Supporting Information

Discovery of IRAK1/4/pan-FLT3 Kinase Inhibitors as Treatments for Acute Myeloid Leukemia

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List of abbreviations. In the accompanying procedures and schemes, abbreviations are used with the following meanings unless otherwise indicated: Ac = acetate; aq, aq. = aqueous; Ar = aryl; BOC, Boc = *t*-butyloxycarbonyl; Bn = benzyl; BSA = bovine serum albumin; Bu = butyl, *t*-Bu = *tert*-butyl; BuLi, *n*-BuLi = *n*-butyllithium; CBZ, Cbz = Benzyloxycarbonyl; conc, conc. = concentrated; *c*-Bu = cyclobutyl; *c*-Pr = cyclopropyl; Cy = cyclohexyl; DAST = (diethylamino)sulfur trifluoride; dba = dibenzylideneacetone; DCM = dichloromethane; DIAD = diisopropylazodicarboxylate; DIBAL, DIBAL-H = diisobutylaluminum hydride; DIEA = diisopropylethylamine; DMAC, DMA = dimethylacetamide; DME = 1,2-dimethoxyethane; DMEM = Dulbecco's modified eagle medium; DMAP = 4-dimethylaminopyridine; DMF = *N,N*-dimethylformamide; DMSO = dimethylsulfoxide; eq. = equivalent(s); EDC = N-[3-(dimethylamino)propyl]-N-ethylcarbodiimide; EDTA = ethylenediaminetetraacetic acid; ESI = electrospray ionization; Et = ethyl; EtOAc = ethyl acetate; EtOH = ethanol; FBS = Fetal Bovine Serum; h, hr = hour; HATU = N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-

ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide; HOAc = acetic acid; HOAt = 3H-[1,2,3]-triazolo[4,5-b]pyridin-3-ol; HOBt = 1H-benzotriazol-1-ol; HPLC = High pressure liquid chromatography; HTRF = homogenous time resolved fluorescence; IPA, i-PrOH = isopropanol; iPr = isopropyl; LAH = lithium aluminum hydride; LCMS = liquid chromatography - mass spectroscopy; LHMDS = lithium bis(trimethylsilyl)amide; Me = methyl; MeOH = methanol; min, min. = minute; μW = microwave; NaHMDS = sodium bis(trimethylsilyl)amide; NIS = 1-iodoopyrrolidine-2,5-dione; NBS = 1-bromopyrrolidine-2,5-dione; NCS = 1-chloropyrrolidine-2,5-dione; NMP = N-methylpyrrolidinone; NMR = nuclear magnetic resonance; OMs, mesyl = methanesulfonyl; Oxone, OXONE = potassium peroxymonosulfate; PBS = phosphate buffered saline; Pd_2dba_3 = *tris*(dibenzylidineacetone)dipalladium; $Pd(dppf)Cl_2$ = [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II); Pd/C = palladium on activated carbon; Ph = phenyl; PMB = 4-methoxybenzyl; PMBCl = 1-(chloromethyl)-4-methoxybenzene; Pr = propyl; Py = pyridyl; QPhos = (1,2,3,4,5-pentaphenyl-1'-(di-tert-butylphosphino)ferrocene; RT, rt = room temperature; RuPhos Pd G3 = (2-dicyclohexylphosphino-2',6'-diisopropoxy-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II)methanesulfonate; sat. = saturated; TBAF = tetrabutylammonium fluoride; TBAI = tetrabutylammonium iodide; *t*-Bu = tert-butyl; TFA = trifluoroacetic acid; THF = tetrahydrofuran; TLC = thin layer chromatography; prep TLC = preparative thin layer chromatography; Tosyl = toluenesulfonyl; triflate, OTf = trifluoromethanesulfonate; triflic = trifluoromethanesulfonic; Xantphos = 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene; XPhos Pd G2 or XPhos-PD-G2 = chloro(2-dicyclohexylphosphino-2′,4′,6′-triisopropyl-1,1′-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II).

IRAK1, IRAK4 and FLT3 Functional Biochemical Assays. Kinase inhibitory data were obtained for compounds **2 – 31** using the Reaction Biology HotSpot® kinase assay protocol described below.[1,2] All assays were run in the presence of 10 μM ATP. This assay uses the isolated kinase enzyme, and is useful for determining competition of the inhibitor for ATP and/or substrates and for measuring the kinetics of enzyme inhibition. It also allows for measuring the relative affinity of binding to the isolated enzyme protein, and hence determines selectivity. Unlike kinase binding assays that measure competition for ATP, the HotSpot® Kinase Assay is a functional assay

that measures catalytic activity; as such it measures relative functional potency regardless of the mechanism of enzyme inhibition. This assay uses the form of the various enzymes that are easiest to express, which may not necessarily be the form of the enzyme that exist in the cell. (Sometimes the carboxy terminus has been truncated to aid in expression, or, if it is a receptor kinase, the enzyme itself is isolated from the other parts of the receptor that are involved in regulating kinase activity.)

The reagent used was as follows: Base Reaction buffer; 20 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 1 mM EGTA, 0.01% Brij35, 0.02 mg/ml BSA, 0.1 mM Na_3VO_4, 2 mM DTT, 1% DMSO. Required cofactors were added individually to each kinase reaction.

The reaction procedure was as follows:

1) Substrates were prepared in freshly prepared Reaction Buffer.

2) Any required cofactors were delivered to the substrate solution above.

3) Kinase was delivered into the substrate solution and gently mixed.

4) Compounds were delivered in 100% DMSO into the kinase reaction mixture by Acoustic technology (Echo550; nanoliter range), followed by incubation for 20 min at room temp.

5) ^{33}P-ATP was delivered into the reaction mixture to initiate the reaction.

6) The mixture was incubated for 2 hours at room temperature.

7) Kinase activity was detected by P81 filter-binding method.

Table S1: IRAK1 and IRAK4 Inhibition for Compounds 1 – 31 & Gilteritinib

	IRAK 1					IRAK 4				
Compd	IC_{50} (nM) Run 1	IC_{50} (nM) Run 2	IC_{50} (nM) Run 3	IC_{50} (nM) Geometric Mean	SEM	IC_{50} (nM) Run 1	IC_{50} (nM) Run 2	IC_{50} (nM) Run 3	IC_{50} (nM) Geometric Mean	SEM
1	19	16	17	17	0.9	3	1	2	1.8	0.6
2	>10000	>10000	>10000	>10000	N.D.	10000	8420	8920	9089.9	466.2
3	2270	6160	5400	4227	1190.4	5950	1240	1080	1997.3	1597.3
4	561	556	645	586	28.9	7	9	9	8.3	0.7
5	6240	8140	7630	7291	567.8	1320	2520	2090	1908.6	351.0

6	>10000	>10000	>10000	>10000	N.D.	927	3180	3020	2072.5	725.8
7	670	1730	1200	1116	306.0	31	94	83	62.3	19.4
8	187	228	224	212	13.1	9	7	6	7.2	0.9
9	>10000	>10000	N.D.	>10000	N.D.	>10000	8420	N.D.	9176	790
10	62	80	135	87	22.0	0.5	0.7	0.7	0.6	0.1
11	21	18	19	19	0.9	0.7	0.1	0.2	0.2	0.2
12	25	25	31	27	2.0	0.6	0.5	0.5	0.5	0.0
13	100	51	42	60	18.0	0.7	0.6	0.6	0.6	0.0
14	11	28	27	20	5.5	0.5	0.5	0.5	0.5	0.0
15	43	71	76	61	10.3	0.5	0.5	0.7	0.6	0.1
16	117	147	161	140	13.0	0.6	0.6	0.6	0.6	0.0
17	26	136	167	84	42.8	0.5	2	2	1.3	0.5
18	15	N.D.	N.D.	N.D.	N.D.	0.5	N.D.	N.D.	N.D.	N.D.
19	141	126	123	130	5.6	4	2	3	2.9	0.6
20	195	249	264	234	21.0	11	11	14	11.9	1.0
21	692	779	770	746	27.6	27	21	29	25.4	2.4
22	592	412	391	457	63.8	6	19	25	14.2	5.6
23	25	19	18	20	2.2	0.5	0.5	0.5	0.5	0.0
24	324	348	309	327	11.4	8	11	10	9.6	0.9
25	386	237	172	251	63.3	4	2	2	2.5	0.7
26	7	28	32	18	7.8	0.5	0.8	0.7	0.7	0.1
27	5	0.5	0.5	1	1.5	0.5	0.5	0.5	0.5	0.0
28	8	50	78	31	20.3	0.5	0.9	0.8	0.7	0.1
29	7	5	6	6	0.6	0.5	0.5	0.5	0.5	0.0
30	81	66	76	74	4.4	1	2	2	1.6	0.3
31	3	6	7	5	1.2	0.5	0.6	0.7	0.6	0.1
Gilter-itinib	120	144	N.D.	131	12	197	221	N.D.	209	12

N.D. = Not determined

Table S2: FLT3 Inhibition for Compounds 1 – 31 & Gilteritinib

	FLT3				
Compd	**IC_{50} (nM) Run 1**	**IC_{50} (nM) Run 2**	**IC_{50} (nM) Run 3**	**IC_{50} (nM) Geometric Mean**	**SEM**
1	0.8	0.9	0.5	0.7	0.1
2	82	59	60	66.2	7.5
3	0.6	3	4	1.9	1.0
4	0.5	0.5	0.5	0.5	0.0
5	0.5	0.7	0.7	0.6	0.1
6	2	12	12	6.6	3.3
7	0.5	0.7	0.6	0.6	0.1
8	0.5	0.5	0.5	0.5	0.0
9	125	60	N.D.	86.6	32.5
10	0.5	0.5	0.5	0.5	0.0
11	0.5	0.5	0.5	0.5	0.0
12	0.6	0.5	0.5	0.5	0.0
13	0.6	0.5	0.5	0.5	0.0
14	0.5	0.5	0.5	0.5	0.0
15	0.5	0.5	0.5	0.5	0.0
16	0.6	0.5	0.5	0.5	0.0
17	0.5	0.5	0.5	0.5	0.0
18	0.5	N.D.	N.D.	N.D.	N.D.
19	1.4	0.5	0.5	0.7	0.3
20	0.8	0.5	0.5	0.6	0.1
21	1.4	0.5	0.5	0.7	0.3
22	0.5	0.5	0.5	0.5	0.0
23	0.7	0.5	0.5	0.6	0.1

24	0.9	0.5	0.5	0.6	0.1
25	0.5	0.5	0.5	0.5	0.0
26	0.5	0.5	0.5	0.5	0.0
27	0.5	0.5	0.5	0.5	0.0
28	0.5	0.5	0.5	0.5	0.0
29	0.5	0.5	0.5	0.5	0.0
30	0.5	0.5	0.5	0.5	0.0
31	0.5	0.5	0.5	0.5	0.0
Gilteritinib	0.9	0.7	N.D.	0.8	0.1

N.D. = Not determined

Kinase Selectivity Profiling of Compounds 1, 28 and 31 in Functional Biochemical Assays. Compounds **1**, **28** and **31** were profiled in a panel of 369 kinase functional biochemical assays using the Reaction Biology HotSpot® kinase assay protocol described above. All assays were run in singlicate (n=1) in the presence of 10 μM ATP.[1,2]

Table S3: Kinase Selectivity Profiling for Compound 1

Kinase	IC_{50} (M)
ABL1	5.86E-10
ABL2/ARG	1.22E-09
ACK1	1.46E-07
AKT1	5.06E-06
AKT2	>1.00E-05
AKT3	>1.00E-05
ALK	3.55E-08
ALK1/ACVRL1	2.78E-08
ALK2/ACVR1	6.35E-08
ALK3/BMPR1A	7.42E-07
ALK4/ACVR1B	4.36E-06
ALK5/TGFBR1	9.49E-06
ALK6/BMPR1B	2.98E-06
ARAF	>1.00E-05
ARK5/NUAK1	8.72E-09
ASK1/MAP3K5	>1.00E-05
Aurora A	4.07E-07
Aurora B	2.11E-07
Aurora C	2.92E-07
AXL	2.45E-08
BLK	6.61E-10
BMPR2	9.75E-07
BMX/ETK	8.95E-09
BRAF	>1.00E-05
BRK	3.82E-08
BRSK1	9.49E-08
BRSK2	4.85E-08
BTK	8.22E-10
c-Kit	2.85E-07
c-MER	1.63E-08
c-MET	>1.00E-05
c-Src	6.02E-10
CAMK1a	2.85E-06
CAMK1b	6.69E-06
CAMK1d	6.91E-07
CAMK1g	>1.00E-05
CAMK2a	4.61E-09
CAMK2b	1.53E-07
CAMK2d	2.16E-08

Kinase	IC_{50} (M)
CAMK2g	1.11E-07
CAMK4	>1.00E-05
CAMKK1	2.35E-07
CAMKK2	7.84E-08
CDC7/DBF4	>1.00E-05
CDK1/cyclin A	1.26E-08
CDK1/cyclin B	1.39E-08
CDK1/cyclin E	3.38E-08
CDK14/cyclin Y (PFTK1)	9.87E-08
CDK16/cyclin Y (PCTAIRE)	1.43E-08
CDK17/cyclin Y (PCTK2)	5.66E-08
CDK18/cyclin Y (PCTK3)	5.04E-08
CDK19/cyclin C	2.17E-08
CDK2/cyclin A	4.79E-08
CDK2/Cyclin A1	1.23E-07
CDK2/cyclin E	1.83E-07
CDK2/cyclin O	7.10E-08
CDK3/cyclin E	4.72E-07
CDK4/cyclin D1	1.16E-06
CDK4/cyclin D3	8.44E-07
CDK5/p25	2.18E-07
CDK5/p35	8.92E-08
CDK6/cyclin D1	4.36E-06
CDK6/cyclin D3	5.58E-09
CDK7/cyclin H	2.24E-07
CDK9/cyclin K	1.20E-07
CDK9/cyclin T1	2.26E-07
CDK9/cyclin T2	9.28E-08
CHK1	2.63E-08
CHK2	5.11E-09
CK1a1	6.94E-06
CK1a1L	>1.00E-05
CK1d	9.79E-06
CK1epsilon	>1.00E-05
CK1g1	6.28E-06
CK1g2	5.67E-06
CK1g3	3.18E-06
CK2a	>1.00E-05
CK2a2	5.99E-06

Table S3: Kinase Selectivity Profiling for Compound 1 (cont.)

Kinase	IC_{50} (M)
CLK1	1.09E-08
CLK2	7.18E-09
CLK3	1.68E-06
CLK4	3.59E-08
COT1/MAP3K8	>1.00E-05
CSK	2.75E-09
CTK/MATK	>1.00E-05
DAPK1	9.42E-06
DAPK2	>1.00E-05
DCAMKL1	>1.00E-05
DCAMKL2	>1.00E-05
DDR1	8.50E-09
DDR2	2.88E-08
DLK/MAP3K12	5.36E-07
DMPK	>1.00E-05
DMPK2	8.73E-07
DRAK1/STK17A	3.26E-07
DYRK1/DYRK1A	5.13E-07
DYRK1B	1.87E-07
DYRK2	2.25E-06
DYRK3	6.81E-07
DYRK4	>1.00E-05
EGFR	1.33E-08
EPHA1	1.62E-07
EPHA2	3.59E-08
EPHA3	5.57E-08
EPHA4	3.74E-08
EPHA5	1.46E-08
EPHA6	1.64E-07
EPHA7	3.44E-07
EPHA8	4.04E-08
EPHB1	1.16E-08
EPHB2	1.83E-08
EPHB3	5.23E-07
EPHB4	1.47E-08
ERBB2/HER2	2.23E-07
ERBB4/HER4	8.89E-08
ERK1	>1.00E-05
ERK2/MAPK1	>1.00E-05

Kinase	IC_{50} (M)
ERK5/MAPK7	>1.00E-05
ERK7/MAPK15	1.04E-08
ERN1/IRE1	2.87E-07
ERN2/IRE2	2.70E-07
FAK/PTK2	7.87E-08
FER	1.00E-08
FES/FPS	2.94E-07
FGFR1	1.50E-08
FGFR2	7.75E-09
FGFR3	3.41E-08
FGFR4	3.90E-07
FGR	<5.08E-10
FLT1/VEGFR1	1.58E-08
FLT3	8.37E-10
FLT4/VEGFR3	1.01E-09
FMS	2.53E-08
FRK/PTK5	5.46E-09
FYN	9.57E-10
GCK/MAP4K2	2.82E-07
GLK/MAP4K3	1.36E-07
GRK1	2.64E-06
GRK2	>1.00E-05
GRK3	>1.00E-05
GRK4	1.88E-06
GRK5	>1.00E-05
GRK6	>1.00E-05
GRK7	2.18E-06
GSK3a	2.75E-06
GSK3b	3.33E-06
Haspin	7.18E-06
HCK	2.44E-09
HGK/MAP4K4	2.82E-08
HIPK1	2.70E-06
HIPK2	4.48E-06
HIPK3	4.06E-06
HIPK4	1.99E-07
HPK1/MAP4K1	1.72E-08
IGF1R	4.75E-06
IKKa/CHUK	>1.00E-05

Table S3: Kinase Selectivity Profiling for Compound 1 (cont.)

Kinase	IC_{50} (M)
IKKb/IKBKB	>1.00E-05
IKKe/IKBKE	3.28E-06
IR	8.14E-07
IRAK1	1.92E-08
IRAK4	2.92E-09
IRR/INSRR	1.03E-06
ITK	4.37E-09
JAK1	6.75E-07
JAK2	2.53E-07
JAK3	5.82E-08
JNK1	>1.00E-05
JNK2	>1.00E-05
JNK3	>1.00E-05
KDR/VEGFR2	4.98E-09
KHS/MAP4K5	3.09E-08
KSR1	>1.00E-05
KSR2	>1.00E-05
LATS1	5.16E-07
LATS2	1.11E-07
LCK	1.61E-09
LCK2/ICK	3.30E-07
LIMK1	1.14E-08
LIMK2	2.59E-07
LKB1	2.94E-07
LOK/STK10	6.02E-08
LRRK2	6.01E-08
LYN	4.96E-10
LYN B	1.09E-09
MAK	1.03E-07
MAPKAPK2	>1.00E-05
MAPKAPK3	>1.00E-05
MAPKAPK5/PRAK	>1.00E-05
MARK1	2.45E-07
MARK2/PAR-1Ba	1.23E-07
MARK3	7.66E-08
MARK4	4.21E-08
MEK1	3.71E-07
MEK2	4.30E-07
MEK3	2.34E-06

Kinase	IC_{50} (M)
MEK5	8.53E-08
MEKK1	>1.00E-05
MEKK2	6.80E-07
MEKK3	7.69E-07
MEKK6	>1.00E-05
MELK	1.26E-07
MINK/MINK1	4.94E-08
MKK4	3.13E-06
MKK6	4.43E-06
MKK7	>1.00E-05
MLCK/MYLK	2.65E-06
MLCK2/MYLK2	2.06E-07
MLK1/MAP3K9	1.76E-08
MLK2/MAP3K10	2.07E-07
MLK3/MAP3K11	2.47E-08
MLK4	1.34E-06
MNK1	3.39E-08
MNK2	2.28E-08
MRCKa/CDC42BPA	>1.00E-05
MRCKb/CDC42BPB	2.83E-06
MSK1/RPS6KA5	3.41E-07
MSK2/RPS6KA4	9.28E-07
MSSK1/STK23	>1.00E-05
MST1/STK4	4.26E-08
MST2/STK3	6.93E-08
MST3/STK24	6.65E-06
MST4	1.86E-06
MUSK	4.35E-07
MYLK3	>1.00E-05
MYLK4	1.27E-07
MYO3A	7.31E-07
MYO3b	2.56E-07
NEK1	6.28E-06
NEK11	>1.00E-05
NEK2	6.19E-06
NEK3	>1.00E-05
NEK4	>1.00E-05
NEK5	>1.00E-05
NEK6	>1.00E-05

Table S3: Kinase Selectivity Profiling for Compound 1 (cont.)

Kinase	IC_{50} (M)
NEK7	>1.00E-05
NEK8	1.30E-06
NEK9	>1.00E-05
NIM1	>1.00E-05
NLK	7.52E-07
OSR1/OXSR1	>1.00E-05
P38a/MAPK14	8.87E-06
P38b/MAPK11	3.84E-06
P38d/MAPK13	4.30E-06
P38g	>1.00E-05
p70S6K/RPS6KB1	1.08E-07
p70S6Kb/RPS6KB2	3.50E-07
PAK1	3.94E-06
PAK2	6.90E-06
PAK3	3.74E-06
PAK4	1.95E-06
PAK5	1.45E-06
PAK6	>1.00E-05
PASK	4.66E-06
PBK/TOPK	>1.00E-05
PDGFRa	1.06E-09
PDGFRb	9.53E-09
PDK1/PDPK1	1.87E-07
PEAK1	5.78E-10
PHKg1	4.60E-09
PHKg2	3.39E-09
PIM1	2.45E-06
PIM2	>1.00E-05
PIM3	3.45E-07
PKA	2.65E-07
PKAcb	6.47E-07
PKAcg	2.20E-06
PKCa	8.94E-07
PKCb1	1.74E-06
PKCb2	6.63E-07
PKCd	3.37E-07
PKCepsilon	6.57E-07
PKCeta	1.13E-06
PKCg	1.44E-06

Kinase	IC_{50} (M)
PKCiota	>1.00E-05
PKCmu/PRKD1	1.03E-07
PKCnu/PRKD3	5.64E-08
PKCtheta	9.96E-08
PKCzeta	>1.00E-05
PKD2/PRKD2	7.72E-08
PKG1a	3.13E-06
PKG1b	3.41E-06
PKG2/PRKG2	>1.00E-05
PKN1/PRK1	2.25E-07
PKN2/PRK2	3.50E-06
PKN3/PRK3	3.84E-07
PLK1	>1.00E-05
PLK2	>1.00E-05
PLK3	>1.00E-05
PLK4/SAK	2.43E-07
PRKX	2.06E-08
PYK2	1.70E-07
RAF1	>1.00E-05
RET	2.07E-09
RIPK2	2.16E-07
RIPK3	3.00E-08
RIPK4	2.09E-07
RIPK5	3.94E-06
ROCK1	5.29E-07
ROCK2	1.24E-06
RON/MST1R	>1.00E-05
ROS/ROS1	5.98E-09
RSK1	7.73E-09
RSK2	2.11E-08
RSK3	8.75E-09
RSK4	2.95E-08
SBK1	7.37E-06
SGK1	4.05E-06
SGK2	>1.00E-05
SGK3/SGKL	>1.00E-05
SIK1	1.68E-09
SIK2	1.29E-09
SIK3	8.65E-08

Table S3: Kinase Selectivity Profiling for Compound 1 (cont.)

Kinase	IC_{50} (M)
SLK/STK2	3.33E-07
SNARK/NUAK2	1.45E-07
SNRK	>1.00E-05
SRMS	2.23E-06
SRPK1	>1.00E-05
SRPK2	>1.00E-05
SSTK/TSSK6	>1.00E-05
STK16	5.91E-06
STK21/CIT	>1.00E-05
STK22D/TSSK1	2.43E-07
STK25/YSK1	6.44E-06
STK32B/YANK2	>1.00E-05
STK32C/YANK3	>1.00E-05
STK33	1.35E-07
STK38/NDR1	1.58E-06
STK38L/NDR2	4.62E-06
STK39/STLK3	3.46E-07
SYK	1.90E-08
TAK1	1.33E-07
TAOK1	2.19E-06
TAOK2/TAO1	>1.00E-05
TAOK3/JIK	2.51E-06
TBK1	1.13E-06
TEC	4.07E-08
TESK1	5.58E-07
TESK2	4.60E-07
TGFBR2	7.89E-08
TIE2/TEK	7.67E-07
TLK1	>1.00E-05
TLK2	6.50E-06
TNIK	4.48E-09
TNK1	7.32E-09
TRKA	1.46E-08
TRKB	1.75E-09
TRKC	7.14E-10
TSSK2	8.08E-06
TSSK3/STK22C	>1.00E-05
TTBK1	>1.00E-05
TTBK2	5.66E-06

Kinase	IC_{50} (M)
TXK	6.82E-09
TYK1/LTK	1.03E-07
TYK2	7.21E-07
TYRO3/SKY	2.56E-07
ULK1	1.73E-07
ULK2	1.63E-07
ULK3	1.40E-07
VRK1	2.07E-05
VRK2	>1.00E-05
WEE1	>1.00E-05
WNK1	>1.00E-05
WNK2	>1.00E-05
WNK3	>1.00E-05
YES/YES1	6.94E-10
YSK4/MAP3K19	>1.00E-05
ZAK/MLTK	7.17E-08
ZAP70	>1.00E-05
ZIPK/DAPK3	6.03E-06

Table S4: Kinase Selectivity Profiling for Compound 28

Kinase	IC_{50} (M)
ABL1	3.37E-09
ABL2/ARG	1.85E-08
ACK1	3.73E-06
AKT1	>1.00E-05
AKT2	>1.00E-05
AKT3	>1.00E-05
ALK	2.73E-08
ALK1/ACVRL1	3.05E-07
ALK2/ACVR1	5.19E-07
ALK3/BMPR1A	>1.00E-05
ALK4/ACVR1B	>1.00E-05
ALK5/TGFBR1	>1.00E-05
ALK6/BMPR1B	5.04E-06
ARAF	>1.00E-05
ARK5/NUAK1	6.26E-08
ASK1/MAP3K5	5.04E-06
Aurora A	2.72E-06
Aurora B	1.68E-07
AURORA C	3.00E-07
AXL	2.87E-08
BLK	2.25E-08
BMPR2	9.62E-06
BMX/ETK	8.95E-08
BRAF	>1.00E-05
BRK	1.90E-07
BRSK1	1.83E-07
BRSK2	3.58E-07
BTK	1.19E-08
c-Kit	2.48E-08
c-MER	4.29E-08
c-MET	>1.00E-05
c-Src	3.95E-09
CAMK1a	>1.00E-05
CAMK1b	>1.00E-05
CAMK1d	6.13E-06
CAMK1g	>1.00E-05
CAMK2a	2.57E-07
CAMK2b	1.11E-06
CAMK2d	4.97E-07

Kinase	IC_{50} (M)
CAMK2g	1.17E-06
CAMK4	>1.00E-05
CAMKK1	1.46E-06
CAMKK2	1.70E-06
CDC7/DBF4	>1.00E-05
CDK1/cyclin A	9.04E-08
CDK1/cyclin B	8.59E-08
CDK1/cyclin E	6.51E-08
CDK14/cyclin Y (PFTK1)	5.07E-06
CDK16/cyclin Y (PCTAIRE)	8.49E-07
CDK17/cyclin Y (PCTK2)	8.21E-07
CDK18/cyclin Y (PCTK3)	1.43E-06
CDK19/cyclin C	2.40E-06
CDK2/CYCLIN A	3.10E-07
CDK2/cyclin A1	6.91E-07
CDK2/CYCLIN E	7.80E-07
CDK2/cyclin E2	3.32E-07
CDK2/cyclin O	5.28E-07
CDK3/cyclin E	1.20E-06
CDK3/cyclin E2	5.26E-07
CDK4/cyclin D1	3.69E-06
CDK4/cyclin D3	1.84E-06
CDK5/P25	3.60E-07
CDK5/p35	4.71E-07
CDK6/cyclin D1	4.80E-06
CDK6/cyclin D3	1.40E-07
CDK7/cyclin H	1.14E-07
CDK8/cyclin C	4.33E-07
CDK9/CYCLIN K	3.06E-06
CDK9/cyclin T1	2.10E-06
CDK9/cyclin T2	2.18E-06
CHK1	1.80E-07
CHK2	4.54E-08
CK1a1	>1.00E-05
CK1a1L	>1.00E-05
CK1d	>1.00E-05
CK1epsilon	>1.00E-05
CK1g1	4.68E-06
CK1g2	3.21E-06

Table S4: Kinase Selectivity Profiling for Compound 28 (cont.)

Kinase	IC_{50} (M)
CK1G3	2.29E-06
CK2a	>1.00E-05
CK2a2	>1.00E-05
CLK1	1.47E-07
CLK2	1.47E-07
CLK3	>1.00E-05
CLK4	8.22E-08
COT1/MAP3K8	>1.00E-05
CSK	2.77E-09
CTK/MATK	>1.00E-05
DAPK1	>1.00E-05
DAPK2	>1.00E-05
DCAMKL1	>1.00E-05
DCAMKL2	>1.00E-05
DDR1	3.06E-08
DDR2	2.37E-07
DMPK	>1.00E-05
DMPK2	1.00E-06
DRAK1/STK17A	4.29E-07
DYRK1/DYRK1A	4.06E-06
DYRK1B	2.05E-06
DYRK2	4.08E-06
DYRK3	4.52E-06
DYRK4	>1.00E-05
EGFR	1.65E-06
EPHA1	1.70E-07
EPHA2	2.71E-08
EPHA3	2.14E-07
EPHA4	3.46E-08
EPHA5	2.99E-08
EPHA6	5.36E-07
EPHA7	1.78E-07
EPHA8	5.96E-08
EPHB1	1.59E-08
EPHB2	2.75E-08
EPHB3	1.04E-06
EPHB4	1.75E-08
ERBB2/HER2	>1.00E-05
ERBB4/HER4	3.02E-06

Kinase	IC_{50} (M)
ERK1	>1.00E-05
ERK2/MAPK1	>1.00E-05
ERK5/MAPK7	>1.00E-05
ERK7/MAPK15	1.98E-06
ERN1/IRE1	>1.00E-05
ERN2/IRE2	2.02E-06
FAK/PTK2	1.35E-07
FER	7.16E-07
FES/FPS	8.37E-07
FGFR1	8.22E-08
FGFR2	3.46E-08
FGFR3	1.09E-07
FGFR4	1.26E-06
FGR	2.66E-09
FLT1/VEGFR1	1.88E-08
FLT3	1.54E-09
FLT4/VEGFR3	6.68E-09
FMS	5.43E-07
FRK/PTK5	4.05E-08
FYN	2.58E-09
GCK/MAP4K2	9.42E-07
GLK/MAP4K3	3.70E-07
GRK1	6.37E-06
GRK2	>1.00E-05
GRK3	>1.00E-05
GRK4	5.00E-06
GRK5	>1.00E-05
GRK6	>1.00E-05
GRK7	4.24E-06
GSK3a	>1.00E-05
GSK3b	>1.00E-05
Haspin	>1.00E-05
HCK	5.97E-09
HGK/MAP4K4	2.12E-07
HIPK1	>1.00E-05
HIPK2	>1.00E-05
HIPK3	>1.00E-05
HIPK4	5.59E-06
HPK1/MAP4K1	2.77E-07

Table S4: Kinase Selectivity Profiling for Compound 28 (cont.)

Kinase	IC_{50} (M)
IGF1R	>1.00E-05
IKKa/CHUK	>1.00E-05
IKKb/IKBKB	>1.00E-05
IKKe/IKBKE	>1.00E-05
IR	9.23E-06
IRAK1	5.28E-08
IRAK4	9.18E-10
IRR/INSRR	4.98E-06
ITK	1.53E-07
JAK1	2.12E-06
JAK2	2.91E-06
JAK3	6.96E-07
JNK1	>1.00E-05
JNK2	>1.00E-05
JNK3	>1.00E-05
KDR/VEGFR2	6.42E-08
KHS/MAP4K5	1.74E-07
KSR1	>1.00E-05
KSR2	>1.00E-05
LATS1	1.78E-06
LATS2	4.87E-07
LCK	2.73E-08
LCK2/ICK	4.84E-06
LIMK1	2.76E-07
LIMK2	4.80E-06
LKB1	8.98E-07
LOK/STK10	1.79E-08
LRRK2	5.25E-07
LYN	7.24E-09
LYN B	4.75E-08
MAK	1.23E-06
MAPKAPK2	>1.00E-05
MAPKAPK3	>1.00E-05
MAPKAPK5/PRAK	>1.00E-05
MARK1	1.47E-06
MARK2/PAR-1Ba	2.01E-06
MARK3	1.33E-06
MARK4	2.09E-06
MAST3	>1.00E-05

Kinase	IC_{50} (M)
MASTL	2.76E-06
MEK1	3.31E-06
MEK2	2.33E-06
MEK3	>1.00E-05
MEK5	4.87E-07
MEKK1	>1.00E-05
MEKK2	3.27E-06
MEKK3	1.15E-06
MEKK6	>1.00E-05
MELK	2.05E-07
MINK/MINK1	1.50E-07
MKK4	5.63E-06
MKK6	>1.00E-05
MKK7	2.92E-06
MLCK/MYLK	>1.00E-05
MLCK2/MYLK2	9.79E-07
MLK1/MAP3K9	1.39E-06
MLK2/MAP3K10	9.12E-07
MLK3/MAP3K11	6.60E-07
MLK4	3.96E-06
MNK1	8.36E-08
MNK2	1.37E-08
MRCKa/CDC42BPA	>1.00E-05
MRCKb/CDC42BPB	>1.00E-05
MSK1/RPS6KA5	>1.00E-05
MSK2/RPS6KA4	4.49E-06
MSSK1/STK23	>1.00E-05
MST1/STK4	1.94E-07
MST2/STK3	3.64E-07
MST3/STK24	7.36E-06
MST4	>1.00E-05
MUSK	1.05E-07
MYLK3	>1.00E-05
MYLK4	8.50E-07
MYO3A	1.05E-06
MYO3b	1.74E-06
NEK1	6.07E-06
NEK11	>1.00E-05
NEK2	>1.00E-05

Table S4: Kinase Selectivity Profiling for Compound 28 (cont.)

Kinase	IC_{50} (M)
NEK3	>1.00E-05
NEK4	>1.00E-05
NEK5	>1.00E-05
NEK6	>1.00E-05
NEK7	>1.00E-05
NEK9	>1.00E-05
NIM1	>1.00E-05
NLK	1.99E-06
OSR1/OXSR1	>1.00E-05
P38a/MAPK14	>1.00E-05
P38b/MAPK11	>1.00E-05
P38d/MAPK13	>1.00E-05
P38g	>1.00E-05
p70S6K/RPS6KB1	1.32E-06
p70S6Kb/RPS6KB2	>1.00E-05
PAK1	2.65E-06
PAK2	3.57E-06
PAK3	1.96E-06
PAK4	1.03E-06
PAK5	1.21E-06
PAK6	>1.00E-05
PASK	>1.00E-05
PBK/TOPK	>1.00E-05
PDGFRa	1.95E-08
PDGFRb	2.12E-08
PDK1/PDPK1	2.61E-06
PHKg1	1.82E-08
PHKg2	1.10E-07
PIM1	9.74E-06
PIM2	>1.00E-05
PIM3	4.63E-06
PKA	7.36E-06
PKAcb	>1.00E-05
PKAcg	>1.00E-05
PKCa	>1.00E-05
PKCb1	>1.00E-05
PKCb2	7.78E-06
PKCd	5.60E-06
PKCepsilon	6.68E-06
PKCeta	>1.00E-05

Kinase	IC_{50} (M)
PKCg	7.56E-06
PKCiota	>1.00E-05
PKCmu/PRKD1	7.12E-08
PKCnu/PRKD3	1.40E-07
PKCtheta	>1.00E-05
PKCzeta	>1.00E-05
PKD2/PRKD2	1.67E-07
PKG1a	>1.00E-05
PKG1b	>1.00E-05
PKG2/PRKG2	>1.00E-05
PKMYT1	4.09E-06
PKN1/PRK1	3.66E-06
PKN2/PRK2	>1.00E-05
PKN3/PRK3	9.85E-07
PLK1	>1.00E-05
PLK2	>1.00E-05
PLK3	>1.00E-05
PLK4/SAK	2.04E-06
PRKX	2.62E-07
PYK2	1.24E-06
RAF1	>1.00E-05
RET	2.35E-09
RIPK2	1.85E-06
RIPK4	6.17E-07
RIPK5	>1.00E-05
ROCK1	1.05E-06
ROCK2	6.16E-07
RON/MST1R	>1.00E-05
ROS/ROS1	5.19E-08
RSK1	6.38E-08
RSK2	9.33E-08
RSK3	1.99E-08
RSK4	8.50E-08
SBK1	>1.00E-05
SGK1	>1.00E-05
SGK2	>1.00E-05
SGK3/SGKL	>1.00E-05
SIK1	7.27E-08
SIK2	1.73E-08
SIK3	5.60E-07

Table S4: Kinase Selectivity Profiling for Compound 28 (cont.)

Kinase	IC_{50} (M)
SLK/STK2	1.47E-06
SNARK/NUAK2	3.87E-07
SNRK	>1.00E-05
SRMS	5.76E-06
SRPK1	>1.00E-05
SRPK2	>1.00E-05
SSTK/TSSK6	>1.00E-05
STK16	9.75E-06
STK21/CIT	4.75E-06
STK22D/TSSK1	1.19E-06
STK25/YSK1	>1.00E-05
STK32B/YANK2	>1.00E-05
STK32C/YANK3	>1.00E-05
STK33	1.82E-06
STK38/NDR1	2.70E-06
STK38L/NDR2	4.19E-06
STK39/STLK3	>1.00E-05
SYK	>1.00E-05
TAK1	1.61E-06
TAOK1	2.27E-06
TAOK2/TAO1	>1.00E-05
TAOK3/JIK	>1.00E-05
TBK1	2.61E-06
TEC	2.70E-07
TESK1	2.88E-06
TESK2	4.82E-07
TGFBR2	5.69E-07
TIE2/TEK	1.55E-06
TLK1	>1.00E-05
TLK2	7.43E-06
TNIK	2.27E-08
TNK1	6.40E-07
TRKA	3.34E-08
TRKB	1.61E-08
TRKC	9.46E-10
TSSK2	>1.00E-05
TSSK3/STK22C	>1.00E-05
TTBK1	>1.00E-05
TTBK2	>1.00E-05
TXK	2.14E-08

Kinase	IC_{50} (M)
TYK1/LTK	8.37E-07
TYK2	3.74E-06
TYRO3/SKY	6.34E-07
ULK1	2.32E-07
ULK2	1.88E-06
ULK3	8.94E-07
VRK1	>1.00E-05
VRK2	>1.00E-05
WEE1	>1.00E-05
WNK1	>1.00E-05
WNK2	>1.00E-05
WNK3	>1.00E-05
YES/YES1	4.59E-09
YSK4/MAP3K19	>1.00E-05
ZAK/MLTK	1.06E-06
ZAP70	>1.00E-05
ZIPK/DAPK3	>1.00E-05

Table S5: Kinase Selectivity Profiling for Compound 31

Kinase	IC_{50} (M)
ABL1	8.99E-10
ABL2/ARG	3.68E-09
ACK1	2.38E-06
AKT1	>1.00E-05
AKT2	>1.00E-05
AKT3	>1.00E-05
ALK	1.33E-08
ALK1/ACVRL1	4.22E-07
ALK2/ACVR1	6.07E-07
ALK3/BMPR1A	9.37E-06
ALK4/ACVR1B	3.42E-06
ALK5/TGFBR1	>1.00E-05
ALK6/BMPR1B	7.15E-06
ARAF	>1.00E-05
ARK5/NUAK1	3.63E-08
ASK1/MAP3K5	2.92E-07
Aurora A	1.08E-06
Aurora B	1.33E-07
AURORA C	1.23E-07
AXL	1.32E-08
BLK	1.16E-08
BMPR2	1.05E-06
BMX/ETK	8.62E-08
BRAF	>1.00E-05
BRK	6.30E-08
BRSK1	9.38E-08
BRSK2	1.07E-07
BTK	1.43E-08
c-Kit	7.26E-09
c-MER	1.14E-08
c-MET	>1.00E-05
c-Src	2.93E-09
CAMK1a	2.56E-06
CAMK1b	6.00E-06
CAMK1d	2.17E-06
CAMK1g	9.42E-06
CAMK2a	3.09E-08
CAMK2b	1.12E-07
CAMK2d	1.62E-08

Kinase	IC_{50} (M)
CAMK2g	1.95E-07
CAMK4	>1.00E-05
CAMKK1	1.83E-07
CAMKK2	2.96E-06
CDC7/DBF4	>1.00E-05
CDK1/cyclin A	4.29E-08
CDK1/cyclin B	4.15E-08
CDK1/cyclin E	2.11E-08
CDK14/cyclin Y (PFTK1)	8.17E-07
CDK16/cyclin Y (PCTAIRE)	1.21E-07
CDK17/cyclin Y (PCTK2)	1.46E-07
CDK18/cyclin Y (PCTK3)	2.32E-07
CDK19/cyclin C	7.58E-07
CDK2/cyclin A	1.20E-07
CDK2/cyclin A1	1.71E-07
CDK2/CYCLIN E	6.22E-08
CDK2/cyclin E2	5.66E-08
CDK2/cyclin O	6.08E-08
CDK3/cyclin E	2.36E-07
CDK3/cyclin E2	2.03E-07
CDK4/cyclin D1	2.16E-07
CDK4/cyclin D3	3.93E-07
CDK5/P25	1.01E-07
CDK5/p35	1.04E-07
CDK6/cyclin D1	2.20E-07
CDK6/cyclin D3	2.21E-08
CDK7/cyclin H	5.33E-08
CDK8/cyclin C	6.59E-08
CDK9/cyclin K	8.13E-08
CDK9/cyclin T1	1.83E-07
CDK9/cyclin T2	8.05E-08
CHK1	5.88E-08
CHK2	8.45E-09
CK1a1	3.94E-06
CK1a1L	>1.00E-05
CK1d	>1.00E-05
CK1epsilon	>1.00E-05
CK1g1	7.99E-07
CK1g2	8.00E-07

Table S5: Kinase Selectivity Profiling for Compound 31 (cont.)

Kinase	IC_{50} (M)
CK1g3	2.01E-06
CK2a	>1.00E-05
CK2a2	>1.00E-05
CLK1	5.42E-08
CLK2	5.55E-08
CLK3	2.89E-06
CLK4	7.06E-08
COT1/MAP3K8	>1.00E-05
CSK	2.08E-09
CTK/MATK	>1.00E-05
DAPK1	>1.00E-05
DAPK2	>1.00E-05
DCAMKL1	9.59E-06
DCAMKL2	4.49E-06
DDR1	8.31E-09
DDR2	4.60E-08
DMPK	>1.00E-05
DMPK2	2.02E-07
DRAK1/STK17A	9.17E-08
DYRK1/DYRK1A	2.85E-07
DYRK1B	9.75E-08
DYRK2	1.78E-06
DYRK3	7.85E-07
DYRK4	>1.00E-05
EGFR	3.31E-07
EPHA1	2.41E-07
EPHA2	3.97E-08
EPHA3	2.27E-07
EPHA4	3.10E-08
EPHA5	1.34E-08
EPHA6	2.03E-07
EPHA7	1.33E-07
EPHA8	3.26E-08
EPHB1	9.38E-09
EPHB2	1.02E-08
EPHB3	5.76E-07
EPHB4	1.13E-08
ERBB2/HER2	3.38E-06
ERBB4/HER4	2.04E-06

Kinase	IC_{50} (M)
ERK1	7.04E-06
ERK2/MAPK1	3.79E-06
ERK5/MAPK7	>1.00E-05
ERK7/MAPK15	1.80E-07
ERN1/IRE1	1.34E-06
ERN2/IRE2	3.85E-07
FAK/PTK2	1.29E-07
FER	2.76E-07
FES/FPS	4.31E-07
FGFR1	8.49E-08
FGFR2	3.76E-08
FGFR3	8.57E-08
FGFR4	5.79E-07
FGR	1.12E-09
FLT1/VEGFR1	4.30E-09
FLT3	5.97E-10
FLT4/VEGFR3	2.85E-09
FMS	5.68E-08
FRK/PTK5	2.50E-08
FYN	5.57E-10
GCK/MAP4K2	3.70E-07
GLK/MAP4K3	3.84E-08
GRK1	1.40E-06
GRK2	>1.00E-05
GRK3	>1.00E-05
GRK4	8.87E-07
GRK5	3.17E-06
GRK6	3.89E-06
GRK7	1.34E-06
GSK3a	3.06E-06
GSK3b	5.55E-06
Haspin	7.25E-06
HCK	4.68E-09
HGK/MAP4K4	2.99E-08
HIPK1	>1.00E-05
HIPK2	>1.00E-05
HIPK3	>1.00E-05
HIPK4	1.64E-06
HPK1/MAP4K1	8.72E-08

Table S5: Kinase Selectivity Profiling for Compound 31 (cont.)

Kinase	IC_{50} (M)
IGF1R	6.46E-06
IKKa/CHUK	4.32E-06
IKKb/IKBKB	7.71E-06
IKKe/IKBKE	1.06E-06
IR	1.85E-06
IRAK1	1.77E-08
IRAK4	8.11E-10
IRR/INSRR	8.81E-07
ITK	6.31E-08
JAK1	3.03E-07
JAK2	9.28E-07
JAK3	2.92E-07
JNK1	>1.00E-05
JNK2	>1.00E-05
JNK3	>1.00E-05
KDR/VEGFR2	9.84E-09
KHS/MAP4K5	7.76E-08
KSR1	>1.00E-05
KSR2	>1.00E-05
LATS1	4.85E-07
LATS2	4.66E-08
LCK	1.72E-08
LCK2/ICK	6.73E-08
LIMK1	1.76E-07
LIMK2	4.44E-06
LKB1	5.16E-07
LOK/STK10	8.14E-09
LRRK2	7.83E-08
LYN	3.49E-09
LYN B	2.78E-08
MAK	2.32E-07
MAPKAPK2	>1.00E-05
MAPKAPK3	>1.00E-05
MAPKAPK5/PRAK	>1.00E-05
MARK1	2.63E-07
MARK2/PAR-1Ba	1.40E-07
MARK3	3.55E-07
MARK4	3.51E-07
MAST3	9.42E-07

Kinase	IC_{50} (M)
MASTL	1.52E-07
MEK1	9.10E-07
MEK2	1.72E-07
MEK3	7.51E-06
MEK5	1.52E-08
MEKK1	>1.00E-05
MEKK2	8.93E-07
MEKK3	3.25E-07
MEKK6	>1.00E-05
MELK	3.42E-08
MINK/MINK1	6.37E-08
MKK4	2.25E-06
MKK6	9.49E-06
MKK7	3.15E-06
MLCK/MYLK	>1.00E-05
MLCK2/MYLK2	2.38E-07
MLK1/MAP3K9	4.51E-07
MLK2/MAP3K10	2.13E-07
MLK3/MAP3K11	1.47E-07
MLK4	1.38E-06
MNK1	4.86E-08
MNK2	7.18E-09
MRCKa/CDC42BPA	6.98E-06
MRCKb/CDC42BPB	4.59E-06
MSK1/RPS6KA5	2.58E-06
MSK2/RPS6KA4	2.10E-06
MSSK1/STK23	>1.00E-05
MST1/STK4	5.40E-08
MST2/STK3	9.10E-08
MST3/STK24	3.34E-06
MST4	3.62E-06
MUSK	4.69E-08
MYLK3	>1.00E-05
MYLK4	5.46E-07
MYO3A	4.96E-07
MYO3b	3.28E-07
NEK1	6.12E-06
NEK11	>1.00E-05
NEK2	6.20E-06

Table S5: Kinase Selectivity Profiling for Compound 31 (cont.)

Kinase	IC_{50} (M)
NEK3	>1.00E-05
NEK4	7.74E-06
NEK5	>1.00E-05
NEK6	>1.00E-05
NEK7	>1.00E-05
NEK9	>1.00E-05
NIM1	>1.00E-05
NLK	2.00E-07
OSR1/OXSR1	>1.00E-05
P38a/MAPK14	>1.00E-05
P38b/MAPK11	2.88E-06
P38d/MAPK13	>1.00E-05
P38g	>1.00E-05
p70S6K/RPS6KB1	6.04E-07
p70S6Kb/RPS6KB2	6.29E-06
PAK1	8.85E-07
PAK3	6.25E-07
PAK4	7.76E-07
PAK5	1.01E-06
PAK6	4.15E-06
PASK	4.73E-06
PBK/TOPK	7.54E-06
PDGFRa	5.89E-09
PDGFRb	2.07E-08
PDK1/PDPK1	1.68E-06
PHKg1	3.09E-09
PHKg2	2.07E-08
PIM1	4.43E-06
PIM2	>1.00E-05
PIM3	1.48E-06
PKA	3.76E-06
PKAcb	5.22E-06
PKAcg	6.12E-06
PKCa	3.90E-06
PKCb1	2.66E-06
PKCb2	1.14E-06
PKCd	6.74E-07
PKCepsilon	1.97E-06
PKCeta	8.60E-07

Kinase	IC_{50} (M)
PKCg	1.30E-06
PKCIOTA	5.22E-06
PKCmu/PRKD1	2.34E-08
PKCnu/PRKD3	4.27E-09
PKCtheta	6.18E-06
PKCzeta	9.49E-06
PKD2/PRKD2	2.11E-08
PKG1a	>1.00E-05
PKG1b	5.54E-06
PKG2/PRKG2	>1.00E-05
PKN1/PRK1	9.28E-08
PKN2/PRK2	1.82E-06
PKN3/PRK3	1.49E-06
PLK1	5.72E-06
PLK2	>1.00E-05
PLK3	>1.00E-05
PLK4/SAK	2.34E-06
PRKX	7.04E-08
PYK2	1.35E-07
RAF1	>1.00E-05
RET	2.97E-09
RIPK2	7.66E-08
RIPK4	3.72E-07
RIPK5	2.92E-06
ROCK1	5.02E-07
ROCK2	1.34E-07
RON/MST1R	>1.00E-05
ROS/ROS1	6.50E-08
RSK1	2.24E-08
RSK2	4.00E-08
RSK3	8.64E-09
RSK4	2.29E-08
SBK1	>1.00E-05
SGK1	8.97E-06
SGK2	1.61E-06
SGK3/SGKL	>1.00E-05
SIK1	9.54E-09
SIK2	1.06E-09
SIK3	2.49E-08

Table S5: Kinase Selectivity Profiling for Compound 31 (cont.)

Kinase	IC_{50} (M)
SLK/STK2	7.49E-07
SNARK/NUAK2	7.56E-08
SNRK	>1.00E-05
SRMS	4.78E-06
SRPK1	>1.00E-05
SRPK2	>1.00E-05
SSTK/TSSK6	>1.00E-05
STK16	4.30E-06
STK21/CIT	1.23E-06
STK22D/TSSK1	2.61E-07
STK25/YSK1	7.61E-06
STK32B/YANK2	5.21E-06
STK32C/YANK3	>1.00E-05
STK33	4.53E-07
STK38/NDR1	5.98E-07
STK38L/NDR2	1.37E-06
STK39/STLK3	1.71E-06
SYK	1.96E-06
TAK1	1.48E-07
TAOK1	1.30E-06
TAOK2/TAO1	7.19E-06
TAOK3/JIK	4.26E-06
TBK1	1.15E-06
TEC	2.18E-07
TESK1	2.40E-06
TESK2	3.71E-07
TGFBR2	6.76E-07
TIE2/TEK	2.87E-07
TLK1	>1.00E-05
TLK2	1.87E-06
TNIK	8.47E-09
TNK1	3.73E-07
TRKA	2.57E-08
TRKB	4.34E-09
TRKC	<5.08E-10
TSSK2	>1.00E-05
TSSK3/STK22C	>1.00E-05
TTBK1	>1.00E-05
TTBK2	>1.00E-05

Kinase	IC_{50} (M)
TXK	1.99E-08
TYK1/LTK	2.81E-07
TYK2	1.03E-06
TYRO3/SKY	1.56E-07
ULK1	9.24E-08
ULK2	1.98E-07
ULK3	3.18E-07
VRK1	>1.00E-05
VRK2	>1.00E-05
WEE1	>1.00E-05
WNK1	>1.00E-05
WNK2	>1.00E-05
WNK3	>1.00E-05
YES/YES1	2.96E-09
YSK4/MAP3K19	>1.00E-05
ZAK/MLTK	2.40E-07
ZAP70	>1.00E-05
ZIPK/DAPK3	>1.00E-05

IRAK1, IRAK4, FLT3 and FLT3 Mutant Binding Assays. Kinase binding data were obtained for compounds **27**, **29** and **31** using the DiscoverX KINOME*scan*® active site-directed competition binding site-directed assay protocol described below.[3] Unlike other kinase competitive binding site assays, KINOME*scan*® assays do not require ATP. As a result, the data report thermodynamic interaction affinities (K_d values), rather than IC_{50} values that are dependent on ATP concentrations. The assay uses a DNA-tagged version of the protein kinase, and an immobilized ligand bound to a solid support. Compounds that directly or indirectly prevent kinase binding to the immobilized ligand reduce the amount of kinase captured on the solid support, which is detected using an ultra-sensitive qPCR method. Affinity constants reported from the assay have been reported to be independent of the immobilized ligand used that is coupled to the solid support.

Kinase-tagged T7 phage strains were prepared in an *E. coli* host derived from the BL21 strain. *E. coli* were grown to log-phase and infected with T7 phage and incubated with shaking at 32 °C until lysis. The lysates were centrifuged and filtered to remove cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 minutes at room temperature to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1% BSA, 0.05% Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1x binding buffer (20% SeaBlock, 0.17x PBS, 0.05% Tween 20, 6 mM DTT). Test compounds were prepared as 111x stocks in 100% DMSO. Kds were determined using an 11-point 3-fold compound dilution series with three DMSO control points. All compounds for Kd measurements are distributed by acoustic transfer (non-contact dispensing) in 100% DMSO. The compounds were then diluted directly into the assays such that the final concentration of DMSO was 0.9%. All reactions were performed in polypropylene 384-well plates. Each was a final volume of 0.02 mL. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05% Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05% Tween 20, 0.5 μM nonbiotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The kinase concentration in the eluates was measured by qPCR.

Binding constants (Kds) were calculated with a standard dose-response curve using the Hill equation. The Hill Slope was set to -1. Curves were fitted using a non-linear least square fit with the Levenberg-Marquardt algorithm.

IRAK4, FLT3 and FLT3 (D835Y) NanoBRET Assays. Kinase cellular potency data were obtained for compounds **27**, **29** and **31** using the Reaction Biology NanoBRET assay protocol described below.[2] The NanoBRET assay measures kinase engagement in real time in the context of the intact cell. Unlike the previously described biochemical kinase assays, the NanoBRET assay measures the binding and activity characteristics under equilibrium conditions using full-length kinases in the presence of cellular concentrations of ATP in live, uncompromised cells. As such, the assay provides a more relevant assessment of kinase potency and selectivity that would be expected to be observed in the native cellular environment, where potency is often considerably lower than that observed in the isolated biochemical assays.[4] The assay uses a Kinase-NanoLuc® fusion vector expressing a kinase protein to which a luciferase tag has been added, a cell-permeant fluorescent NanoBRET™ tracer, a NanoLuc® substrate, and an extracellular NanoLuc® inhibitor. Upon expression of the luciferase-tagged kinase, cells will produce a strong BRET signal only in the presence of the NanoBRET™ tracer. The extracellular NanoLuc® inhibitor ensures that the BRET signal observed emanates only from live cells. Because the BRET signal has tight distance constraints, addition of the test compound will decrease the BRET signal if the compound competes with the NanoBRET™ tracer for binding to the kinase domain. Under the appropriate tracer conditions established by the manufacturer, quantitative intracellular affinity and relative potency can then be determined using Mass Action model equations.

HEK-293 cells were purchased from ATCC. FuGENEHD Transfection Reagent, Kinase-NanoLucfusion plasmids, Transfection Carrier DNA, NanoBRETTracers and dilution buffer, NanoBRETNano-Glo Substrate, Extracellular NanoLucInhibitor were obtained from Promega.

Assays were conducted following Promega assay protocol with some modifications. HEK-293 Cells were transiently transfected with Kinase-NanoLucFusion Vector DNA by FuGENEHD Transfection Reagent. Testing compounds were delivered into 384 well assay plate by Echo 550 (LabcyteInc, Sunnyvale, CA). Transfected cells were harvested and mixed with NanoBRETTracer

Reagent and dispensed into 384 well plates and incubated at 37 ºC in 5% CO_2 cell culture incubator for 1 hour. The NanoBRETNano-Glo Substrate plus Extracellular NanoLucInhibitor Solution were added into the wells of the assay plate and incubated for 2 - 3 minutes at room temperature. The donor emission wavelength (460 nm) and acceptor emission wavelength (600 nm) were measured in the EnVisionplate reader. The BRET Ratios were calculated. BRET Ratio = [(Acceptor sample ÷Donor sample) –(Acceptor no-tracer control ÷Donor no-tracer control)]. The IC_{50} values of compounds were calculated with Prism GraphPad program.

NanoBRET™ Target Engagement Assay Protocol

1. Transient Transfection of HEK-293 Cells NanoLuc® Fusion Vector DNA

1). Cultivate HEK-293 cells (70-80% confluence) appropriately prior to assay. Trypsinize and collect HEK-293 cells.

2). Prepare lipid: DNA complexes as follows:

a. Prepare a 10 µg/ml solution of DNA in Opti-MEM without serum that consists of the following ratios of carrier DNA and DNA encoding NanoLuc® fusion. 9.0 µg/mL of Transfection Carrier DNA, 1.0 µg/mL of NanoLuc fusion vector DNA and 1 mL of Opti-MEM without phenol red. Mix thoroughly.

b. Add 30 µl of FuGENE HD Transfection Reagent into each milliliter of DNA mixture to form lipid: DNA complex.

c. Mix by inversion 10 times.

d. Incubate at ambient temperature for 20 minutes to allow complexes to form.

3). In a sterile, conical tube, mix 1 part of lipid:DNA complex with 20 parts of HEK-293 cells in suspension. Mix gently by inversion 5 times.

4). Dispense cells + lipid: DNA complex into a sterile tissue culture dish and incubate for 22-24 hours.

2. Addition of Test Compounds (dry plate shooting)

Each test compound is delivered from the compound source plate to the wells of 384-well white NBS plate by Echo 550.

3. Preparation of Cells with NanoBRET™ Tracer Reagent

1). Remove medium from dish with transfected HEK-293 cells via aspiration, trypsinize and allow cells to dissociate from the dish.

2). Neutralize trypsin using medium containing serum and centrifuge at 200 × *g* for 5 minutes to pellet the cells. Adjust the cell density to 2 × 105 cells/mL in Opti-MEM without phenol red.

3). Prepare Complete 20X NanoBRET™ Tracer Reagent with Tracer Dilution Buffer.

4). Dispense one part of Complete 20X NanoBRET™ Tracer Reagent to 20 parts of cells in the tube. Mix gently by inversion 10 times.

5). Dispense cell suspension into white, 384-well NBS plates. Incubate the plate at 37 °C, 5% CO2 for 1 hour.

Note: Prepare a separate set of samples without tracer for background correction steps.

4. NanoBRET™ Assay

1). Remove plate from incubator and equilibrate to room temperature for 15 minutes.

2). Prepare 3X Complete Substrate plus Inhibitor Solution in Assay Medium (Opti-MEMR I Reduced Serum Medium, no phenol red) just before measuring BRET.

3). Add 3X Complete Substrate plus Inhibitor Solution to each well of the 384-well plate. Incubate for 2–3 minutes at room temperature.

4). Measure donor emission wavelength (460 nm) and acceptor emission wavelength (600 nm) using the Envision 2104 plate reader.

5. Determination of BRET Ratio

To generate raw BRET ratio values, divide the acceptor emission value (600 nm) by the donor emission value (460 nm) for each sample. To correct for background, subtract the BRET ratio in the absence of tracer (average of no-tracer control samples) from the BRET ratio of each sample.

NanoBRET™ ratio equation:

BRET Ratio = (Acceptor sample ÷ Donor sample)

NanoBRET™ ratio equation, including optional background correction:

BRET Ratio = [(Acceptor sample ÷ Donor sample) – (Acceptor no-tracer control ÷ Donor no-tracer control)]

Normalized Bret Response equation (%):

(BRET Ratio of Compound Treated Sample/BRET Ratio of DMSO Control Sample)*100%

6. Determination of IC_{50} Values

IC_{50} curves are plotted and IC_{50} values are calculated using the GraphPad Prism 4 program based on a sigmoidal dose-response equation.

NF-κB (Pam3SCK4) and NF-κB (IL1B) Assays. Cellular potency data were obtained for compounds **27**, **29** and **31** using the NF-kB assay protocol described below. Activation of NF-kB gene transcription is a downstream signal in the IRAK signaling pathway.[5] Because THP-1 cells do not contain activated FLT3 receptors, measurement of the ability of a FLT3/IRAK1/IRAK4 inhibitor compound to inhibit the NF-kB production reflects the ability to inhibit signaling downstream of blocking signaling through the IRAK1/4 complex, and is not a composite measurement of activity that includes FLT3 kinase inhibition.

THP-1-Blue NF-κB cells (InvivoGen) carrying a stable integrated NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter construct were plated at a concentration of 1 x 10^5 cells per well. The cells were stimulated with Pam3CSK4 (1ng/mL) or hIL1B (1ng/mL). After 10 – 20 minutes, the cells were then treated with vehicle (DMSO) or serial dilutions of the test compounds (10 doses tested for each test compound, with a 1:10 dilution series starting at 1 μM or 3 μM) with a final volume of 200 μL for 24 hours at 37 °C. After 24 hours, the cells were centrifuged and 20 μL supernatant was incubated with 180 μL QUANTI-Blue reagent at 37 °C for 30 – 60 minutes. The levels of NF-κB-induced was measured in a microplate reader at 620 nm.

Table S6: NF-kB Cellular Assay Data for Compounds 27, 29 and 31

	NF-κB (Pam3SCK4)						NF-κB (IL1B)					
Cpd	IC_{50} (nM) Run 1	IC_{50} (nM) Run 2	IC_{50} (nM) Run 3	IC_{50} (nM) Run 4	IC_{50} (nM) Geometric Mean	SEM	IC_{50} (nM) Run 1	IC_{50} (nM) Run 2	IC_{50} (nM) Run 3	IC_{50} (nM) Run 4	IC_{50} (nM) Geometric Mean	SEM
27	3.4	2	2.7	3.9	2.9	0.4	2.7	3.8	6.9	13.8	5.6	2.5
29	25.9	37.3	21.4	N.D.	27.4	4.7	23.5	62.2	36.8	N.D.	37.7	11.4
31	20.5	14.9	20	N.D.	18.3	1.8	14.5	9.7	15.4	N.D.	12.9	1.8

MOLM14 (D835Y) Cell Viability Assay. Cellular potency data were obtained for compounds **27**, **29** and **31** using the MOLM14 (D835Y) cell viability assay protocol described below. The cell line has activated FLT3 receptors that carry additional resistance mutations in the kinase domain (D835Y). Leukemias from patients harboring these kinase domain resistance mutations are resistant to FLT3 inhibitors that do not inhibit the mutant kinase. Because the activated FLT3 receptor drives a mitogenic response, and because there can be a discrepancy between activity in the biochemical kinase assay and in the context of a whole cell, demonstration of antiproliferative activity in these cell lines with compounds known to inhibit the D835Y kinase in biochemical assays provides a more relevant cellular context for demonstration of activity.

MOLM14 (D835Y) cells were grown in RPMI-1640 media supplemented with 20% fetal bovine serum (FBS). For viability/cytotoxicity assessments, cells were seeded into 1536-well white polystyrene tissue culture-treated Greiner plates using a Multidrop Combi dispenser (ThermoFisher), in final volume 5 μL of growth media per well, at a density of 1000 cells per well. After cell addition, 23 nL of test compound were transferred into individual wells (22 doses tested for each test compound, with a 1:2 dilution series starting at 10 μM) via a 1536 pin-tool. Bortezomib (final concentration 2.3 μM) was used as a positive control for cell cytotoxicity. Plates were incubated for 48 hours at standard incubator conditions covered by a stainless steel gasketed lid to prevent evaporation. 48 hours post compound addition, 3 μL of Cell Titer Glo (Promega) were added to each well and plates were incubated at room temperature for 15 minutes with the

stainless-steel lid in place. Luminescence readings were taken using a Viewlux imager (PerkinElmer) with a 2 second exposure time per plate.

Table S7: MOLM14 Cellular Assay Data for Compounds 1, 27, 29, 31 & Gilteritinib

	MOLM14 (D835Y)						MOLM14 (F691L)			
Compd	IC_{50} (nM) Run 1	IC_{50} (nM) Run 2	IC_{50} (nM) Run 3	IC_{50} (nM) Run 4	IC_{50} (nM) Geometric Mean	SEM	IC_{50} (nM) Run 1	IC_{50} (nM) Run 2	IC_{50} (nM) Geometric Mean	SEM
1	4.7	5.6	N.D.	N.D.	5.1	0.5	204	67	117	68.5
27	4.1	0.9	5.3	5.5	3.2	1.1	45.7	37.6	41.5	4.1
29	11.5	2.2	13.3	N.D.	7.0	3.4	57.6	59.6	58.6	1.0
31	10.2	13.3	12.2	1.5	7.1	2.7	45.7	42.2	43.9	1.8
Gilter-itinib	58	60	77	N.D.	65	6.0	N.D.	N.D.	N.D.	N.D.

N.D. = not determined

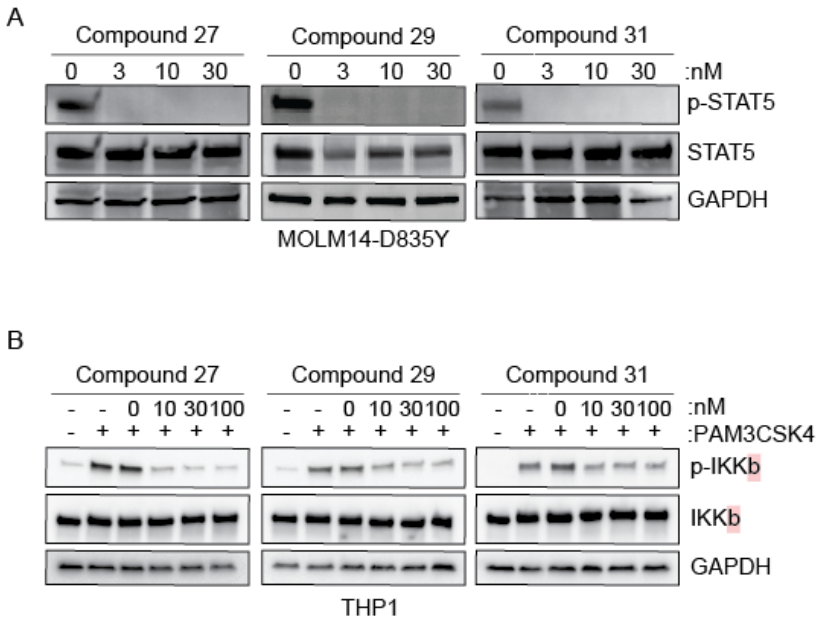
Western Blot Experiments. Western blot experiments were performed using compounds **27**, **29** and **31**, and the cells lines and experimental procedures detailed below.

MOLM14 (D835Y) cells were gifted by the Neil Shah laboratory (UCSF, San Francisco, CA). They were maintained in culture using RPMI 1640 plus 20% fetal bovine serum (FBS) and penicillin/streptomycin (P/S) at 37 °C with 5% CO_2. THP1 cells were purchased from the American Type Culture Collection. THP1 were cultured in RPMI-1640 medium with 10% FBS and 1% penicillin–streptomycin. THP1 cells were pre-treated for 1 hour with the inhibitors, then stimulated with 10 ng/mL of PAM3CSK4 for 1 hour.

Protein lysates were made by lysing cells in cold RIPA lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1% Triton X-100, and 0.1% sodium dodecyl sulfate (SDS)) in the presence of sodium orthovanadate, phenylmethylsulfonyl fluoride (PMSF), and protease and phosphatase inhibitors. Protein concentration was quantified using

bicinchoninic acid (BCA) assay (Pierce, #23225). Protein lysates were separated by SDS-polyacrylamide gel electrophoresis (BIO-RAD), transferred to nitrocellulose membranes (BIO-RAD, #1620112), and immunoblotted. Immunoblotting was performed using the following antibodies: phospho-STAT5 (Cell Signaling, #9351), STAT5 (Cell Signaling, #94205), phospho-IKKa/b (Ser176/180) (Cell Signaling, #2697), IKKb (Cell Signaling, #2370), and GAPDH (Cell Signaling, #5174).

Figure S1: Western Blot Experiments for Compounds 27, 29 and 31.



(A) Immunoblotting of MOLM14 (D835Y) cells treated with the indicated inhibitors for 90 minutes. (B) Immunoblotting of THP1 cells pre-treated with the indicated inhibitors for 1 hour and then stimulated with the TLR2 ligand (PAM3CSK4, 10 ng/ml) for 1 hour to induce NF-kB activation.

In MOLM14 (D835Y) cells, compounds **27**, **29** and **31** inhibit phosphorylation of STAT5 (a substrate of FLT3) at concentrations in the range of 3 – 30 nM. Those concentrations are similar to the IC_{50}s of compounds **27**, **29** and **31** in the MOLM14 (D835Y) assay (Table S7; IC_{50}s 3 – 7 nM). In THP1 cells, compounds **27**, **29** and **31** inhibit phosphorylation of IKKb at concentrations in the range of 10 – 100 nM. Those concentrations are similar to the IC_{50}s of compounds **27**, **29** and **31** in the NF-kB assay when using the same stimulant (PAM3CSK4) to initiate signaling (Table S6; IC_{50}s 3 – 27 nM).

PAMPA Assay. The stirring double-sink PAMPA method patented by Pion Inc. (Billerica, MA) was employed to determine the permeability of compounds via PAMPA passive diffusion. The PAMPA lipid membrane, which consists of an artificial membrane of a proprietary lipid mixture and dodecane (Pion Inc.), was optimized to predict gastrointestinal tract (GIT) passive diffusion permeability. The PAMPA assay was run as outlined below, and as described previously.[6]

PAMPA membrane was immobilized on a plastic matrix of a 96 well donor filter plate placed above a 96 well acceptor plate. Both donor and acceptor wells were buffered to pH 7.4. 10 mM DMSO stock solutions of test compounds were diluted to 0.05 mM in aqueous buffer (pH 7.4) and a concentration of 0.5% DMSO in the final solution. During the 30 minute permeation period test samples in the donor compartment were stirred using Gutbox technology (Pion Inc.) to reduce the unstirred water layer. Test article concentrations in the donor and acceptor compartments were measured using a UV plate reader (Nano Quant, Infinite_200 PRO, Tecan Inc., Mannendorf, Switzerland). Permeability calculations were performed using Pion Inc. software and were expressed in units of 10^{-6} cm/s. PAMPA values of $<10 \times 10^{-6}$ cm/s indicate low passive membrane permeability, values in the range of $10 - 100 \times 10^{-6}$ cm/s indicate moderate passive membrane permeability, and values $>100 \times 10^{-6}$ cm/s indicate high passive membrane permeability.

Caco-2 Assay. Bidirectional Caco-2 (human epithelial colorectal adenocarcinoma) permeability experiments were conducted at Cyprotex US LLC (Watertown and/or Framingham, MA). Caco-2 cells obtained from the American Type Culture Collection (ATCC) were used between passage numbers 40–60. Cells were seeded on to Millipore Multiscreen Caco-2 plates (Millipore) at 1.6×10^5 cells/cm^2. They were cultured for 20 days in Dubecco's modified Eagle's medium, which the media was changed every 2-3 days. On day 20, 21, or 22, the permeability study was performed. Integrity of monolayers were checked for intactness using Trans-epithelial electrical resistance (TEER). Hanks balanced salt solution (HBSS), pH 7.4, buffered with 25 mM 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) and 4.45 mM glucose at 37 ºC was used as the medium in the permeability studies. Cell plates were washed, and test compound was added into assay media at a final concentration of 10 μM (final DMSO concentration 1%). The fluorescent integrity marker lucifer yellow was also included in the dosing solution. Cell plates with test compounds and controls were incubated for 2 hours in an atmosphere of 5% CO_2 with a relative humidity of

95% at 37 °C. Following the incubations, lucifer yellow fluorescence was quantified using a plate reader, and test and control compounds (warfarin, ranitidine, and talinolol) were quantified by liquid chromatography–tandem mass spectrometry (LCMS/MS) to obtain peak area ratios (analyte/internal standard). The permeability coefficients (P_{app}) for the test compounds in the apical to basolateral (A → B) and basolateral to apical (B → A) direction across Caco-2 cells, and the ratio of B → A and A → B permeabilities (efflux ratio), which shows whether the compound undergoes active transport, were calculated.

Liver Microsome Metabolic Stability Assay. The liver microsome metabolic stability assay was run as outlined below, and as described previously.[7] Rat, mouse, and human liver microsome metabolic stability assays were run in triplicate using the substrate depletion method. Incubation and liquid handling were carried out using a Tecan EVO robotic system equipped with EVOware software. Briefly, each reaction mixture (110 μL) consisted of a test compound (1 μM), 0.5 mg/mL of liver microsomes, and NADPH regenerating system (1 μM) in phosphate buffer at pH 7.4. Samples were incubated in 384-well plates at 37 ºC for 0, 5, 10, 15, 30, and 60 minutes. Reactions were stopped by adding cold acetonitrile with internal standard (albendazole). All plates were centrifuged at 3000 rpm for 20 minutes at 4 ºC, and the supernatants were analyzed using the previously described method.[7]

Hepatocyte Stability Assay. The stability in cryopreserved hepatocytes stability experiments were conducted at Cyprotex US LLC (Watertown and/or Framingham, MA). Williams E media supplemented with 2 mM L-glutamine, 25 mM 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), and the test compound (final substrate concentration 1 μM; final DMSO concentration 0.25%) were pre-incubated at 37 °C, prior to adding a suspension of cryopreserved mouse or rat hepatocytes (final cell density 0.5×10^6 viable cells/mL in Williams E media supplemented with 2 mM L-glutamine and 25 mM HEPES) to initiate the reaction. Two control compounds (verapamil and 7-Hydroxy-4-(trifluoromethyl)coumarin) were included for each species, along with the appropriate vehicle control. The reactions were terminated by transferring incubate to an aliquot of ice-cold methanol containing an internal standard at appropriate time points (0, 15, 30, 60, and 120 minutes). The terminated samples were filtered by centrifugation for 1 minute at 500 rcf at 4 °C to remove precipitated proteins. Following filtration and centrifugation,

supernatants were analyzed by liquid chromatography–tandem mass spectrometry (LCMS/MS), to obtain peak area ratios (analyte/internal standard) in order to determine the % remaining of the test compound, to then calculate the half-life and intrinsic clearance values.

Plasma Protein Binding Assays. The plasma protein binding experiments were conducted at Cyprotex US LLC (Watertown and/or Framingham, MA). Test compound (final concentration 5 μM; final DMSO concentration 0.25%) was added in duplicate to 100% mouse, rat, and human plasma (pH 7.4, ± 0.1, adjusted if necessary). The mixture was dialyzed in a RED device (Rapid Equilibrium Dialysis, Pierce) per the manufacturers' instructions against PBS and incubated on an orbital shaker for 4 hours at 37 °C. Positive control compound warfarin was included for each species, along with the appropriate vehicle control. At the end of the incubation, aliquots from both plasma and PBS sides were collected, and were matrix-matrix matched with an appropriate amount of PBS and blank plasma, respectively. Acetonitrile (three volumes) containing an analytical internal standard was added to precipitate the proteins and release the test compound. After filtration, the supernatant was transferred to a new plate and analyzed by liquid chromatography–tandem mass spectrometry (LCMS/MS) to obtain peak area ratios (analyte/internal standard) for determining the fraction unbound.

Rat Pharmacokinetic Assay. Rat pharmacokinetic experiments were conducted as follows: test compounds **27**, **28**, **29** and **31** were formulated as 1.0 mg/mL solutions in 9% DMSO/91% sterile water. Fasted male Sprague-Dawley rats were given either a 1.0 mg/kg i.v. dose (n=3) of test compound solution via a cannula implanted in the femoral vein or a 3.0 mg/kg p.o. dose (n=3) by gavage. Serial blood samples were collected at 5 (i.v. only), 15, and 30 minutes, and at 1, 2, 4, 8, and 24 hours post dose. Plasma was collected by centrifugation, and plasma concentrations of test compound were determined by LCMS/MS following protein precipitation with acetonitrile.

Mouse Pharmacokinetic Assay. Mouse pharmacokinetic experiments were conducted as follows: test compound **31** was formulated as a 0.2 mg/mL solution in 0.9% saline (i.v. dosing) or a 0.3 mg/mL solution in 0.9% saline (p.o. dosing). Fasted CD1 mice were given either a 1.0 mg/kg i.v. dose (n=3) of test compound solution via a cannula implanted in the femoral vein or a 3.0

mg/kg p.o. dose (n=3) by gavage. Serial blood samples were collected at 5, 15, and 30 minutes, and at 1, 2, 4, 8, and 24 hours post dose. Plasma was collected by centrifugation, and plasma concentrations of test compound were determined by LCMS/MS following protein precipitation with acetonitrile.

Dog Pharmacokinetic Assay. Dog pharmacokinetic experiments were conducted as follows: test compound **31** was formulated as a 0.2 mg/mL solution in 0.9% saline (i.v. dosing) or a 0.3 mg/mL solution in 0.9% saline (p.o. dosing). Fasted male beagle dogs were given either a 1.0 mg/kg i.v. dose (n=3) of test compound solution via a cannula implanted in the femoral vein or a 3.0 mg/kg p.o. dose (n=3) by gavage. Serial blood samples were collected at 5, 15, and 30 minutes, and at 1, 2, 4, 8, and 24 hours post dose. Plasma was collected by centrifugation, and plasma concentrations of test compound were determined by LCMS/MS following protein precipitation with acetonitrile.

Mouse MOLM14 FLT3 (ITD, D835Y) Xenograft Assay. The study was approved by the NCI Frederick Animal Care and Use Committee (ACUC). NCI-Frederick is accredited by AAALAC International and follows the Public Health Service Policy for the Care and Use of Laboratory Animals. Animal care was provided in accordance with the procedures outlined in the "Guide for Care and Use of Laboratory Animals (National Research Council; 1996; National Academy Press; Washington, D.C.). Female NSG-SGM3 (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl} Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ) mice aged 8 weeks (Jackson Laboratory, JAX 013062) were intravenously engrafted with MOLM14 FLT3 (ITD, D835Y) acute myeloid leukemia cells at a dose of 5 x 10^4 per mouse. Three weeks post cell injection the mice were randomized into treatment groups (n=10 mice per dosing arm) based on body weight using the Studylog software (Studylog Systems) and dosing started. Mice were administered vehicle control (PBS) IP, 10 mg/kg Gilteritinib IP, or 10 mg/kg compound **31** IP once daily Monday - Friday for 7 weeks. Survival was assessed for the duration of the study. Mice experiencing clinical signs prior to scheduled end-date, including weight loss, lethargy, hunched posture, ruffled coat, or hind limb paralysis were humanely euthanized.

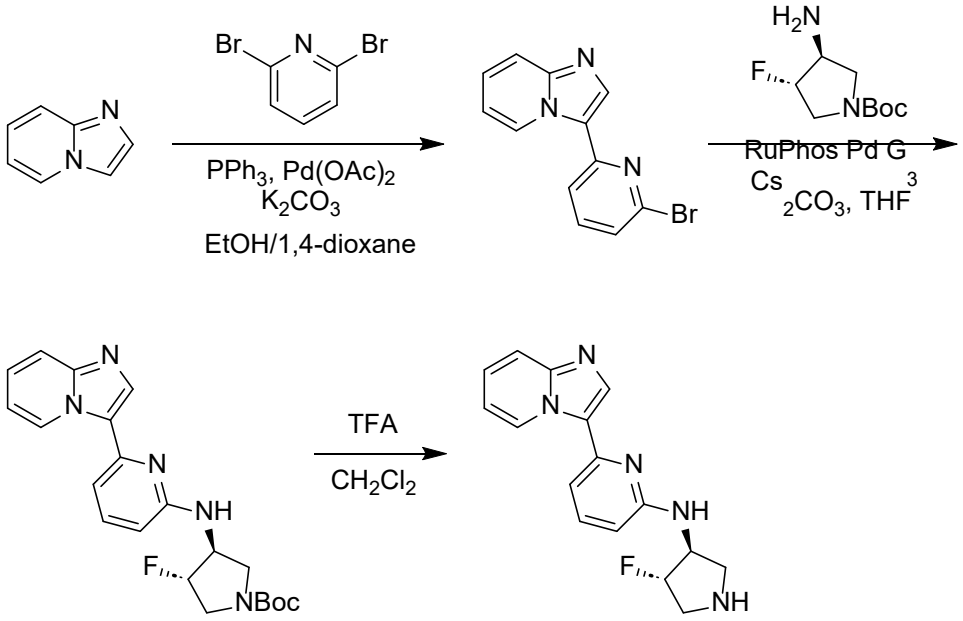
In a separate PK arm of the study, female NSG-SGM3 (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl} Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ) mice aged 8 weeks (Jackson Laboratory, JAX 013062) were administered 10 mg/kg compound **31** IP once daily Monday - Friday for 4 weeks

(n=9 mice per dosing arm). Blood samples were collected at 1 hour post-dose on dosing days 1, 5, 22 and 26, and at 4 hours post-dose on day 5. Plasma was collected by centrifugation, and plasma concentrations of test compound were determined by LCMS/MS following protein precipitation with acetonitrile.

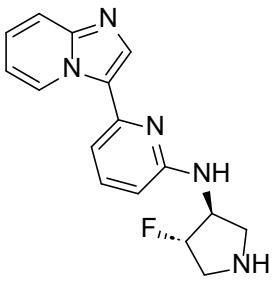
General Synthetic Methods. Unless otherwise stated, all reactions were carried out under an atmosphere of dry nitrogen in dried glassware. Indicated reaction temperatures refer to those of the reaction bath, while room temperature (rt) is noted as 25 ºC. Unless otherwise noted, all solvents were of anhydrous quality purchased from Aldrich Chemical Co. and were used as received. Commercially available starting materials and reagents were purchased from commercial suppliers and were used as received. Analytical thin layer chromatography (TLC) was performed with Sigma Aldrich TLC plates (5 x 20 cm, 60 Å, 250 μm). Visualization was accomplished by irradiation under a 254 nm UV lamp. Chromatography on silica gel was performed using forced flow (liquid) of the indicated solvent system on Biotage KP-Sil pre-packed cartridges and using the Biotage SP-1 automated chromatography system. ^{1}H NMR spectra were recorded on a Varian Inova 400 MHz spectrometer. Chemical shifts are reported in ppm with the solvent resonance as the internal standard (DMSO-d_6 2.50 ppm for ^{1}H). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants, and number of protons. Low resolution mass spectra (electrospray ionization) were acquired on an Agilent Technologies 6130 quadrupole spectrometer coupled to the HPLC system. Unless otherwise noted, all LCMS ions listed are [M+H]. If needed, products were purified via semi-preparative HPLC using the columns and mobile phases noted. Samples were analyzed for purity on an Agilent 1200 series LC/MS equipped with a Luna® C18 reverse phase (3 micron, 3 x 75 mm) column having a flow rate of 0.8 – 1.0 mL/min over a 7-minute gradient and an 8.5 minute run time (Method 1). Unless otherwise noted, the mobile phase was a mixture of acetonitrile (0.025% TFA) and H_2O (0.05% TFA), with temperature maintained at 50 ºC. Purity of final compounds was determined to be >95%, using a 3 μL injection with quantitation by AUC at 220 and 254 nm (Agilent Diode Array Detector). All compounds are >95% pure by HPLC analysis.

Synthesis Schemes. Detailed procedures for the synthesis of these compounds have been reported previously.[8] Compounds **2** – **9** were prepared using general synthesis A and the appropriate commercially available starting materials. Compounds **10** and **11** were prepared using general synthesis B and the appropriate commercially available starting materials. Compounds **12** and **17** – **26** were prepared using general synthesis C and the appropriate commercially available starting materials. Compounds **13**, **14** and **15** were prepared using synthesis routes D, E, and F, respectively. Compounds **16** and **29** were prepared using general synthesis G and the appropriate commercially available starting materials. Compounds **28**, **30** and **31** were prepared using general synthesis H and the appropriate commercially available starting materials.

Scheme S1. General synthesis A of compounds **2** – **9**.

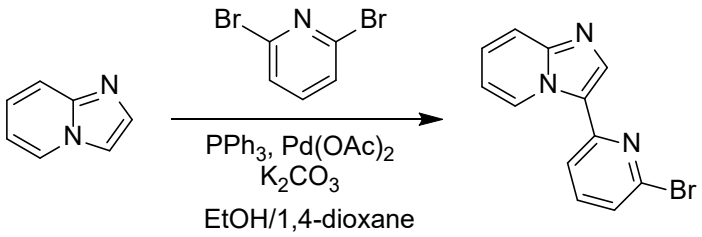


Representative Synthetic Procedure for Compounds 2 – 9.



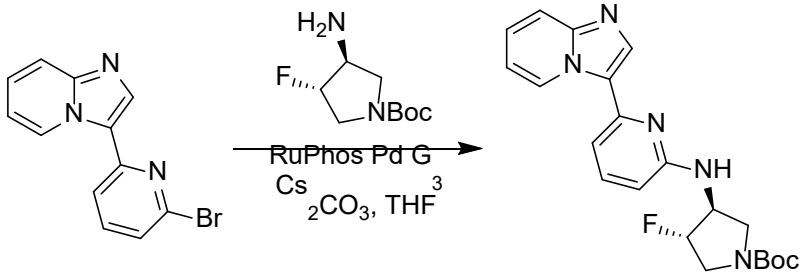
Compound 8, N-((3S,4S)-4-fluoropyrrolidin-3-yl)-6-(imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine

Step A. 3-(6-bromopyridin-2-yl)imidazo[1,2-a]pyridine



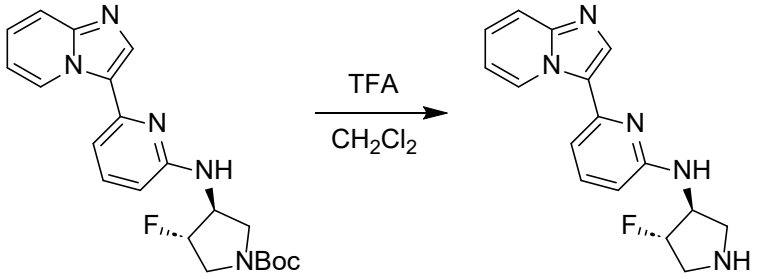
A mixture of imidazo[1,2-a]pyridine (0.300 g, 2.54 mmol), 2,6-dibromopyridine (1.80 g, 7.62 mmol), triphenylphosphine (0.100 g, 0.381 mmol), palladium(II)acetate (0.057 g, 0.254 mmol), and potassium carbonate (1.05 g, 7.62 mmol) in dioxane (6 mL) and ethanol (3 mL) was degassed and purged with nitrogen, and was then stirred at 100 °C for 16 hours under nitrogen atmosphere. The reaction was then cooled to room temperature, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 10% ethyl acetate in petroleum ether) to provide the title compound as a pale yellow solid (0.300 g, 43% yield): LCMS *m/z* calcd for $C_{12}H_8BrN_3$: 274.0 $[M+H]^+$, found 274.0 $[M+H]^+$.

Step B. (3S,4S)-tert-butyl 3-fluoro-4-((6-(imidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)pyrrolidine-1-carboxylate



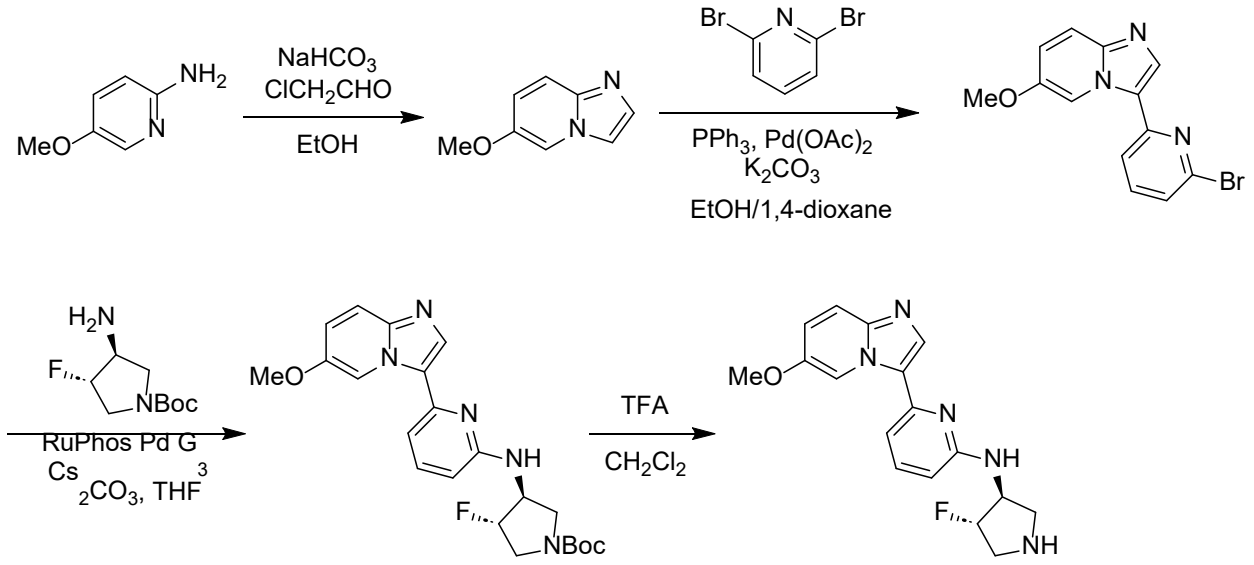
A mixture of tert-butyl (3S,4S)-3-amino-4-fluoro-pyrrolidine-1-carboxylate (0.070 g, 0.342 mmol), 3-(6-bromo-2-pyridyl)imidazo[1,2-a]pyridine (0.094 g, 0.342 mmol), (2-dicyclohexylphosphino-2',6'-diisopropoxy-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II)methanesulfonate (0.029 g, 0.034 mmol), and cesium carbonate (0.335 g, 1.03 mmol) in tetrahydrofuran (3 mL) was degassed and purged with nitrogen, and was then stirred at 80 °C for 4 hours under nitrogen atmosphere. The reaction was then cooled to room temperature, filtered, and concentrated under reduced pressure to provide the title compound which was used in the next step without further purification: LCMS *m/z* calcd for $C_{21}H_{24}FN_5O_2$: 398.2 $[M+H]^+$, found 398.2 $[M+H]^+$.

Step C. N-((3S,4S)-4-fluoropyrrolidin-3-yl)-6-(imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine

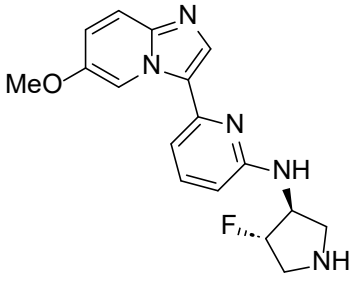


To a solution of tert-butyl (3S,4S)-3-fluoro-4-[(6-imidazo[1,2-a]pyridin-3-yl-2-pyridyl)amino]pyrrolidine-1-carboxylate (0.100 g, 0.252 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (0.770 g, 6.75 mmol, 0.500 mL). The resulting reaction was stirred for 1 hour at room temperature, and was then concentrated under reduced pressure. The resulting crude product was purified by HPLC (Phenomenex Luna C18 column, 10 micron, 250 x 50 mm; 35 – 65% acetonitrile in aqueous 10 mM NH_4HCO_3) to provide the title compound as a pale yellow solid (7.7 mg, 7% yield over two steps): HRMS (ESI^+) *m/z* calcd for $C_{16}H_{16}FN_5$: 298.1463 $[M+H]^+$, found 298.1470; ^1H NMR (400MHz, CD_3OD) δ 10.16 (d, *J* = 7.1 Hz, 1 H), 8.56 (s, 1 H), 8.01 (br s, 1 H), 7.99 (s, 1 H), 7.69 (t, *J* = 7.9 Hz, 1 H), 7.54 – 7.47 (m, 1 H), 7.34 (d, *J* = 7.5 Hz, 1 H), 6.73 (d, *J* = 8.4 Hz, 1 H), 5.59 – 5.37 (m, 1 H), 4.91 (br d, *J* = 6.8 Hz, 1 H), 3.91 (dd, *J* = 6.5, 12.8 Hz, 1 H), 3.78 – 3.72 (m, 1 H), 3.71 – 3.63 (m, 1 H), 3.59 (dd, *J* = 2.6, 12.9 Hz, 1 H).

Scheme S2. General synthesis B of compounds **10** and **11**.

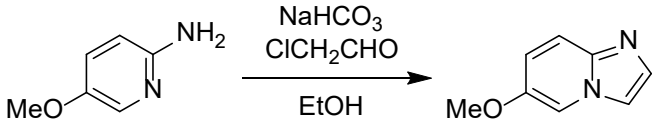


Representative Synthetic Procedure for Compounds 10 and 11.



Compound 10, N-((3S,4S)-4-fluoropyrrolidin-3-yl)-6-(6-methoxyimidazo[1,2-a]pyridin-3-yl)pyridin-2-amine

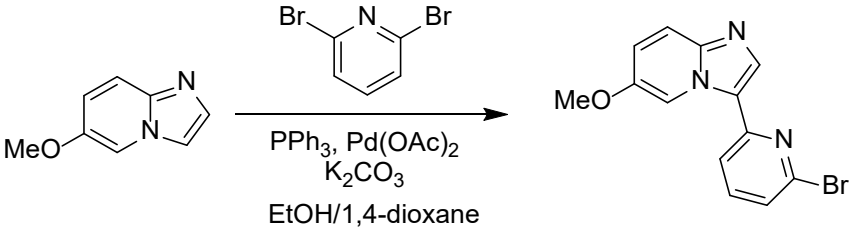
Step A. 6-Methoxyimidazo[1,2-a]pyridine



To a solution of 5-methoxypyridin-2-amine (2.50 g, 20.1 mmol) in ethyl alcohol (20 mL) were added sodium bicarbonate (2.88 g, 34.2 mmol) and 2-chloroacetaldehyde (39.52 g, 201.4 mmol). The mixture was heated at 80 °C for 5 hours, then cooled to room temperature and concentrated under reduced pressure. The resulting residue was diluted with aqueous sodium

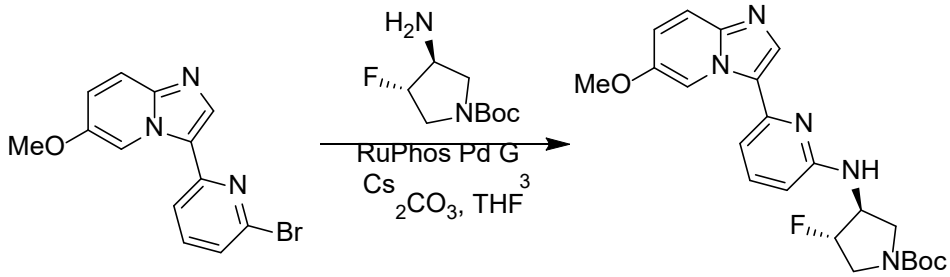
carbonate solution (2 N, 60 mL) and extracted with ethyl acetate (2 x 30 mL). The combined organic extracts were washed with saturated aqueous sodium chloride solution (2 x 20 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The resulting crude product was purified first by flash chromatography on silica gel (0 – 80% methanol in dichloromethane) to give a product that was further purified by HPLC (Xtimate C18 column, 10 micron, 250 x 80 mm; 0 – 30% acetonitrile in aqueous 10 mM NH_4HCO_3) to provide the title compound as a yellow oil (2.50 g, 36% yield): LCMS *m/z* calcd for $C_8H_8N_2O$: 149.1 $[M+H]^+$, found 149.1 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 2.1 Hz, 1 H), 7.49 – 7.43 (m, 3 H), 6.96 (dd, *J* = 2.3, 9.8 Hz, 1 H), 3.76 (s, 3 H).

Step B. 3-(6-bromopyridin-2-yl)-6-methoxyimidazo[1,2-a]pyridine



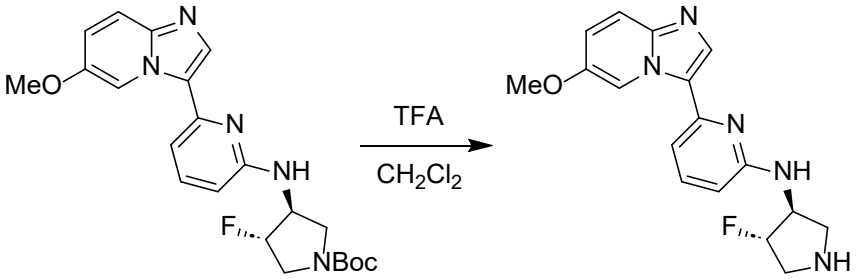
A mixture of 6-methoxyimidazo[1,2-a]pyridine (2.00 g, 13.5 mmol), 2,6-dibromopyridine (15.99 g, 67.49 mmol), triphenylphosphine (0.354 g, 1.35 mmol), palladium acetate (0.303 g, 1.35 mmol) and potassium carbonate (5.60 g, 40.5 mmol) in ethyl alcohol (30 mL) and 1,4-dioxane (60 mL) was degassed and purged with nitrogen, and then heated at 100 °C for 16 hours under nitrogen atmosphere. The reaction mixture was then cooled to room temperature, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 100% ethyl acetate in petroleum ether) to provide the title compound as a pale yellow solid (0.850 g, 21% yield): LCMS *m/z* calcd for $C_{13}H_{10}BrN_3O$: 304.0 $[M+H]^+$, found 304.0 $[M+H]^+$.

Step C. (3S,4S)-tert-butyl 3-fluoro-4-((6-(6-methoxyimidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)pyrrolidine-1-carboxylate



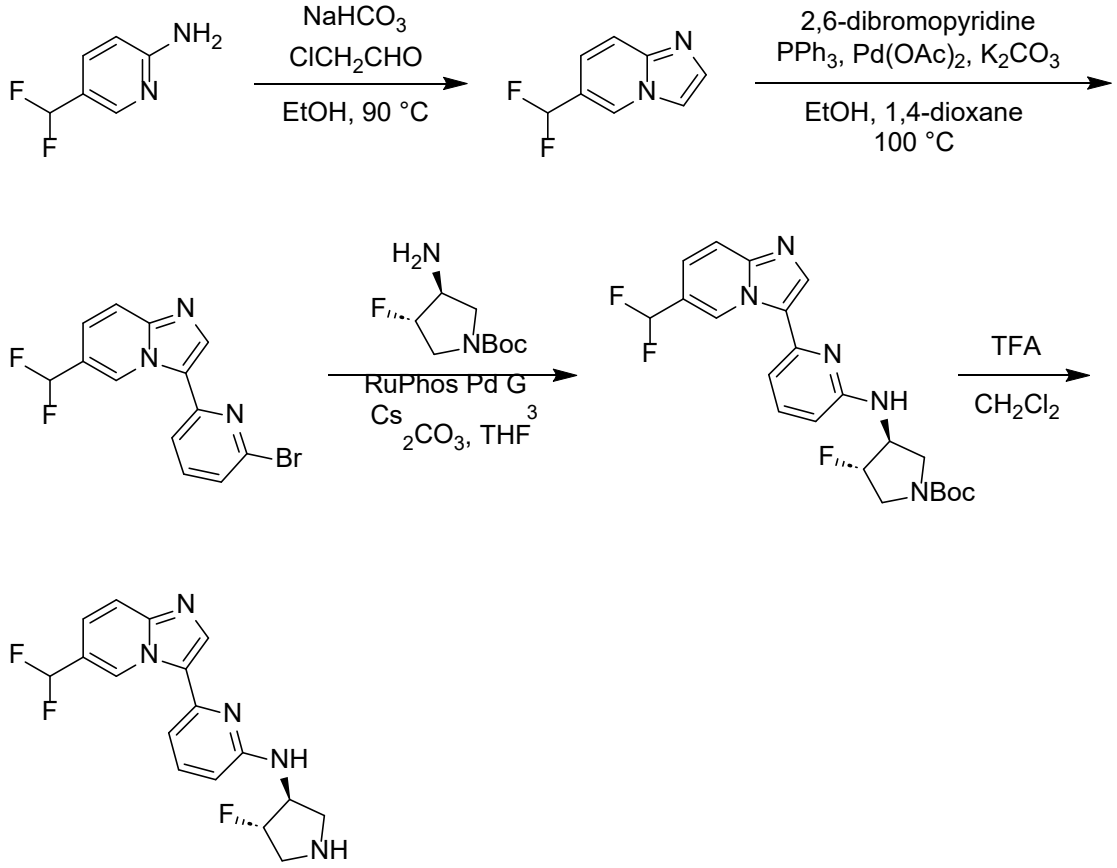
A mixture of 3-(6-bromopyridin-2-yl)-6-methoxyimidazo[1,2-a]pyridine (0.100 g, 0.329 mmol), (3S,4S)-tert-butyl 3-amino-4-fluoropyrrolidine-1-carboxylate (0.067 g, 0.329 mmol), cesium carbonate (0.321 g, 0.986 mmol), (2-dicyclohexylphosphino-2',6'-diisopropoxy-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II)methanesulfonate (0.028 g, 0.033 mmol) in tetrahydrofuran (4.0 mL) was degassed and purged with nitrogen, and was then heated at 80 °C for 2 hours under nitrogen atmosphere. The reaction mixture was then cooled to room temperature, filtered, and concentrated under reduced pressure to provide the title compound which was used without further purification in the next step: LCMS *m/z* calcd for $C_{22}H_{26}FN_5O_3$: 428.2 $[M+H]^+$, found 428.2 $[M+H]^+$.

Step D. N-((3S,4S)-4-fluoropyrrolidin-3-yl)-6-(6-methoxyimidazo[1,2-a]pyridin-3-yl)pyridin-2-amine

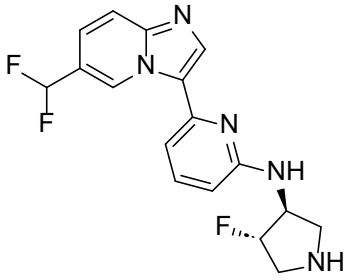


To a solution of (3S,4S)-tert-butyl 3-fluoro-4-((6-(6-methoxyimidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)pyrrolidine-1-carboxylate (0.100 g, 0.234 mmol) in dichloromethane (3 mL) was added trifluoroacetic acid (0.770 g, 6.75 mmol). The resulting mixture was stirred at 20 °C for 2 hours, and was then filtered and concentrated under reduced pressure. The resulting crude product was purified by HPLC (Waters Xbridge OBD C18 column, 10 micron, 150 x 40 mm; 5 – 35% acetonitrile in aqueous 10 mM NH_4HCO_3) to provide the title compound as a pale yellow solid (0.023 g, 23% yield over two steps): HRMS (ESI^+) *m/z* calcd for $C_{17}H_{18}FN_5O$: 328.1568 $[M+H]^+$, found 328.1567; ^1H NMR (400 MHz, CD_3OD) δ 9.34 (d, *J* = 2.2 Hz, 1 H), 7.91 (s, 1 H), 7.55 – 7.38 (m, 2 H), 7.13 – 7.01 (m, 2 H), 6.41 (d, *J* = 7.8 Hz, 1 H), 5.29 – 5.12 (m, 1 H), 4.71 – 4.64 (m, 1 H), 3.81 (s, 3 H), 3.65 – 3.52 (m, 1 H), 3.36 (d, *J* = 2.1 Hz, 1 H), 3.30 – 3.25 (m, 1 H), 3.12 (dd, *J* = 2.9, 12.6 Hz, 1 H).

Scheme S3. General synthesis C of compounds **12** and **17 – 26**.

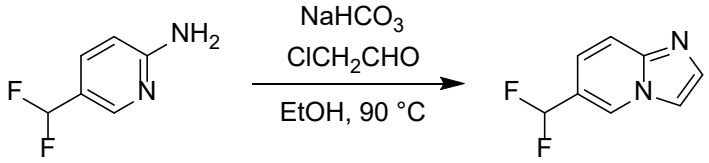


Representative Synthetic Procedure for Compounds 12 and 17 – 26.



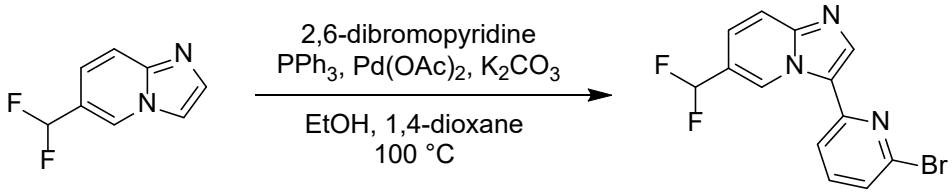
Compound 12, 6-(6-(difluoromethyl)imidazo[1,2-a]pyridin-3-yl)-N-((3S,4S)-4-fluoropyrrolidin-3-yl)pyridin-2-amine

Step A. 6-(difluoromethyl)imidazo[1,2-a]pyridine



To a heavy-walled sealable flask equipped with a magnetic stir bar were added 5-(difluoromethyl)pyridin-2-amine (2.00 g, 13.9 mmol), sodium bicarbonate (2.91 g, 34.7 mmol), ethanol (46 mL), and 2-chloroacetaldehyde (4.86 mL, 34.7 mmol, 45% weight in H_2O), in that order. The vessel was sealed with a Teflon screwcap warmed to 90 °C for 5 hours. The reaction mixture was then cooled to room temperature, filtered through silica, poured into water (20 mL) and extracted with ethyl acetate (2 x 20 mL). The organic extracts were combined, washed with saturated aqueous sodium chloride solution (2 x 20 mL), dried over magnesium sulfate, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 100% ethyl acetate in hexanes) to afford the title compound (2.04 g, 88% yield): LCMS *m/z* calcd for $C_8H_6F_2N_2$: 169.1 $[M+H]^+$, found 169.1 $[M + H]^+$; ^1H NMR (400 MHz, CD_3OD) δ 8.76 (s, 1 H), 7.94 (s, 1 H), 7.70 – 7.60 (m, 2 H), 7.45 (d, J = 9.4 Hz, 1 H), 6.87 (t, J = 55.5 Hz, 1 H).

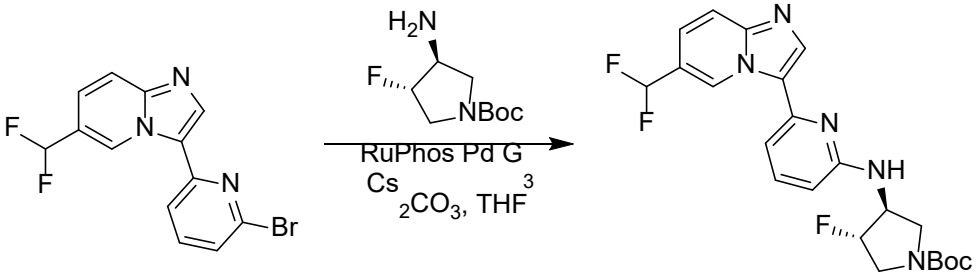
Step B. 3-(6-bromopyridin-2-yl)-6-(difluoromethyl)imidazo[1,2-a]pyridine



To a heavy-walled sealable flask equipped with a magnetic stir bar were added 6-(difluoromethyl)imidazo[1,2-a]pyridine (2.00 g, 11.9 mmol), 2,6-dibromopyridine (4.24 g, 17.9 mmol), triphenylphosphine (0.313 g, 1.19 mmol), potassium carbonate (4.94 g, 35.8 mmol), palladium (II) acetate (0.134 g, 0.596 mmol), 1,4-dioxane (20 mL) and ethanol (10 mL), in that order. The vessel was sealed with a Teflon screw cap and warmed to 100 ºC for 18 hours. The reaction mixture was then cooled to room temperature, diluted with ethyl acetate (25 mL), filtered through silica, and concentrated under reduced pressure. The resulting crude product was then purified by flash chromatography on silica gel (0 – 100% ethyl acetate in hexanes) to deliver the title compound (1.878 g, 49% yield): LCMS *m/z* calcd for $C_{13}H_8BrF_2N_3$: 326.0 $[M+H]^+$, found 325.7 $[M + H]^+$; ^1H NMR (400 MHz, CD_3OD) δ 10.11 – 10.04 (m, 1 H), 8.35 (s, 1 H), 7.92 (dd, J

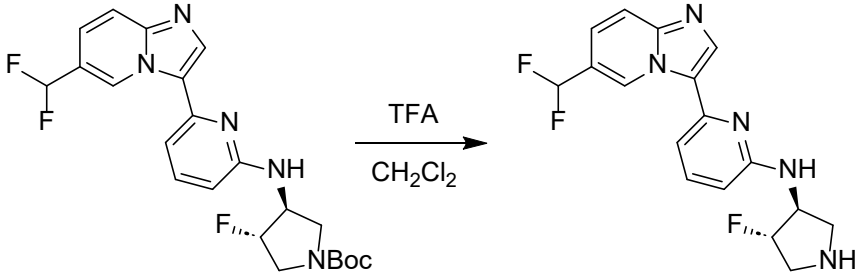
= 7.9, 0.7 Hz, 1 H), 7.72 (t, *J* = 7.9 Hz, 1 H), 7.61 (dd, *J* = 5.3, 1.7 Hz, 1 H), 7.58 – 7.56 (m, 1 H), 7.46 (dd, *J* = 7.9, 0.7 Hz, 1 H), 6.96 (t, *J* = 55.5 Hz, 1 H).

Step C. tert-butyl (3S,4S)-3-((6-(6-(difluoromethyl)imidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)-4-fluoropyrrolidine-1-carboxylate



To a microwave vial equipped with a magnetic stir bar were added 3-(6-bromopyridin-2-yl)-6-(difluoromethyl)imidazo[1,2-a]pyridine (0.094 g, 0.290 mmol), tert-butyl (3S,4S)-3-amino-4-fluoropyrrolidine-1-carboxylate (0.089 g, 0.435 mmol), RuPhos Pd G3 (0.024 g, 0.029 mmol) and cesium carbonate (0.283 g, 0.870 mmol), in that order. The vial was sealed with a pressure release cap and purged with nitrogen for 30 minutes. Tetrahydrofuran (2 mL) was added, and the vial was then warmed in a heating block to 90 ºC for 18 hours. The reaction mixture was then cooled to room temperature, diluted with ethyl acetate (2 x 5 mL), filtered through silica, and concentrated under reduced pressure to yield the title compound (0.196 g, >100% crude yield): LCMS *m/z* calcd for $C_{22}H_{24}F_3N_5O_2$: 448.2 $[M+H]^+$, found 448.2 $[M + H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 10.29 (s, 1 H), 8.20 (s, 1 H), 7.68 – 7.61 (m, 2 H), 7.59 – 7.54 (m, 1 H), 7.29 – 7.14 (m, 1 H), 6.87 (t, J = 55.4 Hz, 1 H), 6.50 (d, J = 8.4 Hz, 1 H), 5.27 (d, J = 50.7 Hz, 1 H), 4.95 – 4.87 (m, 1 H), 4.73 – 4.62 (m, 1 H), 3.92 – 3.52 (m, 4 H), 1.50 (s, 9 H).

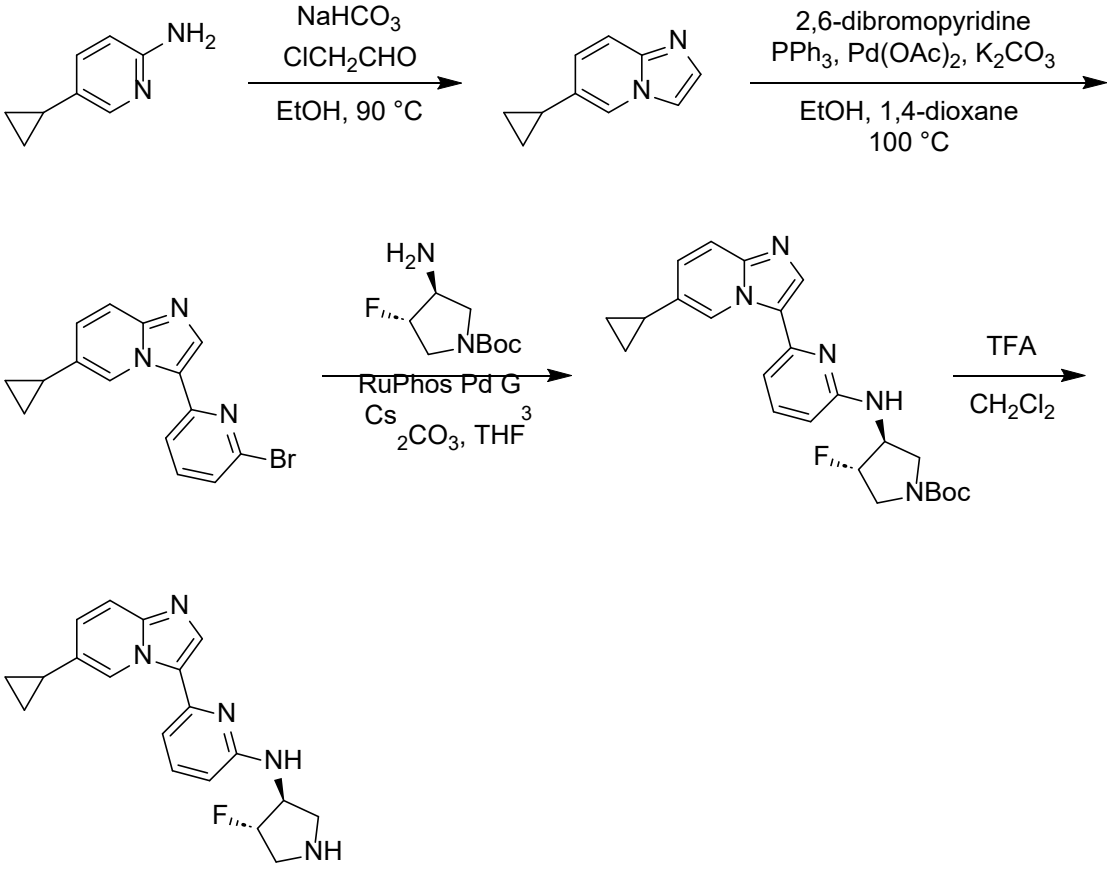
Step D. 6-(6-(difluoromethyl)imidazo[1,2-a]pyridin-3-yl)-N-((3S,4S)-4-fluoropyrrolidin-3-yl)pyridin-2-amine



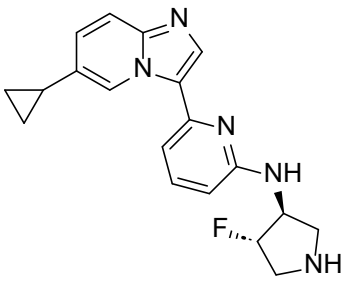
To a microwave vial were added tert-butyl (3S,4S)-3-((6-(6-(difluoromethyl)imidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)-4-fluoropyrrolidine-1-carboxylate (0.196 g, 0.290 mmol theoretical maximum), dichloromethane (2.0 mL), and trifluoroacetic acid (1.0 mL), in that order.

The vial was sealed with a pressure relief cap, and the reaction was stirred overnight at room temperature. The reaction was then concentrated under reduced pressure to afford a crude product that was dissolved in dimethyl sulfoxide (2.0 mL) and purified via HPLC (Agilent XDB C18 column, 5 micron, 30 x 100 mm; 0 – 30% acetonitrile in H_2O each containing 0.1% trifluoroacetic acid) to deliver the title compound (0.041 g, 41% yield over two steps): HRMS (ESI^+) *m/z* calcd for $C_{17}H_{16}F_3N_5$: 348.1431 $[M+H]^+$, found 348.1436; ^1H NMR (400 MHz, CD_3OD) δ 10.31 (d, *J* = 2.4 Hz, 1 H), 8.48 (s, 1 H), 7.97 (d, *J* = 9.4 Hz, 1 H), 7.91 (dd, *J* = 9.5, 1.6 Hz, 1 H), 7.67 (dd, *J* = 8.4, 7.5 Hz, 1 H), 7.35 (dd, *J* = 7.5, 0.7 Hz, 1 H), 7.00 (t, *J* = 55.1 Hz, 1 H), 6.68 (dd, *J* = 8.4, 0.7 Hz, 1 H), 5.62 – 5.40 (m, 1 H), 4.98 – 4.88 (m, 1 H), 3.96 – 3.86 (m, 1 H), 3.80 – 3.56 (m, 4 H), 3.28 – 3.05 (m, 1 H).

Scheme S4. Synthesis of compound **13**.

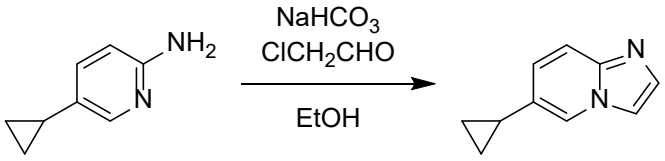


Synthetic Procedure for Compound 13.



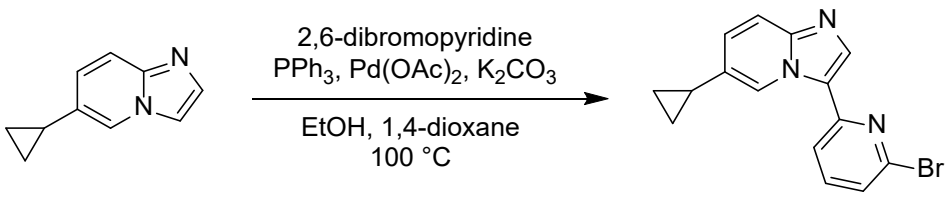
Compound 13, 6-(6-cyclopropylimidazo[1,2-a]pyridin-3-yl)-N-((3S,4S)-4-fluoropyrrolidin-3-yl)pyridin-2-amine

Step A. 6-cyclopropylimidazo[1,2-a]pyridine



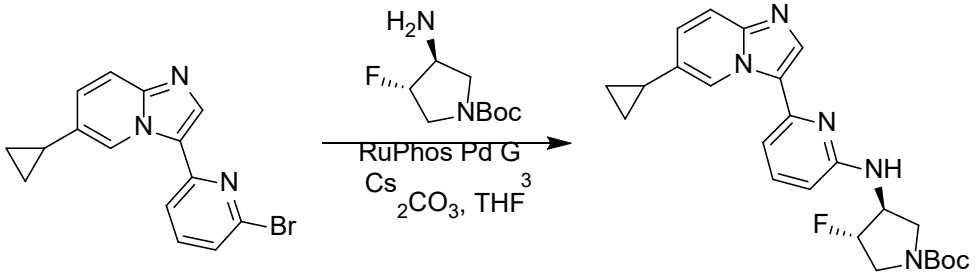
To a heavy-walled sealable flask equipped with a magnetic stir bar were added 5-cyclopropylpyridin-2-amine (2.60 g, 19.4 mmol), sodium bicarbonate (4.07 g, 48.4 mmol), ethanol (50 mL), and 2-chloroacetaldehyde (7.54 milliliters, 48.4 mmol, 45% weight in H_2O), in that order. The vessel was sealed with a Teflon screwcap, and the reaction mixture warmed to 90 °C for 8 hours. The reaction mixture was then cooled to room temperature, filtered through silica, poured into water (20 mL), and extracted with ethyl acetate (2 x 20 mL). The organic extracts were combined, washed with saturated aqueous sodium chloride solution (2 x 20 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 100% ethyl acetate in hexanes) to afford the title compound (2.685 g, 88% yield): LCMS *m/z* calcd for $C_{10}H_{10}N_2$: 159.1 $[M+H]^+$, found 159.0 $[M + H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 8.23 (s, 1 H), 7.73 (d, *J* = 1.4 Hz, 1 H), 7.49 (d, *J* = 1.3 Hz, 1 H), 7.43 (dd, *J* = 9.2, 0.9 Hz, 1 H), 7.08 (dd, *J* = 9.4, 1.8, 0.4 Hz, 1 H), 2.05 – 1.87 (m, 1 H), 1.04 – 0.91 (m, 2 H), 0.82 – 0.62 (m, 2 H).

Step B. 3-(6-bromopyridin-2-yl)-6-cyclopropylimidazo[1,2-a]pyridine



To a heavy-walled sealable flask equipped with a magnetic stir bar were added 6-cyclopropylimidazo[1,2-a]pyridine (2.68 g, 17.0 mmol), 2,6-dibromopyridine (4.02 g, 17.0 mmol), triphenylphosphine (0.445 g, 1.70 mmol), potassium carbonate (7.04 g, 50.9 mmol), palladium (II) acetate (0.190 g, 0.848 mmol), 1,4-dioxane (50 mL), and ethanol (25 mL), in that order. The flask was sealed with a teflon screwcap, and the reaction mixture warmed to 100 ºC for 9 hours. The reaction mixture was then cooled to room temperature, diluted with ethyl acetate (30 mL), filtered through silica, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 100% ethyl acetate in hexanes) to deliver the title compound (1.942 g, 36% yield): LCMS *m/z* calcd for $C_{15}H_{12}BrN_3$: 316.0 $[M+H]^+$, found 315.8 $[M + H]^+$; ^1H NMR (400 MHz, CD_3OD) δ 9.70 (s, 1 H), 8.25 (s, 1 H), 7.90 (d, *J* = 8.0 Hz, 1 H), 7.72 (t, *J* = 7.9 Hz, 1 H), 7.44 (d, *J* = 7.8 Hz, 1 H), 7.31 (d, *J* = 9.3 Hz, 1 H), 7.08 (d, *J* = 9.4 Hz, 1 H), 2.14 – 2.00 (m, 1 H), 1.14 – 1.01 (m, 2 H), 0.84 – 0.77 (m, 2 H).

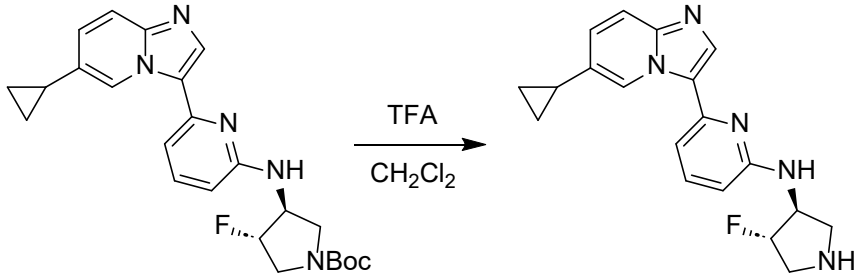
Step C. tert-butyl (3S,4S)-3-((6-(6-cyclopropylimidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)-4-fluoropyrrolidine-1-carboxylate



To a microwave vial equipped with a magnetic stir bar were added 3-(6-bromopyridin-2-yl)-6-cyclopropylimidazo[1,2-a]pyridine (0.256 g, 0.816 mmol), tert-butyl (3S,4S)-3-amino-4-fluoropyrrolidine-1-carboxylate (0.167 g, 0.816 mmol), RuPhos Pd G3 (0.068 g, 0.082 mmol) and cesium carbonate (0.798 g, 2.45 mmol), in that order. The vessel was sealed with a pressure relief cap and flushed with nitrogen for 30 minutes. Tetrahydrofuran (2.5 mL) was then added, and the resulting reaction mixture warmed in a heating block to 90 ºC for 9 hours. The reaction mixture was then cooled to room temperature, diluted with ethyl acetate (2 x 5 mL), filtered through silica, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 100% ethyl acetate in hexanes) to yield the title compound

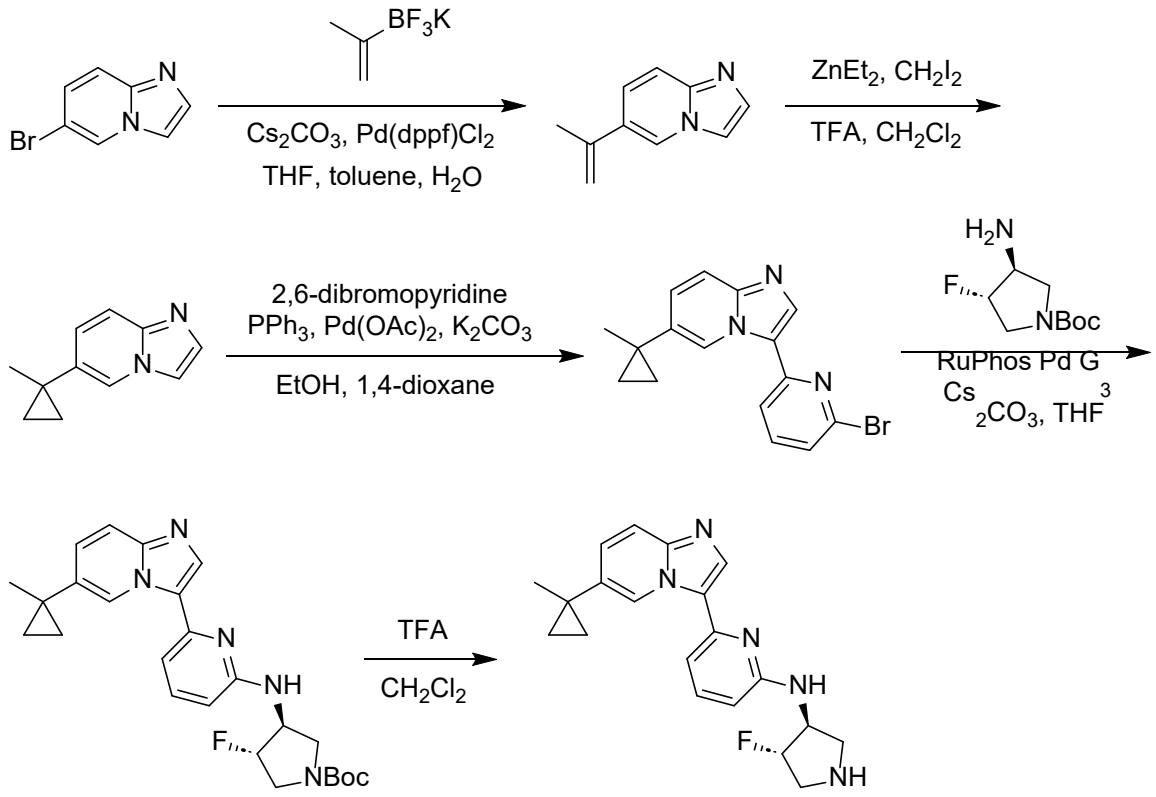
(0.130 g, 36% yield): LCMS *m/z* calcd for $C_{24}H_{28}FN_5O_2$: 438.2 $[M+H]^+$, found 437.9 $[M + H]^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 9.81 (s, 1 H), 8.02 (s, 1 H), 7.67 – 7.63 (m, 1 H), 7.58 – 7.56 (m, 1 H), 7.13 (d, *J* = 7.6 Hz, 1 H), 7.07 (d, *J* = 9.3 Hz, 1 H), 6.45 (d, *J* = 8.3 Hz, 1 H), 5.23 (d, *J* = 51.1 Hz, 1 H), 5.00 – 4.88 (m, 1 H), 4.76 – 4.65 (m, 1 H), 4.01 – 3.32 (m, 4 H), 2.13 – 2.01 (m, 1 H), 1.49 (s, 9 H), 1.02 – 0.94 (m, 2 H), 0.79 – 0.70 (m, 2 H).

Step D. 6-(6-cyclopropylimidazo[1,2-a]pyridin-3-yl)-N-((3S,4S)-4-fluoropyrrolidin-3-yl)pyridin-2-amine

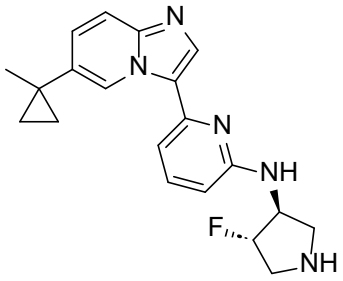


To a microwave vial were added tert-butyl (3S,4S)-3-((6-(6-cyclopropylimidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)-4-fluoropyrrolidine-1-carboxylate (0.123 g, 0.296 mmol), dichloromethane (2.0 mL), and trifluoroacetic acid (1.0 mL), in that order. The vial was sealed with a pressure relief cap, and the reaction was stirred overnight at room temperature. The reaction was then concentrated under reduced pressure to afford a crude product that was dissolved in dimethyl sulfoxide (2.0 mL) and purified via HPLC (Agilent XDB C18 column, 5 micron, 30 x 100 mm; 0 – 30% acetonitrile in water each containing 0.1% trifluoroacetic acid) to deliver the title compound (0.047 g, 50% yield): HRMS (ESI^+) *m/z* calcd for $C_{19}H_{20}FN_5$: 338.1776 $[M+H]^+$, found 338.1781; ^{1}H NMR (400 MHz, CD_3OD) δ 9.90 (s, 1 H), 8.47 (s, 1 H), 7.95 – 7.78 (m, 1 H), 7.75 – 7.60 (m, 2 H), 7.32 (dd, *J* = 7.5, 0.7 Hz, 1 H), 6.73 (dd, *J* = 8.4, 0.7 Hz, 1 H), 5.71 – 5.33 (m, 1 H), 4.99 – 4.89 (m, 2 H), 3.97 – 3.85 (m, 1 H), 3.79 – 3.55 (m, 3 H), 3.49 – 3.33 (m, 1 H), 2.21 (p, *J* = 8.3, 4.9 Hz, 1 H), 1.18 – 0.80 (m, 4 H).

Scheme S5. Synthesis of compound **14**.

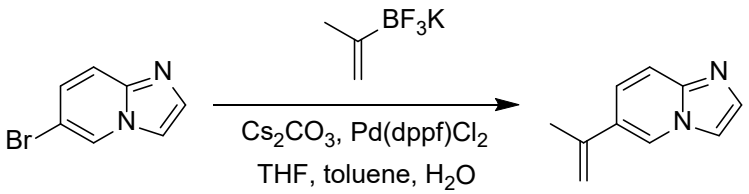


Synthetic Procedure for Compound 14.



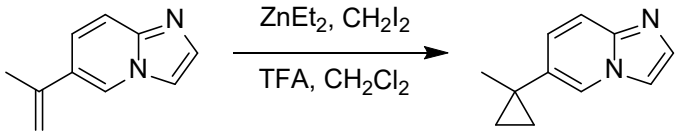
Compound 14, N-((3S,4S)-4-fluoropyrrolidin-3-yl)-6-(6-(1-methylcyclopropyl)imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine

Step A. 6-(prop-1-en-2-yl)imidazo[1,2-a]pyridine



A mixture of 6-bromoimidazo[1,2-a]pyridine (20.0 g, 102 mmol), potassium trifluoro(isopropenyl)boronate (30.04 g, 203.0 mmol), cesium carbonate (99.22 g, 304.5 mmol), and [1,1-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (7.43 g, 10.2 mmol) in toluene (150 mL), tetrahydrofuran (50 mL), and water (50 mL) was degassed and purged with nitrogen, and the reaction mixture was heated at 80 °C for 3 hours under nitrogen atmosphere. The reaction was then cooled to room temperature, diluted with water (200 mL), and extracted with ethyl acetate (3 x 200 mL). The organic extracts were combined, washed with saturated aqueous sodium chloride solution (150 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 20% ethyl acetate in petroleum ether) to provide the title compound as an amber oil (14.01 g, 87% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 – 8.11 (m, 1 H), 7.62 – 7.59 (m, 1 H), 7.57 – 7.53 (m, 2 H), 7.38 (dd, *J* = 1.9, 9.6 Hz, 1 H), 5.43 (s, 1 H), 5.17 – 5.14 (m, 1 H), 2.15 (dd, *J* = 0.6, 1.3 Hz, 3 H).

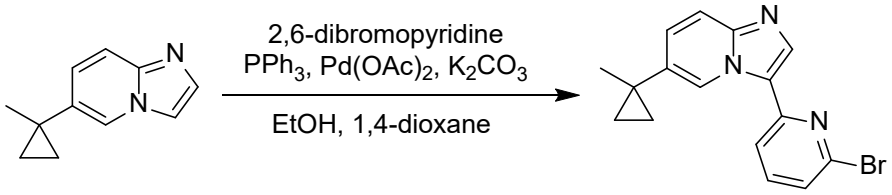
Step B. 6-(1-methylcyclopropyl)imidazo[1,2-a]pyridine



A solution of diethylzinc in n-hexane (1.0 M, 632.1 mL, 632.1 mmol) was added to anhydrous dichloromethane (200 mL) and cooled to 0 °C. A solution of trifluoroacetic acid (46.80 mL, 72.08 g, 632.1 mmol) in dichloromethane (100 mL) was added, and the resulting mixture was stirred at 0 °C for 10 minutes. A solution of CH_2I_2 (152.37 g, 568.90 mmol) in dichloromethane (100 mL) was then added, and the reaction was stirred at 0 °C for an additional 20 minutes. A solution of 6-isopropenylimidazo[1,2-a]pyridine (10.0 g, 63.2 mmol) in dichloromethane (100 mL) was then added, and the resulting mixture was stirred for 16 hours while slowly warming to room temperature. The reaction mixture was then poured into ice water (500 mL), filtered, and extracted with dichloromethane (3 x 300 mL). The organic extracts were combined, washed with saturated aqueous sodium bicarbonate solution (3 x 100 mL), dried over sodium sulfate, filtered,

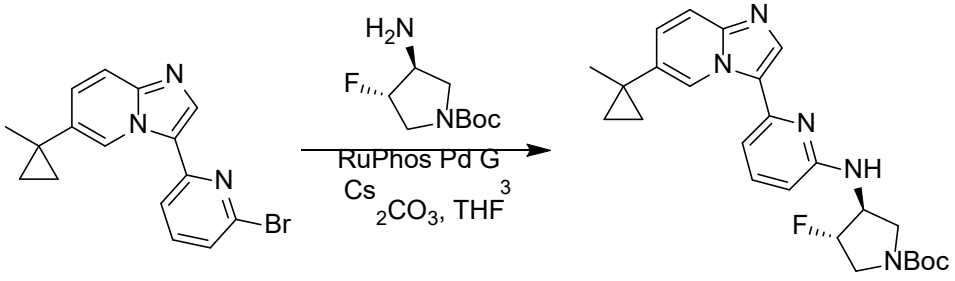
and concentrated under reduced pressure to provide the title compound as a yellow oil (3.50 g, 32% yield): LCMS *m/z* calcd for $C_{11}H_{12}N_2$: 173.1 $[M+H]^+$, found 173.2 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1 H), 7.58 (s, 1 H), 7.52 – 7.50 (m, 2 H), 6.99 (dd, *J* = 1.6, 9.3 Hz, 1 H), 1.36 – 1.30 (m, 3 H), 0.78 – 0.73 (m, 2 H), 0.72 – 0.63 (m, 2 H).

Step C. 3-(6-bromopyridin-2-yl)-6-(1-methylcyclopropyl)imidazo[1,2-a]pyridine



A mixture of 6-(1-methylcyclopropyl)imidazo[1,2-a]pyridine (1.0 g, 5.8 mmol), 2,6-dibromopyridine (6.88 g, 29.0 mmol), triphenylphosphine (0.152 g, 0.581 mmol), palladium(II)acetate (0.130 g, 0.581 mmol) and potassium carbonate (3.21 g, 23.2 mmol) in 1,4-dioxane (16 mL) and ethanol (8 mL) was degassed and purged with nitrogen, then heated at 80 °C for 18 hours under nitrogen atmosphere. The reaction was then cooled to room temperature, diluted with water (30 mL), and extracted with ethyl acetate (3 x 20 mL). The organic extracts were combined, washed with saturated aqueous sodium chloride solution (20 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 60% ethyl acetate in petroleum ether) to provide the title compound as a pale yellow solid (0.601 g, 19% yield): LCMS *m/z* calcd for $C_{16}H_{14}BrN_3$: 328.0 $[M+H]^+$, found 328.0 $[M+H]^+$.

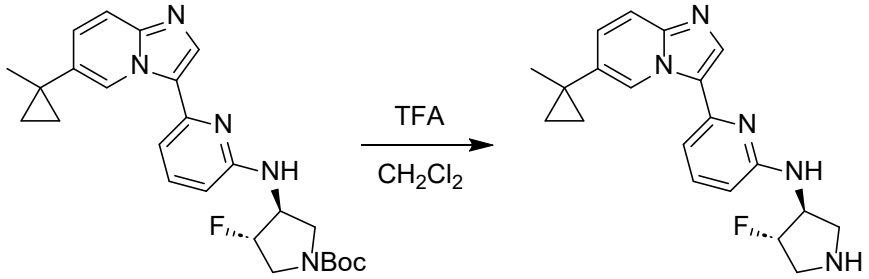
Step D. (3S,4S)-tert-butyl 3-fluoro-4-((6-(6-(1-methylcyclopropyl)imidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)pyrrolidine-1-carboxylate



A mixture of 3-(6-bromo-2-pyridyl)-6-(1-methylcyclopropyl)imidazo[1,2-a]pyridine (0.090 g, 0.274 mmol), tert-butyl (3S,4S)-3-amino-4-fluoro-pyrrolidine-1-carboxylate (0.084 g, 0.411 mmol), cesium carbonate (0.268 g, 0.823 mmol), and (2-dicyclohexylphosphino-2',6'-diisopropoxy-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II)methanesulfonate (0.023 g,

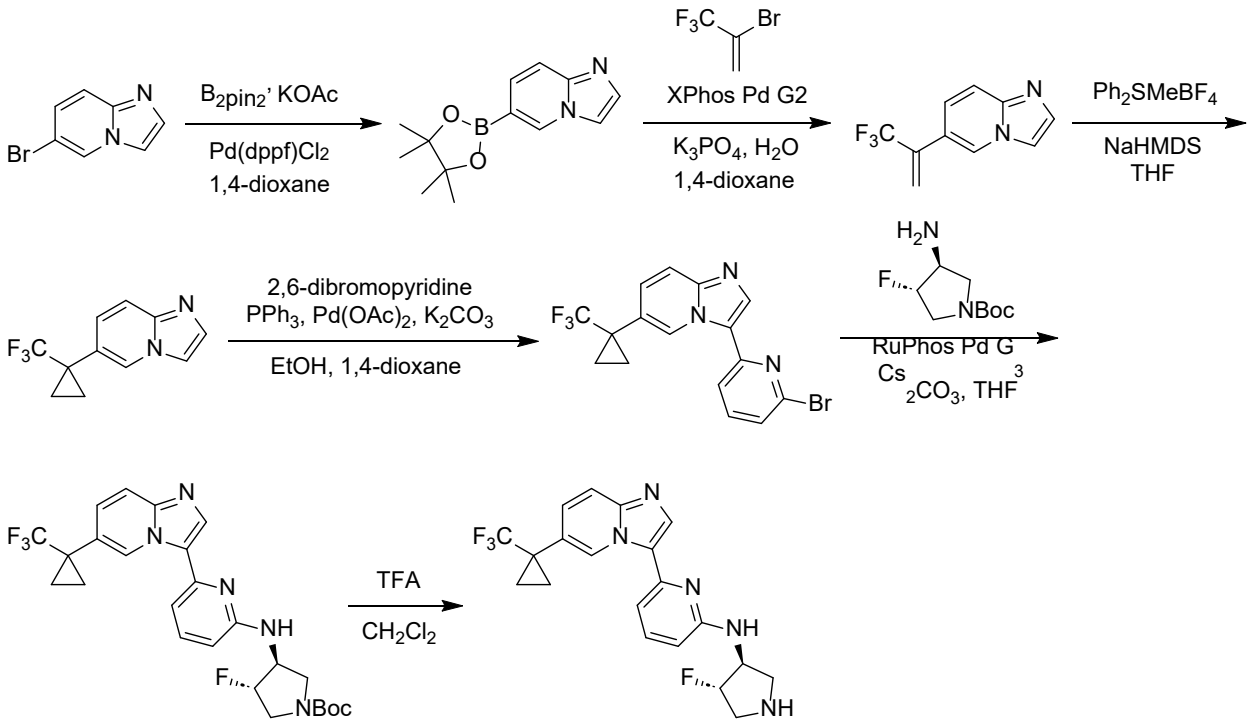
0.027 mmol) in tetrahydrofuran (3.0 mL) was degassed and purged with nitrogen, then heated at 80 °C for 18 hours under nitrogen atmosphere. The reaction was then cooled to room temperature, diluted with water (3 mL), and extracted with ethyl acetate (3 x 3 mL). The organic extracts were combined and concentrated under reduced pressure to give a crude product that was purified by flash chromatography on silica gel (0 – 50% ethyl acetate in petroleum ether) to provide the title compound as a pale yellow oil (0.101 g, 81% yield): LCMS *m/z* calcd for $C_{25}H_{30}FN_5O_2$: 452.2 $[M+H]^+$, found 452.2 $[M+H]^+$.

Step E. N-((3S,4S)-4-fluoropyrrolidin-3-yl)-6-(6-(1-methylcyclopropyl)imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine.

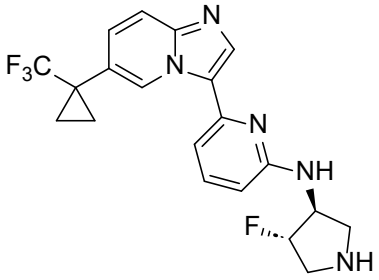


To a solution of tert-butyl (3S,4S)-3-fluoro-4-[[6-[6-(1-methylcyclopropyl)imidazo[1,2-a]pyridin-3-yl]-2-pyridyl]amino]pyrrolidine-1-carboxylate (0.100 g, 0.221 mmol) in dichloromethane (3.0 mL) was added trifluoroacetic acid (1.0 mL). The resulting reaction mixture was stirred at 20 °C for 1 hour. The mixture was then filtered and concentrated under reduced pressure to give a crude product that was purified by HPLC (Kromasil C18 column, 3 micron, 80 x 25 mm; 5 – 35% acetonitrile in water containing 0.1% TFA) to provide the title compound as a white solid (0.062 g, 78% yield): HRMS (ESI^+) *m/z* calcd for $C_{20}H_{22}FN_5$: 352.1932 $[M+H]^+$, found 352.1941; ^1H NMR (400 MHz, CD_3OD) δ 9.86 (s, 1 H), 8.49 (s, 1 H), 8.00 (dd, *J* = 1.5, 9.4 Hz, 1 H), 7.91 (d, *J* = 9.4 Hz, 1 H), 7.70 (t, *J* = 7.9 Hz, 1 H), 7.31 (d, *J* = 7.5 Hz, 1 H), 6.75 (d, *J* = 8.4 Hz, 1 H), 5.60 – 5.34 (m, 1 H), 4.99 – 4.92 (m, 1 H), 3.88 (dd, *J* = 6.0, 12.5 Hz, 1 H), 3.80 – 3.74 (m, 1 H), 3.72 – 3.67 (m, 1 H), 3.66 – 3.61 (m, 1 H), 1.52 (s, 3 H), 1.09 – 0.99 (m, 2 H), 0.95 – 0.85 (m, 2 H).

Scheme S6. Synthesis of compound **15**.

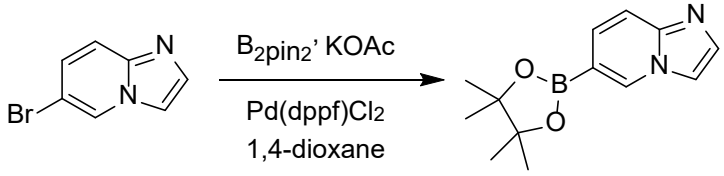


Synthetic Procedure for Compound 15.



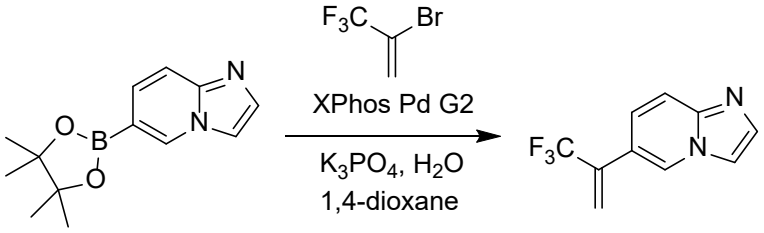
Compound 15, N-((3S,4S)-4-fluoropyrrolidin-3-yl)-6-(6-(1-(trifluoromethyl)cyclopropyl)imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine

Step A. 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)imidazo[1,2-a]pyridine



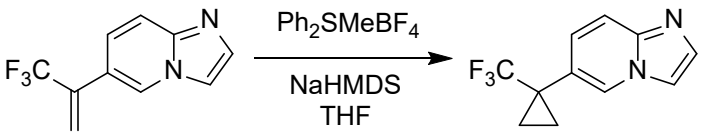
A mixture of 6-bromoimidazo[1,2-a]pyridine (15.0 g, 76.1 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (48.3 g, 190 mmol), potassium acetate (26.15 g, 266.5 mmol), and [1,1-bis(diphenylphosphino)ferrocene]palladium(II)chloride (6.22 g, 7.61 mmol) in 1,4-dioxane (200 mL) was degassed and purged with nitrogen, then heated at 90 °C for 2 hours under nitrogen atmosphere. The reaction mixture was then cooled to room temperature, filtered, and concentrated under reduced pressure to provide the title compound which was used in the next step without further purification: LCMS *m/z* calcd for $C_{13}H_{17}BN_2O_2$: 245.1 $[M+H]^+$, found 245.1 $[M+H]^+$.

Step B. 6-(3,3,3-trifluoroprop-1-en-2-yl)imidazo[1,2-a]pyridine



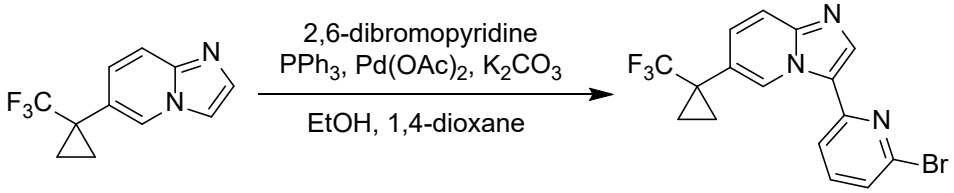
A mixture of 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)imidazo[1,2-a]pyridine (19.0 g, 77.8 mmol), 2-bromo-3,3,3-trifluoro-prop-1-ene (34.04 g, 194.6 mmol), chloro(2-dicyclohexylphosphino-2′,4′,6′-triisopropyl-1,1′-biphenyl)[2-(2′-amino-1,1'-biphenyl)]palladium(II) (3.67 g, 4.67 mmol), and potassium phosphate (83.40 mL, 233.5 mmol, 2.8 M) in 1,4-dioxane (200 mL) and water (85 mL) was degassed and purged with nitrogen, and then heated at 80 °C for 16 hours under nitrogen atmosphere. The reaction mixture was then cooled to room temperature, concentrated under reduced pressure, diluted with water (40 mL), and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were washed with saturated aqueous sodium chloride solution (2 x 100 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 80% ethyl acetate in petroleum ether) to provide the title compound as an amber oil (10.02 g, 61% yield over two steps): LCMS *m/z* calcd for $C_{10}H_7F_3N_2$: 213.1 $[M+H]^+$, found 213.0 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1 H), 7.67 – 7.46 (m, 3 H), 7.21 – 7.16 (m, 1 H), 5.99 (s, 1 H), 5.80 (d, J = 1.4 Hz, 1 H).

Step C. 6-(1-(trifluoromethyl)cyclopropyl)imidazo[1,2-a]pyridine



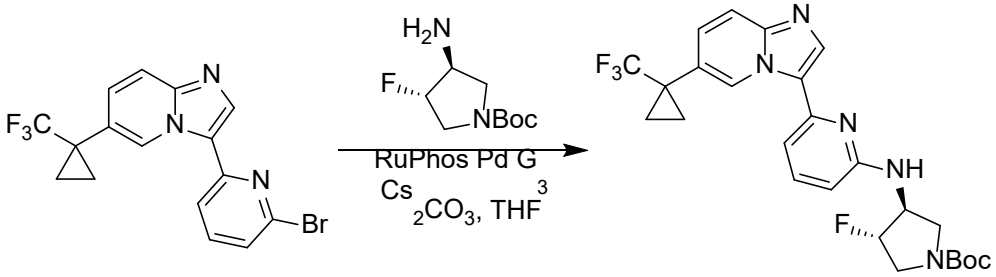
To an oven dried 20 mL vial containing 6-(3,3,3-trifluoroprop-1-en-2-yl)imidazo[1,2-a]pyridine (5.00 g, 23.6 mmol) and methyldiphenylsulfonium tetrafluoroborate (9.29 g, 30.6 mmol) in anhydrous tetrahydrofuran (150 mL) was added a solution of sodium bis(trimethylsilyl)amide in tetrahydrofuran (47.13 mL, 47.14 mmol, 1.0 M) at 0 °C under nitrogen. The reaction mixture was stirred at 0 °C for 10 minutes and then at 20 °C for 2 hours. The reaction mixture was quenched by addition water (40 mL), then extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were washed with saturated aqueous sodium chloride solution (2 x 50 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The resulting crude product was purified by HPLC (Xtimate C18 column, 10 micron, 250 x 80 mm; 20 – 43% acetonitrile in aqueous 10 mM NH_4HCO_3) to provide the title compound as a yellow oil (2.30 g, 43% yield): LCMS *m/z* calcd for $C_{11}H_9F_3N_2$: 227.1 $[M+H]^+$, found 227.1 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1 H), 7.58 (d, *J* = 0.6 Hz, 1 H), 7.54 – 7.48 (m, 2 H), 7.22 – 7.16 (m, 1 H), 1.37 – 1.32 (m, 2 H), 1.03 – 0.97 (m, 2 H).

Step D. 3-(6-bromopyridin-2-yl)-6-(1-(trifluoromethyl)cyclopropyl)imidazo[1,2-a]pyridine



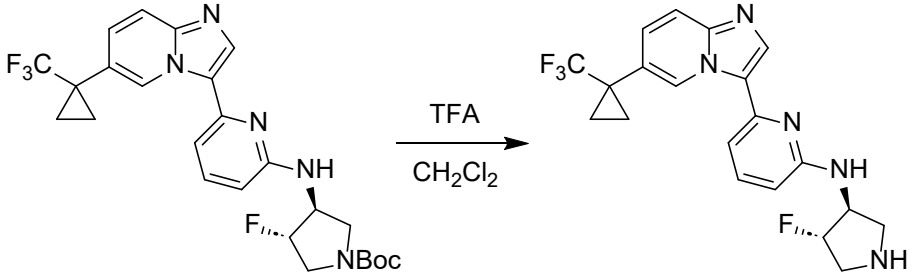
A mixture of 6-(1-(trifluoromethyl)cyclopropyl)imidazo[1,2-a]pyridine (3.50 g, 15.5 mmol), 2,6-dibromopyridine (25.66 g, 108.3 mmol), triphenylphosphine (0.406 g, 1.55 mmol), palladium acetate (0.521 g, 2.32 mmol), and potassium carbonate (6.42 g, 46.4 mmol) in ethyl alcohol (50 mL) and 1,4-dioxane (100 mL) was degassed and purged with nitrogen, then heated at 80 °C for 18 hours under nitrogen atmosphere. The reaction mixture was then cooled to room temperature, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 80% ethyl acetate in petroleum ether) to provide the title compound as a yellow solid (3.00 g, 51% yield): LCMS *m/z* calcd for $C_{16}H_{11}BrF_3N_3$: 382.0 $[M+H]^+$, found 382.1 $[M+H]^+$.

Step E. (3S,4S)-tert-butyl 3-fluoro-4-((6-(6-(1-(trifluoromethyl)cyclopropyl)imidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)pyrrolidine-1-carboxylate



A mixture of 3-(6-bromo-2-pyridyl)-6-[1-(trifluoromethyl)cyclopropyl]imidazo[1,2-a]pyridine (0.120 g, 0.314 mmol), (3S,4S)-tert-butyl 3-amino-4-fluoropyrrolidine-1-carboxylate (0.096 g, 0.471 mmol), cesium carbonate (0.307 g, 0.942 mmol), and (2-dicyclohexylphosphino-2',6'-diisopropoxy-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II)methanesulfonate (0.026 g, 0.031 mmol) in tetrahydrofuran (3 mL) was degassed and purged with nitrogen, then heated at 80 °C for 2 hours under nitrogen atmosphere. The reaction was then cooled to room temperature, filtered, and concentrated under reduced pressure to provide the title compound which was used in the next step without further purification: LCMS *m/z* calcd for $C_{25}H_{27}F_4N_5O_2$: 506.2 $[M+H]^+$, found 506.2 $[M+H]^+$.

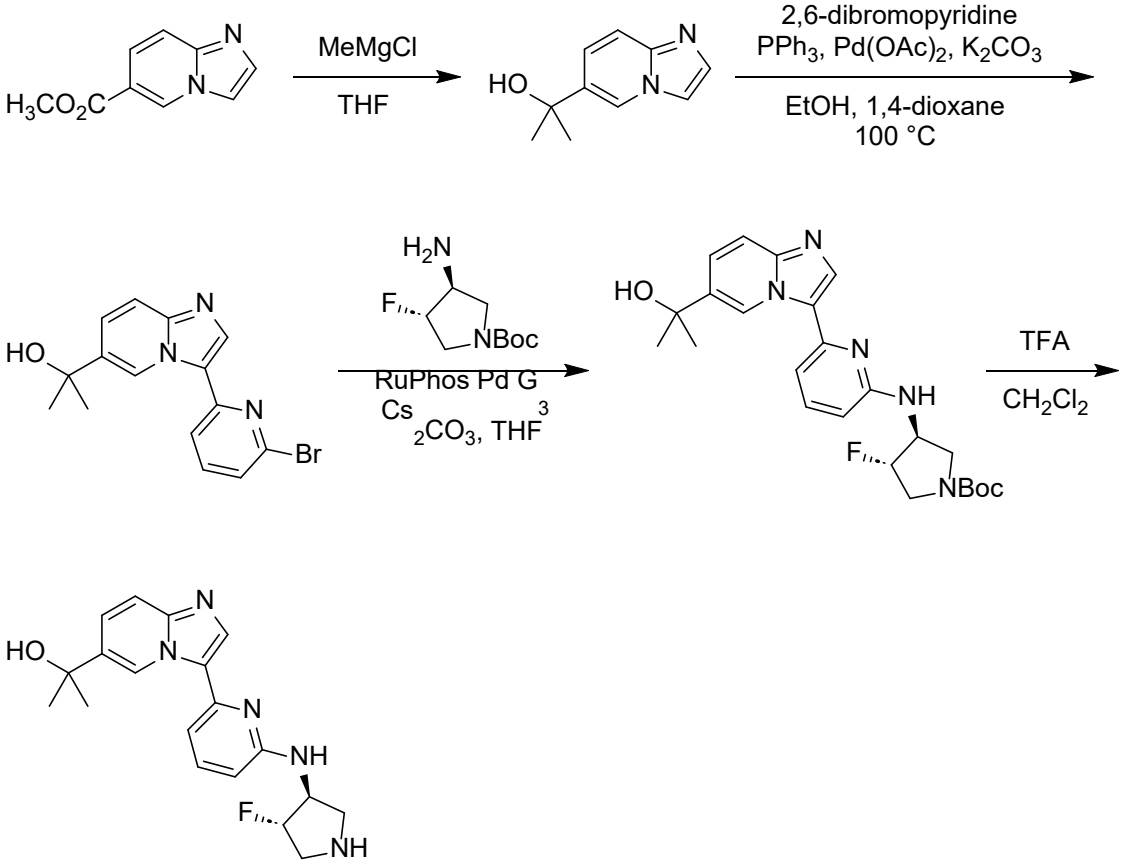
Step F. N-((3S,4S)-4-fluoropyrrolidin-3-yl)-6-(6-(1-(trifluoromethyl)cyclopropyl)imidazo[1,2-a]pyridin-3-yl)pyridin-2-amine



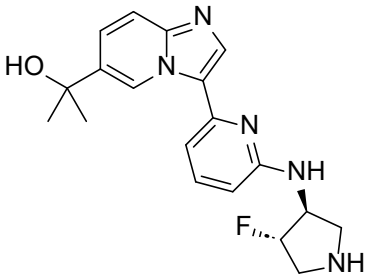
To a solution of (3S,4S)-tert-butyl 3-fluoro-4-((6-(6-(1-(trifluoromethyl)cyclopropyl)imidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)pyrrolidine-1-carboxylate (0.150 g, 0.297 mmol) in dichloromethane (3 mL) was added trifluoroacetic acid (1 mL). The reaction was stirred at 20°C for 1 hour, then concentrated under reduced pressure. The resulting crude product was purified by HPLC (Waters Xbridge Prep OBD C18 column, 10 micron, 150 x 40 mm; 15 – 45% acetonitrile in aqueous 10 mM NH_4HCO_3) to provide the title compound as a white solid (0.022 g, 15% yield over two steps): HRMS (ESI^+) *m/z* calcd for $C_{20}H_{19}F_4N_5$: 406.1649 $[M+H]^+$, found 406.1650; ^1H NMR (400 MHz, CD_3OD) δ 9.98 (s, 1 H), 8.13 (s, 1 H),

7.66 – 7.61 (m, 1 H), 7.59 – 7.50 (m, 2 H), 7.23 (d, *J* = 7.1 Hz, 1 H), 6.53 (d, *J* = 8.2 Hz, 1 H), 5.45 – 5.25 (m, 1 H), 4.90 – 4.87 (m, 1 H), 3.70 (dd, *J* = 5.4, 12.1 Hz, 1 H), 3.63 – 3.46 (m, 2 H), 3.43 – 3.32 (m, 1 H), 1.49 – 1.41 (m, 2 H), 1.31 – 1.16 (m, 2 H).

Scheme S7. General synthesis D of compounds **16** and **29**.

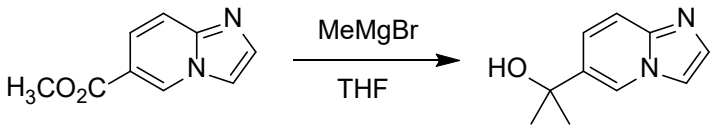


Representative Synthetic Procedure for Compounds 16 and 29.



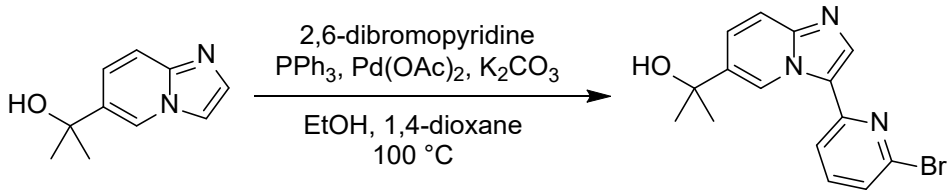
Compound 16, 2-(3-(6-(((3S,4S)-4-fluoropyrrolidin-3-yl)amino)pyridin-2-yl)imidazo[1,2-a]pyridin-6-yl)propan-2-ol

Step A. 2-(imidazo[1,2-a]pyridin-6-yl)propan-2-ol



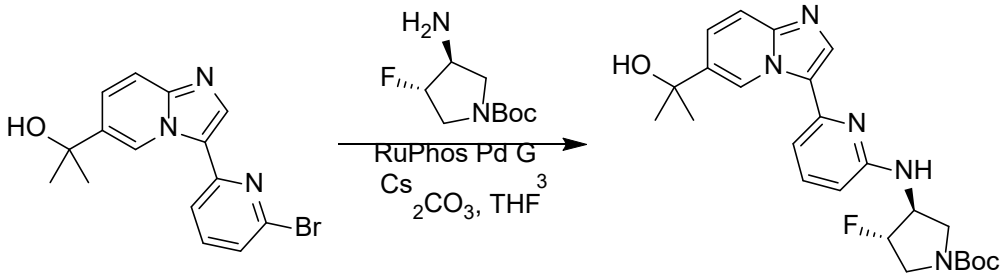
To a cooled 0 ºC solution of methyl imidazo[1,2-a]pyridine-6-carboxylate (1.0 g, 5.7 mmol) in tetrahydrofuran (20 mL) was added a solution of methyl magnesium bromide in diethyl ether (3.0 M, 7.57 mL, 22.7 mmol). The resulting reaction mixture was allowed to warm to 20 °C and stirred for 2 hours. The reaction was then cooled to 0 ºC, quenched by addition of water (20 mL), and extracted with ethyl acetate (2 x 15). The combined organic extracts were washed with saturated aqueous sodium chloride solution (2 x 15 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure to provide the title compound which was used without further purification in the next step: LCMS *m/z* calcd for $C_{10}H_{12}N_2O$: 177.1 $[M+H]^+$, found 177.2 $[M+H]^+$.

Step B. 2-(3-(6-bromopyridin-2-yl)imidazo[1,2-a]pyridin-6-yl)propan-2-ol



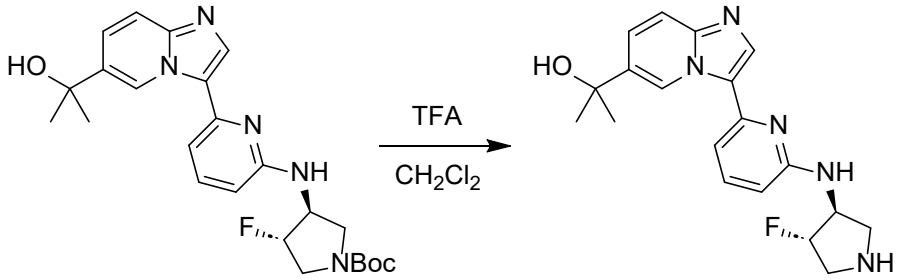
A mixture of 2-(imidazo[1,2-a]pyridin-6-yl)propan-2-ol (0.900 g, 5.11 mmol), 2,6-dibromopyridine (6.05 g, 25.5 mmol), triphenylphosphine (0.152 g, 0.581 mmol), palladium(II)acetate (0.115 g, 0.511 mmol), and potassium carbonate (4.99 g, 15.3 mmol) in ethanol (10 mL) and 1,4-dioxane (20 mL) was degassed and purged with nitrogen, then heated at 100 °C for 18 hours under nitrogen atmosphere. The reaction was filtered, diluted with water (20 mL), and extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were washed with saturated aqueous sodium chloride solution (20 mL) and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 70% ethyl acetate in petroleum ether) to provide the title compound as a pale yellow solid (0.391 g, 23% yield over two steps): LCMS *m/z* calcd for $C_{15}H_{14}BrN_3O$: 332.0 $[M+H]^+$, found 332.0 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 9.94 (s, 1 H), 8.16 – 8.12 (s, 1 H), 7.68 – 7.60 (m, 2 H), 7.59 – 7.48 (m, 2 H), 7.33 – 7.29 (m, 1 H), 1.73 (s, 1 H), 1.70 (s, 6 H).

Step C. (3S,4S)-tert-butyl 3-fluoro-4-((6-(6-(2-hydroxypropan-2-yl)imidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)pyrrolidine-1-carboxylate



A mixture of 2-(3-(6-bromopyridin-2-yl)imidazo[1,2-a]pyridin-6-yl)propan-2-ol (0.090 g, 0.271 mmol), (3S,4S)-tert-butyl 3-amino-4-fluoropyrrolidine-1-carboxylate (0.083 g, 0.406 mmol), cesium carbonate (0.265 g, 0.813 mmol), and (2-dicyclohexylphosphino-2',6'-diisopropoxy-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II)methanesulfonate (0.023 g, 0.027 mmol) in tetrahydrofuran (3 mL) was degassed and purged with nitrogen, and then heated at 80 °C for 2 hours under nitrogen atmosphere. The reaction was then cooled to room temperature, filtered, and concentrated under reduced pressure to provide the title compound which was used without further purification in the next step: LCMS *m/z* calcd for $C_{24}H_{30}FN_5O_3$: 456.2 $[M+H]^+$, found 456.2 $[M+H]^+$.

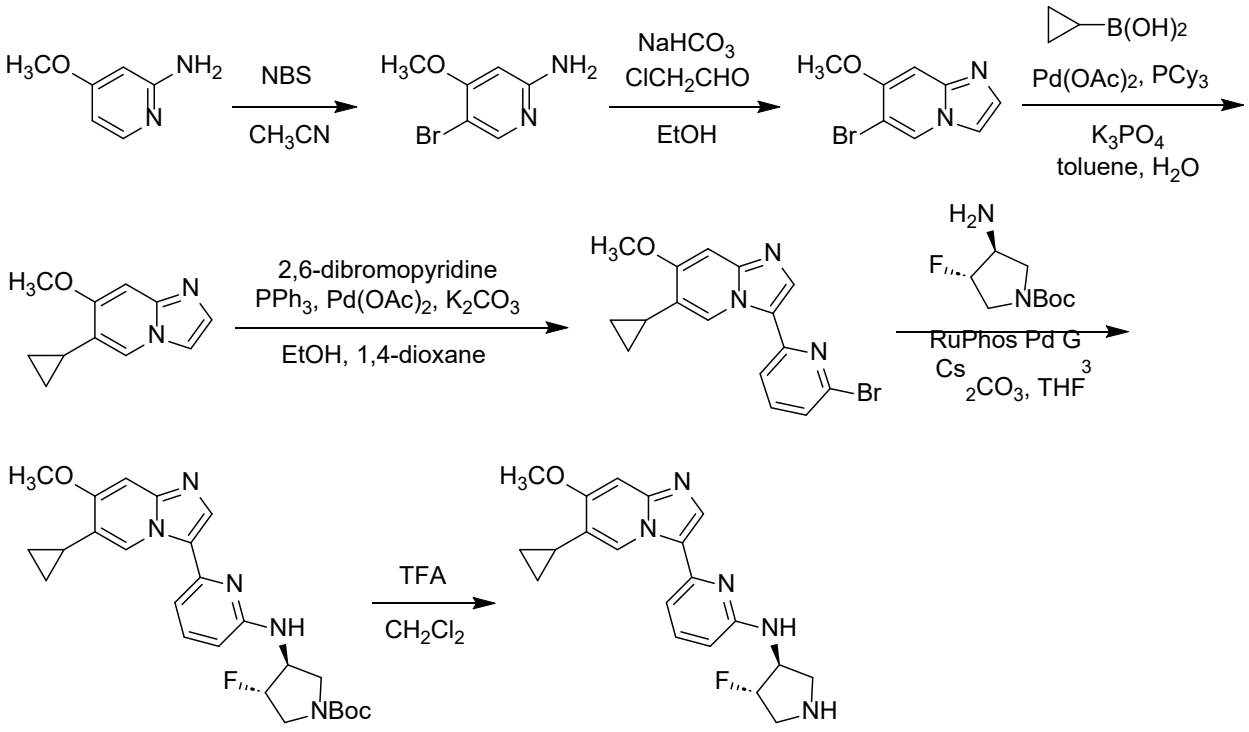
Step D. 2-(3-(6-(((3S,4S)-4-fluoropyrrolidin-3-yl)amino)pyridin-2-yl)imidazo[1,2-a]pyridin-6-yl)propan-2-ol



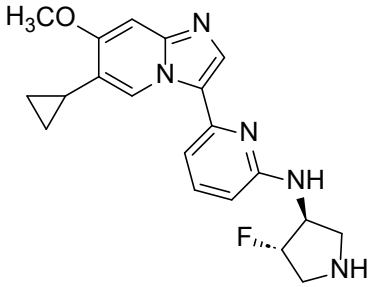
To a solution of (3S,4S)-tert-butyl 3-fluoro-4-((6-(6-(2-hydroxypropan-2-yl)imidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)pyrrolidine-1-carboxylate (0.100 g, 0.220 mmol) in dichloromethane (3 mL) was added trifluoroacetic acid (1 mL). The reaction was stirred at room temperature for 1 hour, then concentrated under reduced pressure to give a crude product that was purified by HPLC (Kromasil C18 column, 3 micron, 80 x 25 mm; 3 – 20% acetonitrile in water containing 0.1% TFA) to provide the title compound as a white solid (0.050 g, 45% yield over two steps): HRMS (ESI^+) *m/z* calcd for $C_{19}H_{22}FN_5O$: 356.1881 $[M+H]^+$, found 356.1890; ^1H NMR

(400 MHz, CD_3OD) δ 10.20 (s, 1 H), 8.51 (s, 1 H), 8.09 (dd, *J* = 1.6, 9.4 Hz, 1 H), 7.94 (d, *J* = 9.4 Hz, 1 H), 7.69 (t, *J* = 7.9 Hz, 1 H), 7.30 (d, *J* = 7.3 Hz, 1 H), 6.73 (d, *J* = 8.3 Hz, 1 H), 5.56 – 5.35 (d, 1 H), 5.03 – 4.92 (m, 1 H), 3.94 (dd, *J* = 5.6, 12.5 Hz, 1 H), 3.81 – 3.60 (m, 3 H), 1.64 (s, 6 H).

Scheme S8. Synthesis of compound **27**.

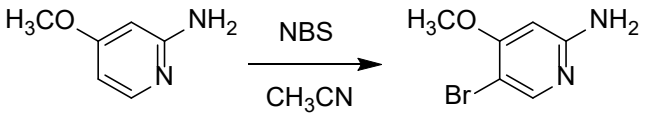


Synthetic Procedure for Compound 27.



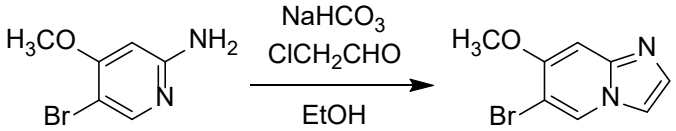
Compound 27, 6-(6-cyclopropyl-7-methoxyimidazo[1,2-a]pyridin-3-yl)-N-((3S,4S)-4-fluoropyrrolidin-3-yl)pyridin-2-amine

Step A. 5-bromo-4-methoxypyridin-2-amine



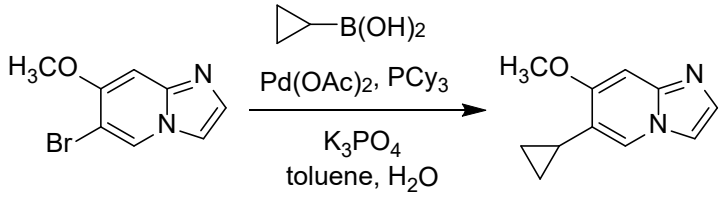
To a cooled 0 ºC solution of 4-methoxypyridin-2-amine (106.0 g, 853.9 mmol) in acetonitrile (2000 mL) was added 1-bromopyrrolidine-2,5-dione (155.01 g, 870.95 mmol). The resulting mixture was stirred for 2 hours while slowly warming to room temperature. The reaction was then concentrated under reduced pressure, diluted with saturated aqueous sodium bicarbonate solution (600 mL), and extracted with dichloromethane (2 x 500 mL). The combined organic extracts were washed with saturated aqueous sodium chloride solution (2 x 300 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure to provide the title compound as a yellow solid (130.0 g, 75% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.84 (s, 1 H), 6.13 (s, 1 H), 6.05 (br s, 2 H), 3.80 (s, 3 H).

Step B. 6-bromo-7-methoxyimidazo[1,2-a]pyridine



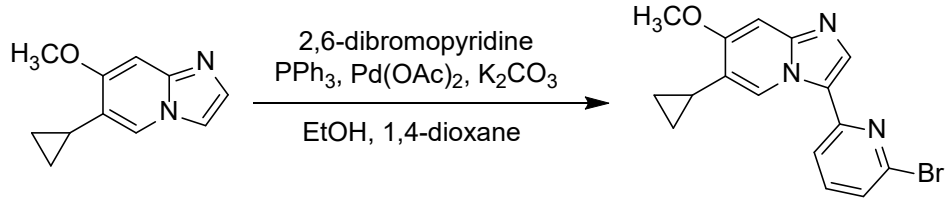
To a solution of 5-bromo-4-methoxy-pyridin-2-amine (10.0 g, 49.3 mmol) and 2-chloroacetaldehyde (48.33 g, 246.3 mmol, 39.61 mL) in ethanol (150 mL) was added sodium bicarbonate (10.34 g, 123.1 mmol). The resulting reaction mixture was heated at 80 °C for 15 hours. The reaction mixture was then cooled to room temperature, diluted with water (100 mL), and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were washed with saturated aqueous sodium chloride solution (2 x 100 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 1% methanol in dichloromethane) to provide the title compound as a tan solid (5.80 g, 53% yield): LCMS *m/z* calcd for $C_8H_7BrN_2O$: 227.0 $[M+H]^+$, found 227.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 – 8.74 (m, 1 H), 7.78 – 7.62 (m, 1 H), 7.44 (s, 1 H), 7.09 (s, 1 H), 3.91 (s, 3 H).

Step C. 6-cyclopropyl-7-methoxyimidazo[1,2-a]pyridine



A mixture of 6-bromo-7-methoxy-imidazo[1,2-a]pyridine (3.60 g, 15.9 mmol), cyclopropylboronic acid (2.72 g, 31.7 mmol), potassium phosphate tribasic (10.10 g, 47.57 mmol), tricyclohexylphosphine (0.445 g, 1.59 mmol), and palladium(II)acetate (0.356 g, 1.59 mmol) in toluene (30 mL) and water (2 mL) was degassed and purged with nitrogen, and was then heated at 100 °C for 15 hours under nitrogen atmosphere. The reaction was then cooled to room temperature, diluted with water (20 mL), and extracted with ethyl acetate (3 x 20 mL). The organic extracts were combined, washed with saturated aqueous sodium chloride solution (30 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 30% ethyl acetate in petroleum ether) to provide the title compound as a yellow oil (2.01 g, 67% yield): LCMS *m/z* calcd for $C_{11}H_{12}N_2O$: 189.1 $[M+H]^+$, found 189.1 $[M+H]^+$.

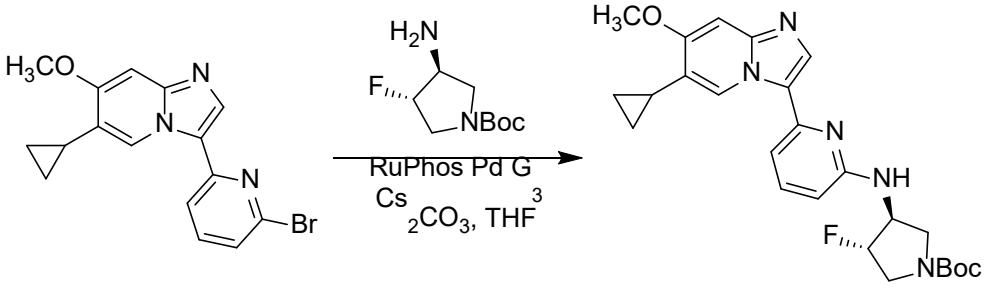
Step D. 3-(6-bromopyridin-2-yl)-6-cyclopropyl-7-methoxyimidazo[1,2-a]pyridine



A mixture of 6-cyclopropyl-7-methoxy-imidazo[1,2-a]pyridine (2.00 g, 10.6 mmol), 2,6-dibromopyridine (10.07 g, 42.50 mmol), palladium(II)acetate (0.239 g, 1.06 mmol), triphenylphosphine (0.418 g, 1.59 mmol), and potassium carbonate (4.41 g, 31.9 mmol) in 1,4-dioxane (20 mL) and ethanol (10 mL) was degassed and purged with nitrogen, and was then heated at 80 °C for 15 hours under nitrogen atmosphere. The reaction was then cooled to room temperature, filtered, diluted with water (30 mL), and extracted with ethyl acetate (3 x 30 mL). The organic extracts were combined, washed with saturated aqueous sodium chloride solution (30 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 100% ethyl acetate in

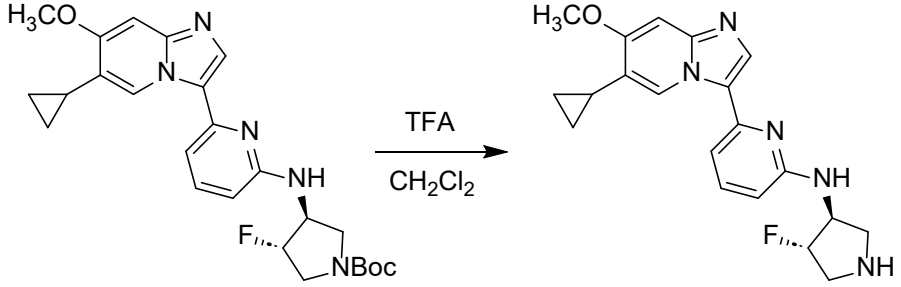
petroleum ether) to provide the title compound as a pale yellow solid (0.902 g, 25% yield): LCMS *m/z* calcd for $C_{16}H_{14}BrN_3O$: 344.0 $[M+H]^+$, found 344.0 $[M+H]^+$.

Step E. (3S,4S)-tert-butyl 3-((6-(6-cyclopropyl-7-methoxyimidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)-4-fluoropyrrolidine-1-carboxylate



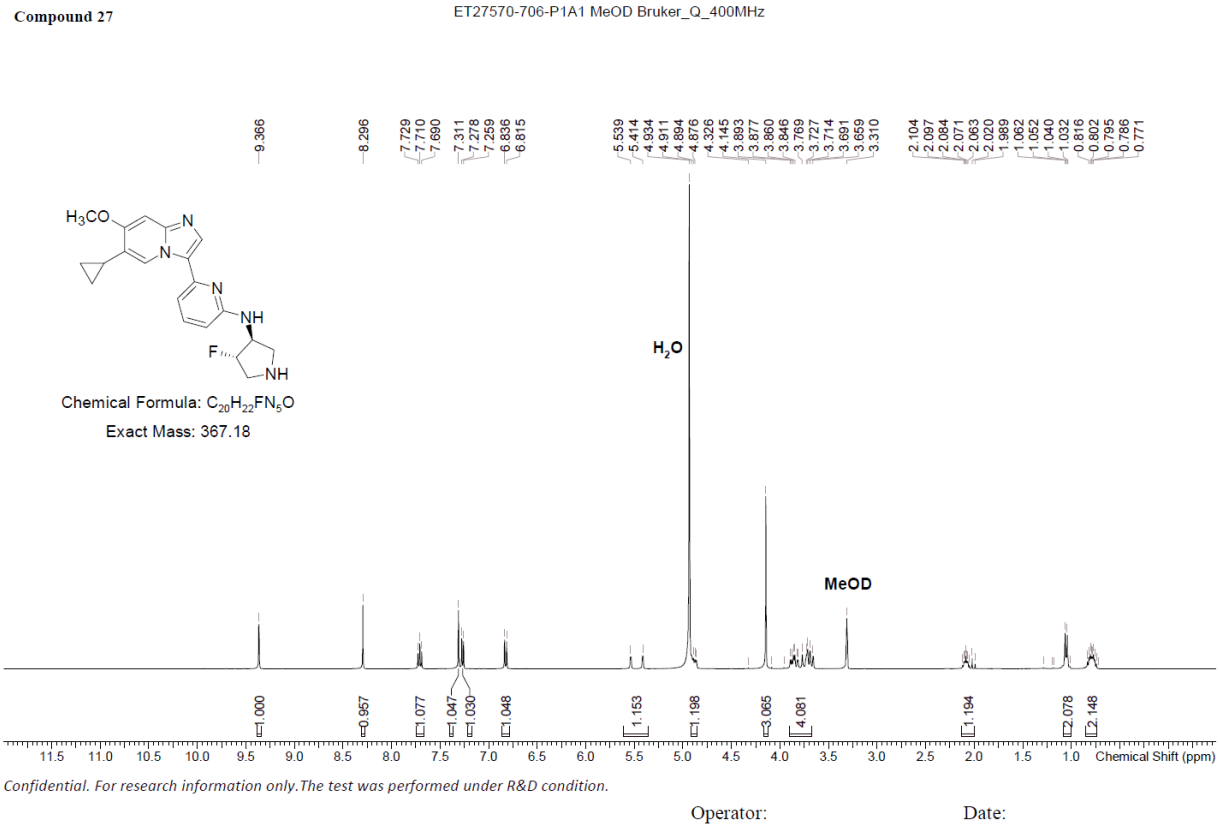
A mixture of 3-(6-bromo-2-pyridyl)-6-cyclopropyl-7-methoxy-imidazo[1,2-a]pyridine (0.075 g, 0.218 mmol), (3S,4S)-tert-butyl 3-amino-4-fluoropyrrolidine-1-carboxylate (0.049 g, 0.240 mmol), (2-dicyclohexylphosphino-2',6'-diisopropoxy-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II)methanesulfonate (0.018 g, 0.022 mmol), and cesium carbonate (0.213 g, 0.654 mmol) in tetrahydrofuran (2 mL) was degassed and purged with nitrogen, and was then heated at 80 °C for 3 hours under nitrogen atmosphere. The reaction was then cooled to room temperature, filtered, and concentrated under reduced pressure to provide the title compound which was used without further purification in the next step: LCMS *m/z* calcd for $C_{25}H_{30}FN_5O_3$: 468.2 $[M+H]^+$, found 468.1 $[M+H]^+$.

Step F. 6-(6-cyclopropyl-7-methoxyimidazo[1,2-a]pyridin-3-yl)-N-((3S,4S)-4-fluoropyrrolidin-3-yl)pyridin-2-amine



To a solution of (3S,4S)-tert-butyl 3-((6-(6-cyclopropyl-7-methoxyimidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)-4-fluoropyrrolidine-1-carboxylate (0.100 g, 0.214 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (1 mL). The resulting reaction was stirred at 20 °C for 1 hour, then concentrated under reduced pressure. The resulting crude product was purified by HPLC (Phenomenex Luna C18 column, 5 micron, 150 x 30 mm; 1 – 25% acetonitrile in water containing 0.1% TFA) to provide the title compound as a white solid (0.011 g, 12%

yield over two steps): HRMS (ESI$^+$) *m/z* calcd for $C_{20}H_{22}FN_5O$: 368.1881 $[M+H]^+$, found 368.1883; ^{1}H NMR (400 MHz, CD_3OD) δ 9.46 (s, 1 H), 8.29 (s, 1 H), 7.67 (t, *J* = 7.9 Hz, 1 H), 7.34 – 7.19 (m, 2 H), 6.75 (d, *J* = 8.3 Hz, 1 H), 5.60 – 5.37 (m, 1 H), 4.88 – 4.80 (m, 1 H), 4.14 (s, 3 H), 3.89 – 3.61 (m, 4 H), 2.16 – 2.02 (m, 1 H), 1.14 – 0.99 (m, 2 H), 0.91 – 0.71 (m, 2 H). ^{13}C NMR (100 MHz, CD_3OD) δ 165.70, 157.95, 144.38, 143.46, 140.73, 128.45, 127.34, 125.37, 122.13, 114.23, 112.08, 95.36 (d, *J* = 181 Hz), 90.90, 57.82, 57.52 (d, *J* = 29 Hz), 51.04 (d, *J* = 23 Hz), 50.11, 9.91, 7.27, 6.91. $[\alpha]^{20}_D$ -28.6° (*c* 1.00, CH_3OH).



LCMS REPORT

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Inj. Vol.         : 1.00  ul
Location          : P1-E-07
Acq  Method       : D:\DATA\2006\200704 5\5_95AB_6min-220.M
Data Filename     : D:\DATA\2006\200704 5\ET27570-706-P1B.D
Instrument        : H
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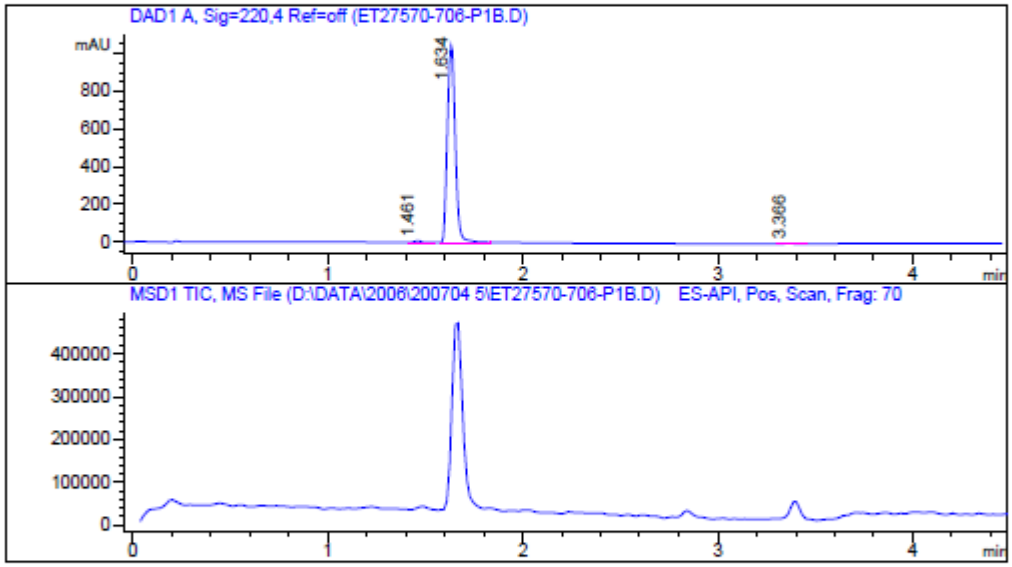


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                            Integration Result
================================================================================
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     2    1.634 2951.516 1048.884    99.037    0.045    99.132
     3    3.366    6.960    2.395     0.226    0.048     0.234
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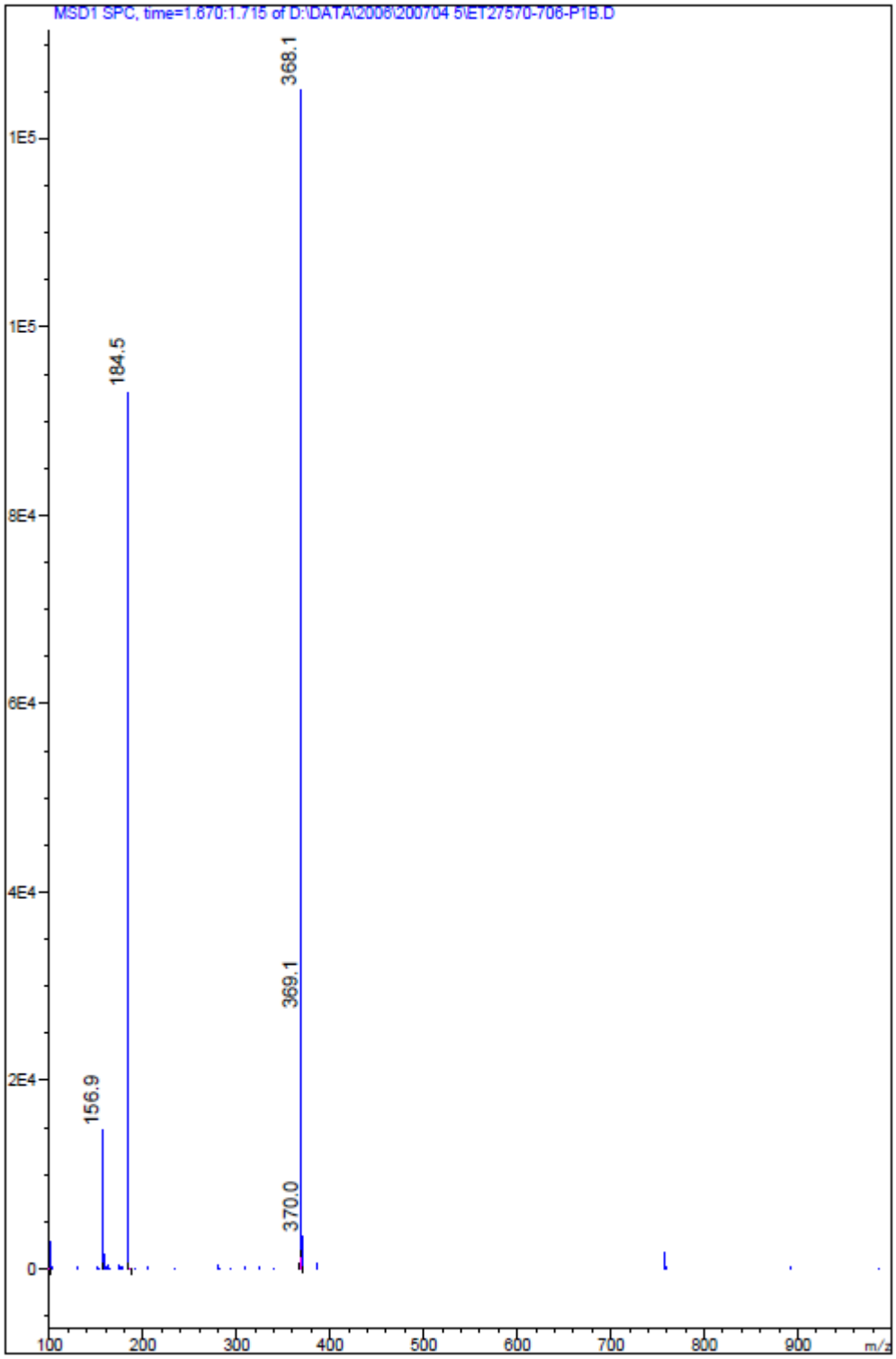
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6E4
4E4
2E4
0
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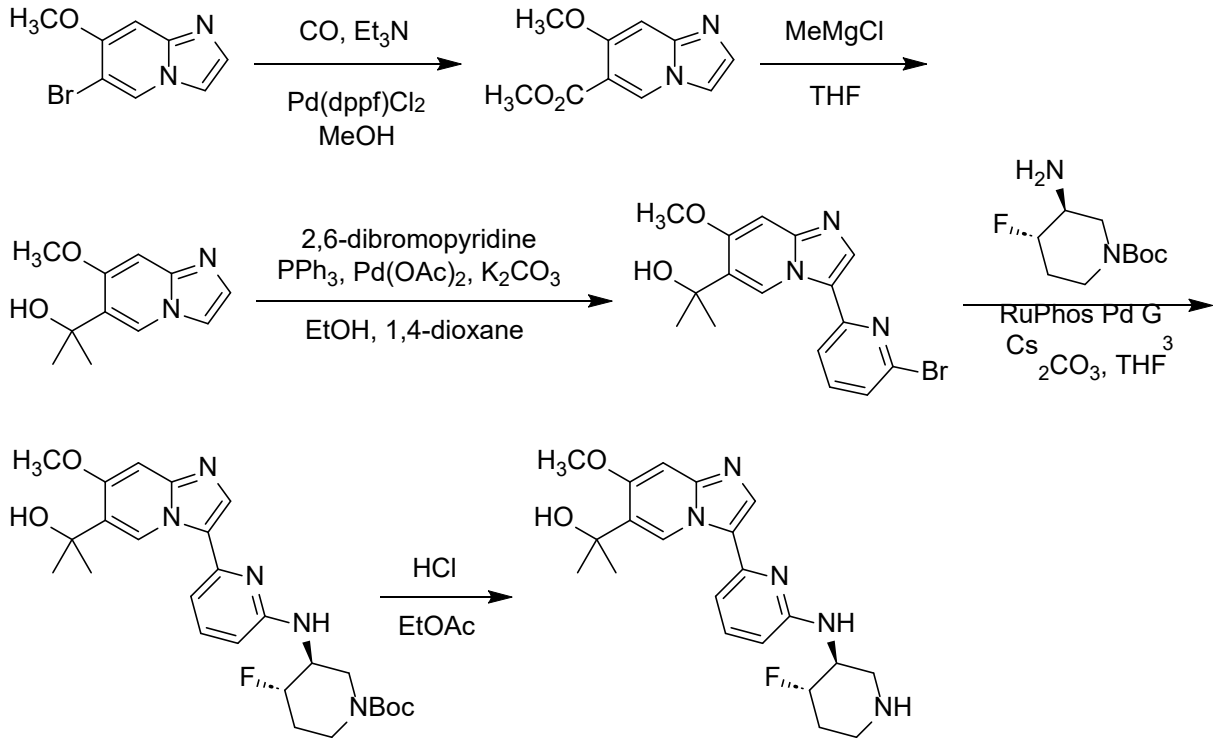
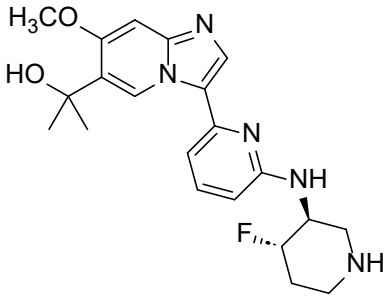
200
300
400
500
600
700
800
900
m/z

Scheme S9. General synthesis E of compounds **28**, **30** and **31**.

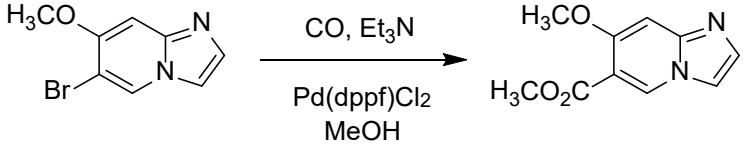


Representative Synthetic Procedure for Compounds 28, 30 and 31.



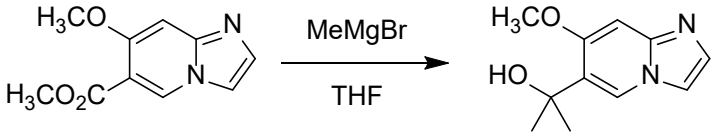
Compound 31, 2-(3-(6-(((3S,4S)-4-fluoropiperidin-3-yl)amino)pyridin-2-yl)-7-methoxyimidazo[1,2-a]pyridin-6-yl)propan-2-ol

Step A. Methyl 7-methoxyimidazo[1,2-a]pyridine-6-carboxylate



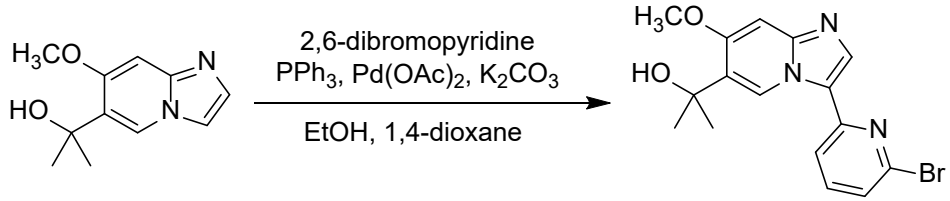
To a solution of 6-bromo-7-methoxy-imidazo[1,2-a]pyridine (8.00 g, 35.2 mmol) in methanol (250 mL) and toluene (250 mL) were added triethylamine (10.70 g, 105.7 mmol) and [1,1-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (2.58 g, 3.52 mmol), in that order. The resulting reaction mixture was heated at 80 ° C under a carbon monoxide atmosphere (3 MPa) for 16 hours. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 20% methanol in ethyl acetate) to provide the title compound as a tan solid (5.02 g, 69% yield): LCMS *m/z* calcd for $C_{10}H_{10}N_2O_3$: 207.1 $[M+H]^+$, found 207.2 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 1 H), 7.54 (s, 1 H), 7.46 (s, 1 H), 6.92 (s, 1 H), 3.92 (s, 3 H), 3.90 (s, 3 H).

Step B. 2-(7-methoxyimidazo[1,2-a]pyridin-6-yl)propan-2-ol



To a cooled 0 ºC solution of methyl 7-methoxyimidazo[1,2-a]pyridine-6-carboxylate (3.00 g, 14.6 mmol) in tetrahydrofuran (100 mL) was added methylmagnesium bromide (19.4 mL, 58.2 mmol, 3.0 M). The resulting reaction mixture was stirred for 2 hours while slowly warming to room temperature. The reaction mixture was then quenched by addition water (20 mL) at 0 °C, and extracted with ethyl acetate (2 x 15 mL). The combined organic extracts were washed with saturated aqueous sodium chloride solution (2 x 15 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to provide the title compound which was used without further purification in the next step.

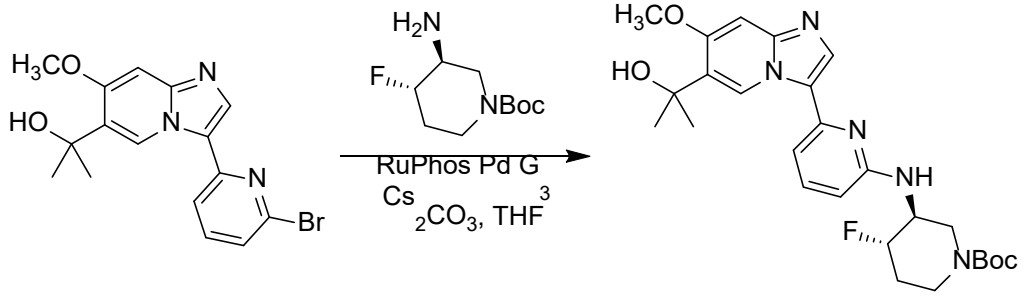
Step C. 2-(3-(6-Bromopyridin-2-yl)-7-methoxyimidazo[1,2-a]pyridin-6-yl)propan-2-ol



A mixture of 2-(7-methoxyimidazo[1,2-a]pyridin-6-yl)propan-2-ol (3.00 g, 14.6 mmol), 2,6-dibromopyridine (10.34 g, 43.64 mmol), triphenylphosphine (0.382 g, 1.45 mmol), palladium acetate (0.327 g, 1.45 mmol), and potassium carbonate (6.03 g, 43.6 mmol) in ethyl alcohol (15 mL) and 1,4-dioxane (30 mL) was degassed and purged with nitrogen, and was then heated at 80 °C for 16 hours under nitrogen atmosphere. The reaction mixture was then cooled to room

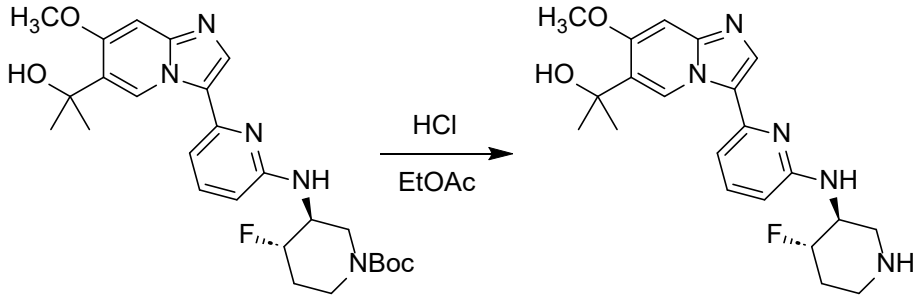
temperature, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 100% ethyl acetate in petroleum ether) to provide the title compound as a tan solid (1.60 g, 42% yield over two steps): LCMS *m/z* calcd for $C_{16}H_{16}BrN_3O_2$: 362.0 $[M+H]^+$, found 362.1 $[M+H]^+$.

Step D. (3R,4S)-tert-butyl 3-((6-(6-(2-hydroxypropan-2-yl)-7-methoxyimidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)-4-(trifluoromethyl)pyrrolidine-1-carboxylate



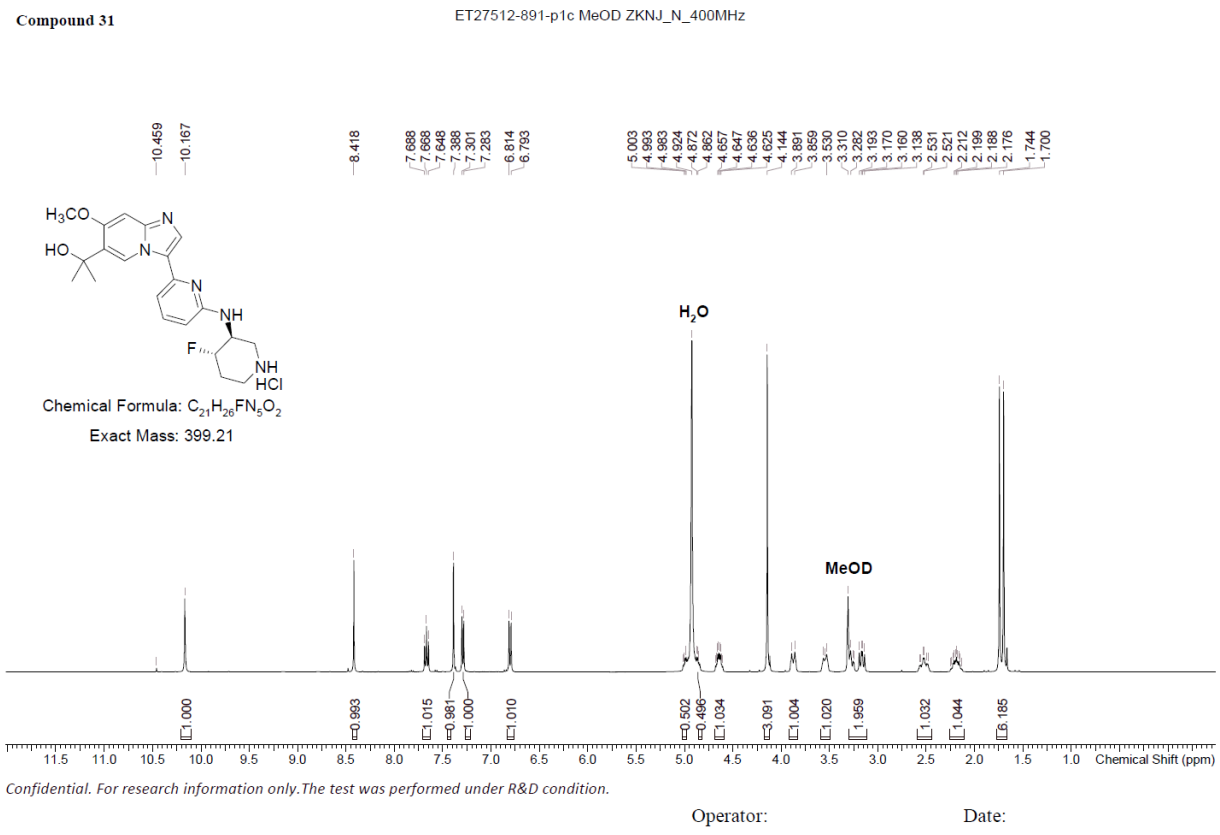
To a stirred solution of 2-[3-(6-bromo-2-pyridyl)-7-methoxy-imidazo[1,2-a]pyridin-6-yl]propan-2-ol (4.60 g, 12.7 mmol) in tetrahydrofuran (50 mL) were added tert-butyl (3S,4S)-3-amino-4-fluoropiperidine-1-carboxylate (2.77 g, 12.7 mmol), (2-dicyclohexylphosphino-2',6'-diisopropoxy-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II)methanesulfonate (1.06 g, 1.27 mmol), and cesium carbonate (10.34 g, 31.75 mmol). The resulting mixture was purged with nitrogen, then heated for 15 hours at 80 °C under nitrogen atmosphere. The reaction mixture was cooled to room temperature, filtered, and concentrated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel (0 – 100 % ethyl acetate in petroleum ether) to provide the title compound as a white solid (3.50 g, 35% yield): LCMS *m/z* calcd for $C_{26}H_{34}FN_5O_4$: 500.3 $[M+H]^+$, found 500.3 $[M+H]^+$.

Step E. 2-(7-methoxy-3-(6-(((3R,4S)-4-(trifluoromethyl)pyrrolidin-3-yl)amino)pyridin-2-yl)imidazo[1,2-a]pyridin-6-yl)propan-2-ol



A mixture of tert-butyl (3S,4S)-4-fluoro-3-[[6-[6-(1-hydroxy-1-methyl-ethyl)-7-methoxy-imidazo[1,2-a]pyridin-3-yl]-2-pyridyl]amino]piperidine-1-carboxylate (2.80 g, 5.60 mmol) in HCl/EtOAc (30 mL, 4 N) was stirred at room temperature for 2 hours, and was then concentrated

under reduced pressure. The resulting crude product was purified by trituration with ethyl acetate (3 x 20 mL) to provide the title compound as a white solid (2.16 g, 85% yield): HRMS (ESI^+) *m/z* calcd for $C_{21}H_{26}FN_5O_2$: 400.2143 $[M+H]^+$, found 400.2123; ^1H NMR (400MHz, CD_3OD) δ 10.20 (s, 1 H), 8.45 (s, 1 H), 7.70 (t, *J* = 7.9 Hz, 1 H), 7.42 (s, 1 H), 7.32 (d, *J* = 7.3 Hz, 1 H), 6.84 (d, *J* = 8.4 Hz, 1 H), 5.06 – 4.87 (m, 1 H), 4.67 (m, 1 H), 4.18 (s, 3 H), 3.91 (br d, *J* = 13.0 Hz, 1 H), 3.62 – 3.52 (m, 1 H), 3.33 – 3.15 (m, 2 H), 2.56 (dt, *J* = 4.7, 15.1 Hz, 1 H), 2.29 – 2.14 (m, 1 H), 1.75 (d, *J* = 17.4 Hz, 6 H). ^{13}C NMR (100 MHz, CD_3OD) δ 163.32, 158.84, 145.91, 143.75, 139.85, 133.06, 128.41, 126.11, 121.97, 112.59, 110.96, 91.99, 88.60 (d, *J* = 180 Hz), 73.02, 57.57, 51.02 (d, *J* = 22 Hz), 46.00 (d, *J* = 5 Hz), 42.45 (d, *J* = 10 Hz), 29.28, 28.99, 28.00 (d, *J* = 22 Hz). $[\alpha]^{20}_D$ +106.1° (*c* 1.00, CH_3OH).



LCMS REPORT

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Injection Date : 28. Mar. 2022
Location : P2-C-09
Inj. Vol. : 0.10 uL
Acq Method : D:\DATA\2203\220328 11\5_95AB_6min-220.M
Data Filename : D:\DATA\2203\220328 11\ET52173-208-P1E.D
Instrument : AN
Instrument:Agilent 1260 HPLC MSD:6120 single quadrupole MSD
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Column Temp: 40 °C
Mobile Phase:A:H2O+TFA(0.04%)
Mobile Phase:B:ACN +TFA(0.02%)
Flow Rate:1.0ml/min

Time	B%	Flow(ml/min)
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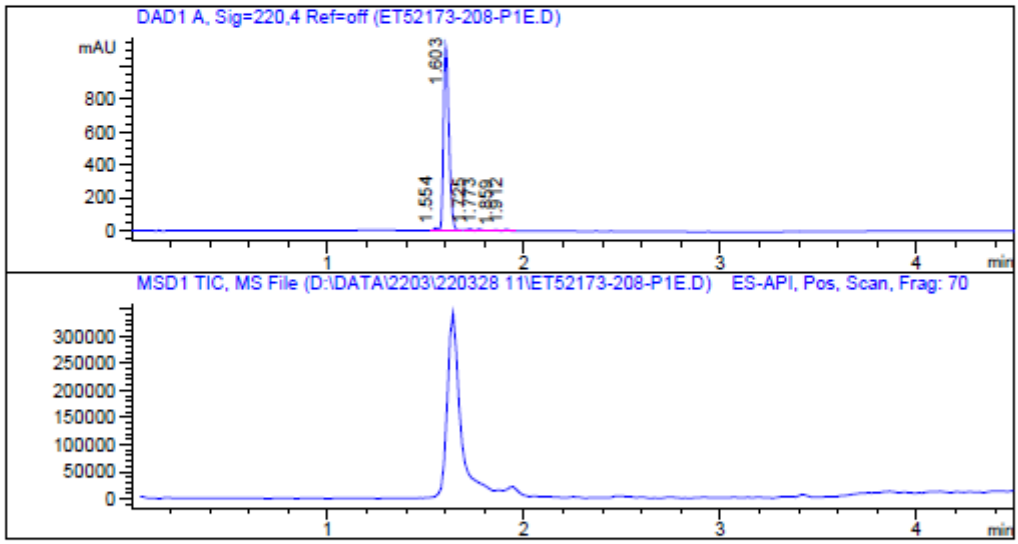
4.00	95	1.0
4.01	5	1.0
4.50	5	1.0



Report

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3	1.725	11.539	0.987	0.025	18.317	0.828
4	1.773	9.221	0.789	0.029	17.158	0.775
5	1.859	4.215	0.361	0.027	7.357	0.332
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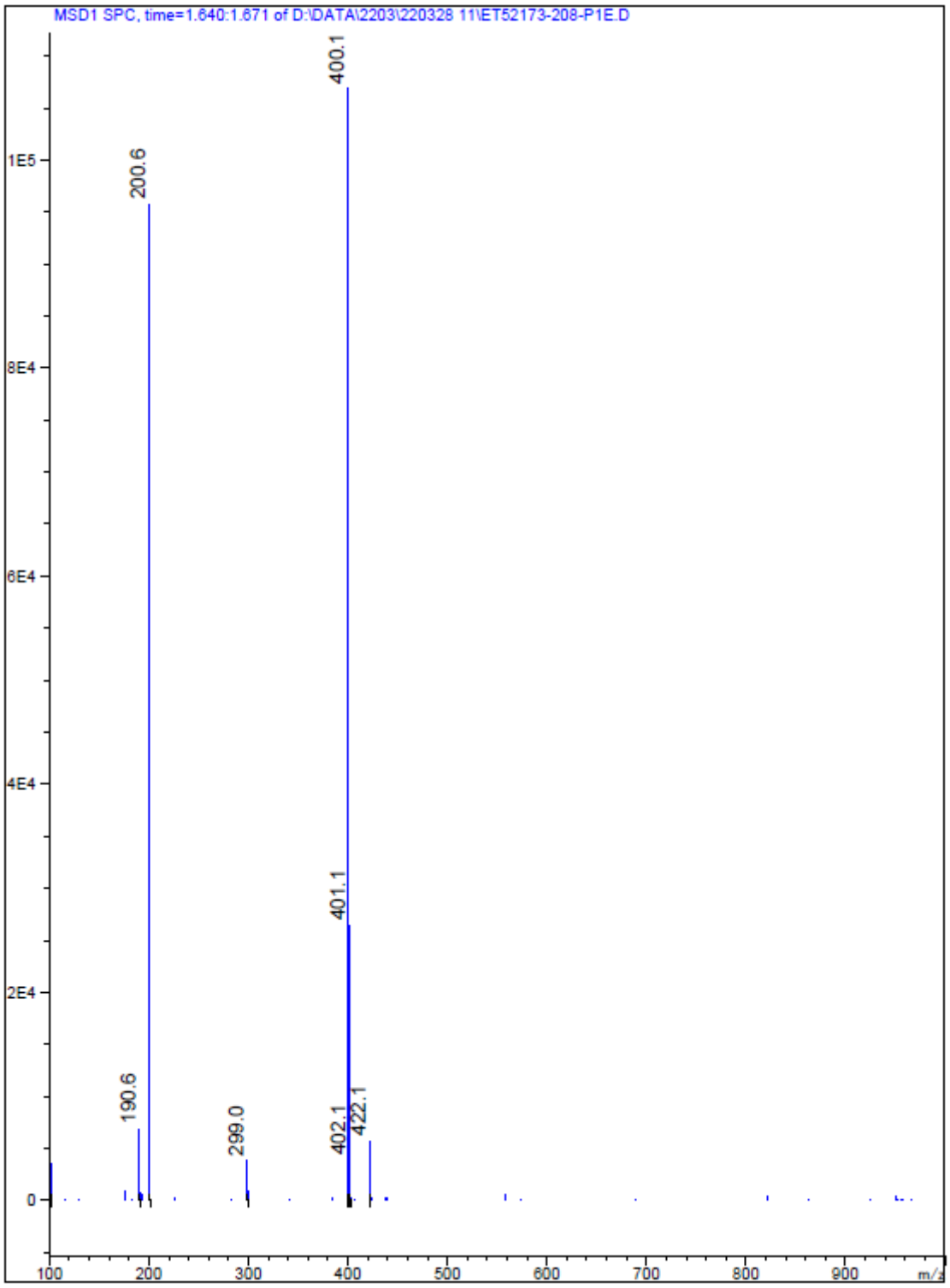
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1E5
8E4
6E4
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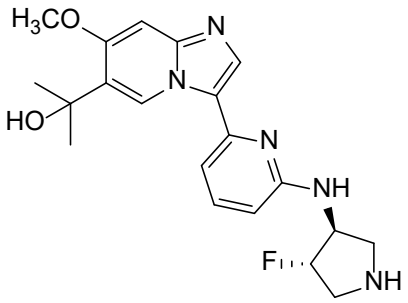
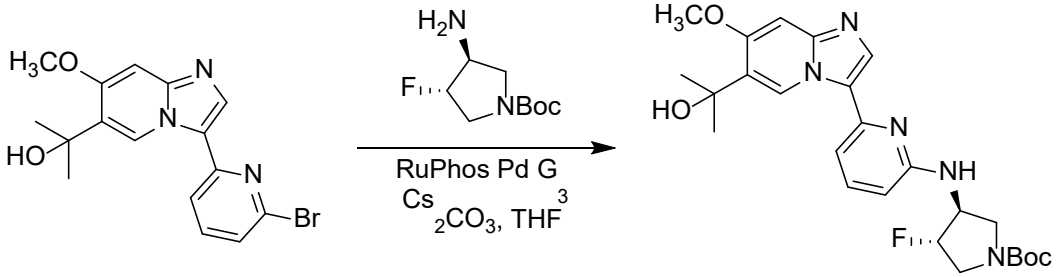
100
200
300
400
500
600
700
800
900
m/z

Compound 28, 2-(3-(6-(((3S,4S)-4-fluoropyrrolidin-3-yl)amino)pyridin-2-yl)-7-methoxyimidazo[1,2-a]pyridin-6-yl)propan-2-ol

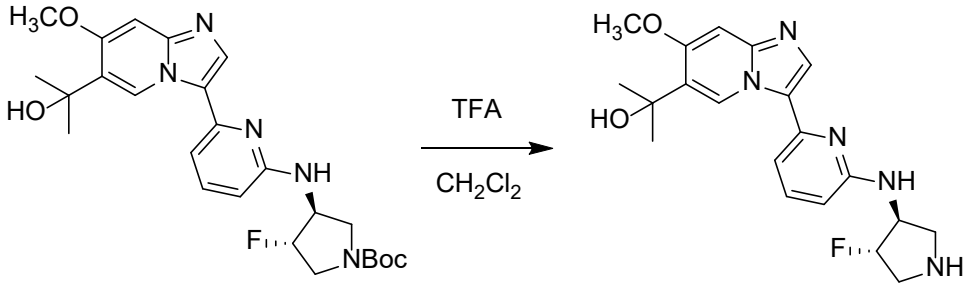


Step A. tert-butyl (3S,4S)-3-fluoro-4-((6-(6-(2-hydroxypropan-2-yl)-7-methoxyimidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)pyrrolidine-1-carboxylate



A mixture of 2-[3-(6-bromo-2-pyridyl)-7-methoxy-imidazo[1,2-a]pyridin-6-yl]propan-2-ol (0.550 g, 1.52 mmol), tert-butyl (3S,4S)-3-amino-4-fluoro-pyrrolidine-1-carboxylate (0.340 g, 1.67 mmol), cesium carbonate (1.24 g, 3.80 mmol) and (2-dicyclohexylphosphino-2',6'-diisopropoxy-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II)methanesulfonate (0.13 g, 0.152 mmol) in tetrahydrofuran (10 mL) was purged with nitrogen, and was then heated at 80 °C for 16 hours. The reaction was then cooled to room temperature, filtered, and concentrated under reduced pressure to provide the title compound which was used without further purification in the next step: LCMS *m/z* calcd for $C_{25}H_{32}FN_5O_4$: 486.2 $[M+H]^+$, found 486.3 $[M+H]^+$.

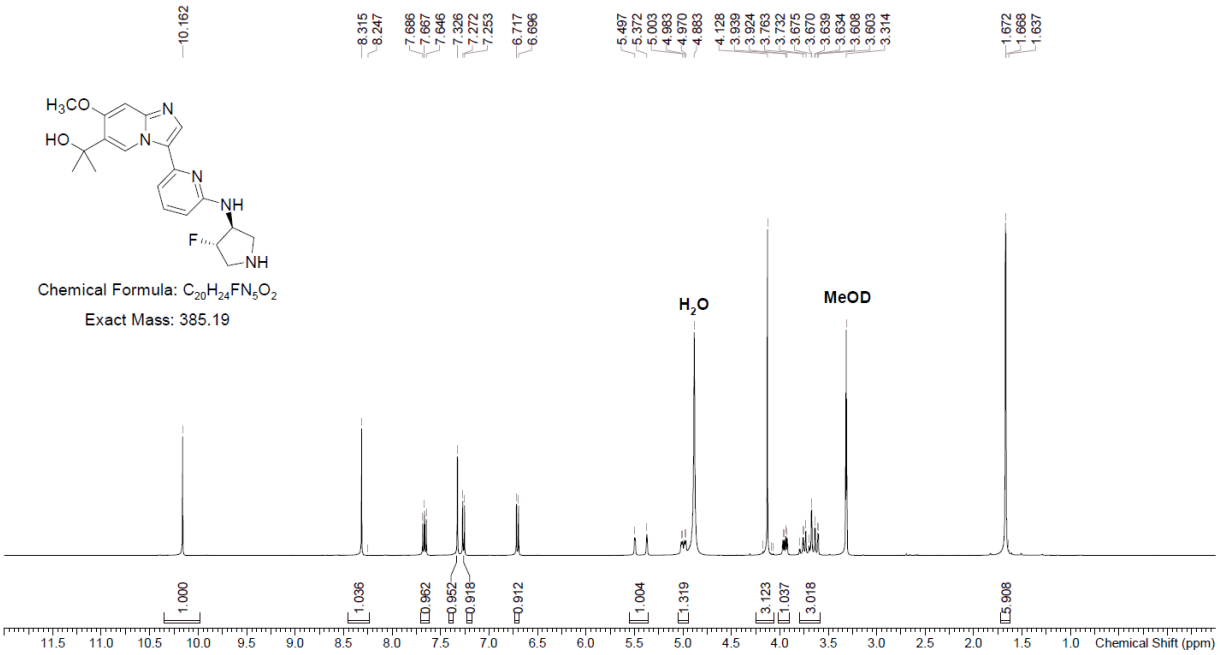
Step B. 2-(3-(6-(((3S,4S)-4-fluoropyrrolidin-3-yl)amino)pyridin-2-yl)-7-methoxyimidazo[1,2-a]pyridin-6-yl)propan-2-ol



To a solution of tert-butyl (3S,4S)-3-fluoro-4-[[6-[6-(1-hydroxy-1-methyl-ethyl)-7-methoxy-imidazo[1,2-a]pyridin-3-yl]-2-pyridyl]amino]pyrrolidine-1-carboxylate (0.501 g, 1.03 mmol) in dichloromethane (8 mL) was added trifluoroacetic acid (2.0 mL). The resulting reaction was stirred at room temperature for 1 hour, and was then concentrated under reduced pressure. The crude product thus obtained was purified by HPLC (Phenomenex Luna C18 column, 10 micron, 150 x 50 mm; 35 – 65% acetonitrile in water containing 0.1% TFA) to provide the title compound as a white solid (0.358 g, 61% yield over two steps): HRMS (ESI^+) *m/z* calcd for $C_{20}H_{24}FN_5O_2$: 386.1987 $[M+H]^+$, found 386.1986; ^1H NMR (400 MHz, CD_3OD) δ 10.15 (s, 1H), 8.30 (s, 1H), 7.66 (t, *J* = 7.6 Hz, 1H), 7.32 (s, 1H), 7.26 (d, *J* = 7.3 Hz, 1H), 6.71 (d, *J* = 8.3 Hz, 1H), 5.51 – 5.35 (m, 1H), 4.99 (br dd, *J* = 5.8, 13.5 Hz, 1H), 4.12 (s, 3H), 3.98 – 3.91 (m, 1H), 3.80 – 3.72 (m, 1H), 3.70 – 3.66 (m, 1H), 3.61 (dd, *J* = 2.3, 12.6 Hz, 1H), 1.67 (d, *J* = 1.8 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 163.50, 158.54, 146.18, 143.80, 139.79, 133.15, 128.49, 126.62, 121.66, 113.24, 111.14, 95.35 (d, *J* = 181 Hz), 91.74, 72.06, 57.36, 57.20 (d, *J* = 29 Hz), 50.97 (d, *J* = 24 Hz), 50.45, 29.49, 29.30. $[\alpha]^{20}_D$ -16.4° (*c* 1.00, CH_3OH).

Compound 28

ET27512-311-p1a MeOD Bruker_B_400MHz



Chemical Formula: $C_{20}H_{24}FN_5O_2$

Exact Mass: 385.19

Operator: Date:

LCMS REPORT

```
Compound ID       : Compound 28
Sample ID         : ET27512-311-p1c
Injection Date    : 2. Dec. 2019
Inj. Vol.         : 1.00  ul
Location          : P2-D-04
Acq  Method       : D:\DATA\1912\191202_HD 17\5_95AB_6min-220.M
Data Filename     : D:\DATA\1912\191202_HD 17\ET27512-311-P1C.D
Instrument        : H
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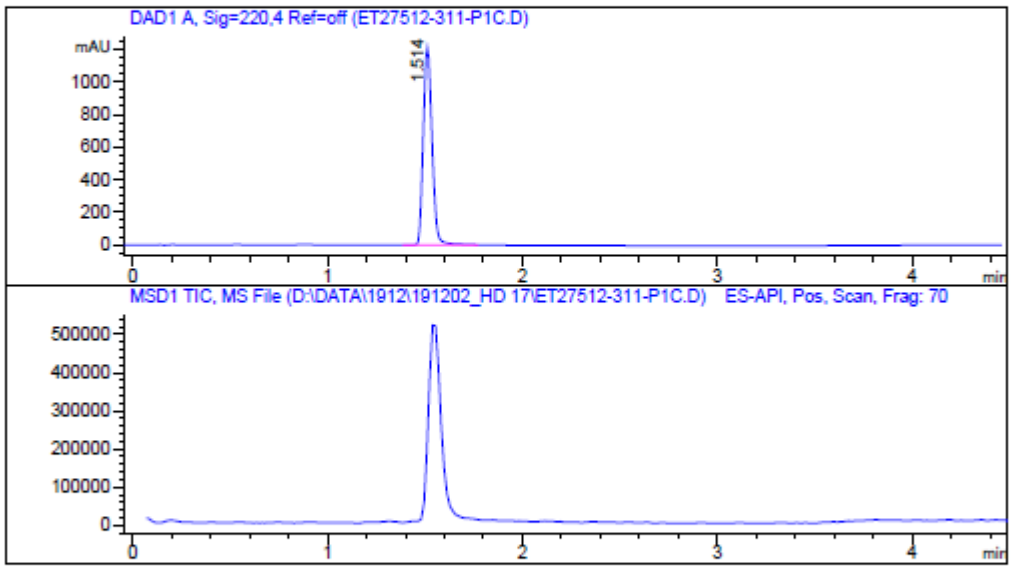
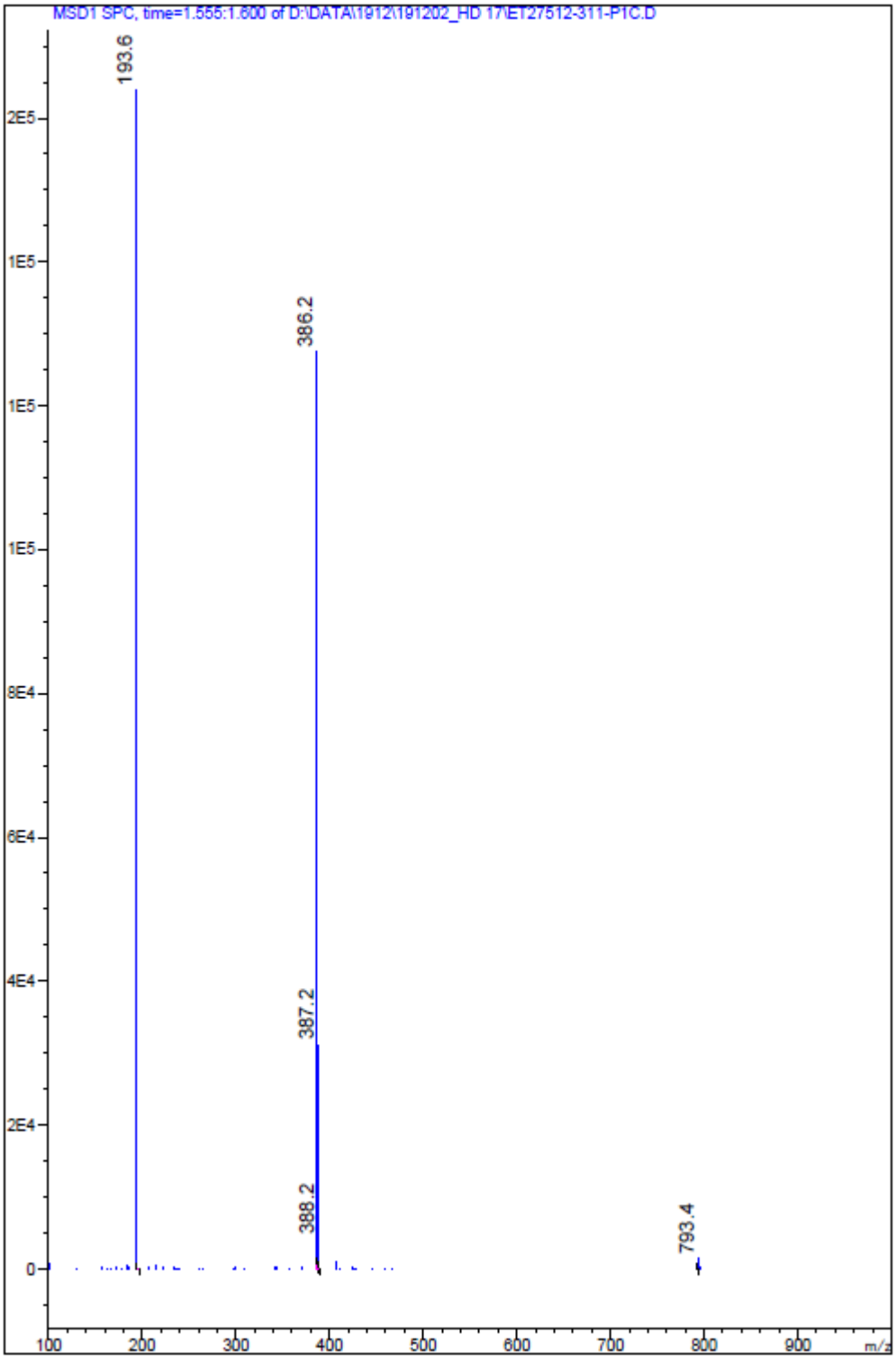
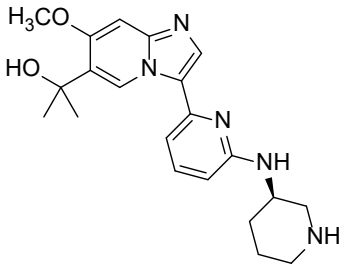


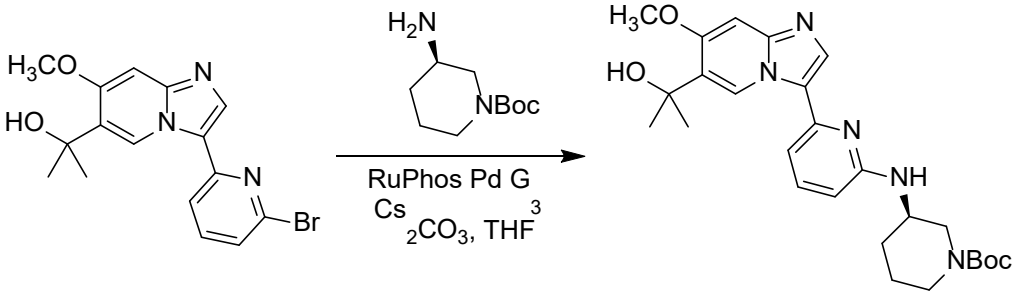

```
==========================================================================
                          Integration Result
==========================================================================
   Signal 1 : DAD1 A, Sig=220,4 Ref=off
   Peak   RT      Area    Height  Height %   Width   Area %
    #    [min]                               [min]
   ----------------------------------------------------------
     1    1.514 3806.272 1213.690  100.000    0.049  100.000
   --- -------- -------- -------- -------- -------- --------
```


MSD1 SPC, time=1.555:1.600 of D:\DATA\1912\191202_HD 17\ET27512-311-P1C.D
193.6
386.2
387.2
388.2
793.4
2E5
1E5
1E5
1E5
8E4
6E4
4E4
2E4
0
100
200
300
400
500
600
700
800
900
m/z

Compound 29, (R)-2-(7-methoxy-3-(6-(piperidin-3-ylamino)pyridin-2-yl)imidazo[1,2-a]pyridin-6-yl)propan-2-ol

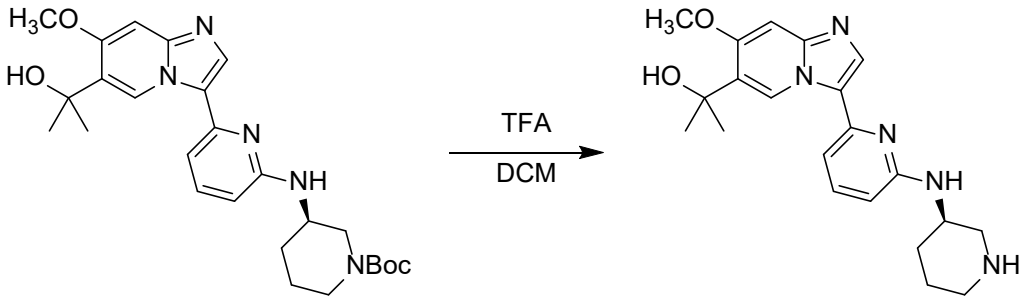


Step A. tert-butyl (R)-3-((6-(6-(2-hydroxypropan-2-yl)-7-methoxyimidazo[1,2-a]pyridin-3-yl)pyridin-2-yl)amino)piperidine-1-carboxylate

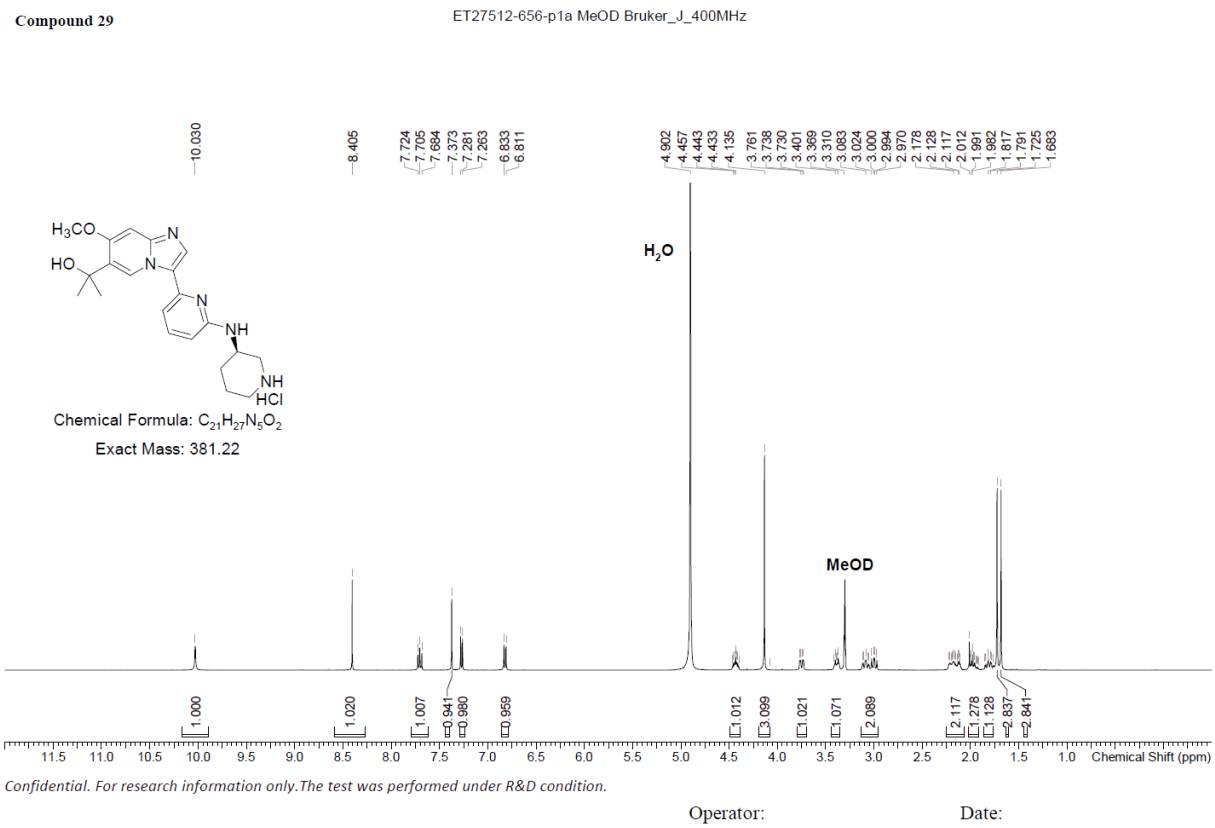


A mixture of 2-[3-(6-bromo-2-pyridyl)-7-methoxy-imidazo[1,2-a]pyridin-6-yl]propan-2-ol (0.501 g, 1.38 mmol), tert-butyl (3R)-3-aminopiperidine-1-carboxylate (0.304 g, 1.52 mmol), cesium carbonate (1.12 g, 3.45 mmol) and (2-dicyclohexylphosphino-2',6'-diisopropoxy-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II)methanesulfonate (0.115 g, 0.138 mmol) in tetrahydrofuran (10 mL) was purged with nitrogen, and was then stirred at 80 °C for 16 hours. The reaction mixture was then cooled to room temperature, filtered, and concentrated under reduced pressure to provide the title compound which was used without further purification in the next step: LCMS *m/z* calcd for $C_{26}H_{35}N_5O_4$: 482.3 $[M+H]^+$, found 482.3 $[M+H]^+$.

Step B. (R)-2-(7-methoxy-3-(6-(piperidin-3-ylamino)pyridin-2-yl)imidazo[1,2-a]pyridin-6-yl)propan-2-ol



To a solution of tert-butyl (3R)-3-[[6-[6-(1-hydroxy-1-methyl-ethyl)-7-methoxy-imidazo[1,2-a]pyridin-3-yl]-2-pyridyl]amino]piperidine-1-carboxylate (0.502 g, 1.04 mmol) in dichloromethane (8 mL) was added trifluoroacetic acid (2.0 mL). The resulting reaction was stirred at room temperature for 1 hour, and was then concentrated under reduced pressure. The resulting crude product was purified by HPLC (Phenomenex Luna C18 column, 10 micron, 250 x 50 mm; 10 – 40% acetonitrile in water containing 0.1% TFA) to provide the title compound as a white solid (0.232 g, 35% yield over two steps): HRMS (ESI^+) *m/z* calcd for $C_{21}H_{27}N_5O_2$: 382.2238 $[M+H]^+$, found 382.2235; ^1H NMR (400 MHz, CD_3OD) δ 10.43 (s, 1H), 8.42 (s, 1H), 7.59 (t, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 4.1 Hz, 1H), 7.25 (d, *J* = 7.4 Hz, 1H), 6.64 (d, *J* = 8.4 Hz, 1H), 4.39 – 4.26 (m, 1H), 4.13 (s, 3H), 3.80 (br d, *J* = 12.8 Hz, 1H), 3.45 – 3.34 (m, 1H), 3.15 – 3.04 (m, 1H), 3.00 – 2.91 (m, 1H), 2.20 – 1.90 (m, 2H), 1.82 – 1.75 (m, 1H), 1.75 (s, 3H), 1.69 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 163.76, 157.12, 143.79, 142.17, 133.59, 127.41, 123.50, 114.07, 113.10, 92.17, 72.65, 57.68, 48.00, 47.19, 44.90, 29.21, 29.16, 29.05, 21.91. $[\alpha]^{20}_D$ +154.9° (*c* 1.00, CH_3OH).



LCMS REPORT

```
Compound ID       : Compound 29
Sample ID         : ET27512-656-p1b
Injection Date    : 8. Jul. 2020
Inj. Vol.         : 1.00  ul
Location          : P1-B-02
Acq  Method       : D:\DATA\2007\200708 16\5_95AB_6min-220.M
Data Filename     : D:\DATA\2007\200708 16\ET27512-656-P1B.D
Instrument        : H
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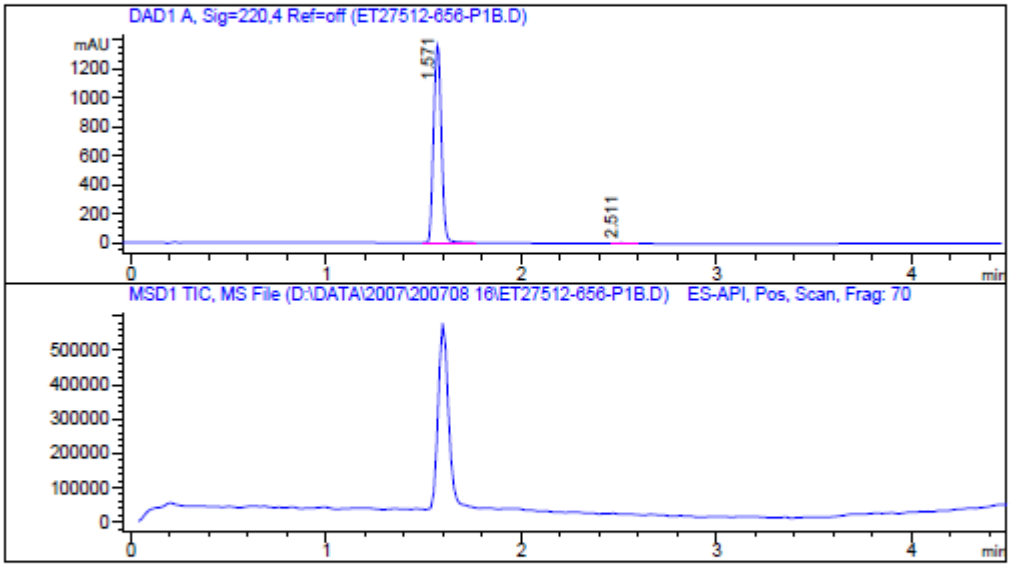
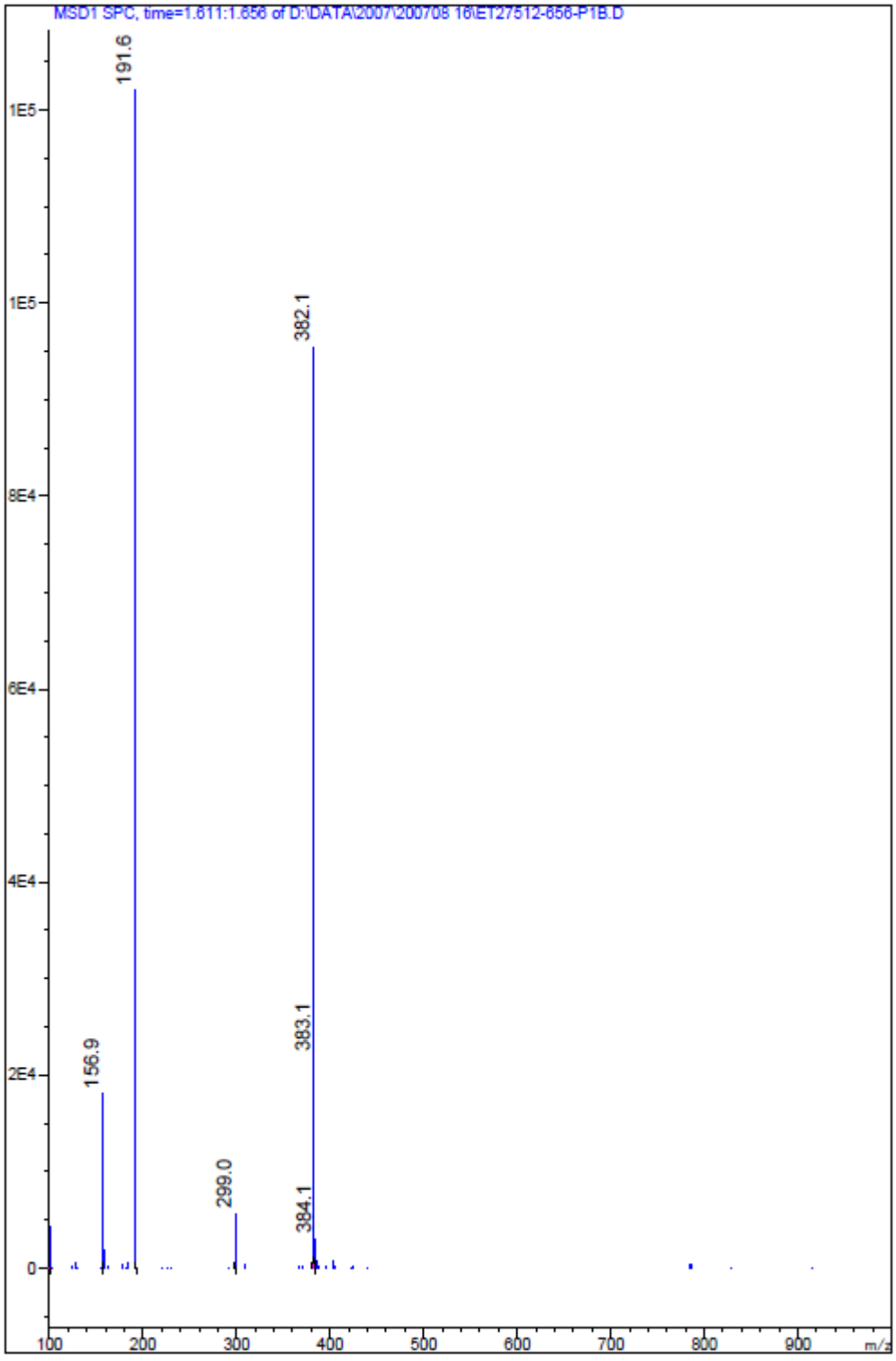


Integration Result

Signal 1 : DAD1 A, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	1.571	3787.050	1364.481	99.780	0.046	99.791
2	2.511	7.919	3.013	0.220	0.043	0.209


MSD1 SPC, time=1.611:1.656 of D:\DATA\2007\200708 16\ET27512-656-P1B.D
191.6
382.1
156.9
383.1
299.0
384.1
1E5
1E5
8E4
6E4
4E4
2E4
0
100
200
300
400
500
600
700
800
900
m/z

References

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(2) For additional information, see the Reaction Biology website: https://www.reactionbiology.com/services/kinase-assays/kinase-screening

(3) For additional information, see the Eurofins/DiscoverX website: https://www.discoverx.com/target-class/kinase/

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