Pyrtriazoles, a Novel Class of Store-Operated Calcium Entry Modulators: Discovery, Biological Profiling and In Vivo Proof-of-Concept Efficacy in Acute Pancreatitis

Beatrice Riva[1,4#], Alessia Griglio[1#], Marta Serafini[1], Celia Cordero-Sanchez[1], Silvio Aprile[1], Rosanna Di Paola[2], Enrico Gugliandolo[2], Dalia Alansary[3], Isabella Biocotino[1], Dmitry Lim[1], Giorgio Grosa[1], Ubaldina Galli[1], Barbara Niemeyer[3], Giovanni Sorba[1], Pier Luigi Canonico[1], Salvatore Cuzzocrea[2], Armando A. Genazzani[1], Tracey Pirali[1,4]*@*

[1] Department of Pharmaceutical Sciences; Università del Piemonte Orientale; Novara, 28100; Italy; [2] Department of Chemical, Biological, Pharmaceutical and Enviromental Sciences; Università di Messina; Messina, 98166; Italy; [3] Department of Molecular Biophysics; Saarland University CIPMM; Homburg, 66421; Germany; [4] ChemICare S.r.l.; Enne3; Novara, 28100; Italy.

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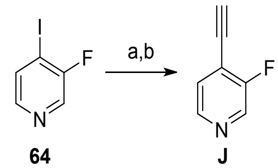
Molecular Formula Strings

Compound	SMILE	Residual SOCE activity (% of Ctrl)	IC50 (10 μM)
1H	O=C(C1=C(C(F)(F)F)N(C2=CC=C(N3N=NC(C4=CC=CC(N)=C4)=C3)C=C2)N=C1)OCC	81.7 ± 12.1	ND
1N	O=C(C1=C(C(F)(F)F)N(C2=CC=C(N3N=NC(C4=CC=C(O)C(OC)=C4)=C3)C=C2)N=C1)OCC	43.2 ± 3.7	ND
1S	O=C(O)C1=CC=CC(C2=CN(C3=CC=C(N4N=CC(C(OCC)=O)=C4C(F)(F)F)C=C3)N=N2)=C1	9.8 ± 0.5	0.6 ± 0.1
1T	O=C(O)C1=CC=C(C2=CN(C3=CC=C(N4N=CC(C(OCC)=O)=C4C(F)(F)F)C=C3)N=N2)C=C1	52.1 ± 4.2	ND
2S	O=C(O)C1=CC=CC(C2=CN(C3=CC=C(N4N=C(C(F)(F)F)C=C4C(F)(F)F)C=C3)N=N2)=C1	53.0 ± 8.7	ND
2T	O=C(O)C1=CC=C(C2=CN(C3=CC=C(N4N=C(C(F)(F)F)C=C4C(F)(F)F)C=C3)N=N2)C=C1	213.0 ± 0.8	ND
3D	O=C(O)CCCN1N=NC(C2=CC=C(N3N=CC(C(OCC)=O)=C3C(F)(F)F)C=C2)=C1	95.5 ± 10.1	ND
3G	O=C(C1=C(C(F)(F)F)N(C2=CC=C(C3=CN(C4=CC=C(Cl)C=C4)N=N3)C=C2)N=C1)OCC	141.6 ± 1.5	ND
3H	O=C(C1=C(C(F)(F)F)N(C2=CC=C(C3=CN(C4=CC=CC(N)=C4)N=N3)C=C2)N=C1)OCC	73.2 ± 3.3	ND
3J	O=C(C1=C(C(F)(F)F)N(C2=CC=C(C3=CN(C4=C(F)C=NC=C4)N=N3)C=C2)N=C1)OCC	81.4 ± 9.2	ND
3S	O=C(O)C1=CC=CC(N2N=NC(C3=CC=C(N4N=CC(C(OCC)=O)=C4C(F)(F)F)C=C3)=C2)=C1	30.0 ± 6.0	ND
3T	O=C(O)C1=CC=C(N2N=NC(C3=CC=C(N4N=CC(C(OCC)=O)=C4C(F)(F)F)C=C3)=C2)C=C1	69.3 ± 10.0	ND
19	O=C(O)C1=CC=CC(NC(C2=CC=C(N3N=CC(C(OCC)=O)=C3C(F)(F)F)C=C2)=O)=C1	> 90%	ND
22	O=C(O)C1=CC=CC(C(NC2=CC=C(N3N=CC(C(OCC)=O)=C3C(F)(F)F)C=C2)=O)=C1	63.5 ± 3.2	ND
23	O=C(O)C1=CC=CC(C2=CN=NN2C3=CC=C(N4N=CC(C(OCC)=O)=C4C(F)(F)F)C=C3)=C1	> 90%	ND
30	O=C(O)C1=CC=CC(C2=CN(C3=CC=C(N4N=CC(C(OC)=O)=C4C(F)(F)F)C=C3)N=N2)=C1	7.9 ± 1.8	ND
31	O=C(O)C1=CC=CC(C2=CN(C3=CC=C(N4N=CC(C(OC(C)C)=O)=C4C(F)(F)F)C=C3)N=N2)=C1	3.5 ± 0.3	3.1 ± 1.3
39	O=C(O)C1=CC=CC(C2=CN(C3=CC=C(N4N=CC(C(OC(CCC)C)=O)=C4C(F)(F)F)C=C3)N=N2)=C1	17.5 ± 1.6	4.4 ± 1.2
40	O=C(O)C1=CC=CC(C2=CN(C3=CC=C(N4N=CC(C(OCCC(C)C)=O)=C4C(F)(F)F)C=C3)N=N2)=C1	> 90%	ND
41	O=C(O)C1=CC=CC(C2=CN(C3=CC=C(N4N=CC(C(OC/C=C(C)/CC/C=C(C)\C)=O)=C4C(F)(F)F)C=C3)N=N2)=C1	> 90%	ND
42	O=C(O)C1=CC=CC(C2=CN(C3=CC=C(N4N=CC(C(OCC5=CC=NC=C5)=O)=C4C(F)(F)F)C=C3)N=N2)=C1	> 90%	ND
43	O=C(O)C1=CC=CC(C2=CN(C3=CC=C(N4N=CC(C(OCC5=CC=CC=C5)=O)=C4C(F)(F)F)C=C3)N=N2)=C1	10.5 ± 1.4	ND
44	O=C(O)C1=CC=CC(C2=CN(C3=CC=C(N4N=CC(C(OCCN5CCOCC5)=O)=C4C(F)(F)F)C=C3)N=N2)=C1	> 90%	ND
46	O=C(O)C1=CC=CC(C2=CN(C3=CC=C(N4N=CC(CO)=C4C(F)(F)F)C=C3)N=N2)=C1	> 90%	ND
48	O=C(O)C1=CC=CC(C2=CN(C3=CC=C(N4N=CC(C(NC(C)C)=O)=C4C(F)(F)F)C=C3)N=N2)=C1	> 90%	ND
49	O=C(O)C1=CC=CC(C2=CN(C3=CC=C(N4N=CC(C(N)=O)=C4C(F)(F)F)C=C3)N=N2)=C1	> 90%	ND
50	O=C(C1=C(C(F)(F)F)N(C2=CC=C(N3N=NC(C4=CC=CC(C(O)=O)=C4)=C3)C=C2)N=C1)O	> 90%	ND
51	O=C(C1=C(C(F)(F)F)N(C2=CC=C(N3N=NC(C4=CC=CC(C(NS(=O)(C)=O)=O)=C4)=C3)C=C2)N=C1)OCC	> 90%	ND
58	O=C(C1=C(C(F)(F)F)N(C2=CC=C(N3N=NC(C4=CC=CC(C(OC)=O)=C4)=C3)C=C2)N=C1)OCC	> 90%	ND
59	O=C(C1=C(C(F)(F)F)N(C2=CC=C(C3=CN(C4=CC=CC(C(C)=O)=C4)N=N3)C=C2)N=C1)OCC	> 90%	ND
60	O=C(C1=C(C(F)(F)F)N(C2=CC=C(N3N=NC(C4=CC=CC(C(N)=O)=C4)=C3)C=C2)N=C1)OCC	72.1 ± 2.5	ND
61	O=C(C1=C(C(F)(F)F)N(C2=CC=C(C3=CN(C4=CC=CC(CO)=C4)N=N3)C=C2)N=C1)OCC	> 90%	ND
62	O=C(C1=C(C(F)(F)F)N(C2=CC=C(N3N=NC(C4=CC=CC(S(=O)(N)=O)=C4)=C3)C=C2)N=C1)OCC	83.6 ± 4.1	ND
63	O=C(C1=C(C(F)(F)F)N(C2=CC=C(N3N=NC(C4=CC=CC(C(NO)=O)=C4)=C3)C=C2)N=C1)OCC	6.4 ± 5.7	ND

Chemistry

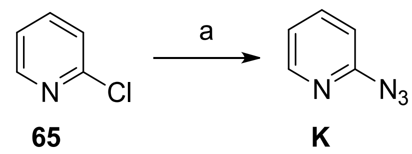
Synthesis and characterization of alkyne **J**



Reagents and conditions: (a) Ethynyltrimethylsilane, DIPEA, CuI, $Pd(PPh_3)_2Cl_2$, DMF, rt, 2 h. (b) TBAF, THF, 0 °C, 30 min, 45%.

*4-Ethynyl-3-fluoropyridine (**alkyne J**)*. **Step 1:** 3-Fluoro-4-iodopyridine (118 mg; 0.53 mmol), DMF (1.2 mL), $Pd(PPh_3)_2Cl_2$ (35 mg; 0.05 mmol), CuI (9.52 mg; 0.05 mmol), DIPEA (0.36 mL; 2.11 mmol) and ethynyltrimethylsilane (0.22 mL; 1.59 mmol) were added in a Schlenk apparatus under nitrogen atmosphere. After 2 h the mixture was filtered over a pad of celite, the volatile was removed under reduced pressure and the reaction was worked up by diluition with ethyl acetate and washed with water (x1). The organic layer was washed with brine, dried over sodium sulfate and evaporated. The crude product was used in the next step without further purification. **Step 2:** 3-fluoro-4-((trimethylsilyl)ethynyl) pyridine was dissolved in THF (3.3 mL) at 0 °C. After 5 min TBAF (0.62 mL; 0.62 mmol) was added. After 30 min the volatile was removed under reduced pressure and the reaction was worked up by dilution with ethyl acetate and washing with water (x1). The crude material was purified by column chromatography using petroleum ether/ethyl acetate 98:2 and then petroleum ether/ethyl acetate 95:5 as eluents yielding 4-ethynyl-3-fluoropyridine (28.9 mg, 0.23 mmol, 45%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.50 (s, 1H), 8.39 (d, J = 4.7 Hz, 1H), 7.38 (d, J = 5.5 Hz, 1H), 3.51 (s, 1H). MS (ESI): m/z: 122 $[M + H]^+$.

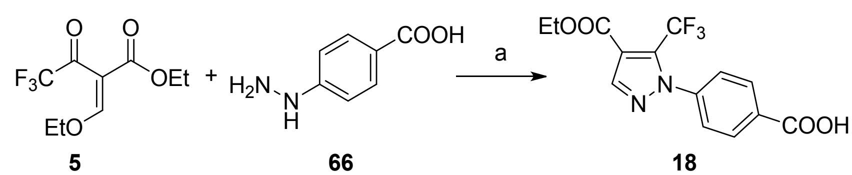
Synthesis and characterization of azide **K**



Reagents and conditions: (a) NaN_3, NH_4Cl, DMF, 110 °C, 10 h, 77%.

*2-azidopyridine, (**azide K**).* To a solution of 2-chloropyridine (2.00 g, 17.6 mmol) in DMF (20 mL) NaN_3 (2.29 g, 35.2 mmol) and NH_4Cl (1.88 g, 35.2 mmol) were added under nitrogen atmosphere. The mixture was heated at 110 °C overnight. The reaction was worked up by dilution with ethyl acetate and washing with water (x4), with brine, dried over sodium sulfate and evaporated to give 2-azidopyridine (1.63 g, 13.55 mmol, 77%) as a pale brown oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.83 (d, *J* = 6.9 Hz, 1H), 8.34 (d, *J* = 6.9 Hz, 1H), 7.65 (t, *J* = 6.9 Hz, 1H), 7.24 (t, *J* = 6.9 Hz, 1H).

Synthesis and characterization of compound **18**

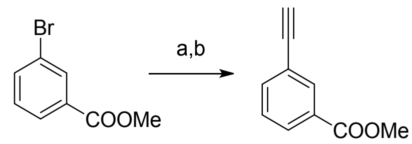


Reagents and conditions: (a) EtOH, THF, rt, 3 h, 93%.

*4-(4-(Ethoxycarbonyl)-5-(trifluoromethyl)-1H-pyrazol-1-yl)benzoic acid, (**18**).*

To a solution of 4-hydrazinylbenzoic acid (1 g, 6.58 mmol) in EtOH (10 mL), THF (2 mL) was added and the reaction mixture was cooled at -8 °C under nitrogen atmosphere. Then (*E*)-ethyl 2-(ethoxymethylene)-4,4,4-trifluoro-3-oxobutanoate (1.28 mL, 6.58 mmol) was added dropwise. After stirring at room temperature for 3 h the mixture was filtered under vacuum and rinsed with heptane to give compound **18** (2 g, 6.10 mmol, 93%) as a dark white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.36 (s, 1H), 8.14 (d, *J* = 7.5 Hz, 2H), 7.71 (d, *J* = 7.5 Hz, 2H), 4.36 (q, *J* = 7.1 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H). MS (ESI): *m/z*: 329 [M + H]$^+$.

Synthesis and characterization of alkyne **52**



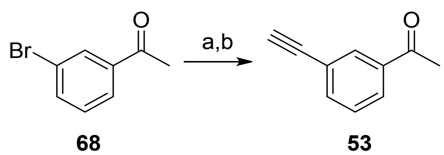
Reagents and conditions: (a) Ethynyltrimethylsilane, DIPEA, CuI, $Pd(PPh_3)_2Cl_2$, toluene, 100 °C, 6 h, 89%. (b) TBAF, CH_3COOH, THF, 0 °C, 1 h, 98%.

*Methyl 3-ethynylbenzoate, (**52**).*

Step 1: To a solution of methyl 3-bromobenzoate (2 g, 9.30 mmol) in toluene (50 mL) DIPEA (6.5 mL, 37.2 mmol), CuI (0.32 g, 1.67 mmol), $Pd(PPh_3)_2Cl_2$ (0.39 g, 0.56 mmol) and ethynyltrimethylsilane (5.25 mL, 37.2 mmol) were added in a Schlenk apparatus. The reaction was stirred at reflux for 6 h under nitrogen atmosphere. Then, the mixture was filtered over a pad of celite and rinsed with ethyl acetate. The organic phase was washed with water (x1), dried over sodium sulfate and evaporated. The crude material was purified by column chromatography using petroleum ether and petroleum ether/ethyl acetate 95:5 as eluents to give methyl 3-((trimethylsilyl)ethynyl)benzoate as a brown oil (1.95 g, 8.30 mmol, 89%). ^{1}H NMR (300 MHz; $CDCl_3$): δ = 8.10 (s, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.59 (d, J = 7.7 Hz, 1H), 7.32 (t, J = 7.7 Hz, 1H), 3.88 (s, 3H), 0.22 (s, 9H).

Step 2: Methyl 3-((trimethylsilyl)ethynyl)benzoate (0.60 g, 2.55 mmol) was dissolved in THF (6 mL). The mixture was cooled at 0 °C and CH_3COOH (175 µL, 3.06 mmol) and TBAF (3.06 mL, 3.06 mmol) were added. The reaction was stirred at 0 °C for 1 h. The volatile was removed under vacuum, ethyl acetate was added and the organic layer was washed with water (x1). After drying over sodium sulfate and evaporation of the solvent, the crude material was purified by column chromatography using petroleum ether and petroleum ether/ethyl acetate 95:5 as eluents to give compound **52** (0.40 g, 2.50 mmol, 98%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.17 (s, 1 H), 7.96 (d, J = 6.5 Hz, 1H), 7.67 (d, J = 6.5 Hz, 1H), 7.40 (t, J = 6.5 Hz, 1H), 3.92 (s, 3H), 3.12 (s, 1H). MS (ESI): m/z: 161 $[M + H]^+$.

Synthesis and characterization of alkyne **53**



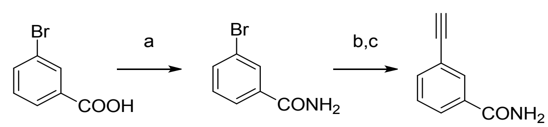
Reagents and conditions: (a) Ethynyltrimethylsilane, TEA, CuI, $Pd(PPh_3)_2Cl_2$, THF, 50 °C, 6 h, 98%. (b) K_2CO_3, MeOH, rt, 1 h, 98%.

*1-(3-Ethynylphenyl)ethanone, (**53**).*

Step 1: To a solution of 1-(3-bromophenyl)ethanone (2 g, 10.05 mmol) in THF (20 mL) TEA (2.1 mL, 15.07 mmol), CuI (7.62 mg, 0.04 mmol), $Pd(PPh_3)_2Cl_2$ (141.08 mg, 0.20 mmol) and ethynyltrimethylsilane (2.78 mL, 20.09 mmol) were added in a Schlenk apparatus. The reaction was stirred at 50 °C for 6 h under nitrogen atmosphere. Then, the mixture was filtered under vacuum and rinsed with ethyl acetate. The volatile was then removed under vacuum and the crude material was purified by column chromatography using petroleum ether/ethyl acetate 98:2 as eluent to give 1-(3-((trimethylsilyl)ethynyl)phenyl)ethanone as a brown oil (2.13 g, 9.85 mmol, 98%). ^{1}H NMR (300 MHz; $CDCl_3$): δ = 7.96 (s, 1H), 7.78 (d, J = 7.7 Hz, 1H), 7.56 (d, J = 7.7 Hz, 1H), 7.28 (t, J = 7.7 Hz, 1H), 3.02 (s, 3H), 0.24 (s, 9H).

Step 2: 1-(3-((Trimethylsilyl)ethynyl)phenyl)ethanone (1.84 g, 8.52 mmol) was dissolved in MeOH (18 mL) and K_2CO_3 (2.96 g, 21.4 mmol) was added. The reaction was stirred for 1 h. The volatile was removed under vacuum, ethyl acetate was added and the organic layer was washed with water (x1). After drying over sodium sulfate and evaporation of the solvent, the crude material was purified by column chromatography using petroleum ether/ethyl acetate 95:5 as eluent to give compound **53** (1.20 g, 8.33 mmol, 98%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.01 (s, 1H), 7.88 (d, J = 7.6 Hz, 1H), 7.61 (d, J = 7.6 Hz, 1H), 7.37 (t, J = 7.6 Hz, 1H), 3.13 (s, 1H), 2.56 (s, 3H). MS (ESI): *m/z*: 145 $[M + H]^+$.

Synthesis and characterization of alkyne **54**



69 **70** **54**

Reagents and conditions: (a) Oxalyl chloride, conc. NH_4OH, THF, DMF, rt, 30 min, 98%. (b) ethynyltrimethylsilane, DIPEA, CuI, $Pd(PPh_3)_2Cl_2$, toluene, 100 °C, 6 h, 48%. (c) TBAF, THF, 0 °C, 30 min, 79%.

*3-Bromobenzamide, (**70**).*

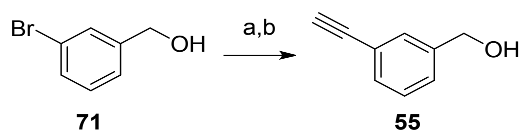
3-Bromobenzoic acid (2 g, 9.95 mmol) was dissolved in THF (20 mL) and oxalyl chloride (1.74 mL, 19.90 mmol) and DMF (62 µL) were added. After stirring at room temperature for 2 h, conc. NH_4OH (12 mL) was added. The reaction was stirred for additional 30 min and then was evaporated, affording compound **70** (2.11 g, 9.75 mmol, 98%) as a white solid. ^{1}H NMR (300 MHz; CD_3OD): δ = 8.04 (s, 1H) 7.84 (d, J = 7.6 Hz, 1H), 7.70 (d, J = 7.6 Hz, 1H), 7.39 (t, J = 7.6 Hz, 1H).

*3-Ethynylbenzamide, (**54**).*

Step 1: 3-((Trimethylsilyl)ethynyl)benzamide was synthesized as previously reported for the preparation of compound **52** in **step 1**, starting from compound **69** (1.30 g, 6.02 mmol). The crude material was subjected to chromatography column using petroleum ether/ethyl acetate 7:3 and petroleum ether/ethyl acetate 5:5 as eluents, yielding 3-((trimethylsilyl)ethynyl)benzamide (0.63 g, 2.91 mmol, 48%) as a brown oil. ^{1}H NMR (300 MHz; $CDCl_3$): δ = 7.88 (s, 1H), 7.76 (d, *J* = 7.9 Hz, 1H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.42 (t, *J* = 7.9 Hz, 1H), 0.26 (s, 9H).

Step 2: 3-((Trimethylsilyl)ethynyl)benzamide (0.63 g, 2.89 mmol) was dissolved in THF (13 mL). The mixture was cooled at 0 °C and TBAF (3.47 mL, 3.47 mmol) was added. The reaction was stirred at 0 °C for 30 min. The volatile was removed under vacuum, water was added and the aqueous layer was extracted with ethyl acetate (x3). The organic phase was dried over sodium sulfate and evaporated. The crude material was purified by column chromatography using petroleum ether/ethyl acetate 5:5 as eluent to give compound **54** (0.33 g, 2.25 mmol, 79%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.91 (s, 1 H), 7.80 (d, *J* = 7.7 Hz, 1H), 7.64 (d, *J* = 7.7 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 1H), 3.13 (s, 1H). MS (ESI): *m/z*: 146 $[M + H]^+$.

Synthesis and characterization of alkyne **55**



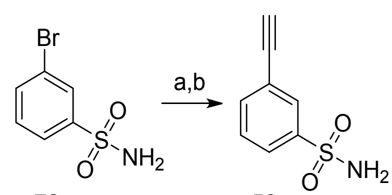
Reagents and conditions: (a) Ethynyltrimethylsilane, PPh_3, $Pd(OAc)_2$, TEA, 94 °C, 1 h, 65%. (b) K_2CO_3, MeOH, rt, 30 min, 49%.

*(3-Ethynylphenyl)methanol, (**55**).*

Step 1: In a Schlenk apparatus (3-bromophenyl)methanol (1.5 g, 8.02 mmol), TEA (7.5 mL), PPh_3 (42.02 mg, 0.16 mmol), $Pd(OAc)_2$ (18.01 mg, 0.08 mmol) and ethynyltrimethylsilane (2.23 mL, 16.04 mmol) were added. The reaction was stirred at 94 °C for 1 h under nitrogen atmosphere. Then, the mixture was filtered under vacuum and rinsed with ethyl acetate. The organic phase was washed with water (3x) and dried over sodium sulfate. The volatile was then removed under vacuum and the crude material was purified by column chromatography using petroleum ether/ethyl acetate 95:5 and petroleum ether/ethyl acetate 9:1 as eluents to give (3-((trimethylsilyl)ethynyl)phenyl)methanol as a brown oil (1.07 g, 5.25 mmol, 65%). ^{1}H NMR (300 MHz; $CDCl_3$): δ = 7.96 (s, 1H), 7.26 (d, J = 7.2 Hz, 1H), 7.18 (d, J = 7.2 Hz, 1H), 6.98 (t, J = 7.2 Hz, 1H), 4.50 (s, 2H), 0.21 (s, 9H).

Step 2: The title compound was synthesized as previously reported for the preparation of compound **53** in **step 2**, starting from (3-((trimethylsilyl)ethynyl)phenyl)methanol (1.07 g, 5.25 mmol). The crude material was subjected to chromatography column using petroleum ether/ethyl acetate 95:5 and petroleum ether/ethyl acetate 9:1 as eluents, yielding compound **55** (0.34 g, 2.52 mmol, 49%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.42 (s, 1H), 7.32 (d, J = 6.2 Hz, 1H), 7.22-7.11 (m, 2H), 4.56 (s, 2H), 3.01 (s, 1H). MS (ESI): *m/z*: 133 $[M + H]^+$.

Synthesis and characterization of alkyne **56**



Reagents and conditions: (a) Ethynyltrimethylsilane, DIPEA, CuI, $Pd(PPh_3)_2Cl_2$, toluene, 100 °C, 6 h, 99%. (b) K_2CO_3, MeOH, rt, 30 min, 89%.

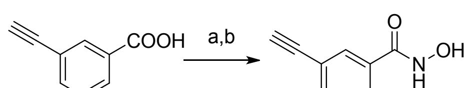
*3-Ethynylbenzenesulfonamide, (**56**).*

Step 1: 3-((Trimethylsilyl)ethynyl)benzenesulfonamide was synthesized as previously reported for the preparation of compound **52** in **step 1**, starting from compound **72** (0.50 g, 2.12 mmol). The crude material was subjected to chromatography column using petroleum ether/ethyl acetate 95:5 and petroleum ether/ethyl acetate 8:2 as eluents, yielding 3-((trimethylsilyl)ethynyl)benzenesulfonamide

(0.53 g, 2.09 mmol, 99%) as a brown oil. ^{1}H NMR (300 MHz; $CDCl_3$): δ = 8.02 (s, 1H), 7.85 (d, *J* = 7.6 Hz, 1H), 7.64 (d, *J* = 7.6 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 1H), 0.25 (s, 9H).

Step 2: The title compound was synthesized as previously reported for the preparation of compound **53** in **step 2**, starting from 3-((trimethylsilyl)ethynyl)benzenesulfonamide (0.53 g, 2.11 mmol). After evaporation, ethyl acetate wad added and the organic phase was washed with 3N HCl, dried over sodium sulfate and evaporated. The crude material was subjected to chromatography column using petroleum ether/ethyl acetate 7:3 as eluent, yielding compound **56** (0.34 g, 1.88 mmol, 89%) as a yellow oil. ^{1}H NMR (300 MHz; CD_3OD): δ = 8.03 (s, 1 H), 7.87 (d, *J* = 7.7 Hz, 1H), 7.67 (d, *J* = 7.7 Hz, 1H), 7.53 (t, *J* = 7.7 Hz, 1H), 3.67 (s, 1H). MS (ESI): *m/z*: 182 $[M + H]^+$.

<u>Synthesis and characterization of alkyne **57**</u>



S **57**

Reagents and conditions: (a) *O*-(Trimethylsilyl)hydroxylamine, TEA, EDCI, dry CH_2Cl_2, rt, 4 h. (b) TBAF, THF, 0 °C, 1 h, 70%.

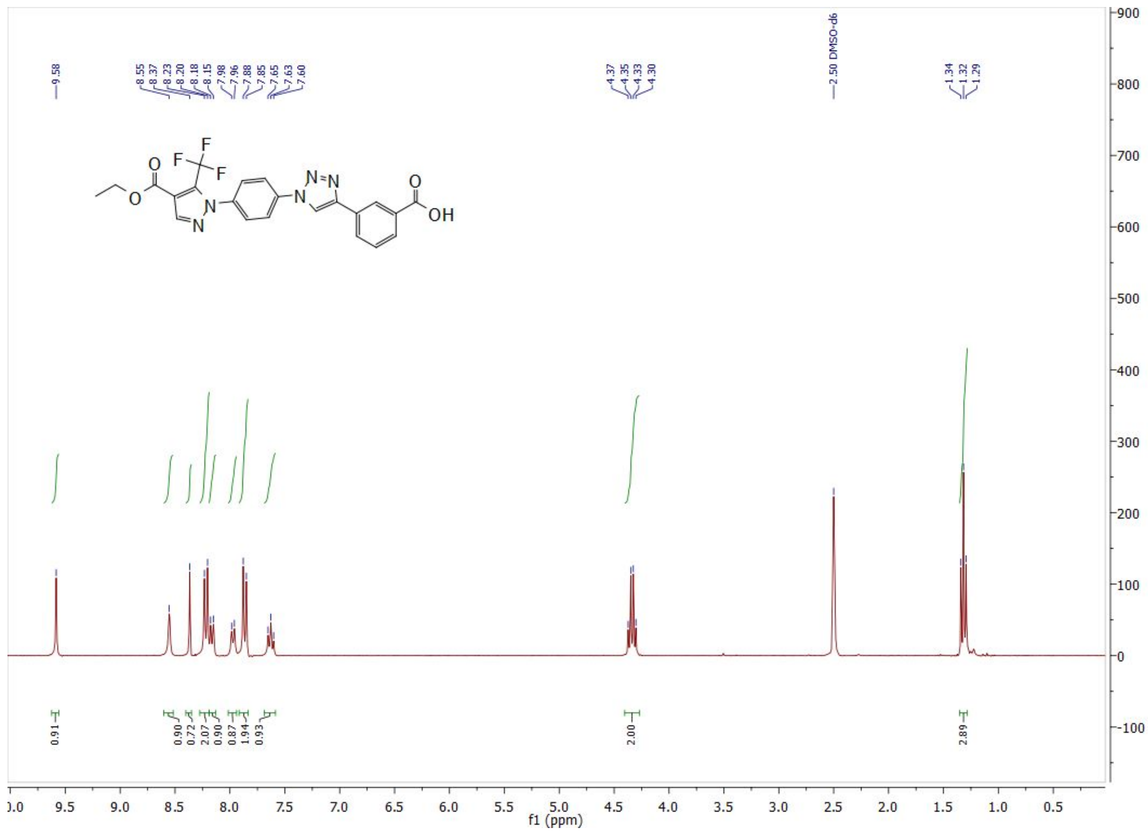
*3-Ethynyl-N-hydroxybenzamide, (**57**).*

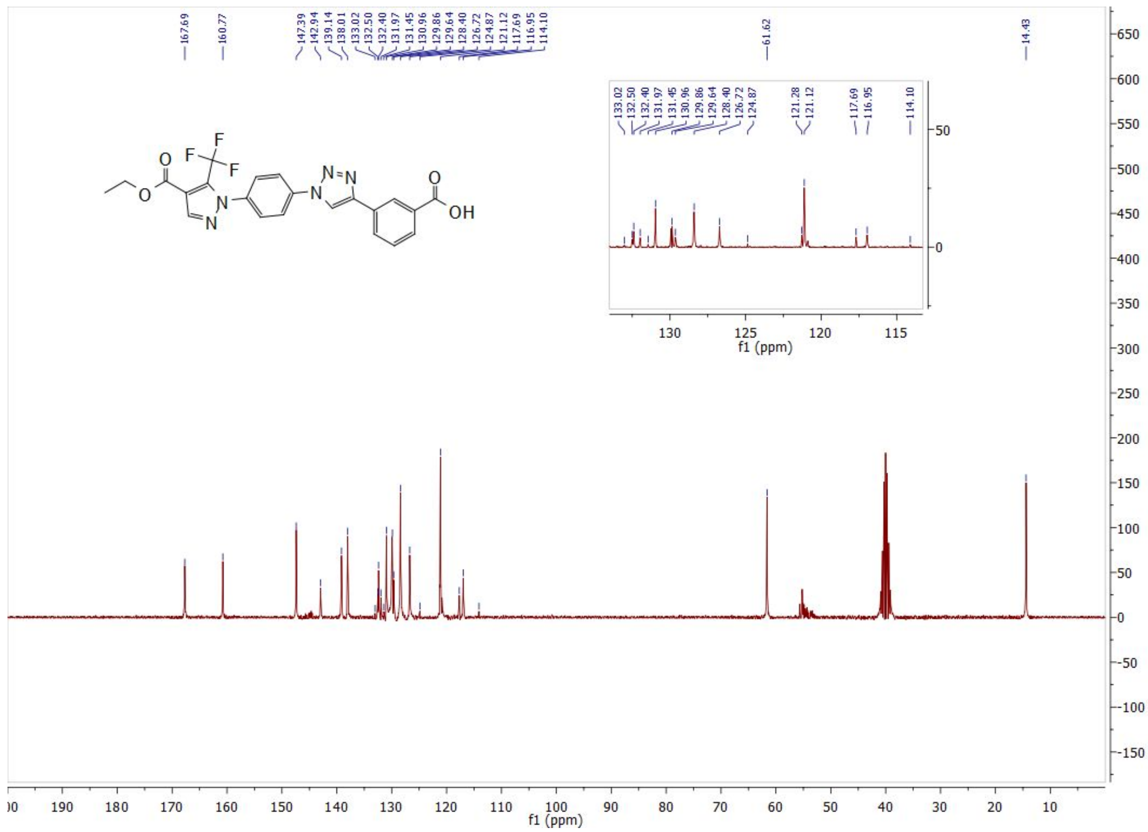
To a solution of alkyne **S** (99 mg, 0.68 mmol) in dry CH_2Cl_2 (1 mL), TEA (94 µL, 0.68 mmol), EDCI (143 mg, 0.68 mmol) and *O*-(trimethylsilyl)hydroxylamine (99.6 mg, 0.68 mmol) were added in order under nitrogen atmosphere. After 4 h, the reaction was finished. The volatile was removed under vacuum and the crude material was dissolved in THF (3.2 mL). The mixture was cooled at 0 °C and TBAF (0.68

mL, 0.68 mmol) was added. The reaction was stirred for 1 h at 0 °C and then ethyl acetate was added. The organic phase was washed with 3N HCl (1x), dried over sodium sulfate and evaporated. The crude material was purified by column chromatography using ethyl acetate and ethyl acetate/methanol 8:2 as eluents, yielding compound **57** (76.6 mg, 0.48 mmol, 70%) as a white solid. ^{1}H NMR (300 MHz; CD_3OD): δ = 7.84 (s, 1H), 7.73 (d, *J* = 7.7 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 1H), 3.56 (s, 1H). MS (ESI): *m/z*: 162 $[M + H]^{+}$.

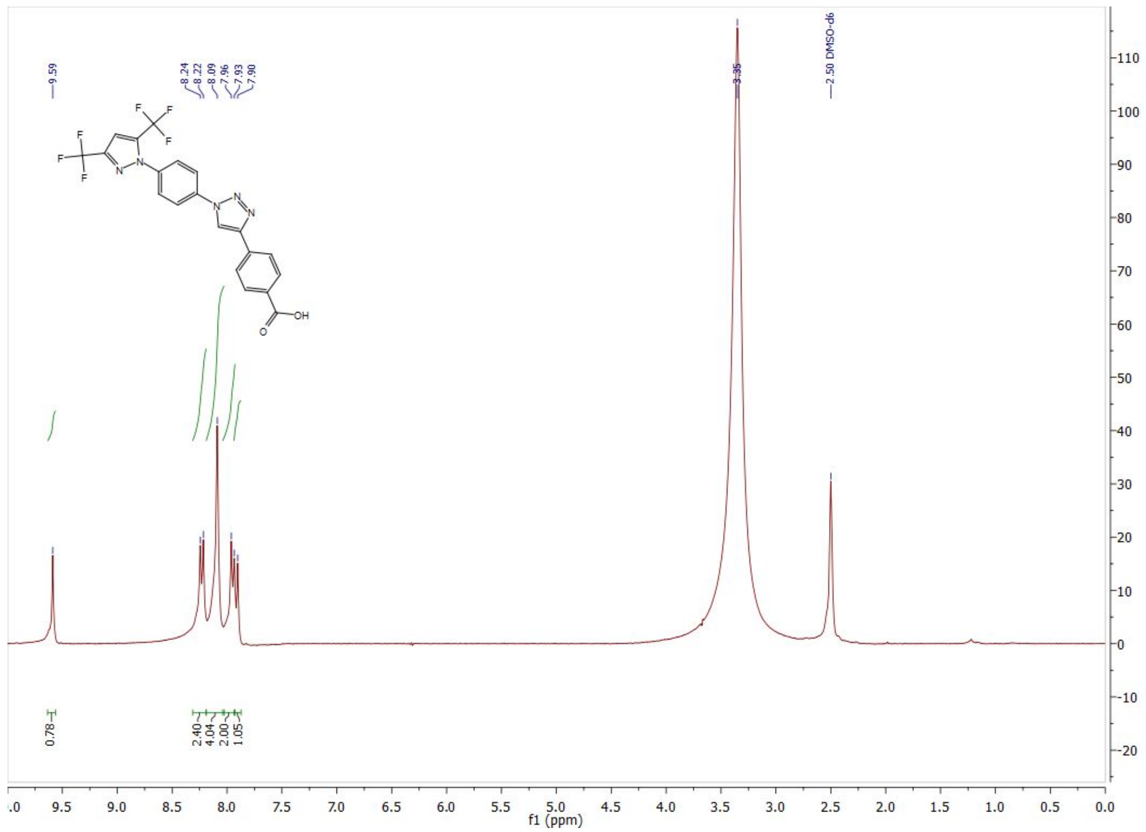
NMR Spectra of compounds 1S, 2T, 3G, 31, 39

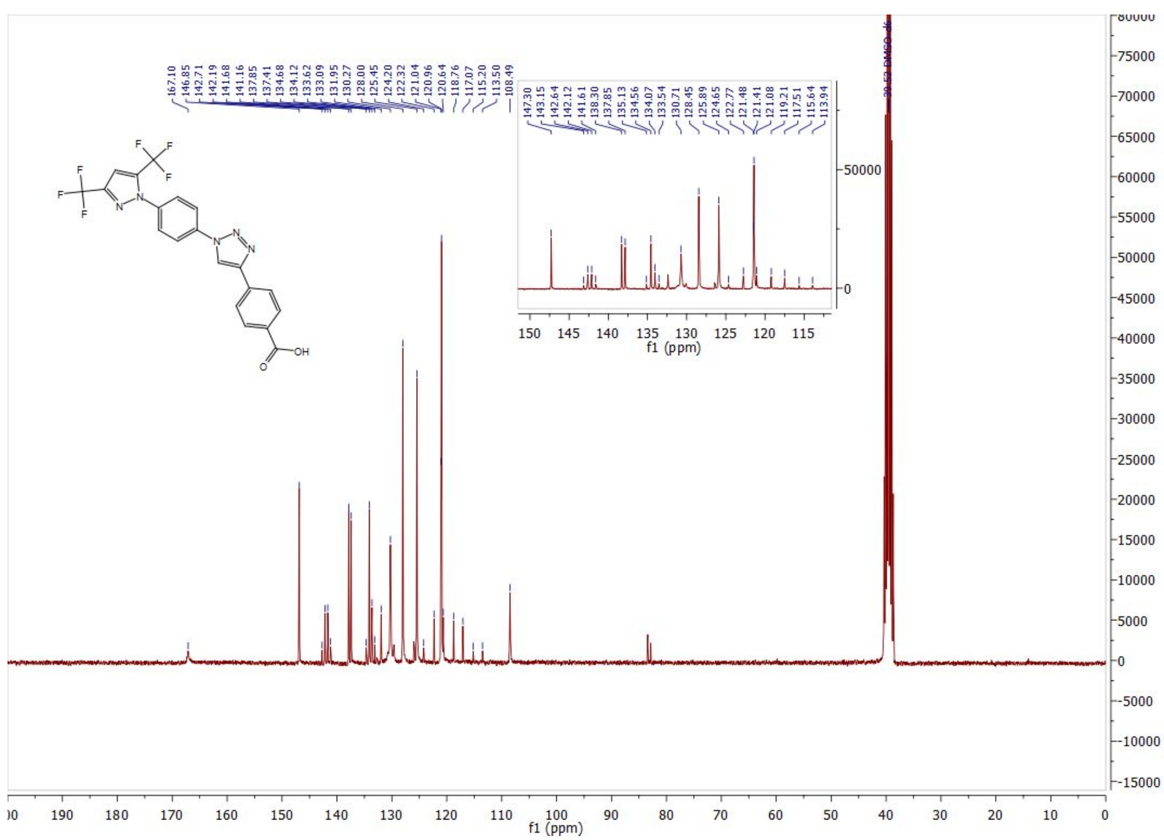
1S: ^{1}H (DMSO-d_6), ^{13}C (DMSO-d_6)



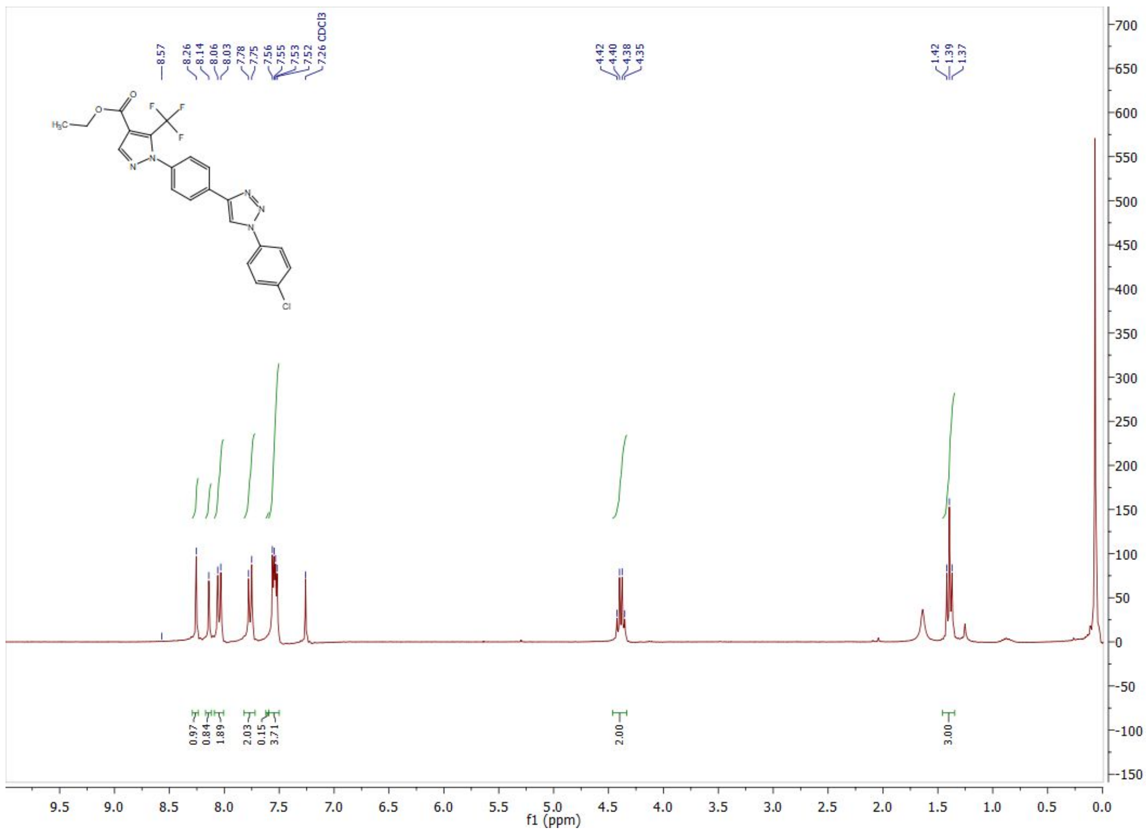


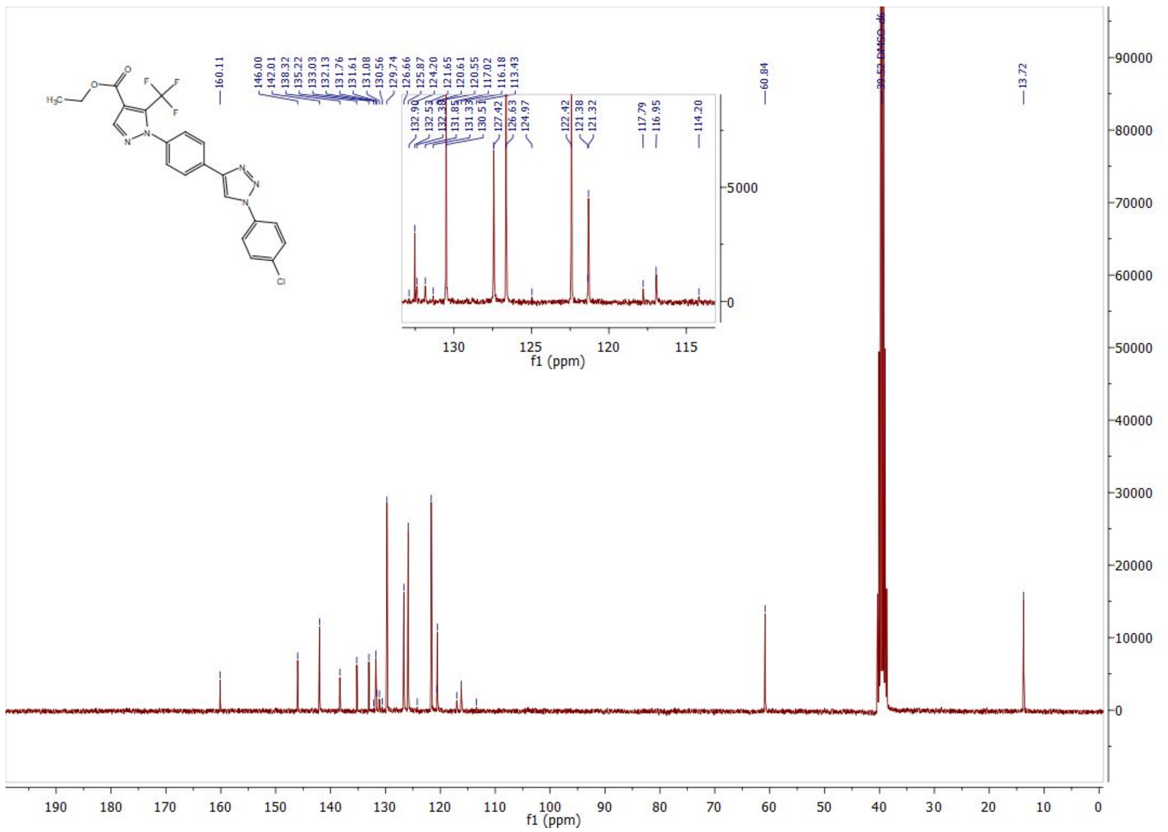
2T: ^{1}H (DMSO-d_6), ^{13}C (DMSO-d_6)


9.59
8.24
8.22
8.09
7.96
7.93
7.90
3.35
2.50 DMSO-d6
0.78
2.40
4.04
2.00
1.05
F
N
OH
O
f1 (ppm)

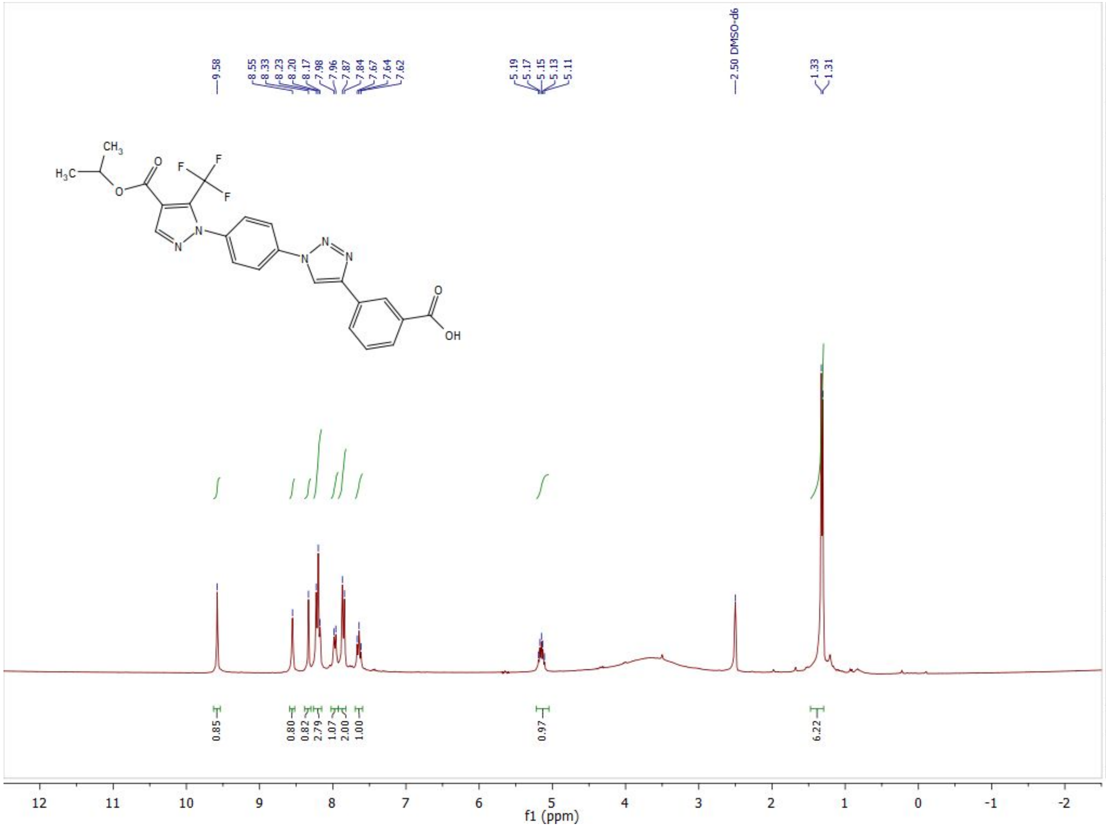

167.10
146.85
142.71
142.19
141.68
141.16
137.85
137.41
134.68
134.12
133.62
133.09
131.95
130.27
128.00
125.45
124.20
122.32
121.04
120.96
120.64
118.76
117.07
115.20
113.50
108.49
147.30
143.15
142.64
142.12
141.61
138.30
137.85
135.13
134.56
134.07
133.54
130.71
128.45
125.89
124.65
122.77
121.48
121.41
121.08
119.21
117.51
115.64
113.94
f1 (ppm)
f1 (ppm)

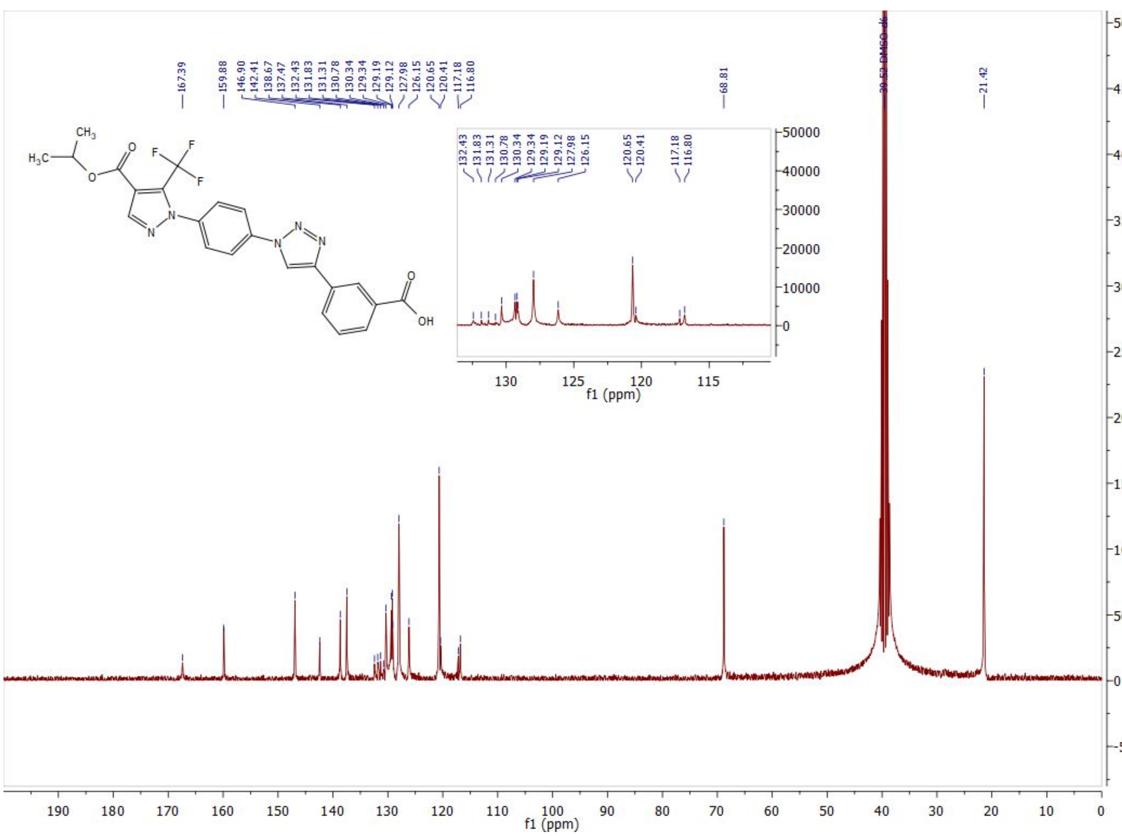
3G: ^{1}H (CDCl$_3$), ^{13}C (DMSO-d_6)



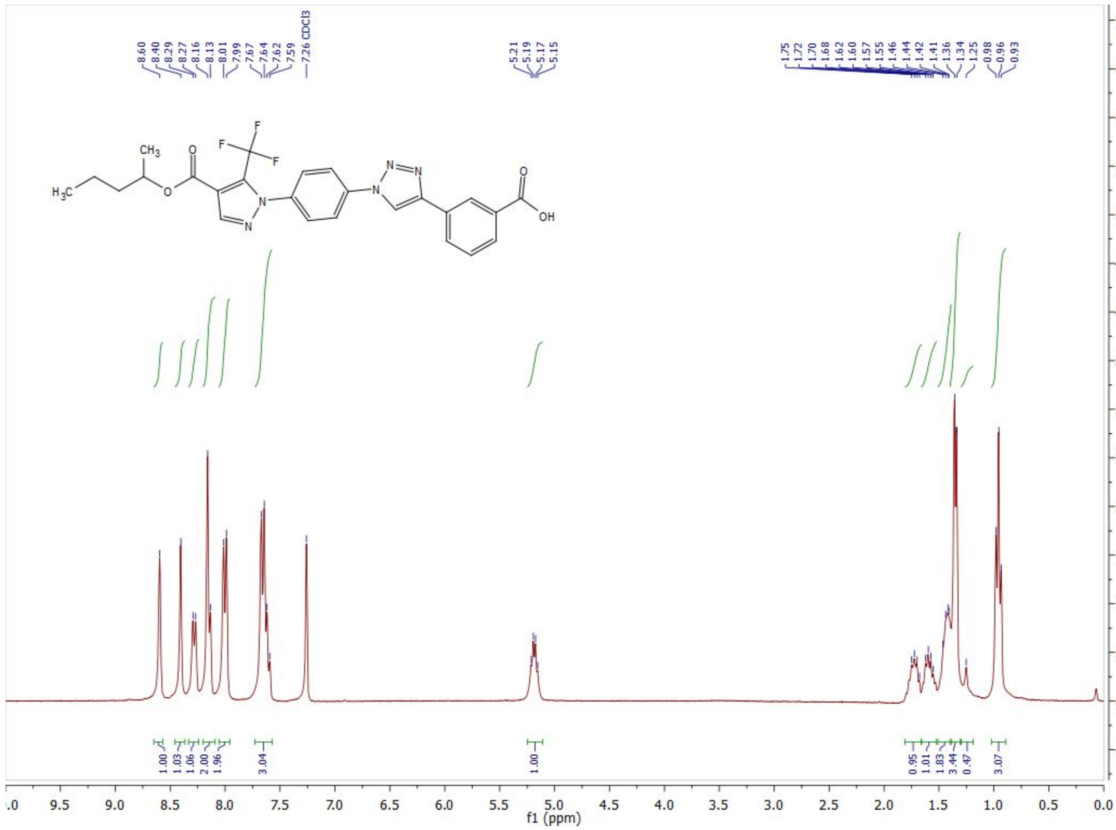


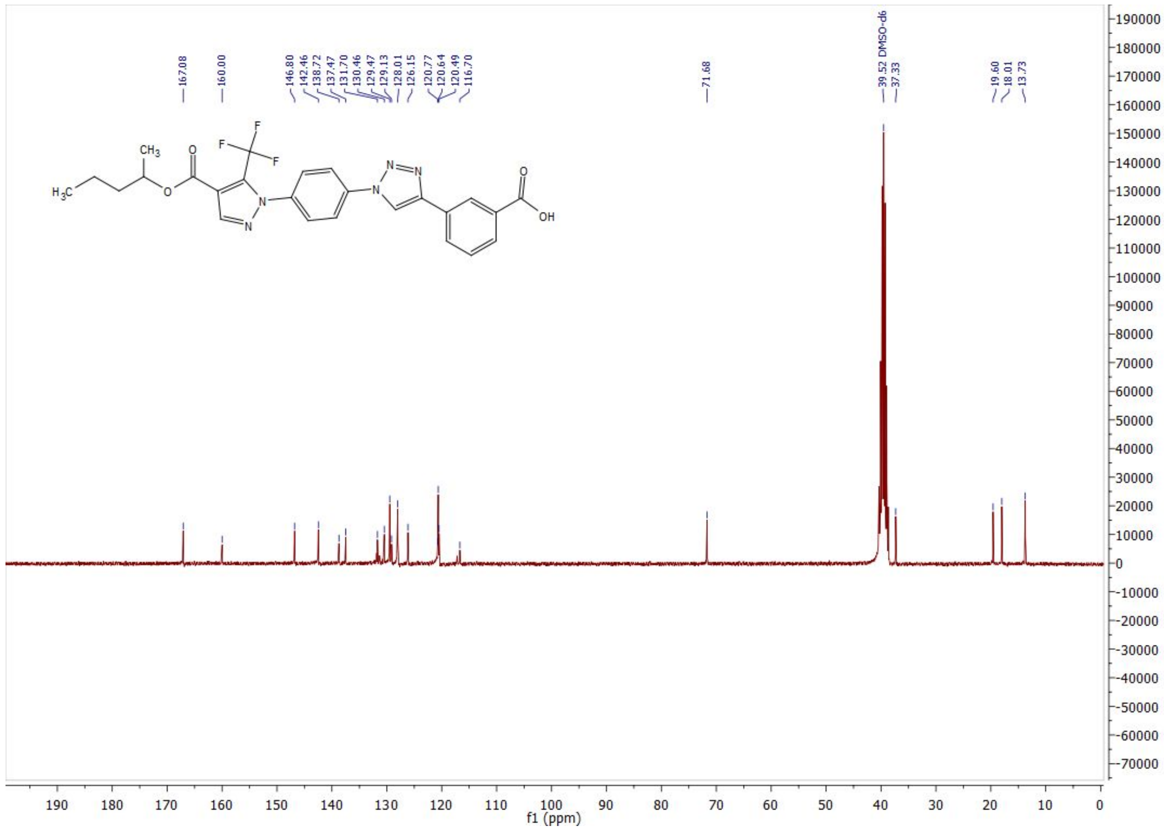
31: ^{1}H (DMSO-d_6),^{13}C (DMSO-d_6)





39: ^{1}H ($CDCl_3$), ^{13}C (DMSO-d_6)





167.08
160.00
146.80
142.46
138.72
137.47
131.70
130.46
129.47
129.13
128.01
126.15
120.77
120.64
120.49
116.70
71.68
39.52 DMSO-d6
37.33
19.60
18.01
13.73
f1 (ppm)

Biology

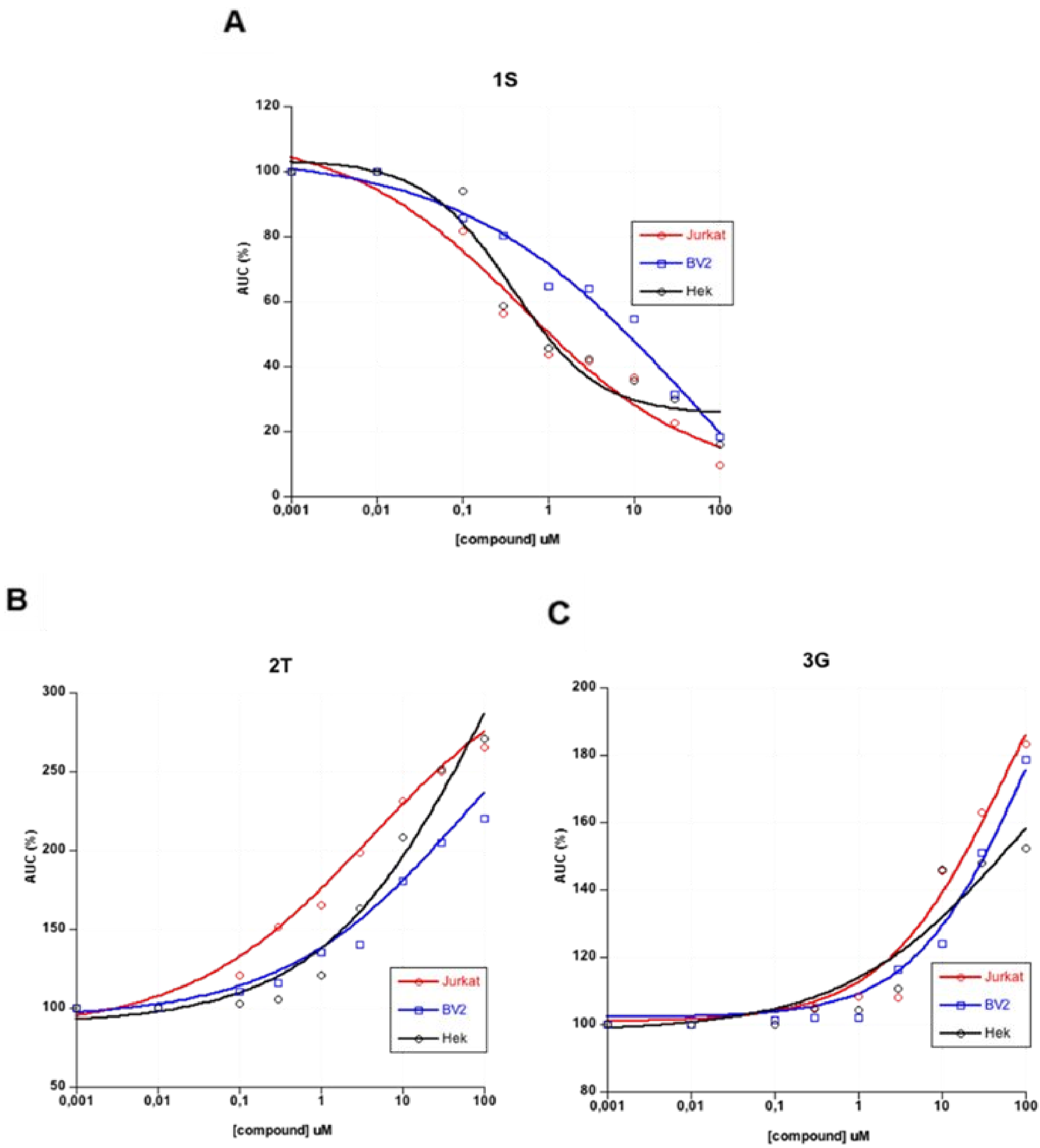


Figure S1. Concentration-response curves of 1S (A), 2T (B) and 3G (C) in Hek, Jurkat and BV-2 cell lines.

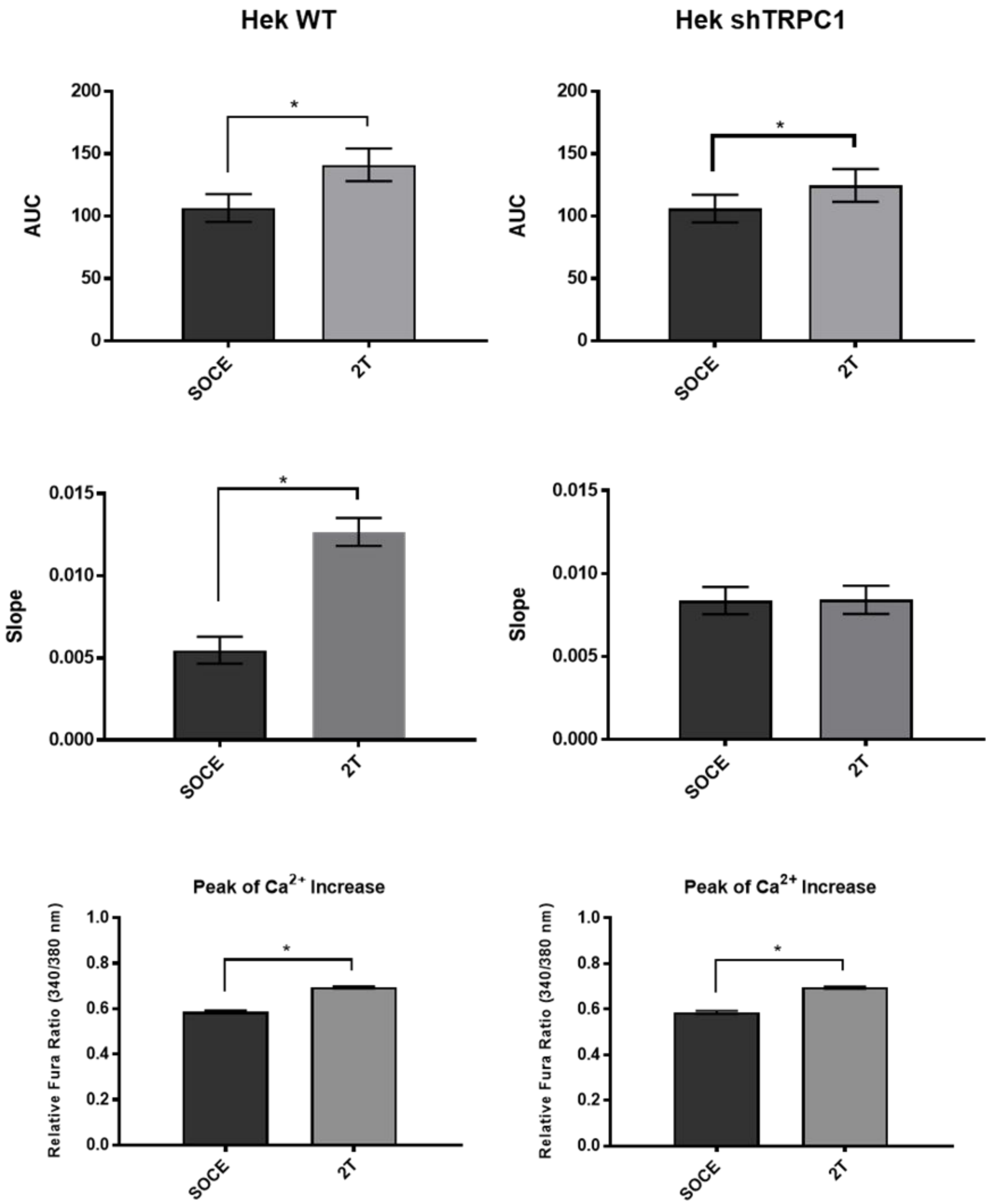


Figure S2. Effect of 2T on the area under the curve, the peak amplitude, and the slope of the Ca^{2+}-rise in wild-type and shTRPC1 Hek cells. Bottom histograms represent AUC, peak amplitude and initial rate. **2T** and **3G** were used at a concentration of 10 μM.

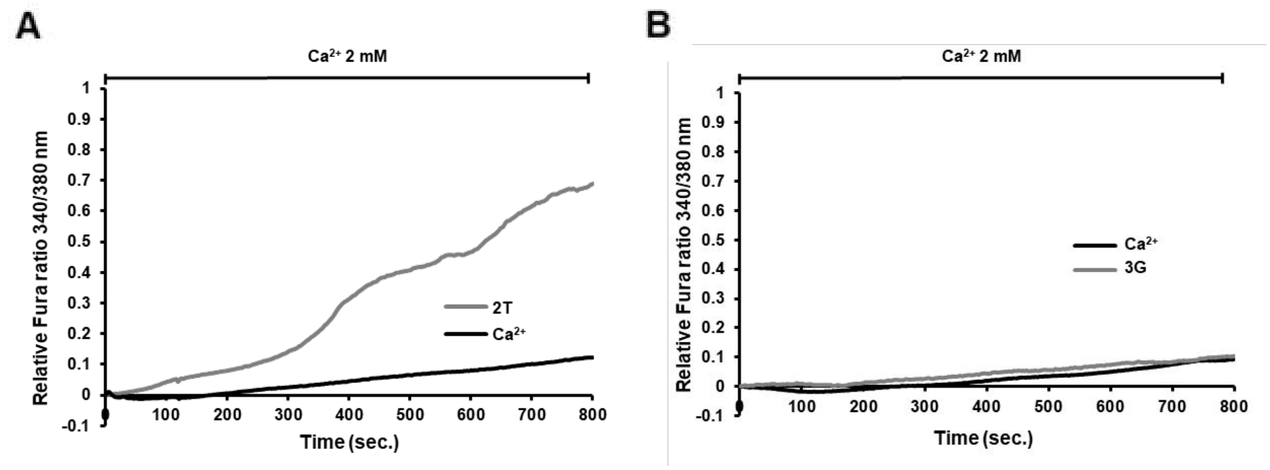


Figure S3. Effect of 2T (A) or 3G (B) (both at 10 μM) under basal conditions, with no Ca^{2+}-emptying. Hek cells were monitored for 800 seconds, as depicted in the traces. Traces are average of 360 cells from 12 plates in four separate experimental days.

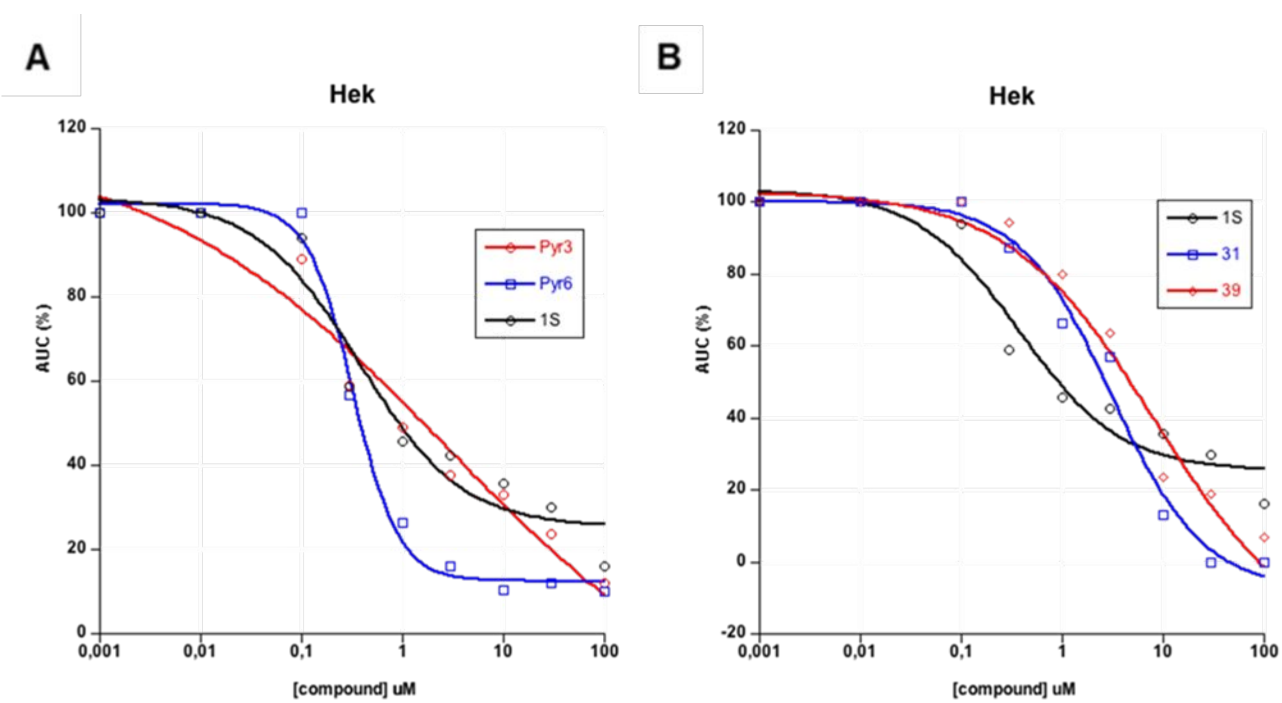


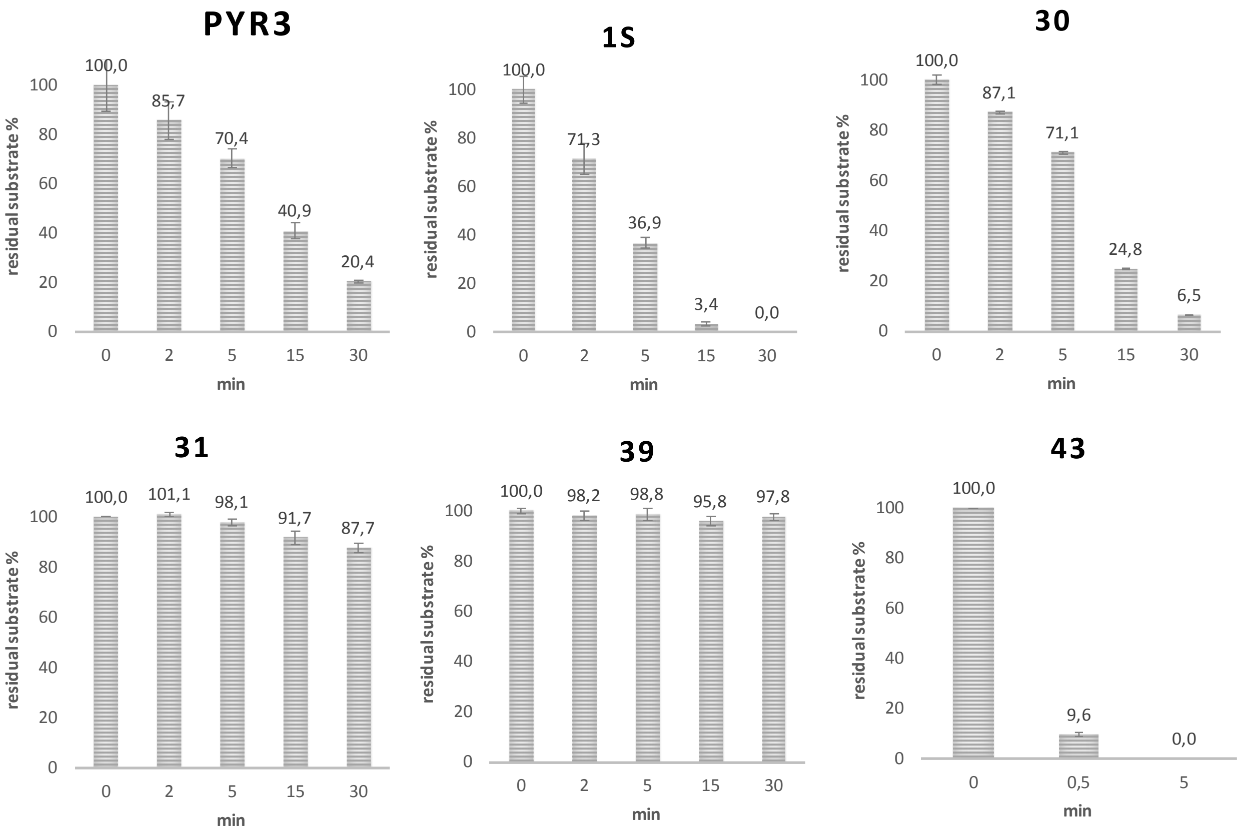
Figure S4. Concentration-response curves of Pyr3, Pyr6 and selected compounds in Hek cells.

Gene Symbol	Mouse Primers	Human Primers
TRPC1	FW 5'-GGACAGCCTCAGACATTCCA-3', RV 5'-CGGGCTAGCTCTTCATAATCA-3'	FW 5'-ATCTCTACCCAAGCCCCATG-3' RV 5'-ACATTATTAGAGCTGGACTGGC-3'
TRPC2	FW 5'-AGAGGCAGAGCTGGAGTTCA-3' RV 5'-GGGGAATTTGAGCTTTTTGTT-3'	-------
TRPC3	FW 5'-GATCAATGCCTACAAGGGACT-3' RV 5'-TGCATTGCATGGAGAGTTTC-3'	FW 5'-CTTACGGCCCTAGAGCTCAG-3' RV 5'-CCAGCACACCCACTACAAAG-3'
TRPC4	FW 5'-ACGCCATCAGAAAAGAGGTG-3' RV 5'-CCAAGATGATGGGTGTGATG-3'	FW 5'-AAGAAGTCGTCGGAGCTGTT-3' RV 5'-TGTCTGGAGTGAATTCAGAGAAC-3'
TRPC5	FW 5'-GAGGTGGTAGGAGCTGTGGA-3' RV 5'-TGCCAACATAATGGGAGTGA-3'	FW 5'-CAGCGTGTATGTGGGTGATG-3' RV 5'-AGAGAACTGCGTGTCCATCA-3'
TRPC6	-------	FW 5'-ATCTGCTCATGGACTCGGAG-3' RV 5'-AACCTTCTCCCCTTCTCACG-3'
TRPC7	-------	FW 5'-CAAGCCTGCGTATTACACCC-3' RV 5'-CTCGTTGAACATGTAGGCGG-3'
STIM1	FW 5'-TCTGAAGAGTCTACCGAAGCAG-3' RV 5'-TGGTAATTGAGGTCTTCCCTTAG-3'	FW 5'-GCCTCAGCCATAGTCACAGT-3' RV 5'-ATGTTACGGACTGCCTCGAA-3'
STIM2	FW 5'-GACGGATGCGATCTGGTG-3' RV 5'-TTCAGTGAAGCAAGGTGGACT-3'	FW 5'-GACGGATGCGAGCTTGTG-3' RV 5'-AAGCATGGTGGACTCAGTGA-3'
Orai1	FW 5'-CCTGGCGCAAGCTCTACTTA-3' RV 5'-TGCAGGCACTAAAGACGATG-3'	FW 5'-GACCTCGGCTCTGCTCTC-3' RV 5'-TGATCATGAGCGCAAACAGG-3'
Orai2	FW 5'-CACTGTCCTGGAGGAAGCTC-3' RV 5'-GGGCTGAGGGTACTGGTACTT-3'	FW 5'- CCCTCCTCTCCGGCTTTG-3' RV 5'- TGATGAGGAGGGCGAACAG-3'
Orai3	FW 5'-GAACCCGGAGGTGGACAG-3' RV 5'-GCTGGAGGCTTTGAGCTTAG-3'	FW 5'-ACGTCTGCCTTGCTCTCG-3' RV 5'-ACCATGAGTGCAAAGAGGTG-3'

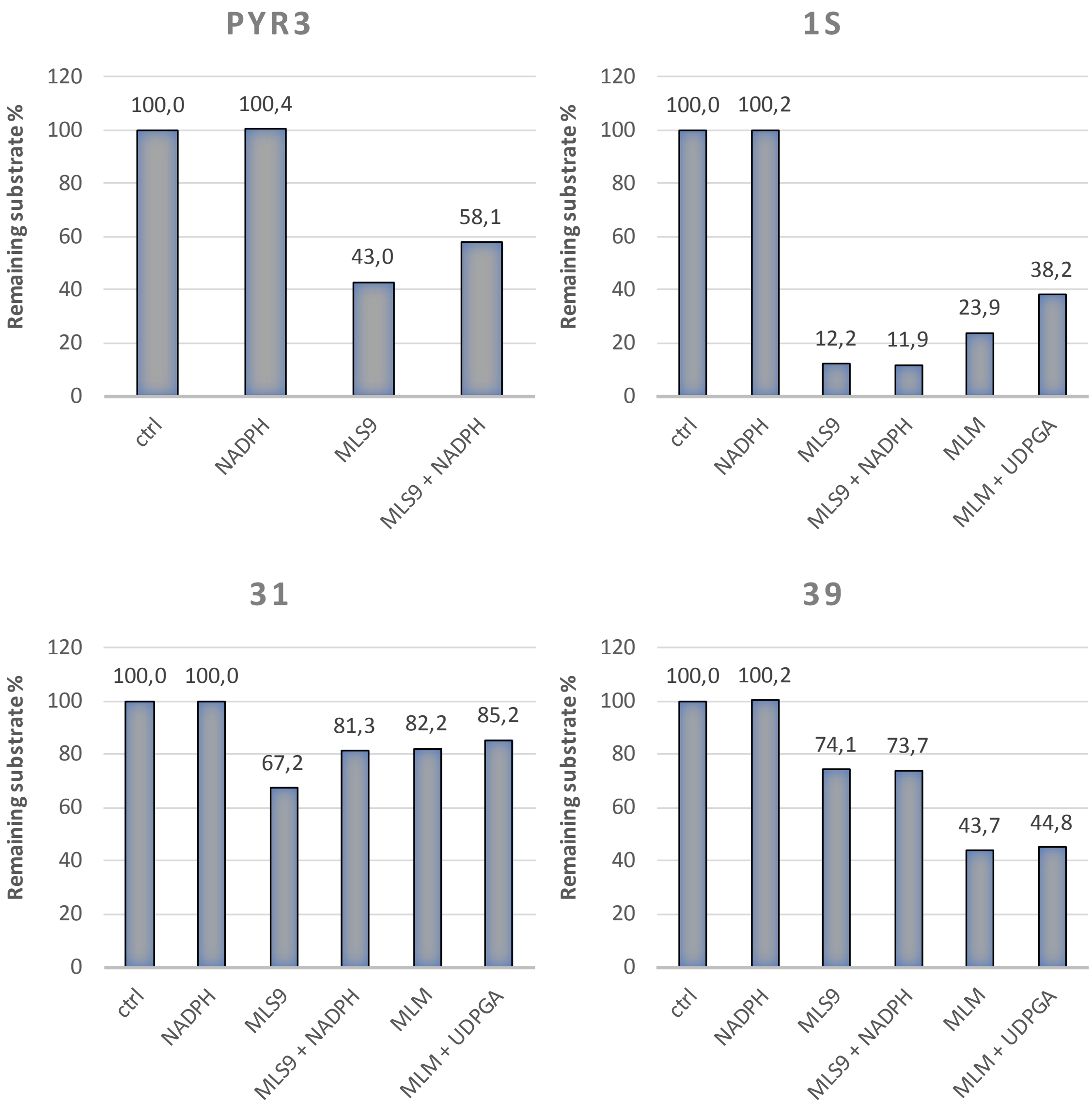
Table S1. List of primers used in the present manuscript to determine transcript levels

Metabolic stability data

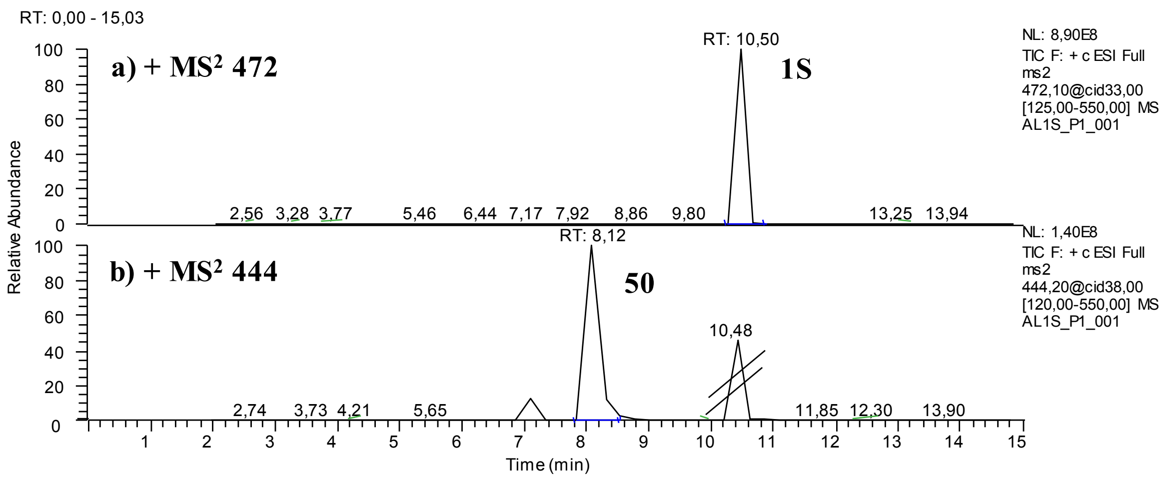
Hydrolytic stability of selected compounds in mouse plasma. All data are expressed as mean (SEM), n = 3 in three independent experiments.

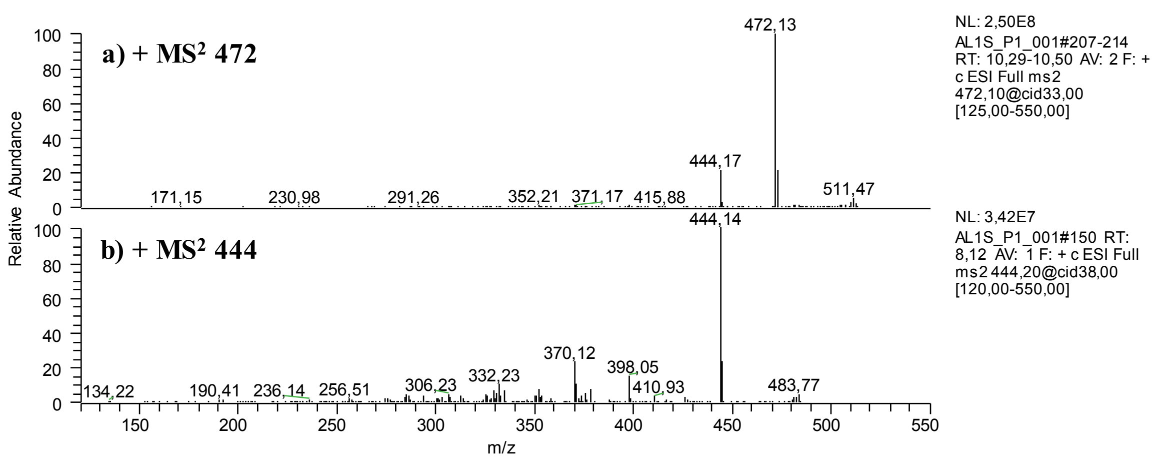


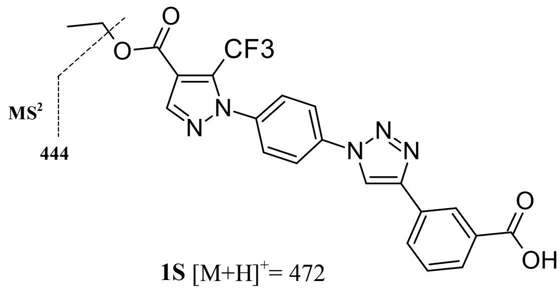
Metabolic stability data of selected compounds in mouse liver S9 (MLS9) and mouse liver microsomes (MLM) fractions.



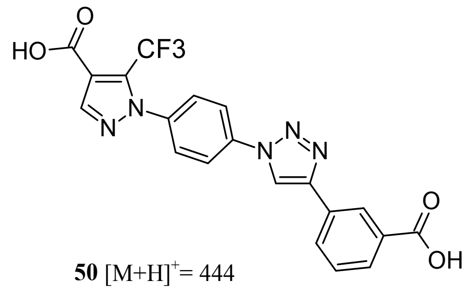
LC-ESI-MS^n analysis of **1S** and its metabolites.





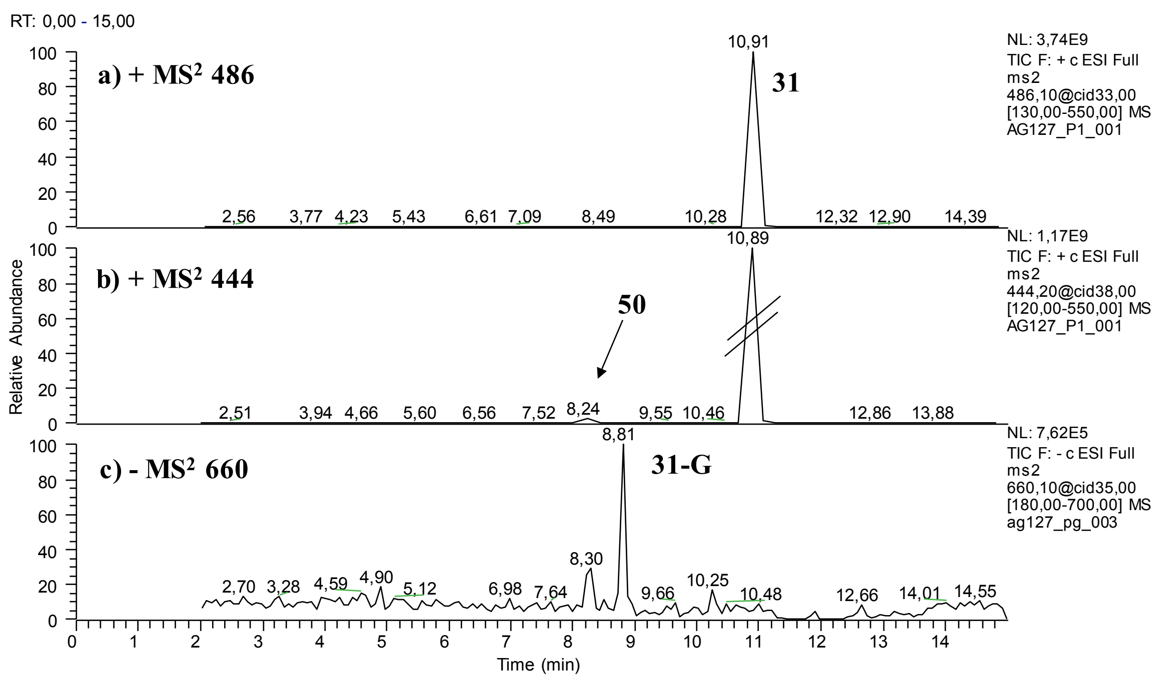


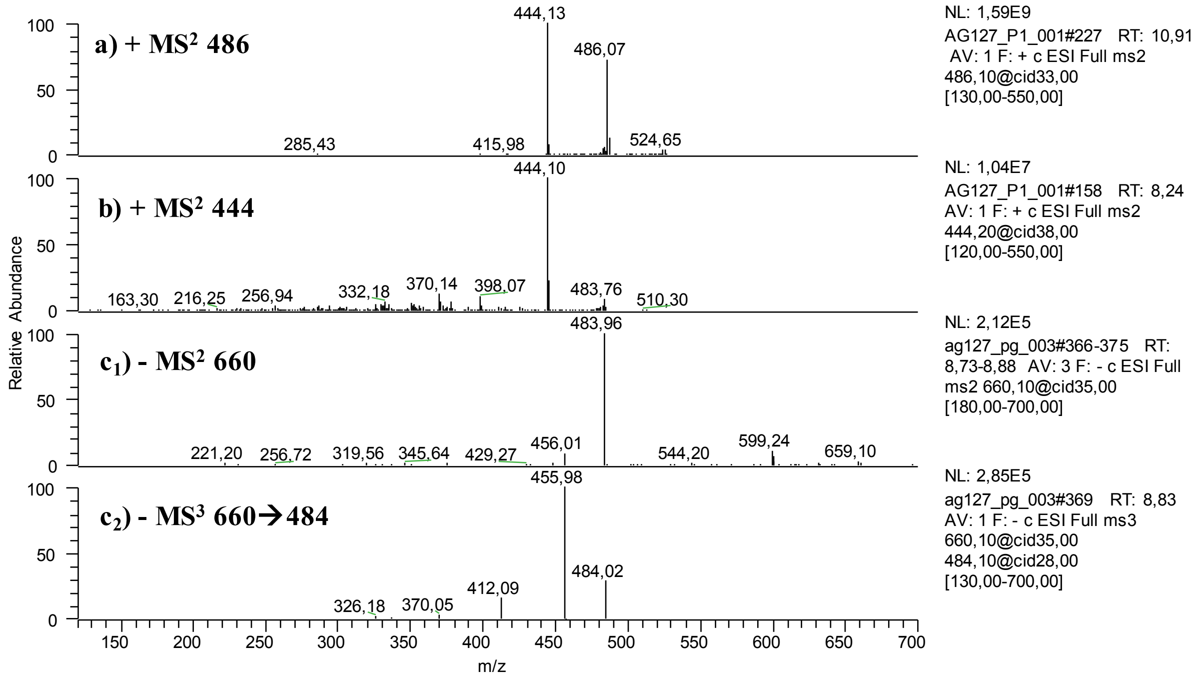
1S [M+H]$^+$= 472

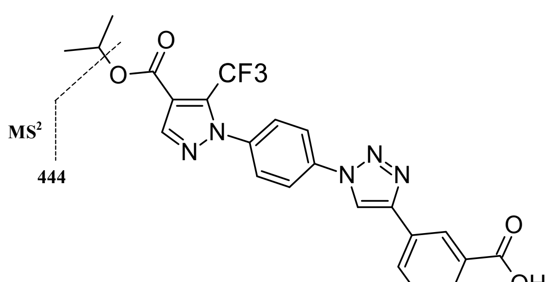


50 [M+H]$^+$= 444

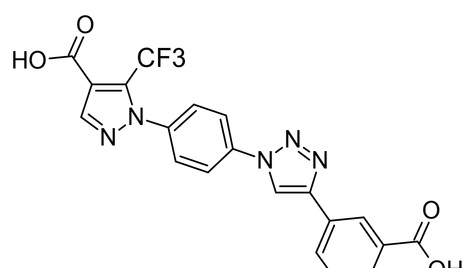
LC-ESI-MS^n analysis of **31** and its metabolites.



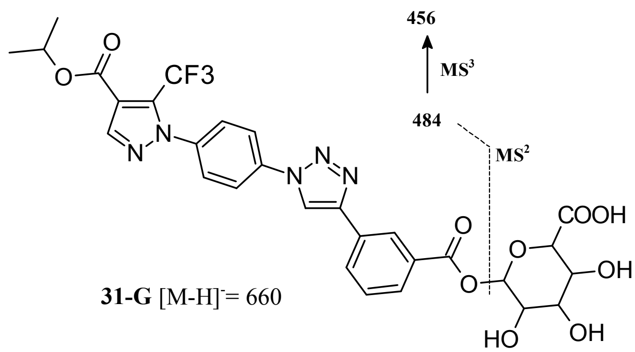




31 $[M+H]^+$= 486

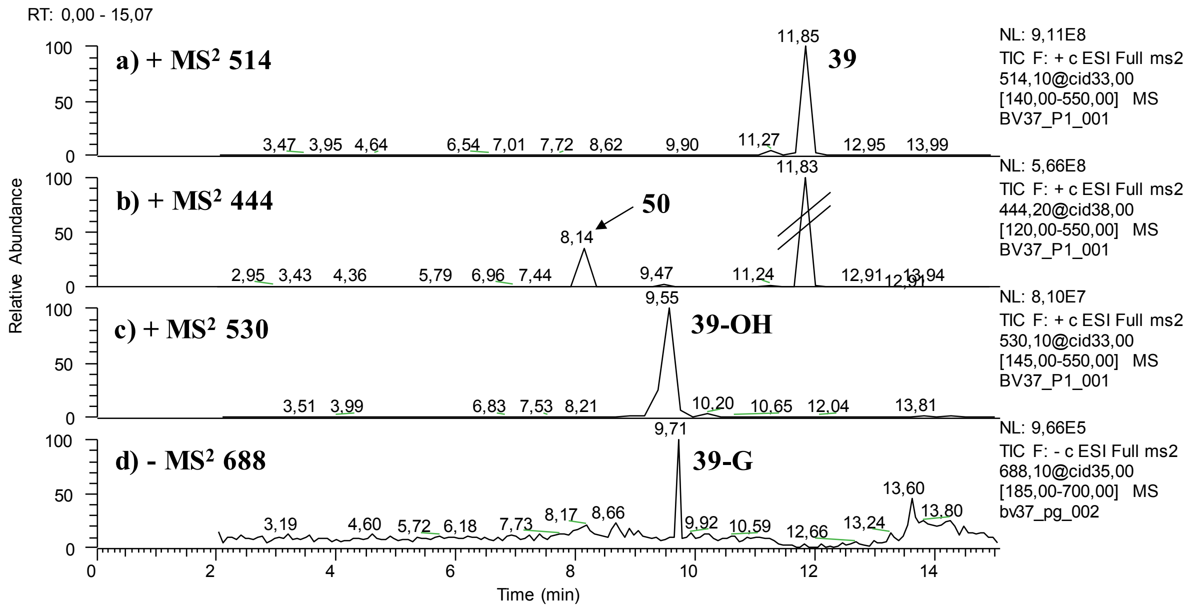


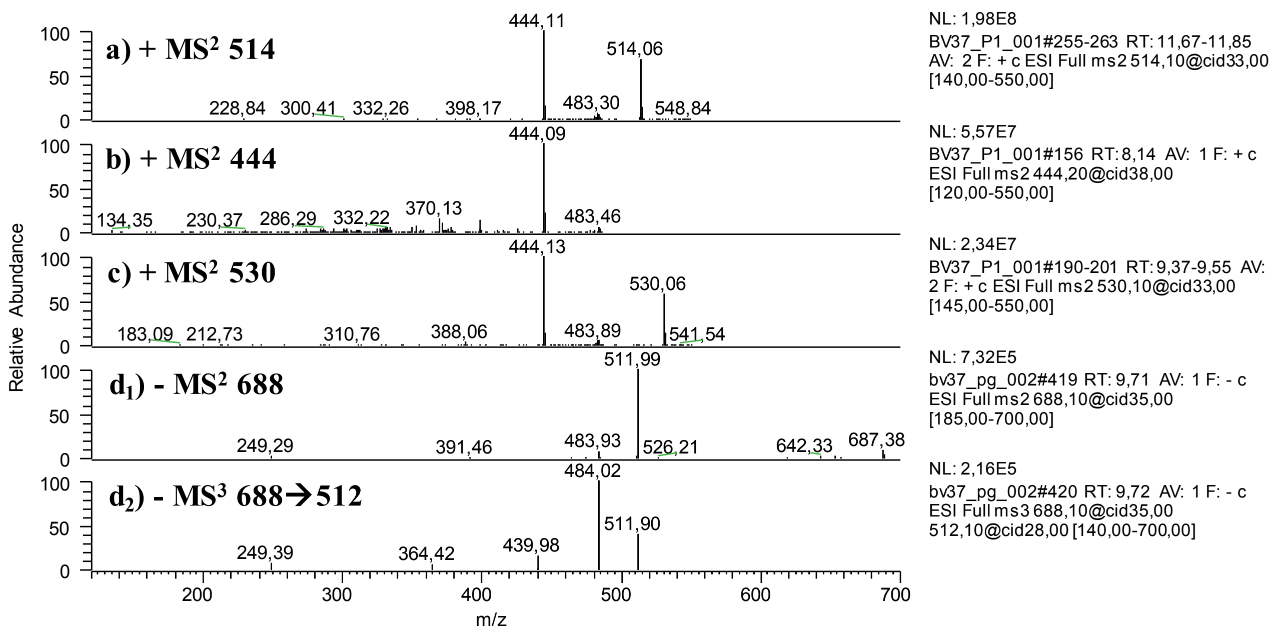
50 $[M+H]^+$= 444

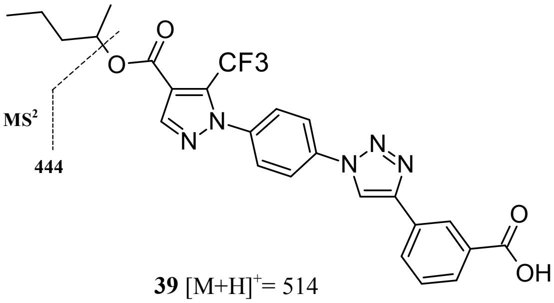


31-G [M-H]$^-$= 660

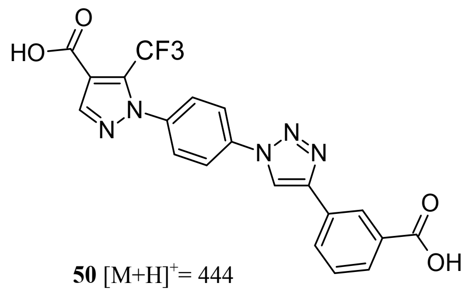
LC-ESI-MS^n analysis of **39** and its metabolites.


RT: 0,00 - 15,07
a) + MS² 514
b) + MS² 444
c) + MS² 530
d) - MS² 688
39
50
39-OH
39-G
Relative Abundance
Time (min)
NL: 9,11E8
TIC F: + c ESI Full ms2 514,10@cid33,00 [140,00-550,00] MS BV37_P1_001
NL: 5,66E8
TIC F: + c ESI Full ms2 444,20@cid38,00 [120,00-550,00] MS BV37_P1_001
NL: 8,10E7
TIC F: + c ESI Full ms2 530,10@cid33,00 [145,00-550,00] MS BV37_P1_001
NL: 9,66E5
TIC F: - c ESI Full ms2 688,10@cid35,00 [185,00-700,00] MS bv37_pg_002

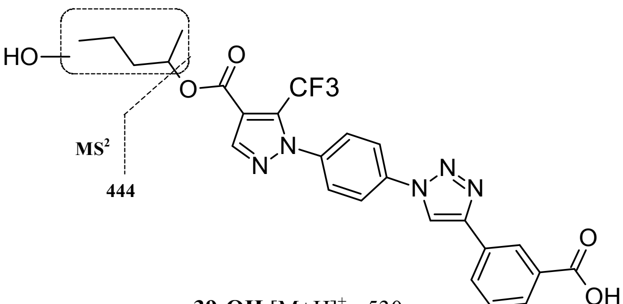

a) + MS² 514
b) + MS² 444
c) + MS² 530
d₁) - MS² 688
d₂) - MS³ 688→512
Relative Abundance
m/z
NL: 1,98E8
BV37_P1_001#255-263 RT: 11,67-11,85 AV: 2 F: + c ESI Full ms2 514,10@cid33,00 [140,00-550,00]
NL: 5,57E7
BV37_P1_001#156 RT: 8,14 AV: 1 F: + c ESI Full ms2 444,20@cid38,00 [120,00-550,00]
NL: 2,34E7
BV37_P1_001#190-201 RT: 9,37-9,55 AV: 2 F: + c ESI Full ms2 530,10@cid33,00 [145,00-550,00]
NL: 7,32E5
bv37_pg_002#419 RT: 9,71 AV: 1 F: - c ESI Full ms2 688,10@cid35,00 [185,00-700,00]
NL: 2,16E5
bv37_pg_002#420 RT: 9,72 AV: 1 F: - c ESI Full ms3 688,10@cid35,00 512,10@cid28,00 [140,00-700,00]



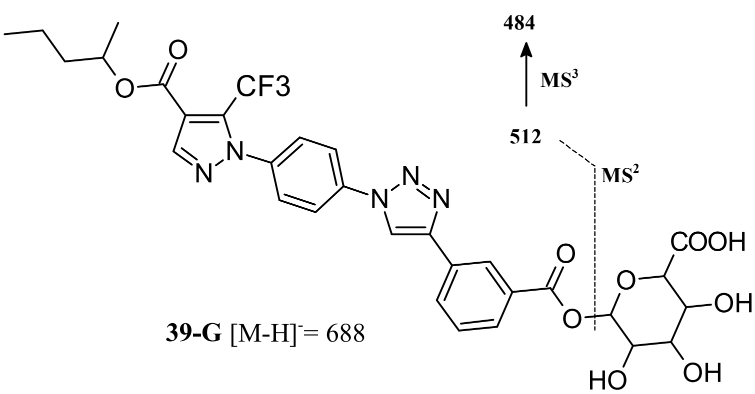
39 $[M+H]^+= 514$



50 $[M+H]^+= 444$



39-OH $[M+H]^+= 530$



39-G $[M-H]^-= 688$

Chromatographic methods

1) LC-UV method for purity and metabolic stability determination

A Shimadzu HPLC system (Shimadzu, Kyoto, Japan), consisting of two LC-10AD Vp module pumps, an SLC-10A Vp system controller, an SIL-10AD Vp autosampler, and a DGU-14-A on-line degasser were used for the analysis. The SPD-M10Avp photodiode array detector was used to detect the analytes. LC-Solution 1.24 software was used to process the chromatograms.

- Column: *Phenomenex Kinetex XB C18, 150 × 4.6 mm (5 µm d.p.)* protected with a SecurityGuard® (Torrance, CA, USA).
- Eluant:

  A: 0.5% trifluoroacetic acid in water.

  B: 0.5% trifluoroacetic acid in acetonitrile.
- Flow rate: 1 mL/min.
- Injection volume: 20 µL.
- Wavelength: 249 nm.
- Gradient program: 0 min [B%=40%], 8 min [B%=90%], 9.5 min [B%=95%], 10 min [B%=40%], 15 min [B%=40%].

2) LC-ESI-MS methods for metabolite characterization and pharmacokinetic analysis

A Thermo Finningan LCQ Deca XP plus system equipped with a quaternary pump, a Surveyor AS autosampler, a Surveyor photodiode array detector and a vacuum degasser was used for LC-MS analyses (Thermo Electron Corporation, Waltham, MA). Data were acquired and processed using Xcalibur® software.

- Column: *Phenomenex Synergi Polar 150 × 3.0 mm (2µm d.p.)* protected with a SecurityGuard® kept at 35 °C (Torrance, CA, USA).
- Eluant: A: 0.2% formic acid in water, B: acetonitrile.
- Flow rate: 0.2 mL/min.
- Injection volume: 5 µL.
- Gradient program for metabolite characterization: 0 min [B%=30%], 9 min [B%=80%], 9.5 min [B%=80%], 10.5 min [B%=30%], 15 min [B%=30%].
- Gradient program for pharmacokinetic study: 0 min [B%=30%], 7 min [B%=80%], 10.5 min [B%=80%], 11 min [B%=30%], 15 min [B%=30%].

The operating conditions of the ion trap mass spectrometer were as follows: *positive mode*, spray voltage, 5.30 kV; source current, 80 µA; capillary temperature, 300 °C; capillary voltage, 21.00 V; tube lens offset, 5.00 V; multipole 1 offset, -5.75 V; multipole 2 offset, -8.50 V; sheath gas flow (N_2), 40 Auxiliary Units. *Negative mode*: spray voltage, 3.40 kV; source current, 80 µA; capillary temperature, 300 °C; capillary voltage, -5.00 V; tube lens offset, -50.00 V; multipole 1 offset, 8.75 V; multipole 2 offset, 17.00V; sheath gas flow (N_2), 40 Auxiliary Units. Data was acquired in full-scan and product ion scan modes (MS^n) using mass scan range *m/z* 105-900. The collision energy was optimized at 28-35%.