Supporting Information

Evaluation of spiropiperidine hydantoins as a novel class of antimalarial agents

Marvin J. Meyers,[a,*] Elizabeth J. Anderson,[a] Sarah A. McNitt,[a] Thomas M. Krenning,[a] Megh Singh,[a] Jing Xu,[b] Wentian Zeng,[b] Limei Qin,[c] Wanwan Xu,[c] Siting Zhao,[c] Li Qin,[c] Christopher S. Eickhoff,[a] Jonathan Oliva,[a] Mary A. Campbell,[a] Stacy D. Arnett,[a] Michael J. Prinsen,[a] David W. Griggs,[a] Peter G. Ruminski,[a] Daniel E. Goldberg,[d] Ke Ding,[e] Xiaorong Liu,[b] Zhengchao Tu,[b] Micky D. Tortorella,[b] Francis M. Sverdrup,[a] and Xiaoping Chen,[c,*]

[a] Center for World Health and Medicine, Saint Louis University School of Medicine, Saint Louis, MO, USA
[b] Drug Discovery Pipeline at the Guangzhou Institutes for Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China
[c] Laboratory of Pathogen Biology, State Key Laboratory of Respiratory Disease, Center for Infection and Immunity, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China
[d] Departments of Medicine and Molecular Microbiology, Washington University in St. Louis, Saint Louis, MO, USA
[e] Key Laboratory of Regenerative Biology, Institute of Chemical Biology, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China

Corresponding Authors
* E-mail: mmeyers8@slu.edu. 1402 South Grand Blvd, M132 Schwitalla Hall, Saint Louis, MO 63104. Phone: 1-314-977-5197; and Email: chen_xiaoping@gibh.ac..cn. #190, Kaiyuan Avenue, Guangzhou Science Park, Guangzhou 510530, China

Table of Contents

General Synthesis Methods. Commercially available reagents and solvents were used without further purification unless stated otherwise. LC-MS analyses were performed on an Agilent 1100 or 1200HPLC/MSD electrospray mass spectrometer in positive ion mode with scan range was 100-1000d. Preparative normal phase chromatography was performed on a Biotage SP1 with prepacked Biotage or Varian silica gel cartridges. Preparative reverse phase HPLC was performed on a Shimadzu LC-20AP or Biotage SP1 equipped with a C18 column and a methanol/water or acetonitrile/water/0.05% TFA gradient. The purity of tested compounds was ≥95% as determined by HPLC analysis (at 210 nm as absorbance at 254 nm is very poor for these compounds) conducted on an Agilent 1100 or 1260 system using a reverse phase C18 column with diode array detector unless stated otherwise. NMR spectra were recorded on a Bruker 400 MHz spectrometer. The signal of the deuterated solvent was used as internal reference. Chemical shifts (δ) are given in ppm and are referenced to residual not fully deuterated solvent signal. Coupling constants (J) are given in Hz.

Compounds **4a-g** and **11c-d** were purchased from ChemBridge (www.hit2lead.com). Compounds **4d** and **4e** were resynthesized as described herein.

3-ethyl-8-[(2-hydroxyphenyl)methyl]-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (4d). A mixture of **12n** (0.15 mmol) and 2-hydroxybenzaldehyde (0.20 mmol) was dissolved in DMF (1 mL) and was stirred at room temp for 30 minutes before the addition of sodium triacetoxyborohydride (0.20 mmol). The reaction stirred at room temp for 24h. A work up of ethyl acetate and saturated bicarbonate solution was performed. The organic layer was dried and condensed, then purified by reverse phase HPLC (10% to 60% acetonitrile/water/0.05% TFA). The isolated compound was then treated with 2M HCl in diethyl ether and 41.0 mg (35% yield overall) of the HCl salt was obtained (>95% purity). White Solid, 40mg, 0.100 mmol, 35% yield, HPLC purity >95%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.21 (s, 1 H), 7.45 (d, J=6.6 Hz, 1 H), 7.24 (t, J=1.0 Hz, 1 H), 6.93 (d, J=7.8 Hz, 1 H), 6.83 (t, J=7.5 Hz, 1H), 4.21 (br. s., 2 H), 3.34 - 3.44 (m, 4 H), 3.31 (d, J=7.1 Hz, 2 H), 3.12 (t, J=1.0 Hz, 2 H), 2.46 - 2.52 (m, 2 H), 1.87 (d, J=13.9 Hz, 2 H), 1.49 (dt,J=13.2, 6.6 Hz, 1 H), 1.32 - 1.42 (m, 2 H), 1.02 (t, J=7.2 Hz, 3 H), 0.85 - 0.85 (m, 1 H), 0.86 (d, J=1.0 Hz, 6 H). ES-MS m/z 375.4 $[M+H]^+$.

8-(5-chloro-2-hydroxybenzyl)-3-ethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (10a). A mixture of 3-ethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (0.26 mmol) and 5-chlorosalicylaldehyde (0.45 mmol) was dissolved in DMF and stirred at room temp for 30 minutes before the addition of sodium triacetoxyborohydride (0.51 mmol). The reaction stirred at room temp under positive nitrogen pressure for 4 hours. The reaction was then quenched with water and the product was extracted with ethyl acetate and saturated sodium bicarbonate. The organic layer was washed twice and condensed yielding a white solid. The product was then purified via reverse phase HPLC (0-60% acetonitrile/water/0.05%TFA). The TFA salt was then treated with HCl in ether and filtered yielding 42 mg final compound as an HCl salt. White solid, 42 mg. HPLC >95% pure. ES-MS m/z 338 (MH^+). ^{1}H NMR (400 MHz, DMSO-d6) d ppm 1.07 (t, J=7.09 Hz, 3 H) 1.84 (q, J=14.70 Hz, 2 H) 2.12 (t, J=1.00 Hz, 2 H) 3.18 (br. q, J=11.00, 11.00, 11.00 Hz, 2 H) 3.38 (t, J=1.00 Hz, 2 H) 3.47 (br. q, J=11.50, 11.50, 11.50 Hz, 2 H) 4.19 (br. d, J=1.00 Hz, 2 H) 6.97 (d, J=8.56 Hz, 1 H) 7.34 (d, J=2.69 Hz, 1 H) 7.36 (d, J=2.69 Hz, 1 H) 7.48 (br. q, J=2.70, 2.70, 2.70 Hz, 1 H) 8.97 (s, 1 H).

8-[(5-chloro-2-hydroxyphenyl)methyl]-1,3-diethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione trifluoroacetate (10b). A mixture of 1,3-diethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (0.15 mmol) and 5-chloro-2-hydroxybenzaldehyde (0.20 mmol) was dissolved in DMF (2 mL) and was stirred at room temp for 30 minutes before the addition of sodium triacetoxyborohydride (0.20 mmol). The reaction stirred under positive nitrogen pressure at room temp for 24h. The mixture was the quenched with water and purified via reverse phase (0-75% acetonitrile/water/0.05%TFA). The isolated fractions were concentrated and dissolved in ether and some precipitate formed yielding 8.2 mg of product. White powder, 8.2 mg, 0.017 mmol, 12% yield, HPLC purity > 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.51 (br. s., 1 H), 7.54 (br. s., 1 H), 7.37 (dd, J=8.7, 2.3 Hz, 1 H), 6.99 (d, J=8.6 Hz, 1 H), 4.30 (br. s., 2 H), 3.46 (br. s., 4 H), 3.40 (q, J=7.3 Hz, 4 H), 2.26 (br. s., 2 H), 1.98 (d, J=14.7 Hz, 2 H), 1.04 - 1.18 (m, 6 H).

Synthesis of 8-(5-chloro-2-hydroxybenzyl)-1-cyclopentyl-3-ethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (10c). See Scheme 2.

ethyl 4-cyano-4-(cyclopentylamino)piperidine-1-carboxylate (14a). A solution of cyclopentylamine hydrochloride (20 mmol) in MeOH (4 mL) and water (4 mL) was treated with ethyl 4-oxopiperidine-1-carboxylate (**13**) (3.02 mL, 20 mmol) at room temp. After 5 min, the reaction was cooled to 0 ºC and treated with a solution of potassium cyanide (1.3 g, 20 mmol) in water (2 mL). The mixture was allowed to warm to room temp overnight. The mixture was diluted with water and extracted with diethyl ether. The organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated to give the title compound as a thick oil (4.57 g, 17.2 mmol, 86% yield). ES-MS m/z 266 $[M+H]^+$, 239 $[M-CN]^+$.

ethyl 1-cyclopentyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (15a). A solution of **14a** (4.55 g, 17.1 mmol) in AcOH (18 mL) was treated with potassium cyanate (2.78 g, 34.3 mmol) in water (6 mL) dropwise. Caution: an exotherm develops near the end of the addition with gas evolution. After the exotherm and evolution of gas subsided (15 min), the reaction was warmed to 50 ºC for 1 h. The reaction was poured into water and extracted with diethyl ether. The organic layers were washed with 1N HCl, satd sodium bicarbonate and brine, dried over sodium sulfate, and concentrated to give the urea intermediate as a thick viscous oil (3.51 g). The thick oil was treated with 10% aq. HCl and stirred at 50 ºC for 2 h. Upon cooling to room temp, the mixture was extracted with ethyl acetate. The organic layer was washed with 10% HCl and brine, dried over sodium sulfate and concentrated to give an oil which slowly solidified on standing. The title compound was obtained as an impure oily waxy solid (2.71 g, 8.76 mmol, 51% yield). ES-MS m/z 310 $[M+H]^+$.

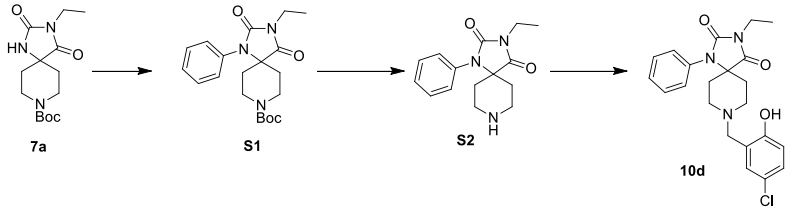
ethyl 1-cyclopentyl-3-ethyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (16a). A solution of **15a** (2.00 g, 6.48 mmol) in DMF was cooled to 0 ºC and treated with sodium hydride (60% dispersion in mineral oil, 520 mg, 13.0 mmol) under nitrogen. After 15 min, the mixture was treated with ethyl iodide (1.05 mL, 13.0 mmol) dropwise. The thick mixture was allowed to warm to room temp overnight. The mixture was quenched with satd ammonium chloride and extracted with ethyl acetate. The organic layers were washed with satd ammonium chloride, dried over sodium sulfate and concentrated to give an oil (2.5 g). The oil was purified by silica gel chromatography (20 to 50% ethyl acetate/heptane) to give the title compound as a clear oil (373 mg, 1.11 mmol, 17%). ES-MS m/z 338 $[M+H]^+$.

1-cyclopentyl-3-ethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (17a). A solution of **16a** (373 mg, 1.11 mmol) in ethanol (12 mL) was treated with KOH (560 mg) and

refluxed for 2 d. The mixture was concentrated and the resulting residue was diluted with 1 N NaOH and extracted with DCM. The organic extracts were dried over magnesium sulfate, filtered and concentrated to give the crude amine as a yellow solid which was converted to the HCl salt with 1N HCl in diethyl ether to give the title compound as a yellow foam (162 mg, 0.537 mmol, 48%) HPLC purity >90%, ES-MS m/z 266 $[M+H]^+$.

8-(5-chloro-2-hydroxybenzyl)-1-cyclopentyl-3-ethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (10c). A mixture of **17a** (50.0 mg, 0.166 mmol) and 5-chloro-2-hydroxybenzaldehyde (33.0 mg, 0.211 mmol) was dissolved in DCE (500 uL) and DMF (500 uL) at room temp. Sodium triacetoxyborohydride (88.8 mg, 0.419 mmol) was added. The solution immediately turned a deep wine color that dissipated within minutes. The reaction was stirred at room temp overnight (20 h). The reaction was quenched with a few drops of water. After 15 min, a couple drops of TFA was added. The solution was concentrated to remove the DCE and then purified by reverse phase HPLC (10 to 70% acetonitrile/water/0.05%TFA). The combined fractions were concentrated to remove the acetonitrile. The aqueous solution was neutralized with satd sodium bicarbonate. The resultant precipitate was filtered and washed with water to give the product as a white solid: 14.5 mg, 0.0357 mmol, 22% yield. HPLC ~90% pure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.19 (dd, J = 2.32, 8.47 Hz, 1H), 7.04 (d, J = 2.01 Hz, 1H), 6.84 (d, J = 8.34 Hz, 1H), 3.87 (s, 2H), 3.41 - 3.56 (m, 4H), 3.01 - 3.21 (m, 2H), 2.05 - 2.21 (m, 2H), 1.85 - 1.97 (m, 3H), 1.75 (d, 5H), 1.51 - 1.64 (m, 3H), 1.20 (t, J = 7.18 Hz, 3H). ES-MS m/z 406.1 (M+H).

Synthesis of 8-(5-chloro-2-hydroxybenzyl)-3-ethyl-1-phenyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (10d).



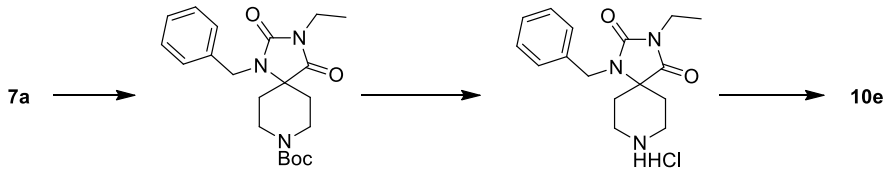
tert-butyl 3-ethyl-2,4-dioxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-carboxylate (S1). *tert*-Butyl 3-ethyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate **(7a)** (1g, 3.36mmol), iodobenzene (2.06g, 10mmol), cuprous iodide (0.64g, 3.36mmol), 2,2,6,6-tetramethyl-3,5-heptanedione (0.62g,3.36mmol) and cesium carbonate (5.5g, 16.88mmol) were dissolved in acetonitrile and DMF (v/v 1:1) under argon atmosphere. The reaction was heated to 110℃ overnight. The mixture was extracted with ethyl acetate. The combined organic phases were dried over sodium sulphate and concentrated in vacuo. The title compound was used without further purification.

3-ethyl-1-phenyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (S2). S1 was dissolved in DCM (20ml) and TFA (20ml) and stirred at room temperature overnight. The mixture was neutralized by Na2CO3 and extracted with dichloromethane. The combined organic phases were dried over sodium sulphate and concentrated in vacuo. The title compound was used without further purification.

8-(5-chloro-2-hydroxybenzyl)-3-ethyl-1-phenyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (10d). S2 (0.47g, 1.72mmol) and 5-Chlorosalicylaldehyde (0.8g, 5.1mmol) were dissolved in ethanol and refluxed overnight. The reaction was cooled to room temperature and sodium cyanoborohydride (0.33g, 5.25mmol) was added. The mixture was stirred overnight. The mixture was concentrated in vacuo and extracted with dichloromethane. The combined organic

phases were dried over sodium sulphate and concentrated in vacuo. The residue was purified by HPLC to give the title compound as a solid (0.18g, 25%yield three steps). HPLC >98% pure. ES-MS m/z 414 (MH^+). ^1H NMR (400 MHz, $CDCl_3$) δ 7.47-7.45 (m, 3H), 7.16-7.14 (m, 2H), 7.08 (d, *J*=8.4, 1H), 6.94 (s, 1H), 6.66 (d, *J*=8.8Hz, 1H), 3.70 (s, 2H), 3.64 (q, *J*=7.2Hz, 2H), 3.04-2.98 (m, 2H), 2.85-2.82 (m, 2H),1.93-1.92 (m, 4H), 1.27 (t, *J*=7.2Hz, 3H).

Synthesis of 1-benzyl-8-(5-chloro-2-hydroxybenzyl)-3-ethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (10e).



tert-butyl 1-benzyl-3-ethyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (S3). To a solution of **7a** (1.0g, 3.36mmol) in dry THF was add potassium tert-butoxide (1.5g, 13.4mmol) and stirred for 30min, then benzyl bromide (0.6g, 3.5mmol) was added. The reaction was stirred at 50℃ overnight. The mixture was extracted with ethyl acetate. The combined organic phases were dried over sodium sulphate and concentrated in vacuo. The residue was purified over a silica column to give the title compound (0.9g, 77%yield).

1-benzyl-3-ethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (S4). S3 (0.9g, 2.32mmol) was dissolved in 18ml of HCl solution in THF (2mol/L) and stirred at room temperature overnight. The mixture was concentrated in vacuo and crystallized to give the title compound as a solid (0.47g, 63%yield).

1-benzyl-8-(5-chloro-2-hydroxybenzyl)-3-ethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (10e). S4 (0.31g, 1.08mmol) and 5-chlorosalicylaldehyde (0.58g, 3.69mmol) were dissolved in ethanol and refluxed overnight. The reaction was cooled to room temperature and sodium cyanoborohydride (0.4g, 6.35mmol) was added. The mixture was stirred overnight. The mixture was concentrated in vacuo and extracted with dichloromethane. The combined organic phases were dried over sodium sulphate and concentrated in vacuo. The residue of 18 was used purified by HPLC to give the title compound as a solid (0.24g, 52%yield). HPLC >98% pure. ES-MS m/z 428 (MH^+). ^1H NMR (400 MHz, $CDCl_3$) δ 7.35-7.26 (m, 5H), 7.11 (dd, *J*=8.8, 2.0Hz, 1H), 6.94 (s, 1H), 6.73 (d, *J*=8.4Hz, 1H), 4.53 (s, 2H), 3.72 (s, 2H), 3.61 (q, *J*=7.2Hz, 2H), 2.94 (t, *J*=11.6Hz, 2H), 2.83-2.80 (m, 2H),1.98-1.90 (m, 2H), 1.63-1.59 (m, 2H), 1.24 (t, *J*=7.2Hz, 3H).

Synthesis of 8-(5-chloro-2-hydroxybenzyl)-3-ethyl-1-phenethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (10f). See Scheme 2.

ethyl 4-cyano-4-(phenethylamino)piperidine-1-carboxylate hydrochloride (14b). A solution of isopentylamine hydrochloride (10 mmol) in MeOH (2 mL) and water (2 mL) was treated with ethyl 4-oxopiperidine-1-carboxylate (1.51 mL, 10 mmol) at room temp. After 5 min, the reaction was cooled to 0 ºC and treated with a solution of potassium cyanide (651 mg, 10 mmol) in water (1.0 mL). The mixture was allowed to warm to room temp overnight. The mixture was diluted with water and extracted with diethyl ether. The organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated to give the crude compound as a clear oil which was purified by silica gel chromatography (30 to 70% EtOAc/heptane). The resultant oil was dissolved in diethyl ether and treated with 9 mL of 1 N HCl in diethyl ether.

The mixture was concentrated and recrystallized from methanol/diethyl ether to give the title compound as a white HCl salt (1.06 g, 3.14 mmol, 31%).

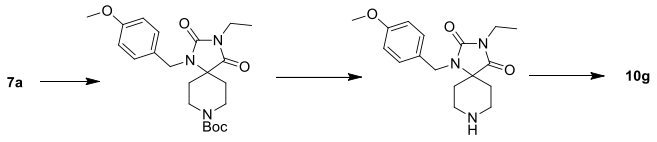
ethyl 2,4-dioxo-1-phenethyl-1,3,8-triazaspiro[4.5]decane-8-carboxylate (15b). A solution of **14b** (500 mg, 1.48 mmol) in AcOH (1.5 mL) was treated with potassium cyanate (240 mg, 2.96 mmol) in water (0.5 mL) and the reaction was warmed to 50 ºC for 1 h. The reaction was poured into water and extracted with ethyl acetate. The organic layers were washed with satd ammonium chloride and brine, dried over sodium sulfate, and concentrated to give the urea intermediate as a viscous semi-solid oil (510 mg). The intermediate was treated with 10% aq. HCl (3 mL) and stirred at 50 ºC for 1 h. Upon cooling to room temp, the ppt was filtered, washed with cold water, and dried to give the title compound was obtained as a white solid (312 mg, 0.904 mmol, 61% yield). HPLC purity >90%.

ethyl 3-ethyl-2,4-dioxo-1-phenethyl-1,3,8-triazaspiro[4.5]decane-8-carboxylate (16b). A solution of **15b** (300 mg, 0.869 mmol) in DMF was cooled to 0 ºC and treated with sodium hydride (60% dispersion in mineral oil, 104 mg, 2.61 mmol) under nitrogen. After 15 min, the mixture was treated with ethyl iodide (0.210 mL, 2.61 mmol) dropwise. The mixture was allowed to warm to room temp overnight. The mixture was quenched with satd ammonium chloride and extracted with ethyl acetate. The organic layers were washed with satd ammonium chloride, dried over sodium sulfate and concentrated. The oil was purified by silica gel chromatography (20 to 60% ethyl acetate/heptane) to give the title compound as a clear viscous oil (251 mg, 0.672 mmol, 77%). HPLC purity ~95%.

3-ethyl-1-phenethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (17b). A solution of **16b** (251 mg, 0.672 mmol) in ethanol (8 mL) was treated with 2.5 N NaOH (2 mL, 5 mmol) and stirred at 80 ºC for 42 h. The mixture was concentrated and the resulting residue was diluted with 1 N NaOH and extracted with DCM. The organic extracts were dried over magnesium sulfate, filtered and concentrated to give the crude product as a 3:1 mixture of product and starting material. The mixture was diluted with diethyl ether and treated with 1N HCl in diethyl ether (1 mL) to give the title compound as a white solid (135 mg, 0.398 mmol, 59%) HPLC purity >95%.

8-(5-chloro-2-hydroxybenzyl)-3-ethyl-1-phenethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (10f). A mixture of **17b** (121 mg, 0.357 mmol) and 5-chloro-2-hydroxybenzaldehyde (56 mg, 0.357 mmol) was suspended in DCE (1.2 mL) and DMF (1.0 mL) at room temp. Sodium triacetoxyborohydride (113 mg, 0.536 mmol) was added. The reaction was stirred at room temp for 2 h. The reaction was quenched with satd sodium bicarbonate and extracted with ethyl acetate. The organic layer was washed with satd sodium bicarbonate, dried over sodium sulfate and concentrated to give the crude product as an oil. The oil was purified by silica gel chromatography (20 to 50% ethyl acetate/heptane). The resultant clear oil was triturated with diethyl ether to give the title compound as a white solid: 59.7 mg, 0.135 mmol, 38% yield. HPLC >98% pure. ES-MS m/z 442 (MH^+). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.18 - 7.37 (m, 6H), 7.12 (dd, *J* = 2.76, 8.60 Hz, 1H), 6.76 (d, *J* = 8.60 Hz, 1H), 3.62 (s, 2H), 3.35 - 3.44 (m, 4H), 2.85 - 2.92 (m, 2H), 2.66 - 2.77 (m, 4H), 1.79 - 1.95 (m, 2H), 1.45 - 1.52 (m, 2H), 1.09 (t, *J* = 7.18 Hz, 3H).

Synthesis of 8-(5-chloro-2-hydroxybenzyl)-3-ethyl-1-(4-methoxybenzyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione (10g).



tert-butyl 3-ethyl-1-(4-methoxybenzyl)-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (S5). To a solution of *tert*-butyl 3-ethyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate **(7a)** (1 g, 3.36 mmol) in dry DMF was add sodium hydride (0.12g, 5mmol) and stirred for 30min, then 4-methoxybenzylchloride (0.53g, 3.38mmol) was added. The reaction was stirred at room temperature overnight. The mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulphate and concentrated in vacuo. The title compound was used without further purification.

3-ethyl-1-(4-methoxybenzyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione (S6). S5 was dissolved in DCM (7.5ml) and TFA (2.5 ml). The solution was stirred at room temperature overnight. The mixture was neutralized by Na2CO3 and extracted with dichloromethane. The combined organic phases were dried over sodium sulphate and concentrated in vacuo. The residue of 5 was purified over a silica column to give the title compound as oil (1.0g, 93%yield two steps, MeOH/DCM=1/10).

8-(5-chloro-2-hydroxybenzyl)-3-ethyl-1-(4-methoxybenzyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione (10g). S6 (0.2g, 0.63mmol) and 5-Chlorosalicylaldehyde (0.14g, 0.89mmol) were dissolved in ethanol and refluxed overnight. The reaction was cooled to room temperature and sodium cyanoborohydride (0.3g, 4.77mmol) was added. The reaction was stirred overnight. The mixture was concentrated in vacuo and extracted with dichloromethane. The combined organic phases were dried over sodium sulphate and concentrated in vacuo. The residue was purified by HPLC to give the title compound as a solid (0.17g, 59%yield). HPLC >98% pure. ES-MS m/z 458 (MH^+). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, *J*=8.4Hz, 2H), 7.11 (d, *J*=8.8Hz, 1H), 6.94 (s, 1H), 6.86 (d, *J*=8.4Hz, 2H), 6.73 (d, *J*=8.8Hz, 1H), 4.47 (s, 2H), 3.79 (s, 3H), 3.72 (s, 2H), 3.59 (q, *J*=7.2Hz, 2H), 2.94 (t, *J*=11.6Hz, 2H), 2.83-2.80 (m, 2H), 1.99-1.91 (m, 2H), 1.60-1.57 (m, 2H), 1.23 (q, *J*=7.2Hz, 3H).

4-((8-(5-chloro-2-hydroxybenzyl)-3-ethyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decan-1-yl)methyl)benzonitrile trifluoroacetate (10h). Tert-butyl 3-ethyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (0.34 mmol) was dissolved in DMF (3 mL) and treated with sodium hydride dispersed in mineral oil (0.4 mmol). The reaction stirred for 15 minutes before the addition of 4-(bromomethyl)benzylnitrile. The reaction stirred at room temperature under positive nitrogen pressure for 2 hours. The mixture was quenched with water and dissolved in ammonium chloride. The organic product was extracted with ethyl acetate. The product was then treated with 20% TFA in dichloromethane to remove the boc protecting group. The mixture stirred for 1 hour yielding the desired compound, 4-({3-ethyl-2,4-dioxo-1,3,8-triazaspiro[4.5]deca-1-yl}methyl)benzonitrile, as a TFA salt.

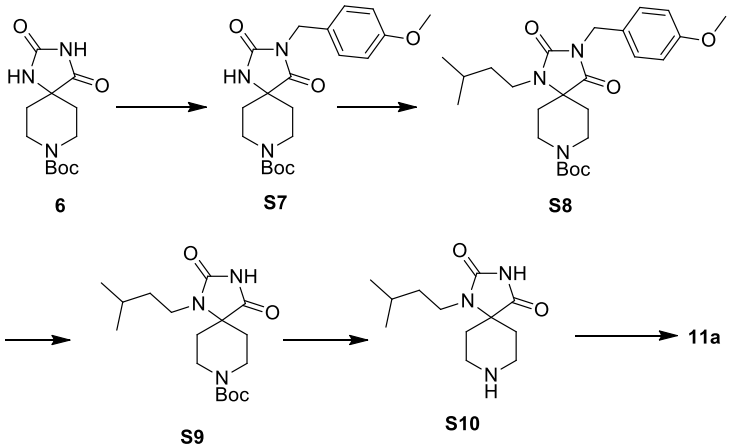
A mixture of 4-({3-ethyl-2,4-dioxo-1,3,8-triazaspiro[4.5]deca-1-yl}methyl)benzonitrile (0.25 mmol) and 5-chlorosalicylaldehyde (0.37 mmol) was dissolved in DMF (4 mL) and stirred for 2 hour before the addition of sodium triacetoxyborohydride (0.48 mmol). The reaction stirred at room temperature under positive nitrogen pressure for 3 days. The mixture was quenched with water and dissolved in saturated sodium bicarbonate. The organic product was extracted with ethyl acetate. The crude product was then purified by reverse phase HPLC. The product was then lyophilized yielding a white fluffy solid. White solid, 58.6 mg,

0.129 mmol, 62.8% yield. HPLC purity > 95%. LC-MS m/z 453 [MH^+]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.15 (t, J=7.21 Hz, 3 H) 1.93 - 2.03 (m, 2 H) 2.08 (d, J=7.83 Hz, 2 H) 3.46 (q, J=7.25 Hz, 6 H) 4.26 (br. d, J=1.00 Hz, 2 H) 4.53 (s, 2 H) 6.96 (d, J=8.80 Hz, 1 H) 7.35 (dd, J=8.56, 2.69 Hz, 1 H) 7.44 - 7.57 (m, 3 H) 7.82 (d, J=8.31 Hz, 3 H).

8-(5-chloro-2-hydroxybenzyl)-3-ethyl-1-(4-(trifluoromethyl)benzyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (10i). Tert-butyl 3-ethyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (0.35 mmol) was dissolved in DMF (2 mL) and treated with sodium hydride dispersed in mineral oil (0.4 mmol). The reaction stirred for 15 minutes before the addition of 4-(trifluoromethyl)benzyl bromide. The reaction stirred at room temperature under positive nitrogen pressure for 24 hours. The mixture was quenched with water and dissolved in ammonium chloride. The organic product was extracted with ethyl acetate. The product was then treated with HCl in ether to remove the boc protecting group. The mixture stirred for 24 hours yielding the desired compound, 3-ethyl-1-{[4-(trifluoromethyl)phenyl]methyl}-1,3,8-triazaspiro[4.5]decane-2,4-dione, as an HCl salt.

A mixture of 3-ethyl-1-{[4-(trifluoromethyl)phenyl]methyl}-1,3,8-triazaspiro[4.5]decane-2,4-dione (0.18 mmol) and 5-chlorosalicylaldehyde (0.33 mmol) was dissolved in DMF (3 mL) and stirred for 1 hour before the addition of sodium triacetoxyborohydride (0.4 mmol). The reaction stirred at room temperature under positive nitrogen pressure for 3 days. The mixture was quenched with water and dissolved in saturated sodium bicarbonate. The organic product was extracted with ethyl acetate. The crude product was then purified by reverse phase HPLC. The purified product was then treated with HCl in ether to yield the final compound as an HCl salt. White solid, 47.6 mg, 0.095 mmol, 56.98% yield. HPLC purity > 95%. ES-MS m/z 495 [MH^+]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.05 - 1.22 (m, 3 H) 1.96 (d, J=14.43 Hz, 2 H) 2.27 - 2.43 (m, 2 H) 3.28 - 3.60 (m, 6 H) 4.24 (br. s., 2 H) 4.56 (s, 2 H) 6.98 (d, J=8.56 Hz, 1 H) 7.34 (dd, J=8.68, 2.57 Hz, 1 H) 7.53 - 7.65 (m, 3 H) 7.71 (d, J=8.31 Hz, 2 H) 10.59 (s, 1 H).

Synthesis of 8-(5-chloro-2-hydroxybenzyl)-1-isopentyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (11a).



tert-butyl 3-(4-methoxybenzyl)-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (S7). To a solution of tert-butyl 2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate **6** (2 g, 7.4 mmol) and potassium carbonate (1.23g, 8.9mmol) in DMF was added 4-methoxybenzylchloride (1.4 g, 9.0 mmol). The mixture was stirred at room temperature overnight. The mixture was

extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulphate and concentrated in vacuo to give the title compound which was used without further purification.

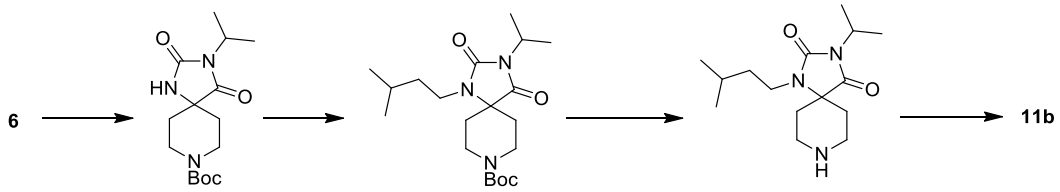
tert-butyl 1-isopentyl-3-(4-methoxybenzyl)-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (S8). To a solution of **S7** in dry DMF was add sodium hydride (0.3g, 12.5mmol) and stirred for 30min followed by isopentyl iodide (1.92g, 10.43mmol) was added. The reaction was stirred at room temperature overnight. The mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulphate and concentrated in vacuo. The title compound was used without further purification.

tert-butyl 1-isopentyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (S9). To a solution of **S8** in acetonitrile (30 ml) was added a solution of ammonium cerium (IV) nitrate (20.38 g, 37.17 mmol) in water (30 ml). The mixture was stirred at room temperature overnight. The residue was purified over a silica column to obtain an impure title compound (3.5g).

1-isopentyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (S10). **S9** (1.4g, impure) was dissolved in DCM (5ml) and TFA (10ml). The solution was stirred at room temperature overnight. The mixture was neutralized by NaHCO3 and extracted with dichloromethane. The combined organic phases were dried over sodium sulphate and concentrated in vacuo. The residue was purified over a silica column to give the title compound (0.6g, 84% yield four steps).

8-(5-chloro-2-hydroxybenzyl)-1-isopentyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (11a). S10 (0.297g, 0.78mmol) and 5-chlorosalicyaldehyde (0.27g, 1.72mmol) were dissolved in ethanol and refluxed overnight. The reaction was cooled to room temperature and sodium cyanoborohydride (0.32g, 4.77mmol) was added. The mixture was stirred overnight. The mixture was concentrated in vacuo and extracted with dichloromethane. The combined organic phases were dried over sodium sulphate and concentrated in vacuo. The residue was purified by HPLC to give the title compound as a solid (0.23g, 50%yield). HPLC >95% pure. ES-MS m/z 380 (MH^+). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (d, *J*=8.8Hz, 1H), 6.97 (s, 1H), 6.75 (d, *J*=8.8Hz, 1H), 3.77 (s, 2H), 3.20-3.18 (m, 2H), 3.00-2.90 (m, 4H), 2.04-1.96 (m, 2H), 1.82-1.79 (m, 2H), 1.65-1.56 (m, 1H), 1.53-1.47 (m, 2H), 0.94 (d, *J*=8.8Hz, 6H).

Synthesis of 8-(5-chloro-2-hydroxybenzyl)-1-isopentyl-3-isopropyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (11b).



tert-butyl 3-isopropyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (S11). To a solution of tert-butyl 2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate **6** (2 g, 7.4 mmol) and potassium carbonate (1.23 g, 8.9 mmol) in DMF was added 2-iodopropane (1.53 g, 9.0 mmol). The reaction was stirred at room temperature overnight. The mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulphate and concentrated in vacuo to give the title compound was used without further purification.

tert-butyl 1-isopentyl-3-isopropyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (S12). To a solution of **S11** (2.3 g, 7.39 mmol) in dry DMF was add sodium

hydride (0.22 g, 9.2 mmol) and stirred for 30min, followed by isopentyl iodide (1.92 g, 10.43 mmol) was added. The reaction was stirred at room temperature overnight. The mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulphate and concentrated in vacuo. The residue was used without further purification.

1-isopentyl-3-isopropyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (S13). tert-butyl 1-isopentyl-3-isopropyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (**S12**) was dissolved in DCM (15 ml) and TFA (15 ml). The solution was stirred at room temperature overnight. The mixture was neutralized by Na2CO3 and extracted with dichloromethane. The combined organic phases were dried over sodium sulphate and concentrated in vacuo. The residue was purified over a silica column (MeOH/DCM=1/10) to give the title compound as oil (1.8 g, 91% yield three steps).

8-(5-chloro-2-hydroxybenzyl)-1-isopentyl-3-isopropyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (11b). S13 (0.24 g, 0.9 mmol) and 5-chlorosalicylaldehyde (0.18 g, 1.15 mmol) were dissolved in ethanol and refluxed overnight. The reaction was cooled to room temperature and sodium cyanoborohydride (0.32 g, 4.77 mmol) was added. The mixture was stirred overnight. The mixture was concentrated in vacuo and extracted with dichloromethane. The combined organic phases were dried over sodium sulphate and concentrated in vacuo. The residue was used purified by HPLC to give the title compound as a solid (0.20 g, 55% yield). HPLC >98% pure. ES-MS m/z 422 (MH^+). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (d, *J*=8.8Hz, 1H), 6.96 (s, 1H), 6.74 (d, *J*=8.4Hz, 1H), 4.31-4.24 (m, 1H), 3.76 (s, 2H), 3.17 (t, *J*=8.0Hz, 2H), 2.99 (t, *J*=11.6Hz, 2H), 2.90-2.88 (m, 2H), 2.01-1.94 (m, 2H), 1.70-1.67 (m, 2H),1.63-1.55 (m, 1H), 1.52-1.47 (m, 2H), 1.38 (d, *J*=6.8Hz, 6H), 0.94 (d, *J*=6.4Hz, 6H).

8-(3-chloro-5-hydroxybenzyl)-3-ethyl-1-isopentyl-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12a). A mixture of **12n** (0.15 mmol) and 3-chloro-5-hydroxybenzaldehyde (0.22 mmol) was dissolved in DMF (2 mL) and stirred for 30 minutes before the addition of sodium triacetoxyborohydride (0.3 mmol). The reaction stirred at room temperature for 24 hours. A second addition of 3-chloro-5-hydroxybenzaldehyde (0.1 mmol) was added to the mixture and stirred for 30 minutes before the addition of sodium triacetoxyborohydride (0.22 mmol). The reaction stirred for another 24 hours. The mixture was quenched with water, dissolved in saturated sodium bicarbonate and the crude product was extracted with ethyl acetate. The crude product was purified by reverse phase HPLC. It was then treated with HCl in ether to give the final purified compound as an HCl salt. White solid, 43.8 mg, 0.107 mmol, 62.94% yield, HPLC purity > 95%. ES-MS m/z 408 [MH^+]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.91 (d, J=6.60 Hz, 6 H) 1.09 (t, J=7.21 Hz, 3 H) 1.38 - 1.50 (m, 2 H) 1.55 (d, J=6.85 Hz, 2 H) 1.96 (d, J=14.67 Hz, 2 H) 2.35 - 2.48 (m, 2 H) 3.10 - 3.24 (m, 2 H) 3.37 - 3.48 (m, 4 H) 4.32 (d, J=4.89 Hz, 2 H) 6.88 - 6.95 (m, 1 H) 7.00 (s, 1 H) 7.20 (s, 1 H) 10.28 (s, 1 H).

8-[(3-chloro-4-hydroxyphenyl)methyl]-3-ethyl-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride hydrochloride (12b). A mixture of **12n** (0.15 mmol) and 3-chloro-4-hydroxybenzaldehyde (0.20 mmol) was dissolved in DMF (2 mL) and was stirred at room temp for 30 minutes before the addition of sodium triacetoxyborohydride (0.20 mmol). The reaction stirred under positive nitrogen pressure at room temp for 24h. The mixture was the quenched with water, dissolved in water/brine, and extracted with DCM washes. The crude was then dissolved into ether and purified by salt formation with 4M HCl in dioxane.

White powder, 10 mg, .03 mmol, 11.3% yield, HPLC purity >95%. ES-MS m/z 408 [MH^+]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 (t, J=7.09 Hz, 6 H) 1.07 (d, J=7.09 Hz, 3 H) 1.42 (br. s., 2 H) 1.55 (d, J=5.38 Hz, 1 H) 1.94 (d, J=9.05 Hz, 2 H) 3.17 (br. s., 3 H) 3.23 - 3.49 (m, 6 H) 4.27 (br. s., 2 H) 7.03 (d, J=7.34 Hz, 1 H) 7.37 (br. s., 1 H) 7.65 (br. s., 1 H) 10.45 (br. s., 1 H) 10.61 (br. s., 1 H).

3-ethyl-8-[(5-fluoro-2-hydroxyphenyl)methyl]-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12c). A mixture of **12n** (0.15 mmol) and 5-bromo-3-fluoro-2-hydroxybenzaldehyde (0.20 mmol) was dissolved in DMF (2 mL) and was stirred at room temp for 30 minutes before the addition of sodium triacetoxyborohydride (0.20 mmol). The reaction stirred under positive nitrogen pressure at room temp for 24h. The mixture was the quenched with water, dissolved in water/brine, and extracted with DCM washes. The crude was then dissolved into ether and purified by HCl salt formation. White powder, 10 mg, mmol, 12.3% yield, HPLC purity > 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.80 - 0.98 (m, 6 H) 1.00 - 1.17 (m, 3 H) 1.42 (d, J=8.07 Hz, 2 H) 1.55 (dt, J=13.14, 6.51 Hz, 1 H) 1.96 (br. s., 2 H) 2.45 (br. s., 2 H) 3.17 (d, J=7.58 Hz, 2 H) 3.34 - 3.50 (m, 5 H) 4.26 (br. s., 2 H) 6.97 (dd, J=8.93, 4.77 Hz, 1 H) 7.15 (td, J=8.50, 3.30 Hz, 1H) 7.41 (d, J=9.05 Hz, 1 H) 10.27 (s, 1 H) 10.55 (br. s., 1 H)

8-[(5-bromo-2-hydroxy-phenyl)methyl]-2-ethyl-4-isopentyl-2,4,8-triazaspiro[4.5]decane-1,3-dione hydrochloride (12d). A mixture of 12n (500 mg, 1.646 mmol, 1.0 equiv) and 5-bromo-2-hydroxy-benzaldehyde (376.6 mg, 1.81 mmol, 1.1 equiv) in DCE (5 mL) was stirred at room temperature under anhydrous conditions for 30 minutes. The solution was then treated with sodium triacetoxyborohydride (~698 mg, roughly 3.29 mmol, 2.0 equiv; solution turned purple upon addition) and the reaction was stirred for 5 days. It was then quenched using 1 mL of a saturated sodium bicarbonate solution, then was extracted with EtOAc. The organic layer was subsequently washed with a saturated solution of sodium chloride, dried with sodium sulfate, filtered and concentrated *in vacuo*. The resulting foam (0.9934 g) was then purified via flash chromatography (10-50% ethyl acetate/heptane). Fractions were concentrated and dissolved with diethyl ether (9 mL). It was then converted to an HCl salt by adding 750 µL of 1 M HCl in diethyl ether. The precipitate was filtered from the solution, yielding the target compound as a white solid in the form of an HCl salt (278 mg, 0.6150 mmol, 35%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (d, J=6.53 Hz, 6 H) 1.07 (t, J=7.15 Hz, 3 H) 1.38 - 1.46 (m, 2 H) 1.48 - 1.60 (m, 1 H) 1.94 (d, J=14.05 Hz, 2 H) 2.41 - 2.48 (m, 2 H) 3.12 - 3.19 (m, 2 H) 3.33 - 3.41 (m, 3 H) 3.41 - 3.47 (m, 2 H) 4.25 (d, J=2.95 Hz, 2 H) 6.95 (d, J=8.72 Hz, 1 H) 7.46 (dd, J=8.72, 2.51 Hz, 1 H) 7.74 (d, J=2.26 Hz, 1 H) 10.67 (s, 1 H) 10.71 (br. s., 1 H). ES-MS m/z 452, 454 (MH^+).

3-ethyl-8-[(2-hydroxy-5-methylphenyl)methyl]-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12e). A mixture of 12n (30.4 mg, 0.100, 1 equiv), and 2-hydroxy-5-methylbenzaldehyde (27.2 mg, 0.200 mmol, 2.0 equiv) dissolved in DMF (2.5 mL) was stirred at room temperature for thirty minutes before the addition of triacetoxyborohydride (53.1 mg, 0.251 mmol, 2.5 equiv; *upon addition, solution turned a hazy yellow). This mixture was then stirred at r.t. for 24 hours, then quenched with a few drops of water. A few drops of TFA were then added and the clear solution was purified by reverse phase HPLC (10-60% acetonitrile/water/0.05% TFA). Pure fractions were neutralized with a solution

of saturated sodium bicarbonate, and extracted twice with 12 mL portions of EtOAc. The combined organic layers were then filtered, dried and concentrated. The resulting oily product was dissolved in diethyl ether (7 mL) and treated with 200 µL HCl (1 M; in diethyl ether). The solvents were then allowed to evaporate, leaving a white solid as the target compound in the form of an HCl salt (27.4 mg, 0.0646 mmol, 64.6%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1 H), 7.27 (s, 1 H), 7.10 (d, J=1.0 Hz, 1 H), 6.86 (d, J=8.2 Hz, 1 H), 4.24 (d, J=4.6 Hz, 2 H), 3.40 - 3.49 (m, 4 H), 3.38 (d, J=7.2 Hz, 2 H), 3.15 (t, J=1.0 Hz, 2 H), 2.32 - 2.44 (m, 2 H), 2.22 (s, 3 H), 1.95 (d, J=13.9 Hz, 2 H), 1.54 (spt, J=1.0 Hz, 1 H), 1.42 (q, J=1.0 Hz, 2 H), 1.08 (t, J=7.2 Hz, 3 H), 0.90 (d, J=6.6 Hz, 6 H). ES-MS m/z 389 (MH^+).

3-ethyl-8-[(2-hydroxy-5-phenylphenyl)methyl]-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12f). A solution of 8-[(5-bromo-2-hydroxyphenyl)methyl]-3-ethyl-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione (**12d**; 100.3 mg, 0.205 mmol, 1.0 equiv), phenylboronic acid (50.0 mg, 0.410 mmol, 2.0 equiv), cesium carbonate (233.3 mg, 0.716 mmol, 3.5 equiv) and tetrakis(triphenylphosphine)palladium(0) (23.7 mg, 0.0205 mmol, 0.1 equiv) in a 1:1 ethanol/toluene solution was sparged with nitrogen and heated to 80 ºC. After stirring for 40 minutes, the solution was cooled to room temperature, and water was added (2 mL) to neutralize excess cesium carbonate and quench the solution. The reaction mixture was then partitioned between two layers of EtOAc and sat. sodium bicarbonate. After separating the organic layer, the aqueous layer was extracted again; the organic layers were then combined, dried, filtered and concentrated *in vacuo*. The crude compound (dark orange oil, 186.2 mg) was then dissolved in 1 mL of acetonitrile/0.05% TFA, treated with a couple drops of TFA and loaded onto a Biotage 40 + M reverse phase HPLC column for purification (10-70% acetonitrile/water + 0.05% TFA gradient). Fractions were then combined and concentrated. The purified residue was then neutralized with a saturated bicarbonate solution and was extracted twice using EtOAc. The combined organic layers were then dried using sodium sulfate, filtered and concentrated yielding 33.8 mg of compound. It was then converted to an HCl salt by dissolving it in 5 mL of diethyl ether and treating it dropwise with 200 µL of 1 M HCl (in diethyl ether). The precipitate was then filtered, yielding the target compound as a white solid in the form of an HCl salt (21.7 mg, 0.0466 mmol, 22%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (d, J=6.53 Hz, 6 H) 1.09 (t, J=1.00 Hz, 3 H) 1.39 - 1.48 (m, 2 H) 1.49 - 1.61 (m, 1 H) 1.95 (d, J=13.61 Hz, 2 H) 2.53 - 2.61 (m, 2 H) 3.14 - 3.23 (m, 2 H) 3.35 - 3.42 (m, 4 H) 3.49 (br. s., 2 H) 4.33 (d, J=1.00 Hz, 2 H) 7.07 (d, J=8.47 Hz, 1 H) 7.28 - 7.35 (m, 1 H) 7.44 (t, J=7.69 Hz, 2 H) 7.62 (dd, J=8.47, 2.26 Hz, 1 H) 7.69 (d, J=7.34 Hz, 2 H) 7.92 (d, J=1.69 Hz, 1 H) 10.41 - 10.49 (m, 1 H). ES-MS m/z 450 (MH^+).

8-[(2-chloro-6-hydroxyphenyl)methyl]-3-ethyl-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12g). A mixture of **12n** (76.1 mg, 0.25 mmol, 1 equiv) and 2-chloro-6-hydroxybenzaldehyde (81.2 mg, 0.52 mmol, 2.07 equiv) was dissolved in DMF (2 mL). It was then stirred at room temperature under anhydrous conditions for 30 minutes before the addition of sodium triacetoxyborohydride (86.5 mg, 0.63 mmol, 2.5 equiv). The reaction stirred at room temperature under anhydrous conditions for 24 h. It was then quenched with water (1 mL). A work up with EtOAc and saturated sodium bicarbonate was used to extract the organic layer, which was then washed twice. The aqueous layer was washed once. The clear organic layer was dried (sodium sulfate), filtered, and condensed yielding a yellow oil of 91 mg. This oil was purified by normal phase HPLC with a silica gel cartridge (0% to 50%

EtOAc/Hexane). Fractions were combined and condensed yielding 112 mg product as a low viscosity transparent yellow oil. It was then treated with 200 μL of 2M HCl, filtered and dried yielding the target compound as an HCl salt (123 mg, 0.277 mmol, 95.6%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.81 - 0.98 (m, 6 H) 1.08 (t, J=7.21 Hz, 3 H) 1.35 - 1.65 (m, 3 H) 1.95 (d, J=13.69 Hz, 2 H) 2.40 - 2.67 (m, 2 H) 3.00 - 3.28 (m, 2 H) 4.40 (br. s., 4 H) 6.92 - 7.18 (m, 4 H) 7.23 - 7.55 (m, 2 H) 10.18 (br. s., 1 H) 11.11 (br. s., 1 H). ES-MS m/z 408 (M+H).

8-[(4-chloro-2-hydroxyphenyl)methyl]-3-ethyl-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12h). A mixture of **12n** (30.3 mg, 0.10 mmol, 1 equiv) and 4-chloro-2-hydroxybenzaldehyde (34.4 mg, 0.21 mmol, 2.07 equiv) was dissolved in DMF (2 mL) and stirred at room temperature for 30 minutes before the addition of sodium triacetoxyborohydride (34.5 mg, 0.1 mmol, 1 equiv). The reaction stirred at room temperature under anhydrous conditions for 24 h. It was then quenched with water (1 mL). A work up with EtOAc and saturated sodium bicarbonate was performed to extract the organic layer, which was then washed twice. The aqueous layer was washed once. The organic layer was dried (sodium sulfate) and condensed yielding 45 mg as a clear oil. The oil was then purified by reverse phase HPLC (0% to 95% acetonitrile/water/0.05% TFA). Fractions were combined yielding the desired compound as a clear oil. This oil was then dissolved in acetonitrile (1 mL) and treated with 2 M HCl in diethyl ether (0.02 mL). A precipitate formed and was resuspended in diethyl ether. It was then filtered yielding the final compound as a white solid HCl salt. (30.2 mg, 0.074 mmol, 64.56% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (s, 1 H), 10.15 (d, J=0.7 Hz, 1 H), 7.77 (d, J=8.8 Hz, 1 H), 7.62 (d, J=8.6 Hz, 2 H), 6.83 - 7.01 (m, 4 H), 4.40 (d, J=4.2 Hz, 1 H), 3.28 - 3.55 (m, 4 H), 3.07 - 3.22 (m, 2 H), 1.98 (d, J=13.4 Hz, 2 H), 1.50 - 1.60 (m, 1 H), 1.38 - 1.48 (m, 2 H), 1.03 - 1.15 (m, 3 H), 0.86 - 0.96 (m, 6 H). ES-MS m/z 408.93 (M+H).

8-[(3-chloro-2-hydroxyphenyl)methyl]-3-ethyl-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12i). A mixture of **12n** (0.15 mmol) and 3-chloro-2-hydroxybenzaldehyde (0.20 mmol) was dissolved in DMF (2 mL) and was stirred at room temp for 30 minutes before the addition of sodium triacetoxyborohydride (0.20 mmol). The reaction stirred under positive nitrogen pressure at room temp for 24h. The mixture was the quenched with water, dissolved in water/brine, and extracted with DCM washes. The crude was then dissolved into ether and purified by HCl salt formation with 4M HCl in dioxane. Clear resin, HCl salt, 40mg, 0.0877 mmol, 43% yield, >94% HPLC purity, ES-MS m/z 408 (M+H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 - 10.23 (m, 1 H), 7.55 (d, J=7.8 Hz, 2 H), 7.02 (t, J=7.8 Hz, 1 H), 4.43 (br. s., 2 H), 3.49 (br. s., 4 H), 3.42-3.46 (m, 2 H), 3.13-3.30 (m, 2 H), 2.50 (d, J=1.7 Hz, 2 H), 2.00 (d, J=14.2 Hz, 2 H), 1.60 (s, 1 H), 1.40-1.53 (m, 2 H), 1.05-1.18 (m, 3 H), 0.90 - 1.02 (m, 6 H).

8-[(3,5-dichloro-2-hydroxyphenyl)methyl]-3-etyl-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12j). A mixture of **12n** (30.3 mg, 0.10 mmol, 1 equiv) and 3,5-dichloro-2-hydroxybenzaldehyde (38.2 mg, 0.20 mmol, 2 equiv) was dissolved in DMF (2 mL) and stirred at room temperature for 30 minutes before the addition of sodium triacetoxyborohydride (52.9 mg, 0.25 mmol, 2.5 equiv). The reaction stirred at room temperature under anhydrous conditions for 24 h. It was then quenched with water (1 mL) then purified by reverse phase HPLC (0% to 75% acetonitrile/water/0.05% TFA). The product was treated with 2

M HCl in diethyl either (0.1 mL) and stirred for 15 minutes before being filtered and dried to yield 50.9 mg product as a HCl salt. Aldehyde was still present in the final product so the solid was then dissolved in minimum amount of DCM and loaded onto an SCX column (2 g). The residual impurities were eluted with 1:1 MeOH/DCM (10 mL). The product was eluted with 2 M Ammonia/MeOH (10 mL). The ammonia eluent was then condensed yielding 30.2 mg final compound. The column was regenerated using 10 mL 5% TFA/CH3CN. The ammonia product was then condensed and dissolved in diethyl ether (1 mL). The product was then treated with 2 M HCl in diethyl ether (0.05 mL) to make an HCl salt. The salt was filtered, yielding the final compound as a white HCl salt. (25.5 mg, 0.0576 mmol, 44.24% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (d, J=2.4 Hz, 1 H), 7.23 (s, 1 H), 3.85 (br. s., 1 H), 3.39 (q, J=7.1 Hz, 4 H), 3.15 - 3.30 (m, 2 H), 2.85 (br. s., 2 H), 1.94 - 2.13 (m, 4 H), 1.72 (d, J=13.2 Hz, 2 H), 1.52 - 1.64 (m, 1 H), 1.36 - 1.49 (m, 2 H), 1.08 (t, J=7.2 Hz, 3 H), 0.92 (d, J=6.6 Hz, 6 H). ES-MS m/z 443.38 (M+H).

8-[(4,5-dichloro-2-hydroxyphenyl)methyl]-3-etyl-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12k). A mixture of **12n** (30.3 mg, 0.10 mmol, 1 equiv) and 4,5-dichloro-2-hydroxybenzaldehyde (38.2 mg, 0.20 mmol, 2 equiv) was dissolved in DMF (2 mL) and stirred at room temperature for 30 minutes before the addition of sodium triacetoxyborohydride (50.8 mg, 0.24 mmol, 2.4 equiv). The reaction stirred at room temperature under anhydrous conditions for 24 h. It was then quenched with water (1 mL) then purified by reverse phase HPLC (10% to 75% acetonitrile/water/0.05% TFA). The product was treated with 2 M HCl in diethyl either (0.1 mL) and stirred for 15 minutes before being filtered and dried to yield 66.7 mg product as a HCl salt. The solid was then dissolved in minimum amount of DCM and loaded onto an SCX column (2 g). The residual impurities were eluted with 1:1 MeOH/DCM (10 mL). The product was eluted with 2 M Ammonia/MeOH (10 mL). The ammonia eluent was then condensed yielding 30.2 mg final compound. The ammonia product was then condensed and dissolved in diethyl ether (1 mL). The product was then treated with 2 M HCl in diethyl ether (0.05 mL) to make an HCl salt. The salt was filtered, yielding the final compound as an off white HCl salt (25.6 mg, 0.0532 mmol, 46.82% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (s, 1 H), 7.00 (s, 1 H), 3.66 (br. s., 1 H), 3.38 (q, J=7.2 Hz, 4 H), 3.09 - 3.25 (m, 2 H), 2.78 (br. s., 4 H), 1.89 - 2.08 (m, 2 H), 1.69 (br. s., 2 H), 1.53 - 1.65 (m, 1 H), 1.34 - 1.51 (m, 2 H), 1.07 (t, J=7.2 Hz, 3 H), 0.91 (d, J=6.6 Hz, 6 H). ES-MS m/z 443.84 (M+H).

8-[(2,3-dichloro-6-hydroxyphenyl)methyl]-3-etyl-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione trifluoroacetate (12l). A mixture of **12n** (45.4 mg, 0.15 mmol, 1 equiv) and 2,3-dichloro-6-hydroxybenzaldehyde (43.0 mg, 0.22 mmol, 1.5 equiv) was dissolved in DMF (2 mL) and stirred at room temperature for 90 minutes before the addition of sodium triacetoxyborohydride (80 mg, 0.37 mmol, 2.5 equiv). The reaction stirred at room temperature under nitrogen atmosphere for 18 h. It was then quenched with water (1 mL) then purified by reverse phase HPLC (10% to 90% acetonitrile/water/0.05% TFA). The solution was then evaporated *in-vacuo* and lyophilized to afford a colorless powder of the desired product as a TFA salt (28.0 mg, 34% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (d, J= 9.05 Hz, 1 H), 7.02 (d, J= 9.05 Hz, 1 H), 4.46 (brs, 1H), 3.48-3.71 (m, 4H), 3.40 (q, J=7.2 Hz, 2H), 3.12 (appt/m, 1H), 2.22-2.38 (m, 2 H), 1.98 (d, J= 14.4 Hz, 2H), 1.50 – 1.62 (m, 1 H), 1.36-1.47 (m, 2H), 1.10 (t, J=7.2 Hz, 3 H), 0.89 (d, J=6.6 Hz, 6 H). ES-MS m/z 442 (^{35Cl}M+H), m/z 444 (^{37Cl}M+H), m/z 464 (^{35Cl}M+Na), and m/z 466 (^{37Cl}M+Na).

8-(5-chloro-2-hydroxybenzoyl)-3-ethyl-1-isopentyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (12m). 3-ethyl-1-isopentyl-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (**12n**) (0.1g, 0.374mmol), 5-chlorosalicylic acid (0.084g, 0.487mmol), EDCI (0.1g, 0.52mmol), HOBt (0.076g, 0.56mmol), Et3N (0.26ml, 1.86mmol) and DMAP (4.5mg, 0.037mmol) were dissolved in DCM. The reaction was stirred at room temperature overnight. The mixture was extracted with DCM. The combined organic phases were washed with citric acid solution, dried over sodium sulphate and concentrated in vacuo. The residue was purified by HPLC to give the title compound as a solid (72mg, 45.6%yield). ES-MS m/z 422 (MH^+). ^{1}H NMR (400 MHz, d^6-DMSO) δ 10.12 (s, 1H), 7.28-7.25 (m, 2H), 6.89 (d, *J*=8.8Hz, 1H), 4.54-4.50 (m, 1H), 3.64-3.58 (m, 1H), 3.42-3.37 (m, 3H), 3.17 (s, 2H), 1.93-1.88 (m, 2H), 1.77-1.74 (m, 1H), 1.64-1.54 (m, 2H), 1.42-1.41 (m, 2H), 1.10 (t, *J*=7.2Hz, 3H), 0.91 (d, *J*=6.4Hz, 6H).

3-ethyl-8-(3-hydroxypropyl)-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12o). A mixture of **12n** (100 mg, 0.329 mmol, 1.0 equiv), bromopropanol (29.5 μL; 0.329 mmol, 1 equiv) and potassium carbonate (181.9 mg, 1.317 mmol, 4 equiv) in acetonitrile (1 mL) was stirred at 60 ºC in a 2 dram vial for 4 days. The mixture was cooled to room temp, diluted with ethyl acetate and filtered through a syringe filter. The filtrate was concentrated to give 99 mg of a clear oil. The oil was dissolved in diethyl ether and filtered again through a syringe filter to remove residual salts. The filtrate was then treated with 1 M HCl in diethyl ether (0.5 mL; white precipitate formed). After drying, white crystalline product was somewhat gummy, so ~6 mL of diethyl ether was added and mixture was slurried for ~24 hours. It was then filtered and dried to give the target compound as an HCl salt (75.7 mg, 0.209 mmol, 60.4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.74 (s, 1 H), 2.65 (t, J=1.0 Hz, 2 H), 2.52 - 2.60 (m, 4 H), 2.43 - 2.49 (m, 2 H), 2.36 (t, J=1.0 Hz, 2 H), 1.67 (dt, J=3.7, 1.8 Hz, 4 H), 1.09 - 1.18 (m, 2 H), 0.98 - 1.08 (m, 2 H), 0.74 (spt, J=1.0 Hz, 1 H), 0.63 (q, J=1.0 Hz, 2 H), 0.27 (t, J=1.0 Hz, 3 H), 0.09 (d, J=6.6 Hz, 6 H). ES-MS m/z 326 (MH^+).

8-[(2-aminopyridin-3-yl)methyl]-3-ethyl-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12p). A mixture of **12n** (100 mg, 0.329 mmol, 1.0 equiv), and 2-amino-3-formylpyridine (81 mg, 0.663 mmol, 2equiv) was dissolved in DMF (2 mL) and was stirred at room temp for 30 minutes before the addition of sodium triacetoxyborohydride (110.8 mg, 0.802 mmol, 2.436 equiv). The reaction was stirred at room temp for 48 h. The reaction was quenched with a few drops of TFA. Solution was then purified by reverse phase HPLC (10 to 60% acetonitrile/water/0.05%TFA). Fractions were combined and were then washed with saturated sodium bicarbonate and extracted with EtOAc. The organic layer was then dried, filtered, and concentrated leaving an oil. The oil was then dissolved in ~5mL of diethyl ether, transferred to a 2 dram vial, and treated dropwise with 330 μL of HCl (1M in diethyl ether). The precipitate was slurried for about 30 minutes, then filtered and dried, yielding the target compound as a an off-white solid in the form of an HCl salt (73.1mg, 0.178 mmol, 54.2%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (d, J=7.1 Hz, 1 H), 8.12 (dd, J=6.2, 1.6 Hz, 1 H), 6.98 (dd, J=7.4, 6.2 Hz, 1 H), 4.45 (br. s., 2 H), 3.52 (br. s., 4 H), 3.40 (q, J=7.2 Hz, 2 H), 3.21 (t, J=1.0 Hz, 2 H), 2.62 (br. s., 2 H), 2.57 - 2.61 (m, 2 H), 1.95 (d, J=13.7 Hz, 2 H), 1.55 (spt, J=1.0 Hz, 1 H), 1.44 (q, J=1.0 Hz, 2 H), 1.10 (t, J=7.2 Hz, 3 H), 0.90 (d, J=6.6 Hz, 6 H). ES-MS m/z 374 (MH^+).

8-((2-amino-5-chloropyridin-3-yl)methyl)-3-ethyl-1-isopentyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (12q)

A mixture of **12n** (0.3 mmol) and 2-amino-5-chloronicotinaldehyde (0.44 mmol) was dissolved in DMF (2 mL) and stirred at room temperature for 30 minutes before the addition of sodium triacetoxyborohydride (0.59 mmol). The reaction stirred under positive nitrogen pressure at room temp for 24 hr. After 24 hours additional 2-amino-5-chloronicotinaldehyde (0.2 mmol) was added to the solution and stirred for 30 minutes before the addition of sodium triacetoxyborohydride (0.3 mmol). The reaction stirred for another 24 hours. The mixture was quenched with water, dissolved in saturated sodium bicarbonate and the crude product was extracted with ethyl acetate. The crude product was purified by reverse phase HPLC yielding the final compound as a TFA salt. The compound was converted to a free base using saturated sodium bicarbonate and extracted with ethyl acetate. Yellow powder, 44.7 mg, 0.109 mmol, 27% yield, >95% HPLC Purity, ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (d, J=6.60 Hz, 6 H) 1.06 (t, J=7.09 Hz, 3 H) 1.37 - 1.48 (m, 1 H) 1.51 - 1.68 (m, 2 H) 1.87 - 2.00 (m, 2 H) 2.63 - 2.75 (m, 4 H) 3.10 - 3.21 (m, 2 H) 3.27 - 3.46 (m, 6 H) 6.20 (s, 2 H) 7.44 (d, J=2.69 Hz, 1 H) 7.87 (d, J=2.69 Hz, 1 H). ES-MS m/z 408.1 (MH^+).

3-ethyl-1-(3-methylbutyl)-8-[(pyridin-2-yl)carbonyl]-1,3,8-triazaspiro[4.5]decane-2,4-dione (12r). To a mixture of **12n** (100 mg, 0.329 mmol, 1.0 equiv) and picolinic acid (0.362 mmol, 1.1 equiv) in DCM (4 mL) was added HOBT (0.494 mmol, 1.5 equiv), EDC and 4-ethylmorpholine (206 μL, 1.646 mmol, 5 equiv). Reaction was stirred at r.t. for 5 days. It was then quenched with a few drops of water, followed by a few drops of TFA. Solution was then purified by reverse phase HPLC (10-60% acetonitrile/water/0.05%TFA). Fractions were then combined and concentrated yielding a clear oil which was then washed with saturated sodium bicarbonate and extracted twice with EtOAc. The organic layer was then dried, filtered and concentrated leaving a clear oil. Oil solidified after ~48 hours, yielding a solid white target compound (74.4 mg, 0.200 mmol, 60.7%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (dq, J=1.0 Hz, 1 H), 7.94 (td, J=7.7, 1.8 Hz, 1 H), 7.63 (dt, J=7.8, 1.1 Hz, 1 H), 7.48 (ddd, J=7.6, 4.9, 1.2 Hz, 1 H), 3.34 - 3.44 (m, 4 H), 3.19 (t, J=1.0 Hz, 2 H), 2.06 - 2.10 (m, 2 H), 1.96 (dt, J=12.5, 6.6 Hz, 2 H), 1.77 - 1.84 (m, 1 H), 1.62 - 1.68 (m, 1 H), 1.52 - 1.62 (m, 1 H), 1.42 (d, J=3.2 Hz, 2 H), 1.09 (t, J=1.0 Hz, 3 H), 0.90 (d, J=6.6 Hz, 6 H). ES-MS m/z 373 (MH^+).

3-ethyl-1-(3-methylbutyl)-8-(quinolin-8-ylmethyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12s). A mixture of **12n** (100.2 mg, 0.33 mmol, 1.0 equiv) and quinolone-8-carboxaldehyde (102.9 mg, 0.655 mmol, 1.985 equiv) was dissolved in DMF (2 mL) and was stirred at room temp for 30 minutes before the addition of sodium triacetoxyborohydride (174.74 mg, 0.824 mmol, 2.5 equiv). After 48 hrs, reaction was quenched with a few drops of water, followed by TFA. The solution was then purified by reverse phase HPLC (10 to 60% acetonitrile/water/0.05%TFA). Fractions were then combined and concentrated, leaving an oily yellowish product. This oil was then washed with saturated sodium bicarbonate and extracted twice with EtOAc. The organic layers were then combined, dried, filtered and concentrated to give a yellow oily product (112.4mg). This oil was then diluted with a minimal amount of diethyl ether and transferred to a 2 dram vial, along with 330 μL of HCl (1 M in diethyl ether). This mixture was slurried for ~30 minutes, then filtered and dried to give the target compound (an off-white solid) as an HCl salt (115.5 mg, 0.260 mmol, 78.7% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (dd, J=4.1, 1.6 Hz, 1 H), 8.52 (dd, J=8.3, 1.7 Hz, 1 H), 8.21 (dd, J=7.1, 1.3 Hz, 1 H), 8.17

(dd, J=8.3, 1.3 Hz, 1 H), 7.75 (dd, J=8.2, 7.2 Hz, 1 H), 7.68 (dd, J=8.3, 4.2 Hz, 1 H), 5.01 (br. s., 2 H), 3.43 - 3.61 (m, 4 H), 3.32 - 3.42 (m, 2 H), 3.17 (t, J=1.0 Hz, 2 H), 2.08 (s, 2 H), 1.92 (d, J=14.7 Hz, 2 H), 1.48 - 1.59 (m, 1 H), 1.41 (d, J=7.9 Hz, 2 H), 1.04 - 1.11 (m, 3 H), 0.88 (d, J=6.4 Hz, 6 H). ES-MS m/z 410 (MH^+).

3-ethyl-1-(3-methylbutyl)-8-(pyridin-2-ylmethyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12t). A mixture of **12n** (100 mg, 0.329 mmol, 1.0 equiv) and 2-bromomethyl pyridine hydrobromide (83.25 mg, 0.329 mmol, 1.0 equiv) and potassium carbonate (227.4 mg, 1.646 mmol, 5 equiv) in acetonitrile (2 mL) was stirred at 60 ºC in a 2 dram vial for 48 hours. After this time, the reaction mixture was diluted with ethyl acetate and filtered through a syringe filter to remove excess potassium carbonate. The filtrate was then concentrated to yield a dark yellow oil. Next the sample was diluted with DMF (~2mL) and a few drops of TFA were added. The solution was then purified by reverse phase HPLC (10 to 60% acetonitrile/water/0.05% TFA). Fractions were combined and concentrated yielding a light yellow oil. The sample was subsequently washed with saturated sodium bicarbonate and extracted twice with EtOAc. Combined organic layers were dried, filtered and concentrated to give a clear oil (116.6mg). This oil was then diluted with a minimal amount of diethyl ether and transferred to a 2 dram vial, along with 360 µL of HCl (1 M in diethyl ether). This mixture was slurried for ~30 minutes, then filtered through a filter funnel and dried to give the target compound, a white solid precipitate, as an HCl salt (84.5 mg, 0.214 mmol, 65%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 - 8.73 (m, 1 H), 7.95 (td, J=7.7, 1.8 Hz, 1 H), 7.69 (d, J=7.8 Hz, 1 H), 7.51 (ddd, J=7.7, 4.9, 1.1 Hz, 1 H), 4.56 (s, 2 H), 3.51 (t, J=1.0 Hz, 4 H), 3.38 (q, J=7.1 Hz, 2 H), 3.19 (t, J=1.0 Hz, 2 H), 2.08 (s, 2 H), 1.95 (d, J=14.1 Hz, 2 H), 1.56 (spt, J=1.0 Hz, 1 H), 1.45 (q, J=1.0 Hz, 2 H), 1.08 (d, J=1.0 Hz, 3 H), 0.91 (d, J=6.6 Hz, 6 H). ES-MS m/z 360 (MH^+).

3-ethyl-8-(1H-indol-7-ylmethyl)-1-(3-methylbutyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12u). A mixture of **12n** (100.5 mg, 0.331 mmol, 1.0 equiv) and 7-formylindole (96.6 mg, 0.665 mmol, 2.012 equiv) was dissolved in DMF (2 mL) and was stirred at room temp for 30 minutes before the addition of sodium triacetoxyborohydride (175.3 mg, 0.827 mmol, 2.5 equiv). After 24 hrs, the reaction was quenched with a few drops of water, followed by TFA. The solution was then purified by reverse phase HPLC (10 to 60% acetonitrile/water/0.05%TFA). The product fraction was concentrated and then washed with sat. sodium bicarbonate and extracted twice with EtOAc. The organic layer was then dried using sodium sulfate, filtered by gravity filtration, and concentrated to yield a red/orange oil. It was then purified by flash chromatograhy (10-90% EtOAc/heptane) and fractions were combined and concentrated yielding 75.3 mg of a clear, light purple oil. This was then dissolved in a minimal amount of diethyl ether, and 280 µL of HCl (1M in diethyl ether) was added. Solution was slurried for ~30 minutes then filtered and dried to give the target compound, a pink powdery solid, as an HCl salt (46.1 mg, 0.106 mmol, 32.2%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.66 (br. s., 1 H), 7.68 (d, J=1.0 Hz, 1 H), 7.48 (br. t, J=1.0, 1.0 Hz, 1 H), 7.38 (d, J=1.0 Hz, 1 H), 7.09 (t, J=1.0 Hz, 1 H), 6.53 (br. q, J=1.0, 1.0, 1.0 Hz, 1 H), 4.68 (br. d, J=1.0 Hz, 2 H), 3.52 (br. t, J=1.0, 1.0 Hz, 4 H), 3.37 (q, J=1.0 Hz, 4 H), 3.16 (br. t, J=1.0, 1.0 Hz, 2 H), 1.95 (br. d, J=1.0 Hz, 2 H), 1.54 (spt, J=1.0 Hz, 1 H), 1.41 (br. q, J=1.0, 1.0, 1.0 Hz, 2 H), 1.07 (t, J=1.0 Hz, 3 H), 0.90 (d, J=1.0 Hz, 6 H). ES-MS m/z 397 (MH^+).

cLogP Calculations

cLogP values were calculated using the cLogP calculators in ChemDraw Ultra 12.0.

In vitro Antimalarial Assays (3D7 and Dd2)

P. falciparum strain 3D7 was cultured according to the method of Trager and Jensen (1976, Science 193:673-675) with minor modifications. Parasites were grown in human erythrocytes (2% hematocrit) in an atmosphere of 5% CO2, 5% O2, 90% N2 in RPMI1640 medium (Gibco) supplemented with 25 mM Hepes buffer (Sigma), 25 mg L^{-1} gentamicin (Gibco), 1 mM Sodium pyruvate (Sigma), 50 mg L^{-1} hypoxanthine (Sigma), 2 g L^{-1} glucose (Sigma), 2.52 g L^{-1} sodium bicarbonate (Sigma) and 5 g L^{-1} Albumax 1 (Gibco). *In vitro* antimalarial activity was determined by the SYBR Green I method described by Smilkstein et al (2004, Antimicrob. Agents Chemother. 48:1803–1806) with modifications (Winter, et al. 2006. Exp. Parasitol. 114:47–56.). Stock solutions of each compound were prepared in DMSO at a concentration of 10 mM and 3-fold serial dilutions prepared in DMSO. Compounds were then diluted 500-fold into culture medium in 96-well storage plates to create 2X drug solutions. Drug solutions (50 µl per well) were transferred in quadruplicate to parasite cultures (50 µl) in 96-well black tissue culture plates for a total volume of 100 µl at 2% hematocrit, 0.5% parasitemia and 0.1% DMSO final concentrations. The plates were then incubated for 72 h at 37 °C. After incubation, 100 µl of lysis buffer containing 0.2 µl ml^{-1} SYBR Green I was added to each well.

After incubation for 1 h at room temperature in the dark, plates were read on a Safire2 (Tecan) plate reader with excitation and emission wavelengths of 497 and 520 nM, respectively. The 50% inhibitory concentrations (IC50s) were determined by nonlinear regression using a four parameter logistic equation (Graph-Pad Prism software).

Antimalarial potency of compounds was determined by this technique for both *Plasmodium falciparum* 3D7 (CQ-sensitive) and Dd2 (multi-drug resistant) strains.

Plasmepsin-2 (PM-II; PM-2) and Plasmpesion-4 (PM-IV; PM-4) Enzyme Inhibition Assays

Plasmepsin-2 (PM-2; Plm II) and Plasmepsin-4 (PM-4; Plm IV) expression and purification were performed following the published protocols (Istvan ES and Goldberg DE, 2005). The final purified protein was activated by diluting the protein to 0.3mg/ml in activating buffer (0.1M citrate pH 4.5,0.1% Tween-20, 50mM dithiothreitol) and incubated at room temperature for 40 min, then the activated enzyme was diluted in assay buffer (50mM sodium acetate pH 4.7, 0.01% Tween-20). The enzymatic inhibition reaction was performed in 384 well plates with a total volume of 20 µl. 10 µl of diluted PM-2 or PM-4 enzyme was added to the 384-well plate except blank wells (blank wells add 10 µL of assay buffer) and 20 nl of serials of diluted 1000 × compounds were added to the wells with 520 Echo® Liquid Handling System (Labcyte Inc.). 10 µl PM-2 peptide substrate (AnaSpec, Cat#, 62050) with assay buffer was then added to final concentration of 20 µM to start the reaction. After incubation the reaction at room temperature for 60 min，the fluorescence intensity at Ex/Em= 360nm/535nm was measured using EnVision multilabel plate reader (Perkin-Elmer). The IC_{50} values were obtained using Graph Pad Prism 4.

β-Secretase (BACE1), Cathepsin D, and Cathepsin E Enzymes Inhibition Assays

The recombinant human BACE1, Cathepsin D, and Cathepsin E enzymes were purchased from R&D Systems (catalog numbers are 931-AS, 1014AS and 1294AS, respectively). The enzymatic inhibition activities Assays were performed in a 384-well plate format using the fluorescence resonance energy transfer (FRET) assay. The concentration of BACE1 and Cathepsin D were 20 ng/µl, and 1 ng/µl of Cathepsin E was used. BACE1 was activated by incubation in assay buffer (100mM sodium acetate pH 4.0) for 15 min at room temperature; CatD and CatE were activated by incubation in assay buffer (0.1 M NaOAc, 0.2 M NaCl, pH 3.5) at room temperature for 30 min. Subsequently, the rhBACE-1 substrate (R&D Systems, catalog# ES004), the Cathepsin D and Cathepsin E substrate ((R&D Systems, Catalog # ES001) were added accordingly to final concentration 20uM to initiate the reaction. After 60 min incubation at room temperature, the time-resolved fluorescence at Ex/Em= 360nm/460nm was measured on an EnVision multilabel plate reader (Perkin-Elmer). The analytical software, GraphPad Prism 5.0 (GraphPad Software, Inc., USA) was used to generate IC_{50} values via non-linear regression analysis.

HepG2 Cytotoxicity Assay

HepG2 cells (ATCC Cat. No. HB-8065) were maintained in DMEM supplemented with 10% fetal bovine serum and L-glutamine. Cells were grown at 37ºC and 5% CO_2 on flasks coated with poly-d-lysine. To assess compounds for potential cytotoxic properties cells were plated at 10,000 cells per well on 96-well poly-d-lysine plates at 10,000 cells per well. HepG2 cells were allowed to adhere for at least 4 hours prior to application of test compounds. Cells were incubated with test compounds for 72 hours before measuring cellular viability. Cellular viability was measured using PrestoBlue® Cell Viability Reagent (Life Technologies). Briefly, 11ul of 10X PrestoBlue reagent was added to 100ul in each assay well. Cells were incubated at 37ºC and 5% CO_2 for 30 minutes prior to reading on Tecan Safire^2, excitation at 560nm, 10 nm bandwidth and emission at 590 nm, 10 nm bandwidth. GraphPad Prism 5.0 (GraphPad Software, Inc., USA) was used to generate IC_{50} values via non-linear regression analysis.

MLM, RLM and HLM Assays

In this protocol, the metabolic stability of **spiropiperidinehydantoin** compounds at 1 µM was determined in mouse liver microsomes (MLM), rat liver microsomes (RLM) or human liver microsomes (HLM). Each test compound was incubated in an aqueous reaction mixture (0.6 mL total volume) consisting of 0.25 µM microsomal protein CYP450 activity, 1.2 mM NADPH, 3.3 mM $MgCl_2$, and 100 mM potassium phosphate buffer (pH 7.4). After incubation at 37◦C for a specific time period (0, 5, 10, 20 and 30 min), a 100-µL aliquot of the reaction was transferred to 200 µL ice cold acetonitrile containing internal standard (100 ng/mL). The quenched reaction mixtures were centrifuged at 3200 rpm for 5 min, and 100 µL of the supernatant were transferred to 96-well plate and analyzed by LC-MS/MS using an Applied Biosystems-Sciex

model API 4000 mass spectrometer. Analyte/internal standard peak area ratios were used to calculate half-life.

Rat Pharmacokinetic (PK) Analysis

Male SD rats, weighing 180-220g (Southern China Medical University,China) were utilized for the studies. The protocol was approved by the Institutional Animal Care and Use Committee at GIBH. Animals were maintained on standard animal chow and water ad libitum, in a climate controlled room(23±1℃, 30–70% relative humidity, a minimum of 10 exchanges of room air per hour and a 12-h light/dark cycle) for one week prior to experiments. The test compound was dissolved in suitable solvent. Pharmacokinetic properties were determined following i.v. and oral administration. Animals were randomly distributed into two experimental groups (n = 4). The oral groups were given 5 mg/kg of the test compound by gastric gavage. The other group was dosed by injection into the tail vein (1mg/kg). After single administration, whole blood samples (100-200 μL) were obtained from the orbital venous plexus at the following time points after dosing: 5,10, 30 min and 1, 2, 3, 4, 6, 8,11 and 24 h(p.o.); 2,10, 30 min and 1, 2, 3, 4, 6, 8, 11 and 24 h (i.v.).Whole blood samples were collected in heparinized tubes. The plasma fraction was immediately separated by centrifugation (8,000 rpm, 6 min, 4 °C) and stored at −20 °C until LC-MS analysis. The rats were humanely euthanasia by carbon dioxide 24 hours after experiment without pain.

Plasma sample analysis. Standard curve sample preparation: The compound was dissolved in DMSO at a concentration of 2mg/ml and diluted with 50% methanol solution to series concentration as follow:20, 50, 100, 500, 1000, 2000, 4000, 6000, 12000, 40000ng/ml. 10μl series concentration solution and 50μl blank plasma were added to 1.5ml tube and vortex for 3min, then 150μl acetonitrile containing internal standard were added and vortex for 5min, finally spin tube in centrifuge at 16000g for 40min at 4°C. **Plasma sample preparation:** The plasma samples were prepared using protein precipitation method.10μl 50% methanol water solution and 50μl plasma samples were added to 1.5ml tube and vortex for 3min, then 150μl acetonitrile containing internal standard were added and vortex for 5min, finally spin tube in centrifuge at 16000g for 40min at 4°C. **LC/MS/MS analysis:** After centrifuge, 100μl supernatant was transfer to the 96 well plate and analyzed by LC-MS/MS using an Applied Biosystems-Sciex model API 3000 mass spectrometer. The pharmacokinetics parameters were calculated by analyzing the compound concentration in plasma samples using the pharmacokinetic software DAS.2.0

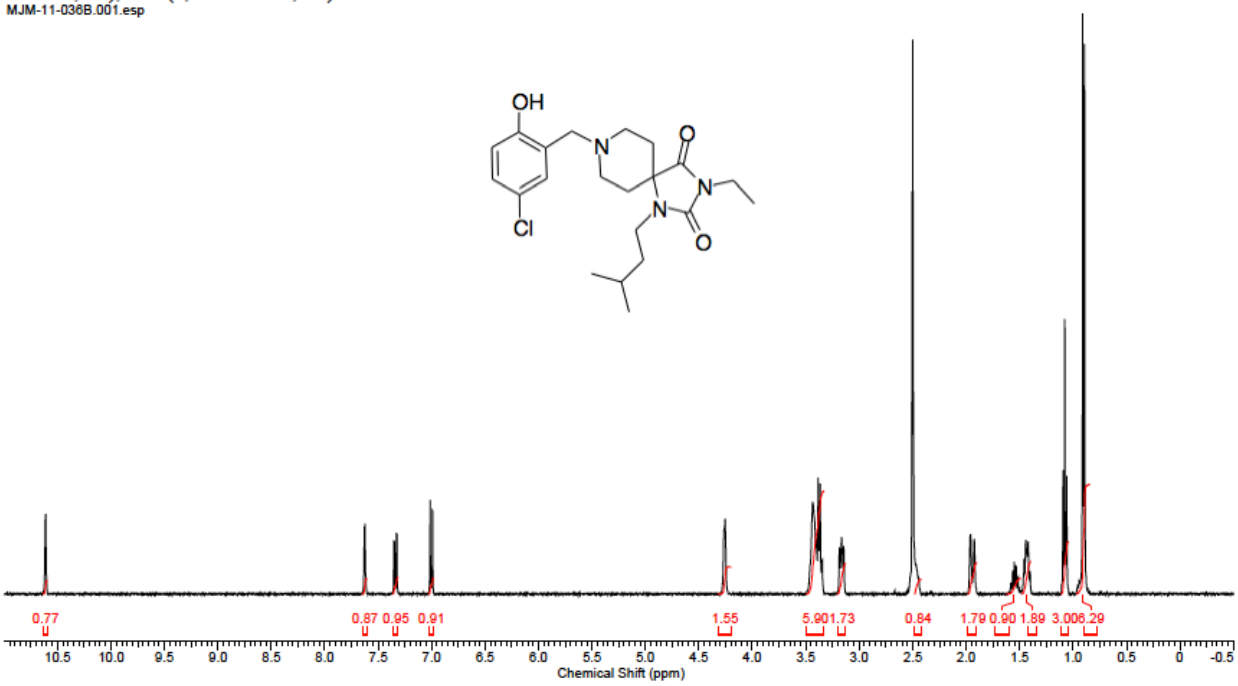
The Institutional Animal Care and Use Committee at the Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, reviewed and approved the animal use in these studies. The animal care and use program, run entirely according to Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALACi) standards, is applying for AAALACi accreditation. The license for using laboratory animals is issued by Guangdong Laboratory Animal monitoring Institute. The Office of Laboratory Animal Welfare (OLAW) has recently re-activated the Animal Welfare Assurance for this institution (OLAW identification number A5748-01).

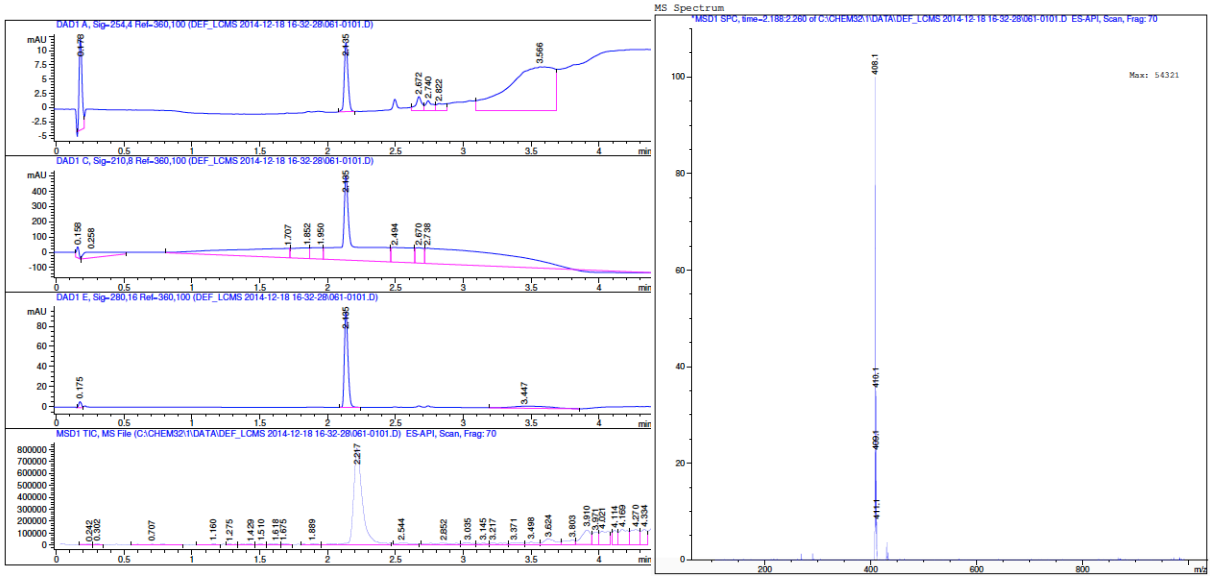
^{1}H NMR and LC-MS spectra for compound 4e (CWHM-123)

Acquisition Time (sec)	3.9846	*Comment*	MJM-11-036B - HCl salt			*Date*	06 Jan 2012 10:53:20
Date Stamp	06 Jan 2012 10:53:20			*File Name*	C:\Users\mmeyers8\Desktop\MJM-11-036B\1\fid		
Frequency (MHz)	400.13	*Nucleus*	1H	*Number of Transients*	16	*Origin*	spect
Original Points Count	32768	*Owner*	mmeyers8	*Points Count*	131072	*Pulse Sequence*	zg30
Receiver Gain	512.00	*SW(cyclical) (Hz)*	8223.68	*Solvent*	DMSO-d6	*Spectrum Offset (Hz)*	2467.5718
Spectrum Type	STANDARD	*Sweep Width (Hz)*	8223.62	*Temperature (degree C)*	25.000		

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.61 (s, 1H), 7.63 (d, J = 2.45 Hz, 1H), 7.34 (dd, J = 2.73, 8.69 Hz, 1H), 7.00 (d, J = 8.85 Hz, 1H), 4.20 - 4.32 (m, 2H), 3.34 - 3.47 (m, 6H), 3.13 - 3.20 (m, 2H), 2.42 - 2.48 (m, 2H), 1.90 - 1.98 (m, 2H), 1.49 - 1.62 (m, 1H), 1.39 - 1.48 (m, 2H), 1.08 (t, J = 7.18 Hz, 3H), 0.90 (d, J = 6.59 Hz, 6H)

MJM-11-036B.001.esp



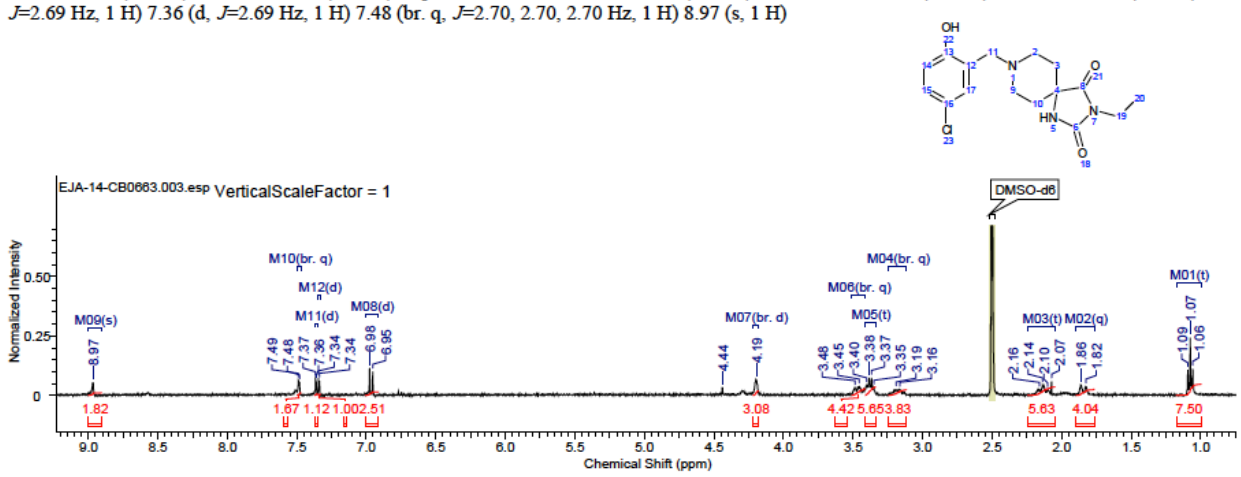


^{1}H NMR spectra for compound 10a

Formula $C_{16}H_{20}ClN_3O_3$	FW 337.8013

Acquisition Time (sec)	4.0894	Comment	5 mm PABBO BB-1H/D Z-GRD Z863001/0012			Date	22 Dec 2014 13:41:52
Date Stamp	22 Dec 2014 13:41:52			File Name	C:\Users\eander39\Desktop\EJA-14-CB0663\3\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	nmr	Points Count	32768	Pulse Sequence	zg30
Receiver Gain	812.00	SW(cyclical) (Hz)	8012.82	Solvent	DMSO-d6	Spectrum Offset (Hz)	2467.6448
Spectrum Type	STANDARD	Sweep Width (Hz)	8012.58	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.07 (t, *J*=7.09 Hz, 3 H) 1.84 (q, *J*=14.70 Hz, 2 H) 2.12 (t, *J*=1.00 Hz, 2 H) 3.18 (br. q, *J*=11.00, 11.00, 11.00 Hz, 2 H) 3.38 (t, *J*=1.00 Hz, 2 H) 3.47 (br. q, *J*=11.50, 11.50, 11.50 Hz, 2 H) 4.19 (br. d, *J*=1.00 Hz, 2 H) 6.97 (d, *J*=8.56 Hz, 1 H) 7.34 (d, *J*=2.69 Hz, 1 H) 7.36 (d, *J*=2.69 Hz, 1 H) 7.48 (br. q, *J*=2.70, 2.70, 2.70 Hz, 1 H) 8.97 (s, 1 H)

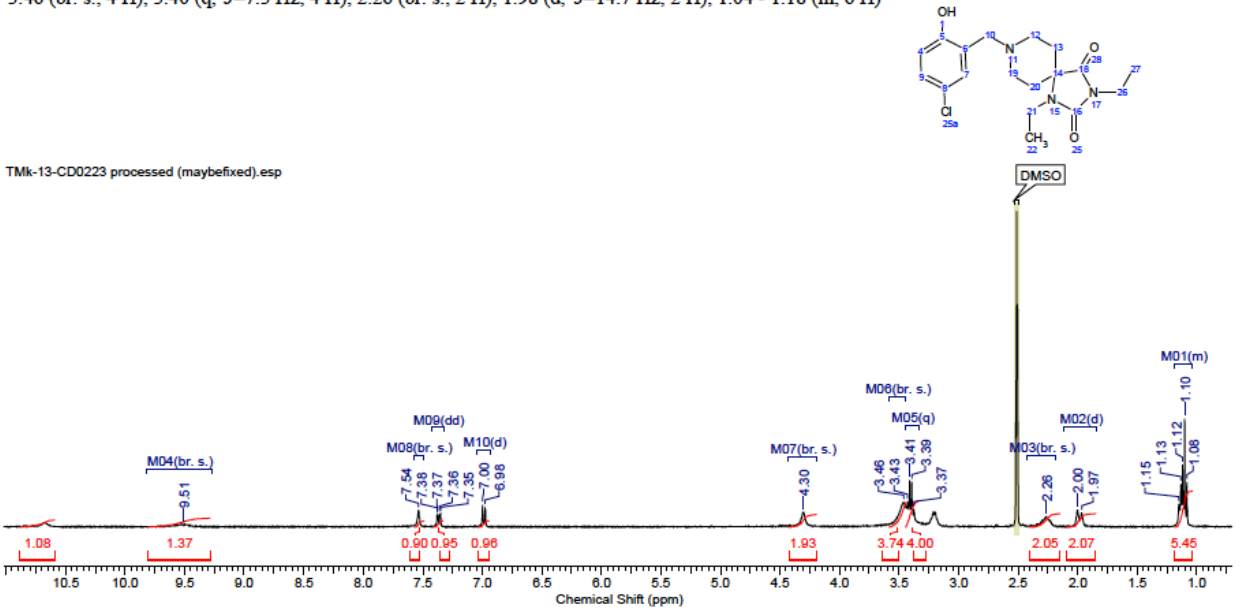


^{1}H NMR spectra for compound 10b

Formula $C_{16}H_{24}ClN_3O_3$	FW 365.8545

Acquisition Time (sec)	4.0894	Comment	5 mm PABBO BB-1H/D Z-GRD Z863001/0012			Date	11 Sep 2013 15:13:52
Date Stamp	11 Sep 2013 15:13:52						
File Name	C:\Documents and Settings\tkrennin\My Documents\Dropbox\Research Folders\raw NMR data - 9.10.13\TMk-13-CD0223\1\fid						
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	nmr	Points Count	32768	Pulse Sequence	zg30
Receiver Gain	812.00	SW(cyclical) (Hz)	8012.82	Solvent	DMSO-d6	Spectrum Offset (Hz)	2470.9668
Spectrum Type	STANDARD	Sweep Width (Hz)	8012.58	Temperature (degree C)	25.000		

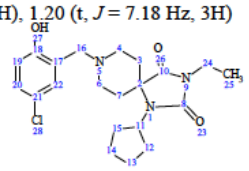
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.51 (br. s., 1 H), 7.54 (br. s., 1 H), 7.37 (dd, *J*=8.7, 2.3 Hz, 1 H), 6.99 (d, *J*=8.6 Hz, 1 H), 4.30 (br. s., 2 H), 3.46 (br. s., 4 H), 3.40 (q, *J*=7.3 Hz, 4 H), 2.26 (br. s., 2 H), 1.98 (d, *J*=14.7 Hz, 2 H), 1.04 - 1.18 (m, 6 H)

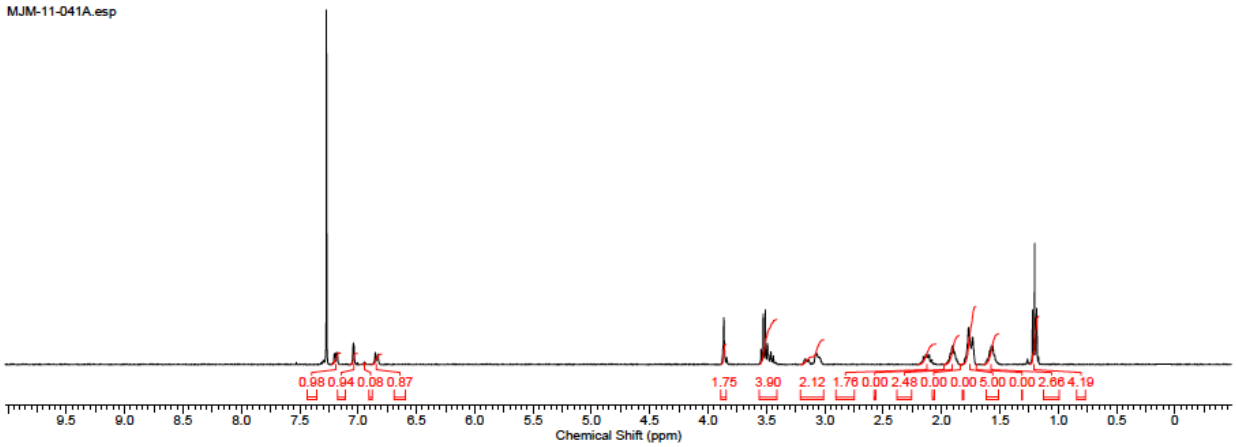


^{1}H NMR spectra for compound 10c

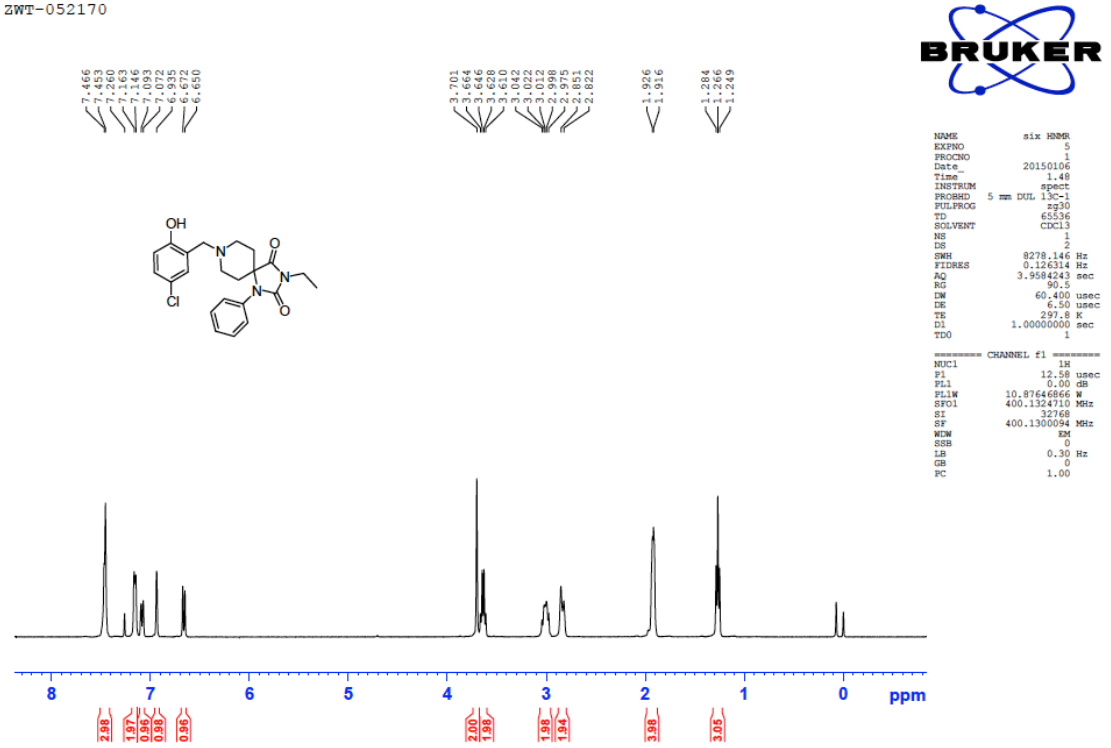
Formula	$C_{21}H_{28}ClN_3O_3$	FW	405.9183				
Acquisition Time (sec)	3.9846	Comment	MTM-11-041A/CDCl3/02-13-2012			Date	13 Feb 2012 15:09:20
Date Stamp	13 Feb 2012 15:09:20						
File Name	\\vmware-host\Shared Folders\Documents\CWHM Projects\Notebooks\CWHM-MJM-11\NMR Data\MJM-11-041A\fid						
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	nmr	Points Count	131072	Pulse Sequence	zg30
Receiver Gain	645.00	SW(cyclical) (Hz)	8223.68	Solvent	CHLOROFORM-d	Spectrum Offset (Hz)	2465.0159
Spectrum Type	STANDARD	Sweep Width (Hz)	8223.62	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, CHLOROFORM-d) δ 7.19 (dd, *J* = 2.32, 8.47 Hz, 1H), 7.04 (d, *J* = 2.01 Hz, 1H), 6.84 (d, *J* = 8.34 Hz, 1H), 3.87 (s, 2H), 3.41 - 3.56 (m, 4H), 3.01 - 3.21 (m, 2H), 2.05 - 2.21 (m, 2H), 1.85 - 1.97 (m, 3H), 1.75 (d, 5H), 1.51 - 1.64 (m, 3H), 1.20 (t, *J* = 7.18 Hz, 3H)

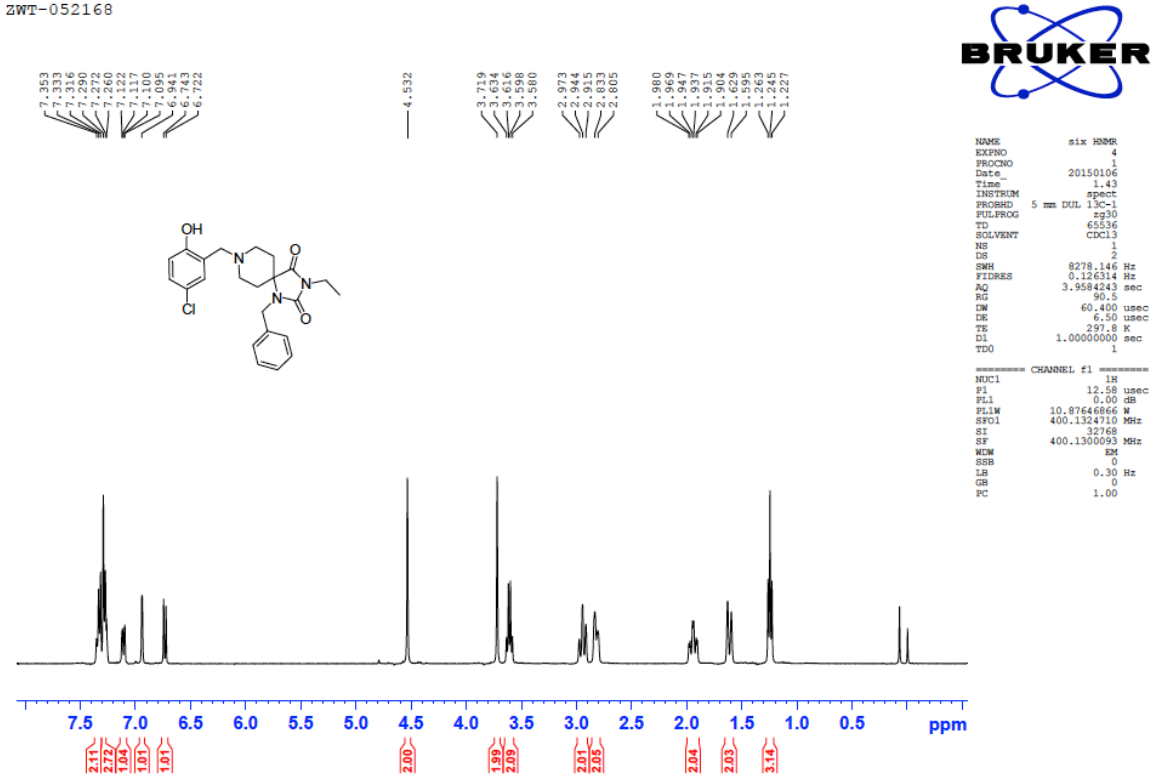




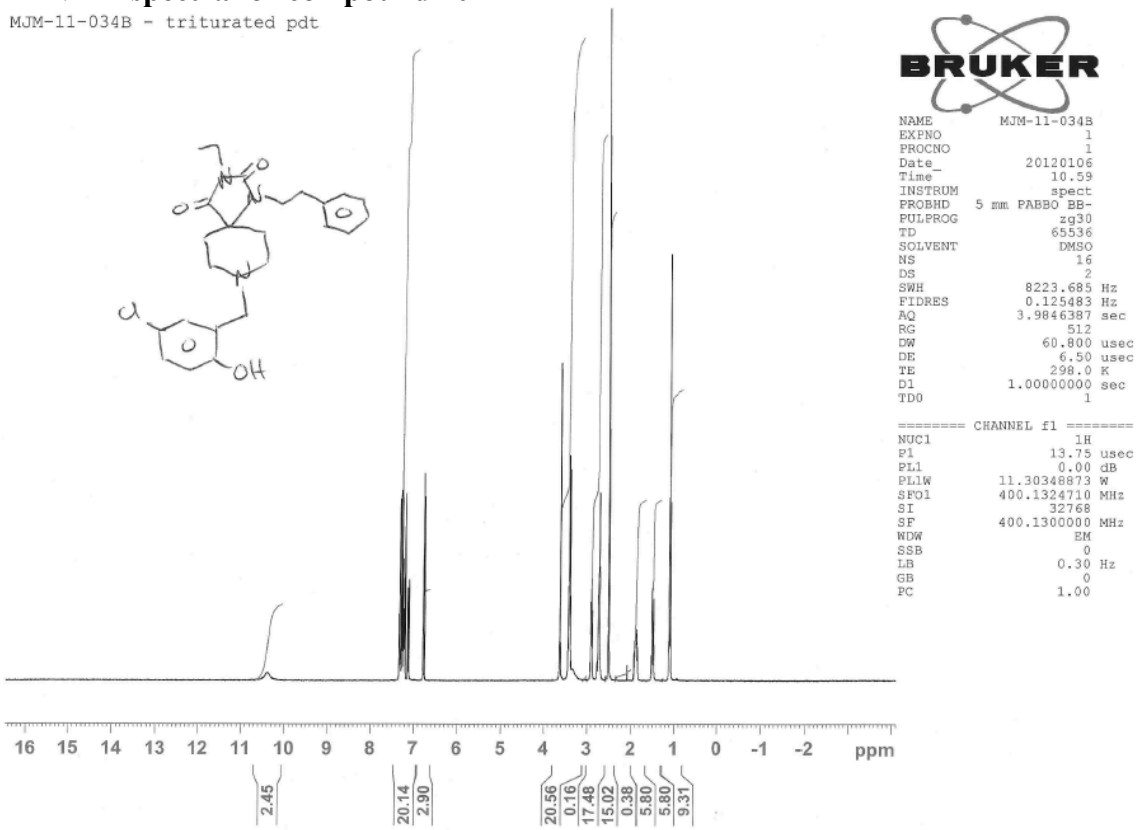
^{1}H NMR spectra for compound 10d



^{1}H NMR spectra for compound 10e

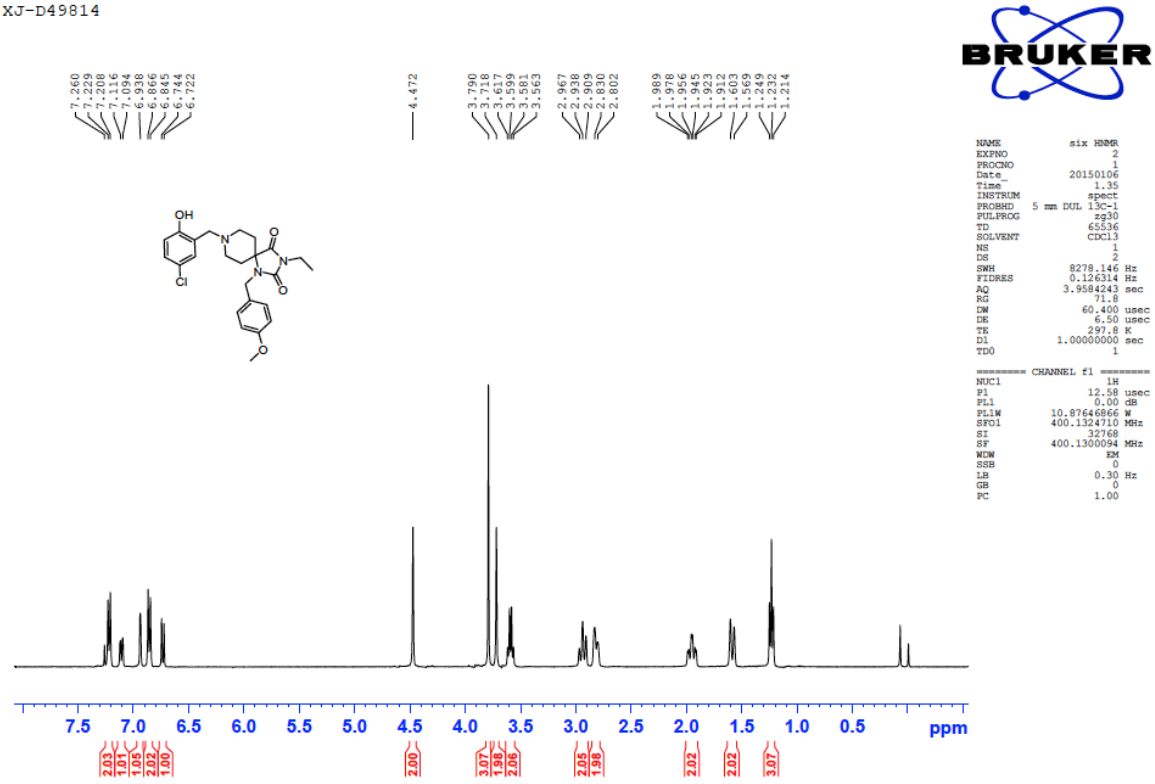


^{1}H NMR spectra for compound 10f



^{1}H NMR spectra for compound 10g

XJ-D49814



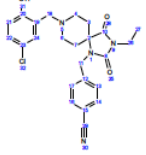

```
NAME                six HNMR
EXPNO                      2
PROCNO                     1
Date_               20150106
Time                   1.35
INSTRUM                spect
PROBHD     5 mm DUL 13C-1
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                         1
DS                         2
SWH                8278.146 Hz
FIDRES             0.126314 Hz
AQ                 3.9584243 sec
RG                      71.8
DW                     60.400 usec
DE                      6.50 usec
TE                    297.8 K
D1                 1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                      1H
P1                     12.58 usec
PL1                     0.00 dB
PL1W           10.87646866 W
SFO1           400.1324710 MHz
SI                      32768
SF             400.1300094 MHz
WDW                       EM
SSB                        0
LB                     0.30 Hz
GB                         0
PC                      1.00
```

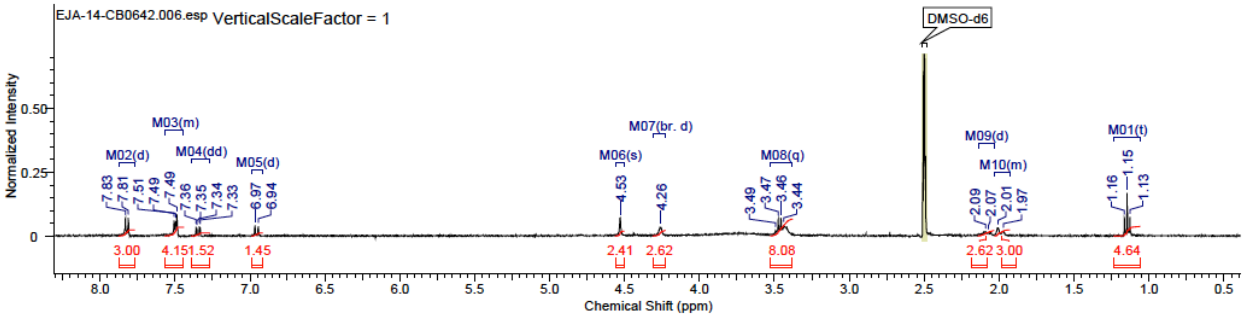
^{1}H NMR spectra for compound 10h

Formula	$C_{24}H_{25}ClN_4O_3$	FW	452.9333

Acquisition Time (sec)	4.0894	*Comment*	5 mm PABBO BB-1H/D Z-GRD Z863001/0012			*Date*	12 Sep 2014 15:41:36
Date Stamp	12 Sep 2014 15:41:36			*File Name*	C:\Users\eander39\Desktop\EJA-14-CB0642\6\fid		
Frequency (MHz)	400.13	*Nucleus*	1H	*Number of Transients*	16	*Origin*	spect
Original Points Count	32768	*Owner*	nmr	*Points Count*	32768	*Pulse Sequence*	zg30
Receiver Gain	912.00	*SW(cyclical) (Hz)*	8012.82	*Solvent*	DMSO-d6	*Spectrum Offset (Hz)*	2466.9111
Spectrum Type	STANDARD	*Sweep Width (Hz)*	8012.58	*Temperature (degree C)*	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.15 (t, *J*=7.21 Hz, 3 H) 1.93 - 2.03 (m, 2 H) 2.08 (d, *J*=7.83 Hz, 2 H) 3.46 (q, *J*=7.25 Hz, 6 H) 4.26 (br. d, *J*=1.00 Hz, 2 H) 4.53 (s, 2 H) 6.96 (d, *J*=8.80 Hz, 1 H) 7.35 (dd, *J*=8.56, 2.69 Hz, 1 H) 7.44 - 7.57 (m, 3 H) 7.82 (d, *J*=8.31 Hz, 3 H)

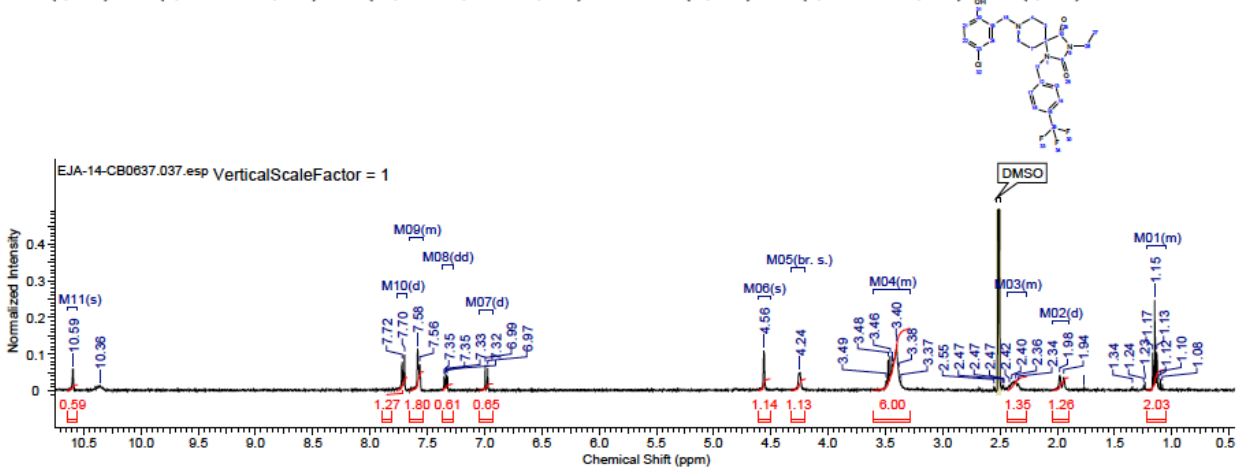




^{1}H NMR spectra for compound 10i

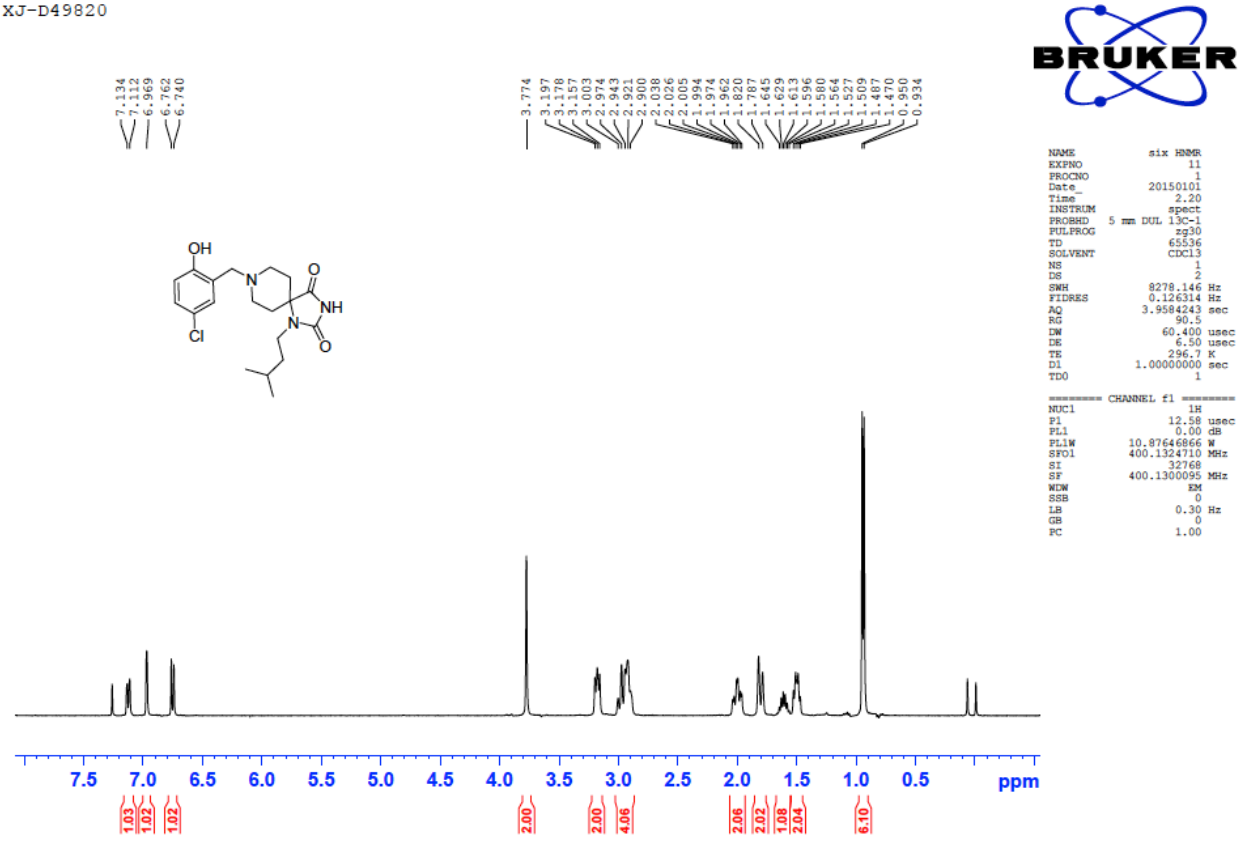
Formula $C_{24}H_{25}ClF_3N_5O_3$		FW	495.9218		
Acquisition Time (sec)	4.0894	**Comment**	5 mm PABBO BB-1H/D Z-GRD Z863001/0012	**Date**	15 Aug 2014 14:58:56
Date Stamp	15 Aug 2014 14:58:56	**File Name**	C:\Users\eander39\Desktop\EJA-14-CB0637\37\fid		
Frequency (MHz)	400.13	**Nucleus**	1H	**Number of Transients**	16	**Origin**	spect
Original Points Count	32768	**Owner**	nmr	**Points Count**	32768	**Pulse Sequence**	zg30
Receiver Gain	812.00	**SW(cyclical) (Hz)**	8012.82	**Solvent**	DMSO-d6	**Spectrum Offset (Hz)**	2470.9666
Spectrum Type	STANDARD	**Sweep Width (Hz)**	8012.58	**Temperature (degree C)**	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.05 - 1.22 (m, 3 H) 1.96 (d, *J*=14.43 Hz, 2 H) 2.27 - 2.43 (m, 2 H) 3.28 - 3.60 (m, 6 H) 4.24 (br. s., 2 H) 4.56 (s, 2 H) 6.98 (d, *J*=8.56 Hz, 1 H) 7.34 (dd, *J*=8.68, 2.57 Hz, 1 H) 7.53 - 7.65 (m, 3 H) 7.71 (d, *J*=8.31 Hz, 2 H) 10.59 (s, 1 H)



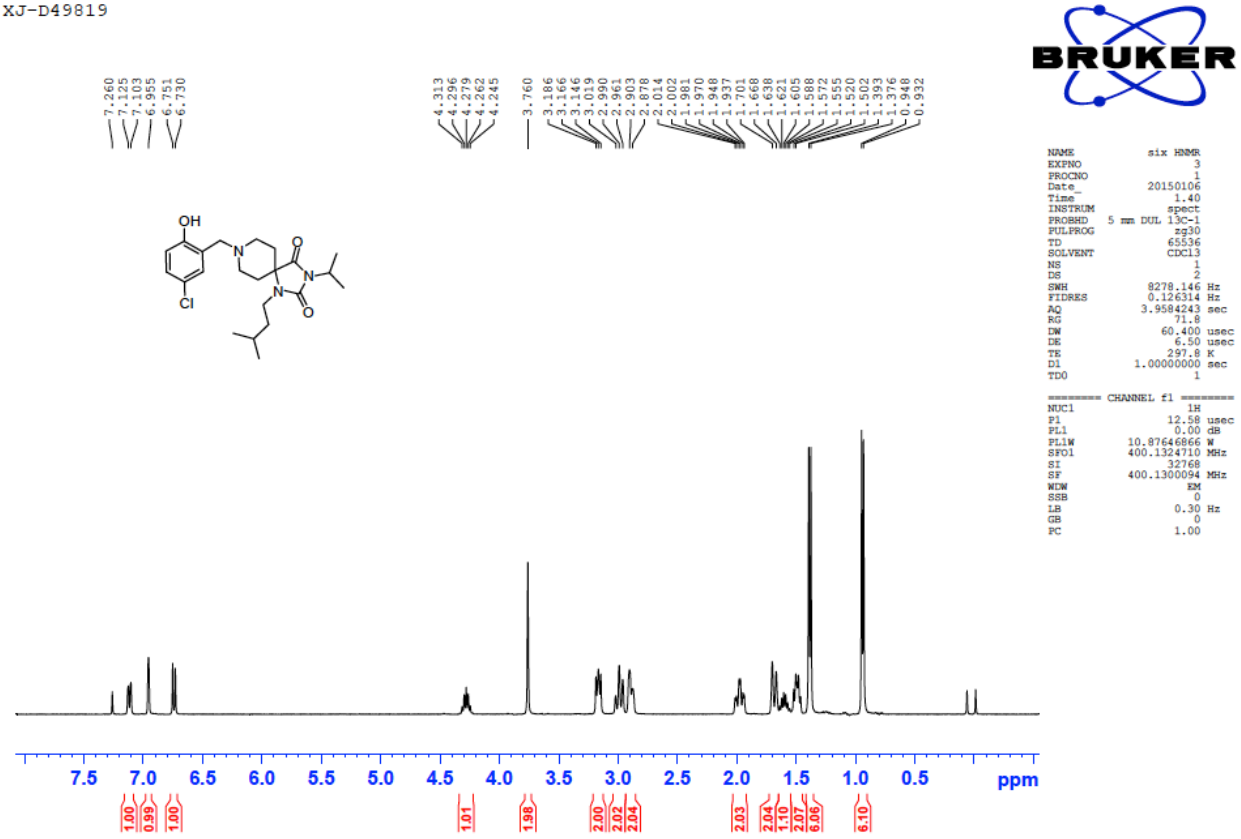
^{1}H NMR spectra for compound 11a

XJ-D49820



^{1}H NMR spectra for compound 11b

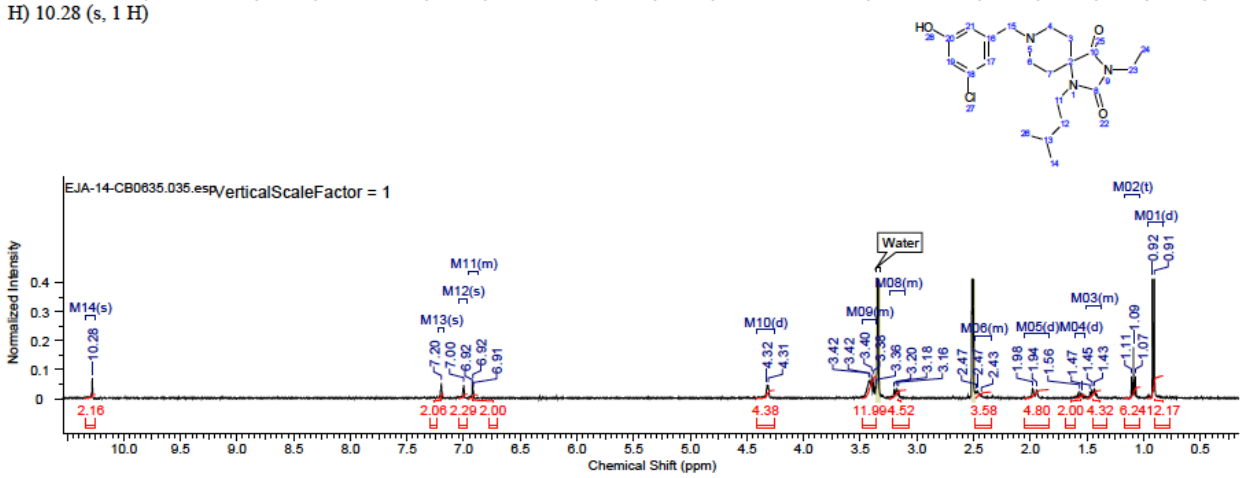
XJ-D49819



^{1}H NMR spectra for compound 12a

Formula $C_{21}H_{30}ClN_3O_3$		*FW* 407.9342	
Acquisition Time (sec) 4.0894	*Comment* 5 mm PABBO BB-1H/D Z-GRD Z863001/0012		*Date* 15 Aug 2014 14:50:24
Date Stamp 15 Aug 2014 14:50:24		*File Name* C:\Users\eander39\Desktop\EJA-14-CB0635\35\fid	
Frequency (MHz) 400.13	*Nucleus* 1H	*Number of Transients* 16	*Origin* spect
Original Points Count 32768	*Owner* nmr	*Points Count* 32768	*Pulse Sequence* zg30
Receiver Gain 812.00	*SW(cyclical) (Hz)* 8012.82	*Solvent* DMSO-d6	*Spectrum Offset (Hz)* 2470.9666
Spectrum Type STANDARD	*Sweep Width (Hz)* 8012.58	*Temperature (degree C)* 25.000	

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.91 (d, J=6.60 Hz, 6 H) 1.09 (t, J=7.21 Hz, 3 H) 1.38 - 1.50 (m, 2 H) 1.55 (d, J=6.85 Hz, 2 H) 1.96 (d, J=14.67 Hz, 2 H) 2.35 - 2.48 (m, 2 H) 3.10 - 3.24 (m, 2 H) 3.37 - 3.48 (m, 4 H) 4.32 (d, J=4.89 Hz, 2 H) 6.88 - 6.95 (m, 1 H) 7.00 (s, 1 H) 7.20 (s, 1 H) 10.28 (s, 1 H)

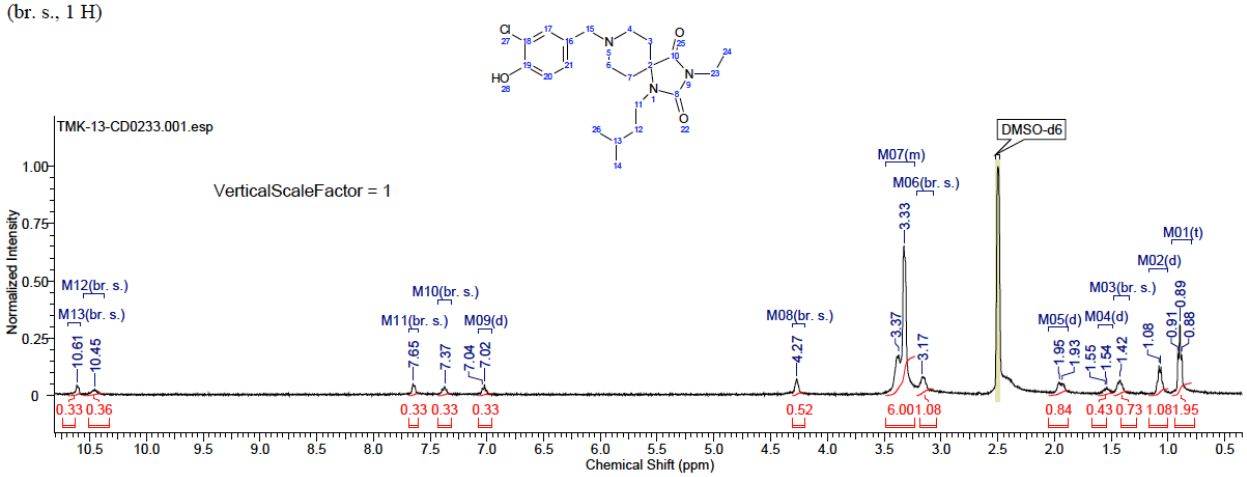


^{1}H NMR spectra for compound 12b

Formula $C_{21}H_{30}ClN_3O_3$	FW	407.9342

Acquisition Time (sec)	4.0894	Comment	5 mm PABBO BB-1H/D Z-GRD Z863001/0012			Date	01 Nov 2013 14:31:12
Date Stamp	01 Nov 2013 14:31:12			File Name	C:\Users\leander39\Desktop\TMK-13-CD0233\1\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	nmr	Points Count	32768	Pulse Sequence	zg30
Receiver Gain	812.00	SW(cyclical) (Hz)	8012.82	Solvent	DMSO-d6	Spectrum Offset (Hz)	2462.9924
Spectrum Type	STANDARD	Sweep Width (Hz)	8012.58	Temperature (degree C)	26.900		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.89 (t, J=7.09 Hz, 6 H) 1.07 (d, J=7.09 Hz, 3 H) 1.42 (br. s., 2 H) 1.55 (d, J=5.38 Hz, 1 H) 1.94 (d, J=9.05 Hz, 2 H) 3.17 (br. s., 3 H) 3.23 - 3.49 (m, 6 H) 4.27 (br. s., 2 H) 7.03 (d, J=7.34 Hz, 1 H) 7.37 (br. s., 1 H) 7.65 (br. s., 1 H) 10.45 (br. s., 1 H) 10.61 (br. s., 1 H)

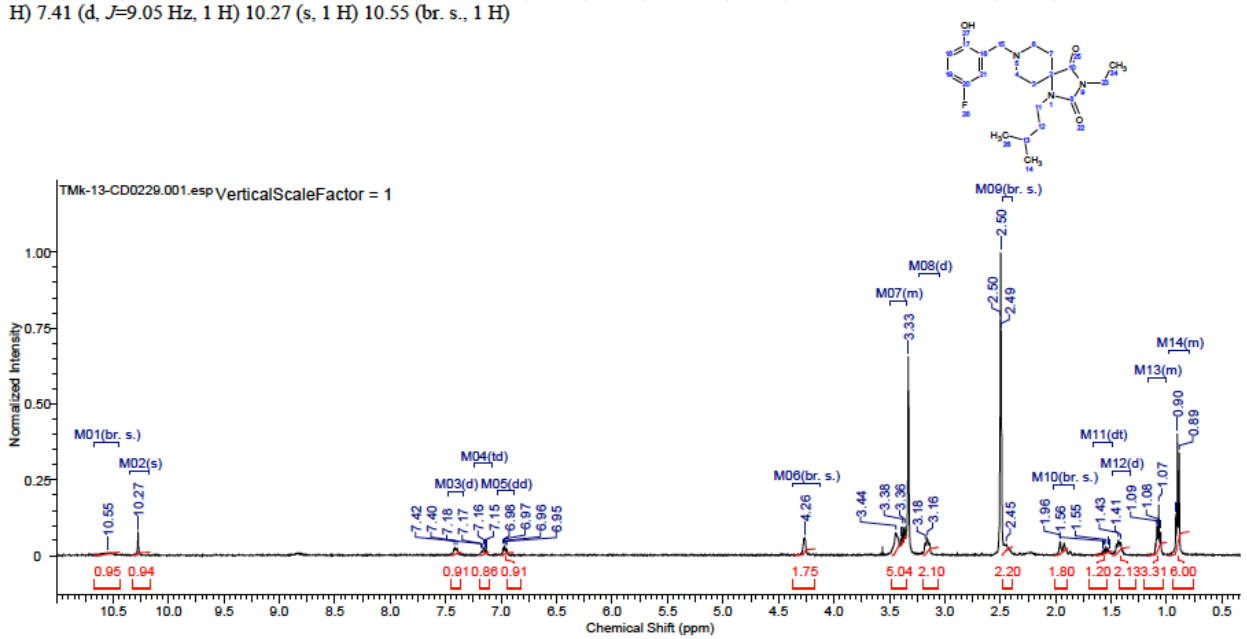


^{1}H NMR spectra for compound 12c

Formula $C_{21}H_{30}FN_3O_3$	FW	391.4796

Acquisition Time (sec)	4.0894	Comment	5 mm PABBO BB-1H/D Z-GRD Z863001/0012			Date	11 Sep 2013 15:03:12
Date Stamp	11 Sep 2013 15:03:12			File Name	C:\Users\tkrennin\Downloads\TMk-13-CD0229\TMk-13-CD0229\1\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	nmr	Points Count	32768	Pulse Sequence	zg30
Receiver Gain	812.00	SW(cyclical) (Hz)	8012.82	Solvent	DMSO-d6	Spectrum Offset (Hz)	2465.1931
Spectrum Type	STANDARD	Sweep Width (Hz)	8012.58	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.80 - 0.98 (m, 6 H) 1.00 - 1.17 (m, 3 H) 1.42 (d, J=8.07 Hz, 2 H) 1.55 (dt, J=13.14, 6.51 Hz, 1 H) 1.96 (br. s., 2 H) 2.45 (br. s., 2 H) 3.17 (d, J=7.58 Hz, 2 H) 3.34 - 3.50 (m, 5 H) 4.26 (br. s., 2 H) 6.97 (dd, J=8.93, 4.77 Hz, 1 H) 7.15 (td, J=8.50, 3.30 Hz, 1 H) 7.41 (d, J=9.05 Hz, 1 H) 10.27 (s, 1 H) 10.55 (br. s., 1 H)

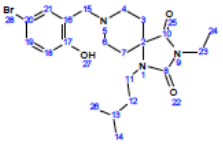


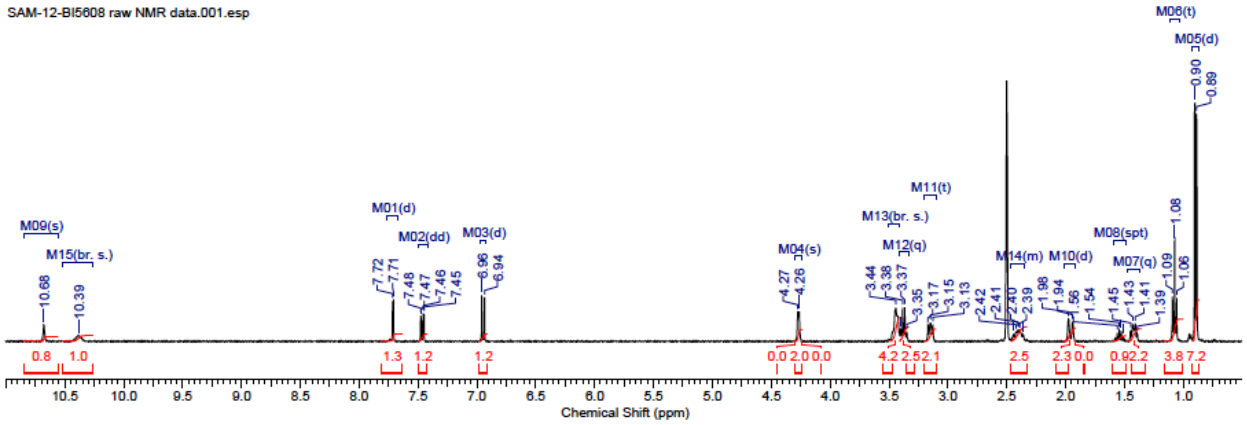
^{1}H NMR spectra for compound 12d

Formula $C_{21}H_{20}BrNO_3$	FW 452.3852		

Acquisition Time (sec)	3.9846	Comment	SAM-12-BI5608 HCl			Date	26 Oct 2012 14:14:08
Date Stamp	26 Oct 2012 14:14:08						
File Name	C:\Users\Sarah\Documents\Data\smcnitt\exp\SAM-12-BI5608\SAM-12-BI5608 raw NMR data\1\fid					Frequency (MHz)	400.13
Nucleus	1H	Number of Transients	16	Origin	spect	Original Points Count	32768
Owner	smcnitt	Points Count	131072	Pulse Sequence	zg30	Receiver Gain	512.00
SW(cyclical) (Hz)	8223.68	Solvent	DMSO-d6	Spectrum Offset (Hz)	2467.5718	Spectrum Type	STANDARD
Sweep Width (Hz)	8223.62	Temperature (degree C)	20.300				

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.68 (s, 1 H), 10.39 (br. s., 1 H), 7.71 (d, *J*=2.6 Hz, 1 H), 7.46 (dd, *J*=8.8, 2.6 Hz, 1 H), 6.95 (d, *J*=8.8 Hz, 1 H), 4.27 (s, 2 H), 3.44 (br. s., 4 H), 3.37 (q, *J*=7.2 Hz, 2 H), 3.15 (t, *J*=1.0 Hz, 2 H), 2.35 - 2.46 (m, 2 H), 1.96 (d, *J*=14.0 Hz, 2 H), 1.54 (spt, *J*=1.0 Hz, 1 H), 1.42 (q, *J*=1.0 Hz, 2 H), 1.08 (t, *J*=7.2 Hz, 3 H), 0.90 (d, *J*=6.6 Hz, 6 H)



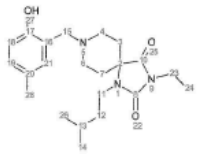


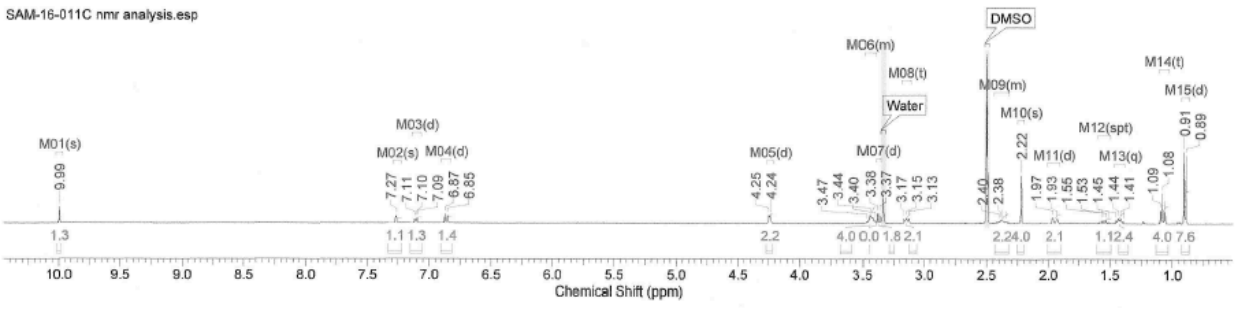
^{1}H NMR spectra for compound 12e

Formula $C_{22}H_{29}NO_3$	FW 387.5157		

Acquisition Time (sec)	3.9846	Comment	SAM-16-011C HCl salt			Date	24 May 2012 12:55:12
Date Stamp	24 May 2012 12:55:12			File Name	C:\Users\Sarah\Documents\Data\smcnitt\exp\SAM-16-011C\1\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	smcnitt	Points Count	131072	Pulse Sequence	zg30
Receiver Gain	645.00	SW(cyclical) (Hz)	8223.68	Solvent	DMSO-d6	Spectrum Offset (Hz)	2467.4465
Spectrum Type	STANDARD	Sweep Width (Hz)	8223.62	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.99 (s, 1 H), 7.27 (s, 1 H), 7.10 (d, *J*=1.0 Hz, 1 H), 6.86 (d, *J*=8.2 Hz, 1 H), 4.24 (d, *J*=4.6 Hz, 2 H), 3.40 - 3.49 (m, 4 H), 3.38 (d, *J*=7.2 Hz, 2 H), 3.15 (t, *J*=1.0 Hz, 2 H), 2.32 - 2.44 (m, 2 H), 2.22 (s, 3 H), 1.95 (d, *J*=13.9 Hz, 2 H), 1.54 (spt, *J*=1.0 Hz, 1 H), 1.42 (q, *J*=1.0 Hz, 2 H), 1.08 (t, *J*=7.2 Hz, 3 H), 0.90 (d, *J*=6.6 Hz, 6 H)



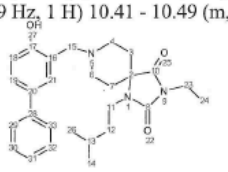


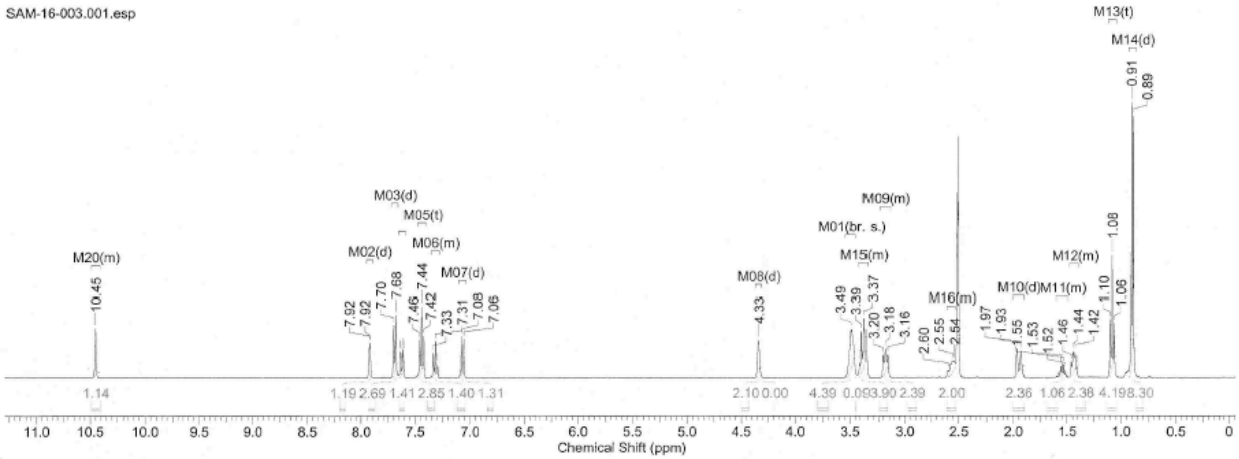
^{1}H NMR spectra for compound 12f

Formula $C_{27}H_{35}N_3O_3$	FW	449.5851

Acquisition Time (sec)	3.9846	Comment	SAM-16-003 - final pdt HCl salt			Date	07 Feb 2012 11:25:20
Date Stamp	07 Feb 2012 11:25:20			File Name	C:\Users\Sarah\Documents\Data\smcnitt\nmr\SAM-16-003\1\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	smcnitt	Points Count	131072	Pulse Sequence	zg30
Receiver Gain	406.00	SW(cyclical) (Hz)	8223.68	Solvent	DMSO-d6	Spectrum Offset (Hz)	2470.9683
Spectrum Type	STANDARD	Sweep Width (Hz)	8223.62	Temperature (degree C)	19.400		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.90 (d, J=6.53 Hz, 6 H) 1.09 (t, J=1.00 Hz, 3 H) 1.39 - 1.48 (m, 2 H) 1.49 - 1.61 (m, 1 H) 1.95 (d, J=13.61 Hz, 2 H) 2.53 - 2.61 (m, 2 H) 3.14 - 3.23 (m, 2 H) 3.35 - 3.42 (m, 4 H) 3.49 (br. s., 2 H) 4.33 (d, J=1.00 Hz, 2 H) 7.07 (d, J=8.47 Hz, 1 H) 7.28 - 7.35 (m, 1 H) 7.44 (t, J=7.69 Hz, 2 H) 7.62 (dd, J=8.47, 2.26 Hz, 1 H) 7.69 (d, J=7.34 Hz, 2 H) 7.92 (d, J=1.69 Hz, 1 H) 10.41 - 10.49 (m, 1 H)



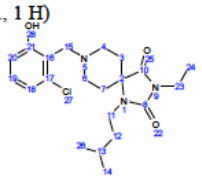


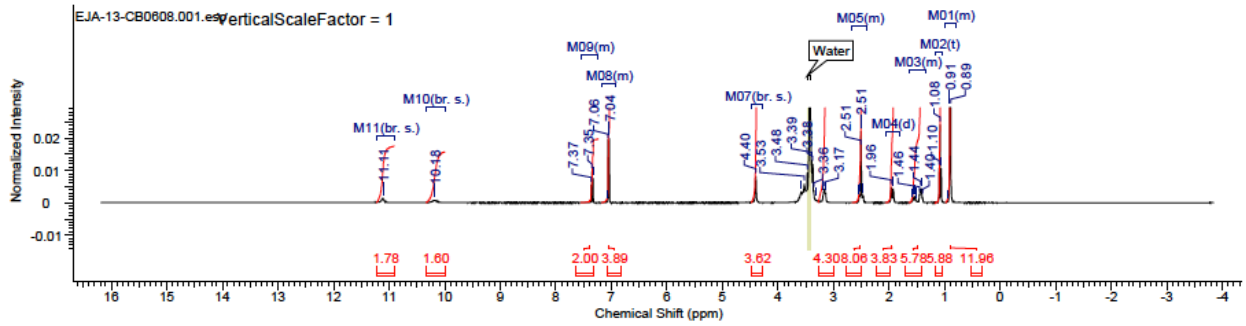
^{1}H NMR spectra for compound 12g

Formula $C_{21}H_{36}ClN_3O_3$	FW	407.9342

Acquisition Time (sec)	4.0894	Comment	5 mm PABBO BB-1H/D Z-GRD Z863001/0012			Date	01 Nov 2013 14:56:48
Date Stamp	01 Nov 2013 14:56:48			File Name	C:\Users\leander39\Desktop\NMR (11.1.13)\EJA-13-CB0608\1\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	32	Origin	spect
Original Points Count	32768	Owner	nmr	Points Count	32768	Pulse Sequence	zg30
Receiver Gain	203.00	SW(cyclical) (Hz)	8012.82	Solvent	DMSO-d6	Spectrum Offset (Hz)	2470.9666
Spectrum Type	STANDARD	Sweep Width (Hz)	8012.58	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.81 - 0.98 (m, 6 H) 1.08 (t, J=7.21 Hz, 3 H) 1.35 - 1.65 (m, 3 H) 1.95 (d, J=13.69 Hz, 2 H) 2.40 - 2.67 (m, 2 H) 3.00 - 3.28 (m, 2 H) 4.40 (br. s., 4 H) 6.92 - 7.18 (m, 4 H) 7.23 - 7.55 (m, 2 H) 10.18 (br. s., 1 H) 11.11 (br. s., 1 H)



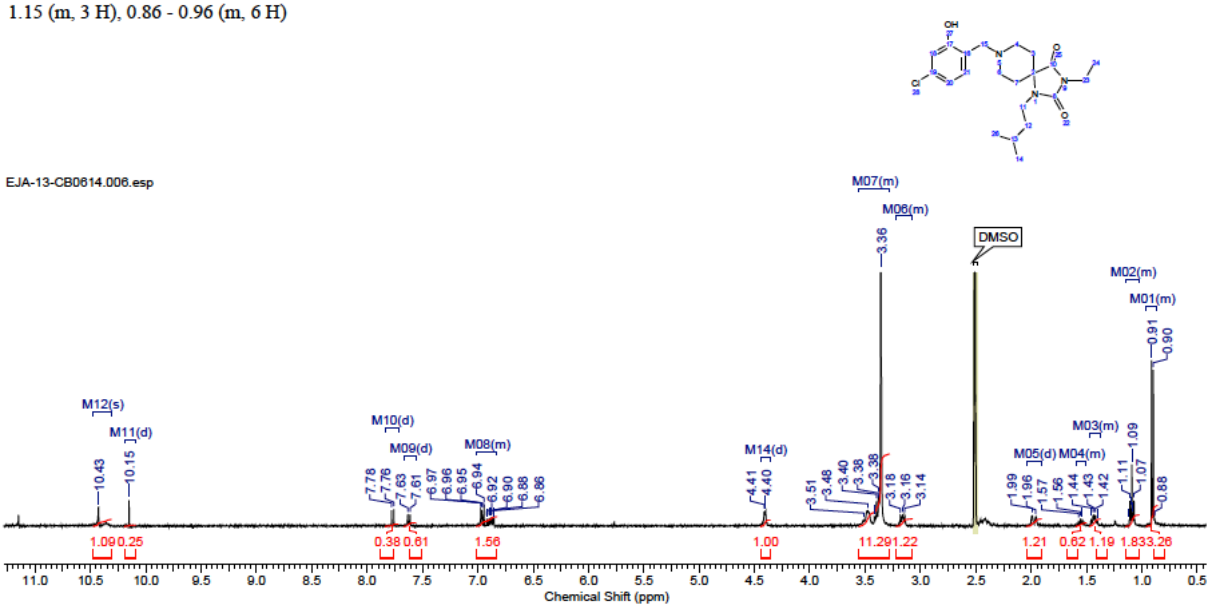


^{1}H NMR spectra for compound 12h

Formula $C_{21}H_{20}ClN_3O_3$	FW 407.9342

Acquisition Time (sec)	4.0894	Comment	5 mm PABBO BB-1H/D Z-GRD Z863001/0012			Date	02 Aug 2013 15:05:20
Date Stamp	02 Aug 2013 15:05:20			File Name	C:\Documents and Settings\tkrennin\Desktop\EJA-13-CB0614\6\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	nmr	Points Count	32768	Pulse Sequence	zg30
Receiver Gain	724.00	SW(cyclical) (Hz)	8012.82	Solvent	DMSO-d6	Spectrum Offset (Hz)	2470.9666
Spectrum Type	STANDARD	Sweep Width (Hz)	8012.58	Temperature (degree C)	22.900		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.43 (s, 1 H), 10.15 (d, J=0.7 Hz, 1 H), 7.77 (d, J=8.8 Hz, 1 H), 7.62 (d, J=8.6 Hz, 2 H), 6.83 - 7.01 (m, 4 H), 4.40 (d, J=4.2 Hz, 1 H), 3.28 - 3.55 (m, 4 H), 3.07 - 3.22 (m, 2 H), 1.98 (d, J=13.4 Hz, 2 H), 1.50 - 1.60 (m, 1 H), 1.38 - 1.48 (m, 2 H), 1.03 - 1.15 (m, 3 H), 0.86 - 0.96 (m, 6 H)

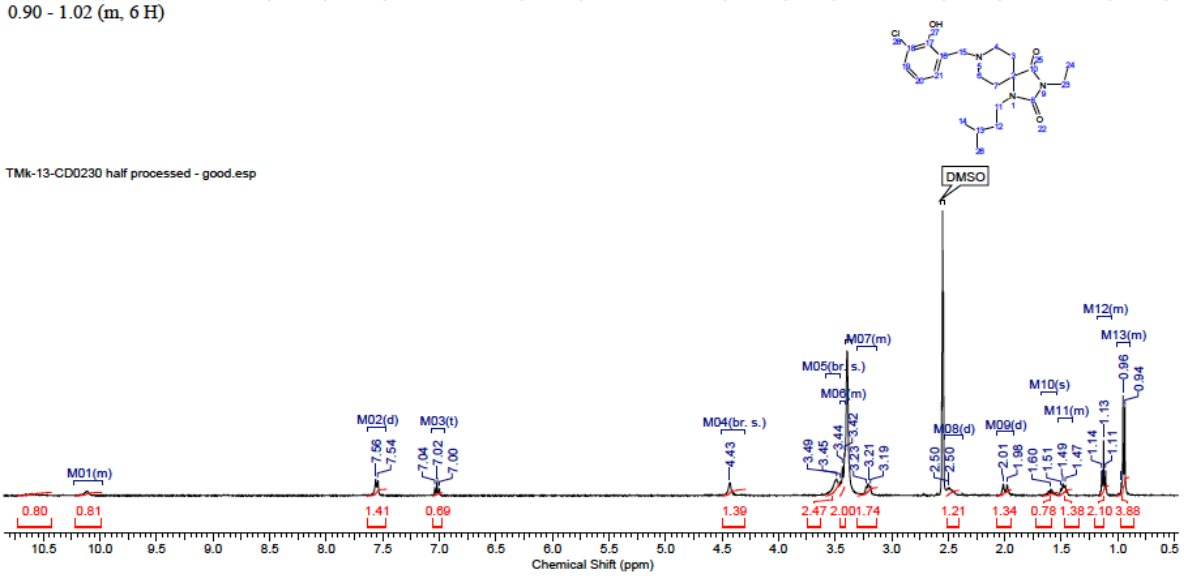


^{1}H NMR spectra for compound 12i

Formula $C_{21}H_{20}ClN_3O_3$	FW 407.9342

Acquisition Time (sec)	4.0894	Comment	5 mm PABBO BB-1H/D Z-GRD Z863001/0012			Date	11 Sep 2013 15:20:16
Date Stamp	11 Sep 2013 15:20:16						
File Name	C:\Documents and Settings\tkrennin\My Documents\Dropbox\Research Folders\raw NMR data - 9.10.13\TMk-13-CD0230\1\fid						
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	nmr	Points Count	32768	Pulse Sequence	zg30
Receiver Gain	812.00	SW(cyclical) (Hz)	8012.82	Solvent	DMSO-d6	Spectrum Offset (Hz)	2486.7119
Spectrum Type	STANDARD	Sweep Width (Hz)	8012.58	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.98 - 10.23 (m, 1 H), 7.55 (d, J=7.8 Hz, 2 H), 7.02 (t, J=7.8 Hz, 1 H), 4.43 (br. s., 2 H), 3.49 (br. s., 4 H), 3.42 - 3.46 (m, 2 H), 3.13 - 3.30 (m, 2 H), 2.50 (d, J=1.7 Hz, 2 H), 2.00 (d, J=14.2 Hz, 2 H), 1.60 (s, 1 H), 1.40 - 1.53 (m, 2 H), 1.05 - 1.18 (m, 3 H), 0.90 - 1.02 (m, 6 H)

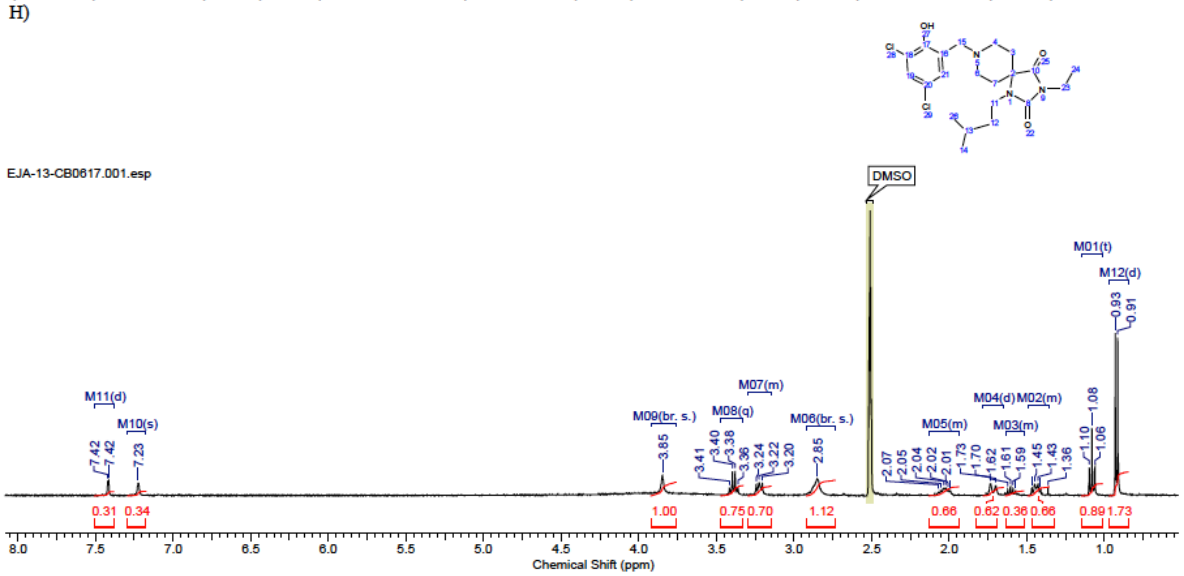


^{1}H NMR spectra for compound 12j

Formula $C_{21}H_{28}ClNO_5$	FW 442.3793

Acquisition Time (sec)	4.0894	Comment	5 mm PABBO BB-1H/D Z-GRD Z863001/0012			Date	11 Sep 2013 16:02:56
Date Stamp	11 Sep 2013 16:02:56						
File Name	C:\DOCUMENTS AND SETTINGS\TKRENNIN\MY DOCUMENTS\DROPBOX\RESEARCH FOLDERS\RAW NMR DATA - 9.10.13\EJA-13-CB0617\1\FID						
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	nmr	Points Count	32768	Pulse Sequence	zg30
Receiver Gain	812.00	SW(cyclical) (Hz)	8012.82	Solvent	DMSO-d6	Spectrum Offset (Hz)	2470.9668
Spectrum Type	STANDARD	Sweep Width (Hz)	8012.58	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.42 (d, J=2.4 Hz, 1 H), 7.23 (s, 1 H), 3.85 (br. s., 1 H), 3.39 (q, J=7.1 Hz, 4 H), 3.15 - 3.30 (m, 2 H), 2.85 (br. s., 2 H), 1.94 - 2.13 (m, 4 H), 1.72 (d, J=13.2 Hz, 2 H), 1.52 - 1.64 (m, 1 H), 1.36 - 1.49 (m, 2 H), 1.08 (t, J=7.2 Hz, 3 H), 0.92 (d, J=6.6 Hz, 6 H)

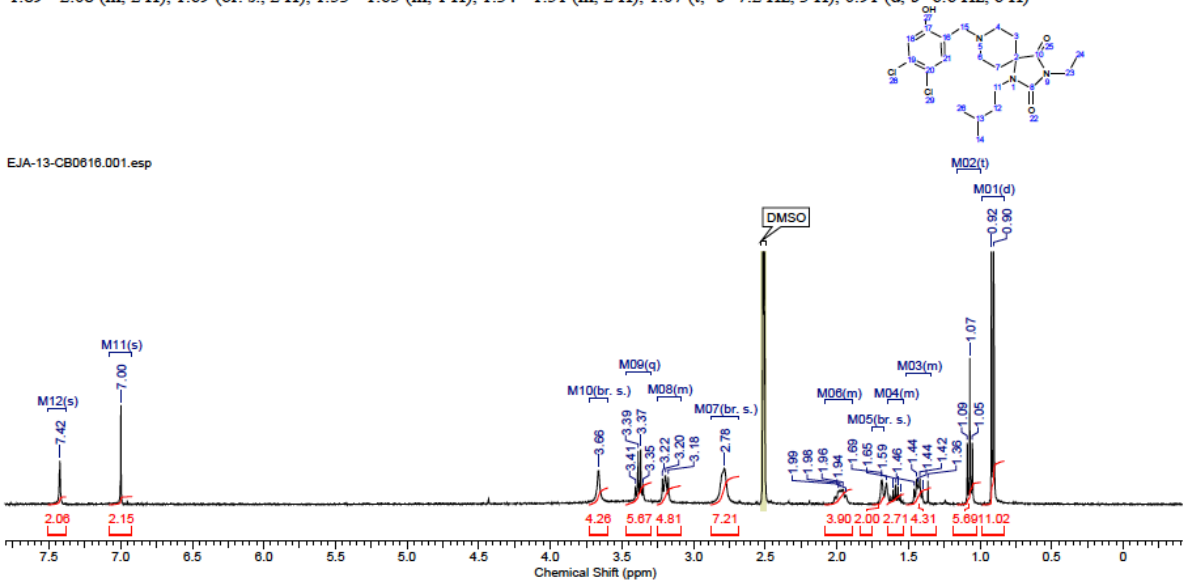


^{1}H NMR spectra for compound 12k

Formula $C_{21}H_{28}ClNO_5$	FW 442.3793

Acquisition Time (sec)	4.0894	Comment	5 mm PABBO BB-1H/D Z-GRD Z863001/0012			Date	11 Sep 2013 15:58:40
Date Stamp	11 Sep 2013 15:58:40						
File Name	C:\DOCUMENTS AND SETTINGS\TKRENNIN\MY DOCUMENTS\DROPBOX\RESEARCH FOLDERS\RAW NMR DATA - 9.10.13\EJA-13-CB0616\1\FID						
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	nmr	Points Count	32768	Pulse Sequence	zg30
Receiver Gain	724.00	SW(cyclical) (Hz)	8012.82	Solvent	DMSO-d6	Spectrum Offset (Hz)	2470.9668
Spectrum Type	STANDARD	Sweep Width (Hz)	8012.58	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.42 (s, 1 H), 7.00 (s, 1 H), 3.66 (br. s., 1 H), 3.38 (q, J=7.2 Hz, 4 H), 3.09 - 3.25 (m, 2 H), 2.78 (br. s., 4 H), 1.89 - 2.08 (m, 2 H), 1.69 (br. s., 2 H), 1.53 - 1.65 (m, 1 H), 1.34 - 1.51 (m, 2 H), 1.07 (t, J=7.2 Hz, 3 H), 0.91 (d, J=6.6 Hz, 6 H)

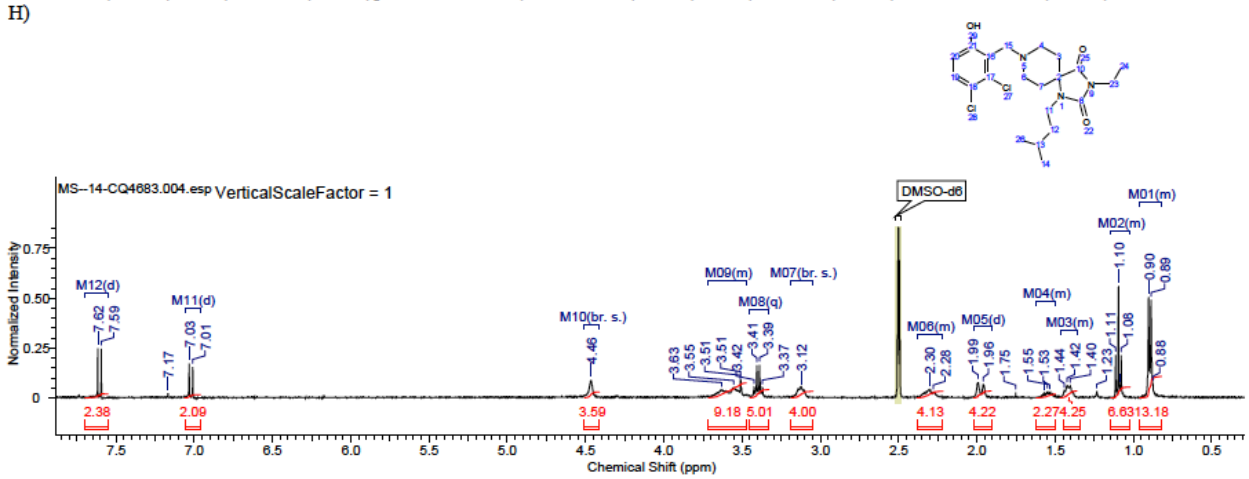


^{1}H NMR spectra for compound 12l

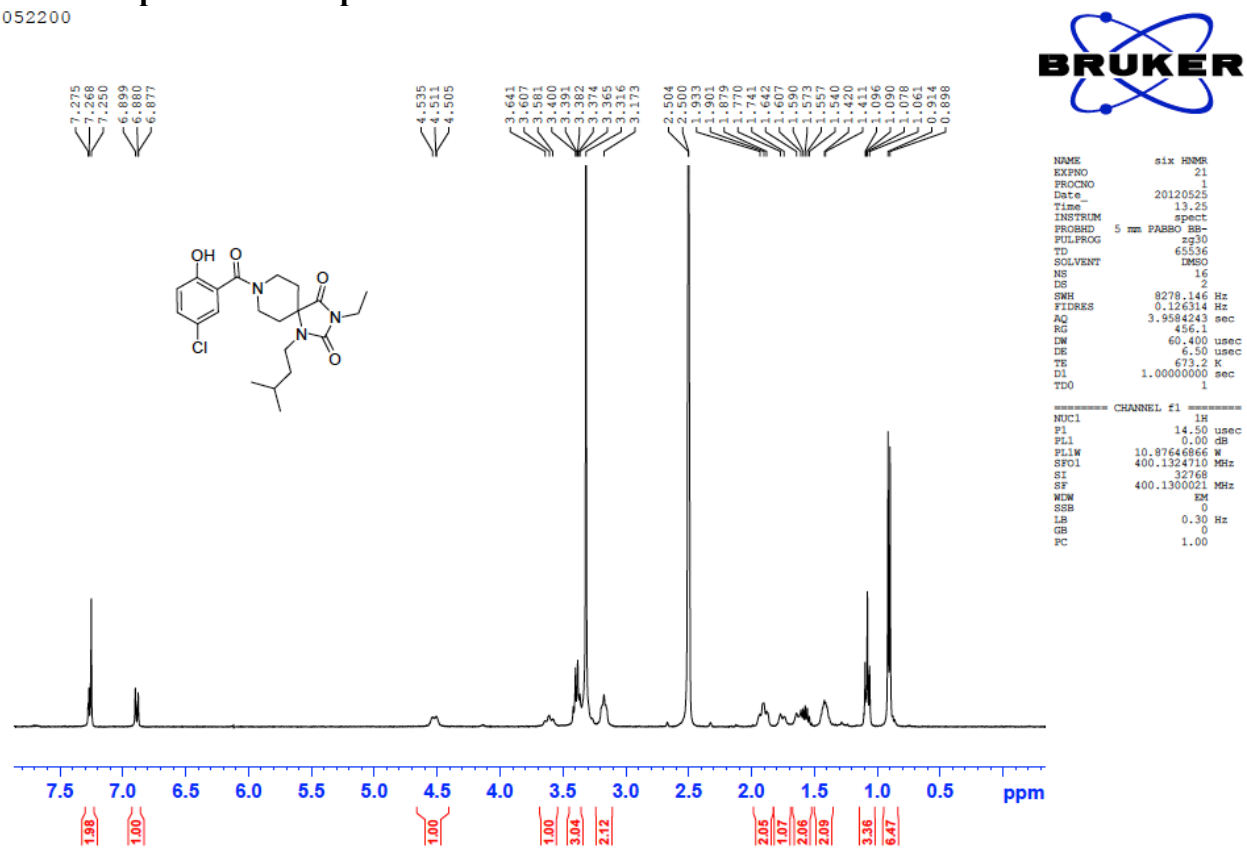
Formula	$C_{21}H_{29}ClN_2O_3$	FW	442.3793

Acquisition Time (sec)	4.0894	Comment	5 mm PABBO BB-1H/D Z-GRD Z863001/0012			Date	22 Dec 2014 13:48:16
Date Stamp	22 Dec 2014 13:48:16			File Name	C:\Users\eander39\Desktop\MS--14-CQ4683\4\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	nmr	Points Count	32768	Pulse Sequence	zg30
Receiver Gain	724.00	SW(cyclical) (Hz)	8012.82	Solvent	DMSO-d6	Spectrum Offset (Hz)	2467.6448
Spectrum Type	STANDARD	Sweep Width (Hz)	8012.58	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.82 - 0.96 (m, 6 H) 1.02 - 1.14 (m, 3 H) 1.36 - 1.47 (m, 2 H) 1.50 - 1.62 (m, 1 H) 1.98 (d, *J*=14.43 Hz, 2 H) 2.22 - 2.38 (m, 2 H) 3.12 (br. s., 4 H) 3.40 (q, *J*=7.09 Hz, 2 H) 3.48 - 3.71 (m, 4 H) 4.46 (br. s., 1 H) 7.02 (d, *J*=9.05 Hz, 1 H) 7.61 (d, *J*=9.05 Hz, 1 H)



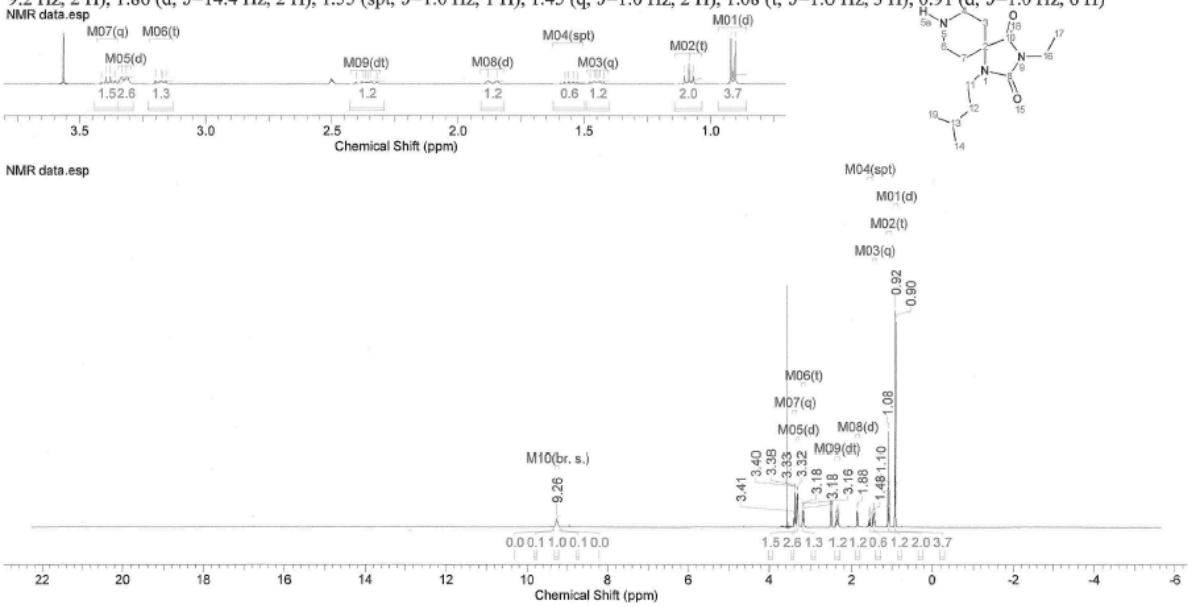
^{1}H NMR spectra for compound 12m



^{1}H NMR spectra for compound 12n

Formula	$C_{14}H_{25}N_3O_2$	FW	267.3672				
Acquisition Time (sec)	1.4680	Comment	SAM-16-000B/DMSO-d6/03-30-12			Date	29 Mar 2012 15:35:12
Date Stamp	29 Mar 2012 15:35:12			File Name	C:\Users\Sarah\Documents\Data\smcnitt\exp\SAM-16-009\NMR data\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	32	Origin	spect
Original Points Count	16384	Owner	nmr	Points Count	131072	Pulse Sequence	zg30
Receiver Gain	322.00	SW(cyclical) (Hz)	11160.71	Solvent	DMSO-d6	Spectrum Offset (Hz)	3332.2715
Spectrum Type	STANDARD	Sweep Width (Hz)	11160.63	Temperature (degree C)	25.000		

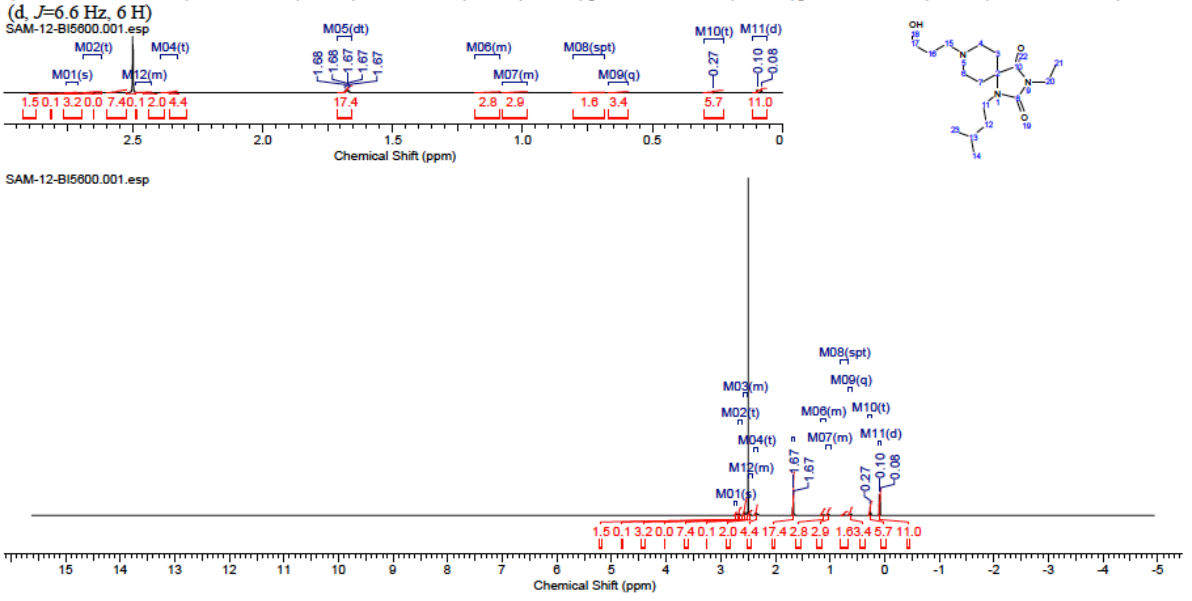
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.26 (br. s., 1 H), 3.39 (q, *J*=7.2 Hz, 2 H), 3.32 (d, *J*=6.9 Hz, 4 H), 3.18 (t, *J*=1.0 Hz, 2 H), 2.36 (dt, *J*=14.1, 9.2 Hz, 2 H), 1.86 (d, *J*=14.4 Hz, 2 H), 1.55 (spt, *J*=1.0 Hz, 1 H), 1.45 (q, *J*=1.0 Hz, 2 H), 1.08 (t, *J*=1.0 Hz, 3 H), 0.91 (d, *J*=1.0 Hz, 6 H)



^{1}H NMR spectra for compound 12o

Formula	$C_{17}H_{31}N_3O_3$	FW	325.4463				
Acquisition Time (sec)	3.9846	Comment	SAM-12-BI5600 HCl salt			Date	24 May 2012 13:03:44
Date Stamp	24 May 2012 13:03:44			File Name	C:\Users\Sarah\Documents\Data\smcnitt\exp\SAM-12-BI5600\1\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	smcnitt	Points Count	131072	Pulse Sequence	zg30
Receiver Gain	512.00	SW(cyclical) (Hz)	8223.68	Solvent	DMSO-d6	Spectrum Offset (Hz)	2137.2366
Spectrum Type	STANDARD	Sweep Width (Hz)	8223.62	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.74 (s, 1 H), 2.65 (t, *J*=1.0 Hz, 2 H), 2.52 - 2.60 (m, 4 H), 2.43 - 2.49 (m, 2 H), 2.36 (t, *J*=1.0 Hz, 2 H), 1.67 (dt, *J*=3.7, 1.8 Hz, 4 H), 1.09 - 1.18 (m, 2 H), 0.98 - 1.08 (m, 2 H), 0.74 (spt, *J*=1.0 Hz, 1 H), 0.63 (q, *J*=1.0 Hz, 2 H), 0.27 (t, *J*=1.0 Hz, 3 H), 0.09 (d, *J*=6.6 Hz, 6 H)

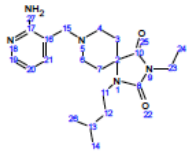


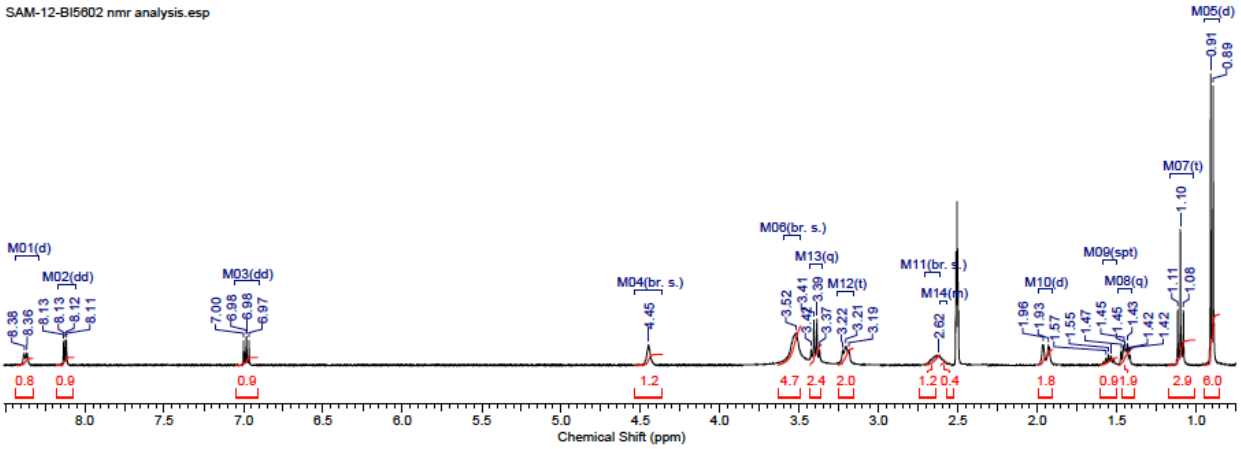
^{1}H NMR spectra for compound 12p

Formula $C_{20}H_{21}N_5O_2$	FW	373.4924

Acquisition Time (sec)	3.9846	Comment	SAM-12-BI5602 HCl salt			Date	24 May 2012 13:20:48
Date Stamp	24 May 2012 13:20:48			File Name	C:\Users\Sarah\Documents\Data\smcnitt\exp\SAM-12-BI5602\1\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	smcnitt	Points Count	131072	Pulse Sequence	zg30
Receiver Gain	512.00	SW(cyclical) (Hz)	8223.68	Solvent	DMSO-d6	Spectrum Offset (Hz)	2469.4541
Spectrum Type	STANDARD	Sweep Width (Hz)	8223.62	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.37 (d, *J*=7.1 Hz, 1 H), 8.12 (dd, *J*=6.2, 1.6 Hz, 1 H), 6.98 (dd, *J*=7.4, 6.2 Hz, 1 H), 4.45 (br. s., 2 H), 3.52 (br. s., 4 H), 3.40 (q, *J*=7.2 Hz, 2 H), 3.21 (t, *J*=1.0 Hz, 2 H), 2.62 (br. s., 2 H), 2.57 - 2.61 (m, 2 H), 1.95 (d, *J*=13.7 Hz, 2 H), 1.55 (spt, *J*=1.0 Hz, 1 H), 1.44 (q, *J*=1.0 Hz, 2 H), 1.10 (t, *J*=7.2 Hz, 3 H), 0.90 (d, *J*=6.6 Hz, 6 H)



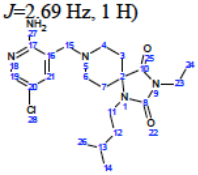


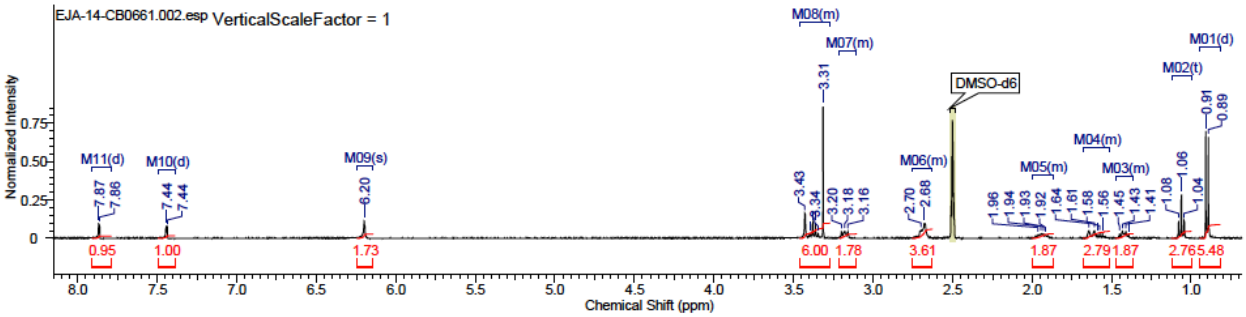
^{1}H NMR spectra for compound 12q

Formula $C_{28}H_{38}ClN_5O_2$	FW	407.9375

Acquisition Time (sec)	4.0894	Comment	5 mm PABBO BB-1H/D Z-GRD Z863001/0012			Date	12 Dec 2014 10:32:00
Date Stamp	12 Dec 2014 10:32:00			File Name	C:\Users\eander39\Desktop\EJA-14-CB0661\2\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	nmr	Points Count	32768	Pulse Sequence	zg30
Receiver Gain	812.00	SW(cyclical) (Hz)	8012.82	Solvent	DMSO-d6	Spectrum Offset (Hz)	2467.6448
Spectrum Type	STANDARD	Sweep Width (Hz)	8012.58	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.90 (d, *J*=6.60 Hz, 6 H) 1.06 (t, *J*=7.09 Hz, 3 H) 1.37 - 1.48 (m, 1 H) 1.51 - 1.68 (m, 2 H) 1.87 - 2.00 (m, 2 H) 2.63 - 2.75 (m, 4 H) 3.10 - 3.21 (m, 2 H) 3.27 - 3.46 (m, 6 H) 6.20 (s, 2 H) 7.44 (d, *J*=2.69 Hz, 1 H) 7.87 (d, *J*=2.69 Hz, 1 H)



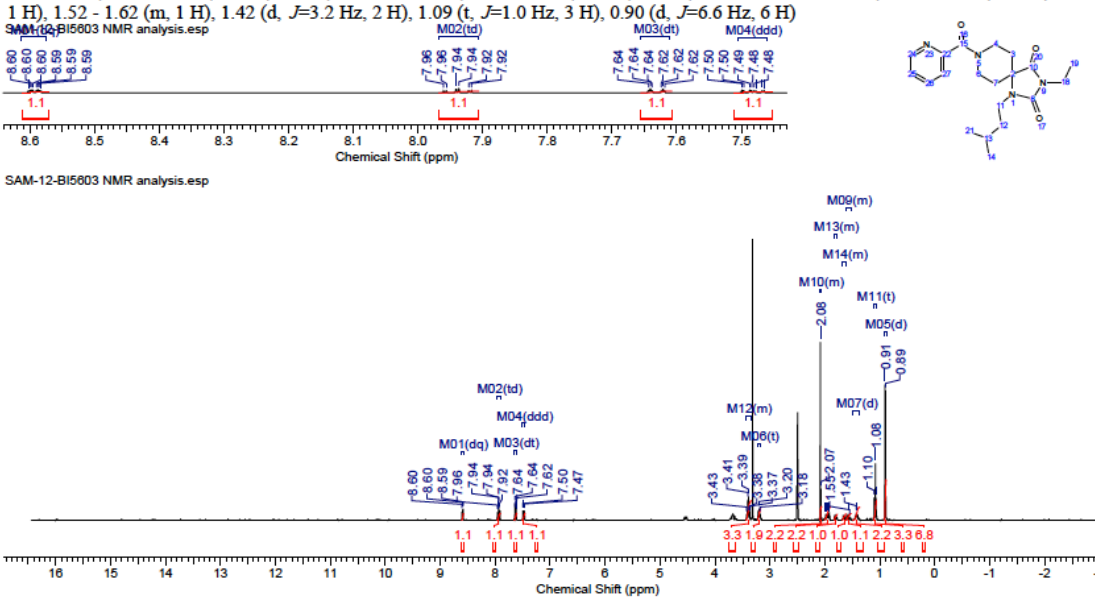


^{1}H NMR spectra for compound 12r

Formula $C_{20}H_{20}N_4O_2$	FW	372.4613

Acquisition Time (sec)	3.9846	Comment	SAM-12-BI5603 final product			Date	22 Jun 2012 11:23:28
Date Stamp	22 Jun 2012 11:23:28			File Name	C:\Users\Sarah\Documents\Data\smcnitt\exp\SAM-12-BI5603\1\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	smcnitt	Points Count	131072	Pulse Sequence	zg30
Receiver Gain	575.00	SW(cyclical) (Hz)	8223.68	Solvent	DMSO-d6	Spectrum Offset (Hz)	2467.5093
Spectrum Type	STANDARD	Sweep Width (Hz)	8223.62	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.59 (dq, J=1.0 Hz, 1 H), 7.94 (td, J=7.7, 1.8 Hz, 1 H), 7.63 (dt, J=7.8, 1.1 Hz, 1 H), 7.48 (ddd, J=7.6, 4.9, 1.2 Hz, 1 H), 3.34 - 3.44 (m, 4 H), 3.19 (t, J=1.0 Hz, 2 H), 2.06 - 2.10 (m, 2 H), 1.96 (dt, J=12.5, 6.6 Hz, 2 H), 1.77 - 1.84 (m, 1 H), 1.62 - 1.68 (m, 1 H), 1.52 - 1.62 (m, 1 H), 1.42 (d, J=3.2 Hz, 2 H), 1.09 (t, J=1.0 Hz, 3 H), 0.90 (d, J=6.6 Hz, 6 H)

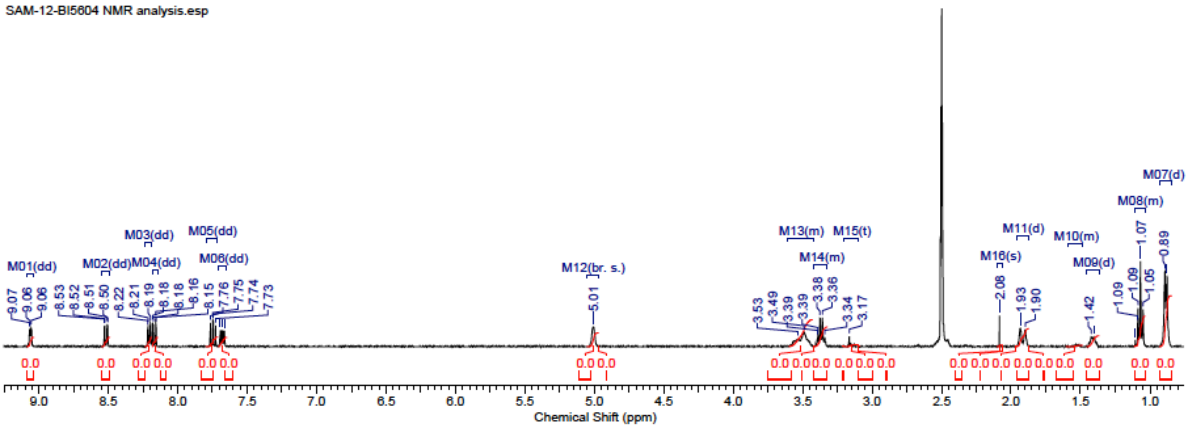


^{1}H NMR spectra for compound 12s

Formula $C_{24}H_{28}N_4O_2$	FW	408.5365

Acquisition Time (sec)	3.9846	Comment	SAM-12-BI5604 HCl salt			Date	22 Jun 2012 11:36:16
Date Stamp	22 Jun 2012 11:36:16			File Name	C:\Users\Sarah\Documents\Data\smcnitt\exp\SAM-12-BI5604\1\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	smcnitt	Points Count	131072	Pulse Sequence	zg30
Receiver Gain	575.00	SW(cyclical) (Hz)	8223.68	Solvent	DMSO-d6	Spectrum Offset (Hz)	2467.4465
Spectrum Type	STANDARD	Sweep Width (Hz)	8223.62	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.06 (dd, J=4.1, 1.6 Hz, 1 H), 8.52 (dd, J=8.3, 1.7 Hz, 1 H), 8.21 (dd, J=7.1, 1.3 Hz, 1 H), 8.17 (dd, J=8.3, 1.3 Hz, 1 H), 7.75 (dd, J=8.2, 7.2 Hz, 1 H), 7.68 (dd, J=8.3, 4.2 Hz, 1 H), 5.01 (br. s., 2 H), 3.43 - 3.61 (m, 4 H), 3.32 - 3.42 (m, 2 H), 3.17 (t, J=1.0 Hz, 2 H), 2.08 (s, 2 H), 1.92 (d, J=14.7 Hz, 2 H), 1.48 - 1.59 (m, 1 H), 1.41 (d, J=7.9 Hz, 2 H), 1.04 - 1.11 (m, 3 H), 0.88 (d, J=6.4 Hz, 6 H)

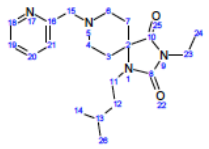


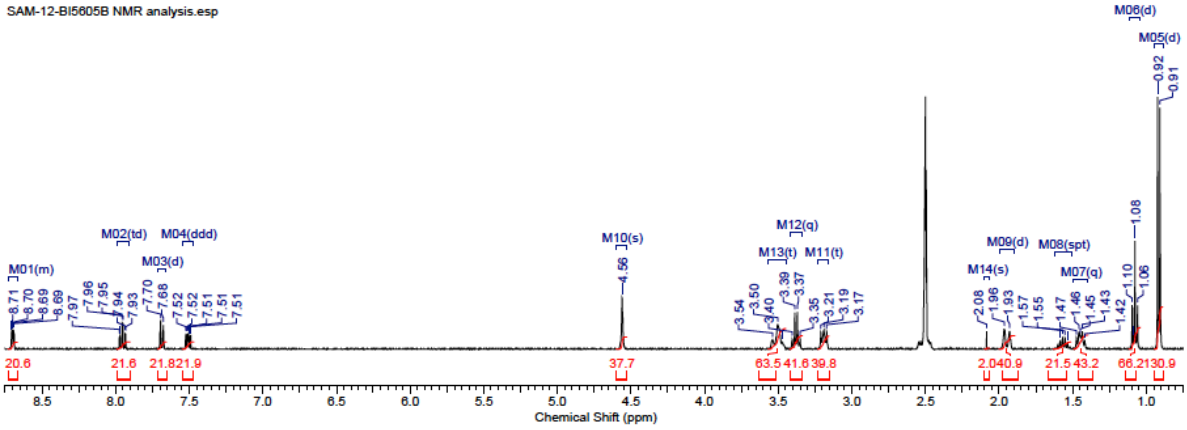
^{1}H NMR spectra for compound 12t

Formula $C_{20}H_{30}N_4O_2$	FW	358.4778

Acquisition Time (sec)	3.9846	Comment	SAM-12-BI5605B HCl salt			Date	22 Jun 2012 11:51:12
Date Stamp	22 Jun 2012 11:51:12			File Name	C:\Users\Sarah\Documents\Data\smcnitt\exp\SAM-12-BI5605B\1\fid		
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	16	Origin	spect
Original Points Count	32768	Owner	smcnitt	Points Count	131072	Pulse Sequence	zg30
Receiver Gain	575.00	SW(cyclical) (Hz)	8223.68	Solvent	DMSO-d6	Spectrum Offset (Hz)	2467.5090
Spectrum Type	STANDARD	Sweep Width (Hz)	8223.62	Temperature (degree C)	25.000		

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.67 - 8.73 (m, 1 H), 7.95 (td, J=7.7, 1.8 Hz, 1 H), 7.69 (d, J=7.8 Hz, 1 H), 7.51 (ddd, J=7.7, 4.9, 1.1 Hz, 1 H), 4.56 (s, 2 H), 3.51 (t, J=1.0 Hz, 4 H), 3.38 (q, J=7.1 Hz, 2 H), 3.19 (t, J=1.0 Hz, 2 H), 2.08 (s, 2 H), 1.95 (d, J=14.1 Hz, 2 H), 1.56 (spt, J=1.0 Hz, 1 H), 1.45 (q, J=1.0 Hz, 2 H), 1.08 (d, J=1.0 Hz, 3 H), 0.91 (d, J=6.6 Hz, 6 H)





^{1}H NMR spectra for compound 12u

Formula $C_{28}H_{40}N_4O_2$	FW	396.5258

Acquisition Time (sec)	3.9846	Comment	SAM-12-BI5606	Date	24 Oct 2012 13:57:04		
Date Stamp	24 Oct 2012 13:57:04						
File Name	C:\Users\Sarah\Documents\Data\smcnitt\exp\SAM-12-BI5606\SAM-12-BI5606 raw NMR data\1\fid					Frequency (MHz)	400.13
Nucleus	1H	Number of Transients	16	Origin	spect	Original Points Count	32768
Owner	smcnitt	Points Count	131072	Pulse Sequence	zg30	Receiver Gain	512.00
SW(cyclical) (Hz)	8223.68	Solvent	DMSO-d6	Spectrum Offset (Hz)	2467.4465	Spectrum Type	STANDARD
Sweep Width (Hz)	8223.62	Temperature (degree C)	25.000				

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.66 (br. s., 1 H), 7.68 (d, J=1.0 Hz, 1 H), 7.48 (br. t, J=1.0, 1.0 Hz, 1 H), 7.38 (d, J=1.0 Hz, 1 H), 7.09 (t, J=1.0 Hz, 1 H), 6.53 (br. q, J=1.0, 1.0, 1.0 Hz, 1 H), 4.68 (br. d, J=1.0 Hz, 2 H), 3.52 (br. t, J=1.0, 1.0 Hz, 4 H), 3.37 (q, J=1.0 Hz, 4 H), 3.16 (br. t, J=1.0, 1.0 Hz, 2 H), 1.95 (br. d, J=1.0 Hz, 2 H), 1.54 (spt, J=1.0 Hz, 1 H), 1.41 (br. q, J=1.0, 1.0, 1.0 Hz, 2 H), 1.07 (t, J=1.0 Hz, 3 H), 0.90 (d, J=1.0 Hz, 6 H)