Supporting Information

Design of 4-oxo-1-aryl-1,4-dihydroquinoline-3-carboxamides as selective negative allosteric modulators of metabotropic glutamate receptor subtype 2

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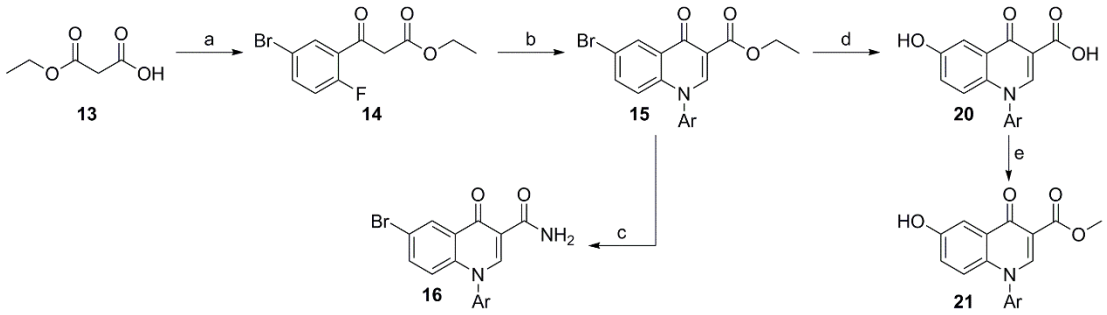
Experimental Procedures and Spectroscopic Data

General. All NMR spectra were recorded on a 400 MHz AMX Bruker NMR spectrometer. ^{1}H and ^{13}C chemical shifts are reported in δ values in ppm downfield with the deuterated solvent as the internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, b = broad, m = multiplet), integration, coupling constant (Hz). Low resolution mass spectra were obtained on an Agilent 6120 or 6150 with ESI source. MS parameters were as follows: fragmentor: 70, capillary voltage: 3000 V, nebulizer pressure: 30 psig, drying gas flow: 13 L/min, drying gas temperature: 350 ºC. Samples were introduced via an Agilent 1290 UHPLC comprised of a G4220A binary pump, G4226A ALS, G1316C TCC, and G4212A DAD with ULD flow cell. UV absorption was generally observed at 215 nm and 254 nm with a 4 nm bandwidth. Column: Waters Acquity BEH C18, 1.0 x 50 mm, 1.7 um. Gradient conditions: 5% to 95% CH_3CN in H_2O (0.1% TFA) over 1.4 min, hold at 95% CH_3CN for 0.1 min, 0.5 mL/min, 55 ºC. High resolution mass spectra were obtained on an Agilent 6540 UHD Q-TOF with ESI source. MS parameters were as follows: fragmentor: 150, capillary voltage: 3500 V, nebulizer pressure: 60 psig, drying gas flow: 13 L/min, drying gas temperature: 275 ºC. Samples were introduced via an Agilent 1200 UHPLC comprised of a G4220A binary pump, G4226A ALS, G1316C TCC, and G4212A DAD with ULD flow cell. UV absorption was observed at 215 nm and 254 nm with a 4 nm bandwidth. Column: Agilent Zorbax Extend C18, 1.8 μm, 2.1 x 50 mm. Gradient conditions: 5% to 95% CH_3CN in H_2O (0.1% formic acid) over 1 min, hold at 95% CH_3CN for 0.1 min, 0.5 mL/min, 40 ºC. For compounds that were purified on a Gilson preparative reversed-phase HPLC, the system comprised of a 333 aqueous pump with solvent-selection valve, 334 organic pump, GX-271 or GX-281 liquid hander, two column switching valves, and a 155 UV detector. UV wavelength for fraction collection was user-

defined, with absorbance at 254 nm always monitored. Method 1: Phenomenex Axia-packed Luna C18, 30 x 50 mm, 5 µm column. Mobile phase: CH_3CN in H_2O (0.1% TFA). Gradient conditions: 0.75 min equilibration, followed by user defined gradient (starting organic percentage, ending organic percentage, duration), hold at 95% CH_3CN in H_2O (0.1% TFA) for 1 min, 50 mL/min, 23 ºC. Method 2: Phenomenex Axia-packed Gemini C18, 50 x 250 mm, 10 um column. Mobile phase: CH_3CN in H_2O (0.1% TFA). Gradient conditions: 7 min equilibration, followed by user defined gradient (starting organic percentage, ending organic percentage, duration), hold at 95% CH_3CN in H_2O (0.1% TFA) for 7 min, 120 mL/min, 23 ºC. All reagents were purchased from Aldrich Chemical Co. and were used without purification. All compounds tested in biological assays were >90% pure as judged at two UV wavelengths (215 nm and 254 nm), and select compounds from each library were checked for confirmation of purity via ^1H and/or ^{13}C NMR. Discrete PK experiments with compound **58** were conducted with a highly pure batch that was judged to be >98% pure by UV (215 nm and 254 nm) and ^1H and ^{13}C NMR.

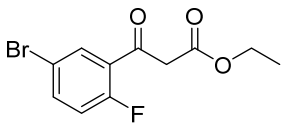
Preparation of Intermediates 15, 16, and 21. The referenced intermediates were prepared via the route pictured immediately below.



Reagents and conditions: (a) *n*-BuLi, 2,2'-bipyridyl, –30 ºC to –5 ºC, then 5-bromo-2-fluoro-benzoyl chloride, –78 ºC to –30 ºC, 67%; (b) *N,N*-dimethylformamide dimethyl acetal, DMF, microwave, 120 ºC, 15 min, then $ArNH_2$, microwave, 150 ºC, 20 min, 60–98%; (c) 7N NH_3 in

MeOH, microwave, 150 ºC, 60 min, 29–99%; (d) KOH, $Pd_2(dba)_3$, *t*-BuXphos, dioxane, H_2O, microwave, 150 ºC, 15 min, 99%; (e) MeOH, con. H_2SO_4, reflux, 74%.

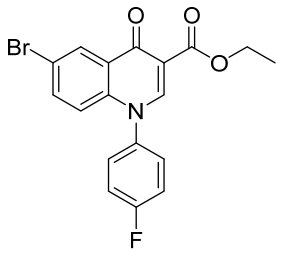
Ethyl 3-(5-bromo-2-fluorophenyl)-3-oxopropanoate (14)



3-Ethoxy-3-oxo-propanoic acid **13** (2.16 mL, 18.3 mmol, 2.00 eq) was dissolved in THF (91 mL) in an oven dried round-bottom flask and 2,2'-bipyridyl (8.00 mg, 0.0512 mmol, 0.0056 eq) was added as an indicator. The reaction was cooled to –30 ºC and *n*-butyllithium (1.6 M in hexanes) (29.0 mL, 45.6 mmol, 4.00 eq) was added dropwise over 20 minutes. Upon final addition the reaction turned red at which point it was allowed to warm to –5 ºC. The reaction was allowed to stir at –5 ºC for 15 minutes, during which time the red color began to dissipate. Enough *n*-butyllithium was added to allow the red color to persist. The reaction was then cooled to –78 ºC and 5-bromo-2-fluoro-benzoyl chloride (2.17 g, 9.14 mmol, 1.00 eq) was added dropwise as a solution in THF (6.9 mL). The reaction was allowed to stir at –78 ºC for 30 minutes and then allowed to warm to –30 ºC and stirred for an additional 30 minutes. The reaction was poured onto ice-cold 1N HCl (92 mL) and the mixture was extracted with ethyl acetate (1x) and DCM (2x). The combined organics were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 1.78 g (67%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.97 (dd, J = 6.5, 2.6 Hz, 1H), 7.91-7.86 (m, 1H), 7.40-7.34 (m, 1H), 4.13-4.07 (m, 4H), 1.15 ppm (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 189.68 (d, J(C,F) = 3.2 Hz), 167.09, 160.26 (d, J(C,F) = 255 Hz), 138.09 (d, J(C,F) = 9.5 Hz), 132.49 (d, J(C,F) = 2.2 Hz), 126.16 (d, J(C,F) =

13.3 Hz), 119.51 (d, *J*(C,F) = 25.1 Hz), 116.64, 60.71, 48.81, 13.92 ppm. HRMS (ESI): calculated for $C_{11}H_{10}BrFO_3$ [M]: 287.9797; found: 287.9794. LCMS R_T = 0.989 min, ES-MS *m/z* = 289.0 $[M+H]^+$.

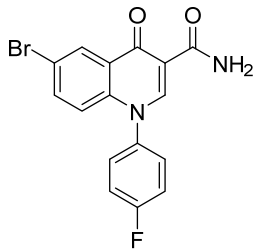
Ethyl 6-bromo-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (15, where Ar = 4-fluorophenyl)



Compound **14** (2.87 g, 9.93 mmol, 1.00 eq) and *N,N*-dimethylformamide dimethyl acetal (1.87 mL, 14.9 mmol, 1.50 eq) were dissolved in DMF (33 mL) in a microwave vial and heated in a microwave reactor at 120 ºC for 15 minutes. To this mixture was then added 4-fluoroaniline (1.41 mL, 14.9 mmol, 1.50 eq) and the reaction was heated in a microwave reactor at 150 ºC for 20 minutes. The reaction mixture was diluted with ethyl acetate and washed with water (2x). The aqueous layers were back-extracted with ethyl acetate and the combined organics were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 3.79 g (98%) of the title compound as yellow solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 8.47 (s, 1H), 8.31 (d, *J* = 2.4 Hz, 1H), 7.81 (dd, *J* = 9.1, 2.4 Hz, 1H), 7.77-7.73 (m, 2H), 7.54-7.50 (m, 2H), 6.92 (d, *J* = 9.0 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 1.25 ppm (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ = 171.80, 163.90, 162.43 (d, *J*(C,F) = 247.5 Hz), 149.02, 139.70, 136.38 (d, *J*(C,F) = 2.8 Hz), 135.37, 130.17 (d, *J*(C,F) = 9.0 Hz), 128.83, 128.15, 120.73, 118.06, 117.26 (d, *J*(C,F) = 23.2 Hz), 110.88, 60.04, 14.21 ppm. HRMS (ESI): calculated for

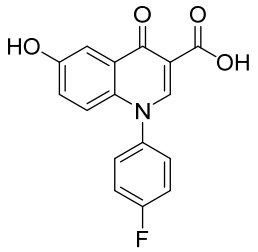
$C_{18}H_{13}BrFNO_3$ [M]: 389.0063; found: 389.0062. LCMS R_T = 0.934 min, ES-MS *m/z* = 390.2 $[M+H]^+$.

6-Bromo-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (16, where Ar = 4-fluorophenyl)



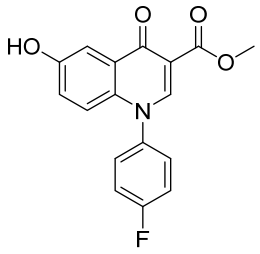
Ethyl 6-bromo-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (1.00 g, 2.56 mmol, 1.00 eq) was suspended in 7N ammonia in methanol (30 mL) in a microwave vial and the reaction was heated in a microwave reactor at 150 ºC for 60 minutes. The reaction was concentrated to afford 881 mg (95%) of the title compound as a brown solid that was used without further purification. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 9.08 (d, *J* = 4.0 Hz, 1H), 8.57 (s, 1H), 8.43 (d, *J* = 2.4 Hz, 1H), 7.85 (dd, *J* = 9.1, 2.4 Hz, 1H), 7.77-7.73 (m, 2H), 7.66 (d, *J* = 4.1 Hz, 1H), 7.56-7.50 (m, 2H), 7.02 ppm (d, *J* = 9.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ = 174.64, 164.83, 162.46 (d, *J*(C,F) = 247.6 Hz), 148.42, 139.85, 136.50 (d, *J*(C,F) = 2.8 Hz), 135.66, 129.97 (d, *J*(C,F) = 9.3 Hz), 128.09, 128.07, 120.86, 118.19, 117.31 (d, *J*(C,F) = 23.3 Hz), 112.07 ppm. HRMS (ESI): calculated for $C_{16}H_{10}BrFN_2O_2$ [M]: 359.9910; found: 359.9909. LCMS R_T = 0.929 min, ES-MS *m/z* = 361.2 $[M+H]^+$.

1-(4-Fluorophenyl)-6-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (20, where Ar = 4-fluorophenyl)



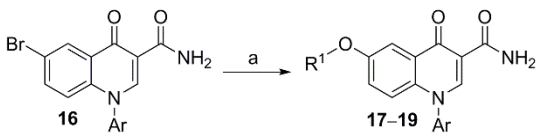
Ethyl 6-bromo-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (500 mg, 1.28 mmol, 1.00 eq), potassium hydroxide (216 mg, 3.84 mmol, 3.00 eq), tris(dibenzylideneacetone)dipalladium(0) (70.4 mg, 0.0769 mmol, 0.060 eq) and 2-di-tert-butylphosphino-2′,4′,6′-triisopropylbiphenyl (65.3 mg, 0.154 mmol, 0.120 eq) were suspended in a mixture of 1,4-dioxane (3.2 mL) and water (3.2 mL) in a microwave vial and heated in a microwave reactor at 150 ºC for 15 minutes. The reaction was neutralized with 2N HCl and the mixture was diluted with ethyl acetate and washed with water. The organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford 383 mg (99%) of the title compound as a brown solid that was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.56 (s, 1H), 7.75-7.72 (m, 2H), 7.67 (d, J = 2.7 Hz, 1H), 7.51 (t, J = 8.7 Hz, 2H), 7.29 (dd, J = 9.3, 2.8 Hz, 1H), 6.99 ppm (d, J = 9.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 177.42, 166.19, 162.52 (d, J(C,F) = 247.9 Hz), 156.36, 147.16, 136.47 (d, J(C,F) = 3.0 Hz), 134.48, 129.90 (d, J(C,F) = 9.3 Hz), 126.61, 124.12, 120.70, 117.12 (d, J(C,F) = 23.2 Hz), 108.10 ppm. HRMS (ESI): calculated for $C_{16}H_{10}FNO_4$ [M]: 299.0594; found: 299.0592. LCMS R_T = 0.744 min, ES-MS m/z = 300.2 $[M+H]^+$.

Methyl 1-(4-fluorophenyl)-6-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (21, where Ar = 4-fluorophenyl)



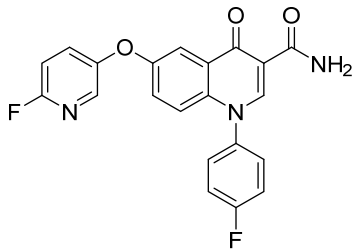
1-(4-Fluorophenyl)-6-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (383 mg, 1.28 mmol, 1.00 eq) was dissolved in methanol (6.4 mL) and concentrated sulfuric acid (0.64 mL) was added dropwise. The reaction was heated to reflux for 3 hours at which point it was cooled and neutralized with a saturated solution of sodium bicarbonate. The mixture was extracted with a solution of 3:1 $CHCl_3$:IPA (2x) and the combined organics were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 296 mg (74%) of the title compound as yellow solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 10.03 (s, 1H), 8.36 (s, 1H), 7.72-7.68 (m, 2H), 7.61 (d, *J* = 2.8 Hz, 1H), 7.49 (t, *J* = 8.7 Hz, 2H), 7.11 (dd, *J* = 9.0, 2.8 Hz, 1H), 6.84 (d, *J* = 9.1 Hz, 1H), 3.71 ppm (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ = 172.60, 165.09, 162.25 (d, *J*(C,F) = 247.3 Hz), 155.80, 147.38, 136.86 (d, *J*(C,F) = 2.9 Hz), 133.49, 130.03 (d, *J*(C,F) = 9.2 Hz), 129.10, 122.17, 119.69, 117.09 (d, *J*(C,F) = 23.1 Hz), 109.35, 108.53, 51.21 ppm. HRMS (ESI): calculated for $C_{17}H_{12}FNO_4$ [M]: 313.0750; found: 313.0749. LCMS R_T = 0.784 min, ES-MS *m/z* = 314.2 $[M+H]^+$.

Preparation of Analogs 17–19. The referenced compounds were prepared via the route pictured immediately below. All compounds were ≥ 95% pure as measured by UV spectroscopy at 215 and 254 nm.



Reagents and conditions: (a) R^1OH, CuI, Cs_2CO_3, $Me_2NCH_2CO_2H$, microwave, 150 ºC, 15 min, 26–56%

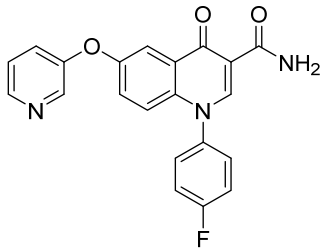
1-(4-Fluorophenyl)-6-((6-fluoropyridin-3-yl)oxy)-4-oxo-1,4-dihydroquinoline-3-carboxamide (19)



6-Bromo-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (46 mg, 0.13 mmol, 1.0 eq), 2-fluoro-5-hyroxypyridine (29 mg, 0.25 mmol, 2.0 eq), cesium carbonate (83 mg, 0.25 mmol, 2.0 eq), copper(I) iodide (2.5 mg, 0.013 mmol, 0.10 eq) and *N*,*N*-dimethylglycine (4.0 mg, 0.038 mmol, 0.30 eq) were dissolved in DMF (1 mL) in a microwave vial and heated in a microwave reactor at 150 ºC for 15 minutes. The reaction was filtered over celite and washed with 5% methanol in DCM. The organics were concentrated and purified using reverse-phase chromatography to afford 13 mg (26%) of title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.12 (d, J = 4.2 Hz, 1H), 8.56 (s, 1H), 8.14 (bs, 1H), 7.85-7.81 (m, 1H), 7.77-7.74 (m, 3H), 7.60 (d, J = 4.1 Hz, 1H), 7.55-7.51 (m, 3H), 7.30 (dd, J = 8.8, 3.3 Hz, 1H), 7.13 ppm (d, J = 9.3 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 175.00, 165.09, 162.42 (d, J(C,F) = 247.2 Hz), 159.20 (d, J(C,F) = 233.7 Hz), 154.54, 150.58 (d, J(C,F) = 4.6 Hz), 147.69, 138.88 (d, J(C,F) = 16.1 Hz), 137.03, 136.71 (d, J(C,F) = 2.9 Hz), 133.67 (d, J(C,F) = 8.8 Hz), 129.95 (d, J(C,F) = 9.2 Hz), 127.95, 124.30, 120.98, 117.27 (d, J(C,F) = 23.2 Hz), 111.83, 111.17 (d, J(C,F) = 5.2 Hz), 110.79 ppm. HRMS (ESI): calculated for $C_{21}H_{13}F_2N_3O_3$ [M]: 393.0925; found: 393.0925. LCMS R_T = 0.910 min, ES-MS m/z = 394.2 $[M+H]^+$.

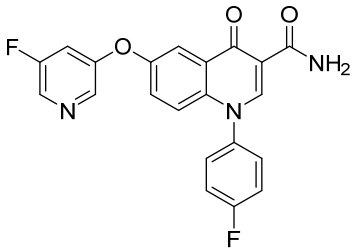
*The following compounds were prepared analogous to compound **19***

1-(4-Fluorophenyl)-4-oxo-6-(pyridin-3-yloxy)-1,4-dihydroquinoline-3-carboxamide (17)



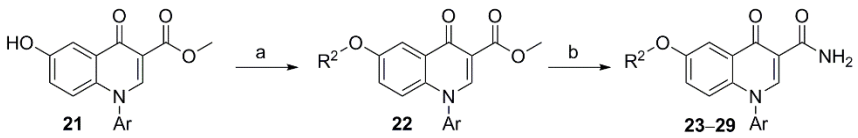
LCMS R_T = 0.685 min, ES-MS *m/z* = 376.2 $[M+H]^+$.

1-(4-Fluorophenyl)-6-((5-fluoropyridin-3-yl)oxy)-4-oxo-1,4-dihydroquinoline-3-carboxamide (18)



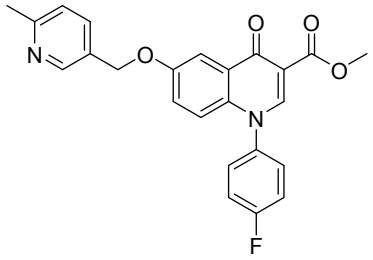
LCMS R_T = 0.904 min, ES-MS *m/z* = 394.2 $[M+H]^+$.

Preparation of Analogs 23–29. The referenced compounds were prepared via the route pictured immediately below. All compounds were ≥ 95% pure as measured by UV spectroscopy at 215 and 254 nm.



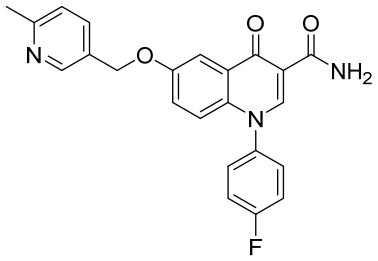
Reagents and conditions: (a) R^2OH, PPh_3, D^tBAD, THF, 40–98%; (b) 7N NH_3 in MeOH, microwave, 150 ºC, 60 min, 29–99%.

Methyl 1-(4-fluorophenyl)-6-((6-methylpyridin-3-yl)methoxy)-4-oxo-1,4-dihydroquinoline-3-carboxylate (22, where R^2 = (6-methylpyridin-3-yl)methyl))



Methyl 1-(4-fluorophenyl)-6-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (11.4 mg, 0.0364 mmol, 1.00 eq) and (6-methylpyridin-3-yl)methanol (8.96 mg, 0.0728 mmol, 2.00 eq) were dissolved in THF (0.5 mL) and cooled to 0 ºC. Triphenylphosphine (21.0 mg, 0.0801 mmol, 2.20 eq) and di-tert-butylazodicarboxylate (13.4 mg, 0.0582 mmol, 1.60 eq) were added, and the reaction was stirred at room temperature until complete by LCMS. The reaction was concentrated and purified using reverse-phase chromatography to afford 6.4 mg (42%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 8.54 (d, *J* = 1.7 Hz, 1H), 8.40 (s, 1H), 7.77-7.71 (m, 4H), 7.50 (t, *J* = 8.7 Hz, 2H), 7.34 (dd, *J* = 9.2, 3.0 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 6.93 (d, *J* = 9.3 Hz, 1H), 5.22 (s, 2H), 3.72 (s, 3H), 2.45 ppm (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ = 172.37, 164.84, 162.31 (d, *J*(C,F) = 247.2 Hz), 157.68, 155.61, 148.46, 147.83, 136.69 (d, *J*(C,F) = 3.0 Hz), 136.07, 135.08, 130.07 (d, *J*(C,F) = 9.2 Hz), 129.06, 128.70, 122.85, 122.55, 119.94, 117.14 (d, *J*(C,F) = 23.1 Hz), 109.21, 107.75, 67.34, 51.29, 23.79 ppm. HRMS (ESI): calculated for $C_{24}H_{19}FN_2O_4$ [M]: 418.1329; found: 418.1333. LCMS R_T = 0.737 min, ES-MS *m/z* = 419.2 $[M+H]^+$.

1-(4-Fluorophenyl)-6-((6-methylpyridin-3-yl)methoxy)-4-oxo-1,4-dihydroquinoline-3-carboxamide (24)

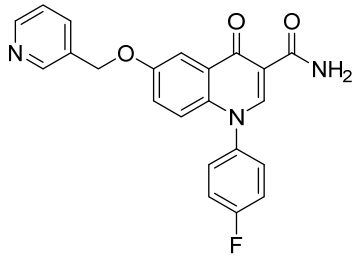


Methyl 1-(4-fluorophenyl)-6-((6-methylpyridin-3-yl)methoxy)-4-oxo-1,4-dihydroquinoline-3-carboxylate (6.8 mg, 0.016 mmol, 1.0 eq) was suspended in 7N ammonia in methanol (1 mL) in a microwave vial and heated in a microwave reactor at 150 ºC for 60 minutes. The reaction was concentrated and purified using reverse-phase chromatography to afford 3 mg (46%) of title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.27 (d, J = 4.4 Hz, 1H), 8.55 (d, J = 1.8 Hz, 1H), 8.51 (s, 1H), 7.86 (d, J = 3.0 Hz, 1H), 7.78-7.71 (m, 3H), 7.59 (d, J = 4.3 Hz, 1H), 7.51 (t, J = 8.7 Hz, 2H), 7.40 (dd, J = 9.3, 3.0 Hz, 1H), 7.27 (d, J = 8.0 Hz, 1H), 7.01 (d, J = 9.3 Hz, 1H), 5.25 (s, 2H), 2.46 ppm (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 175.14, 165.38, 162.34 (d, J(C,F) = 247.2 Hz), 157.71, 155.64, 148.46, 146.86, 136.83 (d, J(C,F) = 2.9 Hz), 136.07, 135.41, 129.92 (d, J(C,F) = 9.2 Hz), 129.06, 127.88, 123.20, 122.88, 120.06, 117.19 (d, J(C,F) = 23.2 Hz), 110.80, 107.26, 67.38, 23.79 ppm. HRMS (ESI): calculated for $C_{23}H_{18}FN_3O_3$ [M]: 403.1332; found: 403.1335. LCMS R_T = 0.659 min, ES-MS m/z = 404.3 $[M+H]^+$.

The following compounds were prepared analogous to compound ***24***

1-(4-Fluorophenyl)-4-oxo-6-(pyridin-3-ylmethoxy)-1,4-dihydroquinoline-3-carboxamide

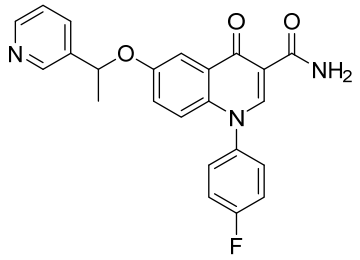
(23)



LCMS R_T = 0.664 min, ES-MS *m/z* = 390.2 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-6-(1-(pyridin-3-yl)ethoxy)-1,4-dihydroquinoline-3-carboxamide

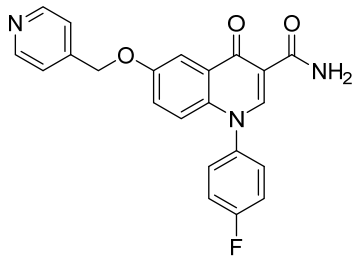
(25)



LCMS R_T = 0.652 min, ES-MS *m/z* = 404.2 $[M+H]^+$.

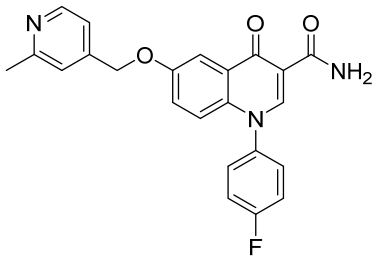
1-(4-Fluorophenyl)-4-oxo-6-(pyridin-4-ylmethoxy)-1,4-dihydroquinoline-3-carboxamide

(26)



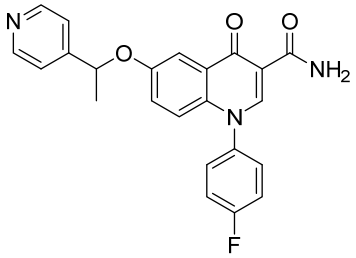
LCMS R_T = 0.663 min, ES-MS *m/z* = 390.2 $[M+H]^+$.

1-(4-Fluorophenyl)-6-((2-methylpyridin-4-yl)methoxy)-4-oxo-1,4-dihydroquinoline-3-carboxamide (27)



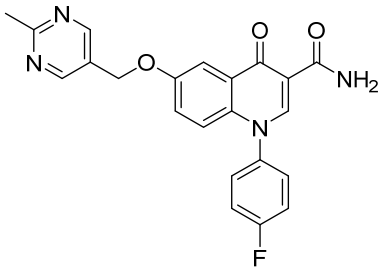
LCMS R_T = 0.669 min, ES-MS *m/z* = 404.3 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-6-(1-(pyridin-4-yl)ethoxy)-1,4-dihydroquinoline-3-carboxamide (28)



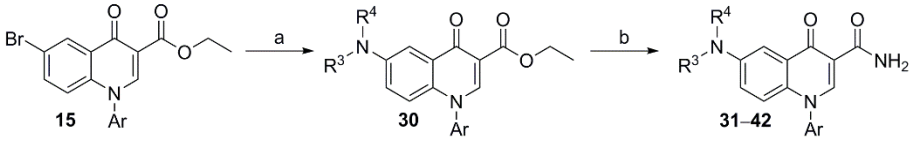
LCMS R_T = 0.694 min, ES-MS *m/z* = 404.2 $[M+H]^+$.

1-(4-fluorophenyl)-6-((2-methylpyrimidin-5-yl)methoxy)-4-oxo-1,4-dihydroquinoline-3-carboxamide (29)



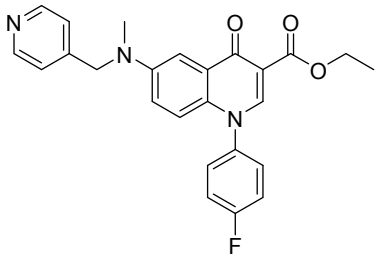
LCMS R_T = 0.763 min, ES-MS *m/z* = 405.2 $[M+H]^+$.

Preparation of Analogs 31–42. The referenced compounds were prepared via the route pictured immediately below. All compounds were ≥ 95% pure as measured by UV spectroscopy at 215 and 254 nm.



Reagents and conditions: (a) HNR^3R^4, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, PhMe, 110 ºC, 8–54%; (b) 7N NH_3 in MeOH, microwave, 150 ºC, 60 min, 29–99%.

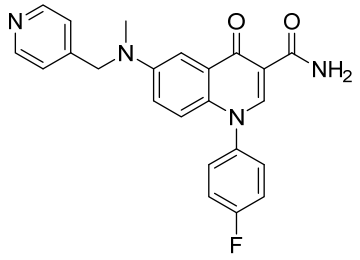
Ethyl 1-(4-fluorophenyl)-6-(methyl(pyridin-4-ylmethyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxylate (30, where Ar = 4-fluorophenyl, R^3 = pyridin-4-ylmethyl, and R^4 = methyl)



Ethyl 6-bromo-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (15 mg, 0.038 mmol, 1.0 eq), *N*-methyl-1-(pyridin-4-yl)methanamine (14 mg, 0.12 mmol, 3.0 eq), cesium carbonate (18 mg, 0.054 mmol, 1.4 eq), tris(dibenzylideneacetone)dipalladium(0) (2.8 mg, 0.0031 mmol, 0.080 eq) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (2.7 mg, 0.046 mmol, 0.12 eq) were dissolved in toluene (1 mL) in a sealed vial and heated at 110 ºC overnight. The reaction was cooled, filtered through celite and washed with 5% methanol in DCM. The organics were concentrated and purified using reverse-phase chromatography to afford 7.0 mg

(42%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.47 (d, J = 5.5 Hz, 2H), 8.29 (s, 1H), 7.70-7.66 (m, 2H), .49-7.45 (m, 2H), 7.36 (d, J = 2.9 Hz, 1H), 7.17 (d, J = 5.5 Hz, 2H), 7.13 (dd, J = 9.3, 3.0 Hz, 1H), 6.80 (d, J = 9.3 Hz, 1H), 4.70 (s, 2H), 4.17 (q, J = 7.03 Hz, 2H), 1.03 ppm (t, J = 7.03 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.66, 164.60, 162.22 (d, J(C,F) = 246.6 Hz), 149.73, 147.77, 146.48, 146.34, 136.81 (d, J(C,F) = 3.0 Hz), 131.91, 130.00 (d, J(C,F) = 9.1 Hz), 128.56, 121.86, 119.24, 118.35, 117.05 (d, J(C,F) = 23.1 Hz), 108.63, 105.21, 59.66, 54.36, 53.76, 14.25 ppm. HRMS (ESI): calculated for $C_{25}H_{22}FN_3O_3$ [M]: 431.1645; found: 431.1646. LCMS R_T = 0.769 min, ES-MS m/z = 432.3 $[M+H]^+$.

1-(4-Fluorophenyl)-6-(methyl(pyridin-4-ylmethyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxamide (37)

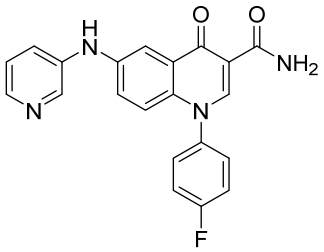


Ethyl 1-(4-fluorophenyl)-6-(methyl(pyridin-4-ylmethyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxylate (20 mg, 0.046 mmol, 1.0 eq) was suspended in 7N ammonia in methanol (3 mL) in a microwave vial and heated in a microwave reactor at 150 ºC for 60 minutes. The reaction was concentrated and purified using reverse-phase chromatography to afford 6.7 mg (36%) of title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.32 (d, J = 4.6 Hz, 1H), 8.47 (d, J = 5.9 Hz, 2H), 8.41 (s, 1H), 7.71-7.68 (m, 2H), 7.50-7.46 (m, 3H), 7.43 (d, J = 3.0 Hz, 1H), 7.22-7.17 (m, 3H), 6.89 (d, J = 9.3 Hz, 1H), 4.73 (s, 2H), 3.17 ppm (s, 3H). ^{13}C NMR (100 MHz,

DMSO-d_6): δ = 175.14, 165.74, 162.24 (d, J(C,F) = 247.0 Hz), 149.74, 147.74, 146.38, 145.56, 136.92 (d, J(C,F) = 3.0 Hz), 132.27, 129.83 (d, J(C,F) = 9.1 Hz), 127.95, 121.86, 119.33, 118.97, 117.07 (d, J(C,F) = 23.1 Hz), 110.06, 104.64, 54.34, 48.58 ppm. HRMS (ESI): calculated for $C_{23}H_{19}FN_4O_2$ [M]: 402.1492; found: 402.1494. LCMS R_T = 0.657 min, ES-MS *m/z* = 403.2 $[M+H]^+$.

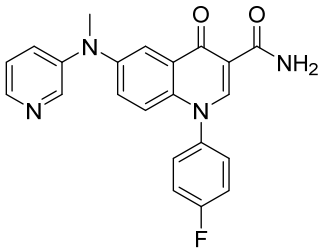
*The following compounds were prepared analogous to compound **37***

1-(4-Fluorophenyl)-4-oxo-6-(pyridin-3-ylamino)-1,4-dihydroquinoline-3-carboxamide (31)



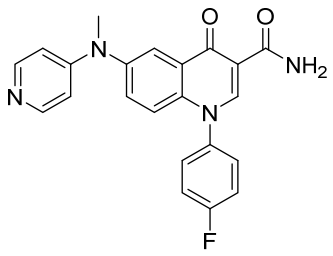
LCMS R_T = 0.635 min, ES-MS *m/z* = 375.2 $[M+H]^+$.

1-(4-Fluorophenyl)-6-(methyl(pyridin-3-yl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxamide (32)



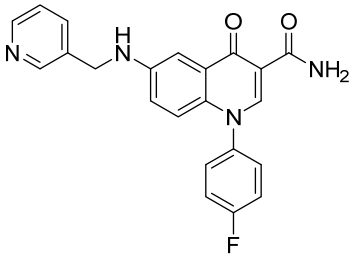
LCMS R_T = 0.664 min, ES-MS *m/z* = 389.2 $[M+H]^+$.

1-(4-Fluorophenyl)-6-(methyl(pyridin-4-yl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxamide (33)



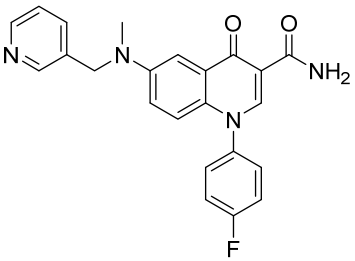
LCMS R_T = 0.569 min, ES-MS *m/z* = 389.2 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-6-((pyridin-3-ylmethyl)amino)-1,4-dihydroquinoline-3-carboxamide (34)



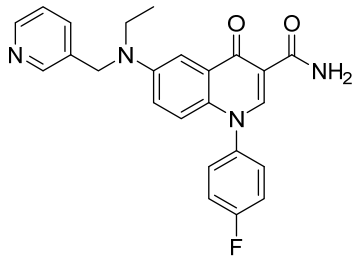
LCMS R_T = 0.639 min, ES-MS *m/z* = 389.3 $[M+H]^+$.

1-(4-Fluorophenyl)-6-(methyl(pyridin-3-ylmethyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxamide (35)



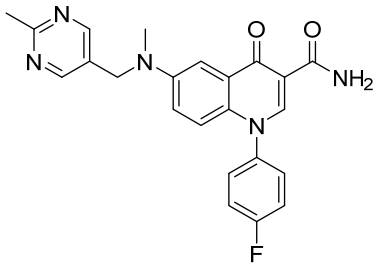
LCMS R_T = 0.678 min, ES-MS *m/z* = 403.2 $[M+H]^+$.

6-(Ethyl(pyridin-3-ylmethyl)amino)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (36)



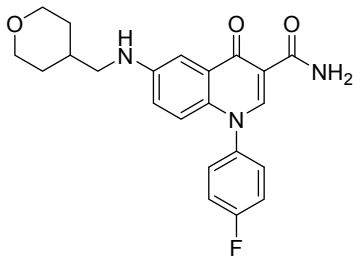
LCMS R_T = 0.672 min, ES-MS *m/z* = 417.2 $[M+H]^+$.

1-(4-Fluorophenyl)-6-(methyl((2-methylpyrimidin-5-yl)methyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxamide (38)



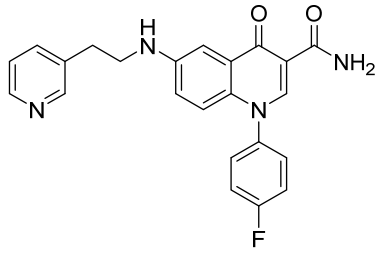
LCMS R_T = 0.752 min, ES-MS *m/z* = 418.2 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-6-(((tetrahydro-2H-pyran-4-yl)methyl)amino)-1,4-dihydroquinoline-3-carboxamide (39)



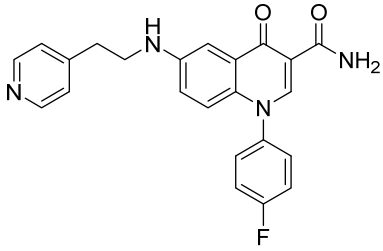
LCMS R_T = 0.811 min, ES-MS *m/z* = 396.2 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-6-((2-(pyridin-3-yl)ethyl)amino)-1,4-dihydroquinoline-3-carboxamide (40)



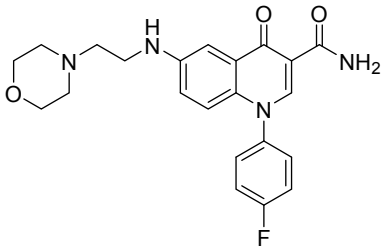
LCMS R_T = 0.624 min, ES-MS *m/z* = 403.2 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-6-((2-(pyridin-4-yl)ethyl)amino)-1,4-dihydroquinoline-3-carboxamide (41)



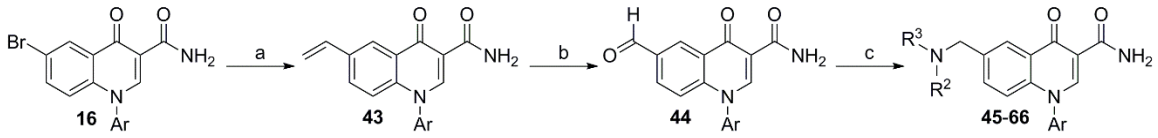
LCMS R_T = 0.599 min, ES-MS *m/z* = 403.2 $[M+H]^+$.

1-(4-Fluorophenyl)-6-((2-morpholinoethyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxamide (42)



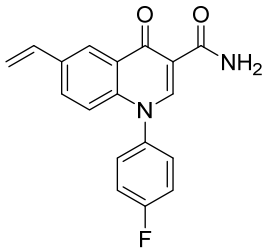
LCMS R_T = 0.547 min, ES-MS *m/z* = 411.2 $[M+H]^+$.

Preparation of Analogs 45–66. The referenced compounds were prepared via the route pictured immediately below. All compounds were ≥ 95% pure as measured by UV spectroscopy at 215 and 254 nm.



Reagents and conditions: (a) H_2CCHBF_3K, Pd(dppf)·CH_2Cl_2, NEt_3, *n*-propanol, 100 ºC, 75–100%; (b) OsO_4, NMO, acetone, H_2O, then $NaIO_4$, 91–99%; (c) HNR^2R^3, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, 7–81%.

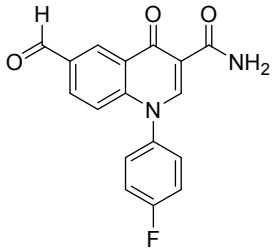
1-(4-Fluorophenyl)-4-oxo-6-vinyl-1,4-dihydroquinoline-3-carboxamide (43, where Ar = 4-fluorophenyl)



To a solution of 6-bromo-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (450 mg, 1.25 mmol, 1.0 eq), triethylamine (174 µL, 1.25 mmol, 1.0 eq), and Pd(dppf)Cl_2·CH_2Cl_2 (18.2 mg, 0.025 mmol, 0.2 eq) in 1-propanol (8.3 mL) was added potassium vinyltrifluoroborate (200 mg, 1.5 mmol, 1.2 eq). The mixture was purged with argon and stirred at 100 ºC for 16 hours. The reaction was filtered through Celite® and washed very well with a 5%MeOH in DCM solution. The filtrate was concentrated *in vacuo* to give 385 mg (100%) of the title compound, which was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.23 (d, J = 4.2 Hz, 1H), 8.55 (s, 1H), 8.36 (d, J = 1.6 Hz, 1H), 7.89 (dd, J = 1.8, 8.9 Hz, 1H),

7.79-7.75 (m, 2H), 7.64 (d, J = 4.2 Hz, 1H), 7.54 (t, J = 8.7 Hz, 2H), 7.03 (d, J = 8.8 Hz, 1H), 6.93 (q, J = 11, 6.6 Hz, 1H), 5.95 (d, J = 17.6 Hz, 1H), 5.39 ppm (d, J = 11 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 175.79, 165.17, 162.36 (d, J(C,F) = 247.0 Hz), 147.77, 140.24, 136.74 (d, J(C,F) = 3.2 Hz), 135.38, 134.17, 130.1, 129.93 (d, J(C,F) = 9.0 Hz), 126.74, 123.72, 118.65, 117.35 (d, J(C,F) = 23.4 Hz), 115.9, 111.72 ppm. HRMS (ESI): calculated for $C_{18}H_{13}FN_2O_2$ [M]: 308.0961; found: 308.0964. LCMS R_T = 0.922 min, ES-MS m/z = 309.2 $[M+H]^+$.

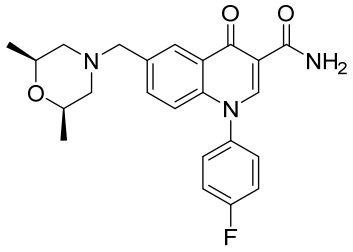
1-(4-Fluorophenyl)-6-formyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (44, where Ar = 4-fluorophenyl)



To a solution of 1-(4-fluorophenyl)-4-oxo-6-vinyl-1,4-dihydroquinoline-3-carboxamide (385 mg, 1.25 mmol, 1.0 eq) in 3:1 acetone/water (8 mL) was added *N*-oxide-4-methylmorpholine (220 mg, 1.87 mmol, 1.5 eq) and osmium tetroxide (6.3 mg, 0.025 mmol, 0.02 eq). After the reaction stirred for one hour, sodium periodate (294 mg, 1.37 mmol, 1.1 eq) was added. After another two hours, the reaction was diluted with EtOAc and washed well with a 10% NaS_2O_3 solution. The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give 365 mg (94%) of the title compound that was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.17 (s, 1H), 9.10 (d, J = 3.8 Hz, 1H), 8.93 (d, J = 1.8 Hz, 1H), 8.62 (s, 1H), 8.12 (dd, J = 1.8, 8.8 Hz, 1H), 7.82-7.78 (m, 2H), 7.74 (d, J = 3.8 Hz, 1H), 7.56 (t, J = 8.8 Hz, 2H), 7.22 ppm (d, J = 8.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 192.23, 175.88, 164.68, 162.49 (d, J(C,F) =

247 Hz), 149.06, 144.23, 136.61 (d, *J*(C,F) = 3.2 Hz), 132.48, 130.98, 130.41, 130.0 (d, *J*(C,F) = 9.3 Hz), 126.54, 119.46, 117.37 (d, *J*(C,F) = 23.3 Hz), 112.78 ppm. HRMS (ESI) calculated for $C_{17}H_{11}FN_2O_3$ [M]: 310.0754; found: 310.0757. LCMS R_T = 0.732 min, ES-MS *m/z* = 311.2 $[M+H]^+$.

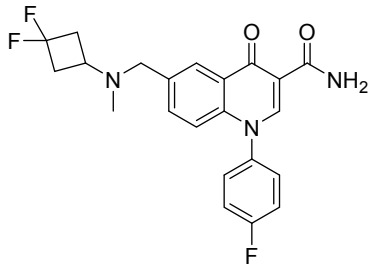
6-((*cis*-2,6-Dimethylmorpholino)methyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (58)



A solution of 1-(4-fluorophenyl)-6-formyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (580 mg, 1.87 mmol, 1.0 eq) in dichloromethane (1 mL), *cis*-2,6-dimethylmorpholine (461 µL, 3.74 mmol, 2.0 eq), and acetic acid (268 µL, 4.67 mmol, 2.5 eq) was stirred for one hour. Sodium triacetoxyborohydride (594 mg, 2.80 mmol, 1.5 eq) was added. After 16 hours, the reaction was concentrated to dryness. Purification by reverse phase HPLC afforded 620 mg (81%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.23 (d, *J* = 4.4 Hz, 1H), 8.55 (s, 1H), 8.26 (d, *J* = 1.4 Hz, 1H), 7.77-7.73 (m, 2H), 7.65 (dd, *J* = 1.8, 8.7 Hz, 1H), 7.59 (d, *J* = 4.3 Hz, 1H), 7.52 (t, *J* = 8.7, 2H), 7.03 (d, *J* = 8.7, 1H), 3.57-3.52 (m, 4H), 2.65 (d, *J* = 10.7 Hz, 2H), 1.68 (t, *J* = 10.7 Hz, 2H), 1.01 ppm (d, *J* = 6.2 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ = 175.8, 165.26, 162.36 (d, *J*(C,F) = 247 Hz), 147.78, 139.9, 136.82 (d, *J*(C,F) = 3.1 Hz), 135.26, 133.91, 129.98 (d, *J*(C,F) = 8.9 Hz), 126.41, 125.85, 118.26, 117.22 (d, *J*(C,F) = 23.0 Hz), 111.59, 70.97, 61.23, 58.81, 18.96 ppm. HRMS (ESI) calculated for $C_{23}H_{24}FN_3O_3$ [M]: 409.1802; found: 409.1804. LCMS R_T = 0.644 min, ES-MS *m/z* = 410.3 $[M+H]^+$.

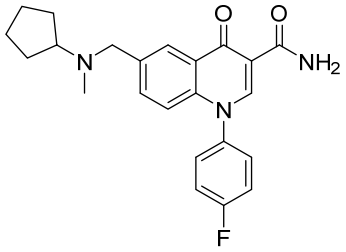
*The following compounds were prepared analogous to compound **58***

6-(((3,3-Difluorocyclobutyl)(methyl)amino)methyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (45)



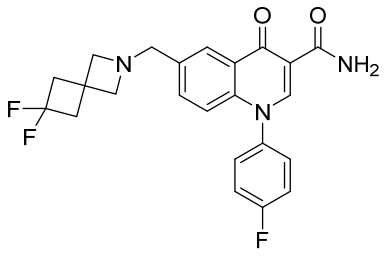
LCMS R_T = 0.590 min, ES-MS *m/z* = 416.2 $[M+H]^+$.

6-((Cyclopentyl(methyl)amino)methyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (46)



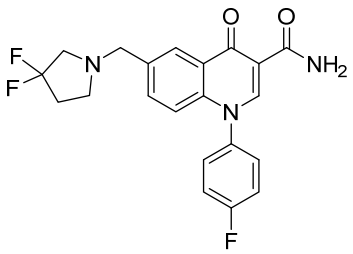
LCMS R_T = 0.622 min, ES-MS *m/z* = 394.2 $[M+H]^+$.

6-((6,6-Difluoro-2-azaspiro[3.3]heptan-2-yl)methyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (47)



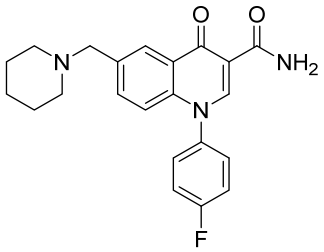
LCMS R_T = 0.792 min, ES-MS *m/z* = 428.2 $[M+H]^+$.

6-((3,3-Difluoropyrrolidin-1-yl)methyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (48)



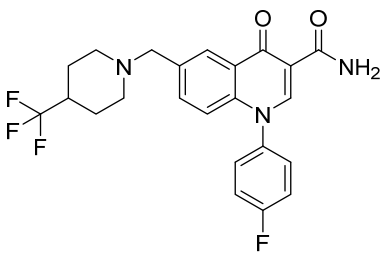
LCMS R_T = 0.688 min, ES-MS *m/z* = 402.2 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-6-(piperidin-1-ylmethyl)-1,4-dihydroquinoline-3-carboxamide (49)



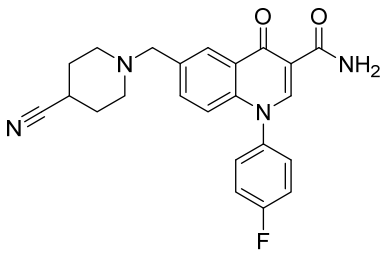
LCMS R_T = 0.633 min, ES-MS *m/z* = 380.4 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-6-((4-(trifluoromethyl)piperidin-1-yl)methyl)-1,4-dihydroquinoline-3-carboxamide (50)



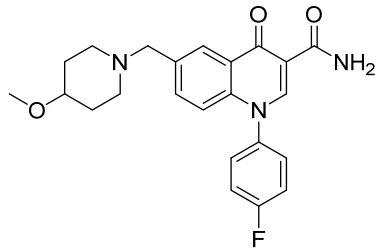
LCMS R_T = 0.788 min, ES-MS *m/z* = 448.2 $[M+H]^+$.

6-((4-Cyanopiperidin-1-yl)methyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (51)



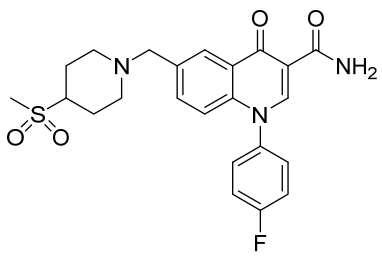
LCMS R_T = 0.591 min, ES-MS *m/z* = 405.2 $[M+H]^+$.

1-(4-Fluorophenyl)-6-((4-methoxypiperidin-1-yl)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (52)



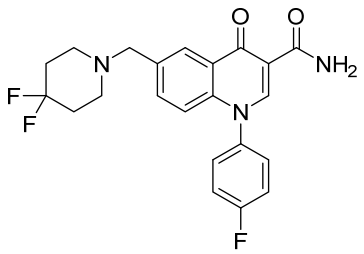
LCMS R_T = 0.595 min, ES-MS *m/z* = 410.2 $[M+H]^+$.

1-(4-Fluorophenyl)-6-((4-(methylsulfonyl)piperidin-1-yl)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (53)



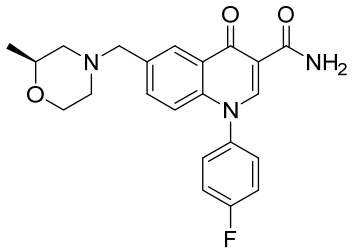
LCMS R_T = 0.555 min, ES-MS *m/z* = 458.2 $[M+H]^+$.

6-((4,4-Difluoropiperidin-1-yl)methyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (54)



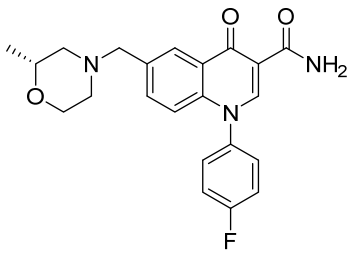
LCMS R_T = 0.733 min, ES-MS *m/z* = 416.2 $[M+H]^+$.

(*S*)-1-(4-Fluorophenyl)-6-((2-methylmorpholino)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (55)



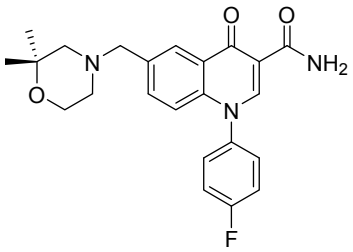
LCMS R_T = 0.611 min, ES-MS *m/z* = 396.4 $[M+H]^+$.

(*R*)-1-(4-Fluorophenyl)-6-((2-methylmorpholino)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (56)



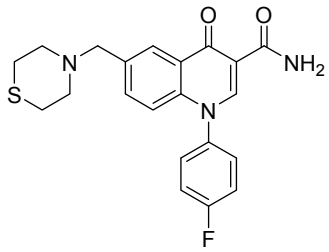
LCMS R_T = 0.610 min, ES-MS *m/z* = 396.4 $[M+H]^+$.

6-((2,2-Dimethylmorpholino)methyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (57)



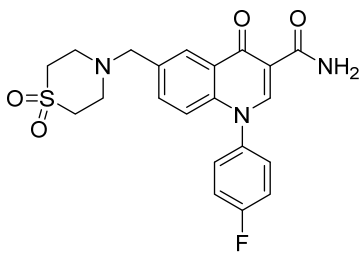
LCMS R_T = 0.638 min, ES-MS *m/z* = 410.4 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-6-(thiomorpholinomethyl)-1,4-dihydroquinoline-3-carboxamide (59)



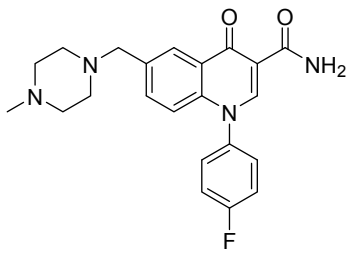
LCMS R_T = 0.620 min, ES-MS *m/z* = 398.4 $[M+H]^+$.

6-((1,1-Dioxidothiomorpholino)methyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (60)



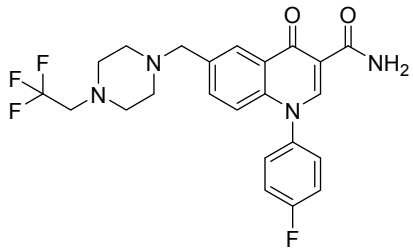
LCMS R_T = 0.739 min, ES-MS *m/z* = 430.2 $[M+H]^+$.

1-(4-Fluorophenyl)-6-((4-methylpiperazin-1-yl)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (61)



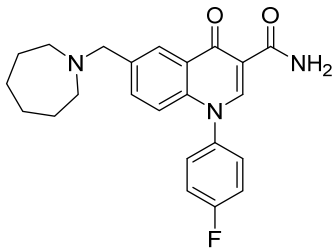
LCMS R_T = 0.578 min, ES-MS *m/z* = 395.2 $[M+H]^+$.

1-(4-fluorophenyl)-4-oxo-6-((4-(2,2,2-trifluoroethyl)piperazin-1-yl)methyl)-1,4-dihydroquinoline-3-carboxamide (62)



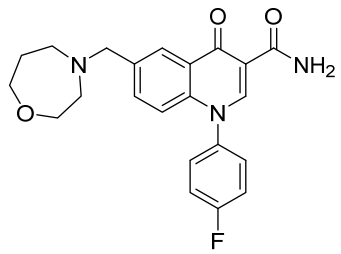
LCMS R_T = 0.792 min, ES-MS *m/z* = 463.2 $[M+H]^+$.

6-(Azepan-1-ylmethyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (63)



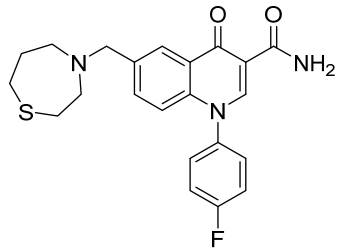
LCMS R_T = 0.642 min, ES-MS *m/z* = 394.2 $[M+H]^+$.

6-((1,4-Oxazepan-4-yl)methyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (64)



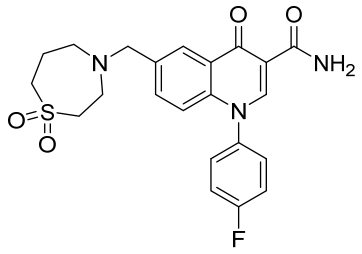
LCMS R_T = 0.569 min, ES-MS *m/z* = 396.2 $[M+H]^+$.

6-((1,4-Thiazepan-4-yl)methyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (65)



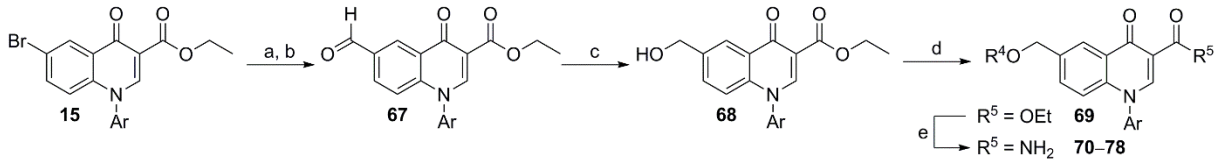
LCMS R_T = 0.545 min, ES-MS *m/z* = 412.2 $[M+H]^+$.

6-((1,1-Dioxido-1,4-thiazepan-4-yl)methyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (66)



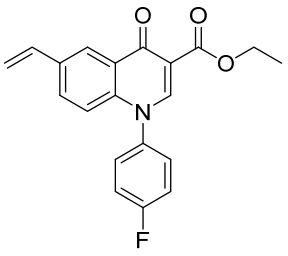
LCMS R_T = 0.598 min, ES-MS *m/z* = 444.2 $[M+H]^+$.

Preparation of Analogs 70–78. The referenced compounds were prepared via the route pictured immediately below. All compounds were ≥ 95% pure as measured by UV spectroscopy at 215 and 254 nm.



Reagents and conditions: (a) H_2CCHBF_3K, $Pd(dppf)\cdot CH_2Cl_2$, NEt_3, *n*-propanol, 100 ºC, 75–100%; (b) OsO_4, NMO, acetone, H_2O, then $NaIO_4$, 91–99%; (c) $NaBH_4$, EtOH, 0 ºC, 36–57%; (d) R^4OH, PPh_3, D^tBAD, THF, 14–98%; (e) 7N NH_3 in MeOH, microwave, 150 ºC, 15 min, 10–94%.

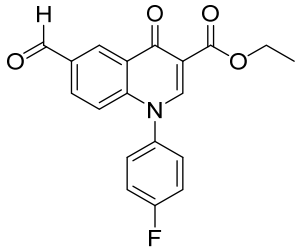
Ethyl 1-(4-fluorophenyl)-4-oxo-6-vinyl-1,4-dihydroquinoline-3-carboxylate



To a solution of ethyl 6-bromo-1-(4-fluorophenyl)-4-oxo-quinoline-3-carboxylate (350 mg, 0.90 mmol, 1.0 eq), triethylamine (125 µL, 0.90 mmol, 1.0 eq), and $Pd(dppf)Cl_2\cdot CH_2Cl_2$ (13.1 mg, 0.018 mmol, 1.0 eq) in 1-Propanol (4.5 mL) was added potassium vinyltrifluoroborate (144 mg, 1.08 mmol, 1.1 eq). The reaction was purged with argon and stirred at 100 ºC for 4 hours. The reaction was filtered through Celite® and washed very well with a 5%MeOH in DCM solution, and the filtrate was concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 240 mg (79%) of the title compound. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.43 (s, 1H),

8.25 (d, J = 1.5 Hz, 1H), 7.82 (dd, J = 1.8, 7.0 Hz, 1H), 7.78-7.75 (m, 2H), 7.53 (t, J = 8.7 Hz, 2H), 6.94 (d, J = 8.8 Hz, 1H), 6.89 (q, J = 11, 6.7 Hz, 1H), 5.89 (d, J = 17.6 Hz, 1H), 5.37 (d, J = 11 Hz, 1H), 4.21 (q, J = 7.1 Hz, 2H), 1.26 ppm (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.94, 164.1, 162.33 (d, J(C,F) = 247.1 Hz), 148.38, 140.08, 136.63 (d, J(C,F) = 2.9 Hz), 135.43, 134.01, 130.13 (d, J(C,F) = 9.2 Hz), 129.79, 127.5, 123.81, 118.5, 117.18 (d, J(C,F) = 23.1 Hz), 115.64, 110.63, 59.89, 14.21 ppm. HRMS (ESI): calculated for $C_{20}H_{16}FNO_3$ [M]: 337.1114; found: 337.1117. LCMS R_T = 0.980 min, ES-MS m/z = 338.2 $[M+H]^+$.

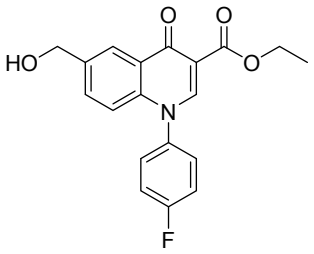
Ethyl 1-(4-fluorophenyl)-6-formyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (67, where Ar = 4-fluorophenyl)



To a solution ethyl 1-(4-fluorophenyl)-4-oxo-6-vinyl-1,4-dihydroquinoline-3-carboxylate (240 mg, 0.71 mmol, 1.0 eq) in 3:1 acetone/water (8 mL) was added *N*-oxide-4-methylmorpholine (125 mg, 1.07 mmol, 1.5 eq) and osmium tetroxide (3.6 mg, 0.014 mmol, 0.02 eq). After the reaction stirred for three hours, sodium periodate (168 mg, 0.78 mmol, 1.1 eq) was added. After another hour, the reaction was diluted with EtOAc and washed well with a 10% NaS_2O_3 solution. The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give 240 mg (99%) of the title compound that was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.1 (s, 1H), 8.78 (d, J = 1.8 Hz, 1H), 8.51 (s, 1H), 8.07 (dd, J = 1.7, 8.6 Hz, 1H), 7.81-7.78 (m, 2H), 7.55 (t, J = 8.6 Hz, 2H), 7.12 (d, J = 8.8 Hz, 1H), 4.23 (q, J = 7.1 Hz, 2H), 1.27 ppm (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 192.19, 172.94, 163.77, 162.49 (d, J(C,F)

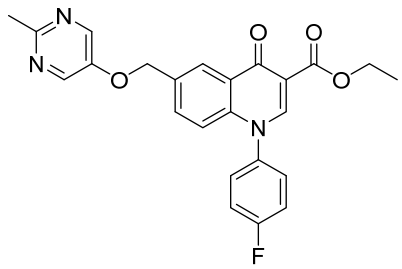
= 247 Hz), 149.49, 144.23, 136.49 (d, *J*(C,F) = 3.0 Hz), 132.37, 130.96, 130.21 (d, *J*(C,F) = 9.0 Hz), 130.19, 127.26, 119.31, 117.33 (d, *J*(C,F) = 23.1 Hz), 111.81, 60.17, 14.21 ppm. HRMS (ESI): calculated for $C_{19}H_{14}FNO_4$ [M]: 339.0907; found: 339.0910. LCMS R_T = 0.846 min, ES-MS *m/z* = 340.2 $[M+H]^+$.

Ethyl 1-(4-fluorophenyl)-6-(hydroxymethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (68, where Ar = 4-fluorophenyl)



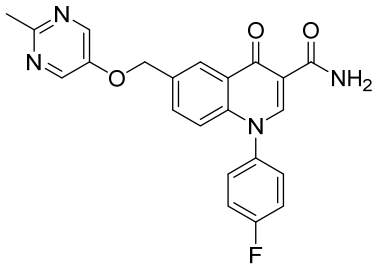
To a solution of ethyl 1-(4-fluorophenyl)-6-formyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (335 mg, 0.99 mmol, 1.0 eq) in ethanol (5 mL) and cooled to 0 ºC was added sodium borohydride (18.7 mg, 0.49 mmol, 0.50 eq). After ten minutes, the reaction was diluted with EtOAc and washed well with water. The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 192 mg (57%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, 1H), 8.25 (s, 1H), 7.76-7.72 (m, 2H), 7.61 (d, *J* = 6 Hz, 1H), 7.54 (t, *J* = 8.64, 8.68 Hz, 2H), 6.94 (d, *J* = 8.68, 1H), 5.42 (t, *J* = 5.68, 5.64 Hz, 1H), 4.62 (d, *J* = 5.48 Hz, 2H), 4.23 (q, *J* = 7.08 Hz, 2H), 1.27 ppm (t, *J* = 7.12, 7.04 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.7, 165.56, 161.98, 148.77, 140.06, 139.23, 136.86 (d, *J*(C,F) = 3.5 Hz), 131.88, 129.67 (d, *J*(C,F) = 9.4 Hz), 128.23, 125.56, 118.01, 117.74 (d, *J*(C,F) = 24 Hz), 111.34, 64.44, 61.20, 14.59 ppm. HRMS (ESI): calculated for $C_{19}H_{16}FNO_4$ [M]: 341.1063; found: 341.1066. LCMS R_T = 0.986 min, ES-MS *m/z* = 342.3 $[M+H]^+$.

Ethyl 1-(4-fluorophenyl)-6-(((2-methylpyrimidin-5-yl)oxy)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (69, where Ar = 4-fluorophenyl and R^4 =2-methylpyrimidin-5-yl)



To a solution of ethyl 1-(4-fluorophenyl)-6-(hydroxymethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (16 mg, 0.047 mmol, 1.0 eq) and 5-hydroxy-2-methylpyrimidine (6.2 mg, 0.056 mmol, 1.2 eq) in THF (1 mL) cooled to 0 ºC was added triphenylphosphine (27.1 mg, 0.1 mmol, 2.1 eq) and D^{t}BAD (17.3 mg, 0.075 mmol,). The reaction was concentrated to dryness after 16 hours of stirring. Purification by reverse phase HPLC afforded 20 mg (98%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 1H), 8.46 (s, 1H), 8.36 (s, 2H), 7.57 (d, J = 8.4 Hz, 1H), 7.44-7.40 (m, 2H), 7.30 (t, J = 8.2 Hz, 2H), 6.97 (d, J = 8.5 Hz, 1H), 5.23 (s, 2H), 4.36 (q, J = 7.1 Hz, 2H), 2.65 (d, 3H), 1.36 ppm (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.25, 165.44, 163.30 (d, J(C,F) = 252 Hz), 160.88, 149.13 (d, J(C,F) = 24 Hz), 144.31, 140.82, 136.61 (d, J(C,F) = 4.0 Hz), 133.01, 131.74, 129.60 (d, J(C,F) = 9.3 Hz), 128.58, 126.83, 125.45, 118.50, 117.89 (d, J(C,F) = 23.9 Hz), 112.08, 70.18, 61.35, 24.80, 14.60 ppm. HRMS (ESI): calculated for $C_{24}H_{20}FN_3O_4$ [M]: 433.1438; found: 433.1440. LCMS R_T = 0.922 min, ES-MS m/z = 434.2 $[M+H]^+$.

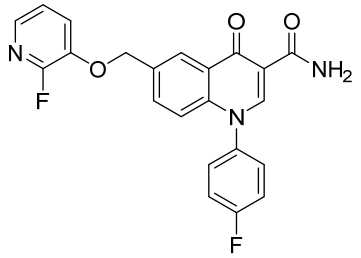
1-(4-Fluorophenyl)-6-(((2-methylpyrimidin-5-yl)oxy)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (78).



Ethyl 1-(4-fluorophenyl)-6-(((2-methylpyrimidin-5-yl)oxy)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (9.4 mg, 0.022 mmol, 1.0 eq) was suspended in 7N ammonia in methanol (800 µL) in a microwave vial and heated in a microwave reactor to 150 ºC for 120 minutes. The reaction was concentrated to dryness and purified using reverse-phase HPLC to afford 7.4 mg (84%) of the title compound. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.26 (d, *J* = 4.1 Hz, 1H), 8.64 (s, 1H), 8.57 (s, 2H), 8.53 (d, *J* = 1.5 Hz, 1H), 7.86 (d, *J* = 1.8 Hz, 1H), 7.84-7.81 (m, 2H), 7.69 (d, *J* = 4.2 Hz, 1H), 7.59 (t, *J* = 8.7 Hz, 2H), 7.16 (d, *J* = 8.8 Hz, 1H), 5.48 (s, 2H), 2.59 ppm (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ = 175.74, 165.15, 162.41 (d, *J*(C,F) = 248 Hz), 159.74, 150.26, 148.14, 144.21, 140.53, 136.77 (d, *J*(C,F) = 2.8 Hz), 133.34, 132.63, 130.01 (d, *J*(C,F) = 9.2 Hz), 126.54, 125.22, 118.77, 117.26 (d, *J*(C,F) = 23.9 Hz), 111.81, 69.07, 24.56 ppm. HRMS (ESI): calculated for $C_{22}H_{17}FN_4O_3$ [M]: 404.1285; found: 404.1288. LCMS R_T = 0.823 min, ES-MS *m/z* = 405.3 $[M+H]^+$.

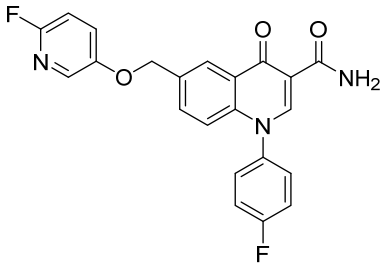
The following compounds were prepared analogous to compound ***78***

1-(4-Fluorophenyl)-6-(((2-fluoropyridin-3-yl)oxy)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (70)



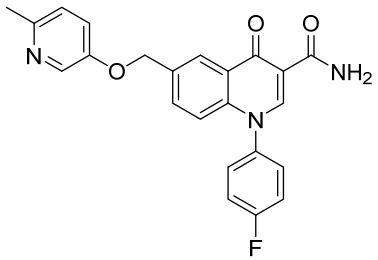
LCMS R_T = 0.937 min, ES-MS *m/z* = 408.3 $[M+H]^+$.

1-(4-fluorophenyl)-6-(((6-fluoropyridin-3-yl)oxy)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (71)



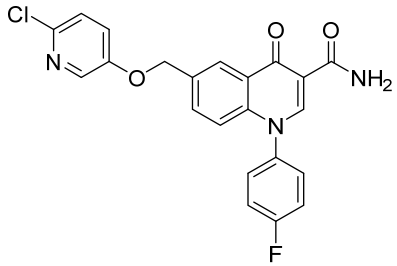
LCMS R_T = 0.949 min, ES-MS *m/z* = 408.3 $[M+H]^+$.

1-(4-fluorophenyl)-6-(((6-methylpyridin-3-yl)oxy)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (72)



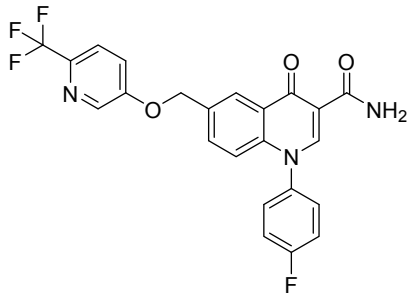
LCMS R_T = 0.697 min, ES-MS *m/z* = 404.2 $[M+H]^+$.

6-(((6-chloropyridin-3-yl)oxy)methyl)-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (73)



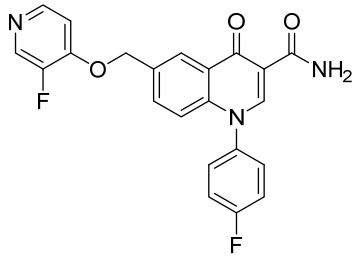
LCMS R_T = 0.823 min, ES-MS *m/z* = 424.2 $[M+H]^+$.

1-(4-fluorophenyl)-4-oxo-6-(((6-(trifluoromethyl)pyridin-3-yl)oxy)methyl)-1,4-dihydroquinoline-3-carboxamide (74)



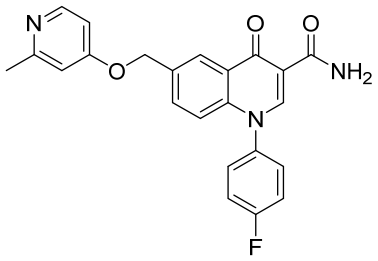
LCMS R_T = 1.054 min, ES-MS *m/z* = 458.2 $[M+H]^+$.

1-(4-fluorophenyl)-6-(((3-fluoropyridin-4-yl)oxy)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (75)



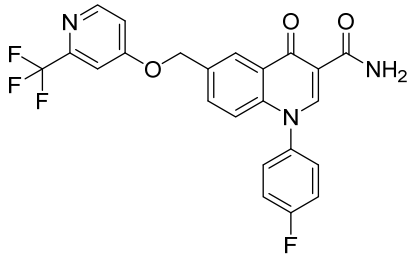
LCMS R_T = 0.707 min, ES-MS *m/z* = 408.2 $[M+H]^+$.

1-(4-fluorophenyl)-6-(((2-methylpyridin-4-yl)oxy)methyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (76)



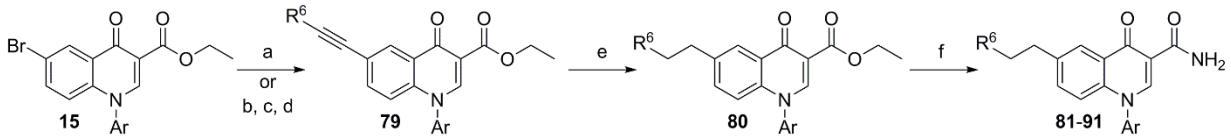
LCMS R_T = 0.687 min, ES-MS *m/z* = 404.3 $[M+H]^+$.

1-(4-fluorophenyl)-4-oxo-6-(((2-(trifluoromethyl)pyridin-4-yl)oxy)methyl)-1,4-dihydroquinoline-3-carboxamide (77)



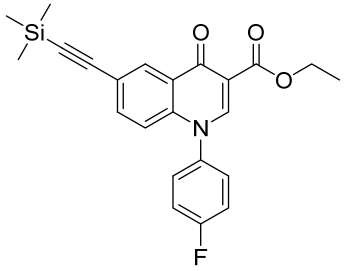
LCMS R_T = 1.026 min, ES-MS *m/z* = 458.2 $[M+H]^+$.

Preparation of Analogs 81–91. The referenced compounds were prepared via the route pictured immediately below. All compounds were ≥ 95% pure as measured by UV spectroscopy at 215 and 254 nm.



Reagents and conditions: (a) R^6CCH, $PdCl_2(PPh_3)_2$, CuI, NEt_3, DMF, microwave, 150 ºC, 15 min, 23–54%; (b) Me_3SiCCH, $PdCl_2(PPh_3)_2$, CuI, NEt_3, DMF, microwave, 150 ºC, 15 min, 83%; (c) TBAF, THF, 70%; (d) R^6Br, $PdCl_2(PPh_3)_2$, CuI, NEt_3, DMF, microwave, 150 ºC, 15 min, 21–47%; (e) 10% Pd/C, MeOH, H_2 (1 atm), 63–99%; (f) 7N NH_3 in MeOH, microwave, 150 ºC, 15 min, 10–94%.

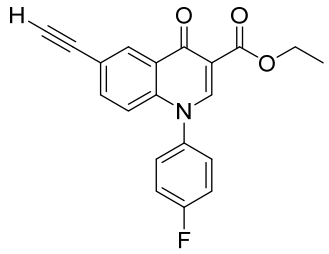
Ethyl 1-(4-fluorophenyl)-4-oxo-6-((trimethylsilyl)ethynyl)-1,4-dihydroquinoline-3-carboxylate (79 where Ar = 4-fluorophenyl and R^6 = $SiMe_3$)



Ethyl 6-bromo-1-(4-fluorophenyl)-4-oxo-quinoline-3-carboxylate (450 mg, 1.15 mmol, 1.0 eq), trimethylsilylacetylene (342 µL, 2.42 mmol, 2.1), $PdCl_2(PPh_3)_2$ (81 mg, 0.12 mmol, 0.10 eq), CuI (44 mg, 0.23 mmol, 0.20 eq), triethylamine (643 µL, 4.61 mmol, 4.0 eq) and DMF (5.8 mL) were added to a microwave vial. The vial was capped and heated in a microwave reactor at 150 ºC for 15 minutes. The reaction was washed with water and brine and extracted with EtOAc (2x). The organics were combined, dried ($MgSO_4$), and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 390 mg (83%) of the title compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (s, 1H), 8.24 (d, J = 1.9 Hz, 1H), 7.76-7.73 (m, 2H), 7.68 (dd, J = 2.0, 8.8 Hz, 1H), 7.52 (t, J = 8.7 Hz, 2H), 6.93 (d, J = 8.8 Hz, 1H), 4.22 (q, J = 7.1 Hz, 2H), 1.25 (t, J = 7.1 Hz, 3H), 0.24 ppm (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.21, 163.88, 162.41 (d, J(C,F) = 247.5 Hz), 148.95, 140.44, 136.38 (d, J(C,F) = 3.0 Hz), 135.06, 130.18 (d, J(C,F) = 9.1

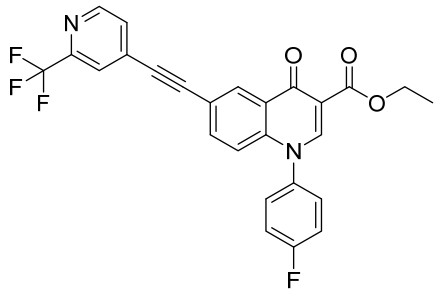
Hz), 129.41, 127.23, 118.74 (d, *J*(C,F) = 5.1 Hz), 117.26 (d, *J*(C,F) = 23.4 Hz), 111.04, 103.8, 95.44, 60.02, 14.21, -0.20 ppm. HRMS (ESI): calculated for $C_{23}H_{22}FNO_3Si$ [M]: 407.1353; found: 407.1358. LCMS R_T = 1.216 min, ES-MS *m/z* = 408.2 $[M+H]^+$.

Ethyl 6-ethynyl-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (79 where Ar = 4-fluorophenyl and R^6 = H)



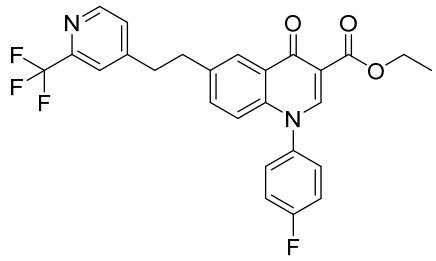
To a solution of ethyl 1-(4-fluorophenyl)-4-oxo-6-((trimethylsilyl)ethynyl)-1,4-dihydroquinoline-3-carboxylate (390 mg, 0.96 mmol, 1.0 eq) in THF (4.8 mL) was added a 1M solution of TBAF in THF (1.05 mL, 1.05 mmol, 1.1 eq). After thirty minutes the reaction was concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 225 mg (70%) of the title compound. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.47 (s, 1H), 8.28 (d, *J* = 1.9 Hz, 1H), 7.78-7.75 (m, 2H), 7.71 (dd, *J* = 2.0, 8.8 Hz, 1H), 7.53 (t, *J* = 8.7 Hz, 2H), 6.97 (d, *J* = 8.8 Hz, 1H), 4.35 (s, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 1.26 ppm (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ = 172.32, 163.97, 162.43 (d, *J*(C,F) = 247 Hz), 149.03, 140.52, 136.40 (d, *J*(C,F) = 3.0 Hz), 135.36, 130.19 (d, *J*(C,F) = 9.2 Hz), 129.40, 127.28, 118.87, 118.35, 117.27 (d, *J*(C,F) = 23 Hz), 111.0, 82.12 (d, *J*(C,F) = 38.2 Hz), 60.06, 48.59, 14.22 ppm. HRMS (ESI) calculated for $C_{20}H_{14}FNO_3$ [M]: 335.0958. found: 335.0962. LCMS R_T = 0.947 min, ES-MS *m/z* = 336.2 $[M+H]^+$.

Ethyl 1-(4-fluorophenyl)-4-oxo-6-((2-(trifluoromethyl)pyridin-4-yl)ethynyl)-1,4-dihydroquinoline-3-carboxylate (79 where Ar = 4-fluorophenyl and R^6 = (2-(trifluoromethyl)pyridin-4-yl).



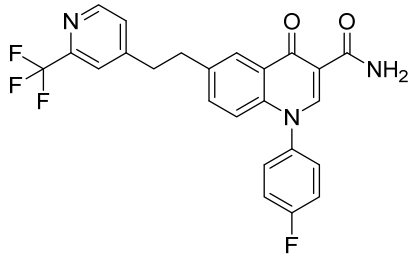
Ethyl 6-ethynyl-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (20 mg, 0.060 mmol, 1.0 eq), 4-bromo-2-(trifluoromethyl)pyridine (16.2 mg, 0.072 mmol, 1.2 eq), CuI (1.1 mg, 0.0063 mmol, 0.11 eq), triethylamine (25 µL, 0.18 mmol, 3.0 eq), $PdCl_2(PPh_3)_2$ (2.7 mg, 0.0038 mmol, 0.63 eq), and DMF (600µL) were added to a small microwave vial. The vial was capped and heated in a microwave reactor at 150 ºC for fifteen minutes. The reaction was filtered through Celite®, washed very well with a 5%MeOH in DCM solution. The filtrate was concentrated *in vacuo*. Purification by reverse phase HPLC afforded 13 mg (45%) of the title compound. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.83 (d, *J* = 4.9 Hz, 1H), 8.48 (s, 1H), 8.47 (d, *J* = 1.8 Hz, 1H), 8.15 (s, 1H), 7.91 (d, *J* = 4.9 Hz, 1H), 7.86 (dd, *J* = 2.0, 8.9 Hz, 1H), 7.82-7.79 (m, 2H), 7.55 (t, *J* = 8.6 Hz, 2H), 7.06 (d, *J* = 8.9 Hz, 1H), 4.22 (q, *J* = 7.09 Hz, 2H), 1.27 ppm (t, *J* = 7.09 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ = 172.25, 163.83, 162.46 (d, *J*(C,F) = 248 Hz), 150.69, 149.12, 146.99 (d, *J*(C,F) = 34.3 Hz), 141.17, 136.39 (d, *J*(C,F) = 2.8 Hz), 135.24, 132.15, 131.47 (d, *J*(C,F) = 10.3 Hz), 130.22 (d, *J*(C,F) = 8.6 Hz), 128.81, 127.39, 122.47 (d, *J*(C,F) = 2.7 Hz), 121.34 (d, *J*(C,F) = 274 Hz), 119.12, 117.29 (d, *J*(C,F) = 22.9 Hz), 117.28, 111.32, 94.43, 86.53, 60.08, 14.21 ppm. HRMS (ESI) calculated for $C_{26}H_{16}F_4N_2O_3$ [M]: 480.1097; found: 480.1102. LCMS R_T = 1.219 min, ES-MS *m/z* = 481.3 $[M+H]^+$.

Ethyl 1-(4-fluorophenyl)-4-oxo-6-(2-(2-(trifluoromethyl)pyridin-4-yl)ethyl)-1,4-dihydroquinoline-3-carboxylate (80 where Ar = 4-fluorophenyl and R^6 = H)



To a solution of ethyl 1-(4-fluorophenyl)-4-oxo-6-((2-(trifluoromethyl)pyridin-4-yl)ethynyl)-1,4-dihydroquinoline-3-carboxylate (13 mg, 0.027 mmol, 1.0 eq) in methanol (2 mL) was added 10% Pd/C (10 mg) under nitrogen gas. After stirring for 18 hours under a hydrogen atmosphere, the reaction was filtered through Celite® and washed very well with a 5%MeOH in DCM solution. The filtrate was concentrated *in vacuo* to give 13.1 mg (99%) of the title compound that was used without further purification. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.64 (d, *J* = 5.0 Hz, 1H), 8.44 (s, 1H), 8.16 (d, *J* = 1.6 Hz, 1H), 7.86 (s, 1H), 7.73-7.70 (m, 2H), 7.62 (d, *J* = 4.8 Hz, 1H), 7.58 (dd, *J* = 1.8, 8.9 Hz, 1H), 7.51 (t, *J* = 8.9 Hz, 2H), 6.90 (d, *J* = 8.6 Hz, 1H), 4.21 (q, *J* = 7.0 Hz, 2H), 3.13-3.04 (m, 4H), 1.26 ppm (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ = 173.15, 164.27, 162.39 (d, *J*(C,F) = 249 Hz), 152.96, 150.05, 148.42, 146.55 (d, *J*(C,F) = 33.7 Hz), 139.14, 138.07, 136.75, 133.44, 130.18 (d, *J*(C,F) = 9.2 Hz), 127.43 (d, *J*(C,F) = 2.6 Hz), 125.41, 123.2, 119.6 (d, *J*(C,F) = 279 Hz), 118.87, 117.27 (d, *J*(C,F) = 23 Hz), 115.89, 110.35, 59.97, 35.87, 34.83, 14.29 ppm. HRMS (ESI) calculated for $C_{26}H_{20}F_4N_2O_3$ [M]: 484.1410; found: 484.1412. LCMS R_T = 1.144 min, ES-MS *m/z* = 485.2 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-6-(2-(2-(trifluoromethyl)pyridin-4-yl)ethyl)-1,4-dihydroquinoline-3-carboxamide (83)

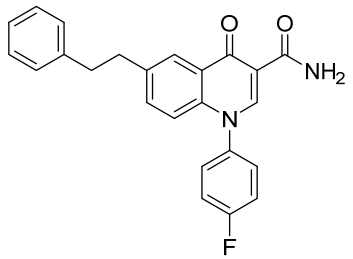


Ethyl 1-(4-fluorophenyl)-4-oxo-6-(2-(2-(trifluoromethyl)pyridin-4-yl)ethyl)-1,4-dihydroquinoline-3-carboxylate (13.1 mg, 0.027 mmol, 1.0 eq) was suspended in 7N ammonia in methanol (1 mL) in a microwave vial and heated to 100 ºC for 18 hours. The reaction was concentrated to dryness and purified using reverse-phase HPLC to afford 5.7 mg (46%) of the title compound. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.26 (d, *J* = 4.3 Hz, 1H), 8.65 (d, *J* = 5.0 Hz, 1H), 8.55 (s, 1H), 8.27 (d, *J* = 1.8 Hz, 1H), 7.88 (s, 1H), 7.76-7.73 (m, 2H), 7.66-7.63 (m, 1H), 7.61 (d, *J* = 4.4 Hz, 2H), 7.53 (t, *J* = 8.7 Hz, 2H), 7.0 (d, *J* = 8.7 Hz, 1H), 3.14-3.07 ppm (m, 4H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ = 175.75, 165.31, 162.36 (d, *J*(C,F) = 247 Hz), 152.91, 150.01, 147.67, 146.51 (d, *J*(C,F) = 34.3 Hz), 139.29, 138.17, 136.83 (d, *J*(C,F) = 2.9 Hz), 133.70, 129.96 (d, *J*(C,F) = 9.2 Hz), 127.39, 126.61, 125.23, 123.17, 119.62 (d, *J*(C,F) = 269 Hz), 118.87, 117.23 (d, *J*(C,F) = 23 Hz), 115.88, 111.45, 35.85, 34.82 ppm. HRMS (ESI) calculated for $C_{24}H_{17}F_4N_3O_2$ [M]: 455.1257; found: 455.1262. LCMS R_T = 1.045 min, ES-MS *m/z* = 456.2 $[M+H]^+$.

The following compounds were prepared analogous to compound ***83****. Compounds* ***81****,* ***82****,* ***84****, and* ***87–91*** *were prepared using a single Sonogashira coupling where* R^6*CCH was the alkyne and* R^6 *was the requisite aryl or heteroaryl ring. The remaining compounds utilized the three-*

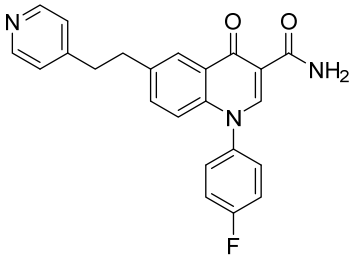
step sequence of (i) Sonogashira coupling with trimethylsilylacetylene, (ii) TBAF deprotection, and (iii) Sonogashira coupling with R^6Br where R^6 was the requisite aryl or heteroaryl ring.

1-(4-Fluorophenyl)-4-oxo-6-phenethyl-1,4-dihydroquinoline-3-carboxamide (81)



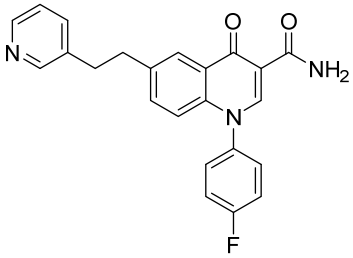
LCMS R_T = 1.121 min, ES-MS *m/z* = 387.2 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-6-(2-(pyridin-4-yl)ethyl)-1,4-dihydroquinoline-3-carboxamide (82)



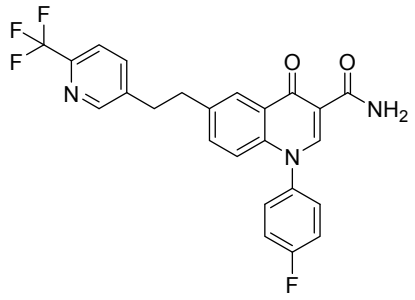
LCMS R_T = 0.654 min, ES-MS *m/z* = 388.2 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-6-(2-(pyridin-3-yl)ethyl)-1,4-dihydroquinoline-3-carboxamide (84)



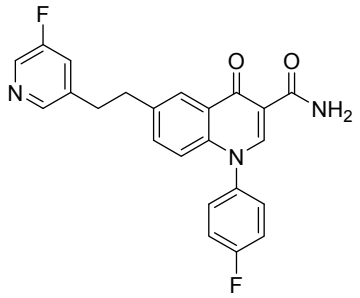
LCMS R_T = 0.655 min, ES-MS *m/z* = 388.2 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-6-(2-(6-(trifluoromethyl)pyridin-3-yl)ethyl)-1,4-dihydroquinoline-3-carboxamide (85)



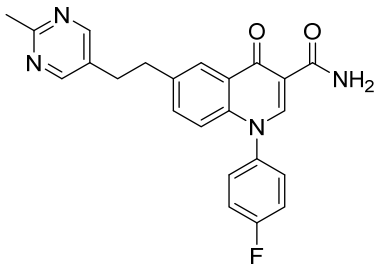
LCMS R_T = 1.071 min, ES-MS *m/z* = 456.2 $[M+H]^+$.

1-(4-Fluorophenyl)-6-(2-(5-fluoropyridin-3-yl)ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (86)



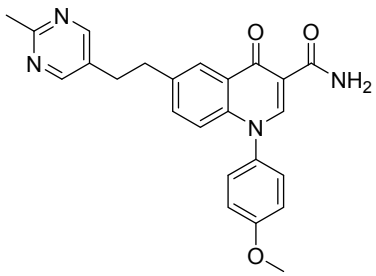
LCMS R_T = 0.855 min, ES-MS *m/z* = 406.4 $[M+H]^+$.

1-(4-Fluorophenyl)-6-(2-(2-methylpyrimidin-5-yl)ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (87)



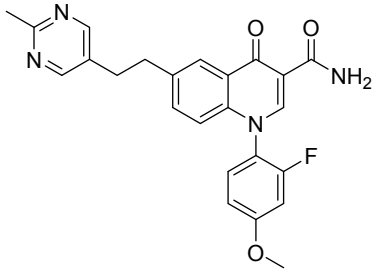
LCMS R_T = 0.761 min, ES-MS *m/z* = 403.2 $[M+H]^+$.

1-(4-Methoxyphenyl)-6-(2-(2-methylpyrimidin-5-yl)ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (88)



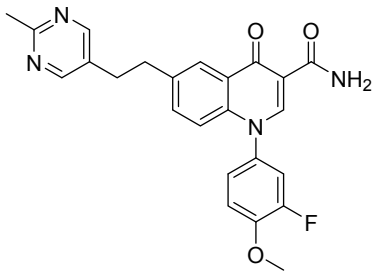
LCMS R_T = 0.791 min, ES-MS *m/z* = 415.2 $[M+H]^+$.

1-(2-Fluoro-4-methoxyphenyl)-6-(2-(2-methylpyrimidin-5-yl)ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (89)



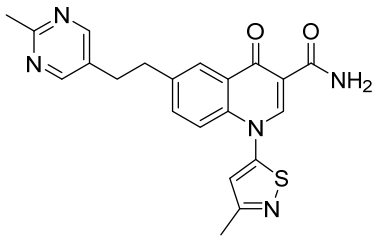
LCMS R_T = 0.806 min, ES-MS *m/z* = 433.3 $[M+H]^+$.

1-(3-Fluoro-4-methoxyphenyl)-6-(2-(2-methylpyrimidin-5-yl)ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (90)



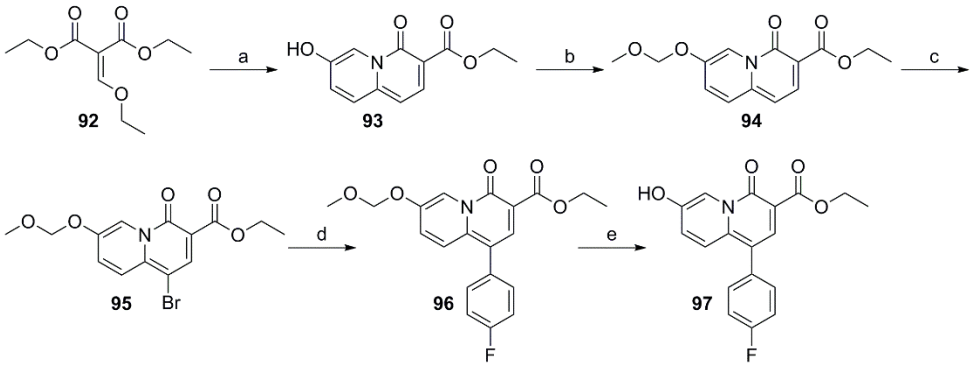
LCMS R_T = 0.798 min, ES-MS *m/z* = 433.2 $[M+H]^+$.

1-(3-Methylisothiazol-5-yl)-6-(2-(2-methylpyrimidin-5-yl)ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (91)



LCMS R_T = 0.714 min, ES-MS *m/z* = 406.2 $[M+H]^+$.

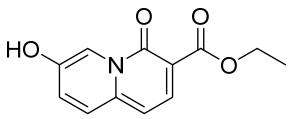
Preparation of Intermediate 97. The referenced intermediate were prepared via the route pictured immediately below.



Reagents and conditions: (a) 5-hydroxy-2-methylpyridine, *n*-BuLi, THF, –78 ºC to –30 ºC, 57%; (b) CH_3OCH_2Cl, DIEA, CH_2Cl_2, 0 ºC to r.t., 96%; (c) NBS, $CHCl_3$, 0 ºC to r.t., 96%; (d) 4-

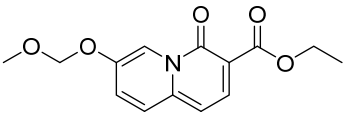
fluorophenylboronic acid, Pd(dppf)·CH_2Cl_2, 1M aq. Na_2CO_3, DME, 90 ºC, 94%; (e) pTSA·H_2O, EtOH, DCE, 80 ºC, 66%.

Ethyl 7-hydroxy-4-oxo-4*H*-quinolizine-3-carboxylate (93)



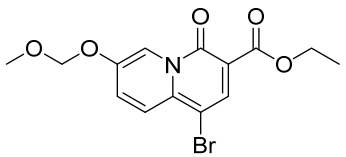
To a solution of 5-hydroxy-2-methylpyridine (4.36 g, 40.0 mmol, 1.0 eq) in THF (320 mL) at –78 ºC was added a solution of *n*-butyllithium (55.3 mL, 88.5 mmol, 2.2 eq). The reaction mixture was stirred at –78 ºC for 30 minutes and 1.5 hours at room temperature. The mixture was then cooled to –78 ºC and a solution of diethyl ethoxymethylenemalonate **92** (8.9 mL, 44 mmol, 1.1 eq) in THF (40 mL) was added dropwise. The mixture was stirred at –30 ºC for 1 hour. A saturated aqueous solution of NH_4Cl was added and the reaction mixture was extracted with DCM (3x). The combined organics were washed with water and dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification using flash chromatography on silica gel to provide the alkylated intermediate as an orange oil (10.4 g) which was subsequently refluxed for 24 h in xylenes (50 mL). The reaction mixture was cooled to room temperature, and the precipitate was filtered and collected using a Buchner funnel. The solid was washed with Et_2O and hexanes and dried in a vacuum oven to afford 5.20 g (57%) of the title compound as a yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (d, J = 2.1 Hz, 1H), 8.02 (d, J = 8.6 Hz, 1H), 7.85 (d, J = 9.2, 1H), 7.57 (dd, J = 9.2, 2.4 Hz, 1H), 6.78 (d, J = 8.6 Hz, 1H), 4.22 (q, J = 7.1 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.2, 154.4, 151.7, 141.5, 136.4, 130.5, 127.3, 112.4, 102.8, 102.7, 60.0. 14.9. HRMS (ESI) calculated for $C_{12}H_{11}NO_4$ [M]: 233.0688; found: 233.0690. LCMS: R_T = 0.664 min, ES-MS m/z = 234.3 $[M+H]^+$.

Ethyl 7-(methoxymethoxy)-4-oxo-4*H*-quinolizine-3-carboxylate (94)



To a solution of compound **93** (4.70 g, 20.2 mmol, 1.0 eq) in DCM (100 mL) at 0 ºC was added chloromethyl methyl ether (2.30 mL, 30.3 mmol, 1.5 eq) followed by *N,N*-diisopropylethylamine (7.02 mL, 40.1 mmol, 2.0 eq). After stirring at room temperature for 1 hour, the reaction mixture was diluted with DCM and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification using flash chromatography on silica gel afforded 5.35 g (96%) of the title compound as a yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.22 (d, J = 2.1 Hz, 1H), 8.32 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 9.3, 1H), 7.46 (dd, J = 9.3, 2.3 Hz, 1H), 6.64 (d, J = 8.4 Hz, 1H), 5.27 (s, 2H), 4.41 (q, J = 7.1 Hz, 2H), 3.51 (s, 3H), 1.42 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 155.5, 149.1, 142.7, 139.1, 128.7, 126.1, 114.1, 106.2, 102.5, 95.4, 60.9, 56.6, 14.5. HRMS (ESI) calculated for $C_{14}H_{15}NO_5$ [M]: 277.0950; found: 277.0954. LCMS: R_T = 0.783 min, ES-MS m/z = 278.4 $[M+H]^+$.

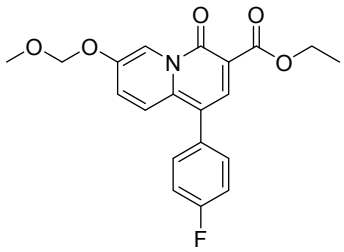
Ethyl 1-bromo-7-(methoxymethoxy)-4-oxo-4*H*-quinolizine-3-carboxylate (95)



To a solution of compound **94** (5.35 g, 19.3 mmol, 1.0 eq) in chloroform (191 mL) at 0 ºC was added *N*-bromosuccinimide (4.12 g, 23.1 mmol, 1.2 eq). After stirring at room temperature for 3 hours, the reaction mixture was diluted with DCM, washed with 10% $Na_2S_2O_3$ solution and saturated $NaHCO_3$ solution. The organics were concentrated *in vacuo*. Purification using flash

chromatography on silica gel provided 5.80 g (84%) the title compound (5.8 g, 84% yield) as a yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (d, J = 2.4 Hz, 1H), 8.51 (s, 1H), 8.05 (d, J = 9.6 Hz, 1H), 7.62 (dd, J = 9.6, 2.4 Hz, 1H), 5.29 (s, 2H), 4.41 (q, J = 7.1 Hz, 2H), 3.50 (s, 3H), 1.42 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.5, 154.5, 149.5, 141.9, 140.2, 130.2, 125.9, 115.4, 106.2, 95.4, 93.0, 61.2, 56.7, 14.5. HRMS (ESI) calculated for $C_{14}H_{14}BrNO_5$ [M]: 355.0055; found: 355.0053. LCMS: R_T = 0.980 min, ES-MS m/z = 358.2 $[M+H]^+$.

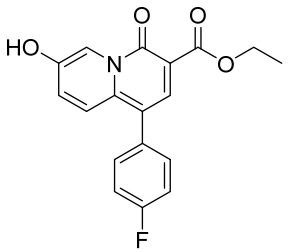
Ethyl 1-(4-fluorophenyl)-7-(methoxymethoxy)-4-oxo-4*H*-quinolizine-3-carboxylate (96)



A suspension of compound **95** (1.05 g, 2.95 mmol, 1.0 eq), 4-fluorophenylboronic acid (825 mg, 5.90 mmol, 2.0 eq), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (215 mg, 0.29 mmol, 0.1 eq), 1M aqueous Na_2CO_3 solution (5.90 mL, 5.90 mmol, 2.0 eq) in 1,2-dimethoxyethane (29.5 mL) was stirred at 90 ºC for 2 hours. After cooling to room temperature, the reaction mixture was diluted with EtOAc and washed with water. The aqueous layer was extracted with EtOAc (2x). The combined extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification using flash chromatography on silica gel afforded 1.30 g (94%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (d, J = 2.4 Hz, 1H), 8.25 (s, 1H), 7.63 (d, J = 9.6 Hz, 1H), 7.43 (dd, J = 9.6, 2.4 Hz, 1H), 7.34 (dd, J = 8.4, 5.5 Hz, 2H), 7.16 (dd, J = 8.5, 8.5 Hz, 2H), 5.28 (s, 2H), 4.41 (q, J = 7.1 Hz, 2H), 3.50 (s, 3H), 1.41 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 162.4 (d, J = 246 Hz, 1C), 154.9, 149.1, 141.0, 140.1, 133.2 (d, J = 3 Hz, 1C), 131.7 (d, J = 8 Hz, 2C), 128.8 (d, J = 24 Hz,

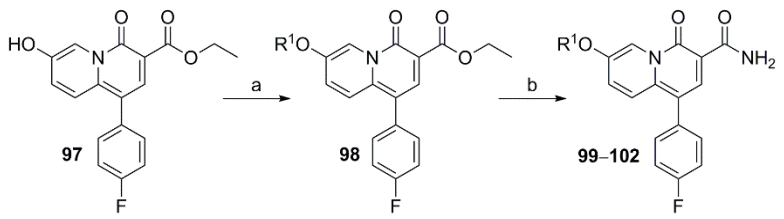
1C), 124.5, 115.9 (d, J = 21 Hz, 2C), 114.9, 114.8, 105.7, 95.3, 61.0 56.6, 14.5. HRMS (ESI) calculated for $C_{20}H_{18}FNO_5$ [M], 371.1169; found 371.1174. LCMS: R_T = 1.079 min, ES-MS *m/z* = 372.2 $[M+H]^+$.

Ethyl 1-(4-fluorophenyl)-7-hydroxy-4-oxo-4*H*-quinolizine-3-carboxylate (97)



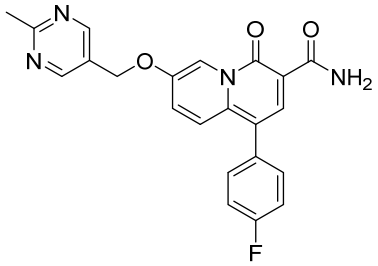
To a solution of compound **96** (1.30 g, 3.47 mmol, 1.0 eq) in ethanol (8.68 mL, 0.2 M) and DCE (8.7 mL) was added p-toluenesulfonic acid monohydrate (330 mg, 1.74 mmol, 0.5 eq). The reaction mixture was stirred at 80 ºC for 2 hours. After cooling to room temperature, the precipitate was filtered and collected using a Buchner funnel. The solid was washed with Et_2O and dried in a vacuum oven to provide 750 mg (66%) of the title as a yellow powder which was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.9 (bs, 1H), 8.97 (d, J = 2.2 Hz, 1H), 7.96 (s, 1H), 7.67 (d, J = 9.5 Hz, 1H), 7.61 (dd, J = 9.5, 2.3 Hz, 1H), 7.49 (dd, J = 8.4, 5.7 Hz, 2H), 7.34 (dd, J = 8.4, 8.4 Hz, 2H), 4.24 (q, J = 7.0 Hz, 2H), 1.28 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9, 162.1 (d, J = 243 Hz, 1C), 153.9, 150.6, 140.1, 137.6, 134.1, 132.2 (d, J = 8 Hz, 2C), 130.3, 125.39, 116.2 (d, J = 21 Hz, 2C), 114.2, 113.3, 102.8, 60.3, 14.8. HRMS (ESI) calculated for $C_{18}H_{14}FNO_4$ [M]: 327.0907; found: 327.0911. LCMS: R_T = 0.963 min, ES-MS *m/z* = 328.2 $[M+H]^+$.

Preparation of Analogs 99–102. The referenced compounds were prepared via the route pictured immediately below.



Reagents and conditions: (a) R^1OH, PPh_3, D^tBAD, THF, 0 ºC to 45 ºC, 38–82%; (b) 7N NH_3 in MeOH, microwave, 150 ºC, 2.0–3.0 h, 26–90%.

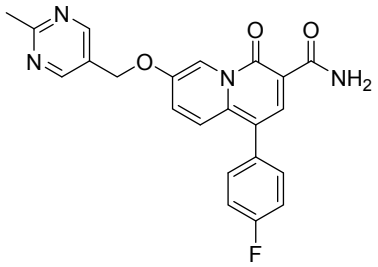
Ethyl 1-(4-fluorophenyl)-7-((2-methylpyrimidin-5-yl)methoxy)-4-oxo-4*H*-quinolizine-3-carboxylate (98 where R^1 = (2-methylpyrimidin-5-yl)methyl)



To a suspension of compound **97** (32.7 mg, 0.10 mmol, 1.0 eq), (2-methylpyrimidin-5-yl)methanol (24.8 mg, 0.20 mmol, 2.0 eq) and triphenylphosphine (73.3 mg, 0.28 mmol, 2.8 eq) in THF (1.0 mL) at 0 ºC was added di-tert-butyl azodicarboxylate (36.8 mg, 0.16 mmol, 1.6 eq). The mixture was stirred at 45 ºC for 15 min. The solvent was removed *in vacuo*. Purification using reversed-phase HPLC provided 30 mg of the title compound contaminated with remaining (2-methylpyrimidin-5-yl)methanol), which was removed following the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (d, J = 2.2 Hz, 1H), 8.73 (s, 2H), 8.23 (s, 1H), 7.62 (d, J = 9.7 Hz, 1H), 7.37-7.30 (m, 3H) 7.14 (dd, J = 8.6, 8.6 Hz, 2H), 5.16 (s, 2H), 4.36 (q, J = 7.1 Hz, 2H), 2.72 (s, 3H), 1.28 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7, 165.9, 162.5 (d, J = 248 Hz, 1C), 156.7 (2C), 155.1, 150.4, 140.7, 140.1, 132.8 (d, J = 3 Hz, 1C), 131.7 (d, J = 8 Hz, 2C), 131.0 128.5, 125.4, 116.0 (d, J = 22 Hz, 2C), 115.6, 110.7, 106.3, 66.3, 61.1, 25.8, 14.4. HRMS,

calculated for $C_{24}H_{20}FN_2O_4$ [M]: 433.1438; found: 433.1441. LCMS: R_T = 1.035 min, ES-MS *m/z* = 434.4 $[M+H]^+$.

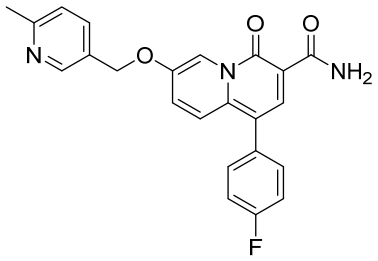
1-(4-Fluorophenyl)-7-((2-methylpyrimidin-5-yl)methoxy)-4-oxo-4*H*-quinolizine-3-carboxamide (**100**)



A solution of ethyl 1-(4-fluorophenyl)-7-((2-methylpyrimidin-5-yl)methoxy)-4-oxo-4*H*-quinolizine-3-carboxylate (30 mg, 0.07 mmol, 1.0 eq) in 7M ammonia in MeOH (2.0 mL) was subjected to a microwave reactor at 150 ºC for 2.5 hours. The solvent was removed *in vacuo*. The crude residue was purified using reversed-phase HPLC system to provide 12 mg (43%) of the title compound. ^{1}H NMR (400 MHz, MeOD) δ 9.09 (s, 1H), 8.79 (s, 2H), 8.33 (s, 1H), 7.71 (d, *J* = 9.7 Hz, 1H), 7.56 (dd, *J* = 9.8, 1.8 Hz, 1H), 7.38 (dd, *J* = 7.8, 5.5 Hz, 2H), 7.16 (dd, *J* = 8.5, 8.5 Hz, 2H), 5.28 (s, 2H), 2.63 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 167.9, 166.0, 162.3 (d, *J* = 244 Hz, 1C), 157.4 (2C), 156.7, 151.1, 139.0, 138.2, 133.8 (d, *J* = 3 Hz, 1C), 132.4 (d, *J* = 8 Hz, 2C), 129.1, 126.7, 125.5, 116.3 (d, *J* = 21 Hz, 2C), 116.2, 111.1, 108.0, 66.5, 25.9. HRMS (ESI) calculated for $C_{22}H_{17}FN_4O_3$ [M]: 404.1285; found: 404.1289. LCMS: R_T = 0.875 min, ES-MS *m/z* = 405.3 $[M+H]^+$.

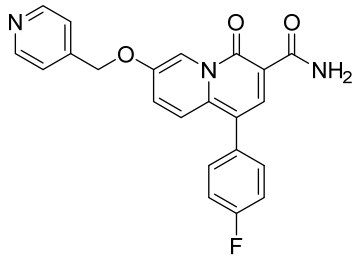
The following compounds were prepared analogous to compound ***100****.*

1-(4-Fluorophenyl)-7-((6-methylpyridin-3-yl)methoxy)-4-oxo-4*H*-quinolizine-3-carboxamide (99)



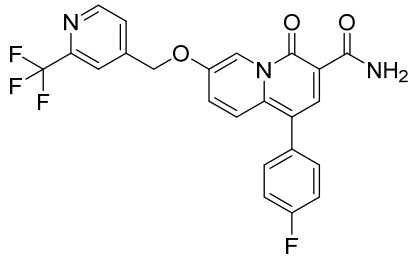
LCMS: R_T = 0.727 min, ES-MS *m/z* = 404.3 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-7-(pyridin-4-ylmethoxy)-4*H*-quinolizine-3-carboxamide (101)



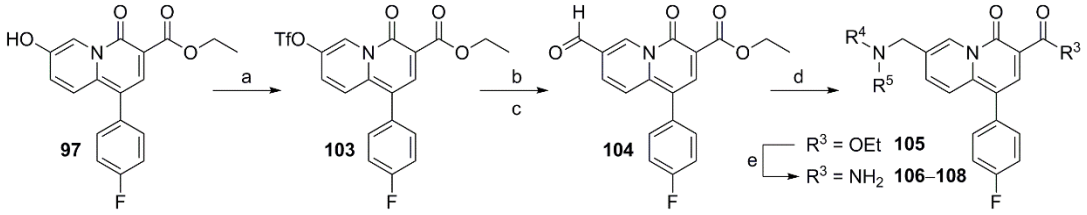
LCMS: R_T = 0.713 min, ES-MS *m/z* = 390.4 $[M+H]^+$.

1-(4-Fluorophenyl)-4-oxo-7-((2-(trifluoromethyl)pyridin-4-yl)methoxy)-4*H*-quinolizine-3-carboxamide (102)



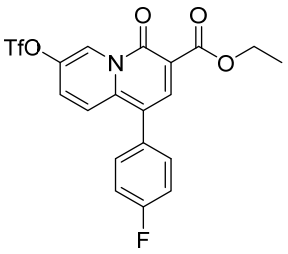
LCMS: R_T = 1.066 min, ES-MS *m/z* = 458.2 $[M+H]^+$.

Preparation of Analogs 106–108. The referenced compounds were prepared via the route pictured immediately below.



Reagents and conditions: (a) $PhN(SO_2CF_3)_2$, NEt_3, CH_2Cl_2, 0 ºC, 96%; (b) H_2CCHBF_3K, $Pd(dppf)\cdot CH_2Cl_2$, NEt_3, *n*-propanol, 90 ºC, 96%; (c) OsO_4, NMO, THF, H_2O, then $NaIO_4$, 70%; (d) HNR^4R^5, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, 27–67%; (e) 7N NH_3 in MeOH, microwave, 150 ºC, 2.0–3.0 h, 26–90%.

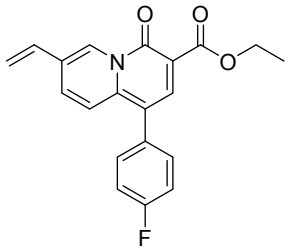
Ethyl 1-(4-fluorophenyl)-4-oxo-7-(((trifluoromethyl)sulfonyl)oxy)-4*H*-quinolizine-3-carboxylate (103)



To a solution of compound **97** (600 mg, 1.83 mmol, 1.0 eq) in DCM (9.16 mL) at 0 ºC was added triethylamine (0.511 mL, 3.67 mmol, 2.0 eq) followed by *N*-phenylbis(trifluoromethane-sulfonimide) (1.31 g, 3.67 mmol, 2.0 eq). The reaction mixture was stirred at 0 ºC for 1 hour. Saturated aqueous $NaHCO_3$ solution (~50 mL) was added. The layers were separated, and the aqueous layer was extracted with DCM (2x). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification using flash chromatography on silica gel provided 811 mg (96%) of the title compound as a yellow powder.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (d, J = 2.2 Hz, 1H), 8.23 (s, 1H), 8.01 (dd, J = 9.9, 2.5 Hz, 1H), 7.78 (d, J = 9.9 Hz, 1H), 7.53 (dd, J = 8.4, 5.6 Hz, 2H), 7.38 (dd, J = 8.8, 8.8 Hz, 2H), 4.29 (q, J = 7.1 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H). LCMS: R_T = 1.201 min, ES-MS m/z = 460.2 $[M+H]^+$.

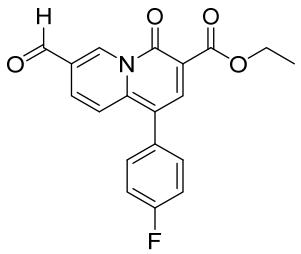
Ethyl 1-(4-fluorophenyl)-4-oxo-7-vinyl-4*H*-quinolizine-3-carboxylate



Compound **103** (344 mg, 0.75 mmol, 1.0 eq), triethylamine (0.209 mL, 1.5 mmol, 2.0 eq), potassium vinyltrifluoroborate **12** (120 mg, 0.90 mmol, 1.2 eq) and [1,1'-bis(diphenylphosphino)-ferrocene]dichloropalladium(II) (27.4 mg, 0.038 mmol, 0.05) were charged to a vial which was evacuated and purged with nitrogen (3x). 1-Propanol (3.75 mL) was added. The vial was sealed, and the reaction mixture was stirred at 90 ºC for 3 hours. After cooling to room temperature, the reaction mixture was filtered through a Celite pad and was washed with EtOAc. The filtrate was concentrated *in vacuo*. Purification by flash chromatography on silica gel provided 243 mg (96%) of the title compound as a yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (s, 1H), 8.14-8.10 (m, 2H), 7.63 (d, J = 9.3 Hz, 1H), 7.52 (dd, J = 8.5, 5.6 Hz, 2H), 7.36 (dd, J = 8.8, 8.8 Hz, 2H), 6.99 (dd, J = 17.6, 11.0 Hz, 1H), 6.05 (d, J = 17.6 Hz, 1H), 5.54 (d, J = 11 Hz, 1H), 4.25 (q, J = 7.1 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.4, 162.2 (d, J = 243 Hz, 1C), 154.5, 143.5, 141.2, 133.7, 133.6, 132.4 (d, J = 8 Hz, 2C), 131.9, 128.1, 127.8, 124.1, 118.5, 116.3 (d, J = 21 Hz, 2C),

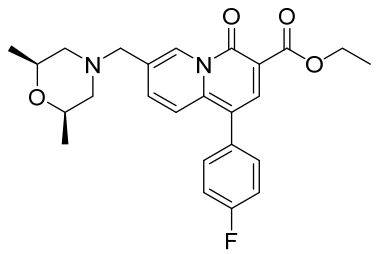
114.2, 105.6, 60.5, 14.8. HRMS (ESI) calculated for $C_{20}H_{16}FNO_3$ [M]: 337.1114; found: 337.1113. LCMS: R_T = 1.109 min, ES-MS *m/z* = 338.2 $[M+H]^+$.

Ethyl 1-(4-fluorophenyl)-7-formyl-4-oxo-4*H*-quinolizine-3-carboxylate (104)



To a suspension of ethyl 1-(4-fluorophenyl)-4-oxo-7-vinyl-4*H*-quinolizine-3-carboxylate (243 mg, 0.72 mmol, 1.0 eq) and 4-methylmorpholine *N*-oxide (127 mg, 1.08 mmol, 1.5 eq) in THF (11 mL) and water (3.6 mL) was added a solution of osmium tetroxide (2.5% in *tert*-BuOH, 0.146 mL, 0.01 mmol, 0.02 eq). The reaction mixture was stirred at room temperature for 3 hours. Sodium periodate (170 mg, 0.79 mmol, 1.1 eq) was added. After stirring for 3 hours at room temperature, the reaction mixture was diluted with EtOAc and washed with 10% aqueous $Na_2S_2O_3$ solution. The layers were separated, and the aqueous layer was extracted with EtOAc (2x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to provide 170 mg (70%) of the crude product, which was used in the subsequent reaction without further purification. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.2 (s, 1H), 9.90 (s, 1H), 8.25 (s, 1H), 7.95 (dd, *J* = 9.4, 1.4 Hz, 1H), 7.68 (d, *J* = 9.2 Hz, 1H), 7.52 (dd, *J* = 8.5, 5.6 Hz, 2H), 7.38 (dd, *J* = 8.8, 8.8 Hz, 2H), 4.29 (q, *J* = 7.1 Hz, 2H), 1.31 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 190.8, 165.1, 162.5 (d, *J* = 230 Hz, 1C), 155.2, 144.9, 144.0, 138.2, 133.2, 132.8 (d, *J* = 75 Hz, 2C), 129.5, 126.3, 124.9, 116.4 (d, *J* = 21 Hz, 2C), 114.9, 107.9, 60.9, 14.7. HRMS (ESI) calculated for $C_{19}H_{14}FNO_4$ [M]: 339.0907; found: 339.0906. LCMS: R_T = 1.027 min, ES-MS *m/z* = 340.2 $[M+H]^+$.

Ethyl 7-((*cis*-2,6-dimethylmorpholino)methyl)-1-(4-fluorophenyl)-4-oxo-4*H*-quinolizine-3-carboxylate (105 where $R^4 = R^5 =$ *cis*–$CH_2CHMeOCHMeCH_2$–)



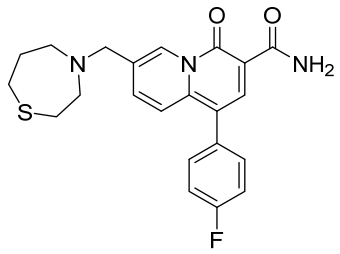
To a solution of compound **104** (15 mg, 0.044 mmol, 1.0 eq) in DCM (0.89 mL) was added acetic acid (0.10 mL) and *cis*-2,6-dimethylmorpholine (27.4uL, 0.22 mmol, 5.0 eq). The reaction mixture was stirred at room temperature for 1 hour. Sodium triacetoxyborohydride (14.1 mg, 0.067 mmol, 1.5 eq) was added, and the reaction was stirred for 15 min. Saturated aqueous $NaHCO_3$ solution was carefully added to the reaction mixture, which was then extracted with DCM (3x). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification using reversed-phase HPLC provided 13 mg (67%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.39 (s, 1H), 8.32 (s, 1H), 7.63 (s, 2H), 7.35 (dd, J = 8.5, 5.4 Hz, 2H), 7.17 (dd, J = 8.6, 8.6 Hz, 2H), 4.42 (q, J = 7.1 Hz, 2H), 3.68-3.61 (m, 2H), 3.55 (s, 2H), 2.65 (d, J = 10.8 Hz, 2H), 1.84 (dd, J = 10.6, 10.6 Hz, 2H), 1.31 (t, J = 7.1 Hz, 3H), 1.12 (d, J = 6.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3, 160.5 (d, J = 247 Hz, 1C), 153.4, 141.7, 139.8, 133.7, 131.1 (d, J = 3 Hz, 1C), 129.8 (d, J = 8 Hz, 2C), 126.6, 125.7, 121.6, 114.0 (d, J = 21 Hz, 2C), 112.8, 104.6, 69.7 (2C), 59.1, 57.7, 57.3, 51.5, 17.2 (2C), 12.6. HRMS (ESI) calculated for $C_{25}H_{27}FN_2O_4$ [M]: 438.1155; found: 438.1158. LCMS: R_T = 0.877 min, ES-MS m/z = 439.4 $[M+H]^+$.

7-((*cis*-2,6-Dimethylmorpholino)methyl)-1-(4-fluorophenyl)-4-oxo-4*H*-quinolizine-3-carboxamide (106)

A solution of ethyl 7-((cis-2,6-dimethylmorpholino)methyl)-1-(4-fluorophenyl)-4-oxo-4*H*-quinolizine-3-carboxylate (13 mg, 0.03 mmol, 1.0 eq) in 7M ammonia in MeOH (2.0 mL) was subjected to a microwave reactor at 150 ºC for 2 hours. The solvent was removed *in vacuo*. Purification of the residue using reversed-phase HPLC provided 7.5 mg (62%) of the title compound. ^{1}H NMR (400 MHz, MeOD) δ 9.38 (s, 1H), 8.47 (s, 1H), 7.84 (dd, J = 9.2 Hz, 1H), 7.79 (d, J = 9.2 Hz, 1H), 7.49 (dd, J = 8.4, 5.5 Hz, 2H), 7.28 (dd, J = 8.7, 8.7 Hz, 2H), 3.75-3.69 (m, 4H), 2.81 (d, J = 11 Hz, 2H), 1.89 (dd, J = 10.7, 10.7 Hz, 2H), 1.13 (d, J = 6.3 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.1, 160.5 (d, J = 244 Hz, 1C), 157.3, 149.1, 143.2, 139.7, 138.3, 135.7, 133.5, 131.7 (d, J = 8 Hz, 2C), 128.5 (d, J = 3 Hz, 1C), 127.9, 124.1, 123.6, 115.5 (d, J = 21 Hz, 2C), 114.8, 107.1, 71.6, 59.0, 17.8 (2C). HRMS (ESI) calculated for $C_{23}H_{24}FN_3O_3$ [M]: 409.1802; found: 409.1804. LCMS: R_T = 0.719 min, ES-MS m/z = 410.4 $[M+H]^+$.

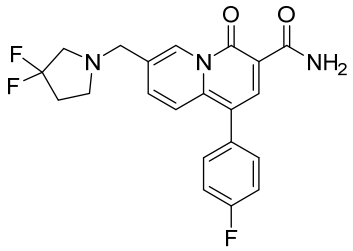
*The following compounds were prepared analogous to compound **106**.*

7-((1,4-Thiazepan-4-yl)methyl)-1-(4-fluorophenyl)-4-oxo-4*H*-quinolizine-3-carboxamide (107)



LCMS: R_T = 0.675 min, ES-MS m/z = 412.3 $[M+H]^+$.

7-((3,3-Difluoropyrrolidin-1-yl)methyl)-1-(4-fluorophenyl)-4-oxo-4*H*-quinolizine-3-carboxamide (108)



LCMS: R_T = 0.718 min, ES-MS *m/z* = 352.3 $[M+H]^+$.

Molecular Pharmacology Methods

$mGlu_2$ and $mGlu_3$ Ca^{2+} flux assays (CRC format)

$G_{\alpha 15}$ HEK293 cells stably expressing rat $mGlu_2$ or $G_{\alpha 15}$/TREx cells stably expressing rat $mGlu_3$ were plated in black-walled, clear-bottomed, poly-D-lysine coated 384-well plates in 20 µL of assay medium (For $mGlu_2$ assay: DMEM containing 10% dialyzed FBS, 20 mM HEPES, and 1 mM sodium pyruvate; For $mGlu_3$ assay: DMEM containing 10% dialyzed FBS, 20 mM HEPES, 25 ng/mL tetracycline, and 1 mM sodium pyruvate) at a density of 12K cells/well ($mGlu_2$) or 20K cells/well ($mGlu_3$). The cells were grown overnight at 37 ºC in the presence of 5% CO_2. The next day, medium was removed and the cells incubated with 20 µL of 2.3 µM Fluo-4 AM prepared as a 2.3 mM stock in DMSO and mixed in a 1:1 ratio with 10% (w/v) pluronic acid F-127 and diluted in assay buffer (Hank's balanced salt solution, 20 mM HEPES, and 2.5 mM probenecid) for 45 minutes at 37 ºC ($mGlu_2$) or 60 minutes at room temperature ($mGlu_3$). Dye was removed, 20 µL of assay buffer was added, and the plate was incubated for 5 ($mGlu_2$) or 10 ($mGlu_3$) minutes at room temperature.

Ca^{2+} flux was measured using the Functional Drug Screening System (FDSS7000, Hamamatsu, Japan). After establishment of a fluorescence baseline for about 3 seconds, the test

compounds were added to the cells, and the response in cells was measured. 2.3 minutes later an EC_{20} concentration of the $mGlu_{2/3}$ receptor agonist glutamate was added to the cells, and the response of the cells was measured for 1.9 minutes; an EC_{80} concentration of agonist was added and readings taken for an additional 1.7 minutes. All test compounds were dissolved and diluted to a concentration of 10 mM in 100% DMSO. Compounds were then serially diluted 1:3 in DMSO into 10 point concentration response curves, transferred to daughter plates, and further diluted into assay buffer to a 2x stock. Calcium fluorescence measures were recorded as fold over basal fluorescence; raw data was then normalized to the maximal response to glutamate. Antagonism of the agonist response of the $mGlu_2$ or $mGlu_3$ receptor was observed as a decrease in response to nearly maximal concentrations of glutamate in the presence of compound compared to the response to glutamate in the absence of compound.

The raw data file containing all time points was used as the data source in the analysis template. This was saved by the FDSS as a tab-delimited text file. Data were normalized using a static ratio function (F/F_0) for each measurement of the total 360 values per well divided by each well's initial value. Data were then reduced to peak amplitudes (Max – Initial Min) using a time range that starts approximately 3 seconds prior to the glutamate EC_{80} addition and continues for approximately 90 seconds. This is sufficient time to capture the peak amplitude of the cellular calcium response. Individual amplitudes were expressed as % EC_{Max} by multiplying each amplitude by 100 and then dividing the product by the mean of the amplitudes derived from the glutamate EC_{Max}-treated wells. IC_{50} values for test compounds were generated by fitting the normalized values versus the log of the test compound concentration (in mol/L) using a 4 parameter logistic equation where none of the parameters were fixed. Each of the three values collected at each concentration of test compound were weighted evenly.

A compound was designated as a negative allosteric modulator (NAM) if the compound showed a concentration-dependent decrease in the glutamate EC_{80} addition. For NAMs, potency (IC_{50}) and maximum response (% Glu Max), i.e. the amplitude of response in the presence of 30 μM test compound as a percentage of the maximal response to glutamate, are reported. For NAMs that show a decrease in the EC_{80} response, but do not hit a plateau, the average of the maximum response at a single concentration (30 μM) was determined (% Glu Max) and potencies were reported as ">10,000 nM". Compounds with no measurable activity are designated as ">30,000 nM" since the top concentration of compound tested in the assay is 30 μM.

$mGlu_1$ and $mGlu_5$ fold-shift selectivity assays

Human $mGlu_1$ TREx293 cells or rat $mGlu_5$ HEK293 cells were plated in black-walled, clear-bottomed, poly-D-lysine coated 384-well plates (BD Biosciences, San Jose, CA) at a density of 20,000 cells/well in 20 μL of assay medium (DMEM supplemented with 10% dialyzed FBS, 20 mM HEPES, and 1 mM sodium pyruvate) containing tetracycline (TET) to induce the $mGlu_1$ expression; 50ng/mL TET was used. The cells were grown overnight at 37 ºC in the presence of 5% CO_2. The next day, cells were washed with assay buffer (Hank's balanced salt solution, 20 mM HEPES, and 2.5 mM probenecid (Sigma-Aldrich, St. Louis, MO)) using an ELX405 microplate washer (BioTek) leaving 20 μL/well. Immediately cells were incubated with 20 μl/well of Fluo-4 AM (Invitrogen) calcium indicator dye solution (1.15 μM final concentration) for 45 m at 37 ºC. The Fluo-4 dye prepared as a DMSO stock, was mixed in a 1:1 ratio with 10% pluronic acid F-127 and then diluted in assay buffer. The dye was then removed and washed with assay buffer using an ELX405, leaving 20 μL/well. Ca^{2+} flux was measured using the Functional

Drug Screening System (FDSS7000, Hamamatsu, Japan). Compounds were diluted into assay buffer to a 2x stock which was applied to cells at t = 3 s. Cells were incubated with the test compounds for 2.3 minutes and then stimulated with varying concentrations of glutamate, and readings taken for an additional 2.6 minutes. Data were collected at 1 Hz. Concentration response curves were generated using a four point logistical equation with XLfit curve fitting software for Excel (IDBS, Guildford, U.K.) or GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

$mGlu_{4/6/7/8}$ fold-shift selectivity assay

Compound **58** activity at the group III mGlu receptors was assessed using thallium flux through G-protein-coupled inwardly rectifying potassium (GIRK) channels, a method that has been described in detail (Niswender et al. *Mol. Pharmacol.* 2008, reference 59). These cell lines were grown in growth media containing 45% DMEM, 45% F-12, 10% FBS, 20 mM HEPES, 2 mM L-glutamine, antibiotic/antimycotic, nonessential amino acids, 700 μg/mL G418, and 0.6 μg/mL puromycin at 37 ºC in the presence of 5% CO_2. Briefly, HEK/GIRK cells expressing rat $mGlu_4$, human $mGlu_6$, rat $mGlu_7$, or rat $mGlu_8$ were plated into 384 well, black-walled, clear-bottom poly-D-lysine coated plates at a density of 15,000 cells/20 μL/well in assay medium and incubated overnight at 37 ºC in the presence of 5% CO_2. The following day, the medium from the cells and 20 μL/well of 1.7 μM concentration of the indicator dye BTC-AM (Invitrogen, Carlsbad, CA) in assay buffer was added. Cells were incubated for 1 hour at room temperature and the dye was replaced with 20 μL/well of assay buffer. After establishment of a fluorescence baseline for about 3 seconds, test compound was added to the cells at 2x final concentration, and the response in cells was measured. 2.3 min later the appropriate concentration of agonist (L-

AP4 for $mGlu_7$, glutamate for all other mGlu receptors) was added and readings taken for an additional 2.6 minutes. Agonists were diluted in thallium buffer (125 mM sodium bicarbonate, 1 mM magnesium sulfate, 1.8 mM calcium sulfate, 5 mM glucose, 12 mM thallium sulfate, 10 mM HEPES) at 5x the final concentration to be assayed. Data were analyzed as described in Niswender et al. *Mol. Pharmacol.* 2008 (reference 59).

M_1 Ca^{2+} flux assay

CHO-K1 cells stably expressing human M_1 receptors were maintained in F-12 supplement (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (Invitrogen), antibiotic/antimycotic (Invitrogen), 20 mM HEPES (Invitrogen), and 500 μg/mL G418 (Mediatech, Manassas, VA) at 37 ºC in the presence of 5% CO_2. Cells were plated at 15,000 cells/20 μL/well in black-walled, clear-bottomed, tissue culture-treated, 384-well plates (Greiner Bio-One, Monroe, NC) in growth media lacking selection antibiotics; cells were grown overnight. The next day, media were washed off and replaced with 20 μL of assay buffer (Hanks' balanced salt solution (Invitrogen), 20 mM HEPES, and 2.5 mM probenecid (Sigma, St. Louis, MO). Then, 2.3 mM Fluo-4 AM calcium dye (Invitrogen) was prepared in DMSO, mixed with equal amounts of 10% (w/v) pluronic acid (Invitrogen), and then diluted in assay buffer 2× the final concentration. Twenty microliters of dye solution was added to cells for a final concentration of 1 μM. Cells were incubated with dye at 37 ºC for 1 h; at 10 min prior to assay, cells were thoroughly washed with assay buffer and incubated with 20 μL of assay buffer at 37 °C. Compound **58** was serially diluted 1:3 into 11-point concentration−response curves in DMSO and transferred to daughter plates by an Echo acoustic plate reformatter (Labcyte, Sunnyvale, CA) and diluted to a 2× final concentration in assay buffer. To measure Ca^{2+} flux, cell plates were loaded onto a Functional

Drug Screening System 6000 (FDSS6000; Hamamatsu Photonics Systems, Hamamatsu, Japan). Baseline fluorescence was established over 3 s (1 Hz; excitation, 470 ±20 nm; emission, 540 ± 30 nm), followed immediately be the addition of 20 µL test compound serial dilutions to the cells; the response was measured for 140 s to determine agonist activity. Immediately afterward, 10 µL of an EC_{20} concentration of acetylcholine (5× final concentration) was added to cells; the response was measured for 86 s to determine PAM activity. Then, 12 µL of an EC_{80} concentration of acetylcholine (5× final concentration) was immediately added to cells; the response was measured for 70 s to determine NAM or antagonist activity. Fluorescence was recorded as fold over basal fluorescence within the same well. Calcium fluorescence traces were reduced to maximal response to acetylcholine per assay window. Potency and maximum response (percentage of 10 µM acetylcholine) for compound were determined using a four-parameter logistical equation in XlFit (ID Business Solutions, Guildford, UK), plugin for Excel (Microsoft, Redmond, WA).

***In-Vitro* DMPK Methods**

Intrinsic clearance in rat liver microsomes

Rat liver microsomes (0.5 mg/mL) and 1 µM test compound were incubated in 100 mM potassium phosphate pH 7.4 buffer with 3 mM $MgCl_2$ at 37 ºC with constant shaking. After a 5 min preincubation, the reaction was initiated by addition of NADPH (1 mM). At selected time intervals (0, 3, 7, 15, 25, and 45 min), 50 µL aliquots were taken and subsequently placed into a 96-well plate containing 150 µL of cold acetonitrile with internal standard (50 ng/mL carbamazepine). Plates were then centrifuged at 3000 rcf (4 ºC) for 10 min, and the supernatant was transferred to a separate 96-well plate and diluted 1:1 with water for LC/MS/MS analysis.

The *in vitro* half-life ($T_{1/2}$, min, Eq. 1), intrinsic clearance (CL_{int}, mL/min/kg, Eq. 2) and subsequent predicted hepatic clearance (CL_{hep}, mL/min/kg, Eq. 3) were determined employing the following equations:

(1) $$T_{1/2} = \frac{Ln(2)}{k}$$

where k represents the slope from linear regression analysis of the natural log percent remaining of test compound as a function of incubation time

(2) $$CL_{\text{int}} = \frac{0.693}{in\,vitro\,T_{1/2}} x \frac{mL\ incubation}{mg\ microsomes} x \frac{45\,mg\,microsomes}{gram\ liver} x \frac{45^{a}\ gram\ liver}{kg\ body\ wt}$$

[a] scale-up factor of 45 for rat

(3) $$CL_{hep} = \frac{Q_h \cdot CL\,\text{int}}{Q_h + CL\,\text{int}}$$

where Q_h (hepatic blood flow, mL/min/kg) is 70 for the rat.

Plasma Protein Binding

The protein binding of each compound was determined in rat plasma via equilibrium dialysis employing HTDialysis Teflon dialysis chamber and cellulose membranes (MWCO 12-14 K) (HTDialysis LLC, Gales Ferry, CT). Plasma was added to the 96 well plate containing test compound and mixed thoroughly for a final concentration of 5 µM. Subsequently, 150 µL of the plasma-compound mixture was transferred to the dialysis chamber, with an accompanying 150 µL of phosphate buffer (25 mM, pH 7.4) on the other side of the membrane. The device plate was sealed and incubated for 4 hours at 37 ºC with shaking. At completion, aliquots from each chamber were diluted 1:1 with either plasma (for the buffer sample) or buffer (for the plasma sample) and transferred to a new 96 well plate, at which time ice-cold acetonitrile containing

internal standard (50 ng/mL carbamazepine) (2 volumes) was added to extract the matrices. The plate was centrifuged (3000 rcf, 10 min) and supernatants transferred and diluted 1:1 (supernatant: water) into a new 96 well plate, which was then sealed in preparation for LC/MS/MS analysis. Each compound was assayed in triplicate within the same 96-well plate. Fraction unbound was determined using the following equation:

$$f_u = \frac{Conc_{buffer}}{Conc_{plasma}}$$

Brain Homogenate Binding

The brain homogenate binding of each compound was determined in brain homogenate via equilibrium dialysis employing HTDialysis Teflon dialysis chamber and cellulose membranes (MWCO 12-14 K) (HTDialysis LLC, Gales Ferry, CT). Brain tissue homogenate was prepared by diluting one volume whole rat brain tissue with three volumes of phosphate buffer (25 mM, pH 7.4). The mixture was then subjected to mechanical homogenation employing a Mini-Beadbeater™ and 1.0 mm Zirconia/Silica Beads (BioSpec Products). Brain homogenate spiked with test compound and mixed thoroughly for a final concentration of 5 µM. Subsequently, 150 µL of the brain homogenate-compound mixture was transferred to the dialysis chamber with an accompanying 150 µL of phosphate buffer (25 mM, pH 7.4) on the other side of the membrane. The block was sealed and incubated for 6 hours at 37 ºC with shaking. At completion, aliquots from each side of the chamber were diluted 1:1 with either brain homogenate (to the buffer side) or buffer (to the brain homogenate side) in a new 96 well plate, at which time ice-cold acetonitrile containing internal standard (50 ng/mL carbamazepine) was added to extract the matrices. The plate was centrifuged (3000 rcf, 10 min) and supernatants transferred and diluted

1:1 (supernatant: water) into a new 96 well plate, which was then sealed in preparation for LC/MS/MS analysis. Each compound was assayed in triplicate within the same 96-well plate. Fraction unbound was determined using the following equation:

$$f_{u,tissue} = \frac{1/D_f}{(1/f_{u,\text{hom}} - 1) + 1/D_f}$$

Where $f_{u,hom}$ represent the measured fraction unbound in the diluted homogenate and D_f represents dilution factor

LC/MS/MS Bioanalysis of Samples from *In Vitro* Assays

Samples were analyzed on a Thermo Electron TSQ Quantum Ultra triple quad mass spectrometer (San Jose, CA) with electrospray ionization (ESI), Shimadzu LC-10ADvp pumps (Columbia, MD), and a Leap Technologies CTC PAL autosampler (Carrboro, NC). Analytes were separated by gradient elution using Fortis C18 (3.0 x 50 mm, 3 µm) columns (Fortis Technologies Ltd, Cheshire, UK) thermostated at 40 ºC. HPLC mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. The gradient started at 30% B after a 0.2 min hold and was linearly increased to 95% B over 0.8 min; hold at 95% B for 0.2 min; returned to 30% B in 0.1 min. The total run time was 1.3 min and the HPLC flow rate was 0.5 mL/min. Compound optimization, data collection and processing was performed using Thermo Electron's QuickQuan software (v2.3) and Xcalibur (v2.0.7 SP1).

P-gp Substrate Assessment Using MDR1-MDCK Cell Monolayers

This study was run by Absorptions Systems®, 436 Creamery Way, Suite 600, Exton, PA 19341 (http://www.absorption.com/). Briefly, MDR1-MDCK cell monolayers were grown to

confluence on collagen-coated, microporous, polycarbonate membranes in 12-well Costar Transwell plates. Details of the plates and their certification are shown below. The permeability assay buffer was Hanks Balanced Salt Solution (HBSS) containing 10 mM HEPES and 15 mM glucose at a pH of 7.4. The buffer in the receiver chamber also contained 1% bovine serum albumin. The dosing solution concentration was 5 µM test article in the assay buffer +/- 1 µM valspodar. Cells were first pre-incubated for 30 minutes with HBSS +/- 1 µM valspodar. Cell monolayers were then dosed on the apical side (A-to-B) or basolateral side (B-to-A) and incubated at 37 ºC with 5% CO_2 in a humidified incubator. Samples were taken from the donor and receiver chambers at 120 minutes. Each determination was performed in duplicate. The flux of co-dosed lucifer yellow was also measured for each monolayer to ensure no damage was inflicted to the cell monolayers during the flux period. All samples were assayed by LC-MS/MS using electrospray ionization. Liquid chromatography was carried out on a Waters ACQUITY UPLC BEH Phenyl 30 x 2.1 mm, 1.7 µm column using an M.P. buffer of 25 mM ammonium formate (pH 3.5), an aqueous reservoir (A) of 90% water and 10% buffer, and an organic reservoir (B) of 90% acetonitrile and 10% buffer with a flow rate of 0.7 mL/min. The gradient program was run from 100% (A) to 100% (B) with a total run time of 1.0 min. Injection volume was 0.2 µL. The mass spectrometer was a PE SCIEX API 4000 with a turbospray interface and was run in multiple reaction monitoring mode. The apparent permeability (Papp) and percent recovery were calculated as follows:

(1) $P_{app} = (dC_r/dt) \times V_r/(A \times C_A)$

Percent recovery = $100 \times ((V_r \times C_r^{final}) + (V_d \times C_d^{final}))/(V_d \times C_N)$

Where,

dC_r/dt is the slope of the cumulative concentration in the receiver compartment versus time in $\mu M \cdot s^{-1}$;

V_r is the volume of the receiver compartment in cm^3;

V_d is the volume of the donor compartment in cm^3;

A is the area of the insert (1.13 cm^2 for 12-well Transwell);

C_A is the average of the nominal dosing concentration and the measured 120 minute donor concentration in μM;

C_N is the nominal concentration of the dosing solution in μM;

C_r^{final} is the cumulative receiver concentration in μM at the end of the incubation period;

C_d^{final} is the concentration of the donor in μM at the end of the incubation period.

Efflux ratio (ER) is defined as P_{app} (B-to-A) / P_{app} (A-to-B)

***In-Vivo* PK Methods**

All rodent PK experiments were conducted in accordance with the National Institute of Health regulations of animal care covered in Principles of Laboratory Animal Care (National Institutes of Health publication 85-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee.

Rodent IV Cassette PK Studies

IV cassette PK experiments in rats were carried out according to methods described previously (Bridges et al. *Pharmacol. Res. Perspect.* 2014; reference 55). Briefly, A cassette of compounds (n = 4–5/cassette) were formulated from 10 mM solutions of compounds in DMSO. In order to reduce the absolute volume of DMSO that was administered, the compounds were

combined and diluted with ethanol and PEG 400 to achieve a final concentration of 0.4–0.5 mg/mL for each compound (2 mg/mL total) administered in each cassette. The final dosing solutions consisted of approximately 10% ethanol, 40% PEG400, and 50% DMSO (v/v). Each cassette dose was administered IV via the jugular vein to two dual-cannulated (carotid artery and jugular vein) adult male Sprague–Dawley rats, each weighing between 250 and 350 g (Harlan, Indianapolis, IN) for a final dose of 0.2–0.25 mg/kg per compound. Whole blood collections via the carotid artery were performed at 0.033, 0.117, 0.25, 0.5, 1, 2, 4, 7, and 24 hours post dose. For tissue distribution studies, animals were euthanized and decapitated at 0.25 hours post dose, and the brains were removed, thoroughly washed in cold phosphate-buffered saline, and immediately frozen on dry ice.

Rodent IP Tissue Distribution Studies

Single time point IP tissue distribution experiments in rodents were carried out according to methods described previously (Bridges et al. *Drug Metab. Dispos*. 2014; reference 63). Briefly, male Sprague–Dawley rats, each weighing between 250 and 350 g, or male CD-1 mice, each weighing between 20-30 g (Harlan, Indianapolis, IN) were dosed with test compound (IP). Formulations were a fine homogeneous suspension in 10% Tween 80 in H_2O for **58**. Animals were euthanized and decapitated, and the brains were removed, thoroughly washed in cold phosphate-buffered saline, and immediately frozen on dry ice. The blood (cardiac puncture) and brain were collected at 0.25 hours post dose. For studies in mice with **109**, compound **109** (20 mg/kg) in 10% Tween 80 in H_2O was dosed one hour prior to compound **58**.

Plasma and Brain Sample Preparation

Plasma was separated by centrifugation (4000 rcf, 4 ºC) and stored at –80 ºC until analysis. On the day of analysis, frozen whole-rat brains were weighed and diluted with 1:3 (w/w) parts of 70:30 isopropanol:water. The mixture was then subjected to mechanical homogenation employing a Mini-Beadbeater™ and 1.0 mm Zirconia/Silica Beads (BioSpec Products) followed by centrifugation. The sample extraction of plasma (20 µL) or brain homogenate (20 µL) was performed by a method based on protein precipitation using three volumes of ice-cold acetonitrile containing an internal standard (50 ng/mL carbamazepine). The samples were centrifuged (3000 rcf, 5 min) and supernatants transferred and diluted 1:1 (supernatant: water) into a new 96 well plate, which was then sealed in preparation for LC/MS/MS analysis.

LC/MS/MS Bioanalysis of Samples from *In Vivo* Assays

In vivo samples were analyzed via electrospray ionization (ESI) on an AB Sciex API-4000 (Foster City, CA) triple-quadrupole instrument that was coupled with Shimadzu LC-10AD pumps (Columbia, MD) and a Leap Technologies CTC PAL auto-sampler (Carrboro, NC). Analytes were separated by gradient elution using a Fortis C18 3.0 x 50 mm, 3 µm column (Fortis Technologies Ltd, Cheshire, UK) thermostated at 40 ºC. HPLC mobile phase A was 0.1% formic acid in water (pH unadjusted), mobile phase B was 0.1% formic acid in acetonitrile (pH unadjusted). The source temperature was set at 500 ºC and mass spectral analyses were performed using multiple reaction monitoring (MRM), with transitions specific for each compound utilizing a Turbo-Ionspray® source in positive ionization mode (5.0 kV spray voltage). The calibration curves were constructed, and linear response was obtained by spiking known amounts of test compound in blank brain homogenate or plasma. All data were analyzed

using AB Sciex Analyst software v1.5.1. The final PK parameters were calculated by noncompartmental analysis using Phoenix (version 6.2) (Pharsight Inc., Mountain View, CA).

Ancillary Pharmacology Profile of Compound 58

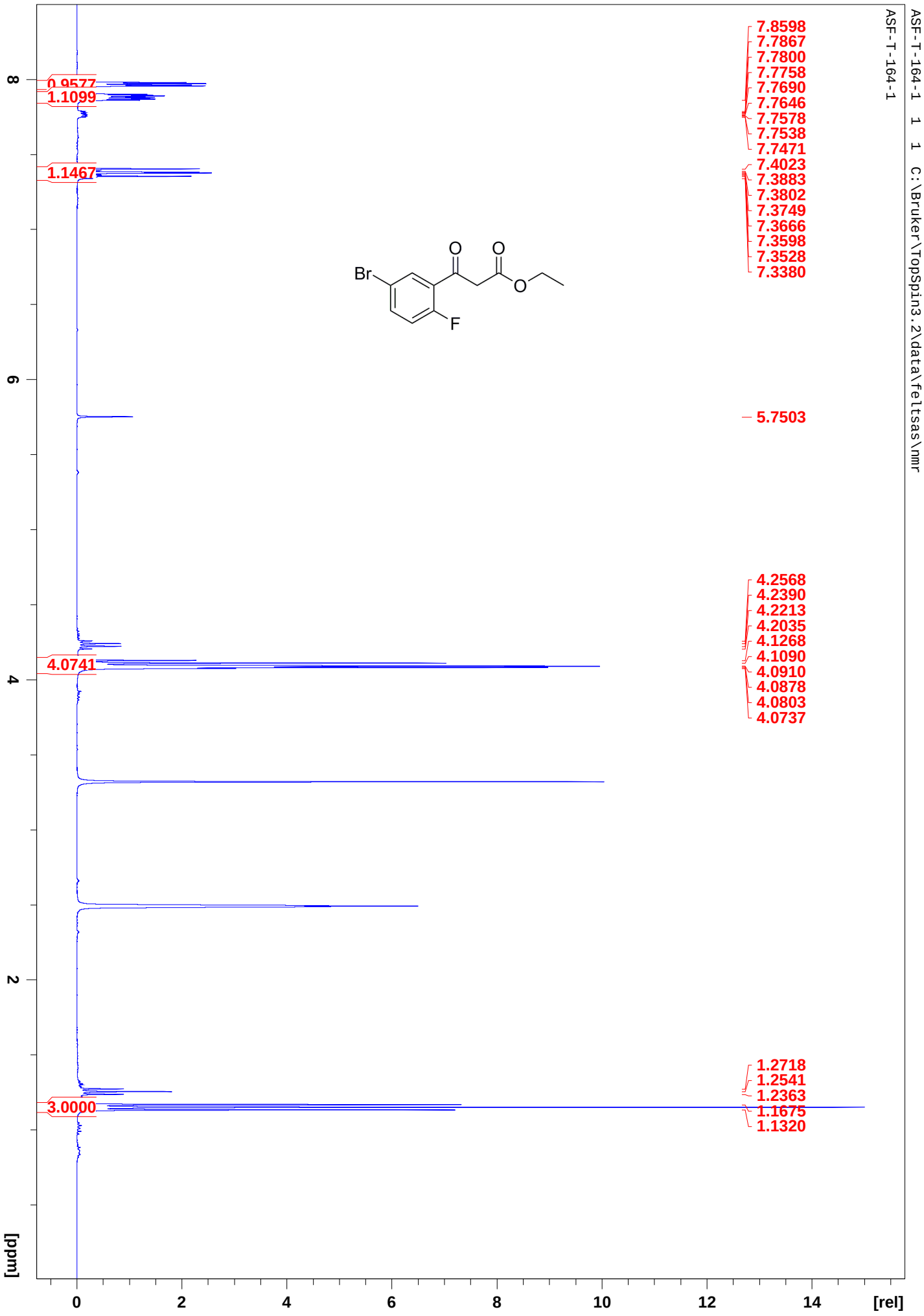
LeadProfilingScreen®, Eurofins Panlabs, Inc. (http://www.eurofinspanlabs.com)

Compound tested at 10 µM

Target	Sp	% Inh	Target	Sp	% Inh
Adenosine A_1	hum	-1	Histamine H_3	hum	-4
Adenosine A_{2A}	hum	3	Imidazoline I_2, central	rat	38
Adenosine A_3	hum	9	Interleukin IL-1	mouse	16
Adrenergic α_{1A}	rat	12	Leukotriene, cysteinyl $CysLT_1$	hum	14
Adrenergic α_{1B}	rat	6	Melatonin MT_1	hum	-1
Adrenergic α_{1D}	hum	10	Muscarinic M_1	hum	1
Adrenergic α_{2A}	hum	49	Muscarinic M_2	hum	4
Adrenergic β_1	hum	-5	Muscarinic M_3	hum	10
Adrenergic β_2	hum	1	Neuropeptide Y Y_1	hum	-1
Androgen AR	rat	6	Neuropeptide Y Y_2	hum	1
Bradykinin B_1	hum	7	Nicotinic acetylcholine	hum	5
Bradykinin B_2	hum	0	Nicotinic acetylcholine α1, bungarotoxin	hum	-3
Ca^{2+} channel L-type, benzothiazepine	rat	-6	Opiate δ_1 (OP1, DOP)	hum	3
Ca^{2+} channel L-type, dihydropyridine	rat	4	Opiate κ(OP2, KOP)	hum	-1
Ca^{2+} channel, N-type	rat	3	Opiate µ(OP3, MOP)	hum	-4
Cannabinoid CB_1	hum	-2	Phorbol ester	mouse	5
Dopamine D_1	hum	5	Platelet activating factor (PAF)	hum	27
Dopamine D_{2S}	hum	8	Potassium Channel [K_{ATP}]	ham	8
Dopamine D_3	hum	6	Potassium Channel hERG	hum	7
Dopamine $D_{4.2}$	hum	11	Prostanoid EP_4	hum	4
Endothelin ET_A	hum	4	Purinergic P_{2X}	rabbit	0
Endothelin ET_B	hum	3	Purinergic P_{2Y}	rat	9
Epidermal Growth Factor (EGF)	hum	0	Rolipram	rat	2
Estrogen ERα	hum	4	Serotonin 5-HT_{1A}	hum	-4
$GABA_A$, flunitrazepam, central	rat	6	Serotonin 5-HT_{2B}	hum	2
$GABA_A$, muscimol, central	rat	-4	Serotonin 5-HT_3	hum	13
$GABA_{B1A}$	hum	1	Sigma σ_1	hum	-6
Glucocorticoid	hum	9	Sodium channel, site 2	rat	3
Glutamate, kainate	rat	3	Tachykinin NK_1	hum	10
Glutamate, NMDA, agonism	rat	1	Thyroid hormone	rat	-6
Glutamate, NMDA, glycine	rat	-3	Transporter, dopamine (DAT)	hum	-9

Target	Sp	% Inh
Glutamate, NMDA, phencyclidine	rat	-6
Histamine H_1	hum	9
Histamine H_2	hum	18

Target	Sp	% Inh
Transporter, GABA	rat	11
Transporter, norepinephrine (NET)	hum	-2
Transporter, serotonin (SERT)	hum	14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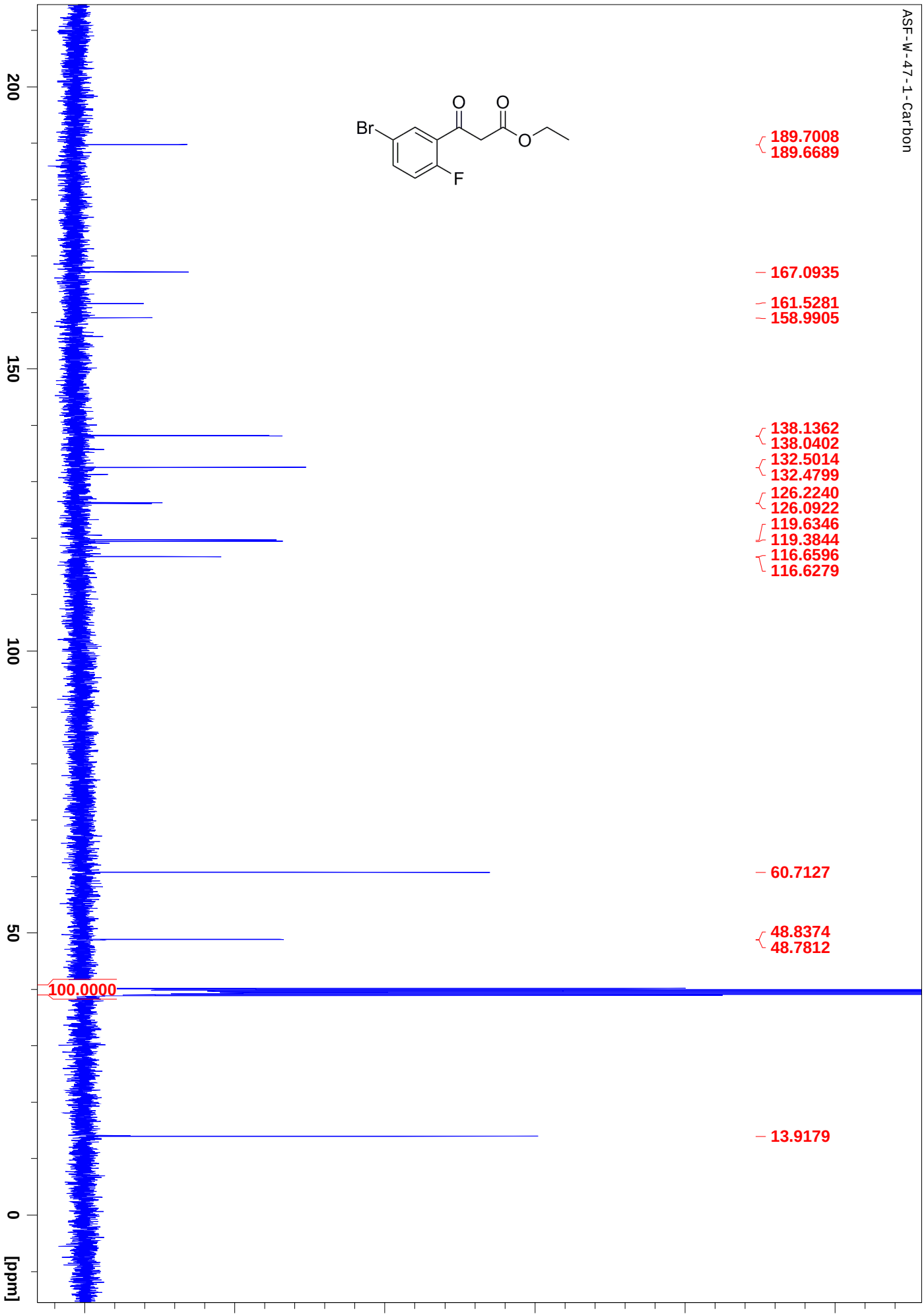
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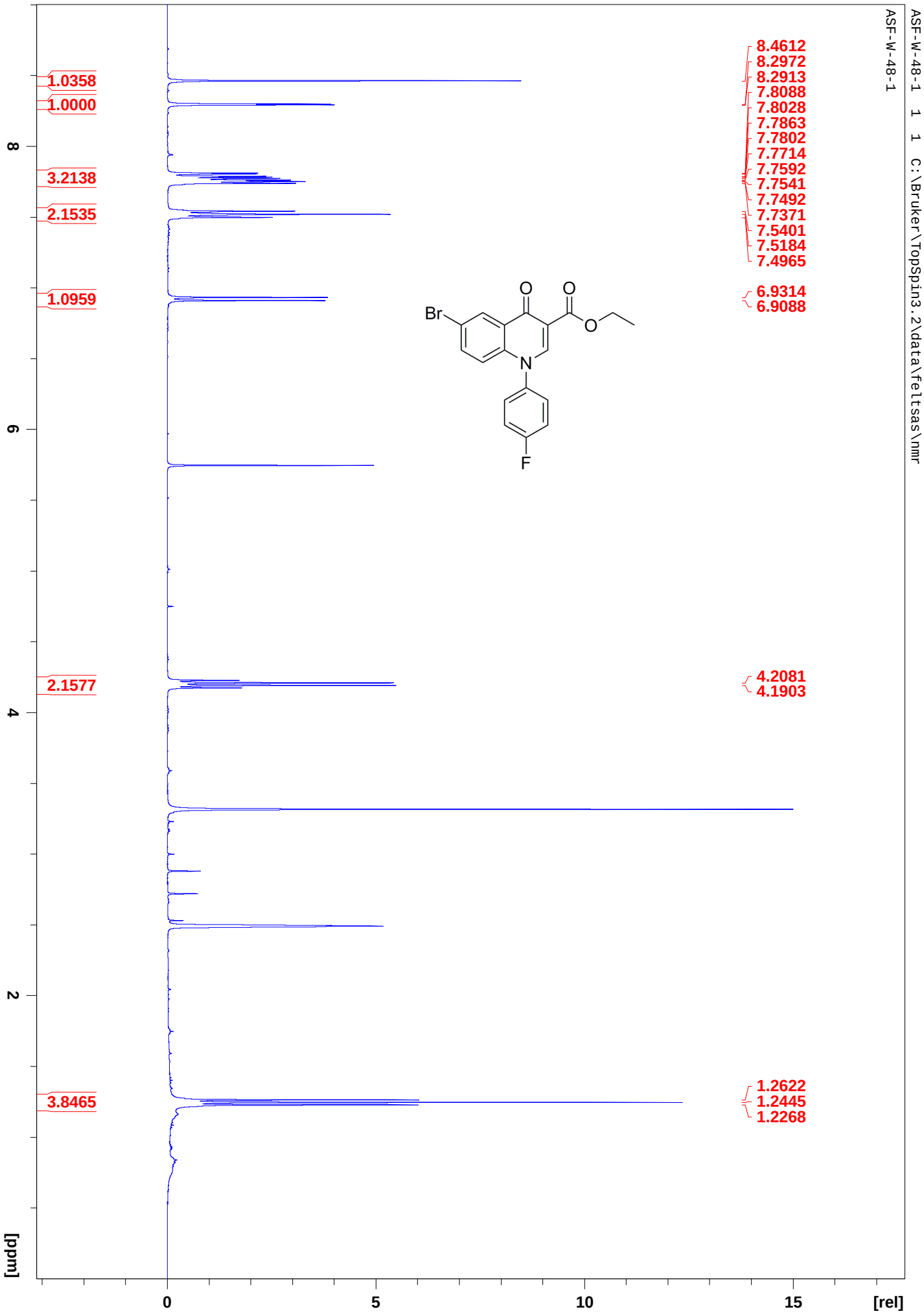
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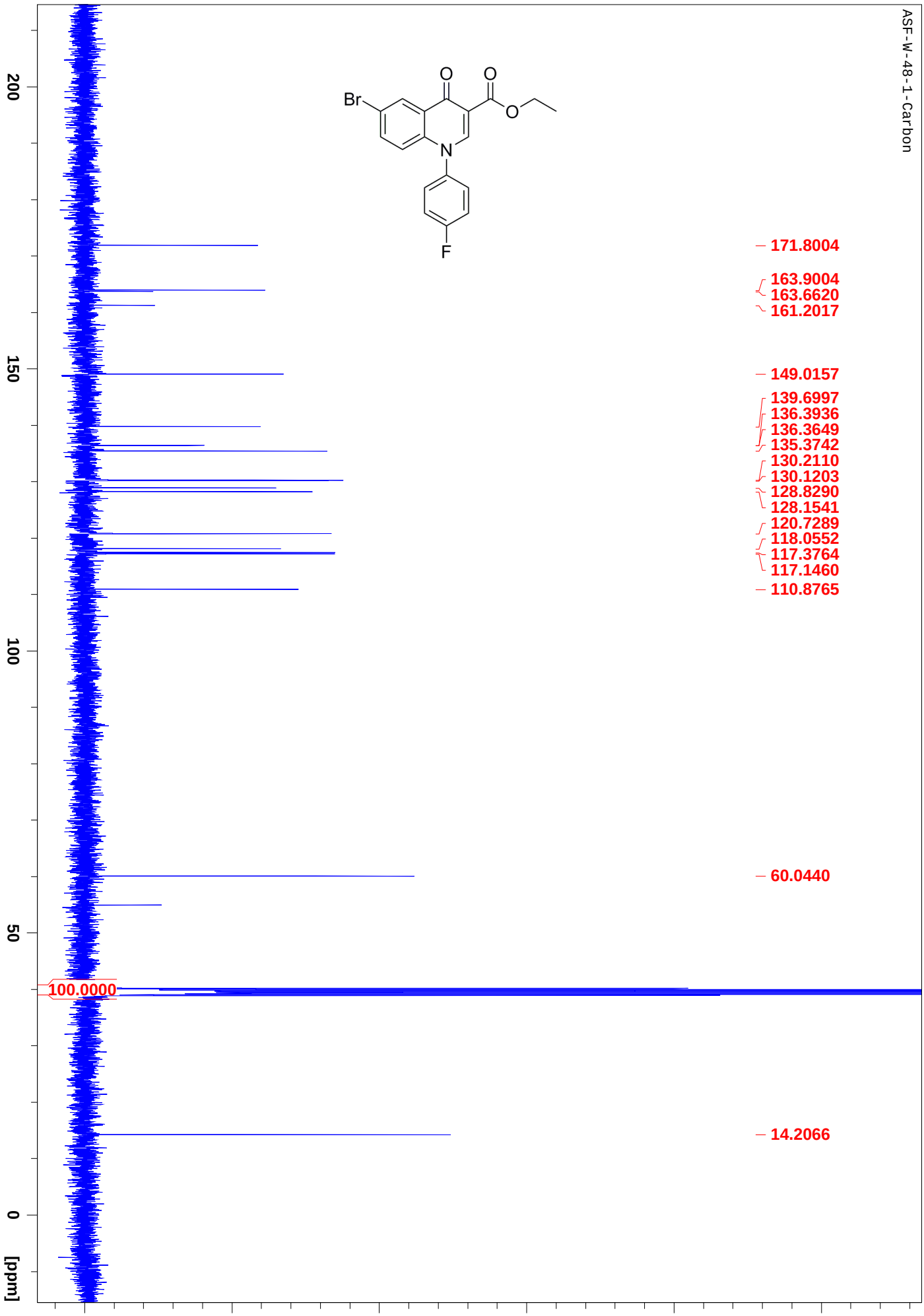
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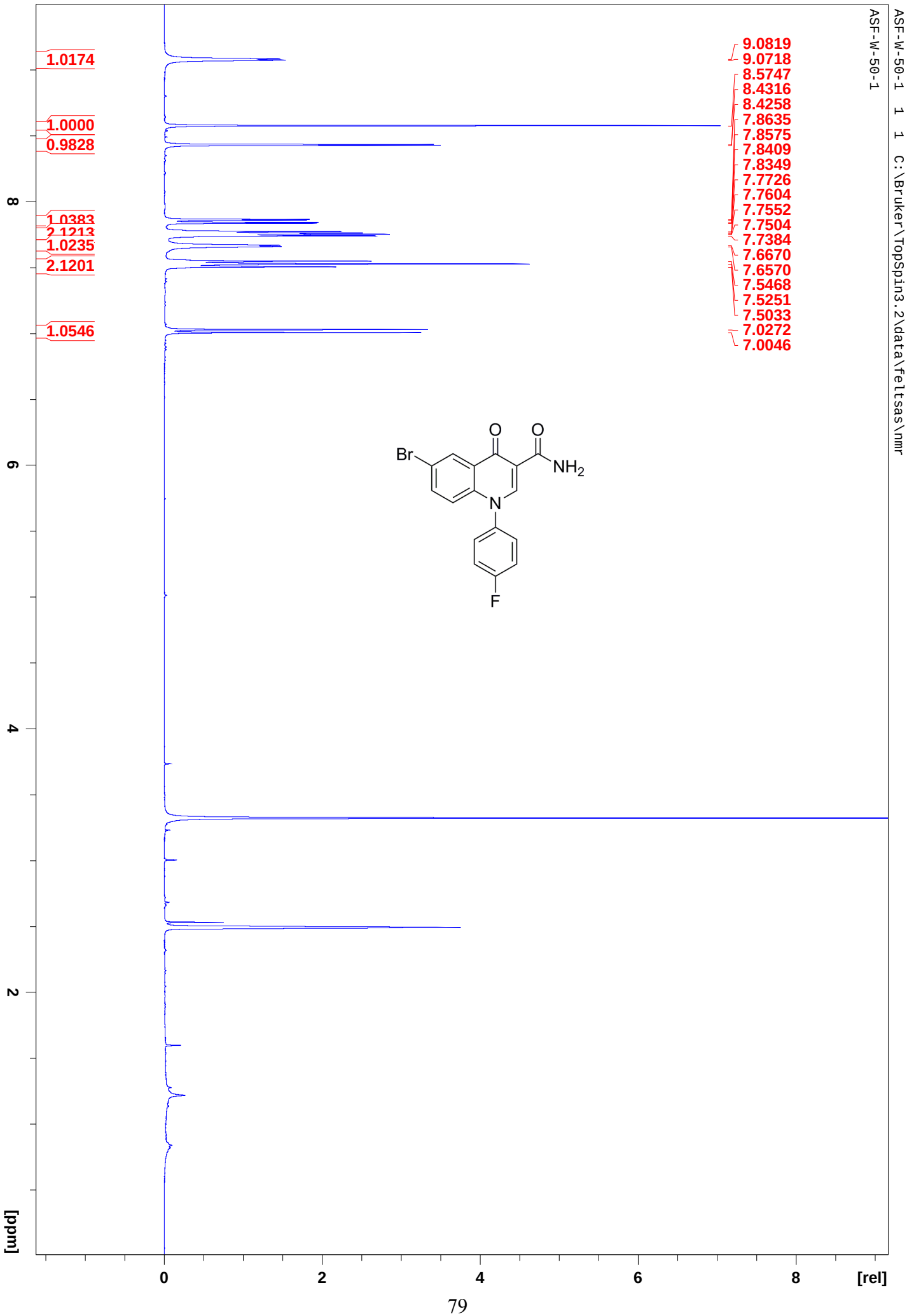
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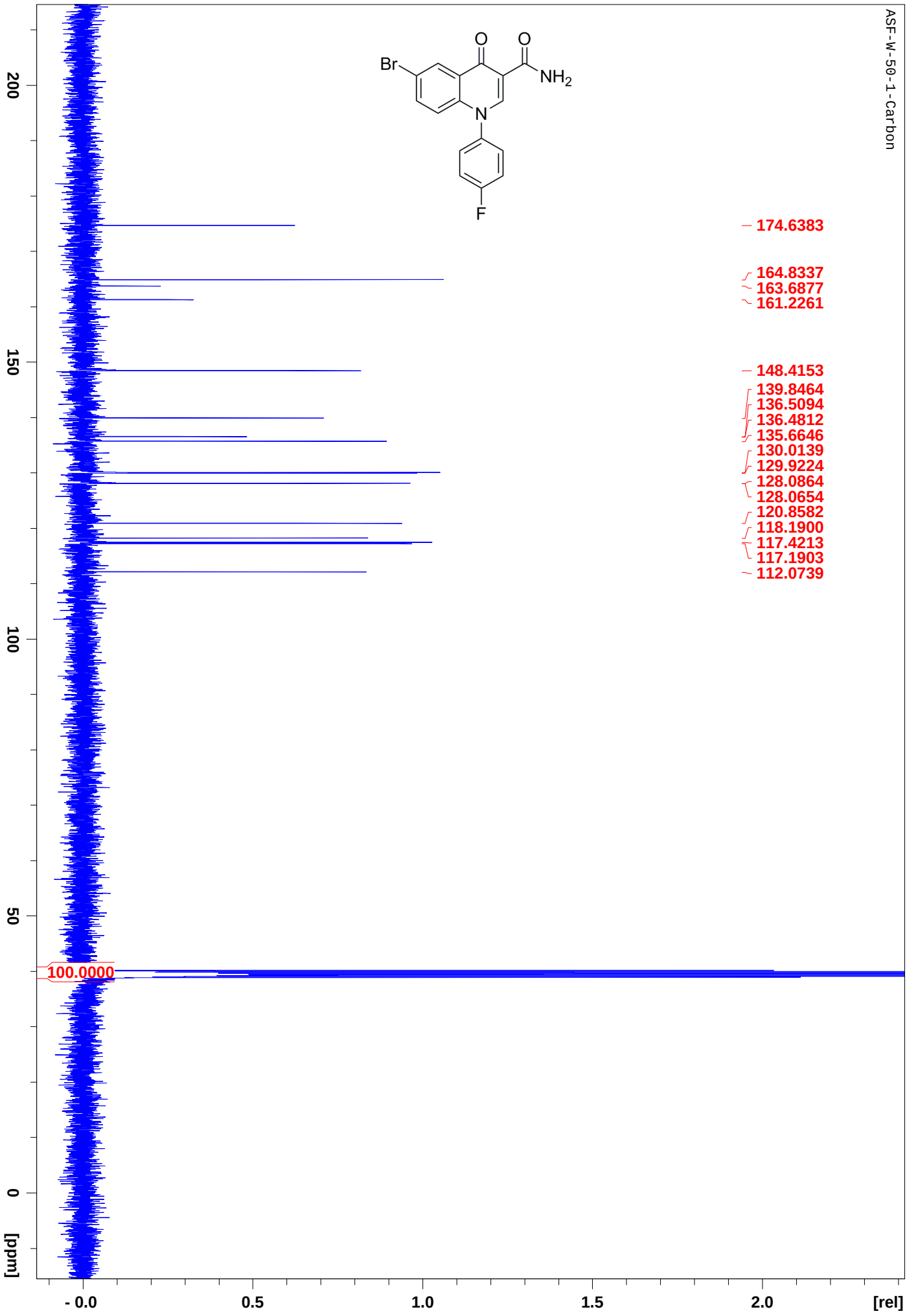
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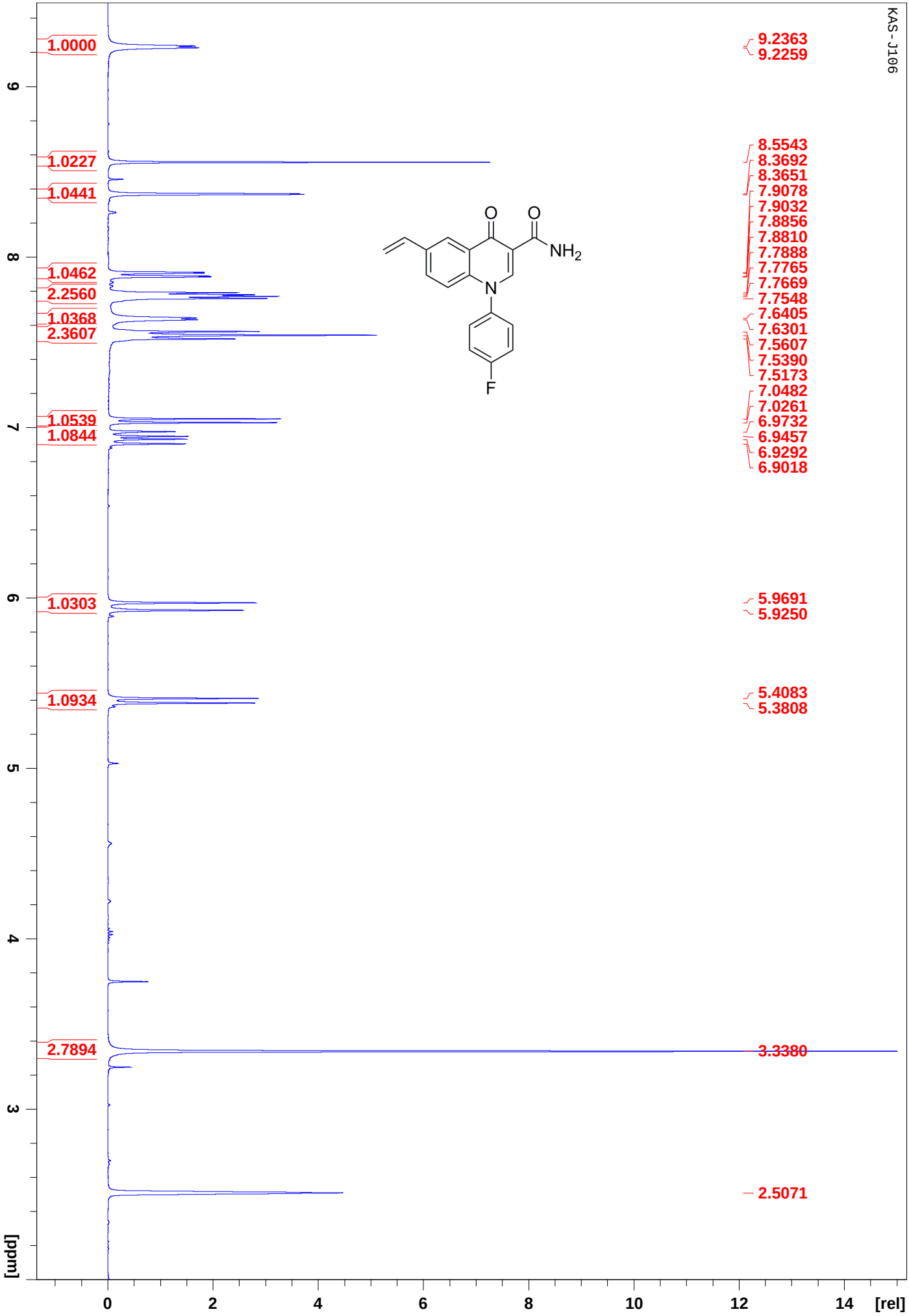
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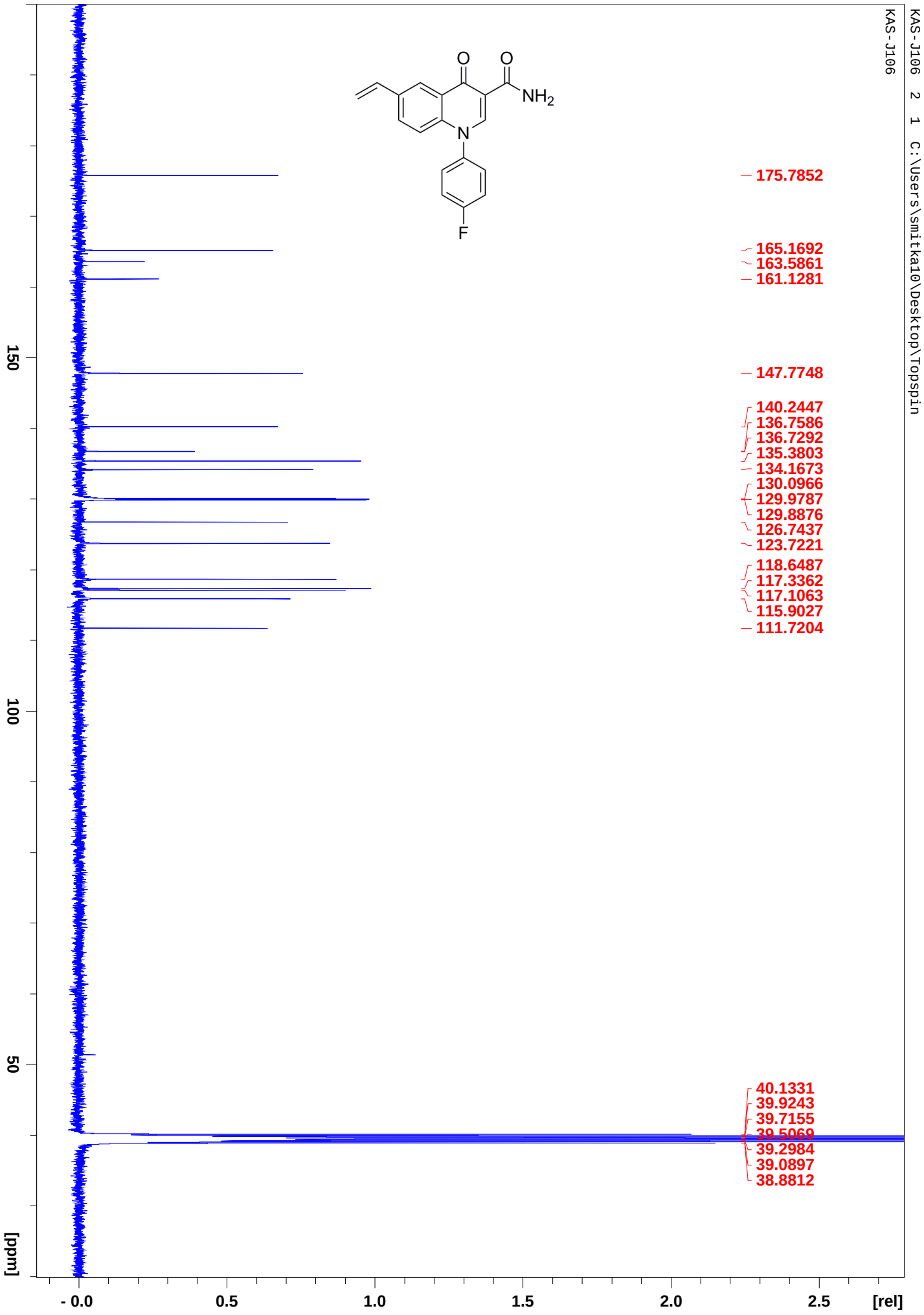
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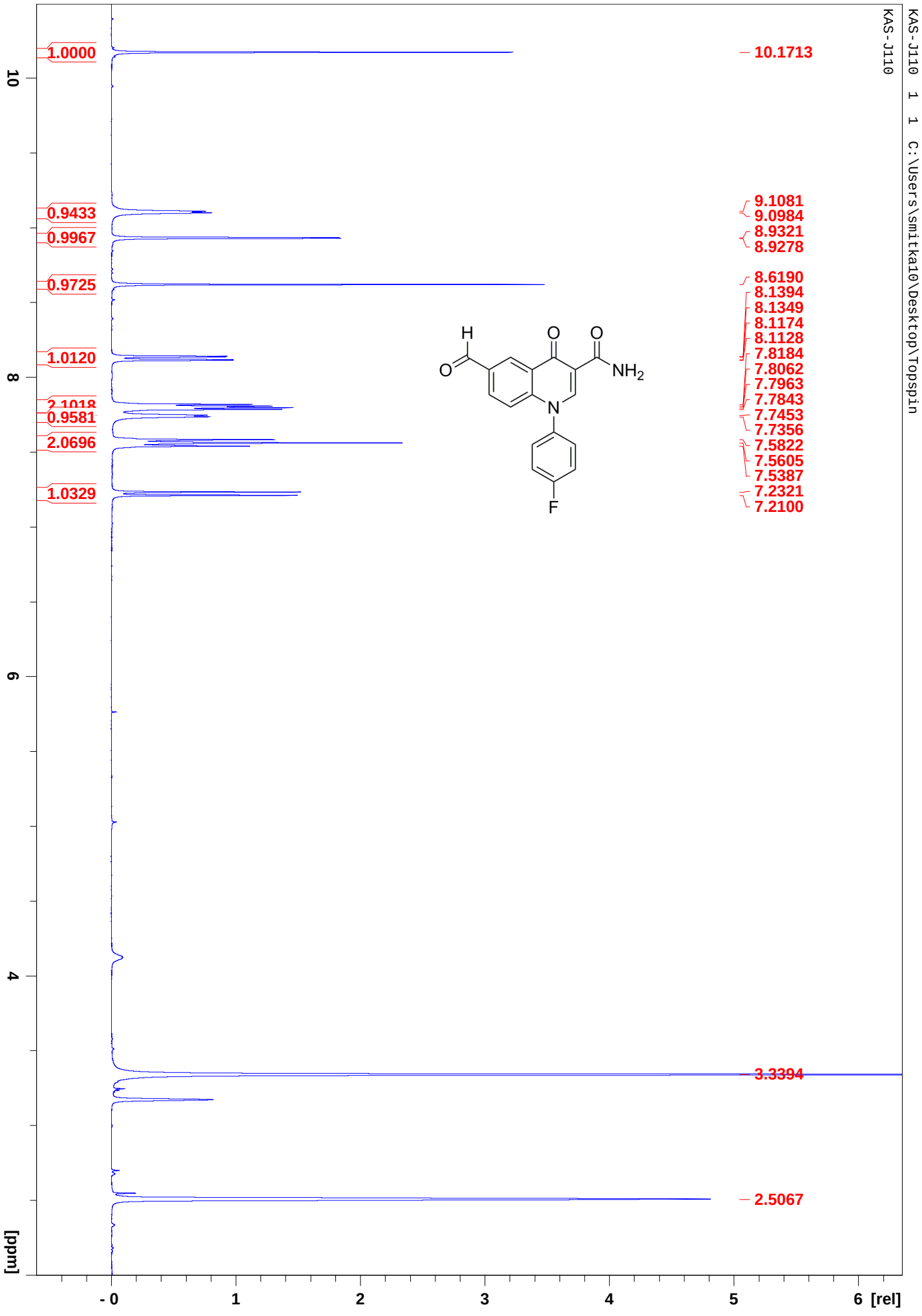
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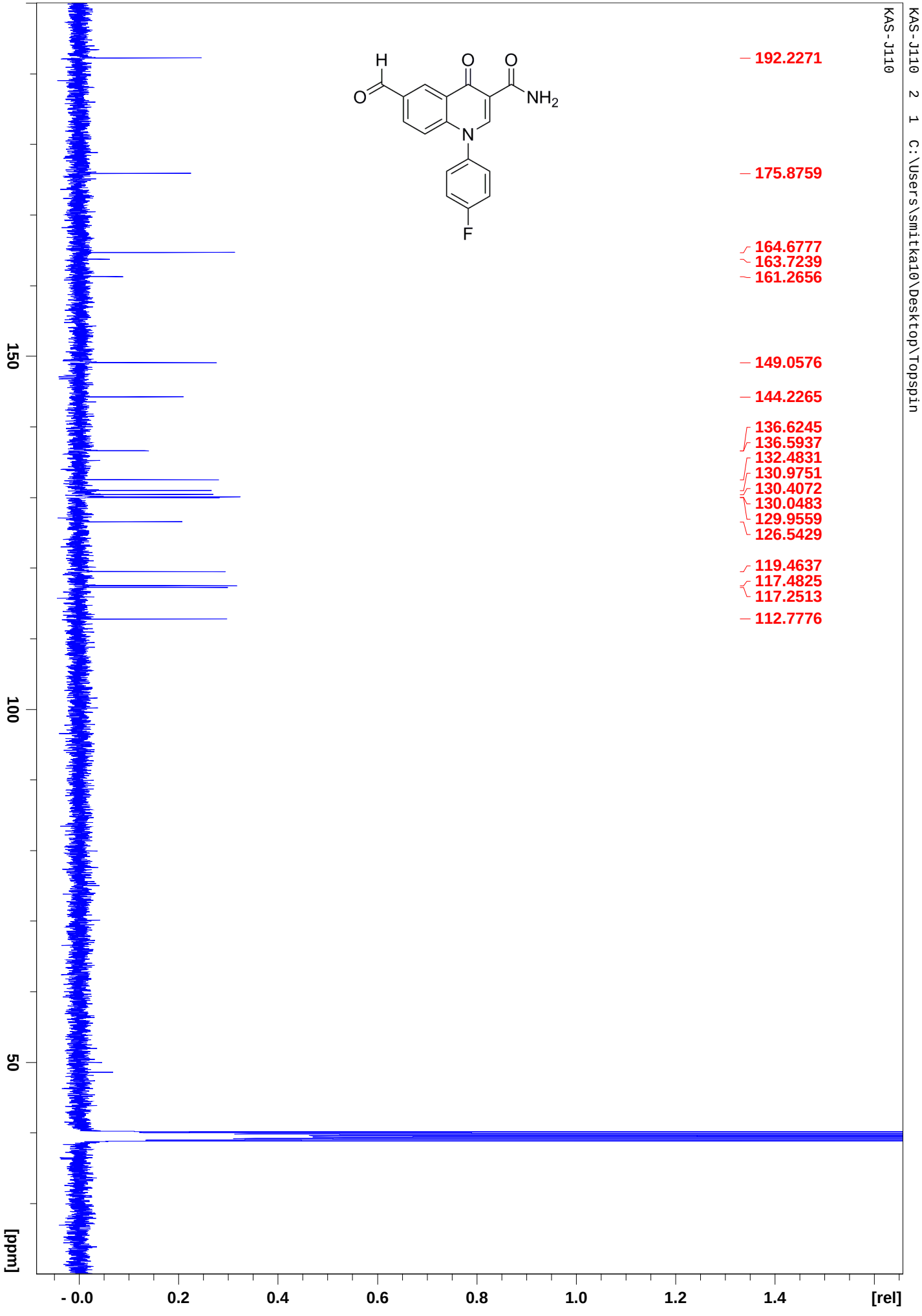
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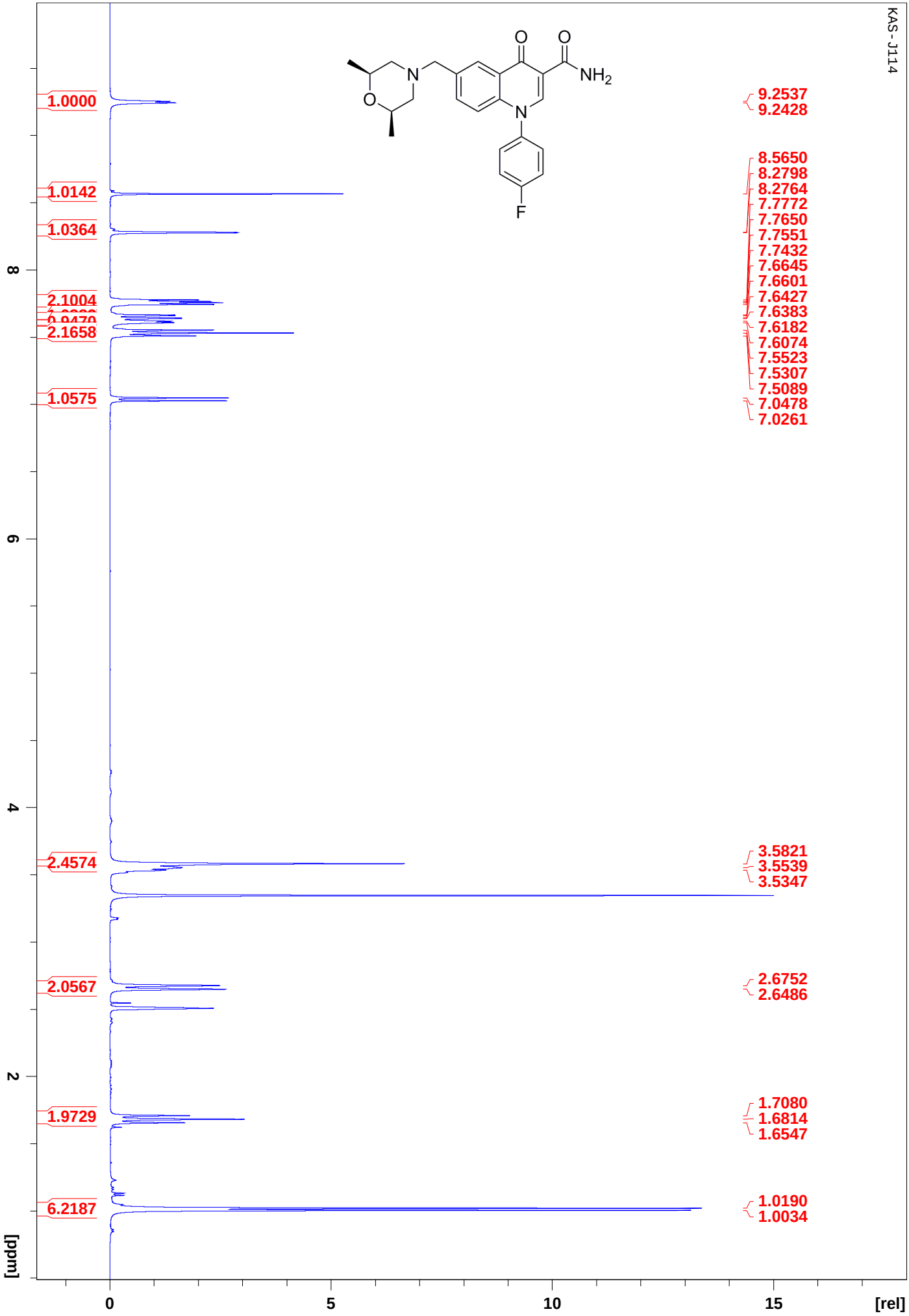
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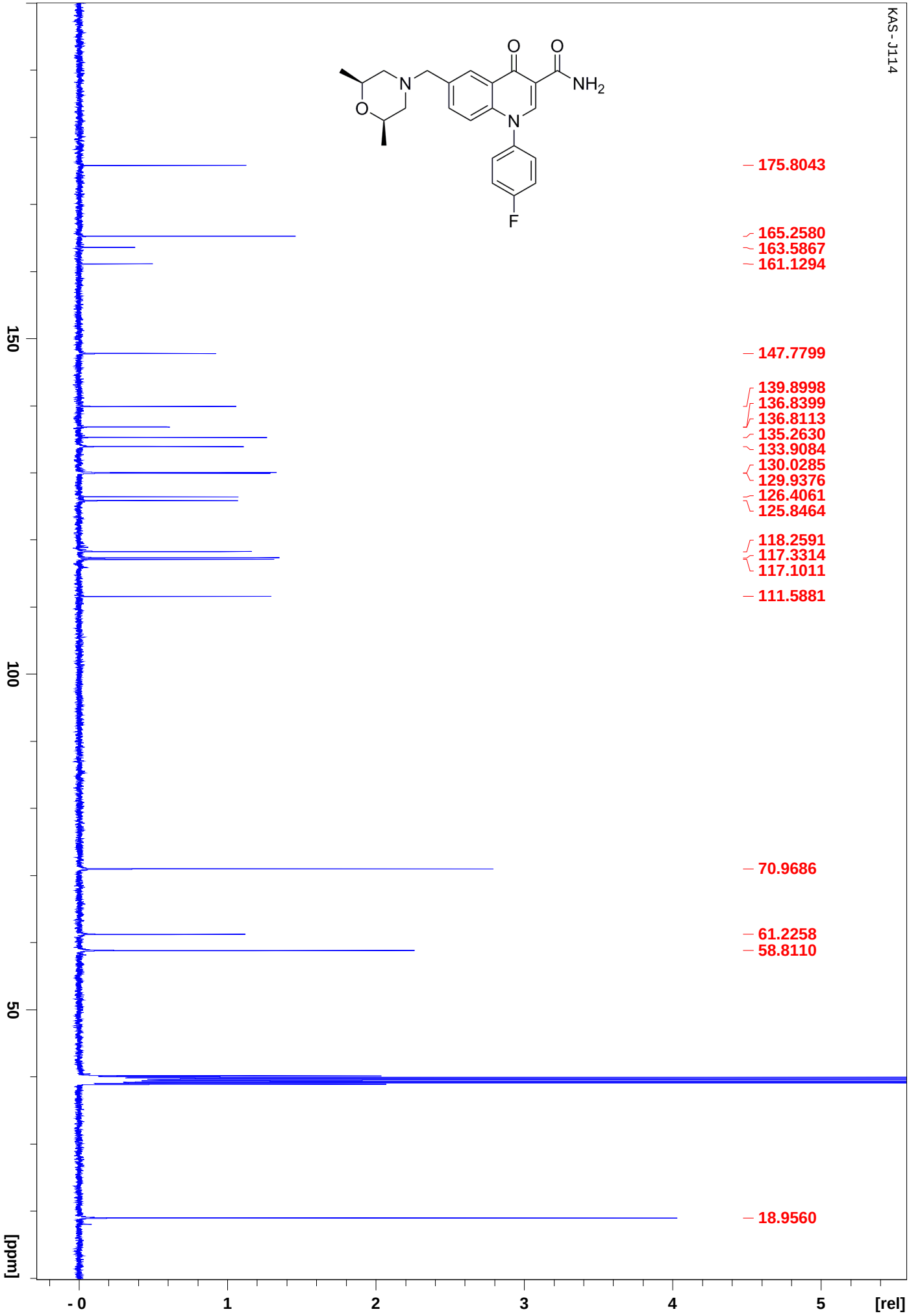
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