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The Synthesis and In Vitro Antibacterial Activity of Conformationally Restricted Quinolone Antibacterial Agents

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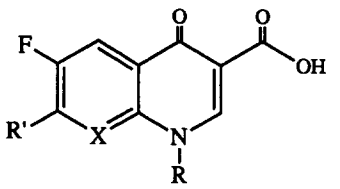
Abstract—Two series of conformationally restricted quinolone antibacterials were synthesized. One series was restricted by formation of a tetrahydrofuran ring between the C-6 position and the C-7 position of the quinolone ring skeleton. The second series achieved conformational rigidity by formation of a tetrahydrofuran ring between the C-7 and the C-8 positions. These compounds were evaluated for their in vitro antibacterial activity. Compounds **19** and **20** were the most active compounds in either series and were about equipotent. Copyright © 1996 Elsevier Science Ltd

Introduction

The quinolones are a group of synthetic antibacterial agents which inhibit the bacterial topoisomerase enzyme DNA gyrase as their mechanism of action. Some members of this class which are currently in clinical use are ofloxacin,[1] ciprofloxacin,[2] and tosufloxacin.[3] A number of structure–activity relationships have been examined for the quinolones.[4] Among these is the increase in activity seen when a fluorine atom is included at position C-6 and when the methyl group at C-7 of nalidixic acid is replaced with a piperazine or other cyclic amine group. In compounds with cyclic amine substituents, there is freedom of rotation around the bond between the amine N-1 and the C-7 position of the quinolone ring, allowing the ring to adopt a variety of conformations. We have prepared two series of compounds in which that conformational mobility has been restricted. This conformational restriction necessitated the replacement of the nitrogen atom at N-1 of the piperazine ring with a carbon atom. Compounds such as rosoxacin[5] and some cycloalkene containing quinolones[6] are known to have good in vitro activity without the presence of a nitrogen atom at the N-1 position of the attached substituent ring.

Chemistry

The two series of conformationally restricted analogues, exemplified by compounds **20** and **32**, were prepared by similar synthetic strategies, but by different routes. In both routes a common synthetic pathway was used to synthesize the key intermediates **7** and **8** prior to the point of the cyclization reaction. These initial common steps are summarized in Scheme 1. In both routes the carboxylic acid **1** or **2** was protected as the oxazoline derivative **3** or **4**.[7] The halide atom (F) for **4** and (Cl) for **3** was then displaced by the anion of *N*-Boc-4-piperidine ethyl ester. The anion was generated by reaction with either LDA or lithium hexamethyldisilazide. The displacement gave **5**



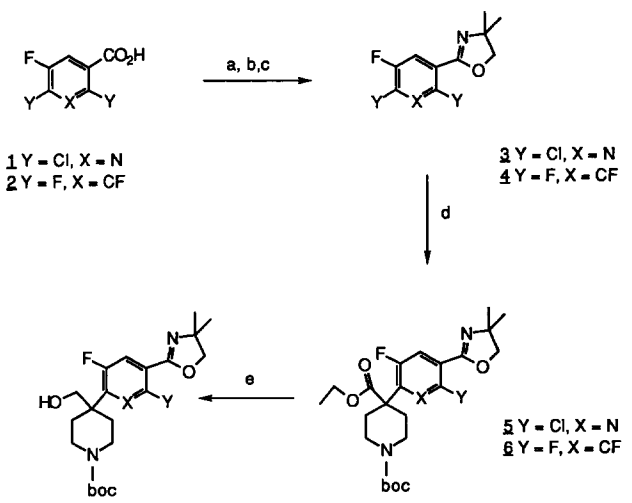
	R'	X	R
Ciprofloxacin	-N NH	CH	
Tosufloxacin	-N NH_2	N	F, F
Ofloxacin	-N N·CH_3	O	
35	-N NH	CH	F, F
36	-N NH	CH	F
37	NH	CH	

or **6** as the major product. The ester group was then selectively reduced with lithium aluminum hydride to the corresponding alcohol **7** or **8**. The oxazoline ring and the *t*-butoxycarbonyl (*t*-Boc) protecting groups were not affected by the reduction conditions.

The intermediate **7** was then converted into the final products **18–20** by the reactions shown in Scheme 2. The alcohol **7** was converted to **9** by an intramolecular cyclization of the alcohol anion generated by reaction

with sodium hydride. The oxazoline and *t*-Boc protecting groups were removed simultaneously by acid hydrolysis with 3 N HCl. The amino group was then reprotected with a benzyloxycarbonyl (Cbz) group giving **10**. The carboxylic acid **10** was converted to the acid chloride **11** by standard procedures. The acid chloride **11** was then reacted with the lithium dianion of monoethylmalonate to give the β-ketoester **12**.[8] The β-ketoester was converted to the enol ether **13**, which was used without purification. Reaction of the enol ether with cyclopropylamine gave **14** and reaction with 2,4-difluoroaniline gave **15**. The enamines **14** or **15** were then cyclized in the presence of sodium hydride to give **16** and **17**, respectively. Removal of the protecting groups by standard methods led to the final products **18** and **19**. The product **20** was obtained from compound **19** by reductive alkylation.[9] These compounds are found in Table 1.

The intermediate **8** was converted into the final products **32–34** by the reactions shown in Scheme 3. The alcohol **8** was converted to **21** by an intramolecular cyclization. The alcohol anion was generated by reaction with sodium hydride. Displacement of the most labile fluorine at position 3 gave **21** as the product. The *t*-Boc and oxazoline protecting groups were then removed by acid hydrolysis in a single step. The amine was reprotected with a Cbz group giving compound **22**. The acid chloride **23** was formed by reaction with thionyl chloride. Treatment of the acid chloride with the dianion of monoethylmalonate, as described in Scheme 2, gave the β-ketoester **24**.[8] The β-ketoester **24** was converted to the enol ether **25** and followed by elaboration to the enamines **26–28** by reaction with the appropriate amines. Cyclization of the enamines with sodium hydride led to **29–31**. The ester and Cbz protecting groups were removed by analogous reactions to those described in Scheme 2 to give the final products **32–34** as seen in Table 1.



Scheme 1. Reagents: (a) $SOCl_2$/cat. DMF/toluene, heat; (b) 2-amino-2-methyl-1-propanol/CH_2Cl_2, 0 °C, rt; (c) $SOCl_2$, rt; (d) for **5**, LDA/*N*-Boc-ethylisonipecotate, −78–0 °C for **6**, LiHMDS/*N*-Boc-ethylisonipecotate, −40 °C; (e) Et_2O/LAH, 0 °C.

Results and Discussion

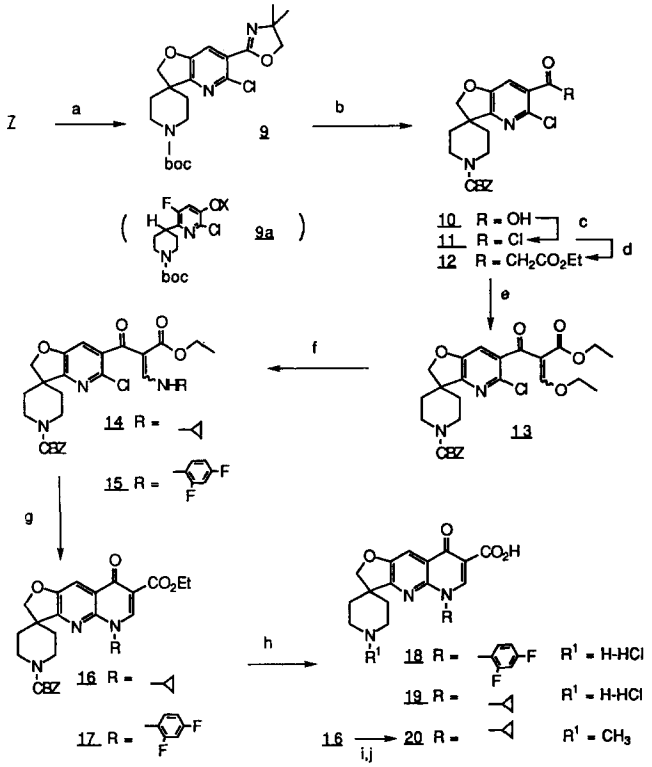
The compounds **18–20** and **32–34** shown in Table 1 were tested for their in vitro antibacterial activity against a variety of gram-positive and gram-negative microorganisms. Ciprofloxacin (CIPRO) was used as a reference standard. The in vitro minimum inhibitory concentrations (MICs) for some representative organisms are summarized in Table 2. Compounds **19** and **20** were the most active compounds in either series and were about equipotent. All of the compounds were significantly less active than ciprofloxacin. The diminished activity of compounds **18–20** and **32–34** is due to two factors: the restricted conformation and the replacement of the basic nitrogen of the amine substituent with an sp^3 hybridized carbon. These compounds are about 10-fold less active than similar analogues reported by Laborde and co-workers, which contain an sp^2 hybridized carbon at the ring juncture.[6] They reported MIC data for a ciprofloxacin analogue **37** which had an sp^3 carbon atom replacing the piperazine nitrogen.[6] Compound **37** cannot be directly compared to **32** due to differences in the microorganisms that were used in the MIC tests. However, some comparisons can be made. Compound **37** had the following MIC (μg/mL) values: *Staphylococcus aureus* 0.8–6.3, *Streptococcus pyogenes* 0.4, *Escherichia coli* 0.2, *Pseudomonas aeruginosa* 1.6, *Klebsiella pneumonia* 0.4 and *Enterococcus* 3.1. The corresponding MIC (μg/mL) values for **32** were: 0.78–12.5, 12.5, 0.78, 1.56–3.1, 0.78

Table 1. Structure of quinolone derivatives

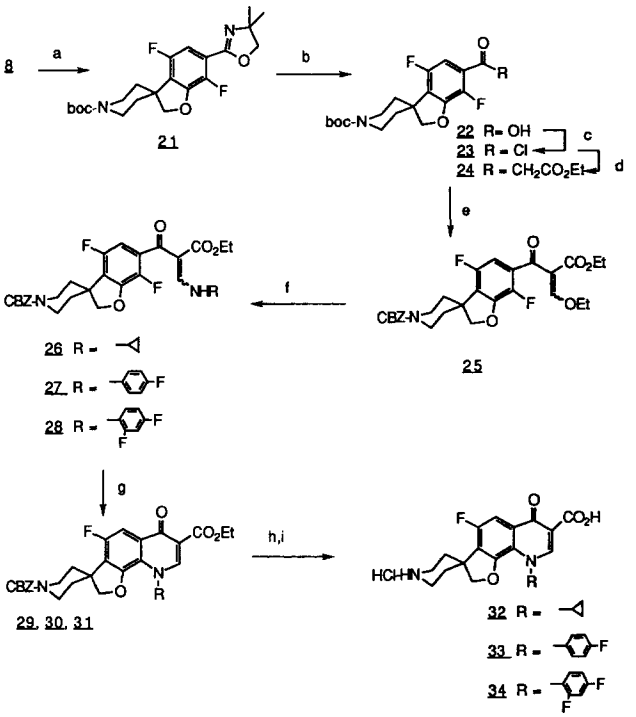
Compound no.	R	R^1	Yield %	Mp, °C[a]	Formula[b]
18	2,4-Difluorophenyl	H·HCl	59	>290	$C_{21}H_{18}ClF_2N_3O_4$
19	Cyclopropyl	H·HCl	48	>270	$C_{18}H_{20}ClN_3O_4 \cdot H_2O$[c]
20	Cyclopropyl	CH_3	72	>270	$C_{19}H_{22}ClN_3O_4 \cdot 1.5H_2O$
32	Cyclopropyl	H·HCl	57	>300	$C_{19}H_{20}ClFN_2O_4 \cdot 1/2H_2O$
33	4-Fluorophenyl	H·HCl	91	270–272	$C_{22}H_{18}ClF_2N_2O_4 \cdot 2.5H_2O$[d]
34	2,4-Difluorophenyl	H·HCl	66	>300	$C_{22}H_{18}ClF_3N_2O_4 \cdot H_2O$[e]

[a]Melting points are uncorrected.
[b]All compounds were analysed for (C,H,N) and gave the analyses indicated within ±0.4% of the theoretical values unless otherwise noted.
[c]H: calcd 5.61, found 5.08.
[d]H: calcd 4.90, found 4.42.
[e]H: calcd 4.16, found 3.23.

and 6.2. In general, compound **32** was fourfold less active than **37**. The conformationally restricted compounds can be compared with their nitrogen-containing counterparts. Compounds **19** and **32** are analogues of ciprofloxacin. Compound **19** was more active than compound **32** and both compounds were on average 10-fold less active than ciprofloxacin. Comparison of **18** and **34** to the conformationally unrestricted analogue **35**[10] showed the conformationally restricted compounds to be between six- and 10-fold less active. A similar difference in activity was seen



Scheme 2. Reagents: (a) NaH/THF, heat; (b) 1.5 N HCl, heat then NaOH/Cbz-Cl; (c) $SOCl_2$ cat. DMF; (d) LDA/monoethylmalonate, −78–0 °C; (e) $(EtO)_3CH/Ac_2O$, heat; (f) for **14** cyclopropylamine, for **15** 2,4-difluoroaniline, CH_2Cl_2, rt; (g) NaH/THF heat; (h) 4 N HCl, reflux; (i) H_2/cat., 88% HCOOH, rt then 37% H_2CO, heat; (j) 4 N HCl reflux.



Scheme 3. Reagents: (a) NaH/THF, rt; (b) 3 N HCl reflux, then NaOH/Cbz-Cl; (c) $SOCl_2$/cat. DMF CH_2Cl_2, 0–75 °C (d) LDA/monoethylmalonate, −78–0 °C; (e) $(EtO)_3CH/Ac_2O$, heat; (f) for **26** cyclopropylamine, for **27** *p*-fluoroaniline, for **28** 2,4-difluoroaniline CH_2Cl_2, rt; (g) NaH/THF, heat; (h) H_2/cat., MeOH then 1.5 N HCl, heat.

Table 2. In vitro antibacterial activities[a]

Organism[b]	MIC (μg/mL)[c]								
	18	**19**	**20**	**32**	**33**	**34**	**35**	**36**	CIP
S. aureus CMX 553	1.56	1.56	0.78	0.78	12.5	6.2	0.1	0.2	0.1
S. aureus A5177	6.2	3.1	3.1	12.5	50	12.5	0.1	0.39	0.78
S. aureus 642A	6.2	1.56	1.56	3.1	25	12.5	—	—	0.39
S. epidermidis 3519	3.1	3.1	3.1	6.2	12.5	6.2	0.1	0.39	0.39
Ent. faecium ATCC 8043	3.1	3.1	3.1	6.2	12.5	6.2	0.39	1.56	0.39
S. bovis A5169	12.5	12.5	25	12.5	12.5	100	0.78	6.2	3.1
S. agalactiae CMX 508	12.5	6.2	12.5	12.5	25	100	0.39	1.56	0.39
S. pyogenes 930	12.5	12.5	12.5	12.5	25	100	0.2	0.78	0.39
E. coli Juhl	0.39	1.56	0.78	0.78	0.78	3.1	0.02	0.05	0.02
E. aerogenes ATCC 13048	0.39	0.39	0.78	1.56	1.56	3.1	0.05	0.2	0.05
K. pneumoniae 8045	0.2	0.78	0.78	0.78	0.78	1.56	0.02	0.02	0.02
P. aeruginosa A5007	3.1	6.2	6.2	3.1	6.2	50	0.78	0.39	0.1
P. aeruginosa K799/WT	1.56	6.2	3.1	1.56	3.1	25	0.2	0.39	0.1
Acinetobacter CMX 669	3.1	12.5	6.2	100	50	25	0.1	0.2	0.39

[a]Structures are shown in Table 1.
[b]Microorganisms: *S. aureus*, *Staphylococcus aureus*; *S. epidermidis*, *Staphylococcus epidermidis*; *E. faecium*, *Enterococcus faecium*; *S. bovis*, *Streptococcus bovis*; *S. agalactiae*, *Streptococcus agalactiae*; *S. pyogenes*, *Streptococcus pyogenes*; *E. coli*, *Escherichia coli*; *E. aerogenes*, *Enterobacter aerogenes*; *K. pneumonia*, *Klebsiella pneumoniae*; *P. aeruginosa*, *Pseudomonas aeruginosa*.
[c]The MIC (Minimum Inhibitory Concentrations) values were determined by the usual twofold agar dilution method using brain–heart infusion agar.

when the monofluorophenyl analogue **36** was compared with its restricted conformation counterpart **33**.[10]

The compounds in the current series showed the difluorophenyl analogues **18** and **34** were more active than the cyclopropyl analogues **32** and **19** against gram-negative organisms. No clear pattern was seen for the gram-positive organisms. The inclusion of a methyl group on the amine substituent in **20** had very little effect on biological activity.

The current series of conformationally restricted analogues showed a significant decrease in biological activity when compared with conformationally unrestricted analogues or to analogues where the nitrogen was replaced with an sp^2 carbon. The current study does not allow the contributions of conformational restriction and nitrogen replacement with carbon to be completely separated. In the case of compound **32** it appears that the restricted rotation decreased the MIC values at least fourfold, in addition to the decrease due to the replacement of the nitrogen atom with an sp^2-hybridized carbon atom.

Experimental

Melting points were taken on a Fisher–Johns melting point apparatus and are uncorrected. Elemental analyses were obtained for all new compounds reported. Carbon, hydrogen, and nitrogen analysis (unless otherwise specified) were within 0.4% of the theoretical values. Microanalyses were performed by the Abbott Analytical Department. The NMR spectra were obtained on a General Electric QE-300 spectrometer. Resonances are reported downfield relative to tetramethylsilane as internal standard. MS were recorded on a Nermag R 30-10 or Hewlett Packard 5985 mass spectrometer in the chemical ionization mode with ammonia or isobutane as the reagent gas. IR spectra were recorded on a Nicolet 60 SX FT IR spectrometer. TLC analyses were performed on 0.25 mm silica gel GF-254 glass plates. Column chromatography was performed on Merck 70–230 mesh silica gel unless otherwise specified.

Synthesis of oxazoline 3. 2,4-Dichloro-5-fluoronicotinic acid (**1**)[11] (20.21 g, 0.096 mol) was suspended in 150 mL toluene to which was subsequently added thionyl chloride (12 mL, 0.138 mol) followed by dimethylformamide (1 mL). The flask was capped with a reflux condenser and drying tube and the mixture heated in an oil bath to 85 °C. After 3 h the resulting solution was concentrated in vacuo and the residue distilled to give the acid chloride as a clear liquid (bp 80 °C at 0.8 torr), 18.57 g (0.081 mol, 84%). A solution of the acid chloride (18.57 g, 0.081 mol) in 50 mL methylene chloride was added dropwise over 50 min to a solution of 2-amino-2-methyl-1-propanol (14.4 g, 0.162 mol) in 75 mL methylene chloride in an ice bath. Upon completion of the addition, the mixture was allowed to warm to rt. After approximately 3 h, the contents of the flask were vacuum filtered and the filtrate concentrated in vacuo. The residue was partitioned between ethyl acetate and brine solution. The aqueous phase was extracted with ethyl acetate and the combined organics dried ($MgSO_4$) and concentrated in vacuo to give 21.91 g (0.078 mol, 96%) of the desired amide as a white solid sufficiently pure for use as isolated. To a solution of the amide (21.91 g, 0.078 mol) in 200 mL of methylene chloride was added a solution of thionyl chloride (15 mL, 0.173 mol) in 20 mL methylene chloride dropwise over 27 min. The mixture was stirred at rt for 22.5 h, then concentrated in vacuo. The residue was cautiously treated with 5% aq sodium bicarbonate and the resulting solid was collected by vacuum filtration and washed with water, then dried in vacuo at rt to give 17.89 g (0.068 mol, 87%) of the target oxazoline **3**. An analytical sample was obtained by flash chromatography on silica gel (ethyl acetate–hexane): mp 48.5–50.5 °C; ^{1}H NMR ($CDCl_3$): δ 1.41 (s, 6H), 4.16 (s, 2H), 7.96 (db, $J = 7.5$ Hz, 1H); MS: *m/z* 263 $(M+H)^+$. Anal. calcd for $C_{10}H_9N_2OCl_2F$: C, 45.63; H, 3.45; N, 10.64. Found: C, 45.53; H, 3.51; N, 10.62.

Synthesis of oxazoline 4. An oven-dried system protected from moisture was charged with 9.5 g (50 mmol) of 2,3,4,5-tetrafluorobenzoic acid (**2**) suspended in 35 mL of dry CH_2Cl_2 to which was added 2 drops of DMF. The reaction mixture was cooled in an ice bath and 14.6 mL (200 mmol) of $SOCl_2$ was added dropwise with stirring. The reaction mixture was stirred at 0 °C for 30 min after the addition was complete. The reaction mixture was then heated at 50 °C for 24 h. The solvent was removed by distillation at atmospheric pressure. The product was distilled under vacuum (6 torr), giving a clear colorless liquid (9.2 g, 86%); bp 53–54 °C. The acid chloride (9.18 g, 43 mmol) was dissolved in 40 mL of dry CH_2Cl_2. The reaction mixture was cooled in an ice bath and 10.4 mL (110 mmol) of 2-amino-2-methyl-1-propanol in 15 mL of dry CH_2Cl_2 was added dropwise with stirring. The mixture was stirred at 0 °C for 30 min after the addition was complete. The reaction was then stirred at rt for 16 h. The resulting precipitate was removed by suction filtration and washed with 20 mL of hexane. The filtrate was concentrated to dryness giving a white solid (10.1 g, 88%). The amide (5.3 g, 20 mmol) was placed in an oven-dried system, protected from moisture. To this was added 6.4 mL (88 mmol) of $SOCl_2$ (exothermic, vigorous gas evolution). After 45 min, the excess $SOCl_2$ was removed in vacuo. The residue was dissolved in 125 mL of CH_2Cl_2 and was washed with 120 mL of 5% $NaHCO_3$ and 120 mL of H_2O. The organic layer was dried over Na_2SO_4, filtered, and the solvent was removed on a rotary evaporator giving **4** as an oil (4.62 g, 93%). The product solidified on standing overnight. NMR ($CDCl_3$): δ 1.41 (s, 6H), 4.13 (s, 2H), 7.55 (m, 1H).

Synthesis of oxazoline 5. To a freshly prepared solution of LDA generated from diisopropylamine (3.1

mL, 22 mmol) and 2.5 M *n*-BuLi (8.3 mL, 21 mmol) in 75 mL anhydrous THF at −78 °C under nitrogen was added a solution of *N-t*-Boc ethylisonipecotate[11] (4.48 g, 18.41 mmol) in 20 mL THF dropwise over 5 min. Upon completion of the addition, the mixture was allowed to stir for 15 min at low temperature before the dropwise addition of a solution of **3** (4.81 g, 18.27 mmol) in 20 mL THF over 13 min. After an additional 10 min at low temperature, the reaction mixture was allowed to warm to ice bath temperature during which time it darkened considerably. After approximately 30 min, the reaction was quenched at ice bath temperature by the addition of 10–15 mL of saturated ammonium chloride solution and was then partitioned between ethyl acetate and brine solution. The aqueous phase was extracted with ethyl acetate (3×) and the combined organics dried ($MgSO_4$) and concentrated in vacuo to give a yellow foam. Purification by flash chromatography on silica gel (ethyl acetate–hexane) gave **5** as a pale yellow foam (6.48 g, 13.79 mmol, 75%): ^{1}H NMR ($CDCl_3$): δ 1.25 (t, 3H, J=7.5 Hz), 1.41 (s, 6H), 1.46 (s, 9H), 2.2–2.3 (m, 4H), 3.43–3.66 (m, 4H), 4.16 (s, 2H), 4.22 (q, 2H, J=7.5 Hz), 7.81 (dd, 1H, J=10.5 Hz); MS: m/z 470 $(M+H)^+$.

Synthesis of oxazoline 6. An oven-dried system under positive N_2 atmosphere was charged with 44 mL of 1.0 M lithium hexamethyldisilazide. The reaction mixture was cooled in an ice bath and 5.14 g (20 mmol) of *N-t*-Boc ethylisonipecotate[12] in 50 mL of dry THF was added dropwise with stirring. The reaction mixture was stirred at 0 °C for 30 min after the addition was complete. The reaction mixture was cooled to −40 °C and 4.94 g (20 mmol) of **4** in 40 mL of dry THF was added dropwise with stirring. The reaction mixture was stirred at −40 °C for 5 h after the addition was complete. The reaction was quenched by pouring into 500 mL of 10% NH_4Cl. The aqueous solution was extracted with CH_2Cl_2 (3×500 mL). The combined organic layers were dried over $MgSO_4$. The solvent was filtered and concentrated on a rotary evaporator giving **6** as a white solid (9.23 g, 95%): NMR ($CDCl_3$): δ 1.22 (t, 3H, J=7.5 Hz), 1.41 (s, 6H), 1.48 (s, 9H), 2.33 (dd, 4H), 3.35 (m, 2H), 3.72 (m, 2H), 4.12 (s, 2H), 4.20 (q, 2H, J=7.5 Hz), 7.40 (ddd, 1H, J=2, 4, 15 Hz); MS: m/z 485 $(M+H)^+$.

Synthesis of oxazoline 7. A solution of **5** (4.33 g, 9.21 mmol) in 50 mL anhydrous ethyl ether was added dropwise over 26 min to a suspension of LAH (665 mg, 17.5 mmol) in 150 mL anhydrous ether cooled in an ice bath under nitrogen. After 1 h the reaction was quenched by the dropwise addition of 25 mL satd ammonium chloride solution to the cold reaction mixture followed by 125 mL of 5% sodium bicarbonate solution with warming to rt. The mixture was transferred to a separatory funnel and the aqueous phase extracted (4×) with ethyl acetate. The combined organics were dried ($MgSO_4$) and concentrated in vacuo to give a yellow foam. The crude product was purified by flash chromatography on silica gel (ethyl acetate–hexane) to give 2.69 g (6.08 mmol, 66%) of oxazoline alcohol **7** as a yellow foam: ^{1}H NMR ($CDCl_3$): δ 1.41 (s, 6H), 1.46 (s, 9H), 1.62–1.75 (m, 2H), 2.35–2.48 (m, 2H), 3.22–3.35 (m, 2H), 3.54–3.67 (m, 2H), 3.9 (s, 2H), 4.15 (s, 2H), 7.83 (db, J=12Hz, 1H); MS: m/z 442 $(M+H)^+$.

Synthesis of oxazoline 8. An oven-dried system under positive N_2 atmosphere was charged with 8.1 g (16.7 mmol) of **6** in 150 mL of dry diethyl ether. The reaction mixture was cooled in an ice bath and 665 mg (17.5 mmol) of $LiAlH_4$ was added in one portion. The reaction mixture was stirred at 0 °C for 4 h and was quenched by cautious addition of 250 mL of 10% NH_4Cl. The solution was extracted with EtOAc (3×250 mL). The combined organic layers were dried over Na_2SO_4. The solution was filtered and the solvent was removed on a rotary evaporator. The product **8** formed a white foam (7.20 g, 97% upon drying on a vacuum pump): NMR ($CDCl_3$): δ 1.40 (s, 6H), 1.45 (s, 9H), 1.54 (s, 1H), 1.65 (m, 2H), 2.60 (d, 2H, J=15 Hz), 2.90 (dd, 2H, J=12 Hz), 3.75 (d, 2H, J=6 Hz), 3.95 (d, 2H, J=15 Hz), 4.10 (s, 2H), 7.20 (ddd, 1H, J=3 Hz, 12 Hz, 6 Hz); MS: m/z 443 $(M+H)^+$.

Synthesis of oxazoline 9. Sodium hydride, as an 80% dispersion in mineral oil (0.348 g, 11.6 mmol) was added in portions to a solution of **7** (3.30 g, 7.47 mmol) in 150 mL of anhydrous THF under nitrogen at rt. The mixture was subsequently heated in an oil bath to 50–60 °C and the reaction was monitored by TLC. After 9.5 h, a slurry of 0.35 g sodium hydride (as above) in 15 mL THF was added via cannulae and the mixture allowed to stir overnight. The flask was subsequently cooled in an ice bath followed by the cautious addition of 5 mL of saturated ammonium chloride and warming to rt. The reaction mixture was partially concentrated in vacuo to remove most of the THF, then partitioned between ethyl acetate (150 mL) and brine solution (550 mL). The aqueous phase was extracted with ethyl acetate (3×50 mL) and the combined organics dried ($MgSO_4$), then concentrated in vacuo to give the crude product mixture of **9** and **9a** as a tan foam. Purification and fractionation was achieved by flash chromatography on silica gel with ethyl acetate–hexane, 0.5 vol. % triethylamine to give **9** (1.43 g, 3.39 mmol, 45%) and **9a** (0.417 g, 1.10 mmol, 13%), both as crystalline solids. An additional 0.336 g of mixed fractions were also recovered. An analytical sample of **9a** was obtained by recrystallization from hexane: mp 102.5–104 °C; ^{1}H NMR ($CDCl_3$): δ 1.4 (s, 6H), 1.48 (s, 9H), 1.7–1.97 (m, 4H), 2.74–2.93 (m, 2H), 3.1–3.23 (m, 1H), 4.15 (s, 2H), 4.18–4.24 (br s, 2H); MS: m/z 412 $(M+H)^+$. Anal. calcd for $C_{20}H_{27}N_3O_3FCl$: C, 58.30; H, 6.62; H, 6.62; N, 10.20. Found: C, 58.49; H, 6.66; N, 10.05. The chromatographed sample of **9** was analytically pure: mp 163.5–166 °C; ^{1}H NMR ($CDCl_3$): δ 1.46 (s, 6H), 1.47 (s, 9H), 1.65 (m, 2H), 1.98–2.10 (m, 2H), 3.0–3.13 (m, 2H), 4.02–4.15 (brm, 2H), 4.14 (s, 2H), 4.50 (s, 2H), 7.39 (s, 1H); MS: m/z 422 $(M+H)^+$. Anal. calcd for $C_{21}H_{28}N_3O_4Cl \cdot H_2O$: C, 57.32; H, 6.88; N, 9.55. Found: C, 57.61; H, 6.47; N, 9.53.

Synthesis of acid 10. A sample of oxazoline **9** (0.194 g, 0.459 mmol) was suspended in 12 mL of 10% HCl and the mixture heated to 90–95 °C for approximately 22 h. The resulting solution was cooled in an ice bath and made basic with solid sodium hydroxide followed by the addition of dioxane (8 mL) and Cbz chloride (0.150 mL, 1.01 mmol). The mixture was allowed to warm to rt. After 1 h the contents of the flask were partitioned between ethyl acetate and 10% aqueous citric acid solution containing sodium chloride. The aqueous phase was extracted with ethyl acetate (2×) and the combined organics were dried ($MgSO_4$) then concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (ethyl acetate–hexane-acetic acid) to give 0.162 g (0.399 mmol, 87%) of compound **10** as a foam. An analytical sample was obtained by recrystallization from ethyl acetate–hexane: mp 165–167 °C; ^{1}H NMR ($CDCl_3$): δ 1.64–1.8 (m, 2H), 2.01–2.15 (m, 2H), 3.16–3.3 (m, 2H), 4.06–4.23 (m, 2H), 4.54 (s, 2H), 5.16 (s, 2H), 7.28–7.4 (m, 5H), 7.62 (s, 1H); MS: *m/z* 403 $(M+H)^+$, m/z 420 $(M+NH_4)^+$. Anal. calcd for $C_{20}H_{19}N_2O_5Cl$: C, 59.62; H, 4.76; N, 6.95. Found: C, 59.49; H, 4.77; N, 6.71.

Synthesis of β-keto ester 12. Spirotricyclic acid **10** (1.11 g, 2.75 mmol) was treated with thionyl chloride (6 mL) followed by two drops of DMF at rt under nitrogen. After 2.5 h, the reaction mixture was concentrated in vacuo and the residue dissolved in toluene, then reconcentrated in vacuo (repeat 1×). The crude acid chloride **11** (quantitative yield) was sufficiently pure for use as isolated. Conversion to the β-ketoester was carried out as previously described.[12] The crude product so obtained was purified by flash chromatography on silica gel (ethyl acetate–hexane) to give 0.91 g 1.92 mmol, 70%) of the desired β-ketoester **12** as an oil: ^{1}H NMR ($CDCl_3$): tautomeric mixture δ 1.26 (t, $J=7$ Hz, 1.8 H), 1.34 (t, $J=7$ Hz, 1.2 H), 1.63–1.80 (m, 2H), 2.0–2.15 (m, 2H), 3.15–3.30 (m, 2H), 4.06 (s, 0.9 H), 4.08–4.20 (m, 2H), 5.62 (s, 0.4 H), 7.24 (s) and 7.25 (s) total 1H, 7.3–7.45 (m, 5H), 12.49 (s, 0.4 H); MS: *m/z* 473 $(M+H)^+$, 490 $(M+NH_4)^+$.

Synthesis of enamine 14. The enol ether of β-ketoester **13** was prepared according to Chu et al.[3] and was used without further purification. The crude enol ether (0.94 mmol) was dissolved in methylene chloride (6 mL) at rt under nitrogen and to this was added cyclopropylamine (0.075 mL, 1.03 mmol). After 2 h at rt, the mixture was concentrated in vacuo to give **14** as a viscous amber-colored oil in quantitative yield: ^{1}H NMR ($CDCl_3$): tautomeric mixture δ 0.7–1.0 (m, 4.7 H), 1.24 (t, 2.3 H), 1.6–1.8 (brm, 2H), 2.0–2.15 (m, 2H), 2.95–3.07 (m, 1H), 3.13–3.30 (m, 2H), 3.62 (q, 1.1 H), 3.99 (q, 0.9 H), 4.07–4.25 (brm, 2H), 4.49 (br s, 2H), 5.15 (s, 2H), 6.89 (s, 0.8 H), 6.96 (s, 0.2 H), 7.28–7.45 (m, 5H), 8.27 (db, $J=13.5$ Hz, 0.8 H), 8.36 (db, $J=15$ Hz, 0.2 H), 9.67 (brdb, 0.2 H), 11.04 (brdb, 0.8 H); MS: *m/z* 540 $(M+H)^+$. Compound **15** was prepared by reacting enol ether **13** (0.96 mmol) with 2,4-difluoroaniline (0.10 mL, 0.96 mmol) also in quantitative yield: ^{1}H NMR ($CDCl_3$): tautomeric mixture δ 0.85 (t), 0.98 (t) and 1.31 (t) total 3H, 1.62–1.8 (brm, 2H), 2.0–2.16 (m, 2H), 3.13–3.30 (m, 2H), 3.73 (q), and 4.24 (q) total 2H, 4.05–4.25 (brm, 2H), 4.52 (s, 2H), 5.15 (s, 2H), 6.85–7.06 (m, 3H), 7.3–7.45 (m, 5H), 8.57 (db, $J=13.5$ Hz) and 8.62 (db, $J=15$ Hz) total 1H, 11.37 (brdb, $J=13.5$ Hz, 0.3 H), 12.69 (brdb, $J=13.5$ Hz, 0.7 H); MS: *m/z* 612 $(M+H)^+$.

Synthesis of naphthyridine 16. Sodium hydride, as an 80% mineral oil dispersion (0.048 g, 1.60 mmol), was added under nitrogen to a solution of enamine **14** (0.420 g, 0.78 mmol) in 25 mL anhydrous THF at rt. The mixture was subsequently warmed to 45–50 °C. After 50 min, TLC analysis showed consumption of **14**. The reaction mixture was cooled to rt. The contents of the flask were partitioned between ether and brine solution. The aqueous phase was extracted with ether (3×) and the combined organics were dried ($MgSO_4$), then concentrated in vacuo to give the crude product as a waxy yellow solid. Recrystallization from ethyl acetate–hexane gave 0.096 g of **16** (0.191 mmol, 24%) as a cream-colored solid: mp 158.5–160 °C; ^{1}H NMR ($CDCl_3$): δ 1.0–1.1 (m, 2H), 1.2–1.32 (m, 2H), 1.42 (t, $J=6$ Hz, 3H), 1.73–1.9 (brm, 2H), 1.97–2.15 (brm, 2H), 3.53–3.7 (m, 3H), 4.02–4.17 (m, 2H), 4.4 (q, 2H), 4.51 (s, 2H), 5.18 (s, 2H), 7.3–7.45 (m, 5H), 8.02 (s, 1H), 8.61 (s, 1H); MS: *m/z* 504 $(M+H)^+$. Anal. calcd $C_{28}H_{29}N_3O_6$: C, 66.78; H, 5.82; N, 8.34. Found: C, 66.32; H, 5.86; N, 8.23. Similarly, **17** was prepared from enamine **15** (0.96 mmol). Purification by flash chromatography on silica gel (ethyl acetate–hexane) gave 0.257 g (0.447 mmol, 47%) of a pale yellow foam: mp poorly defined, 84–94 °C; ^{1}H NMR ($CDCl_3$): δ 1.41 (t, $J=6$ Hz, 3H), 1.56–1.95 (brm, 4H), 3.32–3.5 (brm, 2H), 3.54–3.79 (brm, 2H), 4.41 (q, 2H), 4.47 (s, 2H), 5.14 (s, 2H), 7.0–7.15 (brm, 2H), 7.32–7.48 (m, 6H), 8.05 (s, 1H); MS: *m/z* 576 $(M+H)^+$. Anal. calcd for $C_{31}H_{27}N_3O_6F_2$: C, 64.88; H, 4.74; N, 7.30. Found: C, 64.44; N, 4.64; N, 7.22.

Synthesis of furonaphthyridines 18 and **19**. The fully protected naphthyridine **16** (0.086, 0.171 mmol) was suspended in 4 N HCl (4.5 mL) and heated under reflux overnight. The clear yellow solution was subsequently concentrated in vacuo to a small volume and the product precipitated with THF. Vacuum filtration followed by trituration of the cake with additional THF gave, after drying in vacuo, 38.3 mg (0.101 mmol, 59%) of pure product **19** as a pale yellow powder: mp >290 °C (dec). ^{1}H NMR (TFA): δ 1.41–1.50 (m, 2H), 1.7–1.8 (m, 2H), 2.4–2.68 (m, 4H), 3.65–3.82 (brm, 2H), 4.15–4.35 (brm, 2H), 4.4–4.5 (brm, 1H), 8.22 (s, 1H), 9.46 (s, 1H); MS: *m/z* 342 $(M+H)^+$, 359 $(M+NH_4)^+$. Anal. calcd for $C_{18}H_{20}ClN_3O_4 \cdot H_2O$: C, 54.60; H, 5.61; N, 10.61. Found: C, 54.93; H, 5.08; N, 10.52. In a similar fashion fully protected analogue **17** (0.104 g, 0.182 mmol) was converted to **18**, (39.5 mg, 0.088 mmol, 48%): mp >270 °C (dec); ^{1}H NMR (TFA): δ 2.3–2.45 (m, 4H), 3.47–3.78 (m, 4H), 4.86 (s, 2H), 7.27–7.45 (m, 2H), 7.69–7.84 (m, 1H), 8.29 (s, 1H), 9.48 (s, 1H); MS: *m/z* 414 $(M+H)^+$, 430

$(M+NH_4)^+$. Anal. calcd for $C_{21}H_{18}ClF_2N_3O_4$: C, 56.02; H, 4.04; N, 9.34. Found: C, 55.81; H, 3.90; N, 8.96.

Synthesis of *N*-methyl analogue 20. To a solution of **16** (0.56 g, 0.11 mmol) in 1.5 mL 88% formic acid was added 12.8 mg 5% Pd/C followed by vacuum degassing and exposure to hydrogen (balloon) at rt. After 1 h, the reaction mixture was vacuum filtered through Celite and 0.2 mL 37% formalin solution was added to the filtrate. The mixture was subsequently heated on a steam bath. After 75 min the reaction was complete. The mixture was concentrated in vacuo and the residue dissolved in 3 mL of 4 N HCl, heated on a steam bath for 1 h, then reconcentrated in vacuo. The residue was suspended in THF, then centrifuged. The pellet was triturated with THF, centrifuged, and finally dried in vacuo to give 0.031 g (0.079 mmol, 72%) pure product **20** as a white solid: mp >270 °C (dec); ^{1}H NMR (TFA): mixture of conformers δ 1.4–1.56 (m, 4H), 2.33–2.90 (m, 4H), 3.15–3.29 (m, 3H), 3.84–4.30 (m, 4H), 4.83 (s) and 5.05 (s) total 2H, 8.2 (s) and 8.23 (s) total 1H, 9.42 (s) and 9.5 (s) total 1H; MS: *m/z* 356 $(M+H)^+$, 373 $(M+NH_4)^+$. Anal. calcd for $C_{19}H_{22}ClN_3O_4 \cdot 1.5H_2O$: C, 54.46; H, 6.03; N, 10.03. Found: C, 54.34; H, 5.73; N, 9.77.

Synthesis of oxazoline 21. An oven-dried system under positive N_2 atmosphere was charged with 4.07 g (9.91 mmol) of NaH/mineral oil (washed with dry hexane 3 × 100 mL) covered with 100 mL of dry THF. The flask was cooled in an ice bath and 3.92 g (8.86 mmol) of **8** in 125 mL of dry THF was added dropwise with stirring. The reaction mixture was stirred at rt for 8 h and quenched by pouring the reaction mixture cautiously into 300 mL of 10% NH_4Cl. The solution was extracted with CH_2Cl_2 (3 × 225 mL). The combined organic layers were dried over Na_2SO_4. The solvent was filtered and removed on a rotary evaporator. Drying on a vacuum pump gave **21** as a white solid (3.34 g, 89%): mp 52–53 °C; NMR ($CDCl_3$): δ 1.38 (s, 6H), 1.48 (s, 9H), 1.77 (d, 2H), 2.20 (dt, 2H, J=4.5, 13 Hz), 2.76 (dd, 2H, J=13 Hz), 4.09 (s, 2H), 4.14 (m, 2H), 4.58 (s, 2H), 7.08 (dd, 1H, J=4.5, 9 Hz); MS: *m/z* 423 $(M+H)^+$; IR (KBr): 1680 (C=N), 1640 (C=O) cm^{-1}. Anal. calcd for $C_{22}H_{28}F_2N_2O_4$: C, 62.55; H, 6.68; N, 6.63. Found: C, 63.00; H, 6.85; N, 6.57.

Synthesis of acid 22. A flask was charged with 8.76 g (20.7 mmol) of **21** suspended in 125 mL of 3 N HCl. The reaction mixture was heated at 100 °C for 48 h. After cooling to rt, the reaction mixture was cooled in an ice bath and was adjusted to pH 12–13 with solid NaOH. To this was added 8.9 mL (62 mmol) of benzylchloroformate. The reaction mixture was stirred at 0 °C for 30 min, and then at rt for 12 h. The filtrate was adjusted to pH 1–2 with 1 N HCl and was then extracted with EtOAc (3 × 125 mL). The combined organic layers were then dried over Na_2SO_4. The solvent was filtered and was evaporated in vacuo giving **22** as a tan solid (11.2 g). The product was dissolved in 250 mL of CH_2Cl_2 and was washed with 250 mL 1 N NaOH. The organic layer was dried over $MgSO_4$. The solvent was filtered and concentrated on a rotary evaporator, giving **22** as an oil (8.0 g; 95% yield): MS: *m/z* 421 $(M+NH_4)^+$, 404 $(M+H)^+$.

Synthesis of β-keto ester 24. An oven-dried system protected from moisture was charged with 4.03 g (10 mmol) of **22** suspended in 50 mL of dry CH_2Cl_2. To this was added 2 drops of DMF. The reaction mixture was cooled in an ice bath and 7.2 mL (100 mmol) of $SOCl_2$ was added dropwise with stirring. The reaction mixture was stirred at 0 °C for 1 h and then heated at 45 °C for 24 h. After cooling to rt, the solvent was removed on a rotary evaporator. The product **23** was used immediately in the next step.

An oven-dried system under positive N_2 atmosphere was charged with 2.6 g (20 mmol) of monoethylmalonate dissolved in 20 mL of dry THF. To this was added 1.5 mg of 2,2′-biquinoline as an indicator. The reaction mixture was cooled to −60 °C and *n*-BuLi was added until the purple color persisted at −5 °C. The reaction mixture was recooled to −60 °C and 4.2 g (10 mmol) of **23** in 25 mL of dry THF was added dropwise with stirring. The reaction mixture was stirred at −60 °C for 15 min after the addition was complete and then at rt for 1 h. The reaction was quenched by pouring into 200 mL of 0.5 N HCl. The solution was extracted with EtOAc (3 × 200 mL). The combined organic layers were dried over Na_2SO_4 and then filtered. The solvent was removed on a rotary evaporator. The product **24** weighed 520 mg (80%); NMR ($CDCl_3$): δ 1.35 (t, 2H, J=7 Hz), 1.45 (t, 1H, J=7 Hz), 1.80 (d, 2H), 2.20 (dt, 2H, J=3, 7 Hz), 2.84 (dd, 2H), 3.91 (s, 1H), 3.93 (s, 1H), 4.20 (q, 1.5 H, J=7.5 Hz), 4.30 (q, 0.5 H, J=7.5 Hz), 4.58 (s, 1H), 4.60 (s, 1H), 5.18 (s, 2H), 7.05 (dd, 0.5 H, J=3 Hz), 7.15 (dd, 0.5 H, J=3 Hz), 7.40 (m, 5H). The NMR showed a mixture of the keto and enol forms of the β-keto ester; MS: *m/z* 491 $(M+NH_4)^+$, 473 $(M)^+$.

Synthesis of quinolone 29. An oven-dried system under positive N_2 atmosphere was charged with 314 μL (1.95 mmol) of triethylorthoformate and 457 μL (4.87 mmol) of acetic anhydride. The reaction mixture was heated at 120 °C for 2 h. The excess reagents were removed under vacuum at 50 °C. The product **25** was used immediately in the next step without purification. The enol ether **25** (349 mg, 0.66 mmol) was dissolved in 5 mL of CH_2Cl_2. To this was added 55 μL (0.79 mmol) of cyclopropylamine. The reaction mixture was stirred at rt under a positive N_2 atmosphere for 3 h. The solvent was removed on a rotary evaporator and the product was dried on a vacuum pump. The product **26** was a solid which weighed 356 mg (99%).

An oven-dried system under positive N_2 atmosphere was charged with 33 mg NaH/mineral oil. The NaH was washed with dry hexane (3 × 5 mL) and was covered with 1 mL of dry THF. The reaction mixture was cooled in an ice bath and 365 mg (0.65 mmol) of **26** in 4 mL of dry THF was added dropwise with stirring. After the addition was complete the reaction

mixture was warmed to rt and was then heated at 70 °C for 5 h. The reaction mixture was cooled to rt and was quenched by pouring into 60 mL of 10% NH_4Cl. The solution was extracted with CH_2Cl_2 (3 × 60 mL) and the combined organic layers were dried over Na_2SO_4. The solvent was filtered and was concentrated to dryness on a rotary evaporator. The crude product was purified by column chromatography on 15 g of 70–230 mesh silica gel. The column was eluted with 1% MeOH/CH_2Cl_2. Similar fractions were pooled and concentrated to dryness giving **29** as a white solid (83 mg, 22%); NMR ($CDCl_3$): δ 1.10 (m, 2H), 1.20 (m, 2H), 1.41 (t, 3H, J = 7.5 Hz), 1.83 (d, 2H, J = 15 Hz), 2.32 (dt, 2H), J = 6, 15 Hz), 2.88 (dd, 2H, J = 15 Hz), 3.90 (m, 1H), 4.30 (m, 2H), 4.38 (q, 2H, J = 7.5 Hz), 4.62 (s, 2H), 5.20 (s, 2H), 7.38 (m, 5H), 7.73 (d, 1H, J = 12 Hz), 8.51 (s, 1H); MS: m/z 521 $(M+H)^+$.

Synthesis of quinolone 30. An oven-dried system under positive N_2 atmosphere was charged with 2.36 g (4.5 mmol) of **25** dissolved in 25 mL of dry CH_2Cl_2. To this was added 443 μL (4.7 mmol) of 4–fluoroaniline. The reaction mixture was stirred at rt for 1.5 h. The solvent was removed on a rotary evaporator and the residue was dissolved in 35 mL of dry THF. This solution was treated with 190 mg (4.7 mmol) of NaH. The reaction mixture was heated at 68 °C for 2 h. The system was cooled to rt and was then poured into cold water. The product was isolated by suction filtration and was washed with water and 2:1 Et_2O:hexane, giving **30** as a yellow solid (2.53 g, 98%): NMR ($CDCl_3$): δ 1.38 (t, 3H, J = 7.5 Hz), 1.69 (m, 2H), 2.25 (m, 2H), 2.72 (m, 2H), 4.21 (m, 2H), 4.28 (s, 2H), 4.39 (q, 2H, J = 7.5 Hz), 5.15 (s, 2H), 7.40 (m, 9H), 7.79 (d, 1H, J = 12 Hz), 8.33 (s, 1H); MS: m/z 575 $(M+H)^+$.

Synthesis of quinolone 31. A system under positive N_2 atmosphere was charged with 2.48 g (5.2 mmol) of the enol ether **25** dissolved in 25 mL of dry CH_2Cl_2. To this was added 640 μL (6.3 mmol) of 2,4-difluoroaniline. The reaction mixture was stirred at rt for 20 h. The solvent was removed on a rotary evaporator and the product was dried on a vacuum pump giving **28** as a solid (3.1 g, 96%). An oven-dried system under positive N_2 atmosphere was charged with 233 mg of NaH/mineral oil. The NaH was washed with dry hexane (3 × 8 mL) and was covered with 5 mL of dry THF. The reaction flask was cooled in an ice bath and 3.1 g (5.2 mmol) of **28** in 25 mL of dry THF was added dropwise with stirring. After the addition was complete, the reaction mixture was warmed to rt and was then heated at 65 °C with stirring for 28 h. The reaction was quenched by pouring the cooled reaction mixture into 200 mL of 10% NH_4Cl. The aqueous solution was extracted with CH_2Cl_2 (2 × 200 mL). The combined organic layers were dried over Na_2SO_4. The solution was filtered and the solvent was removed on a rotary evaporator. The product was dried overnight on a vacuum pump, giving **31** as a solid which was recrystallized from EtOH/water giving 2.59 g (84%); NMR ($CDCl_3$): δ 1.40 (t, 3H, J = 7.5 Hz), 1.75 (m, 2H, J = 15 Hz), 2.25 (dt, 2H, J = 6, 15 Hz), 2.73 (d, 2H, J = 15 Hz), 4.22 (m, 2H), 4.35 (s, 2H), 4.40 (q, 2H, J = 7.5 Hz), 5.15 (s, 2H), 7.00 (m, 2H), 7.40 (m, 6 H), 7.77 (d, 1H, J = 12 Hz), 8.25 (s, 1H).

Synthesis of furoquinolone 32. A sample of 75 mg (0.14 mmol) of **29** was dissolved in 25 mL of CH_3OH and was deprotected with 80 mg of 10% Pd/C at rt under 4 atm of hydrogen for 28 h. The catalyst was removed by filtration through a 0.45 μm nylon millipore filter. The solvent was removed on a rotary evaporator giving 56 mg (100%) of a pale yellow solid; NMR ($CDCl_3$): δ 1.11 (m, 2H), 1.20 (m, 2H), 1.42 (t, 3H, J = 7.5 Hz), 1.80 (d, 2H, J = 15 Hz), 2.30 (br s, 1H), 2.35 (dt, 2H, J = 15 Hz), 2.70 (dd, 2H, J = 15 Hz), 3.20 (d, 2H, J = 15 Hz), 3.90 (m, 1H), 4.38 (q, 2H, J = 7.5 Hz), 4.65 (s; 2H), 7.70 (d, 1H, J = 12 Hz), 8.50 (s, 1H). The product (53 mg, 0.14 mmol) was dissolved in 1.5 mL of 1 N HCl. The reaction mixture was heated at 85 °C with stirring for 4 h. The reaction mixture was cooled to rt and the solvent was removed on a rotary evaporator. The product **32** was suspended in 5 mL of 1:1, THF:EtOH and the solid was isolated by suction filtration. The product was dried under vacuum at rt giving **32** as a white solid (31 mg, 57%): mp >300 °C; NMR (TFA): δ 1.52 (m, 4H), 2.30 (m, 2H), 2.90 (m, 2H), 3.41 (m, 2H), 3.81 (m, 2H), 4.60 (m, 1H), 5.07 (s, 2H), 7.92 (d, 1H, J = 12 Hz), 9.28 (s, 1H); MS: m/z 359 $(M-Cl)^+$, 319 $(M-Cl-H_2O)^+$; IR (KBr): 3440 (OH), 1720 (C=O), 1610 (C=O) cm^{-1}. Anal. calcd for $C_{19}H_{22}ClFN_2O_4 \cdot 1/2H_2O$: C, 56.51; H, 5.24; N, 6.94. Found: C, 56.54; H, 5.40; N, 6.80.

Synthesis of furoquinolone 33. A sample of 2.53 g (4.4 mmol) of **30** was dissolved in 250 mL of MeOH and was deprotected with 600 mg of 10% Pd/C under 4 atm of hydrogen at rt for 24 h. The catalyst was removed by filtration through a 0.45 μm millipore filter. The solvent was removed on a rotary evaporator and the product was triturated with diethyl ether, giving a solid (1.12 g, 58%): NMR ($CDCl_3$): δ 1.64–3.40 (m, 9H), 7.18 (m, 2H), 7.36 (m, 2H), 7.74 (m, 1H), 8.34 (s, 1H); MS: m/z 441 $(M+H)^+$. The product from the previous step (1.12 g, 2.5 mmol) was suspended in 25 mL of 1.0 N HCl and was heated at 60 °C with vigorous stirring for 16 h. The reaction mixture was cooled to rt and the product was isolated by suction filtration. The filter cake was washed with water, EtOH, and Et_2O. The product was dried under vacuum at rt giving **33** as an off-white solid (1.04 g, 91%): mp 270–272 °C; NMR (TFA): δ 2.24 (m, 2H), 2.85 (m, 2H), 3.32 (m, 2H), 3.82 (m, 2H), 4.68 (s, 2H), 7.36 (m, 2H), 7.60 (m, 2H), 8.09 (d, 1H, J = 12 Hz), 9.23 (s, 1H); MS: m/z 413 $(M-Cl)^+$. Anal. calcd for $C_{22}H_{18}ClF_2N_2O_4 \cdot 2.5H_2O$: C, 53.50; H, 4.90; N, 5.67. Found: C, 53.62; H, 4.42; N, 5.71.

Synthesis of furoquinolone 34. A sample of **31** (1.93 g, 3.26 mmol) was dissolved in 150 mL of MeOH and was deprotected with 450 mg of 10% Pd/C at rt under 4 atm of hydrogen for 6 h. The catalyst was removed by filtration through a 0.45 μm nylon millipore filter. The solvent was removed on a rotary evaporator and

the product was dried on a vacuum pump giving 1.48 g (99%): NMR ($CDCl_3$): δ 1.40 (t, 3H, $J=7.5$ Hz), 1.65 (m, 2H), 2.25 (m, 3H), 2.55 (dd, 2H, $J=15$ Hz), 2.90 (br s, 1H), 3.11 (m, 2H), 4.30 (s, 2H), 4.40 (q, 2H, $J=7.5$ Hz), 7.00 (m, 2H), 7.41 (m, 1H), 7.75 (d, 1H, $J=12$ Hz), 8.25 (s, 1H); MS: *m/z* 473 $(M+NH_4)^+$, 459 $(M+H)^+$.

A flask was charged with 220 mg (0.48 mmol) of the ester from the previous step and 10 mL of 1.0 N HCl. The reaction mixture was heated at 75 °C with stirring for 4 h. The reaction mixture was cooled to rt and was diluted with 10 mL of water. The resulting precipitate was isolated by suction filtration. The filter cake was washed with 5 mL of water. The product was dried under vacuum at rt. The solid was suspended in 5 mL of EtOH and was heated under reflux for 1.5 h. The system was cooled to rt and the product was isolated by suction filtration. The product was dried under vacuum at rt giving **34** as an off-white solid (147 mg, 66%): mp >300 °C; NMR (TFA): δ 2.25 (m, 2H), 2.90 (m, 2H), 3.35 (m, 2H), 3.82 (m, 2H), 4.72 (s, 2H), 7.20 (m, 2H), 7.70 (m, 1H), 8.10 (d, 1H, $J=10$ Hz), 9.22 (s, 1H); MS: *m/z* 431 $(M-Cl)^+$; IR (KBr): 3420 (OH, NH), 1730 (C=O), 1610 (C=O) cm^{-1}. Anal. calcd for $C_{22}H_{18}ClF_3N_2O_4 \cdot H_2O$: C, 54.50; H, 4.16; N, 5.78. Found: C, 54.12; H, 3.23; N, 5.62.

In vitro antibacterial activity

The in vitro antibacterial activity of the synthesized compounds was tested in a side-by-side comparison with ciprofloxacin (CIP) and determined by conventional agar dilution procedures. The organisms were grown overnight in brain–heart infusion (BHI) broth (Difco 0037-01-6) at 36 °C. Twofold dilutions of the stock solution (2000 μg/mL) of the test compound were made in BHI agar to obtain the test concentration ranging from 200–0.005 μg/mL. The plate was inoculated with approximately 10^4 organisms. It was then incubated at 36 °C for 18 h. The minimal inhibitory concentration (MIC) was the lowest concentration of the test compound that yielded no visible growth on the plate.

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