Supporting Information

Cardiac Troponin Activator CK-963 Increases Fractional Shortening in Rats

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CONTENTS

I. Supporting Information: Chemistry

A. General Information

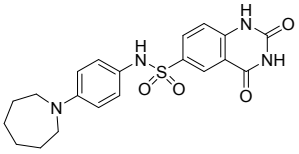
All solvents and reagents were purchased from commercial vendors and used without further purification. ^{1}H NMR were recorded at ambient temperature at 400.13 MHz using a Bruker AVANCE 400 spectrometer. ^{1}H shifts are referenced to the residual protonated solvent signal (δ 2.50 for DMSO-d6, δ 3.31 for MeOH-d4, δ 7.24 for $CDCl_3$). The data are reported as follows: chemical shift in ppm from internal tetramethylsilane on the δ scale, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz), and integration. Mass spectrometry data were obtained using an Agilent LC/MSD Quad VL system. Normal phase liquid chromatography was performed using flash chromatography of the indicated solvent system on EM Reagents silica gel (SiO_2) 60 (230–400 mesh) or using a Biotage Horizon MPLC with Biotage KP-Sil silica gel columns. Reverse phase HPLC purification was performed with an Agilent Series 1100 HPLC equipped with a Phenomenex Gemini C18 Column (5 micron, 150 x 21.2 mm). The typical gradient used for the mobile phase was 20% acetonitrile/water to 90% acetonitrile/water in the presence of 0.1% formic acid over 40 minutes unless otherwise specified. Unless otherwise noted, the purity for compounds was judged to be >95% as determined by ^{1}H NMR and HPLC at 254 nm.

B. General procedure for synthesis of sulfonamides

To a 20-mL scintillation vial was added amine (1.2 equiv), DMF (0-4 mL/mmol), and pyridine (2.0 equiv), followed by sulfonyl chloride. The reactions were generally stirred for 1-24 h. Some were concentrated and purified using reverse phase HPLC (20-90% CH_3CN/H_2O over 35 min), and others were worked up using EtOAc and saturated sodium bicarbonate followed by silica gel purification.

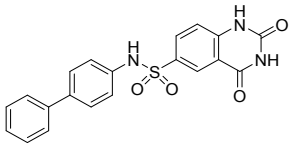
C. Synthetic procedures and characterization

Compounds **2**, **3**, and **6-9** were synthesized using the general procedure for synthesis of sulfonamides.



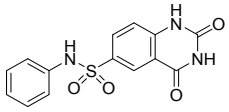
2

N-(4-(Azepan-1-yl)phenyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (2) 1H NMR (400 MHz, DMSO-d6) δ 11.44 (s, 2H), 9.58 (s, 1H), 8.08 (d, J = 2.2 Hz, 1H), 7.73 (dd, J = 8.5, 2.2 Hz, 1H), 7.14 (d, J = 8.6 Hz, 1H), 6.71 (d, J = 8.5 Hz, 2H), 6.43 (d, J = 8.6 Hz, 2H), 3.32 – 3.23 (m, 4H), 1.57 (t, J = 5.6 Hz, 4H), 1.34 (q, J = 3.2 Hz, 4H). LRMS (APCI): calculated for $C_{20}H_{22}N_4O_4S$ 414.1 Da, measured 413.2 m/z $[M - H]^-$.



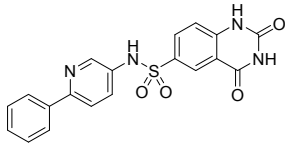
3

N-([1,1'-Biphenyl]-4-yl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (3). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.56 (s, 2H), 10.48 (s, 1H), 8.28 (d, J = 2.2 Hz, 1H), 7.98 (dd, J = 8.7, 2.2 Hz, 1H), 7.62 – 7.52 (m, 4H), 7.41 (dd, J = 8.4, 6.9 Hz, 2H), 7.36 – 7.29 (m, 1H), 7.26 (d, J = 8.7 Hz, 1H), 7.23 – 7.14 (m, 2H). LRMS (APCI): calculated for $C_{20}H_{15}N_3O_4S$ 393.1 Da, measured 392.0 m/z $[M - H]^-$.



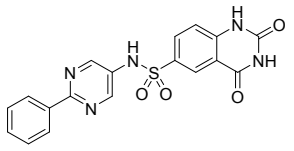
6

2,4-Dioxo-N-phenyl-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (6). 1H NMR (400 MHz, DMSO-d6) δ 11.53 (d, J = 11.8 Hz, 2H), 10.31 (s, 1H), 8.23 (d, J = 2.2 Hz, 1H), 7.92 (dd, J = 8.6, 2.3 Hz, 1H), 7.24 (dt, J = 7.9, 3.5 Hz, 3H), 7.08 (d, J = 7.9 Hz, 2H), 7.03 (t, J = 7.4 Hz, 1H). LRMS (APCI): calculated for $C_{14}H_{11}N_3O_4S$ 317.1 Da, measured 316.0 m/z $[M - H]^-$.



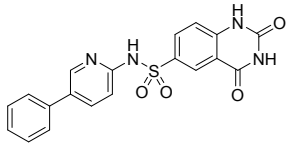
7

2,4-Dioxo-N-(6-phenylpyridin-3-yl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (7). 1H NMR (400 MHz, DMSO-d6) δ 11.56 (d, J = 7.5 Hz, 2H), 10.69 (s, 1H), 8.37 (d, J = 2.7 Hz, 1H), 8.27 (d, J = 2.3 Hz, 1H), 7.97 (ddd, J = 8.7, 3.5, 1.9 Hz, 3H), 7.89 (d, J = 8.7 Hz, 1H), 7.59 (dd, J = 8.6, 2.7 Hz, 1H), 7.45 (dd, J = 8.2, 6.3 Hz, 2H), 7.43 – 7.35 (m, 1H), 7.27 (d, J = 8.7 Hz, 1H). LRMS (APCI): calculated for $C_{19}H_{14}N_4O_4S$ 394.1 Da, measured 395.1 m/z $[M + H]^+$.



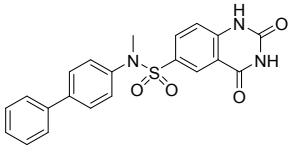
8

2,4-Dioxo-N-(2-phenylpyrimidin-5-yl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (8). 1H NMR (400 MHz, DMSO-d6) δ 11.63 (d, J = 6.1 Hz, 2H), 10.96 (s, 1H), 8.66 (s, 2H), 8.34 (q, J = 4.2, 3.5 Hz, 3H), 8.04 (dd, J = 8.7, 2.3 Hz, 1H), 7.55 (p, J = 3.3 Hz, 3H), 7.33 (d, J = 8.7 Hz, 1H). LRMS (APCI): calculated for $C_{18}H_{13}N_5O_4S$ 395.1 Da, measured 394.1 m/z $[M - H]^-$.



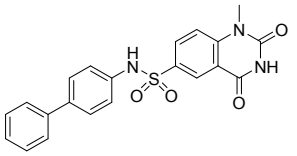
9

2,4-Dioxo-N-(5-phenylpyridin-2-yl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (9). 1H NMR (400 MHz, DMSO-d6) δ 12.93 – 11.12 (br s, 1H), 11.54 – 11.38 (m, 2H), 8.31 (d, J = 2.3 Hz, 2H), 8.03 (ddd, J = 21.2, 8.9, 2.4 Hz, 2H), 7.64 – 7.48 (m, 2H), 7.37 (dd, J = 8.4, 6.8 Hz, 2H), 7.33 – 7.24 (m, 1H), 7.21 (d, J = 8.6 Hz, 1H), 7.15 (d, J = 8.9 Hz, 1H). . LRMS (APCI): molecular ion not detected.



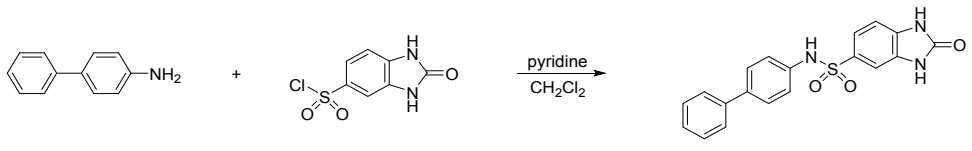
4

***N*-([1,1'-Biphenyl]-4-yl)-*N*-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (4)**. A mixture of *N*-methyl-[1,1'-biphenyl]-4-amine (75 mg, 0.42 mmol, 1 equiv) and pyridine (55 mg, 0.70 mmol, 1.6 equiv) was dissolved in DMF (0.5 mL) and was added to a solution of 2,4-dioxo-1,3-dihydroquinazoline-6-sulfonyl chloride (107 mg, 0.42 mmol, 1 equiv) in DMF (0.5 mL). The reaction mixture was stirred at rt overnight. Methanol (5 mL) was then added to the pink heterogeneous reaction mixture, followed by stirring for 1 h and sonication for 5 min. The resultant solid was filtered and dried to give 87 mg of pale pink solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.68 – 11.52 (m, 2H), 7.96 (d, J = 2.2 Hz, 1H), 7.72 (dd, J = 8.6, 2.2 Hz, 1H), 7.66 (dt, J = 8.6, 2.9 Hz, 4H), 7.47 (t, J = 7.5 Hz, 2H), 7.38 (t, J = 7.3 Hz, 1H), 7.29 (d, J = 8.6 Hz, 1H), 7.25 – 7.18 (m, 2H), 3.16 (s, 3H). LRMS (APCI): calculated for $C_{21}H_{17}N_3O_4S$ 407.1 Da, measured 406.0 m/z $[M - H]^-$.



5

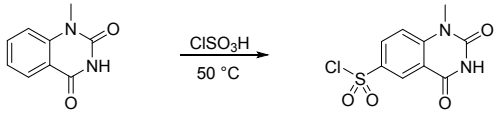
***N*-([1,1'-Biphenyl]-4-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (5)**. A mixture of 4-aminobiphenyl (70 mg, 0.42 mmol, 1 equiv) and pyridine (55 mg, 0.70 mmol, 1.6 equiv) was dissolved in DMF (0.5 mL) and was added to a solution of 1-methyl-2,4-dioxo-3H-quinazoline-6-sulfonyl chloride (114 mg, 0.42 mmol, 1 equiv) in DMF (0.5 mL). The reaction mixture was stirred at rt overnight. Methanol (5 mL) was then added to the pink heterogeneous reaction mixture, followed by stirring for 1 h and sonication for 5 min. The resultant solid was filtered and dried to give 97 mg (55%) of *N*-([1,1'-biphenyl]-4-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide as a pale pink solid. 1H NMR (400 MHz, DMSO-d6) δ 11.82 (s, 1H), 10.52 (s, 1H), 8.37 (d, J = 2.3 Hz, 1H), 8.05 (dd, J = 8.9, 2.3 Hz, 1H), 7.60 – 7.53 (m, 5H), 7.41 (dd, J = 8.4, 6.9 Hz, 2H), 7.34 – 7.28 (m, 1H), 7.24 – 7.18 (m, 2H), 3.41 (s, 3H). LRMS (APCI): calculated for $C_{21}H_{17}N_3O_4S$ 407.1 Da, measured 406.0 m/z $[M - H]^-$.



1

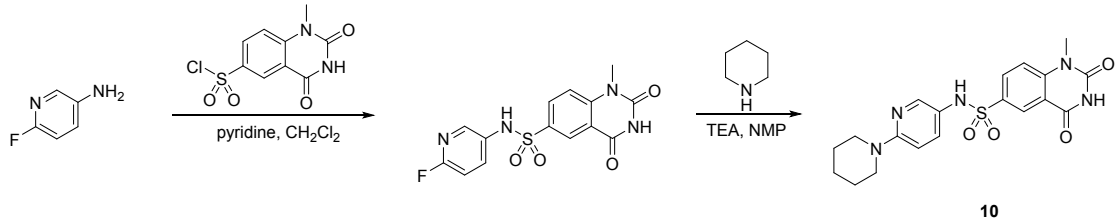
N-([1,1'-Biphenyl]-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazole-5-sulfonamide (1). To a 20-mL scintillation vial was added [1,1'-biphenyl]-4-amine (70 mg, 0.4 mmol, 1.0 equiv), CH_2Cl_2

(2 mL), and pyridine (63 mg, 0.8 mmol, 2.0 equiv), followed by 2-oxo-2,3-dihydro-1H-benzo[d]imidazole-5-sulfonyl chloride (101 mg, 0.44 mmol, 1.1 equiv). The reaction was stirred for 30 min, concentrated, and purified using reverse phase HPLC (20-90% CH_3CN/H_2O over 35 min) to give N-([1,1'-biphenyl]-4-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazole-5-sulfonamide (4 mg, 3%). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.94 (s, 1H), 10.65 (s, 1H), 8.64 (d, J = 2.0 Hz, 1H), 8.17 (dd, J = 8.6, 2.1 Hz, 1H), 7.97 (d, J = 8.6 Hz, 1H), 7.68 (s, 1H), 7.60 – 7.52 (m, 4H), 7.40 (dd, J = 8.4, 6.9 Hz, 2H), 7.35 – 7.26 (m, 1H), 7.23 – 7.15 (m, 2H). LRMS (APCI): calculated for $C_{19}H_{15}N_3O_3S$ 365.1 Da, measured 364.1 m/z $[M-H]^-$.



1-Methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonyl chloride. Chlorosulfonic acid (15 mL) and 1-methyl-3H-quinazoline-2,4-dione (5 g) were added to a 100-mL round bottom flask, and the mixture was heated to 50 °C overnight. The reaction was then cooled to rt and then poured onto ice. The resultant white solid was filtered and dried in vacuo to give 3.06 g (39%) of white solid. 1H NMR (400 MHz, DMSO-d6) δ 11.57 (s, 1H), 8.19 (d, J = 2.1 Hz, 1H), 7.91 (dd, J = 8.6, 2.1 Hz, 1H), 7.38 (d, J = 8.6 Hz, 1H), 3.44 (s, 3H).

Synthesis of 10



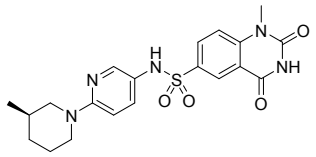
10

Step 1: *N*-(6-Fluoropyridin-3-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide. To a 250-round bottom flask containing 6-fluoropyridin-3-amine (1.22 g, 10.9 mol, 1 equiv), and pyridine (1.78 g, 1.75 mL, 21.8 mmol, 2.0 equiv) at 0 °C was added a suspension of 1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonyl chloride (3.0 g, 10.9 mmol, 1.0 equiv) and CH_2Cl_2 (50 mL). The reaction was stirred at 0 °C for 1 h and then warmed to rt. The reaction was concentrated, and then methanol (35 mL) was added. The mixture was sonicated and the resultant solids filtered to give *N*-(6-fluoropyridin-3-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (3.8 g, 89%) as a tan solid.

Step 2: 1-Methyl-2,4-dioxo-N-(6-(piperidin-1-yl)pyridin-3-yl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (10). To a 5-mL microwave reaction vial was added *N*-(6-fluoropyridin-3-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (100 mg, 0.29 mmol, 1 equiv) dissolved in NMP (3 mL), triethylamine (0.5 mL), and piperidine (102 mg, 1.2 mmol, 4.0 equiv). The microwave tube was sealed, heated to 220 °C, and stirred for 30 min. The reaction was then cooled, followed by addition of formic acid (1 mL) and purified using reverse phase HPLC (20-90% CH_3CN/H_2O over 35 min) to give 1-methyl-2,4-dioxo-N-(6-(piperidin-1-yl)pyridin-3-yl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (61 mg, 52%). ^{1}H NMR (400 MHz, Methanol-d4) δ 8.40 (d, J = 2.3 Hz, 1H), 7.99 (dd, J = 8.8, 2.3 Hz, 1H), 7.65 (d, J = 2.8 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 7.30 (dd, J = 9.1, 2.8 Hz, 1H), 6.68 (d, J = 9.1 Hz, 1H),

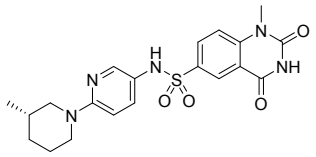
3.55 (s, 3H), 3.41 (t, J = 5.1 Hz, 4H), 1.61 (q, J = 7.8, 7.3 Hz, 6H). LRMS (APCI): calculated for $C_{19}H_{21}N_5O_4S$ 415.3 Da, measured 416.1 m/z $[M + H]^+$.

Compounds **11** and **12** were synthesized using a similar procedure as compound **10**, with the exception that 6-chloropyridin-3-amine was used in the SnAr reaction.



11

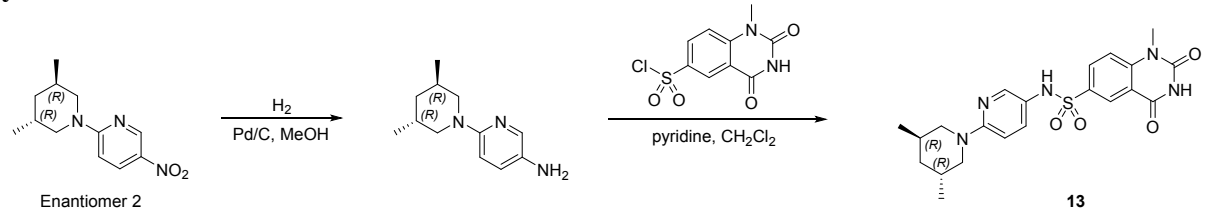
Synthesis of 11. Step 1: Sulfonamide reaction using 6-chloropyridin-3-amine – 2.9 g (85%), white solid. Step 2 SnAr reaction: 17 mg (21 %), to give (*R*)-1-methyl-*N*-(6-(3-methylpiperidin-1-yl)pyridin-3-yl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (**11**) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 11.82 (s, 1H), 9.86 (s, 1H), 8.24 (d, J = 2.3 Hz, 1H), 7.91 (dd, J = 8.9, 2.3 Hz, 1H), 7.68 (d, J = 2.7 Hz, 1H), 7.56 (d, J = 8.9 Hz, 1H), 7.17 (dd, J = 9.1, 2.8 Hz, 1H), 6.71 (d, J = 9.1 Hz, 1H), 4.06 (dd, J = 12.9, 3.8 Hz, 2H), 3.44 (s, 3H), 2.67 (td, J = 12.5, 2.8 Hz, 1H), 2.36 (dd, J = 12.8, 10.6 Hz, 1H), 1.77 – 1.69 (m, 1H), 1.61 (dt, J = 13.1, 3.4 Hz, 1H), 1.49 (dq, J = 13.7, 6.3, 4.7 Hz, 1H), 1.44 – 1.30 (m, 1H), 1.07 (qd, J = 12.3, 3.7 Hz, 1H), 0.86 (d, J = 6.6 Hz, 3H). LRMS (APCI): calculated for $C_{20}H_{23}N_5O_4S$ 429.2 Da, measured 428.2 m/z $[M - H]^-$.



12

Synthesis of 12. Step 1: Sulfonamide reaction using 6-chloropyridin-3-amine – 2.9 g (85%), white solid. Step 2 SnAr reaction: 12 mg (15 %), to give (*S*)-1-methyl-*N*-(6-(3-methylpiperidin-1-yl)pyridin-3-yl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (**12**) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 11.83 (s, 1H), 9.84 (s, 1H), 8.24 (d, J = 2.3 Hz, 1H), 7.91 (dd, J = 8.8, 2.4 Hz, 1H), 7.68 (d, J = 2.8 Hz, 1H), 7.57 (d, J = 8.9 Hz, 1H), 7.17 (dd, J = 9.1, 2.8 Hz, 1H), 6.71 (d, J = 9.1 Hz, 1H), 4.06 (dd, J = 12.7, 3.9 Hz, 2H), 3.44 (s, 3H), 2.67 (td, J = 12.6, 3.0 Hz, 1H), 2.41 – 2.30 (m, 1H), 1.73 (d, J = 12.3 Hz, 1H), 1.61 (dt, J = 13.3, 3.4 Hz, 1H), 1.53 – 1.44 (m, 1H), 1.44 – 1.30 (m, 1H), 1.07 (qd, J = 12.3, 3.6 Hz, 1H), 0.86 (d, J = 6.4 Hz, 3H). LRMS (APCI): calculated for $C_{20}H_{23}N_5O_4S$ 429.2 Da, measured 428.2 m/z $[M - H]^-$.

Synthesis of 13

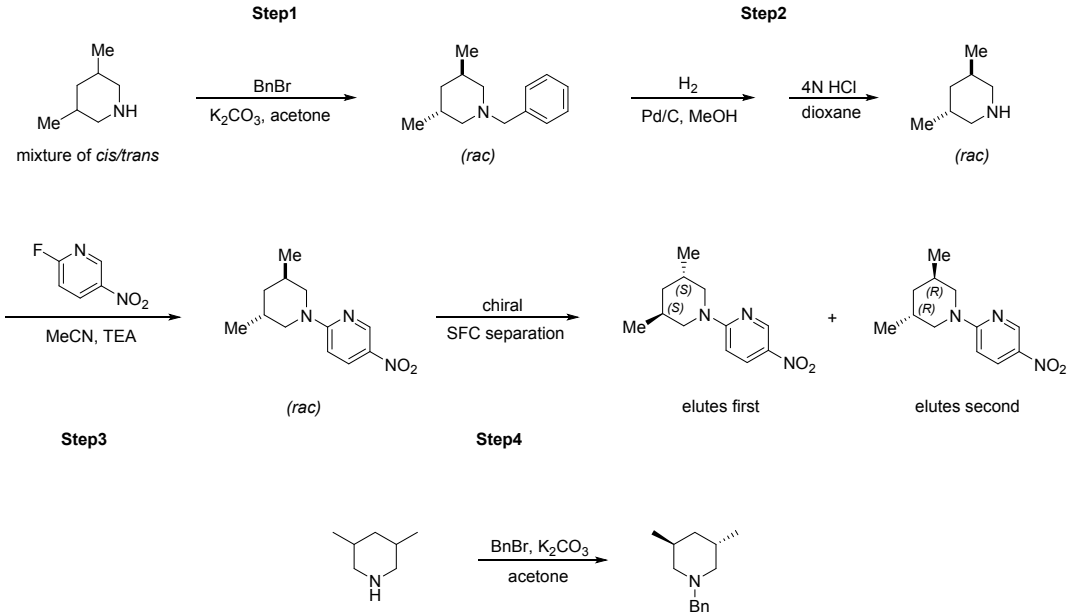


Enantiomer 2 **13**

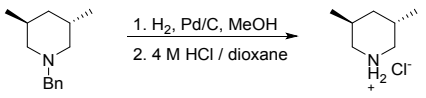
***N*-(6-((3*R*,5*R*)-3,5-Dimethylpiperidin-1-yl)pyridin-3-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (13)**. To a 20-mL scintillation vial was added 2-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)-5-nitropyridine (100 mg, 426 μmol, 1 equiv), 10% Pd/C (28 mg), and

MeOH (5 mL). The reaction was stirred under a hydrogen atmosphere (50 psi) for 20 min. The reaction mixture was then filtered through a pad of celite and concentrated to give 6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-amine (87 mg). To a 20-mL scintillation vial was added 6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-amine (43 mg, 0.2 mmol), CH_2Cl_2 (2 mL) and pyridine (0.2 mL). The reaction was stirred for 1 h, followed by the addition of 20% EtOAc/hexanes (1 mL) and water (5 drops). The resultant solution containing precipitate was sonicated, and the solid then filtered to give *N*-(6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (29 mg, 31%) as an off-white solid. 1H NMR (400 MHz, DMSO-d6) δ 11.83 (s, 1H), 9.90 (s, 1H), 8.25 (d, J = 2.3 Hz, 1H), 7.93 (dd, J = 8.8, 2.3 Hz, 1H), 7.64 (d, J = 2.7 Hz, 1H), 7.57 (d, J = 8.9 Hz, 1H), 7.20 (dd, J = 9.2, 2.7 Hz, 1H), 6.79 (d, J = 9.2 Hz, 1H), 3.51 (dd, J = 12.8, 3.8 Hz, 2H), 3.45 (s, 3H), 3.11 (dd, J = 12.9, 6.9 Hz, 2H), 1.87 (dq, J = 12.7, 6.3 Hz, 2H), 1.40 (t, J = 5.8 Hz, 2H), 0.86 (d, J = 6.8 Hz, 6H). LRMS (APCI): calculated for $C_{21}H_{25}N_5O_4S$ 443.2 Da, measured 444.2 m/z $[M + H]^+$.

Synthesis of 2-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)-5-nitropyridine Intermediate

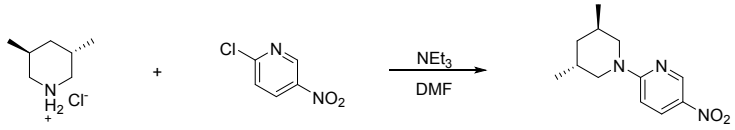


Step 1: *trans*-3,5-Dimethylpiperidine. To a 85/15 mixture of *trans*/*cis* 3,5-dimethyl piperidine (TCI, 320 mL, 2.35 mmol) and K_2CO_3 (960 g, 6.96 mol) in acetone (8 L) was slowly added benzyl bromide (488 mL, 4.08 mol) while using a water bath to control the reaction temperature below 40 °C. The reaction was stirred at rt for 4 d. The reaction was then filtered, and the filtrate washed with acetone (1L). The combined filtrates were concentrated and purified using silica gel chromatography (0-5% diethyl ether in hexanes with 0.2% TEA) to give racemic *trans*-3,5-dimethylpiperidine (200 g, 43%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.34 – 7.15 (m, 5H), 3.54 – 3.28 (m, 2H), 2.37 (d, *J* = 9.1 Hz, 2H), 2.13 – 1.97 (m, 2H), 1.90 (ddp, *J* = 10.0, 6.3, 3.6 Hz, 2H), 1.28 (t, *J* = 5.8 Hz, 2H), 0.95 (d, *J* = 6.8 Hz, 6H). LC/MS (APCI) *m/z* calculated for $C_{14}H_{21}N$ 203.2 Da, measured 204.1 m/z $[M + H]^+$.

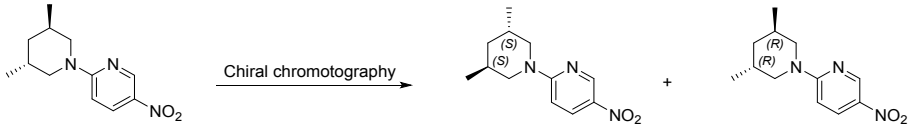


(3*S*,5*S*)-1-benzyl-3,5-dimethylpiperidine

Step 2: *trans*-3,5-Dimethylpiperidin-1-ium chloride. To a solution of racemic *trans*-3,5-dimethylpiperidine (80.0 g, 0.39 mol) in MeOH (500 mL) was added 20% Pd/C (2.1 g, 0.02 mol, Johnson Matthey A402023-20). The reaction was stirred under hydrogen (25 psi) at 45 °C for 12 h and then filtered through celite. To the filtrate was added HCl (4 N in dioxane, 200 mL) followed by concentration to give *trans*-3,5-dimethylpiperidin-1-ium chloride (59.0 g, 100%) as a white solid. ^{1}H NMR (400 MHz, Methanol-d_4) δ 3.14 (dd, J = 12.6, 4.0 Hz, 2H), 2.83 (dd, J = 12.5, 7.0 Hz, 2H), 2.20 – 2.06 (m, 2H), 1.55 (t, J = 5.8 Hz, 2H), 1.07 (d, J = 7.1 Hz, 6H).

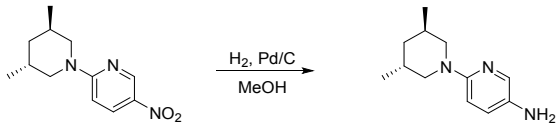


Step 3: 2-(*trans*-3,5-Dimethylpiperidin-1-yl)-5-nitropyridine. To a 2-L round bottom flask was added *trans*-3,5-dimethylpiperidin-1-ium chloride (66.7 g, 446 mmol, 1.2 equiv), 2-chloro-5-nitropyridine (60.1 g, 379 mmol, 1.0 equiv), DMF (250 mL), and triethylamine (137 mL, 1000 mmol, 2.6 equiv). The reaction was heated to 90 °C and stirred overnight. The reaction was then diluted with EtOAc (1 L) and washed three times with brine (200 mL each wash). The organic layer was dried over sodium sulfate, filtered, and concentrated. The resultant crude solid was dissolved in a minimum of EtOAc, followed by the addition of 20% EtOAc/hexanes (50 mL). Hexanes were then added until precipitation was observed, and the reaction suspension was stirred at rt for 14 h. The product was filtered, washed with 20% EtOAc/hexanes, and then dried under vacuum to give 2-(*trans*-3,5-dimethylpiperidin-1-yl)-5-nitropyridine (31.0 g, 29%) as a pale yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.99 (d, J = 2.8 Hz, 1H), 8.12 (ddd, J = 9.6, 2.9, 0.6 Hz, 1H), 6.52 (d, J = 9.6 Hz, 1H), 3.80 (d, J = 12.3 Hz, 2H), 3.36 (dd, J = 13.2, 7.1 Hz, 2H), 2.01 (ddp, J = 10.4, 6.4, 4.0 Hz, 2H), 1.52 (t, J = 5.9 Hz, 2H), 0.94 (d, J = 6.8 Hz, 6H). LC/MS (APCI) *m/z* calculated for $C_{12}H_{17}N_3O_2$ 235.1 Da, measured 236.1 m/z $[M + H]^+$.



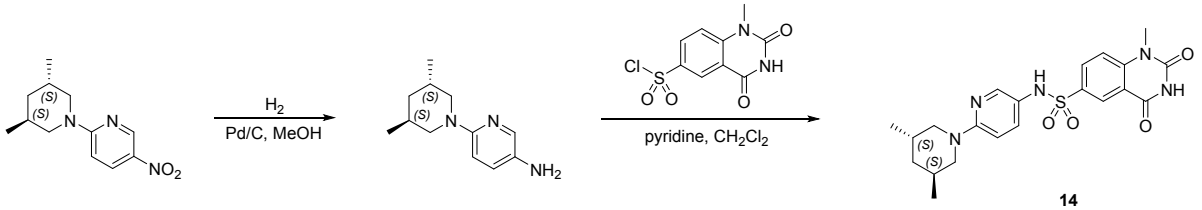
Enantiomer 1 Enantiomer 2

Step 4: 2-((3*S*,5*S*)-3,5-Dimethylpiperidin-1-yl)-5-nitropyridine (enantiomer 1) and 2-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)-5-nitropyridine (enantiomer 2). 2-(*trans*-3,5-dimethylpiperidin-1-yl)-5-nitropyridine (1.1 g) was resolved using chiral SFC (Chiralcel AD-H, 20% (1:1) isopropanol:MeCN/CO_2, 100 bar, 62 mL/min) to give enantiomer 1 (525 mg, $[\alpha]^{20}/_D$ = +41.4° [c 0.95, EtOAc) and enantiomer 2 (520 mg, $[\alpha]^{20}/_D$ = -45.0 (c 0.91, EtOAc). Enantiomers were numbered based on the order of elution, and the absolute stereochemistry of enantiomer 2 was assigned as R,R based on the crystal structure of **19**. Enantiomer 1 was therefore assigned as S, S.



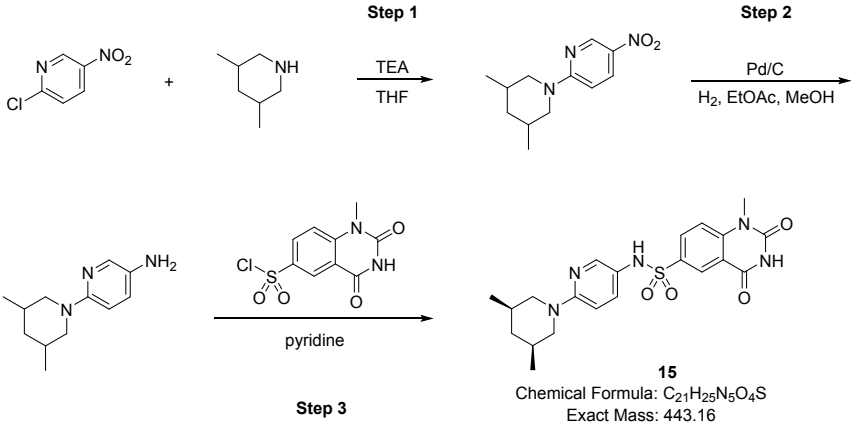
Step 5: Synthesis of 6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-amine. 2-((3*R*,5*R*)-3,5-Dimethylpiperidin-1-yl)-5-nitropyridine (350 mg, 1.49 mmol, 1 equiv) and Pd/C (80 mg, 0.075 mmol, 0.05 equiv.) were suspended in MeOH (35 mL) and stirred under hydrogen (30 psi) for 1 h. The reaction mixture was then filtered through a pad of celite and concentrated to give 6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-amine (300 mg). LC/MS (APCI) *m/z* calculated for $C_{12}H_{19}N_3$ 205.1 Da, measured 206.1 m/z $[M + H]^+$.

Synthesis of 14



***N*-(6-((3*S*,5*S*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (14)** The exact procedure for the synthesis of **13** was followed to synthesize **14**, providing 22 mg (23%) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 11.83 (s, 1H), 9.83 (s, 1H), 8.25 (d, J = 2.3 Hz, 1H), 7.92 (dd, J = 8.8, 2.3 Hz, 1H), 7.65 (d, J = 2.7 Hz, 1H), 7.57 (d, J = 8.9 Hz, 1H), 7.16 (dd, J = 9.1, 2.8 Hz, 1H), 6.72 (d, J = 9.1 Hz, 1H), 3.50 (dd, J = 12.8, 3.7 Hz, 2H), 3.45 (s, 3H), 3.08 (dd, J = 12.8, 6.8 Hz, 2H), 1.86 (dq, J = 10.2, 6.2 Hz, 2H), 1.39 (t, J = 5.8 Hz, 2H), 0.85 (d, J = 6.8 Hz, 6H). LRMS (APCI): calculated for $C_{21}H_{25}N_5O_4S$ 443.2 Da, measured 444.2 m/z $[M + H]^+$.

Synthesis of 15

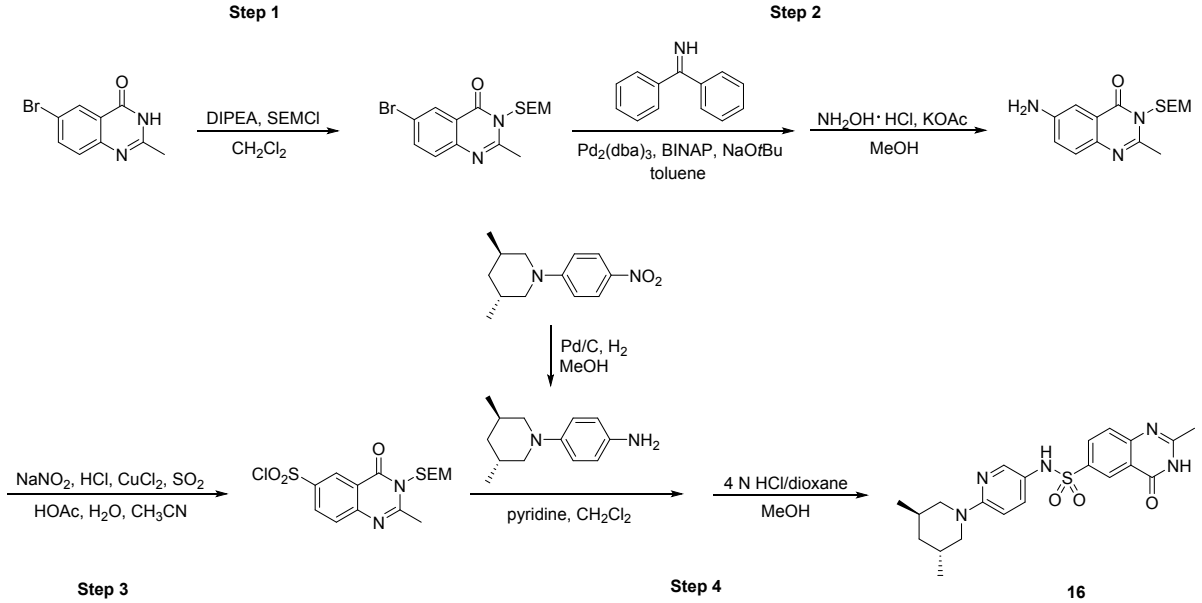


Step 1: 2-(3,5-Dimethylpiperidin-1-yl)-5-nitropyridine. To a 50-mL round bottom flask cooled to 0 °C was added 2-chloro-5-nitropyridine (1.0 g, 6.3 mmol, 1 equiv), 3,5-dimethylpiperidine (1.45 g, 1.70 mL, 12.6 mmol, 2 equiv), triethylamine (0.9 mL, 6.3 mmol, 1 equiv), and THF (20 mL). The reaction was stirred overnight and filtered. The filtrate was washed with brine, dried over sodium sulfate, and concentrated in vacuo to give 1.4 g of crude 2-(3,5-dimethylpiperidin-1-yl)-5-nitropyridine.

Step 2: 6-(3,5-Dimethylpiperidin-1-yl)pyridin-3-amine. To a 100-mL reaction vial was added 2-(3,5-dimethylpiperidin-1-yl)-5-nitropyridine (1.4 g, 6.0 mmol, 1 equiv), methanol (20 mL), ethyl acetate (10 mL), and 10% Pd/C (500 mg). The reaction was stirred under hydrogen (50 psi) for 2 h, followed by filtration, concentration, and purification using silica gel chromatography (0-20% EtOAc/hexanes) to give 6-(3,5-dimethylpiperidin-1-yl)pyridin-3-amine (1.1 g, 90%).

Step 3: *N*-(6-((3*R*,5*S*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide. To a 40-mL scintillation vial was added 6-(3,5-dimethylpiperidin-1-yl)pyridin-3-amine (335 mg, 1.22 mmol, 1 equiv), pyridine (2 mL), and 1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonyl chloride (251 mg, 1.22 mmol, 1 equiv). The reaction was stirred for 30 min at 40 °C, concentrated, and purified using silica gel chromatography and then reverse phase HPLC (20-90% CH_3CN/H_2O over 35 min). The second isomer compound to elute from the column was *N*-(6-((3*R*,5*S*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (204 mg, 38%). 1H NMR (400 MHz, DMSO-d6) δ 11.83 (s, 1H), 9.84 (s, 1H), 8.24 (d, J = 2.3 Hz, 1H), 7.91 (dd, J = 8.9, 2.4 Hz, 1H), 7.68 (d, J = 2.7 Hz, 1H), 7.57 (d, J = 8.9 Hz, 1H), 7.17 (dd, J = 9.1, 2.8 Hz, 1H), 6.72 (d, J = 9.1 Hz, 1H), 4.15 (dd, J = 12.9, 3.9 Hz, 2H), 3.44 (s, 3H), 2.23 – 2.13 (m, 2H), 1.73 (d, J = 12.7 Hz, 1H), 1.48 (ddd, J = 22.4, 7.0, 3.6 Hz, 1H), 1.48 (s, 2H), 0.85 (d, J = 6.6 Hz, 6H). LRMS (APCI): calculated for $C_{21}H_{25}N_5O_4S$ 443.2 Da, measured 442.2 m/z $[M - H]^-$.

Synthesis of 16



Step 1: 6-Bromo-2-methyl-3-((2-(trimethylsilyl)ethoxy)methyl)quinazolin-4(3H)-one. SEMCl (7.5 g. 45 mmol, 1.25 equiv) was added to a stirring solution of 6-bromo-2-methylquinazolin-4(3*H*)-one (8.0 g, 36 mmol, 1 equiv) and DIPEA (8 mL, 45 mmol, 1.25 equiv) in CH_2Cl_2 (200 mL). After 14 h, the reaction was concentrated and purified using silica gel column chromatography (10-30% EtOAc/hexanes) to give 6-bromo-2-methyl-3-((2-

(trimethylsilyl)ethoxy)methyl)quinazolin-4(3H)-one (8.4 g, 64%) as a yellow oil. 1H NMR (400 MHz, Methanol-d4) δ 8.31 (t, J = 1.9 Hz, 1H), 7.92 (ddd, J = 8.6, 2.1, 1.0 Hz, 1H), 7.53 (dd, J = 8.7, 1.2 Hz, 1H), 5.61 (d, J = 1.3 Hz, 2H), 3.83 – 3.58 (m, 2H), 2.70 (d, J = 1.3 Hz, 3H), 1.10 – 0.82 (m, 2H), 0.10 (s, 9H). LRMS (APCI): calculated for $C_{15}H_{21}BrN_2O_2Si$ 368.1 Da, measured 369.1 m/z $[M + H]^+$.

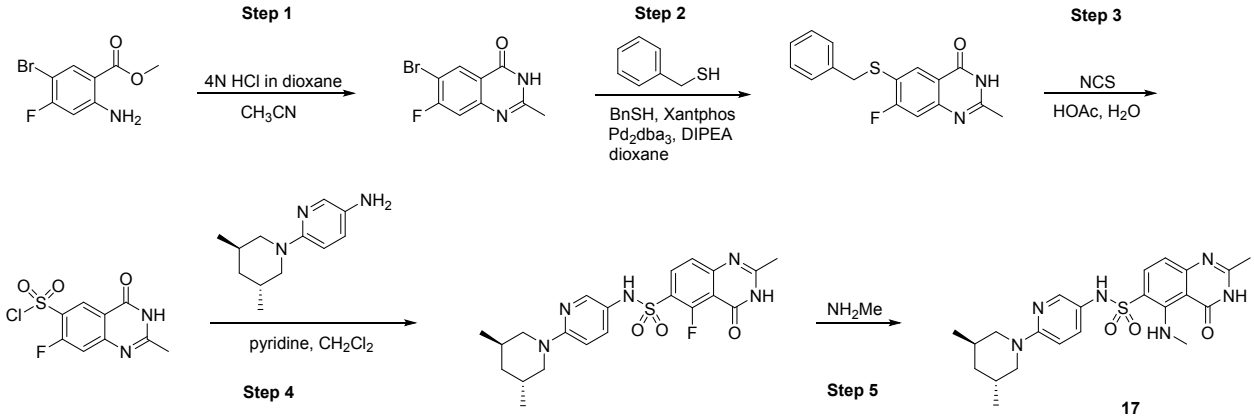
Step 2: 6-Amino-2-methyl-3-((2-(trimethylsilyl)ethoxy)methyl)quinazolin-4(3*H*)-one. To a 250-mL round bottom flask was added 6-bromo-2-methyl-3-((2-(trimethylsilyl)ethoxy)methyl)quinazolin-4(3H)-one (8.4 g, 2.3 mmol, 1.0 equiv), benzophenone imine (4.5, 25 mmol, 1.1 equiv), tris(dibenzylideneacetone)dipalladium (1.05 g, 0.12 mmol, 0.05 equiv), BINAP (2.14 g, 0.35 mmol, 0.15 equiv), sodium *tert*-butoxide (3.1, 32 mmol, 1.4 equiv), and toluene (100 mL). The reaction mixture was heated to reflux and stirred for 1 h. The reaction was then washed with saturated sodium bicarbonate and the organic layer was separated, dried over sodium sulfate, and concentrated. The resultant crude oil was dissolved in methanol (200 mL), followed by the addition of potassium acetate (6.8 g, 6.9 mmol, 3.0 equiv) and hydroxylamine hydrochloride (4.0 g, 58 mmol, 25.0 equiv). The reaction was stirred at rt for 90 min. The reaction was then washed with saturated sodium bicarbonate, and the organic layer was separated, dried over sodium sulfate, and concentrated. The resultant crude oil was purified using silica gel chromatography to give 6-amino-2-methyl-3-((2-(trimethylsilyl)ethoxy)methyl)quinazolin-4(3H)-one (3.3 g, 47%) as a pale yellow solid.

Step 3: 2-Methyl-4-oxo-3-((2-(trimethylsilyl)ethoxy)methyl)-3,4-dihydroquinazoline-6-sulfonyl chloride. To a 100-mL round bottom flask (A) was added copper(II) chloride (0.48 g, 10.8 mmol, 0.3 equiv) and acetic acid (16 mL), and sulfur dioxide was bubbled into the mixture through a gas dispersion tube. In a separate 100-mL round bottom flask (B) was added 6-amino-2-methyl-3-((2-(trimethylsilyl)ethoxy)methyl)quinazolin-4(3H)-one (3.3 g, 10.8 mmol, 1.0 equiv), acetonitrile (10 mL), and concentrated HCl (5 mL). This mixture (B) was cooled to -5 °C, followed by the addition of sodium nitrite (0.75 g, 10.8 mmol, 1.0 equiv) dissolved in water (5 mL). The reaction mixture (B) was stirred for 5 min at -5 °C. The contents of round bottom flask B were then poured in round bottom A, and the combined reaction mixture was stirred for 5 min at -5 °C and then warmed to rt. The reaction was then extracted using EtOAc/water, and the organic layer was separated, washed with saturated sodium bicarbonate solution, dried over sodium sulfate, and concentrated. The resultant crude oil was purified using silica gel chromatography (20-50 % EtOAc/hexanes) to give 2-methyl-4-oxo-3-((2-(trimethylsilyl)ethoxy)methyl)-3,4-dihydroquinazoline-6-sulfonyl chloride (2.75 g, 65%) as a pale orange oil. 1H NMR (400 MHz, Chloroform-d) δ 8.91 (d, J = 2.4 Hz, 1H), 8.26 (dd, J = 8.8, 2.4 Hz, 1H), 7.77 (d, J = 8.7 Hz, 1H), 5.57 (s, 2H), 3.73 – 3.64 (m, 2H), 2.75 (s, 3H), 0.99 – 0.88 (m, 2H), -0.02 (s, 9H).

Step 4: *N*-(6-((3*R*,5*R*)-3,5-Dimethylpiperidin-1-yl)pyridin-3-yl)-2-methyl-5-(methylamino)-4-oxo-3,4-dihydroquinazoline-6-sulfonamide (16). 2-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)-5-nitropyridine (100 mg, 0.5 mmol, 1.0 equiv) and Pd/C (28 mg, 10% Pd by mass, 0.026 mmol, 0.05 equiv) were suspended in MeOH (5 mL) and then stirred under a hydrogen atmosphere (50 psi) for 30 min. The reaction was filtered, concentrated, and dried under high vacuum. The resultant solid was dissolved in pyridine (0.08 g, 1.01 mmol, 2 equiv) and CH_2Cl_2 (2 mL), and 2-methyl-4-oxo-3-((2-(trimethylsilyl)ethoxy)methyl)-3,4-dihydroquinazoline-6-sulfonyl chloride (83 mg,

0.215 mmol, 0.45 equiv) was added. The reaction stirred for 1 h, filtered through a plug of silica (10->30% EtOAc/hexanes), concentrated, and dissolved in MeOH (0.5 mL). HCl (4 N in dioxanes, 3 mL) was then added, and the reaction heated to 90 °C for 10 min. The reaction was cooled to rt, concentrated, and purified using silica gel chromatography (0-10% MeOH/CH_2Cl_2) to give *N*-(6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-2-methyl-5-(methylamino)-4-oxo-3,4-dihydroquinazoline-6-sulfonamide (**16**) as a tan solid (19 mg, 20% over 3 steps). 1H NMR (400 MHz, Methanol-d4) δ 8.43 (d, J = 2.1 Hz, 1H), 7.98 (dd, J = 8.6, 2.1 Hz, 1H), 7.67 (d, J = 8.7 Hz, 1H), 7.56 (d, J = 2.7 Hz, 1H), 7.25 (dd, J = 9.2, 2.7 Hz, 1H), 6.67 (d, J = 9.2 Hz, 1H), 3.51 (dd, J = 12.9, 3.7 Hz, 2H), 3.10 (dd, J = 12.9, 6.9 Hz, 2H), 2.46 (d, J = 1.4 Hz, 3H), 1.92 (ddt, J = 13.0, 10.5, 4.3 Hz, 2H), 1.46 (t, J = 5.8 Hz, 2H), 0.91 (d, J = 6.8 Hz, 6H). LRMS (APCI): calculated for $C_{21}H_{25}N_5O_3S$ 427.2 Da, measured 428.1 m/z $[M + H]^+$.

Synthesis of 17



Step 1: 6-Bromo-7-fluoro-2-methylquinazolin-4(3*H*)-one. To a 1-L round bottom flask was added methyl 2-amino-5-bromo-4-fluorobenzoate (25.0 g, 0.1 mol), 4 N HCl in dioxanes (300 mL), and acetonitrile (350 mL). The mixture was heated to 90 °C and stirred overnight. The reaction was then concentrated, and the resultant solid was resuspended in acetonitrile. Aqueous sodium hydroxide (1 N) was used to adjust the pH to 8-9, and the solid was filtered, washed with cold acetonitrile, and dried in vacuo to give 6-bromo-7-fluoro-2-methylquinazolin-4(3*H*)-one (24.0 g, 93%). 1H NMR (400 MHz, DMSO-d6) δ 12.45 (s, 1H), 8.27 (d, J = 7.7 Hz, 1H), 7.54 (d, J = 10.0 Hz, 1H), 3.32 (s, 3H).

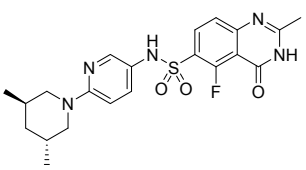
Step 2: 6-(Benzylthio)-7-fluoro-2-methylquinazolin-4(3*H*)-one. 6-Bromo-7-fluoro-2-methylquinazolin-4(3*H*)-one (15.0 g, 58.4 mmol, 1 equiv) was added to a 1-L round bottom flask and dissolved with dioxane (300 mL) and toluene (300 mL), followed by the addition of diisopropylethylamine (15.0 g, 20.3 mL, 116 mol, 2.0 equiv). The mixture was heated to 90 °C, followed by the addition of phenylmethanethiol (7.6 g, 7.3 mL, 61.3 mmol, 1.05 equiv), xantphos (5.1 g, 8.9 mmol, 0.15 equiv) and tris(dibenzylideneacetone)dipalladium (5.3 g, 5.8 mmol, 0.1 equiv). The reaction mixture was heated at 90 °C for 6 h and then cooled to 0 °C. The resultant solid was filtered, washed with water, and dried in vacuo to give 6-(benzylthio)-7-fluoro-2-methylquinazolin-4(3*H*)-one (15.7 g, 90%) as a yellow-green solid. 1H NMR (400 MHz, DMSO-d6) δ 12.28 (s, 1H), 8.26 (s, 1H), 7.95 (d, J = 8.2 Hz, 1H), 7.38 – 7.15 (m, 5H), 4.27 (s, 2H), 2.29 (s, 3H).

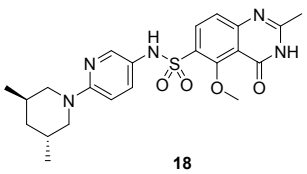
Step 3: 7-Fluoro-2-methyl-4-oxo-3,4-dihydroquinazoline-6-sulfonyl chloride. To a 500-mL round bottom flask was added 6-(benzylthio)-7-fluoro-2-methylquinazolin-4(3H)-one (9.5 g, 31.7 mmol, 1 equiv), acetic acid (200 mL), and water (50 mL). The mixture was cooled with an ice bath to about 0 °C, and *N*-chlorosuccinimide (14.7 g, 110.8 mmol, 3.5 equiv) was added. The reaction mixture was stirred for 5 h at 0 °C, and then at rt for 5 h. The reaction mixture was then extracted using EtOAc (800 mL) and brine (300 mL), and the organic layer was separated, dried over sodium sulfate, and concentrated. The crude product was purified using silica gel chromatography (hexanes/EtOAc) to give 7-fluoro-2-methyl-4-oxo-3,4-dihydroquinazoline-6-sulfonyl chloride (3.9 g, 49%) as a white solid.

Step 4: *N*-(6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-7-fluoro-2-methyl-4-oxo-3,4-dihydroquinazoline-6-sulfonamide. To a 1-L round bottom flask was added 6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-amine (8.0 g, 39 mmol, 1 equiv) and pyridine (200 mL), followed by a suspension of 7-fluoro-2-methyl-4-oxo-3,4-dihydroquinazoline-6-sulfonyl chloride (16.1 g, 58.5 mmol, 1.5 equiv) in methylene chloride (300 mL). The reaction mixture was stirred at rt for 1 h and then concentrated. The crude product was purified using silica gel chromatography (100% Et_2O, then 100% ethyl acetate) to give *N*-(6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-7-fluoro-2-methyl-4-oxo-3,4-dihydroquinazoline-6-sulfonamide (9.5 g, 55%) as a dark purple solid.

Step 5: *N*-(6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-2-methyl-7-(methylamino)-4-oxo-3,4-dihydroquinazoline-6-sulfonamide (**17**). To a 20 mL microwave reaction tube was added *N*-(6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-7-fluoro-2-methyl-4-oxo-3,4-dihydroquinazoline-6-sulfonamide (0.5 g, 1.1 mmol) and methylamine (6 mL, 40% in water). The tube was sealed and the reaction was heated within a microwave reactor at 130 °C for 30 min. The pH of the reaction was then adjusted to 7 followed by extraction with ethyl acetate and brine. The organic layer was dried over sodium sulfate and concentrated. The crude product was purified using reverse phase column chromatography (20-90% CH_3CN/H_2O over 35 min) to give *N*-(6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-2-methyl-7-(methylamino)-4-oxo-3,4-dihydroquinazoline-6-sulfonamide (195 mg, 38%) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 11.94 (s, 1H), 9.80 (s, 1H), 8.08 (s, 1H), 7.61 (d, J = 2.6 Hz, 1H), 7.07 (dd, J = 9.1, 2.7 Hz, 1H), 6.66 (d, J = 9.2 Hz, 1H), 6.62 (s, 1H), 6.32 (q, J = 4.7 Hz, 1H), 3.48 (dd, J = 12.8, 3.7 Hz, 2H), 3.06 (dd, J = 12.8, 6.8 Hz, 2H), 2.88 (d, J = 4.8 Hz, 3H), 2.27 (s, 3H), 1.81 (dddd, J = 13.4, 9.6, 6.7, 3.8 Hz, 2H), 1.36 (t, J = 5.8 Hz, 2H), 0.81 (d, J = 6.8 Hz, 6H). LRMS (APCI): calculated for $C_{22}H_{28}N_6O_3S$ 456.2 Da, measured 457.2 m/z $[M + H]^+$.

Synthesis of 18





18

***N*-(6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-5-methoxy-2-methyl-4-oxo-3,4-dihydroquinazoline-6-sulfonamide** (**18**). To a microwave reaction tube was added *N*-(6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-7-fluoro-2-methyl-4-oxo-3,4-dihydroquinazoline-6-sulfonamide (2.8 g, 6.3 mmol) and sodium methoxide (120 mL of a 25%

solution in methanol). The tube was sealed and the reaction was heated to 130 °C for 35 min. The reaction was then concentrated and purified by reverse phase HPLC (20-90% CH_3CN/H_2O over 35 min) to give a crude solid that was dissolved in ethyl acetate and washed with aqueous sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and concentrated to give *N*-(6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-7-methoxy-2-methyl-4-oxo-3,4-dihydroquinazoline-6-sulfonamide 1.61 g (56%) as an off-white solid. 1H NMR (400 MHz, DMSO-d6) δ 12.33 (s, 1H), 9.55 (s, 1H), 8.22 (s, 1H), 7.67 (d, J = 2.6 Hz, 1H), 7.21 (s, 1H), 7.15 (dd, J = 9.1, 2.7 Hz, 1H), 6.65 (d, J = 9.2 Hz, 1H), 4.03 (s, 3H), 3.45 (dd, J = 12.8, 3.7 Hz, 2H), 3.03 (dd, J = 12.8, 6.8 Hz, 2H), 2.33 (s, 3H), 1.82 (pd, J = 6.4, 3.8 Hz, 2H), 1.36 (t, J = 5.8 Hz, 2H), 0.82 (d, J = 6.8 Hz, 6H). LRMS (APCI): calculated for $C_{22}H_{27}N_5O_4S$ 457.2 Da, measured 458.2 m/z $[M + H]^+$.

Synthesis of CK-963

***N*-(6-((3*R*,5*R*)-3,5-Dimethylpiperidin-1-yl)pyridin-3-yl)-2-methyl-5-(2-morpholinoethoxy)-4-oxo-3,4-dihydroquinazoline-6-sulfonamide (CK-963).** To a 250-mL round bottom flask was added 2-morpholinoethan-1-ol (16.3 g, 15.0 mL, 124 mmol, 1.7 equiv), followed by sodium hydride (60% dispersion in mineral oil, 1.9 g, 72.0 mmol, 1.0 equiv) in small portions with stirring. The reaction mixture was stirred for 1 h, followed by the addition of *N*-(6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-7-fluoro-2-methyl-4-oxo-3,4-dihydroquinazoline-6-sulfonamide (3.2 g, 72.0 mmol). The reaction mixture was transferred to a microwave reaction tube, sealed, and heated in a microwave reactor at 130 °C for 90 min. The reaction mixture was then concentrated and purified using reverse phase HPLC (20-90% CH_3CN/H_2O over 35 min). Ethyl acetate and saturated sodium carbonate solution were used to dissolve the resultant solid, and the organic layer was separated and dried over sodium sulfate to give 1.6 g (40%) of *N*-(6-((3*R*,5*R*)-3,5-dimethylpiperidin-1-yl)pyridin-3-yl)-2-methyl-5-(2-morpholinoethoxy)-4-oxo-3,4-dihydroquinazoline-6-sulfonamide as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 12.35 (s, 1H), 9.18 (s, 1H), 8.15 (s, 1H), 7.62 (d, J = 2.6 Hz, 1H), 7.31 (s, 1H), 7.11 (dd, J = 9.1, 2.8 Hz, 1H), 6.65 (d, J = 9.1 Hz, 1H), 4.47 (t, J = 5.3 Hz, 2H), 3.53 – 3.37 (m, 6H), 3.06 (dd, J = 12.8, 6.8 Hz, 2H), 2.85 – 2.75 (m, 2H), 2.57 – 2.50 (m, 4H), 2.34 (s, 3H), 1.83 (pd, J = 6.4, 3.9 Hz, 2H), 1.37 (t, J = 5.8 Hz, 2H), 0.82 (d, J = 6.8 Hz, 6H). LRMS (APCI): calculated for $C_{27}H_{36}N_6O_5S$ 556.3 Da, measured 557.3 m/z $[M + H]^+$.

X-ray crystallography of CK-963: X-ray crystallography of CK-963 was performed to determine the absolute configuration of the 3,5-dimethylpiperidine substituent (Figure S1). The structure was solved using crystals isolated from a methanol and ethyl acetate solution with data collected at 223K. The absolute configuration of CK-963 was established to be R,R. The crystal structure is a neat form of CK-963. There are two crystallographically independent molecules in the asymmetric unit which differ in conformation. The ethylmorpholine group was disordered in both molecules and were refined over two-positions. In the crystal, hydrogen bonding interactions involve the amide group (N-H…O).

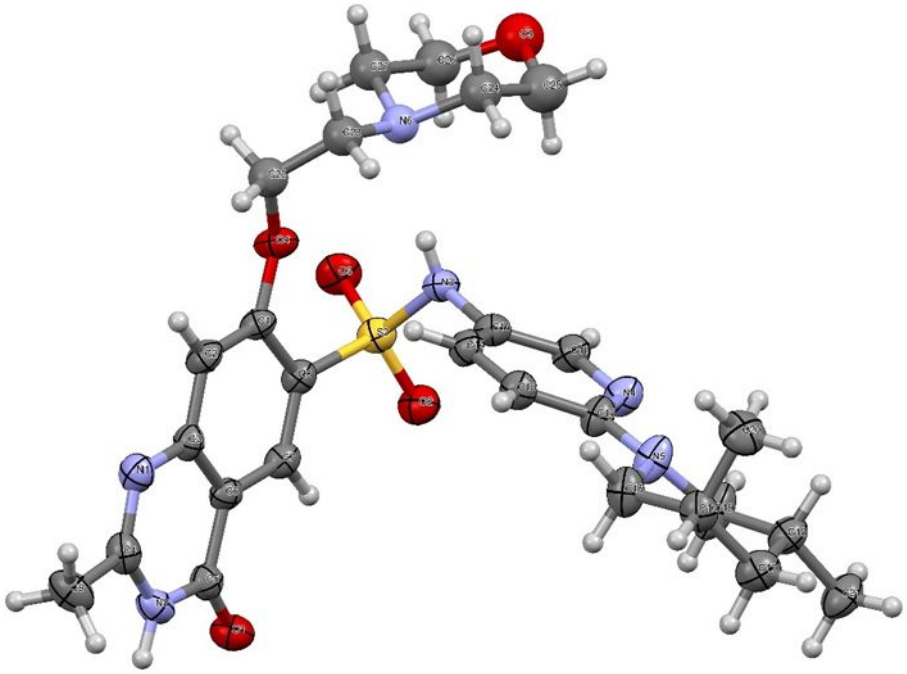


Figure S1. ORTEP drawing of four conformations in the crystal structure of CK-963 neat form, with non-hydrogen atoms in ellipsoids drawn at 50% probability and hydrogen atoms in small circles for clarity. The two crystallographically independent molecules (top and bottom) and disorders of the ethylpiperazine group over two positions (right and left) are shown.

II. Supporting information: Biology and Pharmacology

All animal experiments described in this manuscript were performed in compliance with Institutional Animal Care and Use Committee (IACUC) guidelines.

Cardiac Myofibril Assay. To evaluate the effect of compounds on the ATPase activity of full length cardiac myosin in the context of the native sarcomere, skinned myofibril assays can be performed. Bovine cardiac myofibrils can be obtained by homogenizing bovine cardiac left ventricular tissue in the presence of a detergent such as triton X-100. Such treatment removes membranes and a majority of the soluble cytoplasmic proteins but leaves intact the cardiac sarcomeric acto-myosin apparatus. Myofibril preparations retain the ability to hydrolyze ATP in a Ca^{2+} regulated manner. ATPase activities of such myofibril preparations in the presence and absence of compounds can be assayed at various activating calcium concentrations. Compounds were assessed for their ability to increase the bovine cardiac myofibril ATPase activity rate using

a pyruvate kinase and lactate dehydrogenase-coupled enzyme system that generates adenosine diphosphate (ADP) from adenosine triphosphate (ATP) by oxidizing the reduced form of nicotinamide adenine dinucleotide (NADH) to the oxidized form of nicotinamide adenine dinucleotide (NAD), producing an absorbance change at 340 nm. Myofibril ATPase assays were performed in PM12 buffer (12 mM Pipes, 2 mM $MgCl_2$, 1 mM DTT, pH 6.8) supplemented with 60 mM KCl and ATP at approximately 3-10 times the K_M for the particular myofibril system (0.5 mM ATP for fast skeletal, 0.05 mM ATP for slow skeletal and cardiac). Calcium concentrations were controlled using 0.6 mM EGTA and sufficient $CaCl_2$ to obtain the desired free calcium concentration (calculated using web resource https://somapp.ucdmc.ucdavis.edu/pharmacology/bers/maxchelator/webmaxc/webmaxcS.htm). The calcium concentration in our weekly biochemical assay was determined by the amount of calcium needed to achieve 25% of the maximum calcium-dependent activation in a control experiment. The specific calcium levels varied slightly depending on test occasion but was approximately pCa 6.5. Myofibrils were present at approximately 0.25 mg/mL (fast skeletal) or 1 mg/mL (slow skeletal, cardiac). Absorbance measurements (340 nm) were carried out at approximately 25 °C using either an Envision (Perkin-Elmer) or SpectraMax (Molecular Devices) plate reader. Data analysis was performed with GraphPad Prism V7.04 (GraphPad Software, San Diego, CA).

Table S1. **Standard deviation for cardiac myofibril biochemical data**

compound number	number of tests	cardiac biochemical median (μM)	cardiac standard deviation
1	8	5.5	1.7
2	2	1.1	0.1
3	4	<0.076	<0.004
4	1	>39	-
5	4	<0.076	-
6	2	>39	-
7	1	5.3	-
8	2	>39	-
9	1	18.0	-
10	2	>30	>12.6
11	2	4.3	1.7
12	2	>33	>9.4

13	2	0.5	0.1
14	2	21.9	2.0
15	8	11.1	4.4
16	5	4.5	1.1
17	203	1.4	0.5
18	202	0.7	0.2
19	241	0.7	0.3

Muscle Isoform Selectivity. Compound specificity with respect to muscle type was evaluated by comparing the effect of the compound on calcium stimulated ATPase activity of the following muscle systems: cardiac myofibrils (bovine), slow skeletal myofibrils (bovine masseter), fast skeletal muscle myofibrils (rabbit), and smooth muscle myosin (chicken gizzard) using a 10 point dose response up to a concentration of 40 µM. Muscle isoform selectivity data for CK-963 is shown in Figure S2.

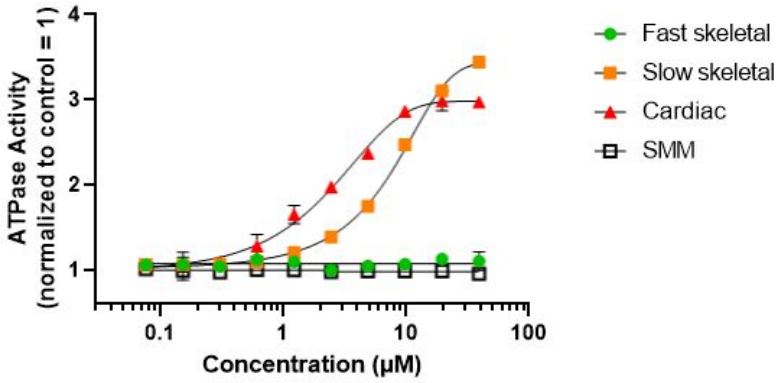


Figure S2. Muscle isoform selectivity data for CK-963.

Rat Cardiomyocyte Assay.

Preparation of adult cardiac ventricular rat myocytes. Adult male Sprague-Dawley rats are anesthetized with a mixture of isoflurane gas and oxygen. Hearts are quickly excised, rinsed, and the ascending aorta cannulated. Continuous retrograde perfusion is initiated on the hearts at a perfusion pressure of 60 cm H_2O. Hearts are first perfused with a nominally Ca^{2+} free modified Krebs solution of the following composition: 113 mM NaCl, 4.7 mM KCl, 0.6 mM KH_2PO_4, 0.6 mM Na_2HPO_4, 1.2 mM $MgSO_4$, 12 mM $NaHCO_3$, 10 mM $KHCO_3$, 30 mM taurine, 5.5 mM glucose, and 10 mM Hepes (all from Sigma). This medium is not recirculated and is continually gassed with 95% O_2/5% CO_2. After approximately 3 minutes, the heart is perfused with modified

Krebs buffer supplemented with collagenase (Worthington) and a 12.5 μM final calcium concentration until the heart becomes sufficiently blanched and soft. The heart is removed from the cannulae, the atria and vessels discarded, and the ventricles are gently torn to small pieces using forceps. The tissue is triturated using pipettes until sufficiently homogenized, and the collagenase is stopped by 10% bovine calf serum, sedimentation, and resuspension in perfusion buffer containing 5% BCS and 12.5 μM $CaCl_2$. Myocytes are made calcium tolerant by stepwise addition of a $CaCl_2$ solution to a final concentration of 1.2 mM. Cells are wash cells in Tyrode's buffer and re-suspend in 50 mL Tyrode's (137 mM NaCl, 3.7 mM KCl, 0.5 mM $MgCl_2$, 11 mM glucose, 4 mM Hepes, and 1.2 mM $CaCl_2$, pH 7.4). Cells are kept for 60 min at 37 °C prior to initiating experiments and used within 5 hours of isolation. Cells are used only if cells QC criteria is met by responding to a standard (>150% of basal) and isoproterenol (ISO; > 250% of basal). Additionally, only cells whose basal contractility is between 3 and 8 % are used in subsequent experiments.

Adult ventricular myocyte contractility experiments. Aliquots of Tyrode buffer containing myocytes are placed in perfusion chambers (series 20 RC-27NE; Warner Instruments) complete with heating platforms. Myocytes are allowed to attach, the chambers heated to 37 °C, and the cells then perfused with 37 °C Tyrode buffer. Myocytes are field stimulated at 1 Hz with platinum electrodes (20% above threshold). Only cells that have clear striations and are quiescent prior to pacing are used for contractility experiments. To determine basal contractility, myocytes are imaged through a 40x objective and using a variable frame rate (60-240 Hz) charge-coupled device camera, and the images are digitized and displayed on a computer screen at a sampling speed of 240 Hz. Frame grabber, myopacer, acquisition, and analysis S52 software for cell contractility are available from IonOptix (Milton, MA). Once cell contraction is stable over time, test compounds (0.01 – 15 μM) are perfused on the myocytes for 5 minutes. Using edge detection strategy, contractility of the myocytes and contraction and relaxation velocities are continuously recorded.

Contractility analysis: Five or more individual myocytes are tested per compound, using two or more different myocyte preparations. For each cell, twenty or more contractility transients at basal (defined as 1 minute prior to compound infusion), and after compound addition (defined as 5 minute after starting compound perfusion) are averaged and compared. These average transients are analyzed using the IonWizard (IonOptix) software to determine changes in diastolic length and

fractional shortening. Fractional shortening is calculated as: ((resting length – length at peak contraction) divided by the resting length). The percent change in fractional shortening from baseline is calculated as: ((post-dose fractional shortening / basal fractional shortening)*100). The percent reduction in fractional shortening from baseline is calculated as: (100 - percent change in fractional shortening from baseline). Maximum contraction and relaxation velocities (um/sec) are also determined. Results from individual cells are averaged and the SEM calculated.

Calcium transient experiments.

Fura loading. Cell permeable Fura-2 (Invitrogen) is dissolved in dry DMSO to a final concentration of 1 mM and added to myocytes to a final concentration of 1 μM. Fura- loading is achieved by incubation for 5 minutes in the dark at room temperature, followed by two washes with Tyrode's buffer separated by a 5 minute incubation period. Simultaneous contractility and calcium measurements are determined within 40 minutes of loading.

Imaging: After baseline data has been collected, a test compound is perfused on cells. Simultaneous contractility and calcium transient ratios are determined at baseline and after compound addition. Cells are digitally imaged and contractility determined as described above, using a red filter in the light path to avoid interference with fluorescent calcium measurements. Acquisition, analysis software and hardware for calcium transient analysis are obtained from IonOptix. The instrumentation for fluorescence measurement includes a xenon arc lamp and a Hyperswitch dual excitation light source that alternates between wavelengths of 340 and 380 nm at 100 Hz by a galvo-driven mirror. A liquid filled light guide delivers the dual excitation light to the microscope and the emission fluorescence is determined using a photomultiplier tube (PMT). The fluorescence system interface routes the PMT signal and the ratios are recorded using the IonWizard acquisition program. Background fluorescence is determined from a cell-free section of the slide and subtracted from the raw calcium signal.

Analysis: For each cell, ten or more contractility and calcium ratio transients before and after compound addition are averaged and compared. These average transients are analyzed using the IonWizard (IonOptix) software to determine changes in diastolic length and fractional shortening as in the contractility analysis above. Additionally, the averaged calcium ratio transients are analyzed to determine changes in diastolic and systolic ratios and the 75% time to baseline (T_{75}).

Data for fractional shortening and calcium transient measurements in adult rat ventricular myocytes for **2** is shown in Table S2.

Table S2: Effect of **2** (10 μM) on simultaneous fractional shortening and calcium transient measurements in adult rat ventricular myocytes

Treatment	N	FS (% of basal)	Cell Length (% of basal)	CV (% of basal)	RV (% of basal)	Time to Peak (% of basal)	Relaxation T_{50} (% of basal)	Diastolic Fura-Ratio	Systolic Fura Ratio	T_{75} (seconds)
Basal	5	100	100	100	100	100	100	0.84 +/- 0.08	1.11 +/- 0.11	0.27 +/- 0.02
10 μM **2**	5	173 +/- 16*	96 +/- 0.8	124 +/- 14	123 +/- 15	133 +/- 9	130 +/- 9	0.81 +/- 0.09	1.04 +/- 0.11*	0.31 +/- 0.02

Basal reference values: diastolic cell length = 140.6 ± 4.6 μm, fractional shortening (FS) = 5.12 ± 0.56 μm, contraction velocity (CV) = 137.1 ± 30.2 μm/sec, relaxation velocity (RV) = 55.4 ± 18.2 μm/sec, time to peak = 0.162 ±.0.023 sec, and time to baseline (T_{50}) = 0.308 ± 0.059 sec.

Echocardiography assessment of acute pharmacodynamic effect on rat cardiac contractility

Assessment of *in vivo* cardiac function by echocardiography was performed in male Sprague Dawley rats (Charles River Laboratories) under isoflurane (1-5%) anesthesia. CK-963 was formulated in 40% hydroxypropyl beta-cyclodextrin in water and administered by intravenous infusion at a continuous rate or by stepwise increases in the infusion rate every 15 minutes over a 80-120 minute period over cumulative dose ranges from 28.5 to 199 mg/kg. Two dimensional M-mode images of the left ventricle in parasternal long axis view and blood samples were collected every 5 -10 minutes over the course of the infusion period. Fractional shortening, the primary measure of cardiac contractility, was determined by analysis of the M-mode images with the following calculation: ((end diastolic diameter – end systolic diameter) / end diastolic diameter x 100). Whole blood samples were centrifuged to obtain the resulting plasma for subsequent compound analysis by LC/MS.

Target Determination: Sarcomere Component Swap Experiment. To determine which component of the sarcomere contained the target, a muscle component swap experiment using rabbit fast skeletal myosin F(S1), bovine cardiac myosin C(S1), rabbit fast skeletal troponin complex F(TF), and bovine cardiac troponin complex C(TF) was performed. These components were used to generate the four possible myosin/troponin complex combinations. Compounds were then assessed for their ability to increase ATPase rate for these reconstituted sarcomeres at a calcium concentration that generates 25% of the maximal response. The mix and match data for CK-963 is shown in Figure S3.

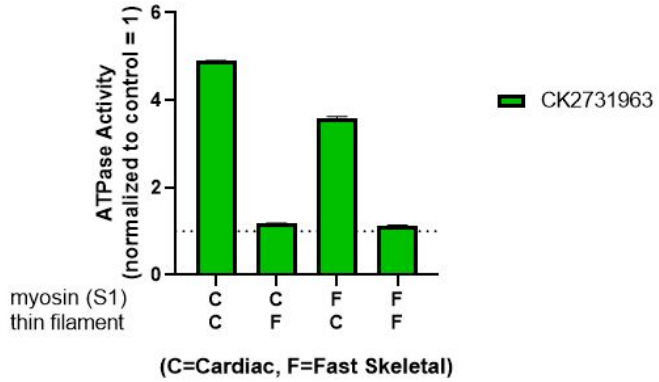


Figure S3. Mix and match data for compound CK-963.

Isothermal Titration Calorimetry Studies. A solution of 450 μM recombinant human 6xHis-ENLYFQG-cNTnC(2-91)-AGAG-cTnI(147-180) was titrated into 30 μM CK-963. Compound and target protein were prepared in 12 mM Pipes, 100 mM KCl, 0.25 mM $CaCl_2$, 5 mM β-mercaptoethanol, and 3% DMSO. Binding reactions were carried out at 25 °C, and the integrated heat from the last two or three injections were averaged and subtracted from all points to control for slight buffer mismatches and heats of dilution. Data were fit to a single binding site model. One representative binding reaction is shown in Figure S3. Parameters for binding of CK-963 to cNTnC-cTnIswitch as measured by isothermal titration calorimetry is shown in Table S4.

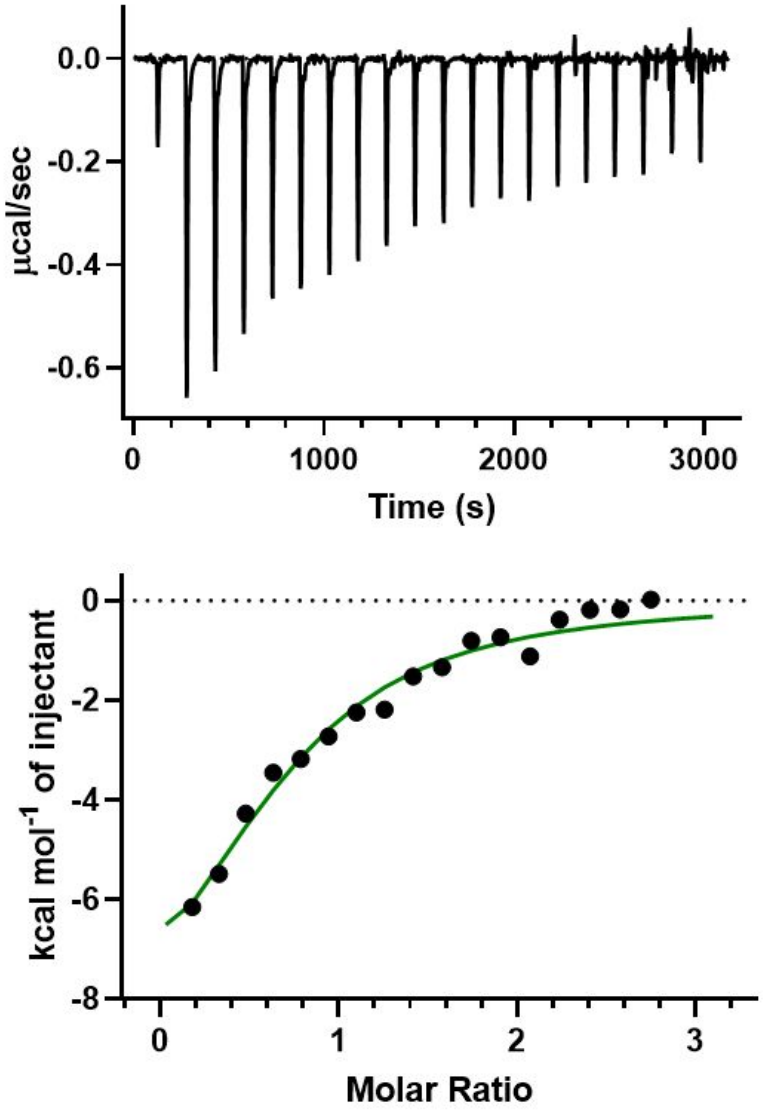


Figure S4. Representative binding reaction of CK-963 to cNTnC-cTnI$_{switch}$.

Table S3: Parameters for binding of CK-963 to cNTnC-cTnI$_{switch}$ as measured by isothermal titration calorimetry

Parameter	Mean± SD (n=7)
Stoichiometry (N, ligand/protein)	1.39 ± 0.19
Dissociation Constant (K_D, µM)	11.5 ± 3.2
Enthalpy (ΔH, kcal/mol)	-10.3 ± 2.1
Entropy (ΔS, e.u.)	-12.0 ± 7.5

III. Supporting information: Safety and ADME/DMPK Assays

In Vitro **Drug Metabolism.** Microsomal turnover assays were conducted by incubating compounds in species specific liver microsome preparations at 37 ºC. Aliquots taken after 0-90 min. of incubation were quenched with acetonitrile and analyzed by HPLC/MS/MS and compared to a control compound.

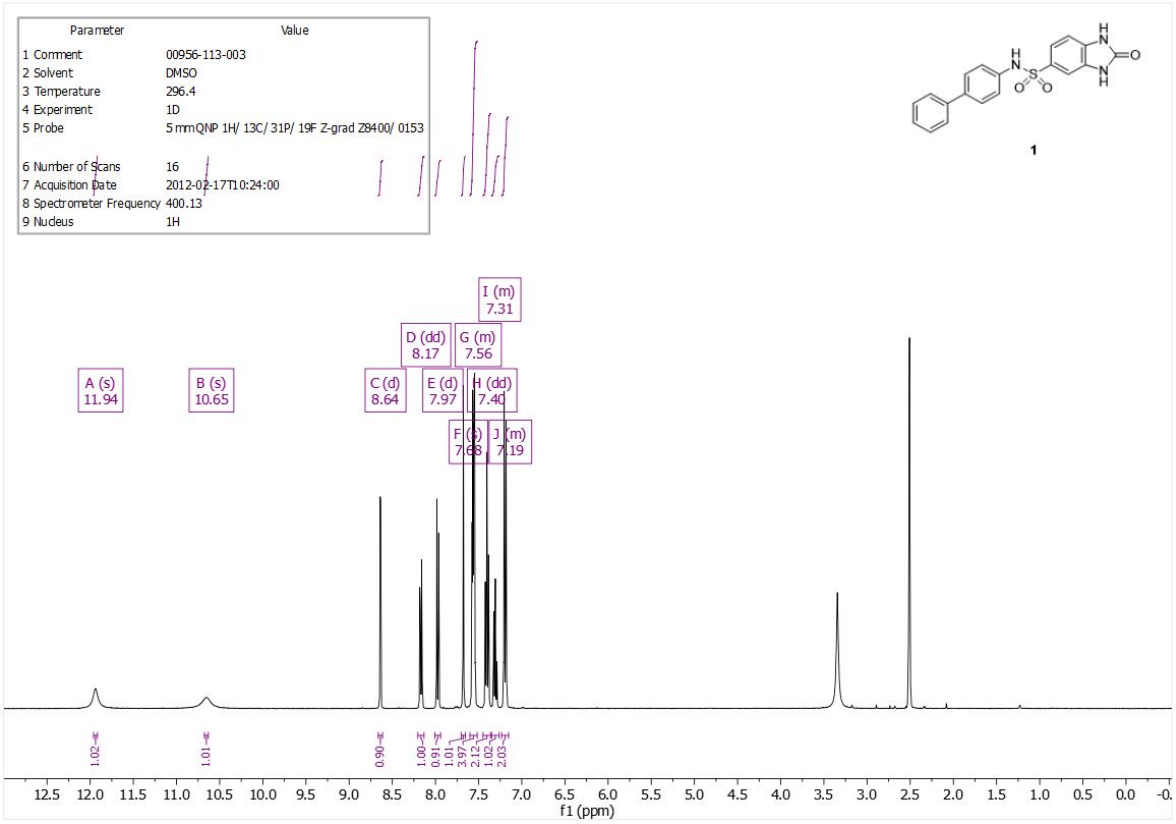
Solubility Assessment. Test article solubility was determined by agitation for 12 hours at room temperature in a 6.8 pH monopotassium phosphate JP2 buffer system followed by filtration and HPLC quantitation of the desired test article relative to a standard, with results expressed in units of μmol.

Protein Binding. Species-specific plasma protein binding measurements of compounds were carried out using equilibrium dialysis with results reported as percentage unbound to plasma for each species. Test compounds were added at 5 μM concentration to the buffer chamber and shaken vs. species-specific plasma for 6 hours at 37 ºC. Samples were taken from both buffer and plasma chambers and analyzed by HPLC to determine concentrations vs. standard solutions.

Pharmacokinetic Assessment. Pharmacokinetic parameters were determined in male Sprague-Dawley rats following single bolus intravenous administration of compound. Blood samples were taken periodically beginning immediately before compound administration to 24 h after administration. The concentrations of compound in blood or plasma samples were analyzed by HPLC relative to a standard.

PDE-3 Inhibition Assay Procedure. Test compounds were plated at various concentrations in 0.5 μl of DMSO in a 384-well white assay plate. 12.5 μl enzyme (phosphodiesterase 3a, (0.0156 μg/mL), SignalChem #p91-31G-10) in assay buffer (50 mM Tris (tris(hydroxymethyl)aminomethane), 5 mM $MgCl_2$, pH 7.5) was added to each well and incubated for 20 min at room temperature. 12.5 μl of substrate buffer (0.2 μM cAMP) in assay buffer (50 mM Tris, 5 mM $MgCl_2$, pH 7.5) was added to each well and incubated for 30 min at room temperature. 12.5 μl stop/detection buffer (stop/detection mix, reconstituted with 10 mL of reconstitution buffer, from PDELight™ HTS cAMP phosphodiesterase kit (LONZA,Cat No LT07-600), with 0.025% antifoam (Anti-Foam 204, Sigma, #A6426-500G)) was added to each well. The plate was incubated for 10 min and then read on a Perkin Elmer EnVision plate reader.

IV. Supporting information: 1H NMR Spectral Data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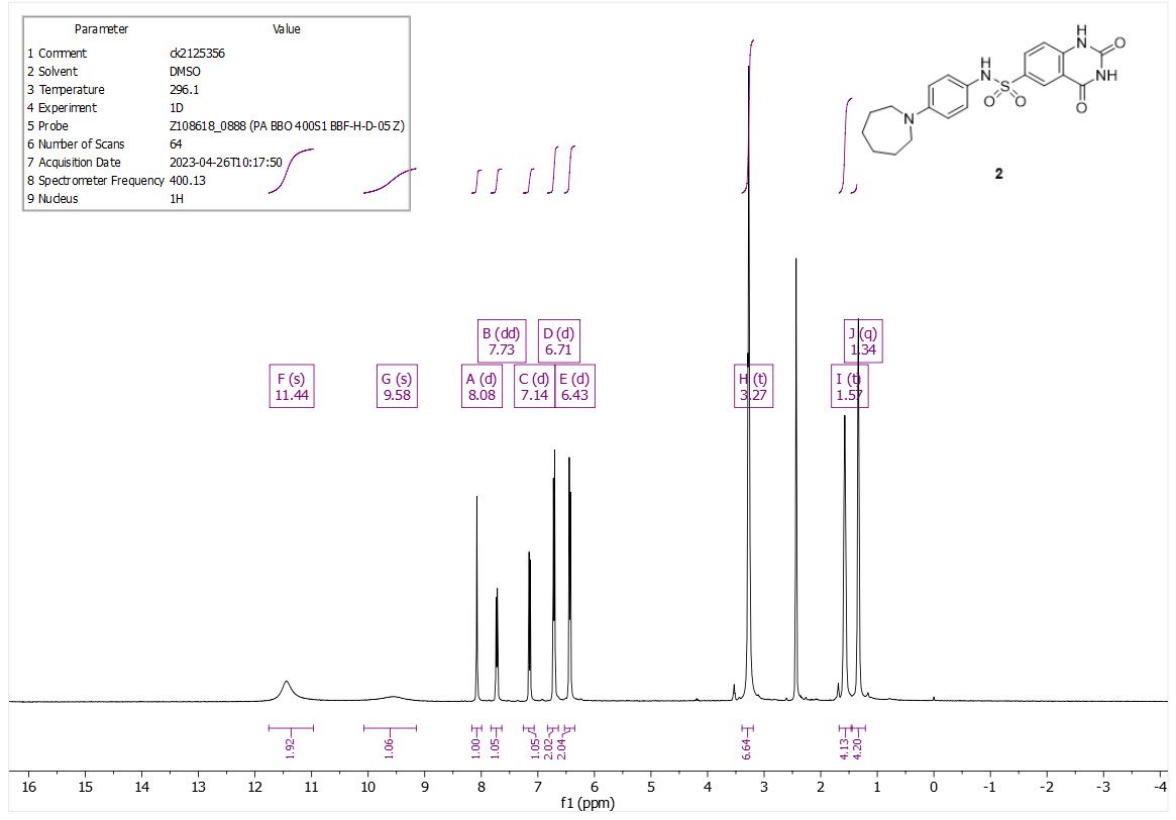
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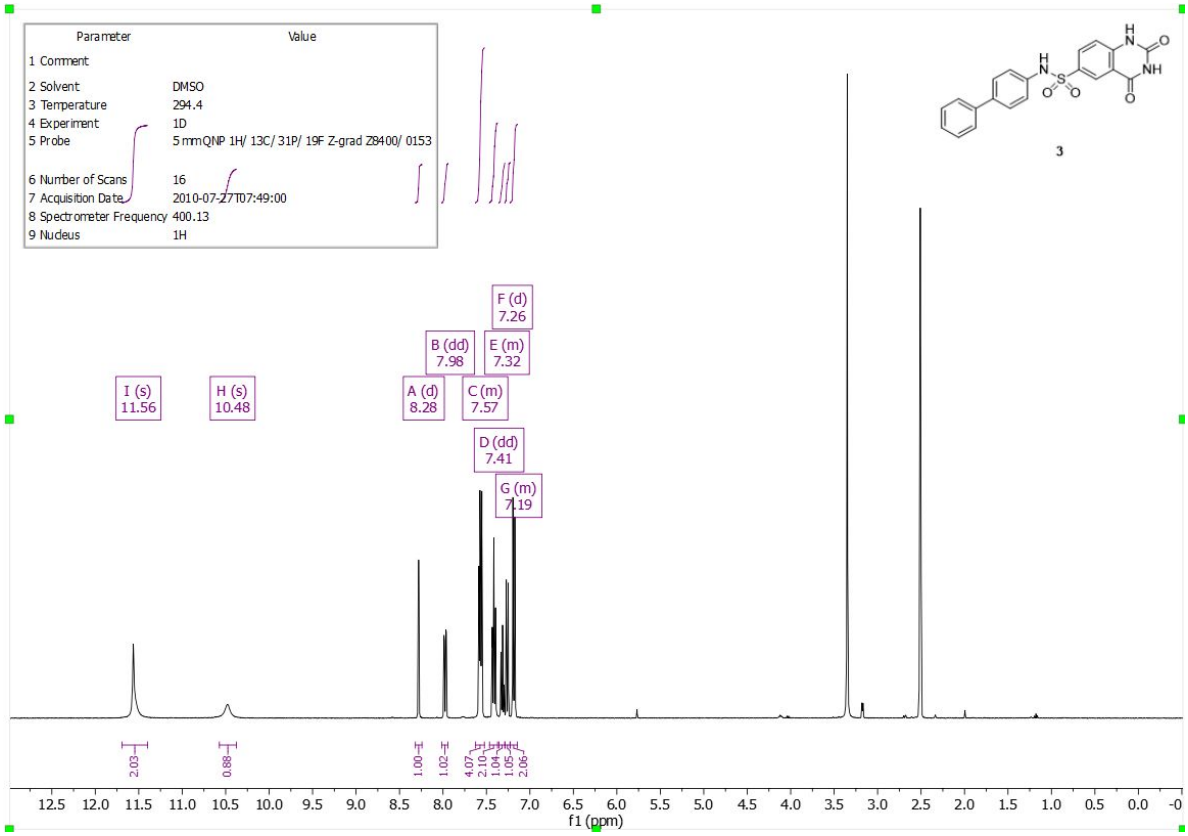
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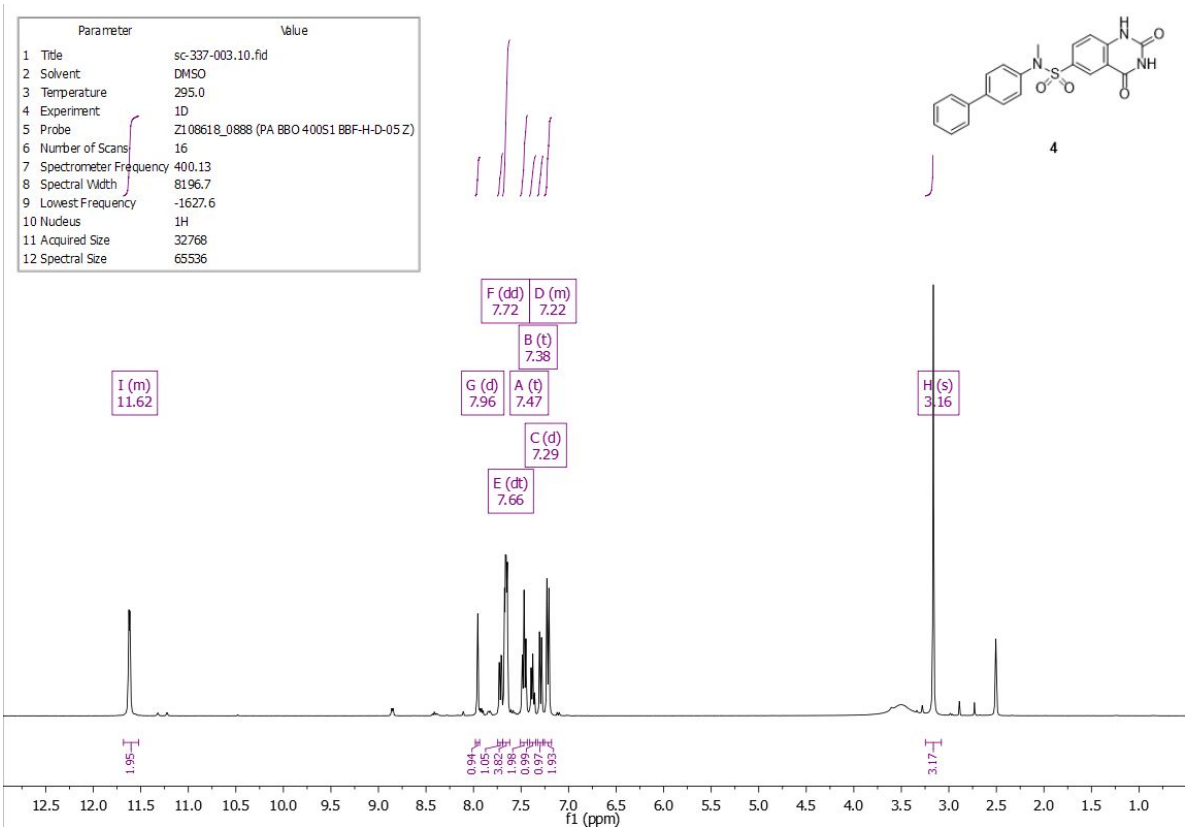
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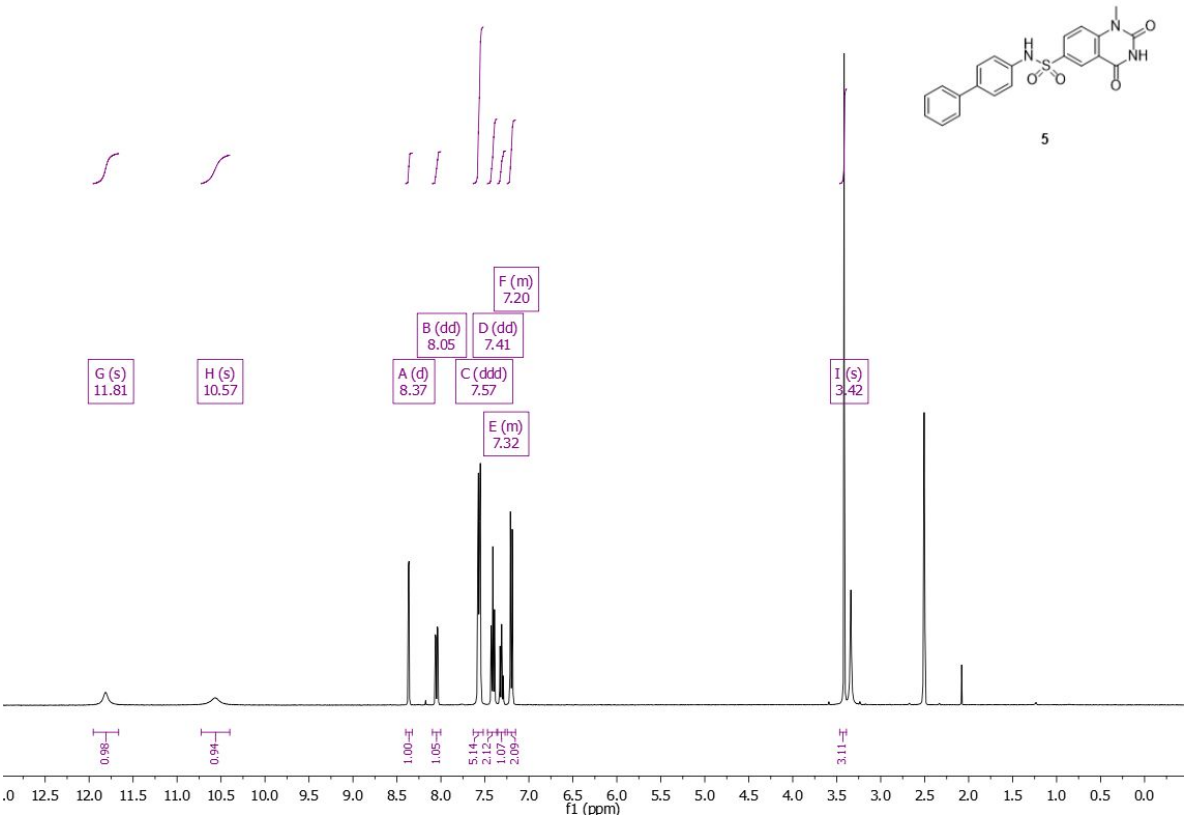
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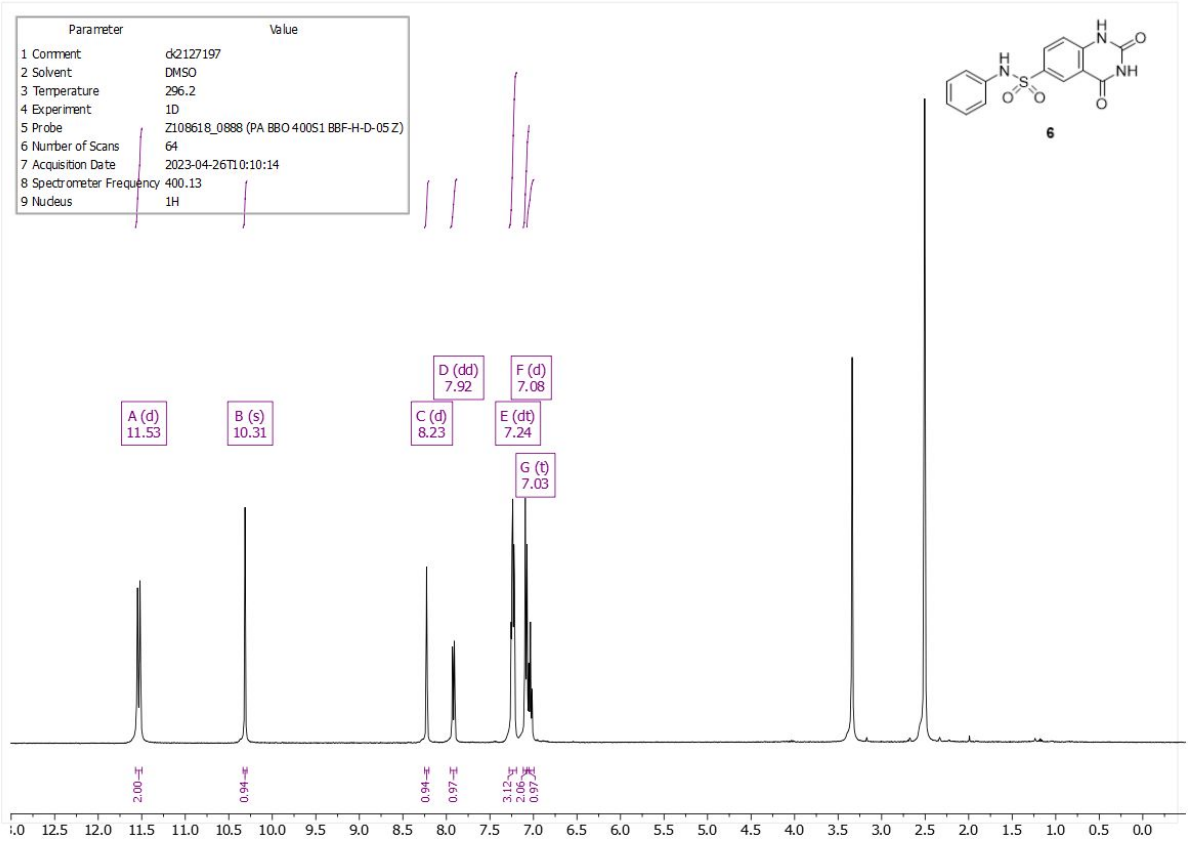
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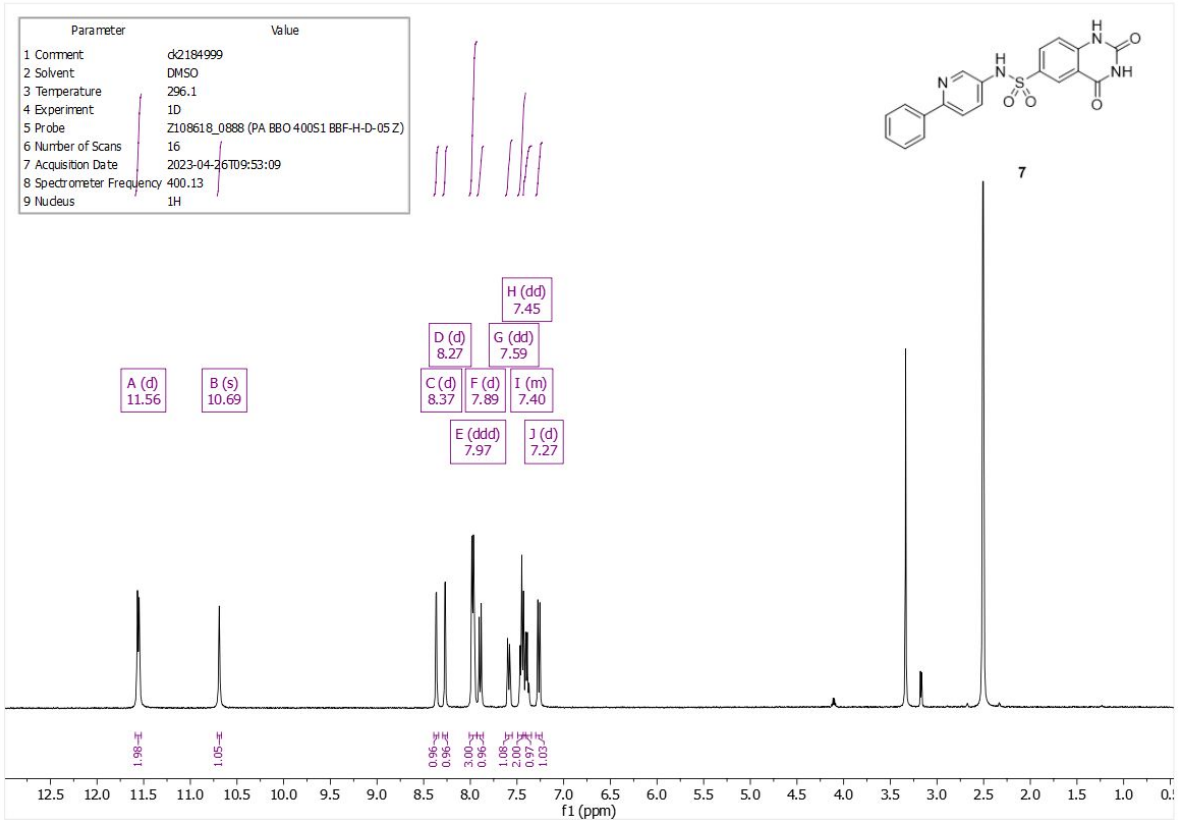
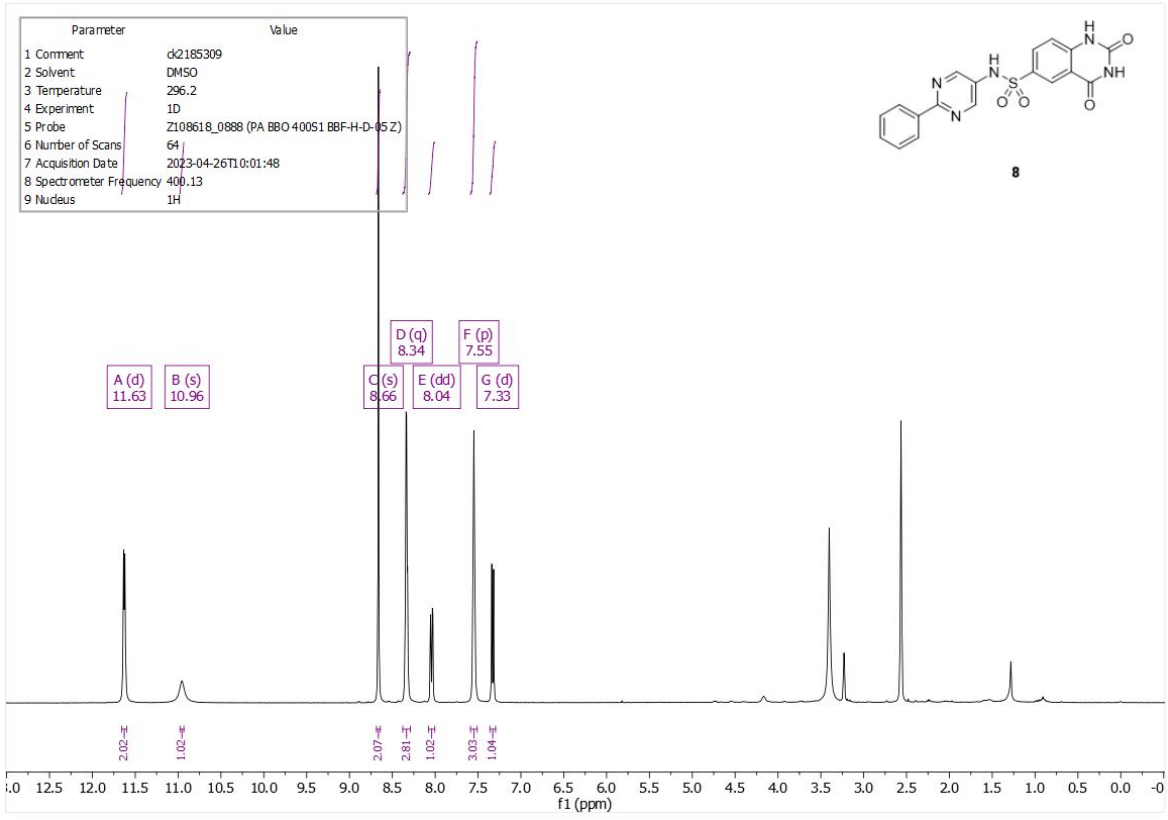
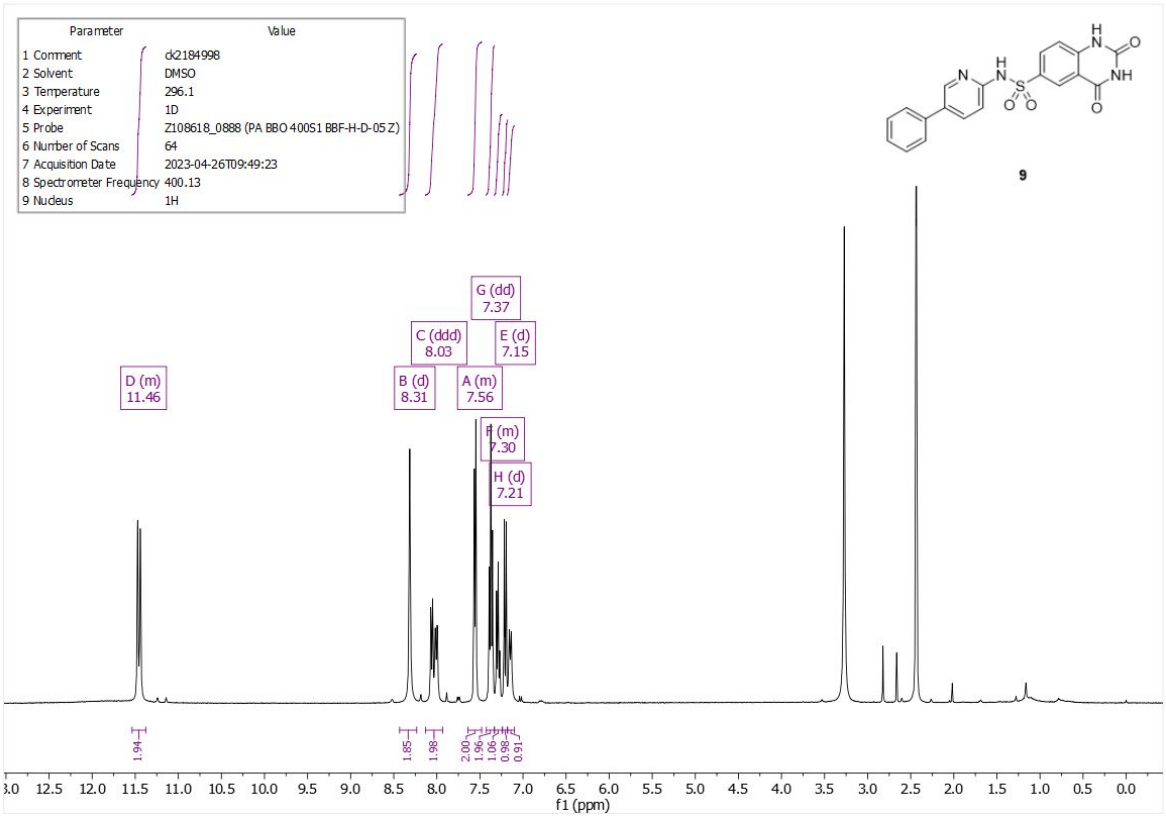
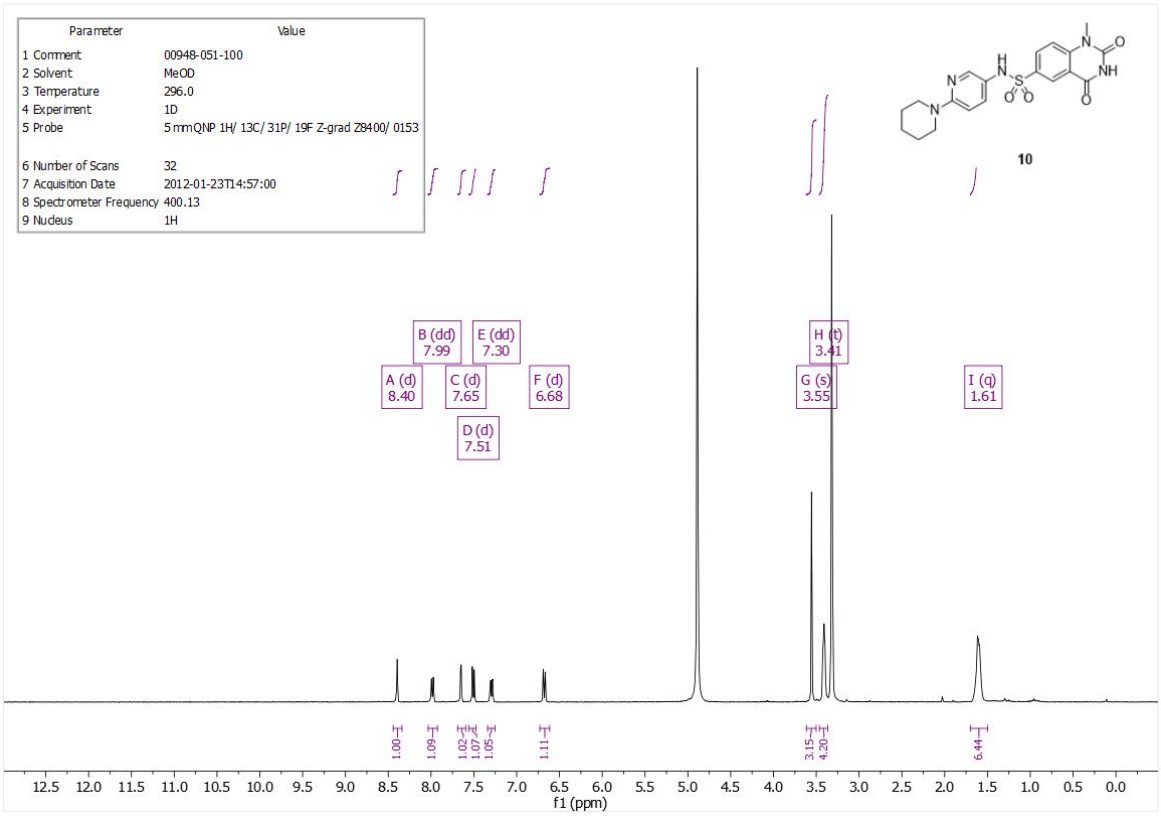
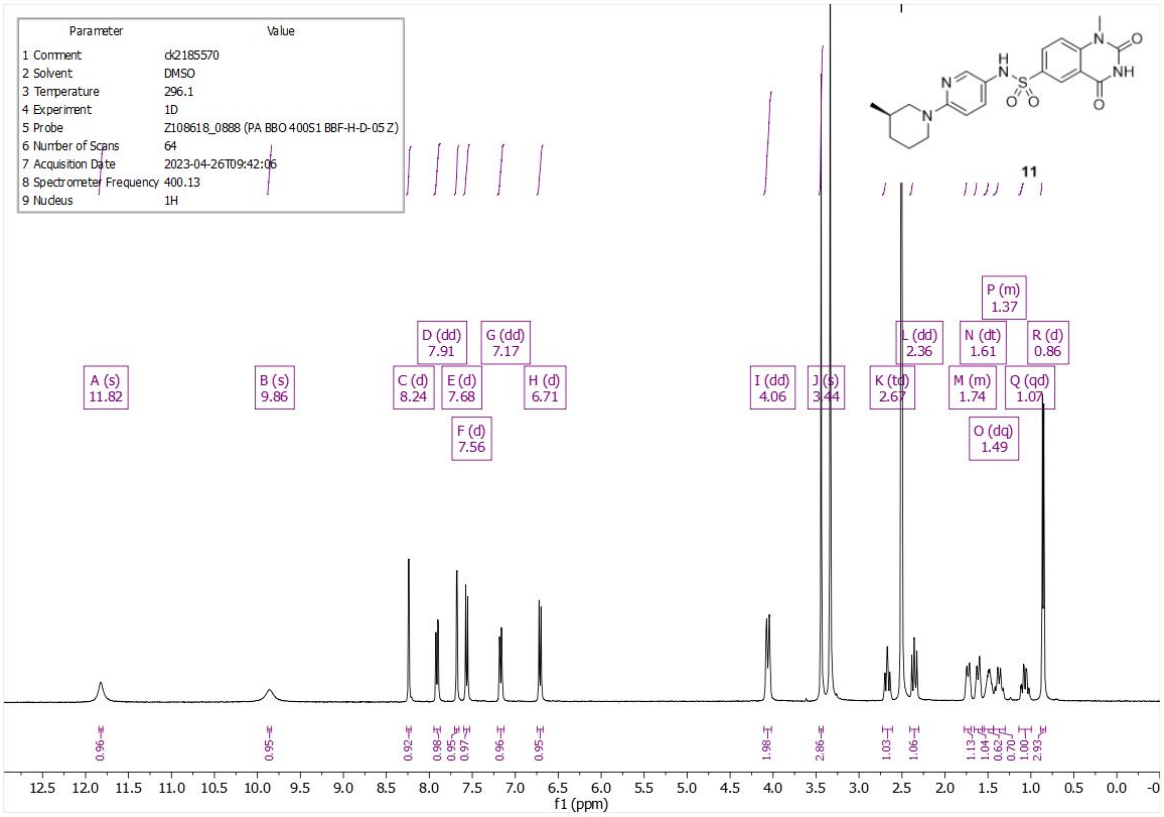
9 Nucleus 1H
6
A (d) 11.53
B (s) 10.31
C (d) 8.23
D (dd) 7.92
E (dt) 7.24
F (d) 7.08
G (t) 7.03
2.00
0.94
0.94
0.97
3.12
2.06
0.97

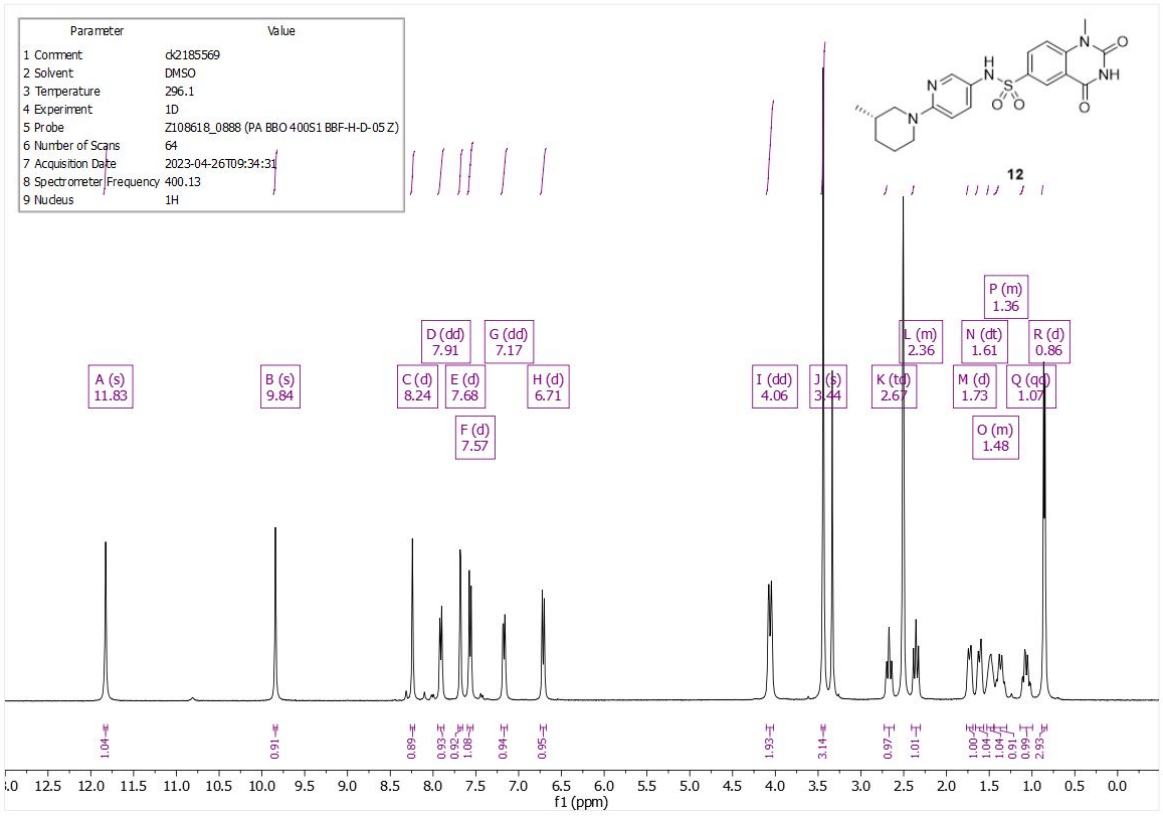

Parameter Value
1 Comment ck2184999
2 Solvent DMSO
3 Temperature 296.1
4 Experiment 1D
5 Probe Z108618_0888 (PA BBO 400S1 BBF-H-D-05 Z)
6 Number of Scans 16
7 Acquisition Date 2023-04-26T09:53:09
8 Spectrometer Frequency 400.13
9 Nucleus 1H
7
A (d) 11.56
B (s) 10.69
D (d) 8.27
C (d) 8.37
F (d) 7.89
H (dd) 7.45
G (dd) 7.59
I (m) 7.40
E (ddd) 7.97
J (d) 7.27
1.98
1.05
0.96
0.96
3.00
0.96
1.08
2.00
0.97
1.03
f1 (ppm)

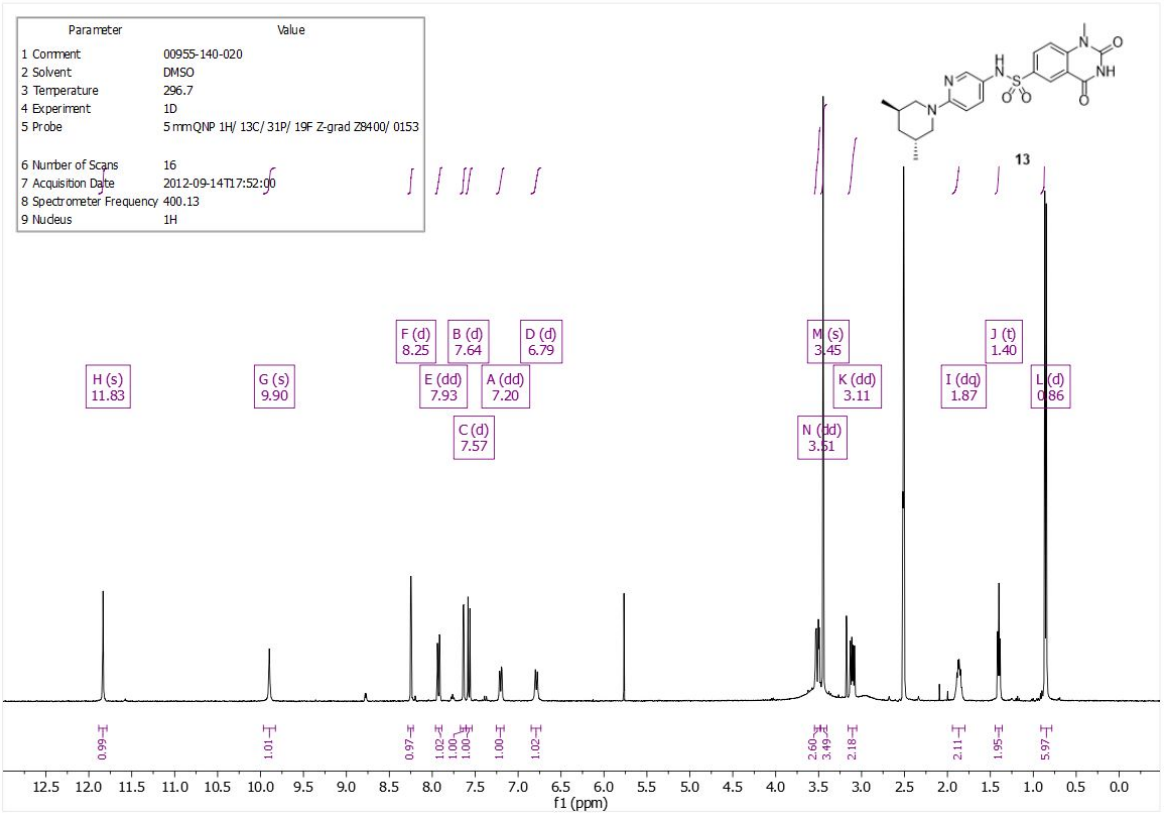

Parameter Value
1 Comment ck2185309
2 Solvent DMSO
3 Temperature 296.2
4 Experiment 1D
5 Probe Z108618_0888 (PA BBO 400S1 BBF-H-D-05 Z)
6 Number of Scans 64
7 Acquisition Date 2023-04-26T10:01:48
8 Spectrometer Frequency 400.13
9 Nucleus 1H
8
A (d) 11.63
B (s) 10.96
D (q) 8.34
C (s) 8.66
E (dd) 8.04
F (p) 7.55
G (d) 7.33
2.02
1.02
2.07
2.81
1.02
3.03
1.04
f1 (ppm)

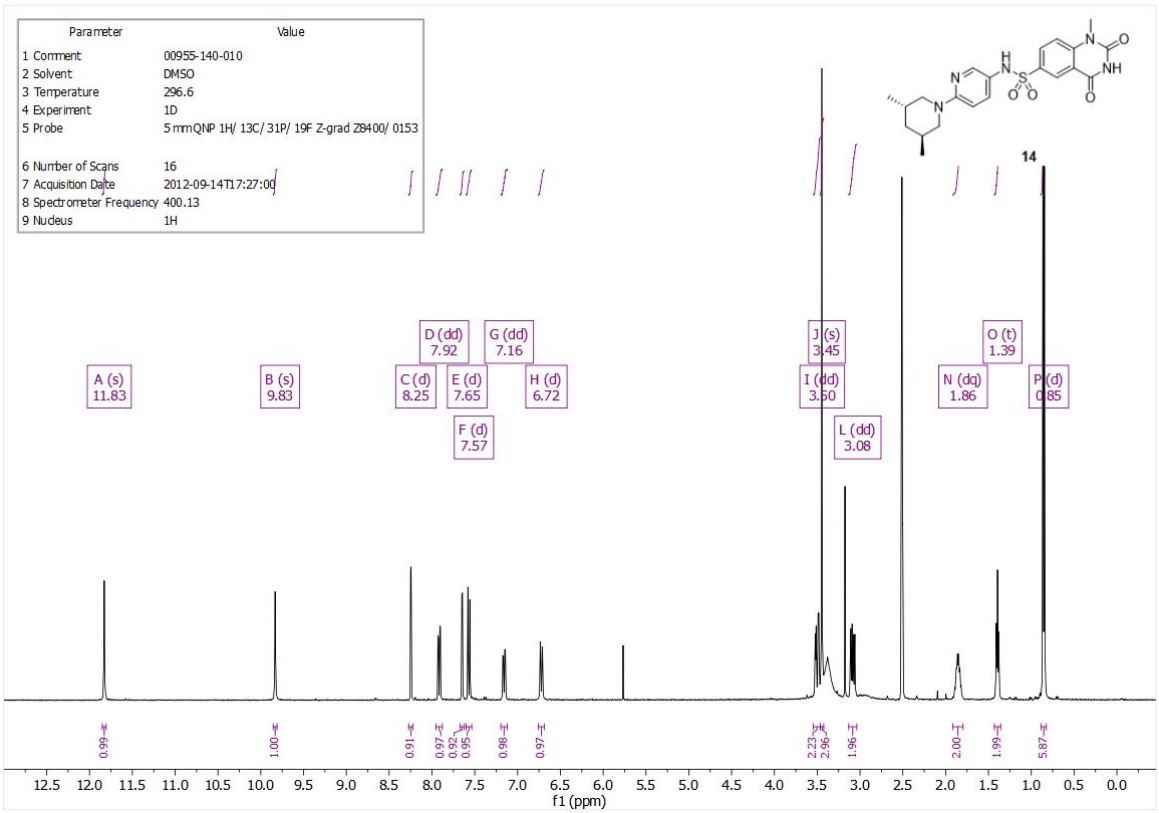

Parameter Value
1 Comment ck2184998
2 Solvent DMSO
3 Temperature 296.1
4 Experiment 1D
5 Probe Z108618_0888 (PA BBO 400S1 BBF-H-D-05 Z)
6 Number of Scans 64
7 Acquisition Date 2023-04-26T09:49:23
8 Spectrometer Frequency 400.13
9 Nucleus 1H
9
G (dd) 7.37
C (ddd) 8.03
E (d) 7.15
D (m) 11.46
B (d) 8.31
A (m) 7.56
F (m) 7.30
H (d) 7.21
1.94
1.85
1.98
2.00
1.96
1.06
0.98
0.91
f1 (ppm)

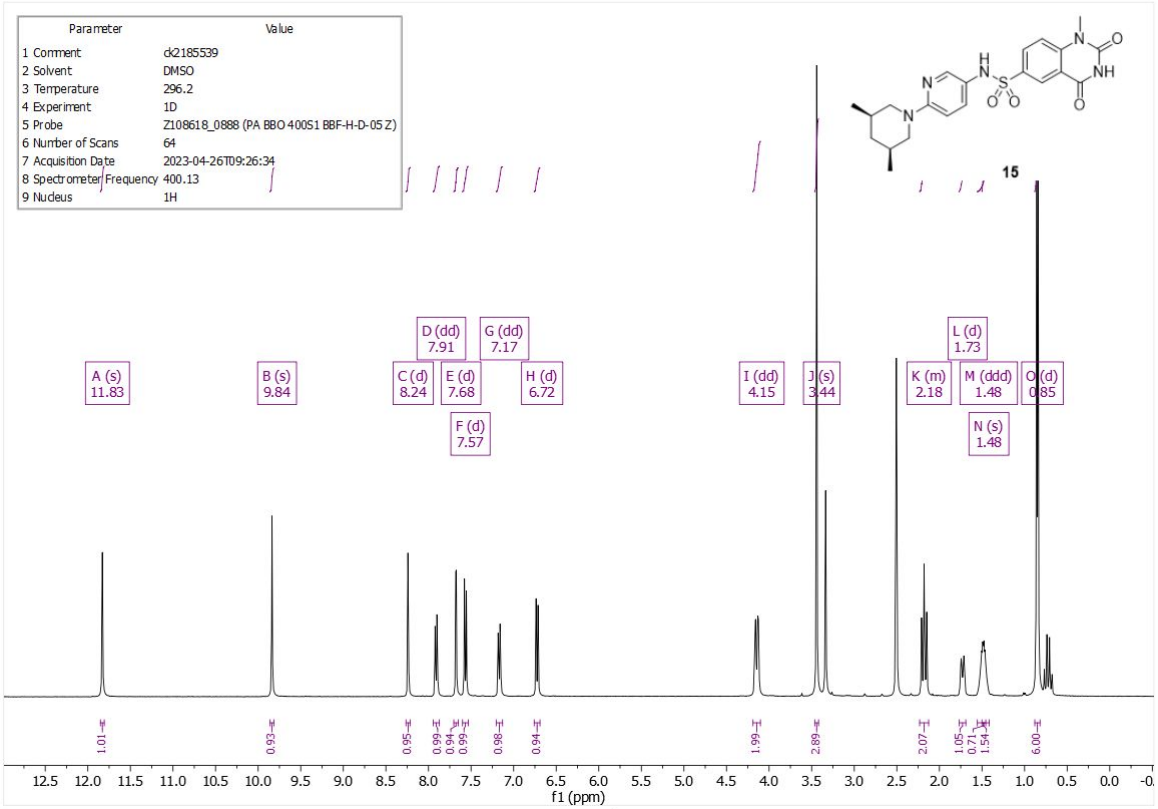

Parameter Value
1 Comment 00948-051-100
2 Solvent MeOD
3 Temperature 296.0
4 Experiment 1D
5 Probe 5 mm QNP 1H/ 13C/ 31P/ 19F Z-grad Z8400/ 0153
6 Number of Scans 32
7 Acquisition Date 2012-01-23T14:57:00
8 Spectrometer Frequency 400.13
9 Nucleus 1H
10
B (dd) 7.99
E (dd) 7.30
H (t) 3.41
A (d) 8.40
C (d) 7.65
F (d) 6.68
G (s) 3.55
I (q) 1.61
D (d) 7.51
1.00
1.09
1.02
1.07
1.05
1.11
3.15
4.20
6.44
f1 (ppm)

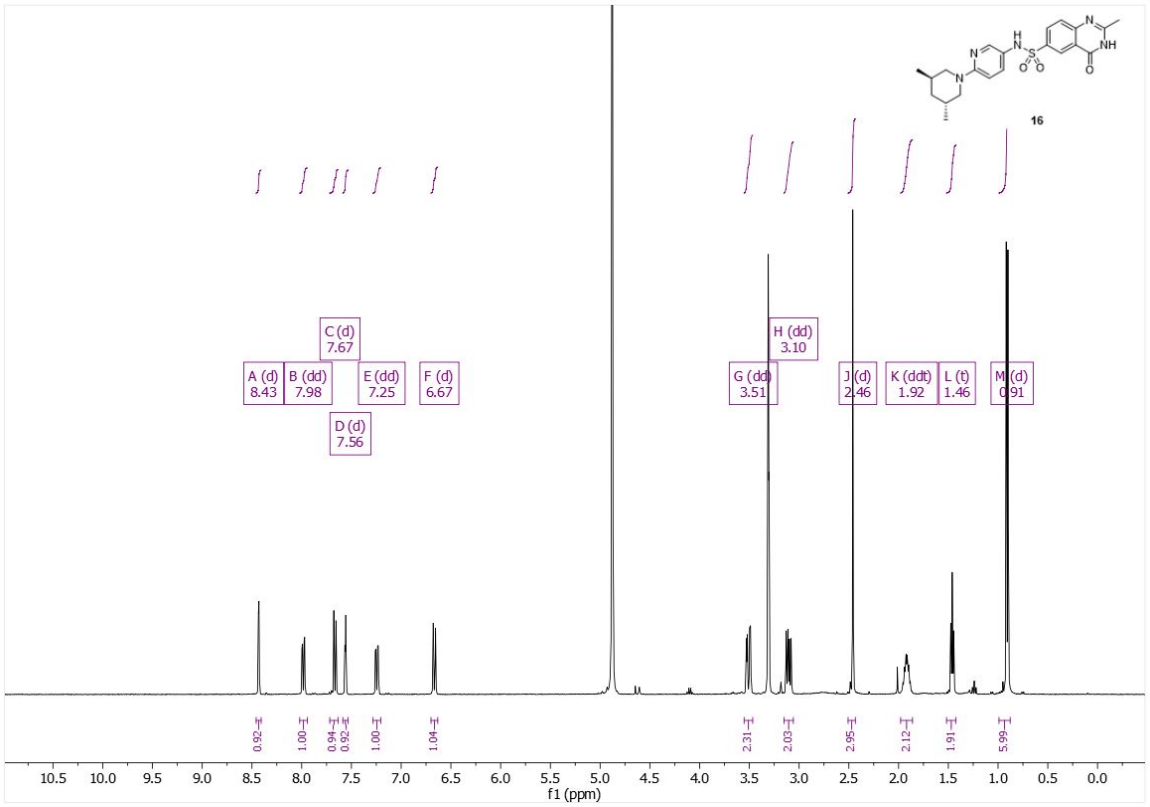

Parameter Value
1 Comment dk2185570
2 Solvent DMSO
3 Temperature 296.1
4 Experiment 1D
5 Probe Z108618_0888 (PA BBO 400S1 BBF-H-D-05 Z)
6 Number of Scans 64
7 Acquisition Date 2023-04-26T09:42:06
8 Spectrometer Frequency 400.13
9 Nucleus 1H
11
A (s) 11.82
B (s) 9.86
C (d) 8.24
D (dd) 7.91
E (d) 7.68
F (d) 7.56
G (dd) 7.17
H (d) 6.71
I (dd) 4.06
J (s) 3.44
K (td) 2.67
L (dd) 2.36
M (m) 1.74
N (dt) 1.61
O (dq) 1.49
P (m) 1.37
Q (qd) 1.07
R (d) 0.86
f1 (ppm)

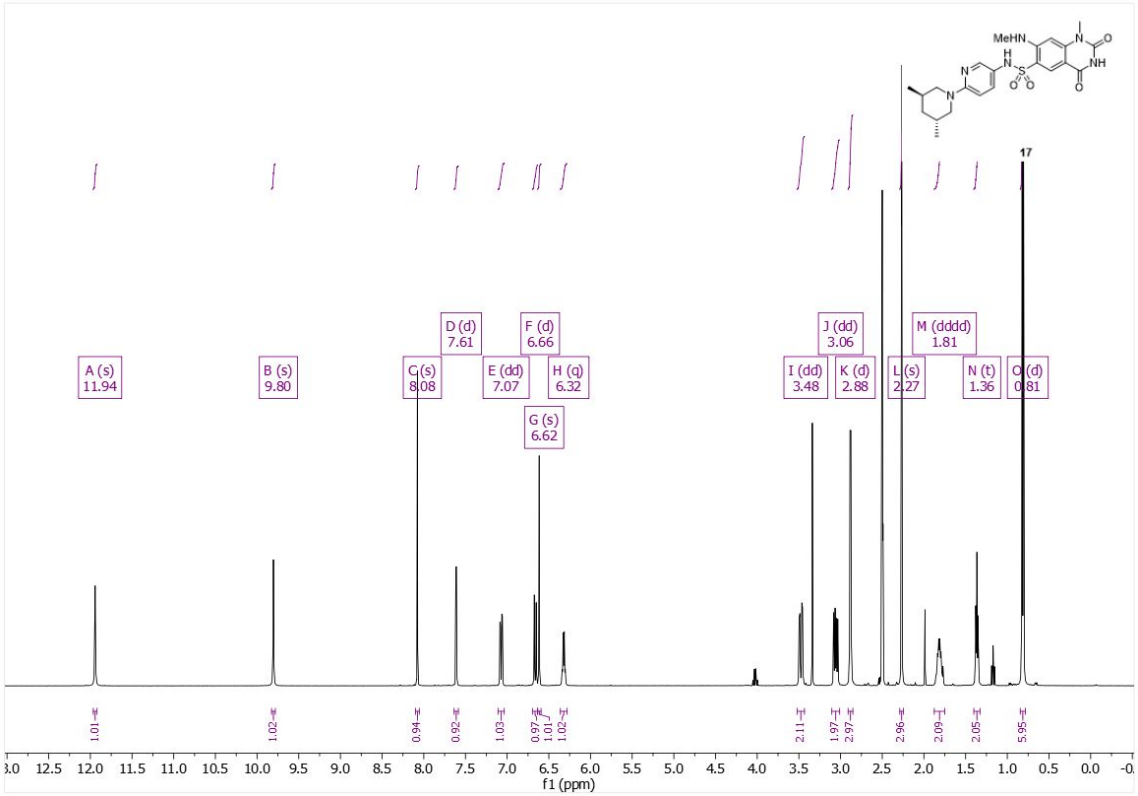

Parameter Value
1 Comment dk2185569
2 Solvent DMSO
3 Temperature 296.1
4 Experiment 1D
5 Probe Z108618_0888 (PA BBO 400S1 BBF-H-D-05 Z)
6 Number of Scans 64
7 Acquisition Date 2023-04-26T09:34:31
8 Spectrometer Frequency 400.13
9 Nucleus 1H
12
A (s) 11.83
B (s) 9.84
C (d) 8.24
D (dd) 7.91
E (d) 7.68
F (d) 7.57
G (dd) 7.17
H (d) 6.71
I (dd) 4.06
J (s) 3.44
K (td) 2.67
L (m) 2.36
M (d) 1.73
N (dt) 1.61
O (m) 1.48
P (m) 1.36
Q (qd) 1.07
R (d) 0.86
f1 (ppm)

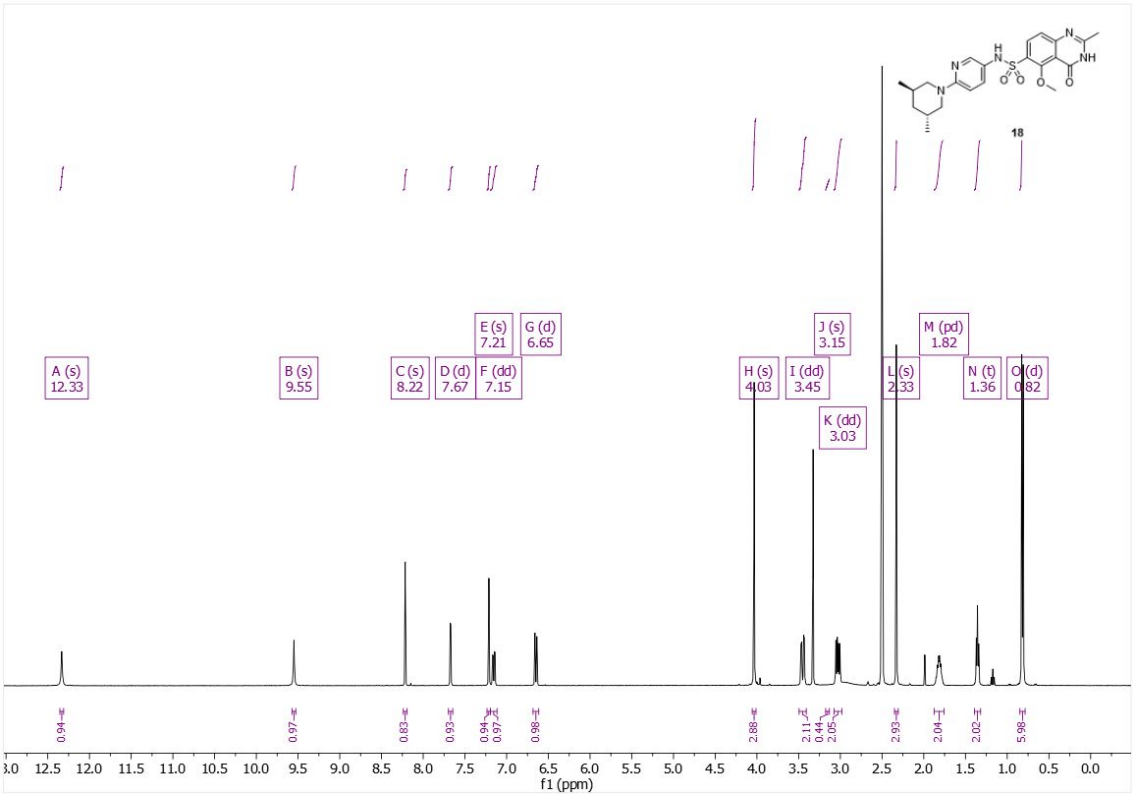

Parameter Value
1 Comment 00955-140-020
2 Solvent DMSO
3 Temperature 296.7
4 Experiment 1D
5 Probe 5 mm QNP 1H/ 13C/ 31P/ 19F Z-grad Z8400/ 0153
6 Number of Scans 16
7 Acquisition Date 2012-09-14T17:52:00
8 Spectrometer Frequency 400.13
9 Nucleus 1H
13
H (s) 11.83
G (s) 9.90
F (d) 8.25
B (d) 7.64
D (d) 6.79
E (dd) 7.93
A (dd) 7.20
C (d) 7.57
M (s) 3.45
K (dd) 3.11
N (dd) 3.51
J (t) 1.40
I (dq) 1.87
L (d) 0.86
f1 (ppm)

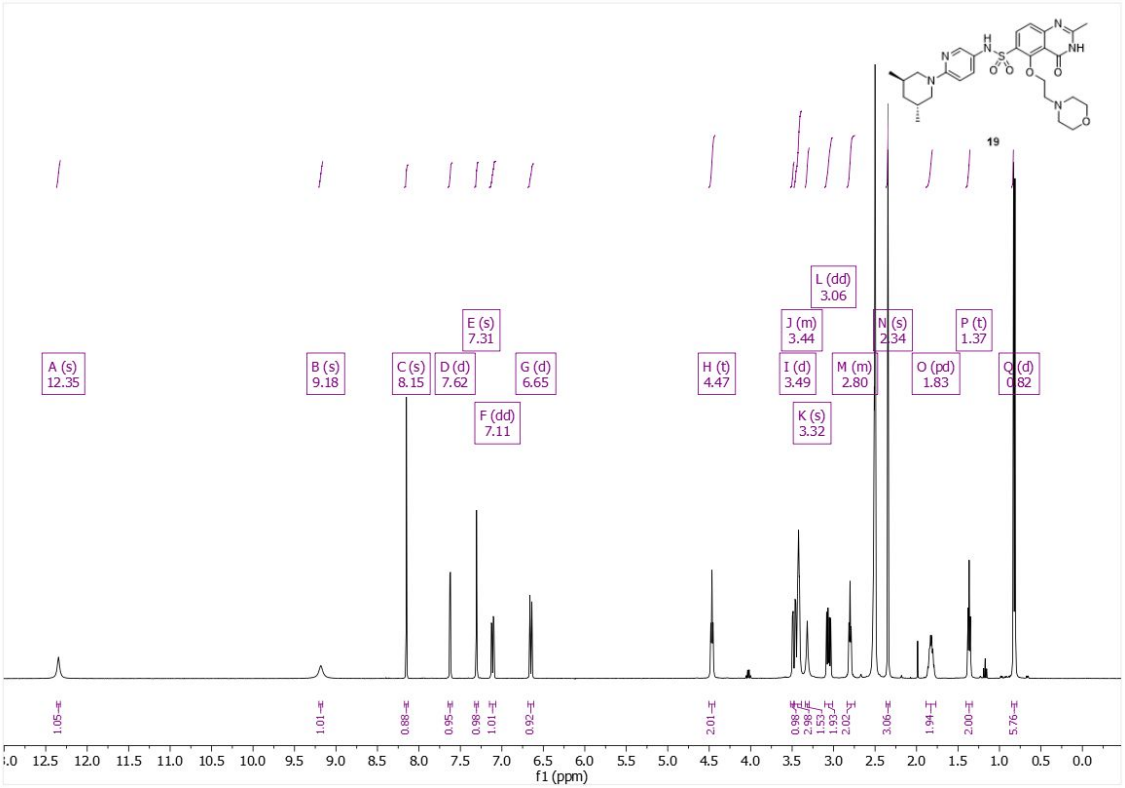

Parameter Value
1 Comment 00955-140-010
2 Solvent DMSO
3 Temperature 296.6
4 Experiment 1D
5 Probe 5 mm QNP 1H/ 13C/ 31P/ 19F Z-grad Z8400/ 0153
6 Number of Scans 16
7 Acquisition Date 2012-09-14T17:27:00
8 Spectrometer Frequency 400.13
9 Nucleus 1H
14
A (s) 11.83
B (s) 9.83
D (dd) 7.92
G (dd) 7.16
C (d) 8.25
E (d) 7.65
H (d) 6.72
F (d) 7.57
J (s) 3.45
I (dd) 3.50
L (dd) 3.08
O (t) 1.39
N (dq) 1.86
P (d) 0.85
f1 (ppm)


Parameter Value
1 Comment dk2185539
2 Solvent DMSO
3 Temperature 296.2
4 Experiment 1D
5 Probe Z108618_0888 (PA BBO 400S1 BBF-H-D-05 Z)
6 Number of Scans 64
7 Acquisition Date 2023-04-26T09:26:34
8 Spectrometer Frequency 400.13
9 Nucleus 1H
15
A (s) 11.83
B (s) 9.84
C (d) 8.24
D (dd) 7.91
E (d) 7.68
F (d) 7.57
G (dd) 7.17
H (d) 6.72
I (dd) 4.15
J (s) 3.44
K (m) 2.18
L (d) 1.73
M (ddd) 1.48
N (s) 1.48
O (d) 0.85
f1 (ppm)


16
A (d) 8.43
B (dd) 7.98
C (d) 7.67
D (d) 7.56
E (dd) 7.25
F (d) 6.67
G (dd) 3.51
H (dd) 3.10
J (d) 2.46
K (ddt) 1.92
L (t) 1.46
M (d) 0.91
f1 (ppm)


MeHN
17
A (s) 11.94
B (s) 9.80
C (s) 8.08
D (d) 7.61
E (dd) 7.07
F (d) 6.66
G (s) 6.62
H (q) 6.32
I (dd) 3.48
J (dd) 3.06
K (d) 2.88
L (s) 2.27
M (dddd) 1.81
N (t) 1.36
O (d) 0.81
f1 (ppm)


18
A (s) 12.33
B (s) 9.55
C (s) 8.22
D (d) 7.67
E (s) 7.21
F (dd) 7.15
G (d) 6.65
H (s) 4.03
I (dd) 3.45
J (s) 3.15
K (dd) 3.03
L (s) 2.33
M (pd) 1.82
N (t) 1.36
O (d) 0.82
f1 (ppm)


19
A (s) 12.35
B (s) 9.18
C (s) 8.15
D (d) 7.62
E (s) 7.31
F (dd) 7.11
G (d) 6.65
H (t) 4.47
I (d) 3.49
J (m) 3.44
K (s) 3.32
L (dd) 3.06
M (m) 2.80
N (s) 2.34
O (pd) 1.83
P (t) 1.37
Q (d) 0.82
f1 (ppm)