Supplementary Material

A Novel Inhibitor of iNOS Dimerization Rescues ER and Mitochondrial Stress in β-cells

Author: Linlin Zhong, Tuan Tran, Tyler D. Baguley, Sang Jun Lee, Adam Henke, Andrew To, Sijia Li, Shan Yu, Fabio A. Grieco, Jason Roland, Peter G Schultz, Decio L Eizirik, Nikki Rogers, Arnab Chatterjee, Matthew S. Tremblay and Weijun Shen

Supplemental Figure S1

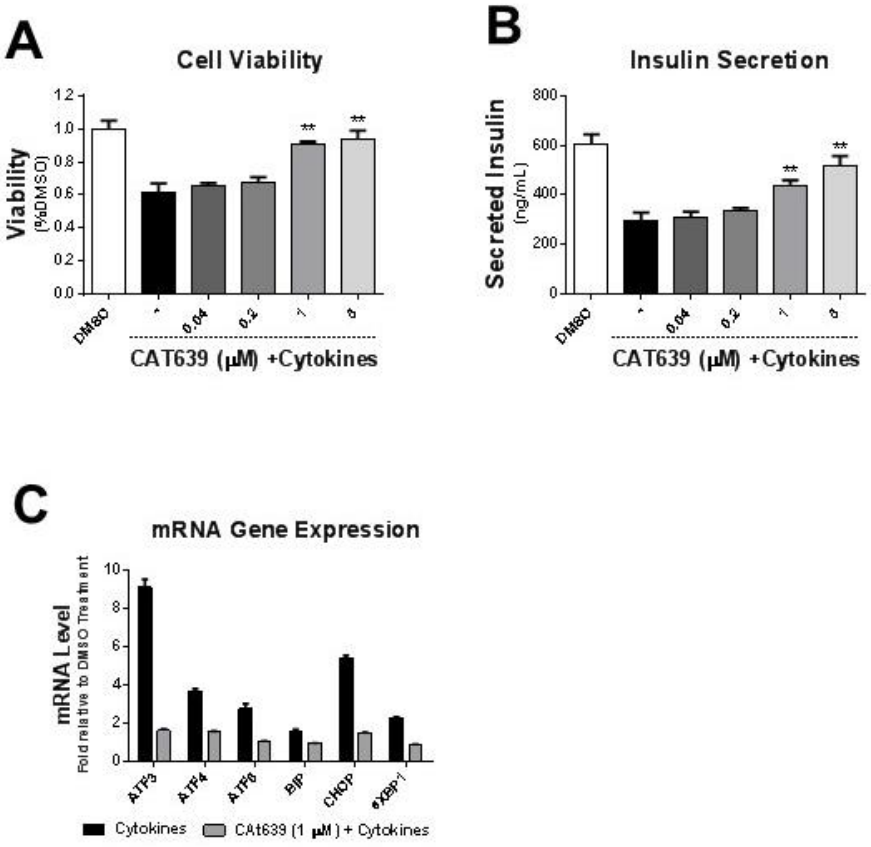


Figure S1. CAT639 had protective effects on INS-1e cells. A) and B) CAT639 rescued cell viability and insulin secretion damaged by cytokines. Cells were pretreated with compounds for 24 h and followed co-treatment with cytokines (10 ng/mL IL-1β, 100 ng/mL IFNγ) for additional 24 h. C) CAT639 recovered cytokine-induced up-regulation of ER stress gene expression. Cells were pretreated with compounds for 8 h and followed co-treatment with cytokines (10 ng/mL IL-1β, 100 ng/mL IFNγ) for additional 8 h.

Supplemental Figure S2

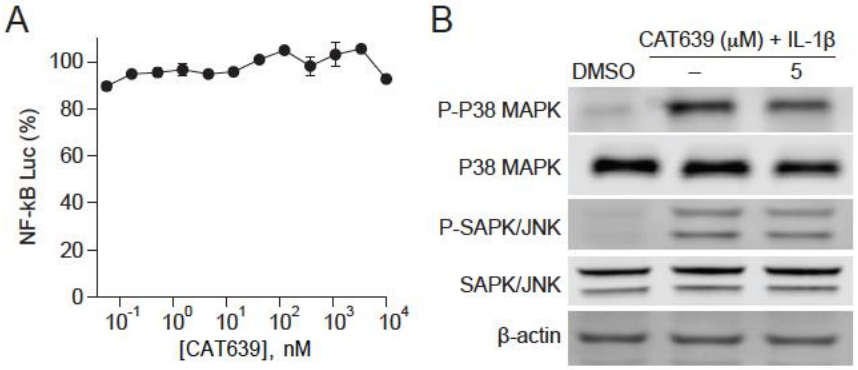


Figure S3. CAT639's effect on NF-kB and MAPK signaling pathway. **A** CAT639 does not block NFκB-activation induced by IL-1β in INS-1e cells (n=3). **B** Cat639 mildly inhibits P38 MAPK phosphorylation induced by IL-1β (n=3).

Supplemental Table S1. IC_{50} of CYP inhibition

IC_{50} (μM)					
	Cyp1A2	Cyp2C9	Cyp2C19	Cyp2D6	Cyp3A4-M
ATV399	32.7	3.15	3.03	7.48	0.082
CAT639	34.3	2.98	6.23	12.2	1.23
CBD504	> 50	3.8	1.19	>50	2.69

Supplemental Figure S3

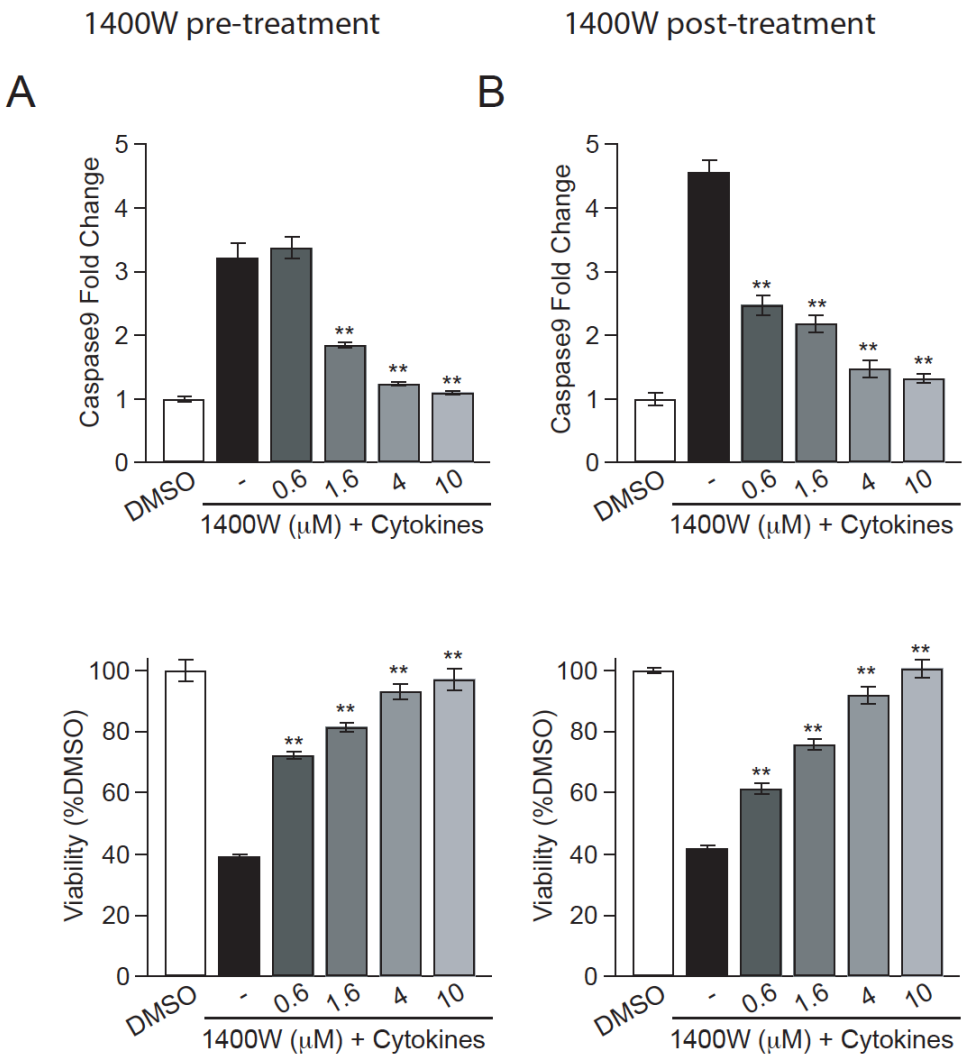
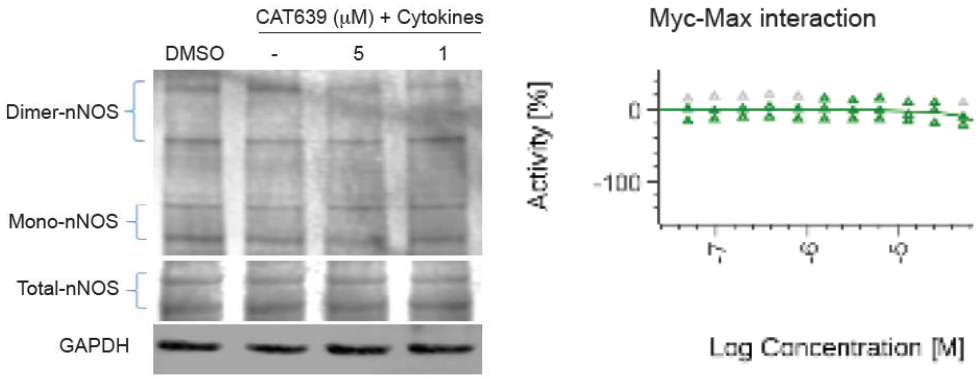


Figure S3. 1400W had both protective effects and therapeutic effects on INS-1e cells. A) and B) Pre-treatment and post-treatment of 1400W decreased caspase 9 activity induced by cytokines (upper panels) and rescued cell viability damaged by cytokines (bottom panels).

Supplemental Figure S4



Supplemental Figure S4 CAT639 destabilized nNOS dimer in SH-SY5Y cells (left panel) but has no effect on cMyc-Max PPI in the protein fragment complimentary assay (PCA). Dimeric proteins were determined by performing low temperature SDS-PAGE gel. Samples lysed in sample buffer with β -ME and boiled for 5 min and GAPDH were used as a loading control (n = 3). PCA was develop to screen for Myc/Max inhibitors and Hek293 cells was overexpressed with cMyc-N-terminal Gaussia Luc (Gluc2) fusion and Max-C-Terminal Gaussia Luc (Gluc1) fusion proteins were develop. When Myc and Max form heterodimer, GLuc is active and luminescence signal is recorded with addition of Luciferase substrate. The results showed that Cat639 did not inhibit the Myc/Max dimerization.

Supplemental Figure S5

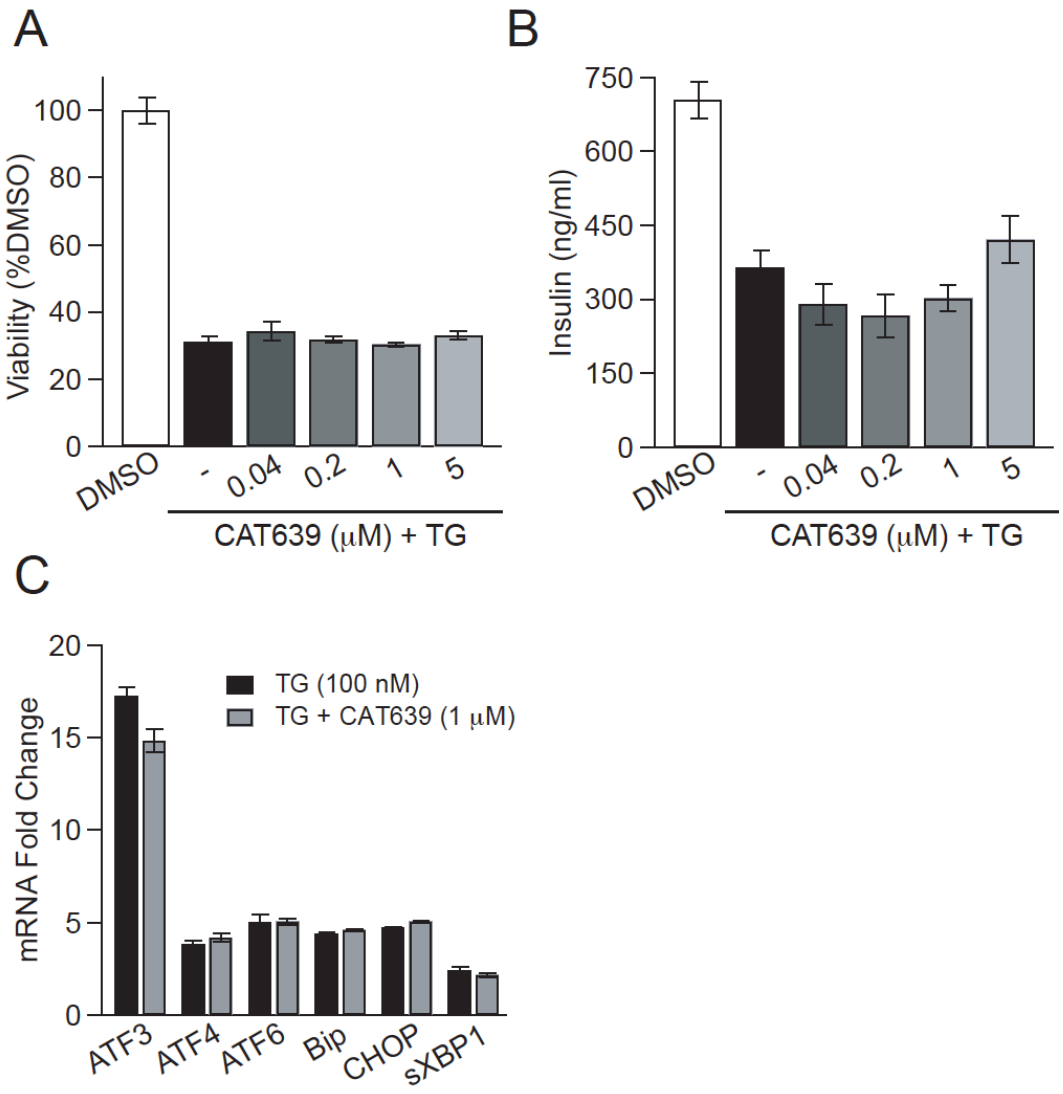
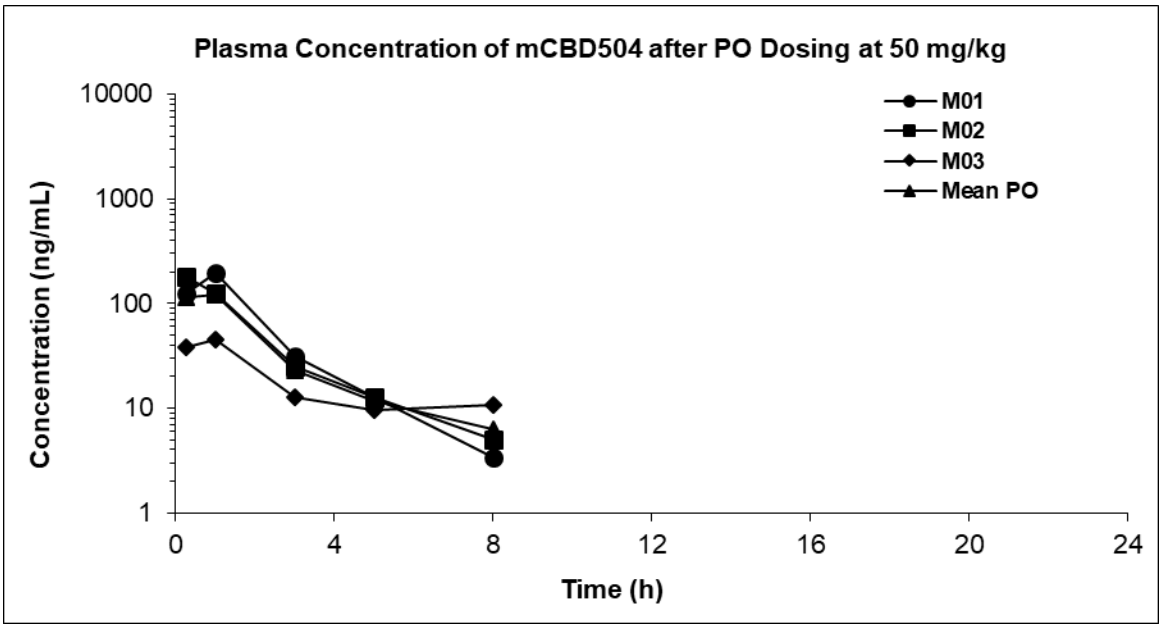


Figure S5. CAT639 had no effect on ER stress induced by thapsigargin. A) CAT639 was not able to rescue cell viability damaged by thapsigargin. B) Thapsigargin-induced decreased insulin secretion was not affected by CAT639. C) CAT639 had no effect on the expression levels of ER stress genes induced by thapsigargin.

Supplementary Figure S6: Mouse PK profile of mCBD504 after PO dosing at 50 mg/kg (n =3)



PK Parameters	Mean PO		SD	CV (%)
Rsq_adj	--	±	--	--
No. points used for $T_{1/2}$	**3.00**	±	--	--
C_{max} (ng/mL)	**141**	±	**82.3**	**58.5**
T_{max} (h)	**0.750**	±	**0.433**	**57.7**
$T_{1/2}$ (h)	**9.85**	±	**13.8**	**141**
T_{last} (h)	**8.00**	±	--	--
$AUC_{0\text{-}last}$ (ng·h/mL)	**278**	±	**123**	**44.2**
$AUC_{0\text{-}inf}$ (ng.h/mL)	**419**	±	**106**	**25.4**
$MRT_{0\text{-}last}$ (h)	**2.17**	±	**0.591**	**27.3**
$MRT_{0\text{-}inf}$ (h)	**12.8**	±	**18.6**	**145**
AUC_{Extra} (%)	**26.9**	±	**40.9**	**152**
$AUMC_{Extra}$ (%)	**43.6**	±	**47.4**	**109**

Supplementary Table S2. Kinase profile of ATV399

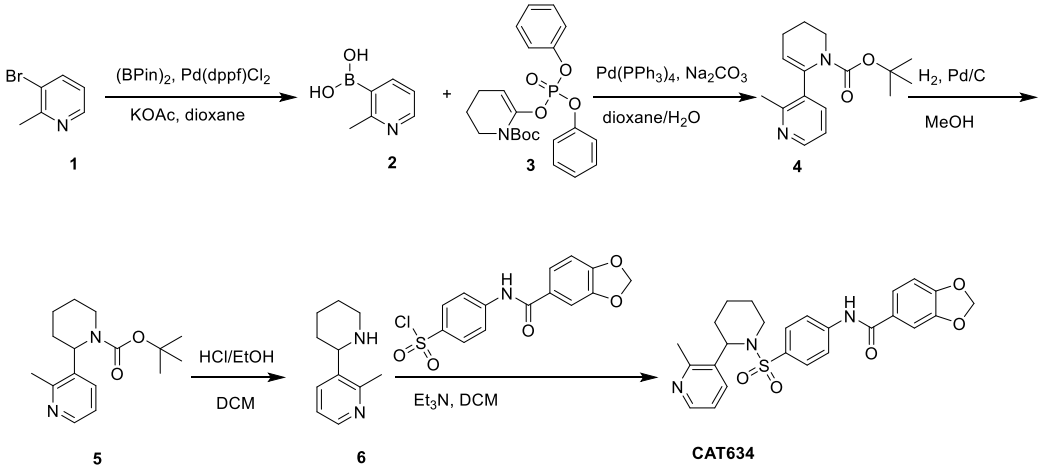
Kinase	Conc. (μM)	sA TV399	Kinase	Conc. (μM)	sA TV399	Kinase	Conc. (μM)	sA TV399
ABL1	10	1	IGF1R	10	-2	NEK2	10	-18
AMP-A1B1G1	10	-5	IKK-BETA	10	2	P38-ALPHA	10	-4
AURORA-A	10	6	IRAK4	10	1	PAK2	10	0
CAMK4	10	-3	ITK	10	5	ALPHA	10	8
CDK1	10	11	JAK1	10	7	PDK1	10	1
CDK2	10	3	JAK2	10	3	ALPHA	10	7
CHEK1	10	0	JNK2	10	-3	PIM-1-KINASE	10	1
CK1-EPSILON	10	6	KDR	10	4	PKC-ALPHA	10	1
CSK	10	20	LCK	10	1	PRKACA	10	2
DAPK1	10	4	MAP2K6	10	1	PRKD2	10	1
DYRK1A	10	4	MAP4K4	10	7	PYK2	10	1
EGFR	10	-3	MAPK1	10	0	ROCK1	10	0
EPH-A2	10	2	MAPKAPK-2	10	1	SGK1	10	-5
FAK	10	-6	MEK1	10	0	SRC	10	4
FGFR1	10	-2	MET	10	1	SYK	10	-5
FLT-3	10	7	MST1	10	3	TYK2	10	-1
GSK-3-BETA	10	1	MST2	10	1			

Supplementary Table S3 Primer sequences

MMP1	CATGTGGGAATCAGAGGTAGAAG	GGAACAGATACGAAGAGGAAACA
Mcl-1	CCACTTTCTTTCTGCCGTGTTA	CCTCCAGCCACCAACTACAT
DP5	GATTGTGCCAGAGCTTCACA	GCCGTGGTGTTACTTGGA
Bcl-2	GTTTCATGGTCCATCCTTG	CATGCGACCTCTGTTTGA
Bcl-XL	GTTTCATGGTCCATCCTTG	TATTGGTGAGTCGGATTGCA
Bid	AGTCCCTCACGTAGGAGAATAG	GACCGTGATTTCCACCAAGA
Noxa	GCTTCTTCTCATCGTGCTCTT	CAGCTCAGGAAGATTGGAGATAAA
ATF4	GGAATGATCTGGAGTGGAAGAC	GCCATCTCCCAGAAAGTGTAATA
ATF3	TCGGATTGAACACTGAGGATTT	GAGATGCGGTCCAGAGTATTTC
ATF6	ACTTCATAATCCTGCCCATTGA	CGAAAGGATCACCTGCTATTAC
BiP	CCCAGATGAGTGTCTCCATTAG	GGATAAGAGAGAGGGAGAGAAGA
Chop	ACTGTCTCAAAGGCGAAAGG	GAGAGTGTTCCAGAAGGAAGTG
sXBP1	CCTGCTTCTGCTGCGGACTCAGCAGACC	AGTGGTGGATTTGGAAGAAGAG
iNOS	CCTCTTGTCTTTGACCCAGTAG	TGGAGCGAGTGGTGGATTG
PDI	AGGACACCACTCTCTGGAATA	CAGGACCTGACCGAAAGATTATAG

Supplemental Experimental Procedures

Synthesis of Compounds



Scheme S1

Synthesis of 2-methylpyridin-3-ylboronic acid (2).

To a solution of 3-bromo-2-methylpyridine (860 mg, 5.0 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (1.5 g, 6.0 mmol) in dioxane (30 mL) were added Pd(dppf)Cl_2 (100 mg, 0.13 mmol) and KOAc (1.47 g, 15 mmol). The mixture was refluxed overnight. The mixture was cooled and diluted with water (30 mL), extracted with EtOAc (3 × 20 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column (DCM/MeOH = 20/1) to give title product (800 mg, crude) as a brown oil.MS (ESI): mass calcd. for $C_6H_8BNO_2$ 137.06, m/z found 138.1 $[M+H]^+$.

Synthesis of *tert*-butyl 6-(2-methylpyridin-3-yl)-3,4-dihydropyridine-1(2H)-carboxylate (4)

To a solution of 2-methylpyridin-3-ylboronic acid (500 mg, crude), *tert*-butyl 6-(diphenoxyphosphoryloxy)-3,4-dihydropyridine-1(2*H*)-carboxylate (862 mg, 2.0 mmol) in dioxane (30 mL) were added a solution of Na_2CO_3 (420 mg, 4 mmol) in water (10 mL) and $Pd(PPh_3)_4$ (100 mg, 0.09 mmol). The mixture was refluxed for 15 h under Ar. The mixture was cooled, diluted with water (30 mL), and extracted with EtOAc (3 ×20 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column (PE/EtOAc = 3/1) to give title product (250 mg, 45%) as a yellow oil. MS (ESI): mass calcd. for $C_{16}H_{22}N_2O_2$ 274.17, m/z found 275.2 $[M+H]^+$.

Synthesis of *tert*-butyl 2-(2-methylpyridin-3-yl)piperidine-1-carboxylate (5).

To a solution of *tert*-butyl 6-(2-methylpyridin-3-yl)-3,4-dihydropyridine-1(2*H*)-carboxylate (250 mg, 0.9 mmol) in methanol (30 mL) was added Pd/C (10% w/w, 50 mg). The mixture was stirred for 48 h under H_2 (1 atm). The mixture was filtered and concentrated. The residue was purified by silica gel column (PE/EtOAc = 3/1) to give title compound (150 mg, 60%) as a yellow oil. MS (ESI): mass calcd. for $C_{16}H_{24}N_2O_2$ 276.18, m/z found 277.2 $[M+H]^+$.

Synthesis of 2-methyl-3-(piperidin-2-yl)pyridine (6).

To a solution of tert-butyl 2-(2-methylpyridin-3-yl)piperidine-1-carboxylate (150 mg, 0.54 mmol) in DCM (10 mL) was added HCl in dioxane (2 M, 20 mL). The mixture was stirred overnight at r. t. The mixture was concentrated to give HCl salt of the title compound (150 mg, crude) as a yellow solid. MS (ESI): mass calcd. for $C_{16}H_{24}N_2O_2$ 276.18, m/z found 277.2 $[M+H]^+$.

Synthesis of *N*-(4-(2-(2-methylpyridin-3-yl)piperidin-1-ylsulfonyl)phenyl)benzo[*d*][1,3]dioxole-5-carboxamide (CAT634).

To a solution of 2-methyl-3-(piperidin-2-yl)pyridine HCl salt (150 mg, crude) and Et_3N (300 mg, 3.0 mmol) in DCM (20 mL) was added a solution of 4-(benzo[*d*][1,3]dioxole-5-carboxamido)benzene-1-sulfonyl chloride (250 mg, 0.74 mmol) in DCM (10 mL) dropwise. The mixture was stirred for 15 h. The mixture was diluted with water (30 mL), extracted with DCM (3 × 20 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column (DCM/MeOH = 20/1) to give title product (43.4 mg, 19% over 2 steps) as a white solid. MS (ESI): mass calcd. for $C_{25}H_{25}N_3O_3S$ 479.15, m/z found 480.1$[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, *J* = 4.1 Hz, 1H), 8.12 (s, 1H), 7.66 (d, *J* = 8.7 Hz, 2H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.52-7.43 (m, 3H), 7.40 (d, *J* = 1.4 Hz, 1H), 7.04-7.00 (m, 1H), 6.89 (d, *J* = 8.1 Hz, 1H), 6.08 (s, 2H), 4.93 (t, *J* = 5.6 Hz, 1H), 3.73-3.56 (m, 2H), 2.56 (s, 3H), 1.86 (q, *J* = 5.8 Hz, 2H), 1.81-1.72 (m, 1H), 1.71-1.44 (m, 3H). HPLC 98% (214 nm), 99% (254 nm).

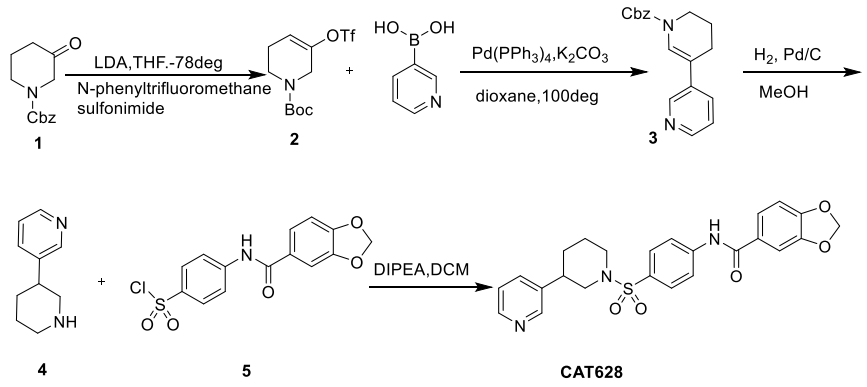
A similar method was used to synthesize **CAT639** and **CAT636**.

CAT639:

White solid, 17.5 mg. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 8.24 (s, 1H), 7.90 (d, *J* = 7.9 Hz, 1H), 7.86-7.78 (m, 4H), 7.51 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.44 (d, *J* = 1.6 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.08 (s, 2H), 5.19 (s, 1H), 3.83 (d, *J* = 13.6 Hz, 1H), 2.97 (s, 1H), 2.68 (s, 3H), 2.13 (d, *J* = 13.6 Hz, 1H), 1.81-1.68 (m, 1H), 1.61-1.45 (m, 2H), 1.42-1.33 (m, 2H). MS (ESI): mass calcd. for $C_{25}H_{25}N_3O_3S$ 479.15, m/z found 480.1 [M+H]+. HPLC 99% (214 nm), 99% (254 nm).

CAT636:

White solid, 18.1 mg. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, *J* = 10.6 Hz, 2H), 8.20 (s, 1H), 7.82 (s, 4H), 7.66 (s, 1H), 7.48 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.42 (d, *J* = 1.5 Hz, 1H), 6.90 (d, *J* = 8.1 Hz, 1H), 6.08 (s, 2H), 5.23 (s, 1H), 3.86 (d, *J* = 13.9 Hz, 1H), 3.04-2.92 (m, 1H), 2.38 (s, 3H), 2.16 (d, *J* = 13.6 Hz, 1H), 1.77-1.65 (m, 1H),1.60-1.30 (m, 4H). MS (ESI): mass calcd. for $C_{25}H_{25}N_3O_3S$ 479.15, m/z found 480.1 [M+H]+. HPLC 98% (214 nm), 98% (254 nm).



Scheme S2

Synthesis of Tert-butyl 3-(trifluoromethylsulfonyloxy)-5,6-dihydropyridine-1(2H)-carboxylate (2).

A solution of compound **1** (1.16g, 5.0 mmol) in THF (5 mL) was added LDA (5.5mmol) in THF (5 mL) at -78°C. After 1h, a solution of N-phenyltrifuoromethanesulfonimide (2.5 g, 6 mmol) in THF (15 mL) was added at -78°C. The mixture was warmed to 0°C and stirred for 3h. The mixture was quenched by water (20 mL), extracted with EtOAc (3 × 20 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column (PE/EA = 1/1) to give title product (580 mg, 35%) as a brown oil.MS (ESI): mass calcd. for $C_{11}H_{16}F_3N_5O$ 331.31, m/z found 332.2 $[M+H]^+$.

Synthesis of benzyl 5-(pyridin-3-yl)-3,4-dihydropyridine-1(2H)-carboxylate (3)

To a solution of pyridin-3-ylboronic acid (215 mg, 1.8 mmol), tert-butyl 3-(trifluoromethylsulfonyloxy)

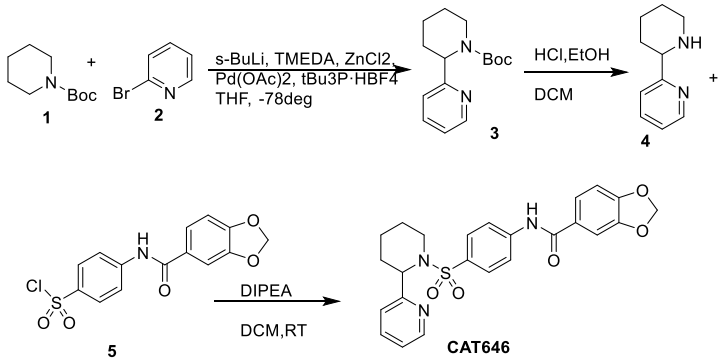
-5,6-dihydropyridine-1(2H)-carboxylate (580 mg, 1.8 mmol) in dioxane (30 mL) were added a solution of Na_2CO_3 (420 mg, 4 mmol) in water (10 mL) and $Pd(PPh_3)_4$ (100 mg, 0.09 mmol). The mixture was refluxed for 15 h under Ar. The mixture was cooled, diluted with water (30 mL), and extracted with EtOAc (3 ×20 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column (PE/EtOAc = 3/1) to give title product (150 mg, 29%) as a yellow solid. MS (ESI): mass calcd. for $C_{18}H_{18}N_2O_2$ 294.35, m/z found 295.2 $[M+H]^+$.

Synthesis of 3-(piperidin-3-yl)pyridine (4).

To a solution of benzyl 5-(pyridin-3-yl)-3,4-dihydropyridine-1(2H)-carboxylate (150 mg, 0.5 mmol) in methanol (20 mL) was added Pd/C (10% w/w, 50 mg). The mixture was stirred for 20 h under H_2 (1 atm). The mixture was filtered and concentrated to give title compound (70 mg, 86%) as a yellow solid. MS (ESI): mass calcd. for $C_{10}H_{14}N_2$162.23, m/z found 163.2 $[M+H]^+$.

Synthesis of N-(4-(3-(pyridin-3-yl)piperidin-1-ylsulfonyl)phenyl)benzo[d][1,3]dioxole-5-carboxamide (CAT628).

To a solution of 3-(piperidin-3-yl)pyridine (70 mg, 0.4mmol) and DIPEA (0.1 mL) in DCM (20 mL) was added a solution of 4-(benzo[*d*][1,3]dioxole-5-carboxamido)benzene-1-sulfonyl chloride (130 mg, 0.4 mmol) in DCM (10 mL) dropwise. The mixture was stirred for 15 h. The mixture was diluted with water (30 mL), extracted with DCM (3 × 20 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column (DCM/MeOH = 20/1) to give title product (23 mg, 12%) as a white solid. MS (ESI): mass calcd. for $C_{24}H_{23}N_3O_5S$ 465.53, m/z found 466.1[M+H]+. ^{1}H NMR (400 MHz, CD_3OD) δ 8.48 (s, 1H), 8.43 (d, *J* = 4.8 Hz, 1H), 7.97 (d, *J* = 8.7 Hz, 2H), 7.79-7.77 (m, 3H), 7.58 (d, *J* = 8.2 Hz, 1H), 7.46 (s, 1H), 7.40 (dd, *J* = 7.6, 5.1 Hz, 1H), 6.97 (d, *J* = 8.1 Hz, 1H), 6.09 (s, 2H), 3.79-3.76 (m, 2H), 2.99-2.94 (m, 1H), 2.53-2.47 (m, 2H), 1.95-1.88 (m, 2H), 1.79-1.73 (m, 1H), 1.63-1.54 (m, 1H). HPLC 97% (214 nm), 96% (254 nm).



Scheme S3

Synthesis of Tert-butyl 2-(pyridin-2-yl)piperidine-1-carboxylate (3).

Sec-BuLi (5 mL, 5.7 mmol) was added to the compound **1** (1g, 5.4 mmol) and TMEDA (0.9 mL, 5.7mmol) in THF (5 mL) at -78°C. After 3h, a solution of $ZnCl_2$ (6 mL, 6 mmol, 1M in Et_2O) in dry THF (5 mL) was added over 10 min. After 30 min, the mixture was warmed to 20 °C. After 30 min, a solution of compound **2** (1.2 g, 7.5 mmol), $Pd(OAc)_2$ (60 mg) and t-Bu_3PHBF_4 (60 mg) was added in one portion. After 15h at 70°C, NH_4OH (10 mL) was added dropwise, followed by Et_2O (50 mL).The organic layer was separated, washed with brine, dried Na_2SO_4 and evaporated. The residue was purified by silica gel column (PE/EtOAc = 3/1) to give title product (150 mg, 10%) as a yellow soid .MS (ESI): mass calcd. for $C_{15}H_{22}N_2O_2$ 262.17, m/z found 263.1 $[M+H]^+$.

Synthesis of 2-(Piperidin-2-yl)pyridine (4).

To a solution of tert-butyl 2-(pyridin-2-yl)piperidine-1-carboxylate (150 mg, 0.57 mmol) in DCM (10 mL) was added HCl in dioxane (2 M, 10 mL). The mixture was stirred 5h at r. t. The mixture was concentrated to give HCl salt of the title compound (100 mg, crude) as a white solid. MS (ESI): mass calcd. for $C_{10}H_{14}N_2$ 162.12, m/z found 163.2 $[M+H]^+$.

Synthesis of N-(4-(2-(pyridin-2-yl)piperidin-1-ylsulfonyl)phenyl)benzo[d][1,3]dioxole-5-carboxamide (CAT646).

To a solution of 2-(piperidin-2-yl)pyridine HCl salt (100 mg, crude) and DIPEA (0.2 mL) in DCM (20 mL) was added a solution of 4-(benzo[*d*][1,3]dioxole-5-carboxamido)benzene-1-sulfonyl chloride (200 mg, 0.59 mmol) in DCM (10 mL) dropwise. The mixture was stirred for 15 h. The mixture was diluted with water (30 mL), extracted with DCM (3 × 20 mL). The

combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column (DCM/MeOH = 20/1) to give title product (41 mg, 15% over 2 steps) as a white solid. MS (ESI): mass calcd. for $C_{24}H_{23}N_3O_5S$465.53, m/z found 466.1[M+H]+. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 4.4 Hz, 1H), 8.12 (s, 1H), 7.81 (brs, 5H), 7.63 (s, 1H), 7.46 (d, *J* = 8.1 Hz, 1H), 7.41 (s, 1H), 7.26 (brs, 1H), 6.89 (d, *J* = 8.1 Hz, 1H), 6.07 (s, 2H), 5.24 (s, 1H), 3.79-3.76 (m, 1H), 3.26-3.20 (m, 1H), 2.53-2.49 (m, 1H), 1.66 (brs, 1H), 1.49-1.47 (m, 2H), 1.41-1.28 (m, 2H). HPLC: 99% (214 nm), 99% (254 nm).

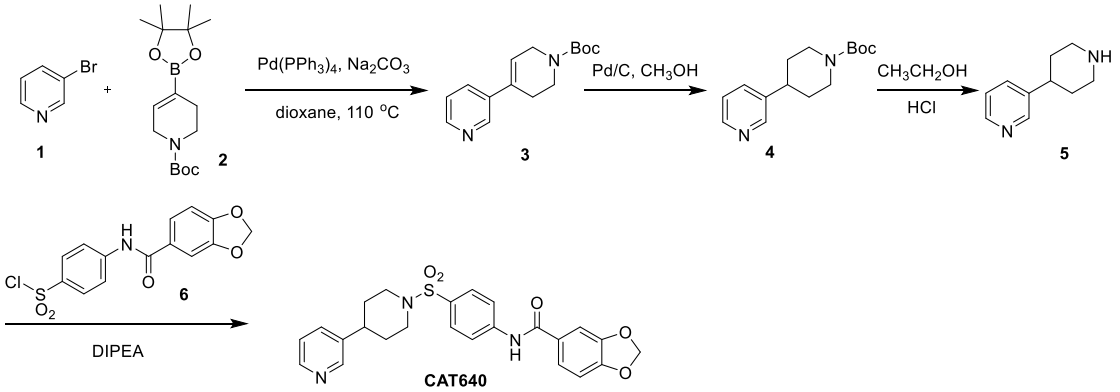
The similar method was used to synthesize **CAT648, CAT635**

CAT648:

White solid 15 mg. MS (ESI): mass calcd. for $C_{24}H_{23}N_3O_5S$ 465.53, m/z found 466.1 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD) δ 8.75 (d, *J* = 5.5 Hz, 2H), 8.00 (d, *J* = 8.9 Hz, 2H), 7.95-7.92 (m, 4H), 7.60 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.47 (d, *J* = 1.7 Hz, 1H), 6.98 (d, *J* = 8.1 Hz, 1H), 6.10 (s, 2H), 5.39 (s, 1H), 3.91-3.87 (m, 1H), 3.18-3.03 (m, 1H), 2.30-2.27 (m, 1H), 1.79 (brs, 1H), 1.62-1.45 (m, 2H), 1.35-1.27 (m, 2H). HPLC: 98% (214 nm), 98% (254 nm).

CAT635:

White solid 22 mg. MS (ESI): mass calcd. for $C_{25}H_{24}N_2O_5S$464.54, m/z found 465.1[M+H]+. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.86 (d, *J* = 8.8 Hz, 2H), 7.78 (d, *J* = 8.8 Hz, 2H), 7.43 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.38 (d, *J* = 1.6 Hz, 1H), 7.37-7.30 (m, 4H), 7.25-7.23 (m, 1H), 6.90 (d, *J* = 8.1 Hz, 1H), 6.08 (s, 2H), 5.28-5.27 (m, 1H), 3.86-3.83 (m, 1H), 3.07-3.00 (m, 1H), 2.24-2.21 (m, 1H), 1.74-1.61 (m, 1H), 1.55-1.23 (m, 4H). HPLC: 97% (214 nm), 96% (254 nm).



Scheme S4

Synthesis of tert-butyl 5',6'-dihydro-[3,4'-bipyridine]-1'(2'H)-carboxylate (3)

The solution of 3-bromopyridine (500 mg, 3.2 mmol), tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2H)-carboxylate (1.2 g, 3.84 mmol), Na_2CO_3 (678 mg, 6.4 mmol), $Pd(PPh_3)_4$ (740 mg, 0.64 mmol) and dioxane (50 mL) was stirred at 110 ^oC under atmosphere of N_2 for overnight. After reaction was completed, the solution was poured into water (100 mL) and extracted with EtOAc (3*50 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by FCC (PE/EtOAC=20/1) to give the product (220 mg, 26%) as yellow oil. MS (ESI): mass calcd. for $C_{15}H_{20}N_2O_2$ 260.15, m/z found 260.9 $[M+H]^+$.

Synthesis of tert-butyl 4-(pyridin-3-yl)piperidine-1-carboxylate (4)

The solution of tert-butyl 5',6'-dihydro-[3,4'-bipyridine]-1'(2'H)-carboxylate (220 mg, 0.85 mmol), Pd/C (30 mg) in CH_3OH (20 mL) was stirred at rt for 2 h under atmosphere of H_2. After reaction, the reaction mixture was filtered and the filtrate was concentrated to give the crude product (190 mg, 85%) as pale yellow oil using for the next step without any purification. MS (ESI): mass calcd. for $C_{15}H_{22}N_2O_2$ 262.17, m/z found 263.0 $[M+H]^+$.

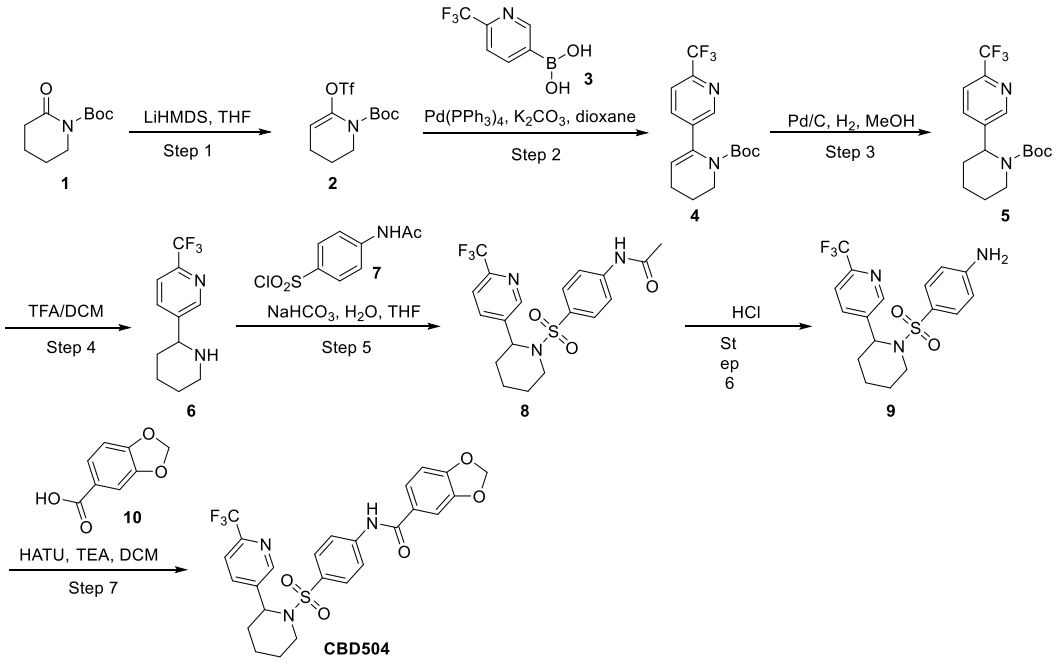
Synthesis of 3-(piperidin-4-yl)pyridine (5)

The intermediate 4 was dissolved in dioxane (5 mL) and added CH_3CH_2OH/HCl solution (0.5 mL). The solution was stirred at rt for 6 h. After reaction, the solution was concentrated under reduced pressure to give the product (50 mg, 42%) as yellow oil using for the next step without any purification. MS (ESI): mass calcd. for $C_{10}H_{14}N_2$ 162.12, m/z found 163.0 $[M+H]^+$.

CAT640:

To a solution of 3-(piperidin-4-yl)pyridine (50 mg, 0.31 mmol) and DIPEA (0.1 mL) in DCM (20 mL) was added a solution of 4-(benzo[d][1,3]dioxole-5-carboxamido)benzene-1-sulfonyl chloride (104 mg, 0.31 mmol) in DCM (10 mL) dropwise. The mixture was stirred for 15 h at rt. The mixture was diluted with water (30 mL), extracted with DCM (3 × 20 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by Prep-HPLC to give the product (7 mg, 4%) as yellow solid. MS (ESI): mass calcd. for $C_{24}H_{23}N_3O_5S$ 465.14, m/z found 465.8 $[M+H]^+$. ^1H NMR (400 MHz, DMSO) δ 10.49 (s, 1H), 8.44 (d, *J* = 1.9 Hz, 1H), 8.41 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.06 (d, *J* = 8.8 Hz, 2H), 7.77 (d,

J = 8.8 Hz, 2H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.62 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.55 (d, *J* = 1.6 Hz, 1H), 7.31 (dd, *J* = 7.8, 4.8 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 1H), 6.16 (s, 2H), 3.79-3.76 (m, 2H), 2.59-2.55 (m, 1H), 2.35-2.27 (m, 2H), 1.85-1.82 (m, 2H), 1.80-1.66 (m, 2H). HPLC: 100% (214 nm), 100% (254 nm).



Scheme S5

Synthesis of tert-butyl 6-(((trifluoromethyl)sulfonyl)oxy)-3,4-dihydropyridine-1(2H)-carboxylate (2)

To a solution of LiHMDS in toluene (87.5 mL, 43.8 mmol) was added dropwise a solution of lactams (7 g, 35 mmol) in THF (80 mL) at -78 °C under nitrogen atmosphere. After addition, the resulting mixture was stirred at the same temperature for 1.5 h. Afterwards a solution of $PhNTf_2$ (15.6 g, 43.8 mmol) in THF (60 mL) was added dropwise, and after 1 h the reaction mixture was allowed to warm to room temperature. After 1 h, a 10% NaOH solution (500 mL) was added. The mixture was extracted with Et_2O (3 x 150 mL) and the combined organic layers were washed with brine (2 x 150 mL), and dried for 30 min over anhydrous K_2CO_3. After filtration and evaporation of the solvent, the crude oil was purified by column chromatography (SiO_2, EtOAc-petroleum ether, 1:5, 1% Et_3N, R_f 0.38) to give the desired product (10.2 g, 90%) as a yellow oil. ^1H NMR (400 MHz, d_6-DMSO, ppm) δ 5.53 (t, *J* = 4 Hz, 1H), 3.50-3.48 (m, 2H),

2.26-2.21 (m, 2H), 1.67-1.64 (m, 2H), 1.43 (s, 9H). MS (ESI): mass calcd. for $C_{11}H_{16}F_3NO_5S$ 331.07, m/z found 332.1$[M+H]^+$.

Synthesis of tert-butyl 6'-(trifluoromethyl)-5,6-dihydro-[2,3'-bipyridine]-1(4H)-carboxylate (4)

A mixture of tert-butyl 6-(((trifluoromethyl)sulfonyl)oxy)-3,4-dihydropyridine-1(2H)-carboxylate (2.25 g, 6.8 mmol), $Pd(PPh_3)_4$ (809 mg, 0.7 mmol), K_2CO_3 (1.88 g, 13.6 mmol) and (6-(trifluoromethyl)pyridin-3-yl)boronic acid (1.34 g, 7 mmol) in dioxane (25 mL) and H_2O (2 mL) was stirred at 110°C under nitrogen atmosphere overnight. The reaction mixture was concentrated to give a residue which was purified by column chromatography (SiO_2, EtOAc-petroleum ether, 1:3, R_f 0.2) to give the desired product (1.72 g, 77%) as a white solid. MS (ESI): mass calcd. for $C_{16}H_{19}F_3N_2O_2$, 328.14, m/z found 329.1$[M+H]^+$.

Synthesis of tert-butyl 2-(6-(trifluoromethyl)pyridin-3-yl)piperidine-1-carboxylate (5)

A mixture of tert-butyl 6'-(trifluoromethyl)-5,6-dihydro-[2,3'-bipyridine]-1(4H)-carboxylate (1.72 g, 5.2 mmol) and Pd/C (200 mg) in MeOH (150 mL) was stirred under H_2 at room temperature overnight. The reaction mixture was filtered and the filterate was concentrated to give crude product (1.43 g, 83%), which was used directly in the next step without further purification. MS (ESI): mass calcd. for $C_{16}H_{21}F_3N_2O_2$, 330.16, m/z found 331.2$[M+H]^+$.

Synthesis of 5-(piperidin-2-yl)-2-(trifluoromethyl)pyridine (6)

A solution of tert-butyl 2-(6-(trifluoromethyl)pyridin-3-yl)piperidine-1-carboxylate (1.43 g, 4.3 mmol) in TFA (5 mL) and DCM (20 mL) was stirred at room temperature for 3 hours. The reaction mixture was concentrated to give crude product as TFA salt (1.25 g), which was used directly in the next step without further purification. MS (ESI): mass calcd. for $C_{11}H_{13}F_3N_2$, 230.10, m/z found 231.1$[M+H]^+$.

Synthesis of N-(4-((2-(6-(trifluoromethyl)pyridin-3-yl)piperidin-1-yl)sulfonyl)phenyl)acetamide (8)

A mixture of 5-(piperidin-2-yl)-2-(trifluoromethyl)pyridine (1.25 g, 5.4 mmol), 4-acetamidobenzenesulfonyl chloride (1.27 g, 5.4 mmol) and $NaHCO_3$ (907 mg, 10.8 mmol) in THF (15 mL) and H_2O (25 mL) was stirred at room temperature overnight. The reaction mixture

was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine (2 x 150 mL), and dried for 30 min over anhydrous Na_2SO_4. After filtration and evaporation of the solvent, the crude oil was purified by column chromatography (SiO_2, EtOAc-petroleum ether, 1:2, R_f 0.2) to give the desired product (752 mg, 32%) as a yellow solid. MS (ESI): mass calcd. for $C_{19}H_{20}F_3N_3O_3S$, 427.12, m/z found 428.1[M+H]+.

Synthesis of 4-((2-(6-(trifluoromethyl)pyridin-3-yl)piperidin-1-yl)sulfonyl)aniline (9)

A mixture of N-(4-((2-(6-(trifluoromethyl)pyridin-3-yl)piperidin-1-yl)sulfonyl)phenyl)acetamide (752 mg, 1.76 mmol) in conc. HCl (5 mL) and H_2O (5 mL) was stirred at reflux for 1 h. The reaction mixture was neutralized with aq. $NaHCO_3$ to pH = 7. The resulting mixture was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (2 x 150 mL), dried for 30 min over anhydrous Na_2SO_4 and concentrated to give the desired product as a yellow solid (310 mg), which was used directly in the next step without further purification. MS (ESI): mass calcd. for $C_{17}H_{18}F_3N_3O_2S$, 385.11, m/z found 386.1$[M+H]^+$.

Synthesis of N-(4-((2-(6-(trifluoromethyl)pyridin-3-yl)piperidin-1-yl)sulfonyl)phenyl)benzo[d][1,3]dioxole- 5-carboxamide (CBD504)

A mixture of 4-((2-(6-(trifluoromethyl)pyridin-3-yl)piperidin-1-yl)sulfonyl)aniline (310 mg, 0.81 mmol), benzo[d][1,3]dioxole-5-carboxylic acid (135 mg, 0.81 mmol), HATU (308 mg, 0.81 mmol) and TEA (164 mg, 1.62 mmol) in DCM (5 mL) was stirred at room temperature overnight. The reaction mixture was concentrated to give a residue which was purified by column chromatography (SiO_2, EtOAc-petroleum ether, 1:1, R_f 0.1) to give the desired product (226 mg, 52%) as a white solid. ^{1}H NMR (400 MHz, d_6-DMSO, ppm) δ 10.47 (s, 1H), 8.74 (s, 1H), 8.06-8.02 (m, 3H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.87(d, *J* = 8.4 Hz, 2H), 7.61 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.54 (d, *J* = 1.6 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 1H), 6.16 (s, 2H), 5.28 (s, 1H), 3.75 (d, *J* = 14.4 Hz, 1H), 3.02 (t, *J* = 13.6 Hz, 1H), 2.20 (d, *J* = 14.8 Hz, 1H), 1.59-1.54 (m, 1H), 1.44-1.42 (m, 2H), 1.24-1.18 (m, 2H). MS (ESI): mass calcd. for $C_{25}H_{22}F_3N_3O_5S$, 533.12, m/z found 534.1[M+H]+. HPLC 99.8% (214 nm), 99.7% (254 nm).

A similar method was used to synthesize the remaining compounds **CBD501, CBE173, CBD505 and CB498.**

CBD501:

MS (ESI): mass calcd. for $C_{25}H_{25}N_3O_6S$ 495.15, m/z found 496.1 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 1.6 Hz, 1H), 8.02 (s, 1H), 7.86 – 7.75 (m, 4H), 7.62 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 1H), 7.38 (s, 1H), 6.89 (d, *J* = 8.1 Hz, 1H), 6.73 (d, *J* = 8.7 Hz, 1H), 6.08 (s, 2H), 5.17 (s, 1H), 3.92 (s, 3H), 3.79 (d, *J* = 14.4 Hz, 1H), 3.03 – 2.93 (m, 1H), 2.13 (d, *J* = 12.8 Hz, 1H), 1.71 – 1.61 (m, 1H), 1.58 – 1.39 (m, 3H), 1.37 – 1.27 (m, 1H).

CBE173:

MS (ESI): mass calcd. for $C_{26}H_{28}N_4O_5S$ 508.18, m/z found 509.1 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.93 (s, 1H), 7.84 (d, *J* = 8.7 Hz, 2H), 7.77 (d, *J* = 8.8 Hz, 2H), 7.53 (d, *J* = 8.6 Hz, 1H), 7.42 (dd, *J* = 8.1 Hz, 1H), 7.38 (d, 1H), 6.90 (d, *J* = 8.1 Hz, 1H), 6.52 (d, *J* = 8.9 Hz, 1H), 6.08 (s, 2H), 5.11 (s, 1H), 3.76 (d, *J* = 14.3 Hz, 1H), 3.09 (s, 6H), 3.04 – 2.95 (m, 1H), 2.11 (d, *J* = 12.4 Hz, 1H), 1.71 – 1.58 (m, 2H), 1.47 – 1.24 (m, 3H).

CBD505:

MS (ESI): mass calcd. for $C_{24}H_{22}ClN_3O_5S$ 499.10, m/z found 500.1 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, *J* = 1.9 Hz, 1H), 8.00 (s, 1H), 7.88 – 7.80 (m, 4H), 7.74 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.45 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.40 (d, *J* = 1.5 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 6.11 (s, 2H), 5.26 – 5.22 (s, 1H), 3.88 – 3.82 (m, 1H), 2.31 – 2.25 (m, 1H), 2.20 – 2.14 (m, 1H), 1.78 – 1.68 (m, 1H), 1.61 – 1.55 (m, 1H), 1.52 – 1.45 (m, 1H), 1.42 – 1.31 (m, 2H).

CBD498:

MS (ESI): mass calcd. for $C_{26}H_{27}N_3O_5S$ 493.17, m/z found 493.8 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD) δ 8.19 (s, 1H), 7.94 (d, *J* = 8.8 Hz, 2H), 7.83 (d, *J* = 8.8 Hz, 2H), 7.59 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.51 (s, 1H), 7.47 (s, 1H), 6.98 (d, *J* = 8.1 Hz, 1H), 6.10 (s, 2H), 5.22 (s, 1H), 3.87 (d, *J* = 13.9 Hz, 1H), 3.06 (d, *J* = 12.1 Hz, 1H), 2.48 (s, 3H), 2.30 (s, 3H), 2.23-2.19 (m, 1H), 1.79-1.74 (m, 1H), 1.60-1.50 (m, 2H), 1.45–1.36 (m, 2H).