DMSO-d_6): δ 171.5 (s), 163.6 (s), 154.2 (dd, J = 250, 8 Hz), 152.9 (dd, J = 249, 13 Hz), 152.8 (s), 152.5 (dd, J = 254, 15 Hz), 149.3 (s), 147.7 (dd, J = 248, 14 Hz), 137.5 (d, J = 10 Hz), 124.6 (d, J = 3 Hz), 124.5 (d, J = 20 Hz), 124.4 (d, J = 16 Hz), 122.6 (dd, J = 15, 4 Hz), 113.9 (d, J = 18 Hz), 111.3 (s), 106.9 (d, J = 21 Hz), 106.0 (t, J = 26 Hz), 79.9 (s), 60.1 (s), 27.9 (s), 14.1 (s). IR: 3269, 1722, 1616, 1533, 1163 cm^{-1}. MS m/z: 481 (M + H)$^+$.

Ethyl 1-(5-*tert*-Butoxycarbonylamino-2,4-difluorophenyl)-4-oxo-6,7,8-trifluoro-1,4-dihydroquinoline-3-carboxylate (12b). Colorless powder (50% yield); mp 181−183 °C. ^{1}H NMR ($CDCl_3$): δ 8.44 (1H, brs), 8.31 (1H, s), 8.17 (1H, t, J = 10.1 Hz), 7.09 (1H, dd, J = 10.5, 8.9 Hz), 6.80 (1H, brs), 4.39 (2H, q, J = 7.3 Hz), 1.52 (9H, s), 1.39 (3H, t, J = 7.3 Hz). ^{13}C NMR ($CDCl_3$): δ 171.4 (s), 164.3 (s), 151.9 (s), 151.5 (dd, J = 250, 10 Hz), 150.8 (s), 148.6 (dd, J = 254, 11 Hz), 143.5 (dd, J = 269, 16 Hz),143.2 (dd, J = 259, 15 Hz), 141.4 (dd, J = 256, 15 Hz), 126.9 (s), 126.2 (d, J = 14 Hz), 124.7 (dd, J = 11, 3 Hz), 124.5 (d, J = 4 Hz), 118.4 (s), 111.7 (s), 109.6 (d, J = 19 Hz), 104.5 (dt, J = 24, 3 Hz), 82.2 (s), 61.4 (s), 28.2 (s), 14.3 (s). IR: 1722, 1608, 1533, 1161 cm^{-1}. MS m/z: 499 (M + H)$^+$.

Ethyl 1-(5-*tert*-Butoxycarbonylamino-2,4-difluorophenyl)-8-chloro-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (12c). Colorless solid (67% yield); mp 185−187 °C. ^{1}H NMR ($CDCl_3$): δ 8.35 (1H, s), 8.34 (1H, dd, J = 10.1, 8.2 Hz), 8.33 (1H, s), 7.06 (1H, dd, J = 10.5, 8.9 Hz), 6.80 (1H, brs), 4.39 (2H, q, J = 7.3 Hz), 1.51 (9H, s), 1.39 (3H, t, J = 7.3 Hz). ^{13}C NMR ($CDCl_3$): δ 171.9 (s), 164.2 (s), 152.3 (dd, J = 241, 12 Hz), 152.0 (s), 151.8 (s), 151.7 (dd, J = 252, 10 Hz), 151.1 (dd, J = 255, 17 Hz), 148.6 (dd, J = 254, 14 Hz), 133.9 (s), 126.4 (dd, J = 14, 5 Hz), 126.2 (d, J = 3 Hz), 124.7 (dd, J = 11, 3 Hz), 119.0 (s), 113.8 (d, J = 12 Hz), 112.2 (d, J = 19 Hz), 111.9 (s), 104.5 (dt, J = 24, 3 Hz), 82.2 (s), 61.4 (s), 28.2 (s), 14.3 (s). IR: 3250, 1739, 1712, 1612, 1529, 1172, 1070 cm^{-1}. MS m/z: 515 and 517 for Cl isotopic peaks of $(M + H)^+$.

Ethyl 8-Bromo-1-(5-*tert*-butoxycarbonylamino-2,4-difluorophenyl)-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (12d). Colorless solid (44% yield); mp 202−203 °C. ^{1}H NMR ($CDCl_3$): δ 8.39 (1H, t, J = 9.4 Hz), 8.37 (1H, s), 8.32 (1H, s), 7.06 (1H, t, J = 9.7 Hz), 6.78 (1H, brs), 4.38 (2H, q, J = 7.3 Hz), 1.51 (9H, s), 1.39 (3H, t, J = 7.3 Hz). ^{13}C NMR ($CDCl_3$): δ 172.0 (s), 164.2 (s), 152.4 (dd, J = 251, 12 Hz), 152.2 (s), 151.9 (dd, J = 253, 16 Hz), 151.8 (s), 151.8 (dd, J = 251, 10 Hz), 148.4 (dd, J = 256, 16 Hz), 135.0 (s), 126.8 (d, J = 3 Hz), 126.0 (dd, J = 14, 4 Hz), 124.9 (dd, J = 11, 4 Hz), 119.4 (s), 114.7 (d, J = 19 Hz), 111.9 (s), 104.6 (dt, J = 24, 3 Hz), 99.9 (d, J = 12 Hz), 82.2 (s), 61.4 (s), 28.1 (s), 14.3 (s). IR: 3219, 1738, 1714, 1610, 1527, 1167 cm^{-1}. MS m/z: 559 and 561 for Br isotopic peaks of $(M + H)^+$.

Ethyl 1-(5-*tert*-Butoxycarbonylamino-2,4-difluorophenyl)-6,7-difluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (12e). Slightly yellow solid (59% yield); mp 191−192 °C. ^{1}H NMR ($CDCl_3$): δ 8.34 (1H, s), 8.32 (1H, s), 8.24 (1H, t, J = 9.6 Hz), 7.10 (1H, dd, J = 10.4, 8.7 Hz), 6.79 (1H, brs), 4.40 (2H, q, J = 7.3 Hz), 1.82 (3H, d, J = 2.7 Hz), 1.52 (9H, s), 1.41 (3H, t, J = 7.3 Hz). ^{13}C NMR ($CDCl_3$): δ 172.7 (s), 164.7 (s), 158.3 (dd, J = 258, 12 Hz), 155.4 (dd, J = 256, 12 Hz), 153.7 (s), 152.6 (dd, J = 242, 15 Hz), 151.5 (s), 148.9 (dd, J = 252, 16 Hz), 136.2 (d, J = 4 Hz), 129.3 (d, J = 16 Hz), 128.8 (s), 127.4 (dd, J = 14, 4 Hz), 126.3 (d, J = 3 Hz), 116.5 (d, J = 19 Hz), 112.7 (d, J = 19 Hz), 111.7 (s), 106.1 (dt, J = 26, 3 Hz), 81.6 (s), 61.3 (s), 28.1 (s), 14.3 (s), 11.5 (d, J = 7 Hz). IR: 3049, 1736, 1714, 1616, 1520, 1159 cm^{-1}. MS m/z: 495 $(M + H)^+$.

1-(5-Amino-2,4-difluorophenyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (10a). The naphthyridine-3-carboxylate derivative **9a** (1.9 g, 3.82 mmol) was added to a mixture of acetic acid (10 mL) and aqueous 6 M HCl (5 mL). The reaction mixture was heated to reflux for 6 h, and then, H_2O (50 mL) was added. After it was cooled, the precipitate was collected by filtration and washed with H_2O, EtOH, and Et_2O to give **10a** as yellow needles (1.17 g, 84%); mp >270 °C. ^{1}H NMR (DMSO-d_6): δ 8.98 (1H, s), 8.76 (1H, d, J = 7.4 Hz), 7.43 (1H, t, J = 10.6 Hz), 7.02 (1H, dd, J = 8.8, 7.7 Hz), 5.46 (2H, s). ^{13}C NMR (DMSO-d_6): δ 177.4 (s), 164.4 (s), 152.2 (d, J = 259 Hz), 150.6 (s), 150.0 (dd, J = 244, 11 Hz), 147.1 (dd, J = 240, 11 Hz), 144.9 (s), 142.7 (d, J = 23 Hz), 134.0 (d, J = 15 Hz), 123.0 (d, J = 20 Hz), 122.5 (dd, J = 15, 4 Hz), 121.4 (d, J = 3 Hz), 114.7 (s), 110.3 (s), 104.5 (t, J = 24 Hz). IR: 3472, 1741, 1612, 1525, 1406, 1255, 1147 cm^{-1}. MS m/z: 370 $(M + H)^+$. Anal. ($C_{15}H_7ClF_3N_3O_3$) C, H, N.

1-(5-Amino-2-fluorophenyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic Acid (10b). Yellow solid (82% yield); mp >290 °C. ^{1}H NMR (DMSO-d_6): δ 8.91 (1H, s), 8.75 (1H, d, J = 7.8 Hz), 7.19 (1H, t, J = 9.6 Hz), 6.81−6.79 (2H, m), 5.57 (2H, s). ^{13}C NMR (DMSO-d_6): δ 177.4 (s), 164.4 (s), 152.2 (d, J = 260 Hz), 150.4 (s), 144.9 (s), 143.8 (d, J = 255 Hz), 142.7 (d, J = 22 Hz), 126.6 (d, J = 14 Hz), 123.0 (d, J = 20 Hz), 121.4 (d, J = 4 Hz), 116.6 (d, J = 21 Hz), 116.5 (d, J = 20 Hz), 114.4 (s), 113.9 (s), 110.3 (s). IR: 3474, 1743, 1618, 1518, 1404, 1251, 1049 cm^{-1}. MS m/z: 352 (M + H)$^+$. Anal. ($C_{15}H_8ClF_2N_3O_3$) C, H, N.

1-(3-Amino-4-fluorophenyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic Acid (10c). Yellow solid (94% yield); mp >270 °C. ^{1}H NMR (DMSO-d_6): δ 8.79 (1H, s), 8.76 (1H, d, J = 7.4 Hz), 7.22 (1H, dd, J = 11.0, 8.7 Hz), 6.95 (1H, dd, J = 7.8, 2.7 Hz), 6.74 (1H, ddd, J = 8.7, 4.1, 2.7 Hz), 5.62 (2H, s). ^{13}C NMR (DMSO-d_6): δ 177.5 (s), 164.6 (s), 152.0 (d, J = 260 Hz), 150.6 (d, J = 241 Hz), 150.2

(s), 145.5 (s), 142.4 (d, J = 23 Hz), 137.4 (d, J = 15 Hz), 135.9 (s), 122.6 (d, J = 21 Hz), 121.5 (d, J = 5 Hz), 115.5 (d, J = 20 Hz), 114.4 (s), 114.3 (d, J = 7 Hz), 109.4 (s). IR: 3481, 3377, 1741, 1616, 1516, 1404, 1249, 1049 cm^{-1}. MS m/z: 352 (M + H)$^+$. Anal. ($C_{15}H_8ClF_2N_3O_3$) C, H, N.

1-(5-Amino-2,3,4-trifluorophenyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic Acid (10d). Colorless solid (96% yield); mp >242 °C (decomposed). ^{1}H NMR (DMSO-d_6): δ 9.04 (1H, s), 8.77 (1H, d, J = 7.3 Hz), 6.87 (1H, dt, J = 8.2, 1.6 Hz), 5.84 (2H, s). ^{13}C NMR (DMSO-d_6): δ 177.4 (s), 164.3 (s), 152.3 (d, J = 260 Hz), 150.6 (s), 144.8 (s), 142.7 (d, J = 22 Hz), 139.8 (dt, J = 235, 14 Hz), 139.4 (dd, J = 244, 11 Hz), 137.2 (dd, J = 241, 12 Hz), 134.5 (d, J = 11 Hz), 123.2 (s), 123.1 (d, J = 20 Hz), 121.4 (d, J = 4 Hz), 110.5 (s), 108.8 (s). IR: 3472, 3379, 1743, 1616, 1523, 1257, 1155 cm^{-1}. MS m/z: 388 (M + H)$^+$. Anal. ($C_{15}H_6ClF_4N_3O_3$) C, H, N.

1-(3-Amino-2,4-difluorophenyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic Acid (10e). Yellowish solid (66% yield); mp 244−248 °C. ^{1}H NMR (DMSO-d_6): δ 8.96 (1H, s), 8.76 (1H, d, J = 7.3 Hz), 7.14 (1H, dd, J = 9.1, 8.2 Hz), 7.02 (1H, dt, J = 8.2, 5.5 Hz), 5.69 (2H, s). ^{13}C NMR (DMSO-d_6): δ 177.3 (s), 164.4 (s), 152.3 (d, J = 259 Hz), 151.5 (dd, J = 242, 8 Hz), 150.8 (s), 146.0 (dd, J = 235, 10 Hz), 144.9 (s), 142.8 (d, J = 23 Hz), 126.6 (dd, J = 18, 15 Hz), 123.5 (dd, J = 11, 3 Hz), 123.0 (d, J = 21 Hz), 121.3 (d, J = 4 Hz), 113.3 (d, J = 8 Hz), 111.1 (d, J = 20 Hz), 110.2 (s). IR: 3344, 3072, 1734, 1616, 1568, 1402, 1253, 1170, 1053 cm^{-1}. MS m/z: 370 and 372 for Cl isotopic peaks of (M + H)$^+$. Anal. ($C_{15}H_7ClF_3N_3O_3$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (13a). Colorless solid (95% yield); mp >270 °C. ^{1}H NMR (DMSO-d_6): δ 8.87 (1H, s), 8.34 (1H, dd, J = 10.1, 8.7 Hz), 7.50 (1H, t, J = 10.5 Hz), 7.45 (1H, dd, J = 11.5, 6.4 Hz), 7.04 (1H, t, J = 8.2 Hz), 5.54 (2H, s). ^{13}C NMR (DMSO-d_6): δ 176.8 (s), 164.9 (s), 153.6 (dd, J = 266, 15 Hz), 150.5 (dd, J = 246, 12 Hz), 150.3 (s), 148.4 (dd, J = 252, 14 Hz), 146.9 (dd, J = 241, 12 Hz), 138.2 (d, J = 11 Hz), 134.8 (d, J = 15 Hz), 122.6 (d, J = 11 Hz), 122.3 (d, J = 6 Hz), 114.0(s), 113.5 (d, J = 18 Hz), 108.7 (s), 107.8 (d, J = 24 Hz), 105.3 (t, J = 25 Hz). IR: 3371, 1705, 1616, 1529, 1284, 1138 cm^{-1}. MS m/z: 353 (M + H)$^+$. Anal. ($C_{16}H_8F_4N_2O_3$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-4-oxo-6,7,8-trifluoro-1,4-dihydroquinoline-3-carboxylic Acid (13b). Colorless solid (94% yield); mp 256−261 °C. ^{1}H NMR (DMSO-d_6): δ 8.72 (1H, s), 8.24 (1H, t, J = 8.7 Hz), 7.44 (1H, t, J = 10.6 Hz), 7.13 (1H, t, J = 8.5 Hz). ^{13}C NMR (DMSO-d_6): δ 175.6 (s), 164.5 (s), 152.0 (s), 150.2 (dd, J = 245, 11 Hz), 148.2 (dd, J = 255, 10 Hz), 147.3 (dd, J = 240, 11 Hz), 143.4 (dd, J = 275, 14 Hz), 141.4 (dd, J = 275, 17 Hz), 133.6 (dd, J = 15, 2 Hz), 127.9 (s), 125.0 (dd, J = 14, 4 Hz), 122.1 (d, J = 6 Hz), 114.1 (s), 108.9 (s), 108.3 (d, J = 21 Hz), 104.4 (t, J = 25 Hz). IR: 3356, 1726, 1620, 1521, 1234, 1145 cm^{-1}. MS m/z: 371 (M + H)$^+$. Anal. ($C_{16}H_7F_5N_2O_3$·1/3H_2O) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-8-chloro-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (13c). Colorless solid (85% yield); mp 225−227 °C. ^{1}H NMR (DMSO-d_6): δ 8.69 (1H, s), 8.40 (1H, dd, J = 9.7, 8.7 Hz), 7.42 (1H, dd, J = 11.0, 10.1 Hz), 7.08 (1H, dd, J = 8.7, 7.8 Hz). ^{13}C NMR (DMSO-d_6): δ 175.9 (s), 164.4 (s), 152.9 (s), 151.2 (dd, J = 254, 7 Hz), 151.0 (dd, J = 246, 12 Hz), 148.6 (dd, J = 241, 12 Hz), 148.1 (dd, J = 243, 15 Hz), 134.4 (s), 132.2 (d, J = 14 Hz), 125.5 (dd, J = 10, 4 Hz), 124.0 (d, J = 7 Hz), 115.6 (s), 112.6 (d, J = 18 Hz), 112.4 (d, J = 19 Hz), 109.1 (s), 104.7 (t, J = 25 Hz). IR: 3346, 1722, 1614, 1514, 1273, 1165, 1109 cm^{-1}. MS m/z: 387 and 389 for Cl isotopic peaks of (M + H)$^+$. Anal. ($C_{16}H_7ClF_4N_2O_3$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-8-bromo-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (13d). Colorless solid (87% yield); mp 228−232 °C. ^{1}H NMR (DMSO-d_6): δ 8.68 (1H, s), 8.40 (1H, dd, J = 9.6, 8.7 Hz), 7.42 (1H, dd, J = 11.0, 10.0 Hz), 7.08 (1H, t, J = 8.2 Hz), 5.94 (2H, brs). ^{13}C NMR (DMSO-d_6): δ 176.1 (s), 164.4 (s), 153.0 (s), 152.0 (dd, J = 251, 16 Hz), 150.8 (dd, J = 246, 11 Hz), 148.3 (dd, J = 239, 10 Hz), 148.0 (dd, J = 254, 17 Hz), 135.7 (s), 133.3 (d, J = 15 Hz), 125.1 (d, J = 14 Hz), 124.4 (d, J = 6 Hz), 115.2 (s), 113.2 (d, J = 18 Hz), 109.1 (s), 104.7 (t, J = 25 Hz), 101.3 (d, J = 23 Hz). IR: 3333, 1716, 1620, 1512, 1271, 1151 cm^{-1}. MS m/z: 431 and 433 for Br isotopic peaks of (M + H)$^+$. Anal. ($C_{16}H_7BrF_4N_2O_3$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-6,7-difluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (13e). Colorless solid (96% yield); mp 264−267 °C. ^{1}H NMR (DMSO-d_6): δ 8.68 (1H, s), 8.24 (1H, t, J = 9.6 Hz), 7.45 (1H, t, J = 10.5 Hz), 7.10 (1H, dd, J = 8.7, 7.8 Hz), 5.45 (2H, brs), 1.86 (3H, d, J = 2.7 Hz). ^{13}C NMR (DMSO-d_6): δ 176.8 (s), 164.7 (s), 152.4 (dd, J = 251, 14 Hz), 152.4 (s), 150.2 (dd, J = 246, 11 Hz), 148.3 (dd, J = 251, 16 Hz), 147.2 (dd, J = 237, 11 Hz), 137.2 (d, J = 5 Hz), 134.2 (d, J = 14 Hz), 126.5 (dd, J = 14, 4 Hz), 123.3 (d, J = 5 Hz), 118.3 (d, J = 17 Hz), 114.2 (s), 110.8 (d, J = 18 Hz), 108.2 (s), 104.9 (t, J = 25 Hz), 10.8 (d, J = 8 Hz). IR: 3337, 1724, 1620, 1527, 1280, 1143 cm^{-1}. MS m/z: 367 (M + H)$^+$. Anal. ($C_{17}H_{10}F_4N_2O_3$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-7-[(3*S*)-3-aminiopyrrolidin-1-yl]-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic Acid (3). The 1,8-naphthyridine-3-carboxylic acid derivative **10a** (1.3 g, 3.52 mmol), (3*S*)-aminopyrrolidine (0.60 g, 6.97 mmol), and Et_3N (0.60 g, 5.94 mmol) were dissolved in DMF (7.0 mL). The solution was stirred at 90 °C for 1 h. After addition of EtOH (25 mL), the solution was heated at 90 °C for 10 min and allowed to cool. The precipitate was collected by filtration and washed with EtOH and Et_2O. The crude solid was purified by reprecipitaiton from its DMF solution with EtOH to obtain 1.4 g of **3** as a colorless solid (95% yield); mp 260−266 °C (decomposed). ^{1}H NMR (DMSO-d_6): δ 8.66 (1H, s), 8.01 (1H, d, J = 12.8 Hz), 7.35 (1H, t, J = 9.7 Hz), 6.97 (1H, t, J = 8.3 Hz), 5.34 (2H, s), 4.01−3.30 (5H, m), 1.92 (1H, brs), 1.64 (1H, brs). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-d): δ 176.9 (d, J = 3 Hz), 165.3 (s), 149.6 (dd, J = 244, 11 Hz), 149.2 (d, J = 14 Hz), 147.6 (s), 147.1 (dd, J = 240, 12 Hz), 146.1 (d, J = 259 Hz), 146.1 (s), 133.7 (d, J = 15 Hz), 123.3 (d, J = 14 Hz), 117.8 (d, J = 21 Hz), 114.5 (s), 110.7 (s), 108.8 (s), 104.1 (t, J = 24 Hz), 52.3 (s), 46.4 (s). IR: 1633, 1523, 1469, 1271, 823 cm^{-1}. MS m/z: 420 (M + H)$^+$. Anal. ($C_{19}H_{16}F_3N_5O_3$·2.25H_2O) C, H, N.

1-(5-Amino-2-fluorophenyl)-7-[(3*S*)-3-aminiopyrrolidin-1-yl]-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic Acid (14). Colorless solid (85% yield); mp 217−219 °C. ^{1}H NMR (DMSO-d_6): δ 8.61 (1H, s), 8.01 (1H, d, J = 12.4 Hz), 7.11 (1H, t, J = 9.7 Hz), 6.74−6.70 (2H, m), 5.29 (2H, s), 3.95−3.20 (5H, m), 1.89 (1H, brs), 1.59 (1H, brs). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-d): δ 176.9 (d, J = 3 Hz), 165.3 (s), 148.7 (d, J = 237 Hz), 148.6 (d, J = 12 Hz), 147.3 (s), 146.1 (d, J = 259 Hz), 146.0 (s), 144.7 (s), 127.3 (d, J = 15 Hz), 117.7 (d, J = 22 Hz), 116.4 (s), 116.2 (d, J = 20 Hz), 113.5(s), 110.7 (s), 108.7 (s), 51.7 (s), 48.4 (s), 46.0 (s). IR: 1633, 1512, 1456, 1269, 821 cm^{-1}. MS m/z: 402 (M + H)$^+$. Anal. ($C_{19}H_{17}F_2N_5O_3$·2H_2O) C, H, N.

1-(3-Amino-4-fluorophenyl)-7-[(3*S*)-3-aminiopyrrolidin-1-yl]-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic Acid (15). Colorless powder (87% yield); mp 262−265 °C. ^{1}H NMR (DMSO-d_6 with 1 equiv TFA-d): δ 8.57 (1H, s), 8.12 (1H, d, J = 12.4 Hz), 8.02 (3H, brs), 7.17 (1H, dd, J = 11.5, 8.3 Hz), 6.91 (1H, dd, J = 8.3, 2.7 Hz), 6.73−6.68 (1H, m), 5.51 (2H, s), 3.95−3.20 (5H, m), 2.12 (1H, brs), 2.02 (1H, brs). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-d): δ 176.7 (d, J = 3 Hz), 165.6 (s), 150.1 (d, J = 239 Hz), 148.4 (d, J = 13 Hz), 147.2 (s), 146.4 (s), 145.9 (d, J = 259 Hz), 137.1 (d, J = 15 Hz), 136.5 (s), 117.7 (d, J = 19 Hz), 115.1 (d, J = 19 Hz), 114.5-(s), 114.4 (d, J = 7 Hz), 111.1 (d, J = 3 Hz), 108.1 (s), 51.7 (s), 46.1 (s). IR: 1712, 1635, 1512, 1265, 1207, 808 cm^{-1}. MS m/z: 402 (M + H)$^+$. Anal. ($C_{19}H_{17}F_2N_5O_3$·2H_2O) C, H, N.

7-[(3*S*)-3-Aminiopyrrolidin-1-yl]-1-(5-Amino-2,3,4-trifluorophenyl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic Acid (16). Colorless solid (69% yield); mp >268 °C (decomposed). ^{1}H NMR (DMSO-d_6): δ 8.76 (1H, s), 8.09 (1H, d, J = 12.9 Hz), 6.81 (1H, brs), 5.74 (2H, s), 4.05−3.41 (5H, m), 2.14 (1H, brs), 1.91 (1H, brs). ^{13}C NMR (DMSO-d_6 with 1

equiv TFA-*d*): δ 177.0 (d, *J* = 2 Hz), 165.7 (s), 148.6 (d, *J* = 14 Hz), 147.1 (s), 146.1 (d, *J* = 258 Hz), 145.9 (d, *J* = 3 Hz), 139.7 (ddd, *J* = 245, 17, 14 Hz), 139.0 (dd, *J* = 243, 12 Hz), 137.1 (dd, *J* = 242, 11 Hz), 134.3 (d, *J* = 10 Hz), 124.0 (d, *J* = 8 Hz), 117.9 (d, *J* = 21 Hz), 110.6(s), 108.9 (s), 108.6 (s), 52.3 (s), 46.7 (s). IR: 1635, 1510, 1464, 1219, 821 cm^{-1}. MS *m/z*: 438 $(M + H)^+$. Anal. ($C_{19}H_{17}F_2N_5O_3 \cdot 2.75H_2O$) C, H, N.

1-(3-Amino-2,4-difluorophenyl)-7-[(3*S*)-3-aminiopyrrolidin-1-yl]-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic Acid (17). Yellowish solid (76% yield); mp >268 °C (decomposed). ^1H NMR (DMSO-d_6 with 1 equiv TFA-*d*): δ 8.70 (1H, s), 8.13 (2H, s), 8.12 (1H, d, *J* = 12.3 Hz), 7.10 (1H, t, *J* = 9.6 Hz), 6.83 (1H, dd, *J* = 13.8, 8.2 Hz), 4.10−3.35 (5H, m), 2.20 (1H, brs), 2.03 (1H, brs). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-*d*): δ 176.9 (s), 165.3 (s), 151.1 (dd, *J* = 242, 8 Hz), 148.6 (d, *J* = 12 Hz), 147.7 (s), 146.2 (d, *J* = 10 Hz), 146.1 (d, *J* = 257 Hz), 146.0 (dd, *J* = 247, 12 Hz), 126.3 (dd, *J* = 17, 16 Hz), 124.1 (dd, *J* = 11, 2 Hz), 117.7 (d, *J* = 20 Hz), 113.3 (s), 110.8 (s), 110.7 (s), 108.7 (s), 51.7 (s), 46.0 (s). IR: 1728, 1639, 1550, 1467, 1271, 1176, 815 cm^{-1}. MS *m/z*: 420 $(M + H)^+$. Anal. ($C_{19}H_{16}F_3N_5O_3 \cdot 2.25H_2O$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-7-[(3*S*)-3-aminiopyrrolidin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (20). Colorless solid (97% yield); mp 213−221 °C (decomposed). ^1H NMR (DMSO-d_6 with 1 equiv TFA-*d*): δ 8.67 (1H, s), 8.03 (2H, s), 7.93 (1H, d, *J* = 13.8 Hz), 7.51 (1H, t, *J* = 9.7 Hz), 7.03 (1H, dd, *J* = 14.7, 7.8 Hz), 5.96 (1H, d, *J* = 7.4 Hz), 3.90 (1H, s), 3.73 (1H,s), 3.58−3.42 (3H, m), 2.28−2.20 (1H, m), 2.04 (1H, brs). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-*d*): δ 176.3 (s), 165.6 (s), 150.1 (dd, *J* = 245, 11 Hz), 149.9 (dd, *J* = 248, 4 Hz), 148.4 (s), 146.9 (dd, *J* = 239, 10 Hz), 141.4 (dd, *J* = 12, 4 Hz), 139.4 (d, *J* = 6 Hz), 134.8 (d, *J* = 14 Hz), 122.9 (dd, *J* = 14, 3 Hz), 114.8 (d, *J* = 7 Hz), 114.1 (t, *J* = 3 Hz), 111.1 (d, *J* = 23 Hz), 107.5(s), 105.2 (t, *J* = 24 Hz), 100.2 (s), 53.0 (d, *J* = 8 Hz), 49.4 (s), 47.3 (s), 28.6 (s). IR: 3470, 1716, 1631, 1520, 1147, 819 cm^{-1}. MS *m/z*: 419 $(M + H)^+$. Anal. ($C_{20}H_{17}F_3N_4O_3 \cdot 2.75H_2O$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-7-[(3*S*)-3-aminiopyrrolidin-1-yl]-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (21). Colorless solid (95% yield); mp 204−210 °C (decomposed). ^1H NMR (DMSO-d_6 with 1 equiv TFA-*d*): δ 8.53 (1H, s), 8.04 (2H, s), 7.85 (1H, d, *J* = 13.7 Hz), 7.39 (1H, t, *J* = 10.1 Hz), 7.10 (1H, t, *J* = 8.2 Hz), 3.90−3.55 (5H, br), 2.19−2.14 (1H, m), 1.99−1.95 (1H, m). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-*d*): δ 175.7 (s), 165.0 (s), 151.4 (dd, *J* = 250, 6 Hz), 150.9 (s), 149.7 (dd, *J* = 244, 11 Hz), 146.9 (dd, *J* = 239, 11 Hz), 140.8 (dd, *J* = 247, 8 Hz), 133.8 (d, *J* = 15 Hz), 131.6 (dd, *J* = 14, 11 Hz), 128.6 (s), 126.9 (d, *J* = 15 Hz), 115.6 (d, *J* = 9 Hz), 113.6 (s), 107.4 (s), 107.0 (d, *J* = 24 Hz), 104.2 (dt, *J* = 25, 4 Hz), 54.6 (s), 49.2 (s), 40.4 (s), 29.0 (s). IR: 3319, 1732, 1624, 1523, 1253, 1155, 819 cm^{-1}. MS *m/z*: 437 $(M + H)^+$. Anal. ($C_{20}H_{16}F_4N_4O_3 \cdot H_2O$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-7-[(3*S*)-3-aminiopyrrolidin-1-yl]-8-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (22). Colorless solid (93% yield); mp >205 °C (decomposed). ^1H NMR (DMSO-d_6 with 1 equiv TFA-*d*): δ 8.58 (1H, s), 8.07 (2H, s), 8.04 (1H, d, *J* = 13.2 Hz), 7.39 and 7.38 (1H, t, *J* = 10.5 Hz), 7.02 and 6.98 (1H, t, *J* = 8.3 Hz), 3.90−3.45 (5H, br), 2.29−2.21 (1H, m), 1.99−1.91 (1H, m). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-*d*): δ 176.1 (s), 164.7 (s), 155.8 and 154.7 (d, *J* = 253 Hz), 152.3 (s), 149.8 and 149.7 (dd, *J* = 245, 11 Hz), 146.8 and 146.7 (dd, *J* = 239, 12 Hz), 141.2 and 141.1 (d, *J* = 14 Hz), 136.5 and 136.4 (s), 134.3 and 134.1 (d, *J* = 15 Hz), 126.9 (dd, *J* = 13, 4 Hz), 121.3 (d, *J* = 6 Hz), 115.3 (d, *J* = 4 Hz), 113.3(s), 110.5 (d, *J* = 22 Hz), 108.4 (s), 104.5 and 104.4(t, *J* = 24 Hz), 54.5 (s), 49.2 (s), 40.4 (s), 29.6 (s). IR: 1716, 1635, 1452, 1261, 1109 cm^{-1}. MS *m/z*: 453 and 455 for Cl isotopic peaks of $(M + H)^+$. Anal. ($C_{20}H_{16}ClF_3N_4O_3 \cdot 4H_2O$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydro-1,8-naphthyridine-3-carboxylic Acid Dihydrocloride (18). The 1,8-naphthyridine-3-carboxylic acid derivative **10a** (185 mg, 0.50 mmol), piperazine (86 mg, 1.0 mmol), and Et_3N (100 mg, 1.0 mmol) were dissolved in DMSO (1.5 mL). The solution was stirred at 70 °C for 1 h. After addition of EtOH (25 mL), the solution was heated at 90 °C for 10 min and allowed to cool. The precipitate was collected by filtration and washed with EtOH and Et_2O. The crude solid was dissolved in aqueous 10% (v/v) HCl (10 mL). After the solvent was evaporated, the residue was purified by reprecipitaiton from its DMF solution with EtOH to obtain 183 mg of **18** as a yellow solid (74% yield); mp 197−199 °C. ^1H NMR (DMSO-d_6): δ 9.50 (2H, s), 8.82 (1H, s), 8.24 (1H, d, *J* = 12.8 Hz), 7.42 (1H, t, *J* = 10.5 Hz), 7.08 (1H, t, *J* = 8.2 Hz), 3.80 (4H, t, *J* = 5.1 Hz), 3.15 and 3.13 (4H, t, *J* = 5.1 Hz). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-*d*): δ 177.1 (s), 165.1 (s), 150.6 (dd, *J* = 246, 11 Hz), 149.7 (d, *J* = 10 Hz), 148.3 (dd, *J* = 242, 11 Hz), 148.2 (s), 147.2 (d, *J* = 265 Hz), 145.2 (s), 131.4 (dd, *J* = 12, 3 Hz), 123.0 (dd, *J* = 14, 4 Hz), 119.8 (d, *J* = 22 Hz), 116.3(s), 112.8 (d, *J* = 3 Hz), 109.0 (s), 104.5 (t, *J* = 25 Hz), 43.3 (d, *J* = 8 Hz), 41.9 (s). IR: 1726, 1631, 1452, 1277, 1033, 810 cm^{-1}. MS *m/z*: 420 $(M + H)^+$. Anal. ($C_{19}H_{16}F_3N_5O_3 \cdot 2HCl \cdot 0.5H_2O$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic Acid (23). The quinolone-3-carboxylic acid derivative **13a** (190 mg, 0.50 mmol), piperazine (85 mg, 1.0 mmol), and Et_3N (100 mg, 1.0 mmol) were dissolved in DMSO (1.5 mL). The solution was stirred at 70 °C for 1 h. After EtOH (25 mL) was added, the solution was heated at 90 °C for 10 min and allowed to cool. The precipitate was collected by filtration and washed with EtOH and Et_2O. The residue was purified by reprecipitaiton from its DMF solution with EtOH to obtain 70 mg of **23** as a colorless solid (63% yield); mp 167−171 °C. ^1H NMR (DMSO-d_6): δ 8.73 (1H, s), 7.97 (1H, d, *J* = 13.3 Hz), 7.52 (1H, t, *J* = 10.5 Hz), 7.07 (1H, t, *J* = 8.2 Hz), 6.43 (1H, d, *J* = 6.9 Hz), 5.56 (2H, s), 3.02 (4H, s), 2.81 (4H, s). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-*d*): δ 176.7 (s), 165.3 (s), 152.8 (d, *J* = 251 Hz), 150.2 (dd, *J* = 245, 11 Hz), 149.3 (s), 146.8 (dd, *J* = 241, 11 Hz), 144.7 (d, *J* = 11 Hz), 138.5 (s), 134.8 (dd, *J* = 15, 2 Hz), 122.8 (dd, *J* = 14, 3 Hz), 119.2 (d, *J* = 7 Hz), 114.1(s), 111.5 (d, *J* = 24 Hz), 108.1 (s), 106.1 (s), 105.2 (t, *J* = 24 Hz), 46.2 (s), 42.5 (s), 40.4 (s). IR: 3308, 1720, 1618, 1520, 1271, 1145, 823 cm^{-1}. MS *m/z*: 419 $(M + H)^+$. Anal. ($C_{20}H_{17}F_3N_4O_3 \cdot H_2O$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-6,8-difluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic Acid (24). Colorless solid (85% yield); mp 262−265 °C. ^1H NMR (DMSO-d_6): δ 8.54 (1H, s), 7.88 (1H, d, *J* = 11.9 Hz), 7.40 (1H, t, *J* = 10.6 Hz), 7.09 (1H, t, *J* = 8.3 Hz), 5.45 (2H, s), 3.14 (4H, s), 2.83−2.77 (4H, m). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-*d*): δ 175.9 (s), 164.7 (s), 154.6 (dd, *J* = 250, 5 Hz), 151.4 (s), 149.8 (dd, *J* = 244, 11 Hz), 146.9 (dd, *J* = 246, 8 Hz), 145.8 (dd, *J* = 253, 7 Hz), 133.8 (d, *J* = 15 Hz), 132.8 (dd, *J* = 15, 13 Hz), 128.0 (d, *J* = 5 Hz), 126.5 (d, *J* = 14 Hz), 120.8 (d, *J* = 9 Hz), 113.8(s), 108.0 (s), 107.2 (d, *J* = 23 Hz), 104.4 (t, *J* = 24 Hz), 47.3 (s), 43.3 (d, *J* = 9 Hz). IR: 1722, 1628, 1525, 1273, 1141 cm^{-1}. MS *m/z*: 437 $(M + H)^+$. Anal. ($C_{20}H_{16}F_4N_4O_3 \cdot H_2O$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-8-chloro-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic Acid (25). Colorless solid (65% yield); mp >232 °C (decomposed). ^1H NMR (DMSO-d_6): δ 8.53 (1H, s), 8.06 (1H, d, *J* = 11.9 Hz), 7.36 (1H, t, *J* = 10.5 Hz), 6.96 (1H, t, *J* = 8.7 Hz), 5.40 (2H, s), 3.14 (4H, s), 2.82−2.78 (4H, m). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-*d*): δ 176.2 (d, *J* = 2 Hz), 164.5 (s), 156.2 (d, *J* = 252 Hz), 152.7 (s), 149.8 (dd, *J* = 245, 11 Hz), 147.1 (dd, *J* = 249, 11 Hz), 142.6 (d, *J* = 16 Hz), 135.4 (s), 134.2 (dd, *J* = 15, 2 Hz), 126.7 (dd, *J* = 14, 4 Hz), 123.9 (d, *J* = 9 Hz), 120.5 (d, *J* = 5 Hz), 113.7(s), 111.2 (d, *J* = 24 Hz), 108.3 (s), 104.4 (t, *J* = 24 Hz), 47.4 (s), 43.4 (s). IR: 1725, 1620, 1525, 1263, 1113 cm^{-1}. MS *m/z*: 453 and 455 for Cl isotopic peaks of $(M + H)^+$. Anal. ($C_{20}H_{16}ClF_3N_4O_3 \cdot H_2O$) C, H, N.

7-(3-Aminoazetidin-1-yl)-1-(5-amino-2,4-difluorophenyl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic Acid (19). The 1,8-naphthyridine-3-carboxylic acid derivative **10a** (556 mg, 1.5 mmol), 3-aminoazetidine dihydrochloride (320 mg, 2.2 mmol), and Et_3N (1.04 g, 8.0 mmol) were dissolved

in MeCN (200 mL). The solution was stirred at 80 °C for 15 h. After EtOH (25 mL) was added, the solution was heated at 90 °C for 10 min and allowed to cool. The precipitate was collected by filtration and washed with EtOH and Et_2O. The crude solid was purified by reprecipitaiton from its DMF solution with EtOH to obtain 720 mg of **19** as a colorless solid (91% yield); mp 168−171 °C. ^{1}H NMR (DMSO-d_6): δ 8.68 (1H, s), 8.02 (1H, d, J = 11.5 Hz), 7.35 (1H, t, J = 10.5 Hz), 6.94 (1H, dd, J = 10.8, 7.6 Hz), 5.34 (2H, brs), 4.60−3.96 (4H, br), 3.85−3.83 (1H, m). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-d): δ 177.1 (s), 165.2 (s), 150.2 (d, J = 15), 149.7 (dd, J = 243, 11 Hz), 147.7 (s),147.3 (dd, J = 240, 12 Hz), 146.4 (s), 146.3 (d, J = 257 Hz), 133.6 (dd, J = 15, 2 Hz), 123.1 (dd, J = 14, 4 Hz), 117.4 (d, J = 18 Hz), 114.7(s), 111.4 (s), 108.9 (s), 104.0 (t, J = 24 Hz), 55.0 (br), 41.2 (s). IR: 3072, 1651, 1525, 1255, 1141, 891 cm^{-1}. MS m/z: 406 $(M + H)^+$. Anal. ($C_{18}H_{14}F_3N_5O_3 \cdot 1.5H_2O$) C, H, N.

7-(3-Aminoazetidin-1-yl)-1-(5-amino-2,4-difluorophenyl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (26). Colorless solid (74% yield); mp >183 °C (decomposed). ^{1}H NMR (DMSO-d_6): δ 8.64 (1H, s), 7.86 (1H, d, J = 12.8 Hz), 7.51 (1H, t, J = 10.6 Hz), 7.02 (1H, t, J = 8.3 Hz), 5.74 (1H, d, J = 7.3 Hz), 5.54 (2H, s), 4.28−4.16 (2H, m), 3.83−3.78 (1H, m), 3.67−3.58 (2H, m). ^{13}C NMR (DMSO-d_6): δ 176.4 (s), 165.5 (s), 150.1 (dd, J = 246, 10 Hz), 150.0 (d, J = 246 Hz), 148.5 (s), 146.9 (dd, J = 240, 11 Hz), 143.5 (d, J = 14 Hz), 139.2 (s), 134.8 (d, J = 15 Hz), 122.8 (dd, J = 14, 4 Hz), 115.5 (d, J = 6 Hz), 114.1 (s), 110.6 (d, J = 20 Hz), 107.5(s), 105.2 (t, J = 24 Hz), 98.7 (d, J = 4 Hz), 57.3 (d, J = 10 Hz), 40.8 (s). IR: 3036, 1711, 1630, 1525, 1240, 1143, 825 cm^{-1}. MS m/z: 405 $(M + H)^+$. Anal. ($C_{19}H_{15}F_3N_4O_3 \cdot 0.4H_2O$) C, H, N.

7-(3-Aminoazetidin-1-yl)-1-(5-amino-2,4-difluorophenyl)-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (27). Colorless solid (74% yield); mp >203 °C (decomposed). ^{1}H NMR (DMSO-d_6): δ 8.45 (1H, s), 7.77 (1H, d, J = 12.4 Hz), 7.38 (1H, t, J = 10.6 Hz), 7.07 (1H, t, J = 8.3 Hz), 5.44 (2H, s), 4.42 (2H, brs), 3.91 (2H, brs), 3.77−3.73 (1H, m). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-d): δ 175.7 (s), 165.0 (s), 150.7 (s), 149.9 (dd, J = 246, 8 Hz), 149.6 (dd, J = 244, 10 Hz), 146.9 (dd, J = 239, 12 Hz), 139.0 (dd, J = 245, 7 Hz), 133.9 (d, J = 15 Hz), 132.6 (dd, J = 15, 12 Hz), 128.1 (d, J = 3 Hz), 126.6 (d, J = 15 Hz), 114.9 (s), 113.6(s), 107.4 (s), 106.9 (d, J = 21 Hz), 104.5 (t, J = 25 Hz), 59.5 (s), 42.1 (s). IR: 2980, 1622, 1527, 1257, 1145, 825 cm^{-1}. MS m/z: 423 $(M + H)^+$. Anal. ($C_{19}H_{14}F_4N_4O_3 \cdot 0.75H_2O$) C, H, N.

7-(3-Aminoazetidin-1-yl)-1-(5-amino-2,4-difluorophenyl)-8-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (4). Colorless solid (70% yield); mp >270 °C (decomposed). ^{1}H NMR (DMSO-d_6): δ 8.42 (1H, s), 7.87 (1H, d, J = 13.8 Hz), 7.32 (1H, t, J = 10.7 Hz), 6.96 (1H, t, J = 8.2 Hz), 5.32 (s, 1H), 4.69−4.63 (2H, m), 4.10−4.03 (2H, m), 3.75 (1H, brs). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-d): δ 175.8 (s), 164.7 (s), 151.8 (s), 150.2 (d, J = 249 Hz), 149.0 (dd, J = 244, 10 Hz), 146.9 (dd, J = 239, 12 Hz), 142.0 (d, J = 12 Hz), 136.6 (s), 134.2 (d, J = 14 Hz), 126.9 (dd, J = 15, 2 Hz), 117.7 (d, J = 8 Hz), 113.4 (d, J = 6 Hz), 110.0 (d, J = 23 Hz), 108.1 (s), 104.5 (d, J = 7 Hz), 104.3 (t, J = 24 Hz), 60.8 (d, J = 6 Hz), 60.7 (d, J = 8 Hz), 41.4 (d, J = 5 Hz). IR: 3331, 1718, 1618, 1521, 1452, 1255, 1143, 891 cm^{-1}. MS m/z: 439 $(M + H)^+$. Anal. ($C_{19}H_{14}ClF_3N_4O_3 \cdot 0.75H_2O$) C, H, N.

7-(3-Aminoazetidin-1-yl)-1-(5-amino-2,4-difluorophenyl)-8-bromo-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (28). The quinoline-3-carboxylic acid derivative **13d** (120 mg, 0.27 mmol), 3-aminoazetidine dihydrochloride (73 mg, 0.5 mmol), LiCl (50 mg, 1.2 mmol), and Et_3N (300 mg, 3.0 mmol) were dissolved in DMSO (0.6 mL). The solution was stirred at 60 °C for 2 h. After addition of Et_2O (15 mL), the supernatant was removed by decantation. To the residue was added H_2O (3 mL). The precipitate was collected by filtration and washed with EtOH and Et_2O. The crude solid was purified by reprecipitaiton from its DMF solution with EtOH to obtain 110 mg of **28** as a yellow solid (82% yield); mp >206 °C (decomposed). ^{1}H NMR (DMSO-d_6): δ 8.43 (1H, s), 7.86 (1H, d, J = 13.2 Hz), 7.36 (1H, t, J = 10.6 Hz), 6.89 (1H, t, J = 8.7 Hz), 5.39 (2H, s), 4.65 (2H, s), 4.00 (2H, s), 3.75−3.66 (1H, m). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-d): δ 175.9 (s), 164.8 (s), 152.0 (s), 150.3 (d, J = 251 Hz), 149.7 (dd, J = 244, 11 Hz), 146.7 (dd, J = 239, 12 Hz), 144.4 (d, J = 11 Hz), 138.3 (s), 134.5 (d, J = 14 Hz), 126.6 (dd, J = 13, 4 Hz), 118.3 (d, J = 8 Hz), 113.3 (s), 110.4 (d, J = 24 Hz), 108.3 (s), 104.6 (t, J = 25 Hz), 92.4 (d, J = 4 Hz), 61.0 (s), 60.8 (s), 41.0 (s). IR: 3072, 1718, 1620, 1523, 1143, 869 cm^{-1}. MS m/z: 483 and 485 for Br isotopic peaks of $(M + H)^+$. Anal. ($C_{19}H_{14}BrF_3N_4O_3 \cdot 1.5H_2O$) C, H, N.

7-(3-Aminoazetidin-1-yl)-1-(5-amino-2,4-difluorophenyl)-6-fluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (29). Colorless solid (69% yield); mp 203−205 °C. ^{1}H NMR (DMSO-d_6): δ 8.46 (1H, s), 7.76 (1H, d, J = 13.7 Hz), 7.40 (1H, t, J = 10.6 Hz), 7.02 (1H, t, J = 8.1 Hz), 5.46 (2H, s), 4.46−4.35 (2H, m), 3.86 (1H, m), 3.75−3.67 (2H, m), 1.63 (3H, s). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-d): δ 176.5 (s), 165.3 (s), 151.4 (s), 150.8 (d, J = 257 Hz), 149.4 (dd, J = 245, 11 Hz), 146.1 (dd, J = 240, 12 Hz), 145.7 (d, J = 10 Hz), 139.3 (s), 134.7 (d, J = 14 Hz), 127.6 (dd, J = 13, 3 Hz), 118.1 (d, J = 8 Hz), 113.1 (d, J = 4 Hz), 113.0 (s), 108.7 (d, J = 23 Hz), 107.5 (s), 104.7 (t, J = 24 Hz), 60.4 (s), 60.2 (s), 41.1 (s), 17.1 (s). IR: 3094, 1718, 1628, 1523, 1248, 1145, 821 cm^{-1}. MS m/z: 419 $(M + H)^+$. Anal. ($C_{20}H_{17}F_3N_4O_3 \cdot 1.5H_2O$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-8-chloro-6-fluoro-7-(3-methylaminoazetidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (30). Colorless solid (52% yield); mp 220−224 °C. ^{1}H NMR (DMSO-d_6): δ 8.44 (1H, s), 7.87 (1H, d, J = 13.8 Hz), 7.36 (1H, t, J = 10.5 Hz), 6.95 (1H, t, J = 8.7 Hz), 5.41 (2H, s), 4.67−4.55 (2H, m), 4.15−4.06 (2H, m), 3.48−3.41 (1H, m), 2.20 (3H, s). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-d): δ 175.8 (s), 164.8 (s), 151.9 (s), 150.1 (d, J = 249 Hz), 149.7 (dd, J = 244, 10 Hz), 146.9 (dd, J = 239, 12 Hz), 141.9 (d, J = 12 Hz), 136.5 (s), 134.2 (d, J = 15 Hz), 126.8 (dd, J = 13, 4 Hz), 117.6 (d, J = 8 Hz), 113.3 (s), 110.1 (d, J = 22 Hz), 108.1 (s), 105.0 (d, J = 6 Hz), 104.4 (t, J = 24 Hz), 59.4 (s), 48.8 (s), 30.2 (s). IR: 3335, 1718, 1622, 1525, 1263, 883 cm^{-1}. MS m/z: 453 and 455 for Cl isotopic peaks of $(M + H)^+$. Anal. ($C_{20}H_{16}ClF_3N_4O_3 \cdot 1.5H_2O$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-8-chloro-7-(3-ethylaminoazetidin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (31). Colorless solid (71% yield); mp 208−209 °C. ^{1}H NMR (DMSO-d_6): δ 8.43 (1H, s), 7.86 (1H, d, J = 14.2 Hz), 7.36 (1H, t, J = 10.6 Hz), 6.96 (1H, t, J = 8.5 Hz), 5.42 (2H, s), 4.68−4.60 (2H, m), 4.15−4.08 (2H, m), 3.58−3.50 (1H, m), 2.47 (2H, q, J = 6.9 Hz), 0.97 (3H, t, J = 6.9 Hz). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-d): δ 175.8 (s), 164.8 (s), 151.9 (s), 150.1 (d, J = 249 Hz), 149.7 (dd, J = 245, 11 Hz), 146.9 (dd, J = 238, 12 Hz), 141.9 (d, J = 12 Hz), 136.5 (s), 134.2 (d, J = 16 Hz), 126.8 (dd, J = 13, 4 Hz), 117.6 (d, J = 7 Hz), 113.3 (t, J = 6 Hz), 110.1 (d, J = 23 Hz), 108.1 (s), 105.0 (d, J = 6 Hz), 104.4 (t, J = 25 Hz), 59.6 (s), 47.2 (s), 11.0 (s). IR: 1622, 1525, 1263, 1147, 817 cm^{-1}. MS m/z: 467 $(M + H)^+$. Anal. ($C_{21}H_{18}ClF_3N_4O_3 \cdot 0.5H_2O$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-8-chloro-7-(3-dimethylaminoazetidin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (32). Yellowish solid (63% yield); mp >256 °C (decomposed). ^{1}H NMR (DMSO-d_6): δ 8.45 (1H, s), 7.87 (1H, d, J = 13.6 Hz), 7.37 (1H, t, J = 10.6 Hz), 6.95 (1H, t, J = 8.3 Hz), 5.40 (2H, s), 4.57−4.51 (2H, m), 4.25−4.17 (2H, m), 3.05−2.96 (1H, m), 2.07 (6H, s). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-d): δ 175.8 (s), 164.8 (s), 151.9 (s), 150.2 (d, J = 249 Hz), 149.8 (dd, J = 245, 11 Hz), 147.0 (dd, J = 239, 11 Hz), 141.8 (d, J = 12 Hz), 136.4 (s), 134.2 (d, J = 16 Hz), 126.8 (dd, J = 14, 4 Hz), 117.8 (d, J = 8 Hz), 113.4 (s), 110.1 (d, J = 23 Hz), 108.0 (s), 105.4 (d, J = 6 Hz), 104.4 (t, J = 25 Hz), 58.3 (s), 55.8 (d, J = 5 Hz). IR: 3391, 1718, 1624, 1529, 1446, 1226, 1157 cm^{-1}. MS m/z: 467 and 469 for Cl isotopic peaks of $(M + H)^+$. Anal. ($C_{21}H_{18}ClF_3N_4O_3$) C, H, N.

1-(5-Amino-2,4-difluorophenyl)-7-(3-aminio-3-methylazetidin-1-yl)-8-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (33). Colorless solid (96% yield); mp 252−257 °C. ^{1}H NMR (DMSO-d_6): δ 8.43 (1H, s), 7.86 (1H, d, J = 13.7 Hz), 7.36 (1H, t, J = 10.5 Hz), 6.95 (1H, t, J = 8.7

Hz), 5.42 (2H, s), 4.33−4.27 (2H, m), 4.21−4.14 (2H, m), 1.34 (3H, s). ^{13}C NMR (DMSO-d_6 with 1 equiv TFA-d): δ 175.8 (s), 164.8 (s), 151.9 (s), 150.3 (d, J = 250 Hz), 149.7 (dd, J = 244, 10 Hz), 146.9 (dd, J = 239, 12 Hz), 142.1 (d, J = 12 Hz), 134.2 (d, J = 14 Hz), 126.9 (dd, J = 14, 3 Hz), 117.5 (d, J = 8 Hz), 115.6 (s), 110.0 (d, J = 23 Hz), 108.0 (s), 105.1 (d, J = 6 Hz), 104.3 (t, J = 24 Hz), 65.9 (d, J = 7 Hz), 50.1 (s), 22.0 (s). IR: 1730, 1624, 1523, 1452, 885 cm^{-1}. MS m/z: 453 and 455 for Cl isotopic peaks of $(M + H)^+$. Anal. ($C_{20}H_{16}ClF_3N_4O_3 \cdot 0.5H_2O$) C, H, N.

In Vitro Antibacterial Activity. Newly synthesized compounds (**3**, **4**, and **14**−**33**) and reference compounds (**1**, **2**, **34**, and **35**) were evaluated for their in vitro antibacterial activity against representative organisms; Gram (+) organisms including *S. aureus* 209P, *S. aureus* smith, MRSA W200, *Staphylococcus epidermidis* IFO12293, *Enterococcus faecalis* IFO12580, *E. faecalis* ATCC19433, *E. faecium* W1140, *Micrococcus luteus* IFO12708, *Bacillus subtilis* ATCC6633, *E. faecium* (VanA) ATCC51559, *Enterococcus casseliflavus* (VanC) ATCC51559, *E. faecalis* (VanB) ATCC51299, and *Enterococcus gallinarum* (VanC) ATCC49573, and Gram (−) organisms including *E. coli* NIHJ-JC2, *E. coli* NIHJ, *Citrobacter freundii* IFO1268, *Klebsiella pneumoniae* KC-1, *K. pneumoniae* DT-S, *Salmonella typhimurium* IFO13245, *Enterobacter cloacae* IFO13535, *Proteus vulgaris* IFO3167, *Serratia marcescens* IFO3736, *Morganella morganii* W1026, *Providencia rettgeri* W1008, *P. aeruginosa* IFO3445, *P. aeruginosa* E-2, and *P. aeruginosa* 15846. The antibacterial activities of compounds **1**, **4**, **34**, and **35** were determined against quinolone susceptible *S. pneumoniae* W1466 and three quinolone resistant isolates including MRSA W9501, *P. aeruginosa* W348, and *E. coli* W9523. The minimum inhibitory concentrations (MICs) were determined using standard agar dilution method as described by the National Committee for Clinical Laboratory Standards.[26] Two-fold dilution of the test compound in the range of 128−0.002 μg/mL was carried out in Muller Hinton Medium (Difco). For *S. pneumoniae*, 10% horse blood was added to Muller Hinton Medium. The plate was inoculated with approximately 10^4 organisms and then incubated at 37 °C for 18 h. The MIC was the lowest concentration of the test compound that yielded no visible growth on the plate.

Crystallographic Study of 31·TsOH·EtOH. A crystal suitable for X-ray crystallographic analysis was obtained from a DMSO solution containing an equimolar TsOH by using the vapor diffusion method with EtOH. Colorless prisms of **31**·TsOH·EtOH ($C_{30}H_{32}ClF_3N_4O_7S$, M_r = 685.11) with approximate dimensions of 0.25 mm × 0.10 mm × 0.10 mm was mounted in a loop and used for data collection. All measurements were made on a Rigaku RAXIS-RAPID Imaging Plate diffract meter with graphite monochronomated Mo Kα radiation. Indexing was performed from three oscillations, which were exposed for 0.6 min. The camera radius was 127.40 mm. Readout was performed in the 0.100 mm pixel mode. The data were collected at a temperature of −180 ± 1 °C to a maximum 2θ value of 59.9°. A total of 44 images, corresponding to 220.0° oscillation angles, were collected with two different goniometer settings. Exposure time was 4.00 min per degree. The structure was solved by the direct methods (SIR 97) and expanded using Fourier techniques (DIRDIF 94). All calculations were performed using the PROCESS-AUTO program package. Crystal data for **31**·TsOH·EtOH: monoclinic, space group $P2_1/c$ (no. 14), a = 15.502 (1) Å, b = 8.5274(5) Å, c = 23.390(1) Å, β = 106.599(2)°, V = 2963.1(3) Å^3, Z = 4, D_{calcd} = 1.536 g/cm^3, $2\theta_{max}$ = 59.9°, total no. of reflections = 33 174. The nonhydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 8378 observed reflections ($I > -3.00\sigma(I)$, $2\theta < 59.94°$) and 415 variable parameters and converged (largest parameter shift was 0.00 times its esd) with unweighted and weighted agreement factors of R (= $\sum(F_o^2 - F_c^2)/\sum F_o^2$) = 0.088, R_w (= $(\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2)^{0.5}$) = 0.107, and $R1 = \sum||F_o| - |F_c||/\sum|F_o|$ = 0.051 for $I > 2.0\sigma(I)$.

Molecular Modeling Study. All calculations were performed using the CAChe WorkSystem Version 5.0 (Oxford Molecular Limited). The X-ray crystallographic data of **31** were used as starting geometries of the quinolones **4** and **26**−**29**. By concerning their main species at physiological pH, calculations were performed as their zwitterionic species. The structures of the these compounds are refined by performing a preoptimization calculation in Mechanics using Augmented MM3, followed by an optimized geometry calculation in a molecular orbital package (MOPAC 2002) using a Hamiltonian, PM3 parameters, and including solvent effects of water simulated by Conductor-like Screening model (COSMO).

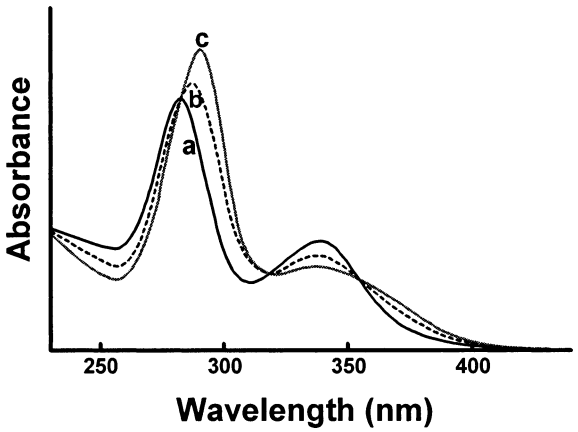


Figure 3. UV−pH profile for 10 μM **4** at 35 °C with I = 0.10 (NaCl) in aqueous solution: (a) at pH 7.4, λ_{max} = 282 (ϵ = 3.6 × 10^4) and 340 nm (ϵ = 1.6 × 10^4); (b) at pH 5.2; (c) at pH 3.4, λ_{max} = 290 (ϵ = 4.4 × 10^4) and 337 nm (ϵ = 1.2 × 10^4).

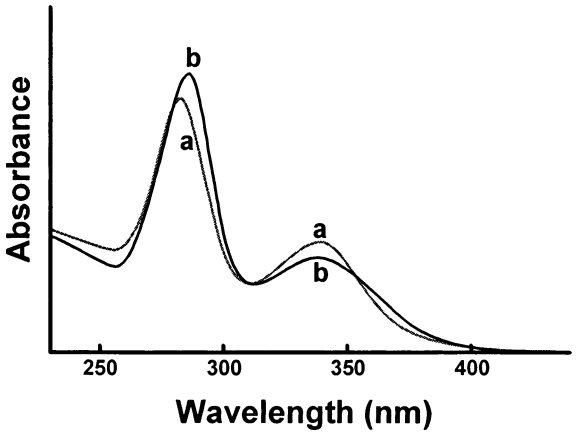


Figure 4. UV absorption spectra for 10 μM **4** at 35 °C and pH 7.4 with I = 0.10 (NaCl) in aqueous solution: (a) in the absence of Mg^{2+} ion; (b) in the presence of 20 mM Mg^{2+} ion, λ_{max} = 285 (ϵ = 4.0 × 10^4) and 337 nm (ϵ = 1.4 × 10^4).

Measurements of pK_a Values and Dissociation Constants of 4 and 27 in Aqueous Solution. Deprotonation constants (pK_a) of monoprotonated species for **4** and **27** were estimated by using pKalc 5.0 (PALLAS version 3.0, CompuDrug International Inc.). Almost the same values were obtained for 3-carboxylic acid, and protonated aminoazetidine is ca. 5.6 and 9.7, respectively. To define the protonation of the 3-carboxylate, spectrophotometric pH titrations with 10 μM ligand (**4** and **27**) were conducted at 35 °C with I = 0.10 (NaCl) in aqueous solution. With decreasing pH, the UV absorption band at ca. 340 nm decreased while another band at ca. 280 nm increased (see Figure 3 for **4**). The UV−pH profile for **4** gave a pK_a value of 5.4 ± 0.1, which is almost the same as the calculated value for the carboxylic acid. The pK_a value for the carboxylic acid of **27** was similarly determined to be 5.4 ± 0.1.

In the presence of excess amounts of Mg^{2+} ion at 35 °C and pH 7.4 with I = 0.10 (NaCl), the ligands **4** and **27** showed similar UV spectral changes as shown for the carboxylate protonation (see Figure 4). The addition of $MgCl_2$ (0.2, 0.6, 1.4,

3.0, 6.0, 10.0, and 20.0 mM) did not change the solution pH of 7.4 for the 10 μM ligands, indicating that the Mg^{2+} complexation occurs without protonation and deprotonation (e.g., $-NH_3^+$ to $-NH_2$). A typical UV absorption spectrum for **4** with 20 mM Mg^{2+} ion is shown in Figure 2b. A well-known double reciprocal plot of the absorbances at 340 nm against concentrations of Mg^{2+} ion gave a line, from which a Mg^{2+} dissociation constant of 2.0 ± 0.2 mM is estimated as $-1/x$-intercept. Compound **27** gave almost the same dissociation constants of 2.1 ± 0.2 mM.

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Supporting Information Available: Data for the X-ray crystal structure determination of **31**·TsOH·EtOH and the table of in vitro antibacterial activity. These materials are available free of charge via the Internet at http://pubs.acs.org.

References

(1) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. Structure−Activity Relationships of Antibacterial 6,7- and 7,8-Disubstituted 1-Alkyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acids. *J. Med. Chem.* **1980**, *23*, 1358−1363.

(2) Wise, R.; Andrews, J. M.; Edwards, L. J. In Vitro Activity of Bay 09867, A New Quinoline Derivative, Compared with Those of Other Antimicrobial Agents. *Antimicrob. Agents Chemother.* **1983**, *23*, 559−564.

(3) Takahata, M.; Otsuki, M.; Nishino, T. *In Vitro* and *In Vivo* Activities of T-3262, a New Pyridonecarboxylic Acid. *J. Antimicrob. Chemother.* **1988**, *22*, 143−154.

(4) Hayakawa, I.; Hiramatsu, T.; Tanaka, Y. Synthesis and Antibacterial Activities of Substituted 7-Oxo-2,3-dihydro-7*H*-pyrido-[1,2,3-de][1,4]benzoxazine-6-carboxylic Acids. *Chem. Pharm. Bull.* **1984**, *32*, 4907−4913.

(5) Domagala, J. M. Structure−Activity and Structure-Side-Effect Relationships for the Quinolone Antibacterials. *J. Antimicrob. Chemother.* **1994**, *33*, 685−706.

(6) (a) Domagala, J. M.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Nicols, M. J.; Solomon, M.; Worth, D. F. 1-Substituted 7-{3-[(Ethylamino)methyl]-1-pyrrolidinyl}-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acids. New Quantitative Structure−Activity Relationships at N1 for the quinolone Antibacterials. *J. Med. Chem.* **1988**, *31*, 991−1001. (b) Chu, D. T. W.; Fernandes, P. B. Structure−Activity Relationships of the Fluoroquinolones. *Antimicrob. Agents Chemother.* **1989**, *33*, 131−135.

(7) (a) Neu, H. C.; Novelli, A.; Chin, N. X. Comparative In Vitro Activity of a New Quinolone, AM-1091. *Antimicrob. Agents Chemother.* **1989**, *33*, 1036−1041. (b) Suto, M. J.; Domagala, J. M.; Roland, G. E.; Mailloux, G. B.; Cohen, M. A. Fluoroquinolones: Relationships between Structural Variations, Mammalian Cell Cytotoxicity, and Antibacterial Activity. *J. Med. Chem.* **1992**, *35*, 4745−4750.

(8) (a) Man, I.; Murphy, J.; Ferguson, J. Fluoroquinolone Phototoxicity: A Comparison of Moxifloxacine and Lomefloxacin in Normal Volunteers. *J. Antimicrob. Chemother.* **1999**, *43* (Suppl. B), 77−82. (b) Dalhoff, A.; Peterson, U.; Endermann, R. In Vitro Activity of BAY 12−8039, A New 8-Methoxyquinolone. *Chemotherapy* **1996**, *42* (6), 410−425. (c) Sanchez, J. P.; Gogliotti, R. D.; Domagala, J. M.; Gracheck, S. J.; Huband, M. D.; Sesnie, J. A.; Cohen, M. A.; Shapiro, M. A. The Synthesis, Structure−Activity, and Structure-Side Effect Relationships of a Series of 8-Alkoxy- and 5-Amino-8-alkoxyquinolone Antibacterial Agents. *J. Med. Chem.* **1995**, *38*, 4478−4487.

(9) Sanchez, J. P.; Bridges, A. J.; Bucsh, R.; Domagala, J. M.; Gogliotti, R. D.; Hagen, S. E.; Heifetz, C. L.; Joannides, E. T.; Sesnie, J. C.; Shapiro, M. A.; Szotek, D. L. New 8-(Trifluoromethyl)-Substituted Quinolones. The Benefits of the 8-Fluoro Group with Reduced Phototoxic Risk. *J. Med. Chem.* **1992**, *35*, 361−367.

(10) (a) Li, Q.; Chu, D. T. W.; Claiborne, A.; Cooper, C. S.; Lee, C. M.; Raye, K.; Berst, K. B.; Donner, P.; Wang, W.; Hasvold, L.; Fung, A.; Ma, Z.; Tufano, M.; Flamm, R. K.; Shen, L. L.; Baranowski, J.; Nilius, A. M.; Alder, J. D.; Meulbroek, J. A.; Marsh, K.; Crowell, D.; Hui, Y.; Seif, L.; Melcher, L. M.; Henry, R.; Spanton, S.; Faghih, R.; Klein, L. L.; Tanaka, S. K.; Plattner, J. J. Synthesis and Structure−Activity Relationships of 2-Pyridones: A Novel Series of Potent DNA Gyrase Inhibitors as Antibacterial Agents. *J. Med. Chem.* **1996**, *39*, 3070−3088. (b) Ma, Z.; Chu, D. T. W.; Cooper, C. S.; Li, Q.; Fung, A. K. L.; Wang, S.; Shen, L. L.; Flamm, R. K.; Nilius, A. M.; Alder, J. D.; Meulbroek, J. A.; Or, Y. S. Synthesis and Antimicrobial Activity of 4*H*-4-Oxoquinolidine Derivatives: Consequences of Structural Modification at the C-8 Position. *J. Med. Chem.* **1999**, *42*, 4202−4213.

(11) (a) Pfaller, M. A.; Jones, R. N.; Doern, G. V. Bacterial Pathogens Isolated from Patients with Bloodstream Infections: Frequencies of Occurrence and Antimicrobial Susceptibility Patterns from The SENTRY Antimicrobial Surveillance Program. *Antimicrob. Agents Chemother.* **1998**, *42*, 1762−1770. (b) Lehner, S. A. Clinical Impact of Antibiotic Resistance. *Adv. Exp. Med. Biol.* **1998**, *456*, 7−15. (c) Livermore, D. M. Epidemiology of antibiotic resistance. *Intensive Care Med.* **2000**, *26* (Suppl. 1), S14−S21.

(12) (a) Norrby, S. R.; Lietman, P. S. Safety and Tolerability of Fluoroquinolones. *Drugs* **1993**, *45* (Suppl. 3), 59−64. (b) Martinez, L. J.; Sik, R. H.; Chignell, C. F. Fluoroquinolone Antimicrobials: Singlet Oxygen, Superoxide and Phototoxicity. *Photochem. Photobiol.* **1998**, *67*, 399−403. (c) Blodeau, J. M. Expanded Activity and Utility of the New Fluoroquinolones: A Review. *Clin. Ther.* **1999**, *21*, 3−40.

(13) Kahn, J. B. Quinolone-induced QT Interval Prolongation: A Not-so-unexpected Class Effect. *J. Antimicrob. Chemother.* **2000**, *46*, 847−848.

(14) Kimura, Y.; Atarashi, S.; Kawakami, K.; Sato, K.; Hayakawa, I. (Fluorocyclopropyl)quinolones. 2. Synthesis and Stereochemical Structure−Activity Relationships of Chiral 7-(7-Amino-5-azaspiro[2.4]heptan-5-yl)-1-(2-fluorocyclopropyl)quinolone Antibacterial Agents. *J. Med. Chem.* **1994**, *37*, 3344−3352.

(15) Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Pihuleac, E.; Nordeen, C. W.; Maleczka, R. E., Jr.; Pernet, A. G. Synthesis and Structure−Activity Relationships of Novel Arylfluoroquinolone Antibacterial Agents. *J. Med. Chem.* **1985**, *28*, 1558−1564.

(16) Yazaki, A.; Hayashi, N. (Wakunaga Pharmaceutical Co., Ltd.). Unpublished results.

(17) Frigola, J.; Pares, J.; Corbera, J.; Vano, D.; Merce, R.; Torrens, A.; Mas, J.; Valenti, E. 7-Azetidinylquinolones as Antibacterial Agents. Synthesis and Structure−Activity Relationships. *J. Med. Chem.* **1993**, *36*, 801−810.

(18) (a) Chatterjee, S. S.; Shoeb, A. General Method for the Synthesis of Substituted Azetidines. *Synthesis* **1973**, 153−154. (b) Chatterjee, S. S. Synthesis and Reaction of 3-Azetidinone. *Tetrahedron Lett.* **1972**, 5063−5064. (c) Okutani, T.; Kaneko, T.; Masuda, K. Studies on Azetidine Derivatives. 1. Synthesis of 3-Substituted Azetidine Derivatives. *Chem. Pharm. Bull.* **1974**, *22*, 1490−1497.

(19) Renau, T. E.; Snchez, J. P.; Domagala, J. M. The Synthesis of 3-Bromo-2,4,5-trifluorobenzoic Acids and its Conversion to 8-Bromoquinolonecarboxylic Acids. *J. Heterocycl. Chem.* **1996**, *33*, 1407−1411.

(20) Gooding, B. B.; Jones, R. N. In Vitro Antimicrobial Activity of CP-99, 219, A Novel Azabicyclo-naphthyridone. *Antimicrob. Agents Chemother.* **1993**, *37*, 349−353.

(21) (a) Stahlmann, R.; Lode, H. Toxicity of quinolones. *Drugs* **1999**, *58* (Suppl. 2), 37−42. (b) Snyder, R. D.; Cooper, C. D. Photogenotoxicity of Fluoroquinolones in Chinese Hamster V79 Cells: Dependency on Acitve Topoisomerase II. *Phototoxicity Photobiol.* **1999**, *69* (3), 288−293.

(22) Turel, I. The Interaction of Metal Ions with Quinolone Antibacterial Agents. *Coord. Chem. Rev.* **2002**, *232*, 27−47.

(23) Shen, L. L.; Mitscher, L. A.; Sharma, P. N.; O'Donnell, T. J.; Chu, D. W. T.; Cooper, C. S.; Rosen, T.; Pernet, A. G. Mechanism of Inhibition of DNA Gyrase by Quinolone Antibacterials: A Cooperative Drug DNA Binding Model. *Biochemistry* **1989**, *28*, 3886−3894.

(24) Chen, Z. F.; Xiong, R. G.; Zuo, J. L.; Guo, Z.; You, X. Z.; Fun, H. K. X-ray Crystal Structurers of Mg^{2+} and Ca^{2+} Dimers of The Antibacterial Drug Norfloxacin. *J. Chem. Soc. Dalton Trans.* **2000**, 4013−4014.

(25) (a) Sissi, C.; Andreolli, M.; Cecchetti, V.; Fravolini, A.; Gatto, B.; Palumbo, M. Mg^{2+}-Mediated Binding of 6-Substituted Quinolones to DNA: Relevance to Biological Activity. *Bioorg. Med. Chem.* **1998**, *6*, 1555−1561. (b) Marshall, A. J.; Piddock, L. J. Interaction of Divalent Cations, Quinolones and Bacteria. *J. Antimicrob. Chemother.* **1994**, *34*, 465−483. (c) Palu, G.; Valisena, S.; Ciarrocchi, G.; Gatto, B.; Palumbo, M. Quinolone Binding to DNA is Mediated by Magnesium Ions. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *6*, 1555−1561.

(26) National Committee for Clinical and Laboratory Standards. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. *Approved Standard*, 5th ed.; NCCLS: U.S.A., 2000; M7-A5, Vol. 20, No. 2.

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