Supporting Information for

Discovery of a highly selective and potent TRPC3 inhibitor with high metabolic stability and low toxicity

Sicheng Zhang[‡,1], Luis O. Romero[‡,2, 3], Shanshan Deng[1], Jiaxing Wang,[1] Yong Li,[4] Lei Yang[4],David J. Hamilton[5], Duane D. Miller[1], Francesca-Fang Liao[6], Julio F. Cordero-Morales[,2], Zhongzhi Wu[*,1], Wei, Li[*,1]*

[1] Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN 38163, United States

[2] Department of Physiology, University of Tennessee Health Science Center, Memphis, TN 38163, United States

[3] Integrated Biomedical Sciences Graduate Program, College of Graduate Health Sciences, Memphis, TN, 38163, United States

[4] Department of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis, TN 38105, United States

[5] Department of Comparative Medicine, College of Graduate Health Sciences, the University of Tennessee Health Science Center, Memphis, TN 38163, United States

[6] Department of Pharmacology, the University of Tennessee Health Science Center, Memphis, TN 38103, United States

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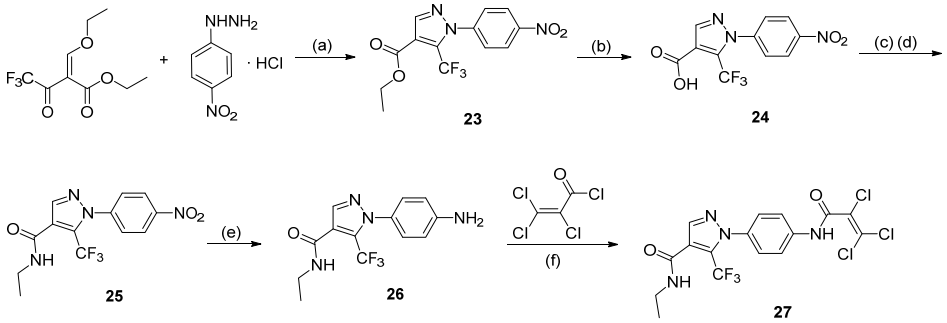
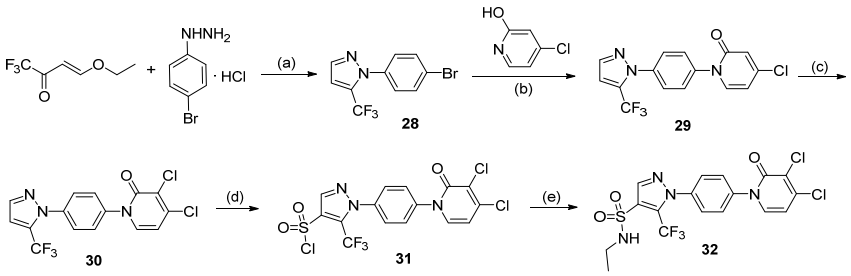
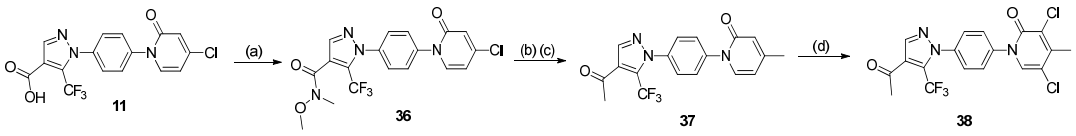


Scheme S1. Synthesis of compound **27**. Reagents and conditions: (a) EtOH, 60°C, 6 h; (b) 1 M KOH (aq.), EtOH, H_2O, rt, 8 h; (c) $SOCl_2$, CH_2Cl_2, reflux for 4 h; (d) ethylamine, TEA, r.t., 12 h; (e) H_2, Pd/C, MeOH, r.t., 8 h; (f) THF, r.t., 12 h.



Scheme S2. Synthesis of sulfonamide compound **32**. Reagents and conditions: (a) EtOH, 60 °C, 6 h; (b) 4-chloropridinone, CuI, DMCDA, K_2CO_3, toluene, reflux, 12 h; (c) NCS, DMF, 100 °C, 8 h; (d) $ClSO_3OH$, 160 °C, 12 h; (e) ethylamine, TEA, r.t., 12 h.



Scheme S3. Synthesis of ketone compound **37** and **38**. Reagents and conditions: (a) Oxalyl chloride, DMF, CH_2Cl_2, 0 ℃–r.t.; (b) *N,O*-dimethylhydroxyamine hydrochloride, TEA, CH_2Cl_2, 0 ℃–r.t.; (c) MeMgBr, THF, 0 ℃–r.t. (d) NCS, DMF, 100 ℃, 8 h.

Experimental section

General chemistry. All solvents and chemical reagents were purchased from commercial sources and used directly without further purification. Glassware was oven-dried before use. All reactions were performed under an argon atmosphere. TLC was performed on silica gel 60 GF254 and monitored under UV light. Flash chromatography was performed on 230-400 mesh silica gel (Fisher Scientific). NMR spectra were obtained on a Bruker Ascend 400 (Billerica, MA) spectrometer. HR-MS were obtained on Waters Acquity UPLC linked to a Waters Acquity Photodiode Array Detector and Waters qTof mass detector. The purities of all compounds reported herein with biological data showed purities ≥95% as determined by the HPLC study performed on Agilent 1100 with Kinetex® F5 (4.6 × 250 mm, 5 μm) column using a mixture of solvent methanol/water at a flow rate of 1 mL/min and monitoring by UV absorption at 240 nm wavelength. Chemical shifts are given in ppm. Tetramethylsilane (TMS) is used as an internal reference for NMR spectra taken in DMSO-d_6 or chloroform-*d*. All coupling constants (*J*) are given in Hertz (Hz).

General procedure for cyclization reaction to form pyrazole derivatives.

To a stirred solution of substituted phenylhydrazine hydrochloride (18.74 mmol) in ethanol (20 mL) was added ethoxymethylene compound (12.49 mmol). The mixture was stirred and heated to reflux for 6 hours under argon atmosphere. Then, the solvent was removed under reduced pressure. The yellow solid was stirred in 30 mL of saturated $NaHCO_3$ solution and extracted with ethyl acetate (3x50 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered and concentrated to dryness under reduced pressure. The yellow oily residue was subjected to flash column chromatography (silica gel, CH_2Cl_2) to afford the pyrazoles as pale yellow solids.

Ethyl 1-(4-bromophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (1). 77.2% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.63 (d, *J* = 8.8 Hz, 2H), 7.32 (d, *J* = 8.8 Hz, 2H), 4.38 (q, *J* = 7.2 Hz, 2H), 1.38 (t, *J* = 7.2 Hz, 3H).

Ethyl 1-(4-nitrophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (23). 82.1% yield; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 8.38 (d, *J* = 8.8 Hz, 2H), 8.18 (s, 1H), 7.66 (d, *J* = 8.8 Hz, 2H), 4.40 (q, *J* = 7.2 Hz, 2H), 1.40 (t, *J* = 7.2 Hz, 3H).

1-(4-bromophenyl)-5-(trifluoromethyl)-1H-pyrazole (28). The crude compound was used directly in next step without further purication.

General procedure for coupling of pyrazole derivatives with substituted pyridones.

Pyrazole (3.32 mmol), pyridone (3.32 mmol), CuI (0.66 mmol), *trans-N,N'*-dimethylcyclohexane-1,2-diamine (0.66 mmol) and K_2CO_3 (6.63 mmol) were mixed together in 20 mL of anhydrous toluene at room temperature under argon. The reaction mixture was stirred and heated to reflux overnight under argon. The reaction was quenched by addition of 100 mL of water and extracted with ethyl acetate (3x50 mL). Organic layer was separated, washed with water, dried over anhydrous $MgSO_4$, filtered and evaporated to an orange oil which was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 19/1 v/v) to afford products as white solids.

Ethyl 1-(4-(4-chloro-2-oxopyridin-1(2*H*)-yl)phenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (2). 64.7% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (s, 1H), 7.57 – 7.52 (m, 4H), 7.33 (dd, J_1 = 7.6 Hz, J_2 = 0.4 Hz, 1H), 6.74 (dd, J_1 = 2 Hz, J_2 = 0.4 Hz, 1H), 6.34 (dd, J_1 = 7.6 Hz, J_2 = 2 Hz, 1H), 4.39 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.86, 147.47, 142.81, 141.01, 139.31, 137.31, 132.82, 132.42, 127.37, 126.87, 120.41, 117.67, 117.22, 108.54, 61.4, 14.1. HRMS (ESI): calcd for $C_{18}H_{13}ClF_3N_3O_3$ 412.0670 $[M + H]^+$, found 412.0664 (-1.5 ppm). Purity: 99.0% by HPLC (Rt = 4.3 min).

Ethyl 1-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (3). 76% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.58 (s, 4H), 7.47 – 7.43 (m, 1H), 7.38 – 7.37 (m, 1H), 6.71 (d,), 6.34 – 6.30 (m, 1H), 4.39 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.9, 142.7, 141.9, 140.2, 139.0, 137.4, 132.8, 132.4, 127.5, 126.7, 122.1, 120.7, 120.4, 117.7, 117.1, 106.5, 61.4, 14.1. HRMS (ESI): calcd for $C_{18}H_{14}F_3N_3NaO_3$ 400.0879 $[M + H]^+$, found 400.0873 (-1.5 ppm). Purity: 97.2% by HPLC (Rt = 3.62 min).

Ethyl 1-(4-(3,5-dichloro-2-oxopyridin-1(2*H*)-yl)phenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (7). 40% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H); 7.64 (d, J = 2.4 Hz, 1 H), 7.61 – 7.55 (m, 4H), 7.42 (d, J = 2.4 Hz, 1H), 4.39 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.8, 157.1, 142.9, 140.8, 139.6, 138.9, 138.4, 133.3, 129.2, 128.3, 127.3, 127.0, 120.4, 117.7, 117.3, 112.0, 61.5, 14.1. HRMS (ESI): calcd for $C_{18}H_{13}Cl_2F_3N_3O_3$ 446.0281 $[M + H]^+$, found 446.0300 (4.3 ppm). Purity: 96.8% by HPLC (Rt = 4.87 min).

Ethyl 1-(4-(5-chloro-2-oxopyridin-1(2*H*)-yl)phenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (8). 32.4% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (s, 1H); 7.55-7.54 (m, 4H), 7.43 (dd, J_1 = 6.8 Hz, J_2 = 0.8 Hz, 1H), 7.38 (dd, J_1 = 9.6 Hz, J_2 = 2.8 Hz,1H), 6.66 (dd, J_1 = 9.6 Hz, J_2 = 0.8 Hz,1H), 4.39 (q, J = 7.2 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.8, 160.4, 142.8, 141.2, 141.1, 139.3, 134.7, 132.8, 128.0, 127.3, 126.9, 122.9, 120.8, 120.4, 117.2, 113.2, 61.4, 14.1. HRMS (ESI): calcd for $C_{18}H_{13}ClF_3N_3NaO_3$ 434.0490 $[M + Na]^+$, found 434.0490 (0 ppm). Purity: 95.4% by HPLC (Rt = 4.26 min).

4-chloro-1-(4-(5-(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)pyridin-2(1H)-one (29). 42.2% yield for two steps; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 1.2 Hz, 1H), 7.65 (d, J = 8.7 Hz, 2H), 7.52 (d, J = 8.8 Hz, 2H), 7.33 (d, J = 7.4 Hz, 1H), 6.86 (d, J = 1.6 Hz, 1H), 6.74 (d, J = 2.0 Hz, 1H), 6.34 (dd, J = 7.4, 2.3 Hz, 1H).

Chlorination of pyridone ring of 2 to afforded compounds 4-6 and 30.

Compound **2** (1.52 g, 3.69 mmol) and *N*-chlorosuccinimide (0.54 g, 4.06 mmol) were dissolved in 50 mL of anhydrous DMF at room temperature under argon. The reaction mixture was stirred and heated to 100 ºC for 4 hours. The reaction was hydrolyzed by addition of 100 mL of water and extracted with ethyl acetate (3×50 mL). Organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, filtered and evaporated to an orange oil which was purified by column chromatography (silica gel, CH_2Cl_2/Acetone = 19/1 *v*/*v*) to give compound **4-6** and **30** as white solid products.

Ethyl 1-(4-(3,4,5-trichloro-2-oxopyridin-1(2*H*)-yl)phenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (4). 14.1% yield; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (s, 1H), 8.36 (s, 1H), 7.73 (dt, J_1 = 6.6 Hz, J_2 = 5.7 Hz, 4H), 4.33 (dt, J = J_1 = 7.1 Hz, J_2 = 5.8 Hz, 2H), 1.31 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.7, 156.3,

142.9, 142.8, 140.9, 139.5, 136.1, 128.8, 128.6, 127.5, 125.1, 120.9, 116.8, 110.6, 61.6, 14.4. HRMS (ESI): calcd for $C_{18}H_{11}Cl_3F_3N_3NaO_3$ 501.9710 $[M + Na]^+$, found 501.9704 (-1.2 ppm). Purity: 95.7% by HPLC (Rt = 6.52 min).

Ethyl 1-(4-(4,5-dichloro-2-oxopyridin-1(2*H*)-yl)phenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (5). 21.3% yield; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (s, 1H), 8.36 (s, 1H), 7.71 (dt, J_1 = 6.5 Hz, J_2 = 5.7 Hz, 4H), 6.98 (s, 1H), 4.33 (q, J = 7.1 Hz, 2H), 1.31 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.8, 159.3, 145.4, 142.9, 140.8, 139.3, 138.3, 132.3, 131.9, 128.5, 127.4, 120.5, 118.0, 116.8, 110.9, 61.6, 39.7, 39.5, 39.3, 14.4. HRMS (ESI): calcd for $C_{18}H_{13}Cl_2F_3N_3O_3$ 446.0281 $[M + H]^+$, found 446.0268 (-2.9 ppm). Purity: 99.5% by HPLC (Rt = 5.3 min).

Ethyl 1-(4-(3,4-dichloro-2-oxopyridin-1(2*H*)-yl)phenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (6). 48.5% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.62 – 7.51 (m, 4H), 7.30 (d, J = 7.5 Hz, 1H), 6.47 (d, J = 7.5 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.8, 157.7, 144.3, 142.8, 140.9, 139.6, 134.3, 132.9, 132.5, 127.3, 126.9, 125.8, 120.4, 117.7, 117.3, 108.3, 77.4, 77.0, 76.7, 61.5, 14.1. HRMS (ESI): calcd for $C_{18}H_{13}Cl_2F_3N_3O_3$ 446.0281 $[M + H]^+$, found 446.0269 (-2.7 ppm). Purity: 97.3% by HPLC (Rt = 5.08 min).

3,4-dichloro-1-(4-(5-(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)pyridin-2(1H)-one (30). 67.6% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.66 (d, J = 8.7 Hz, 2H), 7.53 (d, J = 8.7 Hz, 2H), 7.30 (d, J = 7.5 Hz, 1H), 6.87 (s, 1H), 6.45 (d, J = 7.5 Hz, 1H).

Fluorination of pyridone ring of 2 to form ethyl 1-(4-(4-chloro-3-fluoro-2-oxopyridin-1(2*H*)-yl)phenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (9). A mixture of compound **2** (2.05 g, 5 mmol) and Selectfluor [1-(chloromethyl)-4-fluoro-1,4-diazoniabicyclo[2.2.2]octanebis(tetrafluoroborate)] (3.55 g, 10 mmol) in MeCN (50 mL) was stirred at 85 ℃ for 2 h. The reaction was cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in EtOAc (100 mL) and washed by water (3×50 mL). The extract was dried over $MgSO_4$ and concentrated under reduced pressure to give the crude material that was purified by silica gel flash column chromatography (CH_2Cl_2/acetone = 19/1, *v*/*v*) to give the offwhite solid in 24.7% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.65 – 7.51 (m, 4H), 7.18 (dd, J_1 = 7.6 Hz, J_2 = 1.9 Hz, 1H), 6.36 (dd, J_1 = 7.5 Hz, J_2 = 5.9 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H).

Bromination of pyridone ring of 2 to form ethyl 1-(4-(3,5-dibromo-4-chloro-2-oxopyridin-1(2*H*)-yl)phenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (10). Ethyl 1-(4-(4-chloro-2-oxopyridin-1(2*H*)-yl)phenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (**2**) (1.52 g, 3.69 mmol) and *N*-bromosuccinimide (0.49 g, 3.69 mmol) were dissolved in 50 mL of anhydrous DMF at room temperature under argon. The reaction mixture was stirred at room temperature overnight. The reaction was quenched by addition of 100 mL of water and extracted with ethyl acetate (3×50 mL). Organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, filtered and evaporated to an orange oil which was purified by column chromatography (silica gel, CH_2Cl_2/acetone = 19/1 *v*/*v*) to give **10** as a white solid in 68.3% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.70 (s, 1H), 7.60 (d, J = 8.8 Hz, 2H), 7.55 (d, J = 8.8 Hz, 2H), 4.39 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H).

General procedure for hydrolysis of the esters to carboxylic acids.

The ester compound (1.17 mmol) was dissolved in 10 mL of ethanol at room temperature. To this solution was added 2.33 mL of 1M KOH solution at room temperature. The resulted mixture was stirred at room temperature for 8 hours. The reaction solution was neutralized to PH = 6 by adding saturated NH_4Cl solution and extracted with Ethyl acetate (3×50 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered and concentrated to dryness under reduced pressure. The crude solids were used directly in next step without further purification.

General procedure for synthesis of amides.

Thionyl chloride (1M in CH_2Cl_2, 1.4 mL) was added dropwise to a solution of carboxylic acid (1.15 mmol) in CH_2Cl_2 (20 mL)/DMF (0.1 mL). The reaction mixture was stirred at reflux for 4 h. The volatile was removed under reduced pressure and the residue with ethylamine (0.69 mL of 2M THF solution, 1.38 mmol) were mixed together in 40 mL of anhydrous THF at 0 ºC in an ice bath. Triethylamine (0.35 g, 3.45 mmol) was added dropwise with stirring and the resulting mixture was slowly warmed to room temperature and stirred overnight. The solvent was evaporated, and the residue purified by column chromatography using a mixture of CH_2Cl_2/MeOH (19/1, v/v) to afford the desired compounds as white solids.

1-(4-(4-chloro-2-oxopyridin-1(2*H*)-yl)phenyl)-*N*-ethyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (17). 70.3% yield for two steps; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (t, J = 5.5 Hz, 1H), 8.16 (s, 1H), 7.87 (d, J = 7.4 Hz, 1H), 7.66 (s, 4H), 6.72 (d, J = 2.3 Hz, 1H), 6.50 (dd, J_1 = 7.4 Hz, J_2 = 2.3 Hz, 1H), 3.31 – 3.20 (m, 2H), 1.11 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6, 160.3, 147.0, 141.2, 140.2, 139.0, 130.1, 129.7, 128.4, 127.1, 122.3, 121.2, 119.2, 107.7, 34.3, 15.0. HRMS (ESI): calcd for $C_{18}H_{15}ClF_3N_4O_2$ 411.0830 $[M + H]^+$, found 411.0836 (1.5 ppm). Purity: 97.5% by HPLC (Rt = 3.06 min).

1-(4-(3,4,5-trichloro-2-oxopyridin-1(2*H*)-yl)phenyl)-*N*-ethyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (18). 55.7% yield for two steps; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (t, J = 5.5 Hz, 1H), 8.44 (s, 1H), 8.17 (s, 1H), 7.84 – 7.57 (m, 4H), 3.32 – 3.19 (m, 2H), 1.12 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.3, 156.4, 142.8, 140.6, 140.2, 139.5, 136.1, 130.2, 129.8, 128.6, 127.2, 125.1, 122.4, 121.1, 118.4, 110.6, 34.3, 15.0. HRMS (ESI): calcd for $C_{18}H_{13}Cl_3F_3N_4O_2$ 479.0051 $[M + H]^+$, found 479.0050 (-0.2 ppm). Purity: 95.9% by HPLC (Rt = 3.89 min).

1-(4-(4,5-dichloro-2-oxopyridin-1(2*H*)-yl)phenyl)-*N*-ethyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (19). 63.0% yield for two steps; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.59 (t, J = 5.2 Hz, 1H), 8.17 (s, 1H), 7.89 (d, J = 7.6 Hz, 1H), 7.70 (s, 4H), 6.70 (d, J = 7.2 Hz, 1H), 3.29-3.23 (m, 2H), 1.12 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.3, 159.4, 145.4, 140.5, 140.2, 139.2, 138.3, 130.1, 129.7, 128.6, 127.1, 122.4, 121.1, 120.5, 118.4, 110.8, 34.3, 15.0. HRMS (ESI): calcd for $C_{18}H_{14}Cl_2F_3N_4O_2$ 445.0440 $[M + H]^+$, found 445.0444 (0.9 ppm). Purity: 99.5% by HPLC (Rt = 3.45 min).

1-(4-(3,4-dichloro-2-oxopyridin-1(2*H*)-yl)phenyl)-*N*-ethyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (20). 67.6% yield for two steps; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (t, J = 5.5 Hz, 1H), 8.35 (s, 1H), 8.15 (s, 1H),

7.77 – 7.58 (m, 4H), 6.97 (s, 1H), 3.30 – 3.19 (m, 2H), 1.10 (t, J = 7.2 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.5, 157.6, 144.2, 141.2, 140.2, 139.2, 137.5, 130.2, 129.8, 128.4, 127.3, 123.5, 122.2, 121.1, 118.4, 107.8, 34.4, 14.9. HRMS (ESI): calcd for $C_{18}H_{13}Cl_2F_3N_4NaO_2$ 467.0260 $[M + Na]^+$, found 467.0259 (-0.2 ppm). Purity: 97.5% by HPLC (Rt = 3.35 min).

1-(4-(4-chloro-3-fluoro-2-oxopyridin-1(2*H*)-yl)phenyl)-*N*-ethyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (21). 71.6% yield for two steps; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (t, J = 5.5 Hz, 1H), 8.16 (s, 1H), 7.74 (dd, J_1 = 7.6 Hz, J_2 = 1.9 Hz, 1H), 7.69 (s, 4H), 6.59 (dd, J_1 = 7.5 Hz, J_2 = 6.3 Hz, 1H), 3.30 – 3.20 (m, 2H), 1.10 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.4, 154.9, 154.6, 149.7, 147.3, 140.5, 140.2, 139.3, 134.6, 134.5, 130.2, 129.8, 129.0, 128.4, 127.3, 122.3, 121.1, 118.4, 106.3, 34.4, 14.9. ^{19}F NMR (376 MHz, DMSO-d_6) δ -54.38, -134.40. HRMS (ESI): calcd for $C_{18}H_{14}Cl_1F_4N_4O_2$ 429.0736 $[M + H]^+$, found 429.0738 (0.5 ppm). Purity: 96.2% by HPLC (Rt = 3.12 min).

1-(4-(3,5-dibromo-4-chloro-2-oxopyridin-1(2*H*)-yl)phenyl)-*N*-ethyl--5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (22). 48.8% yield for two steps; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.70 (s, 1H), 7.59 (d, J = 8.7 Hz, 2H), 7.53 (d, J = 8.9 Hz, 2H), 6.01 (t, J = 5.1 Hz, 1H), 3.65 – 3.34 (m, 2H), 1.25 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.7, 156.8, 147.4, 140.2, 140.1, 139.5, 135.6, 127.3, 126.9, 121.7, 120.7, 118.9, 118.0, 100.0, 77.3, 77.0, 76.7, 35.1, 14.7. HRMS (ESI): calcd for $C_{18}H_{13}Br_2ClF_3N_4O_2$ 568.9025 $[M + H]^+$, found 568.9033 (1.4 ppm). Purity: 95.8% by HPLC (Rt = 3.93 min).

***N*-ethyl-1-(4-nitrophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (25).** 86.9% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.62 (t, J = 5.2 Hz, 1H), 8.43 (d, J = 7.2 Hz, 2H), 8.24 (s, 1H), 7.84 (d, J = 8.8 Hz, 2H), 3.28-3.25 (m, 2H), 1.12 (t, J = 7.2 Hz, 3H).

1-(4-aminophenyl)-N-ethyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (26). Compound **25** (1.00 g, 3.05 mmol) was dissolved in 50 mL of methanol and 10 mL of ethyl acetate at room temperature. To this solution was added Pd/C (0.10 g, 10% Pd base). The reaction vessel was vacuumed and hydrogen gas was introduced by a hydrogen balloon at room temperature. The reaction mixture was stirred at room temperature under hydrogen atmosphere for 12 hours and filtered through a celite. The solution was evaporated to dryness. The crude was subjected to flash column chromatography (silica gel, CH_2Cl_2/MeOH = 19/1 *v*/*v*) to afford the desired amino compound as a white solid product (0.80 g, 88.0%). ^{1}H NMR (400 MHz, DMSO-d_6): 8.48 (t, J = 5.6 Hz, 1H), 7.97 (s, 1H), 7.06 (d, J = 8.8 Hz, 2H), 6.62 (d, J = 8.8 Hz, 2H), 5.59 (s, 2H), 3.26-3.19 (m, 2H), 1.09 (t, J = 7.2 Hz, 3H).

***N*-ethyl-1-(4-(2,3,3-trichloroacrylamido)phenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (27).** 2,3,3-trichloroacrylic acid (0.44 g, 2.561 mmol) was dissolved in 10 mL of anhydrous methylene chloride at room temperature under nitrogen. Thionyl chloride (0.90 g, 7.53 mmol) and three drops of DMF were added to the above solution *via* a syringe at room temperature. The reaction mixture was stirred and heated to reflux for 4 h. Then, the volatile was removed under reduced pressure to give the corresponding acyl chloride as a yellow oil which was dissolved in 10 mL of anhydrous THF. This acyl chloride THF solution was added to the THF (20 mL) solution of compound **26** (0.75 g, 2.51 mmol) at room temperature. The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was subjected to flash column

chromatography (silica gel, CH_2Cl_2/MeOH = 9/1 *v*/*v*) to afford the desired product as a white solid (0.95 g, 82.2%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.33 (s, 1H), 8.56 (t, J = 5.2 Hz, 1H), 8.10 (s, 1H), 7.81 (d, J = 8.2 Hz, 2H), 7.53 (d, J = 8.3 Hz, 2H), 3.31 – 3.18 (m, 2H), 1.11 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.4, 159.3, 139.9, 139.3, 135.4, 130.0, 129.6, 127.4, 124.6, 123.8, 122.9, 122.0, 121.2, 120.5, 118.5, 34.3, 15.0. HRMS (ESI): calcd for $C_{16}H_{13}Cl_3F_3N_4O_2$ 455.0051 $[M + H]^+$, found 455.0053 (0.4 ppm). Purity: 96.4% by HPLC (Rt = 3.74 min).

1-(4-(3,4-dichloro-2-oxopyridin-1(2*H*)-yl)phenyl)-5-(trifluoromethyl)-1H-pyrazole-4-sulfonyl chloride (31). Compound **30** (374 mg, 1 mmol) was dissolved in chlorosulfonic acid (5 mL) with vigorous stirring at room temperature and heated to 140 ℃ for 12 h. After cooling to room temperature, the reaction mixture was added dropwise to finely ground ice, filtered at 0°C and dried under reduced pressure to afford the sulfonyl chloride as pale-yellow solid which was used directly in next step.

1-(4-(3,4-dichloro-2-oxopyridin-1(2*H*)-yl)phenyl)-*N*-ethyl-5-(trifluoromethyl)-1*H*-pyrazole-4-sulfonamide (32). The title compound was prepared following the general procedure described above for the amide formation using ethylamine as the reactant. 25.3% overall yield in two steps; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (s, 1H), 8.04 – 7.94 (m, 2H), 7.81 – 7.65 (m, 4H), 7.22 (s, 1H), 3.04 – 2.88 (m, 2H), 1.05 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.0, 141.1, 140.8, 140.5, 139.4, 139.4, 132.1, 131.7, 128.6, 127.0, 126.0, 121.6, 119.0, 117.8, 110.6, 37.8, 15.5. HRMS (ESI): calcd for $C_{17}H_{13}Cl_2F_3N_4NaO_3S$ 502.9930 $[M + Na]^+$, found 502.9940 (2.0 ppm). Purity: 97.5% by HPLC (Rt = 3.18 min).

1-(4-(4-chloro-2-oxopyridin-1(2H)-yl)phenyl)-N-methoxy-N-methyl-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (36). A solution of oxalyl chloride (152 mg, 1.2 mmol) in 5 mL CH_2Cl_2 was added dropwise at 0 °C to a solution of **11** (1.92 g, 5 mmol) in CH_2Cl_2 (50 mL)/DMF (0.5 mL). Mixture was allowed to come to room temperature and after stirring for 4 h, *N*,*O*-dimethylhydroxylamine hydrochloride (730 mg, 7.5 mmol) in CH_2Cl_2 (20 mL) and TEA (1.01 g, 10 mmol) were then added. The reaction mixture was stirred at room temperature for 5 h. The reaction was quenched by adding water. The organic layer was separated, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH = 19/1 *v*/*v*) to afford product as a white solid (85.5% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.64 (d, J = 8.7 Hz, 2H), 7.54 (d, J = 8.7 Hz, 2H), 7.34 (d, J = 7.4 Hz, 1H), 6.74 (d, J = 2.2 Hz, 1H), 6.35 (dd, J_1 = 7.4 Hz, J_2 = 2.2 Hz, 1H), 3.61 (s, 3H), 3.38 (s, 3H).

1-(4-(4-acetyl-5-(trifluoromethyl)-1*H*-pyrazol-1-yl)phenyl)-4-methylpyridin-2(1*H*)-one (37). Compound **36** (854 mg, 2 mmol) was dissolved in 20 mL of anhydrous THF at room temperature under argon. Methylmagnesium bromide (6 mL of 1M THF solution, 6 mmol) was added *via* a syringe at room temperature under argon. The reaction solution was stirred at room temperature overnight. Then, the reaction was quenched by adding 50 mL of water. The resulted solution was extracted with ethyl acetate (3×30 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude solid was purified by flash column chromatography (silica gel, CH_2Cl_2/Acetone = 19/1 v/v) to give a white solid (72.7% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (s, 1H), 7.68 – 7.59 (m, 5H), 6.33 (s, 1H), 6.22 (dd, J_1 = 7.1, J_2 = 1.7 Hz, 1H), 2.58 (s, 3H), 2.19 (s,

3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 192.0, 161.3, 152.6, 143.3, 142.2, 138.7, 138.0, 128.2, 127.2, 124.8, 120.9, 119.1, 118.2, 108.8, 30.0, 21.3. HRMS (ESI): calcd for $C_{18}H_{15}F_3N_3O_2$ 362.1111 $[M + H]^+$, found 362.1122 (3.0 ppm). Purity: 96.0% by HPLC (Rt = 3.96 min).

1-(4-(4-acetyl-5-(trifluoromethyl)-1*H*-pyrazol-1-yl)phenyl)-3,5-dichloro-4-methylpyridin-2(1*H*)-one (38). The title compound was prepared following the general procedure described above for the chlorination of pyridone ring. 68.2% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.58 (d, J = 1.6 Hz, 4H), 7.45 (s, 1H), 2.59 (s, 3H), 2.50 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.1, 156.8, 146.7, 142.0, 140.9, 139.4, 131.6, 131.2, 127.3, 126.9, 126.8, 125.0, 120.5, 117.8, 114.7, 29.7, 18.6. HRMS (ESI): calcd for $C_{18}H_{12}Cl_2F_3N_3NaO_2$ 452.0151 $[M + Na]^+$, found 452.0136 (-3.3 ppm). Purity: 95.6% by HPLC (Rt = 4.23 min).

Cell culture and electrophysiology.

Human embryonic kidney 293 (HEK293; catalog number CRL-1573) cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37 °C and 5% CO_2. Constructs cloned in pMO (a pcDNA3-modified vector) were co-transfected with GFP-pMO, using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Macroscopic currents in the whole-cell patch clamp configuration were recorded 18–24 h post-transfection. The extracellular solution contained 140 mM NaCl, 6 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES, and 10 mM glucose (pH 7.4). Pipettes were made of borosilicate glass (outer diameter, 1.5 mm; inner diameter, 1.10 mm; Sutter Instruments) and fire-polished with a resistance between 2.8 and 4.0 megaohms when filled with an intracellular solution that contained 140 mM CsCl, 5 mM EGTA, and 10 mM HEPES, pH 7.2. For whole-cell currents recorded in the presence of extracellular Ca^{2+}, the extracellular solution contained 140 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, and 10 mM HEPES (pH 7.4), and the pipette solution contained 140 mM CsCl, 5 mM EGTA, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES (pH 7.2). Currents were recorded with a Multiclamp 700B amplifier (Molecular Devices) using a 1-s ramp from -100 to 100 mV. Agonist and antagonists perfused were dissolved in the bath solution prior experiments: TRP channel agonists Allyl isothiocyanate (AITC; TRPA1), capsaicin (TRPV1), and menthol (TRPM8) were obtained from Sigma, GSK1016790A (TRPV4) from Cayman chemicals, OAG (TRPC3) from Avanti Lipids, and GSK1702934A (TRPC3, TRPC6 and TRPC7) from Tocris Bioscience. Antagonist Pyr3 was obtained from Tocris Bioscience, all other antagonists were synthesized in house. Data were acquired with a sampling rate of 20 kHz, low-pass filtered (10 kHz), and analyzed offline using Clampfit v10.4.2.0 (Molecular Devices).

Calcium imaging.

HEK 293 cells were loaded with Fluo-4 AM (1 μM; Invitrogen) per the manufacturer's protocols. Images were acquired and analyzed with CellSens Dimension (Olympus LS). The "No Ca^{2+}" solution contained 140 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, 5 mM EGTA, 10 mM glucose and 10 mM HEPES (pH = 7.4); and the solution with calcium contained: 140 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, 3 mM $CaCl_2$, 10 mM glucose and 10 mM HEPES (pH

= 7.4). Thapsigargin (Sigma-Aldrich), compound 20 and Pyr3 were dissolved in DMSO prior addition to the corresponding solution prior experiments.

Data analysis.

Electrophysiology results were expressed as means ± SD. Data were plotted, and sigmoidal fitting was done using OriginPro (from OriginLab), with the following Boltzmann function:

$$f_{(x)} = A_2 + \frac{A_1 - A_2}{1 + e^{(^{(X - X_o)}/_{dX})}} \qquad (1),$$

where A_2 = final value, A_1 = initial value; X_o = center, and dX = time constant.

Human TRPC3 expression and purification (NP_003296.1)

A DNA construct containing His8-MBP-hTRPC3b with a conserved single-point mutation, I395V, was cloned into the pFastbac1 expression vector. Recombinant baculovirus was obtained following the manufacturer's protocol (Bac-to-Bac expression system, Invitrogen). Sf9 cells were infected with recombinant baculovirus and harvested by centrifugation 72 h after infection. The cell pellet from 0.8 L of culture was resuspended and lysed with a high-pressure homogenizer (Avestin) in a hypotonic buffer (36.5 mm sucrose, 50 mm Tris, and 4 mm TCEP, pH 8) and supplemented with protease inhibitors (1 mm phenylmethanesulfonyl fluoride, 3 mg/ml aprotinin, 3 mg/ml leupeptin, and 1 mg/mL pepstatin). Cell debris was collected by low-speed centrifugation (8,000 × g for 15 min at 4 °C). Membranes were collected by ultracentrifugation (100,000 × g for 30 min at 4 °C).

Membranes were solubilized in Buffer A1 (300 mm NaCl, 4 mm TCEP, and 50 mm Tris, pH 8) supplemented with protease inhibitors. Protein was extracted with 1% digitonin (Millipore) with gentle stirring for 2 h. The detergent-insoluble material was removed by centrifugation (150,000 × g for 45 min at 4 °C), and the supernatant was incubated with amylose resin (New England Biolabs) with gentle stirring for 3 h. After loading onto the column and collecting the flow-through, the resin was washed with 10 column volumes of Buffer B1 (300 mm NaCl, 4 mm TCEP, 0.1% digitonin, and 50 mm Tris, pH 8). Afterward, the protein was eluted with Buffer B1 supplemented with 20 mm maltose. The protein then was digested with ProTEV Plus protease (Promega) at 4 °C overnight to remove the MBP tag. Cleaved protein was further purified by size-exclusion chromatography on a Superose 6 10/300 GL column (GE Healthcare) pre-equilibrated with Buffer C1 (200 mm NaCl, 4 mm TCEP, 40 μm GDN, and 50 mm Tris, pH 8). Peak fractions corresponding to the tetrameric channel were collected and concentrated to ~1.2 mg/ml for LC-MS analysis.

Metabolic stability assay

NADPH regenerating agent solutions A and B were obtained from BD Gentest (Woburn, MA). Pooled human, mice, and rat liver microsomes were purchased from XenoTech (human) and Fisher Scientific CO (mice and rats). Ninety-

six deep well plates were obtained from Midsci (St. Louis, MO). Ninety-six analytical plates were obtained from Corning Incorporated (Acton, MA).

Sample preparation. The procedure for microsomal stability was modified from published literatures[1, 2] A set of incubation times of 0, 15, 30, 60, 120, and 240 min were used. DMSO stock solutions of test compounds were prepared at 10 mM concentration. Human, mouse, and rat liver microsomal solution was prepared by adding 0.058 mL of concentrated human or mouse liver microsomes (20 mg/mL protein concentration) to 1.756 mL of 0.1 M potassium phosphate buffer (pH 7.4) and 5 µL of 0.5 M EDTA to make a 0.6381 mg/mL (protein) microsomal solution. NADPH regenerating agent contained 0.113 mL of NADPH A, 0.023 mL of NADPH B, and 0.315 mL of 0.1 M potassium phosphate buffer (pH 7.4). 2.2 µL of each test compound diluted solution was each added directly to 1.79 mL of liver microsomal solution. This solution was mixed and 90 µL was transferred to 6-time points plates (each in triplicate wells). For the Time 0 plate, 225 µL of cold acetonitrile with internal standard (4 µg/mL warfarin) was added to each well, followed by addition of NADPH regenerating agent (22.5 µL) and no incubation. For other five time points' plate, NADPH regenerating agent (22.5 µL) was added to each well to initiate the reaction, the plate was incubated at 37° C for required time, followed by quenching of the reaction by adding 225 µL of cold acetonitrile with internal standard (4 µg/mL warfarin) to each well. All of the plates were sealed and mixed well at 600 rpm for 10 min and were centrifuged at 4000 rpm for 20 min. The supernatants (120 µL) were transferred to analytical plates for analysis by LC-MS. The metabolic stability is evaluated via the half-life from least-squares fit of the multiple time points based on first-order kinetics.

UPLC/MS/UV System. LC-MS chromasolv grade acetonitrile (ACN) was purchased from Fisher Scientific (Loughborough, UK). LC-MS chromasolv grade methanol, ammonium bicarbonate, and formic acid were obtained from Sigma-Aldrich (St. Louis, MO). Milli-Q water as an ultrapure laboratory-grade water was used in aqueous mobile phase.

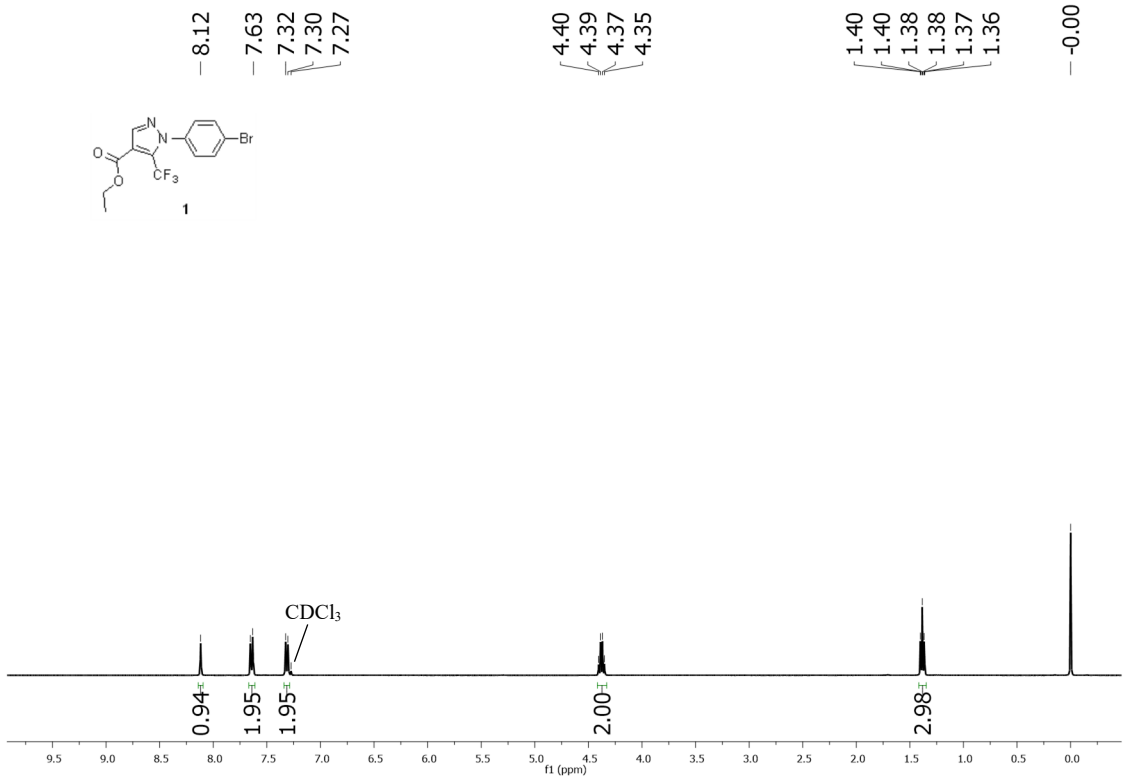
Chromatographic separation was performed on an Acquity UPLC BEH C18 1.7 µm, 2.1 × 50 mm column (Waters Corporation, Milford, MA) using an Acquity ultra-performance liquid chromatography system. Data were acquired using Masslynx v. 4.1 and analyzed using the Quanlynx software suite. This was coupled to an SQ mass spectrometer. The total flow rate was 0.9 mL/min. The sample injection volume was 10 µL. The UPLC column was maintained at 55 °C. Samples were separated on the column under acidic mobile phase (solvent A was 0.1% formic acid in MilliQ H_2O and solvent B was 0.1% formic acid in ACN). The gradient program started at 90% A, changed to 70% A over 0.2 min, to 95 % B over 1.4 minutes, held for 0.35 minutes, then to 90% A over 0.05 minutes. The mass spectrometer was operated in positive-ion mode with electrospray ionization. The conditions were as follows: capillary voltage 3.1 kV, cone voltage 30 V, source temperature 150 °C, desolvation temperature 350 °C, desolvation gas 800 L/hr, cone gas 50 L/hr. A full scan range from m/z = 100-1000 in 0.2 s was used to acquire MS data. A single ion recording mass spectrometry for parent compound and their metabolites were used to determine the quantification of the samples.

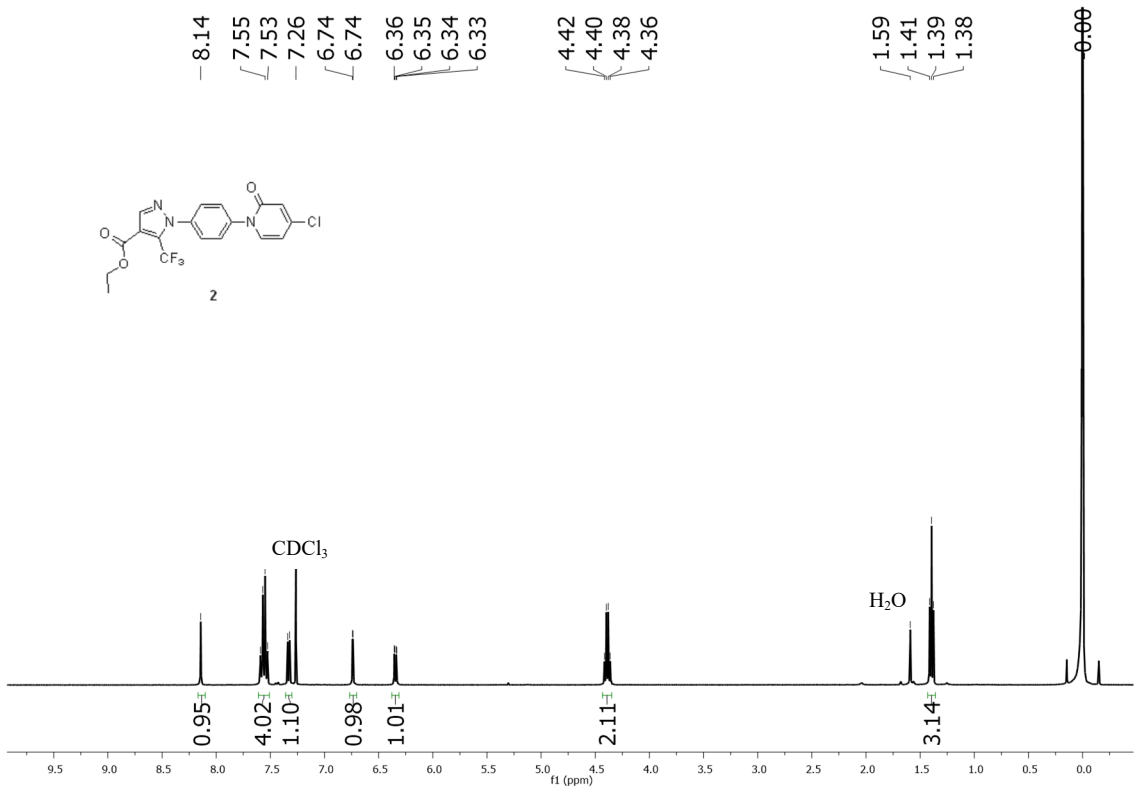
***In vivo* toxicity study.**

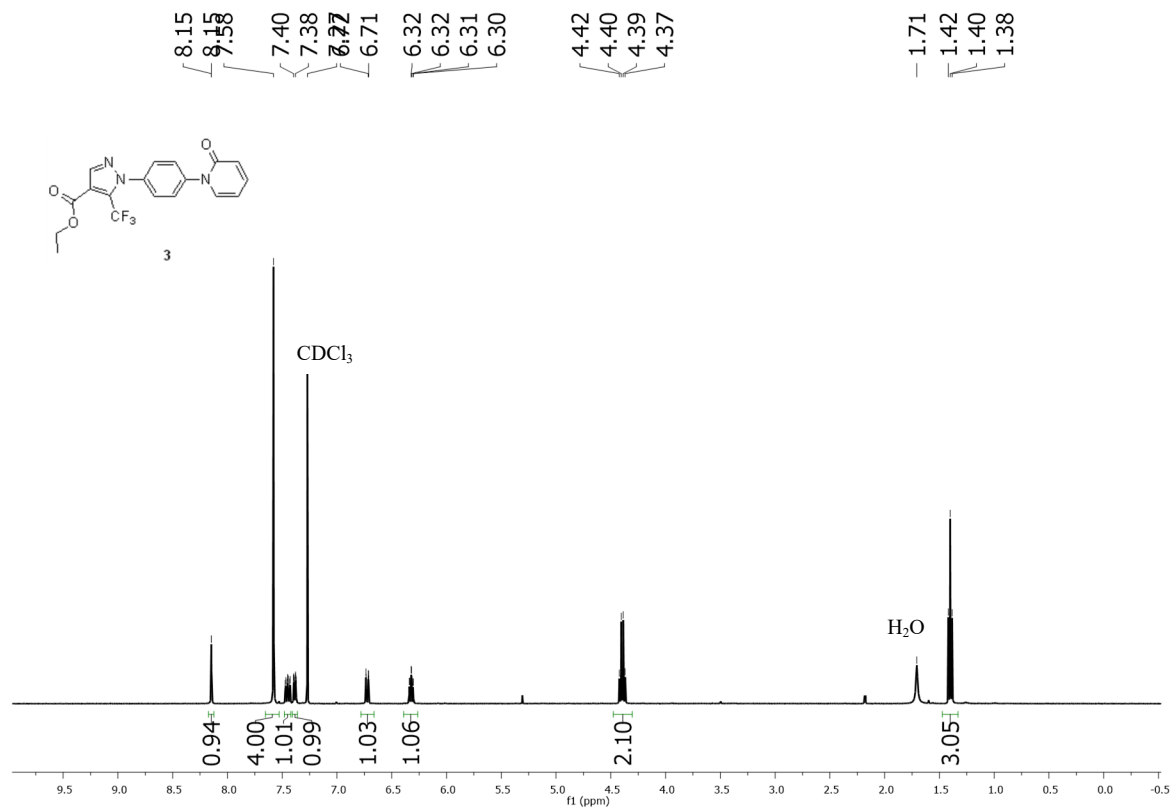
The *in vivo* study was performed in compliance with the NIH Animal Use Guidelines and protocol approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Tennessee Health Science Center (UTHSC, Memphis, TN). NSG mice at 5-8 weeks (n = 2) and ICR mice at 12 weeks (n = 3) were housed with a 12:12-hour light-dark cycle and fed a breeder chow diet. Daily IP injections of 200 mg/kg of compound **20**, **27**, or Pyr3 were administered for five consecutive injections in five mice, respectively, which were followed to monitor the mortality rate. Body weights of the mice were recorded daily.

Primary neuronal culture preparation. Primary hippocampal neurons were prepared from E17 Sprague Dawley rat embryos, seeded in 24-well plates and cultured in coverslips precoated with Poly-D-Lysine in neurobasal medium supplemented with 0.8 mM L-glutamine and B27 for over 14-16 days (DIV). Serum-free conditioned medium (CM) containing naturally secreted Aβ oligomers was collected from cultures of 7PA2/CHO cells [3] which contained 2-5 nM of soluble Abeta based on ELISA. At the end of the compound treatment, neurons on coverslips were fixed, permeabilized and immunostained by anti-MAP2 antibody (1:1000. Sigma-Aldrich, St Louis, MO.) followed by Alexa Fluor™ Plus 488 (1:1000. Invitrogen, Carlsbad, CA) and counterstained by DAPI. Dendrite length were quantified using NIH ImageJ software (Bethesda, MD), bases on 50 dendrites from 3 independent experiments, as performed previously.[4]

[1]H NMR data for pyrazole analogs



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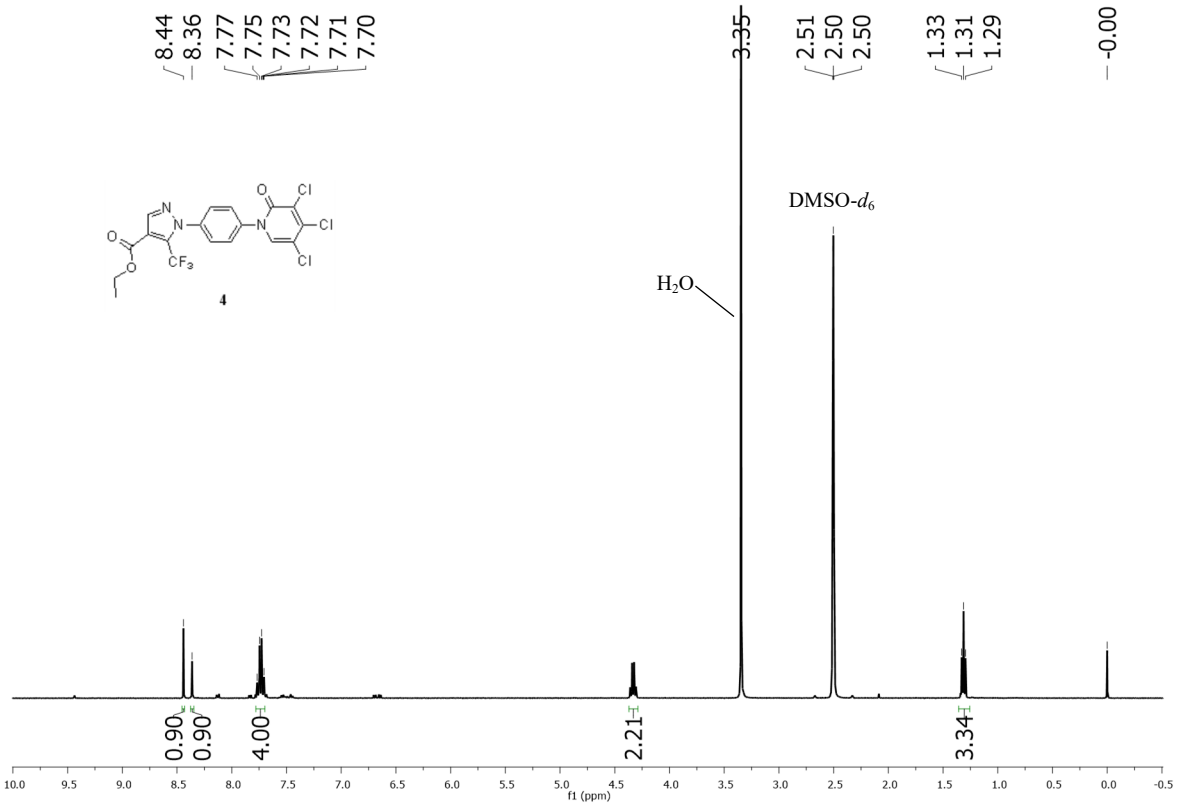
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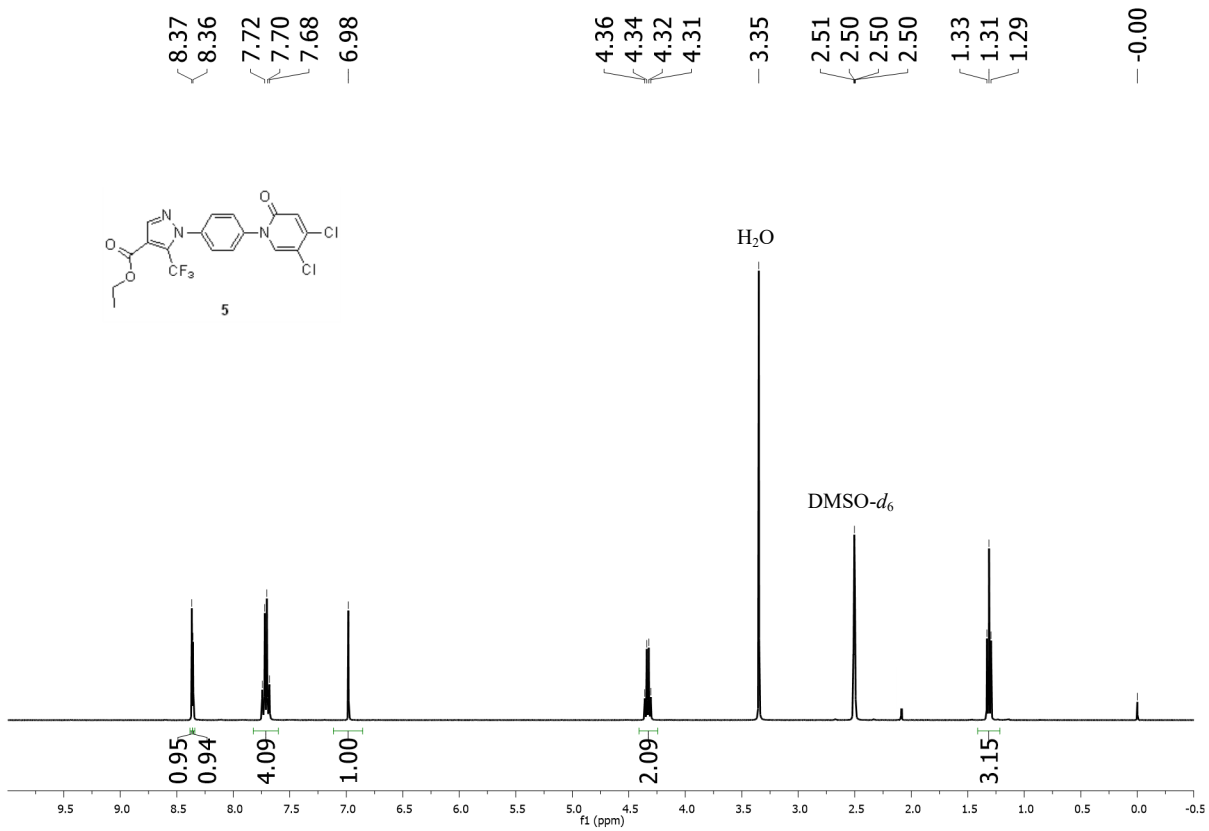
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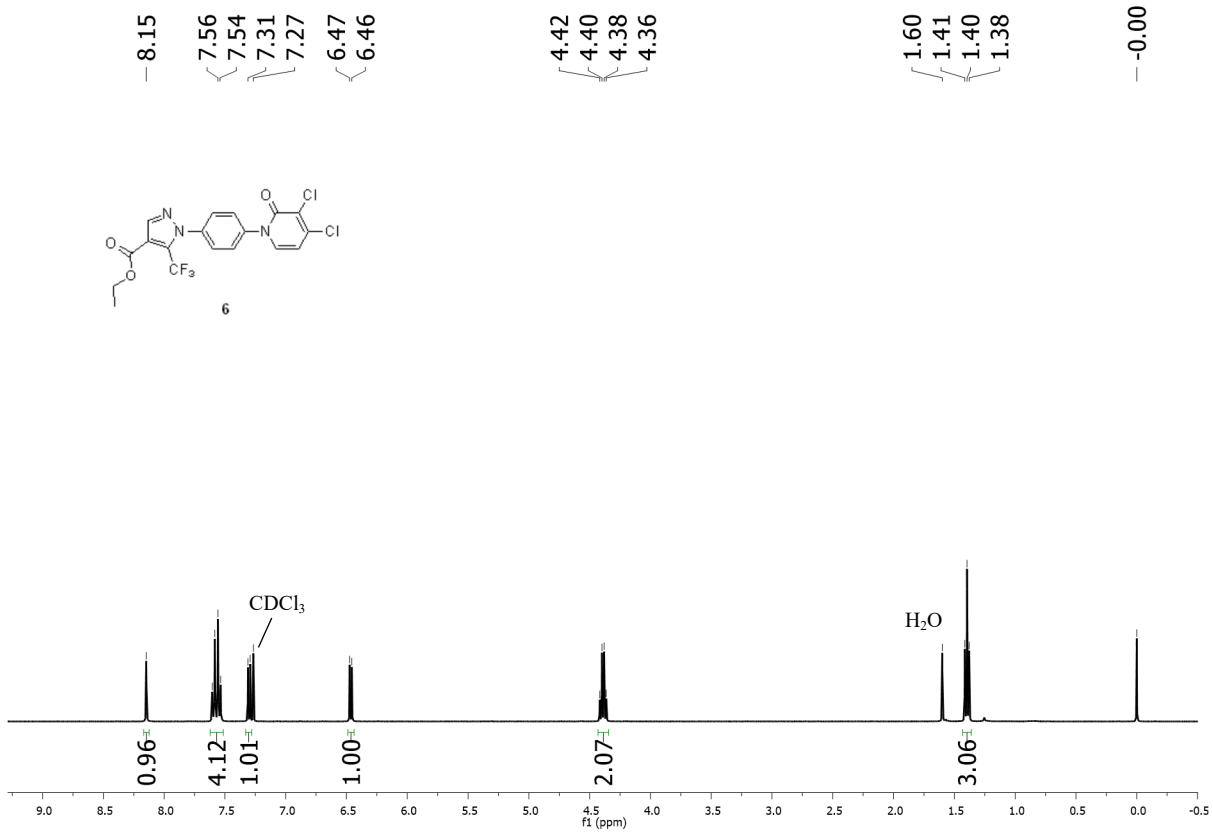
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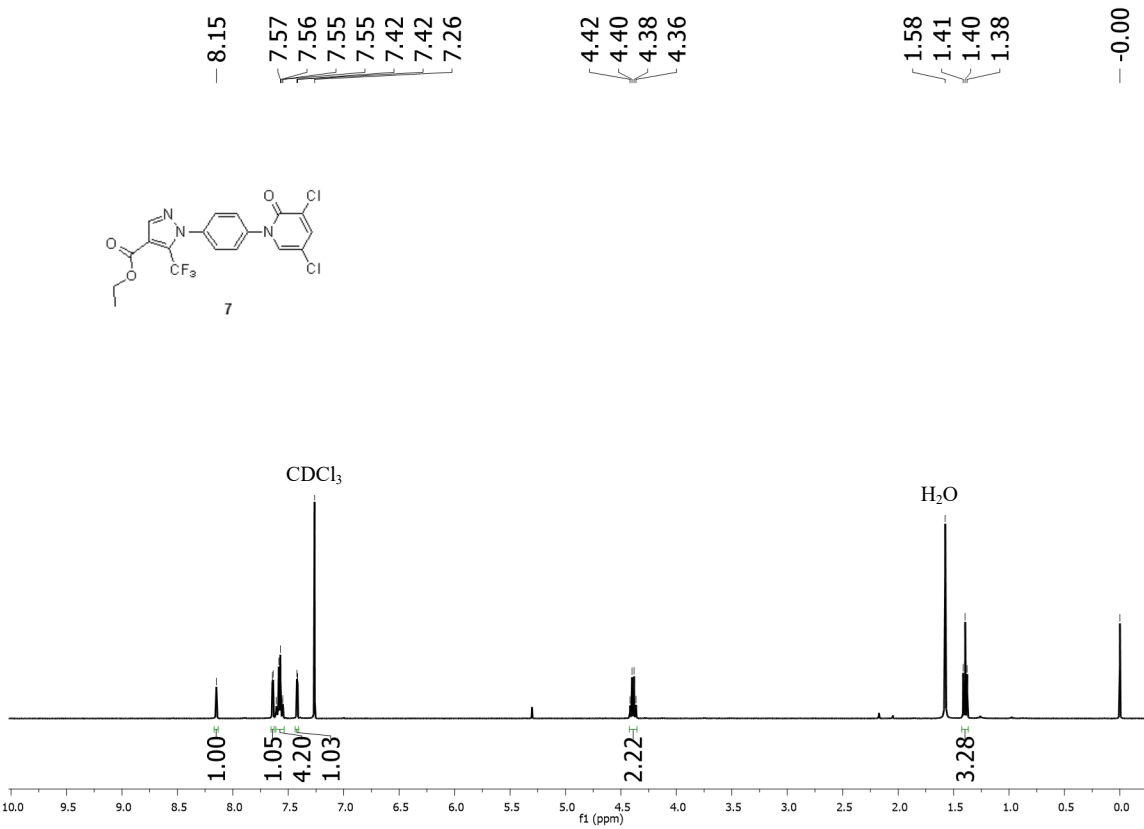
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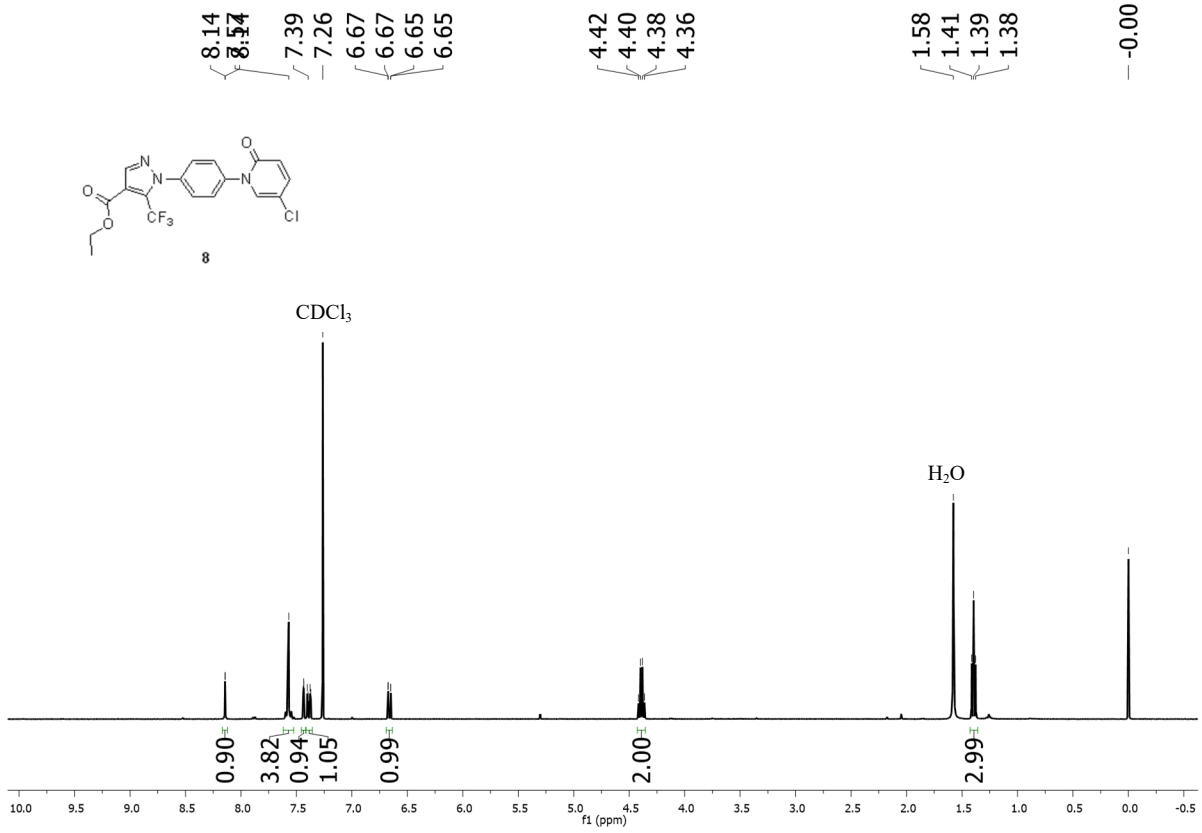
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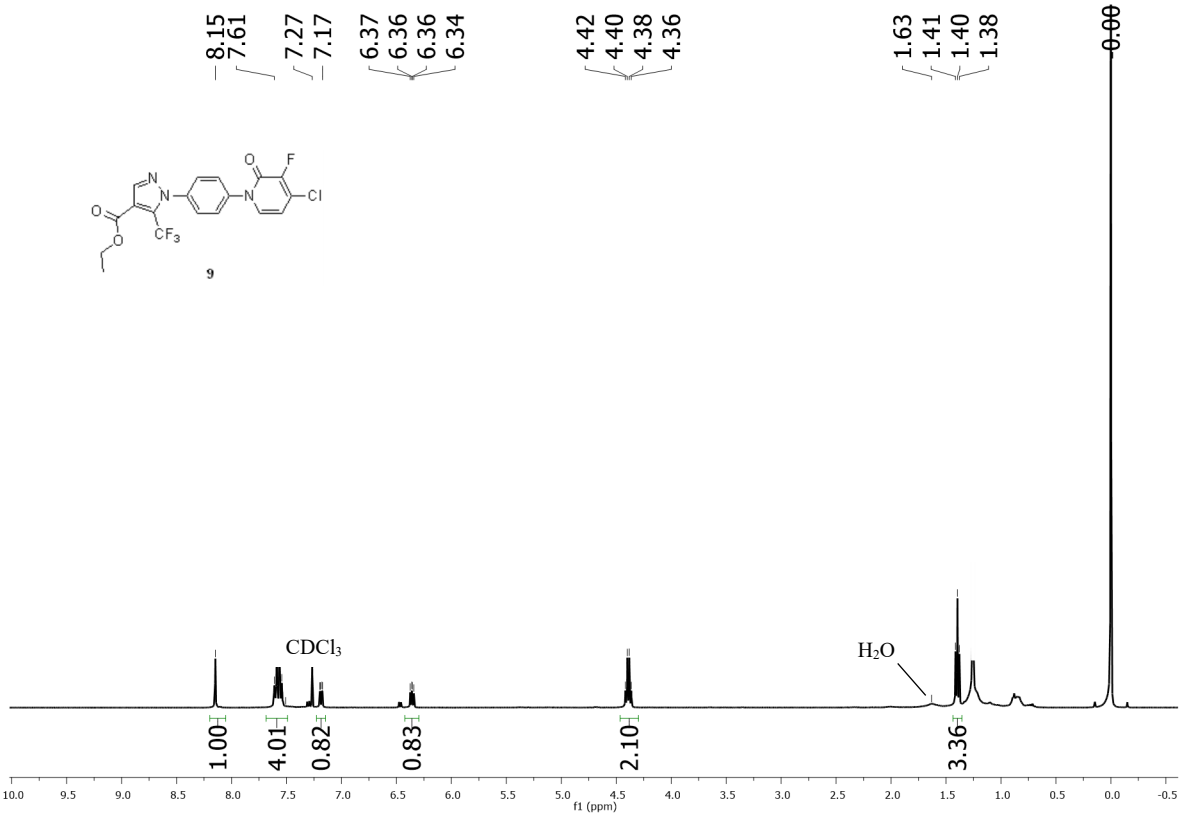
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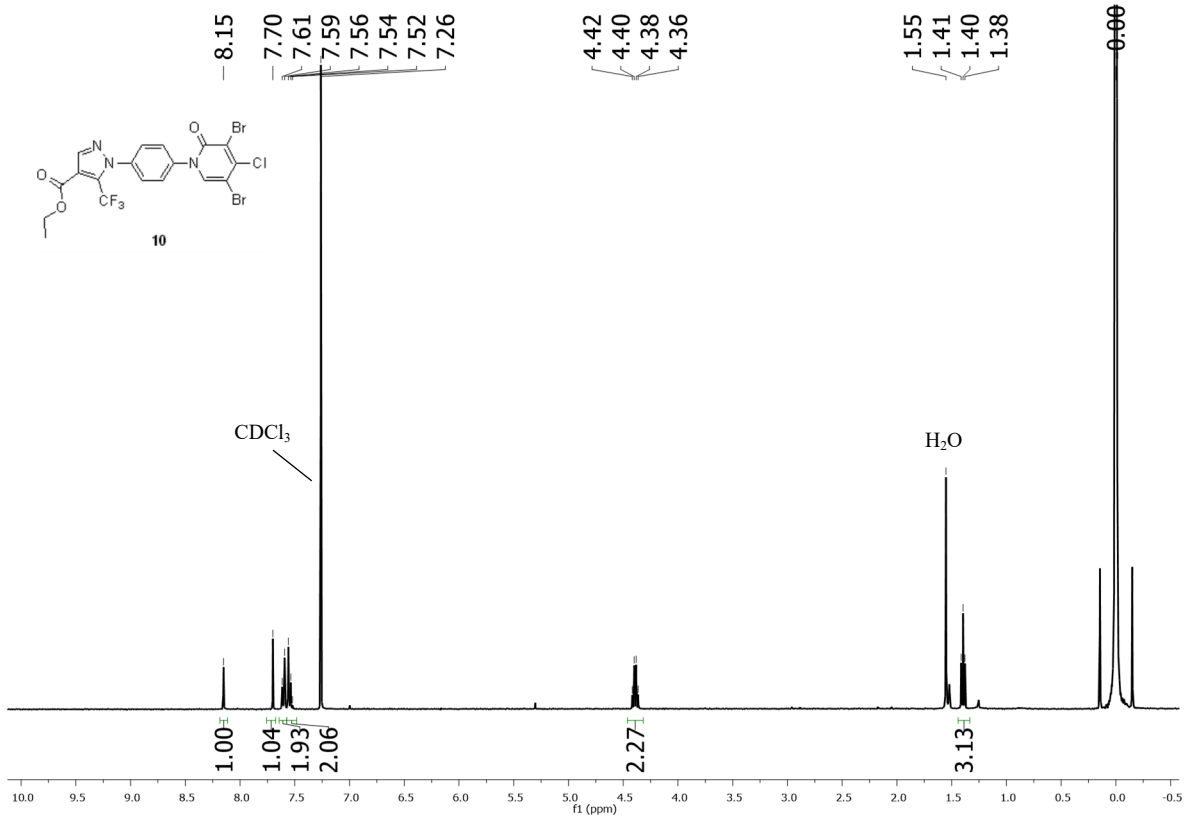
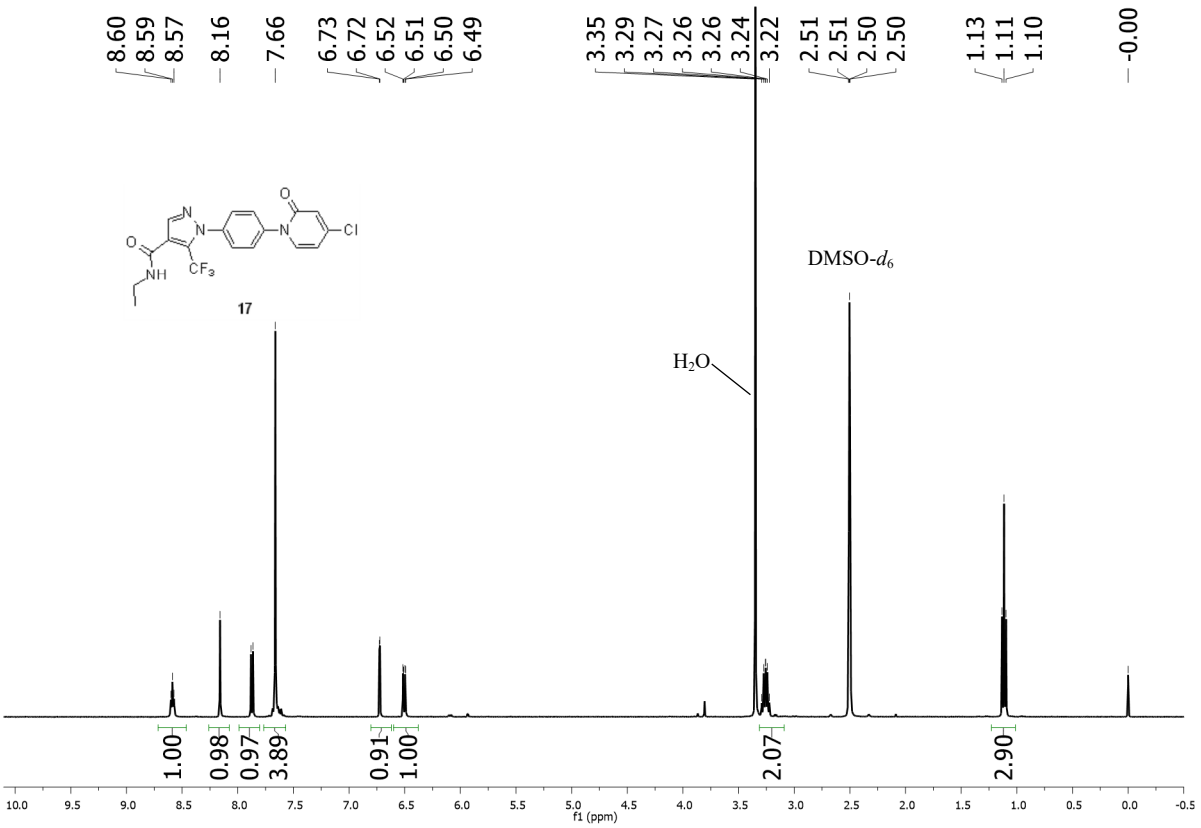
3.36
f1 (ppm)

9


8.15
7.70
7.61
7.59
7.56
7.54
7.52
7.26
4.42
4.40
4.38
4.36
1.55
1.41
1.40
1.38
0.00
CDCl3
H2O
1.00
1.04
1.93
2.06
2.27
3.13
f1 (ppm)

10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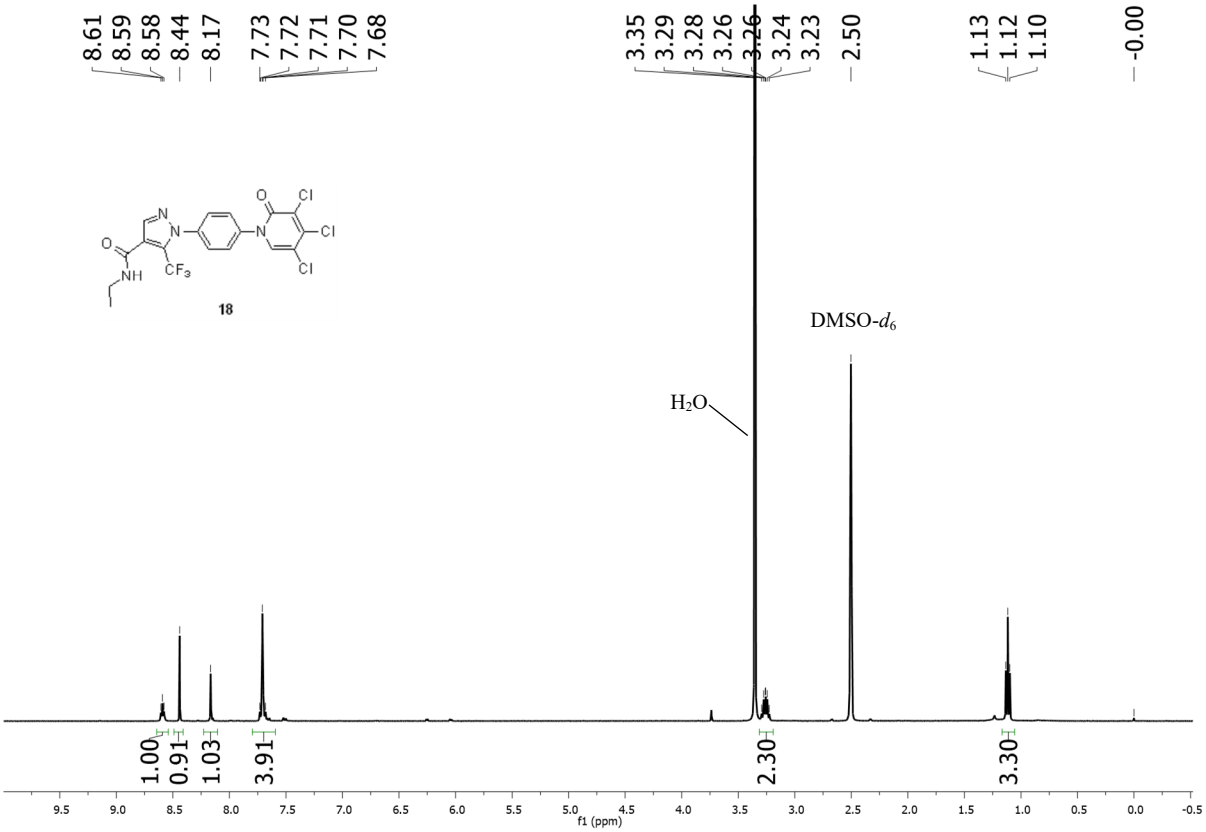
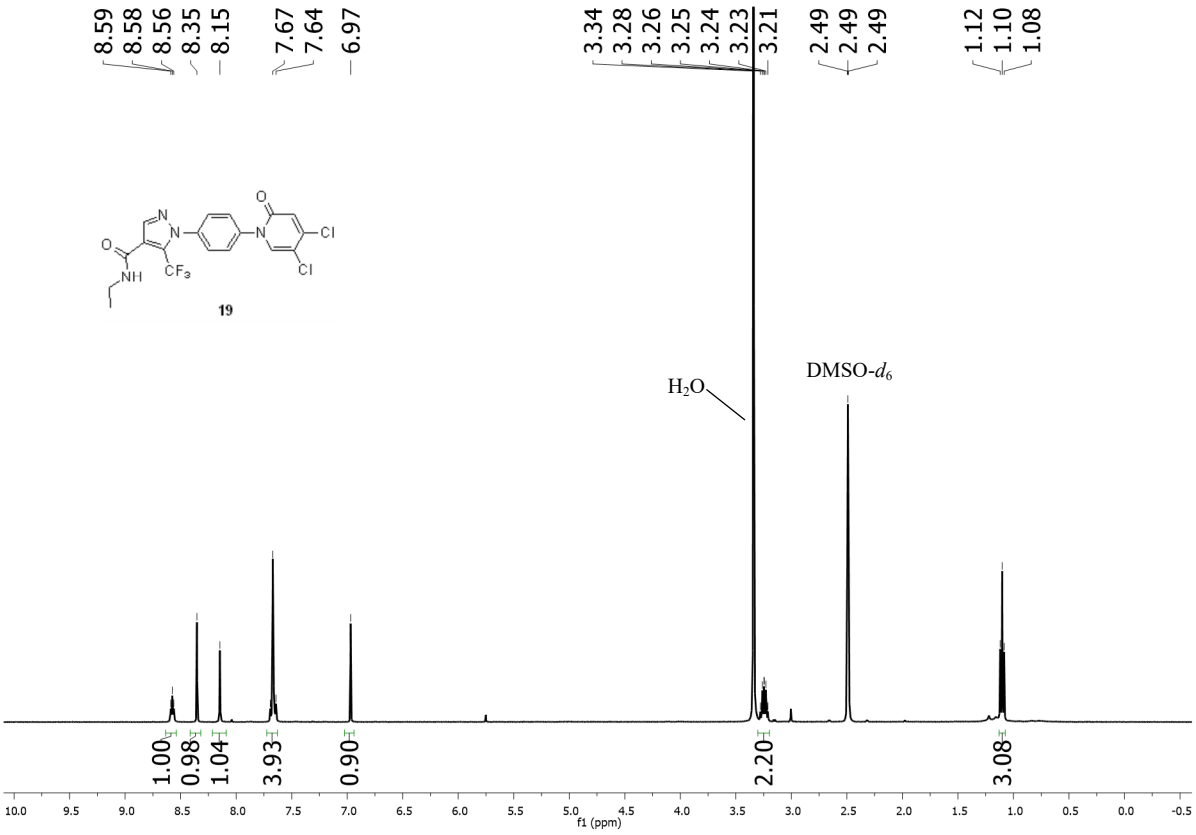
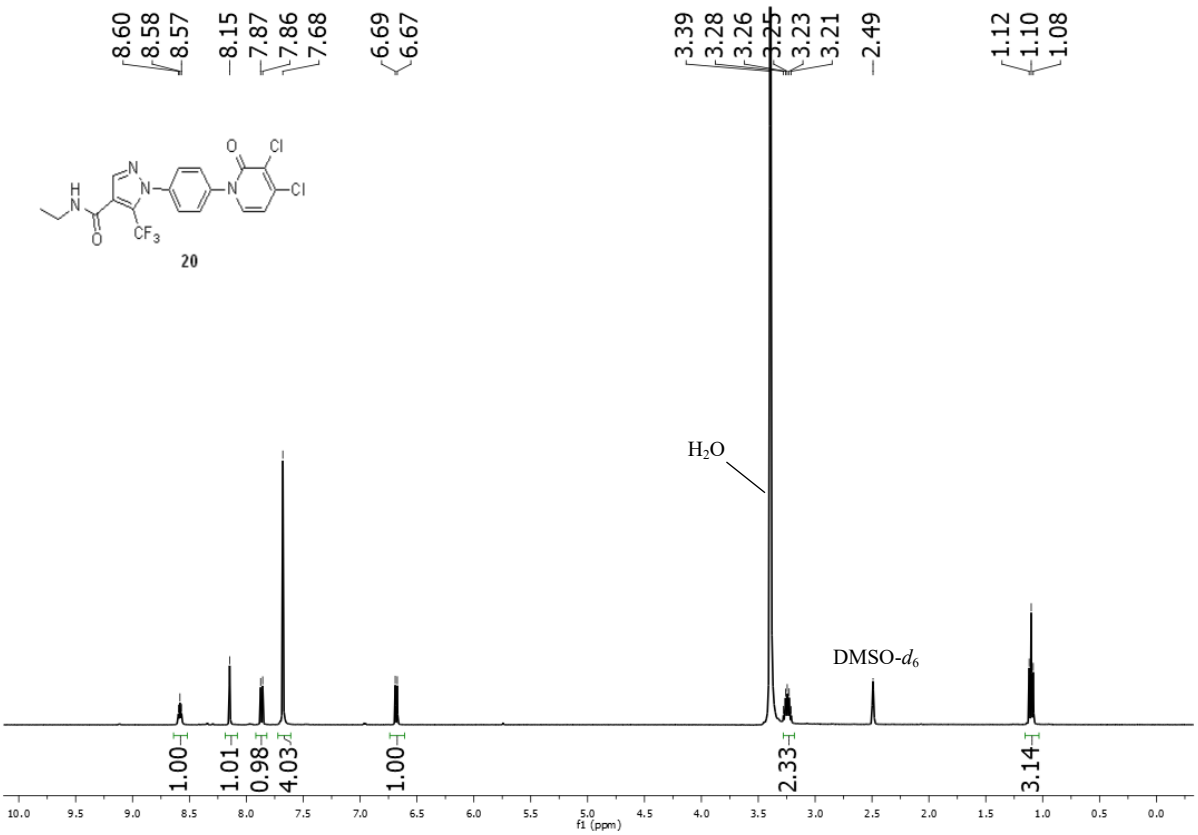
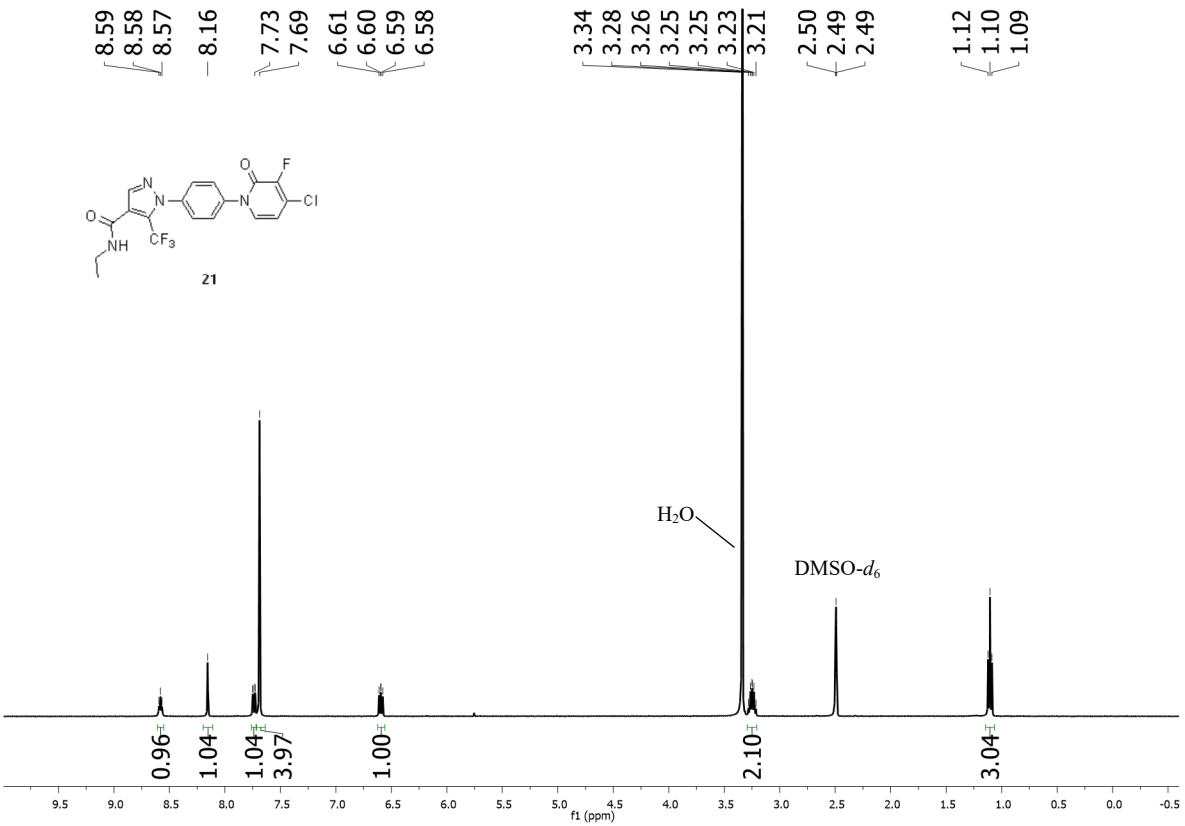
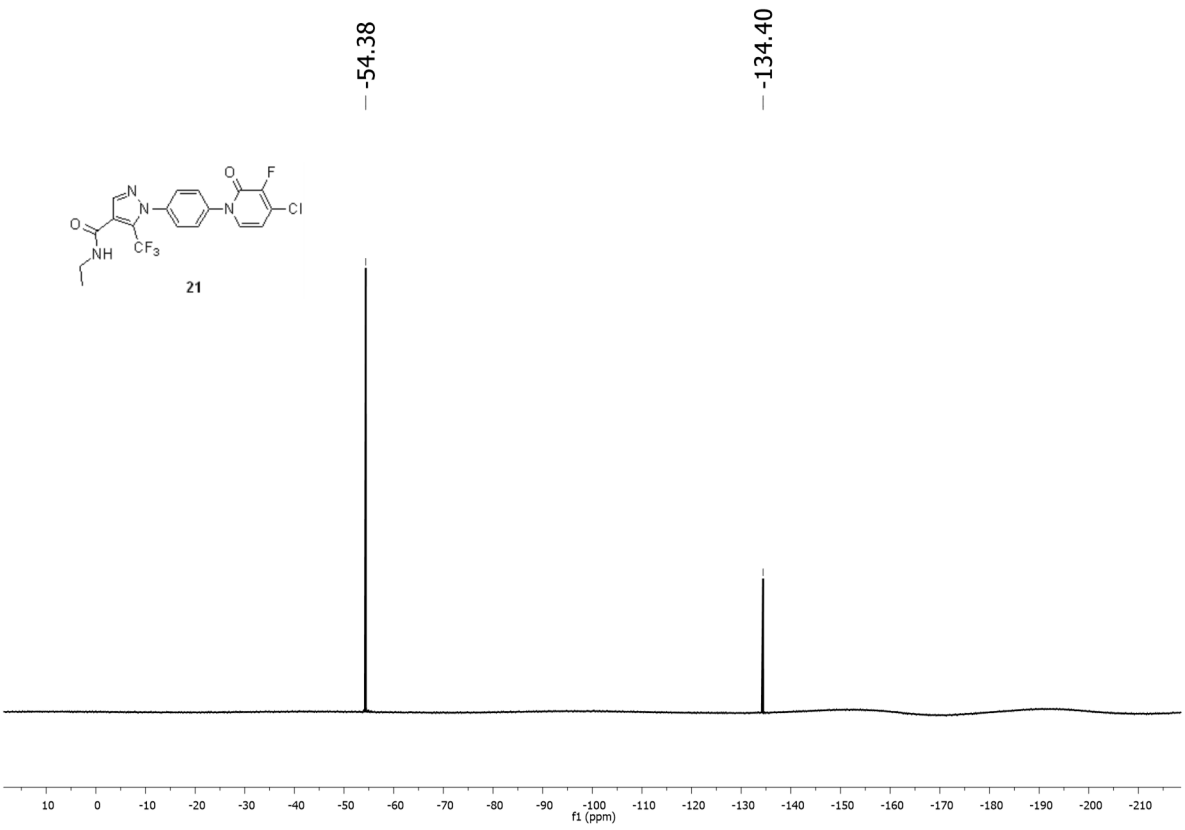
8.59
8.57
8.16
7.66
6.73
6.72
6.52
6.51
6.50
6.49
3.35
3.29
3.27
3.26
3.26
3.24
3.22
2.51
2.51
2.50
2.50
1.13
1.11
1.10
-0.00
17
DMSO-d_6
H_2O
1.00
0.98
0.97
3.89
0.91
1.00
2.07
2.90
f1 (ppm)

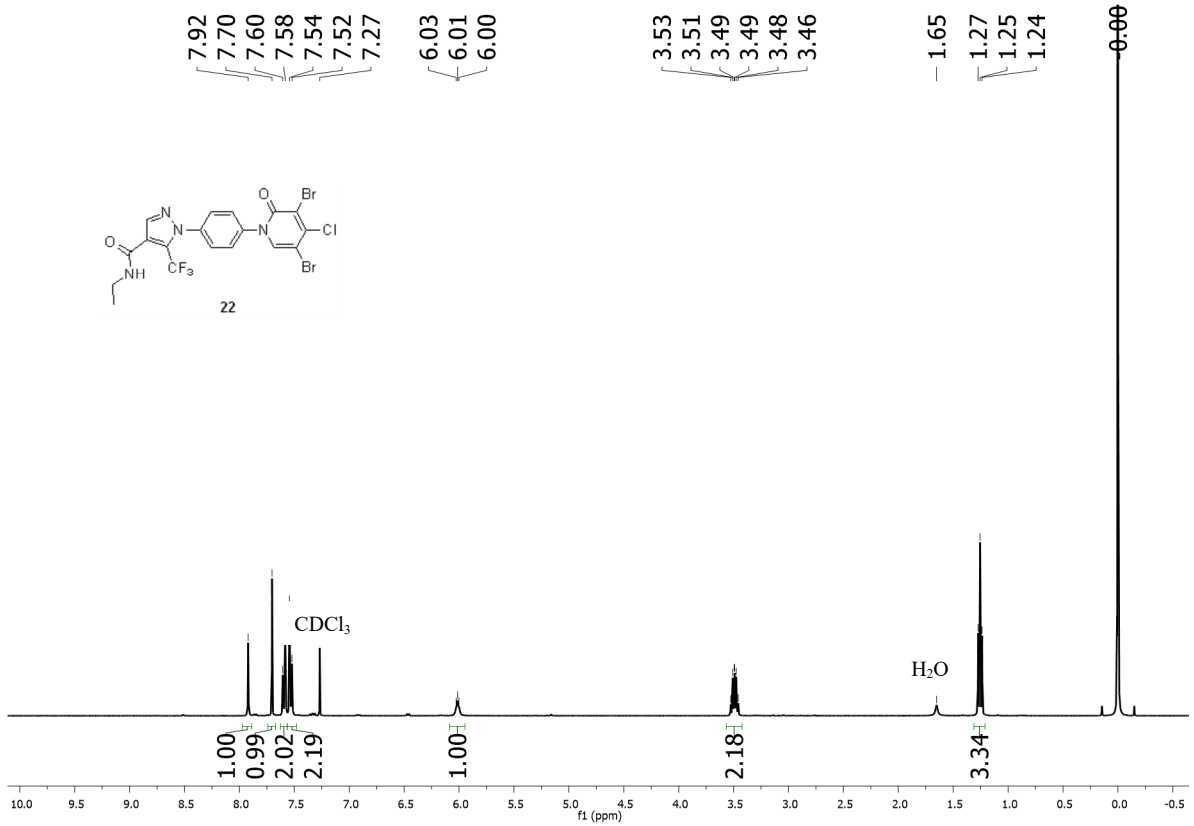

8.61
8.59
8.58
8.44
8.17
7.73
7.72
7.71
7.70
7.68
3.35
3.29
3.28
3.26
3.26
3.24
3.23
2.50
1.13
1.12
1.10
-0.00
18
DMSO-d_6
H_2O
1.00
0.91
1.03
3.91
2.30
3.30
f1 (ppm)

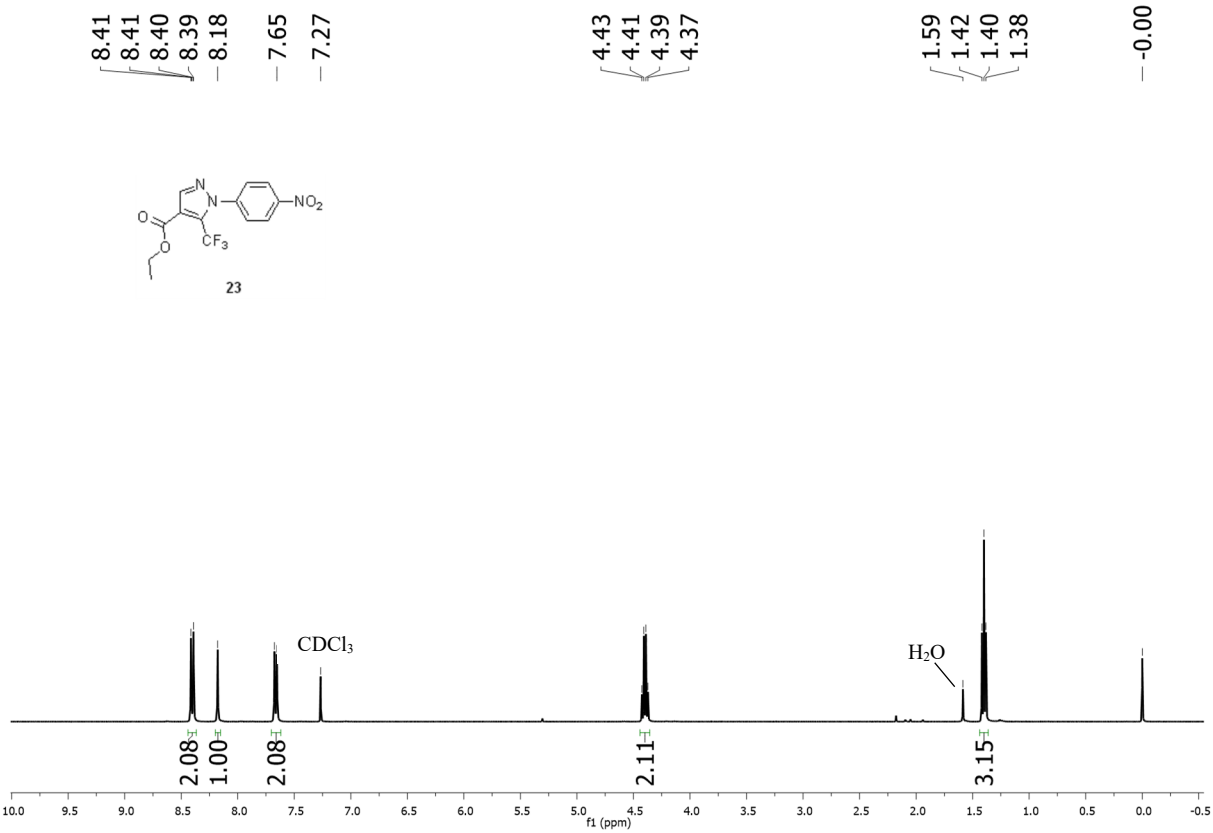

8.59
8.58
8.56
8.35
8.15
7.67
7.64
6.97
3.34
3.28
3.26
3.25
3.24
3.23
3.21
2.49
2.49
2.49
1.12
1.10
1.08
19
H2O
DMSO-d6
1.00
0.98
1.04
3.93
0.90
2.20
3.08
f1 (ppm)


8.60
8.58
8.57
8.15
7.87
7.86
7.68
6.69
6.67
3.39
3.28
3.26
3.25
3.23
3.21
2.49
1.12
1.10
1.08
20
H2O
DMSO-d6
1.00
1.01
0.98
4.03
1.00
2.33
3.14
f1 (ppm)

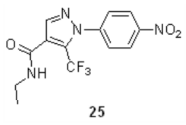

8.59
8.58
8.57
8.16
7.73
7.69
6.61
6.60
6.59
6.58
3.34
3.28
3.26
3.25
3.25
3.23
3.21
2.50
2.49
2.49
1.12
1.10
1.09
21
H2O
DMSO-d6
0.96
1.04
1.04
3.97
1.00
2.10
3.04
f1 (ppm)


-54.38
-134.40
21
f1 (ppm)

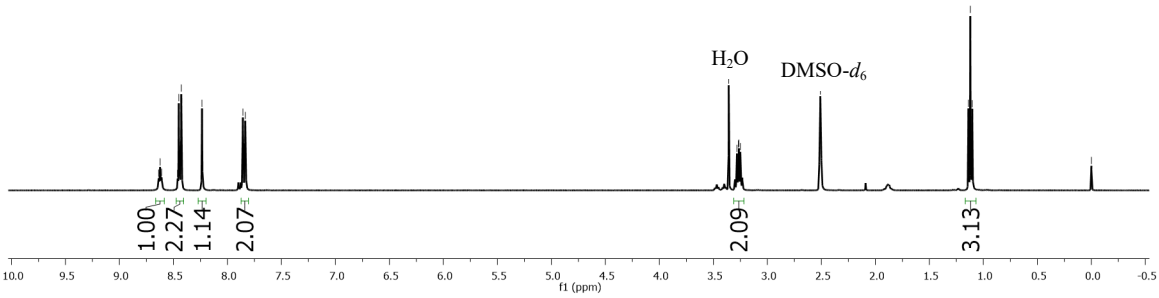

7.92
7.70
7.60
7.58
7.54
7.52
7.27
6.03
6.01
6.00
3.53
3.51
3.49
3.49
3.48
3.46
1.65
1.27
1.25
1.24
0.00
Br
O
Cl
N
N
O
NH
CF3
Br
22
CDCl3
H2O
1.00
0.99
2.02
2.19
1.00
2.18
3.34
f1 (ppm)

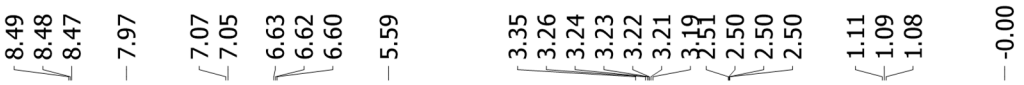

8.41
8.41
8.40
8.39
8.18
7.65
7.27
4.43
4.41
4.39
4.37
1.59
1.42
1.40
1.38
-0.00
N
N
NO2
O
O
CF3
23
CDCl3
H2O
2.08
1.00
2.08
2.11
3.15
f1 (ppm)

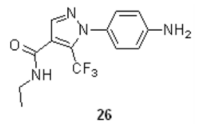

8.45
8.44
8.44
8.43
8.43
8.42
8.24
7.86
7.83
3.36
3.28
3.27
3.26
3.25
2.51
2.51
2.51
2.50
1.14
1.12
1.10
-0.00


O
N
N
NO2
NH
CF3

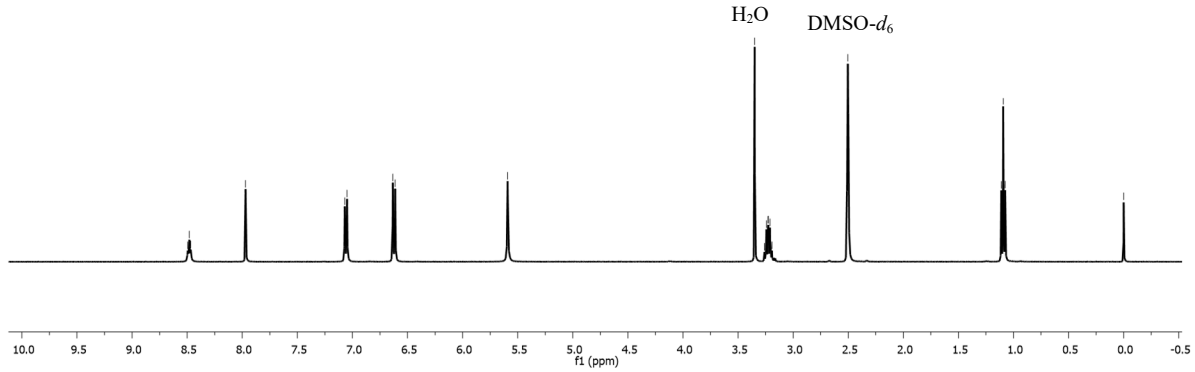
25

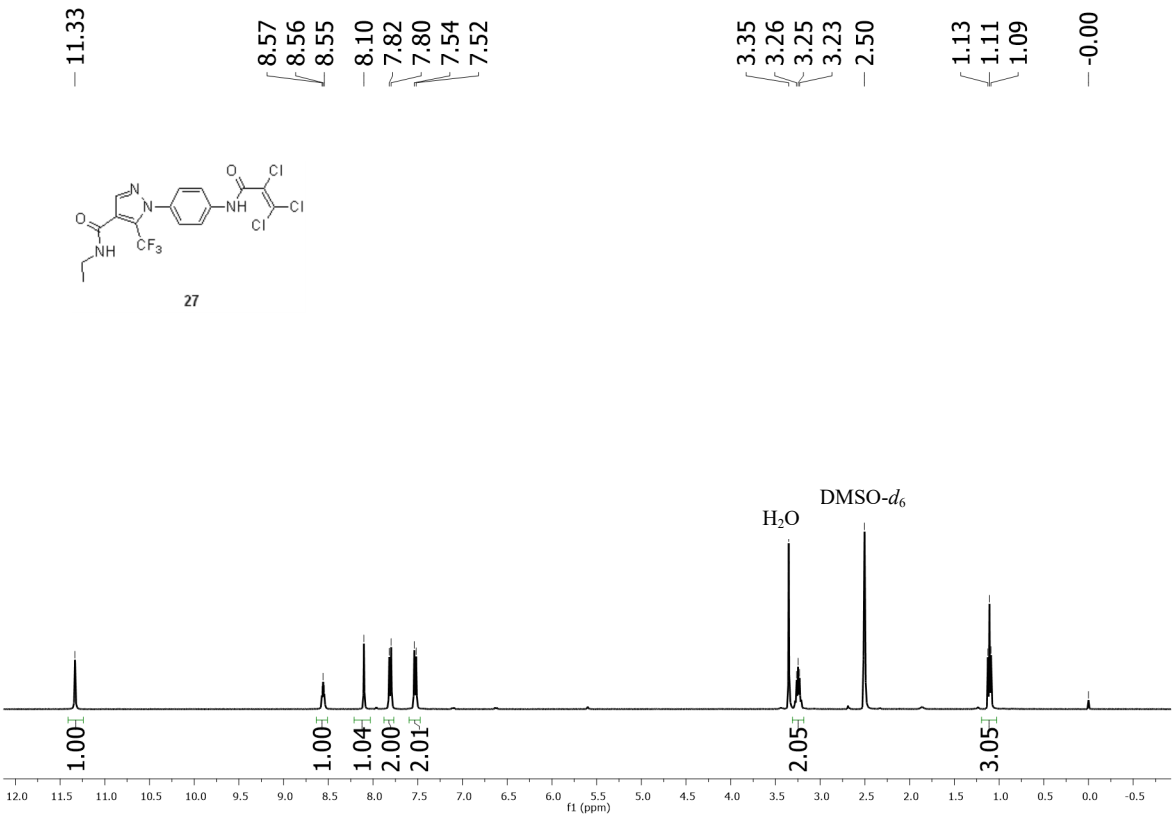

H2O
DMSO-d6
1.00
2.27
1.14
2.07
2.09
3.13
f1 (ppm)

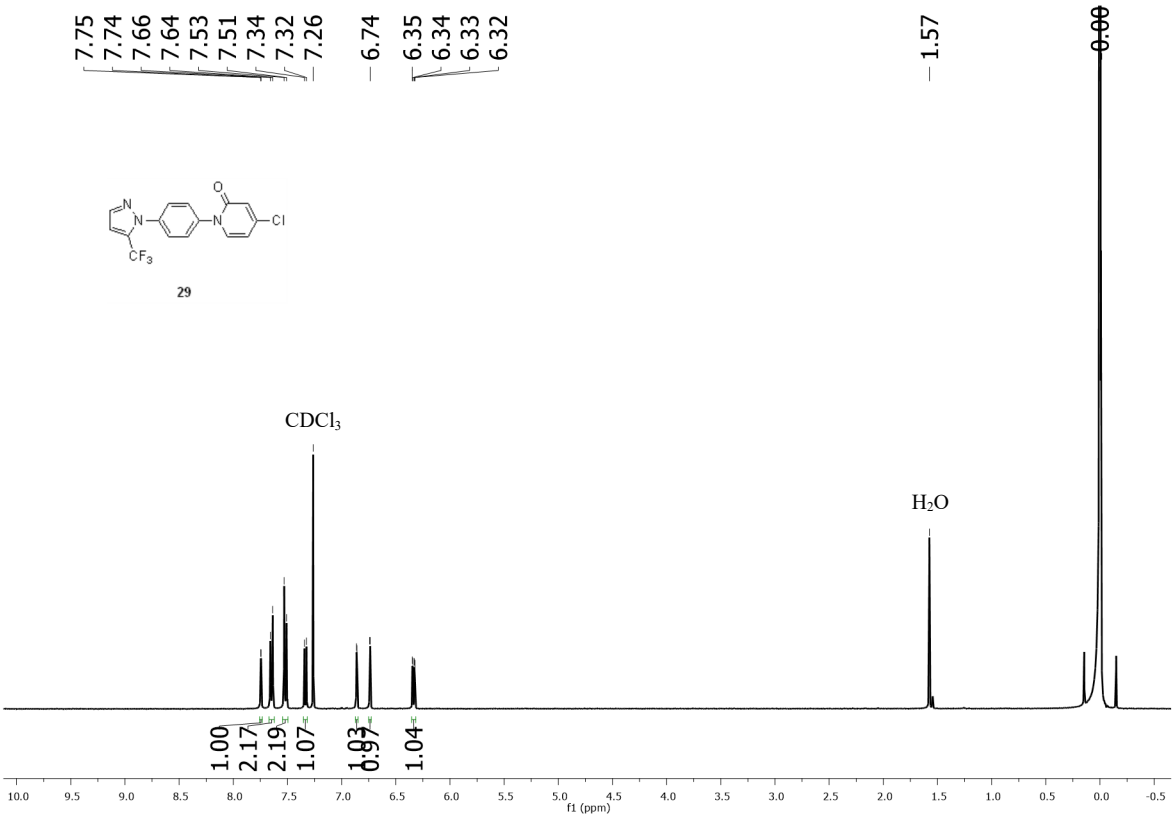

8.49
8.48
8.47
7.97
7.07
7.05
6.63
6.62
6.60
5.59
3.35
3.26
3.24
3.23
3.22
3.21
3.19
2.51
2.50
2.50
2.50
1.11
1.09
1.08
-0.00

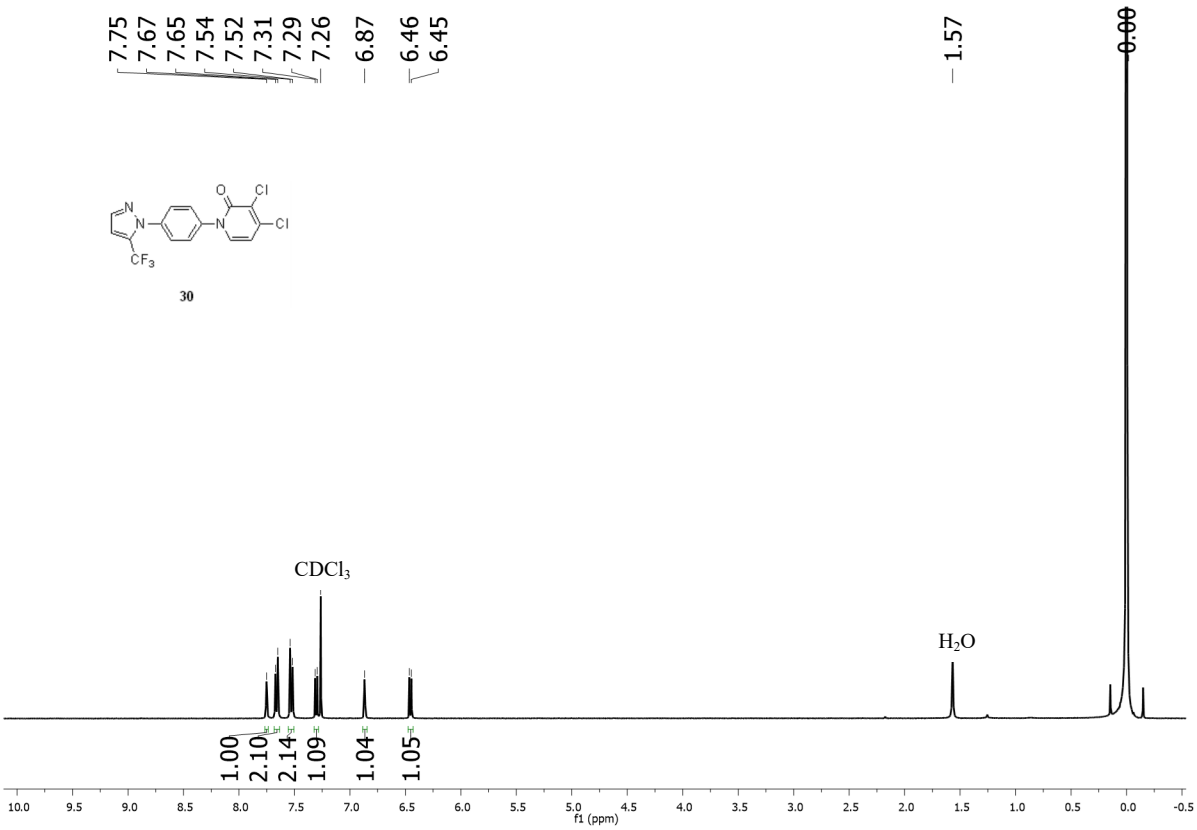

O
N
N
NH2
NH
CF3

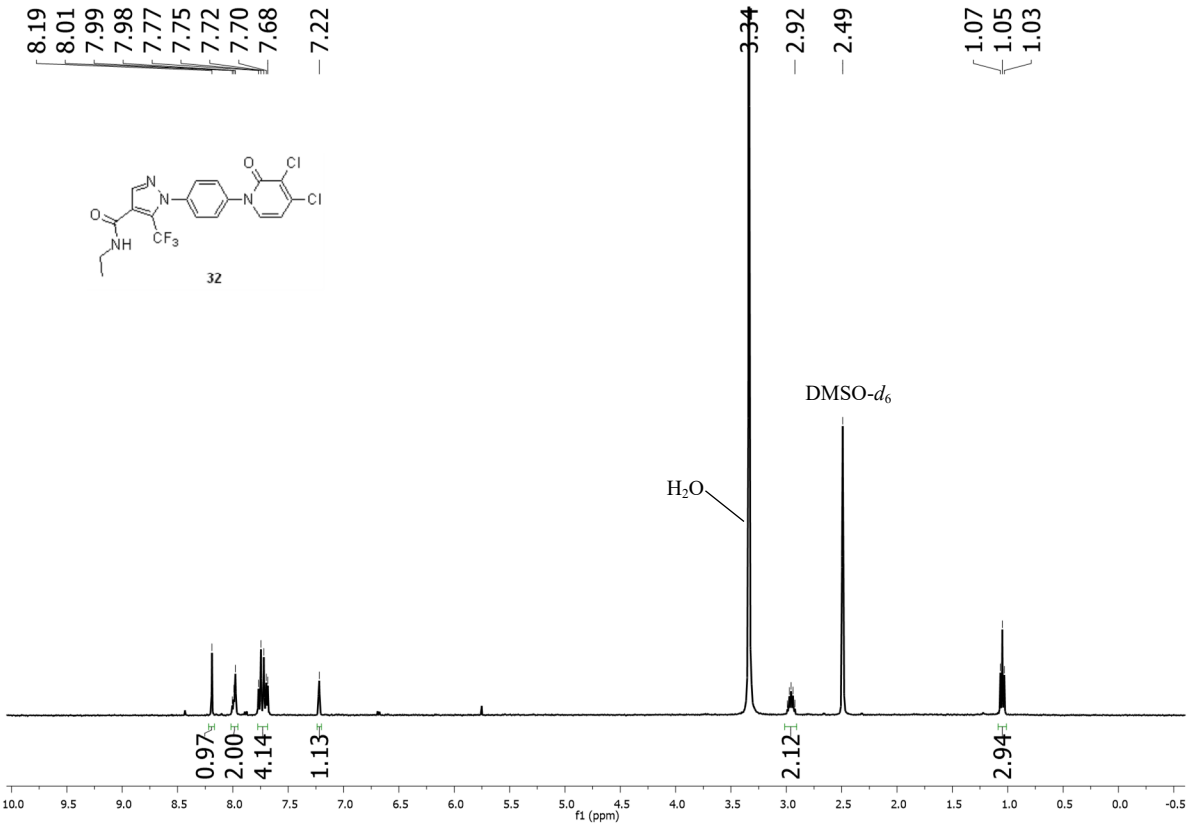
26

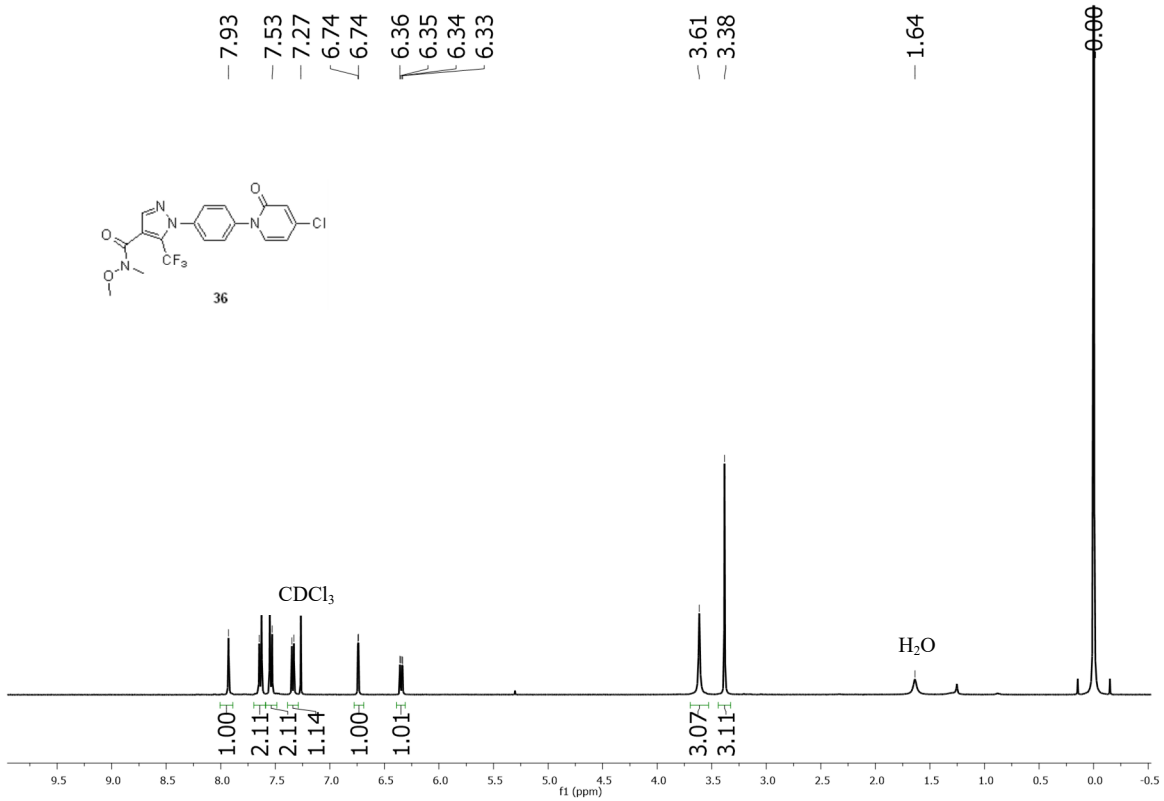

H2O
DMSO-d6
f1 (ppm)

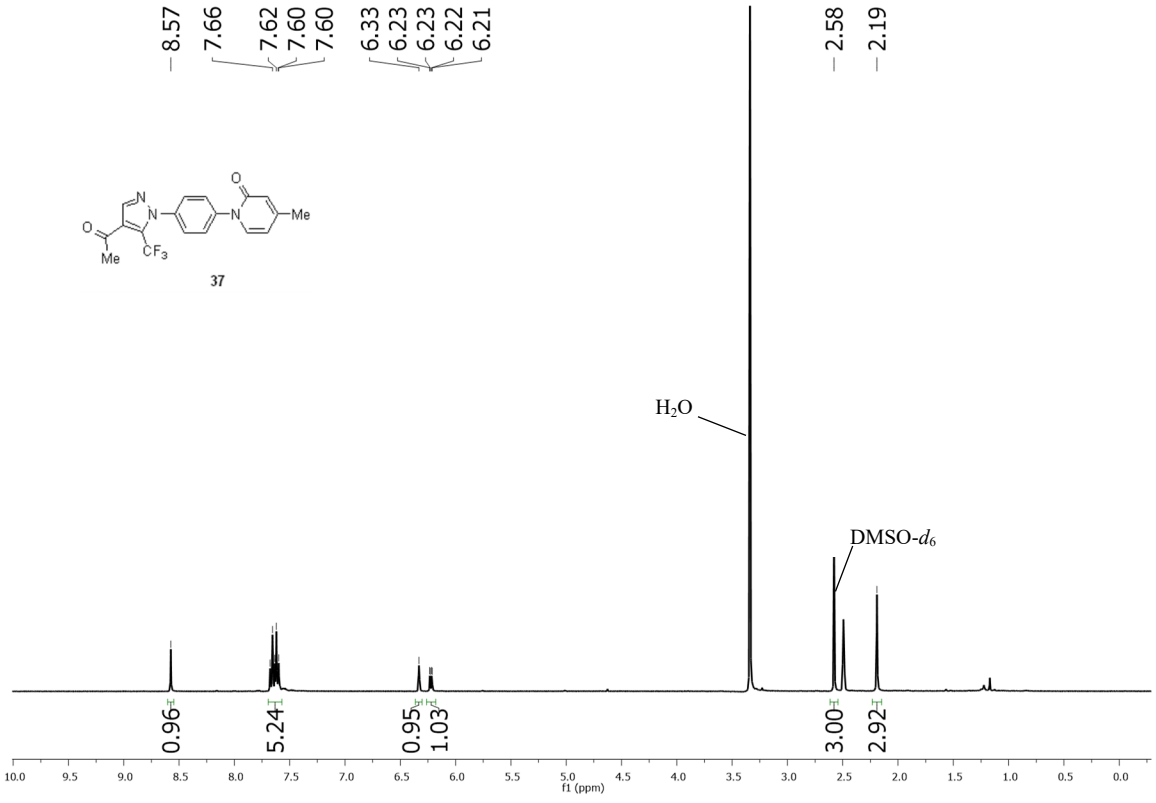

11.33
8.57
8.56
8.55
8.10
7.82
7.80
7.54
7.52
3.35
3.26
3.25
3.23
2.50
1.13
1.11
1.09
-0.00
27
DMSO-d_6
H_2O
1.00
1.00
1.04
2.00
2.01
2.05
3.05
f1 (ppm)

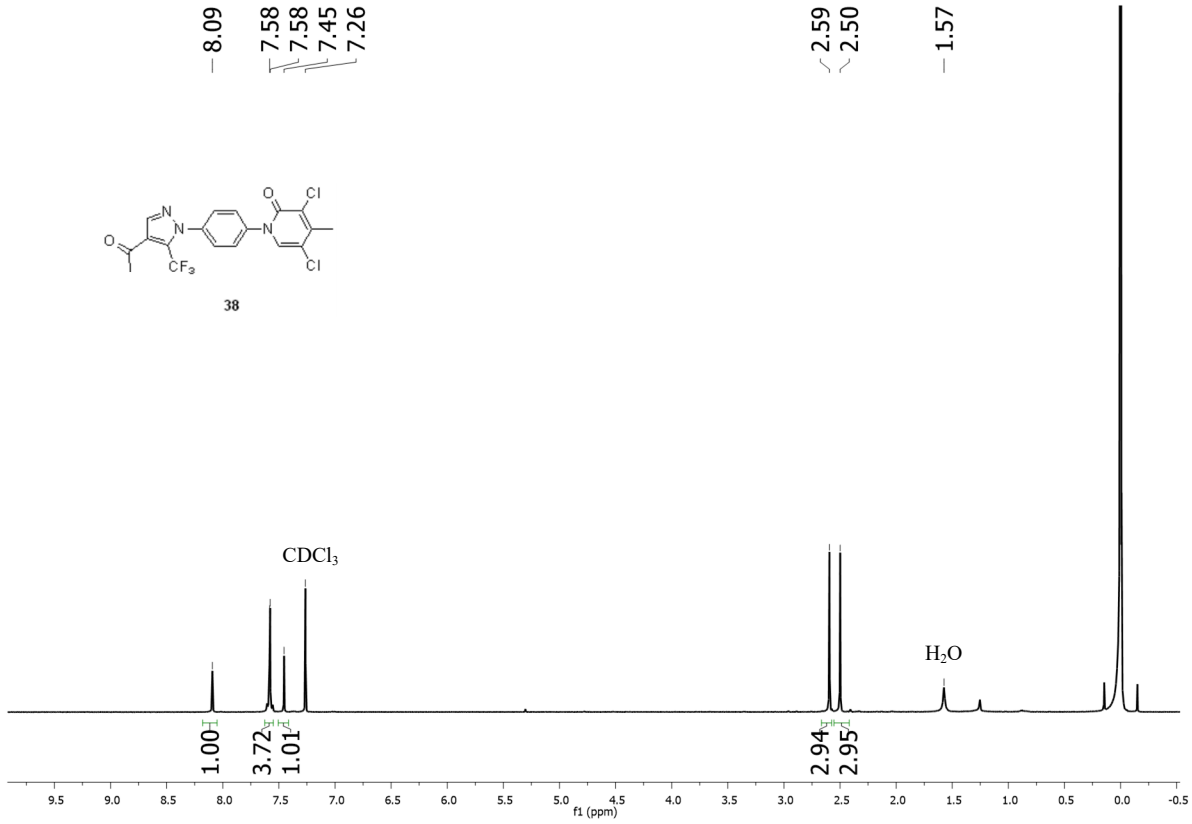

7.75
7.74
7.66
7.64
7.53
7.51
7.34
7.32
7.26
6.74
6.35
6.34
6.33
6.32
1.57
0.00
29
$CDCl_3$
H_2O
1.00
2.17
2.19
1.07
1.03
0.97
1.04
f1 (ppm)


7.75
7.67
7.65
7.54
7.52
7.31
7.29
7.26
6.87
6.46
6.45
1.57
0.00
30
CDCl3
H2O
1.00
2.10
2.14
1.09
1.04
1.05
f1 (ppm)

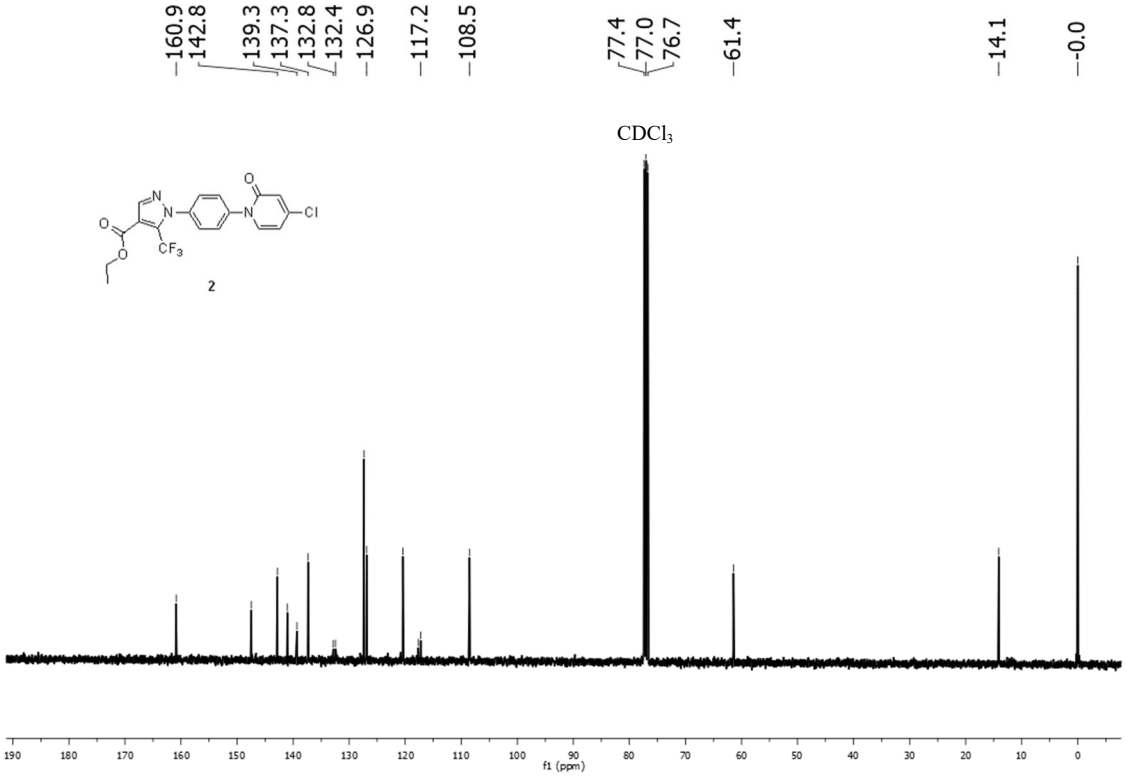

8.19
8.01
7.99
7.98
7.77
7.75
7.72
7.70
7.68
7.22
3.34
2.92
2.49
1.07
1.05
1.03
32
DMSO-d6
H2O
0.97
2.00
4.14
1.13
2.12
2.94
f1 (ppm)

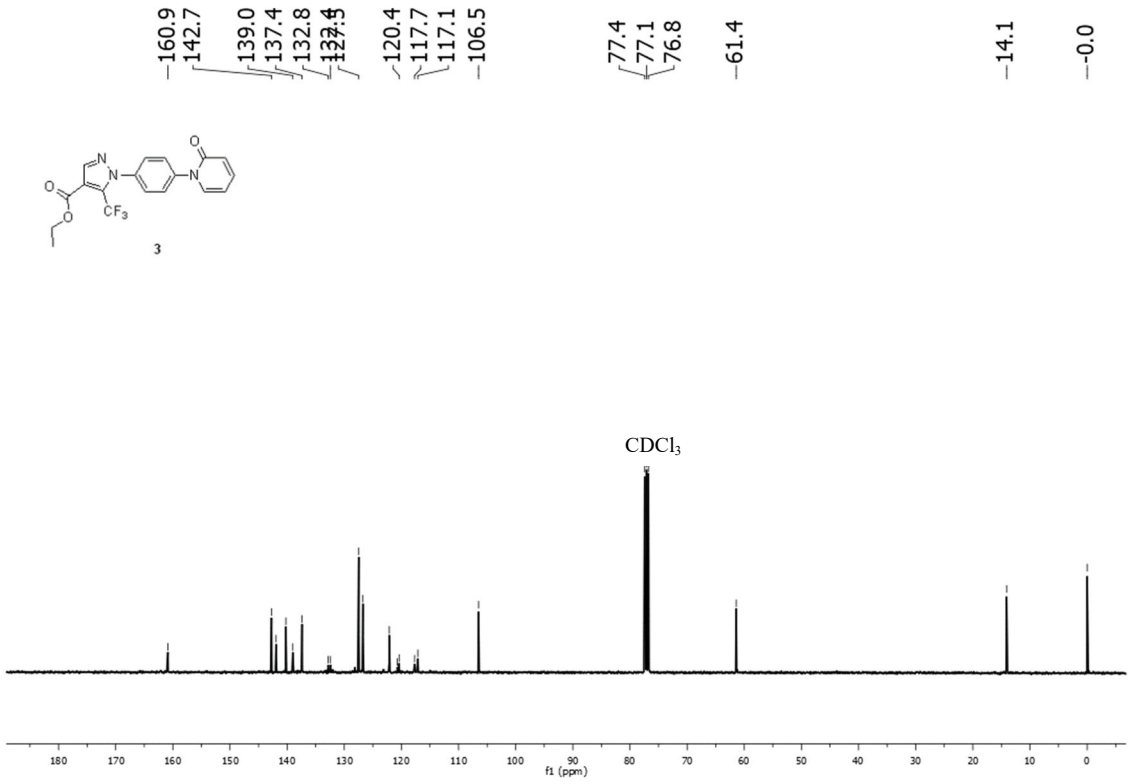

7.93
7.53
7.27
6.74
6.74
6.36
6.35
6.34
6.33
3.61
3.38
1.64
0.00
36
CDCl3
H2O
1.00
2.11
2.11
1.14
1.00
1.01
3.07
3.11
f1 (ppm)

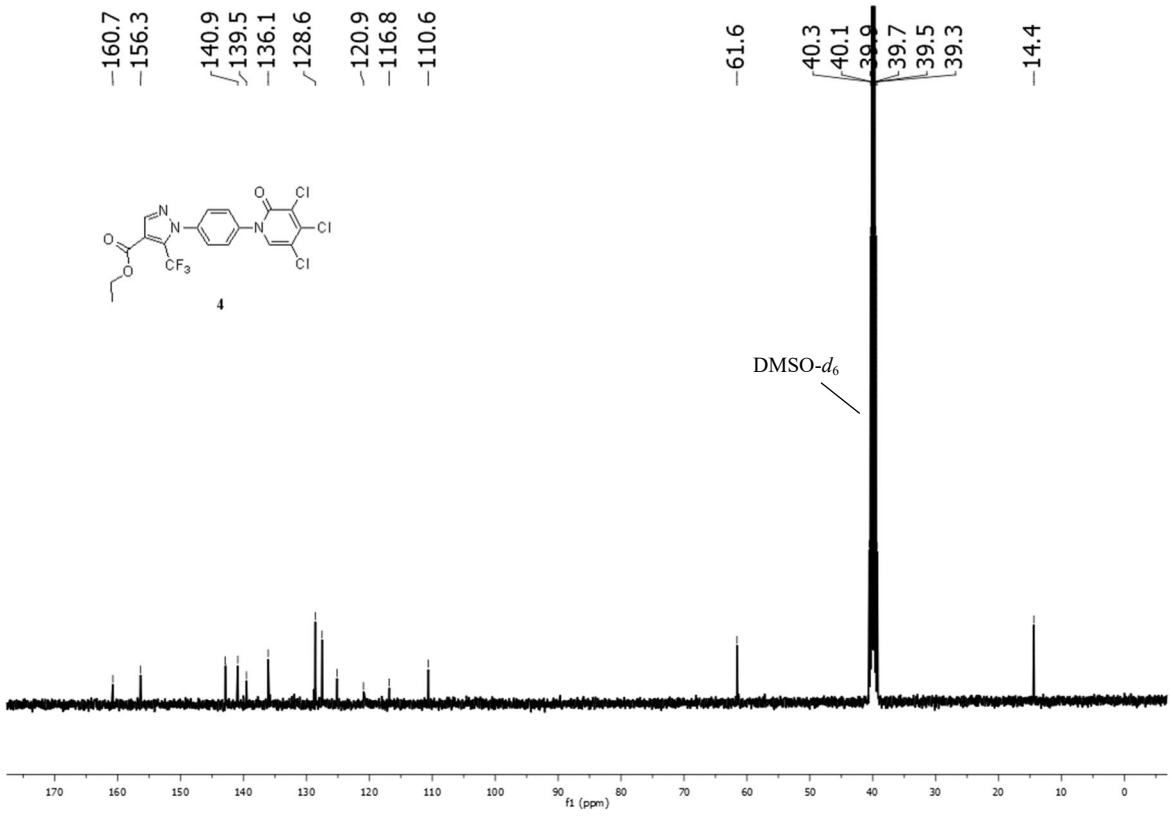

8.57
7.66
7.62
7.60
7.60
6.33
6.23
6.23
6.22
6.21
2.58
2.19
37
H2O
DMSO-d6
0.96
5.24
0.95
1.03
3.00
2.92
f1 (ppm)


8.09
7.58
7.58
7.45
7.26
2.59
2.50
1.57
38
CDCl3
H2O
1.00
3.72
1.01
2.94
2.95
f1 (ppm)

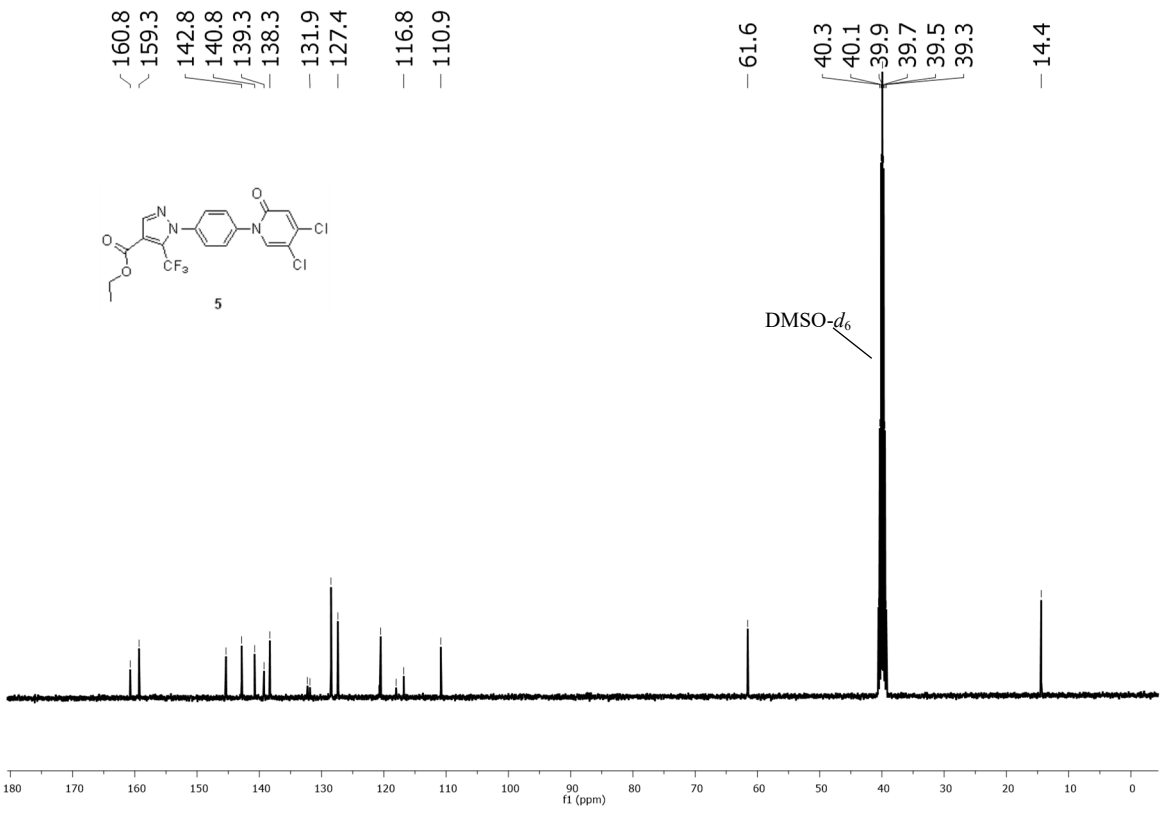
^{13}C NMR data for the key pyrazole compounds



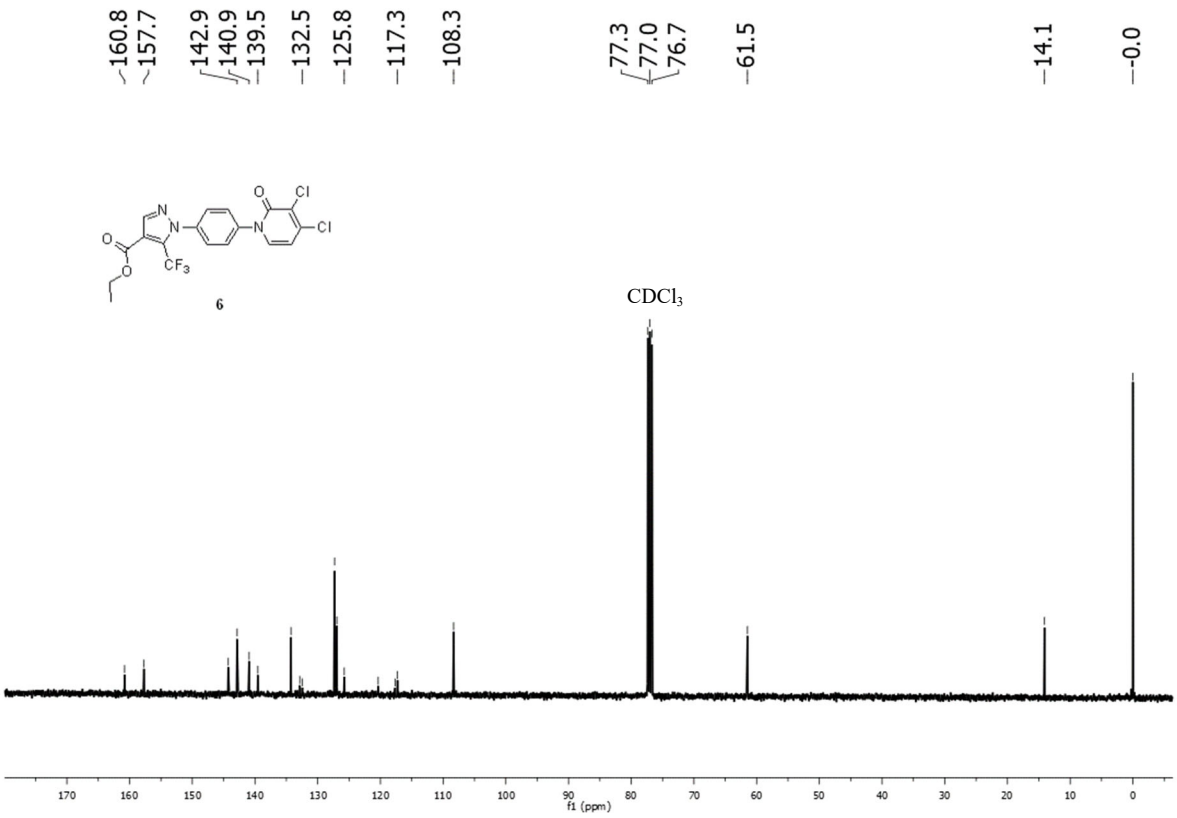


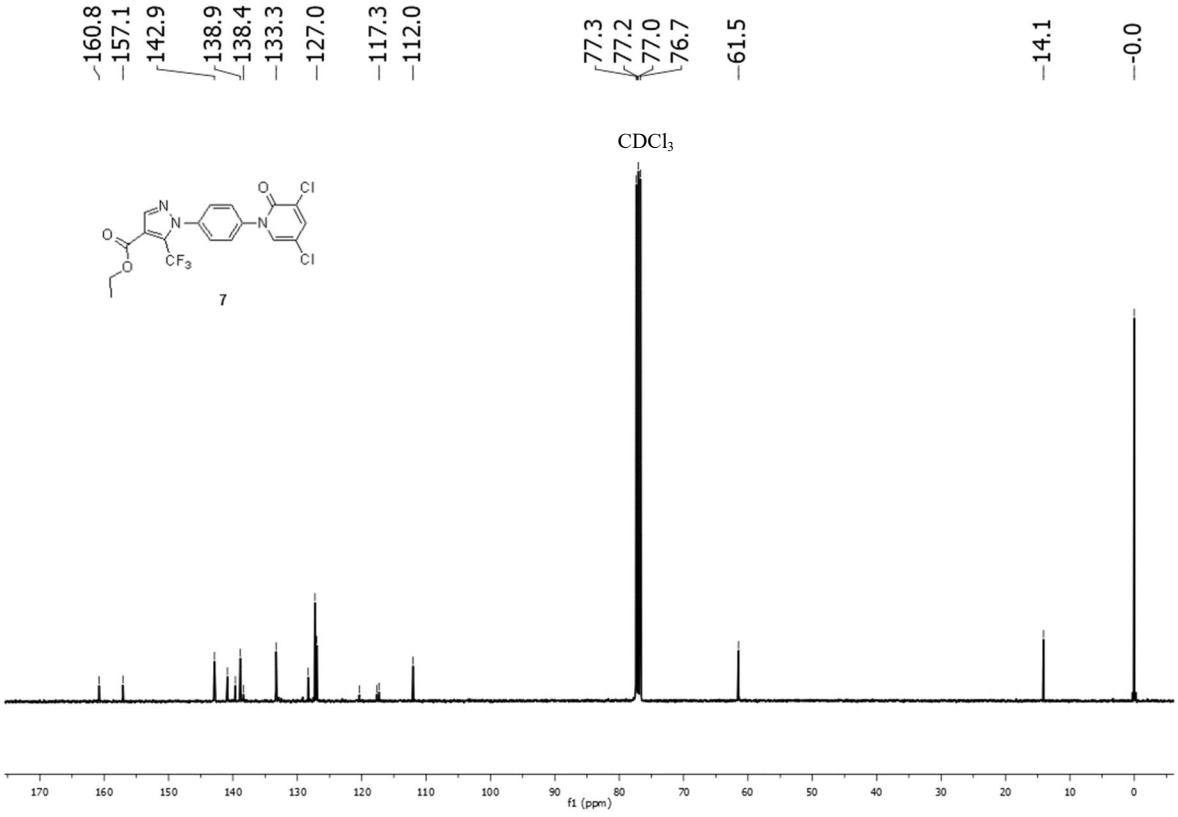

160.7
156.3
140.9
139.5
136.1
128.6
120.9
116.8
110.6
61.6
40.3
40.1
39.9
39.7
39.5
39.3
14.4
DMSO-d_6
f1 (ppm)

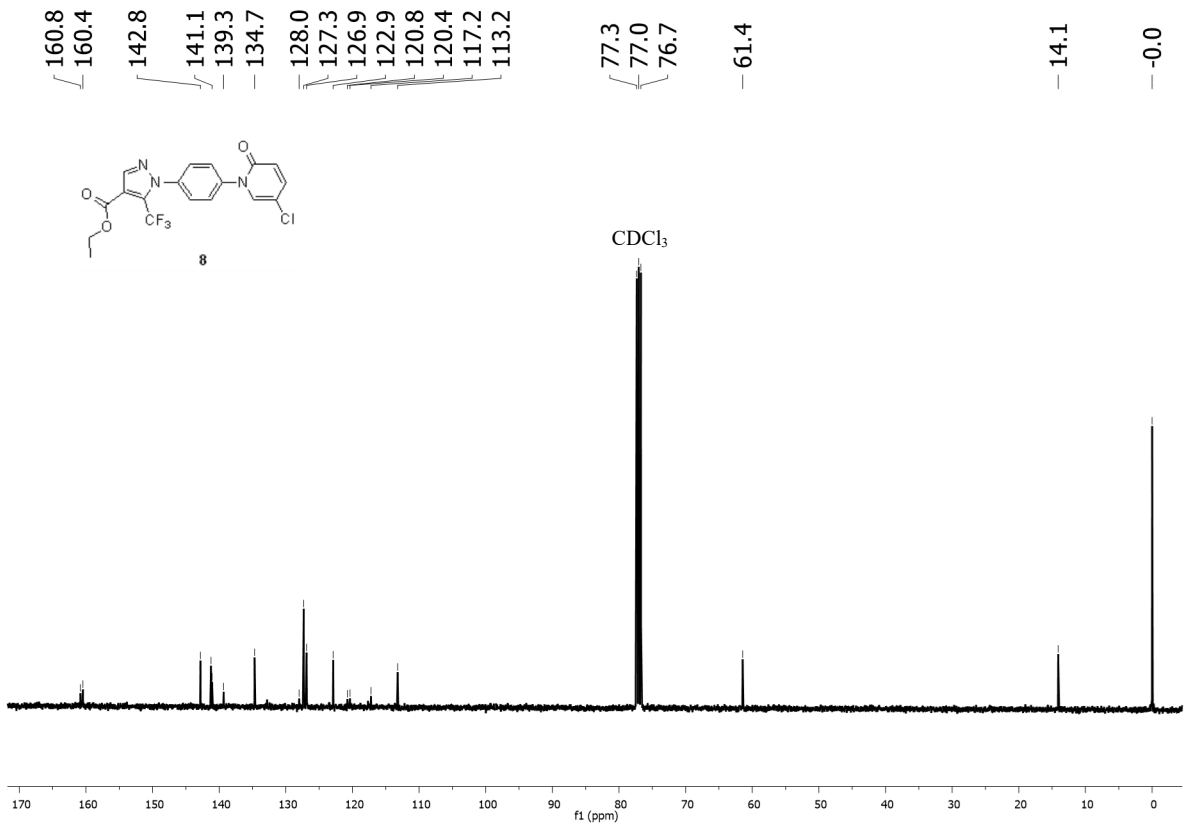
4

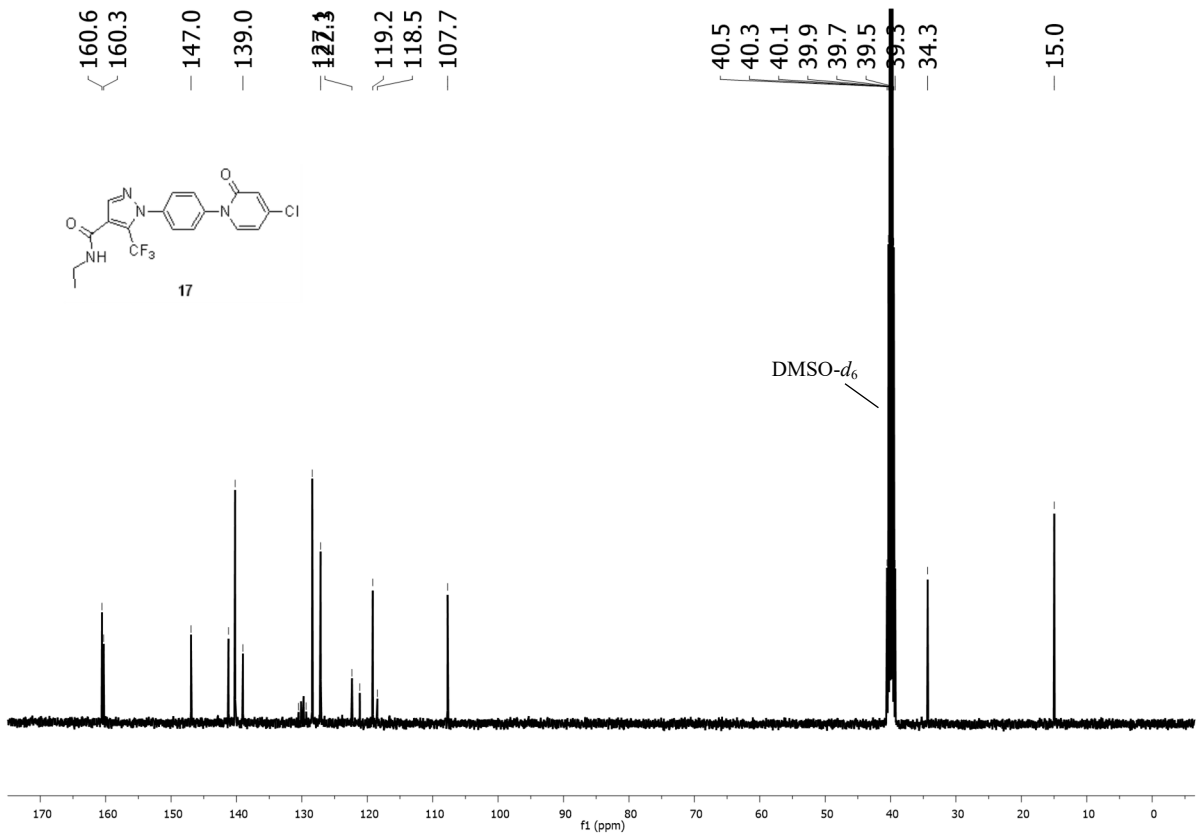

160.8
159.3
142.8
140.8
139.3
138.3
131.9
127.4
116.8
110.9
61.6
40.3
40.1
39.9
39.7
39.5
39.3
14.4
DMSO-d_6
f1 (ppm)

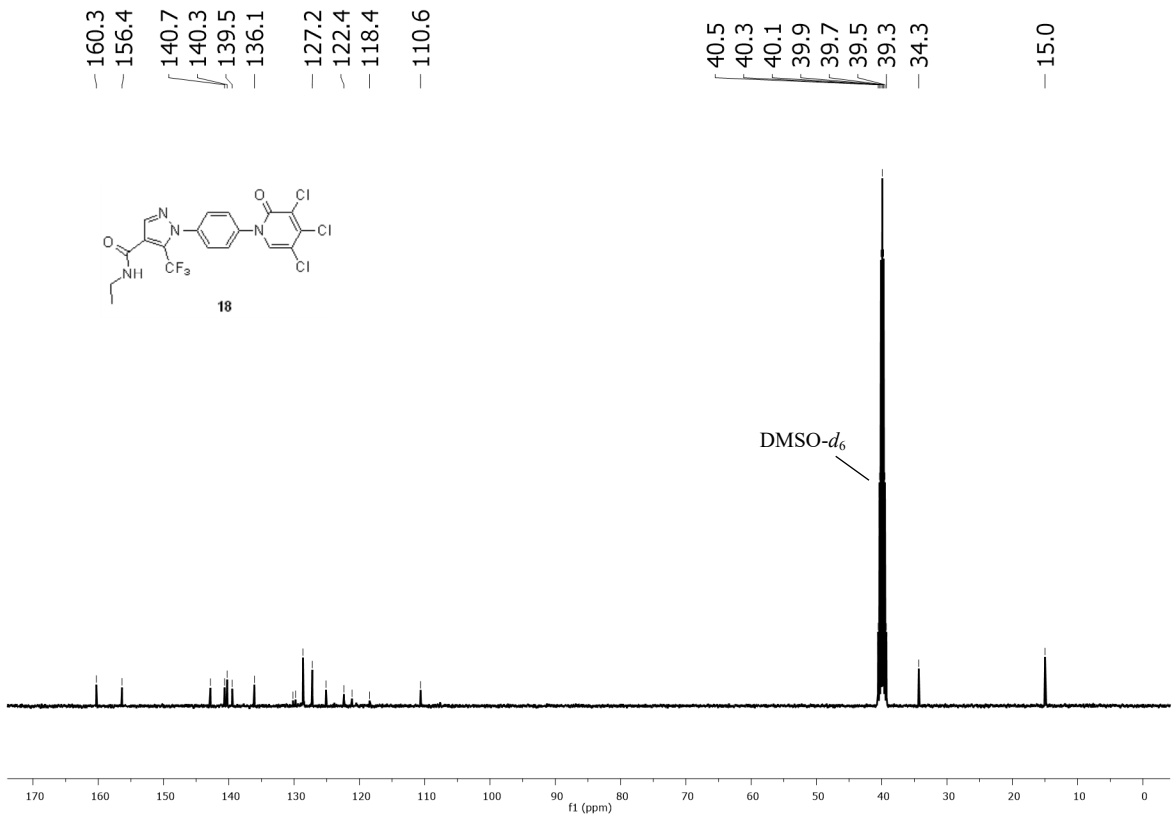
5

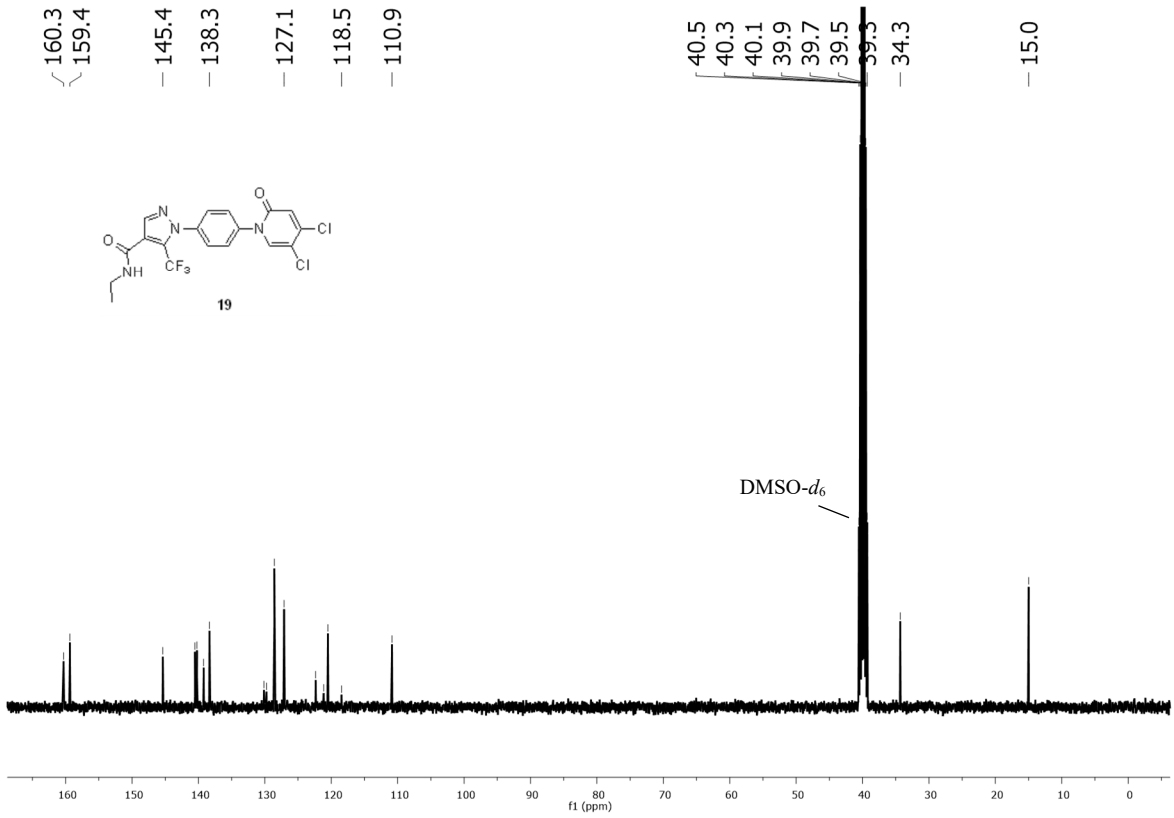

160.8
157.7
142.9
140.9
139.5
132.5
125.8
117.3
108.3
77.3
77.0
76.7
61.5
14.1
0.0
CDCl3
6
f1 (ppm)

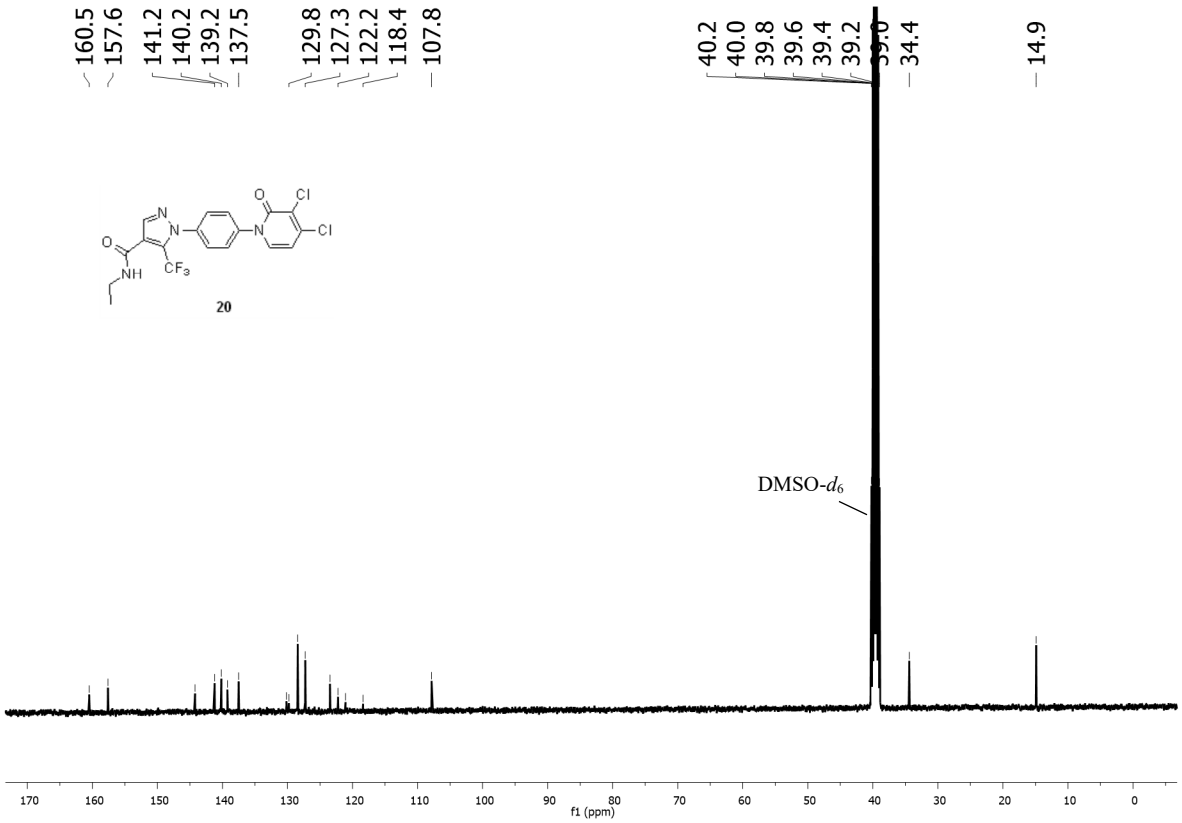

160.8
157.1
142.9
138.9
138.4
133.3
127.0
117.3
112.0
77.3
77.2
77.0
76.7
61.5
14.1
0.0
CDCl3
7
f1 (ppm)


160.8
160.4
142.8
141.1
139.3
134.7
128.0
127.3
126.9
122.9
120.8
120.4
117.2
113.2
77.3
77.0
76.7
61.4
14.1
-0.0
CDCl3
8
f1 (ppm)

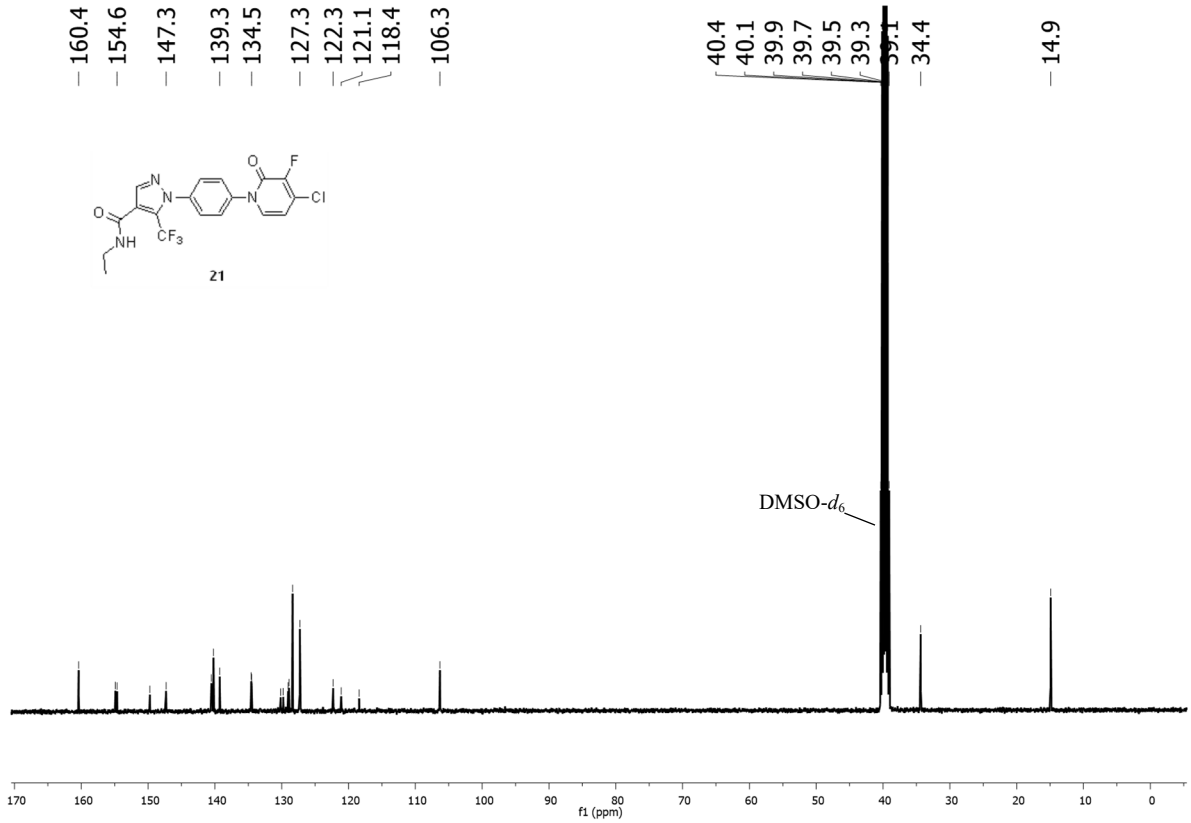

160.6
160.3
147.0
139.0
119.2
118.5
107.7
40.5
40.3
40.1
39.9
39.7
39.5
34.3
15.0
DMSO-d6
17
f1 (ppm)


160.3
156.4
140.7
140.3
139.5
136.1
127.2
122.4
118.4
110.6
40.5
40.3
40.1
39.9
39.7
39.5
39.3
34.3
15.0
18
DMSO-d_6
f1 (ppm)


160.3
159.4
145.4
138.3
127.1
118.5
110.9
40.5
40.3
40.1
39.9
39.7
39.5
39.3
34.3
15.0
19
DMSO-d_6
f1 (ppm)


160.5
157.6
141.2
140.2
139.2
137.5
129.8
127.3
122.2
118.4
107.8
40.2
40.0
39.8
39.6
39.4
39.2
39.0
34.4
14.9
DMSO-d_6
f1 (ppm)

20


160.4
154.6
147.3
139.3
134.5
127.3
122.3
121.1
118.4
106.3
40.4
40.1
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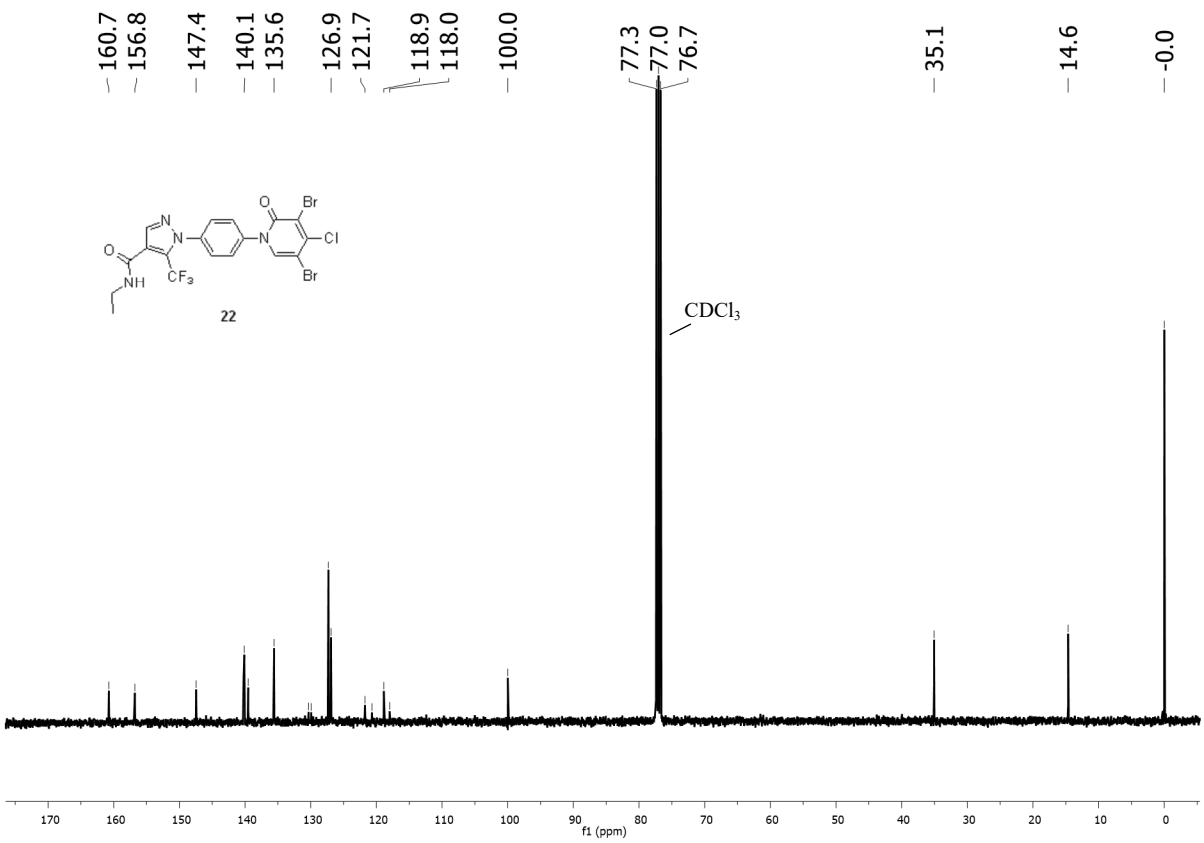
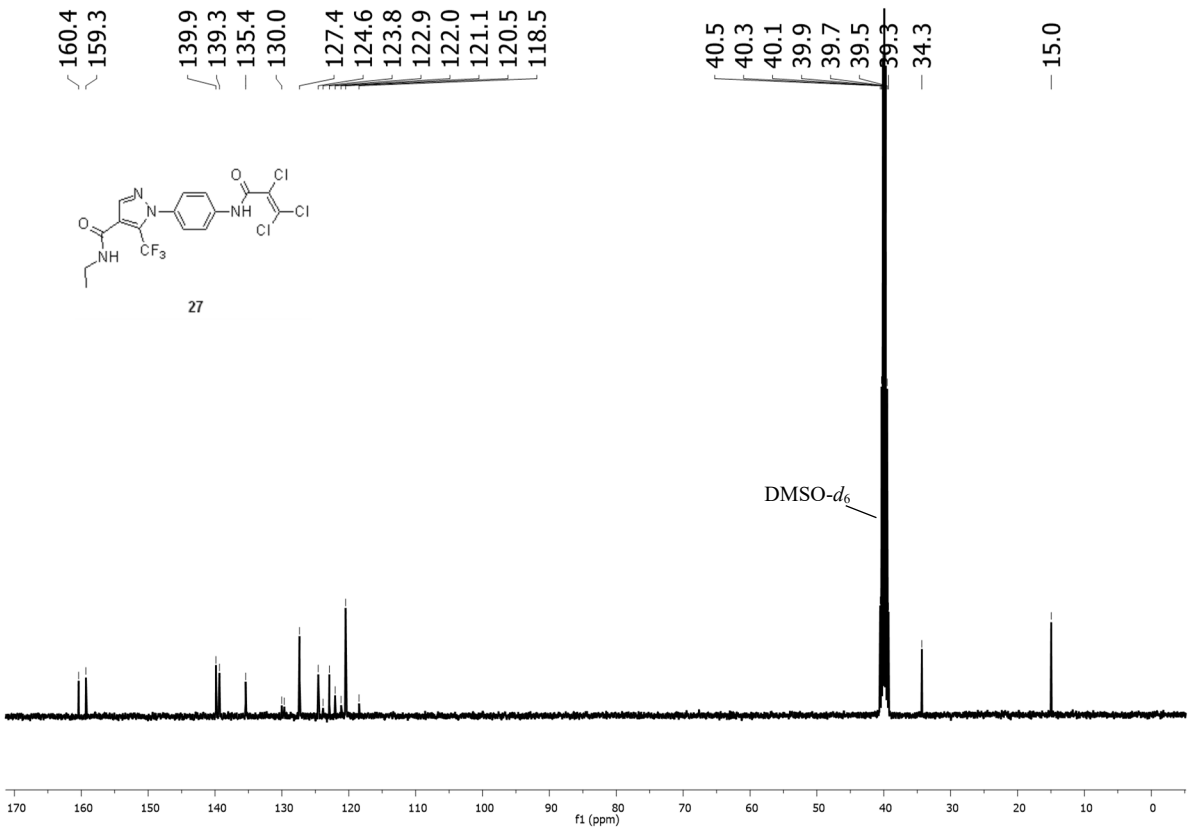
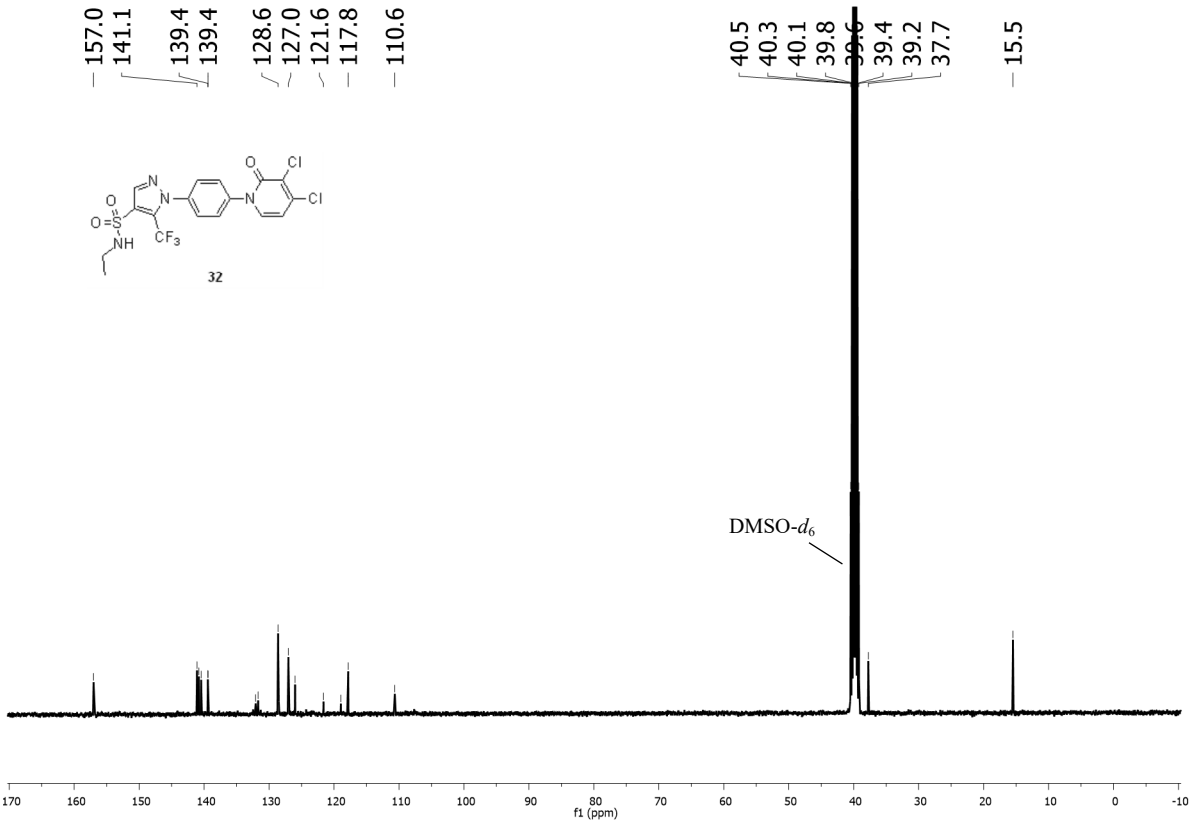
34.4
14.9
DMSO-d_6
f1 (ppm)

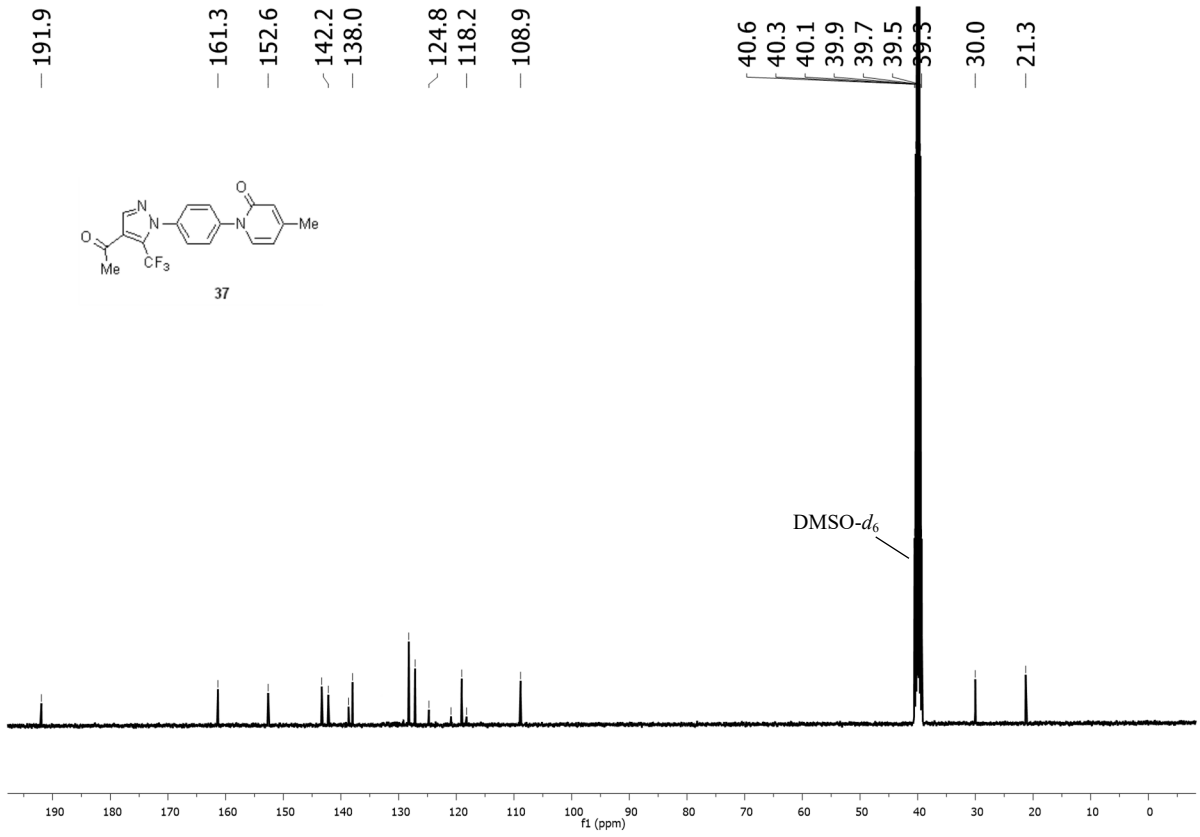
21


160.7
156.8
147.4
140.1
135.6
126.9
121.7
118.9
118.0
100.0
77.3
77.0
76.7
35.1
14.6
-0.0
CDCl3
22
f1 (ppm)

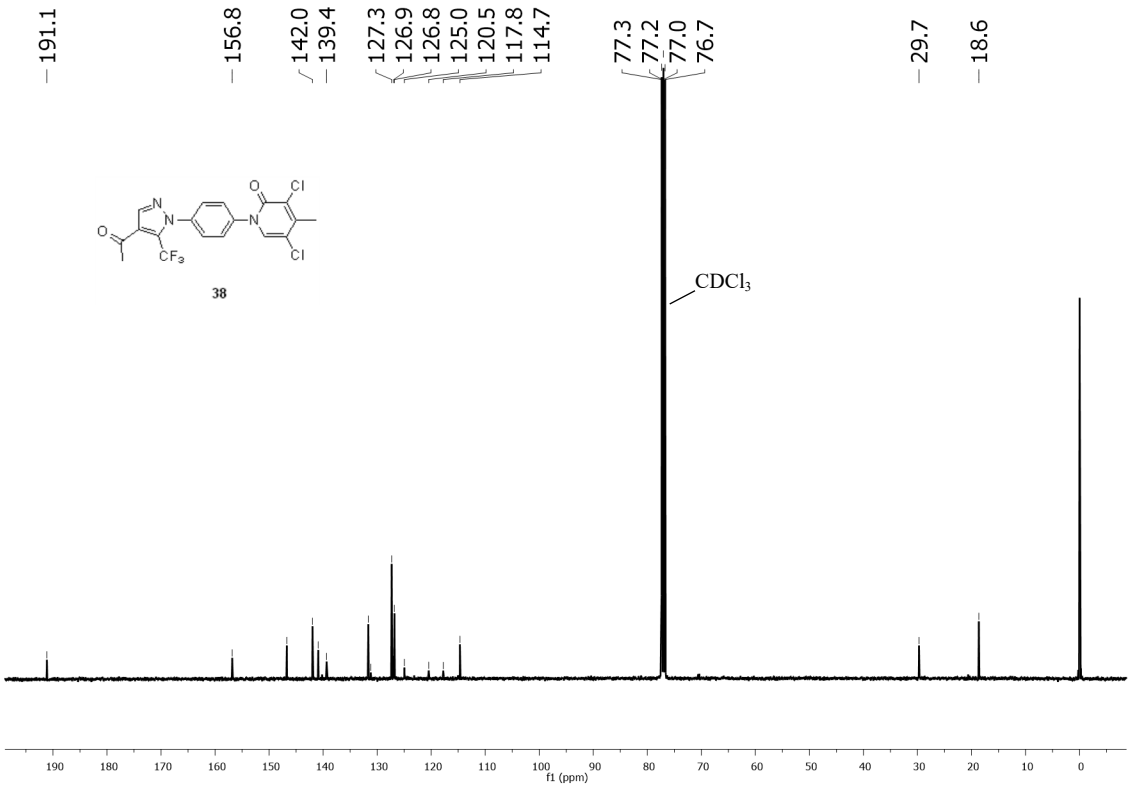

160.4
159.3
139.9
139.3
135.4
130.0
127.4
124.6
123.8
122.9
122.0
121.1
120.5
118.5
40.5
40.3
40.1
39.9
39.7
39.5
39.3
34.3
15.0
DMSO-d6
27
f1 (ppm)


157.0
141.1
139.4
139.4
128.6
127.0
121.6
117.8
110.6
40.5
40.3
40.1
39.8
39.6
39.4
39.2
37.7
15.5
DMSO-d_6
f1 (ppm)

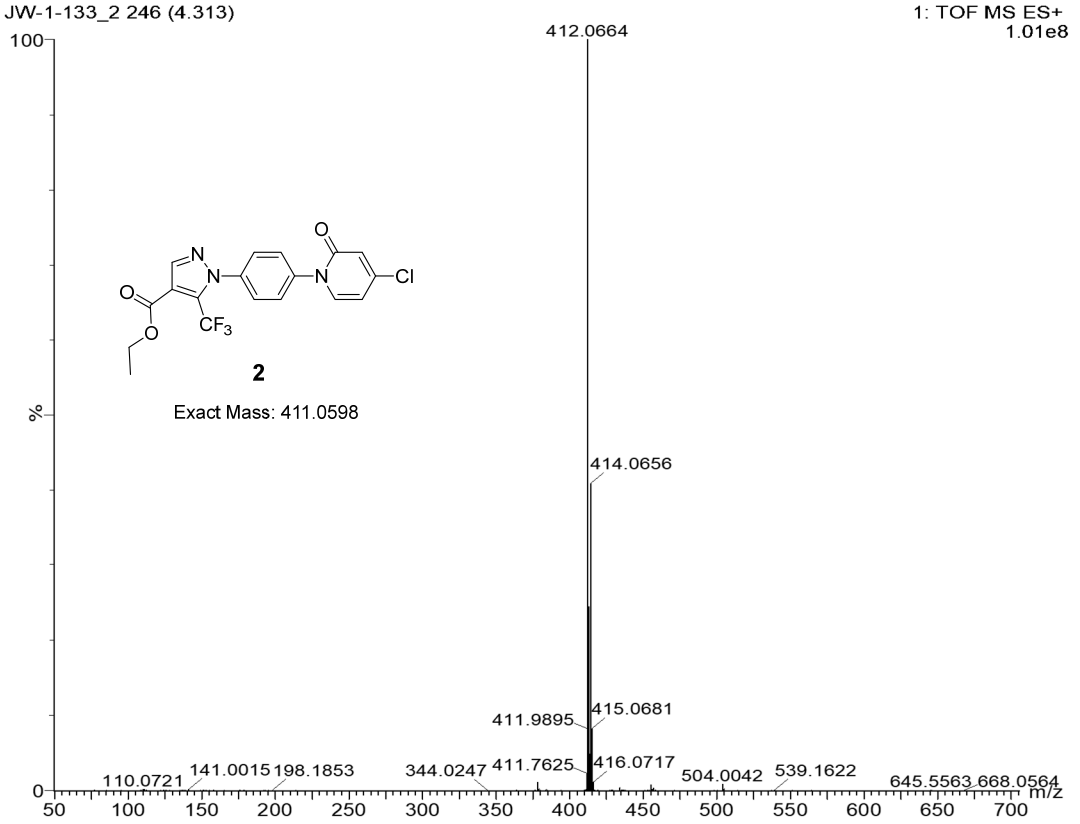
32

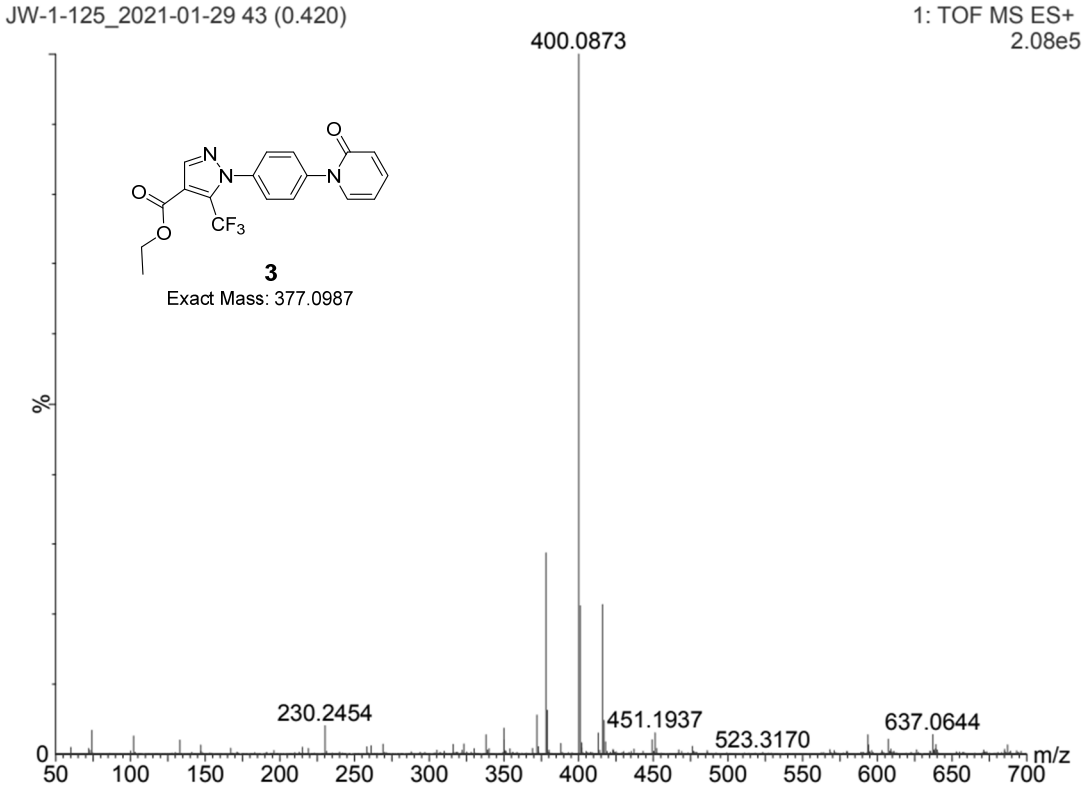

191.9
161.3
152.6
142.2
138.0
124.8
118.2
108.9
40.6
40.3
40.1
39.9
39.7
39.5
39.3
30.0
21.3
DMSO-d_6
f1 (ppm)

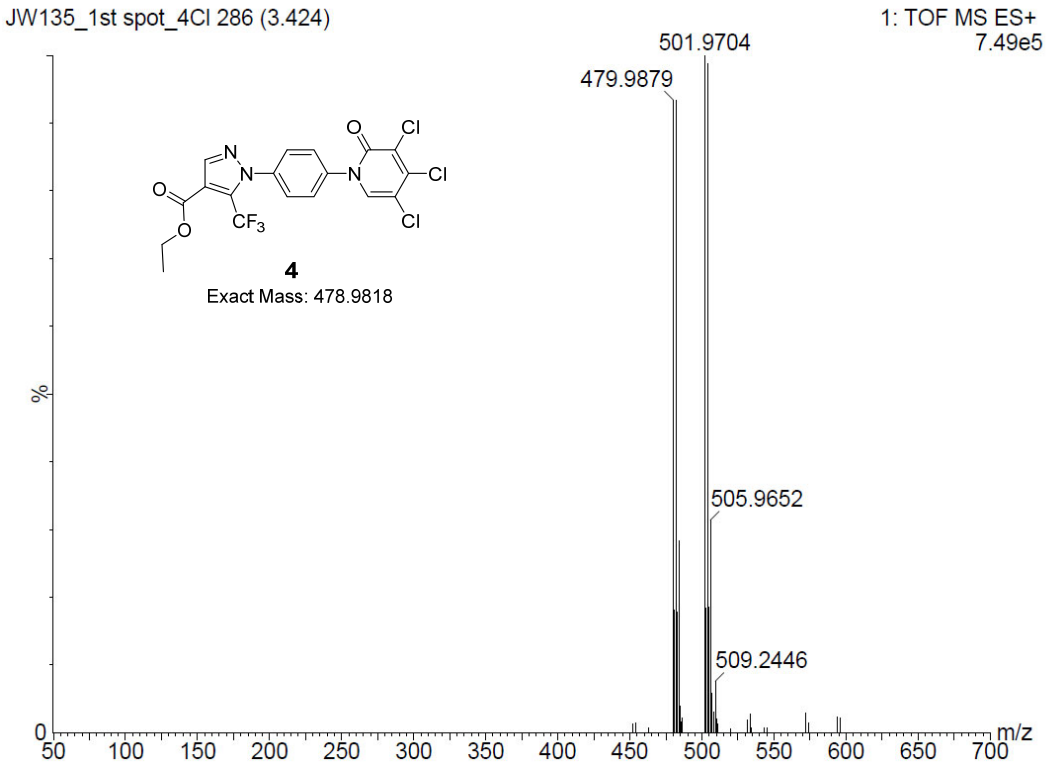
37

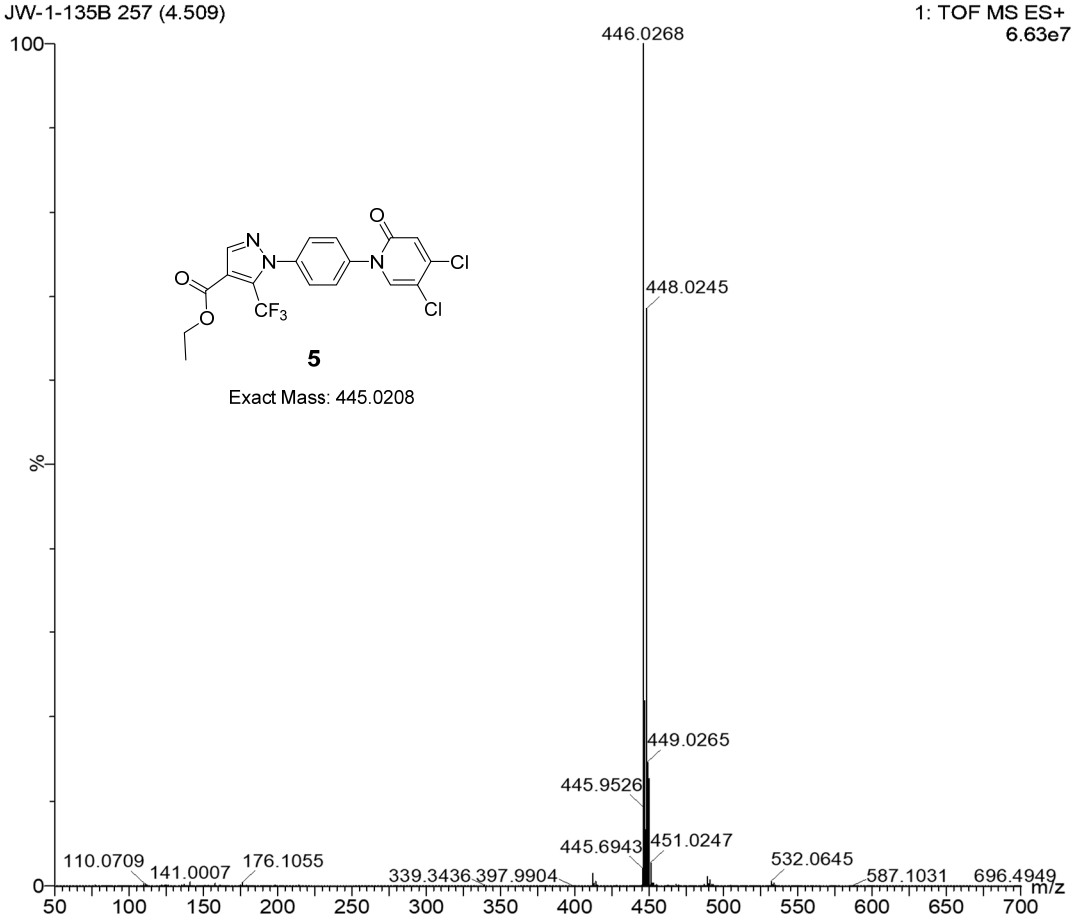

191.1
156.8
142.0
139.4
127.3
126.9
126.8
125.0
120.5
117.8
114.7
77.3
77.2
77.0
76.7
29.7
18.6
CDCl3
38
f1 (ppm)

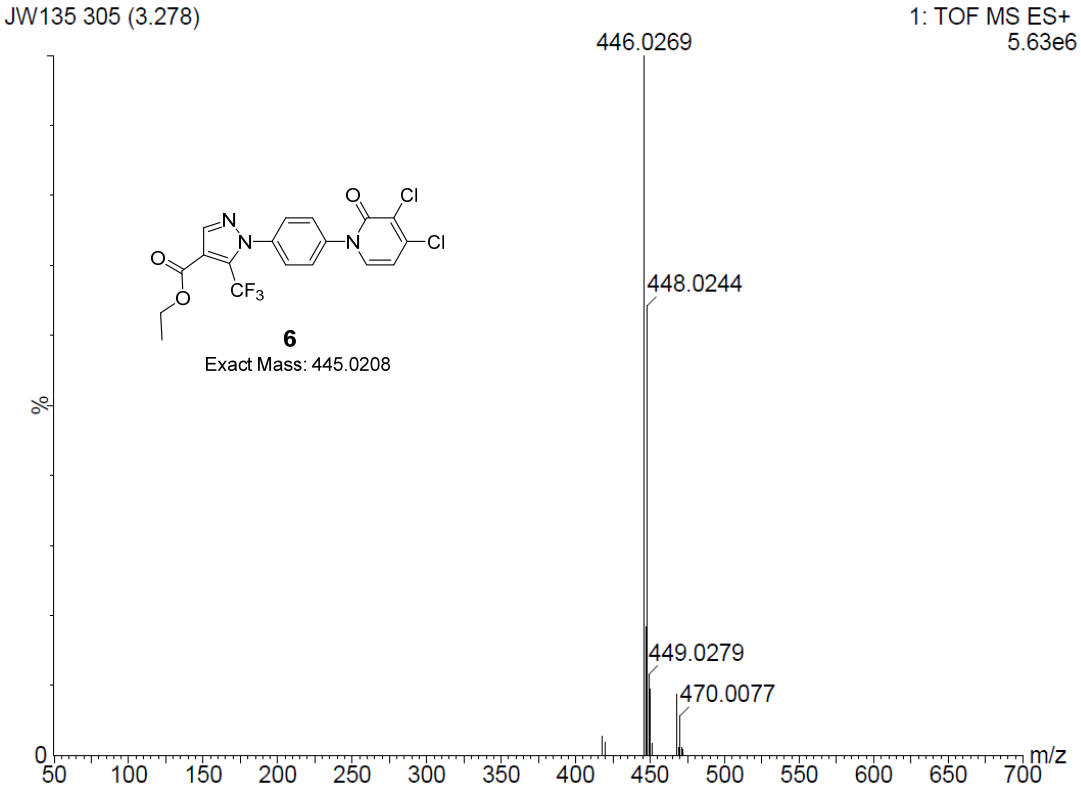
HR-MS spectra for the key pyrazole compounds

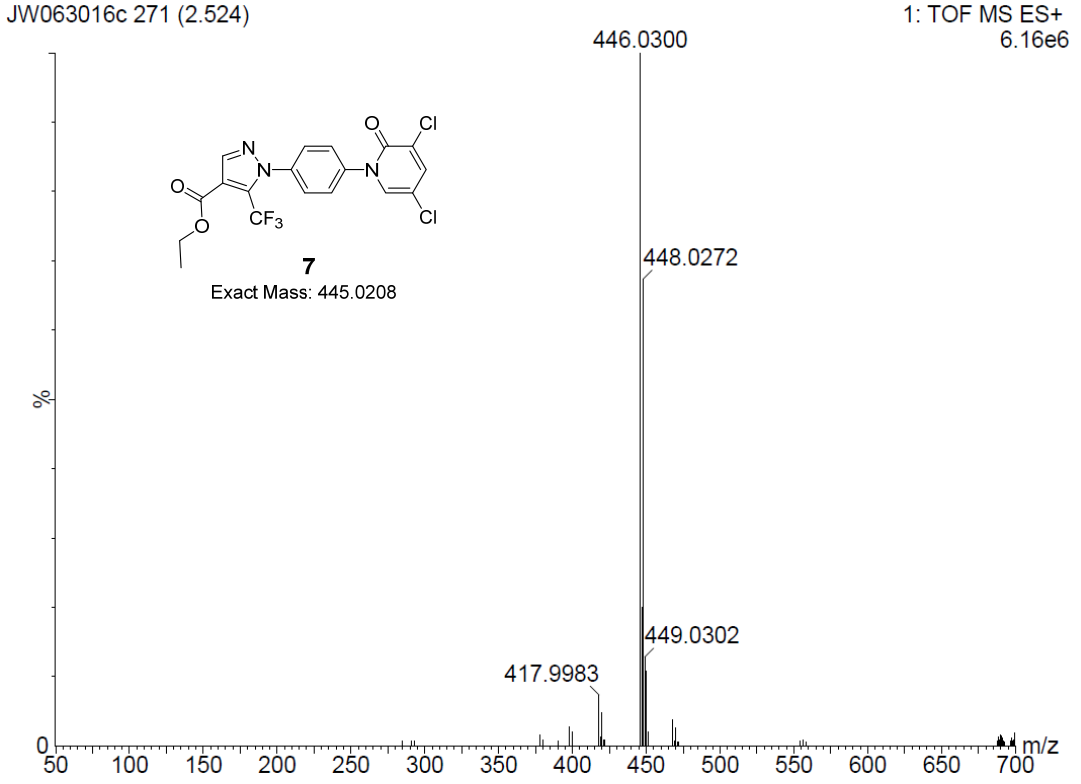


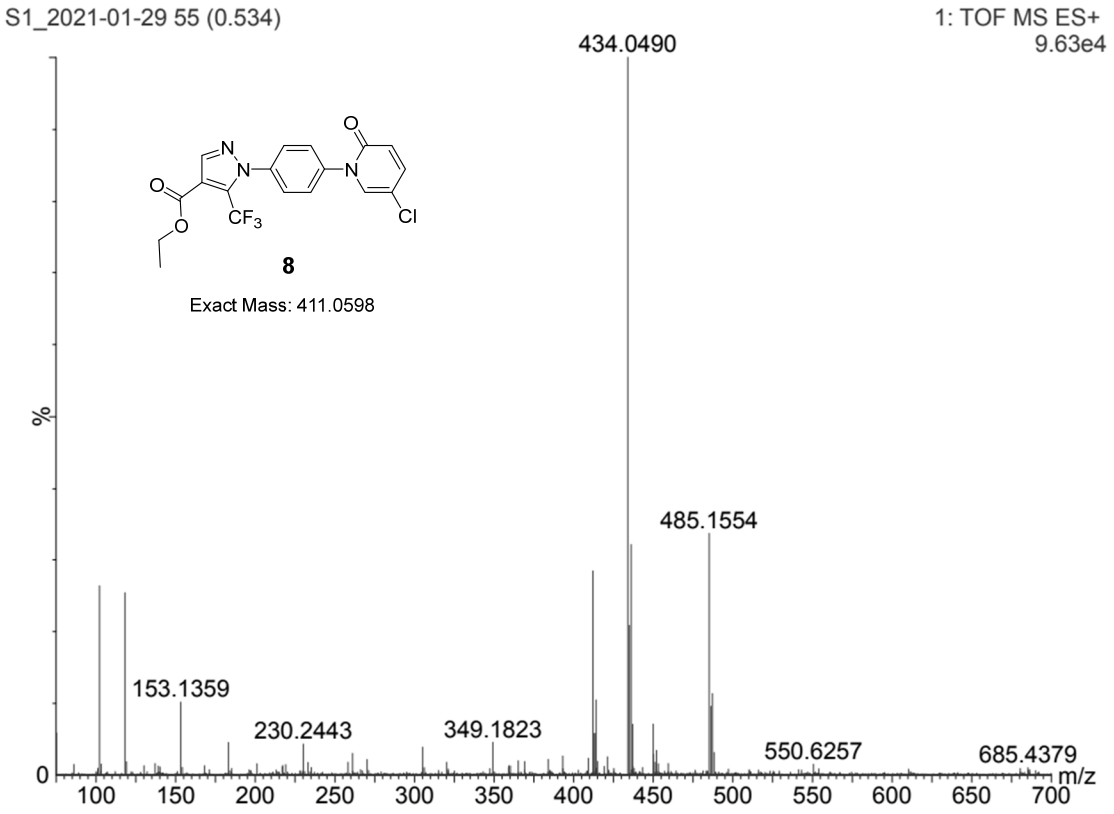


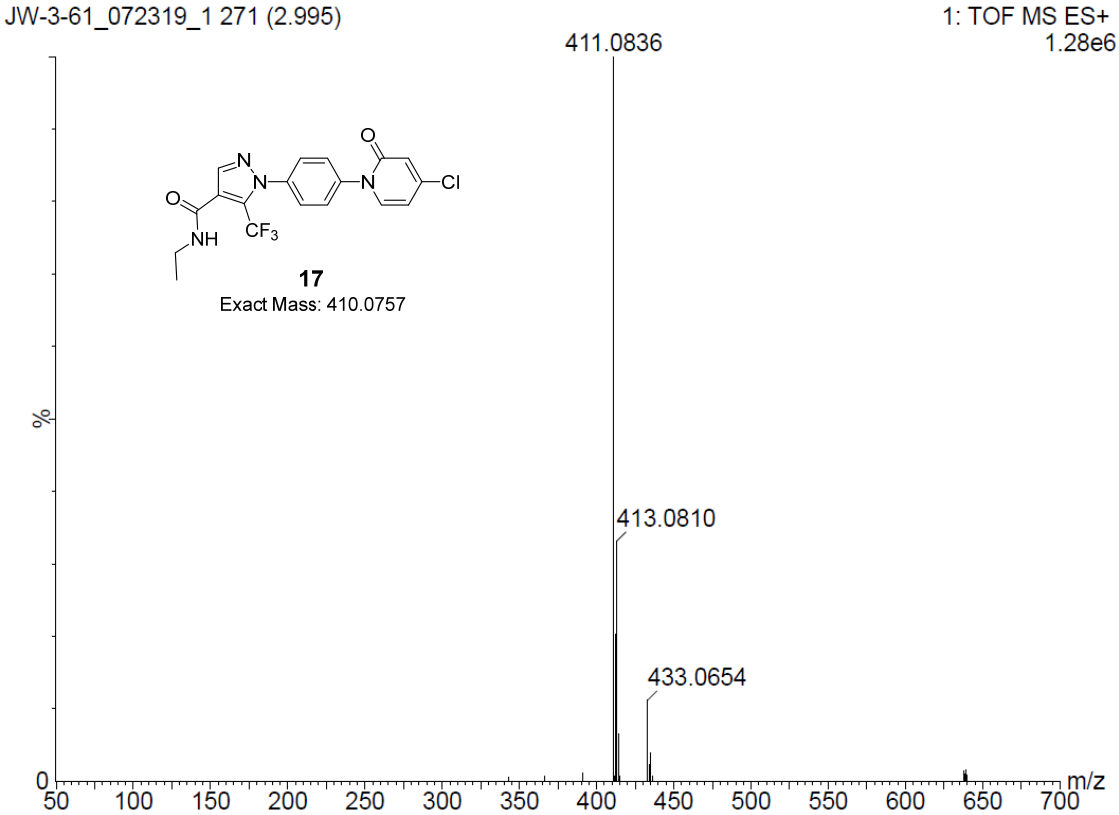

JW135_1st spot_4Cl 286 (3.424)
1: TOF MS ES+
7.49e5
501.9704
479.9879
505.9652
509.2446
%
0
50 100 150 200 250 300 350 400 450 500 550 600 650 700
m/z
4
Exact Mass: 478.9818

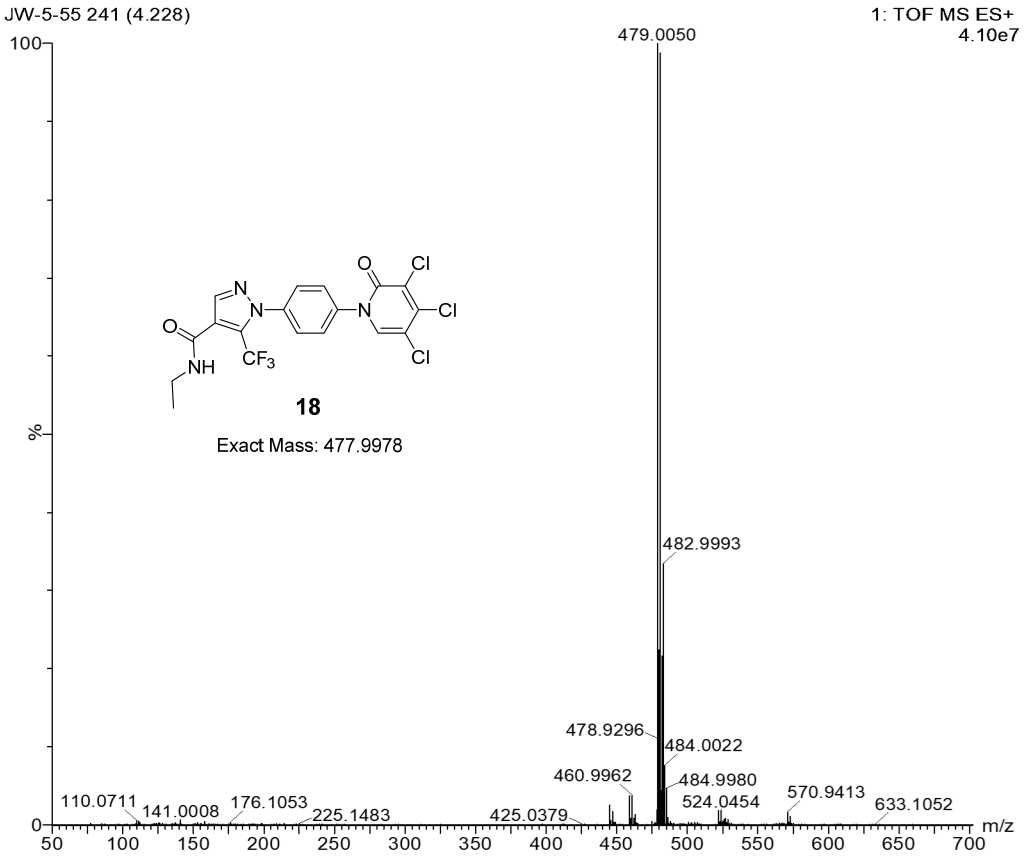

JW-1-135B 257 (4.509)
1: TOF MS ES+
6.63e7
100
446.0268
448.0245
449.0265
445.9526
445.6943
451.0247
110.0709
141.0007
176.1055
339.3436
397.9904
532.0645
587.1031
696.4949
%
0
50 100 150 200 250 300 350 400 450 500 550 600 650 700
m/z
5
Exact Mass: 445.0208

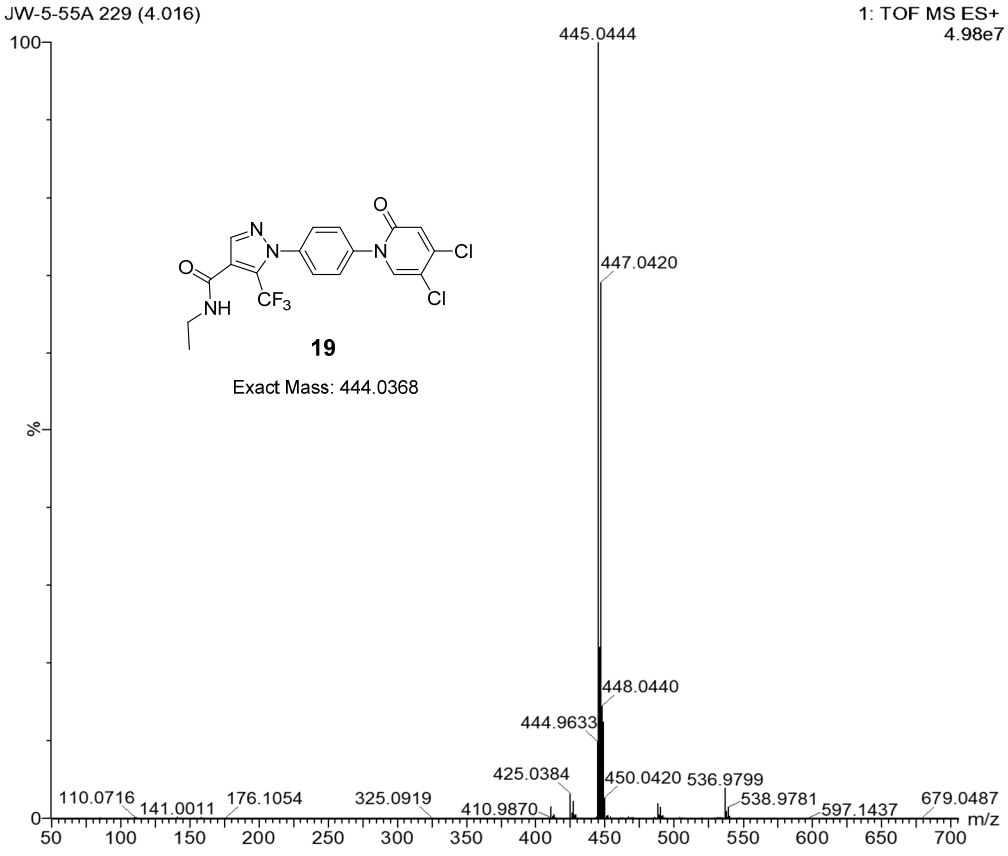

JW135 305 (3.278)
1: TOF MS ES+
5.63e6
446.0269
448.0244
449.0279
470.0077
%
0
50 100 150 200 250 300 350 400 450 500 550 600 650 700
m/z
6
Exact Mass: 445.0208

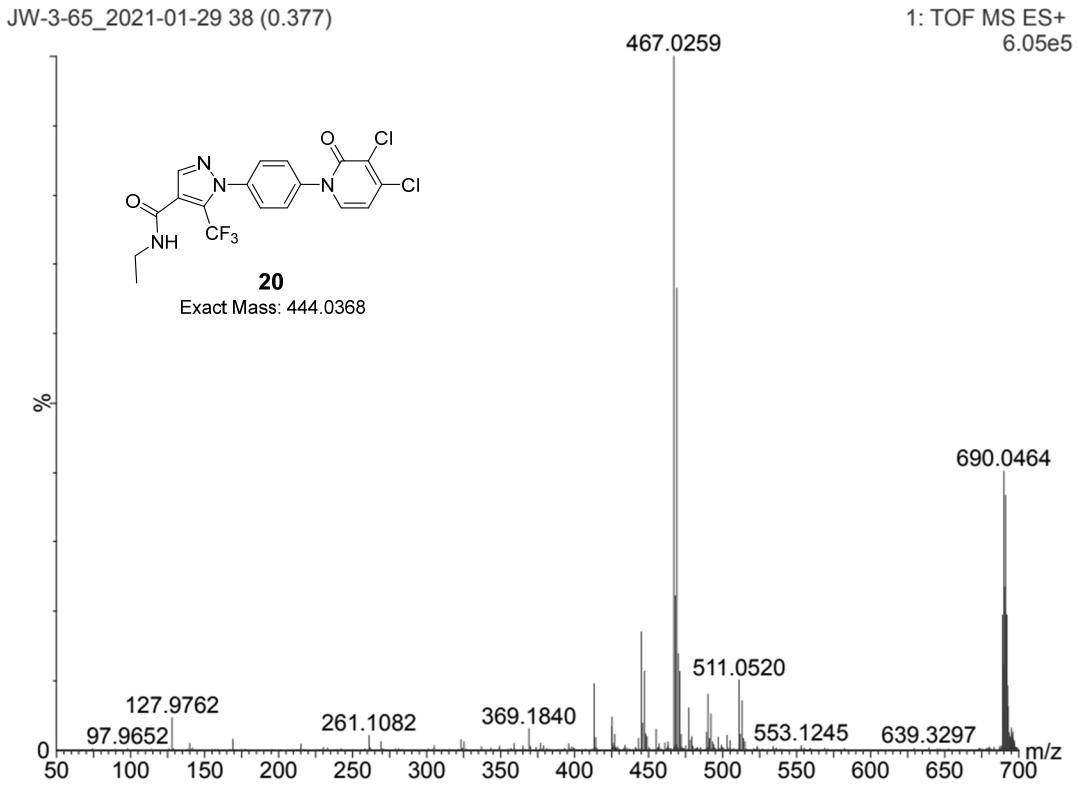

JW063016c 271 (2.524)
1: TOF MS ES+
6.16e6
446.0300
448.0272
449.0302
417.9983
%
0
50 100 150 200 250 300 350 400 450 500 550 600 650 700
m/z
7
Exact Mass: 445.0208

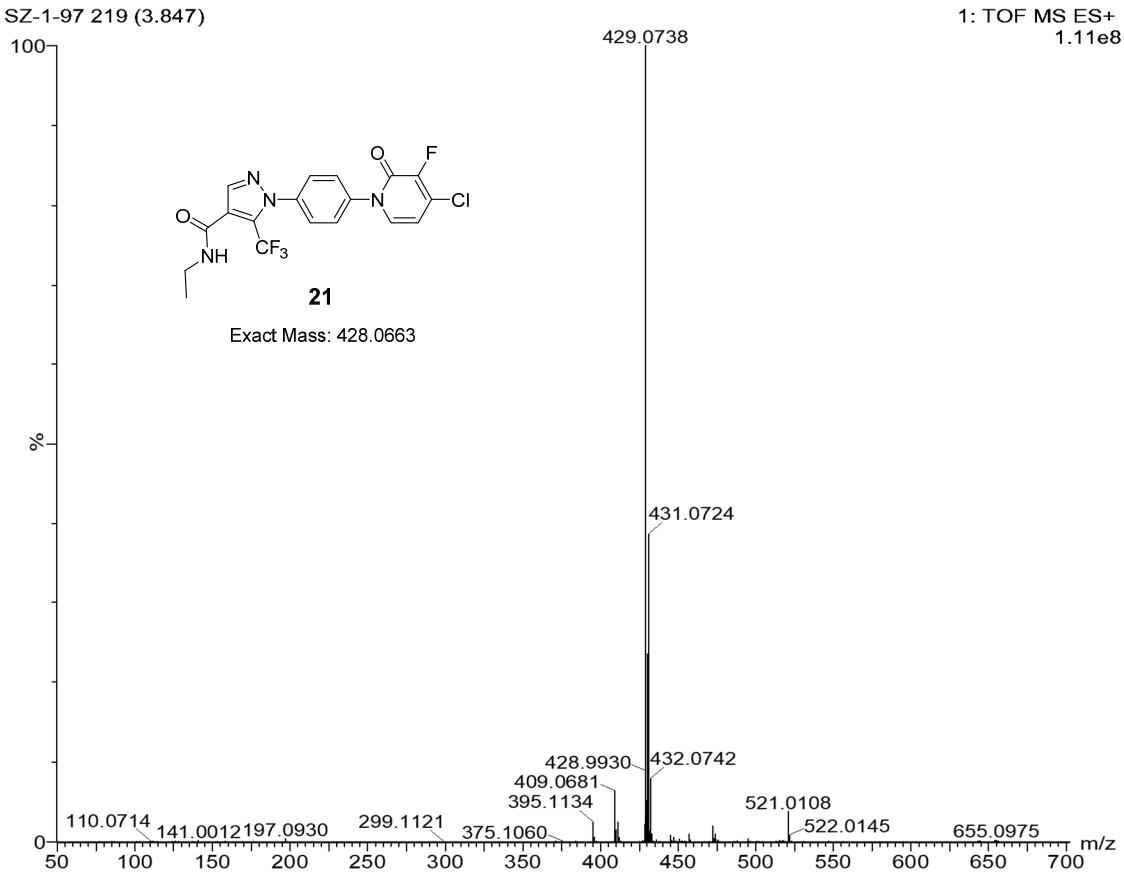

S1_2021-01-29 55 (0.534)
1: TOF MS ES+
9.63e4
434.0490
485.1554
153.1359
230.2443
349.1823
550.6257
685.4379
%
m/z
0
100 150 200 250 300 350 400 450 500 550 600 650 700
8
Exact Mass: 411.0598

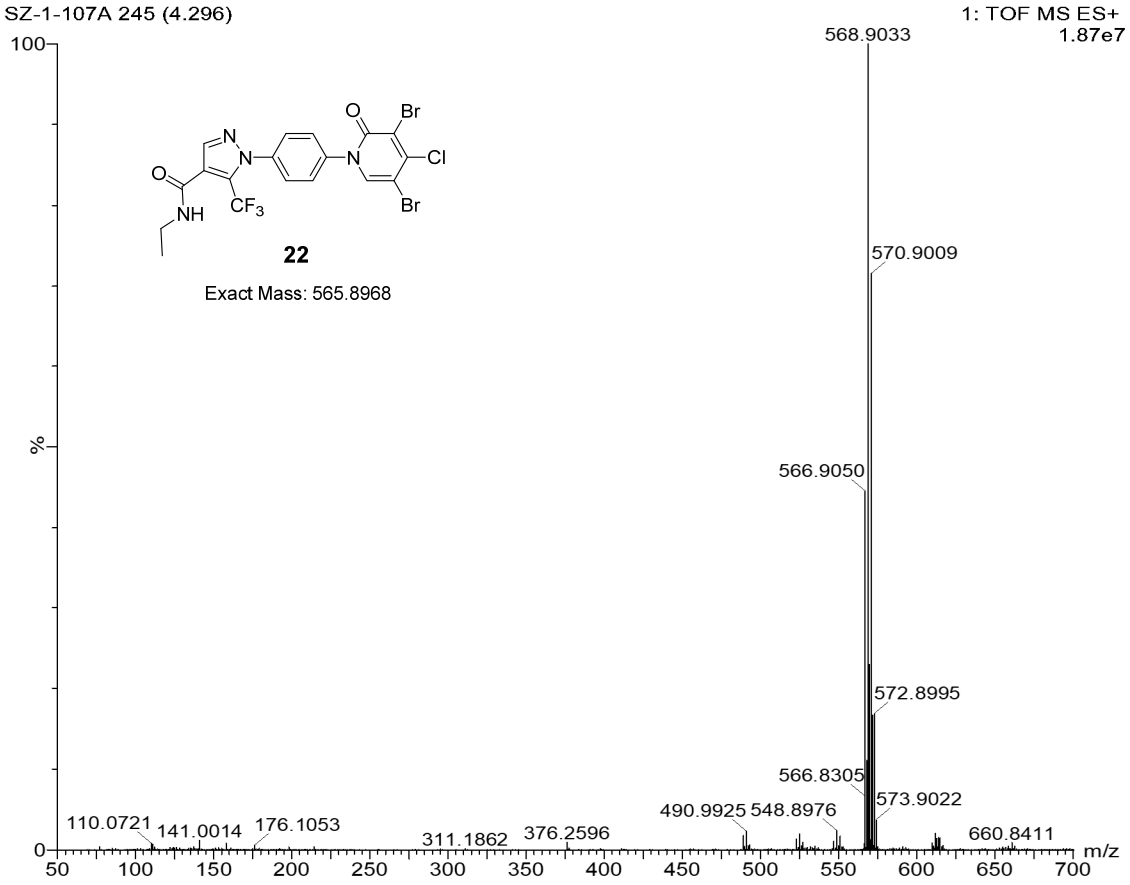

JW-3-61_072319_1 271 (2.995)
1: TOF MS ES+
1.28e6
411.0836
413.0810
433.0654
%
m/z
0
50 100 150 200 250 300 350 400 450 500 550 600 650 700
17
Exact Mass: 410.0757

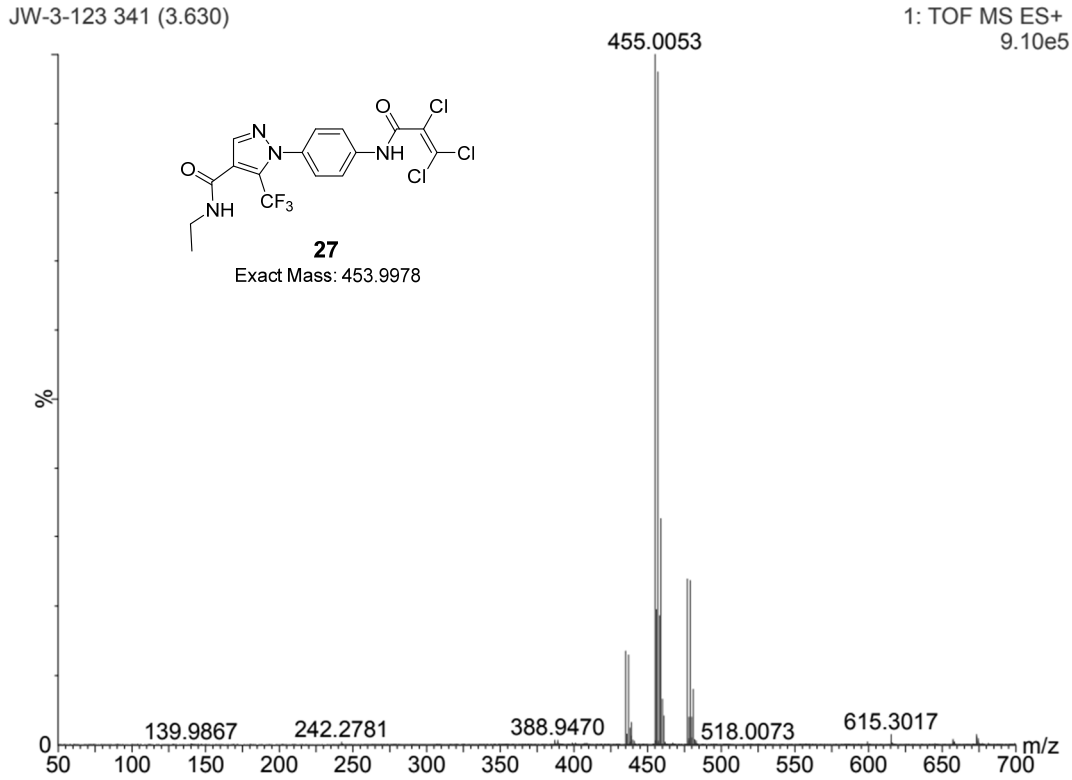

JW-5-55 241 (4.228)
1: TOF MS ES+
4.10e7
479.0050
482.9993
478.9296
484.0022
460.9962
484.9980
524.0454
570.9413
633.1052
110.0711
141.0008
176.1053
225.1483
425.0379
%
m/z
18
Exact Mass: 477.9978

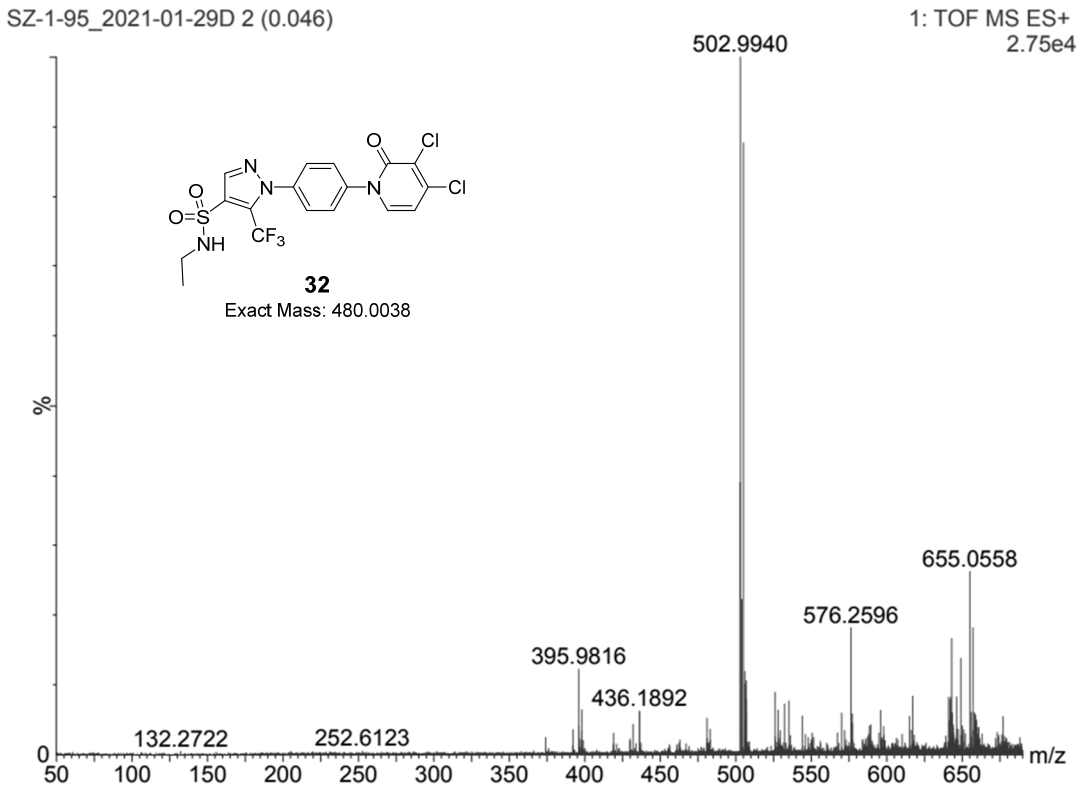

JW-5-55A 229 (4.016)
1: TOF MS ES+
4.98e7
445.0444
447.0420
448.0440
444.9633
425.0384
450.0420
536.9799
538.9781
597.1437
679.0487
110.0716
141.0011
176.1054
325.0919
410.9870
%
m/z
19
Exact Mass: 444.0368

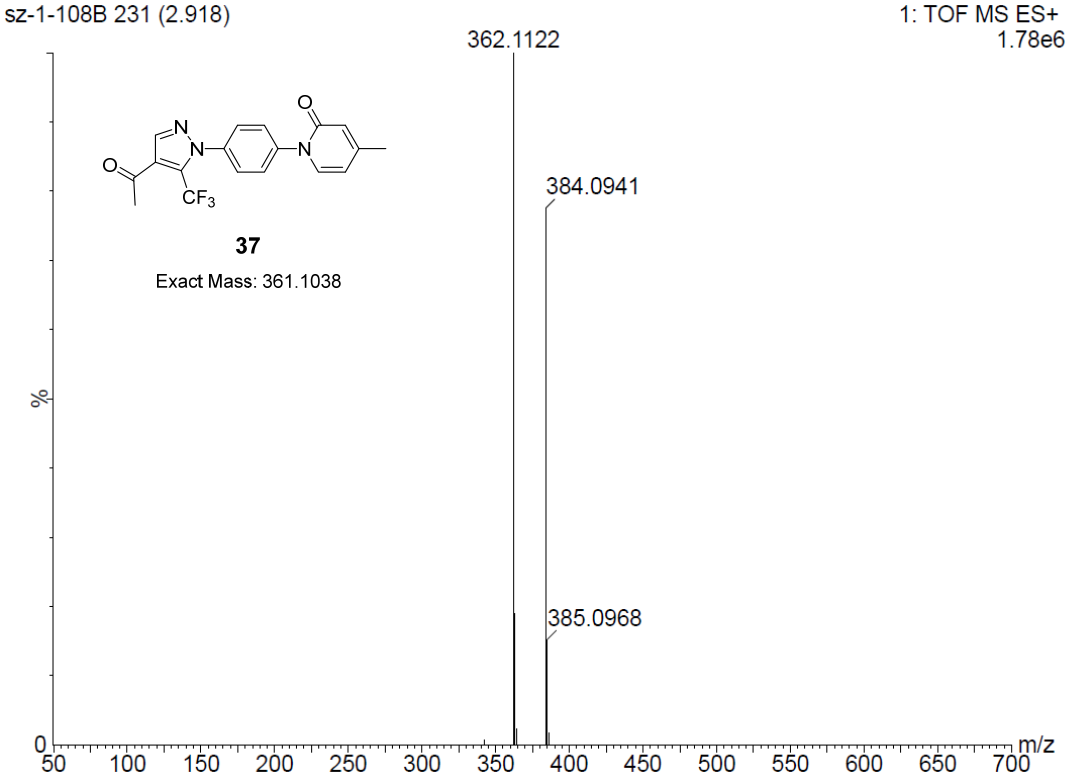

JW-3-65_2021-01-29 38 (0.377)
1: TOF MS ES+
6.05e5
467.0259
690.0464
511.0520
127.9762
97.9652
261.1082
369.1840
553.1245
639.3297
%
m/z
20
Exact Mass: 444.0368

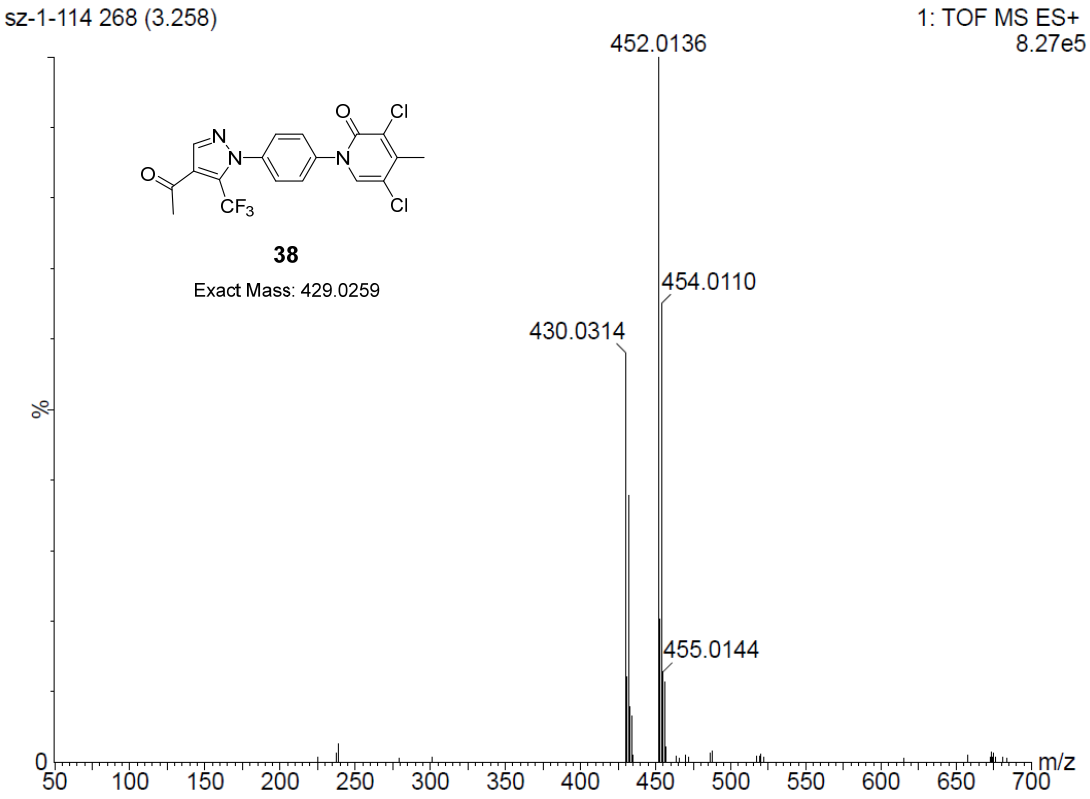

SZ-1-97 219 (3.847)
1: TOF MS ES+
1.11e8
429.0738
431.0724
428.9930
432.0742
409.0681
395.1134
521.0108
522.0145
110.0714
141.0012
197.0930
299.1121
375.1060
655.0975
%
m/z
21
Exact Mass: 428.0663


SZ-1-107A 245 (4.296)
1: TOF MS ES+
1.87e7
100
%
0
568.9033
570.9009
566.9050
572.8995
566.8305
573.9022
548.8976
490.9925
110.0721
141.0014
176.1053
311.1862
376.2596
660.8411
m/z
50 100 150 200 250 300 350 400 450 500 550 600 650 700
22
Exact Mass: 565.8968

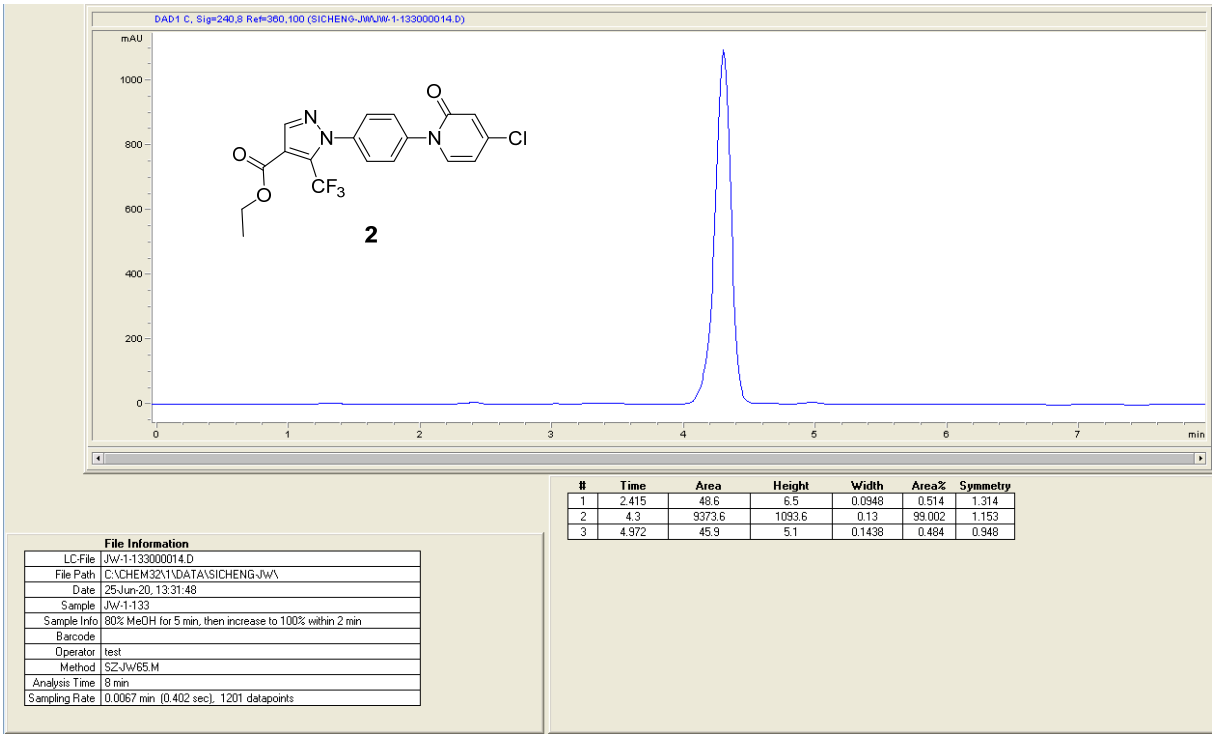

JW-3-123 341 (3.630)
1: TOF MS ES+
9.10e5
%
0
455.0053
139.9867
242.2781
388.9470
518.0073
615.3017
m/z
50 100 150 200 250 300 350 400 450 500 550 600 650 700
27
Exact Mass: 453.9978


SZ-1-95_2021-01-29D 2 (0.046)
1: TOF MS ES+
2.75e4
502.9940
655.0558
576.2596
395.9816
436.1892
132.2722
252.6123
%
0
50 100 150 200 250 300 350 400 450 500 550 600 650
m/z
32
Exact Mass: 480.0038


sz-1-108B 231 (2.918)
1: TOF MS ES+
1.78e6
362.1122
384.0941
385.0968
%
0
50 100 150 200 250 300 350 400 450 500 550 600 650 700
m/z
37
Exact Mass: 361.1038


sz-1-114 268 (3.258)
1: TOF MS ES+
8.27e5
452.0136
454.0110
430.0314
455.0144
%
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
m/z
38
Exact Mass: 429.0259

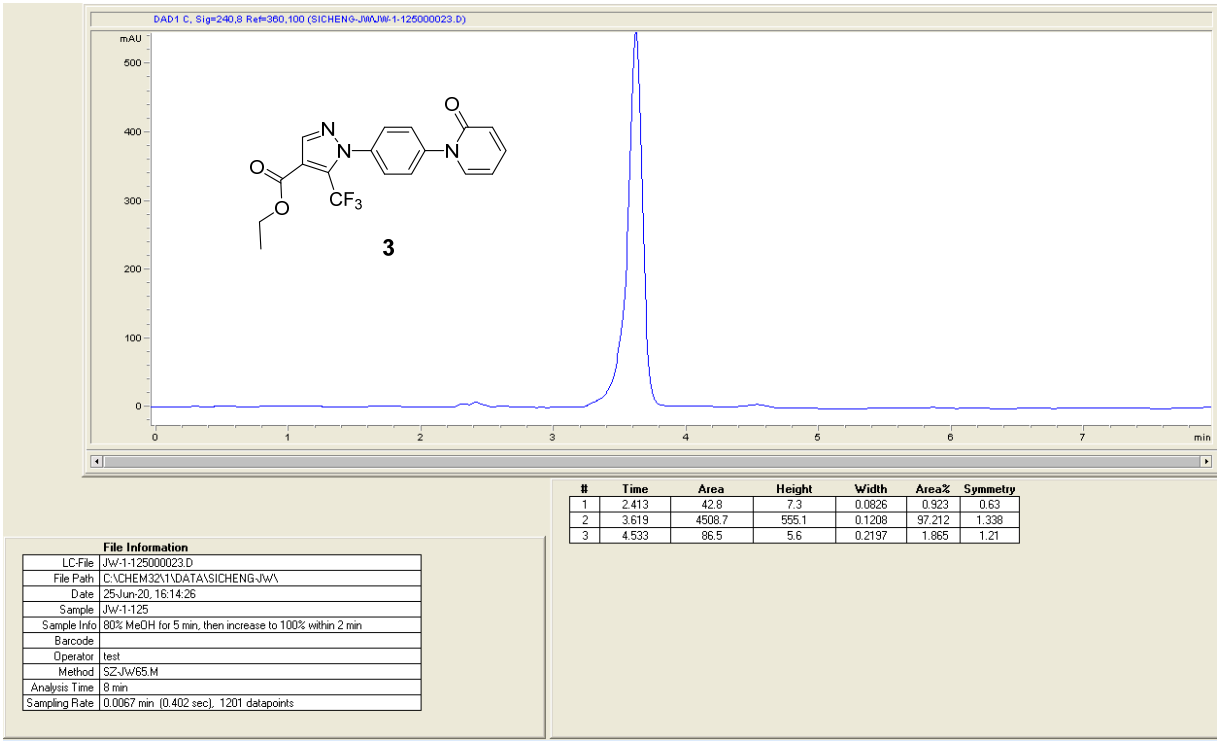
HPLC Purity of the key pyrazole compounds



#	Time	Area	Height	Width	Area%	Symmetry
1	2.415	48.6	6.5	0.0948	0.514	1.314
2	4.3	9373.6	1093.6	0.13	99.002	1.153
3	4.972	45.9	5.1	0.1438	0.484	0.948

File Information

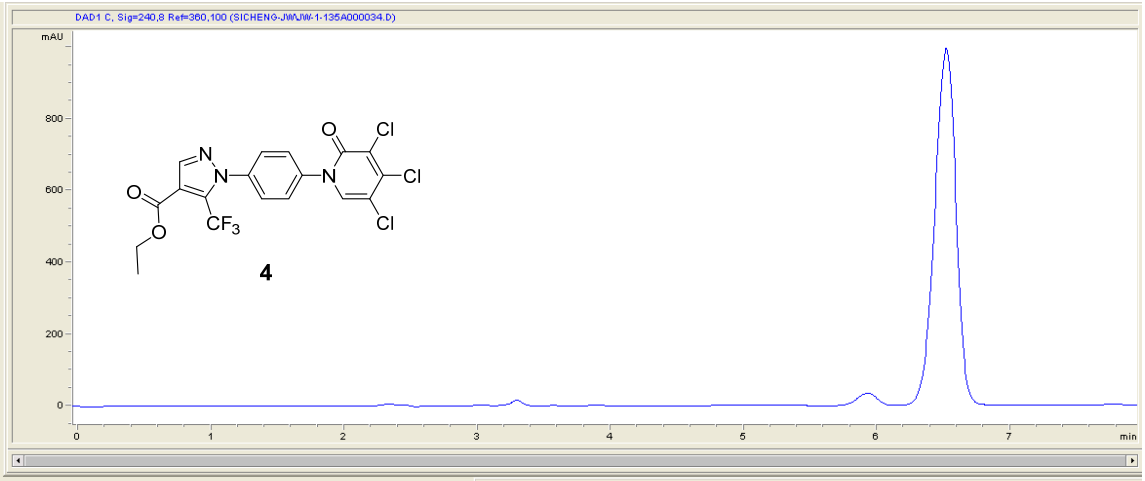
LC-File	JW-1-133000014.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 13:31:48
Sample	JW-1-133
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZ-JW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



#	Time	Area	Height	Width	Area%	Symmetry
1	2.413	42.8	7.3	0.0826	0.923	0.63
2	3.619	4508.7	555.1	0.1208	97.212	1.338
3	4.533	86.5	5.6	0.2197	1.865	1.21

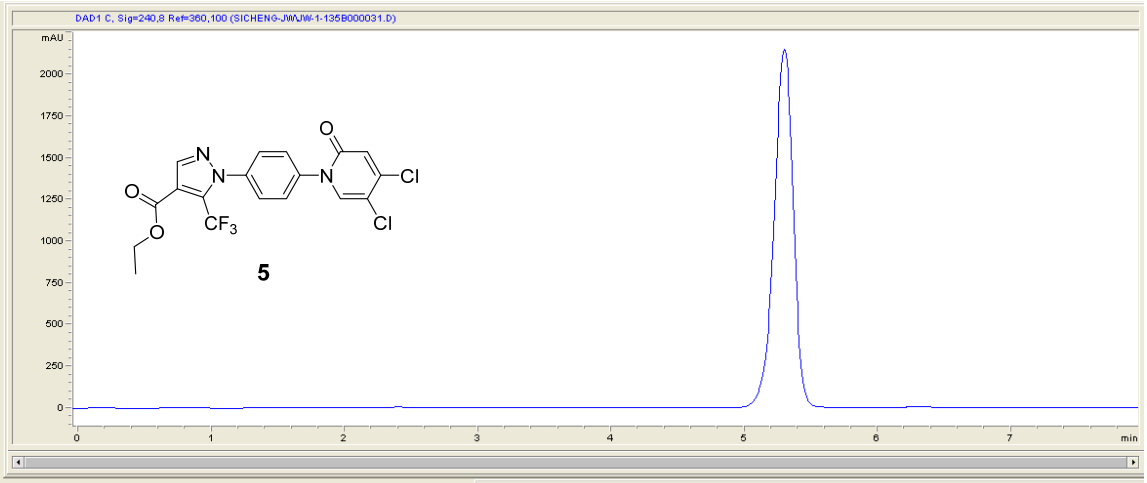
File Information

LC-File	JW-1-125000023.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 16:14:26
Sample	JW-1-125
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZ-JW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



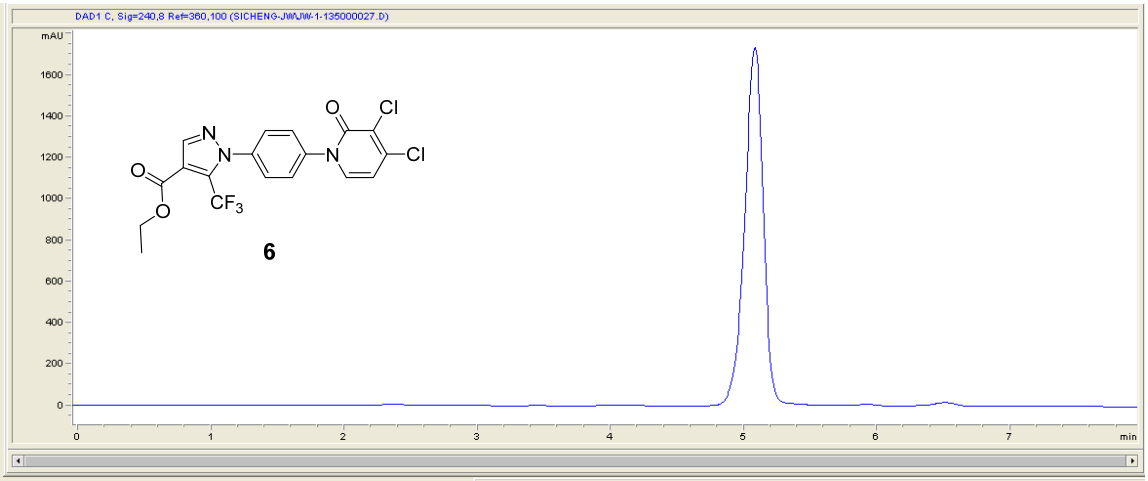
#	Time	Area	Height	Width	Area%	Symmetry
1	3.297	127.9	17.3	0.1061	1.104	1.233
2	5.931	369.4	34.7	0.1683	3.188	1.114
3	6.523	11087.6	995.3	0.176	95.708	1.176

File Information	
LC-File	JW-1-135A000034.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 20:19:10
Sample	JW-1-135A
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZ-JW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



#	Time	Area	Height	Width	Area%	Symmetry
1	5.3	21670.1	2147.6	0.1577	99.538	1.149
2	6.311	100.5	9	0.1757	0.462	0.993

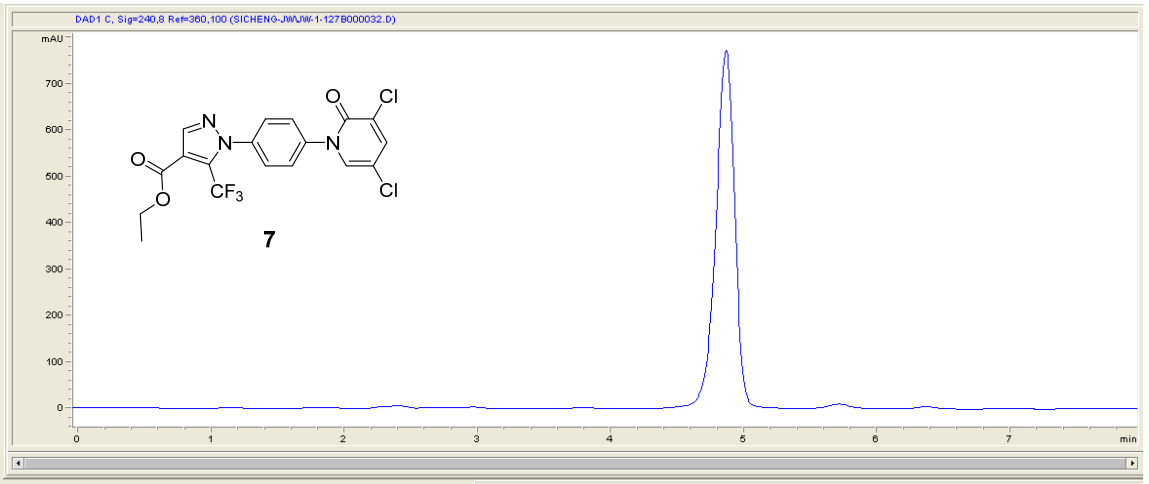
File Information	
LC-File	JW-1-135B000031.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 19:25:38
Sample	JW-1-135B
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZ-JW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



#	Time	Area	Height	Width	Area%	Symmetry
1	2.36	66.5	6.7	0.1275	0.378	0.857
2	5.084	17116.3	1736.8	0.151	97.336	1.166
3	5.927	135.3	9.7	0.2073	0.769	1.38
4	6.516	266.7	19.1	0.2071	1.517	1.572

File Information

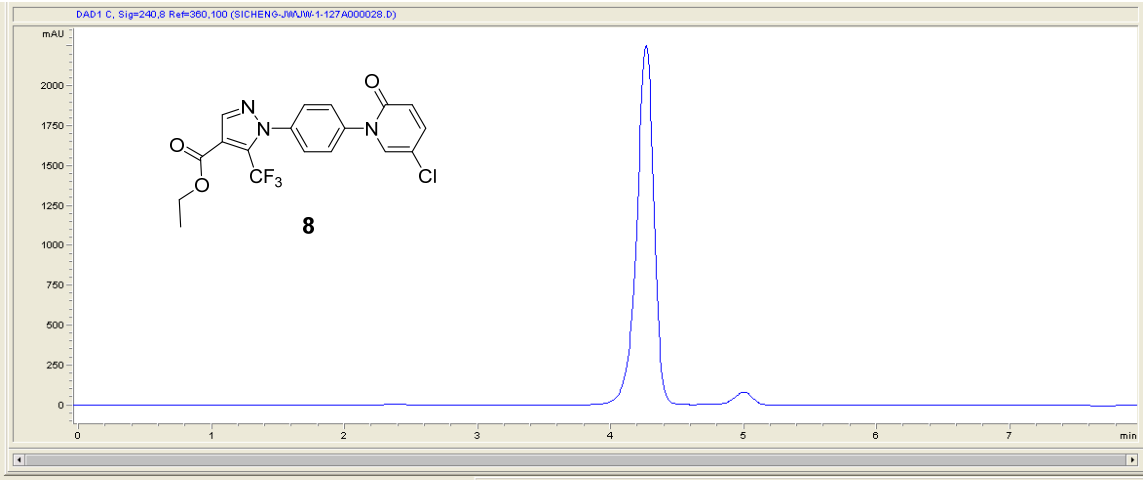
LC-File	JW-1-135000027.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 17:53:47
Sample	JW-1-135
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZ-JW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



#	Time	Area	Height	Width	Area%	Symmetry
1	2.371	51.8	5.5	0.1218	0.650	0.377
2	4.866	7718.3	773.2	0.1525	96.808	1.193
3	5.716	141.8	10.6	0.1984	1.778	0.938
4	6.368	60.9	5	0.181	0.764	0.988

File Information

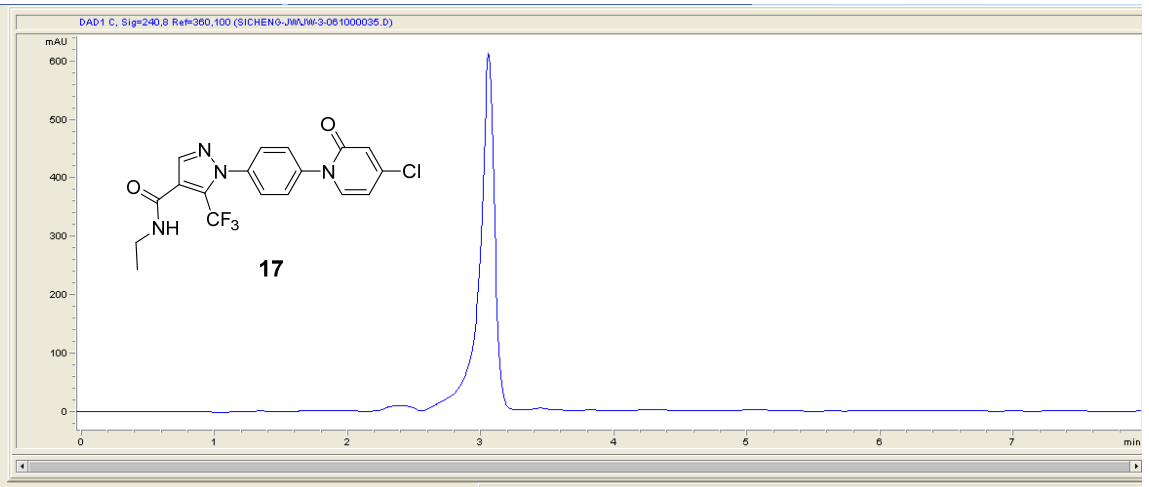
LC-File	JW-1-127B000032.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 19:42:04
Sample	JW-1-127B
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZ-JW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



#	Time	Area	Height	Width	Area%	Symmetry
1	4.264	20515.7	2256.6	0.1401	95.453	1.187
2	4.999	977.2	83.8	0.174	4.547	1.292

File Information

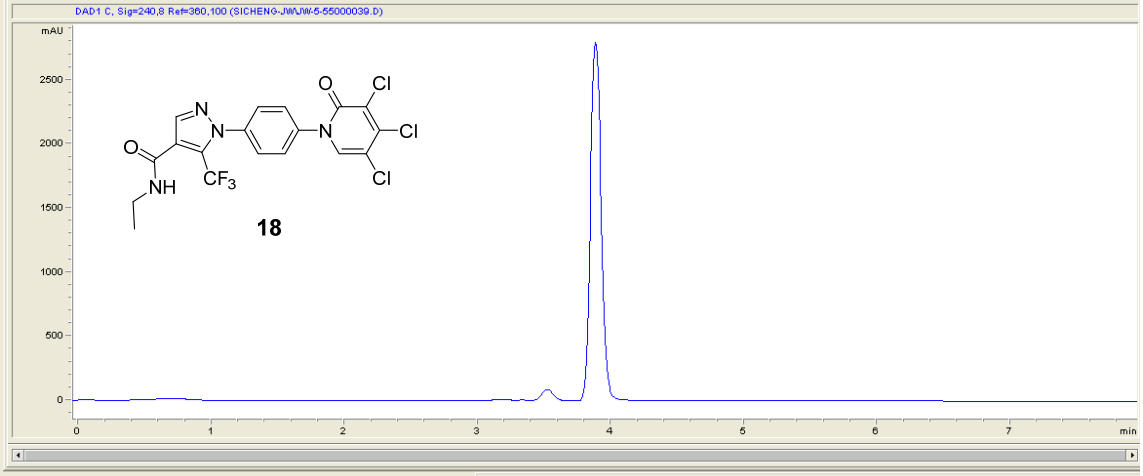
LC-File	JW-1-127A000028.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 18:09:04
Sample	JW-1-127A
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZJW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



#	Time	Area	Height	Width	Area%	Symmetry
1	2.354	59.5	9.2	0.0937	1.190	3.981
2	2.422	67.1	9.4	0.1155	1.342	0.571
3	3.056	4871.3	612.3	0.1128	97.468	1.634

File Information

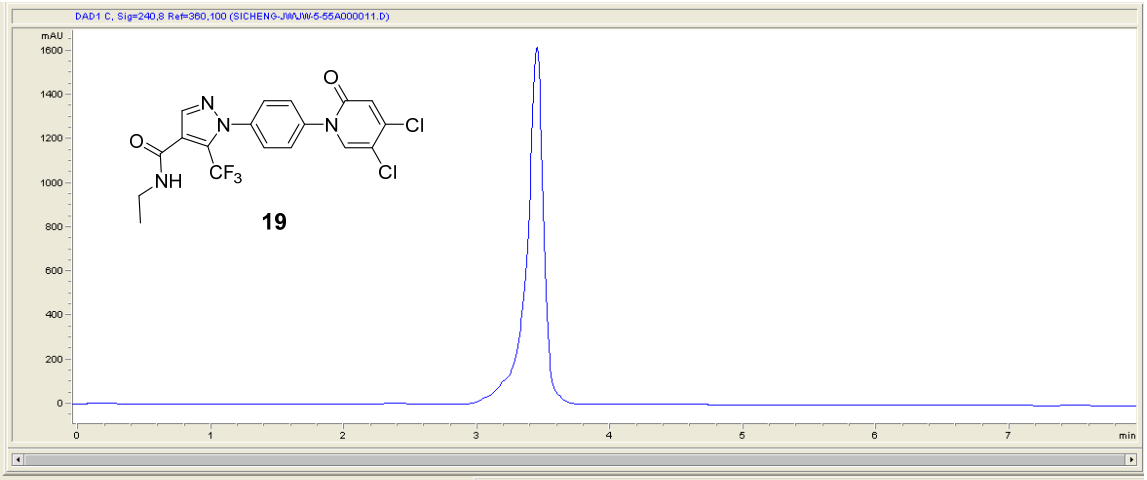
LC-File	JW-3-061000035.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 20:43:32
Sample	JW-3-061
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZJW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



#	Time	Area	Height	Width	Area%	Symmetry
1	3.202	104.2	11.2	0.1266	0.636	1.971
2	3.525	564	88.9	0.102	3.442	0.995
3	3.886	15718.7	2798	0.0895	95.923	0.854

File Information

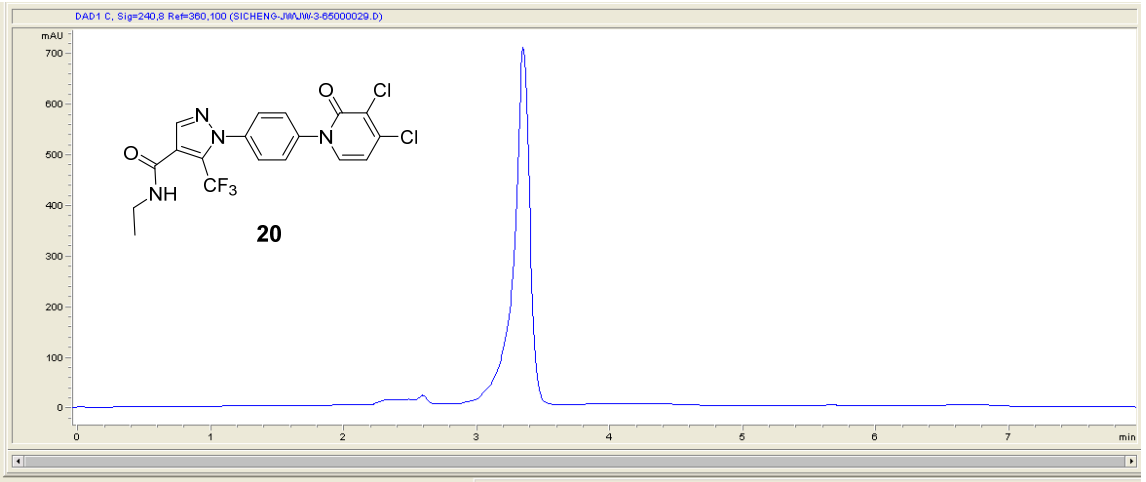
LC-File	JW-5-55000039.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	26-Jun-20, 00:28:10
Sample	JW-5-55
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZJW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



#	Time	Area	Height	Width	Area%	Symmetry
1	2.372	72.4	6.2	0.1501	0.494	1.079
2	3.448	14579.2	1617.1	0.1292	99.506	1.519

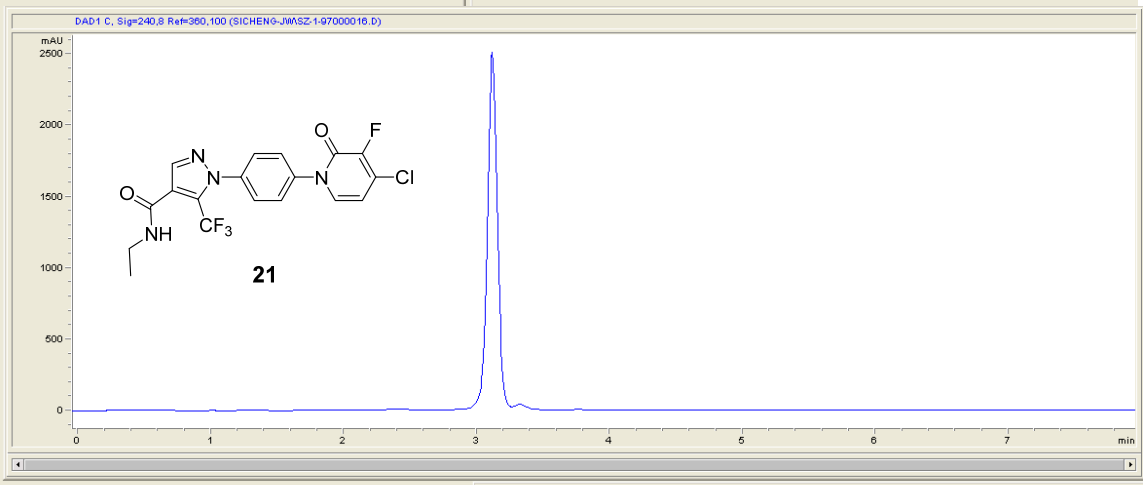
File Information

LC-File	JW-5-55A000011.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 11:59:03
Sample	JW-5-55A
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZJW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



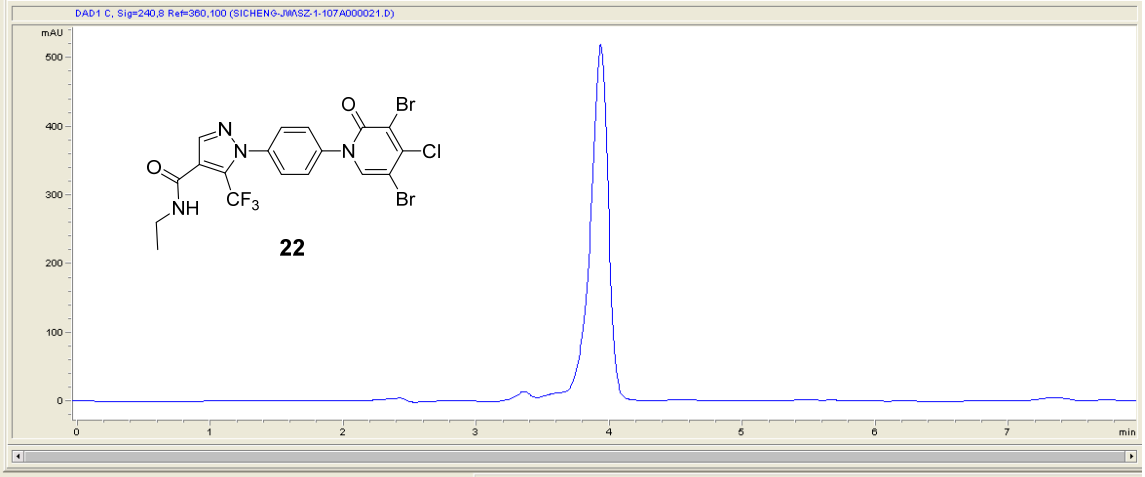
#	Time	Area	Height	Width	Area%	Symmetry
1	2.592	159.9	21.7	0.102	2.533	0.625
2	3.347	6150.7	708.9	0.1252	97.467	1.591

File Information	
LC-File	JW-3-65000029.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 18:53:24
Sample	JW-3-65
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZ-JW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



#	Time	Area	Height	Width	Area%	Symmetry
1	2.362	52.3	8.8	0.0836	0.377	4.089
2	2.444	63.8	9	0.0949	0.460	1.026
3	3.116	13334.7	2508.9	0.08	96.219	0.96
4	3.323	344.7	44.3	0.1108	2.487	0.49
5	3.764	63.2	7.1	0.1258	0.456	0.705

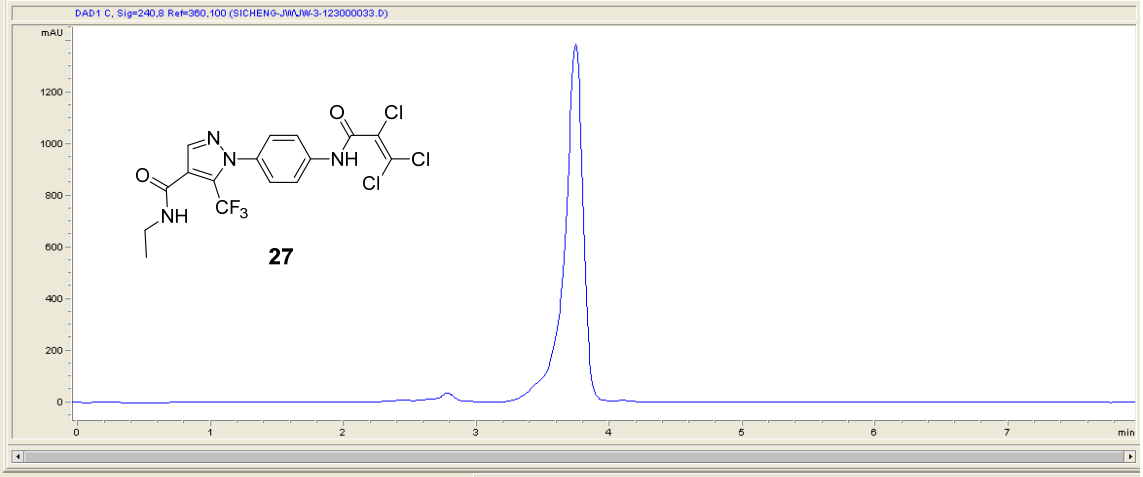
File Information	
LC-File	SZ-1-97000016.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 14:01:41
Sample	SZ-1-97
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZ-JW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



#	Time	Area	Height	Width	Area%	Symmetry
1	3.355	135.9	15.1	0.1267	2.585	1.308
2	3.933	5035.2	519.8	0.143	95.753	1.419
3	7.349	87.4	5.2	0.2643	1.662	1.45

File Information

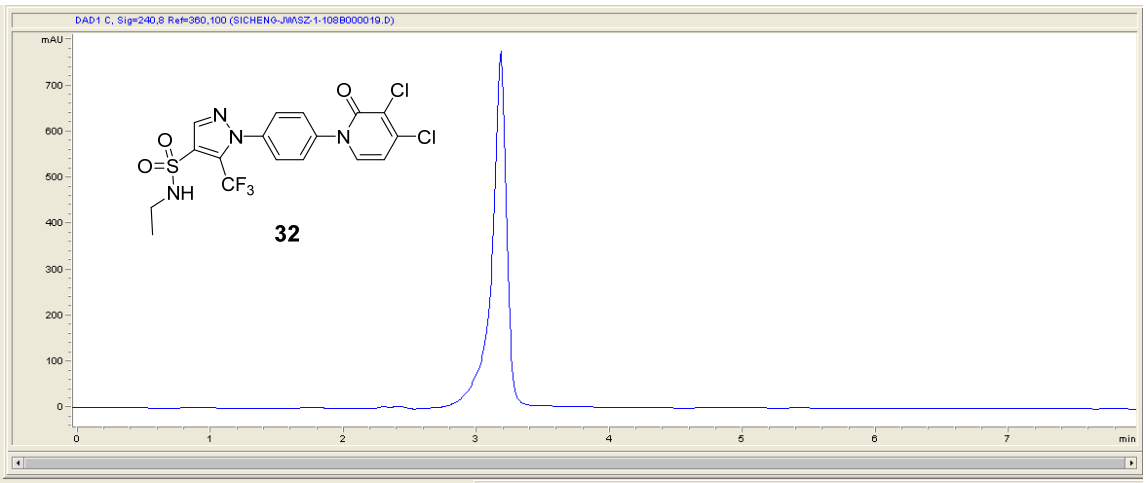
LC-File	SZ-1-107A000021.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 15:37:37
Sample	SZ-1-107A
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZ-JW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



#	Time	Area	Height	Width	Area%	Symmetry
1	2.442	88.6	7.3	0.1552	0.624	1.681
2	2.779	377.5	35.2	0.1512	2.658	1.36
3	3.744	13688.8	1382.9	0.1455	96.397	1.564
4	4.098	45.5	5.3	0.1306	0.321	1.074

File Information

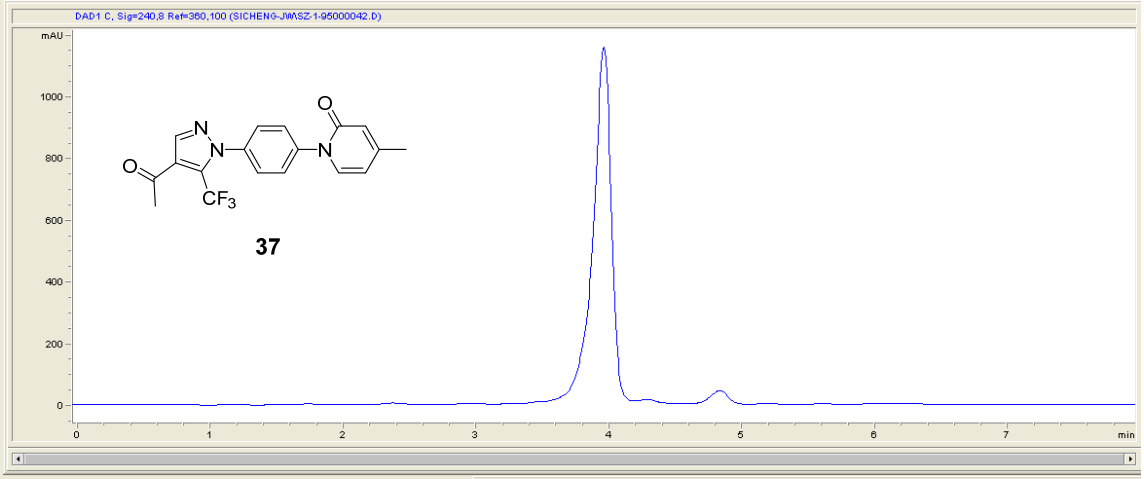
LC-File	JW-3-123000033.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 20:02:18
Sample	JW-3-123
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZ-JW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



#	Time	Area	Height	Width	Area%	Symmetry
1	2.301	32.4	5.6	0.0793	0.510	2.145
2	2.418	40.3	5.7	0.0982	0.635	1.179
3	3.18	6186.6	776.2	0.113	97.522	1.554
4	3.508	84.5	7	0.1667	1.332	0.411

File Information

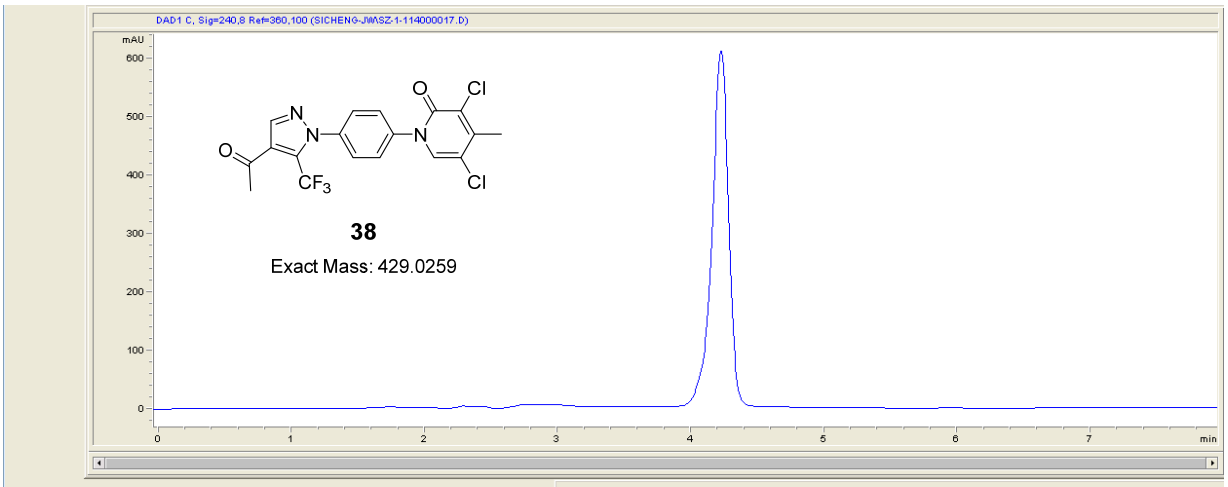
LC-File	SZ-1-108B000019.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 15:05:19
Sample	SZ-1-108B
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZ-JW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



#	Time	Area	Height	Width	Area%	Symmetry
1	3.959	11572.3	1158.6	0.1465	96.023	1.531
2	4.83	479.3	46	0.1575	3.977	1.24

File Information

LC-File	SZ-1-95000042.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	26-Jun-20, 01:17:24
Sample	SZ-1-95
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZ-JW65.M
Analysis Time	8 min
Sampling Rate	0.0067 min (0.402 sec), 1201 datapoints



#	Time	Area	Height	Width	Area%	Symmetry
1	2.286	27.7	5	0.0762	0.491	1.547
2	2.826	217.8	7.9	0.3874	3.854	0.572
3	4.226	5404.8	610.8	0.1352	95.655	1.221

File Information

LC-File	SZ-1-114000017.D
File Path	C:\CHEM32\1\DATA\SICHENG-JW\
Date	25-Jun-20, 14:16:47
Sample	SZ-1-114
Sample Info	80% MeOH for 5 min, then increase to 100% within 2 min
Barcode	
Operator	test
Method	SZJW65.M
Analysis Time	7.993 min
Sampling Rate	0.0067 min (0.402 sec), 1200 datapoints

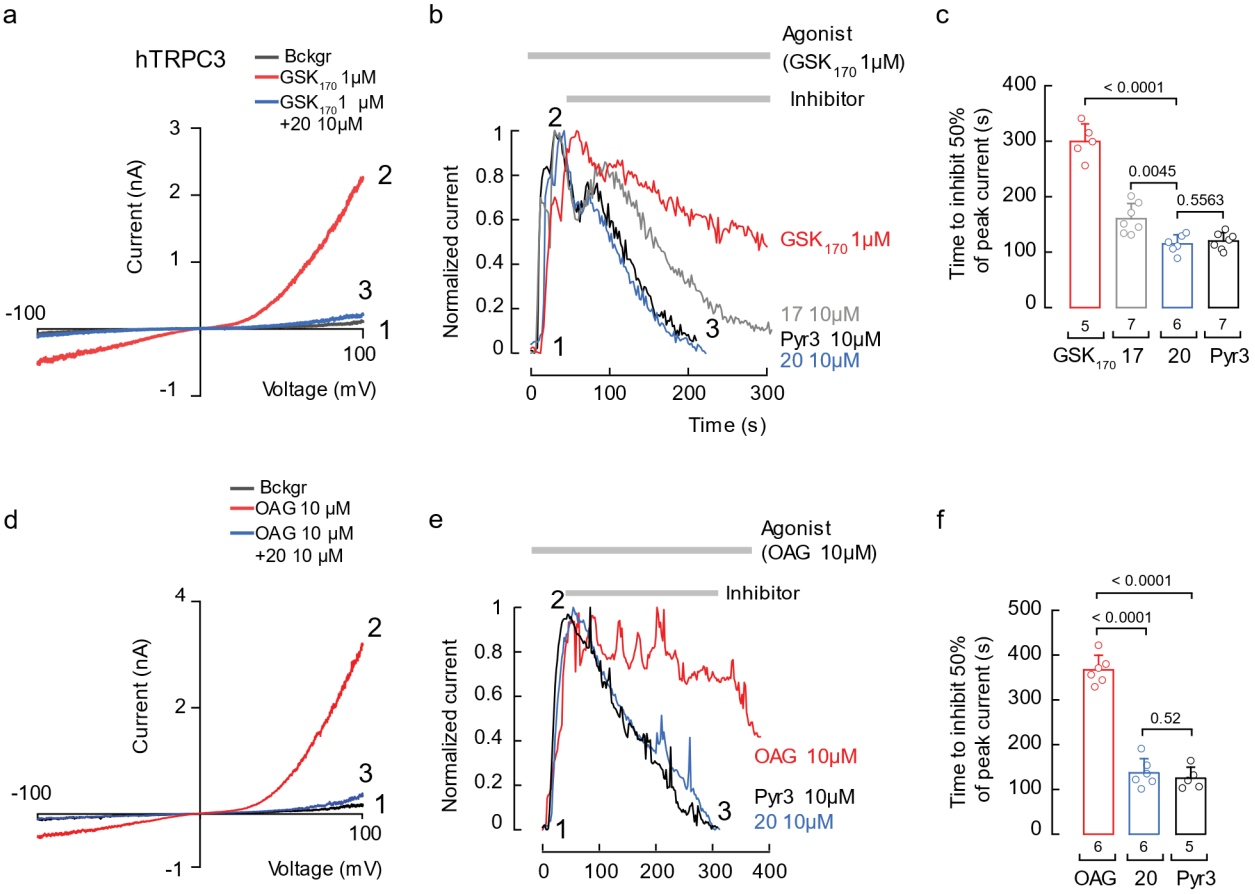


Figure S1. (a) Representative whole-cell recording from a HEK293 cell over-expressing hTRPC3 challenged by (1) voltage ramp alone, (2) GSK_{170} (agonist; 1 μM) alone, and (3) 1 μM GSK_{170} + 10 μM compound **20**. (b): Time course of peak currents taken from consecutive ramps depicting the events of (a), for Pyr3 (black), compound **20** (blue), compound **17** (gray), and GSK_{170} (1 μM) alone. All traces correspond to independent cells and preparations. (c): Time it takes for the response of hTRPC3 to GSK_{170} to decay to 50% of its peak (red), and the same when also adding 10 μM compound **17** (gray), compound **20** (blue), and Pyr3 to inhibit 50% of peak current elicited by 1 μM GSK_{170}. Bars are mean ± SD. n is denoted above the x-axis. Two-way Unpaired t-test and Mann-Whitney test, *p* values are noted above the bars. (d) Representative whole-cell recording from a HEK293 cell over-expressing hTRPC3 challenged by (1) voltage ramp alone, (2) OAG (agonist; 10 μM) alone, and (3) 10 μM OAG + 10 μM compound **20**. (e): Time course of peak currents taken from consecutive ramps depicting the events of (d), for Pyr3 (black), compound **20** (blue), and OAG (10 μM) alone. All traces correspond to independent cells and preparations. (f): Time it takes for the response of hTRPC3 to OAG to decay to 50% of its peak (red), and the same when also adding 10 μM compound **20** (blue), and Pyr3 to inhibit 50% of peak current elicited by 10 μM OAG. GSK_{170}: GSK1702934A; OAG: 1-Oleoyl-2-Acetyl-sn-Glycerol. Bars are mean ± SD. n is denoted above the *x*-axis. Two-way Unpaired *t*-test, *p* values are noted above the bars.

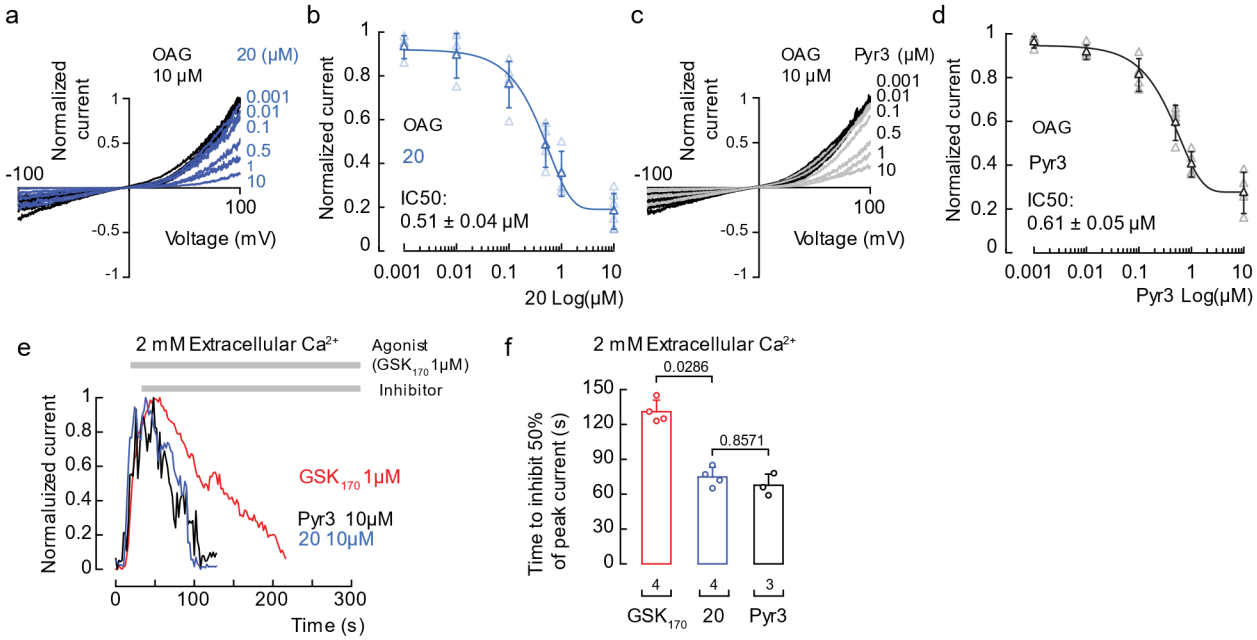


Figure S2. (a) Representative traces of hTRPC3 currents activated by 10 µM OAG and inhibited at different concentrations of compound **20** (blue traces). Each concentration was tested on independent cells and normalized against its internal control (maximum amplitude) to avoid tachyphylaxis. (b) Dose-response profile of hTRPC3 currents elicited by 10 µM OAG and inhibited at different concentrations of compound **20**. Circles represent mean ± SD. (c) Representative traces of hTRPC3 currents activated by 10 µM OAG and inhibited at different concentrations of Pyr3 (gray traces). Each concentration was tested on independent cells and normalized against its internal control (maximum amplitude) to avoid tachyphylaxis. (d) Dose-response profile of hTRPC3 currents elicited by 10 µM OAG and inhibited at different concentrations of Pyr3. Circles represent mean ± SD. (e) Time course of peak currents taken from consecutive ramps for hTRPC3 currents activated with GSK_{170} (red), 10 µM Pyr3 (black) or 10 µM compound **20** (blue), in the presence of 2 mM Ca^{2+} (2 mM). All traces correspond to independent cells and preparations. (f) Average time it takes for the response of hTRPC3 activated by 1 µM GSK_{170} to decay to 50% of its maximum currents (red), or when inhibiting with 10 µM compound **20** (blue) or Pyr3. GSK_{170}: GSK1702934A; OAG: 1-Oleoyl-2-Acetyl-sn-Glycerol. Bars represent mean ± SD. n are denoted above the x-axis. Mann-Whitney test, *p* values are noted above the bars.

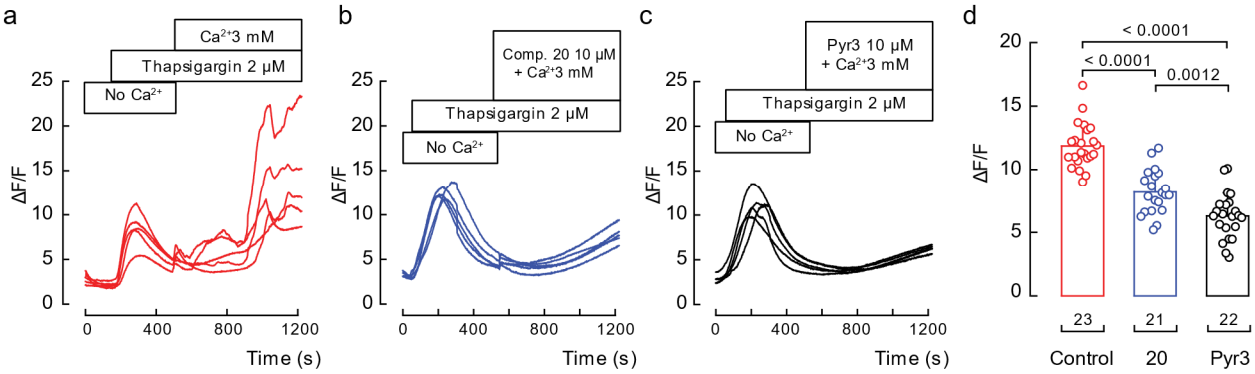


Figure S3. (a-c) Representative traces corresponding to normalized (ΔF/F) fluorescence intensity of HEK293 cells in a bath solution free of Ca^{2+} challenged with: thapsigargin (2 µM) and 3 mM Ca^{2+} (red) with **20** (10 µM; blue) or Pyr3 (10 µM; black). (d) Normalized fluorescence intensity of HEK293 cells in a bath solution free of Ca^{2+} challenged with: thapsigargin (2 µM) and 3 mM Ca^{2+} (red) with **20** (10 µM; blue) or Pyr3 (10 µM; black) at t = 1200 s. Bars are mean ± SD. n is denoted above the *x*-axis. Two-way unpaired *t*-test, *p* values are noted above the bars.

References

1. Di, L.; Kerns, E. H.; Li, S. Q.; Petusky, S. L. High throughput microsomal stability assay for insoluble compounds. *Int. J. Pharm.* **2006,** *317*, 54-60.
2. Di, L.; Kerns, E. H.; Ma, X. J.; Huang, Y.; Carter, G. T. Applications of high throughput microsomal stability assay in drug discovery. *Comb. Chem. High Throughput Screen.* **2008,** *11*, 469-476.
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