Impact of Atropisomerism on a Non-Steroidal Glucocorticoid Receptor Agonist - Supplemental Information

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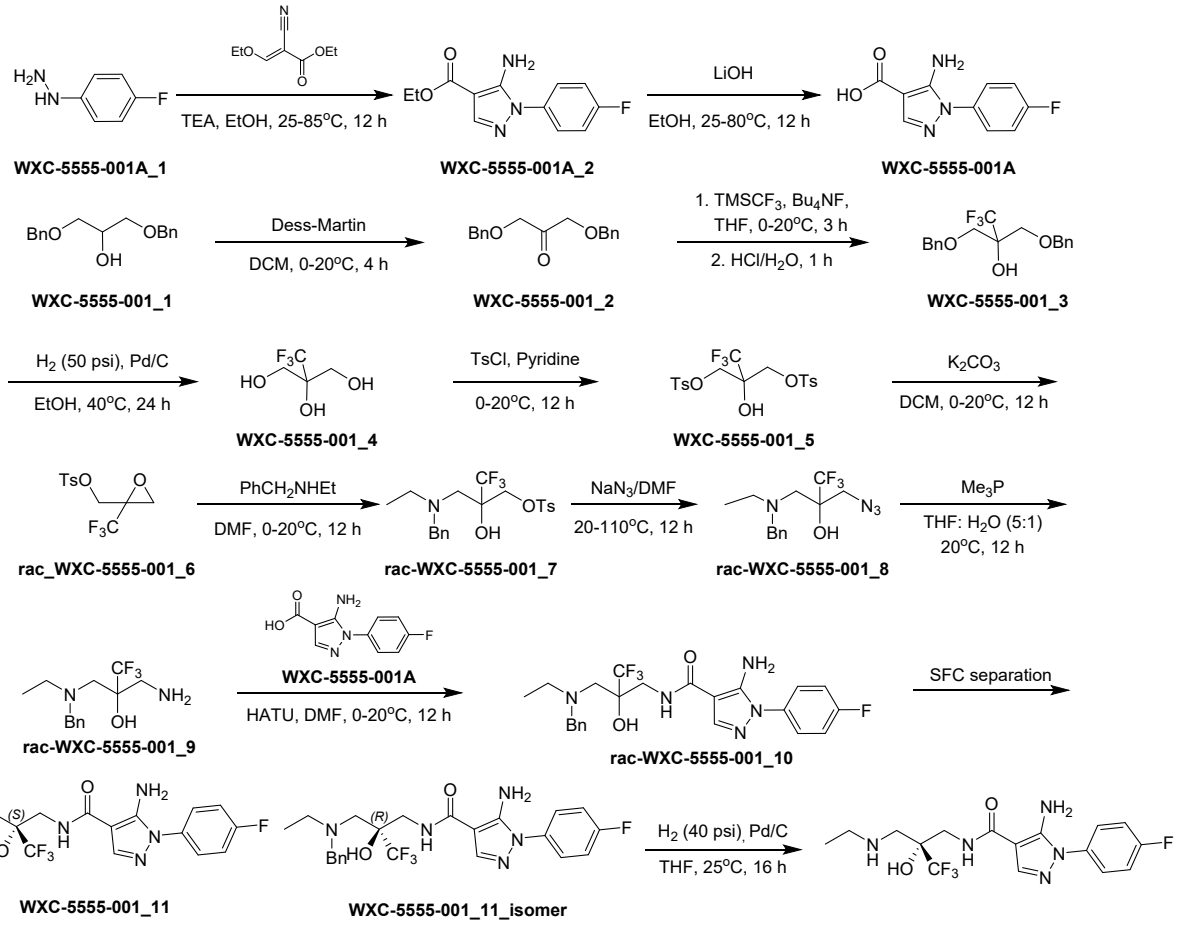
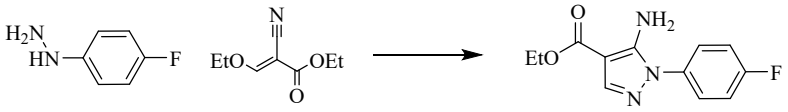
Synthesis of (R)-5-amino-N-(2-((ethylamino)methyl)-3,3,3-trifluoro-2-hydroxypropyl)-1-(4-fluorophenyl)-1H-pyrazole-4-carboxamide

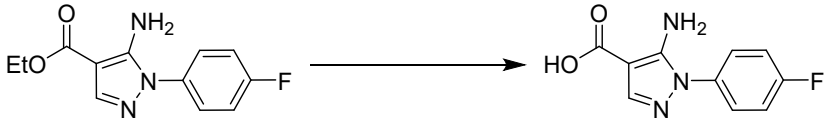


Ethyl 5-amino-1-(4-fluorophenyl)-1H-pyrazole-4-carboxylate.



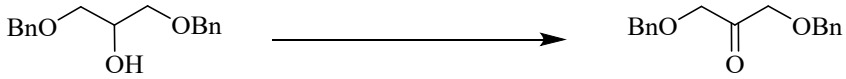
To a solution of (E)-ethyl 2-cyano-3-ethoxyacrylate (10 g, 59.1 mmol) and (4-fluorophenyl)hydrazine (9.61 g, 59.1 mmol) in ethanol (EtOH, 100 mL) was added triethylamine (8.24 mL, 59.1 mmol) at 25 ˚C. The reaction was stirred at 85 ˚C for 12 h. Four identical reactions were set up and the five reactions combined, cooled to 25 °C and concentrated to afford the title compound (73.7 g, 98 % yield) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.27 (t, *J*=7.07 Hz, 3 H) 4.21 (q, *J*=7.09 Hz, 2 H) 6.32 (s, 2 H) 7.37 (t, *J*=8.82 Hz, 2 H) 7.52 - 7.60 (m, 2 H) 7.69 (s, 1 H). ^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -114.20 (s, 1F).

5-Amino-1-(4-fluorophenyl)-1H-pyrazole-4-carboxylic acid.



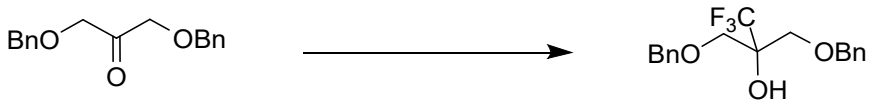
To a solution of ethyl 5-amino-1-(4-fluorophenyl)-1H-pyrazole-4-carboxylate (18.4 g, 72.3 mmol) in ethanol (EtOH, 200 mL) was added lithium hydroxide, H_2O (200 mL, 400 mmol) at 25 ˚C. The reaction was stirred at 80 ˚C for 12 h. Three identical reactions were set up and the four reactions combined, cooled to 25 °C and concentrated. The mixture was adjusted to pH 7 with 1 M HCl (1500 mL), filtered, and the filter cake concentrated to afford the title compound (61 g, 93 % yield) as a yellow solid. LCMS (Method a, Table S1) Rt: 0.313 min; MS m/z = 222.1 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 6.27 (br s, 2 H) 7.36 (t, *J*=8.82 Hz, 2 H) 7.54 - 7.60 (m, 2 H) 7.66 (s, 1 H).

1,3-Bis(benzyloxy)propan-2-one.



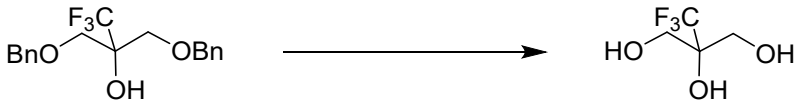
To a solution of 1,3-bis(benzyloxy)propan-2-ol (19g, 69.8 mmol) in DCM (190 mL) was added Dess-Martin Periodinane (1, 1, 1-tris (acetyloxy)-1, 1-dihydro-1, 2-benzodioxol-3-(1H)-one) (35.5 g, 84 mmol) at 0 ˚C under N_2. The mixture was stirred at 25 ˚C for 4 h. Nine identical reactions were set up and the ten reactions combined, filtered and concentrated to afford a residue that was subjected to purification by chromatography on silica gel (petroleum ether/ethyl acetate = 10/1 to 2/1) to afford the title compound (141 g, 67 % yield) as a colourless oil. LCMS (Method a, Table S1) Rt: 0.505 min; MS m/z = 304.3 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.26 (s, 4 H) 4.59 (s, 4 H) 7.31 - 7.40 (m, 10 H).

3-(Benzyloxy)-2-((benzyloxy)methyl)-1,1,1-trifluoropropan-2-ol.



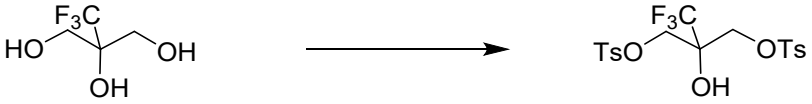
To a solution of 1,3-bis(benzyloxy)propan-2-one (20 g, 70.3 mmol) and (trifluoromethyl) trimethylsilane (20.0 g, 141 mmol) in THF (200 mL) was added tetrabutylammonium fluoride trihydrate (4.4 g, 14.1 mmol) at 0°C under N_2 and the mixture stirred at 20 ˚C for 3 h under N_2. Five identical reactions were set up and the six reactions combined, and hydrochloric acid (1M, 1200 mL) added, and the mixture stirred for 1 h. The mixture was extracted with ethyl acetate (3 X 300 mL), the organic layers combined and concentrated to afford a residue that was subjected to purification by chromatography on silica gel (petroleum ether/ethyl acetate=1/0 to 5/1) to afford the title compound (105 g, 69 % yield) as a yellow oil. LCMS (Method a, Table S1) Rt: 0.560 min; MS m/z = 363.2 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.38 (s, 1 H) 3.72 (s, 4 H) 4.60 (s, 4 H) 7.28 - 7.41 (m, 10 H). ^{19}F NMR (376 MHz, $CDCl_3$) δ ppm -78.17 (s, 1 F).

2-(Trifluoromethyl)propane-1,2,3-triol.



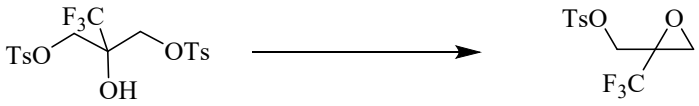
To a solution of 3-(benzyloxy)-2-((benzyloxy)methyl)-1,1,1-trifluoropropan-2-ol (21 g, 58.6 mmol) and Pd/C (6.24 g, 5.9 mmol) in ethanol (EtOH, 210 mL) at 20 ˚C, then the mixture was stirred at 40 ˚C for 24 h under H_2 (50 psi). Four identical reactions were set up and the five reactions combined, filtered and concentrated to afford a crude residue that was triturated with ethyl acetate (500 mL), filtered and evaporated to dryness to afford the title compound (46.9 g, 98 % yield) as a colourless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.54 (br s, 4 H) 4.87 (br s, 2 H) 5.62 (s, 1 H). ^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -75.64 (s, 1 F).

2-Hydroxy-2-(trifluoromethyl)propane-1,3-diyl bis(4-methylbenzenesulfonate).



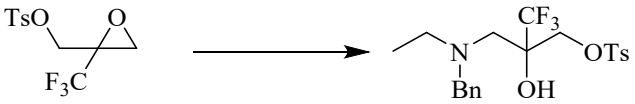
To a solution of 2-(trifluoromethyl)propane-1,2,3-triol (6.9 g, 40.9 mmol) in pyridine (40 mL, 495 mmol) was added p-toluenesulfonyl chloride (31.2 g, 164 mmol) at 0 ˚C under N_2 and the mixture stirred at 20 ˚C for 12 h under N_2. Four identical reactions were set up and the five reactions combined, poured into hydrochloric acid (1M, 1000 mL) and extracted with ethyl acetate (3 X 500 mL). The combined organic layers were washed with saturated brine (500 mL), dried (Na_2SO_4), filtered, and concentrated to afford a crude residue that was subjected to purification by chromatography on silica gel (petroleum ether/ethyl acetate = 10/1 to 3/1) to afford the title compound (140 g, 99 % yield) as a yellow oil. LCMS (Method a, Table S1) Rt: 0.523 min; MS m/z = 491.3 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.78 (d, J = 8.4 Hz, 4H), 7.38 (d, J = 8.0 Hz, 4H), 4.18 (s, 4H), 2.47 (s, 6H). ^{19}F NMR (376 MHz, $CDCl_3$) δ ppm -77.77 (s, 1 F).

(2-(Trifluoromethyl)oxiran-2-yl)methyl 4-methylbenzenesulfonate.



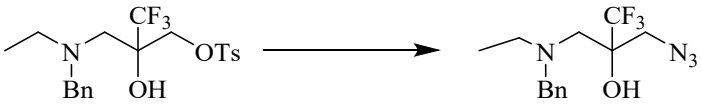
To a solution of 2-hydroxy-2-(trifluoromethyl)propane-1,3-diyl bis(4-methylbenzenesulfonate) (20 g, 39.5 mmol) in DCM (200 mL) was added K_2CO_3 (38.2 g, 277 mmol) at 0 ˚C under N_2 and the mixture stirred at 20 ˚C for 12 h under N_2. Five identical reactions were set up and the six reactions combined, filtered and concentrated to afford a residue that was subjected to purification by chromatography on silica gel (petroleum ether/ethyl acetate = 10/1 to 3/1) to afford the title compound (66.5 g, 94 % yield) as a colourless oil. LCMS (Method a, Table S1) Rt: 0.484 min; MS m/z = 338.2 $[M+MeCN]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.4 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 4.47 - 4.38 (m, 1H), 4.33 - 4.23 (m, 1H), 3.14 (d, *J* = 4.6 Hz, 1H), 3.05 - 2.95 (m, 1H), 2.47 (s, 3H). ^{19}F NMR (377 MHz, $CDCl_3$) δ ppm -74.86 (s, 1F).

2-((Benzyl(ethyl)amino)methyl)-3,3,3-trifluoro-2-hydroxypropyl 4-methylbenzenesulfonate.



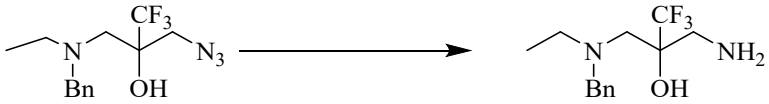
To a solution of (2-(trifluoromethyl)oxiran-2-yl)methyl 4-methylbenzenesulfonate (14 g, 46.7 mmol) in DMF (48 mL) was added N-benzylethanamine (12.6 g, 93 mmol) at 0 ˚C under N_2 and the mixture stirred at 20 ˚C for 12 h under N_2 protection and concentrated to afford the title compound (17.23 g, 85 % yield) as a colourless oil that was used directly in the next step. LCMS (Method b, Table S1) Rt: 0.662 min; MS m/z = 432.1 $[M+H]^+$.

3-Azido-2-((benzyl(ethyl)amino)methyl)-1,1,1-trifluoropropan-2-ol.



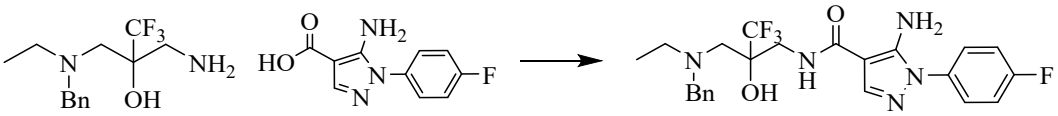
To a solution of 2-((benzyl(ethyl)amino)methyl)-3,3,3-trifluoro-2-hydroxypropyl 4-methylbenzenesulfonate (17.23 g, 39.9 mmol) in DMF (80 mL) was added sodium azide (3.25 g, 50.0 mmol) at 20 ˚C under N_2 protection and the mixture stirred at 110 ˚C for 12 h under N_2. The mixture was poured into aqueous $NaHCO_3$ (700 mL), extracted with ethyl acetate (3 X 200 mL), dried (Na_2SO_4), filtered and concentrated to afford the title compound (12.07 g, 100 % yield) as a colourless oil that was used directly in the next step. LCMS (Method b, Table S1) Rt: 1.044 min; MS m/z = 303.1 $[M+H]^+$.

3-Amino-2-((benzyl(ethyl)amino)methyl)-1,1,1-trifluoropropan-2-ol.



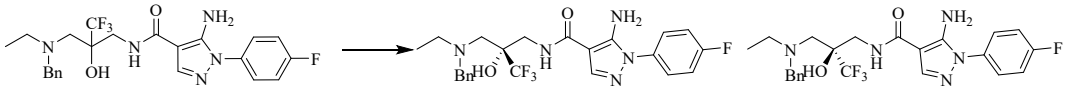
To a solution of 3-azido-2-((benzyl(ethyl)amino)methyl)-1,1,1-trifluoropropan-2-ol (12.1 g, 39.9 mmol) in THF (90 mL) and water (18 mL) was added trimethylphosphine (47.9 mL, 47.9 mmol) at 20 ˚C and the mixture stirred at 20 ˚C for 12 h. Two identical reactions were set up and the three reactions combined, poured into water (1500 mL), extracted with ethyl acetate (3 X 500 mL), dried (Na_2SO_4), filtered and concentrated to afford a residue that was subjected to purification by chromatography on silica (petroleum ether/ethyl acetate = 10/1 to 2/1) to afford the title compound (25 g, 72 % yield) as a colourless oil. LCMS (Method c, Table S1) Rt: 0.728 min; MS m/z = 277.2 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.39 - 7.21 (m, 5H), 3.90 (d, *J* = 13.5 Hz, 1H), 3.64 (d, *J* = 13.5 Hz, 1H), 2.95 (d, *J* = 13.4 Hz, 1H), 2.91 - 2.84 (m, 1H), 2.78 - 2.65 (m, 3H), 2.64 - 2.53 (m, 1H), 1.06 (t, *J* = 7.1 Hz, 3H). ^{19}F NMR (377 MHz, $CDCl_3$) δ ppm -79.79 (s, 1F).

5-Amino-N-(2-((benzyl(ethyl)amino)methyl)-3,3,3-trifluoro-2-hydroxypropyl)-1-(4-fluorophenyl)-1H-pyrazole-4-carboxamide.



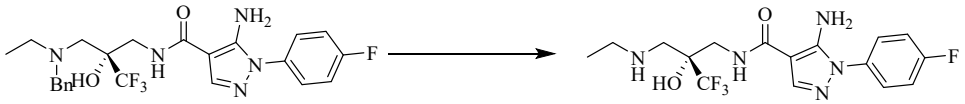
To a solution of 5-amino-1-(4-fluorophenyl)-1H-pyrazole-4-carboxylic acid (4.67 g, 20.6 mmol) and N, N-diisopropylethylamine (9.01 mL, 51.6 mmol) in THF (60 mL) was added HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) (9.41 g, 24.76 mmol) at 0 °C and the mixture stirred at 0 °C for 0.5 h. 3-Amino-2-((benzyl(ethyl)amino)methyl)-1,1,1-trifluoropropan-2-ol (6.0 g, 20.6 mmol) in THF (6 mL) was added at 0 °C under N_2 and the mixture stirred at 20 °C for 12 h. Five identical reactions were set up and the six reactions combined, filtered and concentrated to afford a residue that was subjected to purification by chromatography on silica gel (petroleum ether: ethyl acetate =1: 1 to 0:1) to afford the title compound (18 g, 43 % yield) as a yellow solid. LCMS (Method a, Table S1) Rt: 0.351 min; MS m/z = 480.3 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.07 (br t, J = 5.7 Hz, 1H), 7.91 (s, 1H), 7.62 - 7.51 (m, 2H), 7.44 - 7.30 (m, 6H), 7.28 - 7.21 (m, 1H), 6.47 (s, 1H), 6.37 (s, 2H), 3.89 - 3.74 (m, 2H), 3.61 (d, J = 13.6 Hz, 1H), 3.45 (br dd, J = 6.3, 14.6 Hz, 1H), 2.81 - 2.70 (m, 2H), 2.59 (td, J = 7.0, 13.6 Hz, 1H), 2.49 - 2.43 (m, 1H), 0.96 (t, J = 7.0 Hz, 3H). ^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -77.00 (s, 1F), -114.69 (s, 1F).

(S)-5-Amino-N-(2-((benzyl(ethyl)amino)methyl)-3,3,3-trifluoro-2-hydroxypropyl)-1-(4-fluorophenyl)-1H-pyrazole-4-carboxamide and (R)-5-amino-N-(2-((benzyl(ethyl)amino)methyl)-3,3,3-trifluoro-2-hydroxypropyl)-1-(4-fluorophenyl)-1H-pyrazole-4-carboxamide.



5-Amino-N-(2-((benzyl(ethyl)amino)methyl)-3,3,3-trifluoro-2-hydroxypropyl)-1-(4-fluorophenyl)-1H-pyrazole-4-carboxamide (30 g, 59.4 mmol) was purified by SFC (column: daicel chiralpak 250 mm*50 mm,10 um; mobile phase: A: CO_2; B: IPA; B%: 45%-45%, 2.90 min) to afford (S)-title compound (13 g, 91% yield) as white solid SFC (Method d, Table S1) Rt: 3.633 min; 100 % e.e. and afford (R)-title compound (12 g, 84 % yield) as white solid, SFC (Method d, Table S1) Rt: 4.435 min; 100 % e.e..

(R)-5-Amino-N-(2-((ethylamino)methyl)-3,3,3-trifluoro-2-hydroxypropyl)-1-(4-fluorophenyl)-1H-pyrazole-4-carboxamide.



To a solution of (S)-5-amino-N-(2-((benzyl(ethyl)amino)methyl)-3,3,3-trifluoro-2-hydroxypropyl)-1-(4-fluorophenyl)-1H-pyrazole-4-carboxamide (10.0 g, 20.9 mmol) and Pd/C (2.2 g, 2.1 mmol) in THF (100 mL) at 25 ˚C and the mixture stirred at 25 ˚C for 16 h under H_2 40 psi). LCMS showed the starting material was completed and the product was generated. An identical reaction was set up and the two reactions combined, filtered and concentrated to afford a residue that was triturated with n-heptane (80 mL), filtered, and concentrated to afford the title compound (9 g, 92 % yield) as a white solid. LCMS (Method e, Table S1) Rt: 1.472 min; MS m/z = 390.2 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.08 (br t, J = 6.0 Hz, 1H), 7.94 (s, 1H), 7.62 - 7.52 (m, 2H), 7.43 - 7.29 (m, 2H), 6.36 (s, 2H), 6.16 (br s, 1H), 3.67 (dd, J = 6.2, 14.2 Hz, 1H), 3.46 (dd, J = 5.9, 14.1 Hz, 1H), 2.78 - 2.66 (m, 2H), 2.56 (br t, J = 7.3 Hz, 2H), 1.92 (br s, 1H), 1.01 (t, J = 7.1 Hz, 3H). ^{19}F NMR (377 MHz, DMSO-d_6) δ ppm -77.28 (s, 1F), -114.74 (s, 1F). SFC (Method d, Table S1) Rt: 2.478 min; 100 % e.e. Specific rotation=12.75.

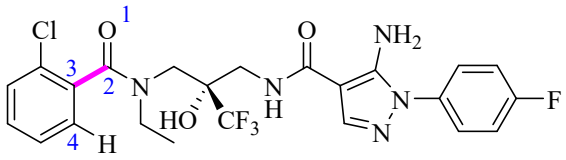
Table S1. LCMS and SFC conditions

Methods	Description

a	Column: Halo C18, 3.0 * 30mm, 5μm; Mobile Phase A: 0.04 % TFA in H_2O; Mobile Phase B: 0.02 % TFA in MeCN; Gradient: 10 % - 100 % B in 0.5 min, hold for 0.4 min; Flow rate: 2.0 mL/min.
b	Column: Halo C18, 3.0 * 30mm,5μm; Mobile Phase A: 0.04 % TFA in H_2O; Mobile Phase B: 0.02 % TFA in MeCN; Gradient: 5 % - 95 % B in 0.7 min, hold for 0.5 min; Flow rate: 1.5 mL/min.
c	Column: Halo C18, 3.0*30mm,5μm; Mobile Phase A: 0.04 % TFA in H_2O; Mobile Phase B: 0.02 % TFA in MeCN; Gradient: 5 % - 95 % B in 2.5 min, hold for 0.5 min; Flow rate: 1.0 mL/min.
d	Column: Chiralpak AD-3, 150×4.6 mm I.D., 3 um; Mobile phase A: CO_2; Mobile Phase B: IPA (0.2 % NH_3 (7 M in MeOH), v/v); Gradient: 10 % B for 0.5 min, 10 % - 50 % B in 3 min, hold for 1 min; Flow rate: 2.5 mL/min.
e	Column: Halo C18, 3.0 * 30mm, 5μm; Mobile Phase A: 0.04 % TFA in H_2O; Mobile Phase B: 0.02 % TFA in MeCN; Gradient: 5 % B for 0.4 min, 5 % - 95 % B in 2.6 min, hold for 1 min; Flow rate: 1.0 mL/min.

Torsion Angle Scan Protocol

Take compound 4 as an example to calculate the torsional scan energy.



4

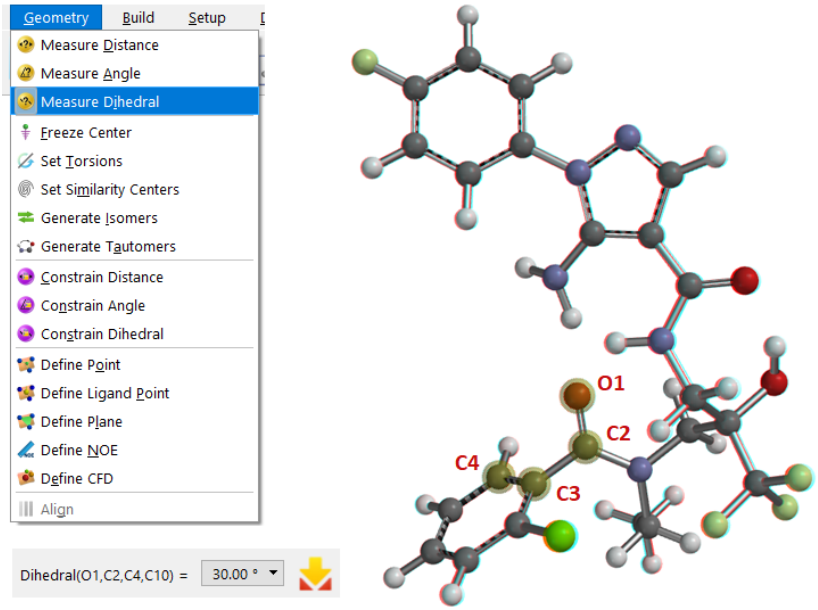
Software: **Spartan'20**

Protocol for torsion angel scan.

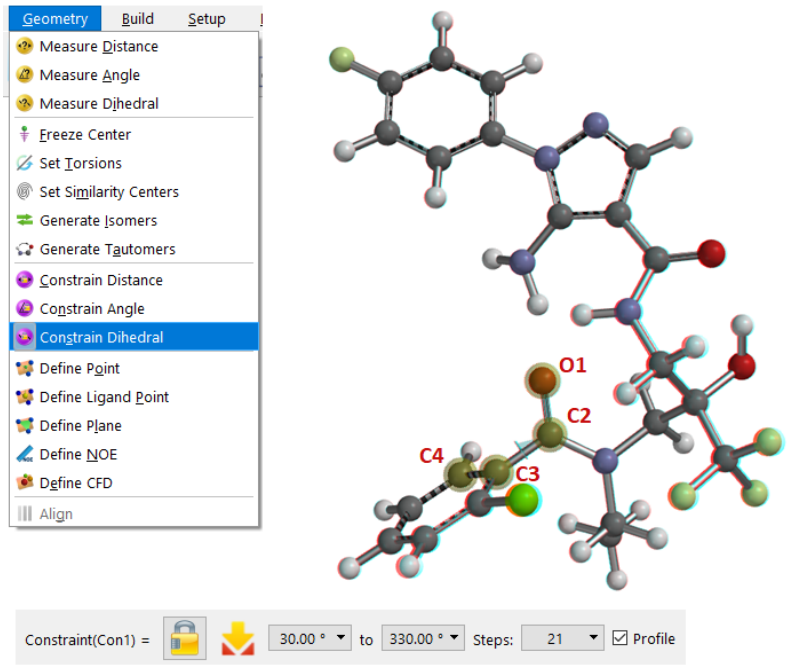
First, the low-energy conformation of compound 4 was calculated using MMFF (Spartan'20). Then from the menu, choose Geometry, select Measure Dihedral, click on the O1-C2-C3-C4 atoms in order, and select the dihedral angle to rotate. The torsion scan was then set to calculate from a dihedral angle of 30º to 330º, with an increment of 15º, in 21 steps (excluding the conformers with dihedral angle of 0º and 360º which will have the chloro and ethyl group crashing into one another) using Hartree-Fock 6-31G**method (gas phase).

The step-by-step procedure is described as below.

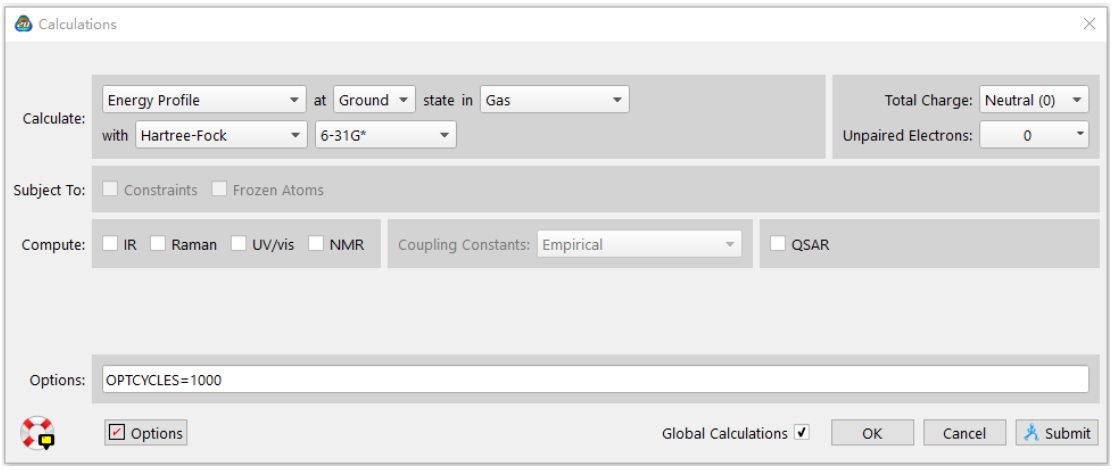
1. Build the molecule (compound 4). Select **Measure Dihedral** from the **Geometry** menu, *click* on O1-C2-C3-C4 atoms in order, *click* inside the box to the right of **Dihedral...** and enter **30** (30°).



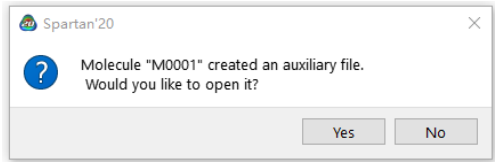
2. Select **Constrain Dihedral** from the **Geometry** menu. Select the O1-C2-C3-C4 torsion (*click* on O1-C2-C3-C4 atoms in order), and then *click* on  at the bottom right of the screen. The ic on will change to  indicating that a dihedral constraint is to be applied. *Check* the box to the left of **Profile** at the bottom right of the screen. This will result in two additional text boxes. Leave 30° in the original (leftmost) box alone, change the value in the box to the right of **to** to 330°. **Steps** should be 21.



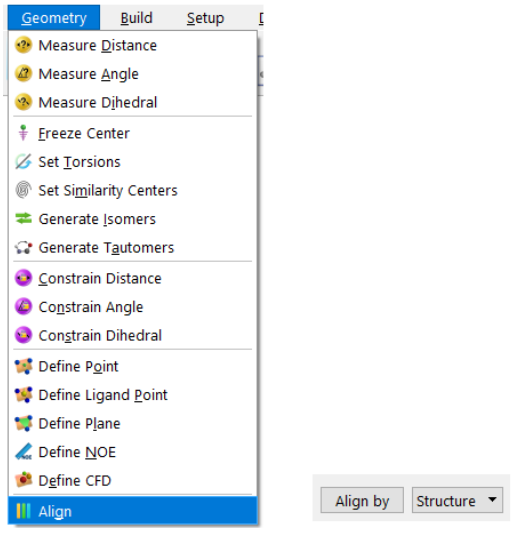
3. Select **Calculations...** from the **Setup** menu and specify **Energy Profile** from the top menu to the right of **Calculate**, and **Hartree-Fock,** and **6-31G*** from the three bottom menus. *Click* on **Submit** and named as **compound 4**.



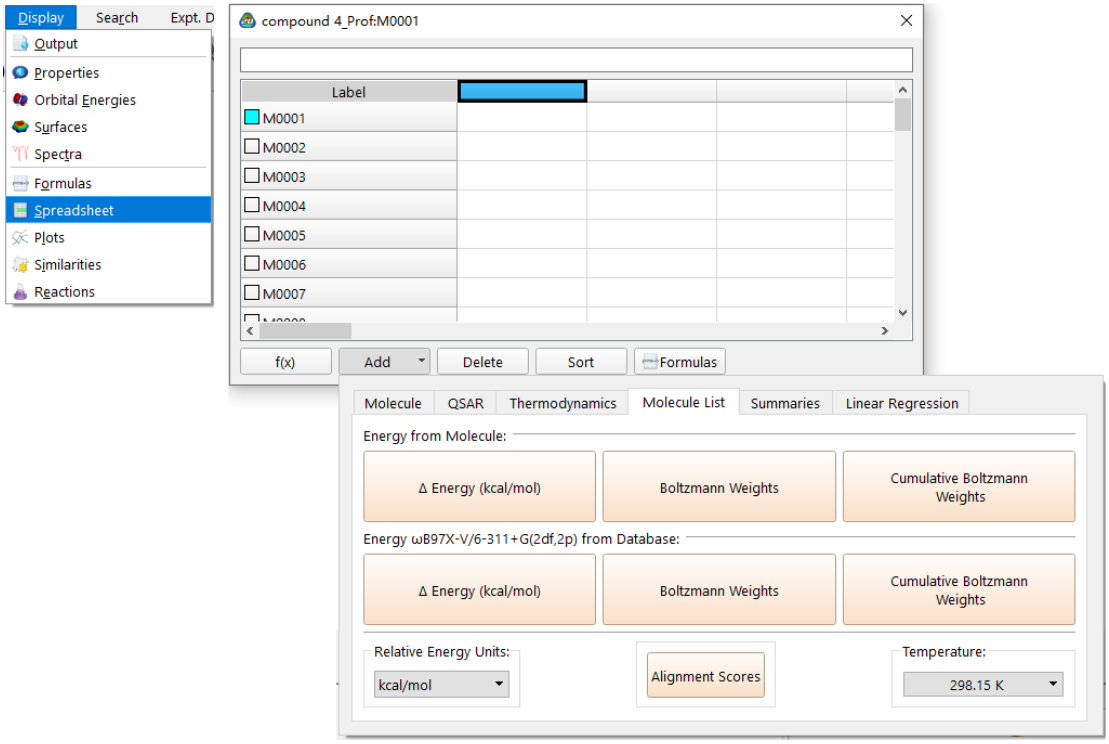
4. When the calculations have completed, they will go into a file named ***compound 4.Prof.M0001***. A prompt will ask you if you want to open this file. *Click* on **Yes**.



5. Align the conformers. Select **Align** from the **Geometry** menu, select **Structure** from the **Align by** menu at the bottom right of the screen and, one after the other, *click* on atoms of amide group. Then *click* on the **Align by** button at the bottom right of the screen.



6. Select **Spreadsheet** from the **Display** menu, First *click* on the label (**M0001**) for the top entry in the spreadsheet (the 30° conformer), then *click* on the header cell for the leftmost blank column, and finally, *click* on **Add...** at the bottom of the spreadsheet. Select **rel. E (kcal/mol)** from the quantities in the **Molecule List** tab, and *click* on the spreadsheet to release the dialog.



To enter the dihedral angle constraints, select **Constrain Dihedral** from the **Geometry** menu, *click* on the constraint marker attached to compound 4 and *click* on  at the bottom of the screen (to the right of the value of the dihedral angle). *Click* on  .

7. Select **Plots** from the **Display** menu. *Click* on  at the top of the (empty) plot pane and select **Constraint(Con1)** from the **X Axis** menu and **rel. E(kcal/mol)** from the **Y Axes** list and then *click* **Create**. Then a curve of torsional scan can be obtained.

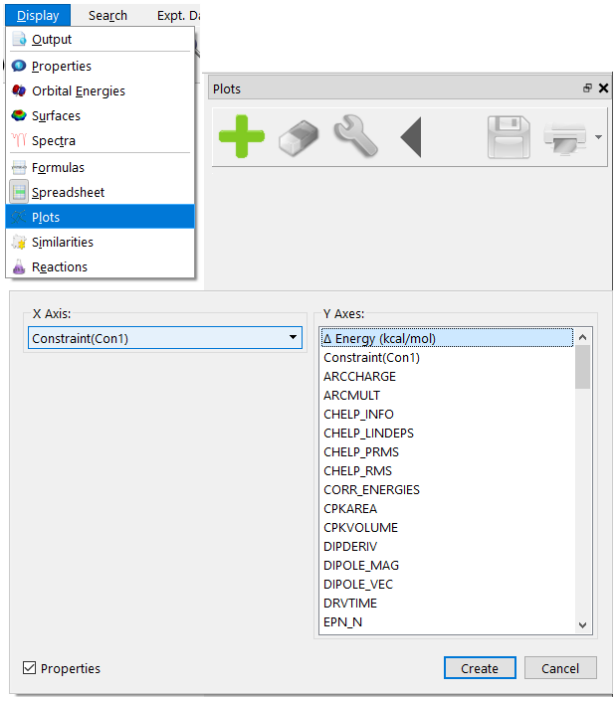
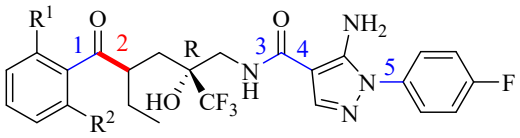


Table S2. Torsion Rotation Energy Barrier of Tertiary Amide Bond 2.



Cpd	R^1	R^2	Stereo chemistry	Bond 2 Torsion Rotation Energy Barrier / kcal mol^{-1}
1	H	H	S	16.65
2	F	H	S	31.31
3	Me	H	S	26.26
4	Cl	H	S	22.60
5	Et	H	S	25.13
6	F	F	S	17.93
7	F	Me	S	23.32
8	F	Cl	S	24.15
9	Me	Me	S	24.95
10	Me	Cl	S	26.94
11	Cl	Cl	S	39.96
12	Cl	Cl	R	35.05
13a	Me	Et	S	28.94
13b	Me	Et	S	26.99
14a	Cl	Et	S	26.30
15	Et	Et	S	26.94

GR Assay Protocol

Material	Vendor	Cat #	Lot #
DMEM with phenol red	Invitrogen	11960051	1896954
FBS	Hyclone	SV30087.03	18019393
DMEM without phenol red	Gibco	31053028	1919102
Charcoal stripped FBS	SERANA	S-FBS-US-065	15100113FBS
Assay plate	Corning	3610	NA
Dual-Glo	Promega	E2920	NA
P/S	Hyclone	SV30010	J19007
GlutaMax	Gibco	35050-061	1848033

Medium:

Cell culture medium: 88% DMEM with phenol red, 10% FBS, 1% P/S and 1% GlutaMax, 900ug/ml G418, 75 ug/mL Hygromycin.

Cell culture medium: 89% DMEM without phenol red, 10% charcoal stripped FBS and 1% GlutaMax.

Procedure:

Day 1:

1. 3 or 4-fold serial dilute compound to get 10 doses and transfer 125 nL of each concentration to compound plate as the plate layout using Echo.

2. Seed 30000 cells in 25 uL medium to each well of assay plate, place at 5% CO2, 37℃ for 24 h.

Day 2:

1. Add 25 uL luciferase to assay plate, shake at room temperature for 20 minutes, and read on Envision.

2. Add 25 uL Stop & Glo reagent to assay plate, shake at room temperature for 20 minutes, and read on Envision.

Figure S1. SFC spectra of compound 14

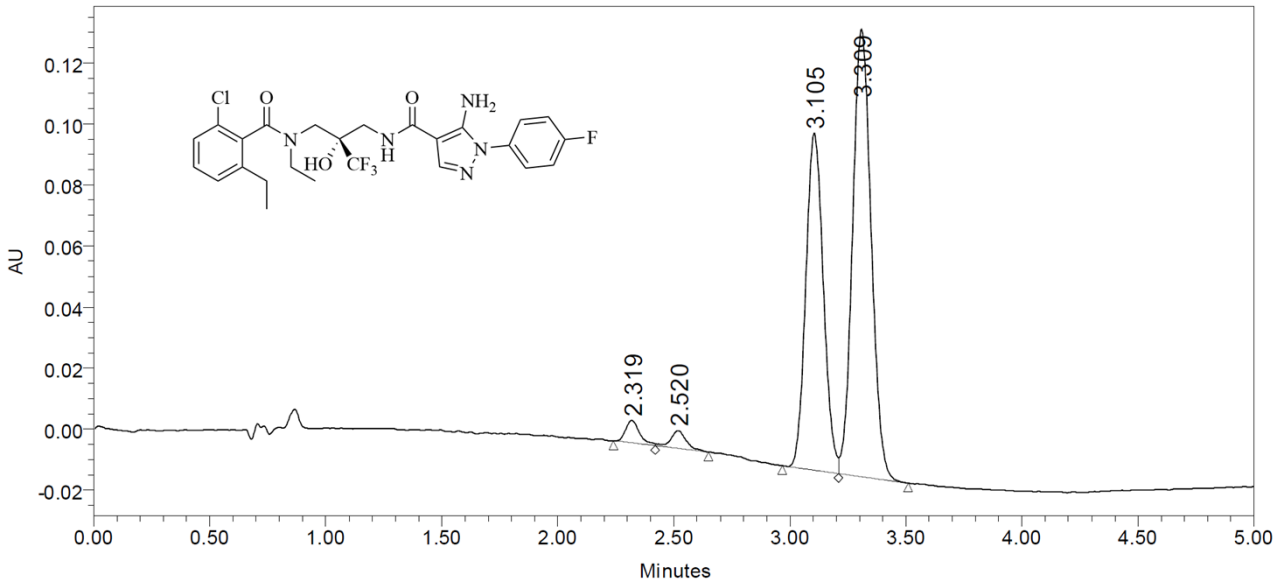


Figure S2. Torsional scan of compound 4

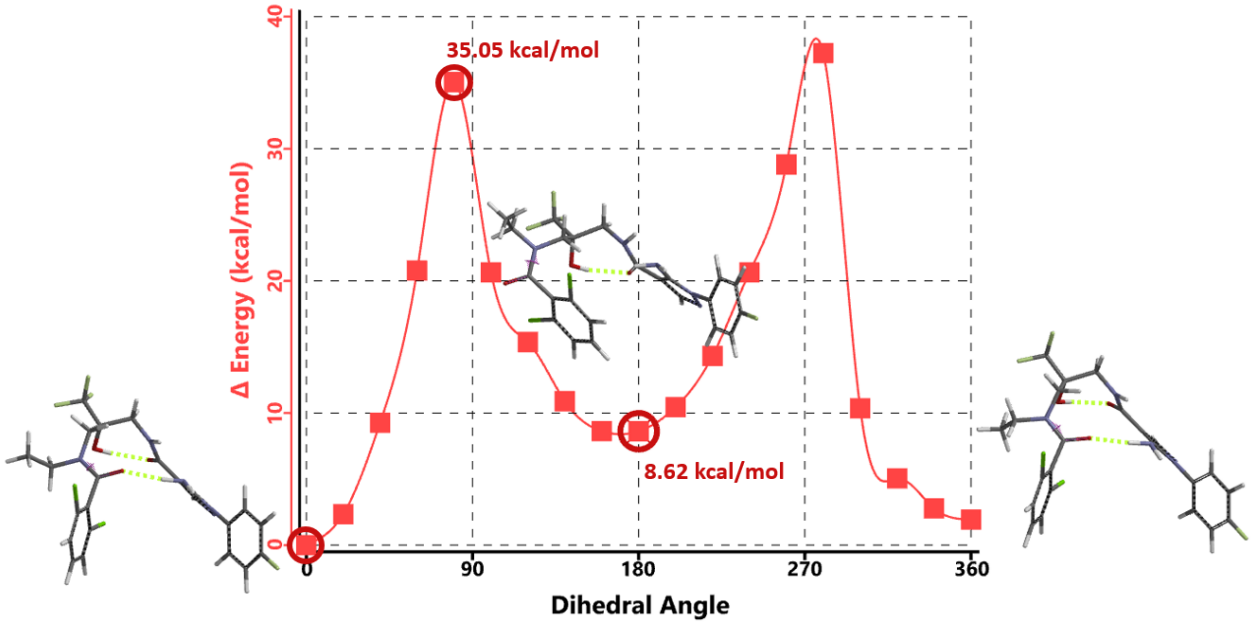
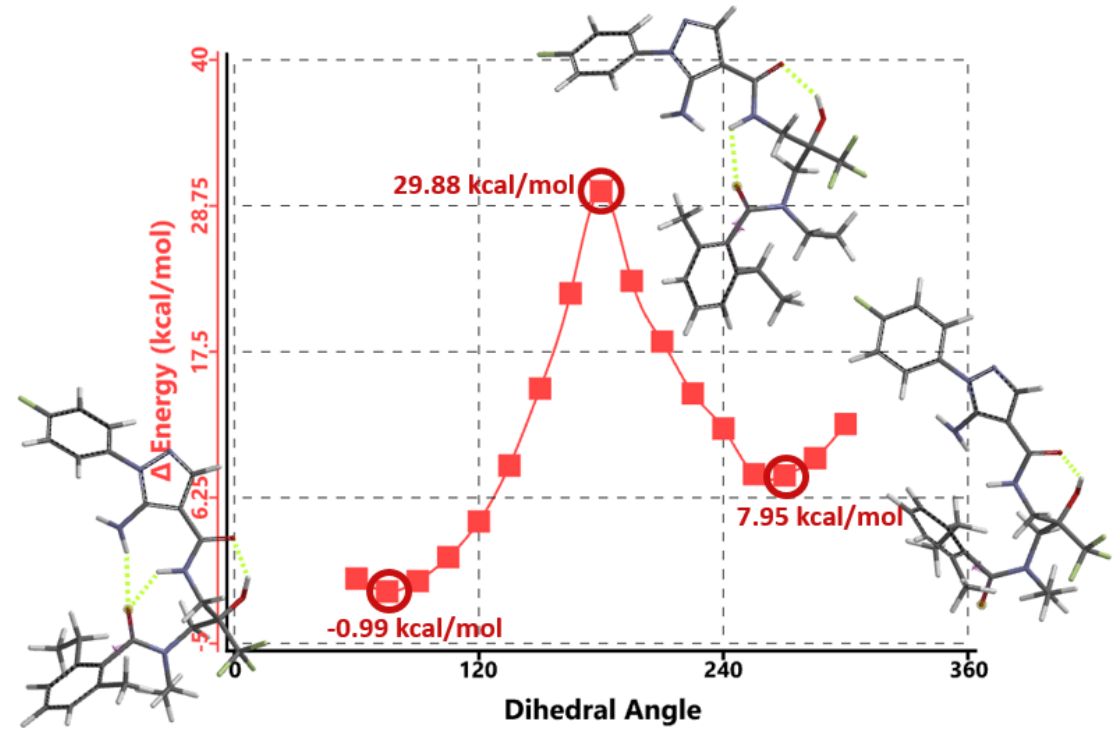


Figure S3. Torsional scan of compound 13



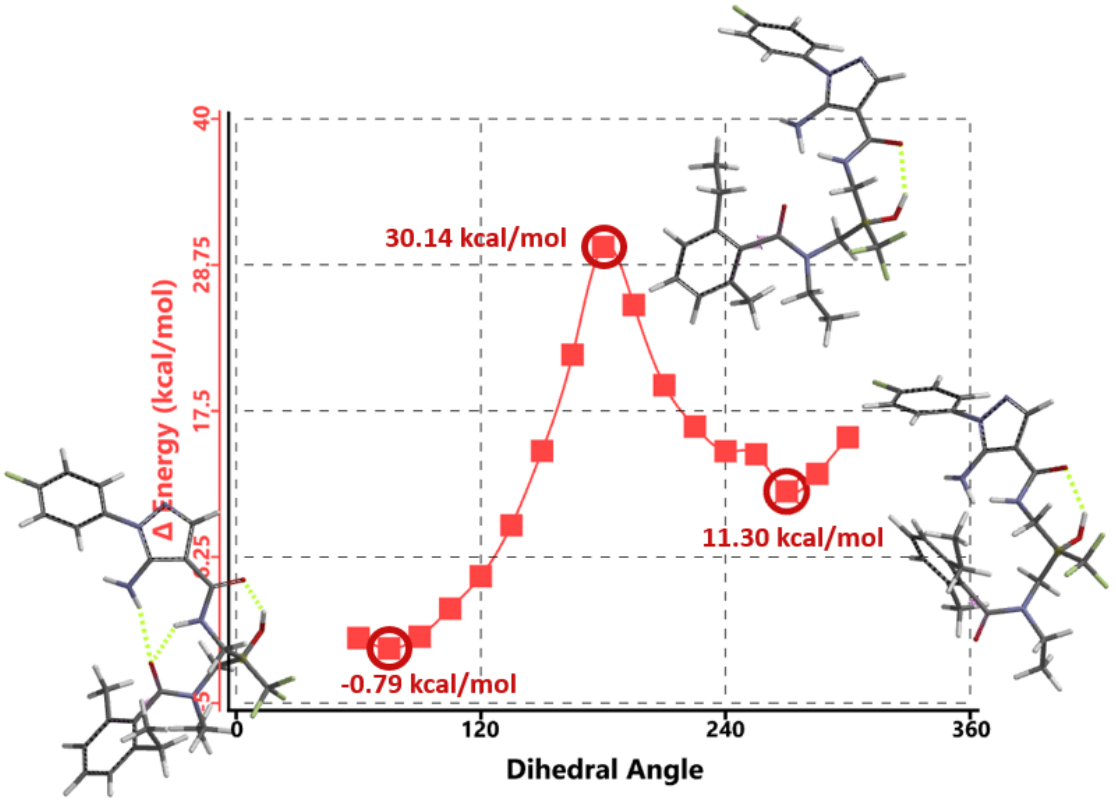
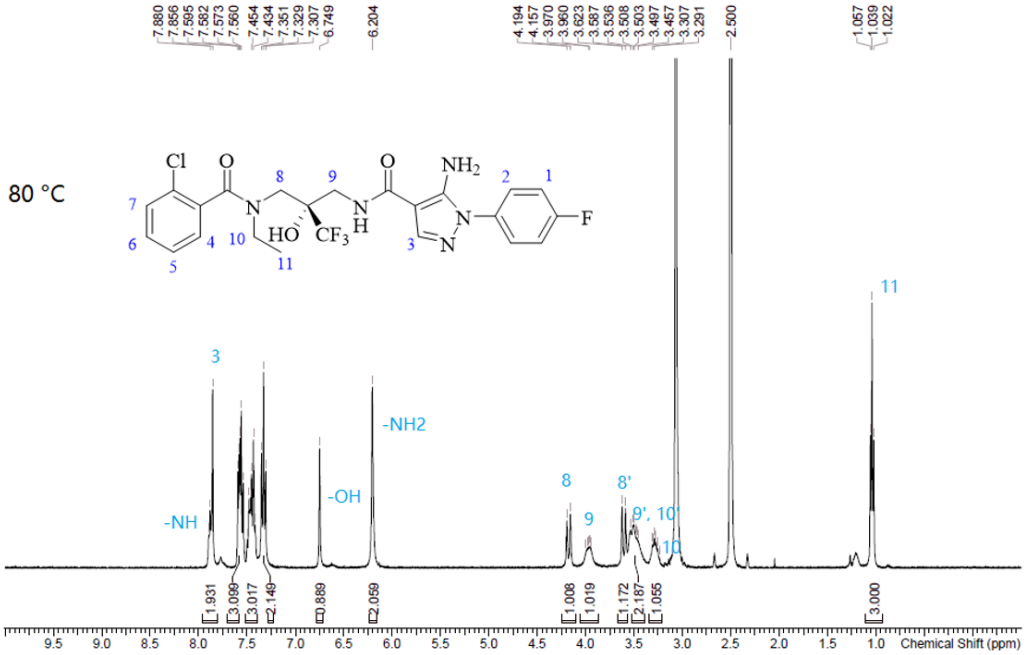


Figure S4. ^{1}H NMR of compound 4 at 80 °C



Molecular Docking Protocol

Molecular Docking was performed using the Schrodinger GLIDE SP program (Schrodinger Suite 2023-4). The structural model was built based on crystal structure of Glucocorticoid Receptor LBD bound to GSK866 (PDB code: 3E7C). All water molecules and ion in the complex were removed prior to docking. “Protein Preparation Wizard” module in Schrodinger was used to process the protein structure, including assigning protonation states (pH=7.4) and structure minimization. “LigPrep” module was employed for preprocessing of the small molecule structures, which contains generating stereoisomers, assigning tautomer states, and protonation states (pH=7.4). During docking the protein structure was kept rigid. After obtaining the docking poses, MM-GBSA calculation was performed on the high-scoring docking pose to obtain the binding free energy (MMGBSA dG) using force field OPLS4.

The crystal structure (PDB code: 3E7C) used for structural modeling is a complex of glucocorticoid receptor and GSK866, which is close analog of our compound series. So the GSK866 binding mode is used as a reference to determine binding sites and predict binding mode of our compounds. The PDB structure 3E7C has two mutations, F602Y and C638G. The minimum distances between the Cα atoms on these two residues and the heavy atoms of the small molecule are over 8.0 Å and 5.0 Å, respectively. Besides, the side chains are oriented away from the small molecule. So these two mutations have no significant effect on the binding site.

Figure S5. Molecular Docking of methyl-ethyl compound 13 using Schrodinger GLIDE SP program in crystal structure PDB code 3E7C a) Compound 13a; b) Compound 13b.

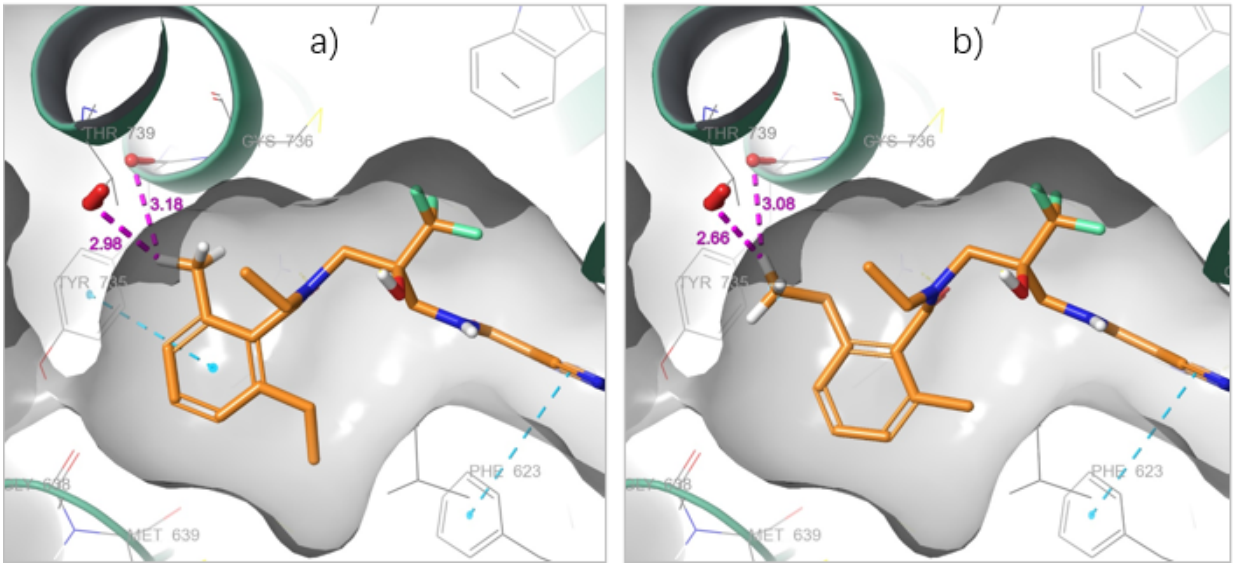


Figure S6. Configuration assignment for compounds 13a and 13b.

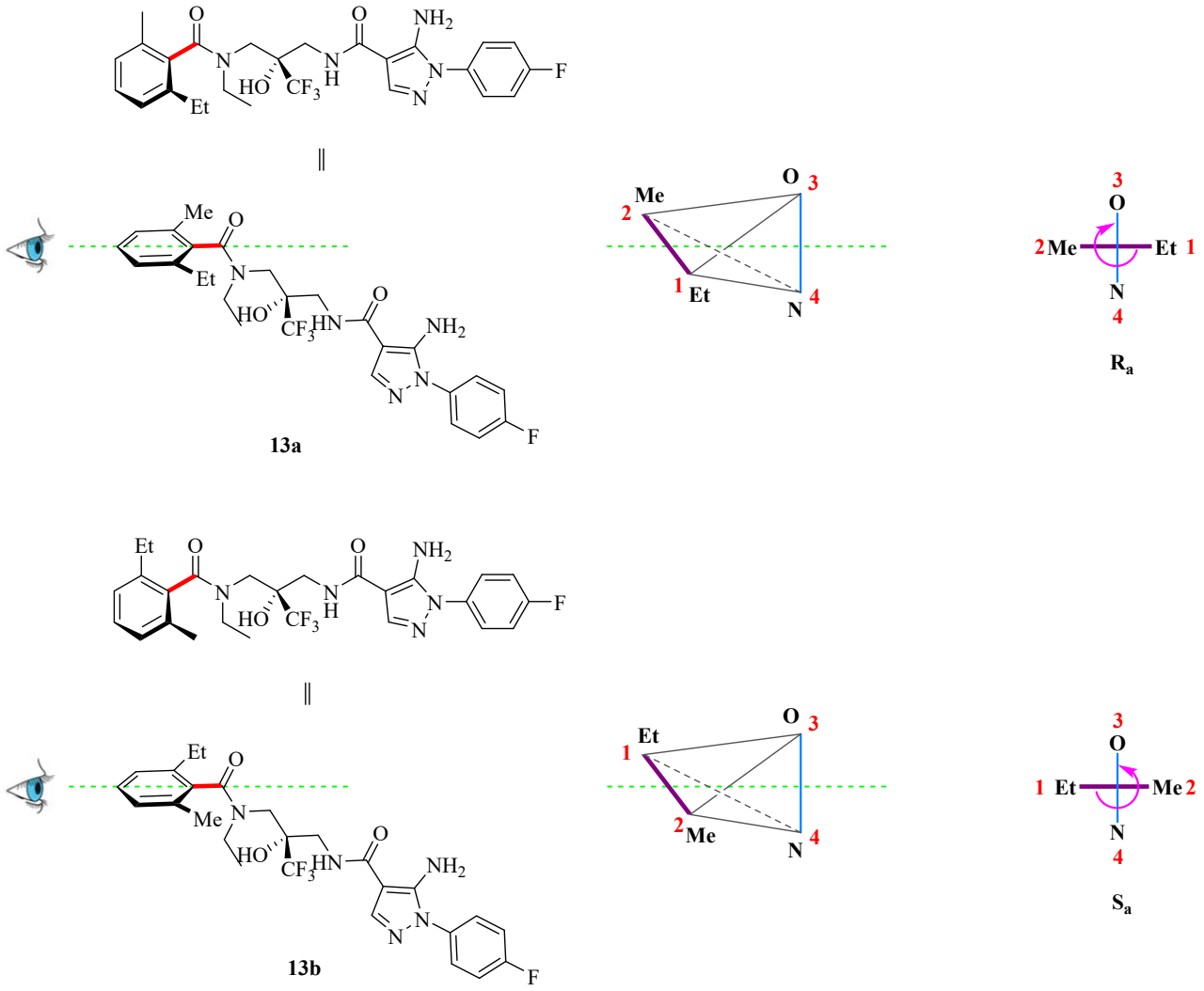
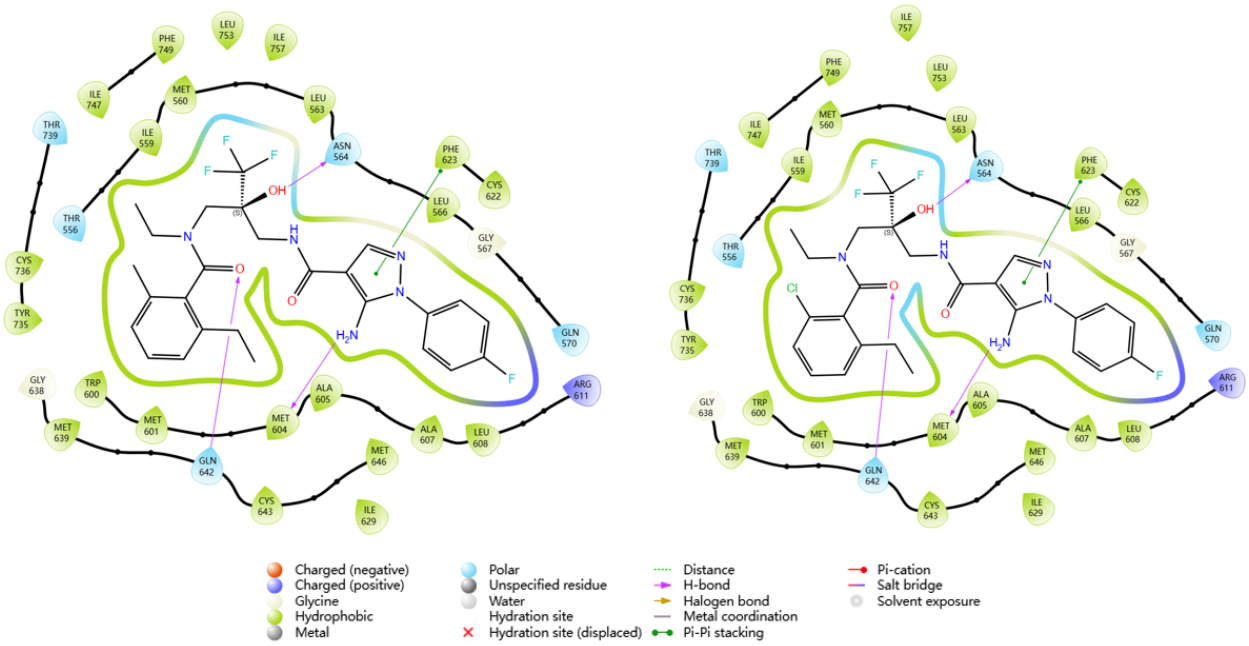


Figure S7. 2D Ligand interaction diagram of 13a and 14b


Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure