Supporting Information for

Overcoming Target-Mediated Quinolone Resistance in Topoisomerase IV by Introducing Metal Ion-Independent Drug-Enzyme Interactions

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Supplementary Table S1. Full chemical, library, and abbreviated names of compounds used in this study.

Name used in this study	Library name	Chemical name
Ciprofloxacin		1-Cyclopropyl-6-fluoro-1,4-dihydro-7-(1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid
Cipro-dione	UIHS-IIa-249	3-amino-7-(1-piperazinyl)-1-cyclopropyl-6-fluoro-2,4(1H,3H)-quinazolinedione
8-Methyl-cipro	UILI-2-89	1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-7-(1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid
8-Methyl-cipro-dione	UILI-2-87	3-amino-7-(1-piperazinyl)-1-cyclopropyl-6-fluoro-8-methyl-2,4(1H,3H)-quinazolinedione
8-Methyl-cipro-NA-dione	UILI-2-75	7-(1-piperazinyl)-1-cyclopropyl-6-fluoro-8-methyl-2,4(1H,3H)-quinazolinedione
8-Methoxy-cipro	UIHS-IIa-101	1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-7-(1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid
8-Methoxy-cipro-dione	UIHS-IIa-253	3-amino-7-(1-piperazinyl)-1-cyclopropyl-6-fluoro-8-methoxy-2,4(1H,3H)-quinazolinedione
Moxifloxacin		1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid
Moxi-dione	UING-5-157, Lot UIHS-IIa-251	3-amino-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-1-cyclopropyl-6-fluoro-8-methoxy-2,4(1H,3H)-quinazolinedione
8-Methyl-moxi	UIHS-IIa-45	1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid
8-Methyl-moxi-dione	UILI-2-81	3-amino-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-1-cyclopropyl-6-fluoro-8-methyl-2,4(1H,3H)-quinazolinedione
8-Methyl-moxi-NA-dione	UILI-2-83	7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-1-cyclopropyl-6-fluoro-8-methyl-2,4(1H,3H)-quinazolinedione
8-H-moxi	UIHS-IIa-239	1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid
8-H-moxi-dione	UIHS-IIa-247	3-amino-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-1-cyclopropyl-6-fluoro-2,4(1H,3H)-quinazolinedione
CP-115,955	CP-115,955	1-Cyclopropyl-6-fluoro-1,4-dihydro-7-(4-hydroxyphenyl)-4-oxo-3-quinolinecarboxylic acid
8-Methyl-3'-(AM)P-FQ	UIHS-I-303	1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-7-[(3S)-3-(aminomethyl)-1-pyrrolidinyl]-4-oxo-3-quinolinecarboxylic acid
8-Methyl-3'-(AM)P-dione	UIJR1-048, Lot UILI-2-95	3-amino-7-[(3S)-3-(aminomethyl)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-8-methyl-2,4(1H,3H)-quinazolinedione
8-Methyl-3'-(AM)P-NA-dione	UILI-2-97	7-[(3S)-3-(aminomethyl)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-8-methyl-2,4(1H,3H)-quinazolinedione
8-Methoxy-3'-(AM)P-FQ	UING-5-249, Lot UIHS-IIa-77	1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(3S)-3-(aminomethyl)-1-pyrrolidinyl]-4-oxo-3-quinolinecarboxylic acid
8-Methoxy-3'-(AM)P-dione	UING-5-207, Lot UIHS-IIa-229	3-amino-7-[(3S)-3-(aminomethyl)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-8-methoxy-2,4(1H,3H)-quinazolinedione
8-H-3'-(AM)P-FQ	UIHS-IIIa-35	1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[(3S)-3-(aminomethyl)-1-pyrrolidinyl]-4-oxo-3-quinolinecarboxylic acid
8-H-3'-(AM)P-dione	UIHS-IIa-245	3-amino-7-[(3S)-3-(aminomethyl)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-2,4(1H,3H)-quinazolinedione

PREPARATION AND CHARACTERIZATION OF COMPOUNDS EMPLOYED IN THIS STUDY

General Methods.

All reagents were purchased from commercial sources and used without further purification. Semi-preparative HPLC separations to purify final compounds were carried out with a Phenomenox-Luna 5u PFP (150 mm × 21.2 mm) column connected to a Shimadzu system that was equipped with two LC-10AT pumps (one for solvent A and one for solvent B), SPD-M10Avp photodiode array detector, and SCL-10Avp system controller. The system was connected to a Dell Optiplex 755 and controlled by Shimadzu EZStart Version 7.4 software. All synthetic derivatives were characterized by nuclear magnetic resonance (NMR) and mass spectrometry. Routine NMR spectra were obtained for ^{1}H and ^{19}F using a Bruker Ultrashield 300 MHz instrument at ambient temperature. Chemical shifts are reported in parts per million from low to high field and referenced to residual solvent. Standard abbreviations indicating multiplicity are used as follows: br s = broad, d = doublet, m = multiplet, s = singlet, and t = triplet. In many cases DMSO was used as the solvent (DMSO-δ_6 – 2.50). Low resolution mass spectrometry (LRMS) was determined using a Thermo LCQ Deca mass spectrometer with electrospray ionization (ESI) and quadrupole ion trap mass analyzer.

All final compounds were purified >95%, as determined by analytical high-performance liquid chromatography (HPLC). The analytical HPLC analysis was determined using a Shimadzu system equipped with LC-20AT pump, DGU-14A degasser, CBM-20A system controller, and SPD-M10A vp photodiode array detector. The system was connected to a Dell Optiplex GX400 PC and controlled by Shimadzu Client/Server Version 7.4 software. A Restek Allure PFP Propyl (150 mm x 4.6 mm) 5u column was used as stationary phase, while mobile phase consisted of solvent A (water, buffered 0.1% TFA) and solvent B (acetonitrile, buffered 0.1% TFA). Gradient elution used the following program: from t = 0 min [solvent A (0.95 mL/min), solvent B (0.05 mL/min)] to t = 30 min [solvent A (0.05 mL/min), solvent B (0.95 mL/min)], to t = 35 min [solvent A (0.05 mL/min), solvent B (0.95 mL/min)], to t = 40 [solvent A (0.95 mL/min), solvent B (0.05 mL/min)]. Analytical HPLC was used to monitor reactions, as well as to determine purity for final products.

Specific Methods.

Each compound synthesized in this work followed established procedures for nucleophilic aromatic substitution of the C-7 position on the requisite fluoroquinolone or quinazolinedione core structures (Figure S1). The 8-H (core A) and 8-methoxy (core B) fluoroquinolone core structures were purchased from Acros and 3B Scientific, respectively. The 8-methyl fluoroquinolone core structure (core C) was synthesized following procedures previously described.[1] Dione core structures D-G were synthesized as previously reported.[2] We have reported[3] and used well-established methods[2a] for addition of C-7 groups to one or more fluoroquinolone and/or quinazolinedione core structures for each of the three C-7 groups studied in this work.

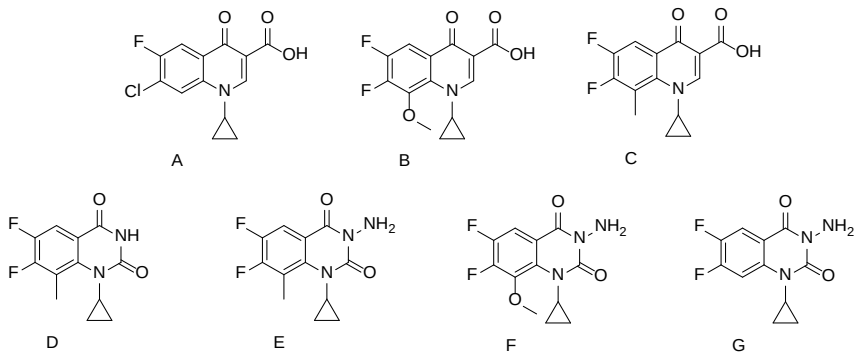
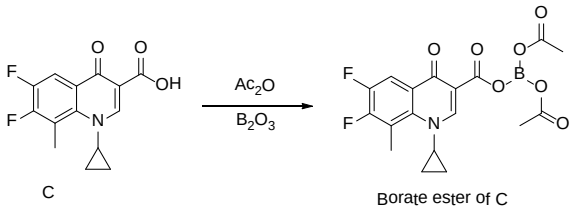


Figure S1. Fluoroquinolone cores A-C and quinazolinedione cores D-G used for nucleophilic aromatic substitution on C-7. **A**. 7-chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid; **B**. 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid; **C**. 1-cyclopropyl-6,7-difluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid; **D**. 1-cyclopropyl-6,7-difluoro-8-methyl-2,4(1*H*,3*H*)-quinazolinedione; **E**. 3-amino-1-cyclopropyl-6,7-difluoro-8-methyl-2,4(1*H*,3*H*)-quinazolinedione; **F**. 3-amino-1-cyclopropyl-6,7-difluoro-8-methoxy-2,4(1*H*,3*H*)-quinazolinedione; **G**. 3-amino-1-cyclopropyl-6,7-difluoro-2,4(1*H*,3*H*)-quinazolinedione.

Fluoroquinolone cores A-C were converted to the corresponding borate esters using a previously reported method.[1] For example, FQ core C was converted to the borate ester of FQ core C as shown in Scheme S1 by the following procedure: Boric acid (Fisher, 0.34 g, 5.34 mmol) was added portionwise to acetic anhydride (Aldrich, 12.14 mL) at 100 °C during one hour. FQ core C (1.25 g, 4.47 mmol) was added to the clear solution, and the mixture was heated to 100 °C for one hour. The reaction mixture was concentrated *in vacuo*, diisopropyl ether (Fisher, 25 mL) was added, and a whitish tan precipitate formed. The precipitate was collected and dried to yield the borate ester (95%). Spectral data were consistent with published literature values.[1] The other borate esters of FQ cores A and B were synthesized in a similar manner. After conversion, the borate esters of the FQ cores were used immediately for coupling.



Scheme S1. Conversion of FQ core C to the corresponding borate ester.

STRUCTURAL CHARACTERIZATION DATA FOR THE COMPOUNDS SYNTHESIZED IS AS FOLLOWS:

8-methyl-cipro (UILI-2-89). The borate ester of FQ core C (20 mg, 0.0716 mmol) was added to piperazine (Acros, 15.3 mg, 0.1685mmol) and stirred at 130 ^{o}C in DMSO (anhydrous, 0.3 mL) for 12 hours. The product was then isolated and purified with semi-preparative HPLC (45%). Previous synthesis does not contain spectral data.[1] Of note, it is included in a variety of patents, one of which are listed here.[4] ^{1}H NMR (300 MHz, DMSO-d_6) δ = 14.83 (br s, 1H), 8.82 (br s, 2H), 7.86 (d, J = 12 Hz, 1H), 4.38 (m, 1H), 3.41 (s, 3H), 3.25 (m, 4H), 2.79 (s, 3H), 1.17 (m, 2H), 0.89 (m, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -121.82. LRMS (ESI) calcd for ($M+H^+$) 346.25, found 346.17.

8-methoxy-cipro (UIHS-IIa-101). The borate ester of FQ core B (28.55 mg, 0.0675 mmol) and piperazine (Acros, 14.5 mg, 0.1685 mmol) were stirred at room temperature in DMSO (anhydrous, 0.3 mL) for four hours. Aqueous sodium hydroxide (3%, 0.5 mL) was added and allowed to stir for two hours. The resulting product was purified as the trifluoroacetate salt by semi-preparative HPLC (96%). While it was previously reported in a patent,[5] no spectral data were provided. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 14.87 (br s, 1H), 8.87 (br s, 1H), 8.74 (s, 1H), 7.82 (d, J = 12 Hz, 1H), 4.17 (m, 1H), 3.81 (s, 3H), 3.51 (m, 4H), 3.28 (m, 4H), 0.98-1.18 (m, 4H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -120.23. LRMS (ESI) calcd for ($M+H^+$) 362.15, found 362.13.

8-methyl-cipro-dione (UILI-2-87). The quinazolinedione core E (20 mg, 0.0749 mmol) was combined with piperazine (Acros, 16 mg, 0.187 mmol) and triethylamine (Fisher, 20 µL) and stirred at 130 ^{o}C in DMSO (anhydrous, 0.3 mL) for 12 hours. The product was then isolated and purified with semi-preparative HPLC (68%). While the compound was previously reported and evaluated,[6] no spectral data were provided. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.84 (br s, 2H), 7.53 (d, J = 12 Hz, 1H), 3.44 (m, 1H), 3.34 (s, 4H), 3.26 (m, 4H), 2.54 (s, 3H), 1.04 (m, 2H), 0.51 (m, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -127.21. LRMS (ESI) calcd for ($M+H^+$) 334.24, found 334.27.

8-methoxy-cipro-dione (UIHS-IIa-253). The quinazolinedione core F (20 mg, 0.0706 mmol) was combined with piperazine (Acros, 15.2 mg, 0.1776 mmol) and triethylamine (Fisher, 20 µL) in DMSO (0.3 mL) and stirred at 80 ^{o}C for 3 hours. The product was then isolated and purified as the di-trifluoroacetate salt with semi-preparative HPLC (25%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.933 (br s, 2H), 7.47 (d, J = 11.8 Hz, 1H), 3.69 (s, 3H), 3.46 (m, 4H), 3.27 (m, 5H), 0.96 (m, 2H), 0.58 (m, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -126.29. LRMS (ESI) calcd for ($M+H^+$) 350.16, found 350.24.

Cipro-dione (UIHS-IIa-249). The quinazolinedione core G (20 mg, 0.079 mmol) was combined with piperazine (Acros, 17.0 mg, 0.1975 mmol) and triethylamine (Fisher, 20 µL) and stirred at 80 ^{o}C in DMSO (anhydrous, 0.3 mL) for one hour. The product was purified as the di-trifluoroacetate salt by semi-preparative HPLC (22%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.96 (br s, 2H), 7.67 (d, J = 12.5 Hz, 1H), 7.18 (d, J = 7.1 Hz, 1H), 3.43 (m, 4H), 3.32 (m, 4H), 2.97 (m, 1H), 1.25 (m, 2H), 0.84 (m, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -128.47. LRMS (ESI) calcd for ($M+H^+$) 320.15, found 320.14.

8-methyl-cipro-NA-dione (UILI-2-75). The quinazolinedione core D (20 mg, 0.062 mmol) was combined with piperazine (Acros, 13.2 mg, 0.155 mmol) and triethylamine (Fisher, 20 µL) and stirred at 130 °C in DMSO (anhydrous, 0.3 mL) overnight. The product was then isolated and purified with semi-preparative HPLC (61%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.84 (br s, 2H), 7.53 (d, J = 12 Hz, 1H), 3.64 (m, 5H), 3.23 (m, 4H), 1.00 (m, 2H), 0.51 (m, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -127.75. LRMS (ESI) calcd for ($M+H^+$) 319.23, found 319.25.

8-H-moxi (UIHS-IIa-239). The borate ester of FQ core A (15 mg, 0.0366 mmol), cis-octahydropyrrolo[3,4*b*]-pyridine (3B Scientific, 13.9 mg, 0.1099 mmol), and triethylamine (Fisher, 20 µL) were heated to 90 °C in DMSO (anhydrous, 0.4 mL) and stirred for one hour. Aqueous sodium hydroxide (3%, 1 mL) was added and allowed to stir for two hours. The resulting product was purified as the di-trifluoroacetate salt by semi-preparative HPLC (78%). While it was previously reported in a patent,[7] no spectral data were provided. Additionally, it was evaluated in some other work[8] and was first published as 'Bay y3114'.[7] ^{1}H NMR (300 MHz, DMSO-d_6) δ = 15.48 (br s, 1H), 9.13 (br m, 1H), 8.61 (s, 1H), 7.86 (d, J = 14.5 Hz, 1H), 7.18 (d, J = 7.2 Hz, 1H), 3.90-4.07 (m, 2H), 3.59-3.81 (m, 4H), 3.24 (m, 1H), 2.96 (m, 1H), 2.75 (m, 1H), 1.66-1.84 (m, 4H), 1.32 (m, 1H), 1.15 (m, 1H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -127.71. LRMS (ESI) calcd for ($M+H^+$) 372.17, found 372.21.

8-methyl-moxi (UIHS-IIa-45). The borate ester of FQ core C (50 mg, 0.1225 mmol) and cis-octahydropyrrolo[3,4b]pyridine (3B Scientific, 27.8 mg, 0.3675 mmol) were stirred in DMSO (anhydrous, 0.5 mL) at 100 °C for 21 hours. Aqueous sodium hydroxide (3%, 1.0 mL) was added after the mixture was removed from heat and stirred for 30 min, cooled, and re-acidified. The product was purified as the di-trifluoroacetate salt by semi-preparative HPLC (16%). While it was previously reported in a patent,[9] no spectral data were provided. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 15.11 (br s, 1H), 9.34 (m, 1H), 8.79 (s, 1H), 8.56 (m, 1H), 7.72 (d, J = 13.4 Hz, 1H), 4.33 (m, 1H), 4.07 (m, 1H), 3.93 (m, 1H), 3.72 (m, 1H), 3.51 (m, 2H), 3.25 (m, 1H), 2.91 (m, 1H), 2.71 (m, 1H), 2.62 (s, 3H), 1.73 (m, 4H), 1.20 (m, 1H), 0.94 (m, 1H), 0.81 (m, 1H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -121.67. LRMS (ESI) calcd for ($M+H^+$) 386.19, found 386.21.

8-H-moxi-dione (UIHS-IIa-247). The quinazolinedione core G (20 mg, 0.079 mmol) was combined with cis-octahydropyrrolo[3,4b] pyridine (3B Scientific, 24.9 mg, 0.1975 mmol) and triethylamine (Fisher, 20 µL) and stirred at 80 °C in DMSO (anhydrous, 0.3 mL) for 1.5 hours. The product was then isolated and purified as the di-trifluoroacetate salt by semi-preparative HPLC (69%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 9.13 (br m, 1H), 8.58 (br m, 1H), 7.54 (d, J = 13.9 Hz, 1H), 6.71 (d, J = 7.5 Hz, 1H), 3.93 (m, 2H), 3.66 (m, 3H), 3.23 (m, 1H), 2.92 (m, 2H), 2.72 (m, 1H), 1.75 (m, 4H), 1.25 (m, 2H), 0.81 (m, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -135.16. LRMS (ESI) calcd for ($M+H^+$) 360.19, found 360.29.

8-methyl-moxi-dione (UILI-2-81). The quinazolinedione core E (20 mg, 0.0747 mmol) was combined with cis-octahydropyrrolo[3,4b] pyridine (3B Scientific, 11.4 mg, 0.1872 mmol) and triethylamine (Fisher, 20 µL) and stirred at 130 °C in DMSO (anhydrous, 0.3 mL) for 12 hours. The product was then isolated and purified with semi-preparative HPLC (64%). While it was previously reported in a patent,[10] no spectral data were provided. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 9.03 (br m, 1H), 8.27 (br m, 1H), 7.44 (d, J = 13.5 Hz, 1H), 3.93 (m, 2H), 3.42 (m, 2H), 3.34 (m, 2H), 3.01 (m, 2H), 2.67 (m, 1H), 2.43 (d, J = 8.4 Hz,

3H), 1.73 (m, 4H), 1.05 (m, 2H), 0.53 (m, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -127.02. LRMS (ESI) calcd for ($M+H^+$) 374.27, found 374.31.

Moxi-dione (UING-5-157, Lot UIHS-IIa-251). The quinazolinedione core F (20 mg, 0.0706 mmol), cis-octahydropyrrolo[3,4*b*]pyridine (3B Scientific, 22.4 mg, 0.1776 mmol), and triethylamine (Fisher, 20 µL) were combined in DMSO (anhydrous, 0.3 mL) and stirred at 80 °C for three hours. The product was purified as the di-trifluoroacetate salt by semi-preparative HPLC (54%). Spectral data were consistent with literature values[3a] and the compound was evaluated in other work.[8b]

8-methyl-moxi-NA-dione (UILI-2-83). The quinazolinedione core D (20 mg, 0.079 mmol) was combined with cis-octahydropyrrolo[3,4b] pyridine (3B Scientific, 25 mg, 0.1975 mmol) and triethylamine (Fisher, 20 µL) and stirred at 130 °C in DMSO (anhydrous, 0.3 mL) for 12 hours. The product was then isolated and purified with semi-preparative HPLC (70%). While it was previously reported in a patent,[11] no spectral data were provided. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.32 (br m, 1H), 9.04 (br m, 1H), 8.30 (br m, 1H), 7.38 (d, J = 13.5 Hz, 1H), 3.93 (m, 2H), 3.62 (m, 1H), 3.44 (m, 4H), 2.95 (m, 1H), 2.68 (m, 1H), 2.49 (s, 3H), 1.17 (m, 4H), 1.05 (m, 2H), 0.55 (m, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -127.62. LRMS (ESI) calcd for ($M+H^+$) 359.26, found 359.26.

8-methyl-3'-(AM)P-dione (UIJR1-048, Lot UILI-2-95). Made as previously described.[10, 12] Spectral data were consistent with literature values[12] and the compound was evaluated in other work.[8b, 13]

8-H-3'-(AM)P-dione (UIHS-IIa-245). The quinazolinedione core G (20 mg, 0.079 mmol), (*R*)-3-N-Boc-aminomethyl pyrrolidine (Asta Tech, Inc., 39.6 mg, 0.1975 mmol), and triethylamine (Fisher, 20 µL) were combined in DMSO (anhydrous, 0.3 mL) and stirred at 80 °C for three hours. Trifluoroacetic acid (concentrated, 1 mL) was added and stirred for one hour. The resulting product was purified as the di-trifluoroacetate salt by semi-preparative HPLC (28%). While it was previously reported in literature,[10] no spectral data were provided. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.92 (br s, 3H), 7.52 (d, J = 13.8 Hz, 1H), 6.72 (d, J = 7.5 Hz, 1H), 3.49 (m, 4H), 3.35 (m, 1H), 2.97 (m, 2H), 2.87 (m, 1H), 2.15 (m, 1H), 1.78 (m, 1H), 1.24 (m, 2H), 0.83 (m, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -134.52. LRMS (ESI) calcd for ($M+H^+$) 334.17, found 334.26.

8-methoxy-3'-(AM)P-dione (UING-5-207, Lot UIHS-IIa-229). Made as previously described.[3a] Spectral data were consistent with literature values[3a] and the compound was evaluated in other work.[8b, 12, 14]

8-methyl-3'-(AM)P-FQ (UIHS-I-303). The borate ester of FQ core C (50 mg, 0.1225 mmol) and (*R*)-3-N-Boc-aminomethyl pyrrolidine (AstaTech Inc., 44.1 mg, 0.3675 mmol) were stirred at 100 °C in DMSO (anhydrous, 0.5 mL) for 21 hours. Aqueous sodium hydroxide (3%, 0.8 mL) was added after the mixture was removed from heat and stirred for three hours. After three hours, trifluoroacetic acid (conc., 1 mL) was added and stirred overnight. The product was purified as the di-trifluoroacetate salt by semi-preparative HPLC (31%). Previous synthesis does not contain spectral data.[1] ^{1}H NMR (300 MHz, DMSO-d_6) δ = 15.10 (s, 1H), 8.80 (s, 1H), 7.88 (br s, 3H), 7.73 (d, 1H), 4.33 (m, 1H), 3.56 (m, 4H), 3.39 (m, 1H), 2.97 (m, 2H), 2.57 (s, 3H), 2.15 (m, 1H), 1.75 (m, 1H), 1.19 (m, 2H), 0.88 (m, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -122.2. LRMS (ESI) calcd for ($M+H^+$) 360.17, found 360.10.

8-H-3'-(AM)P-FQ (UIHS-IIIa-35). The borate ester of FQ core A (15 mg, 0.0366 mmol), (*R*)-3-N-Boc-aminomethyl pyrrolidine (Asta Tech, Inc., 22 mg, 0.1099 mmol), and triethylamine (Fisher, 20 µL) were heated to 90 °C in DMSO (anhydrous, 0.3 mL) and stirred for one hour. Aqueous sodium hydroxide (3%, 1 mL) was added and allowed to stir for two hours. The resulting product was purified as the di-trifluoroacetate salt by semi-preparative HPLC (92%). While it was previously reported in literature,[15] no spectral data were provided. Additionally, it was evaluated in some other work.[16] ^{1}H NMR (300 MHz, DMSO-d_6) δ = 15.506 (s, 1H), 8.58 (s, 1H), 7.93 (br m, 3H), 7.81 (d, J = 14.2 Hz, 1H), 7.06 (d, J = 5.8 Hz, 1H), 3.81-3.59 (m, 4H), 3.44 (m, 1H), 2.99 (m, 2H), 2.60 (m, 1H), 2.18 (m, 1H), 1.82 (m, 1H), 1.30 (m, 2H), 1.16 (m, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -127.09. LRMS (ESI) calcd for ($M+H^+$) 346.16, found 346.21.

8-methoxy-3'-(AM)P-FQ (UING-5-249, Lot UIHS-IIa-77). Made as previously described.[3a] Spectral data were consistent with literature values[17] and the compound was evaluated in other work.[3a, 8b, 12, 14]

8-methyl-3'-(AM)P-NA-dione (UILI-2-97). The quinazolinedione core D (20 mg, 0.079 mmol) was combined with (*R*)-3-N-Boc-aminomethyl pyrrolidine (Asta Tech, Inc., 39.6 mg, 0.196 mmol) and triethylamine (Fisher, 20 µL) and stirred at 130 ^{o}C in DMSO (anhydrous, 0.25 mL) for 12 hours. Trifluoroacetic acid (conc., 1 mL) was added and stirred for 1 hour. The product was then isolated and purified with semi-preparative HPLC (60%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.30 (s, 1H), 7.97 (br m, 3H), 7.36 (d, J = 13.2 Hz, 1H), 3.71 (m, 2H), 3.46 (m, 3H), 3.31 (s, 2H), 2.95 (m, 2H), 2.36 (s, 3H), 2.10 (m, 1H), 1.74 (m, 1H), 1.03 (m, 2H), 0.49 (m, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ = -127.02. LRMS (ESI) calcd for ($M+H^+$) 333.45, found 333.23.

^{1}H NMR SPECTRA AND HPLC CHROMATOGRAMS:

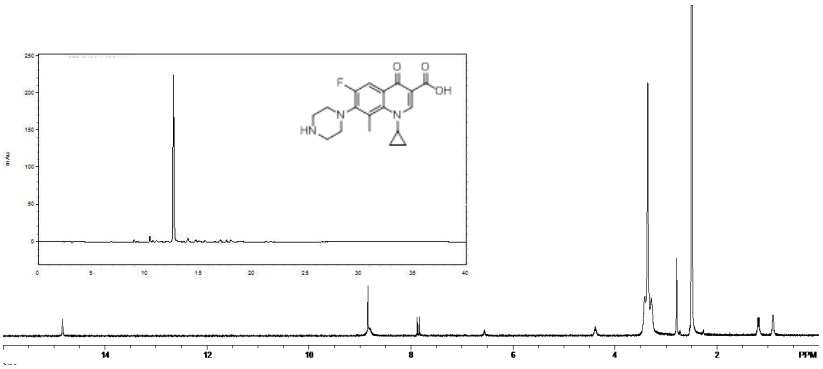


Figure S2. ^{1}H NMR of **8-methyl-cipro** (UILI-2-89) in DMSO; HPLC inset (300 nm).

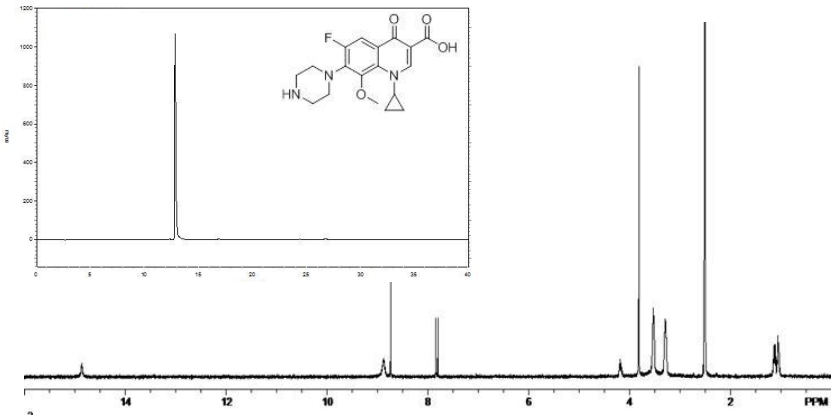


Figure S3. ^{1}H NMR of **8-methoxy-cipro** (UIHS-IIa-101) in DMSO; HPLC inset (300 nm).

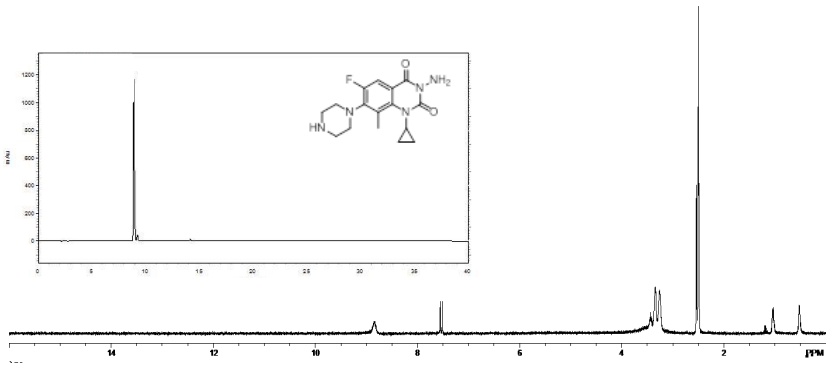


Figure S4. ^{1}H NMR of **8-methyl-cipro-dione** (UILI-2-87) in DMSO; HPLC inset (300 nm).

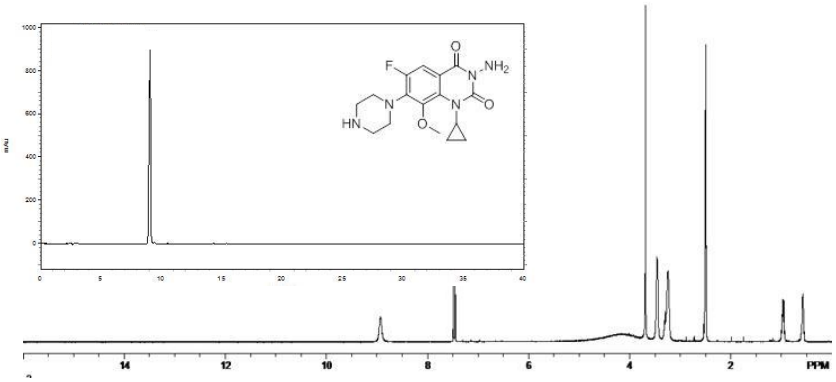


Figure S5. ^{1}H NMR of **8-methoxy-cipro-dione** (UIHS-IIa-253) in DMSO; HPLC inset (300 nm).

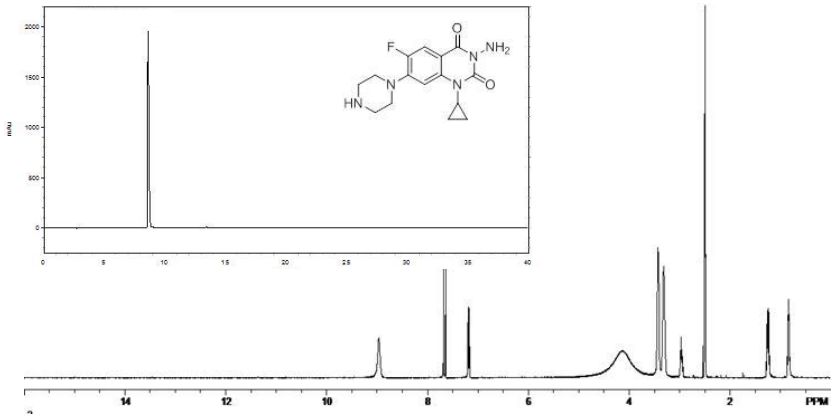


Figure S6. ^{1}H NMR of **cipro-dione** (UIHS-IIa-249) in DMSO; HPLC inset (300 nm).

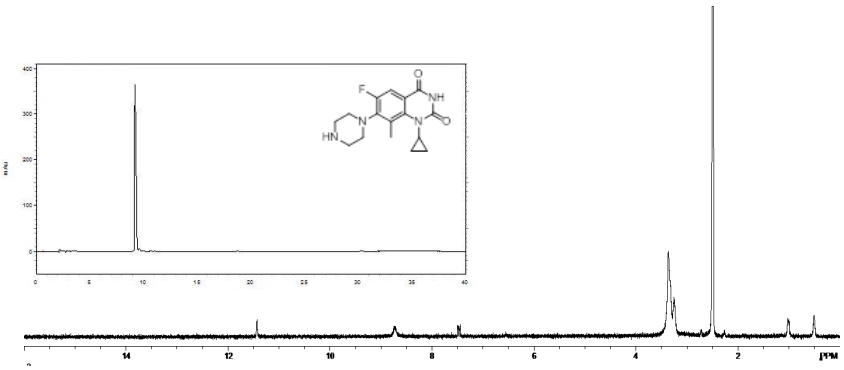


Figure S7. ^{1}H NMR of **8-methyl-cipro-NA-dione** (UILI-2-75) in DMSO; HPLC inset (300 nm).

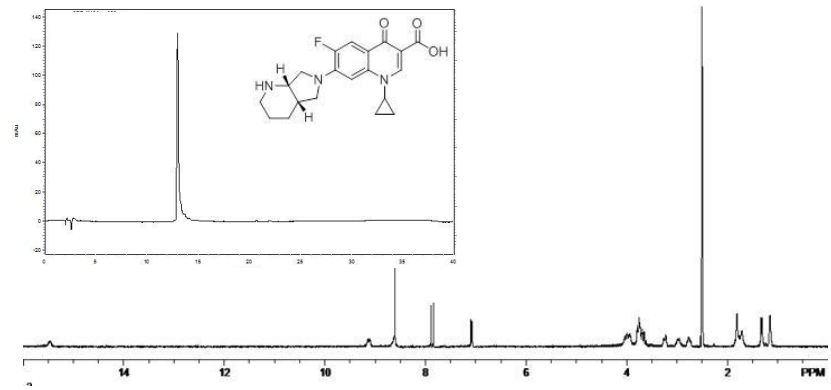


Figure S8. ^{1}H NMR of **8-H-moxi** (UIHS-IIa-239) in DMSO; HPLC inset (300 nm).

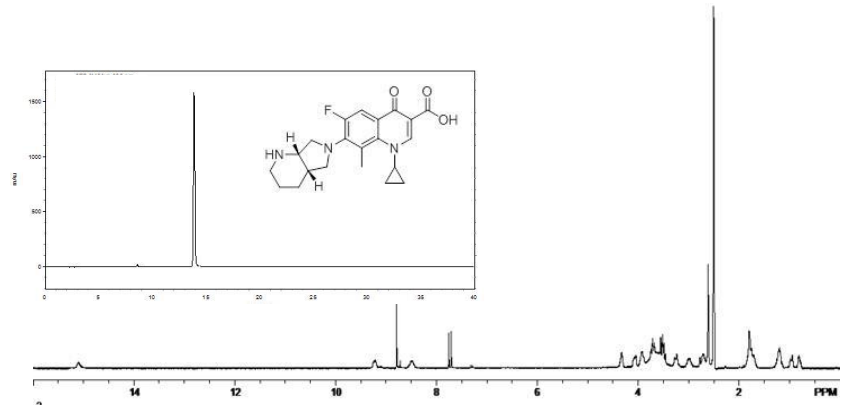


Figure S9. ^{1}H NMR of **8-methyl-moxi** (UIHS-IIa-45) in DMSO; HPLC inset (300 nm).

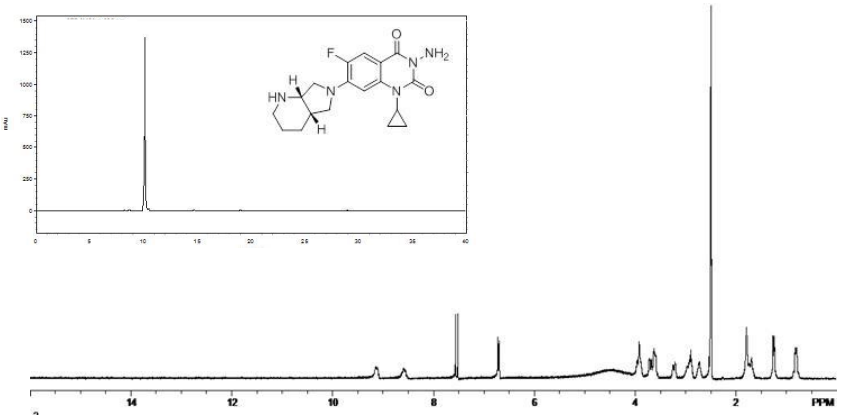


Figure S10. ^{1}H NMR of **8-H-moxi-dione** (UIHS-IIa-247) in DMSO; HPLC inset (300 nm).

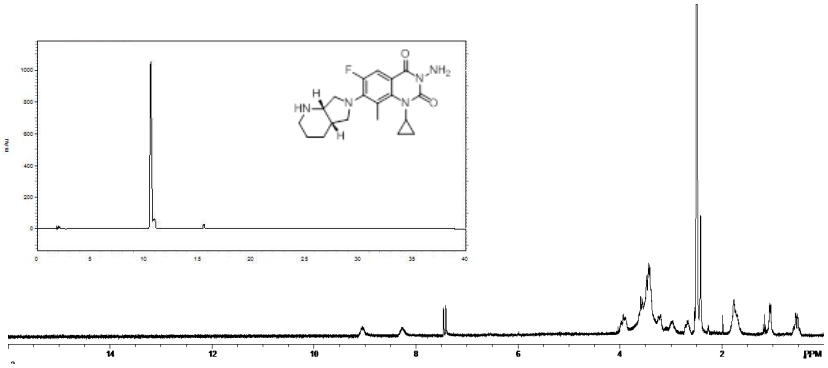


Figure S11. ^{1}H NMR of **8-methyl-moxi-dione** (UILI-2-81) in DMSO; HPLC inset (300 nm).

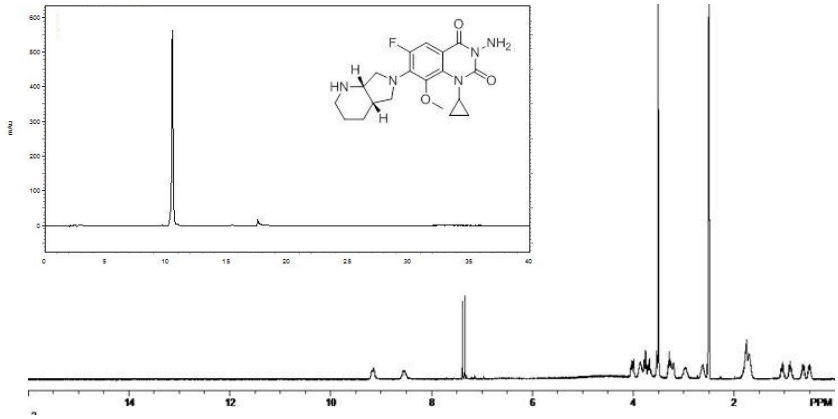


Figure S12. ^{1}H NMR of **moxi-dione** (UING-5-157, Lot UIHS-IIa-251) in DMSO; HPLC inset (300 nm).

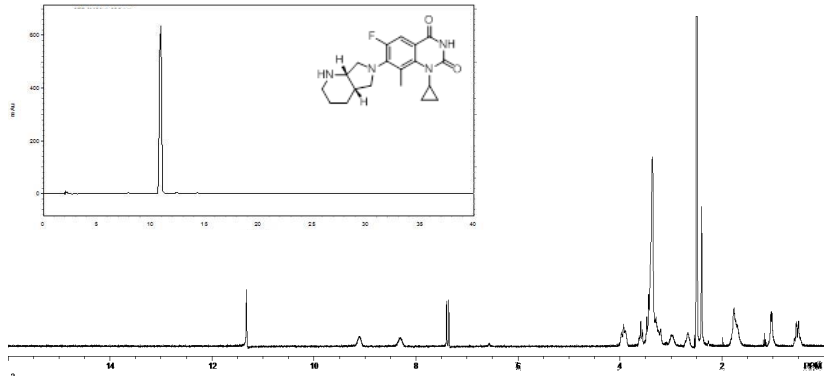


Figure S13. ^{1}H NMR of **8-methyl-moxi-NA-dione** (UILI-2-83) in DMSO; HPLC inset (300 nm).

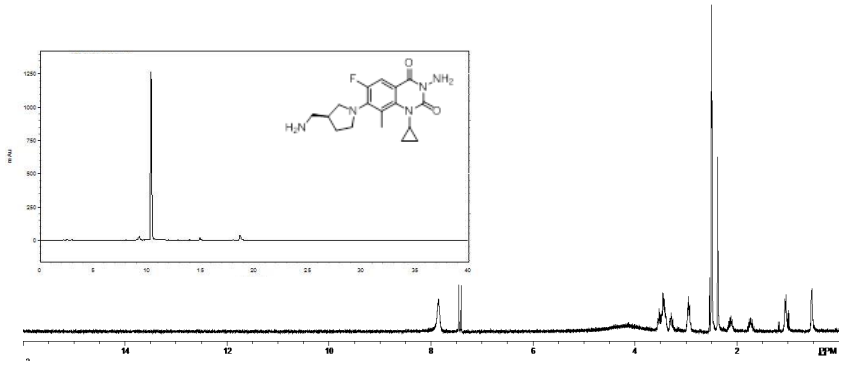


Figure S14. ^{1}H NMR of **8-methyl-3'-(AM)P-dione** (UIJR1-048, Lot UILI-2-95) in DMSO; HPLC inset (300 nm).

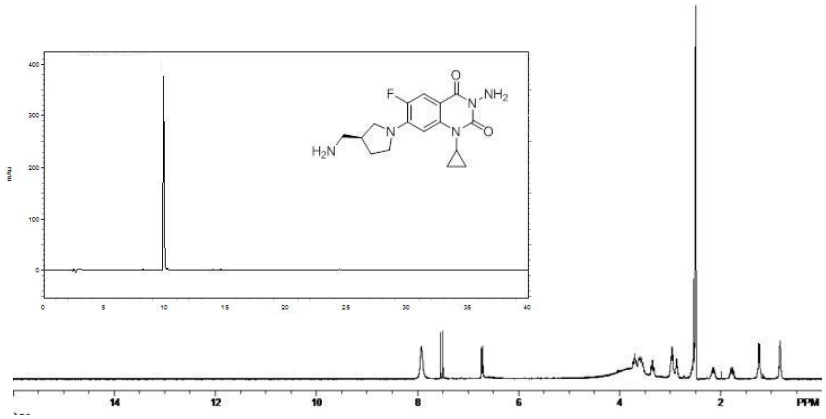


Figure S15. ^{1}H NMR of **8-H-3'-(AM)P-dione** (UIHS-IIa-245) in DMSO; HPLC inset (300 nm).

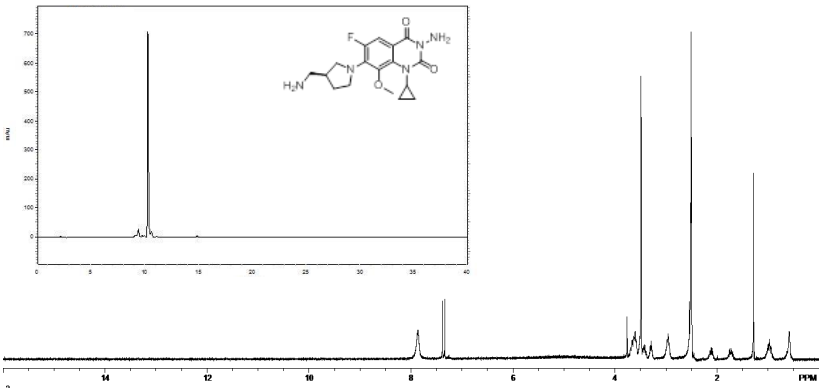


Figure S16. ^{1}H NMR of **8-methoxy-3'-(AM)P-dione** (UING-5-207, Lot UIHS-IIa-229) in DMSO; HPLC inset (300 nm).

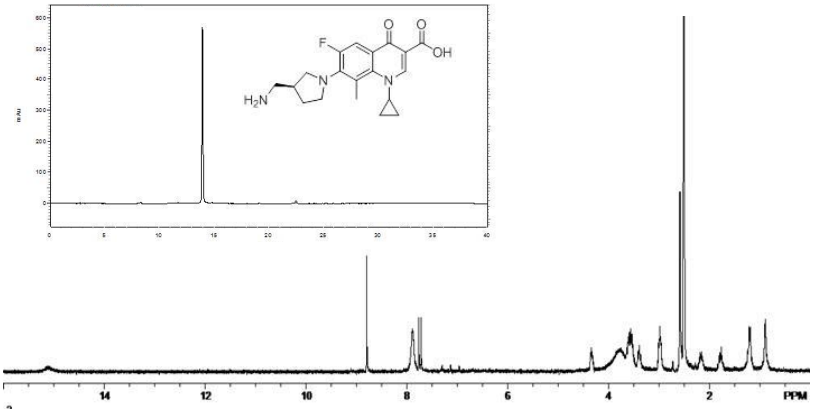


Figure S17. ^{1}H NMR of **8-methyl-3'-(AM)P-FQ** (UIHS-I-303) in DMSO; HPLC inset (300 nm).

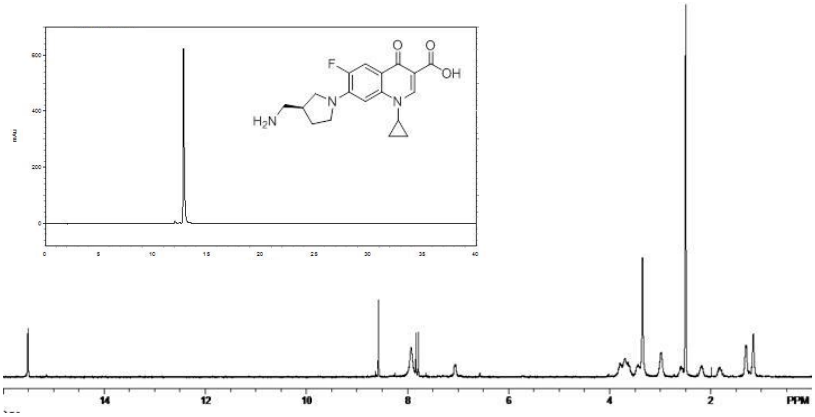


Figure S18. ^{1}H NMR of **8-H-3'-(AM)P-FQ** (UIHS-IIIa-35) in DMSO; HPLC inset (300 nm).

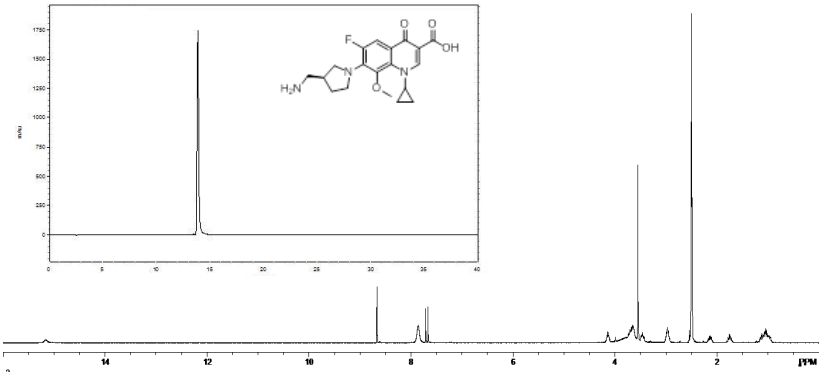


Figure S19. ^{1}H NMR of **8-methoxy-3'-(AM)P-FQ** (UING-5-249, Lot UIHS-IIa-77) in DMSO; HPLC inset (300 nm).

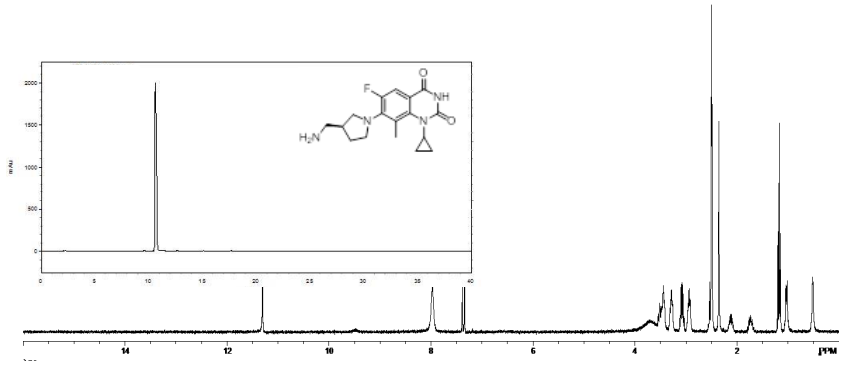


Figure S20. ^{1}H NMR of **8-methyl-3'-(AM)P-NA-dione** (UILI-2-97) in DMSO; HPLC inset (300 nm).

CONSTRUCTION AND PURIFICATION OF TYPE II TOPOISOMERASES

***Bacillus anthracis* topoisomerase IV.** Wild-type genes were PCR-amplified from *B. anthracis* Sterne 34F2 chromosomal DNA and cloned into the pET15b expression vector (Novagen), which added a 6×His tag to the N-terminus of the expressed protein. Mutant genes were generated by QuikChange (Stratagene) site-directed mutagenesis, and recombinant topoisomerase IV subunits were individually expressed in *E. coli* strain BL21(DE3). The resulting proteins were purified by affinity chromatography,[18] dialyzed into 20 mM Tris-HCl (pH 7.5), 200 mM NaCl, and 20% glycerol, and stored at -80 °C. In all assays, topoisomerase IV was used as a 1:1 mixture of GrlA:GrlB.

Generation of hTop2A$^{M762S/M766E}$. The YEpWOB6 expression vector containing the gene for human topoisomerase IIα with a 6×His tag inserted at the C-terminus of the enzyme[19] was the gift of Joseph E. Deweese (Lipscomb University). Mutations in the His-tagged human topoisomerase IIα gene were generated by QuikChange Lightning (Agilent) site-directed mutagenesis using the primer pair 5'-GTCTTCTTATCATCATGGT GAGTCGTCACTAATGGAGACCATTATCAATTTGGC and 5'-GCCAAATTGATAATGGTCTCCATTAGTGACGACTCACCAT GATGATAAGAAGAC (mutated positions are underlined). The mutated gene was sequenced for accuracy using the primer 5'-GGTTTGAAACCAGGTCAGAG.

Purification of His-tagged human topoisomerase IIα. Yeast cells were lysed in Y-PER Plus Reagent (Thermo) containing 1 mM β-mercaptoethanol and protease inhibitors as described by the manufacturer. Unless otherwise noted, the following purification steps were carried out at 4 °C. After addition of 500 mM NaCl, the lysate was centrifuged for 10 min at 19000 × *g*. The cleared lysate was subjected to batch binding to Ni-NTA agarose beads (Qiagen) for 30 min. The beads were then washed for 30 min in wash buffer 1 [1 M NaCl, 30 mM imidazole, 20 mM Tris–HCl (pH 7.9), 1 mM β-mercaptoethanol, and protease inhibitors] followed by four 1 min washes at RT in wash buffer 2 (wash buffer 1 containing 500 mM NaCl rather than 1 M NaCl). Mutant human topoisomerase IIα was eluted with 12 mL elution buffer [500 mM NaCl, 1 M imidazole, 20 mM Tris–HCl (pH 7.9), 1 mM β-mercaptoethanol, and protease inhibitors], collecting 1 mL fractions. Fractions 2-11 were injected into a 20k MWCO Slide-a-Lyzer dialysis cassette (Thermo) and dialyzed for 4 hours into dialysis buffer 1 [750 mM KCl, 50 mM Tris–HCl (pH 7.7), 100 μM Na_2EDTA, and 500 μM DTT] and for 16 hours into dialysis buffer 2 (dialysis buffer 1 containing 40% glycerol).

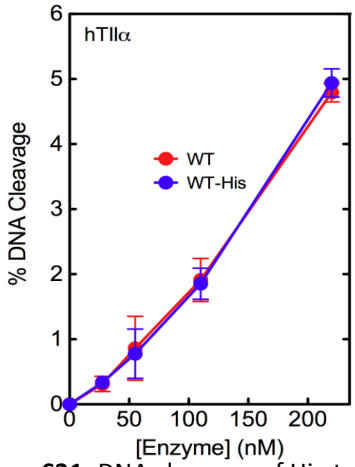


Figure S21. DNA cleavage of His-tagged (WT-His) and untagged (WT) human topoisomerase IIα. Cleavage was as described in the text, except that 5 mM $MgCl_2$ was replaced with 5 mM $CaCl_2$ in the cleavage buffer. Error bars represent the standard deviation of three independent experiments.

REFERENCES FOR SUPPORTING INFORMATION

1. Miyamoto, H.; Ueda, H.; Otsuka, T.; Aki, S.; Tamaoka, H.; Tominaga, M.; Nakagawa, K., Studies on antibacterial agents. III. Synthesis and antibacterial activities of substituted 1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic acids. *Chemical & Pharmaceutical Bulletin* **1990,** *38* (9), 2472-2475.
2. (a) Beylin, V.; Boyles, D. C.; Curran, T. T.; Macikenas, D.; Parlett, R. V.; Vrieze, D., The Preparation of Two, Preclinical Amino-quinazolinediones as Antibacterial Agents. *Organic Process Research & Development* **2007,** *11*, 441-449; (b) Tran, T. P.; Ellsworth, E. L.; Watson, B. M.; Sanchez, J. P.; Showalter, H. D. H.; Rubin, J. R.; Sier, M. A.; Yip, J.; Nguyen, D. Q.; Bird, P.; Singh, R., A Facile Synthesis of Substituted 3-Amino-1*H*-Quinazoline -2,4-Diones. *Journal of Heterocyclic Chemistry* **2005,** *42*, 669-674.
3. (a) German, N.; Malik, M.; Rosen, J. D.; Kerns, R. J., Use of Gyrase Resistance Mutants to Guide Selection of 8-Methoxy-Quinazoline-2,4-Diones. *Antimicrobial Agents and Chemotherapy* **2008,** *52* (11), 3915-3921; (b) Malik, M.; Marks, K. R.; Schwanz, H. A.; German, N.; Drlica, K.; Kerns, R. J., Effect of N-1/C-8 Ring Fusion and C-7 Ring Structure on Fluoroquinolone Lethality. *Antimicrobial Agents and Chemotherapy* **2010,** *54* (12), 5214-5221.
4. Masuzawa, K.; Suzue, S.; Hirai, K.; Ishizaki, T. Quinolonecarboxylic acid derivatives and their preparation. 04894458, 1990.
5. Masuzawa, K.; Suzue, S.; Hirai, K.; Ishizaki, T. Preparation of 8-alkoxyquinolonecarboxylic acids as antibacterials with selective toxicity. EP230295A2, 1987.
6. Hutchings, K. M.; Tran, T. P.; Ellsworth, E. L.; Watson, B. M.; Sanchez, J. P.; Showalter, H. D. H.; Stier, M. A.; Shapiro, M.; Joannides, E. T.; Huband, M.; Nguyen, D. Q.; Maiti, S.; Li, T.; Tailor, J.; Thomas, G.; Ha, C.; Singh, R., Synthesis and antibacterial activity of the C-7 side chain of 3-aminoquinazolinediones. *Bioorganic & Medicinal Chemistry Letters* **2008,** *18* (18), 5087-5090.
7. Petersen, U.; Krebs, A.; Schenke, T.; Philipps, T.; Grohe, K.; Bremm, K. d.; Endermann, R.; Metzger, K. G.; Haller, I. Preparation of (diazabicyclononyl)quinolones and related compounds as antibacterials. EP550903A1, 1993.
8. (a) Malik, M.; Lu, T.; Zhao, X.; Singh, A.; Hattan, C. M.; Domagala, J.; Kerns, R.; Drlica, K., Lethality of Quinolones against *Mycobacterium smegmatis* in the Presence or Absence of Chloramphenicol. *Antimicrobial Agents and Chemotherapy* **2005,** *49* (5), 2008-2014; (b) Malik, M.; Marks, K. R.; Mustaev, A.; Zhao, X.; Chavda, K.; Kerns, R. J.; Drlica, K., Fluoroquinolone and Quinazolinedione Activities against Wild-Type and Gyrase Mutant Strains of *Mycobacterium smegmatis*. *Antimicrobial Agents and Chemotherapy* **2011,** *55* (5), 2335-2343; (c) Wetzstein, H. G.; Hallenbach, W., Tuning of antibacterial activity of a cyclopropyl fluoroquinolone by variation of the substituent at position C-8. *J Antimicrob Chemoth* **2011,** *66* (12), 2801-2808; (d) Cianchetta, G.; Mannhold, R.; Cruciani, G.; Baroni, M.; Cecchetti, V., Chemometric studies on the bactericidal activity of quinolones via an extended VolSurf approach. *Journal of Medicinal Chemistry* **2004,** *47* (12), 3193-3201.
9. Takemura, M.; Takahashi, H.; Kawakami, K.; Namba, K.; Tanaka, M.; Miyauchi, R. Preparation of oxoquinolinecarboxylic acid, oxonaphthyridinecarboxylic acid, and pyridobenzoxazinecarboxylic acid derivatives as antibacterial agents. WO2001058876A1, 2001.
10. Bird, P.; Ellsworth, E. L.; Nguyen, D. Q.; Sanchez, J. P.; Showalter, H. D. H.; Singh, R.; Stier, M. A.; Tran, T. P.; Watson, B. M.; Yip, J. Preparation of 3-aminoquinazoline-2,4-dione antibacterial agents. WO2001053273A1, 2001.
11. Ellsworth, E. L.; Showalter, H. D. H.; Powell, S. A.; Sanchez, J. P.; Kerschen, J. A.; Stier, M. A.; Tran, T. P. Preparation of quinazolinediones as antibacterial agents for quinolone-resistant bacteria. WO2002102793A2, 2002.

12. Oppegard, L. M.; Steck, K. R.; Rosen, J. D.; Schwanz, H. A.; Drlica, K.; Kerns, R. J.; Hiasa, H., Comparison of *In Vitro* Activities of Fluoroquinolone-Like 2,4- and 1,3-Diones. *Antimicrobial Agents and Chemotherapy* **2010,** *54* (7), 3011-3014.
13. Aldred, K. J.; McPherson, S. A.; Wang, P.; Kerns, R. J.; Graves, D. E.; Turnbough, C. L., Jr.; Osheroff, N., Drug interactions with *Bacillus anthracis* topoisomerase IV: biochemical basis for quinolone action and resistance. *Biochemistry* **2012,** *51* (1), 370-381.
14. (a) Malik, M.; Hoatam, G.; Chavda, K.; Kerns, R. J.; Drlica, K., Novel Approaches for Comparing the Abilities of Quinolones to Restrict the Emergence of Resistant Mutants during Quinolone Exposure. *Antimicrobial Agents and Chemotherapy* **2010,** *54* (1), 149-156; (b) Malik, M.; Chavda, K.; Zhao, X. L.; Shah, N.; Hussain, S.; Kurepina, N.; Kreiswirth, B. N.; Kerns, R. J.; Drlica, K., Induction of Mycobacterial Resistance to Quinolone Class Antimicrobials. *Antimicrobial Agents and Chemotherapy* **2012,** *56* (7), 3879-3887.
15. Sanchez, J. P.; Domagala, J. M.; Hagen, S. E.; Heifetz, C. L.; Hutt, M. P.; Nichols, J. B.; Trehan, A. K., Quinolone antibacterial agents. Synthesis and structure-activity relationships of 8-substituted quinoline-3-carboxylic acids and 1,8-naphthyridine-3-carboxylic acids. *Journal of Medicinal Chemistry* **1988,** *31* (5), 983-991.
16. (a) Domagala, J. M.; Hagen, S. E.; Joannides, T.; Kiely, J. S.; Laborde, E.; Schroeder, M. C.; Sesnie, J. A.; Shapiro, M. A.; Suto, M. J.; Vanderroest, S., Quinolone antibacterials containing the new 7-[3-(1-aminoethyl)-1-pyrrolidinyl] side chain: the effects of the 1-aminoethyl moiety and its stereochemical configurations on potency and in vivo efficacy. *Journal of Medicinal Chemistry* **1993,** *36* (7), 871-882; (b) Hagen, S. E.; Domagala, J. M.; Gracheck, S. J.; Sesnie, J. A.; Stier, M. A.; Suto, M. J., Synthesis and Antibacterial Activity of New Quinolones Containing a 7-[3-(1-Amino-1-Methylethyl)-1-Pyrrolidinyl] Moiety - Gram-Positive Agents with Excellent Oral Activity and Low Side-Effect Potential. *Journal of Medicinal Chemistry* **1994,** *37* (6), 733-738; (c) Suto, M. J.; Domagala, J. M.; Roland, G. E.; Mailloux, G. B.; Cohen, M. A., Fluoroquinolones - Relationships between Structural Variations, Mammalian-Cell Cytotoxicity, and Antimicrobial Activity. *Journal of Medicinal Chemistry* **1992,** *35* (25), 4745-4750.
17. Murphy, S. T.; Case, H. L.; Ellsworth, E.; Hagen, S.; Huband, M.; Joannides, T.; Limberakis, C.; Marotti, K. R.; Ottolini, A. M.; Rauckhorst, M.; Starr, J.; Stier, M.; Taylor, C.; Zhu, T.; Blaser, A.; Denny, W. A.; Lu, G. L.; Smaill, J. B.; Rivault, F., The synthesis and biological evaluation of novel series of nitrile-containing fluoroquinolones as antibacterial agents. *Bioorganic & Medicinal Chemistry Letters* **2007,** *17* (8), 2150-2155.
18. Dong, S., McPherson, S. A., Wang, Y., Li, M., Wang, P., Turnbough, C. L., Jr., and Pritchard, D. G. (2010) Characterization of the enzymes encoded by the anthrose biosynthetic operon of Bacillus anthracis, J. Bacteriol. 192, 5053-5062.
19. Oestergaard, V. H., Bjergbaek, L., Skouboe, C., Giangiacomo, L., Knudsen, B. R., and Andersen, A. H. (2004) The transducer domain is important for clamp operation in human DNA topoisomerase II alpha, J. Biol. Chem. 279, 1684-1691.