Supporting Information

Structure-Based Development of Isoform Selective Inhibitors of Casein Kinase 1ε vs. Casein Kinase 1δ

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Table S1. X-ray data collection and refinement statistics (CK1δ-SR-3029 & CK1δ-SR-4133)

Complex	**CK1d - SR3029**	**CK1d - SR4133**
PDB accession code	**6RCG**	**6RCH**
Data Collection		
Resolution[a] (Å)	29.92-1.40 (1.48-1.40)	19.72-1.45 (1.53-1.45)
Spacegroup	*C* 2	*P* 2_1
Cell dimensions	*a* = 88.0, *b* = 53.6, *c* = 72.2 Å	*a* = 49.1, *b* = 74.1, *c* = 89.0 Å
	α, γ = 90.0°, *β* = 113.5°	*α, γ* = 90.0°, *β* = 103.2°
No. unique reflections[a]	58,592 (8,620)	108,708 (15,481)
Completeness[a] (%)	96.7 (97.5)	99.0 (97.1)
I/σI[a]	12.0 (2.4)	6.5 (3.1)
R_{merge}[a] (%)	0.051 (0.462)	0.171 (0.439)
CC (1/2)	0.998 (0.870)	0.978 (0.858)
Redundancy[a]	3.6 (3.5)	5.7 (5.4)
Refinement		
No. atoms in refinement	2,456/ 70/ 389	4,915/ 38/ 841
B factor (P/L/O)[b] (Å²)	22/ 18/ 35	14/ 17/ 28
R_{fact} (%)	14.2	15.5
R_{free} (%)	18.5	17.8
rms deviation bond[c] (Å)	0.016	0.016
rms deviation angle[c] (°)	1.7	1.6
Molprobity Ramachandran		
Favour (%)	98.29	98.28
Outlier (%)	0	0

[a] Values in brackets show the statistics for the highest resolution shells.

[b] P/L/O indicate protein, ligand molecule, and other (water and solvent molecules), respectively.

[c] rms indicates root-mean-square.

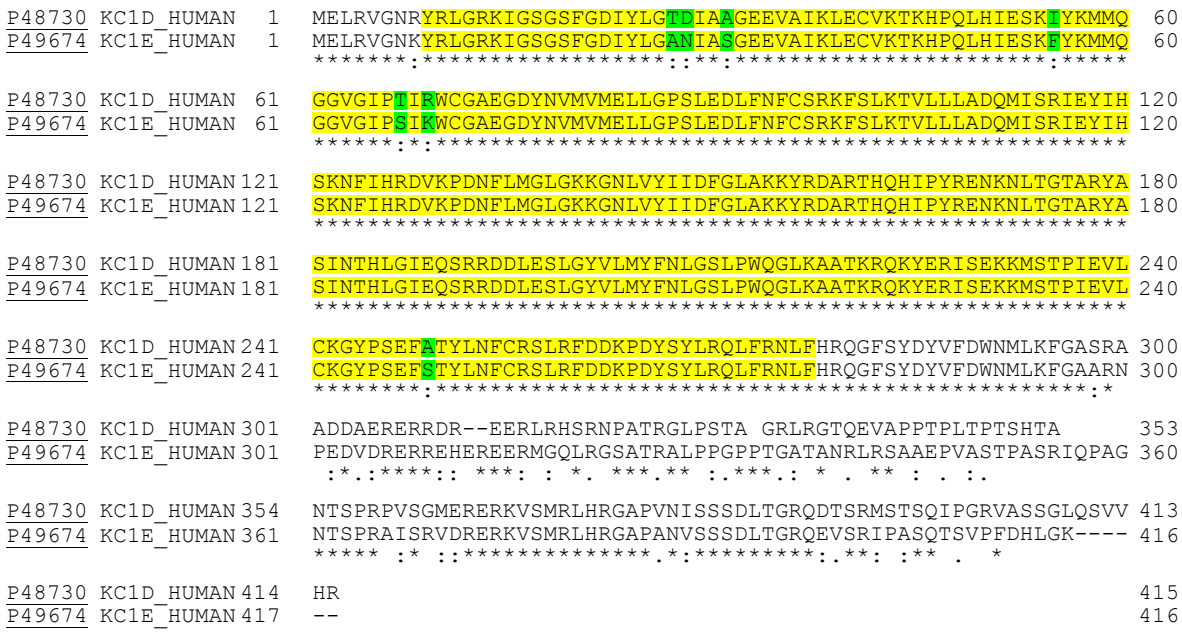
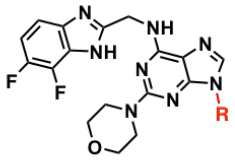
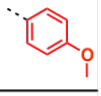


Figure S1. Sequence alignment of CK1δ and CK1ε. Sequences of human CK1δ (UniProtKB code: P48730) and CK1ε (UniProtKB code: P49678) were obtained from the server (**www.uniprot.org**) and sequence alignment was conducted by using the Clustal Omega program in the server.[1-2] Catalytic domains of CK1δ and CK1ε are highlighted in yellow, and seven different amino acids in the catalytic domains of CK1δ and CK1ε are highlighted in green.



IC_{50} (nM)	SR3029	SR4133	SR4310
R =			
CK1α	>10,000	>10,000	>10,000
CK1Y1	>10,000	>10,000	>10,000
CK1Y2	>10,000	>10,000	>10,000
CK1Y3	>10,000	>10,000	>10,000
CK1δ	44	>10,000	1,670
CK1ε	260	61	160

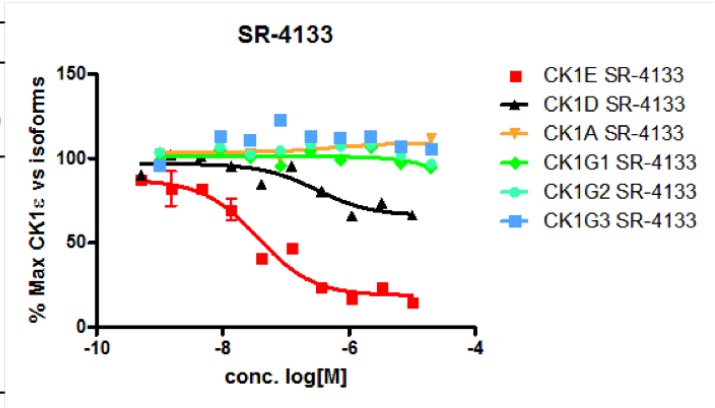


Figure S2. Specificity of SR-4133 to CK1ε vs. CK1 isoforms. SR-4133 and SR-4310 were assayed to CK1α, CK1γ1, CK1γ2, and CK1γ3 by Reaction Biology Kinase Assay Services.

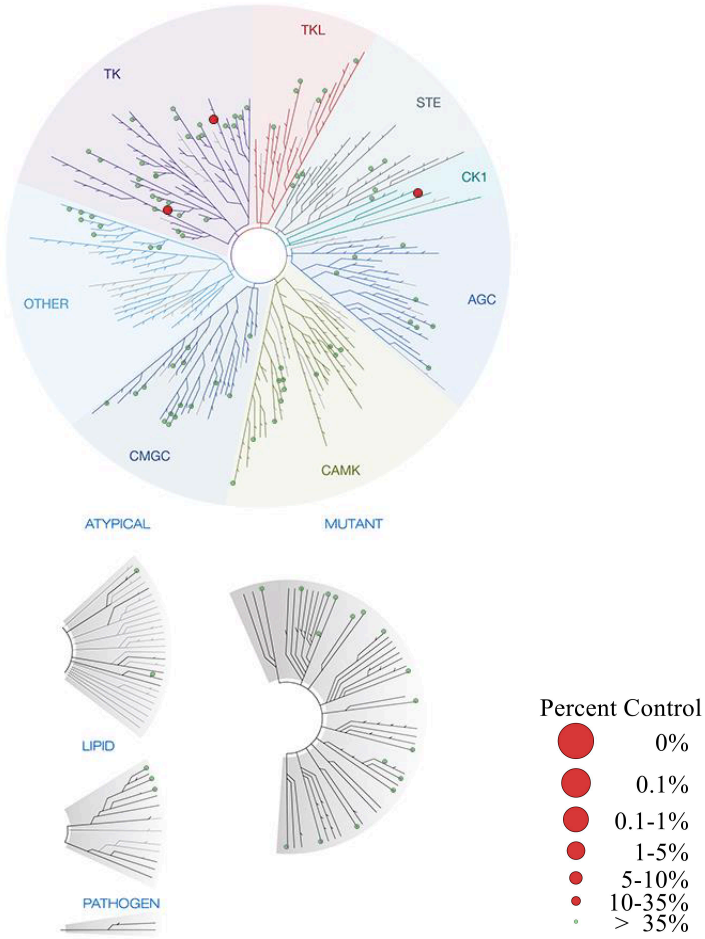


Figure S3. Specificity of SR-4133 to CK1ε vs. 97 human kinome from the KINOMEscan profiling assay at 10 μM.

Table S2. Selectivity profiling data for SR-4133

Enzyme	Percent activity remaining when dosed with 10 µM SR-4133
ABL1(E255K)-phosphorylated	66
ABL1(T315I)-phosphorylated	83
ABL1-nonphosphorylated	87
ABL1-phosphorylated	76
ACVR1B	73
ADCK3	100
AKT1	100
AKT2	90
ALK	97
AURKA	76
AURKB	73
AXL	95
BMPR2	78
BRAF	84
BRAF(V600E)	74
BTK	76
CDK11	86
CDK2	84
CDK3	100
CDK7	67
CDK9	79
CHEK1	90
CSF1R	80
CSNK1D	30
CSNK1G2	76
DCAMKL1	62
DYRK1B	78
EGFR	73

EGFR(L858R)	90
EPHA2	74
ERBB2	65
ERBB4	95
ERK1	91
FAK	97
FGFR2	93
FGFR3	79
FLT3	41
GSK3B	64
IGF1R	87
IKK-alpha	96
IKK-beta	76
INSR	82
JAK2(JH1domain-catalytic)	100
JAK3(JH1domain-catalytic)	95
JNK1	67
JNK2	66
JNK3	55
KIT	62
KIT(D816V)	89
KIT(V559D,T670I)	83
LKB1	100
MAP3K4	87
MAPKAPK2	100
MARK3	89
MEK1	70
MEK2	91
MET	79
MKNK1	82
MKNK2	78
MLK1	94

p38-alpha	85
p38-beta	85
PAK1	81
PAK2	87
PAK4	95
PCTK1	44
PDGFRA	53
PDGFRB	14
PDPK1	92
PIK3C2B	87
PIK3CA	70
PIK3CG	76
PIM1	98
PIM2	100
PIM3	100
PKAC-alpha	91
PLK1	97
PLK3	71
PLK4	84
PRKCE	84
RAF1	94
RET	93
RIOK2	83
ROCK2	73
RSK2(Kin.Dom.1-N-terminal)	64
SNARK	86
SRC	90
SRPK3	39
TGFBR1	92
TIE2	71
TRKA	34
TSSK1B	98

TYK2(JH1domain-catalytic)	100
ULK2	75
VEGFR2	72
YANK3	85
ZAP70	100

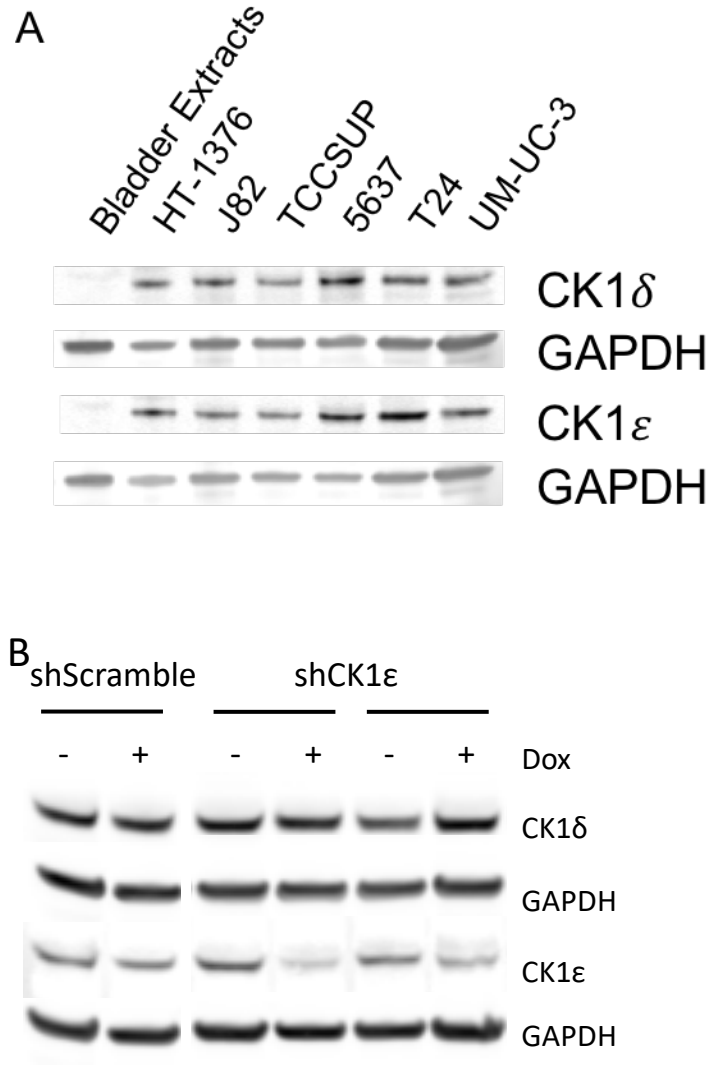


Figure S4. (A) CK1ε protein expression in indicated bladder cancer cell lines, (B) CK1δ and CK1ε protein expression in CK1ε doxycycline inducible knockdown J82 cells with 2 different shRNA. A. The basal expression of CK1ε was examined by Western blot analysis on a panel of bladder cancer cell lines (HT-1376, J82, TCCSUP, 5637, T24, and UM-UC-3). GAPDH expression was used as a loading control. B. J82 bladder cancer cell line was transduced with doxycycline inducible ShRNA against CK1ε or scramble sequence (Sc). Knockdown of CK1ε was confirmed by western blot after 2 days of treatment with 1 μg/ml doxycycline.

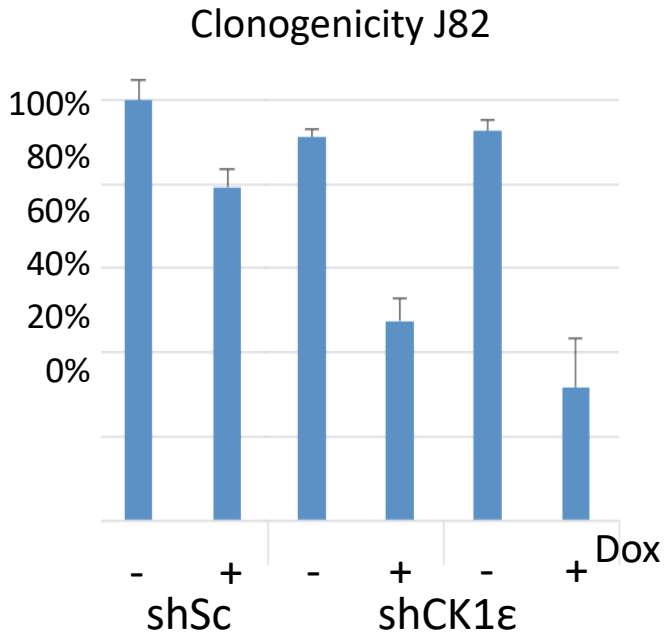


Figure S5. Clonogenic growth and survival of CK1ε-doxycycline inducible silencing J82 cells. Colony formation assay of cells expressing doxycycline-inducible shRNA against CK1ε and scramble was assessed with 1 µg/ml doxycycline treatment. % of colonies was then calculated depending on the shScramble cell line without doxycycline.

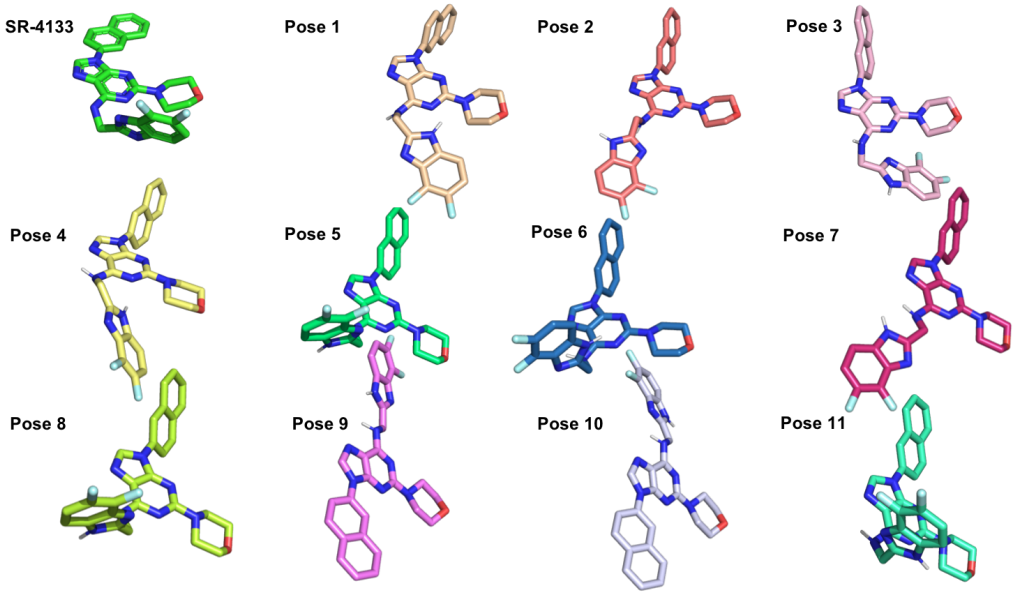


Figure S6. The binding poses of SR-4133 in the ATP-binding site of CK1ε from AutoDock-GPU, which were used for MD simulations. All poses are oriented the same way as the original binding pose of SR-4133 in the X-ray co-crystal structure of CK1δ.

Table S3. MM/GBSA binding affinity energies of 11 poses of SR-4133 complexed in CK1ε.

	$\Delta G_{binding}$ (kcal/mol)
Pose 1	-42.40
Pose 2	-45.24
Pose 3	-42.37
Pose 4	-41.95
Pose 5	-43.29
Pose 6	-40.17
Pose 7	-42.77
Pose 8	-41.01
Pose 9	-35.60
Pose 10	-26.89
Pose 11	-47.02

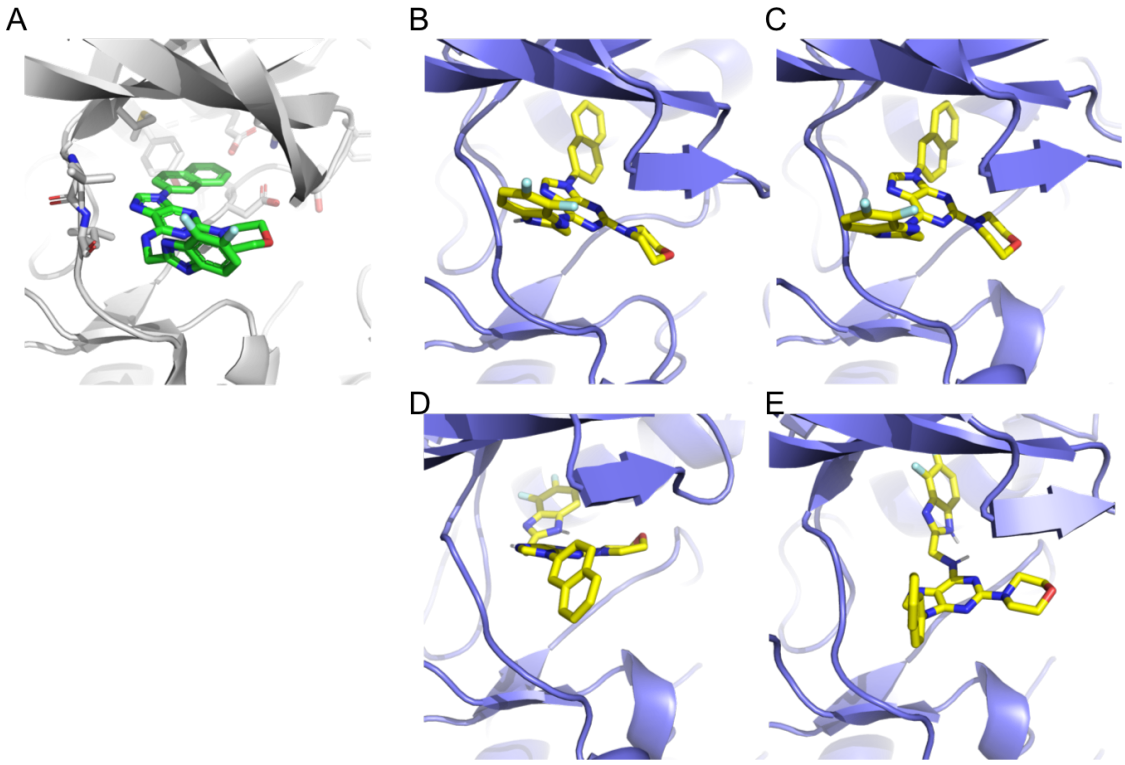


Figure S7. The binding orientations of SR-4133 in the ATP-binding site of CK1. (A) The binding orientation of SR-4133 in the X-ray co-crystal structure of CK1δ - SR-4133 complex (pdb code: 6RCH); (B) (C) (D) (E) The binding orientations of SR-4133 from 100 ns MD simulations of CK1ε - SR-4133 complexes obtained from molecular docking studies. Pose 2 (B) and pose 11 (C) have the highest binding affinities in MM/GBSA calculation (Table S3). Pose 9 (D) and pose 10 (E) have the lowest binding affinities in MM/GBSA calculation (Table S3).

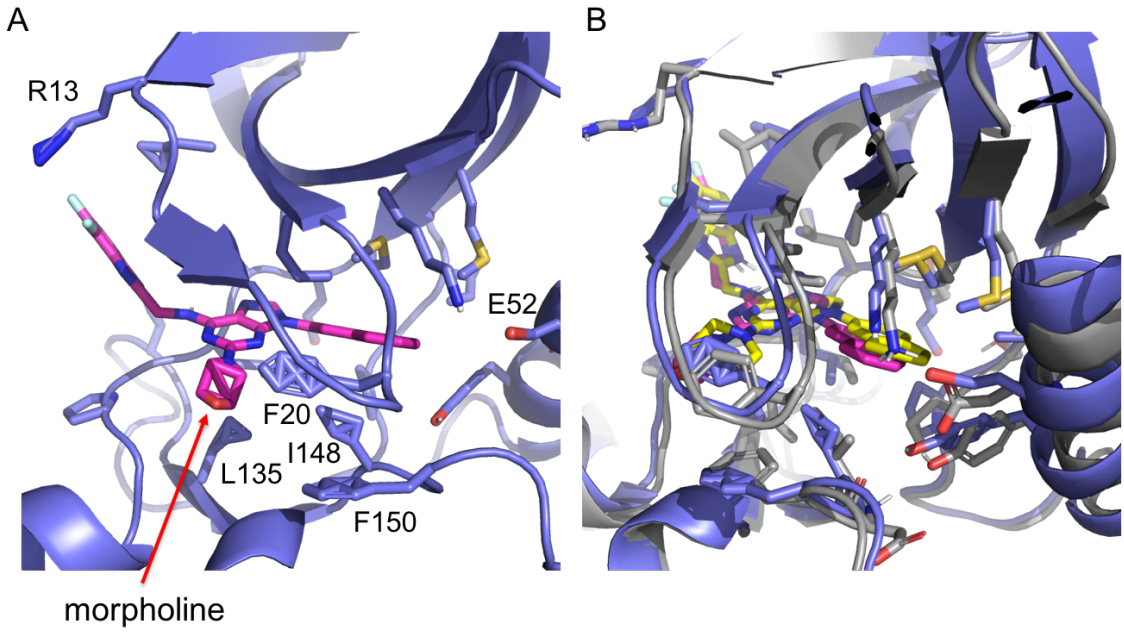


Figure S8. The averaged structure of CK1ε - SR-4133 complex from MD simulations (800 ns – 1 μs). (A) and its overlay to the structure with the lowest energy from the 1 μs MD simulations (B). SR-4133 and CK1ε in the averaged structure are magenta and purple, and SR-4133 and CK1ε in the structure with the lowest energy are yellow and gray, respectively.

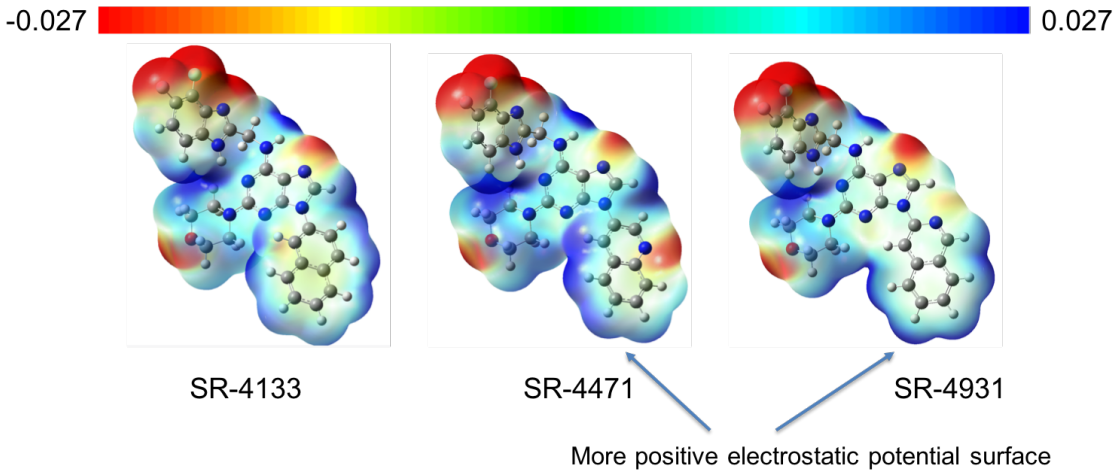


Figure S9. Electrostatic potential surfaces of SR-4133, SR-4471, and SR-4931. GaussView 6 was used to generate the electrostatic potential surfaces by using checkpoint files obtained from the geometry optimization of each ligand in B3LYP/6-311+G**. SR-4471 and SR-4931 show more positive electrostatic potentials on the outer ring of quinoline/isoquinoline ring than SR-4133.

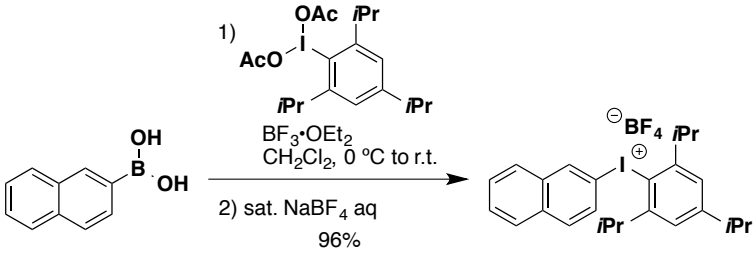
Chemistry, General Experimental Details.

Commercially available reagents were used without further purification. Tetrahydrofuran and dichloromethane were purified by passing through a solvent column composed of activated A-1 alumina. Anhydrous acetone was purchased from Aldrich Chemical Company. Triethylamine and diisopropylethylamine were purified by distillation from calcium hydride. Unless indicated otherwise, all reactions were conducted under an atmosphere of argon using flame-dried or oven-dried (140 °C) glassware. The term "concentrated under reduced pressure" refers to the removal of solvents and other volatile materials using a rotary evaporator with the water bath temperature below 40 °C, followed by the removal of residual solvent at high vacuum (< 0.2 mbar). Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a commercial instrument at 400 MHz. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded at 100 MHz and 175 MHz. The proton signal for residual non-deuterated solvent (δ 7.26 for $CHCl_3$, 2.50 for dimethyl sulfoxide) was used as an internal reference for ^{1}H NMR spectra. For ^{13}C NMR spectra, chemical shifts are reported relative to the δ 77.1 resonance of $CHCl_3$ and δ 39.52 resonance of dimethyl sulfoxide. Coupling constants are reported in Hz. Infrared (IR) spectra were recorded as films on a commercial FTIR instrument. High-resolution mass spectra were recorded on a commercial high-resolution mass spectrometer. Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F254 glass plates precoated with a 0.25 mm thickness of silica gel. The TLC plates were visualized with UV light and/or by staining with $KMnO_4$. Column chromatography was generally performed using Kieselgel 60 (230-400 mesh) silica gel, typically using a 50-100:1 weight ratio of silica gel to crude product. All compounds are >95% pure by HPLC analysis.

General procedure A for Preparation of iodonium tetrafluoroborate

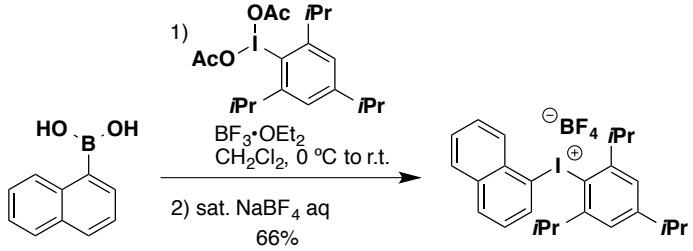
To a solution of the 1-naphthalene boronic acid (6.28 mmol) in CH_2Cl_2 (100 ml) was added BF_3•OEt_2 (6.93 mmol) at 0 ºC. The reaction mixture was stirred for 10 min before the addition of a solution of 2-iodo-1,3,5-triisopropylbenzene diacetate (6.71 mmol) in CH_2Cl_2 (20 ml) dropwise over 10 minutes. The reaction was allowed to warm to r.t. over the course of 2 h and then 100 ml saturated aq. $NaBF_4$ solution was added with rapid stirring and the stirring continued for 30 minutes. After this time the phases were separated, and the aqueous layer was extracted twice with CH_2Cl_2. The combined organics dried over $NaSO_4$ and passed through a short silica pad (30 g), eluted with CH_2Cl_2 (300 mL) to remove byproduct, followed by CH_2Cl_2/MeOH (300 mL, 20:1), to elute the product. The iodonium tetrafluoroborate was precipitated from Et_2O. The solid was filtered, washed with Et_2O, and dried under a vacuum.

2-Naphthyl-(2,4,6-tri-*iso*propylphenyl)iodonium tetrafluoroborate (2)



Synthesized according to general procedure A from 2-naphthlene boronic acid. *Iodonium tetrafluoroborate was* obtained as a brown solid (3.33 g, 96%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.74 (d, J = 1.4 Hz, 1H), 8.07 (d, J = 4.4 Hz, 1H), 8.02 (m, 2H), 7.90 (dd, J = 9.2, 2.0 Hz, 1H), 7.69 (m, 2H), 7.31 (s, 2H), 3.52 (sept., J = 6.7 Hz, 2H), 2.96 (sept., J = 6.9 Hz, 1H), 1.25 (d, J = 6.8 Hz, 12H), 1.19 (d, J = 6.8 Hz, 6H).; ^{13}C NMR (100 MHz, DMSO-d_6, δ):154.2, 151.2 (x2), 135.2, 133.9, 133.2, 131.6, 129.1, 128.9, 128.1 (x2), 128.0, 124.7 (x2), 123.4, 112.0, 38.7 (x2), 33.4, 24.1 (x4), 23.5 (x2).

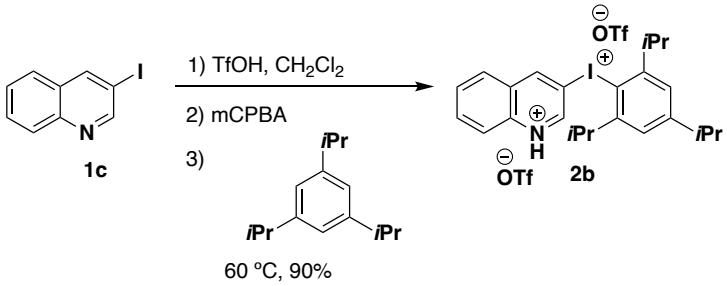
1-Napthyl-(2,4,6-tri-*iso*propylphenyl)iodonium tetrafluoroborate (2a)



Synthesized according to general procedure A from 1-naphthlene boronic acid. *Iodonium tetrafluoroborate was* obtained as a brown solid (2.25 g, 66%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.26 (d, J = 8.0 Hz, 2H),

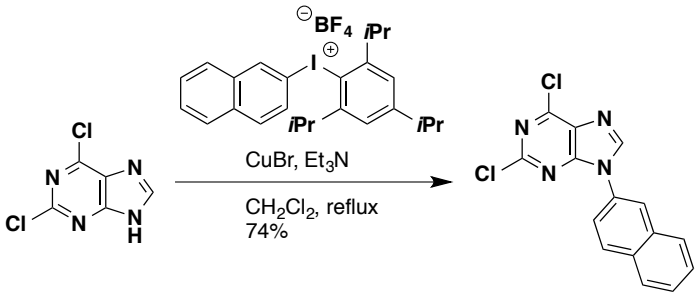
8.23 (d, *J* = 8.4 Hz, 1H), 8.08 (d, *J* = 7.6 Hz, 1H), 7.88 (m, 1H), 7.75 (m, 1H), 7.59 (t, *J* = 7.8 Hz, 1H), 7.26 (s, 2H), 3.50 (sept., *J* = 6.7 Hz, 2H), 3.02 (sept., *J* = 6.9 Hz, 1H), 1.17 (d, *J* = 6.8 Hz, 6H), 1.14 (d, *J* = 6.4 Hz, 12H).; ^{13}C NMR (100 MHz, DMSO-d_6, δ): 154.0, 151.2 (x2), 136.3, 134.3, 133.2, 130.9, 129.5, 129.4, 128.6, 128.0, 127.8, 124.8 (x2), 124.3, 118.0, 39.4 (x2), 33.2, 24.1 (x4), 23.5 (x2).= 6.8 Hz, 6H), 1.14 (d, *J* = 6.4 Hz, 12H).; ^{13}C NMR (100 MHz, DMSO-d_6, δ): 154.0, 151.2 (x2), 136.3, 134.3, 133.2, 130.9, 129.5, 129.4, 128.6, 128.0, 127.8, 124.8 (x2), 124.3, 118.0, 39.4 (x2), 33.2, 24.1 (x4), 23.5 (x2).

3-((2,4,6-triisopropylphenyl)iodonio)quinolin-1-ium bistrifluoromethanesulfonate (2b)



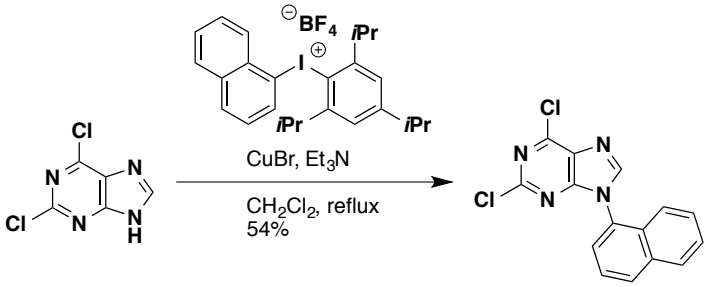
To a solution of 3-iodoquinoline (2.4 g, 9.5 mmol, 1 eq.) in CH_2Cl_2 (50 mL, 0.2 M) was added triflic acid (3.4 mL, 38.5 mmol, 4 eq.). This mixture was stirred at RT for 5 minutes. Then, mCPBA (3.23 g, 14.1 mmol, 1.5 eq.) was added followed by triisoppropylbenzene (2.6 mL, 10.8 mmol, 1.1 eq). [Note: mCPBA *must* be added first.] This mixture was heated to 60 ºC and stirred for 30 min. The cooled reaction mixture was concentrated under reduced pressure, triturated in Et_2O, and stirred at 0 ºC for 2 hr. The resulting solid was collected by filtration, dried under a vacuum, and used without further purification. The product was isolated as a white solid, 6.52 g, 90% yield. ^{1}H NMR (400 MHz, Methanol-d_4) δ 9.19 – 9.11 (m, 2H), 8.18 – 7.96 (m, 3H), 7.83 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H), 7.36 (s, 2H), 3.62 – 3.45 (m, 2H), 3.01 (hept, *J* = 6.9 Hz, 1H), 1.33 (d, *J* = 6.7 Hz, 12H), 1.26 (d, *J* = 6.8, 6H).*J* = 6.8, 6H).

2,6-dichloro-9-(naphthlen-2-yl)-*9H*-purine (4)



To a solution of 2,6-dichloropurine (507.7 mg, 2.69 mmol) in CH_2Cl_2 (11 mL, 0.25 M) was added 2-Naphthyl-(2,4,6-tri-*iso*propylphenyl)iodonium tetrafluoroborate (2.22 g, 4.08 mmol), CuBr (47.2 mg, 0.329 mmol) and triethylamine (940 μL, 6.74 mmol). The reaction mixture was stirred at reflux condition for 2.5 h. Then the reaction mixture was cooled to r.t. and concentrated *in vacuo*. The crude material was purified by flash chromatography (Hexane/EtOAc = 2/1 to 1/1) to give 624.8 mg (1.98 mmol, 74%) of the compound as a yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 9.22 (s, 1H), 8.39 (d, J = 2.0 Hz, 1H), 8.21 (d, J = 8.8 Hz, 1H), 8.07 (m, 2H), 7.95 (dd, J = 8.4, 2.2 Hz, 1H), 7.66 (m, 2H).; ^{13}C NMR (100 MHz, DMSO-d_6, δ): 153.0, 151.6, 150.2, 147.6, 132.7, 132.3, 131.2, 131.0, 129.7, 128.1, 127.9, 127.4, 127.2, 122.3, 122.0.= 8.4, 2.2 Hz, 1H), 7.66 (m, 2H).; ^{13}C NMR (100 MHz, DMSO-d_6, δ): 153.0, 151.6, 150.2, 147.6, 132.7, 132.3, 131.2, 131.0, 129.7, 128.1, 127.9, 127.4, 127.2, 122.3, 122.0.

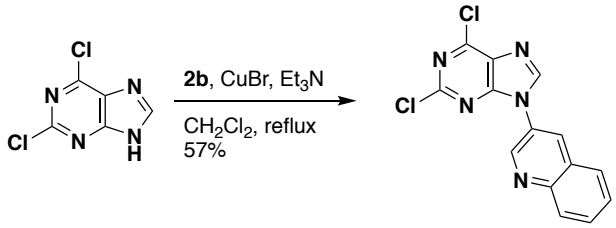
2,6-dichloro-9-(naphthlen-1-yl)-*9H*-purine (4a)



To a solution of 2,6-dichloropurine (505.4 mg, 2.67 mmol) in CH_2Cl_2 (11 mL, 0.25 M) was added 1-Napthyl-(2,4,6-tri-*iso*propylphenyl)iodonium tetrafluoroborate (2.14 g, 3.93 mmol), CuBr (45.5 mg, 0.317 mmol) and triethylamine (930 μL, 6.67 mmol). The reaction mixture was stirred at reflux condition for 2.5 h. Then the reaction mixture was cooled to r.t. and concentrated *in vacuo*. The crude material was purified by flash chromatography (Hexane/EtOAc = 2/1) to give 452.1 mg (1.43 mmol, 54%) of title compound as a brown powder. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 9.03 (s, 1H), 8.25 (d, J = 8.0 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 7.80 (dd, J = 7.2, 1.3 Hz, 1H), 7.74 (app t, J = 7.8 Hz, 1H), 7.67 (m, 1H), 7.56 (m, 1H), 7.45 (dd,

J = 8.4, 0.8 Hz, 1H).; ^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ): 154.7, 151.5, 150.2, 148.9, 133.7, 130.7, 130.6, 129.3, 129.0, 128.4, 127.9, 127.2, 126.2, 125.6, 122.3.

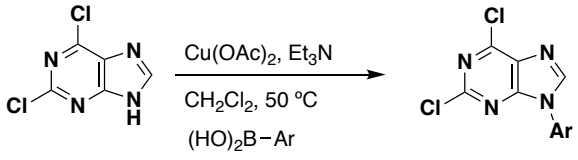
3-(2,6-dichloro-9H-purin-9-yl)quinoline (4b)



3 **4b**

Synthesized by following the procedure for **4** or **4a**, and the product was isolated as a white solid (500 mg, 57% yield). ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ): 9.33 (d, *J* = 2.5 Hz, 1H), 9.28 (s, 1H), 8.86 (dd, *J* = 2.5, 0.8 Hz, 1H), 8.17 (ddd, *J* = 8.1, 1.9, 1.0 Hz, 2H), 7.91 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H), 7.77 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H).

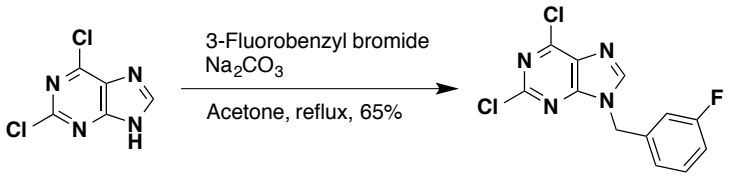
General procedure B – Chan-Lam Boronic Acid Coupling



3

To a solution of 2,6-dichloropurine (**3**, 1 eq.) in CH_2Cl_2 (0.25 M) was added arylboronic acid (2 eq.), triethylamine (3 eq.), and copper acetate (2 eq.). The reaction mixture was refluxed at 50 ºC for 1 h, then cooled to 25 ºC and filtered over celite washing 3 times with CH_2Cl_2. The filtrate was dry-loaded onto a silica column and purified by flash chromatography to give the coupled product.

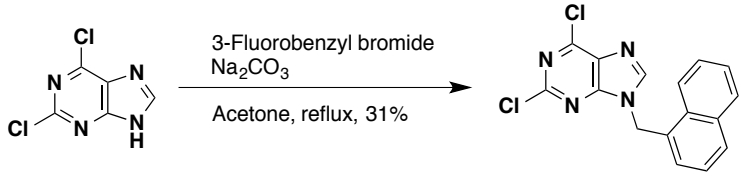
2,6-dichloro-9-(3-fluorobenzyl)-*9H*-purine (5)



To a solution of 2,6-dichloropurine (1.0 g, 5.29 mmol) in acetone (22 mL, 0.24 M) was added sodium carbonate (1.16 g, 10.94 mmol). The reaction mixture was stirred at reflux condition for 20 minutes and was added 3-fluorobenzyl bromide (700 μL, 5.71 mmol). Then the reaction mixture was stirred at reflux condition for 3 h. The reaction mixture was cooled to r.t. and passed through a Celite pad, washed with

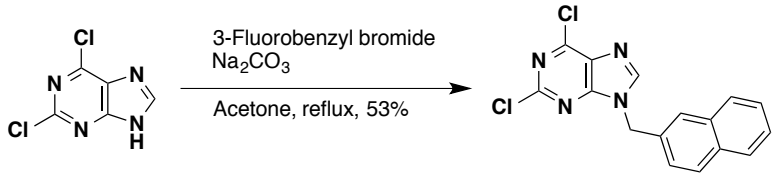
EtOAc. The filtrate was concentrated *in vacuo*. The crude material was purified by flash chromatography (100/1: CH_2Cl_2/MeOH) to give 1.02 g (3.43 mmol, 65%) of title compound as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.40 (s, 1H), 7.3 (m, 1H), 7.00 (m, 1H), 6.91 (m, 1H), 6.82 (m, 1H), 5.71 (s, 2H).; ^{13}C NMR (100 MHz, DMSO-d_6, δ): 162.2 (d, J = 242.9 Hz), 153.4, 151.1, 149.8, 148.4, 138.3 (d, J = 7.2 Hz), 130.9 (d, J = 8.0 Hz) 130.6, 123.7 (d, J = 2.2 Hz), 115.0 (d, J = 21.1 Hz), 114.6 (d, J = 21.8 Hz), 46.5.

2,6-dichloro-9-(naphthalen-1-ylmethyl)-*9H*-purine (5a)



To a solution of 2,6-dichloropurine (861 mg, 4.56 mmol) in acetone (19 mL, 0.24 M) was added sodium carbonate (1.04 g, 9.81 mmol). The reaction mixture was stirred at reflux condition for 20 minutes and was added 1-(chloromethyl)-naphthalene (850 μL, 5.11 mmol). Then the reaction mixture was stirred at reflux condition for 17 h. The reaction mixture was cooled to r.t. and passed through a Celite pad, washed with EtOAc. The filtrate was concentrated *in vacuo*. The crude material was purified by flash chromatography (100/1: CH_2Cl_2/MeOH) to give 470.6 mg (1.43 mmol, 31%) of title compound as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.93-7.87 (m, 3H), 7.86 (s, 1H), 7.56-7.42 (m, 4H), 5.82 (s, 2H).; ^{13}C NMR (100 MHz, DMSO-d_6, δ): 153.6, 151.2, 149.9, 148.5, 133.3, 130.9, 130.6, 130.1, 128.7 (x2), 126.9, 126.3, 125.7, 125.6, 122.9, 45.1.

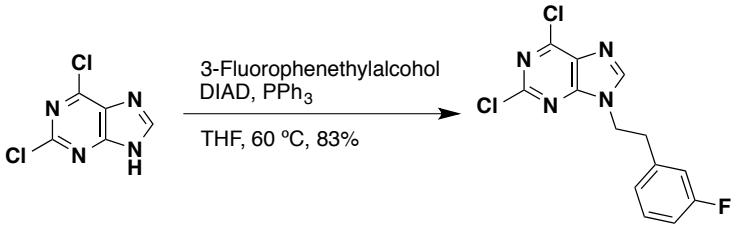
2,6-dichloro-9-(naphthalen-2-ylmethyl)-*9H*-purine (5b)



To a solution of 2,6-dichloropurine (1.09 g, 5.71 mmol) in acetone (24 mL, 0.24 M) was added sodium carbonate (1.20 g, 11.32 mmol). The reaction mixture was stirred at reflux condition for 20 minutes and was added 2-(bromomethyl)-naphthalene (1.39 g, 6.29 mmol). Then the reaction mixture was stirred at reflux condition for 2 h. The reaction mixture was cooled to r.t. and passed through a Celite pad, washed with EtOAc. The filtrate was concentrated *in vacuo*. The crude material was purified by flash chromatography (100/1: CH_2Cl_2/MeOH) to give 986.1 mg (3.00 mmol, 53%) of title compound as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.09 (s, 1H), 7.86-7.79 (m, 3H),7.76 (brs, 1H), 7.54-7.49 (m, 2H),

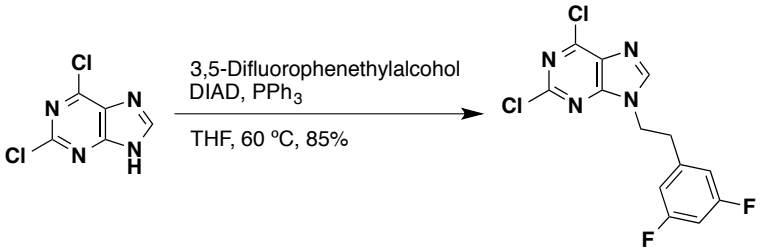
7.36 (dd, J = 8.4, 1.8 Hz, 1H), 5.55 (s, 2H).; ^{13}C NMR (100 MHz, DMSO-d_6, δ): 153.5, 151.1, 149.8, 148.5, 133.1, 132.8, 132.5, 130.6, 128.5, 127.8, 127.6, 126.5, 126.4, 126.3, 125.4, 47.7.

2,6-dichloro-9-(3-fluorophenethyl)-*9H*-purine (6)



To a solution of 2,6-dichloropurine (497.9 mg, 2.63 mmol) in THF (26 mL, 0.1 M) was added 3-fluorophenylethylalcohol (650 μL, 5.24 mmol), PPh_3 (1.38 g, 5.26 mmol) and DIAD (1.0 mL, 5.16 mmol). The reaction mixture was stirred at 60 ºC for 16 h. Then the reaction mixture was cooled to r.t. and concentrated *in vacuo*. The crude material was purified by flash chromatography (Hexane/EtOAc = 1/1) to give the product 673.8 mg (2.17 mmol, 83%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.54 (s, 1H), 7.29-7.23 (m, 1H), 7.08-6.99 (m, 2H), 6.92 (d, J = 7.6 Hz, 1H), 4.53 (t, J = 7.0 Hz, 1H), 3.18 (t, J = 7.0 Hz, 1H).; ^{13}C NMR (100 MHz, DMSO-d_6, δ):162.2 (d, J = 241.4 Hz), 153.4, 150.9, 149.5, 148.3, 140.3 (d, J = 7.3 Hz), 130.3 (d, J = 4.4 Hz), 130.2 (d, J = 4.4 Hz), 124.9 (d, J = 2.9 Hz), 115.6 (d, J = 21.1 Hz), 113.5 (d, J = 21.0 Hz), 44.9, 34.4.= 21.1 Hz), 113.5 (d, J = 21.0 Hz), 44.9, 34.4.

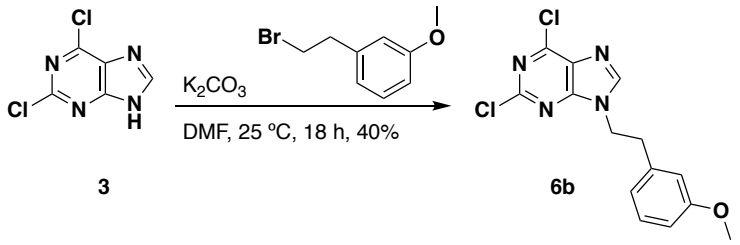
2,6-dichloro-9-(3,5-difluorophenethyl)-*9H*-purine (6a)



To a solution of 2,6-dichloropurine (505.2 mg, 2.67 mmol) in THF (28 mL, 0.1 M) was added 3,5-difluorophenethylalcohol (849.8 mg, 5.37 mmol), PPh_3 (1.43 g, 5.68 mmol) and DIAD (1.1 mL, 5.68 mmol). The reaction mixture was stirred at 60 ºC for 16 h. Then the reaction mixture was cooled to r.t. and concentrated *in vacuo*. The crude material was purified by flash chromatography (Hexane/EtOAc = 2/1) to give the product 750.9 mg (2.28 mmol, 85%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.54

(s, 1H), 7.06 (m, 1H), 6.93 (m, 2H), 4.54 (d, J = 6.8 Hz, 2H), 3.18 (d, J = 6.8 Hz, 2H).; ^{13}C NMR (100 MHz, DMSO-d_6, δ):162.4 (d, J = 244.4 Hz), 162.2 (d, J = 244.4 Hz), 153.4, 150.9, 149.5, 148.3, 142.1 (t, J = 9.5 Hz,), 130.3, 112.1 (d, J = 24.7 Hz, x2), 102.2 (t, J = 25.8 Hz), 44.6, 34.3.

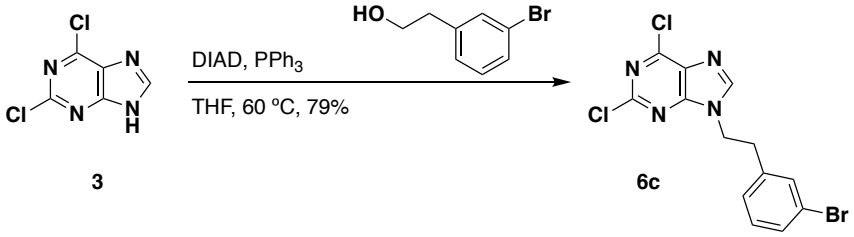
2,6-dichloro-9-(3-methoxyphenethyl)-9H-purine (6b)



3 6b

To a solution of **3** (790 mg, 4.2 mmol, 1 eq.) in DMF (17 mL, 0.25 M) was added potassium carbonate (880 mg, 6.3 mmol, 1.5 eq) and the alkyl bromide (680 µL, 4.3 mmol, 1.05 eq). The mixture was stirred overnight at 25 ºC then subjected to column chromatography. Product isolated as a tan solid, 530 mg, 40% yield. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.66 (s, 1H), 7.25 – 7.14 (m, 1H), 6.82 – 6.74 (m, 1H), 6.59 (dt, J = 6.9, 1.6 Hz, 2H),4.51 (t, J = 6.8 Hz, 2H), 3.75 (s, 3H), 3.14 (t, J = 6.8 Hz, 2H).

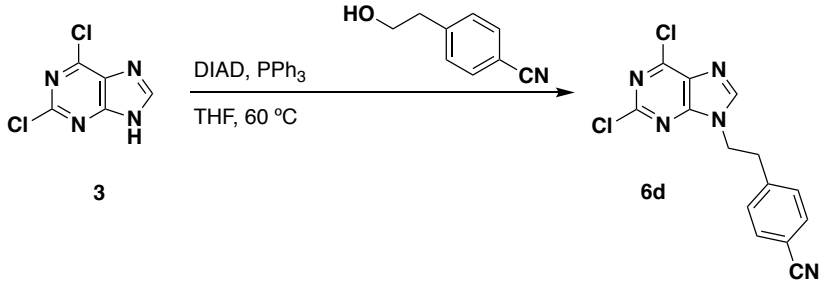
9-(3-bromophenethyl)-2,6-dichloro-9H-purine (6c)



3 6c

Synthesized by following the procedure for **6a**, and the product was isolated as a tan solid, 800 mg, 79% yield. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.76 (s, 1H), 7.39 (ddd, J = 8.0, 2.0, 1.0 Hz, 1H), 7.29 (t, J = 1.9 Hz, 1H), 7.14 (t, J = 7.8 Hz, 1H), 6.92 (ddd, J = 7.6, 1.7, 1.0 Hz, 1H), 4.50 (t, J = 7.0 Hz, 2H), 3.17 (t, J = 7.0 Hz, 2H).

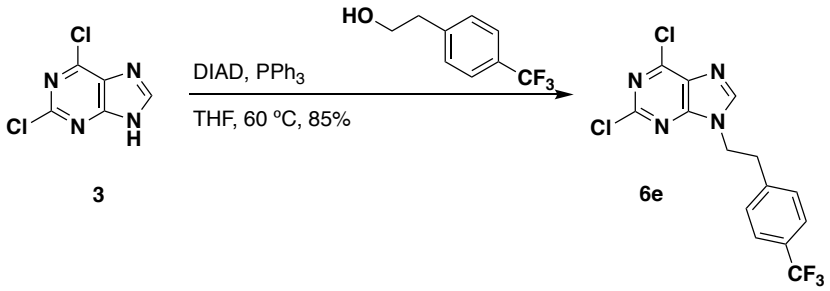
4-(2-(2,6-dichloro-9H-purin-9-yl)ethyl)benzonitrile (6d)



3 6d

Synthesized by following the procedure for **6a**, and the product was isolated as a mixture with triphenylphosphine oxide in a 1:2 product:TPPO ratio. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.56 (s, 1H), 7.71 (d, J = 8.3 Hz, 2H), 7.66 – 7.51 (m, 32H - TPPO), 7.35 (d, J = 8.3 Hz, 2H), 4.55 (t, J = 6.9 Hz, 2H), 3.25 (t, J = 6.9 Hz, 2H). = 6.9 Hz, 2H).

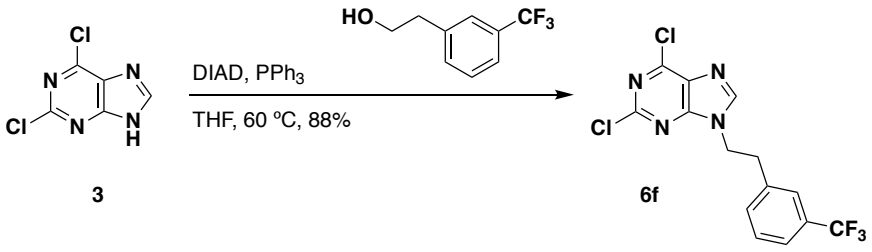
2,6-dichloro-9-(4-(trifluoromethyl)phenethyl)-9H-purine (6e)



3 6e

Synthesized by following the procedure for **6a**, and the product was isolated as a white solid, 810 mg, 85% yield. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.58 (s, 1H), 7.66 – 7.55 (m, 2H), 7.37 (d, J = 8.0 Hz, 2H), 4.55 (t, J = 7.0 Hz, 2H), 3.26 (t, J = 7.0 Hz, 2H).

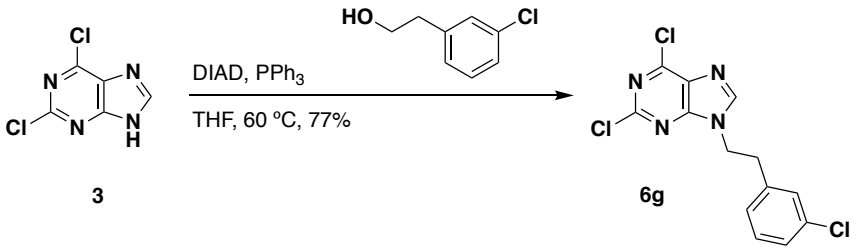
2,6-dichloro-9-(3-(trifluoromethyl)phenethyl)-9H-purine (6f)



3 6f

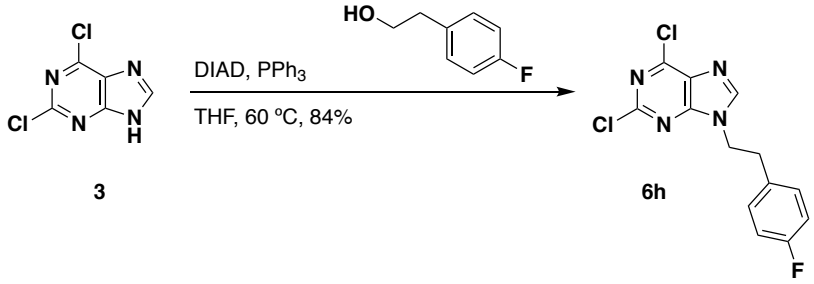
Synthesized by following the procedure for **6a**, and the product was isolated as a white solid, 630 mg, 88% yield. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.61 (s, 1H), 7.56 – 7.37 (m, 4H), 4.56 (t, J = 7.0 Hz, 2H), 3.26 (t, J = 7.0 Hz, 2H).

2,6-dichloro-9-(3-chlorophenethyl)-9H-purine (6g)



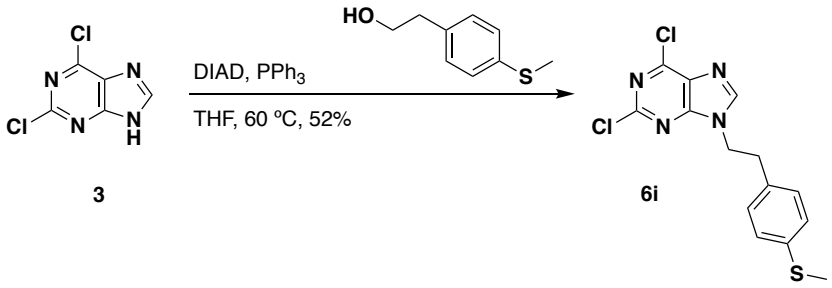
Synthesized by following the procedure for **6a**, and the product was isolated as a white solid, 705 mg, 77% yield. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.57 (s, 1H), 7.29 (dd, J = 1.9, 1.0 Hz, 1H), 7.26 – 7.22 (m, 2H), 7.06 (ddd, J = 4.9, 3.6, 1.6 Hz, 1H), 4.52 (t, J = 7.0 Hz, 2H), 3.16 (t, J = 7.0 Hz, 2H).

2,6-dichloro-9-(4-fluorophenethyl)-9H-purine (6h)



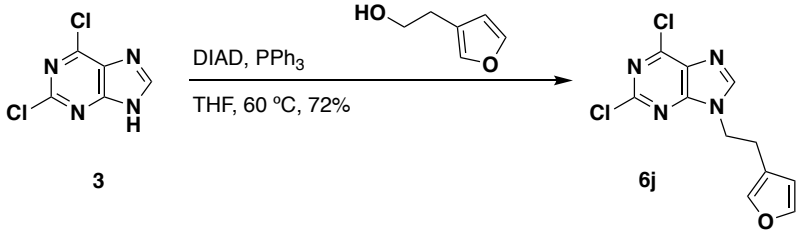
Synthesized by following the procedure for **6a**, and the product was isolated as a white solid, 700 mg, 84% yield. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.54 (s, 1H), 7.16 (ddt, J = 8.7, 5.3, 2.6 Hz, 2H), 7.11 – 7.00 (m, 2H), 4.49 (t, J = 7.0 Hz, 2H), 3.15 (t, J = 7.0 Hz, 2H).

2,6-dichloro-9-(4-(methylthio)phenethyl)-9H-purine (6i)



Synthesized by following the procedure for **6a**, and the product was isolated as a white solid, 350 mg, 52% yield. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.52 (s, 1H), 7.17 – 7.11 (m, 2H), 7.10 – 7.05 (m, 2H), 4.48 (t, J = 7.1 Hz, 2H), 3.12 (t, J = 7.1 Hz, 2H), 2.23 (s, 3H).

2,6-dichloro-9-(2-(furan-3-yl)ethyl)-9H-purine (6j)

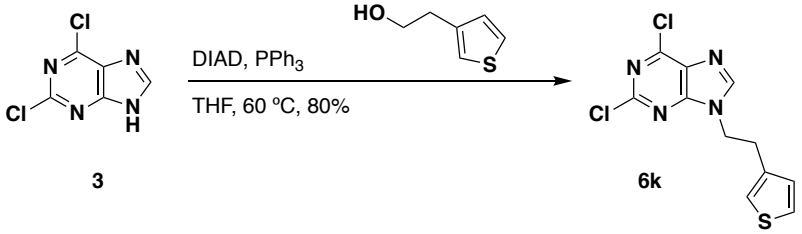


3 6j

Synthesized by following the procedure for **6a**, and the product was isolated as a white solid, 390 mg, 72% yield.

^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.59 (s, 1H), 7.56 (t, J = 1.7 Hz, 1H), 7.41 (dq, J = 1.8, 0.9 Hz, 1H), 6.37 (dd, J = 1.8, 0.9 Hz, 1H), 4.45 (t, J = 7.0 Hz, 2H), 3.03 – 2.95 (m, 2H).

2,6-dichloro-9-(2-(thiophen-3-yl)ethyl)-9H-purine (6k)

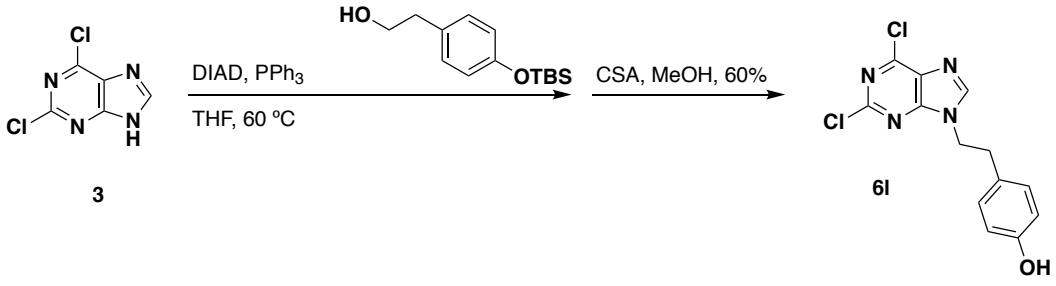


3 6k

Synthesized by following the procedure for **6a**, and the product was isolated as a white solid, 655 mg, 80% yield., 655 mg, 80% yield.

^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.53 (s, 1H), 7.45 (dd, J = 4.9, 2.9 Hz, 1H), 7.15 (dt, J = 2.9, 1.0 Hz, 1H), 6.97 (dd, J = 4.9, 1.3 Hz, 1H), 4.51 (t, J = 7.1 Hz, 2H), 3.22 – 3.14 (m, 2H).

4-(2-(2,6-dichloro-9H-purin-9-yl)ethyl)phenol (6l)

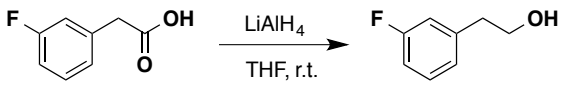


3 6l

Synthesized by following the procedure for **6a**, and the product was isolated as a mixture with DIAD byproduct in a 1:1.5 product:DIAD ratio. To a solution of this mixture (1.34g of mixture, 2.3 mmol, 1 eq.) in methanol (12 mL, 0.2M) was added camphorsulfonic acid (722 mg, 3.1 mmol, 1.3 eq.). This solution was stirred at room temperature for 3 h then quenched with a saturated aqueous $NaHCO_3$ solution. This

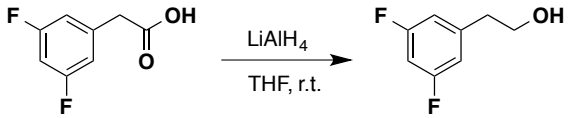
mixture was extracted 2 times with CH_2Cl_2, and the combined organic layers were dried over Na_2SO_4 and subjected to flash chromatography. Product isolated as a white solid, 500 mg, 60% yield over 2 steps. ^1H NMR (400 MHz, DMSO-*d*$_6$, δ): 9.24 (s, 1H), 8.49 (s, 1H), 6.90 (d, *J* = 8.4 Hz, 2H), 6.62 (d, *J* = 8.4 Hz, 2H), 4.43 (t, *J* = 7.1 Hz, 2H), 3.02 (t, *J* = 7.1 Hz, 2H).

3-Fluorophenethylalcohol



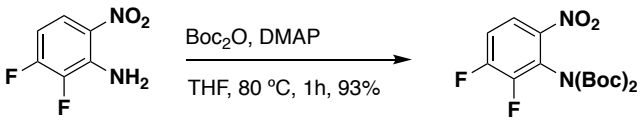
To a solution of $LiAlH_4$ (1.46 g, 38.47 mmol) in THF (40 mL) was added 3-fluorophenylacetic acid (2.31 g, 14.99 mmol) in THF (14 mL) at 0 ºC. After stirring for 2 h at room temperature, the reaction was quenched with H_2O (1.2 mL), 10% NaOH aq. (2.0 mL) and H_2O (1.2 mL). The resulting solution was stirred for 1 h at r.t., and passed through Celite pad, washed with CH_2Cl_2. The filtrate was concentrated *in vacuo*. The crude product was used for subsequent reaction without purification. The analytical data was identified with the literature.[3]

3,5-Difluorophenethylalcohol



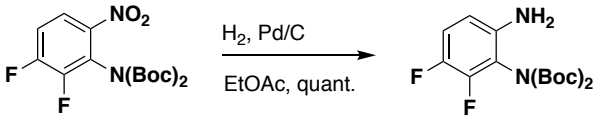
To a solution of $LiAlH_4$ (1.18 g, 31.09 mmol) in THF (30 mL) was added 3,5-difluorophenylacetic acid (2.05 g, 11.9 mmol) in THF (13 mL) at 0 ºC. After stirring for 4 h at room temperature, the reaction was quenched with H_2O (1.0 mL), 10% NaOH aq. (1.5 mL) and H_2O (1.0 mL). The resulting solution was stirred for 1 h at r.t., and passed through Celite pad, washed with CH_2Cl_2. The filtrate was concentrated *in vacuo*. The crude product was used for subsequent reaction without purification. The analytical data was identified with the literature.[4]

***N*-*N*-tert-butoxycarbonyl-2,3-difluoro-6-nitroaniline**



To a solution of 2,3-difluoro-6-nitroaniline (1000 mg, 5.7 mmol, 1 eq.) in THF (60 mL, 0.1 M) was added Boc anhydride (3760 mg, 17.2 mmol, 3 eq.), and DMAP (70 mg, 0.6 mmol, 0.1 eq). This mixture was refluxed at 80ºC for 1 hour until the consumption of **IV-23** was observed by LCMS. The mixture was dried under reduced pressure and subjected to flash chromatography (0% EtOAc in Hexanes to 10% EtOAc in Hexanes). Rf in 10% EtOAc in Hexanes = 0.5. Material isolated as a tan solid, 2000 mg, 93% yield. LCMS Method 2 time = 6.9 min.

***N*-*N*-tert-butoxycarbonyl-3,4-difluorobenzene-1,2-diamine**



To a solution of *N*-*N*-tert-butoxycarbonyl-2,3-difluoro-6-nitroaniline (2000 mg, 5.3 mmol, 1 eq.) in ethyl acetate (30 mL, 0.2 M) was added palladium on carbon. The reaction was sealed in a pressure reactor and purged 3 times with argon. Subsequently, the vessle was purged 3 times with H_2 at 30 psi. The vessel was filled a final time with 30 psi of H_2 and stirred at this pressure overnight. The next day the vessle was purged 3 times with argon and opened. The mixture was filtered over celite, washing 3 times with ethyl acetate. The filtrate was dried down under reduced pressure and used without further purification. Material isolated as a tan solid, 1800 mg, quantative yield. LCMS Method 1 time = 6.3 min. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.07 (dt, J = 10.3, 9.0 Hz, 1H), 6.46 (ddd, J = 9.2, 4.8, 2.1 Hz, 1H), 5.21 (s, 2H), 1.36 (s, 18H); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 149.93, 147.25, 147.12, 144.84, 144.71, 142.90, 142.88, 141.99, 141.87, 139.69, 139.57, 116.40, 116.22, 113.56, 113.43, 108.99, 108.96, 108.93, 108.89, 82.12, 27.40.

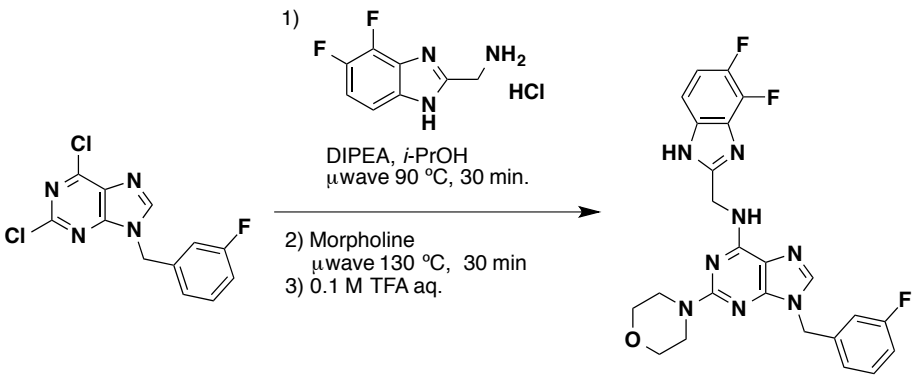
General procedure C for the Preparation of the final compounds (yoshi comp)

A 2-5 mL Biotage microwave vial was charged with 2,6-dichloropurine analogues (1.0 mmol), 2-(methylamino)-4,5-difluorobenzimidazole • HCl (1.1 mmol), *N,N*-diisopropylethylamine (5.0 mmol,), and isopropanol (2.5 mL). The vial was resealed and the reaction was heated to 90 °C for 30 minutes in the microwave unit. The cooled vial was then placed on a rotary evaporator and the reaction mixture was concentrated. Morpholine (2.5 mL) was added to the vial containing the concentrated crude mixture. The vial was resealed and the reaction was heated to 130 °C for 30 minutes in the microwave unit. The cooled reaction mixture was concentrated on a rotary evaporator and then the crude product was purified by flash chromatography on silica gel to give desired compound. Then, 0.1 M TFA aq. (1.0 mmol) was added to product (1.0 mmol) and sonicated for 30 min. Followed by freeze under –78 ºC, dried using lyophilizer.

General procedure D for Preparation of the final compounds (jamie comp)

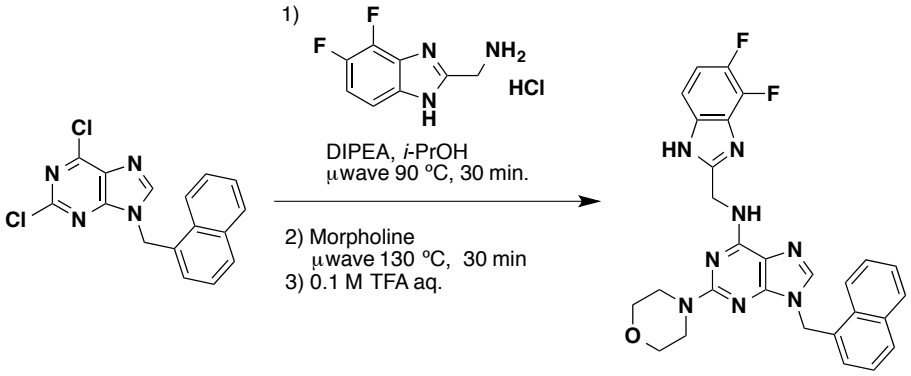
A Biotage microwave vial was charged with N9 substituted 2,6-dichloropurine (1 eq.), 2-(methylamino)-4,5-difluorobenzimidazole • HCl (1.1 eq.), *N,N*-diisopropylethylamine (5 eq), and isopropanol (0.4 M). The vial was sealed and the reaction was heated at 90 °C for 30 minutes in the microwave unit. The cooled vial was then placed on a rotary evaporator and the reaction mixture was concentrated. Morpholine (0.4 M) was added to a microwave vial containing the concentrated crude mixture. The vial was sealed and the reaction was heated at 130 °C for 30 min in the microwave unit. The cooled reaction mixture was concentrated on a rotary evaporator and then the crude product was purified by flash chromatography or HPLC to give the final product.

***N*-((4,5-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-9-(3-fluorobenzyl)-2-morpholino-9Hpurin-6-amine trifluoroacetate salt (SR-3448)**



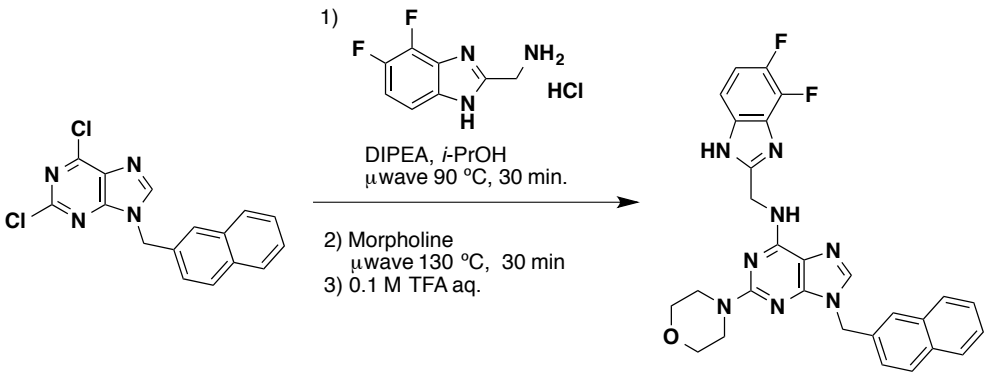
Synthesized according to general procedure C from 2,6-dichloro-9-(3-fluorobenzyl)-*9H*-purine in 49% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.37 (d, J = 9.2 Hz, 1H), 8.28 (brs, 1H), 8.20 (brs, 1H), 7.97 (m, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.57 (m, 2H), 7.49 (app t, J = 7.4 Hz, 1H), 7.38 (d, J = 6.8 Hz, 1H), 7.30-7.21 (m, 2H), 5.76 (s, 2H), 4.86 (brs, 2H), 3.51 (brs, 4H), 3.46 (brs, 4H).; ^{13}C NMR (175 MHz, DMSO-d_6, δ): 162.1 (d, J = 242.2 Hz), 158.5, 158.4 (q, J = 36.9 Hz), 155.7, 153.0, 150.7, 146.1 (d, J = 8.8 Hz), 144.8 (d, J = 9.3 Hz), 139.3, 138.0, 133.1, 130.7 (d, J = 8.6 Hz), 128.1, 124.1 (d, J = 2.1 Hz), 115.9 (q, J = 288.7 Hz), 114.9 (d, J = 21.7 Hz), 114.7 (d, J = 21.0 Hz), 112.1 (d, J = 21.0 Hz), 110.4, 108.7, 65.8 (x2), 45.8, 44.5 (x2), 38.3.

_N_-((4,5-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-2-morpholino-9-(naphthalen-1-ylmethyl)-9Hpurin-6-amine trifluoroacetate salt (SR-3449)



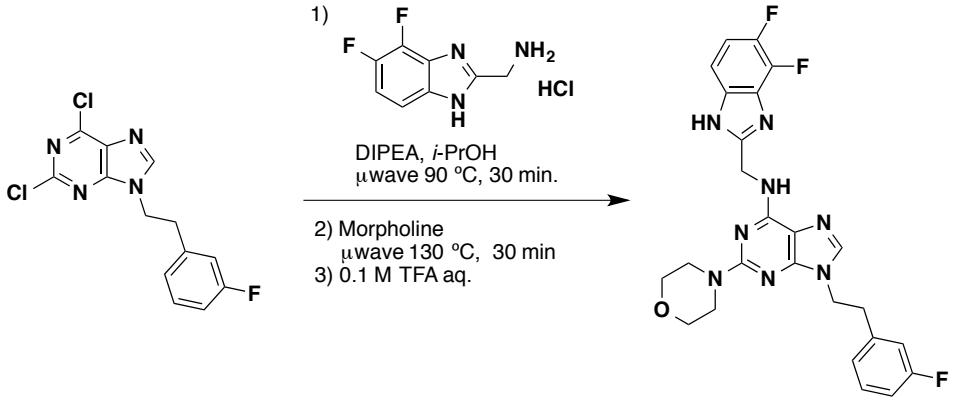
Synthesized according to general procedure C from 2,6-dichloro-9-(naphthalen-1-ylmethyl)-*9H*-purine in 57% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.30 (brs, 1H), 8.25 (s, 1H), 7.39 (m, 2H), 7.31-7.10 (m, 7H), 5.29 (s, 2H), 4.86 (s, 2H), 3.49 (brs, 4H), 3.46 (brs, 4H).; ^{13}C NMR (175 MHz, DMSO-d_6, δ): 158.5, 158.4 (q, J = 36.9 Hz), 155.7, 153.8, 150.9, 146.1 (d, J = 9.5 Hz), 144.7 (d, J = 9.5 Hz), 139.5 (d, J = 15.3 Hz), 138.3, 138.0 (d, J = 15.2 Hz), 133.3, 132.1, 130.5, 128.6 (d, J = 4.2 Hz), 128.5, 126.7 (d, J = 5.1 Hz), 126.1, 125.5, 116.0 (q, J = 288.9 Hz), 111.9 (d, J = 20.8 Hz), 110.7, 108.6, 65.8 (x2), 44.5 (x2), 44.0, 38.3.

_N_-((4,5-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-2-morpholino-9-(naphthalen-2-ylmethyl)-9Hpurin-6-amine trifluoroacetate salt (SR-3450)



Synthesized according to general procedure C from 2,6-dichloro-9-(naphthalen-2-ylmethyl)-*9H*-purine in 58% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.23 (brs, 2H), 7.90-7.85 (m, 4H), 7.53-7.48 (m, 3H), 7.27-7.18 (m, 2H), 5.43 (s, 2H), 4.84 (s, 2H), 3.52 (brs, 4H), 3.46 (brs, 4H).; ^{13}C NMR (175 MHz, DMSO-d_6, δ): 158.5, 158.4 (q, *J* = 36.9 Hz), 155.7, 153.2, 150.9, 145.9 (d, *J* = 9.5 Hz), 144.6 (d, *J* = 9.5 Hz), 139.6 (d, *J* = 13.7 Hz), 138.2, 134.2, 133.5, 132.7, 132.4, 128.3, 127.7, 127.6, 126.8, 126.5, 126.3, 126.0, 115.7 (q, *J* = 289.6 Hz), 111.5 (d, *J* = 20.8 Hz), 111.1, 108.4, 65.8 (x2), 46.5, 44.5 (x2), 38.4.

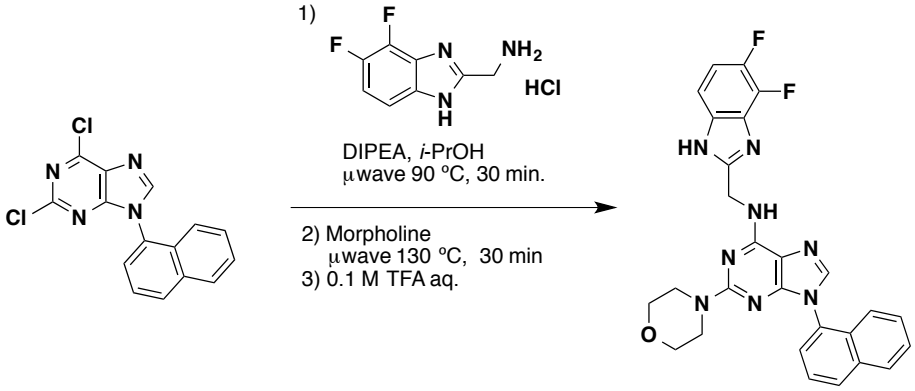
_N_-((4,5-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-9-(3-fluorophenethyl)-2-morpholino-9Hpurin-6-amine trifluoroacetate salt (SR-3451)



Synthesized according to general procedure C from 2,6-dichloro-9-(3-fluorophenethyl)-*9H*-purine in 72% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.26 (brs, 1H), 8.07 (s, 1H), 7.32-7.21 (m, 3H), 7.08-7.00 (m, 2H), 6.96 (d, *J* = 7.6 Hz, 1H), 4.86 (s, 2H), 4.35 (t, *J* = 7.0 Hz, 2H), 3.52 (s, 4H), 3.50 (s, 4H), 3.15 (t, *J* = 7.0 Hz, 2H).; ^{13}C NMR (175 MHz, DMSO-d_6, δ): 162.6 (d, *J* = 241.3 Hz), 158.9, 158.8 (q, *J* = 36.4 Hz), 156.0, 153.0, 151.1, 146.4 (d, *J* = 9.5 Hz), 145.1 (d, *J* = 9.3 Hz), 141.2 (d, *J* = 7.2 Hz), 140.0 (d, *J* = 15.2 Hz), 138.6 (d, *J* = 13.7 Hz), 138.4, 134.0, 130.7 (d, *J* = 8.6 Hz), 129.3, 125.3, 116.0 (q, *J* = 289.4 Hz), 115.9 (d, *J* = 20.8 Hz), 113.8 (d, *J* = 20.3 Hz), 112.1 (d, *J* = 20.1 Hz), 110.0, 109.0, 66.3

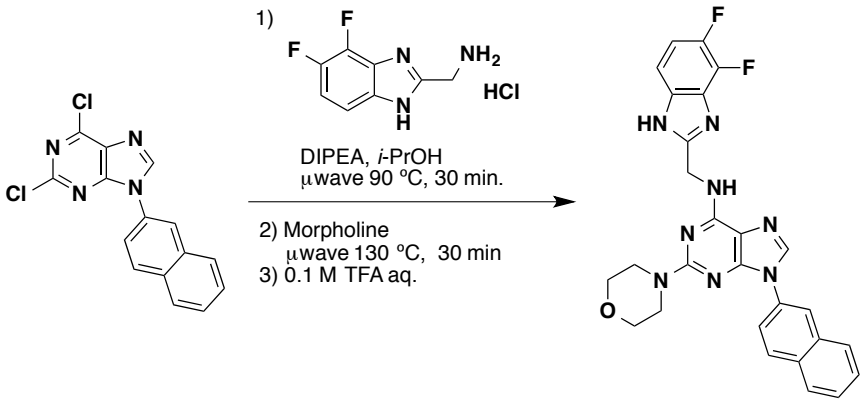
(x2), 44.9 (x2), 44.6, 38.8, 34.8.; HRMS (ESI-TOF) calculated for $C_{25}H_{24}N_8OF_3$ $(M+H)^+$ 509.2025, found 509.2022.

***N*-((4,5-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-2-morpholino-9-(naphthalen-1-yl)-9Hpurin-6-amine (SR-4132)**



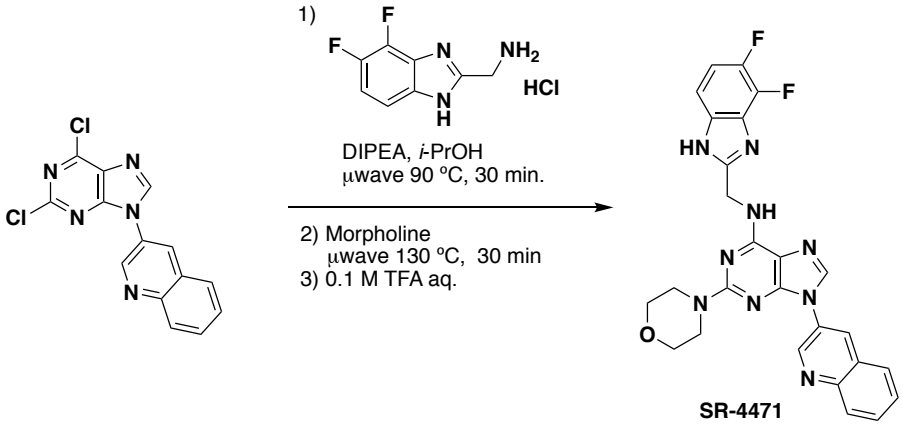
Synthesized according to general procedure C from 2,6-dichloro-9-(naphthlen-1-yl)-*9H*-purine in 53% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ): 8.37 (brs, 1H), 8.13 (s, 1H), 8.11 (dd, *J* = 14.0, 8.2 Hz), 7.69 (app t, *J* = 7.6 Hz), 7.64-7.60 (m, 2H), 7.57- 7.53 m, 1H), 7.39 (d, *J* = 8.4 Hz), 7.31-7.20 (m, 2H), 4.89 (s, 2H), 3.34 (s, 4H), 3.29 (s, 4H); ^{13}C NMR (175 MHz, DMSO-*d*$_6$, δ): 158.6, 158.3, (q, *J* = 36.4 Hz), 156.1, 154.1, 152.5. 146.0 (d, *J* = 9.5 Hz), 144.6 (q, *J* = 9.3 Hz), 139.6 (d, *J* = 15.2 Hz), 139.2, 138.2 (d, *J* = 15.2 Hz), 133.8, 133.5, 131.2, 129.3 (d, *J* = 7.4 Hz), 128.3, 127.2, 126.8, 125.7, 125.4, 122.7, 115.7 (q, *J* = 289.6 Hz), 112.9, 111.5 (d, *J* = 20.8 Hz), 108.4, 65.7 (x2), 44.3 x2).

***N*-((4,5-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-2-morpholino-9-(naphthalen-2-yl)-9Hpurin-6-amine (SR-4133)**



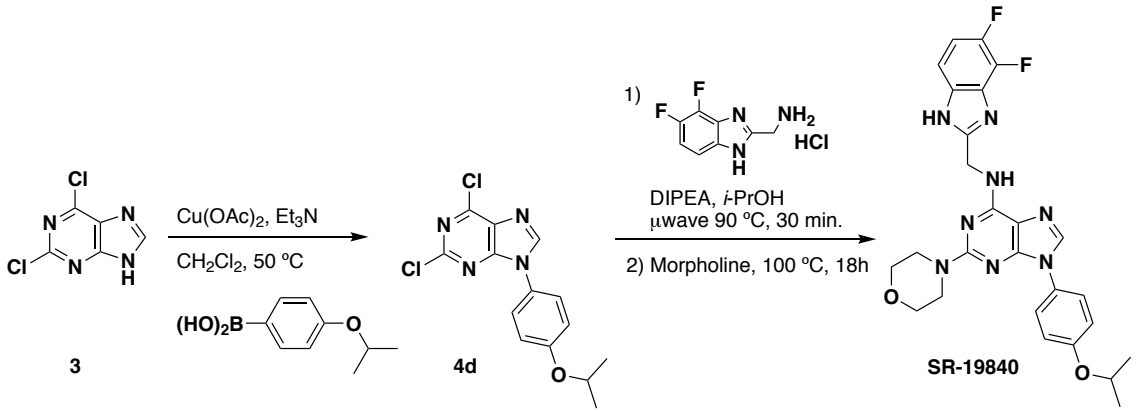
Synthesized according to general procedure C from 2,6-dichloro-9-(naphthlen-2-yl)-*9H*-purine in 74% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.48 (s, 1H), 8.46 (s, 1H), 8.33 (brs,1H), 8.13-8.06 (m, 2H), 8.00-7.99 (m, 2H), 7.62-7.55 (m, 2H), 7.29-7.19 (m, 2H), 4.87 (s, 2H), 3.52 (s, 4H), 3.49 (s, 4H).; ^{13}C NMR (175 MHz, DMSO-d_6, δ): 158.6, 158.3 (q, J = 35.2 Hz), 156.0, 154.1, 150.9, 146.0 (d, J = 9.5 Hz), 144.6 (d, J = 9.3 Hz), 139.5 (d, J = 14.4 Hz), 138.1 (d, J = 14.4 Hz), 137.2, 133.4, 133.1, 133.0, 131.4, 129.2, 127.9, 127.7, 127.0, 126.4, 121.1, 119.8, 115.6 (q, J = 289.4 Hz), 113.9, 111.7 (d, J = 21.0 Hz), 108.4, 65.9, 44.5.; HRMS (ESI-TOF) calculated for $C_{27}H_{23}N_8OF_2$ $(M+H)^+$ 513.1963, found 513.1971.

N-((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-2-morpholino-9-(quinolin-3-yl)-9H-purin-6-amine (SR-4471)



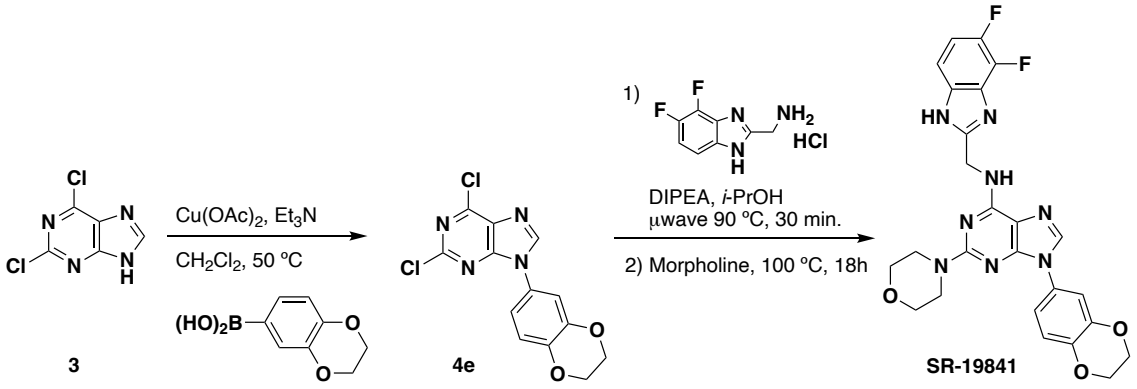
Synthesized according to general procedure C from 3-(2,6-dichloro-9H-purin-9-yl)quinoline (white solid, 120 mg, 67% yield). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 9.49 (d, J = 2.5 Hz, 1H), 8.87 (d, J = 2.5 Hz, 1H), 8.54 (s, 1H), 8.40 (s, 1H), 8.09 (td, J = 7.4, 6.6, 1.2 Hz, 2H), 7.83 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.71 (ddd, J = 8.1, 6.8, 1.2 Hz, 1H), 7.32 – 7.17 (m, 2H), 4.88 (s, 2H), 3.50 (d, J = 16.9 Hz, 8H); ^{13}C NMR (151 MHz, DMSO-d, δ): 159.25, 159.14, 159.01, 158.77, 156.43, 154.65, 151.33, 146.53 (d, J = 9.5 Hz), 146.10, 145.73, 139.36 (d, J = 251.3 Hz), 137.25, 133.89, 130.32, 129.13, 128.75, 128.17, 127.89, 117.19, 115.26, 114.27, 112.07 (d, J = 20.3 Hz), 108.83, 66.32, 44.92, 38.81; HRMS (ESI-TOF) calculated for $C_{26}H_{22}N_9OF_2$ $(M+H)^+$ 514.1915, found 514.1898.

N-((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-9-(4-isopropoxyphenyl)-2-morpholino-9H-purin-6-amine (SR-19840)



Synthesized according to the general procedure B followed by the general procedure D (100 ºC, oil bath heating, for 18 h in step 2). Product isolated as a white solid (63 mg, 77% yield from **4d**, 11% yield from **3** to **4d**). ^{1}H NMR (400 MHz, DMSO-*d*, δ): 8.28 (d, *J* = 14.3 Hz, 2H), 7.76 – 7.64 (m, 2H), 7.34 – 7.15 (m, 2H), 7.12 – 7.00 (m, 2H), 4.90 (s, 2H), 4.66 (hept, *J* = 6.0 Hz, 1H), 3.47 (s, 8H), 1.28 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (151 MHz, DMSO-*d*$_6$, δ): 158.55, 156.27, 156.00, 154.06, 150.68, 145.18 (dd, *J* = 234.8, 9.3 Hz), 139.00 (dd, *J* = 250.4, 14.9 Hz), 137.19, 133.74, 129.47, 128.15, 123.98, 116.12, 113.54, 111.28 (d, *J* = 21.2 Hz), 108.19, 69.54, 65.87, 44.48, 38.41, 21.77; HRMS (ESI-TOF) calculated for $C_{26}H_{27}N_8O_2F_2$ $(M+H)^+$ 521.2225, found 521.2206.

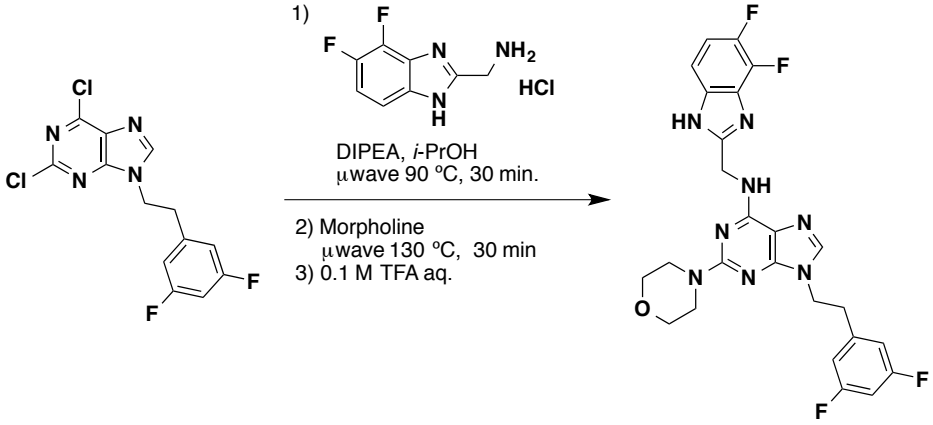
N-((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-9-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-morpholino-9H-purin-6-amine (SR-19841)



Synthesized according to the general procedure B followed by the general procedure D (100 ºC, oil bath heating, for 18 h in step 2). ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ): 8.29 (s, 2H), 7.42 (d, *J* = 2.6 Hz, 1H), 7.36 – 7.21 (m, 3H), 7.01 (d, *J* = 8.7 Hz, 1H), 4.89 (s, 2H), 4.29 (s, 4H), 3.46 (s, 8H); ^{13}C NMR (151 MHz, DMSO-*d*$_6$, δ): 170.04, 158.98, 156.47, 154.49, 151.05, 150.19, 146.83 – 144.78 (m), 144.01, 143.78, 143.14, 142.78, 137.61, 133.63, 131.01, 130.11, 129.35, 127.75, 127.26, 124.31, 123.40, 117.98, 115.69, 114.08,

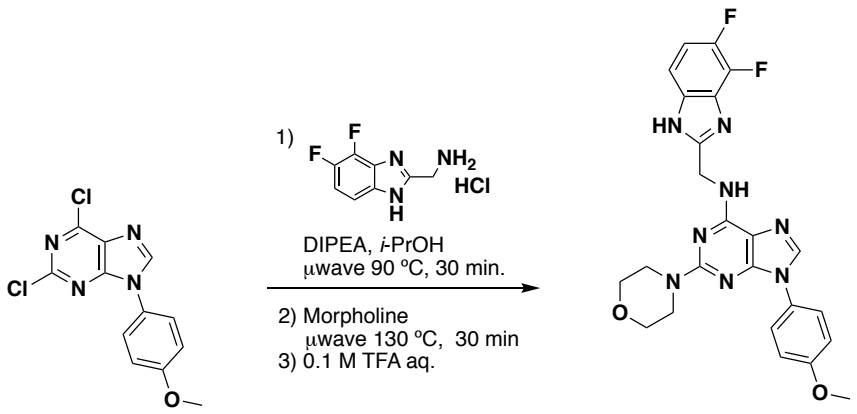
111.84, 108.77, 66.32, 64.70, 64.55, 44.95, 38.84; HRMS (ESI-TOF) calculated for $C_{25}H_{23}N_8O_3F_2$ $(M+H)^+$ 521.1861, found 521.1852.

_N_-((4,5-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-9-(3,5-difluorophenethyl)-2-morpholino-9Hpurin-6-amine (SR-4290)



Synthesized according to general procedure C from 2,6-dichloro-9-(3,5-difluorophenethyl)-*9H*-purine in 74% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.15 (brs, 1H), 7.91 (s, 1H), 7.28-7.18 (m, 2H), 7.08-7.03 (m, 1H), 6.95-6.91 (m, 1H), 4.83 (s, 2H), 4.33 (t, J = 7.0 Hz), 3.51 (brs, 8H), 3.15 (t, J = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 162.3 (d, J = 244.3 Hz), 162.2 (t, J = 244.3 Hz), 158.5 (q, J = 35.4 Hz), 158.4, 155.7, 153.0, 150.8, 145.9 (d, J = 9.5 Hz),.144.5 (d, J = 9.5 Hz), 142.6 (t, J = 9.0 Hz), 139.7 (d, J = 15.2 Hz), 138.3 (d, J = 15.2 Hz), 138.1, 133.8, 129.4, 115.9 (d, J = 290.4 Hz), 112.0 (d, J = 4.4 Hz), 111.9 (d, J = 4.4 Hz), 110.8, 108.3, 102.0 (t, J = 25.3 Hz), 65.9 (x2), 44.5 (x2), 43.5, 38.4.

N-((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-9-(4-methoxyphenyl)-2-morpholino-9H-purin-6-amine (SR-4310)



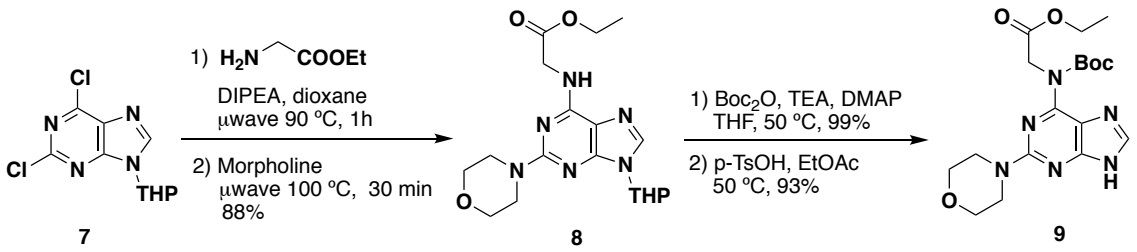
Synthesized according to general procedure C from 2,6-dichloro-9-(4-methoxyphenyl)-9*H*-purine in 72% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.28 (s, 1H), 7.84 – 7.65 (m, 2H), 7.35 – 7.17 (m, 2H), 7.13 – 7.05 (m, 2H), 4.87 (s, 2H), 3.81 (s, 3H), 3.46 (m, 8H). HRMS (ESI-TOF) calculated for $C_{24}H_{23}N_8O_2F_2$ $(M+H)^+$ 493.1912, found 493.193.

2,6-dichloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (7)

3 7

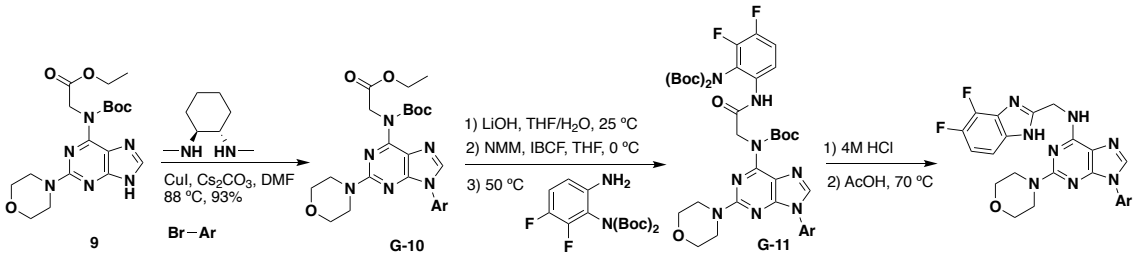
To a solution of 2,6-dichloropurine (5000 mg, 26.5 mmol, 1 eq.) was added *p*-TsOH monohydrate (50 mg, 1 mmol, 0.01 eq.). This mixture was heated to 50 ºC and 3,4-dihydro-2*H*-pyran was added at this temperature. After stirring 30 min at 50 ºC, the mixture is cooled to 25 ºC, dried down under reduced pressure, and subjected to flash chromatography (0% EtOAc in Hexanes to 10% EtOAc in Hexanes). Material isolated as a tan solid, 7000 mg, 50% yield. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.94 (s, 1H), 5.74 (dd, J = 10.8, 2.3 Hz, 1H), 4.02 (m, 1H), 3.82 – 3.69 (m, 1H), 2.26 (m, 1H), 2.06 – 1.93 (m, 2H), 1.77 (m, 1H), 1.60 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6, δ): 152.68, 151.23, 149.93, 146.35, 130.54, 81.66, 67.76, 29.71, 24.38, 22.03.

Ethyl N-(tert-butoxycarbonyl)-N-(2-morpholino-9H-purin-6-yl)glycinate (9)



The compound (**8**, white solid) was prepared using the General method D with glycine ethyl ester. To a solution of **8** (1200 mg, 3.1 mmol, 1 eq.) in THF (15 mL, 0.2 M) was added Boc anhydride (1350 mg, 6.1 mmol, 2 eq.), triethylamine (470 μL, 3.4 mmol, 1.1 eq.) and DMAP (38 mg, 0.3 mmol, 0.1 eq.). This mixture is heated to 50 ºC overnight. The cooled reaction mixture was concentrated under reduced pressure and subjected to flash chromatography to produce Boc-protected **8** as an off-white solid (1500 mg, 99% yield). To a solution of Boc-protected **8** (1400 mg, 2.9 mmol, 1 eq.) in ethanol (30 mL, 0.1 M) was added *p*-TsOH (600 mg, 3.1 mmol, 1.1 eq.). This mixture was heated to 50 ºC until the consumption of the reactant was observed by LCMS. A small amount of product without the Boc protecting group is also observed during the reaction conditions. The cooled reaction mixture was concentrated under reduced pressure and subjected to flash chromatography (0% MeOH in CH_2Cl_2 to 10% MeOH in CH_2Cl_2). Material isolated as a tan solid, 1100 mg, 93% yield. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.19 (s, 1H), 4.52 (s, 2H), 4.13 (q, J = 7.2 Hz, 2H), 3.70 – 3.54 (m, 11H), 1.43 (s, 9H), 1.18 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6, δ): 169.64, 158.30, 158.06, 153.11, 150.03, 141.95, 128.53, 125.98, 115.48, 82.49, 66.46, 66.40, 61.04, 60.71, 48.69, 45.14, 45.05, 27.96, 14.53.

General procedure E for the Preparation of SR-4931, SR-4932, and SR-4933



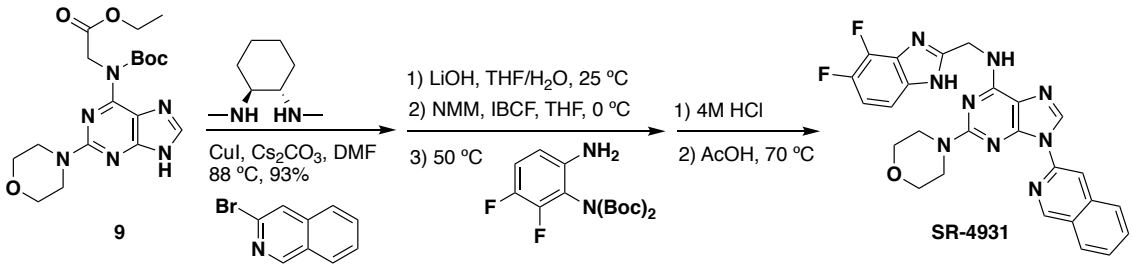
Aryl bromide coupling. A Biotage microwave vial containing a solution of the substituted purine **9** (1 eq.), aryl bromide (2 eq.), and cesium carbonate (3 eq.) in DMF (0.1 M) was degassed by bubbling argon through the mixture for 30 min. The trans-*N,N'*-dimethylcyclohexane-1,2-diamine and copper iodide were subsequently added and degassed another 5 min. The vial was sealed and heated overnight at 88 ºC in an oil bath. The next day water was added to the cooled mixture which was subsequently extracted 3 times with ethyl acetate. After washing with brine, the combined organic layers were dried over sodium sulfate and purified using flash chromatography to give the N9 substituted compound **G-10**.

Saponification of Glycine Ethyl Ester Substituted Purines. To a solution of **G-10** (1 eq.) in THF (0.2 M) was added a 2.2 M solution of LiOH in water (4 eq.). This mixture was stirred at 25 ºC until the consumption of starting material was observed by LCMS. After this point, 1 M HCl was added, and the product was extracted 3 times with ethyl acetate. After washing with slightly acidic brine, the combined organic layers were dried over sodium sulfate. After drying under reduced pressure, the product was used without further purification.

Amide coupling. To a solution of the product from Saponification (1 eq.) in THF (0.2 M) was added N-methylmorpholine (1.4 eq.). This mixture was cooled to 0 ºC and isobutyl chloroformate (1.2 eq.) was added dropwise. This mixture was stirred at 0 ºC for 15 min. After this point, a solution of *N*-*N*-tert-butoxycarbonyl-3,4-difluorobenzene-1,2-diamine (1.2 eq.) in THF (0.2 M) was added at 0 ºC then heated to 50 ºC overnight. The cooled reaction mixture was concentrated under reduced pressure and then the crude product was partially purified by flash chromatography to give the final product as a TFA salt (**G-11**).

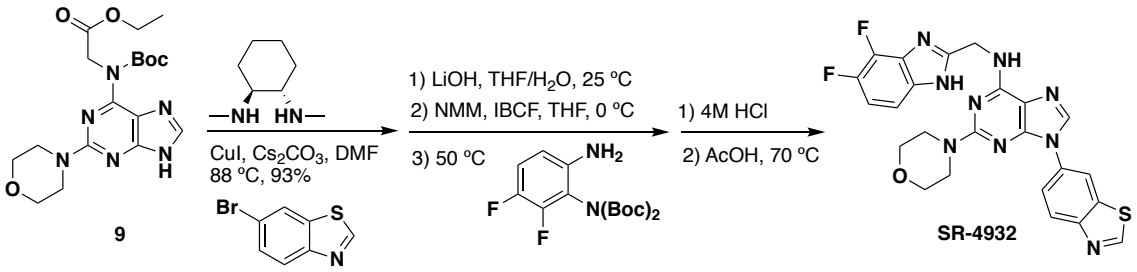
Cyclization of Substituted Glycine Derivatives to Benzimidazoles. To a mixture of the starting material **G-11** in 4 M HCl in dioxane (0.2 M) was added 4 M HCl in water (0.2 M) This mixture was stirred until the complete removal of all Boc protecting groups was observed by LCMS. After drying under reduced pressure, acetic acid is added, and the mixture is heated to 70 ºC overnight. The cooled reaction mixture was concentrated under reduced pressure and then purified by HPLC to give the final product.

N-((4,5-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-9-(isoquinolin-3-yl)-2-morpholino-9H-purin-6-amine (SR-4931)



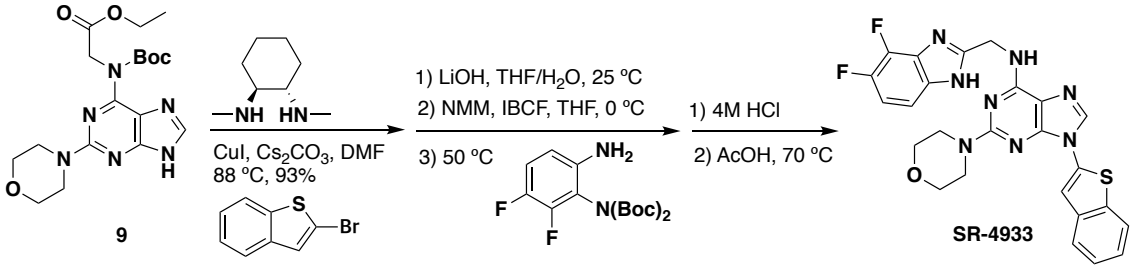
Synthesized according to the general procedure E with 3-bromoisoquinoline. ^{1}H NMR (600 MHz, DMSO-*d*$_6$, δ): 9.35 (s, 1H), 8.90 (s, 1H), 8.75 (s, 1H), 8.34 (s, 1H), 8.20 (d, *J* = 8.2 Hz, 1H), 8.18 – 8.09 (m, 1H), 7.88 – 7.81 (m, 1H), 7.69 (dd, *J* = 8.2, 6.9 Hz, 1H), 7.26 (dd, *J* = 8.8, 3.8 Hz, 1H), 7.20 (ddd, *J* = 11.1, 8.7, 7.0 Hz, 1H), 4.98 – 4.75 (m, 2H), 3.62 (s, 8H); ^{13}C NMR (151 MHz, DMSO-*d*$_6$, δ): 159.17, 158.95, 158.72, 158.48, 156.39, 154.72, 152.60, 152.16, 150.78, 145.56 (dd, *J* = 234.0, 9.6 Hz), 144.16, 137.35, 136.05, 132.05, 131.51, 128.42, 127.69, 127.47, 126.52, 115.10, 111.44, 109.45, 108.43, 107.01, 66.46, 45.11, 39.02; HRMS (ESI-TOF) calculated for $C_{26}H_{22}N_9OF_2$ $(M+H)^+$ 514.1915, found 514.1916.

9-(benzo[d]thiazol-6-yl)-N-((4,5-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-2-morpholino-9H-purin-6-amine (SR-4932)



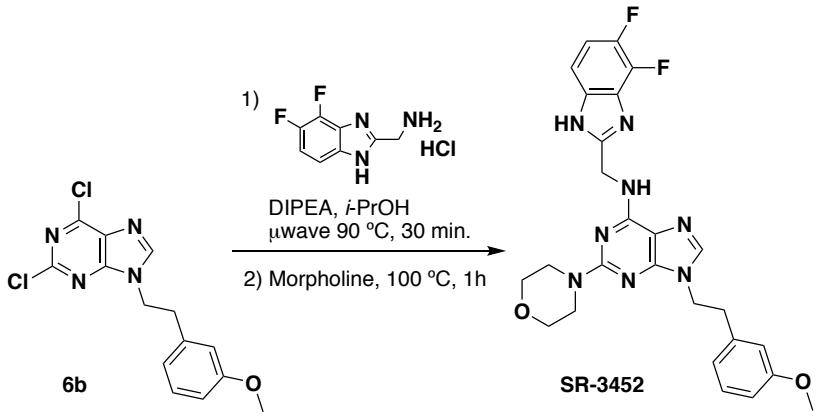
Synthesized according to the general procedure E with 6-bromobenzo[*d*]thiazole. ^{1}H NMR (600 MHz, DMSO-*d*$_6$, δ): 9.51 (s, 1H), 8.64 (d, *J* = 2.1 Hz, 1H), 8.47 (s, 1H), 8.35 (d, *J* = 8.7 Hz, 1H), 8.32 – 8.24 (m, 1H), 8.06 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.25 (dd, *J* = 8.9, 3.8 Hz, 1H), 7.19 (ddd, *J* = 11.4, 8.7, 7.1 Hz, 1H), 4.95 – 4.79 (m, 2H), 3.50 (d, *J* = 26.7 Hz, 8H); ^{13}C NMR (151 MHz, DMSO-*d*$_6$, δ): 174.27, 159.13, 158.78, 156.43, 155.14, 154.79, 154.01, 151.27, 145.50 (dd, *J* = 234.2, 9.8 Hz), 137.69, 134.57, 132.31, 130.08, 123.81, 120.51, 116.73, 114.57, 111.35 (d, *J* = 21.3 Hz), 108.29 (d, *J* = 23.5 Hz), 66.35, 44.99, 39.06; HRMS (ESI-TOF) calculated for $C_{24}H_{20}N_9OF_2S$ $(M+H)^+$ 520.1480, found 520.1479.

9-(benzo[b]thiophen-2-yl)-N-((4,5-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-2-morpholino-9H-purin-6-amine (SR-4933)



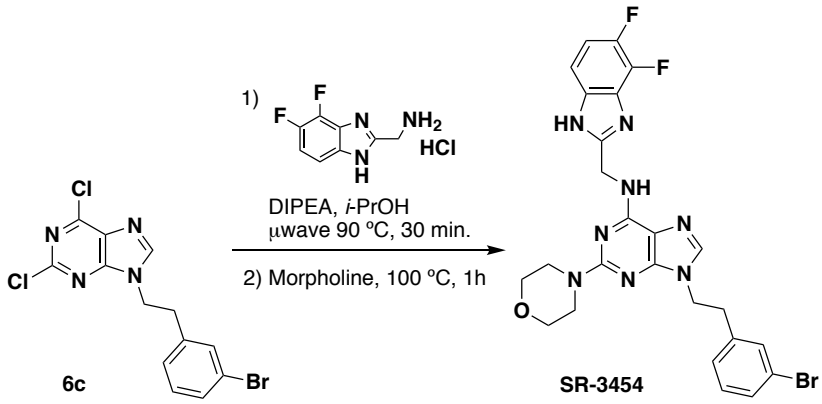
Synthesized according to the general procedure E with 2-bromobenzo[*b*]thiophene. ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ): 8.53 (d, *J* = 9.5 Hz, 1H), 8.42 (s, 1H), 8.00 (d, *J* = 7.9 Hz, 1H), 7.83 (d, *J* = 6.7 Hz, 2H), 7.38 (dt, *J* = 23.7, 7.4 Hz, 2H), 7.24 (ddt, *J* = 20.0, 11.3, 6.2 Hz, 2H), 4.89 (s, 2H), 3.55 (d, *J* = 32.1 Hz, 8H); ^{13}C NMR (151 MHz, DMSO-*d*$_6$, δ): 159.10, 156.32, 154.69, 150.58, 145.61 (dd, *J* = 234.3, 9.8 Hz), 139.57 (dd, *J* = 249.1, 13.9 Hz), 137.66, 136.60, 136.40, 136.31, 134.38, 130.27, 125.54, 124.86 (d, *J* = 2.9 Hz), 123.77, 122.87, 117.28, 115.34, 113.69 (d, *J* = 22.6 Hz), 112.24, 111.65 (d, *J* = 20.9 Hz), 108.60, 66.36, 45.01, 38.99; HRMS (ESI-TOF) calculated for $C_{25}H_{21}N_8OF_2S$ $(M+H)^+$ 519.1527, found 519.1526.

N-((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-9-(3-methoxyphenethyl)-2-morpholino-9H-purin-6-amine (SR-3452)



Synthesized according to general procedure D from **6b** (heating in oil bath for 60 min) in 70% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ): 8.20 (s, 1H), 7.97 (s, 1H), 7.32 – 7.11 (m, 3H), 6.80 – 6.67 (m, 3H), 4.84 (s, 2H), 4.31 (t, *J* = 7.1 Hz, 2H), 3.51 (m, 8H), 3.69 (s, 3H), 3.09 (t, *J* = 7.1 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-*d*$_6$, δ): 159.76, 158.84 (d, *J* = 36.0 Hz), 156.14, 152.29 (d, *J* = 327.7 Hz), 145.68 (d, *J* = 234.8 Hz), 140.38, 139.93, 138.55, 134.30, 129.95, 121.36, 117.27, 115.33, 114.71, 112.46, 111.83 (d, *J* = 20.9 Hz), 108.80, 66.33, 55.33, 44.95, 44.67, 38.90, 35.17; HRMS (ESI-TOF) calculated for $C_{26}H_{27}N_8O_2F_2$ $(M+H)^+$ 521.2225, found 521.2244.

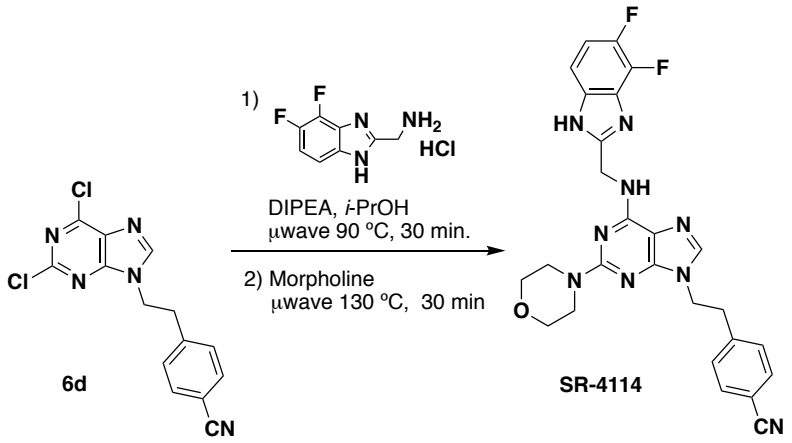
9-(3-bromophenethyl)-N-((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-2-morpholino-9H-purin-6-amine (SR-3454)



Synthesized according to general procedure D from **6c** (heating in oil bath for 60 min) in 63% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ): 8.20 (s, 1H), 7.99 (s, 1H), 7.46 (t, *J* = 1.8 Hz, 1H), 7.39 (ddd, *J* = 7.9, 2.1, 1.1 Hz, 1H), 7.32 – 7.16 (m, 3H), 7.11 (dt, *J* = 7.6, 1.3 Hz, 1H), 4.84 (s, 2H), 4.32 (t, *J* = 7.0 Hz, 2H), 3.50 (s, 8H), 3.12 (t, *J* = 7.0 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-*d*$_6$, δ): 159.76, 159.20, 158.96, 158.72, 156.14, 152.29 (d, *J* = 327.7 Hz), 145.68 (d, *J* = 234.8 Hz), 140.38, 139.93, 138.55 , 134.30,

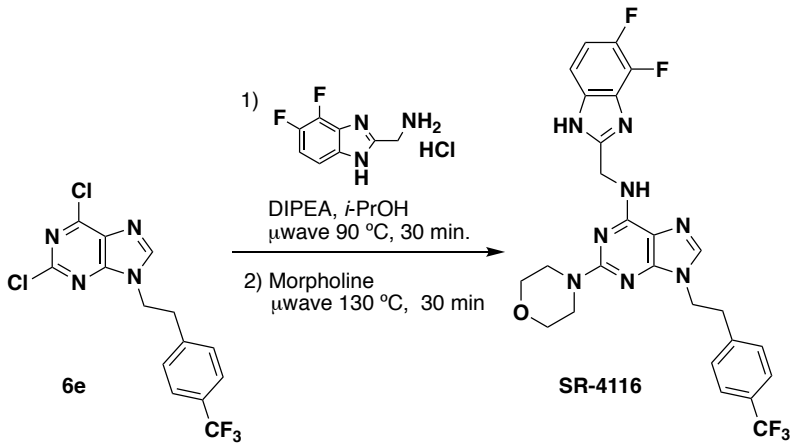
129.95, 121.36, 117.27, 115.33, 114.71, 112.46, 111.83 (d, J = 20.9 Hz), 108.80, 66.33, 55.33, 44.95, 44.67, 38.90, 35.17; HRMS (ESI-TOF) calculated for $C_{25}H_{24}N_8OF_2Br$ $(M+H)^+$ 569.1225, found 569.1239.

4-(2-(6-(((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)amino)-2-morpholino-9H-purin-9-yl)ethyl)benzonitrile (SR-4114)



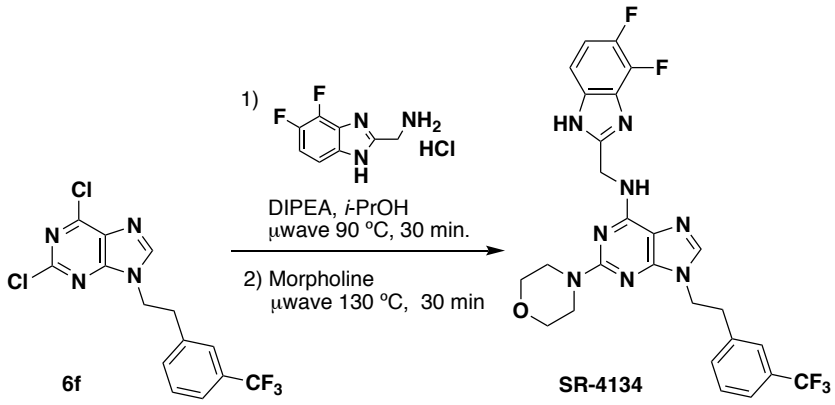
Synthesized according to general procedure D from **6d** in 60% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.16 (s, 1H), 7.91 (s, 1H), 7.72 (d, J = 8.3 Hz, 2H), 7.38 – 7.31 (m, 2H), 7.31 – 7.16 (m, 2H), 4.83 (s, 2H), 4.34 (t, J = 6.8 Hz, 2H), 3.49 (s, 8H), 3.21 (t, J = 6.9 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6, δ): 158.74, 158.50, 158.30 (d, J = 9.9 Hz), 158.03, 155.76, 153.09, 150.85, 145.18 (dd, J = 234.4, 9.8 Hz), 144.18, 138.11, 133.84, 132.29, 129.90, 118.87, 116.86, 114.92, 111.32 (d, J = 20.8 Hz), 109.39, 108.28, 65.89, 44.46, 43.65, 38.42, 34.92; HRMS (ESI-TOF) calculated for $C_{26}H_{24}N_9OF_2$ $(M+H)^+$ 516.2072, found 516.2065.

N-((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-2-morpholino-9-(4-(trifluoromethyl)phenethyl)-9H-purin-6-amine (SR-4116)



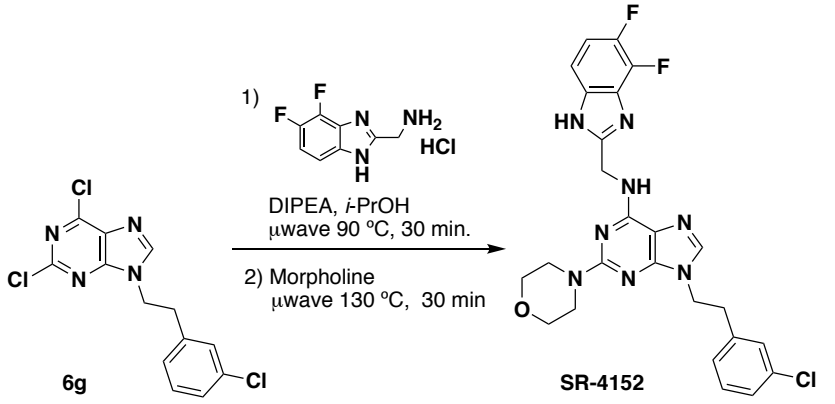
Synthesized according to general procedure D from **6e** in 75% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ): 8.15 (s, 1H), 7.90 (s, 1H), 7.62 (d, *J* = 8.1 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.31 – 7.15 (m, 2H), 4.82 (s, 2H), 4.34 (t, *J* = 7.0 Hz, 2H), 3.49 (s, 8H), 3.22 (t, *J* = 7.0 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-*d*$_6$, δ): 158.66, 158.43, 155.78, 153.10, 150.86, 145.18 (dd, *J* = 234.6, 9.4 Hz), 143.05, 138.15, 133.86, 129.61, 127.61 – 126.76 (m), 125.24 (d, *J* = 3.7 Hz), 123.45, 121.65, 117.03, 115.09, 114.30, 113.15, 111.29 (d, *J* = 21.1 Hz), 108.27, 65.88, 44.48, 43.85, 38.45, 34.65; HRMS (ESI-TOF) calculated for $C_{26}H_{24}N_8OF_5$ $(M+H)^+$ 559.1993, found 559.2005.

N-((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-2-morpholino-9-(3-(trifluoromethyl)phenethyl)-9H-purin-6-amine (SR-4134)



Synthesized according to general procedure D from **6f** in 72% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.13 (s, 1H), 7.90 (s, 1H), 7.56 (d, J = 10.8 Hz, 2H), 7.52 – 7.39 (m, 2H), 7.30 – 7.15 (m, 2H), 4.82 (s, 2H), 4.34 (t, J = 7.0 Hz, 2H), 3.49 (s, 8H), 3.22 (t, J = 7.0 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6, δ): 158.41, 155.80, 153.08, 146.00, 145.93, 144.44, 144.38, 139.3, 139.54, 138.24, 133.85, 132.97, 129.46, 129.39, 129.25, 129.04, 128.84, 126.94, 125.36, 125.32, 125.13, 123.32, 123.28, 121.52, 111.40, 111.26, 108.31, 65.87, 63.32, 44.46, 43.85, 42.84, 40.03, 38.44, 34.59; HRMS (ESI-TOF) calculated for $C_{26}H_{24}N_8OF_5$ $(M+H)^+$ 559.1993, found 559.1992.

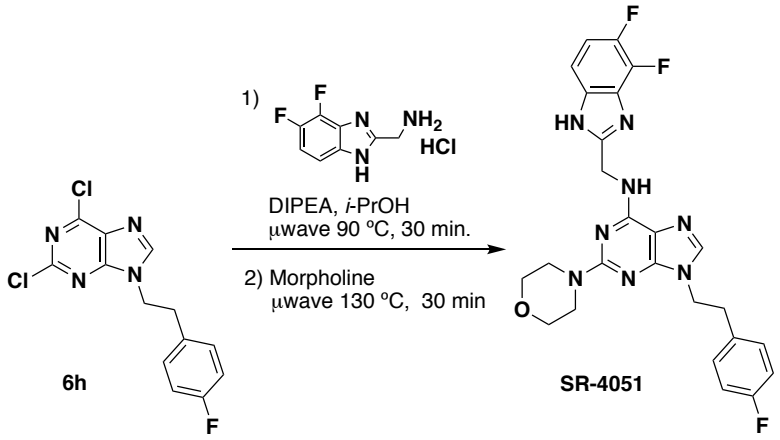
9-(3-chlorophenethyl)-N-((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-2-morpholino-9H-purin-6-amine (SR-4152)



Synthesized according to general procedure D from **6g** in 63% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.17 (s, 1H), 7.94 (s, 1H), 7.34 – 7.13 (m, 5H), 7.13 – 7.04 (m, 1H), 4.83 (s, 2H), 4.32 (t, J = 7.0 Hz, 2H), 3.50 (s, 8H), 3.13 (t, J = 7.0 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6, δ): 158.96, 158.72,

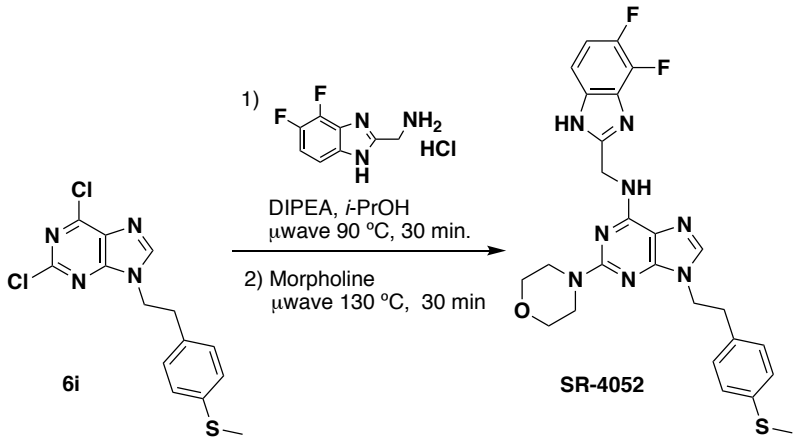
158.49, 158.25, 155.72, 152.92, 150.77, 145.22 (dd, J = 234.8, 9.4 Hz), 140.57, 139.85 (d, J = 15.5 Hz), 138.07, 133.77, 133.05, 130.22, 129.46, 128.63, 128.49, 127.50, 126.56, 111.40 (d, J = 21.5 Hz), 110.54, 108.33, 65.90, 44.48, 43.91, 38.43, 34.43; HRMS (ESI-TOF) calculated for $C_{25}H_{24}N_8OF_2Cl$ $(M+H)^+$ 525.1730, found 525.1755.

N-((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-9-(4-fluorophenethyl)-2-morpholino-9H-purin-6-amine (SR-4051)



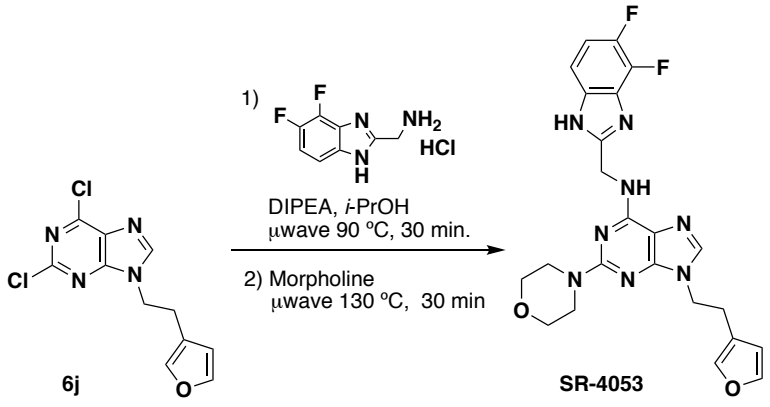
Synthesized according to general procedure D from **6h** in 80% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.18 (s, 1H), 7.92 (s, 1H), 7.31 – 7.13 (m, 4H), 7.13 – 7.03 (m, 2H), 4.83 (s, 2H), 4.29 (t, J = 7.0 Hz, 2H), 3.51 (s, 9H), 3.10 (t, J = 7.1 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6, δ): 162.30, 160.69, 159.40, 159.17, 158.93, 158.70, 156.13, 153.29, 151.17, 145.67 (dd, J = 234.8, 9.7 Hz), 139.43 (d, J = 274.6 Hz), 134.54, 134.20, 131.02 (d, J = 8.4 Hz), 129.86, 117.38, 115.63 (d, J = 20.9 Hz), 111.86 (d, J = 21.0 Hz), 110.73, 108.79, 66.33, 44.92, 38.88, 34.37; HRMS (ESI-TOF) calculated for $C_{25}H_{24}N_8OF_3$ $(M+H)^+$ 509.2025, found 509.2022.

N-((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-9-(4-(methylthio)phenethyl)-2-morpholino-9H-purin-6-amine (SR-4052)



Synthesized according to general procedure D from **6i** in 60% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.16 (s, 1H), 7.91 (s, 1H), 7.31 – 7.17 (m, 2H), 7.20 – 7.05 (m, 4H), 4.83 (s, 2H), 4.28 (t, J = 6.9 Hz, 2H), 3.51 (s, 8H), 3.07 (t, J = 7.1 Hz, 2H), 2.42 (s, 3H); ^{13}C 13C NMR (151 MHz, DMSO-d_6, δ): 159.42, 159.18, 158.94, 158.70, 156.08, 153.14, 151.11, 145.70 (dd, J = 235.2, 9.8 Hz), 138.48, 136.53, 134.93, 134.16, 129.78, 126.56, 117.33, 115.40, 111.94 (d, J = 21.4 Hz), 110.27, 108.85, 66.32, 44.91, 38.87, 34.58, 15.20; HRMS (ESI-TOF) calculated for $C_{26}H_{27}N_8OF_2S$ $(M+H)^+$ 537.1997, found 537.2012.

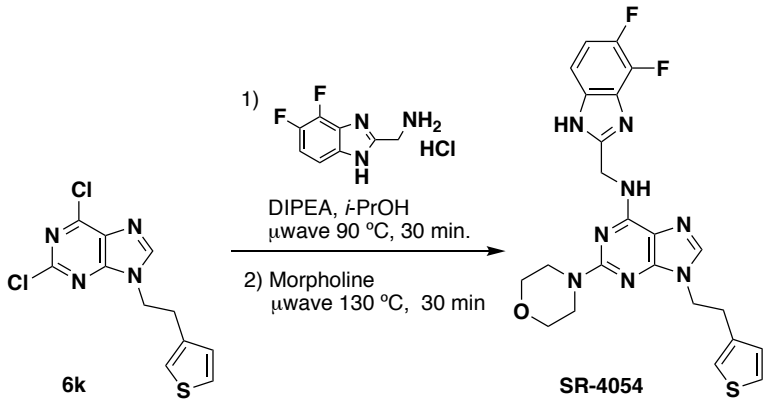
N-((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-9-(2-(furan-3-yl)ethyl)-2-morpholino-9H-purin-6-amine (SR-4053)



Synthesized according to general procedure D from **6j** in 80% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.19 (s, 1H), 8.00 (s, 1H), 7.57 (t, J = 1.7 Hz, 1H), 7.45 – 7.40 (m, 1H), 7.31 – 7.15 (m, 2H), 6.36 (dd, J = 1.8, 0.9 Hz, 1H), 4.84 (s, 2H), 4.26 (t, J = 7.0 Hz, 2H), 3.51 (m, 8H), 2.94 (t, J = 6.9 Hz, 2H);

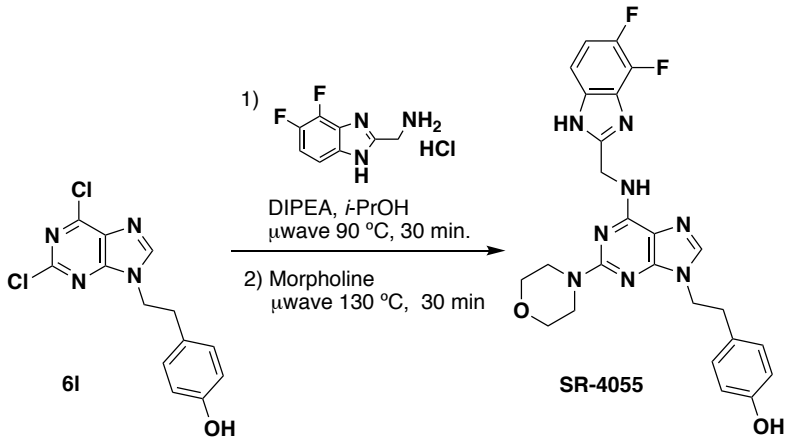
^{13}C NMR (151 MHz, DMSO-d_6, δ): 159.42, 159.18, 158.94, 158.70, 156.08, 153.14, 151.11, 145.70 (dd, J = 235.2, 9.8 Hz), 138.48, 136.53, 134.93, 134.16, 129.78, 126.56, 117.33, 115.40, 111.94 (d, J = 21.4 Hz), 110.27, 108.85, 66.32, 44.91, 38.87, 34.58, 15.20; HRMS (ESI-TOF) calculated for $C_{23}H_{23}N_8O_2F_2$ $(M+H)^+$ 481.1912, found 481.1898.

N-((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)-2-morpholino-9-(2-(thiophen-3-yl)ethyl)-9H-purin-6-amine (SR-4054)



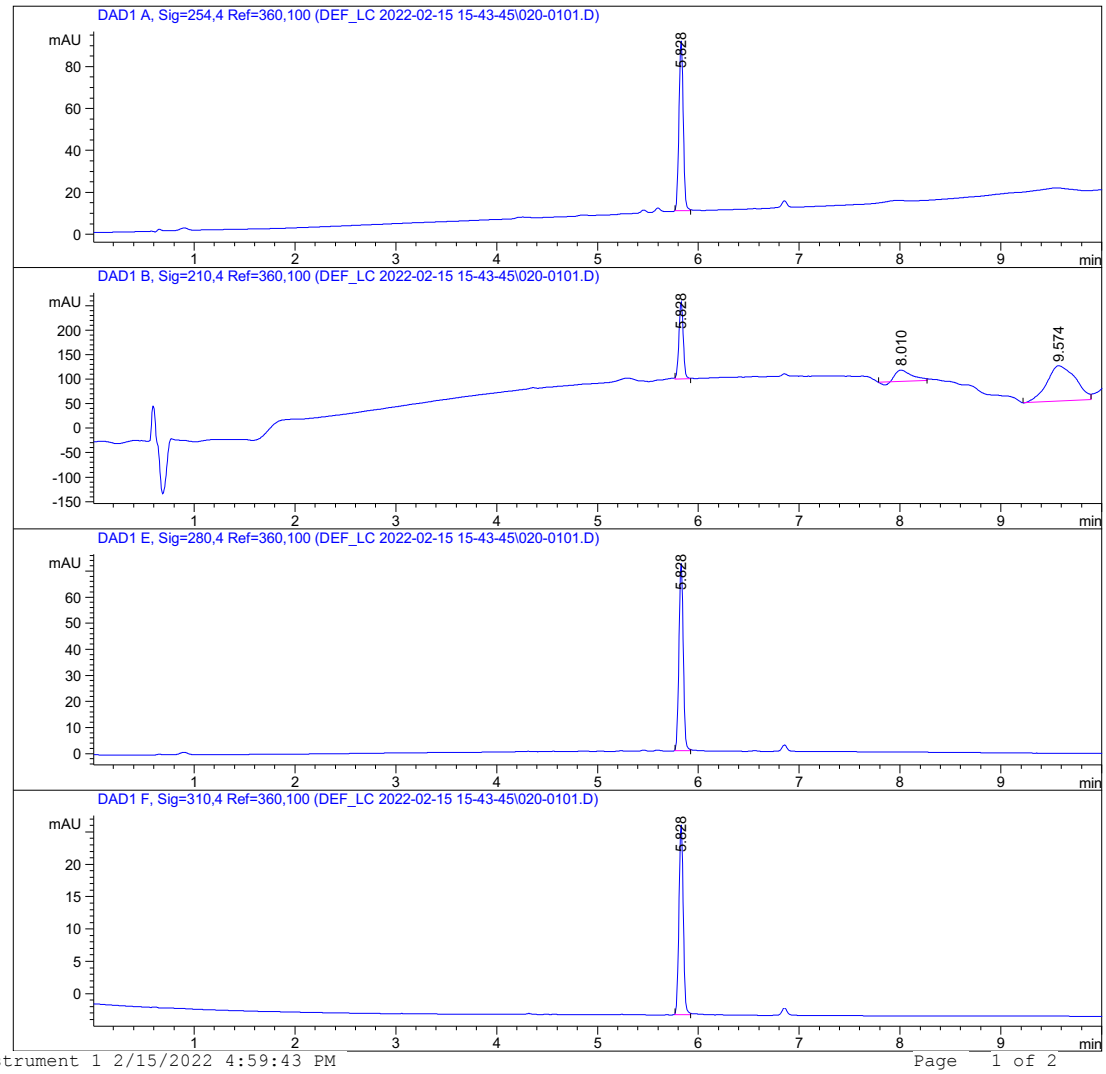
Synthesized according to general procedure D from **6k** in 70% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.19 (s, 1H), 7.97 (s, 1H), 7.47 (dd, J = 4.9, 2.9 Hz, 1H), 7.32 – 7.15 (m, 3H), 6.97 (dd, J = 4.9, 1.3 Hz, 1H), 4.84 (s, 2H), 4.32 (t, J = 7.1 Hz, 2H), 3.51 (m, 8H), 3.14 (t, J = 7.1 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6, δ): 158.97, 158.73, 158.49, 158.25, 155.65, 152.70, 150.65, 145.28 (dd, J = 235.1, 9.6 Hz), 138.08, 133.72, 129.26, 128.25, 126.37, 122.06, 118.80, 116.86, 114.93, 112.99, 111.54 (d, J = 21.3 Hz), 109.73, 108.43, 65.89, 44.49, 43.79, 38.44, 29.38; HRMS (ESI-TOF) calculated for $C_{23}H_{23}N_8OF_2S$ $(M+H)^+$ 497.1684, found 497.1684.

4-(2-(6-(((6,7-difluoro-1H-benzo[d]imidazol-2-yl)methyl)amino)-2-morpholino-9H-purin-9-yl)ethyl)phenol (SR-4055)



Synthesized according to general procedure D from **6l** in 55% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 9.22 (s, 1H), 8.19 (s, 1H), 7.95 (s, 1H), 7.31 – 7.16 (m, 2H), 6.98 – 6.90 (m, 2H), 6.69 – 6.60 (m, 2H), 4.84 (s, 2H), 4.24 (t, J = 7.1 Hz, 2H), 3.51 (m, 8H), 2.98 (t, J = 7.2 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6, δ): 159.36, 159.12, 158.89, 158.66, 156.45, 156.06, 153.13, 151.06, 145.66 (dd, J = 234.7, 9.6 Hz), 140.57 – 137.50 (m), 134.27, 130.07, 128.22, 117.41, 115.72, 115.47, 111.84 (d, J = 20.7 Hz), 110.17, 108.79, 66.33, 45.29, 44.92, 38.91, 34.37; HRMS (ESI-TOF) calculated for $C_{25}H_{25}N_8O_2F_2$ $(M+H)^+$ 507.2069, found 507.2067.

HPLC trace for SR-4133



Instrument 1 2/15/2022 4:59:43 PM Page 1 of 2

Molecular Formula Strings

Molecule Name	SMILES String
SR-3029a	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5C6=CC=CC(F)=C6)=N2
SR-4132	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5C6=CC=CC7=C6C=CC=C7)=N2
SR-4133	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5C6=CC=C(C=CC=C7)C7=C6)=N2
SR-4310	COC(C=C1)=CC=C1N(C=N2)C3=C2C(NCC4=NC(C(F)=C(F)C=C5)=C5N4)=NC(N6CCOCC6)=N3
SR-19840	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5C6=CC=C(OC(C)C)C=C6)=N2
SR-19841	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5C6=CC=C(OCCO7)C7=C6)=N2
SR-4932	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5C6=CC=C(N=CS7)C7=C6)=N2
SR-4971	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5C6=CN=C(C=CC=C7)C7=C6)=N2
SR-4931	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5C6=NC=C(C=CC=C7)C7=C6)=N2
SR-4933	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5C6=CC(C=CC=C7)=C7S6)=N2
SR-3448	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CC6=CC=CC(F)=C6)=N2
SR-3449	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CC6=CC=CC7=C6C=CC=C7)=N2
SR-3450	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CC6=CC=C(C=CC=C7)C7=C6)=N2
SR-3451	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CCC6=CC=CC(F)=C6)=N2
SR-4290	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CCC6=CC(F)=CC(F)=C6)=N2
SR-3452	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CCC6=CC=CC(OC)=C6)=N2
SR-3454	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CCC6=CC=CC(Br)=C6)=N2
SR-4114	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CCC6=CC=C(C#N)C=C6)=N2
SR-4116	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CCC6=CC=C(C(F)(F)F)C=C6)=N2
SR-4134	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CCC6=CC=CC(C(F)(F)F)=C6)=N2
SR-4152	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CCC6=CC=CC(Cl)=C6)=N2
SR-4051	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CCC6=CC=C(F)C=C6)=N2
SR-4052	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CCC6=CC=C(SC)C=C6)=N2
SR-4053	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CCC6=COC=C6)=N2
SR-4054	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CCC6=CSC=C6)=N2
SR-4055	FC(C=C1)=C(F)C2=C1NC(CNC3=NC(N4CCOCC4)=NC5=C3N=CN5CCC6=CC=C(O)C=C6)=N2

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