Supporting Information

Discovery of BAY-985 a highly selective TBK1/IKKε inhibitor

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HTS

3.05 million compounds of the Bayer compound library were screened using a cell-based TBK1/IKKε activity assay. The reporter cell line expressed luciferase under the control of multiple interferon-stimulated response elements (ISREs). Co-incubation with poly dA:dT stimulates TBK/ IKK driven ISRE activation and hence luciferase expression. Luciferase activity was read out 20 hours after poly dA:dT stimulation. 39719 hits that showed a minimum of 40% inhibition of the luciferase signal at 10 µM were filtered for hits of the previous biochemical HTS campaign and undesirable structural features. The remaining 38026 hits were retested in the primary assay at two different concentrations (20 µM and 4 µM). To test for toxic compounds or transcriptional, translational or luciferase inhibitors a cell line of the same cell background was used that constitutively expressed luciferase. 30699 hits showed significant reduction of the luciferase signal in the control cell line. This high number can mostly be attributed to toxicity which is not unlikely in an experimental setting of 20 hours incubation time. Subsequently, hits were selected that showed a minimum of 20% inhibition at 4 µM and 30% at 20 µM which resulted in 3046 hits. IC_{50} values were obtained using the primary assay in a poly dA:dT stimulated as well as an unstimulated setting. Hits that were able to inhibit both the activated signal as well as the unstimulated background signal were considered as true TBK1/ IKK inhibitors. The reason for this selection was that in theory hits that only act on stimulated cells could also inhibit upstream pathway members such as TLR or TRAF. This led to a final hit list of 961 compounds. All hits in the hit list were tested in biochemical TBK1 and IKKε assays before entering the hit-to-lead phase.

Assay descriptions

Biochemical assays

Inhibition of TBK1, IKKε, CDK9, FLT3, and RSK4 was assessed in TR-FRET-based kinase activity inhibition assays using recombinant human enzymes and suitable biotinylated peptides as substrates. Detailed descriptions of the assays are provided below.

General procedure for sample preparation and data evaluation

Compounds were tested in duplicate at 11 concentrations (20 µM, 5.7 µM, 1.6 µM, 0.47 µM, 0.13 µM, 38 nM, 11 nM, 3.1 nM, 0.89 nM, 0.25 nM, 0.073 nM), the dilution series prepared separately before the assay at the level of 100-fold concentrated solutions in DMSO by serial dilutions. The different kinase assays were performed in black 1536-well microtiter plates (Greiner Bio-One) in a 5 µL assay volume with 50 nL copies of the same dilution series.

The amount of phosphorylated biotinylated peptide substrates was evaluated via quantification of their complexes with the detection reagents by measurement of the resonance energy transfer from the europium chelate to streptavidin-XL. Therefore, the fluorescence emissions at 620 nm and 665 nm after excitation at 350 nm were measured using a TR-FRET reader [PHERAstar Plus (BMG LABTECH) or ViewLux (Perkin Elmer)]. The ratio of the emissions at 665 nm and at 620 nm was taken as the measure for the amount of phosphorylated substrate. The data were normalized (enzyme reaction without inhibitor = 0% inhibition, all other assay components but no enzyme = 100% inhibition). IC_{50} values were calculated by four-parameter logistic fitting using the Screener software package (Genedata, Switzerland).

TBK1 low ATP/high ATP assay

Recombinant full-length N-terminal His-tagged human TBK1 (Life Technologies, cat. no. PR5618B) was used as enzyme and the biotinylated peptide biotin-Ahx-GDEDFSSFAEPG [C-terminus in amide form, Biosyntan (Berlin-Buch, Germany)] as substrate.

For the assay, 2 µL of a solution of TBK1 in aqueous assay buffer [50 mM HEPES pH 7.0, 10 mM $MgCl_2$, 1.0 mM dithiothreitol (DTT), 0.05% (w/v) bovine serum albumin (BSA), 0.01% (v/v) Nonidet P40 (Sigma), protease inhibitor mixture (complete EDTA-free, Roche; 1 tablet/5 mL)] was added to the solution of the test compound and the mixture was incubated for 15 min at 22 °C to allow pre-binding of the test compound to the enzyme before the start of the kinase reaction. Then, the kinase reaction was started by the addition of 3 µL of a solution of adenosine triphosphate [ATP, 16.7 µM (=> final concn in the 5 µL assay volume was 10 µM) for the low ATP assay or 1.67 mM (=> final concn was 1 mM) for the high ATP assay] and substrate (1.67 µM => final concn in the 5 µL assay volume was 1 µM) in assay buffer and the resulting mixture was incubated for 30 min at 22 °C. The concentration of TBK1 was adjusted depending on the activity of the enzyme lot and was chosen appropriate to have the assay in the linear range; typical concentrations were 0.03 µg/mL in the low ATP assay and 0.003 µg/mL in the high ATP assay. The reaction was stopped by the addition of 3 µL of a solution of TR-FRET detection reagents [0.33 µM streptavidin-XL665 (Cisbio Bioassays, Codolet, France), 2.5 nM anti-phosphoserine antibody (Merck Millipore, STK antibody, cat. no. 35-002), and 1.25 nM LANCE EU-W1024 labeled anti-mouse IgG antibody (Perkin Elmer, product no. AD0077)] in an aqueous EDTA solution [167 mM EDTA, 0.13% (w/v) BSA in 100 mM HEPES/NaOH pH 7.5]. The resulting mixture was incubated for 1 h at 22 °C to allow the formation of a complex between the phosphorylated biotinylated peptide and the detection reagents before measurement.

IKKε assay

A recombinant fusion protein of GST (N-terminal) and full-length human IKKε (Life Technologies, cat. no. PV4876) was used as enzyme with a typical concentration of 0.01 µg/mL in the assay. Substrate, buffer, and all other assay conditions were the same as in the TBK1 low ATP assay described above.

CDK9 assay

A complex of recombinant full-length His-tagged human CDK9 and CycT1 (Life Technologies, cat. no. PV4131) was used as enzyme and the biotinylated peptide biotin-Ttds-YISPLKSPYKISEG [C-terminus in amide form, Jerini Peptide Technologies (Berlin)] as substrate.

For the assay, 2 µL of a solution of CDK9/CycT1 in aqueous assay buffer [50 mM Tris HCl pH 8.0, 10 mM $MgCl_2$, 1.0 mM DTT, 0.1 mM sodium orthovanadate, 0.01% (v/v) Nonidet P40] was added to the solution of the test compound and the mixture was incubated for 15 min at 22 °C. Then, the kinase reaction was started by the addition of 3 µL of a solution of ATP (16.7 µM => final concn in the 5 µL assay volume was 10 µM) and substrate (1.25 µM => final concn was 0.75 µM) in assay buffer and the resulting mixture was incubated for 25 min at 22 °C. The concentration of CDK9/CycT1 was adjusted depending on the activity of the enzyme lot and was chosen appropriate to have the assay in the linear range; typical concentrations were in the range of 1 µg/mL. The reaction was stopped by the addition of 3 µL of a solution of TR-FRET detection reagents [333 nM streptavidin-XL665, 1.67 nM anti-RB(pSer807/pSer811) antibody (BD Pharmingen, no. 558389), and 2 nM LANCE EU-W1024 labeled anti-mouse IgG antibody] in an aqueous EDTA solution [167 mM EDTA, 0.2% (w/v) BSA in 100 mM HEPES pH 7.5]. The resulting mixture was incubated for 1 h at 22 °C to allow the formation of a complex between the phosphorylated biotinylated peptide and the detection reagents before measurement.

FLT3 assay

N-Terminal GST-tagged, recombinant catalytic domain of human FLT3 (amino acids 564–end, Merck Millipore, cat. no. 14-500) was used as enzyme and the biotinylated peptide biotin-Ahx-GGEEEEYFELVKKKK (C-terminus in amide form, Biosyntan) as substrate.

For the assay, 2 µL of a solution of FLT3 in aqueous assay buffer [25 mM HEPES pH 7.5, 10 mM $MgCl_2$, 2 mM DTT, 5 mM ß-glycerophosphate, 0.5 mM EGTA 0.01% (v/v) Triton X-100 (Sigma)] was added to the solution of the test compound and the mixture was incubated for 15 min at 22 °C. Then, the kinase reaction was started by the addition of 3 µL of a solution of ATP (16.7 µM => final concn in the 5 µL assay volume was 10 µM) and substrate (1.67 µM => final concn was 1 µM) in assay buffer and the resulting mixture was incubated for 45 min at 22 °C. The concentration of FLT3 was adjusted depending on the activity of the enzyme lot and was chosen appropriate to have the assay in the linear range; typical concentrations were in the range of 0.2 nM. The reaction was stopped by the addition of 3 µL of a solution of TR-FRET detection reagents (333 nM streptavidin-XL665 and 5 nM PT66-Tb-cryptate, a terbium cryptate labeled anti-phosphotyrosine antibody from Cisbio Bioassays) in an aqueous EDTA solution [83 mM EDTA, 0.1% (w/v) BSA in 50 mM HEPES pH 7.5]. The resulting mixture was incubated for 1 h at 22 °C to allow the formation of a complex between the phosphorylated biotinylated peptide and the detection reagents before measurement.

RSK4 assay

A complex of recombinant full-length His-tagged human RSK4 (Merck Millipore, cat. no. 14-702) was used as enzyme and the biotinylated peptide biotin-Ahx-KKLNRTLSFAEPG (C-terminus in amide form, Biosyntan) as substrate.

For the assay, 2 µL of a solution of RSK4 in aqueous assay buffer [20 mM MOPS pH 7.0, 10 mM $MgCl_2$, 1.0 mM DTT, 1 mM EDTA, 0.001% (w/v) BSA, 0.01% (v/v) Brij-35] was added to the solution of the test compound and the mixture was incubated for 15 min at 22 °C. Then, the kinase reaction was started by the addition of 3 µL of a solution of ATP (16.7 µM => final concn in the 5 µL assay volume was 10 µM) and substrate (1.67 µM => final concn was 1 µM) in assay buffer and the resulting mixture was incubated for 30 min at 22 °C. The concentration of RSK4 was adjusted depending on the activity of the enzyme lot and was chosen appropriate to have the assay in the linear range; a typical concentration was 0.03 nM. The reaction was stopped by the addition of 3 µL of a solution of TR-FRET detection reagents [167 nM streptavidin-XL665, 2.5 nM anti-phosphoserine antibody (Merck Millipore, STK antibody, cat. no. 35-002), and 1.25 nM LANCE EU-W1024 labeled anti-mouse IgG antibody] in an aqueous EDTA solution [167 mM EDTA, 0.2% (w/v) BSA in 50 mM HEPES pH 7.5]. The resulting mixture was incubated for 1 h at 22 °C to allow the formation of a complex between the phosphorylated biotinylated peptide and the detection reagents before measurement.

Cellular assays

TBK1/Ikkepsilon activity assay (for HTS)

For the assay An MDA-MB231 cell line containing a stably integrated ISRE-Luciferase reporter construct (MDA-MB231 ISRE-TA-Luc2, clone 6. Source: NMI Reutlingen) was used and Poly I:C (InvivoGen, # tlrl-pic) was the agonist. In brief, 4-5 µl of cells in assay medium (DMEM/Hams F12 with L-Glutamin, w/o Phenole red (Gibco, #21041), FCS 10% (FCS Gold, PAA), Penicillin/Streptomycin (Gibco BRL # 15140-114)) containing 100 ng/ml Poly I:C were dispensed into assay wells containing compound dilution. Cell concentration was 3000 cells/well. After 20h incubation at 37°C, 5%CO_2 and 95% humidity the incubation was stopped by the addition of 2-2.5 µl luciferase reagent (Promega Steady-Glo, #E2550) and luciferase activity was measured after 20 min pre-incubation (Pherastar Plus (BMG Labtechnologies) or Viewlux (Perkin-Elmer)). The data

were normalised (luciferase signal after Poly I:C stimulation = 0 % inhibition, unstimulated cells = 100 % inhibition). The IC_{50} values were calculated by four-parameter logistic fitting using the Screener software package (Genedata, Switzerland).

Interference Assay (For HTS)

To exclude toxic compounds or general inhibitors of the transcriptional or translational machinery or inhibitors of luciferase activity an MDA-MB231 cell line was used that carried a CMV-promoter driven luciferase gene, hereby constitutively expressing luciferase. For the assay 4-5 µl of MDA-MB231 cells consitutively expressing luciferase (MDA-MB231_694, Bayer) in assay medium contatining Poly I:C (see above) were dispensed into wells containing compound dilution at 750 cells/well. After 20h incubation at 37°C, 5%CO_2 and 95% humidity the incubation was stopped by the addition of 2-2.5 µl luciferase reagent (Promega Steady-Glo, #E2550) and luciferase activity was measured after 20 min pre-incubation (Pherastar Plus (BMG Labtechnologies) or Viewlux (Perkin-Elmer)). The data were normalised (luciferase signal after Poly I:C stimulation = 0 % inhibition, Cells + 10 µM Staurosporin or 10 µM Actinomycin = 100 % inhibition).

pIRF3 cell-based mechanistic assay

MDA-MB231 mIRF3 cells were plated at 10000 cells/well in 384-well microtiter plates in 30 µL growth medium/well. The following day, the medium was exchanged for phenol red free medium and test compounds were added to the cells using a D300 Digital Dispenser (Tecan, Germany). After 1 h, cells were transfected with 5µg/mL Poly(I:C) (InvivoGen, Toulouse, France) using Lipofectamine 2000 (Life Technologies) according to the manufacturer's protocol. After 1 h, IRF3 phospho-S385/386 was measured (Cisbio Bioassays, Codolet, France) using a PHERAstar reader (BMG LABTECH, Germany) at two wavelengths (665 nm and 620 nm). IC_{50} values were calculated by four-parameter logistic fitting using the Screener software package (Genedata, Switzerland).

Cell culture and proliferation

Cell culture. ACHN and SK-MEL-2 cell lines were obtained from the ATCC, Manassas, USA. Both cell lines were grown in Earle's MEM with stable glutamine (Biochrom) supplemented with 10% fetal calf serum (Biochrom).

MDA-MB231 mIRF3 cells were generated by lentiviral transduction of MDA-MB231 cells (ATCC, Manassas, USA) with the coding sequence for human IRF3 (NM_001571) under the control of a CMV promoter. The cells were grown in DMEM/Ham's F12 (Biochrom) supplemented with 10% fetal calf serum (Biochrom). Monoclonal lines were selected with 10 µg/mL hygromycin.

Cell line identity was confirmed by STR DNA typing at DSMZ.

Proliferation. For proliferation assays, cells were plated in white 384-well microtiter plates (Corning, Germany) at 300 (ACHN) and 800 (SK-MEL-2) cells/well, respectively, in 50 µL medium. The following day, test compounds were added to the cells using a D300 Digital Dispenser (Tecan, Germany). After 96 h, cell numbers were determined using CellTiter-Glo solution (Promega). Luminescence was read on a VICTOR reader (Perkin Elmer). The luminescence signal was compared to the signal measured on sister plates on the day of compound addition (time zero).

Standard deviations

Table 1

Compd	TBK1		IKKε	pIRF3	SK-MEL-2	ACHN
	IC_{50} (nM) (low ATP)	**IC_{50} (nM) (high ATP)**	**IC_{50} (nM)**	**IC_{50} (nM)**	**IC_{50} (nM)**	**IC_{50} (nM)**
1	29 ± 12 (*n=8*)	618 ± 136 (*n=8*)	17 ± 4 (*n=8*)	2030 ± 975 (*n=2*)	n.d.	n.d.
2	93 ± 22 (*n=4*)	1390 ± 221 (*n=4*)	80 ± 8 (*n=4*)	n.d.	3360 ± 6930(*n=5*)	>33000(*n=3*)
3	129 ± 64 (*n=12*)	2270 ± 1270 (*n=12*)	168 ± 73 (*n=12*)	2750 ± 1580 (*n=2*)	2180 [b] (*n=1*)	9670 ± 7780 (*n=5*)
4	273 ± 70 (*n=3*)	>20000 ± 1000 (*n=3*)	914 ± 36 (*n=2*)	n.d.	n.d.	n.d.
5	764 ± 95 (*n=2*)	14700 ± 921 (*n=2*)	940 ± 54 (*n=2*)	n.d.	n.d.	n.d.
6	1440 [b] (*n=1*)	16700 [b] (*n=1*)	n.d.	n.d.	n.d.	n.d.
7	1060 [b] (*n=1*)	5110 [b] (*n=1*)	n.d.	n.d.	n.d.	n.d.
8	281 ± 34 (*n=2*)	5290 ± 734 (*n=2*)	1170 ± 82 (*n=2*)	5160 [b] (*n=1*)	>30000 [b](*n=1*)	350 [b] (*n=1*)
9	98 ± 9 (*n=2*)	2040 ± 381 (*n=2*)	730 ± 137 (*n=2*)	4780 [b](*n=1*)	1600 [b] (*n=1*)	381 [b] (*n=1*)
10	188 ± 27 (*n=2*)	3740 ± 500 (*n=2*)	n.d.	>30000 [b](*n=1*)	>30000 [b] (*n=1*)	>30000 [b] (*n=1*)
11	148 ± 115 (*n=3*)	1270 ± 79 (*n=3*)	154 ± 6 (*n=2*)	n.d.	n.d.	n.d.
12	115 ± 8 (*n=2*)	1920 ± 109 (*n=2*)	226 ± 7 (*n=2*)	n.d.	n.d.	n.d.
13	146 ± 2 (*n=2*)	2660 ± 69 (*n=2*)	433 ± 20 (*n=2*)	n.d.	n.d.	n.d.
14	429[b] (*n=1*)	5070 [b] (*n=1*)	n.d.	n.d.	n.d.	n.d.

n.d.: not determined. [b] single measurement

Table 2

Compd	TBK1		IKKε	pIRF3	SK-MEL-2	ACHN
	IC_{50} (nM) (low ATP)	**IC_{50} (nM) (high ATP)**	**IC_{50} (nM)**	**IC_{50} (nM)**	**IC_{50} (nM)**	**IC_{50} (nM)**
3	129 ± 64 (*n=12*)	2270 ± 1270 (*n=12*)	168 ± 73 (*n=12*)	2750 ± 1580 (*n=2*)	2180 [b] (*n=1*)	9670 ± 7780 (*n=5*)
15	34 ± 5 (*n=4*)	829 ± 301 (*n=4*)	593 ± 5 (*n=4*)	2270 ± 910 (*n=2*)	n.d.	n.d.
16	106 ± 10 (*n=6*)	2490 ± 794 (*n=6*)	200 ± 35 (*n=6*)	3020 ± 1620 (*n=2*)	n.d.	n.d.
17	19 ± 5 (*n=12*)	372 ± 139 (*n=12*)	37 ± 6 (*n=12*)	1210 ± 344 (*n=5*)	3530 ± 1620 (*n=5*)	8980 ± 8890 (*n=8*)
18	1210 ± 269 (*n=2*)	>20000 (*n=2*)	3880 ± 1150 (*n=2*)	>10000 ± 1000 (*n=3*)	>25000 ± 2500 (*n=2*)	>30000 (*n=2*)

n.d.: not determined. [b] single measurement

Table 3 (part 1/2)

Compd	TBK1		IKKε	pIRF3	SK-MEL-2	ACHN
	IC_{50} (nM) (low ATP)	**IC_{50} (nM) (high ATP)**	**IC_{50} (nM)**	**IC_{50} (nM)**	**IC_{50} (nM)**	**IC_{50} (nM)**
3	129 ± 64 (*n=12*)	2270 ± 1270 (*n=12*)	168 ± 73 (*n=12*)	2750 ± 1580 (*n=2*)	2180 [b] (*n=1*)	9670 ± 7780 (*n=5*)
17	19 ± 5 (*n=12*)	372 ± 139 (*n=12*)	37 ± 6 (*n=12*)	1210 ± 344 (*n=5*)	3530 ± 1620 (*n=5*)	8980 ± 8890 (*n=8*)
19	58 ± 14 (*n=3*)	>20000 [b] (*n=1*)	41 ± 17 (*n=4*)	>30000 [b] (*n=1*)	n.d.	n.d.
20	72 ± 2. (*n=2*)	>20000 (*n=2*)	89 ± 2 (*n=2*)	n.d.	n.d.	n.d.
21	55 [b] (*n=1*)	2100 [b] (*n=1*)	197 [b] (*n=1*)	1960 [b] (*n=1*)	n.d.	n.d.
22	50 ± 5(*n=2*)	530 ± 14 (*n=2*)	98 ± 31 (*n=2*)	1580 ± 608 (*n=2*)	n.d.	n.d.
23	5 ± 1 (*n=6*)	137 ± 64 (*n=6*)	7 ± 2 (*n=6*)	368 ± 209 (*n=3*)	221 [b] (*n=1*)	7900 ± 3680 (*n=3*)
24	1 ± 0.5 (*n=4*)	18 ± 2 (*n=2*)	6 ± 0.2 (*n=2*)	131 ± 108 (*n=7*)	408 ± 2000 (*n=6*)	5270 ± 5560 (*n=10*)
25	2 ± 0.5 (*n=4*)	6 ± 0.3 (*n=2*)	4 ± 0.05 (*n=2*)	93 [b] (*n=1*)	3710 [b] (*n=1*)	5420 ± 992 (*n=3*)
26	54 ± 13 (*n=2*)	391 ± 19 (*n=2*)	15 ± 1 (*n=2*)	840 [b] (*n=1*)	n.d.	n.d.
27	23 ± 1 (*n=2*)	388 ± 63 (*n=2*)	35 ± 8 (*n=2*)	1260 [b] (*n=1*)	337 [b] (*n=1*)	2240 [b] (*n=1*)
28	15 ± 0.5 (*n=2*)	434 ± 5 (*n=2*)	16 ± 2 (*n=2*)	773	172 ± 27 (*n=4*)	15300 ± 7300 (*n=6*)
29	2 ± 0.1 (*n=2*)	17 ± 2 (*n=2*)	4 ± 0.2 (*n=2*)	140 ± 90 (*n=7*)	476 ± 238 (*n=7*)	2010 ± 1610 (*n=13*)
30	0.7 ± 0.3 (*n=6*)	7 ± 3 (*n=6*)	2 ± 1 (*n=5*)	63 ± 48 (*n=6*)	883 ± 651 (*n=14*)	13400 ± 7470 (*n=17*)
31	2 ± 0.5 (*n=2*)	5 ± 0.4 (*n=2*)	4 ± 0.5 (*n=2*)	66 ± 25 (*n=2*)	470 ± 440 (*n=7*)	3870 ± 2360 (*n=4*)
32	10 ± 0.8 (*n=2*)	155 ± 2 (*n=2*)	10 ± 1 (*n=2*)	1990 [b] (*n=1*)	245 ± 1440 (*n=7*)	29800 [b] (*n=1*)
33	9 ± 4 (*n=6*)	167 ± 104 (*n=6*)	10 ± 2 (*n=5*)	213 ± 184 (*n=3*)	246 ± 139 (n=9)	9690 ± 3790 (*n=10*)
34	2 ± 0.6 (*n=17*)	30 ± 11 (*n=20*)	2 ± 0.5 (*n=13*)	74 ± 51 (*n=11*)	900 ± 623 (n=29)	7260 ± 5140 (*n=18*)
35	2 ± 0.2 (*n=2*)	50 ± 5 (*n=2*)	n.d.	119 ± 51 (*n=3*)	3970 [b] (*n=1*)	7960 ± 8276 (*n=7*)
36	1140 ± 99 (*n=2*)	>20000 (*n=2*)	1280 ± 40 (*n=2*)	3890 [b] (*n=1*)	6330 ± 9020 (*n=2*)	1110 [b] (*n=1*)

n.d.: not determined. [b] single measurement

Table 3 (part 2/2)

Compd	CDK9	FLT3	RSK4
	IC_{50} (nM)	**IC_{50} (nM)**	**IC_{50} (nM)**
3	455± 142 (*n=12*)	37 ± 16 (*n=12*)	8910 ± 3360 (*n=2*)
17	1520 ± 429 (*n=10*)	30 ± 8 (*n=11*)	2360± 318 (*n=6*)
19	n.d.	69 ± 12 (*n=4*)	n.d.
20	>20000 (*n=2*)	173 ± 18 (*n=2*)	n.d.
21	240[b](*n=1*)	11 ± 0.2 (*n=2*)	11200 ± 159 (*n=2*)
22	1460± 391 (*n=2*)	17 ± 0.5 (*n=2*)	8850 ± 68 (*n=2*)
23	9190± 886 (*n=6*)	9 ± 4 (*n=6*)	4120 ± 840 (*n=8*)
24	>20000 (*n=2*)	18 ± 7 (*n=4*)	3220 ± 267 (*n=4*)
25	>20000 (*n=2*)	357 ± 340 (*n=4*)	325 ± 361 (*n=4*)
26	71 ± 25 (*n=2*)	0.3± 0.02 (*n=2*)	1060 ± 121 (*n=2*)
27	263 ± 34 (*n=2*)	1 ± 0.2 (*n=2*)	2830 ± 207 (*n=2*)
28	320 ± 38 (*n=2*)	9 ± 0.4 (*n=2*)	434 ± 2 (*n=2*)
29	2130 ± 44 (*n=2*)	10 ± 0.8 (*n=2*)	170 ± 14 (*n=2*)
30	5110 ± 1600 (*n=2*)	5 ± 3 (*n=6*)	192 ± 55 (*n=6*)
31	16100 ± 2360 (*n=2*)	13 ± 0.3 (*n=2*)	1260 ± 122 (*n=2*)
32	n.d.	6 ± 0.5 (*n=2*)	177 ± 28 (*n=2*)
33	14400 ± 1010 (*n=2*)	114 ± 38 (*n=6*)	528 ± 154 (*n=6*)
34	n.d.	123 ± 39 (*n=20*)	276 ± 56 (*n=20*)
35	824 ± 176 (*n=2*)	1 ± 0.02 (*n=2*)	7 ± 1 (*n=2*)
36	n.d.	536 ± 32 (*n=2*)	6110 ± 745 (*n=2*)

n.d.: not determined. [b] single measurement

DiscoverX KINOME*scan* panels

In red the kinases showing 65% inhibition or more

Table S1: DiscoverX KINOME*scan* @ 100 nM for compound 3

DiscoverX gene symbol	% control
AAK1	61
ABL1(E255K)-phosphorylated	67
ABL1(F317I)-nonphosphorylated	55
ABL1(F317I)-phosphorylated	60
ABL1(F317L)-nonphosphorylated	67
ABL1(F317L)-phosphorylated	69
ABL1(H396P)-nonphosphorylated	100
ABL1(H396P)-phosphorylated	64
ABL1(M351T)-phosphorylated	68
ABL1(Q252H)-nonphosphorylated	66
ABL1(Q252H)-phosphorylated	73
ABL1(T315I)-nonphosphorylated	55
ABL1(T315I)-phosphorylated	49
ABL1(Y253F)-phosphorylated	68
ABL1-nonphosphorylated	54
ABL1-phosphorylated	68
ABL2	94
ACVR1	93
ACVR1B	100
ACVR2A	83
ACVR2B	87
ACVRL1	95
ADCK3	85
ADCK4	97
AKT1	86
AKT2	89
AKT3	95
ALK	90
ALK(C1156Y)	88
ALK(L1196M)	81
AMPK-alpha1	92
AMPK-alpha2	100
ANKK1	56
ARK5	86
ASK1	80
ASK2	91

DiscoverX gene symbol	% control
MAP3K1	67
MAP3K15	64
MAP3K2	89
MAP3K3	57
MAP3K4	91
MAP4K2	47
MAP4K3	100
MAP4K4	94
MAP4K5	82
MAPKAPK2	100
MAPKAPK5	74
MARK1	86
MARK2	90
MARK3	100
MARK4	86
MAST1	89
MEK1	66
MEK2	76
MEK3	88
MEK4	100
MEK5	**4.7**
MEK6	82
MELK	74
MERTK	86
MET	68
MET(M1250T)	40
MET(Y1235D)	74
MINK	**35**
MKK7	94
MKNK1	67
MKNK2	70
MLCK	**19**
MLK1	76
MLK2	88
MLK3	100
MRCKA	96

DiscoverX gene symbol	% control
AURKA	59
AURKB	64
AURKC	68
AXL	58
BIKE	49
BLK	90
BMPR1A	100
BMPR1B	72
BMPR2	81
BMX	76
BRAF	97
BRAF(V600E)	95
BRK	80
BRSK1	90
BRSK2	85
BTK	79
BUB1	96
CAMK1	74
CAMK1D	88
CAMK1G	80
CAMK2A	88
CAMK2B	75
CAMK2D	90
CAMK2G	87
CAMK4	100
CAMKK1	96
CAMKK2	74
CASK	77
CDC2L1	94
CDC2L2	92
CDC2L5	100
CDK11	93
CDK2	94
CDK3	100
CDK4-cyclinD1	97
CDK4-cyclinD3	100
CDK5	88
CDK7	56
CDK8	81
CDK9	87
CDKL1	71
CDKL2	93
CDKL3	81

DiscoverX gene symbol	% control
MRCKB	87
MST1	87
MST1R	75
MST2	58
MST3	97
MST4	100
MTOR	100
MUSK	87
MYLK	88
MYLK2	84
MYLK4	77
MYO3A	91
MYO3B	100
NDR1	49
NDR2	98
NEK1	83
NEK10	77
NEK11	94
NEK2	89
NEK3	56
NEK4	94
NEK5	85
NEK6	100
NEK7	100
NEK9	100
NIK	87
NIM1	100
NLK	100
OSR1	67
p38-alpha	88
p38-beta	98
p38-delta	100
p38-gamma	86
PAK1	95
PAK2	94
PAK3	78
PAK4	94
PAK6	90
PAK7	100
PCTK1	72
PCTK2	94
PCTK3	89
PDGFRA	99

DiscoverX gene symbol	% control
CDKL5	87
CHEK1	89
CHEK2	92
CIT	67
CLK1	39
CLK2	40
CLK3	92
CLK4	**32**
CSF1R	91
CSF1R-autoinhibited	84
CSK	79
CSNK1A1	85
CSNK1A1L	100
CSNK1D	99
CSNK1E	99
CSNK1G1	78
CSNK1G2	60
CSNK1G3	87
CSNK2A1	84
CSNK2A2	72
CTK	91
DAPK1	90
DAPK2	87
DAPK3	97
DCAMKL1	83
DCAMKL2	100
DCAMKL3	98
DDR1	98
DDR2	64
DLK	99
DMPK	72
DMPK2	80
DRAK1	**21**
DRAK2	**28**
DYRK1A	41
DYRK1B	92
DYRK2	56
EGFR	74
EGFR(E746-A750del)	91
EGFR(G719C)	92
EGFR(G719S)	97
EGFR(L747-E749del, A750P)	74
EGFR(L747-S752del, P753S)	90

DiscoverX gene symbol	% control
PDGFRB	100
PDPK1	92
PFCDPK1(P.falciparum)	74
PFPK5(P.falciparum)	71
PFTAIRE2	100
PFTK1	100
PHKG1	97
PHKG2	88
PIK3C2B	88
PIK3C2G	70
PIK3CA	90
PIK3CA(C420R)	66
PIK3CA(E542K)	76
PIK3CA(E545A)	58
PIK3CA(E545K)	86
PIK3CA(H1047L)	64
PIK3CA(H1047Y)	74
PIK3CA(I800L)	65
PIK3CA(M1043I)	70
PIK3CA(Q546K)	78
PIK3CB	66
PIK3CD	73
PIK3CG	89
PIK4CB	79
PIM1	92
PIM2	94
PIM3	81
PIP5K1A	64
PIP5K1C	86
PIP5K2B	99
PIP5K2C	100
PKAC-alpha	85
PKAC-beta	100
PKMYT1	85
PKN1	94
PKN2	100
PKNB(M.tuberculosis)	72
PLK1	92
PLK2	78
PLK3	81
PLK4	57
PRKCD	86
PRKCE	98

DiscoverX gene symbol	% control
EGFR(L747-T751del,Sins)	90
EGFR(L858R)	84
EGFR(L858R,T790M)	86
EGFR(L861Q)	79
EGFR(S752-I759del)	72
EGFR(T790M)	53
EIF2AK1	95
EPHA1	85
EPHA2	80
EPHA3	86
EPHA4	100
EPHA5	88
EPHA6	100
EPHA7	92
EPHA8	90
EPHB1	94
EPHB2	62
EPHB3	100
EPHB4	99
EPHB6	84
ERBB2	95
ERBB3	100
ERBB4	92
ERK1	80
ERK2	75
ERK3	69
ERK4	96
ERK5	96
ERK8	81
ERN1	68
FAK	94
FER	100
FES	100
FGFR1	80
FGFR2	74
FGFR3	69
FGFR3(G697C)	86
FGFR4	90
FGR	95
FLT1	83
FLT3	68
FLT3(D835H)	39
FLT3(D835Y)	39

DiscoverX gene symbol	% control
PRKCH	96
PRKCI	98
PRKCQ	100
PRKD1	87
PRKD2	100
PRKD3	84
PRKG1	100
PRKG2	83
PRKR	82
PRKX	90
PRP4	92
PYK2	93
QSK	86
RAF1	93
RET	88
RET(M918T)	97
RET(V804L)	98
RET(V804M)	89
RIOK1	48
RIOK2	54
RIOK3	65
RIPK1	2.2
RIPK2	77
RIPK4	77
RIPK5	77
ROCK1	91
ROCK2	83
ROS1	100
RPS6KA4(Kin.Dom.1-N-terminal)	100
RPS6KA4(Kin.Dom.2-C-terminal)	80
RPS6KA5(Kin.Dom.1-N-terminal)	89
RPS6KA5(Kin.Dom.2-C-terminal)	89
RSK1(Kin.Dom.1-N-terminal)	66
RSK1(Kin.Dom.2-C-terminal)	92
RSK2(Kin.Dom.1-N-terminal)	87
RSK2(Kin.Dom.2-C-terminal)	72
RSK3(Kin.Dom.1-N-terminal)	100
RSK3(Kin.Dom.2-C-terminal)	99
RSK4(Kin.Dom.1-N-terminal)	82
RSK4(Kin.Dom.2-C-terminal)	92
S6K1	64
SBK1	61
SGK	**16**

DiscoverX gene symbol	% control
FLT3(ITD)	62
FLT3(K663Q)	75
FLT3(N841I)	42
FLT3(R834Q)	87
FLT3-autoinhibited	92
FLT4	90
FRK	79
FYN	99
GAK	89
GCN2(Kin.Dom.2,S808G)	100
GRK1	92
GRK4	100
GRK7	89
GSK3A	85
GSK3B	70
HASPIN	90
HCK	100
HIPK1	80
HIPK2	75
HIPK3	76
HIPK4	93
HPK1	100
HUNK	92
ICK	76
IGF1R	76
IKK-alpha	61
IKK-beta	58
IKK-epsilon	**40**
INSR	59
INSRR	100
IRAK1	27
IRAK3	58
IRAK4	48
ITK	89
JAK1(JH1domain-catalytic)	88
JAK1(JH2domain-pseudokinase)	100
JAK2(JH1domain-catalytic)	65
JAK3(JH1domain-catalytic)	89
JNK1	73
JNK2	65
JNK3	58
KIT	90
KIT(A829P)	90

DiscoverX gene symbol	% control
SgK110	100
SGK2	40
SGK3	74
SIK	93
SIK2	95
SLK	76
SNARK	71
SNRK	89
SRC	84
SRMS	73
SRPK1	100
SRPK2	100
SRPK3	100
STK16	**18**
STK33	79
STK35	96
STK36	97
STK39	92
SYK	99
TAK1	56
TAOK1	90
TAOK2	85
TAOK3	93
TBK1	**31**
TEC	94
TESK1	90
TGFBR1	95
TGFBR2	89
TIE1	64
TIE2	100
TLK1	79
TLK2	87
TNIK	90
TNK1	90
TNK2	90
TNNI3K	95
TRKA	79
TRKB	80
TRKC	98
TRPM6	62
TSSK1B	73
TTK	92
TXK	85

DiscoverX gene symbol	% control
KIT(D816H)	94
KIT(D816V)	58
KIT(L576P)	74
KIT(V559D)	85
KIT(V559D,T670I)	87
KIT(V559D,V654A)	100
KIT-autoinhibited	79
LATS1	100
LATS2	94
LCK	100
LIMK1	89
LIMK2	90
LKB1	93
LOK	89
LRRK2	73
LRRK2(G2019S)	90
LTK	100
LYN	74
LZK	100
MAK	100

DiscoverX gene symbol	% control
TYK2(JH1domain-catalytic)	75
TYK2(JH2domain-pseudokinase)	100
TYRO3	100
ULK1	56
ULK2	61
ULK3	58
VEGFR2	80
VRK2	86
WEE1	94
WEE2	96
WNK1	83
WNK3	100
YANK1	84
YANK2	100
YANK3	82
YES	87
YSK1	91
YSK4	**0.1**
ZAK	83
ZAP70	86

Table S2: DiscoverX KINOME*scan* @ 100 nM for compound 24

DiscoverX gene symbol	% control
AAK1	85
ABL1(E255K)-phosphorylated	63
ABL1(F317I)-nonphosphorylated	98
ABL1(F317I)-phosphorylated	86
ABL1(F317L)-nonphosphorylated	86
ABL1(F317L)-phosphorylated	84
ABL1(H396P)-nonphosphorylated	73
ABL1(H396P)-phosphorylated	75
ABL1(M351T)-phosphorylated	82
ABL1(Q252H)-nonphosphorylated	100
ABL1(Q252H)-phosphorylated	77
ABL1(T315I)-nonphosphorylated	97
ABL1(T315I)-phosphorylated	88
ABL1(Y253F)-phosphorylated	84
ABL1-nonphosphorylated	97
ABL1-phosphorylated	68
ABL2	92
ACVR1	100
ACVR1B	88
ACVR2A	100
ACVR2B	100
ACVRL1	71
ADCK3	87
ADCK4	96
AKT1	100
AKT2	67
AKT3	100
ALK	90
ALK(C1156Y)	85
ALK(L1196M)	89
AMPK-alpha1	97
AMPK-alpha2	100
ANKK1	41
ARK5	99
ASK1	100
ASK2	91
AURKA	90
AURKB	57
AURKC	82
AXL	86
BIKE	78

DiscoverX gene symbol	% control
MAK	79
MAP3K1	97
MAP3K15	98
MAP3K2	99
MAP3K3	83
MAP3K4	87
MAP4K2	62
MAP4K3	84
MAP4K4	100
MAP4K5	100
MAPKAPK2	100
MAPKAPK5	89
MARK1	97
MARK2	91
MARK3	87
MARK4	95
MAST1	72
MEK1	81
MEK2	82
MEK3	81
MEK4	100
MEK5	37
MEK6	96
MELK	100
MERTK	87
MET	89
MET(M1250T)	84
MET(Y1235D)	100
MINK	70
MKK7	93
MKNK1	93
MKNK2	44
MLCK	99
MLK1	95
MLK2	71
MLK3	100
MRCKA	96
MRCKB	86
MST1	100
MST1R	**35**
MST2	100

DiscoverX gene symbol	% control
BLK	100
BMPR1A	81
BMPR1B	89
BMPR2	80
BMX	87
BRAF	88
BRAF(V600E)	91
BRK	89
BRSK1	77
BRSK2	100
BTK	95
BUB1	69
CAMK1	92
CAMK1B	100
CAMK1D	90
CAMK1G	99
CAMK2A	100
CAMK2B	87
CAMK2D	97
CAMK2G	93
CAMK4	74
CAMKK1	92
CAMKK2	91
CASK	85
CDC2L1	97
CDC2L2	88
CDC2L5	100
CDK11	80
CDK2	92
CDK3	99
CDK4	82
CDK4-cyclinD1	94
CDK4-cyclinD3	73
CDK5	98
CDK7	61
CDK8	100
CDK9	96
CDKL1	93
CDKL2	97
CDKL3	84
CDKL5	90
CHEK1	74
CHEK2	100

DiscoverX gene symbol	% control
MST3	87
MST4	84
MTOR	98
MUSK	91
MYLK	88
MYLK2	90
MYLK4	100
MYO3A	89
MYO3B	100
NDR1	80
NDR2	93
NEK1	91
NEK10	100
NEK11	95
NEK2	97
NEK3	87
NEK4	98
NEK5	100
NEK6	97
NEK7	100
NEK9	100
NIK	85
NIM1	82
NLK	83
OSR1	92
p38-alpha	96
p38-beta	90
p38-delta	63
p38-gamma	98
PAK1	94
PAK2	90
PAK3	100
PAK4	92
PAK6	88
PAK7	80
PCTK1	85
PCTK2	100
PCTK3	84
PDGFRA	69
PDGFRB	90
PDPK1	100
PFCDPK1(P.falciparum)	97
PFPK5(P.falciparum)	97

DiscoverX gene symbol	% control
CIT	**7.7**
CLK1	**30**
CLK2	**19**
CLK3	91
CLK4	42
CSF1R	63
CSF1R-autoinhibited	100
CSK	92
CSNK1A1	95
CSNK1A1L	97
CSNK1D	91
CSNK1E	98
CSNK1G1	92
CSNK1G2	100
CSNK1G3	99
CSNK2A1	86
CSNK2A2	100
CTK	89
DAPK1	91
DAPK2	92
DAPK3	95
DCAMKL1	71
DCAMKL2	77
DCAMKL3	75
DDR1	100
DDR2	100
DLK	100
DMPK	100
DMPK2	95
DRAK1	**0.7**
DRAK2	**2.8**
DYRK1A	76
DYRK1B	86
DYRK2	71
EGFR	100
EGFR(E746-A750del)	91
EGFR(G719C)	98
EGFR(G719S)	94
EGFR(L747-E749del, A750P)	96
EGFR(L747-S752del, P753S)	80
EGFR(L747-T751del,Sins)	97
EGFR(L858R)	98
EGFR(L858R,T790M)	91

DiscoverX gene symbol	% control
PFTAIRE2	100
PFTK1	90
PHKG1	100
PHKG2	93
PIK3C2B	89
PIK3C2G	76
PIK3CA	85
PIK3CA(C420R)	89
PIK3CA(E542K)	89
PIK3CA(E545A)	82
PIK3CA(E545K)	88
PIK3CA(H1047L)	95
PIK3CA(H1047Y)	74
PIK3CA(I800L)	79
PIK3CA(M1043I)	76
PIK3CA(Q546K)	95
PIK3CB	100
PIK3CD	92
PIK3CG	83
PIK4CB	100
PIKFYVE	100
PIM1	90
PIM2	100
PIM3	96
PIP5K1A	89
PIP5K1C	51
PIP5K2B	100
PIP5K2C	91
PKAC-alpha	100
PKAC-beta	100
PKMYT1	81
PKN1	89
PKN2	100
PKNB(M.tuberculosis)	93
PLK1	96
PLK2	91
PLK3	85
PLK4	81
PRKCD	85
PRKCE	100
PRKCH	100
PRKCI	92
PRKCQ	42

DiscoverX gene symbol	% control
EGFR(L861Q)	95
EGFR(S752-I759del)	93
EGFR(T790M)	86
EIF2AK1	92
EPHA1	95
EPHA2	91
EPHA3	88
EPHA4	92
EPHA5	97
EPHA6	85
EPHA7	94
EPHA8	91
EPHB1	93
EPHB2	96
EPHB3	88
EPHB4	85
EPHB6	64
ERBB2	100
ERBB3	98
ERBB4	86
ERK1	96
ERK2	94
ERK3	95
ERK4	100
ERK5	97
ERK8	79
ERN1	84
FAK	95
FER	100
FES	92
FGFR1	84
FGFR2	89
FGFR3	93
FGFR3(G697C)	92
FGFR4	95
FGR	85
FLT1	90
FLT3	**34**
FLT3(D835H)	23
FLT3(D835V)	20
FLT3(D835Y)	26
FLT3(ITD)	54
FLT3(ITD,D835V)	31

DiscoverX gene symbol	% control
PRKD1	70
PRKD2	100
PRKD3	77
PRKG1	100
PRKG2	86
PRKR	100
PRKX	100
PRP4	97
PYK2	100
QSK	82
RAF1	87
RET	98
RET(M918T)	86
RET(V804L)	84
RET(V804M)	89
RIOK1	100
RIOK2	93
RIOK3	99
RIPK1	53
RIPK2	90
RIPK4	94
RIPK5	84
ROCK1	87
ROCK2	81
ROS1	100
RPS6KA4(Kin.Dom.1-N-terminal)	96
RPS6KA4(Kin.Dom.2-C-terminal)	79
RPS6KA5(Kin.Dom.1-N-terminal)	100
RPS6KA5(Kin.Dom.2-C-terminal)	98
RSK1(Kin.Dom.1-N-terminal)	63
RSK1(Kin.Dom.2-C-terminal)	95
RSK2(Kin.Dom.1-N-terminal)	92
RSK2(Kin.Dom.2-C-terminal)	73
RSK3(Kin.Dom.1-N-terminal)	51
RSK3(Kin.Dom.2-C-terminal)	93
RSK4(Kin.Dom.1-N-terminal)	97
RSK4(Kin.Dom.2-C-terminal)	94
S6K1	89
SBK1	64
SGK	88
SgK110	100
SGK2	88
SGK3	84

DiscoverX gene symbol	% control
FLT3(ITD,F691L)	21
FLT3(K663Q)	42
FLT3(N841I)	21
FLT3(R834Q)	99
FLT3-autoinhibited	88
FLT4	95
FRK	91
FYN	100
GAK	94
GCN2(Kin.Dom.2,S808G)	78
GRK1	95
GRK2	100
GRK3	100
GRK4	86
GRK7	87
GSK3A	100
GSK3B	90
HASPIN	82
HCK	100
HIPK1	77
HIPK2	100
HIPK3	84
HIPK4	94
HPK1	97
HUNK	100
ICK	97
IGF1R	91
IKK-alpha	99
IKK-beta	89
IKK-epsilon	**6.6**
INSR	100
INSRR	87
IRAK1	56
IRAK3	67
IRAK4	71
ITK	76
JAK1(JH1domain-catalytic)	100
JAK1(JH2domain-pseudokinase)	70
JAK2(JH1domain-catalytic)	80
JAK3(JH1domain-catalytic)	87
JNK1	79
JNK2	79
JNK3	88

DiscoverX gene symbol	% control
SIK	94
SIK2	51
SLK	96
SNARK	87
SNRK	100
SRC	100
SRMS	67
SRPK1	100
SRPK2	96
SRPK3	89
STK16	78
STK33	100
STK35	83
STK36	93
STK39	100
SYK	93
TAK1	78
TAOK1	76
TAOK2	79
TAOK3	76
TBK1	**3.3**
TEC	89
TESK1	93
TGFBR1	84
TGFBR2	74
TIE1	48
TIE2	83
TLK1	89
TLK2	92
TNIK	86
TNK1	100
TNK2	100
TNNI3K	92
TRKA	57
TRKB	86
TRKC	91
TRPM6	96
TSSK1B	61
TSSK3	88
TTK	83
TXK	95
TYK2(JH1domain-catalytic)	85
TYK2(JH2domain-pseudokinase)	94

DiscoverX gene symbol	% control
KIT	70
KIT(A829P)	94
KIT(D816H)	100
KIT(D816V)	80
KIT(L576P)	95
KIT(V559D)	78
KIT(V559D,T670I)	54
KIT(V559D,V654A)	90
KIT-autoinhibited	85
LATS1	100
LATS2	75
LCK	96
LIMK1	100
LIMK2	96
LKB1	78
LOK	91
LRRK2	59
LRRK2(G2019S)	61
LTK	100
LYN	89
LZK	100

DiscoverX gene symbol	% control
TYRO3	100
ULK1	**4**
ULK2	39
ULK3	97
VEGFR2	87
VPS34	100
VRK2	95
WEE1	100
WEE2	99
WNK1	68
WNK2	100
WNK3	83
WNK4	100
YANK1	91
YANK2	100
YANK3	87
YES	100
YSK1	100
YSK4	**4.7**
ZAK	89
ZAP70	48

Table S3: DiscoverX KINOME*scan* @ 100 nM for compound 34 (BAY-985)

DiscoverX gene symbol	% control
AAK1	86
ABL1(E255K)-phosphorylated	79
ABL1(F317I)-nonphosphorylated	75
ABL1(F317I)-phosphorylated	66
ABL1(F317L)-nonphosphorylated	95
ABL1(F317L)-phosphorylated	100
ABL1(H396P)-nonphosphorylated	55
ABL1(H396P)-phosphorylated	77
ABL1(M351T)-phosphorylated	100
ABL1(Q252H)-nonphosphorylated	56
ABL1(Q252H)-phosphorylated	93
ABL1(T315I)-nonphosphorylated	100
ABL1(T315I)-phosphorylated	100
ABL1(Y253F)-phosphorylated	86
ABL1-nonphosphorylated	58
ABL1-phosphorylated	71
ABL2	94
ACVR1	90
ACVR1B	96
ACVR2A	100
ACVR2B	100
ACVRL1	100
ADCK3	100
ADCK4	100
AKT1	100
AKT2	100
AKT3	100
ALK	100
ALK(C1156Y)	100
ALK(L1196M)	100
AMPK-alpha1	100
AMPK-alpha2	100
ANKK1	100
ARK5	100
ASK1	100
ASK2	100
AURKA	100
AURKB	71
AURKC	88

DiscoverX gene symbol	% control
MAK	81
MAP3K1	100
MAP3K15	100
MAP3K2	81
MAP3K3	83
MAP3K4	87
MAP4K2	100
MAP4K3	100
MAP4K4	100
MAP4K5	100
MAPKAPK2	97
MAPKAPK5	100
MARK1	100
MARK2	100
MARK3	100
MARK4	100
MAST1	57
MEK1	98
MEK2	97
MEK3	96
MEK4	100
MEK5	**14**
MEK6	99
MELK	100
MERTK	99
MET	100
MET(M1250T)	79
MET(Y1235D)	100
MINK	83
MKK7	95
MKNK1	100
MKNK2	74
MLCK	99
MLK1	100
MLK2	100
MLK3	100
MRCKA	100
MRCKB	100
MST1	100

DiscoverX gene symbol	% control
AXL	91
BIKE	87
BLK	100
BMPR1A	99
BMPR1B	96
BMPR2	87
BMX	100
BRAF	100
BRAF(V600E)	100
BRK	100
BRSK1	100
BRSK2	93
BTK	100
BUB1	100
CAMK1	95
CAMK1B	97
CAMK1D	95
CAMK1G	99
CAMK2A	100
CAMK2B	100
CAMK2D	100
CAMK2G	100
CAMK4	100
CAMKK1	100
CAMKK2	100
CASK	85
CDC2L1	97
CDC2L2	91
CDC2L5	98
CDK11	96
CDK2	100
CDK3	100
CDK4	100
CDK4-cyclinD1	100
CDK4-cyclinD3	95
CDK5	98
CDK7	82
CDK8	100
CDK9	91
CDKL1	82
CDKL2	100
CDKL3	93
CDKL5	100

DiscoverX gene symbol	% control
MST1R	87
MST2	78
MST3	100
MST4	100
MTOR	100
MUSK	99
MYLK	93
MYLK2	99
MYLK4	90
MYO3A	100
MYO3B	87
NDR1	70
NDR2	100
NEK1	90
NEK10	57
NEK11	100
NEK2	100
NEK3	75
NEK4	100
NEK5	97
NEK6	93
NEK7	80
NEK9	95
NIK	96
NIM1	87
NLK	100
OSR1	96
p38-alpha	100
p38-beta	100
p38-delta	99
p38-gamma	83
PAK1	100
PAK2	100
PAK3	100
PAK4	100
PAK6	94
PAK7	88
PCTK1	100
PCTK2	94
PCTK3	100
PDGFRA	100
PDGFRB	71
PDPK1	100

DiscoverX gene symbol	% control
CHEK1	100
CHEK2	100
CIT	78
CLK1	98
CLK2	100
CLK3	95
CLK4	100
CSF1R	77
CSF1R-autoinhibited	77
CSK	100
CSNK1A1	87
CSNK1A1L	93
CSNK1D	98
CSNK1E	100
CSNK1G1	100
CSNK1G2	100
CSNK1G3	100
CSNK2A1	82
CSNK2A2	87
CTK	100
DAPK1	91
DAPK2	100
DAPK3	100
DCAMKL1	55
DCAMKL2	84
DCAMKL3	97
DDR1	100
DDR2	91
DLK	100
DMPK	100
DMPK2	95
DRAK1	47
DRAK2	72
DYRK1A	100
DYRK1B	96
DYRK2	100
EGFR	91
EGFR(E746-A750del)	100
EGFR(G719C)	100
EGFR(G719S)	83
EGFR(L747-E749del, A750P)	84
EGFR(L747-S752del, P753S)	93
EGFR(L747-T751del,Sins)	100

DiscoverX gene symbol	% control
PFCDPK1(P.falciparum)	85
PFPK5(P.falciparum)	100
PFTAIRE2	100
PFTK1	100
PHKG1	100
PHKG2	100
PIK3C2B	99
PIK3C2G	91
PIK3CA	94
PIK3CA(C420R)	100
PIK3CA(E542K)	69
PIK3CA(E545A)	100
PIK3CA(E545K)	50
PIK3CA(H1047L)	86
PIK3CA(H1047Y)	100
PIK3CA(I800L)	81
PIK3CA(M1043I)	100
PIK3CA(Q546K)	78
PIK3CB	81
PIK3CD	90
PIK3CG	95
PIK4CB	100
PIKFYVE	93
PIM1	100
PIM2	100
PIM3	100
PIP5K1A	100
PIP5K1C	100
PIP5K2B	94
PIP5K2C	100
PKAC-alpha	100
PKAC-beta	100
PKMYT1	100
PKN1	100
PKN2	100
PKNB(M.tuberculosis)	55
PLK1	100
PLK2	75
PLK3	64
PLK4	88
PRKCD	100
PRKCE	91
PRKCH	100

DiscoverX gene symbol	% control
EGFR(L858R)	94
EGFR(L858R,T790M)	100
EGFR(L861Q)	100
EGFR(S752-I759del)	90
EGFR(T790M)	56
EIF2AK1	100
EPHA1	81
EPHA2	85
EPHA3	97
EPHA4	100
EPHA5	93
EPHA6	91
EPHA7	92
EPHA8	100
EPHB1	100
EPHB2	100
EPHB3	100
EPHB4	100
EPHB6	91
ERBB2	84
ERBB3	71
ERBB4	100
ERK1	100
ERK2	94
ERK3	93
ERK4	87
ERK5	93
ERK8	100
ERN1	100
FAK	100
FER	100
FES	100
FGFR1	100
FGFR2	99
FGFR3	97
FGFR3(G697C)	69
FGFR4	100
FGR	100
FLT1	69
FLT3	55
FLT3(D835H)	65
FLT3(D835V)	14
FLT3(D835Y)	50

DiscoverX gene symbol	% control
PRKCI	93
PRKCQ	100
PRKD1	77
PRKD2	100
PRKD3	100
PRKG1	100
PRKG2	86
PRKR	100
PRKX	91
PRP4	100
PYK2	91
QSK	97
RAF1	99
RET	91
RET(M918T)	100
RET(V804L)	78
RET(V804M)	87
RIOK1	100
RIOK2	100
RIOK3	82
RIPK1	81
RIPK2	64
RIPK4	79
RIPK5	64
ROCK1	100
ROCK2	84
ROS1	100
RPS6KA4(Kin.Dom.1-N-terminal)	100
RPS6KA4(Kin.Dom.2-C-terminal)	100
RPS6KA5(Kin.Dom.1-N-terminal)	100
RPS6KA5(Kin.Dom.2-C-terminal)	100
RSK1(Kin.Dom.1-N-terminal)	91
RSK1(Kin.Dom.2-C-terminal)	95
RSK2(Kin.Dom.1-N-terminal)	86
RSK2(Kin.Dom.2-C-terminal)	88
RSK3(Kin.Dom.1-N-terminal)	100
RSK3(Kin.Dom.2-C-terminal)	100
RSK4(Kin.Dom.1-N-terminal)	92
RSK4(Kin.Dom.2-C-terminal)	99
S6K1	92
SBK1	84
SGK	94
SgK110	100

DiscoverX gene symbol	% control
FLT3(ITD)	78
FLT3(ITD,D835V)	35
FLT3(ITD,F691L)	79
FLT3(K663Q)	50
FLT3(N841I)	83
FLT3(R834Q)	100
FLT3-autoinhibited	79
FLT4	100
FRK	100
FYN	100
GAK	100
GCN2(Kin.Dom.2,S808G)	100
GRK1	88
GRK2	88
GRK3	89
GRK4	100
GRK7	100
GSK3A	100
GSK3B	68
HASPIN	83
HCK	100
HIPK1	69
HIPK2	79
HIPK3	100
HIPK4	100
HPK1	100
HUNK	100
ICK	100
IGF1R	100
IKK-alpha	87
IKK-beta	88
IKK-epsilon	**14**
INSR	57
INSRR	91
IRAK1	98
IRAK3	77
IRAK4	100
ITK	100
JAK1(JH1domain-catalytic)	100
JAK1(JH2domain-pseudokinase)	100
JAK2(JH1domain-catalytic)	86
JAK3(JH1domain-catalytic)	86
JNK1	57

DiscoverX gene symbol	% control
SGK2	95
SGK3	76
SIK	100
SIK2	100
SLK	100
SNARK	100
SNRK	100
SRC	100
SRMS	82
SRPK1	71
SRPK2	100
SRPK3	85
STK16	86
STK33	100
STK35	100
STK36	100
STK39	86
SYK	91
TAK1	88
TAOK1	100
TAOK2	100
TAOK3	100
TBK1	**1.5**
TEC	100
TESK1	88
TGFBR1	100
TGFBR2	100
TIE1	83
TIE2	99
TLK1	88
TLK2	100
TNIK	81
TNK1	82
TNK2	100
TNNI3K	100
TRKA	45
TRKB	92
TRKC	100
TRPM6	100
TSSK1B	100
TSSK3	100
TTK	100
TXK	81

DiscoverX gene symbol	% control
JNK2	64
JNK3	81
KIT	74
KIT(A829P)	47
KIT(D816H)	100
KIT(D816V)	95
KIT(L576P)	76
KIT(V559D)	71
KIT(V559D,T670I)	81
KIT(V559D,V654A)	90
KIT-autoinhibited	95
LATS1	100
LATS2	98
LCK	100
LIMK1	100
LIMK2	100
LKB1	73
LOK	100
LRRK2	86
LRRK2(G2019S)	100
LTK	100
LYN	100
LZK	100

DiscoverX gene symbol	% control
TYK2(JH1domain-catalytic)	100
TYK2(JH2domain-pseudokinase)	100
TYRO3	100
ULK1	79
ULK2	90
ULK3	57
VEGFR2	93
VPS34	100
VRK2	100
WEE1	100
WEE2	87
WNK1	100
WNK2	100
WNK3	100
WNK4	100
YANK1	100
YANK2	100
YANK3	100
YES	95
YSK1	100
YSK4	**14**
ZAK	73
ZAP70	100

Pharmacokinetic studies

Caco2 Permeability Assay.

Cell culture: Caco-2 cells (purchased from DSMZ Braunschweig, Germany) were seeded at a density of 2.5 x 10^5 cells per well on 24-well insert plates, 0.4 µm pore size, 0.3 cm^2 (Costar) and grown for 13-15 days in DMEM medium supplemented with 10 % fetal calf serum (FCS), 1 % GlutaMAX (100x, GIBCO), 100 U/mL penicillin, 100 µg/mL streptomycin (GIBCO) and 1 % non-essential amino acids (100 x). Cells were maintained at 37 °C in a humidified 5 % CO^2 atmosphere. Medium was changed every 2-3 days. Evaluation of Caco-2 permeability in a bidirectional transport assay: The bidirectional transport assay was done in 24-well insert plates using a robotic system (Tecan). Before running the bidirectional transport assay, culture medium was replaced by transport medium (FCS-free HEPES-carbonate transport puffer, pH 7.2). For assessment of monolayer integrity the transepithelial electrical resistance (TEER) was measured. Only monolayers with a TEER of at least 400 Ω*cm^2 were used. Test compounds were pre-dissolved in DMSO and added either to the apical or basolateral compartment in final concentration of 2 µM. Evaluation was done in triplicates. Before and after 2 h of incubation at 37 °C samples were taken from both compartments and analyzed after precipitation with methanol by LC/MS-MS. The apparent permeability coefficient (Papp) was calculated both for the apical to basolateral (A→B) and the basolateral to apical (B→A) direction using following equation: Papp = (Vr/P0)(1/S)(P2/t) Where Vr is the volume of medium in the receiver chamber, P0 is the measured peak area of the test drug in the donor chamber at t = 0, S the surface area of the monolayer, P2 is the measured peak area of the test drug in the acceptor chamber after 2 h of incubation, and t is the incubation time. The efflux ratio basolateral (B) to apical (A) was calculated by dividing Papp(B-A) by Papp(A-B).

In vitro metabolic stability in liver microsomes.

The in vitro metabolic stability of test compounds was determined by incubating them at 1 µM in a suspension of liver microsomes in 100 mM phosphate buffer, pH 7.4 (NaH2PO4 x H2O + Na2HPO4 x 2H2O) and at a protein concentration of 0.5 mg/mL at 37 °C. The microsomes were activated by adding a co-factor mix containing 8 mM Glucose-6-Phosphat, 4 mM MgCl2, 0.5 mM NADP and 1 IU/ml G-6-P-Dehydrogenase in phosphate buffer, pH 7.4. The metabolic assay was started shortly afterwards by adding the test compound to the incubation at a final volume of 1 mL. Organic solvent in the incubations was limited to ≤0.01 % dimethylsulfoxide (DMSO) and ≤1% acetonitrile. During incubation, the microsomal suspensions were continuously shaken at 580 rpm and aliquots were taken at 2, 8, 16, 30, 45 and 60 min, to which equal volumes of cold methanol were immediately added. Samples were frozen at -20 °C overnight, subsequently centrifuged for 15 minutes at 3000 rpm and the supernatant was analyzed with an Agilent 1200 HPLC-system with LC/MS-MS detection. The half-life of a test compound was determined from the concentration-time plot. From the half-life the intrinsic clearances and the hepatic in vivo blood clearance (CL) and maximal oral bioavailability (Fmax) were calculated using the 'well stirred' liver model together with the additional parameters liver blood flow, specific liver weight and microsomal protein content. The following parameter values were used: Liver blood flow – 5.4 L/h/kg mouse; 4.2 L/h/kg rat; 2.1 L/h/kg dog and 1.32 L/h/kg human. Specific liver weight – are 43, 32, 39 and 21 g/kg body weight for mouse, rat, dog and human, respectively. Microsomal protein content 40 mg/g (for all species)

In vitro metabolic stability in hepatocytes.

Hepatocytes from Han/Wistar rats were isolated via a 2-step perfusion method. After perfusion, the liver was carefully removed from the rat: the liver capsule was opened and the hepatocytes were gently shaken out into a Petri dish with ice-cold Williams' medium E (WME). The resulting cell suspension was filtered through sterile gaze in 50 ml falcon tubes and centrifuged at 50 × g for 3 min at room temperature. The cell pellet was resuspended in 30 ml WME and centrifuged twice through a Percoll® gradient at 100 × g. The hepatocytes were washed again with WME and resuspended in medium containing 5 % FCS. Cell viability was determined by trypan blue exclusion. For the metabolic stability assay liver cells were distributed in WME containing 5 % FCS to glass vials at a density of 1.0×10^6 vital cells/ml. The test compound was added to a final concentration of 1 µM. During incubation, the hepatocyte suspensions were continuously shaken at 580 rpm and aliquots were taken at 2, 8, 16, 30, 45 and 90 min, to which equal volumes of cold methanol were immediately added. Samples were frozen at -20 °C overnight, subsequently centrifuged for 15 minutes at 3000 rpm and the supernatant was analyzed with an Agilent 1200 HPLC-system with LC/MS-MS detection. The half-life of a test compound was determined from the concentration-time plot. From the half-life the intrinsic clearances and the hepatic in vivo blood clearance (CL) and maximal oral bioavailability (Fmax) were calculated using the 'well stirred' liver model together with the additional parameters liver blood flow, specific liver weight and amount of liver cells in vivo and in vitro. Same parameters for liver blood flow and specific liver weight as described above were used; Liver cells in vivo 1.1×10^8 cells/g liver, liver cells in vitro 1.0 x 106/ml.

In vivo pharmacokinetics in rats.

All animal experiments were conducted in accordance with the German Animal Welfare Law and approved by local authorities. For in vivo pharmacokinetic (PK) experiments test compounds were administered to male Wistar rats intravenously at doses of 0.3 to 0.5 mg/kg and intragastral at doses of 0.6 to 1 mg/kg formulated as solutions using solubilizers such as PEG400 in well-tolerated amounts. For PK after intravenous (i.v.) administration test compounds were given as i.v. bolus and blood samples were taken at 2 min, 8 min, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h after dosing. For pharmacokinetics after intragastral (i.g.) administration test compounds were given i.g. to fasted rats and blood samples were taken at 5 min, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h after dosing. Blood was collected into Lithium-Heparintubes (Monovetten® , Sarstedt) and centrifuged for 15 min at 3000 rpm. An aliquot of 100 µL from the supernatant (plasma) was taken and precipitated by addition of 400 µL cold acetonitril and frozen at -20 °C over night. Samples were subsequently thawed and centrifuged at 3000 rpm, 4°C for 20 minutes. Aliquots of the supernatants were taken for analytical testing using an Agilent 1200 HPLC-system with LCMS/MS detection. PK parameters were based on the plasma concentration time data and calculated (e.g., using the linear-log trapezoidal rule for AUC estimation) with an excel based program. PK parameters derived from concentration-time profiles after i.v.: CLplasma: Total plasma clearance of test compound (in L/kg/h); CLblood: Total blood clearance of test compound: CLplasma*Cp/Cb (in L/kg/h) with Cp/Cb being the ratio of concentrations in plasma and blood. PK parameters calculated from concentration time profiles after i.g.: Cmax: Maximal plasma concentration (in mg/L); Cmaxnorm: Cmax divided by the administered dose (in kg/L); Tmax: Time point at which Cmax was observed (in h). Parameters calculated from both, i.v. and i.g. concentration-time profiles: AUCnorm: Area under the concentration-time curve from t=0h to infinity (extrapolated) divided by the administered dose (in kg*h/L); AUC(0-tlast)norm: Area

under the concentration-time curve from t=0h to the last time point for which plasma concentrations could be measured divided by the administered dose (in kg*h/L); Tt1/2: apparent half-life (in h); F: oral bioavailability: AUCnorm after intragastral administration divided by AUCnorm after intravenous administration (in %).

In vivo pharmacology

Animals

All animal experiments were conducted in accordance with the German Animal Welfare Act Law and approved by local authorities. Experiments were initiated after an acclimatization period of at least 7 days. Mice were kept in a 12 hours light/dark cycle, food and water were available ad libitum, and the housing temperature was 23 °C.

In vivo study in the cell line derived SK-MEL-2 xenograft model

The in vivo antitumor efficacy and tolerability of 34 at 200 mg/kg applied orally (po=per os) twice per day (bid) continuously as monotherapy was evaluated in the cell line derived human SK-MEL-2 melanoma xenograft model in female NMRI nude mice (5–6 weeks, 20–22 g, Taconic). Cancer cells from mid-log phase (70%) cultures were harvested and inoculated subcutaneously by injection of 100 µL cell suspension into the flank of mice. When tumors reached a predetermined size of 36 mm2, mice were randomized into treatment and control groups (n = 10 mice/group), and treatment was started. 34 was formulated in PEG 400/EtOH/water (60:10:30). The oral application volume was 10 mL/kg and the time interval between the two applications per day was 6–7 hours.

Tumor response was assessed by measuring tumor area (length × width) using a caliper. Animal body weight was monitored as a measure of treatment-related toxicity. Tumor area and body weight were determined three times per week. Changes in body weight throughout the study compared to initial body weight at treatment start were considered a measure of treatment-related toxicity (>10% = critical, treatment on hold until recovery; >20% = toxic, termination). Study was terminated on day 111 after tumor cell inoculation (= 35 treatment days). Tumor growth inhibition is presented as the T/C ratio (treatment/control), calculated with tumor areas or tumor weights at study end. Relative tumor growth inhibition based on tumor area (T/Crel.area) was calculated using the formula [(tumor area of treatment group at day of termination) – (tumor area of treatment group at day before first treatment)]/[(tumor area of vehicle group at dayof termination) – (tumor area of vehicle group at day before first treatment)]. Tumor growth inhibition based on tumor weight (T/C tumor weight) was calculated using the formula (tumor weight of treatment group at day x)/(tumor weight of vehicle group at day of termination). Compounds having a T/C below 0.5 were defined as effective (T/C <0.3 = good activity, T/C 0.3-0.7 = moderate activity, T/C 0.7-0.9 = weak activity). Statistical analysis was assessed using SigmaStat software. A one-way analysis of variance was performed and differences to the control were compared by a pair-wise comparison procedure (Dunn’s method).

Synthesis of compounds 1–35 and BAY-440

General Information

All reagents and solvents were used as purchased, unless otherwise specified. The Purity of the compounds was determined by UPLC-MS or 1H-NMR. All final products were at least 95% pure, as determined by UPLC or ^{1}H NMR.

Materials

NMR

^{1}H NMR and ^{13}C spectra were recorded on Bruker Avance III HD spectrometers operating at 300, 400, or 500 MHz. The chemical shifts (δ) reported are given in parts per million (ppm), and the coupling constants (J) are in Hertz (Hz). The residual solvent peak was used as a reference (1H NMR DMSO: 2.50 ppm). The spin multiplicities are reported as s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad.

UPLC-MS Method 1

Instrument: Waters Acquity UPLC-MS Single Quad; column: Acquity UPLC BEH C18 1.7 µm, 50 x 2.1 mm; eluent A: water + 0.1 vol% formic acid (99%), eluent B: acetonitrile; gradient: 0–1.6 min 1–99% B, 1.6–2.0 min 99% B; flow: 0.8 mL/min; temperature: 60 °C; DAD scan: 210–400 nm.

UPLC-MS Method 2

Instrument: Waters Acquity UPLC-MS Single Quad; column: Acquity UPLC BEH C18 1.7 µm, 50 x 2.1 mm; eluent A: water + 0.2 vol% aq NH_3 (32%), eluent B: acetonitrile; gradient: 0–1.6 min 1–99% B, 1.6–2.0 min 99% B; flow: 0.8 mL/min; temperature: 60 °C; DAD scan: 210–400 nm.

UPLC-MS Method 3

Instrument: Waters AutoPurification LC-MS Single Quad; column: Waters XBridge C18 5 µm, 100 x 30 mm; eluent A: water + 0.2 vol% aq NH_3 (32%), eluent B: acetonitrile; gradient: 0–5.5 min 5–100% B; flow: 70 mL/min; temperature: 25 °C; DAD scan: 210–400 nm.

UPLC-MS Method 4

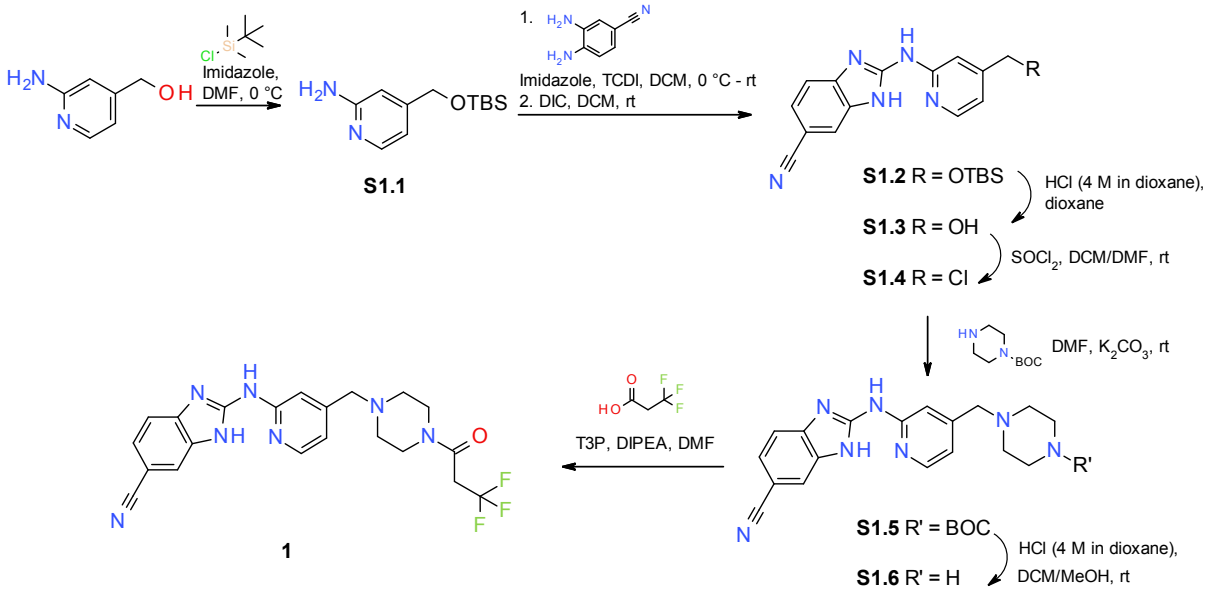
Instrument: Waters Acquity UPLCMS SingleQuad; Column: Acquity UPLC BEH C18 1.7 µm, 50x2.1mm; eluent A: water + 0.1 vol % formic acid (99%), eluent B: acetonitrile; gradient: 0-1.6 min 1-99% B, 1.6-2.0 min 99% B; flow 0.8 ml/min; temperature: 60 °C; DAD scan: 210-400 nm.

Abbreviations and acronyms

sat.: saturated; DIPEA: *N*,*N*-diisopropylethylamine; HATU: *N*-[(dimethylamino)(3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yl)methylene]-*N*-methylmethanaminium hexafluorophosphate 1-oxide; T3P: 2,4,6-tripropyl-1,3,5,2λ^5,4λ^5,6λ^5-trioxatriphosphinane-2,4,6-trione; TCDI: 1,1'-thiocarbonyldiimidazole; EDCI: *N*-[3-(dimethylamino)propyl]-*N*'-ethylcarbodiimide hydrochloride; DIC: *N*,*N*'-diisopropylcarbodiimide; PyBOP: (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate.

Experimental procedures

Scheme S1



4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)pyridin-2-amine (S1.1)

tert-Butyl(chloro)dimethylsilane (12.1 g, 97% purity, 78.1 mmol) and 1*H*-imidazole (5.32 g, 78.1 mmol) were dissolved in DMF (60 mL) and the mixture was cooled to 0 °C. (2-Aminopyridin-4-yl)methanol (10.0 g, 97% purity, 78.1 mmol) was added and the mixture was warmed to rt overnight, then concentrated under reduced pressure. The residue was diluted with water and EtOAc, and the organic phase was washed with water, dried over Na_2SO_4 and filtered. The filtrate was purified by flash chromatography to give **S1.1** (10 g, 54% yield).

LC-MS (Method 2): ^{t}R (min) = 1.30. MS (ESI+): *m*/*z* = 239[M + H]$^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.80 (d, *J* = 5.1 Hz, 1H), 6.33–6.42 (m, 2H), 5.85 (s, 2H), 4.56 (s, 2H), 0.87–0.96 (m, 9H), 0.05–0.12 (m, 6H).

2-{[4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)pyridin-2-yl]amino}-1*H*-benzimidazole-6-carbonitrile (S1.2)

Step 1:

1*H*-Imidazole (771 mg, 11.3 mmol) and TCDI (13.5 g, 90% purity, 68.0 mmol) were dissolved in DCM (200 mL), 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)pyridin-2-amine (**S1.1**, 13.5 g, 56.6 mmol) in DCM (210 mL) was added dropwise at 0 °C, and the mixture was stirred for 72 h at rt. Then, 3,4-diaminobenzonitrile (9.33 g, 97% purity, 68.0 mmol) was added and the mixture was stirred for 2 h at rt. The reaction mixture was diluted with water and the aqueous phase was extracted three times with DCM. The organic layer was dried over Na_2SO_4 and filtered, and the filtrate was used without further purification.

Step 2:

The filtrate and DIC (12 mL, 79 mmol) were stirred overnight at rt. The reaction mixture was quenched with sat. aq NH_4Cl solution and the mixture was stirred for 30 min at rt. The aqueous phase was extracted with EtOAc. The organic layer was filtered through a silica column, then concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc) to give **S1.2** (1.3 g, 6% yield).

LC-MS (Method 2): ^{t}R (min) = 1.51. MS (ESI+): *m*/*z* = 380 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.28–12.61 (m, 1H), 10.75–11.12 (m, 1H), 8.27 (br s, 1H), 7.71–7.92 (m, 1H), 7.29–7.50 (m, 2H), 7.26 (s, 1H), 6.91 (br d, *J* = 4.6 Hz, 1H), 4.75 (s, 2H), 0.92–1.02 (m, 9H), 0.08–0.18 (m, 6H).

2-{[4-(Hydroxymethyl)pyridin-2-yl]amino}-1*H*-benzimidazole-6-carbonitrile hydrochloride (S1.3)

2-{[4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)pyridin-2-yl]amino}-1*H*-benzimidazole-6-carbonitrile (**S1.2**, 6.80 g, 17.9 mmol) was dissolved in 1,4-dioxane (170 mL), then treated with HCl (90 mL, 4.0 M in 1,4-dioxane, 360 mmol), and the mixture was stirred for 10 h at rt. The suspension was diluted with Et_2O, and the solid was collected by filtration, washed with a mixture of 1,4-dioxane and Et_2O, and dried under reduced pressure at 60 °C to give **S1.3** (6 g), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 0.82. MS (ESI+): *m*/*z* = 266$[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.39 (d, *J* = 5.3 Hz, 1H), 8.00 (s, 1H), 7.63–7.85 (m, 2H), 7.42 (s, 1H), 7.17 (dd, *J* = 5.6, 1.0 Hz, 1H), 4.61 (s, 2H), 3.49–3.64 (m, 3H).

2-{[4-(Chloromethyl)pyridin-2-yl]amino}-1*H*-benzimidazole-6-carbonitrile (S1.4)

2-{[4-(Hydroxymethyl)pyridin-2-yl]amino}-1*H*-benzimidazole-6-carbonitrile hydrochloride (**S1.3**, 4.00 g, 13.3 mmol) was suspended in a mixture of DCM (10 mL) and DMF (10 mL), $SOCl_2$ (1.9 mL, 27 mmol) was added dropwise and the suspension was stirred overnight at rt. The reaction mixture was added dropwise to half-sat. aq K_2CO_3 solution and stirred for 1 h at rt. Then, the solid was collected by filtration, and the filter cake was washed with water and EtOH, and dried under reduced pressure at 60 °C to give **S1.4** (3 g, 80% yield), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 1.04. MS (ESI+): *m*/*z* = 284 $[M + H]^+$.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.46 (br s, 1H), 11.05 (br s, 1H), 8.34 (d, *J* = 5.3 Hz, 1H), 7.81 (s, 1H), 7.47–7.65 (m, 1H), 7.37–7.47 (m, 1H), 7.26 (s, 1H), 7.05 (dd, *J* = 5.3, 1.3 Hz, 1H), 4.78 (s, 2H).

tert-Butyl 4-({2-[(6-cyano-1*H*-benzimidazol-2-yl)amino]pyridin-4-yl}methyl)piperazine-1-carboxylate (S1.5)

2-{[4-(Chloromethyl)pyridin-2-yl]amino}-1*H*-benzimidazole-6-carbonitrile (**S1.4**, 5.00 g, 17.6 mmol), *tert*-butyl piperazine-1-carboxylate (6.56 g, 35.2 mmol) and K_2CO_3 (12.78 g, 88.1 mmol) were suspended in DMF (150 mL) and the mixture was stirred for 5 h at rt. The reaction mixture was concentrated under reduced pressure, the residue was diluted with DCM, and the organic phase was washed with water and concentrated under reduced pressure. The crude material was purified by flash chromatography (DCM/MeOH). The product was diluted in warm EtOAc and stirred for 10 min at rt. The solid was collected by filtration, washed with EtOAc and then hexane, and dried under reduced pressure at 60 °C to give **S1.5** (6.1 g, 80% yield), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 1.23. MS (ESI+): *m*/*z* = 434 $[M + H]^+$.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.51 (br d, *J* = 9.8 Hz, 1H), 10.71–11.20 (m, 1H), 8.30 (br d, *J* = 4.5 Hz, 1H), 7.71–7.96 (m, 1H), 7.45 (br s, 1H), 7.63 (br s, 1H), 7.19 (s, 1H), 6.98 (br d, *J* = 4.9 Hz, 1H), 3.52 (s, 2H), 3.29–3.38 (m, 5H), 2.37 (br t, *J* = 4.8 Hz, 4H), 1.40 (s, 9H).

2-({4-[(Piperazin-1-yl)methyl]pyridin-2-yl}amino)-1*H*-benzimidazole-6-carbonitrile hydrochloride (S1.6)

tert-Butyl 4-({2-[(6-cyano-1*H*-benzimidazol-2-yl)amino]pyridin-4-yl}methyl)piperazine-1-carboxylate (**S1.5**, 6.10 g, 14.1 mmol) was dissolved in a mixture of DCM (300 mL) and MeOH (150 mL), treated with HCl (53 mL, 4.0 M in 1,4-dioxane, 210 mmol), and the mixture was stirred overnight at rt. The reaction mixture was concentrated under reduced pressure to give **S1.6** (7.1 g), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 0.87. MS (ESI+): *m*/*z* = 334 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.80 (br s, 2H), 8.53 (d, *J* = 5.3 Hz, 1H), 8.02 (s, 1H), 7.68–7.88 (m, 2H), 7.56 (s, 2H), 4.38 (br s, 2H), 3.43 (s, 4H), 3.32 (s, 1H), 1.99 (s, 1H).

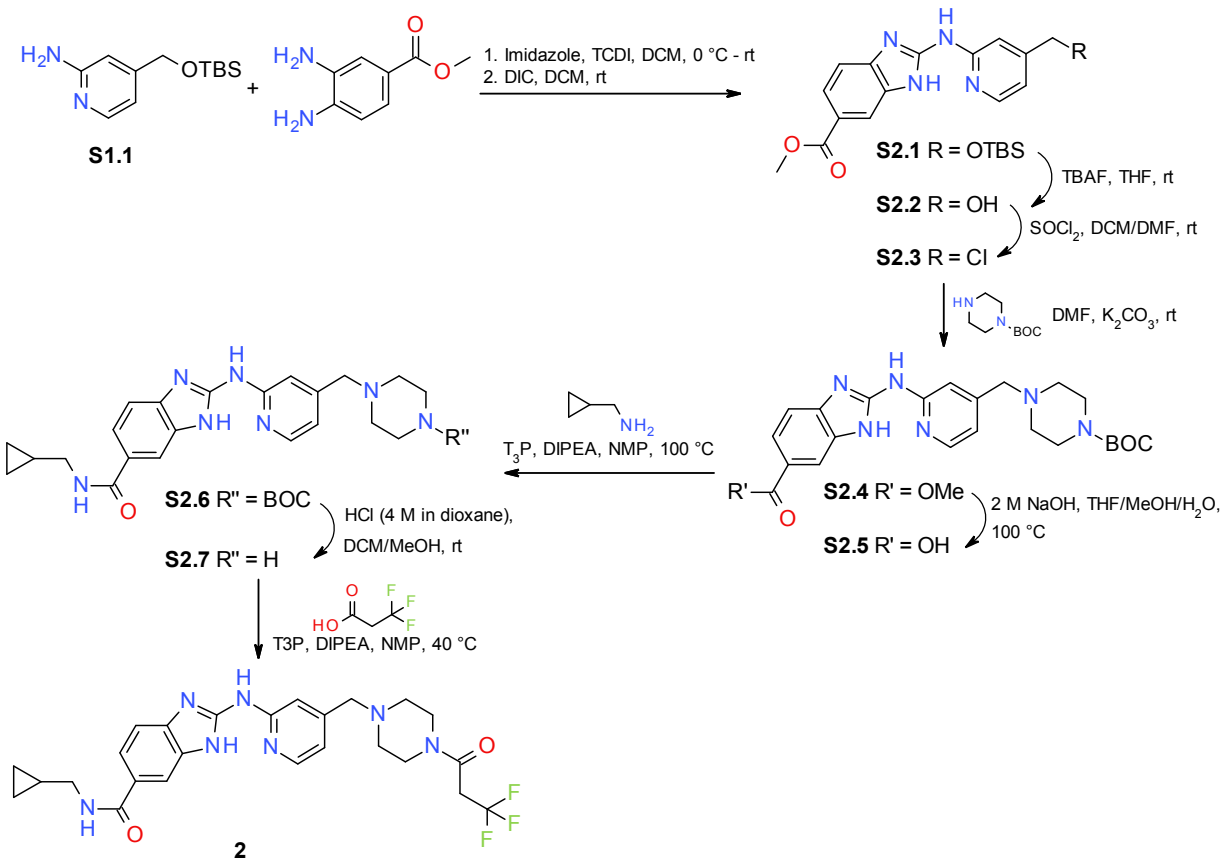
2-[(4-{[4-(3,3,3-Trifluoropropanoyl)piperazin-1-yl]methyl}pyridin-2-yl)amino]-1*H*-benzimidazole-6-carbonitrile (1)

2-({4-[(Piperazin-1-yl)methyl]pyridin-2-yl}amino)-1*H*-benzimidazole-6-carbonitrile hydrochloride (**S1.6**, 1.00 g, 2.70 mmol), T3P (2.8 mL, 50% purity, 4.9 mmol), 3,3,3-trifluoropropanoic acid (410 µL, 98% purity, 4.6 mmol) and DIPEA (2.4 mL, 14 mmol) were dissolved in DMF (50 mL) and the mixture was stirred overnight at rt. The reaction mixture was concentrated under reduced pressure, the residue was diluted with a mixture of DCM and MeOH (100:1), and washed twice with water. The organic layer was concentrated under reduced pressure and the crude material was purified by flash chromatography (DCM/MeOH). The impure product was suspended in warm EtOH and stirred for 10 min at rt. The suspension was filtered and the collected solid was washed with EtOH and hexane, and dried under reduced pressure at 60 °C to give **1** (700 mg, 58%, 100% purity by UPLC).

LC-MS (Method 2): ^{t}R (min) = 1.03. MS (ESI+): *m*/*z* = 444 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.47 (br s, 1H), 10.90 (br s, 1H), 8.29 (br d, *J* = 4.8 Hz, 1H), 7.72–7.93 (m, 1H), 7.63 (br s, 1H), 7.43 (br s, 1H), 7.20 (s, 1H), 6.97 (br d, *J* = 4.8 Hz, 1H), 3.63 (q, *J* = 11.0 Hz, 2H), 3.40–3.55 (m, 6H), 2.40 (dt, *J* = 16.6, 4.7 Hz, 4H).

Scheme S2



Methyl 2-{[4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)pyridin-2-yl]amino}-1*H*-benzimidazole-6-carboxylate (S2.1)

Step 1:

1*H*-Imidazole (457 mg, 6.71 mmol) and TCDI (9.30 g, 90% purity, 47.0 mmol) were dissolved in DCM (180 mL), cooled to 0 °C and 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)pyridin-2-amine (**S1.1**, 8.00 g, 33.6 mmol) dissolved in DCM (100 mL) was added dropwise. The mixture was stirred at rt for 16 h. Methyl 3,4-diaminobenzoate (8.62 g, 97% purity, 50.3 mmol) was added and the mixture was stirred for 16 h at rt. The mixture was diluted with water and extracted with DCM. The organic layer was washed three times with water and once with sat. NaCl solution. Then, it was dried, filtered and concentrated under reduced pressure.

Step 2:

The residue was dissolved in DCM (200 mL), DIC (6.0 mL, 39 mmol) was added and the mixture was stirred for 88 h at rt. Sat. NH_4Cl solution was added and the mixture was stirred for 1 h at rt. The layers were

separated and the organic phase was dried, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (DCM/MeOH) to give **S2.1** (14.3 g, 75% purity, 87% yield).

LC-MS (Method 1): ^{t}R (min) = 1.34. MS (ESI+): *m*/*z* = 413 $[M + H]^+$.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.33 (br s, 1H), 10.90 (td, *J* = 1.70, 8.10 Hz, 1H), 8.25 (d, *J* = 5.27 Hz, 1H), 7.88–8.17 (m, 1H), 7.67–7.73 (m, 1H), 7.31–7.60 (m, 1H), 7.25 (s, 1H), 6.88 (d, *J* = 5.09 Hz, 1H), 4.73 (s, 2H), 3.83 (d, *J* = 1.32 Hz, 3H), 0.90–0.98 (m, 9H), 0.07–0.14 (m, 6H).

Methyl 2-{[4-(hydroxymethyl)pyridin-2-yl]amino}-1*H*-benzimidazole-6-carboxylate (S2.2)

Methyl 2-{[4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)pyridin-2-yl]amino}-1*H*-benzimidazole-6-carboxylate (**S2.1**, 14.3 g, 75% purity, 26.0 mmol) was dissolved in THF (500 mL), TBAF (39 mL, 1.0 M in THF, 39 mmol) was added and the mixture was stirred overnight at rt. Sat. $NaHCO_3$ solution was added and the mixture was extracted with EtOAc. The organic layer was dried and concentrated under reduced pressure. The residue was stirred in EtOH to give **S2.2** (4.79 g, 62% yield), which was used without further purification.

LC-MS (Method 1): ^{t}R (min) = 0.71. MS (ESI+): *m*/*z* = 299 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.39 (br s, 1H), 10.73–10.91 (m, 1H), 8.24 (d, *J* = 5.31 Hz, 1H), 7.94–8.20 (m, 1H), 7.70 (br d, *J* = 7.83 Hz, 1H), 7.33–7.57 (m, 1H), 7.18 (s, 1H), 6.90 (d, *J* = 5.05 Hz, 1H), 5.42 (t, *J* = 5.68 Hz, 1H), 4.51 (d, *J* = 5.31 Hz, 2H), 3.83 (s, 3H).

Methyl 2-{[4-(chloromethyl)pyridin-2-yl]amino}-1*H*-benzimidazole-6-carboxylate (S2.3)

Methyl 2-{[4-(hydroxymethyl)pyridin-2-yl]amino}-1*H*-benzimidazole-6-carboxylate (**S2.2**, 4.79 g, 16.1 mmol) was dissolved in anhydrous DCM (70 mL) and DMF (100 mL). $SOCl_2$ (2.3 mL, 32 mmol) was added dropwise and the mixture was stirred for 72 h at rt. Sat. $NaHCO_3$ solution was added, and the mixture was stirred for 15 min at rt then extracted with EtOAc. The organic layer was washed three times with water, dried and concentrated under reduced pressure to give **S2.3** (4.46 g, 88% yield), which was used without further purification.

LC-MS (Method 1): ^{t}R (min) = 0.90. MS (ESI+): *m*/*z* = 317 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.34 (br s, 1H), 10.97 (br s, 1H), 8.34 (d, *J* = 5.32 Hz, 1H), 7.91–8.21 (m, 1H), 7.73 (br d, *J* = 8.11 Hz, 1H), 7.36–7.60 (m, 1H), 7.28 (s, 1H), 7.05 (dd, *J* = 1.01, 5.32 Hz, 1H), 4.79 (s, 2H), 3.85 (s, 3H).

Methyl 2-[(4-{[4-(*tert*-butoxycarbonyl)piperazin-1-yl]methyl}pyridin-2-yl)amino]-1*H*-benzimidazole-6-carboxylate (S2.4)

Methyl 2-{[4-(chloromethyl)pyridin-2-yl]amino}-1*H*-benzimidazole-6-carboxylate (**S2.3**, 2.00 g, 6.31 mmol) was dissolved in DMF (120 mL), and K_2CO_3 (4.36 g, 31.6 mmol) and *tert*-butyl piperazine-1-carboxylate (2.64 g, 14.2 mmol) were added. The mixture was stirred for 40 h at rt, then diluted with water and extracted with EtOAc. The organic layer was washed three times with half-sat. NaCl solution, dried and concentrated under reduced pressure. The residue was stirred in DCM/hexane to give **S2.4** (2.76 g, 91% yield), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 1.23. MS (ESI+): *m*/*z* = 467 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.39 (br s, 1H), 10.83 (br s, 1H), 8.28 (d, *J* = 5.32 Hz, 1H), 7.90–8.20 (m, 1H), 7.72 (br d, *J* = 7.86 Hz, 1H), 7.34–7.61 (m, 1H), 7.17 (s, 1H), 6.95 (d, *J* = 5.07 Hz, 1H), 3.85 (s, 3H), 3.51 (s, 2H), 3.35 (br s, 4H), 2.36 (br t, *J* = 4.94 Hz, 4H), 1.40 (s, 9H).

2-[(4-{[4-(*tert*-Butoxycarbonyl)piperazin-1-yl]methyl}pyridin-2-yl)amino]-1*H*-benzimidazole-6-carboxylic acid (S2.5)

Methyl 2-[(4-{[4-(*tert*-butoxycarbonyl)piperazin-1-yl]methyl}pyridin-2-yl)amino]-1*H*-benzimidazole-6-carboxylate (**S2.4**, 12.0 g, 25.7 mmol) was dissolved in THF (130 mL), MeOH (380 mL) and water (130 mL), NaOH (130 mL, 2.0 M, 260 mmol) was added and the mixture was stirred overnight at 100 °C. The mixture was neutralized with 2 M HCl (pH 7), then concentrated under reduced pressure to give **S2.5** (26.4 g), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 0.66. MS (ESI+): *m*/*z* = 453 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.39 (br s, 1H), 10.75 (br s, 1H), 8.23 (d, *J* = 5.30 Hz, 1H), 8.01 (br s, 1H), 7.69 (br d, *J* = 8.08 Hz, 1H), 7.23–7.36 (m, 2H), 6.86–6.93 (m, 1H), 3.49 (s, 2H), 3.28–3.38 (m, 4H), 2.29–2.39 (m, 4H), 1.38 (s, 9H).

tert-Butyl 4-{[2-({6-[(cyclopropylmethyl)carbamoyl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (S2.6)

2-[(4-{[4-(*tert*-Butoxycarbonyl)piperazin-1-yl]methyl}pyridin-2-yl)amino]-1*H*-benzimidazole-6-carboxylic acid (**S2.5**, 1.20 g, 48% purity, 1.26 mmol), T3P (1.3 mL, 50% purity, 2.3 mmol), cyclopropylmethanamine

(220 µL, 98% purity, 2.5 mmol) and DIPEA (880 µL, 5.0 mmol) were dissolved in *N*-methylpyrrolidone (9.5 mL, 99 mmol) and the mixture was heated for 5 h at 100 °C. The reaction mixture was diluted with EtOAc and washed with sat. aq $NaHCO_3$, water and brine. The organic phase was filtered through a silicone filter and the filtrate was concentrated under reduced pressure. The residue was suspended in a mixture of DCM and hexane, and the suspension was stirred for 10 min at rt. The solid was collected by filtration and washed with hexane to give **S2.6** (580 mg, 91% yield), which was used without further purification.

LC-MS (Method 1): ^{t}R (min) = 0.89. MS (ESI+): *m*/*z* = 506 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.22 (br s, 1H), 10.68 (br s, 1H), 8.36 (br d, *J* = 17.2 Hz, 1H), 8.26 (d, *J* = 5.1 Hz, 1H), 7.87–8.04 (m, 1H), 7.59 (br d, *J* = 7.6 Hz, 1H), 7.28–7.53 (m, 1H), 7.19 (s, 1H), 6.93 (d, *J* = 4.8 Hz, 1H), 3.50 (s, 2H), 3.35 (br s, 4H), 3.16 (t, *J* = 6.3 Hz, 2H), 2.36 (br t, *J* = 4.9 Hz, 4H), 1.40 (s, 9H), 0.98–1.11 (m, 1H), 0.37–0.49 (m, 2H), 0.18–0.29 (m, 2H).

N-(Cyclopropylmethyl)-2-({4-[(piperazin-1-yl)methyl]pyridin-2-yl}amino)-1*H*-benzimidazole-6-carboxamide hydrochloride (S2.7)

tert-Butyl 4-{[2-({6-[(cyclopropylmethyl)carbamoyl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]-methyl}piperazine-1-carboxylate (**S2.6**, 575 mg, 1.14 mmol) was dissolved in a mixture of MeOH (5.9 mL) and DCM (14 mL), treated with HCl (5.7 mL, 4.0 M in 1,4-dioxane, 23 mmol) and the suspension was stirred for 3 h at rt. The reaction mixture was diluted with Et_2O and hexane, and the solid was collected by filtration and dried under reduced pressure at 60 °C to give **S2.7** (505 mg, 90% purity), which was used without further purification.

LC-MS (Method 1): ^{t}R (min) = 0.75. MS (ESI+): *m*/*z* = 406$[M + H]^+$.

^{1}H NMR (300 MHz, DMSO-d_6) δ = 9.49–9.85 (m, 1H), 8.69 (t, *J* = 5.65 Hz, 1H), 8.51 (d, *J* = 5.09 Hz, 1H), 8.12 (s, 1H), 7.86 (dd, *J* = 1.51, 8.48 Hz, 1H), 7.68 (d, *J* = 8.48 Hz, 1H), 7.52 (s, 2H), 4.30 (br s, 2H), 3.33–3.45 (m, 4H), 3.21–3.30 (m, 2H), 3.11–3.20 (m, 4H), 0.97–1.12 (m, 1H), 0.38–0.49 (m, 2H), 0.18–0.28 (m, 2H).

N-(Cyclopropylmethyl)-2-[(4-{[4-(3,3,3-trifluoropropanoyl)piperazin-1-yl]methyl}pyridin-2-yl)amino]-1*H*-benzimidazole-6-carboxamide (2)

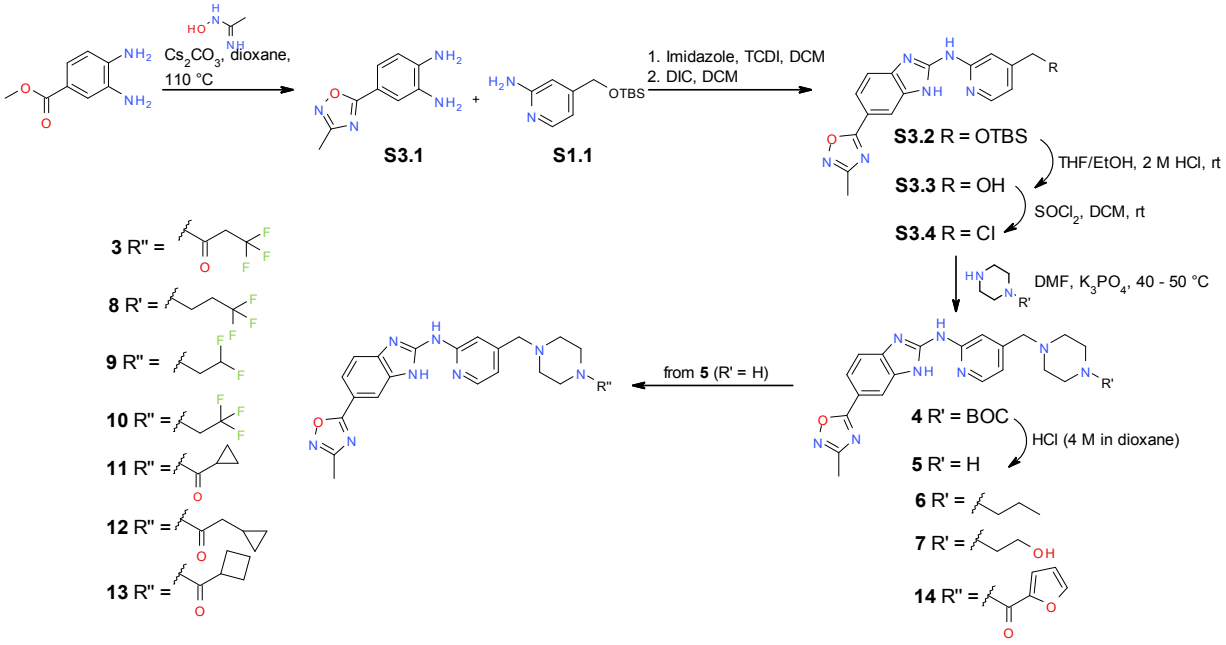
N-(Cyclopropylmethyl)-2-({4-[(piperazin-1-yl)methyl]pyridin-2-yl}amino)-1*H*-benzimidazole-6-carboxamide hydrochloride (**S2.7**, 100 mg, 209 µmol), T3P (220 µL, 50% purity, 380 µmol), 3,3,3-trifluoropropanoic acid (39 µL, 98% purity, 420 µmol) and DIPEA (180 µL, 1.0 mmol) were dissolved in *N*-methylpyrrolidone (2 mL) and the mixture was stirred for 30 h at 40 °C. The reaction mixture was cooled to rt, diluted with EtOAc and washed with sat. aq $NaHCO_3$ and then water. The organic layer was washed

with half-sat. NaCl solution and filtered through a water-resistant filter, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (DCM/MeOH) and then preparative HPLC (acetonitrile/NH_3 in water) to give **2** (21 mg, 99% purity by UPLC, 18% yield).

LC-MS (Method 2): ^{t}R (min) = 1.02. MS (ESI+): *m/z* = 516 $[M + H]^+$.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.24 (br s, 1H), 10.71 (br s, 1H), 8.39 (br s, 1H), 8.26 (d, *J* = 5.1 Hz, 1H), 7.86–8.03 (m, 1H), 7.58 (br d, *J* = 7.7 Hz, 1H), 7.28–7.52 (m, 1H), 7.19 (s, 1H), 6.93 (d, *J* = 5.1 Hz, 1H), 3.64 (q, *J* = 11.1 Hz, 2H), 3.42–3.54 (m, 6H), 3.14 (t, *J* = 6.2 Hz, 2H), 2.40 (td, *J* = 4.82 , 16.22 Hz, 4H) 0.97–1.11 (m, 1H), 0.36–0.49 (m, 2H), 0.19–0.27 (m, 2H).

Scheme S3



4-(3-Methyl-1,2,4-oxadiazol-5-yl)benzene-1,2-diamine (S3.1)

Methyl 3,4-diaminobenzoate (5.00 g, 30.1 mmol), *N*-hydroxyethanimidamide (5.28 g, 95% purity, 67.7 mmol) and cesium carbonate (9.80 g, 30.1 mmol) were stirred in 1,4-dioxane (50 mL) overnight at 110 °C. The reaction mixture was cooled to rt, diluted with water and the aqueous layer was extracted with DCM/propan-2-ol. The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was stirred with water at 60 °C. The suspension was filtered and the collected solid was dried under reduced pressure at 60 °C to give **S3.1** (3.02 g, 95% purity, 50% yield), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 0.65. MS (ESI+): *m/z* = 191 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.21 (d, *J* = 2.03 Hz, 1H), 7.16 (dd, *J* = 2.03, 8.11 Hz, 1H), 6.59 (d, *J* = 8.11 Hz, 1H), 5.43 (s, 2H), 4.83 (s, 2H), 2.31 (s, 3H).

N-[4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)pyridin-2-yl]-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-amine (S3.2)

Step 1:

1*H*-Imidazole (143 mg, 2.10 mmol) and TCDI (2.18 g, 90% purity, 11.0 mmol) were dissolved in DCM (10 mL) under argon, 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)pyridin-2-amine (**S1.1**, 2.50 g, 10.5 mmol) dissolved in DCM (20 mL) was added and the mixture was stirred overnight at rt. A solution of 4-(3-methyl-1,2,4-oxadiazol-5-yl)benzene-1,2-diamine (**S3.1**, 2.06 g, 97% purity, 10.5 mmol) in DCM (20 mL) was added and the mixture was stirred for 20 h at rt. Then, it was diluted with water and extracted with DCM. The organic layer was dried over a silicone filter and the crude material was purified by flash chromatography (DCM/MeOH) to give the thiourea intermediate (4.7 g, 95% yield).

Step 2:

The thiourea intermediate was dissolved in DCM (46 mL), DIC (3.0 mL, 20 mmol) was added and the mixture was stirred overnight at rt under argon. Additional DIC (3.0 mL, 20 mmol) was added and the mixture was stirred for 3 h at rt. The reaction mixture was concentrated under reduced pressure. The residue was diluted with EtOAc and washed with water. The organic layer was dried through a silicone filter and concentrated under reduced pressure. The crude material was purified by flash chromatography (hexane/EtOAc). The product was suspended in EtOH and stirred for 30 min. The suspension was filtered and the collected solid was washed with EtOH and hexane, and concentrated under reduced pressure at 60 °C to give **S3.2** (2.95 g, 60% yield).

LC-MS (Method 2): ^{t}R (min) = 1.52. MS (ESI+): *m*/*z* = 437[M + H]$^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.44 (s, 1H), 10.90–11.15 (m, 1H), 8.20–8.33 (m, 2H), 8.01 (br s, 1H), 7.75–7.87 (m, 1H), 7.68 (br d, *J* = 7.9 Hz, 1H), 7.49 (br d, *J* = 8.4 Hz, 1H), 7.27 (s, 1H), 6.82–6.96 (m, 1H), 4.76 (s, 2H), 2.41 (s, 3H), 0.96 (s, 9H), 0.09–0.17 (m, 6H).

(2-{[6-(3-Methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methanol (S3.3)

N-[4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)pyridin-2-yl]-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-amine (**S3.2**, 1.70 g, 3.89 mmol) was dissolved in a mixture of THF (51 mL) and EtOH (17 mL). HCl (5.8 mL, 2.0 M in water, 12 mmol) was added and the mixture was stirred for 1 h at rt. The mixture

was diluted with water and extracted with hexane/EtOAc (1:1). The aqueous phase was adjusted to pH 6 with NaOH (2 M). The aqueous layer was lyophilized and the residue was diluted with $CHCl_3$ (150 mL) and concentrated under reduced pressure to give **S3.3** (2.2 g), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 0.86. MS (ESI+): *m*/*z* = 323 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.23 (br s, 1H), 8.23 (d, *J* = 5.3 Hz, 1H), 8.08 (s, 1H), 7.76 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.48–7.59 (m, 3H), 6.91 (d, *J* = 5.1 Hz, 1H), 5.59 (br s, 1H), 4.53 (s, 2H), 2.39 (s, 3H).

N-[4-(Chloromethyl)pyridin-2-yl]-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-amine (S3.4)

(2-{[6-(3-Methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methanol (**S3.3**, 2.20 g, 57% purity, 3.89 mmol) was suspended in DCM (100 mL) and $SOCl_2$ (850 µL, 11.6 mmol) was added. The reaction mixture was stirred for 72 h at rt. Then, it was carefully quenched with sat. $NaHCO_3$ solution, and the aqueous phase was extracted with DCM/MeOH (10:1) and $CHCl_3$/MeOH (5:1). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was suspended in EtOH and stirred for 20 min. The solid was collected by filtration, washed with EtOH and dried under reduced pressure at 60 °C to give **S3.4** (900 mg, 68% yield), which was used without further purification.

LC-MS (Method 1): ^{t}R (min) = 0.89. MS (ESI+): *m*/*z* = 341 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.43 (br s, 1H), 10.88–11.17 (m, 1H), 8.65 (d, *J* = 6.34 Hz, 1H), 8.36 (d, *J* = 5.32 Hz, 1H), 7.81 (br d, *J* = 7.35 Hz, 1H), 7.51 (d, *J* = 0.76 Hz, 1H), 7.27 (s, 1H), 7.11 (dd, *J* = 1.65, 6.21 Hz, 1H), 7.06 (br d, *J* = 5.07 Hz, 1H), 4.79 (s, 2H), 2.41–2.42 (m, 1H), 2.40 (s, 2H).

tert-Butyl 4-[(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (4)

N-[4-(Chloromethyl)pyridin-2-yl]-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-amine (**S3.4**, 5.00 g, 14.7 mmol), *tert*-butyl piperazine-1-carboxylate (5.47 g, 29.3 mmol) and potassium phosphate (10.1 g, 73.4 mmol) were suspended in DMF (130 mL) and the mixture was stirred overnight at 50 °C. Then, the mixture was cooled to rt and filtered. The solid was washed with DMF. The filtrate was concentrated under reduced pressure. The residue was diluted with DCM and washed with water and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified by

flash chromatography (EtOAc/MeOH). The product was suspended in warm EtOAc and the solid was collected by filtration, washed with EtOAc and dried under reduced pressure to give **4** (4.29 g, 58 % yield).

LC-MS (Method 2): ^{t}R (min) = 1.24. MS (ESI+): *m*/*z* = 491 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.46 (br s, 1H), 10.79–10.97 (m, 1H), 8.30 (br d, *J*=4.82 Hz, 1H), 7.94–8.25 (m, 1H), 7.75–7.87 (m, 1H), 7.48 (br d, *J*=8.11 Hz, 1H), 7.40–7.71 (m, 1H), 7.17 (s, 1H), 6.97 (br d, *J*=4.56 Hz, 1H), 3.51 (s, 2H), 3.35 (br s, 4H), 2.40 (s, 3H), 2.36 (br t, *J*=4.94 Hz, 4H), 1.39 (s, 9H).

6-(3-Methyl-1,2,4-oxadiazol-5-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (5)

tert-Butyl 4-[(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)-methyl]piperazine-1-carboxylate (**4**, 4.29 g, 8.74 mmol) was dissolved in DCM (56 mL) and MeOH (10 mL), HCl (22 mL, 4.0 M in 1,4-dioxane, 87 mmol) was added and the mixture was stirred for 2 h at rt. Then, it was filtered and the collected solid was washed with DCM to give **5** (4.56 g), which was used without further purification (99% purity by UPLC).

LC-MS (Method 2): ^{t}R (min) = 0.92. MS (ESI+): *m*/*z* = 391 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.12 (br s, 1H), 9.64 (br s, 2H), 8.53 (br d, *J* = 5.32 Hz, 1H), 8.33 (s, 1H), 8.05 (dd, *J* = 1.65, 8.49 Hz, 1H), 7.82 (d, *J* = 8.36 Hz, 1H), 7.54 (br s, 2H), 4.31 (br s, 3H), 3.56 (s, 2H), 3.19–3.45 (m, 8H), 2.43 (s, 3H).

3,3,3-Trifluoro-1-{4-[(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazin-1-yl}propan-1-one (3)

6-(3-Methyl-1,2,4-oxadiazol-5-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**5**, 3.55 g, 92% purity, 7.65 mmol) was dissolved in DMA (50 mL), treated with 3,3,3-trifluoropropanoic acid (1.0 mL, 98% purity, 11 mmol), DIPEA (8.0 mL, 46 mmol) and PyBOP (5.97 g, 11.5 mmol), and the mixture was stirred for 1 h at rt. The reaction mixture was diluted with water and the solid was collected by filtration. The filtrate was extracted with DCM/MeOH (9:1). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was diluted with EtOAc and the organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue and the solid were combined, dissolved in DCM/MeOH (9:1) and the solution was

filtered through a basic column. The filtrate was concentrated under reduced pressure to give **3** (2.98 g, 78% yield, 100% purity by UPLC), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 1.07. MS (ESI+): *m*/*z* = 501[M + H]$^{+}$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.47 (br s, 1H), 10.90 (br s, 1H), 8.30 (d, *J* = 5.3 Hz, 1H), 7.98–8.28 (m, 1H), 7.80 (br d, *J* = 8.1 Hz, 1H), 7.42–7.76 (m, 1H), 7.20 (s, 1H), 6.98 (d, *J* = 5.1 Hz, 1H), 3.65 (q, *J* = 10.9 Hz, 2H), 3.44–3.60 (m, 6H), 2.33–2.47 (m, 7H).

6-(3-Methyl-1,2,4-oxadiazol-5-yl)-*N*-{4-[(4-propylpiperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine (6)

N-[4-(Chloromethyl)pyridin-2-yl]-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-amine (**S3.4**, 51.1 mg, 150 µmol) was dissolved in DMF (3 mL), 1-propylpiperazine (38.5 mg, 300 µmol) and K_2CO_3 (104 mg, 750 µmol) were added, and the suspension was stirred overnight at 40 °C. The reaction mixture was cooled to rt, the suspension was filtered and the filtrate was purified by preparative HPLC to give **6** (4.8 mg, 7% yield).

LC-MS (Method 4): ^{t}R (min) = 0.62 MS (ESI+): *m*/*z* = 435[M + H]$^{+}$.

2-{4-[(2-{[6-(3-Methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazin-1-yl}ethan-1-ol (7)

Compound **7** was synthesized analogously to **6**, from *N*-[4-(chloromethyl)pyridin-2-yl]-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-amine (**S3.4**, 51.1 mg, 150 µmol) and 2-(piperazin-1-yl)ethan-1-ol (39.1 mg, 300 µmol) in 18% yield (11.7 mg).

LC-MS (Method 4): ^{t}R (min) = 0.66 MS (ESI+): *m*/*z* = 433[M + H]$^{+}$.

6-(3-Methyl-1,2,4-oxadiazol-5-yl)-*N*-(4-{[4-(3,3,3-trifluoropropyl)piperazin-1-yl]methyl}pyridin-2-yl)-1*H*-benzimidazol-2-amine (8)

Compound **8** was synthesized analogously to **9**, from 6-(3-methyl-1,2,4-oxadiazol-5-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**5**, 150 mg, 351 µmol) and 3-bromo-1,1,1-trifluoropropane (124 mg, 703 µmol) with a reaction temperature of 80 °C in 23% yield (39.3 mg).

LC-MS (Method 2): ^{t}R (min) = 1.17. MS (ESI+): *m*/*z* = 487 [M + H]$^{+}$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.47 (br s, 1H), 10.89 (br s, 1H), 8.28 (d, *J* = 5.3 Hz, 1H), 7.97–8.26 (m, 1H), 7.80 (br d, *J* = 7.9 Hz, 1H), 7.43–7.73 (m, 1H), 7.13–7.22 (m, 1H), 6.91–7.02 (m, 1H), 3.45 - 3.54 (m, 2H), 2.51 - 2.55 (m, 4H), 2.34 - 2.48 (m, 11H).

N-(4-{[4-(2,2-Difluoroethyl)piperazin-1-yl]methyl}pyridin-2-yl)-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-amine (9)

6-(3-Methyl-1,2,4-oxadiazol-5-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**5**, 150 mg, 351 µmol) was suspended in DMF (1.4 mL). DIPEA (140 µL, 780 µmol) and 2,2-difluoroethyl trifluoromethanesulfonate (75.2 mg, 351 µmol) were added and the mixture was stirred for 1.5 h at rt. The reaction mixture was diluted with EtOAc and water. The aqueous layer was extracted with EtOAc. The organic layer was filtered through a silicone filter and concentrated under reduced pressure. The residue was diluted with EtOH and the resulting suspension was filtered, washed with EtOH and the solid dried under reduced pressure at 50 °C. The filtrate was concentrated under reduced pressure and purified by flash chromatography (EtOAc/MeOH). The purified filtrate and solid were combined to give **9** (93 mg, 95% purity, 56% yield).

LC-MS (Method 2): ^{t}R (min) = 1.10. MS (ESI+): *m/z* = 455 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.47 (br s, 1H), 10.91 (br s, 1H), 8.29 (br d, *J* = 4.8 Hz, 1H), 7.95–8.26 (m, 1H), 7.81 (br d, *J* = 8.6 Hz, 1H), 7.43–7.72 (m, 1H), 7.10–7.22 (m, 1H), 6.95 (br d, *J* = 4.6 Hz, 1H), 5.91–6.35 (m, 1H), 3.49 (s, 2H), 2.73 (td, *J* = 15.7, 4.3 Hz, 2H), 2.53–2.65 (m, 4H), 2.42 (br s, 4H), 2.40 (s, 3H).

6-(3-Methyl-1,2,4-oxadiazol-5-yl)-*N*-(4-{[4-(2,2,2-trifluoroethyl)piperazin-1-yl]methyl}pyridin-2-yl)-1*H*-benzimidazol-2-amine (10)

Compound **10** was synthesized analogously to **9** from 6-(3-methyl-1,2,4-oxadiazol-5-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**5**, 150 mg, 351 µmol) and 2,2,2-trifluoroethyl trifluoromethanesulfonate (51 µL, 350 µmol) in 59% yield (92.8 mg, 100% purity by UPLC)

LC-MS (Method 2): ^{t}R (min) = 1.22. MS (ESI+): *m/z* = 473 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.39–12.62 (m, 1H), 10.77–11.05 (m, 1H), 8.29 (br d, *J* = 5.1 Hz, 1H), 7.98–8.25 (m, 1H), 7.75–7.86 (m, 1H), 7.42–7.72 (m, 1H), 7.16 (s, 1H), 6.95 (br d, *J* = 4.3 Hz, 1H), 3.49 (s, 2H), 3.18 (q, *J* = 10.4 Hz, 2H), 2.66 (br s, 4H), 2.42 (br s, 4H), 2.40 (s, 3H).

Cyclopropyl{4-[(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazin-1-yl}methanone (11)

6-(3-Methyl-1,2,4-oxadiazol-5-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**5**, 70.0 mg, 164 µmol), T3P (170 µL, 50% purity, 300 µmol), cyclopropanecarboxylic acid (23 µL, 98% purity, 280 µmol) and DIPEA (140 µL, 820 µmol) were dissolved in DMF (1.6 mL) and the mixture was stirred overnight at rt. The reaction mixture was diluted with water and the aqueous phase was extracted three times with EtOAc. The combined organic layers were washed with half-sat. NaCl solution, filtered over a silicone filter and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography to give **11** (36 mg, 98% purity by UPLC, 43% yield).

LC-MS (Method 2): ^{t}R (min) = 1.05. MS (ESI+): *m*/*z* = 459 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.46 (br s, 1H), 10.88 (br s, 1H), 8.30 (d, *J* = 5.3 Hz, 1H), 7.95–8.27 (m, 1H), 7.80 (br d, *J* = 7.8 Hz, 1H), 7.45–7.73 (m, 1H), 7.21 (s, 1H), 6.99 (d, *J* = 5.1 Hz, 1H), 3.71 (br s, 2H), 3.45–3.59 (m, 4H), 2.35–2.46 (m, 6H), 1.96 (tt, *J* = 7.7, 4.8 Hz, 1H), 0.66–0.78 (m, 4H).

2-Cyclopropyl-1-{4-[(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazin-1-yl}ethan-1-one (12)

Compound **12** was synthesized analogously to **11**, from 6-(3-methyl-1,2,4-oxadiazol-5-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**5**, 70.0 mg, 164 µmol) and cyclopropylacetic acid (28.5 mg, 98% purity, 279 µmol) in 25% yield (22.0 mg, 97% purity by UPLC).

LC-MS (Method 2): ^{t}R (min) = 1.08. MS (ESI+): *m*/*z* = 473 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.45 (br s, 1H), 10.87 (br s, 1H), 8.29 (d, *J* = 5.3 Hz, 1H), 7.94–8.28 (m, 1H), 7.80 (br d, *J* = 8.1 Hz, 1H), 7.42–7.77 (m, 1H), 7.20 (s, 1H), 6.97 (d, *J* = 4.8 Hz, 1H), 3.52 (s, 2H), 3.41–3.51 (m, 4H), 2.40 (s, 6H), 2.25 (d, *J* = 6.8 Hz, 2H), 0.79–1.03 (m, 1H), 0.38–0.50 (m, 2H), 0.04–0.19 (m, 2H).

Cyclobutyl{4-[(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazin-1-yl}methanone (13)

Compound **13** was synthesized analogously to **11**, from 6-(3-methyl-1,2,4-oxadiazol-5-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**5**, 70.0 mg, 164 µmol) and cyclobutanecarboxylic acid (28.5 mg, 98% purity, 279 µmol) in 43% yield (33.0 mg, 99% purity by UPLC).

LC-MS (Method 2): ^{t}R (min) = 1.11. MS (ESI+): *m*/*z* = 473 $[M + H]^+$.

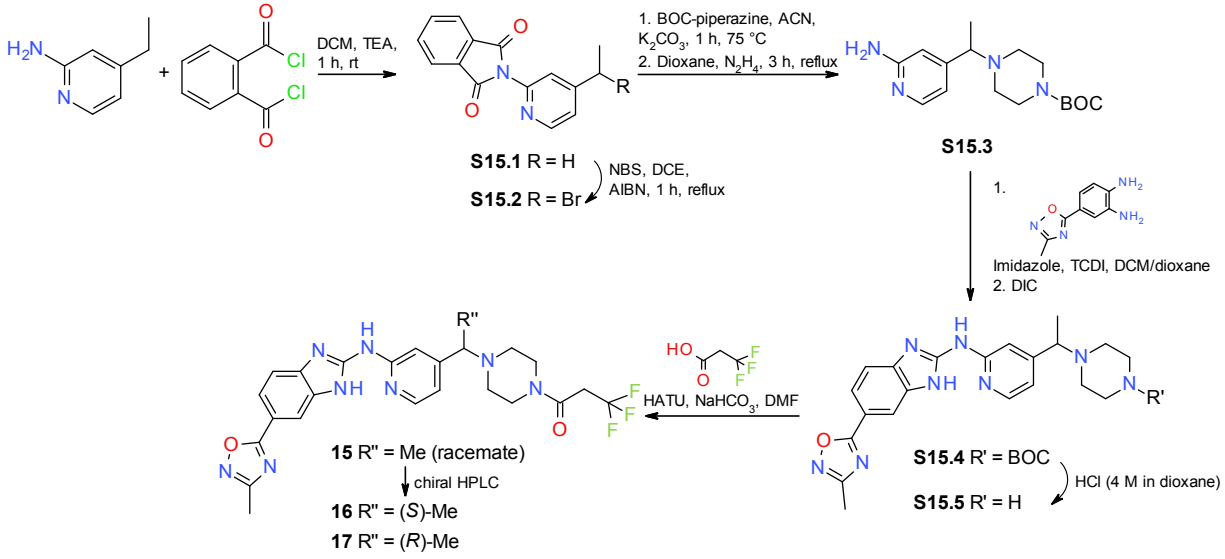
^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.44 (br s, 1H), 10.86 (br s, 1H), 8.28 (d, *J* = 5.3 Hz, 1H), 7.97–8.26 (m, 1H), 7.79 (br d, *J* = 8.3 Hz, 1H), 7.49 (br s, 1H), 7.18 (s, 1H), 6.95 (d, *J* = 5.1 Hz, 1H), 3.50 (s, 2H), 3.47 (br s, 2H), 3.31–3.37 (m, 3H), 2.39 (s, 3H), 2.32–2.37 (m, 4H), 2.01–2.20 (m, 4H), 1.78–1.94 (m, 1H), 1.67–1.76 (m, 1H).

(Furan-2-yl){4-[(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazin-1-yl}methanone (14)

Compound **14** was synthesized analogously to **6**, from *N*-[4-(chloromethyl)pyridin-2-yl]-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-amine (**S3.4**, 51.1 mg, 150 µmol) and (furan-2-yl)(piperazin-1-yl)methanone (54.1 mg, 300 µmol) in 28 % yield (20.7 mg) .

LC-MS (Method 4): ^{t}R (min) = 0.73 MS (ESI+): *m*/*z* = 485[M + H]$^+$.

Scheme S4



2-(4-Ethylpyridin-2-yl)-1*H*-isoindole-1,3(2*H*)-dione (S15.1)

To a solution of 4-ethylpyridin-2-amine (20.0 g, 164 mmol) in DCM (600 mL) was added dropwise benzene-1,2-dicarbonyl dichloride (26 mL, 180 mmol) and triethylamine (60 mL, 430 mmol). The mixture was stirred for 1 h at rt, then washed three times with water. The organic layer was dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (Et_2O) to give **S15.1** (37.0 g, 90% yield).

LC-MS (Method 2): ^{t}R (min) = 1.03. MS (ESI+): *m*/*z* = 253 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (dd, *J* = 0.76, 5.07 Hz, 1H), 7.97 – 8.01 (m, 2H), 7.92 – 7.96 (m, 2H), 7.42 – 7.44 (m, 1H), 7.40 (dd, *J* = 1.52, 5.07 Hz, 1H), 2.72 (q, *J* = 7.60 Hz, 2H), 1.23 (t, *J* = 7.60 Hz, 3H).

2-[4-(1-Bromoethyl)pyridin-2-yl]-1*H*-isoindole-1,3(2*H*)-dione (S15.2)

2-(4-Ethylpyridin-2-yl)-1*H*-isoindole-1,3(2*H*)-dione (**S15.1**, 24.7 g, 97.9 mmol), NBS (19.0 g, 107 mmol) and AIBN (800 mg, 4.87 mmol) were stirred in DCE (300 mL) for 1 h at reflux. The mixture was washed three times with water and the organic layer was dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residue was stirred in Et_2O, filtered and dried to give **S15.2** (27.8 g, 86% yield), which was used without further purification.

LC-MS (Method 1): ^{t}R (min) = 1.11. MS (ESI+): *m*/*z* = 331 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64 (d, *J* = 5.07 Hz, 1H), 7.98 – 8.04 (m, 2H), 7.91 – 7.97 (m, 2H), 7.70 (d, *J* = 1.52 Hz, 1H), 7.65 (dd, *J* = 1.65, 5.20 Hz, 1H), 5.53 (q, *J* = 6.84 Hz, 1H), 2.00 (d, *J* = 6.84 Hz, 3H).

tert-Butyl 4-[1-(2-aminopyridin-4-yl)ethyl]piperazine-1-carboxylate (S15.3)

Step 1:

2-[4-(1-Bromoethyl)pyridin-2-yl]-1*H*-isoindole-1,3(2*H*)-dione (**S15.2**, 41.7 g, 126 mmol), *tert*-butyl piperazine-1-carboxylate (53.0 g, 285 mmol) and K_2CO_3 (22.0 g, 159 mmol) were stirred in acetonitrile (200 mL) for 1 h at 75 °C. The mixture was diluted with water and extracted four times with Et_2O. The combined organic layers were dried with $MgSO_4$, filtered and concentrated under reduced pressure.

LC-MS (Method 2): ^{t}R (min) = 1.37. MS (ESI+): *m*/*z* = 623 $[M + H]^+$.

Step 2:

tert-Butyl 4-[1-(2-{2-[4-(*tert*-butoxycarbonyl)piperazin-1-ylcarbonyl]benzamido}pyridin-4-yl)-ethyl]piperazine-1-carboxylate (91.6 g, 77% purity, 113 mmol) was dissolved in 1,4-dioxane (350 mL) and hydrazine hydrate (50 mL, 1.0 mol) was added. The mixture was stirred for 3 h at reflux, then filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in Et_2O and washed four times with water. The organic layer was dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc) to give **S15.3** (32.35 g, 93% yield).

LC-MS (Method 2): ^{t}R (min) = 1.02. MS (ESI+): *m*/*z* = 307 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (d, *J* = 5.32 Hz, 1H), 6.42 (dd, *J* = 1.27, 5.32 Hz, 1H), 6.35 (s, 1H), 5.81 (s, 2H), 3.16 – 3.31 (m, 5H), 2.17 – 2.40 (m, 4H), 1.34 – 1.42 (m, 9H), 1.21 (d, *J* = 6.59 Hz, 3H).

tert-Butyl 4-[1-(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)ethyl]piperazine-1-carboxylate (S15.4)

Step 1:

To a solution of TCDI (7.90 g, 44.3 mmol) and 1*H*-imidazole (320 mg, 4.7 mmol) in DCM (40 mL) was added dropwise a solution of *tert*-butyl 4-[1-(2-aminopyridin-4-yl)ethyl]piperazine-1-carboxylate (**S15.3**, 12.9 g, 42.1 mmol) in DCM (30 mL). After stirring for 4 h at rt, the mixture was added to a solution of 4-(3-methyl-1,2,4-oxadiazol-5-yl)benzene-1,2-diamine (**S3.1**, 8.10 g, 42.6 mmol) in 1,4-dioxane (80 mL) and stirring was continued for 2 h at 40 °C. The reaction mixture was transferred to the next step without workup.

LC-MS (Method 2): ^{t}R (min) = 1.30. MS (ESI+): *m*/*z* = 539 $[M + H]^+$.

Step 2:

To the reaction mixture of step 1, DIC (10 mL, 64.6 mmol) was added and the mixture was stirred at 45 °C for 16 h, then washed twice with water. The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (cyclohexane/EtOAc/THF). The residue was suspended in Et_2O. The precipitated crystals were collected by filtration and dried in vacuo to give **S15.4** (10.8 g, 51% yield).

LC-MS (Method 2): ^{t}R (min) = 1.32. MS (ESI+): *m*/*z* = 505 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.48 (br s, 1H), 10.72 – 11.04 (m, 1H), 8.30 (d, *J* = 5.07 Hz, 1H), 7.96 – 8.25 (m, 1H), 7.81 (br d, *J* = 7.60 Hz, 1H), 7.36-7.73 (m, 1H), 7.17 (s, 1H), 6.98 (d, *J* = 5.32 Hz, 1H), 3.46 (q, *J* = 6.76 Hz, 1H), 3.33 (br s, 3H), 2.41 (s, 5H), 2.26 – 2.34 (m, 2H), 1.38 (s, 9H), 1.29 (d, *J* = 6.59 Hz, 3H).

6-(3-Methyl-1,2,4-oxadiazol-5-yl)-*N*-{4-[1-(piperazin-1-yl)ethyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S15.5)

tert-Butyl 4-[1-(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)-ethyl]piperazine-1-carboxylate (**S15.4**, 16.1 g, 31.9 mmol) was dissolved in DCM (80 mL) and MeOH (40 mL), HCl (80 mL, 4.0 M in 1,4-dioxane, 320 mmol) was added and the mixture was stirred for 4 h at rt.

Then, it was concentrated under reduced pressure to give **S15.5** (18.3 g, 83% purity), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 1.01. MS (ESI+): *m*/*z* = 405 $[M + H]^+$.

rac-3,3,3-Trifluoro-1-{4-[1-(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)ethyl]piperazin-1-yl}propan-1-one (15)

6-(3-Methyl-1,2,4-oxadiazol-5-yl)-*N*-{4-[1-(piperazin-1-yl)ethyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**S15.5**, 18.3 g, 83% purity, 31.8 mmol) was dissolved in DMF (402 mL), and 3,3,3-trifluoropropanoic acid (12.2 g, 95.4 mmol), $NaHCO_3$ (13.4 g, 159 mmol) and HATU (36.3 g, 95.4 mmol) were added. The mixture was stirred for 2 h at rt. Water was added and the mixture was stirred for 30 min at rt. Sat. $NaHCO_3$ solution was added to the mixture which was then extracted with EtOAc. The organic phase was washed three times with half-sat. NaCl solution, dried and concentrated under reduced pressure. The residue was purified by flash chromatography to give **15** (13.5 g, 83% yield, 100% purity by UPLC).

LC-MS (Method 2): ^{t}R (min) = 1.10. MS (ESI+): *m*/*z* = 515 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.48 (br s, 1H), 10.91 (br s, 1H), 8.31 (d, *J* = 5.32 Hz, 1H), 7.99–8.27 (m, 1H), 7.75–7.85 (m, 1H), 7.44–7.73 (m, 1H), 7.18 (s, 1H), 6.99 (d, *J* = 5.32 Hz, 1H), 3.63 (q, *J* = 10.90 Hz, 2H), 3.39–3.54 (m, 5H), 2.29–2.49 (m, 7H), 1.30 (d, *J* = 6.59 Hz, 3H).

3,3,3-Trifluoro-1-{4-[(1*S*)-1-(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)ethyl]piperazin-1-yl}propan-1-one (16)

Racemate **15** (750 mg) was separated by chiral HPLC to give enantiomer **16** (275 mg, 95% purity by NMR, 33% yield) and enantiomer **17** (data below).

HPLC conditions: instrument: Labomatic HD5000, Labocord-5000; Gilson GX-241, Labcol Vario 4000; column: Chiralpak IA 5 µm, 250 x 30 mm; eluent A: MTBE, eluent B: EtOH; isocratic: 90% A + 10% B; flow: 40.0 mL/min; UV: 325 nm. ^{t}R (min) = 15.4–18.6.

$[\alpha]_D^{20}$ –35 (*c* = 1, in DMSO).

LC-MS (Method 2): ^{t}R (min) = 1.13. MS (ESI+): *m*/*z* = 515 $[M + H]^+$.

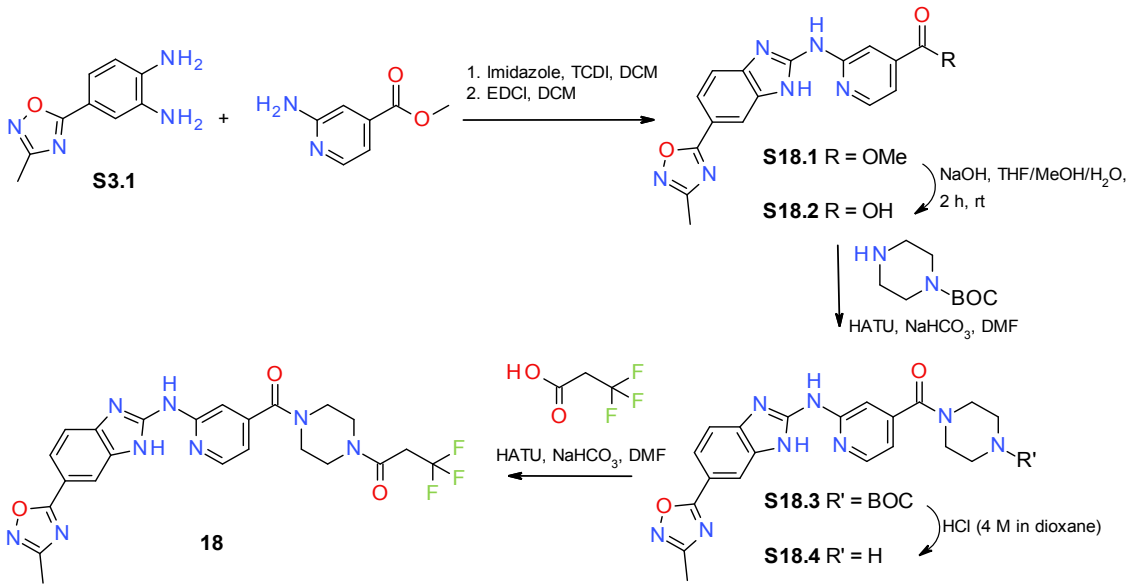
3,3,3-Trifluoro-1-{4-[(1*R*)-1-(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)ethyl]piperazin-1-yl}propan-1-one (17)

Enantiomer **17** (270 mg, 95% purity by NMR, 36% yield).

$[\alpha]_D^{20}$ +37 (*c* = 1, in DMSO).

LC-MS (Method 2): ^tR (min) = 1.13. MS (ESI+): *m/z* = 515 $[M + H]^+$.

Scheme S5



Methyl 2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridine-4-carboxylate (S18.1)

Step 1:

1*H*-Imidazole (35.8 mg, 526 µmol) and TCDI (562 mg, 3.15 mmol) were dissolved in anhydrous DCM (34 mL) under argon. The solution was cooled to 0 °C and methyl 2-aminopyridine-4-carboxylate (400 mg, 2.63 mmol) was added. The mixture was stirred for 2 d at rt. 4-(3-Methyl-1,2,4-oxadiazol-5-yl)benzene-1,2-diamine (**S3.1**, 500 mg, 2.63 mmol) was added and the mixture was stirred for 4 h at rt. The mixture was filtered, washed with water and dried under reduced pressure (520 mg, 93% purity, 48% yield).

LC-MS (Method 2): ^tR (min) = 0.96. MS (ESI+): *m/z* = 385 $[M + H]^+$.

Step 2:

The intermediate from step 1 and EDCI (259 mg, 1.35 mmol) were stirred in DCM (24 mL) under argon overnight at rt. The mixture was diluted with sat. NH_4Cl solution and filtered. The solid was stirred in water

overnight, the resulting suspension was filtered and washed with water, and the solid was dried under reduced pressure at 60 °C to give **S18.1** (140.5 mg, 97% purity, 28% yield), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 1.00. MS (ESI–): *m*/*z* = 349 $[M - H]^-$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.41 (br s, 1H), 11.07 – 11.33 (m, 1H), 8.53 (d, *J* = 5.32 Hz, 1H), 7.98 – 8.37 (m, 1H), 7.75 – 7.87 (m, 2H), 7.48 – 7.72 (m, 1H), 7.42 (dd, *J* = 1.01, 5.32 Hz, 1H), 3.92 (s, 3H), 2.40 (s, 3H).

2-{[6-(3-Methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridine-4-carboxylic acid (S18.2)

Methyl 2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridine-4-carboxylate (**S18.1**, 121 mg, 345 µmol) was stirred in THF (4.0 mL)/MeOH (1.3 mL)/water (1.3 mL) (3:1:1). NaOH (1.7 mL, 2.0 M, 3.4 mmol) was added and the mixture was stirred for 2 h at rt. Then, the mixture was acidified with citric acid (10%). The resulting suspension was concentrated until the organic solvent had evaporated, then was filtered, washed with water and dried under reduced pressure at 60 °C to give **S18.2** (138 mg, 70% purity, 83% yield), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 0.53. MS (ESI+): *m*/*z* = 337 $[M + H]^+$.

tert-Butyl 4-[(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)carbonyl]piperazine-1-carboxylate (S18.3)

2-{[6-(3-Methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridine-4-carboxylic acid (**S18.2**, 83.0 mg, 247 µmol), *tert*-butyl piperazine-1-carboxylate (69.0 mg, 370 µmol), HATU (141.0 mg, 370 µmol) and $NaHCO_3$ (62.2 mg, 740 µmol) were stirred in DMF (3.3 mL) overnight at rt. The mixture was filtered, washed with DCM and the filtrate was concentrated under reduced pressure to give **S18.3** (335 mg), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 1.09. MS (ESI+): *m*/*z* = 505 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (d, *J* = 5.07 Hz, 1H), 8.09 (br s, 1H), 7.74 (br d, *J* = 8.36 Hz, 1H), 7.33 – 7.67 (m, 2H), 6.95 (br d, *J* = 5.07 Hz, 1H), 3.62 (br s, 2H), 3.44 (br s, 2H), 3.35-3.40 (m, 2H), 2.39 (s, 3H), 1.39-1.43 (m, 9H).

(2-{[6-(3-Methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)(piperazin-1-yl)methanone hydrochloride (S18.4)

tert-Butyl 4-[(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)-carbonyl]piperazine-1-carboxylate (**S18.3**, 335 mg, 664 µmol) was stirred in DCM (4.2 mL) and MeOH (2.1 mL), HCl (830 µL, 4.0 M in 1,4-dioxane, 3.3 mmol) was added and the mixture was stirred overnight at rt. The mixture was concentrated under reduced pressure to give **S18.4** (335 mg), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 0.79. MS (ESI–): *m*/*z* = 403 $[M - H]^-$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (br s, 2H), 8.53 (d, *J* = 5.07 Hz, 1H), 8.28 (s, 1H), 7.99 (dd, *J* = 1.39, 8.49 Hz, 1H), 7.75 (d, *J* = 8.36 Hz, 1H), 7.42 (s, 1H), 7.24 (d, *J* = 5.07 Hz, 1H), 3.86 (br s, 2H), 3.55 – 3.62 (m, 2H), 3.04-3.31 (m, 4H), 2.43 (s, 3H).

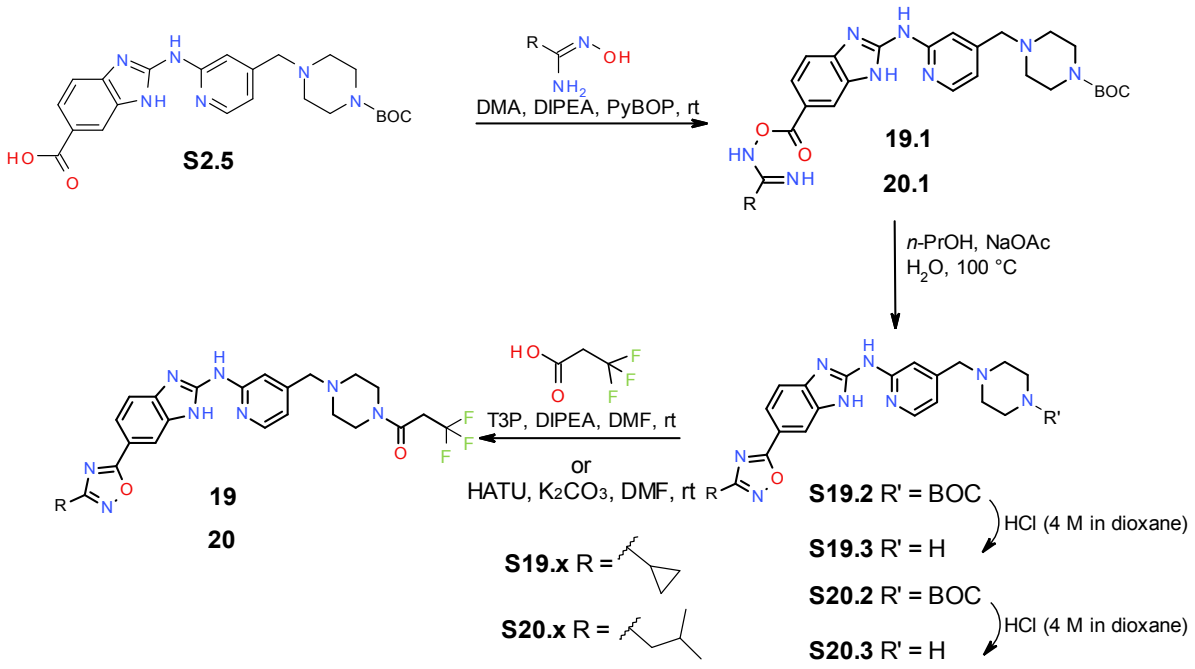
3,3,3-Trifluoro-1-{4-[(2-{[6-(3-methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)carbonyl]piperazin-1-yl}propan-1-one (18)

(2-{[6-(3-Methyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)(piperazin-1-yl)-methanone hydrochloride (**S18.4**, 111 mg,), 3,3,3-trifluoropropanoic acid (57 µL, 650 µmol), $NaHCO_3$ (109 mg, 1.30 mmol) and HATU (246 mg, 648 µmol) were stirred in DMF (2.5 mL) overnight at rt. The residue was purified by preparative HPLC (Method 3) to give **18** (18.0 mg, 90% purity, 15% yield).

LC-MS (Method 2): ^{t}R (min) = 0.94. MS (ESI–): *m*/*z* = 513 $[M - H]^-$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (d, *J* = 5.32 Hz, 1H), 8.17 (br s, 1H), 7.82 (dd, *J* = 1.77, 8.36 Hz, 1H), 7.57 (br s, 1H), 7.27 (br s, 1H), 7.03 (dd, *J* = 1.14, 5.20 Hz, 1H), 4.03 – 4.16 (m, 1H), 3.42 – 3.82 (m, 8H), 2.86 – 2.94 (m, 1H), 2.40 (s, 3H)

Scheme S6



tert-Butyl 4-{[2-({6-[({[cyclopropyl(imino)methyl]amino}oxy)carbonyl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (S19.1)

2-[(4-{[4-(*tert*-Butoxycarbonyl)piperazin-1-yl]methyl}pyridin-2-yl)amino]-1*H*-benzimidazole-6-carboxylic acid (**S2.5**, 18.6 g, 47% purity, 19.3 mmol) was dissolved in DMA (440 mL), *N*-hydroxycyclopropancarboximidanide (5.8 g, 57.9 mmol), DIPEA (67 mL, 390 mmol) and PyBOP (30.2 g, 58.0 mmol) were added and the mixture was stirred overnight at rt. Sat. $NaHCO_3$ solution was added and the mixture was extracted with EtOAc. The organic phase was washed three times with half-sat. NaCl solution, dried and concentrated under reduced pressure. The residue was purified by flash chromatography to give **S19.1** (7.50 g, 73% yield).

LC-MS (Method 2): ^{t}R (min) = 1.12. MS (ESI+): *m/z* = 535 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.30 (br s, 1H), 10.79 (br s, 1H), 8.27 (d, *J* = 5.32 Hz, 1H), 8.05–8.23 (m, 1H), 7.78–7.87 (m, 1H), 7.32–7.60 (m, 1H), 7.19 (s, 1H), 6.95 (d, *J* = 5.07 Hz, 1H), 6.26 (br s, 2H), 3.51 (s, 2H), 3.34–3.41 (m, 4H), 2.36 (br t, *J* = 4.82 Hz, 4H), 1.52 (tt, *J* = 5.32, 8.36 Hz, 1H), 1.40 (s, 9H), 0.82–0.89 (m, 2H), 0.72–0.81 (m, 2H).

tert-Butyl 4-[(2-{[6-(3-cyclopropyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (S19.2)

tert-Butyl 4-{[2-({6-[({[cyclopropyl(imino)methyl]amino}oxy)carbonyl]-1*H*-benzimidazol-2-yl}-amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (**S19.1**, 7.50 g, 14.0 mmol) was dissolved in propan-1-ol (330 mL), NaOAc (1.27 g, 15.4 mmol) and water (170 mL) were added and the mixture was stirred for 72 h at 100 °C. Then, the mixture was concentrated, diluted with DCM/EtOH (9:1), washed with half-sat. $NaHCO_3$ solution and sat. NaCl solution, dried and concentrated under reduced pressure. The residue was purified by flash chromatography to give **S19.2** (6.40 g, 88% yield).

LC-MS (Method 2): ^{t}R (min) = 1.36. MS (ESI+): *m*/*z* = 517 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.50 (br s, 1H), 10.89 (br s, 1H), 8.28 (d, *J* = 5.32 Hz, 1H), 7.95–8.24 (m, 1H), 7.76 (br d, *J* = 7.86 Hz, 1H), 7.44–7.65 (m, 1H), 7.20 (s, 1H), 6.97 (d, *J* = 5.32 Hz, 1H), 3.51 (s, 2H), 3.35–3.39 (m, 4H), 2.37 (br t, *J* = 4.94 Hz, 4H), 2.11–2.22 (m, 1H), 1.40 (s, 9H), 1.07–1.14 (m, 2H), 0.97–1.03 (m, 2H).

6-(3-Cyclopropyl-1,2,4-oxadiazol-5-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S19.3)

tert-Butyl 4-[(2-{[6-(3-cyclopropyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (**S19.2**, 6.40 g, 12.4 mmol) was dissolved in DCM (87 mL) and MeOH (17 mL), HCl (31 mL, 4.0 M in 1,4-dioxane, 120 mmol) was added and the mixture was stirred for 2 h at rt. The mixture was concentrated and stirred in MeOH to give **S19.3** (6.11 g), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 1.05. MS (ESI+): *m*/*z* = 417 $[M + H]^+$.

1-{4-[(2-{[6-(3-Cyclopropyl-1,2,4-oxadiazol-5-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazin-1-yl}-3,3,3-trifluoropropan-1-one (19)

6-(3-Cyclopropyl-1,2,4-oxadiazol-5-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**S19.3**, 100 mg, 221 µmol), 3,3,3-trifluoropropanoic acid (34 µL, 98% purity, 380 µmol), DIPEA (190 µL, 1.1 mmol) and T3P (230 µL, 50% purity in DMF, 400 µmol) were stirred in DMF (2.1 mL) overnight at rt. The mixture was diluted with water and extracted with EtOAc. The organic layer was

washed three times with half-sat. NaCl solution, dried and concentrated. The residue was purified by flash chromatography to give **19** (19.0 mg, 96% purity by UPLC, 15% yield).

LC-MS (Method 1): ^{t}R (min) = 0.92. MS (ESI+): *m*/*z* = 527 $[M + H]^+$.

^{1}H NMR (300 MHz, DMSO-d_6) δ 12.48 (br s, 1H), 10.93 (br s, 1H), 8.29 (d, *J* = 5.27 Hz, 1H), 7.92 – 8.25 (m, 1H), 7.73 – 7.81 (m, 1H), 7.37 – 7.69 (m, 1H), 7.18 (s, 1H), 6.97 (br d, *J* = 5.09 Hz, 1H), 3.65 (q, *J* = 10.93 Hz, 2H), 3.40 – 3.55 (m, 6H), 2.32 – 2.46 (m, 4H), 2.09 – 2.23 (m, 1H), 1.06 – 1.17 (m, 2H), 0.94 – 1.04 (m, 2H).

tert-Butyl 4-[(2-{[6-({[(3-methylbutanimidoyl)amino]oxy}carbonyl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (S20.1)

2-[(4-{[4-(*tert*-Butoxycarbonyl)piperazin-1-yl]methyl}pyridin-2-yl)amino]-1*H*-benzimidazole-6-carboxylic acid (**S2.5**, 400 mg, 46% purity, 407 µmol) was dissolved in *N*-methylpyrrolidone (9.2 mL), *N*'-hydroxy-3-methylbutanimidamide (142 mg, 1.22 mmol), DIPEA (1.4 mL, 8.1 mmol) and PyBOP (635 mg, 1.22 mmol) were added and the mixture was stirred for 1 h at rt. Sat. $NaHCO_3$ solution was added and the mixture was extracted with EtOAc. The organic layer was washed three times with half-sat. NaCl solution, dried and concentrated under reduced pressure. The residue was purified by flash chromatography (DCM/MeOH) to give **S20.1** (190 mg, 85% yield).

LC-MS (Method 2): ^{t}R (min) = 1.23. MS (ESI+): *m*/*z* = 551 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.25–12.35 (m, 1H), 10.68–10.84 (m, 1H), 8.26 (d, *J* = 5.31 Hz, 1H), 8.07–8.23 (m, 1H), 7.76–7.87 (m, 1H), 7.31–7.56 (m, 1H), 7.18 (s, 1H), 6.94 (br d, *J* = 3.28 Hz, 1H), 6.27–6.41 (m, 2H), 3.50 (s, 2H), 3.34 (br s, 4H), 2.35 (br t, *J* = 4.80 Hz, 4H), 1.94 – 2.05 (m, 3H),1.39 (s, 9H), 0.93 (d, *J* = 6.06 Hz, 6H).

tert-Butyl 4-{[2-({6-[3-(2-methylpropyl)-1,2,4-oxadiazol-5-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (S20.2)

Compound **S20.2** was synthesized analogously to **S19.2**, from *tert*-butyl 4-[(2-{[6-({[(3-methylbutanimidoyl)amino]oxy}carbonyl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (**S20.1**, 190 mg, 345 µmol) in 72% yield (133.0 mg).

LC-MS (Method 2): ^{t}R (min) = 1.51. MS (ESI+): *m*/*z* = 533 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.48 (br s, 1H), 10.89 (br s, 1H), 8.29 (d, *J* = 5.07 Hz, 1H), 7.97–8.27 (m, 1H), 7.81 (br d, *J* = 7.60 Hz, 1H), 7.45–7.73 (m, 1H), 7.20 (s, 1H), 6.97 (d, *J* = 5.07 Hz, 1H), 3.52 (s, 2H), 3.35–

3.39 (m, 4H), 2.64 (d, *J* = 7.10 Hz, 2H), 2.35–2.40 (m, 4H), 2.14 (quin d, *J* = 6.70, 13.50 Hz, 1H), 1.40 (s, 9H), 0.99 (d, *J* = 6.59 Hz, 6H).

6-[3-(2-Methylpropyl)-1,2,4-oxadiazol-5-yl]-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S20.3)

Compound **S20.3** was synthesized analogously to **S19.3**, from *tert*-butyl 4-{[2-({6-[3-(2-methylpropyl)-1,2,4-oxadiazol-5-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (**S20.2**, 133 mg, 250 µmol) in quantitative yield.

LC-MS (Method 2): ^{t}R (min) = 1.20. MS (ESI+): *m*/*z* = 433 $[M + H]^+$.

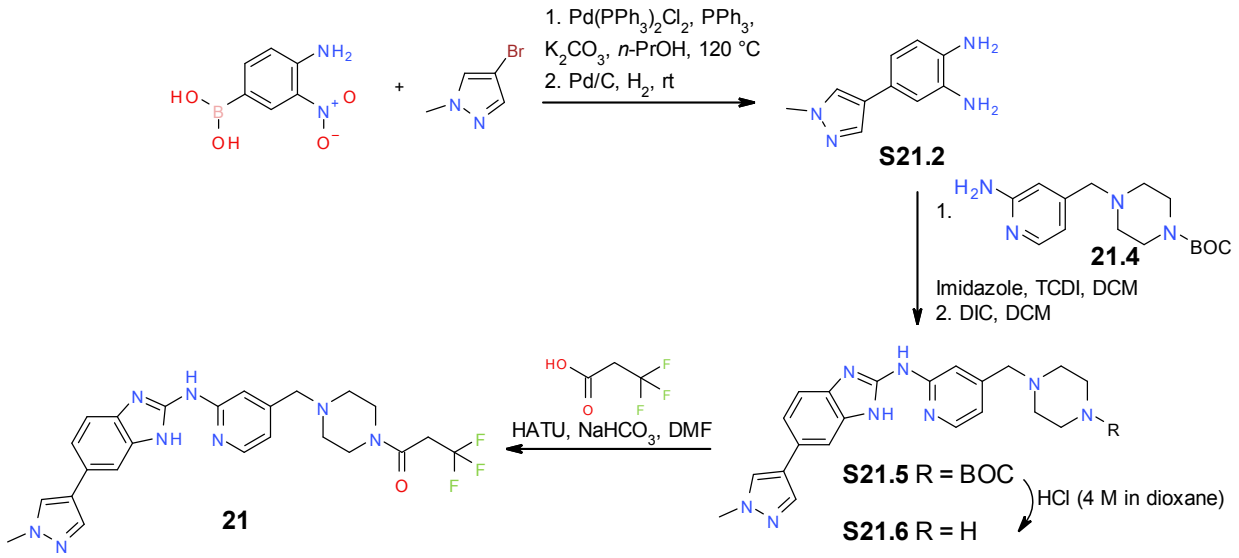
3,3,3-Trifluoro-1-(4-{[2-({6-[3-(2-methylpropyl)-1,2,4-oxadiazol-5-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazin-1-yl)propan-1-one (20)

6-[3-(2-Methylpropyl)-1,2,4-oxadiazol-5-yl]-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**S20.3**, 139 mg, 296 µmol) was dissolved in DMF (3.0 mL), and 3,3,3-trifluoropropanoic acid (55 µL, 98% purity, 590 µmol), K_2CO_3 (205 mg, 1.48 mmol) and HATU (225 mg, 593 µmol) were added. The mixture was stirred for 72 h at rt. Water was added and the mixture was stirred for 30 min at rt. Sat. $NaHCO_3$ solution was added and the mixture was extracted with EtOAc. The organic layer was washed three times with half-sat. NaCl solution, dried and concentrated under reduced pressure. The residue was purified by flash chromatography (DCM/MeOH) to give **20** (40.0 mg, 100% purity by UPLC, 22% yield).

LC-MS (Method 2): ^{t}R (min) = 1.33. MS (ESI+): *m*/*z* = 543 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.48 (br s, 1H), 10.83–10.99 (m, 1H), 8.30 (d, *J* = 5.32 Hz, 1H), 8.00–8.27 (m, 1H), 7.81 (br d, *J* = 8.36 Hz, 1H), 7.45–7.72 (m, 1H), 7.20 (s, 1H), 6.98 (d, *J* = 5.32 Hz, 1H), 3.65 (q, *J* = 10.90 Hz, 2H), 3.54 (s, 2H), 3.50 (td, *J* = 4.75, 14.57 Hz, 4H), 2.64 (d, *J* = 7.10 Hz, 2H), 2.41 (td, *J* = 4.72, 16.41 Hz, 4H), 2.14 (quin d, *J* = 6.82, 13.53 Hz, 1H), 0.99 (d, *J* = 6.84 Hz, 6H).

Scheme S7



4-(1-Methyl-1*H*-pyrazol-4-yl)-2-nitroaniline (S21.1)

4-Amino-3-nitrophenylboronic acid (2.00 g, 11.0 mmol), 4-bromo-1-methyl-1*H*-pyrazole (1.1 mL, 99% purity, 11 mmol), $Pd(PPh_3)_2Cl_2$ (386 mg, 550 µmol), PPh_3 (144 mg, 550 µmol) and K_2CO_3 (16 mL, 2.0 M, 33 mmol) were dissolved in propan-1-ol (50 mL) and the mixture was stirred for 1 h at 120 °C. Then, the mixture was diluted with water and extracted with EtOAc. The organic layer was concentrated and purified by flash chromatography (hexane/EtOAc) to give **S21.1** (1.78 g, 74% yield).

LC-MS (Method 2): ^{t}R (min) = 0.82. MS (ESI+): *m/z* = 219 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.08 (s, 1H), 8.06 (d, *J* = 2.02 Hz, 1H), 7.79 (d, *J* = 0.76 Hz, 1H), 7.63 (dd, *J* = 2.15, 8.72 Hz, 1H), 7.39 (s, 2H), 7.03 (d, *J* = 8.59 Hz, 1H), 3.83 (s, 3H).

4-(1-Methyl-1*H*-pyrazol-4-yl)benzene-1,2-diamine (S21.2)

4-(1-Methyl-1*H*-pyrazol-4-yl)-2-nitroaniline (**S21.1**, 3.28 g, 15.0 mmol) was dissolved in EtOH (15 mL) and DCM (35 mL), 10% Pd/C (800 mg, 752 µmol) was added and the mixture was stirred under hydrogen atmosphere for 72 h at rt. The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (DCM/MeOH) to give **S21.2** (2.17 g, 77% yield).

LC-MS (Method 2): ^{t}R (min) = 0.56. MS (ESI+): *m/z* = 189 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.74 (s, 1H), 7.52 (d, *J* = 0.76 Hz, 1H), 6.66 (d, *J* = 2.02 Hz, 1H), 6.54–6.58 (m, 1H), 6.44–6.49 (m, 1H), 4.39 (br d, *J* = 11.87 Hz, 4H), 3.80 (s, 3H).

4-(Bromomethyl)pyridin-2-amine hydrobromide (21.3)

(2-Aminopyridin-4-yl)methanol (60.0 g, 483 mmol) was stirred in HBr (560 mL, 47% purity, 4.8 mol) overnight at 120 °C. The mixture was concentrated, then diluted with EtOH. The resulting suspension was filtered, and washed with EtOH/hexane (1:1) and hexane. The solid was dried under reduced pressure to give **S21.3** (80.04 g, 62% yield), which was used without further purification.

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.26 (br dd, *J* = 4.06, 9.38 Hz, 1H), 8.12 (br s, 2H), 7.93 (d, *J* = 6.59 Hz, 1H), 7.02 (d, *J* = 1.01 Hz, 1H), 6.87 (dd, *J* = 1.77, 6.59 Hz, 1H), 4.68 (s, 2H).

tert-Butyl 4-[(2-aminopyridin-4-yl)methyl]piperazine-1-carboxylate (S21.4)

4-(Bromomethyl)pyridin-2-amine hydrobromide (**S21.3**, 79.0 g, 295 mmol) was dissolved in acetonitrile (620 mL), and K_2CO_3 (122 g, 884 mmol) and *tert*-butyl piperazine-1-carboxylate (54.9 g, 295 mmol) were added. The mixture was stirred overnight at rt, then diluted with water and extracted twice with EtOAc. The combined organic phases were washed with half-sat. NaCl, dried and concentrated under reduced pressure. The residue was stirred in hexane, filtered and the solid was dried under reduced pressure to give **S21.4** (75.07 g, 87% yield), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 0.98. MS (ESI+): *m*/*z* = 293 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75 – 7.88 (m, 1H), 6.41 (dd, *J* = 1.39, 5.20 Hz, 1H), 6.38 (s, 1H), 5.83 (s, 2H), 3.34 (s, 4H), 2.26 – 2.34 (m, 4H), 1.38 (s, 9H).

tert-Butyl 4-[(2-{[6-(1-methyl-1*H*-pyrazol-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (S21.5)

Step 1:

1*H*-Imidazole (157 mg, 2.31 mmol) and TCDI (2.16 g, 95% purity, 11.5 mmol) were dissolved in DCM (120 mL), cooled to 0 °C, *tert*-butyl 4-[(2-aminopyridin-4-yl)methyl]piperazine-1-carboxylate (**S21.4**, 3.37 g, 11.5 mmol) dissolved in DCM (50 mL) was added and the mixture was stirred overnight at rt. 4-(1-Methyl-1*H*-pyrazol-4-yl)benzene-1,2-diamine (**S21.2**, 2.17 g, 11.5 mmol) dissolved in DCM (50 mL) was added to the mixture which was then stirred for 2 h at rt. Water was added and the phases were separated. The organic layer was dried and filtered.

Step 2:

DIC (5.3 mL, 34 mmol) was added to the solution from step 1 and the mixture was stirred overnight at rt. Sat. NH_4Cl solution was added and and the mixture was stirred for 30 min at rt. The layers were separated and the organic layer was concentrated. The residue was purified by flash chromatography to give **S21.5** (900 mg, 16% yield).

LC-MS (Method 2): ^{t}R (min) = 1.17. MS (ESI+): *m*/*z* = 489 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.04 (br d, *J* = 1.01 Hz, 1H), 10.58 (br s, 1H), 8.25 (d, *J* = 5.32 Hz, 1H), 7.97–8.09 (m, 1H), 7.73–7.84 (m, 1H), 7.49–7.64 (m, 1H), 7.27–7.47 (m, 1H), 7.16–7.27 (m, 2H), 6.88–6.94 (m, 1H), 3.87 (s, 3H), 3.50 (s, 2H), 3.34 (s, 4H), 2.26–2.42 (m, 4H), 1.36–1.45 (m, 9H).

6-(1-Methyl-1*H*-pyrazol-4-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S21.6)

tert-Butyl 4-[(2-{[6-(1-methyl-1*H*-pyrazol-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]-piperazine-1-carboxylate (**S21.5**, 900 mg, 1.84 mmol) was dissolved in DCM (41 mL) and HCl (4.6 mL, 4.0 M in 1,4-dioxane, 18 mmol) was added. The mixture was stirred for 4 h at rt. Then, the mixture was concentrated to give **S21.6** (930 mg), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 0.86. MS (ESI+): *m*/*z* = 389 $[M + H]^+$.

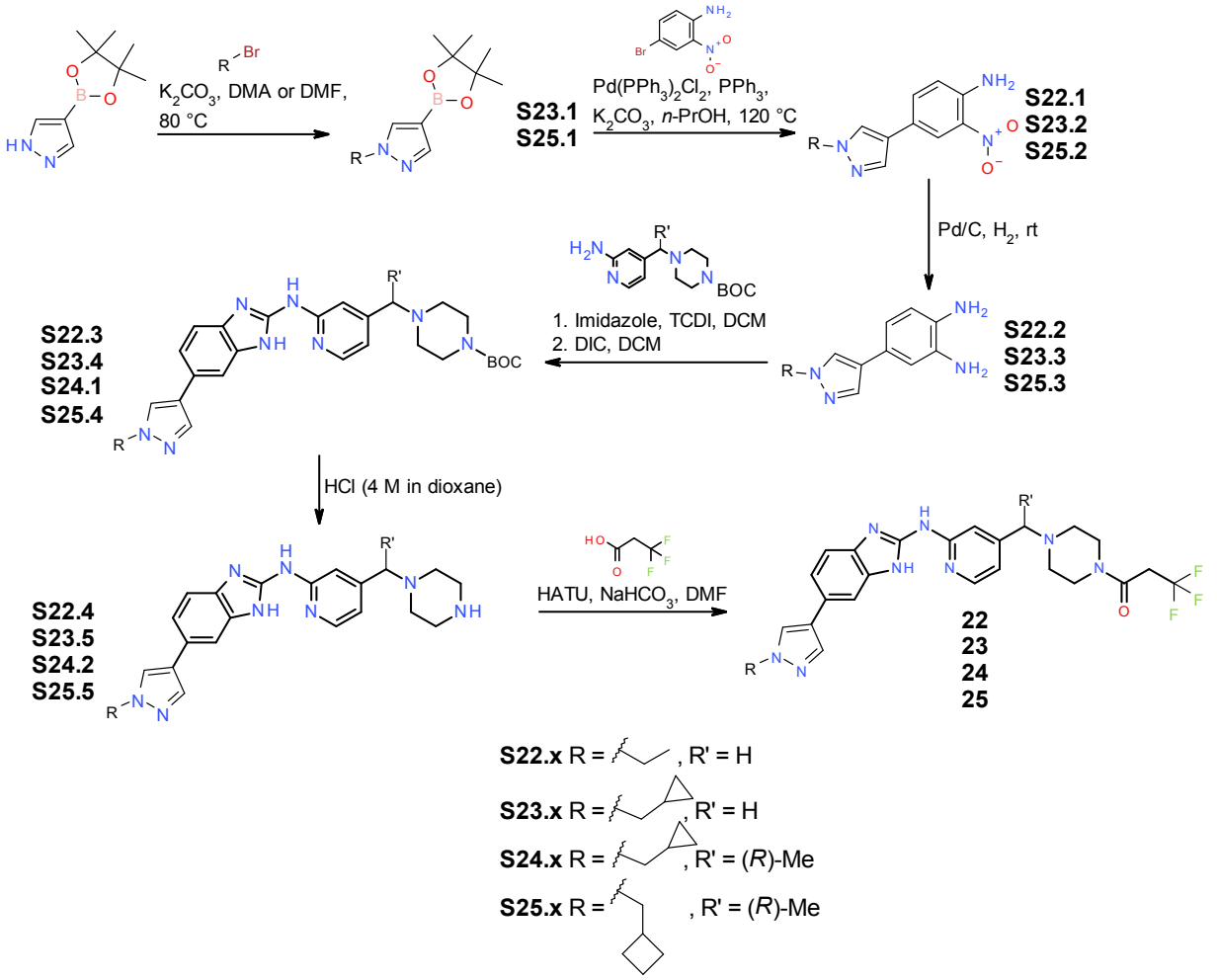
3,3,3-Trifluoro-1-{4-[(2-{[6-(1-methyl-1*H*-pyrazol-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazin-1-yl}propan-1-one (21)

6-(1-Methyl-1*H*-pyrazol-4-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**S21.6**, 120 mg, 80% purity, 208 µmol) was dissolved in DMF (4.6 mL), 3,3,3-trifluoropropanoic acid (28 µL, 98% purity, 310 µmol), $NaHCO_3$ (105 mg, 1.25 mmol) and HATU (119 mg, 312 µmol) were added and the mixture was stirred overnight at rt. Water was added and the mixture was extracted with EtOAc. The organic layer was concentrated and purified by flash chromatography (DCM/MeOH) to give **21** (46.0 mg, 90% purity, 40% yield).

LC-MS (Method 2): ^{t}R (min) = 0.98. MS (ESI+): *m*/*z* = 499 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.94–12.17 (m, 1H), 10.48–10.70 (m, 1H), 8.26 (d, *J* = 5.32 Hz, 1H), 8.03 (br s, 1H), 7.78 (br s, 1H), 7.29–7.64 (m, 2H), 7.16–7.27 (m, 2H), 6.92 (d, *J* = 5.07 Hz, 1H), 3.87 (s, 3H), 3.66 (q, *J* = 10.90 Hz, 2H), 3.45–3.56 (m, 6H), 2.41 (td, *J* = 4.59, 16.67 Hz, 4H).

Scheme S8



4-(1-Ethyl-1*H*-pyrazol-4-yl)-2-nitroaniline (S22.1)

Compound **S22.1** was synthesized analogously to **S21.1**, from 4-bromo-2-nitroaniline (2.00 g, 9.22 mmol) and 1-ethyl-1*H*-pyrazol-4-ylboronic acid (2.17 g, 95% purity, 14.7 mmol) with stirring for 6 h at 120 °C in 61% yield (1.31 g, 90% purity)

LC-MS (Method 2): ^{t}R (min) = 0.64. MS (ESI+): *m/z* = 233 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.17 (d, *J* = 0.76 Hz, 1H), 8.10 (d, *J* = 2.03 Hz, 1H), 7.82 (d, *J* = 0.76 Hz, 1H), 7.66 (dd, *J* = 2.15, 8.74 Hz, 1H), 7.43 (s, 2H), 7.04 (d, *J* = 8.62 Hz, 1H), 4.13 (q, *J* = 7.27 Hz, 2H), 1.40 (t, *J* = 7.35 Hz, 3H).

4-(1-Ethyl-1*H*-pyrazol-4-yl)benzene-1,2-diamine (S22.2)

Compound **S22.2** was synthesized analogously to **S21.2**, from 4-(1-ethyl-1*H*-pyrazol-4-yl)-2-nitroaniline (**S22.1**, 1.30 g, 5.60 mmol) with stirring for 2 h at rt.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80 (s, 1H), 7.54 (s, 1H), 6.68 (d, *J* = 1.77 Hz, 1H), 6.58 (dd, *J* = 2.03, 7.86 Hz, 1H), 6.47 (d, *J* = 7.86 Hz, 1H), 4.43 (s, 2H), 4.41 (s, 2H), 4.09 (q, *J* = 7.35 Hz, 2H), 1.37 (t, *J* = 7.22 Hz, 3H).

tert-Butyl 4-[(2-{[6-(1-ethyl-1*H*-pyrazol-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (S22.3)

Compound **S22.3** was synthesized analogously to **S21.5**, from *tert*-butyl 4-[(2-aminopyridin-4-yl)methyl]piperazine-1-carboxylate (**S21.4**, 850 mg, 2.91 mmol) and 4-(1-ethyl-1*H*-pyrazol-4-yl)benzene-1,2-diamine (**S22.2**, 706 mg, 3.49 mmol).

LC-MS (Method 2): ^{t}R (min) = 1.25. MS (ESI+): *m*/*z* = 503 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (br s, 1H), 10.57 (br s, 1H), 8.24 (d, *J* = 5.58 Hz, 1H), 8.02–8.15 (m, 1H), 7.71 – 7.85 (m, 1H), 7.48–7.68 (m, 1H), 7.20 – 7.47 (m, 2H), 7.17 (s, 1H), 6.90 (dd, *J* = 1.14, 5.20 Hz, 1H), 4.14 (q, *J* = 7.35 Hz, 2H), 3.49 (s, 2H), 3.35–3.40 (m, 2H), 2.35 (br t, *J* = 4.82 Hz, 4H), 1.35–1.46 (m, 12H).

6-(1-Ethyl-1*H*-pyrazol-4-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S22.4)

Compound **S22.4** was synthesized analogously to **S21.6**, from *tert*-butyl 4-[(2-{[6-(1-ethyl-1*H*-pyrazol-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (**S22.3**, 145 mg, 288 µmol) with DCM/MeOH (3:1) as solvent and stirring for 2 h at rt.

LC-MS (Method 2): ^{t}R (min) = 0.91. MS (ESI+): *m*/*z* = 403 $[M + H]^+$.

1-{4-[(2-{[6-(1-Ethyl-1*H*-pyrazol-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazin-1-yl}-3,3,3-trifluoropropan-1-one (22)

Compound **22** was synthesized analogously to **21**, from 6-(1-ethyl-1*H*-pyrazol-4-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**S22.4**, 115 mg, 80% purity, 210 µmol) and 3,3,3-trifluoropropanoic acid (28 µL, 310 µmol) in 41% yield (44 mg, 96 % purity by UPLC).

LC-MS (Method 2): ^{t}R (min) = 1.05. MS (ESI+): *m*/*z* = 513 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.03 (br s, 1H), 10.59 (br s, 1H), 8.26 (d, *J* = 5.07 Hz, 1H), 8.02–8.15 (m, 1H), 7.79 (br d, *J* = 16.48 Hz, 1H), 7.50–7.66 (m, 1H), 7.17–7.47 (m, 3H), 6.92 (dd, *J* = 1.01, 5.32 Hz, 1H), 4.15 (q, *J* = 7.10 Hz, 2H), 3.66 (q, *J* = 11.07 Hz, 2H), 3.44–3.56 (m, 6H), 2.41 (td, *J* = 4.91, 16.29 Hz, 4H), 1.42 (t, *J* = 7.35 Hz, 3H).

1-(Cyclopropylmethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (S23.1)

4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (6.00 g, 30.9 mmol) was dissolved in DMF (60 mL), K_2CO_3 (12.8 g, 92.8 mmol) and (bromomethyl)cyclopropane (18.0 mL, 186 mmol) were added and the mixture was stirred for 5 h at 80 °C. The reaction mixture was cooled to rt and diluted with water. The aqueous phase was extracted with EtOAc. The organic layer was washed with brine, filtered through a silicone filter and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc) to give **S23.1** (4.05 g, 53% yield).

LC-MS (Method 1): ^{t}R (min) = 1.15. MS (ESI+): *m*/*z* = 249 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96 (s, 1H), 7.57 (s, 1H), 3.96 (d, *J* = 7.10 Hz, 2H), 1.18–1.30 (m, 13H), 0.47–0.55 (m, 2H), 0.32–0.38 (m, 2H).

4-[1-(Cyclopropylmethyl)-1*H*-pyrazol-4-yl]-2-nitroaniline (S23.2)

Compound **S23.2** was synthesized analogously to **S21.1**, from 4-bromo-2-nitroaniline (2.62 g, 12.1 mmol) and 1-(cyclopropylmethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (**S23.1**, 4.10 g, 95% purity, 15.7 mmol) with stirring for 2 h at 120 °C.

LC-MS (Method 2): ^{t}R (min) = 1.02. MS (ESI+): *m*/*z* = 259 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (d, *J* = 0.76 Hz, 1H), 8.10 (d, *J* = 2.03 Hz, 1H), 7.81 (d, *J* = 0.76 Hz, 1H), 7.66 (dd, *J* = 2.15, 8.74 Hz, 1H), 7.43 (s, 2H), 7.04 (d, *J* = 8.62 Hz, 1H), 3.95 (d, *J* = 7.10 Hz, 2H), 1.19–1.32 (m, 1H), 0.50–0.57 (m, 2H), 0.35–0.41 (m, 2H).

4-[1-(Cyclopropylmethyl)-1*H*-pyrazol-4-yl]benzene-1,2-diamine (S23.3)

Compound **S23.3** was synthesized analogously to **S21.2**, from 4-[1-(cyclopropylmethyl)-1*H*-pyrazol-4-yl]-2-nitroaniline (**S23.2**, 2.60 g, 10.1 mmol) with only EtOH as solvent and stirring for 6 h at rt in quantitative yield

LC-MS (Method 2): ^{t}R (min) = 0.61. MS (ESI+): *m*/*z* = 229[M + H]$^{+}$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.84 (d, *J* = 0.76 Hz, 1H), 7.56 (d, *J* = 0.76 Hz, 1H), 6.72 (d, *J* = 2.03 Hz, 1H), 6.61 (dd, *J* = 2.03, 7.86 Hz, 1H), 6.51 (d, *J* = 7.86 Hz, 1H), 4.70 (br s, 3H), 3.93 (d, *J* = 7.10 Hz, 2H), 3.34 (s, 1H), 1.17–1.31 (m, 1H), 0.46–0.58 (m, 2H), 0.32-–0.40 (m, 2H).

tert-Butyl 4-{[2-({6-[1-(cyclopropylmethyl)-1*H*-pyrazol-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (S23.4)

Compound **S23.4** was synthesized analogously to **S21.5**, from *tert*-butyl 4-[(2-aminopyridin-4-yl)methyl]piperazine-1-carboxylate (**S21.4**, 1.14 g, 3.89 mmol) and 4-[1-(cyclopropylmethyl)-1*H*-pyrazol-4-yl]benzene-1,2-diamine (**S23.3**, 1.10 g, 97% purity, 4.67 mmol).

LC-MS (Method 2): ^{t}R (min) = 1.28. MS (ESI+): *m*/*z* = 529[M + H]$^{+}$.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.02 (br s, 1H), 10.57 (br s, 1H), 8.24 (d, *J* = 5.32 Hz, 1H), 8.03–8.17 (m, 1H), 7.74–7.87 (m, 1H), 7.49–7.69 (m, 1H), 7.21–7.46 (m, 2H), 7.17 (s, 1H), 6.90 (d, *J* = 5.32 Hz, 1H), 3.97 (d, *J* = 6.84 Hz, 2H), 3.49 (s, 2H), 3.31–3.39 (m, 7H), 2.35 (br t, *J* = 4.94 Hz, 4H), 1.39 (s, 19H), 1.21-1.33 (m, 1H), 0.52-–0.58 (m, 2H), 0.36–0.43 (m, 2H).

6-[1-(Cyclopropylmethyl)-1*H*-pyrazol-4-yl]-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S23.5)

Compound **S23.5** was synthesized analogously to **S21.6**, from *tert*-butyl 4-{[2-({6-[1-(cyclopropylmethyl)-1*H*-pyrazol-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (**S23.4**, 355 mg, 672 µmol) with stirring for 2 h at rt.

LC-MS (Method 2): ^{t}R (min) = 1.01. MS (ESI+): *m*/*z* = 429[M + H]$^{+}$.

1-(4-{[2-({6-[1-(Cyclopropylmethyl)-1*H*-pyrazol-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazin-1-yl)-3,3,3-trifluoropropan-1-one (23)

Compound **23** was synthesized analogously to **21**, from 6-[1-(cyclopropylmethyl)-1*H*-pyrazol-4-yl]-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**S23.5**, 160 mg, 344 µmol) and 3,3,3-trifluoropropanoic acid (132 mg, 1.03 mmol) with stirring for 2 h at rt in 56% (104 mg, 96% purity by UPLC).

LC-MS (Method 2): ^{t}R (min) = 1.11. MS (ESI+): *m*/*z* = 539[M + H]$^{+}$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.03 (br s, 1H), 10.59 (br s, 1H), 8.26 (d, *J* = 5.07 Hz, 1H), 8.05–8.16 (m, 1H), 7.74–7.84 (m, 1H), 7.51–7.67 (m, 1H), 7.22–7.48 (m, 2H), 7.19 (s, 1H), 6.92 (dd, *J* = 1.01, 5.32 Hz, 1H), 3.98 (d, *J* = 7.10 Hz, 2H), 3.66 (q, *J* = 11.07 Hz, 2H), 3.43–3.55 (m, 6H), 2.41 (td, *J* = 4.75, 16.35 Hz, 4H), 1.28 (t quin, *J* = 4.78, 7.56 Hz, 1H), 0.52–0.59 (m, 2H), 0.38–0.44 (m, 2H).

tert-Butyl 4-[(1*R*)-1-(2-aminopyridin-4-yl)ethyl]piperazine-1-carboxylate (39)

tert-Butyl 4-[1-(2-aminopyridin-4-yl)ethyl]piperazine-1-carboxylate (**S15.3**, 625 g, 90% purity, 1836 mmol) was dissolved in propan-2-ol (13.5 L) and warmed to 40 °C, a solution of (2*R*,3*R*)-2,3-bis(benzoyloxy)butanedioic acid (330 g, 917 mmol) in propan-2-ol (7.5 L) was added dropwise and the suspension was stirred overnight at rt. The mixture was filtered through Fisherbrand MF200 glass microfiber filter paper. The solid was dissolved in EtOAc (5 L) and washed with half-sat. aq K_2CO_3 solution (5 L). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was purified by preparative HPLC to give **39** (152 g, 27 % yield, enantiomeric ratio: 94:6).

The protocol was repeated using the 152 g batch and 123 g were obtained with an enantiomeric ratio of 98.8:1.2

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.81 (d, *J*=5.32 Hz, 1H), 6.39–6.46 (m, 1H), 6.36 (s, 1H), 5.81 (s, 2H), 3.28 (br s, 4H), 3.22 (q, *J*=6.67 Hz, 1H), 2.17–2.38 (m, 4H), 1.21 (d, *J*=6.84 Hz, 3H).

tert-Butyl 4-{(1*R*)-1-[2-({6-[1-(cyclopropylmethyl)-1*H*-pyrazol-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]ethyl}piperazine-1-carboxylate (S24.1)

Compound **S24.1** was synthesized analogously to **S21.5**, from *tert*-butyl 4-[(1*R*)-1-(2-aminopyridin-4-yl)ethyl]piperazine-1-carboxylate (**39**, 1.24 g, 4.05 mmol) and 4-[1-(cyclopropylmethyl)-1*H*-pyrazol-4-yl]benzene-1,2-diamine (**S23.3**, 1.14 g, 97% purity, 4.86 mmol).

LC-MS (Method 2): ^{t}R (min) = 1.32. MS (ESI+): *m*/*z* = 543 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.03 (br s, 1H), 10.56 (br s, 1H), 8.24 (d, *J*=5.32 Hz, 1H), 8.01–8.20 (m, 1H), 7.73–7.87 (m, 1H), 7.49–7.68 (m, 1H), 7.20–7.47 (m, 2H), 7.16 (s, 1H), 6.92 (dd, *J*=1.01, 5.32 Hz, 1H), 3.98 (d, *J*=6.84 Hz, 2H), 3.43 (q, *J*=6.42 Hz, 1H), 3.32 (br s, 4H), 2.24–2.45 (m, 4H), 1.38 (s, 9H), 1.28 (d, *J*=6.59 Hz, 4H), 0.50–0.59 (m, 2H), 0.35–0.44 (m, 2H).

6-[1-(Cyclopropylmethyl)-1*H*-pyrazol-4-yl]-*N*-{4-[(1*R*)-1-(piperazin-1-yl)ethyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S24.2)

Compound **S24.2** was synthesized analogously to **S21.6**, from *tert*-butyl 4-{(1*R*)-1-[2-({6-[1-(cyclopropylmethyl)-1*H*-pyrazol-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]ethyl}piperazine-1-carboxylate (**S24.1**, 0.39 g, 0.71 mmol) with DCM/MeOH (2:1) as solvent and stirring for 2 h at rt in quantitative yield.

LC-MS (Method 2): ^{t}R (min) = 1.00. MS (ESI+): *m*/*z* = 443 $[M + H]^+$.

1-(4-{1-[2-({6-[1-(Cyclopropylmethyl)-1*H*-pyrazol-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]ethyl}piperazin-1-yl)-3,3,3-trifluoropropan-1-one (24)

Compound **24** was synthesized analogously to **21**, from 6-[1-(cyclopropylmethyl)-1*H*-pyrazol-4-yl]-*N*-{4-[(1*R*)-1-(piperazin-1-yl)ethyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**S24.2**, 120 mg, 251 µmol) and 3,3,3-trifluoropropanoic acid (96.2 mg, 752 µmol) with stirring for 2 h at rt, in 44 % yield (61 mg, 95% purity by NMR).

$[\alpha]_D^{20}$ +30.4 (*c* = 1, in DMSO).

LC-MS (Method 2): ^{t}R (min) = 1.15. MS (ESI+): *m*/*z* = 553 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.03 (br s, 1H), 10.57 (br s, 1H), 8.25 (d, *J*=5.32 Hz, 1H), 8.03–8.17 (m, 1H), 7.70–7.86 (m, 1H), 7.49–7.67 (m, 1H), 7.20–7.48 (m, 2H), 7.17 (s, 1H), 6.93 (dd, *J*=1.14, 5.45 Hz, 1H), 3.98 (d, *J*=7.10 Hz, 2H), 3.62 (q, *J*=10.98 Hz, 2H), 3.41–3.52 (m, 5H), 2.28–2.47 (m, 4H), 1.24–1.34 (m, 4H), 0.50–0.58 (m, 2H), 0.33–0.44 (m, 2H)

1-(Cyclobutylmethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (S25.1)

Compound **S25.1** was synthesized analogously to **S23.1**, from 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (2.00 g, 10.3 mmol) and (bromomethyl)cyclobutane (2.3 mL, 21 mmol) with stirring overnight at 80 °C in 80 % yield (2.4 g, 90% purity).

LC-MS (Method 1): ^{t}R (min) = 1.24. MS (ESI+): *m*/*z* = 263 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.90 (d, *J*=0.76 Hz, 1H), 7.55 (s, 1H), 4.12 (d, *J*=7.35 Hz, 2H), 2.65–2.80 (m, 1H), 1.88–2.00 (m, 2H), 1.66–1.88 (m, 4H), 1.24 (s, 12H).

4-[1-(Cyclobutylmethyl)-1*H*-pyrazol-4-yl]-2-nitroaniline (S25.2)

Compound **S25.2** was synthesized analogously to **S21.1**, from 4-bromo-2-nitroaniline (1.60 g, 7.37 mmol) and 1-(cyclobutylmethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (**S25.1**, 1.93 g, 7.37 mmol) with stirring for 2 h at 120 °C in 85% yield (1.80 g, 95% purity)

LC-MS (Method 2): ^{t}R (min) = 1.13. MS (ESI+): *m*/*z* = 273 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.12 (d, *J*=0.76 Hz, 1H), 8.08 (d, *J*=2.03 Hz, 1H), 7.80 (d, *J*=1.01 Hz, 1H), 7.65 (dd, *J*=2.15, 8.74 Hz, 1H), 7.42 (s, 2H), 7.03 (d, *J*=8.62 Hz, 1H), 4.11 (d, *J*=7.35 Hz, 2H), 2.75 (spt, *J*=7.52 Hz, 1H), 1.93–2.05 (m, 2H), 1.72–1.90 (m, 4H).

4-[1-(Cyclobutylmethyl)-1*H*-pyrazol-4-yl]benzene-1,2-diamine (S25.3)

Compound **S25.3** was synthesized analogously to **S21.2**, from 4-[1-(cyclobutylmethyl)-1*H*-pyrazol-4-yl]-2-nitroaniline (**S25.2**, 1.80 g, 6.61 mmol) with only EtOH as solvent and stirring for 3 h at rt.

LC-MS (Method 2): ^{t}R (min) = 0.87. MS (ESI+): *m*/*z* = 243 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.76 (s, 1H), 7.54 (d, *J*=0.76 Hz, 1H), 6.68 (d, *J*=2.03 Hz, 1H), 6.58 (dd, *J*=2.03, 7.86 Hz, 1H), 6.48 (d, *J*=7.86 Hz, 1H), 4.46 (br s, 4H), 4.08 (d, *J*=7.35 Hz, 2H), 3.17 (d, *J*=5.07 Hz, 1H), 2.67–2.81 (m, 1H), 1.92–2.03 (m, 2H), 1.68–1.89 (m, 4H).

tert-Butyl 4-{(1*R*)-1-[2-({6-[1-(cyclobutylmethyl)-1*H*-pyrazol-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]ethyl}piperazine-1-carboxylate (S25.4)

Compound **S25.4** was synthesized analogously to **S21.5**, from *tert*-butyl 4-[(1*R*)-1-(2-aminopyridin-4-yl)ethyl]piperazine-1-carboxylate (**39**, 550 mg, 1.79 mmol) and 4-[1-(cyclobutylmethyl)-1*H*-pyrazol-4-yl]benzene-1,2-diamine (**S25.3**, 538 mg, 97% purity, 2.15 mmol). Step 2 was performed with EDCI instead of DIC. **S25.4** was obtained in 67% yield (680 mg).

LC-MS (Method 2): ^{t}R (min) = 1.40. MS (ESI+): *m*/*z* = 557 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.02 (br s, 1H), 10.55 (br s, 1H), 8.24 (d, *J*=5.32 Hz, 1H), 7.98–8.11 (m, 1H), 7.73–7.84 (m, 1H), 7.19–7.65 (m, 3H), 7.16 (s, 1H), 6.92 (dd, *J*=1.14, 5.45 Hz, 1H), 4.13 (br d, *J*=7.10 Hz, 2H), 3.32 (br s, 4H), 2.78 (spt, *J*=7.39 Hz, 1H), 2.24-2.44 (m, 4H), 1.95-2.06 (m, 2H), 1.75-1.90 (m, 4H), 1.38 (s, 10H), 1.28 (d, *J*=6.84 Hz, 3H).

6-[1-(Cyclobutylmethyl)-1*H*-pyrazol-4-yl]-*N*-{4-[(1*R*)-1-(piperazin-1-yl)ethyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S25.5)

Compound **S25.5** was synthesized analogously to **S21.6**, from *tert*-butyl 4-{(1*R*)-1-[2-({6-[1-(cyclobutylmethyl)-1*H*-pyrazol-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]ethyl}piperazine-1-carboxylate (**S25.4**, 600 mg, 1.08 mmol) with DCM/MeOH (9:1) as solvent and stirring overnight at rt.

LC-MS (Method 2): ^{t}R (min) = 1.12. MS (ESI+): *m*/*z* = 457 $[M + H]^+$.

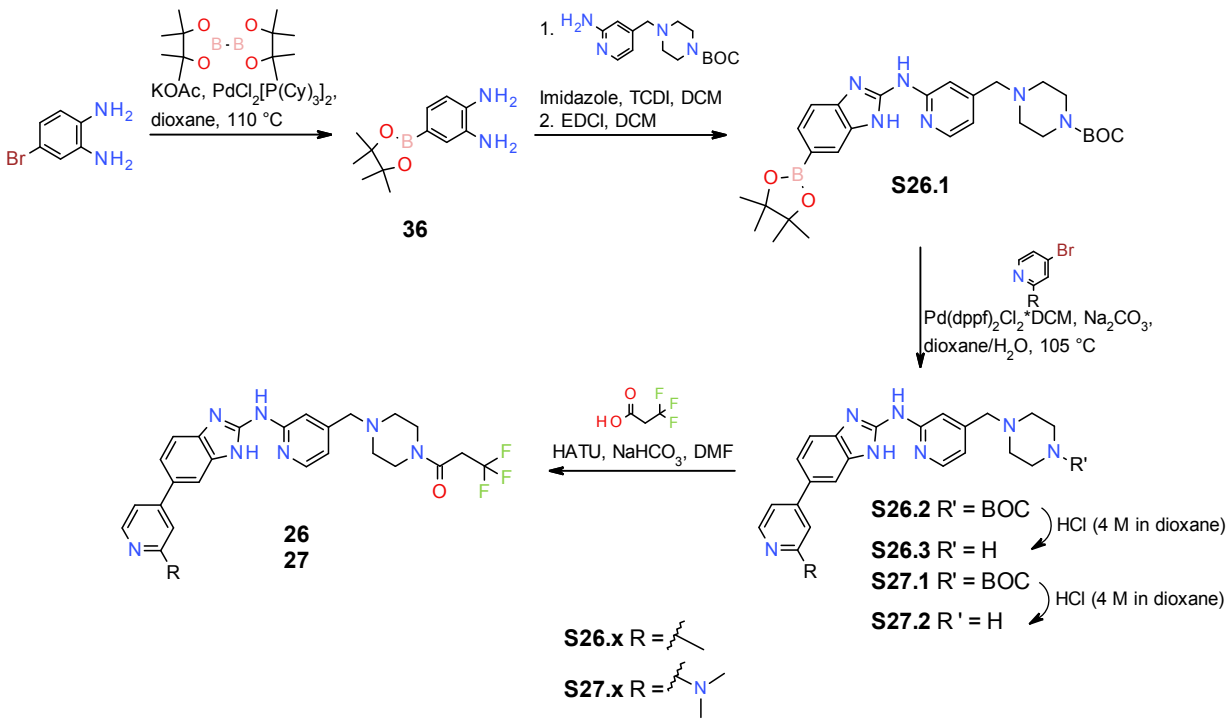
1-(4-{(1*R*)-1-[2-({6-[1-(Cyclobutylmethyl)-1*H*-pyrazol-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]ethyl}piperazin-1-yl)-3,3,3-trifluoropropan-1-one (25)

Compound **25** was synthesized analogously to **21**, from 6-[1-(cyclobutylmethyl)-1*H*-pyrazol-4-yl]-*N*-{4-[(1*R*)-1-(piperazin-1-yl)ethyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (crude **S25.5**, 150 mg, 61% purity, 162 µmol) and 3,3,3-trifluoropropanoic acid (32.0 mg, 242 µmol) with stirring overnight at rt.

LC-MS (Method 2): ^{t}R (min) = 1.24. MS (ESI+): *m*/*z* = 567 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.03 (br s, 1H), 10.57 (br s, 1H), 8.25 (d, *J*=5.32 Hz, 1H), 8.04 (br s, 1H), 7.78 (br s, 1H), 7.57 (br s, 1H), 7.37 (br s, 1H), 7.23 (br d, *J*=7.60 Hz, 1H), 7.17 (s, 1H), 6.93 (br d, *J*=5.32 Hz, 1H), 4.13 (br d, *J*=7.35 Hz, 2H), 3.62 (q, *J*=10.98 Hz, 2H), 3.40–3.52 (m, 4H), 2.78 (td, *J*=7.45, 14.76 Hz, 1H), 2.28–2.46 (m, 4H), 1.95–2.07 (m, 2H), 1.73–1.93 (m, 4H), 1.30 (br d, *J*=6.59 Hz, 3H).

Scheme S9



4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzene-1,2-diamine (36)

4-Bromobenzene-1,2-diamine (25.0 g, 134 mmol) was dissolved in 1,4-dioxane (1.0 L). 4,4,4',4',5,5,5',5'-Octamethyl-2,2'-bi-1,3,2-dioxaborolane (40.7 g, 160 mmol), KOAc (65.6 g, 668 mmol) and $Pd[P(Cy)_3]_2Cl_2$ (4.93 g, 6.68 mmol) were added and the mixture was stirred overnight at 110 °C. The mixture was filtered, washed with DCM and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc) to give **36** (22.68 g, 98% purity, 71% yield).

LC-MS (Method 2): ^{t}R (min) = 0.89. MS (ESI+): *m/z* = 235 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.88 (d, *J* = 1.27 Hz, 1H), 6.76 (dd, *J* = 1.27, 7.60 Hz, 1H), 6.45 (d, *J* = 7.60 Hz, 1H), 4.82 (s, 2H), 4.37 (br s, 2H), 1.23 (s, 12H).

tert-Butyl 4-[(2-{[6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (S26.1)

Step 1:

1*H*-Imidazole (174 mg, 2.56 mmol), TCDI (2.40 g, 13.5 mmol) and *tert*-butyl 4-[(2-aminopyridin-4-yl)methyl]piperazine-1-carboxylate (**S21.4**, 3.75 g, 12.8 mmol) were dissolved in DCM (75 mL) and the mixture was stirred at 0 °C for 30 min, then stored in a fridge overnight. 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzene-1,2-diamine (**36**, 3.00 g, 12.8 mmol) dissolved in DCM (9 mL) was added and the mixture was stirred at rt overnight. The mixture was diluted with water and extracted three times with DCM. The combined organic layers were washed with water and brine, dried and concentrated under reduced pressure.

Step 2:

The crude material was dissolved in DCM (150 mL) and EDCI (2.80 g, 14.6 mmol) was added. The mixture was stirred for 2 d under nitrogen atmosphere, then diluted with water and extracted three times with DCM. The combined organic layers were washed with water and brine, dried and concentrated under reduced pressure. The crude product was stirred in EtOAc, the mixture was filtered, and the precipitate was dried at 50 °C under reduced pressure to give **S26.1** (3.69 g, 47% yield), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 1.44. MS (ESI+): *m*/*z* = 535 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.05–12.21 (m, 1H), 10.67 (br s, 1H), 8.24 (d, *J* = 5.32 Hz, 1H), 7.61–7.88 (m, 1H), 7.10–7.49 (m, 3H), 6.91 (d, *J* = 5.32 Hz, 1H), 3.49 (s, 2H), 3.34–3.39 (m, 4H), 2.35 (br t, *J* = 4.82 Hz, 4H), 1.39 (s, 9H), 1.30 (s, 12H).

tert-Butyl 4-[(2-{[6-(2-methylpyridin-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (S26.2)

tert-Butyl 4-[(2-{[6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (**S26.1**, 500 mg, 936 µmol), 4-bromo-2-methylpyridine (225 mg, 1.31 mmol), Pd(dppf)$_2$Cl$_2$·DCM (115 mg, 140 µmol) and Na_2CO_3 (297 mg, 2.81 mmol) were dissolved in 1,4-dioxane (4.5 mL) and water (910 µL). The mixture was stirred at 105 °C for 19 h. Then, the mixture was diluted with DCM, filtered and the filtrate was concentrated. The residue was purified by flash chromatography (DCM/EtOH) to give **S26.2** (388 mg, 83% yield).

LC-MS (Method 2): ^{t}R (min) = 1.24. MS (ESI+): *m*/*z* = 500 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.02–12.28 (m, 1H), 10.67 (br s, 1H), 8.24 (d, *J*=5.32 Hz, 1H), 7.60–7.88 (m, 1H), 7.27–7.54 (m, 2H), 7.09–7.23 (m, 1H), 6.91 (d, *J*=5.30 Hz, 1H), 3.49 (s, 2H), 3.34–3.38 (m, 2H), 2.35 (br t, *J*=4.82 Hz, 4H), 1.39 (s, 9H), 1.30 (s, 12H).

6-(2-Methylpyridin-4-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S26.3)

Compound **S26.3** was synthesized analogously to **S21.6**, from *tert*-butyl 4-[(2-{[6-(2-methylpyridin-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (**S26.2**, 388 mg, 777 µmol) with DCM/MeOH (9:1) as solvent and stirring overnight at rt.

LC-MS (Method 2): ^{t}R (min) = 0.92. MS (ESI+): *m*/*z* = 400 [M + H]$^+$.

3,3,3-Trifluoro-1-{4-[(2-{[6-(2-methylpyridin-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazin-1-yl}propan-1-one (26)

Compound **26** was synthesized analogously to **21**, from 6-(2-methylpyridin-4-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**S26.3**, 81.0 mg, 186 µmol) and 3,3,3-trifluoropropanoic acid (35.7 mg, 279 µmol) with stirring for 2 d at rt in 31 % yield (33 mg, 95% purity by NMR).

LC-MS (Method 2): ^{t}R (min) = 1.05. MS (ESI+): *m*/*z* = 510 [M + H]$^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.17–12.29 (m, 1H), 10.73 (br s, 1H), 8.45 (br d, *J* = 4.05 Hz, 1H), 8.28 (d, *J* = 5.32 Hz, 1H), 7.73–7.94 (m, 1H), 7.41–7.63 (m, 4H), 7.20 (s, 1H), 6.95 (br d, *J* = 4.82 Hz, 1H), 3.66 (q, *J* = 10.98 Hz, 2H), 3.53 (s, 2H), 3.44–3.52 (m, 4H), 2.53 (s, 3H), 2.41 (td, *J* = 4.94, 16.73 Hz, 4H).

tert-Butyl 4-{[2-({6-[2-(dimethylamino)pyridin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (S27.1)

Compound **S27.1** was synthesized analogously to **S26.2**, from *tert*-butyl 4-[(2-{[6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (**S26.1**, 500 mg, 936 µmol) and 4-bromo-*N*,*N*-dimethylpyridin-2-amine (245 mg, 1.22 mmol) with DME as the only solvent and stirring for 6 h at 150 °C.

LC-MS (Method 2): ^{t}R (min) = 1.33. MS (ESI+): *m*/*z* = 529 [M + H]$^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.19 (br d, *J*=13.43 Hz, 1H), 10.61–10.73 (m, 1H), 8.26 (d, *J*=5.58 Hz, 1H), 8.10 (br d, *J*=5.07 Hz, 1H), 7.67–7.92 (m, 1H), 7.36–7.61 (m, 2H), 7.18 (br s, 1H), 6.93 (d, *J*=5.07 Hz, 1H), 6.79–6.90 (m, 2H), 3.50 (s, 2H), 3.35–3.41 (m, 4H), 3.09 (s, 5H), 2.32–2.41 (m, 4H), 1.39 (s, 9H).

6-[2-(Dimethylamino)pyridin-4-yl]-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S27.2)

Compound **S27.2** was synthesized analogously to **S21.6**, from *tert*-butyl 4-{[2-({6-[2-(dimethylamino)pyridin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (**S27.1**, 12.7 mg, 24.0 µmol) with DCM/MeOH (2:1) as solvent and stirring overnight at rt.

LC-MS (Method 2): ^{t}R (min) = 1.01. MS (ESI–): *m*/*z* = 427 $[M - H]^-$.

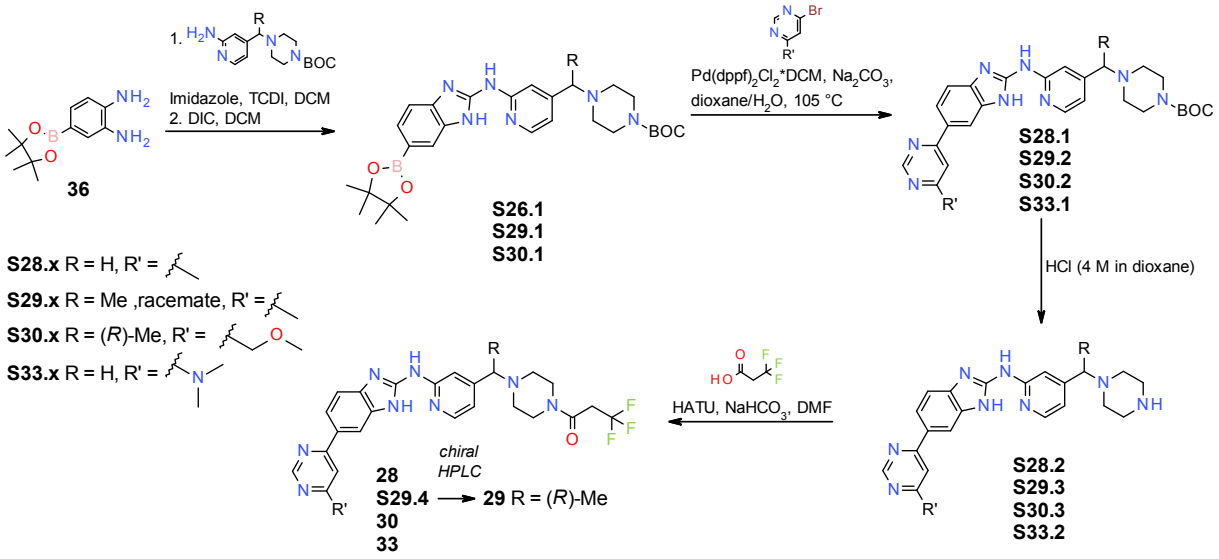
1-(4-{[2-({6-[2-(Dimethylamino)pyridin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazin-1-yl)-3,3,3-trifluoropropan-1-one (27)

Compound **27** was synthesized analogously to **21**, from 6-[2-(dimethylamino)pyridin-4-yl]-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**S27.2**, 353 mg, 704 µmol) and 3,3,3-trifluoropropanoic acid (190 µL, 2.1 mmol) with stirring overnight at rt in 22% yield (95% purity by UPLC)

LC-MS (Method 2): ^{t}R (min) = 1.14. MS (ESI+): *m*/*z* = 539 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.13–12.25 (m, 1H), 10.61–10.74 (m, 1H), 8.27 (d, *J*=5.32 Hz, 1H), 8.06–8.14 (m, 1H), 7.67–7.92 (m, 1H), 7.44–7.62 (m, 1H), 7.37–7.44 (m, 1H), 7.20 (br s, 1H), 6.94 (d, *J*=5.07 Hz, 1H), 6.78–6.91 (m, 2H), 3.65 (q, *J*=10.98 Hz, 2H), 3.43–3.55 (m, 6H), 3.02–3.18 (m, 6H), 2.40 (td, *J*=4.91, 16.79 Hz, 4H).

Scheme S10



tert-Butyl 4-[(2-{[6-(6-methylpyrimidin-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (S28.1)

Compound **S28.1** was synthesized analogously to **S26.2**, from *tert*-butyl 4-[(2-{[6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (**S26.1**, 500 mg, 936 µmol) and 4-bromo-6-methylpyrimidine (291 mg, 1.68 mmol).

LC-MS (Method 2): ^{t}R (min) = 1.20. MS (ESI+): *m*/*z* = 501 $[M + H]^+$.

6-(6-Methylpyrimidin-4-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S28.2)

Compound **S28.2** was synthesized analogously to **S21.6**, from *tert*-butyl 4-[(2-{[6-(6-methylpyrimidin-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (**S28.1**, 480 mg, 959 µmol) with DCM/MeOH (9:1) as solvent and stirring overnight at rt.

LC-MS (Method 2): ^{t}R (min) = 0.86. MS (ESI+): *m*/*z* = 401 $[M + H]^+$.

3,3,3-Trifluoro-1-{4-[(2-{[6-(6-methylpyrimidin-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazin-1-yl}propan-1-one (28)

Compound **28** was synthesized analogously to **21**, from 6-(6-methylpyrimidin-4-yl)-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**S28.2**, 150 mg, 80% purity, 300 µmol) and

3,3,3-trifluoropropanoic acid (57.6 mg, 449 µmol) with stirring overnight at rt in 36% yield (60 mg, 92% purity by UPLC).

LC-MS (Method 2): ^{t}R (min) = 1.04. MS (ESI+): *m*/*z* = 511 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.87–12.57 (m, 1H), 10.60–11.02 (m, 1H), 9.02 (d, *J*=1.01 Hz, 1H), 8.28 (d, *J*=5.32 Hz, 1H), 8.15–8.47 (m, 1H), 7.84–8.06 (m, 2H), 7.38–7.69 (m, 1H), 7.21 (s, 1H), 6.95 (d, *J*=5.07 Hz, 1H), 3.65 (q, *J*=11.07 Hz, 2H), 3.44–3.57 (m, 7H), 2.85 (s, 2H), 2.40 (td, *J*=4.82, 16.73 Hz, 4H).

tert-Butyl 4-[1-(2-{[6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)ethyl]piperazine-1-carboxylate (S29.1)

Compound **S29.1** was synthesized analogously to **S26.1**, from *tert*-butyl 4-[1-(2-aminopyridin-4-yl)ethyl]piperazine-1-carboxylate (**S15.3**, 10.3 g, 33.5 mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene-1,2-diamine (**36**, 7.84 g, 33.5 mmol).

LC-MS (Method 2): ^{t}R (min) = 1.49. MS (ESI+): *m*/*z* = 549 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.97–12.20 (m, 1H), 10.47–10.73 (m, 1H), 8.25 (d, *J* = 5.58 Hz, 1H), 7.73–7.91 (m, 1H), 7.25–7.51 (m, 2H), 7.10–7.22 (m, 1H), 6.87–6.96 (m, 1H), 3.43 (q, *J* = 6.67 Hz, 1H), 3.32 (br s, 4H), 2.35–2.44 (m, 2H), 2.24–2.34 (m, 2H), 1.37 (s, 12H), 1.30 (s, 12H).

tert-Butyl 4-[1-(2-{[6-(6-methylpyrimidin-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)ethyl]piperazine-1-carboxylate (S29.2)

Compound **S29.2** was synthesized analogously to **S26.2**, from *tert*-butyl 4-[1-(2-{[6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)ethyl]piperazine-1-carboxylate (**S29.1**, 1.00 g, 1.82 mmol) and 4-bromo-6-methylpyrimidine (631 mg, 3.65 mmol).

LC-MS (Method 2): ^{t}R (min) = 1.24. MS (ESI+): *m*/*z* = 515 $[M + H]^+$.

6-(6-Methylpyrimidin-4-yl)-*N*-{4-[1-(piperazin-1-yl)ethyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S29.3)

Compound **S29.3** was synthesized analogously to **S21.6**, from *tert*-butyl 4-[1-(2-{[6-(6-methylpyrimidin-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)ethyl]piperazine-1-carboxylate (**S29.2**, 700 mg, 1.36 mmol) with DCM/MeOH (6:1) as solvent and stirring for 3 h at rt.

LC-MS (Method 2): ^{t}R (min) = 0.89. MS (ESI+): *m*/*z* = 415 $[M + H]^+$.

3,3,3-Trifluoro-1-{4-[1-(2-{[6-(6-methylpyrimidin-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)ethyl]piperazin-1-yl}propan-1-one (S29.4)

Compound **S29.4** was synthesized analogously to **21**, from 6-(6-methylpyrimidin-4-yl)-*N*-{4-[1-(piperazin-1-yl)ethyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**S29.3**, 300 mg, 724 µmol) and 3,3,3-trifluoropropanoic acid (139 mg, 1.09 mmol) with stirring overnight at rt.

LC-MS (Method 2): ^{t}R (min) = 1.04. MS (ESI+): *m*/*z* = 525 $[M + H]^+$.

3,3,3-Trifluoro-1-{4-[(1*R*)-1-(2-{[6-(6-methylpyrimidin-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)ethyl]piperazin-1-yl}propan-1-one (29)

Racemate **S29.4** (55 mg) was separated by chiral HPLC to give compound **29** (16.0 mg, 99% purity, 29% yield).

HPLC conditions: instrument: Labomatic HD5000, Labocord-5000; Gilson GX-241, Labcol Vario 4000; column: Chiralpak IA 5 µm, 250 x 30 mm; eluent A: MTBE + 0.1 vol% diethylamine (99%), eluent B: acetonitrile; isocratic: 50% A + 50% B; flow: 60.0 mL/min; UV: 325 nm. ^{t}R (min) = 7.00–8.80.

$[\alpha]_D^{20}$ +35 (*c* = 1, in DMSO).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.31 (br d, *J* = 16.48 Hz, 1H), 10.78 (br s, 1H), 9.02 (d, *J* = 1.01 Hz, 1H), 8.29 (d, *J* = 5.32 Hz, 1H), 8.17–8.43 (m, 1H), 7.85–8.03 (m, 2H), 7.40–7.64 (m, 1H), 7.18 (s, 1H), 6.97 (br d, *J* = 4.56 Hz, 1H), 3.63 (q, *J* = 11.07 Hz, 2H), 3.42–3.53 (m, 5H), 2.30–2.47 (m, 5H), 1.31 (d, *J* = 6.84 Hz, 3H), 1.11 (s, 2H).

tert-Butyl 4-[(1*R*)-1-(2-{[6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)ethyl]piperazine-1-carboxylate (S30.1)

Compound **S30.1** was synthesized analogously to **S26.1**, from *tert*-butyl 4-[(1*R*)-1-(2-aminopyridin-4-yl)ethyl]piperazine-1-carboxylate (**39**, 3.67 g, 12.0 mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene-1,2-diamine (**36**, 2.80 g, 12.0 mmol). The first mixture of step 1 was stored in a freezer for 36 h. **S30.1** was obtained in 45% yield (2.50 g).

tert-Butyl 4-{(1*R*)-1-[2-({6-[6-(methoxymethyl)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]ethyl}piperazine-1-carboxylate (S30.2)

Compound **S30.2** was synthesized analogously to **S26.2**, from *tert*-butyl 4-[(1*R*)-1-(2-{[6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)ethyl]piperazine-1-carboxylate (**S30.1**, 330 mg, 602 µmol) and 4-chloro-6-(methoxymethyl)pyrimidine (286 mg, 1.80 mmol).

LC-MS (Method 2): ^{t}R (min) = 1.23. MS (ESI+): *m*/*z* = 545 $[M + H]^+$.

6-[6-(Methoxymethyl)pyrimidin-4-yl]-*N*-{4-[(1*R*)-1-(piperazin-1-yl)ethyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S30.3)

Compound **S30.3** was synthesized analogously to **S21.6**, from *tert*-butyl 4-{(1*R*)-1-[2-({6-[6-(methoxymethyl)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]ethyl}piperazine-1-carboxylate (**S30.2**, 280 mg, 514 µmol) with DCM/MeOH (2:1) as solvent and stirring overnight at rt.

LC-MS (Method 2): ^{t}R (min) = 0.92. MS (ESI+): *m*/*z* = 445 $[M + H]^+$.

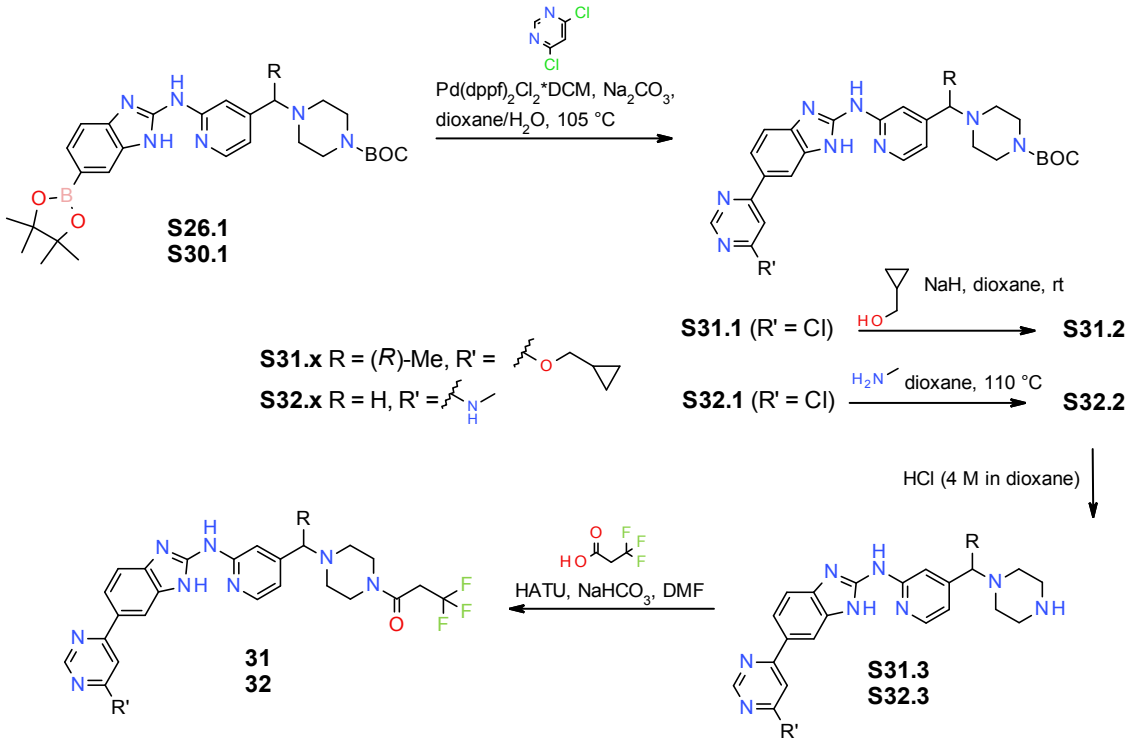
3,3,3-Trifluoro-1-(4-{(1*R*)-1-[2-({6-[6-(methoxymethyl)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]ethyl}piperazin-1-yl)propan-1-one (30)

Compound **30** was synthesized analogously to **21**, from 6-[6-(methoxymethyl)pyrimidin-4-yl]-*N*-{4-[(1*R*)-1-(piperazin-1-yl)ethyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (crude **S30.3**, 280 mg) and 3,3,3-trifluoropropanoic acid (130 µL, 1.5 mmol) with stirring for 3 h at rt in 37% yield (105 mg, 100% purity by UPLC).

LC-MS (Method 2): ^{t}R (min) = 1.07. MS (ESI+): *m*/*z* = 555 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.23–12.46 (m, 1H), 10.70–10.89 (m, 1H), 9.09 (s, 1H), 8.29 (d, *J* = 5.32 Hz, 1H), 8.13–8.47 (m, 1H), 7.83–8.03 (m, 2H), 7.40–7.67 (m, 1H), 7.17 (s, 1H), 6.97 (br d, *J* = 4.82 Hz, 1H), 4.55 (s, 2H), 3.62 (q, *J* = 11.07 Hz, 2H), 3.40–3.53 (m, 8H), 2.22–2.46 (m, 4H), 1.30 (d, *J* = 6.59 Hz, 3H).

Scheme S11



tert-Butyl 4-[(1*R*)-1-(2-{[6-(6-chloropyrimidin-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)ethyl]piperazine-1-carboxylate (S31.1)

Compound **S31.1** was synthesized analogously to **S26.2**, from *tert*-butyl 4-[(1*R*)-1-(2-{[6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)ethyl]piperazine-1-carboxylate (**S30.1**, 1.00 g, 85% purity, 1.55 mmol) and 4,6-dichloropyrimidine (693 mg, 4.65 mmol) with stirring overnight at 120 °C.

LC-MS (Method 2): ^{t}R (min) = 1.38. MS (ESI+): *m*/*z* = 535 [M + H]$^{+}$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.26–12.45 (m, 1H), 10.70–10.91 (m, 1H), 9.01 (s, 1H), 8.13–8.49 (m, 3H), 8.00 (br d, *J* = 8.36 Hz, 1H), 7.41–7.64 (m, 1H), 7.17 (s, 1H), 6.96 (br s, 1H), 3.45 (q, *J* = 6.42 Hz, 1H), 3.29–3.33 (m, 4H), 2.22–2.45 (m, 5H), 1.38 (s, 9H), 1.29 (d, *J* = 6.59 Hz, 3H).

tert-Butyl 4-{(1*R*)-1-[2-({6-[6-(cyclopropylmethoxy)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]ethyl}piperazine-1-carboxylate (S31.2)

tert-Butyl 4-[(1*R*)-1-(2-{[6-(6-chloropyrimidin-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)-ethyl]piperazine-1-carboxylate (**S31.1**, 60.0 mg, 112 µmol) was dissolved in 1,4-dioxane (770 µL) and 60%

NaH (26.9 mg, 673 µmol) was added portionwise. Cyclopropylmethanol (53 µL, 670 µmol) was added dropwise to the mixture which was then stirred for 30 min at rt. The reaction mixture was diluted with EtOAc and quenched with water. The layers were separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried and concentrated under reduced pressure to give **S31.2** (64.2 mg, 95% purity, 95% yield), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 1.47. MS (ESI+): *m*/*z* = 571 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.21–12.40 (m, 1H), 10.61–10.80 (m, 1H), 8.74–8.81 (m, 1H), 8.27 (d, *J* = 5.07 Hz, 1H), 8.15–8.40 (m, 1H), 7.92 (br d, *J* = 6.59 Hz, 1H), 7.31–7.65 (m, 2H), 7.17 (br s, 1H), 6.95 (br d, *J* = 4.31 Hz, 1H), 4.23 (d, *J* = 7.10 Hz, 2H), 3.45 (q, *J* = 6.76 Hz, 1H), 2.24–2.45 (m, 4H), 1.38 (s, 9H), 1.25–1.34 (m, 4H), 0.55–0.62 (m, 2H), 0.34–0.41 (m, 2H).

6-[6-(Cyclopropylmethoxy)pyrimidin-4-yl]-*N*-{4-[(1*R*)-1-(piperazin-1-yl)ethyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S31.3)

Compound **S31.3** was synthesized analogously to **S21.6**, from *tert*-butyl 4-{(1*R*)-1-[2-({6-[6-(cyclopropylmethoxy)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]ethyl}piperazine-1-carboxylate (**S31.2**, 62.0 mg, 109 µmol) with DCM/MeOH (9:1) as solvent, cooling to 0 °C before addition of HCl and stirring overnight at rt.

LC-MS (Method 2): ^{t}R (min) = 1.25. MS (ESI+): *m*/*z* = 471 $[M + H]^+$.

1-(4-{(1*R*)-1-[2-({6-[6-(Cyclopropylmethoxy)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]ethyl}piperazin-1-yl)-3,3,3-trifluoropropan-1-one (31)

Compound **31** was synthesized analogously to **21**, from 6-[6-(cyclopropylmethoxy)pyrimidin-4-yl]-*N*-{4-[(1*R*)-1-(piperazin-1-yl)ethyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (**S31.3**, 46.0 mg, 81% purity, 64.2 µmol) and 3,3,3-trifluoropropanoic acid (8.5 µL, 96 µmol) with stirring overnight at rt in 43% yield (18 mg, 95% purity by NMR).

LC-MS (Method 2): ^{t}R (min) = 1.30. MS (ESI+): *m*/*z* = 581 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.21–13.31 (m, 1H), 10.68–10.81 (m, 1H), 8.77 (d, *J* = 1.01 Hz, 1H), 8.28 (d, *J* = 5.32 Hz, 1H), 8.13–8.43 (m, 1H), 7.86–7.98 (m, 1H), 7.32–7.61 (m, 2H), 7.17 (br d, *J* = 4.56 Hz, 1H), 6.96 (br s, 1H), 4.23 (d, *J* = 7.10 Hz, 2H), 3.62 (q, *J* = 10.90 Hz, 2H), 3.40–3.53 (m, 5H), 2.27–2.47 (m, 5H), 1.30 (d, *J* = 6.84 Hz, 4H), 0.55–0.63 (m, 2H), 0.34–0.42 (m, 2H).

tert-Butyl 4-[(2-{[6-(6-chloropyrimidin-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (S32.1)

Compound **S32.1** was synthesized analogously to **S26.2**, from *tert*-butyl 4-[(2-{[6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (**S26.1**, 1.50 g, 2.81 mmol) and 4,6-dichloropyrimidine (1.25 g, 8.42 mmol) with stirring overnight at 110 °C.

LC-MS (Method 2): ^{t}R (min) = 1.33. MS (ESI+): *m*/*z* = 521 $[M + H]^+$.

tert-Butyl 4-{[2-({6-[6-(methylamino)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (S32.2)

tert-Butyl 4-[(2-{[6-(6-chloropyrimidin-4-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (**S32.1**, 400 mg, 70% purity, 537 µmol) and methylamine (1.3 mL, 2.0 M in THF, 2.7 mmol) were stirred in 1,4-dioxane (5.3 mL) overnight at 110 °C. The mixture was filtered and the filtrate was concentrated under reduced pressure to give **S32.2** (330 mg, 57% purity, 68% yield), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 1.13. MS (ESI+): *m*/*z* = 516 $[M + H]^+$.

6-[6-(Methylamino)pyrimidin-4-yl]-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S32.3)

Compound **S32.3** was synthesized analogously to **S21.6**, from *tert*-butyl 4-{[2-({6-[6-(methylamino)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (**S32.2**, 6.50 mg, 12.6 µmol) with DCM/MeOH (2:1) as solvent and stirring for 1 h at rt.

LC-MS (Method 2): ^{t}R (min) = 0.81. MS (ESI+): *m*/*z* = 414 $[M + H]^+$.

3,3,3-Trifluoro-1-(4-{[2-({6-[6-(methylamino)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazin-1-yl)propan-1-one (32)

Compound **32** was synthesized analogously to **21**, from 6-[6-(methylamino)pyrimidin-4-yl]-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (crude **S32.3**, 100 mg) and

3,3,3-trifluoropropanoic acid (50 µL, 570 µmol) with stirring overnight at rt in 10% yield (11 mg, 100% purity by UPLC).

LC-MS (Method 2): ^{t}R (min) = 0.95. MS (ESI+): *m*/*z* = 526 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.23 (br d, *J* = 10.65 Hz, 1H), 10.71 (br s, 1H), 8.47 (br s, 1H), 8.27 (d, *J* = 5.32 Hz, 1H), 8.22 (br s, 0.5H), 7.76 (br s, 0.5H), 7.34–7.58 (m, 1H), 7.15–7.32 (m, 2H), 6.94 (d, *J* = 5.07 Hz, 1H), 6.84–6.92 (m, 1H), 3.65 (q, *J* = 10.90 Hz, 2H), 3.49–3.56 (m, 4H), 3.44–3.49 (m, 2H), 2.86 (d, *J* = 4.56 Hz, 3H), 2.40 (dt, *J* = 16.22, 4.69 Hz, 4H).

tert-Butyl 4-{[2-({6-[6-(dimethylamino)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (S33.1)

Compound **S33.1** was synthesized analogously to **S26.2**, from *tert*-butyl 4-[(2-{[6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (**S26.1**, 187 mg, 350 µmol) and 6-bromo-*N*,*N*-dimethylpyrimidin-4-amine (106 mg, 525 µmol) with stirring overnight at 110 °C.

LC-MS (Method 2): ^{t}R (min) = 1.20. MS (ESI+): *m*/*z* = 530 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.16–12.29 (m, 1H), 10.64–10.76 (m, 1H), 8.52 (d, *J* = 1.27 Hz, 1H), 8.26 (d, *J* = 5.07 Hz, 1H), 8.10–8.35 (m, 1H), 7.89 (br t, *J* = 7.22 Hz, 1H), 7.35–7.60 (m, 1H), 7.15–7.23 (m, 1H), 7.00–7.14 (m, 1H), 6.93 (br d, *J* = 5.32 Hz, 1H), 3.50 (s, 2H), 3.35 (br s, 4H), 3.15 (s, 6H), 2.36 (br t, *J* = 4.94 Hz, 4H), 1.40 (s, 9H).

6-[6-(Dimethylamino)pyrimidin-4-yl]-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S33.2)

Compound **S33.2** was synthesized analogously to **S21.6**, from *tert*-butyl 4-{[2-({6-[6-(dimethylamino)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (**S33.1**, 30.0 mg, 56.6 µmol) with DCM/MeOH (4:1) as solvent and stirring over the weekend at rt.

LC-MS (Method 2): ^{t}R (min) = 0.88. MS (ESI+): *m*/*z* = 430 $[M + H]^+$.

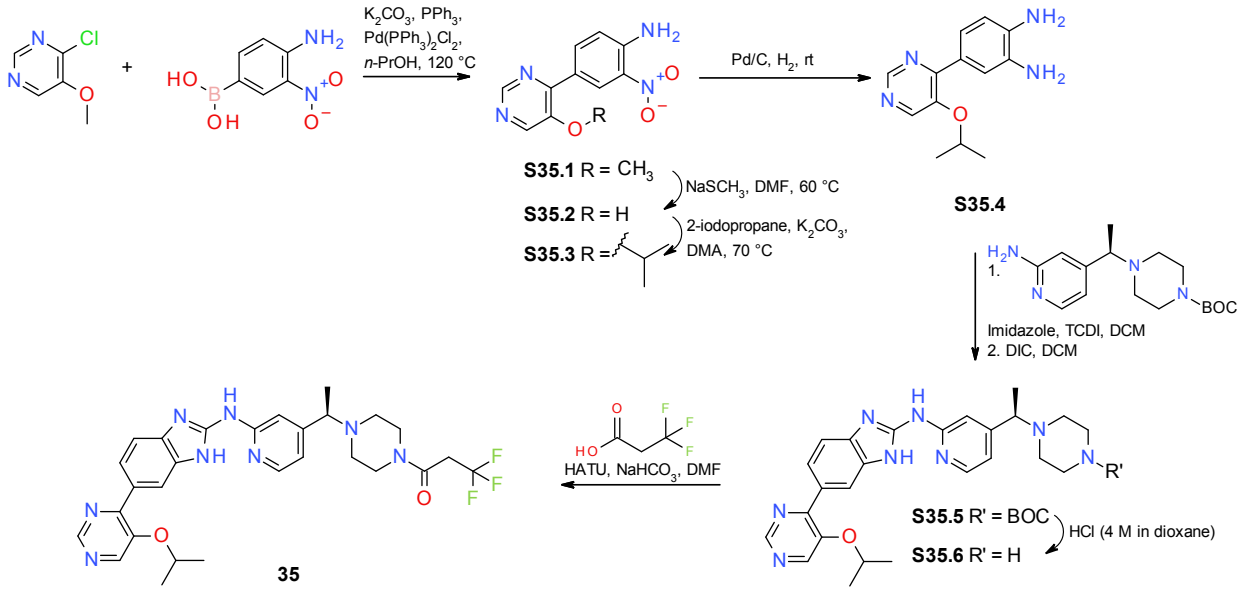
1-(4-{[2-({6-[6-(Dimethylamino)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazin-1-yl)-3,3,3-trifluoropropan-1-one (33)

Compound **33** was synthesized analogously to **21**, from 6-[6-(dimethylamino)pyrimidin-4-yl]-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (crude **S33.2**, 60.0 mg, 111 µmol) and 3,3,3-trifluoropropanoic acid (29 µL, 330 µmol) with stirring for 90 min at rt in 32% yield (20 mg, 100% purity by UPLC).

LC-MS (Method 2): ^{t}R (min) = 1.03. MS (ESI+): *m/z* = 540 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.09–12.38 (m, 1H), 10.72 (br d, *J* = 1.01 Hz, 1H), 8.52 (d, *J* = 0.76 Hz, 1H), 8.07–8.36 (m, 2H), 7.89 (br d, *J* = 7.86 Hz, 1H), 7.34–7.64 (m, 1H), 7.20 (s, 1H), 7.01–7.14 (m, 1H), 6.94 (d, *J* = 5.07 Hz, 1H), 3.65 (q, *J* = 11.07 Hz, 2H), 3.43–3.56 (m, 6H), 3.15 (s, 6H), 2.28–2.44 (m, 4H).

Scheme S12



4-(5-Methoxypyrimidin-4-yl)-2-nitroaniline (S35.1)

Compound **S35.1** was synthesized analogously to **S21.1**, from 4-chloro-5-methoxypyrimidine (1.10 g, 98% purity, 7.46 mmol) and 4-amino-3-nitrophenylboronic acid (2.44 g, 13.4 mmol) with stirring for 3 h at reflux.

LC-MS (Method 2): ^{t}R (min) = 0.85. MS (ESI+): *m/z* = 247 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.97 (d, *J* = 2.03 Hz, 1H), 8.81 (s, 1H), 8.65 (s, 1H), 8.25 (dd, *J* = 2.15, 9.00 Hz, 1H), 7.83 (s, 2H), 7.11 (d, *J* = 9.12 Hz, 1H), 4.03 (s, 3H).

4-(4-Amino-3-nitrophenyl)pyrimidin-5-ol (S35.2)

4-(5-Methoxypyrimidin-4-yl)-2-nitroaniline (**S35.1**, 1.40 g, 5.69 mmol) was dissolved in DMF (21 mL), sodium methanethiolate (1.99 g, 28.4 mmol) was added and the mixture was stirred for 3 h at 60 °C. Sat. NaCl solution was added to the mixture which was then extracted with $CHCl_3$/MeOH (9:1). The aqueous layer was dried under reduced pressure to give **S35**. which was used without further purification.

LC-MS (Method 1): ^tR (min) = 0.70. MS (ESI+): m/z = 233 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.88 (d, J = 1.77 Hz, 1H), 8.71 (dd, J = 2.03, 8.87 Hz, 1H), 7.78 (s, 1H), 7.58 (s, 1H), 7.43 (s, 2H), 6.95 (d, J = 9.12 Hz, 1H).

2-Nitro-4-{5-[(propan-2-yl)oxy]pyrimidin-4-yl}aniline (S35.3)

4-(4-Amino-3-nitrophenyl)pyrimidin-5-ol (crude **S35.2**) was dissolved in DMA (30 mL), K_2CO_3 (2.36 g, 17.1 mmol) and 2-iodopropane (850 µL, 8.5 mmol) were added and the mixture was stirred at 70 °C for 1 h. Water was added to the mixture which was then extracted with EtOAc. The organic layer was washed with half-sat. NaCl solution, dried and concentrated. The crude product was purified by flash chromatography to give **S35.3** (822 mg, 53% yield over two steps).

LC-MS (Method 1): ^tR (min) = 1.00. MS (ESI+): m/z = 275 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.13 (d, J = 2.03 Hz, 1H), 8.79 (s, 1H), 8.66 (s, 1H), 8.29 (dd, J = 2.15, 9.00 Hz, 1H), 7.83 (s, 2H), 7.11 (d, J = 9.12 Hz, 1H), 4.93 (sept, J = 6.04 Hz, 1H), 1.38 (d, J = 6.08 Hz, 6H).

4-{5-[(Propan-2-yl)oxy]pyrimidin-4-yl}benzene-1,2-diamine (S35.4)

Compound **S35.4** was synthesized analogously to **S21.2**, from 2-nitro-4-{5-[(propan-2-yl)oxy]pyrimidin-4-yl}aniline (**S35.3**, 865 mg, 3.15 mmol) with stirring for 2 h at rt in quantitative yield.

LC-MS (Method 1): ^tR (min) = 0.63. MS (ESI+): m/z = 245 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.67 (s, 1H), 8.46 (s, 1H), 7.52 (d, J = 2.03 Hz, 1H), 7.46 (dd, J = 2.15, 8.24 Hz, 1H), 6.55 (d, J = 8.11 Hz, 1H), 4.81–5.08 (m, 4H), 4.75 (td, J = 5.99, 12.10 Hz, 1H), 1.32 (d, J = 6.08 Hz, 6H).

tert-Butyl 4-[(1*R*)-1-{2-[(6-{5-[(propan-2-yl)oxy]pyrimidin-4-yl}-1*H*-benzimidazol-2-yl)amino]pyridin-4-yl}ethyl]piperazine-1-carboxylate (S35.5)

Compound **S35.5** was synthesized analogously to **S21.5**, from *tert*-butyl 4-[(1*R*)-1-(2-aminopyridin-4-yl)ethyl]piperazine-1-carboxylate (**39**, 755 mg, 2.47 mmol) and 4-{5-[(propan-2-yl)oxy]pyrimidin-4-yl}benzene-1,2-diamine (**S35.4**, 745 mg, 97% purity, 2.96 mmol).

LC-MS (Method 2): ^{t}R (min) = 1.31. MS (ESI+): *m*/*z* = 559[M + H]$^{+}$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.24 (br s, 1H), 10.62–10.76 (m, 1H), 8.80 (s, 1H), 8.62 (s, 1H), 8.14–8.40 (m, 2H), 7.84–8.00 (m, 1H), 7.36–7.60 (m, 1H), 7.17 (s, 1H), 6.94 (d, *J* = 5.58 Hz, 1H), 4.84 (td, *J* = 6.05, 11.98 Hz, 1H), 3.45 (q, *J* = 6.67 Hz, 1H), 3.27–3.32 (m, 4H), 2.25–2.44 (m, 4H), 1.25–1.42 (m, 18H).

N-{4-[(1*R*)-1-(Piperazin-1-yl)ethyl]pyridin-2-yl}-6-{5-[(propan-2-yl)oxy]pyrimidin-4-yl}-1*H*-benzimidazol-2-amine hydrochloride (S35.6)

Compound **S35.6** was synthesized analogously to **S21.6**, from *tert*-butyl 4-[(1*R*)-1-{2-[(6-{5-[(propan-2-yl)oxy]pyrimidin-4-yl}-1*H*-benzimidazol-2-yl)amino]pyridin-4-yl}ethyl]piperazine-1-carboxylate (**S35.5**, 450 mg, 805 µmol) with DCM/MeOH (9:1) as solvent and stirring for 2 h at rt.

LC-MS (Method 2): ^{t}R (min) = 0.98. MS (ESI+): *m*/*z* = 456[M + H]$^{+}$.

3,3,3-Trifluoro-1-{4-[(1*R*)-1-{2-[(6-{5-[(propan-2-yl)oxy]pyrimidin-4-yl}-1*H*-benzimidazol-2-yl)amino]pyridin-4-yl}ethyl]piperazin-1-yl}propan-1-one (35)

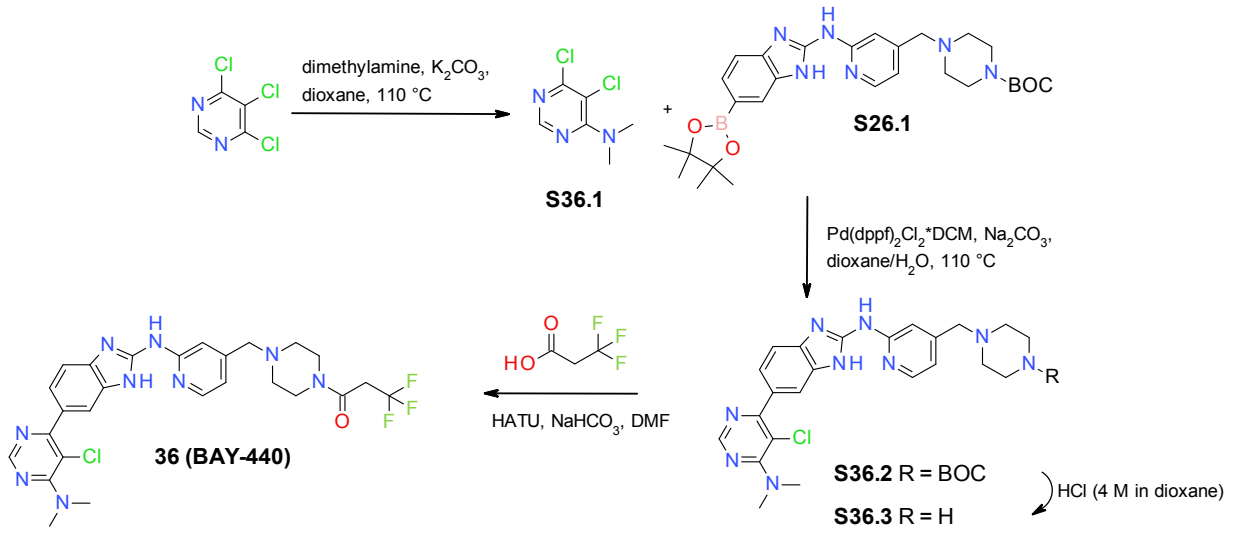
Compound **35** was synthesized analogously to **21**, from *N*-{4-[(1*R*)-1-(piperazin-1-yl)ethyl]pyridin-2-yl}-6-{5-[(propan-2-yl)oxy]pyrimidin-4-yl}-1*H*-benzimidazol-2-amine hydrochloride (**S35.6**, 160 mg, 62% purity, 200 µmol) and 3,3,3-trifluoropropanoic acid (77.0 mg, 601 µmol) with stirring for 2 h at rt in 47% yield (53 mg, 100% purity by UPLC).

$[\alpha]_D^{20}$ +30 (*c* = 1, in DMSO).

LC-MS (Method 2): ^{t}R (min) = 1.13. MS (ESI+): *m*/*z* = 569 [M + H]$^{+}$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.25 (br s, 1H), 10.72 (br s, 1H), 8.80 (s, 1H), 8.62 (s, 1H), 8.14–8.41 (m, 2H), 7.84–7.99 (m, 1H), 7.36–7.60 (m, 1H), 7.18 (s, 1H), 6.95 (dd, *J* = 0.89, 5.45 Hz, 1H), 4.85 (sept, *J* = 6.00 Hz, 1H), 3.62 (q, *J* = 11.07 Hz, 2H), 3.39–3.53 (m, 5H), 2.28–2.47 (m, 4H), 1.36 (br d, *J* = 5.83 Hz, 6H), 1.30 (d, *J* = 6.59 Hz, 3H).

Scheme S13



5,6-Dichloro-*N*,*N*-dimethylpyrimidin-4-amine (S36.1)

4,5,6-Trichloropyrimidine (500 mg, 2.73 mmol), dimethylamine (1.5 mL, 2.0 M, 3.0 mmol) and K_2CO_3 (414 mg, 3.00 mmol) were stirred in 1,4-dioxane (3.2 mL) overnight at 110 °C. The mixture was diluted with water and the aqueous mixture was extracted three times with DCM. The organic layer was dried over a silicone filter and concentrated under reduced pressure to give **36.1** (493 mg, 80% purity, 75% yield), which was used without further purification.

LC-MS (Method 2): ^{t}R (min) = 1.05. MS (ESI+): *m*/*z* = 192 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.30 (s, 1H), 3.19 (s, 6H).

tert-Butyl 4-{[2-({6-[5-chloro-6-(dimethylamino)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (S36.2)

S36.2 was synthesized analogously to **S26.2**, from *tert*-butyl 4-[(2-{[6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-benzimidazol-2-yl]amino}pyridin-4-yl)methyl]piperazine-1-carboxylate (**S26.1**, 445 mg, 833 µmol) and 5,6-dichloro-*N*,*N*-dimethylpyrimidin-4-amine (**S36.1**, 480 mg, 2.50 mmol) with stirring overnight at 110 °C.

LC-MS (Method 2): ^{t}R (min) = 1.30. MS (ESI+): *m*/*z* = 564 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.18–12.36 (m, 1H), 10.62–10.78 (m, 1H), 8.50 (s, 1H), 8.26 (d, *J* = 5.32 Hz, 1H), 7.65–7.97 (m, 1H), 7.32–7.60 (m, 3H), 7.17 (s, 1H), 6.93 (d, *J* = 5.07 Hz, 1H), 3.50 (s, 2H), 3.35 (br s, 3H), 3.18 (s, 6H), 2.33–2.40 (m, 4H), 1.39 (s, 9H).

6-[5-Chloro-6-(dimethylamino)pyrimidin-4-yl]-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (S36.3)

S36.3 was synthesized analogously to **S21.6**, from *tert*-butyl 4-{[2-({6-[5-chloro-6-(dimethylamino)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazine-1-carboxylate (**S36.2**, 230 mg, 408 µmol) with DCM/MeOH (2:1) as solvent and stirring overnight at rt.

LC-MS (Method 2): ^{t}R (min) = 1.01. MS (ESI+): *m*/*z* = 464 $[M + H]^+$.

1-(4-{[2-({6-[5-Chloro-6-(dimethylamino)pyrimidin-4-yl]-1*H*-benzimidazol-2-yl}amino)pyridin-4-yl]methyl}piperazin-1-yl)-3,3,3-trifluoropropan-1-one (36, BAY-440)

36 was synthesized analogously to **21**, from 6-[5-chloro-6-(dimethylamino)pyrimidin-4-yl]-*N*-{4-[(piperazin-1-yl)methyl]pyridin-2-yl}-1*H*-benzimidazol-2-amine hydrochloride (crude **S36.3**, 110 mg) and 3,3,3-trifluoropropanoic acid (51 µl, 580 µmol) with stirring overnight at rt in 75% yield (90 mg, 95% purity by NMR).

LC-MS (Method 2): ^{t}R (min) = 1.14. MS (ESI+): *m*/*z* = 574 $[M + H]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.19–12.34 (m, 1H), 10.64–10.79 (m, 1H), 8.50 (s, 1H), 8.27 (d, *J*=5.32 Hz, 1H), 7.64–7.95 (m, 1H), 7.32–7.59 (m, 2H), 7.19 (s, 1H), 6.94 (dd, *J* = 0.89, 5.20 Hz, 1H), 3.65 (q, *J* = 10.98 Hz, 2H), 3.43–3.57 (m, 6H), 3.18 (s, 6H), 2.40 (td, *J* = 4.82, 16.48 Hz, 4H).

Crystallography

Crystallization and Structure Determination

The previously published TBK1 crystallization construct comprising human TBK1 amino acids 1–657 and the mutation S172E was purified from Hi5 insect cells infected with recombinant baculovirus, as described previously.[1] Small molecule inhibitors were resuspended in DMSO to a concentration of 10 mM. For crystallization, 100 µM TBK1 was mixed with 160 µM inhibitor. Crystals in the $P3_221$ space group were grown by hanging-drop vapor diffusion at 21 °C by mixing equal volumes of protein and precipitation solution containing 100 mM HEPES pH 7.5 and 5–8% PEG 8000 or PEG 6000 or PEG 4000. For each compound, 20–50 crystals were screened for diffraction quality at the ESRF (beamline ID30A-1). Diffraction data were processed using XDS[2] and imported into CCP4 format using AIMLESS[3].

X-ray crystal structures of TBK1 were determined by molecular replacement in Phaser[4] using a previously determined structure of TBK1 (PDB code 4IWO) as search model. Difference Fourier methods were used to calculate $F_o–F_c$ and $2F_o–F_c$ difference density maps. For parametrization, 3D models for the inhibitors were generated using the program Discovery Studio (Dassault Systèmes BIOVIA, San Diego, USA) and parameter files were generated using the software PRODRG.[5] The inhibitors were built into the electron density maps using the program COOT.[6] Atomic coordinates, B factors, and SMOL occupancies were refined using PHENIX[7] for **2** and BUSTER[8] for **24** and **35**. Refined coordinates were validated according to standard stereochemical criteria (Table S4).

Supplementary Figure S1

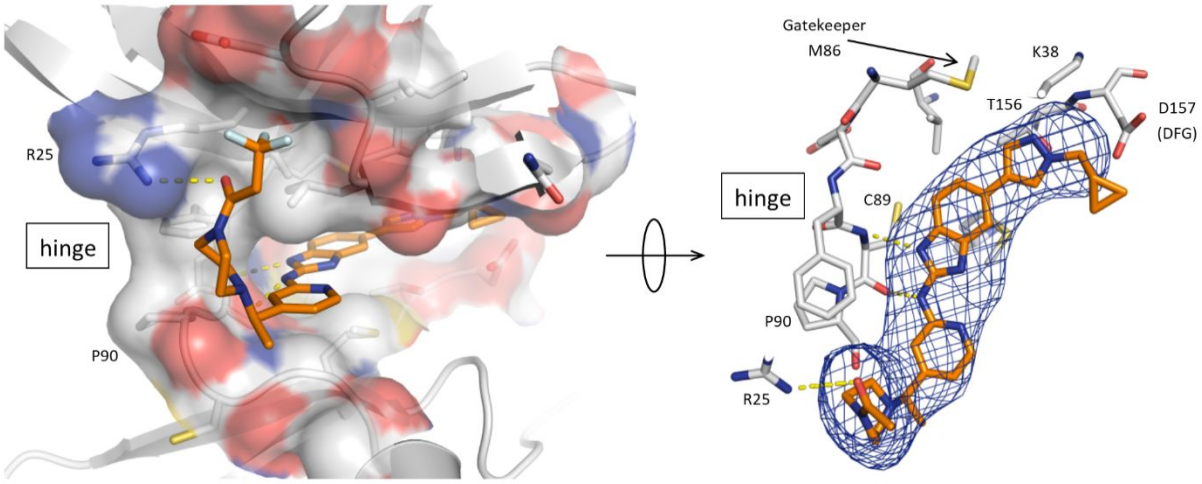


Figure S1. Crystal structure of TBK1 in complex with **24** (PDB accession code 6RSU). Left, view into the ATP site of TBK1, with the protein shown in surface representation (transparent). Right, view rotated and surface omitted for clarity. Hydrogen-bond interactions between the inhibitor **24** and TBK1 shown as yellow dotted lines. The 3.3 Å resolution mFo-DFc electron density omit map contoured at 2 σ around the inhibitor is shown in blue.

Crystallographic Data Collection and Refinement Statistics

	TBK1: compound **2**	TBK1: compound **24**	TBK1:compound **35**
PDB accession code	6RSR	6RST	6RSU
Data collection			
Space group	$P3_221$	$P3_221$	$P3_221$
Cell dimensions			
a, *b*, *c* (Å)	136.20, 136.20, 86.62	136.35, 136.35, 87.19	136.56, 136.56, 87.19
Wavelength (Å)	1.033	0.966	0.966
Resolution (Å)	48.75–3.15	50.00–3.29	48.90–2.99
R_{meas} (%)	9.4 (204)*	9.6 (114)*	7.4 (123)*
$I/\sigma I$	13.0 (0.93)*	14.3 (1.8)*	15.7 (1.6)*
CC(1/2)	40.9	57.9	61.3
Completeness (%)	99.7 (98.8)*	99.5 (99.2)*	99.4 (96.9)*
Redundancy	8.1 (8.0)*	6.4 (6.5)*	6.7 (6.7)*
Refinement			
No. reflections	13358	14377	19151
R_{work}/R_{free}	0.27/0.30	0.20/0.23	0.21/0.26
No. atoms	5099	5106	5107
Protein	5062	5066	5066
Ligand	37	40	41
B factors (Å^2)			
Protein	132.6	147.9	137.4
Ligand	103.5	120.5	103.5
rms deviations			
Bond lengths (Å)	0.002	0.010	0.010
Bond angles (°)	0.505	0.91	0.88

*Values in parentheses are for the highest-resolution shell.

Table S4. Crystallographic Data Collection and Refinement Statistics

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