Supplementary Material

Discovery of PF-5190457, a potent, selective and orally bioavailable GHS-R1a inverse agonist clinical candidate

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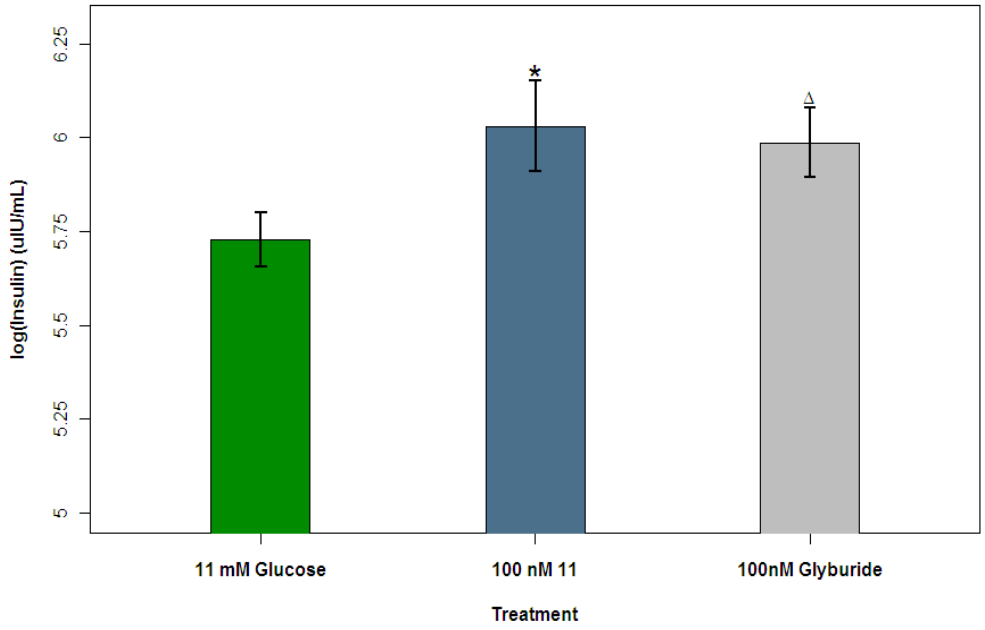


* p <0.05; Δ p<0.1

Figure S1: Glucose-stimulated insulin secretion in human dispersed islet static culture following incubation with **11** at 100nM. Data are expressed as arithmetic mean ± standard error of the mean. The sulfonylurea glibenclamide (glyburide) was run as a positive control, and significantly increased insulin secretion above the 11.2 mM glucose control with a confidence value of p <0.1. When tested at 100 nM, **11** significantly increased insulin secretion above the 11.2 mM glucose control with a significance level of p < 0.05.

$CHRM_2$ β-arrestin Muscarinic Functional Assay

CHO-K1 $CHRM_2$ β-arrestin cells (DiscoveRx) were cultured in HAMs F12 containing 10% heat inactivated FBS, 100 unit/mL penicillin/streptomycin, 2 mM L-glutamine, 300 µg/mL hygromycin, and 800 µg/mL geneticin G418 and incubated in a humidified chamber at 37 °C with 5% CO_2. Twenty four hours prior to assay, the cells were seeded into 384-well plates (Greiner, 781098) at a density of 6000 cells per well in assay media (Opti-MEM with 2% charcoal dextran FBS). The test compounds were serially diluted and added to the cell plates for a 15 min incubation followed by the addition of an EC_{80} concentration (2-3 µM) of oxotremorine S and further 90 min incubation. All reagents were diluted in assay media and all incubations were performed at 37 °C with 5% CO_2. Each plate contained control wells (with diluent but no

compound) to define the upper and lower limits for the assay signal. The positive control wells (100% inhibition of agonist challenge) did not contain the EC_{80} concentration of oxotremorine S and the negative control wells (0% inhibition of agonist challenge) contained an EC_{80} concentration of oxotremorine S. Substrate working solution from the PathHunter kit (DiscoveRx, 93-0001) was added to the cell plates and incubated for 60 min. The chemiluminescence was then read on an Envision 2104 reader (Perkin Elmer). Data analysis was performed using a proprietary software package. Briefly, the percent inhibition for each compound concentration was calculated using the control wells on the plate. An IC_{50} was determined using a standard 4-parameter fit algorithm which was then converted to a K_b value using the Cheng Prussoff equation as follows: $K_b = IC_{50} /((1 + ([agonist] / agonist\ EC_{50}))$ where the [agonist] is the EC_{80} concentration of oxotremorine S used in the assay and agonist EC_{50} was experimentally determined from an oxotremorine S titration.

Human Islet Assay

Preparation of Human Whole Islets

Islets were decanted from the transport vessel into sterile 50 mL polypropylene conical tubes, brought up with supplier recommended culture media and spun 2 minutes at 1000 rpm at room temperature. Supernatant was aspirated and islets were resuspended in media. 5,000 IEQ's were pipetted into each 10 cm^2 sterile suspension culture dish (Corning 25070-100), 10mls media/plate. The dishes are then placed in a 37 °C, 5% CO_2 cell culture incubator overnight.

Test Compound Preparation

The test compounds were solubilized in 100% dimethylsulfoxide (DMSO) at a concentration of 30 mM. Compound dilutions were made in assay buffer (11.5 mM NaCl, 0.5 mM KCl, 2.4 mM $NaHCO_3$, 2.2 mM $CaCl_2$, 1 mM $MgCl_2$, 24 mM Hepes, 0.25 % BSA) in 0.03% DMSO final.

Whole Islet Insulin Secretion Assay

To evaluate the effect of test compound on glucose-stimulated insulin secretion (GSIS), human whole islets were incubated in assay buffer containing 2.8 mM glucose for 45 minutes at 37 °C to stabilize insulin secretion; islets were then treated with 11.2 mM glucose in the presence and absence of compound for one hour at 37 °C. Following incubation, samples were tested for the amount of insulin secreted into the media. 2.8 mM glucose was run to show that the islets were glucose-responsive by comparing the secretion to islets incubated in 11.2 mM glucose. The

sulfonylurea glyburide was run as a positive control at 1 μM in 11.2 mM glucose. If positive controls failed data was not reported.

Dispersed Islet Insulin Secretion Assay

Islets were enzymatically dispersed and placed in a 37 °C, 5% CO_2 cell culture incubator overnight. The following day, dispersed islets were incubated in assay buffer containing 2.8 mM glucose for 45 minutes at 37 °C to stabilize insulin secretion. Dispersed islets were then incubated with compounds for one hour at 37°C. Following incubation, samples were tested for the amount of insulin secreted into the media. The sulfonylurea glyburide was run as a positive control at 100 nM in 11.2 mM glucose. If positive controls failed data was not reported.

Safety Assays:

Compound **16h** was devoid of genotoxicity findings in the presence or absence of metabolic activation. Compound **16h** did not induce micronuclei in Chinese Hamster Ovary cells after 24 h treatment or after 3 h treatment with metabolic activation. In addition, **16h** was found to be safe in a cytotoxicity assay (transformed human liver epithelial cell assay, THLE, IC_{50} 262 μM)[1] and clean in a hepatic cellular imaging (human hepatocyte imaging assay technology, HIAT) assay.[2]

Table S1. Summary Table of **16h** Pharmacokinetics Observed in Nonclinical Species[a]

Species	**Dose (mg/kg)**	**Fed/ Fasted**	**Route**	**C_{max} (ng/mL)**	**T_{max} (h)**	**$AUC_{(0-\infty)}$ (ng*h/mL)**	**T½ (h)**	**CL_p (mL/min/ mg)**	**Vd_{ss} (L/kg)**	**%F**	**%F_a**
Rat	1[a]	Fed	IV	-	-	203	1.23	83.7	5.88	-	-
Rat	10[c]	Fasted	PO	303	0.25	596		-	-	29.4	100
Dog	1[b]	Fed	IV	-	-	1850	7.67	9.05	4.98	-	-
Dog	5[c]	Fasted	PO	928	2.0	8530		-	-	92.2	100
Monkey	1[b]	Fed	IV	-	-	1340	3.67	12.5	1.68	-	-
Monkey	5[c]	Fasted	PO	424	2.0	1650		-	-	24.7	34.5

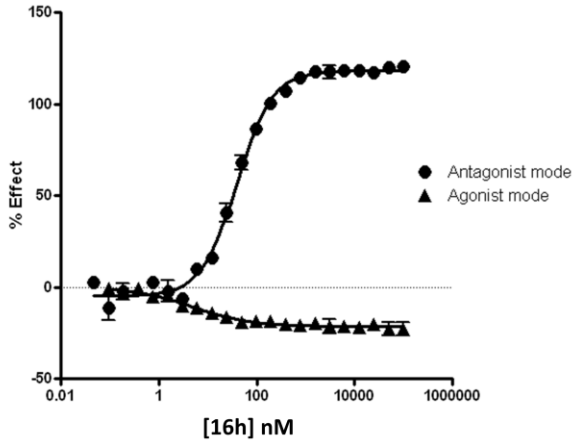
[a] The *in vitro* plasma protein binding (fu) of **16h** for rat, dog and monkey was 0.33, 0.42, and 0.15 respectively.

[b] Vehicle was 12% w/w sulfobutyl ether beta-cyclodextrin (SBECD) in 50 mM citrate buffer

[c] Formulation was prepared in 0.5% methylcellulose using a mortar and pestle

C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which the highest drug concentration occurs; AUC = Area under the curve; T½= Half-life; CL_p = Plasma clearance; Vd_{ss} = Volume of distribution at steady state; F = Bioavailability; F_a = Fraction of drug absorbed in the gut.

Figure S2 Functional Activity of **16h** using Eu-GTP assay



Potency	Inverse Agonist mode (EC_{50})	Antagonist mode (K_i)
nM (95% C.I.)	4.85 (3.53 – 6.65)	6.57 (5.01 – 8.62)
n	16	16

The EC_{50} and K_i values are reported as the geometric mean with confidence interval (C.I.) for the indicated number of determinations.

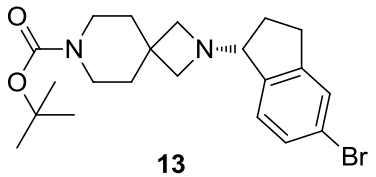
General Methods:

All chemicals, reagents and solvents were purchased from commercial sources and used without further purification. All reactions were performed under an atmosphere of nitrogen unless otherwise noted. Proton (^{1}H NMR) nuclear magnetic spectra were recorded with 400 MHz and 500 MHz Varian spectrometers. Chemical shifts are reported in parts per million relative to the residual solvent signals (i.e. for $CDCl_3$ δH = 7.27 ppm, for DMSO-d_6 δH = 2.50 ppm and for

CD_3OD δH = 4.78 and 3.31 ppm). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet. Mass spectrometry (MS) was performed via atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) sources. Liquid chromatography mass spectrometry (LCMS) was performed on an Agilent 1100 Series (Waters Atlantis C18 column, 4.6 x 50 mm, 5 μm; 95% water/acetonitrile linear gradient to 5% water/acetonitrile over 4 min, hold at 5% water/ 95% acetonitrile to 5.0 min, trifluoroacetic acid modifier (0.05%); flow rate of 2.0 mL/ min) and ultra pressure liquid chromatography mass spectrometry (UPLC-MS) was performed on a Waters Acquity System (Waters Acquity HSS T3 C18 column, 2.1 x 50 mm, 1.7 μm; 95% water/acetonitrile linear gradient to 5% water/acetonitrile over 1.0 min, hold at 5% water/95% acetonitrile to 1.5 min, formic acid modifier (0.1%) or ammonia modifier (0.1%); flow rate of 1.25 mL/min. Silica gel chromatography was performed using a medium pressure Biotage or ISCO system using columns pre-packaged by various commercial vendors including Biotage and ISCO. Whatman pre-coated silica gel plates (250 μm) were used for analytical TLC. Accurate Mass Spectrometry analyses (HRMS) were conducted on an Agilent 6220 TOF mass spectrometer (Agilent Technologies, Wilmington, DE) in positive or negative electrospray mode. The mass accuracy was calculated for all observed isotopes against the theoretical mass ions derived from the chemical formula using MassHunter software (Agilent Technologies, Wilmington, DE).

Preparation of Intermediates

tert-Butyl 2-[(1R)-5-bromo-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]nonane-7-carboxylate (**13**)

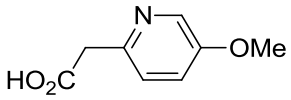


13

To a solution of (1R)-5-bromoindan-1-amine (1835 g, 8.66 mol) in anhydrous MeOH (24 L) was added *tert*-butyl 4-(chloromethyl)-4-formylpiperidine-1-carboxylate (2310 g, 8.83 mol). The mixture was stirred at 50 °C for 16 h, and cooled to room temperature. Sodium cyanoborohydride (1000 g, 15.9 mol) in THF (15 L) was added via a syringe pump over 2 h. The

mixture was stirred at 60 °C for 24 h under nitrogen with a vent into a bleach bath. The reaction was cooled to 20 °C and transferred via a cannula into a vessel containing 2.5M NaOH (24 L), and CH_2Cl_2 (30 L). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 5 L). The aqueous layer was treated to destruct residual sodium cyanoborohydride. The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The crude material was slurried in methyl *tert*-butyl ether (MTBE) (7 L) by stirring at 40 °C for 1 h and at room temperature for 1 h. The resultant solid was filtered, and washed with MTBE (2 x 500 mL) and dried in a vacuum oven at 50 °C to give the title product as a white crystals (3657 g, 90%). ^{1}H NMR (500 MHz, CD_3OD): δ 7.42 (s, 1 H), 7.32 (d, *J* = 10 Hz, 1 H), 7.26 (d, *J* = 10.0 Hz, 1 H), 4.01–3.97 (m, 1 H), 3.36 (s, 4 H), 3.28–3.30 (m, 2 H), 3.24 (d, *J* = 10.0 Hz, 2 H), 3.09–3.03 (m, 1 H), 2.86–2.80 (m, 1 H), 2.25–2.18 (m, 1 H), 1.92–1.86 (m, 1 H), 1.72–1.70 (m, 4 H), 1.45 (s, 9 H); ^{13}C NMR (125 MHz, CD_3OD): δ 155.3, 147.3, 142.7, 129.1, 127.9, 126.1, 119.2, 80.0, 71.3, 62.2, 51.5, 35.5, 33.8, 29.9, 29.1, 27.5; HRMS (CI): *m/z* 421.1480 (calc 421.1485 for $C_{21}H_{29}BrN_2O_2$ + H); $[\alpha]_D^{20}$ = +39.6 deg (c = 1.06 mg/mL, MeOH); ee 99% (checked by chiral SFC, compared to racemic **13**).

(5-Methoxypyridin-2-yl)acetic acid (**14a**)



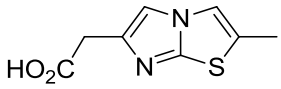
14a

A solution of *tert*-butyl cyanoacetate (3.75 mL, 26.2 mmol) in anhydrous 1,4-dioxane (20 mL) was purged with nitrogen. Potassium *tert*-butoxide (21.5 mL, 21.5 mmol, 1M in THF) was added, and after stirring for 5 min, 2-bromo-5-methoxypyridine (2 g, 10.7 mmol) in 1,4-dioxane (4 mL) was added followed by 1,1'-bis(diphenylphosphino)ferrocene palladium dichloride-CH_2Cl_2 (1:1 complex) (239 mg, 0.29 mmol). The mixture was heated at 70 °C for 18 h, and a second portion of catalyst (120 mg, 0.15 mmol) was added. After heating for an additional 3 h, the mixture was cooled to room temperature and 2N acetic acid (80 mL) was added. The mixture was filtered, washing with water (2x) and the crude product was dried in a current of air. The filtrate was extracted twice with ethyl acetate. The combined extracts were washed with brine, dried (Na_2SO_4), and concentrated to afford a black oil which was combined with the above solid

to yield 3.46 g of crude *tert*-butyl cyano(5-methoxypyridin-2-yl)acetate. This material was used directly in the next step.

The crude *tert*-butyl cyano(5-methoxypyridin-2-yl)acetate (3.46 g) was suspended in a mixture of water (45 mL) and concentrated HCl (45 mL). The mixture was heated at 60 °C for 1 h and at reflux for 18 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resultant residue was dissolved in water (~50-70 mL) and 2N NaOH was added to adjust the pH to ~14. The aqueous solution was washed with Et_2O and was the pH was adjusted to ~4 with 2N HCl, and concentrated to dryness to afford a white solid. This solid was triturated in hot THF (3x) and the combined supernatants were cooled in water-icebath to initiate crystallization. After 20 min, the resultant solid was collected by filtration washing with heptanes to afford 1.25 g of **17** as a white solid. A second crop of product precipitated from the filtrate, to afford total of 1.75 g (98%) of **17** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (d, 1 H), 7.33-7.36 (m, 1 H), 7.27 (d, 1 H), 3.81 (s, 3 H), 3.66 (s, 2 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 154.8, 147.7, 136.9, 125.0, 121.8, 56.2, 43.1; HRMS: *m/z* 168.0582 (calc 168.0582 for $C_8H_9NO_3$ + H).

(2-Methylimidazo[2,1-b][1,3]thiazol-6-yl)acetic acid hydrochloride (**14b**)



14b

A solution of bromine (436 g, 2.73 mol) in acetic acid (750 mL) was added to a solution of ethyl 3-oxobutanoate (355 g, 2.73 mol) in acetic acid (1000 mL). The mixture was stirred at room temperature for 72 h and concentrated under reduced pressure at 45 °C to remove the acetic acid. The residue was partitioned between CH_2Cl_2 (400 mL) and water (250 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (2 x 300 mL), water (300 mL), brine (125 mL) and dried ($MgSO_4$). The solution was filtered and concentrated to give ethyl 4-bromo-3-oxobutanoate as a yellow oil (421 g).

To a solution of 2-amino-5-methylthiazole (150 g, 1.31 mol) in acetone (1500 mL) was slowly added ethyl 4-bromo-3-oxobutanoate (345 g, 1.65 mol). The temperature of the reaction mixture was maintained between 22–40 °C. The mixture turned into a thick paste whereupon acetone

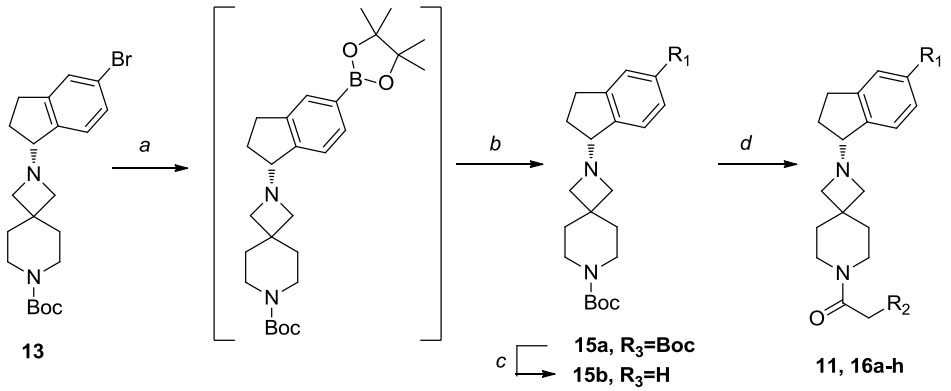
(300 mL) was added to facilitate stirring. After stirring at room temperature for 18 h, the mixture was filtered and the filter cake was washed with acetone to afford a white solid. The solid was washed with hexanes and dried in a vacuum oven at 40 °C for 4 h to give 4-(2-amino-5-methyl-thiazol)-3-oxobutyric acid ethyl ester hydrobromide (272 g).

To 4-(2-amino-5-methyl-thiazol)-3-oxobutyric acid ethyl ester hydrobromide (272 g, 0.84 mol) was added anhydrous ethanol (675 mL) and the mixture was heated at 90 °C for 2 h, during which the mixture became homogeneous. The reaction mixture was concentrated to give a brown semi-solid which was triturated with EtOH to provide a white fluffy solid that was collected by filtration. The solids were washed with Et_2O and dried under reduced pressure at 40 °C for 4 h to afford ethyl (2-methylimidazo[2,1-b][1,3]thiazol-6-yl)acetate hydrobromide (226 g).

Ethyl (2-methylimidazo[2,1-b][1,3]thiazol-6-yl)acetate hydrobromide (226 g, 0.74 mol) was dissolved in water (350 mL) and the pH of the solution was adjusted to ~7 by addition of K_2CO_3 (51.0 g, 0.37 mol). The aqueous solution was extracted with CH_2Cl_2 (300 mL) and the organic layer was washed with brine (150 mL), dried ($MgSO_4$), filtered and concentrated to give ethyl (2-methylimidazo[2,1-b][1,3]thiazol-6-yl)acetate as a brown oil (151.3 g).

Ethyl (2-methylimidazo[2,1-b][1,3]thiazol-6-yl)acetate (151.3 g, 0.67 mol) was dissolved in 10% aqueous HCl (435 mL) and the mixture was heated at reflux for 2 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure to give a yellow oil. EtOH (100 mL) and Et_2O (200 mL) were added and the resulting white precipitate was collected by filtration and dried under reduced pressure for 18 h to give 144.3 g (93%) of (2-methylimidazo[2,1-b][1,3]thiazol-6-yl)acetic acid hydrochloride) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.85 (s, 1 H), 7.81 (s, 1 H), 3.88 (s, 2 H), 2.48 (s, 3 H); LCMS (ESI) *m/z* 197.1 $(M+H)^+$.

General Scheme for the Synthesis of Compounds 11, 16a-h



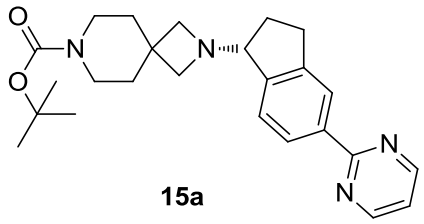
(a) Bis(pinacolato)diboron, $Pd(dppf)Cl_2$ (1.6 mol%), KOAc, dioxane, 110 °C, 1 h (b) R^1-Cl or R^1-Br, $Pd(dppf)Cl_2$ (2.5 mol %), K_2CO_3 (aq), dioxane, 110 °C, 3 h; (c) HCl or TFA; (d) $R^2CH_2CO_2H$ (**14a, b**), HBTU or CDI, DIEA or TEA.

General Procedure for 1-Pot 2-Step Suzuki Coupling

To a vial containing *tert*-butyl 2-[(*R*)-5-bromo-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]nonane-7-carboxylate **13** (2.42 mmol) was added bis(pinacolato)diboron (2.61 mmol), potassium acetate (12.12 mmol) and $Pd(dppf)Cl_2$ (0.039 mmol). The vial was purged with a stream of N_2, and anhydrous 1,4-dioxane (12 mL) was added. The resulting mixture was purged with N_2 for three times, and the mixture was heated at 110 °C for 1 h. The aryl halide (R^1-Cl or R^1-Br) (2.61 mmol), $Pd(dppf)Cl_2$ (0.062 mmol), and 9 mL of 2M aqueous K_2CO_3 solution (de-oxygenated by bubbling through N_2 for 15 min) were added. The mixture was purged with N_2 three times and heated at 110 °C for 3 h. The mixture was cooled to room temperature and the organic layer was separated from the aqueous layer. The organic layer was filtered through a thin plug of silica gel, and rinsed with *i*PrOH (5 mL) followed by MeOH (5 mL). The filtrate and the washings were concentrated under reduced pressure to give the crude product that was purified by silica gel chromatography eluting with a gradient of 0–10% MeOH in CH_2Cl_2 to afford **15**.

Representative Intermediates 15

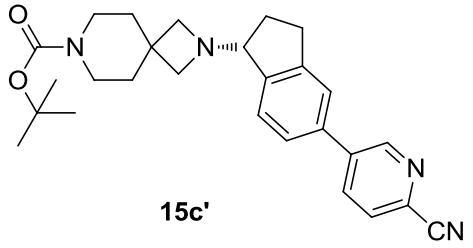
tert-Butyl 2-[(1R)-5-pyrimidin-2-yl-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]nonane-7-carboxylate (**15a**)



15a

^{1}H NMR (500 MHz, CD_3OD) δ 8.83 (d, *J* = 5.0 Hz, 2 H), 8.29 (s, 1 H), 8.24 (d, J = 10.0 Hz, 1 H), 7.48 (d, *J* = 10.0 Hz, 1 H), 7.34 (t, *J* = 5.0 Hz, 1 H), 4.10–4.08 (m, 1 H), 3.37 (s, 4 H), 3.32–3.30 (m, 2 H), 3.29–3.28 (m, 2 H), 3.17–3.11 (m, 1 H), 2.94–2.88 (m, 1 H), 2.31–2.24 (m, 1 H), 1.97–1.91 (m, 1 H), 1.74–1.72 (m, 4 H), 1.45 (s, 9 H); ^{13}C NMR (125 MHz, CD_3OD): δ 164.7, 157.5, 155.3, 145.6, 145.2, 137.6, 126.4, 124.5, 119.5, 99.9, 79.8, 71.7, 62.3, 51.0, 35.6, 33.9, 30.0, 29.3, 27.5; HRMS (CI): *m/z* 421.2587 (calc 421.2525 for $C_{25}H_{32}N_4O_2$ + H).

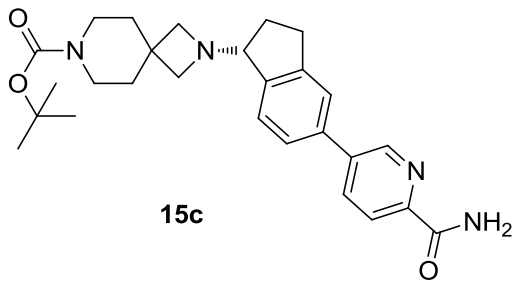
tert-Butyl 2-[(*R*)-5-(6-cyanopyridin-3-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]nonane-7-carboxylate (**15c'**)



15c'

^{1}H NMR (500 MHz, CD_3OD) δ 8.98 (d, *J* = 2.2 Hz, 1 H), 8.23 (dd, *J* = 8.1, 2.2 Hz, 1 H), 7.93 (d, *J* = 8.1 Hz, 1 H), 7.65 (s, 1 H), 7.59–7.47 (m, 2 H), 4.10 (d, *J* = 2.7 Hz, 1 H), 3.37–3.31 (m, 6 H), 3.30–3.26 (m, 2 H), 3.21–3.11 (m, 1 H), 2.98–2.88 (m, 1 H), 2.34–2.22 (m, 1 H), 2.00–1.90 (m, 1 H), 1.79–1.69 (m, 4 H), 1.46 (s, 9 H); LCMS (ESI): *m/z* 445.5 $(M+H)^+$.

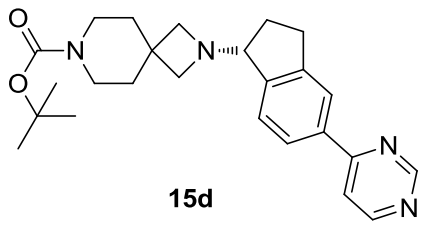
tert-Butyl 2-[(1R)-5-(6-carbamoylpyridin-3-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]nonane-7-carboxylate (**15c**)



15c

To a solution of water (150 mL) and urea hydrogen peroxide (916 mg, 9.45 mmol) was added NaOH (220 mg, 5.51 mmol) at room temperature. Once a clear solution was observed, the reaction mixture was placed in water-ice bath and fitted with an addition funnel. A solution of *tert*-butyl 2-[(*R*)-5-(6-cyanopyridin-3-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]nonane-7-carboxylate (**15c'**) (700 mg, 1.58 mmol) in EtOH (50 mL) was added dropwise via the funnel over 30 min. The reaction was warmed to room temperature and was stirred for 18 h. The mixture was filtered and the solids were washed with water (50 mL) and air-dried to give 440 mg (60%) of the title compound as an off-white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (s, 1 H), 8.23 (d, J = 8.0 Hz, 1 H), 8.01–7.97 (m, 1 H), 7.46 (s, 1 H), 7.38 (s, 2 H), 5.56–5.52 (m, 1 H), 3.97–3.92 (m, 1 H), 3.35–3.29 (m, 4 H), 3.18–3.04 (m, 4 H), 2.91–2.80 (m, 1 H), 2.21–2.10 (m, 1 H), 1.99–1.88 (m, 1 H), 1.73–1.69 (m, 4 H), 1.43 (s, 9 H); LCMS (ESI) *m/z* 463.1 $(M+H)^+$.

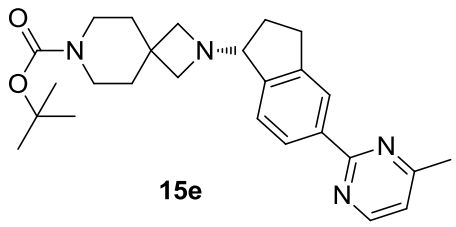
tert-Butyl 2-[(*R*)-5-(pyrimidin-4-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]nonane-7-carboxylate (**15d**)



15d

^{1}H NMR (400 MHz, CD_3OD) δ 9.16 (d, *J* = 1.2 Hz, 1 H), 8.77 (d, *J* = 5.5 Hz, 1 H), 8.08 (s, 1 H), 8.05–7.99 (m, 1 H), 7.97 (dd, *J* = 5.5, 1.4 Hz, 1 H), 7.53 (d, *J* = 8.0 Hz, 1 H), 4.23–4.13 (m, 1 H), 3.46–3.39 (m, 2 H), 3.41–3.34 (m, 6 H), 3.23–3.07 (m, 1 H), 3.00–2.86 (m, 1 H), 2.37–2.22 (m, 1 H), 2.03–1.92 (m, 1 H), 1.78–1.67 (m, 4 H), 1.50–1.39 (m, 9 H); LCMS (ESI): *m/z* 421.9 $(M+H)^+$.

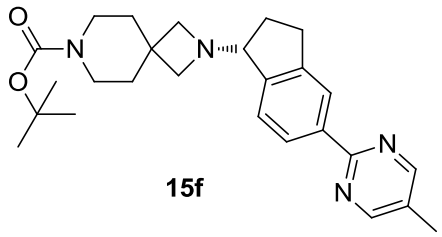
tert-Butyl 2-[(*R*)-5-(4-methylpyrimidin-2-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]nonane-7-carboxylate (**15e**)



15e

^{1}H NMR (400 MHz, CD_3OD) δ 8.66 (d, *J* = 5.1 Hz, 1 H), 8.33–8.17 (m, 2 H), 7.50 (d, *J* = 8.0 Hz, 1 H), 7.26 (dd, *J* = 5.1, 0.5 Hz, 1 H), 4.16–4.08 (m, 1 H), 3.44–3.35 (m, 3 H), 3.34 (dt, *J* = 3.3, 1.6 Hz, 6 H), 3.22–3.10 (m, 1 H), 3.01–2.88 (m, 1 H), 2.61 (d, *J* = 0.4 Hz, 3 H), 2.38–2.24 (m, 1 H), 2.02–1.91 (m, 1 H), 1.79–1.71 (m, 3 H), 1.48 (s, 9 H); LCMS (ESI): *m/z* 435.1$(M+H)^+$.

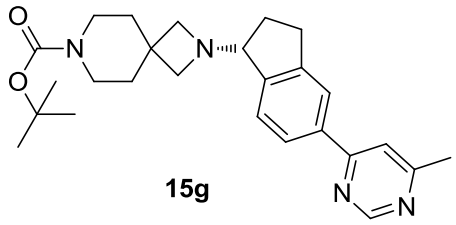
tert-Butyl 2-[(*R*)-5-(5-methylpyrimidin-2-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]nonane-7-carboxylate (**15f**)



15f

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66–8.52 (m, 2 H), 8.34–8.11 (m, 2 H), 7.36 (d, *J* = 8.0 Hz, 1 H), 3.94–3.92 (m, 1 H), 3.40–3.26 (m, 4 H), 3.22–3.11 (m, 4 H), 3.09–3.01 (m, 1 H), 2.88–2.79 (m, 1 H), 2.30 (s, 3 H), 2.20–2.10 (m, 1 H), 1.94–1.86 (m, 1 H), 1.76–1.60 (m, 4 H), 1.43 (s, 9 H); LCMS (ESI): *m/z* 435.4 $(M+H)^+$.

tert-Butyl 2-[(1R)-5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]nonane-7-carboxylate (**15g**)



15g

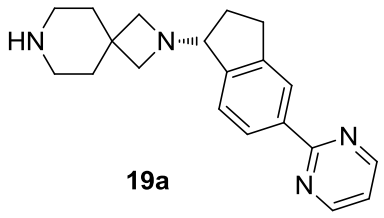
^{1}H NMR (500 MHz, CD_3OD) δ 9.00 (s, 1 H), 8.03 (s, 1 H), 7.98 (d, *J* = 5.0 Hz, 1 H), 7.86 (s, 1 H), 7.49 (d, *J* = 10.0 Hz, 1 H), 4.07–4.04 (m, 1 H), 3.36 (s, 4 H), 3.32–3.29 (m, 2 H), 3.26 (d, *J* = 5.0 Hz, 2 H), 3.16–3.10 (m, 1 H), 2.93–2.87 (m, 1 H), 2,57 (s, 3 H), 2.29–2.23 (m, 1 H), 1.96–1.90 (m, 1 H), 1.72–1.67 (m, 4 H), 1.45 (s, 9 H); ^{13}C NMR (125 MHz, CD_3OD): δ 167.9, 164.4, 157.9, 155.3, 146.2, 145.7, 136.4, 125.6, 125.0, 123.7, 116.9, 79.8, 71.6, 62.3, 48.0, 35.6, 33.9, 30.1, 29.3, 27.5, 22.7; HRMS (CI): *m/z* 435.2753 (calc 435.2755 for $C_{26}H_{34}N_4O_2$ + H).

General Procedure For N-Boc Deprotection

To a flask containing intermediate **15** (1.39 mmol) was added 4N HCl in 1,4-dioxane (8 mL) at room temperature resulting in formation of a yellow precipitate. The mixture was stirred at room temperature for 40 min. Anhydrous MeOH (4 mL) was added. The resulting homogeneous solution was stirred at room temperature for 4 h. Solvent and excess HCl were removed and dried under reduced pressure to afford the intermediate **19**. The crude product was taken onto the next step without further purification.

Representative Intermediates 19

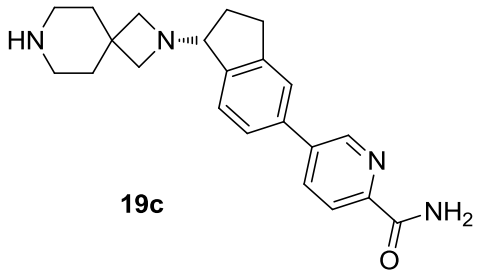
2-[(1R)-5-Pyrimidin-2-yl-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]nonane dihydrochloride (**19a**)



19a

^{1}H NMR (400 MHz, CD_3OD) δ 8.94 (d, J = 4.7 Hz, 2 H), 8.41 (s, 1 H), 8.36 (d, J = 8.0 Hz, 1 H), 7.75–7.73 (m, 1 H), 7.51 (t, J = 4.7 Hz, 1 H), 5.08–5.07 (m, 1 H), 4.47–4.45 (m, 1 H), 4.21 (s, 2 H), 4.13–4.10 (m, 1 H), 3.23–3.19 (m, 5 H), 3.13–3.09 (m, 1 H), 2.58–2.52 (m, 1 H), 2.22–2.14 (m, 5 H); LCMS (APCI) *m/z* 321.4 $(M+H)^+$.

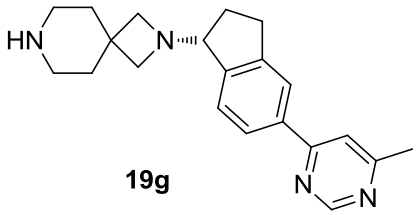
5-[(1R)-1-(2,7-Diazaspiro[3.5]non-2-yl)-2,3-dihydro-1*H*-inden-5-yl]pyridine-2-carboxamide dihydrochloride (**19c**)



19c

^{1}H NMR (400 MHz, CD_3OD) δ 9.07–9.05 (m, 1 H), 8.46–8.44 (m, 1 H), 8.31–8.29 (m, 1 H), 7.81 (s, 1 H), 7.81–7.79 (m, 2 H), 5.10–5.08 (m, 1 H), 4.48–4.46 (m, 1 H), 4.24 (s, 2 H), 4.16–4.14 (m, 1 H), 3.35–3.33 (m, 1 H), 3.22–3.18 (m, 4 H), 3.16–3.11 (m, 1 H), 2.66–2.56 (m, 1 H), 2.26–2.22 (m, 5 H); LCMS (ESI) *m/z* 363.1 $(M+H)^+$.

2-[(1R)-5-(6-Methylpyrimidin-4-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]nonane dihydrochloride (**19g**)



19g

^{1}H NMR (400 MHz, CD_3OD) δ 9.36 (s, 1 H), 8.46 (s, 1 H), 8.34–8.30 (m, 2 H), 7.84 (d, *J* = 8.0 Hz, 1 H), 5.12–5.10 (m, 1 H), 4.50–4.47 (m, 1 H), 4.23–4.14 (m, 3 H), 3.49–3.37 (m, 1 H), 3.24–3.19 (m, 4 H), 3.13–3.11 (m, 1 H), 2.80 (s, 3 H), 2.60–2.57 (m, 1 H), 2.23–2.16 (m, 5 H); LCMS (ESI) *m/z* 335.2 $(M+H)^+$.

Procedure A for Amide Coupling

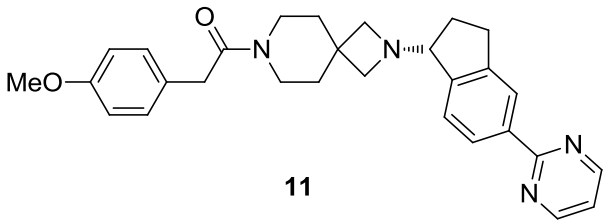
To a suspension of the amine hydrochloride **19a-f** (1.22 mmol) in CH_2Cl_2 (10 mL) was added triethylamine (492 mg, 4.90 mmol) at room temperature. Once the mixture became homogenous, it was added to a solution of the acid (**17** or **18**, 1.28 mmol) in CH_2Cl_2 (3 mL). The mixture was stirred for 5 min and (1*H*-benzotriazol-1yloxy)(dimethylamino)-N,N-dimethylmethaniminium hexafluorophosphate (HBTU) (462 mg, 1.22 mmol) in DMF (2 mL) was added. The reaction was stirred at room temperature for 1.5 h. Saturated aqueous $NaHCO_3$ (10 mL) and CH_2Cl_2 (50 mL) were added, and the organic layer was washed with saturated brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was dissolved in CH_3CN (5 mL) and the solution was heated at 100 °C for 1 h. The mixture was cooled to room temperature and the resulting solids were collected by vacuum filtration to afford compounds **11**, **16a-f**.

Procedure B for Amide Coupling

To a mixture of the acid (**17** or **18**, 53.2 mmol) in CH_2Cl_2 (225 mL) was added 1,1'-carbonyldiimidazole (CDI) (8.63 g, 53.2 mmol) and the reaction was stirred for 2 h at room temperature. In a separate flask, triethylamine (28.3 mL, 203 mmol) was added to a mixture of amine hydrochloride **19g** (50.7 mmol) in CH_2Cl_2 (113 mL). The acid-CDI solution was added to the amine solution and the reaction mixture was stirred at room temperature for 2 h. Aqueous NaOH (1N, 80 mL) and water (100 mL) were added and the mixture was stirred for 10 min. The aqueous layer was washed with CH_2Cl_2 (150 mL). The combined organic layers were washed with aqueous NH_4Cl (3x), and concentrated under reduced pressure. The residue was treated with EtOAc (150 mL) and stirred at 50 °C until a homogeneous solution was obtained. The solution was cooled to room temperature with stirring, and the resultant thick slurry was diluted with EtOAc (50 mL) and heptanes (50 mL). The slurry was stirred for 1 h and filtered under nitrogen to afford compounds **16g-h**.

Compounds 11, 16a-h

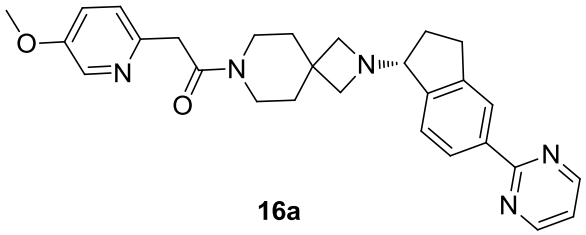
2-(4-Methoxyphenyl)-1-{2-[(1R)-5-(pyrimidin-2-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]non-7-yl}ethanone (**11**)



11

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (d, *J* = 6.0 Hz, 2 H), 8.28 (s, 1 H), 8.24 (d, *J* = 8.0 Hz, 1 H), 7.35 (d, *J* = 8.0 Hz, 1 H), 7.17–7.12 (m, 3 H), 6.83 (d, J = 8.0 Hz, 2 H), 3.97–3.94 (m, 1 H), 3.78 (s, 3 H), 3.65 (s, 2 H), 3.52 (t, *J* = 6.0 Hz, 2 H), 3.33 (t, *J* = 6.0 Hz, 2 H), 3.15–3.02 (m, 5 H), 2.90–2.82 (m, 1 H), 2.21–2.12 (m,1 H), 1.95–1.87 (m, 1 H), 1.72–1.65 (m, 2 H), 1.58–1.52 (m, 2 H); HRMS (CI): *m/z* 469.2614 (calc 469.2615 for $C_{29}H_{32}N_4O_2$ + H); $[\alpha]_D^{20}$ = +43.2 deg (c = 8 mg/mL, MeOH).

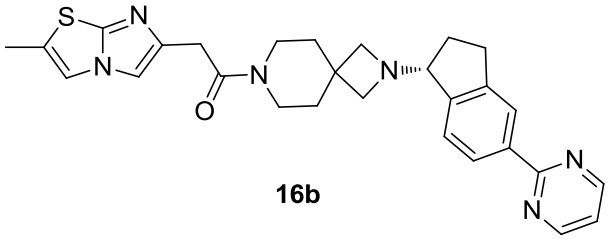
2-(5-Methoxypyridin-2-yl)-1-{2-[(1R)-5-(pyrimidin-2-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]non-7-yl}ethanone (**16a**)



16a

^{1}H NMR (400 MHz, CD_3OD) δ 8.80 (d, *J* = 3.8 Hz, 2 H), 8.26 (br s, 1 H), 8.22 (d, *J* = 7.6 Hz, 1 H), 8.13 (d, *J* = 3.8 Hz, 1 H), 7.45 (d, *J* = 8.0 Hz, 1 H), 7.36–7.30 (m, 2 H), 7.25 (d, *J* = 8.0 Hz, 1 H), 3.97–3.94 (m, 1 H), 3.78 (s, 3 H), 3.65 (s, 2 H), 3.52 (q, *J* = 6.0 Hz, 2 H), 3.38–3.30 (m, 2 H), 3.15–3.02 (m, 5 H), 2.90–2.82 (m, 1 H), 2.21–2.12 (m,1 H), 1.95–1.87 (m, 1 H), 1.72–1.65 (m, 2 H), 1.58–1.52 (m, 2 H); HRMS (CI): *m/z* 470.2546 (calc 470.2551 for $C_{28}H_{31}N_5O_2$ + H);

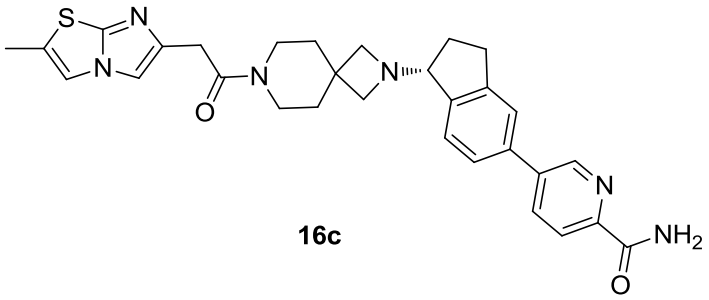
2-(2-Methylimidazo[2,1-b][1,3]thiazol-6-yl)-1-{2-[(1R)-5-(pyrimidin-2-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]non-7-yl}ethanone (**16b**)



16b

^{1}H NMR (400 MHz, CD_3OD) δ 8.85 (d, *J* = 6.0 Hz, 2 H), 8.26 (s, 1 H), 8.22 (d, *J* = 8.0 Hz, 1 H), 7.46 (d, *J* = 8.0 Hz, 1 H), 7.40 (br s, 2 H), 7.32 (t, *J* = 6.0 Hz, 1 H), 5.05–4.99 (m, 1 H), 4.43–4.33 (m, 1 H), 4.22–4.09 (m, 3 H), 3.67–3.53 (m, 4 H), 3.33 (s, 2 H), 3.23 (t, *J* = 6.0 Hz, 1 H), 3.13–3.04 (m, 1 H), 2.65–2.55 (m, 1 H), 2.53 (s, 3 H), 2.26–2.18 (m, 1 H), 2.02–1.83 (m, 4 H); HRMS (CI): *m/z* 499.2269 (calc 499.2275 for $C_{28}H_{30}N_6OS$ + H).

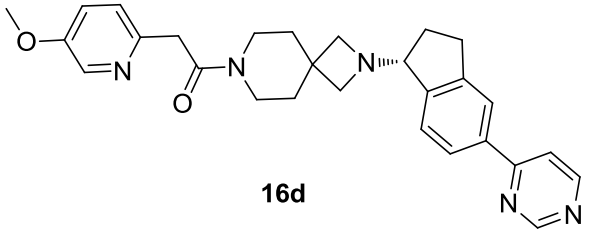
5-[(1R)-1-{7-[(2-Methylimidazo[2,1-b][1,3]thiazol-6-yl)acetyl]-2,7-diazaspiro[3.5]non-2-yl}-2,3-dihydro-1*H*-inden-5-yl]pyridine-2-carboxamide (**16c**)



16c

^{1}H NMR (400 MHz, CD_3OD) δ 8.85 (t, *J* = 1.7 Hz, 1 H), 8.14 (s, 2 H), 7.60 (s, 1 H), 7.55–7.44 (m, 2 H), 7.40 (s, 2 H), 4.12–4.03 (m, 1 H), 3.75 (s, 2 H), 3.58–3.50 (m, 4 H), 3.37–3.30 (m, 4 H), 3.19–3.06 (m, 1 H), 2.96–2.83 (m, 1 H), 2.40 (s, 3 H), 2.32–2.17 (m, 1 H), 1.98–1.86 (m, 1 H), 1.77–1.68 (m, 4 H); LCMS (ESI) *m/z* 541.7 $(M+H)^+$.

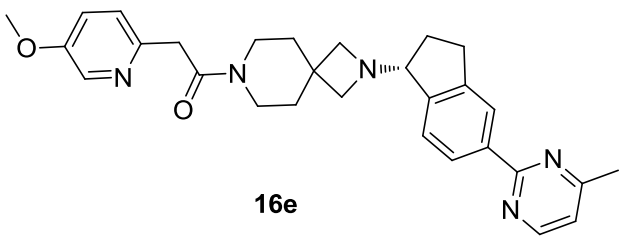
2-(5-Methoxypyridin-2-yl)-1-{2-[(1R)-5-(pyrimidin-4-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]non-7-yl}ethanone (**16d**)



16d

^{1}H NMR (400 MHz, CD_3OD) δ 9.16 (s, 1 H), 8.76 (d, *J* = 6.0 Hz, 1 H), 8.15 (d, *J* = 6.0 Hz, 1 H), 8.07 (s, 1 H), 8.04–7.94 (m, 2 H), 7.51 (d, *J* = 8.1 Hz, 1 H), 7.39–7.33 (m, 1 H), 7.30–7.23 (m, 1 H), 4.11–4.07 (m, 1 H), 3.87 (s, 2 H), 3.85 (s, 3 H), 3.54–3.50 (m, 4 H), 3.39–3.33 (m, 2 H), 3.32–3.26 (m, 2 H), 3.20–3.09 (m, 1 H), 2.97–2.87 (m, 1 H), 2.34–2.21 (m, 1 H), 2.00–1.88 (m, 1 H), 1.78–1.64 (m, 4 H),; LCMS (ESI) *m/z* 470.2 $(M+H)^+$.

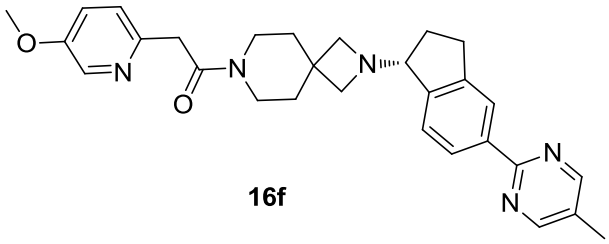
2-(5-Methoxypyridin-2-yl)-1-{2-[(1R)-5-(4-methylpyrimidin-2-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]non-7-yl}ethanone (**16e**)



16e

^{1}H NMR (400 MHz, CD_3OD) δ 9.49 (d, *J* = 4.8 Hz, 1 H), 9.01 (s, 1 H), 8.98–8.94 (m, 2 H), 8.17 (d, *J* = 5.2 Hz, 1 H), 8.11 (dd, *J* = 8.6, 2.8 Hz, 1 H), 8.07 (d, *J* = 5.2 Hz, 1 H), 7.97 (d, *J* = 8.6 Hz, 1 H), 4.72–4.65 (m, 1 H), 4.61 (s, 3 H), 4.58 (s, 2 H), 4.26–4.16 (m, 4 H), 4.12 (s, 3 H), 3.94–3.87 (m, 2 H), 3.88–3.85 (m, 3 H), 3.68–3.56 (m, 1 H), 2.94–2.82 (m, 1 H), 2.71–2.60 (m, 1 H), 2.35–2.32 (m, 4 H); LCMS (ESI) *m/z* 484.6 $(M+H)^+$.

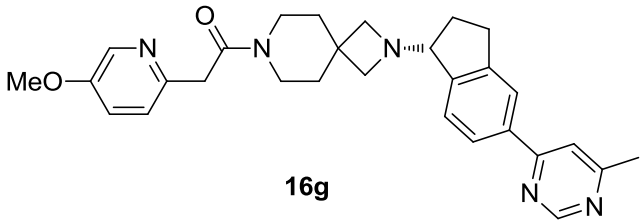
2-(5-Methoxypyridin-2-yl)-1-{2-[(1R)-5-(5-methylpyrimidin-2-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]non-7-yl}ethanone (**16f**)



16f

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 2 H), 8.25–8.20 (m, 2 H), 8.14 (d, 1 H), 7.38 (d, *J* = 7.8 Hz, 1 H), 7.20–7.12 (m, 2 H), 4.16–4.13 (m, 1 H), 3.81 (s, 3 H), 3.77 (s, 2 H), 3.50–3.41 (m, 4 H), 3.37–3.27 (m, 4 H), 3.10–3.01 (m, 1 H), 2.91–2.83 (m, 1 H), 2.32 (s, 3 H), 2.27–2.19 (m, 1 H), 2.00–1.92 (m, 1 H), 1.70–1.60 (m, 4 H); HRMS (CI): *m/z* 484.2703 (calc 484.2707 for $C_{29}H_{33}N_5O_2$ + H).

2-(5-Methoxypyridin-2-yl)-1-{2-[(1R)-5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]non-7-yl}ethanone (**16g**)

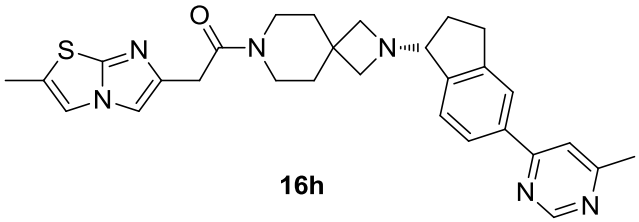


16g

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.04 (s, 1 H), 8.14 (s, 1 H), 7.88 (s, 1 H), 7.80 (d, *J* = 10.0 Hz, 1 H), 7.49 (s, 1 H), 7.31 (d, *J* = 10.0 Hz, 1 H), 7.19 (d, *J* = 10.0 Hz, 1 H), 7.11–7.08 (m, 1 H), 3.89–3.88 (m, 1 H), 3.79 (s, 2 H), 3.76 (s, 3 H), 3.46–3.39 (m, 4 H), 3.09–2.98 (m, 5 H), 2.82–2.76 (m, 1 H), 2.50 (s, 3 H), 2.13–2.06 (m, 1 H), 1.89–1.83 (m, 1 H), 1.64–1.62 (m, 2 H), 1.55–1.53 (m, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.9, 167.5, 164.1, 158.8, 154.6, 147.9, 146.5,

145.5, 136.8, 136.5, 125.5, 125.4, 125.1, 123.8, 121.5, 116.6, 70.9, 62.3, 55.8, 43.2, 39.5, 36.5, 35.9, 34.6, 29.6, 24.5; HRMS (CI): *m/z* 484.2637 (calc 484.2634 for $C_{29}H_{33}N_5O_2$ +H); $[\alpha]_D^{20}$ = +55.0 deg (c = 1 mg/mL, MeOH).

2-(2-Methylimidazo[2,1-b][1,3]thiazol-6-yl)-1-{2-[(1R)-5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1*H*-inden-1-yl]-2,7-diazaspiro[3.5]non-7-ylethanone (**16h**)



16h

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.05 (s, 1 H), 7.98 (d, *J* = 8.5 Hz, 1 H), 7.96 (s, 1 H), 7.59 (d, *J* = 1.7 Hz, 1 H), 7.46 (s, 1 H), 7.42 (d, *J* = 8.5 Hz, 1 H), 3.92–3.87 (m, 1 H), 3.63 (s, 2 H), 3.46–3.44 (m, 2 H), 3.40–3.38 (m, 2 H), 3.12–3.11 (m, 2 H), 3.02–2.96 (m, 4 H), 2.89–2.79 (m, 1 H), 2.50 (s, 3 H), 2.37 (s, 3 H), 2.11–2.04 (m, 1 H), 1.89–1.83 (m, 1 H), 1.60–1.56 (m, 4 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.6, 168.2, 163.2, 158.9, 147.4, 145.6, 141.4, 136.3, 125.7, 125.6, 123.9, 117.3, 116.9, 111.6, 70.9, 62.3, 43.8, 39.3, 36.7, 35.9, 35.2, 34.7, 30.7, 29.6, 24.5, 14.2; HRMS (CI): *m/z* 513.2431 (calc 513.2431 for $C_{29}H_{32}N_6OS$ + H; $[\alpha]_D^{20}$ = +45.3 deg (c = 2.5 mg/mL, MeOH), ee >99.7% (determined using chiral SFC, based on comparison to enantiomer of **16h**, synthesized starting from corresponding enantiomer of **13**).

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