Supporting information

Discovery of Imidazoisoindole Derivatives as Highly Potent and Orally Active Indoleamine-2,3-dioxygenase Inhibitors

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Biological Assays

IDO1 enzymatic assay

Human IDO1 gene purchased from Origene (SC126221) was transferred to Pet30a plasmids by gene cloning, and transferred to competent Escherichia coli Rosseta. This IDO1 gene was scale-up cultured in liquid LB (Luria-Bertani) medium which was prepared according to "Molecular Cloning A Laboratory Manual" (Sambrook, J.; Russell, D. W.). The bacteria were collected and broken by the ultrasonic wave. The purified IDO1 was obtained through the column by elution.

24 μL of enzyme (IDO1) was diluted 100 times with 50 mM KPB to 2400 μL. The concentration of enzyme solution was 2.6ng/μL. A 96 well reaction plate (AXYGEN, PCR-96-FLT-C, hereinafter referred to as the reaction plate) was added with the enzyme solution at 24 μL/well. The blank well was added with 24 μL of KPB [Preparation of KPB buffer (50mM): 6.805 g of KH_2P0_4 was weighed by an analytical balance, and placed into a 1000 ml of beaker, deionized water was added with a measuring cylinder to 900 ml, the pH was adjusted to 6.5 by 1M KOH, then the mixture was introduced into a 1 L measuring cylinder, and water was added to 1 L. It was stored at 4°C]. 1 μL of a compound or DMSO was added into the corresponding wells in the reaction plate. Preparation of solution A: 200 μL of 500 mM sodium ascorobate was added with 1050 μL of KPB, then the mixture was mixed uniformly for 3 seconds at the maximum speed in a turbine mixer. Solution B: 100 μL of 10 mM tryptophan was added with 100 μL of 100000 unit/ml catalase, 5 μL of 10 mM methylene blue, and 1050 μL of KPB sucessively, then the mixture was mixed uniformly for 3 seconds at the maximum speed in a turbine mixer. 1200 μL of solution A and 1200 μL of solution B were taken and mixed uniformly for 3 seconds at the maximum speed in a turbine mixer. The mixture was added to the reaction plate at 24μL/well. The reaction plate was placed in a plate centrifuge and centrifuged for 15 seconds at the maximum speed, so the reaction liquids were converged to the bottom. The reaction mixture was mixed uniformly for 30 seconds on a shaker, and incubated for 1 hour at 37°C in a constant temperature incubator. In the reaction plate, 30% (W/V) trichloroacetic acid was added at 10 μL/well, then the mixture was incubated for 15 minutes at 65°C in a incubator. The reaction plate was centrifuged in a centrifuge for 5 minutes at 4700RPM at room temperature. 40 μL of the supernatant was transferred from the reaction plate to the corresponding 96 wells test plate (Corning, #3599) by a

multi-channel pipette. 2% (W/V) 4-(dimethylamino)benzaldehyde/glacial acetic acid solution was added at 40 μL/well, then the mixture was mixed uniformly for 1 minute on a shaker at the maximum speed. After incubation for 2 minutes at room temperature, the absorbance at 480 nm was read on Synergy HT (BIOTEK).

TDO enzymatic assay

Human TDO gene purchased from Suzhou Genewiz Biological Technology Co., Ltd. (U32989.1) was transferred to competent Escherichia coli Rosseta. This TDO gene was scale-up cultured in liquid LB (Luria-Bertani) medium which was prepared according to "Molecular Cloning A Laboratory Manual" (Sambrook, J.; Russell, D. W.). The bacteria were collected and broken by the ultrasonic wave. The purified TDO was obtained through the column by elution.

24 μL of enzyme (TDO) was diluted 100 times with 50 mM KPB to 2400 μL. The concentration of enzyme solution was 2.6ng/μL. A 96 well reaction plate (AXYGEN, PCR-96-FLT-C) (hereinafter referred to as the reaction plate) was added with the enzyme solution at 24 μL/well. The blank well was added with 24 μL of KPB [Preparation of KPB buffer (50mM): 6.805 g of KH_2PO_4 was weighed by an analytical balance, and placed into a 1000 ml of beaker, deionized water was added with a measuring cylinder to 900 ml, the pH was adjusted to 6.5 by 1M KOH, then the mixture was introduced into a 1 L measuring cylinder, and water was added to 1 L. It was stored at 4°C]. 1 μL of a compound or DMSO was added into the corresponding wells in the reaction plate. Preparation of solution A: 200 μL of 500 mM sodium ascorobate was added with 1050 μL of KPB, then the mixture was mixed uniformly for 3 seconds at the maximum speed in a turbine mixer. Solution B: 100 μL of 10 mM tryptophan was added with 100 μL of 100000 unit/ml catalase, 5 μL of 10 mM methylene blue, and 1050 μL of KPB sucessively, then the mixture was mixed uniformly for 3 seconds at the maximum speed in a turbine mixer. 1200 μL of solution A and 1200 μL of solution B were taken and mixed uniformly for 3 seconds at the maximum speed in a turbine mixer. The mixture was added to the reaction plate at 24 μL/well. The reaction plate was placed in a plate centrifuge and centrifuged for 15 seconds at the maximum speed, so the reaction liquids were converged to the bottom. The reaction mixture was mixed uniformly for 30 seconds on a shaker, and incubated for 1 hour at 37°C in a constant

temperature incubator. In the reaction plate, 30% (W/V) trichloroacetic acid was added at 10 μL/well, then the mixture was incubated for 15 minutes at 65°C in a incubator. The reaction plate was centrifuged in a centrifuge for 5 minutes at 4700RPM at room temperature. 40 μL of the supernatant was transferred from the reaction plate to the corresponding 96 wells test plate (Corning, #3599) by a multi-channel pipette. 2% (W/V) 4-(dimethylamino)benzaldehyde/glacial acetic acid solution was added at 40 μL/well, then the mixture was mixed uniformly for 1 minute on a shaker at the maximum speed. After incubation for 2 minutes at room temperature, the absorbance at 480 nm was read on Synergy HT (BIOTEK).

Hela cellular assay

This method is used to determine the inhibition effect of the test compounds on the activity of IDO in Hela cells. (Note: indoleamine 2,3-dioxygenase (IDO) is expressed in the Hela cell line and induced by interferon gamma (INFγ))

Hela cell suspension was prepared with a fresh cell medium, and added into a 96 cell plate with 100 μL culture system at 10000 cells/well，then incubated for 24 hours in 5% carbon dioxide at 37°C. The supernatant was removed, serum-free OMEM high glucose medium was added at 90 μL/well, then the test compounds contained in the culture medium with INFγ and tryptophan were added at 10 μL/well (the final concentration: 10000, 1000, 100, 10, 1, 0.1 nM), the mixture was incubated for 48 hours in 5% carbon dioxide at 37°C. 80 μL of the supernate was transferred from the 96-well cell culture plate to a 96 well round-bottomed plate, then 30% *(WN)* trichloroacetic acid was added at 16μL/well, then the mixture was incubated for 25 minutes at 65°C in an incubator. The reaction plate was centrifuged in a centrifuge for 5minutes at 4700RPM. 50 μL of the supernatant was transferred from the reaction plate to a 96-well flatbottomed transparent plate by a multi-channel pipette. 2% *(WN)* 4-(dimethylamino)benzaldehyde/glacial acetic acid solution was added at 50 μL/well, then the mixture was mixed uniformly for 1 minute on a shaker. After incubation for 2 minutes at room temperature, the absorbance at 480 nm was read on Synergy HT Reader.

Plasma protein bonding

Mouse, dog and human plasma protein bonding of test compound was determined via Rapid Equilibrium Dialysis (RED) system according to method described in

literatures.

Wan H, Rehngren M. High throughput screening of protein binding by equilibrium dialysis combined with liquid chromatography and mass spectrometry. *J.Chromatogr A*. 2006, 1102, 125-134.
Jones R, Williams G, Sohal B, et al.Validation of a rapid equilibrium dialysis approach for the measurement of plasma protein binding. *J.Pharm.Sci.* 2008, 97, 4586-4595.

Liver microsome stability

Stability of test compound in rat and human liver microsome was determined according to method described in literatures.

Masimirembwa CM, Bredberg U, et al. Metabolic stability for drug discovery and development. Clin Pharmacokinet, 2003, 42(6): 515-528.
Brian Davies, Tim Morris. Physiological Paramaters in Laboratory Animals and Humans. Pharmaceutical Research, 1993,Vol. 10, No. 7:1093-1095.

hERG patch clamp assay

hERG inhibitory activity of test compound was determined by the Automated Patch-Clamp system according to method described in literature.

Kutchinsky, J; Friis, S.; Asmild, M., et al. Characterization of potassium channel modulators with QPatch automated patch-clamp technology: system characteristics and performance. Assay Drug Dev. Technol. 2003, 1, 685-693.

CYP inhibition assay

The inhibitory IC50 values of tested compounds for five major P450 enzymes were determined in human liver microsome (BD Gentest) according to method described in literatures.

Li, G., Huang,K., Nikolic, D., and B. van Breemen, R., High-Throughput Cytochrome P450 Cocktail Inhibition Assay for Assessing Drug-Drug and Drug-Botanical Interactions. Drug Metab Dispos 43:1670–1678, 2015.
9. FDA Guidance for Industry: Drug Interaction Studies-Study Design, Data Analysis, and Implications for Dosing and Labeling, 2006.

Pharmacokinetic Assays

Pharmacokinetics in the rats, mice and dogs

Compound **25** was administrated with its suspension in the mixture of 0.5% CMC-Na for p.o and its solution in the mixture of 1% DMSO+99% saline for i.v., respectively.

Sprague-Dawley rats (160–180 g) were obtained from Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China, certificate no. SCXK-2008-0016) and housed with free access to food and water. After a 12 h fast, two males and two females were administrated by oral gavage at a dose of 10 mg/kg compound **25** suspension. The other two males and two females were administrated by intravenous injection at a dose of 1 mg/kg compound **25**. Blood samples (0.1 mL) were collected via the posterior orbital venous plexus at times of 0.083, 0.25, 0.5, 1, 2, 4, 8, 11 and 24 h after administration. Plasma was separated by immediate centrifugation and was kept at -20 °C until analyzed.

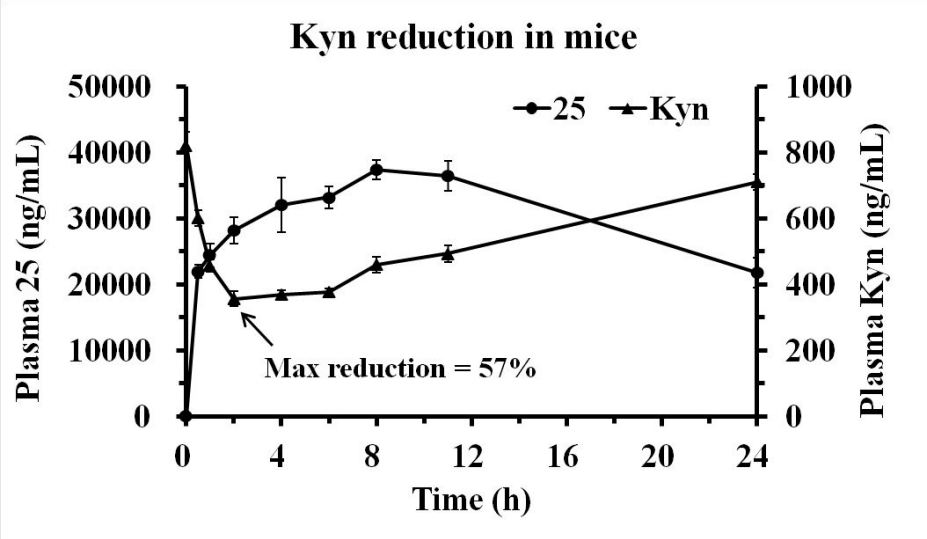
c57bl/6 mice (20-25 g) were obtained from Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China, certificate no. SCXK-2008-0016) and housed with free access to food and water. After a 12 h fast, nine females were administrated by oral gavage at a dose of 10 mg/kg compound **25** suspension. The other nine females were administrated by intravenous injection at a dose of 2 mg/kg compound **25**. Blood samples (0.2 mL) were collected via the posterior orbital venous plexus at times of 0.083, 0.25, 0.5, 1, 2, 4, 8, 11 and 24 h after administration. Plasma was separated by immediate centrifugation and was kept at -20 °C until analyzed.

Beagle dogs were obtained from Suzhou Xishan Zhongke Laboratory Animal Co., Ltd. After a 12 h fast, two males and two females were administrated by oral gavage at a dose of 2 mg/kg compound **25** suspension. The other two males and two females were administrated by intravenous injection at a dose of 0.5 mg/kg compound **25**. Blood samples (1.0 mL) were collected via the forelimb vein at times of 0.083, 0.25, 0.5, 1, 2, 4, 8, 11 and 24 h after administration. Plasma was separated by immediate centrifugation and was kept at -20 °C until analyzed.

In Vivo Pharmacodynamic Study

The test compound was administrated (i.g.) to C57BL/6 mice (6 animals, 3 male+3 female/group). Blood samples were collected at 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 11.0, 24.0 h after dosing. The plasma concentrations of test compound and kynurenine were measured. Kynurenine reduction ratio = C_0-C_t/C_0 × 100%.

Figure S1. Kynurenine reduction of oral administration of **25** in mice.



In Vivo Efficacy Study

MC38 Xenograft model in hPD1 transgenic mouse

Xenograft model was developed in hPD1 transgenic mice with human MC38 cancer cell line purchased from American Type Culture Collection (USA). MC38 cells (1×10^5) were implanted s.c. into the hind flank region of each mouse and allowed to grow to the designated size (c.a. 100 mm^3) before administration of test compound. Six or seven mice were allocated into each treatment group according to the size of tumors. For the combination group, the test compound was given orally at various dose levels twice daily for 14 days and SHR-1210 (produced by Shanghai Hengrui Pharmaceutical Co., Ltd.) was given intraperitoneally every other day for 8 times.

Tumor response was determined by measurement of tumors with a digital caliper twice weekly. Tumor volume (mm^3) was estimated from the formula: Tumor volume = 1/2 (length × $(width)^2$). Treated animals were checked daily for treatment related toxicity/mortality. Body weights were collected on each group of animals before the initiation of treatment (Wt1) and then again following the last treatment dose (Wt2). The difference in body weight (Wt2-Wt1) provides a measure of treatment-related

toxicity. Additional weights were recorded at each measurement date to monitor toxicity.

Study was terminated when mean tumor volume exceeded 2000mm^3 for any group according to the guidelines of IACUC.

Computational Methods

Small Molecule Preparation

Molecules were constructed in MOE and ionized using MOE's WashMDB function, and hydrogens were added. The small molecule conformation was minimized to a gradient of 0.01 in the MMFF94S force field using distance dependent dielectric constant of 1.

Protein Preparation

Chain A of the IDO-1 crystal structure (PDB: 2D0T) was used for all docking runs. Waters greater than 4.5Å from the protein were removed before protons were added and the H-bonding network optimised using MOE's Protonate3D protocol. The Amber10:EHT force field in MOE was used, and iron was parametrized in the Fe^{3+} state. A stepwise minimization followed for residues within 8 Å of the ligand using a quadratic force constant (10) to tether the atoms to their starting geometries.

Docking Calculation

The active site was defined using dummy atoms created by MOE's Site Finder protocol. MOE general docking mode, where the ligand sampling was set flexible, was used for the docking. All other parameters were left at their default values. The top-scored conformation was selected as the best docking result.

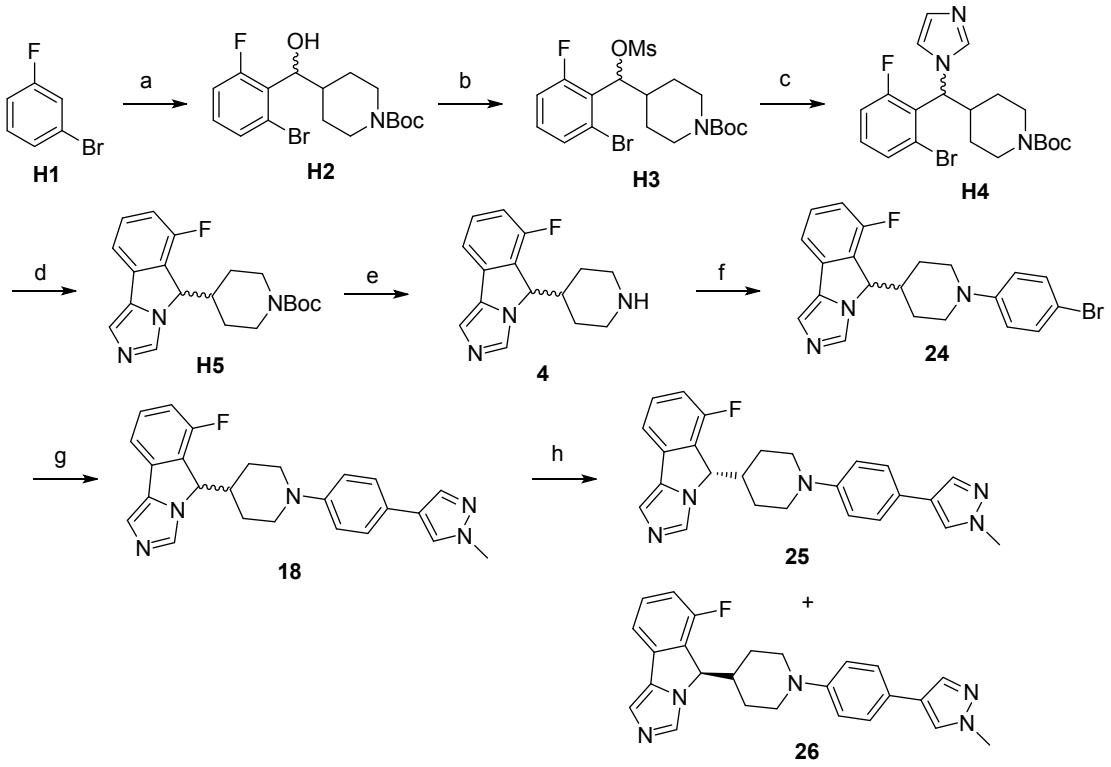
General Experimental Procedures and Analytical Characterization Methods

All purchased starting materials were used without further purification.
^{1}HNMR spectra were acquired on a Bruker Avance-400 spectrometer (400 MHz), with tetramethylsilane (TMS) as an internal standard; chemical shifts are expressed in parts per million (ppm, δ units). Mass spectra were obtained on an ACQUITY UPLC-SQD (ESI) from Waters Corporation (U.S.). Most masses were reported as those of the protonated parent ions. High-resolution mass spectra (HRMS) were recorded on a Thermo Q Exactive instrument (ESI). Where noted, compounds were determined to be >95% pure by analytical reverse-phase HPLC. HPLC conditions: an isocratic program using 60-80% methanol, 40-20% water, and 0.1% aqueous ammonia was employed on a Gemini C18 column (250 mm, 4.6 mm). The flow rate was 1.0 mL/min, and UV detection was at 214 and 254 nm. Chiral separation and chiral analysis were performed on HPLC equipped with Daicel chiral columns. Melting point was measured on a SGW X-4 melting point detector.

Abbreviations used
DMSO: dimethyl sulfoxide; DMF: dimethyl formamide; DCM: dichloromethane; THF: tetrahydrofuran; DME: 1,2-Dimethoxyethane; TEA: triethylamine; MsCl: Methanesulfonyl chloride; BINAP: 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl; TFA: trifluoroacetic acid; dba: dibenzylideneacetone

Experimental Procedures and Analytical Data for key compounds

Scheme S1. Synthesis of compound 25[a]



[a]Reagents and conditions: (a) (1) LDA, THF, –78°C, 1 h; (2) tert-butyl 4-formylpiperidine-1-carboxylate, –78°C, 1 h; (b) NaH, THF, MsCl, reflux, 48 h; (c) imidazole, NaH, DMF, 100°C, 12 h; (d) $Pd(OAc)_2$, PPh_3, Cy_2NMe, DMF, 100°C, 5 h; (e) CH_2Cl_2, TFA, rt, 1 h; (f)1,4-dibromobenzene, $Pd_2(dba)_3$, BINAP, *t*BuONa, toluene, 80°C, 12 h; (g) 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole, $Pd(dppf)Cl_2$, Na_2CO_3, DME/H_2O, microwave, 120°C, 40 min; (h) chiral separation.

(S)-6-fluoro-5-(1-(4-(1-methyl-1H-pyrazol-4-yl)phenyl)piperidin-4-yl)-5H-imidazo[5,1-a]isoindole (25)

Step A: tert-butyl 4-((2-bromo-6-fluorophenyl)(hydroxy)methyl)piperidine-1–carboxylate (**H2)**

LDA (32.5 mL, 65 mmol, 2M in THF) was added to 50 mL of tetrahydrofuran, then 25 mL of a pre-prepared solution of 1-bromo-3-fluorobenzene **H1** (8.75 g, 50.0 mmol) in tetrahydrofuran was added dropwise at -78°C, and the resulting mixture was stirred for 1 hour at -78°C. Then 25 mL of a pre-prepared solution of tert-butyl 4-formylpiperidine-1-carboxylate (8.75 g, 50.0 mmol) in tetrahydrofuran was added dropwise at -78°C. The reaction was continuely stirred for 1 hour at -78°C. After the

completion of the reaction, 25 mL of methanol was added dropwise to quench the reaction at -78° C, and the reaction solution was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography with ethyl acetate/hexane to give the desired product (16.3 g, 84%) as yellow syrup solid.

LC-MS (ESI) *m*/*z*: calcd for $C_{17}H_{23BrFNO_3}$ $[M+H]^+$ 387.1, 389.1; found 333.0, 334.0 [M-55]

HNMR (400 MHz, $CDCl_3$) δ 7.42-7.40 (m, 1H), 7.20-7.15 (m, 1H), 7.11-7.06 (m, 1H), 4.93 (d, *J* = 9 Hz, 1H), 4.25-4.22 (m, 1H), 4.12-4.09 (m, 1H), 2.78-2.71 (m, 1H), 2.67-2.60 (m, 1H), 2.22-2.18 (m, 1H), 1.49 (s, 9H), 1.36-1.30 (m, 3H), 1.23-1.19 (m, 1H).

Step B: tert-butyl 4-((2-bromo-6-fluorophenyl)((methylsulfonyl)oxy)methyl) piperidine-1-carboxylate (**H3)**

H2 (15 g, 38.6 mmol) was dissolved in 100 mL of dichloromethane, and the resulting mixture was cooled to 0°C. Triethylamine (7.82 g, 77.3 mmol) was added. Then methylsufonyl chloride (6.63 g, 57.9 mmol) were added dropwise. The reaction system was stirred for 1 hour at 0°C. After the reaction was completed, the reaction solution was washed with water (50 mL × 3), dried over sodium anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to give the desired product (18 g) as brown viscous solid, which was used directly in the next step without further purification.

LC-MS (ESI) *m*/*z*: calcd for $C_{18}H_{25}BrFNO_5S$ $[M+H]^+$ 466.1, 468.1; found 314.0, 316.0 [M-152]

HNMR (400 MHz, $CDCl_3$) δ 7.48-7.46 (m, 1H), 7.30-7.27 (m, 1H), 7.17-7.13 (m, 1H), 5.86 (d, *J* = 10 Hz, 1H), 4.24 (d, *J* = 13.6 Hz, 1H), 4.14 (d, *J* = 12.4 Hz, 1H), 2.84 (s, 3H), 2.76-2.69 (m, 1H), 2.64-2.61 (m, 1H), 2.19-2.15 (m, 1H), 1.49 (s, 9H), 1.48-1.40 (m, 3H), 1.27-1.24 (m, 1H).

Step C: tert-butyl 4-((2-bromo-6-fluorophenyl)(1*H*-imidazol-1-yl)methyl)piperidine-1-carboxylate (**H4)**

H3 (2.0 g, 4.28 mmol) was dissolved in 5 mL of acetonitrile, imidazole (2.9 g, 42.8 mmol) and N,N-diisopropylethylamine (5.5 g, 42.8 mmol) were added. The resulting mixture was stirred in microwave for 1 hour at 120°C. After the reaction was completed, the reaction solution was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography with

dichloromethane/methanol to give the desired product (0.85 g, 47%) as light brown oil.

LC-MS (ESI) *m/z*: calcd for $C_{20}H_{25}BrFN_3O_2$ $[M+H]^+$ 438.1, 440.1; found 438.0, 440.0

Step D: tert-butyl 4-(6-fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)piperidine-1-carboxylate (**H5)**

H4 (1.90 g, 4.33 mmol), N,N-dicyclohexylmethylamine (1.35 g, 6.93 mmol) and triphenylphosphine (908 mg, 3.46 mmol) were dissolved in 10 mL of N,N-dimethylformamide. Palladium acetate (390 mg, 1.74 mmol) was added under argon atmosphere. The reaction mixture was stirred for 5 hours at 100°C. After the reaction was completed, the reaction solution was concentrated under reduced pressure, and the resulting residue was purified by silica gel column chromatography with ethyl acetate/hexane to give the desired product (1.30 g, 84%) as yellow viscous solid.

LC-MS (ESI) *m/z*: calcd for $C_{20}H_{24}FN_3O_2$ $[M+H]^+$ 358.2; found 358.1

HNMR (400 MHz, $CDCl_3$) δ 8.12 (bs, 1H), 7.41-7.39 (m, 1H), 7.32 (s, 1H), 7.07-7.02 (m, 1H), 5.47 (bs, 1H), 4.25 (bs, 1H), 4.06 (bs, 1H), 2.73 (bs, 1H), 2.51-2.45 (m, 1H), 1.77-1.74 (m, 1H), 1.57-1.44 (m, 1H), 1.41 (s, 9H), 1.22-1.19 (m, 1H), 0.88-0.82 (m, 1H).

Step E: 6-fluoro-5-(piperidin-4-yl)-5*H*-imidazo[5,1-*a*]isoindole ditrifluoroacetate (**4)**

H5 (1.30 g, 3.64 mmol) was dissolved in 5 mL of dichloromethane, then 5 mL of trifluoroacetate was added dropwise. The resulting mixture was stirred for 1 hour at room temperature. After the reaction was completed, the reaction solution was concentrated under reduced pressure to give the desired product (1.77 g) as brown viscous solid, which was used directly in the next step without further purification.

LC-MS (ESI) *m/z*: calcd for $C_{15}H_{16}FN_3$ $[M+H]^+$ 258.1; found 258.3

HNMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.39-7.34 (m, 1H), 7.32-7.30 (m, 1H), 7.19 (s, 1H), 6.98-6.93 (m, 1H), 5.32 (bs, 1H), 3.33-3.30 (m, 1H), 3.13-3.10 (m, 1H), 2.80-2.74 (m, 1H), 2.66-2.60 (m, 1H), 2.47-2.42 (m, 1H), 1.86-1.73 (m, 2H), 1.25-1.20 (m, 1H), 1.00-0.97 (m, 1H).

Step F: 5-(1-(4-bromophenyl)piperidin-4-yl)-6-fluoro-5*H*-imidazo[5,1-*a*]isoindole (**24)**

Compound **4** (1.45 g, 3 mmol) was dissolved in 30 mL of toluene, 1,4-dibromobenzene (1.41 g, 6 mmol), then (+-)2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (187 mg, 0.3 mmol), sodium tert-butoxide (1.15 g, 12 mmol) and tri(dibenzylideneacetone)dipalladium (275 mg, 0.3 mmol) were added under argon atmosphere. The reaction system was stirred for 12 hours at 80° C. After the reaction was completed, the filtrate was concentrated under reduced pressure, and the resulting residue was purified by silica gel column chromatography with dichloromethane/methanol to give the desired product (670 mg, 50%) as yellow solid.
LC-MS (ESI) *m/z*: calcd for $C_{21}H_{19}BrFN_3$ $[M+H]^+$ 412.1, 414.1; found 412.3, 414.3

Step G: 6-fluoro-5-(1-(4-(1-methyl-1H-pyrazol-4-yl)phenyl)piperidin-4-yl)-5*H*-imidazo[5,1-*a*]isoindole (**18)**
Compound **24** (165 mg, 0.4 mmol) was dissolved in 5 mL of 1,2-dimethoxyethane, then 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan -2-yl)-1H-pyrazole (166 mg, 0.8 mmol), sodium carbonate (127 mg, 1.2 mmol) and water (0.5 mL) were added. After mixing uniformly, [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (29 mg, 0.04 mmol) was added and the reaction system was stirred for 40 minutes at 120°C in microwave under argon atmosphere. After the reaction was completed, 50 mL of ethyl acetate and 20 mL of water were added. Two phase were separated, and the aqueous phase was extracted with ethyl acetate (30 mL). The organic phases were combined, washed with saturated sodium chloride solution (40 mL), dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure, and the resulting residue was purified by high performance liquid chromatography to give the desired product (10 mg, 6.06%) as a white solid.
LC-MS (ESI) *m/z*: calcd for $C_{25}H_{24}FN_5$ $[M+H]^+$ 414.2; found 414.4.
HRMS (ESI) $[M+H]^+$, calcd for $C_{25}H_{24}FN_5$ 414.2016, found 414.2038.
^{1}HNMR (400 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.96 (s, 1H), 7.71 (s, 1H), 7.48-7.46 (m, 2H), 7.35 (d, *J* = 8.5 Hz, 2H,), 7.22 (s, 1H), 7.16-7.13 (m, 1H), 6.85 (d, *J* = 8.6 Hz, 2H), 5.71 (s, 1H), 3.83 (s, 3H), 3.74 (d, *J* = 10.6 Hz, 1H), 3.61 (d, *J* = 10.6 Hz, 1H), 2.67 (t, *J* = 12 Hz, 1H), 2.55-2.52 (m, 1H), 2.36 (t, *J* = 12 Hz, 1H), 1.79-1.76 (m, 1H), 1.70-1.65 (m, 1H), 1.19-1.16 (m, 1H), 0.90-0.87 (m, 1H).

Step H: (S)-6-fluoro-5-(1-(4-(1-methyl-1H-pyrazol-4-yl)phenyl)piperidin-4-yl)-5*H*-imidazo[5,1-*a*]isoindole (**25)** and
(R)-6-fluoro-5-(1-(4-(1-methyl-1H-pyrazol-4-yl)phenyl)piperidin-4-yl)-5*H*-

imidazo[5,1-*a*]isoindole (**26)**

Compound **18** was submitted to chiral separation by the following conditions.

Separation conditions: chiral column CHIRALPAK IF, mobile phase: dichloromethane: methanol=70:30, flow rate: 30 mL/min

Relevant fractions were collected and concentrated under reduced pressure to give the undesired enantiomer **26** (700 mg, 1.69 mmol) with a yield of 74% and desired enantiomer **25** (640 mg, 1.54 mmol) with a yield of 67%.

26:

LC-MS (ESI) *m*/*z*: calcd for $C_{25}H_{24}FN_5$ $[M+H]^+$ 414.2; found 414.4.

Chiral HPLC analysis: retention time, 2.466 min, ee>99.0% (chromatographic column: CHIRALPAK ID; mobile phase: DCM/MeOH/TEA =80/20/0.1(V/V/V)

25:

LC-MS (ESI) *m*/*z*: calcd for $C_{25}H_{24}FN_5$ $[M+H]^+$ 414.2; found 414.4.

HRMS (ESI) $[M+H]^+$, calcd for $C_{25}H_{24}FN_5$ 414.2016, found 414.2025.

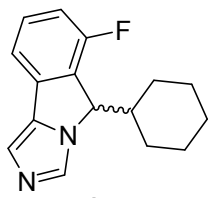
Chiral HPLC analysis: retention time, 4.122 min, ee>99.0% (chromatographic column: CHIRALPAK ID; mobile phase: DCM/MeOH/TEA=80/20/0.1(V/V/V))

M.P. = 259-260°C

^{1}HNMR (400 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.94 (s, 1H), 7.70 (s, 1H), 7.48-7.46 (m, 2H), 7.34 (d, *J* = 8.5 Hz, 2H,), 7.22 (s, 1H), 7.16-7.13 (m, 1H), 6.85 (d, *J* = 8.6 Hz, 2H), 5.69 (s, 1H), 3.82 (s, 3H), 3.73 (d, *J* = 12 Hz, 1H), 3.60 (d, *J* = 12 Hz, 1H), 2.66 (t, *J* = 12 Hz, 1H), 2.55-2.52 (m, 1H), 2.36 (t, *J* = 12 Hz, 1H), 1.78-1.75 (m, 1H), 1.68-1.63 (m, 1H), 1.19-1.16 (m, 1H), 0.91-0.87 (m, 1H).

^{13}CNMR is not available due to the poor solubility of **25** in DMSO and other commonly used deuterated solvents.

5-cyclohexyl-6-fluoro-5*H*-imidazo[5,1-*a*]isoindole (1)

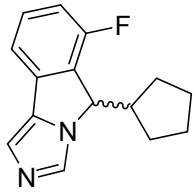


1

Prepared in a fashion similar to that used for the synthesis of **H5**. LC-MS (ESI) *m*/*z*: calcd for $C_{16}H_{17}FN_2$ $[M+H]^+$ 357.1; found 357.6. ^{1}H NMR (400MHz, DMSO-d_6) δ 9.38 (s, 1H), 8.00 (s, 1H), 7.73-7.71 (m, 1H), 7.66-7.60 (m, 1H), 7.41-7.37 (m, 1H),

5.96(s, 1H), 2.37-2.31 (m, 1H),1.77-1.70(m, 1H), 1.62-1.60(m, 1H), 1.32-1.18 (m, 4H), 1.08-1.02 (m, 1H), 0.68-0.58 (m, 1H).

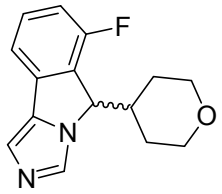
5-cyclopentyl-6-fluoro-5*H*-imidazo[5,1-*a*]isoindole (2)



2

Prepared in a fashion similar to that used for the synthesis of **H5**. LC-MS (ESI) *m*/*z*: calcd for $C_{15}H_{15}FN_2$ $[M+H]^+$ 243.1; found 243.4. ^{1}H NMR (400MHz, DMSO-d_6) δ 9.36 (s, 1H), 7.98(s, 1H), 7.73-7.71 (m, 1H), 7.65-7.60 (m, 1H), 7.39-7.35 (m, 1H), 6.11-6.10(m, 1H), 2.82-2.77(m, 1H), 1.95-1.87 (m, 1H), 1.64-1.51 (m, 3H), 1.47-1.39 (m, 3H), 1.25-1.24 (m, 1H), 0.84-0.79 (m, 1H).

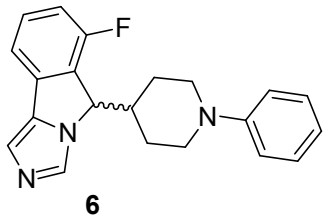
6-fluoro-5-(tetrahydro-2*H*-pyran-4-yl)-5*H*-imidazo[5,1-*a*]isoindole (3)



3

Prepared in a fashion similar to that used for the synthesis of **H5**. LC-MS (ESI) *m*/*z*: calcd for $C_{15}H_{15}FN_2O$ $[M+H]^+$ 259.3; found 259.1. ^{1}H NMR (400MHz, DMSO-d_6) δ 7.99 (s, 1H), 7.50-7.46 (m, 1H), 7.22 (s, 1H), 7.16-7.11(m, 1H), 5.63 (d, J = 2.1 Hz, 1H), 3.91-3.88 (m, 1H), 3.76-3.72(m, 1H), 3.25-3.19(m, 1H), 1.61-1.57(m, 2H), 1.34-1.24 (m, 2H), 1.00-0.97 (m, 1H), 0.81-0.73 (m, 1H).

6-fluoro-5-(1-phenylpiperidin-4-yl)-5*H*-imidazo[5,1-*a*]isoindole (6)

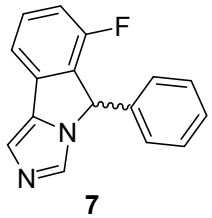


6

Prepared in a fashion similar to that used for the synthesis of **24**. LC-MS (ESI) *m*/*z*: calcd for $C_{21}H_{20}FN_3$ $[M+H]^+$ 334.2; found 334.3. ^{1}H NMR (400 MHz, CD_3OD): δ 9.35(s, 1H), 7.93 (s, 1H), 7.75-7.73 (m, 1H), 7.68-7.66 (m, 1H), 7.58-7.52 (m, 5H),

7.46-7.42 (m, 1H), 7.36-7.34 (m, 1H), 6.14 (d, J = 2.1 Hz, 1H), 3.76-3.73 (m, 1H), 3.68-3.61 (m, 2H), 3.60-3.52 (m, 1H), 3.00-2.94 (m, 1H), 2.10-2.06 (m, 2H), 1.73-1.62 (m, 2H)

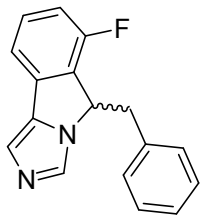
6-fluoro-5-phenyl-5*H*-imidazo[5,1-*a*]isoindole (7)



7

Prepared in a fashion similar to that used for the synthesis of **H5**. LC-MS (ESI) m/z: calcd for $C_{16}H_{11}FN_2$ $[M+H]^+$ 251.1; found 251.3. ^{1}H NMR (400MHz, DMSO-d_6) δ 9.11 (s, 1H), 7.97 (s, 1H), 7.80-7.78 (m, 1H), 7.66-7.64 (m, 1H), 7.47-7.42 (m, 3H), 7.34-7.26 (m, 3H), 7.04 (s, 1H).

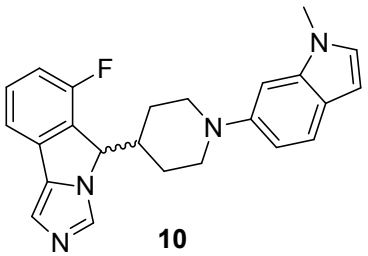
5-benzyl-6-fluoro-5*H*-imidazo[5,1-*a*]isoindole (8)



8

Prepared in a fashion similar to that used for the synthesis of **H5**. LC-MS (ESI) m/z: calcd for $C_{17}H_{13}FN_2$ $[M+H]^+$ 265.1; found 265.3. ^{1}H NMR (400MHz, DMSO-d_6) δ 7.60 (s, 1H), 7.40-7.36 (m, 1H), 7.32-7.30 (m, 1H), 7.14-7.09 (m, 4H), 7.05 (s, 1H), 6.90-6.88 (m, 2H), 5.95 (t, J = 5 Hz, 1H), 3.70-3.66 (m, 1H), 3.23-3.19 (m, 1H).

6-fluoro-5-(1-(1-methyl-1*H*-indol-6-yl)piperidin-4-yl)-5*H*-imidazo[5,1-*a*]isoindole (10)

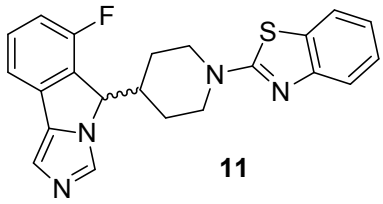


10

Prepared in a fashion similar to that used for the synthesis of **24**. LC-MS (ESI) m/z:

calcd for $C_{24}H_{23}FN_4$ $[M+H]^+$ 387.2; found 387.3. ^1H NMR (400MHz, DMSO-d_6) δ 8.01 (s, 1H), 7.55-7.45 (m, 2H), 7.34-7.33 (m, 1H), 7.25(s, 1H), 7.20-7.10 (m, 2H), 6.83 (s, 1H), 6.74-6.71 (m, 1H), 6.25 (d, *J* = 3 Hz, 1H), 5.70 (d, *J* = 2 Hz, 1H), 3.75-3.63 (m, 4H), 3.55-3.53 (m, 1H), 2.71-2.65 (m, 1H), 2.56-2.51 (m, 1H), 2.36-2.34 (m, 1H), 1.79-1.73 (m, 2H), 1.23-1.20 (m, 1H) , 0.99-0.95 (m, 1H).

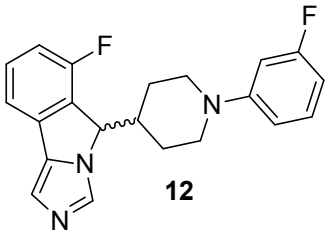
2-(4-(6-fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)piperidin-1-yl)benzo[*d*]thiazole (11)



11

Prepared in a fashion similar to that used for the synthesis of **24**. LC-MS (ESI) *m*/*z*: calcd for $C_{22}H_{19}FN_4S$ $[M+H]^+$ 391.1; found 391.3. ^1H NMR (400MHz, DMSO-d_6) δ 9.48 (s, 1H), 8.06 (s, 1H), 7.80-7.62 (m, 3H), 7.46-7.38 (m, 2H), 7.26-7.23 (m, 1H), 7.07-7.05 (m, 1H), 6.14 (s, 1H), 4.15-4.12 (m, 1H), 4.02-4.00 (m, 1H), 3.27-3.21(m, 1H), 3.15-3.09 (m, 1H), 2.75-2.69 (m, 1H), 1.90-1.87 (m, 1H), 1.61-1.57 (m, 1H), 1.36-1.33 (m, 1H), 1.04-1.00 (m, 1H).

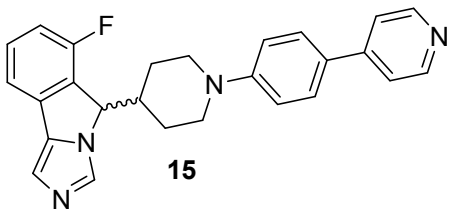
6-fluoro-5-(1-(3-fluorophenyl)piperidin-4-yl)-5*H*-imidazo[5,1-*a*]isoindole (12)



12

Prepared in a fashion similar to that used for the synthesis of **18**. LC-MS (ESI) *m*/*z*: calcd for $C_{21}H_{19}F_2N_3$ $[M+H]^+$ 352.2; found 352.4. ^1H NMR (400MHz, DMSO-d_6) δ 9.48 (s, 1H), 8.05 (s, 1H), 7.76-7.74 (m, 1H), 7.68-7.63 (m, 1H), 7.44-7.39 (m, 1H), 7.18-7.16 (m, 1H), 6.71-6.67 (m, 2H), 6.52-6.49 (m, 1H), 6.11 (s, 1H), 3.81-3.78 (m, 1H), 3.72-3.68 (m, 1H), 2.75-2.70 (m, 1H), 2.61-2.54(m, 1H), one proton overlapped with residual DMSO, 1.79-1.76 (m, 1H), 1.57-1.49 (m, 1H), 1.30-1.27 (m, 1H), 1.04-0.96 (m, 1H).

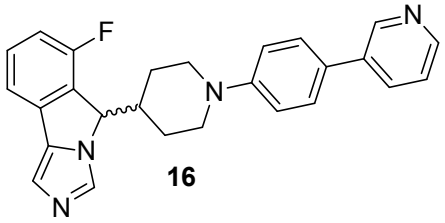
6-fluoro-5-(1-(4-(pyridin-4-yl)phenyl)piperidin-4-yl)-5*H*-imidazo[5,1-*a*]isoindole (15)



15

Prepared in a fashion similar to that used for the synthesis of **18**. LC-MS (ESI) *m*/*z*: calcd for $C_{26}H_{23}FN_4$ $[M+H]^+$ 411.2; found 411.4. ^1H NMR (400MHz, DMSO-d_6) δ 8.53-8.52 (m, 2H), 7.96 (s, 1H), 7.67-7.61 (m, 4H), 7.49-7.45 (m, 2H), 7.22 (s, 2H), 7.17-7.12 (m, 2H), 5.71 (s, 1H), 3.93-3.90 (m, 1H), 3.80-3.77 (m, 1H), 2.81-2.76 (m, 1H), 2.66-2.63 (m, 1H), 2.48-2.41 (m, 1H), 1.80-1.77 (m, 1H), 1.66-1.62 (m, 1H), 1.34-1.30 (m, 1H), 0.89-0.84 (m, 1H).

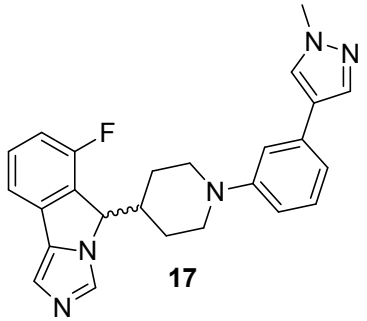
6-fluoro-5-(1-(4-(pyridin-3-yl)phenyl)piperidin-4-yl)-5*H*-imidazo[5,1-*a*]isoindole (16)



16

Prepared in a fashion similar to that used for the synthesis of **18**. LC-MS (ESI) *m*/*z*: calcd for $C_{26}H_{23}FN_4$ $[M+H]^+$ 411.2; found 411.4. ^1H NMR (400MHz, DMSO-d_6) δ 8.82 (s, 2H), 8.47 (s, 1H), 7.98 (s, 2H), 7.67(t, 1H), 7.57 (d, 2H), 7.49 (s, 1H), 7.41 (s, 1H), 7.23 (s, 1H), 7.15 (d, 1H), 6.99 (d, 2H), 5.71 (s, 1H), 3.86 (d, 1H), 3.74 (d, 1H), 2.76 (t, 1H), 2.62 (t, 1H), 2.42-2.34 (m, 1H), 1.79 (d, 1H), 1.70-1.62(m, 1H), 1.32 (d, 1H), 0.91-0.88 (m, 1H).

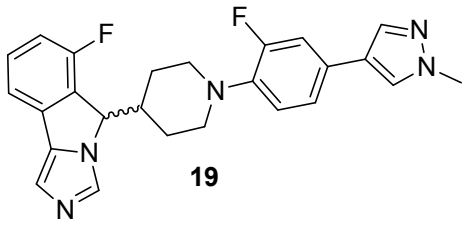
6-fluoro-5-(1-(3-(1-methyl-1*H*-pyrazol-4-yl)phenyl)piperidin-4-yl)-5*H*-imidazo[5, 1-*a*]isoindole (17)



17

Prepared in a fashion similar to that used for the synthesis of **18**. LC-MS (ESI) *m*/*z*: calcd for $C_{25}H_{24}FN_3$ $[M+H]^+$ 414.2; found 414.4. ^1H NMR (400MHz,CD_3OD) δ 8.20-8.18 (m, 2H), 8.02 (s, H), 7.95-7.93 (m, 2H), 7.90-7.88 (m, 1H), 7.77-7.70 (m, 3H), 7.39-7.37 (m, 1H), 6.69 (d, *J* = 2.4 Hz, 1H), 3.99 (s, 3H), one proton overlapped with residual methonal, 3.17-3.14 (m, 1H), 2.83-2.80 (m, 1H), 2.72-2.69 (m, 1H), 2.52-2.47 (m, 1H), 2.09-2.06 (m, 1H), 1.86-1.83 (m, 1H), 1.26-1.23 (m, 1H), 1.02-0.98 (m, 1H).

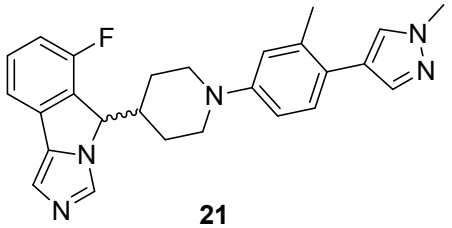
6-fluoro-5-(1-(2-fluoro-4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)piperidin-4-yl)-5*H*-imidazo[5,1-*a*]isoindole (19)



19

Prepared in a fashion similar to that used for the synthesis of **18**. LC-MS (ESI) *m*/*z*: calcd for $C_{25}H_{23}F_2N_5$ $[M+H]^+$ 432.2; found 432.3. ^1H NMR (400MHz, DMSO-d_6) δ 8.06 (s, 2H), 7.80 (s, 1H), 7.44-7.58 (m, 2H), 7.22-7.40 (m, 3H), 7.13-7.22 (m, 1H), 6.91-7.00 (m, 1H), 5.71 (s, 1H), 3.83 (s, 3H), 3.40(d, 1H), 3.25(d, 1H), 2.72 (t, 1H), 2.56 (t, 1H), 2.22-2.33 (m, 2H), 1.19 (d, 1H), 0.90-1.00 (m, 2H)

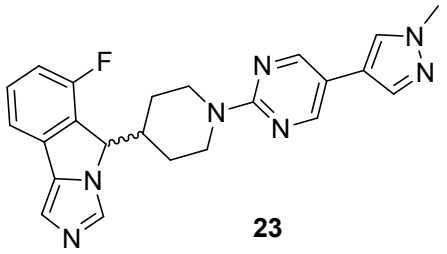
6-fluoro-5-(1-(3-methyl-4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)piperidin-4-yl)-5*H*-imidazo[5,1-*a*]isoindole (21)



21

Prepared in a fashion similar to that used for the synthesis of **18**. LC-MS (ESI) *m*/*z*: calcd for $C_{26}H_{26}FN_5$ $[M+H]^+$ 428.2; found 428.3. ^1H NMR (400MHz, DMSO-d_6) δ 8.39 (s, 1H), 7.79 (s, 1H), 7.57-7.45 (m, 5H), 7.23-7.20 (m, 1H), 7.16-7.14 (m, 1H), 6.76-6.70 (m, 1H), 5.81 (d, *J* = 1.6 Hz, 1H), 3.85 (s, 3H), 3.76-3.73 (m, 1H), 3.63-3.60 (m, 1H), one proton overlapped with residual DMSO, 2.43-2.40 (m, 1H), 2.28 (s, 3H), 1.80-1.77 (m, 1H), 1.66-1.63 (m, 1H), 1.22-1.19 (m, 1H), 0.94-0.90 (m, 1H).

6-fluoro-5-(1-(5-(1-methyl-1*H*-pyrazol-4-yl)pyrimidin-2-yl)piperidin-4-yl)-5*H*-imidazo[5,1-*a*]isoindole (23)



23

Prepared in a fashion similar to that used for the synthesis of **18**. LC-MS (ESI) *m*/*z*: calcd for $C_{23}H_{22}FN_7$ $[M+H]^+$ 416.2; found 416.4. ^{1}H NMR (400MHz, DMSO-d_6) δ 9.47 (s, 1H), 8.55 (s, 2H), 8.05-8.04 (m, 1H), 7.79-7.74 (m, 2H), 7.69-7.65 (m, 1H), 7.44-7.39 (m, 1H), 5.76 (s, 1H), 4.83-4.80 (m, 1H), 4.68-4.65 (m, 1H), 3.85 (s, 3H), 2.91-2.88 (m, 1H), 2.78-2.75 (m, 1H), 2.71-2.65 (m, 1H), 1.86-1.83 (m, 1H), 1.46-1.43 (m, 1H),1.29-1.26 (m, 1H), 0.86-0.82 (m, 1H).

SHHRP-NMR BRUKER *AVANCE II*

Comment	50011406-6059061-01			*Acquisition Time (sec)*	3.9846	*Temperature (degree C)*	20.000	*Frequency (MHz)*	400.13
Nucleus	1H	*Number of Transients*	16	*Original Points Count*	32768	*Owner*	topspin3	*Points Count*	65536
Pulse Sequence	zg30	*Receiver Gain*	203.00	*SW(cyclical) (Hz)*	8223.68	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	2470.9683	*Sweep Width (Hz)*	8223.56	*Date*	06 Mar 2015 15:34:24				

50011406-6059061-01.001.1r.esp

H2

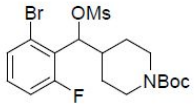
Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50011407-6059073-01			*Acquisition Time (sec)*	3.9846	*Temperature (degree C)*	20.000	*Frequency (MHz)*	400.13
Nucleus	1H	*Number of Transients*	16	*Original Points Count*	32768	*Owner*	topspin3	*Points Count*	65536
Pulse Sequence	zg30	*Receiver Gain*	181.00	*SW(cyclical) (Hz)*	8223.68	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	2470.9683	*Sweep Width (Hz)*	8223.56	*Date*	06 Mar 2015 15:42:56				

50011407-6059073-01.001.1r.esp

H3



Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

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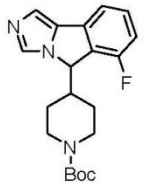
Pulse Sequence	zg30	***Receiver Gain***	203.00	***SW(cyclical) (Hz)***	8223.68	***Solvent***	CHLOROFORM-d		
Spectrum Offset (Hz)	2456.6399	***Sweep Width (Hz)***	8223.56	***Date***	17 Mar 2015 15:57:52				

50011652-6059097-01.001.1r.esp



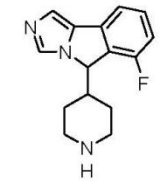
H5

Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50011653-6059103-01			***Acquisition Time (sec)***	3.9846	***Temperature (degree C)***	21.400	***Frequency (MHz)***	400.13
Nucleus	1H	***Number of Transients***	16	***Original Points Count***	32768	***Owner***	topspin3	***Points Count***	65536
Pulse Sequence	zg30	***Receiver Gain***	256.00	***SW(cyclical) (Hz)***	8223.68	***Solvent***	CHLOROFORM-d		
Spectrum Offset (Hz)	2456.6399	***Sweep Width (Hz)***	8223.56	***Date***	17 Mar 2015 16:04:16				

50011653-6059103-01.001.1r.esp



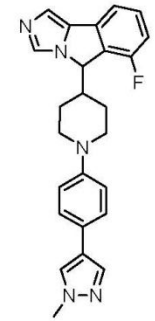
compound 4

Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50013865-6220043-01			***Acquisition Time (sec)***	3.9846	***Temperature (degree C)***	20.000	***Frequency (MHz)***	400.13
Nucleus	1H	***Number of Transients***	64	***Original Points Count***	32768	***Owner***	topspin3	***Points Count***	65536
Pulse Sequence	zg30	***Receiver Gain***	287.00	***SW(cyclical) (Hz)***	8223.68	***Solvent***	DMSO-d6	***Spectrum Offset (Hz)***	2470.9683
Sweep Width (Hz)	8223.56	***Date***	10 Jun 2015 11:35:28						

50013865-6220043-01.001.1r.esp



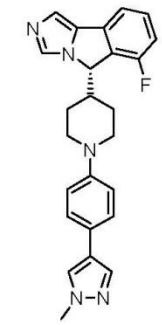
compound 18

Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50013119-6313019-01			***Acquisition Time (sec)***	3.9846	***Temperature (degree C)***	24.000	***Frequency (MHz)***	400.13
Nucleus	1H	***Number of Transients***	32	***Original Points Count***	32768	***Owner***	topspin3	***Points Count***	65536
Pulse Sequence	zg30	***Receiver Gain***	228.00	***SW(cyclical) (Hz)***	8223.68	***Solvent***	DMSO-d6	***Spectrum Offset (Hz)***	2467.1641
Sweep Width (Hz)	8223.56	***Date***	12 May 2015 17:04:00						

50013119-6313019-01.001.1r.esp



compound 25

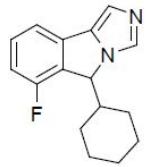
Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50006346-5842085-01			***Acquisition Time (sec)***	3.9846	***Temperature (degree C)***	20.500	***Frequency (MHz)***	400.13
Nucleus	1H	***Number of Transients***	64	***Original Points Count***	32768	***Owner***	topspin3	***Points Count***	65536
Pulse Sequence	zg30	***Receiver Gain***	406.00	***SW(cyclical) (Hz)***	8223.68	***Solvent***	DMSO-d6	***Spectrum Offset (Hz)***	2470.9683
Sweep Width (Hz)	8223.56	***Date***	25 Jul 2014 12:28:48						

50006346-5842085-01.001.1r.esp

compound 1



Chemical Formula: $C_{16}H_{17}FN_2$

Molecular Weight: 256.32

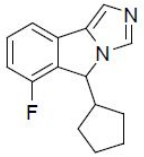
Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50006752-5881001-01			***Acquisition Time (sec)***	3.9846	***Temperature (degree C)***	20.100	***Frequency (MHz)***	400.13
Nucleus	1H	***Number of Transients***	16	***Original Points Count***	32768	***Owner***	topspin3	***Points Count***	65536
Pulse Sequence	zg30	***Receiver Gain***	512.00	***SW(cyclical) (Hz)***	8223.68	***Solvent***	DMSO-d6	***Spectrum Offset (Hz)***	2470.9683
Sweep Width (Hz)	8223.56	***Date***	13 Aug 2014 09:16:48						

50006752-5881001-01.001.1r.esp

compound 2



Chemical Formula: $C_{15}H_{15}FN_2$

Molecular Weight: 242.30

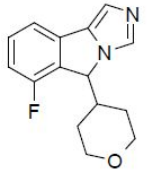
Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50006986-5881022-01			*Acquisition Time (sec)*	3.9846	*Temperature (degree C)*	21.700	*Frequency (MHz)*	400.13
Nucleus	1H	*Number of Transients*	64	*Original Points Count*	32768	*Owner*	topspin3	*Points Count*	65536
Pulse Sequence	zg30	*Receiver Gain*	456.00	*SW(cyclical) (Hz)*	8223.68	*Solvent*	DMSO-d6	*Spectrum Offset (Hz)*	2470.9683
Sweep Width (Hz)	8223.56	*Date*	21 Aug 2014 11:01:20						

50006986-5881022-01.001.1r.esp

compound 3



Chemical Formula: $C_{15}H_{15}FN_2O$

Molecular Weight: 258.30

Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50011653-6059103-01			***Acquisition Time (sec)***	3.9846	***Temperature (degree C)***	21.400	***Frequency (MHz)***	400.13
Nucleus	1H	***Number of Transients***	16	***Original Points Count***	32768	***Owner***	topspin3	***Points Count***	65536
Pulse Sequence	zg30	***Receiver Gain***	256.00	***SW(cyclical) (Hz)***	8223.68	***Solvent***	CHLOROFORM-d		
Spectrum Offset (Hz)	2456.6399	***Sweep Width (Hz)***	8223.56	***Date***	17 Mar 2015 16:04:16				

50011653-6059103-01.001.1r.esp

compound 4

Operater : ***Supervisor :***

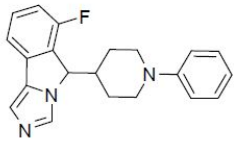
SHHRP-NMR BRUKER *AVANCE II*

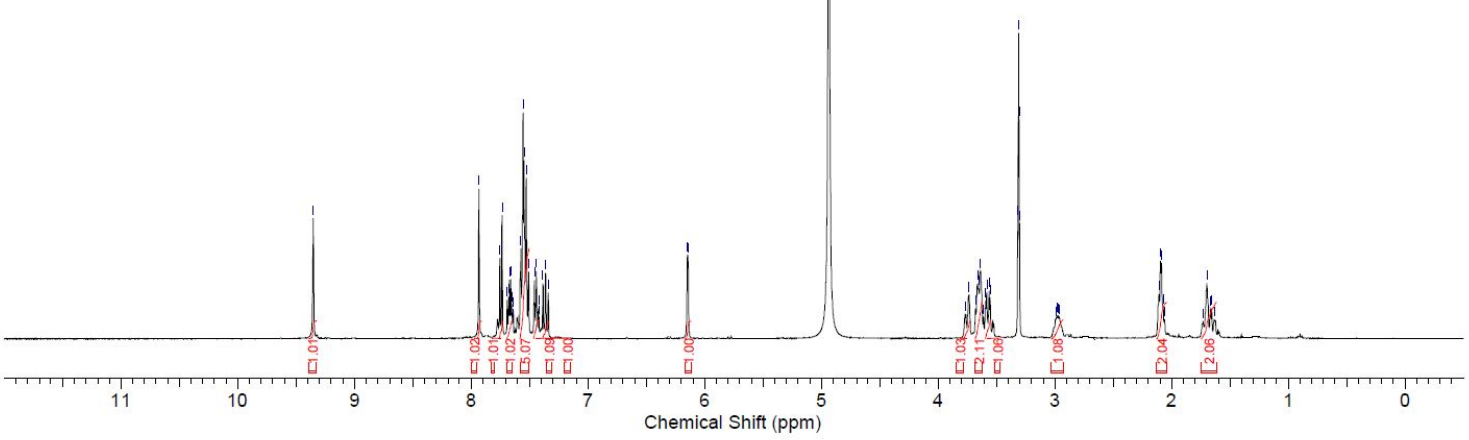
Comment	50006478-5836154-01			*Acquisition Time (sec)*	3.9846	*Temperature (degree C)*	21.700	*Frequency (MHz)*	400.13
Nucleus	1H	*Number of Transients*	32	*Original Points Count*	32768	*Owner*	topspin3	*Points Count*	65536
Pulse Sequence	zg30	*Receiver Gain*	203.00	*SW(cyclical) (Hz)*	8223.68	*Solvent*	METHANOL-d4		
Spectrum Offset (Hz)	2463.3811	*Sweep Width (Hz)*	8223.56	*Date*	30 Jul 2014 13:54:08				

50006478-5836154-01.001.1r.esp

9.3535 | 7.9338 7.7544 7.7356 7.6798 7.6597 7.5754 7.5565 7.5471 7.5290 7.4411 7.3405 | 6.1478 6.1425 | 4.9364 | 3.7644 3.6609 3.6518 3.6415 3.6355 3.5838 3.5631 3.3178 3.3100 3.3059 3.3018 2.9795 2.7468 2.7333 2.1073 2.0951 2.0869 2.0716 2.0609 1.6974 1.6686 1.6582

compound 6





Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50005830-5765094-01			*Acquisition Time (sec)*	3.9846	*Temperature (degree C)*	24.000	*Frequency (MHz)*	400.13
Nucleus	1H	*Number of Transients*	64	*Original Points Count*	32768	*Owner*	topspin3	*Points Count*	65536
Pulse Sequence	zg30	*Receiver Gain*	322.00	*SW(cyclical) (Hz)*	8223.68	*Solvent*	DMSO-d6	*Spectrum Offset (Hz)*	2470.9683
Sweep Width (Hz)	8223.56	*Date*	03 Jul 2014 10:46:24						

50005830-5765094-01.001.1r.esp

compound 7

Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50006432-5830034-01			***Acquisition Time (sec)***	3.9846	***Temperature (degree C)***	21.600	***Frequency (MHz)***	400.13
Nucleus	1H	***Number of Transients***	16	***Original Points Count***	32768	***Owner***	topspin3	***Points Count***	65536
Pulse Sequence	zg30	***Receiver Gain***	406.00	***SW(cyclical) (Hz)***	8223.68	***Solvent***	DMSO-d6	***Spectrum Offset (Hz)***	2470.9683
Sweep Width (Hz)	8223.56	***Date***	15 Jul 2014 10:37:52						

50006432-5830034-01.001.1r.esp

compound 8

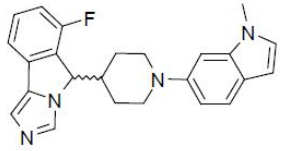
Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50010275-6097055-01			*Acquisition Time (sec)*	3.9846	*Temperature (degree C)*	24.000	*Frequency (MHz)*	400.13
Nucleus	1H	*Number of Transients*	64	*Original Points Count*	32768	*Owner*	topspin3	*Points Count*	65536
Pulse Sequence	zg30	*Receiver Gain*	128.00	*SW(cyclical) (Hz)*	8223.68	*Solvent*	DMSO-d6	*Spectrum Offset (Hz)*	2470.9683
Sweep Width (Hz)	8223.56	*Date*	08 Jan 2015 17:36:00						

50010275-6097055-01.001.1r.esp

compound 10



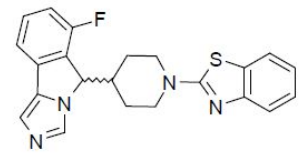
Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50010274-6097034-01			***Acquisition Time (sec)***	3.9846	***Temperature (degree C)***	24.000	***Frequency (MHz)***	400.13
Nucleus	1H	***Number of Transients***	64	***Original Points Count***	32768	***Owner***	topspin3	***Points Count***	65536
Pulse Sequence	zg30	***Receiver Gain***	228.00	***SW(cyclical) (Hz)***	8223.68	***Solvent***	DMSO-d6	***Spectrum Offset (Hz)***	2470.9683
Sweep Width (Hz)	8223.56	***Date***	08 Jan 2015 17:27:28						

50010274-6097034-01.001.1r.esp

compound 11



Operater : ***Supervisor :***

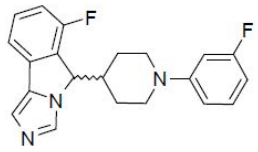
SHHRP-NMR BRUKER *AVANCE II*

Comment	50008195-5970046-01			*Acquisition Time (sec)*	3.9846	*Temperature (degree C)*	24.000	*Frequency (MHz)*	400.13
Nucleus	1H	*Number of Transients*	64	*Original Points Count*	32768	*Owner*	topspin3	*Points Count*	65536
Pulse Sequence	zg30	*Receiver Gain*	322.00	*SW(cyclical) (Hz)*	8223.68	*Solvent*	DMSO-d6	*Spectrum Offset (Hz)*	2466.7876
Sweep Width (Hz)	8223.56	*Date*	30 Oct 2014 10:16:32						

50008195-5970046-01( ).001.1r.esp

9.4809 8.0496 7.7633 7.7441 7.6827 7.6629 7.6516 7.4399 7.4167 7.3941 7.1793 7.1605 6.7070 6.6898 6.6606 6.5273 6.5066 6.4875 6.1134 4.61 3.8121 3.7823 3.7152 3.6854 2.7553 2.7258 2.6963 2.6433 2.6142 2.5815 2.5386 2.5000 1.7903 1.7599 1.5708 1.5435 1.5184 1.4889 1.3011 1.2703 1.0446 1.0138 0.9897 0.9618

compound 12



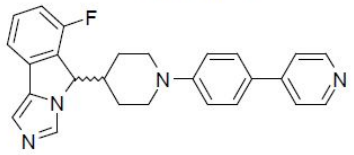
Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50008398-5952070-01			***Acquisition Time (sec)***	3.9846	***Temperature (degree C)***	24.000	***Frequency (MHz)***	400.13
Nucleus	1H	***Number of Transients***	64	***Original Points Count***	32768	***Owner***	topspin3	***Points Count***	65536
Pulse Sequence	zg30	***Receiver Gain***	406.00	***SW(cyclical) (Hz)***	8223.68	***Solvent***	DMSO-d6	***Spectrum Offset (Hz)***	2470.9683
Sweep Width (Hz)	8223.56	***Date***	10 Nov 2014 11:35:28						

50008398-5952070-01.001.1r.esp

compound 15



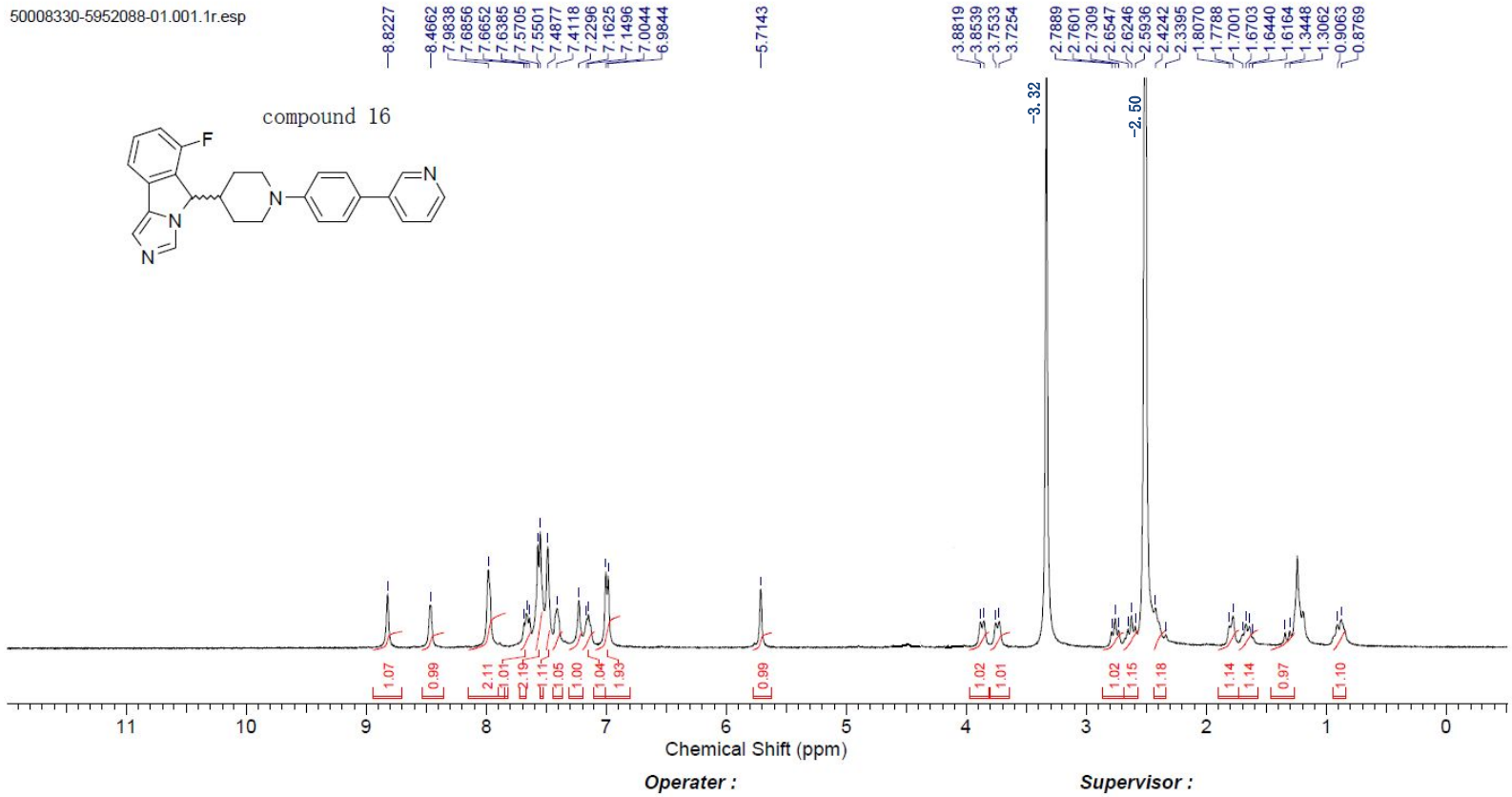
Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50008330-5952088-01			*Acquisition Time (sec)*	3.9846	*Temperature (degree C)*	24.000	*Frequency (MHz)*	400.13
Nucleus	1H	*Number of Transients*	128	*Original Points Count*	32768	*Owner*	topspin3	*Points Count*	65536
Pulse Sequence	zg30	*Receiver Gain*	456.00	*SW(cyclical) (Hz)*	8223.68	*Solvent*	DMSO-d6	*Spectrum Offset (Hz)*	2470.9683
Sweep Width (Hz)	8223.56	*Date*	06 Nov 2014 09:59:28						

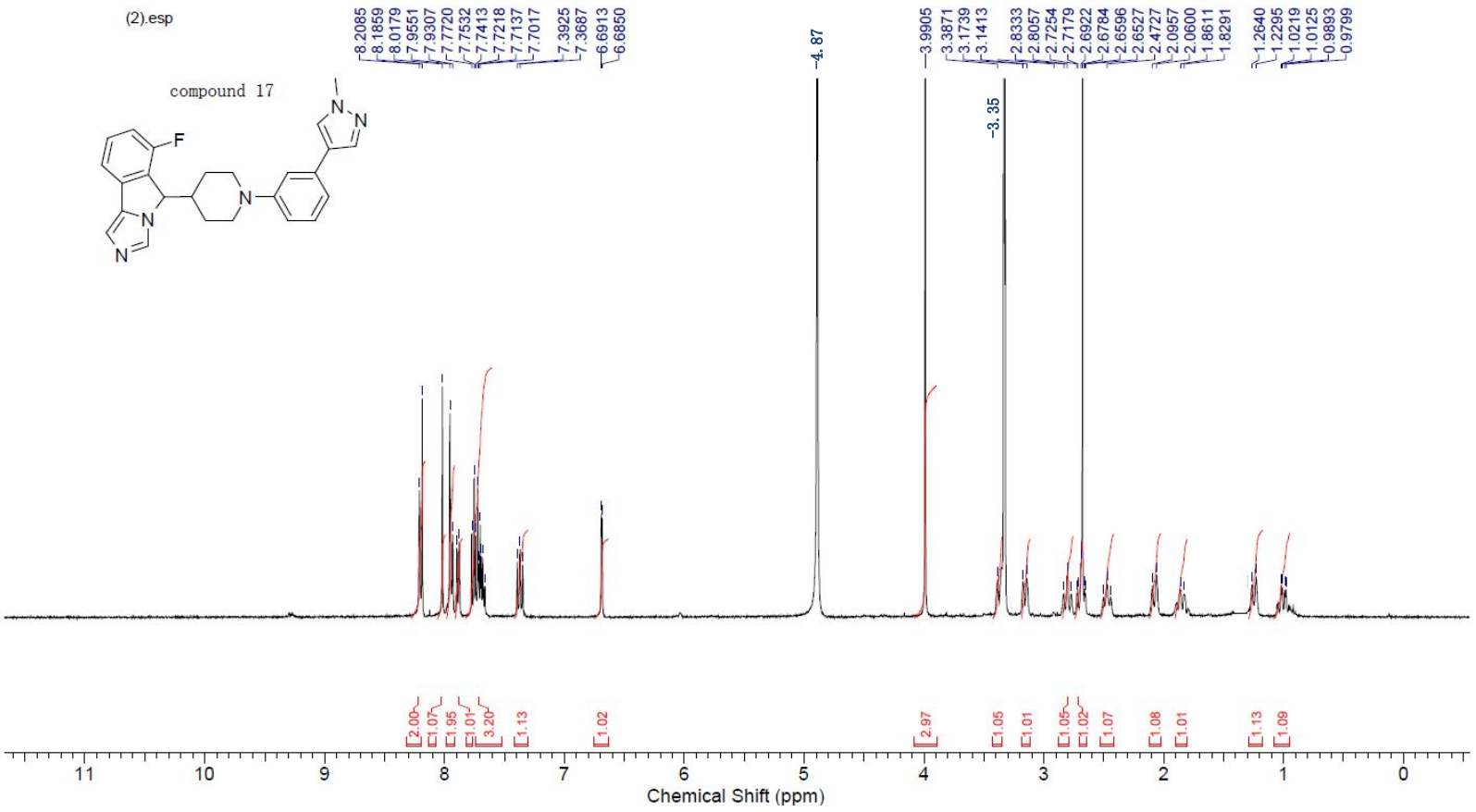
50008330-5952088-01.001.1r.esp

compound 16



Operater : ***Supervisor :***

Comment	50008196-5977022-01			*Acquisition Time (sec)*	3.9846	*Temperature (degree C)*	24.000	*Frequency (MHz)*	400.13
Nucleus	1H	*Number of Transients*	128	*Original Points Count*	32768	*Owner*	topspin3	*Points Count*	32768
Pulse Sequence	zg30	*Receiver Gain*	287.00	*SW(cyclical) (Hz)*	8223.68	*Solvent*	METHANOL-d4		
Spectrum Offset (Hz)	2470.9683	*Sweep Width (Hz)*	8223.43	*Date*	30 Oct 2014 11:24:48				

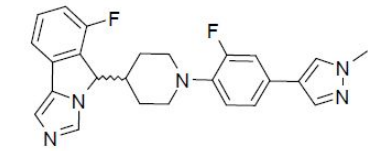


SHHRP-NMR BRUKER *AVANCE II*

Comment	50010411-6097094-02			*Acquisition Time (sec)*	3.9846	*Temperature (degree C)*	24.000	*Frequency (MHz)*	400.13
Nucleus	1H	*Number of Transients*	64	*Original Points Count*	32768	*Owner*	topspin3	*Points Count*	65536
Pulse Sequence	zg30	*Receiver Gain*	287.00	*SW(cyclical) (Hz)*	8223.68	*Solvent*	DMSO-d6	*Spectrum Offset (Hz)*	2470.9683
Sweep Width (Hz)	8223.56	*Date*	15 Jan 2015 10:42:08						

50010411-6097094-02.001.1r.esp

compound 19

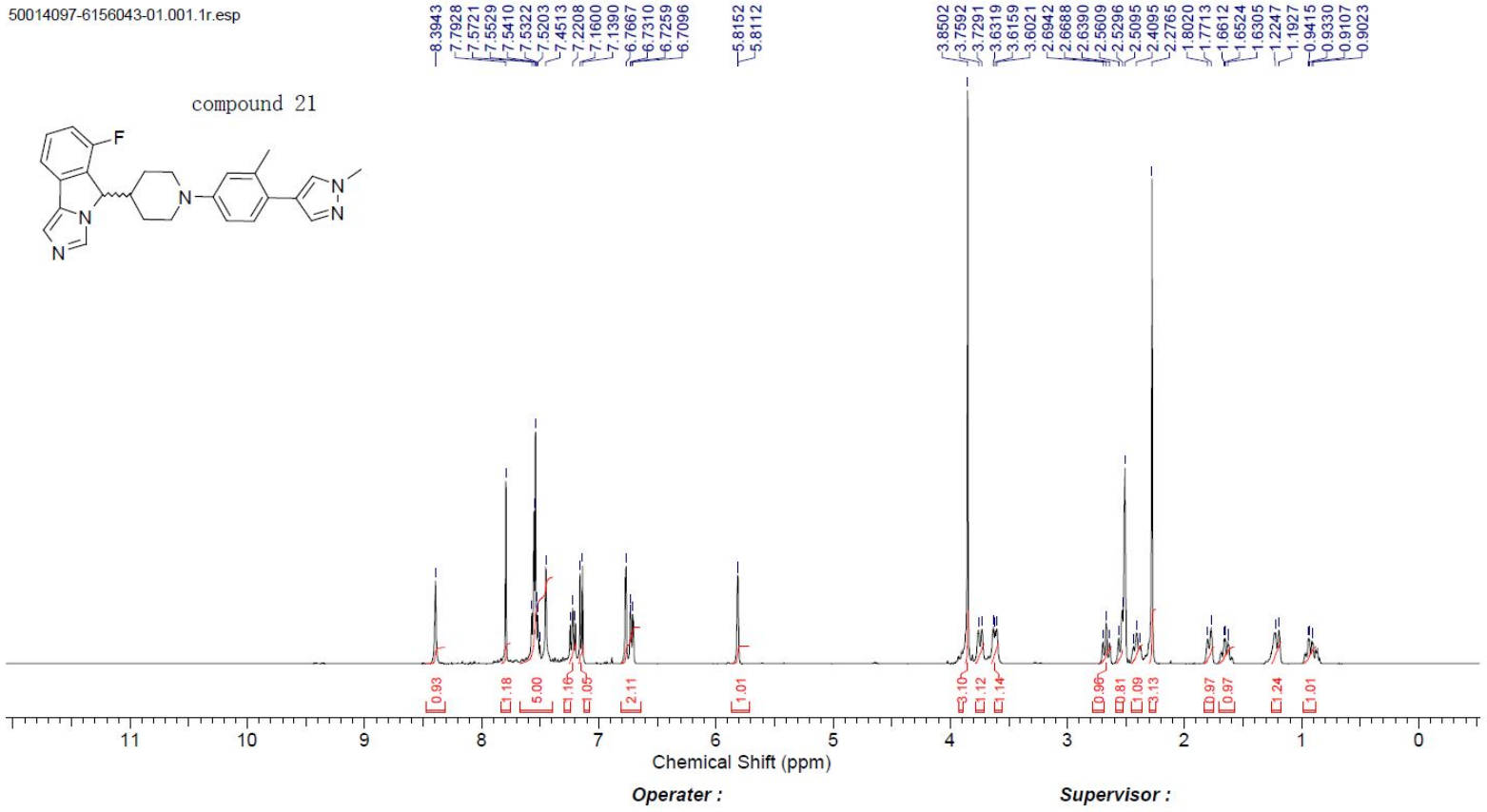


Operater : ***Supervisor :***

SHHRP-NMR BRUKER *AVANCE II*

Comment	50014097-6156043-01			***Acquisition Time (sec)***	3.9846	***Temperature (degree C)***	20.300	***Frequency (MHz)***	400.13
Nucleus	1H	***Number of Transients***	64	***Original Points Count***	32768	***Owner***	topspin3	***Points Count***	65536
Pulse Sequence	zg30	***Receiver Gain***	203.00	***SW(cyclical) (Hz)***	8223.68	***Solvent***	DMSO-d6	***Spectrum Offset (Hz)***	2470.9683
Sweep Width (Hz)	8223.56	***Date***	19 Jun 2015 17:38:08						

50014097-6156043-01.001.1r.esp



Operater : ***Supervisor :***

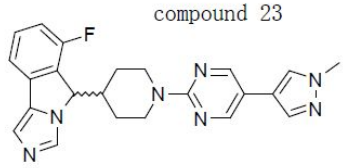
SHHRP-NMR BRUKER *AVANCE II*

Comment	50008678-6018040-01			*Acquisition Time (sec)*	3.9846	*Temperature (degree C)*	24.000	*Frequency (MHz)*	400.13
Nucleus	1H	*Number of Transients*	128	*Original Points Count*	32768	*Owner*	topspin3	*Points Count*	65536
Pulse Sequence	zg30	*Receiver Gain*	512.00	*SW(cyclical) (Hz)*	8223.68	*Solvent*	DMSO-d6	*Spectrum Offset (Hz)*	2470.9683
Sweep Width (Hz)	8223.56	*Date*	19 Nov 2014 16:40:32						

50008678-6018040-01.001.1r.esp

9.4688 8.5499 8.0516 8.0400 7.7947 7.7593 7.7405 7.6865 7.6665 7.6549 7.4422 7.4187 7.3958 5.7635 4.8277 4.7957 4.6856 4.6549 3.98 3.8483 2.9401 2.9097 2.8811 2.8146 2.7842 2.7554 2.7130 2.6823 2.6516 1.8575 1.8265 1.4630 1.4332 1.2874 0.8606 0.8493 0.8267 0.8198

compound 23



3.35

1.18 1.96 2.10 2.00 1.09 1.09 1.04 1.13 1.12 9.17 1.18 1.12 1.12 1.15 1.13 1.04 1.17

11 10 9 8 7 6 5 4 3 2 1 0

Chemical Shift (ppm)

Operater : ***Supervisor :***