Supporting Information

Discovery of Spirocyclic Aldosterone Synthase Inhibitors as Potential Treatments for Resistant Hypertension

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List of abbreviations. In the following procedures and schemes, abbreviations are used with the following meanings unless otherwise indicated: ACTH = adrenocorticotropic hormone; aq. = aqueous; BSA = bovine serum albumin; Bu = butyl, *t*-Bu = *tert*-butyl; DMEM = Dulbecco's modified eagle medium; DMSO = dimethylsulfoxide; eq. = equivalent(s); EDTA = ethylenediaminetetraacetic acid; ESI = electrospray ionization; Et = ethyl; EtOAc = ethyl acetate; EtOH = ethanol; FBS = Fetal Bovine Serum; h, hr = hour; HCl = hydrochloric acid; HPLC = High pressure liquid chromatography; HRMS = high resolution mass spectrometry; HTRF = homogenous time resolved fluorescence; LCMS = liquid chromatography - mass spectrometry; Me = methyl; min. = minute; NMR = nuclear magnetic resonance; PBS = phosphate buffered saline; Ph = phenyl; Pr = propyl; Py = pyridyl; RT, rt = room temperature; sat. = saturated; RuPhos Indoline Pre-catalyst = Chloro-(2-Dicyclohexylphosphino-2′,6′-diisopropoxy-1,1′-biphenyl)[2-(2-aminoethyl)phenyl]palladium(II) - methyl-t-butyl ether adduct; SFC = supercritical fluid chromatography; *t*-Bu = tert-butyl; TFA = trifluoroacetic acid; THF = tetrahydrofuran; TLC = thin layer chromatography.

Human CYP11B2 and CYP11B1 Assays. V79 cell lines stably expressing the either the human CYP11B2 or the human CYP11B1 enzyme were generated using a standard transfection

protocol. V79 cells were transfected with plasmids pTriEx3-Hygro-hCYP11B2 or pTriEx3-Hygro-hCYP11B1 using Lipofectamine2000 reagent. V79 cells that stably express the human CYP11B2 or human CYP11B1 enzyme were selected for and maintained in DMEM supplemented with 10% FBS and 400 μg/mL hygromycin for ~2 weeks. Single cell clones were generated by infinite dilution in DMEM supplemented with 10% FBS and 400 μg/mL hygromycin until single colonies were obtained. Clones V79-hCYP11B2-CLE9 and V79-hCYP11B1-8C7 were determined to produce the most aldosterone and cortisol, respectively, and were selected for inhibitor screening. For testing of inhibitors, cells were harvested at 80% confluency with 0.5% Trypsan-EDTA, washed once in PBS, and reconstituted in DMEM + 0.1% BSA media at a cell concentration of 400,000 cells / mL. 25 μL of cells were added to a 384-well tissue culture treated plate and mixed with 0.25 μL of inhibitor or DMSO (1% final DMSO concentration) for 1 hour at 37 °C, 5% CO_2. After pre-incubation with inhibitor, the reaction was initiated by adding 5 μL of substrate (final concentration of 125 nM 11-deoxycorticosterone for the CYP11B2 assay or 250 nM 11-deoxycortisol for the CYP11B1 assay). The reaction was carried out for 3 hours at 37 °C, 5% CO_2 and was stopped by harvesting the supernatants. The amount of product in the supernatant (aldosterone for CYP11B2 assay and cortisol for the CYP11B1 assay) was measured using HTRF-based assay kit (Aldosterone HTRF-CisBio #64ALDPEB, Cortisol HTRF-CisBio #63IDC002-CORT). IC_{50}'s for the inhibitor were determined by plotting the amount of product formed against the concentration of inhibitor using sigmoidal dose-response curve (variable slope) fit in GraphPad.

Human CYP17 Assay: COS were maintained in DMEM supplemented with 10% fetal calf serum. For transfection of COS-7, cells were grown to 70% confluency and transfected with pTrieX3-Hygro-Human CYP17 using Lipofectamine 2000 according to the manufacturer's instructions. For testing of inhibitors, cells were harvested at 48 h post transfection with 0.5% Trypsan-EDTA, washed once in PBS, and reconstituted in DMEM + 0.1% BSA media at a cell concentration of 800,000 cells / mL. 100 μL of cells were added to a 96 well tissue culture treated plate and mixed with 1.0 μL of inhibitor or DMSO (1% final DMSO concentration) for 1 hour at 37 °C, 5% CO_2. After pre-incubation with inhibitor, the reaction was initiated by adding 5 μL of substrate (final concentration 360 nM 17-hydroxypregnenolone). The reaction was carried out for 3 hours at 37 °C, 5% CO_2 and was stopped by harvesting the supernatants. The

amount of dehydroepiandrosterone (DHEA) product in the supernatant was measured using EIA-based assay kit (DHEA EIA kit (Enzo Life Sciences, cat no. 901-093). IC_{50}'s for the inhibitor were determined by plotting the amount of product formed against the concentration of inhibitor using sigmoidal dose-response curve(variable slope) fit in GraphPad.

CYP19 Assay: CYP19 assay was performed using the CYP19/MFC High Throughput Inhibitor Screening Kit according to manufacturer's instructions. Briefly, inhibitor was first dissolved in 100% acetonitrile, then 6 μL of inhibitor was mixed with 144 μL of NADPH-cofactor mix (16.25 μM NADP, 825 μM MgCl2, 825 μM Glucose-6-Phosphate, 0.4 Units/mL Glucose-6-Phosphate Dehydrogenase). The inhibitor was furthered titratrated against the same NADPH-cofactor mix. 100 μL of inhibitor / NADPH-cofactor mix was added to 96 well plate and incubated at 37 °C for 10 minutes. The assay was initiated by adding100 μL of enzyme and substrate mix (15 nM CYP19 and 50 μM MFC in 0.1 M Phosphate buffer, pH 7.4). The reaction was carried out for 30 minutes at 37 °C and was stopped by adding 75 μL stop solution (0.5 M Tris Base). The amount of product formed was detected by measuring the HFC metabolite at 409 nm excitation / 530 nm emission wavelengths.

Human In Vitro CYP11B2 and CYP11B1 Data for Compounds 1 – 16

Cpd	hCYP11B2 (IC_{50}, nM)	N	SEM	hCYP11B1 (IC_{50}, nM)	n	SEM
1	108.2	1		1674	1	
2	554.8	1		1573	1	
3	13.4	1		386.9	1	
4	703.9	1		786.9	1	
5	41.6	1		1595	1	
6	228.0	2	10.8	1996	2	137.5
7	>8333	1		>8333	1	
8	>10000	2		1308.0	2	231.5
9	1031	2	18.9	>1000	2	
10	1134	1		>8333	1	
11	94	2	3.2	4460.0	2	1283.0
12	34.2	2	4.6	1739	2	116.2
13	72.8	2	8.3	2192.0	2	427.0
14	31.5	2	5.1	1544.0	2	352.4
15	448.4	2	94.1	>10000	1	
16	12.8	3	0.9	354.9	2	1.9

Activity of Selected Compounds at Hepatic CYP and Ion Channel Targets

Cpd	CYP11B2 IC_{50} (nM)	CYP3A4 IC_{50} (nM)	CYP2C9 IC_{50} (nM)	CYP2D6 IC_{50} (nM)	MK-0499 IC_{50}[a] (nM)	Nav1.5 IC_{50} (nM)	Cav1.2 IC_{50} (nM)
1	108	11440	>50000	>50000	22050	12790	10820
3	13	31580	>50000	>50000	>60000	>30000	>30000
6	228	19720	>50000	>50000	17970	>30000	>30000
14	32	>50000	33690	>50000	>60000	>30000	>30000
16	13	>50000	>50000	>50000	>60000	>30000	>30000

[a)] This is binding assay that measures displacement of ^{35}S-labeled MK-0499, a known hERG blocker.

Activity of Lead Compounds vs Related CYPs (11B1, 17, 19 and 21)

Cpd	CYP11B2 IC_{50} (nM)	CYP11B1 IC_{50} (nM)	CYP17 IC_{50} (nM)	CYP19A1 IC_{50} (nM)	CYP21 IC_{50} (nM)
14	32	1544	>10000	7679	>10000

Determination of Intrinsic Clearance. Reactions were carried out in a 96-well plate (Thermo Fisher Scientific Inc., Waltham, MA). The reaction mixture (450 µL), containing 100 mM potassium phosphate buffer, pH 7.4, rat liver microsomes (0.25 mg/mL), and the test compound (0.3 mM), was preincubated at 37 °C for 10 minutes. Reactions were initiated by the addition of NADPH (50 µL, 10 mM) to the incubation mixture. At time points 5, 15, 30, and 45 min, 50-µL aliquots of the reaction mixture were quenched with 200 µL of acetonitrile with 0.1% v/v formic acid and an internal standard cocktail. The samples were centrifuged at 4 °C for 10 min at 3,000 rpm. The supernatant was transferred to a 96-well plate for analysis using a generic LC/MS method. The unscaled microsomal intrinsic clearance was estimated using the equation $Cl_{int,\ unscaled} = K_e \times$ (volume of incubation/mg microsomal protein), where K_e is the first-order rate constant describing the disappearance of parent compound in the incubation and can be obtained from regressing the initial slope of the natural log of the analyte area/internal standard area (designated as C at an appointed time t) versus time (min) profile.

Rat Pharmacokinetic Assays. Rat pharmacokinetic experiments were conducted as follows: test compounds were typically formulated as 1.0 mg/mL solutions in mixtures of ethanol/PEG200/water (10:40:50, v/v/v). Fasted male Sprague-Dawley rats were given either a 0.50 mg or 1.0 mg/kg i.v. (n = 2) dose of test compound solution via a cannula implanted in the femoral vein. Additionally fasted male Sprahue-Dawley rats were either given a 1.0 mg or 2.0

mg/kg p.o. dose by gavage (n = 3). Serial blood samples were collected at 5 (i.v. only), 15, and 30 minutes, and at 1, 2, 4, 6, and 8 hours post dose. Plasma was collected by centrifugation, and plasma concentrations of test compound were determined by LC-MS/MS following protein precipitation with acetonitrile. All animal studies followed protocols that were approved by our Institutional Animal Care and Use Committee.

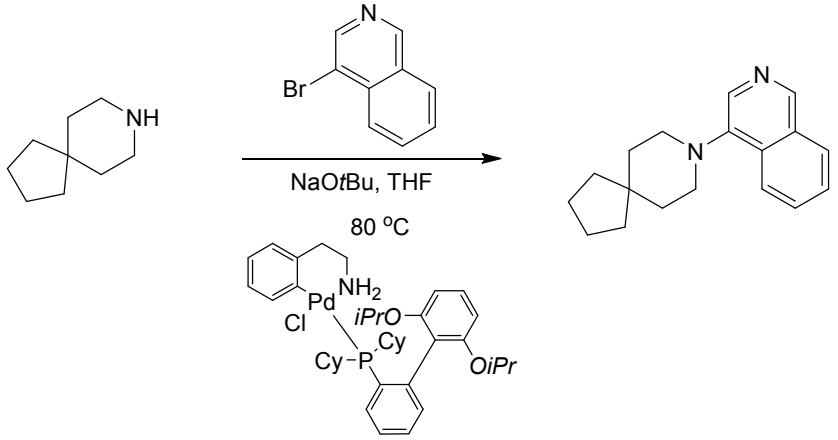
Molecular modeling. The structure of CYP11B2 was obtained from PDB files 4zgx, 4dvq, and 4fdh. Inhibitors were docked to the CYP11B2 active sites using both GOLD (Cambridge Crystallographic Data Centre, Cambridge, UK) and Posit (version 3.1.0.5, OpenEye Scientific Software, Inc., Santa Fe, NM, USA, www.eyesopen.com). The top poses from these methods were inspected visually, and the pose most consistent with available crystal structures was selected.

General Synthetic Procedures. Unless otherwise noted, commercially available materials were used without further purification. Air or moisture sensitive reactions were carried out under a nitrogen or argon atmosphere. Anhydrous solvents were obtained from Sigma-Aldrich and used as received. Flash chromatography was performed using pre-packed silica gel cartridges using Biotage Horizon or Biotage SP-1 instruments equipped with UV detectors. Preparative HPLC was performed using a SunFire Prep C18 OBD column (5 μM, 19 x 100 mm i.d.) on Gilson instruments equipped with UV detectors. Chemical reactions were monitored by LCMS, and the purity and identity of the reaction products were assayed by LCMS (electrospray ionization) and NMR. LCMS spectra were recorded on an Agilent 1100 series instrument equipped with an Xterra MS C18 column (3.5 μM, 3.0 x 50 mm i.d.) and UV detector. ^{1}H NMR spectra were recorded on a Varian or Brunker 500 HHz spectrometer, and are internally referenced to residual protio solvent signals. Data for ^{1}H NMR are reported with chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, ddd = doublet of doublet of doublet t = triplet, q = quartet, m = multiplet, br s = broad singlet, br m = broad multiplet), coupling constant (Hz), and integration. Unless otherwise noted, all LCMS ions listed are [M + H]. All temperatures are degrees Celsius

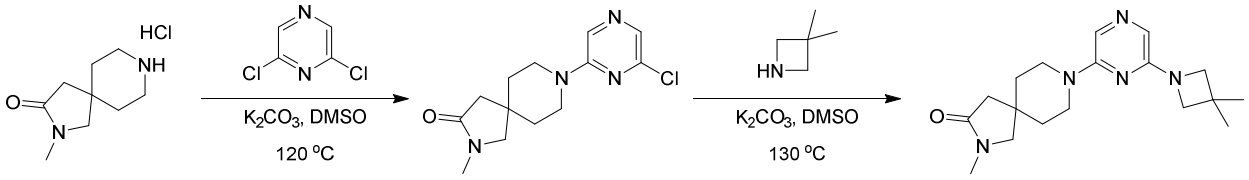
unless otherwise noted. All final compounds with reported biological data were determined to be >95% purity based on LC-MS and NMR data unless otherwise noted.

Synthesis Schemes. Compounds **1** – **10** were prepared using general synthesis A and the appropriate commercially available starting materials. Compounds **11 - 13** were synthesized using general synthesis B. Compounds **14 and 16** were synthesized using general synthesis C and bromide **X**, with the appropriate starting material. Compound **15** was prepared using general synthesis C and **Y.**

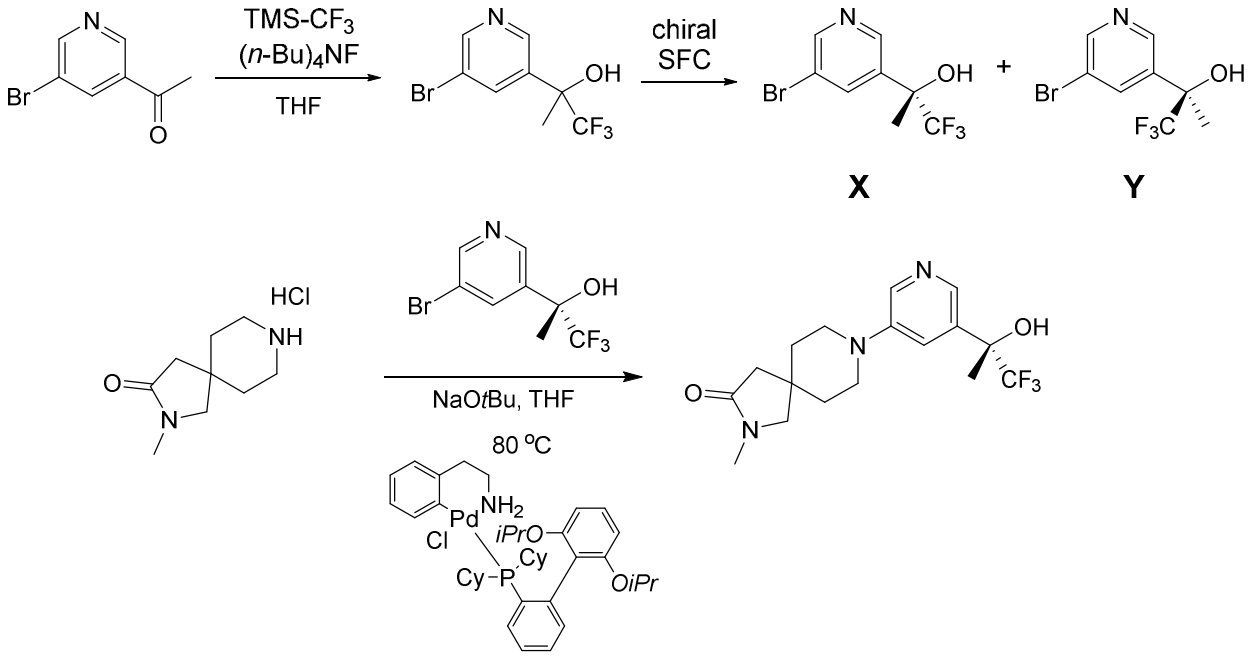
Scheme 1. General synthesis A of spirocyclic compounds **1 – 10.**



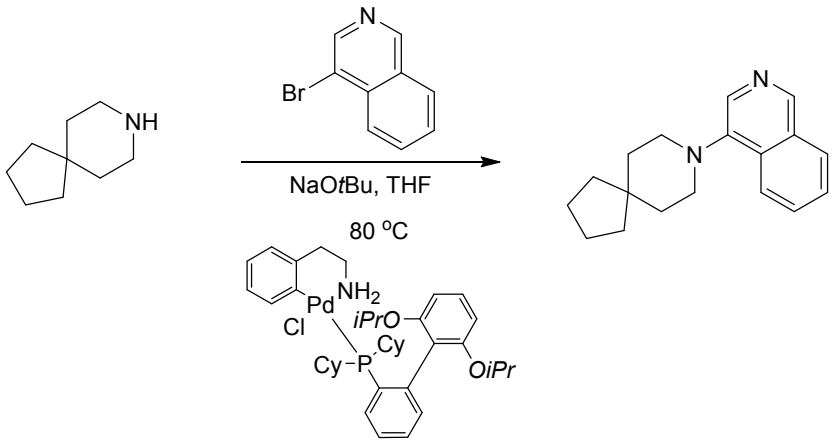
Scheme 2. General synthesis B of spirocyclic compound **11 - 13.**



Scheme 3. General synthesis C of spirocyclic compounds **14 – 16.**



Example 1: Exemplification of General Synthesis A: Preparation of Compound **1**.



To a vial with 8-azaspiro[4.5]decane (50 mg, 0.359 mmol) and 4-bromoisoquinoline (90 mg, 0.431 mmol) was added RuPhos Indoline Pre-catalyst (13.08 mg, 0.018 mmol) and sodium tert-butoxide (69.0 mg, 0.718 mmol). This mixture was then evacuated and backfilled with nitrogen before dry, degassed tetrahydrofuran (1.8 mL) was added and the reaction was heated at 80 °C for 18 h. The reaction was cooled to room temperature and then poured into brine (5 mL). It was extracted with ethyl acetate (3 x 10 mL). The combined organics were washed with brine then dried over sodium sulfate. The crude product was purified by HPLC (C18 column, 0 to

50% acetonitrile/water, both 0.1% v/v trifluoroacetic acid. The fractions containing product were combined and then freeze-dried to provide the title compound: HRMS (Positive ESI) m/z 267.1872 (267.1817 calcd for C18H22N2); ^{1}H NMR (500 MHz, CD_3OD) δ 8.86 (d, *J* = 0.8 Hz, 1H), 8.12 (dq, *J* = 8.5, 0.9 Hz, 1H), 8.05 (s, 1H), 8.03 (dt, *J* = 8.1, 0.9 Hz, 1H), 7.76 (ddd, *J* = 8.4, 6.9, 1.3 Hz, 1H), 7.64 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H), 3.10 (dd, *J* = 7.3, 3.9 Hz, 4H), 1.75 (t, *J* = 5.5 Hz, 4H), 1.71 – 1.62 (m, 4H), 1.62 – 1.48 (m, 4H); ^{13}C NMR (125 MHz, CD_3OD) δ 147.82, 146.37, 133.03, 132.15, 131.28, 130.70, 129.17, 128.62, 123.86, 52.07, 41.97, 39.20, 25.29.

Compound 2: 3-(isoquinolin-4-yl)-3-azaspiro[5.5]undecane was prepared by the common procedure as compound 1 starting with 3-azaspiro[5.5]undecane [CAS 180-44-9] and 4-bromoisoquinoline. LCMS m/z 280.89 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.21 (s, 1H), 8.36 – 8.18 (m, 2H), 8.15 (s, 1H), 8.05 (ddd, *J* = 8.5, 7.0, 1.2 Hz, 1H), 7.89 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H), 3.31 – 3.13 (m, 4H), 1.85 – 1.69 (m, 4H), 1.49 (s, 10H).

Compound 3: 8-(isoquinolin-4-yl)-2-oxa-8-azaspiro[4.5]decane was prepared by the common procedure for compound 1 starting with 2-oxa-8-azaspiro[4.5]decane hydrochloride [CAS 479195-19-2] and 4-bromoisoquinoline. LCMS m/z 269.26 $[M + H]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 8.90 (d, *J* = 0.8 Hz, 1H), 8.15 (dq, *J* = 8.5, 0.9 Hz, 1H), 8.10 (s, 1H), 8.06 (dt, *J* = 8.1, 0.9 Hz, 1H), 7.79 (ddd, *J* = 8.4, 6.9, 1.3 Hz, 1H), 7.67 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H), 3.92 (t, *J* = 7.2 Hz, 2H), 3.67 (s, 2H), 3.14 (d, *J* = 6.2 Hz, 4H), 2.01 – 1.79 (m, 6H). ^{13}C NMR (125 MHz, CD_3OD) δ 148.13, 146.11, 133.02, 132.37, 131.45, 130.71, 129.23, 128.71, 123.76, 78.90, 68.25, 52.09, 42.76, 36.51.

Compound 4: 8-(isoquinolin-4-yl)-1-oxa-8-azaspiro[4.5]decane was prepared by the common procedure for compound 1 starting with 1-oxa-8-azaspiro[4.5]decane hydrochloride [CAS 3970-79-4] and 4-bromoisoquinoline. LCMS m/z 268.85 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.32 – 8.21 (m, 2H), 8.15 (s, 1H), 8.06 (ddd, *J* = 8.5, 7.0, 1.2 Hz, 1H), 7.90 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H), 3.91 (t, *J* = 6.7 Hz, 2H), 3.36 (ddd, *J* = 12.5, 8.5, 4.4 Hz, 2H), 3.27 (dt, *J* = 11.9, 4.6 Hz, 2H), 2.11 – 1.88 (m, 6H), 1.84 (dd, *J* = 8.4, 6.5 Hz, 2H).

Compound 5: 7-(isoquinolin-4-yl)-2-oxa-7-azaspiro[3.5]nonane was prepared by the common procedure for compound 1 starting with 2-oxa-7-azaspiro[3.5]nonane oxalate [CAS 1379811-94-5] and 4-bromoisoquinoline. LCMS m/z 255.16 $[M + H]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 8.89 (s, 1H), 8.13 (d, *J* = 8.49 Hz, 1H), 8.06 – 8.04 (m, 2H), 7.78 (td, *J* = 1.07, 7.66 Hz, 1H), 7.66 (t, *J* = 7.80 Hz, 1H), 4.54 (s, 4H), 3.06 (bs, 4H), 2.15 (t, *J* = 5.24 Hz, 4H).

Compound 6: 8-(isoquinolin-4-yl)-2-methyl-2,8-diazaspiro[4.5]decan-3-one was prepared by the common procedure for compound 1 starting with 2-methyl-2,8-diazaspiro[4.5]decan-3-one hydrochloride [CAS 1380300-72-0] and 4-bromoisoquinoline. LCMS m/z 295.78 $[M + H]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 9.81 (s, 1H), 8.59 (d, *J* = 6.6 Hz, 1H), 8.55 – 8.50 (m, 1H), 8.33 (d, *J* = 8.3 Hz, 1H), 8.26 (t, *J* = 7.6 Hz, 1H), 8.07 (t, *J* = 7.7 Hz, 1H), 3.37 (s, 2H), 3.27 – 3.20 (m, 4H), 2.84 (s, 3H), 2.40 (s, 2H), 1.89 – 1.85 (m, 4H).

Compound 7: 8-(isoquinolin-4-yl)-2-methyl-2,8-diazaspiro[4.5]decan-1-one was prepared using the common procedure for compound 1 starting with 2-methyl-2,8-diazaspiro[4.5]decan-1-one hydrochloride [CAS 848580-34-7] and 4-bromoisoquinoline. LCMS m/z 296.19 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.34 – 8.25 (m, 3H), 8.09 (ddd, J = 8.4, 7.0, 1.3 Hz, 1H), 7.92 (ddd, J = 8.1, 6.9, 1.1 Hz, 1H), 3.55 (dt, J = 12.5, 3.8 Hz, 2H), 3.49 – 3.38 (m, 2H), 3.04 (td, J = 12.1, 2.4 Hz, 2H), 2.94 (s, 3H), 2.35 (ddd, J = 13.3, 11.8, 4.1 Hz, 2H), 2.12 (t, J = 7.0 Hz, 2H), 1.68 (dt, J = 12.0, 2.8 Hz, 2H).

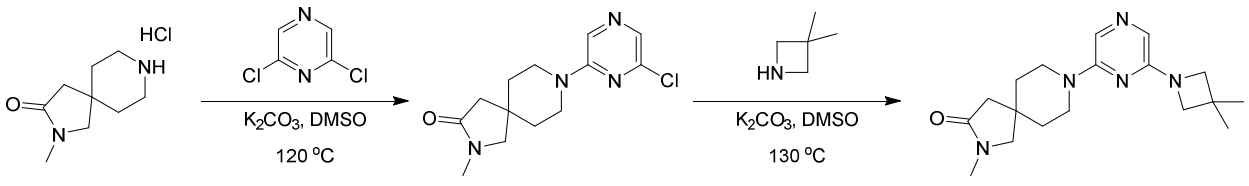
Compound 8: 8-(isoquinolin-4-yl)-2,8-diazaspiro[4.5]decane was prepared using the common procedure for compound 1 starting with tert-butyl 2,8-diazaspiro[4.5]decane-2-carboxylate [CAS 336191-17-4] and 4-bromoisoquinoline. The product from this reaction was treated with 4N HCl in 1,4-dioxane (5.0 eq) at room temperature. After stirring for 1 hour the starting material was consumed and the product was present by LCMS. The reaction was concentrated and then purified by column chromatography on a C18 reverse-phase column eluting with 0-60% acetonitrile/water (+ 0.05% trifluoroacetic acid). The fractions containing product were combined and concentrated to afford the title compound: LCMS m/z 267.87 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.12 (s, 1H), 8.22 (d, *J* = 8.3 Hz, 1H), 8.13 (d, *J* = 8.5 Hz, 1H), 8.04 (s,

1H), 7.99 (t, *J* = 8.1 Hz, 1H), 7.82 (t, *J* =8.0, 1H), 3.32 (t, *J* = 7.1 Hz, 2H), 3.12 (bs, 6H), 1.93 (t, *J* = 7.6 Hz, 2H), 1.88 – 1.85 (m, 4H).

Compound 9: 1-(8-(isoquinolin-4-yl)-2,8-diazaspiro[4.5]decan-2-yl)ethan-1-one was prepared by dissolving the title compound **8**, 8-(isoquinolin-4-yl)-2,8-diazaspiro[4.5]decane (0.04 mmol), in pyridine (0.2M) and adding acetic anhydride (1.20 eq) at room temperature. After stirring for 4 hours the reaction was complete as determined by the consumption of starting material by LCMS. The pyridine solution was neutralized with a saturated aqueous ammonium chloride solution (2 mL) and then extracted with ethyl acetate (3 x 5 mL). The combined organics were washed with brine, dried over sodium sulfate, filtered and concentrated. The crude residue was purified by column chromatography on a C18 reverse-phase column, eluting with 0-50% acetonitrile/water (+ 0.05% trifluoroacetic acid). The fractions containing product were combined and concentrated to afford the title compound: LCMS m/z 310.24 $[M + H]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 9.35 (s, 1H), 8.45 (d, *J* = 8.3 Hz, 1H), 8.40 (d, *J* = 8.5 Hz, 1H), 8.20 (ddd, *J* = 8.4, 7.0, 1.3 Hz, 1H), 8.17 (s, 1H), 8.01 (dd, *J* = 8.2, 7.1 Hz, 1H), 3.67 (t, *J* = 7.2 Hz, 1H), 3.56 (t, *J* = 7.3 Hz, 1H), 3.52 (s, 1H), 3.45 (s, 1H), 3.36 (tt, *J* = 12.4, 4.8 Hz, 2H), 3.27 – 3.18 (m, 2H), 2.09 (d, *J* = 9.9 Hz, 3H), 2.04 (t, *J* = 7.2 Hz, 1H), 1.96 (tdd, *J* = 10.4, 7.2, 4.6 Hz, 5H).

Compound 10: tert-butyl 8-(isoquinolin-4-yl)-2,8-diazaspiro[4.5]decane-2-carboxylate was prepared using the common procedure for compound 1 starting with tert-butyl 2,8-diazaspiro[4.5]decane-2-carboxylate [CAS 336191-17-4] and 4-bromoisoquinoline.
LCMS m/z 368.26 $[M + H]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 9.32 (s, 1H), 8.42 (d, *J* = 8.36 Hz, 1H), 8.37 (d, *J* = 8. 54 Hz, 1H), 8.18 – 8.14 (m, 2H), 7.98 (t, *J* = 7.57 Hz, 1H), 3.44 (dd, *J* = 7.50, 15.2 Hz, 2H), 3.37 – 3.31 (m, 4 H), 3.26 – 3.16 (m, 2H), 1.91 (bs, 6H), 1.46 (s, 9H).

Example 2: Exemplification of General Synthesis B: Preparation of Compound **11**.



Step A. 8-(6-chloropyrazin-2-yl)-2-methyl-2,8-diazaspiro[4.5]decan-3-one

A solution of 2-methyl-2,8-diazaspiro[4.5]decan-3-one hydrochloride (200 mg, 1.189 mmol), 2,6-dichloropyrazine (213 mg, 1.427 mmol) and potassium carbonate (657 mg, 4.76 mmol) in DMSO (5944 µl) was heated to 120 °C. After two hours, the reaction was cooled to room temperature. The solution was acidified with 1N HCl (2 mL) and then passed through a syringe filter. The solution was purified by HPLC (C18 column, 10 to 100% acetonitrile/water, both 0.1% v/v trifluoroacetic acid. The fractions containing product were combined and concentrated to afford the title compound: LCMS m/z 281.09 $[M + H]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 8.1 (s, 1H), 7.71 (s, 1H), 3.76 – 3.71 (m, 2H), 3.63 – 3.58 (m, 2H), 3.34 (s, 2H), 2.84 (s, 3H), 2.37 (s, 2H), 1.72 – 1.70 (m, 4H).

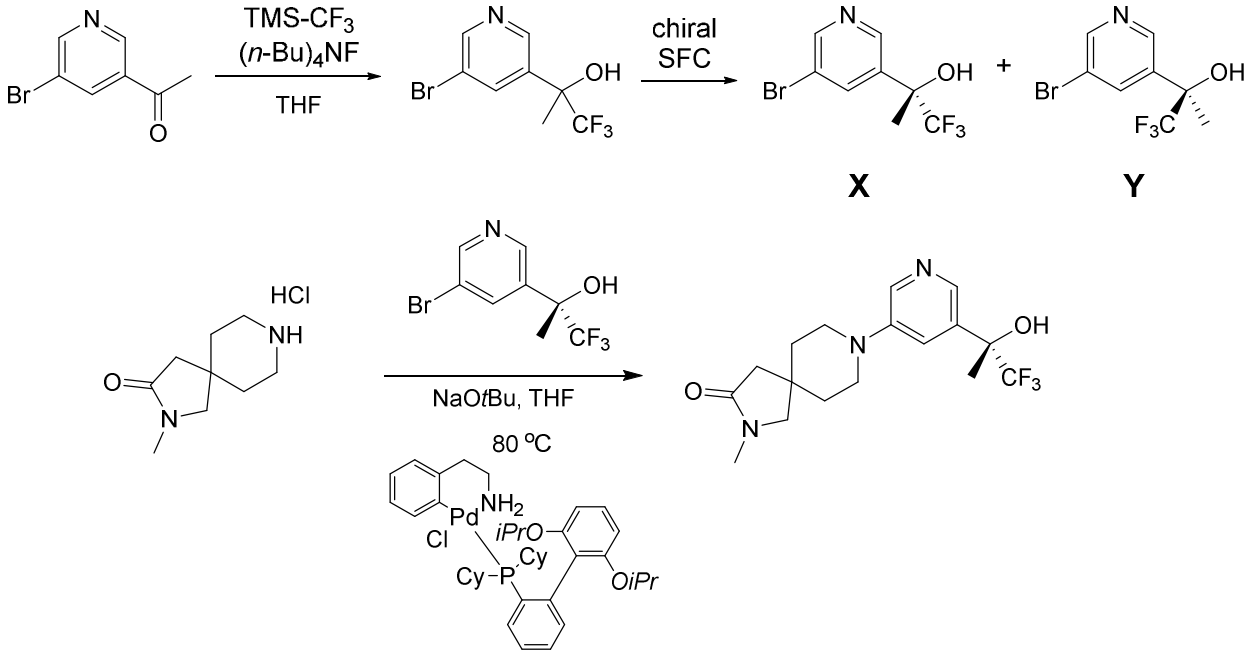
Step B. 8-(6-(3,3-dimethylazetidin-1-yl)pyrazin-2-yl)-2-methyl-2,8-diazaspiro[4.5]decan-3-one

A solution of 8-(6-chloropyrazin-2-yl)-2-methyl-2,8-diazaspiro[4.5]decan-3-one (20 mg, 0.071 mmol), 3,3-dimethylazetidine (7.28 mg, 0.085 mmol) and potassium carbonate (19.69 mg, 0.142 mmol) in DMSO (297 µl) was heated to 130 °C for 72 hours. The solution was passed through a syringe filter and then purified by HPLC (C18 column, 10 to 100% acetonitrile/water, both 0.1% v/v trifluoroacetic acid. The fractions containing product were combined and concentrated to remove the acetonitrile. The resulting solution was neutralized with saturated aqueous sodium bicarbonate solution and then extracted with ethyl acetate. The combined organics were dried over sodium sulfate, filtered and concentrated to afford the title compound: LCMS m/z $[M + H]^+$ 330.31; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (s, 1H), 6.91 (s, 1H), 3.78 (s, 4H), 3.77 – 3.72 (m, 2H), 3.46 (ddd, J = 13.8, 8.2, 4.2 Hz, 2H), 3.25 (s, 2H), 2.88 (s, 3H), 2.45 – 2.31 (m, 2H), 1.79 – 1.60 (m, 4H), 1.36 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 175.9, 157.5, 156.6, 108.3, 107.1, 69.8, 63.4, 61.2, 43.7, 42.7, 36.5, 36.2, 33.7, 30, 27.3, 26.6, 26.5.

Compound 12: 8-(6-(3,3-dimethylazetidin-1-yl)pyrazin-2-yl)-2,8-diazaspiro[4.5]decan-3-one was prepared using the common procedure for compound 11 starting with 2,8-diazaspiro[4.5]decan-3-one [CAS 561314-57-6]. LCMS m/z 316.27 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (s, 1H), 6.92 (s, 1H), 6.16 (s, 1H), 3.78 (s, 4H), 3.73 (ddd, J = 13.9, 6.4, 4.3 Hz, 2H), 3.49 (ddd, J = 13.9, 8.1, 4.0 Hz, 2H), 3.28 (s, 2H), 2.33 (s, 2H), 1.73 (qt, J = 13.3, 6.7 Hz, 4H), 1.35 (s, 6H).

Compound 13: 8-(6-(3,3-dimethylazetidin-1-yl)pyrazin-2-yl)-1,8-diazaspiro[4.5]decan-2-one was prepared using the common procedure for compound 11 starting with 1,8-diazaspiro[4.5]decan-2-one [CAS 911103-15-6]. LCMS m/z 317.10 $[M + H]^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 7.43 (s, 1H), 7.08 (s, 1H), 3.99 – 3.96 (m, 2H), 3.71 (s, 4H) 3.44 – 3.38 (m, 2H), 2.64 (t, *J* = 8.3, 2H), 2.06 (t, *J* = 8.3, 2H), 1.92 – 1.87 (m, 2H), 1.80 – 1.74 (m, 2H), 0.89 – 0.84 (m, 6H).

Example 3: Exemplification of General Synthesis C: Preparation of Compound **14**.



Step A. (*R*)- and (*S*)-2-(5-bromopyridin-3-yl)-1,1,1-trifluoropropan-2-ol

To a flask containing 3-acetyl-5-bromopyridine (2.27 g, 11.4 mmol) was added a solution of (trifluoromethyl)trimethylsilane in tetrahydrofuran (0.5 M, 40 mL, 20 mmol) at 0 °C. A solution of tetrabutylammonium fluoride in tetrahydrofuran (1.0 M, 11.4 mL, 11.4 mmol) was then added, and the reaction stirred at room temperature until the reaction was complete. The reaction was then concentrated under reduced pressure, diluted with ethyl acetate, and washed with water and saturated aqueous sodium bicarbonate solution. The organic layer was separated, dried over sodium sulfate, filtered and concentrated to give a residue that was purified by flash

chromatography on silica gel (10% to 50% ethyl acetate / hexanes linear gradient) to provide the racemic title compound: LCMS m/z 269.85 $[M + 2 + H]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 8.70 (s, 1H), 8.65 (1H), 8.13 (s, 1H), 1.81 (s, 3H). The racemic title compound was resolved by supercritical fluid chromatography on a chiral AD column, eluting with 10% ethanol:CO_2. Data for the fast-eluting (*R)*-enantiomer, **compound Y** (retention time = 2.68 min): LCMS m/z 271.85 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) 8.71 (s, 1H), 8.68 (d, *J* = 2.0 Hz, 1H), 8.10 (s, 1H), 1.82 (s, 3H). Data for the slower-eluting (*S*)-enantiomer, **compound X** (retention time = 2.95 min): LCMS m/z 271.83 $[M + H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) 8.71 (s, 1H), 8.68 (s, 1H), 8.10 (s, 1H), 1.81 (s, 3H).[1]

Step B. (S)-2-methyl-8-(5-(1,1,1-trifluoro-2-hydroxypropan-2-yl)pyridin-3-yl)-2,8-diazaspiro[4.5]decan-3-one

To a vial with 2-methyl-2,8-diazaspiro[4.5]decan-3-one hydrochloride (60 mg, 0.293 mmol) and (S)-2-(5-bromopyridin-3-yl)-1,1,1-trifluoropropan-2-ol (95 mg, 0.352 mmol) was added RuPhos Indoline Pre-catalyst (10.68 mg, 0.015 mmol) followed by sodium tert-butoxide (56.3 mg, 0.586 mmol). This mixture was then evacuated and backfilled with nitrogen before dry, degassed tetrahydrofuran (1.4 mL) was added. The reaction was then heated at 80 °C for 18 h. The reaction was cooled to room temperature and then poured into brine (5 mL). It was extracted with ethyl acetate (3 x 10 mL). The combined organics were washed with water and brine then dried over sodium sulfate. The crude product was purified by HPLC (C18 column, 0 to 50% acetonitrile/water, both 0.1% v/v trifluoroacetic acid. The fractions containing product were combined and then free-dried to provide the title compound: HRMS (Positive ESI) m/z 358.1745 (358.1698 calcd for $C_{17}H_{22}F_3N_3O_2H$); $[\alpha]_D$ -7.2°, (c.033, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.39 (d, *J* = 2.8 Hz, 1H), 8.28 (d, *J* = 1.5 Hz, 1H), 8.14 (t, *J* = 2.1 Hz, 1H), 3.57 (ddt, *J* = 10.6, 7.3, 4.3 Hz, 2H), 3.52 – 3.41 (m, 2H), 3.36 (s, 2H), 2.86 (d, *J* = 0.8 Hz, 3H), 2.39 (d, *J* = 0.9 Hz, 2H), 1.86 – 1.75 (m, 7H); ^{13}C NMR (125 MHz, CD_3OD) δ 175.70, 149.86, 142.08, 128.88, 128.32, 128.07, 127.66, 125.39, 60.99, 45.33, 43.39, 36.03, 35.55, 29.90, 22.69.

Compound 15. (R)-2-methyl-8-(5-(1,1,1-trifluoro-2-hydroxypropan-2-yl)pyridin-3-yl)-2,8-diazaspiro[4.5]decan-3-one was prepared using the general procedure for compound 14 starting with 2-methyl-2,8-diazaspiro[4.5]decan-3-one hydrochloride and **compound Y,** (R)-2-(5-bromopyridin-3-yl)-1,1,1-trifluoropropan-2-ol.

LCMS m/z 357.89 $[M + H]^+$; ^{1}H NMR (500 MHz, CD^3OD) δ 8.41 (s, H), 8.27 (s, H), 8.16 (s, H), 3.61 – 3.55 (m, 2 H), 3.51 – 3.45 (m, 2 H), 3.39 (s, 2 H), 2.87 (s, 3 H), 2.42 (s, 2 H), 1.86 – 1.77 (m, 7 H).

Compound 16. (S)-2-(5-(2-oxa-8-azaspiro[4.5]decan-8-yl)pyridin-3-yl)-1,1,1-trifluoropropan-2-ol was prepared using the general procedure for compound 14 starting with 2-oxa-8-azaspiro[4.5]decane hydrochloride and **compound X**, (S)-2-(5-bromopyridin-3-yl)-1,1,1-trifluoropropan-2-ol.
HRMS (Positive ESI) m/z 331.1640 (331.1589 calcd for $C_{16}H_{21}F_3N_2O_2H$, M + H); $[\alpha]_D$ -2.9°, (c.034, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.36 (dd, J = 2.8, 0.6 Hz, 1H), 8.24 (d, J = 1.5 Hz, 1H), 8.12 (t, J = 2.0 Hz, 1H), 3.89 (t, J = 7.2 Hz, 2H), 3.60 (s, 2H), 3.53 (ddd, J = 14.6, 7.1, 4.7 Hz, 2H), 3.49 – 3.40 (m, 2H), 1.87 (t, J = 7.2 Hz, 2H), 1.83 – 1.79 (m, 3H), 1.75 (t, J = 5.8 Hz, 4H); ^{13}C NMR (125 MHz, CD_3OD) δ 149.9, 142.1, 129.0, 128.4, 128.0, 125.4, 78.4, 68.7, 46.2, 42.5, 37.6, 34.8, 22.7.

References

(1) This reference provides the X-ray crystallographic analysis of bromide **X**; an ORTEP representation and associated data are included in this reference. Hoyt, S. B.; Petrilli, W.; London, C.; Liang, G.-B.; Tata, J.; Hu, Q.; Yin, L.; van Koppen, C. J.; Hartmann, R. W.; Struthers, M.; Cully, D.; Wisniewski, T.; Ren, N.; Bopp, C.; Sok, A.; Cai, T.-Q.; Stribling, S.; Pai, L.-Y.; Ma, X.; Metzger, J.; Verras, A.; McMasters, D.; Chen, Q.; Tung, E.; Tang, W.; Salituro, G.; Buist, N.; Clemas, J.; Zhou, G.; Gibson, J.; Maxwell, C. A.; Lassman, M.; McLaughlin, T.; Castro-Perez, J.; Szeto, D.; Forrest, G.; Hajdu, R.; Rosenbach, M.; Xiong, Y. Discovery of triazole CYP11B2 inhibitors with *in Vivo* activity in rhesus monkeys. *ACS Med. Chem. Lett.* **2015**, *6*, 861 – 865.