Supporting Information

Impact of minor structural modifications on properties of a series of mTOR inhibitors

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ChromLogD

Reference and test compounds are solubilized in methanol at 0.5 mM. An aliquot was injected onto the UPLC/UV system and the retention times (RT) were measured with different mobile phases at pH 6.5. A calibration curve was performed with reference compounds by plotting the highest RT of each compound. The RT measured for each test compound was converted to Chromatographic Hydrophobicity Index (CHI) value by using the calibration curve established with reference compounds.[1] The CHI was then converted to ChromLogD using the equation established by Young: CHI*0.0857 – 2.[2]

Solubility in H_2O

Compounds from a stock solution in DMSO (10mM) were dissolved at 100µM into a phosphate buffer solution pH7.4 and were shaken during 24H at 20°C.

After filtration and dilution, the samples were quantified using a High performance liquid chromatography method by comparison from standards.

Human microsome metabolic stability

Human liver microsomes are used (pooled from a 35 individuals for human). Incubations are performed at a test compound concentration of 1µM at 37°C with 0.5mg protein/mL in presence of phase I metabolism cofactors. The final DMSO concentration in the incubation is 0.1 %. Control incubations are also performed in lysed microsomes to reveal any non-enzymatic degradation. Control compound (phase 1 metabolism) is included. Samples (65µL) are removed from the incubation mixture at 0 and 15 min and added to acetonitrile:water (75:25), containing internal standard, (175µL) to stop the reaction. Samples are analyzed by LC-MS/MS. % Parent Compound at 15 min is calculated.

Human hepatocytes metabolic stability

Suspension of cryopreserved human hepatocytes was used (pooled from a 10 individuals). Incubations were performed at a test compound concentration of 1µM at 37°C and 5% CO2. The cell density was 0.5x 106 viable cells/ml. The final DMSO concentration in the incubation is 0.1 %. Control incubations were also performed in lysed cells to reveal any non-enzymatic degradation. Two control compounds (phase 1 and 2 metabolism) were included with each species. Samples (65µl) were removed from the incubation mixture at 0, 5, 15, 30, 60 and 90 min (control sample at 90min only) and added to acetonitrile:water (75:25), containing internal standard, (175µl) to stop the reaction. Samples were analyzed by LC-MS/MS. Half-life and intrinsic clearance were calculated.

Biochemical assay

mTOR biochemical LanthaScreen™ assays were performed in a 10 µL volume in low-volume 384-well plates (Corning 4514). According to the manufacturer (Lifetechnologies), the concentration of substrate was 400 nM, and the 1x kinase reaction buffer consisted of 50 mM HEPES pH 7.5, 0.01% Tween 20, 1 mM EGTA, 10 mM $MnCl_2$, and 2 mM DTT. Reactions were allowed to proceed for 1 hour (linear phase) at room temperature in the presence and in the absence of compounds at 1% DMSO before a 10 µL preparation of EDTA (20 mM) and Tb-labeled antibody (4 nM) in TR-FRET dilution buffer were added. The final concentrations of antibody and EDTA in the assay were 2 nM and 10 mM respectively. Plates were allowed to incubate at room temperature for at least 30 minutes before being red on a plate reader configured for LanthaScreen™ TR-FRET.

For PI3Kα, enzymatic reaction was performed in a 10 µL volume, using low-volume 384-well plates (Corning 4514). The concentration of substrate (PIP2) was 10 µM, and the 1X kinase reaction buffer consisted of proprietary EUROFIN buffer plus 5 mM DTT. Kinase reaction was allowed to proceed for 30 minutes (linear phase) at room temperature before a 10 µL preparation of STOP solution (STOP A and B) and Detection mix (DMC A, B and C) were added. Plate was incubated at room temperature for at least 2 hours before being read on a plate reader configured for TR-FRET.

Cellular assay

A431 cells were seeded on poly-L-lysine coated plates at 25000 cells/well in DMEM medium. Before experiments cells were starved and maintained in culture for 24 hours. The day of the experiment cells were treated with increasing compounds concentrations (final DMSO%=0.1) for 3 hours and level of phosphorylated S6RP (Ser235/236) and AKT (Ser473) were measured by HTRF (CISBIO) kit following protocols of the provider.

DiscoveRx ScanMax Kinome Binding Scan

Compounds were tested at 10 µM concentration against a panel of 442 known protein kinases. Data are presented as percent of control activity remaining. (0% indicates very tight binders, 100% indicates no binding).

		Percent Control								
DiscoveRx Gene Symbol	**Entrez Gene Symbol**	**4a**	**3e**	**3j**	**3k**	**6a**	**1**	**6e**	**3b**	**4b**
AAK1	AAK1	92	67	81	27	49	77	73	81	49
ABL1(E255K)-phosphorylated	ABL1	92	29	22	0.8	66	2.4	74	57	93
ABL1(F317I)-nonphosphorylated	ABL1	95	90	79	79	84	94	100	100	100
ABL1(F317I)-phosphorylated	ABL1	66	50	54	41	73	21	100	100	100
ABL1(F317L)-nonphosphorylated	ABL1	93	81	33	62	60	35	100	100	100
ABL1(F317L)-phosphorylated	ABL1	78	31	15	20	46	4.6	100	89	99
ABL1(H396P)-nonphosphorylated	ABL1	78	26	13	0.25	17	0.15	74	65	70
ABL1(H396P)-phosphorylated	ABL1	82	49	28	0.1	49	3.4	87	69	100
ABL1(M351T)-phosphorylated	ABL1	84	56	36	0.4	50	5.3	100	92	100
ABL1(Q252H)-nonphosphorylated	ABL1	80	15	11	0.65	46	0.4	86	46	93
ABL1(Q252H)-phosphorylated	ABL1	90	39	22	0.3	19	5	100	100	100
ABL1(T315I)-nonphosphorylated	ABL1	98	100	80	2.7	81	64	100	100	100
ABL1(T315I)-phosphorylated	ABL1	90	75	80	0.8	78	44	93	96	99
ABL1(Y253F)-phosphorylated	ABL1	71	47	22	0.15	41	6	100	89	100
ABL1-nonphosphorylated	ABL1	54	29	16	0.25	64	2.8	54	44	80
ABL1-phosphorylated	ABL1	72	42	24	0.2	82	4.3	72	71	87
ABL2	ABL2	96	63	74	21	100	36	97	93	100
ACVR1	ACVR1	88	96	81	62	100	0.55	100	100	91
ACVR1B	ACVR1B	100	96	84	88	87	13	86	90	100
ACVR2A	ACVR2A	85	95	98	100	100	3.8	76	97	93
ACVR2B	ACVR2B	88	98	99	98	100	6.3	54	80	86
ACVRL1	ACVRL1	67	73	72	96	100	24	89	100	94
ADCK3	CABC1	85	60	31	99	95	90	87	86	91
ADCK4	ADCK4	99	100	68	68	100	75	84	95	79
AKT1	AKT1	95	100	88	100	100	67	97	98	100
AKT2	AKT2	92	100	75	83	98	80	86	100	100
AKT3	AKT3	100	100	100	100	96	44	100	84	91
ALK	ALK	100	99	86	31	72	78	100	100	100
ALK(C1156Y)	ALK	100	84	90	26	64	33	97	90	87
ALK(L1196M)	ALK	93	95	99	35	72	92	97	97	92

AMPK-alpha1	PRKAA1	80	83	86	69	100	92	100	100	100
AMPK-alpha2	PRKAA2	87	98	81	87	100	79	77	100	100
ANKK1	ANKK1	76	88	54	9.9	64	95	100	100	100
ARK5	NUAK1	100	92	91	33	66	95	100	89	100
ASK1	MAP3K5	95	99	99	23	100	100	100	100	96
ASK2	MAP3K6	100	100	91	49	70	99	100	100	98
AURKA	AURKA	99	91	90	16	100	63	95	92	100
AURKB	AURKB	100	99	63	26	90	72	100	94	98
AURKC	AURKC	58	74	79	33	73	69	79	96	89
AXL	AXL	100	90	61	19	91	34	87	86	96
BIKE	BMP2K	15	52	70	16	25	63	59	78	75
BLK	BLK	67	34	18	5.8	87	3.1	88	97	100
BMPR1A	BMPR1A	87	81	100	94	92	18	83	99	100
BMPR1B	BMPR1B	69	62	41	8.1	83	0.05	93	86	93
BMPR2	BMPR2	99	97	89	19	42	53	98	79	100
BMX	BMX	95	93	95	41	85	76	88	99	100
BRAF	BRAF	71	53	43	57	88	36	100	82	63
BRAF(V600E)	BRAF	72	43	22	55	80	21	96	80	57
BRK	PTK6	83	73	19	75	100	26	68	99	96
BRSK1	BRSK1	96	91	85	90	100	99	100	100	96
BRSK2	BRSK2	81	89	85	92	94	71	72	85	100
BTK	BTK	98	100	95	6.4	93	100	100	100	100
BUB1	BUB1	77	72	77	16	57	86	100	99	100
CAMK1	CAMK1	68	73	71	12	98	57	66	88	48
CAMK1B	PNCK	100	100	100	24	100	66	80	100	79
CAMK1D	CAMK1D	83	78	67	19	94	53	77	85	72
CAMK1G	CAMK1G	95	82	86	54	100	87	100	100	76
CAMK2A	CAMK2A	98	100	78	85	100	46	90	91	96
CAMK2B	CAMK2B	93	100	84	89	100	60	89	90	88
CAMK2D	CAMK2D	92	86	76	84	97	52	94	94	86
CAMK2G	CAMK2G	96	79	68	80	94	38	67	87	64
CAMK4	CAMK4	61	80	82	100	99	68	93	97	100
CAMKK1	CAMKK1	100	91	100	100	83	73	80	79	75
CAMKK2	CAMKK2	86	74	73	67	90	65	77	83	58
CASK	CASK	78	81	45	70	67	31	99	81	94
CDC2L1	CDK11B	89	96	86	92	100	100	93	99	91
CDC2L2	CDC2L2	100	100	98	94	97	94	98	92	66
CDC2L5	CDK13	100	94	91	87	77	100	100	100	100
CDK11	CDK19	85	98	86	85	99	21	100	96	100
CDK2	CDK2	100	100	93	100	98	81	83	90	100
CDK3	CDK3	100	94	100	100	91	85	86	92	93
CDK4	CDK4	100	100	100	100	94	92	100	100	100
CDK4-cyclinD1	CDK4	99	90	99	89	66	100	100	100	100
CDK4-cyclinD3	CDK4	93	100	97	95	73	100	100	97	100
CDK5	CDK5	90	92	93	100	92	89	87	85	100
CDK7	CDK7	93	100	79	93	69	64	98	93	100

CDK8	CDK8	100	100	95	100	100	42	100	100	100
CDK9	CDK9	87	100	87	93	100	100	98	99	100
CDKL1	CDKL1	80	60	70	91	97	66	85	59	88
CDKL2	CDKL2	89	89	96	100	94	55	93	93	98
CDKL3	CDKL3	79	78	79	97	85	72	81	82	100
CDKL5	CDKL5	85	90	94	100	100	100	100	100	100
CHEK1	CHEK1	97	96	88	59	100	92	100	93	81
CHEK2	CHEK2	91	81	79	37	80	81	88	96	55
CIT	CIT	84	76	81	89	76	76	79	88	86
CLK1	CLK1	89	65	65	24	57	71	92	81	85
CLK2	CLK2	96	95	63	27	40	64	71	79	82
CLK3	CLK3	80	91	84	69	100	68	97	84	100
CLK4	CLK4	72	93	62	46	62	76	84	86	100
CSF1R	CSF1R	100	42	12	0.05	30	0.45	100	79	59
CSF1R-autoinhibited	CSF1R	100	90	72	0.1	37	6.2	100	77	97
CSK	CSK	94	99	95	20	100	56	75	86	91
CSNK1A1	CSNK1A1	87	56	22	54	100	5.1	58	87	95
CSNK1A1L	CSNK1A1L	84	69	25	54	100	15	96	79	100
CSNK1D	CSNK1D	71	57	5.3	60	100	3.4	98	72	77
CSNK1E	CSNK1E	69	43	1.2	58	100	0.5	100	66	85
CSNK1G1	CSNK1G1	94	98	95	78	99	93	75	86	93
CSNK1G2	CSNK1G2	76	70	60	48	100	65	98	100	100
CSNK1G3	CSNK1G3	78	62	55	67	100	56	100	90	79
CSNK2A1	CSNK2A1	100	100	97	66	70	95	100	96	100
CSNK2A2	CSNK2A2	100	86	98	74	86	92	81	88	73
CTK	MATK	91	98	79	62	72	54	100	73	98
DAPK1	DAPK1	99	94	82	61	93	74	75	84	83
DAPK2	DAPK2	100	88	72	65	96	63	65	86	92
DAPK3	DAPK3	88	90	85	62	100	88	80	85	96
DCAMKL1	DCLK1	78	92	90	100	81	88	90	77	94
DCAMKL2	DCLK2	100	100	94	99	89	99	100	98	75
DCAMKL3	DCLK3	78	36	45	54	100	39	94	90	100
DDR1	DDR1	88	43	24	78	100	3.7	100	89	66
DDR2	DDR2	100	76	50	67	94	0.85	100	100	89
DLK	MAP3K12	97	99	100	66	11	71	20	48	71
DMPK	DMPK	66	72	100	84	98	100	91	92	89
DMPK2	CDC42BPG	93	97	93	92	100	68	100	95	81
DRAK1	STK17A	89	92	78	25	100	95	83	100	100
DRAK2	STK17B	93	85	74	23	100	86	73	85	100
DYRK1A	DYRK1A	89	80	80	70	80	92	100	92	100
DYRK1B	DYRK1B	85	85	84	95	87	72	84	89	68
DYRK2	DYRK2	83	81	79	77	96	100	100	100	94
EGFR	EGFR	75	84	82	100	100	67	100	97	97
EGFR(E746-A750del)	EGFR	75	57	60	89	86	45	52	69	56
EGFR(G719C)	EGFR	91	79	65	60	75	57	78	81	37
EGFR(G719S)	EGFR	68	38	66	76	84	53	82	80	95

EGFR(L747-E749del, A750P)	EGFR	66	78	63	82	94	61	100	100	86
EGFR(L747-S752del, P753S)	EGFR	93	100	78	100	94	59	69	83	52
EGFR(L747-T751del,Sins)	EGFR	90	56	52	80	85	50	97	72	99
EGFR(L858R)	EGFR	68	73	62	78	97	47	91	93	86
EGFR(L858R,T790M)	EGFR	95	77	81	51	56	37	90	89	100
EGFR(L861Q)	EGFR	27	67	57	92	72	52	80	74	84
EGFR(S752-I759del)	EGFR	87	67	69	84	76	36	77	46	94
EGFR(T790M)	EGFR	98	100	98	64	64	44	86	73	81
EIF2AK1	EIF2AK1	77	80	67	83	91	100	100	100	100
EPHA1	EPHA1	83	91	83	24	98	40	100	100	98
EPHA2	EPHA2	84	96	97	67	96	74	93	100	100
EPHA3	EPHA3	92	88	91	39	99	93	98	88	86
EPHA4	EPHA4	76	96	91	83	100	97	95	93	98
EPHA5	EPHA5	89	92	89	65	100	76	77	78	99
EPHA6	EPHA6	76	97	91	59	100	79	100	91	96
EPHA7	EPHA7	99	100	97	40	100	92	94	94	100
EPHA8	EPHA8	91	95	91	93	100	49	98	97	100
EPHB1	EPHB1	84	69	71	28	100	90	95	90	96
EPHB2	EPHB2	77	80	82	56	81	61	53	66	97
EPHB3	EPHB3	81	100	87	94	100	87	98	91	97
EPHB4	EPHB4	81	94	75	57	100	96	100	100	100
EPHB6	EPHB6	70	42	33	6.6	49	4.8	100	93	78
ERBB2	ERBB2	63	34	2.2	87	92	18	100	100	44
ERBB3	ERBB3	100	100	87	45	78	53	100	96	100
ERBB4	ERBB4	95	96	93	77	100	74	74	95	100
ERK1	MAPK3	92	93	80	89	100	65	86	97	100
ERK2	MAPK1	88	94	81	97	100	77	88	90	94
ERK3	MAPK6	89	96	91	97	100	98	96	95	87
ERK4	MAPK4	83	92	89	89	100	100	97	98	100
ERK5	MAPK7	92	100	89	98	100	98	91	100	87
ERK8	MAPK15	82	68	90	65	98	83	99	87	78
ERN1	ERN1	80	87	86	59	70	91	100	100	100
FAK	PTK2	94	98	93	92	96	60	60	74	97
FER	FER	79	98	91	91	97	91	98	96	91
FES	FES	57	93	78	80	100	93	100	98	93
FGFR1	FGFR1	85	72	39	11	74	10	77	90	97
FGFR2	FGFR2	82	73	38	16	79	29	98	98	96
FGFR3	FGFR3	91	91	60	18	84	36	84	100	84
FGFR3(G697C)	FGFR3	76	91	53	11	88	36	79	94	97
FGFR4	FGFR4	89	82	49	37	100	37	70	92	97
FGR	FGR	77	78	68	28	96	12	91	91	100
FLT1	FLT1	98	53	67	34	95	24	87	96	94
FLT3	FLT3	97	46	45	4.7	72	2.1	77	77	64
FLT3(D835H)	FLT3	80	64	61	7	39	15	94	82	76
FLT3(D835V)	FLT3	100	47	41	2.1	10	22	100	100	55
FLT3(D835Y)	FLT3	60	46	40	3.8	49	32	88	70	82

FLT3(ITD)	FLT3	89	34	58	9.3	56	23	82	92	98
FLT3(ITD,D835V)	FLT3	100	94	71	0.8	20	80	100	100	100
FLT3(ITD,F691L)	FLT3	96	92	84	2	25	100	100	100	76
FLT3(K663Q)	FLT3	90	43	45	6.5	80	13	74	87	96
FLT3(N841I)	FLT3	99	95	56	0	71	21	72	86	82
FLT3(R834Q)	FLT3	88	75	79	27	81	53	88	92	100
FLT3-autoinhibited	FLT3	64	47	46	26	72	5.6	100	94	95
FLT4	FLT4	51	77	89	1.1	76	86	89	100	91
FRK	FRK	80	57	58	78	95	41	71	100	92
FYN	FYN	57	72	44	38	84	17	77	100	97
GAK	GAK	19	7.8	6.2	9.6	24	4.5	86	54	20
GCN2(Kin.Dom.2,S808G)	EIF2AK4	99	88	81	100	100	96	98	100	100
GRK1	GRK1	91	93	89	60	69	72	92	84	96
GRK2	ADRBK1	89	93	100	40	100	100	97	100	99
GRK3	ADRBK2	100	100	100	96	81	82	92	81	95
GRK4	GRK4	78	80	100	83	67	88	87	100	71
GRK7	GRK7	88	92	82	51	62	77	100	100	98
GSK3A	GSK3A	100	85	97	87	60	76	92	70	93
GSK3B	GSK3B	100	100	97	92	83	100	100	92	100
HASPIN	GSG2	73	53	11	33	48	13	100	100	97
HCK	HCK	48	41	33	10	89	9.6	96	86	82
HIPK1	HIPK1	76	62	59	58	83	43	78	59	82
HIPK2	HIPK2	100	100	100	84	72	73	100	100	100
HIPK3	HIPK3	100	97	92	91	78	59	100	88	100
HIPK4	HIPK4	76	64	51	89	96	38	69	82	83
HPK1	MAP4K1	98	93	60	38	99	68	98	100	100
HUNK	HUNK	96	96	81	100	100	100	100	100	100
ICK	ICK	100	100	98	85	71	100	100	100	92
IGF1R	IGF1R	77	86	86	55	100	81	91	100	100
IKK-alpha	CHUK	96	95	89	27	82	78	96	89	98
IKK-beta	IKBKB	97	92	89	26	55	82	96	97	97
IKK-epsilon	IKBKE	80	72	53	32	61	51	100	100	100
INSR	INSR	80	84	98	24	69	27	89	74	80
INSRR	INSRR	100	84	99	58	88	64	100	100	100
IRAK1	IRAK1	100	97	88	20	66	36	100	83	100
IRAK3	IRAK3	96	100	88	65	100	55	91	83	77
IRAK4	IRAK4	100	99	90	28	67	84	100	93	97
ITK	ITK	95	74	86	52	93	90	94	89	98
JAK1(JH1domain-catalytic)	JAK1	92	91	86	74	77	100	100	100	93
JAK1(JH2domain-pseudokinase)	JAK1	13	12	20	0.2	0.25	45	1.9	3.2	15
JAK2(JH1domain-catalytic)	JAK2	94	94	87	0.95	15	76	79	74	93
JAK3(JH1domain-catalytic)	JAK3	96	88	99	5.3	1	87	23	17	84
JNK1	MAPK8	100	85	47	17	57	66	86	89	89
JNK2	MAPK9	74	60	17	64	57	52	97	95	91
JNK3	MAPK10	81	77	31	36	60	67	100	100	96
KIT	KIT	98	93	67	7.6	76	3.2	91	93	62

KIT(A829P)	KIT	96	75	50	20	35	36	100	100	100
KIT(D816H)	KIT	100	100	84	11	60	28	86	89	100
KIT(D816V)	KIT	94	61	40	1.4	44	9.8	81	83	93
KIT(L576P)	KIT	100	95	64	1.2	76	4.9	92	99	59
KIT(V559D)	KIT	100	96	59	5.1	59	1.5	81	92	57
KIT(V559D,T670I)	KIT	100	100	74	9.2	100	38	100	100	79
KIT(V559D,V654A)	KIT	96	91	94	25	95	57	71	78	100
KIT-autoinhibited	KIT	95	81	70	15	81	12	100	88	90
LATS1	LATS1	56	91	76	100	100	90	100	99	100
LATS2	LATS2	79	76	82	74	67	71	100	92	100
LCK	LCK	76	29	15	21	54	2.8	87	78	74
LIMK1	LIMK1	99	94	99	75	99	53	100	100	96
LIMK2	LIMK2	89	96	87	87	97	79	97	89	80
LKB1	STK11	73	89	87	90	91	81	100	97	64
LOK	STK10	80	70	68	4.3	100	35	99	100	97
LRRK2	LRRK2	100	94	0	43	67	57	89	73	87
LRRK2(G2019S)	LRRK2	100	100	100	73	55	100	100	100	100
LTK	LTK	75	79	75	54	100	44	88	89	100
LYN	LYN	74	73	71	53	80	32	59	74	95
LZK	MAP3K13	100	100	100	100	100	82	82	92	93
MAK	MAK	97	76	52	95	100	70	95	91	100
MAP3K1	MAP3K1	98	100	85	47	81	89	100	96	100
MAP3K15	MAP3K15	100	100	88	39	60	90	100	96	100
MAP3K2	MAP3K2	95	86	60	2.3	33	22	100	79	100
MAP3K3	MAP3K3	84	66	51	4.2	45	11	92	77	87
MAP3K4	MAP3K4	59	99	75	69	95	100	100	100	87
MAP4K2	MAP4K2	88	94	93	21	66	62	100	100	100
MAP4K3	MAP4K3	99	100	100	40	98	62	95	87	97
MAP4K4	MAP4K4	89	97	99	71	100	84	85	95	100
MAP4K5	MAP4K5	93	100	100	81	99	79	93	97	92
MAPKAPK2	MAPKAPK2	93	100	100	100	100	88	99	100	95
MAPKAPK5	MAPKAPK5	89	87	80	95	87	93	100	96	100
MARK1	MARK1	92	96	91	77	91	57	56	65	100
MARK2	MARK2	97	100	88	44	79	43	62	75	100
MARK3	MARK3	93	95	100	89	100	87	96	100	100
MARK4	MARK4	79	88	84	67	96	69	80	80	99
MAST1	MAST1	100	100	100	94	63	55	100	95	68
MEK1	MAP2K1	82	67	26	28	73	0.2	99	85	99
MEK2	MAP2K2	67	72	35	35	84	0.55	87	89	100
MEK3	MAP2K3	86	78	56	28	69	42	100	90	96
MEK4	MAP2K4	74	66	30	31	59	38	100	96	91
MEK5	MAP2K5	80	61	37	3.8	36	0.2	93	79	83
MEK6	MAP2K6	81	100	85	67	87	65	77	87	91
MELK	MELK	91	85	66	78	100	24	81	96	100
MERTK	MERTK	78	53	60	15	93	65	91	96	71
MET	MET	93	87	76	47	100	55	83	98	100

MET(M1250T)	MET	100	100	100	84	100	72	99	100	87
MET(Y1235D)	MET	82	77	72	59	94	40	77	83	94
MINK	MINK1	98	90	87	37	57	55	96	89	80
MKK7	MAP2K7	100	100	100	96	68	100	100	100	100
MKNK1	MKNK1	86	94	93	88	72	100	100	100	100
MKNK2	MKNK2	73	64	55	54	66	18	95	92	75
MLCK	MYLK3	95	95	86	64	100	59	99	100	100
MLK1	MAP3K9	95	89	81	12	96	59	72	92	100
MLK2	MAP3K10	82	49	57	53	76	68	86	77	89
MLK3	MAP3K11	77	92	71	21	89	46	74	83	98
MRCKA	CDC42BPA	85	94	78	82	100	68	84	91	98
MRCKB	CDC42BPB	93	91	99	100	95	95	100	100	87
MST1	STK4	81	87	87	64	91	54	96	94	100
MST1R	MST1R	90	88	70	79	100	80	97	81	94
MST2	STK3	100	100	78	66	73	71	90	85	78
MST3	STK24	100	82	70	47	81	44	74	83	85
MST4	MST4	100	100	87	64	71	52	100	95	100
MTOR	MTOR	0	0	0	0	0	0	0	0	0
MUSK	MUSK	100	87	100	96	99	100	100	100	100
MYLK	MYLK	74	59	60	48	77	30	94	98	90
MYLK2	MYLK2	100	41	96	97	100	93	81	96	100
MYLK4	MYLK4	100	100	86	62	78	69	91	93	98
MYO3A	MYO3A	94	94	88	93	93	31	88	92	92
MYO3B	MYO3B	59	89	70	100	100	85	96	97	100
NDR1	STK38	90	94	77	74	75	39	78	86	90
NDR2	STK38L	94	94	91	63	100	45	98	90	100
NEK1	NEK1	84	89	81	81	100	95	61	100	84
NEK10	NEK10	92	74	77	1.6	50	100	100	100	100
NEK11	NEK11	100	99	100	92	87	92	85	100	100
NEK2	NEK2	88	94	80	93	88	81	94	92	90
NEK3	NEK3	87	81	80	75	67	67	93	75	83
NEK4	NEK4	99	94	82	73	86	93	100	94	98
NEK5	NEK5	85	90	82	74	94	100	100	89	76
NEK6	NEK6	97	93	97	96	100	79	63	76	95
NEK7	NEK7	77	86	71	75	100	87	91	89	100
NEK9	NEK9	93	99	82	87	97	75	84	78	99
NIK	MAP3K14	89	80	93	25	81	85	76	81	100
NIM1	MGC42105	99	100	91	88	77	80	100	96	94
NLK	NLK	57	44	18	79	98	27	100	97	83
OSR1	OXSR1	71	69	72	42	83	100	100	100	100
p38-alpha	MAPK14	97	100	84	96	93	82	86	99	100
p38-beta	MAPK11	70	83	82	83	99	98	78	100	100
p38-delta	MAPK13	100	85	80	96	100	64	100	73	92
p38-gamma	MAPK12	95	41	71	79	96	62	100	76	100
PAK1	PAK1	83	90	87	76	88	53	86	81	96
PAK2	PAK2	83	94	82	70	54	0	69	48	94

PAK3	PAK3	76	100	92	86	100	60	92	93	99
PAK4	PAK4	92	88	72	10	93	71	71	77	92
PAK6	PAK6	91	91	84	17	94	72	81	89	83
PAK7	PAK7	91	91	86	12	98	94	95	97	93
PCTK1	CDK16	92	96	100	100	77	100	100	96	100
PCTK2	CDK17	87	100	83	94	100	100	100	100	96
PCTK3	CDK18	96	100	92	100	94	96	93	89	71
PDGFRA	PDGFRA	61	43	23	15	40	12	100	95	99
PDGFRB	PDGFRB	98	34	33	8.3	50	0.8	94	85	44
PDPK1	PDPK1	89	97	87	49	94	58	92	72	89
PFCDPK1(P.falciparum)	CDPK1	93	68	29	46	88	2.4	97	87	92
PFPK5(P.falciparum)	MAL13P1.279	99	96	100	91	66	100	100	100	100
PFTAIRE2	CDK15	79	98	80	96	94	88	97	82	97
PFTK1	CDK14	99	100	100	97	90	84	96	90	71
PHKG1	PHKG1	96	100	94	89	100	86	87	100	97
PHKG2	PHKG2	97	88	73	72	96	62	76	86	80
PIK3C2B	PIK3C2B	35	44	13	49	80	47	97	70	35
PIK3C2G	PIK3C2G	2.4	0.7	2.7	16	66	41	100	20	7.4
PIK3CA	PIK3CA	47	28	2.6	37	86	0	83	4.5	55
PIK3CA(C420R)	PIK3CA	40	28	1.9	24	83	0	94	6.6	73
PIK3CA(E542K)	PIK3CA	50	31	1.4	29	71	0.05	86	6.9	60
PIK3CA(E545A)	PIK3CA	36	26	1.6	23	100	0	81	5.4	79
PIK3CA(E545K)	PIK3CA	33	21	1.5	24	83	0	65	6.4	66
PIK3CA(H1047L)	PIK3CA	27	10	0	7.7	51	0	100	0	27
PIK3CA(H1047Y)	PIK3CA	48	18	4.6	27	100	0	79	1.4	82
PIK3CA(I800L)	PIK3CA	26	4.2	0	6.5	20	0	47	2.4	41
PIK3CA(M1043I)	PIK3CA	44	36	2.5	21	74	0	100	0.7	40
PIK3CA(Q546K)	PIK3CA	41	27	1.8	31	68	0	77	7.7	78
PIK3CB	PIK3CB	47	39	3.1	43	66	0.5	100	9.6	73
PIK3CD	PIK3CD	45	34	13	53	88	1.2	100	12	93
PIK3CG	PIK3CG	2.3	0.75	0	0	28	0	75	0.05	3.7
PIK4CB	PI4KB	2.8	2.2	2.3	1.6	0.55	5.5	25	3.8	1.3
PIKFYVE	PIKFYVE	83	68	64	0.3	84	88	100	100	80
PIM1	PIM1	100	100	94	100	99	93	80	85	91
PIM2	PIM2	99	99	76	93	100	92	100	92	97
PIM3	PIM3	93	95	75	76	96	85	92	81	91
PIP5K1A	PIP5K1A	100	100	100	77	61	69	90	74	92
PIP5K1C	PIP5K1C	100	100	72	62	32	72	87	87	70
PIP5K2B	PIP4K2B	68	99	94	91	73	100	100	100	96
PIP5K2C	PIP4K2C	38	27	20	5.9	62	62	100	100	30
PKAC-alpha	PRKACA	96	100	100	100	99	19	94	86	100
PKAC-beta	PRKACB	99	54	89	100	100	21	100	91	91
PKMYT1	PKMYT1	89	78	91	90	93	88	84	95	100
PKN1	PKN1	97	100	96	85	98	85	100	95	81
PKN2	PKN2	100	89	92	66	93	99	87	91	87
PKNB(M.tuberculosis)	pknB	99	87	93	9.3	70	28	90	71	78

PLK1	PLK1	92	94	92	82	87	95	100	100	100
PLK2	PLK2	71	79	73	31	100	97	100	100	100
PLK3	PLK3	87	97	96	88	85	75	91	81	100
PLK4	PLK4	100	100	96	25	84	30	68	81	91
PRKCD	PRKCD	84	83	88	76	100	68	91	99	100
PRKCE	PRKCE	93	85	77	73	91	35	100	100	79
PRKCH	PRKCH	77	97	76	70	100	46	97	93	100
PRKCI	PRKCI	83	48	54	68	92	36	88	48	88
PRKCQ	PRKCQ	97	100	94	93	100	85	96	57	71
PRKD1	PRKD1	97	43	33	100	100	70	91	100	91
PRKD2	PRKD2	98	88	55	100	100	84	100	95	98
PRKD3	PRKD3	100	78	43	100	100	84	96	83	97
PRKG1	PRKG1	88	52	95	100	98	95	89	85	91
PRKG2	PRKG2	81	64	71	38	83	88	100	85	100
PRKR	EIF2AK2	81	65	70	57	87	50	64	85	94
PRKX	PRKX	100	95	92	98	100	93	99	87	84
PRP4	PRPF4B	100	88	83	77	100	96	98	98	79
PYK2	PTK2B	71	65	73	62	100	81	86	82	94
QSK	KIAA0999	100	99	92	90	74	79	100	99	100
RAF1	RAF1	77	66	57	77	100	59	61	81	100
RET	RET	92	88	53	1.9	61	3.8	70	78	90
RET(M918T)	RET	83	37	46	1.2	61	3.7	100	95	80
RET(V804L)	RET	96	45	92	19	67	91	99	99	83
RET(V804M)	RET	98	94	86	22	74	61	88	83	94
RIOK1	RIOK1	93	49	77	32	25	80	75	72	72
RIOK2	RIOK2	100	100	82	55	75	90	100	100	100
RIOK3	RIOK3	100	79	89	39	16	71	72	74	82
RIPK1	RIPK1	94	94	94	90	96	87	92	92	90
RIPK2	RIPK2	62	17	11	76	79	4.3	72	92	53
RIPK4	RIPK4	100	98	90	4.7	92	72	100	100	100
RIPK5	DSTYK	56	70	57	27	90	61	100	96	66
ROCK1	ROCK1	100	95	88	61	79	18	89	100	99
ROCK2	ROCK2	87	82	84	69	84	22	99	100	95
ROS1	ROS1	86	91	84	83	95	48	82	79	97
RPS6KA4(Kin.Dom.1-N-terminal)	RPS6KA4	97	42	93	100	100	77	89	85	82
RPS6KA4(Kin.Dom.2-C-terminal)	RPS6KA4	87	84	79	65	83	93	100	87	100
RPS6KA5(Kin.Dom.1-N-terminal)	RPS6KA5	75	100	86	100	100	73	96	100	100
RPS6KA5(Kin.Dom.2-C-terminal)	RPS6KA5	92	90	87	66	94	100	100	94	94
RSK1(Kin.Dom.1-N-terminal)	RPS6KA1	100	100	70	17	84	76	100	97	77
RSK1(Kin.Dom.2-C-terminal)	RPS6KA1	90	72	38	63	98	42	94	84	77
RSK2(Kin.Dom.1-N-terminal)	RPS6KA3	84	81	72	14	54	70	99	90	100
RSK2(Kin.Dom.2-C-terminal)	RPS6KA3	85	90	29	78	100	100	100	100	100
RSK3(Kin.Dom.1-N-terminal)	RPS6KA2	90	98	93	81	90	96	100	74	81
RSK3(Kin.Dom.2-C-terminal)	RPS6KA2	100	100	100	100	100	69	73	70	89
RSK4(Kin.Dom.1-N-terminal)	RPS6KA6	78	79	53	7.8	23	99	100	78	100
RSK4(Kin.Dom.2-C-terminal)	RPS6KA6	90	46	6.7	62	91	7	72	75	68

S6K1	RPS6KB1	74	79	71	48	81	80	97	87	100
SBK1	SBK1	92	75	82	68	75	95	100	100	94
SGK	SGK1	100	100	100	89	65	80	100	86	100
SgK110	SgK110	73	100	73	100	100	100	100	100	81
SGK2	SGK2	100	100	100	86	82	99	100	96	100
SGK3	SGK3	61	64	67	45	87	96	96	93	93
SIK	SIK1	98	93	88	48	100	50	74	80	96
SIK2	SIK2	100	94	100	41	92	66	100	91	81
SLK	SLK	94	100	76	7.7	93	31	89	92	98
SNARK	NUAK2	67	70	60	4.8	26	65	100	91	97
SNRK	SNRK	86	72	75	80	96	100	100	100	97
SRC	SRC	79	76	74	6.5	71	19	89	74	89
SRMS	SRMS	83	80	71	43	81	42	100	96	87
SRPK1	SRPK1	100	83	77	17	24	78	74	74	85
SRPK2	SRPK2	84	65	89	97	100	100	78	100	100
SRPK3	SRPK3	78	72	72	73	80	89	86	99	71
STK16	STK16	76	79	69	7	90	3.1	84	90	81
STK33	STK33	99	100	99	97	100	92	94	100	90
STK35	STK35	100	100	63	56	99	6.3	98	90	100
STK36	STK36	80	73	28	90	90	79	84	85	85
STK39	STK39	100	100	90	65	64	65	100	85	94
SYK	SYK	90	88	72	33	73	76	79	67	82
TAK1	MAP3K7	67	72	46	4	56	71	100	88	100
TAOK1	TAOK1	85	74	35	96	72	10	100	100	87
TAOK2	TAOK2	70	83	81	99	90	5.6	90	91	96
TAOK3	TAOK3	81	94	90	100	81	71	100	100	99
TBK1	TBK1	84	78	84	18	81	66	66	69	93
TEC	TEC	95	94	93	40	100	91	91	94	100
TESK1	TESK1	83	97	63	61	100	8.9	89	84	89
TGFBR1	TGFBR1	91	94	72	81	100	7.8	56	85	69
TGFBR2	TGFBR2	89	5.9	48	32	100	14	82	100	38
TIE1	TIE1	100	88	44	73	94	51	100	100	93
TIE2	TEK	92	36	51	68	94	40	80	98	100
TLK1	TLK1	98	96	83	85	91	76	79	80	75
TLK2	TLK2	83	86	82	75	98	87	79	86	95
TNIK	TNIK	92	86	84	18	64	46	77	76	84
TNK1	TNK1	100	100	89	47	97	37	95	90	90
TNK2	TNK2	84	81	76	51	99	83	100	100	90
TNNI3K	TNNI3K	94	100	75	38	100	53	71	94	93
TRKA	NTRK1	97	73	45	7.5	38	3.9	81	74	100
TRKB	NTRK2	100	100	65	12	62	12	95	83	100
TRKC	NTRK3	100	100	87	29	38	12	100	100	100
TRPM6	TRPM6	98	83	57	54	77	89	96	91	100
TSSK1B	TSSK1B	100	92	100	96	100	93	95	100	100
TSSK3	TSSK3	100	91	97	73	90	86	98	94	93
TTK	TTK	86	57	4.9	47	100	15	81	68	62

TXK	TXK	75	83	80	31	93	36	100	100	97
TYK2(JH1domain-catalytic)	TYK2	100	100	100	8.1	11	93	70	68	98
TYK2(JH2domain-pseudokinase)	TYK2	59	51	46	1.6	27	57	84	82	81
TYRO3	TYRO3	51	92	41	51	84	51	100	100	95
ULK1	ULK1	69	71	79	72	76	100	100	100	100
ULK2	ULK2	99	100	92	72	75	77	96	91	100
ULK3	ULK3	90	94	71	8.2	69	84	100	96	99
VEGFR2	KDR	73	85	74	14	75	43	100	97	100
VPS34	PIK3C3	47	65	75	71	69	95	100	74	75
VRK2	VRK2	74	96	97	36	3.8	100	100	100	73
WEE1	WEE1	90	97	99	100	100	62	72	81	88
WEE2	WEE2	82	62	66	77	95	87	94	81	97
WNK1	WNK1	84	76	4.9	79	82	1.6	100	92	85
WNK2	WNK2	73	71	0	100	63	16	100	100	78
WNK3	WNK3	90	83	1.6	83	91	16	100	92	98
WNK4	WNK4	100	100	11	100	97	4.8	100	100	93
YANK1	STK32A	71	63	55	28	82	99	100	99	95
YANK2	STK32B	77	98	87	88	100	79	85	93	100
YANK3	STK32C	82	93	70	70	100	81	89	100	81
YES	YES1	75	82	58	27	98	43	100	100	99
YSK1	STK25	79	97	81	70	96	41	100	99	100
YSK4	MAP3K19	90	40	28	0.25	29	35	91	96	53
ZAK	ZAK	78	87	90	44	100	69	100	99	95
ZAP70	ZAP70	95	99	100	61	73	94	100	93	93

General synthetic methods

^{1}H NMR spectra were recorded on a BRUKER 400 MHz spectrometer. Chemical shifts are reported as δ values downfield from internal TMS in appropriate organic solutions. The abbreviations used for explaining the multiplicities were as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High resolution mass (ESI HRMS) was recorded on a Thermofisher Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. The relative purity and the mass of the products were confirmed by LC/MS (220 nm to 420 nm) on a Waters acquity uplc photodiode array detector system using the following conditions: Column, BEH C18 50*2.1 mm; 1.8µm ; Solvent A, water 0.1% formic acid or water ammonium carbonate 2 g/l; Solvent B, CH3CN; flow rate, 0.8 ml/min; run time, 2.2 min; gradient, from 5 to 95% solvent B; mass detector, Waters SQ detector. All compounds were purified by LC/MS on a waters Autopurification system using the following conditions : Column, Xbridge C18 150*30mm, 5µm; Solvent A, water 0.1% formic acid or water ammonium carbonate 2 g/l; Solvent B, CH3CN; flow rate, 50 ml/min; run time, 10 or 15 min; with adapted isocratic elution mode; mass detector, Waters ZQ detector.

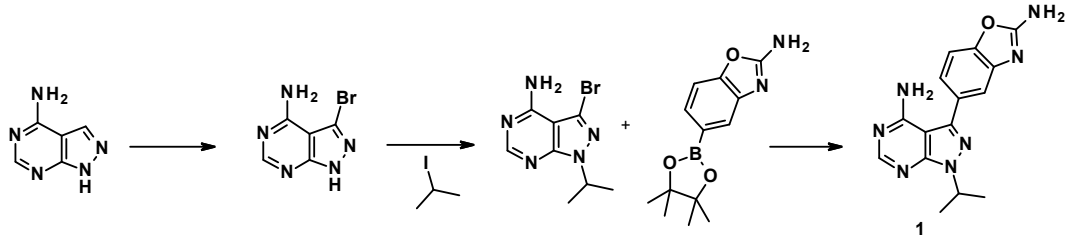
Abbreviations

AcOH	acetic acid	EtOAc	ethyl acetate
NH_3	ammonia	Et_2O	diethyl ether
HCl	hydrochloric acid	THF	tetrahydrofuran
MeOH	methanol	DMSO	dimethyl sulfoxide
MeCN	acetonitrile	HCl	hydrochloric acid
Na_2SO_4	sodium sulfate	DMF	*N,N*-dimethylformamide
HPLC	high performance liquid chromatography		

Preparation of compounds shown in Table 1. R_1 exploration

Preparation of 5-(4-Amino-1-isopropyl-1H-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (1)

Compound **1** was prepared by employing the following sequence:



3-Bromo-1-isopropyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-amine

N-Bromosuccinimide (6.92 g, 38.9 mmol, 1.05 eq.) was added to a suspension of 1*H*-pyrazolo[3,4-d]pyrimidin-4-ylamine (5.00 g, 37.0 mmol, 1.00 eq.) in DMF (50 ml) and the reaction mixture was heated at 60 °C for 3 hours, cooled to room temperature and the solid was collected by filtration, washed with diethyl ether and dried to a constant weight to afford the title compound (7.92 g, quant.) as a beige solid: LCMS (t_R=0.70 min., purity= 96.7%), ESI+ m/z 214, 216$(M+H)^+$. The compound was directly engaged in the next step without further purification.

2-Iodopropane (5.54 ml, 55.5 mmol, 1.50 eq.) was added to a stirred solution of 3-bromo-1*H*-pyrazolo[3,4-d]pyrimidin-4-ylamine (7.92 g, 37.0 mmol, 1.00 eq.) and cesium carbonate (36.17 g, 110 mmol, 3.00 eq.) in DMF (30.0 ml). The reaction mixture was stirred at room temperature for 16 h, filtered and the filtrate was purified by flash chromatography (silica gel, DCM-MeOH (0-20%). The fractions were evaporated and the residue crystallised from heptane / EtOAc. The solid was collected by filtration and dried to a constant weight to afford the title compound (5.75 g, 61%) as a beige solid: LCMS (t_R=0.79 min., purity= 99.8%), ESI+ m/z 256, 257 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42-1.44 (d, *J* = 6.7 Hz, 6H), 4.94-5.01 (q, *J* = 6.7 Hz, 1H), 8.20 (s, 1H).

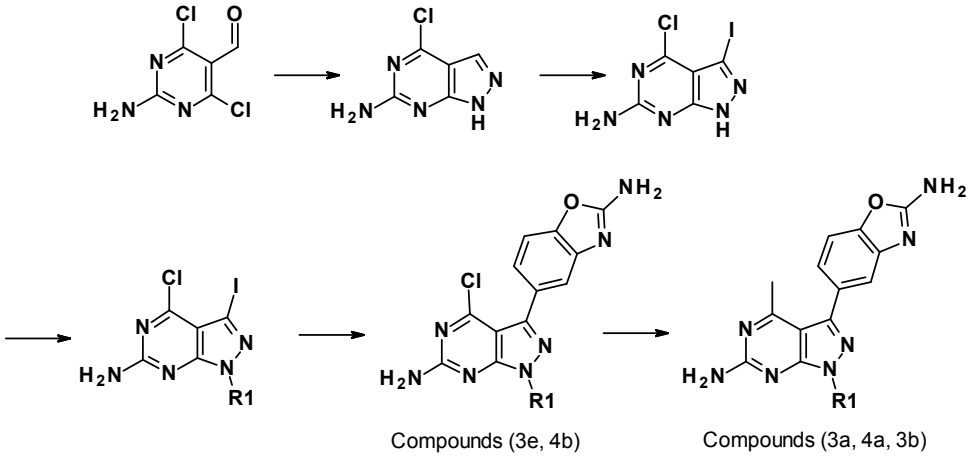
5-(4-Amino-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (1)
Tetrakis(triphenylphosphine)palladium(0) (2.08 g, 1.80 mmol, 0.08 eq.) was added to a mixture of 3-bromo-1-isopropyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-ylamine (5.75 g, 22.5 mmol, 1.00 eq.), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[d]oxazol-2-amine (7.59 g, 29.2 mmol, 1.30 eq.) and sodium carbonate (11.9 g, 110 mmol, 5.00 eq.) in 1,4-dioxane (86.3 mL) et water (28.8 mL) : The reaction mixture was heated at 110 °C for 2 h 45 min, cooled to room temperature and filtered through a pad of Celite. The filtrate was concentrated and the residue was triturated with DCM. The solid was recuperated by filtration, washed with EtOAc and dried to a constant weight to afford the title compound (**1**, 6.42g, 92%) as a pale pink solid that was used without further purification: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.49 (d, *J* = 6.7 Hz, 6H), 5.05 (p, *J* = 6.7 Hz, 1H), 7.24 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.41 (d, *J* = 1.7 Hz, 1H), 7.46 (d, J = 8.1 Hz, 1H), 7.52 (s, 2H), 8.23 (s, 1H).

HRMS (ESI) calculated for $C_{15}H_{15}N_7O$ $[M+H]^+$ 310.1411, found 310.14096.

Preparation of :

- **5-(6-Amino-4-chloro-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (3e)**
- **5-(6-Amino-1-isopropyl-4-methyl-1*H*-pyrazolo[3,4-d]pyrimidin-3-yl)benzo[d]oxazol-2-amine (3a)**
- **5-(6-Amino-1-butyl-4-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (4b)**
- **5-(6-Amino-1-butyl-4-methyl-1*H*-pyrazolo[3,4-d]pyrimidin-3-yl)benzo[d]oxazol-2-amine (4a)**
- **5-(6-Amino-4-ethyl-1-isopropyl-1*H*-pyrazolo[3,4-d]pyrimidin-3-yl)benzo[d]oxazol-2-amine (3b)**

Compound **3e, 3a, 4a** and **3b** were prepared by employing the following sequence:



4-Chloro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine

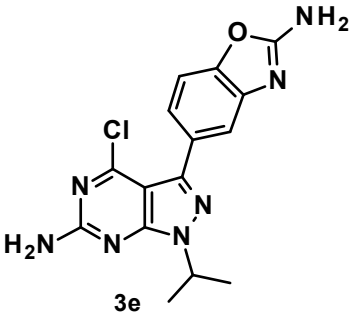
2-Amino-4,6-dichloropyrimidine-5-carbaldehyde (30.0 g, 156 mmol, 1.0 equiv) and triethylamine (30.5 mL, 219 mmol, 1.15 equiv) were dissolved into a mixture of THF/H_2O 3.5/1. Hydrazine solution 98 wt % in water (9.10 mL, 188 mmol, 1.0 equiv) was added dropwise over a period of 20 min. The mixture was stirred at 60 °C for 1 h 30 min and left at room temperature overnight affording a heterogeneous mixture. After filtration of the orange precipitate, the filtrate was concentrated in under vacuum to give the desired product (15.2 g, 89.6 mmol, 57%) that was used without further purification: LCMS (t_R=0.78 min., purity= 100%), ESI+ m/z 170 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.15 (br s, 2H), 7.96 (s, 1H), 13.25 (s, 1H).

4-Chloro-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine

To a solution of 4-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (7.0 g, 41.28 mmol, 1.0 equiv) in DMF (105 mL, 0.4 M) was added *N*-iodosuccinimide (18.57g, 82.56 mmol, 2.0 equiv) at room temperature. The mixture was heated at 80°C until full conversion by HPLC-MS of the starting material and cooled down to 25°C to be transferred over a 1L solution of cold water. The precipitate formed was filtered and dried to obtain the desired product as a yellow solid (12.0 g, 98%): LCMS (t_R=1.08 min., purity= 99%), ESI+ m/z 296 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.31-7.48 (br s, 2H), 13.51 (s, 1H).

Generic alkylation procedure to afford 4-amino-3-iodo-1-alkyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine analogues

To a solution of 4-chloro-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine in DMF (0.34 M) was added cesium carbonate (1.05 equiv) and the desired alkylation reagent (R_1-X, 1.05 equiv.). The mixture was stirred at room temperature until completion of the reaction by HPLC-MS and then diluted with EtOAc and water. The separated aqueous phase was extracted with EtOAc and the combined organic layers were washed with water, brine and dried over Na_2SO_4. After filtration, the solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography to yield desired alkylated derivative.



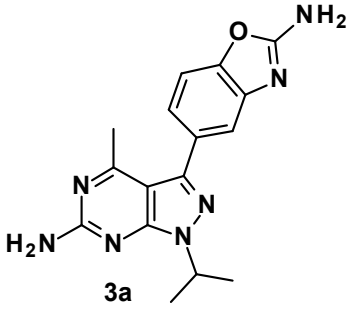
3e

5-(6-Amino-4-chloro-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (3e)

To a stirred suspension of 3-iodo-4-chloro-1*H*-pyrazolo[3,4-d]pyrimidin-6-ylamine (1.00 g, 3.64 mmol, 1.00 eq.) and cesium carbonate (2.37 g; 7.27 mmol; 2.00 eq.) in DMF (10 ml), was added 2-iodopropane (0.38 ml;,3.82 mmol, 1.05 eq.) and the reaction mixture was stirred for 3 h and 30 minutes at room temperature. Water was added and the reaction mixture was extracted into EtOAc. The organic phases were washes with water, dried over $MgSO_4$ and concentrated to dryness. The residue was purified by flash chromatography (silica gel, heptane/ EtOAc 1:1) to afford the title compound (880 mg, 76%): LCMS (t_R=0.97 min., purity= 95%), ESI+ m/z 318 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.38 (d, J = 6.6 Hz, 6H), 2.63 (s, 3H), 4.80 (hept, J = 6.6 Hz, 1H), 6.91 (s, 2H).

In a microwave tube, was added 4-chloro-3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-d]pyrimidin-6-ylamine (0.50 g, 1.48 mmol, 1.00 eq.) and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[d]oxazol-2-amine (0.58 g, 2.22 mmol, 1.50 eq.) to a mixture of 1,4-dioxane (5.28 ml) and a 2.0 M aqueous solution of potassium carbonate (2.22 ml, 4.44 mmol, 3.00 eq.). The reaction mixture was degassed and purged with argon. 1,1'-Bis(diphenylphosphino)ferrocenepalladium(II) dichloride, dichloromethane (121 mg, 0.15 mmol, 0.10 eq.) was added rapidly and the reaction mixture was heated at 100 °C for 60 minutes, cooled to room temperature and EtOAc was added. The reaction mixture was filtered, concentrated to dryness and purified by mass-triggered preparative LCMS (acidic conditions) to afford the title compound (**3e**, 210 mg, 41%): LCMS (t_R=1.03 min., purity= 97%), ESI+ m/z 344 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.47 (d, J = 6.7 Hz, 6H), 4.91 (h, J = 6.7 Hz, 1H), 7.22 – 7.32 (m, 3H), 7.41 (d, J = 8.2 Hz, 1H), 7.44 – 7.50 (m, 3H).

HRMS (ESI) calculated for $C_{16}H_{14}ClN_7O$ $[M+H]^+$ 344.1021, found 344.1019.



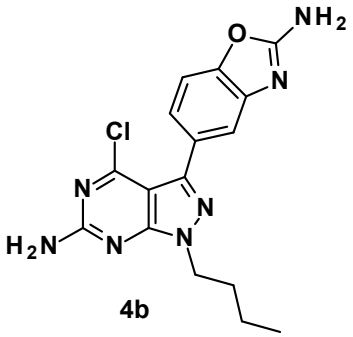
3a

5-(6-Amino-1-isopropyl-4-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (3a)

5-(6-Amino-4-chloro-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (**3e**, 50.0 mg, 0.15 mmol, 1.00 eq.) was dissolved in THF (250 µl) and bis(tri-*t*-butylphosphine)palladium(0) (14.9 mg, 0.03 mmol, 0.20 eq.) was added at 0 °C. The reaction mixture was degassed and purged with argon and 1M solution of dimethylzinc in heptane (436 µl, 0.44 mmol, 3.00 eq.) was added

keeping the temperature at 0 °C. The reaction mixture was stirred at room temperature overnight, quenched with a saturated aqueous solution of ammonium chloride and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated to dryness. The residue was purified by mass-triggered preparative HPLC (acidic method) to afford the title compound (**3a**, 14.3 mg, 30%) as a beige solid: LCMS (t_R=0.92 min., purity= 97%), ESI+ m/z 324 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42-1.46 (d, J = 7.1 Hz, 6H), 2.35 (s, 3H), 4.87-4.94 (hp, J = 7.1 Hz, 1H), 6.72-6.76 (br s, 2H), 7.22-7.26 (d, J = 8.1 Hz, 1H), 7.38.7.42 (overlapping s and d, 2H), 7.46-7.52 (br s, 2H).

HRMS (ESI) calculated for $C_{16}H_{17}N_7O$ $[M+H]^+$ 324.1567, found 324.1567.



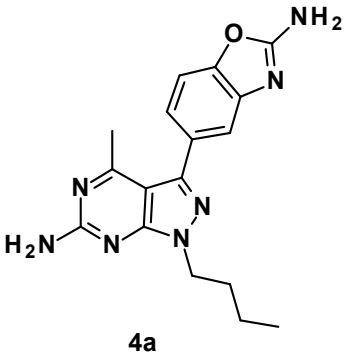
4b

5-(6-Amino-1-butyl-4-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (4b)

4-Chloro-3-iodo-*1H*-pyrazolo[3,4-d]pyrimidin-6-amine (4.00 g, 4.06 mmol, 1.00 eq.) was dissolved in DMF (14.4 ml) and cesium carbonate (1.32 g, 4.06 mmol, 1.00 eq.) was added followed by 1-iodobutane (0.55 ml, 4.87 mmol, 1.20 eq.) at room temperature for 18 h. Water was added and the reaction mixture was extracted into EtOAc. The suspension was filtered and the organic phase was separated, washed with water and dried over $MgSO_4$ and concentrated to dryness. The residue was purified by flash chromatography (silica gel, heptane:EtOAc 8:2) to afford the alkylated intermediate (593 mg, 42%) as a yellow soilid: LCMS (t_R=1.22 min., purity= 99%), ESI+ m/z 352 $(M+H)^+$.

In a microwave tube, was added 1-butyl-4-chloro-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (543 mg, 1.54 mmol, 1.00 eq.), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[*d*]oxazol-2-amine amine (603 mg, 2.32 mmol, 1.50 eq.) to a mixture of 1,4-dioxane (5.28 ml) and a 2.0 M aqueous solution of potassium carbonate (2.32 ml, 4.63 mmol, 3.00 eq.). The reaction mixture was degassed and purged with argon. 1,1'-Bis(diphenylphosphino)ferrocene-palladium(II) dichloride, dichloromethane (126 mg, 0.15 mmol, 0.10 eq.) was added rapidly and the reaction mixture was heated at 100 °C for 60 minutes, cooled to room temperature, concentrated and purified directly by mass-triggered preparative LCMS (acidic conditions) to afford the title compound (**4b**, 171 mg, 31%): LCMS (t_R=1.08 min., purity= 99%), ESI+ m/z 358 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 0.82 (t, J = 7.3 Hz, 2H), 1.83 (h, J = 7.3 Hz, 2H), 4.21 (t, J = 7.3 Hz, 1H), 7.20 – 7.27 (overlapping signals br s and d, 3H), 7.35 (d, J = 8.2 Hz, 1H), 7.46 - 7.49 (overlapping signals br s and s, 3H).

HRMS (ESI) calculated for $C_{16}H_{14}ClN_7O$ $[M+H]^+$ 344.1021, found 344.1019.

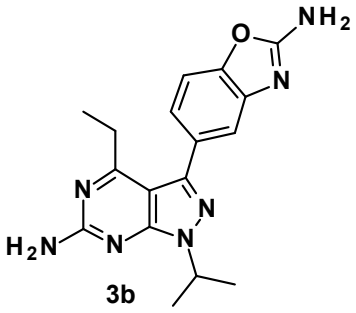


4a

5-(6-Amino-1-butyl-4-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (4a)

5-(6-amino-1-butyl-4-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (80.0 mg, 0.22 mmol, 1.00 eq.) was dissolved in THF (500 µl) and bis(tri-*t*-butylphosphine)palladium(0) (13.0 mg, 0.01 mmol, 0.05 eq.) was added at 0 °C. The reaction mixture was degassed and purged with argon and a solution of dimethylzinc in THF (0.37 ml, 0.45 mmol, 2.00 eq.) was added keeping the temperature at 0 °C. The reaction mixture was stirred at room temperature overnight, quenched with a saturated aqueous solution of ammonium chloride and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated to dryness. The residue was purified by mass-triggered preparative HPLC (acidic method) to afford the title compound (**4a**, 51.1 mg, 70%) as an orange solid. LCMS (t_R=3.19 min., purity= 97%, [C1-ACN-Full Range 7.5 min, positive ionization, UV 254 nm]), ESI+ m/z 338 $(M+H)^+$; Rf = 0.15 (Hex/EtOAc:1/1) [SiO2 ; UV, KMnO4];^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (t, J = 7.4 Hz, 3H), 1.36 – 1.19 (m, 2H), 1.71 – 1.85 (m, 2H), 2.35 (s, 3H), 4.18 (t, J = 7.0 Hz, 2H), 6.78 (s, 2H), 7.16 – 7.27 (m, 1H), 7.34 – 7.44 (m, 2H), 7.49 (s, 2H).

HRMS (ESI) calculated for $C_{16}H_{19}N_7O$ $[M+H]^+$ 337.1651, found 337.1650.



3b

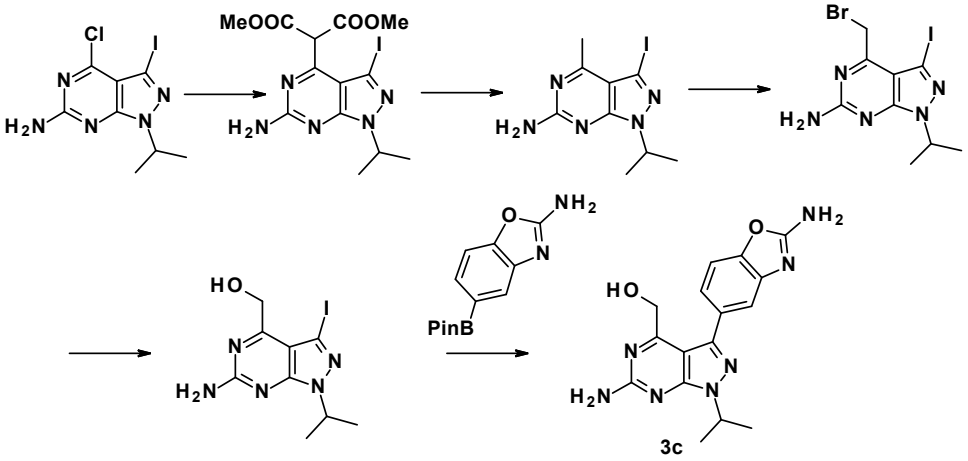
5-(6-Amino-4-ethyl-1-isopropyl-1*H*-pyrazolo[3,4- *d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (3b)

5-(6-amino-4-chloro-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine **3e** (75.0 mg, 0.22 mmol, 1.00 eq.) was dissolved in THF (2.00 ml). The reaction mixture was degassed and purged with argon then cooled to 0 °C. Bis(tri-*t*-butylphosphine)palladium(0) (22.3 mg, 0.04 mmol, 0.20 eq.) was added followed by diethylzinc (0.87 ml, 1.00 M, 0.87 mmol;, 4.00 eq.)**.** The reaction mixture was stirred at room temperature for 1 h, quenched with a saturated aqeous solution of NH_4Cl, extracted into EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated to dryness. The residue was purified by mass-triggered preparative HPLC to afford the title compound (**3b**, 15.1 mg, 21%) as a pale yellow solid: LCMS (t_R=0.93 min., purity= 99%), ESI+ m/z 338 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02 (t, J = 7.5 Hz, 3H), 1.45 (d, J = 6.7 Hz, 6H), 2.63 – 2.71 (m, 2H), 4.90 (h, J = 6.7 Hz, 1H), 6.74 (s, 2H), 7.17 (dd, J = 8.2, 1.7 Hz, 1H), 7.36 (d, J = 1.6 Hz, 1H), 7.41 (d, J = 8.2 Hz, 1H), 7.49 (s, 2H).

HRMS (ESI) calculated for $C_{17}H_{19}N_7O$ $[M+H]^+$ 338.1724, found 338.1721.

Preparation of (6-Amino-3-(2-aminobenzo[*d*]oxazol-5-yl)-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)methanol (3c)

The title compound **3c** was prepared using the following sequence:



Dimethyl 2-(6-amino-3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)malonate

To a stirred suspension of 4-chloro-3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-ylamine (2.00 g, 5.93 mmol, 1.00 eq.) and cesium carbonate (4.63 g, 14.2 mmol, 2.40 eq.) in DMSO (13.2 ml) at room temperature, was added dimethyl malonate (1.88 g, 14.2 mmol, 2.40 eq.). The resulting suspension was heated at 100 °C for 2 h 15 min to afford a yellow suspension. The reaction mixture was cooled to room temperature and water was added. The reaction mixture was extracted into EtOAc and the organic phase was recuperated, washed with water, dried over $MgSO_4$ and concentrated to dryness to afford the title compound (2.60 g, 95%) as a yellow solid that was used without further purification: LCMS (t_R=1.08 min., purity= 94%), ESI+ m/z 434 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43 (d, J = 6.6 Hz, 6H), 3.78 (s, 6H), 4.78-4.86 (hept, J = 6.6 Hz, 1H), 5.62 (s, 1H), 7.13 (br s, 2H).

3-Iodo-1-isopropyl-4-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine

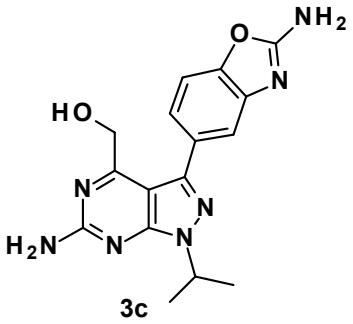
Dimethyl 2-(6-amino-3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl)malonate (2.40 g; 5.54 mmol; 1.00 eq.) was suspended in 6N HCl (aq) (52 ml) and heated at 50 °C for 4 hours to afford a yellow solution. The reaction mixture was cooled to 0 °C and 8N NaOH was added to neutral pH and the reaction mixture was extracted into EtOAc. The organic phases was dried over $MgSO_4$ and concentrated to dryness to afford the title compound (1.60 g, 91%) as an orange solid: LCMS (t_R=0.97 min., purity= 95%), ESI+ m/z 318 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42 (d, J = 6.7 Hz, 6H), 2.64 (s, 3H), 4.74-4.84 (hept, J = 6.7 Hz, 1H), 6.91 (s, 2H).

(6-Amino-3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)methanol

To a stirred solution of 3-iodo-1-isopropyl-4-methyl-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (730 mg, 2.30 mmol, 1.00 eq.) in DCM (7.30 ml), were added *N*-bromosuccinimide (409 mg, 2.30 mmol, 1.00 eq.) and benzoyl peroxide (27.9 mg, 0.12 mmol, 0.05 eq) and the reaction mixture was heated at 70

°C for 10 h. **The** reaction mixture was evaporated to dryness, dissolved in DMF and purified directly by mass-triggered preparative LCMS to afford the title compound (480 mg, 53%) as an orange solid : LCMS (t_R=1.19 min., purity= 97%), ESI+ m/z 396 $(M+H)^+$. 4-(Bromomethyl)-3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine was directly engaged in the next step without further purification.

3-Iodo-1-isopropyl-4-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (480 mg,1.21 mmol, 1.00 eq.) was dissolved in DMSO (4.8 ml) and $NaHCO_3$ (305 mg, 3.64 mmol, 3.00 eq.) was added and the reaction mixture was stirred at 50 °C for 16 h. The reaction mixture was purified directly by mass-triggered preparative LCMS to afford the title compound (60.0 mg, 15%) as a beige solid: LCMS (t_R=0.96 min., purity= 97%), ESI+ m/z 334 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.40 (d, *J* = 6.7 Hz, 6H), 4.83 (dd, *J* = 6.1, 4.2 Hz, 3H), 5.14 (t, *J* = 5.6 Hz, 1H), 7.03 (s, 2H).



3c

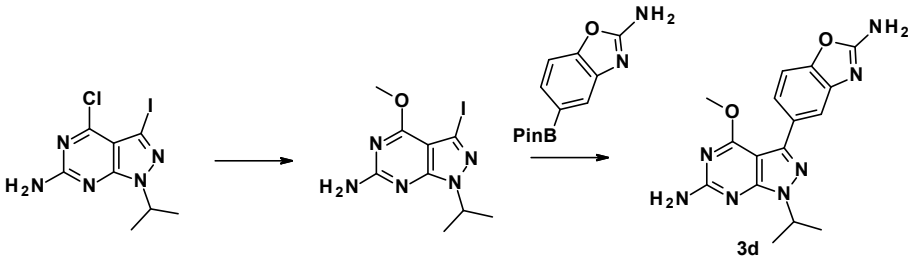
(6-Amino-3-(2-aminobenzo[d]oxazol-5-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)methanol (3c)

In a microwave tube was added (6-amino-3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)methanol (60.0 mg, 0.18 mmol, 1.00 eq.), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[d]oxazol-2-amine (70.3 mg, 0.27 mmol, 1.50 eq.), a 2.0 M aqueous solution of potassium carbonate (0.27 ml, 0.54 mmol, 3.00 eq.) and 1,4-dioxane (0.48 ml). The reaction mixture was degassed and purged with argon before 1,1'-bis(diphenylphosphino)ferrocenepalladium(II) dichloride.dichloromethane (14.7 mg, 0.02 mmol, 0.10 eq.) was added. The reaction mixture was heated at 100 °C for 30 min. The reaction mixture was cooled to room temperature wans purified directly by mass-triggered preparativeLCMS (acidic conditions) to afford the title compound (**3c**, 11.1 mg, 18.2%): LCMS (t_R=0.87 min., purity= 99%), ESI+ m/z 340 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.47 (d, *J* = 6.7 Hz, 6H), 4.49 (d, *J* = 5.5 Hz, 2H), 4.93 (h, *J* = 6.6 Hz, 1H), 5.01 (t, *J* = 5.5 Hz, 1H), 6.88 (s, 2H), 7.27 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.42 – 7.54 (m, 3H).

HRMS (ESI) calculated for $C_{16}H_{17}N_7O_2$ $[M+H]^+$ 340.1516, found 340.1511

Preparation of:5-(6-Amino-1-isopropyl-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (3d)

The title compound **3d** was prepared using the following sequence:

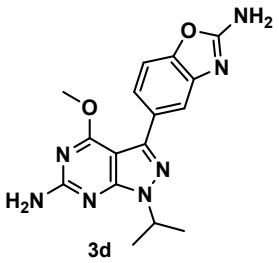


3d

3-Iodo-1-isopropyl-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine

To a stirred solution of 4-chloro-3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (300 mg, 0.89 mmol, 1.00 eq.) in MeOH (2.0 ml) was added 30% solution of sodium methoxide in MeOH (0.49 ml, 2.67 mmol, 3.00 eq.). The reaction mixture was stirred at room temperature for 30 minutes, quenched with a saturated solution of ammonium chloride and extracted into EtOAc. The organic phases were washed with brine, dried over $MgSO_4$ and concentrated to dryness to afford the title compound (280 mg, 95%) as an orange solid: LCMS (t_R=1.18 min., purity= 99%), ESI+ m/z 334 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.37 (d, J = 6.6 Hz, 6H), 3.97 (s, 3H), 4.76 (h, J = 6.6 Hz, 1H), 6.88 (s, 2H)

5-(6-Amino-1-isopropyl-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (3d)



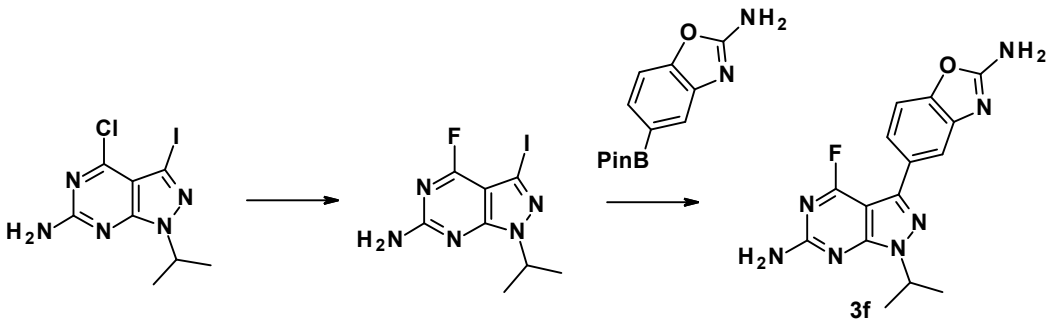
3d

In a microwave tube, 3-Iodo-1-isopropyl-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (98.0 mg, 0.29 mmol, 1.00 eq.) and a 2.0M aqueous solution of potassium carbonate (0.44 ml, 0.88 mmol, 3.00 eq.) in 1,4-dioxane (1.03 ml) were degassed and purged with argon. 1,1'-Bis(diphenylphosphino)ferrocenepalladium(II) dichloride.dichloromethane (24.0 mg, 0.03 mmol, 0.10 eq.) was added followed by 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[*d*]oxazol-2-amine (115 mg, 0.44 mmol, 1.50 eq.) and the reaction mixture was heated at 100 °C for 30 min. The reaction mixture was cooled to room temperature. EtOAc was added and the reaction mixture filtered and the filtrate was concentrated and purified by mass-triggered preparative LCMS (basic conditions) to afford the title compound (**3d**, 34 mg, 34%) as a pale yellow solid: LCMS (t_R=0.90 min., purity= 97%), ESI+ m/z 340 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.46 (d, J = 6.6 Hz, 6H), 3.99 (s, 3H), 4.88 (h, J = 6.7 Hz, 1H), 6.76 (s, 2H), 7.37 (d, J = 8.3 Hz, 1H), 7.42 (s, 2H), 7.59 (dd, J = 8.3, 1.7 Hz, 1H), 7.75 (d, J = 1.6 Hz, 1H).

HRMS (ESI) calculated for $C_{16}H_{17}N_7O_4$ $[M+H]^+$ 340.1516, found 340.1515

Preparation of 5-(6-Amino-4-fluoro-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[d]oxazol-2-amine (3f)

The title compound **3f** was prepared using the following sequence:



3f

5-(6-Amino-4-fluoro-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[d]oxazol-2-amine (3f)

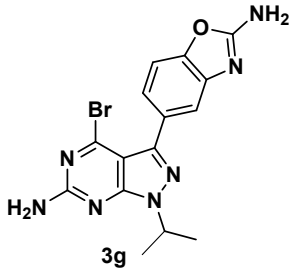
To a stirred solution of 4-chloro-3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (300 mg, 0.89 mmol, 1.00 eq.) in THF (3.00 ml) was added triethylamine (0.15 ml, 1.07 mmol, 1.20 eq.) followed by a 1.0 M solution of tetrabutylammonium fluoride (0.89 ml, 0.89 mmol, 1.00 eq.). The reaction mixture was heated at 80 °C for 11 , cooled to room temperature and diluted with EtOAc. The reaction mixture was washed with brine and the organic phase separated, dried over $MgSO_4$ and concentrated to dryness. The residue was purified by mass-triggered preparative LCMS to afford the title compound (150 mg, 53%) as a yellow solid: LCMS (t_R=1.09 min., purity= 99%), ESI+ m/z 322 $(M+H)^+$; $)^+$; ^{1}H NMR (DMSO-d_6) δ 1.42 (d, *J* = 6.7 Hz, 6H), 4.81 (h, *J* = 6.7 Hz, 1H), 7.38 (br s, 2H).

In a microwave tube, 4-fluoro-3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (20.0 mg, 0.06 mmol, 1.00 eq.) was added to a mixture of a 2.0 M aqueous solution of potassium carbonate (0.09 ml, 0.19 mmol, 3.00 eq.) and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[*d*]oxazol-2-amine amine (24.3 mg, 0.09 mmol, 1.50 eq.) in 1,4-dioxane (0.24 ml). The reaction mixture was degassed and purged with argon. 1,1'-Bis(diphenylphosphino)ferrocene-palladium(II) dichloride, dichloromethane (5.1 mg, 0.01 mmol, 0.10 eq.) was added and the reaction mixture was heated at 100 °C for 30 minutes, cooled to room temperature and MeOH was added. The reaction mixture was filtered, concentrated and purified directly by mass-triggered preparative LCMS (basic conditions) to afford the title compound **3f** (6 mg, 31%)**.** δ 1.49 (d, *J* = 6.6 Hz, 6H), 4.92 (h, *J* = 6.9 Hz, 1H), 7.25 (s, 2H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.51 (d, *J* = 4.7 Hz, 3H), 7.65 (s, 1H).

HRMS (ESI) calculated for $C_{16}H_{26}N_3O_4$ $[M+H]^+$ 328.1317, found 328.1310.

Preparation of 5-(6-Amino-4-bromo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (3g)

The title compound **3g** was prepared starting directly from previously described **3e**.



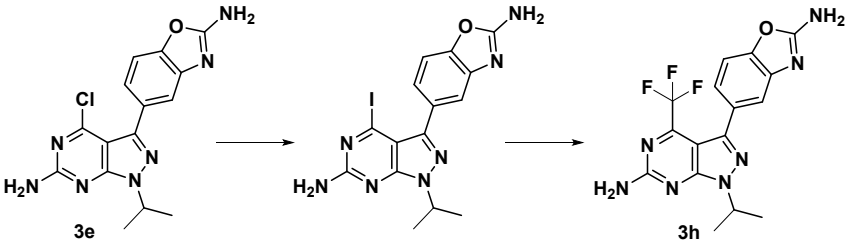
3g

5-(6-Amino-4-chloro-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)benzo[d]oxazol-2-amine (200 mg, 0.29 mmol, 1.00 eq.) was dissolved in DCM (1 ml). A 1.0 M solution of BBr_3 in DCM (0.58 ml, 0.58 mmol, 2.00 eq.) was added and the reaction mixture was stirred at room temperature for 45 minutes under argon. The reaction mixture was quenched with water and extracted into DCM. The organic phases were combined, dried over $MgSO_4$ and concentrated to dryness. The residue was purified by mass-triggered preparative LCMS (acidic conditions) to afford the title compound (5.8 mg, 5%) as an orange solid: LCMS (t_R=3.71 min., purity= 91.4%), ESI+ m/z 388 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.46 (d, *J* = 6.7 Hz, 6H), 4.90 (h, *J* = 6.7 Hz, 1H), 7.25 (td, *J* = 8.2, 2.2 Hz, 3H), 7.40 (d, *J* = 8.3 Hz, 1H), 7.43 (d, *J* = 1.7 Hz, 1H), 7.48 (s, 2H).

HRMS (ESI) calculated for $C_{15}H_{14}BrN_7O$ $[M+H]^+$ 388.0516, found 388.1070.

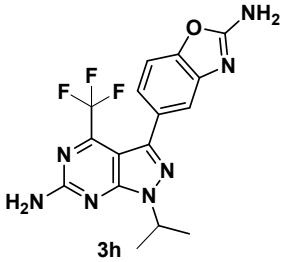
Preparation of 5-(6-Amino-1-isopropyl-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[d]oxazol-2-amine (3h)

The title compound **3h** was prepared following the sequence below:



5-(6-Amino -4-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine

To a stirred suspension of 5-(6-amino-4-chloro-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine and sodium iodide (1.74 g, 11.6 mmol, 20 eq.) at 0°C, was added hydriodic acid (2.00 ml, 40% w/w, 8.73 mmol, 15 eq.) and the suspension was stirred at room temperature for 4 h. The reaction mixture was diluted with DCM and pour onto a saturated aqueous solution of $NaHCO_3$. The aqueous phase was extracted with DCM. The organic phases were combined, washed with a 1M solution of sodium thiosulfate, water, dried over $MgSO_4$ and concentrated to dryness to afford the title compound (150 mg, 59%) as a beige solid: LCMS (t_R=2,70 min., purity= 97%), ESI+ m/z 436 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.46 (d, J = 6.7 Hz, 6H), 4.90 (h, J = 6.7 Hz, 1H), 7.18 (m, 1H), 7.28 (m, 2H), 7.40 (m, 2H), 7.47 (br s, 2H).



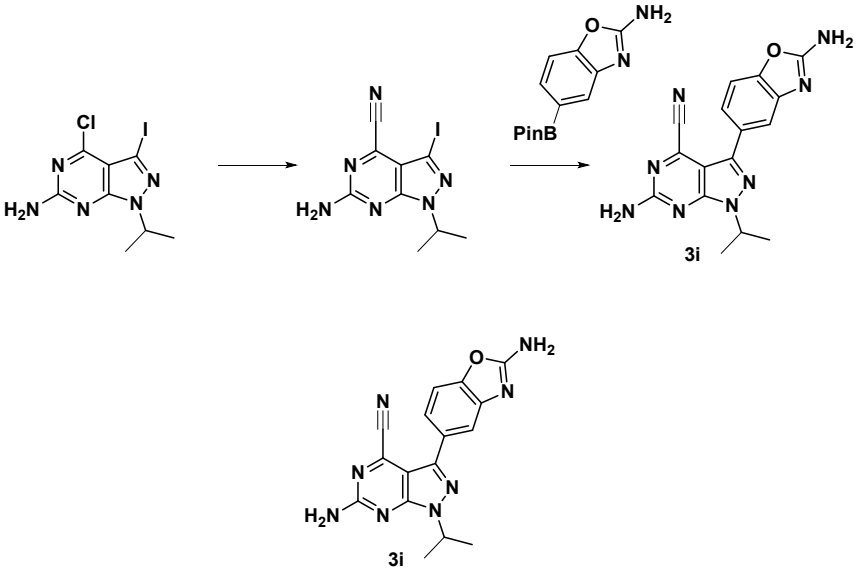
5-(6-Amino-1-isopropyl-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (3h)

To a stirred solution of 5-(6-amino-4-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[d]oxazol-2-amine (70.0 mg, 0.11 mmol, 1.00 eq.) in DMF (1.37 ml) under argon, was added (1,10-phenanthroline)(trifluoromethyl)copper(I) (59.9 mg, 0.19 mmol, 1.70 eq.) and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was filtered through a pad of Celite and concentrated to dryness. The residue was purified by mass-triggered preparative LCMS (acidic conditions), concentrated to dryness and the solid triturated in a methanolic ammonia solution. The solid was collected by filtration and dried to a constant weight to afford the title compound (**3h**, 10.3 mg, 24%) as a beige solid: LCMS (t_R=0.99 min., purity= 97%), ESI+ m/z 378 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.48 (d, J = 6.7 Hz, 6H), 4.98 (h, J = 6.7 Hz, 1H), 7.07 (dd, J = 8.1, 1.6 Hz, 1H), 7.26 (d, J = 1.6 Hz, 1H), 7.39 (d, J = 8.1 Hz, 1H), 7.47 (d, J = 12.3 Hz, 4H).

HRMS (ESI) calculated for $C_{16}H_{14}F_3N_7O$ $[M+H]^+$ 378.1285, found 378.1281.

Preparation of: 6-Amino-3-(2-aminobenzo[*d*]oxazol-5-yl)-1-isopropyl-1H-pyrazolo[3,4-*d*]pyrimidine-4-carbonitrile (3i)

The title compound **3i** was prepared following the sequence below:



6-Amino-3-(2-aminobenzo[*d*]oxazol-5-yl)-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4-carbonitrile (3i)

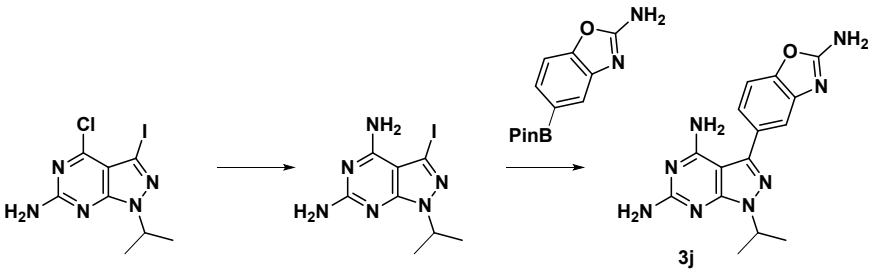
To a stirred solution of 4-chloro-3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (500 mg, 1.48 mmol, 1.00 eq.) and 18-CROWN-6 (235 mg, 0.89 mmol, 0.60 eq.) in MeCN (5.0 ml), was added potassium cyanide (96.5 mg, 1.48 mmol, 1.00 eq.) and the reaction mixture was stirred under reflux for 1 h 40 min. The reaction mixture was diluted with brine (pH was basique), and the the aqueous phase was extracted with EtOAc. The organic phases were combinedm washed with brine, dried over $MgSO_4$ and concentrated to dryness to afford the title compound (474 mg, 85%) as a yellow soild that was used without further purification: LCMS (t_R=1.11 min., purity= 94%), ESI+ m/z 329 $(M+H)^+$.

In a microwave tube, 6-amino-3-iodo-1-isopropyl-*1H*-pyrazolo[3,4-*d*]pyrimidine-4-carbonitrile (40.0 mg, 0.12 mmol, 1.00 eq., 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[d]oxazol-2-amine (47.6 mg, 0.18 mmol, 1.50 eq.) was added to a mixture of a 2.0 M aqueous solution of potassium carbonate (0.18 ml, 0.37 mmol, 3.00 eq.) and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[*d*]oxazol-2-amine amine (24.3 mg, 0.09 mmol, 1.50 eq.) and in 1,4-dioxane (0.42 ml). The reaction mixture was degassed and purged with argon. 1,1'-Bis(diphenylphosphino)ferrocene-palladium(II) dichloride, dichloromethane (10 mg, 0.01 mmol, 0.10 eq.) was added and the reaction mixture was heated at 90 °C for 16 h, cooled to room temperature filtered through a pad of Celite and concentrated to dryness. The residue was purified directly by mass-triggered preparative LCMS (acidic conditions) to afford the title compound (**3i**, 5.0 mg, 12%) as a yellow solid: LCMS (t_R=0.94min., purity= 98%), ESI+ m/z 335 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.49 (d, J = 6.7 Hz, 6H), 4.94 (h, J = 6.7 Hz, 1H), 7.41 (dd, J = 8.3, 1.7 Hz, 1H), 7.46 (d, J = 8.2 Hz, 3H), 7.53 (s, 2H), 7.60 (d, J = 1.9 Hz, 1H).

HRMS (ESI) calculated for $C_{16}H_{14}N_8O$ $[M+H]^+$ 335.1363, found 335.1360.

Preparation of 3-(2-Aminobenzo[d]oxazol-5-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidine-4,6-diamine (3j)

The title compound **3j** was prepared following the sequence below:



3-iodo-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidine-4,6-diamine

A suspension of 4-chloro-3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-ylamine (1.50 g, 4.44 mmol, 1.00 eq.) in 1,4-dioxane (7.95 ml) and ammonia (7.10 ml, 32.00 % w/w, 13.33 mmol, 3.00 eq.) was heated at 100°C for 4 h and allowed to cool to room temperature during the night. The solid formed was collected by filtration and dried to a constant weight to afford the title compoud (1.38 g, 97%) as a white solid: : LCMS (t_R=0.70min., purity= 98%), ESI+ m/z 319 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.36 (d, *J* = 6.6 Hz, 6H), 4.73 (h, *J* = 6.6 Hz, 1H), 6.26 (br s, 2H), 7.02 (br s, 2H).

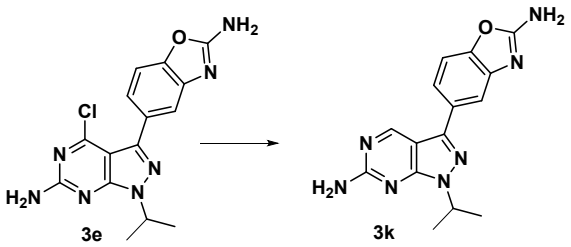
3-(2-Aminobenzo[d]oxazol-5-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidine-4,6-diamine (3j)

In a microwave tube, were added 3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (700 mg, 2.20 mmol, 1.00 eq.), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[*d*]oxazol-2-amine (858 mg, 3.30 mmol, 1.50 eq.) and a 2.0 M aqueous solution of potassium carbonate (3.30 ml, 6.60 mmol, 3.00 eq.) in 1,4-dioxane (7.35 ml) and the reaction mixture was degassed and purged with argon. 1,1'-Bis(diphenylphosphino)ferrocenepalladium(ii) dichloride, dichloromethane (180 mg, 0.22 mmol, 0.10 eq.) was added and the reaction mixture was heated at 100 °C under microwave irradiation. The reaction mixture was allowed to cool to room temperature, filtered and the filtrated was diluted with EtOAc and water. The organic phase was washed with brine, filtered and dried over $MgSO_4$ and concentrated to dryness. The residue was suspended in DCM and collected by filtration then suspended in not MeOH in the presence of activated charcoal. The suspension was filtered and a solid crystalised that was collected by filtration, dried to a constant weight to afford the title compound (**3j**, 285 mg, 40%) as an off-white solid: LCMS (t_R=0.66min., purity= 100%), ESI+ m/z 325 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.43 (d, *J* = 6.7 Hz, 6H), 4.79-4.85 (p, *J* = 6.7 Hz, 1H), 6.14 (s, 2H), 7.18-7.21 (dd, *J* = 8.1-1.7 Hz, 1H), 7.36 (d, *J* = 1.7 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 1H), 7.50 (s, 2H).

HRMS (ESI) calculated for $C_{15}H_{16}N_8O$ $[M+H]^+$ 325.1520, found 325.1518.

Preparation of 3-(2-Aminobenzo[*d*]oxazol-5-yl)-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (3k)

The title compound **3k** was prepared directly from previously described **3e**:



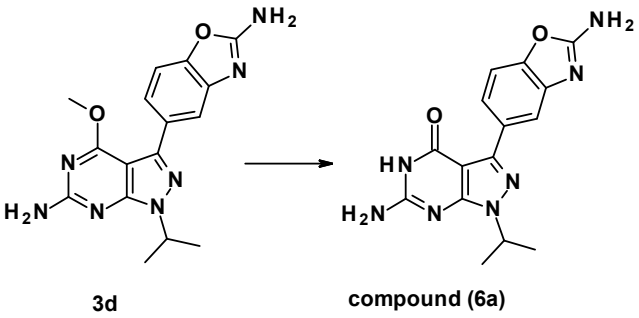
5-(6-Amino-4-chloro-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (30 mg, 0.09 mmol,1.00 eq.) was dissolved in MeOH (0.30 ml) and the reaction mixture was degassed then 10% Pd-C (18.6 mg, 0.01 mmol, 0.10 eq.) was added. The reaction mixture was degassed and purged with hydrogen and stirred at room temperature for 48 h. The reaction mixture was filtered through a pad of Celite, concentrated to dryness and purified by mass-triggered preparative LCMS (basic conditions) to afford the title compound (**3k**, 7.9 mg, 29%): LCMS (t_R=0.86 min., purity= 100%), ESI+ m/z 310 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.41 (d, J = 6.7 Hz, 6H), 4.77-4.83 (hp, J = 6.7 Hz, 1H), 6.12 (s, 2H), 7.14-7.19 (dd, J = 8.1-1.7 Hz, 1H), 7.34 (d, J = 1.7 Hz, 1H), 7.43 (d, J = 8.1 Hz, 1H), 7.49 (s, 2H), 8.72 (s, 1H).

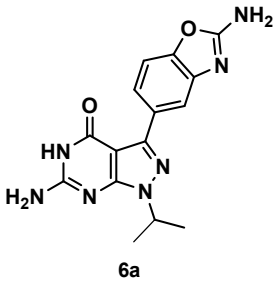
HRMS (ESI) calculated for $C_{15}H_{15}N_7O$ $[M+H]^+$ 310.1411, found 310.1409

Preparation of compounds shown in Table 3. Pyrazolopyrimidones exploration

Preparation of 6-Amino-3-(2-aminobenzo[*d*]oxazol-5-yl)-1-isopropyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (6a)

The title compound **6a** was prepared directly from previously described **3d**:





6-Amino-3-(2-aminobenzo[*d*]oxazol-5-yl)-1-isopropyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (6a)

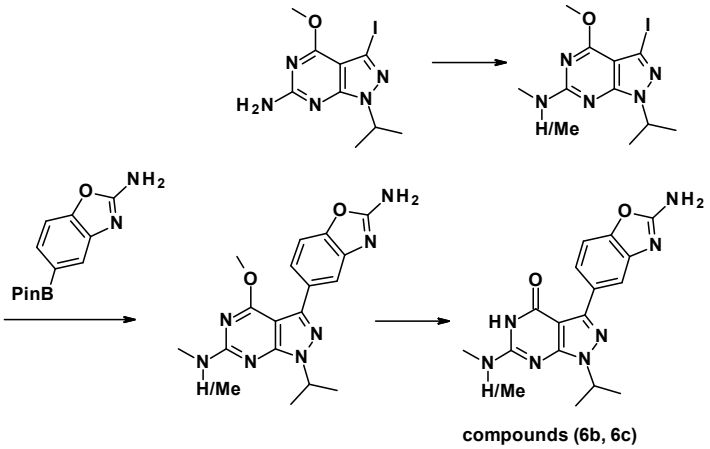
5-(6-Amino-1-isopropyl-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine **3d** (67 mg, 0.20 mmol, 1.00 eq.) was dissolved in DCM (0.5 ml) and cooled down to 0°C for 30 min. A 1.0 M solution of BBr_3 in DCM (0.33 ml, 0.33 mmol, 3.00 eq.) was added and the reaction mixture was stirred at room temperature overnight under argon. The reaction mixture was quenched with water and extracted into DCM. The organic phases were combined, dried over $MgSO_4$ and concentrated to dryness. The residue was purified by mass-triggered preparative LCMS (acidic conditions) to afford the title compound (**6a**, 3.3 mg, 5%) as an orange solid. LCMS (purity= 97%), ESI+ m/z 326 $(M+H)^+$; ^1H NMR (DMSO-d_6) δ 1.43 (d, J = 6.6 Hz, 6H), 4.79 (h, J = 6.7 Hz, 1H), 6.67 (s, 2H), 7.32 (d, J = 8.5 Hz, 1H), 7.38 (s, 2H), 8.00 (dd, J = 8.4, 1.7 Hz, 1H), 8.24 (d, J = 1.7 Hz, 1H), 10.68 (s, 1H).

HRMS (ESI) calculated for $C_{15}H_{15}N_7O_2$ $[M+H]^+$ 326.1360, found 326.1359.

Preparation of:

- **3-(2-Aminobenzo[*d*]oxazol-5-yl)-1-isopropyl-6-(methylamino)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (6b)**
- **3-(2-Aminobenzo[*d*]oxazol-5-yl)-6-(dimethylamino)-1-isopropyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (6c)**

The compounds **6b** and **6c** were prepared following the sequence below:



compounds (6b, 6c)

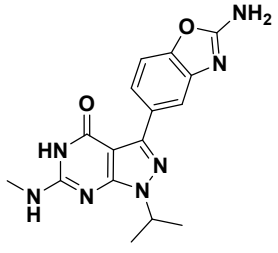
3-iodo-1-isopropyl-4-methoxy-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine

To a stirred solution of 3-Iodo-1-isopropyl-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (100 mg, 0.30 mmol, 1.00 eq.) in anhydrous THF (1.0 ml) under argon was added at 0°C iodomethane (20.6 µl, 0.33 mmol, 1.10 eq.). The reaction mixture was stirred at 0°C for 5 min before the addition of sodium hydride (13.21 ⍰⍰, 0.33 mmol, 1.10 eq.). Then, the reaction mixture was stirred at room temperature for 6 hours, quenched with a saturated solution of ammonium chloride and extracted into EtOAc. The organic phases were washed with brine, dried over $MgSO_4$ and concentrated to dryness. The residue was purified by flash chromatography (silica gel, heptane:EtOAc 9:1) to afford the title compound (51 mg, 49%) as an orange solid: LCMS (purity= 97%), ESI+ m/z 348 $(M+H)^+$; ^1H NMR (DMSO-d_6) δ 1.39 (d, J = 6.7 Hz, 6H), 2.82 (d, J = 4.4 Hz, 3H), 3.96 (s, 3H), 4.82 (s, 1H), 7.32 (s, 1H)

5-(1-isopropyl-4-methoxy-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine

In a microwave tube, 3-iodo-1-isopropyl-4-methoxy-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (50.0 mg, 0.22 mmol, 1.00 eq.) and a 2.0M aqueous solution of potassium carbonate (0.22 ml, 0.43 mmol, 3.00 eq.) in 1,4-dioxane (0.8 ml) were degassed and purged with argon. 1,1'-Bis(diphenylphosphino)ferrocenepalladium(II) dichloride.dichloromethane (11.8 mg, 0.01 mmol, 0.10 eq.) was added followed by 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[*d*]oxazol-2-amine (56.2 mg, 0.22 mmol, 1.50 eq.) and the reaction mixture was heated at 100 °C for 30 min. The reaction mixture was cooled to room temperature. Water and EtOAc were added and the organic phase was extracted with EtOAc. The organic phases were washed with brine, dried over $MgSO_4$ and concentrated to dryness to afford the title compound (87 mg, 99%). 5-(1-isopropyl-4-methoxy-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine was directly used in the next step without further purification. ; LCMS (purity= 97%), ESI+ m/z 354 $(M+H)^+$.

3-(2-Aminobenzo[*d*]oxazol-5-yl)-1-isopropyl-6-(methylamino)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (6b)

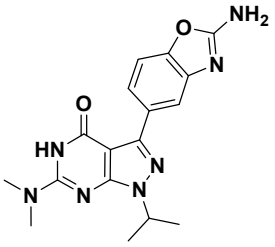


6b

5-(1-isopropyl-4-methoxy-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (67 mg, 0.11 mmol, 1.00 eq.) was dissolved in DCM (0.4 ml) and cooled down to 0°C for 30 min. A 1.0 M solution of BBr_3 in DCM (0.33 ml, 0.33 mmol, 3.00 eq.) was added and the reaction mixture was stirred at room temperature overnight under argon. The reaction mixture was quenched with water and extracted into DCM. The organic phases were combined, dried over $MgSO_4$ and concentrated to dryness. The residue was purified by mass-triggered preparative LCMS (acidic conditions) to afford the title compound (2.0 mg, 5%) as a white solid. LCMS (purity= 97%), ESI+ m/z 340 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.45 (d, J = 6.7 Hz, 6H), 2.86 (d, J = 4.7 Hz, 3H), 4.85 (h, J = 6.7 Hz, 1H), 6.40 (s, 1H), 7.32 (d, J = 8.3 Hz, 1H), 7.38 (s, 2H), 8.00 (dd, J = 8.3, 1.7 Hz, 1H), 8.24 (d, J = 1.7 Hz, 1H), 10.65 (s, 1H).

HRMS (ESI) calculated for $C_{16}H_{17}N_7O_2$ $[M+H]^+$ 340.1516, found 340.1515.

Preparation of 3-(2-Aminobenzo[*d*]oxazol-5-yl)-6-(dimethylamino)-1-isopropyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (6c)



6c

3-iodo-1-isopropyl-4-methoxy-*N*,*N*-dimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine

To a stirred solution of 3-Iodo-1-isopropyl-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (150 mg, 0.45 mmol, 1.00 eq.) in anhydrous THF (1.1 ml) under argon was added at 0°C iodomethane (60 µl, 0.99 mmol, 2.20 eq.). The reaction mixture was stirred at 0°C for 5 min before the addition of sodium hydride (39.6 mg, 0.99 mmol, 2.20 eq.). Then, the reaction mixture was stirred at room temperature for 6 hours, quenched with a saturated solution of ammonium chloride and extracted into EtOAc. The organic phases were washed with brine, dried over $MgSO_4$ and concentrated to dryness. The residue was purified by flash chromatography (silica gel, heptane:EtOAc 9:1) to afford the title compound (154 mg, 95%) as an orange solid: LCMS (purity= 97%), ESI+ m/z 362 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.40 (d, J = 6.7 Hz, 6H), 3.17 (s, 6H), 4.01 (s, 3H), 4.84 (p, J = 6.6 Hz, 1H).

3-(2-Aminobenzo[*d*]oxazol-5-yl)-6-(dimethylamino)-1-isopropyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (6c)

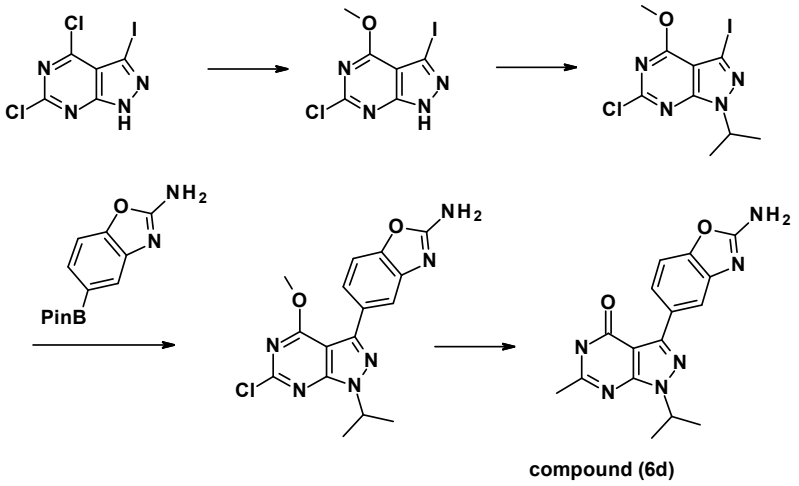
In a microwave tube, 3-iodo-1-isopropyl-4-methoxy-*N*,*N*-dimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (165.0 mg, 0.46 mmol, 1.00 eq.) and a 2.0M aqueous solution of potassium carbonate (685 µl, 1.37 mmol, 3.00 eq.) in 1,4-dioxane (2.5 ml) were degassed and purged with argon. 1,1'-Bis(diphenylphosphino)ferrocenepalladium(II) dichloride.dichloromethane (37.3 mg, 0.05 mmol, 0.10 eq.) was added followed by 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[*d*]oxazol-2-amine (178 mg, 0.69 mmol, 1.50 eq.) and the reaction mixture was heated at 100 °C for 30 min. The reaction mixture was cooled to room temperature. Water and EtOAc were added and the organic phase was extracted with EtOAc. The organic phases were washed with brine, dried over $MgSO_4$ and concentrated to dryness to afford the title compound (167mg, 99%). 5-(6-(dimethylamino)-1-isopropyl-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[d]oxazol-2-amine was directly used in the next step without further purification; LCMS (purity= 97%), ESI+ m/z 368 $(M+H)^+$.

To 5-(6-(dimethylamino)-1-isopropyl-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[d]oxazol-2-amine (95.0 mg, 0.26 mmol, 1.00 eq.) was added a 4M solution of hydrogen chloride in dioxane (0.65 ml, 2.59 mmol, 10.0 eq.). The mixture was stirred for 2 hours at room temperature and cooled down to 0°C to be quenched with a saturated solution of $NaHCO_3$ and extracted into AcOEt. The organic phases were combined, dried over $MgSO_4$ and concentrated to dryness. The residue was precipitated in DMF, filtrated and washed with water to obtain the title compound (**6d**, 16.8 mg, 18%) as a white solid. LCMS (purity= 97%), ESI+ m/z 354 $(M+H)^+$; ^1H NMR (DMSO-d_6) δ 1.45 (d, J = 6.7 Hz, 6H), 3.11 (s, 6H), 4.84 (h, J = 6.7 Hz, 1H), 7.34 (d, J = 8.3 Hz, 1H), 7.38 (s, 2H), 7.99 (dd, J = 8.4, 1.7 Hz, 1H), 8.25 (d, J = 1.7 Hz, 1H), 10.60 (s, 1H).

HRMS (ESI) calculated for $C_{17}H_{19}N_7O_2$ $[M+H]^+$ 354.1673, found 354.1670.

Preparation of 3-(2-Aminobenzo[d]oxazol-5-yl)-1-isopropyl-6-methyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (6d)

Compound **6d** was prepared following the sequence below:



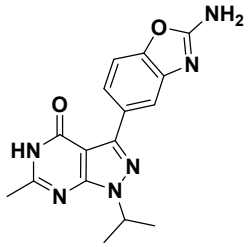
5-(6-chloro-1-isopropyl-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine

To a stirred solution of 4,6-dichloro-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidine (400.0 mg, 1.27 mmol, 1.00 eq.) in MeOH (2.4 ml) was added 30% solution of sodium methoxide in MeOH (0.25 ml, 1.33 mmol, 1.05 eq.). The reaction mixture was stirred at room temperature overnight, quenched with a saturated solution of ammonium chloride and extracted into EtOAc. The organic phases were washed with brine, dried over $MgSO_4$ and concentrated to dryness to afford the title compound (385 mg, 97%) as a white solid: LCMS (purity= 99%), ESI+ m/z 311 $(M+H)^+$. 6-Chloro-3-iodo-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine was engaged in the next step without further purification.

2-Iodopropane (106.3 ml, 1.06 mmol, 1.10 eq.) was added to a stirred solution of 6-Chloro-3-iodo-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (7.92 g, 37.0 mmol, 1.00 eq.) and cesium **carbonate** (346.0 mg, 1.06 mmol, 1.10 eq.) in DMF (3 ml). The reaction mixture was stirred at room temperature for 2 hours, filtered and the filtrate was purified by flash chromatography over silica gel. The fractions were evaporated and the residue crystallised from heptane/EtOAc. The solid was collected by filtration and dried to a constant weight to afford the title compound (170 mg, 49%) as a beige solid:

LCMS (purity= 99%), ESI+ m/z 353 $(M+H)^+$. 6-Chloro-3-iodo-1-isopropyl-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin was engaged in the next step without further purification.

In a microwave tube, 6-Chloro-3-iodo-1-isopropyl-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (170.0 mg, 0.48 mmol, 1.00 eq.) and a 2.0M aqueous solution of potassium carbonate (724 µl, 1.45 mmol, 3.00 eq.) in 1,4-dioxane (1.7 ml) were degassed and purged with argon. 1,1'-Bis(diphenylphosphino)ferrocenepalladium(II) dichloride.dichloromethane (39.4 mg, 0.05 mmol, 0.10 eq.) was added followed by 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[*d*]oxazol-2-amine (188 mg, 0.72 mmol, 1.50 eq.) and the reaction mixture was heated at 100 °C for 30 min. The reaction mixture was cooled to room temperature. Water and EtOAc were added and the organic phase was extracted with EtOAc. The organic phases were washed with brine, dried over $MgSO_4$ and concentrated to dryness. The residue was purified by mass-triggered preparative LCMS (acidic conditions) to afford the title compound (29.0 mg, 16%) as a white solid.; LCMS (purity= 97%), ESI+ m/z 359 $(M+H)^+$. ^{1}H NMR (DMSO-d_6) δ 1.53 (d, J = 6.6 Hz, 6H), 4.12 (s, 3H), 5.03 – 5.12 (m, 1H), 7.44 (d, J = 8.3 Hz, 1H), 7.50 (s, 2H), 7.61 (dd, J = 8.3, 1.8 Hz, 1H), 7.77 (d, J = 1.7 Hz, 1H).



6d

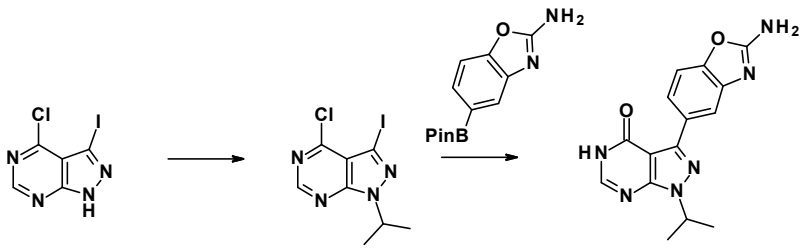
3-(2-Aminobenzo[d]oxazol-5-yl)-1-isopropyl-6-methyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (6d)

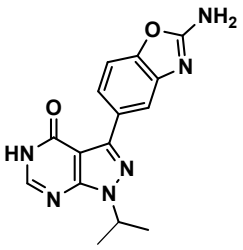
5-(6-chloro-1-isopropyl-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]oxazol-2-amine (29.0 mg, 0.08 mmol, 1.00 eq.) was dissolved in THF (150 µl) and bis(tri-*t*-butylphosphine)palladium(0) (8.3 mg, 0.02 mmol, 0.20 eq.) was added at 0 °C. The reaction mixture was degassed and purged with argon and 1M solution of dimethylzinc in heptane (245 µl, 0.24 mmol, 3.00 eq.) was added keeping the temperature at 0 °C. The reaction mixture was stirred at room temperature overnight, quenched with a saturated aqueous solution of ammonium chloride and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated to dryness. A 4M solution of hydrogen chloride in dioxane (0.20 ml, 0.81 mmol, 10.0 eq.) was added to the residue and the mixture stirred for 5 hours at room temperature. The crude mixture is diluted with DMF and purified by mass-triggered preparative HPLC (acidic method) to afford the title compound (3.0 mg, 11%) as a white solid: LCMS (purity= 97%), ESI+ m/z 339 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.47 (d, J = 6.6 Hz, 6H), 2.38 (s, 3H), 5.00 (p, J = 6.6 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.42 (s, 2H), 8.03 (dd, J = 8.3, 1.7 Hz, 1H), 8.26 (d, J = 1.7 Hz, 1H), 12.07 (s, 1H).

HRMS (ESI) calculated for $C_{16}H_{16}N_6O_2$ $[M+H]^+$ 325.1408, found 325.1404.

Preparation of 3-(2-aminobenzo[*d*]oxazol-5-yl)-1-isopropyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (6e)

The compounds **6e** was prepared following the sequence below:





6e

3-(2-aminobenzo[*d*]oxazol-5-yl)-1-isopropyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (6e)

2-Iodopropane (690 ml, 6.93 mmol, 1.05 eq.) was added to a stirred solution of commercially available 6-Chloro-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidine (1.85 g, 6.60 mmol, 1.00 eq.) and cesium carbonate (4.30 g, 13.19 mmol, 2.0 eq.) in DMF (35 ml)**.** The reaction mixture was stirred at room temperature for 2 hours, filtered and the filtrate was purified by flash chromatography over silica gel. The fractions were evaporated and the residue crystallised from heptane / EtOAc. The solid was collected by filtration and dried to a constant weight to afford the title compound (1.80 g, 82%) as a beige solid: LCMS (purity= 95%), ESI+ m/z 323 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.51 (d, J = 6.6 Hz, 6H), 5.04 (p, J = 6.7 Hz, 1H), 9.43 (s, 1H). 6-Chloro-3-iodo-1-isopropyl-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin was engaged in the next step without further purification.

In a microwave tube, 4-chloro-3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (92.0 mg, 0.29 mmol, 1.00 eq.) and a 2.0M aqueous solution of potassium carbonate (430 µl, 0.86 mmol, 3.00 eq.) in 1,4-dioxane (1.4 ml) were degassed and purged with argon. 1,1'-Bis(diphenylphosphino)ferrocenepalladium(II) dichloride.dichloromethane (23.29 mg, 0.03 mmol, 0.10 eq.) was added followed by 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[*d*]oxazol-2-amine (111.3 mg, 0.43 mmol, 1.50 eq.) and the reaction mixture was heated at 100 °C for 4 hours to obtain complete hydrolysis of the chloride. The reaction mixture was cooled to room temperature. Water and EtOAc were added and the organic phase was extracted with EtOAc. The organic phases were washed with brine, dried over $MgSO_4$ and concentrated to dryness. The residue was purified by mass-triggered preparative LCMS (acidic conditions) to afford the title compound (**6e**, 10.0 mg, 11%) as a white solid.; LCMS (purity= 97%), ESI+ m/z 311 $(M+H)^+$. ^{1}H NMR (DMSO-d_6) δ 1.51 (d, J = 6.6 Hz, 6H), 5.04 (p, J = 6.7 Hz, 1H), 7.38 (d, J = 8.3 Hz, 1H), 7.44 (s, 2H), 8.02 (dd, J = 8.4, 1.8 Hz, 1H), 8.08 (s, 1H), 8.25 (d, J = 1.7 Hz, 1H), 12.20 (s, 1H).

HRMS (ESI) calculated for $C_{15}H_{14}N_6O_2$ $[M+H]^+$ 311.1251, found 311.1250.

[1] Valko, K.; Bevan, C.; Reynolds, D. Chromatographic hydrophobicity index by fast-gradient RP-HPLC: A high-throughput alternative to logP/logD. *Anal. Chem.* **1997**, *69*, 2022-2029.

[2] Young, R. J.; Green, D. V. S.; Luscombe, C. N.; Hill, A. P. Getting physical in drug discovery II: the impact of chromatographic hydrophobicity measurements and aromaticity. *Drug Disc. Today* **2011**, *16(17/18)*, 822-830