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Research paper

Probing structural requirements for human topoisomerase I inhibition by a novel *N*1-Biphenyl fluoroquinolone

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ABSTRACT

Fluoroquinolones substituted with *N*-1 biphenyl and napthyl groups were discovered to act as catalytically inhibitors of human topoisomerases I and II, and to possess anti-proliferative activity *in vivo*. Structural requirements for these novel quinolones to inhibit catalytic activity of human topoisomerase I have not been explored. In this work novel derivatives of the *N*-1 biphenyl fluoroquinolone were designed, synthesized and evaluated to understand structural requirements of the *C*-3 carboxylic acid, *C*-6 fluorine, *C*-7 aminomethylpyrrolidine, *C*-8 methoxy, and the *N*-1 biphenyl functional groups for hTopoI inhibition. Characterization of each analog for inhibition of hTopoI catalytic inhibition reveals critical insight into structural requirements of these novel quinolones for activity. Additionally, results of DNA binding and modeling studies suggest that *N*-1 biphenyl fluoroquinolones intercalate between the DNA base pairs with the *N*-1 biphenyl functional group, rather than the quinolone core, and that this mode of DNA intercalation contributes to inhibition of hTopoI by these novel structures. The results presented here support further development and evaluation of *N*-1 biphenyl fluoroquinolone analogs as a novel class of anti-cancer agents that act through catalytic inhibition of hTopoI.

1. Introduction

DNA topoisomerases are ubiquitous enzymes that control the topology of DNA [1–5]. They play critical roles in many biological processes that involve DNA, such as DNA replication, chromosome segregation, and transcription. There are two types of topoisomerases, type I and type II [1–5]. Type I topoisomerases cleave one DNA strand of duplex DNA to create a nick, passing the other DNA strand through the nick, and then resealing the broken strand, whereas type II topoisomerases cleave both DNA strands of duplex DNA to create a double-strand break (DSB), passing another segment of duplex DNA through the DSB, and then resealing the broken DNA strands. Type I topoisomerases are further divided into type IA, type IB, and type IC subtypes. Type IA subtype includes topoisomerase III and bacterial topoisomerase I (Topo I). Eukaryotic Topo I belongs to the type IB subtype and a unique type I enzyme, topoisomerase V, from *Methanopyrus kandleri* is the only known type IC topoisomerase [6]. There are two subtypes of type II topoisomerases, type IIA and type IIB. Topoisomerase VI from archaea, some plants, and a few bacteria are type IIB topoisomerases. All other type II topoisomerases, including bacterial DNA gyrase and topoisomerase IV, as well as eukaryotic topoisomerase II (Topo II), belong to the type IIA subtype. Type IIA topoisomerases are cellular targets of clinically important antibacterial (e.g. fluoroquinolones) and anticancer (e.g. etoposide) drugs [7–12].

Topoisomerase inhibitors are classified as either 'topoisomerase poisons' or 'catalytic inhibitors' based on their modes of action [7–12]. Topoisomerase poisons bind a topoisomerase-DNA complex after DNA has been cleaved, block DNA religation, and trap a covalent topoisomerase-DNA catalytic intermediate as a topoisomerase-drug-DNA ternary complex. Thus, topoisomerase poisons convert an essential enzyme into a cytotoxic covalently-attached protein adduct. Ternary complex formation initiates cytotoxic events, including the inhibition of DNA replication and the generation of DSBs. Clinically successful antibacterial and anticancer drugs are topoisomerase poisons [7–12]. These include fluoroquinolones, which poison DNA gyrase and topoisomerase IV,

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and etoposide and doxorubicin, which poison human Topo II (hTopo II), as well as irinotecan, which poisons human Topo I (hTopo I). Although topoisomerase poisoning is an effective cell-killing mechanism, it is also the cause of various genotoxicities, including the development of therapy-related secondary cancer [13,14] because it induces DNA breaks.

Catalytic topoisomerase inhibitors interfere with steps other than the strand breakage-religation step to inhibit the catalytic activity of a topoisomerase [8,11,12,15,16]. Simocyclinone D8 and aclarubicin inhibit the DNA binding step [17–19], and aminocoumarins and bisdioxopiperazines are ATPase inhibitors [20–23]. Simocyclinone D8 and aminocoumarins target DNA gyrase and topoisomerase IV whereas aclarubicin and bisdioxopiperazines target hTopo II [8,11,12,15,16]. Unlike topoisomerase poisons, these catalytic inhibitors do not induce DNA breaks. However, their clinical success is limited primarily due to their lower potency compared to topoisomerase poisons as well as other liabilities independent of mechanism of action.

In previous studies we discovered that N1-biphenyl fluoroquinolone UITT-3-217 (**217**, Fig. 1) and N1-napthyl fluoroquinolone UITT-3-227 (**227**) could inhibit the catalytic activity of both hTopo I and hTopo II [24,25]. Fluoroquinolones are normally selective poisons of bacterial type II topoisomerases [7–12]. However, a few fluoroquinolones, such as voreloxin and CP-115,955, have been shown to poison hTopo II [26–28]. Thus, a modest inhibitory effect of **217** and **227** on hTopo II activity (IC_{50} values for 217 and 227 in a decatenation assay were 127.6 μM and 164.6 μM, respectively) was not unexpected [24]. However, it was completely unexpected to find that **217** could inhibit the catalytic activity of hTopo I (IC_{50} values for **217** and **227** in a relaxation assay were 26.0 μM and 43.7 μM, respectively) and that its activity against hTopo I was significantly higher than that against hTopo II [25]. Although the levels of topoisomerase inhibition by **217** appeared to be modest, the IC_{50} value of **217** in relaxation assay was similar to that (27 μM) of camptothecin [29]. These fluoroquinolones did not poison either hTopo I or hTopo II. To our knowledge, this is the first fluoroquinolone that is capable of inhibiting the catalytic activity of type I topoisomerases. The 60 DTP Human Tumor Cell Line Screen at the National Cancer Institute (NCI) showed that **217** and **227** exhibited significant growth inhibition (the mean GI_{50} values of 217 and 227 to be 1.9 μM and 3.1 μM, respectively) and the GI_{50} values of both **217** and **227** are significantly lower than that of etoposide [25]. A proof of concept efficacy study in mice with **217** and **227** using a colon cancer (HT-29) xenograft model showed that **217** inhibited the proliferation of colon cancer *in vivo* as well as fluorouracil, one of the first line drugs used for colon cancer treatment [25]. Compound **227** also exhibited activity but was not as effective as **217** in the xenograft model.

Toward advancing the translation of **217**, we designed and synthesized structural derivatives of **217** in order to determine the structural requirements of this novel N1-biphenyl fluoroquinolone to act as a catalytic inhibitor of hTopo I activity (Fig. 1). In addition, each compound prepared was evaluated for ability to inhibit the relaxation activity of hTopo I. These studies identified preferred structures at the *N*-1, *C*-3, *C*-7, and *C*-8 positions. Docking and modeling experiments suggested that **217** may intercalate into DNA through either the quinolone core or the N1-biphenyl group. An antibacterial fluoroquinolone moxifloxacin intercalates into DNA only through the quinolone core. Thus we propose a model that for the unique ability of N1-biphenyl fluoroquinolones to inhibit hTopo I activity is due to the DNA intercalation through the N1-biphenyl group.

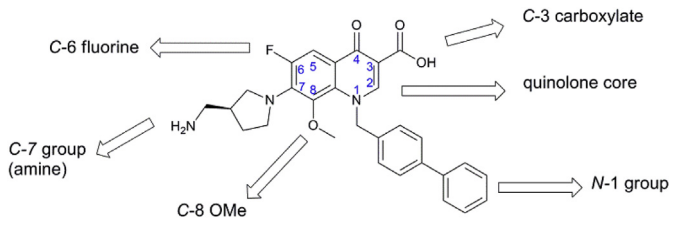


Fig. 1. Structural requirements for hTopo I inhibition by 217. Role of structural elements examined in this study are shown.

2. Results and discussion

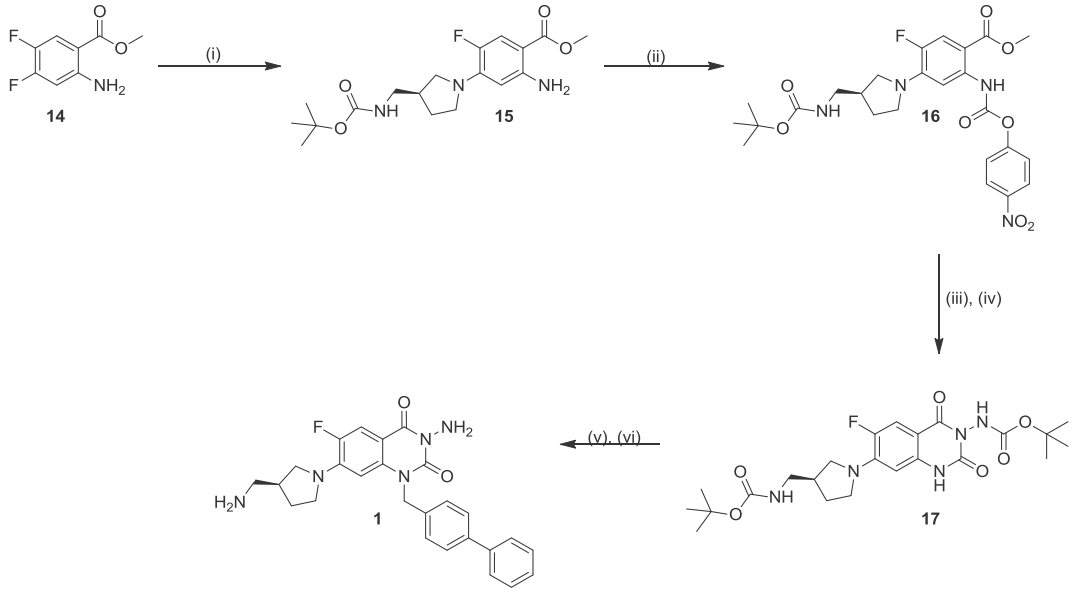
2.1. Chemistry

Synthesis of a quinazoline-2,4-dione derivative with *N*-1 1,1′-biphenyl-4-ylmethyl and naphthalen-2-ylmethyl substitutions, cognate derivatives of fluoroquinolones **217** and **227**, using traditional top-down or bottom-up approaches to ring closing was unsuccessful. The acidic nature of the benzylic protons for these *N*-1 substituents prevents ring closing using either of the traditional approaches. Therefore, as outlined in Scheme 1, we developed a novel route to obtain advanced intermediate **17** which is amenable to rapid derivatization at the *N*-1 position. Briefly, commercially available methyl 2-amino-4,5-difluorobenzoate (**14**) was reacted with (R)-(+)-3-(Boc)-amino)pyrrolidine in presence of base to give intermediate **15**. The aniline moiety of **15** was reacted with 4-nitrophenylchloroformate to give carbamate **16**, upon which tert-butylcarbazate was employed to displace 4-nitrophenol followed by cyclization in the presence of base to give Boc-protected quinazoline-2,4-dione (**17**). Alkylation of **17** with bromomethylbiphenyl, followed by deprotection of *C*-7 substituent Boc group under acidic conditions gave the desired quinazoline-2,4-dione UICK-II-215a (**1**).

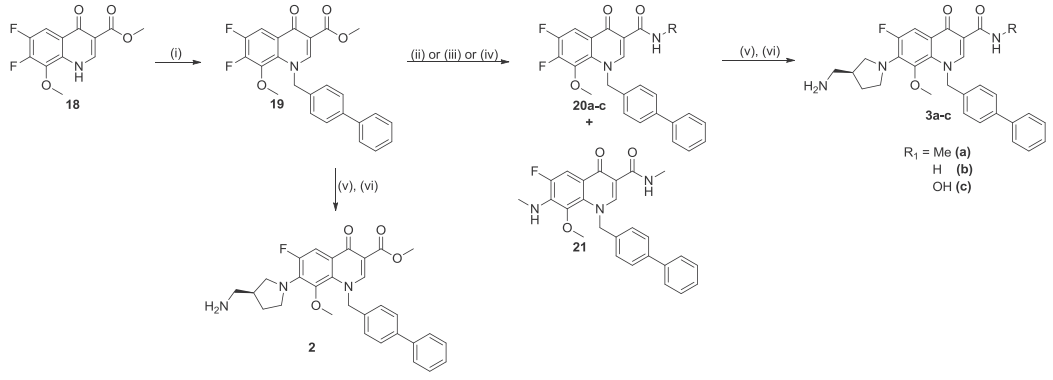
The synthesis and chemical analysis of **217** and advanced intermediates (**18, 19** and **22**) have been reported previously [24]. Synthesis of *C*-3 modified derivatives using this synthetic route and these intermediates is shown (Scheme 2). First, aniline **18** was alkylated with a biphenyl alkyl halide at the *N*-1 position under basic conditions, yielding ester intermediate **19**. The methyl ester **19** was dissolved in methylamine (neat), 2 mM ammonia in methanol, or 1 N hydroxylamine in EtOH to form the *C*-3 *N*-methyl amides **20a** and **21**, amide **20b**, or hydroxamic acid **20c**, respectively. The *C*-7 aminomethylpyrrolidine (AMP) group was added via S_N-aromatic substitution of the *C*-7 fluorine. Subsequent treatment with aqueous hydrochloric acid removed the Boc protecting group to afford **3 a-c** (Scheme 2). The *C*-3 ester analog **2** was synthesized from intermediate **19** by direct S_NAr substitution of the *C*-7 fluorine with the AMP group followed by treatment with aqueous hydrochloric acid to remove the Boc protecting group (Scheme 2).

Ester **19** was employed as common intermediate to synthesize a series of *C*-7 substituted analogs of **217**. Ester hydrolysis using lithium hydroxide yields carboxylic acid **22**, as shown in Scheme 3. The *C*-7 substituents were subsequently added through S_NAr substitution of the *C*-7 fluorine with various amines to afford **4 a-i**. Analogs **4 c-i** required subsequent treatment with aqueous hydrochloric acid to remove the Boc protecting group (Scheme 3).

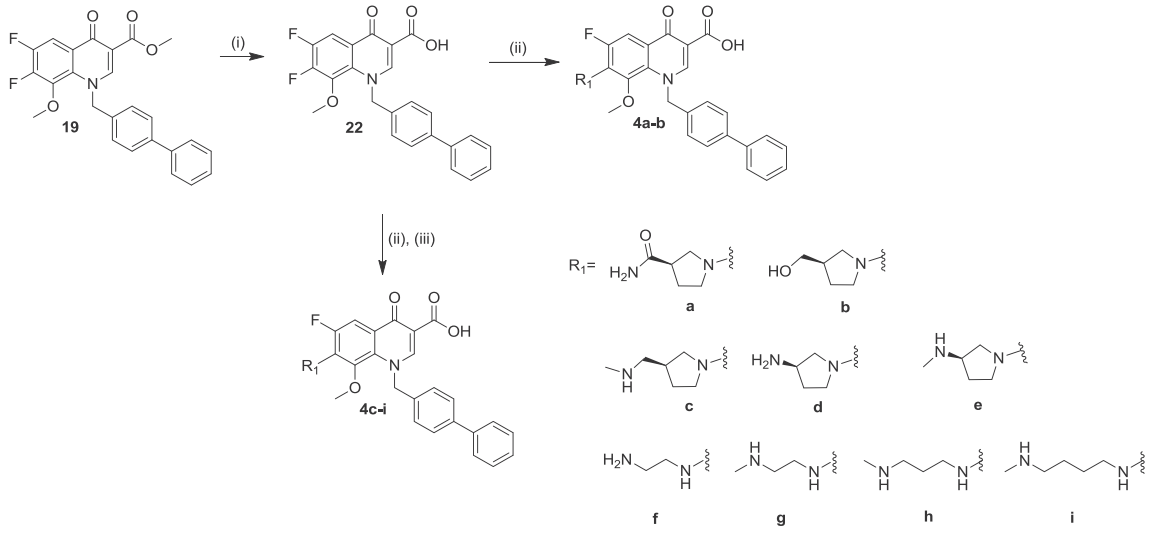
Analogs of **217** having *C*-8 hydrogen in place of *C*-8 methoxy were synthesized through an alternate route for quinolone core formation. Commercially available 2,4,5-trifluoro-β-oxobenzenepropanoic acid ethyl ester, **23**, was refluxed in triethylorthoformate and acetic anhydride to afford enol ether intermediate **24** (Scheme 4). Enol ether **24** was then substituted with 4-(aminomethyl) biphenyl and subsequently treated with 18-crown-6 to effect cyclization to obtain the quinolone ethyl ester **25**. Hydrolysis of ester **25** under basic conditions with lithium hydroxide affords



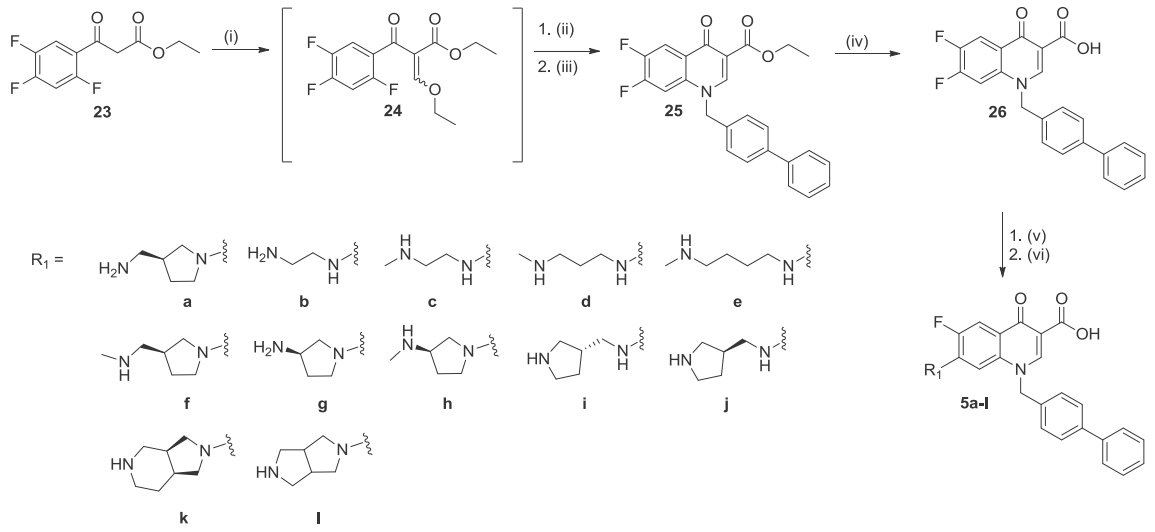
Scheme 1. Synthetic route for quinazoline-2,4-dione analog UICK-II-215a (**1**). (i) (R)-(+)-3-(Boc)-amino)pyrrolidine, TEA, DMF, 80 °C. (ii) 4-nitrophenyl-chloroformate, DIPEA, DCM, rt. (iii) t-butylcarbazate, DIPEA, THF, reflux. (iv) K_2CO_3, MeOH, 50 °C. (v) 4-bromomethylbiphenyl, K_2CO_3, THF, reflux. (vi) TFA, reflux.



Scheme 2. Synthetic route for series of *C*-3 modified, *C*-8 methoxy fluoroquinolones **2**, **3 a-c**, and **21**. Reagents and conditions: (i) 4-(bromomethyl)-1,1′-biphenyl, K_2CO_3, CAN; (ii) 40% Methylamine in H_2O, ACN, 60 °C; (iii) Ammonia in MeOH, 35 °C; (iv) hydroxylamine•HCl in EtOH, room temperature (rt); (v) (R)-(+)-3-(Boc)-amino)pyrrolidine, TEA, DMSO, 60 °C; and (vi) 3 N HCl, ACN, rt.



Scheme 3. Synthetic route for *N*-1 biphenyl, *C*-7 modified derivatives **4 a-i**. (i) 1% LiOH, THF, rt. (ii) 1° or 2° amine, TEA, DMSO or ACN, rt - 60 °C. (iii) TFA, rt or 3 N HCl, ACN, rt.



Scheme 4. Synthetic route for series of C-8 hydrogen, C-6 fluorine analogs **5 a-l**. (i) Ac_2O, triethylorthoformate, reflux. (ii) [1,1′-biphenyl]-4-ylmethanamine, 1,4-dioxane, rt. (iii) K_2CO_3, 18-crown-6, 85 °C. (iv) 1% LiOH, THF, rt - 40 °C. (v) 1° or 2° amine, TEA or DIPEA, ACN or DMSO, 35–70 °C. (vi) 3 N or 4 N HCl, ACN, rt.

carboxylic acid **26**. Intermediate **26** was then substituted at *C*-7 with selected primary or secondary amines to give **5 a-l**. Subsequent treatment with aqueous hydrochloric acid was required to remove Boc protecting groups, when present, to afford **5 a-k**.

Two *C*-6 des fluoro derivatives of **217** were synthesized beginning from commercially available 2,4-difluorobenzoic acid, **27**, which on reaction with ethyl potassium malonate afforded propanate intermediate **28** (Scheme 5). Ethyl 3-(2,4-difluorophenyl)-3-oxopropanoate, **28**, was then refluxed in triethylorthoformate and acetic anhydride to give enol ether intermediate **29**, which was substituted with 4-(aminomethyl)biphenyl or (4-(1H-pyrazol-1-yl) phenyl)methanamine and subsequently treated with sodium hydroxide to effect cyclization to furnish ethyl esters **30 a-b**. The ethyl esters were hydrolyzed using lithium hydroxide to give carboxylic acids **31 a-b**, to which the AMP group was added substituted at the *C*-7 position, followed by treatment with aqueous hydrochloric acid to remove the Boc protecting group, to afford **6 a-b**.

A series of *C*-8 OMe, *N*-1 bis-aryl derivatives was synthesized starting from intermediate **18** (Scheme 6). The secondary aniline (*N*-1) of **18** was alkylated with the selected bis-aryl alkyl halides under basic conditions to yield compounds **32 a-c**. Hydrolysis of methyl ester under basic conditions yields carboxylic acids **33 a-c**. Subsequent S_NAr substitution of the *C*-7 fluorine with Boc-AMP or Boc-(R)-aminopyrrolidine (Boc-AP), followed by treatment with aqueous hydrochloric acid to remove the Boc protecting groups, afforded derivatives **7 a-c** and **8c**, respectively (Scheme 6).

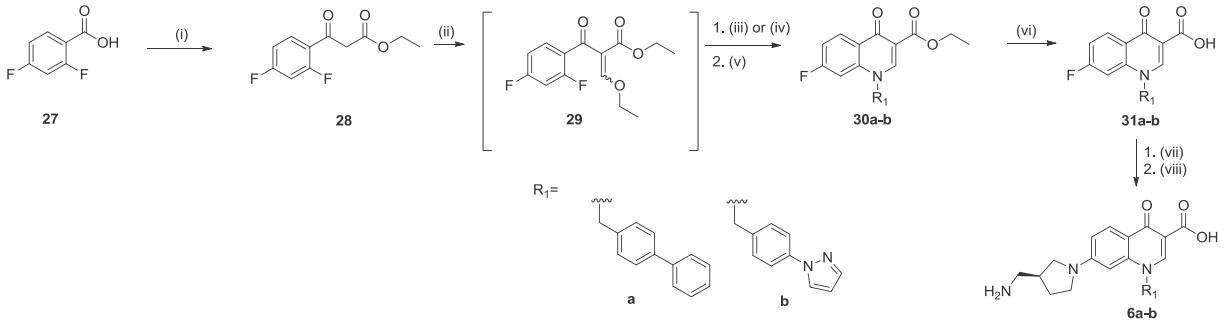
Various *C*-8 H, *N*-1 bis-aryl derivatives were synthesized from intermediate **24**. Substitution of enol ether **24** with the appropriate amine, followed by treatment with sodium hydride to afford cyclization, and subsequent ester hydrolysis with lithium hydroxide affords intermediates **9**–**13**. Intermediate **9** was substituted at *C*-7 with various secondary amines under weakly basic conditions to give **9 a-f**. Subsequent treatement with aqueous hydrochloric acid was required to remove *C*-7 Boc protecting groups, when present, to afford analogs **9 a-e**. Similarly, the AMP group was substituted at the *C*-7 position of carboxylic intermediates **10**–**13**, affording **10a**, **11a**, **12a**, and **13a**. Subsequent treatement with aqueous hydrochloric acid was required to remove the Boc protecting group, as shown in Scheme 7.

2.2. Activities of 217 derivatives against hTopo I

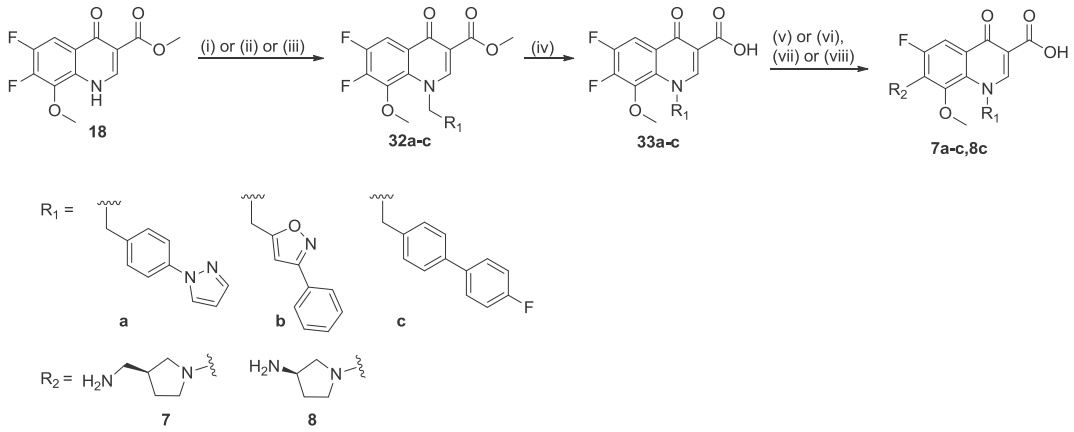
2.2.1. The quinolone core is not required for catalytic inhibiton of hTopo I

First, we examined if the quinolone core structure is essential for the ability of *N*-1-biphenyl quinolones to inhibit the catalytic activity of hTopo I. An *N*-1-biphenyl quinazoline-2,4-dione UICK-2-215a (**1**), which lacks a *C*-3-carboxylate group, and has a *N*-3-amino group, was synthesized and found to be as active as **217** (Table 1).

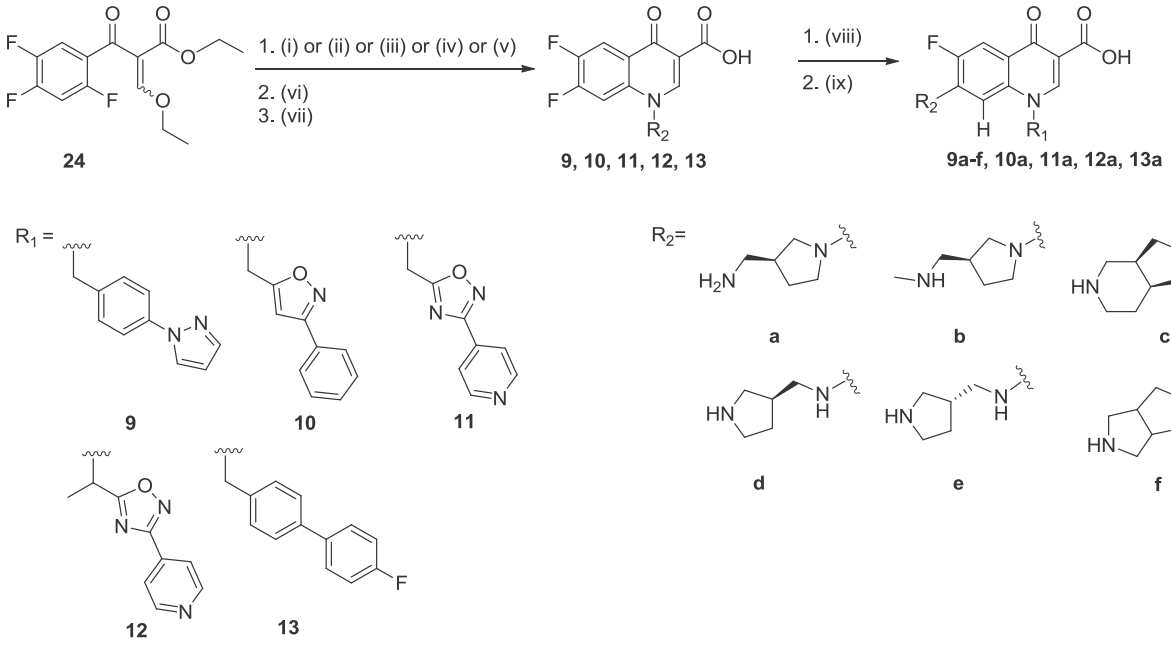
To further evaluate the role and/or requirement of the *C*-3-



Scheme 5. Synthetic route for series of *C*-8 hydrogen, *C*-6 hydrogen analogs **6 a-b**. (i) Ethyl potassium malonate, TEA, oxalyl chloride, ACN, DCM, 0 °C to rt. (ii) Ac_2O, triethylorthoformate, 120 °C. (iii) [1,1′-biphenyl]-4-ylmethanamine, MeOH, 0 °C to rt. (iv) (4-(1H-pyrazol-1-yl)phenyl)methanamine, MeOH, 0 °C to rt. (v) NaH, dioxane, 0 °C to rt. (vi) 1% LiOH, THF, 40 °C. (vii) (R)-(+)-3-(Boc)-amino)pyrrolidine, DIPEA, ACN or DMSO, 35–70 °C. (viii) 3 N HCl, ACN, rt.



Scheme 6. Synthetic route for *C*-8 methoxy, *N*-1 aryl fluoroquinolone derivates, **8 a-c**, **9c**. (i) 1-(4-(bromomethylphenyl)-1H-pyrazole, K_2CO_3, ACN, 45 °C. (ii) 5-(bromomethyl)-3-phenylisoxazole, K_2CO_3, ACN, 45 °C. (iii) 4-fluoro-4′-methyl-1,1′-biphenyl, K_2CO_3, DMF, 50 °C. (iv) 1% LiOH, THF, rt. (v) (R)-tert-butyl(pyrrolidin-3-ylmethyl)carbamate, TEA, DMSO or ACN, rt - 60 °C. (vi) (R)-(+)-3-(Boc-amino)pyrrolidine, TEA, ACN. (vii) TFA, rt. (viii) 4 N HCl, ACN, rt.



Scheme 7. Synthetic route for *C*-8 hydrogen, *N*-1 bis-aryl fluoroquinolone analogs **9a-f**, **10a**, **11a**, **12a**, and **13a**. (i) 4-(1H-pyrazol-1-yl)phenyl methanamine, MeOH, 0 °C to rt. (ii) 3-phenylisoxazol-5-yl methylamine, MeOH, 0 °C to rt. (iii) 3-(4-pyridinyl)-1,2,4-oxadiazole-5-methanamine, MeOH, rt. (iv) 1-(3-pyridin-4-yl-1,2,4-oxadiazol-5-yl)ethanamine, MeOH, 0 °C to rt. (v) (4′-fluoro-[1,1′-biphenyl]-4-yl)methanamine, MeOH, rt. (vi) NaH, dioxane, 0 °C to rt. (vii) 1% LiOH, THF, rt. (viii) 2° amine, TEA or DIPEA, DMSO, 40° - 70 °C. (ix) 4 N HCl, ACN, rt.

carboxylic acid for hTopo I inhibition, the methyl ester [UIJD-2-224 (**2**)], N-methyl amide [UIJD-2-242 (**3a**), hydroxamic acid [UIJD-2-246 (**3c**)] and amide [UIJD-2-251 (**3b**)] analogs of **217** were compared in the relaxation assay with hTopo I (Table 1). Similar to the quinazoline-2,4-dione (**1**), the *C*-3-hydroxamic acid (**3c**) was as active as **217**. However, *C*-3- methyl ester (**2**), *N*-methyl amide (**3a**), and amide (**3b**) were 3- to 7-fold less active than **217**. These results demonstrated that the *C*-3-carboxylate group was not required for the catalytic inhibition of hTopo I activity by *N*-1-biphenyl compounds, but it could modulate the activity.

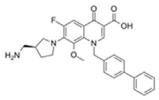
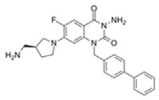
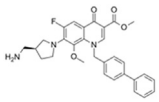
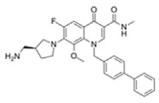
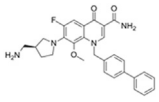
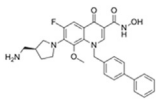
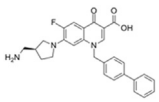
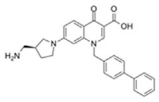
Since quinazoline-2,4-dione (**1**), which has a *C*-8-hydrogen (*C*-8-H) (C8-H) in place of *C*-8-methoxy (OMe), was as active as **217**, the *C*-8-H, N1-biphenyl fluoroquinolone CK-2-065 (**5a**) was synthesized in order to initially determine potential effect of the *C*-8-OMe substituent. The relaxation assay with hTopo I demonstrated that fluoroquinolone **5a** was slightly more active against hTopo I than **217** (Fig. 2 and Table 1), suggesting that the *C*-8-OMe group was not required for the inhibition of hTopoI. In fact, the hydrogen appeared to be preferred over a methoxy group at the *C*-8 position.

Substitution of the quinolone core with a *C*-6 fluorine dramatically enhances antibacterial potency. However, it was not clear if a *C*-6 fluorine would or would not play a role in the catalytic inhibition of hTopoI. To directly examine the role of the *C*-6 fluorine, UIPC-2-086B (**6a**), a *C*-6 desfluoro quinolone analog of **5a**, was synthesized. The comparison of the IC_{50} value of **6a** with that of **5a** showed that the loss of the C6-fluorine resulted in an approximate 4-fold reduction in inhibitory potency against hTopoI (Table 1). Thus, the C6-fluorine can affect, although is not essential for, the inhibition of hTopo I by N1-biphenyl quinolones.

2.2.2. C7 derivatives of N1-biphenyl fluoroquinolones

Primary amines are susceptible to metabolism due to *N*-

Table 1
Effect of changes in the quinolone core on hTopo I inhibition.

Compound	Structure	IC_{50} (μM)[a]
217		26.0 ± 0.5[b]
215a/**1**		25.9 ± 5.4
224/**2**		71.4 ± 1.6
242/**3a**		82.1 ± 4.6
251/**3b**		184.2 ± 6.9
246/**3c**		35.9 ± 3.6
065/**5a**		15.6 ± 1.1
086B/**6a**		61.2 ± 0.9

[a] The IC_{50} values against hTopo I were determined in the relaxation assays [25].
[b] Data from the previous study [25].

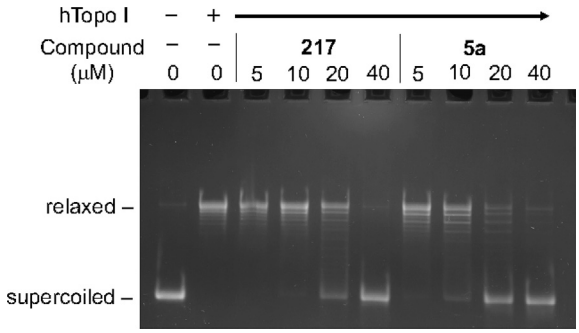


Fig. 2. Relaxation assay for hTopo I Inhibition. The activities of **217** and **5a** against hTopo I were determined in the relaxation assays for hTopo I [25]. Representative results are shown here.

acetylation and oxidation by monoaminoxidase and CYP450 [30,31]. Thus, the primary amine on the *C*-7-AMP group is a predicted structural liability due to metabolic instability. Here, we looked to determine the structural requirements of the *C*-7 functional group while concomitantly improving likely metabolic stability of the *C*-7 side chain. Removal of the *C*-7-AMP group (UIJD-2-214B (**22**) and UIJD-2-215B (**21**)) completely abolished ability to inhibit hTopo I (Table 2). Thus, we introduced substitutions for the

Table 2
Effects of C7 modifications on hTopo I inhibition.

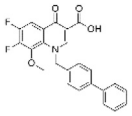
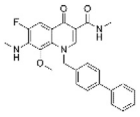
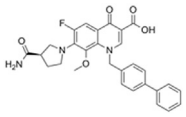
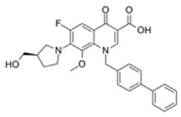
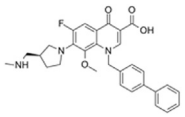
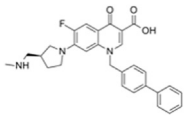
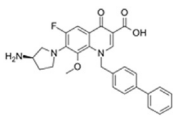
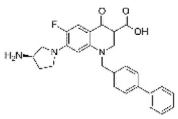
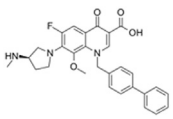
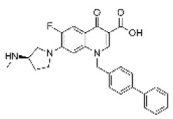
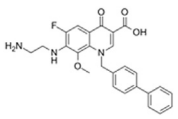
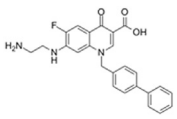
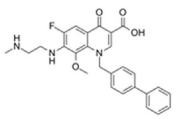
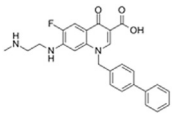
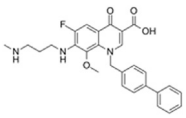
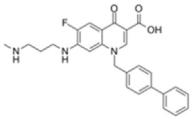
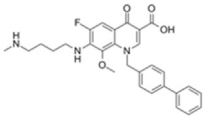
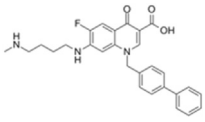
Compounds	Structure	hTopo I inhibition[a]
214B/**22**		None[b]
215B/**21**		None[b]
115/**4a**		> 200[c]
116/**4b**		> 200[c]
086/**4c**		154.6 ± 26.7
097/**5f**		167.6 ± 22.3
226/**4d**		92.2 ± 0.6
117/**5g**		63.6 ± 6.6
228/**4e**		57.7 ± 6.6
118/**5h**		34.8 ± 0.3
264/**4f**		> 200[c]
281B/**5b**		72.8 ± 7.7
276B/**4g**		> 200[c]

Table 2 (*continued*)

Compounds	Structure	hTopo I inhibition[a]
282B/**5c**		> 200[c]
275B/**4h**		101.0 ± 1.7
269B/**5d**		63.6 ± 6.0
285B/**4i**		> 200[c]
271B/**5e**		88.3 ± 1.1

[a] IC_{50} values (μM) determined in the relaxation assay.
[b] None, no inhibition was detected at 200 μM.
[c] Compounds with IC_{50} values > 200 exhibited at least 10% inhibition at 200 μM.

primary amine of AMP with either a secondary amine, or non-amine, as well as various substitutions of the entire pyrrolidine ring.

First, the effect of changing the primary amine on the *C*-7-AMP group to an amide [UIJD-2-115 (**4a**)] or hydroxyl [UIJD-2-116 (**4b**)] on activity against hTopo I were assessed. Neither the amide (**4a**) nor hydroxyl (**4b**) derivatives retained hTopo I inhibitory activity (Table 2). Combined, these results showed the terminal amine on the *C*-7-AMP group is critical for hTopo I inhibition.

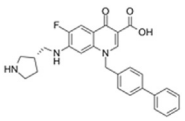
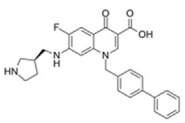
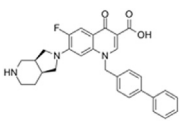
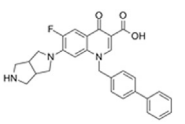
Next, we replaced the pyrrolidine ring with a more flexible alkyl chain with both C8-OMe [UIJD-1-264 (**4f**), UIJD-2-276B (**4g**), UIJD-2-275B (**4h**), and UIJD-2-285B (**4i**)] and C8-H [UIJD-2-281B (**5b**), UIJD-2-282B (**5c**), UIJD-2-269B (**5d**), and UIJD-2-271B (**5e**)] (Table 2). Additionally, the effect of the distance of the C7 amine from the quinolone core was examined by placing the amine at 4, 5, or 6 atoms away from the quinolone core. Compounds **4f** and **4g**, as well as **5b** and **5c**, have either a primary (**4f** and **5b**) or secondary amine (**4g** and **5c**) placed at 4 atoms away from the quinolone core. Compounds **4h** and **5d**, and **4i** and **5e** have a secondary amine placed at 5 and 6 atoms away from the quinolone core, respectively. All of these analogs with a flexible *C*-7 side chain had 4-fold or greater reduced activity as compared to their parent compound, either **217** or **5a** (Table 2), indicating that a certain rigidity of the *C*-7 side chain is likely important for optimal catalytic inhibition of hTopo I. Although their activities were reduced, the analogs with a secondary amine placed at 5 atoms away from the quinolone core (**4h** and **5d**) exhibited the highest activity, whereas analogs with a secondary amine placed at 4 atoms away from the quinolone core (**4g** and **5c**) exhibited lowest activity. As was the case with **5a** relative to **217** (Table 1), the *C*-7 flexible analogs with the *C*-8-H core were more active than those with the *C*-8-OMe core (Table 2).

We also substituted the terminal primary amine of the AMP group with an *N*-methyl amine [UIJD-2-086 (**4c**)] and [UICK-II-097 (**5f**)]. Although the activity of *N*-methyl derivatives (**4c**) and (**5f**) was significantly affected, both exhibited equivalent hTopoI inhibitory activity (Table 2). Furthermore, the *C*-7-AMP group of **217** was substituted with either an aminopyrrolidine [UIJD-2-226 (**4d**), with *C*-8-OMe] or [UIJD-3-117 (**5g**) with *C*-8-H] and a (methylamino) pyrrolidine [UIJD-2-228 (**4e**) with *C*-8-OMe] or [UIJD-3-118 (**5h**) with *C*-8-H] to evaluate the required positioning of the *C*-7 amine on the pyrrolidine ring. The activity of the *C*-8-OMe analogs, with *C*-7-(methylamino) pyrrolidine substituted (**4e**) was about 2-fold lower than that of **217**, whereas the activity of the *C*-7-aminopyrrolidine (**4d**) was more than 3-fold lower than that of **217** (Table 2). The activity of the *C*-8-H analogs, with *C*-7-aminopyrrolidine substituted (**5g**) is 4-fold lower than **5a**, whereas the *C*-7-(methylamino)pyrrolidine analog (**5h**) was only 2-fold lower than that of **5a**. Both the *C*-8-H analogs **5g** and **5h** had improved inhibition of hTopoI as compared to the cognate *C*-8-OMe analogs **4d** and **4e**. However, all modifications to the AMP resulted in reduced inhibition of hTopo I.

Results described above demonstrated that structural derivatives having a *C*-8-H were more potent inhibitors of hTopo I than those substituted at *C*-8 with a methoxy group. Therefore, additional *C*-8-H derivatives having a secondary amine on the *C*-7 functional group were synthesized and evaluated [UICK-4-089 (**5l**), UICK-4-091 (**5k**), UICK-4-093 (**5i**), UICK-4-095 (**5j**). This panel of **5a** analogs was designed to determine if a secondary amine with more rigid moieties could maintain activity against hTopo I. Two *C*-7-pyrrolidine(methanamine) isomers (**5i** and **5j**) were synthesized to compare the stereochemistry requirement, if any, at the *C*-7 position. Both isomers had essentially identical activity against hTopo I (Table 3). Furthermore, their activities were somewhat similar to the activity of the *C*-7 *N*-methylpropylenediamine (**5d**) analog with a secondary amine placed at 5 atoms from the core by a flexible alkyl chain. Thus, this data suggests that there is no rigid three-dimensional orientation requirement for the secondary amine at the *C*-7 position.

Compound **5k**, which has the more rigid diazabicyclononane group substituted at the *C*-7 position, was 4-fold less active than the cognate *C*-7-AMP derivative, **5a** (Table 3). However, **5k** was nearly 3-fold more active than the *C*-7 *N*-methyl AMP analog, **5f**, which also bears a secondary amine. These results further suggest

Table 3
Effects of C7 modifications on hTopo I inhibition.

Compounds	Structure	hTopo I inhibition[a]
093/**5i**		103.9 ± 8.4
095/**5j**		100.4 ± 5.0
091/**5k**		61.0 ± 6.2
089/**5l**		33.3 ± 1.0

[a] IC_{50} values (μM) determined in the relaxation assay.

that increased rigidity of the *C*-7 side chain may improve the activity of *N*-1-biphenyl fluoroquinolones against hTopo I. To test this possibility, compound **5l** with a rigid diazabicyclooctane group at the *C*-7 position was synthesized. Compound **5l** turned out to be one of the most active analogs tested in this study (Table 3), with only a 2-fold loss in activity compared to **5a**. Since **5l** is expected to have improved metabolic stability, it will be further tested for its *in vitro* and *in vivo* anti-proliferative activity as compared to parent derivative **217**.

2.2.3. N1 derivatives

As compared to typical antibacterial fluoroquinolones, the N1-biphenyl modified derivatives studied above had generally poor aqueous solubility, which limited the dosing range in the *in vivo* studies [25]. To improve aqueous solubility while concomitantly determining the structural requirements of the *N*-1 functional group for hTopo I inhibition, we substituted the phenyl rings of the biphenyl group with a pyrazole [UIJD-2-292B (**7a**) and UIJD-2-290B (**9a**)], oxazole [UIJD-2-294B (**7b**) and UIJD-2-286B (**10a**)], or pyridinyl diazaoxazole [UIJD-3-130 (**11a**) and UIJD-3-067C (**12a**)]. The *N*-1 pyrazole (**7a**) and oxazole (**7b**) analogs having a *C*-8-OMe substituent displayed a 7-fold or greater loss in hTopo I inhibition as compared to *N*-1 biphenyl **217** (Table 4). The *N*-1 pyrazole (**9a**) and oxazole (**10a**) analogs, with unsubstituted C8-H, had a 4-fold to 6-fold loss in hTopo I inhibition as compared to *N*-1 biphenyl **5a**. Neither of the *N*-1 diazaoxazole pyridine analogs (**11a** and **12a**) inhibited hTopoI. These results demonstrated that the 6-membered bicyclic rings are likely preferred for hTopoI inhibition over the 5-membered heterocyclic rings. Again, derivatives unsubstituted at the C-8 position (*C*-8-H) had lower IC_{50} values compared to the cognate *C*-8-OMe derivatives (Table 4), supporting our earlier finding that *C*-8-H is preferred over *C*-8-OMe for hTopo I inhibition.

While these modifications at the *N*-1 position did not improve hTopoI inhibitory activity, the *N*-1 heterocyclic analogs were designed to improve aqueous solubility of the parent compounds. Of the *N*-1 heterocyclic analogs that inhibited hTopo I, the *N*-1 pyrazole analog **9a** was predicted to have the best aqueous solubility based on the calculated logP (Table S1). Combining preferred structural modifications, we synthesized UIPC-II-159B (**6b**), a *C*-6 desfluoro quinolone analog of *N*-1 pyrazole **9a**. Unlike **6a**, which exhibited 4-fold lower activity than its cognate *C*-6-fluorine analog **7a**, **6b** had a slightly higher activity than **9a** (Table 4). These results support the previous finding that the *C*-6-fluorine is not essential for the inhibition of hTopo I, and that structural change to *C*-6 affects whether a given *N*-1 substituent will increase or decrease potency.

An overriding consideration of **217** as a novel catalytic inhibitor of hTopo I is that the biphenyl group is predicted to be a promiscuous protein binding group and a structural liability, susceptible to metabolism [32,33]. Thus, *N*-1 4′-fluoro biphenyl derivatives, UIJD-1-277 (**7c**), UIJD-2-244 (**8c**), and UIJD-3-133 (**13a**) were designed to protect a predicted site of metabolism on the biphenyl group (Figure S2), and evaluated (Table 4). The 4-fluorobiphenyl (**7c**) analog exhibited 2-fold lower activity than parent compound **217**. The inhibition of hTopo I by the 4-fluorobiphenyl derivative also having a *C*-7-aminopyrrolidine group (**8c**) was slightly better than that of the cognate *N*-1-biphenyl (**4d**) derivative (Table 4). The *in vivo* stability of **7c** and **8c**, together with **217** and **4d**, will be tested in future studies to determine if, in fact, these modifications improve metabolic stability of the *N*-1-biphenyl functionality (Figure S1 and S2).

A series of *N*-1-pyrazole, *C*-7-secondary amine derivatives, UIJD-2-299 (**9f**), UIJD-2-304B (**9b**), UIJD-2-300B (**9c**), UIJD-2-303B (**9d**), and UIJD-2-298B (**9e**), were synthesized to combine structural features likely to both improve aqueous solubility and *C*-7

Table 4
Effects of *N*-1 modifications on hTopo I inhibition.

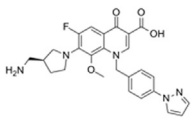
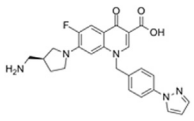
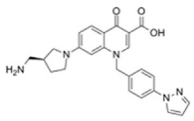
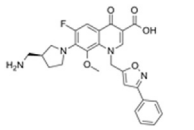
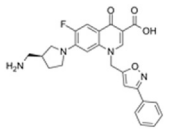
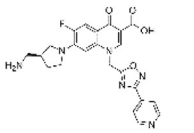
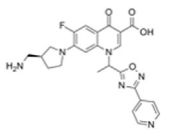
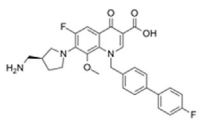
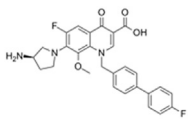
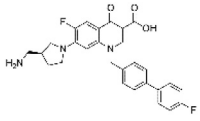
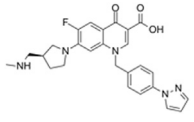
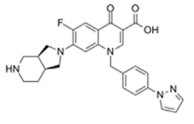
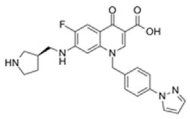
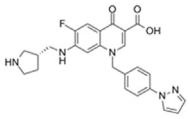
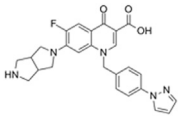
Compounds	Structure	hTopo I inhibition[a]
292B/**7a**		>200
290B/**9a**		91.6 ± 6.5
159B/**6b**		61.0 ± 3.7
294B/**7b**		178.4 ± 7.9
286B/**10a**		58.3 ± 2.8
130/**11a**		>200
067C/**12a**		>200
277/**7c**		60.9 ± 5.4
244/**8c**		58.6 ± 4.6
133/**13a**		69.6 ± 3.0
304B/**9b**		>200
300B/**9c**		31.3 ± 1.7

Table 4 (*continued*)

Compounds	Structure	hTopo I inhibition[a]
303B/**9d**		187.4 ± 4.2
298B/**9e**		>200
299/**9f**		172.5 ± 4.0

[a] IC_{50} values (μM) determined in the relaxation assay.

metabolic stability. Compound **9f**, having a *C*-7-diazabicyclooctane group to increase rigidity at the *C*-7 position exhibited a 2-fold loss in hTopoI inhibitory activity compared to **9a**, with the *C*-7-AMP group that contains a primary amine (Table 4). The pyrrolidine methylamine isomers **9d** and **9e**, as well as *N*-methyl pyrrolidine-methanamine, **9b**, had reduced hTopo I inhibition as compared to the cognate *C*-7-AMP compound, **9a**. We found that compound **9c**, having a *C*-7-diazabicyclononane group, to be the best inhibitor of hTopo I as compared to **9a**, with a 2-fold improvement of hTopo I inhibition (Table 4). Thus, **9c** together with **5l**, will be tested for *in vitro* and *in vivo* anti-proliferative activity in future studies.

2.3. A model for the interaction of 217 with DNA

We have previously showed that **217** can intercalate into DNA [25]. To gain the insight into how **217** may interact with double-stranded DNA in a way that is different from antibacterial fluoroquinolones, we conducted a series of qualitative docking experiments. We first examined if docking could predict (recapitulate) the interaction of Moxifloxacin, a control fluoroquinolone, into a predefined intercalation site in a 12 bp sequence of dsDNA d(CGCGAATTCGCG; PDB ID:1g3x) As expected, the quinolone core of Moxifloxacin intercalated into the DNA intercalation site (Fig. 3C). Next, we examined the docking of **217** into the same DNA. Similar to Moxifloxacin, the quinolone core of **217** could intercalate into DNA (Fig. 1A). Interestingly, the N1-biphenyl group was also found to intercalate into the DNA intercalation site (Fig. 1B). Analysis of the top 10 docked poses showed that the quinolone core and N1-biphenyl group of **217** intercalated into DNA with equivalent occurrence (data not shown). The top 10 docked poses of Moxifloxacin showed intercalation only through the quinolone core. We then conducted another set of docking experiments using a 15 bp sequence of dsDNA d(AGGTCACGGTGGCCA) having four predefine sites of intercalation, and found that **217** intercalated into DNA through either the quinolone core (Fig. S3) or the N1-biphenyl group (Fig. S4) with equivalent occurrence. In contrast, Moxifloxacin intercalated into DNA only through the quinolone core (Fig. S5). Thus, docking experiments suggest a significant difference between quinolone-based topo II poisons and the quinlone-based catalytic inhibitors of hTopo I here is that the hTopo I catalytic inhibitors possess two modes of DNA intercalation.

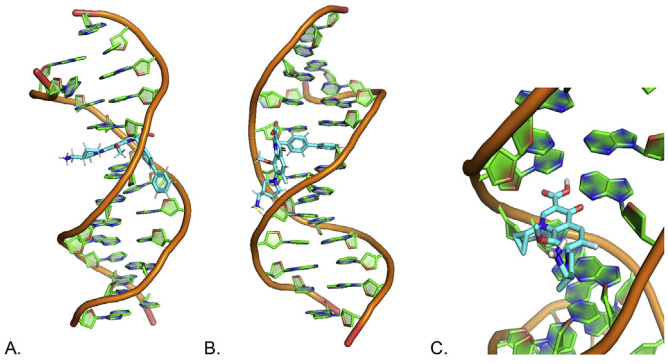


Fig. 3. Docking of 217 and moxifloxacin into double-stranded DNA. Either **217** or moxifloxacin was modeled into a predefined intercalation site of a dodecamer d(CGCGAATTCGCG) (PDB ID: 1g3x) using the program MOE 2016.08. Out of the top 10 docked poses, **217** intercalated into the DNA through the quinolone core (panel A) and *N*-1-biphenyl group (panel B) with equivalent occurrence. Moxifloxacin (panel C) intercalated only through the quinolone core.

Previous studies determined the binding mode of Norfloxacin to calf thymus DNA (Ct-DNA) and reported significant quenching of the fluoroquinolone fluorescence spectra upon addition of Ct-DNA, and an observed bathochromic shift upon addition of Ct-DNA to Norfloxacin indicating the fluoroquinolone core intercalates between the DNA base pairs. We evaluated Ct-DNA binding of N1-biphenyl fluoroquinolones, **217** and **5a,** at several ratios of DNA to FQ and no bathochromic shift was observed under similar conditions to those reported with Norfloxacin (Fig. S6 and S7). Modest fluorescence quenching was observed upon addition of Ct-DNA, indicating FQ-DNA binding, however the quenching of fluoroquinolone fluorescence was much lower than observed with antibacterial fluoroquinolones. We therefore conducted additional experiments to evaluate DNA binding of the N1-biphenyl fluoroquinolones, **217** and **5a,** with a 27-bp sequence, d(CCTTACGTG-CATAGTCATTCATGACCG), of dsDNA previously used to determine binding affinity of several clinically used and novel fluoroquinolones. The fluorescence spectra and intensity of **217** and **5a** were measured in the absence and presence of dsDNA, fluoroquinolone fluorescence was quenched in a concentration-dependent manner upon addition of DNA (Fig. 4A and B). However, a bathochromic shift was not observed upon addition of dsDNA (Figure S8 and S9). Because the innate fluoroquinolone fluorescence is attributed to the quinolone core, a bathochromic shift is observed upon DNA intercalation by the quinolone core. Thus, these results further suggest that the *N*-1-biphenyl group over the quinolone core is primarily responsible for DNA intercalation of either **217** or **5a**.

Because **217** and its analogs act differently from normal fluoroquinolones, it seems possible that the second mode of DNA intercalation, through the *N*-1 biphenyl group, may be important for activity as a catalytic inhibitor of hTopo I. Unlike normal fluoroquinolones, a quinazoline-2,4-dione has shown not to intercalate into DNA. Thus, the activity of **1** to inhibit hTopo I (Table 1) supports our model that intercalation through the *N*-1 bis-aryl group is important for inhibition of hTopo I activity by the compounds under investigation here. The added *N*-1 aryl functionality provides an additional intercalative moiety and may convert these fluoroquinolones to be threading intercalators, whereas moxifloxacin and other quinolone-based topoisomerase poisons are partial or classic intercalators, blocking interaction with the minor groove of DNA.

Guided by structural effects on activity of derivatives to inhibit hTopo I and the binding observations, interactions, and docking of quinolones with DNA, we propose a model for how **217** and its derivatives here may inhibit hTopo I activity. The N1-biphenyl fluoroquinolone intercalates through the *N*-1-biphenyl group, which would position the quinolone core in the minor groove of DNA, where the *C*-7-group with required basic (cationic) amine is

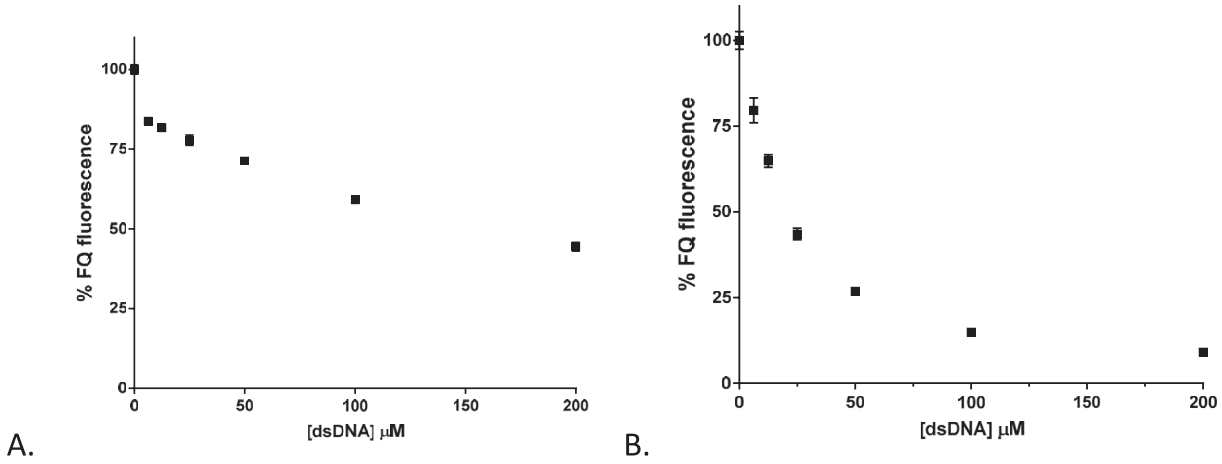


Fig. 4. Representative fluorescence intensities of 217 and 5a in the absence and presence of increasing concentrations of 27-bp sequence of dsDNA. The fluorescence intensity of either **217** (panel A) or **5a** (panel B) [25 μM] were measured in the absence and presence of increasing concentrations of dsDNA [0–200 μM]. Reported as n = 3, error bars represent S.E.M.

positioned to interact with a phosphate of the DNA backbone (Fig. 3B). The presence of the quinolone core and/or the interaction of the *C*-7 side chain with DNA may interfere with the binding of hTopo I to DNA and/or the passing of one DNA strand through a nick in the other strand by hTopo I. This unique mode of binding allows the *N*-1 bis-aryl fluoroquinolones to act as a hTopo I catalytic inhibitors.

3. Conclusion

Topoisomerase poisons trap topoisomerase-DNA covalent complexes as topoisomerase-drug-DNA ternary complexes, which leads to an increase in the level of topoisomerase-catalyzed double-strand breaks. This mode of action (topoisomerase poisoning) is an effective cell-killing mechanism, but it also causes genotoxicities, including the development of therapy-related secondary cancer. In the present study we describe initial structural requirements for, and initial structural effects of, novel fluoroquinolone-derived structures that act as catalytic inhibitors of hTopo I. Catalytic inhibitors of hTopo I do not increase DNA breaks and thus lack a significant liability of hTopo poisons. Results demonstrate that structure-function relationships for catalytic inhibition of hTopo I do not compare in any way to known SAR for antibacterial fluoroquinolones or to SAR for fluoroquinolones that poison hTopo II. Moreover, we demonstrate a unique mode of DNA binding, that being threading intercalation, is likely central to the mechanism by which these novel anticancer compounds act to inhibit hTopo I catalytic activity. Combined, the results of this study provide the structural and functional basis for the continued design, synthesis and study of structural derivatives of this new type of hTopo I inhibitor toward the further development of a new class of anticancer agents.

4. Experimental section

4.1. General experimental protocol

All commercially available chemicals and reagents were used without any further purification unless otherwise indicated. Reaction progress was monitored by thin-layer chromatography (TLC) on silica gel 60 ADAMANT plates with fluorescence indicator (254 nm) or by analytical HPLC. All analytical HPLC analyses were performed using a Shimadzu system comprised of an LC-20AT pump, DGU-14A degasser, CBM-20A system controller, and a SPD-M10AVP photodiode array detector. The HPLC was operated using Shimadzu Client/Server Version 7.4 software installed on a Dell Optiplex GX400 PC. The column used was Restek Allure PFP Propyl or Restek C-18 unless otherwise indicated. The mobile phase was composed of water buffered with 0.1% Trifluoroacetic acid (TFA) and acetonitrile also buffered with 0.1% TFA. The HPLC analyses were obtained by running a gradient of 5%–95% buffered acetonitrile over 30 min with a flow rate of 1.000 mL/min. Flash column chromatography was carried out with the indicated solvents using silica gel 230–400 mesh size. ^{1}H were recorded at 300 or 400 MHz and ^{19}F NMR spectra were recorded at 282 MHz and reported in ppm using appropriate deuterated solvents. Either a Bruker Ultrashield 300 MHz or Bruker DRX 400 MHz instrument was used to collect NMR spectra. The chemical shifts are reported in ppm relative to tetramethylsilane and solvent. Mass spectrometry data were obtained in most cases using a Thermo LCQ Deca mass spectrometer utilizing ESI ionization and quadrupole ion trap mass analyzer. Final products were purified using reverse-phase semi-preparative HPLC. The Shimadzu system was composed of the following parts: two LC-10AT pumps (one for each solvent), SPD-M10AVP photodiode array detector, and an SCL-10AVP system controller. The HPLC system was operated using Shimadzu EZStart Version 7.4 control software installed on a Dell Optiplex 755 PC. The stationary phase used for separation was a Phenomenex Luna PFP(2) or C-18 reverse phase column. The mobile phase was composed of water buffered with 0.1% TFA and acetonitrile also buffered with 0.1% TFA. Various gradients were used for separation of products, but commonly a 5%–95% buffered acetonitrile gradient over 60 min was used. All final products are >95% pure determined by analytical HPLC as shown in Table S2.

4.2. Synthesis

4.2.1. Methyl (S)-2-amino-4-(3-(((tert-butoxycarbonyl)amino) methyl)pyrrolidin-1-yl)-5-fluorobenzoate (*UICK-II-189, 15*)

Methyl 2-amino-4,5-difluorobenzoate (**14**) (250 mg, 1.33 mmol) was dissolved in 4 mL DMF. TEA (2.5 eq) and *R*-tert-butyl(pyrrolidin-3-yl methyl) carbamate (298.5 mg, 1.47 mmol) were added and

stirred at 80 °C for 5 h. The reaction mixture was dissolved in 20 mL DCM and washed with 10 mL water three times. The organic layer was concentrated by rotary evaporation. The residual DMF was removed by placing under high vacuum overnight. The residue was purified by silica gel chromatography using gradient from 3:1 Hexanes:EtOAc to 1:1 Hexanes:EtOAc to yield pure **UICK-II-189** (**15**) Yield 73%. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.43 (d, *J* = 15.4 Hz, 1H), 5.73 (d, *J* = 7.8 Hz, 1H), 4.70 (bs, 1H, exchangeable), 3.82 (s, 3H), 3.53 (m, 3H), 3.20 (m, 3H), 2.47 (m, 1H), 2.08 (m, 1H), 1.71 (m, 1H), 1.47 (s, 9H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −141.00 (s, 1F). MS (ESI) calculated for $(M + H)^+$ 368.19, found 368.0.

*4.2.2. Methyl (S)-4-(3-(((tert-butoxycarbonyl)amino)methyl) pyrrolidin-1-yl)-5-fluoro-2-(((4-nitrophenoxy)carbonyl)amino) benzoate (**UICK-II-193, 16**)*

UICK-II-189 (**15**) (97.6 mg, 0.32 mmol) and N,N-diisopropylamine (60 μL, 0.35 mmol) were dissolved in 7 mL DCM and allowed to stir for 10 min 4-Nitrophenylchloroformate (70.4 mg, 0.349 mmol) was added and the reaction was stirred for 30 min. White ppt is observed. 25 mL DCM was added to dissolve the precipitate and the reaction mixture washed with 15 mL water three times. The organic layer was dried over Na_2SO_4, concentrated *in vacuo*, and purified by silica gel chromatography using a gradient 5:1 hexanes:EtOAc to pure EtOAc, to give pure **UICK-II-193** (**16**). Yield 90% ^{1}H NMR (300 MHz, $CDCl_3$) δ = 11.21 (bs, 1H, exchangeable), 8.30 (dd, *J* = 9.6, 2.6 Hz, 2H), 7.65 (d, *J* = 9.7 Hz, 1H), 7.62 (d, *J* = 11.5 Hz, 1H), 7.41 (dd, *J* = 9.6, 2.6 Hz, 2H), 4.70 (bs, 1H), 3.91 (s, 3H), 3.64 (m, 3H), 3.23 (m, 3H), 2.48 (m, 1H), 2.10 (m, 1H), 1.72 (m, 1H), 1.46 (s, 9H). ^{19}F NMR (282 MHz, CDCl3) δ −135.10 (s, 1F). MS (ESI) calculated for $(M + H)^+$ 533.69, found 533.20.

*4.2.3. tert-butyl (S)-((1-(3-((tert-butoxycarbonyl)amino)-6-fluoro-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-7-yl)pyrrolidin-3-yl) methyl)carbamate (**UICK-II-197, 17**)*

UICK-II-189 (**16**) (95 mg, 0.179 mmol), N,N-diisopropylamine (34 μL, 0.196 mmol) and tert-butyl carbazate (117.8 mg, 0.895 mmol) were dissolved in 10 mL of tetrahydrofuran and set to reflux for 2 h. Reaction complete as observed on TLC. Reaction mixture was cooled, tetrahydrofuran was removed *in vacuo* and the dark yellow residue was dissolved in 20 mL methanol. K_2CO_3 (25 mg, 0181 mmol) was added and the reaction mixture was set to stir at 50 °C overnight. The reaction was diluted with 25 mL DCM and washed with 15 mL water thrice. The organic layers were combined, dried over Na_2SO_4, concentrated *in vacuo* and purified by silica gel chromatography using a gradient from 9:1 hexanes: EtOAc to 1:1 hexanes:EtOAc to give pure **UICK-II-197** (**17**). Quantitative yield. ^{1}H NMR (300 MHz, DMSO) δ = 11.24 (bs, 1H), 9.46 (s, 1H), 7.42 (d, *J* = 14.1 Hz, 1H), 7.06 (bs, 1H), 6.24 (d, *J* = 7.5 Hz, 1H), 3.46 (m, 4H), 3.00 (m, 2H), 2.38 (m, 1H), 2.00 (m, 1H), 1.69 (m, 1H), 1.43 (s, 9H), 1.38 (s, 9H). ^{19}F NMR (282 MHz, DMSO) δ = −133.75 (s, 1F). MS (ESI) calculated for $(M + K)^+$ 532.20, found 532.65.

*4.2.4. (S)-1-([1,1′-biphenyl]-4-ylmethyl)-3-amino-7-(3-(aminomethyl)pyrrolidin-1-yl)-6-fluoroquinazoline-2,4(1H,3H)-dione (1) (**UICK-II-215a**)*

UICK-II-197 (**17**) (60 mg, 0.122 mmol), 4-bromomethylbiphenyl (32.5 mg, 0.132 mmol) and K_2CO_3 (19 mg, 0.136 mmol) were dissolved in THF (5 mL) and set to reflux for 24 h. TFA (2 mL) was added and the solution was refluxed for further 2 h. The solution was concentrated *in vacuo*, diluted with water and the product (**1**) purified by preparatory HPLC. Yield 16%. ^{1}H NMR (300 MHz, DMSO) δ = 7.89 (bs, 2H), 7.49 (m, 10H), 6.31 (d, *J* = 7.2 Hz, 1H), 5.41 (s, 1H), 4.44 (bs, 2H), 3.45 (m, 4H), 2.90 (m, 2H), 2.44 (m, 1H), 2.09 (m, 1H), 1.74 (m, 1H). ^{19}F NMR (282 MHz, DMSO) δ = −134.10 (s, 1F). MS (ESI) calculated for $(M + H)^+$ 460.21, found 460.05. Retention time (analytical HPLC) = 16.7 min.

*4.2.5. (S)-Methyl 1-([1,1′-biphenyl]-4-ylmethyl)-7-(3-(aminomethyl)pyrrolidin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate **(UIJD-II-224, 2)***

UIJD-II-219 (19) (59 mg, 0.14 mmol) was added to a flame dried round bottom flask and place under vacuum. 1 mL of anhydrous DMSO was added under argon atmosphere and stirred. Distilled TEA (60 μL, 0.4 mmol) and Boc-AMP (42.8 mg, 0.21 mmol) were added and the reaction mixture was heated to 60 °C for 30 h 5 mL of cold water was added and the precipitate was filtered and washed three times with cold water. The crude reaction product was dissolved in 1 mL of 3 N aqueous HCl and 1 mL of distilled ACN. The reaction mixture was stirred for 48 h at room temperature and the crude product purified by semi-preparative HPLC (C-18, 20–95% ACN over 30 min). Affording 14.5 mg of pure **7** (**UIJD-II-224**), 20.7% yield.^{1}H NMR (400 MHz, MeOD) δ 8.96 (s, 1H), 7.77 (d, *J* = 14.1 Hz, 1H), 7.60–7.52 (m, 4H), 7.44–7.36 (m, 2H), 7.34–7.27 (m, 1H), 7.23 (d, *J* = 8.3 Hz, 2H), 5.87 (dd, *J* = 63.8, 15.4 Hz, 2H), 3.92 (s, 3H), 3.71–3.63 (m, 2H), 3.53 (ddd, *J* = 7.3, 6.6, 3.6 Hz, 1H), 3.50 (s, 3H), 3.46 (ddd, *J* = 10.5, 5.9, 1.8 Hz, 1H), 3.07 (d, *J* = 7.2 Hz, 2H), 2.55 (dt, *J* = 11.0, 5.5 Hz, 1H), 2.25–2.16 (m, 1H), 1.74 (dq, *J* = 16.6, 8.2 Hz, 1H). ^{19}F NMR (300 MHz, $CDCl_3$) δ −121.79 to −122.05 (m). Retention time (analytical HPLC) = 19.7 min. MS ESI calculated for $(M + H)^+$ 516.22, found 516.2.

*4.2.6. 1-([1,1′-biphenyl]-4-ylmethyl)-6,7-difluoro-8-methoxy-N-methyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (**UIJD-II-215A, 20a**)*

UIJD-II-196 (**19**) (90 mg, 0.21 mmol) was added to a flame dried RBF under argon atmosphere. 1 mL of ACN was added and stirred, followed by 40% methylamine in H_2O (200 μL, 2 mmol). The reaction was heated to 60 °C for 16 h 10 mL of $NaHCO_3$ was added and the organic solvent removed by rotary evaporation. The compound was reconstituted in DCM and then extracted three times. The residue was purified by silica gel flash chromatography using a gradient from hexanes to ethyl acetate, producing 35.5 mg pure **UIJD-II-215A** (**20a**), 40.8% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.73 (d, *J* = 4.2 Hz, 1H), 8.82 (s, 1H), 8.24–8.01 (m, 1H), 7.57 (dd, *J* = 7.2, 5.5 Hz, 4H), 7.49–7.33 (m, 3H), 7.13 (d, *J* = 8.0 Hz, 2H), 5.91–5.71 (m, 2H), 3.78–3.61 (m, 3H), 3.12–2.95 (m, 3H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −135.64 (dd, *J* = 21.4, 10.1 Hz), −142.77 (dd, *J* = 21.4, 7.1 Hz). Retention time (analytic HPLC) = 23.5 min. MS ESI calculated $(M + H)^+$ 435.14, found 435.2.

*4.2.7. 1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-8-methoxy-N-methyl-7-(methylamino)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**UIJD-II-215B, 21**)*

UIJD-II-196 (**19**) (90 mg, 0.21 mmol) was added to a flame dried RBF under argon atmosphere. 1 mL of ACN was added and stirred, followed by methylamine (200 μL, 2 mmol). The reaction was heated to 60 °C for 16 h 10 mL of $NaHCO_3$ was added and the organic solvent removed by rotary evaporation. The compound was reconstituted in DCM and then extracted three times. The residue was purified by silica gel flash chromatography using a gradient from hexanes to ethyl acetate, yielding 34 mg pure **UIJD-II-215B** (**21**), 37% yield. ^{1}H NMR (300 MHz, Acetone) δ 9.78 (d, *J* = 4.9 Hz, 1H), 8.82 (s, 1H), 7.70 (d, *J* = 13.4 Hz, 1H), 7.62–7.57 (m, 4H), 7.43 (t, *J* = 7.4 Hz, 2H), 7.33 (t, *J* = 7.3 Hz, 1H), 7.14 (d, *J* = 8.2 Hz, 2H), 5.85 (s, 2H), 3.64 (s, 3H), 3.44 (s, 1H), 2.97 (t, *J* = 4.4 Hz, 3H), 2.85 (d, *J* = 4.8 Hz, 3H). ^{19}F NMR (300 MHz, DMSO) δ −129.73 to −130.14 (m). Retention time (analytic HPLC) = 21.8 min. MS ESI calculated for $(M + Na)^+$ 468.18, found 468.16.

*4.2.8. (S)-1-([1,1′-biphenyl]-4-ylmethyl)-7-(3-(aminomethyl) pyrrolidin-1-yl)-6-fluoro-8-methoxy-N-methyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (**UIJD-II-242B) (3a**)*

UIJD-II-215A (**20a**) (55 mg, 0.13 mmol) was placed in a flame dried RBF under argon atmosphere, 1 mL of DMSO was added with stirring. To the reaction flask distilled TEA (53 μL, 0.38 mmol) and Boc-AMP (50.73 mg, 0.25 mmol) was added. The reaction was heated to 60 °C for 24 h, 5 mL of cold water was added. The precipitate was filtered and washed 3 times with 5 mL of cold water. The crude reaction mixture was then dissolved in 2 mL of ACN and 2 mL of 3 N HCl and stirred at 25 °C for 20 h. The reaction mixture was diluted with water and purified by preparatory HPLC (C-18, 30–95% ACN over 20 min), giving 16.5 mg, **UIJD-II-242B** (**3a**), 25% yield over two steps. ^{1}H NMR (400 MHz, MeOD) δ 8.94 (s, 1H), 7.76 (d, *J* = 14.1 Hz, 1H), 7.57–7.49 (m, 4H), 7.41–7.35 (m, 2H), 7.32–7.27 (m, 1H), 7.22 (d, *J* = 8.3 Hz, 2H), 5.87 (dd, *J* = 67.3, 15.5 Hz, 2H), 3.69 (ddd, *J* = 9.4, 8.0, 4.0 Hz, 2H), 3.55–3.50 (m, 1H), 3.48 (d, *J* = 8.0 Hz, 3H), 3.46–3.40 (m, 1H), 3.10–3.04 (m, 2H), 2.98 (s, 3H), 2.57 (dt, *J* = 14.5, 7.3 Hz, 1H), 2.21 (dt, *J* = 10.7, 6.7 Hz, 1H), 1.75 (dq, *J* = 12.2, 8.3 Hz, 1H).^{19}F NMR (282 MHz, MeOD) δ −122.16 (d, *J* = 14.7 Hz). Retention time (analytical HPLC): 18.45. MS ESI calculated $(M + H)^+$ 515.24, found 515.2.

*4.2.9. (S)-1-([1,1′-biphenyl]-4-ylmethyl)-7-(3-(aminomethyl) pyrrolidin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxamide (**UIJD-II-251, 3b**)*

UIJD-II-196 (**19**) (55 mg, 0.13 mmol) under argon atmosphere, was dissolved in 6.5 mL of 2 mM ammonia in methanol and stirred at 35 °C for 72 h. The organic solvent was removed by rotary evaporation and the product reconstituted in DCM and extracted from water, the combined organic layers were concentrated by rotary evaporation. The crude product was used without further purification. **UIJD-II-249** (**20b**) (40 mg, 0.1 mmol) placed in flame dried RBF, under argon atmosphere 1 mL of anhydrous DMSO was added with stirring, followed by distilled TEA (100 μL, 0.72 mmol) and Boc-AMP (38 mg, 0.19 mmol). The reaction was heated to 60 °C for 48 h. The DMSO was removed by rotary evaporation. The crude reaction mixture was dissolved in 2 mL of distilled ACN and 2 mL of 3 N aqueous HCl. The reaction mixture was stirred at 25 °C for 42 h. The reaction mixture was diluted with water and purified by preparatory HPLC (C-18, 50–95% ACN over 30 min) Pure **UIJD-II-251** (**3b**) was isolated 6.6 mg, 13.8% yield over 2 steps. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.68 (d, *J* = 14.0 Hz, 1H), 7.59 (t, *J* = 10.0 Hz, 4H), 7.47–7.29 (m, 3H), 7.23 (d, *J* = 8.1 Hz, 2H), 5.86 (q, *J* = 15.3 Hz, 2H), 4.51 (s, 1H), 3.57 (dd, *J* = 13.4, 5.7 Hz, 2H), 3.47 (s, 3H), 3.37 (dd, *J* = 15.2, 9.0 Hz, 2H), 2.90 (s, 2H), 2.09 (dd, *J* = 10.6, 4.3 Hz, 1H), 1.75–1.66 (m, 1H).^{19}F NMR (282 MHz, DMSO) δ −122.44 (d, *J* = 13.6 Hz). Retention time (analytical HPLC) = 17.4 min. MS ESI calculated $(M + H)^+$ 501.22, found 501.23.

*4.2.10. (S)-1-([1,1′-biphenyl]-4-ylmethyl)-7-(3-(aminomethyl) pyrrolidin-1-yl)-6-fluoro-N-hydroxy-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxamide (**UIJD-II-246, 3c**)*

UIJD-II-196 (**19**) (70 mg, 0.16 mmol) was dissolved in 1 N hydroxylamine·HCl in EtOH (3.2 mL) and TEA was added (0.8 mmol, 112 μL). The reaction was stirred at 25 °C for 72 h, and the pH was adjusted as needed to pH 9–10 as the reaction progressed. The EtOH was removed by rotary evaporation and the product reconstituted in DCM and precipitated out of ether. The crude precipitated product was used without further purification. **UIJD-II-217** (**20c**) (50 mg, 0.12 mmol) was added to a flame dried round bottom flask under argon atmosphere and 1 mL of anhydrous DMSO was added. Distilled TEA (60 μL, 0.45 mmol) and Boc-AMP (46 mg, 0.23 mmol) was added with stirring. The reaction mixture was heated to 65 °C for 17 h. DMSO was removed by rotary evaporation and the crude product was stirred in 3 mL of distilled ACN and 3 mL of 3 N HCl at 25 °C. The product was purified by preparatory HPLC (C-18, 20–95% ACN over 35 min). Affording 22 mg of UIJD-II-246 (**3c**), 36% yield over two steps. ^{1}H NMR (300 MHz, DMSO) δ 11.66 (s, 1H), 8.90 (s, 1H), 7.68 (d, *J* = 14.1 Hz, 1H), 7.61 (d, *J* = 8.3 Hz, 4H), 7.43 (t, *J* = 7.5 Hz, 2H), 7.35 (d, *J* = 7.3 Hz, 1H), 7.22 (d, *J* = 8.2 Hz, 2H), 5.89 (q, *J* = 15.6 Hz, 2H), 3.62–3.53 (m, 3H), 3.48 (s, 3H), 3.43–3.31 (m, 2H), 2.98–2.84 (m, 2H), 2.08 (dd, *J* = 7.8, 4.7 Hz, 1H), 1.76–1.67 (m, 1H). ^{19}F NMR (282 MHz, DMSO) δ −121.96 to −122.25 (m). Retention time (analytical HPLC) = 21.8 min. MS ESI calculated $(M + H)^+$ 517.22, found 517.22.

*4.2.11. (R)-1-([1,1′-biphenyl]-4-ylmethyl)-7-(3-carbamoylpyrrolidin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4 dihydroquinoline-3-carboxylic acid (**UIJD-II-115, 4a**)*

UIJD-II-111 (**22**) (27 mg, 0.1 mmol) was dissolved in 1 mL of anhydrous DMSO under inert conditions. TEA (100 μL, 0.72 mmol) and (R)-pyrrolidin3-carboxamide were added to the solution and stirred for 4 h at 60 °C. The solution was diluted in a 1:1 mixture of ACN to H_2O and the product purified by preparatory HPLC (PFP, 50–95% ACN over 25 min). Affording 18 mg of pure **UIJD-II-115** (**4a**), 64% yield. ^{1}H NMR (400 MHz, DMSO) δ 9.16–8.98 (m, 1H), 7.69 (d, *J* = 13.9 Hz, 1H), 7.61–7.57 (m, 3H), 7.47 (s, 1H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.2 Hz, 1H), 7.23 (d, *J* = 8.2 Hz, 2H), 5.92 (dd, *J* = 30.0, 15.4 Hz, 2H), 3.69–3.61 (m, 1H), 3.51 (d, *J* = 8.2 Hz, 1H), 3.43 (d, *J* = 12.1 Hz, 3H), 3.11–3.02 (m, 1H), 2.98–2.89 (m, 1H), 2.10–1.95 (m, 1H), 1.17 (t, *J* = 7.1 Hz, 2H). ^{19}F NMR (282 MHz, DMSO) δ −73.49 (d, *J* = 19.6 Hz), −120.57 (s). Retention time (analytical HPLC) = 19.7 min. MS ESI calculated $(M + H)^+$ 516.19, found 516.2.

*4.2.12. (R)-1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-7-(3-(hydroxymethyl)pyrrolidin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(UIJD-II-116), 4b**)*

UIJD-II-111B (**22**) (40 mg, 0.1 mmol) was dissolved in 5 mL of distilled ACN. D-β-propinol (24 mg, 0.24 mmol) and distilled TEA (66 μL, 0.48 mmol) were added to the solution and stirred under argon atmosphere for 36 h at 50 °C. The solution was diluted with water and the product purified by preparatory HPLC (PFP, 65–95% ACN over 25 min). After purification 11 mg of pure **UIJD-II-116** (**4b**) was obtained, 22% yield. ^{1}H NMR (400 MHz, DMSO) δ 9.10 (s, 1H), 7.70 (d, *J* = 14.0 Hz, 1H), 7.63–7.57 (m, 4H), 7.44 (dd, *J* = 10.3, 4.8 Hz, 2H), 7.38–7.32 (m, 1H), 7.25 (d, *J* = 8.3 Hz, 2H), 5.94 (dd, *J* = 30.9, 15.2 Hz, 2H), 3.52 (ddd, *J* = 11.6, 9.5, 4.2 Hz, 3H), 3.46 (s, 3H), 3.45–3.32 (m, 3H), 2.33 (dt, *J* = 14.5, 7.2 Hz, 1H), 1.96 (dt, *J* = 11.3, 5.8 Hz, 1H), 1.64 (dq, *J* = 12.2, 7.8 Hz, 1H). ^{19}F NMR (282 MHz, DMSO) δ −120.50 (s). Retention time (analytical HPLC) = 21.7 min. MS ESI calculated $(M + H)^+$ 503.19, found 503.2.

*4.2.13. (S)-1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-8-methoxy-7-(3-((methylamino)methyl)pyrrolidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(UIJD-II-086, 4c)***

UIJD-II-061B (**22**) (35 mg, 0.09 mmol) was dissolved in 3 mL of distilled ACN. (R)-tert-butyl methyl(pyrrolidin-3-ylmethyl)carbamate (12.4 mg, 0.06 mmol) and distilled TEA (16 μL, 0.12 mmol) were added to the reaction flask and stirred under argon atmosphere for 4 days at 60 °C. Trifluoroacetic acid (2 mL) was added to the reaction flask and stirred at room temperature for 12 h. The solution was diluted with water and the product purified by preparatory HPLC (PFP, 50–85% ACN over 25 min). Pure **UIJD-II-086** was obtained, 12.3 mg, 28% yield over two steps. ^{1}H NMR (400 MHz, DMSO) δ 15.17 (s, 1H), 9.10 (s, 1H), 8.79 (s, 2H), 7.71 (d, *J* = 13.9 Hz, 1H), 7.60 (d, *J* = 8.2 Hz, 4H), 7.42 (t, *J* = 7.7 Hz, 2H), 7.33 (t, *J* = 7.3 Hz, 1H), 7.24 (d, *J* = 8.2 Hz, 2H), 5.93 (dd, *J* = 31.4, 15.3 Hz, 2H), 3.60 (t, *J* = 7.6 Hz, 1H), 3.52 (d, *J* = 8.0 Hz, 1H), 3.47 (s, 3H), 3.39 (d, *J* = 7.2 Hz, 3H), 2.99 (d, *J* = 6.4 Hz, 2H), 2.54 (t, *J* = 4.8 Hz, 3H), 2.14–2.02 (m,

1H), 1.71 (dd, $J = 12.1$, 8.0 Hz, 1H). ^{19}F NMR (282 MHz, DMSO) δ −120.45 (d, J = 13.3 Hz). Retention time (analytical HPLC) = 23.9 min MS ESI calculated $(M + H)^+$ 516.22, found 515.22.

*4.2.14. (R)-1-([1,1′-biphenyl]-4-ylmethyl)-7-(3-aminopyrrolidin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(UIJD-II-226B, 4d)***

UIJD-II-214B (**22**) (64 mg, 0.15 mmol) placed in flame dried round bottom flask, 1 mL of anhydrous DMSO was added and stirred under inert conditions. 70 μL (0.5 mmol) of TEA and Boc-AMP (46.3 mg, 0.25 mmol) were added and the reaction heated to 60 °C for 24 h. To the crude reaction product, 2 mL of ACN and 2 mL of 3 N aqueous HCl were added and stirred at 25 °C for 22 h. The crude product was purified by preparatory HPLC (C-18, 10–95 over 40 min) Yielding 12.5 mg of pure **UIJD-II-226**, 17%, over 2 steps. ^{1}H NMR (400 MHz, MeOD) δ 8.97 (s, 1H), 7.77 (d, $J = 13.6$ Hz, 1H), 7.54 (d, $J = 7.2$ Hz, 4H), 7.38 (t, $J = 7.3$ Hz, 2H), 7.31 (dd, $J = 13.6$, 6.6 Hz, 1H), 7.24 (s, 2H), 5.89 (s, 2H), 4.09–3.81 (m, 2H), 3.74 (s, 2H), 3.56 (d, $J = 9.3$ Hz, 3H), 3.51 (dd, $J = 12.6$, 6.0 Hz, 1H), 2.41 (s, 1H), 2.10 (s, 1H). ^{19}F NMR (282 MHz, DMSO) δ −120.22 (d, J = 12.7 Hz). Retention time (analytical HPLC) = 19.8 min. MS ESI calculated $(M + H)^+$ 488.19, found 488.2.

*4.2.15. (R)-1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-8-methoxy-7-(3-(methylamino)pyrrolidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(UIJD-II-228B, 4e)***

UIJD-II-214B (**22**) (66 mg, 0.16 mmol) was added to a flame dried round bottom flask under inert conditions. 1 mL of anhydrous DMSO was added and stirred. Distilled TEA (70 μL, 0.5 mmol) and Boc-AMP (50 mg, 0.24 mmol) were then added and the reaction mixture was heated to 60 °C for 24 h 5 mL of cold water was added and the precipitate was collected, filtered, and washed three times with 5 mL of cold water. To the crude reaction product 2 mL of ACN and 2 mL of 3 N aqueous HCl were added at 25 °C. The reaction was stirred for 24 h. The reaction mixture was diluted with water and purified by preparatory HPLC (C-18, 10–95% ACN over 40 min). Yielding pure **UIJD-II-228B** (**4e**) 26 mg, 39% yield over two steps. ^{1}H NMR (400 MHz, MeOD) δ 8.93 (s, 1H), 7.69 (d, $J = 13.5$ Hz, 1H), 7.53 (d, $J = 7.9$ Hz, 4H), 7.38 (t, $J = 7.3$ Hz, 2H), 7.28 (dd, $J = 19.6$, 12.4 Hz, 3H), 5.88 (s, 2H), 3.86 (d, $J = 26.0$ Hz, 2H), 3.73 (s, 3H), 3.55 (s, 3H), 2.75 (s, 3H), 2.44 (s, 1H), 2.16 (s, 1H).^{19}F NMR (282 MHz, DMSO) δ −121.37 (d, J = 13.4 Hz). Retention time (analytical HPLC) = 20.7 min. MS ESI calculated $(M + H)^+$ 502.2, found 502.2.

*4.2.16. 1-([1,1′-biphenyl]-4-ylmethyl)-7-((2-aminoethyl)amino)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(UIJD-II-264B, 4f)***

UIJD-II-214B (**22**) (50 mg, 0.1 mmol) was added to a flamed dried round bottom flask and 1 mL of anhydrous DMSO was added to the reaction flask with stirring under argon atmosphere, followed by distilled TEA (50 μL, 0.36 mmol) and ethylene diamine (36.5 μL, 0.23 mmol). The reaction was heated to 60 °C and stirred for 7 h 5 mL of cold water was added and the precipitate was filtered and washed three times with 5 mL cold water. The crude precipitate was dissolved in 2 mL of distilled ACN and 2 mL of 3 N HCl and stirred for 24 h at 25 °C. The ACN was removed by rotary evaporation, the remaining aqueous solution was extracted three times with DCM and concentrated *in vacuo*. The pure product was collected from the frit, affording 25.2 mg of **UIJD-II-264B** (**4f**), 44% over 2 steps. ^{1}H NMR (400 MHz, DMSO) δ 15.23 (s, 1H), 9.06 (s, 1H), 8.24 (s, 3H), 7.77 (d, $J = 12.9$ Hz, 1H), 7.59 (d, $J = 8.2$ Hz, 4H), 7.37 (dt, $J = 35.8$, 7.3 Hz, 3H), 7.18 (d, $J = 8.2$ Hz, 2H), 6.41 (t, $J = 5.3$ Hz, 1H), 5.93 (s, 2H), 3.71 (s, 3H), 3.65 (d, $J = 5.9$ Hz, 2H), 2.95 (d, $J = 5.8$ Hz, 2H).^{19}F NMR (282 MHz, DMSO) δ −126.65 (d, $J = 12.9$ Hz). Retention time (analytical HPLC) = 18.8 min. MS ESI calculated $(M + H)^+$ 462.18, found 462.18.

*4.2.17. 1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-8-methoxy-7-((2-(methylamino)ethyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(UIJD-II-276B, 4g)***

UIJD-II-219B (**22**) (50 mg, 0.12 mmol) was placed in a flame dried RBF under inert conditions. 200 μL of anhydrous DMSO was added, followed by distilled TEA (82 μL, 0.59 mmol) and n-boc, n-methyl ethylene diamine (41 μL, 0.24 mmol). The reaction mixture was stirred and heated to 60 °C for 17 h. Cold water (2 mL) was added to the reaction mixture and the precipitate was filtered and washed three times with cold water (2 mL). The crude reaction product was dissolved in 4 mL of distilled ACN and 2 mL of 3 N HCl and stirred for 24 h. The reaction mixture was diluted with water and purified by preparatory HPLC (C-18, 40–95% ACN over 35 min). Yielding 17 mg of pure **UIJD-II-276B** (**4g**), 33% over two steps. ^{1}H NMR (400 MHz, DMSO) δ 9.08 (s, 1H), 8.85 (s, 2H), 7.79 (d, $J = 12.9$ Hz, 1H), 7.59 (d, $J = 8.1$ Hz, 4H), 7.42 (t, $J = 7.6$ Hz, 2H), 7.34 (d, $J = 7.4$ Hz, 1H), 7.16 (d, $J = 8.3$ Hz, 2H), 6.40 (d, $J = 5.4$ Hz, 1H), 5.93 (s, 2H), 3.71 (s, 3H), 3.65 (d, $J = 5.4$ Hz, 2H), 3.05–2.97 (m, 2H), 2.47 (d, $J = 5.4$ Hz, 3H). 19F NMR (282 MHz, DMSO) δ −126.55 (d, J = 19.6 Hz). Retention time (analytical HPLC) = 19.7 min. MS ESI calculated $(M + H)^+$ 476.19, found 476.19.

*4.2.18. 1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-8-methoxy-7-((3-(methylamino)propyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(UIJD-II-275B, 4h)***

UIJD-II-219B (**22**) (37.6 mg, 0.09 mmol) was added to a flame dried RBF under inert conditions. Anhydrous DMSO (100 μL) was added with stirring, followed by distilled TEA (100 μL, 0.71 mmol) and Boc-n-Me-1,3,-diaminopropane (40 mg, 0.17 mmol). The reaction was heated to 60 °C and stirred for 48 h. Cold water (5 mL) was added to the reaction mixture and the precipitate filtered and washed three times with cold water (5 mL). To the crude reaction product 3 mL of 3 N HCl and 6 mL of distilled ACN were added and stirred at 25 °C for 24hr. The crude product was purified by preparatory HPLC (C-18, 40–95% ACN over 40 min). Yielding 13 mg of pure **UIJD-II-275B** (**4h**), 29% yield over two steps. ^{1}H NMR (400 MHz, DMSO) δ 9.04 (s, 1H), 8.72 (s, 2H), 7.77 (d, $J = 13.1$ Hz, 1H), 7.59 (d, $J = 8.2$ Hz, 4H), 7.42 (t, $J = 7.6$ Hz, 2H), 7.33 (t, $J = 7.3$ Hz, 1H), 7.16 (d, $J = 8.2$ Hz, 2H), 6.46 (s, 1H), 5.93 (s, 2H), 3.68 (s, 3H), 3.42 (d, $J = 2.1$ Hz, 2H), 2.86–2.76 (m, 2H), 2.45 (t, $J = 5.4$ Hz, 3H), 1.83–1.75 (m, 2H).^{19}F NMR (282 MHz, $CDCl_3$) δ −127.30 to −127.74 (m). Retention time (analytical HPLC) = 20.2 min. MS ESI calculated $(M + H)^+$ 490.21, found 490.21.

*4.2.19. 1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-8-methoxy-7-((4-(methylamino)butyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-285B, 4i**)*

UIJD-II-219B (**22**) (46 mg, 0.11 mmol) was placed in a flame dried round bottom flask, under inert conditions. Anhydrous DMSO (500 μL) was added with stirring, followed by distilled TEA (100 μL, 0.71 mmol) and tert-butyl N-(4-aminopropyl)-N-methyl (45 μL, 0.22 mmol). The reaction was heated to 40 °C for 18 h. Cold water (5 mL) was added and the precipitate filtered and washed three times with cold water (5 mL). To the crude reaction product 2 mL of 3 N HCl and 6 mL of ACN were added and stirred at 25 °C for 24 h. The crude product was purified by preparatory HPLC (C-18, 40–95% ACN over 40 min). Affording 24 mg of pure **UIJD-II-285B** (**4i**), 43% over two steps. ^{1}H NMR (400 MHz, DMSO) δ 9.05 (s, 1H), 8.82 (s, 2H), 7.75 (d, $J = 13.0$ Hz, 1H), 7.63–7.55 (m, 4H), 7.42 (dd, $J = 10.4$, 4.8 Hz, 2H), 7.33 (ddd, $J = 7.3$, 6.0, 1.0 Hz, 1H), 7.14 (d, $J = 8.3$ Hz, 2H), 6.40 (s, 1H), 5.92 (s, 2H), 3.66 (s, 3H), 3.33 (t, $J = 5.5$ Hz, 2H), 2.76–2.64 (m, 2H), 2.36 (t, $J = 5.4$ Hz, 3H), 1.53 (dt, $J = 15.2$, 7.6 Hz, 2H), 1.45–1.33 (m, 2H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −127.58

to −127.83 (m). Retention time (analytical HPLC) = 20.8. MS ESI calculated $(M + H)^+$ 504.22, found 504.22.

*4.2.20. Ethyl 1-([1,1′-biphenyl]-4-ylmethyl)-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate. (**UICK-III-195, 25**)*

Commercially available ethyl 3-oxo-3-(2,4,5-trifluorophenyl) propanoate (**23**) (5.0 g, 20.31 mmol) was refluxed in triethylorthoformate (5.0 mL, 30.5 mmol) and acetic anhydride (5.8 mL, 60.9 mmol) for 4 h to yield enol ether **24**. After the reaction was complete, the mixture was concentrated *in vacuo* and then dissolved in 1,4-dioxane (170 mL). 4-Phenylbenzylamine (3.5 g, 19.3 mmol) was added and the reaction stirred at room temperature for 6 h K_2CO_3 (4.2 g, 30.5 mmol) and 18-crown-6 (1.0 g, 4.1 mmol) was added and the reaction was heated to 85 °C and stirred for an additional 2 h. The reaction mixture was then concentrated *in vacuo* and the resultant precipitate was washed with water and then purified by flash chromatography using a gradient starting with 1% MeOH in DCM and ending with 7% MeOH in DCM to give pure compound **25** (**UICK-III-195**). Yield = 6.4 g, 75% over 3 steps. ^{1}H NMR (300 MHz, DMSO) δ 8.98 (s, 1H), 8.11 (dd, J = 10.5, 9.1 Hz, 1H), 7.92 (dd, J = 12.2, 6.6 Hz, 1H), 7.66 (dd, J = 12.1, 7.7 Hz, 4H), 7.45 (t, J = 7.5 Hz, 2H), 7.36 (m, 3H), 5.72 (s, 2H), 4.26 (q, J = 7.1 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H). ^{19}F NMR (282 MHz, DMSO) δ −124.64 (dd, J = 23.4, 9.2 Hz, 1F), −135.28 (ddd, J = 17.6, 10.7, 6.3 Hz, 1F). MS ESI calculate for $(M + H)^+$ 420.14, found 420.14. Retention time (analytical HPLC) = 22.8 min.

*4.2.21. 1-([1,1′-biphenyl]-4-ylmethyl)-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UICK-III-197**, **26**)*

UICK-III-195 (**25**) (6.4 g, 15.3 mmol) was dissolved in 250 mL of 1:1 THF: 1% aqueous LiOH and stirred at room temperature for 24 h. The reaction was diluted with water and acidified to pH 1 with HCl. The aqueous layer was extracted three times with 300 mL DCM. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give pure **UICK-III-197** (**26**) in quantitative yield. ^{1}H NMR (300 MHz, DMSO) δ 14.80 (bs, exchangeable, 1H), 9.34 (s, 1H), 8.30 (dd, J = 10.2, 8.8 Hz, 1H), 8.17 (dd, J = 12.3, 6.6 Hz, 1H), 7.65 (dd, J = 10.6, 7.8 Hz, 4H), 7.42 (m, 5H), 5.91 (s, 2H). ^{19}F NMR (282 MHz, DMSO) δ −126.00 (m, 1F), −137.14 (m, 1F). MS ESI calculate for $(M + H)^+$ 392.11, found 391.98. Retention time (analytical HPLC) = 23.9 min.

*4.2.22. (S)-1-([1,1′-biphenyl]-4-ylmethyl)-7-(3-(aminomethyl) pyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UICK-II-065, 5a**)*

UICK-III-197 (1.5 g, 3.8 mmol) was dissolved in 40 mL ACN to which was added Boc-(*R*)-aminomethylpyrrolidine (921.1 mg, 4.600 mmol) and DIPEA (1 mL, 5.7 mmol). The reaction was set to stir at 70 °C for 4 h. 25 mL 4 N HCl was then added and the reaction was stirred for additional 12 h. The resultant precipitate was filtered and washed with ACN to give pure **UICK-II-065** (**5a**) (Batch CK-III-199). Yield 1.5 g, 77%. ^{1}H NMR (300 MHz, DMSO) δ 15.61 (s, 1H, exchangeable), 9.19 (s, 1H), 8.16 (bs, 2H), 7.80 (d, J = 14.3 Hz, 1H), 7.66 (dd, J = 12.5, 7.9 Hz, 4H), 7.42 (m, 5H), 6.67 (d, J = 7.5 Hz, 1H), 5.83 (s, 2H), 3.68 (m, 1H), 3.48 (m, 3H), 2.90 (m, 2H), 2.55 (m, 1H), 2.11 (m, 1H), 1.79 (m, 1H). ^{19}F NMR (282 MHz, DMSO) δ −126.75 (s, 1F). MS ESI calculated for $(M + H^+)$ 472.20, found 472.20. Retention time (analytical HPLC) = 19.0 min.

*4.2.23. 1-([1,1′-biphenyl]-4-ylmethyl)-7-((2-aminoethyl)amino)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-281B, 5b**)*

UIJD-II-267A (**26**) (50 mg, 0.12 mmol) was added to a flame dried RBF under inert conditions. Anhydrous DMSO (500 μL) was added with stirring, followed by Boc-ethylene diamine (41 μL, 0.26 mmol) and distilled TEA (89 μL, 0.64 mmol). The reaction mixture was heated to 45 °C for 18 h. Cold water (5 mL) was added and the precipitate was filtered and washed three times with cold water (5 mL). To the crude reaction product 3 mL of 4 N HCl and 6 mL of distilled ACN was added and stirred for 19 h at 25 °C. The organic solvent was removed by rotary evaporation and the remaining aqueous solution frozen and lyophilized. **UIJD-II-281B** (**5b**) was isolated as a pure product collecting 21 mg, 40% yield over two steps. ^{1}H NMR (400 MHz, DMSO) δ 9.17 (s, 1H), 7.83 (d, J = 11.7 Hz, 1H), 7.68–7.60 (m, 4H), 7.44 (t, J = 8.4 Hz, 4H), 7.35 (dd, J = 8.3, 6.4 Hz, 1H), 6.89 (d, J = 7.2 Hz, 1H), 5.89 (s, 2H), 3.50 (q, J = 5.9 Hz, 2H), 3.05 (s, 2H).^{19}F NMR (282 MHz, $CDCl_3$) δ −131.78 to −132.05 (m). Retention time (analytical HPLC) = 18.0 min. MS ESI calculated $(M + H)^+$ 432.16, found 432.17.

*4.2.24. 1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-7-((2-(methylamino)ethyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-282B, 5c**)*

UIJD-II-267A (**26**) (50 mg, 0.13 mmol) was added to a flame dried RBF under inert conditions. Anhydrous DMSO (500 μL) was added with stirring, followed by Boc-N-Me ethylene diamine (45 μL, 0.24 mmol) and distilled TEA (89 μL, 0.64 mmol). The reaction was heated to 45 °C for 18 h, cold water was added (5 mL). The precipitate was then filtered and washed three times. The crude product was then dissolved in 6 mL of distilled ACN. 3 mL of 4 N HCl was added and stirred for 24 h at 25 °C. The ACN was removed by rotary evaporation and the aqueous solution was frozen and lyophilized. **UIJD-II-282B** (**5c**) was obtained as a pure product 25 mg, 44% yield over two steps. ^{1}H NMR (400 MHz, DMSO) δ 15.61 (s, 1H), 9.16 (s, 1H), 8.87 (s, 2H), 7.83 (d, J = 11.7 Hz, 1H), 7.69–7.60 (m, 4H), 7.43 (dd, J = 8.0, 6.9 Hz, 4H), 7.34 (t, J = 7.3 Hz, 1H), 7.09 (d, J = 2.1 Hz, 1H), 6.91 (d, J = 7.2 Hz, 1H), 5.93 (s, 2H), 3.56 (dd, J = 11.6, 5.8 Hz, 2H), 2.96 (s, 2H), 2.47 (s, 3H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −131.68 to −131.96 (m). Retention time (analytical HPLC) = 18.7 min. MS ESI calculated $(M + H)^+$ 446.18, found 446.19.

*4.2.25. 1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-7-((3-(methylamino)propyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-269B, 5d**)*

UIJD-II-267A (**26**) (40 mg, 0.1 mmol) was added to a flame dried round bottom flask under inert conditions. Anhydrous DMSO (1 mL) was added with stirring, followed by distilled TEA (70 μL, 0.5 mmol) and Boc-n-me-1,3,-diaminopropane (45 mg, 0.2 mmol). The reaction mixture was heated to 60 °C for 24hrs. Cold water (5 mL) was added and the precipitate washed three times with cold water (5 mL) and concentrated *in vacuo*. To the crude reaction product 2 mL of 4 N HCl and 4 mL of distilled ACN were added and stirred for 5 h at 25 °C. The ACN was removed by rotary evaporation and the aqueous solution frozen and lyophilized. **UIJD-II-269B** (**5d**) was collected as a pure product 27 mg, 54% yield over two steps. ^{1}H NMR (400 MHz, DMSO) δ 9.13 (s, 1H), 7.80 (d, J = 11.8 Hz, 1H), 7.69–7.61 (m, 4H), 7.47–7.40 (m, 4H), 7.34 (t, J = 7.3 Hz, 1H), 6.80 (d, J = 7.2 Hz, 1H), 5.87 (s, 2H), 3.30 (dd, J = 12.3, 6.2 Hz, 2H), 2.90–2.81 (m, 2H), 2.43 (t, J = 5.2 Hz, 3H), 1.80 (dd, J = 13.6, 7.0 Hz, 2H).^{19}F NMR (282 MHz, $CDCl_3$) δ −132.24 to −132.45 (m). Retention time (analytical HPLC) = 19.2 min. MS ESI calculated $(M + H)^+$ 460.20, found 460.20.

*4.2.26. 1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-7-((4-(methylamino)butyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**JD-II-271B, 5e**)*

UIJD-II-267A (**26**) (35 mg, 0.1 mmol) was added to a flame dried round bottom flask under inert conditions. Anhydrous DMSO (1 mL) was added with stirring, followed by distilled TEA (37 μL, 0.27 mmol) and tert-butyl N-(4-aminopropyl)-N-methyl (36 mg,

0.18 mmol). The reaction was heated to 60 °C and stirred for 24 h. 5 mL of cold water was added to the reaction mixture and the precipitate filtered and washed three times with cold water. The crude reaction product was dissolved in 6 mL of distilled ACN and 3 mL of 4 N HCl and stirred for 21 h. The ACN was removed by rotary evaporation and the remaining aqueous solution frozen and lyophilized. **UIJD-II-271B** (**5e**) was collected as pure product 30 mg, 71% yield over two steps. ^{1}H NMR (400 MHz, DMSO) δ 9.15 (s, 1H), 7.79 (d, J = 11.9 Hz, 1H), 7.70 (d, J = 8.3 Hz, 2H), 7.67–7.62 (m, 2H), 7.45 (t, J = 7.6 Hz, 2H), 7.36 (dd, J = 9.6, 5.1 Hz, 3H), 6.65 (d, J = 7.2 Hz, 1H), 5.85 (s, 2H), 3.16 (dd, J = 12.6, 6.5 Hz, 2H), 2.61 (q, J = 12.7 Hz, 2H), 2.32 (t, J = 5.3 Hz, 3H), 1.55–1.46 (m, 2H), 1.34–1.25 (m, 2H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −132.46 to −132.79 (m). Retention time (analytical HPLC) = 19.5 min. MS ESI calculated $(M + H)^+$ 474.21, found 474.21.

*4.2.27. (S)-1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-7-(3-((methylamino)methyl)pyrrolidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UICK-IV-097, 5f**)*

UICK-III-197 (**26**) (100 mg, 0.26 mmol) was dissolved in 2 mL of DMSO to which was added (methylamino)methyl pyrrolidine (82.3 mg, 0.38 mmol) and DIPEA (89 μL, 0.51 mmol). The reaction was set to stir at 40 °C for 24 h. To the reaction mixture 4 mL of 3 N HCl was added and stirred for 24 h. To the reaction mixture water was added and the resulting mixture was centrifuged and the supernatant removed. The remaining precipitant was washed with water and centrifuged again. The resulting precipitate was lyophilized. Yield 91.8 mg, 68%. ^{1}H NMR (300 MHz, DMSO) δ 15.59 (s, 1H), 9.19 (s, 1H), 8.92 (bs, 1H, exchangeable), 7.82 (d, J = 14.3 Hz, 1H), 7.66 (m, 4H), 7.45 (m, 4H), 7.37 (d, J = 7.2 Hz, 1H), 6.68 (d, J = 7.5 Hz, 1H), 5.83 (s, 2H), 3.70 (m, 1H), 3.53 (m, 3H), 3.00 (m, 2H), 2.61 (m, 1H), 2.55 (s, 3H), 2.16 (m, 1H), 1.78 (m, 1H). ^{19}F NMR (282 MHz, DMSO) δ −126.79 (s, 1F). MS ESI calculated for $(M + H^+)$ 486.2187, found 486.2162. Retention time (analytical HPLC) = 20.0 min.

*4.2.28. (R)-1-([1,1′-biphenyl]-4-ylmethyl)-7-(3-aminopyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-III-117, 5g**)*

UILK-I-17 (**26**) (50 mg, 0.13 mmol) was dissolve in DMSO (1 mL) and (R)-tert-Butyl [[pyrrolidin-3-yl]methyl]carbamate (49 mg, 0.26 mmol) and DIPEA (100 μL) were added. The reaction was heated to 30 °C and stirred for 20 h. To the crude reaction product 1 mL of 4 N aqueous hydrochloric acid and 4 mL of ACN were added and stirred for 24 h. The ACN was removed by rotatory evaporation and the remaining aqueous layer was frozen and lyophilized. Pure **UIJD-III-117** (**5g**) was isolated, 20 mg, 32% over 2 steps. ^{1}H NMR (300 MHz, MeOD) δ 9.22 (s, 1H), 8.48 (exchangeable) (s, 3H), 7.85 (d, J = 14.4 Hz, 1H), 7.67 (dd, J = 13.7, 8.0 Hz, 4H), 7.45 (t, J = 7.6 Hz, 4H), 7.37 (d, J = 7.1 Hz, 1H), 6.72 (d, J = 7.7 Hz, 1H), 5.85 (s, 2H), 3.97–3.77 (m, 2H), 3.69–3.46 (m, 3H), 2.26 (dt, J = 14.7, 7.2 Hz, 1H), 2.16–1.97 (m, 1H).^{19}F NMR (282 MHz, MeOD) δ −126.70 to −127.04 (m). MS ESI calculated for (M + H)+458.18, found 458.18. Retention time (analytical HPLC) = 18.6min.

*4.2.29. (R)-1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-7-(3-(methylamino)pyrrolidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-III-118, 5h**)*

UILK-I-17 (**26**) (50 mg, 0.13 mmol) was dissolve in DMSO (1 mL) and (R)-tert-Butyl [[pyrrolidin-3-yl]methyl]carbamate (53 mg, 0.26 mmol) and DIPEA (100 μL) were added. The reaction was heated to 30 °C and stirred for 21 h. To the crude reaction product 1 mL of 4 N aqueous hydrochloric acid and 5 mL of ACN were added and stirred for 24 h. The ACN was removed by rotatory evaporation and the remaining aqueous layer was frozen and lyophilized. Pure **UIJD-III-118** (**5h**) was isolated, 60 mg, 75%. ^{1}H NMR (300 MHz, MeOD) δ 9.45 (exchangeable) (bs, 2H), 9.22 (s, 1H), 7.86 (d, J = 14.2 Hz, 1H), 7.67 (dd, J = 15.0, 7.8 Hz, 3H), 7.49–7.41 (m, 3H), 7.37 (d, J = 7.2 Hz, 1H), 6.75 (d, J = 7.4 Hz, 1H), 5.86 (s, 2H), 3.85–3.66 (m, 4H), 3.58–3.46 (m, 1H), 2.59 (m, 3H), 2.31 (m, 2H). ^{19}F NMR (282 MHz, MeOD) δ −126.52 to −126.83 (m). MS ESI calculated for $(M + H)^+$ 472.2, found 472.2. Retention time (analytical HPLC) = 19.5 min.

*4.2.30. (R)-1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-4-oxo-7-((pyrrolidin-3-ylmethyl)amino)-1,4-dihydroquinoline-3-carboxylic acid (**UICK-IV-093, 5i**)*

UICK-III-197 (**26**) (100 mg, 0.26 mmol) was dissolved in 2 mL DMSO to which was added (S)-3-(Aminomethyl)-1-N-Boc-pyrrolidine (76.9 mg, 0.38 mmol) and DIPEA (89 μL, 0.51 mmol). The reaction was set to stir at 40 °C for 1.5 h. To the reaction mixture 1 mL of 6 N HCl was added and stirred for 18 h. To the reaction mixture water was added and the resulting mixture was centrifuged and the supernatant removed. The remaining precipitant was washed with water and centrifuged again. The resulting precipitate was lyophilized and pure **UICK-IV-093** (**5i**) was isolated, 95.5 mg, 68%. ^{1}H NMR (300 MHz, DMSO) δ 9.15 (s, 1H), 9.03 (bs, 1H, exchangeable), 7.82 (d, J = 11.9 Hz, 1H), 7.67 (m, 4H), 7.42 (m, 5H), 7.22 (bs, 1H, exchangeable), 6.81 (d, J = 7.1 Hz, 1H), 5.88 (s, 2H), 3.29 (m, 2H), 3.16 (m, 2H), 2.92 (m, 2H), 2.36 (m, 1H), 1.87 (m, 1H), 1.57 (m, 1H). ^{19}F NMR (282 MHz, DMSO) δ −132.24 (s, 1F). MS ESI calculated for $(M + H)^+$ 472.20, found 472.20. Retention time (analytical HPLC) = 19.1 min.

*4.2.31. 1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-7-((3aS,7aS)-hexahydro-1H-pyrrolo[3,4-c]pyridin-2(3H)-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UICK-IV-091, 5k**)*

UICK-III-197 (**26**) (100 mg, 0.26 mmol) was dissolved in 4 mL DMSO to which was added tert-butyl(3aR, 7aS)-octahydro-5H-pyrrolo [3,4-c] pyridine-5-carboxylate (87 mg, 0.38 mmol) and DIPEA (89 μL, 0.51 mmol). The reaction was set to stir at 40 °C for 1.5 h. To the reaction mixture 1 mL of 6 N HCl was added and stirred for 18 h. To the reaction mixture water was added and the resulting mixture was centrifuged and the supernatant removed. The remaining precipitant was washed with water and centrifuged again. The resulting precipitate was lyophilized and pure **UICK-IV-091** (**5k**) was isolated 97 mg, 70%.^{1}H NMR (300 MHz, DMSO) δ 15.60 (bs, 1H, exchangeable), 9.19 (s, 1H), 8.96 (bs, 1H, exchangeable), 7.82 (d, J = 14.2 Hz, 1H), 7.66 (dd, J = 14.8, 8.1 Hz, 4H), 7.42 (m, 5H), 6.69 (d, J = 7.5 Hz, 1H), 5.82 (s, 2H), 3.67 (s, 1H), 3.53 (m, 3H), 3.09 (m, 4H), 2.60 (m, 2H), 1.88 (m, 1H), 1.69 (m, 1H). ^{19}F NMR (282 MHz, DMSO) δ −126.90 (s, 1F). MS ESI calculated for $(M + H)^+$ 498.22, found 498.22. Retention time (analytical HPLC) = 19.9 min.

*4.2.32. (S)-1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-4-oxo-7-((pyrrolidin-3-ylmethyl)amino)-1,4-dihydroquinoline-3-carboxylic acid (**UICK-IV-095, 5j**)*

UICK-III-197 (**26**) (100 mg, 0.26 mmol) was dissolved in 2 mL DMSO to which was added (R)-tert-butyl-3-(aminomethyl)pyrrolidine-1-carboxylate (76.9 mg, 0.38 mmol) and DIPEA (89 μL, 0.51 mmol). The reaction was set to stir at 40 °C for 1.5 h. To the reaction mixture 1 mL of 6 N HCl was added and stirred for 18 h. To the reaction mixture water was added and the resulting mixture was centrifuged and the supernatant removed. The remaining precipitant was washed with water and centrifuged again. The resulting precipitate was lyophilized and pure **UICK-IV-095** (**5j**) was isolated 97.2 mg, 69% ^{1}H NMR (300 MHz, DMSO) δ 9.15 (s, 1H), 8.97 (bs, 1H, exchangeable), 7.82 (d, J = 11.9 Hz, 1H), 7.67 (m, 4H), 7.39 (m, 5H), 7.21 (bs, 1H, exchangeable), 6.81 (d, J = 7.2 Hz, 1H), 5.88 (s, 2H), 3.28 (m, 2H), 3.17 (m, 2H), 2.92 (m, 2H), 2.34 (m, 1H), 1.85 (m, 1H), 1.57 (m, 1H). ^{19}F NMR (282 MHz, DMSO) −132.26 (s, 1F). MS ESI calculated for $(M + H)^+$ 472.20, found 472.20. Retention

time (analytical HPLC) = 19.1 min.

4.2.33. 1-([1,1′-biphenyl]-4-ylmethyl)-6-fluoro-7-(hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UICK-IV-089, 5l**)

UICK-III-197 (100 mg, 0.26 mmol) was dissolved in 4 mL DMSO to which was added octahydropyrrolo [3,4-c] pyrrole (72 mg, 0.64 mmol) and DIPEA (115 μL, 0.67 mmol). The reaction was set to stir at 40 °C for 4 h. To the reaction mixture water was added and the resulting mixture was centrifuged and the supernatant removed. The remaining precipitant was washed with water and centrifuged again. The resulting precipitate was lyophilized and pure **UICK-IV-089** (**5l**) was isolated, 100 mg, 74%. ^{1}H NMR (300 MHz, DMSO) δ 9.37 (bs, 1H, exchangeable), 9.22 (s, 1H), 7.85 (d, J = 14.2 Hz, 1H), 7.67 (m, 4H), 7.42 (m, 5H), 6.78 (d, J = 7.4 Hz, 1H), 5.84 (s, 2H), 3.58 (m, 4H), 3.40 (m, 2H), 3.08 (m, 4H). ^{19}F NMR (282 MHz, DMSO) δ −124.68 (s, 1F). MS ESI calculated for (M + H)+ 484.20, found 484.20. Retention time (analytical HPLC) = 19.8 min.

4.2.34. Ethyl 3-(2,4-difluorophenyl)-3-oxopropanoate (**UIPC-II-078, 28**)

Commercially available ethyl potassium malonate (622 mg, 1 eq.) was dissolved in distilled ACN to which was added trimethylamine (509 μL, 1 eq.) and allowed to stir at 0 °C. To this was added magnesium chloride (414 mg, 1.2 eq.) and the reaction was allowed to stir at RT for 4 h. In another RBF commercially available 2,4-difluorobenzoic acid (**27**) (300 mg, 1 eq.) was dissolved in dry DCM at 0 °C. To this was added oxalyl chloride (192 μL, 1.3 eq.) and DMF (13 μL, 0.1 eq.) at 0 °C and the reaction was allowed to stir at RT for 2 h. Upon completion of 2 h, the reaction was slowly added to the first RBF contacting the ethyl potassium malonate reaction and upon complete addition, the reaction mixture was allowed to stir at RT for 16 h. Upon completion, the reaction was concentrated, washed with 4 N HCl and the extracted thrice with 25 mL ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* and then purified by flash chromatography using a gradient of hexanes:ethyl acetate to afford compound **UIPC-II-078** (**28**) in 70% yield. ^{1}H NMR (300 MHz, Chloroform-d) δ 7.97 (dtd, J = 34.9, 8.7, 6.6 Hz, 1H), 7.06–6.82 (m, 2H), 4.37–4.14 (m, 2H), 3.97 (d, J = 3.7 Hz, 2H), 1.43–1.17 (m, 3H). 19F NMR (282 MHz, CDCl3) δ −100.10 (dt, J = 20.5, 7.7 Hz), −104.98 to −105.38 (m), −105.97 (d, J = 9.1 Hz). MS ESI calculate for (M + H)$^+$ 229.06, found 229.06.

4.2.35. Ethyl 1-([1,1′-biphenyl]-4-ylmethyl)-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**UIPC-II-083, 30a**)

Ethyl 3-(2,4-difluorophenyl)-3-oxopropanoate (**28**) (777 mg, 3.4 mmol) was refluxed in triethylorthoformate (1.42 mL, 8.5 mmol) and acetic anhydride (1.30 mL, 13.6 mmol) for 24 h to yield the intermediate enol ether (**29**). After the reaction was complete, the mixture was concentrated *in vacuo* and then dissolved in ethanol. To this was added [1,1′-biphenyl]-4-ylmethanamine (750 mg, 4.08 mmol) and the reaction was allowed to stir at room temperature for 2 h. The reaction was concentrated *in vacuo* and purified by flash chromatography using a gradient of dichloromethane:methanol. The reaction intermediate was then dissolved in dioxanes, sodium hydride was added at 0 °C and the reaction was allowed to stir at the same temperature for 1 h. Once complete, the reaction was concentrated *in vacuo* and diluted with water. The precipitated product (**30a**) was filtered, dried and used for the next step. Yield: 84%. ^{1}H NMR (300 MHz, CDCl3) δ 8.65 (s, 1H), 8.58 (dd, J = 9.0, 6.4 Hz, 1H), 7.66–7.54 (m, 4H), 7.53–7.30 (m, 4H), 7.23 (d, J = 9.4 Hz, 1H), 7.14 (ddd, J = 8.9, 8.0, 2.3 Hz, 1H), 7.04 (dd, J = 10.3, 2.2 Hz, 1H), 5.40 (s, 2H), 4.44 (q, J = 7.1 Hz, 2H), 1.44 (t, J = 7.1 Hz, 3H). MS ESI calculate for (M + H)$^+$ 402.1455, found 402.14. Retention time (analytical HPLC) = 22.1 min.

4.2.36. 1-([1,1′-biphenyl]-4-ylmethyl)-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIPC-II-085, 31a**)

UIPC-II-083 (**30a**) (100 mg, 0.26 mmol) was dissolved in 30 mL of 1:1 THF: 1% aqueous LiOH and stirred at 40 °C for 2 h. The reaction was diluted with water and acidified to pH 1 with HCl. The aqueous layer was extracted three times with 20 mL EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give **UIPC-02-083** (**31a**) in quantitative yield which was used for next step without purification. MS ESI calculate for (M + H)$^+$ 374.11, found 374.11. Retention time (analytical HPLC) = 22.9 min.

4.2.37. (S)-1-([1,1′-biphenyl]-4-ylmethyl)-7-(3-(aminomethyl) pyrrolidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**PC-II-086B) (6a)**

UIPC-II-085 (**31a**) (100 mg, 0.26 mmol) was dissolved in 5 mL ACN to which was added Boc-(R)-aminomethylpyrrolidine (64 mg, 0.32 mmol) and DIPEA (0.07 mL, 0.40 mmol). The reaction was set to stir at 50 °C for 48 h. Once complete, 3 mL 4 N HCl was added and the reaction was stirred for additional 12 h. The crude product was purified on a C-18 reverse phase HPLC column, 5–95% ACN over 30 min to give **6a** in 72% yield over 2 steps. ^{1}H NMR (300 MHz, MeOD) δ 8.75 (s, 1H), 8.13 (d, J = 9.1 Hz, 1H), 7.81–7.20 (m, 9H), 6.84 (d, J = 9.0 Hz, 1H), 6.36 (s, 1H), 5.63 (s, 2H), 3.51 (d, J = 29.4 Hz, 3H), 3.14 (d, J = 30.4 Hz, 3H), 2.86–2.59 (m, 1H), 2.43–2.20 (m, 1H), 2.06–1.77 (m, 1H). MS ESI calculated for (M + H)$^+$ 454.20, found 454.20. Retention time (analytical HPLC) = 18.1 min.

4.2.38. Ethyl 1-(4-(1H-pyrazol-1-yl)benzyl)-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**UIPC-II-151C, 30b**)

Ethyl 3-(2,4-difluorophenyl)-3-oxopropanoate (**UIPC-II-278**) (**28**) (60 mg, 0.26 mmol) was refluxed in triethylorthoformate (0.110 mL, 0.65 mmol) and acetic anhydride (0.10 mL, 1.05 mmol) for 24 h to yield the intermediate enol ether (**29**). After the reaction was complete, the mixture was concentrated *in vacuo* and then dissolved in ethanol. To this was added (4-(1H-pyrazol-1-yl) phenyl)methanamine (55 mg, 0.30 mmol) and the reaction was allowed to stir at room temperature for 2 h. The reaction was concentrated *in vacuo* and purified by flash chromatography using a gradient of dichloromethane:methanol. The reaction intermediate was then dissolved in dioxanes, sodium hydride (13 mg, 0.52 mmol) was added at 0 °C and the reaction was allowed to stir for 1 h. Once complete, the reaction was concentrated *in vacuo* and diluted with water and extracted thrice with 10 mL ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford **30b** in 89% yield. **30b** was used for the next step without further purification. MS ESI calculate for (M + H)$^+$ 392.136, found 392.13. Retention time (analytical HPLC) = 18.1 min.

4.2.39. 1-(4-(1H-pyrazol-1-yl)benzyl)-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIPC-II-157, 31b**)

UIPC-II-151C (**30b**) (70 mg, 0.17 mmol) was dissolved in 20 mL of 1:1 THF to 1% aqueous LiOH and stirred at 40 °C for 2 h. The reaction was diluted with water and acidified to pH 1 with HCl. The aqueous layer was extracted three times with 20 mL EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give **UIPC-II-157** (**31b**) in quantitative yield which was used for next step without purification. ^{1}H NMR (300 MHz, DMSO) δ 14.99 (s, 1H), 9.32 (s, 1H), 8.59–8.34 (m, 2H), 7.92–7.65 (m, 4H), 7.59–7.36 (m, 3H), 6.63–6.41 (m, 1H), 5.95–5.77 (m, 2H). 19F NMR (282 MHz, DMSO) δ −102.26

(dd, J = 18.4, 7.2 Hz). MS ESI calculate for $(M + H)^+$ 364.10, found 364.10. Retention time (analytical HPLC) = 18.9 min.

4.2.40. (S)-7-(3-(aminomethyl)pyrrolidin-1-yl)-1-(4-(cyclopenta-2,4-dien-1-yl)benzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIPC-II–159B, 6b**)

UIPC-II-157 (**31b**) (40 mg, 0.11 mmol) was dissolved in 3 mL DMSO to which was added Boc-(R)-aminomethylpyrrolidine (33 mg, 0.16 mmol) and DIPEA (0.04 mL, 0.2 mmol). The reaction was set to stir at 50 °C for 1 h. Once complete, 3 mL 4 N HCl was added and the reaction was stirred for additional 12 h. The crude product was purified on a C-18 reverse phase HPLC column, 5–95% ACN over 30 min to give **6b** in 51% yield over 2 steps. ^{1}H NMR (300 MHz, MeOD) δ 9.44 (s, 18H), 8.30 (dd, J = 16.5, 6.0 Hz, 36H), 7.93–7.66 (m, 54H), 7.62–7.46 (m, 34H), 7.22 (dd, J = 9.4, 1.9 Hz, 18H), 6.67 (d, J = 2.0 Hz, 18H), 6.55 (dd, J = 2.6, 1.9 Hz, 17H), 5.99 (s, 34H), 3.60 (ddd, J = 26.8, 18.4, 9.4 Hz, 54H), 3.39 (d, J = 1.7 Hz, 3H), 3.28–3.24 (m, 5H), 3.13 (dd, J = 9.9, 7.4 Hz, 37H), 2.72 (dd, J = 15.1, 7.4 Hz, 18H), 2.36 (dd, J = 11.2, 5.8 Hz, 19H), 2.03–1.80 (m, 18H). MS ESI calculated for $(M + H)^+$ 442.20, found 442.20. Retention time (analytical HPLC) = 14.7 min.

4.2.41. Methyl 1-(4-(1H-pyrazol-1-yl)benzyl)-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (**UIJD-II-291A, 32a**)

UIJD-II-202 (**18**) (55 mg, 0.2 mmol) was added to a flame dried round bottom flask and placed under vacuum. Under argon atmosphere 1-(4-(bromomethyl)phenyl)-1H-pyrazole (99 mg, 0.42 mmol) and K_2CO_3 (85 mg, 0.61 mmol) were added. Distilled ACN (1.5 mL) was added to the reaction flask and the mixture was stirred at 45 °C for 20 h. The crude reaction product was purified by silica gel flash chromatography using a gradient of hexanes to ethyl acetate, yielding 25 mg of pure **UIJD-II-291A** (**32a**), 33% yield. ^{1}H NMR (300 MHz, DMSO) δ 8.60–8.42 (m, 1H), 8.12 (dd, J = 10.0, 8.7 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.72 (d, J = 8.5 Hz, 3H), 7.17 (d, J = 8.5 Hz, 2H), 6.56–6.39 (m, 1H), 5.82–5.62 (m, 2H), 4.04–3.84 (m, 3H), 3.78–3.58 (m, 3H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −135.54 (dd, J = 21.5, 10.2 Hz), −142.98 to −143.35 (m). MS ESI calculated $(M + H)^+$ 426.12, found 426.14. Retention time (analytical HPLC) = 18.9 min.

4.2.42. (S)-1-(4-(1H-pyrazol-1-yl)benzyl)-7-(3-(aminomethyl) pyrrolidin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-292B, 7a**)

UIJD-II-291A (**32a**), (25 mg, 0.06 mmol) was placed in a reaction flask and 1.5 mL of 1% LiOH and 3 mL of distilled THF were added. The reaction mixture was stirred for 3.5 h. The solvent was removed by rotary evaporation and the crude product used without further purification. The carboxylic acid (**33a**) was then placed in a flame dried round bottom flask under vacuum. Under argon atmosphere DMSO (800 μL) was added with stirring, followed by tert-butyl (pyrrolidin-3-ylmethyl)carbamate (18.5 mg, 0.09 mmol) and triethylamine (45 μL, 0.31 mmol). The reaction mixture was heated to 45 °C and stirred for 24 h. The reaction mixture was diluted with 1:1 ACN to water and purified on a C-18 reverse phase HPLC column, 25–95% ACN over 30 min. To the purified product 1 mL of ACN and 1 mL of 4 N HCl were added and the reaction was stirred at RT for 10 h, to remove the *tert*-butyloxycarbonyl protecting group. The purified product was lyophilized and 10 mg of **UIJD-II-292B** (**7a**) was obtained, 34% yield over 3 steps. ^{1}H NMR (300 MHz, DMSO) δ 9.12 (s, 1H), 8.53–8.37 (m, 1H), 8.27 (s, 3H), 7.77 (d, J = 8.3 Hz, 2H), 7.69 (d, J = 13.4 Hz, 2H), 7.30 (d, J = 8.4 Hz, 2H), 6.60–6.41 (m, 1H), 5.91 (q, J = 15.1 Hz, 2H), 3.76–3.51 (m, 2H), 3.48 (s, 3H), 3.46–3.30 (m, 2H), 2.99–2.83 (m, 2H), 2.56 (d, J = 7.4 Hz, 1H), 2.09 (dd, J = 11.5, 5.9 Hz, 1H), 1.74–1.64 (m, 1H). ^{19}F NMR (300 MHz, DMSO) −120.44 (d, J = 13.5 Hz). MS ESI calculated $(M + H)^+$ 492.2, found 492.2. Retention time (analytical HPLC) = 23 min.

4.2.43. Methyl 6,7-difluoro-8-methoxy-4-oxo-1-((3-phenylisoxazol-5-yl)methyl)-1,4-dihydroquinoline-3-carboxylate (**UIJD-II-293A, 32b**)

UIJD-II-202 (**18**), (150 mg, 0.55 mmol) was placed in a flame dried round bottom flask under vacuum. Under argon atmosphere, 5-(bromomethyl)-3-phenylisoxazole (278 mg, 1.17 mmol) and dried K_2CO_3 (231 mg, 1.7 mmol) were added to the reaction flask. Distilled ACN (1.5 mL) was then added and the reaction mixture stirred at 45 °C for 24 h. The crude product was purified by silica gel flash chromatography using a gradient of hexanes to ethyl acetate, yielding 185 mg of **UIJD-II-293A** (**32b**), 77% yield. ^{1}H NMR (300 MHz, DMSO) δ 8.88 (s, 1H), 7.97–7.89 (m, 1H), 7.84 (m, 2H), 7.51–7.44 (m, 3H), 7.00 (s, 1H), 5.97 (s, 2H), 3.85 (d, J = 1.9 Hz, 3H), 3.78 (s, 3H). ^{19}F NMR (300 MHz, DMSO) δ −135.56 (m), −143.03 (m). MS ESI calculated $(M + H)^+$ 429.12, found 429.18. Retention time (analytical HPLC) = 20.8 min.

4.2.44. (S)-7-(3-(aminomethyl)pyrrolidin-1-yl)-6-fluoro-8-methoxy-4-oxo-1-((3-phenylisoxazol-5-yl)methyl)-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-294B, 7b**)

To **UIJD-II-293A** (**32b**), (180 mg, 0.43 mmol) 2 mL of 1% LiOH and 4 mL of distilled THF were added and stirred at room temperature for 6 h. The solvent was removed by rotary evaporation and the crude product used without further purification. Carboxylic acid **UIJD-II-293B** (**33b**), (180 mg, 0.43 mmol) was placed in a flame dried flask under vacuum, the reaction flask was placed under argon atmosphere and anhydrous DMSO was added (1 mL) with stirring. Next, (R)-tert-butyl (pyrrolidin-3-ylmethyl)carbamate (129 mg, 0.6 mmol) and triethylamine (300 μL, 2.15 mmol) were added. The reaction was heated to 40 °C for 24 h, the crude reaction product was dissolved in 4 mL of 1:1 ACN to water and purified on a C-18 reverse phase HPLC column, 25–95% ACN over 30 min. To the purified product 1 mL of ACN and 1 mL of 4 N HCl were added and the reaction was stirred at 25 °C for 12 h, to remove the *tert*-butyloxycarbonyl protecting group. The purified product was lyophilized and 12 mg of **UIJD-II-294B** (**7b**)**,** was obtained, 7% yield over 3 steps. ^{1}H NMR (400 MHz, DMSO) δ 9.07 (s, 1H), 7.99 (s, 3H), 7.83–7.78 (m, 2H), 7.74 (d, J = 13.8 Hz, 1H), 7.46 (d, J = 3.5 Hz, 3H), 7.01 (s, 1H), 6.12–5.99 (m, 2H), 3.61–3.55 (m, 2H), 3.53 (d, J = 8.0 Hz, 1H), 3.50–3.44 (m, 1H), 3.41 (s, 3H), 3.37 (d, J = 7.6 Hz, 1H), 2.94–2.85 (m, 2H), 2.07 (dd, J = 11.6, 5.3 Hz, 1H), 1.72–1.63 (m, 1H). ^{19}F NMR (300 MHz, DMSO) δ −120.43 to −120.63 (m). MS ESI calculated $(M + H)^+$ 493.2, found 493.19. Retention time (analytical HPLC) 18.3 min.

4.2.45. Methyl 6,7-difluoro-1-((4′-fluoro-[1,1′-biphenyl]-4-yl) methyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (**UIJD-I-265, 32c**)

UIJD-I-207 (**18**) (27.2 mg, 0.1 mmol), 4-fluoro-4′-methyl-1,1′-biphenyl (Scheme S1, **37**) (80 mg, 0.3 mmol) and K_2CO_3 (414.9 mg, 0.3 mmol) were added to a flame dried reaction flask. The reaction flask was flushed three times with argon and the reagents were dissolved in 2 mL of anhydrous DMF at 50 °C. The reaction was stirred for 20 h. The DMF was removed by rotary evaporation, $NaHCO_3$ was added and the aqueous phase was extracted three times with 10 mL of DCM. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was then purified by normal phase silica gel flash chromatography using a gradient of hexanes to ethyl acetate. Yielding pure, **UIJD-I-265 (32c)**, 37 mg, 81% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (s, 1H), 8.20 (ddd, J = 18.8, 9.3, 7.5 Hz, 1H), 7.57–7.50 (m, 4H), 7.16 (ddd, J = 10.1, 8.5, 5.4 Hz, 5H), 5.76 (s, 2H), 3.96 (s, 3H), 3.72 (d, J = 2.3 Hz,

3H). ^{19}F NMR (282 MHz, Acetone) δ −114.87 (td, J = 8.4, 4.9 Hz), −135.37 (dd, J = 21.2, 10.4 Hz), −142.73 to −143.35 (m). MS ESI calculated $(M + H)^+$ 453.12, found 453.65. Retention time (analytical HPLC) = 23.4 min.

*4.2.46. (S)-7-(3-(aminomethyl)pyrrolidin-1-yl)-6-fluoro-1-((4′-fluoro-[1,1′-biphenyl]-4-yl)methyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-I-277, 7c**)*

UIJD-I-265 (**32c**), (37 mg, 0.08 mmol), was dissolved in 1% LiOH in THF (1:2) and stirred at 25 °C for 2 h. The THF was removed by rotary evaporation and then 1 mL of 4 N HCl was added to acidify to pH of 1. The aqueous phase was extracted three times with DCM, washed with brine, dried over Na_2SO_4 filtered and concentrated *in vacuo*. The crude product (**33c**) was used in the next step without purification. **UIJD-I-271** (**33c**), (50 mg, 0.11 mmol) and (R)-tert-butyl (pyrrolidin-3-ylmethyl)carbamate (270 μL, 0.23 mmol) were placed in a flame dried flask. 1 mL of anhydrous DMSO was added under inert conditions. Finally, triethylamine (466 μL, 0.34 mmol) was added and stirred at 25 °C for 42 h. Without purification, 2 mL of trifluoroacetic acid was added and the mixture was stirred for 24 h at 25 °C. The solution was then diluted with water and purified by semi-preparative HPLC (PFP). Pure **UIJD-I-277** (**7c**) was isolated, 20 mg, 48% over 3 steps. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.13 (s, 1H), 7.73 (d, J = 13.9 Hz, 1H), 7.65 (dd, J = 8.8, 5.6 Hz, 2H), 7.59 (d, J = 8.3 Hz, 2H), 7.31–7.22 (m, 4H), 5.94 (d, J = 9.9 Hz, 2H), 3.64–3.58 (m, 2H), 3.47 (s, 3H), 3.42–3.37 (m, 3H), 2.97–2.88 (m, 2H), 2.08 (dd, J = 8.2, 6.2 Hz, 1H), 1.75–1.65 (m, 1H).^{19}F NMR (300 MHz, MeOD) δ −118.28 (ddd, J = 19.0, 11.2, 5.6 Hz), −122.35 to −122.62 (m).). MS ESI calculated M + H^+ 520.20, found 519.95. Retention time (analytical HPLC) = 20.8 min.

*4.2.47. (R)-7-(3-aminopyrrolidin-1-yl)-6-fluoro-1-((4′-fluoro-[1,1′-biphenyl]-4-yl)methyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-244, 8c**)*

UIJD-I-271 (**33c**), (16 mg, 0.36 mmol) was placed in a flame dried round bottom flask with oven dried stir bar and dissolved in 1 mL of ACN. Under argon atmosphere distilled TEA (152 μL, 1.09 mmol) and (R)-tert-butyl pyrrolidin-3-ylcarbamate (18 mg, 0.09 mmol) were added with stirring. The reaction was heated to 60 °C for 27 h. The ACN was removed by rotary evaporation. The crude product was reconstituted in DCM, extracted three times from water and dried *in vacuo*. To the crude reaction product 2 mL of 3 N HCl and 2 mL of ACN was added and stirred at RT for 24 h. The crude product was purified on a PFP propyl reverse phase HPLC column, 20–95% ACN over 25 min. **UIJD-II-244** (**8c**), was isolated as the pure product, 8.1 mg, 44% yield over 2 steps. ^{1}H NMR (400 MHz, MeOD) δ 8.99 (s, 1H), 7.82 (d, J = 13.7 Hz, 1H), 7.56 (dd, J = 14.5, 8.8 Hz, 4H), 7.23 (d, J = 7.8 Hz, 2H), 7.13 (t, J = 8.7 Hz, 2H), 5.90 (s, 2H), 3.96 (d, J = 0.4 Hz, 1H), 3.86 (dd, J = 10.7, 5.6 Hz, 1H), 3.77–3.70 (m, 2H), 3.58 (s, 3H), 3.56–3.46 (m, 1H), 2.41 (dd, J = 13.1, 6.4 Hz, 1H), 2.07 (dd, J = 12.6, 5.9 Hz, 1H). ^{19}F NMR (300 MHz, MeOD) δ −117.37 to −117.58 (m), −121.26 (d, J = 14.3 Hz). MS ESI calculated $(M + H)^+$ 506.18, found 506.2. Retention time (analytical HPLC) = 20.7 min.

*4.2.48. 1-(4-(1H-pyrazol-1-yl)benzyl)-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-289C, 9**)*

UIJD-II-270 (**24**), (70 mg, 0.23 mmol) was dissolved in 500 μL of MeOH with stirring and cooled to 0 °C. (4-(1H-pyrazol-1-yl)phenyl) methanamine (48 mg, 0.28 mmol) was added, the reaction allowed to warmed to room temperature, and stirred for 30 min. The MeOH was removed by rotary evaporation and concentrated *in vacuo*. Crude product was dissolved in 500 μL of dioxane and cooled to 0 °C. After 5 min, sodium hydride was added (22 mg, 0.92 mmol) and the reaction allowed to warm to RT. The reaction was stirred for 30 min before 5 mL of cold water was added. The resulting precipitate was collected, filtered, and washed three times with cold water. The crude product was dried *in vacuo*. Next, the ester intermediate was dissolved in a 1% LiOH in THF solution with stirring and heated to 40 °C. After 1 h the reaction was complete. The THF was removed by rotary evaporation. The aqueous layer was acidified to pH = 1 and the precipitate collected, filtered and washed three times with 1 mL of water. The carboxylic **UIJD-II-289C** (**9**), was collected 43.1 mg, 50% over three steps. ^{1}H NMR (300 MHz, DMSO) δ 9.09 (s, 1H), 8.47 (d, J = 2.6 Hz, 1H), 8.23–8.12 (m, 1H), 7.98 (dd, J = 12.2, 6.7 Hz, 1H), 7.83 (d, J = 8.6 Hz, 2H), 7.73 (d, J = 1.7 Hz, 1H), 7.37 (d, J = 8.5 Hz, 2H), 6.56–6.50 (m, 1H), 5.74 (s, 2H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −129.80 (s), −140.73 (s). MS ESI calculated $(M + Na)^+$ 404.08, found 404.08. Retention time (analytical HPLC) = 20.4 min.

*4.2.49. (S)-1-(4-(1H-pyrazol-1-yl)benzyl)-7-(3-(aminomethyl) pyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-290B, 9a**)*

UIJD-II-289C (**9**)**,** (40 mg, 0.11 mmol) was placed in a flame dried flask with an oven dried stir bar and dissolved in 1 mL of anhydrous DMSO. Under argon atmosphere distilled TEA (73 μL, 0.524 mmol) and Boc-AMP (31.5 mg, 0.15 mmol) were added and stirred. The reaction was warmed to 60 °C for 2 h, 5 mL of cold water was added. The resulting precipitate was collected and washed three times with 5 mL of water. The crude reaction mixture was dissolved in 2 mL of 4 N HCl and 2 mL of ACN with stirring. After 20 h the reaction was complete, the ACN was removed and remaining aqueous layer was lyophilized. Pure **UIJD-II-290B** (**9a**), was collected 13.1 mg, 27% yield over two steps. ^{19}F NMR (282 MHz, DMSO) δ −126.78 (d, J = 12.5 Hz ^{1}H NMR (400 MHz, DMSO) δ 9.16 (s, 1H), 8.47 (d, J = 2.5 Hz, 1H), 7.81 (m, 3H), 7.72 (d, J = 1.6 Hz, 1H), 7.48 (d, J = 8.6 Hz, 2H), 6.63 (d, J = 7.5 Hz, 1H), 6.54–6.49 (m, 1H), 5.80 (s, 2H), 3.70–3.63 (m, 2H), 3.46–3.36 (m, 3H), 2.92–2.85 (m, 2H), 2.11 (dd, J = 11.6, 5.3 Hz, 1H), 1.80–1.72 (m, 1H). ^{19}F NMR (282 MHz, DMSO) δ −126.67 to −126.85 (m).). MS ESI calculated $(M + H)^+$ 462.19, found 462.19. Retention time (analytical HPLC) = 15.71 min.

*4.2.50. (S)-1-(4-(1H-pyrazol-1-yl)benzyl)-6-fluoro-7-(3-((methylamino)methyl)pyrrolidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-304B,9b**)*

UIJD-II-301C (**9**), (50 mg, 0.13 mmol) was added to a flame dried round bottom flask with an oven dried stir bar and dissolved in anhydrous DMSO (1 mL) with stirring. Under argon atmosphere (R)-tert-butyl methyl(pyrrolidin-3-ylmethyl)carbamate (34 mg, 0.15 mmol) and distilled TEA (55 μL, 0.39 mmol) were added. The reaction was stirred at 40 °C for 24 h. Cold water (5 mL) was added to the reaction mixture, the precipitate was collected, filtered, and washed three times with 2 mL of water. To the crude reaction product 2 mL of 4 N HCl and 2 mL of ACN were added and stirred for 12 h at room temperature. The ACN was removed by rotary evaporation and the remaining aqueous layer lyophilized. Pure **UIJD-II-304B** (**9b**), was collected 32 mg, 50% over two steps. ^{1}H NMR (400 MHz, DMSO) δ 15.57 (s, 1H), 9.19 (s, 1H), 9.15 (s, 1H), 8.47 (d, J = 2.5 Hz, 1H), 7.80 (dd, J = 17.1, 11.4 Hz, 3H), 7.71 (d, J = 1.7 Hz, 1H), 7.50 (d, J = 8.5 Hz, 2H), 6.64 (d, J = 7.4 Hz, 1H), 6.55–6.49 (m, 1H), 5.81 (s, 2H), 3.66 (t, J = 7.5 Hz, 1H), 3.53 (s, 1H), 3.48–3.40 (m, 2H), 2.97 (s, 2H), 2.65 (dd, J = 13.9, 7.2 Hz, 1H), 2.53 (d, J = 1.7 Hz, 3H), 2.14 (td, J = 11.9, 6.7 Hz, 1H), 1.78 (dt, J = 17.1, 6.3 Hz, 1H). ^{19}F NMR (282 MHz, DMSO) δ −122.36 to −122.65 (m). MS ESI calculated $(M + H)^+$ 476.2, found 476.21. Retention time (analytical HPLC) = 16.2 min.

*4.2.51. 1-(4-(1H-pyrazol-1-yl)benzyl)-6-fluoro-7-((3aS,7aS)-hexahydro-1H-pyrrolo[3,4-c]pyridin-2(3H)-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-300B, 9c**)*

UIJD-II-297C (**9**), (34 mg, 0.01 mmol) was placed in a flame dried round bottom flask with an oven dried stir bar and dissolved in 1 mL of anhydrous DMSO. Under argon atmosphere (3aS, 7aR)-tert-butyl hexahydro-1H-pyrrolo[3,4-c]pyridine-5(6H)-carboxylate (30.3 mg, 0.13 mmol) and distilled TEA (62 μL, 0.45 mmol) were added. The reaction mixture was heated to 45 °C for 5 h. Cold water (5 mL) was added and the resulting precipitate was collected and washed three times with 2 mL of water. To the crude reaction product 1 mL of 4 N HCl and 2 mL of ACN were added and the reaction was stirred for 5 h at room temperature. The purified product was lyophilized and 18 mg of **UIJD-II-300B** (**9c**), was collected, 42% yield over two steps. ^{1}H NMR (400 MHz, DMSO) δ 15.57 (s, 1H), 9.60 (d, J = 50.1 Hz, 1H), 9.26 (d, J = 20.8 Hz, 1H), 9.15 (s, 1H), 8.50–8.42 (m, 1H), 7.91–7.74 (m, 3H), 7.72 (d, J = 1.6 Hz, 1H), 7.48 (d, J = 8.5 Hz, 2H), 6.66 (d, J = 7.5 Hz, 1H), 6.56–6.43 (m, 1H), 5.79 (d, J = 2.4 Hz, 1H), 3.92–3.68 (m, 1H), 3.49 (d, J = 5.0 Hz, 2H), 3.25–2.94 (m, 6H), 2.67–2.58 (m, 1H), 1.94–1.81 (m, 1H), 1.68 (m, 1H). ^{19}F NMR (300 MHz, DMSO) δ −120.65 (s). MS ESI calculated $(M + H)^+$ 488.2, found 488.21. Retention time (analytical HPLC) = 16.2 min.

*4.2.52. (S)-1-(4-(1H-pyrazol-1-yl)benzyl)-6-fluoro-4-oxo-7-((pyrrolidin-3-ylmethyl)amino)-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-303B, 9d**)*

UIJD-II-301C (**9**), (50 mg, 0.13 mmol) was placed in a flame dried round bottom flask with an oven dried stir bar and dissolved in 1 mL of anhydrous DMSO. Under argon atmosphere (R)-tert-butyl 3-(aminomethyl)pyrrolidine-1-carboxylate (31.5 mg, 0.16 mmol) and distilled TEA (55 μL, 0.39 mmol) were added. The reaction mixture was heated to 70 °C for 20 h. Cold water (5 mL) was added and the resulting precipitate was collected and washed three times with 2 mL of water. To the crude reaction product 1 mL of 4 N HCl and 2 mL of ACN were added and the reaction was stirred for 4 h at room temperature. The crude product was purified on a PFP propyl reverse phase HPLC column, 30–95% ACN over 30 min. The purified product was lyophilized and 12.5 mg of **UIJD-II-303B** (**9d**), was collected, 20% yield over two steps. ^{1}H NMR (300 MHz, DMSO) δ 9.43 (s, 1H), 9.15 (s, 1H), 8.64–8.45 (m, 1H), 7.84 (dd, J = 17.4, 10.2 Hz, 2H), 7.73 (d, J = 1.6 Hz, 1H), 7.48 (d, J = 8.6 Hz, 2H), 7.23 (s, 1H), 6.79 (d, J = 7.1 Hz, 1H), 6.56–6.50 (m, 1H), 5.87 (s, 3H), 3.46–3.25 (m, 2H), 3.17 (ddd, J = 19.2, 10.9, 7.6 Hz, 2H), 3.06–2.82 (m, 2H), 2.42–2.25 (m, 1H), 1.96–1.79 (m, 1H), 1.65–1.49 (m, 1H).^{19}F NMR (300 MHz, DMSO) δ −130.18 (dd, J = 11.4, 7.0 Hz). MS ESI calculated $(M + H)^+$ 462.19, found 462.19. Retention time (analytical HPLC) = 15.5 min.

*4.2.53. (R)-1-(4-(1H-pyrazol-1-yl)benzyl)-6-fluoro-4-oxo-7-((pyrrolidin-3-ylmethyl)amino)-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-298B, 9e**)*

UIJD-II-297C (**9**), (50 mg, 0.13 mmol) was placed in a flame dried round bottom flask with an oven dried stir bar and dissolved in 1 mL of anhydrous DMSO. Under argon atmosphere (S)-3-(aminomethyl)-1-boc-pyrrolidine (39.4 mg, 0.19 mmol) and distilled TEA (200 μL, 0.66 mmol) were added. The reaction mixture was heated to 70 °C for 20 h. Cold water (5 mL) was added and the resulting precipitate was collected and washed three times with 2 mL of water. To the crude reaction product 1 mL of 4 N HCl and 2 mL of ACN were added and the reaction was stirred for 7 h at room temperature. The crude product was purified on a PFP propyl reverse phase HPLC column, 30–95% ACN over 30 min. The purified product was lyophilized and 7.1 mg of **UIJD-II-298B** (**9e**), was collected, 12% yield over two steps. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.39 (s, 1H), 8.28 (d, J = 2.4 Hz, 1H), 8.03 (d, J = 11.5 Hz, 1H), 7.84 (d, J = 8.1 Hz, 2H), 7.77 (d, J = 1.7 Hz, 1H), 7.50 (d, J = 8.1 Hz, 2H), 6.88 (d, J = 6.4 Hz, 1H), 6.64–6.47 (m, 1H), 6.04 (s, 2H), 3.44 (d, J = 3.0 Hz, 2H), 3.40–3.35 (m, 1H), 3.26 (dd, J = 3.2, 1.6 Hz, 1H), 3.18–2.93 (m, 2H), 2.44 (ddd, J = 9.6, 5.3, 2.2 Hz, 1H), 2.12–1.98 (m, 1H), 1.71 (ddd, J = 15.9, 13.7, 8.8 Hz, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −130.06 to −130.55 (m). MS ESI calculated $(M + H)^+$ 462.19, found 462.19. Retention time (analytical HPLC) = 15.6 min.

*4.2.54. 1-(4-(1H-pyrazol-1-yl)benzyl)-6-fluoro-7-(hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-299, 9f**)*

UIJD-II-297C (**9**), (45 mg, 0.12 mmol) was placed in a flame dried round bottom flask with an oven dried stir bar and dissolved in 2 mL of anhydrous DMSO. Under argon atmosphere octahydropyrrolo[3,4-c]pyrrole (33.7 mg, 0.3 mmol) and distilled TEA (54 μL, 0.4 mmol) were added. The reaction mixture was heated to 40 °C for 3 h. Cold water (5 mL) was added and the resulting precipitate was collected and washed three times with 2 mL of water. The purified product was lyophilized and 5.2 mg of **UIJD-II-299** (**9f**), was collected, 9% yield over two steps. ^{1}H NMR (400 MHz, DMSO) δ 9.53 (s, 1H), 9.17 (d, J = 2.5 Hz, 1H), 8.47 (d, J = 1.9 Hz, 1H), 7.88–7.79 (m, 4H), 7.72 (dd, J = 2.9, 1.6 Hz, 1H), 7.46 (dd, J = 8.3, 5.2 Hz, 2H), 6.70 (dd, J = 31.1, 7.3 Hz, 1H), 6.52 (dd, J = 4.3, 2.6 Hz, 1H), 5.81 (s, 2H), 4.22–4.14 (m, 1H), 3.94 (d, J = 14.0 Hz, 1H), 3.73–3.69 (m, 2H), 3.65 (d, J = 7.8 Hz, 2H), 3.40 (d, J = 4.9 Hz, 1H), 3.23 (d, J = 1.7 Hz, 1H), 3.05 (dd, J = 13.4, 6.1 Hz, 2H). ^{19}F NMR (300 MHz, DMSO) δ −124.61 to −124.77 (m). MS ESI calculated $(M + H)^+$ 474.19, found 474.19. Retention time (analytical HPLC) = 15.8 min.

*4.2.55. 6,7-difluoro-4-oxo-1-((3-phenylisoxazol-5-yl)methyl)-1,4-dihydroquinoline-3 carboxylic acid (**UIJD-II-280, 10**)*

UIJD-II-270 (**24**), (87 mg, 0.29 mmol) was dissolved in MeOH (500 μL) with stirring and the reaction mixture was cooled to 0 °C. Next, (3-phenylisoxazol-5-yl)methanamine (60 mg, 0.35 mmol) and warmed to room temperature and stirred for 3 h. The MeOH was removed by rotary evaporation and concentrated *in vacuo*. The crude product was dissolved in dioxane (3 mL) and cooled to 0 °C and stirred for 5 min before sodium hydride was added (13.7 mg, 0.57 mmol). The reaction mixture was allowed to warm to room temperature and was stirred for 1 h 5 mL of cold water was added, the resulting precipitate collected, and washed three times with 2 mL of water. The crude product was then dissolved in a 1% LiOH in THF solution (6 mL) the reaction mixture was stirred for 1 h at 40 °C. The organic layer was removed by rotary evaporation and the product was extracted three times with DCM. The resulting product was concentrated *in vacuo*, yielding 30 mg of **UIJD-II-280** (**10**), 27% over three steps. ^{1}H NMR (300 MHz, DMSO) δ 14.67 (s, 5H), 9.33 (s, 6H), 8.40–8.26 (m, 10H), 7.81 (dd, J = 6.7, 3.0 Hz, 8H), 7.57–7.40 (m, 17H), 7.09 (s, 6H), 6.13 (s, 11H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −125.68 to −125.95 (m), −137.11 to −137.36 (m). MS ESI calculated $(M + Na)^+$ 405.07, found 405.07. Retention time (analytical HPLC) = 22.2 min.

*4.2.56. (S)-7-(3-(aminomethyl)pyrrolidin-1-yl)-6-fluoro-4-oxo-1-((3-phenylisoxazol-5-yl)methyl)-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-II-286B, 10a**)*

UIJD-II-280 (**10**), (30 mg, 0.08 mmol) was placed in a flame dried RBF under argon. Anhydrous DMSO (500 μL) was added with stirring, followed by distilled TEA (54 μL, 0.39 mmol) and Boc-AMP (23 mg, 0.11 mmol). The reaction was heated to 45 °C and stirred for 22 h. The crude product was concentrated *in vacuo* and dissolved in 4 mL of distilled ACN and 2 mL of 4 N HCl. The reaction mixture was stirred for 18 h. The ACN was removed by rotary evaporation and the remaining aqueous solution was frozen and lyophilized. Pure

UIJD-II-286B (**10a**) was collected, 19.1 mg, 53% yield. ^{1}H NMR (400 MHz, DMSO) δ 9.12 (s, 1H), 8.06 (s, 3H), 7.88–7.79 (m, 3H), 7.52–7.43 (m, 3H), 7.21 (s, 1H), 6.71 (d, J = 7.3 Hz, 1H), 6.07 (s, 2H), 3.75 (t, J = 7.2 Hz, 1H), 3.60 (s, 1H), 3.52 (dd, J = 16.8, 8.1 Hz, 1H), 2.95–2.85 (m, 2H), 2.61–2.52 (m, 1H), 2.14 (dd, J = 11.5, 5.1 Hz, 1H), 1.78 (dd, J = 12.4, 8.1 Hz, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −126.59 to −126.85 (m). MS ESI calculated $(M + H)^+$ 463.17, found 463.17. Retention time (analytical HPLC) = 17.4 min.

*4.2.57. (S)-7-(3-(aminomethyl)pyrrolidin-1-yl)-6-fluoro-4-oxo-1-((3-(pyridin-4-yl)-1,2,4-oxadiazol-5-yl)methyl)-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-III-130,11a**)*

UIJD-III-116 (**24**) (104 mg, 0.34 mmol) was dissolved in methanol (5 mL), cooled to 0 °C and [3-pyridin-4-yl-1,2,4-oxadiazol-5-yl] methanamine (103 mg, 0.41 mmol) was added. The reaction stirred at room temperature for 48 h. The reaction mixture was then concentrated *in vacuo* and the resultant crude product was used without further purification. The crude product was dissolved in dioxane (5 mL), cooled to 0 °C, and sodium hydride (24 mg, 1.0) mmol) was added. The reaction was allowed to warm to room temperature was stirred for 28 h. The reaction mixture was concentrated *in vacuo* and the crude product was used without further purification. To the crude intermediate 5 mL of 1% LiOH and 10 mL of THF were added and the reaction stirred for 25 min. The crude product was concentrated *in vacuo* and used in the next step without purification. The crude intermediate was dissolve in DMSO (1 mL) and (R)-tert-Butyl [[pyrrolidin-3-yl]methyl]carbamate (34 mg, 0.18 mmol) and DIPEA (100 μL) were added. The reaction was heated to 45 °C and stirred for 1 h. To the crude reaction product 2 mL of 4 N aqueous hydrochloric acid and 4 mL of ACN were added and stirred for 24 h. The boc deprotected product was purified by semi-preparative HPLC on a C-18 column, 10–50% over 30 min. Pure **UIJD-III-130** (**11a**) was isolated, 20 mg, 7% yield over 5 steps. ^{1}H NMR (300 MHz, DMSO) δ 9.13 (s, 1H), 8.78 (d, J = 6.0 Hz, 2H), 7.87 (dt, J = 5.9, 4.0 Hz, 4H), 6.66 (d, J = 7.3 Hz, 1H), 6.34 (s, 2H), 3.84–3.71 (m, 1H), 3.62–3.45 (m, 3H), 3.44–3.35 (m, 2H), 2.97–2.86 (m, 1H), 2.18–2.03 (m, 1H), 1.75 (m, 1H).^{19}F NMR (282 MHz, $CDCl_3$) δ −126.65 (m). MS ESI calculated for (M + H)+ 465.16, found 465.16. Retention time (analytical HPLC) = 12.5 min.

*4.2.58. 7-((S)-3-(aminomethyl)pyrrolidin-1-yl)-6-fluoro-4-oxo-1-(1-(3-(pyridin-4-yl)-1,2,4-oxadiazol-5-yl)ethyl)-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-III-067C, 12a**)*

UIJD-II-296A (**24**), (300 mg, 0.99 mmol) was dissolved in MeOH (3 mL) and cooled to 0 °C. 1-(3-(pyridin-4-yl)-1,2,4-oxadiazol-5-yl) ethanamine (272 mg, 0.89 mmol) was added, the reaction allowed to warm to room temperature, and stirred for 1 h. The MeOH was removed by rotary evaporation and the product concentrated *in vacuo*. To the crude product 1 mL of dioxane was added and the reaction mixture cooled to 0 °C. After 5 min, sodium hydride (48 mg, 1.99 mmol) was added and stirred for 24 h to facilitate ring closing. The reaction product was concentrated and used in the next step without purification, 5 mL of 1% LiOH and 10 mL THF were added and the reaction stirred at rt for 1 h. The crude product was concentrated *in vacuo*. The hydrolyzed product (**12**) was placed in a flame dried round bottom flask with an oven dried stir bar and dissolved in 1 mL of anhydrous DMSO. Under argon atmosphere distilled TEA (418 μL, 3.0 mmol) and Boc-AMP (237.9 mg, 1.18 mmol) were added to the reaction mixture and heated to 40 °C for 24hrs. Upon reaction completion 3 mL of ACN and 3 mL of 4 N HCl were added and stirred for 24 h. The crude product was purified on a PFP propyl reverse phase HPLC column, 20–95% ACN over 30 min. Pure **UIJD-III-067C** (**12a**), was collected 18 mg, 4% yield over 5 steps. ^{1}H NMR (400 MHz, DMSO) δ 8.99 (s, 1H), 8.85 (d, J = 5.2 Hz, 3H), 8.16 (s, 3H), 8.00 (d, J = 4.9 Hz, 2H), 7.87 (d, J = 14.1 Hz, 1H), 6.97 (s, 1H), 5.01 (s, 3H), 3.78–3.70 (m, 1H), 3.60 (s, 1H), 3.51 (dd, J = 16.6, 8.1 Hz, 1H), 3.46–3.37 (m, 1H), 2.87 (s, 2H), 2.62–2.51 (m, 1H), 2.11 (s, 1H), 1.77 (dd, J = 17.2, 8.7 Hz, 1H). ^{19}F NMR (300 MHz, DMSO) −126.74 (ddd, J = 12.7, 8.8, 4.6 Hz). MS ESI calculated $(M + H)^+$ 479.18, found 479.18. Retention time (analytical HPLC) = 13.4 min.

*4.2.59. (S)-7-(3-(aminomethyl)pyrrolidin-1-yl)-6-fluoro-1-((4′-fluoro-[1,1′-biphenyl]-4-yl)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**UIJD-III-133, 13a**)*

UIJD-III-116 (**24**) (170 mg, 0.58 mmol) was dissolved in methanol (10 mL), cooled to 0 °C and (4′-fluoro-[1,1′-biphenyl]-4-yl) methanamine (Scheme S2, **40**) (140 mg, 0.7 mmol) was added. The reaction stirred at room temperature for 48 h. The reaction mixture was then concentrated *in vacuo* and the resultant crude product was used without further purification. The crude intermediate was dissolved in dioxane (10 mL), cooled to 0 °C, and sodium hydride (50 mg, 2.1 mmol) was added to facilitate ring closing. The reaction was allowed to warm to room temperature was stirred for 28 h. The reaction mixture was concentrated *in vacuo* and the crude product was used without purification. The crude product was dissolve in DMSO (1 mL) and (R)-tert-Butyl [[pyrrolidin-3-yl]methyl]carbamate (223 mg, 1.14 mmol) and DIPEA (100 μL) were added. The reaction was heated to 45 °C and stirred for 24 h. To the crude reaction product 15 mL of 4 N aqueous hydrochloric acid and 15 mL of ACN were added and stirred for 48 h. The boc deprotected product was purified by semi-preparative HPLC on a C-18 column, 30–95% over 30 min. **UIJD-III-133** (**13a**) was isolated, 60 mg, 20% yield over 4 steps.^{1}H NMR (300 MHz, MeOD) δ 9.19 (s, 1H), 8.14 (exchangeable) (s, 3H), 7.81 (d, J = 14.2 Hz, 1H), 7.73–7.62 (m, 4H), 7.48–7.39 (m, 2H), 7.27 (m, 2H), 6.67 (d, J = 7.4 Hz, 1H), 5.82 (s, 2H), 3.53 (m, 1H), 3.44 (m, 2H), 2.90 (t, 2H), 2.12 (m, 1H), 1.80–1.74 (m, 1H). ^{19}F NMR (282 MHz, DMSO) δ −115.13 (m), −126.61 to −126.89 (m). MS ESI calculated for $(M + H^+)$ 490.19, found 490.19. Retention time (analytical HPLC) = 19.8 min.

4.3. Relaxation assay for eukaryotic Topo I

hTopo I purchased from Inspiralis was used in this study. One unit of hTopo I was defined as the amount of topoisomerase required to completely relax 0.3 μg of the negatively-supercoiled pBR322 plasmid DNA under the conditions described below.

Relaxation reaction mixtures (20 μL) contained 50 mM Tris-HCl (pH 7.5 at 23 °C), 100 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 50 μg/mL bovine serum albumin (BSA), 0.3 μg of the supercoiled plasmid DNA, 1 unit of hTopo I, and the various concentrations of fluoroquinolones. Reaction mixtures were incubated at 37 °C for 15 min and terminated by adding ethylenediaminetetraacetic acid (EDTA) to 25 mM. The DNA products were analyzed by electrophoresis through vertical 1.2% agarose gels at 2 V/cm for 15 h in TAE buffer. Gels were stained with 0.5 μg/mL ethidium bromide, and then photographed and quantified using a MyECL Imager (Thermo Fisher Scientific).

4.4. Docking

Docking was preformed using the program MOE 2016.08. The DNA receptor sequences d(CGCGAATTCGCG) [PDB ID: 1G3X] and d(AGGTCACGGTGGCCA) [PDB ID: 2MG8] were downloaded from the protein data bank (http://rcsb.org/pdb). The solvent, molecular ions, and co-crystallized compounds were removed from the receptor. The protonate 3D function was used to prepare the receptor. This function determines the lowest potential energy configuration, places hydrogens and assigns the ionization state throughout the system. Next the site finder function in MOE was used to determine

potential sites for ligand binding docking calculations. Corresponding atoms within 4.5 A of the binding sites were selected and all other atoms deselected. For receptor 1G3X, site 1 and 2 were chosen. For receptor 1DNH, site 1 was chosen. The active site is defined as the selected receptor atoms. Ligand conformations are generated with the bond rotation method. These are then placed in the site with the triangle matcher method, and ranked with the London dG scoring function. The retain option is set to 30 and specifies the number of poses to pass to refinement which is set to rigid receptor, for energy minimization in the pocket, before rescoring with the GBVI/WSAdGscoring function.

The fluoroquinolones were drawn in ChemDraw, converted to a mol file and appended to a MOE ligand database. The molecules were prepared using the database wash application in MOE. This application rebalances the protonation states at pH = 7.0, adds or deletes explicit hydrogen atoms, regenerates the 3D coordinates, and places molecules in the lowest energy conformation state.

4.5. DNA binding assay

The fluorescence spectra and intensities were measured on a Perkin Elmer EnVision multi-label plate reader using UV-Star 96-well black, clear bottom plates. The final volume in each well was 100 μL. DNA purity and concentration was determined on a BioTek Synergy 2 multi-detection microplate reader. Gen5 reader software and the nucleic acid quantification protocol was used. All experiments were done in presence of 10 mM Tris-HCl buffer (pH = 7.4) at room temperature. Appropriate fluoroquinolone excitation and max emission wavelengths were determined in the absence of DNA. All stock solutions of the tested compounds were prepared in DMSO and then diluted in 10 mM Tris-HCl buffer (pH = 7.4), the final DMSO content did not exceed 5%. Data is reported as technical replicates, n = 3. The error bars represent standard error of the means shown for each of the three trials. Data was plotted and analyzed with GraphPad Prism 7 software. Data was normalized to fluoroquinolone fluorescence in the absence of DNA (100%) and appropriate blanks corresponding to the buffer were subtracted to correct the background fluorescence. The difference in the fluorescence observed at each DNA concentration from the initial fluorescence of the drug and plotted as the concentration of DNA in μM versus the percent (%) fluoroquinolone (FQ) fluorescence compared to untreated control. The 27-bp sequence d(CCTTACGTGCATAGTCATTCATGACCG) was purchased from IDT as the complimentary ssDNA, DNA was annealed per IDT protocols. The concentration of ssDNA stock solutions was determined spectrophotometrically using the appropriate molar extinction coefficient for each ssDNA sequence. Briefly, ssDNA complimentary strands were incubated in a 1:1 ratio heated to 95 °C for 2 min and then allowed to cool to room temperature.

Ct-DNA was purchased from Worthington Biochemical Corporation, as lyophilized dsDNA. The Ct-DNA was suspended in 10 mM Tris-HCl buffer (pH = 7.4) at 4 °C and was mixed over 24 h to produce a homogenous solution. The purity of DNA was recorded by using the absorbance ratio A_{260}/A_{280}. The attenuance ratio was between 1.8 and 1.9 and required no further purification. Various concentrations of Ct-DNA were used and the concentration was determined spectrophotometrically using the average molar extinction coefficient value of 6600 M^{-1} cm^{-1} of a single nucleotide at 260 nm. To determine if a bathochromic shift is observed upon addition of Ct-DNA the fluoroquinolone concentration was 25 μM and the Ct-DNA concentration was 0 or 400 μM. The fluorescence spectra of the fluoroquinolone was obtain from 320 nm–600 nm.

4.6. Calculated logP

For Canvas:

The AlogP values of the compounds were calculated using Canvas cheminformatics (part of Schrodinger Suite), which were used preliminarily to predict the lipophilicity of the synthesized compounds. The compounds were imported into a canvas database and their AlogP values were calculated by utilizing the Physicochemical Descriptors utility within the Molecular Properties calculation Table 1. (Schrödinger Release 2018–3: Canvas, Schrödinger, LLC, New York, NY, 2018.)

For MOE:

The AlogP/clogP/clogD values of the compounds were calculated using Cheminformatics tools in Molecular Operating Environment (Chemical Computing Group), which were used preliminarily to predict the lipophilicity of the synthesized compounds. The compounds were imported into a MOE database followed by energy minimization and protonation. Their AlogP/clogP/clogD values were calculated by utilizing the Molecular Descriptors utility1 [34].

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Appendix A. Supplementary data

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