Minor structural change to tertiary sulfonamide RORc ligands led to opposite mechanisms of action

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Supplementary Data

A. Experimental Methods for Structures of RORc

The RORc ligand binding domain was expressed and purified as described (Fauber et. al, *Bioorg. Med. Chem. Lett*. **2013**, *23*, 6604). RORc co-crystals of compound **3** were grown in the presence of a 10-fold molar excess of coactivator peptide (RHKILHRLLQEGSPS) and 1 mM ligand mixed 1:1 with reservoir solution (0.2M sodium formate, 0.1M Bis Tris propane buffer (pH 6.5), and 20% PEG 3350). Crystals of the RORc-compound **2** complex were grown over 10 days at 19°C in hanging drops composed of 1 μL protein solution (5mg/mL in 20 mM Tris-HCl buffer (pH 7.0), 200 mM NaCl, 4 % glycerol, 5 mM DTT, and 1 mM compound **2**) and 1 μL reservoir solution (200 mM sodium citrate and 15% PEG 3350). Crystals were flash cooled in liquid nitrogen, with 25% glycerol as a cryoprotectant. Diffraction data were collected at the Advanced Light Source beamline 5.0.2 (compound **3**) and Canadian Light Source at beamline CMCF1-08ID (compound **2**) and were processed with autoXDS or HKL2000. The structures were solved by molecular replacement and were manually rebuilt using COOT followed by refinement with Refmac5. Each structure contained two molecules in the asymmetric unit. During refinement, both data sets were found to be merohedrally twinned. The data from compound **3** had a twin fraction of 0.45 and were described by the twin law k, h, -l. The data from compound **2** had a twin fraction of ~0.25 and were described by the twin law h,

-h-k, -l. For the structure of compound **2**, the B-factors and density commencing at residue 468 and continuing through to the C-terminal regions of both monomers in the crystallographic asymmetric unit suggests this region has some level of static disorder. In particular, strong density for an alternative position of residues 490-502 was present between the two monomers in the asymmetric unit. This density was modeled as chain P. It is not possible to unambiguously assign this density to a particular monomer but based on the position of the termini, it likely represents an alternate conformation of chain B. Crystallization data and refinement statistics are summarized in Supplemental Table S1. These structures have been deposited in the RCSB and assigned accession codes 4WQP and 4WPF.

Table S1. X-ray Data Collection and Refinement statistics

	RORc : **3**	RORc : **2**
Data collection		
Space group	$P4_1$	$P6_1$
Cell dimensions		
a, *b*, *c* (Å)	61.7, 61.7, 155.2	99.7, 99.7, 129.6
α, β, γ (°)	90, 90, 90	90, 90, 120
Resolution (Å)[a]	35-2.2 (2.24-2.2)	50-1.986 (1.993-1.986)
R_{sym}(%)[b]	11.0 (75.4)	5.7 (62.0)
$\langle I/\sigma I \rangle$	8.3 (0.91)	22.5 (3.7)
Completeness (%)	97.4 (85.5)	98.9 (97.4)
Redundancy	2.8 (2.3)	7.7 (7.8)
Refinement		
Resolution (Å)	35-2.2	50-1.986
No. reflections	21,308	49,776
R_{work} / R_{free} [c]	17.5, 22.7	15.0, 18.0
No. atoms		
Protein	4418	3882
Ligand	62	62
Water	249	261
Residual *B*-factors		
Protein	28.8	25
Ligand	47.4	43
Water/ion	33.4	44
R.m.s. deviations		
Bond lengths (Å)	0.005	0.007
Bond angles (°)	0.834	1.044

[a] Values in parenthesis are for the highest resolution shell.
[b] $R_{sym} = \Sigma||I| - |\langle I \rangle||/ \Sigma|\langle I \rangle|$, where I is the intensity of a single observation and $\langle I \rangle$ the average intensity for symmetry equivalent observations.
[c] $R_{work} = \Sigma|F_o-F_c|/ \Sigma|F_o|$, where F_o and F_c are observed and calculated structure factor amplitudes, respectively. R_{free} is calculated as R for 5% of reflections sequestered from refinement.

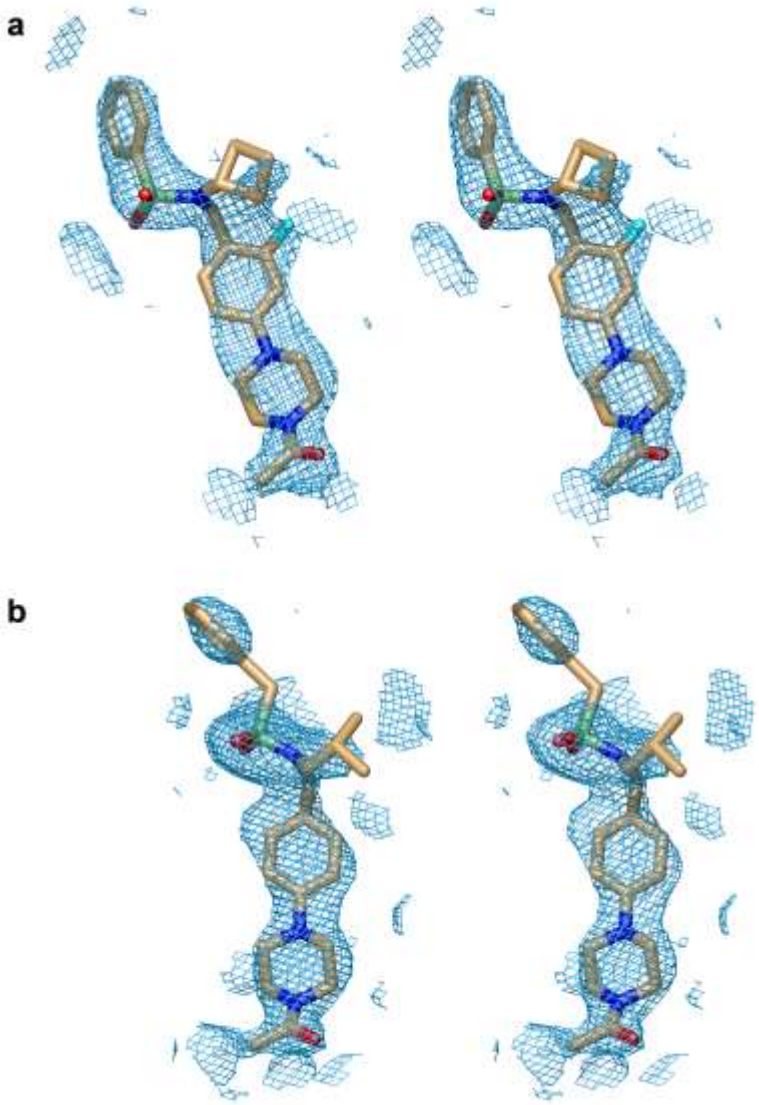


Figure S1. Electron density maps. Divergent eye stereo diagram of the unbiased $2F_o$-F_c difference electron density maps for **3** (a) and **2** (b) calculated prior to addition of the compounds to the models and displayed contoured at 0.7σ.

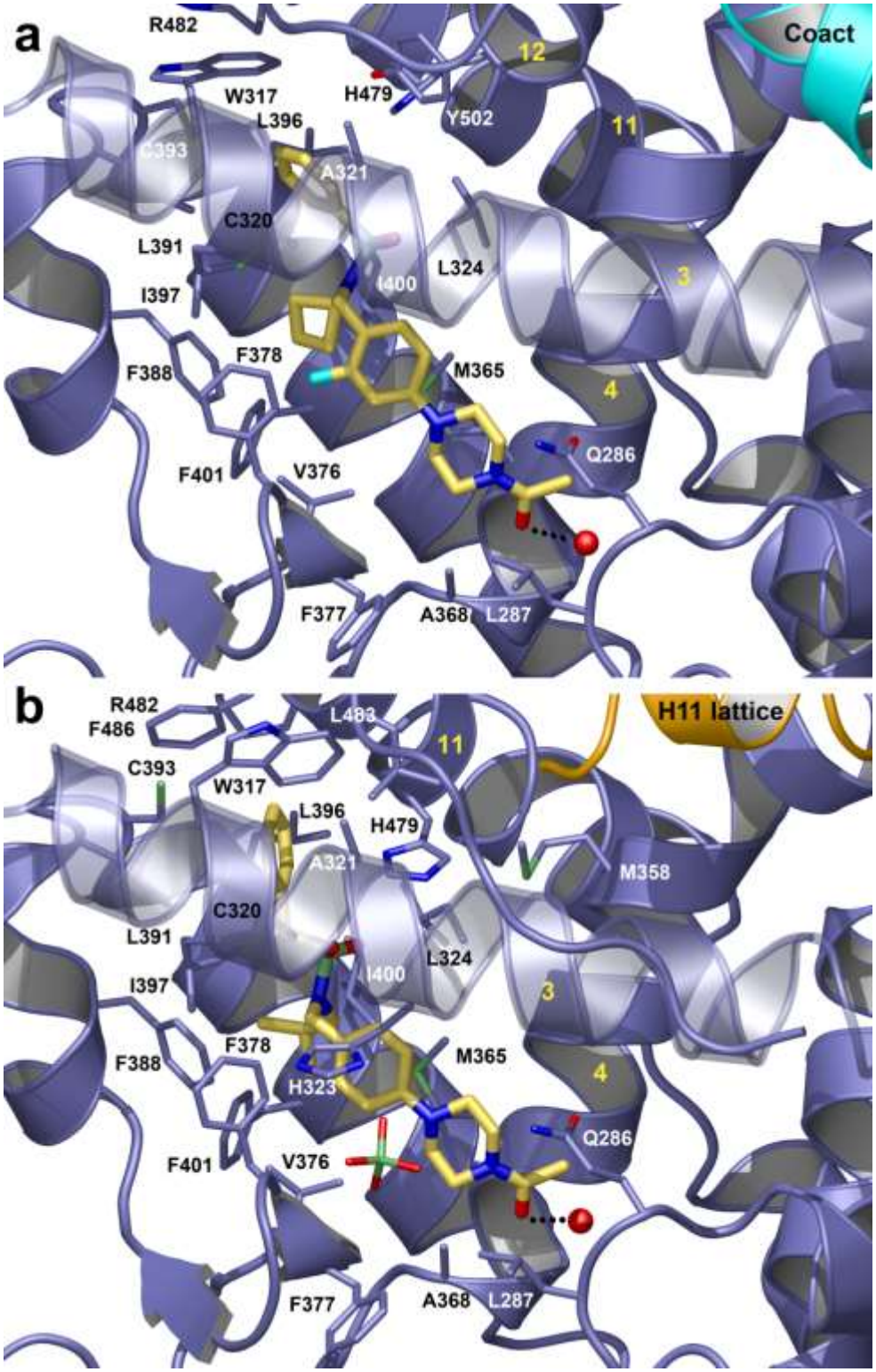


Figure S2. Binding modes of agonist and inverse agonists within the ligand binding pocket of RORc. The crystal structure of the agonist **3** (a) stabilized helix 12 and shows a co-activator peptide (cyan) bound to the receptor. In the inverse agonist **2** structure (b), the helix 11 was displaced, helix 11´ was unwound, and helix 12 was disordered, eliminating the docking site for co-activator proteins to bind. Instead, a partially ordered helix 11 from a neighboring protein monomer in the crystal lattice (orange) filled the co-activator binding site.

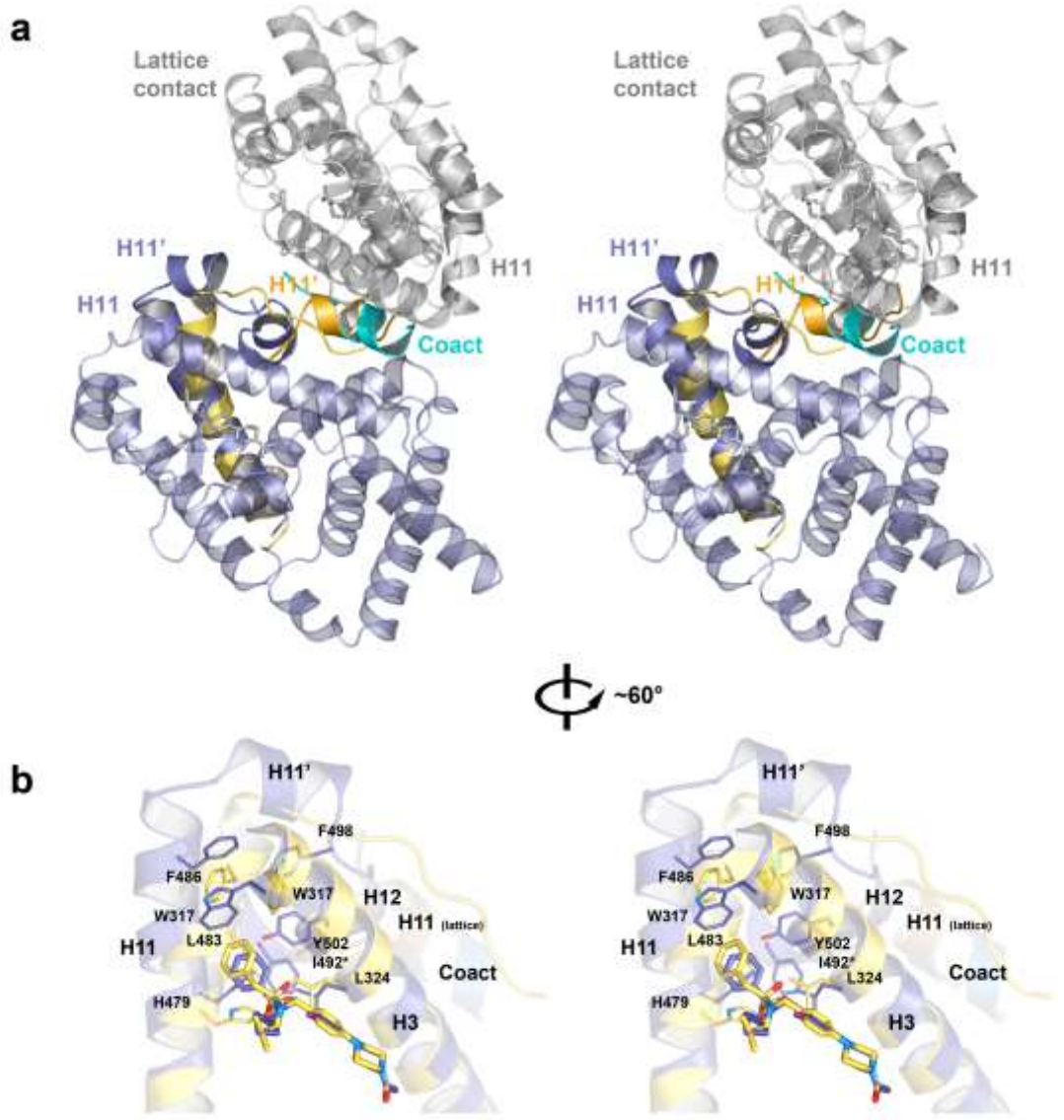


Figure S3. Inverse agonist 2 disrupted RORc C-terminus and the binding sites for helix 12 and coactivator. Divergent eye stereo diagram of RORc agonist **3** (blue ribbon) recruited co-activator proteins (a), represented by the co-crystallized co-activator peptide (cyan). Binding of the inverse agonist **2** disrupted the position of helix 11 (gold ribbon), causing helix 11´ and 12 to unwind and become completely disordered, respectively. In the crystal lattice of the inverse agonist complex, a neighboring protein monomer (gray ribbon) extended its helix 11´ (orange ribbon) to occupy a portion of the binding site vacated by helix 11 and 12 of the reference protein monomer. Multiple amino acid sidechains rearranged to re-pack the hydrophobic cluster vacated by the expulsion of helices 11´ and 12 in the presence of the inverse agonist **2** (b).

B. Purity and LCMS Analysis of Sulfonamide Library Compounds

Compound	MW	LC-MS ESI$^+$ $[M+1]^+$	LC-MS Purity (254 nm)
3	445.55	446.1	97.3%
4	459.58	460.1	100%
9	473.6	474.1	96.5%
10	463.54	464.1	97.6%
11	477.57	478.1	96.5%
12	463.54	464.1	98.8%
13	477.57	478.1	97.0%
14	463.54	464.1	98.7%
15	477.57	478.1	96.6%
16	480.0	480.0	100%
17	494.02	494.0	100%
18	480.0	480.0	96.2%
19	494.02	494.0	100%
20	480.0	480.1	100%
21	494.02	494.1	100%
22	514.44	514.0	97.6%
23	528.47	528.0	100%
24	481.53	482.1	90.1%
25	475.58	476.0	100%
26	473.6	474.1	100%
27	470.56	471.1	94.5%
28	475.58	476.1	98.3%
29	514.44	514.0	100%
30	511.56	512.1	100%
31	529.55	530.1	91.7%
32	513.55	514.1	94.6%
33	501.66	502.1	100%

C. Characterization of Key Compounds 3, 4, 10 and 11

***N*-(4-(4-Acetylpiperazin-1-yl)-2-fluorobenzyl)-*N*-cyclobutylbenzenesulfonamide (3).**
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.81 (d, *J* = 7.4 Hz, 1H), 7.62 – 7.42 (m, 4H), 6.70 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.52 (dd, *J* = 13.1, 2.5 Hz, 1H), 4.36 (s, 2H), 4.24 (p, *J* = 9.1 Hz, 1H), 3.82 – 3.70 (m, 2H), 3.66 – 3.57 (m, 2H), 3.17 (dt, *J* = 14.9, 5.2 Hz, 4H), 2.14 (s, 3H), 2.00 – 1.86 (m, 4H), 1.56 – 1.41 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.0, 160.5 (d, J_{C-F} = 246.9 Hz), 151.6 (d, J_{C-F} = 10.3 Hz), 140.1, 132.5, 130.4 (d, J_{C-F} = 5.3 Hz), 129.1, 127.0, 116.5 (d, J_{C-F} = 14.2 Hz), 112.0, 102.7 (d, J_{C-F} = 24.9 Hz), 52.8, 49.1, 48.8, 46.0, 41.2 (d, J_{C-F} = 4.5 Hz), 41.1, 28.9, 21.3, 15.0; HRMS calcd for $C_{23}H_{28}FN_3O_3S$ $[M+H]^+$ 446.1908, found 446.1918.

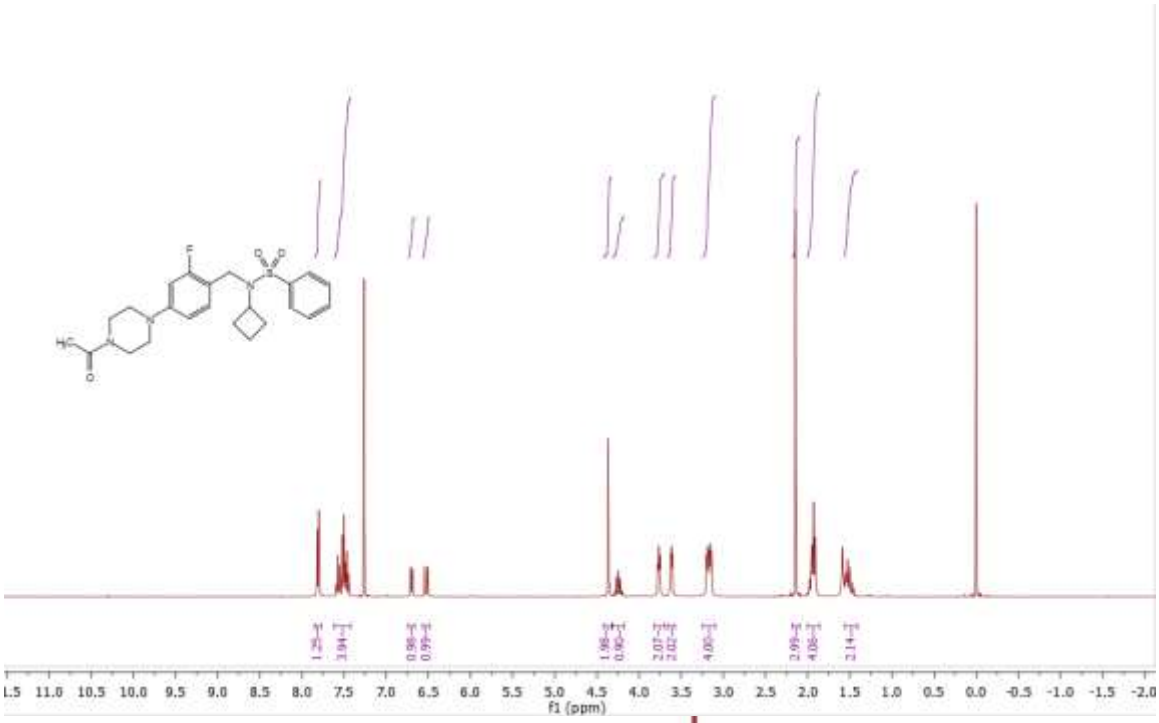
***N*-(4-(4-Acetylpiperazin-1-yl)-2-fluorobenzyl)-*N*-cyclobutyl-1-phenylmethanesulfonamide (4).**
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.43 – 7.31 (m, 6H), 6.65 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.51 (dd, *J* = 13.2, 2.4 Hz, 1H), 4.20 – 4.05 (m, 5H), 3.81 – 3.70 (m, 2H), 3.65 – 3.55 (m, 2H), 3.23 – 3.09 (m, 4H), 2.13 (s, 3H), 2.08 – 1.95 (m, 2H), 1.95 – 1.83 (m, 2H), 1.57 – 1.32 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.0, 160.6 (d, J_{C-F} = 244.7 Hz), 151.6 (d, J_{C-F} = 9.4 Hz), 130.8 (J_{C-F} = 6.1 Hz), 130.7, 129.0, 128.8, 128.7, 116.2 (d, J_{C-F} = 17.0 Hz), 111.9 (d, J_{C-F} = 2.7 Hz), 102.6 (d, J_{C-F} = 26.1 Hz), 59.0, 52.7, 49.0, 48.7, 46.0, 41.5 (d, J_{C-F} = 3.4 Hz), 41.1, 29.4, 21.3, 14.5; HRMS calcd for $C_{24}H_{30}FN_3O_3S$ $[M+H]^+$ 460.2065, found 460.2078.

***N*-(4-(4-Acetylpiperazin-1-yl)-2-fluorobenzyl)-*N*-cyclobutyl-2-fluorobenzenesulfonamide (10).**

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.88 (td, *J* = 7.5, 1.8 Hz, 1H), 7.60 – 7.50 (m, 1H), 7.44 (t, *J* = 8.8 Hz, 1H), 7.26 – 7.21 (m, 1H), 7.21 – 7.16 (m, 1H), 6.69 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.52 (dd, *J* = 13.1, 2.4 Hz, 1H), 4.55 (s, 2H), 4.35 – 4.22 (m, 1H), 3.81 – 3.72 (m, 2H), 3.68 – 3.57 (m, 2H), 3.16 (dt, *J* = 14.8, 5.4 Hz, 4H), 2.14 (s, 3H), 2.07 – 1.82 (m, 4H), 1.54 – 1.38 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.0, 160.5 (d, $J_{C\text{-}F}$ = 245.4 Hz), 158.7 (d, $J_{C\text{-}F}$ = 245.2 Hz), 151.6 (d, $J_{C\text{-}F}$ = 9.8 Hz), 134.7 (d, $J_{C\text{-}F}$ = 8.7 Hz), 130.9, 130.3 (d, $J_{C\text{-}F}$ =6.0 Hz), 128.8 (d, $J_{C\text{-}F}$ = 13.8 Hz), 124.3 (d, $J_{C\text{-}F}$ = 3.4 Hz), 117.1 (d, $J_{C\text{-}F}$ = 22.1 Hz), 116.6 (d, $J_{C\text{-}F}$ = 14.5 Hz), 112.0 (d, $J_{C\text{-}F}$ = 3.8 Hz), 102.7 (d, $J_{C\text{-}F}$ = 26.2 Hz), 52.3, 49.1, 48.8, 46.0, 41.2 (d, $J_{C\text{-}F}$ = 4.5 Hz), 41.1, 28.8, 21.3, 14.9; HRMS calcd for $C_{23}H_{27}F_2N_3O_3S$ $[M+H]^+$ 464.1814, found 464.1830.

***N*-(4-(4-Acetylpiperazin-1-yl)-2-fluorobenzyl)-*N*-cyclobutyl-1-(2-fluorophenyl)methanesulfonamide (11).**

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.49 (td, *J* = 7.6, 1.8 Hz, 1H), 7.43 – 7.31 (m, 2H), 7.18 (td, *J* = 7.5, 1.3 Hz, 1H), 7.12 (ddd, *J* = 9.6, 8.2, 1.2 Hz, 1H), 6.65 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.51 (dd, *J* = 13.2, 2.5 Hz, 1H), 4.28 (s, 2H), 4.23 (s, 2H), 4.15 – 4.01 (m, 1H), 3.80 – 3.71 (m, 2H), 3.67 – 3.55 (m, 2H), 3.21 – 3.08 (m, 4H), 2.13 (s, 3H), 2.10 – 1.95 (m, 2H), 1.93 – 1.81 (m, 2H), 1.54 – 1.46 (m, 1H), 1.46 – 1.32 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.0, 161.1 (d, $J_{C\text{-}F}$ = 252.1 Hz), 160.6 (d, $J_{C\text{-}F}$ = 245.7 Hz), 151.7 (d, $J_{C\text{-}F}$ = 10.7 Hz), 132.8 (d, $J_{C\text{-}F}$ = 3.1 Hz), 130.7 (d, $J_{C\text{-}F}$ = 12.6 Hz), 130.7, 124.5 (d, $J_{C\text{-}F}$ = 3.5 Hz), 116.7 (d, $J_{C\text{-}F}$ = 15.4 Hz), 116.1 (d, $J_{C\text{-}F}$ = 14.5 Hz), 115.7 (d, $J_{C\text{-}F}$ = 22.5 Hz), 112.0 (d, $J_{C\text{-}F}$ = 2.0 Hz), 102.5 (d, $J_{C\text{-}F}$ = 26.2 Hz), 52.9, 51.4, 49.0, 48.7, 46.0, 41.4 (d, $J_{C\text{-}F}$ = 3.7 Hz), 41.1, 29.3, 21.3, 14.5; HRMS calcd for $C_{24}H_{29}F_2N_3O_3S$ $[M+H]^+$ 478.1970, found 478.1979.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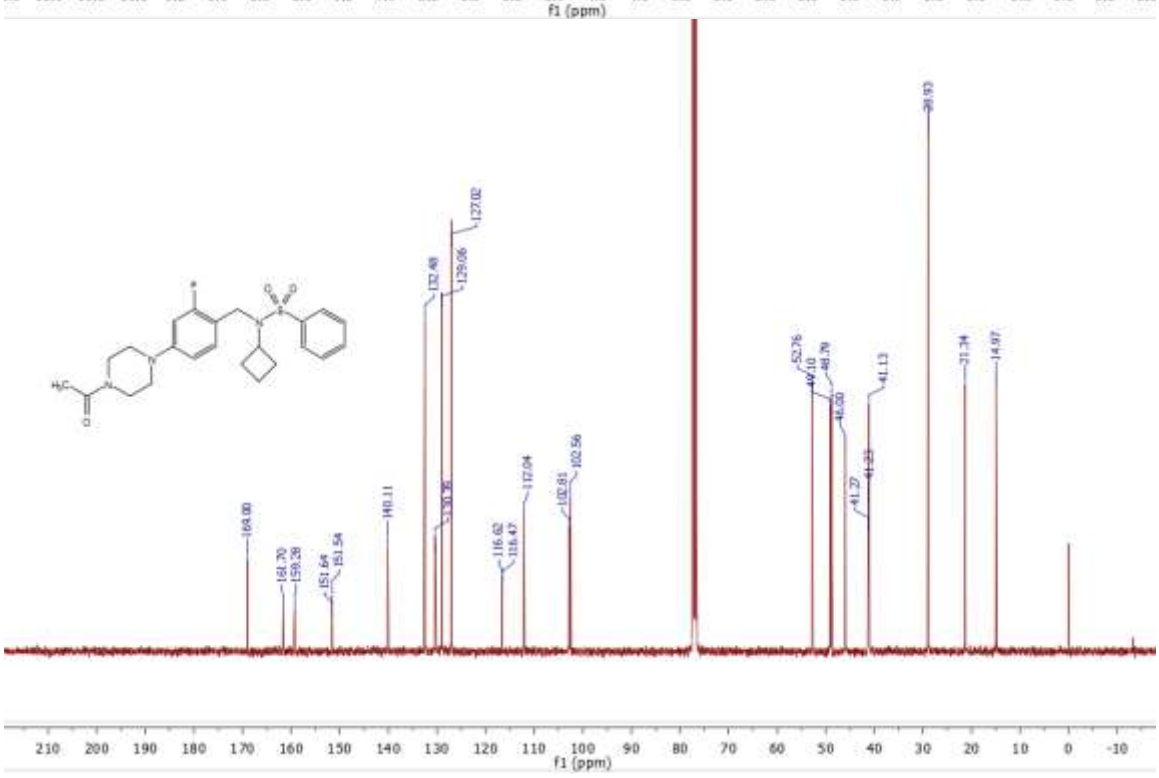
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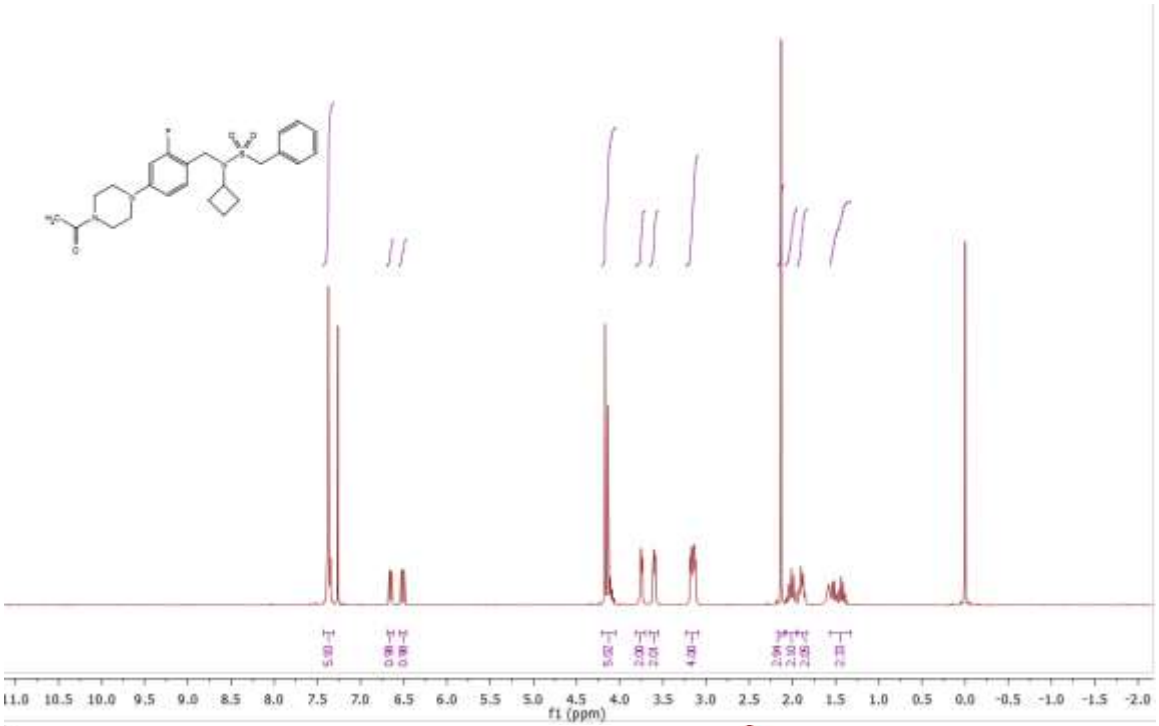
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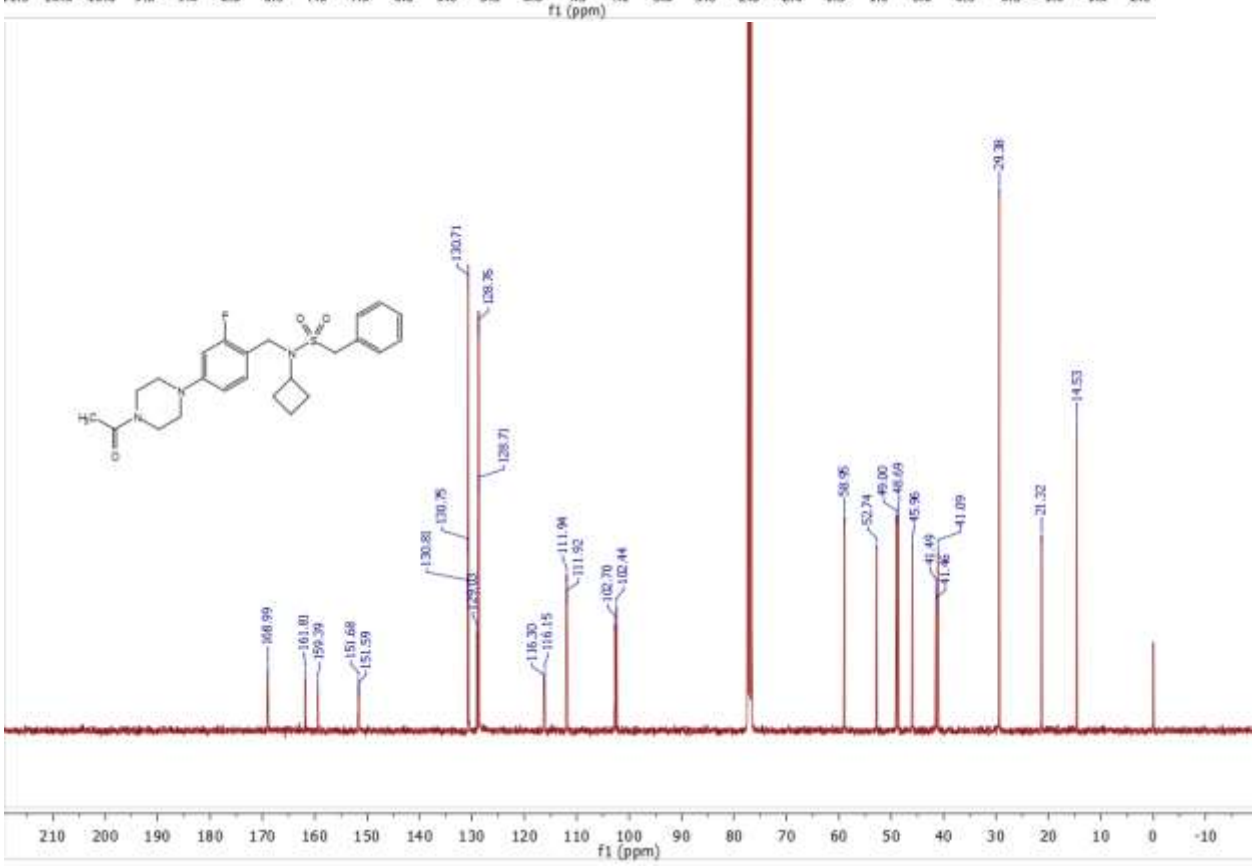
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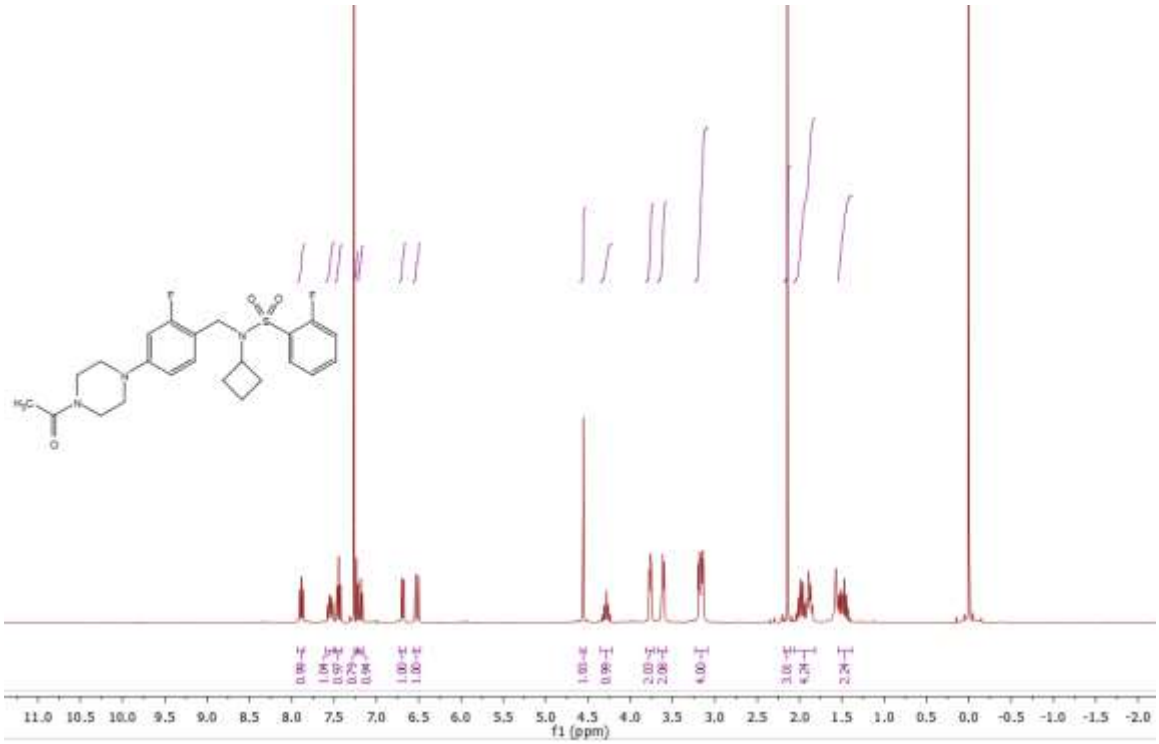
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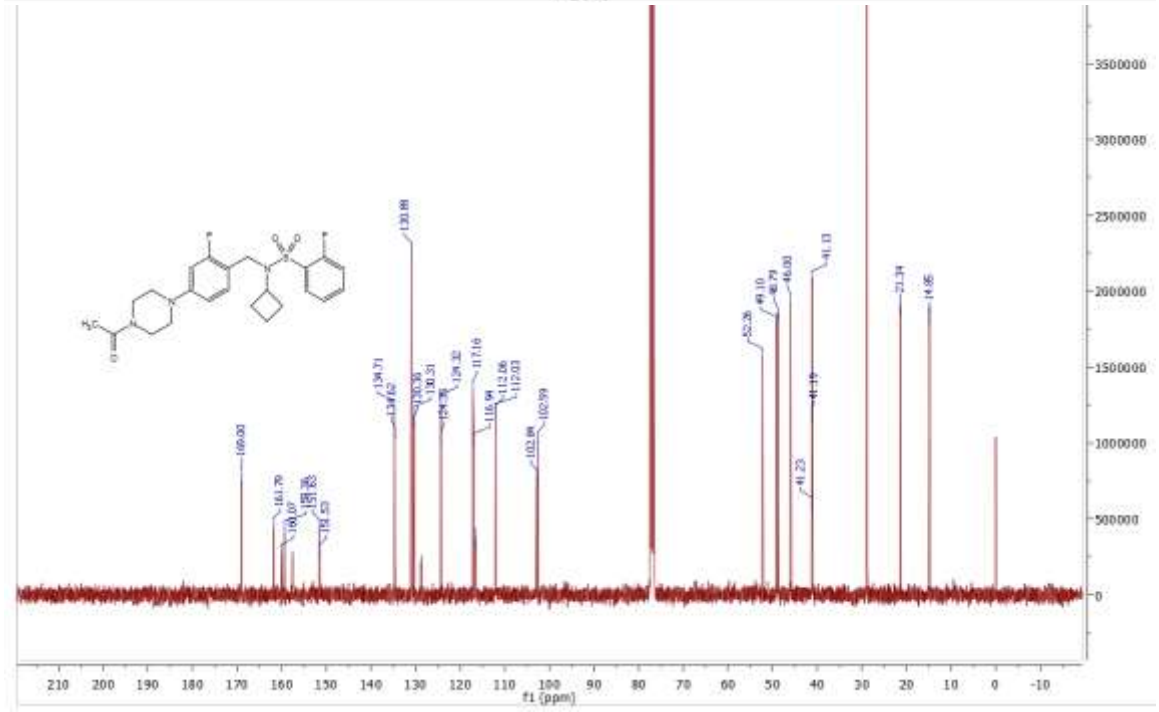
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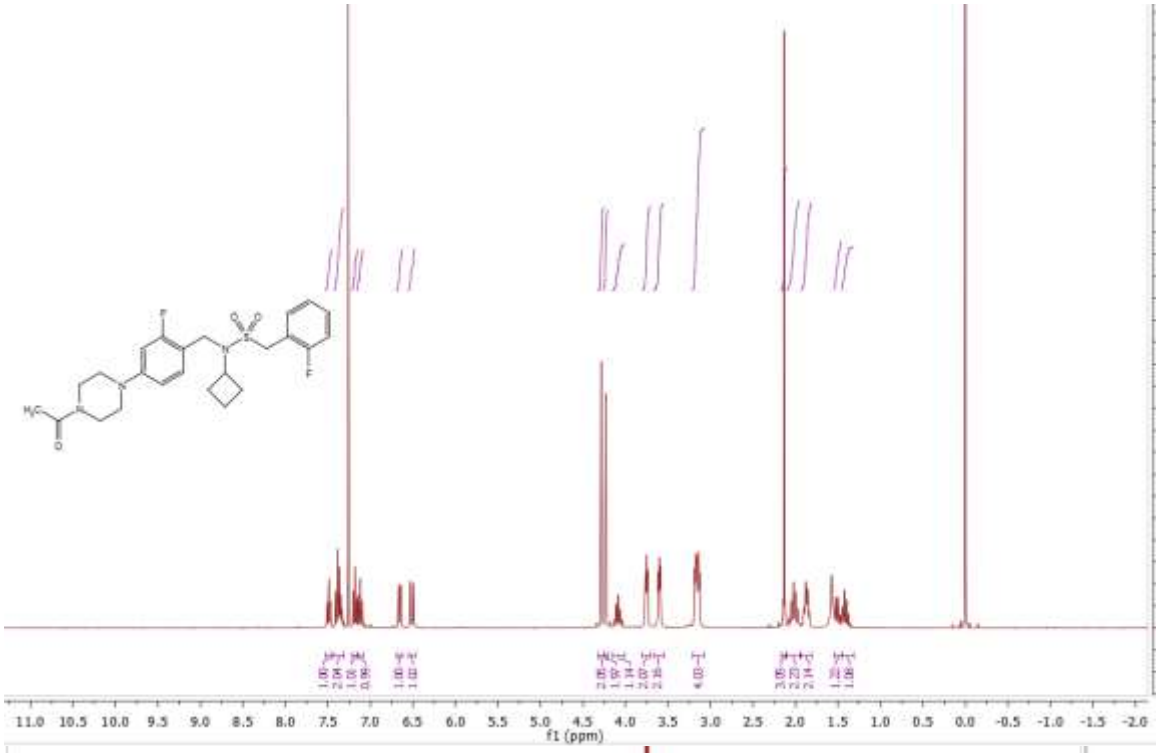
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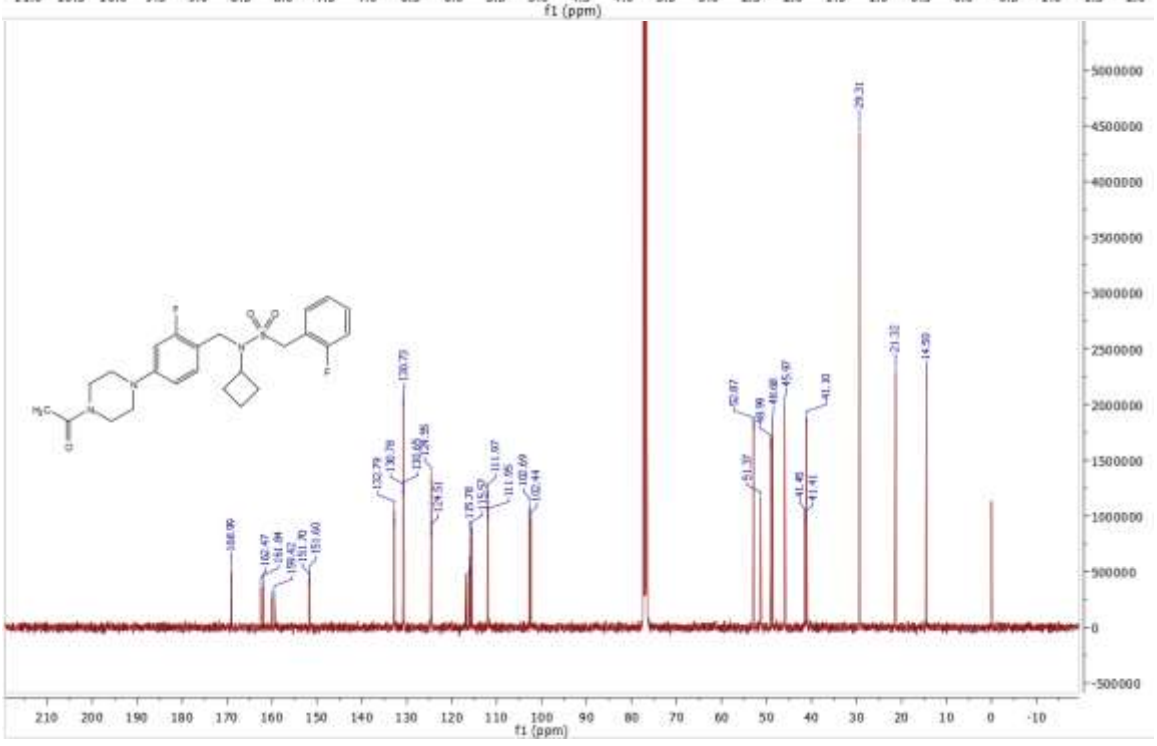
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