Topoisomerase Inhibitors Addressing Fluoroquinolone Resistance in Gram-Negative Bacteria

Colin K. Skepper,,† Duncan Armstrong,§ Carl J. Balibar,† Daniel Bauer,‡ Cornelia Bellamacina,† Bret M. Benton,† Dirksen Bussiere,† Gianfranco De Pascale,† Javier De Vicente,† Charles R. Dean,† Bhavesh Dhumale,⊥ L. Mark Fisher,¶ John Fuller,† Mangesh Fulsunder,⊥ Lauren M. Holder,† Cheng Hu,† Bhavin Kantariya,⊥ Guillaume Lapointe,† Jennifer A. Leeds,† Xiaolin Li,† Peichao Lu,† Anatoli Lvov,§ Sylvia Ma,† Shravanthi Madhavan,† Swapnil Malekar,† David McKenney,† Wosenu Mergo,† Louis Metzger,† Heinz E. Moser,† Daniel Mutnick,† Jonas Noeske,† Colin Osborne,† Ashish Patel,⊥ Darshit Patel,⊥ Tushar Patel,⊥ Krunal Prajapati,⊥ Katherine R. Prosen,† Folkert Reck,† Daryl L. Richie,† Alice Rico,† Mark R. Sanderson,‖ Shailesh Satasia,⊥ William S. Sawyer,† Jogitha Selvarajah,¶ Nirav Shah,⊥ Kartik Shanghavi,⊥ Wei Shu,† Katherine V. Thompson,† Martin Traebert,‡ Anand Vala,⊥ Lakhan Vala,⊥ Dennis A. Veselkov,‖ Jason Vo,† Michael Wang,† Marcella Widya,† Sarah L. Williams,† Yongjin Xu,† Qin Yue,† Richard Zang,† Bo Zhou† and Alexey Rivkin†*

†Novartis Institutes for BioMedical Research, Emeryville, California 94608, United States

‡Novartis Institutes for BioMedical Research, 4002 Basel, Switzerland

[§]Novartis Institutes for BioMedical Research, Cambridge, Massachusetts 02139, United States

[⊥] Piramal Discovery Solutions, Pharmaceutical Special Economic Zone, Sarkhej Bavla Highway, Ahmedabad, Gujarat 382213, India

[¶] Molecular and Clinical Sciences Research Institute, St George's, University of London, London SW17 0RE, UK

[‖] Randall Centre for Cell and Molecular Biophysics, King's College, Guy's Campus, London Bridge, London SE1 1UL, UK

Abstract

Since their discovery over five decades ago, quinolone antibiotics have found enormous success as broad spectrum agents that exert their activity through dual inhibition of bacterial DNA gyrase and topoisomerase IV. Increasing rates of resistance, driven largely by target-based mutations in the GyrA/ParC Quinolone Resistance Determining Region, have eroded the utility and threaten the future use of this vital class of antibiotics. Herein we describe the discovery and optimization of a series of 4-(aminomethyl)quinolin-2(1*H*)-ones, exemplified by **34**, that inhibit bacterial DNA gyrase and topoisomerase IV and display potent activity against ciprofloxacin-resistant Gram-negative pathogens. X-ray crystallography reveals that **34** occupies the classical quinolone binding site in the topoisomerase IV-DNA cleavage complex, but does not form significant contacts with residues in the Quinolone Resistance Determining Region.

Introduction

Quinolone antibiotics trace their history to two parallel discovery efforts at Imperial Chemical Industries (ICI) and Sterling Drug over 50 years ago.[1] From these landmark studies emerged one

of the most successful classes of antibacterial agents with nearly 30 analogs approved for clinical use, and more still in the pipeline at various pharmaceutical companies.[2-8]

Quinolone antibiotics exert their activity by binding to and stabilizing DNA cleavage complexes formed by two essential bacterial enzymes: DNA gyrase and topoisomerase IV. This results in blockade of DNA replication. Subsequent downstream events lead to induction of the SOS response, chromosome fragmentation and cell death.[9, 10] Structural characterization of DNA-enzyme-quinolone ternary complexes has revealed that quinolones intercalate between DNA base pairs at two neighboring cleavage sites in the gyrase or topoisomerase IV heterotetramer complex.[11-13] Additional studies support a binding mode in which the keto-acid functionality of bound quinolones is chelated to a Mg^{2+} cation which in turn forms water mediated hydrogen bonds to residues in the quinolone resistance determining region (QRDR) of GyrA and ParC (e.g., Ser83 and Asp87 in *Escherichia coli* GyrA).[14-18] This interaction appears to be critical for the quinolone mechanism of action and may account for the fact that, despite 50 years of medicinal chemistry research, the overwhelming majority of quinolone antibiotics retain a carbonyl at C4 and carboxylate at C3 (as suggested by Wohlkonig *et al*).[14] In fact, this arrangement of substituents at C3 and C4 has been widely regarded as optimal and essential for the antibacterial activity of quinolones.[19] As with all classes of antibiotics, resistance to quinolones has emerged and continues to develop. While several mechanisms have been described, a major driver of high-level resistance is amino acid substitutions in DNA gyrase and topoisomerase IV at residues that are responsible for mediating the water-metal ion bridge interaction described above.[20] As a result, the current and long-term utility of quinolones for treatment of many infections caused by both Gram-positive and Gram-negative bacteria has already been eroded.[21]

The global problem of antibiotic resistance and the need for new and effective antibiotic drug candidates is well-documented.[22-28] Bacterial DNA gyrase and topoisomerase IV are clinically validated targets for chemotherapy, and quinolone antibiotics possess many attractive features including bactericidal activity against a broad spectrum of pathogens, rapid killing kinetics and oral bioavailability. As such, there is significant interest in identifying inhibitors of these enzymes that are able to overcome target-based resistance to quinolones.[29-31] In a series of seminal publications and patents Pfizer described the discovery and optimization of 3-aminoquinazolinediones[32-34] (e.g., compound **2**, Figure 1) that are capable of binding to and stabilizing DNA gyrase/topoisomerase IV cleavage complexes in the absence of the key water-metal ion bridge interaction that characterizes classical quinolone binding.[14-18, 35, 36] Lead compounds in this series exhibited little or no cross-resistance with quinolones. Importantly, these results demonstrated that the C3 carboxylate/C4 carbonyl arrangement present in nearly all quinolone antibiotics (see ciprofloxacin, Figure 1) is not strictly required for antibacterial activity. More recently, researchers from the Innovative Medicines Initiative European Gram-Negative Antibacterial Engine (IMI-ENABLE) consortium reported a series of imidazopyrazinones (IPYs) that target the quinolone binding pocket of bacterial DNA gyrase and topoisomerase IV without formation of the water-metal ion bridge.[37, 38] Representative compounds from this series showed partial cross-resistance with quinolones. Interestingly, the influence of QRDR mutations on the activity of IPYs was found to be intermediate between 3-aminoquinazolinediones and quinolone antibiotics.

Within Novartis, antibacterial drug discovery efforts were broadly focused on pathogens and resistance phenotypes identified as major threats to human health by the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO).[22, 23] As a part of these

efforts, we initiated an early stage medicinal chemistry program around compound **3** (Figure 1), which was identified as a hit during phenotypic screening for inhibitors of bacterial cell growth. Target identification efforts indicated that compounds in this series were inhibitors of DNA gyrase/topoisomerase IV, however the SAR proved to be extremely steep and we were unable to improve antibacterial potency to levels sufficient for a lead optimization program. The structural similarity to Pfizer's quinazolinediones, however, led us to consider a scaffold-morphing approach with the classical quinolone scaffold (e.g., **1**, ciprofloxacin).[39]

Structural and biochemical studies with quinazolinediones suggest that while this chemotype is incapable of forming a water-metal ion bridge with the target enzymes, it derives potency and affinity through additional interactions around the C7 amine moiety and a hydrogen bond between the C2 carbonyl oxygen and Arg117 (*Streptococcus pneumoniae* ParC numbering).[12, 35, 36] We hypothesized that by simply moving the C4 carbonyl of quinolones such as **1** to the C2 position (e.g., **4,** Figure 1), we would be able to generate compounds that take advantage of similar interactions and thus retain potency against both wild type (WT) and quinolone-resistant bacteria. We further postulated that completely removing the C3 carboxylate (e.g., **5**, Figure 1) could generate compounds that make no binding interactions at all with the GyrA/ParC QRDR region. Importantly, if this strategy proved successful it would provide a unique opportunity to explore SAR at the C3 and C4 positions of the quinolone scaffold which have remained largely untouched over 50 years of research.[19]

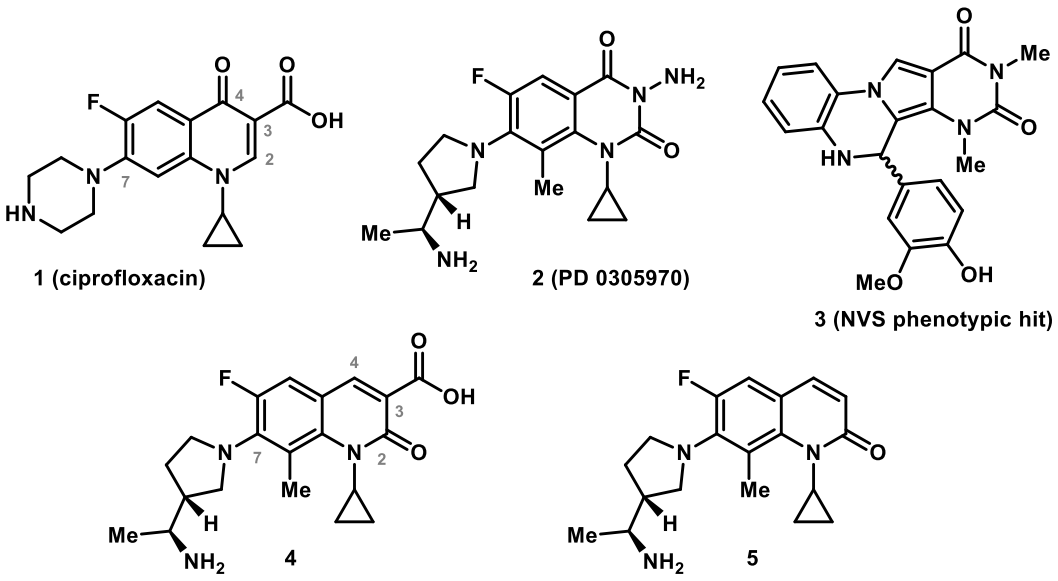


Figure 1. Structures of the fluoroquinolone antibiotic ciprofloxacin, quinazolinedione PD 0305970 (Pfizer), Novartis phenotypic hit **3** and initial lead compounds **4** and **5**.

Results and Discussion

SAR Development of the Quinolin-2(1*H*)-one Scaffold Our efforts commenced with the synthesis and profiling of compounds **4** and **5**. Biochemical assays were established that used size-exclusion chromatography to monitor the introduction of supercoils into relaxed DNA and decatenation of kinetoplast DNA by *E. coli* DNA gyrase and topoisomerase IV respectively (see Experimental Section for details). Against DNA gyrase from *E. coli*, compound **4** was less potent than both ciprofloxacin (**1**) and PD 0305970 (**2**), while **5** (which lacks the C3 carboxylic acid) exhibited potency more comparable to **1** and **2** (Table 1). Against *E. coli* topoisomerase IV, compound **5** was again more potent than **4** and approached the activity seen with compound **2**.

Against a selection of clinically relevant Gram-negative bacteria, compounds **4** and **5** exhibited encouraging levels of cell growth inhibition (Table 1). Robust activity was observed, in particular, against *E. coli* and *Klebsiella pneumoniae*. Importantly, both **4** and **5** exhibited a minimal shift in

MIC against an engineered efflux-deficient *Pseudomonas aeruginosa* strain harboring QRDR mutations in gyrase and topoisomerase IV (MIC values increased by 8X for **4** and 2X for **5** compared to 128X for ciprofloxacin). However, there was clearly a significant loss of intrinsic cellular activity for compounds **4** and **5** relative to fluoroquinolones such as ciprofloxacin (**1**). We thus embarked upon a program that was focused upon optimization of activity against Gram-negative bacteria.

Table 1. Biochemical and cellular activity of **1**, **2**, **4** and **5**.

		Compounds			
		1	**2**	**4**	**5**
Biochemical IC_{50} (μM)	*E. coli* Gyrase	0.49	0.20	2.91	0.43
	E. coli Topo IV	2.71	0.22	4.80	0.39
MIC (μg/mL)	*E. coli* WT	0.008	1	4	1
	K. pneumoniae WT	0.03	2	8	4
	P. aeruginosa WT	0.125	4	32	16
	P. aeruginosa Δ*mexB*/Δ*mexX*	0.03	1	8	4
	P. aeruginosa Δ*mexB*/Δ*mexX* FQ-R[a]	4	2	64	8

[a] GyrA T83I, ParC S87L

Historically, achieving sufficient cellular activity against Gram-negative bacteria has been one of the major hurdles limiting the success of many preclinical antibacterial research programs.[40-42] This is due in part to the complexity of the Gram-negative cell envelope which includes an outer membrane that restricts permeation of large and/or lipophilic molecules and an inner membrane that opposes influx of more polar molecules. These physical barriers work in concert with multi-drug efflux pumps to create a significant barrier to the accumulation of small molecule drugs in Gram-negative microorganisms.[43-51] This is a particularly acute problem for inhibitors that must

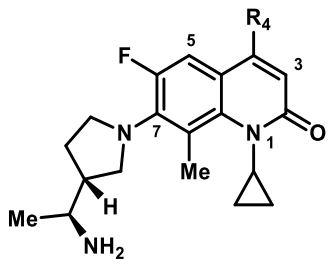
reach targets within the cytoplasm. Simultaneous optimization of cell permeability, efflux, on-target potency and drug-like properties continues to be a major challenge for the field.[52-57]

With this in mind, compound **5** was selected as the starting point for our efforts based on its superior baseline cellular activity and reduced impact of QRDR mutations on antimicrobial activity relative to **4**. Compound **5** also provided a unique opportunity to explore SAR at both the C3 and C4 positions of the scaffold. At the time this work was initiated there was scant knowledge about the characteristics of this region of the binding pocket in the absence of the water-metal ion bridge since the Pfizer quinazolinedione series maintained a C4 carbonyl group.

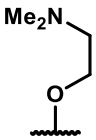
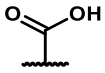
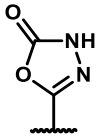
Small lipophilic substituents such as chlorine were well tolerated at C4 (e.g., **6**, Table 2), however compound **7** was substantially less active. The latter may be ascribed to the relatively acidic nature and hydration of the hydroxyl (pK_a = 5.7), which unlike the carboxylic acid of **4** cannot be masked by an internal hydrogen bond to the C2 carbonyl and may contribute to poor inner membrane permeability. Alternatively, while spectroscopic data for compound **7** was qualitatively similar to related C4-*O*-alkoxy derivatives, we cannot rule out the possibility that the C2-hydroxy/C4-carbonyl tautomer of **7** could exist in the bacterial cellular environment, and may contribute to the poor antibacterial activity observed. Capping the hydroxyl of **7** with a methyl group (**8**) restored some potency, while the nitrile (**9**) was 2-4 fold more potent against Gram-negative bacteria than compound **5**. The corresponding aminomethyl analog **10**, accessed by reduction of the nitrile, proved to be the most potent compound of the series. The introduction of a second, weakly basic amine at C4 often served to improve Gram-negative potency, particularly against *P. aeruginosa* (see Table 4 for additional examples of this effect). MIC values for **10** and related analogs against *E. coli* Δ*acrB* revealed a moderate efflux liability that was later partially addressed through modifications at the C7 position (see Table 4 below). Replacing the C4 amine

with an alcohol (**11**) was detrimental to antibacterial activity and appeared to further increase susceptibility to efflux in *E. coli*. The weakly basic amine could, however, be accommodated on a longer chain which presumably projects further into the binding pocket without significant loss of potency (**12**). A carboxylic acid (**13**) was deleterious to antimicrobial activity, possibly due to poor inner membrane permeability (carboxylate pK_a = 2.2). While smaller substituents at C4 were generally preferred for Gram-negative antibacterial activity, some larger groups were tolerated as exemplified by **14**, which retained some weak activity against *E. coli* WT.

Table 2. Antibacterial SAR at position 4 of the quinolin-2(1*H*)-one scaffold.



Cmpd	R_4	MIC (μg/mL)					
		***E. coli* WT**	***E. coli* Δ*acrB***	***K. pneumoniae* WT**	***P. aeruginosa* WT**	***P. aeruginosa* Δ*mexB* Δ*mexX***	***P. aeruginosa* Δ*mexB* Δ*mexX* FQ-R[a]**
5	H	1	0.06	4	16	4	8
6	Cl	2	0.06	4	8	4	8
7	OH	>32	16	>32	>32	>32	>32
8	OMe	32	1	16	32	8	16

9		1	≤0.03	2	4	1	1
10		1	0.06	4	2	0.5	2
11		16	0.25	32	16	4	8
12		1	-	4	8	4	8
13		>32	-	>32	>32	>32	>32
14		8	-	32	>32	8	32

[a] GyrA T83I, ParC S87L

Upon additional profiling of compound **10** it became apparent that the advantages conveyed by the presence of a weakly basic amine at C4 extended beyond an improvement in Gram-negative antibacterial potency. Relative to compound **5**, **10** displayed improved solubility and a reduction in plasma protein binding and hERG inhibition (Table 3). Both compounds exhibited low to moderate intrinsic clearance in liver microsomes, however **10** suffered from reduced permeability across mammalian cell membranes as gauged by CACO-2 P_{app}. Despite this, the aminomethyl substituent at C4 appeared to provide a good balance of potency and *in vitro* properties, prompting us to retain this group for subsequent SAR studies.

Table 3. *In vitro* profile of compounds **5** and **10**.

	5	**10**
$logD_{7.4}$	0.74	-0.29
Solubility (μg/mL, PBS)	498	>555
Plasma Protein Binding (% bound, mouse/rat/human)	81/75/90	56/18/17
hERG IC_{50} (μM)	48.8[a]	>300[b]
CACO-2 P_{app} A-B (efflux ratio)	16.7 (1.5)	0.44 (18)
CL_{int} in Liver Microsomes (μL/min/mg, rat/human)	10 / <7.7	<7.7 / 14

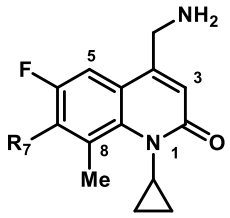
[a] Determined using manual patch clamp electrophysiology (see Experimental Section for details). [b] Determined using QPatch automated electrophysiology (see Experimental Section for details).

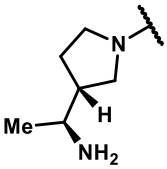
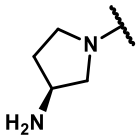
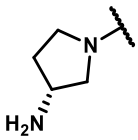
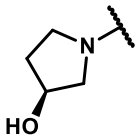
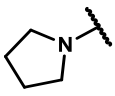
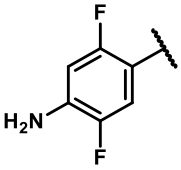
The C7 position of the classical quinolone scaffold is remarkably tolerant of a wide variety of substituents. Similarly, we found the C7 position of our scaffold to be more tolerant of structural changes than other positions. However, the scope of substituents that provided highly active compounds was significantly smaller than that described for quinolone antibiotics.

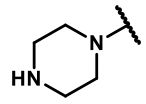
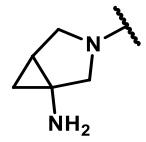
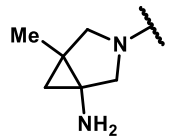
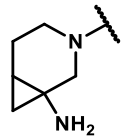
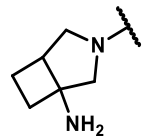
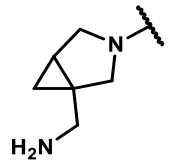
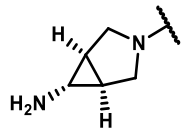
In general, substituted pyrrolidines proved to be the most suitable substituents at C7. 3*S*-aminopyrrolidine **15** was almost equally active relative to **10**, however inversion of the amine stereocenter resulted in ≥4-fold loss of *in vitro* potency (**16**, Table 4). Hydroxypyrrolidine **17** was also well tolerated and provided a boost in potency against *E. coli* and *K. pneumoniae*. An unsubstituted pyrrolidine at the C7 position provided a compound (**18**) with comparable potency against *E. coli* and *K. pneumoniae* but substantially reduced activity against *P. aeruginosa*. Aromatic substituents (e.g., **19**) often provided potent compounds but this came at the expense of higher $logD_{7.4}$ and poor solubility (data not shown). Compounds **18** and **19** support the observation that compounds with only a single basic amine often exhibited reduced activity against *P.*

aeruginosa compared to compounds with two (e.g., **10**) despite equal or better activity against *E. coli*. Six-membered saturated rings (e.g., piperazine, compound **20**) offered no improvement in antimicrobial activity despite the fact that this group is present in ciprofloxacin. This result suggested a divergence in SAR from known quinolone antibiotics. A minor breakthrough was realized with the identification of [3.1.0] bicyclic amines **21** and **22**, which were the most potent compounds of this series against *E. coli*. Compound **21** also showed reduced susceptibility to efflux relative to **10** as evidenced by a 4-fold shift between MIC values against *E. coli* WT and Δ*acrB* (compared to 16-fold for **10**). Further modification of the [3.1.0] azabicyclic moiety generally led to loss of antibacterial activity, which was strongly dependent on appropriate positioning of the amine. For example, the related [4.1.0] and [3.2.0] bicyclic amines (**23** and **24**) were much less potent, and moving the amine to alternate locations on the [3.1.0] ring system provided similar results (**25**-**26**). Given the comparable *in vitro* activity of **21** and **22**, we elected to retain the less lipophilic [3.1.0] moiety of **21** for subsequent SAR studies.

Table 4. Antibacterial SAR at position 7 of the quinolin-2(1*H*)-one scaffold.



Cmpd	R_7	MIC (μg/mL)					
		***E. coli* WT**	***E. coli* Δ*acrB***	***K. pneumoniae* WT**	***P. aeruginosa* WT**	***P. aeruginosa* Δ*mexB* Δ*mexX***	***P. aeruginosa* Δ*mexB* Δ*mexX* FQ-R[a]**
10		1	0.06	4	2	0.5	2
15		2	0.5	2	4	0.5	2
16		16	2	8	>32	8	32
17		0.5	≤0.03	1	4	0.25	1
18		1	0.06	2	32	4	8
19		0.25	≤0.03	1	8	2	8

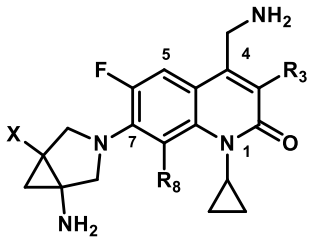
20		4	1	2	8	1	2
21		0.25	0.06	0.5	4	0.5	1
22		0.125	≤0.03	0.25	4	1	2
23		4	1	4	16	4	16
24		2	0.25	4	8	2	4
25		8	1	32	32	4	16
26		1	0.25	4	16	1	2

[a] GyrA T83I, ParC S87L

With an optimized C7 moiety established, we explored the C3 and C8 positions in the hope of deriving additional antibacterial potency. Removal of the methyl group from the C8 position resulted in a 4-fold reduction in potency (**27**, Table 5), and switching the methyl group for other small, polar groups provided similar results (**28**-**30**). The C3 position was tolerant of a variety of small substituents including CH_3, Cl and OH (although activity was lost with groups larger than CH_3). However, addition of a fluorine substituent led to a further 4 to 8-fold improvement in the

MICs against WT *E. coli*, *K. pneumoniae* and *P. aeruginosa* (**34**). Whether this improvement in activity was due to an increase in on-target potency, an increase in intracellular accumulation, or a combination of both factors was not immediately clear.

Table 5. Optimization of substitution at positions 3 and 8 of the quinolin-2(1*H*)-one scaffold.



Cmpd	X	R_3	R_8	MIC (μg/mL)					
				E. coli WT	*E. coli* Δ*acrB*	*K. pneumoniae* WT	*P. aeruginosa* WT	*P. aeruginosa* Δ*mexB* Δ*mexX*	*P. aeruginosa* Δ*mexB* Δ*mexX* FQ-R[a]
20	H	H	CH_3	0.25	0.06	0.5	4	0.5	1
27	H	H	H	1	0.5	4	16	2	4
28	H	H	CN	1	0.125	2	16	2	4
29	CH_3	H	OCH_3	1	0.25	4	32	8	16
30	H	H	OCF_2H	2	1	8	>32	8	32
31	H	CH_3	CH_3	0.25	0.125	1	8	1	2
32	H	OH	CH_3	0.5	0.25	1	2	0.5	2
33	H	Cl	CH_3	0.25	0.06	0.5	8	1	2
34	H	F	CH_3	0.06	≤0.03	0.125	1	0.25	0.5

[a] GyrA T83I, ParC S87L

Structural Characterization of the Binding Mode of 34 to *K. pneumoniae* topoisomerase IV

During the course of the optimization campaign, we developed a co-crystallization system with topoisomerase IV from *K. pneumoniae*. Topoisomerase IV is a tetrameric enzyme formed by two ParC and two ParE subunits. For structural studies, we followed a previously employed approach to generate a fusion protein, ParE-ParC, from the respective subunits of *K. pneumoniae.*[18] The resulting single polypeptide is a fusion protein from the C-terminal portion of the ParE (residues 390-631) and N-terminal portion of the ParC (residues 1-490) subunits of *K. pneumoniae* topoisomerase IV.[18] This enabled us to solve several co-crystal structures with inhibitor and DNA bound, including compound **34** (Figure 2 and Supplementary Figures 1 and 2). The DNA duplex was an uninterrupted 26-mer of the symmetrized E-site DNA.[18] Because of the size and inherent flexibility of the complex, achieving near-atomic level diffraction proved very challenging and most data sets had diffraction limits between 3-3.50 Å.

The complex formed between the ParE-ParC fusion protein, the homoduplex DNA and compound **34** resulted in crystals that diffracted to 3.20 Å resolution and contained two copies of a ParE-ParC fusion protein dimer in the asymmetric unit, each bound to one homoduplex DNA. Unbiased electron density for compound **34** was observed in all four compound binding sites (Supplementary Figure 1). The following description is based on observations made of the compound binding site with the best electron density. Only small variations in the binding mode are observed in the other copies present in the asymmetric unit.

Inspection of the electron density revealed that **34** occupies the quinolone binding site as expected with the compound intercalating between base pairs and stabilizing the cleaved DNA. A covalent phosphotyrosine bond was observed between the catalytic Tyr120 of ParC and the 5′-phosphate of the bound DNA at the break site, thus affording a view of the biologically relevant

cleavage complex (in contrast to structures of ternary complexes formed with pre-cut DNA; see D. A. Veselkov *et al*.[18] for a comparison of structures solved with intact and pre-cut DNA). Binding affinity for compound **34** is driven in part by π-stacking interactions with the base pairs located above and below the ligand. A number of suboptimal intermolecular interactions between **34** and neighboring residues of topoisomerase IV are also present, generally characterized by relatively large interatomic distances. The C2 carbonyl oxygen is posed to interact with the side chain of Arg119 (ParC subunit), similar to the reported *S. pneumoniae* topoisomerase IV-DNA co-structure with PD 0305970.[12] In this case, however, the interatomic distance is too long to invoke a productive interaction. The C4 aminomethyl substituent occupies a relatively open portion of the binding pocket that would normally accommodate the water-metal ion bridge interaction involved in classical fluoroquinolone binding. Only poor electron density is observed for the C4 aminomethyl substituent, which does not permit placing this group accurately and therefore no direct intermolecular interactions can be postulated. No specific interactions were observed between the nearby C3 fluorine atom and the protein, however its close proximity to Ser80 of ParC could explain the detrimental effect of a larger substituent at this position. It is possible that the fluorine atom contributes to an improved stacking interaction with DNA through electronic effects on the quinolin-2(1*H*)-one core, which would explain the boost in potency conveyed by that substituent. With respect to the crucial C7 amine, the moderate resolution of the structure (3.2 Å) meant that the preferred stereochemistry and precise orientation was difficult to determine. Finally, the primary amine is positioned to form weak interactions with the backbone carbonyl of K442 and the side chain of E461 (ParE subunit). The boost in potency observed for the [3.1.0] bicyclic group (as compared to a simple aminopyrrolidine such as **15**, for example) is potentially driven by a conformational preference that places the primary amine in an optimal location for binding.

However, improved bacterial inner membrane permeability due to the reduced pK_a of the amine may also have a significant impact.

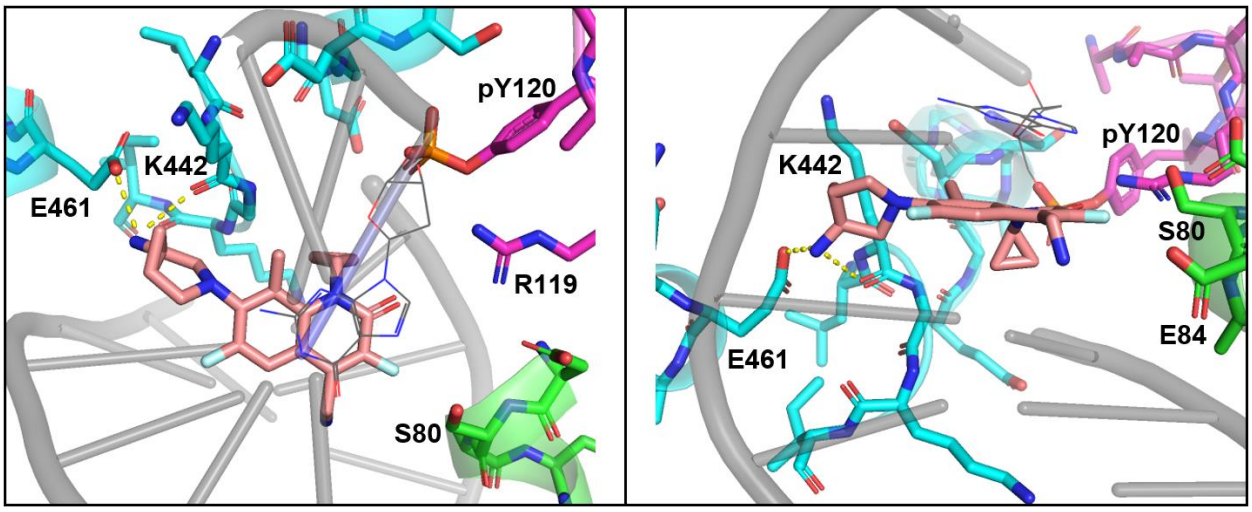


Figure 2. Binding site view of the topoisomerase IV cleavage complex from *K. pneumoniae* with compound **34** bound (PDB ID: 6WAA). DNA nucleotides of the cleavage site are shown in gray. ParC and ParE residues of one ParE-ParC fusion protein are shown in green and cyan, respectively. ParC residues from the second ParE-ParC fusion protein, which provide the catalytic tyrosine, are shown in magenta. Residues further out from the binding site were omitted for clarity.

***In Vitro* ADMET Profile of Compound 34** Further *in vitro* profiling data for **34** is shown in Table 6. Biochemical activity against DNA gyrase and topoisomerase IV from *E. coli* was comparable to ciprofloxacin and well balanced (IC_{50} = 1.25 and 0.81 μM respectively). The two amines proved to be weakly basic as expected with experimentally determined pK_a values of 8.0 and 7.6. Over the course of our optimization effort, this pK_a range was generally associated with good Gram-negative antibacterial potency. Solubility and plasma protein binding values were comparable to clinically used fluoroquinolone antibiotics. Importantly, **34** displayed CACO-2 permeability that was significantly higher than starting point **10**, likely driven by a reduction in the

amine pK_a of the C7 moiety and an associated increase in $logD_{7.4}$. Unfortunately, it appeared that this increase in lipophilicity was also associated with a reduction in microsomal stability (particularly in rat microsomes) and a marginal *in vitro* safety profile.

Compound **34** was found to exhibit moderate levels of cytotoxicity against three human cell lines (EC_{50} = 82, 40 and 29 μM respectively against HepG2, K562 and MT4 cells). Further *in vitro* evaluation of **34** for phototoxicity potential using the 3T3 neutral red uptake (NRU) test produced a photo-irritation factor (PIF) value of 621. The measured cellular IC_{50} in the presence of irradiation with simulated sunlight was 1.61 μM, while in the absence of irradiation the IC_{50} exceeded the solubility limit of the compound (1000 μM). The solubility limit was thus used in lieu of a measured IC_{50} in the absence of irradiation to calculate the PIF ($PIF = IC_{50(-irr)}/ IC_{50(+irr)}$). While a PIF value of 621 was considered high, it also reflected the very high solubility of compound **34**. Although **34** was not evaluated *in vivo* for phototoxicity, Schümann *et al*. have demonstrated that for a range of systemic drug candidates, the probability of *in vivo* phototoxicity correlates well with the magnitude of the PIF value obtained from the 3T3 NRU assay.[58] Notably, this study made use of the fluoroquinolone sparfloxacin as a positive control. Sparfloxacin produced an *in vitro* PIF value of >82 and was found to produce significant and dose-dependent phototoxicity in mice. Phototoxicity has also been observed clinically with a number of fluoroquinolones, and has severely limited the use of certain members of this class (e.g., clinafloxacin, sparfloxacin).[59, 60]

Fluoroquinolones exhibit a range of photochemical activation and degradation pathways that depend strongly on substitution pattern and on the reaction medium.[61] It has been suggested that fluoroquinolone phototoxicity may be caused by toxic photochemical degradation products, the generation of reactive oxygen species, or reactive intermediates such as aryl cations (arising from

dehalogenation) and/or radicals (arising from oxidation reactions induced by fluoroquinolones in the triplet state).[62-64] Compared with quinolones that have a single fluorine at the C6 position, difluorinated quinolones (i.e. fluorine at C6 and C8) are markedly more photolabile, with unimolecular defluorination constituting a major photodegradation pathway. The pendant alkyl groups at C7 and N1 can also participate in secondary intramolecular reactions with excited state species and reactive intermediates, thereby influencing the photochemical fate of quinolones.

Without further experimental data it is not possible to confirm if compound **34** exhibits comparable photochemical reactivity patterns. However, in order to derive additional SAR around phototoxicity for this series, compounds **15** and **21** were evaluated in the 3T3 NRU test. Compound **21**, which lacks the C3 fluorine atom present in **34**, produced a PIF value of 104. Like **34**, the IC_{50} of **21** in the absence of irradiation exceeded the solubility limit of the compound (316 μM). In the presence of irradiation, however, compound **21** produced an IC_{50} of 3.03 μM, only marginally higher than **34** ($IC_{50(+irr)}$ = 1.61 μM). Interestingly, compound **15**, which lacks the C3 fluorine and features a simple 3-aminopyrrolidine at C7, produced a PIF value of 1.7 ($IC_{50(+irr)}$ = 288 μM). Thus, it appears that phototoxicity of this series (as assessed by the 3T3 NRU test) is strongly influenced by the nature of the C7 substituent and to a lesser extent by halogenation at C3.

Compound **34** also exhibited increased hERG channel inhibition relative to **10** (IC_{50} = 114 μM compared to >300 μM).[65] Like the *in vitro* phototoxicity results, the observed *in vitro* hERG inhibition data was viewed with some concern given the clinical history of certain members of the quinolone class. Moxifloxacin, for example, has a similar reported hERG IC_{50} and produces QT prolongation in humans at therapeutic doses.[66-68] Based on reported data for fluoroquinolones, we anticipated that the plasma free C_{max} for an efficacious gyrase/topoisomerase IV inhibitor would be roughly 5-10 μM. A hERG IC_{50} of 114 μM would thus fall significantly short of the preferred

100-fold safety margin.[69] Many compounds in this series (including compound **34**), feature a basic amine flanked by a lipophilic aromatic group, which broadly fits the accepted hERG pharmacophore model for channel blockers. The reduced hERG inhibition exhibited by compound **10** is likely driven by reduced lipophilicity ($logD_{7.4}$ = -0.29 for **10**, compared with 1.1 for **34**). Increasing polarity represents a reasonable strategy for addressing hERG inhibition in future analogs, and one which has been applied successfully in the context of many drug discovery efforts.[70] Other well-known strategies, including removal of basic amines and aromatic groups, reduction of amine pKa, and introduction of acidic groups (c.f. fluoroquinolones) may also be successful in reducing hERG inhibition, but come with the risk of undesirable effects on other compound properties (e.g. antibacterial activity, solubility, lipophilicity and fluoroquinolone cross-resistance). Although analogs with improved ADMET properties were identified during the course of the optimization effort, we were unable to identify compounds that combined these attributes with the necessary antibacterial potency or spectrum.

Table 6. *In vitro* ADMET profile of **34**.

Assay	34
E. coli Gyrase IC_{50} (μM)	1.25
E. coli Topo IV IC_{50} (μM)	0.81
$logD_{7.4}$	1.1
pKa	8.0, 7.6
Solubility (μg/mL, PBS)	1276
CL_{int} in Liver Microsomes (μL/min/mg, mouse/rat/human)	40 / 117 / 18
Plasma Protein Binding (% bound, mouse/rat/human)	32 / 58 / 35
CACO-2 P_{app} A-B (cm/sec) [efflux ratio]	10.4 [1.8]
hERG IC_{50} (μM)	114[a]
Cytotoxicity EC_{50} (μM, HepG2 / K562 / MT4)	82 / 40 / 29
PIF (3T3 NRU)	621

[a] Determined using QPatch automated electrophysiology with a long incubation protocol (see Experimental Section for details).

Microbiological Profile Of Compound 34 Table 7 shows the microbiological profile of **34** and ciprofloxacin against a panel of 126 clinical isolates, 70% of which were resistant to ciprofloxacin.[71] Against *Enterobacteriaceae* (*n* = 96) **34** displayed a MIC_{90} of 8 μg/mL, whereas the ciprofloxacin MIC_{90} was >32 μg/mL. Against isolates of *E. coli* (*n* = 30) and *K. pneumoniae* (*n* = 30) MIC_{90} values were 2 and 16 μg/mL, respectively. This level of *in vitro* activity was again substantially better than ciprofloxacin (MIC_{90} = >32 μg/mL against *E. coli* and *K. pneumoniae*). As expected, **34** was less active against *P. aeruginosa* (MIC_{90} = 32 μg/mL). The frequency of single step mutant selection was low; no resistant mutants were selected when $>10^{10}$ cells of *E. coli* ATCC 25922 were plated on Mueller-Hinton agar containing 2X (0.12 μg/mL), 4X (0.25 μg/mL) or 8X (0.5 μg/mL) multiples of the MIC of compound **34** (mutant frequency $<10^{-10}$). The

low frequency of single step resistance for **34** may reflect balanced targeting of both DNA gyrase and topoisomerase IV in *E. coli*, consistent with similar levels of activity against both enzymes in the biochemical assay (Table 6).[72]

Table 7. Microbiological profile of **34**: performance against a Gram-negative challenge panel (126 clinical isolates, 70% ciprofloxacin-resistant).

Organism	# isolates	MIC (μg/mL)					
		1 (ciprofloxacin)			**34**		
		Range	**MIC_{50}**	**MIC_{90}**	**Range**	**MIC_{50}**	**MIC_{90}**
Enterobacteriaceae*	All species (96)	≤0.03->32	8	>32	0.06->32	1	8
	E. coli (30)	≤0.03->32	>32	>32	0.06-8	1	2
	K. pneumoniae (30)	≤0.03->32	8	>32	0.25-32	4	16
P. aeruginosa	30	0.125->32	8	>32	1->32	8	32

* 6 *Citrobacter* spp., 10 *Enterobacter* spp., 30 *E. coli*, 30 *K. pneumoniae*, 5 *Klebsiella oxytoca*, 4 *Morganella morganii,* 5 *Proteus mirabilis*, 6 *Serratia marcescens*

Given the promising potency of **34** against *Enterobacteriaceae*, we anticipated that a primary indication for a putative clinical candidate would be in complicated urinary tract infections (cUTI). It has been shown previously that *in vitro* antibacterial activity of some fluoroquinolone antibiotics is influenced by the pH of the test medium.[73] It therefore became a priority to assess the antibacterial activity of **34** at a slightly acidic pH in order to mimic the environment in the urinary tract. As shown in Table 8, ciprofloxacin is 16-fold less active against *E. coli* WT at pH 5.8 compared to pH 7.4 (MIC = 0.125 μg/mL vs 0.008 μg/mL respectively). Analogs such as **5** and **17**, which feature a single basic amine, displayed a similar loss of activity at pH 5.8. Compounds

with two basic amines, however, were affected to a greater extent. The pH-dependent reduction of activity was particularly severe for **34**, which was 512-fold less active against *E. coli* WT at pH 5.8 compared to pH 7.4 (MIC = 32 μg/mL vs 0.06 μg/mL). Although monobasic compounds were more tolerant of acidic pH, such compounds had insufficient antibacterial potency and spectrum to be progressed further.

Table 8. Effect of pH on antimicrobial activity of selected compounds.

Compound	*E. coli* WT MIC (μg/mL)		MIC Fold Shift
	pH 7.4	pH 5.8	
1 (ciprofloxacin)	0.008	0.125	16
5	1	16	16
10	1	>32	≥64
15	2	>32	≥32
17	0.5	8	16
21	0.25	32	128
34	0.06	32	512

In Vivo Profile of Compound 34 The *in vivo* PK and efficacy of compound **34** is shown in Table 9. In both rat and mouse PK studies compound **34** exhibited moderate to high CL, consistent with *in vitro* microsomal stability results. This likely drives the low oral bioavailability observed with **34**. However, dose-normalized oral exposure was broadly similar to ciprofloxacin, and was somewhat higher in mice. In a neutropenic mouse thigh infection model using fluoroquinolone-sensitive *E. coli* ATCC 25922, the static dose was determined. At the initiation of therapy, mice had a cfu/thigh of approximately $\log_{10}$ 6 which increased to >$\log_{10}$ 9.5 cfu/thigh 24 hours later in vehicle treated animals (see Supplementary Figure 3). **34** resulted in a static dose of 45.5 mg/kg/day compared to 6.4 mg/kg/day for ciprofloxacin. This closely parallels the difference in *in*

vitro potency (MIC) between the two compounds and demonstrates that compounds in this series are capable of achieving *in vivo* efficacy against Gram-negative bacteria.

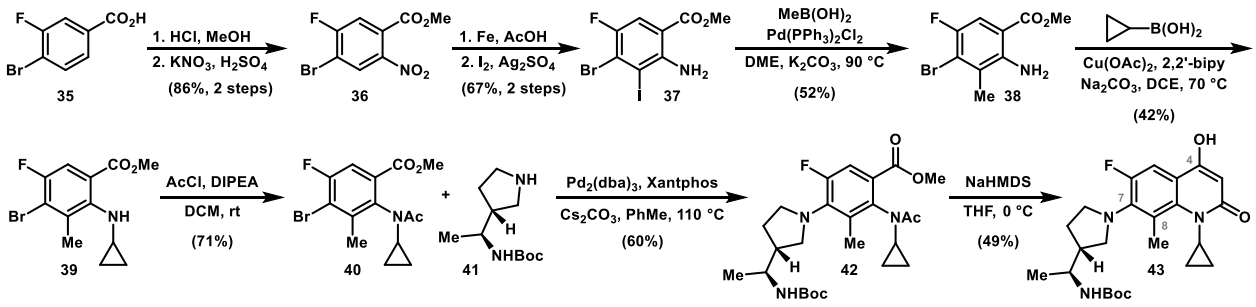
Table 9. PK and *in vivo* efficacy of **34** in comparison to ciprofloxacin (**1**).

Pharmacokinetics (PK)				
	1 (ciprofloxacin)		**34**	
	Mouse[a]	**Rat**[b]	**Mouse**[c]	**Rat**[d]
CL (mL/min·kg)	63	40	50	71
Vss (L/kg)	2.2	3.4	5.1	24.1
$T_{1/2\ term.\ i.v.}$ (h)	0.46	1.2	1.7	8.2
AUC (μM·h) / dose (mg/kg) p.o.	0.13	0.29	0.31	0.10
C_{max} (μM) / dose (mg/kg) p.o.	0.06	0.14	0.10	0.01
T_{max} p.o. (h)	0.25	0.5	0.5	1.3
Oral BA (%F)	19	23	36	15
Neutropenic Murine Thigh Infection Model				
	1[e]		**34**[f]	
MIC (*E. coli* ATCC 25922, μg/mL)	0.008		0.06	
Static Dose (mg/kg/day)	6.4		45.5	

Compounds were administered as hydrochloride salts in solution using the following vehicles and doses: [a] Vehicle = 0.9% NaCl; dose = 5 mg/kg I.V. and 10 mg/kg P.O. [b] Vehicle = DI water; dose = 5 mg/kg I.V. and 10 mg/kg P.O. [c] Vehicle = 20% PEG300 + 5% Solutol in D5W; dose = 5 mg/kg I.V. and P.O. [d] Vehicle = 20% PEG300 + 5% Solutol in D5W; dose = 5 mg/kg I.V. and 10 mg/kg P.O. [e] Vehicle = DI water; multiple dose levels, subcutaneous route of administration. [f] Vehicle = 40% PEG300 + 10% Solutol HS-15 in D5W; multiple dose levels, subcutaneous route of administration. See Experimental Section for further details.

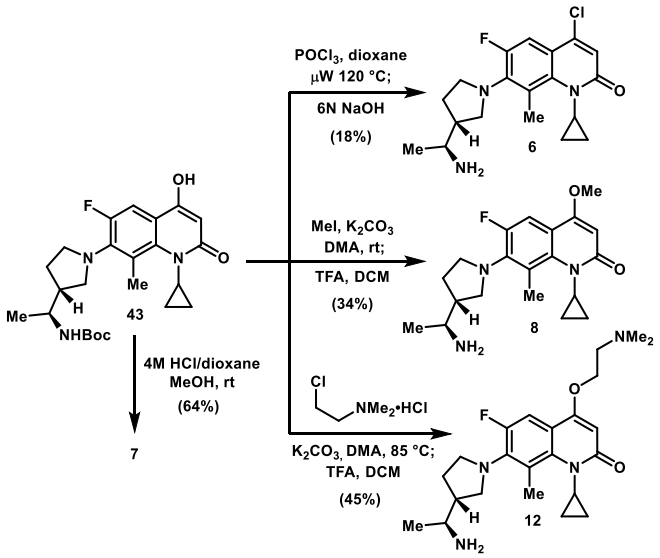
Chemistry A detailed description of the synthesis of compounds **4** and **5** is provided in the Experimental Section, and followed synthetic routes similar to those described below. Initial SAR exploration at C4 was enabled by the synthesis of key intermediate **43**, equipped with an oxidative handle for further functionalization (Scheme 1). Starting from commercially available arene **35**, standard protocols were employed to effect sequential esterification, nitration, reduction, and halogenation to provide iodide **37** in four steps and 57% overall yield. The methyl group at C8 was next installed by Suzuki reaction with methyl boronic acid, while the aniline in **38** was engaged in a Chan–Lam coupling to provide cyclopropylamine **39**.[74-76] Following acetylation, bromide **40** was leveraged to append functionalized pyrrolidine **41** at C7 via Buchwald–Hartwig coupling, catalyzed by the combination of $Pd_2(dba)_3$ and Xantphos in toluene at 110 °C.[77-80] Construction of the quinolin-2(1*H*)-one core was then accomplished by subjection of **42** to base-mediated cyclization, delivering **43** in an overall 9-step sequence.

Scheme 1. Synthesis of key intermediate toward C4-substituted analogs.



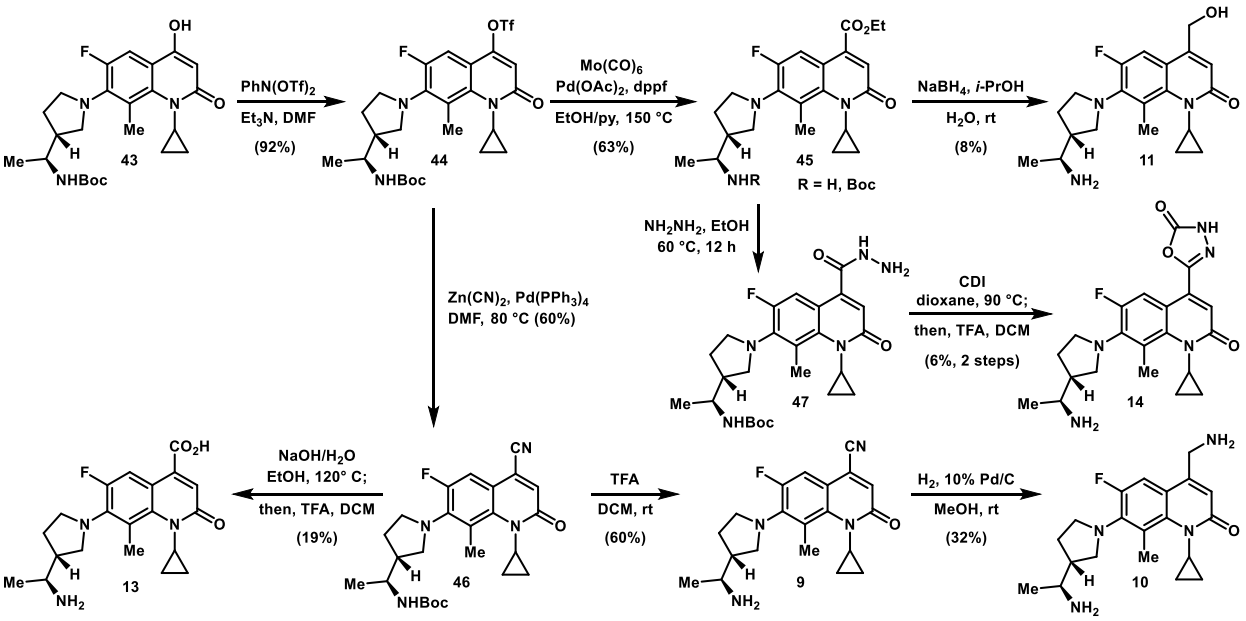
With a robust route to access **43**, analogs containing oxidation at C4 were prepared in short order as shown in Scheme 2. Boc deprotection gave hydroxy derivative **7**, while treatment with $POCl_3$ effected chlorination and concomitant deprotection to provide **6**. Ethers **8** and **12** were accessed via alkylations with methyl iodide and 2-dimethylaminoethyl chloride, respectively.

Scheme 2. Direct functionalization of C4-OH.



The divergent synthesis of analogs with carbon-based functionality at C4 is illustrated in Scheme 3. Conversion of **43** to triflate **44** enabled installation of an ethyl ester (**45**, obtained as a partially de-protected mixture) *via* palladium-catalyzed carbonylation,[81, 82] or a nitrile (**46**) *via* a Negishi-type cyanation reaction.[83] Reduction of the deprotected ester (**45**, R = H) afforded hydroxymethyl derivative **11**. Alternatively, heating the protected ester with hydrazine in EtOH provided the acyl hydrazide **47**, which was cyclized with CDI in dioxane at 90 °C to give oxadiazolone **14** after Boc deprotection. The versatile nitrile **46** was used to access **10** (*via* deprotection and hydrogenation) and **13** (via hydrolysis of the cyano group and Boc deprotection with TFA).

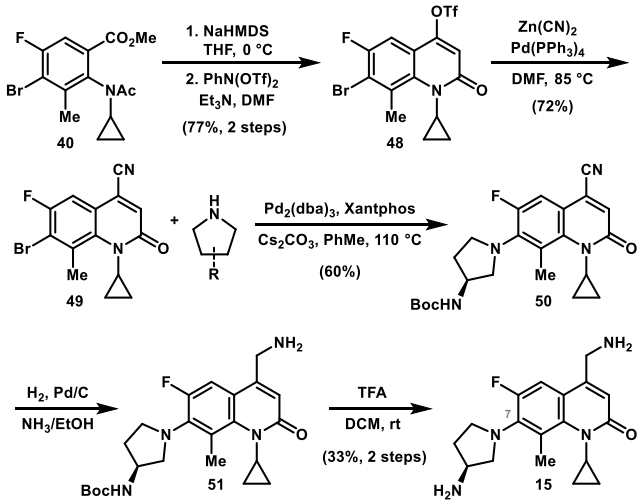
Scheme 3. Generation of C4-carbon-based analogs via triflate functionalization.



Having established the C4 aminomethyl substituent as optimal, attention turned to adapting the synthetic route to facilitate rapid SAR exploration at the C7 position by introducing the amine at a later stage in the synthesis (Scheme 4). To this end, N-acetylated bromide **40** was cyclized to form the quinolin-2(1*H*)-one core as before, followed by treatment with *N*-phenyl triflamide to give **48** in 77% yield over the two steps. Triflate **48** underwent chemoselective Negishi cyanation, leaving the bromide in **49** intact and poised for subsequent Buchwald–Hartwig coupling. A variety of C7 amine derivatives were prepared in this manner as exemplified by **50**, obtained in 60% yield after reaction with *tert*-butyl (*S*)-pyrrolidin-3-ylcarbamate. The reaction conditions shown in Scheme 4 were slightly modified as necessary to accommodate more difficult amine substrates (see Experimental Section for details).[79, 84, 85] Next, the aminomethyl substituent at C4 was revealed upon hydrogenation of the cyano group, for which the best results were consistently obtained when ammonia/methanol was used as solvent. A final Boc deprotection efficiently generated a series of differentially functionalized C7 analogs **15**-**26** (see Table 4). Compound **19**

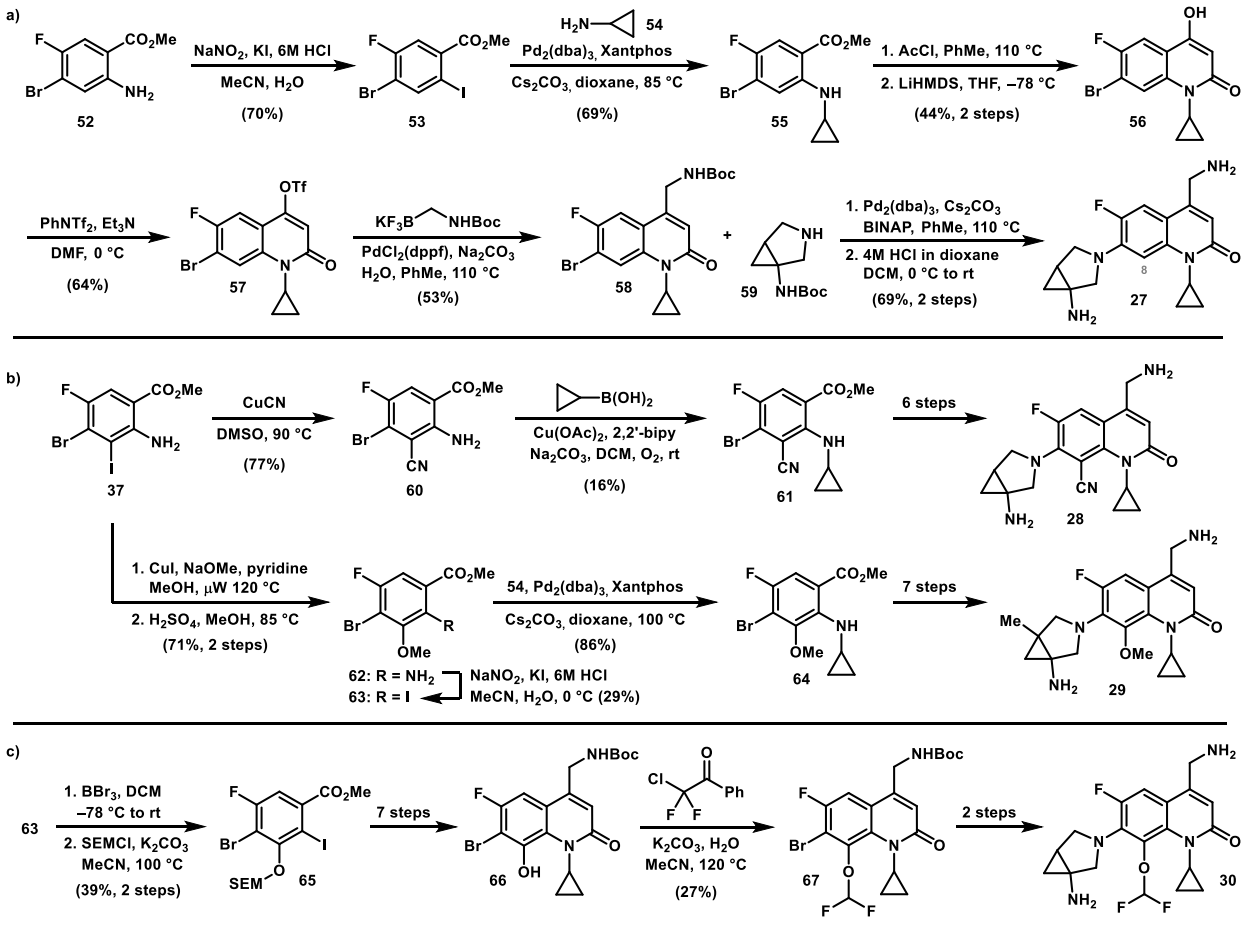
was prepared *via* Suzuki coupling with the appropriate arylboronic acid.[86, 87] The requisite azabicyclic amines for compounds **22**-**23** were prepared using an intramolecular Kulinkovich-style reaction reported by Laroche *et al*.[88] (see Experimental Section for details).

Scheme 4. Divergent synthesis of C7 analogs.



Whereas the C7 position was ultimately amenable to SAR exploration in a divergent fashion, the preparation of compounds with alternative substituents at C3 and C8 required multiple adaptations at an earlier point in the synthetic route. However, efforts directed at developing new sequences also provided an opportunity to improve upon the previously established strategy. For instance, an alternative 2-step procedure was identified to afford **55** by conversion of aniline **52** (see Scheme 1, intermediate not drawn) to iodide **53** via Sandmeyer reaction,[89, 90] followed by Buchwald–Hartwig coupling with cyclopropylamine, which proved higher yielding and generally more reliable than the previously utilized Chan–Lam reaction (Scheme 5a). Likewise, it was found that Suzuki reaction with triflate **57** and *N*-Boc aminomethyl potassium trifluoroborate proceeded smoothly to afford **58** in 53% yield, thus establishing a more convenient protocol for direct introduction of the aminomethyl group at C4.[91] Compound **27** lacking the C8 methyl group was then obtained by coupling with pyrrolidine **59** and double Boc deprotection.

Scheme 5. Adapted synthetic routes toward C8 analogs.

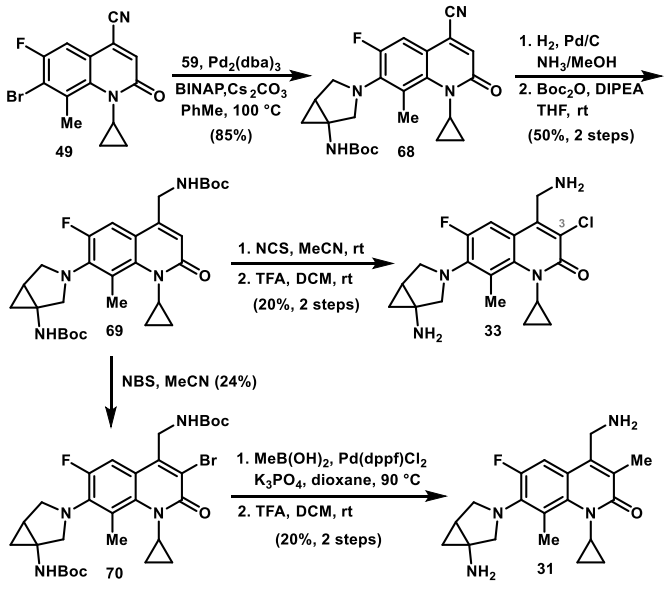


The synthetic routes toward C8 analogs **28** and **29** diverge at an early stage. Aryl cyanide **60** was produced in 77% yield by exposure of iodide **37** to CuCN in DMSO at elevated temperature (Scheme 5b). In this case, Chan–Lam coupling was utilized, albeit in low yield to access intermediate **61**, which was elaborated to **28** through a sequence analogous to that described in Scheme 1 (see Experimental Section for details). Aryl iodide **37** was also used to prepare C8-methoxy intermediate **62**, which was converted to cyclopropylamine **64** using the same strategy employed for **27**. Compound **29** was then accessed by carrying **64** through the 7-step synthetic sequence outlined in Scheme 4. Fortunately, iodide **63** could also be leveraged toward the synthesis of analog **30**; demethylation and SEM protection gave **65**, which was elaborated to **66** in 7 steps

(Scheme 5c). The free phenol was then reacted with difluorocarbene generated from $PhCOCF_2Cl$ to produce **67** in serviceable yield.[92] Finally, coupling with pyrrolidine **59** under our standard conditions and double Boc deprotection delivered C8 difluoromethoxy analog **30**.

The syntheses of C3 analogs **31** and **33** are shown in Scheme 6. Pyrrolidine **59** was coupled to aryl bromide **49** in 85% yield, followed by conversion of the C4 cyano group to the corresponding aminomethyl via hydrogenation in NH_3/MeOH with subsequent Boc protection to give **69**. This common intermediate was chlorinated at C3 upon exposure to NCS in acetonitrile, leading to **33**. Alternatively, **69** could be brominated and subjected to a Suzuki reaction with methyl boronic acid, affording **31** after deprotection.

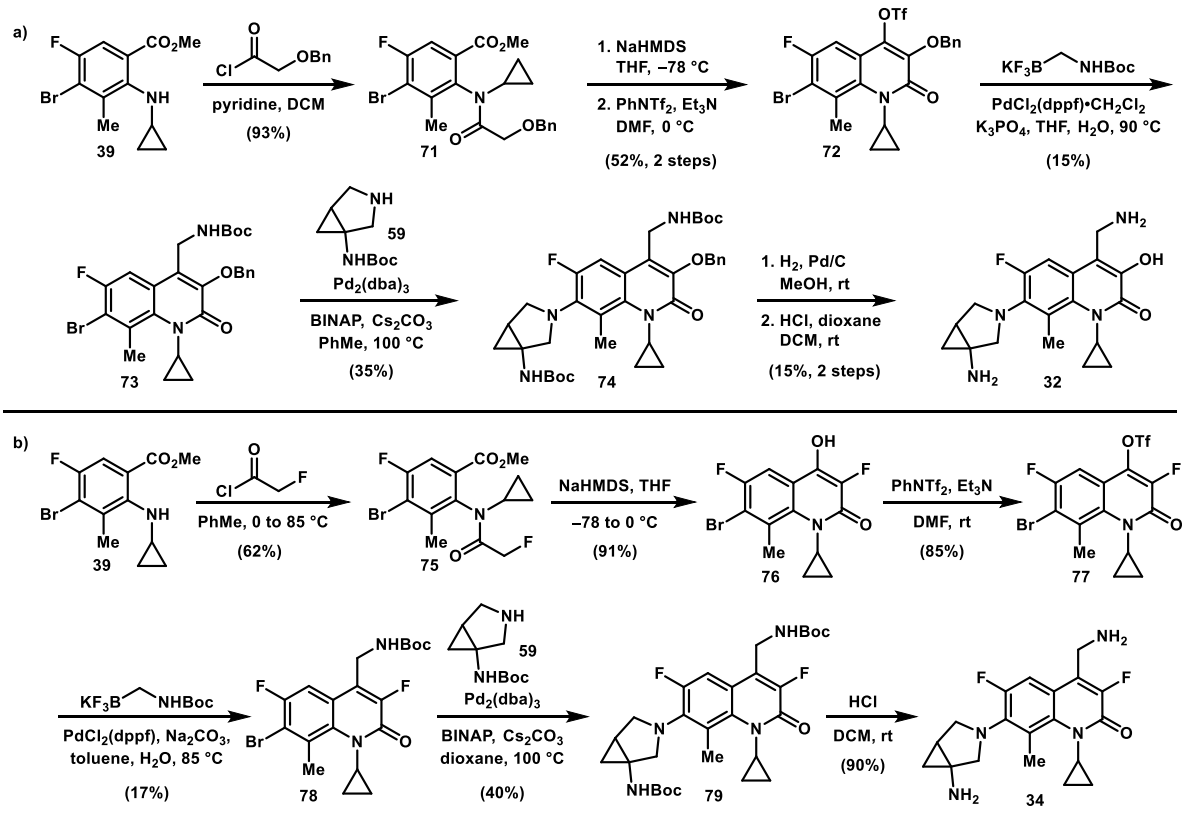
Scheme 6. Synthesis of C3-Cl and Me analogs.



Incorporation of either OH or F substituents at C3 required yet another synthetic route modification to build in an additional oxidation state prior to formation of the quinolin-2(1*H*)-one core; this was accomplished from cyclopropylamine **39** via acylation with either 2-benzyloxy or 2-fluoroacetyl chloride to generate **71** and **75**, respectively (Scheme 7a/b). Each of these

intermediates was progressed through the established 4-step sequence involving cyclization, triflation, Suzuki, and Buchwald–Hartwig coupling reactions to yield advanced intermediates **74** (C3-OH) and **79** (C3-F) respectively. Fluoride **34** was obtained after Boc deprotection of **79**, while hydrogenolysis was required to remove the benzyl group from **74**, thus providing **32** after Boc deprotection.

Scheme 7. Synthesis of C3-OH and C3-F analogs **32** and **34**.



Conclusions

After decades of clinical success quinolone antibiotics remain a crucial component of our antibacterial armamentarium. Unfortunately, resistance—driven by target-based QRDR mutations that disrupt quinolone binding—poses a serious threat to their continued use. In an effort to address this situation we elected to prioritize the discovery of small molecules that target WT and QRDR mutant forms of bacterial DNA gyrase and topoisomerase IV. Based on a scaffold-morphing

approach we were ultimately able to identify inhibitors that lacked both the C3 carboxylate and the C4 carbonyl responsible for engaging in the key water-metal ion bridge that anchors classical quinolones in the gyrase/topoisomerase IV cleavage complex. Compounds in this series displayed minimal cross-resistance with quinolones in spite of an intrinsic loss of potency. Through a concerted SAR exploration effort we were successful in optimizing activity against Gram-negative bacteria *en route* to **34**, which demonstrated promising activity against Gram-negative bacteria and *in vivo* efficacy against *E. coli*. Unfortunately, however, further optimization of antibacterial potency proved extremely challenging. A number of *in vitro* safety issues were subsequently identified for compound **34**, including hERG inhibition, cytotoxicity, and phototoxicity. Compound **34**, and related analogs, also suffered from a severe loss of antibacterial potency at pH 5.8, suggesting that clinical utility in the cUTI setting would be compromised. Although some of these issues were resolved in other analogs from this series, we were ultimately unable to identify compounds that married such properties with sufficient antibacterial potency and spectrum of activity. These challenges prompted us to halt further development of the series. It is our hope, however, that these studies may point the way toward development of more efficacious inhibitors that can help to address the ever-growing need for new agents to treat drug-resistant Gram-negative infections.

EXPERIMENTAL SECTION

Chemistry

General: Unless otherwise noted, all key compounds possess a purity of at least 95% as assessed by analytical reversed phase HPLC and/or LC/MS.

Typical conditions for analytical HPLC: High performance liquid chromatography (HPLC) using a Waters UPLC instrument (Milford, MA). HPLC solvent A was 100% Water with 0.1% trifluoroacetic acid (TFA) and solvent B was 100% acetonitrile with 0.1% TFA from EMD Chemicals Inc. The instrument was a Waters ACQUITY UPLC system with 1.2 mL/min flow rate; column Kinetex-C18, 2.6 µm, 2.1 x 50 mm from Phenomenex, column temperature: 50 °C; gradient: 2-88% solvent B over 9.79 min. Compounds were detected by ultraviolet light (UV) absorption at either 220 or 254 nm.

Typical conditions for LC/MS analysis: HPLC/mass spectrometric analysis (LC/MS) was performed on a Waters ACQUITY UPLC system equipped with a ZQ 2000 or SQD MS system; Column: Kinetex by Phenomenex, 2.6 µm, 2.1 x 50mm, column temperature: 50 °C; gradient: 2-88% (or 0-45%, or 65-95%) solvent B over a 1.29 min or 9.79 min period; flow rate 1.2 mL/min. Compounds were detected by a Waters Photodiode Array Detector. All masses were reported as those of the protonated parent ions, molecular weight range 150-850; cone Voltage 20 V. For select bromine-containing intermediates, MS data is reported as $[M+2]^+$, reflecting the mass of the heavier isotope $[M+H]^+$.

Alternative LC/MS method (compounds **27**, **30**, **32**): BEH 1.7µm C18 50x2.1mm; column temperature: ambient; mobile phase: 5mM ammonium acetate in 0.1% formic acid in water (solvent A) and 0.1% formic acid in MeCN (solvent B); gradient: 5%-100% solvent B over 2.5

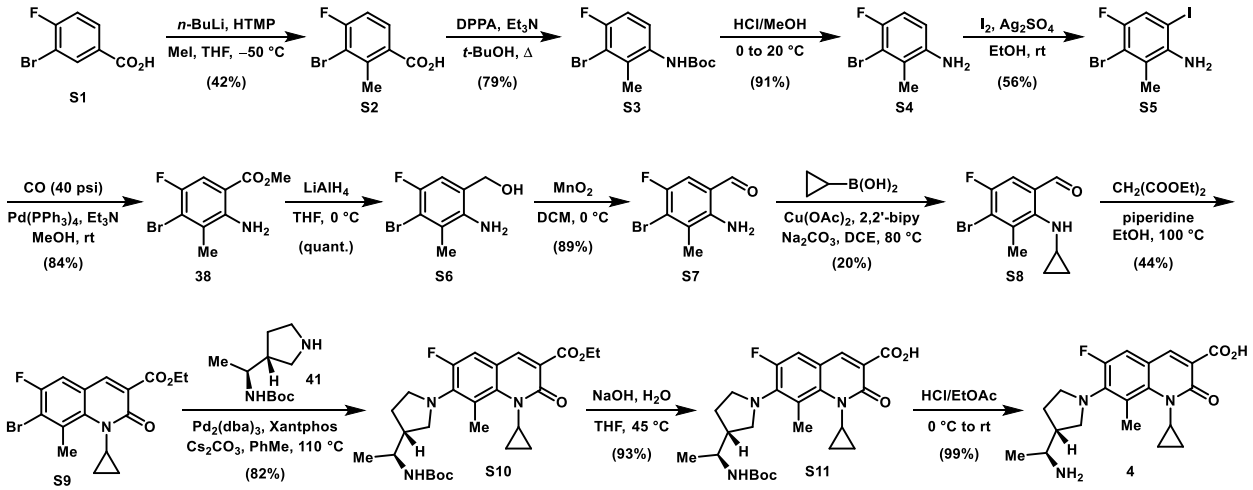
minutes and holding at 100% for 0.8 minutes with a flow rate of 0.55 mL/min. Compounds were detected by ESI-MS in positive ion mode and UV absorption at 254 nm.

High resolution ESI-MS data were recorded using a LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific) with electrospray ionization source. The resolution of the MS system was approximately 30000. The analyte compound was infused into the mass spectrometer by UPLC (Acquity, Waters) from sample probe. The separation was performed on Acquity UPLC BEH C18 1x50 mm column at 0.15 mL/min flow rate with the gradient from 5% to 95% in 2.8 min. Solvent A was Water with 0.1% Trifluoroacetic acid and solvent B was 75% Methanol and 25% Isopropyl alcohol with 0.1% Trifluoroacetic acid. The mass accuracy of the system was found to be <5 ppm.

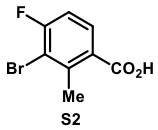
NMR spectra were run on Varian 400 or Bruker 400 and 500 MHz NMR spectrometers. Spectra were measured at 298K and were referenced using the solvent peak.

Preparative separations were carried out using a Combiflash Rf system (Teledyne Isco, Lincoln, NE) with RediSep silica gel cartridges (Teledyne Isco, Lincoln, NE) or SiliaSep silica gel cartridges (Silicycle Inc., Quebec City, Canada) or by flash column chromatography using silica gel (230-400 mesh) packing material, or by HPLC using a Waters 2767 Sample Manager, C-18 reversed phase Sunfire column, 30X50 mm, flow 75 mL/min. Typical solvents employed for the Combiflash Rf system and flash column chromatography were dichloromethane, methanol, ethyl acetate, hexane, heptane, acetone, aqueous ammonia (or ammonium hydroxide), and triethyl amine. Typical solvents employed for the reverse phase HPLC are varying concentrations of acetonitrile and water with 0.1% trifluoroacetic acid.

Synthesis of 7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid hydrochloride (4)

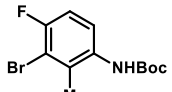


3-Bromo-4-fluoro-2-methylbenzoic acid (S2)



To a stirred solution of *n*-butyllithium (1024 mL, 2560 mmol, 2.5M in hexanes) was added 2,2,6,6-tetramethylpiperidine (436.0 mL, 2560 mmol) in THF (2300 mL) at −5 °C. After stirring for 15 min the mixture was cooled to −60 °C. A solution of 3-bromo-4-fluorobenzoic acid (255.0 g, 1160 mmol) in THF (700 mL) was added dropwise and the mixture was stirred at this temperature for 2.5 h. The mixture was then treated with MeI (291.0 mL, 4640 mmol) at −70 °C. The resulting solution was allowed to warm to 20 °C within 30 mins. Water was added to quench the reaction, and the resulting solution was washed with EtOAc and then acidified with 4M HCl. The resulting mixture was extracted with EtOAc, the organic layer dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude benzoic acid, which was re-crystallized from toluene to give the title compound (114 g, 42% yield) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (dd, J = 8.8, 5.6 Hz, 1H), 7.28 (t, J = 8.4 Hz, 1H), 2.60 (s, 3H).

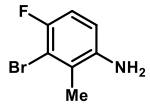
***tert*-Butyl (3-bromo-4-fluoro-2-methylphenyl)carbamate (S3)**



S3

To a solution of 3-bromo-4-fluoro-2-methylbenzoic acid (6.00 g, 25.8 mmol) in *t*-BuOH (49 mL) was added TEA (7.20 mL, 51.6 mmol) and DPPA (6.30 mL, 28.4 mmol) at 15 °C. The mixture was then allowed to reflux for 1 h. After the reaction was complete, the reaction mixture was concentrated to give the crude product, which was purified by chromatography on silica gel (10% EtOAc/PE) to afford the title compound (6.20 g, 79% yield) as a white solid. LCMS (m/z): 248.0 [M–55]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (br s, 1H), 7.27 (dd, J = 9.2, 5.6 Hz, 1H), 7.16 (t, J = 8.4 Hz, 1H), 2.26 (s, 3H), 1.43 (s, 9H).

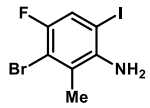
3-Bromo-4-fluoro-2-methylaniline (S4)



S4

To a mixture of *tert*-butyl (3-bromo-4-fluoro-2-methylphenyl)carbamate (6.20 g, 20.4 mmol) in MeOH (5.00 mL) was added HCl (4M in MeOH, 25.5 mL, 102 mmol) at 0 °C. The reaction mixture was stirred at rt for 30 min. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (20 mL). The slurry was extracted with EtOAc, the organic layer dried with Na_2SO_4, filtered and concentrated to give the title compound (3.80 g, 91% yield) as a white solid. LCMS (m/z): 204.0 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.91 (t, J = 8.4 Hz, 1H), 6.63 (dd, J = 8.8, 5.2 Hz, 1H), 5.04 (br s, 2H), 2.18 (s, 3H).

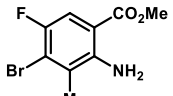
3-Bromo-4-fluoro-6-iodo-2-methylaniline (S5)



S5

A solution of 3-bromo-4-fluoro-2-methylaniline (2.30 g, 11.3 mmol) in EtOH (15 mL) was added dropwise to a mixture of I_2 (2.90 g, 11.3 mmol) and Ag_2SO_4 (3.50 g, 11.3 mmol) in EtOH (35 mL) at rt. The resulting solution was then stirred at rt for 2 h. After completion the reaction mixture was filtered and the filtrate was concentrated. The residue was re-dissolved in DCM. The organic phase was washed with 5% aq. NaOH and water, dried over Na_2SO_4, filtered and concentrated to give a residue, which was purified by column chromatography on silica gel (0-10% EtOAc/PE), providing the title compound (2.10 g, 56% yield) as a black solid. LCMS (m/z): 329.9 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, J = 7.6 Hz, 1H), 4.02 (br s, 2H), 2.36 (s, 3H).

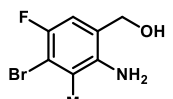
Methyl 2-amino-4-bromo-5-fluoro-3-methylbenzoate (38)



38

To a solution of 3-bromo-4-fluoro-6-iodo-2-methylaniline (2.00 g, 6.10 mmol) in MeOH (20 mL) was added $Pd(PPh_3)_4$ (36.0 mg, 0.031 mmol) and TEA (1.70 mL, 12.2 mmol). The mixture was stirred under a CO (40 psi) atmosphere for 30 mins. After completion the reaction mixture was concentrated to give a crude product which was purified by chromatography on silica gel (0-5% EtOAc/PE) affording the title compound (1.40 g, 84% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, J = 9.6 Hz, 1H), 5.83 (br s, 2H), 3.87 (s, 3H), 2.32 (s, 3H).

(2-Amino-4-bromo-5-fluoro-3-methylphenyl)methanol (S6)

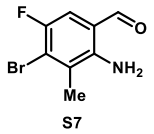


S6

To a solution of methyl 2-amino-4-bromo-5-fluoro-3-methylbenzoate (1.47 g, 5.61 mmol) in THF (10 mL) was added LAH (234 mg, 6.17 mmol) at 0 °C. After stirring at 0 °C for 20 mins,

another batch of LAH (117 mg, 3.09 mmol) was added. The reaction mixture was further stirred at 0 °C for 30 mins. After completion of the reaction, water was added, the resulting suspension dried over anhydrous Na_2SO_4 and the solid filtered. The filtrate was concentrated to give the desired product (1.32 g, quantitative yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.99 (d, J = 9.6 Hz, 1H), 5.29 (t, J = 5.6 Hz, 1H), 4.83 (br s, 2H), 4.38 (d, J = 9.6 Hz, 1H), 2.23 (s, 3H).

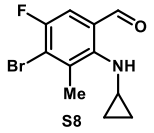
2-Amino-4-bromo-5-fluoro-3-methylbenzaldehyde (S7)



S7

To a solution of (2-amino-4-bromo-5-fluoro-3-methylphenyl)methanol (1.32 g, 5.64 mmol) in DCM (60 mL) was added MnO_2 (3.68 g, 42.3 mmol) at 0 °C. After stirring at 10 °C for 30 mins, another batch of MnO_2 (1.84 g, 21.2 mmol) was added. The reaction mixture was further stirred at 0 °C for 30 mins. The suspension was filtered and the filtrate was concentrated to give the desired product (1.17 g, 89% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.80 (s, 1H), 7.52 (d, J = 8.8 Hz, 1H), 7.15 (br s, 2H), 2.23 (s, 3H).

4-Bromo-2-(cyclopropylamino)-5-fluoro-3-methylbenzaldehyde (S8)

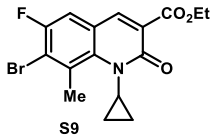


S8

To a solution of 2-amino-4-bromo-5-fluoro-3-methylbenzaldehyde (1.70 g, 7.30 mmol) in DCE (30 mL) was added cyclopropylboronic acid (1.25 g, 14.6 mmol), $Cu(OAc)_2$ (1.33 g, 7.30 mmol), bipyridine (1.14 g, 7.30 mmol) and Na_2CO_3 (1.55 g, 14.6 mmol). The mixture was stirred at 80 °C for 40 mins. After completion the mixture was filtered and the filtrate was concentrated to give the crude product, which was purified by flash chromatography (5% EtOAc/PE), providing the title compound as a yellow oil (400 mg, 20% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (s, 1H),

7.49 (d, J = 8.8 Hz, 1H), 7.31 (br s, 1H), 2.95–2.85 (m, 1H), 2.50 (s, 3H), 0.75–0.70 (m, 2H), 0.50–0.45 (m, 2H).

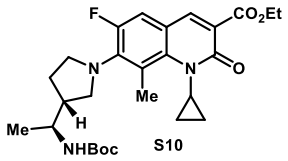
Ethyl 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (S9)



S9

To a solution of 4-bromo-2-(cyclopropylamino)-5-fluoro-3-methylbenzaldehyde (1.40 g, 5.20 mmol) in EtOH (30 mL) was added diethyl malonate (1.67 g, 10.4 mmol) and piperidine (1.77 g, 20.8 mmol). The mixture was refluxed at 100 °C for 16 h. The mixture was concentrated to give the crude product, which was purified by flash chromatography (20% EtOAc/PE) to give the title compound as a yellow solid (840 mg, 44% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.17 (d, J = 7.2 Hz, 1H), 4.40 (q, J = 7.2 Hz, 2H), 3.52–3.45 (m, 1H), 2.78 (s, 3H), 1.41 (t, J = 7.2 Hz, 3H), 1.28–1.20 (m, 2H), 0.60–0.50 (m, 2H).

Ethyl 7-((*R*)-3-((*S*)-1-((tert-butoxycarbonyl)amino)ethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (S10)

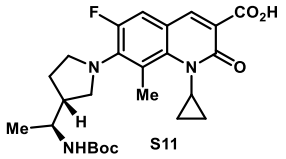


S10

To a solution of ethyl 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (250 mg, 0.68 mmol) in toluene (50 mL) was added *tert*-butyl ((*S*)-1-((*R*)-pyrrolidin-3-yl)ethyl)carbamate (291 mg, 1.36 mmol), Cs_2CO_3 (666 mg, 2.04 mmol), xantphos (59.0 mg, 0.10 mmol) and $Pd_2(dba)_3$ (31.1 mg, 0.03 mmol) at rt. The reaction mixture was stirred at 110 °C for 12 h. After completion the mixture was dissolved in water (20 mL) and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated to give a crude product, which was

purified by column chromatography on silica gel (0-50% EtOAc/PE) affording the desired product (280 mg, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.05 (d, *J* = 12.4 Hz, 1H), 4.51 (br s, 1H), 4.41 (q, *J* = 7.2 Hz, 2H), 3.80–3.70 (m, 1H), 3.68–3.60 (m, 1H), 3.54–3.45 (m, 4H), 2.45 (s, 3H), 2.12–2.10 (m, 1H), 2.11–2.07 (m, 1H), 1.80–1.70 (m, 1H), 1.47–1.40 (m, 12H), 1.30–1.26 (m, 5H), 0.60–0.56 (m, 2H).

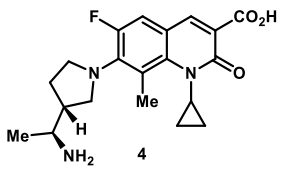
7-((*R*)-3-((*S*)-1-((tert-Butoxycarbonyl)amino)ethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (S11)



S11

To a solution of ethyl 7-((*R*)-3-((*S*)-1-((*tert*-butoxycarbonyl)amino)ethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (200 mg, 0.40 mmol) in THF/H_2O (8 mL, 1:1) was added NaOH (32.0 mg, 0.80 mmol). The reaction mixture was stirred at 45 °C for 4 h. After the reaction was complete, the reaction mixture was acidified to pH = 5 and extracted with EtOAc. The organic phase was dried over Na_2SO_4 and concentrated to give the desired product (175 mg, 93% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64 (s, 1H), 7.64 (d, *J* = 13.6 Hz, 1H), 7.20–7.17 (m, 1H), 4.48–4.40 (m, 2H), 4.20–4.10 (m, 2H), 3.69–3.60 (m, 1H), 3.35–3.25 (m, 2H), 2.89–2.75 (m, 1H), 2.60–2.50 (m, 1H), 2.38 (s, 3H), 1.50–1.40 (s, 11H), 1.30–1.24 (m, 3H), 0.70–0.62 (m, 2H).

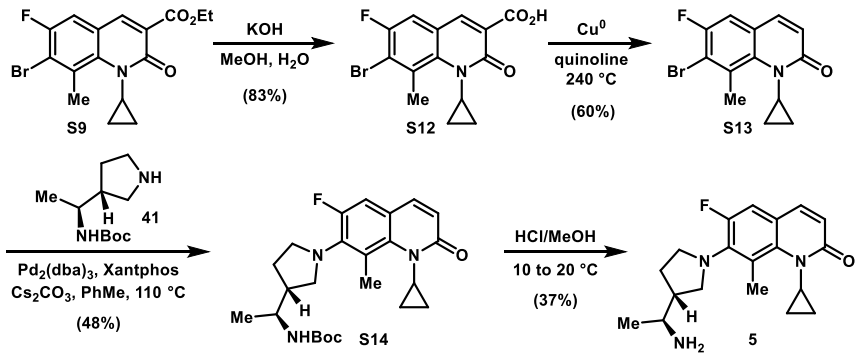
7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid hydrochloride (4)



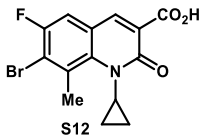
4

To a solution of 7-((*R*)-3-((*S*)-1-((*tert*-butoxycarbonyl)amino)ethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (180 mg, 0.38 mmol) in EtOAc (10 mL) was added HCl (1M in EtOAc, 10 mL) at 0 °C. The reaction mixture was stirred at rt for 3 h. After completion of the reaction, the organic layer was concentrated to give a crude product which was purified by reverse phase HPLC, affording the product (140 mg, 99% yield). HRMS (ESI) calc'd for $C_{20}H_{25}FN_3O_3$ $[M+H]^+$ 374.1874, found 374.1869. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (s, 1H), 8.25 (br s, 3H), 7.66 (d, J = 13.6 Hz, 1H), 3.78–3.70 (m, 1H), 3.60–3.40 (m, 4H), 3.30–3.20 (m, 1H), 2.45–2.35 (m, 4H), 2.20–2.10 (m, 1H), 1.80–1.70 (m, 1H), 1.30–1.20 (m, 5H), 0.62–050 (m, 2H).

Synthesis of 7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one hydrochloride (5)



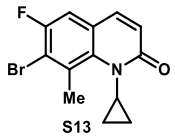
7-Bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (S12)



To a solution of ethyl 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (300 mg, 0.81 mmol) in MeOH (12 mL) and H_2O (3 mL) was added KOH (91.4 mg, 1.63 mmol) at 10 °C. The resulting mixture was stirred at 40 °C for 2 h. After completion the

reaction mixture was acidified to pH 4 by the addition of 2N HCl. The resulting solution was extracted with DCM, and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the title compound (230 mg, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.16 (br s, 1H), 8.68 (s, 1H), 7.33 (d, *J* = 6.4 Hz, 1H), 3.65–3.60 (m, 1H), 2.86 (s, 3H), 1.40–1.30 (m, 2H), 0.70–0.60 (m, 2H).

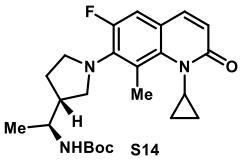
7-Bromo-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one (S13)



S13

To a solution of 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (230 mg, 0.68 mmol) in quinoline (2 mL) was added Cu (42.9 mg, 0.68 mmol) at 10 °C. The resulting mixture was stirred at 240 °C for 30 mins. The reaction mixture was cooled to 10 °C and water was added. The resulting suspension was extracted with EtOAc, the organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography on silica gel (0-50% EtOAc/PE) to give the title compound as a yellow solid (120 mg, 60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, *J* = 9.6 Hz, 1H), 7.08 (d, *J* = 7.6 Hz, 1H), 6.61 (d, *J* = 9.6 Hz, 1H), 3.53–3.48 (m, 1H), 2.78 (s, 3H), 1.30–1.20 (m, 2H), 0.58–0.54 (m, 2H).

***tert*-Butyl ((*S*)-1-((*R*)-1-(1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)pyrrolidin-3-yl)ethyl)carbamate (S14)**

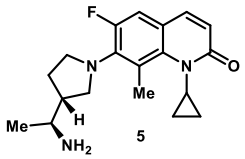


S14

To a solution of 7-bromo-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one (50.0 mg, 0.17 mmol) in toluene (3 mL) was added *tert*-butyl ((*S*)-1-((*R*)-pyrrolidin-3-yl)ethyl)carbamate (45.4

mg, 0.21 mmol) and Cs_2CO_3 (138.0 mg, 0.42 mmol), followed by xantphos (12.3 mg, 0.021 mmol) and $Pd_2(dba)_3$ (6.5 mg, 0.007 mmol). The resulting mixture was stirred at 110 °C under nitrogen for 16 h. The reaction mixture was concentrated, the residue was dissolved in water, and the slurry was extracted with DCM. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to afford a crude product, which was purified by preparative TLC to give the title compound (35.0 mg, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 9.2 Hz, 1H), 6.94 (d, *J* = 12.4 Hz, 1H), 6.45 (d, *J* = 9.6 Hz, 1H), 4.63 (d, *J* = 8.0 Hz, 1H), 3.80–3.73 (m, 1H), 3.60–3.35 (m, 5H), 2.76–2.68 (m, 1H), 2.49 (s, 3H), 2.32–2.27 (m, 1H), 2.10–2.00 (m, 1H), 1.43 (s, 9H), 1.22 (d, *J* = 6.8 Hz, 3H), 1.25–1.15 (m, 2H), 0.60–0.50 (m, 2H).

7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one hydrochloride (5)

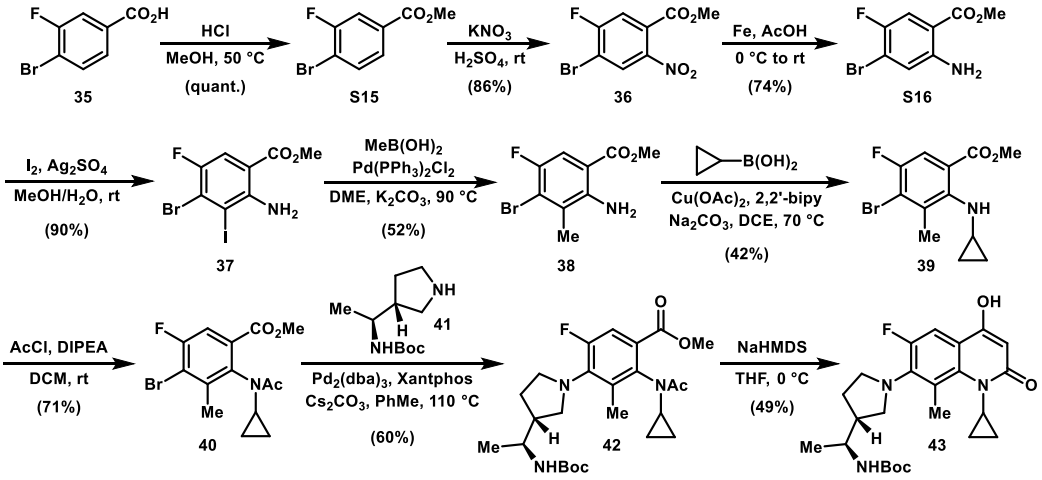


5

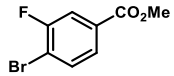
To a solution of *tert*-butyl ((*S*)-1-((*R*)-1-(1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)pyrrolidin-3-yl)ethyl)carbamate (35.0 mg, 0.08 mmol) in MeOH (2 mL) was added HCl (4M in MeOH, 2 mL) at 10 °C. The resulting mixture was stirred at rt for 1 h. The reaction mixture was concentrated, and the residue was purified by reverse phase HPLC to give the title compound (10.9 mg, 37% yield). HRMS (ESI) calc'd for $C_{19}H_{25}FN_3O$ $[M+H]^+$ 330.1976, found 330.1972. ^{1}H NMR (400 MHz, D_2O) δ 7.63 (d, *J* = 9.2 Hz, 1H), 7.25 (d, *J* = 12.0 Hz, 1H), 6.49 (d, *J* = 9.2 Hz, 1H), 3.90–3.83 (m, 1H), 3.82–3.75 (m, 2H), 3.70–3.60 (m, 1H), 3.55–3.40 (m, 2H), 2.90–2.80 (m, 1H), 2.56 (s, 3H), 2.40–2.30 (m, 1H), 2.07–1.96 (m, 1H), 1.40 (d, *J* = 6.4 Hz, 3H), 1.20–1.15 (m, 2H), 0.50–0.40 (m, 2H).

Preparation of compounds 6–15.

Compounds **6–15** were prepared from intermediate **43**, which was synthesized according to the scheme below:

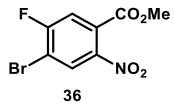


Methyl 4-bromo-3-fluorobenzoate (S15)



A solution of 4-bromo-3-fluorobenzoic acid (350 g, 1.60 mol) in HCl (4M in MeOH, 2 L) was stirred at 50 °C for 3 h. The reaction mixture was concentrated to give the title compound (372 g, quant. yield) as a gray solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78–7.64 (m, 3H), 3.93 (s, 3H).

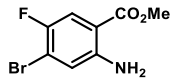
Methyl 4-bromo-5-fluoro-2-nitrobenzoate (36)



To a solution of methyl 4-bromo-3-fluorobenzoate (372 g, 1.60 mol) in H_2SO_4 (1000 mL) was added KNO_3 (170 g, 1.68 mol) at 0 °C. The mixture was then stirred at rt for 2 h. The reaction mixture was quenched with ice water and then extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to give the crude product, which was purified by column chromatography on silica gel (5% EtOAc/PE) affording the title compound (380 g, 86% yield) as

a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 5.6 Hz, 1H), 7.45 (d, J = 7.6 Hz, 1H), 3.94 (s, 3H).

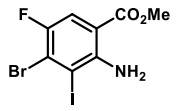
Methyl 2-amino-4-bromo-5-fluorobenzoate (S16)



S16

To a solution of methyl 4-bromo-5-fluoro-2-nitrobenzoate (95.0 g, 0.34 mol) in MeOH (1000 mL) was added AcOH (102 g, 1.70 mol). Fe power (95.4 g, 1.70 mol) was added slowly at 0 °C. After stirred at rt for 1 h, the reaction mixture was filtered. The filtrate was concentrated, and the residue was purified by column chromatography on silica gel (5% EtOAc/PE) to give the title compound (62.5 g, 74% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 9.6 Hz, 1H), 6.88 (d, J = 5.6 Hz, 1H), 5.62 (br s, 2H), 3.88 (s, 3H).

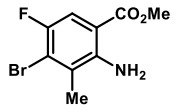
Methyl 2-amino-4-bromo-5-fluoro-3-iodobenzoate (37)



37

To a solution of methyl 2-amino-4-bromo-5-fluorobenzoate (50.0 g, 0.20 mol) in MeOH (480 mL) was added water (400 mL) and Ag_2SO_4 (100 g, 0.32 mol). A solution of I_2 (81.2 g, 0.32 mol) in THF (480 mL) was added at rt. After stirring at rt for 30 min, the solvent was evaporated and the resulting suspension was filtered. The filtrate was extracted with EtOAc, the organic phase was dried over anhydrous Na_2SO_4 and concentrated to give the crude product, which was purified by column chromatography on silica gel (5% EtOAc/PE) affording the title compound (68.0 g, 90% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 9.6 Hz, 1H), 6.55 (br s, 2H), 3.90 (s, 3H).

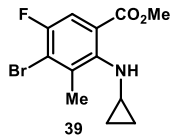
Methyl 2-amino-4-bromo-5-fluoro-3-methylbenzoate (38)



38

To a solution of methyl 2-amino-4-bromo-5-fluoro-3-iodobenzoate (80.0 g, 214 mmol) in DME (1170 mL) was added water (200 mL), $CH_3B(OH)_2$ (77.0 g, 1.28 mol), K_2CO_3 (59.0 g, 428 mmol) and $Pd(PPh_3)_2Cl_2$ (15.0 g, 2.14 mol). The reaction mixture was stirred at 60 °C for 18 h and then at 90 °C for 6 h. The reaction was quenched with water and extracted with EtOAc. The organic phase was dried over Na_2SO_4, concentrated and then purified by column chromatography on silica gel (2% EtOAc/PE), followed by recrystallization with PE/EtOAc to give the title compound (29.0 g, 52% yield) as a yellow solid. LCMS (m/z): 262.0 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 9.6 Hz, 1H), 5.83 (br s, 2H), 3.87 (s, 3H), 2.32 (s, 3H).

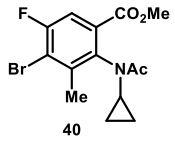
Methyl 4-bromo-2-(cyclopropylamino)-5-fluoro-3-methylbenzoate (39)



39

To a solution of methyl 2-amino-4-bromo-5-fluoro-3-methylbenzoate (5.00 g, 19.2 mmol) in DCE (50 mL) was added Na_2CO_3 (4.07 g, 38.4 mmol), 2,2'-bipyridine (2.90 g, 19.2 mmol), $Cu(OAc)_2$ (3.50 g, 19.2 mmol) and cyclopropylboronic acid (3.50 g, 19.2 mmol) at 10 °C. The reaction mixture was stirred at 10 °C for 20 min and then at 70 °C for 5 h. The reaction mixture was filtered and concentrated. The residue was purified by column chromatography on silica gel (2% EtOAc/PE) to give the title compound (2.40 g, 42% yield) as a yellow oil. LCMS (m/z): 302.2 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 9.0 Hz, 1H), 7.22 (br s, 1H), 3.85 (s, 3H), 2.71 (tt, *J* = 6.8, 3.5 Hz, 1H), 2.57 (s, 3H), 0.68–0.62 (m, 2H), 0.49–0.45 (m, 2H).

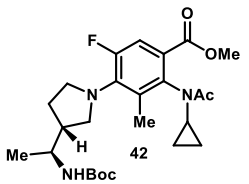
Methyl 4-bromo-2-(*N*-cyclopropylacetamido)-5-fluoro-3-methylbenzoate (40)



40

To a solution of methyl 4-bromo-2-(cyclopropylamino)-5-fluoro-3-methylbenzoate (13.0 g, 43.0 mmol) in DCM (260 mL) was added DIPEA (27.8 g, 215 mmol) and acetyl chloride (33.8 g, 430 mmol). The reaction mixture was stirred at rt for 20 minutes. The reaction mixture was poured into ice water and the phases were separated. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (0-50% EtOAc/PE) to give the title compound (10.5 g, 71% yield) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63–7.53 (m, 1H), 3.90–3.80 (m, 3H), 3.15–3.01 (m, 1H), 2.41 (s, 2H), 2.34 (s, 1H), 2.27 (s, 2H), 1.76 (s, 1H), 0.93–0.83 (m, 1H), 0.79–0.60 (m, 3H), 0.47–0.27 (m, 1H).

Methyl 4-((*R*)-3-((*S*)-1-((*tert*-butoxycarbonyl)amino)ethyl)pyrrolidin-1-yl)-2-(*N*-cyclopropylacetamido)-5-fluoro-3-methylbenzoate (42)

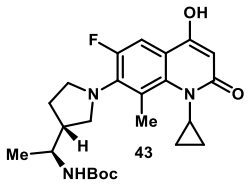


42

To a suspension of methyl 4-bromo-2-(*N*-cyclopropylacetamido)-5-fluoro-3-methylbenzoate (6.00 g, 17.5 mmol) in toluene (180 mL) was added *tert*-butyl ((*S*)-1-((*R*)-pyrrolidin-3-yl)ethyl) carbamate (6.72 g, 31.5 mol), Cs_2CO_3 (11.4 g, 35.0 mmol), xantphos (3.00 g, 5.25 mmol) and $Pd_2(dba)_3$ (1.60 g, 1.75 mmol) at 10 °C. The resulting mixture was purged with nitrogen for 5 min and heated to 110 °C for 24 h. The reaction mixture was filtered and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (50% EtOAc/PE) to give the title compound (5.00 g, 60% yield) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.45 (m, 1H), 4.61 (br s, 1H), 3.85–3.80 (m, 3H), 3.75–3.65 (m, 1H), 3.40–3.26 (m, 3H), 3.12–3.05 (m,

1H), 2.44–2.37 (m, 2H), 2.29–2.23 (m, 1H), 2.13 (s, 2H), 2.08–2.02 (m, 3H), 1.79–1.72 (m, 2H), 1.66 (s, 1H), 1.43 (s, 9H), 1.22–1.16 (m, 3H), 0.89–0.79 (m, 1H), 0.75–0.61 (m, 3H), 0.45–0.30 (m, 1H).

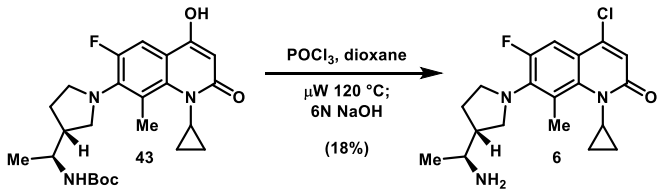
***tert*-Butyl ((*S*)-1-((*R*)-1-(1-cyclopropyl-6-fluoro-4-hydroxy-8-methyl-2-oxo-1, 2-dihydroquinolin-7-yl)pyrrolidin-3-yl)ethyl)carbamate (43)**



43

To a solution of methyl 4-((*R*)-3-((*S*)-1-((*tert*-butoxycarbonyl)amino)ethyl)pyrrolidin-1-yl)-2-(*N*-cyclopropylacetamido)-5-fluoro-3-methylbenzoate (5.00 g, 10.5 mmol) in THF (150 mL) was added NaHMDS (52.4 mL, 52.4 mmol, 1M in THF) at 10 °C followed by stirring for 20 min. The mixture was quenched with water and acidified to pH 4 with 1M HCl. The resulting suspension was extracted with EtOAc, and the organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by HPLC to give the title compound (2.30 g, 49% yield) as a yellow solid. LCMS (m/z): 447.2 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD) δ 7.40 (d, *J* = 13.6 Hz, 1H), 6.71 (d, *J* = 8.8 Hz, 1H), 5.79 (s, 1H), 3.62 (d, *J* = 6.5 Hz, 2H), 3.48–3.38 (m, 4H), 2.54 (s, 3H), 2.40–2.29 (m, 1H), 2.11 (ddd, *J* = 11.4, 7.3, 3.9 Hz, 1H), 1.80–1.68 (m, 1H), 1.45 (s, 9H), 1.25–1.10 (m, 5H), 0.55–0.44 (m, 2H).

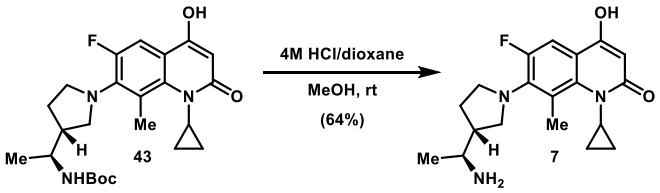
7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-4-chloro-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (6)



43 6

A solution of *tert*-butyl ((*S*)-1-((*R*)-1-(1-cyclopropyl-6-fluoro-4-hydroxy-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)pyrrolidin-3-yl)ethyl)carbamate (65.0 mg, 0.15 mmol) and phosphoryl trichloride (0.03 mL, 0.15 mmol) in dioxane (2 mL) was stirred at room temperature for 2 minutes and then heated to 120 °C via microwave for 20 minutes. The reaction was cooled to room temperature and treated with ice and 6N NaOH. The reaction was extracted with ethyl acetate, the organic phase was collected, dried over $MgSO_4$, filtered, concentrated, and purified by prep HPLC to afford the title compound (16.0 mg, 18% yield). HRMS (ESI) calc'd for $C_{19}H_{24}ClFN_3O$ $[M+H]^+$ 364.1586, found 364.1582. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.83 (br s, 2H), 7.39 (d, J = 13.6 Hz, 1H), 6.67 (s, 1H), 3.58 (q, J = 8.2 Hz, 1H), 3.51–3.35 (m, 4H), 3.32–3.25 (m, 1H), 2.44 (s, 3H), 2.38 (q, J = 8.6, 8.5 Hz, 1H), 2.15–2.05 (m, 1H), 1.80–1.68 (m, 1H), 1.27 (d, J = 6.4 Hz, 3H), 1.19–1.07 (m, 2H), 0.46–0.38 (m, 2H).

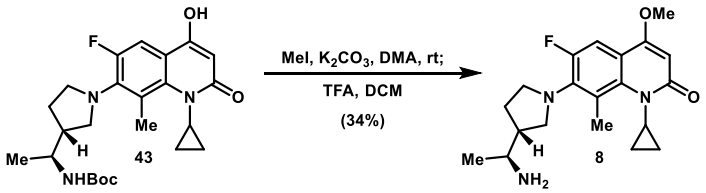
7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-hydroxy-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (7)



To a solution of *tert*-butyl ((*S*)-1-((*R*)-1-(1-cyclopropyl-6-fluoro-4-hydroxy-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)pyrrolidin-3-yl)ethyl)carbamate (50.0 mg, 0.11 mmol) in MeOH (1.1 mL) was added HCl (4M in dioxane, 1.1 mL) and the solution was stirred at rt for 16 h. The reaction solution was concentrated and purified by reverse phase HPLC to give the desired product (42.0 mg, 64% yield). HRMS (ESI) calc'd for $C_{19}H_{25}FN_3O_2$ $[M+H]^+$ 346.1925, found 346.1922. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.27 (s, 1H), 7.82 (s, 3H), 7.28 (d, J = 13.3 Hz, 1H), 5.65 (s, 1H), 3.51–3.39 (m, 2H), 3.36–3.29 (m, 3H), 3.29–3.21 (m, 1H), 2.43 (s, 3H), 2.41–2.32 (m, 1H),

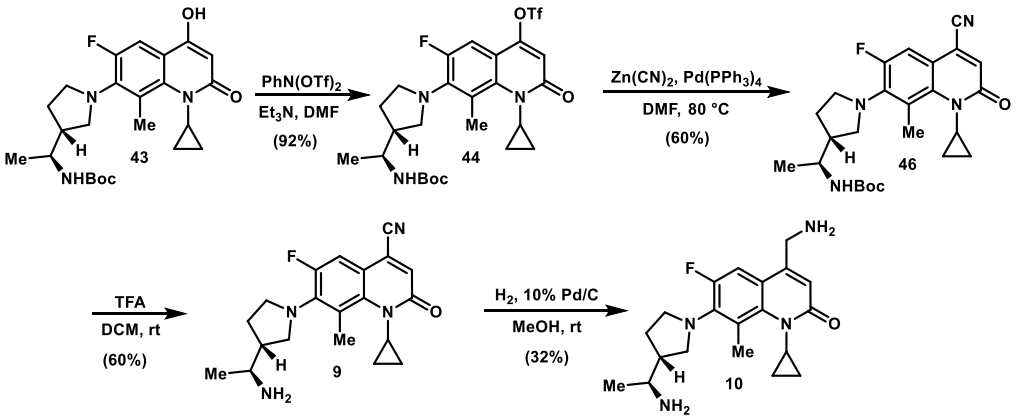
2.12–2.04 (m, 1H), 2.07 (s, 3H), 1.80–1.66 (m, 1H), 1.26 (d, *J* = 6.6 Hz, 3H), 1.13–1.00 (m, 2H), 0.34 (p, *J* = 4.8 Hz, 2H).

7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-methoxy-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (8)

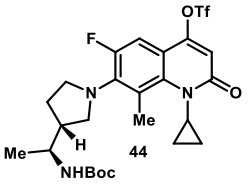


A mixture of *tert*-butyl ((*S*)-1-((*R*)-1-(1-cyclopropyl-6-fluoro-4-hydroxy-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)pyrrolidin-3-yl)ethyl)carbamate (17.0 mg, 0.04 mmol), iodomethane (10.9 mg, 0.08 mmol), potassium carbonate (10.6 mg, 0.08 mmol) and 18-crown-6 (41.0 mg, 0.15 mmol) in DMA (1 mL) was stirred at rt overnight. The reaction was poured into brine and extracted with ethyl acetate. The organic phase was collected, dried over $MgSO_4$, filtered and concentrated. The crude material was diluted with dichloromethane (1 mL) and treated with trifluoroacetic acid (1 mL) for 30 minutes. The reaction was concentrated and purified by prep HPLC to afford the desired material (6.0 mg, 34% yield). HRMS (ESI) calc'd for $C_{20}H_{27}FN_3O_2$ $[M+H]^+$ 360.2082, found 360.2077. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (br s, 2H), 7.29 (d, *J* = 13.5 Hz, 1H), 5.83 (s, 1H), 3.86 (s, 3H), 3.53–3.45 (m, 2H), 3.44–3.23 (m, 4H), 2.55 (p, *J* = 2.0 Hz, 2H), 2.44 (s, 3H), 2.42–2.31 (m, 1H), 2.13–2.01 (m, 1H), 1.79–1.66 (m, 1H), 1.26 (d, *J* = 6.5 Hz, 3H), 1.14–0.99 (m, 2H), 0.43–0.29 (m, 2H).

Preparation of compounds 9–10.

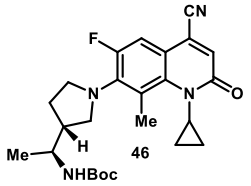


7-((*R*)-3-((*S*)-1-((*tert*-Butoxycarbonyl)amino)ethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl trifluoromethanesulfonate (44)



To a ice-cold solution of *tert*-butyl ((*S*)-1-((*R*)-1-(1-cyclopropyl-6-fluoro-4-hydroxy-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)pyrrolidin-3-yl)ethyl)carbamate (170 mg, 0.38 mmol) and triethylamine (160 μL, 1.15 mmol) in DMF (1.2 mL) was added dropwise 1,1,1-trifluoro-*N*-phenyl-*N*-((trifluoromethyl)sulfonyl)methanesulfonamide (164 mg, 0.46 mmol) in DMF (1 mL). The reaction was stirred for 1 hour and then poured into water, extracted with ethyl acetate and washed with brine. The organic phase was collected, dried over $MgSO_4$, filtered, and concentrated. The crude material was purified by silica gel column chromatography (0-100% EtOAc/heptane) to afford the desired material (202 mg, 92% yield). LCMS (m/z): 478.2 [M-Boc+H]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (d, *J* = 12.9 Hz, 1H), 6.49 (s, 1H), 4.48 (d, *J* = 7.1 Hz, 1H), 3.84–3.70 (m, 1H), 3.65 (q, *J* = 8.8 Hz, 1H), 3.55–3.39 (m, 4H), 2.47 (s, 3H), 2.33–2.23 (m, 1H), 2.09 (dtd, *J* = 14.0, 6.8, 2.8 Hz, 1H), 1.80–1.69 (m, 1H), 1.44 (s, 9H), 1.30–1.24 (m, 2H), 1.23 (d, *J* = 6.7 Hz, 3H), 1.19–1.10 (m, 1H), 0.66–0.52 (m, 2H).

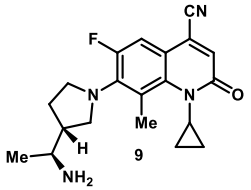
***tert*-Butyl ((*S*)-1-((*R*)-1-(4-cyano-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)pyrrolidin-3-yl)ethyl)carbamate (46)**



46

A solution of 7-((*R*)-3-((*S*)-1-((*tert*-butoxycarbonyl)amino)ethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl trifluoromethanesulfonate (113 mg, 0.20 mmol), tetrakis(triphenylphosphine)palladium (2.25 mg, 1.95 μmol) and dicyanozinc (13.7 mg, 0.12 mmol) in DMF (650 μl) was heated at 80 °C for 2 days. The reaction was cooled to room temperature and partitioned between brine and ethyl acetate. The organic phase was collected, dried over $MgSO_4$, filtered, concentrated and purified by silica gel chromatography (0-100% EtOAc/heptane) to afford the desired material (54.5 mg, 60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 12.7 Hz, 1H), 6.85 (s, 1H), 4.48 (d, *J* = 9.2 Hz, 1H), 3.81–3.70 (m, 1H), 3.65 (q, *J* = 7.1 Hz, 1H), 3.54–3.40 (m, 4H), 2.46 (s, 3H), 2.29 (q, *J* = 8.3 Hz, 1H), 2.14–3.05 (m, 1H), 1.75 (dq, *J* = 12.0, 8.9 Hz, 1H), 1.44 (s, 9H), 1.32–1.24 (m, 2H), 1.23 (d, *J* = 6.6 Hz, 3H), 0.62–0.48 (m, 2H).

7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile trifluoroacetic acid (9)

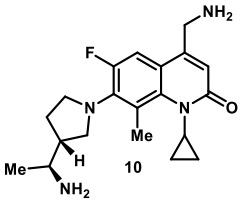


9

A solution of *tert*-butyl ((*S*)-1-((*R*)-1-(4-cyano-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)pyrrolidin-3-yl)ethyl)carbamate (26.4 mg, 0.06 mmol) in dichloromethane (1 mL) was treated with trifluoroacetic acid (1 mL) and stirred for 30 minutes at room temperature.

The reaction was concentrated and purified by prep HPLC to afford the title compound (13.0 mg, 60% yield). HRMS (ESI) calc'd for $C_{20}H_{24}FN_4O$ $[M+H]^+$ 355.1929, found 355.1924. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.84 (br s, 2H), 7.24 (d, J = 12.9 Hz, 1H), 7.12 (s, 1H), 3.65–3.56 (m, 1H), 3.54–3.44 (m, 3H), 3.39 (t, J = 9.0 Hz, 1H), 3.33–3.24 (m, 1H), 2.43 (s, 3H), 2.43–2.31 (m, 2H), 2.15–2.07 (m, 1H), 1.81–1.68 (m, 1H), 1.27 (d, J = 6.5 Hz, 3H), 1.21–1.10 (m, 1H), 0.44 (ddd, J = 10.5, 6.2, 4.5 Hz, 2H).

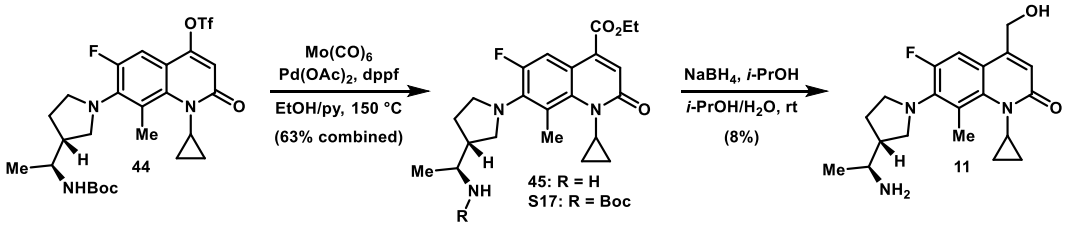
7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (10)



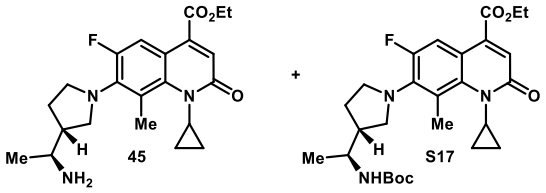
10

A mixture of 7-((*R*)-3-((*S*)-1-aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile (13.7 mg, 0.020 mmol), Pd/C (10%, 50% in water, 10.0 mg), and acetic acid (100 µL) in methanol (2.00 mL) was sparged with H_2 (g) for 5 minutes and then left under an atmosphere of H_2 (g) for 4 hours. The suspension was filtered over Celite, concentrated, and purified by prep HPLC to give the title compound (3.0 mg, 32% yield). LCMS (m/z): 359.5 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (br s, 2H), 7.86 (br s, 2H), 7.38 (d, J = 14.1 Hz, 1H), 6.44 (s, 1H), 4.23 (dd, J = 5.4, 4.7 Hz, 2H), 3.55 (dd, J = 8.6, 7.5 Hz 1H), 3.50–3.24 (m, 6H), 2.45 (s, 3H), 2.43–2.35 (m, 1H), 2.14–2.05 (m, 1H), 1.79–1.68 (m, 1H), 1.27 (d, J = 6.5 Hz, 3H), 1.22–1.09 (m, 2H), 0.38–0.26 (m, 2H).

Preparation of 7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-(hydroxymethyl)-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (11)

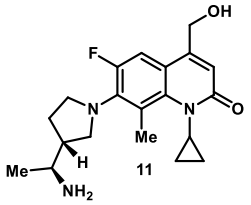


Ethyl 7-((*R*)-3-((*S*)-1-aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carboxylate (45)



A solution of 7-((*R*)-3-((*S*)-1-((*tert*-butoxycarbonyl)amino)ethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl trifluoromethanesulfonate (193 mg, 0.34 mmol), palladium acetate (7.50 mg, 0.03 mmol), dppf (18.5 mg, 0.03 mmol), molybdenum hexacarbonyl (44.0 mg, 0.17 mmol), EtOH (1.00 mL) and pyridine (0.250 mL) was heated at 150 °C via microwave for 20 minutes. The reaction was concentrated, diluted with ethyl acetate, and washed with water. The organic phase was collected, dried over $MgSO_4$, filtered, concentrated, and purified by silica gel column chromatography (0-100% EtOAc/heptane, then 0-10% MeOH/DCM) to afford deprotected ester **45** (34.0 mg, 25% yield) along with the corresponding Boc protected amine **S17** (64.0 mg, 38% yield). LCMS (m/z) for **45**: 402.1 $[M+H]^+$.

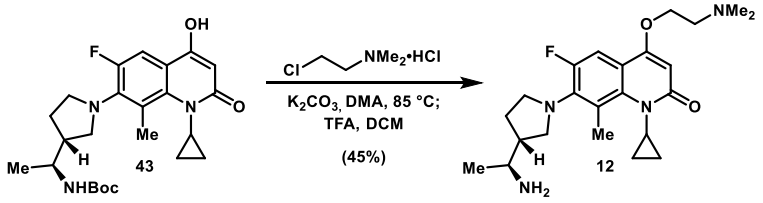
7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-(hydroxymethyl)-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (11)



11

A solution of the ester (cmpd **45**, 34.0 mg, 0.08 mmol) in *i*-PrOH (0.7 mL)/water (0.15 mL) was treated with sodium borohydride (11.3 mg, 0.30 mmol) and stirred at rt for 4 hours. The reaction was quenched with acetic acid, concentrated under reduced pressure and purified by reverse phase prep HPLC to afford the title compound (4.0 mg, 8% yield). LCMS (m/z): 360.4 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (br s, 2H), 7.24 (d, J = 13.9 Hz, 1H), 6.42 (s, 1H), 4.59 (s, 2H), 3.53–3.22 (m, 6H), 3.17 (s, 1H), 2.45 (s, 3H), 2.43–2.33 (m, 1H), 2.09 (ddt, J = 14.9, 7.4, 3.0 Hz, 1H), 1.78–1.68 (m, 1H), 1.26 (d, J = 6.5 Hz, 3H), 1.11 (tq, J = 10.1, 5.1 Hz, 2H), 0.40–0.29 (m, 2H).

7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-4-(2-(dimethylamino)ethoxy)-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (12)

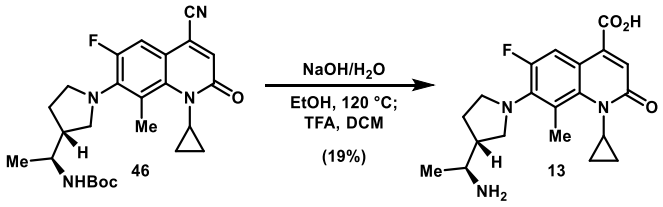


43 12

A suspension of *tert*-butyl ((*S*)-1-((*R*)-1-(1-cyclopropyl-6-fluoro-4-hydroxy-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)pyrrolidin-3-yl)ethyl)carbamate (59.4 mg, 0.133 mmol), 2-chloro-*N*,*N*-dimethylethanamine hydrochloride (19.2 mg, 0.13 mmol), potassium carbonate (55.3 mg, 0.40 mmol), and 18-crown-6 (211 mg, 0.80 mmol) in DMA (444 µl) was heated at 85 °C and stirred overnight. The reaction was poured into brine and extracted with ethyl acetate. The organic phase was collected, dried over $MgSO_4$, filtered, and concentrated. The crude residue was dissolved in

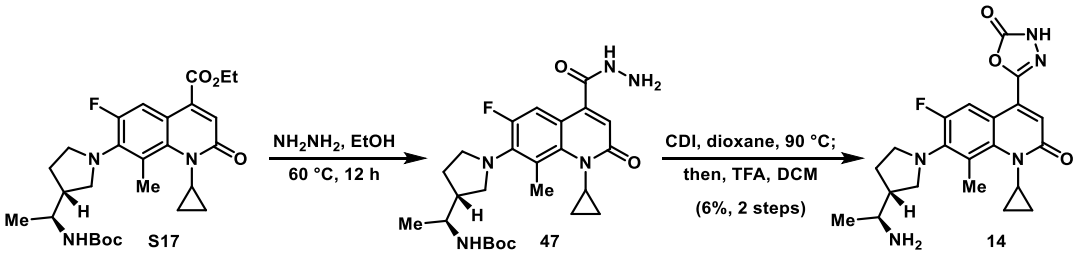
DCM (0.5 mL) and treated with TFA (1 mL). The reaction was stirred 1 hour and then concentrated under reduced pressure. The crude residue was purified by prep HPLC to afford the title compound (45.2 mg, 45% yield). LCMS (m/z): 417.1 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD) δ 7.61 (d, *J* = 13.3 Hz, 1H), 5.95 (s, 1H), 4.47 (dd, *J* = 5.6, 4.0 Hz, 2H), 3.74–3.68 (m, 2H), 3.65–3.54 (m, 2H), 3.50–3.40 (m, 3H), 3.40–3.33 (m, 1H), 3.01 (s, 6H), 2.56 (s, 3H), 2.49 (q, *J* = 8.5 Hz, 1H), 2.28–2.18 (m, 1H), 1.89–1.78 (m, 1H), 1.40 (d, *J* = 6.6 Hz, 3H), 1.27–1.14 (m, 2H), 0.55–0.41 (m, 2H).

Preparation of 7-((*R*)-3-((*S*)-1-Aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carboxylic acid trifluoroacetic acid (13)

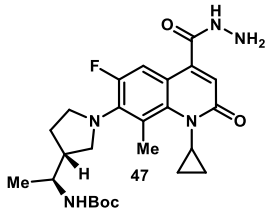


A solution of *tert*-butyl ((*S*)-1-((*R*)-1-(4-cyano-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)pyrrolidin-3-yl)ethyl)carbamate (27.7 mg, 0.06 mmol) in 10% aqueous ethanol (2.00 mL) was treated with sodium hydroxide (12.2 mg, 0.30 mmol) and stirred at 120 °C for 30 minutes. The reaction was concentrated under reduced pressure, dissolved in DCM (1.00 mL) and treated with TFA (1.00 mL). After 30 minutes, the reaction was concentrated and the desired product was isolated by prep HPLC (4.0 mg, 19% yield, purity = 87%) along with the corresponding primary amide (8.0 mg, 38% yield). LCMS (m/z) for **13**: 374.0 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.83 (br s, 2H), 7.73 (d, *J* = 14.7 Hz, 1H), 6.74 (s, 1H), 3.60–3.51 (m, 1H), 3.53–3.44 (m, 2H), 3.43–3.30 (m, 2H), 3.30–3.20 (m, 1H), 2.44 (s, 3H), 2.42–2.34 (m, 2H), 2.14–2.04 (m, 1H), 1.80–1.68 (m, 1H), 1.27 (d, *J* = 6.5 Hz, 3H), 1.17–1.06 (m, 2H), 0.43–0.33 (m, 2H).

Preparation of 7-((R)-3-((S)-1-aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-4-(5-oxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)quinolin-2(1*H*)-one trifluoroacetic acid (14)

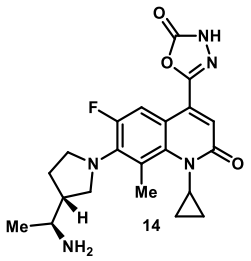


***tert*-butyl ((S)-1-((R)-1-(1-cyclopropyl-6-fluoro-4-(hydrazinecarbonyl)-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)pyrrolidin-3-yl)ethyl)carbamate (47)**



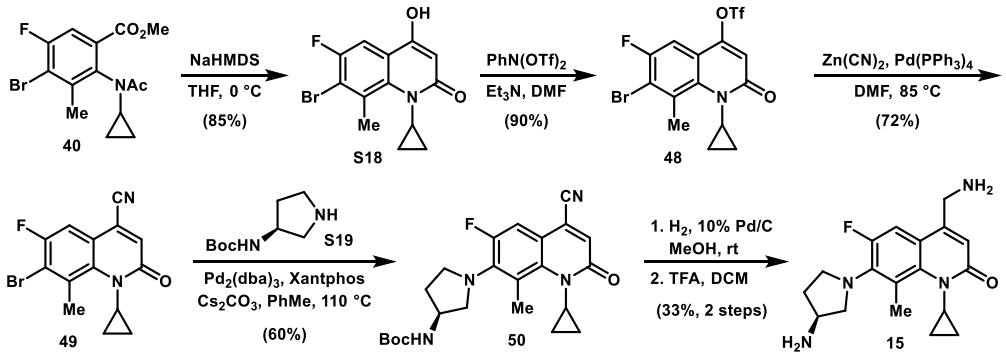
A solution of ethyl 7-((*R*)-3-((*S*)-1-((*tert*-butoxycarbonyl)amino)ethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carboxylate (48.1 mg, 0.096 mmol) and hydrazine (0.015 mL, 0.479 mmol) in ethanol (2 mL) was stirred for 12 hours at 60 °C. The reaction cooled to room temperature and was concentrated under reduced pressure to afford the desired material, which was used without further purification. LCMS (m/z): 488.1 $[M+H]^+$.

7-((*R*)-3-((*S*)-1-aminoethyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methyl-4-(5-oxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)quinolin-2(1*H*)-one trifluoroacetic acid (14)

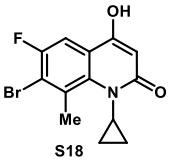


A solution of **47** (51.6 mg, 0.106 mmol) and carbonyldiimidazole (34.32 mg, 0.212 mmol) in dioxane (1 mL) was stirred for 2 days at 90 °C. The reaction was concentrated and then diluted with dichloromethane (1 mL) and treated with trifluoroacetic acid (1 mL) and aged for 30 minutes. The reaction was concentrated under reduced pressure and then purified by prep HPLC to afford the title compound (4.9 mg, 6% yield, 2 steps, purity = 90%). LCMS (m/z): 414.1 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d6) δ 13.03 (s, 1H), 8.08 (d, *J* = 15.0 Hz, 1H), 7.80 (bs, 2H), 6.72 (s, 1H), 3.68 - 3.16 (m, 6H), 2.45 (s, 3H), 2.42 - 2.34 (m, 1H), 2.10 (m, 1H), 1.75 (m, 1H), 1.27 (d, *J* = 6.5 Hz, 3H), 1.15 (m, 2H), 0.42 (m, 2H).

Preparation of (*S*)-4-(Aminomethyl)-7-(3-aminopyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one hydrochloride (15)



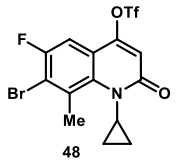
7-Bromo-1-cyclopropyl-6-fluoro-4-hydroxy-8-methylquinolin-2(1*H*)-one (S18)



To an ice-cold solution of methyl 4-bromo-2-(*N*-cyclopropylacetamido)-5-fluoro-3-methylbenzoate (595 mg, 1.73 mmol) in THF (12 mL) was added dropwise NaHMDS (8.64 mL, 8.64 mmol, 1M in THF). After 30 minutes of stirring, 1N HCl was added dropwise until pH 2 was reached. A solid precipitated, was filtered and washed with water. The aqueous phase was extracted with ethyl acetate and the organic phase collected, dried over $MgSO_4$, filtered, and

concentrated under reduced pressure. The extracted material was combined with the solid filtrate to give the title compound (461 mg, 85% yield). LCMS (m/z): 313.9 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.60 (br s, 1H), 7.50 (d, J = 8.6 Hz, 1H), 5.78 (s, 1H), 3.39 (tt, J = 6.8, 4.0 Hz, 1H), 2.71 (s, 3H), 1.11–1.03 (m, 2H), 0.38–0.32 (m, 2H).

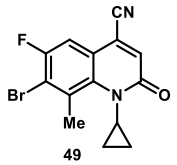
7-Bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl trifluoromethanesulfonate (48)



48

A ice-cold mixture of 7-bromo-1-cyclopropyl-6-fluoro-4-hydroxy-8-methylquinolin-2(1*H*)-one (287 mg, 0.92 mmol) and triethylamine (384 µl, 2.76 mmol) in DMF (2.5 mL) was treated dropwise with 1,1,1-trifluoro-*N*-phenyl-*N*-((trifluoromethyl)sulfonyl)methanesulfonamide (394 mg, 1.10 mmol) in DMF (1 mL) followed by stirring for 1 hour. The reaction was poured into water, extracted with ethyl acetate and washed with brine. The organic phase was collected, dried over $MgSO_4$, filtered, and concentrated. The crude material was purified by silica gel column chromatography (0-100% EtOAc/heptane) to afford the desired material (369 mg, 90% yield). LCMS (m/z): 446.0 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, J = 7.8 Hz, 1H), 6.68 (s, 1H), 3.51 (tt, J = 6.8, 4.0 Hz, 1H), 2.81 (s, 3H), 1.30–1.22 (m, 2H), 0.63–0.56 (m, 2H).

7-Bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile (49)

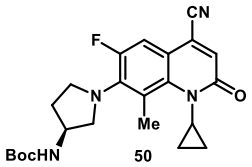


49

A solution of 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl trifluoromethanesulfonate (238 mg, 0.54 mmol), tetrakis(triphenylphosphine)palladium (31.0 mg,

0.03 mmol), and dicyanozinc (33.0 mg, 0.28 mmol) in DMF (1.8 mL) was heated at 85 °C for 18 hours. The reaction was treated with water and extracted with ethyl acetate. The organic phase was collected, washed with brine, dried over $MgSO_4$, filtered, concentrated, and purified by silica gel column chromatography (0-100% EtOAc/heptane) to afford the desired material (125 mg, 72% yield). LCMS (m/z): 322.9 $[M+H]^+$.

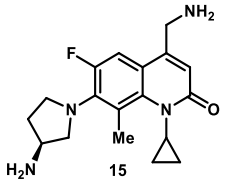
(*S*)-*tert*-Butyl (1-(4-cyano-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)pyrrolidin-3-yl)carbamate (50)



50

A solution of 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile (159 mg, 0.50 mmol), xantphos (43.0 mg, 0.07 mmol), (*S*)-*tert*-butyl pyrrolidin-3-ylcarbamate (138 mg, 0.74 mmol), tris(dibenzylideneacetone)dipalladium(0) (22.7 mg, 25.0 μmol) and cesium carbonate (323 mg, 0.99 mmol) in toluene (1.65 mL) was heated at 110 °C overnight. The reaction was poured into water and extracted with ethyl acetate. The organic phase was washed with brine, dried over $MgSO_4$, filtered, concentrated, and purified by silica gel column chromatography (0-80% EtOAc/heptane) to afford the desired compound (126 mg, 60% yield). LCMS (m/z): 427.1 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, *J* = 12.5 Hz, 1H), 6.87 (s, 1H), 4.75 (s, 1H), 4.35 (s, 1H), 3.81–3.73 (m, 1H), 3.61 (q, *J* = 8.0 Hz, 1H), 3.49 (tt, *J* = 7.4, 4.2 Hz, 2H), 3.30 (dd, *J* = 10.0, 4.3 Hz, 1H), 2.48 (s, 3H), 2.33 (dq, *J* = 13.5, 6.9 Hz, 1H), 1.92 (td, *J* = 12.9, 5.6 Hz, 1H), 1.47 (s, 9H), 0.88 (t, *J* = 6.9 Hz, 2H), 0.56 (q, *J* = 4.1 Hz, 2H).

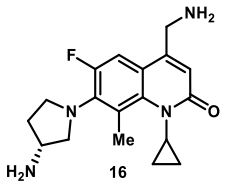
(*S*)-4-(Aminomethyl)-7-(3-aminopyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one hydrochloride (15)



15

A mixture of (*S*)-*tert*-butyl (1-(4-cyano-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)pyrrolidin-3-yl)carbamate (126 mg, 0.30 mmol) in 2M NH_3 in EtOH (5 mL) was treated with Pd/C (10% dry wt, 50% water, 95.0 mg, 0.09 mmol) and sparged with hydrogen for 5 minutes. The reaction was left under an atmosphere of H_2 for 2 hours. The reaction was filtered over Celite, concentrated under reduced pressure, diluted with DCM (1 mL) and treated with TFA (2 mL). After 1 hour, the reaction was concentrated under reduced pressure and purified by reverse phase HPLC to give the title compound (44.0 mg, 33% yield, 2 steps). Re-lyophilizing with 1N HCl provided the HCl salt. HRMS (ESI) calc'd for $C_{18}H_{24}FN_4O$ $[M+H]^+$ 331.1929, found 331.1925. ^{1}H NMR (400 MHz, CD_3OD) δ 7.38 (d, *J* = 13.5 Hz, 1H), 6.54 (s, 1H), 4.34 (s, 2H), 3.99 (p, *J* = 5.7 Hz, 1H), 3.82 (ddd, *J* = 10.8, 6.3, 1.8 Hz, 1H), 3.63 (q, *J* = 8.1, 7.5 Hz, 1H), 3.53 (dt, *J* = 10.2, 3.4 Hz, 3H), 2.60 (s, 3H), 2.49 (dtd, *J* = 13.6, 7.9, 5.9 Hz, 1H), 2.09 (ddt, *J* = 13.3, 8.0, 5.5 Hz, 1H), 1.27–1.15 (m, 2H), 0.51–0.42 (m, 2H).

(*R*)-4-(Aminomethyl)-7-(3-aminopyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (16)

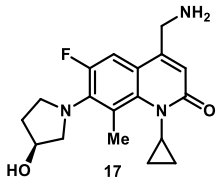


16

16 (0.09 g, 10% yield, 2 steps) was prepared from 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile **49** (105 mg, 0.33 mmol) and (*R*)-*tert*-butyl pyrrolidin-3-

ylcarbamate (91.0 mg, 0.49 mmol) according to the procedure used for compound **15**. LCMS (m/z): 331.2 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (br s, 2H), 8.10 (br s, 2H), 7.41 (d, J = 14.1 Hz, 1H), 6.46 (s, 1H), 4.23 (q, J = 6.9 Hz, 2H), 3.95–3.87 (m, 1H), 3.71–3.65 (m, 1H), 3.56–3.44 (m, 4H), 2.50 (s, 3H), 2.37–2.30 (m, 1H), 2.02–1.96 (m, 1H), 1.18–1.11 (m, 2H), 0.34–0.30 (m, 2H).

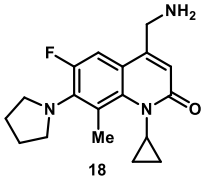
(*S*)-4-(Aminomethyl)-1-cyclopropyl-6-fluoro-7-(3-hydroxypyrrolidin-1-yl)-8-methylquinolin-2(1*H*)-one hydrochloride (17)



17

17 (62.2 mg, 18% yield, 2 steps) was prepared from 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile **49** (300 mg, 0.93 mmol) and (*S*)-3-((*tert*-butyldimethylsilyl)oxy)pyrrolidine (376 mg, 1.87 mmol) according to the procedure used for compound **15** (omitting the Boc deprotection step). HRMS (ESI) calc'd for $C_{18}H_{23}FN_3O_2$ $[M+H]^+$ 332.1769, found 332.1767. ^{1}H NMR (400 MHz, CD_3OD) δ 7.30 (d, J = 13.9 Hz, 1H), 6.46 (s, 1H), 4.53 (tt, J = 5.1, 2.8 Hz, 1H), 4.36–4.28 (m, 2H), 3.84 (p, J = 6.3, 5.2 Hz, 2H), 3.58–3.35 (m, 2H), 2.53 (s, 3H), 2.27–2.12 (m, 1H), 1.99 (ddd, J = 11.6, 6.8, 3.6 Hz, 1H), 1.34–1.17 (m, 2H), 0.54–0.41 (m, 2H).

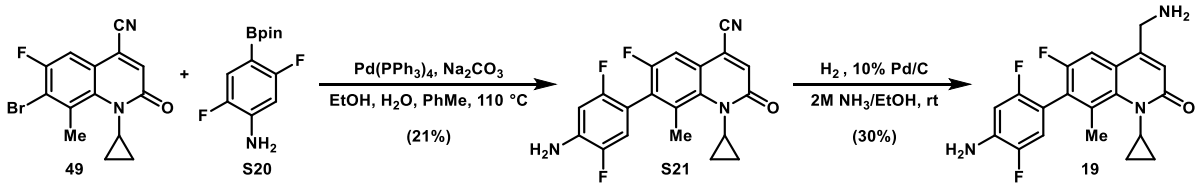
4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methyl-7-(pyrrolidin-1-yl)quinolin-2(1*H*)-one (18)



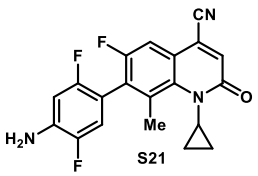
18

18 (17.8 mg, 14% yield, 2 steps) was prepared from 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile **49** (75 mg, 0.23 mmol) and pyrrolidine (39 µL, 0.47 mmol) according to the procedure used for compound **15** (omitting the Boc deprotection step). HRMS (ESI) calc'd for $C_{18}H_{23}FN_3O$ $[M+H]^+$ 316.1820, found 316.1820. ^{1}H NMR (500 MHz, DMSO-*d*6) δ 8.33 (br s, 3H), 7.35 (d, J = 14.2 Hz, 1H), 6.41 (s, 1H), 4.26 - 4.17 (m, 2H), 2.42 (s, 3H), 1.98 - 1.88 (m, 4H), 1.21 - 1.09 (m, 2H), 0.35 - 0.28 (m, 2H).

Preparation of 7-(4-Amino-2,5-difluorophenyl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (19)



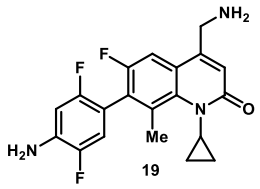
7-(4-Amino-2,5-difluorophenyl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile (S21)



A mixture of 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile (85.0 mg, 0.20 mmol), 2,5-difluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (68.0 mg, 0.26 mmol), tetrakis(triphenylphosphine)palladium (92.0 mg, 0.08 mmol), and sodium carbonate (84.0 mg, 0.80 mmol) in toluene (1 mL)/water (0.35 mL)/ethanol (0.35 mL) was sealed and maintained at 110 °C for 18 hours. The reaction was cooled to room temperature, diluted with water, ethyl acetate, and the phases separated. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was

purified by silica gel column chromatography (0-100% EtOAc/heptane) to afford the product (16.0 mg, 21% yield) along with an equimolar amount of the dehalogenated starting material. LCMS (m/z): 369.9 $[M+H]^+$.

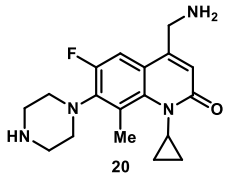
7-(4-Amino-2,5-difluorophenyl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (19)



19

A mixture of 7-(4-amino-2,5-difluorophenyl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile (41.0 mg, 0.05 mmol) in 2M NH_3 in EtOH (4 mL) was treated with Pd/C (10% dry wt, 50% water, 17.4 mg, 0.02 mmol) and sparged with hydrogen for 5 minutes. The reaction was left under an atmosphere of H_2 for 2 hours. The reaction was then filtered over Celite and concentrated under reduced pressure. The residue was purified by reverse phase HPLC to give the desired compound (6.0 mg, 30% yield). LCMS (m/z): 374.1 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD) δ 7.46 (d, J = 9.6 Hz, 1H), 6.93 (dd, J = 11.1, 6.4 Hz, 1H), 6.69 (dd, J = 11.1, 7.5 Hz, 1H), 6.65 (s, 1H), 4.41 (s, 2H), 3.58 (dq, J = 6.8, 3.4 Hz, 1H), 2.52 (s, 3H), 1.36–1.17 (m, 2H), 0.60–0.49 (m, 2H).

4-(Aminomethyl)-1-cyclopropyl-6-fluoro-8-methyl-7-(piperazin-1-yl)quinolin-2(1*H*)-one trifluoroacetic acid (20)

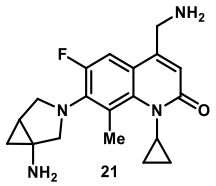


20

20 (7.5 mg, 6% yield, 2 steps) was prepared from 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile **49** (81 mg, 0.25 mmol) and benzyl piperazine-1-

carboxylate (83 mg, 0.38 mmol) according to the procedure used for compound **15**. LCMS (m/z): 331.1 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (br s, 1H), 8.36 (br s, 2H), 7.45 (d, J = 13.1 Hz, 1H), 6.49 (s, 1H), 4.22 (s, 2H), 3.53–3.42 (m, 4H), 3.30–3.17 (m, 5H), 2.58 (s, 3H), 1.20–1.10 (m, 2H), 0.35–0.22 (m, 2H).

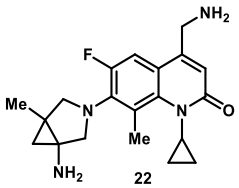
7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (21)



21

21 (7.0 mg, 6% yield, 2 steps) was prepared from 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile **49** (60.0 mg, 0.30 mmol) and *tert*-butyl (3-azabicyclo[3.1.0]hexan-1-yl)carbamate (75.0 mg, 0.23 mmol) according to the procedure used for compound **15**. HRMS (ESI) calc'd for $C_{19}H_{24}FN_4O$ $[M+H]^+$ 343.1929, found 343.1929. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.65 (br s, 2H), 8.37 (br s, 2H), 7.43 (d, J = 13.6 Hz, 1H), 6.55 (br s, 2H), 4.23 (s, 2H), 3.73 (d, J = 8.9 Hz, 1H), 3.69–3.60 (m, 1H), 3.54 (d, J = 9.1 Hz, 1H), 3.50–3.42 (m, 1H), 3.23–3.19 (m, 1H), 2.43 (s, 3H), 1.94 (ddd, J = 8.5, 4.6, 3.4 Hz, 1H), 1.24 (dd, J = 8.8, 5.7 Hz, 1H), 1.18–1.07 (m, 3H), 0.36–0.27 (m, 2H).

***rac*-7-((1*S*,5*R*)-1-Amino-5-methyl-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (22)**

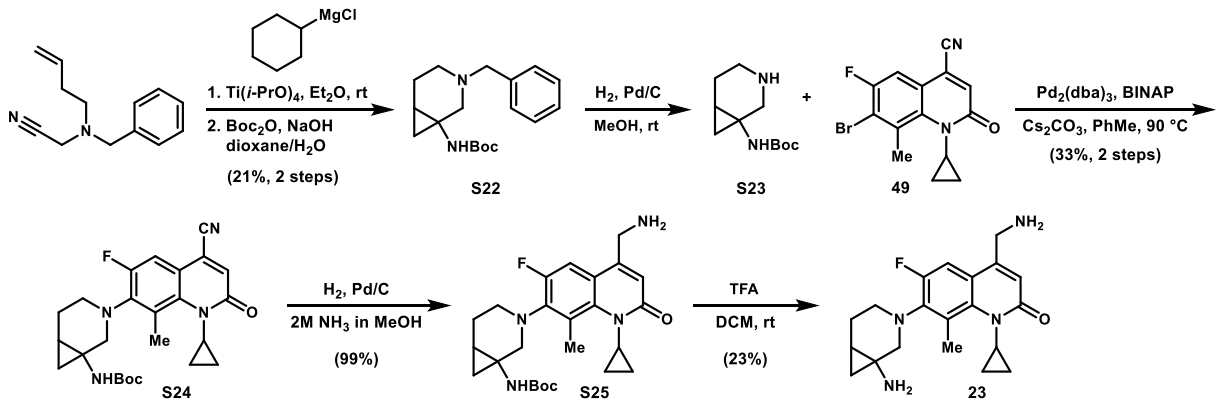


22

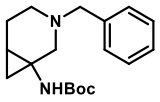
22 (9.0 mg, 5% yield, 2 steps) was prepared from 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile **49** (80.0 mg, 0.22 mmol), BINAP (21 mg, 0.03 mmol) as

ligand instead of xantphos, and *tert*-butyl *rac*-((1*S*,5*R*)-5-methyl-3-azabicyclo[3.1.0]hexan-1-yl)carbamate (66.0 mg, 0.31 mmol) according to the procedure used for compound **15**. *tert*-butyl *rac*-((1*S*,5*R*)-5-methyl-3-azabicyclo[3.1.0]hexan-1-yl)carbamate was prepared using procedures analogous to those described for **23** below. LCMS (m/z): 357.4 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD) δ 7.32 (d, *J* = 13.5 Hz, 1H), 6.55 (s, 1H), 4.13 (s, 2H), 3.63 (d, *J* = 9.0 Hz, 1H), 3.59–3.47 (m, 3H), 3.38 (d, *J* = 9.2 Hz, 1H), 2.53 (s, 3H), 1.32 (s, 3H), 1.25–1.17 (m, 2H), 1.13 (d, *J* = 5.2 Hz, 1H), 0.67 (d, *J* = 5.1 Hz, 1H), 0.52–0.42 (m, 2H).

Preparation of 7-(1-amino-3-azabicyclo[4.1.0]heptan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one (23)



***tert*-butyl (3-benzyl-3-azabicyclo[4.1.0]heptan-1-yl)carbamate (S22)**

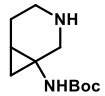


S22

A solution of 2-(benzyl(but-3-en-1-yl)amino)acetonitrile (500 mg, 2.50 mmol) and titanium (IV) isopropoxide (1.48 ml, 4.99 mmol) in ether (15 ml) was slowly treated with a 2M solution of cyclohexylmagnesium chloride (9.99 ml, 9.99 mmol) over 1 hour. The reaction mixture was then stirred at rt for 1 more hour. The crude product was then cooled to 0 °C and treated with 2M NaOH (15 mL) and allowed to stir for 1 hour. Inorganic precipitates were filtered off through a pad of Celite, and the filter cake was washed thoroughly with ether. The combined filtrates were

washed with brine and dried with $MgSO_4$. The solvent was removed under reduced pressure. The residue was dissolved in dioxane (2.5 mL) then treated 1N NaOH (2 mL) and di-tert-butyl dicarbonate (1079 mg, 4.94 mmol) and stirred at room temperature overnight. The reaction was diluted with ethyl acetate and water. Phases were separated and the organic phase collected, dried (MgSO4), filtered, concentrated, and purified by silica gel column chromatography eluting with a linear gradient of 0-60% ethyl acetate in heptane to afford the desired product (157 mg, 21% yield, 2 steps). LCMS (m/z): 303.1 [M+H]+.

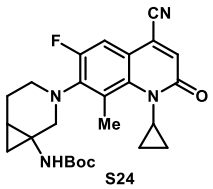
***tert*-butyl (3-azabicyclo[4.1.0]heptan-1-yl)carbamate (S23)**



S23

A mixture of *tert*-butyl (3-benzyl-3-azabicyclo[4.1.0]heptan-1-yl)carbamate (155 mg, 0.513 mmol) and 10% Pd/C (54.5 mg, 0.051 mmol) in MeOH (5 mL) was sparged with H_2 for 5 minutes and then left under an atmosphere (balloon) of H_2 overnight. The reaction was then filtered over an acrodisc filter and concentrated under reduced pressure to give the desired amine, which was used without further purification. LCMS (m/z): 213.1 $[M+H]^+$.

***tert*-butyl (3-(4-cyano-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-azabicyclo[4.1.0]heptan-1-yl)carbamate (S24)**

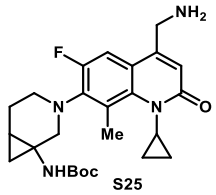


S24

A microwave vial was charged with **49** (100 mg, 0.311 mmol), *tert*-butyl (3-azabicyclo[4.1.0]heptan-1-yl)carbamate (99 mg, 0.467 mmol), cesium carbonate (304 mg, 0.934 mmol), $Pd_2(dba)_3$ (57.0 mg, 0.062 mmol) and BINAP (78 mg, 0.125 mmol). The vial was placed

under vaccum and backfilled with N_2 3 times, then toluene (2 mL) was added. The mixture was stirred at 90 °C overnight. The crude reaction mixture was filtered through a disposable filter funnel and volatiles were evaporated under reduced pressure. SiO_2 flash chromatography (0-100% EtOAc/heptane) provided the desired product (46.3 mg, 33 % yield, 2 steps) as a yellow dense oil. LCMS (m/z): 453.2 $[M+H]^+$.

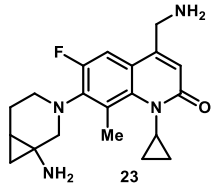
***tert*-butyl (3-(4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-azabicyclo[4.1.0]heptan-1-yl)carbamate (S25)**



S25

A mixture of *tert*-butyl (3-(4-cyano-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-azabicyclo[4.1.0]heptan-1-yl)carbamate (45 mg, 0.099 mmol) in 2M NH_3 in MeOH (2 mL) was treated with Pd/C (10% dry wt, 50% water, 63.5 mg, 0.030 mmol). The flask was partially evacuated and back-filled with H_2 5 times, then stirred under a balloon of hydrogen for ~60 minutes. The flask was partially evacuated and back-filled with N_2 5 times, then the reaction mixture was passed through a 1 µm syringe filter and concentrated under reduced pressure to give the desired product (45 mg, 99 % yield) as a yellow oil, which was used without further purification. LCMS (m/z): 457.3 $[M+H]^+$.

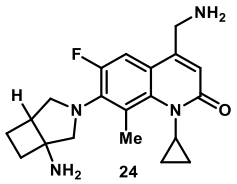
7-(1-amino-3-azabicyclo[4.1.0]heptan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (23)



23

A mixture of *tert*-butyl (3-(4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-azabicyclo[4.1.0]heptan-1-yl)carbamate (45.7 mg, 0.1 mmol) in DCM (1 mL) and TFA (1 mL) was stirred for 30 minutes. Volatiles were evaporated under reduced pressure. The residue was dissolved in water/H_2O and purified by preparative RP-HPLC (0.1% TFA in MeCN/H_2O) to give the desired product (11 mg, 23% yield). HRMS (ESI) calc'd for $C_{20}H_{25}FN_4O$ $[M+H]^+$ 357.2085, found 357.2080. ^{1}H NMR (400 MHz, Methanol-*d*4) δ 7.40 (d, *J* = 12.8 Hz, 1H), 6.56 (s, 1H), 4.35 (s, 2H), 3.73 – 3.60 (m, 2H), 3.60 – 3.52 (m, 1H), 3.10 – 3.02 (m, 1H), 2.65 (s, 3H), 2.27 – 2.16 (m, 1H), 2.05 – 1.92 (m, 1H), 1.61 – 1.51 (m, 1H), 1.35 – 1.18 (m, 4H), 0.50 – 0.42 (m, 2H).

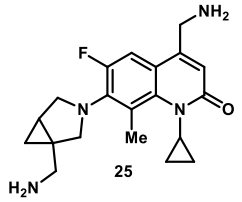
***rac*-7-((1*S*,5*R*)-1-Amino-3-azabicyclo[3.2.0]heptan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (24)**



24

24 (13.5 mg, 7% yield, 2 steps) was prepared from 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile **49** (80.0 mg, 0.22 mmol), BINAP (21.0 mg, 0.03 mmol) as ligand instead of xantphos and benzyl *rac*-((1*S*,5*R*)-3-azabicyclo[3.2.0]heptan-1-yl)carbamate (55.0 mg, 0.22 mmol) according to the procedure used for compound **15**. HRMS (ESI) calc'd for $C_{20}H_{26}FN_4O$ $[M+H]^+$ 357.2085, found 357.2080. ^{1}H NMR (400 MHz, CD_3OD) δ 7.41 (d, *J* = 13.3 Hz, 1H), 6.57 (s, 1H), 4.37 (d, *J* = 1.2 Hz, 2H), 3.78–3.71 (m, 1H), 3.71–3.55 (m, 5H), 3.54–3.45 (m, 1H), 3.11–3.00 (m, 1H), 2.77 (s, 3H), 2.45–2.36 (m, 3H), 1.93 (dd, *J* = 10.5, 3.6 Hz, 1H), 1.29–1.20 (m, 2H), 0.56–0.44 (m, 2H).

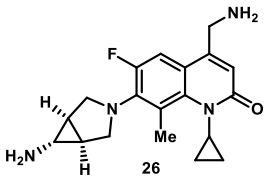
***rac*-4-(Aminomethyl)-7-(1-(aminomethyl)-3-azabicyclo[3.1.0]hexan-3-yl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (25)**



25

25 (4.0 mg, 3% yield, 2 steps) was prepared from 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile **49** (80.0 mg, 0.22 mmol), BINAP (21.0 mg, 0.03 mmol) as ligand instead of xantphos and *tert*-butyl ((3-azabicyclo[3.1.0]hexan-1-yl)methyl)carbamate hydrochloride (78.0 mg, 0.31 mmol) according to the procedure used for compound **15**. HRMS (ESI) calc'd for $C_{20}H_{26}FN_4O$ $[M+H]^+$ 357.2085, found 357.2080. ^{1}H NMR (400 MHz, CD_3OD) δ 7.34 (d, *J* = 13.3 Hz, 1H), 6.52 (s, 1H), 4.34 (d, *J* = 1.2 Hz, 2H), 3.72 (d, *J* = 9.6 Hz, 1H), 3.68–3.57 (m, 2H), 3.57–3.50 (m, 1H), 3.46–3.36 (m, 2H), 3.08 (d, *J* = 13.6 Hz, 1H), 2.54 (s, 3H), 1.70 (dt, *J* = 7.8, 3.9 Hz, 1H), 1.30–1.19 (m, 2H), 1.10 (t, *J* = 4.7 Hz, 1H), 0.93 (dt, *J* = 8.1, 4.2 Hz, 2H), 0.52–0.42 (m, 2H).

***rac*-7-((1*R*,5*S*,6*s*)-6-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (26)**

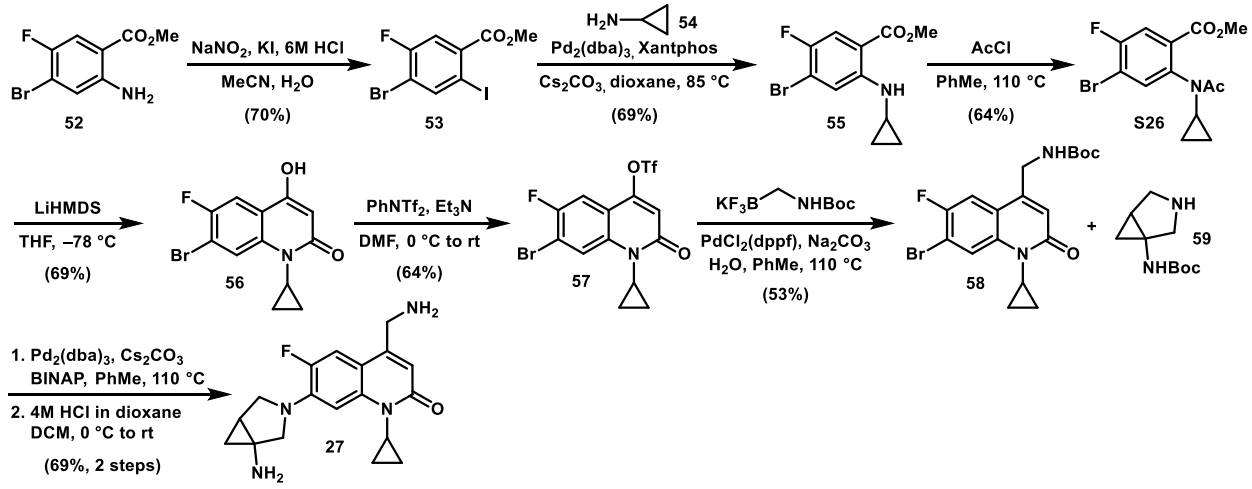


26

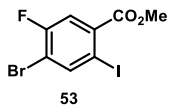
26 (14.3 mg, 11% yield, 2 steps) was prepared from 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile **49** (75.0 mg, 0.22 mmol), BINAP (58.0 mg, 0.09 mmol) as ligand instead of xantphos and *tert*-butyl 3-azabicyclo[3.1.0]hexan-1-ylcarbamate (70.0 mg, 0.35 mmol) according to the procedure used for compound **15**. HRMS (ESI) calc'd for

$C_{19}H_{24}FN_4O$ $[M+H]^+$ 343.1929, found 343.1923. ^1H NMR (400 MHz, CD_3OD) δ 7.38 (d, J = 13.2 Hz, 1H), 6.56 (s, 1H), 4.37 (d, J = 1.2 Hz, 2H), 3.70 (d, J = 9.4 Hz, 2H), 3.62–3.52 (m, 3H), 2.86 (t, J = 2.2 Hz, 1H), 2.57 (s, 3H), 2.08 (q, J = 1.9 Hz, 2H), 1.30–1.17 (m, 2H), 0.56–0.42 (m, 2H).

Preparation of 7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoroquinolin-2(1*H*)-one hydrochloride (27)



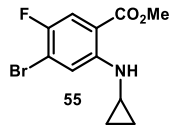
Methyl 4-bromo-5-fluoro-2-iodobenzoate (53)



Methyl 2-amino-4-bromo-5-fluorobenzoate (2.37 g, 9.54 mmol) was dissolved in acetonitrile (15 mL) and 6M HCl (48 mL). To the heterogeneous yellow mixture at 0 °C was added dropwise a solution of sodium nitrite (0.69 g, 10.0 mmol) in water (20 mL). The resulting mixture was stirred for 1 hour at 0 °C to give an almost clear, yellow solution. A solution of potassium iodide (3.17 g, 19.1 mmol) in water (20 mL) was added dropwise and the mixture was allowed to warm slowly to rt overnight. The reaction mixture was diluted with water and extracted with EtOAc. The combined organic extracts were washed with saturated aqueous $Na_2S_2O_3$, brine, dried over $MgSO_4$, filtered

and concentrated under reduced pressure. The desired product was purified silica gel column chromatography (0-5% EtOAc/heptane) to give the desired product (2.40 g, 70% yield).

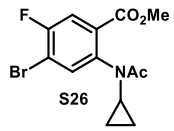
Methyl 4-bromo-2-(cyclopropylamino)-5-fluorobenzoate (55)



55

To methyl 4-bromo-5-fluoro-2-iodobenzoate (5.00 g, 13.9 mmol) in dioxane (50 mL) were added Cs_2CO_3 (9.05 g, 27.8 mmol), xantphos (1.21 g, 2.01 mmol), $Pd_2(dba)_3$ (0.64 g, 0.70 mmol), cyclopropylamine (1.59 g, 27.8 mmol) and the reaction mixture was stirred at 85 °C for 7 hours. The reaction mixture was cooled to room temperature, diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated to afford a crude residue. The crude residue was purified by silica gel column chromatography (0-5% EtOAc/hexanes) to afford the desired product (2.75 g, 69% yield). LCMS (m/z): 290.3 $[M+2]^+$.

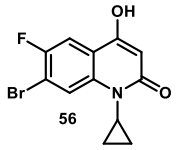
Methyl 4-bromo-2-(*N*-cyclopropylacetamido)-5-fluorobenzoate (S26)



S26

Methyl 4-bromo-2-(cyclopropylamino)-5-fluorobenzoate (2.75 g, 9.55 mmol) was dissolved in toluene (30 mL) at room temperature. Acetyl chloride (1.49 g, 19.1 mmol) was added and the reaction mixture was stirred at 110 °C for 3 hours. The reaction mixture was cooled to room temperature, diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford a crude residue. The crude residue was purified by silica gel column chromatography (0-5% EtOAc/hexanes) to afford the desired product (2.00 g, 64% yield). LCMS (m/z): 332.3 $[M+2]^+$.

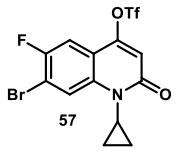
7-Bromo-1-cyclopropyl-6-fluoro-4-hydroxyquinolin-2(1*H*)-one (56)



56

Methyl 4-bromo-2-(*N*-cyclopropylacetamido)-5-fluorobenzoate (1.00 g, 3.03 mmol) was dissolved in dry THF (10 mL) and the reaction mixture was cooled to –78 °C. LiHMDS (1.0 M solution in THF, 6.06 mL, 6.06 mmol) was added dropwise and the reaction mixture was stirred at –78 °C for 1 hour. The reaction mixture was quenched with water at 0 °C, acidified with HCl and extracted with EtOAc. The organic layer was washed with water and concentrated to afford the desired product (0.62 g, 69% yield). LCMS (m/z): 299.9 $[M+2]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.67 (s, 1H), 8.05 (d, J = 6.0 Hz, 1H), 7.67 (d, J = 9.0 Hz, 1H), 5.84 (s, 1H), 2.85 (ddd, J = 10.8, 7.0, 4.1 Hz, 1H), 1.24 (q, J = 7.2 Hz, 2H), 0.76–0.66 (m, 2H).

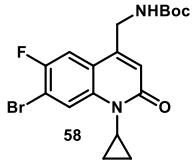
7-Bromo-1-cyclopropyl-6-fluoro-2-oxo-1,2-dihydroquinolin-4-yl trifluoromethane sulfonate (57)



57

7-Bromo-1-cyclopropyl-6-fluoro-4-hydroxyquinolin-2(1*H*)-one (0.62 g, 2.08 mmol) and TEA (0.63 g, 6.25 mmol) were dissolved in DMF (8 mL) and the reaction mixture was stirred at 0 °C for 30 minutes. A solution of *N*-phenyl-bis(trifluoromethanesulfonimide) (0.59 g, 1.66 mmol) in DMF (1 mL) was added dropwise and the reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford the product (0.57 g, 64% yield). LCMS (m/z): 432.4 $[M+2]^+$.

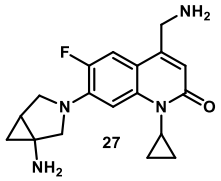
***tert*-Butyl ((7-bromo-1-cyclopropyl-6-fluoro-2-oxo-1,2-dihydroquinolin-4-yl)methyl)carbamate (58)**



58

A mixture of 7-Bromo-1-cyclopropyl-6-fluoro-2-oxo-1,2-dihydroquinolin-4-yl trifluoromethane sulfonate (0.47 g, 1.09 mmol), potassium (((*tert*-butoxycarbonyl)amino)methyl)trifluoroborate (0.52 g, 2.19 mmol) and Na_2CO_3 (0.35 g, 3.28 mmol) in toluene (10 mL) and water (0.5 mL) was degassed with nitrogen for 5 minutes. $PdCl_2$(dppf) (0.08 g, 0.11 mmol) was added and the reaction mixture was stirred at 110 °C for 5 hours. The reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford the crude residue. The crude residue was purified by silica gel column chromatography (45-60% EtOAc/hexanes) to afford the desired product (0.24 g, 53% yield). LCMS (m/z): 413.5 $[M+2]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 6.1 Hz, 1H), 7.45 (d, *J* = 9.1 Hz, 1H), 6.67 (s, 1H), 4.93 (s, 1H), 4.47 (d, *J* = 6.0 Hz, 2H), 2.97–2.88 (m, 1H), 1.48 (d, *J* = 11.7 Hz, 9H), 1.44 –1.37 (m, 2H), 0.91 (q, *J* = 7.3 Hz, 2H).

7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoroquinolin-2(1*H*)-one hydrochloride (27)

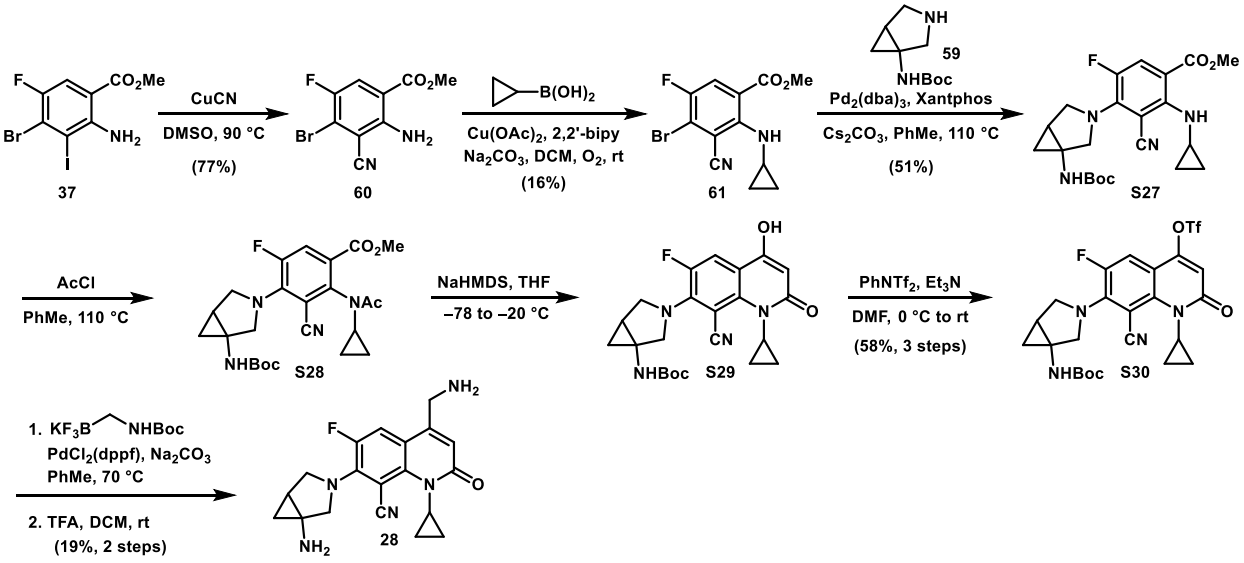


27

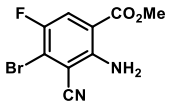
tert-Butyl ((7-bromo-1-cyclopropyl-6-fluoro-2-oxo-1,2-dihydroquinolin-4-yl)methyl)carbamate (0.210 g, 0.51 mmol), *tert*-butyl (3-azabicyclo[3.1.0]hexan-1-yl)carbamate

(0.20 g, 1.02 mmol), cesium carbonate (0.50 g, 1.53 mmol), Pd_2dba_3 (0.09 g, 0.10 mmol) and BINAP (0.13 g, 0.20 mmol) were combined in a sealed tube, which was evacuated and back-filled with nitrogen. Previously degassed toluene (5 mL) was added and the reaction mixture was stirred at 110 °C for 5 hours in a sealed tube. The reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford a crude residue. The crude product was purified by silica gel column chromatography (45-60% EtOAc/Hexane) to afford the desired protected amine. The Boc protected material was dissolved in DCM (5 mL) and cooled to 0 °C. HCl (4M in dioxane, 1.0 mL) was added and the reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was concentrated to afford a crude product, which was triturated with diethyl ether to afford the title compound (0.140 g, 69% yield, 2 steps). HRMS (ESI) calc'd for $C_{18}H_{22}FN_4O$ $[M+H]^+$ 329.1772, found 329.1771. ^{1}H NMR (400 MHz, CD_3OD) δ 7.53 (d, *J* = 14.8 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 6.47 (s, 1H), 4.38 (s, 2H), 4.26 (dd, *J* = 9.5, 2.8 Hz, 1H), 3.87 (d, *J* = 8.1 Hz, 1H), 3.76–3.67 (m, 2H), 3.04–2.97 (m, 1H), 2.18 (dt, *J* = 8.9, 4.4 Hz, 1H), 1.48–1.35 (m, 3H), 1.18–1.10 (m, 1H), 0.86 (dd, *J* = 8.9, 7.4 Hz, 2H).

Preparation of 7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-2-oxo-1,2-dihydroquinoline-8-carbonitrile trifluoroacetic acid (28)

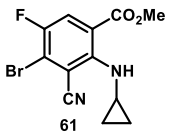


Methyl 2-amino-4-bromo-3-cyano-5-fluorobenzoate (60)



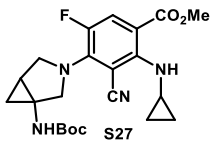
To methyl 2-amino-4-bromo-5-fluoro-3-iodobenzoate (5.00 g, 13.4 mmol) in DMSO (50 mL) was added CuCN (2.40 g, 26.7 mmol) followed by stirring at 90 °C overnight. The reaction mixture was diluted with cold water, filtered through Celite and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated. The crude residue was purified by silica gel column chromatography (2.5% EtOAc/hexanes) to afford the desired product (2.80 g, 77% yield). LCMS (m/z): 273.1 $[M+H]^+$.

Methyl 4-bromo-3-cyano-2-(cyclopropyl amino)-5-fluorobenzoate (61)



To methyl 2-amino-4-bromo-3-cyano-5-fluorobenzoate (2.80 g, 10.2 mmol) in DCM (60 mL) was added cyclopropyl boronic acid (1.80 g, 20.4 mmol), 2,2'-bipyridine (3.20 g, 20.4 mmol), $Cu(OAc)_2$ (3.70 g, 20.4 mmol) and Na_2CO_3 (3.2 g, 30.7 mmol). Oxygen (gas) was purged continuously and the reaction mixture was stirred at room temperature for 3 days. The reaction mixture was filtered through celite, washed with DCM and the filtrate was concentrated. The crude residue was purified by silica gel column chromatography (0-5% EtOAc/hexanes) to afford the desired product (0.50 g, 16% yield). 1.5 g of starting material was recovered. LCMS (m/z): 313.1 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 8.36 (s, 1H), 7.98 (d, J = 9.1 Hz, 1H), 3.83 (d, J = 3.9 Hz, 3H), 3.06 (dd, J = 6.4, 3.5 Hz, 1H), 0.92–0.86 (m, 2H), 0.71–0.66 (m, 2H).

Methyl 4-(1-((*tert*-butoxycarbonyl)amino)-3-azabicyclo[3.1.0]hexan-3-yl)-3-cyano-2-(cyclopropylamino)-5-fluorobenzoate (S27)

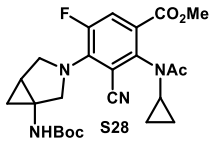


S27

A suspension of methyl 4-bromo-3-cyano-2-(cyclopropyl amino)-5-fluorobenzoate (280 mg, 0.89 mmol), Cs_2CO_3 (870 mg, 2.68 mmol), *tert*-butyl (azabicyclo[3.1.0]hexan-1-yl)carbamate (354 mg, 1.79 mmol) in toluene (6 mL) was degassed for 5 minutes. $Pd_2(dba)_3$ (163 mg, 0.18 mmol) and BINAP (222 mg, 0.36 mmol) were added and the reaction mixture was stirred at 110 °C for 15 hours. The reaction mixture was quenched with cold water and extracted with EtOAc. The combined organic layers were washed with brine, dried over sodium sulfate and concentrated to afford a crude residue, which was purified by silica gel column chromatography (0-10% EtOAc/hexanes) giving the desired product (210 mg, 51% yield). LCMS (m/z): 431.6 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, 1H), 7.61 (d, J = 15.2 Hz, 1H), 5.06 (s, 1H), 4.03–4.00 (m,

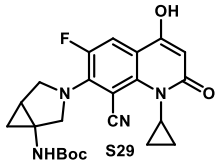
2H), 3.84–3.80 (m, 5H), 3.14–3.10 (m, 1H), 1.74–1.71 (m, 1H), 1.50 (s, 9H), 1.05–1.02 (m, 2H), 0.95–0.85 (m, 2H), 0.63–0.60 (m, 2H).

Methyl 4-(1-((*tert*-butoxycarbonyl)amino)-3-azabicyclo[3.1.0]hexan-3-yl)-3-cyano-2-(*N*-cyclopropylacetamido)-5-fluorobenzoate (S28)



Methyl 4-(1-((*tert*-butoxycarbonyl)amino)-3-azabicyclo[3.1.0]hexan-3-yl)-3-cyano-2-(cyclopropylamino)-5-fluorobenzoate (190 mg, 0.44 mmol) was dissolved in toluene (2 mL), acetyl chloride (0.06 mL, 0.88 mmol) was added and the reaction mixture was stirred at 110 °C for 4 hours. The reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with $NaHCO_3$, dried over sodium sulfate and concentrated to afford the desired product.

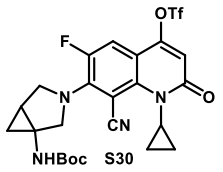
***tert*-Butyl (3-(8-cyano-1-cyclopropyl-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-7-yl)-3-azabicyclo[3.1.0]hexan-1-yl)carbamate (S29)**



NaHMDS (1M in THF, 0.31 mL, 0.31 mmol) was added dropwise to a solution of methyl 4-(1-((*tert*-butoxycarbonyl)amino)-3-azabicyclo[3.1.0]hexan-3-yl)-3-cyano-2-(*N*-cyclopropylacetamido)-5-fluorobenzoate (48.0 mg, 0.10 mmol) in THF (2 mL) at –78 °C. After 3 hours at this temperature, the reaction mixture was allowed to warm to –20 °C over the next 45 minutes. The reaction was quenched by dropwise addition of saturated aqueous NH_4Cl at –78 °C. Upon warming to rt, the mixture was diluted with water and extracted with EtOAc. The combined

organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. LCMS (m/z): 441.2 $[M+H]^+$.

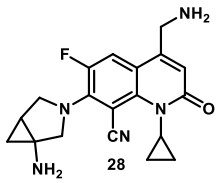
7-(1-((*tert*-Butoxycarbonyl)amino)-3-azabicyclo[3.1.0]hexan-3-yl)-8-cyano-1-cyclopropyl-6-fluoro-2-oxo-1,2-dihydroquinolin-4-yl trifluoromethanesulfonate (S30)



S30

tert-Butyl (3-(8-cyano-1-cyclopropyl-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinolin-7-yl)-3-azabicyclo[3.1.0]hexan-1-yl)carbamate (92.0 mg, 0.21 mmol) and Et_3N (63.0 mg, 0.63 mmol) were dissolved in DMF (1 mL) and the reaction mixture was stirred at 0 °C for 10 minutes. A solution of *N*-phenyl-bis(trifluoromethanesulfonimide) (89.0 mg, 0.25 mmol) in DMF (1 mL) was added dropwise and the reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated. The crude residue was purified by silica gel column chromatography (0-100% EtOAc/hexanes) to afford the desired product (69.0 mg, 58% yield, 3 steps). LCMS (m/z): 573.1 $[M+H]^+$.

7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-2-oxo-1,2-dihydroquinoline-8-carbonitrile trifluoroacetic acid (28)

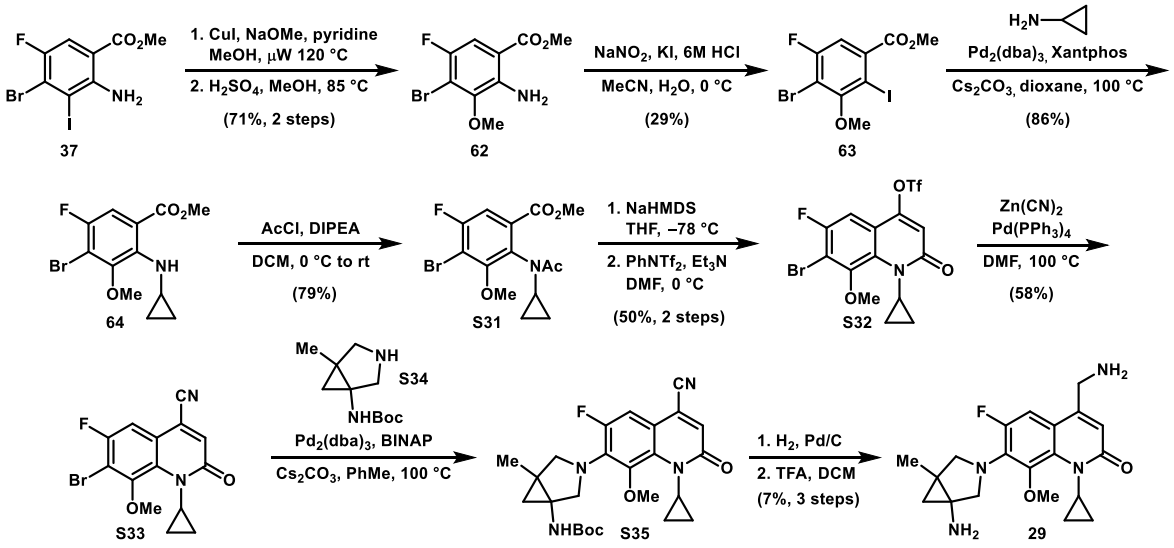


28

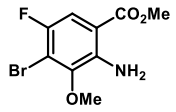
A mixture of 7-(1-((*tert*-Butoxycarbonyl)amino)-3-azabicyclo[3.1.0]hexan-3-yl)-8-cyano-1-cyclopropyl-6-fluoro-2-oxo-1,2-dihydroquinolin-4-yl trifluoromethanesulfonate (69.0 mg, 0.12 mmol), potassium (((*tert*-butoxycarbonyl)amino)methyl)trifluoroborate (57.0 mg, 0.24 mmol) and

Na_2CO_3 (38.0 mg, 0.36 mmol) in toluene (1.2 mL) and water (0.05 mL) was degassed with nitrogen for 5 minutes. $PdCl_2$(dppf) (20.0 mg, 0.02 mmol) was added and the reaction mixture was stirred at 70 °C overnight. The reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford the crude residue. The crude residue was purified by silica gel column chromatography (0-100% EtOAc/heptane) to afford the protected amine. The Boc protected material was dissolved in DCM (1 mL) and TFA (0.5 mL) was added. The reaction mixture was stirred at room temperature for 20 minutes. The reaction mixture was concentrated to afford a crude product, which was purified by prep-HPLC to afford the title compound (14.0 mg, 19% yield, 2 steps). HRMS (ESI) calc'd for $C_{19}H_{21}FN_5O$ $[M+H]^+$ 354.1725, found 354.1722. ^1H NMR (400 MHz, CD_3OD) δ 7.76 (d, *J* = 14.1 Hz, 1H), 6.60 (s, 1H), 4.36 (d, *J* = 0.9 Hz, 2H), 4.16 (d, *J* = 10.1 Hz, 1H), 4.04 (d, *J* = 9.8 Hz, 2H), 3.90 (d, *J* = 10.5 Hz, 1H), 3.55–3.47 (m, 1H), 2.11–2.04 (m, 1H), 1.48–1.39 (m, 2H), 1.37–1.30 (m, 1H), 1.27–1.20 (m, 1H), 0.78–0.70 (m, 2H).

Preparation of 7-(1-Amino-5-methyl-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methoxyquinolin-2(1*H*)-one trifluoroacetic acid (29)



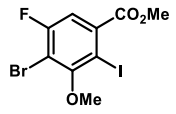
Methyl 2-amino-4-bromo-5-fluoro-3-methoxybenzoate (62)



62

Methyl 2-amino-4-bromo-5-fluoro-3-iodobenzoate (1.00 g, 2.65 mmol) was dissolved in methanol (15 mL) and CuI (0.10 g, 0.53 mmol) was added, followed by stirring for 5 minutes. NaOMe (1.43 g, 26.5 mmol) and pyridine (2 mL) were added and the reaction mixture was stirred at 110 °C for 1 hour under microwave irradiation. The reaction mixture was quenched with cold water, acidified to pH 2 by the addition of 1N HCl and the suspension was extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford the corresponding acid which was triturated with pentane:diethyl ether (1:1). The acid was dissolved in methanol (120 mL), concentrated H_2SO_4 (12 mL) was added and the reaction mixture was stirred at 85 °C for 24 hours. The reaction mixture was quenched with ice-water and extracted with DCM. The organic layer was washed with brine, dried over sodium sulfate and concentrated to give a crude residue, which was purified by column chromatography (0-20% EtOAc/hexanes), affording the desired product (4.50 g, 71% yield). LCMS (m/z): 279.9 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41 (d, J = 9.7 Hz, 1H), 6.69 (s, 2H), 3.82 (s, 3H), 3.75 (s, 3H).

Methyl 4-bromo-5-fluoro-2-iodo-3-methoxybenzoate (63)

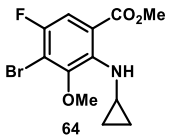


63

Methyl 2-amino-4-bromo-5-fluoro-3-methoxybenzoate (4.50 g, 16.2 mmol) was dissolved in acetonitrile (27 mL), 6M HCl (58.5 mL) was added and the reaction mixture was stirred at room temperature for 10 minutes. The reaction mixture was cooled to 0 °C and $NaNO_2$ (1.17 g, 17.0 mmol) in water (18 mL) was added followed by stirring at 0 °C for 1 hour. KI (5.37 g, 32.4 mmol)

in water (36 mL) was added dropwise and the reaction mixture was stirred at 0 °C for 30 minutes. The reaction mixture was quenched with cold water and extracted with EtOAc. The organic layer was washed with saturated aqueous sodium thiosulfate solution, dried over sodium sulfate and concentrated to afford a crude residue. The crude residue was purified by silica gel column chromatography (10% EtOAc/hexanes) to afford the desired product (1.80 g, 29% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (d, J = 8.5 Hz, 1H), 3.87 (s, 3H), 3.83 (s, 3H).

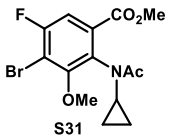
Methyl 4-bromo-2-(cyclopropylamino)-5-fluoro-3-methoxybenzoate (64)



64

Methyl 4-bromo-5-fluoro-2-iodo-3-methoxybenzoate (6.00 g, 15.4 mmol) and Cs_2CO_3 (15.0 g, 46.3 mmol) were suspended in dioxane (60 mL) and degassed with nitrogen for 10 minutes. Pd_2dba_3 (0.70 g, 0.70 mmol), xantphos (1.30 g, 2.30 mmol) and cyclopropyl amine (2.20 mL, 30.8 mmol) were added and the reaction mixture was stirred at 100 °C for 16 hours. The reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford a crude residue, which was purified by silica gel column chromatography (0-10% EtOAc/hexanes), providing the desired product (4.20 g, 86% yield). LCMS (m/z): 320.13 $[M+2]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J = 9.2 Hz, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 3.03–3.00 (m, 1H), 0.74–0.69 (m, 2H), 0.52–0.48 (m, 2H).

Methyl 4-bromo-2-(*N*-cyclopropylacetamido)-5-fluoro-3-methoxybenzoate (S31)

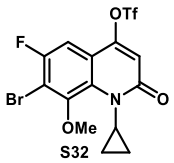


S31

Methyl 4-bromo-2-(cyclopropylamino)-5-fluoro-3-methoxybenzoate (2.90 g, 9.10 mmol) was dissolved in DCM (45 mL) and cooled to 0 °C. DIPEA (7.92 mL, 45.5 mmol) and acetyl chloride

(6.50 mL, 91.1 mmol) were added and the reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was quenched with saturated $NaHCO_3$ solution and extracted with DCM. The combined organic layers were washed with brine, dried over sodium sulfate and concentrated to afford a crude residue, which was purified by silica gel column chromatography (25-40% EtOAc/hexanes) providing the desired product (2.60 g, 79% yield). LCMS (m/z): 362.4 $[M+2]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, J = 6.8 Hz, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.19–3.15 (m, 1H), 2.42 (s, 3H), 0.99–0.97 (m, 1H), 0.90–0.79 (m, 2H), 0.68–0.65 (m, 1H).

7-Bromo-1-cyclopropyl-6-fluoro-8-methoxy-2-oxo-1,2-dihydroquinolin-4-yl trifluoromethanesulfonate (S32)

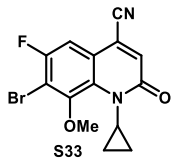


S32

Methyl 4-bromo-2-(*N*-cyclopropylacetamido)-5-fluoro-3-methoxybenzoate (2.30 g, 6.30 mmol) was dissolved in THF (25 mL) and cooled to –78 °C. NaHMDS (1.0 M in THF, 19.2 mL, 19.2 mmol) was added dropwise and the reaction mixture was stirred at –78 °C for 1 hour. The reaction mixture was quenched with ice-water, acidified to pH 2 by the addition of 1N HCl and extracted with 5% MeOH in DCM solution. The combined organic layer was dried over sodium sulfate and concentrated to afford the desired cyclized product. The quinolone was dissolved in DMF (20 mL) and cooled to 0 °C. TEA (2.56 mL, 18.3 mmol), $PhNTf_2$ (2.61 g, 7.31 mmol) were added and the reaction mixture was stirred at room temperature for 3 hours. The reaction mixture was quenched with cold water and extracted with EtOAc. The organic layer was washed with cold water, brine, dried over sodium sulfate and concentrated to give a crude residue, which was purified by silica gel column chromatography (0-15% EtOAc/hexanes) providing the desired product (1.50 g, 50%

yield, 2 steps). LCMS (m/z): 462.3 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (d, J = 8.4 Hz, 1H), 6.90 (s, 1H), 3.75 (s, 3H), 3.40 –3.38 (m, 1H), 1.16–1.10 (m, 2H), 0.54–0.50 (m, 2H).

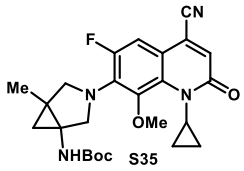
7-Bromo-1-cyclopropyl-6-fluoro-8-methoxy-2-oxo-1,2-dihydroquinoline-4-carbonitrile (S33)



S33

A solution of 7-bromo-1-cyclopropyl-6-fluoro-8-methoxy-2-oxo-1,2-dihydroquinolin-4-yl trifluoromethanesulfonate (0.80 g, 1.74 mmol) and $Zn(CN)_2$ (0.22 g, 1.91 mmol) in DMF (12 mL) was degassed with nitrogen for 10 minutes. $Pd(PPh_3)_4$ (0.10 g, 0.09 mmol) was added and the reaction mixture was stirred at 100 °C for 16 hours. The reaction mixture was quenched with cold water and extracted with EtOAc. The combined organic layers were washed with brine, dried over sodium sulfate and concentrated to give a crude residue, which was purified by silica gel column chromatography (0-15% EtOAc/hexanes), affording the desired product (0.58 g, 58% yield). LCMS (m/z): 339.3 $[M+2]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 (d, J = 7.6 Hz, 1H), 7.10 (s, 1H), 3.80 (s, 3H), 3.46–3.40 (m, 1H), 1.31–1.26 (m, 2H), 0.65–0.60 (m, 2H).

***tert*-Butyl (3-(4-cyano-1-cyclopropyl-6-fluoro-8-methoxy-2-oxo-1,2-dihydroquinolin-7-yl)-5-methyl-3-azabicyclo[3.1.0]hexan-1-yl)carbamate (S35)**

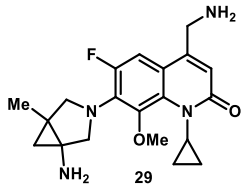


S35

A vial containing 7-bromo-1-cyclopropyl-6-fluoro-8-methoxy-2-oxo-1,2-dihydroquinoline-4-carbonitrile (100 mg, 0.30 mmol), *tert*-butyl (5-methyl-3-azabicyclo[3.1.0]hexan-1-yl)carbamate (94.0 mg, 0.45 mmol), BINAP (74.0 mg, 0.12 mmol), tris(dibenzylideneacetone)dipalladium(0)

(54.3 mg, 0.06 mmol) and cesium carbonate (290 mg, 0.89 mmol) was sealed, evacuated and back-filled with N_2. Toluene (3 mL) was added and the mixture was heated to 100 °C for 6 hours. Upon cooling to room temperature the mixture was filtered and volatiles were evaporated under reduced pressure. Silica gel flash chromatography (0-100% EtOAc/heptane) provided the desired product (200 mg). LCMS (m/z): 469.4 $[M+H]^+$.

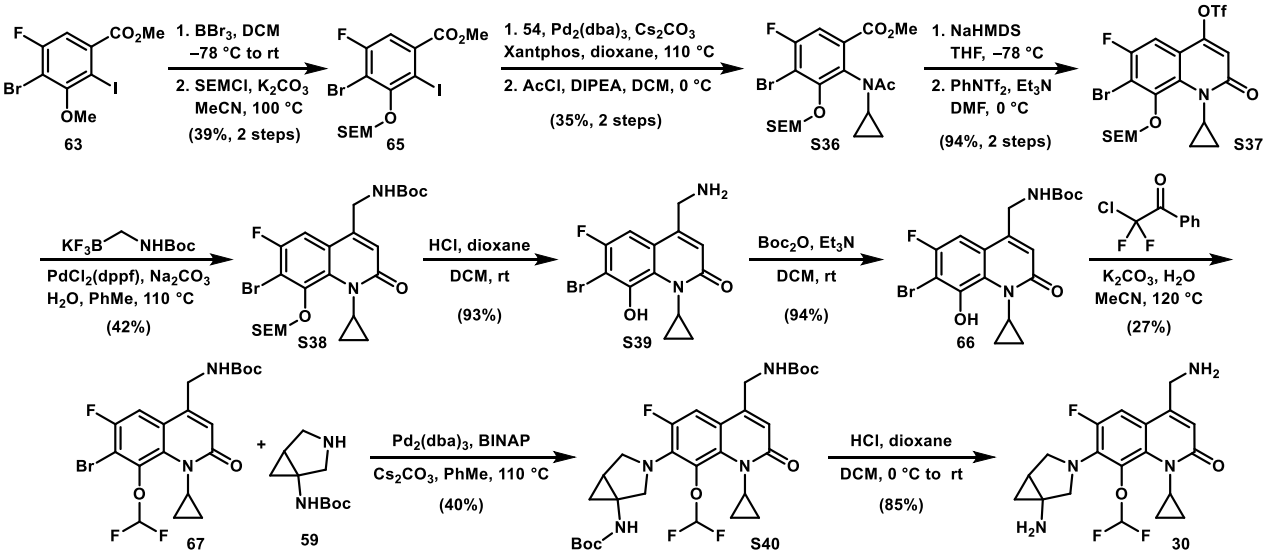
7-(1-Amino-5-methyl-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-8-methoxyquinolin-2(1*H*)-one trifluoroacetic acid (29)



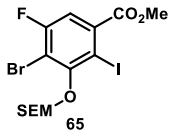
29

A mixture of *tert*-butyl (3-(4-cyano-1-cyclopropyl-6-fluoro-8-methoxy-2-oxo-1,2-dihydroquinolin-7-yl)-5-methyl-3-azabicyclo[3.1.0]hexan-1-yl)carbamate (200 mg, 0.43 mmol) in 2M NH_3 in MeOH (8.5 mL) was treated with Pd/C (10%, 50% in water, 136 mg, 0.06 mmol). The flask was partially evacuated and backfilled with H_2 several times, then stirred under a balloon of H_2 for 1 hour. The reaction mixture was then filtered and volatiles were evaporated under reduced pressure. The crude product was dissolved in a mixture of DCM (2 mL) and TFA (2 mL) and stirred at room temperature for 45 minutes. Volatiles were evaporated under reduced pressure. The residue was concentrated twice from MeOH-toluene. Preparative prep-HPLC provided the product (14.9 mg, 7% yield, 3 steps) as a dark yellow powder. HRMS (ESI) calc'd for $C_{20}H_{26}FN_4O_2$ $[M+H]^+$ 373.2034, found 373.2030. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72 (s, 3H), 8.37 (s, 3H), 7.37 (d, J = 13.9 Hz, 1H), 6.45 (s, 1H), 4.25–4.17 (m, 2H), 3.80 (d, J = 9.4 Hz, 1H), 3.71 (d, J = 9.3 Hz, 1H), 3.64 (d, J = 9.8 Hz, 1H), 3.48 (s, 3H), 3.42 (d, J = 8.8 Hz, 1H), 3.36–3.29 (m, 1H), 1.36 (s, 3H), 1.10–0.98 (m, 4H), 0.42–0.31 (m, 2H).

Synthesis of 7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-8-(difluoromethoxy)-6-fluoroquinolin-2(1*H*)-one hydrochloride (30)



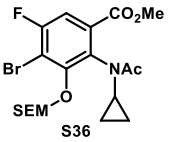
Methyl 4-bromo-5-fluoro-2-iodo-3-((2- (trimethylsilyl)ethoxy)methoxy) benzoate (65)



Methyl 4-bromo-5-fluoro-2-iodo-3-methoxybenzoate (1.80 g, 4.60 mmol) was dissolved in DCM (20 mL) and cooled to –78 °C. BBr_3 (1M in DCM, 36.8 mL, 36.8 mmol) was added and the reaction mixture was stirred at room temperature for 18 hours. The reaction mixture was quenched with ice-water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford the crude residue. The crude residue was purified by silica gel column chromatography (0-10% EtOAc/hexanes) to afford the demethylated product. The corresponding phenol was dissolved in acetonitrile (6 mL). K_2CO_3 (0.73 g, 5.30 mmol) and SEM-Cl (0.42 g, 2.50 mmol) were added and the reaction mixture was stirred at 100 °C for 1 hour. The reaction mixture was filtered, washed with EtOAc, and concentrated to afford the crude desired product (0.90 g, 39% yield, 2 steps). The crude product was used in the next step without

further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.6 Hz, 1H), 5.30 (s, 2H), 4.11–4.01 (m, 2H), 3.96 (s, 3H), 1.11–1.00 (m, 2H), 0.06 (s, 9H).

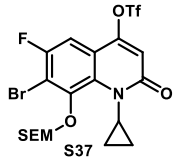
Methyl 4-bromo-2-(*N*-cyclopropylacetamido)-5-fluoro-3-((2-(trimethylsilyl)ethoxy)methoxy)benzoate (S36)



S36

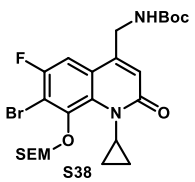
Methyl 4-bromo-5-fluoro-2-iodo-3-((2-(trimethylsilyl)ethoxy)methoxy) benzoate (0.90 g, 1.78 mmol), Cs_2CO_3 (0.86 g, 2.65 mmol) and xantphos (0.15 g, 0.27 mmol) were suspended in dioxane (28 mL). $Pd_2(dba)_3$ (0.08 g, 0.09 mmol) and cyclopropyl amine (0.20 g, 3.50 mmol) were added and the reaction mixture was stirred at 110 °C for 8 hours. The reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated. The crude residue was purified by silica gel column chromatography (0-5% EtOAc/hexanes) affording the cyclopropyl aniline. The aniline was dissolved in DCM (10 mL) and cooled to 0 °C. DIPEA (0.44 g, 3.46 mmol) and acetyl chloride (0.27 g, 3.46 mmol) were added and the reaction mixture was stirred at room temperature for 4 hours. The reaction mixture was quenched with water and extracted with DCM. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford the crude residue. The crude residue was purified by silica gel column chromatography (20-30% EtOAc/hexanes) to afford the desired product (0.30 g, 35% yield, 2 steps). LCMS (m/z): 346.2 $[M–SEM+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, *J* = 8.6 Hz, 1H), 5.10 (d, *J* = 5.0 Hz, 1H), 5.02 (d, *J* = 5.0 Hz, 1H), 3.90 (d, *J* = 1.9 Hz, 2H), 3.85 (s, 3H), 3.22–3.19 (m, 1H), 2.41 (s, 3H), 1.28 (d, *J* = 2.8 Hz, 2H), 1.03 (d, *J* = 3.3 Hz, 2H), 0.84–0.81 (m, 1H), 0.68–0.64 (m, 1H), 0.06 (s, 9H).

7-Bromo-1-cyclopropyl-6-fluoro-2-oxo-8-((2-(trimethylsilyl)ethoxy)methoxy)-1,2-dihydroquinolin-4-yl trifluoromethanesulfonate (S37)



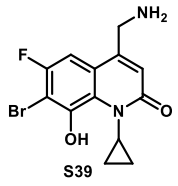
Methyl 4-bromo-2-(*N*-cyclopropylacetamido)-5-fluoro-3-((2-(trimethylsilyl)ethoxy)methoxy) benzoate (0.30 g, 0.63 mmol) was dissolved in THF (10 mL) and cooled to –78 °C. NaHMDS (1.0 M in THF, 1.90 mL, 1.90 mmol) was added dropwise and the reaction mixture was stirred at –78 °C for 1 hour. The reaction mixture was quenched with ice-water, acidified to pH 2 by the addition of 1N HCl and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford the desired cyclized product. The crude heterocycle was dissolved in DMF (5 mL) and cooled to 0 °C. Et_3N (0.10 g, 1.00 mmol) and $PhNTf_2$ (0.19 g, 0.50 mmol) were added and the reaction mixture was stirred at room temperature for 1.5 hours. The reaction mixture was quenched with cold water and extracted with EtOAc. The organic layer was washed with cold water, brine, dried over sodium sulfate and concentrated to afford a crude residue. The crude residue was purified by silica gel column chromatography (0-20% EtOAc/hexanes) to afford the desired product (0.19 g, 94% yield, 2 steps). LCMS (m/z): 578.4 $[M+2]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.6 Hz, 1H), 6.72 (s, 1H), 5.12 (s, 2H), 3.87–3.82 (m, 2H), 3.45 (s, 1H), 1.28–1.26 (m, 2H), 0.97–0.91 (m, 2H), 0.64 (s, 2H), 0.02 (s, 9H).

***tert*-Butyl ((7-bromo-1-cyclopropyl-6-fluoro-2-oxo-8-((2-(trimethylsilyl)ethoxy)methoxy)-1,2-dihydroquinolin-4-yl)methyl)carbamate (S38)**



7-bromo-1-cyclopropyl-6-fluoro-2-oxo-8-((2-(trimethylsilyl)ethoxy)methoxy)-1,2-dihydroquinolin-4-yl trifluoromethanesulfonate (0.59 g, 10.2 mmol), potassium (((*tert*-butoxycarbonyl)amino)methyl)trifluoroborate (0.49 g, 20.5 mmol) and Na_2CO_3 (0.27 g, 2.56 mmol) were suspended in toluene (18 mL) and water (0.1 mL) and the reaction mixture was degassed with nitrogen for 5 minutes. $PdCl_2$(dppf) (0.04 g, 0.51 mmol) was added and the reaction mixture was stirred at 110 °C for 9 hours. The reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford a crude residue, which was purified silica gel column chromatography (20-25% EtOAc/hexanes), providing the desired product (0.24 g, 42% yield). LCMS (m/z): 559.6 $[M+2]^+$.

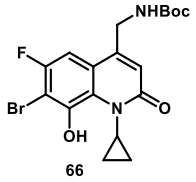
4-(Aminomethyl)-7-bromo-1-cyclopropyl-6-fluoro-8-hydroxyquinolin-2(1*H*)-one (S39)



S39

tert-Butyl ((7-bromo-1-cyclopropyl-6-fluoro-2-oxo-8-((2-(trimethylsilyl)ethoxy) methoxy)-1,2-dihydroquinolin-4-yl)methyl)carbamate (0.12 g, 9.55 mmol) was dissolved in DCM (2 mL) at room temperature. HCl (4M in dioxane, 1 mL) was added and the reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was concentrated to afford a crude residue, which was purified by trituration with pentane, affording the desired product (65.0 mg, 93% yield). LCMS (m/z): 329.4 $[M+2]^+$.

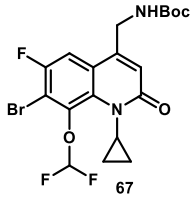
***tert*-Butyl ((7-bromo-1-cyclopropyl-6-fluoro-8-hydroxy-2-oxo-1,2-dihydro quinolin-4-yl)methyl)carbamate (66)**



66

4-(Aminomethyl)-7-bromo-1-cyclopropyl-6-fluoro-8-hydroxyquinolin-2(1*H*)-one (65.0 mg, 0.20 mmol) was dissolved in DCM (2 mL) and the reaction mixture was cooled to 0 °C. Et_3N (0.12 g, 1.22 mmol) and $(Boc)_2O$ (59.0 mg, 0.27 mmol) were added and the reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was concentrated to afford a crude residue which was purified by trituration with pentane, affording the desired product (80.0 mg, 94% yield). LCMS (m/z): 429.5 $[M+2]^+$.

***tert*-Butyl ((7-bromo-1-cyclopropyl-8-(difluoromethoxy)-6-fluoro-2-oxo-1,2-dihydroquinolin-4-yl)methyl)carbamate (67)**

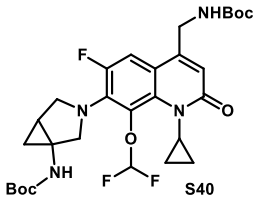


67

tert-Butyl ((7-bromo-1-cyclopropyl-6-fluoro-8-hydroxy-2-oxo-1,2-dihydro quinolin-4-yl)methyl)carbamate (100 mg, 0.23 mmol) was dissolved in acetonitrile at room temperature. K_2CO_3 (1.29 g, 9.36 mmol) in water (3 mL) and 2-chloro-2,2-difluoro-1-phenylethan-1-one (178 mg, 0.94 mmol) were added and the reaction mixture was stirred at 120 °C for 24 hours. The reaction mixture was quenched with cool water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford a crude residue, which

was purified by silica gel column chromatography (15-20% EtOAc/Hexane), providing the desired product (0.03 g, 27% yield). LCMS (m/z): 479.1 $[M+2]^+$.

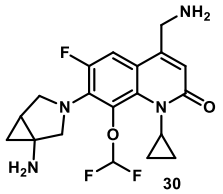
***tert*-Butyl ((7-(1-((*tert*-butoxycarbonyl)amino)-3-azabicyclo[3.1.0]hexan-3-yl)-1-cyclopropyl-8-(difluoromethoxy)-6-fluoro-2-oxo-1,2-dihydroquinolin-4-yl)methyl) carbamate (S40)**



S40

tert-Butyl ((7-bromo-1-cyclopropyl-8-(difluoromethoxy)-6-fluoro-2-oxo-1,2-dihydroquinolin-4-yl)methyl)carbamate (30.0 mg, 0.06 mmol), *tert*-butyl (3-azabicyclo[3.1.0]hexan-1-yl)carbamate (25.0 mg, 0.13 mmol), cesium carbonate (61.0 mg, 0.19 mmol), $Pd_2(dba)_3$ (11.0 mg, 0.01 mmol) and BINAP (16.0 mg, 0.03 mmol) were combined in a sealed tube which was evacuated and back-filled with nitrogen. Toluene (0.6 mL) was added and the reaction mixture was stirred at 110 °C for 12 hours. The reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford a crude residue, which was purified by prep TLC (50% EtOAc/hexanes), affording the desired product (15.0 mg, 40% yield). LCMS (m/z): 595.9 $[M+H]^+$.

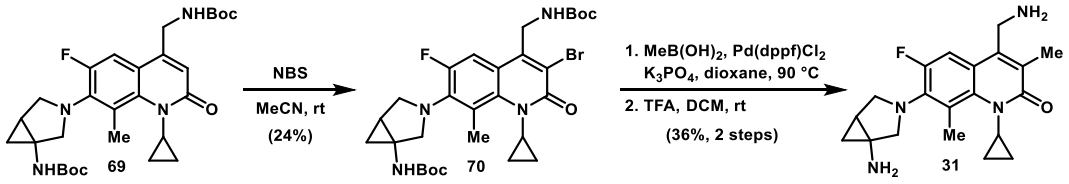
7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-8-(difluoromethoxy)-6-fluoroquinolin-2(1*H*)-one hydrochloride (30)



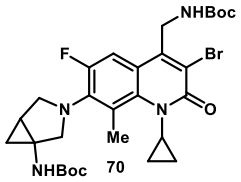
30

tert-Butyl ((7-(1-((*tert*-butoxycarbonyl)amino)-3-azabicyclo[3.1.0]hexan-3-yl)-1-cyclopropyl-8-(difluoromethoxy)-6-fluoro-2-oxo-1,2-dihydroquinolin-4-yl)methyl) carbamate (15.0 mg, 0.03 mmol) was dissolved in DCM (1 mL) and cooled at 0 °C. HCl (4M in dioxane, 0.5 mL) was added and the reaction mixture was stirred at room temperature for 3 hours. The reaction mixture was concentrated to afford the crude product, which was triturated with diethyl ether, providing the desired product (10.0 mg, 85% yield, purity = 82%). HRMS (ESI) calc'd for $C_{19}H_{22}F_3N_4O_2$ $[M+H]^+$ 395.1689, found 395.1682. ^{1}H NMR (400 MHz, CD_3OD) δ 7.53 (d, *J* = 13.6 Hz, 1H), 6.57 (s, 1H), 4.38 (s, 2H), 4.00 (d, *J* = 9.6 Hz, 1H), 3.88–3.80 (m, 2H), 3.73 (d, *J* = 10.1 Hz, 1H), 3.47 (s, 1H), 2.03 (d, *J* = 4.2 Hz, 1H), 1.35–1.27 (m, 2H), 1.23–1.18 (m, 2H), 0.57 (d, *J* = 2.9 Hz, 2H).

Preparation of 7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-3,8-dimethylquinolin-2(1*H*)-one trifluoroacetic acid (31)



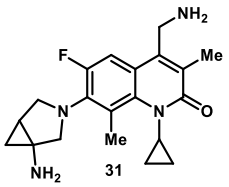
***tert*-Butyl ((3-bromo-7-(1-((*tert*-butoxycarbonyl)amino)-3-azabicyclo[3.1.0]hexan-3-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl)methyl)carbamate (70)**



A mixture of *tert*-butyl ((7-(1-((*tert*-butoxycarbonyl)amino)-3-azabicyclo[3.1.0]hexan-3-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl)methyl)carbamate (100 mg, 0.18 mmol) in MeCN (1 mL) was treated with *N*-bromosuccinimide (49.0 mg, 0.24 mmol). After stirring 30 minutes at rt, the reaction was concentrated and the crude material was purified by silica

gel flash chromatography (0-100% EtOAc/heptane) to provide the desired product (27.0 mg, 24% yield) as a yellow oil. LCMS (m/z): 623.4 $[M+2]^+$.

7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-3,8-dimethylquinolin-2(1*H*)-one trifluoroacetic acid (31)

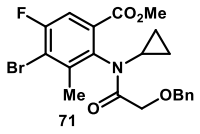


31

A solution of *tert*-butyl ((3-bromo-7-(1-((*tert*-butoxycarbonyl)amino)-3-azabicyclo[3.1.0]hexan-3-yl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl)methyl)carbamate (27.0 mg, 0.04 mmol), methylboronic acid (13.0 mg, 0.22 mmol), $PdCl_2$(dppf) CH_2Cl_2 adduct (7.10 mg, 8.69 µmol) and K_3PO_4 (18.4 mg, 0.09 mmol) in dioxane (0.9 mL) was stirred at 90 °C for 18 hours. Upon cooling to rt the mixture was diluted with water and extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated to give a dark brown oil. To a mixture of the protected bis-amine in DCM (0.5 mL) was added TFA (0.5 mL), followed by stirring at rt for 1 hour. The solution was concentrated and the crude material was purified by prep-HPLC, affording the desired product (9.3 mg, 36% yield, 2 steps) as an off-white powder. LCMS (m/z): 357.4 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD) δ 7.47 (d, *J* = 13.6 Hz, 1H), 4.38 (s, 2H), 3.83 (d, *J* = 8.8 Hz, 1H), 3.77 (dd, *J* = 9.4, 2.8 Hz, 1H), 3.66 (d, *J* = 8.9 Hz, 1H), 3.62–3.53 (m, 1H), 3.37 (d, *J* = 9.5 Hz, 1H), 2.56 (s, 3H), 2.30 (s, 3H), 2.03–1.96 (m, 1H), 1.38–1.33 (m, 1H), 1.32–1.25 (m, 1H), 1.25–1.18 (m, 2H), 0.45–0.38 (m, 2H).

Preparation of 7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-3-hydroxy-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (32)

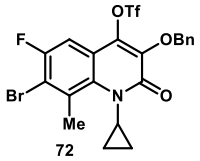
Methyl 2-(2-(benzyloxy)-*N*-cyclopropylacetamido)-4-bromo-5-fluoro-3-methylbenzoate (71)



71

Benzyloxyacetyl chloride (0.39 mL, 2.48 mmol) was added dropwise to a mixture of methyl 2-(*N*-cyclopropylacetamido)-4-bromo-5-fluoro-3-methylbenzoate (150 mg, 0.50 mmol) and pyridine (0.20 mL, 2.48 mmol) in DCM (3 mL) at 0 °C. The solution was stirred at rt for 3 hours. The mixture was diluted with DCM and washed with saturated aqueous NH_4Cl, saturated $NaHCO_3$, and brine, then dried over $MgSO_4$ and concentrated under reduced pressure. Purification by silica gel column chromatography (0-60% EtOAc/heptane) provided the desired product as a yellow oil (209 mg, 93% yield). LCMS (m/z): 452.2 $[M+2]^+$.

3-(Benzyloxy)-7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl trifluoromethanesulfonate (72)

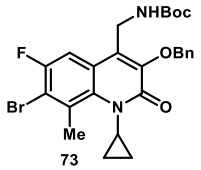


72

Methyl 2-(2-(benzyloxy)-*N*-cyclopropylacetamido)-4-bromo-5-fluoro-3-methylbenzoate (113 mg, 0.25 mmol) was dissolved in THF (2.5 mL) and cooled to –78 °C. NaHMDS (1M in THF, 0.75 mL, 0.75 mmol) was added dropwise and the reaction mixture was stirred at –78 °C for 30 minutes. The reaction mixture was quenched with ice-water, acidified to pH 2 by the addition of 1N HCl and extracted with EtOAc. The organic layer was washed with brine, dried over sodium

sulfate and concentrated to afford the desired heterocycle. The crude material was dissolved in DMF (1.25 mL) and cooled to 0 °C. Et_3N (76.0 mg, 0.75 mmol) and $PhNTf_2$ (107 mg, 0.30 mmol) were added and the reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was quenched with cold water and extracted with EtOAc. The organic layer was washed with cold water, brine, dried over sodium sulfate and concentrated to afford a crude residue, which was purified by silica gel column chromatography (0-40% EtOAc/hexanes), providing the desired product (0.071 g, 52% yield). LCMS (m/z): 551.9 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.50 (dd, *J* = 9.1, 7.3 Hz, 2H), 7.41–7.32 (m, 3H), 7.19 (d, *J* = 8.1 Hz, 1H), 5.54 (s, 2H), 3.55 (tt, *J* = 7.0, 4.0 Hz, 1H), 2.79 (s, 3H), 1.26 (q, *J* = 6.9 Hz, 2H), 0.62–0.48 (m, 2H).

***tert*-Butyl ((3-(benzyloxy)-7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl)methyl)carbamate (73)**

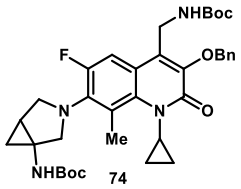


73

A suspension of 3-(benzyloxy)-7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl trifluoromethanesulfonate (0.50 g, 0.90 mmol), K_3PO_4 (0.58 g, 2.70 mmol), potassium (((*tert*-butoxycarbonyl)amino)methyl)trifluoroborate (0.43 g, 1.81 mmol) in THF (5 mL) and water (0.05 mL) was degassed with argon for 30 minutes. $PdCl_2$(dppf)·DCM adduct (22.0 mg, 0.03 mmol) was added and the reaction mixture was stirred at 90 °C for 7 hours. The reaction mixture was quenched with cold water and extracted with EtOAc. The combined organic layers were washed with brine, dried over sodium sulfate and concentrated to afford a crude residue, which was purified by silica gel column chromatography (0-25% EtOAc/hexanes), providing the desired product (0.14 g, 15% yield). LCMS (m/z): 431.4 [M–Boc+H]. ^1H NMR (400 MHz,

DMSO-d_6) δ 7.50–7.30 (m, 6H), 5.35 (s, 2H), 4.30 (d, *J* = 6.0 Hz, 2H), 3.81–3.79 (m, 1H), 3.61–3.59 (m, 1H), 2.80 (s, 3H), 1.45 (s, 9H), 0.90–0.88 (m, 2H), 0.57–0.55 (m, 2H).

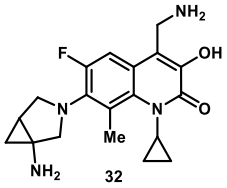
***tert*-Butyl (3-(3-(benzyloxy)-4-(((*tert*-butoxycarbonyl)amino)methyl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-azabicyclo[3.1.0]hexan-1-yl)carbamate (74)**



74

A suspension of *tert*-butyl ((3-(benzyloxy)-7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl)methyl)carbamate (130 mg, 0.24 mmol), Cs_2CO_3 (240 mg, 0.73 mmol), *tert*-butyl (azabicyclo[3.1.0]hexan-1-yl)carbamate (73.0 mg, 0.37 mmol) in toluene (5 mL) was degassed for 5 minutes. $Pd_2(dba)_3$ (11.0 mg, 0.01 mmol) and BINAP (15.0 mg, 0.02 mmol) were added and the reaction mixture was stirred at 100 °C for 15 hours. The reaction mixture was quenched with cold water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford a crude residue, which was purified by silica gel column chromatography (50% EtOAc/hexanes), providing the desired product (55.0 mg, 35% yield). LCMS (m/z): 649.5 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.28 (m, 6H), 5.32 (s, 2H), 4.28 (d, *J* = 5.6 Hz, 2H), 3.93–3.91 (m, 1H), 3.81–3.79 (m, 1H), 3.68–3.66 (m, 1H), 3.57–3.55 (m, 1H), 3.26–3.23 (m 1H), 2.47 (s, 3H), 1.71–1.68 (m, 1H), 1.57 (s, 9H), 1.49 (s, 9H), 1.30–1.20 (m, 2H), 0.91–0.88 (m, 2H), 0.59–0.56 (m, 2H).

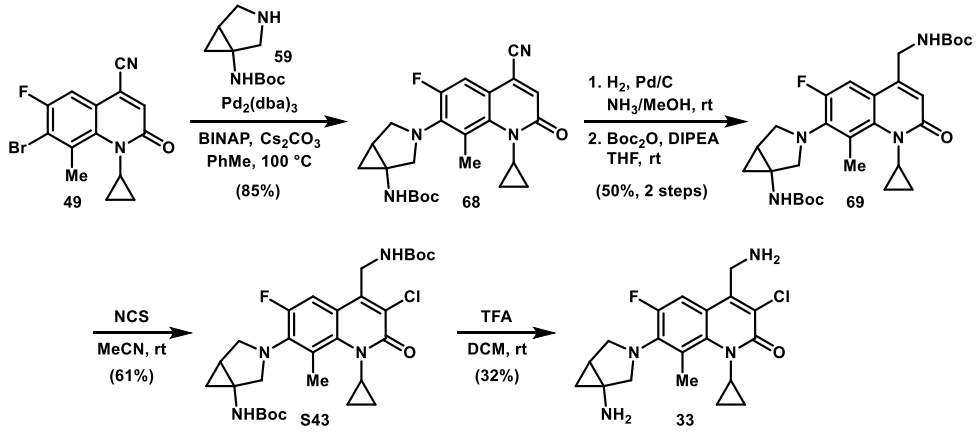
7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-6-fluoro-3-hydroxy-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (32)



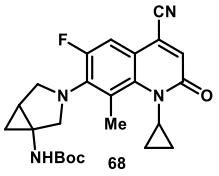
32

tert-Butyl (3-(3-(benzyloxy)-4-(((*tert*-butoxycarbonyl)amino)methyl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-azabicyclo[3.1.0]hexan-1-yl)carbamate (55.0 mg, 0.08 mmol) was dissolved in MeOH (0.5 mL). Pd/C (10%, 50% in water, 6.00 mg) was added and the reaction mixture was stirred at room temperature for 2 hours under a hydrogen atmosphere. The reaction mixture was filtered through Celite and the filtrate was concentrated to afford the deprotected hydroxyl. The protected bis-amine was dissolved in DCM (0.5 mL) and cooled to 0 °C. HCl (4.0 M in dioxane, 0.3 mL) was added and the reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was concentrated to afford a crude residue, which was purified by prep HPLC providing the desired product (3.50 mg, 15% yield, purity = 91%). LCMS (m/z): 359.4 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.38 (d, J = 13.2 Hz, 1H), 4.32 (s, 2H), 3.82–3.57 (m 4H), 3.37 (s, 2H), 2.54 (s, 3H), 2.01–1.99 (m, 1H), 1.40–1.20 (m 4H), 0.57–0.54 (m, 2H).

Preparation of 7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-3-chloro-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (33)

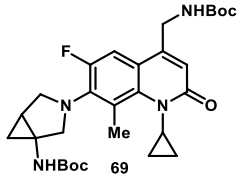


***tert*-Butyl (3-(4-cyano-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-azabicyclo[3.1.0]hexan-1-yl)carbamate (68)**



A suspension of 7-bromo-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinoline-4-carbonitrile (250 mg, 0.78 mmol), *tert*-butyl 3-azabicyclo[3.1.0]hexan-1-ylcarbamate (309 mg, 1.56 mmol), BINAP (194 mg, 0.31 mmol), Pd_2dba_3 (143 mg, 0.16 mmol) and cesium carbonate (761 mg, 2.34 mmol) in toluene (7.8 mL) was heated to 100 °C for 5.5 hours. Upon cooling to room temperature the mixture was filtered and concentrated. The crude material was purified by silica gel column chromatography (0-100% EtOAc/heptane) to give the product (650 mg, 85% yield). LCMS (m/z): 439.4 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, J = 12.1 Hz, 1H), 6.87 (s, 1H), 3.94–3.82 (m, 1H), 3.75–3.67 (m, 1H), 3.59–3.52 (m, 1H), 3.52–3.44 (m, 1H), 3.40–3.30 (m, 1H), 2.45 (s, 3H), 1.73 (s, 1H), 1.54 (s, 9H), 1.27–1.20 (m, 2H), 1.17–1.10 (m, 1H), 1.06–0.97 (m, 1H), 0.60–0.47 (m, 2H).

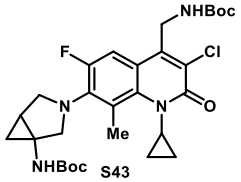
***tert*-Butyl (3-(4-(((*tert*-butoxycarbonyl)amino)methyl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-azabicyclo[3.1.0]hexan-1-yl)carbamate (69)**



69

A mixture of *tert*-butyl (3-(4-cyano-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-azabicyclo[3.1.0]hexan-1-yl)carbamate (650 mg, 1.48 mmol) in 2M NH_3 in MeOH (60 mL) was treated with Pd/C (10%, 50% water, 947 mg, 0.45 mmol). The flask was partially evacuated and back-filled with H_2 5 times, then stirred under a balloon of H_2 for 1 hour. The reaction mixture was filtered through a plug of celite and concentrated under reduced pressure to give the desired primary amine as a yellow-brown oil. The amine was dissolved in THF (7.4 mL) was treated with DIPEA (0.8 mL, 4.45 mmol) and Boc anhydride (0.45 mL, 1.93 mmol) followed by stirring at room temperature for 1 hour. The mixture was diluted with saturated aqueous NH_4Cl and extracted with EtOAc. The combined organic extracts were washed with saturated brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Silica gel column chromatography (0-100% EtOAc/heptane) provided the title compound (448 mg, 50% yield, 2 steps) as a yellow foam. LCMS (m/z): 543.4 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.10 (d, *J* = 13.2 Hz, 1H), 6.47 (s, 1H), 5.04 (s, 1H), 4.86–4.74 (m, 1H), 4.44–4.31 (m, 2H), 3.88–3.75 (m, 1H), 3.67 (d, *J* = 8.6 Hz, 1H), 3.56–3.39 (m, 2H), 3.28 (d, *J* = 9.3 Hz, 1H), 2.45 (s, 3H), 1.69 (s, 1H), 1.47 (s, 18H), 1.21–1.12 (m, 3H), 1.03–0.96 (m, 1H), 0.54–0.47 (m, 2H).

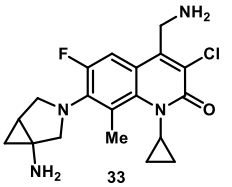
***tert*-Butyl (3-(4-(((*tert*-butoxycarbonyl)amino)methyl)-3-chloro-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-azabicyclo[3.1.0]hexan-1-yl)carbamate (S43)**



S43

A mixture of *tert*-butyl (3-(4-(((*tert*-butoxycarbonyl)amino)methyl)-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-azabicyclo[3.1.0]hexan-1-yl)carbamate (176 mg, 0.32 mmol) in MeCN (1.6 mL) was treated with *N*-chlorosuccinimide (130 mg, 0.97 mmol) followed by stirring for 4 hours at room temperature. Volatiles were evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, then dried over Na_2SO_4, filtered and concentrated under reduced pressure. Silica gel column chromatography (0-100% EtOAc/heptane) provided the title compound (127 mg, 61% yield) as a pale yellow foam. LCMS (m/z): 577.3 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.55–7.44 (m, 1H), 5.04 (br s, 1H), 4.83 (br s, 1H), 4.62 (d, *J* = 5.7 Hz, 2H), 3.83 (br s, 1H), 3.68 (d, *J* = 8.7 Hz, 1H), 3.61–3.45 (m, 2H), 3.31 (d, *J* = 9.3 Hz, 1H), 2.44 (s, 3H), 1.70 (br s, 1H), 1.55–1.38 (m, 18H), 1.22–1.14 (m, 3H), 1.04–0.96 (m, 1H), 0.56–0.48 (m, 2H).

7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-3-chloro-1-cyclopropyl-6-fluoro-8-methylquinolin-2(1*H*)-one trifluoroacetic acid (33)



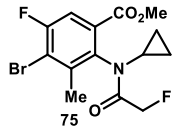
33

A mixture of *tert*-butyl (3-(4-(((*tert*-butoxycarbonyl)amino)methyl)-3-chloro-1-cyclopropyl-6-fluoro-8-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-3-azabicyclo[3.1.0]hexan-1-yl)carbamate (67

mg, 0.12 mmol) in DCM (0.6 mL) and TFA (0.6 mL) was stirred for 20 minutes at room temperature. Volatiles were evaporated under reduced pressure and the oily residue was concentrated twice from MeOH-toluene then subjected to preparative HPLC to give the product (22.8 mg, 32% yield). LCMS (m/z): 377.2 $[M+H]^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 7.55 (d, *J* = 13.5 Hz, 1H), 4.52 (s, 2H), 3.87–3.82 (m, 1H), 3.82–3.76 (m, 1H), 3.74–3.69 (m, 1H), 3.69–3.63 (m, 1H), 3.44–3.40 (m, 1H), 2.56 (s, 3H), 2.03–1.97 (m, 1H), 1.36–1.22 (m, 4H), 0.52–0.42 (m, 2H).

Preparation of 7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-3,6-difluoro-8-methylquinolin-2(1H)-one hydrochloride (34)

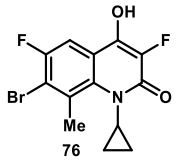
Methyl 4-bromo-2-(*N*-cyclopropyl-2-fluoroacetamido)-5-fluoro-3-methylbenzoate (75)



75

Methyl 4-bromo-2-(cyclopropylamino)-5-fluoro-3-methylbenzoate (3.50 g, 11.6 mmol) was dissolved in toluene (15 mL) at 0 °C. 2-Fluoro acetyl chloride (1.11 g, 11.6 mmol) was added and the reaction mixture was stirred at 85 °C for 4 hours. The reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution and brine, then dried over sodium sulfate and concentrated. The crude residue was purified by silica gel column chromatography (25-30% EtOAc/hexanes) to afford the desired product (3.00 g, 62% yield). LCMS (m/z): 364.4 $[M+2]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71–7.55 (m, 1H), 5.40 (ddd, *J* = 47.0, 37.4, 14.2 Hz, 1H), 4.53 (ddd, *J* = 76.7, 47.1, 13.5 Hz, 1H), 3.97–3.84 (m, 3H), 3.19–3.03 (m, 1H), 2.48–2.22 (m, 3H), 0.97–0.38 (m, 4H).

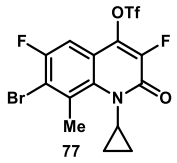
7-Bromo-1-cyclopropyl-3,6-difluoro-4-hydroxy-8-methylquinolin-2(1H)-one (76)



76

Methyl 4-bromo-2-(*N*-cyclopropyl-2-fluoroacetamido)-5-fluoro-3-methylbenzoate (3.00 g, 8.20 mmol) was dissolved in THF (20 mL) and cooled to –78 °C. NaHMDS (1M in THF, 24.9 mL, 24.9 mmol) was added dropwise and the reaction mixture was stirred at –78 °C for 30 minutes. The reaction mixture was then stirred at room temperature for 1 hour. The reaction mixture was quenched with ice-water and washed with EtOAc. The aqueous layer was acidified with 1N HCl and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated to afford a crude residue, which was purified by trituration with pentane, providing the desired product (2.50 g, 91% yield). LCMS (m/z): 332.3 $[M+2]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (s, 1H), 7.59 (d, J = 8.7 Hz, 1H), 3.50 (s, 1H), 2.73 (s, 3H), 1.12 (d, J = 6.2 Hz, 2H), 0.43 (s, 2H).

7-Bromo-1-cyclopropyl-3,6-difluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl trifluoro methanesulfonate (77)

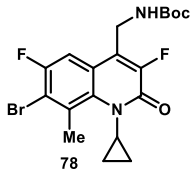


77

7-Bromo-1-cyclopropyl-3,6-difluoro-4-hydroxy-8-methylquinolin-2(1H)-one (2.50 g, 7.50 mmol) was dissolved in DMF (14 mL) at 0 °C. Triethylamine (2.29 g, 22.7 mmol) and $PhNTf_2$ (4.00 g, 11.4 mmol) in DMF (14 mL) were added at 0 °C and the reaction mixture was stirred at room temperature for 4 hours. The reaction mixture was quenched with ice-water and extracted with EtOAc. The organic layer was washed with cold water and brine, then dried over sodium

sulfate and concentrated to afford a crude residue, which was purified by silica gel column chromatography (0-5% EtOAc/hexanes), providing the desired product (3.00 g, 85% yield). LCMS (m/z): 464.4 $[M+2]^+$.

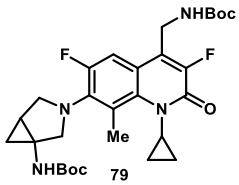
***tert*-Butyl ((7-bromo-1-cyclopropyl-3,6-difluoro-8-methyl-2-oxo-1,2-dihydro quinolin-4-yl)methyl)carbamate (78)**



78

A solution of 7-bromo-1-cyclopropyl-3,6-difluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl trifluoro methanesulfonate (1.50 g, 3.20 mmol), potassium (((*tert*-butoxycarbonyl)amino)methyl) trifluoroborate (1.53 g, 6.40 mmol) and Na_2CO_3 (0.86 g, 8.10 mmol) in toluene (45 mL) and water (0.15 mL) was degassed with nitrogen for 5 minutes. $PdCl_2$(dppf) (0.12 g, 0.16 mmol) was added and the reaction mixture was stirred at 85 °C for 5 hours. The reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated. The crude residue was purified by silica gel column chromatography (0-50% EtOAc/hexanes) to afford the desired product (0.50 g, 17% yield). LCMS (m/z): 445.5 $[M+2]^+$.

***tert*-Butyl ((7-(1-((*tert*-butoxycarbonyl)amino)-3-azabicyclo[3.1.0]hexan-3-yl)-1-cyclopropyl-3,6-difluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl)methyl)carbamate (79)**

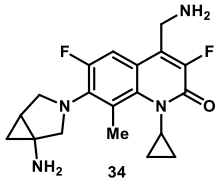


79

A suspension of *tert*-butyl ((7-bromo-1-cyclopropyl-3,6-difluoro-8-methyl-2-oxo-1,2-dihydro quinolin-4-yl)methyl)carbamate (0.22 g, 0.50 mmol), *tert*-butyl (3-azabicyclo[3.1.0]hexan-1-

yl)carbamate (0.20 g, 0.99 mmol), cesium carbonate (0.49 g, 1.49 mmol), Pd_2dba_3 (0.09 g, 0.10 mmol) and BINAP (0.12 g, 0.20 mmol) in dioxane (3.6 mL) was stirred at 100 °C for 6 hours. The reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated. The crude residue was purified by prep HPLC purification to afford the desired product (0.08 g, 40% yield). LCMS (m/z): 561.8 $[M+H]^+$. ^1H NMR (400 MHz, MeOD) δ 7.47 (d, *J* = 13.2 Hz, 1H), 4.46 (s, 2H), 3.84 (s, 1H), 3.61 (d, *J* = 20.3 Hz, 3H), 3.35 (s, 1H), 2.54 (s, 3H), 1.67 (s, 1H), 1.46 (s, 18H), 1.21 (d, *J* = 19.9 Hz, 3H), 0.98 (s, 1H), 0.53 (s, 2H).

7-(1-Amino-3-azabicyclo[3.1.0]hexan-3-yl)-4-(aminomethyl)-1-cyclopropyl-3,6-difluoro-8-methylquinolin-2(1H)-one hydrochloride (34)



34

tert-Butyl ((7-(1-((*tert*-butoxycarbonyl)amino)-3-azabicyclo[3.1.0]hexan-3-yl)-1-cyclopropyl-3,6-difluoro-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl)methyl)carbamate (50.0 mg, 0.01 mmol) was dissolved in dioxane (3 mL) at 0 °C. HCl (4M in dioxane, 3.0 mL) was added and the reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was concentrated and the crude residue was triturated with diethyl ether to afford the desired product (35.0 mg, 90% yield). HRMS (ESI) calc'd for $C_{19}H_{23}F_2N_4O$ $[M+H]^+$ 361.1834, found 361.1832. ^1H NMR (400 MHz, MeOD) δ 7.54 (d, *J* = 13.2 Hz, 1H), 4.41 (s, 2H), 3.86 (d, *J* = 8.9 Hz, 1H), 3.80 (d, *J* = 9.9 Hz, 1H), 3.66 (s, 1H), 3.43–3.36 (m, 2H), 2.60 (s, 3H), 2.03 (s, 1H), 1.38–1.26 (m, 4H), 0.54 (s, 2H).

***In vitro* and *in vivo* Biology**

General: IC_{50} values obtained from the GyrA/B and ParC/E biochemical assays are reported as the geometric mean of 2 or more replicates, with the exception of compound **34** against E. coli GyrA/B (single replicate). For MIC data, the most frequently occurring value from three or more replicates is reported. In cases where only two replicates were available, or where two values occurred with identical frequency, the higher of the two values is reported. MIC data is based on two or more separate experiments with the exception of compounds **6**-**8**, **11**-**14** and **18**.

Biochemical Assay

Purification of *E. coli* GyrA, GyrB, ParC and ParE: Large scale expression cultures of *E. coli* cells carrying expression plasmid of recombinant full-length GyrA, GyrB, ParC and ParE (T7 promoter C-terminal 6xHis tag) were grown separately using standard procedures (1-6 liters). When cultures reached late log-phase, incubation temperature was lowered to 18 ºC and protein expression was induced by adding IPTG to 1 mM with growth continuing overnight. Cells were collected by centrifugation, resuspended in 5x volume of IMAC buffer A (50 mM Tris-HCl pH7.5; 300 mM NaCl; 2 mM $MgCl_2$; 10% (v/v) glycerol; 1 mM TCEP) containing 15 mM imidazole, Benzonase endonuclease (EMD Millipore) and protease inhibitors (Roche cOmplete, EDTA-free), and lysed by sonication. The total lysate was centrifuged to obtain the soluble lysate, which was applied to a column of Ni Sepharose FF (GE Healthcare Life Sciences) equilibrated with 6% (v/v) IMAC buffer B (50 mM Tris-HCl pH 7.5; 300 mM NaCl; 2 mM MgCl; 10% (v/v) glycerol; 1 mM TCEP; 250 mM imidazole). The column was washed with the same buffer and developed with a linear gradient to 100% (v/v) IMAC buffer B over 20 column volumes.

Fractions were analyzed by SDS-PAGE and pooled accordingly, concentrated and applied to a Superdex 200 (GE Healthcare Life Sciences) size-exclusion column equilibrated with 50 mM Tris-

HCl pH7.5, 50 mM KCl, 2 mM $MgCl_2$, 10% (v/v) glycerol; 1 mM TCEP. Fractions were pooled based on analysis by SDS-PAGE, made to 2 mM spermidine, quantified by Bradford assay, aliquoted, flash-frozen in liquid nitrogen, and stored at -80 ºC for reconstitution prior to biochemical assays.

***E. coli* gyrase DNA supercoiling assay**: Tetrameric *E. coli* GyrAB was prepared by incubation of 10 µM of each subunit in GyrAB assay buffer (50 mM Tris-HCl pH7.5, 10 mM $MgCl_2$, 30 mM KCl, 10% glycerol, 0.005% BSA, 0.005% CHAP, 75 mM ammonium acetate , 0.5 mM EDTA and 1 mM DTT) at room temperature for 60 minutes.

10 nM tetramer of *E. coli* GyrAB was pre-incubated with compound for 30 minutes at room temperature before 40 µg/mL (final concentration) relaxed DNA (TopoGEN TG2035-3) and 1 mM (final concentration) ATP in GyrAB assay buffer were added for a reaction time of 2 hours. The reaction was quenched with 0.1% (final) SDS.

Supercoiled DNA generated by *E. coli* GyrAB was monitored by size exclusion chromatography. An Agilent 1100 HPLC containing a diode array detector was used to acquire data. The instrument was controlled using the Agilent Chemstation software. A 10 µL aliquot of each sample was injected onto a Waters Acquity BEH200 (1.7 µm particle, 4.6 x 300 mm) column. Separation occurred using an isocratic gradient with 100 mM sodium phosphate, pH 6.8, as mobile phase. Detection was by diode array at 260 nm. To increase assay throughput, the SEC analysis was multiplexed, by staggering injections of samples to effectively analyze four samples in one run. Samples were injected every 4 minutes using separate 4 min long isocratic gradient, with the fourth sample injected being subjected to a full 40 minute gradient to elute all peaks (see HPLC conditions below). Peak height was used to calculate percent inhibition.

To reduce column degradation and clogging, two 10 µL injections of Pierce universal nuclease (100 kU) were run through the column on a 120 minute isocratic gradient at a flow rate of 10 µL/min, followed by 3 blank injections using the same gradient.

Multiplexed HPLC pump and autosampler conditions for samples 1*n*-3*n*

Sample 1*n*-3*n* pump parameters	
Time (min)	Flow Rate (mL/min)
0	0.1
4	0.1

NOTE: Injection overlap at 2.5 minutes

Multiplexed HPLC pump and autosampler conditions for samples 4*n*

Sample 4*n* pump parameters	
Time (min)	Flow Rate (mL/min)
0	0.1
20	0.1
20.1	0.2
37	0.2
37.1	0.1
40	0.1

***E. coli* Topoisomerase IV DNA decatenation assay**: *E. coli* ParCE tetramer was prepared by incubation of 10 µM of each subunit in ParCE assay buffer (50 mM Tris-HCl pH7.5, 10 mM $MgCl_2$, 50 mM KCl, 100 mM KGlu, 1 mM EDTA, 0.01% Tween 20, 4 mM DTT, 0.01% BSA) at room temperature for 60 minutes.

1.25 nM *E. coli* ParCE tetramer was pre-incubated with compound for 30 minutes at room temperature before 10 µg/mL (final concentration) kinetoplast DNA (TopoGEN TG2013-3) and 1 mM (final concentration) ATP in ParCE assay buffer were added for a reaction time of 1 hour. The reaction was quenched with 0.1% (final) SDS.

Decatenated kinetoplast DNA generated by *E coli* ParCE was monitored by size exclusion chromatography. An Agilent 1200 capillary HPLC containing a variable wavelength detector was used to acquire data. The instrument was controlled using the Agilent MassHunter Acquisition software. An 8 µL aliquot of each sample was injected onto a Sepax Zenix size exclusion chromatography guard column (3 µm particle, 4.6 x 50 mm). The separation of the decatenated DNA from the UV sensitive buffer components was achieved on a 7 minute isocratic gradient at 600µL per minute. 100 mM sodium phosphate pH 6.8 was used as the mobile phase. Diode array detector (DAD) detection was performed at 260 nm.

Data were analyzed and LC peaks integrated using Agilent ChemStation software (Revision B.0.03.02-SR2). Manual integration was used to adjust integration in the event of additional peak integration. Area under the curve of decatenated kDNA was used to calculate percent inhibition.

Antibacterial Susceptibility Testing

Antibacterial Activity Testing: Antibacterial activity was assessed using a broth microdilution assay following the recommended methodology of the Clinical and Laboratories Institute (CLSI M07).[93] Performance of the assay was monitored by testing ciprofloxacin against laboratory quality control strains in accordance with guidelines of the CLSI (CLSI M100).[71]

Selection of single step spontaneous mutants. *E. coli* ATCC 25922 was used for selection experiments. Single-step selection was performed on cation-adjusted Mueller-Hinton agar plates containing test compounds at 2X, 4X and 8X multiples of the MIC. Dilutions of cell suspension

were also plated on drug-free medium to enumerate CFU/mL. Plates were incubated at 37 ºC for up to 72 hours and CFU were counted. Mutant frequencies were calculated by dividing the number of colonies on drug containing plates by the number of CFU plated.

Bacterial strains: Wild type and laboratory engineered mutant strains are described in Table 9. In *P. aeruginosa*, a C248T mutation in *gyrA* (encoding T83I) together with a C260G mutation in *parC* (encoding S87L) imparts a 256-fold decrease in susceptibility to ciprofloxacin.[94] These mutations were introduced onto the genome of the efflux deficient *P. aeruginosa* strain NB52023 as follows: first, a 1 kb *gyrA* fragment encompassing the C248T mutation was amplified from a genome sequenced *P. aeruginosa* clinical isolate using primers SR73 (5′-ggccagtgccaagctattgaacgaggcgactgga-3′) and SR75 (5′-acgaattcgagctcgggcagctcggtgatgataa-3′). This was inserted into the gene replacement vector pEX18Ap,[95] that had been digested with HindIII/KpnI, using the In-Fusion HD cloning kit (Takara) according to the supplied instructions. The resulting vector was mobilized into strain NB52023 as described previously[96] and merodiploids were selected on L-agar containing irgasan and carbenicillin. Merodiploids were resolved on L-agar containing sucrose and 0.125 µg/mL ciprofloxacin, and the presence of the *gyrA* C248T mutation was confirmed by PCR and sequencing. Next, a 1 kb *parC* fragment encompassing C260G was amplified from a genome sequenced *P. aeruginosa* clinical isolate using primers SR80 (5′-ggccagtgccaagcttgaccttcctgctcgatacc-3′) and SR82 (5′-acgaattcgagctcgccggtctcgtagaccttctg-3′), which was inserted into pEXAp. The resulting suicide vector was used to introduce the mutation into NB52023 *gyrA* C248T as described above, except that merodiploids were resolved on L-agar containing sucrose and 2 µg/mL ciprofloxacin. The presence of *parC* C260G was confirmed by PCR and sequencing. The resulting *gyrA parC* double mutant was designated NB52023-CDK0006.

$MIC_{50/90}$ Panel: A panel consisting of 126 Gram-negative clinical isolates, including 96 *Enterobacteriaceae* and 30 *P. aeruginosa*, was assembled from the Novartis collection. The isolates were acquired between 2000 and 2016 from various geographic regions and included the following Enterobacteriaceae species: *Citrobacter* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella oxytoca*, *K. pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, and *Serratia marcescens*.

Table 9. Wild type and mutant bacterial strains used in this study

Novartis Strain Code	Referred to as	Description	Source or Reference
E. coli			
NB27001	*E. coli* WT	ATCC 25922	ATCC
NB27178	*E. coli*-Δ*acrB*	BW25113 *ΔacrB*, JW0451-2	Ref[97]
K. pneumoniae			
NB29002	*K. pneumoniae* WT	ATCC 43816	ATCC
P. aeruginosa			
NB52019	*P. aeruginosa* WT	K767; PAO1, prototroph	Ref[98]
NB52023	*P. aeruginosa* Δ*mexB* Δ*mexXY*	K1542; K767 Δ*mexB* Δ*mexXY*	Ref[99]
NB52023-CDK0006	*P. aeruginosa* Δ*mexB*Δ*mexX* FQ-R	NB52023 *gyrA* T83I *parC* S87L	This study (also see Ref[100])

Animal studies

All studies were approved by the Institutional Animal Care and Use Committee of Novartis Institutes for BioMedical Research, Inc. (Emeryville, CA, USA). Female CD-1 mice (17–20 g; Envigo, Livermore, CA, USA) and male Wistar-Han rats (250-300 g, CRL, Raleigh, North Carolina, USA) were kept under controlled conditions with food and water ad libitum. Pharmacokinetic studies were performed in mice and rat with drug concentrations determined by LC-MS/MS. All analysis was performed using Phoenix WinNonLin 6.4 to generate PK

parameters. The AUC was obtained from the measured plasma concentrations versus time using the trapezoidal method.

***In Vivo* PK Studies:** All animal studies were conducted under a Novartis-Emeryville IACUC approved protocol in compliance with Animal Welfare Act regulations and the Guide for the Care and Use of Laboratory Animals. Animals were fasted overnight and dosed IV and PO at the targeted doses in a solution of 20% PEG300 and 5% Solutol in D5W or saline water. The whole blood was collected into vials containing EDTA at post-dose 0.083, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 hours, and immediately placed on dry ice until all samples were stored in –80 °C freezer. Prior to analysis, the samples were thawed on ice. Ten microliters of each blood sample were added to 80 µL of acetonitrile containing Internal Standard (CHIRO73911) and centrifuged 3450 xg at 10 °C for 20 minutes. A 70 µL aliquot of the supernatant was transferred into a microtiter plate and dried down at 40 °C, and the residue was reconstituted with 100 µL of 0.1% FA in water. An aliquot of each sample was injected into the liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS) system for analysis. Non-compartmental PK analysis was conducted using Phoenix WinNonLin 6.4 professional ^{TM} (6.4) (Certara, inc., Menlo Park, CA) to generate pharmacokinetic parameters.

***In Vivo* Pharmacology Studies**

Inoculum preparation: An overnight culture of *E. coli* ATCC 25922 was prepared by inoculating 25 mL Mueller–Hinton Broth with 20 µL of bacterial strain from frozen stock. The culture was allowed to grow for 16–18 h at 37°C with agitation at 150 rpm (I2400 Incubator Shaker; New Brunswick Scientific). An aliquot was taken, centrifuged and the bacterial pellet resuspended in sterile saline (0.9% w/v). Using optical density: CFU correlations, the inoculum was prepared at 2×10^7 CFU/mL by diluting with sterile saline.

Neutropenic murine thigh infection: Animals (4 mice/group) were rendered neutropenic by intraperitoneal injections of 150 and 100 mg/kg cyclophosphamide (Sigma–Aldrich, St Louis, MO, USA), on days −4 and −1 respectively prior to infection. Two hours prior to treatment (−2 h), 50 µL of bacterial inoculum was administered into the left gastrocnemius muscle via an intramuscular injection. At time 0 h, animals were treated via the subcutaneous (sc) route of administration with doses from 2.5-120 mg/kg. At 0 h a cohort of animals was sacrificed via CO_2 to determine the bacterial levels at the start of treatment. The remaining animals were euthanized 24 h after the start of therapy. The infected thighs were excised and homogenized in sterile saline (0.9% w/v) until the tissue was completely homogenized. The homogenates were serially diluted in sterile saline before dilutions were plated on TSA plates, incubated overnight at 37°C, the colonies and. CFU/thigh determined.

Calculation of static dose: The static dose (mg/kg/day) is that which is required to keep the bacterial load at the same level as when therapy was initiated. Dose/day was plotted against $\log_{10}$ CFU and analysed using a four parametric logistic curve (SigmaPlot 12.0; SyStat Software, San Jose, CA, USA). The static dose was calculated using the following equation: $\log_{10}$ static dose = $\{\text{Log}_{10}\,[E/(E_{max} - E)]/N\} + \log_{10} ED_{50}$, where E is the control growth ($\log_{10}$ change in CFU per thigh in untreated controls after the 24 h period of study), E_{max} is the maximum effect, ED_{50} is the dose required to achieve 50% of E_{max}, and N is the slope of the dose–effect curve.

***In vitro* ADMET Assays**

$LogD_{7.4}$ determination: Compound stock solutions (10 µL, 10 mM in DMSO) were placed in each well of a 96 well plate. 5 µL of 2 mM Halodipine (logD = 3.00) was added to each well as an internal standard (IS) and mixed. DMSO was removed using a lyophilizer (overnight). 250 µL of water (PBS, pH = 7.4)-saturated octanol was added to each well followed by 250 µL of octanol-

saturated water (PBS, pH = 7.4) and the plate was vortexed overnight. 100 µL of the octanol phase was removed from each well, diluted 1:100 with DMSO and 1 uL was injected into a LC/UV/qTOF system (RP column). 100 µL of the water phase was removed from each well and 10 µL was injected into the LC/UV/qTOF system (RP column). Data was processed with ProfileLynx. Mass chromatograms were integrated, corrected for dilution and injection volumes and, finally, corrected using area ratio of internal standard. IS peak areas were adjusted to fit the theoretical concentration for aqueous and octanol phases for the known logD value.

LogD was calculated according to the following:

Analyte logD = log(Ratio_oct/Ratio_aq)

Ratio_oct = (Undiluted analyte_peak area/Adjusted IS_peak area)oct

Ratio_aq = (Undiluted analyte_peak area/Adjusted IS_peak area)aq

Solubility determination: 20 µL of 10 mM DMSO compound stock solution was transferred into a 96 deep well "sample plate". 5 µL of 10 mM DMSO stock solution was transferred to a second "compound standard plate". The sample plate was placed in a Multi-Tainer MT-4 container (FTS Systems) and freeze-dried overnight to remove DMSO. 100 µL of PBS (pH 7.0) was added to the dried compound in the sample plate and 95 µL of DMSO to the standard plate. The sample plate was sonicated in a water bath for 10 min. The two plates were then placed onto a VWR orbital shaker to equilibrate for 24 hours at room temperature, then centrifuged at 4000 rpm for 30 min. 10 µL aliquots of supernatant from the sample plate were removed and diluted 5-fold. Compound standard and sample were injected into a UPLC/UV/CLND/MS system to generate multi detector qualitative and quantitive analytical data. Data was processed with Xcalibur. CLND equimolar response was used for measuring compound concentration of DMSO solution and UV270 nm or MS relative ratio for was used for solubility determination.

pK_a Measurements: pK_a values were determined using the UV-metric method as described previously.[101] UV-metric ionization constants were determined on the commercial Spectral Gradient Analyzer (SGA) or T3 instrument (Sirius Analytical Ltd., sirius-analytical.com) as described by Allen et al.[102] Test compounds were diluted to 0.04 mM in a cosolvent mixture and titrated three times in 20–40% wt methanol. The titrations were performed at 25 °C and 0.15 M ionic strength, from pH 2 to 12 or 12 to 2 (with delta pH of 0.2) depending on the acidic or basic nature of the test compound. A linear buffer was added to allow fast pH stabilization after each titrant addition. Wavelengths from 230 to 450 nm were typically monitored for UV absorbance change due to the ionization state of the compound. Target factor analysis (TFA) was used to calculate apparent pK_as from the multiwavelength absorption data at a given percent of cosolvent (psK_a), followed by Yasuda–Shedlovsky extrapolation to 0% methanol to provide the aqueous pK_a. Acid/base assignment was performed based on the slope of extrapolation.

Metabolic stability in liver microsomes: Test compounds at 1 μM in duplicate were incubated at 37 ºC in the presence of 0.5 mg/mL liver microsomal protein suspension, 1 mM NADPH, 1 mM UDPGA, 3 mM $MgCl_2$, and 25 μg alamethicine per mg of microsomal protein. Sequential samples were collected at 0, 5, 15, and 30 minutes of the incubation. DMSO final concentration in the incubation was 0.01%. Bioanalysis was carried out using semi-quantitative LC-MS/MS method in MRM mode. Then compound concentration (Peak area ratio to internal standard) against time is plotted to obtain elimination rate constant (k) for intrinsic clearance estimation: Intrinsic Clearance = (-k) / [microsome protein].

Compounds were tested in the following number of individual experiments (replicates). For compounds with more than one replicate, data is presented as an average: compound **5**: 4 replicates; compound **10**: 2 replicates; compound **34**: 1 replicate.

***In vitro* plasma protein binding:** Compounds were spiked into plasma of selected species (male CD-1 mouse, male Sprague Dawley rat or pooled human plasma, all in lithium heparin preparations, BioreclamationIVT) in the "plasma chamber" of a Rapid Equilibrium Device (RED, Thermo Scientific) plate at a final concentration of 10 μM (1% DMSO in the incubation), and allowed to reach equilibrium between the "plasma chamber" and "buffer chamber" (100 mM PBS) for four hours in a shaking incubator (37 ºC, 800 rpm). Compounds were tested in duplicates. At the end of incubation, samples were collected from plasma and buffer chambers followed by bioanalysis using LC-MS/MS. The percent plasma protein binding of a test compound was estimated using the following equation:

$$PPB\ (\%) = 100 \times (1 - C_{buffer} / C_{plasma})$$

Where C_{buffer} and C_{plasma} are compound concentrations in the buffer and plasma chambers at equilibrium, respectively.

Recovery of a compound after the incubation was also calculated as % Recovery:

$$Recovery\ (\%) = 100 \times (C_{buffer} \times V_{buffer} + C_{plasma} \times V_{plasma}) / (C_0 \times V_{plasma})$$

Where V_{buffer} and V_{plasma} are incubation volumes in buffer and plasma chambers, respectively. C_0 is the initial compound concentration in plasma at the beginning of the incubation.

The data presented for compounds **5** and **10** represents the results of a single experiment. Data for compound **34** represents the average of two individual experiments (replicates).

CACO-2 Permeability: CACO-2 cells were cultured for 21 days on 96-well transwell plates. Test compound solutions were prepared by diluting 7 μL of 2 mM DMSO stock solution in transport buffer to a final volume of 1.4 mL (final compound concentration was 10 μM). Test compound solutions were transferred to either the apical or basolateral chambers of the 96-well transwell plates (the donor wells). Transport buffer was then added to the respective receiver wells.

Plates were then incubated for 2 hours at 37 °C with shaking/vortex. Donor wells were sampled at $t = 0$. Apical and basolateral wells were sampled at $t = 2$ hours. Samples were analyzed by LC-MS-MS. Transport from A-B and B-A was measured in triplicate. Apparent compound permeability is calculated as follows:

$$P_{app} = (\partial Q/\partial t)*(1/AC_0)$$

where $\partial Q/\partial t$ is the total amount of compound transported to the recipient chamber per unit time (e.g., nmol/sec); A is the surface area of the transport membrane (cm^2) and C_0 is the initial compound concentration in the donor chamber (e.g., nmol/mL). P_{app} is expressed as cm/sec.

Compounds were tested in the following number of individual experiments (replicates). For compounds with more than one replicate, data is presented as an average: compound **5**: 2 replicates; compound **10**: 2 replicates; compound **34**: 1 replicate.

hERG Manual Patch Clamp Electrophysiology: HEK293 cells stably transfected with the alpha-subunits of the hERG (University of Wisconsin, USA) were continuously maintained and passaged using standard cell culture media (Gibco-BRL, Switzerland). For experiments, the cells were plated onto sterile glass coverslips in 35 mm^2 dishes at a density of 1.1-1.5 x 10^5 cells per dish. The dishes were stored in a humidified and gassed (5% CO_2) incubator at 37ºC until use.

The effect on hERG currents was assessed by means of the patch clamp technique in the whole-cell configuration at 35 ± 2°C. The corresponding vehicle for all superfusion concentrations was 0.1% DMSO. The vehicle effect was investigated in 5 cells. The effect of the positive controls 100 nM E-4031 (Calbiochem, Switzerland) was investigated in 2 cells. Cells were exposed to the test item for approximately 10 min. hERG tail currents were elicited by voltage jumps from -75 mV to +10 mV (500 ms) and then to -40 mV (500 ms) at 0.1 Hz. The composition of the extracellular solutions was [mM]: NaCl 137; KCl 4; $CaCl_2$ 1.8; $MgCl_2$ 1.0; D-glucose 10; N-2-

hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) 10; pH 7.4 (adjusted with 5 M NaOH). The composition of the pipette solutions was (mM): KCl 130; $MgCl_2$ 1.0; Ethylene glycol-bis(ß-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) 5; Mg-ATP 5; HEPES 10; pH 7.2 (adjusted with 1 M KOH). The compound effect on the currents was corrected by the mean vehicle rundown which was observed (n=5) by the treatment with extracellular solution containing 0.1 % DMSO. Data capturing and analysis was performed by using Pulse (Heka Electronics, Germany) and Excel.

The data presented for compounds **5** represents the results of a single experiment.

Automated Electrophysiology: *hERG* expressing cell lines were produced *in-house* at Novartis using CHO-K1 T-Rex™ inducible plasmid system (Invitrogen) as described previously.[103] Cell lines were maintained in Ham's F12 Nutrient Mixture containing 10% FBS, blasticidin (10 mg/mL; InvivoGen), hygromycin B (200 mg/mL; InvivoGen), Zeocin (200 mg/mL, Invitrogen) and neomycin (200 mg/mL, Invitrogen) using SelecT™ automated cell culture system (TAP Biosystems, Cambridge, UK). *hERG* and *$hCa_v1.2$* channels expression was induced with tetracycline (0.25-1 µg/ml, Invitrogen) at least 24 hours prior to the experiment.

hERG currents were recorded using the Qpatch automated patch clamp systems (Sophion Bioscience Inc., North Brunswick, NJ) in the whole (single) cell configuration. hERG expressing CHO-K1 cells were harvested with Detachin (Genlantis) and stored in the modified serum-free SFM-2 media (life Technologies) at room temperature. The extracellular solution contained (in mM) NaCl (145), KCl (4), $MgCl_2$ (1), $CaCl_2$ (2), and HEPES (10), pH 7.4 with NaOH. The intracellular solution contained KCl (135), $MgCl_2$ (1.75), $CaCl_2$ (5.4), EGTA (10), K2-ATP (4), and HEPES (10), pH 7.2 with KOH. After whole cell configuration was achieved, the cell was held at -90 mV, and a 0.1-sec pulse to -50 mV was delivered to measure the leaking current, which

was subtracted from the tail current on-line. Then the cell was depolarized to +20 mV for 4 seconds (pre-pulse), followed by a 4-sec test pulse to -50 mV to reveal hERG tail current. To monitor changes in the current amplitude, this voltage protocol was repeatedly applied every 20 sec. Test compounds were first diluted in DMSO for six dose-response experiments and then dissolved in the extracellular solution using Freedom EVO liquid handling robotic system (Tecan, Männedorf, Switzerland). The final DMSO concentration in samples was 0.3% v/v. Amitriptyline (Sigma) was tested as a positive control. Data were analyzed using *in-house* developed MatLab-based program (MathWorks, Natick, MA).

hERG QPatch testing for compound **34** was performed using a long incubation protocol (10 minutes per concentration point). To avoid current rundown during prolonged incubation, current was measured in duplicate ([11 and 100 μM] and [33 and 300 μM] concentration pairs) in different cells simultaneously. The results of these measurements were combined into one DR-plot. Data were analyzed manually using Sophion QPatch Assay software.

The data presented for compounds **10** and **34** represents the results of a single experiment.

Cytotoxicity assay: K562 (human leukemia cells), Molt-4 (T-lymphoblast derived from acute lymphoblastic leukemia) and HepG2 (human hepatocellular carcinoma cells) were used to determine compound cytotoxicity. Cell viability was quantified by using CellTiter-Glo®, which measures ATP release based on the mono-oxygenation of luciferin catalyzed by Mg^{2+}, ATP and molecular oxygen.

Cell line maintenance media - K562 cells were maintained in Iscove's Modification of Dulbecco's Modified Eagle's Medium (IMDM) supplemented with 1% penicillin/streptomycin and 10% heat–inactivated fetal bovine serum (FBS). Molt-4 cells were maintained in Roswell Park Memorial Institute (RPMI 1640) culture medium supplemented with 1% penicillin/streptomycin

and 10% heat-inactivated FBS. HepG2 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM)-F12 supplemented with 1% penicillin/streptomycin and 10% heat–inactivated FBS.

Cells were counted using a hemocytometer and diluted to $2x10^4$ cells/mL for K562, $1x10^5$ cells/mL for Molt-4, and $5x10^4$ cells/mL for HepG2 in the appropriate culture media. 50 µL of cell suspension were added to each well of the 384-well assay plate containing 1 µL of serial diluted compound (100 µM top concentration, 3.16x dilution factor) using the BioTek Multiflo-Stacker liquid dispenser. Plates were incubated 72 hours at 37 ºC, 5% CO_2 and 90% humidity. 25 µLof 1X CellTiter-Glo® were then added to each well of the assay plate. Plates were incubated for 10 minutes at room temperature before reading on the PolarStar-Omega plate reader using the Luminescence settings and a gain of 3300. The 50% cytotoxic concentrations (CC_{50}) were determined. When multiple CC_{50} were determined for the same compound the average is reported. If one or more CC_{50} for the same compound showed a value > 100 µM (top concentration tested), 100 µM was used as the value in the average calculation. Compound **34** was tested in the following number of individual experiments (replicates): 3 (HepG2 and K562), 2 (MT4).

***In vitro* 3T3 NRU phototoxicity test:** *In vitro* phototoxicity assessment was conducted as described previously.[58] Mouse Balb/c 3T3 fibroblast cells were treated with the test item for one hour and, subsequently, either irradiated with simulated sunlight for 50 minutes (normalized to 1.7 ± 0.1 mW/cm^2 of UVA, resulting in a normalized radiation dose of 5.1 ± 0.3 J/cm^2 UVA), or kept in the dark for 50 minutes. After medium replacement and approximately 24 hours recovery cell viability was assessed using the Neutral Red Uptake (NRU) endpoint. IC_{50} values were derived from the concentration-response curves obtained with and without irradiation and used to calculate the PIF (Photo Irritation Factor, PIF) as follows:

$PIF = IC_{50\ (-irr)} / IC_{50\ (+irr)}$

In the case of compounds **15**, **21** and **34**, an IC_{50} was not achieved in the absence of irradiation. As a surrogate, the solubility limit during incubation was used (500 μM for **15**, 316 μM for **21** and 1000 μM for **34**). In parallel, the known phototoxic compound chlorpromazine was tested as a positive control.

X-ray Crystallography Studies

Cloning, protein expression and purification: A *K. pneumoniae* Topoisomerase IV ParE-ParC fusion gene was made by fusing the C-terminal domain of *parE* (residues 390-631) and the N-terminal domain of *parC* (residues 1-490) linked by a 2 amino acid Glu-Phe linker. The resulting construct (Kp ParEC) was inserted into a pETite vector (Lucigen) harboring a C- terminal 6 X-histidine tag. OverExpress C41 (DE3) cells (Lucigen) were then transformed with this construct and grown at 37 °C in Terrific Broth. Protein expression was induced with 100 μM IPTG. Cells were pelleted and resuspended in 20 mM Tris pH 8.0, 200 mM NaCl, 10% glycerol, 2 mM TCEP, 1 mM EDTA and Roche protease inhibitor and lysed in a pneumatic cell homogenizer. The cleared lysate was supplemented with imidazole and $MgCl_2$ to final concentrations of 10 mM and 1.5 mM, respectively, and loaded on a immobilized metal chelate affinity column and eluted with increasing imidazole concentrations. The fusion protein was then further purified on a Q Sepharose ion exchange column equilibrated with 20 mM Tris pH 8.0, 10% glycerol and 2 mM TCEP and eluted with column equilibration buffer including 1 M NaCl. Protein containing fractions were pooled and further purified on a Superdex 200 SEC column equilibrated with 20 mM Tris pH 7.5, 100 mM NaCl, and 2 mM TCEP. The fusion protein (MW ~84 kDa) was concentrated to 7.6 mg/mL, flash frozen in liquid nitrogen and stored in –80 °C for up to 6 months. A total of 22 mg of Kp ParEC with >95% purity was purified from 2 L of cell culture.

Crystallography: HPLC purified symmetric DNA oligo 5′-TTACGTTGTATGATCATACAACGTAA-3′ was ordered from IDT and dissolved in 10 mM Tris-HCl pH 7.5 and 50 mM NaCl. The DNA was annealed by denaturing at 95 °C and slow cooling to room temperature in a thermos flask over 48 hours to form a 1 mM solution of symmetric duplex DNA. The protein-DNA-compound ternary complex was made by mixing 23 uM (4 mg/mL) Kp ParEC dimer with 28 μM duplex DNA and 0.2 mM compound **34** and incubated overnight at 17 °C. Complex crystals were grown by the sitting drop vapor diffusion method at 18 °C and appeared after 24 to 48 hours. 200 nL complex solution was mixed with 100 nL crystallization solution (20 mM $MgCl_2$, 40 mM LiCl, 2 mM hexamine cobalt chloride, 40 mM sodium cacodylate trihydrate pH 5.5, and 30% MPD) and crystals were frozen in liquid nitrogen directly from the drop. Diffraction data were collected at the beamline 5.0.2 at the Advanced Light Source in Berkeley and processed with XDS. The structure was solved by molecular replacement using PDB 5EIX as starting model using Phaser.[104] The model was improved to convergence by iterative rounds of model rebuilding in Coot[105, 106] and refinement using phenix.refine of the Phenix suite.[107, 108]

ASSOCIATED CONTENT

Supporting Information

The following files are available free of charge.

Crystallographic data table for the structure of *K. pneumoniae* topoisomerase IV bound to compound **34**; unbiased electron-density difference map (F_{obs}-F_{calc}) for **34** bound to *K. pneumoniae* topoisomerase IV; interaction diagram of compound **34** bound to topoisomerase IV from *K. pneumoniae*; plot of CFU/thigh determined for each dose of compound **34** in the

neutropenic murine thigh infection model; SEC chromatograms showing separation of supercoiled and relaxed DNA and detection of decatenated kDNA; LC/MS traces of all final compounds (PDF)

Molecular Formula Strings (CSV)

Accession Codes

Structural coordinates for compound **34** have been deposited in the RCSB Protein Data Bank under the accession codes 6WAA. Authors will release the atomic coordinates and experimental data upon article publication.

AUTHOR INFORMATION

Corresponding Author

*E-mail: colin.skepper@novartis.com. Phone: +1 510 879 9317

Author Contributions

Alexey Rivkin was the Project Team Leader for this drug discovery program. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

This work was funded by Novartis AG.

ACKNOWLEDGMENTS

The authors thank Haiying He, Zhigan Jiang and Jianhua Xia (WuXi AppTec) for synthesis of early compounds in this series including **4** and **5**; Shengtian Yang for NMR structure elucidation support; Weiping Jia, Heidi Struble, Dazhi Tang and Alice Wan Wang for analytical and

separations support; Linhong Yang for pKa determination; Colin Lorentzen, Kent Wong and Linda Xiao for *in vitro* ADME assays; Srijan Ranjitkar for construction of the bacterial strain *P. aeruginosa* Δ*mexB*Δ*mexX* FQ-R. Johanne Blais is gratefully acknowledged for assistance in writing and editing this manuscript.

ABBREVIATIONS

ADMET: Absorption, Distribution, Metabolism, Excretion, Toxicity; BA: bioavailability; BINAP: 2,2′-Bis(diphenylphosphino)-1,1′-binaphthalene; C_{max}: maximum plasma concentration; CDI: carbonyl diimidazole; CL: clearance; CL_{int}: intrinsic clearance; DIPEA: *N*,*N*-diisopropylethylamine; DPPF: 1,1′-ferrocenediyl-bis(diphenylphosphine); FQ: fluoroquinolone; FQ-R: fluoroquinolone resistant; %F: percent orally bioavailable; fAUC: free (unbound) area under the curve; LiHMDS: lithium bis(trimethylsilyl)amide; MeCN: acetonitrile; NaHMDS: sodium bis(trimethylsilyl)amide; QRDR: quinolone resistance determining region; QT interval: the time that elapses between the beginning of the Q wave and the end of the T wave in an electrocardiogram; SAR: structure-activity relationship; SEMCl: 2-(Trimethylsilyl)ethoxymethyl chloride; T_{max}: time at which maximum plasma concentration was reached; Vss: volume of distribution.

REFERENCES

1. Bisacchi, G. S. Origins of the quinolone class of antibacterials: an expanded "discovery story". *J. Med. Chem.* **2015,** *58*, 4874-4882.

2. Da Silva, A.; De Almeida, M.; De Souza, M.; Couri, M. Biological activity and synthetic methodologies for the preparation of fluoroquinolones, a class of potent antibacterial agents. *Curr. Med. Chem.* **2003,** *10*, 21-39.

3. Emmerson, A. M.; Jones, A. M. The quinolones: decades of development and use. *J. Antimicrob. Chemother.* **2003,** *51 Suppl 1*, 13-20.

4. Mitscher, L. A. Bacterial topoisomerase inhibitors: quinolone and pyridone antibacterial agents. *Chem. Rev.* **2005,** *105*, 559-592.

5. Andriole, V. T. The quinolones: past, present, and future. *Clin. Infect. Dis.* **2005,** *41 Suppl 2*, S113-119.

6. Wagman, A. S.; Wentland, M. P. Quinolone antibacterial agents. In *Comprehensive Medicinal Chemistry II*, Taylor, J. B.; Triggle, D. J., Eds. Elsevier: Oxford, 2007; pp 567-596.

7. Jacoby, G. A.; Hooper, D. C. Review of the quinolone family. In *Antibiotic Discovery and Development*, Dougherty, T. J.; Pucci, M. J., Eds. Springer: Boston, MA, 2012; pp 119-146.

8. Naeem, A.; Badshah, S. L.; Muska, M.; Ahmad, N.; Khan, K. The current case of quinolones: synthetic approaches and antibacterial activity. *Molecules* **2016,** *21*, 268.

9. Drlica, K.; Malik, M.; Kerns, R. J.; Zhao, X. Quinolone-mediated bacterial death. *Antimicrob. Agents Chemother.* **2008,** *52*, 385-392.

10. Drlica, K.; Hiasa, H.; Kerns, R.; Malik, M.; Mustaev, A.; Zhao, X. Quinolones: action and resistance updated. *Curr. Top. Med. Chem.* **2009,** *9*, 981-998.

11. Laponogov, I.; Sohi, M. K.; Veselkov, D. A.; Pan, X.-S.; Sawhney, R.; Thompson, A. W.; McAuley, K. E.; Fisher, L. M.; Sanderson, M. R. Structural insight into the quinolone–DNA cleavage complex of type IIA topoisomerases. *Nat. Struct. Mol. Biol.* **2009,** *16*, 667.

12. Laponogov, I.; Pan, X.-S.; Veselkov, D. A.; McAuley, K. E.; Fisher, L. M.; Sanderson, M. R. Structural basis of gate-DNA breakage and resealing by type II topoisomerases. *PLOS ONE* **2010,** *5*, e11338.

13. Bax, B. D.; Chan, P. F.; Eggleston, D. S.; Fosberry, A.; Gentry, D. R.; Gorrec, F.; Giordano, I.; Hann, M. M.; Hennessy, A.; Hibbs, M.; Huang, J.; Jones, E.; Jones, J.; Brown, K. K.; Lewis, C. J.; May, E. W.; Saunders, M. R.; Singh, O.; Spitzfaden, C. E.; Shen, C.; Shillings, A.; Theobald, A. J.; Wohlkonig, A.; Pearson, N. D.; Gwynn, M. N. Type IIA topoisomerase inhibition by a new class of antibacterial agents. *Nature* **2010,** *466*, 935.

14. Wohlkonig, A.; Chan, P. F.; Fosberry, A. P.; Homes, P.; Huang, J.; Kranz, M.; Leydon, V. R.; Miles, T. J.; Pearson, N. D.; Perera, R. L.; Shillings, A. J.; Gwynn, M. N.; Bax, B. D. Structural basis of quinolone inhibition of type IIA topoisomerases and target-mediated resistance. *Nat. Struct. Mol. Biol.* **2010,** *17*, 1152.

15. Aldred, K. J.; McPherson, S. A.; Wang, P.; Kerns, R. J.; Graves, D. E.; Turnbough, C. L.; Osheroff, N. Drug interactions with *Bacillus anthracis* topoisomerase IV: biochemical basis for quinolone action and resistance. *Biochemistry* **2012,** *51*, 370-381.

16. Aldred, K. J.; McPherson, S. A.; Turnbough, C. L., Jr; Kerns, R. J.; Osheroff, N. Topoisomerase IV-quinolone interactions are mediated through a water-metal ion bridge: mechanistic basis of quinolone resistance. *Nucleic Acids Res.* **2013,** *41*, 4628-4639.

17. Aldred, K. J.; Breland, E. J.; Vlckova, V.; Strub, M. P.; Neuman, K. C.; Kerns, R. J.; Osheroff, N. Role of the water-metal ion bridge in mediating interactions between quinolones and *Escherichia coli* topoisomerase IV. *Biochemistry* **2014,** *53*, 5558-5567.

18. Veselkov, D. A.; Laponogov, I.; Pan, X.-S.; Selvarajah, J.; Skamrova, G. B.; Branstrom, A.; Narasimhan, J.; Prasad, J. V. N. V.; Fisher, L. M.; Sanderson, M. R. Structure of a quinolone-stabilized cleavage complex of topoisomerase IV from *Klebsiella pneumoniae* and comparison with a related *Streptococcus pneumoniae* complex. *Acta Cryst. D Struct. Biol.* **2016,** *72*, 488-496.

19. Domagala, J. M.; Hagen, S. E. Structure-activity relationships of the quinolone antibacterials in the new millenium: some things change and some do not. In *Quinolone Antimicrobial Agents, 3rd Edition*, Hooper, D. C.; Rubinstein, E., Eds. ASM Press: Washington, D.C., 2003; pp 3-18.

20. Aldred, K. J.; Kerns, R. J.; Osheroff, N. Mechanism of quinolone action and resistance. *Biochemistry* **2014,** *53*, 1565-1574.

21. Hooper, D. C.; Jacoby, G. A. Topoisomerase inhibitors: fluoroquinolone mechanisms of action and resistance. *Cold Spring Harb. Perspect. Med.* **2016,** *6*.

22. Antibiotic Resistance Threats in the United States, 2019. https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf (accessed Mar 25, 2020).

23. Antimicrobial Resistance: Global Report on Surveillance 2014. http://www.who.int/drugresistance/documents/surveillancereport/en/ (accessed May 24, 2019).

24. A Scientific Roadmap for Antibiotic Discovery. https://www.pewtrusts.org/en/research-and-analysis/reports/2016/05/a-scientific-roadmap-for-antibiotic-discovery (accessed Mar 20, 2019).

25. Alanis, A. J. Resistance to antibiotics: are we in the post-antibiotic era? *Arch. Med. Res.* **2005,** *36*, 697-705.

26. Hwang, A. Y.; Gums, J. G. The emergence and evolution of antimicrobial resistance: Impact on a global scale. *Bioorg. Med. Chem.* **2016,** *24*, 6440-6445.

27. Overbye, K.; Barrett, J. Antibiotics: Where did we go wrong? *Drug Discov. Today* **2005,** *10*, 45-52.

28. Spellberg, B.; Guidos, R.; Gilbert, D.; Bradley, J.; Boucher, H. W.; Scheld, W. M.; Bartlett, J. G.; Edwards, J., Jr. The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. *Clin. Infect. Dis.* **2008,** *46*, 155-164.

29. Tse-Dinh, Y. C. Targeting bacterial topoisomerases: how to counter mechanisms of resistance. *Future Med. Chem.* **2016,** *8*, 1085-1100.

30. Basarab, G. S. Four ways to skin a cat: inhibition of bacterial topoisomerases leading to the clinic. In *Topics in Medicinal Chemistry: Antibacterials*, Fisher, J. F.; Mobashery, S.; Miller, M. J., Eds. Springer International Publishing: Cham, 2017; Vol. 25, pp 165-188.

31. Mayer, C.; Janin, Y. L. Non-quinolone inhibitors of bacterial type IIA topoisomerases: a feat of bioisosterism. *Chem. Rev.* **2014,** *114*, 2313-2342.

32. Tran, T. P.; Ellsworth, E. L.; Stier, M. A.; Domagala, J. M.; Hollis Showalter, H. D.; Gracheck, S. J.; Shapiro, M. A.; Joannides, T. E.; Singh, R. Synthesis and structural–activity relationships of 3-hydroxyquinazoline-2,4-dione antibacterial agents. *Bioorg. Med. Chem. Lett.* **2004,** *14*, 4405-4409.

33. Ellsworth, E. L.; Tran, T. P.; Showalter, H. D.; Sanchez, J. P.; Watson, B. M.; Stier, M. A.; Domagala, J. M.; Gracheck, S. J.; Joannides, E. T.; Shapiro, M. A.; Dunham, S. A.; Hanna, D. L.; Huband, M. D.; Gage, J. W.; Bronstein, J. C.; Liu, J. Y.; Nguyen, D. Q.; Singh, R. 3-aminoquinazolinediones as a new class of antibacterial agents demonstrating excellent antibacterial activity against wild-type and multidrug resistant organisms. *J. Med. Chem.* **2006,** *49*, 6435-6438.

34. Tran, T. P.; Ellsworth, E. L.; Sanchez, J. P.; Watson, B. M.; Stier, M. A.; Showalter, H. D.; Domagala, J. M.; Shapiro, M. A.; Joannides, E. T.; Gracheck, S. J.; Nguyen, D. Q.; Bird, P.;

Yip, J.; Sharadendu, A.; Ha, C.; Ramezani, S.; Wu, X.; Singh, R. Structure-activity relationships of 3-aminoquinazolinediones, a new class of bacterial type-2 topoisomerase (DNA gyrase and topo IV) inhibitors. *Bioorg. Med. Chem. Lett.* **2007,** *17*, 1312-1320.

35. Pan, X. S.; Gould, K. A.; Fisher, L. M. Probing the differential interactions of quinazolinedione PD 0305970 and quinolones with gyrase and topoisomerase IV. *Antimicrob. Agents Chemother.* **2009,** *53*, 3822-3831.

36. Aldred, K. J.; Schwanz, H. A.; Li, G.; McPherson, S. A.; Turnbough, C. L., Jr.; Kerns, R. J.; Osheroff, N. Overcoming target-mediated quinolone resistance in topoisomerase IV by introducing metal-ion-independent drug-enzyme interactions. *ACS Chem. Biol.* **2013,** *8*, 2660-2668.

37. Jeannot, F.; Taillier, T.; Despeyroux, P.; Renard, S.; Rey, A.; Mourez, M.; Poeverlein, C.; Khichane, I.; Perrin, M.-A.; Versluys, S.; Stavenger, R. A.; Huang, J.; Germe, T.; Maxwell, A.; Cao, S.; Huseby, D. L.; Hughes, D.; Bacqué, E. Imidazopyrazinones (IPYs): non-quinolone bacterial topoisomerase inhibitors showing partial cross-resistance with quinolones. *J. Med. Chem.* **2018,** *61*, 3565-3581.

38. Germe, T.; Vörös, J.; Jeannot, F.; Taillier, T.; Stavenger, R. A.; Bacqué, E.; Maxwell, A.; Bax, B. D. A new class of antibacterials, the imidazopyrazinones, reveal structural transitions involved in DNA gyrase poisoning and mechanisms of resistance. *Nucleic Acids Res.* **2018,** *46*, 4114-4128.

39. de Vicente Fidalgo, J.; He, H.; Hu, C.; Jiang, Z.; Li, X.; Lu, P.; Mergo, W.; Mutnick, D.; Reck, F.; Rivkin, A.; Skepper, C. K.; Wang, X. M.; Xia, J.; Xu, Y. Quinolone derivatives as antibacterials. U.S. Patent 10,160,726 B2, December 25, 2018.

40. Payne, D. J.; Gwynn, M. N.; Holmes, D. J.; Pompliano, D. L. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nat. Rev. Drug Discov.* **2007,** *6*, 29-40.

41. Silver, L. L. Challenges of antibacterial discovery. *Clin. Microbiol. Rev.* **2011,** *24*, 71-109.

42. Tommasi, R.; Brown, D. G.; Walkup, G. K.; Manchester, J. I.; Miller, A. A. ESKAPEing the labyrinth of antibacterial discovery. *Nat. Rev. Drug Discov.* **2015,** *14*, 529-542.

43. Nikaido, H. Prevention of drug access to bacterial targets: permeability barriers and active efflux. *Science* **1994,** *264*, 382-388.

44. Hancock, R. E. W. The bacterial outer membrane as a drug barrier. *Trends Microbiol.* **1997,** *5*, 37-42.

45. Nikaido, H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev.* **2003,** *67*, 593-656.

46. Li, X.-Z.; Nikaido, H. Efflux-mediated drug resistance in bacteria. *Drugs* **2004,** *64*, 159-204.

47. Kumar, A.; Schweizer, H. P. Bacterial resistance to antibiotics: active efflux and reduced uptake. *Adv. Drug Del. Rev.* **2005,** *57*, 1486-1513.

48. Lomovskaya, O.; Zgurskaya, H. I.; Totrov, M.; Watkins, W. J. Waltzing transporters and 'the dance macabre' between humans and bacteria. *Nat. Rev. Drug Discov.* **2007,** *6*, 56-65.

49. Delcour, A. H. Outer membrane permeability and antibiotic resistance. *Biochim. Biophys. Acta* **2009,** *1794*, 808-816.

50. Zgurskaya, H. I.; Lopez, C. A.; Gnanakaran, S. Permeability barrier of Gram-negative cell envelopes and approaches to bypass it. *ACS Infect. Dis.* **2015,** *1*, 512-522.

51. Silver, L. L. A Gestalt approach to Gram-negative entry. *Bioorg. Med. Chem.* **2016,** *24*, 6379-6389.

52. O'Shea, R.; Moser, H. E. Physicochemical properties of antibacterial compounds: implications for drug discovery. *J. Med. Chem.* **2008,** *51*, 2871-2878.

53. Manchester, J. I.; Buurman, E. T.; Bisacchi, G. S.; McLaughlin, R. E. Molecular determinants of AcrB-mediated bacterial efflux implications for drug discovery. *J. Med. Chem.* **2012,** *55*, 2532-2537.

54. Brown, D. G.; May-Dracka, T. L.; Gagnon, M. M.; Tommasi, R. Trends and exceptions of physical properties on antibacterial activity for Gram-positive and Gram-negative pathogens. *J. Med. Chem.* **2014,** *57*, 10144-10161.

55. Tommasi, R.; Iyer, R.; Miller, A. A. Antibacterial drug discovery: some assembly required. *ACS Infect. Dis.* **2018,** *4*, 686-695.

56. Richter, M. F.; Hergenrother, P. J. The challenge of converting Gram-positive-only compounds into broad-spectrum antibiotics. *Ann. N. Y. Acad. Sci.* **2019,** *1435*, 18-38.

57. Reck, F.; Jansen, J. M.; Moser, H. E. Challenges of antibacterial drug discovery. *Arkivoc* **2019,** *4*, 227-244.

58. Schümann, J.; Boudon, S.; Ulrich, P.; Loll, N.; Garcia, D.; Schaffner, R.; Streich, J.; Kittel, B.; Bauer, D. Integrated preclinical photosafety testing strategy for systemically applied pharmaceuticals. *Toxicol. Sci.* **2014,** *139*, 245-256.

59. Owens, R. C., Jr.; Ambrose, P. G. Antimicrobial safety: focus on fluoroquinolones. *Clin. Infect. Dis.* **2005,** *41*, S144-S157.

60. Stahlmann, R.; Lode, H. Toxicity of quinolones. *Drugs* **1999,** *58*, 37-42.

61. Albini, A.; Monti, S. Photophysics and photochemistry of fluoroquinolones. *Chem. Soc. Rev.* **2003,** *32,* 238-250.

62. Soldevila, S.; Cuquerella, M. C.; Bosca, F. Understanding of the Photoallergic Properties of Fluoroquinolones: Photoreactivity of Lomefloxacin with Amino Acids and Albumin. *Chem. Res. Toxicol.* **2014,** *27,* 514-523.

63. Soldevila, S.; Bosca, F. Photoreactivity of Fluoroquinolones: Nature of Aryl Cations Generated in Water. *Org. Lett.* **2012,** *14,* 3940-3943.

64. Cuquerella, M. C.; Miranda, M. A.; Boscá, F. Generation of Detectable Singlet Aryl Cations by Photodehalogenation of Fluoroquinolones. *J. Phys. Chem. B* **2006,** *110,* 6441-6443.

65. Compound 34 was tested in the hERG QPatch assay using a non-standard long incubation protocol (see Experimental Section for details). Under the standard conditions, steady state was not reached for some recordings. This can result in underestimation of the true IC50.

66. Kang, J.; Wang, L.; Chen, X.-L.; Triggle, D. J.; Rampe, D. Interactions of a series of fluoroquinolone antibacterial drugs with the human cardiac K+ channel HERG. *Mol. Pharmacol.* **2001,** *59,* 122-126.

67. Culley, C. M.; Lacy, M. K.; Klutman, N.; Edwards, B. Moxifloxacin: clinical efficacy and safety. *Am. J. Health Syst. Pharm.* **2001,** *58,* 379-388.

68. Darpo, B.; Nebout, T.; Sager, P. T. Clinical evaluation of QT/QTc prolongation and proarrhythmic potential for nonantiarrhythmic drugs: the international conference on harmonization of technical requirements for registration of pharmaceuticals for human use E14 guideline. *J. Clin. Pharmacol.* **2006,** *46,* 498-507.

69. Redfern, W. S.; Carlsson, L.; Davis, A. S.; Lynch, W. G.; MacKenzie, I.; Palethorpe, S.; Siegl, P. K. S.; Strang, I.; Sullivan, A. T.; Wallis, R.; Camm, A. J.; Hammond, T. G.

Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovasc. Res.* **2003,** *58*, 32-45.

70. Cavalluzzi, M. M.; Imbrici, P.; Gualdani, R.; Stefanachi, A.; Mangiatordi, G. F.; Lentini, G.; Nicolotti, O. Human ether-à-go-go-related potassium channel: exploring SAR to improve drug design. *Drug Discov. Today* **2020,** *25*, 344-366.

71. Clinical Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing: 28th edition; 2018.

72. Takei, M.; Fukuda, H.; Kishii, R.; Hosaka, M. Target preference of 15 quinolones against *Staphylococcus aureus*, based on antibacterial activities and target inhibition. *Antimicrob. Agents Chemother.* **2001,** *45*, 3544-3547.

73. Emrich, N.-C.; Heisig, A.; Stubbings, W.; Labischinski, H.; Heisig, P. Antibacterial activity of finafloxacin under different pH conditions against isogenic strains of *Escherichia coli* expressing combinations of defined mechanisms of fluoroquinolone resistance. *J. Antimicrob. Chemother.* **2010,** *65*, 2530-2533.

74. Chan, D. M. T.; Monaco, K. L.; Wang, R.-P.; Winters, M. P. New N- and O-arylations with phenylboronic acids and cupric acetate. *Tetrahedron Lett.* **1998,** *39*, 2933-2936.

75. Lam, P. Y. S.; Clark, C. G.; Saubern, S.; Adams, J.; Winters, M. P.; Chan, D. M. T.; Combs, A. New aryl/heteroaryl C-N bond cross-coupling reactions via arylboronic acid/cupric acetate arylation. *Tetrahedron Lett.* **1998,** *39*, 2941-2944.

76. Evans, D. A.; Katz, J. L.; West, T. R. Synthesis of diaryl ethers through the copper-promoted arylation of phenols with arylboronic acids. An expedient synthesis of thyroxine. *Tetrahedron Lett.* **1998,** *39*, 2937-2940.

77. Guram, A. S.; Rennels, R. A.; Buchwald, S. L. A simple catalytic method for the conversion of aryl bromides to arylamines. *Angew. Chem. Int. Ed.* **1995,** *34*, 1348-1350.

78. Louie, J.; Hartwig, J. F. Palladium-catalyzed synthesis of arylamines from aryl halides. Mechanistic studies lead to coupling in the absence of tin reagents. *Tetrahedron Lett.* **1995,** *36*, 3609-3612.

79. Wolfe, J. P.; Wagaw, S.; Buchwald, S. L. An improved catalyst system for aromatic carbon−nitrogen bond formation: the possible involvement of bis(phosphine) palladium complexes as key intermediates. *J. Am. Chem. Soc.* **1996,** *118*, 7215-7216.

80. Guari, Y.; van Es, D. S.; Reek, J. N. H.; Kamer, P. C. J.; van Leeuwen, P. W. N. M. An efficient, palladium-catalysed, amination of aryl bromides. *Tetrahedron Lett.* **1999,** *40*, 3789-3790.

81. Kaiser, N.-F. K.; Hallberg, A.; Larhed, M. In situ generation of carbon monoxide from solid molybdenum hexacarbonyl. A convenient and fast route to palladium-catalyzed carbonylation reactions. *J. Comb. Chem.* **2002,** *4*, 109-111.

82. Georgsson, J.; Hallberg, A.; Larhed, M. Rapid palladium-catalyzed synthesis of esters from aryl halides utilizing Mo(CO)6 as a solid carbon monoxide source. *J. Comb. Chem.* **2003,** *5*, 350-352.

83. Tschaen, D. M.; Desmond, R.; King, A. O.; Fortin, M. C.; Pipik, B.; King, S.; Verhoeven, T. R. An improved procedure for aromatic cyanation. *Synth. Commun.* **1994,** *24*, 887-890.

84. Maiti, D.; Fors, B. P.; Henderson, J. L.; Nakamura, Y.; Buchwald, S. L. Palladium-catalyzed coupling of functionalized primary and secondary amines with aryl and heteroaryl halides: two ligands suffice in most cases. *Chem. Sci.* **2011,** *2*, 57-68.

85. Bruno, N. C.; Tudge, M. T.; Buchwald, S. L. Design and preparation of new palladium precatalysts for C–C and C–N cross-coupling reactions. *Chem. Sci.* **2013,** *4*, 916-920.

86. Suzuki, A. Overview of the Suzuki protocol with B. In *Handbook of Organopalladium Chemistry for Organic Synthesis*, Negishi, E., Ed. John Wiley & Sons: New York, 2003; pp 249-262.

87. Miyaura, N. Metal-catalyzed cross-coupling reactions of organoboron compounds with organic halides. In *Metal-Catalyzed Cross-Coupling Reactions, Second Edition*, de Meijere, A.; Diederich, F., Eds. Wiley-VCH: Weinheim, 2004; pp 41-123.

88. Laroche, C.; Bertus, P.; Szymoniak, J. Titanium-mediated synthesis of bicyclic cyclopropylamines from unsaturated nitriles. *Tetrahedron Lett.* **2003,** *44*, 2485-2487.

89. Heaney, H.; Millar, I. T. Triphenylene. *Org. Synth.* **1960,** *40*, 105.

90. Clive, D.; Angoh, A. G.; Bennett, S. M. Radical spirocyclization: synthesis of an appropriately oxygenated spiro compound related to the antitumor antibiotic fredericamycin A. *J. Org. Chem.* **1987,** *52*, 1339-1342.

91. Molander, G. A.; Shin, I. Synthesis and Suzuki–Miyaura cross-coupling reactions of potassium Boc-protected aminomethyltrifluoroborate with aryl and hetaryl halides. *Org. Lett.* **2011,** *13*, 3956-3959.

92. Zhang, L.; Zheng, J.; Hu, J. 2-Chloro-2,2-difluoroacetophenone: a non-ODS-based difluorocarbene precursor and its use in the difluoromethylation of phenol derivatives. *J. Org. Chem.* **2006,** *71*, 9845-9848.

93. Clinical Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: 11th edition; 2018.

94. Bruchmann, S.; Dötsch, A.; Nouri, B.; Chaberny, I. F.; Häussler, S. Quantitative contributions of target alteration and decreased drug accumulation to *Pseudomonas aeruginosa* fluoroquinolone resistance. *Antimicrob. Agents Chemother.* **2013,** *57*, 1361.

95. Hoang, T. T.; Karkhoff-Schweizer, R. R.; Kutchma, A. J.; Schweizer, H. P. A broad-host-range Flp-*FRT* recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked *Pseudomonas aeruginosa* mutants. *Gene* **1998,** *212*, 77-86.

96. Caughlan, R. E.; Sriram, S.; Daigle, D. M.; Woods, A. L.; Buco, J.; Peterson, R. L.; Dzink-Fox, J.; Walker, S.; Dean, C. R. Fmt bypass in *Pseudomonas aeruginosa* causes induction of MexXY efflux pump expression. *Antimicrob. Agents Chemother.* **2009,** *53*, 5015.

97. Baba, T.; Ara, T.; Hasegawa, M.; Takai, Y.; Okumura, Y.; Baba, M.; Datsenko, K. A.; Tomita, M.; Wanner, B. L.; Mori, H. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol.* **2006,** *2*, 2006.0008.

98. Masuda, N.; Ohya, S. Cross-resistance to meropenem, cephems, and quinolones in Pseudomonas aeruginosa. *Antimicrob. Agents Chemother.* **1992,** *36*, 1847-1851.

99. Fraud, S.; Campigotto, A. J.; Chen, Z.; Poole, K. MexCD-OprJ multidrug efflux system of *Pseudomonas aeruginosa*: involvement in chlorhexidine resistance and induction by membrane-damaging agents dependent upon the AlgU stress response sigma factor. *Antimicrob. Agents Chemother.* **2008,** *52*, 4478-4482.

100. Ross, A. G.; Benton, B. M.; Chin, D.; De Pascale, G.; Fuller, J.; Leeds, J. A.; Reck, F.; Richie, D. L.; Vo, J.; LaMarche, M. J. Synthesis of ciprofloxacin dimers for evaluation of bacterial permeability in atypical chemical space. *Bioorg. Med. Chem. Lett.* **2015,** *25*, 3468-3475.

101. Gedeck, P.; Lu, Y.; Skolnik, S.; Rodde, S.; Dollinger, G.; Jia, W.; Berellini, G.; Vianello, R.; Faller, B.; Lombardo, F. Benefit of retraining pKa models studied using internally measured data. *J. Chem. Inf. Model.* **2015,** *55*, 1449-1459.

102. Allen, R. I.; Box, K. J.; Comer, J. E. A.; Peake, C.; Tam, K. Y. Multiwavelength spectrophotometric determination of acid dissociation constants of ionizable drugs. *J. Pharm. Biomed. Anal.* **1998,** *17*, 699-712.

103. Cao, X.; Lee, Y. T.; Holmqvist, M.; Lin, Y.; Ni, Y.; Mikhailov, D.; Zhang, H.; Hogan, C.; Zhou, L.; Lu, Q.; Digan, M. E.; Urban, L.; Erdemli, G. Cardiac ion channel safety profiling on the IonWorks Quattro automated patch clamp system. *Assay Drug Dev. Technol.* **2010,** *8*, 766-780.

104. McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J. Phaser crystallographic software. *J. Appl. Crystallogr.* **2007,** *40*, 658-674.

105. Emsley, P.; Cowtan, K. Coot: model-building tools for molecular graphics. *Acta Cryst. D Struct. Biol.* **2004,** *60*, 2126-2132.

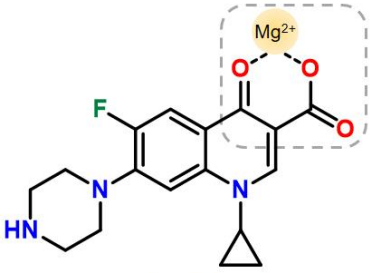
106. Emsley, P.; Lohkamp, B.; Scott, W. G.; Cowtan, K. Features and development of Coot. *Acta Cryst. D Struct. Biol.* **2010,** *66*, 486-501.

107. Adams, P. D.; Afonine, P. V.; Bunkoczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L.-W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P. H. PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Cryst. D Struct. Biol.* **2010,** *66*, 213-221.

108. Liebschner, D.; Afonine, P. V.; Baker, M. L.; Bunkoczi, G.; Chen, V. B.; Croll, T. I.; Hintze, B.; Hung, L.-W.; Jain, S.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R. D.; Poon, B. K.;

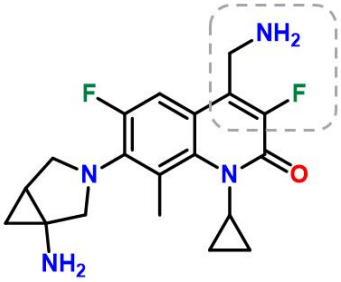
Prisant, M. G.; Read, R. J.; Richardson, J. S.; Richardson, D. C.; Sammito, M. D.; Sobolev, O. V.; Stockwell, D. H.; Terwilliger, T. C.; Urzhumtsev, A. G.; Videau, L. L.; Williams, C. J.; Adams, P. D. Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix. *Acta Cryst. D Struct. Biol.* **2019,** *75*, 861-877.

Table of Contents Graphic



ciprofloxacin

Potency driven by Mg^{2+}-dependent QRDR interactions



34

Minimal fluoroquinolone cross-resistance