PYRIDONES AS HIGHLY SELECTIVE, NONCOVALENT INHIBITORS OF T790M DOUBLE-MUTANTS OF EGFR

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Page Information

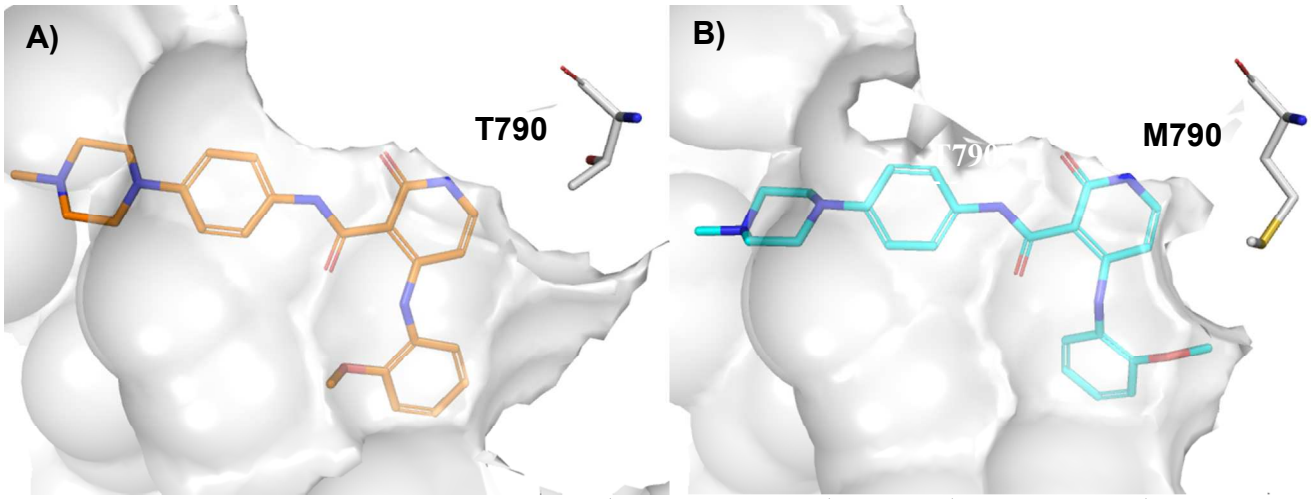


Figure S1. Compound **13** crystalized with either wtEGFR (Figure S1A in orange) or TMLR (Figure S1B in cyan) with the protein's surface in grey.

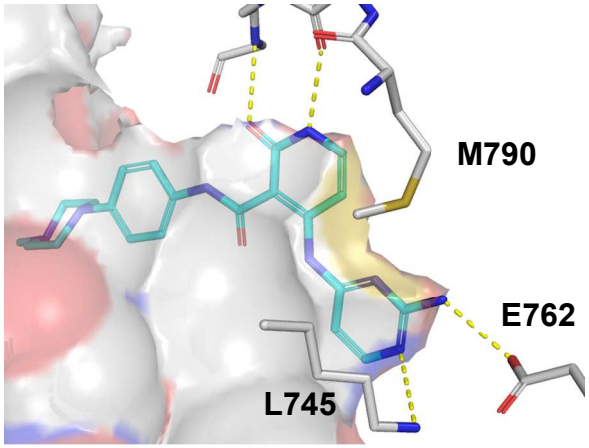


Figure S2. Compound **19** crystalized with TMLR. The protein's surface is shown and colored by electrostatic potential. Hydrogen bonds between the pyrimidine N and the catalytic Lysine, L745, as well as the pyrimidine NH_2 and glutamic acid E762 are shown in yellow.

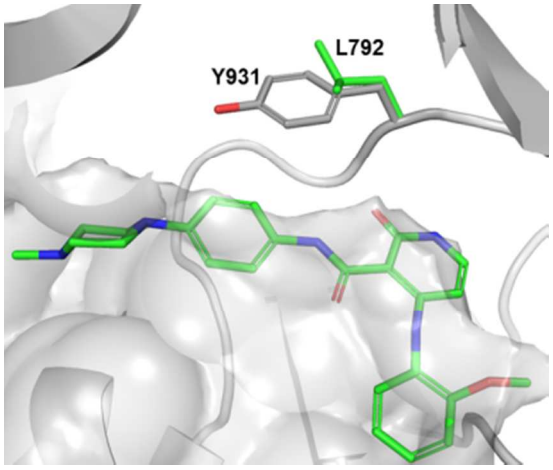


Figure S3. Overlay of compound **13** crystalized with TMLR (green) highlighting L792 with an aligned crystal structure of JAK2 (grey) showing Y931 and the protein's surface in grey.

Table S1. Percent inhibition for compound 1 for full panel of 220 kinases.

Kinase[a]	Percent Inhibition at 1 μM
ACVR1B	13.4
ACVR2B	42
AKT1	0.3
AKT2	3.8
ALK2	93.8
ARK5	88.5
ASK1	3.6
Abl	96.2
Aurora A	53.8
Aurora B	75.4
Axl	81.8
B-Raf	-1
BMPR1A	31.1
BTK	87.3
Blk	91.3
Bmx	85.2
BrSK1	19.1
Brk	47.6
CAMKK1	79.3
CAMKK2	68.6
CDK1/cyclin B	4.7
CDK2/cyclin A	-0.7
CDK5/p25	-0.4
CDK7/cyclin H	40.2
CDK8/cyclin C	6.9
CDK9/cyclinT1	16.1
CHK1	46.1
CHK2	59.6
CK1_alpha1	13.4
CK1_delta	10.2
CK1 epsilon1	31.3
CK1 gamma1	16.9
CK1 gamma2	32
CK2 alpha1	6.2
CLK1	75.8
CLK2	75.8
CLK3	5.3
CLK4	56
CSF1R	91
CSK	54.5
CaMKI	39.5
CaMKII beta	37.4
CaMKI delta	48

CamKII alpha	14.4
CamKIV	17.3
Cot	34.1
DAPK1	64.4
DCAMKL2	27.7
DDR1	47.5
DMPK	68.3
DNA-PK	9.4
DRAK1	79.2
DYRK1A	7.8
DYRK3	3.3
DYRK4	0
EGFR	91
EGFR(T790M,L858R)	91.8
ERK2	10.8
EphA1	69.8
EphA3	20.5
EphA7	64.3
EphA8	78.3
EphB1	73.1
EphB3	9.5
ErbB2	37.8
ErbB4	87.3
FAK	96.7
FGFR1	89.2
FGFR3	83.8
FGFR4	68.5
Fes	79.6
Fgr	101.7
Flt1	60.4
Flt3	93.3
Flt4	91.6
Frk	93.9
GRK2	23.8
GRK3	12.7
GRK5	2.9
GRK6	30.7
GSK3 alpha	14.3
GSK3 beta	23.6
HIPK1	21.9
HIPK2	49.6
HIPK4	56.9
Hyl	17.9
IGF1R	60.2
IKK alpha	0.9

IKK beta	-2.3
IKK epsilon	31.8
IRAK1	69.5
IRAK4	2.6
IRR	90.1
ITK	70.8
InsR	81
JAK1	80.2
JAK2	93
JAK3	88.3
JNK1 alpha1	39.9
JNK2	23.2
JNK3	29.3
KDR	89.2
KHS1	99.1
Kit	10.8
LIMK1	13.3
LRRK2	92.5
LTK	83.5
Lck	95.8
Lyn	95.6
MAP4K4	105.2
MAPKAPK2	0
MAPKAPK3	-4.3
MARK1	70.8
MARK3	89.8
MEK1	43.5
MEK3	31.3
MEKK2	78.5
MELK	98.4
MKK6	18.6
MKNK1	3.8
MKNK2	18.9
MLK1	67.5
MLK2	27.3
MRCK alpha	16.4
MSK1	25.3
MSSK1	24.6
MST1	94.1
MST2	63.9
MST3	38
MST4	57
MYLK(smMLCK)	58.1
MYLK3(caMLCK)	22.1
Mer	73.8

Met	14.4
Mink1	109.1
MuSK	98.7
NEK1	15.9
NEK4	20.1
NEK6	4.4
NEK9	-1.1
NLK	2.4
PAK1	20
PAK3	13.8
PAK4	79.4
PAK6	7.7
PASK	6.7
PDGFR alpha	61.5
PDK1(direct)	53.5
PI3K-A	0.3
PI3K-G	-5.1
PIM1	-3.1
PKA	27.6
PKC alpha	27.4
PKC_beta1	-0.1
PKC delta	8.1
PKC epsilon	24.9
PKC eta	-8.1
PKC theta	13.4
PKC zeta	15.6
PKD1	75.3
PKG1_alpha	59
PLK1	23.3
PLK2	34
PLK3	8
PRAK	9.4
PRK1	6
PRKAA1	65.5
PhK_gamma1	64.8
PhK_gamma2	44.8
PrKX	50.5
RAF1(Y340D,Y341D)	3.3
RIPK2	62.3
ROCK1	13.2
ROCK2	4.5
RSK1	34.3
RSK2	35.7
RSK3	30.1
Ret	101.3

Ron	21.6
Ros	93.8
Rse	68.5
SGK1	0.3
SGK2	-2.4
SGK3	1.9
SIK2	65.5
SLK	60.3
SPHK1	15.1
SRPK1	21.9
STK16	92.3
STK33	79.3
Src	89.2
Srm	77.5
Syk	86.3
TAK1-TAB1	83
TAO1	13.3
TBK1	73.6
TEC	11.4
TGFBR1	70.1
TNK2	91.8
TSSK1	88.4
TTK	81.5
TXK	70.6
TYK2	99.5
Tie2	45.3
TrkA	91.9
TrkB	96.4
WEE1	50.9
WNK2	42.3
YSK1	39
Yes	99.3
ZAK	9.9
ZAP-70	12.8
ZIPK	44.4
eEF-2K	9.8
mTOR	1.9
p38 alpha(direct)	25.5
p38 beta	12.6
p38 delta	16.2
p38 gamma	15.8
p70S6K	7.5

[a]ATP concentration at Kmapp. Data show inhibition of single replicates.

Table S2. Percent Inhibition for compounds 13 and 37 for selected kinases at 1 μM.

Kinase[a]	1	13	19	36	37
TMLR Ki (μM)	0.065	0.019	0.004	0.031	0.088
Aurora B	75%	52%	67%	21%	15%
B-Raf	ND	17%	43%	ND	-6%
CDK2/cyclin A	-1%	2%	96%	ND	4%
CHK1	46%	19%	90%	ND	-3%
EGFR(T790M,L858R)	92%	97%	96%	ND	81%
ERK2	11%	6%	12%	ND	10%
Flt3	93%	87%	100%	ND	34%
IKK_epsilon	32%	8%	86%	ND	2%
JAK2	93%	92%	83%	76%	71%
KDR	89%	88%	95%	81%	15%
Lck	96%	70%	72%	67%	14%
MEK1	ND	20%	46%	ND	6%
p70S6K	8%	7%	36%	ND	6%
Src	89%	48%	27%	ND	25%
TrkA	92%	92%	92%	ND	56%

[a]ATP concentration at Kmapp. Data show inhibition of single replicates.

Table S3. PDB data used in the generation of Figures

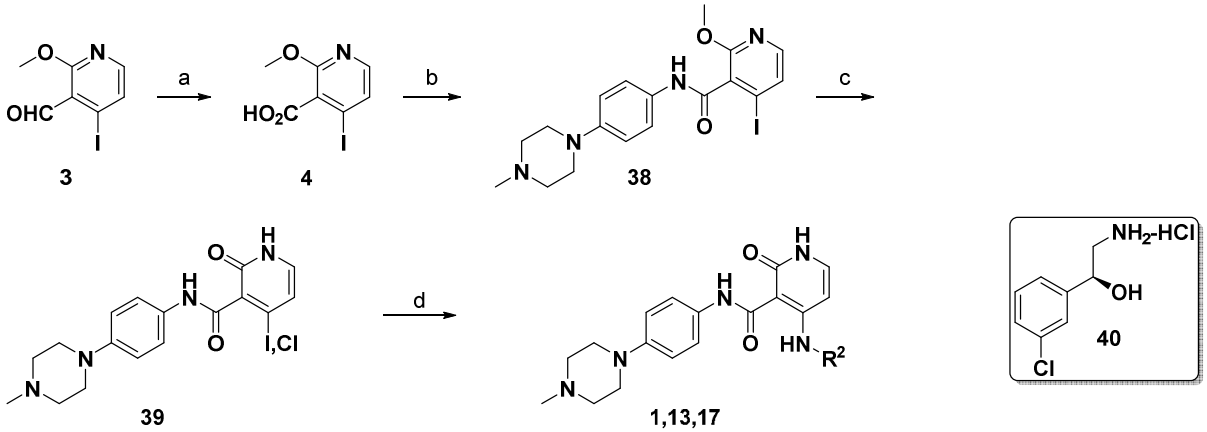
	Compound 13/ EGFR	Compound 13/ EGFR_TMLR_SER1	Compound 19/ EGFR_TMLR_SER1	Compound 2/ EGFR_TMLR_SER1
PDB accession	5EM8	5EM7	5EM6	5EM5
Data				
X-ray source	ALS 5.0.2	SSRL 12-2	ALS 5.0.1	ALS 5.0.2
Wavelength (Å)	1.0000	0.9795	0.9774	0.999993
Resolution range (Å)	46.7-2.8(2.9-2.8)	39.0-2.8(2.9-2.8)	59.7-2.8(2.9-2.8)	47.1-2.6(2.7-2.6)
Space group	I23	I23	I23	I23
Unit cell *a*=*b*=*c* (Å)	147.661	146.105	146.249	148.968
Unit cell α=β=γ (°)	90	90	90	90
Total reflections	172460	42507	145352	220625
Unique reflections	13327 (1339)	12247 (1210)	13262 (1319)	16103 (1602)
Multiplicity	12.9 (13.1)	3.5 (3.4)	11.8 (12.0)	13.7 (13.9)
Completeness (%)	100 (100)	96.7 (97.3)	100 (100)	100 (100)
Mean I/sigma(I)	43.2 (5.0)	15.7 (1.7)	28.6 (5.6)	30.3 (5.0)
Wilson B-factor	78.16	68.39	46.91	56.95
R-symm	0.055 (0.588)	0.072 (0.736)	0.106 (0.487)	0.095 (0.686)
Refinement				
Refs for R-free	569	526	568	673
R-work	0.189	0.189	0.195	0.212
R-free	0.229	0.216	0.229	0.223
no. non-H atoms	2449	2474	2464	2438
macromolecules	2417	2405	2422	2397
ligands	32	69	36	33
water	0	0	6	8
Protein residues	303	300	301	298
RMS(bonds) (Å)	0.010	0.009	0.009	0.008
RMS(angles) (°)	1.10	1.04	1.04	1.00
ϕ/ψ favored (%)	95	96	95	96
Ave. B-factor (Å2)	88.3	74	50.8	67.3
macromolecules	88.0	74.0	50.7	67.5
ligands	107.7	75.4	56.9	60.9
solvent	-	-	32.4	50.2

Experimental Section

Experimental procedures for kinetic solubility experiments. Compounds were dissolved in DMSO to a concentration of 10 mM. These solutions were diluted into PBS buffer (pH 7.2, composed with NaCl, KCl, Na_2HPO_4, and KH_2PO_4) to a final compound concentration of 100 µM, DMSO concentration of 2%, at pH 7.4. The samples were shaken for 24 h at room temperature followed by filtration. LC/CLND was used to determine compound concentration in the filtrate, with the concentration calculated by a caffeine calibration curve and the sample's nitrogen content. An internal standard compound was spiked into each sample for accurate quantification.

General. Unless otherwise indicated, all reagents and solvents were purchased from commercial sources and used without further purification. Moisture or oxygen sensitive reactions were conducted under an atmosphere nitrogen gas. Unless otherwise stated, ^{1}H NMR spectra were recorded at room temperature using Varian Unity Inova Bruker AVANCE III UltraShield-Plus Digital NMR spectrometer at indicated frequencies. Chemical shifts are expressed in ppm relative to an internal standard, tetramethylsilane (=0.00 ppm). The following abbreviations are used: br = broad signal, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet. Purification by silica gel chromatography was carried out using a CombiFlash by Teledyne ISCO system with prepacked cartridges. Purification by reverse-phase high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) was also used. All final compounds were purified to ≥95% chemical purity as determined by HPLC with UV detection at 254 nm.

Scheme S1. Synthesis of compounds **1**, **13** and **17**.[a]



[a]Reagents and conditions: (a) 2-methyl-2-butene, $NaClO_2$, $NaPO_4H_2$-H_2O; (b) Thionyl chloride, NH_2R^1; (c) HCl; (d) NH_2R^2, TEA, Δ.

4-Iodo-2-methoxy-pyridine-3-carboxylic acid (4). To a 250mL round bottom flask equipped with a stir bar was added 4-iodo-2-methoxy-pyridine-3-carbaldehyde (42.60 g, 162.0 mmol) and *tert*-butanol (55 equiv., 8908 mmol). To this cooled solution at 0°C was added a solution of 2-methyl-2-butene (485.9 mmol), sodium dihydrogen phosphate hydrate (404.9 mmol) and sodium chlorite (323.9 mmol) in water (408.5 mL) and the mixture was stirred for 10 minutes. The reaction mixture was poured into 500mL of 1N formic acid and partitioned with ethyl acetate. The aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over magnesium sulfate, filtered and concentrated. The residue was brought up in 1N NaOH, cooled to 0°C and acidified with concentrated HCl to pH ~2. This mixture was washed with ethyl acetate (2x), re-acidified with concentrated HCl and extracted with ethyl acetate an additional time. The combined organic phases were washed with brine, dried over magnesium sulfate, filtered and concentrated to give 4-iodo-2-methoxy-pyridine-3-carboxylic acid (45.2g, quant.) as a white powder.

4-Iodo-2-methoxy-N-(4-(4-methylpiperazin-1-yl)phenyl)nicotinamide (38). Thionyl chloride (30 mL) and a catalytic amount of DMF was added to a stirred suspension of 4-iodo-2-methoxy-nicotinic acid (14 g, 50.2 mmol) in DCM (300 mL) and the reaction was heated 2 hours at 45°C. The reaction was cooled to room temperature and concentrated *in vacuo* to give the crude title compound 14 g (crude) as a yellow solid, which was used in the next step without further purification.

To a stirred solution of 4-(4-methylpiperazin-1-yl)aniline (11 g, 57.51 mmol) in DCM was added TEA (14.3 g, 142 mmol). A solution of 4-iodo-2-methoxynicotinoyl chloride (14 g, 47.1 mmol) in DCM was then added via cannula over 10 minutes. The reaction mixture was stirred overnight at room temperature and then washed with brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give a residue which was purified by flash chromatography on silica gel (gradient: 5-100% EtOAc/hexanes) to provide the title compound (18 g, 85%) as a yellow solid. LCMS (ESI): [M+H]+ 453.

4-Iodo-N-(4-(4-methylpiperazin-1-yl) phenyl)-2-oxo-1, 2-dihydropyridine-3-carboxamide dihydrochloride and **4-Chloro-N-(4-(4-methylpiperazin-1-yl) phenyl)-2-oxo-1, 2-dihydropyridine-3-carboxamide dihydrochloride (39)**. A solution of 4-iodo-2-methoxy-N-(4-(4-methylpiperazin-1-yl)phenyl)nicotinamide (10 g, 22.1mmol) in 1,4-dioxane (300 mL, saturated with HCl

gas) was stirred at 110 °C for 3 h. The resulting mixture was cooled to rt and concentrated in vacuo. The residue was washed with ether and the solids were collected by filtration to provide the title compounds (12 g, crude) as a yellow solid, which was used in the next step without further purification. LCMS (ESI): [M+H]+ 439 and 347.

(S)-2-Amino-1-(3-chlorophenyl) ethanol hydrochloride (40). To a stirred solution of (S)-1-methyl-3,3-diphenyl-hexahydropyrrolo[1,2-c][1,3,2]oxazaborole (0.3 mL, 1M in toluene) and BH_3 (2.7 mL, 1 M in THF) in THF (50 mL) was added 2-chloro-1-(3-chlorophenyl)ethan-1-one (5.0 g, 26.5mmol) and BH_3 (13.2 mL, 1 M in THF). The resulting solution was stirred at 20 °C for 10 minutes. The reaction was then quenched at 0 °C by addition of MeOH (8.0 mL) and anhydrous ether saturated with hydrogen chloride (2 mL). The solvent was removed in vacuo. The residue was dissolved in ether (15 mL), washed with brine and saturated sodium bicarbonate solution and dried over $MgSO_4$. Removal of the solvent *in vacuo* provided crude (S)-2-Chloro-1-(3-chlorophenyl) ethanol (4.45 g, 88%) as a brown oil, which was used in the next step without further purification. GCMS (ESI): [M]+ 190.

A solution of (S)-2-chloro-1-(3-chlorophenyl) ethanol (4 g, 20.9 mmol) in methanol (80 mL, saturated with NH_3) was stirred for 5d at room temperature. The resulting mixture was concentrated in vacuo. The residue was washed with ether and collected by filtration. The crude product was purified by re-crystallization from ethanol / ethyl acetate to provide (S)-2-amino-1-(3-chlorophenyl) ethanol hydrochloride (1.14 g, 32% yield, 100% ee) as a white solid. LCMS (ESI): [M+H]+ 172. 1HNMR (300MHz, CD_3OD): δ 7.45 (s, 1 H), 7.35-7.27 (m, 3H), 5.03-4.89 (m, 1H), 3.19-3.12 (m, 1H), 2.99-2.91 (m, 1 H).

(S)-4-(2-(3-Chlorophenyl)-2-hydroxyethylamino)-N-(4-(4-methylpiperazin-1-yl)phenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (1). To a solution of 4-iodo-N-[4-(4-methylpiperazin-1-yl)phenyl]-2-oxo-1,2-dihydropyridine-3-carboxamide dihydrochloride and 4-chloro-N-(4-(4-methylpiperazin-1-yl)phenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide dihydrochloride (300 mg, 0.59 mmol, calculated with iodine isomer) in CH_3CN (10 mL) at rt was added TEA (1 mL). The resulting solution was stirred at rt for 20 min and then (1S)-2-amino-1-(3-chlorophenyl) ethan-1-ol hydrochloric (184 mg, 0.88 mmol) was added. The resulting solution was stirred at 70 °C for 23 h and then concentrated *in vacuo*. The crude product was purified by Prep-HPLC to provide the title compound (160 mg, 57%) as an off-white solid. LCMS (ESI): [M+H]+ 482. 1H NMR (300 MHz, DMSO-d6): δ 12.78 (s, 1H), 11.06 (s, 1H), 10.83 (s, 1H), 7.47-7.24 (m, 7H), 6.86-6.83 (d, J = 9.0 Hz, 2H), 6.04-6.01 (d, J = 7.5 Hz, 1H), 5.85-5.83 (d, J = 4.8 Hz, 1H), 4.79-4.76 (m, 1H), 3.52-3.44 (m, 1H),3.34-3.28 (m, 1H), 3.04-3.01 (m, 4H), 2.45-2.38 (m, 4H), 2.17 (s, 3H).

N-(4-(4-Methylpiperazin-1-yl)phenyl)-2-oxo-4-(phenylamino)-1,2-dihydropyridine-3-carboxamide (7). ^{1}H NMR (300 MHz, DMSO-d6) δ 12.90 (s, 1H), 12.47 (s, 1H), 11.44 (m, 1H), 7.49-7.43 (m, 4H), 7.37-7.34 (m, 4H), 7.31-7.26 (m, 2H), 6.08-6.05 (m, 1H), 3.10 – 3.07 (m, 4H), 2.54 – 2.45 (m, 4H), 2.27 (s, 3H). MS (ESI): m/z = 403.9 [M+1]+.

4-(2-Methoxyphenylamino)-N-(4-(4-methylpiperazin-1-yl)phenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (13). To a solution of 4-iodo-N-(4-(4-methylpiperazin-1-yl)phenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide dihydrochloride and 4-chloro-N-(4-(4-methylpiperazin-1-yl)phenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide dihydrochloride (250 mg, 0.49 mmol, calculate with iodine isomer) in DMF (2 mL) was added 2-methoxybenzenamine (120 mg, 0.97 mmol) and TEA (1 mL). The resulting solution was reacted at 150 °C for 1 h with microwave radiation, diluted with water and the solid were collected by filtration and washed with water. The crude product was purified by Prep-HPLC to provide the title compound 158 mg (75%) as a light yellow solid. LCMS (ESI): [M+H]+ 434. 1H NMR (300 MHz, DMSO-d6): δ 12.87(s, 1H), 12.16 (s, 1H), 11.37-11.35 (d, J = 5.4 Hz, 1H), 7.47-7.44 (d, J = 8.7 Hz, 2H), 7.33-7.24 (m, 3H), 7.14-7.16 (d, J = 7.5 Hz, 1H), 7.03-6.89 (m, 3H), 5.95-5.92 (d, J = 7.2 Hz, 1H), 3.81 (s, 3H), 3.10-3.07 (m, 4H), 2.46-2.43 (m, 4H), 2.21 (s, 3H).

N-(4-(4-methylpiperazin-1-yl)phenyl)-2-oxo-4-(pyrimidin-4-ylamino)-1,2-dihydropyridine-3-carboxamide (17). To a solution of a mixture of 4-iodo-N-(4-(4-methylpiperazin-1-yl)phenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide dihydrochloride and 4-chloro-N-(4-(4-methylpiperazin-1-yl)phenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide dihydrochloride (400 mg, 0.78 mmol, calculate with iodine isomer) in 1,4-dioxane (4 mL) was added pyrimidin-4-amine (142 mg, 1.49 mmol), $Pd(dba)_3CHCl_3$ (106 mg, 0.1 mmol), Xantphos (100 mg, 0.17 mmol) and Cs_2CO_3 (1.88 g, 5.77 mmol). The resulting solution was reacted at 160 oC for 3 h with microwave radiation. The reaction was diluted with water, extracted dichloromethane. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by Prep-HPLC to provide the title compound 8.9 mg (3%) as a yellow solid. LCMS (ESI): [M+H]+ 406. 1H NMR (300 MHz, DMSO-d6): δ 13.79 (s, 1H), 13.04 (s, 1H), 12.08 (s, 1H), 8.85 (s, 1H), 8.53-8.51 (d, J = 5.7 Hz, 1H), 8.07-8.05 (d, J = 7.5 Hz, 1H), 7.64-7.62 (d, J = 5.7 Hz, 1H), 7.47-7.44 (d, J = 9.0 Hz, 2H), 7.11-7.09 (m, 1H), 6.91-6.88 (d, J = 9.0 Hz, 2H), 3.08-3.05 (m, 4H), 2.46-2.43 (m, 4H), 2.22 (s, 3H).

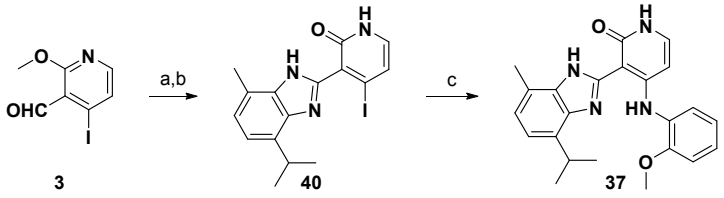
4-((2-Aminopyrimidin-4-yl)amino)-N-(4-(4-methylpiperazin-1-yl)phenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (19). ^{1}H NMR (300 MHz, DMSO-d6) δ 13.46 (s, 1H), 13.1 (s, 1H), 11.92 (br s, 1H), 8.17-8.04 (m, 2H), 7.53-7.47 (m, 3H), 6.95-6.92 (m, 2H), 6.45 (s, 2H), 6.19-6.18 (m, 1H), 3.12 – 3.09 (m, 4H), 2.50 – 2.44 (m, 4H), 2.22 (s, 3H). MS (ESI): m/z = 420.9 [M+1]+.

4-(2-Methoxyanilino)-N-[4-(1-methyl-4-piperidyl)phenyl]-2-oxo-1H-pyridine-3-carboxamide (29). To a 100 mL round bottom flask equipped with a stir bar was added 4-iodo-2-methoxy-pyridine-3-carboxylic acid (1.314 mmol, 366.6 mg) and dichloromethane (5 mL). After dissolution, the mixture was cooled to 0°C and 2M oxalyl chloride in dichloromethane (1.2 equiv., 1.58 mmol) was added followed by DMF (0.01 mL, 0.1 mmol). The reaction was allowed to warm to room temperature and stirred for 1h. The reaction was concentrated to dryness to give 4-iodo-2-methoxy-pyridine-3-carbonyl chloride which was taken on directly. 4-Iodo-2-methoxy-pyridine-3-carbonyl chloride was dissolved in dichloromethane (5 mL) and N-ethyldiisopropylethylamine

(1.5 equiv., 1.97 mmol) was added. 4-(1-Methyl-4-piperidyl)aniline (1.2 equiv., 1.57 mmol) was added and the reaction stirred at room temperature overnight. The reaction was then concentrated and brought up in ethyl acetate and water. The organic phase was washed with brine, dried over magnesium sulfate, filtered and concentrated to give the crude material. Silica gel chromatography (100g, 0 to 10% methanol in dichloromethane) then gave 4-iodo-2-methoxy-N-[4-(1-methyl-4-piperidyl)phenyl]pyridine-3-carboxamide (0.34g, 57%) as a clear glass. MS (ESI): m/z = 452.6 [M+1]+.

To a 250mL round bottom flask equipped with a stir bar was added 4-iodo-2-methoxy-N-[4-(1-methyl-4-piperidyl)phenyl]pyridine-3-carboxamide (0.34 g, 0.75 mmol) and HCl (37 mass%) in H2O (50 mL). The solution was heated to 50°C and stirred at this temperature for 18h. The reaction was concentrated under reduced pressure to give 4-iodo-N-[4-(1-methyl-4-piperidyl)phenyl]-2-oxo-1H-pyridine-3-carboxamide as a pale yellow solid, which was taken on without further purification. A reaction vessel charged with 4-iodo-N-[4-(1-methyl-4-piperidyl)phenyl]-2-oxo-1H-pyridine-3-carboxamide (330 mg, 0.75 mmol) in acetonitrile (3 mL) and 2-methoxyaniline (1.13 mmol) was capped and heated under microwave conditions for 20 minutes at 150°C. The reaction was cooled to room temperature, concentrated to dryness and purified by preparative HPLC to give 4-(2-methoxyanilino)-N-[4-(1-methyl-4-piperidyl)phenyl]-2-oxo-1H-pyridine-3-carboxamide (96.3 mg, 30% yield) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 13.05 (s, 1H), 12.10 (s, 1H), 11.38 (d, J = 5.0 Hz, 1H), 7.55 (d, J = 8.3 Hz, 2H), 7.31 (td, J = 14.6, 12.9, 6.6 Hz, 7H), 7.21 (d, J = 8.3 Hz, 2H), 7.16 (d, J = 8.2 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 5.95 (d, J = 7.4 Hz, 1H), 3.82 (s, 3H), 3.16 – 3.08 (m, 2H), 2.61 – 2.52 (m, 2H), 2.47 – 2.37 (m, 5H), 1.89 – 1.64 (m, 5H).MS (ESI): m/z = 433.2 [M+1]+.

Scheme S2. Synthesis of compound **37**.[a]



[a]Reagents and conditions: (a) $R^1(NH_2)_2$, MeOH, Δ; (b) HCl; (c) NH_2-2-OMe-Ph, DIEA, Δ.

4-Iodo-3-(4-isopropyl-7-methyl-1H-benzo[d]imidazol-2-yl)pyridin-2(1H)-one (40). 3-Isopropyl-6-methyl-benzene-1,2-diamine (60 mg, 0.37 mmol), 4-iodo-2-methoxy-pyridine-3-carbaldehyde (0.73 mmol) and methanol (5 mL) were heated to 75 °C for 22h. The reaction was concentrated and purified by silica gel chromatography (0% to 50% ethyl acetate in heptanes) to give 2-(4-iodo-2-methoxy-3-pyridyl)-4-isopropyl-7-methyl-1H-benzimidazole (126 mg, 85% yield). This material was brought up in concentrated HCl (15 mL) and heated to 50 °C for 3d. The reaction was concentrated to afford 4-iodo-3-(4-isopropyl-7-methyl-1H-benzo[d]imidazol-2-yl)pyridin-2(1H)-one and was taken on as is.

3-(4-Isopropyl-7-methyl-1H-benzimidazol-2-yl)-4-(2-methoxyanilino)-1H-pyridin-2-one (37). 4-Iodo-3-(4-isopropyl-7-methyl-1H-benzimidazol-2-yl)-1H-pyridin-2-one (101 mg, 0.26 mmol), 2-methoxyaniline (0.39 mmol), N,N-diisopropylethylamine (0.77 mmol) and n-butanol (5 ml) was combined and heated under microwave conditions at 150 °C for 60 min. The reaction was then cooled to room temperature and purified by silica gel chromatography (0% to 3% methanol in dichloromethane) to give the desired product in 79% yield (100 mg). ^{1}H NMR (400 MHz, DMSO-d6) δ 12.96 (d, J = 86.8 Hz, 1H), 12.75 (d, J = 16.9 Hz, 1H), 11.47 (s, 1H), 7.46 – 7.32 (m, 2H), 7.29 – 7.16 (m, 2H), 7.03 (tt, J = 7.5, 1.7 Hz, 1H), 6.96 (d, J = 10.0 Hz, 2H), 6.29 (dd, J = 8.4, 7.5 Hz, 1H), 3.87 (d, J = 10.2 Hz, 3H), 3.50 (p, J = 6.9 Hz, 0.5H), 3.25 (p, J = 7.0 Hz, 0.5H), 2.52 (d, J = 11.1 Hz, 3H), 1.36 (dd, J = 6.9, 1.4 Hz, 6H). MS (ESI): m/z = 408.1 [M+1]+.

Enzymatic assays. Experiments were carried out as previously described.[1-2]

In Vitro Microsome Metabolic Stability. Experiments were carried out as previously described.[3]

Cellular assays. Experiments were carried out as previously described.[4]

Expression, purification and crystallization EGFR kinase domain TMLR. Crystallographic methods and the production and use of wtEGFR and TMLR proteins were as previously described.[4,5]

References

[1] Lee, H. J.; Schaefer, G.; Heffron, T. P.; Shao, L.; Ye, X.; Sideris, S.; Malek, S.; Chan, E.; Merchant, M.; La, H.; Ubhayakar, S.; Yauch, R. L.; Pirazzoli, V.; Politi, K.; Settleman, J. "Noncovalent wild-type-sparing inhibitors of EGFR T790M." *Cancer Discovery* **2013**, *3*, 168–181.

[2] Hurley, C.; Kulagowski, J.; Zak, M. "Tricyclic heterocyclic compounds, compositions and methods of use thereof as JAK inhibitors." WO/2013/007768.

[3] Halladay, J. S.; Wong, S.; Jaffer, S. M.; Sinhababu, A. K.; Khojasteh-Bakht, S. C. "Metabolic stability screen for drug discovery using cassette analysis and column switching." *Drug Metab. Lett*. **2007**, *1*, 67–72.

[4] Heald, R.; Bowman, K. K.; Bryan, M. C.; Burdick, D.; Chan, B.; Chan, E.; Chen, Y.; Clausen, S.; Eigenbrot, C.; Elliott, R.; Hanan, E. J.; Jackson, P.; Knight, J.; La, H.; Lainchbury, M.; Malek, S.; Mann, S.; Merchant, M.; Mortara, K.; Purkey, H.; Schaefer, G. Schmidt, S.; Seward, E.; Sideris, S.; Shao, L.; Wang, S.; Yeap, K.; Yen, I.; Yu, C.; Heffron, T. P. "Noncovalent Mutant Selective Epidermal Growth Factor Receptor Inhibitors: A Lead Optimization Case History". *J. Med. Chem.*, **2015**, *58*, 8877-8895.

[5] Hanan, E. J.; Eigenbrot, C.; Bryan, M. C.; Burdick, D. J.; Chan, B. K.; Chen, Y.; Dotson, J.; Heald, R. A.; Jackson, P. S.; La, H.; Lainchbury, M. D.; Malek, S.; Purkey, H. E.; Schaefer, G.; Schmidt, S.; Seward, E. M.; Sideris, S.; Tam, C.; Wang, S.; Yeap, S. K.; Yen, I.; Yin, J.; Yu, C.; Zilberleyb, I.; Heffron, T. P. *J. Med. Chem.* **2014**, *57*, 10176.