A Prodrug Approach toward the Development of Water Soluble Fluoroquinolones and Structure−Activity Relationships of Quinoline-3-carboxylic Acids

William R. Baker, Shaopei Cai, Martin Dimitroff,* Liming Fang, Kay K. Huh, David R. Ryckman, Xiao Shang, Ribhi M. Shawar, and Joseph H. Therrien

Chiron Corporation, 201 Elliott Avenue West, Seattle, Washington 98119

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A fluoroquinolone prodrug, PA2808, was prepared and shown to convert to the highly active parent drug PA2789. In vitro and in vivo activation of PA2808 by alkaline phosphatase was demonstrated using disk diffusion and rat lung infection models. The water solubility of PA2808 showed a marked increase compared to PA2789 over a pH range suitable for aerosol drug delivery. A total of 48 analogues based on PA2789 were prepared and screened against a panel of Gram-positive and Gram-negative pathogens. Incorporating a cyclopropane-fused pyrrolidine (amine **g**) at C-7 resulted in some of the most active analogues.

Introduction

Interest in the development of new quinolone antibacterial agents remains high, nearly 40 years after the clinical introduction of nalidixic acid (Figure 1).[1] The incorporation of a C-7 piperazinyl group and, most importanly, a C-6 fluorine substituent gave rise to a class of compounds known as fluoroquinolones. These compounds proved to be more potent than nalidixic acid and served as a template for a large number of analogues. More recently developed fluoroquinolones such as ciprofloxacin, ofloxacin, and tosufloxacin are examples from this important class of therapeutically useful drugs.[2] Many fluoroquinolones have microbiological activity similar to that of the aminoglycosides tobramycin, gentimicin, and amikacin and, as such, are clinically important. In general, fluoroquinolones exhibit high intracellular penetration and have relatively long half-lives with good oral bioavailability. These factors all contribute to the continuing efforts of researchers to develop novel and more potent fluoroquinolones.

The mode of action of fluoroquinolones has been determined to involve the inhibition of the bacterial enzymes DNA topoisomerase IV and DNA gyrase (topoisomerase II).[3] Both of these enzymes play a vital role in DNA replication. The bidirectional nature of DNA replication is such that the daughter DNA molecules become catenated after replication and therefore must be cut and religated for the daughter chromosomes to separate. The necessary separation of the interlinked DNA helices is carried out by topoisomerase IV while DNA gyrase allows the continuation of DNA replication. Fluoroquinolone inhibition of DNA gyrase and topoisomerase IV during DNA replication is a result of the formation of a reversible complex of topoisomerase, cleaved DNA, and quinolone molecule. This results in the degradation of chromosomal DNA into fragments by exonucleases, leading to termination of replication and eventual cell death.

* Author to whom correspondence should be addressed. Tel: (206) 664-6032. Fax: (206)282-5065. E-mail: martin_dimitroff@chiron.com.

As part of our continuing program to develop effective antibacterial agents for lung infections, the known fluoroquinolone PA2789 (Figure 2) was chosen as a platform to determine the feasibility of producing a fluoroquinolone antibacterial agent suitable for aerosol delivery. Aerosolized antibiotic therapy has been demonstrated effective in respiratory-compromised patients, specifically the use of TOBI in cystic fibrosis (CF) patients.

PA2789 was first synthesized in 1985 as part of an investigation to determine the steric effect of N-1 substituents on antibacterial activity.[4] In the course of the study, a series of 4′-*N*-1-aryl-substituted fluoroquinolones were prepared with H, F, Br, Cl, OH, OCH_3, and CH_3 groups at the para position. The *p*-hydroxy derivative (PA2789) proved to be the most potent compound in this series (MIC μg/mL *Staphylococcus aureus* 0.05; *Escherichia coli* 0.1). Although PA2789 demonstrated excellent MICs against a panel of both Gram-positive and Gram-negative organisms, it was later determined to have poor oral bioavailability. This was, however, a desirable property for our current program as our goal was to deliver active compound to the lungs and minimize systemic exposure from any compound entering the stomach during inhalation. The low oral bioavailability of PA2789 precludes high systemic levels of PA2789 during aerosol delivery, confining the drug to pulmonary tissue. An issue that needed to be addressed in order to use PA2789 as an aerosol was the compound's low water solubility. It was decided that modifications to PA2789 would be needed in order to increase the water solubility. The phenolic group at the N-1 position of PA2789 was attractive as a modification point allowing for the synthesis of analogues with potentially improved water solubility. We envisaged that a prodrug of PA2789 containing a phosphate group would be suitable for aerosol delivery due to the ability of the phosphate group to impart greater water solubility as well as be cleaved by ubiquitous alkaline phosphatase releasing the active molecule (PA2789) at the site of infection. The use of a phosphate group was also

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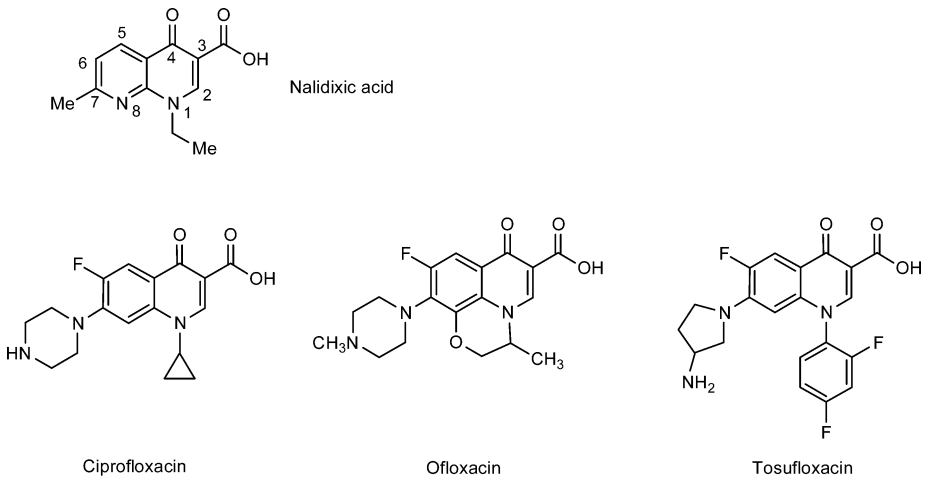


Figure 1. Quinolone numbering and structures of some commercial fluoroquinolones.

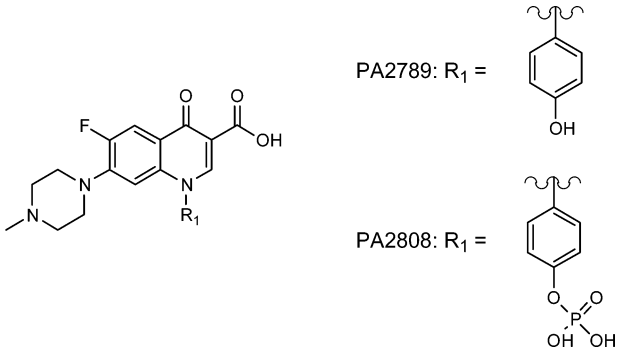


Figure 2. Structures of PA2789 and PA2808.

attractive in light of literature reports showing that cystic fibrosis sufferers have elevated alkaline phosphatase levels in their lung tissue.[5] Jung and co-workers recently described the preparation of an arylglycosidic prodrug of PA2789 to take advantage of the bacterial sugar-dependent phosphotransferase system but reported no activity data for this compound.[6] Along with presenting the synthesis and biological activity of PA2808, a water soluble fluoroquinolone prodrug, the present paper describes the preparation and reports the activity of a variety of PA2789 analogues. While we were naturally interested in the activity of our analogues against *Pseudomonas aeruginosa* (due to this organism's presence in CF patients), a panel consisting of both Gram-positive and Gram-negative organisms was used for activity evaluation.

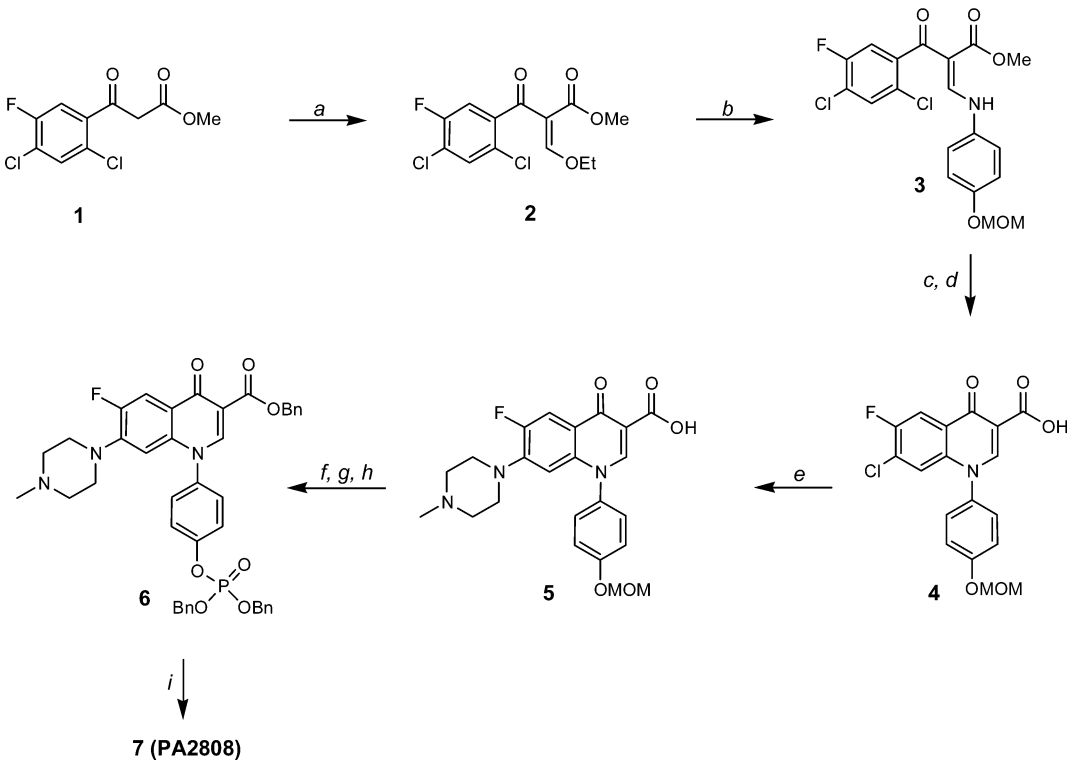
Chemistry

PA2808 Preparation. One of our early syntheses of PA2808 is shown in Scheme 1. The synthesis was based on an approach that is widely used at present for preparing fluoroquinolones.[7]

The synthesis required only two steps from commercially available oxopropionic ester **1** to produce key methylene intermediate **3**. Treatment with sodium hydride resulted in ring closure to give the fluoroquinolone **4**. Hydrolysis of the ester group is necessary to allow for *N*-methylpiperazine substitution at the C-7 chlorine. Following piperazine substitution and re-esterification, the MOM group was removed and the resultant hydroxyl group converted to the benzyl phosphate ester to give dibenzyl phosphate **6**. Removal of the benzyl groups by hydrogenation in the presence of palladium afforded PA2808. Although this synthesis was relatively straightforward, an alternate route that avoided the need to deprotect and reprotect the acid moiety was desired. We also preferred to use 4-aminophenol directly and, therefore, avoid the additional steps involved in synthesizing 4-methoxymethoxyaniline from 4-nitrophenol. Our efforts (Scheme 2) toward the development of a more efficient and scalable synthesis of PA2808 benefited from published reports by Wemple describing the use of magnesium chloride/triethylamine to metalate potassium ethyl malonate in order to produce β-oxo esters in high yield and high purity.[8]

Formation of β-ketoester **9** was achieved by acylation of potassium ethyl malonate with 2,4,5-trifluorobenzoyl chloride in acetonitrile to give a mixture of keto and enol tautomers in 90% yield and sufficient purity to be used as is for the subsequent step. In a three-step one-pot sequence analogous to that described for Scheme 1, ester **9** was treated with triethyl orthoformate in acetic anhydride to give an ethoxymethylene intermediate. Solvent removal under reduced pressure followed by treatment with 4-aminophenol gave an arylmethylene intermediate, which was then subjected to basic conditions in order to effect ring closure to give compound **10**. This facile reaction was accomplished using potassium carbonate and heating the mixture at 95 °C for 70 min, affording fluoroquinolone **10** in an overall yield of 74% for the three steps. Phosphorylation of the arylhydroxyl group or the *N*-methylpiperazine substitution reaction may be carried out on fluoroquinolone **10**. Both transformations gave comparable yields for the two-step sequence to afford the monobenzyl ester **13**. In our hands we found that forming dibenzyl phosphate **11** first was preferred as this compound had greater organic solvent solubility compared to the corresponding piperazine-substituted phenol **12**. Preparation of benzyl phosphate **11** required forming the sodium salt of **10**, followed by treatment with dibenzylphosphoryl chloride in DMF. Reaction with *N*-methylpiperazine in DMSO afforded monobenzyl phosphate **13** in 20% yield for the two steps. The loss of a benzyl group during the *N*-methylpiperazine substitution reaction was not entirely unexpected as demonstrated by literature reports describing the use of amines to deprotect phosphates.[9] It was unexpected that using the standard benzylation

Scheme 1[a]



[a] Conditions: (a) triethyl orthoformate, acetic anhydride, 130 °C, 2 h; (b) 4-methoxymethoxyaniline, CH_2Cl_2, rt, 30 min; (c) sodium hydride, DME, 80 °C, 2 h; (d) NaOH, EtOH, reflux, 3 h; (e) *N*-methylpiperazine, DMSO, 130 °C, 40 min; (f) benzyl alcohol, DMAP, DCC, CH_2Cl_2, 50 °C, 2 h; (g) 4 M HCl, CH_2Cl_2, 0 °C, 30 min; (h) dibenzylphosphoryl chloride, sodium hydride, DMF, 0 °C → rt, 3 h; (i) H_2, Pd/C, MeOH, rt, 1 h.

conditions we were not able to isolate any dibenzylated material. Apparently, the presence of the piperazine moiety results in the loss of a benzyl group. Hydrolysis of ethyl ester **13** with LiOH in methanol, followed by phosphate deprotection under standard hydrogenolysis conditions, afforded PA2808 (**7**).

Preparation of PA2789 Analogues. The fluoroquinolone skeleton provides a number of sites suitable for generating analogues. A brief survey of the literature showed that the carboxylic acid and the C-4 carbonyl group are essential for gyrase activity, while variations at the N-1 substituent, C-7 amino side chain, and C-8 atom can lead to increased activity and better pharmacokinetics. An excellent review of the SAR and structure–side effect relationship for the quinolones by Domagala highlights these trends.[5] The vast majority of fluoroquinolone analogues reported, however, have either a cyclopropyl or an aryl substituent at N-1. With these trends in mind, along with the PK data for the lung residence time for PA2789 and PA2808 (vide infra), we initiated a program to produce a series of analogues based on PA2789. Anecdotal evidence from numerous PK studies by our researchers working on other compounds and programs suggested that polar molecules have a longer lung residence time compared to less polar molecules. It was decided to retain the phenolic N-1 substituent found in PA2789 and to vary the C-7 amino side chain with the aim of imparting greater overall polarity to the analogues. We were also interested in producing a number of novel fluoroquinolones with an N-1 aryl substituent in order to evaluate their antibacterial activity. In addition, two series of analogues related to PA2789, with modifications at C-8, were prepared using the same series of C-7 amino side chains. The three scaffolds (referred to as core I, II, and III) are shown in Figure 3 along with the C-7 substituents that were used in this study (Figure 4).

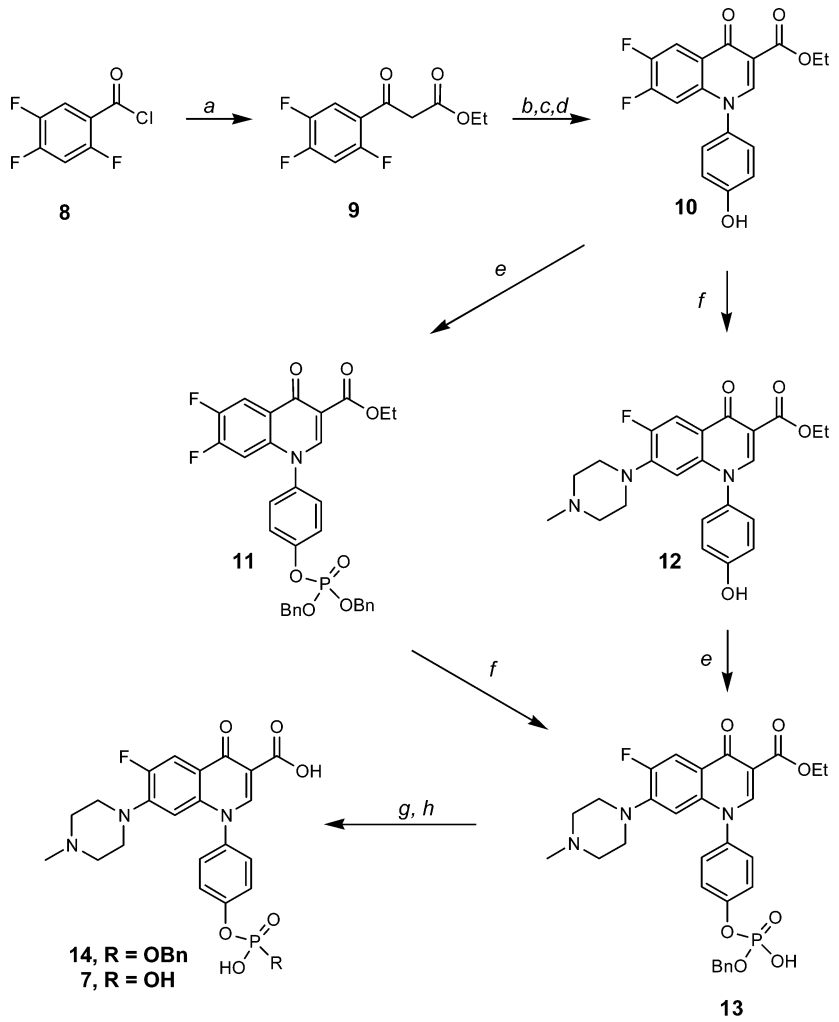
Amino side chains were chosen from those found on a selection of commercially available fluoroquinolones or compounds that have progressed to late-stage development. We hoped that these side chains would retain biological activity for our analogues, while imparting these novel fluoroquinolones with additional overall polarity and/or water solubility.

Core I. The method used to prepare fluoroquinolone core I analogues is shown in Scheme 3.

Ester hydrolysis using 4 N HCl gave the desired acids **A**. This was followed by amine substitution in the presence of triethylamine in DMF to afford the desired fluoroquinolones **B** in good yields. Elaboration to final products involved treatment of **B** with 4 N HCl in dioxane to give the corresponding HCl salts. Using the above procedure, 17 amines were successfully coupled to core I. Amine **c** was coupled using core I directly, followed by ester hydrolysis. Coupling of amines **b**, **k**, and **m1** to core I was not successful using either method. The isolated yields of the core I analogues are shown in Table 1.

Core II. The C-7 side chain of fluoroquinolones continues to be a site targeted for analogue studies. This is undoubtedly due to the large number of amines that are commercially available (or that are easily synthesized) thus allowing for SAR studies. The C-8 group has also been scrutinized in an effort to determine how the nature of the substituent at this location affects activity. As a result of a number of studies, the substituent at

Scheme 2[a]



[a] Conditions: (a) potassium ethyl malonate, CH_3CN, triethylamine, $MgCl_2$, rt, 18 h; (b) triethyl orthoformate, acetic anhydride, reflux, 4 h; (c) 4-aminophenol, DMSO, rt, 18 h; (d) potassium carbonate, DMSO, 95 °C, 70 min; (e) dibenzylphosphoryl chloride, DBU, DMF, 0 °C → rt, 3 h; (f) *N*-methylpiperazine, DMSO, 130 °C, 40 min; (g) lithium hydroxide, MeOH/H_2O, rt, 5 h; (h) H_2, Pd/C, MeOH, rt, 1 h.

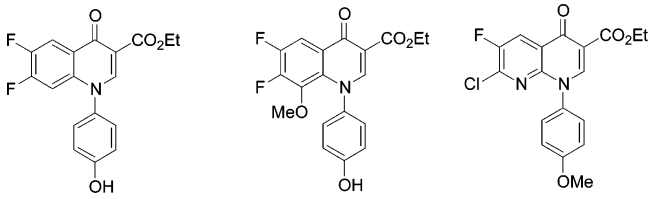


Figure 3. Fluoroquinolone cores used for analogues.

C-8 has been implicated as one of the major contributors to phototoxicity and in vitro genetic toxicity sometimes observed with fluoroquinolones.[10] The highest phototoxicity is seen when the C-8 atom bears a halogen and the least when the substituent at this position is O-alkyl. The order of decreasing phototoxicity is CF > CCl > N > CH > CCF_3 > COR. The picture for genetic toxicity is somewhat less clear with substituents at C-8, where the nature of the C-7 side chain and C-8 substituent all play a role in the molecule's in vitro genetic toxicity. In any event, we undertook to prepare a series of PA2789-based analogues with a methoxy substituent at the C-8 position and test them against our panel of pathogens. The synthesis of core II is outlined in Scheme 4.

Preparation of core II began with commercially available 2,4,5-trifluoro-3-hydroxybenzoic acid (**16**). Following protection of the phenol and acid groups with methyl iodide, the resultant ester **17** was converted to acid chloride **19** by hydrolysis of the ester functionality and subjecting the resultant acid **18** to oxalyl chloride. An analogous series of transformations used for the preparation of PA2789 and PA2808 (see Scheme 2) provided core II.

The preparation of analogues using core II was somewhat more complicated than that for core I (Scheme 5).

Following a procedure described by Sanchez and coworkers, core II was converted to the more reactive BF_2 ester in two steps.[11] The phenol group was first protected as the acetate **20** followed by carboxylic acid group activation via the corresponding BF_2 ester **21**. The basic conditions used in the subsequent amine substitution step removed both the BF_2 and acetyl protecting group of the amines used. For those amines that were Boc-protected, deprotection under standard conditions proved to be somewhat problematic. The material after the Boc deprotection was typically a dark brown oil from which solid material was difficult to isolate.

The BF_2 ester proved reactive under basic conditions to the majority of amines used. Amines containing a

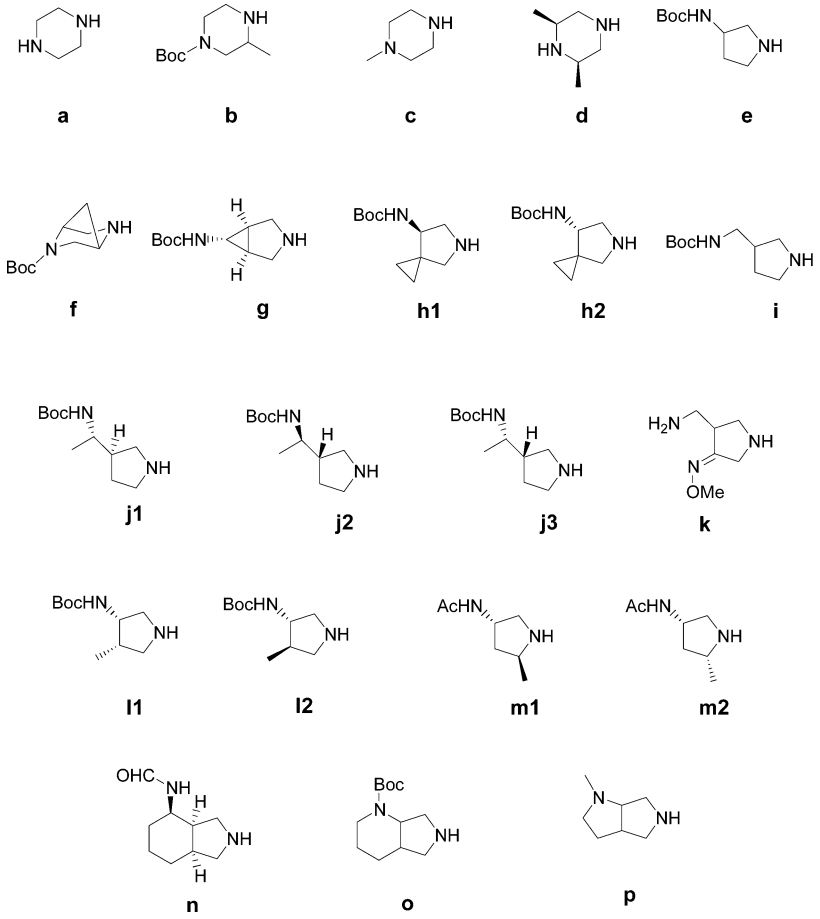
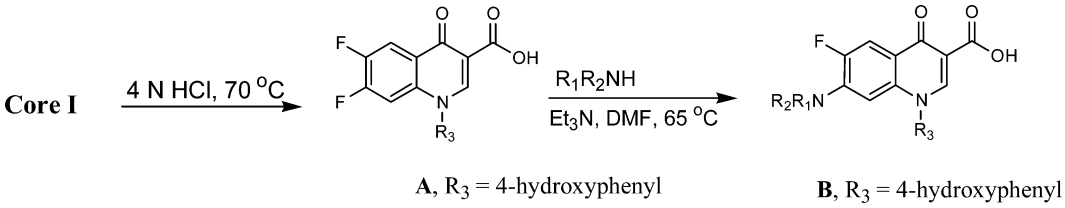


Figure 4. Amines used in analogue studies.

Scheme 3



methyl group adjacent to the nucleophilic nitrogen atom (**b**, **m1**, **m2**) did not give any coupled product, possibly due to unfavorable steric interactions between the C-8 methoxy group and the incoming amine. It was interesting to note that amine **f** was coupled successfully to core II. In this case, the bridged methylene group may not result in an unfavorable steric interaction with the C-8 methoxy group. The structures and yields of the core II analogues are shown in Table 2.

Core III. The 1,8-naphthyridine skeleton of core III was chosen as a candidate in our study based on reports from the late 1980s wherein it was established that the in vivo potency of the naphthyridines was considerably superior to that of the C-8H quinolones.[12] It has been suggested that the greater in vivo potency is a result of the better blood levels often associated with the 1,8-naphthyridines. The preparation of core III is described in Scheme 6 starting from commercially available pyridine **23** following the literature procedure of Bouzard and co-workers.[13]

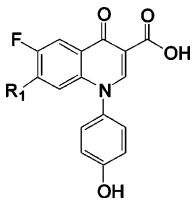
Two different methods (Scheme 7) were employed to attach the amines to the core III structure.

In the first method core III and the amine were combined in the presence of triethylamine in DMF. Ester **25** was then hydrolyzed under basic conditions followed by removal of the phenol protecting group using boron tribromide. For those amines with a Boc protecting group, the BBr_3 phenol deprotection step resulted in concomitant loss of the Boc group and, thus, no further steps were required. In the second method used, both the methyl protecting group and the ester were removed with BBr_3. The resultant fluoroquinolone acid **28** was coupled with the amine in the presence of triethylamine in DMF to give the desired product **29**. For amines with a Boc protecting group, 4 N HCl in dioxane was used to give the product as the HCl salt. The structures and yields of the core III analogues are shown in Table 3.

Results and Discussion

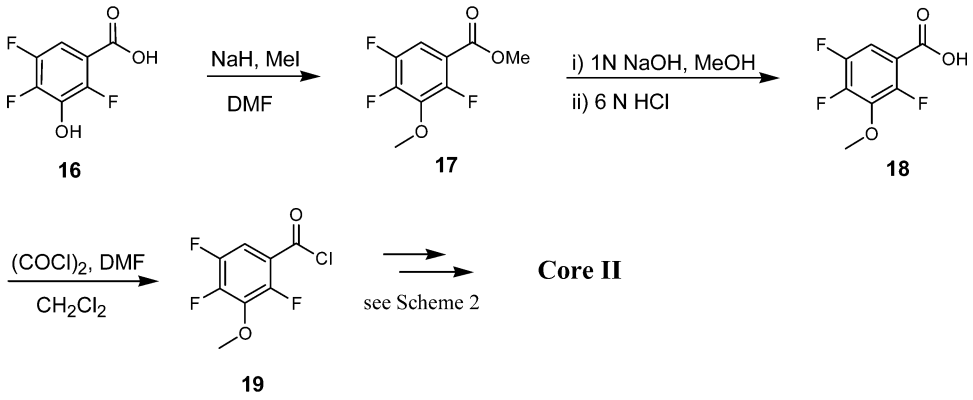
A. PA2808 Water Solubility. Solubility studies of PA2808 were carried out at various pHs at room temperature. The data (Figure 5) shows a clear pH dependency with the water solubility increasing dra-

Table 1.



compound	R_1	yield	compound	R_1	yield
30		83 %	**39**		75 %
31		41 %	**40**		79 %
32		93 %	**41**		67 %
33		72 %	**42**		67 %
34		75 %	**43**		67 %
35		89 %	**44**		98 %
36		65 %	**45**		47 %
37		68 %	**46**		76 %
38		73 %	**47**		79 %

Scheme 4



matically over a narrow range. The water solubility of PA2808 at pH 7.6, 8.0, and 8.3 was found to be 5 mg/mL, 30 mg/mL, and 60 mg/mL, respectively. These results demonstrated that a significant increase in solubility can be achieved at pHs that are considered physiologically acceptable (inhaled aerosols that fall outside of the pH range 4.5−8.7 can cause coughing or bronchoconstriction).[14]

B. Evaluation of in Vitro Activity of PA2808 and Conversion to PA2789. PA2808 was tested against a panel of organisms along with PA2789 and ciprofloxacin (Table 4).

As expected, PA2789 displayed excellent activity against the organisms tested and produced lower MICs compared to ciprofloxacin for *S. aureus and Enterococcus faecalis*. The prodrug PA2808 also showed activity

Scheme 5

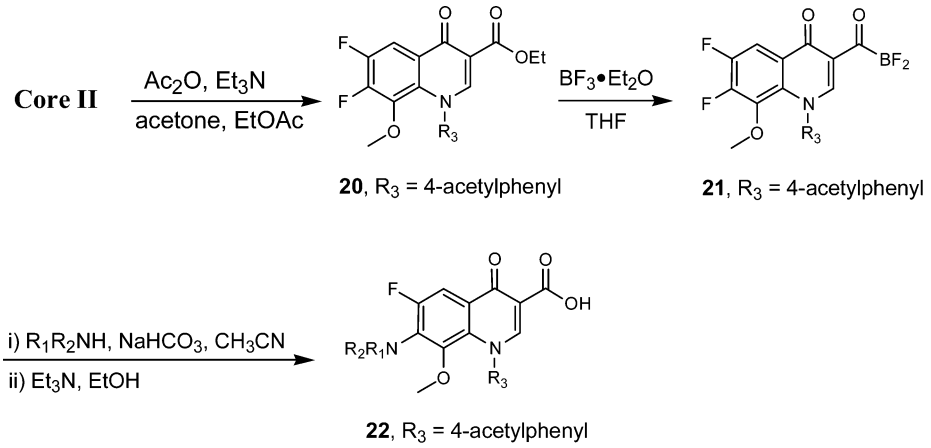
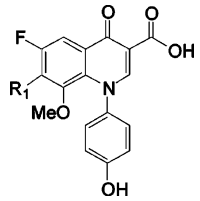
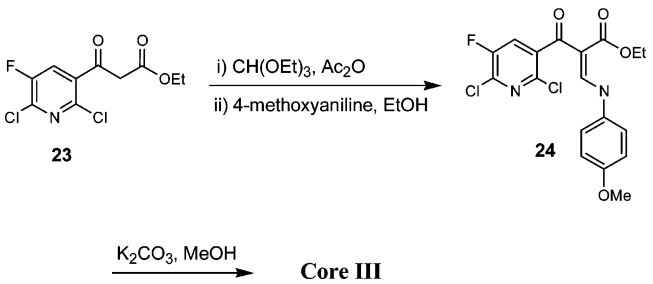


Table 2.



compound	R_1	yield	compound	R_1	yield
48		49 %	**54**		23 %
49		35 %	**55**		43 %
50		41 %	**56**		36 %
51		32 %	**57**		22 %
52		30 %	**58**		36 %
53		38 %	**59**		48 %

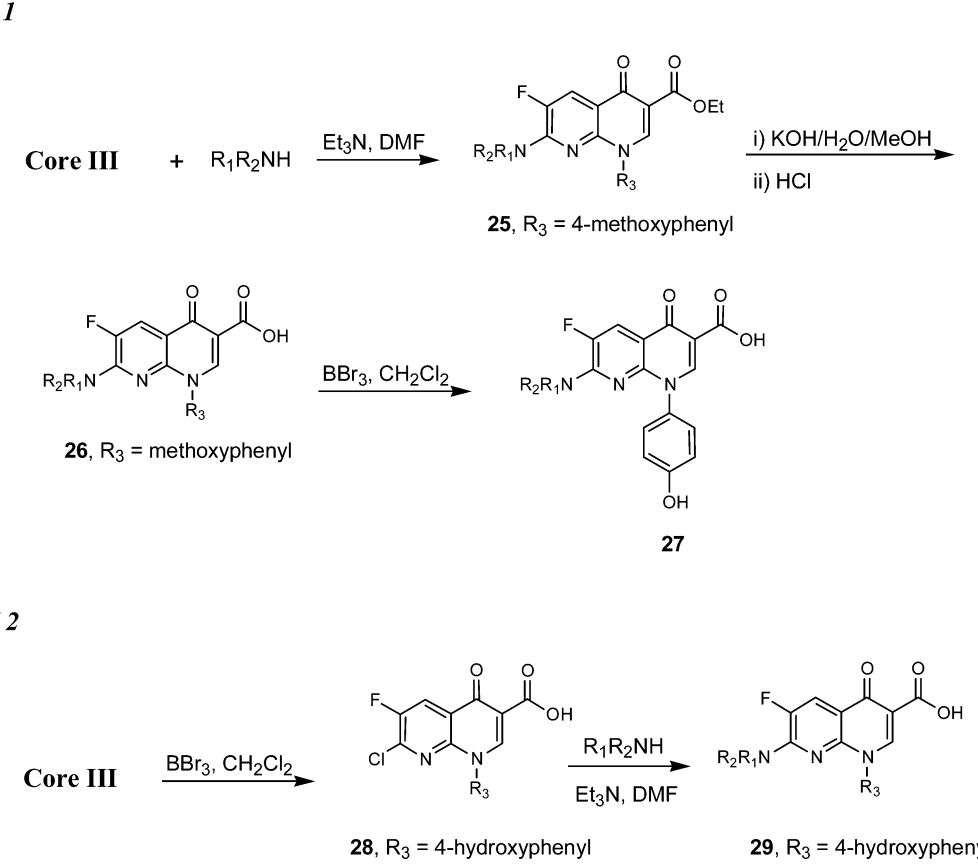
Scheme 6



against *E. coli*, but the activity was poor against *P. aeruginosa and E. faecalis*.

With PA2808 in hand it was necessary to demonstrate that activation to PA2789 would occur after in vitro exposure to alkaline phosphatase and also that the phosphate group imparted greater water solubility. Initial PA2808 activation measurements were conducted using alkaline phosphatase (ALP) or rat lung homogenate. These studies were carried out at physiological pH (7.4) and at optimum pH for ALP activity (10.4). A filter paper disk assay was employed composed of PA2808 treated with ALP, PA2808 treated with rat lung homogenate, or PA2808 with no treatment. Disks were placed on plates inoculated with *P. aeruginosa* or *E. coli*. ALP or rat lung ALP conversion of PA2808 to the active drug (PA2789) should result in a larger inhibition zone compared with untreated PA2808. Untreated PA2808 and PA2789 were used as comparators, rat lung without PA2808 was used as a negative control,

Scheme 7



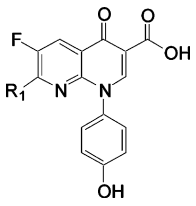
and disks containing 10 μg of tobramycin served as quality controls. The results are shown in Table 5.

Exposure to ALP or rat lung homogenate resulted in conversion of PA2808 to an active form (likely PA2789, vide infra) as observed by an increased zone of inhibition for *P. aeruginosa* compared to untreated PA2808, which showed no inhibition. PA2808 alone was active against *E. coli* without any ALP or rat lung. Exposure to ALP or rat lung, however, resulted in increased inhibition zone size against *E. coli*. The rat lung treatment control did not show any inhibition zone, and the tobramycin QC was in the expected range. There was very little difference in the size of the inhibition zone between the regular lung and the perfused lung. This is consistent with studies showing that the majority of ALP exists in lung tissue as compared with plasma.[5] Evidence that PA2808 was being converted to PA2789 by ALP (pure enzyme) and ALP in the rat lung homogenate was obtained from HPLC studies, performed at pH 7.4 and 10.4. Stock solutions of PA2808 were prepared and demonstrated to be stable in the absence of ALP at both pHs. Exposure to ALP or rat lung homogenate produced a new peak in the HPLC traces at a retention time corresponding to that of PA2789 reference standard.

C. PA2808 Activation by Rat Lung Homogenate. An isolated perfused rat lung model was used to demonstrate conversion of PA2808 to PA2789 in rat lung. Male Sprague−Dawley rats were used as the animal model. After the rats had been anesthetized, tracheal and pulmonary cannulation was performed. The lungs were isolated and mounted in an artificial glass thorax with Krebs buffer circulating at a rate of 15 mL/min. A dose of PA2808 (0.1 mL, 10 mg/mL) was administered to the lungs by using a dosing cartridge. Two hundred microliter aliquots were removed from the drug reservoir at 1, 2, 5, 10, 15, 20, and 30 min and replaced with an equal amount of buffer. The lungs were collected at the end of the experiment and analyzed for drug concentrations. The amounts of PA2808 and PA2789 transported across lung epithelium were calculated from concentrations in the perfusate. The amounts of PA2808 and PA2789 in the perfusate after 30 min were found to be 348 μg and 174 μg, respectively. The amounts of PA2808 and PA2789 found in the lung after 30 min were 157 μg and 68.2 μg, respectively.

D. PA2808 in Vivo Antibacterial Efficacy. Two studies were performed to evaluate and compare the in vivo efficacy of PA2789 and PA2808. The first study consisted of a mouse ED_{50} model wherein a single 100-fold LD_{50} dose of *E. coli* ATCC 25922 was injected intraperitoneally. Groups of five female Balb/c mice were then treated intravenously over a five-dose range (0.5, 1.0, 2.0, 3.0, 5.0 mg/kg) with PA2789 and PA2808 and observed for the following 5 days. The second study was a rat lung infection model using *E. coli* infected (ATCC 25922) agarose beads. PA2808 was administrated by tail vein injection over a three-dose range (1.0, 5.0, 10.0 mg/kg) twice a day for 3 days. The rat lungs were then harvested and homogenized and CFU counts performed. The results from the mouse systemic infection model produced an estimated ED_{50} of 0.74 mg/kg for PA2808 and an estimated ED_{50} of 0.75 mg/kg for PA2789. The earlier in vitro study (see Table 4), however, had demonstrated that PA2808 was less active than the parent PA2789. These results suggest that PA2808 is converted to PA2789 upon exposure to

Table 3.



compound	R_1	yield	compound	R_1	yield
60		12 %	**69**		85 %
61		36 %	**70**		66 %
62		47 %	**71**		78 %
63		40 %	**72**		73 %
64		35 %	**73**		55 %
65		26 %	**74**		74 %
66		30 %	**75**		29 %
67		86 %	**76**		17 %
68		58 %	**77**		22 %

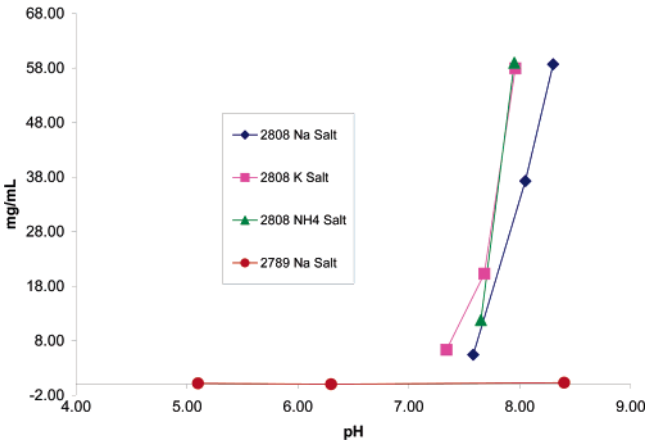


Figure 5. Water solubility of various salts of PA2808 and PA2789.

systemic circulation. The results from the rat lung infection model showed that an intravenous dose of PA2808 at 5 mg/kg produced a greater than 2 log reduction of *E. coli* and that total clearance of the infection was achieved using PA2808 at 10 mg/kg.

Table 4. MICs of PA2808 and PA2789 against Primary Test Panel

organism	MIC (μg/mL) ciprofloxacin	PA2789[a]	PA2808[b]
S. aureus			
methicillin susceptible	0.25	0.1	1.56
methicillin resistant	0.5	0.1	0.78
E. faecalis	1	0.78	25
E. coli	0.015	0.05	0.2
P. aeruginosa	0.5	0.78	50

[a] PA-2789Z0 lot 3110 (2789.0.2). [b] PA-2808N3 lot 3139 (2808.1.2).

E. Rat Intravenous and Inhalation Pharmacokinetic Studies. The intravenous study consisted of a dose of PA2808 administered via tail vein injection, followed by sampling of plasma and lung samples at various time points. The results of the IV study suggest that ALP in rat plasma rapidly converts PA2808 to PA2789. Five minutes after injection of PA2808, the concentration of PA2789 in both plasma and lung was 8.07 μg/mL while the concentration of PA2808 in the plasma was 1.51 μg/mL. Concentration of PA2808 in the lung was below the limit of detection. In the inhalation

Table 5. PA2808 Activation Conditions and Disk Inhibition Zone Size[a]

drug and conditions	inhibition zone size (mm) *P. aeruginosa* 27853	*E. coli* 25922
PA2808		
pH 7.4 buffer	7.0	19.4
pH 10.4 buffer	7.0	19.5
pH 7.4 + ALP	11.6	22.8
pH 10.4 + ALP	16.1	24.7
pH 7.4 + perfused lung	19.7	25.5
pH 7.4 + regular lung	20.6	26.6
PA2789		
pH 7.4 buffer	23.8	27.8
pH 10.4 buffer	24.9	28.2
lungs (no drug added)		
perfused lung pH 7.4	7.0	7.0
regular lung pH 7.4	7.0	7.0
tobramycin QC	22.1	19.4
expected range	19−25	18−26

[a] Filter paper disk size is 7 mm; 7 mm value indicates no inhibition.

Table 6. Antibacterial Activity of Core I Analogues[a] (MIC, μg/mL)

no. (amine used)	*S. aureus* Stau 29213	Stau 33591 MRSA	*E. faecalis* Enfa 29212	*E. coli* Esco 25922	*P. aeruginosa* Psae 27853
30 (**a**)	0.78	0.4	6.25	0.4	1.56
31 (**c**)	0.1	0.2	0.78	<0.05	0.78
32 (**d**)	0.2	0.4	3.13	0.1	3.13
33 (**e**)	0.4	0.4	3.13	0.4	1.56
34 (**f**)	0.78	0.78	12.5	1.56	6.25
35 (**g**)	0.1	0.1	1.56	0.2	3.13
36 (**h1**)	0.4	0.78	6.25	0.78	6.25
37 (**h2**)	0.2	0.1	0.78	0.2	1.56
38 (**i**)	0.4	0.78	6.25	1.56	25
39 (**j1**)	0.4	0.4	6.25	1.56	25
40 (**j2**)	1.56	1.56	25	3.13	50
41 (**j3**)	0.4	0.78	3.13	1.56	25
42 (**l1**)	0.2	0.2	1.56	0.2	1.56
43 (**l2**)	0.2	0.2	1.56	0.2	1.56
44 (**m2**)	0.78	0.78	12.5	3.13	50
45 (**n**)	0.2	0.2	1.56	3.13	12.5
46 (**o**)	0.4	0.4	3.13	1.56	6.25
47 (**p**)	0.2	0.4	3.13	0.2	6.25

[a] All compounds were also tested against Cipro resistant strains as well as *E. faecium*. MICs were ≥25 μg/mL except **31**: 12.5 μg/mL (*E. coli*-Cipro R), 6.25 μg/mL (Psae-Cipro R).

study, rats were exposed to a solution of PA2808 at a concentration of 50 mg/mL for 30 min using the Battelle system. Following the exposure, plasma and lungs were sampled at five time points. As expected, much greater lung concentrations for both PA2808 and PA2789 were found following inhalation as compared to iv administration. The concentration of PA2789 in the lung and plasma at the first time point (immediately after administration was stopped) was 39.73 μg/mL and 1.78 μg/mL, respectively. The rat lung concentration of PA2808 at the same time point was 14.22 μg/mL, and the concentration in the plasma was below the limit of detection.

F. Antibacterial Activity of Core I Analogues. A total of 18 fluoroquinolones containing the core I skeleton were tested against a panel consisting of both Gram-positive and Gram-negative organisms. The MIC results are summarized in Table 6.

All of the analogues were inactive against those organisms possessing ciprofloxacin resistance. A number of analogues had activity comparable to that of the parent compound PA2789 (**31**) with respect to *S. aureus*, but most displayed less activity against *P. aeruginosa* than PA2789. Overall, compounds **35** and **37** showed good activity against all non-cipro resistant pathogens in the panel.

Table 7. Antibacterial Activity of Core II Analogues[a] (MIC, μg/mL)

no. (amine used)	*S. aureus* Stau 29213	Stau 33591 MRSA	*E. faecalis* Enfa 29212	*E. coli* Esco 25922	*P. aeruginosa* Psae 27853
48 (**a**)	0.2	0.4	25	0.78	6.25
49 (**c**)	0.4	0.4	3.13	0.1	3.13
50 (**d**)	3.13	6.25	50	1.56	50
51 (**e**)	1.56	1.56	25	1.56	6.25
52 (**f**)	6.25	3.13	>50	6.25	12.5
53 (**g**)	0.1	0.1	1.56	0.1	1.56
54 (**h2**)	0.78	0.78	6.25	0.4	3.13
55 (**i**)	3.13	3.13	25	6.25	50
56 (**l1**)	0.78	0.78	6.25	0.78	3.13
57 (**l2**)	0.78	0.78	12.5	0.78	6.25
58 (**o**)	0.78	0.78	12.5	1.56	6.25
59 (**p**)	0.78	1.56	12.5	0.4	6.25

[a] All compounds were also tested against Cipro resistant strains as well as *E. faecium*. MICs were ≥25 μg/mL for all except **53**: 12.5 μg/mL (Psae-Cipro R).

Table 8. Antibacterial Activity of Core III Analogues[a] (MIC, μg/mL)

no. (amine used)	*S. aureus* Stau 29213	Stau 33591 MRSA	*E. faecalis* Enfa 29212	*E. coli* Esco 25922	*P. aeruginosa* Psae 27853
60 (**a**)	1.56	1.56	12.5	1.56	3.13
61 (**b**)	0.78	0.78	6.25	0.78	6.25
62 (**c**)	0.2	0.2	1.56	0.05	3.13
63 (**d**)	3.13	6.25	12.5	0.4	6.25
64 (**e**)	0.78	0.78	>50	1.56	1.56
65 (**f**)	1.56	3.13	>50	1.56	3.13
66 (**g**)	0.2	0.2	0.78	0.1	0.78
67 (**h1**)	0.2	0.4	0.78	0.2	0.78
68 (**h2**)	1.56	1.56	25	1.56	3.13
69 (**i**)	0.4	1.56	3.13	3.13	6.25
70 (**j1**)	1.56	1.56	6.25	1.56	12.5
71 (**j2**)	1.56	1.56	12.5	6.25	12.5
72 (**j3**)	0.78	0.78	3.13	3.13	3.13
73 (**l1**)	0.4	0.4	1.56	0.4	0.78
74 (**l2**)	0.4	0.4	1.56	0.4	0.78
75 (**n**)	0.4	0.4	3.13	3.13	6.25
76 (**o**)	0.4	0.4	3.13	1.56	1.56
77 (**p**)	0.2	0.2	1.56	0.1	6.25

[a] All compounds were also tested against Cipro resistant strains as well as *E. faecium*. MICs were ≥25 μg/mL for all except **62**, **66**, **67**: 12.5 μg/mL (Psae-Cipro R).

G. Antibacterial Activity of Core II Analogues. A total of 12 analogues were prepared using core II. As with the core I analogues, the fused pyrrolidine−cyclopropylamine (amine **g**) imparted good activity against the pathogens tested. Some activity against ciprofloxacin resistant *P. aeruginosa* was demonstrated, although no definite trends with respect to greater activity in a side-by-side comparison with the core I analogues were observed (Table 7).

H. Antibacterial Activity of Core III Analogues. Core III was successfully reacted with all amines except **k**, **m1**, and **m2**. The series of analogues using core III proved to be somewhat more active against ciprofloxacin resistant *P. aeruginosa*; however, no MICs lower than 12.5 were obtained. The analogue prepared with the

cyclopropane-fused pyrrolidine (amine **g**) again proved to be among the most active in this series (Table 8).

Conclusions

The development of PA2808, a prodrug of the highly active fluoroquinolone PA2789, demonstrated that water solubility could be increased by the conversion of the free hydroxyl group of PA2789 into a phosphate group. In addition, in vitro and in vivo tests confirmed that PA2808 is converted into PA2789 after phosphate cleavage by alkaline phosphatase. A mouse ED_{50} model confirmed the conversion of PA2808 to PA2789 after systemic exposure with an estimated ED_{50} of 0.75 mg/kg. The rat lung infection model confirmed the conversion of PA2808 to PA2789 in lung tissue and demonstrated significant efficacy of PA2808 at 5 mg/kg. A total of 48 analogues based on PA2789 were prepared and screened against a panel of Gram-positive and Gram-negative pathogens. These new compounds all displayed some activity, the extent of which being determined by the nature of the C-7 side chain. Incorporating the cyclopropane-fused pyrrolidine (amine **g**) at C-7 resulted in some of the most active analogues regardless of which core was used. The C-8 substituent apparently plays a relatively minor role in determining the antibacterial efficacy of the compounds prepared for this study. Investigations into determining the effect the substituents at C-7 and C-8 have with respect to water solubility and increasing lung residence time compared with PA2789 are ongoing and will be reported in due course.

Experimental Section

Biological General Procedures. i. Minimum Inhibitory Concentration (MIC). Minimum inhibitory concentration (MIC; μg/mL) of test drug against reference bacterial strains was determined by the broth microdilution method using procedures in accordance with approved standards of the National Committee for Clinical Laboratory Standards. Microtiter trays were prepared to contain serial 2-fold dilutions of the test drug in cation-adjusted Mueller−Hinton broth. The trays were inoculated with the test organisms at $\sim 5 \times 10^5$ CFU/mL and incubated at 35 °C for 18−24 h. The trays were examined visually at the end of incubation. The MIC was determined to be the lowest concentration that resulted in complete inhibition of growth (absence of visible turbidity).

ii. Activation by Pure Enzyme. The ALP and rat lung treated and nontreated PA2808 were put onto filter paper disks and then put into plates which were inoculated with *P. aeruginosa* or *E. coli*. If the ALP or ALP in rat lung converts PA2808 to an active drug (hopefully to its parent drug PA2789), the ALP or rat lung treated PA2808 will have a larger inhibition zone than untreated PA2808. PA2808 was incubated with ALP or homogenized rat lung at 37 °C for 2 h. The untreated PA2808 and PA2789 were used as the comparators. The lungs without drug were used as negative control. Tobramycin was used as QC.

The agar-disk diffusion method was used to evaluate the conversion of PA2808 to the active form as a result of exposure to ALP. If PA2808 exposed to ALP or rat lung homogenate converts to an active drug (the parent drug), then the treated PA2808 will have a bigger inhibition zone than untreated PA2808.

For testing ALP, phosphate buffer solution (buffered at either pH of 7.4 or 10.4) containing 1000 μg/mL of PA2808 was incubated with 0.2 unit/mL of ALP and incubated at 37 °C for 2 h. At the end of incubation, 10 μL of the above solutions (treated or untreated) was placed onto a sterile filter paper disk, and the disk was used within 10 min of preparation. Solutions of PA2808 or PA2789 without ALP were used to prepare disks in the same way for comparison. Disks containing enzyme alone were also prepared and served as negative controls. Commercial disks containing 10 μg of tobramycin were used for quality control of the experiment. A suspension of the organism to be tested was prepared to a density matching that of a 0.5 McFarland, and a swab was used to spread this suspension evenly on the surface of Mueller−Hinton agar plate. The prepared disks were applied on the agar plate, and the plate was incubated at 37 °C for 18−24 h. At the end of incubation, the inhibition zone around the disk was measured to the nearest tenth of a millimeter.

iii. Activation by Rat Lung Homogenate. For testing lung homogenates, rat lungs were obtained and washed by perfusion with Krebs solution (no bovine serum albumin) to remove blood and then placed in 3 mL of sterile deionized water for each gram of lung. The lungs were homogenized and stored at −20 °C until use. A stock solution containing 2500 μg/mL of PA2808 was added to the above homogenized lung suspension resulting in a final concentration of 1000 μg/mL of PA2808. This resulted in a 1:10 final dilution of rat lung content. Samples were incubated at 37 °C for 2 h. At the end of incubation, 10 μL of the above solutions (treated or untreated) was placed onto a sterile filter paper disk, which was used within 10 min of preparation and applied to the agar plates as described above.

iv. Conversion by Alkaline Phosphatase. For testing ALP, 50 μL of PA2808 stock solution (2500 μg/mL) was added to 100 μL of 0.2 unit/mL of ALP (buffered at pH 10.4) and incubated at 37 °C for 1 h. A solution of PA2808 without addition of ALP was processed similarly and served as the control. At the end of incubation, an equal volume of 100% methanol was added to stop the enzymatic activity. Samples were subjected to HPLC, and the tracings were compared.

v. Conversion by Rat Lung Homogenate. For testing rat lung homogenates, 50 μL of PA2808 was added to 100 μL of homogenized rat lung buffered at pH 7.4 and processed as described above. Samples were incubated at 37 °C for 1 h. A solution of PA2808 without addition of rat lung was processed similarly and served as the control. At the end of incubation, an equal volume of 100% methanol was added to stop the enzymatic activity. Samples were subjected to HPLC, and the tracing was measured.

Chemical General Procedures. General Procedure for Core I Analogues. Hydrolysis of Core I Ester. A mixture of 10 g of core I in 4 N HCl (1 L) was stirred at 70 °C for 48 h and then further heated at 80 °C for 12 h. The solution was cooled, filtered, washed with water, and then dissolved in DMF (150 mL). The resultant solution was filtered through Celite and concentrated in vacuo. The solid was washed with water, ethanol, and diethyl ether to give 6.3 g of the product as the HCl salt.

Coupling Step. A mixture of the core I acid (1 mmol, 317 mg), amine (1.2 mmol), and triethylamine (1.2 mmol) in DMF (10 mL) was stirred at 65 °C for 48 h. The mixture was concentrated in vacuo, then treated with water, and stirred overnight at room temperature. The resultant solid was filtered and washed with water.

Boc Deprotection. The product from the amine coupling step above was dissolved in 4 N HCl in dioxane (15 mL) and water (5 mL). The solution was stirred at room temperature for 12 h and then concentrated in vacuo. The residue was purified by crystallization (MeOH/aectone) to give the final product.

Formamide Deprotection (for Amine n). A mixture of amine **n** coupled product (440 mg) in concentrated HCl (10 mL) was refluxed for 1 h and then cooled to room temperature. The resultant solid was filtered and washed with water and the solid recrystallized from MeOH/acetone to give the final product.

General Procedure for Core II Analogues. Synthesis of Acetyl-Protected Core II. Core II (12.4 g, 33 mmol) was dissolved in EtOAc:acetone (2:1, 300 mL) followed by treatment with triethylamine (25 mL, 179 mmol) and acetic anhydride (12.5 mL, 132 mmol). The mixture was stirred at

room temperature for 12 h. The mixture was concentrated in vacuo and the residue dissolved in EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated to give **20** as a white solid (10.3 g, 75% yield).

Synthesis of BF_2 Ester. To the acetyl-protected core II **20** (10.3 g, 24.7 mmol) dissolved in THF (120 mL) was added BF_3OEt (10 mL, 79 mmol). The mixture was heated to reflux and held at this temperature for 12 h. The solution was cooled, and diethyl ether was added. The resultant white precipitate was filtered and washed with diethyl ether to give BF_2 ester **21** (8.45 g, 78% yield).

Coupling Step. To a mixture of BF_2 ester **21** (500−600 mg) in acetonitrile was added the amine (1.2 equiv) and $NaHCO_3$ (4 equiv). The mixture was heated to 65 °C and maintained at this temperature for 12 h. The mixture was cooled and concentrated in vacuo. The residue was dissolved in ethanol (20 mL) and treated with triethylamine (2. 5 mL) and this mixture heated at 65 °C for 3 h. The mixture was concentrated in vacuo and the residue treated with water. The resultant solid **22** was washed with water and the product used in the next step without further purification.

Boc Deprotection. The product from the amine coupling step above was dissolved in THF (10 mL) followed by 4 N HCl in dioxane (3 mL). The solution was stirred at room temperature for 12 h, filtered, and recrystallized from *n*-propanol/ether, ethanol/ether, or methanol/ether to give the final product.

General Procedure for Core III Analogues. Method 1. Coupling Step. To a mixture of core III (500−600 mg) in DMF (9 mL) were added amine (1.2 equiv) and triethylamine (4 equiv). The reaction mixture was heated to 65 °C and maintained at this temperature for 12 h. The mixture was concentrated in vacuo, water added to the resultant residue, and the precipitate filtered. The product **25** was purified by column chromatography using $MeOH/CH_2Cl_2$ as eluent.

Ester Hydrolysis. The coupled product **25** was dissolved in a minimum amount of CH_2Cl_2 followed by 6 N KOH in MeOH and stirred at room temperature for 12 h. The mixture was cooled using an ice bath and treated slowly with 6 N HCl until the solution was pH 2. The resultant precipitate **26** was filtered and washed with ether.

Phenol Deprotection. Acid **26** from the above reaction was dissolved in CH_2Cl_2 and cooled to −75 °C. To this solution was added BBr_3, and the reaction mixture was stirred and allowed to warm to room temperature slowly. After TLC analysis had indicated that no starting material remained, the mixture was cooled to −75 °C and water added in order to quench excess reagent. The resultant solid **27** was filtered, washed with ether, and recrystallized from *n*-propanol.

Method 2. Core III Deprotection and Hydrolysis. The core III ester (9.75 g, 26 mmol) was dissolved in CH_2Cl_2 (250 mL) and cooled to −75 °C. To this solution was added BBr_3 (8 mL, 35 mmol), and the reaction mixture was stirred and allowed to warm to room temperature slowly. After TLC analysis had indicated that no starting material remained, the mixture was cooled to −75 °C and water added in order to quench excess reagent. The resultant solid acid **28** was filtered, washed with ether, and recrystallized from acetone to give 5.27 g (60% yield) of desired product.

Coupling Step. To a mixture of acid **28** (500−600 mg) in DMF (9 mL) was added amine (1.2 equiv) and triethylamine (2.5 equiv). The reaction mixture was heated to 65 °C and maintained at this temperature for 12 h. The mixture was concentrated in vacuo, water added to the resultant residue, and the precipitate filtered off to give **29**, which was used in the next step without further purification.

Boc Deprotection. The product from the amine coupling step above was dissolved in THF (10 mL) followed by 4 N HCl in dioxane (3 mL). The solution was stirred at room temperature for 12 h, filtered, and recrystallized from *n*-propanol/ether, ethanol/ether, or methanol/ether to give the final product. If no solid formed, the mixture was concentrated in vacuo and the residue washed with diethyl ether and crystallized as described above. The product from the amine coupling step above was dissolved in THF (10 mL) followed by 4 N HCl in dioxane (3 mL). The solution was stirred at room temperature for 12 h, filtered, and the precipitate recrystallized from *n*-propanol/ether, ethanol/ether, or methanol/ether to give the final product.

Ethyl 2,4,5-Trifluoro-α-oxobenzenepropanoate, 9. A stirred solution of potassium ethyl malonate (13 g, 76 mmol) in anhydrous acetonitrile (120 mL) under nitrogen was cooled to 10−15 °C. To this mixture was added triethylamine (10.4 mL, 74.6 mmol) followed by magnesium chloride (8.8 g, 93 mmol), and stirring continued at 20−25 °C for 2.5 h. The resultant slurry was recooled to 0 °C and 2,4,5-trifluorobenzoyl chloride (**8**) (4.8 mL, 37 mmol) added dropwise over 15 min followed by the addition of triethylamine (1.0 mL, 7.2 mmol). The mixture was stirred at room temperature for 18 h and then concentrated under reduced pressure to remove acetonitrile. Toluene (60 mL) was added and the mixture concentrated under reduced pressure. More toluene (60 mL) was added and the mixture stirred and cooled to 10−15 °C. Aqueous HCl (13%, 75 mL) was added while the temperature was kept below 25 °C. The aqueous layer was separated and the organic layer washed with aqueous HCl (12%, 2 × 65 mL) followed by water (2 × 50 mL) and then concentrated under reduced pressure to give a mixture of trifluorobenzoyl acetates as keto and enol tautomers (pale yellow oil) in 90% yield: ^{1}H NMR (enol form, 400 MHz, $CDCl_3$) δ 1.34 (3H, t, J = 7.2 Hz), 4.26 (2H, q, J = 7.2 Hz), 5.84 (1H, s), 7.00 (1H, m), 7.74 (1H, m), 12.71 (1H, s), (keto form, 400 MHz, $CDCl_3$) δ 1.26 (3H, t, J = 7.2 Hz), 3.94 (2H, d, J = 3.8 Hz), 4.21 (2H, q, J = 7.2 Hz), 7.02 (1H, m), 7.82 (1H, m).

Ethyl 6,7-Difluoro-1,4-dihydro-1-(4-hydroxyphenyl)-4-oxoquinoline-3-carboxylate, 10. To a stirred solution of ethyl 2,4,5-trifluoro-α-oxobenzenepropanoate (**9**) (9.1 g, 37 mmol) in acetic anhydride (10 mL, 103 mmol) at room temperature was added triethyl orthoformate (9.0 mL, 55 mmol). The mixture was heated to reflux and maintained at this temperature for 4 h. The mixture was cooled to 45 °C and then concentrated under reduced pressure as the temperature was increased to 98 °C. The residual oil was diluted with dimethyl sulfoxide (50 mL) and treated with 4-aminophenol (10 g, 92 mmol) in portions over 10 min while the temperature was maintained between 15 and 20 °C. After the addition was complete, dimethyl sulfoxide (50 mL) was added and the mixture stirred for 18 h at room temperature. The mixture was treated with potassium carbonate (2.1 g, 15 mmol) and then heated to 95 °C and maintained at this temperture for 70 min. The mixture was filtered hot and the resultant solid washed with warm dimethyl sulfoxide (50 mL) followed by water washes (4 × 50 mL) and dried under reduced pressure (60 °C) for 24 h to give a yellow solid in 74% yield: mp 280 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 (3H, t, J = 7.0 Hz), 4.15 (2H, q, J = 7.0 Hz), 6.89 (1H, m), 6.94 (2H, d, J = 8.3 Hz), 7.41 (2H, d, J = 8.3 Hz), 8.04 (1H, m), 8.35 (1H, s), 10.11 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.7, 59.6, 105.0, 110.2, 111.6, 116.3, 119.3, 123.9, 137.5, 144.6, 153.2, 165.8, 189.0.

Ethyl 1,4-Dihydro-6-fluoro-1-(4-hydroxyphenyl)-7-(4-methyl-1-piperazinyl)-4-oxoquinoline-3-carboxylate, 12. A stirred solution of ethyl 6,7-difluoro-1,4-dihydro-1-(4-hydroxyphenyl)-4-oxoquinoline-3-carboxylate (**10**) (1.0 g, 2.9 mmol) in dimethyl sulfoxide (5 mL) was heated to 115 °C and treated with *N*-methylpiperazine (1.6 mL, 14 mmol). The mixture was stirred for 90 min, allowed to cool, and then filtered. The solid was washed with warm dimethyl sulfoxide (20 mL) and water (4 × 50 mL) and dried under reduced pressure to give a pale yellow solid in 65% yield: mp 263 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.22 (3H, t, J = 7.1 Hz), 2.15 (3H, s), 2.39 (4H, s), 3.00 (4H br s), 4.18 (2H, q, J = 7.1 Hz), 6.32 (1H, m), 6.99 (2H, d, J = 8.6 Hz), 7.43 (2H, m), 7.78 (1H, d, J = 13.4 Hz), 8.29 (1H, s), 10.10 (1H, br s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.7, 38.7, 57.6, 104.8, 109.1, 116.3, 117.2, 118.6, 119.6, 135.8, 136.5, 138.5, 140.9, 146.8, 153.5, 165.0, 188.2.

Ethyl 6,7-Difluoro-1,4-dihydro-1-(4-dibenzylphosphonophenyl)-4-oxoquinoline-3-carboxylate, 11. A stirred

solution of ethyl 6,7-difluoro-1,4-dihydro-1-(4-hydroxyphenyl)-4-oxoquinoline-3-carboxylate (**10**) (1.0 g, 2.9 mmol) in a 1:1 dimethylformamide:tetrahydrofuran mixture (15 mL) was cooled to 0 °C and treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (0.45 mL, 3.0 mmol) dropwise. The mixture was stirred for 10 min and then treated with dibenzylphosphoryl chloride (18 mL, 0.26 M in benzene) over 10 min and stirred for 18 h at room temperature. The mixture was concentrated under reduced pressure and the resultant oil purified by silica gel chromatography (EtOAc:MeOH, 9:1) to give a pale yellow solid in 45% yield: mp 101 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (3H, t, J = 7.1 Hz), 4.46 (2H, q, J = 7.1 Hz), 5.30 (4H, m), 6.84 (1H, m), 7.40 (4H, m), 8.40 (1H, m), 8.52 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.7, 59.6, 71.3, 108.0, 111.6, 116.4, 119.3, 125.5, 127.1, 128.7, 135.4, 139.6, 140.7, 141.0, 146.7, 153.6, 156.0, 166.3, 187.0.

Ethyl 1,4-Dihydro-6-fluoro-7-(4-methyl-1-piperazyl)-4-oxoquinoline-1-[4-O-(phosphoric acid monobenzyl ester)phenyl]-3-carboxylate, 13. A stirred solution of ethyl 6,7-difluoro-1,4-dihydro-1-(4-dibenzylphosphonophenyl)-4-oxoquinoline-3-carboxylate (**11**) (1.0 g, 2.3 mmol) in a 1:1 dimethylformamide:tetrahydrofuran mixture (15 mL) was cooled to 0 °C and treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (0.45 mL, 3.0 mmol) dropwise. The mixture was stirred for 10 min and then treated with dibenzylphosphoryl chloride (18 mL, 0.26 M in benzene) over 10 min and stirred for 18 h at room temperature. The mixture was concentrated under reduced pressure and the resultant oil purified by silica gel chromatography (EtOAc:MeOH, 9:1) to give a pale yellow solid in 30% yield: mp 210 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.27 (3H, t, J = 7.1 Hz), 2.80 (3H, s), 2.96 (2H, br m), 3.18 (2H, br m), 3.46 (4H, br m), 4.21 (2H, q, J = 7.1 Hz), 5.02 (2H, m), 6.43 (1H, d), 7.38 (5H, m), 7.41 (2H, m), 7.62 (2H, m), 7.88 (1H, d, J = 13.4 Hz), 8.37 (1H, s), 10.22 (1H, br s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.6, 38.8, 57.6, 59.5, 71.5, 105.6, 110.6, 116.3, 117.5, 118.0, 119.3, 127.3, 127.4, 128.7, 135.8, 136.2, 138.0, 140.9, 144.6, 152.7, 165.3, 188.5.

1,4-Dihydro-6-fluoro-7-(4-methyl-1-piperazinyl)-4-oxoquinoline-1-[4-O-(phosphoric acid monobenzyl ester)-phenyl]-3-carboxylic Acid, 14. To a stirred solution of ethyl 1,4-dihydro-6-fluoro-7-(4-methyl-1-piperazyl)-4-oxoquinoline-1-[4-O-(phosphoric acid monobenzyl ester)phenyl]-3-carboxylate (**13**) (0.50 g, 0.84 mmol) in 2:1 methanol:water (30 mL) at room temperature was added lithium hydroxide monohydrate (0.14 g, 3.3 mmol). The mixture was stirred for 5 h, filtered, and concentrated under reduced pressure to give a white solid in 81% yield: mp 280 °C (dec); ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.20 (3H, s), 2.44 (2H, br s), 2.97 (2H, br s), 4.93 (2H, m), 6.39 (1H, d, J = 7.0 Hz), 7.23 (5H, m), 7.35 (4H, m), 7.90 (1H, d, J = 13.3 Hz), 8.58 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 38.7, 57.7, 71.3, 105.6, 108.0, 116.6, 117.8, 118.1, 119.5, 126.3, 127.4, 128.2, 135.8, 136.9, 138.4, 146.6, 156.2, 171.5, 189.0.

1,4-Dihydro-6-fluoro-7-(4-methyl-1-piperazinyl)-4-oxoquinoline-1-[4-O-(phosphoric acid)phenyl]-3-carboxylic Acid, 7. To a stirred solution of ethyl 1,4-dihydro-6-fluoro-7-(4-methyl-1-piperazyl)-4-oxoquinoline-1-[4-O-(phosphoric acid monobenzyl ester)phenyl]-3-carboxylate (**13**) (7.45 g, 12.5 mmol) in 3:1 methanol:water (3 mL) was added palladium (3.57 g, 10% on activated carbon). The mixture was placed under a hydrogen atmosphere and stirred at room temperature for 18 h. The mixture was filtered and concentrated under reduced pressure. The residue was triturated with 10% acetic acid and filtered. The solid was redissolved in 10% sodium bicarbonate and triturated with 10% acetic acid. The solid was filtered to give a light yellow solid (3.84 g, 64.3%) that contained traces of PA2798. This material was further purified by preparatory HPLC to give analytically pure material: mp >300 °C (dec); ^{1}H NMR (400 MHz, CD_3OD) δ 2.30 (3H, s), 2.54 (4H, m), 3.06 (4H, m), 6.48 (1H, d, J = 7.1 Hz), 7.30 (2H, d, J = 8.6 Hz), 7.60 (2H, d, J = 8.6 Hz), 7.98 (1H, d, J = 13.3 Hz), 8.44 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 38.7, 57.4, 104.5, 109.4, 116.2, 117.3, 118.5, 119.0, 135.2, 136.9, 138.6, 146.6, 156.0, 170.3, 187.2. Anal. Calcd for $C_{21}H_{21}FN_3O_7P$: C, 52.84; H, 4.43; N, 8.80. Found: C, 52.61; H, 4.63; N, 8.56.

Core I, II, and III Analogues. 6-Fluoro-1-(4-hydroxyphenyl)-4-oxo-7-piperazin-1-yl-1,4-dihydroquinoline-3-carboxylic acid, 30: mp >300 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.78 (4H, m), 2.95 (4H, m), 6.44 (1H, d, J = 8.0 Hz), 7.00 (2H, d, J = 9.0 Hz), 7.48 (2H, d, J = 9.0 Hz), 7.95 (1H, d, J = 13.0 Hz), 8.55 (1H, s), 10.25 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 45.1, 50.4, 106.4, 107.1, 110.7, 110.9, 116.5, 118.3, 128.4, 131.2, 139.6, 145.6, 145.7, 148.5, 151.9, 153.9, 158.6, 165.8, 176.5; MS (ESI$^+$) 384.15 (M + 1). Anal. Calcd for $C_{20}H_{18}FN_3O_4 \cdot H_2O$: C, 59.85; H, 5.02; N, 10.47; Found: C, 59.79; H, 4.81; N, 10.29.

7-(3,5-Dimethylpiperazin-1-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 32: mp >300 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.23 (6H, d, J = 6.5 Hz), 2.80 (2H, m), 3.39 (2H, m), 3.55 (2H, d, J = 11 Hz), 6.51 (1H, d, J = 7.5 Hz), 7.03 (2H, m), 7.50 (2H, m), 8.03 (1H, d, J = 13 Hz), 8.58 (1H, s), 9.05 (1H, br s), 9.47 (1H, br s), 10.25 (1H, s), 15.07 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 15.3, 50.5, 51.5, 107.3, 107.4, 111.0, 111.3, 116.5, 119.3, 119.4, 128.3, 131.0, 139.2, 143.5, 143.6, 148.9, 151.5, 154.0, 158.7, 165.7, 176.5; MS (ESI$^+$) 411.89 (M + 1). Anal. Calcd for $C_{22}H_{22}FN_3O_4 \cdot 2H_2O \cdot HCl$: C, 56.28; H, 5.45; N, 8.95; Cl, 7.55. Found: C, 56.21; H, 5.05; N, 8.71; Cl, 7.41.

7-(3-Aminopyrrolidin-1-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 33: mp 265 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.06 (1H, m), 2.21 (1H, m), 3.37 (1H, m), 3.46 (1H, m), 3.55 (1H, m), 3.67 (1H, m), 3.86 (1H, m), 6.00 (1H, d, J = 7.5 Hz), 7.02 (2H, d, J = 9.0 Hz), 7.45 (2H, d, J = 9.0 Hz), 7.91 (1H, d, J = 14.0 Hz), 8.28 (3H, s), 8.48 (1H, s), 10.30 (1H, s), 15.40 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.6, 47.3, 49.2, 52.9, 101.4, 106.7, 110.6, 110.9, 115.0, 116.5, 128.3, 131.2, 140.1, 140.9, 141.0, 148.2, 148.6, 151.0, 158.7, 166.0, 176.1; MS (ESI$^+$) 384.04 (M + 1). Anal. Calcd for $C_{20}H_{18}FN_3O_4 \cdot H_2O \cdot 0.9HCl$: C, 55.32; H, 4.85; N, 9.68; Cl, 7.35. Found: C, 55.10; H, 4.74; N, 9.41; Cl, 7.07.

7-(2,5-Diazabicyclo[2.2.1]hept-2-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 34: mp 285 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.91 (1H, d, J = 11.0 Hz), 2.10 (1H, d, J = 10.0 Hz), 3.20 (2H, m), 3.47 (1H, d, J = 11.0 Hz), *3.55* (1H, d, J = 10.0 Hz), 4.39 (1H, s), 4.70 (1H, s), 6.04 (1H, d, J = 7.5 Hz), 7.02 (2H, d, J = 8.0 Hz), *7.45* (2H, d, J = 8.0), 7.95 (1H, d, J = 14.0 Hz), 8.49 (1H, s), 9.14 (1H, br s), 9.67 (1H, br s), 10.27 (1H, s), 15.3 (1H, br); ^{13}C NMR (125 MHz, DMSO-d_6) δ 35.6, 49.8, 53.2, 56.5, 57.6, 57.7, 101.6, 106.8, 111.0, 111.2, 115.5, 116.4, 128.2, 131.1, 139.7, 139.8, 140.0, 148.4, 148.7, 150.7, 158.7, 165.9, 176.2; MS (ESI$^+$) 396.06 (M + 1). Anal. Calcd for $C_{21}H_{20}FN_3O_4 \cdot H_2O \cdot 0.95HCl$: C, 56.30; H, 4.71; N, 9.38; Cl, 7.52. Found: C, 56.45; H, 4.40; N, 9.19; Cl, 7.49.

7-(6-Amino-3-aza-bicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 35: mp 240−242 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.12 (2H, s), 2.44 (1H, s), 3.46 (2H, m), 3.57 (2H, m), 7.03 (2H, m), 7.45 (2H, m), 7.86 (1H, d, J = 14.0 Hz), 8.40 (3H, s), 8.46 (1H, s), 10.29 (1H, s), 15.40 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 30.8, 40.7, 40.9, 60.8, 60.9, 112.4, 116.7, 120.7, 120.9, 125.2, 126.5, 138.3, 141.2, 149.9, 150.9, 151.1, 158.2, 158.5, 161.0, 168.7, 176.0, 186.1; MS (ESI$^+$) 396.05 (M + 1). Anal. Calcd for $C_{21}H_{18}FN_3O_4 \cdot 2H_2O \cdot 1.05HCl$: C, 55.40; H, 4.75; N, 9.23; Cl, 8.18. Found: C, 55.47; H, 4.69; N, 8.72; Cl, 8.15.

7-[(7*R)-7-Amino-5-aza-spiro[2.4]hept-5-yl]-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 36:** mp 250 °C (dec); $[\alpha]^D_{20}$ = +56.2 (c = 4.96, DMF); ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.60−0.90 (3H, m), 1.00−1.10 (1H, m), 3.10 (1H, m), 3.30 (1H, m), 3.60 (1H, m), 3.90 (2H, m), 6.00 (1H, d, J = 8.5 Hz), 7.02 (2H, m), 7.45 (2H, m), 7.94 (1H, d, J = 14.0 Hz), 8.20 (3H, br), 8.48 (1H, s), 10.26 (1H, s), 15.40 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 4.86, 14.24, 23.62, 53.9, 54.3, 55.4, 101.2, 106.7, 110.7, 110.9, 115.0, 116.5, 128.3, 131.2, 140.1, 140.9, 141.0, 148.2, 148.8, 150.7, 158.6, 166.0, 176.2; MS (ESI$^+$) 410.05 (M + 1). Anal.

Calcd for $C_{22}H_{20}FN_3O_4 \cdot 1.8H_2O \cdot HCl$: C, 55.25; H, 5.18; N, 8.79; Cl, 7.41. Found: C, 55.07; H, 4.82; N, 8.42; Cl, 7.10.

7-[(7*S)-7-Amino-5-aza-spiro[2.4]hept-5-yl]-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 37:** mp 245 °C (dec); $[\alpha]^D_{20} = -56.2$ (c = 5.05, DMF); ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.60−0.90 (3H, m), 1.00−1.10 (1H, m), 3.10 (1H, m), 3.30 (1H, m), 3.60 (1H, m), 3.90 (2H, m), 6.00 (1H, d, J = 8.5 Hz), 7.02 (2H, m), 7.45 (2H, m), 7.94 (1H, d, J = 14.0 Hz), 8.20 (3H, br), 8.48 (1H, s), 10.25 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 4.86, 14.24, 23.62, 53.9, 54.3, 55.4, 101.2, 106.7, 110.7, 110.9, 115.0, 116.5, 128.3, 131.2, 140.1, 140.9, 141.0, 148.2, 148.8, 150.7, 158.6, 166.0, 176.2; MS (ESI$^+$) 410.05 (M + 1). Anal. Calcd for $C_{22}H_{20}FN_3O_4 \cdot 1.8H_2O \cdot HCl$: C, 55.25; H, 5.18; N, 8.79; Cl, 7.41. Found: C, 55.11; H, 4.86; N, 8.47; Cl, 7.43.

7-(3-Aminomethyl-pyrrolidin-1-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 38: mp 255 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.75 (1H, m), 2.10 (1H, m), 2.50 (1H, m), 2.90 (2H, m), 3.20−3.40 (3H, m), 3.60 (1H, m), 6.02 (1H, d, J = 7.5 Hz), 7.02 (2H, d, J = 9.0 Hz), 7.44 (2H, d, J = 9.0 Hz), 7.85 (1H, d, J = 14.0 Hz), 8.08 (3H, br s), 8.43 (1H, s), 10.25 (1H, s), 15.4 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.14, 36.3, 40.5, 48.7, 52.6, 101.9, 106.6, 110.5, 110.7, 114.5, 116.5, 128.2, 131.3, 140.2, 141.3, 141.4, 148.0, 148.8, 150.8, 158.6, 166.0, 176.0; MS (ESI$^+$) 398.05 (M + 1). Anal. Calcd for $C_{21}H_{20}FN_3O_4 \cdot 0.6H_2O \cdot 1.1HCl$: C, 56.26; H, 5.01; N, 9.37; Cl, 8.70. Found: C, 56.13; H, 4.91; N, 9.05; Cl, 8.96.

7-[(3*R)-3-[(1*S**)-1-Aminoethyl]pyrrolidin-1-yl]-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 39:** mp >300 °C; $[\alpha]^D_{20} = -49.0$ (c = 0.517, DMF); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.18 (3H, d, J = 7.0 Hz), 1.74 (1H, m), 2.13 (1H, m), 2.39 (1H, m), 3.20−3.45 (4H, m), 3.55 (1H, m), 6.01 (1H, d, J = 8.0 Hz), 7.03 (2H, d, J = 9.0 Hz), 7.45 (2H, d, J = 9.0 Hz), 7.84 (1H, d, J = 14.0 Hz), 8.17 (3H, br s), 8.44 (1H, s), 10.29 (1H, s), 15.40 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.4, 27.6, 42.2, 48.7, 49.3, 51.6, 100.9, 106.6, 110.5, 110.7, 114.6, 116.5, 128.2, 131.3, 140.1, 141.2, 141.3, 148.0, 148.8, 150.7, 158.6, 166.0, 176.0; MS (ESI$^+$) 412.05 (M + 1). Anal. Calcd for: $C_{22}H_{22}FN_3O_4 \cdot 0.5H_2O \cdot 0.95HCl$: C, 58.07; H, 5.30; N, 9.23; Cl, 7.40. Found: C, 58.08; H, 5.17; N, 9.15; Cl, 7.23.

7-[(3*S)-3-[(1*R**)-1-Aminoethyl]pyrrolidin-1-yl]-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 40:** mp >300 °C; $[\alpha]^D_{20} = +51.8$ (c = 0.513, DMF); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.18 (3H, d, J = 7.0 Hz), 1.74 (1H, m), 2.13 (1H, m), 2.39 (1H, m), 3.20−3.45 (4H, m), 3.55 (1H, m), 6.01 (1H, d, J = 8.0 Hz), 7.03 (2H, d, J = 9.0 Hz), 7.45 (2H, d, J = 9.0 Hz), 7.84 (1H, d, J = 14.0 Hz), 8.17 (3H, br s), 8.44 (1H, s), 10.29 (1H, s), 15.40 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.4, 27.6, 42.2, 48.7, 49.3, 51.6, 100.9, 106.6, 110.5, 110.7, 114.6, 116.5, 128.2, 131.3, 140.1, 141.2, 141.3, 148.0, 148.8, 150.7, 158.6, 166.0, 176.0; MS (ESI$^+$) 412.05 (M + 1). Anal. Calcd for: $C_{22}H_{22}FN_3O_4 \cdot 0.5H_2O \cdot HCl$: C, 57.83; H, 5.29; N, 9.20; Cl, 7.76. Found: C, 58.08; H, 5.20; N, 9.33; Cl, 7.62.

7-[(3*S)-3-[(1*S**)-1-Aminoethyl]pyrrolidin-1-yl]-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 41:** mp >300 °C; $[\alpha]^D_{20} = +38.7$ (c = 0.503, DMF); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.22 (3H, d, J = 7.0 Hz), 1.72 (1H, m), 2.03 (1H, m), 2.39 (1H, m), 3.2−3.45 (4H, m), 3.62 (1H, m), 6.04 (1H, d, J = 8.0 Hz), 7.02 (2H, d, J = 9.0 Hz), 7.45 (2H, d, J = 9.0 Hz), 7.86 (1H, d, J = 13.0 Hz), 8.14 (3H, br s), 8.43 (1H, s), 10.27 (1H, s), 15.45 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.8, 27.7, 43.0, 49.3, 49.9, 53.2, 101.6, 107.3, 111.3, 111.5, 115.4, 117.3, 128.9, 132.1, 140.9, 142.0, 142.1, 148.8, 149.5, 151.5, 159.3, 166.8, 176.8; MS (ESI$^+$) 412.06 (M + 1). Anal. Calcd for $C_{22}H_{22}FN_3O_4 \cdot 0.5H_2O \cdot HCl$: C, 57.83; H, 5.29; N, 9.20; Cl, 7.76. Found: C, 57.97; H, 5.21; N, 9.04; Cl, 7.53.

7-[(3*S,4*S**)-3-Amino-4-methylpyrrolidin-1-yl]-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 42:** mp 257 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.05 (3H, d, J = 6.5 Hz), 2.6 (1H, m), 3.3 (1H, m), 3.5−3.85 (4H, m), 5.97 (1H, d, J = 7.5 Hz), 7.03 (2H, m), 7.45 (2H, m), 7.90 (1H, d, J = 14.0 Hz), 8.3 (3H, br s), 8.47 (1H, s), 10.2 (1H, br s), 15 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 11.6, 34.2, 52.2, 53.3, 53.4, 101.1, 106.7, 110.7, 110.9, 114.9, 116.5, 128.3, 131.2, 140.1, 140.9, 141.0, 148.1, 148.8, 150.7, 158.7, 166.0, 176.1; MS (ESI$^+$) 398.04 (M + 1). Anal. Calcd for: $C_{21}H_{20}FN_3O_4 \cdot 2H_2O \cdot HCl$: C, 55.38; H, 5.18; N, 9.23; Cl, 7.78. Found: C, 55.22; H, 4.94; N, 9.01; Cl, 7.87.

7-[(3*S,4*R**)-3-Amino-4-methylpyrrolidin-1-yl]-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 43:** mp >300 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.09 (3H, d, J = 7.0 Hz), 2.4 (1H, m), 3.0 (1H, m), 3.45 (2H, m), 3.65−3.8 (2H, m), 5.99 (1H, d, J = 7.5 Hz), 7.04 (2H, d, J = 9.0 Hz), 7.46 (2H, d, J = 9.0 Hz), 7.88 (1H, d, J = 14.0 Hz), 8.45 (4H, br s), 10.3 (1H, b rs), 15.4 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 15.7, 36.0, 52.2, 54.3, 54.5, 101.3, 106.7, 110.7, 110.9, 115.1, 116.5, 128.3, 131.2, 140.1, 140.8, 140.9, 148.2, 148.8, 150.8, 158.6, 166.0, 176.1; MS (ESI$^+$) 398.03 (M + 1). Anal. Calcd for: $C_{21}H_{20}FN_3O_4 \cdot 2H_2O \cdot HCl$: C, 56.73; H, 5.13; N, 9.45; Cl, 6.78. Found: C, 56.60; H, 4.79; N, 9.18; Cl, 6.77.

7-[(2*R,4*S**)-4-Amino-2-methylpyrrolidin-1-yl]-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, 44:** mp 265 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.05 (3H, d, J = 6 Hz), 1.50 (1H, in), 1.80 (3H, s), 2.40 (1H, m), 3.30 (1H, m), 3.55 (1H, m), 3.80 (1H, m 4.15 (1H, m 6.07 (1H, d, J = 7.5 Hz), 7.00 (2H, m), 7.47 (2H, m), 7.90 (1H, d, J = 14.0 Hz), 8.08 (1H, d, J = 6.5 Hz), 8.49 (1H, s), 10.16 (1H, s), 15.40 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.0, 22.5, 38.6, 47.1, 54.0, 55.1, 103.0, 106.6, 110.7, 110.9, 115.4, 116.2, 116.6, 128.4, 131.3, 139.9, 140.8, 140.9, 148.1, 149.7, 151.6, 158.5, 166.0, 169.2, 176.2. MS(ESI$^+$) 440.13 (M + 1). Anal. Calcd for $C_{23}H_{22}FN_3O_5 \cdot 0.5H_2O$: C, 61.60; H, 5.17; N, 9.37. Found: C, 61.46; H, 4.95; N, 9.04.

7-[(4*R)-4-Aminooctahydroisoindol-2-yl]-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 45:** mp 270 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.00−1.10 (1H, m), 1.20−1.30 (1H, m), 1.40−1.60 (2H, m), 1.70−1.80 (2H, m), 2.25 (1H, m), 2.70 (1H, m), 2.95 (1H, m), 3.30−3.50 (3H, m), 3.65 (1H, m), 6.02 (1H, d, J = 7.0 Hz), 7.02 (2H, m), 7.45 (2H, m), 7.88 (1H, d, J = 14.0 Hz), 8.22 (3H, br s), 8.42 (1H, s), 10.25 (1H, s), 15.4 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.6, 24.4, 25.3, 36.5, 47.2, 48.6, 55.5, 100.6, 106.6, 110.6, 114.6, 128.2, 131.4, 140.2, 141.8, 141.9, 148.1, 150.9, 159.0, 166.1, 176.0; MS (ESI$^+$) 438.11 (M + 1). Anal. Calcd for: $C_{24}H_{24}FN_3O_4 \cdot 1.5H_2O \cdot HCl$: C, 57.54; H, 5.63; N, 8.39; Cl, 7.08. Found: C, 57.66; H, 5.53; N, 8.27; Cl, 6.92.

6-Fluoro-1-(4-hydroxyphenyl)-7-(octahydropyrrolo[3,4-*b*]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 46: mp >300 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.60−1.80 (4H, m), 2.62 (1H, m), 2.87 (1H, m), 3.14 (1H, m), 3.47 (3H, m), 3.75 (2H, m), 6.00 (1H, d, J = 7.5 Hz), 7.03 (2H, m), 7.45 (2H, m), 7.90 (1H, d, J = 14.0 Hz), 8.46 (1H, s), 8.73 (1H, br), 8.60 (1H, br s), 10.29 (1H, s), 15.4 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.2, 20.2, 33.8, 41.6, 49.9, 52.8, 54.1, 101.1, 106.7, 110.7, 110.9, 114.9, 116.5, 128.2, 131.3, 140.1, 141.0, 141.1, 148.2, 148.5, 151.0, 158.6, 166.0, 176.1; MS (ESI$^+$) 424.11 (M + 1). Anal. Calcd for $C_{23}H_{22}FN_3O_4 \cdot 0.4H_2O \cdot 1.15HCl$: C, 58.46; H, 5.11; N, 8.89; Cl, 8.63. Found: C, 58.76; H, 5.40; N, 8.57; Cl, 8.72.

6-Fluoro-1-(4-hydroxyphenyl)-7-(1-methylhexahydropyrrolo[3,4-*b*]pyrrol-5-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, 47: mp >300 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.55 (1H, m), 1.95 (1H, m), 2.21 (3H, s), 2.24 (1H, m), 2.80 (2H, m), 2.95 (1H, m), 3.10 (1H, m), 3.30−3.45 (3H, m), 6.04 (1H, d, J = 7.0 Hz), 7.00 (2H, m), 7.45 (2H, m), 7.88 (1H, d, J = 14.0 Hz), 8.46 (1H, s), 10.15 (1H, s), 15.40 (1H, s); MS (ESI$^+$) 424.15 (M + 1). Anal. Calcd for $C_{23}H_{22}FN_3O_4 \cdot 0.1H_2O$: C, 64.96; H, 5.26; N, 9.88. Found: C, 64.83; H, 5.28; N, 9.89.

6-Fluoro-1-(4-hydroxyphenyl)-8-methoxy-4-oxo-7-piperazin-1-yl-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 48: mp 238 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ

3.16 (4H, m), 3.23 (3H, s), 3.43 (4H, m), 6.88 (2H, d, J = 9.0 Hz), 7.41 (2H, d, J = 9.0 Hz), 7.90 (1H, d, J = 12.0 Hz), 8.37 (1H, s), 9.30 (1H, br s), 9.60 (1H, br s), 9.98 (1H, s), 14.80 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 43.2, 46.9, 55.9, 61.9, 106.5, 106.6, 115.3, 121.7, 121.8, 126.3, 133.3, 136.2, 138.0, 138.1, 145.6, 151.4, 154.7, 157.1, 157.6, 165.4, 176.5; MS (ESI$^+$) 413.6 (M + 1). Anal. Calcd for $C_{21}H_{20}FN_3O_5 \cdot 1.7H_2O \cdot 1.2HCl$: C, 51.71; H, 5.08; N, 8.61; Cl, 8.72. Found: C, 51.82; H, 5.01; N, 8.59; Cl, 8.78.

6-Fluoro-1-(4-hydroxyphenyl)-8-methoxy-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 49: mp 268 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.80 (3H, s), 3.23 (3H, s), 3.05−3.60 (8H, m), 6.88 (2H, m), 7.42 (2H, m), 7.90 (1H, d, J = 12.0 Hz), 8.37 (1H, s), 9.97 (1H, s), 10.85 (1H, br s), 14.8 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 42.2, 46.9, 46.9, 48.5, 52.8, 61.9, 106.5, 106.6, 115.2, 121.8, 121.9, 126.3, 133.3, 136.2, 137.6, 137.7, 145.6, 151.4, 154.8, 156.8, 157.5, 165.4, 176.5; MS (ESI$^+$) 428.16 (M + 1). Anal. Calcd for $C_{22}H_{22}FN_3O_5 \cdot 0.6H_2O \cdot HCl$: C, 55.66; H, 5.14; N, 8.85; Cl, 7.47. Found: C, 55.77; H, 5.21; N, 8.42; Cl, 7.62.

7-(3,5-Dimethylpiperazin-1-yl)-6-fluoro-1-(4-hydroxyphenyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, 50: mp 265 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.93 (6H, d, J = 6.5 Hz), 2.60 (2H, m), 2.85 (2H, m), 3.12 (2H, m), 3.16 (3H, s), 3.29 (1H, m), 6.87 (2H, m), 7.40 (2H, m), 7.82 (1H, d, J = 12.0 Hz), 8.32 (1H, s), 9.85 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.0, 50.6, 50.9, 56.7, 61.6, 106.2, 106.5, 115.2, 120.5, 126.3, 133.5, 136.5, 139.1, 144.9, 151.0, 154.7, 157.2, 157.3, 165.6, 176.4; MS (ESI$^+$) 442.2 (M + 1). Anal. Calcd for $C_{23}H_{24}FN_3O_5 \cdot 0.7H_2O$: C, 60.84; H, 5.64; N, 9.25. Found: C, 60.96; H, 5.57; N, 9.00.

7-(3-Aminopyrrolidin-1-yl)-6-fluoro-1-(4-hydroxyphenyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 51: mp 213 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.00 (1H, m), 2.25 (1H, m), 3.06 (3H, s), 3.40−3.90 (4H, m), 4.35 (1H, m), 6.87 (2H, d, J = 9.0 Hz), 7.42 (2H, m), 7.80 (1H, d, J = 14.0 Hz), 8.17 (3H, br s), 8.33 (1H, s), 9.91 (1H, s), 15.05 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.5, 29.3, 48.9, 54.0, 55.9, 61.0, 106.3, 106.5, 115.3, 117.8, 117.8, 125.8, 126.0, 133.8, 136.1, 136.2, 136.5, 140.7, 150.9, 152.0, 154.5, 157.4, 165.7, 176.2; MS (ESI$^+$) 414.1 (M + 1). Anal. Calcd for $C_{21}H_{20}FN_3O_5 \cdot H_2O \cdot HCl$: C, 53.91; H, 4.95; N, 8.98; Cl, 7.58. Found: C, 54.17; H, 4.71; N, 8.76; Cl, 7.37.

7-(2,5-Diazabicyclo[2.2.1]hept-2-yl)-6-fluoro-1-(4-hydroxyphenyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 52: mp 223 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.95 (1H, m), 2.06 (1H, m), 3.03 (3H, s), 3.30−3.40 (3H, m), 3.67 (1H, m), 4.35 (1H, m), 4.74 (1H, m), 6.85 (1H, m), 6.91 (1H, m), 7.22 (1H, m), 7.54 (1H, m), 7.85 (1H, d, J = 14.0 Hz), 8.32 (1H, s), 9.97 (1H, s), 9.00−9.80 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 35.6, 51.3, 55.3, 56.8, 59.4, 60.0, 106.4, 106.8, 107.0 115.2, 115.3, 117.5, 117.6, 125.4, 126.5, 133.6, 135.5, 135.6, 136.3, 139.6, 150.1, 151.1, 152.5, 157.4, 165.6, 176.2; MS (ESI$^+$) 426.16 (M + 1). Anal. Calcd for $C_{22}H_{20}FN_3O_5 \cdot 1.5H_2O \cdot 1.2HCl$: C, 53.25; H, 4.92; N, 8.47; Cl, 8.57. Found: C, 53.12; H, 4.54; N, 8.21; Cl, 8.45.

7-(6-Amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(4-hydroxyphenyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 53: mp 206 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.98 (2H, m), 2.40 (1H, m), 3.03 (3H, s), 3.48 (2H, m), 3.58 (2H, m), 6.87 (2H, d, J = 8.5 Hz), 7.40 (2H, d, J = 8.5 Hz), 7.81 (1H, d, J = 13.0 Hz), 8.25 (3H, br s), 8.34 (1H, s), 9.91 (1H, s), 15 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.5, 29.4, 50.8, 61.4, 106.2, 106.5, 106.5, 115.3, 119.7, 119.7, 126.1, 133.6, 135.7, 135.8, 136.4, 143.8, 151.1, 153.6, 156.1, 157.4, 165.6, 176.4; MS (ESI$^+$) 426.13 (M + 1).

7-[(7*R)-7-Amino-5-aza-spiro[2.4]hept-5-yl]-6-fluoro-1-(4-hydroxyphenyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 54:** mp 242 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.70 (1H, m), 0.76 (2H, m), 1.11 (2H, m), 3.09 (3H, s), 3.18 (1H, d, J = 10.0 Hz), 3.59 (1H, d, J = 11.5 Hz), 4.05 (2H, m), 6.88 (2H, d, J = 16.0 Hz), 7.27 (1H, d, J = 7.5 Hz), 7.51 (1H, d), 7.79 (1H, d, J = 14.0 Hz), 8.32 (1H, s), 8.37 (3H, s), 9.98 (1H, s), 15.08 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 5.1, 13.5, 24.1, 25.4, 55.0, 55.3, 55.3, 55.9, 61.1, 64.8, 106.4, 106.6, 115.2, 117.8, 117.8, 125.4, 126.3, 133.8, 136.1, 136.3, 136.4, 140.5, 140.5, 151.0, 151.9, 154.4, 157.4, 165.7, 176.2; MS (APCI$^+$) 440.0 (100%). Anal. Calcd for $C_{23}H_{22}FN_3O_5 \cdot 1.3H_2O \cdot 1.5HCl$: C, 53.38; H, 5.08; N, 8.12; Cl, 10.28. Found: C, 53.09; H, 4.96; N, 7.79; Cl, 10.07.

7-(3-Aminomethyl-pyrrolidin-1-yl)-6-fluoro-1-(4-hydroxyphenyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 55: mp 215 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.68 (1H, m), 2.07 (1H, m), 2.48 (1H, m), 2.88 (2H, m), 3.05 (3H, s), 3.32 (1H, m), 3.50−3.60 (3H, m), 6.88 (2H, m), 7.40 (2H, m), 7.76 (1H, d, J = 14.0 Hz), 8.11 (3H, br s), 8.30 (1H, s), 9.97 (1H, s), 15.14 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.9, 36.7, 40.6, 50.1, 54.2, 60.6, 106.2, 106.4, 115.3, 117.3, 117.4, 125.7, 126.1, 133.8, 136.5, 136.7, 140.5, 150.9, 152.2, 154.7, 157.4, 165.7, 176.1; MS (ESI$^+$) 428.14 (M + 1). Anal. Calcd for $C_{22}H_{22}FN_3O_5 \cdot 1.5H_2O \cdot 1.3HCl$: C, 52.65; H, 5.28; N, 8.37; Cl, 9.18. Found: C, 52.75; H, 5.02; N, 8.09; Cl, 9.00.

7-[(3*S,4*S**)-3-Amino-4-methyl-pyrrolidin-1-yl]-6-fluoro-1-(4-hydroxyphenyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 56:** mp 226 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.07 (5H, m), 3.42 (7H, m, J = 7.0 Hz), 3.71 (2H, m), 6.89 (2H, m), 7.32 (1H, m), 7.45 (1H, m), 7.78 (1H, d, J = 14.0 Hz), 8.27 (2H, br s), 9.94 (1H, s), 15.10 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 11.6, 25.4, 34.3, 51.9, 54.2, 55.3, 55.4, 55.9, 61.1, 61.9, 106.3, 106.6, 115.3, 117.5, 117.5, 125.7, 126.1, 126.9, 133.8, 136.3, 136.4, 136.4, 140.1, 140.2, 150.9, 151.7, 154.2, 157.4, 165.7, 176.2, 176.2; MS (APCI$^+$) 428.1. Anal. Calcd for $C_{22}H_{22}FN_3O_5 \cdot 1.4H_2O \cdot HCl$: C, 54.02; H, 5.32; N, 8.59; Cl, 7.25. Found: C, 54.09; H, 5.16; N, 7.60; Cl, 7.27.

7-(3-Amino-4-methylpyrrolidin-1-yl)-6-fluoro-1-(4-hydroxyphenyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 57: mp 208 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.12 (3H, d, J = 7.0 Hz), 2.38 (1H, m), 3.06 (3H, s), 3.43 (3H, m), 3.61 (1H, m), 3.68 (1H, m), 3.74 (2H, m), 6.89 (2H, t, J = 9.5 Hz), 7.31 (1H, d, J = 8.0 Hz), 7.48 (1H, d, J = 8.0 Hz), 7.78 (1H, d, J = 14.0 Hz), 8.32 (1H, s), 8.56 (3H, s), 10.02 (1H, s), 15.07 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 15.1, 25.4, 36.5, 53.8, 54.3, 56.3, 56.4, 61.0, 61.9, 106.3, 106.6, 115.2, 115.4, 117.7, 117.8, 125.5, 126.3, 133.8, 136.0, 136.1, 136.4, 140.6, 140.7, 151.0, 152.0, 154.5, 157.5, 165.7, 176.2, 176.2; MS (ESI$^+$) 428.1 (100%) Anal. Calcd for $C_{22}H_{22}FN_3O_5 \cdot 3.3H_2O \cdot 2.1HCl$: C, 46.90; H, 5.49; N, 7.46; Cl, 13.21. Found: C, 46.78; H, 4.81; N, 6.61; Cl, 13.22.

6-Fluoro-1-(4-hydroxyphenyl)-8-methoxy-7-(octahydropyrrolo[3,4-*b*]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 58: mp 220 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.60−1.80 (4H, m), 2.54 (1H, m), 2.92 (1H, m), 3.07 (3H, s), 3.14 (1H, m), 3.50 (1H, m), 3.65 (1H, m), 3.75 (2H, m), 3.85 (1H, m), 6.80 (2H, m), 7.27 (1H, m), 7.52 (1H, m), 7.79 (1H, d, J = 14.0 Hz), 8.32 (1H, s), 8.65 (1H, br s), 9.67 (1H, br s), 9.94 (1H, s), 15.13 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.5, 20.4, 33.9, 41.3, 51.7, 51.8, 53.8, 54.0, 61.1, 106.3, 106.6, 115.3, 117.2, 117.2, 125.4, 126.3, 133.8, 136.5, 136.6, 139.5, 139.6, 150.9, 151.4, 153.9, 157.4, 165.7, 176.1; MS (ESI$^+$) 454.1 (M + 1). Anal. Calcd for $C_{24}H_{24}FN_3O_5 \cdot 1.6H_2O \cdot 1.07HCl$: C, 55.30; H, 5.47; N, 8.06; Cl, 7.28. Found: C, 55.29; H, 5.19; N, 7.89; Cl, 7.15.

6-Fluoro-1-(4-hydroxyphenyl)-8-methoxy-7-(1-methylhexahydro-pyrrolo[3,4-*b*]pyrrol-5-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid HCl salt, 59: mp 230 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.78 (1H, m), 2.34 (1H, m), 2.80 (3H, d, J = 4.5 Hz), 3.05−3.15 (2H, m), 3.16 (3H, s), 3.30 (1H, m), 3.48 (1H, m), 3.60−3.75 (2H, m), 3.85 (1H, m), 4.03 (1H, m), 6.91 (2H, m), 7.23 (1H, m), 7.55 (1H, m), 7.83 (1H, d, J = 13.0 Hz), 8.34 (1H, s), 10.12 (1H, s), 11.08 (1H, br s), 14.99 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.9, 41.0, 51.9, 55.3, 55.8, 62.0, 70.9, 106.4, 106.5, 106.6, 115.2, 115.4, 119.6, 125.3, 126.5, 133.5, 135.9, 136.0, 136.2, 142.9, 151.2, 153.1, 155.5, 157.5, 165.6, 176.3; MS (ESI$^+$) 454.25 (M + 1).

6-Fluoro-1-(4-hydroxyphenyl)-4-oxo-7-piperazin-1-yl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid, 60 (prepared by method 1): mp >300 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.12 (4H, br s), 3.44 (1H, m, J = 7.0 Hz), 3.77 (4H, br s), 6.93 (2H, d, J = 4.5 Hz), 7.39 (2H, d, J = 3.0 Hz), 8.22 (1H, d, J = 13.5 Hz), 8.64 (1H, s), 9.30 (2H, s), 10.05 (1H, s), 15.02 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 12.0, 43.4, 43.4, 108.3, 113.3, 113.3, 115.4, 119.7, 119.9, 128.3, 131.2, 145.8, 148.1, 148.4, 149.4, 149.5, 157.9, 165.5, 176.8; MS (ESI$^+$) 385.2 (M + 1). Anal. Calcd for $C_{17}H_{17}FN_4O_4 \cdot 1.35H_2O \cdot HCl$: C, 51.27; H, 4.69; N, 12.59; F, 4.51; Cl, 7.96. Found: C, 51.35; H, 4.62; N, 12.27; F, 4.64; Cl, 8.17.

6-Fluoro-1-(4-hydroxyphenyl)-7-(2-methylpiperazin-1-yl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 61: mp >300 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24 (3H, d, J = 7 Hz), 2.90−3.50 (5H, m), 6.94 (2H, m), 7.40 (2H, m), 8.20 (1H, d, J = 13.0 Hz), 8.64 (1H, s), 9.20 (1H, br s), 9.50 (1H, br s), 10.04 (1H, s), 15.00 (1H, br s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.7, 37.8, 41.8, 45.5, 47.2, 108.3, 113.1, 115.4, 119.6, 119.9, 128.3, 131.2, 145.2, 145.9, 148.1, 148.7, 149.2, 149.3, 157.9, 165.5, 176.8; MS (ESI$^+$) 399.06 (M + 1). Anal. Calcd for $C_{20}H_{19}FN_4O_4 \cdot 0.8H_2O \cdot HCl$: C, 53.47; H, 4.85; N, 12.47; Cl, 7.89. Found: C, 53.54; H, 4.60; N, 12.04; Cl, 7.96.

6-Fluoro-1-(4-hydroxyphenyl)-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 62: mp >275 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.73 (3H, s), 3.05 (2H, m), 3.45 (4H, m), 4.20 (2H, m), 6.94 (2H, d, J = 4.5 Hz), 7.40 (2H, d, J = 4.5 Hz), 8.25 (1H, d, J = 12.5 Hz), 8.66 (1H, s), 10.04 (1H, s), 11.07 (1H, s), 15.00 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 40.6, 42.8, 44.2, 52.3, 109.0, 114.3, 116.0, 116.1, 120.6, 120.8, 129.0, 131.9, 146.4, 146.7, 148.8, 149.0, 149.9, 150.0, 158.4, 16.1, 177.5, 229.9; MS (ESI$^+$) 399.3 (M + 1). Anal. Calcd for $C_{20}H_{19}FN_4O_4 \cdot H_2O \cdot HCl$: C, 53.04; H, 4.90; N, 12.37; F, 4.20. Found: C, 52.76; H, 4.66; N, 12.14; F, 4.33.

7-(3,5-Dimethylpiperazin-1-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 63 (prepared by method 1): mp >280 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.14 (6H, d, J = 6.5 Hz), 3.15 (5H, m, J = 13.5 Hz), 4.12 (2H, m, J = 13.5 Hz), 6.95 (2H, d, J = 4.5 Hz), 7.40 (2H, d, J = 4.5 Hz), 8.24 (1H, d, J = 13.0 Hz), 8.69 (1H, s), 9.08 (1H, s), 9.52 (1H, s), 10.04 (1H, s), 15.02 (1H,s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 15.2, 38.5, 38.7, 48.9, 50.2, 108.3, 115.2, 115.3, 119.9, 128.5, 131.1, 145.8, 148.1, 148.5, 149.2, 158.1, 165.5, 176.9; MS (ESI$^+$) 413.1 (M + 1). Anal. Calcd for $C_{21}H_{21}FN_4O_4 \cdot 0.5H_2O \cdot HCl$: C, 55.09; H, 5.06; N, 12.24; Cl, 7.76. Found: C, 55.01; H, 4.93; N, 12.08; Cl, 7.84.

7-(3-Aminopyrrolidin-1-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 64: mp 302 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.20 (1H, br s), 2.20 (1H, br s), 3.40−4.40 (5H, br s), 6.93 (2H, m), 7.38 (2H, m), 8.10 (1H, d, J = 12.5 Hz), 8.26 (3H, br s), 8.56 (1H, s), 10.04 (1H, s), 15.30 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 40.8, 108.8, 111.7, 116.1, 118.2, 118.4, 148.2, 149.1, 149.2, 158.5, 166.4; MS (ESI$^+$) 385.3 (M + 1). Anal. Calcd for $C_{19}H_{17}FN_4O_4 \cdot H_2O \cdot HCl$: C, 52.00; H, 4.59; N, 12.77; Cl, 8.08. Found: C, 52.32; H, 4.33; N, 12.74; Cl, 8.51.

7-(2,5-Diazabicyclo[2.2.1]hept-2-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 65: mp > 290 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.92 (1H, m, J = 11.0 Hz), 2.07 (1H, m, J = 10.5 Hz), 3.29 (6H, m), 4.43 (1H, s), 6.93 (2H, d, J = 8.5 Hz), 7.38 (2H, d, J = 9 Hz), 8.17 (1H, d, J = 12.5 Hz), 8.60 (1H, s), 9.11 (1H, s), 9.68 (1H, s), 10.02 (1H, s), 15.19 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 52.0, 56.8, 108.2, 111.7, 115.3, 118.0, 118.2, 128.2, 131.2, 146.4, 147.7, 157.8, 165.6, 176.7; MS (APCI$^-$) 395.1 (M − 1). Anal. Calcd for $C_{20}H_{17}FN_4O_4 \cdot 0.85H_2O \cdot HCl$: C, 53.60; H, 4.43; N, 12.50; Cl, 7.91. Found: C, 53.53; H, 4.23; N, 12.34; Cl, 8.02.

7-(6-Amino-3-azabicyclo[3.1.0]hex-3-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 66: mp 225 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.13 (2H, s), 2.40 (1H, s), 3.64 (4H, m), 6.97 (2H, d, J = 4.8 Hz), 7.35 (2H, d, J = 4.2 Hz), 8.02 (1H, d, J = 12.6 Hz), 8.53 (3H, m, J = 4.2 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 8.41, 31.0, 45.3, 49.8, 108.0, 111.0, 111.1, 115.3, 117.5, 117.7, 128.2, 131.3, 144.8, 146.4, 146.8, 147.4, 148.5, 148.6, 157.9, 165.6, 176.5, 176.6; MS (APCI$^+$) 397.1 (M + 1). Anal. Calcd for: $C_{20}H_{17}FN_4O_4 \cdot H_2O \cdot HCl$: C, 51.41; H, 4.41; N, 11.99; Cl, 11.00. Found: C, 51.67; H, 4.41; N, 11.88; Cl, 10.99.

7-(7-Amino-5-azaspiro[2.4]hept-5-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 67: mp 250 °C (dec); $[\alpha]^D_{20}$ = + 8.5 (c = 0.500, DMF). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.60−0.90 (3H, m), 1.05−1.10 (1H, m), 3.30−4.20 (5H, br s), 6.93 (2H, d, J = 9.0 Hz), 7.40 (2H, d, J = 9.0 Hz), 8.12 (1H, d, J = 12.6 Hz), 8.10−8.40 (3H, br s), 8.57 (1H, s), 10.02 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 4.8, 14.3, 52.8, 108.1, 111.1, 115.4, 117.6, 117.9, 128.2, 131.4, 144.2, 146.6, 147.6, 148.4, 157.7, 165.7, 176.6; MS (ESI$^+$) 410.99 (M + 1). Anal. Calcd for $C_{21}H_{19}FN_4O_4 \cdot 0.5H_2O \cdot HCl$: C, 55.21; H, 4.85; N, 12.26; Cl, 7.76. Found: C, 55.51; H, 4.91; N, 12.10; Cl, 7.57.

7-(7-Amino-5-azaspiro[2.4]hept-5-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 68: mp 250 °C (dec); $[\alpha]^D_{20}$ = − 9.3 (c = 0.500, DMF). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.60−0.90 (3H, m), 1.05−1.10 (1H, m), 3.30−4.20 (5H, br s), 6.93 (2H, d, J = 9.0 Hz), 7.40 (2H, d, J = 9.0 Hz), 8.12 (1H, d, J = 12.3 Hz), 8.24 (3H, br s), 8.57 (1H, s), 10.02 (1H, s), 15.28 (1H, br s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 4.8, 14.3, 52.8, 108.1, 111.1, 115.4, 117.6, 117.9, 128.2, 131.4, 144.2, 146.5, 147.6, 148.3, 148.4, 157.7, 165.7, 176.6; MS (ESI$^+$) 410.98 (M + 1). Anal. Calcd for $C_{21}H_{19}FN_4O_4 \cdot 4H_2O \cdot HCl$: C, 53.43; H, 4.87; N, 11.87; Cl, 7.51. Found: C, 53.54; H, 4.57; N, 11.60; Cl, 7.77.

7-(3-Aminomethyl-pyrrolidin-1-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 69: mp 280 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.70 (1H, br s), 1.90 (1H, br s), 2.40 (1H, br s), 2.80 (2H, br s), 3.04.(4H, br s), 6.87 (2H, d, J = 8.5 Hz), 7.31 (2H, d, J = 8.5 Hz), 7.94 (1H, d, J = 12.5 Hz), 8.05 (3H, br s), 8.47 (1H, s), 9.97 (1H, s), 15.27 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 40.4, 47.6, 51.3, 108.0, 111.7, 115.3, 117.2, 117.3, 128.2, 131.4, 144.8, 146.7, 146.9, 147.3, 148.4, 148.5, 157.7, 165.7, 176.5; MS (ESI$^+$) 399.04 (M + 1). Anal. Calcd for $C_{20}H_{19}FN_4O_4 \cdot H_2O \cdot HCl$: C, 53.04; H, 4.90; N, 12.37; Cl, 7.83. Found: C, 52.91; H, 4.67; N, 12.16; Cl, 8.00.

7-[3-(1-Aminoethyl)pyrrolidin-1-yl]-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid, HCl salt, 70: mp >300 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.16 (3H, d, J = 5.5 Hz), 1.70 (1H, br s), 2.10 (1H, br s), 2.30 (1H, br s), 3.04 (5H, br s), 6.92 (2H, d, J = 8.5 Hz), 7.38 (2H, d, J = 8.5 Hz), 8.03 (1H, d, J = 12.5 Hz), 8.05 (3H, br s), 8.57 (1H, s), 9.96 (1H, s), 15.34 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.5, 48.8, 48.8, 108.0, 110.7, 115.2, 117.3, 117.5, 128.3, 131.4, 144.8, 146.6, 146.9, 147.3, 148.2, 148.3, 157.7, 165.7, 176.5; MS (ESI$^+$) 413.05 (M + 1). Anal. Calcd for $C_{21}H_{21}FN_4O_4 \cdot 1.5H_2O \cdot HCl$: C, 53.00; H, 5.29; N, 11.77; Cl, 7.45. Found: C, 53.34; H, 5.15; N, 11.61; Cl, 7.34.

7-[3-(1-Aminoethyl)pyrrolidin-1-yl]-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 71: mp >300 °C; $[\alpha]^D_{20}$ = + 16.1 (c = 0.500, DMF). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.16 (3H, d, J = 5.0 Hz), 1.70 (1H, br s), 2.10 (1H, br s), 2.30 (1H, br s), 3.00−4.00 (5H, br s), 6.91 (2H, m), 7.38 (2H, m), 8.05 (1H, d, J = 12.5 Hz), 8.03 (3H, br s), 8.57 (1H, s), 9.96 (1H, s), 15.34 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.5, 48.8, 48.8, 108.0, 110.7, 115.2, 117.3, 117.5, 128.3, 131.4, 144.6, 146.7, 147.2, 147.3, 148.2, 148.3, 157.8, 165.7, 176.6; MS (ESI$^+$) 413.05 (M + 1). Anal. Calcd for $C_{21}H_{21}FN_4O_4 \cdot H_2O \cdot HCl$: C, 54.02; H, 5.18; N, 12.00; Cl, 7.59. Found: C, 53.83; H, 5.02; N, 11.77; Cl, 7.43.

7-[3-(1-Aminoethyl)pyrrolidin-1-yl]-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 72: mp >300 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.22 (3H, d, J = 6.4 Hz), 1.70 (1H, m), 2.00 (1H, m), 2.40 (1H, m), 3.20−4.20 (5H, m), 6.92 (2H, m), 7.39 (2H, m), 8.06 (1H, d, J = 12.4 Hz), 8.09 (3H, br), 8.55 (1H, s), 9.99 (1H, s), 15.35 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ

16.9, 26.0, 48.1, 48.4, 50.7, 108.0, 110.8, 115.3, 117.3, 117.5, 128.2, 131.5, 144.6, 146.7, 147.1, 147.3, 148.3, 148.4, 157.7, 165.7, 176.5; MS (ESI$^+$) 413.05 (M + 1). Anal. Calcd for $C_{21}H_{21}FN_4O_4 \cdot 0.8H_2O \cdot HCl$: C, 54.44; H, 5.13; N, 12.09; Cl, 7.65. Found: C, 54.43; H, 4.81; N, 11.98; Cl, 7.86.

7-(3-Amino-4-methylpyrrolidin-1-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 73: mp >300 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.05 (3H, d, J = 6.5 Hz), 2.6 (1H, m), 3.30−4.20 (5H, m), 6.94 (2H, m), 7.39 (2H, m), 8.09 (1H, d, J = 12.5 Hz), 8.27 (3H, br s), 8.55 (1H, s), 10.05 (1H, s), 15.3 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 11.6, 108.1, 111.0, 115.4, 117.6, 117.8, 128.2, 131.4, 144.6, 146.6, 147.2, 147.6, 148.3, 148.4, 157.8, 165.7, 176.6; MS (ESI$^+$) 399.1 (M + 1). Anal. Calcd for $C_{20}H_{19}FN_4O_4 \cdot 1.2H_2O \cdot HCl$: C, 52.63; H, 4.95; N, 12.27; Cl, 7.77. Found: C, 52.88; H, 4.71; N, 12.13; Cl, 7.34.

7-(3-Amino-4-methylpyrrolidin-1-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 74: mp >300 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.06 (3H, d, J = 7.0 Hz), 2.40 (1H, m), 3.30−4.40 (5H, br s), 6.93 (2H, m), 7.39 (2H, m), 8.11 (1H, d, J = 12.5 Hz), 8.28 (3H, br s), 8.56 (1H, s), 10.01 (1H, s), 15.3 (1H, br s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 40.4, 108.0, 110.7, 115.3, 117.2, 117.3, 128.2, 131.5, 144.9, 146.7, 146.9, 147.3, 148.4, 148.5, 157.7, 165.8, 176.5; MS (ESI$^+$) 399.04 (M + 1). Anal. Calcd for $C_{20}H_{19}FN_4O_4 \cdot 1.2H_2O \cdot HCl$: C, 52.63; H, 4.95; N, 12.27; Cl, 7.77. Found: C, 52.49; H, 4.57; N, 11.96; Cl, 7.55.

7-(4-Aminooctahydroisoindol-2-yl)-6-fluoro-1-(4-hydroxyphenyl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 75: mp 285 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.00−1.10 (1H, m), 1.20−1.30 (1H, m), 1.40−1.60 (2H, m), 1.60−1.80 (2H, m), 2.20 (1H, br s), 2.70 (1H, br s), 3.00−4.00 (4H, m), 6.93 (2H, d, J = 9.0 Hz), 7.38 (2H, d, J = 9.0 Hz), 8.04 (1H, d, J = 12.3 Hz), 8.31 (3H, br s), 8.52 (1H, s), 10.00 (1H, br s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.7, 24.4, 25.2, 35.2, 45.7, 48.6, 54.8, 108.0, 110.8, 115.4, 117.2, 117.5, 128.2, 131.5, 144.2, 146.7, 147.3, 147.6, 149.0, 149.2, 157.7, 165.7, 176.5; MS (ESI$^+$) 439.2 (M + 1). Anal. Calcd for $C_{23}H_{23}FN_4O_4 \cdot H_2O \cdot 1.5HCl$: C, 54.02; H, 5.19; N, 10.96; Cl, 10.72. Found: C, 53.93; H, 5.07; N, 10.76; Cl, 10.64.

6-Fluoro-1-(4-hydroxyphenyl)-7-(octahydropyrrolo-[3,4-*b*]pyridin-6-yl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 76 (prepared by method 1): mp >290 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.69 (4H, m), 2.70 (1H, m), 2.83 (1H, m), 3.42 (4H, m), 3.86 (3H, m), 6.94 (2H, d, J = 7.0 Hz), 7.39 (2H, d, J = 8.5 Hz), 8.10 (1H, d, J = 13.0 Hz), 8.57 (1H, s), 8.81 (1H, s), 9.38 (1H, s), 10.04 (1H, s), 15.29 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 16.7, 20.0, 35.0, 42.1, 48.1, 52.6, 52.8, 108.1, 111.0, 115.4, 128.2, 131.4, 146.5, 147.5, 157.7, 165.7, 176.6, 176.6; MS (ESI$^+$) 425.2 (M + 1). Anal. Calcd for $C_{22}H_{21}FN_4O_4 \cdot HCl$: C, 57.33; H, 4.81; N, 12.16; Cl, 7.69. Found: C, 57.18; H, 4.89; N, 2.01; Cl, 7.44.

6-Fluoro-1-(4-hydroxyphenyl)-7-(1-methylhexahydropyrrolo[3,4-*b*]pyrrol-5-yl)-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid HCl salt, 77: mp >280 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.74 (1H, m), 2.33 (1H, m,), 2.81 (3H, d, J = 4.0 Hz), 3.14 (2H, m), 3.57 (2H, m), 3.73 (2H, m,), 4.08 (1H, m), 4.18 (1H, m), 6.94 (2H, d, J = 5.0 Hz), 7.41 (2H, d, J = 4.5 Hz), 8.12 (1H, d, J = 13.0 Hz), 8.58 (1H, s), 10.01 (1H, s), 10.94 (1H, s), 15.24 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 49.3, 49.4, 53.1, 53.2, 55.5, 71.0, 108.1, 111.4, 111.4, 115.4, 117.8, 118.0, 128.2, 131.4, 144.8, 146.3, 147.3, 147.7, 148.3, 148.5, 157.8, 165.7, 176.6; MS (ESI$^+$) 425.0 (M + 1). Anal. Calcd for $C_{22}H_{21}FN_4O_4 \cdot 0.8H_2O \cdot HCl$: C, 55.59; H, 5.00; N, 11.79; F, 4.00; Cl, 7.46. Found: C, 55.20; H, 4.80; N, 11.49; F, 4.38; Cl, 7.85.

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