Discovery of FLT3 inhibitors for the treatment of acute myeloid leukemia

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General introduction

On cancer

Cancer is used as an umbrella term for diseases in which cells in a multicellular organism are behaving differently than their tissue of origin due to a variety of root causes.[1–3] In these diseases this generally goes along with uncontrolled cell growth and cell proliferation, which in turn results in a diverse set of problems for the host organism, most prominently death. This makes cancer one of the leading causes of death throughout the world.[1,4] While the development of many types of cancers is attributable to epidemiological factors, such as smoking,[5,6] alcohol consumption,[7,8] solar radiation[9,10] and nutritional preferences,[11,12] there are also general risk factors, namely age, partially through buildup of other risk factors.[13] While the root causes vary, they all seem to cause mutations or genome rearrangements in different types of cells.[14] Some of these mutations ultimately lead to the development of malignant cells, which are defined by six hallmarks: *sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis and activating invasion and metastasis.*[1,2,15] The occurrence of malignant cells seems to be governed by an evolutionary process, starting out with small genetic alterations, leading through acquired genetic instability and clone selection to the above described characteristics.[16] This furthermore suggests that each cancer might be, at least on some level, unique. Most cancer cells constitute of numerous genetic abnormalities and the question which genetic alterations are responsible for malignancy is subject to some debate and is, depending on the type of cancer, not definitely answered.[17] To differentiate between mutations responsible for malignancy and the ones that are not, the terms driver and passenger mutations have been defined.[18] Driver mutations have a causal relation to oncogenesis, the formation of cancer and are frequently additionally responsible for cancer maintenance. To use the screening for, and identification of mutations as a starting point for

the development of targeted treatments, it is crucial to distinguish between driver and passenger mutations.[18] Responsible for a vast cellular signaling network, mutations in protein kinases have frequently been named driver mutations in cancer progression.[19]

Protein kinases and kinase inhibitors

Post-translational modifications (PTMs) are covalent chemical modifications of proteins that occur during or after protein-synthesis. This process ensures the possibility for regulation of activity and function of cellular processes. There are a multitude of different enzyme classes described that facilitate PTMs and among them, protein kinases take up a prominent role.[20]

Protein kinases are a closely related class of enzymes that are characterized by their common function to phosphorylate other proteins, most commonly on serine, threonine or tyrosine residues.[21,22] The reverse process of this, the dephosphorylation is controlled by phosphatases. The transfer of the phosphate group causes structural changes in the target enzyme, directly influencing its activity or localization. If the target enzyme is also a kinase, a concerted signaling pathway may be created, which are often called kinase signaling cascades.[23] Through this system, cells can react or adapt to extracellular stimuli, involving many different cellular processes such as metabolic pathways or gene transcription. There are over 500 human kinases described in the human genome and many of them have been associated with driver mutations in cancer progression and as such qualify as drug targets.[19,24] Not surprisingly, a sizeable portion of these kinases are involved in signaling pathways connected to cell proliferation and apoptosis.[25–27]

This led to extensive efforts to develop compounds that can selectively inhibit kinase signaling in aberrant pathways. Currently, 38 kinase inhibitors (KIs) have been approved by the authorities.[26] The large number of kinases and the fact that most of KIs bind in the highly conserved adenosine triphosphate (ATP) binding pocket make the efforts to selectively inhibit one kinase in a complex cellular environment difficult. While there are more and less selective KIs, most clinically investigated KIs tend to inhibit at least several kinases.[28,29] While profiling KIs in any complex environment, such as cells, off-target activity has always to be considered in the interpretation of the results.

Acute myeloid leukemia and FLT3

Acute myeloid leukemia (AML) is a group of hematopoietic disorders that are characterized by failure in differentiation and abnormal growth of hematopoietic stem cells, resulting in high levels of non-functional blood cells termed blast cells in the blood stream, leading to impaired blood flow, especially in capillaries, resulting in high mortality.[30–33] Prognosis varies, but relapse rates remain high and especially older patients have a poor prognosis due to them not being able to cope with the standard chemotherapy.[34,35] Untreated AML leads in almost all

cases to death within weeks to months and incident rates vary most prominently with age, steadily increasing to a peak at around 80 years. The median age of a newly diagnosed AML patient is 65 years old and the incident rate < 65 years olds is 1.8 and ≥ 65 years old is 17 per 100.000 persons (data from the U.S., 2000 - 2003).[36] Newly diagnosed AML is genetically diverse and several genetic aberrations are deemed clinically relevant, amongst them aberrations in nucleophosmin (NPM1) and CCAAT/enhancer-binding protein alpha (CEBPA) as well as fms-like tyrosine kinase 3 (FLT3).[33,34]

FLT3, classified as a type 3 receptor tyrosine kinase together with PDGFR, c-KIT and FMS, consists of an extracellular domain of five immunoglobulin-like domains, a transmembrane domain, a cytosolic juxtamembrane domain and two intracellular kinase insert domain linked tyrosine kinase-domains and is normally expressed in myeloid and lymphoid progenitor cells.[37–39] Upon extracellular activation by the FLT3 ligand (FL), FLT3 activates, like other members of the type 3 receptor tyrosine kinases, downstream signaling pathways via PI3K, AKT, STAT5, mTOR, RAS and ERK.[39,40] 20 to 30% of AML patients harbor an internal tandem duplication mutation (ITD) in the juxtamembrane domain of FLT3 rendering the FLT3 activation and signaling FL binding-independent. The continued signaling of the mutated FLT3 receptor, possibly through alternate pathways than the wild-type counterpart, is thought to drive the proliferation of mutated cells growth-factor independent.[41]

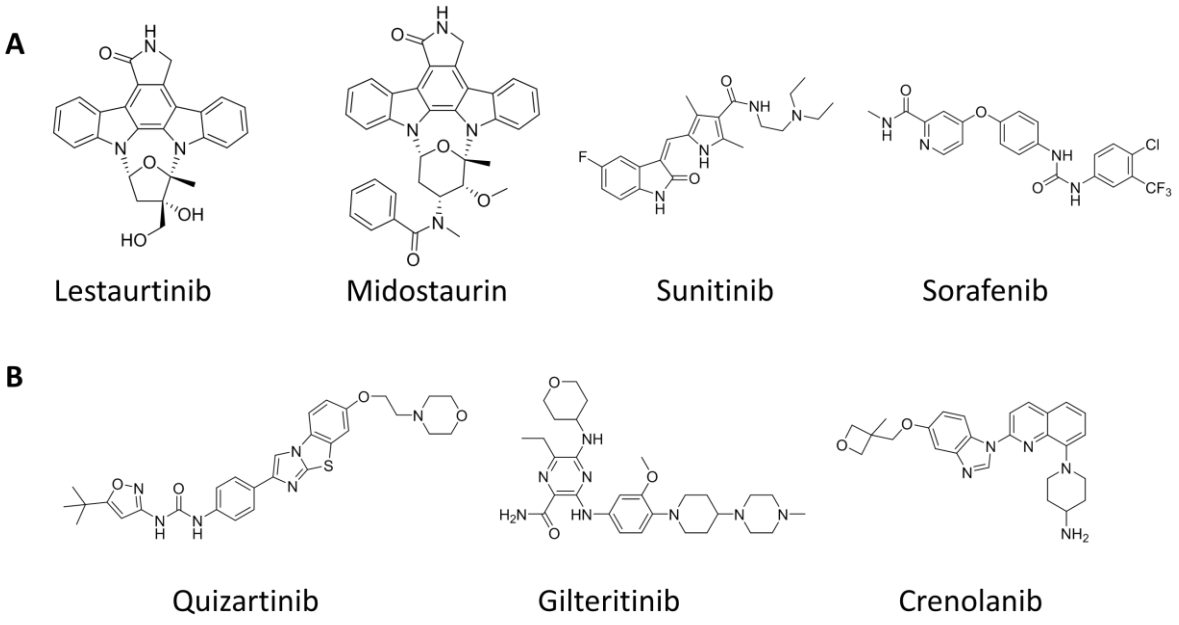

Figure 1: Clinically investigated first- (A) and second-generation (B) FLT3 inhibitors.

Numerous FLT3 inhibitors have been described and the most promising of them have been investigated in clinical trials.[42] The to date clinically investigated inhibitors include the so called first generation FLT3 inhibitors, which were originally developed for other targets and repurposed as FLT3 inhibitors. First generation inhibitors are lestaurtinib, midostaurin, sunitinib and sorafenib (Figure 1A). Second generation inhibitors, such as quizartinib, crenolanib and gilteritinib, were specifically developed as FLT3 inhibitors (Figure 1B). While all

of these drugs are highly potent FLT3 inhibitors, their clinical efficacy varies substantially, which can be partially explained by different off-target and pharmacokinetic profiles.[42–44] Different possible treatment regimens were investigated in clinical trials, including kinase inhibitor monotherapy or in combination with standard chemotherapy. Efficacy of the monotherapy trials varied from short lived partial response[45] to promising in the cases of quizartinib, crenolanib and gilteritinib.[43] The benefits of combination treatment, together with existing treatment regimens vary in a similar fashion from virtually non-existent with lestaurtinib[46] to sufficient to obtain market approval, as seen in midostaurin.[47,48] A substantial number of clinical trials, are still ongoing and it remains to be seen if any drug candidates can show enough benefit for patients to warrant approval.

Complicating FLT3-ITD AML treatment is the emergence of drug resistance conferring mutations after FLT3 inhibitor treatment. This provides strong evidence that FLT3 mutations are driver mutations in AML progression, thereby validating FLT3 as a valuable target in AML treatment.[31,49,50] Numerous mutations have been described, most notably in the amino acid residues of the activation loop (most frequently D835) and the gatekeeper residue (F691).[49–52] These mutations cause structural changes, thereby decreasing inhibitor binding activity. To treat these emerging mutations, or to preemptively prevent their emergence at relapse, new chemical matter is needed that retains activity against the numerous possible mutant forms of FLT3, either as a single agent or as combination treatment.[49,53,54]

Aim and outline of this thesis

The aim of this thesis is the discovery and optimization of novel FLT3 inhibitors that can be used to develop potential new treatments of drug-resistant acute myeloid leukemia (AML), as well as chemical biological techniques that facilitate their discovery.

Chapter 2 brings together modern techniques and describes the development of a chemical proteomics-based assay to determine kinase inhibitor off-target landscape in living cells.[55,56] In this study five clinically investigated FLT3 inhibitors, namely sunitinib, quizartinib, crenolanib, gilteritinib and midostaurin, were tested for their off-target profile in two different AML cell lines: MV4-11 and U937. For numerous proteins cellular inhibitor-target engagement could be confirmed, and moreover new off-targets were discovered and subsequently validated. **Chapter 3** shows the comprehensive structure-activity relationship of a H-89-derived chemical series as FLT3 inhibitors for treatment of AML. Extensive structure-activity-relationships of the H-89 series provided insight into the binding mode, as well as the development of highly active, sub-nanomolar FLT3 inhibitors. **Chapter 4** describes the high throughput screening of more than 230,000 compounds to discover new chemical matter as FLT3 inhibitors that are less vulnerable to known clinically observed FLT3 mutations in the ATP-binding pocket. After the initial *in vitro* screen against FLT3, two deselection screens, dose response evaluation against FLT3 and an intensive literature study, 21 compounds were selected and tested in a total of seven human and murine cell lines to ensure activity against

mutant variations of the FLT3 gene. This culminated in the discovery of SPCE00476_01 and NP_004099_001. **Chapter 5** describes the optimization the novel hits discovered in Chapter 4. Design, synthesis and testing of a large array of compounds did not only lead to a good understanding of the structure-activity relationship of the chemical series, but also led to the discovery of cellular active, sub-nanomolar FLT3 inhibitors with appropriate physico-chemical properties, such as molecular weight and lipophilic efficiency. Two compounds were selected and further profiling of their off-target activity in AML cells using the chemical proteomics assay developed in Chapter 2, and pharmacokinetic properties in mice. **Chapter 6** summarizes this thesis and suggests possible future directions for AML research.

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Chapter 2

Chemical proteomics enables cellular selectivity profiling of clinical FLT3 inhibitors*

Introduction

Acute myeloid leukemia (AML) is an aggressive form of blood cancer in elderly people with poor prognosis. In approximately 20-30% of AML patients an internal tandem duplication (ITD) in the juxtamembrane domain of the Fms-like tyrosine kinase 3 (FLT3) receptor has been identified as a driver mutation. This modification of the FLT3 gene results in growth factor independent proliferation of immature blasts cells, thereby fatally disrupting normal hematopoietic function.[1–4] For this reason, kinase inhibitors targeting FLT3 have been developed for the treatment of AML.[5] Several compounds, including sunitinib, quizartinib, crenolanib, gilteritinib and midostaurin (Figure 1), have been clinically tested in AML patients,[6,7] resulting in the recent approval, by the FDA, of midostaurin in combination with current treatment (cytarabine and daunorubicin).[8,9]

* The data presented in this chapter was gathered in collaboration with Eva van Rooden, Ruud Wijdeven, Laura de Paus, Hengyi You, Marjolein Quik, Tom van der Wel, Elliot D. Mock, Hermen S. Overkleeft, Jacques Neefjes and Mario van der Stelt.

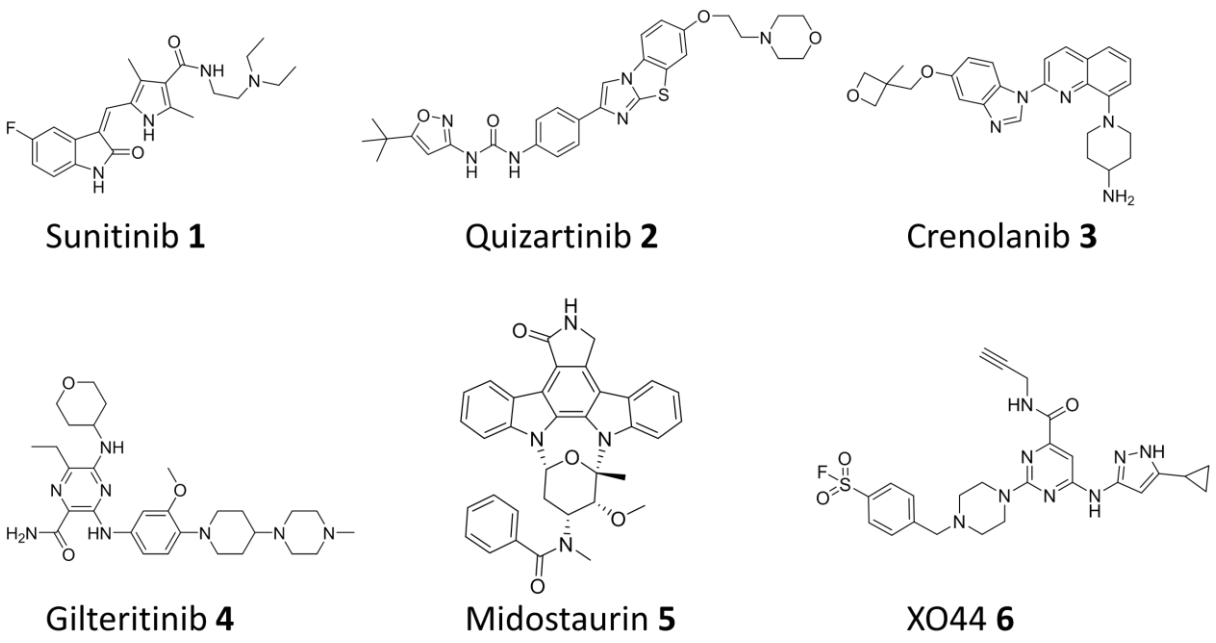


Figure 1: Chemical structures of the used compounds.

Most kinase inhibitors interact with the structurally and functionally conserved ATP-binding site, which is present in all 518 canonical human protein kinases. It is well-established that kinase inhibitors bind multiple proteins, and that this may affect their efficacy and toxicity.[10] Detailed investigation of the target-interaction landscape of kinase inhibitors is, therefore, important to understand their cellular and molecular mode of action. To this end, binding and activity assays with purified (recombinant) kinases or their catalytic domains are widely applied, however these assays do not recapitulate the cellular environment in which post-translational modifications, endogenous ligands, substrate levels, and protein-protein interactions may alter enzyme activity.[11–13] To address this issue, chemical proteomics methods have recently been developed to study target engagement in complex native (cellular) environments. These mass spectrometry (MS)-based assays make use of reversible bead-immobilized kinase inhibitors,[14] irreversible ATP-biotin probes,[15] photoaffinity-based probes,[16] or an irreversible pan-kinase probe (XO44, Figure 1).[12] The MS-based studies capture endogenously expressed kinases from complex, native proteomes and using inhibitors in competition experiments can be used to generate an off-target profile. For example, XO44, a fluorosulfonyl-based probe that covalently and irreversibly reacts with a conserved lysine-omega-amine, allowed the identification of 133 different kinases in Jurkat cells and was used to map the cellular target-interaction landscape of the approved drug dasatinib.[12]

The selectivity profile of the clinical FLT3 inhibitors has previously been investigated using binding-, activity- and Kinobeads-assays,[10,14] but the cellular target interaction profile has not been defined. This chapter reports the cellular target engagement landscape of five different FLT3 inhibitors (sunitinib, quizartinib, crenolanib, gilteritinib and midostaurin) in MV4-11 cells, a widely used cellular model for AML that harbors the FLT3-ITD mutant, and the lymphoma cell line U937. A recently, in-house developed label-free chemical proteomics protocol was

employed in which data-independent acquisition and ion mobility separation was used to increase peptide identification and the number of samples analyzed in the same experiment.[17]

Results

First, the activity of sunitinib, quizartinib, crenolanib, gilteritinib and midostaurin was confirmed in a biochemical assay using commercially available purified recombinantly expressed human FLT3 with a time-resolved fluorescence resonance energy transfer assay. All five inhibitors demonstrated strong inhibition with a half maximum inhibitory concentration (IC_{50}) in the low to sub-nanomolar range (Table 1). Crenolanib appeared to be the most potent inhibitor (pIC_{50} = 9.60 ± 0.07), whereas quizartinib the weakest inhibitor (pIC_{50} = 8.30 ± 0.07) in our assay. To determine the cellular activity of the clinical compounds, a cell proliferation assay using the FLT3-dependent AML cell line MV4-11, and as a control, the histiocytic lymphoma cell line (that is not dependent on FLT3 activity) U937 was performed (Table 1). All five inhibitors inhibited MV4-11 proliferation with nanomolar potency (pIC_{50}: 7.7 – 8.5), whereas the U937 cells were not sensitive to the inhibitors ($pIC_{50} \leq 5.5$), except for the pan-kinase inhibitor midostaurin (pIC_{50} = 6.85 ± 0.06). Altogether, these results confirm that the five compounds are potent, cellularly active FLT3 inhibitors inhibiting the growth of AML cells.

Table 1: Summary of pIC_{50} ± SEM *in vitro* (FLT3) and cellular (MV4-11 and U937).

	Sunitinib	**Quizartinib**	**Crenolanib**	**Gilteritinib**	**Midostaurin**	**XO44**
	1	**2**	**3**	**4**	**5**	**6**
pIC50 FLT3	9.01 ± 0.10	8.30 ± 0.07	9.60 ± 0.07	8.55 ± 0.07	8.84 ± 0.10	7.98 ± 0.11
pIC50 MV4-11	7.78 ± 0.04	8.55 ± 0.06	7.84 ± 0.07	7.81 ± 0.07	8.06 ± 0.04	6.90 ± 0.05
pIC50 U937	< 5	5.55 ± 0.13	< 5	5.10 ± 0.12	6.85 ± 0.06	n.a.

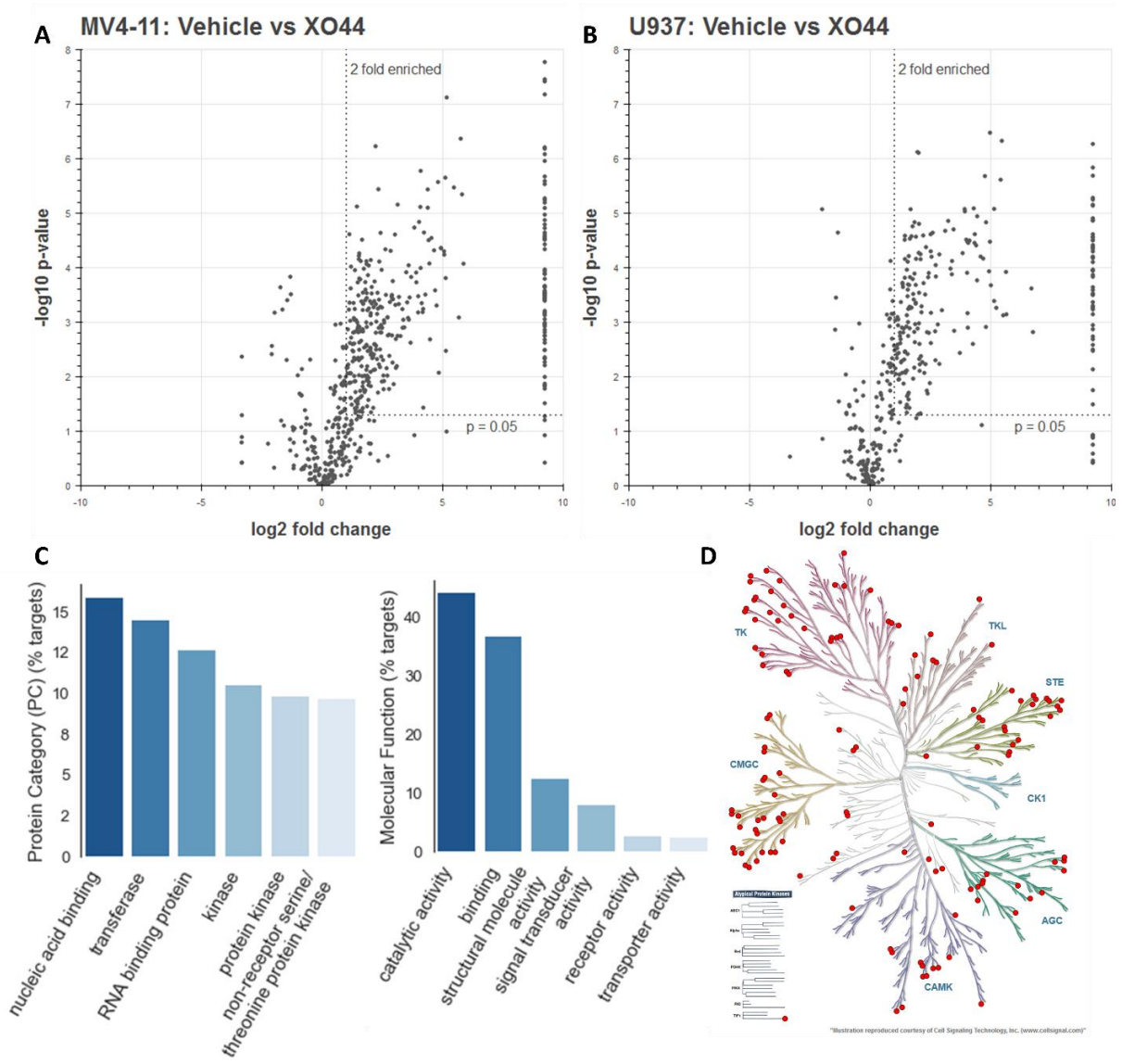


Figure 2: Analysis of the identified targets of XO44 in MV4-11 and U937 cells at 1 µM XO44. (A/B) Volcano plot of the label-free quantification signal from IsoQuant, vehicle vs XO44 treated MV4-11 and U937 cells. To enable plotting of all identified targets, infinite fold change (XO44 treated divided by vehicle control) was set to 600 and a fold change value of 0 was set to 0.1. Proteins are named targets of XO44, if there was a > 2-fold enrichment of quantification value, the probability associated with a Student's t-test (two-tailed distribution; two-sample equal variance) was < 0.05 and the conditions for the Benjamini–Hochberg correction with an FDR of 10% were fulfilled. (C) Targets found were analyzed by gene ontology using the panther protein-classification system (www.pantherdb.com). (D) From the targets of XO44, target kinases were selected according to annotations in Uniprot (transfer of P containing group (EC number Filter "2.7.-.-"). 147 unique kinases could be identified and were plotted on the canonical kinome-tree (13 targets could not be placed). (Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com))

To determine the selectivity profile of the inhibitors in MV4-11 and U937 cells, a chemical proteomics experiment using XO44 as a cell-permeable pan-kinase probe was performed. To this end, XO44 was synthesized according to previously reported procedures (See experimental part for synthesis and chemical analysis).[12] MV4-11 and U937 cells (7 x 10^6 cells/mL) were incubated with 1 µM XO44 or vehicle (DMSO) for 30 min, subsequently lysed

and subjected to a Cu(I)-catalyzed alkyne-azide [2+3] cycloaddition ("click"-reaction) with a biotin-azide.[18] Probe targets were enriched via pull-down with avidin-agarose beads and digested with trypsin. The resulting peptides were analyzed by LC-MS/MS and quantified using PLGS and IsoQuant software. Probe targets were selected using a > 2-fold enrichment of quantification value, depending on whether the probability associated with a Student's t-test (two-tailed distribution; two-sample equal variance) was < 0.05 testing positive control versus negative control samples and the conditions for the Benjamini–Hochberg correction with an FDR of 10% were fulfilled (Figure 2A/B). Kinase targets were selected from this according to annotations in Uniprot (transfer of P containing group (EC number Filter "2.7.-.-"). In total 114 and 118 kinases were identified in MV4-11 and U937 cells, respectively. 150 unique kinases could be identified and 82 kinases were identified in both cell lines. Proteins from all kinase sub-families were identified (Figure 2D). Peptides derived from FLT3-ITD were identified, albeit just below the quantification limit using the standard statistical parameters, therefore the presence of the FLT3-ITD construct in MV4-11 cells was confirmed using genomic polymerase chain reaction (PCR) (SI). Furthermore, 415 non-kinase proteins, including ATP-binding proteins ATP-dependent RNA helicase DDX3Y, ADP/ATP translocase 2 and ATP synthase subunit alpha (mitochondrial), were also identified as probe targets. All targets were analyzed by gene ontology using the panther protein-classification system (www.pantherdb.com) (Figure 2C).

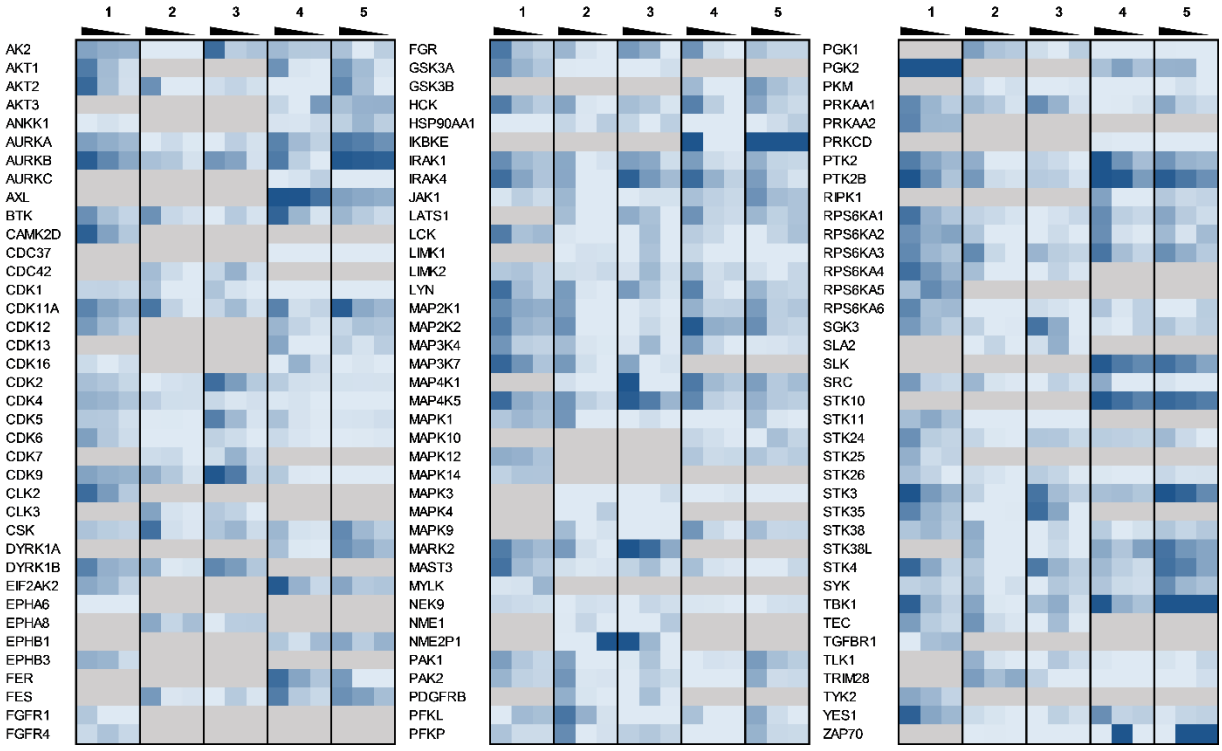


Figure 3: Heatmap of kinase inhibitor (**1** – **5**) targets in MV4-11. Kinase Targets were selected as described earlier. The heatmap shows the ratio of label free quantification signal from IsoQuant of inhibitor pretreated samples at three concentrations (100, 10 and 1 µM), normalized by only XO44 treatment after subtraction of negative control signal (e.g. 1: no difference in competitor and probe treated sample (light blue) 0: full competition of probe by the inhibitor (dark blue)).

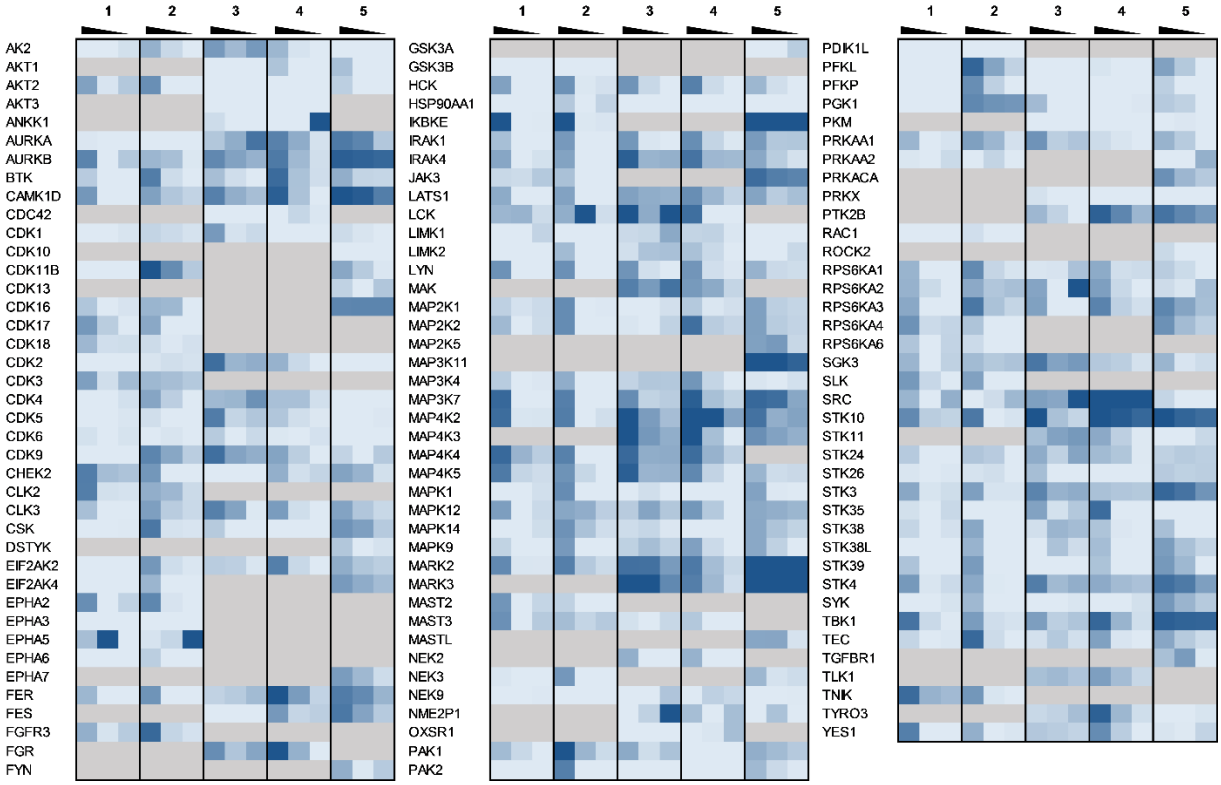


Figure 4: Heatmap of kinase inhibitor (**1** – **5**) targets in U937. Kinase Targets were selected as described earlier. The heatmap shows the ratio of label free quantification signal from IsoQuant of inhibitor pretreated samples at three concentrations (100, 10 and 1 µM), normalized by only XO44 treatment after subtraction of negative control signal (e.g. 1: no difference in competitor and probe treated sample (light blue) 0: full competition of probe by the inhibitor (dark blue)).

Next, the five clinical FLT3 inhibitors were tested at 1, 10 and 100 µM in a competitive chemical proteomics format using a 60 min pre-incubation-time before XO44 was added to MV4-11 or U937 cells for 30 min. These concentrations were chosen to represent 10x the IC_{50}-values (maximal efficacy) observed in the cellular proliferation assays. Kinases displaying > 50% reduction in probe labeling in a dose-dependent manner were designated as drug-inhibited targets. All targets for the five compounds in each cell line are summarized as heat maps in Figure 3 and Figure 4, as well as plotted as volcano-plots (SI Figure 1). Sunitinib and quizartinib were the most selective FLT3 inhibitors targeting PGK2 and PGK1 at 1 µM, respectively and in a dose-dependent fashion (SI). Crenolanib, gilteritinib and midostaurin were less selective and inhibited 12, 7, and 14 kinases at 1 µM in a dose-dependent fashion in MV4-11 and U937 cells (Table 2) respectively. Our data is in line with previously reported binding assays for midostaurin and sunitinib as reported in the ChEMBL database (SI).[19] Of note, at 1 µM midostaurin, a concentration that blocks the growth of both MV4-11 and U937 cells, AURKA, AURKB, IKBKE, PTK2B, STK3, STK10 and TBK1 were highly engaged in both cell lines. In U937 cells and at 10x IC_{50}-values, the compounds were significantly less selective (Table 2). At this high concentration, the clinical compounds inhibited 24 up to 39 kinases. TBK1, STK10, MAP4K2, MARK2 and MAP3K7 were inhibited by all clinical inhibitors at least at one of the tested concentrations.

Table 2: Summary of the identified targets for the corresponding inhibitors (**1** – **5**) at 10x IC50 on cell proliferation. Targets are selected if 50% outcompeted compared to 1 µM probe treatment without competitor.

1 µM in MV4-11 and U937 (10x IC_{50})

1		2		3		4		5	
MV4-11	**U937**	**MV4-11**	**U937**	**MV4-11**	**U937**	**MV4-11**	**U937**	**MV4-11**	**U937**
PGK2		NME2P1	PGK1		AK2	AKT3	STK10	AURKA	JAK3
			EPHA5		MAK	AXL	SRC	AURKB	TBK1
					SGK3	PTK2B	ANKK1	IKBKE	STK3
					MARK2	SLK		PTK2B	MAP3K11
					CDK4	STK10		STK10	CDK16
					SRC			STK3	STK10
					STK11			TBK	PTK2B
					LCK			ZAP70	AURKB
					RPS6KA2				CAMK1D
					MARK3				MARK3
					NME2P1				MARK2
					AURKA				IKBKE

100 µM in U937 (10x IC_{50})

1		2		3		4		5	
AURKB	MAP4K5	AKT2	MAP4K4	AK2	MAP3K7	ANKK1	MAP3K4	AURKA	MAP4K3
CAMK1D	MAPK12	BTK	MAP4K5	AURKA	MAP4K2	AURKA	MAP3K7	AURKB	MARK2
CDK17	MARK2	CDK11B	MAPK1	AURKB	MAP4K3	AURKB	MAP4K2	CAMK1D	MARK3
CHEK2	MAST2	CDK9	MAPK14	CAMK1D	MAP4K4	BTK	MAP4K3	CDK16	PRKACA
CLK2	MAST3	CHEK2	MAPK9	CDK1	MAP4K5	CAMK1D	MAP4K4	CSK	PTK2B
EPHA2	RPS6KA4	CSK	MARK2	CDK2	MARK2	CLK3	MAP4K5	EIF2AK4	RPS6KA3
EPHA5	SLK	EIF2AK2	NEK3	CDK4	MARK3	EIF2AK2	MAPK12	EPHA7	RPS6KA4
IKBKE	STK10	EPHA2	PAK1	CDK5	NME2P1	FER	MARK2	FER	STK10
LYN	STK4	EPHA5	PAK2	CDK9	PRKAA1	FES	MARK3	FES	STK3
MAP3K7	TBK1	FER	PFKL	CLK3	RPS6KA2	FGR	PTK2B	IKBKE	STK38L
MAP4K2	TNIK	FGFR3	PFKP	FGR	SGK3	HCK	RPS6KA3	IRAK1	STK39
MAP4K4	YES1	HCK	PGK1	HCK	SRC	IRAK1	SRC	JAK3	STK4
		IKBKE	RPS6KA1	IRAK1	STK10	IRAK4	STK10	MAP2K5	SYK
		IRAK1	RPS6KA3	IRAK4	STK11	LATS1	STK35	MAP3K11	TBK1
		LCK	STK10	LCK	STK3	LCK	TBK1	MAP3K7	TGFBR1
		LYN	STK39	LYN	STK4	LYN	TYRO3	MAP4K2	
		MAP2K1	TBK1	MAK	TBK1	MAK	YES1		
		MAP2K2	TEC			MAP2K2			
		MAP3K7	TNIK						
		MAP4K2							

Furthermore, several previously not reported targets could be identified, amongst them AK2, MAK and SGK3 for crenolanib, SRC, SLK and STK10 for gilteritinib, as well as CDK16 for midostaurin. All of these except AK2 were available to be tested at KinaseProfiler™ from Eurofins, where in a radiometric assay, residual protein activity was determined at 1 and 10 µM. The results from this study are shown in Table 3. All three gilteritinib targets (SRC, SLK and STK10) show full inhibition at 1 and 10 µM. Crenolanib and midostaurin show light (1 µM) and moderate (10 µM) inhibition towards their examined targets.

Table 3: Percent (%) residual kinase activity as tested in a radiometric assay at Eurofins KinaseProfiler™.

	Crenolanib			**Gilteritinib**			**Midostaurin**	
µM	**1**	**10**		**1**	**10**		**1**	**10**
MAK	99 ± 12	65 ± 3	**SRC**	3 ± 1	1 ± 0	**CDK16**	94 ± 1	55 ± 3
SGK3	89 ± 10	60 ± 8	**SLK**	-3 ± 1	-2 ± 1			
			STK10	2 ± 1	-1 ± 0			

Discussion

The determination of the cellular target interaction landscapes of experimental drugs is important to assess their safety profile and to guide their clinical development. Using activity-based proteomics this lab has previously mapped the cellular target interaction landscape of the drug BIA 10-2474 that killed a volunteer in a phase 1 study. BIA 10-2474 was a significantly more promiscuous lipase inhibitor in living human neuronal cells compared to its biochemical selectivity profile.[13] In the field of kinases, several methods, including cellular thermal shift assay (CETSA),[20] energy transfer assays (nano-BRET),[21] and chemical proteomics[12] have recently been developed and applied to map selectivity profiles of clinical compounds in live cells. The value of these techniques is that they capture cell permeability of the compound and take into account the physiological complexity of full-length kinases and their regulation by the native cellular environment. For example, most biochemical kinase selectivity panels operate at ATP concentrations close to the K_M (10-100 µM), whereas cellular ATP concentrations exceeds 1 mM in the nucleus and cytosol where kinases are active. This leads to a shift in potency for kinase engagement with ATP-competitive inhibitors, which alters their selectivity profile.

In the present study the cellular target engagement profile of five clinical FLT3 kinase inhibitors was determined using chemical proteomics. By using the chemical probe XO44 to covalently capture kinases in living MV4-11 and U937 cells, in combination with mass spectrometry-based detection, over 140 kinases were identified. Competitive activity-based proteomics assays were performed with sunitinib, quizartinib, crenolanib, gilteritinib and midostaurin, kinase inhibitors that are considered in the treatment of AML. As was also noted previously for dasatinib[12,21] and crizotinib,[21] the compounds displayed an improved selectivity profile in living cells compared to biochemical assays. The high cellular ATP levels likely

explains improved selectively. Alternatively, and despite choosing kinetically balanced conditions, it cannot be completely ruled out that the covalent XO44 out-competed the reversible inhibitors. Furthermore, kinases that are absent or have an expression level below the detection limit of our assay will also not be evaluated in the current setup. The inclusion of multiple different cell lines and development of new broad-spectrum activity-based probes with a different target kinase profile may improve the coverage of the kinome.

Ideally, the identified target-ligand interaction profiles should be validated by orthogonal techniques.[13,17,22] Indeed, many cellular off-targets were previously identified in biochemical assays (for instance 12 out of 14 targets for midostaurin are listed in the ChEMBL database).[23] MARK2 and MARK3 were confirmed as targets for crenolanib,[14] while AXL and PTK2B were previously reported as targets for gilteritinib. In fact, gilteritinib is being developed as a dual FLT3/AXL inhibitor.[24] In contrast, it was previously reported that crenolanib is not active against LCK,[14] which might indicate that LCK is a false negative hit or that cellular regulation of LCK activity is required to observe the effect of crenolanib. Furthermore, unprecedented off-targets were also identified by the chemical proteomics assay, such as CDK16 for midostaurin, SRC, SLK and STK10 for gilteritinib and AK2, MAK and SGK3 for crenolanib. Of interest, AK2 plays a central role in hematopoiesis[25] and biallelic mutations in AK2 cause a strong decrease in protein expression and results in reticular dysgenesis, which might make AK2 an undesirable off-target.[26] Of these newly described targets, SRC, SLK and STK10 could be confirmed as targets of gilteritinib and partial inhibition of CDK16 (midostaurin) as well as MAK and SGK3 (crenolanib) in an orthogonal *in vitro* assay could be shown. While some reported off-targets for the five kinase inhibitors tested can be confirmed, we also identified others, which will require further biological validation. Especially the inhibition of AK2 by crenolanib is of importance in view of its potential toxicological side-effects.

Of note, the off-target profile of the assessed inhibitors may provide additional information about their cellular mode of action that may even add to the anti-cancer effects observed. For example, AURKA and AURKB are inhibited by midostaurin in MV4-11 and U937 cells, but these kinases are also under investigation as potential therapeutic targets for hematological cancers.[27,28] This may suggest that they could contribute to the efficacy of midostaurin. It is important to understand the off-targets of the anti-AML kinase inhibitors, as these are now finding their way into the clinic. The off-targets can explain some of the clinical effects and side-effects observed in the treatment of patients with these drugs.

Experimental

In vitro FRET based FLT3 assay

In a 384-wells plate (PerkinElmer 384 Flat White), 5 µL kinase/peptide mix (0.06 ng/µL FLT3 (Life Technologies; PV3182; Lot: 1614759F), 200 nM peptide (PerkinElmer; Lance® Ultra ULightTM TK-peptide; TRFO127-M; Lot: 2178856)) in assay buffer (50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 0.01% Tween-20, 2 mM DTT) was dispensed. Separately inhibitor solutions (10 µM – 0.1 pM) were prepared in assay buffer containing 400 µM ATP and 1% DMSO. 5 µL of these solutions were dispensed and the plate was incubated in the dark at room temperature. After 90 minutes the reaction was quenched by the addition of 10 µL of 20 mM EDTA containing 4 nM antibody (PerkinElmer; Lance® Eu-W1024-anti-phosphotyrosine(PT66); AD0068; Lot: 2342358). After mixing, samples were incubated for 60 minutes in the dark. The FRET fluorescence was measured on a Tecan Infinite M1000 Pro plate reader (excitation 320 nm, emission donor 615 nm, emission acceptor 665 nm). Data was processed using Microsoft Excel 2016, pIC_{50} values were fitted using GraphPad Prism 7.0. Final assay concentrations during reaction: 200 µM ATP, 0.03 ng/µL FLT3, 100 nM Lance TK-peptide, 0.5% DMSO. Compounds were tested in n=2 and N=2.

In situ testing of kinase inhibitors

Cell lines were obtained as a gift from Linda Smit (VUMC, Amsterdam, the Netherlands) and were tested on regular basis for mycoplasma contamination. To evaluate inhibitor effect on cell proliferation MV4-11 and U937 cells were grown in RPMI, supplemented with 10% fetal bovine serum in an incubator at 37°C under 5% CO_2 atmosphere. For viability assays, 10,000 cells were seeded per well in a 96-wells plate and inhibitors were added at the indicated concentration. After three days, cell viability was measured using the Cell Titer Blue viability assay (Promega) and fluorescence was measured using the Clariostar (BMG Labtech). Relative survival was normalized to the untreated control and corrected for background signal. Data was processed using Microsoft Excel 2016, pIC_{50} values were fitted using GraphPad Prism 7.0. Experiments were performed in either n=3 or n=2.

Genomic DNA extraction and PCR

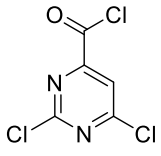
U937 and MV4-11 cells (1 x 10^6) were harvested by centrifugation (1,000 g, 5 min). Medium was removed and cell pellet was suspended in 50 µL QuickExtract™ (Epicentre). Samples were incubated at 65°C for 6 min, mixed by vortexing and then incubated at 98°C for 2 min. Genomic DNA extracts were diluted in sterile water and directly used in PCR reactions. Genomic PCR reactions were performed on 5 µL isolated genomic DNA extract using Phusion High-Fidelity DNA Polymerase (Thermo Fisher) in Phusion HF buffer with 10 pmol primers (forward: GCAATTTAGGTATGAAAGCCAGC, reverse: CTTTCAGCATTTTGACGGCAACC) in a total volume of 50 µL. Amplicons were purified using NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel) and analyzed by Sanger sequencing (Macrogen).[29]

Synthetic procedures

Solvents were purchased from Biosolve, Sigma Aldrich or Fluka and, if necessary dried over 3Å or 4Å molecular sieves. Reagents purchased from chemical suppliers were used without further purification, unless stated otherwise. O_2- or H_2O-sensitive reactions were performed under argon or nitrogen atmosphere and/or under exclusion of H_2O. Reactions were followed by thin layer chromatography and was performed using TLC Silica gel 60 F_{245} on aluminium

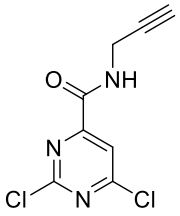
sheets, supplied by Merck. Compounds were visualized by UV absorption (254 nm) or spray reagent (permanganate (5 g/L $KMnO_4$, 25 g/L K_2CO_3)). TLCMS was measured on a thin layer chromatography-mass spectrometer (Advion, Eppression L CMS; Advion, Plate Express). ^1H and ^{13}C-NMR spectra were performed on one of the following Bruker spectrometers: DPX 300 NMR spectrometer (300 MHz), equipped with 5mm-BBO-z-gradient-probe; AV-400 NMR spectrometer (400 MHz), equipped with 5mm-BBO-z-gradient-probe; AV-500 NMR spectrometer (500 MHz), equipped with BBFO-z-gradient-probe; AV-600 NMR spectrometer (600 MHz), equipped with 5mm-Cryo-z-gradient probe. NMR spectra were measured in deuterated methanol, chloroform or DMSO and were referenced to the residual protonated solvent signals as internal standards (chloroform-*d* = 7.260 (^1H), 77.160 (^{13}C); methanol-d_4 = 3.310 (^1H), 49.000 (^{13}C); DMSO-d_6 = 2.500 (^1H), 39.520 (^{13}C)). Signals multiplicities are written as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), p (pentet) or m (multiplet). Coupling constants (*J*) are given in Hz. Preparative HPLC (Waters, 515 HPLC pump M; Waters, 515 HPLC pump L; Waters, 2767 sample manager; Waters SFO System Fluidics Organizer; Waters Acquity Ultra Performance LC, SQ Detector; Waters Binary Gradient Module) was performed on a Phenomenex Gemini column (5 µM C18, 150 x 4.6 mm) or a Waters XBridgeTM column (5 µM C18, 150 x 19 mm). Diode detection was done between 210 and 600 nm. Gradient: ACN in (H_2O + 0.2% TFA). HRMS (Thermo, Finnigan LTQ Orbitrap; Thermo, Finnigan LTQ Pump; Thermo, Finnigan Surveyor MS Pump PLUS Thermo, Finnigan Surveyor Autosampler; NESLAB, Merlin M25). Data acquired through direct injection of 1 mM of the sample in ACN/H_2O/*t*-BuOH (1:1:1), with mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas low 10, capillary temperature 275°C) with resolution R = 60.000 at m/z = 400 (mass range = 150-2000) and dioctylphtalate (m/z = 391.28428) as lock mass. Gradient: ACN in (H_2O + 0.1% TFA). All tested compounds were checked for purity by LCMS liquid chromatography-mass spectrometer, either on a Thermo (Thermo Finnigan LCQ Advantage Max; Thermo Finnigan Surveyor LC-pump Plus; Thermo Finnigan Surveyor Autosampler Plus; Thermo Finnigan Surveyor PDA Plus Detector; Phenomenex Gemini column (5 µm C18, 50 x 4.6 mm)) or a Waters (Waters 515 HPLC pump M; Waters 515 HPLC pump L; Waters 2767 sample manager; Waters SFO System Fluidics Organizer; Waters Acquity Ultra Performance LC, SQ Detector; Waters binary gradient module; Phenomenex Gemini column (5 µm C18, 150 x 4.6 mm)) system and were determined to be >95% pure by integrating UV intensity recorded.

2,6-Dichloropyrimidine-4-carbonyl chloride (7)



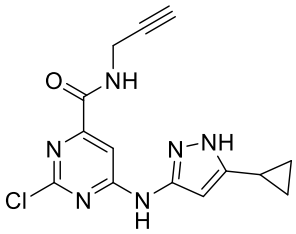
A round-bottom flask was charged with orotic acid (15.6 g, 100 mmol, 1 eq) dissolved in phosphorus oxychloride (46 mL, 500 mmol, 5 eq) and 10 drops DMF. After stirring at reflux for 19 h, *n*-hexane (250 mL) was added, the mixture was stirred vigorously for 10 min and transferred into a separator funnel containing H_2O (100 mL). The layers were separated and the organic layer was washed with brine (1x100 mL), dried over $MgSO_4$ and concentrated under reduced pressure to yield the product (12.8 g, 60%) which was used without further purification. ^1H NMR (500 MHz, chloroform-*d*) δ 8.00 (s, 1H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 167.17, 165.27, 161.56, 158.19, 119.70.

2,6-Dichloro-*N*-(prop-2-yn-1-yl)pyrimidine-4-carboxamide (8)



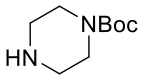
A round-bottom flask was charged with 2,6-dichloropyrimidine-4-carbonyl chloride (7) (1.15 g, 5 mmol, 1 eq) dissolved in dry DCM (50 mL) was cooled to -78°C. After addition of Et_3N (0.91 mL, 6.5 mmol, 1.3 eq) and dropwise addition of propargyl amine (0.33 mL, 5.15 mmol, 1 eq), the mixture was stirred for 3.5 h, quenched by addition of H_2O (50 mL) and diluted with DCM (60 mL). The phases were separated and the organic layer was washed with H_2O (1x50 mL) and brine (1x75 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting crude product was purified via flash-column chromatography (SiO_2, 5% → 50% EtOAc in pentane) to yield the product (0.88 g, 76%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.09 (s, 1H), 4.21 (d, *J* = 2.6 Hz, 2H), 2.57 (t, *J* = 2.5 Hz, 1H). ^{13}C NMR (126 MHz, methanol-d_4) δ 165.41, 161.59, 161.40, 160.68, 119.26, 79.76, 72.40, 29.71.

2-Chloro-6-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)-*N*-(prop-2-yn-1-yl)pyrimidine -4-carboxamide (9)



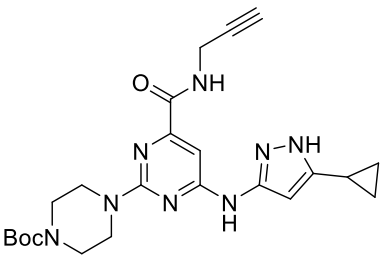
A vial was charged with 2,6-dichloro-*N*-(prop-2-yn-1-yl)pyrimidine-4-carboxamide (8) (229 mg, 1.00 mmol, 1 eq), 5-cyclopropyl-1*H*-pyrazol-3-amine (138 mg, 1.12 mmol, 1.1 eq), DIPEA (0.2 mL, 1.15 mmol, 1.15 eq) dissolved in THF (2 mL). After stirring for 45 h at RT, the mixture was diluted with methanol and concentrated onto celite. The crude product was purified via flash-column chromatography (SiO_2, dry-loading, 50% → 80% EtOAc in pentane) to yield the product (111 mg, 35%). ^{1}H NMR (400 MHz, methanol-d_4) δ 7.47 (bs, 1H), 6.28 (bs, 1H), 4.14 (d, *J* = 2.5 Hz, 2H), 2.61 (t, *J* = 2.5 Hz, 1H), 1.97 – 1.85 (m, 1H), 1.08 – 0.94 (m, 2H), 0.78 – 0.71 (m, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 164.28, 161.22, 148.48, 94.65, 80.13, 72.28, 30.76, 29.61, 8.25, 7.65. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.76 min; *m/z* : 317 $[M+1]^+$.

***tert*-Butyl piperazine-1-carboxylate (10)**



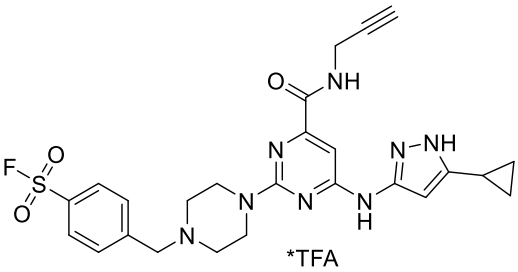
A round bottom flask was charged with *n*-butanol (45 mL), 2 M aqueous NaOH (7.5 mL) and piperazine (3.17 g, 14.5 mmol, 1 eq). After dropwise addition of Boc_2O (3.16 g, 36.7 mmol, 2.5 eq) dissolved in *n*-butanol (5 mL), the mixture was stirred for 60 min at RT and then concentrated under reduced pressure. H_2O (10 mL) was added and di-substituted piperazine was removed by filtration. The filtrate was extracted with DCM (3x30mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to yield the product (1.96 g, 73%) without further purification. ^{1}H NMR (500 MHz, methanol-d_4) δ 3.39 (bs, 4H), 2.81 – 2.70 (m, 4H), 1.46 (s, 9H). ^{13}C NMR (126 MHz, methanol-d_4) δ 156.34, 81.15, 45.89, 44.71, 28.68.

tert-Butyl 4-(4-((5-cyclopropyl-1_H_-pyrazol-3-yl)amino)-6-(prop-2-yn-1-ylcarbamoyl) pyrimidin-2-yl) piperazine-1-carboxylate (11)



A vial was charged with 2-chloro-6-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)-*N*-(prop-2-yn-1-yl)pyrimidine -4-carboxamide (9) (96 mg, 0.30 mmol, 1 eq), *tert*-butyl piperazine-1-carboxylate (10) (78 mg, 0.42 mmol, 1.4 eq) and DIPEA (0.13 mL, 0.76 mmol, 2.5 eq) dissolved in *n*-butanol (2.5 mL). After stirring for 11 h at 100°C, the mixture was diluted with methanol and concentrated onto celite. The crude product was purified via flash-column chromatography (SiO_2, dry-loading, 30% → 70% EtOAc in pentane) to yield the product (168 mg, quant.). ^{1}H NMR (500 MHz, methanol-d_4) δ 6.83 (bs, 1H), 6.09 (bs, 1H), 4.14 (d, *J* = 2.5 Hz, 2H), 3.86 – 3.81 (m, 4H), 3.50 (bs, 4H), 2.59 (t, *J* = 2.5 Hz, 1H), 1.93 – 1.86 (m, 1H), 1.49 (s, 9H), 1.03 – 0.95 (m, 2H), 0.76 – 0.71 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 166.37, 163.33, 162.52, 157.81, 157.28, 156.56, 156.37, 95.01, 94.65, 81.42, 80.63, 71.96, 44.90, 44.18, 29.46, 28.68, 8.27, 7.72. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.41 min; *m/z* : 467 $[M+1]^+$.

4-((4-(4-((5-Cyclopropyl-1_H_-pyrazol-3-yl)amino)-6-(prop-2-yn-1-ylcarbamoyl) pyrimidin-2-yl)piperazin-1-yl)methyl)benzenesulfonyl fluoride (6, XO44)



Step 1: *tert*-butyl 4-(4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)-6-(prop-2-yn-1-ylcarbamoyl) pyrimidin-2-yl)piperazine-1-carboxylate (11) (168 mg, 0.46 mmol, 1 eq) was dissolved in chloroform (8 mL) and TFA (2 mL). After stirring the mixture for 75 min, it was concentrated under reduced pressure. The resulting crude product was used in Step 2 without further purification.

Step 2: A vial was charged with crude product from Step 1 (109 mg, 0.30 mmol, 1 eq), 4-(bromomethyl)benzenesulfonyl fluoride (140 mg, 0.55 mmol, 1.8 eq) and DIPEA (0.13 mL, 0.75 mmol, 2.5 eq) dissolved in DMF (1 mL) and DCM (4 mL). The resulting mixture was stirred at RT for 80 min and concentrated under reduced pressure. The residue was redissolved in EtOAc (20 mL) and washed with saturated aqueous Na_2CO_3 (1x20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting crude product was purified by preparative HPLC (Gemini C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the product as a TFA salt after lyophilisation (58 mg, 30%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.23 (d, *J* = 8.4 Hz, 2H), 7.91 (d, *J* = 8.4 Hz, 2H), 6.96 (s, 1H), 6.04 (s, 1H), 4.55 (s, 2H), 4.14 (d, *J* = 2.5 Hz, 2H), 4.08 (bs, 4H), 3.39 (bs, 4H), 2.60 (t, *J* = 2.5 Hz, 1H), 1.96 – 1.89 (m, 1H), 1.03 – 0.98 (m, 2H), 0.77 – 0.72 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.50, 161.31, 160.35, 156.12, 147.08, 138.33, 133.41, 132.77 (d, *J* = 23.9 Hz), 129.09, 128.12 (d, *J* = 92.3 Hz), 95.36, 93.03, 81.29, 72.82, 58.10, 51.02, 40.90, 28.34, 8.24, 7.13. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 8.27 min; *m/z* : 539 $[M+1]^+$. HRMS calculated for $C_{25}H_{28}FN_8O_3S$ 539.19836 $[M+1]^+$, found 539.19822.

Chemical proteomics assay protocol

The assay protocol is adapted from an earlier publication.[17]

Preparation of Reagents and Stocks

Acetonitrile(ACN): ULC/MS grade (Biosolve, cat. no. 012041)

Chloroform($CHCl_3$): (Sigma, cat. no. 32211-M)

DMSO: (Sigma, cat. no. 34943-M)

Formic acid(FA): LC-MS grade (Actuall Chemicals, art. nr. 8060128A1)

Methanol: Reagent grade (Sigma, cat. no. 32213-M) or ULC/MS grade (Biosolve, cat. no. 136841)

Pierce™ Avidin Agarose: (Thermo Scientific, cat. no. 20219 20225)

Benzonase stock: Dilute benzonase nuclease (Santa Cruz Biotechnology, cat. no. sc-202391) to 10 U/µL. Store at -20℃.

$CaCl_2$ stock: Dissolve 147 mg calcium chloride dihydrate ($CaCl_2 \cdot 2\ H_2O$; Merck Millipore, cat. no. 102382) in 1 mL water to prepare a 1M $CaCl_2$ solution. Store the stock at room temperature.

DTT stock: Dissolve 1.54 g dithiothreitol (DTT; BioChemica, cat. no A1101) in water to a final volume of 10 mL for a 1M solution. Aliquots can be stored at -20 °C for up to 3 months.

IAA stock: Dissolve 92 mg iodoacetamide (IAA; Sigma, cat. no. I6125) in 1 mL water for a final concentration of 0.5 M. Light-sensitive. Prepare directly before use.

$MgCl_2$ stock: Dissolve 2.0 g of magnesium chloride hexahydrate ($MgCl_2 \cdot 6\ H_2O$; Acros Organics, cat. no. 413415000) in water to a final volume of 10 mL for a 1 M solution. Store the stock at room temperature.

NaCl stock: Dissolve 0.58 g of sodium chloride (NaCl; Chem-Lab, art. no. CL00.1423) in water to a final volume of 10 mL for a 1 M solution. Store the stock at room temperature.

NH_4HCO_3 buffer: Dissolve 198 mg ammonium bicarbonate (NH_4HCO_3; Fluka, cat. no. 09830) in water to a final volume of 10 mL for a 250 mM solution. Prepare directly before use.

PBS stock: Dilute 10x PBS solution ten times in Milli-Q water. Prepare the solution in a laminar hood. Store the stock at room temperature.

SDS stock: Prepare a 10% wt/vol sodium dodecyl sulfate (SDS; MP Biomedicals cat. no. 811032) solution in water. Store the stock at room temperature.

Tris stock: Prepare 1M tris(hydroxymethyl)aminomethane (Tris; Acros Organics cat. no. 16762) in water. Adjust pH to 8 with HCl. Store the stock at room temperature.

Trypsin solution: Dissolve 20 µg of trypsin (sequencing grade; Promega, cat. no. V5111) in 40 µL 1 mM HCl (hydrochloric acid; Sigma, cat. no. 30721-M) solution (diluting 37% HCl (~12 M) into water, pH=3) to get a final concentration of 0.5 µg/µL. Store at -20℃ and avoid freeze-thaw cycles.

Yeast enolase stock: Dissolve 1 nmol yeast enolase (Waters, product no. 186002325. SwissProt P00924) in 1 mL 3% acetonitrile in water (vol%).

Biotin-Azide: Dissolve solid biotin-azide (Cayman Chemical, product no. 13040, CAS: *908007-17-0*) in DMSO to reach a 4 mM concentration.

$CuSO_4$: Dissolve solid $CuSO_4(H_2O)_5$ in H_2O to reach a 100 mM concentration.

Na Ascorbate: Dissolve solid Na Ascorbate in H_2O to reach a 1 M concentration.

THPTA: Dissolve tris-hydroxypropyltriazolylmethylamine in DMSO to reach a 100 mM concentration.

Preparation of Mixes

- **LC-MS sample solution(2mL)**

0.1% (v/v) FA	2 µL	FA
3% (v/v) ACN	60 µL	ACN
97% (v/v) H_2O	1900 µL	MilliQ
20 fmol/µL enolase	40 µL	yeast enolase stock

Note: Prepare directly before use.

- **OB-Dig buffer(6mL)**

100 mM Tris	600 µL	Tris stock
100 mM NaCl	600 µL	NaCl stock
1 mM $CaCl_2$	6 µL	$CaCl_2$ stock
2% (v/v) ACN	120 µL	ACN

Note: Prepare directly before use

- **PBS/SDS**

0.5% (w/v) SDS	50 mL	SDS stock
99.5% (w/v) PBS	950 mL	PBS stock

Note: Prepare the solution in a laminar hood. Add SDS stock in the end. Store the stock at room temperature.

- **Stage tip solution A(100mL)**

0.5%(v/v) FA	500 µL	FA
99.5% (v/v) H_2O	100 mL	MilliQ

- **Stage tip solution B(100mL)**

0.5%(v/v) FA	500 µL	FA
80%(v/v) ACN	80 mL	ACN
20%(v/v) H_2O	20 mL	MilliQ

- **Urea buffer (10 mL)**

6M urea	3.6 g	Urea, Sigma, cat. no. 33247
25mM NH_4HCO_3	1 mL	NH_4HCO_3 buffer
In water	10 mL	MilliQ

Note: Prepare directly before use.

Cell treatment

Incubation

MV4-11 or U937 cells were cultured in IMDM or RPMI1640 (Sigma Aldrich) supplemented with stable glutamine and phenolred (PAA), 10% new born calf serum or 10% fetal calf serum (Biowest), penicillin and streptomycin at 37°C with 5% CO_2. Cell passage was performed every 2-3 days, growing these suspension cells at 2-10x10^5 cells/mL in 50 mL medium.

1) Count the cells: mix the cell suspension homogenously with a 25 mL pipette. Then take 3 mL cell suspension from the cell culture flask and transfer to 15 mL centrifuge tube. Mix 10 µL cell suspension from the centrifuge tube with 10 µL trypan blue. Pipette 10 µL of the mixture into a counting chamber. Count live/dead cells using a Biorad TC10 cell counter. Make sure to gently vortex the tube before counting.
2) Collect enough cell solution in a 50 mL centrifuge tube to obtain a final amount of 7×10^6 cells per sample. Collect the cells by centrifugation (10 min, 300 g) and discard the supernatant.
3) Add serum-free medium to the centrifuge tube and re-suspend the cell pellet gently with a 25 mL pipette to reach a final concentration of 7×10^6 cells/mL.
4) Add 1 mL of cell solution per sample to 1.5 mL Eppendorf tubes.

Note: To minimize the sample difference between samples, first add 500 µL from the first one to the last one. Then gently vortex the centrifuge tubes and put it upside down to homogenize the cell solution. Then add the other 500 µL cell solution from the last one to the first one.

5) Add 10 µL of DMSO or inhibitor (pre-diluted in DMSO to reach the desired final concentration) depending on the condition. Each condition should be tested in triplicate. Of note, one positive control replicate in kinase17_raw had to excluded due to large parts of the sample being lost during chloroform/methanol precipitation.
6) Puncture a hole with a needle on a new Eppendorf cap and install this cap to the original Eppendorf.
7) Start the first incubation at 37°C with 5% of CO_2 for 60 min.
8) Add 1 µL of DMSO or probe depending on the condition. Start second incubation at 37°C with 5% of CO_2 for 30 min.
9) Harvest cells by centrifuging the samples (10 min, 300 g). Remove the supernatant with vacuum suction device with a yellow pipette tip at its tip.
10) Snap freeze the pellet in liquid nitrogen.

Cell lysis

11) Thaw the samples on ice.
12) Dilute benzonase solution to 25 U/mL in MilliQ.
13) Add 225 µL benzonase solution to each sample and lyse cells by pipetting up and down with a 200 µL pipet until all the pellet is gone. Incubate on ice for 10 min.

14) Continue lysis by adding 25 µL 10% SDS solution to the samples (resulting in a concentration of 1% SDS in the sample). Incubate the samples at 95°C for 5 min. Spin down shortly when the samples cool down to room temperature.

Click reaction

15) Prepare 25 µL click mix per sample according to the list:

Reagents, Stock Conc.	Dilution factor	Volume (µL)
$CuSO_4 \cdot 5H_2O$, 100 mM	10×	2.5
Na Ascorbate, 1 M	17.86×	1.4
THPTA, 100 mM	50×	0.5
Biotin-Azide, 4 mM	10×	2.5
Milli Q water		18.1
	Total	25

Add the reagent according to the order on the list. Vortex gently after each reagent. The final color should be lightly yellow-golden.

16) Add 25 µL click mix to each sample. Incubate at 37°C while shaking (300 rpm) for 1 h, followed by a short centrifugation.

Methanol/chloroform precipitation

17) Add 225 µL Milli Q water to each sample for a final volume of 500 µL.

18) Add 666 µL MeOH and vortex.

19) Add 166 µL $CHCl_3$ and vortex.

20) Add 150 µL water and vortex; this should result in a cloudy suspension.

21) Pellet the precipitated protein by centrifugation (10 min, 1500 g), this should result in a floating pellet.

22) Remove the upper and lower layer without disturbing pellet (this works best by holding the tube at the angle of 45° to stick the protein pellet against the side).

23) Add 600 µL MeOH to the pellet.

24) Resuspend the pellet by sonication with a probe sonicator (10 sec, 30% amplitude); this should result in a suspension without any visible protein pellet left.

25) Pellet the protein by centrifugation (5 min, 18,400 g) and remove supernatant.

26) Add 250 µL urea buffer to each sample.

27) Resuspend pellet by thoroughly pipetting up and down with a yellow pipette tip (~10 times).

28) Snap freeze the samples and store the samples at -20 °C until required

Reduction, alkylation and avidin enrichment

29) Thaw the samples on ice.

30) Add 2.5 µL DTT stock, vortex, spin down briefly and incubate for 15 min at 65°C while shaking (600 rpm).

31) Let samples cool down to room temperature (at least 5 min).

32) Add 20 µL IAA solution, vortex and incubate for 30 min at room temperature in the dark.

33) Add 70 µL 10% SDS solution, vortex and incubate for 5 min at 65°C. Spin down shortly after the samples cool down to room temperature.

34) For 24 samples remove 1.2 mL avidin beads from a 50% slurry (50 µL per sample) and divide over four 15 mL tubes (300 µL per tube).

Note: Be sure to properly homogenize the slurry before pipetting.

35) Wash beads once with 10 mL PBS. Pellet beads by centrifugation (2 min, 2500 *g*) and remove the supernatant with a suction pump.

36) Homogenize the beads in 6 mL PBS per tube.

37) For 24 samples prepare 24 tubes (15 mL) with 2 mL PBS and 1 mL beads from step 36. Add each individual sample from step 33 to one of these tubes.

38) Incubate while rotating at low speed using an overhead shaker for at least 3 h at room temperature.

Wash beads

39) Pellet beads by centrifugation (2 min, 2500 *g*) and remove the supernatant.

Note: Be careful not to suck up the beads. Leave a little liquid above the beads.

40) Wash the beads once with 6 mL PBS/SDS, followed by three times with 6 mL PBS. Pellet beads by centrifugation (2 min, 2500 *g*) and remove the supernatant.

On-bead digestion

41) Add 250 µL OB-Dig buffer to the beads and transfer to a 1.5 mL low binding tube.

42) Add 1 µL trypsin solution per sample

43) Digest overnight at 37 °C with vigorous shaking (950 rpm).

LCMS sample preparation

44) Spin down shortly and add 12.5 µL formic acid per sample, briefly vortex and spin down.

45) Filter off the beads with a bio-spin column by centrifugation (2 min, 600 *g*) and collect the flow-through in a 2 mL tube.

46) Condition the stage tips, load the sample and wash the sample following this centrifugation scheme.

Note: Flow-through from conditioning, loading, and washing can be discarded. Elution should be done in a low binding tube. Centrifugation speed and duration are merely estimates. Solutions should have entirely run through without drying of the column

Conditioning 1	50 µL MeOH	7 min 650 g
Conditioning 2	50 µL Stage tip solution B	7 min 650 g
Conditioning 3	50 µL Stage tip solution A	7 min 650 g
Loading	Sample from step 45	15 min 750 g
Washing	100 µL Stage tip solution A	10 min 750 g
Switch to the low binding tube		
Elution	100 µL Stage tip solution B	10 min 750 g

47) Evaporate the solvent in a SpeedVac at 45°C for 3 hours.

The samples can be stored at -20°C until required.

48) Reconstitute sample in 50 µL of LC-MS sample solution.

49) Prepare a QC sample: pool 2 µL from each sample.

LC-MS analysis

50) Inject 5 µL of the sample onto the UPLC-IMS-MS system (see LC-MS Analysis).

LC-MS Analysis

The peptides were measured as described previously for the NanoACQUITY UPLC System coupled to SYNAPT G2-Si high definition mass spectrometer [17]. A trap-elute protocol, where 5 µL of the digest is loaded on a trap column (C_{18} 100 Å, 5 µM, 180 µM x 20 mm, Waters) followed by elution and separation on the analytical column (HSS-T3 C18 1.8 µM, 75 µM x 250 mm, Waters). The sample is brought onto this column at a flow rate of 10 µL/min with 99.5% solvent A for 2 min before switching to the analytical column. Peptide separation is achieved using a multistep concave gradient based on gradients previously described [30]. The column is re-equilibrated to initial conditions after washing with 90% solvent B. The rear seals of the pump are flushed every 30 min with 10% (vol/vol) ACN. [Glu1]-fibrinopeptide B (GluFib) is used as a lock mass compound. The auxiliary pump of the LC system is used to deliver this peptide to the reference sprayer (0.2 µL/min). A UDMSe method is set up as described in Distler et al. Briefly, the mass range is set from 50 to 2,000 Da with a scan time of 0.6 seconds in positive, resolution mode. The collision energy is set to 4 V in the trap cell for low-energy MS mode. For the elevated energy scan, the transfer cell collision energy is ramped using drift-time specific collision energies. The lock mass is sampled every 30 seconds. For raw data processing, PLGS (v3.0.3) was used. The MS^E identification workflow was performed with the parameters summarized in SI Table 1 to search the human proteome from Uniprot (uniprot-homo-sapiens-trypsin-reviewed-2016_08_29.fasta). For the MV4-11 experiments, the canonical sequence of FLT3 was replaced with the ITD sequence stated in the SI. Protein quantification was performed using ISOQuant (v1.5). The parameter settings used are summarized in SI Table 2.

Quantification and statistical analysis

All data analysis starting from the ISOQuant output has been performed in Python. The corresponding Jupyter notebook file with all the used functions is included in the Supporting Information. To calculate the Student's t-test the script depends on the RPy2 2.9.0 package. The following packages are optional for plotting and API queries Bokeh (0.12.10), chembl_webresource_client. Heatmaps were created with Microsoft Excel.

Probe targets were selected based on the following cut-offs:

i) 2-fold enrichment of quantification value from negative (vehicle treated) to positive control (1 µM XO44).

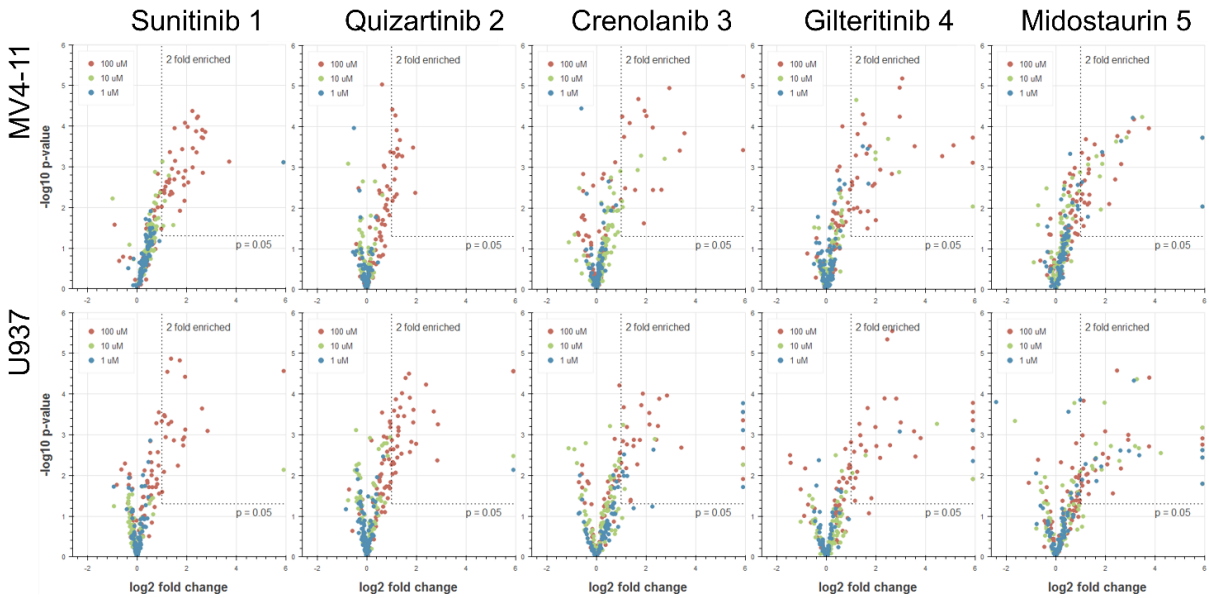
ii) Probability associated with a Student's t-test (two-tailed distribution; two-sample equal variance) < 0.05 testing positive control vs negative control samples.

iii) Benjamini–Hochberg correction with an FDR of 10%.

iv) Annotated as proteins transferring Phosphate containing groups in the Uniprot-database (EC number Filter "2.7.-.-"). Downloaded on 21-12-2017 (http://www.uniprot.org/uniprot/?query=reviewed:yes%20AND%20organism:%22Homo%20sapiens%20(Human)%20[9606]%22+ec:2.7.-.-).

Kinase inhibitor targets were selected as follows: kinases displaying > 50% reduction in probe labeling comparing inhibitor pre-treated sample with positive control (1 µM XO44) after subtraction of negative control signal (vehicle treated).

Supplementary Information



SI Figure 1: Competition with sunitinib, quizartinib, crenolanib, gilteritinib and midostaurin in living cells. Volcano plot of the label free quantification signal from IsoQuant for target kinases, pretreated with three different inhibitor concentrations in two cell lines. To enable plotting of all targets, infinite fold change (XO44 treated divided by competitor treated) was set to 60. A kinase was named a target if there was at least 50% reduction in quantification signal from probe treated samples vs inhibitor pretreated sample.

SI Table 1: The PLGS parameter settings used.

Parameter	**value**
Lock mass m/z	785.8426
Low energy threshold	150 counts
Elevated energy threshold	30 counts
Digest reagent	trypsin
Max missed cleavages	2
Modifications	Fixed carbamidomethyl C, variable oxidation M
FDR less than	1%
Fragments/peptide	2
Fragments/protein	5
Peptides/protein	1

SI Table 2: The ISOQuant parameter settings used.

parameter	**value**
isoquant.pluginQueue.name	design project and run ISOQuant analysis
process.peptide.deplete.PEP_FRAG_2	false
process.peptide.deplete.CURATED_0	false
process.peptide.statistics.doSequenceSearch	false
process.emrt.minIntensity	1000
process.emrt.minMass	500
process.emrt.rt.alignment.match.maxDeltaMass.ppm	10
process.emrt.rt.alignment.match.maxDeltaDriftTime	2
process.emrt.rt.alignment.normalizeReferenceTime	false
process.emrt.rt.alignment.maxProcesses	24
process.emrt.rt.alignment.referenceRun.selectionMethod	AUTO
process.emrt.clustering.preclustering.orderSequence	MTMTMT
process.emrt.clustering.preclustering.maxDistance.mass.ppm	6.06E-6
process.emrt.clustering.preclustering.maxDistance.time.min	0.202
process.emrt.clustering.preclustering.maxDistance.drift	2.02
process.emrt.clustering.distance.unit.mass.ppm	6.0E-6
process.emrt.clustering.distance.unit.time.min	0.2
process.emrt.clustering.distance.unit.drift.bin	2
process.emrt.clustering.dbscan.minNeighborCount	1
process.identification.peptide.minReplicationRate	2
process.identification.peptide.minScore	5.5
process.identification.peptide.minOverallMaxScore	5.5
process.identification.peptide.minSequenceLength	6
process.identification.peptide.acceptType.PEP_FRAG_1	true
process.identification.peptide.acceptType.IN_SOURCE	false
process.identification.peptide.acceptType.MISSING_CLEAVAGE	false
process.identification.peptide.acceptType.NEUTRAL_LOSS_H20	false
process.identification.peptide.acceptType.NEUTRAL_LOSS_NH3	false
process.identification.peptide.acceptType.PEP_FRAG_2	true
process.identification.peptide.acceptType.DDA	true
process.identification.peptide.acceptType.VAR_MOD	true

process.identification.peptide.acceptType.PTM	true
process.annotation.peptide.maxSequencesPerEMRTCluster	1
process.annotation.protein.resolveHomology	true
process.annotation.peptide.maxFDR	0.01
process.annotation.useSharedPeptides	all
process.normalization.lowess.bandwidth	0.3
process.normalization.orderSequence	XPIR
process.normalization.minIntensity	3000
process.quantification.peptide.minMaxScorePerCluster	5.5
process.quantification.peptide.acceptType.IN_SOURCE	false
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process.quantification.peptide.acceptType.NEUTRAL_LOSS_NH3	false
process.quantification.peptide.acceptType.PEP_FRAG_1	true
process.quantification.peptide.acceptType.PEP_FRAG_2	false
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process.quantification.minPeptidesPerProtein	2
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process.quantification.absolute.standard.fmol	50
process.quantification.topx.allowDifferentPeptides	true
process.quantification.absolute.standard.entry	ENO1_YEAST
process.quantification.absolute.standard.fmol	50
process.quantification.maxProteinFDR	0.01

Used Sequence FLT3-ITD in MV4-11:

>sp|F3LT00ITD1|FLT3_MV411 Receptor-type tyrosine-protein kinase FLT3 OS=Homo sapiens GN=FLT3_MV411 PE=1 SV=2
MPALARDGGQLPLLVVFSAMIFGTITNQDLPVIKCVLINHKNNDSSVGKSSSYPMVSESPEDLGCALRPQSSGTVYEAAAVEVDVS
ASITLQVLVDAPGNISCLWVFKHSSLNCQPHFDLQNRGVVSMVILKMTETQAGEYLLFIQSEATNYTILFTVSIRNTLLYTLRRPYF
RKMENQDALVCISESVPEPIVEWVLCDSQGESCKEESPAVVKKEEKVLHELFGTDIRCCARNELGRECTRLFTIDLNQTPQTTLPQ
LFLKVGEPLWIRCKAVHVNHGFGLTWELENKALEEGNYFEMSTYSTNRTMIRILFAFVSSVARNDTGYYTCSSSKHPSQSALVTI
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CCAYNSLGTSCETILLNSPGPFPFIQDNISFYATIGVCLLFIVVLTLLICHKYKKQFRYESQLQMVQVTGSSDNEYFYVDFREYEYDH
VDFREYEYDLKWEFPRENLEFGKVLGSGAFGKVMNATAYGISKTGVSIQVAVKMLKEKADSSEREALMSELKMMTQLGSHENIV
NLLGACTLSGPIYLIFEYCCYGDLLNYLRSKREKFHRTWTEIFKEHNFSFYPTFQSHPNSSMPGSREVQIHPDSDQISGLHGNSFHS
EDEIEYENQKRLEEEEDLNVLTFEDLLCFAYQVAKGMEFLEFKSCVHRDLAARNVLVTHGKVVKICDFGLARDIMSDSNYVVRG
NARLPVKWMAPESLFEGIYTIKSDVWSYGILLWEIFSLGVNPYPGIPVDANFYKLIQNGFKMDQPFYATEEIYIIMQSCWAFDSRK
RPSFPNLTSFLGCQLADAEEAMYQNVDGRVSECPHTYQNRRPFSREMDLGLLSPQAQVEDS

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Chapter 3

Comprehensive structure-activity-relationship of azaindoles as highly potent FLT3 inhibitors*

Introduction

Acute myeloid leukemia (AML) is a cancer of the blood and bone marrow that is characterized by a failure in differentiation of stem cells during hematopoiesis, resulting in flooding of the bloodstream with immature myeloid blood cells. These blast cells fatally disrupt normal hematopoietic function and their abundance in blood obstruct the normal flow in capillaries resulting in a high mortality.[1,2] While in younger patients cure rates can reach up to 35-40%, elderly patients, who are often unable to cope with the intensive chemotherapy regimen, do not experience this benefit.[3] AML is a genetically diverse disease, but in 20-30% of patients an internal tandem duplication (ITD) in the juxtamembrane domain of the Fms-like tyrosine kinase 3 (FLT3) receptor has been identified as a driver mutation.[4,5] The validation of FLT3 as a drug target led to clinical development of several small molecule inhibitors, culminating in the recent FDA approval of midostaurin for treatment of FLT3-dependent AML in conjunction with standard treatment.[6–9] Although the initial response to treatment with FLT3 inhibitors shows therapeutic promise, many AML patients relapse due to the emergence of drug-resistant cancer cells.[10–12] Resistance-inducing mutations have thus far been observed in

* The data presented in this chapter was gathered in collaboration with Berend Gagestein, Jordi F. Keijzer, Nora Liu, Ruud H. Wijdeven, Eelke B. Lenselink, Adriaan W. Tuin, Adrianus M. C. H. van den Nieuwendijk, Gerard J. P. van Westen, Constant A. A. van Boeckel, Herman S. Overkleeft, Jacques Neefjes, Mario van der Stelt.

treatments with several FLT3 inhibitors, among which the highly potent experimental drug quizartinib.[12–14] The discovery of new chemical entities to target FLT3 represents, therefore, a medical need.

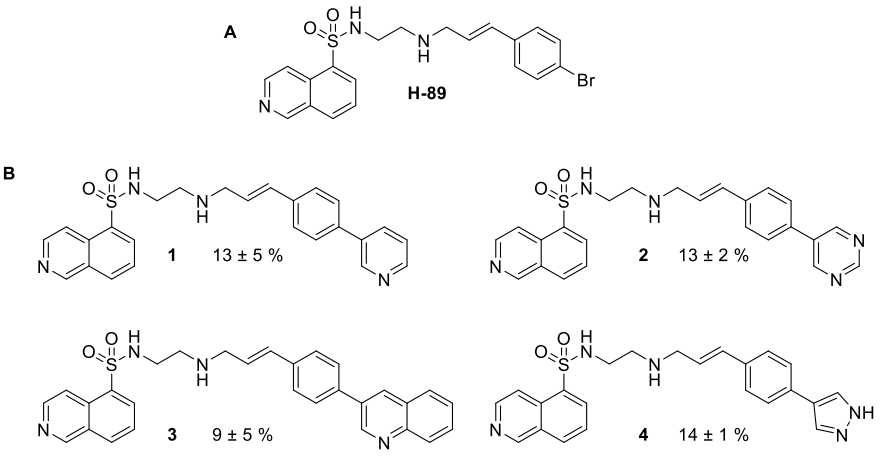


Figure 1: FLT3 screening hits (**1-4**) from an H-89 library.[15] Data represent residual *in vitro* FLT3 activity at 2 µM.

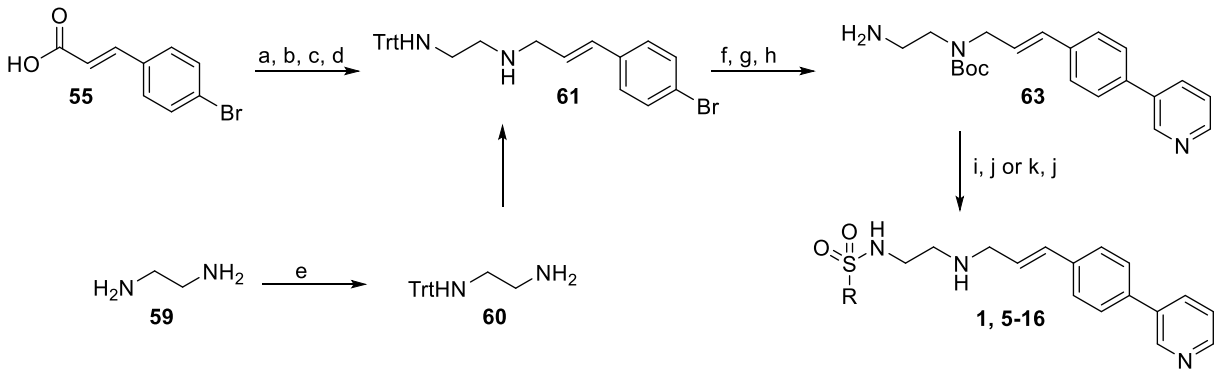
N-[2-(*p*-Bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89) is a prototypical and intensely-studied kinase inhibitor (Figure 1A). It was one of the first non-natural, synthetic inhibitors that competitively inhibited the binding of ATP to the structurally conserved binding domain of cAMP-dependent protein kinase (PKA).[16,17] The binding mode of H-89 to PKA has been studied in great detail at the atomic level using crystallization studies.[18] This contributed to the understanding of kinase function and provided general principles to develop drug-like kinase inhibitors. The isoquinoline sulfonamide mimics the binding mode of adenosine. The nitrogen of the isoquinoline ring forms a crucial H-bond bridge to the backbone of Val-123, located in the hinge region of PKA.[18] This binding mode of H-89 is not specific to PKA, but has also been observed with Haspin, as shown in structural data (PDB: 3FMD). Furthermore, H-89 activity has been shown for several other kinases, including S6K1, MSK1 and ROCK-II.[19,20] Consequently, H-89 is used as a starting point in several drug discovery programs. For example, this lab has previously described the use of H-89 and its analogs as RAC-alpha serine/threonine-protein kinase (AKT1) inhibitors to combat bacterial infections, such as *Salmonella typhimurium* and *Mycobacterium tuberculosis*.[15,21] During the hit optimization program of H-89 analogs as AKT1 inhibitors, four compounds (**1**-**4**) were identified that demonstrated substantial activity against FLT3 (Figure 1B).[15] In this chapter the optimization and structure-activity relationships of H-89-derived compounds as new FLT3 inhibitors is presented.

Results and Discussion

To confirm the structure and activity of compound **1**, the synthesis was started with the commercially available building blocks as outlined in Scheme 1. After methylation and

reduction, the resulting alcohol was exchanged for a chlorine and a trityl protected ethylenediamine linker was introduced via nucleophilic substitution. Subsequent Boc-protection, Suzuki-coupling with 3-pyridinylboronic acid and trityl-deprotection yielded the primary amine, which could be coupled with isoquinoline sulfonyl chloride to provide the desired product **1**. The activity of compound **1** was confirmed in a biochemical assay using purified, recombinantly expressed human FLT3 with a time-resolved fluorescence resonance energy transfer (FRET) method. Compound **1** showed potent inhibition with a half maximum inhibitory concentration (IC_{50}) in the low nanomolar range (pIC_{50} = 8.02 ± 0.05), which was comparable to the inhibitory activity of the reference inhibitor quizartinib (pIC_{50} = 8.30 ± 0.07). Compound **1** demonstrated favorable physico-chemical properties with a molecular weight (MW) of 445 and a logD (pH 7.4) of 1.5.[22] This resulted in a lipophilic efficiency (LipE = pIC_{50} - logD) of 6.5.[23] In summary, compound **1** was defined as an excellent starting point to develop new FLT3 inhibitors.

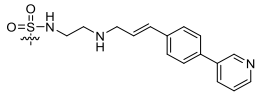
Scheme 1: Synthetic route towards the derivatives **1**, **5**-**16**.[a]

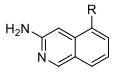
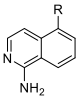
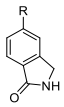
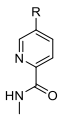


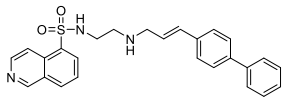
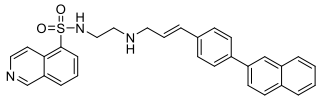
[a]Reagents and conditions: (a) K_2CO_3, dimethyl sulfate, ACN, 80°C, overnight; (b) DIBAL-H, toluene, -80 – 0°C; (c) $SOCl_2$, DCM, RT; (d) **60**, K_2CO_3, ACN, 70°C, 2 h; (e) TrtCl, K_2CO_3, RT, 40 min; (f) $NaHCO_3$, Boc_2O, THF, RT, overnight; (g) 3-pyridinylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, DCM/DMF, 85°C, 6 h; (h) TFA, TES, DCM 0°C – RT, 5 h; (i) heteroaryl-bromide, $K_2S_2O_5$, HCOONa, $Pd(OAc)_2$, PPh_3, 1,10-phenanthroline, DMSO then DiPEA, **63**, NBS, THF, 0°C – RT, 1 h; (j) TFA, $CHCl_3$, 1 h; (k) aryl-sulfonylchloride, Et_3N, DCM/DMF, 0°C – RT.

A topological exploration of the structure-activity relationship of isoquinolinesulfonamides was employed guided by the observed binding mode of H-89 in other kinases.[18] First, the isoquinoline substituent was replaced by various other hinge binding moieties inspired by kinase drugs, including indolones (sunitinib and nintedanib),[24–27] aminoisoquinolines (crizotinib and palbociclib),[24,28,29] indazoles (axitinib)[24,30] and picolinamides (sorafenib).[24,31,32] The analogs (**5**-**16**) were synthesized in a similar manner as compound **1** using a palladium-catalyzed sulfination of heteroaryl halides and subsequent coupling with the primary amine as shown in Scheme 1.[33] Interestingly, compounds **5**-**12** displayed similar or slightly weaker activity compared to compound **1** with a range of pIC_{50}s between 7.6 and 8.0 (Table 1). Indazolone **6** was the most potent compound of the series with a pIC_{50} of 8.01 ± 0.08.

Table 1: *In vitro* FLT3 activity and LipE of compounds **1** – **18**.

R = 

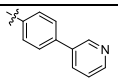
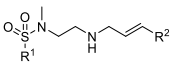
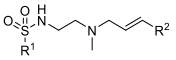
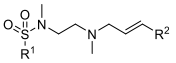
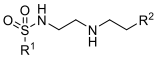
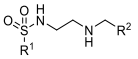
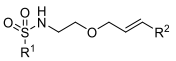
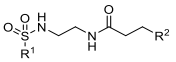
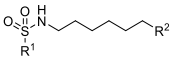
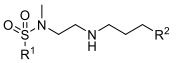
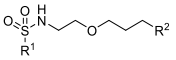
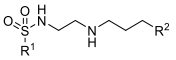
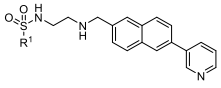
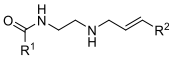
Entry		pIC_{50} ± SEM	LipE	Entry		pIC_{50} ± SEM	LipE
1		8.02 ± 0.05	6.5	**11**		7.71 ± 0.10	6.4
5		7.70 ± 0.11	7.0	**12**		7.62 ± 0.16	6.3
6		8.01 ± 0.08	6.6	**13**		7.21 ± 0.14	4.6
7		7.77 ± 0.09	6.6	**14**		6.19 ± 0.15	6.2
8		7.74 ± 0.11	7.0	**15**		8.07 ± 0.07	6.6
9		7.32 ± 0.12	6.6	**16**		7.57 ± 0.18	6.6
10		7.86 ± 0.10	7.6				

Entry		pIC_{50} ± SEM	LipE
17		< 5	n.a.
18		< 5	n.a.

Moreover, substantially more polar groups such as picolinamide were well tolerated (as observed in compound **10**), resulting in a high lipE of 7.6. Surprisingly, the nitrogen atom, which plays an important role in the hinge binding to other kinases, was not required for activity. Compounds **13** and **14** retained activity with a pIC_{50} of 7.21 ± 0.34 and 6.19 ± 0.15, respectively. The same was true for the nitro and amino phenyl derivatives **15** and **16**. All together, these results suggested that the binding orientation of the isoquinolinesulfonamides might be different than the one of H-89 in PKA. It was envisioned that the nitrogen atom of the pyridyl ring could act as a potential H-bond acceptor to interact with the hinge region,

which may potentially explain the activity of compounds **13**-**16**. To test this hypothesis compounds (**17**-**18**), in which the pyridine ring was substituted for a carbacycle, were synthesized (SI Scheme 1). The pIC_{50} of these novel derivatives dropped to < 5 (Table 1). This suggested that the nitrogen in the pyridine is indeed important for the interaction with FLT3 and the isoquinolinesulfonamide may have a flipped binding orientation in the ATP-pocket of FLT3 compared to PKA.

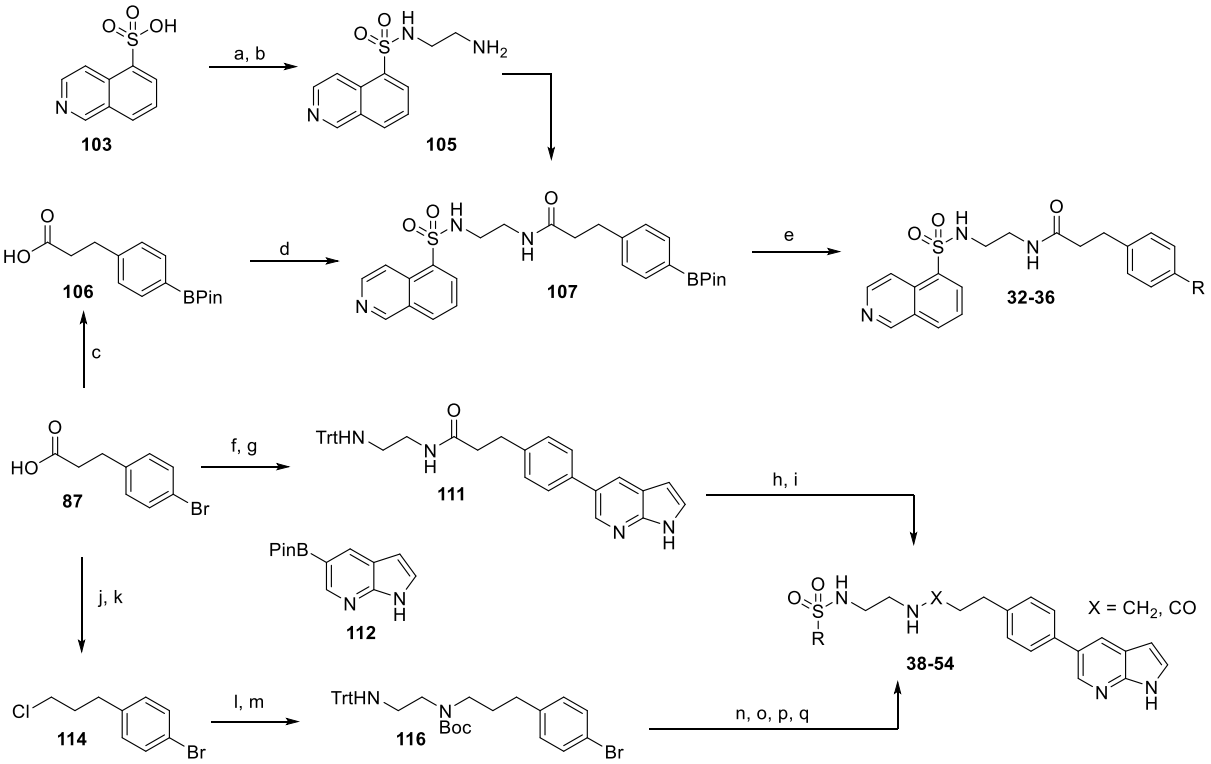
Table 2: FLT3 activity and LipE of compounds **19** – **31**.

	R^1 =  **R^2 =** 		
Entry		**pIC_{50} ± SEM**	**LipE**
19		6.74 ± 0.26	5.0
20		6.87 ± 0.20	4.6
21		6.90 ± 0.19	4.3
22		6.57 ± 0.21	5.4
23		6.80 ± 0.17	5.6
24		8.08 ± 0.09	5.3
25		7.49 ± 0.14	5.3
26		6.77 ± 0.17	2.2
27		7.32 ± 0.15	5.5
28		7.41 ± 0.13	4.3
29		8.05 ± 0.07	6.4
30		6.25 ± 0.21	3.7
31		< 5	n.a.

To further understand the SAR of our chemical series, the importance of the linker between the isoquinoline and the pyridyl moieties was investigated (**19**-**31**). The results from this study are summarized in Table 2. The synthetic schemes for these compounds (**19**-**31**) are shown in

the SI (SI Scheme 1-4). Several analogs were made to investigate possible hydrogen bond donor capability of the sulfonamide and secondary amine group. To this end, the nitrogens of sulfonamide (**19**), amine (**20**) or both (**21**) were substituted with a methyl group. This led to a > 10-fold drop in potency for all compounds, which indicated that these NH donors could be important for the interaction with FLT3. Next, the linker length between the secondary amine and the phenyl was investigated. Compounds with reduced length of one (**22**) and two (**23**) methylene groups showed decreased activity. The importance of the basicity of the linker moiety was tested by replacing the amine with an ether (**24**), amide (**25**), or a methylene (**26**) containing linker. **24** and **25** were equally active as the corresponding amine derivative, while **26** was > 10-fold less active (Table 2). These results suggested that the basic center of the linker is not required. Of note, reduction of the double bond (**27** - **29**) in the linker resulted in an almost identical inhibitory activity as the parent compound, whereas increasing the conformational restriction in compound **30** reduced its activity. This indicated that the reduced conformational flexibility by the double bond in compound **1** is not beneficial for its activity as has recently been noted for other kinase inhibitors.[34] Finally, the substitution of the sulfonamide for an amide did result in an inactive compound (**31**) (pIC_{50} < 5), which could possibly be due to a difference in the spatial orientation of the (sulfon)amide substituents. These data indicate that a flexible linker of 6 atoms with or without a basic amine is optimal between the sulfonamide and phenyl-pyridyl rings.

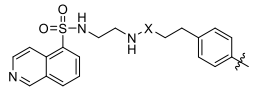
Scheme 2: Synthetic route towards the derivatives **32** - **36** and **38** - **54**.[a]

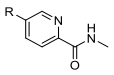


[a]Reagents and conditions: (a) $SOCl_2$, DMF, reflux, 4 h; (b) ethylenediamine, DCM, 0°C – RT; (c) B_2Pin_2, KOAc, $Pd(dppf)Cl_2$, 1,4-dioxane, 100°C, overnight; (d) **105**, EDC, HOBt, DiPEA, DCM, 4 h; (e) heteroaryl-bromide, $Pd(PPh_3)_4$, K_2CO_3, DMF, 85°C, overnight; (f) **60**, EDC, HOBt, DiPEA, DCM, 4 h; (g) **112**, $Pd(PPh_3)_4$, K_2CO_3, DMF, 90°C; (h) TFA, TES, DCM, 0°C – RT, 16 h; (i) aryl-sulfonylchloride, Et_3N, DCM/DMF, 0°C – RT, 16 h; (j) $NaBH_4$, BF_3, THF, 0° - RT, 16 h; (k) $SOCl_2$, DMF, 0°C – RT, 19 h; (l) **60**, K_2CO_3, ACN, 70°C, 72 h; (m) $NaHCO_3$, Boc_2O, THF, RT, 36 h; (n) **112**, $Pd(PPh_3)_4$, K_2CO_3, DMF, 90°C; (o) TFA, TES, DCM, 0°C – RT, 20 h; (p) aryl-sulfonylchloride, Et_3N, DCM/DMF, 0°C – 30°C, 16 h (q) TFA, DCM, 0°C – RT, 16 h.

Having established the optimal linker features, an additional array of compounds (**32**-**37**) was synthesized in which the pyridyl ring was replaced with other (substituted) heteroaryls to optimize the hinge-binding interaction (Scheme 2 and SI Scheme 5). In contrast to the isoquinoline replacements, a wide range of activities was observed (pIC_{50}: 5 – 8.9) (Table 3). While the picolinamide variations (**34**-**35**) were inactive (pIC_{50} < 5), the azaindoles **36** and **37** demonstrated a significantly increased pIC_{50} of 8.87 ± 0.06. and 8.78 ± 0.05, respectively. Of note, **37** demonstrated a LipE of 6.7. Altogether, the optimization of the potential hinge-binding pyridyl moiety resulted in the discovery of the azaindoles as a potent FLT3 inhibitor scaffold.

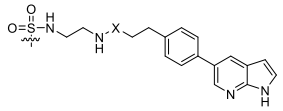
Table 3: FLT3 activity and LipE of compounds **32** - **37**.

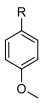
R = 

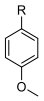
Entry		X	pIC_{50} ± SEM	LipE
32		CO	6.82 ± 0.14	4.8
33		CO	7.63 ± 0.11	5.6
34		CO	< 5	n.a.
35		CO	< 5	n.a.
36		CO	8.87 ± 0.06	6.2
37		CH_2	8.78 ± 0.05	6.7

Next, a matched-molecular pair analysis was performed using the azaindole scaffold with amide (**38**-**49**) and amine linker (**50**-**54**) series.[35] The goal was to study the influence of the substitution pattern of the phenyl ring.[36] Compounds (**38**-**54**) were prepared as shown in Scheme 2. Compounds with electron-withdrawing groups, such as Cl (**39**), *p*-NO_2 (**43**), *p*-F (**45**), or electron donating groups (*p*-Me (**41**) and *p*-OMe (**42**)) both displayed high potency (pIC_{50} > 8.0). No correlation could be found between the Hammett constants of the substituents and the activity of the compounds (SI Figure 1). In fact, non-substituted compound **38** was the most potent compound identified in this study with a pIC_{50} of 9.49 ± 0.08. The matched-molecular pair analysis of LipE values of the amine and amide series showed good correlation, which supports the hypothesis that both series bind in a similar fashion to FLT3 (Figure 2).

Table 4: FLT3 activity and LipE of compounds **38** - **54**

R = 

Entry		X	pIC_{50} ± SEM	LipE
38		CO	9.49 ± 0.08	6.5
39		CO	8.62 ± 0.05	5.0
40		CO	8.39 ± 0.05	4.1
41		CO	8.67 ± 0.06	5.2
42		CO	8.74 ± 0.08	5.8
43		CO	8.80 ± 0.08	5.9
44		CO	8.72 ± 0.06	5.1
45		CO	9.39 ± 0.18	6.2
46		CO	9.32 ± 0.09	5.7
47		CO	8.16 ± 0.08	3.9
48		CO	7.97 ± 0.09	5.0
49		CO	8.37 ± 0.09	4.5
50		CH_2	8.88 ± 0.06	6.5
51		CH_2	8.36 ± 0.08	6.4

52		CH_2	8.13 ± 0.09	4.5
53		CH_2	8.69 ± 0.07	5.9
54		CH_2	8.62 ± 0.10	6.3

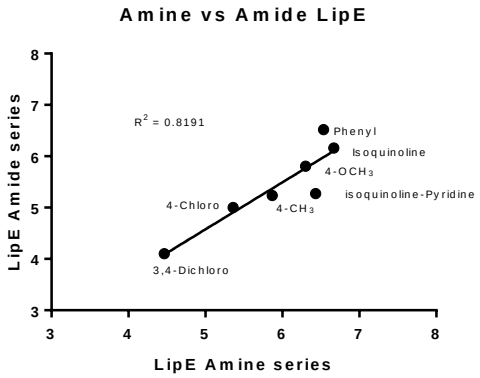


Figure 2: Matched molecular pair analysis of amine and amide containing compounds. Data shows a high correlation (R^2 = 0.82), indicating a similar binding mode for both linker series.

Finally, to explain our structure activity relationships a structure based study was performed with compound **1** and compound **38** using a published DFG-out crystal structure (4RT7), and a DFG-in model (see methods). Induced fit docking was performed in combination with an previously established binding pose metadynamics protocol[45], in order to determine a feasible binding mode. On the basis of these results and overlap in binding mode with quizartinib (SI Figure 2) it was established that compound **1** and compound **38** bind DFG-out (Figure 3A). The pyridine moiety of **1** is engaged in a hydrogen bond interaction with the backbone of C694 (hinge) and the adjacent phenyl engages in a π-interaction with F691 (Figure 3A). Moreover, in the induced fit docking, no poses were observed in which the isoquinoline interacted with the hinge of FLT3. As shown in Figure 3B the resulting docking pose of **38** is similar to the binding mode of **1** with an additional hydrogen-bond-interaction to C694, which may explain the increased potency. To conclude, the observed binding mode is in agreement with the obtained structure activity relations.

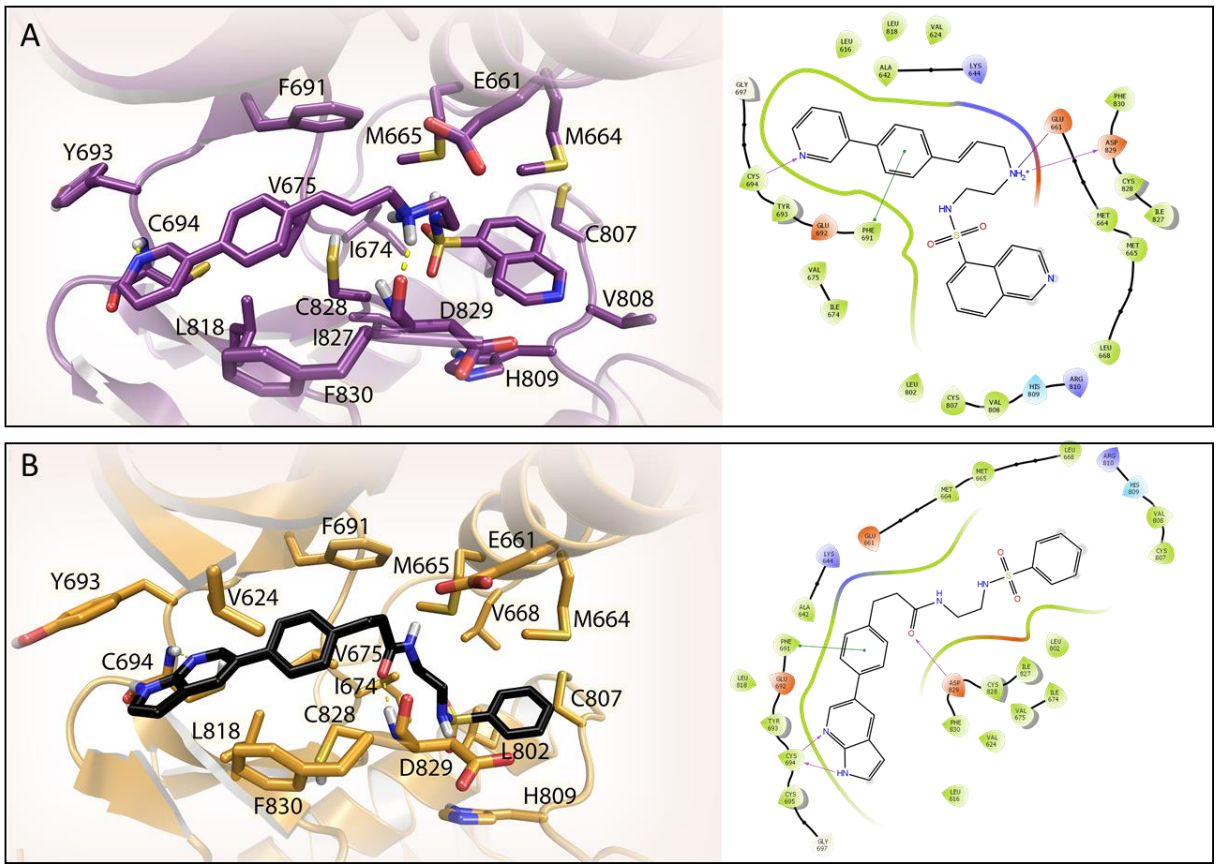


Figure 3: Proposed "flipped" binding mode of **1** and **38** in FLT3. (A-left) **1** and (B-left) **38** docked in FLT3 crystal structure (PDB: 4RT7) On the right a 2D-interaction diagram is shown depicting the interactions between the ligand and FLT3.

In summary, azaindole **38** was identified as a new, highly potent inhibitor of FLT3-ITD with favorable physico-chemical properties. Our structure-activity relationships and modeling studies suggest that **38** has an alternative flipped binding mode compared to other kinase inhibitors derived from the prototypical kinase inhibitor H-89. **38** forms an excellent starting point for further lead optimization studies to obtain clinical candidates to modulate FLT3-ITD in AML patients.

Experimental

Biochemical Evaluation of FLT3 inhibitors

In a 384-wells plate (PerkinElmer 384 Flat White), 5 µL kinase/peptide mix (0.06 ng/µL FLT3 (Life Technologies; PV3182; Lot: 1614759F), 200 nM peptide (PerkinElmer; Lance® Ultra ULightTM TK-peptide; TRFO127-M; Lot: 2178856)) in assay buffer (50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 0.01% Tween-20, 2 mM DTT) was dispensed. Separately inhibitor solutions (10 µM – 0.1 pM) were prepared in assay buffer containing 400 µM ATP and 1% DMSO. 5 µL of these solutions were dispensed and the plate was incubated in the dark at room temperature. After 90 minutes the reaction was quenched by the addition of 10 µL of 20 mM EDTA containing 4 nM antibody (PerkinElmer; Lance® Eu-W1024-anti-phosphotyrosine(PT66); AD0068; Lot: 2342358). After mixing, samples were incubated for 60 minutes in the dark. The FRET fluorescence was measured on a Tecan Infinite M1000 Pro plate reader (excitation 320 nm, emission donor 615 nm, emission acceptor 665 nm). Data was processed using Microsoft Excel 2016, pIC_{50} values were fitted using GraphPad Prism 7.0. Final assay concentrations during reaction: 200 µM ATP, 0.03 ng/µL FLT3, 100 nM Lance TK-peptide, 0.5% DMSO. Compounds were tested in n=2 and N=2.

Structure based modeling on FLT3

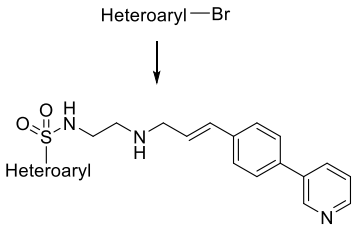
All structure based modeling was performed in the Schrödinger suite (Schrödinger Release 2017-4: Maestro, Schrödinger, LLC, New York, NY, 2017). Crystal structures were prepared using the protein preparation wizard,[37] ligands were prepared using LigPrep.[38] Both the DFG-out structure co-crystalized with quizartinib (4RT7)[39] and a DFG-in model were used in order to dock our initial compound **1**. The DFG-in model was constructed on the basis of 4RT7 and 3LCD, in a similar fashion as has been done before,[40] using the knowledge based potential in prime.[41, 42] Docking was done using induced fit docking and using H-bond constraints on C694.[43] In order to determine to correct binding pose, induced fit docking was followed by the conformer cluster script, using the Kelley criterion[44] to determine the optimal number of clusters. The highest scoring poses of every cluster were used in a previously published workflow to determine binding poses[45], which is based on metadynamics. The highest scoring pose was selected by adding the Metadynamics CompScore to the docking score. Based on this workflow the highest scoring pose was visualized and rendered using PyMol.[46]

Synthetic Procedures

Solvents were purchased from Biosolve, Sigma Aldrich or Fluka and, if necessary dried over 3Å or 4Å molecular sieves. Reagents purchased from chemical suppliers were used without further purification, unless stated otherwise. Oxygen or H_2O sensitive reactions were performed under argon or nitrogen atmosphere and/or under exclusion of H_2O. Reactions were followed by thin layer chromatography which was performed using TLC silica gel 60 F_{245} on aluminium sheets, supplied by Merck. Compounds were visualized by UV absorption (254 nm) or spray reagent (permanganate (5 g/L $KMnO_4$, 25 g/L K_2CO_3)). TLCMS was measured with a thin layer chromatography-mass spectrometer (Advion, Eppression LCMS; Advion, Plate Express). ^{1}H- and ^{13}C-NMR spectra were performed on one of the following Bruker spectrometers: DPX 300 NMR spectrometer (300 MHz), equipped with 5mm-BBO-z-gradient-probe; AV-400 NMR spectrometer (400 MHz), equipped with 5mm-BBO-z-gradient-probe; AV-500 NMR spectrometer (500 MHz), equipped with BBFO-z-gradient-probe; AV-600 NMR spectrometer (600 MHz), equipped with 5mm-Cryo-z-gradient probe. NMR spectra were

measured in deuterated methanol, chloroform or DMSO and were referenced to the residual protonated solvent signals as internal standards (chloroform-*d* = 7.260 (^{1}H), 77.160 (^{13}C); methanol-d_4 = 3.310 (^{1}H), 49.000 (^{13}C); DMSO-d_6 = 2.500 (^{1}H), 39.520 (^{13}C)). Signals multiplicities are written as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), p (pentet) or m (multiplet). Coupling constants (*J*) are given in Hz. Preparative HPLC (Waters, 515 HPLC pump M; Waters, 515 HPLC pump L; Waters, 2767 sample manager; Waters SFO System Fluidics Organizer; Waters Acquity Ultra Performance LC, SQ Detector; Waters Binary Gradient Module) was performed on a Phenomenex Gemini column (5 µM C18, 150 x 4.6 mm) or a Waters XBridgeTM column (5 µM C18, 150 x 19 mm). Diode detection was done between 210 and 600 nm. Gradient: ACN in (H_2O + 0.2% TFA). HRMS (Thermo, Finnigan LTQ Orbitrap; Thermo, Finnigan LTQ Pump; Thermo, Finnigan Surveyor MS Pump PLUS Thermo, Finnigan Surveyor Autosampler; NESLAB, Merlin M25). Data acquired through direct injection of 1 mM of the sample in ACN/H_2O/*t*-BuOH (1:1:1), with mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas low 10, capillary temperature 275°C) with resolution R = 60.000 at m/z = 400 (mass range = 150-2000) and dioctylphtalate (m/z = 391.28428) as lock mass. All tested compounds were checked for purity by HPLC, either on a Thermo (Thermo Finnigan LCQ Advantage Max; Thermo Finnigan Surveyor LC-pump Plus; Thermo Finnigan Surveyor Autosampler Plus; Thermo Finnigan Surveyor PDA Plus Detector; Phenomenex Gemini column (5 µm C18, 50 x 4.6 mm)) or a Waters (Waters 515 HPLC pump M; Waters 515 HPLC pump L; Waters 2767 sample manager; Waters SFO System Fluidics Organizer; Waters Acquity Ultra Performance LC, SQ Detector; Waters binary gradient module; Phenomenex Gemini column (5 µm C18, 150 x 4.6 mm)) system and were determined to be >95% pure by integrating UV intensity recorded.

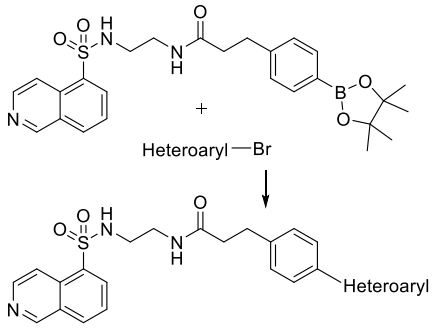
General procedure A: Sulfonamide coupling



<u>Step 1:</u> A glass vial was charged with corresponding bromo-heteroaryl compound (0.20 mmol, 1 eq), potassium metabisulfite (88 mg, 0.40 mmol, 2 eq), tetrabutylammonium bromide (70 mg, 0.22 mmol, 1.1 eq), sodium formate (15 mg, 0.22 mmol, 1.1 eq), palladium(II) acetate (5 mg, 0.02 mmol, 0.1 eq), triphenylphosphine (16 mg, 0.06 mmol, 0.3 eq), 1,10-phenanthroline (11 mg, 0.06 mmol, 0.3 eq). After sealing, the vial was flushed with argon for 30 min and the reagents were suspended in dry, degassed DMSO (1 mL) and the reaction mixture was stirred for 4 h at 70°C. After cooling to RT *N*,*N*-Diisopropylethylamine (70 µL, 0.40 mmol, 2 eq) and a solution of *tert*-butyl (*E*)-(2-aminoethyl)(3-(4-(pyridin-3-yl)phenyl)allyl)carbamate (**63**) (106 mg, 0.30 mmol, 1.5 eq) in dry THF (1 mL) were added and the reaction mixture was cooled to 0°C. Subsequently a solution of *N*-bromosuccinimide (62 mg, 0.40 mmol, 2 eq) in dry THF (1 mL) was added and the reaction mixture was allowed to come to RT. After stirring for 1 h the reaction was quenched by adding H_2O (1 mL) and brine (2 mL). The resulting mixture was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 0% → 5% MeOH in DCM) to yield the desired Boc-protected product, which was used directly in step 2.

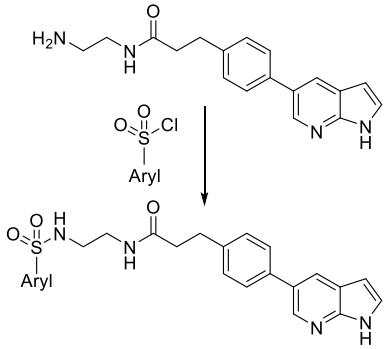
Step 2: The Boc-protected product was dissolved in chloroform (1.6 mL) and cooled to 0°C. After drop-wise addition of TFA (0.4 mL), the reaction mixture was allowed to come to RT and stirred for 1 h. Chloroform (10 mL) was added to the reaction mixture and subsequently concentrated in vacuum. After co-evaporating with chloroform (1x10 mL), the residue was purified by reverse phase HPLC.

General procedure B: Suzuki Coupling



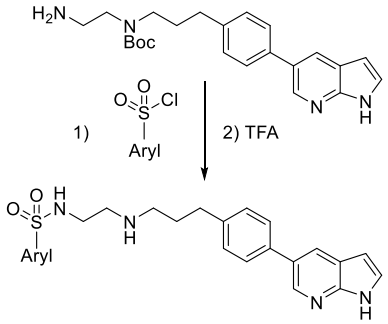
A glass vial was charged with the corresponding bromo-heteroaryl compound (0.15 mmol, 1.5 eq), *N*-(2-(isoquinoline-5-sulfonamido)ethyl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanamide (**107**) (51 mg, 0.10 mmol, 1 eq) and $Pd(PPh_3)_4$ (6 mg, 0.005 mmol, 0.05 eq). The vial was put under an argon atmosphere and degassed DMF (0.35 mL) and 2 M degassed aqueous K_2CO_3 (0.125 mL, 0.25 mmol, 2.5 eq) were added. The reaction mixture was stirred at 85°C overnight, diluted with DCM (10 mL) and half-saturated aq. $NaHCO_3$ solution (10 mL), extracted with DCM (3x10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by reverse phase HPLC.

General procedure C: Sulfonamide formation



3-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(2-aminoethyl)propanamide (**113**) (50 mg, 0.16 mmol, 1.0 eq) and Et_3N (45 µL,0.32 mmol, 2.0 eq) were dissolved in DMF (1.6 mL). The reaction mixture was cooled to 0°C and corresponding sulfonylchloride (194.6 µmol, 1.2 eq) dissolved in DCM (1.6 mL) or DMF (1.6 mL) was added. After 15 min the mixture was warmed up to RT and stirred for 5-16 h. The mixture was quenched with saturated aqueous $NaHCO_3$ (50 mL), the phases were separated and the aqueous layer was extracted with DCM or with a mixture of 10% MeOH in $CHCl_3$ (3x40 mL). The combined organic layers were washed with brine (1x100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified via flash column chromatography and preparative HPLC.

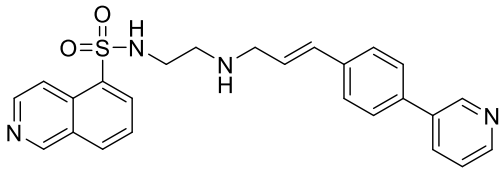
General Procedure D: Sulfonamide formation and debocylation



Step 1: *tert*-Butyl (3-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)propyl)(2-aminoethyl) carbamate (**117**) (90 mg, 228.3 µmol, 1.0 eq) and Et_3N (63 µL, 456.3 µmol, 2.0 eq) were dissolved in DCM (1 mL). The mixture was cooled to 0°C, corresponding sulfonylchloride (0.27 mmol, 1.2 eq) dissolved in DCM (1 mL) was added and the mixture was allowed to warm up and stirred at 30°C until full conversion was confirmed by TLC (4 – 40 h). The mixture was quenched with saturated aqueous $NaHCO_3$ (50 mL), the phases were separated and the aqueous layer was extracted with DCM (3x70 mL). The combined organic layers were washed with brine (1x120 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by flash-column-chromatography (SiO_2, dry-loading, 5% → 7% (10% of sat. aqueous NH_3 in MeOH) in DCM) and used in step 2.

Step 2: The product from step 1 was dissolved in DCM (1 mL) and subsequently cooled to 0°C. TFA (250 µL) was added dropwise to the solution and warmed to RT and stirred for 19 h. The mixture was diluted with 15 mL $CHCl_3$ and concentrated under reduced pressure. The resulting crude was purified by flash-column-chromatography and preparative HPLC to yield the desired compound after lyophilisation.

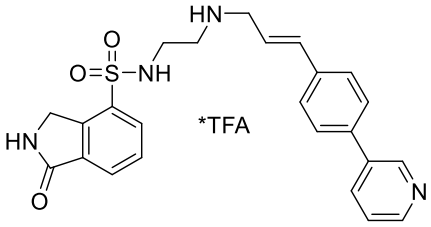
(*E*)-*N*-(2-((3-(4-(Pyridin-3-yl)phenyl)allyl)amino)ethyl)isoquinoline-5-sulfonamide (1)



A round-bottom-flask was charged with *tert*-butyl (*E*)-(2-(isoquinoline-5-sulfonamido)ethyl)(3-(4-(pyridin-3-yl)phenyl) allyl)carbamate (**64**) (610 mg, 1.12 mmol, 1 eq) dissolved in $CHCl_3$ (50 mL). After cooling the solution to 0°C and dropwise addition of TFA (12.5 mL), it was allowed to warm to RT and stirred for 30 min. The reaction was quenched by slow addition of sat. aqueous Na_2CO_3 solution (70 mL) until a pH of ~12 was reached and the mixture was extracted with DCM (3x50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 0% → 15% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the desired product (329 mg, 66%). ^{1}H NMR (400 MHz, methanol-d_4) δ 9.32 (d, *J* = 0.7 Hz, 1H), 8.80 (dd, *J* = 2.3, 0.7 Hz, 1H), 8.61 (d, *J* = 6.2 Hz, 1H), 8.55 (d, *J* = 6.2 Hz, 1H), 8.50 (dd, *J* = 4.9, 1.5 Hz, 1H), 8.47 (dd, *J* = 7.4, 1.2 Hz, 1H), 8.33 (d, *J* = 8.3 Hz, 1H), 8.11 – 8.06 (m, 1H), 7.81 – 7.74 (m, 1H), 7.62 (d, *J* = 8.3 Hz, 2H), 7.53 – 7.48 (m, 1H), 7.46 (d, *J* = 8.3 Hz, 2H), 6.44 (d, *J* = 15.9 Hz, 1H), 6.17 (dt, *J* = 15.9, 6.5 Hz, 1H), 3.21 (dd, *J* = 6.5, 1.1 Hz, 2H), 3.03 (t, *J* = 6.4 Hz, 2H), 2.60 (t, *J* = 6.4 Hz, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 154.33, 148.68, 148.12, 144.87, 138.41, 138.10, 137.49, 136.36, 136.24, 134.88, 134.75, 132.65, 132.60, 130.62, 128.72, 128.25, 128.21, 127.72, 125.49, 119.15, 68.12, 51.73, 43.06. HRMS calculated for $C_{25}H_{25}N_4O_2S$ 445.16927 $[M+H]^+$, found

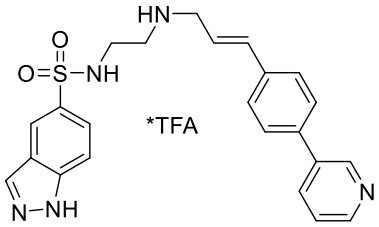
445.16891. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 5.17 min; *m/z* : 445 $[M+H]^+$.

(*E*)-1-Oxo-*N*-(2-((3-(4-(pyridin-3-yl)phenyl)allyl)amino)ethyl)isoindoline-4-sulfonamide (5)



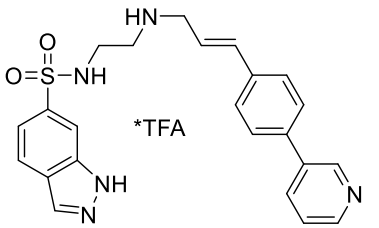
The title compound was synthesized from 4-bromoisoindolin-1-one following general procedure A on a 0.2 mmol scale and purified by preparative HPLC (XBridge, C_{18}, 0% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (27 mg, 24%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.98 (s, 1H), 8.66 (d, *J* = 4.8 Hz, 1H), 8.45 (dt, *J* = 8.1, 1.6 Hz, 1H), 8.07 (dd, *J* = 17.2, 7.6 Hz, 2H), 7.82 – 7.72 (m, 4H), 7.66 (d, *J* = 8.3 Hz, 2H), 6.95 (d, *J* = 15.9 Hz, 1H), 6.41 (dt, *J* = 15.7, 7.2 Hz, 1H), 4.76 (s, 2H), 3.90 (d, *J* = 7.1 Hz, 2H), 3.23 (s, 4H). ^{13}C NMR (151 MHz, methanol-d_4) δ 170.09, 144.52, 143.92, 141.94, 138.51, 137.84, 137.75, 136.29, 136.01, 134.98, 134.39, 130.70, 129.02, 127.63, 127.55, 127.28, 125.41, 119.05, 49.03, 46.21, 45.73, 38.79. HRMS calculated for $C_{24}H_{25}N_4O_3S$ 449.16419 $[M+H]^+$, found 449.16397. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 5.59 min; *m/z* : 449 $[M+H]^+$.

(*E*)-*N*-(2-((3-(4-(Pyridin-3-yl)phenyl)allyl)amino)ethyl)-1*H*-indazole-5-sulfonamide (6)



The title compound was synthesized from 5-bromo-1*H*-indazole following general procedure A on a 0.2 mmol scale and purified by preparative HPLC (XBridge, C_{18}, 0% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (11 mg, 10%). ^{1}H NMR (600 MHz, methanol-d_4) δ 9.03 (s, 1H), 8.69 (d, *J* = 4.7 Hz, 1H), 8.54 (d, *J* = 8.1 Hz, 1H), 8.43 – 8.42 (m, 1H), 8.25 (s, 1H), 7.88 – 7.84 (m, 2H), 7.79 (d, *J* = 8.3 Hz, 2H), 7.74 (d, *J* = 8.9 Hz, 1H), 7.68 (d, *J* = 8.3 Hz, 2H), 6.96 (d, *J* = 15.9 Hz, 1H), 6.42 (dt, *J* = 15.8, 7.2 Hz, 1H), 3.90 (d, *J* = 7.1 Hz, 2H), 3.22 (t, *J* = 5.5 Hz, 2H), 3.17 (t, *J* = 5.5 Hz, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 145.10, 144.55, 142.82, 140.89, 139.64, 139.03, 137.93, 137.01, 136.53, 132.92, 129.00, 128.74, 127.18, 125.40, 123.58, 123.43, 120.67, 112.49, 50.36, 47.65, 40.33. HRMS calculated for $C_{23}H_{24}N_5O_2S$ 434.16452 $[M+H]^+$, found 434.16414. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 5.80 min; *m/z* : 434 $[M+H]^+$.

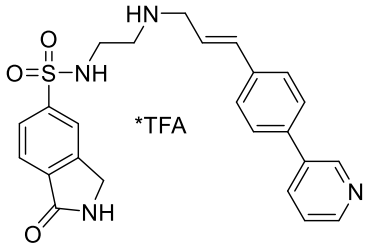
(*E*)-*N*-(2-((3-(4-(Pyridin-3-yl)phenyl)allyl)amino)ethyl)-1*H*-indazole-6-sulfonamide (7)



The title compound was synthesized from 6-bromo-1*H*-indazole following general procedure A on a 0.2 mmol scale and purified by preparative HPLC (XBridge C_{18}, 10% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (27 mg, 25%). ^{1}H NMR (600 MHz, methanol-d_4) δ 9.02 (d, *J* = 1.9 Hz, 1H), 8.71 – 8.68 (m, 1H), 8.54 (dt, *J* = 8.1, 1.6 Hz, 1H), 8.20 (d, *J* = 0.9 Hz, 1H), 8.15 (s, 1H), 8.03 – 7.96 (m, 1H), 7.86 (dd, *J* = 8.1, 5.4 Hz, 1H), 7.79 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.3 Hz, 2H), 7.62 (dd, *J* = 8.5, 1.5 Hz, 1H), 6.96 (d, *J* = 15.9 Hz, 1H), 6.42 (dt, *J* = 15.8, 7.2 Hz, 1H), 3.90 (d, *J* = 7.2 Hz, 2H), 3.22 (d, *J* = 4.9 Hz, 2H), 3.19 (d, *J* = 4.7 Hz, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 143.67, 143.12, 139.51, 139.06, 138.23, 137.65, 137.34, 136.51, 135.59, 133.75, 127.59, 127.33, 125.77, 125.07, 122.03, 119.22, 117.78, 110.40, 48.97, 46.24, 38.96. HRMS calculated for $C_{23}H_{24}N_5O_2S$

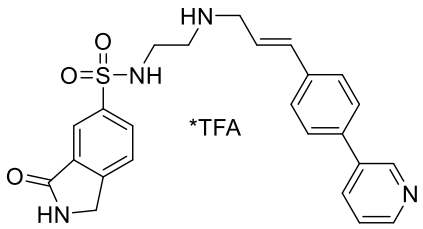
434.16452 $[M+H]^+$, found 434.16410. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 6.02 min; *m/z* : 434 $[M+H]^+$.

(*E*)-1-Oxo-*N*-(2-((3-(4-(pyridin-3-yl)phenyl)allyl)amino)ethyl)isoindoline-5-sulfonamide (8)



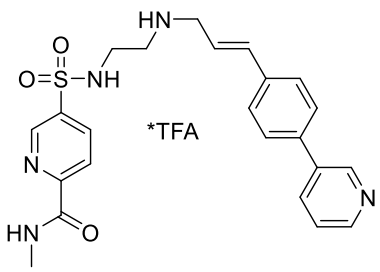
The title compound was synthesized from 5-bromoisoindolin-1-one following general procedure A on a 0.2 mmol scale and purified by preparative HPLC (Gemini C_{18}, 0% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (9 mg, 8%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.99 (s, 1H), 8.67 (d, *J* = 5.0 Hz, 1H), 8.48 (d, *J* = 7.7 Hz, 1H), 8.13 (s, 1H), 8.03 (d, *J* = 8.5 Hz, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.84 – 7.80 (m, 1H), 7.78 (d, *J* = 8.2 Hz, 2H), 7.68 (d, *J* = 8.3 Hz, 2H), 6.97 (d, *J* = 15.9 Hz, 1H), 6.45 – 6.37 (m, 1H), 4.56 (s, 2H), 3.91 (d, *J* = 7.1 Hz, 2H), 3.22 (s, 4H). ^{13}C NMR (126 MHz, methanol-d_4) δ 170.33, 145.49, 145.25, 144.82, 142.88, 137.93, 137.60, 137.53, 136.56, 136.15, 136.06, 127.59, 127.33, 126.64, 125.14, 124.05, 122.52, 118.94, 49.10, 46.30, 45.60, 39.02. HRMS calculated for $C_{24}H_{25}N_4O_3S$ 449.16419 $[M+H]^+$, found 449.16390. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 5.35 min; *m/z* : 449 $[M+H]^+$.

(*E*)-3-Oxo-*N*-(2-((3-(4-(pyridin-3-yl)phenyl)allyl)amino)ethyl)isoindoline-5-sulfonamide (9)



The title compound was synthesized from 6-bromoisoindolin-1-one following general procedure A on a 0.2 mmol scale and purified by preparative HPLC (XBridge, C_{18}, 0% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (16 mg, 14%). ^{1}H NMR (600 MHz, methanol-d_4) δ 9.04 (s, 1H), 8.70 (d, *J* = 5.0 Hz, 1H), 8.57 (d, *J* = 8.2 Hz, 1H), 8.27 (s, 1H), 8.13 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.88 (dd, *J* = 8.1, 5.4 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.79 (d, *J* = 8.3 Hz, 2H), 7.69 (d, *J* = 8.3 Hz, 2H), 6.97 (d, *J* = 15.9 Hz, 1H), 6.47 – 6.37 (m, 1H), 4.57 (s, 2H), 3.91 (d, *J* = 7.2 Hz, 2H), 3.26 – 3.22 (m, 2H), 3.22 – 3.17 (m, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 171.74, 150.24, 144.77, 144.25, 141.27, 141.26, 139.78, 139.04, 138.01, 136.84, 134.58, 131.36, 129.02, 128.76, 127.30, 126.15, 123.29, 120.70, 50.40, 47.67, 47.05, 40.34. HRMS calculated for $C_{24}H_{25}N_4O_3S$ 449.16419 $[M+H]^+$, found 449.16386. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 5.39 min; *m/z* : 449 $[M+H]^+$.

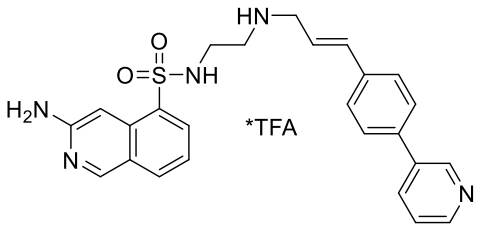
(*E*)-*N*-Methyl-5-(*N*-(2-((3-(4-(pyridin-3-yl)phenyl)allyl)amino)ethyl)sulfamoyl) picolinamide (10)



The title compound was synthesized from 5-bromo-*N*-methylpicolinamide following general procedure A on a 0.2 mmol scale and purified by preparative HPLC (XBridge C_{18}, 0% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (17 mg, 15%). ^{1}H NMR (600 MHz, methanol-d_4) δ 9.07 (d, *J* = 2.0 Hz, 1H), 9.03 (s, 1H), 8.70 (d, *J* = 5.2 Hz, 1H), 8.57 (d, *J* = 8.2 Hz, 1H), 8.40 (dd, *J* = 8.2, 2.2 Hz, 1H), 8.27 (d, *J* = 8.2 Hz, 1H), 7.88 (dd, *J* = 8.0, 5.4 Hz, 1H), 7.79 (d, *J* = 8.3 Hz, 2H), 7.68 (d, *J* = 8.3 Hz, 2H), 6.97 (d, *J* = 15.9 Hz, 1H), 6.42 (dt, *J* = 15.7, 7.2 Hz, 1H), 3.91 (d, *J* = 7.1 Hz, 2H), 3.29 – 3.23 (m, 4H), 2.98 (s, 3H). ^{13}C NMR (151 MHz,

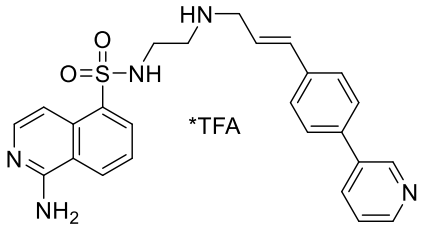
methanol-d_4) δ 165.72, 154.28, 148.04, 144.92, 144.39, 141.12, 139.78, 139.70, 139.10, 137.95, 137.76, 136.94, 129.01, 128.76, 127.25, 123.41, 120.64, 50.43, 47.67, 40.30, 26.53. HRMS calculated for $C_{23}H_{26}N_5O_3S$ 452.17509 $[M+H]^+$, found 452.17469. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 5.62 min; *m/z* : 452 $[M+H]^+$.

(*E*)-3-Amino-*N*-(2-((3-(4-(pyridin-3-yl)phenyl)allyl)amino)ethyl)isoquinoline-5-sulfonamide (11)



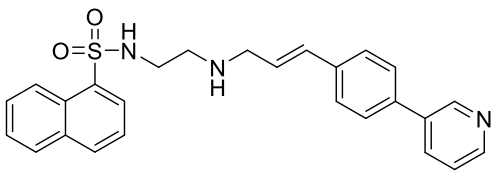
The title compound was synthesized from 5-bromoisoquinolin-3-amine following general procedure A on a 0.2 mmol scale and purified by preparative HPLC (XBridge C_{18}, 10% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (19 mg, 17%). ^{1}H NMR (400 MHz, methanol-d_4) δ 9.06 (s, 1H), 8.98 (s, 1H), 8.72 (d, *J* = 5.2 Hz, 1H), 8.61 (d, *J* = 8.2 Hz, 1H), 8.29 (d, *J* = 7.3 Hz, 1H), 8.13 (d, *J* = 8.2 Hz, 1H), 7.92 (dd, *J* = 8.0, 5.5 Hz, 1H), 7.80 (d, *J* = 8.3 Hz, 2H), 7.68 (d, *J* = 8.3 Hz, 2H), 7.60 (s, 1H), 7.36 (t, *J* = 7.8 Hz, 1H), 6.94 (d, *J* = 15.9 Hz, 1H), 6.46 – 6.36 (m, 1H), 3.89 (d, *J* = 7.2 Hz, 2H), 3.19 (m, *J* = 8.6, 4.4 Hz, 4H). ^{13}C NMR (101 MHz, methanol-d_4) δ 156.42, 150.93, 144.36, 143.86, 141.72, 139.95, 138.95, 138.10, 136.93, 136.62, 136.47, 136.13, 132.35, 129.04, 128.77, 127.47, 124.30, 122.66, 120.79, 99.56, 50.39, 47.72, 40.16. HRMS calculated for $C_{25}H_{26}N_5O_2S$ 460.18017 $[M+H]^+$, found 460.17998. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 5.51 min; *m/z* : 460 $[M+H]^+$.

(*E*)-1-Amino-*N*-(2-((3-(4-(pyridin-3-yl)phenyl)allyl)amino)ethyl)isoquinoline-5-sulfonamide (12)



The title compound was synthesized from 5-bromoisoquinolin-1-amine following general procedure A on a 0.2 mmol scale and purified by preparative HPLC (XBridge C_{18}, 0% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (32 mg, 28%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.96 (s, 1H), 8.71 (d, *J* = 8.4 Hz, 1H), 8.64 (d, *J* = 4.3 Hz, 1H), 8.60 (d, *J* = 8.7 Hz, 1H), 8.41 (dt, *J* = 8.1, 1.8 Hz, 1H), 7.94 – 7.88 (m, 2H), 7.79 – 7.73 (m, 4H), 7.66 (d, *J* = 8.3 Hz, 2H), 6.95 (d, *J* = 15.9 Hz, 1H), 6.41 (dt, *J* = 15.8, 7.2 Hz, 1H), 3.90 (d, *J* = 7.1 Hz, 2H), 3.22 (s, 4H). ^{13}C NMR (151 MHz, methanol-d_4) δ 156.25, 146.32, 145.70, 139.44, 139.12, 139.05, 137.61, 137.58, 137.46, 137.05, 135.36, 131.46, 130.51, 128.98, 128.93, 128.65, 126.65, 121.02, 120.43, 109.12, 50.47, 47.69, 40.15. HRMS calculated for $C_{25}H_{26}N_5O_2S$ 460.18017 $[M+H]^+$, found 460.18005. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 5.45 min; *m/z* : 460 $[M+H]^+$.

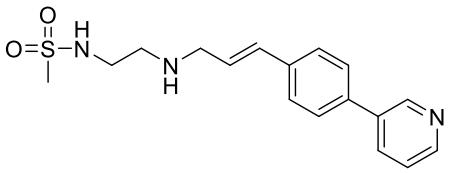
(*E*)-*N*-(2-((3-(4-(Pyridin-3-yl)phenyl)allyl)amino)ethyl)naphthalene-1-sulfonamide (13)



To a solution of *tert*-butyl (*E*)-(2-(naphthalene-1-sulfonamido)ethyl)(3-(4-(pyridin-3-yl)phenyl) allyl) carbamate (**65**) (0.270 g, 0.50 mmol, 1 eq) in DCM (5 mL) at 0 °C was added TFA (1 mL). The reaction was allowed to warm to RT and stirred for 1 h before it was concentrated under reduced pressure and re-dissolved in DCM (20 mL) and sat. aqueous Na_2CO_3 solution (20 mL). The organic layer was

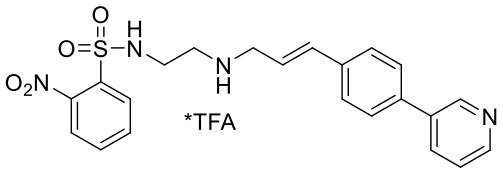
collected and the aqueous layer extracted with DCM (4x20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2 (neutralized with 1% Et_3N in DCM), 1.25% → 1.5% MeOH in DCM) to yield the product (0.18 g, 81%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.85 (d, *J* = 1.9 Hz, 1H), 8.70 (d, *J* = 8.6 Hz, 1H), 8.58 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.29 (dd, *J* = 7.3, 1.1 Hz, 1H), 8.05 (d, *J* = 8.2 Hz, 1H), 7.93 (d, *J* = 7.9 Hz, 1H), 7.89 – 7.84 (m, 1H), 7.67 – 7.61 (m, 1H), 7.59 – 7.54 (m, 1H), 7.53 – 7.49 (m, 3H), 7.39 – 7.33 (m, 3H), 6.35 (d, *J* = 15.9 Hz, 1H), 6.06 (dt, *J* = 15.9, 6.3 Hz, 1H), 3.17 (bs, 2H), 3.09 (dd, *J* = 6.3, 1.1 Hz, 2H), 3.03 – 2.98 (m, 2H), 2.66 – 2.61 (m, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 148.45, 148.07, 136.81, 136.69, 136.20, 134.52, 134.30, 134.29, 134.22, 130.69, 129.82, 129.20, 128.50, 128.42, 128.18, 127.26, 127.01, 126.95, 124.45, 124.26, 123.71, 50.87, 47.34, 42.51. HRMS calculated for $C_{26}H_{26}N_3O_2S$ 444.17402 $[M+H]^+$, found 444.17370. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 90% ACN in H_2O 0.2% TFA, 10 min): t_R = 5.32 min; *m/z* : 444 $[M+H]^+$.

(*E*)-*N*-(2-((3-(4-(Pyridin-3-yl)phenyl)allyl)amino)ethyl)methanesulfonamide (14)



A round-bottom-flask was charged with *tert*-butyl (*E*)-(2-(isoquinoline-5-sulfonamido)ethyl)(3-(4-(pyridin-3-yl)phenyl) allyl)carbamate (**66**) (107 mg, 0.25 mmol, 1 eq) dissolved in $CHCl_3$ (8 mL). After cooling the solution to 0°C and dropwise addition of TFA (2 mL), it was allowed to warm to RT and stirred for 60 min. The reaction was quenched by slow addition of sat. aqueous Na_2CO_3 solution (12 mL) until a pH of ~12 was reached and the mixture was extracted with DCM (3x10 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 0% → 15% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (52 mg, 63%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.80 (d, *J* = 2.3 Hz, 1H), 8.50 (dd, *J* = 4.9, 1.4 Hz, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.51 (dd, *J* = 8.0, 4.9 Hz, 1H), 6.65 (d, *J* = 15.9 Hz, 1H), 6.40 (dt, *J* = 15.9, 6.5 Hz, 1H), 3.46 – 3.42 (m, 2H), 3.23 (t, *J* = 6.3 Hz, 2H), 2.96 (s, 3H), 2.80 (t, *J* = 6.3 Hz, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 148.66, 148.11, 138.58, 138.15, 137.49, 136.26, 132.78, 129.03, 128.26, 128.23, 125.49, 51.91, 49.46, 43.26, 39.68. HRMS calculated for $C_{17}H_{22}N_3O_2S$ 332.14272 $[M+H]^+$, found 332.14267. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 4.49 min; *m/z* : 332 $[M+H]^+$.

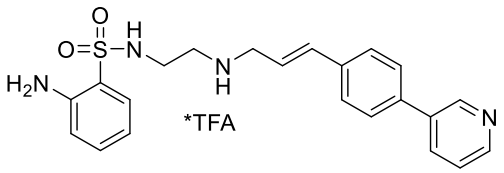
(*E*)-2-Nitro-*N*-(2-((3-(4-(pyridin-3-yl)phenyl)allyl)amino)ethyl)benzenesulfonamide (15)



To a solution of *tert*-butyl (*E*)-(2-((2-nitrophenyl)sulfonamido)ethyl)(3-(4-(pyridin-3-yl)phenyl)allyl)carbamate (**67**) (0.347 g, 0.64 mmol, 1 eq) dissolved in $CHCl_3$ (4.8 mL) at 0 °C was added dropwise TFA (1.2 mL). The reaction was allowed to warm to RT and stirred for 2 h before it was concentrated under reduced pressure. It was re-dissolved in DCM (20 mL) and sat. aqueous Na_2CO_3 solution (20 mL). The organic layer was collected and the aqueous layer extracted with DCM (3x20 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated under reduced pressure and purified by preparative HPLC (Gemini, C_{18}, 10% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (11 mg, 3%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.99 (s, 1H), 8.86 (bs, 2H), 8.63 (dd, *J* = 3.5, 1.3 Hz, 1H), 8.36 (d, *J* = 5.4 Hz, 1H), 8.23 (d, *J* = 6.9 Hz, 1H), 8.03 (dt, *J* = 6.1, 3.2 Hz, 2H), 7.91 (dd, *J* = 5.9,

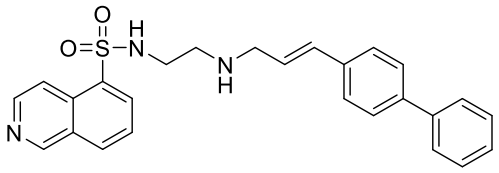
3.3 Hz, 2H), 7.81 (d, *J* = 8.2 Hz, 2H), 7.61 (d, *J* = 8.3 Hz, 3H), 6.87 (d, *J* = 15.9 Hz, 1H), 6.40 – 6.30 (m, 1H), 3.81 (d, *J* = 5.1 Hz, 2H), 3.23 (d, *J* = 6.1 Hz, 2H), 3.10 (s, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 148.40, 147.98, 136.77, 136.61, 136.06, 134.11, 133.57, 133.39, 132.69, 131.07, 130.73, 128.68, 127.20, 126.98, 125.25, 123.65, 51.03, 47.46, 43.16. HRMS calculated for $C_{22}H_{23}N_4O_4S$ 439.14345 $[M+H]^+$, found 439.14302. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 6.71min; *m/z* : 439 $[M+H]^+$.

(*E*)-2-Amino-*N*-(2-((3-(4-(pyridin-3-yl)phenyl)allyl)amino)ethyl) benzenesulfonamide (16)



(*E*)-2-Nitro-*N*-(2-((3-(4-(pyridin-3-yl)phenyl) allyl) amino) ethyl)benzenesulfonamide (**15**) (84 mg, 0.19 mmol, 1 eq) was dissolved in EtOH (0.32 mL), AcOH (0.32 mL) and H_2O (0.16 mL) after which iron powder (30 mg) was added and the vial was sonicated for 2.5 h. The mixture was basified with aqueous NaOH (1 M, 5.5 mL) solution, concentrated under reduced pressure, re-suspended in DCM (5 mL) and sat. aqueous Na_2CO_3 (5 mL) and filtered over filter paper. The filter was rinsed with sat. aqueous Na_2CO_3 (50 mL) and the filtrate was extracted with DCM (5x40 mL). The combined organic layers were dried over $MgSO_4$, concentrated under reduced pressure and purified by preparative HPLC (XBridge C_{18}, 10% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (48 mg, 48%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.00 (d, *J* = 1.8 Hz, 1H), 8.78 (bs, 2H), 8.65 (dd, *J* = 4.9, 1.5 Hz, 1H), 8.26 (d, *J* = 8.1 Hz, 1H), 7.86 – 7.79 (m, 3H), 7.65 – 7.59 (m, 3H), 7.50 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.31 – 7.27 (m, 1H), 6.88 – 6.82 (m, 2H), 6.64 (t, *J* = 7.0 Hz, 1H), 6.34 (dt, *J* = 15.8, 6.9 Hz, 1H), 4.37 (bs, 2H), 3.83 – 3.72 (m, 2H), 3.08 – 2.96 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 147.26, 146.41, 146.19, 136.36, 136.18, 135.59, 135.56, 135.41, 133.90, 129.13, 127.40, 127.35, 124.52, 120.42, 118.72, 117.12, 115.31, 48.38, 45.42, 38.47. HRMS calculated for $C_{22}H_{25}N_4O_2S$ 409.16927 $[M+H]^+$, found 409.16884. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 90% ACN in H_2O 0.2% TFA, 10 min): t_R = 4.62 min; *m/z* : 409 $[M+H]^+$.

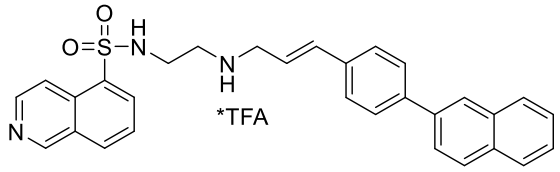
(*E*)-*N*-(2-((3-([1,1'-Biphenyl]-4-yl)allyl)amino)ethyl)isoquinoline-5-sulfonamide (17)



To a solution of *tert*-butyl (*E*)-(3-([1,1'-biphenyl]-4-yl)allyl)(2-(isoquinoline-5-sulfonamido) ethyl) carbamate (**71**) (0.387 g, 0.70 mmol, 1 eq) in DCM (3.1 mL) at 0 °C was added TFA (3.1 mL) after which the mixture was allowed to warm to RT. After stirring for 30 min it was concentrated under reduced pressure, re-dissolved in sat. aqueous $NaHCO_3$ (30 mL) and DCM (30 mL), the organic layer was collected and the aqueous layer extracted with DCM (3x30 mL). The combined organic layers were washed with brine (1x50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was purified via flash-column-chromatography (SiO_2, 3% → 4% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the desired product (0.150 g, 48%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.35 (d, *J* = 0.8 Hz, 1H), 8.71 (d, *J* = 6.1 Hz, 1H), 8.48 – 8.42 (m, 2H), 8.18 (d, *J* = 8.2 Hz, 1H), 7.72 – 7.64 (m, 1H), 7.63 – 7.58 (m, 2H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.40 – 7.32 (m, 3H), 6.40 (d, *J* = 15.9 Hz, 1H), 6.08 (dt, *J* = 15.9, 6.4 Hz, 1H), 3.31 (bs, 2H), 3.17 (dd, *J* = 6.4, 1.3 Hz, 2H), 3.01 (dd, *J* = 6.4, 4.8 Hz, 2H), 2.69 (dd, *J* = 6.4, 4.9 Hz, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 153.49, 145.39, 140.69, 140.44, 135.78, 134.27, 133.70, 133.49, 131.49, 131.36, 129.13, 128.92, 127.46, 127.40, 127.39, 127.02, 126.81, 126.02, 117.30, 51.01, 47.18, 42.41. HRMS calculated for $C_{26}H_{26}N_3O_2S$ 444.17402

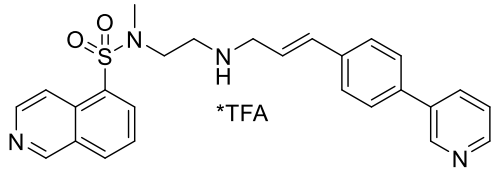
$[M+H]^+$, found 444.17354. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 90% ACN in H_2O 0.2% TFA, 10 min): t_R = 6.27 min; *m/z* : 444 $[M+H]^+$.

(*E*)-*N*-(2-((3-(4-(Naphthalen-2-yl)phenyl)allyl)amino)ethyl)isoquinoline-5-sulfonamide (18)



To a solution of *tert*-butyl (*E*)-(2-(isoquinoline-5-sulfonamido)ethyl)(3-(4-(naphthalene-2-yl)phenyl)allyl)carbamate (**72**) (0.339 g, 0.62 mmol, 1 eq) in DCM (3.1 mL) at 0 °C was added TFA (3.1 mL) after which the mixture was allowed to warm to RT. After stirring for 30 min it was concentrated under reduced pressure, re-dissolved in sat. aqueous $NaHCO_3$ (30 mL) and DCM (30 mL), the organic layer was collected and the aqueous layer extracted with DCM (3x30 mL). The combined organic layers were washed with brine (1x50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude was purified by flash-column-chromatography (SiO_2, 3% → 4% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (XBridge C_{18}, 25% → 50% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the desired compound as a TFA salt after lyophilisation (23 mg, 6%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.74 (d, *J* = 6.1 Hz, 3H), 8.48 (d, *J* = 8.2 Hz, 1H), 8.44 – 8.41 (m, 2H), 8.38 (dd, *J* = 7.4, 1.1 Hz, 1H), 8.28 – 8.25 (m, 1H), 8.02 (t, *J* = 8.6 Hz, 2H), 7.95 (d, *J* = 7.8 Hz, 1H), 7.91 – 7.84 (m, 4H), 7.60 (d, *J* = 8.3 Hz, 2H), 7.58 – 7.51 (m, 2H), 6.84 (d, *J* = 15.9 Hz, 1H), 6.30 (dt, *J* = 15.8, 7.0 Hz, 1H), 3.81 – 3.76 (m, 2H), 3.10 – 3.00 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.46, 144.67, 139.92, 136.67, 136.49, 134.63, 133.91, 133.72, 133.30, 132.88, 132.34, 130.31, 128.72, 128.55, 128.22, 127.51, 127.31, 127.30, 126.50, 126.27, 125.16, 124.82, 119.70, 117.04, 48.43, 45.42, 38.69. HRMS calculated for $C_{30}H_{28}N_3O_2S$ 494.18967 $[M+H]^+$, found 494.18922. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 90% ACN in H_2O 0.2% TFA, 10 min): t_R = 6.80 min; *m/z* : 494 $[M+H]^+$.

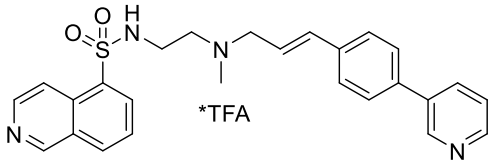
(*E*)-*N*-Methyl-*N*-(2-((3-(4-(pyridin-3-yl)phenyl)allyl)amino)ethyl)isoquinoline-5-sulfonamide (19)



A solution of *tert*-butyl (*E*)-(2-(*N*-methylisoquinoline-5-sulfonamido)ethyl)(3-(4-(pyridine-3-yl)phenyl)allyl)carbamate (**75**) (0.768 g, 1.4 mmol, 1 eq) in $CHCl_3$ (10.4 mL) and TFA (2.6 mL) was stirred for 1.5 h. The reaction mixture was concentrated under reduced pressure and re-dissolved in sat. aqueous Na_2CO_3 solution (20 mL) and DCM (20 mL) by stirring vigorously until both phases became clear. The organic layer was collected and the aqueous layer extracted with DCM (3x20 mL), after which the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 0% → 10% (10% of sat. aqueous NH_3 in MeOH) in DCM) and then further by preparative HPLC (XBridge C_{18}, 0% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (44 mg, 5%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.54 (s, 1H), 9.05 (s, 1H), 8.90 (bs, 2H), 8.72 (d, *J* = 6.2 Hz, 1H), 8.69 (s, 2H), 8.53 (d, *J* = 8.1 Hz, 1H), 8.47 (d, *J* = 6.1 Hz, 1H), 8.37 (d, *J* = 7.4 Hz, 1H), 7.91 (t, *J* = 7.8 Hz, 1H), 7.84 (d, *J* = 7.4 Hz, 2H), 7.71 (bs, 1H), 7.65 (d, *J* = 7.8 Hz, 2H), 6.88 (d, *J* = 15.9 Hz, 1H), 6.44 – 6.35 (m, 1H), 3.86 – 3.81 (m, 2H), 3.44 (t, *J* = 6.4 Hz, 2H), 3.26 – 3.18 (m, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.88, 146.69, 145.68, 145.07, 137.10, 136.54, 136.35, 136.23, 136.20, 134.81, 133.87, 132.43, 131.44, 129.27, 127.87, 127.84, 127.18, 125.35, 121.03, 117.60, 48.96, 46.15,

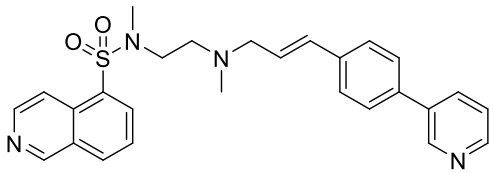
43.95, 35.48, 31.71. HRMS calculated for $C_{26}H_{27}N_4O_2S$ 459.18492 $[M+H]^+$, found 459.18464. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 90% ACN in H_2O 0.2% TFA, 10 min): t_R = 4.12 min; *m/z* : 459 $[M+H]^+$.

(*E*)-*N*-(2-(Methyl(3-(4-(pyridin-3-yl)phenyl)allyl)amino)ethyl)isoquinoline-5-sulfonamide (20)



(*E*)-*N*-(2-((3-(4-(pyridin-3-yl)phenyl)allyl) amino) ethyl) isoquinoline-5-sulfonamide (**1**) (158 mg, 0.35 mmol, 1 eq), formaldehyde in H_2O (36%, 30 µL, 0.39 mmol, 1.1 eq) and $NaHB(OAc)_3$ (188 mg, 0.89 mmol, 2.5 eq) were dissolved in THF (13 mL) and MeOH (2 mL) and after activated molecular sieves (3 Å) were added to the reaction, it was stirred under argon atmosphere for 16 h. The reaction was quenched with sat. aqueous NH_4Cl (2.5 mL), H_2O (7.5 mL), diluted with sat. aqueous Na_2CO_3 (25 mL) and Et_2O (30 mL) after which the organic phase was collected and the aqueous layer extracted with DCM (3x20 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated under reduced pressure and purified by preparative HPLC (Gemini C_{18}, 0% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (11 mg, 5%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.79 (bs, 1H), 9.52 (s, 1H), 9.04 (s, 1H), 8.74 (d, *J* = 6.1 Hz, 1H), 8.71 – 8.66 (m, 1H), 8.49 (t, *J* = 5.8 Hz, 2H), 8.42 (d, *J* = 6.1 Hz, 1H), 8.39 (dd, *J* = 7.4, 1.0 Hz, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 7.87 (t, *J* = 7.8 Hz, 1H), 7.83 (d, *J* = 8.3 Hz, 2H), 7.70 – 7.66 (m, 1H), 7.65 (s, *J* = 8.4 Hz, 2H), 6.89 (d, *J* = 15.8 Hz, 1H), 6.40 (dt, *J* = 15.6, 7.2 Hz, 1H), 3.94 (dd, *J* = 19.5, 6.4 Hz, 2H), 3.30 – 3.21 (m, 1H), 3.21 – 3.11 (m, 3H), 2.81 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.35, 146.67, 145.63, 144.42, 138.38, 136.35, 136.31, 135.67, 135.54, 133.97, 133.70, 133.02, 130.36, 128.71, 127.69, 127.35, 126.59, 124.79, 118.39, 117.13, 57.12, 53.29, 40.06, 37.38. HRMS calculated for $C_{26}H_{27}N_4O_2S$ 459.18592 $[M+H]^+$, found 459.18460. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 90% ACN in H_2O 0.2% TFA, 10 min): t_R = 5.23 min; *m/z* : 459 $[M+H]^+$.

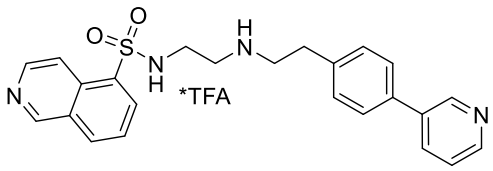
(*E*)-*N*-Methyl-*N*-(2-(methyl(3-(4-(pyridin-3-yl)phenyl)allyl)amino) ethyl)isoquinoline-5-sulfonamide (21)



To a solution of (*E*)-*N*-methyl-*N*-(2-((3-(4-(pyridin-3-yl)phenyl)allyl)amino)ethyl)isoquinoline-5-sulfonamide (**19**) (0.261 g, 0.57 mmol, 1 eq), formaldehyde in H_2O (36%, 48 µL, 0.63 mmol, 1.1 eq) and $NaHB(OAc)_3$ (300 mg, 1.4 mmol, 2.5 eq) were dissolved in THF (21 mL) and MeOH (3.5 mL) and after activated molecular sieves (3 Å) were added to the reaction, it was stirred under argon atmosphere for 16 h. The reaction was was quenched with sat. aqueous NH_4Cl (2.5 mL), H_2O (7.5 mL), diluted with sat. aqueous Na_2CO_3 (25 mL) and Et_2O (30 mL) after which the organic phase was collected and the aqueous layer extracted with DCM (3x40 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure and the resulting residue was purified via flash-column-chromatography (SiO_2, 2% → 4% MeOH in DCM, 0.5% Et_3N) to yield the product (222 mg, 82%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.33 (s, 1H), 8.86 (d, *J* = 2.1 Hz, 1H), 8.68 (d, *J* = 6.1 Hz, 1H), 8.59 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.52 (d, *J* = 6.1 Hz, 1H), 8.39 (d, *J* = 7.3 Hz, 1H), 8.18 (d, *J* = 8.2 Hz, 1H), 7.88 (dt, *J* = 7.9, 1.8 Hz, 1H), 7.68 (t, *J* = 7.8 Hz, 1H), 7.55 (d, *J* = 8.2 Hz, 2H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.36 (dd, *J* = 7.9, 4.8 Hz, 1H), 6.53 (d, *J* = 15.9 Hz, 1H), 6.21 (dt, *J* = 15.8, 6.6 Hz, 1H), 3.37 (t, *J* = 6.9 Hz, 2H), 3.17 (d, *J* = 6.6

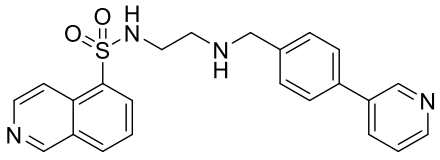
Hz, 2H), 2.92 (s, 3H), 2.61 (t, *J* = 6.9 Hz, 2H), 2.27 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 153.24, 148.53, 148.15, 145.11, 136.85, 136.75, 136.13, 134.13, 133.78, 133.55, 133.47, 132.12, 131.86, 129.17, 127.60, 127.32, 127.05, 125.88, 123.64, 117.83, 60.37, 54.88, 47.65, 42.41, 34.99. HRMS calculated for $C_{27}H_{29}N_4O_2S$ 473.20057 $[M+H]^+$, found 473.20031. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 90% ACN in H_2O 0.2% TFA, 10 min): t_R = 4.25 min; *m/z* : 473 $[M+H]^+$.

***N*-(2-((4-(Pyridin-3-yl)phenethyl)amino)ethyl)isoquinoline-5-sulfonamide (22)**



2-(4-(Pyridin-3-yl)phenyl)ethan-1-ol (**77**) (96 mg, 0.48 mmol, 1 eq) was dissolved in DCM (5 mL) to which was added Dess–Martin periodinane (0.24 g, 0.58 mmol, 1.2 eq). The reaction was stirred for 2 h before it was quenched using aqueous $Na_2S_2O_3$ (3 mL), then diluted with sat. aqueous Na_2CO_3 (30 mL) and DCM (15 mL) after which the organic layer was collected and the aqueous layer extracted with DCM (5x20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure after which a silica filtration with 50% EtOAc in pentane and concentrating under reduced pressure afforded the crude aldehyde. It was re-dissolved in dry THF (2.6 mL) together with *N*-(2-aminoethyl)isoquinoline-5-sulfonamide (**105**) (0.13 g, 0.52 mmol, 1.1 eq), glacial acetic acid (15 µL, 0.26 mmol, 0.5 eq), $NaHB(OAc)_3$ (0.11 g, 0.52 mmol, 1.2 eq) and activated molecular sieves (3 Å). The reaction was stirred under argon atmosphere for 16 h after which it was diluted with sat. aqueous Na_2CO_3 (10 mL) and Et_2O (10 mL). The organic layer was collected and the aqueous layer extracted with DCM (3x10 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated under reduced pressure and purified by preparative HPLC (Gemini C_{18}, 0% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (13 mg, 5%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.54 (d, *J* = 0.8 Hz, 1H), 8.99 (d, *J* = 2.1 Hz, 1H), 8.74 (d, *J* = 6.1 Hz, 1H), 8.69 – 8.59 (m, 3H), 8.50 (d, *J* = 8.2 Hz, 1H), 8.44 (t, *J* = 5.8 Hz, 2H), 8.39 (dd, *J* = 7.4, 1.2 Hz, 1H), 8.29 (d, *J* = 8.1 Hz, 1H), 7.92 – 7.85 (m, 1H), 7.76 (d, *J* = 8.3 Hz, 2H), 7.67 (dd, *J* = 8.0, 5.0 Hz, 1H), 7.40 (d, *J* = 8.3 Hz, 2H), 3.22 (bs, 2H), 3.06 (s, 4H), 2.99 – 2.91 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.36, 146.43, 145.56, 144.44, 137.47, 136.35, 136.02, 134.77, 133.95, 133.75, 132.96, 130.38, 129.57, 128.72, 127.25, 126.59, 124.78, 117.14, 47.51, 46.28, 38.59, 31.20. HRMS calculated for $C_{24}H_{25}N_4O_2S$ 433.16927 $[M+H]^+$, found 433.16897. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 4.89 min; *m/z* : 433 $[M+H]^+$.

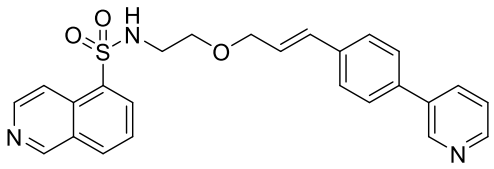
***N*-(2-((4-(Pyridin-3-yl)benzyl)amino)ethyl)isoquinoline-5-sulfonamide (23)**



To a solution *tert*-butyl (2-(isoquinoline-5-sulfonamido)ethyl)(4-(pyridin-3-yl)benzyl)carbamate (**81**) (0.290 g, 0.56 mmol, 1 eq) in DCM (4 mL) at 0°C was added TFA (1 mL). The reaction was allowed to warm to RT and stirred for 2 h before the solvents were removed under reduced pressure. $CHCl_3$ (5 mL) and sat. aqueous Na_2CO_3 solution (10 mL) were added and the mixture was stirred vigorously until both phases became clear. The organic layer was collected and the aqueous layer extracted with $CHCl_3$ (3x15 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 6% → 8% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (174 mg, 74%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.46 (s, 1H), 8.87

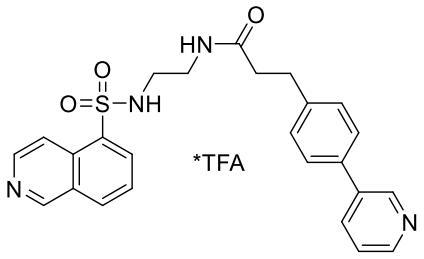
(d, *J* = 2.4 Hz, 1H), 8.68 (d, *J* = 6.0 Hz, 1H), 8.56 (dd, *J* = 4.7, 1.6 Hz, 1H), 8.45 – 8.40 (m, 2H), 8.35 (dd, *J* = 7.4, 1.1 Hz, 1H), 8.08 – 8.02 (m, 1H), 7.85 – 7.78 (m, 1H), 7.60 (d, *J* = 8.2 Hz, 2H), 7.51 – 7.44 (m, 1H), 7.25 (d, *J* = 8.1 Hz, 2H), 3.52 (s, 2H), 3.32 (bs, 2H), 2.91 (t, *J* = 6.5 Hz, 2H), 2.43 (t, *J* = 6.6 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.38, 148.30, 147.53, 144.56, 140.55, 135.42, 135.29, 134.91, 133.91, 133.35, 132.42, 130.34, 128.67, 128.50, 126.54, 126.40, 123.85, 117.15, 51.92, 47.76, 42.35. HRMS calculated for $C_{23}H_{23}N_4O_2S$ 419.15362 $[M+H]^+$, found 419.15328. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 4.58 min; *m/z* : 419 $[M+H]^+$.

(*E*)-*N*-(2-((3-(4-(Pyridin-3-yl)phenyl)allyl)oxy)ethyl)isoquinoline-5-sulfonamide (24)



To a solution of (*E*)-2-((3-(4-(pyridin-3-yl)phenyl)allyl)oxy)ethan-1-amine (**84**) (95 mg, 0.37 mmol, 1 eq) and Et_3N (62 µL, 0.45 mmol, 1.2 eq) in DCM (11.6 mL) at 0°C was added dropwise an isoquinoline-5-sulfonyl chloride solution which was prepared by extracting from a solution of isoquinoline-5-sulfonyl chloride hydrochloride (**104**) (0.12 g, 0.45 mmol, 1.2 eq) in sat. aqueous $NaHCO_3$ with DCM (3x1 mL). The reaction was allowed to warm to RT and stirred for 2 h before it was quenched with aqueous NaOH (1 M, 1 mL) and subsequently diluted with sat. aqueous Na_2CO_3 solution (20 mL). The organic phase was collected and the aqueous layer was extracted with DCM (3x20 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated under reduced pressure and the crude was purified via flash-column-chromatography (SiO_2, 2% → 5% (10% of sat. aqueous NH_3 in MeOH) in DCM) and the further by preparative HPLC (C_{18}, 10% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (32 mg, 19%). ^{1}H NMR (600 MHz, methanol-d_4) δ 9.46 (s, 1H), 9.13 (s, 1H), 8.82 – 8.69 (m, 3H), 8.65 (d, *J* = 5.5 Hz, 1H), 8.61 – 8.55 (m, 1H), 8.43 (d, *J* = 7.4 Hz, 1H), 8.08 – 7.99 (m, 1H), 7.89 (dd, *J* = 12.6, 5.1 Hz, 1H), 7.77 (d, *J* = 8.3 Hz, 2H), 7.52 (d, *J* = 7.1 Hz, 2H), 6.45 (d, *J* = 15.9 Hz, 1H), 6.10 (dt, *J* = 15.9, 5.7 Hz, 1H), 3.85 (d, *J* = 5.7 Hz, 2H), 3.38 (t, *J* = 5.3 Hz, 2H), 3.21 (t, *J* = 5.2 Hz, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 153.01, 143.08, 142.86, 142.60, 142.06, 140.85, 139.77, 137.70, 135.58, 135.08, 134.79, 133.65, 131.74, 130.51, 128.84, 128.64, 128.62, 128.56, 127.97, 120.68, 72.04, 69.80, 43.92. HRMS calculated for $C_{25}H_{24}N_3O_3S$ 446.15329 $[M+H]^+$, found 446.15301. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 6.77 min; *m/z* : 446 $[M+H]^+$.

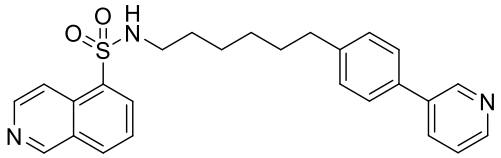
***N*-(2-(Isoquinoline-5-sulfonamido)ethyl)-3-(4-(pyridin-3-yl)phenyl)propanamide (25)**



A vial was charged with 3-(4-bromophenyl)-*N*-(2-(isoquinoline-5-sulfonamido)ethyl)propanamide (**91**) (374 mg, 0.81 mmol, 1 eq), pyridin-3-ylboronic acid (149 mg, 1.21 mmol, 1.5 eq) and $Pd(PPh_3)_4$ (10 mg, 0.01 mmol, 0.01 eq) dissolved in DCM (0.8 mL) and DMF (1.8 mL). The vial is put under an argon atmosphere and degassed aqueous K_2CO_3 (2 M, 1.0 mL, 2.02 mmol, 2.5 eq) was added. The reaction mixture was stirred at 85°C for 2.5 h, filtered over celite, concentrated under reduced pressure and purified by preparative HPLC (XBridge C_{18}, 0% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (27 mg, 6%). ^{1}H NMR (600 MHz, methanol-d_4) δ 9.53 (s, 1H), 9.08 (s, 1H), 8.77 – 8.71 (m, 2H), 8.67 (q, *J* = 6.4 Hz, 2H), 8.52 (dd, *J* = 7.4, 1.1 Hz, 1H), 8.47 (d, *J* = 8.2 Hz, 1H), 8.02 (dd, *J* = 8.1, 5.6 Hz, 1H), 7.91 – 7.87 (m, 1H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.40

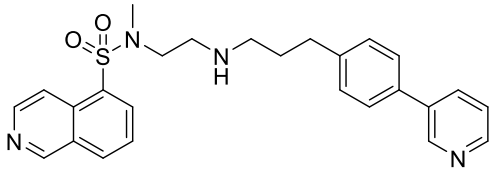
(d, *J* = 8.3 Hz, 2H), 3.15 (t, *J* = 6.3 Hz, 2H), 2.93 (t, *J* = 6.4 Hz, 4H), 2.42 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 175.17, 153.05, 144.47, 143.63, 142.21, 142.09, 142.02, 141.27, 136.83, 135.80, 135.36, 133.49, 133.45, 130.76, 130.52, 128.65, 128.49, 128.09, 120.50, 43.07, 40.27, 38.29, 32.27. HRMS calculated for $C_{25}H_{25}N_4O_3S$ 461.16419 $[M+H]^+$, found 461.16406. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 5.58 min; *m/z* : 461 $[M+H]^+$.

***N*-(6-(4-(Pyridin-3-yl)phenyl)hexyl)isoquinoline-5-sulfonamide (26)**



To a solution of 6-(4-(pyridin-3-yl)phenyl)hexan-1-amine (**97**) (62 mg, 0.24 mmol, 1 eq) and Et_3N (41 µL, 0.30 mmol, 1.25 eq) in DCM (1.2 mL) at 0°C was added dropwise an isoquinoline-5-sulfonyl chloride solution which was prepared by extracting from a solution of isoquinoline-5-sulfonyl chloride hydrochloride (**104**) (77 mg, 0.29 mmol, 1.2 eq) in sat. aqueous $NaHCO_3$ with DCM (2x0.7 mL). The reaction was allowed to warm to RT and after 3 h of stirring it was concentrated onto Celite and purified via flash-column-chromatography (SiO_2, 0% → 10% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (105 mg, 98%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.47 (s, 1H), 8.87 (d, *J* = 2.4 Hz, 1H), 8.70 (d, *J* = 6.1 Hz, 1H), 8.55 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.45 (d, *J* = 6.1 Hz, 1H), 8.42 (d, *J* = 8.2 Hz, 1H), 8.33 (d, *J* = 7.3 Hz, 1H), 8.08 – 8.02 (m, 2H), 7.82 (t, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 2H), 7.47 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.24 (d, *J* = 8.1 Hz, 2H), 2.79 (q, *J* = 6.6 Hz, 2H), 2.45 (t, *J* = 7.5 Hz, 2H), 1.36 – 1.29 (m, 2H), 1.27 – 1.20 (m, 2H), 1.10 – 0.97 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.36, 148.13, 147.42, 144.49, 142.32, 135.51, 135.08, 134.39, 133.92, 133.28, 132.38, 130.39, 129.01, 128.67, 126.72, 126.41, 123.86, 117.25, 42.19, 34.50, 30.55, 28.76, 27.92, 25.56. HRMS calculated for $C_{26}H_{28}N_3O_2S$ 446.18967 $[M+H]^+$, found 446.18926. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 90% ACN in H_2O 0.2% TFA, 10 min): t_R = 5.69 min; *m/z* : 446 $[M+H]^+$.

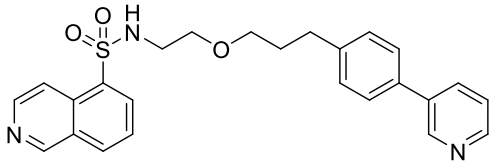
***N*-Methyl-*N*-(2-((3-(4-(pyridin-3-yl)phenyl)propyl)amino)ethyl)isoquinoline-5-sulfonamide (27)**



Acetyl chloride (35 µL, 0.49 mmol, 3 eq) was added to a vial containing MeOH (2.3 mL) and after 10 minutes of stirring (*E*)-*N*-methyl-*N*-(2-((3-(4-(pyridin-3-yl) phenyl) allyl)amino)ethyl)isoquinoline-5-sulfonamide (**19**) (75 mg, 0.16 mmol, 1 eq) and Pd/C (30 w%, 22 mg) were added and the vial was sealed. The mixture was degassed and H_2 gas was bubbled through under vigorous stirring for 1 h. The reaction was stirred for another 16 h under H_2 atmosphere until full conversion, after which aqueous NaOH (1 M, 1 mL) was added to neutralize the acid. The mixture was dried over $MgSO_4$, filtered and concentrated onto Celite. The resulting crude was purified via flash-column-chromatography (SiO_2, dry-loading, 0% → 10% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (12 mg, 16%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.34 (s, 1H), 8.84 (d, *J* = 1.7 Hz, 1H), 8.69 (d, *J* = 6.2 Hz, 1H), 8.58 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.51 (d, *J* = 6.1 Hz, 1H), 8.39 (dd, *J* = 7.4, 1.1 Hz, 1H), 8.21 (d, *J* = 8.2 Hz, 1H), 7.89 – 7.84 (m, 1H), 7.70 (t, *J* = 7.6 Hz, 1H), 7.51 (d, *J* = 8.2 Hz, 2H), 7.36 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 2H), 3.30 (t, *J* = 6.2 Hz, 2H), 2.89 (s, 3H), 2.82 (t, *J* = 6.2 Hz, 2H), 2.73 – 2.58 (m, 4H), 1.91 – 1.74 (m, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 153.40, 148.40, 148.34, 145.32, 142.14, 136.58, 135.53, 134.29, 133.81, 133.79, 133.40, 131.98, 129.28, 129.25, 127.24, 126.02, 123.66, 117.77, 49.55, 49.06, 47.21, 34.97, 33.21, 31.50. HRMS calculated for $C_{26}H_{29}N_4O_2S$ 461.20057 $[M+H]^+$, found

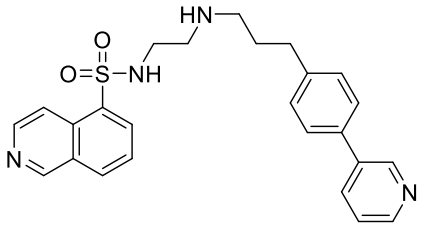
461.20029. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 90% ACN in H_2O 0.2% TFA, 10 min): t_R = 4.07 min; *m/z* : 461 $[M+H]^+$.

_N_-(2-(3-(4-(Pyridin-3-yl)phenyl)propoxy)ethyl)isoquinoline-5-sulfonamide (28)



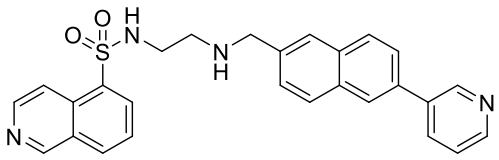
(*E*)-*N*-(2-((3-(4-(pyridin-3-yl)phenyl)allyl)oxy) ethyl) isoquinoline-5-sulfonamide (**24**) (38 mg, 0.086 mmol, 1 eq), *p*-toluenesulfonyl hydrazide (48 mg, 0.26 mmol, 3 eq) and NaOAc (21 mg, 0.26 mmol, 3 eq) were suspended in THF (0.9 mL) and heted to reflux for 3 days with daily addition of both *p*-toluenesulfonyl hydrazide and NaOAc (3x0.17 mmol). It was then diluted with sat. aqueous Na_2CO_3 and extracted with DCM (3x5 mL) after which the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified via flash-column-chromatography (SiO_2, 1% → 5% (10% of sat. aqueous NH_3 in MeOH) in DCM) and then further by preparative HPLC (C_{18}, 10% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (13 mg, 34%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.51 (s, 1H), 9.06 (d, *J* = 2.1 Hz, 1H), 8.73 – 8.69 (m, 2H), 8.50 (d, *J* = 6.2 Hz, 1H), 8.48 – 8.42 (m, 2H), 8.39 (dd, *J* = 7.4, 1.2 Hz, 1H), 8.27 (t, *J* = 5.8 Hz, 1H), 7.85 (dd, *J* = 8.1, 7.5 Hz, 1H), 7.79 (dd, *J* = 8.0, 5.3 Hz, 1H), 7.71 (d, *J* = 8.3 Hz, 2H), 7.29 (d, *J* = 8.3 Hz, 2H), 3.25 (t, *J* = 5.6 Hz, 2H), 3.10 (t, *J* = 6.4 Hz, 2H), 3.02 (q, *J* = 5.7 Hz, 2H), 2.50 – 2.45 (m, 2H), 1.54 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.92, 144.52, 143.90, 143.59, 142.77, 138.16, 137.00, 135.43, 133.41, 132.90, 132.56, 130.68, 129.19, 128.64, 127.02, 126.63, 125.43, 117.74, 69.17, 68.49, 42.29, 31.09, 30.41. HRMS calculated for $C_{25}H_{26}N_3O_3S$ 448.16894 $[M+H]^+$, found 448.16847. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 90% ACN in H_2O 0.2% TFA, 10 min): t_R = 5.01 min; *m/z* : 448 $[M+H]^+$.

_N_-(2-((3-(4-(Pyridin-3-yl)phenyl)propyl)amino)ethyl)isoquinoline-5-sulfonamide (29)



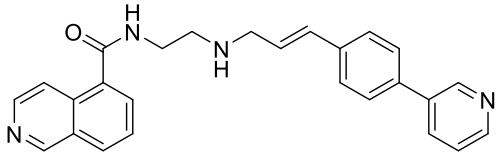
A round-bottom-flask was charged with 3-(4-(pyridin-3-yl)phenyl)propanal (**90**) (167 mg, 0.79 mmol, 1 eq), *N*-(2-aminoethyl)isoquinoline-5-sulfonamide (**105**) (397 mg, 1.58 mmol, 2 eq) and $NaHB(OAc)_3$ (318 mg, 1.58 mmol, 2 eq) suspended in DCM (79 mL). The reaction mixture was stirred overnight and half sat. aqueous Na_2CO_3 (80 mL) was added and the product was extracted with DCM (3x80 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure and the resulting residue was purified via flash-column-chromatography (SiO_2, 1% → 4% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (220 mg, 62%). ^{1}H NMR (400 MHz, methanol-d_4) δ 9.34 (s, 1H), 8.75 (d, *J* = 2.2 Hz, 1H), 8.61 (d, *J* = 6.2 Hz, 1H), 8.54 (d, *J* = 6.2 Hz, 1H), 8.47 (dd, *J* = 4.9, 1.3 Hz, 1H), 8.45 (d, *J* = 7.4 Hz, 1H), 8.33 (d, *J* = 8.2 Hz, 1H), 8.02 (dt, *J* = 8.0, 1.8 Hz, 1H), 7.77 (t, *J* = 7.8 Hz, 1H), 7.53 (d, *J* = 8.1 Hz, 2H), 7.47 (dd, *J* = 8.0, 4.9 Hz, 1H), 7.25 (d, *J* = 8.1 Hz, 2H), 2.98 (t, *J* = 6.3 Hz, 2H), 2.61 – 2.51 (m, 4H), 2.45 – 2.36 (m, 2H), 1.63 (p, *J* = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 154.32, 148.45, 148.13, 144.90, 143.60, 138.40, 136.34, 136.23, 136.03, 134.82, 134.69, 132.58, 130.60, 130.26, 128.05, 127.69, 125.40, 119.12, 49.55, 49.45, 43.02, 33.96, 32.03. HRMS calculated for $C_{25}H_{27}N_4O_2S$ 447.18492 $[M+H]^+$, found 447.18461. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 5.25 min; *m/z* : 447 $[M+H]^+$.

_N_-(2-(((6-(Pyridin-3-yl)naphthalen-2-yl)methyl)amino)ethyl)isoquinoline-5-sulfonamide (30)



A vial containing *tert*-butyl ((6-bromonaphthalen-2-yl)methyl)(2-(isoquinoline-5-sulfonamido) ethyl)carbamate (**102**) (0.448 g, 0.79 mmol, 1 eq), $Pd(PPh_3)_4$ (18 mg, 0.016 mmol, 0.02 eq) and pyridine-3-boronic acid (0.14 g, 1.2 mmol, 1.5 eq) was sealed and flushed with argon, after which a deoxygenated mixture of DCM (0.8 mL), DMF (1.7 mL) and aqueous K_2CO_3 solution (2M, 1 mL, 2.0 mmol, 2.5 eq) was added. After stirring at 80°C for 4 h, the mixture was cooled to ambient temperature, concentrated under reduced pressure, diluted with EtOAc, filtered over silica and concentrated again. It was re-dissolved in DCM (8 mL) and TFA (1.6 mL) and stirred for 4 h before the reaction was neutralized with sat. aqueous Na_2CO_3 solution (30 mL). DCM (30 mL) was added and the mixture was stirred vigorously until two clear phases were formed. The organic layer was collected and the aqueous layer was extracted with DCM (5x30 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 2% → 4% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (301 mg, 81%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.27 (s, 1H), 8.92 (d, *J* = 2.1 Hz, 1H), 8.62 – 8.56 (m, 2H), 8.47 (d, *J* = 6.1 Hz, 1H), 8.42 (d, *J* = 7.3 Hz, 1H), 8.09 (d, *J* = 8.2 Hz, 1H), 7.96 (d, *J* = 7.9 Hz, 1H), 7.91 (s, 1H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.64 – 7.57 (m, 2H), 7.54 (s, 1H), 7.38 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.26 (d, *J* = 8.4 Hz, 1H), 4.03 (bs, 2H), 3.69 (s, 2H), 3.10 – 3.04 (m, 2H), 2.70 (t, *J* = 5.6 Hz, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 153.26, 148.34, 148.26, 144.98, 137.72, 136.47, 134.74, 134.66, 134.46, 133.42, 133.21, 132.70, 131.21, 128.96, 128.64, 128.50, 126.97, 126.10, 125.92, 125.85, 125.19, 123.76, 117.31, 53.18, 47.68, 42.54. HRMS calculated for $C_{27}H_{25}N_4O_2S$ 469.16927 $[M+H]^+$, found 469.16903. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 90% ACN in H_2O 0.2% TFA, 10 min): t_R = 4.17 min; *m/z* : 469 $[M+H]^+$.

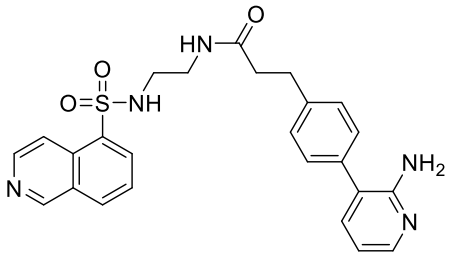
(*E*)-*N*-(2-((3-(4-(Pyridin-3-yl)phenyl)allyl)amino)ethyl)isoquinoline-5-carboxamide (31)



A round-bottom-flask was charged with *tert*-butyl (*E*)-(2-(isoquinoline-5-carboxamido)ethyl) (3-(4-(pyridin-3-yl)phenyl) allyl)carbamate (**73**) (57 mg, 0.112 mmol, 1 eq) dissolved in $CHCl_3$ (4 mL). After cooling the solution to 0°C and dropwise addition of TFA (1 mL), it was allowed to warm to RT and stirred for 60 min. The reaction was quenched by slow addition of sat. aqueous Na_2CO_3 solution (10 mL) until a pH of ~12 was reached and the mixture was extracted with DCM (3x10 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 0% → 10% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (25 mg, 55%). ^{1}H NMR (400 MHz, methanol-d_4) δ 9.28 (s, 1H), 8.79 (d, *J* = 2.1 Hz, 1H), 8.50 (dd, *J* = 4.9, 1.4 Hz, 1H), 8.46 (d, *J* = 6.1 Hz, 1H), 8.24 – 8.19 (m, 2H), 8.09 (dt, *J* = 8.0, 1.9 Hz, 1H), 8.04 – 7.99 (m, 1H), 7.75 – 7.69 (m, 1H), 7.62 (d, *J* = 8.3 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.50 (dd, *J* = 8.0, 4.9 Hz, 1H), 6.69 (d, *J* = 15.9 Hz, 1H), 6.44 (dt, *J* = 15.9, 6.5 Hz, 1H), 3.67 (t, *J* = 6.4 Hz, 2H), 3.53 (d, *J* = 6.5 Hz, 2H), 2.98 (t, *J* = 6.4 Hz, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 169.53, 152.33, 147.35, 146.78, 142.47, 137.17, 136.77, 136.23, 134.89, 133.17, 132.89, 131.72, 130.34, 130.18, 128.84, 127.42, 126.92, 126.90, 126.73, 124.12, 118.65, 50.64, 47.56, 39.07. HRMS calculated for $C_{26}H_{25}N_4O$ 409.20229 $[M+H]^+$, found

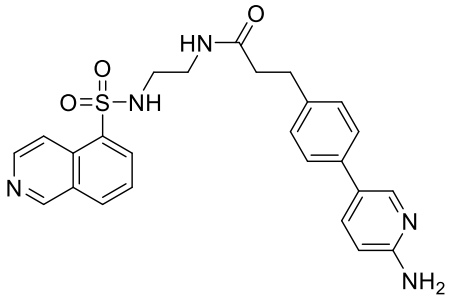
409.20208. LCMS (ESI, Waters, C_{18}, linear gradient, 5% → 50% ACN in H_2O 0.2% TFA, 10 min): t_R = 4.69 min; *m/z* : 409 $[M+H]^+$.

3-(4-(2-Aminopyridin-3-yl)phenyl)-*N*-(2-(isoquinoline-5-sulfonamido) ethyl)propanamide (32)



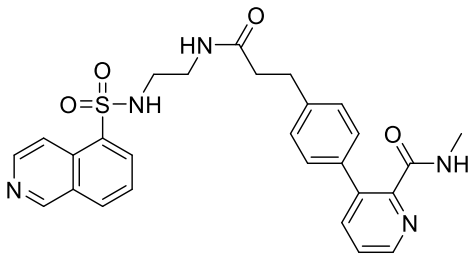
The title compound was synthesized from 3-bromopyridin-2-amine following general procedure B on a 0.29 mmol scale and purified by preparative HPLC (Gemini C_{18}, 10% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (52 mg, 38%). ^{1}H NMR (600 MHz, methanol-d_4) δ 9.44 (s, 1H), 8.63 (d, *J* = 6.2 Hz, 1H), 8.59 (d, *J* = 6.2 Hz, 1H), 8.48 (dd, *J* = 7.3, 1.1 Hz, 1H), 8.42 (d, *J* = 8.2 Hz, 1H), 7.88 (dd, *J* = 6.4, 1.6 Hz, 1H), 7.86 – 7.83 (m, 1H), 7.81 (dd, *J* = 7.3, 1.2 Hz, 1H), 7.36 (s, 4H), 7.00 (t, *J* = 6.8 Hz, 1H), 3.17 (t, *J* = 6.3 Hz, 2H), 2.93 (t, *J* = 6.3 Hz, 2H), 2.89 (t, *J* = 7.7 Hz, 2H), 2.40 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 175.24, 154.38, 153.72, 145.07, 143.82, 143.63, 143.59, 136.55, 135.68, 135.14, 135.07, 132.99, 132.77, 130.61, 129.79, 128.20, 128.13, 119.81, 114.36, 43.09, 40.28, 38.26, 32.38. HRMS calculated for $C_{25}H_{26}N_5O_3S$ 476.17509 $[M+H]^+$, found 476.17485. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.70 min; *m/z* : 476 $[M+H]^+$.

3-(4-(6-Aminopyridin-3-yl)phenyl)-*N*-(2-(isoquinoline-5-sulfonamido) ethyl)propanamide (33)



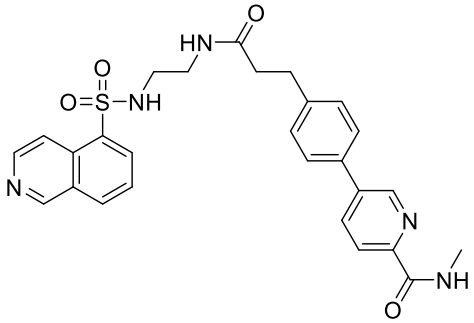
The title compound was synthesized from 5-bromopyridin-2-amine following general procedure B on a 0.1 mmol scale and purified by preparative HPLC (Gemini C_{18}, 10% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (24 mg, 50%). ^{1}H NMR (600 MHz, methanol-d_4) δ 9.44 (s, 1H), 8.64 (d, *J* = 6.2 Hz, 1H), 8.58 (d, *J* = 6.2 Hz, 1H), 8.46 (dd, *J* = 7.3, 1.1 Hz, 1H), 8.42 (d, *J* = 8.2 Hz, 1H), 8.22 (dd, *J* = 9.3, 2.3 Hz, 1H), 8.05 (d, *J* = 2.1 Hz, 1H), 7.84 (dd, *J* = 8.2, 7.3 Hz, 1H), 7.50 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 8.3 Hz, 2H), 7.08 (dd, *J* = 9.3, 0.8 Hz, 1H), 3.14 (t, *J* = 6.3 Hz, 2H), 2.92 – 2.85 (m, 4H), 2.38 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 175.27, 154.78, 153.84, 144.47, 143.80, 142.84, 136.58, 135.02, 134.99, 133.77, 133.21, 132.96, 130.64, 130.46, 128.07, 127.66, 127.33, 119.74, 115.09, 43.06, 40.26, 38.43, 32.24. HRMS calculated for $C_{25}H_{26}N_5O_3S$ 476.17509 $[M+H]^+$, found 476.17485. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.75 min; *m/z* : 476 $[M+H]^+$.

3-(4-(3-((2-(Isoquinoline-5-sulfonamido)ethyl)amino)-3-oxopropyl)phenyl)-*N*-methylpicolinamide (34)



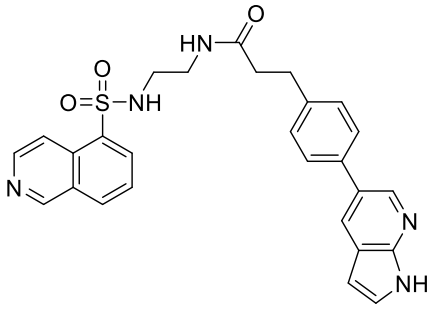
The title compound was synthesized from 3-bromo-*N*-methylpicolinamide following general procedure B on a 0.1 mmol scale and purified by preparative HPLC (Gemini C_{18}, 10% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (15 mg, 29%). ^{1}H NMR (600 MHz, methanol-d_4) δ 9.40 (s, 1H), 8.62 (d, *J* = 6.2 Hz, 1H), 8.55 (d, *J* = 6.3 Hz, 2H), 8.45 (dd, *J* = 7.3, 1.0 Hz, 1H), 8.38 (d, *J* = 8.2 Hz, 1H), 7.83 – 7.78 (m, 2H), 7.55 (dd, *J* = 7.8, 4.8 Hz, 1H), 7.27 (d, *J* = 8.2 Hz, 2H), 7.21 (d, *J* = 8.2 Hz, 2H), 3.15 (t, *J* = 6.4 Hz, 2H), 2.89 (t, *J* = 6.4 Hz, 2H), 2.86 (t, *J* = 7.6 Hz, 2H), 2.77 (s, 3H), 2.36 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 175.36, 170.25, 154.02, 152.51, 148.26, 144.18, 142.01, 140.40, 137.67, 137.38, 136.48, 134.94, 134.90, 132.84, 130.65, 129.60, 129.50, 127.91, 126.33, 119.57, 43.05, 40.36, 38.67, 32.43, 26.43. HRMS calculated for $C_{27}H_{28}N_5O_4S$ 518.18565 $[M+H]^+$, found 518.18541. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.09 min; *m/z* : 518 $[M+H]^+$.

5-(4-(3-((2-(Isoquinoline-5-sulfonamido)ethyl)amino)-3-oxopropyl)phenyl)-*N*-methylpicolinamide (35)



The title compound was synthesized from 5-bromo-*N*-methylpicolinamide following general procedure B on a 0.1 mmol scale and purified by preparative HPLC (Gemini C_{18}, 10% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (26 mg, 50%). ^{1}H NMR (600 MHz, methanol-d_4) δ 9.50 (s, 1H), 8.81 (s, 1H), 8.68 – 8.63 (m, 2H), 8.49 (dd, *J* = 7.4, 1.1 Hz, 1H), 8.44 (d, *J* = 8.2 Hz, 1H), 8.14 – 8.06 (m, 2H), 7.89 – 7.84 (m, 1H), 7.59 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 8.2 Hz, 2H), 3.16 (t, *J* = 6.4 Hz, 2H), 2.99 (s, 3H), 2.89 (t, *J* = 7.3 Hz, 4H), 2.40 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 175.29, 167.27, 153.16, 149.51, 147.91, 143.02, 142.26, 140.22, 136.83, 136.39, 135.98, 135.63, 135.28, 133.43, 130.52, 130.41, 128.55, 128.31, 123.00, 120.40, 43.03, 40.32, 38.49, 32.34, 26.41. HRMS calculated for $C_{27}H_{28}N_5O_4S$ 518.18565 $[M+H]^+$, found 518.18522. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.41 min; *m/z* : 518 $[M+H]^+$.

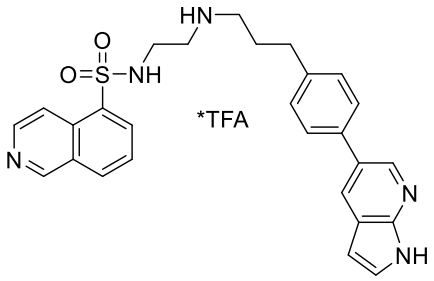
3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(2-(isoquinoline-5-sulfonamido) ethyl)propanamide (36)



The title compound was synthesized from 5-bromo-1*H*-pyrrolo[2,3-*b*]pyridine following general procedure B on a 0.1 mmol scale and purified by preparative HPLC (Gemini C_{18}, 10% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (36 mg, 72%). ^{1}H NMR (600 MHz, methanol-d_4) δ 9.53 (s, 1H), 8.72 (d, *J* = 6.4 Hz, 1H), 8.65 (d, *J* = 6.4 Hz, 1H), 8.59 (d, *J* = 1.8 Hz, 1H), 8.54 – 8.50 (m, 2H), 8.45 (d, *J* = 8.2 Hz, 1H), 7.89 (t, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 3.5 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.33 (d,

J = 8.1 Hz, 2H), 6.76 (d, *J* = 3.5 Hz, 1H), 3.16 (t, *J* = 6.4 Hz, 2H), 2.94 – 2.89 (m, 4H), 2.42 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 173.94, 151.16, 141.78, 140.76, 139.63, 135.65, 134.97, 134.91, 134.67, 134.04, 132.38, 132.21, 129.63, 129.02, 128.99, 128.64, 127.51, 127.03, 124.37, 119.51, 101.97, 41.68, 38.90, 37.13, 30.89. HRMS calculated for $C_{27}H_{26}N_5O_3S$ 500.17509 $[M+H]^+$, found 500.17487. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.19 min; *m/z* : 486 $[M+H]^+$.

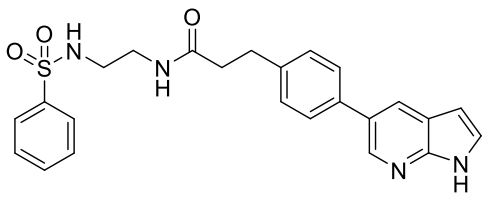
_N_-(2-((3-(4-(1_H_-Pyrrolo[2,3-_b_]pyridin-5-yl)phenyl)propyl)amino)ethyl)isoquinoline-5-sulfonamide (37)



Step 1: A round-bottom-flask was charged with 3-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)phenyl) propan-1-ol (**109**) (182 mg, 0.72 mmol, 1 eq) dissolved in DCM (4 mL). After addition of Dess–Martin periodinane (337 mg, 0.79 mmol, 1.1 eq) the reaction-mixture was stirred for 60 min and the reaction mixture was quenched with sat. aqueous $NaHCO_3$ (5 mL) and aqueous $Na_2S_2O_3$ (1 M, 5 mL). The product was extracted with DCM (3x15 mL), the combined organic layers dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was used without further purification in step 2.

Step 2: A round-bottom-flask was charged with crude from step 1 (181 mg, 0.72 mmol, 1 eq), *N*-(2-aminoethyl)isoquinoline-5-sulfonamide (**105**) (364 mg, 1.45 mmol, 2 eq) and $NaHB(OAc)_3$ (307 mg, 1.45 mmol, 2 eq) suspended in DCM (8 mL). After addition of AcOH (90 µL, 1.45 mmol, 2 eq) the reaction mixture was stirred overnight, diluted with DCM (10 mL) and sat. aqueous Na_2CO_3 (10 mL) and extracted with DCM (3x25 mL). The combined organic layers were dried over Na_2SO_4, filtered, concentrated under reduced pressure and the resulting residue was purified by preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (53 mg, 12% over 2 steps). ^{1}H NMR (400 MHz, methanol-d_4) δ 9.63 (s, 1H), 8.78 (d, *J* = 6.5 Hz, 1H), 8.71 – 8.68 (m, 2H), 8.63 – 8.53 (m, 3H), 7.96 (t, *J* = 7.9 Hz, 1H), 7.70 – 7.64 (m, 3H), 7.41 (d, *J* = 8.1 Hz, 2H), 6.82 (d, *J* = 3.5 Hz, 1H), 3.17 (bs, 4H), 3.14 – 3.07 (m, 2H), 2.80 (t, *J* = 7.7 Hz, 2H), 2.13 – 2.04 (m, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 151.11, 140.95, 140.47, 139.42, 135.46, 134.88, 134.68, 134.51, 133.88, 133.08, 132.49, 129.61, 129.09, 129.07, 129.02, 127.69, 127.25, 125.01, 119.53, 102.23, 47.07, 47.04, 38.66, 31.73, 27.32. HRMS calculated for $C_{27}H_{28}N_5O_2S$ 486.19582 $[M+H]^+$, found 486.19561. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.19 min; *m/z* : 486 $[M+H]^+$.

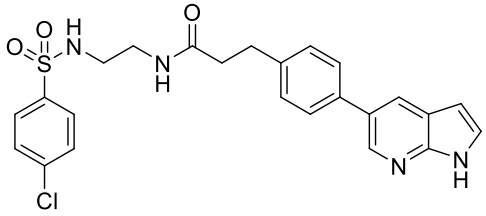
3-(4-(1_H_-Pyrrolo[2,3-_b_]pyridin-5-yl)phenyl)-_N_-(2-(phenylsulfonamido)ethyl) propanamide (38)



The title compound was synthesized from benzenesulfonyl chloride following general procedure C and purified by flash-column-chromatography (SiO_2, 2% → 8% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini C_{18}, 30% → 40% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (48 mg, 66%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.70 (d, *J* = 1.4 Hz, 1H), 8.57 (s, 1H), 7.82 – 7.78 (m, 2H), 7.66 (d, *J* = 3.5 Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 2H), 7.57 (d, *J* = 7.2 Hz, 1H), 7.52 (t, *J* = 7.4 Hz, 2H), 7.36 (d, *J* = 8.1 Hz, 2H), 6.82 (d, *J* = 3.5 Hz, 1H), 3.20 (t, *J* = 6.4

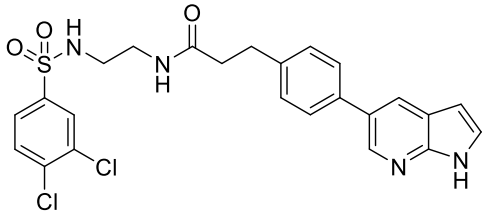
Hz, 2H), 2.96 (t, *J* = 7.6 Hz, 2H), 2.87 (t, *J* = 6.4 Hz, 2H), 2.50 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 175.39, 162.13, 142.45, 142.12, 141.68, 135.93, 135.17, 134.70, 133.64, 131.18, 130.47, 130.23, 128.48, 127.90, 126.49, 103.69, 43.26, 40.28, 38.61, 32.35. HRMS calculated for $C_{24}H_{25}N_4O_3S$ 449.16419 $[M+H]^+$, found 449.16412. LCMS (ESI, Thermo, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.73 min; *m/z* : 449 $[M+H]^+$.

3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(2-((4-chlorophenyl)sulfonamido) ethyl)propanamide (39)



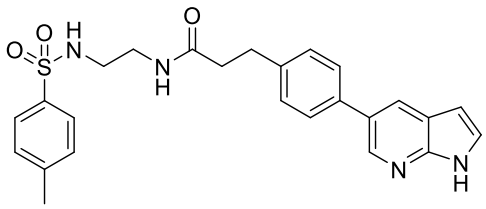
The title compound was synthesized from 4-chlorophenylsulfonylchloride following general procedure C and purified by flash-column-chromatography (SiO_2, 0% → 4% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini C_{18}, 30% → 40% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (38 mg, 49%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.69 (s, 1H), 8.47 (d, *J* = 2.0 Hz, 1H), 8.15 (d, *J* = 1.9 Hz, 1H), 7.91 (t, *J* = 5.8 Hz, 1H), 7.84 – 7.76 (m, 3H), 7.66 (d, *J* = 8.6 Hz, 2H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.53 – 7.48 (m, 1H), 7.27 (d, *J* = 8.1 Hz, 2H), 6.49 (dd, *J* = 3.2, 1.6 Hz, 1H), 3.08 (q, *J* = 6.5 Hz, 2H), 2.82 (t, *J* = 7.7 Hz, 2H), 2.76 (d, *J* = 6.9 Hz, 2H), 2.36 (t, *J* = 7.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.56, 147.96, 141.38, 139.89, 139.19, 137.29, 136.74, 129.39, 128.85, 128.45, 128.04, 126.90, 126.75, 125.82, 119.67, 100.11, 42.01, 38.42, 36.92, 30.57. HRMS calculated for $C_{24}H_{24}ClN_4O_3S$ 483.12522 $[M+H]^+$, found 483.12522. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.64 min; *m/z* : 483 $[M+H]^+$.

3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(2-((3,4-dichlorophenyl) sulfonamido)ethyl)propanamide (40)



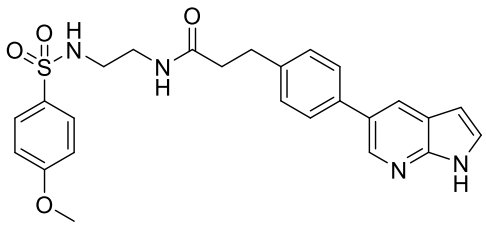
The title compound was synthesized from 3,4-dichlorobenzenesulfonyl chloride following general procedure C and purified by flash-column-chromatography (SiO_2, 0% → 5% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini C_{18}, 35% → 45% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (40 mg, 48%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 8.47 (d, *J* = 2.1 Hz, 1H), 8.16 (d, *J* = 1.9 Hz, 1H), 7.97 (d, *J* = 2.1 Hz, 1H), 7.95 – 7.90 (m, 2H), 7.87 (d, *J* = 8.4 Hz, 1H), 7.74 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.59 (d, *J* = 8.2 Hz, 2H), 7.52 – 7.48 (m, 1H), 7.28 (d, *J* = 8.2 Hz, 2H), 6.49 (dd, *J* = 3.4, 1.8 Hz, 1H), 3.09 (q, *J* = 6.5 Hz, 2H), 2.81 (p, *J* = 7.2, 6.6 Hz, 4H), 2.41 – 2.33 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.59, 147.91, 141.33, 140.70, 139.83, 136.73, 135.53, 132.15, 131.71, 128.84, 128.30, 128.04, 126.91, 126.76, 126.69, 125.87, 119.71, 100.12, 42.03, 38.41, 36.91, 30.57. HRMS calculated for $C_{24}H_{23}Cl_2N_4O_3S$ 517.08624 $[M+H]^+$, found 517.08602. LCMS (ESI, Thermo, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 9.07 min; *m/z* : 517 $[M+H]^+$.

3-(4-(1*H*-Pyrrolo[2,3-b]pyridin-5-yl)phenyl)-*N*-(2-((4-methylphenyl) sulfonamido)ethyl)propanamide (41)



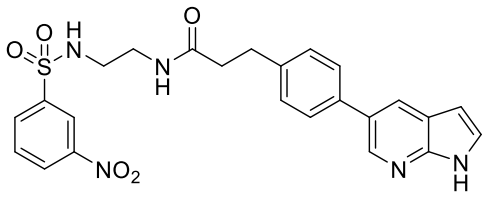
The title compound was synthesized from *p*-tosylsulfonylchloride following general procedure C and purified by preparative HPLC (Gemini, C_{18}, 30% → 40% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (71 mg, 95%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.88 (s, 1H), 8.52 (d, *J* = 2.1 Hz, 1H), 8.27 (d, *J* = 2.0 Hz, 1H), 7.91 (t, *J* = 5.8 Hz, 1H), 7.65 (d, *J* = 8.2 Hz, 2H), 7.62 – 7.58 (m, 3H), 7.57 – 7.54 (m, 1H), 7.36 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 8.2 Hz, 2H), 6.55 (dd, *J* = 3.3, 1.9 Hz, 1H), 3.07 (q, *J* = 6.6 Hz, 2H), 2.82 (t, *J* = 7.7 Hz, 2H), 2.70 (q, *J* = 6.6 Hz, 2H), 2.38 – 2.35 (m, 2H), 2.34 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.57, 146.64, 142.72, 140.17, 140.14, 137.39, 136.28, 129.69, 128.94, 128.17, 127.48, 127.13, 126.84, 126.56, 120.55, 100.53, 42.07, 36.93, 30.61, 20.97. HRMS calculated for $C_{25}H_{27}N_4O_3S$ 463.17984 $[M+H]^+$, found 463.17975. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.47 min; *m/z* : 463 $[M+H]^+$.

3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(2-((4-methoxyphenyl) sulfonamido)ethyl)propanamide (42)



The title compound was synthesized from 4-methoxybenzenesulfonyl chloride following general procedure C and purified by preparative HPLC (Gemini, C_{18}, 30% → 40% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (64 mg, 83%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.98 (s, 1H), 8.55 (d, *J* = 1.9 Hz, 1H), 8.34 (d, *J* = 1.9 Hz, 1H), 7.91 (t, *J* = 5.8 Hz, 1H), 7.73 – 7.68 (m, 2H), 7.61 (d, *J* = 8.2 Hz, 2H), 7.59 – 7.57 (m, 1H), 7.52 (t, *J* = 6.0 Hz, 1H), 7.29 (d, *J* = 8.2 Hz, 2H), 7.12 – 7.05 (m, 2H), 6.58 (dd, *J* = 3.4, 1.8 Hz, 1H), 3.80 (s, 3H), 3.07 (q, *J* = 6.5 Hz, 2H), 2.83 (t, *J* = 7.7 Hz, 2H), 2.70 (q, *J* = 6.5 Hz, 2H), 2.37 (t, *J* = 7.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.60, 162.18, 145.82, 140.36, 139.35, 136.00, 131.92, 128.98, 128.72, 128.24, 127.94, 127.83, 126.91, 121.10, 114.39, 100.79, 55.66, 42.10, 38.45, 36.94, 30.63. HRMS calculated for $C_{25}H_{27}N_4O_4S$ 479.17475 $[M+H]^+$, found 479.17450. LCMS (ESI, Thermo, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.85 min; *m/z* : 479 $[M+H]^+$.

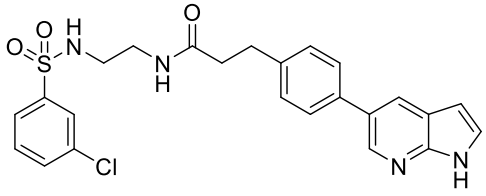
3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(2-((3-nitrophenyl)sulfonamido) ethyl)propanamide (43)



The title compound was synthesized from 3-nitrobenzenesulfonyl chloride following general procedure C and purified by flash-column-chromatography (SiO_2, 5% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini C_{18}, 30% → 40% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (33 mg, 41%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.73 (d, *J* = 1.8 Hz, 1H), 8.62 – 8.53 (m, 2H), 8.42 (dd, *J* = 8.2, 1.4 Hz, 1H), 8.18 (d, *J* = 7.9 Hz, 1H), 7.80 (t, *J* = 8.0 Hz, 1H), 7.67 (d, *J* = 3.5 Hz, 1H), 7.64 (d, *J* = 8.2 Hz, 2H), 7.38 (d, *J* = 8.2 Hz, 2H), 6.84 (d, *J* = 3.5 Hz, 1H), 3.21 (t, *J* = 6.4 Hz, 2H), 2.97 (t, *J* = 7.6 Hz, 2H), 2.93 (t, *J* = 6.4 Hz, 2H),

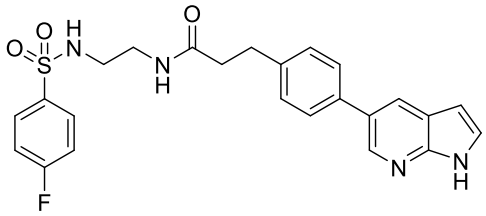
2.51 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 175.41, 149.72, 143.98, 142.55, 141.77, 135.80, 135.07, 134.77, 133.58, 132.03, 131.23, 130.65, 130.50, 128.49, 128.00, 126.75, 122.79, 103.82, 43.24, 40.27, 38.56, 32.32. HRMS calculated for $C_{24}H_{24}N_5O_5S$ 494.14927 $[M+H]^+$, found 494.14886. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.36 min; *m/z* : 494 $[M+H]^+$.

3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(2-((3-chlorophenyl)sulfonamido) ethyl)propanamide (44)



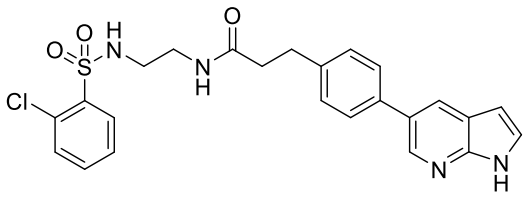
The title compound was synthesized from 3-chlorobenzenesulfonyl chloride following general procedure C and purified by flash-column-chromatography (SiO_2, 5% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini C_{18}, 30% → 40% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (14 mg, 18%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.76 (s, 1H), 8.48 (d, *J* = 2.0 Hz, 1H), 8.21 (d, *J* = 2.0 Hz, 1H), 7.92 (t, *J* = 5.7 Hz, 1H), 7.85 (t, *J* = 5.9 Hz, 1H), 7.78 (t, *J* = 1.7 Hz, 1H), 7.76 – 7.68 (m, 2H), 7.64 – 7.56 (m, 3H), 7.54 – 7.49 (m, 1H), 7.28 (d, *J* = 8.1 Hz, 2H), 6.55 – 6.49 (m, 1H), 3.09 (q, *J* = 6.5 Hz, 2H), 2.82 (t, *J* = 7.7 Hz, 2H), 2.79 – 2.73 (m, 2H), 2.36 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.82, 147.31, 142.29, 140.78, 140.11, 136.57, 133.99, 132.57, 131.48, 128.99, 128.21, 127.29, 126.89, 126.65, 126.17, 125.32, 120.26, 100.47, 42.10, 38.53, 37.00, 30.67. HRMS calculated for $C_{24}H_{24}ClN_4O_3S$ 483.12522 $[M+H]^+$, found 483.12498. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.53 min; *m/z* : 483 $[M+H]^+$.

3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(2-((4-fluorophenyl)sulfonamido) ethyl)propanamide (45)



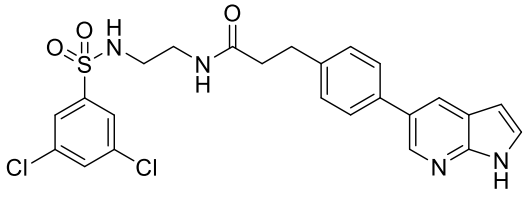
The title compound was synthesized from 4-fluorobenzenesulfonyl chloride following general procedure C and purified by flash-column-chromatography (SiO_2, 5% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini, C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (18 mg, 24%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.72 (s, 1H), 8.48 (d, *J* = 2.1 Hz, 1H), 8.19 (d, *J* = 2.0 Hz, 1H), 7.92 (t, *J* = 5.8 Hz, 1H), 7.87 – 7.80 (m, 2H), 7.73 (t, *J* = 6.0 Hz, 1H), 7.58 (d, *J* = 8.2 Hz, 2H), 7.52 – 7.49 (m, 1H), 7.45 – 7.37 (m, 2H), 7.27 (d, *J* = 8.2 Hz, 2H), 6.51 (dd, *J* = 3.4, 1.8 Hz, 1H), 3.08 (q, *J* = 6.5 Hz, 2H), 2.82 (t, *J* = 7.7 Hz, 2H), 2.74 (q, *J* = 6.5 Hz, 2H), 2.36 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.84, 164.22 (d, *J* = 250.8 Hz), 147.57, 141.03, 140.07, 136.76 (d, *J* = 3.1 Hz), 136.66, 129.62 (d, *J* = 9.5 Hz), 129.00, 128.20, 127.20, 126.89, 126.42, 120.11, 116.50 (d, *J* = 22.6 Hz), 100.42, 42.10, 38.55, 37.02, 30.69. HRMS calculated for $C_{24}H_{24}FN_4O_3S$ 467.15477 $[M+H]^+$, found 467.15439. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.24 min; *m/z* : 467 $[M+H]^+$.

3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(2-((2-chlorophenyl) sulfonamido)ethyl)propanamide (46)



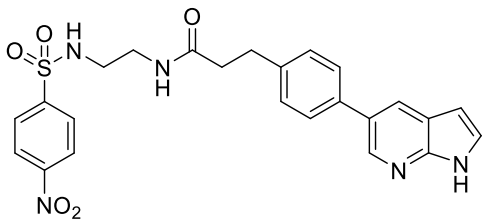
The title compound was synthesized from 2-chlorobenzenesulfonyl chloride following general procedure C and purified by flash-column-chromatography (SiO_2, 5% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (38 mg, 49%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.66 (d, *J* = 1.8 Hz, 1H), 8.55 (d, *J* = 1.6 Hz, 1H), 8.03 – 7.98 (m, 1H), 7.64 (d, *J* = 3.5 Hz, 1H), 7.62 (d, *J* = 8.2 Hz, 2H), 7.56 – 7.53 (m, 2H), 7.47 – 7.40 (m, 1H), 7.36 (d, *J* = 8.2 Hz, 2H), 6.81 (d, *J* = 3.5 Hz, 1H), 3.21 (t, *J* = 6.4 Hz, 2H), 2.97 (t, *J* = 7.6 Hz, 2H), 2.91 (t, *J* = 6.4 Hz, 2H), 2.50 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 175.42, 142.57, 142.36, 138.97, 136.14, 135.66, 134.97, 134.30, 132.90, 132.70, 132.09, 131.15, 130.46, 130.29, 128.47, 128.39, 126.22, 103.57, 43.13, 40.31, 38.64, 32.34. HRMS calculated for $C_{24}H_{24}ClN_4O_3S$ 483.12522 $[M+H]^+$, found 483.12502. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.28 min; *m/z* : 483 $[M+H]^+$.

3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(2-((3,5-dichlorophenyl) sulfonamido)ethyl)propanamide (47)



The title compound was synthesized from 3,5-dichlorobenzenesulfonyl chloride following general procedure C and purified by flash-column-chromatography (SiO_2, 5% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini, C_{18}, 30% → 40% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (29 mg, 35%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.78 (s, 1H), 8.49 (s, 1H), 8.22 (s, 1H), 7.99 (t, *J* = 5.9 Hz, 1H), 7.96 – 7.90 (m, 2H), 7.76 (d, *J* = 1.9 Hz, 2H), 7.59 (d, *J* = 8.2 Hz, 2H), 7.52 (t, *J* = 2.7 Hz, 1H), 7.28 (d, *J* = 8.2 Hz, 2H), 6.53 (dd, *J* = 3.0, 1.6 Hz, 1H), 3.10 (q, *J* = 6.4 Hz, 2H), 2.87 – 2.77 (m, 4H), 2.37 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.85, 147.08, 143.59, 140.57, 140.15, 136.51, 135.22, 132.26, 129.00, 128.22, 127.37, 126.90, 125.19, 120.40, 100.54, 42.13, 38.52, 37.00, 30.68. HRMS calculated for $C_{24}H_{23}Cl_2N_4O_3S$ 517.08624 $[M+H]^+$, found 517.08618. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.00 min; *m/z* : 517 $[M+H]^+$.

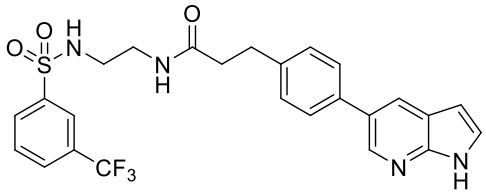
3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(2-((4-nitrophenyl) sulfonamido)ethyl)propanamide (48)



The title compound was synthesized from 4-nitrobenzenesulfonyl chloride following general procedure C and purified by flash-column-chromatography (SiO_2, 5% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini, C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (29 mg, 36%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.87 (s, 1H), 8.51 (d, *J* = 1.8 Hz, 1H), 8.40 (d, *J* = 8.8 Hz, 2H), 8.28 (d, *J* = 1.6 Hz, 1H), 8.09 (t, *J* = 5.9 Hz, 1H), 8.03 (d, *J* = 8.8 Hz, 2H), 7.94 (t, *J* = 5.7 Hz, 1H),

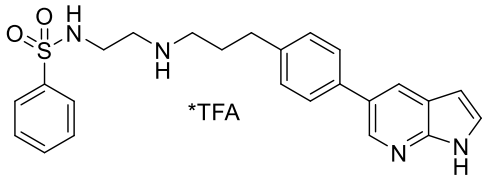
7.60 (d, *J* = 8.1 Hz, 2H), 7.56 – 7.52 (m, 1H), 7.28 (d, *J* = 8.1 Hz, 2H), 6.55 (dd, *J* = 3.3, 1.6 Hz, 1H), 3.09 (q, *J* = 6.4 Hz, 2H), 2.82 (m, 4H), 2.36 (t, *J* = 7.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.88, 149.69, 146.44, 146.07, 140.28, 139.94, 136.27, 129.03, 128.27, 128.18, 127.65, 127.47, 126.95, 124.75, 120.82, 100.74, 42.11, 38.62, 36.99, 30.68. HRMS calculated for $C_{24}H_{24}N_5O_5S$ 494.14927 $[M+H]^+$, found 494.14870. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.39 min; *m/z* : 494 $[M+H]^+$.

3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(2-((3-(trifluoromethyl) phenyl)sulfonamido)ethyl)propanamide (49)



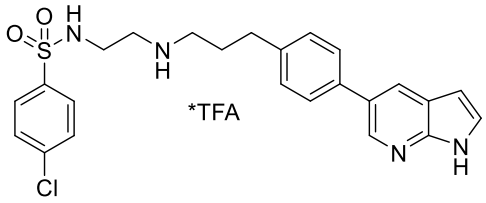
The title compound was synthesized from 3-(trifluoromethyl)benzenesulfonyl chloride following general procedure C and purified by flash-column-chromatography (SiO_2, 5% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini, C_{18}, 30% → 40% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (25 mg, 30%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.52 (d, *J* = 7.1 Hz, 2H), 8.11 – 8.04 (m, 2H), 7.90 (d, *J* = 7.8 Hz, 1H), 7.74 (t, *J* = 7.8 Hz, 1H), 7.62 – 7.56 (m, 3H), 7.35 (d, *J* = 8.1 Hz, 2H), 6.73 (d, *J* = 3.5 Hz, 1H), 3.22 (t, *J* = 6.4 Hz, 2H), 2.96 (t, *J* = 7.6 Hz, 2H), 2.91 (t, *J* = 6.4 Hz, 2H), 2.51 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 175.48, 144.23, 143.31, 142.02, 137.42, 136.80, 132.70, 131.61, 131.53, 131.00, 130.34, 130.22, 130.18, 129.59, 128.42, 125.16, 124.69, 103.06, 43.22, 40.32, 38.67, 32.36. HRMS calculated for $C_{25}H_{24}F_3N_4O_3S$ 517.15157 $[M+H]^+$, found 517.15101. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.82 min; *m/z* : 517 $[M+H]^+$.

***N*-(2-((3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)propyl)amino)ethyl) benzenesulfonamide (50)**



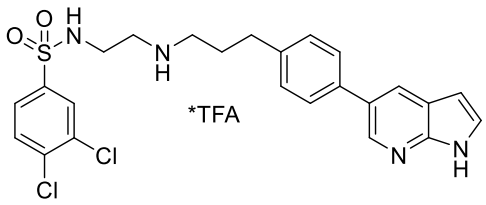
The title compound was synthesized from benzenesulfonyl chloridefollowing general procedure D and purified by flash-column-chromatography (SiO_2, 7% → 10% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini, C_{18}, 23% → 26% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound after lyophilisation (4 mg, 3%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.50 (d, *J* = 1.7 Hz, 1H), 8.46 (d, *J* = 1.9 Hz, 1H), 7.90 – 7.86 (m, 2H), 7.70 – 7.64 (m, 3H), 7.63 – 7.57 (m, 2H), 7.54 (d, *J* = 3.5 Hz, 1H), 7.40 (d, *J* = 8.2 Hz, 2H), 6.69 (d, *J* = 3.5 Hz, 1H), 3.18 – 3.14 (m, 2H), 3.14 – 3.07 (m, 4H), 2.81 (t, *J* = 7.6 Hz, 2H), 2.08 (dd, *J* = 9.3, 6.3 Hz, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 145.70, 141.11, 140.70, 138.89, 137.72, 134.17, 131.30, 130.72, 130.48, 130.27, 129.10, 128.60, 128.12, 124.30, 102.63, 49.46, 48.37, 40.18, 33.15, 28.75. HRMS calculated for $C_{24}H_{27}N_4O_2S$ 435.18492 $[M+H]^+$, found 435.18503. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.61 min; *m/z* : 435 $[M+H]^+$.

_N_-(2-((3-(4-(1_H_-Pyrrolo[2,3-_b_]pyridin-5-yl)phenyl)propyl)amino)ethyl)-4-chlorobenzenesulfonamide (51)



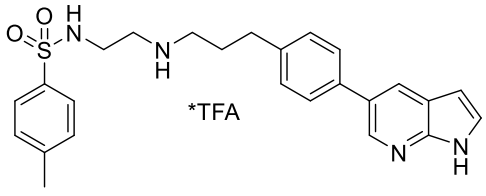
The title compound was synthesized from 4-chlorobenzenesulfonyl chloride on a 127 µmol scale following general procedure D and purified by flash-column-chromatography (SiO_2, 5% → 10% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini C_{18}, 25% → 28% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (13 mg, 18%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.47 (d, J = 1.9 Hz, 1H), 8.38 (d, J = 2.0 Hz, 1H), 7.87 – 7.83 (m, 2H), 7.66 – 7.59 (m, 4H), 7.51 (d, J = 3.5 Hz, 1H), 7.38 (d, J = 8.2 Hz, 2H), 6.65 (d, J = 3.5 Hz, 1H), 3.17 – 3.13 (m, 4H), 3.12 – 3.07 (m, 2H), 2.80 (t, J = 7.6 Hz, 2H), 2.12 – 2.04 (m, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 146.46, 140.93, 140.38, 139.69, 139.52, 138.02, 132.76, 130.68, 130.55, 130.22, 129.88, 128.77, 128.57, 123.81, 102.40, 49.28, 48.33, 40.17, 33.14, 28.74. HRMS calculated for $C_{24}H_{26}ClN_4O_2S$ 469.14595 $[M+H]^+$, found 469.14604. LCMS (ESI, Thermo, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.65 min; *m/z* : 469 $[M+H]^+$.

_N_-(2-((3-(4-(1_H_-Pyrrolo[2,3-_b_]pyridin-5-yl)phenyl)propyl)amino)ethyl)-3,4-dichlorobenzenesulfonamide (52)



The title compound was synthesized from 3,4-dichlorobenzenesulfonyl chloride on a 80 µmol scale following general procedure D and purified by flash-column-chromatography (SiO_2, 5% → 9% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini, C_{18}, 29% → 32% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (23 mg, 47%). ^{1}H NMR (500 MHz, chloroform-d) δ 10.38 (bs, 1H), 8.50 (d, J = 2.0 Hz, 1H), 8.12 (d, J = 2.0 Hz, 1H), 7.97 (d, J = 2.1 Hz, 1H), 7.68 (dd, J = 8.4, 2.1 Hz, 1H), 7.55 – 7.50 (m, 3H), 7.37 (d, J = 3.5 Hz, 1H), 7.23 (d, J = 8.1 Hz, 2H), 6.55 (d, J = 3.5 Hz, 1H), 3.08 – 3.04 (m, 2H), 2.77 – 2.73 (m, 2H), 2.66 (t, J = 7.6 Hz, 2H), 2.59 (t, J = 7.2 Hz, 2H), 2.51 (bs, 2H), 1.80 (p, J = 7.4 Hz, 2H). ^{13}C NMR (126 MHz, chloroform-d) δ 147.78, 141.92, 140.65, 140.06, 137.36, 137.22, 133.74, 131.23, 129.61, 129.12, 129.00, 127.52, 127.48, 126.20, 126.00, 120.59, 101.14, 48.93, 48.30, 42.55, 33.22, 31.54. HRMS calculated for $C_{24}H_{25}Cl_2N_4O_2S$ 503.10698 $[M+H]^+$, found 503.10711. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.33 min; *m/z* : 503 $[M+H]^+$.

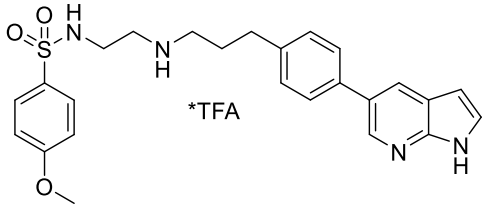
_N_-(2-((3-(4-(1_H_-Pyrrolo[2,3-_b_]pyridin-5-yl)phenyl)propyl)amino)ethyl)-4-methylbenzenesulfonamide (53)



The title compound was synthesized from *p*-tosyl chloride following general procedure D and purified by flash-column-chromatography (SiO_2, 4% → 7% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini, C_{18}, 24% → 27% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (8 mg, 6%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.46 (d, J = 1.5 Hz, 1H), 8.33 (d, J = 1.9 Hz, 1H), 7.75 (d, J = 8.2 Hz, 2H), 7.64 (d, J = 8.1 Hz, 2H), 7.49 (d, J = 3.4 Hz, 1H), 7.42 – 7.35 (m, 4H), 6.62 (d, J = 3.5 Hz, 1H), 3.15 (t, J = 5.8 Hz, 2H), 3.12 – 3.05 (m, 4H), 2.80 (t, J =

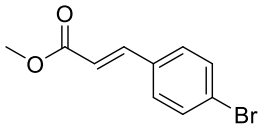
7.6 Hz, 2H), 2.42 (s, 3H), 2.08 (p, *J* = 7.8 Hz, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 147.09, 145.35, 140.79, 140.36, 138.28, 137.72, 130.97, 130.60, 130.19, 129.96, 128.56, 128.43, 128.19, 123.42, 102.21, 48.35, 48.34, 40.14, 33.14, 28.72, 21.44. HRMS calculated for $C_{25}H_{29}N_4O_2S$ 449.20057 $[M+H]^+$, found 449.20051. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.83 min; *m/z* : 449 $[M+H]^+$.

_N_-(2-((3-(4-(1_H_-Pyrrolo[2,3-_b_]pyridin-5-yl)phenyl)propyl)amino)ethyl)-4-methoxybenzenesulfonamide (54)



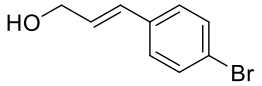
The title compound was synthesized from 4-methoxybenzenesulfonyl chloride following general procedure D and purified by flash-column-chromatography (SiO_2, 4% → 7% (10% of sat. aqueous NH_3 in MeOH) in DCM) and preparative HPLC (Gemini, C_{18}, 24% → 27% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA salt after lyophilisation (19 mg, 14%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.52 (s, 1H), 8.51 – 8.50 (m, 1H), 7.82 – 7.78 (m, 2H), 7.67 (d, *J* = 8.2 Hz, 2H), 7.57 (d, *J* = 3.5 Hz, 1H), 7.40 (d, *J* = 8.2 Hz, 2H), 7.11 – 7.06 (m, 2H), 6.71 (d, *J* = 3.5 Hz, 1H), 3.86 (s, 3H), 3.17 – 3.13 (m, 2H), 3.11 – 3.06 (m, 4H), 2.83 – 2.78 (m, 2H), 2.12 – 2.03 (m, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 164.78, 145.02, 141.26, 138.17, 137.42, 132.01, 131.93, 130.78, 130.33, 130.30, 129.37, 128.60, 124.72, 115.53, 102.83, 56.24, 48.31 (2C), 40.12, 33.14, 28.71. HRMS calculated for $C_{25}H_{29}N_4O_3S$ 465.19549 $[M+H]^+$, found 465.19543. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.70 min; *m/z* : 465 $[M+H]^+$.

Methyl (*E*)-3-(4-bromophenyl)acrylate (56)



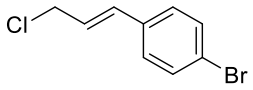
A round-bottom-flask was charged with (*E*)-3-(4-bromophenyl)acrylic acid (18.2 g, 80 mmol, 1 eq) and K_2CO_3 (55.3 g, 400 mmol, 5 eq). After suspending in ACN (120 mL), dimethyl sulfate (8.0 mL, 84 mmol, 1.05 eq) was added dropwise and the mixture was stirred at 80°C for 20 h. The reaction mixture was filtered and concentrated under reduced pressure to yield the product (quant.) without further purification. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.62 (d, *J* = 16.0 Hz, 1H), 7.55 – 7.49 (m, 2H), 7.41 – 7.35 (m, 2H), 6.42 (d, *J* = 16.0 Hz, 1H), 3.81 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 167.29, 143.62, 133.44, 132.29, 129.58, 124.69, 118.64, 51.95.

(*E*)-3-(4-Bromophenyl)prop-2-en-1-ol (57)



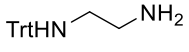
A round-bottom-flask was charged with methyl (*E*)-3-(4-bromophenyl)acrylate (**56**) (19.3 g, 80 mmol, 1 eq) and dissolved in toluene (300 mL). After cooling to -80°C, diisobutylaluminium hydride solution (1 M, 176 mL, 176 mmol, 2.2 eq) was added dropwise and the reaction mixture was allowed to warm to 0°C. The mixture was quenched with EtOAc (80 mL) and diluted with Et_2O (150 mL). H_2O (7.2 mL), aqueous NaOH (10%, 7.2 mL) and H_2O (18 mL) were added sequentially and the mixture was stirred at RT overnight. Drying over Na_2SO_4, filtering and concentration under reduced pressure yielded the product (14.9 g, 86%) which was used without further purification. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.44 (d, *J* = 8.1 Hz, 2H), 7.24 (d, *J* = 8.4 Hz, 2H), 6.56 (d, *J* = 15.9 Hz, 1H), 6.44 – 6.24 (m, 1H), 4.32 (s, 1H), 1.54 (s, 1H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 135.77, 131.84, 129.93, 129.46, 128.11, 121.58, 63.66.

(*E*)-1-Bromo-4-(3-chloroprop-1-en-1-yl)benzene (58)



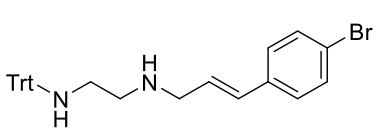
A round-bottom-flask was charged with (*E*)-3-(4-bromophenyl)prop-2-en-1-ol (**57**) (14.9 g, 70 mmol, 1 eq) dissolved in DCM (230 mL). Thionyl chloride (15.2 mL) was added dropwise and the evolving gas was neutralized with aqueous $NaHCO_3$ solution. After confirming complete conversion with TLC, the reaction mixture was concentrated under reduced pressure and co-evaporated with DCM to yield the product (16.2 g, quant.) without further purification. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.45 (d, *J* = 8.5 Hz, 2H), 7.25 (d, *J* = 8.5 Hz, 2H), 6.60 (d, *J* = 15.6 Hz, 1H), 6.31 (dt, *J* = 15.6, 7.1 Hz, 1H), 4.22 (dd, *J* = 7.1, 1.2 Hz, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 134.97, 133.03, 131.94, 128.35, 125.82, 122.29, 45.28.

***N*^{1}-Tritylethane-1,2-diamine (60)**



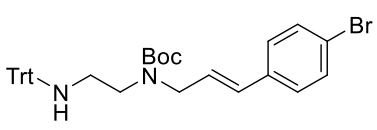
A round-bottom-flask was charged with ethylenediamine (267 mL, 4 mol, 10 eq), K_2CO_3 (66.3 g, 440 mmol, 1.1 eq) suspended in DCM (700 mL) and a solution of (chloromethanetriyl)tribenzene (111.5 g, 400 mmol, 1 eq) in DCM (700 mL) was added dropwise over 40 min. The reaction-mixture was stirred overnight at RT, filtered, concentrated under reduced pressure and co-evaporated with toluene to yield the product (122.8 g, quant.) which was used without further purification. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.48 (d, *J* = 7.6 Hz, 6H), 7.26 (t, *J* = 7.7 Hz, 6H), 7.17 (t, *J* = 7.3 Hz, 3H), 2.79 (t, *J* = 5.9 Hz, 2H), 2.21 (t, *J* = 6.0 Hz, 2H), 1.51 (bs, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 146.24, 128.76, 127.89, 126.34, 70.77, 46.60, 42.89.

(*E*)-*N*^{1}-(3-(4-Bromophenyl)allyl)-*N*^{2}-tritylethane-1,2-diamine (61)



A round-bottom-flask was charged with *N*^{1}-tritylethane-1,2-diamine (**60**) (72.6 g, 240 mmol, 4 eq), (*E*)-1-bromo-4-(3-chloroprop-1-en-1-yl)benzene (**58**) (13.9 g, 60 mmol, 1 eq) and K_2CO_3 (9.1 g, 66 mmol, 1.1 eq) suspended in ACN (1200 mL). The reaction mixture is stirred at 70°C for 2 h. After confirming complete conversion with TLC the reaction mixture was filtrated and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 10% → 40% EtOAc in pentane, 1% Et_3N) to yield the product (22.4 g, 75%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.50 – 7.45 (m, 6H), 7.43 – 7.38 (m, 2H), 7.29 – 7.14 (m, 11H), 6.43 (d, *J* = 16.0 Hz, 1H), 6.24 (dt, *J* = 15.9, 6.2 Hz, 1H), 3.32 (dd, *J* = 6.2, 1.5 Hz, 2H), 2.79 – 2.73 (m, 2H), 2.34 – 2.27 (m, 2H), 1.91 (bs, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 146.16, 136.12, 131.72, 130.17, 129.32, 128.76, 127.90, 126.36, 121.16, 70.86, 51.56, 49.66, 43.24.

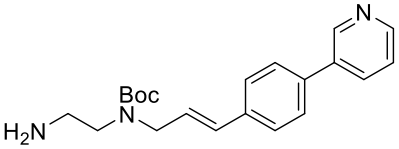
***tert*-Butyl (*E*)-(3-(4-bromophenyl)allyl)(2-(tritylamino)ethyl)carbamate (62)**



A flask was charged with (*E*)-*N*^{1}-(3-(4-bromophenyl)allyl)-*N*^{2}-tritylethane-1,2-diamine (**61**) (22.4 g, 45.0 mmol, 1 eq), di-tert-butyl dicarbonate (11.8 g, 54.0 mmol, 1.2 eq) and $NaHCO_3$ (4.54 g, 54.0 mmol, 1.2 eq) suspended in THF (150 mL). The reaction-mixture was stirred overnight at RT and after confirming complete conversion with TLC, sat. aqueous $NaHCO_3$ (300 mL) was added. The phases were separated and the aqueous layer was extracted with DCM (3x200 mL). The combined organic layers were washed with brine (1x200 mL), dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 1% → 8% EtOAc in pentane) to yield the product (20.6 g, 76%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.49 (d, *J* = 8.5

Hz, 2H), 7.38 (d, *J* = 7.2 Hz, 6H), 7.30 (d, *J* = 8.6 Hz, 2H), 7.25 (t, *J* = 7.7 Hz, 6H), 7.16 (t, *J* = 7.3 Hz, 3H), 6.39 (d, *J* = 15.9 Hz, 1H), 6.20 (dt, *J* = 15.9, 6.0 Hz, 1H), 3.93 (d, *J* = 5.7 Hz, 2H), 3.30 (t, *J* = 6.4 Hz, 2H), 2.63 (bs, *J* = 9.8 Hz, 1H), 2.19 (q, *J* = 6.8 Hz, 2H), 1.36 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.02, 155.35, 145.14, 140.73, 139.09, 137.60, 137.49, 136.91, 136.61, 135.32, 129.62, 88.45, 87.92, 79.64, 56.49, 52.01, 37.39.

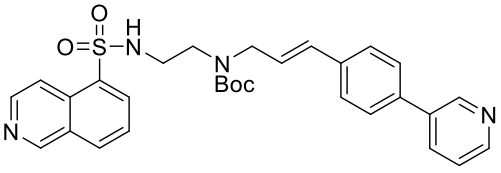
tert-Butyl (_E_)-(2-aminoethyl)(3-(4-(pyridin-3-yl)phenyl)allyl)carbamate (63)



Step 1: A round-bottom-flask was charged with *tert*-butyl (*E*)-(3-(4-bromophenyl)allyl)(2-(tritylamino)ethyl)carbamate (**62**) (19.8 g, 33.20 mmol, 1 eq), 3-pyridinylboronic acid (6.1 g, 49.80 mmol, 1.5 eq) and $Pd(PPh_3)_4$ (0.415 g, 0.33 mmol, 0.01 eq) dissolved in DCM (34 mL) and DMF (73 mL). After addition of aqueous K_2CO_3 (2 M, 41.5 mL, 83.0 mmol, 2.5 eq) the reaction mixture was heated to 85°C for 6 h and after confirming complete conversion with TLC the reaction mixture was filtered over celite and concentrated under reduced pressure. Excess reagents were removed via silica flash column chromatography, eluting with a gradient from 10% to 100% EtOAc in pentane. The resulting product was then directly used in step 2.

Step 2: A round bottom flask was charged with *tert*-butyl (*E*)-(3-(4-(pyridin-3-yl)phenyl)allyl)(2-(tritylamino)ethyl)carbamate (19.78 g, 33.20 mmol, 1 eq) and dissolved in DCM (1025 mL). The flask was cooled down to 0°C and after adding TFA (15.35 mL, 199.20 mmol, 6 eq) the solution turns bright yellow and turns colorless again after addition of triethylsilane (42.42 mL, 265 mmol, 8 eq). The solution was allowed to warm to RT and was stirred for 5 h. The reaction was basified by adding sat. aqueous Na_2CO_3 (300 mL). The phases were separated and the aqueous layer was extracted with DCM (5x300 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 2% → 10% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (9.61 g, 82% over 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (d, *J* = 2.0 Hz, 1H), 8.56 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.15 – 7.99 (m, 1H), 7.72 (d, *J* = 8.3 Hz, 2H), 7.57 (d, *J* = 8.3 Hz, 2H), 7.48 (dd, *J* = 7.9, 4.8 Hz, 1H), 6.53 (d, *J* = 15.8 Hz, 1H), 6.34 (bs, 1H), 3.98 (s, 2H), 3.39 (bs, 2H), 2.94 (t, *J* = 6.5 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 148.53, 147.52, 136.27, 136.13, 135.06, 133.91, 131.09, 130.71, 127.11, 126.28, 123.94, 79.43, 49.36, 48.54, 44.35, 37.62, 28.09. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.37 min; *m/z* : 354 $[M+H]^+$.

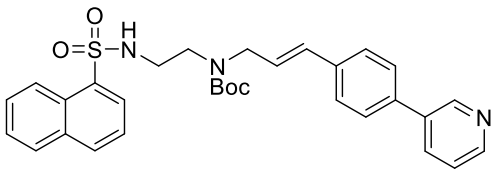
tert-Butyl (_E_)-(2-(isoquinoline-5-sulfonamido)ethyl)(3-(4-(pyridin-3-yl)phenyl)allyl) carbamate (64)



A round-bottom-flask equipped with an addition funnel was charged with *tert*-butyl (*E*)-(2-aminoethyl)(3-(4-(pyridin-3-yl)phenyl)allyl) carbamate (**63**) (500 mg, 1.41 mmol, 1 eq) and Et_3N (0.47 mL, 3.39 mmol, 2.4 eq) dissolved in DCM (14 mL). Isoquinoline-5-sulfonyl chloride (**104**) (0.45 g) was dissolved in sat. aqueous $NaHCO_3$ (5 mL) and extracted with DCM (3x4 mL). The resulting solution was dried over Na_2SO_4, filtered, transferred into the addition funnel and after cooling the reaction mixture to 0°C added dropwise. The reaction mixture was allowed to warm to RT and after stirring for 60 min sat. aqueous $NaHCO_3$ (50 mL) was added. The mixture was extracted with DCM (3x50 mL), the combined organic layers were dried over

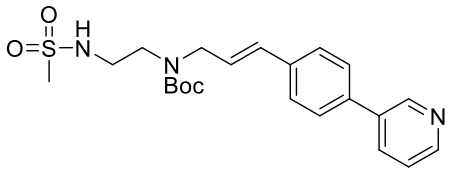
Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 1% → 10% MeOH in DCM) to yield the product (0.61 g, 66%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.34 (s, 1H), 8.86 (d, *J* = 1.8 Hz, 1H), 8.65 (d, *J* = 6.0 Hz, 1H), 8.60 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.46 – 8.36 (m, 2H), 8.17 (d, *J* = 8.2 Hz, 1H), 7.89 (dt, *J* = 7.9, 2.0 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.55 (d, *J* = 8.2 Hz, 2H), 7.43 (d, *J* = 8.3 Hz, 2H), 7.38 (dd, *J* = 7.6, 4.5 Hz, 1H), 6.43 (d, *J* = 15.9 Hz, 1H), 6.17 – 6.06 (m, 1H), 3.91 (d, *J* = 6.0 Hz, 2H), 3.36 (t, *J* = 5.0 Hz, 2H), 3.13 (s, 2H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 153.38, 148.58, 148.12, 145.33, 137.16, 136.34, 136.18, 134.32, 133.58, 133.21, 131.61, 131.34, 129.15, 127.43, 127.24, 125.92, 125.76, 123.78, 117.39, 80.98, 50.63, 46.64, 42.98, 28.48. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.12 min; *m/z* : 545 $[M+H]^+$.

tert-Butyl (_E_)-(2-(naphthalene-1-sulfonamido)ethyl)(3-(4-(pyridin-3-yl)phenyl) allyl) carbamate (65)



To a solution of *tert*-butyl (*E*)-(2-aminoethyl) (3-(4-(pyridin-3-yl)phenyl)allyl)carbamate (**63**) (0.181 g, 0.51 mmol, 1 eq) and Et_3N (100 µL, 0.72 mmol, 1.4 eq) in DCM (5.1 mL) at 0 °C was added dropwise a solution of 1-naphthalenesulfonyl chloride (0.12 g, 0.56 mmol 1.1 eq) in DCM (5.6 mL). It was allowed to warm to RT and stirred for 30 min before sat. aqueous Na_2CO_3 (20 mL) was added. The organic layer was collected and the aqueous layer extracted with DCM (2x20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 0.5% → 0.7% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (0.270 g, 98%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.85 (s, 1H), 8.66 (d, *J* = 8.3 Hz, 1H), 8.58 (d, *J* = 4.1 Hz, 1H), 8.22 (d, *J* = 7.2 Hz, 1H), 8.02 (d, *J* = 8.1 Hz, 1H), 7.94 – 7.84 (m, 2H), 7.62 – 7.55 (m, 2H), 7.55 – 7.49 (m, 2H), 7.45 (t, *J* = 7.8 Hz, 1H), 7.42 – 7.33 (m, 3H), 6.38 (d, *J* = 15.8 Hz, 1H), 6.28 (s, 1H), 6.07 (dt, *J* = 15.8, 6.2 Hz, 1H), 3.86 (d, *J* = 5.8 Hz, 2H), 3.33 (s, 2H), 3.11 (s, 2H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 148.40, 147.97, 136.86, 136.42, 136.16, 134.29, 134.25, 134.17, 131.27, 129.47, 129.08, 128.34, 128.15, 127.27, 127.16, 126.89, 125.89, 124.56, 124.12, 123.73, 80.55, 50.45, 46.61, 42.52, 28.40.

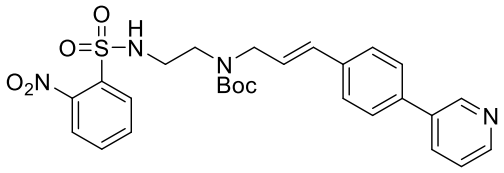
tert-Butyl (_E_)-(2-(methylsulfonamido)ethyl)(3-(4-(pyridin-3-yl)phenyl)allyl) carbamate (66)



A round-bottom-flask was charged with *tert*-butyl (*E*)-(2-aminoethyl)(3-(4-(pyridin-3-yl)phenyl)allyl) carbamate (**63**) (100 mg, 0.28 mmol, 1 eq) and Et_3N (80 µL, 0.57 mmol, 2 eq) dissolved in DCM (2.8 mL). After cooling the mixture to 0°C a solution of methane sulfonyl chloride (25 µL, 0.31 mmol, 1.2 eq) in DCM (2.8 mL) was added dropwise and the reaction was slowly allowed to warm to RT. After 50 min half sat. aqueous $NaHCO_3$ solution (4 mL) was added, the mixture was extracted with DCM (3x5 mL), the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 0% → 15% MeOH in DCM) to yield the product (107 mg, 88%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.83 (d, *J* = 1.9 Hz, 1H), 8.56 (dd, *J* = 4.8, 1.5 Hz, 1H), 7.86 (dt, *J* = 7.9, 1.9 Hz, 1H), 7.53 (d, *J* = 8.2 Hz, 2H), 7.46 (d, *J* = 8.3 Hz, 2H), 7.35 (dd, *J* = 7.9, 4.8 Hz, 1H), 6.50 (d, *J* = 15.8 Hz, 1H), 6.20 (d, *J* = 15.4 Hz, 1H), 4.03 (bs, 2H), 3.45 (bs, 2H), 3.31 (bs, 2H), 2.93 (s, 3H), 1.47 (s, 9H). ^{13}C NMR (101 MHz,

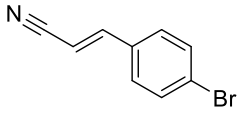
chloroform-*d*) δ 156.59, 148.48, 148.07, 137.05, 136.40, 136.15, 134.26, 131.51, 127.38, 127.20, 125.83, 123.71, 80.77, 50.35, 46.75, 42.42, 40.39, 28.48.

tert-Butyl (*E*)-(2-((2-nitrophenyl)sulfonamido)ethyl)(3-(4-(pyridin-3-yl)phenyl)allyl) carbamate (67)



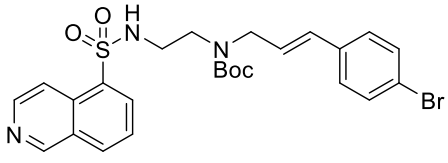
To a solution of *tert*-butyl (*E*)-(2-aminoethyl) (3-(4-(pyridin-3-yl)phenyl)allyl)carbamate (**63**) (0.335 g, 0.95 mmol, 1 eq) and Et_3N (170 µL, 1.3 mmol, 1.4 eq) in DCM (8 mL) at 0 °C was added dropwise a solution of 2-nitrobenzenesulfonyl chloride (0.23 g, 1.1 mmol, 1.1 eq) in DCM (4 mL). The reaction was allowed to warm to RT and stirred for 1 h before it was washed with H_2O (2x20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 0.5% → 0.7% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (0.404 g, 79%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.89 – 8.84 (m, 1H), 8.58 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.06 (d, *J* = 7.0 Hz, 1H), 7.92 – 7.85 (m, 1H), 7.79 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.71 – 7.61 (m, 2H), 7.55 (d, *J* = 8.0 Hz, 2H), 7.45 (d, *J* = 8.2 Hz, 2H), 7.39 – 7.34 (m, 1H), 6.49 (d, *J* = 15.9 Hz, 1H), 6.31 (s, 1H), 6.19 (dt, *J* = 15.8, 6.1 Hz, 1H), 4.00 (d, *J* = 5.7 Hz, 2H), 3.50 – 3.42 (m, 2H), 3.31 (s, 2H), 1.48 (s, 9H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 148.40, 147.96, 147.93, 136.88, 136.40, 136.05, 134.18, 133.56, 132.69, 131.31, 130.80, 127.25, 127.15, 125.91, 125.23, 123.68, 80.60, 50.52, 46.60, 42.55, 28.36. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.33 min; *m/z* : 539 $[M+H]^+$.

(*E*)-3-(4-Bromophenyl)acrylonitrile (69)



Diethyl cyanomethylphosphonate (35.43 g, 200 mmol, 1 eq) was added slowly to a solution of NaH (8.80 g, 220 mmol, 1.1 eq) in DMF (900 mL) at 0°C. After the mixture was allowed to stir for 30 min, a solution of 4-bromobenzaldehyde (40.70 g, 220 mmol, 1.1 eq) dissolved in DMF (100 mL) was added dropwise. The mixture was allowed to warm up to RT, stirred overnight and quenched by addition of saturated aqueous $NaHSO_3$ (800 mL). After further dilution with H_2O (800 mL), the mixture was extracted with Et_2O (4x600 mL). The combined organic layers were washed with sat. aqueous $NaHSO_3$ and brine, before being dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude was purified via flash-column-chromatography (SiO_2, 10% EtOAc in pentane) to yield the product (25.4 g, 61%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.51 (d, *J* = 8.8 Hz, 2H), 7.33 – 7.29 (m, 3H), 5.89 (d, *J* = 16.8 Hz, 1H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 149.89, 132.12, 132.03, 128.52, 125.26, 117.67, 96.84.

tert-Butyl (*E*)-(3-(4-bromophenyl)allyl)(2-(isoquinoline-5-sulfonamido)ethyl) carbamate (70)

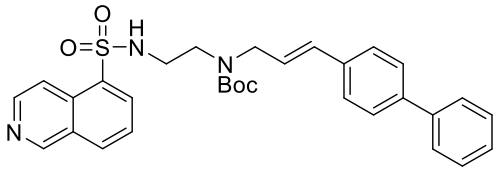


<u>Step 1:</u> A solution of (*E*)-3-(4-bromophenyl)acrylonitrile (**69**) (10.40 g, 50 mmol, 1 eq) in Et_2O (250 mL) was cooled to -87°C, before DiBAL-H in hexanes (1 M, 100 mL, 100 mmol, 2 eq) was added dropwise and the reaction was allowed to warm up to 0°C. After stirring at 0°C for 2 h the mixture was cooled to -100°C, followed by rapid addition of MeOH (100 mL) and after 5 min stirring a solution of *N*-(2-aminoethyl)isoquinoline-5-sulfonamide (**105**) (25.18 g, 100 mmol, 2 eq) in MeOH (100 mL) was added dropwise. The resulting mixture was allowed

to warm up to RT and stirred overnight. After cooling to 0°C, $NaBH_4$ (3.78 g, 100 mmol, 2 eq) was added and the mixture was stirred for 4 h and then diluted with aqueous NaOH (2 M, 250 mL). The phases were separated and the aqueous layer was extracted with DCM (3x250 mL). The combined organic layers were washed with H_2O (3x250 mL) and brine, before being dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was used in step 2 without further purification.

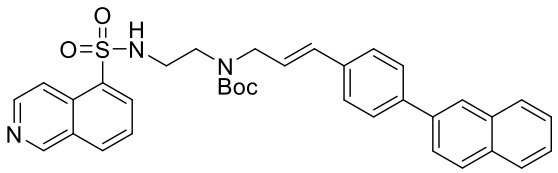
Step 2: The crude product from step 1 was dissolved in THF (250 mL) and cooled to 0°C before Boc_2O (27.28 g, 125 mmol, 2.5 eq) was added and the reaction was allowed to warm up to RT and stirred overnight. The reaction mixture was diluted with H_2O and extracted with EtOAc (4x250 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (2x250 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified via flash-column-chromatography (SiO_2, 0.1% → 2% MeOH in DCM) to yield the product (14.9 g, 55%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.32 (s, 1H), 8.59 (d, *J* = 6.4 Hz, 1H), 8.44 (d, *J* = 6.0 Hz, 1H), 8.36 (d, *J* = 7.2 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.38 (s, 2H), 7.15 (d, *J* = 7.6 Hz, 2H), 6.77 (bs, 1H), 6.30 (d, *J* = 16 Hz, 1H), 6.06 – 5.99 (m, 1H), 3.87 (d, *J* = 5.2 Hz, 2H), 3.35 (bs, 2H), 3.12 (bs, 2H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 152.93, 144.54, 135.13, 134.33, 133.23, 132.82, 131.43, 131.00, 128.80, 127.70, 125.72, 121.23, 117.25, 80.38, 50.16, 46.45, 42.11, 29.41, 28.12.

tert-Butyl (_E_)-(3-([1,1'-biphenyl]-4-yl)allyl)(2-(isoquinoline-5-sulfonamido)ethyl)carbamate (71)



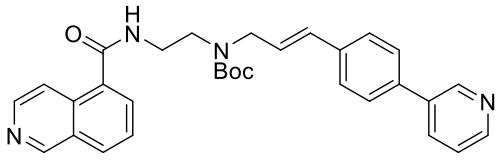
A vial containing *tert*-butyl (*E*)-(3-(4-bromophenyl)allyl)(2-(isoquinoline-5- sulfon amido) ethyl)carbamate (**70**) (0.55 g, 1.0 mmol, 1 eq), $Pd(PPh_3)_4$ (13 mg, 0.012 mmol, 0.012 eq) and phenylboronic acid (0.182 g, 1.5 mmol, 1.5 eq) was sealed and flushed with argon, after which a deoxygenated mixture of DCM (1 mL), DMF (2.2 mL) and aqueous K_2CO_3 (2 M, 1.25 mL, 2.5 mmol, 2.5 eq) was added. After stirring at 90 °C for 21 h, the mixture was cooled to ambient temperature, concentrated under reduced pressure, re-suspended with EtOAc, filtered over silica and concentrated again. The resulting crude was purified via flash-column-chromatography (SiO_2, 45% → 65% EtOAc in pentane) to yield the product (0.378 g, 70%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.29 (s, 1H), 8.58 (d, *J* = 6.1 Hz, 1H), 8.46 (d, *J* = 6.1 Hz, 1H), 8.36 (dd, *J* = 7.4, 1.1 Hz, 1H), 8.07 (d, *J* = 8.1 Hz, 1H), 7.61 – 7.48 (m, 5H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.38 – 7.29 (m, 3H), 6.85 (t, *J* = 5.7 Hz, 1H), 6.40 (d, *J* = 15.9 Hz, 1H), 6.10 – 6.01 (m, 1H), 3.90 (s, 2H), 3.35 (s, 2H), 3.12 (s, 2H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 156.32, 153.07, 144.70, 140.34, 140.31, 135.34, 134.48, 133.31, 132.97, 131.61, 131.12, 129.16, 128.93, 128.75, 127.32, 127.13, 126.76, 125.83, 124.95, 117.41, 80.43, 50.45, 46.57, 42.31, 28.28. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.63 min; *m/z* : 544 $[M+H]^+$.

tert-Butyl (_E_)-(2-(isoquinoline-5-sulfonamido)ethyl)(3-(4-(naphthalen-2-yl)phenyl)allyl)carbamate (72)



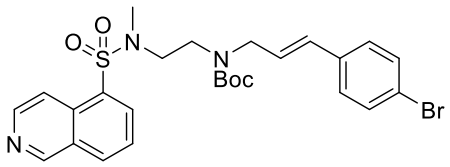
A vial containing *tert*-butyl (*E*)-(3-(4-bromophenyl)allyl)(2-(isoquinoline-5- sulfon amido)ethyl)carbamate (**70**) (0.55 g, 1.0 mmol, 1 eq), $Pd(PPh_3)_4$ (13 mg, 0.012 mmol, 0.012 eq) and 2-naphthaleneboronic acid (0.26 mg, 1.5 mmol, 1.5 eq) was sealed and flushed with argon, after which a deoxygenated mixture of DCM (1 mL), DMF (2.2 mL) and aqueous K_2CO_3 (2 M, 1.25 mL, 2.5 mmol, 2.5 eq) was added. After stirring at 90 °C for 21 h, the mixture was cooled to ambient temperature, concentrated under reduced pressure, re-suspended with EtOAc, filtered over silica and concentrated again. The crude was purified via flash-column-chromatography (SiO_2, 40% → 70% EtOAc in pentane) to yield the desired product (0.370 g, 62%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.31 (s, 1H), 8.63 (d, *J* = 6.0 Hz, 1H), 8.42 (d, *J* = 5.7 Hz, 1H), 8.37 (dd, *J* = 7.4, 1.0 Hz, 1H), 8.11 (d, *J* = 8.2 Hz, 1H), 8.04 (s, 1H), 7.93 – 7.82 (m, 3H), 7.74 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.67 (d, *J* = 8.2 Hz, 2H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.51 – 7.46 (m, 2H), 7.41 (d, *J* = 8.3 Hz, 2H), 6.43 (d, *J* = 15.9 Hz, 1H), 6.38 (s, 1H), 6.13 – 6.02 (m, 1H), 3.90 (d, *J* = 5.8 Hz, 2H), 3.35 (s, 2H), 3.11 (s, 2H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 171.12, 162.61, 153.17, 144.91, 140.27, 137.77, 135.55, 134.66, 133.66, 133.34, 132.99, 132.65, 131.72, 131.24, 129.04, 128.51, 128.19, 127.63, 127.47, 126.95, 126.36, 126.01, 125.86, 125.49, 125.20, 117.47, 80.51, 50.56, 46.68, 42.48, 28.37. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 8.24 min; *m/z* : 594 $[M+H]^+$.

tert-Butyl (_E_)-(2-(isoquinoline-5-carboxamido)ethyl)(3-(4-(pyridin-3-yl)phenyl) allyl)carbamate (73)



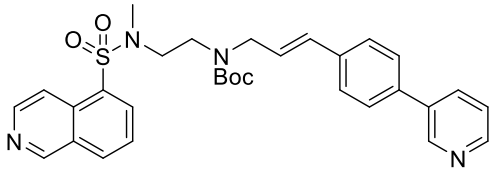
A round bottom flask was charged with isoquinoline-5-carboxylic acid (50 mg, 0.29 mmol, 1.05 eq) suspended in $SOCl_2$ (2 mL). After addition of 3 drops of DMF the reaction was heated to 70°C for 60 min and excess $SOCl_2$ was removed under reduced pressure. The resulting solid was re-dissolved in DCM (3 mL) and after addition of DiPEA (140 µL, 0.41 mmol, 3 eq) a solution of *tert*-butyl(*E*)-(2-aminoethyl)(3-(4-(pyridin-3-yl)phenyl) allyl) carbamate (**63**) (97 mg, 0.275 mmol, 1 eq) and DMAP (3 mg, 0.03 mmol, 0.1 eq) dissolved in DCM (5 mL) was added dropwise at 0°C. The reaction mixture was allowed to warm up to RT. After 75 min half saturated aqueous $NaHCO_3$ solution (10 mL) was added, the mixture was extracted with DCM (3x15 mL), the combined organic layers were washed with brine (1x40 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 0% → 15% MeOH in DCM) to yield the product (57 mg, 41%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.26 (s, 1H), 8.85 (s, 1H), 8.62 – 8.51 (m, 2H), 8.36 – 8.16 (m, 1H), 8.03 (d, *J* = 8.2 Hz, 1H), 7.88 (d, *J* = 7.7 Hz, 2H), 7.62 – 7.33 (m, 7H), 6.55 (d, *J* = 15.9 Hz, 1H), 6.32 – 6.19 (m, 1H), 4.09 (bs, 2H), 3.73 (d, *J* = 5.3 Hz, 2H), 3.62 (bs, 2H), 1.41 (s, 9H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 168.47, 157.28, 152.82, 148.61, 148.18, 144.24, 137.20, 136.37, 136.16, 134.26, 133.35, 132.83, 131.68, 130.61, 129.46, 128.84, 127.45, 127.21, 126.24, 125.80, 123.73, 118.58, 80.77, 50.08, 45.59, 40.30, 28.42. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.67 min; *m/z* : 509 $[M+H]^+$.

tert-Butyl (_E_)-(3-(4-bromophenyl)allyl)(2-(_N_-methylisoquinoline-5-sulfonamido)ethyl) carbamate (74)



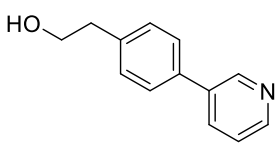
To a solution of (*E*)-(3-(4- bromophenyl)allyl)(2-(isoquinoline-5-sulfon amido)ethyl)carbamate (**70**) (2.51 g, 4.6 mmol, 1 eq), and cesium carbonate (2.2 g, 6.9 mmol, 1.5 eq) in DMF (45 mL) was added methyl iodide (357 µL, 5.7 mmol, 1.25 eq). The mixture was stirred for 21 h and concentrated under reduced pressure at 75°C, after which the resulting solids were re-dissolved in DCM (100 mL) and H_2O (100 mL). The organic layer was collected and the aqueous layer extracted with DCM (2x100 mL), the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was purified via flash-column-chromatography (SiO_2, 40% → 65% EtOAc in pentane) to yield the product (2.57 g, quant.). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.33 (s, 1H), 8.64 (d, *J* = 6.1 Hz, 1H), 8.49 – 8.38 (m, 1H), 8.26 (s, 1H), 8.18 (d, *J* = 8.2 Hz, 1H), 7.68 – 7.53 (m, 1H), 7.49 – 7.35 (m, 2H), 7.24 (d, *J* = 7.9 Hz, 2H), 6.41 (d, *J* = 15.5 Hz, 1H), 6.14 (dt, *J* = 15.9, 6.3 Hz, 1H), 3.99 (s, 2H), 3.51 – 3.24 (m, 4H), 2.93 – 2.85 (m, 3H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 155.37, 153.27, 145.16, 135.51, 133.59, 133.33, 131.74, 130.84, 129.17, 129.06, 127.99, 126.16, 125.88, 121.57, 121.51, 117.64, 80.27, 50.12, 47.69, 44.90, 35.11, 28.44. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.51 min; *m/z* : 560, 562 $[M+1]^+$.

tert-Butyl (_E_)-(2-(_N_-methylisoquinoline-5-sulfonamido)ethyl)(3-(4-(pyridin-3-yl)phenyl)allyl)carbamate (75)



tert-Butyl (*E*)-(3-(4-bromophenyl)allyl)(2-(*N*-methyl isoquinoline-5-sulfonamido)ethyl)carbamate (**74**) (2.57 g, 4.6 mmol, 1 eq), $Pd(PPh_3)_4$ (106 mg, 0.092 mmol, 0.02 eq) and pyridine-3-boronic acid (0.85 g, 6.9 mmol, 1.5 eq) were dissolved in a deoxygenated mixture of DCM (4.6 mL), DMF (10.1 mL) and aqueous K_2CO_3 solution (2 M, 5.7 mL, 11.4 mmol, 2.5 eq) and the reaction was heated under argon atmosphere to 80°C for 17 h. After cooling to ambient temperature, the mixture was concentrated under reduced pressure, diluted with EtOAc, filtered over silica and concentrated again. The residue was purified via flash-column-chromatography (SiO_2, 0% → 2% MeOH in EtOAc) to yield the product (1.91 g, 74%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.33 (d, *J* = 0.4 Hz, 1H), 8.87 (d, *J* = 1.9 Hz, 1H), 8.64 (d, *J* = 6.2 Hz, 1H), 8.59 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.46 (bs, 1H), 8.29 (bs, 1H), 8.17 (d, *J* = 8.2 Hz, 1H), 7.89 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.64 – 7.53 (m, 3H), 7.50 (d, *J* = 7.9 Hz, 2H), 7.37 (dd, *J* = 7.7, 4.9 Hz, 1H), 6.53 (d, *J* = 15.6 Hz, 1H), 6.23 (dt, *J* = 15.8, 6.3 Hz, 1H), 4.04 (s, 2H), 3.56 – 3.21 (m, 4H), 2.93 (t, *J* = 7.8 Hz, 3H), 1.48 (s, 9H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 155.38, 154.99, 153.23, 148.50, 148.07, 145.15, 136.99, 136.44, 136.03, 134.12, 133.55, 133.33, 131.72, 131.26, 129.13, 127.31, 127.15, 126.06, 125.83, 123.62, 117.61, 80.22, 50.16, 48.08, 44.89, 35.07, 28.42. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.38 min; *m/z* : 559 $[M+1]^+$.

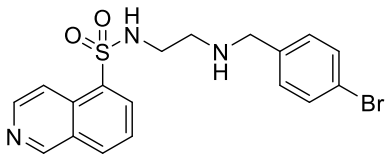
2-(4-(Pyridin-3-yl)phenyl)ethan-1-ol (77)



A vial containing 2-(4-bromophenyl)ethanol (420 µL, 3.0 mmol, 1 eq), $Pd(PPh_3)_4$ (69 mg, 0.060 mmol, 0.02 eq) and pyridine-3-boronic acid (0.55 g, 4.5 mmol, 1.5 eq) was sealed and flushed with argon, after which a deoxygenated mixture of DCM (3 mL), DMF (6.6 mL) and

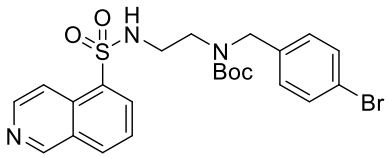
aqueous K_2CO_3 solution (2 M, 3.8 mL, 7.6 mmol, 2.5 eq) was added. After stirring at 80°C for 3 h, the mixture was cooled to ambient temperature, concentrated under reduced pressure, diluted with EtOAc, filtered over silica and concentrated again. The residue was purified via flash-column-chromatography (SiO_2, 80% → 100% EtOAc in pentane) to yield the product (96 mg, 16%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.78 (s, 1H), 8.55 (d, *J* = 4.7 Hz, 1H), 7.86 (d, *J* = 7.9 Hz, 1H), 7.59 – 7.43 (m, 2H), 7.36 (d, *J* = 7.9 Hz, 3H), 3.91 (t, *J* = 6.7 Hz, 2H), 2.97 – 2.90 (m, 2H), 2.80 (bs, 1H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 148.20, 148.07, 139.18, 136.47, 135.76, 134.31, 132.14, 129.85, 123.63, 63.40, 38.95.

_N_-(2-((4-Bromobenzyl)amino)ethyl)isoquinoline-5-sulfonamide (79)



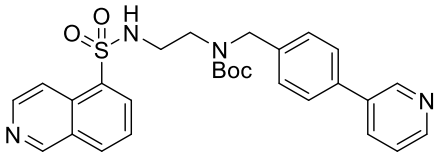
4-Bromobenzaldehyde (0.283 g, 1.5 mmol, 1 eq) and *N*-(2-aminoethyl)isoquinoline-5-sulfonamide (**105**) (0.77 g, 3.1 mmol, 2.05 eq) were dissolved in THF (15 mL). Subsequently, activated molecular sieves (3 Å), glacial acetic acid (87 µL, 1.5 mmol, 1 eq) and $NaHB(OAc)_3$ (0.65 g, 3.1 mmol, 2.05 eq) were added and the reaction was stirred for 18 h. Sat. aqueous Na_2CO_3 solution (35 mL) and Et_2O (40 mL) were added, the organic layer was collected and the aqueous layer extracted with DCM (3x50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 6% → 8% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (633 mg, quant.). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.32 (s, 1H), 8.61 (d, *J* = 6.1 Hz, 1H), 8.45 (d, *J* = 6.1 Hz, 1H), 8.41 (dd, *J* = 7.3, 1.0 Hz, 1H), 8.17 (d, *J* = 8.2 Hz, 1H), 7.67 (t, *J* = 7.8 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 2H), 6.98 (d, *J* = 8.3 Hz, 2H), 4.77 (bs, 2H), 3.51 (s, 2H), 3.08 – 2.97 (m, 2H), 2.64 (t, *J* = 5.6 Hz, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 153.28, 144.90, 138.03, 134.33, 133.58, 133.24, 131.45, 131.19, 129.74, 128.97, 126.03, 120.96, 117.31, 52.25, 47.48, 42.32.

***tert*-Butyl (4-bromobenzyl)(2-(isoquinoline-5-sulfonamido)ethyl)carbamate (80)**



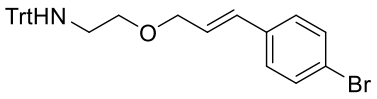
To a solution of *N*-(2-((4-bromobenzyl)amino) ethyl)isoquinoline-5-sulfonamide (**79**) (0.633 g, 1.5 mmol, 1 eq) and $NaHCO_3$ (0.14 g, 1.7 mmol, 1.1 eq) in THF (15 mL) at 0°C was added di-*tert*-butyl dicarbonate (0.36 g, 1.7 mmol, 1.1 eq). The reaction was allowed to warm to RT and stirred for 2 h before it was diluted with sat. aqueous Na_2CO_3 solution (30 mL) and DCM (30 mL). The organic layer was collected and the aqueous layer was extracted with DCM (3x20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 50% → 80% EtOAc in pentane) to yield the product (0.570 g, 73%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.36 (s, 1H), 8.63 (d, *J* = 6.1 Hz, 1H), 8.39 (d, *J* = 6.0 Hz, 1H), 8.35 (dd, *J* = 7.4, 1.2 Hz, 1H), 8.21 (d, *J* = 8.1 Hz, 1H), 7.68 (t, *J* = 7.7 Hz, 1H), 7.39 – 7.31 (m, 2H), 6.97 (s, 2H), 6.45 (s, 1H), 4.27 (s, 2H), 3.31 (s, 2H), 3.00 (s, 2H), 1.41 (s, 9H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 153.28, 145.06, 136.90, 134.31, 133.55, 133.20, 131.72, 131.22, 129.09, 128.88, 125.98, 121.28, 117.38, 81.13, 51.31, 46.76, 42.37, 28.35.

tert-Butyl (2-(isoquinoline-5-sulfonamido)ethyl)(4-(pyridin-3-yl)benzyl)carbamate (81)



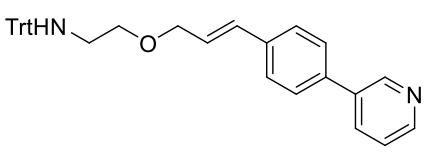
A vial containing *tert*-butyl (4-bromobenzyl)(2-(isoquinoline-5-sulfonamido)ethyl)carbamate (**80**) (0.633 g, 1.1 mmol, 1 eq), $Pd(PPh_3)_4$ (38 mg, 0.033 mmol, 0.03 eq) and pyridine-3-boronic acid (0.20 g, 1.6 mmol, 1.45 eq) was sealed and flushed with argon, after which a deoxygenated mixture of DCM (1.1 mL), DMF (2.2 mL) and aqueous K_2CO_3 solution (2 M, 1.4 mL, 2.8 mmol, 2.5 eq) was added. After stirring at 80°C for 26 h, the mixture was cooled to ambient temperature, concentrated under reduced pressure, diluted with EtOAc, filtered over silica and concentrated again. The residue was purified via flash-column-chromatography (SiO_2, 80% → 100% EtOAc in pentane) to yield the product (0.289 g, 51%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.33 (s, 1H), 8.81 (s, 1H), 8.66 – 8.54 (m, 2H), 8.43 (d, *J* = 5.9 Hz, 1H), 8.38 (dd, *J* = 7.3, 0.9 Hz, 1H), 8.16 (d, *J* = 8.0 Hz, 1H), 7.86 (d, *J* = 7.1 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.39 (dd, *J* = 7.8, 4.9 Hz, 1H), 7.22 (s, 2H), 6.87 (s, 1H), 4.40 (s, 2H), 3.36 (s, 2H), 3.08 (s, 2H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 153.23, 148.44, 148.03, 145.02, 137.93, 136.81, 136.12, 134.58, 134.36, 133.38, 133.04, 131.24, 129.07, 127.94, 127.31, 125.88, 123.72, 117.41, 80.95, 51.48, 46.72, 42.27, 28.35. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.50 min; *m/z* : 519 $[M+1]^+$.

(*E*)-2-((3-(4-Bromophenyl)allyl)oxy)-*N*-tritylethan-1-amine (82)



To a solution of 2-(tritylamino)ethan-1-ol (**60**) (0.91 g, 3.0 mmol, 1.11 eq) in ACN (20 mL) was added NaH (60% in mineral oil, 0.12 g, 3.0 mmol) and (*E*)-1-bromo-4-(3-chloroprop-1-en-1-yl)benzene (**58**) (0.63 g, 2.7 mmol, 1 eq), after which the reaction was stirred at 70°C for 4 h. The solvents were removed under reduced pressure and the mixture was re-dissolved in DCM (60 mL) and washed with sat. aqueous $NaHCO_3$ (40 mL) after which the organic layer was collected and the aqueous layer extracted with DCM (4x25 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 3% → 7% EtOAc in pentane) to yield the product (0.492 g, 37%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.51 – 7.46 (m, 6H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.28 (d, *J* = 7.3 Hz, 6H), 7.23 (d, *J* = 8.5 Hz, 2H), 7.18 (t, *J* = 7.3 Hz, 3H), 6.51 (d, *J* = 16.0 Hz, 1H), 6.23 (dt, *J* = 15.9, 5.8 Hz, 1H), 4.06 (dd, *J* = 5.8, 1.2 Hz, 2H), 3.61 (t, *J* = 5.3 Hz, 2H), 2.38 (t, *J* = 5.2 Hz, 2H), 2.07 (bs, 1H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 146.20, 135.80, 131.79, 130.87, 128.82, 128.11, 127.94, 127.23, 126.39, 121.52, 71.29, 70.79, 70.65, 43.38.

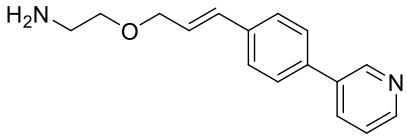
(*E*)-2-((3-(4-(Pyridin-3-yl)phenyl)allyl)oxy)-*N*-tritylethan-1-amine (83)



(*E*)-2-((3-(4-bromophenyl)allyl)oxy)-*N*-trityl ethan-1-amine (**82**) (0.491 g, 0.86 mmol, 1 eq), $Pd(PPh_3)_4$ (20 mg, 0.017 mmol, 0.02 eq) and pyridine-3-boronic acid (0.16 g, 1.3 mmol, 1.5 eq) were dissolved in a deoxygenated mixture of DCM (0.9 mL), DMF (1.9 mL) and aqueous K_2CO_3 solution (2 M, 1.1 mL, 2.2 mmol, 2.5 eq) and the reaction was stirred under argon atmosphere at 80°C for 3 h and at 70 °C for 16 h. After cooling to ambient temperature, the mixture was concentrated under reduced pressure, diluted with EtOAc, filtered over silica and concentrated again. The residue was purified via flash-column-chromatography (SiO_2, 40% → 60% EtOAc in pentane) to yield the product (0.383 g, 78%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.85 (d, *J* = 2.0 Hz, 1H), 8.55 (dd, *J* = 4.8, 1.5 Hz, 1H), 7.82 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.54 – 7.44

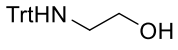
(m, 10H), 7.32 – 7.29 (m, 1H), 7.28 – 7.20 (m, 6H), 7.16 (t, *J* = 7.3 Hz, 3H), 6.61 (d, *J* = 16.0 Hz, 1H), 6.31 (dt, *J* = 15.9, 5.8 Hz, 1H), 4.11 – 4.06 (m, 2H), 3.63 (t, *J* = 5.3 Hz, 2H), 2.40 (t, *J* = 5.3 Hz, 2H), 2.19 (bs, 1H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 148.51, 148.16, 146.11, 136.89, 136.65, 136.10, 134.07, 131.22, 128.72, 127.85, 127.26, 127.19, 127.09, 126.29, 123.58, 71.29, 70.71, 70.49, 43.32.

(*E*)-2-((3-(4-(Pyridin-3-yl)phenyl)allyl)oxy)ethan-1-amine (84)



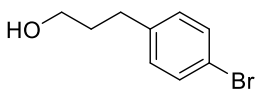
To a solution of (*E*)-2-((3-(4-(pyridin-3-yl)phenyl)allyl)oxy)-*N*-tritylethan-1-amine (**83**) (0.322 g, 0.65 mmol, 1 eq) in DCM (20 mL) was added dropwise TFA (0.30 mL), after which triethylsilane (0.83 mL, 5.2 mmol, 8 eq) was added and the reaction was stirred for 1 h. It was quenched with sat. aqueous Na_2CO_3 (30 mL), the organic layer was collected and the aqueous layer extracted with DCM (3x25 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure after which filtration over silica (DCM, 0.5% Et_3N) yielded the product without further purification (103 mg, 62%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.85 (dd, *J* = 2.4, 0.7 Hz, 1H), 8.58 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.89 – 7.82 (m, 1H), 7.58 – 7.47 (m, 4H), 7.35 (ddd, *J* = 7.9, 4.8, 0.8 Hz, 1H), 6.66 (d, *J* = 16.0 Hz, 1H), 6.37 (dt, *J* = 15.9, 6.0 Hz, 1H), 4.20 (dd, *J* = 6.0, 1.4 Hz, 2H), 3.55 (t, *J* = 5.2 Hz, 2H), 2.92 (t, *J* = 5.2 Hz, 2H), 1.43 (bs, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 148.49, 148.13, 136.93, 136.56, 136.07, 134.07, 131.49, 127.24, 127.18, 126.89, 123.57, 72.68, 71.56, 41.99.

2-(Tritylamino)ethan-1-ol (86)



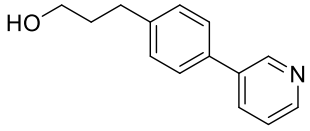
To a solution of trityl chloride (1.39 g, 5.0 mmol, 1 eq) and K_2CO_3 (0.76 g, 5.5 mmol, 1.1 eq) in DCM (17 mL) at 0°C was added dropwise ethanolamine (1.51 mL, 25.0 mmol, 5 eq). The reaction was allowed to warm to RT and stirred for 3 h before it was mixed with sat. aqueous $NaHCO_3$ (15 mL) and H_2O (15 mL). The organic layer was collected and the aqueous layer extracted with DCM (3x30 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 15% → 25% EtOAc in pentane) to yield the product (1.52 g, quant.). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.49 – 7.44 (m, 6H), 7.31 – 7.24 (m, 6H), 7.22 – 7.16 (m, 3H), 3.68 (t, *J* = 5.2 Hz, 2H), 2.34 (t, *J* = 5.2 Hz, 2H), 2.04 (bs, 1H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 145.89, 128.74, 128.02, 126.52, 70.71, 62.76, 45.76.

3-(4-Bromophenyl)propan-1-ol (88)



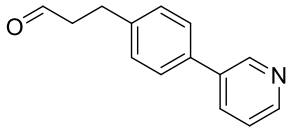
A round-bottom-flask was charged with 3-(4-bromophenyl) propanoic acid (1.00 g, 4.37 mmol, 1 eq) dissolved in dry THF (8.8 mL). After cooling the solution to 0°C $NaBH_4$ (331 mg, 8.73 mmol, 2 eq) was added in small portions and thereafter $BF_3{\cdot}Et_2O$ (1.10 mL, 8.73 mmol, 2 eq) was added dropwise. The resulting mixture was stirred overnight and then quenched by slowly adding MeOH (6 mL), aqueous HCl (1 M, 5 mL) and brine (50 mL). The mixture was then extracted with EtOAc (3x50 mL), the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was re-dissolved in DCM and filtered over Celite to yield the product (0.92 g, 97%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.40 (d, *J* = 8.4 Hz, 2H), 7.07 (d, *J* = 8.4 Hz, 2H), 3.66 (t, *J* = 6.4 Hz, 2H), 2.70 – 2.61 (m, 2H), 1.92 – 1.80 (m, 2H), 1.54 (s, 1H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 140.88, 131.55, 130.33, 119.70, 62.10, 34.11, 31.56.

3-(4-(Pyridin-3-yl)phenyl)propan-1-ol (89)



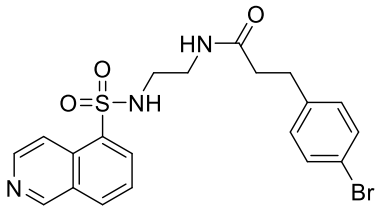
A round-bottom-flask was charged with 3-(4-bromophenyl)propan-1-ol (**88**) (406 mg, 1.89 mmol, 1 eq), pyridin-3-ylboronic acid (348 mg, 2.83 mmol, 1.5 eq) and $Pd(PPh_3)_4$ (20 mg, 0.02 mmol, 0.01 eq) dissolved in DCM (1.9 mL) and DMF (4.2 mL). The flask was put under an argon atmosphere and aqueous K_2CO_3 (2 M, 2.36 mL, 4.73 mmol, 2.5 eq) was added. The reaction mixture was stirred at 85°C for 2.5 h, filtered over celite and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 50% → 90% EtOAc in pentane) to yield the product (296 mg, 74%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.83 (d, *J* = 1.9 Hz, 1H), 8.57 (dd, *J* = 4.8, 1.3 Hz, 1H), 7.88 (dt, *J* = 7.9, 1.9 Hz, 1H), 7.51 (d, *J* = 8.1 Hz, 2H), 7.37 (dd, *J* = 7.8, 4.9 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 2H), 3.72 (t, *J* = 6.4 Hz, 2H), 2.83 – 2.74 (m, 2H), 2.59 (s, 1H), 1.94 (dt, *J* = 13.9, 6.4 Hz, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 148.12, 148.09, 142.25, 136.70, 135.37, 134.49, 129.34, 127.23, 123.75, 62.13, 34.28, 31.85.

3-(4-(Pyridin-3-yl)phenyl)propanal (90)



A round-bottom-flask was charged with 3-(4-(pyridin-3-yl)phenyl)propan-1-ol (**89**) (265 mg, 1.19 mmol, 1 eq) dissolved in DCM (4 mL). After addition of Dess–Martin periodinane (553 mg, 1.30 mmol, 1.1 eq) the reaction-mixture was stirred for 60 min, diluted with DCM (10 mL) and quenched with aqueous $Na_2S_2O_3$ (1 M, 15 mL). The mixture was then extracted with DCM (3x15 mL), the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 40% → 80% EtOAc in pentane) to yield the product (204 mg, 74%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.85 (t, *J* = 1.2 Hz, 1H), 8.84 (d, *J* = 1.9 Hz, 1H), 8.59 (dd, *J* = 4.8, 1.5 Hz, 1H), 7.87 (dt, *J* = 7.9, 2.2 Hz, 1H), 7.52 (d, *J* = 8.2 Hz, 2H), 7.42 – 7.35 (m, 1H), 7.32 (d, *J* = 8.2 Hz, 2H), 3.02 (t, *J* = 7.5 Hz, 2H), 2.84 (t, *J* = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 201.41, 148.23, 148.05, 140.59, 136.46, 135.86, 134.46, 129.18, 127.41, 123.73, 45.23, 27.79.

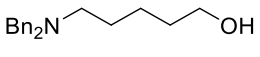
3-(4-Bromophenyl)-*N*-(2-(isoquinoline-5-sulfonamido)ethyl)propanamide (91)



A round-bottom-flask was charged with 3-(4-bromophenyl)propanoic acid (200 mg, 0.87 mmol, 1.05 eq), *N*-(2-aminoethyl)isoquinoline-5-sulfonamide (**105**) (208 mg, 0.83 mmol, 1 eq), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (184 mg, 0.96 mmol, 1.15 eq) and hydroxybenzotriazole (130 mg, 0.96 mmol, 1.15 eq) suspended in DCM (9 mL). After addition of DiPEA (0.23 mL, 1.31 mmol, 1.5 eq) the reaction mixture was stirred for 4 h, quenched with half sat. aqueous $NaHCO_3$ (10 mL) and extracted with DCM (3x10 mL). The combined organic layers were washed with brine (1x50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 0.5% → 4% MeOH in DCM) to yield the desired product (0.41 g, quant.). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.36 (s, 1H), 8.67 (d, *J* = 6.1 Hz, 1H), 8.45 – 8.38 (m, 2H), 8.22 (d, *J* = 8.2 Hz, 1H), 7.75 – 7.68 (m, 1H), 7.35 (d, *J* = 8.3 Hz, 2H), 7.00 (d, *J* = 8.3 Hz, 2H), 6.20 (t, *J* = 5.5 Hz, 1H), 6.12 (t, *J* = 5.5 Hz, 1H), 3.30 (q, *J* = 5.7 Hz, 2H), 3.01 (q, *J* = 5.7 Hz, 2H), 2.83 (t, *J* = 7.6 Hz, 2H), 2.36 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 173.29, 153.31, 145.07, 139.63, 134.20, 133.91, 133.48, 131.69, 131.31, 130.22, 129.16, 126.19, 120.18, 117.45, 43.47, 39.56, 37.89, 30.92. LCMS (ESI,

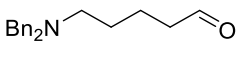
C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.39 min; *m/z* : 462, 464 $[M+1]^+$.

5-(Dibenzylamino)pentan-1-ol (93)



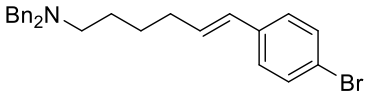
5-Amino-1-pentanol (0.57 mL, 5.0 mmol, 1 eq) and benzaldehyde (1.28 mL, 13 mmol, 2.6 eq) were dissolved in dry THF (50 mL), after which $NaH(OAc)_3$ (3.2 g, 15 mmol, 3 eq) and activated molecular sieves (3 Å) were added. The mixture was stirred for 23 h before sat. aqueous Na_2CO_3 (150 mL) and Et_2O (100 mL) were added, the organic layer was collected and the aqueous layer extracted with DCM (3x50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified via flash-column-chromatography (SiO_2, 1% → 10% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (1.37 g, 96%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.39 – 7.19 (m, 10H), 3.59 – 3.53 (m, 6H), 2.41 (t, *J* = 7.1 Hz, 2H), 1.57 – 1.48 (m, 2H), 1.48 – 1.40 (m, 2H), 1.38 – 1.27 (m, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 140.05, 128.91, 128.26, 126.87, 63.06, 58.44, 53.28, 32.62, 26.88, 23.39.

5-(Dibenzylamino)pentanal (94)



A solution of oxalyl chloride (0.8 mL, 9.5 mmol, 3.2 eq) in DCM (15 mL) was cooled to -78 °C under argon atmosphere, after which DMSO (1.3 mL, 18 mmol, 6 eq) was added dropwise, 15 min later a solution of 5-(dibenzylamino)pentan-1-ol (**93**) (0.857 g, 3.0 mmol, 1 eq) in DCM (5 mL) was added dropwise and 1 h later Et_3N (3.4 mL, 24 mmol, 8 eq) was added dropwise after which the reaction was allowed to warm to RT over 1 h. The reaction was quenched with sat. aqueous NH_4Cl (2 mL), diluted with sat. aqueous $NaHCO_3$ (100 mL) and DCM (50 mL), after which the organic layer was collected and the aqueous layer extracted with DCM (3x60 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified via flash-column-chromatography (SiO_2, 10% → 30% EtOAc in pentane) to yield the product (0.774 g, 93%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.67 (s, 1H), 7.38 – 7.33 (m, 4H), 7.33 – 7.27 (m, 4H), 7.26 – 7.20 (m, 2H), 3.53 (s, 4H), 2.42 (t, *J* = 6.8 Hz, 2H), 2.27 (td, *J* = 7.3, 1.7 Hz, 2H), 1.71 – 1.56 (m, 2H), 1.56 – 1.47 (m, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 202.87, 139.93, 128.92, 128.31, 126.96, 58.51, 52.70, 43.59, 26.55, 19.69.

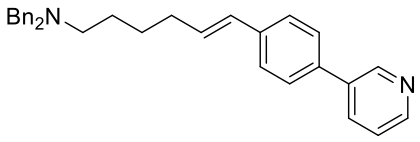
(*E*)-*N*,*N*-Dibenzyl-6-(4-bromophenyl)hex-5-en-1-amine (95)



To a solution of NaH (60% in mineral oil, 6 mg, 0.15 mmol, 1.05 eq) in dry THF (0.2 mL) at 0°C was added dropwise a solution of diethyl (4-bromobenzyl)phosphonate (44 mg, 0.14 mmol, 1 eq) in dry THF (0.2 mL) and the reaction was allowed to warm to RT over 1 h. The solution was cooled to 0°C and a solution of 5-(dibenzylamino)pentanal (**94**) (40 mg, 0.14 mmol, 1 eq) in dry THF (0.4 mL) was added dropwise before the reaction was allowed to warm to RT. After 16 h the reaction was quenched with sat. aqueous NH_4Cl (0.5 mL) and diluted with sat. aqueous $NaHCO_3$ (10 mL) and DCM (10 mL), after which the organic layer was collected and the aqueous layer was extracted with DCM (3x10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified via flash-column-chromatography (SiO_2, 5% → 20% EtOAc in pentane) to yield the product (30 mg, 48%). ^{1}H NMR (400 MHz, chloroform-d) δ 7.41 – 7.39 (m, 1H), 7.37 (dd, *J* = 6.7, 4.5 Hz, 5H), 7.33 – 7.27 (m, 4H), 7.25 – 7.19 (m, 2H), 7.19 – 7.14 (m, 2H), 6.23 (d, *J* = 15.9 Hz, 1H), 6.14 (dt, *J* = 15.8, 6.5 Hz, 1H), 3.54 (s, 4H), 2.42 (t, *J* = 7.0 Hz, 2H), 2.09 (q, *J* = 6.7 Hz, 2H), 1.60 – 1.50 (m, 2H), 1.49 –

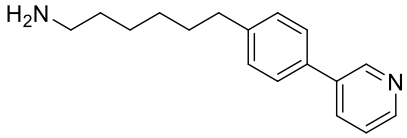
1.39 (m, 2H). ^{13}C NMR (101 MHz, chloroform-d) δ 140.11, 136.91, 131.98, 131.63, 128.89, 128.84, 128.28, 127.59, 126.88, 120.49, 58.48, 53.18, 32.87, 26.83, 26.63.

(*E*)-*N*,*N*-Dibenzyl-6-(4-(pyridin-3-yl)phenyl)hex-5-en-1-amine (96)



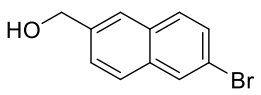
(*E*)-*N*,*N*-dibenzyl-6-(4-bromophenyl)hex-5-en-1-amine (**95**) (0.272 g, 0.63 mmol, 1 eq), $Pd(PPh_3)_4$ (44 mg, 0.038 mmol, 0.06 eq) and pyridine-3-boronic acid (0.12 g, 0.94 mmol, 1.5 eq) were dissolved in a deoxygenated mixture of DCM (0.6 mL), DMF (1.4 mL) and aqueous K_2CO_3 (2 M, 0.8 mL, 1.6 mmol, 2.5 eq) and the reaction was stirred at 80°C for 27 h under argon atmosphere. After cooling to ambient temperature, the mixture was concentrated under reduced pressure, diluted with EtOAc, filtered over silica and concentrated again. The resulting residue was purified via flash-column-chromatography (SiO_2, 20% → 50% EtOAc in pentane) to yield the product (224 mg, 83%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.85 (d, *J* = 1.7 Hz, 1H), 8.57 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.85 (ddd, *J* = 7.9, 2.3, 1.7 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 8.3 Hz, 2H), 7.39 – 7.36 (m, 4H), 7.36 – 7.33 (m, 1H), 7.33 – 7.28 (m, 4H), 7.25 – 7.19 (m, 2H), 6.34 (d, *J* = 15.9 Hz, 1H), 6.23 (dt, *J* = 15.8, 6.7 Hz, 1H), 3.55 (s, 4H), 2.44 (t, *J* = 6.9 Hz, 2H), 2.14 (q, *J* = 6.8 Hz, 2H), 1.57 (dt, *J* = 14.0, 6.9 Hz, 2H), 1.53 – 1.42 (m, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 148.42, 148.24, 140.10, 137.92, 136.41, 136.16, 134.13, 132.00, 129.27, 128.89, 128.27, 127.28, 126.87, 126.71, 123.64, 58.46, 53.19, 32.95, 26.91, 26.63.

6-(4-(Pyridin-3-yl)phenyl)hexan-1-amine (97)



A vial containing (*E*)-*N*,*N*-dibenzyl-6-(4-(pyridin-3-yl)phenyl)hex-5-en-1-amine (**96**) (0.204 g, 0.47 mmol, 1 eq) in a mixture of *t*-BuOH/dioxane/H_2O (1:1:0.1, 5 mL) was flushed with argon. $Pd(OH)_2$ (30 wt%, 61 mg) was added and the vial was sealed. The mixture was flushed with H_2 gas for 1 h under vigorous stirring and heated to 50 °C for 3 days under H_2 atmosphere, with periodical H_2 flushing (3x1 h). The mixture was concentrated under reduced pressure and purified via flash-column-chromatography (SiO_2, 15% → 40% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (75 mg, 63%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.84 (dd, *J* = 2.3, 0.7 Hz, 1H), 8.56 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.88 – 7.81 (m, 1H), 7.55 – 7.46 (m, 2H), 7.37 – 7.30 (m, 1H), 7.30 – 7.24 (m, 2H), 3.53 (bs, 2H), 2.75 (t, *J* = 7.2 Hz, 2H), 2.65 (t, *J* = 7.6 Hz, 2H), 1.66 (dd, *J* = 10.2, 4.7 Hz, 2H), 1.58 – 1.46 (m, 2H), 1.43 – 1.33 (m, 4H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 148.19, 148.18, 142.83, 136.55, 135.16, 134.16, 129.16, 127.01, 123.54, 41.61, 35.52, 32.29, 31.33, 29.04, 26.71.

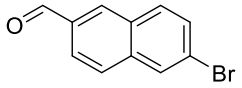
(6-Bromonaphthalen-2-yl)methanol (99)



To a solution of 6-bromo-2-naphthoic acid (1.48 g, 5.9 mmol, 1 eq) dissolved in dry THF (75 mL) at 0°C was added dropwise a lithium aluminium hydride solution (2.4 M, 5 mL, 12 mmol, 2 eq). The reaction was allowed to warm to RT, and after 1 h of stirring it was quenched by addition of H_2O (0.5 mL), queous NaOH (10%, 1 mL) solution and H_2O (3 mL), after which it was stirred for 16 h. The mixture was dried by addition of $MgSO_4$, filtered and the filtrate was concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 10% → 40% EtOAc in pentane) to yield the product (0.872 g, 63%). ^{1}H NMR (400 MHz, chloroform-d) δ 7.99 (s, 1H), 7.79 – 7.72 (m, 2H), 7.69 (d, *J* = 8.7 Hz, 1H), 7.55 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.53 –

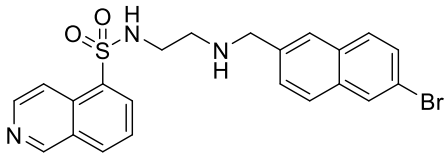
7.46 (m, 1H), 4.84 (s, 2H), 1.79 (s, 1H). ^{13}C NMR (101 MHz, chloroform-d) δ 138.94, 134.07, 131.89, 129.90, 129.71, 129.67, 127.55, 126.29, 125.41, 119.95, 65.37.

6-Bromo-2-naphthaldehyde (100)



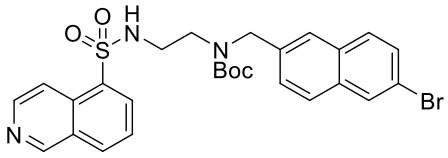
(6-bromonaphthalen-2-yl)methanol (**99**) (0.106 g, 0.45 mmol, 1 eq) and Dess–Martin periodinane (0.23 g, 0.54 mmol, 1.2 eq) were dissolved in DCM (5.4 mL) and subsequently stirred for 1 h at RT. Aqueous $Na_2S_2O_3$ solution (1 M, 10 mL) was added to quench excess reagent, after which the mixture was diluted with H_2O (10 mL). The phases were separated, the aqueous layer was extracted with DCM (3x20 mL), the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 5% → 6% EtOAc in pentane) to yield the product (0.105 g, quant.). ^{1}H NMR (400 MHz, chloroform-*d*) δ 10.13 (s, 1H), 8.27 (s, 1H), 8.04 (s, 1H), 7.96 (d, *J* = 8.4 Hz, 1H), 7.82 (t, *J* = 9.6 Hz, 2H), 7.68 – 7.58 (m, 1H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 191.93, 137.31, 134.36, 134.18, 131.11, 131.05, 130.70, 130.32, 128.23, 124.06, 123.67.

***N*-(2-(((6-Bromonaphthalen-2-yl)methyl)amino)ethyl)isoquinoline-5-sulfonamide (101)**



6-bromo-2-naphthaldehyde (**100**) (0.538 g, 2.3 mmol, 1 eq) and *N*-(2-aminoethyl)isoquinoline-5-sulfonamide (**105**) (1.2 g, 4.6 mmol, 2 eq) were dissolved in dry THF (23 mL) by sonication, after which glacial acetic acid (0.13 mL, 2.3 mmol, 1 eq) and $NaHB(OAc)_3$ (0.97 g, 4.6 mmol, 2 eq) were added and the reaction was stirred for 19 h at RT. The reaction was diluted with EtOAc (100 mL), sat. aqueous K_2CO_3 solution (100 mL) was added, the phases separated and the aqueous layer was extracted with EtOAc (3x50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 1.5% → 2.5% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (788 mg, 74%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.19 (s, 1H), 8.53 (d, *J* = 6.1 Hz, 1H), 8.45 (d, *J* = 6.1 Hz, 1H), 8.36 (d, *J* = 7.3 Hz, 1H), 7.95 (d, *J* = 8.2 Hz, 1H), 7.81 (s, 1H), 7.55 – 7.43 (m, 3H), 7.43 – 7.36 (m, 2H), 7.15 (d, *J* = 9.4 Hz, 1H), 4.21 (bs, 2H), 3.58 (s, 2H), 3.03 (t, *J* = 5.6 Hz, 2H), 2.62 (t, *J* = 5.6 Hz, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 152.99, 144.50, 137.49, 134.17, 133.25, 133.19, 132.96, 131.32, 130.91, 129.37, 129.12, 129.10, 128.66, 127.09, 126.88, 125.93, 125.78, 119.25, 117.17, 52.80, 47.56, 42.38.

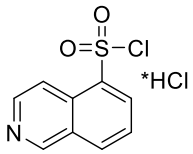
***tert*-Butyl ((6-bromonaphthalen-2-yl)methyl)(2-(isoquinoline-5-sulfonamido) ethyl)carbamate (102)**



A solution of *N*-(2-(((6-bromonaphthalen-2-yl)methyl)amino)ethyl)isoquinoline-5-sulfonamide (**101**) (0.788 g, 1.7 mmol, 1 eq) and $NaHCO_3$ (0.17 g, 2.0 mmol, 1.2 eq) in THF (8.4 mL) was cooled to 0°C after which di-*tert*-butyl dicarbonate (0.55 g, 2.5 mmol, 1.5 eq) were added and the reaction was allowed to warm to RT. After stirring for 24 hours sat. aqueous $NaHCO_3$ solution (20 mL) and DCM (20 mL) were added after which the organic layer was collected and the aqueous layer was extracted with DCM (3x20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 40% → 70% EtOAc in pentane) to yield the product (0.448 g, 46%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.33 (s, 1H), 8.63 (d, *J* = 5.4 Hz, 1H), 8.37 (s, 1H), 8.26 (s, 1H), 8.13 (d, *J* = 8.2 Hz, 1H), 7.95 (s, 1H), 7.62 (d, *J* = 3.3 Hz, 1H), 7.60

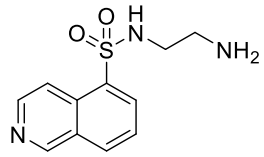
(d, *J* = 3.1 Hz, 1H), 7.58 – 7.52 (m, 2H), 7.50 (s, 1H), 7.24 (s, 1H), 6.22 (s, 1H), 4.46 (s, 2H), 3.35 (s, 2H), 3.01 (s, 2H), 1.44 (s, 9H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 153.35, 145.26, 135.83, 134.36, 133.84, 133.52, 133.17, 131.69, 131.27, 129.84, 129.48, 129.10, 127.82, 127.75, 126.29, 125.96, 125.86, 124.89, 120.03, 117.39, 81.28, 51.98, 46.66, 42.63, 28.47.

Isoquinoline-5-sulfonyl chloride (104)



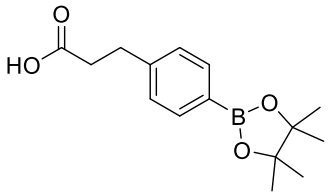
A flask was charged with isoquinoline-5-sulfonic acid (3.20 g, 15.30 mmol, 1 eq) dissolved in $SOCl_2$ (20 mL). After addition of DMF (0.5 mL) the mixture was heated to reflux for 4 h. Excess $SOCl_2$ was removed under reduced pressure, the resulting solid was re-suspended in DCM, filtered over a glass-filter and washed with DCM to yield the product (3.83 g, 95%). Due to the unstable nature of the product it was used without further purification.

***N*-(2-Aminoethyl)isoquinoline-5-sulfonamide (105)**



A solution of isoquinoline-5-sulfonic acid (4.01 g, 19.16 mmol, 1 eq) and catalytic DMF (0.1 mL) in $SOCl_2$ (25 mL) was stirred under reflux for three hours. The mixture was filtered over a glass filter and the resulting white powder was washed thoroughly with DCM and dried under reduced pressure. It was dissolved in a 4°C sat. aqueous $NaHCO_3$ solution and extracted with DCM (3x40 mL). The combined organic layers were dried over $MgSO_4$, filtered and added dropwise over half an hour to an ice cold solution of ethylenediamine (7.6 mL, 114 mmol, 6 eq) in DCM (200 mL). The reaction was allowed to warm to RT and 1.5 h later sat. aqueous Na_2CO_3 (200 mL) were added. The mixture was extracted with DCM (3x150 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was purified via flash-column-chromatography (SiO_2, 3% → 10% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the desired product (3.78 g, 79%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.37 (s, 1H), 8.68 (d, *J* = 6.1 Hz, 1H), 8.48 – 8.41 (m, 2H), 8.22 (d, *J* = 8.2 Hz, 1H), 7.76 – 7.66 (m, 1H), 2.96 (dd, *J* = 6.5, 4.8 Hz, 2H), 2.77 (dd, *J* = 6.5, 4.8 Hz, 2H), 2.66 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 153.41, 145.21, 134.50, 133.67, 133.38, 131.37, 129.16, 126.08, 117.38, 45.25, 40.91. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 0.8 min; *m/z* : 252 $[M+1]^+$.

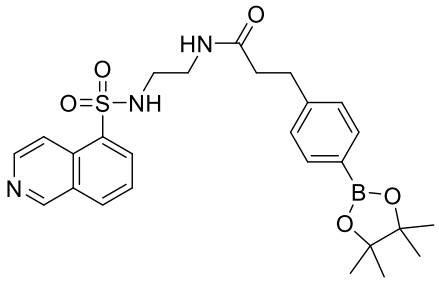
3-(4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoic acid (106)



A round-bottom-flask was charged with 3-(4-bromophenyl)propanoic acid (2.00 g, 8.73 mmol, 1 eq), bis(pinacolato)diboron (3.33 g, 13.10 mmol, 1.5 eq), potassium acetate (4.28 g, 43.65 mmol, 5 eq) and [1,1'-bis (diphenylphosphino)ferrocene]dichloropalladium (357 mg, 0.44 mmol, 0.05 eq) suspended in dry and degassed 1,4-dioxane (44 mL). The reaction mixture was degassed for 30 min by passing N_2 through it while sonicating and stirred at 100°C overnight. The resulting black solution was concentrated in vacuum, re-suspended in EtOAc (100 mL) and extracted with aqueous NaOH (2 M, 3x100 mL). The combined aqueous layers where acidified to pH ~4 with conc. aqueous HCl and extracted with EtOAc (3x100 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentration under reduced pressure yielded the product (2.47 g, quant.), which was used without further purification. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.75 (d, *J* = 8.0 Hz, 1H), 7.22

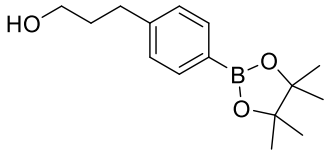
(d, *J* = 7.9 Hz, 1H), 2.97 (t, *J* = 7.8 Hz, 1H), 2.68 (t, *J* = 7.8 Hz, 1H), 1.34 (s, 12H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 178.18, 143.60, 135.23, 127.85, 83.88, 35.39, 30.91, 25.00.

_N_-(2-(Isoquinoline-5-sulfonamido)ethyl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanamide (107)



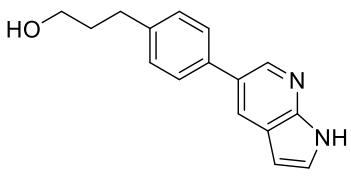
A round-bottom-flask was charged with 3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl) propanoic acid (**106**) (1.00 g, 3.62 mmol, 1 eq), *N*-(2-aminoethyl)isoquinoline-5-sulfonamide (**105**) (0.96 g, 3.80 mmol, 1.05 eq), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (764 mg, 3.98 mmol, 1.1 eq) and hydroxybenzotriazole (538 mg, 3.98 mmol, 1.1 eq) suspended in DCM (36 mL). After addition of DiPEA (0.95 mL, 5.43 mmol, 1.5 eq) the reaction mixture was stirred for 4 h, diluted with H_2O (100 mL) and extracted with DCM (3x100 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 3% → 10% MeOH in DCM) to yield the product (1.45 g, 79%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.35 (s, 1H), 8.70 (d, *J* = 6.0 Hz, 1H), 8.44 – 8.36 (m, 2H), 8.20 (d, *J* = 8.2 Hz, 1H), 7.75 – 7.66 (m, 3H), 7.14 (d, *J* = 7.9 Hz, 2H), 5.92 (dt, *J* = 11.4, 5.6 Hz, 2H), 3.24 (q, *J* = 5.7 Hz, 2H), 2.98 (q, *J* = 5.6 Hz, 2H), 2.88 (t, *J* = 7.5 Hz, 2H), 2.37 (t, *J* = 7.6 Hz, 2H), 1.33 (s, 12H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 173.49, 153.29, 145.16, 143.99, 135.22, 134.39, 133.80, 133.42, 131.35, 129.17, 127.92, 126.15, 117.54, 83.94, 75.17, 43.45, 39.67, 38.09, 31.89, 25.01. LCMS (ESI, Thermo, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.81 min; *m/z* : 510 $[M+1]^+$.

3-(4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propan-1-ol (108)



A round-bottom-flask was charged with 3-(4-bromophenyl)propan-1-ol (**88**) (916 mg, 4.26 mmol, 1 eq), bis (pinacolato)diboron (1.63 g, 6.39 mmol, 1.5 eq), potassium acetate (2.09 g, 21.29 mmol, 5 eq) and [1,1'-bis (diphenylphosphino) ferrocene]dichloropalladium (174 mg, 0.21 mmol, 0.05 eq). The flask was put under argon atmosphere and after the reactants were suspended in 1,4-dioxane (22 mL) the mixture was heated to 100°C overnight and then concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 0% → 20% EtOAc in pentane) to yield the product (1.03 g, 92%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.74 (d, *J* = 7.4 Hz, 2H), 7.22 (d, *J* = 7.4 Hz, 2H), 3.66 (t, *J* = 6.3 Hz, 2H), 2.72 (t, *J* = 7.6 Hz, 2H), 1.89 (p, *J* = 6.7 Hz, 2H), 1.34 (s, 12H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 145.40, 135.09, 128.05, 83.80, 62.35, 34.20, 32.40, 24.98.

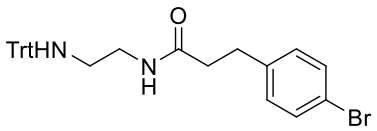
3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)propan-1-ol (109)



A round-bottom-flask was charged with 3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propan-1-ol (**108**) (0.51 g, 1.95 mmol, 1 eq), 5-bromo-7-azindole (0.58 g, 2.92 mmol, 1.5 eq) and $Pd(PPh_3)_4$ (112 mg, 0.097 mmol, 0.05 eq). The flask was put under an argon atmosphere and degassed DMF (7 mL) and degassed aqueous K_2CO_3 solution (2 M, 2.43 mL, 4.88 mmol, 2.5 eq) were added. After the reaction mixture was stirred at 85°C overnight, sat. aqueous $NaHCO_3$ (40 mL) was added and the product was extracted with DCM (3x40 mL). The combined organic

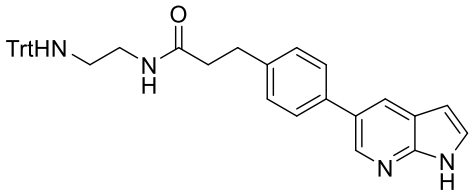
layers were washed with brine (1x100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, 50% → 70% EtOAc in pentane) to yield the desired product (0.248 g, 51%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.69 (s, 1H), 8.49 (d, *J* = 1.9 Hz, 1H), 8.20 – 8.13 (m, 1H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.51 – 7.48 (m, 1H), 7.29 (d, *J* = 8.0 Hz, 2H), 6.49 (s, 1H), 4.50 (t, *J* = 5.0 Hz, 1H), 3.45 (q, *J* = 6.1 Hz, 2H), 2.70 – 2.62 (m, 2H), 1.75 (p, *J* = 6.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 147.96, 141.40, 140.81, 136.48, 128.96, 128.15, 126.85, 126.76, 125.82, 119.68, 100.10, 60.11, 34.29, 31.25.

3-(4-Bromophenyl)-*N*-(2-(tritylamino)ethyl)propanamide (110)



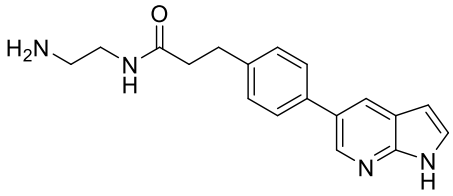
3-(4-Bromophenyl) propionic acid (3.00 g, 13.10 mmol, 1.05 eq), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (3.63 g, 13.72 mmol, 1.1 eq), hydroxybenzotriazole (1.85 g, 13.7 mmol, 1.1 eq) and N^1-tritylethane-1,2-diamine (**60**) (3.77 g, 12.47 mmol, 1.0 eq) were dissolved in DCM (130 mL). DiPEA (3.26 mL, 18.71 mmol, 1.5 eq) was added and the mixture stirred for 16 h at RT. The mixture was quenched with saturated aqueous $NaHCO_3$ (300 mL). The phases were separated and the aqueous layer was extracted with DCM (3x200 mL). The combined organic layers were washed with brine (1x250 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified via flash-column-chromatography (SiO_2, 20% → 60% EtOAc in pentane) to yield the product (4.52 g, 71%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.45 – 7.39 (m, 6H), 7.37 – 7.31 (m, 2H), 7.30 – 7.24 (m, 7H), 7.22 – 7.17 (m, 3H), 7.09 – 7.03 (m, 2H), 5.68 (s, 1H), 3.33 (q, *J* = 6.0 Hz, 2H), 2.92 (t, *J* = 7.6 Hz, 2H), 2.44 (t, *J* = 7.6 Hz, 2H), 2.26 (t, *J* = 6.1 Hz, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 171.82, 145.75, 140.01, 131.70, 130.26, 128.64, 128.05, 126.61, 120.16, 43.55, 40.15, 38.39, 31.13. TLCMS (ESI): *m/z* : 513 $[M+1]^+$.

3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(2-(tritylamino)ethyl) propanamide (111)



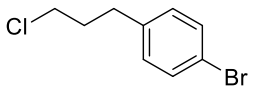
3-(4-Bromophenyl)-*N*-(2-(tritylamino) ethyl) propanamide (**110**) (1.00 g, 1.95 mmol, 1.0 eq), 5-(4,4,5,5- tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrrolo[2,3-*b*]pyridine (710 mg, 2.92 mmol, 1.5 eq) and $Pd(PPh_3)_4$ (45 mg, 0.039 mmol, 0.02 eq) were dissolved in deoxygenated DMF (8 mL) and aqueous K_2CO_3 (2 M, 2.43 mL, 4.87 mmol, 2.5 eq). The mixture was stirred for 18 h at 85°C. The reaction mixture was filtered over silica, washed with EtOAc and concentrated under reduced pressure. The resulting residue was purified via flash-column-chromatography (SiO_2, 50% → 100% EtOAc in pentane) to yield the product (0.91 g, 85%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 10.41 – 10.07 (m, 1H), 8.54 – 8.52 (m, 1H), 8.09 (d, *J* = 1.8 Hz, 1H), 7.53 (d, *J* = 8.1 Hz, 2H), 7.45 (d, *J* = 7.4 Hz, 6H), 7.40 (s, 1H), 7.34 (d, *J* = 8.1 Hz, 2H), 7.32 – 7.25 (m, 7H), 7.20 (t, *J* = 7.2 Hz, 3H), 6.59 – 6.57 (m, 1H), 5.87 (s, 1H), 3.40 (q, *J* = 5.8 Hz, 2H), 3.07 (t, *J* = 7.6 Hz, 2H), 2.58 (t, *J* = 7.6 Hz, 2H), 2.32 (t, *J* = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 172.18, 148.09, 145.71, 142.18, 139.81, 137.59, 129.52, 128.98, 128.57, 127.96, 127.55, 127.29, 126.50, 125.81, 120.35, 101.20, 70.82, 43.48, 40.12, 38.58, 31.37. TLCMS (ESI): *m/z* : 551 $[M+1]^+$.

3-(4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)-*N*-(2-aminoethyl)propanamide (113)



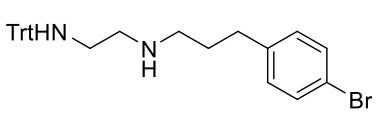
3-(4-(1*H*-pyrrolo[2,3-b]pyridin-5-yl)phenyl)-*N*-(2-(tritylamino) ethyl) propanamide (**111**) (0.827 g, 1.50 mmol, 1.0 eq) was dissolved in DCM (48 mL). TFA (0.67 mL, 9.01 mmol, 6.0 eq) was added dropwise over 10 min at 0°C. Subsequently, triethylsilane (1.92 mL, 12.0 mmol, 8.0 eq) was added to the reaction mixture and it was stirred for 16 h at RT. The mixture was quenched with sat. aqueous Na_2CO_3 (250 mL). The phases were separated and the aqueous layer was extracted with a mixture of 5% MeOH in $CHCl_3$ (3x100 mL). The combined organic layers were washed with brine (1x250 mL), dried over Na_2SO_4, filtered and concentrated onto celite under reduced pressure. The resulting residue was purified via flash-column-chromatography (SiO_2, 10% → 25% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (0.404 g, 86%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.35 (d, *J* = 2.0 Hz, 1H), 8.08 (s, 1H), 7.47 (d, *J* = 7.8 Hz, 2H), 7.36 (d, *J* = 3.5 Hz, 1H), 7.23 (d, *J* = 7.9 Hz, 2H), 6.47 (d, *J* = 3.5 Hz, 1H), 3.18 (d, *J* = 6.2 Hz, 2H), 2.90 (t, *J* = 7.5 Hz, 2H), 2.61 (t, *J* = 6.2 Hz, 2H), 2.48 (t, *J* = 7.5 Hz, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 175.45, 148.67, 142.12, 140.97, 138.57, 130.41, 130.08, 128.31, 128.22, 127.72, 122.25, 101.71, 42.74, 41.82, 38.87, 32.46. TLCMS (ESI): *m/z* : 309 $[M+H]^+$.

1-Bromo-4-(3-chloropropyl)benzene (114)



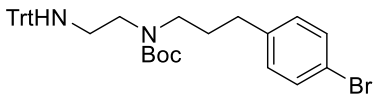
3-(4-Bromophenyl)propan-1-ol (**88**) (3.5 g, 15 mmol, 1.0 eq) was dissolved in DMF (30 mL). The solution was cooled to 0°C and thionyl chloride (2.36 mL, 32.54 mmol, 2.2 eq) was added and the resulting mixture stirred for 19 h at RT. The mixture was quenched with H_2O (1x100 mL) and washed with H_2O (2x100 mL). The phases were separated and the combined aqueous layers were extracted with Et_2O (2x100 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The resulting residue was purified via flash-column-chromatography (SiO_2, 100% pentane) to yield the product (3.75 g, 99%). ^{1}H NMR (300 MHz, chloroform-*d*) δ 7.46 – 7.37 (m, 2H), 7.08 (d, *J* = 8.4 Hz, 2H), 3.51 (t, *J* = 6.4 Hz, 2H), 2.74 (t, *J* = 7.4 Hz, 2H), 2.12 – 1.98 (m, 2H). ^{13}C NMR (75 MHz, chloroform-*d*) δ 139.74, 131.68, 130.44, 120.04, 44.11, 33.90, 32.24.

***N*^{1}-(3-(4-Bromophenyl)propyl)-*N*^{2}-tritylethane-1,2-diamine (115)**



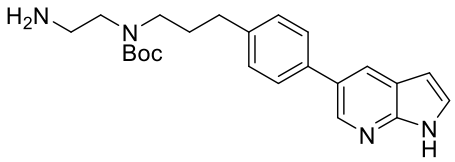
1-Bromo-4-(3-chloropropyl)benzene (**114**) (3.70 g, 15.8 mmol, 1.0 eq), *N*^{1}-tritylethane-1,2-diamine (**60**) (14.37 g, 47.53 mmol, 3.0 eq) and K_2CO_3 (4.38 g, 31.69 mmol, 2.0 eq) were suspended in ACN (55 mL). The mixture was heated to 70°C and stirred for 72 h. The reaction mixture was cooled to RT, filtered and the solvent removed under reduced pressure. The reaction mixture was concentrated onto celite and purified via flash-column-chromatography (SiO_2, dry-loading, 0.5% → 3% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (5.56 g, 70%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.46 (dt, *J* = 8.5, 1.9 Hz, 6H), 7.39 – 7.34 (m, 2H), 7.29 – 7.23 (m, 6H), 7.20 – 7.14 (m, 3H), 7.04 (d, *J* = 8.4 Hz, 2H), 2.71 (t, *J* = 5.9 Hz, 2H), 2.61 – 2.56 (m, 2H), 2.56 – 2.51 (m, 2H), 2.28 (t, *J* = 5.9 Hz, 2H), 1.88 (bs, 2H), 1.77 (p, *J* = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 146.21, 141.10, 131.49, 130.26, 128.78, 127.90, 126.36, 119.61, 70.87, 50.13, 48.94, 43.07, 33.06, 31.49. TLCMS (ESI): *m/z* : 499 $[M+H]^+$.

tert-Butyl (3-(4-bromophenyl)propyl)(2-(tritylamino)ethyl)carbamate (116)



N^1-(3-(4-Bromophenyl)propyl)-N^2-tritylethane-1,2-diamine (**115**) (5.51 g, 11.0 mmol, 1.0 eq), di-*tert*-butyl dicarbonate (3.86 g, 17.6 mmol, 1.6 eq) and $NaHCO_3$ (1.11 g, 13.2 mmol, 1.2 eq) were dissolved in THF (37 mL). The reaction mixture was stirred for 36 h at RT. The mixture was quenched with sat. aqueous $NaHCO_3$ (300 mL). The phases were separated and the aqueous layer was extracted with DCM (3x200 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The resulting residue was purified via flash-column-chromatography (SiO_2, 5% → 40% Et_2O in pentane) to yield the product (6.62 g, quant.). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.47 – 7.41 (m, 6H), 7.37 (d, *J* = 8.1 Hz, 2H), 7.29 – 7.21 (m, 6H), 7.17 (t, *J* = 7.2 Hz, 3H), 7.00 (d, *J* = 8.3 Hz, 2H), 3.28 (s, 2H), 3.17 (s, 2H), 2.54 – 2.44 (m, 2H), 2.27 (bs, 2H), 1.74 (p, *J* = 7.7 Hz, 2H), 1.62 (bs, 1H), 1.50 – 1.30 (m, 9H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 155.72, 146.08, 140.88, 131.51, 130.18, 128.66, 127.95, 126.40, 119.66, 79.53, 70.84, 48.01, 47.41, 42.56, 32.74, 29.91, 28.54. TLCMS (ESI): *m/z* : 599 $[M+H]^+$.

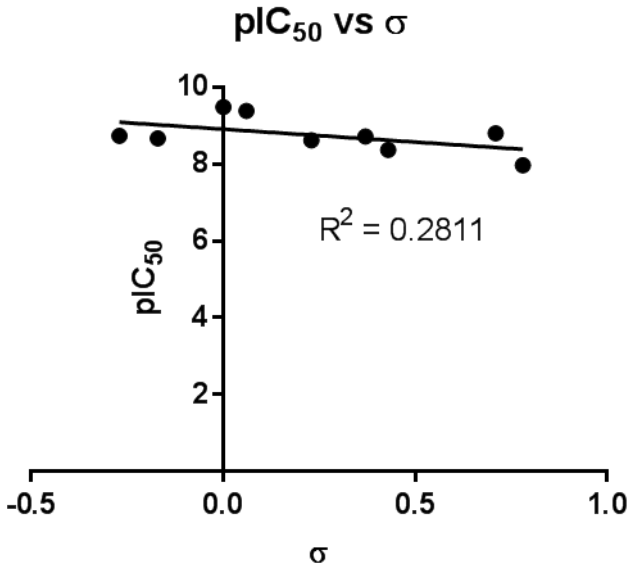
tert-Butyl (3-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)phenyl)propyl)(2-aminoethyl) carbamate (117)



<u>Step 1:</u> *tert*-Butyl (3-(4-bromophenyl)propyl)(2-(tritylamino)ethyl)carbamate (**116**) (6.62 g, 11.04 mmol, 1.0 eq), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (4.03 g, 16.56 mmol, 1.5 eq) and $Pd(PPh_3)_4$ (255 mg, 0.26 mmol, 0.02 eq) were dissolved in deoxygenated DMF (48 mL) and aqueous K_2CO_3 (2 M, 13.80 mL, 26.60 mmol, 2.5 eq). The mixture was stirred for 17 h at 90°C and then filtered over celite and silica. The resulting residue was purified via flash-column-chromatography (SiO_2, 50% → 100% Et_2O in pentane) and used directly in the following step.

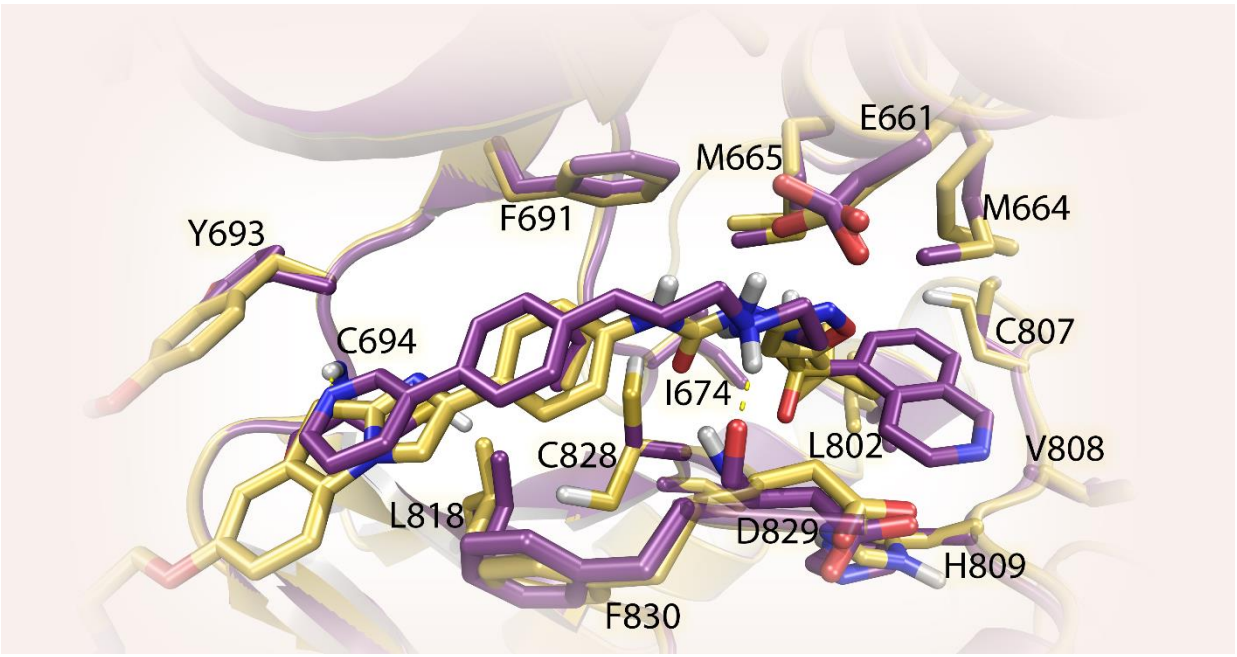
<u>Step 2:</u> Crude product from step 1 (2.90 g, 5.27 mmol, 1.0 eq) was dissolved in DCM (163 mL) and cooled to 0°C. TFA (2.35 mL) was added dropwise and after 10 min, triethylsilane (6.73 mL, 42.13 mmol, 8.0 eq) was added. The mixture was stirred for 20 h at RT and was then quenched with sat. aqueous $NaHCO_3$ (150 mL). The phases were separated and the aqueous layer was extracted with DCM (3x100 mL). The combined organic layers were washed with brine (1x200 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified via flash-column-chromatography (SiO_2, dry-loading, 7% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the desired product (0.903 g, 21% over 2 steps). ^{1}H NMR (600 MHz, chloroform-*d*, 330K) δ 10.22 (s, 1H), 8.54 (d, *J* = 2.1 Hz, 1H), 8.09 (d, *J* = 2.1 Hz, 1H), 7.53 (d, *J* = 8.1 Hz, 2H), 7.36 (d, *J* = 3.5 Hz, 1H), 7.27 (d, *J* = 8.1 Hz, 2H), 6.53 (d, *J* = 3.5 Hz, 1H), 3.31 – 3.25 (m, 4H), 2.85 (t, *J* = 6.6 Hz, 2H), 2.69 – 2.63 (m, 2H), 1.92 (p, *J* = 7.7 Hz, 2H), 1.59 (bs, *J* = 35.1 Hz, 2H), 1.45 (d, *J* = 6.7 Hz, 9H). ^{13}C NMR (151 MHz, chloroform-*d*, 330K) δ 156.11, 148.40, 142.42, 140.70, 137.57, 129.92, 128.99, 127.59, 127.25, 125.80, 120.51, 101.29, 79.70, 50.59, 47.83, 41.06, 33.06, 30.33, 28.66.

Supplementary Information



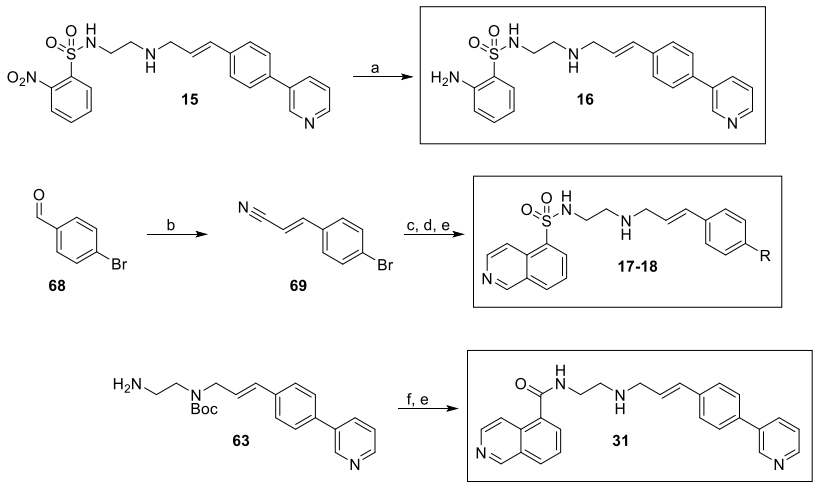
Substituent	σ
H	0
4-Cl	0.23
4-CH_3	-0.17
4-OCH_3	-0.27
3-NO_2	0.71
3-Cl	0.37
4-F	0.06
4-NO_2	0.78
3-CF_3	0.43

SI Figure 1: Plot of pIC_{50}-values of the amide series versus the corresponding substituent σ-values and the used σ-values for each substituent.[36]



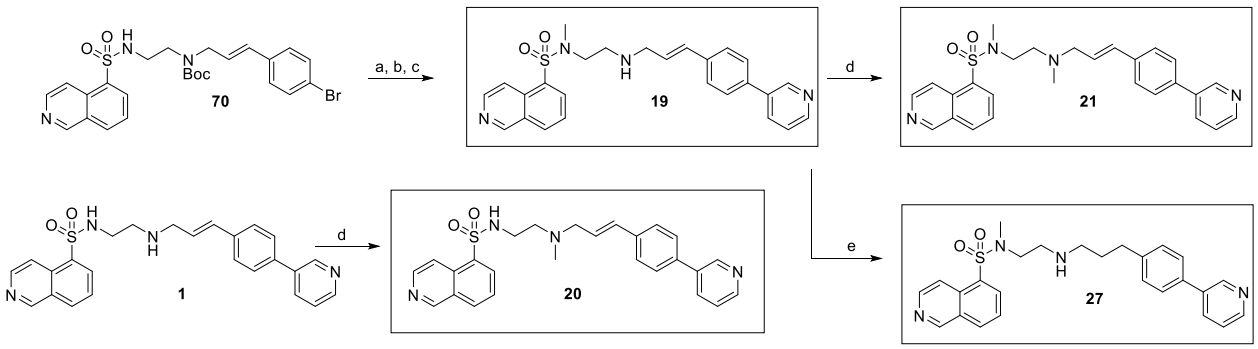
SI Figure 2: Proposed binding mode of **1** (purple) overlaid with the crystal structure of FLT3 co-crystalized with quizartinib (yellow) (PDB: 4RT7).

SI Scheme 1: Synthetic route towards the derivatives **16** - **18**, **31**.[a]



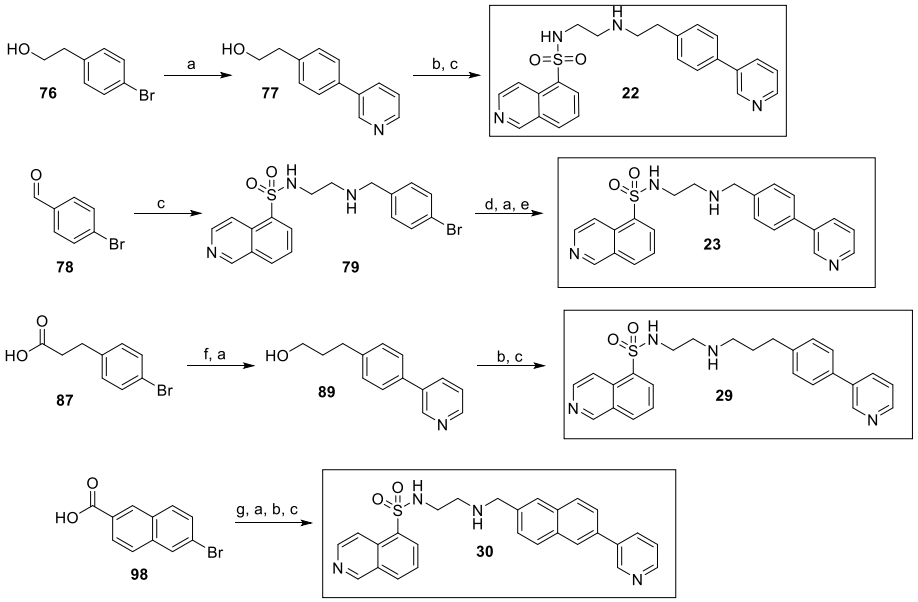
[a]Reagents and conditions: (a) Fe, AcOH, EtOH/H_2O; (b) diethyl cyanomethylphosphonate, NaH, DMF, 0°C – RT; (c) DiBAL-H, Et_2O, -80°C – 0°C, then **105**, $NaBH_4$, MeOH, -100°C – 0°C, then Boc_2O; (d) arylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, DMF/DCM/H_2O, 90°C; (e) TFA, DCM, 0°C – RT; (f) isoquinoline-5-carboxylic acid, $SOCl_2$, then DiPEA, DMAP, substrate, DCM, 0°C – RT.

SI Scheme 2: Synthetic route towards the derivatives **19** – **21**, **27**.[a]



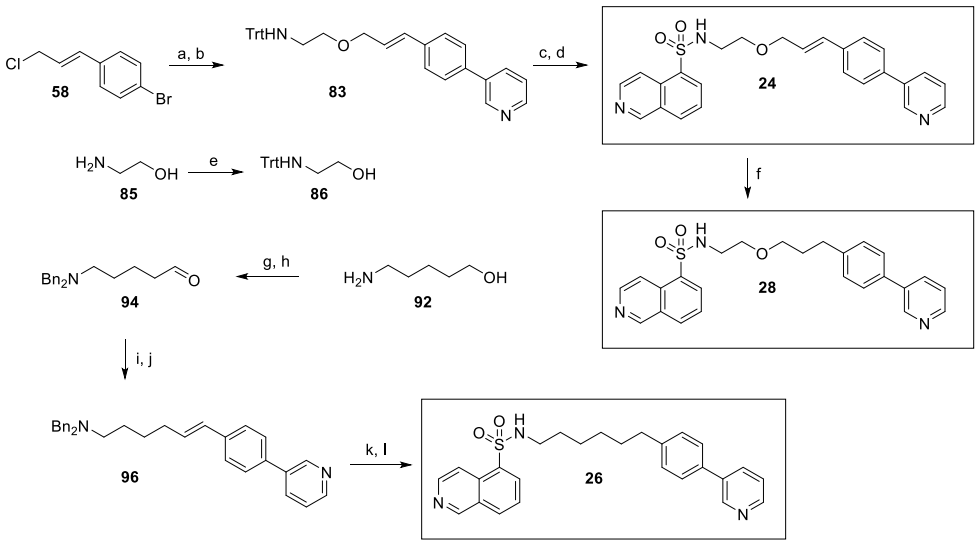
[a]Reagents and conditions: (a) MeI, Cs_2CO_3, DMF, RT; (b) arylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, DMF/DCM/H_2O, 80°C; (c) TFA, $CHCl_3$, 0°C – RT; (d) formaldehyde, $NaHB(OAc)_3$, THF/MeOH, RT; (e) Pd/C, H_2, MeOH.

SI Scheme 3: Synthetic route towards the derivatives **22**, **23**, **29** and **30**.[a]



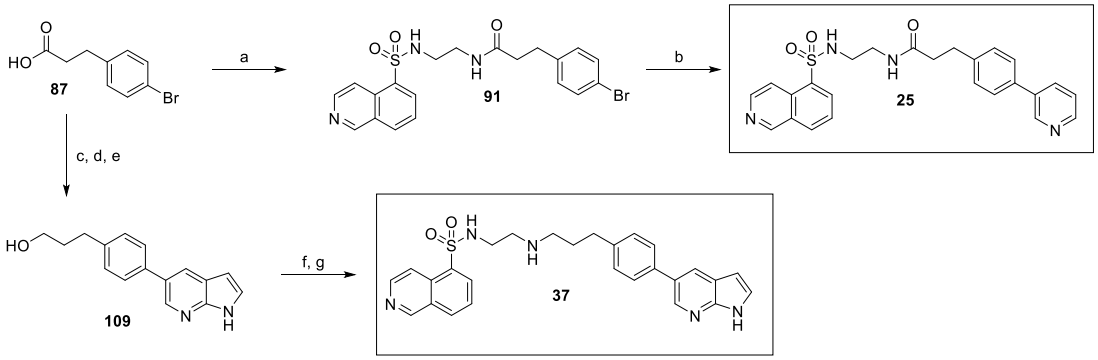
[a]Reagents and conditions: (a) arylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, DMF/DCM/H_2O, 90°C; (b) DMP, DCM, 0°C – RT; (c) **105**, $NaHB(OAc)_3$, THF or DCM, RT; (d) Boc_2O, $NaHCO_3$, THF, RT; (e) TFA, DCM, 0°C – RT; (f) $NaBH_4$, BF_3, THF, 0° - RT; (g) $LiAlH_4$, THF, 0° - RT.

SI Scheme 4: Synthetic route towards the derivatives **24**, **26**, **28**.[a]



[a]Reagents and conditions: (a) **86**, NaH, ACN, 70°C; (b) arylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, DMF/DCM/H_2O, 80°C; (c) TFA, TES, DCM, 0°C – RT; (d) **104**, Et_3N, DCM, 0°C – RT; (e) TrtCl, K_2CO_3, DCM, RT; (f) *p*-toluenesulfonyl hydrazide, NaOAc, THF, 66°C; (g) benzaldehyde, $NaBH(OAc)_3$, THF, RT; (h) oxalyl chloride, DMSO, Et_3N, DCM, -80°C – RT; (i) diethyl (4-bromobenzyl)phosphonate, NaH, THF, 0°C – RT; (j) arylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, DMF/DCM/H_2O, 80°C; (k) $Pd(OH)_2$, *t*-BuOH/1,4-dioxane/H_2O, H_2, RT; (l) **104**, Et_3N, DCM, 0°C – RT.

SI Scheme 5: Synthetic route towards the derivatives **25** and **37**.[a]



[a]Reagents and conditions: (a) **105**, EDC, HOBt, DiPEA, DCM, RT; (b) arylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, DMF/DCM/H_2O, 85°C; (c) $NaBH_4$, BF_3, THF, 0° - RT; (d) B_2Pin_2, KOAc, $Pd(dppf)Cl_2$, 1,4-dioxane, 100°C, overnight; (e) arylbromide, $Pd(PPh_3)_4$, K_2CO_3, DMF/DCM/H_2O, 85°C; (f) DMP, DCM, 0°C – RT; (g) **105**, $NaHB(OAc)_3$, DCM, RT.

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Chapter 4

Discovery of new mutant-active FLT3 inhibitors by high throughput screening*

Introduction

Acute myeloid leukemia (AML) is a disorder of hematopoietic stem-cells, in which during hematopoiesis cell differentiation is impaired, leading to immature blood cells flooding the bloodstream.[1] If untreated, this malignancy leads to death within weeks to months, especially for older patients who cannot cope with the severe standard chemotherapy treatment.[1] Several genetic alterations have been discovered, among them an internal tandem duplication (ITD) in the juxtamembrane domain of the Fms-like tyrosine kinase 3 (FLT3) receptor.[2] This mutation, FLT3-ITD, has been identified as a driver mutation in cancer progression, enabling growth factor independent cell proliferation.[3–5] This led to an extensive effort to discover FLT3 inhibitors for the use in AML treatment,[6,7] which resulted in the recent approval of midostaurin for treatment of AML patients with FLT3-ITD mutations. Other clinically investigated drugs, such as sorafenib or quizartinib, suffered from the emergence of treatment resistant clones possibly due to evolutionary selection during inhibitor treatment.[8–12] Several of these mutations that impaired the drug binding to the FLT3 kinase domain were identified, affecting binding affinity by either direct exchange of amino acids interacting with the drug (F691 mutations)[12,13], or a structural resistance, destabilizing the conformation the FLT3 kinase domain that is binding to the drug (D835 mutations)[11,13,14]. A schematic representation of the location of these mutations within the FLT3 receptor is shown in Figure 1.

* The data presented in this chapter was gathered in collaboration with Ruud H. Wijdeven, Jan van Groningen, Helma Rutjes, Constant A. A. van Boeckel, Herman S. Overkleeft, Jacques Neefjes and Mario van der Stelt.

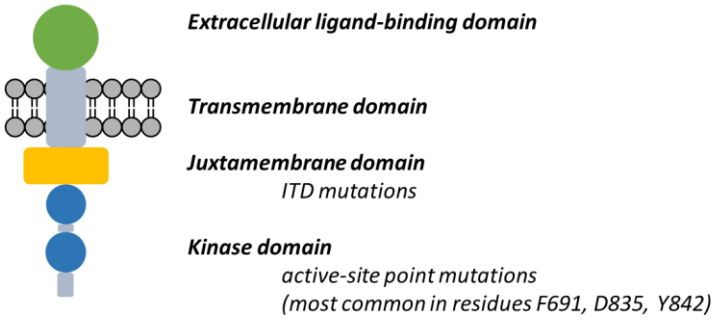


Figure 1: Schematic representation of the FLT3 receptor.

Since molecules rely on specific interactions with an enzyme for effective inhibition, it is unlikely that one single drug can counter all potential mutations that confer resistance to different drugs. This exemplifies the need for multiple structurally diverse chemotypes to treat AML that are active against wild type FLT3 as well as currently known mutations of FLT3, to successfully treat AML.

To this end, a screening strategy was devised to identify such new molecules. In the first stage a high throughput screen (HTS) was performed to identify novel inhibitors of wild type FLT3. After triaging, a selected set of hits was tested on cell lines, to ensure cellular activity as well as activity against cell lines carrying FLT3 F691 and D835 mutants.

To confirm cellular activity of compounds, MV4-11 cells were used which originated from a patient with biphenotypic B-myelomonocytic leukemia. Its proliferation is FLT3-signaling dependent and continuous inhibition of FLT3 in MV4-11 cells over several days leads to apoptosis.[4] To check for general off-target activity, the U937 cell line was chosen, which does not depend on FLT3 signaling. U937 cells are hematopoietic cells derived from a patient with histiocytic lymphoma.[15] Here, treatment with selective FLT3 inhibitors is not expected to lead to apoptosis.

To investigate the effects of mutant FLT3 Ba/F3 cells are used. Ba/F3 is a murine hematopoietic cell line dependent on the presence of interleukin 3 (IL-3) for proliferation.[16] However, Ba/F3 cell proliferation can be rendered IL-3 independent by using retroviruses to induce the stable expression of constitutively active tyrosine kinase or other oncogenes.[17] This oncogene-dependent signaling was exploited to generate Ba/F3 cell lines dependent on FLT3-ITD and/or F691L, D835H and D835Y point mutants.[16,18] The Ba/F3 wild type and the Ba/F3 FLT3-ITD variations were used to further profile the hits.

Results and Discussion

In vitro screening campaign

A fluorescence resonance energy transfer (FRET) based assay, using recombinantly expressed kinase, was used to screen for FLT3 active molecules (Figure 2A).[19] After adaption of the assay for a 1,536 well plate format, the search for new chemical entities was started by screening a library of 231,152 compounds against wild type-FLT3 in 3 days at a concentration of 10 µM. The results from the *in vitro* screening as well as the selection criteria are summarized in Figure 3. The quality of the data was ensured by monitoring Z' and S/B per plate. These showed that the assay is robust with values of ~0.8 and 6-13, respectively. 4,262 primary active compounds were found using cut-off criteria of a Z-score of ≤ -4 (~20-25% effect). The primary actives were retested in the same assay (Z-score of ≤ -4 corresponding to ~35% effect). This resulted in 1,400 confirmed actives, which corresponds to a hit rate of 0.61%.

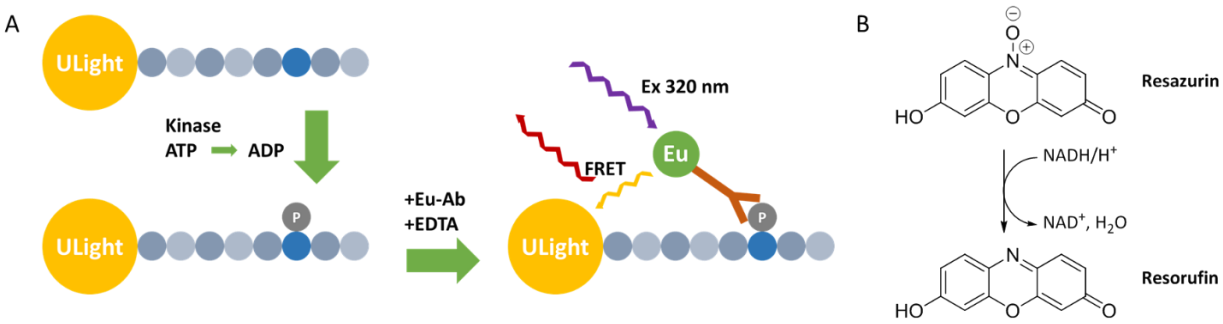


Figure 2: Used assays in the screening process: (A) Schematic representation of the used commercial FRET-based FLT3 *in vitro* assay. A peptide chain, tagged with an ULight acceptor fluorophore is phosphorylated by the kinase. Subsequent addition and binding of an europium donor fluorophore-tagged antibody, specific for the phosphorylated peptide brings donor and acceptor into close proximity, enabling FRET measurement. (B) The resazurin/resorufin redox reaction, the basis of the used alamarBlue cell viability assay is dependent on metabolic activity of the tested cells.

To further reduce the number of hits and to deprioritize pan-kinase inhibitors, deselection assays were performed against RAC-alpha serine/threonine-protein kinase (AKT1) and cAMP-dependent protein kinase (PKA) at 1.25 and 12.5 µM. In the deselection assay only 34 and 9 compounds showed activity of > 40% against AKT1 and PKA, respectively. These compounds were removed from the list. Subsequent analysis of the hit list for common pan-assay interference compounds (PAINS)[20,21] and intellectual property (IP) situation resulted in 141 compounds.

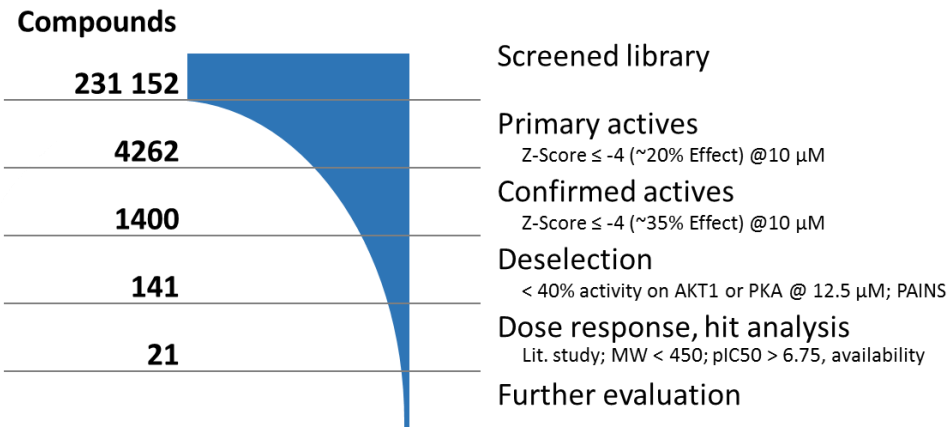


Figure 3: Hit triaging during the high-throughput screening.

The remaining 141 compounds were tested in a dose-response assay against FLT3 to determine inhibitory potency, followed by a detailed literature analysis for known kinase activity and freedom-to-operate with respect to IP. After further analysis of compound availability and purity, compounds were selected on basis of the following cut-offs: MW < 450; FLT3 pIC_{50} > 6.5. This resulted in a list with 21 compounds (Table 1).

Cellular evaluation of the qualified hit list

The 21 selected hits were further profiled in proliferation assays using seven different cell lines (MV4-11, U937, Ba/F3, Ba/F3-FLT3-ITD and Ba/F3 with FLT-ITD with the following mutations F691L, D835H and D835Y). The alamarBlue proliferation assay is based on the metabolic redox conversion of the dye resazurin to resorufin in living cells (Figure 2B).[22,23] The results of this study are shown in Table 1. This qualified hit list shows a diverse set of kinase inhibitors, including crenolanib (**19**), a known FLT3 inhibitor, which was added as positive control. On basis of this data, as well as the *in vitro* potency and general physicochemical properties, the hit list was carefully analyzed to make a selection of compounds exhibiting a balanced profile that could be further optimized.

The list includes compounds, such as **18**, that showed almost equal activity against all cell lines, regardless of FLT3 dependency, suggesting off-target mediated cytotoxicity. These compounds were not further considered. In addition, other compounds (e.g. **2**, **8** and **17)** were deselected based on their low cellular activity in the FLT3 dependent cell lines (pIC_{50} < 6).

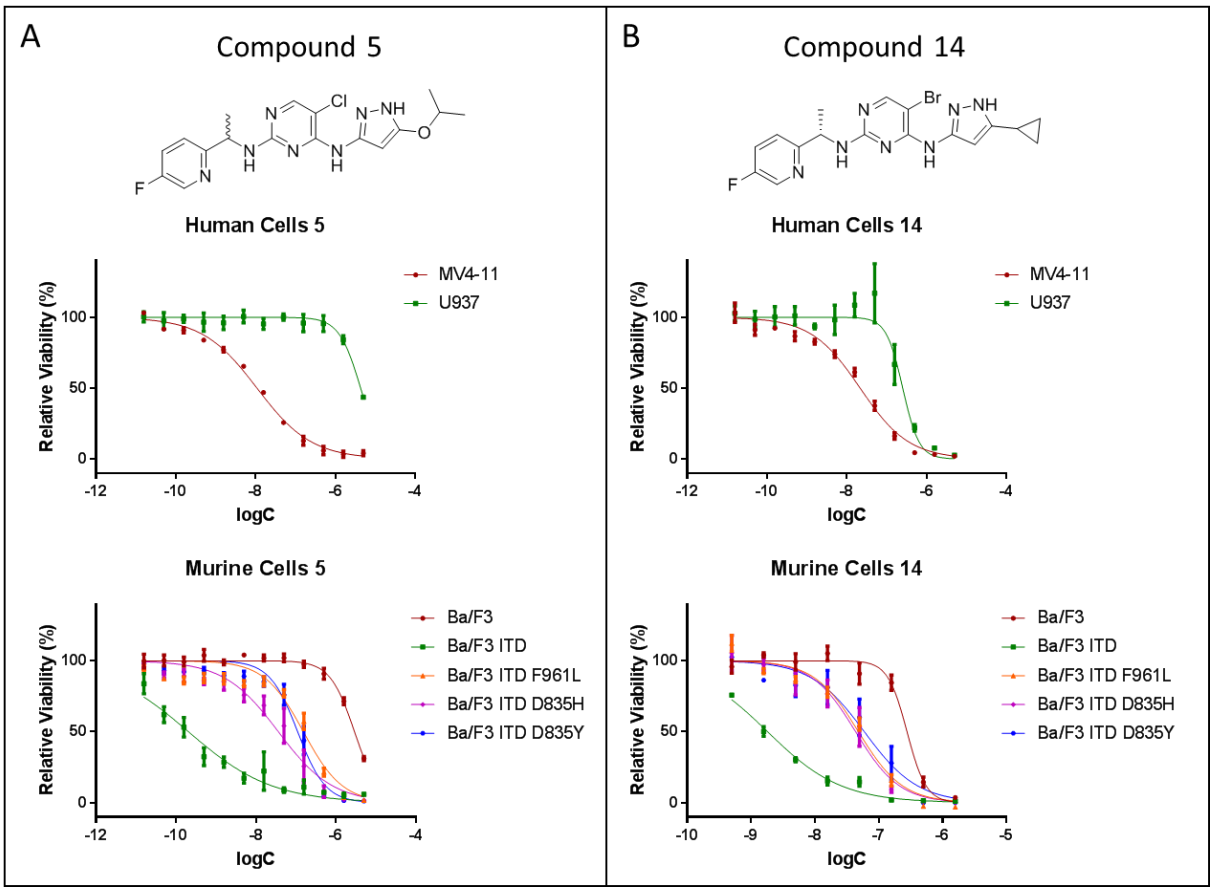


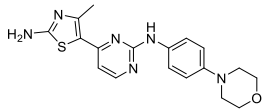
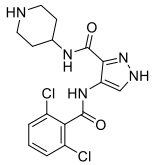
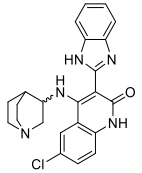
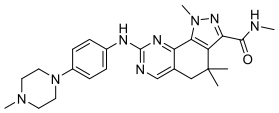
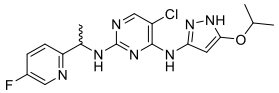
Figure 4: Summary of activity data of the selected hit compounds. Dose-response curves show individual data points ± SEM.

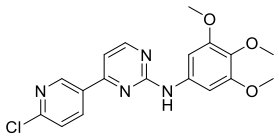
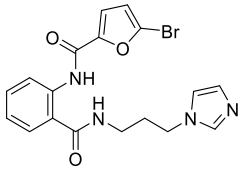
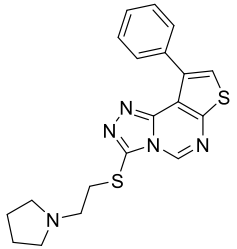
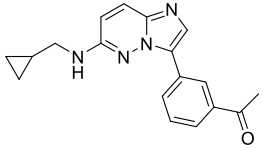
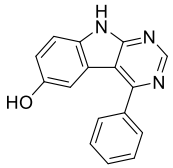
Finally, hits **5** (SPCE000476_01, 5-chloro-N^2-(1-(5-fluoropyridin-2-yl)ethyl)-N^4-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidine-2,4-diamine, Figure 4A) and **14** (NP_004099_001, (*S*)-5-bromo-N^4-(5-cyclopropyl-1*H*-pyrazol-3-yl)-N^2-(1-(5-fluoropyridin-2-yl)ethyl)pyrimidine-2,4-diamine, Figure 4B) were selected for further hit optimization (Chapter 5) on basis of their high activity against ITD mutations (MV4-11 and Ba/F3-ITD), acceptable point-mutant activity and good physico chemical parameters, such as low molecular weight (**5**: 392 and **14**: 418 g/mol) and satisfactory LipE (**5**: 4.9 and **14**: 4.4).

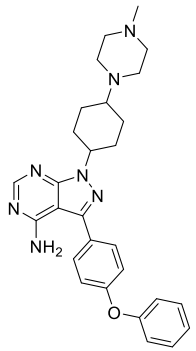
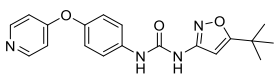
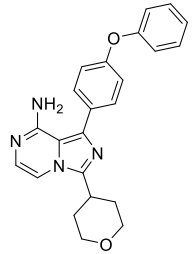
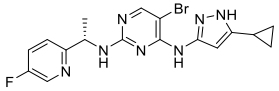
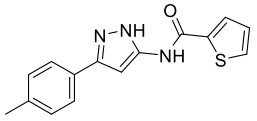
Conclusion

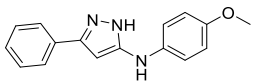
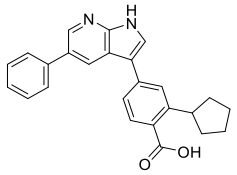
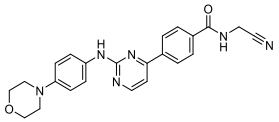
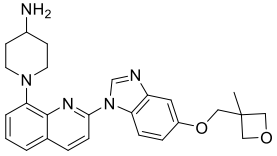
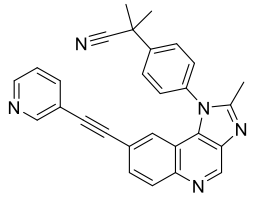
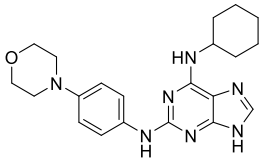
More than 231,000 compounds have been successfully screened in a 1,536-well format against wildtype FLT3. After hit confirmation and two deselection assays against AKT1 and PKA a dose-response assay was performed with 141 hit compounds, resulting in a qualified hit list of 21 compounds. These compounds were further evaluated on their anti-proliferative effects on seven cell lines. This led to the selection of compounds **5** and **14**, which displayed the most optimal profile and structure for further optimization (Chapter 5).

Table 1: Summary of the 21 molecules as FLT3 inhibitors, which were in-depth evaluated. *In vitro* pIC_{50} FLT3 was measured using a FRET based assay, all other pIC_{50}-values are effects on cell proliferation, measured using the alamarBlue viability assay, based on a resazurin/resorufin redox reaction. cLogP and tPSA were calculated with PerkinElmer ChemDraw 16. LipE = pIC_{50} (*in vitro* FLT3) – cLogP.

			pIC_{50} ± SEM										
						Ba/F3							
Entry	**Code**	**Structure**	***in vitro* FLT3**	**MV4-11**	**U937**	***wt***	**FLT3 ITD**	**FLT3 ITD F691L**	**FLT3 ITD D835H**	**FLT3 ITD D835Y**	**MW (Da)**	**cLogP**	**LipE**	**tPSA (Å²)**
1	SPCE000338_01		7.6	7.9 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	7.0 ± 0.1	7.4 ± 0.1	6.9 ± 0.1	6.8 ± 0.1	368	2.5	5.0	88
2	SPCE000368_01		6.8	6.7 ± 0.1	6.8 ± 0.1	5.7 ± 0.1	6.0 ± 0.1	6.2 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	382	0.3	6.5	95
3	SPCE000415_01		8.5	9.6 ± 0.1	7.6 ± 0.1	7.2 ± 0.1	9.5 ± 0.1	8.2 ± 0.1	9.0 ± 0.1	8.6 ± 0.1	420	4.0	4.4	69
4	SPCE000442_01		7.9	8.1 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	8.5 ± 0.1	7.2 ± 0.1	7.4 ± 0.1	7.0 ± 0.1	461	3.1	4.9	88
5	SPCE000476_01		8.4	8.0 ± 0.1	5.4 ± 0.1	5.5 ± 0.1	9.7 ± 0.1	6.8 ± 0.1	7.4 ± 0.1	7.0 ± 0.1	392	3.5	4.9	95

Entry	Code	Structure	pIC50 ± SEM								MW (Da)	cLogP	LipE	tPSA (Å²)
			in vitro FLT3	MV4-11	U937	Ba/F3								
						wt	FLT3 ITD	FLT3 ITD F691L	FLT3 ITD D835H	FLT3 ITD D835Y				
6	NP_000948_001		7.2	6.7 ± 0.1	6.5 ± 0.1	6.2 ± 0.1	6.8 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.2 ± 0.1	373	3.0	4.3	77
7	SPCA067086_01		7.1	7.1 ± 0.1	< 5	< 5	7.0 ± 0.1	6.0 ± 0.1	6.8 ± 0.1	6.9 ± 0.1	417	2.3	4.8	83
8	SPCA067975_01		7.0	6.5 ± 0.1	< 5	< 5	6.3 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	5.9 ± 0.1	382	3.8	3.2	44
9	CO_002775_001		7.6	7.3 ± 0.1	5.6 ± 0.1	< 5	6.7 ± 0.1	< 5	6.5 ± 0.1	6.8 ± 0.1	306	3.3	4.3	57
10	NP_000412_001		8.0	7.4 ± 0.1	5.8 ± 0.1	5.7 amb	7.3 ± 0.1	6.5 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	261	3.5	4.5	57

Entry	Code	Structure	pIC$_{50}$ ± SEM								MW (Da)	cLogP	LipE	tPSA (Å^2)
			in vitro FLT3	MV4-11	U937	Ba/F3								
						wt	FLT3 ITD	FLT3 ITD F691L	FLT3 ITD D835H	FLT3 ITD D835Y				
11	NP_001016_001		8.2	7.9 ± 0.1	5.4 ± 0.1	5.5 ± 0.2	7.3 ± 0.1	6.3 ± 0.1	6.9 ± 0.1	6.7 ± 0.1	484	3.6	4.6	82
12	NP_004035_001		8.1	na	< 5	< 5	7.8 ± 0.2	6.3 ± 0.1	7.0 ± 0.1	6.5 ± 0.1	352	4.1	3.9	84
13	NP_004039_001		7.1	6.9 ± 0.1	5.2 ± 0.1	5.5 ± 0.3	6.8 ± 0.1	6.4 ± 0.1	6.6 ± 0.1	6.8 ± 0.1	386	4.4	2.7	72
14	NP_004099_001		7.9	7.7 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	8.7 ± 0.1	7.3 ± 0.1	7.4 ± 0.1	7.2 ± 0.1	418	3.5	4.4	86
15	SPCA043560_01		7.0	7.0 ± 0.1	5.0 ± 0.1	< 5	6.3 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	283	3.9	3.1	53

Entry	Code	Structure	pIC50 ± SEM							MW (Da)	cLogP	LipE	tPSA (Å²)
			in vitro FLT3	Ba/F3									
				MV4-11	U937	*wt*	FLT3 ITD	FLT3 ITD F691L	FLT3 ITD D835H	FLT3 ITD D835Y				
16	SPCA053729_01		6.8	6.5 ± 0.1	< 5	< 5	6.1 ± 0.1	5.8 ± 0.1	5.4 ± 0.2	< 5	265	4.6	2.2	46
17	SPCE000191_01		8.3	6.1 ± 0.1	5.8 ± 0.1	< 5	6.6 ± 0.1	5.6 ± 0.2	6.0 ± 0.1	6.1 ± 0.1	382	5.7	2.6	62
18	SPCE000396_01		7.2	6.6 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	414	2.2	5.0	102
19	SPCE000437_01		9.0	9.3 ± 0.1	5.8 ± 0.1	6.0 ± 0.1	8.4 ± 0.1	7.1 ± 0.1	7.7 ± 0.1	7.9 ± 0.1	444	3.4	5.6	76
20	SPCE000468_01		7.3	6.6 ± 0.1	7.0 ± 0.1	6.2 ± 0.2	6.6 ± 0.2	6.5 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	428	5.5	1.8	64
21	SPCE000480_01		7.2	7.2 ± 0.1	6.9 ± 0.1	7.5 ± 0.5	7.8 ± 0.5	8.1 ± 0.7	8.2 ± 0.6	8.2 ± 0.6	393	4.9	2.3	86

Experimental

Final conditions primary screen and active confirmation

20 nL compound stock dissolved in DMSO or only DMSO were dispensed in a 1536 well plate (white polystyrene NBS microplate, Corning, cat# 3729). After addition of 2 µL assay buffer (50 mM HEPES (pH 7.5), 1 mM EGTA, 10 mM $MgCl_2$, 0.01% Tween-20, 2 mM DTT) and 2 µL 0.75 µg/mL FLT3 (BPS Bioscience, cat# 40225, lot# 141201) dissolved in assay buffer, the plate was centrifuged at 187 g for 30 s and incubated in the dark for 30 min. 2 µL of a mix of 600 µM ATP, 12.5 nM peptide (PerkinElmer; Lance® Ultra ULight™ TK-peptide; cat# TRFO127-M; lot# 1934454)) and 4 nM antibody (PerkinElmer; Lance® Eu-W1024-anti-phosphotyrosine(PT66); cat# AD0068;lot# 1889053) were added and the plate was again centrifuged at 187 g for 30 s and incubated in the dark for 90 min. The FRET was measured on a Envision plate reader (excitation: 337 nm, emission filter donor: 615 nm, emission filter: acceptor 665 nm).

Final conditions deselection assay AKT1

2.5 nL or 25 nL compound stock dissolved in DMSO or only DMSO were dispensed in a 1536 well plate (white polystyrene NBS microplate, Corning, cat# 3729). After addition of 2 µL assay buffer (50 mM HEPES (pH 7.5), 1 mM EGTA, 10 mM $MgCl_2$, 0.01% Tween-20, 2 mM DTT) and 2 µL 0.5 nmol/min AKT1 (SignalChem, cat# A16-10G-10, lot# W293-3) dissolved in assay buffer, the plate was centrifuged at 187 g for 30 s and incubated in the dark for 30 min. 2 µL of a mix of 200 µM ATP, 35 nM peptide (PerkinElmer; ULight™ phospho-40S-ribosomal protein S6 peptide; cat# TRF0129-D; lot# 1908909)) and 4 nM antibody (PerkinElmer; Eu-anti-phosph-rpS6, cat# TRF0217-D, lot# 1759011) were added and the plate was again centrifuged at 187 g for 30 s and incubated in the dark for 30 min. The FRET was measured on a Envision plate reader (excitation: 337 nm, emission filter donor: 615 nm, emission filter: acceptor 665 nm).

Final conditions deselection assay PKA

2.5 nL or 25 nL compound stock dissolved in DMSO or only DMSO were dispensed in a 1536 well plate (white polystyrene NBS microplate, Corning, cat# 3729). After addition of 2 µL assay buffer (50 mM HEPES (pH 7.5), 1 mM EGTA, 10 mM $MgCl_2$, 0.01% Tween-20, 2 mM DTT) and 2 µL 3.2 nmol/min PKAcα (SigalChem, cat# PS1-10G-10, lot# Q210-2) dissolved in assay buffer, the plate was centrifuged at 187 g for 30 s and incubated in the dark for 30 min. 2 µL of a mix of 200 µM ATP, 45 nM peptide (PerkinElmer; ULight™ phospho-40S-ribosomal protein S6 peptide; cat# TRF0129-D; lot# 1908909)) and 4 nM antibody (PerkinElmer; Eu-anti-phosph-rpS6, cat# TRF0217-D, lot# 1759011) were added and the plate was again centrifuged at 187 g for 30 s and incubated in the dark for 30 min. The FRET was measured on a Envision plate reader (excitation: 337 nm, emission filter donor: 615 nm, emission filter: acceptor 665 nm).

In situ testing of kinase inhibitors

To evaluate inhibitor effect on cell proliferation MV4-11, U937 and Ba/F3 cell lines were grown in RPMI, supplemented with 10% fetal bovine serum in an incubator at 37°C under 5% CO_2 atmosphere. Ba/F cells (wild-type) were grown in the presence of IL-3 (10 ng/mL, PeproTech). For viability assays, 10,000 cells were seeded per well in a 96-wells plate and inhibitors were added at the indicated concentration. After three days, cell viability was measured using the Cell Titer Blue (alamarBlue) viability assay (Promega) and fluorescence was measured using

the Clariostar (BMG Labtech). Relative survival was normalized to the untreated control and corrected for background signal. Data was processed using Microsoft Excel 2016, pIC_{50} values were fitted using GraphPad Prism 7.0. Experiments were performed in either n=3 or n=2.

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Chapter 5

Discovery and development of pyrrolo-pyrimidines as mutant active FLT3 inhibitors*

Introduction

Acute myeloid leukemia (AML) is a cancer of the blood, characterized by excessive proliferation of immature hematopoietic cells and high mortality.[1–3] 20-30% of AML patients harbor an internal tandem duplication (ITD) in the fms-like tyrosine kinase 3 (FLT3) gene, which is thought to enable growth-factor independent proliferation.[4–6] FLT3-ITD has been validated as a target for AML treatment, as evidenced by the approval of midostaurin as a FLT3 inhibitor.[7–9] Nevertheless, successful AML therapy remains a challenge due to the emergence of treatment-resistant point-mutations in the FLT3 tyrosine kinase domain.[9–11] Hence, there is a need for new chemical matter that also inhibits the treatment-resistant FLT3 kinase activity.

To this end a high throughput screen was performed to identify new chemical entities that inhibit FLT3 (as described in Chapter 4). This led to the discovery of SPCE00476_01 and NP_004099_001 (Figure 1A) as qualified hits. In this Chapter, the hit confirmation, optimization of the potency, physico-chemical properties and cellular activity against several mutant FLT3 proteins is described (Figure 1B).

* The data presented in this chapter was gathered in collaboration with Laura de Paus, Ruud H. Wijdeven, Hengyi You, Hugo van Kessel, Maxime A. Siegler, Constant A. A. van Boeckel, Herman S. Overkleeft, Jacques Neefjes and Mario van der Stelt.

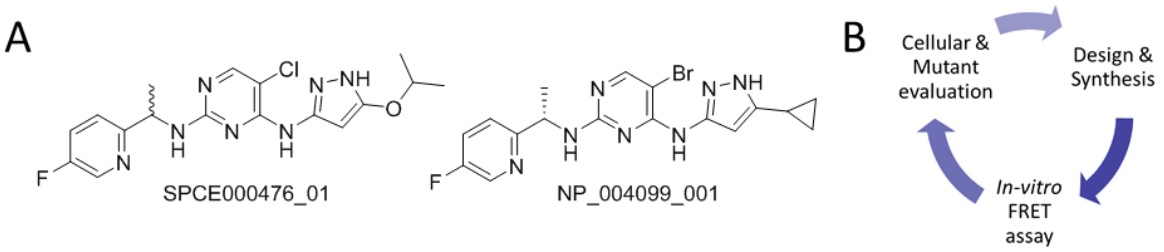


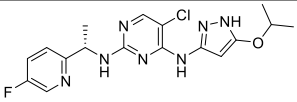
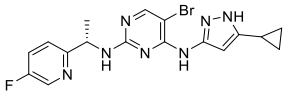
Figure 1: (**A**) The hits discovered in the high-throughput screening campaign described in chapter 4. (**B**) General development strategy employed.

Results and discussion

Confirmation of screening hits

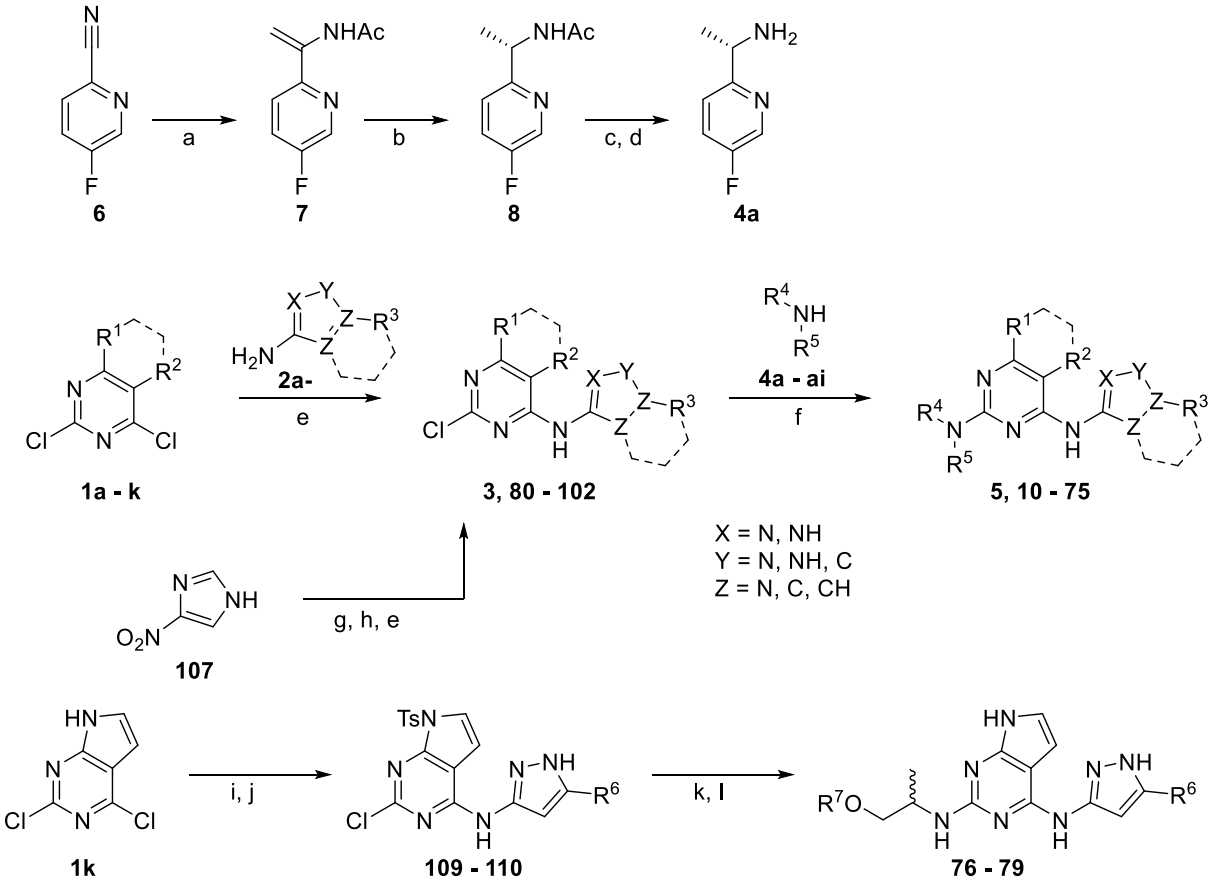
First, SPCE00476_01 and NP_004099_001 were resynthesized to confirm their structure and activity. The synthesis was performed following known literature procedures.[12–16] The general synthetic strategy to produce these compounds is summarized in Scheme 1. In short, SPCE00476_01 was synthesized by coupling the core building block 2,4,5-trichloropyrimidine (**1a**) with 5-isopropoxy-1*H*-pyrazol-3-amine (**2a**) via a nucleophilic aromatic substitution reaction (S_NA_R). The resulting building block (**3**) was reacted with (*S*)-1-(5-fluoropyridin-2-yl)ethan-1-amine (**4a**) in a second S_NA_R, which resulted in the desired compound (SPCE00476_01), subsequently renamed **5**. The chiral amine **4a** was synthesized from 5-fluoropicolinonitrile (**6**), starting with methyl-Grignard reagent, followed by acetylation to yield the corresponding protected enamine **7** (in Scheme 1).[12] The enamine was reduced using a chiral rhodium catalyst to yield **8**. Deprotection resulted in the desired chiral amine **4a**. This synthesis provided a moderate enantiomeric ratio of 76% in favor of the required *S*-enantiomer. This was considered sufficient for confirmation of the activity and subsequent structure-activity studies. NP_004099_001 was synthesized in a similar fashion, starting from 5-bromo-2,4-dichloropyrimidine (**1b**) and 5-cyclopropyl-1*H*-pyrazol-3-amine (**2b**) and was subsequently named **10**.

Table 1: Bioactivities of the resynthesized initial screening hits.

	Structure	pIC_{50} ± SEM							
					Ba/F3				
		in vitro FLT3	MV4-11	U937	wt	FLT3 ITD	FLT3 ITD F691L	FLT3 ITD D835H	FLT3 ITD D835Y
5		8.2 ± 0.1	7.2 ± 0.1	5.0 ± 0.2	< 5	7.3 ± 0.2	5.8 ± 0.1	6.7 ± 0.1	6.3 ± 0.1
10		8.5 ± 0.1	7.7 ± 0.1	6.4 ± 0.1	6.2 ± 0.2	7.9 ± 0.2	6.9 ± 0.1	7.1 ± 0.2	6.8 ± 0.2

The two hits were tested in the biochemical FLT3 and cellular assays (MV4-11[6], U937[17] and Ba/F3 derived[18–20]). The activity of the two compounds was confirmed (Table 1). Of note, **5** showed significantly less toxicity towards the U937 and Ba/F3 wt cells compared to **10** and was less active in the cell lines harboring the point-mutant derivatives. This might suggest that off-target activity contributes to the cellular activity of **10**.

Scheme 1: Synthetic strategies used in the synthesis of the FLT3 inhibitors presented in this chapter.[a]

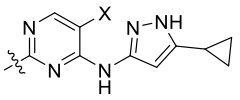


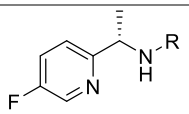
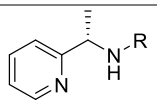
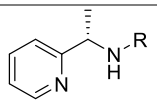
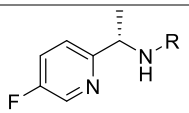
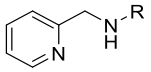
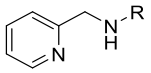
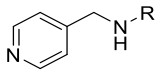
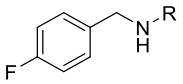
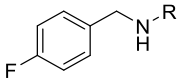
[a]Reagents and conditions: (a) MeMgBr, THF, 0°C, then Ac_2O, RT; (b) (+)-1,2-Bis((2*S*,5*S*)-2,5-diethylphospholano) benzene(cyclooctadiene)rhodium trifluoromethanesulfonate, MeOH, 10 bar H_2, RT; (c) DMAP, Boc_2O, THF, 50°C, then LiOH, H_2O, RT; (d) TFA, $CHCl_3$, 0°C – RT; (e) Heterocycle-amine, Et_3N or DIPEA; (f) alkyl-amine, DIPEA; (g) Alkyl-tosylate or alkyl halide, K_2CO_3, ACN; (h) Pd/C, H_2, EtOH; (i) TsCl, tetrabutylammonium hydrogen sulfate, DCM, H_2O, RT; (j) heterocycle-amine, Et_3N, ACN, 100°C; (k) alkyl-amine, DIPEA, *n*-butanol, 160°C; (l) NaOH, MeOH, 1,4-dioxane, H_2O, 0°C – RT.

Structure activity relationship studies

The SAR study was initiated by using a disjunctive approach in which functional groups were deleted from hit compound **10**. To this end a series of compounds (**11** – **20**) was synthesized and tested. The bioactivities of the compounds are summarized in Table 2.

Table 2: Structure-activity relation study of left-hand side of **10**.

R = 

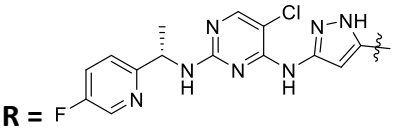
	Structure	X	pIC$_{50}$ ± SEM							
						Ba/F3				
			in vitro FLT3	MV4-11	U937	wt	FLT3 ITD	FLT3 ITD F691L	FLT3 ITD D835H	FLT3 ITD D835Y
11		Cl	8.44 ± 0.06	7.6 ± 0.1	6.5 ± 0.1	6.4 ± 0.2	7.8 ± 0.1	6.9 ± 0.2	7.1 ± 0.2	6.8 ± 0.2
12		Br	8.49 ± 0.07	7.7 ± 0.1	6.2 ± 0.2	5.8 ± 0.3	7.9 ± 0.2	6.9 ± 0.1	7.2 ± 0.2	6.9 ± 0.2
13		Cl	8.50 ± 0.08	7.5 ± 0.1	6.4 ± 0.1	5.8 ± 0.4	8.0 ± 0.1	7.0 ± 0.2	7.3 ± 0.2	7.0 ± 0.2
14		H	8.44 ± 0.09	7.3 ± 0.1	6.8 ± 0.1	6.8 ± 0.3	7.7 ± 0.2	6.9 ± 0.2	6.9 ± 0.2	6.9 ± 0.2
15		Cl	7.92 ± 0.05	7.4 ± 0.1	5.5 ± 0.2	5.2 ± 0.6	7.2 ± 0.2	6.5 ± 0.2	6.9 ± 0.2	6.7 ± 0.2
16		Br	8.01 ± 0.05	7.3 ± 0.1	5.3 ± 0.3	5.3 ± 0.5	7.2 ± 0.2	6.5 ± 0.2	6.9 ± 0.2	6.7 ± 0.2
17		Cl	7.62 ± 0.10	6.8 ± 0.2	5.7 ± 0.1	< 5	6.6 ± 0.3	6.1 ± 0.3	6.4 ± 0.2	6.4 ± 0.2
18		Br	6.60 ± 0.09				ND			
19		Cl	6.72 ± 0.11				ND			
20		Cl	7.84 ± 0.08	7.5 ± 0.1	5.2 amb.	< 5	7.4 ± 0.2	6.7 ± 0.2	7.0 ± 0.2	6.9 ± 0.2

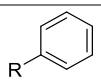
The substitution of a bromine (**10**) to a chlorine (**11**) or its removal (**14**) did not affect the biochemical or cellular activity of the hit. The same effect was observed for the removal of the para-fluoro substituent on the pyridyl ring (**12** and **13**). Removal of the chiral methyl on the benzyl carbon (**15** and **16**) resulted in a small loss of activity in the *in vitro* assay and in the Ba/F3 ITD, but not for MV4-11, cellular assays. Moving the pyridine nitrogen to the para-position (**17**) or its substitution by a carbon atom (**18** and **19**) resulted in a substantial loss of activity. Remarkably, substituting the chiral pyridine-amine group for an isopropyl amine provided a potent compound (pIC$_{50}$ = 7.8 ± 0.1), while substantially reducing the molecular

weight by almost 30% from 418 (original hit, **10**) to 293 g/mol (**20**). All together, these results indicated that the pyridine ring substituent does not make any significant interactions with the FLT3 binding pocket.

Next, the size of the binding pocket accommodating the alkyl substituent on the pyrazole moiety was investigated (Table 3). Smaller substituents, such as hydrogen (**21**) or methyl (**22**) as well as larger substituents (e.g. *tert*-butyl (**23**) and phenyl (**24**)) showed substantially decreased activity against FLT3. The cyclobutyl analog (**25**) showed an increase in potency compared to the hit. This indicated that the cyclopropyl group has an almost optimal fit with a small lipophilic pocket.

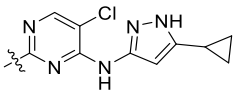
Table 3: SAR study of the cyclopropyl analogs of **10**.

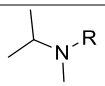
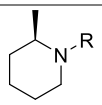
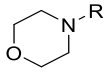
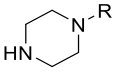
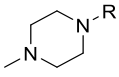
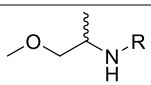
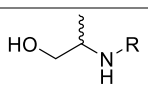
R = 

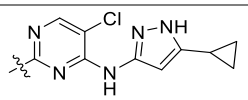
	Structure	**pIC$_{50}$ ± SEM**							
					Ba/F3				
		***in vitro* FLT3**	**MV4-11**	**U937**	**wt**	**FLT3 ITD**	**FLT3 ITD F691L**	**FLT3 ITD D835H**	**FLT3 ITD D835Y**
21		6.22 ± 0.12				ND			
22		7.38 ± 0.10	6.6 ± 0.2	6.5 ± 0.1	6.5 ± 0.4	6.9 ± 0.2	6.8 ± 0.3	6.4 ± 0.2	6.3 ± 0.3
23		5.94 ± 0.10				ND			
24		7.11 ± 0.09	6.8 ± 0.2	5.2 ± 0.1	< 5	6.8 ± 0.2	6.0 ± 0.3	6.4 ± 0.2	6.1 ± 0.3
25		8.85 ± 0.15	7.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.4	8.2 ± 0.2	7.0 ± 0.2	7.4 ± 0.2	7.1 ± 0.2

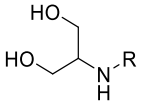
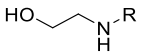
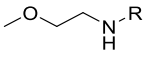
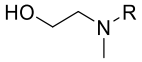
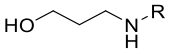
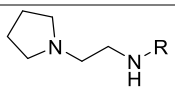
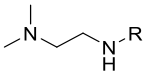
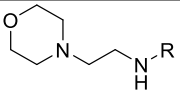
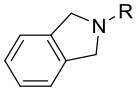
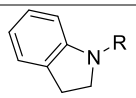
In view of the remarkable activity of the isopropyl analog (**20**), a series of amine analogs (**26** - **51**) was synthesized and evaluated (Table 4). Alkylation (**26**, **27**) and cyclization (**28** - **35**) of the amine did not significantly alter the activity of the compounds, indicating that the N-H group does not form an H-bond interaction with the protein. Of note, pyrrolidine analog (**28**) showed a significant increase in activity in the *in vitro* assay, but this did not translate into increased cellular activity. As observed previously for the hit compound, introduction of chiral methyl substituents (**30** - **35**) on the cyclic amines did not improve the potency of the compounds.

Table 4: Structure-activity relationship investigation of the amine tail substituent.

R = 

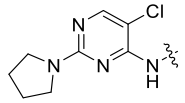
	Structure	pIC$_{50}$ ± SEM							
					Ba/F3				
		in vitro FLT3	MV4-11	U937	wt	FLT3 ITD	FLT3 ITD F691L	FLT3 ITD D835H	FLT3 ITD D835Y
26		7.70 ± 0.13	6.8 ± 0.1	5.1 amb.	5.2 ± 0.2	6.9 ± 0.1	6.0 ± 0.2	6.6 ± 0.2	6.4 ± 0.1
27		7.51 ± 0.12	6.5 ± 0.1	5.5 ± 0.2	< 5	6.3 ± 0.2	5.5 ± 0.2	6.0 ± 0.2	5.6 ± 0.2
28		8.39 ± 0.12	7.4 ± 0.1	5.4 ± 0.1	< 5	7.1 ± 0.2	6.3 ± 0.2	6.8 ± 0.2	6.6 ± 0.2
29		7.93 ± 0.10	6.8 ± 0.2	5.3 ± 0.4	< 5	6.8 ± 0.2	6.0 ± 0.3	6.3 ± 0.2	6.4 ± 0.2
30		7.78 ± 0.09	7.2 ± 0.2	5.6 ± 0.2	< 5	7.1 ± 0.3	6.3 ± 0.2	6.7 ± 0.2	6.7 ± 0.2
31		7.81 ± 0.15				ND			
32		7.90 ± 0.08				ND			
33		7.44 ± 0.09	6.5 ± 0.2	5.1 amb.	< 5	6.5 ± 0.3	5.6 ± 0.2	6.1 ± 0.2	5.9 ± 0.2
34		7.75 ± 0.11				ND			
35		7.42 ± 0.11				ND			
36		6.82 ± 0.18				ND			
37		6.87 ± 0.12				ND			
38		6.90 ± 0.18				ND			
39		7.78 ± 0.09	7.2 ± 0.2	5.4 ± 0.2	5.5 ± 0.2	7.2 ± 0.1	6.5 ± 0.2	6.9 ± 0.1	6.7 ± 0.1
40		7.51 ± 0.06	7.1 ± 0.1	5.1 amb.	5.1 amb.	7.3 ± 0.1	6.3 ± 0.2	7.1 ± 0.2	6.9 ± 0.1

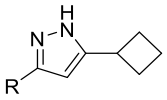
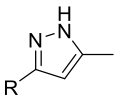
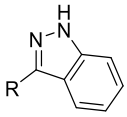
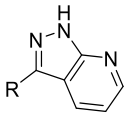
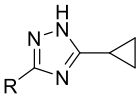
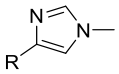
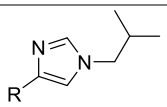
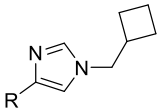
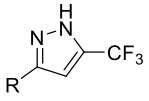
R = 

	Structure	pIC$_{50}$ ± SEM							
		in vitro FLT3	MV4-11	U937	Ba/F3 wt	Ba/F3 FLT3 ITD	Ba/F3 FLT3 ITD F691L	Ba/F3 FLT3 ITD D835H	Ba/F3 FLT3 ITD D835Y
41		7.08 ± 0.07	6.7 ± 0.1	5.0 amb.	< 5	6.5 ± 0.2	5.6 ± 0.2	6.4 ± 0.2	6.3 ± 0.1
42		7.08 ± 0.10	6.6 ± 0.2	< 5	< 5	6.3 ± 0.2	5.1 amb.	6.1 ± 0.1	6.0 ± 0.2
43		7.57 ± 0.12	6.7 ± 0.1	5.2 ± 0.1	< 5	6.4 ± 0.2	5.6 ± 0.2	6.2 ± 0.2	6.2 ± 0.2
44		7.60 ± 0.11	6.8 ± 0.1	< 5	< 5	6.5 ± 0.2	5.5 ± 0.2	6.4 ± 0.1	6.3 ± 0.1
45		7.57 ± 0.08	7.0 ± 0.1	5.1 amb.	< 5	6.6 ± 0.2	5.7 ± 0.2	6.5 ± 0.1	6.4 ± 0.2
46		6.78 ± 0.14	6.0 ± 0.2	< 5	< 5	5.5 ± 0.3	5.3 ± 0.2	5.4 ± 0.2	5.5 ± 0.2
47		6.57 ± 0.13	6.0 ± 0.2	< 5	< 5	5.4 ± 0.2	5.1 ± 0.2	5.2 amb.	5.1 amb.
48		6.81 ± 0.09	6.1 ± 0.2	< 5	< 5	5.6 ± 0.2	5.2 ± 0.2	5.4 ± 0.3	5.4 ± 0.3
49		7.23 ± 0.15	6.5 ± 0.1	5.3 ± 0.2	< 5	6.3 ± 0.2	5.7 ± 0.2	5.8 ± 0.2	5.8 ± 0.2
50		7.00 ± 0.09				ND			
51		7.00 ± 0.16	6.6 ± 0.1	5.1 amb.	< 5	6.4 ± 0.2	5.5 ± 0.2	6.3 ± 0.2	5.7 ± 0.2

To improve the solubility of the compounds, the left hand side substituent was replaced by various solubilizers (**36** - **48**). Morpholines (**36**, **48**) and piperazines (**37**, **38**) or other basic amines (**46**, **47**) were, however, not preferred and resulted in a 10-fold drop in activity. Interestingly, more flexible ethanolamine derivatives (**39** - **45**) retained activity (pIC$_{50}$ > 7). Compounds **49**, **50** and **51** retained strong inhibitor activity in the biochemical assay (pIC$_{50}$ > 7.5), but showed very low activity in the Ba/F3 cells, especially in the cell line expressing the F691L mutation (pIC$_{50}$ < 6). **39** and **40**, with a propanol-2-amine substituent were among the most potent compounds with a favorable cellular activity profile, retaining strong anti-proliferative activity for most of the mutant variations.

Table 5: Investigation of the pyrazole moiety.

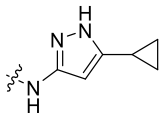
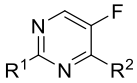
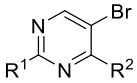
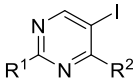
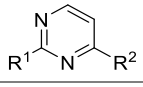
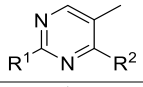
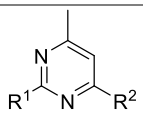
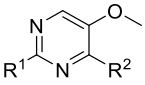
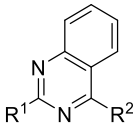
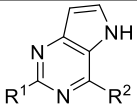
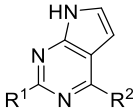
R = 

	Structure	pIC$_{50}$ ± SEM							
					Ba/F3				
		in vitro FLT3	MV4-11	U937	wt	FLT3 ITD	FLT3 ITD F691L	FLT3 ITD D835H	FLT3 ITD D835Y
52		7.38 ± 0.10	6.8 ± 0.1	5.1 amb.	< 5	6.9 ± 0.1	5.8 ± 0.2	6.7 ± 0.2	6.4 ± 0.1
53		6.84 ± 0.08	5.9 ± 0.2	ND	< 5	5.5 ± 0.1	5.7 ± 0.2	5.5 ± 0.2	5.6 ± 0.1
54		5.28 ± 0.14				ND			
55		5.46 ± 0.10				ND			
56		7.44 ± 0.08	6.3 ± 0.1	6.8 ± 0.1	6.1 ± 0.2	6.2 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.3 ± 0.1
57		5.88 ± 0.18				ND			
58		5.86 ± 0.13				ND			
59		6.34 ± 0.06				ND			
60		6.47 ± 0.13				ND			
61		5.56 ± 0.12				ND			

Compounds **52** – **54** show that the head group alkyl substituents follow the same general trend as with the original tail fragment, albeit the cyclobutyl residue demonstrated a somewhat lower activity compared to the cyclopropyl (Table 5). **55** and **56** were synthesized to investigate the effect of an annulated aromatic ring. Interestingly, while the phenyl derivative completely lost activity, the pyridyl retained its activity. Of note, the cellular activity was very

similar across the whole panel of cell lines. Inhibition of U937 cell growth was even stronger than the reduced MV4-11 cell proliferation, indicating that this effect was independent of FLT3 inhibition. Perhaps a change in binding mode, due to the introduction of an additional hydrogen bond donor-acceptor pair in **56** is responsible for additional kinase inhibitory activity. Replacing the pyrazole with a 1, 2, 4-triazole (**57**) or imidazole (**58** - **60**) resulted in loss of activity. Finally, introducing electron-withdrawing groups (i.e. CF_3) on the pyrazole (**61**) also led to a reduction of activity.

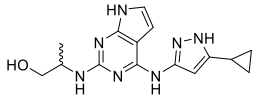
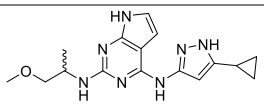
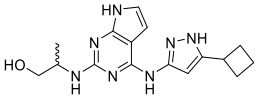
Table 6: Optimization of the scaffold of **10**.

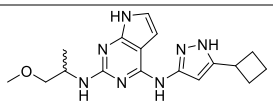
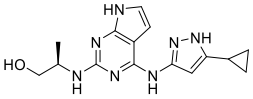
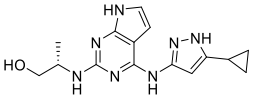
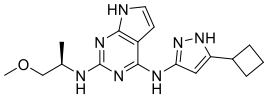
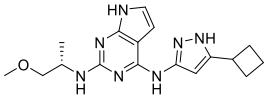
		R^1 =  , R^2 = 							
		pIC_{50} ± SEM							
					Ba/F3				
	Structure	***in vitro* FLT3**	**MV4-11**	**U937**	**wt**	**FLT3 ITD**	**FLT3 ITD F691L**	**FLT3 ITD D835H**	**FLT3 ITD D835Y**
62		7.47 ± 0.15	6.7 ± 0.1	6.0 amb.	5.2 ± 0.3	6.7 ± 0.1	5.8 ± 0.2	6.3 ± 0.1	6.2 ± 0.1
63		7.56 ± 0.11	6.7 ± 0.1	5.1 amb.	< 5	6.8 ± 0.2	5.9 ± 0.2	6.5 ± 0.2	6.3 ± 0.1
64		6.43 ± 0.12				ND			
65		7.50 ± 0.10	7.0 ± 0.1	6.7 ± 0.1	6.3 ± 0.1	7.0 ± 0.2	6.6 ± 0.1	6.8 ± 0.2	6.7 ± 0.1
66		7.46 ± 0.08	6.9 ± 0.1	6.0 amb.	5.6 ± 0.2	6.9 ± 0.1	6.1 ± 0.2	6.5 ± 0.2	6.4 ± 0.1
67		7.73 ± 0.12	6.9 ± 0.1	6.5 amb.	6.4 ± 0.2	6.7 ± 0.1	6.3 ± 0.1	6.5 ± 0.1	6.3 ± 0.1
68		8.17 ± 0.14	7.1 ± 0.1	5.4 ± 0.2	5.2 ± 0.4	7.3 ± 0.1	6.3 ± 0.2	6.8 ± 0.2	6.6 ± 0.1
69		8.84 ± 0.07	7.5 ± 0.1	6.4 ± 0.1	6.1 ± 0.2	7.3 ± 0.1	6.6 ± 0.1	7.0 ± 0.1	7.0 ± 0.1
70		8.66 ± 0.09	7.1 ± 0.1	6.5 amb.	6.2 ± 0.1	7.2 ± 0.2	6.5 ± 0.1	6.9 ± 0.2	6.7 ± 0.1
71		9.23 ± 0.14	7.8 ± 0.1	6.3 ± 0.1	5.6 ± 0.2	7.7 ± 0.1	6.7 ± 0.1	7.4 ± 0.1	7.3 ± 0.1

Next, the central core pyrimidine of **10** was investigated (Table 6). Compounds **62** – **65** were synthesized and tested to explore the influence of the halogen on the scaffold. The original chloro-substituted pyrimidine (**28**) was the most active compound, followed by the almost equipotent fluoro (**62**), bromo (**63**) and hydrogen substitution (**65**). The iodo substituted compound (**64**) lost approximately 100-fold in activity compared to **28**. The cellular activities were comparable, but **65** lacking a halogen showed substantial inhibition of the cellular proliferation of control cell lines U937 and Ba/F3. Introducing electron-donating substituents, such as methyl (**66**, **67**) and methoxy (**68**) groups, did not improve the potency in the biochemical or cellular assays compared to **28**. Of note, annulated ring systems, mimicking the adenosine-base in ATP, such as **69**, **70** and **71**, demonstrated substantially increased potency. **71** reached even subnanomolar potency (pIC_{50} of 9.2 ± 0.1) in the biochemical assay, which was accompanied by good cellular activity.

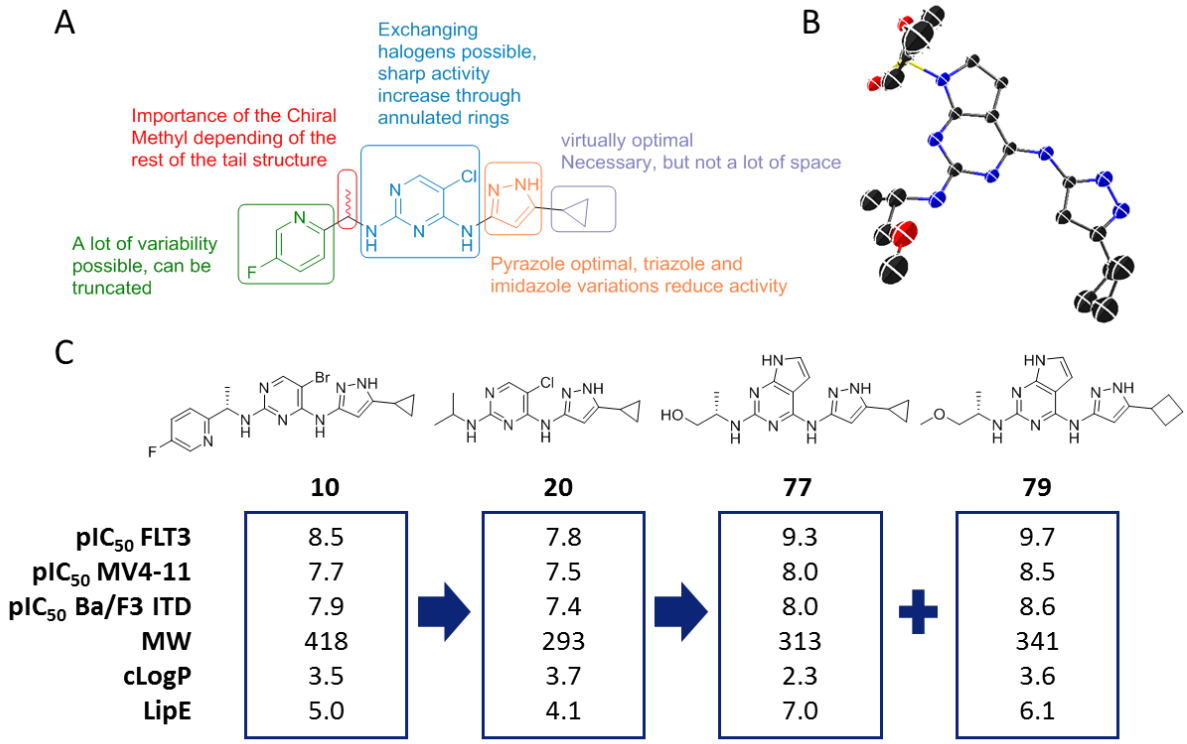
In the final round of optimization, the optimal core (7*H*-pyrrolo[2,3-*d*]pyrimidine) was combined with methoxypropanol-2-amine or propanol-2-amine as the best substituents at the left hand side with cyclopropylpyrazoloamine or cyclobutylpyrazoloamine on the right hand side. This led to the synthesis of **72** - **75** (Table 7). The biochemical potency for these compounds was high and ranged from pIC_{50} 8.7 to 9.1. The cellular activity as measured in the MV4-11 anti-proliferation assay was also excellent (pIC_{50} 7.8 - 8.3). Importantly, **75** also demonstrated high cellular activity against the mutant cell lines. As a last step the chirality of the tail group methyl was revisited. For this purpose **72** and **75** were chosen. **75** was the most active compound *in vitro* and across all cell lines. **72** exhibits slightly lower activity, but also reduced off-target toxicity (Ba/F3 wt) and decreased lipophilicity. For both compounds the two enantiomers were synthesized from enantio pure building blocks (**76** – **79**). A clear preference for the *S*-enantiomer (**77** and **79**) was observed with subnanomolar biochemical activities and a good cellular profile.

Table 7: Combination of optimal substituents.

		pIC_{50} ± SEM							
					Ba/F3				
	Structure	***in vitro* FLT3**	**MV4-11**	**U937**	**wt**	**FLT3 ITD**	**FLT3 ITD F691L**	**FLT3 ITD D835H**	**FLT3 ITD D835Y**
72		8.70 ± 0.10	7.8 ± 0.1	< 5	5.3 ± 0.3	7.4 ± 0.1	6.6 ± 0.1	7.4 ± 0.1	7.2 ± 0.1
73		8.76 ± 0.09	7.9 ± 0.1	5.1 ± 0.3	6.0 ± 0.2	8.0 ± 0.1	7.0 ± 0.1	7.7 ± 0.1	7.5 ± 0.1
74		8.94 ± 0.10	8.0 ± 0.1	< 5	5.1 amb.	7.8 ± 0.1	6.5 ± 0.1	7.6 ± 0.1	7.4 ± 0.1

	Structure	pIC_{50} ± SEM							
					Ba/F3				
		in vitro FLT3	MV4-11	U937	wt	FLT3 ITD	FLT3 ITD F691L	FLT3 ITD D835H	FLT3 ITD D835Y
75		9.12 ± 0.06	8.3 ± 0.1	5.1 ± 0.3	6.0 amb.	8.2 ± 0.1	7.1 ± 0.1	8.0 ± 0.1	7.8 ± 0.1
76		8.64 ± 0.09	7.5 ± 0.1	5.7 ± 0.2			ND		
77		9.31 ± 0.08	8.0 ± 0.1	6.4 ± 0.2	6.1 ± 0.1	8.0 ± 0.1	6.8 ± 0.1	7.6 ± 0.1	7.5 ± 0.1
78		8.99 ± 0.07	7.7 ± 0.1	5.9 amb.			ND		
79		9.67 ± 0.06	8.5 ± 0.1	6.7 ± 0.1	6.4 ± 0.1	8.6 ± 0.1	7.2 ± 0.1	8.2 ± 0.1	8.1 ± 0.1

To confirm the position of the nitrogen atoms in the series, a X-ray study with a crystal from the tosyl-protected intermediate was performed (Figure 2B). This clearly showed the expected substitution pattern.



	10	20	77	79
pIC_{50} FLT3	8.5	7.8	9.3	9.7
pIC_{50} MV4-11	7.7	7.5	8.0	8.5
pIC_{50} Ba/F3 ITD	7.9	7.4	8.0	8.6
MW	418	293	313	341
cLogP	3.5	3.7	2.3	3.6
LipE	5.0	4.1	7.0	6.1

Figure 2: (A) Summary of the established structure-activity relationship in this chapter. (B) Crystal structure of tosyl-protected **79** to confirm the configuration. (C) Milestones in the development process of the series.

Compounds **77** and **79** were selected for further profiling (Figure 2C), because they are subnanomolar FLT3 inhibitors with single digit nanomolar potency in the cellular assays. Furthermore, they retained activity on the cells expressing the mutant FLT3 proteins, while having favorable physico-chemical properties and keeping general cellular toxicity to a minimum (Figure 3).

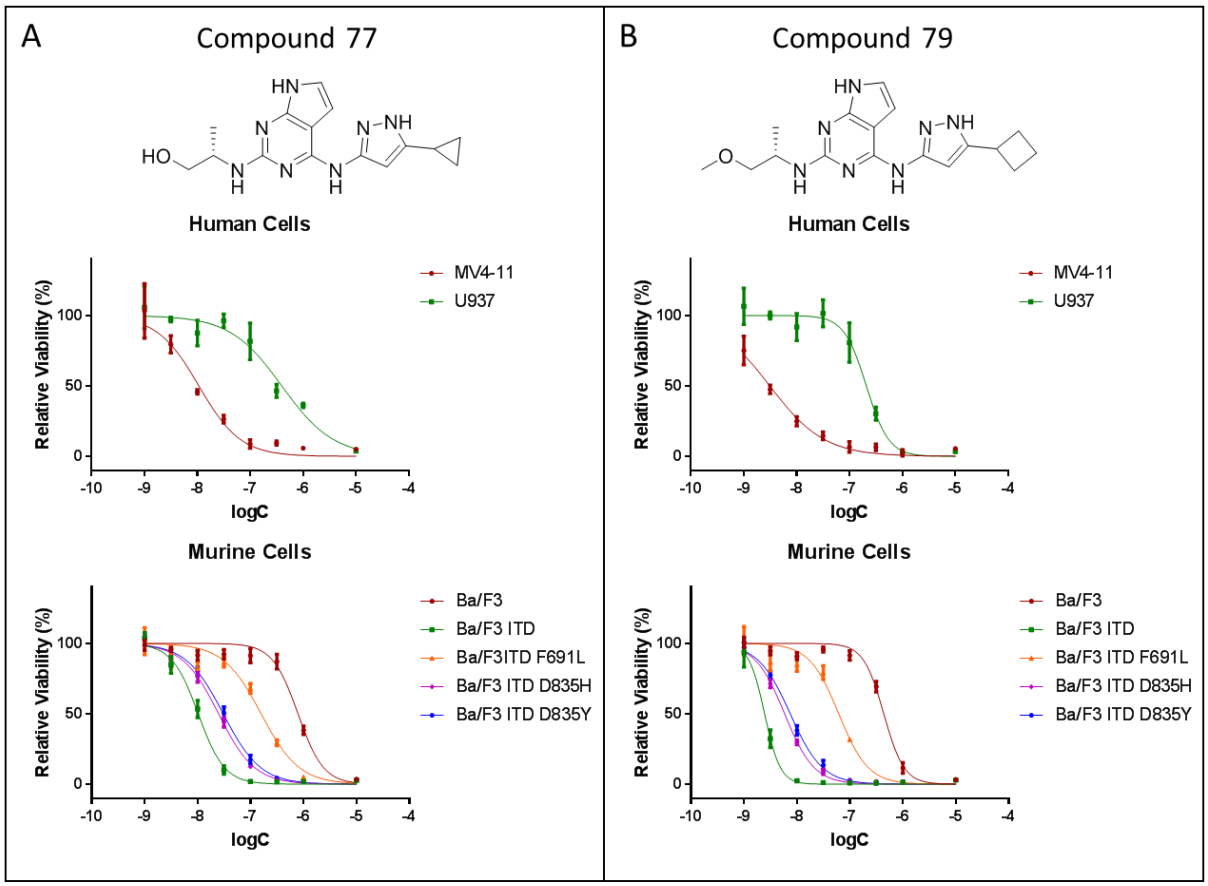


Figure 3: Summary of activity data of the two potent inhibitors X and Y, selected to be further profiled.

In situ selectivity testing using chemical proteomics

The cellular selectivity profile of compound **77** and **79** was determined using chemical proteomics (Chapter 2).[21,22] In this experiment a total of 77 and 53 kinases were identified in MV4-11 and U937 cells, respectively, using the same cut-offs as described in Chapter 2. Next, the two compounds were tested at three different concentrations, 1, 10 and 100 µM in MV4-11 and U937 cells. The results from this study are summarized as a heatmap (Figure 4) and as volcano plots (SI Figure 2). Table 8 lists the off-targets that were dose-dependently inhibited and displayed an IC_{50} < 1 uM. **77** inhibited 19 targets, whereas 34 targets were inhibited by **79**. The target selection procedure is outlined in detail in chapter 2. AURKA, AURKB, CSNK2A1, FYN, GSK3A, GSK3B, IRAK4, JAK1, MAP3K7, MARK2, PTK2, PTK2B, RPS6KA6, SLK, STK11, TBK1 and TEC were inhibited by both compounds. Some of them have been named as drug targets or oncogenes for various disorders, among them AURKB,[23] FYN,[24] IRAK4[25] and MARK2.[26] Furthermore **79** also strongly inhibited tyrosine-protein kinase receptor UFO (AXL), which is being investigated as a target for AML treatment.[27–29]

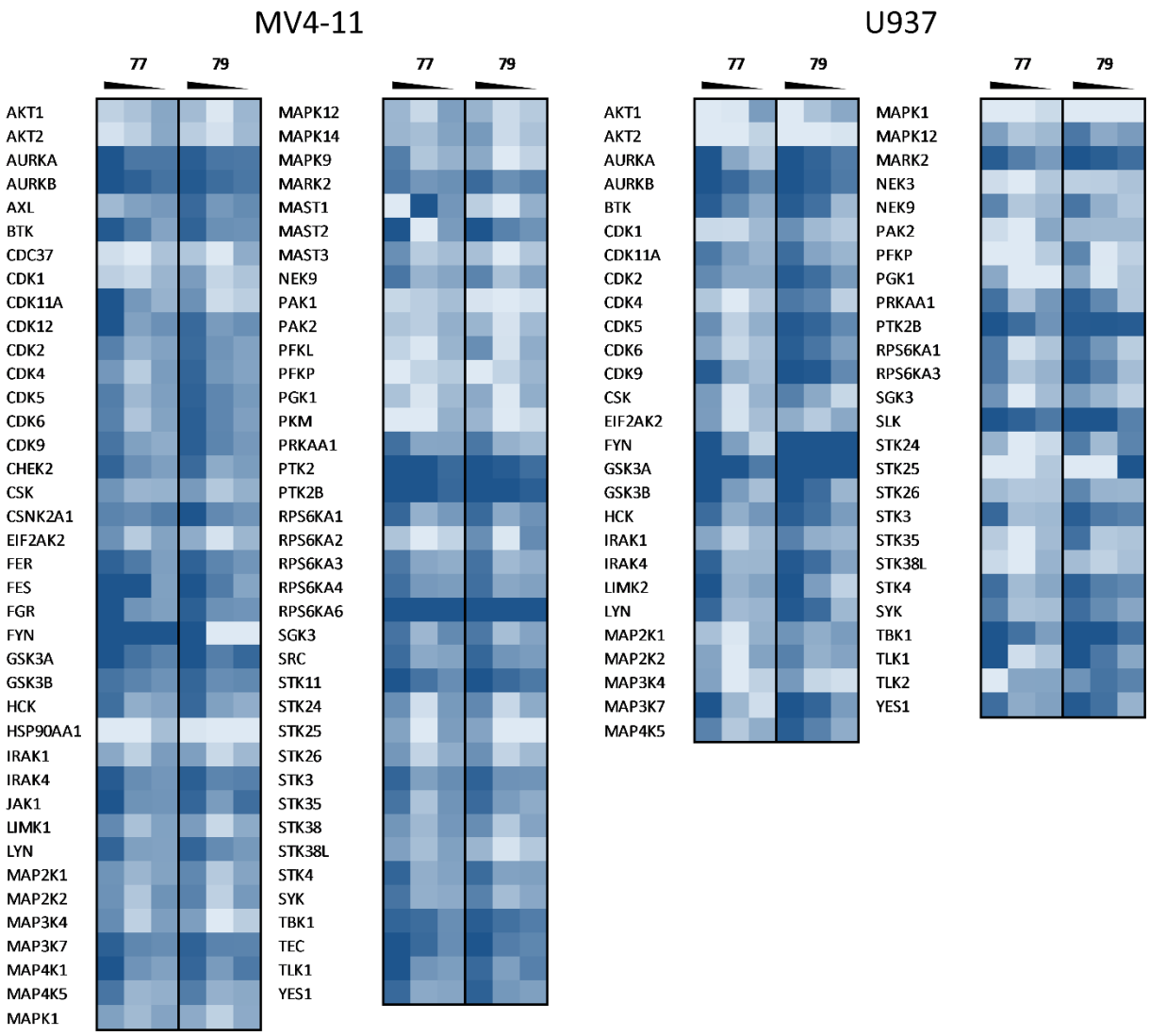


Figure 4: Heatmap of kinase targets of **77** and **79** in MV4-11 and U937. The heatmap shows the ratio of label-free quantification signal from IsoQuant of inhibitor pretreated samples at three concentrations (100, 10 and 1 µM), normalized by only XO44 treatment after subtraction of negative control signal (e.g. 1: no difference in competitor and probe treated sample (light blue) 0: full competition of probe by the inhibitor (dark blue)).

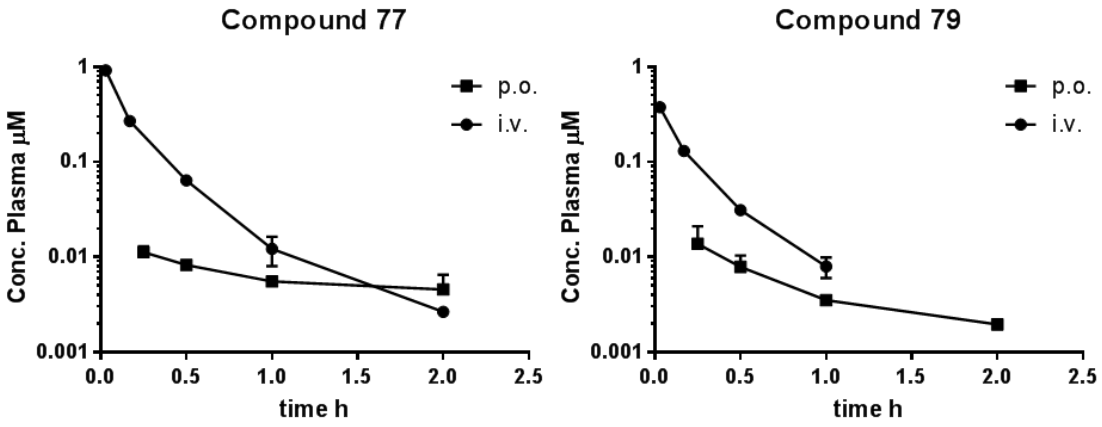
Table 8: Identified targets of **77** and **79** at 1 µM competitor. Targets were selected if there was at least 50% reduction in quantification signal from probe treated samples vs inhibitor pretreated sample in all three concentrations.

Compound 77				**Compound 79**			
MV4-11		**U937**		**MV4-11**		**U937**	
AURKA	MARK2	AURKB	PTK2B	AURKA	LYN	AURKA	MAP3K7
AURKB	PTK2	GSK3A	SLK	AURKB	MAP3K7	AURKB	MARK2
CSNK2A1	PTK2B	MARK2	TBK1	AXL	MAP4K1	CDK2	PTK2B
FYN	RPS6KA4			BTK	MARK2	CDK5	SLK
GSK3A	RPS6KA6			CDK12	MAST2	CDK6	STK3
GSK3B	STK11			CDK5	PTK2	CDK9	STK4
IRAK4	TBK1			CDK9	PTK2B	FYN	TBK1
JAK1	TEC			CSNK2A1	RPS6KA6	GSK3A	TLK2
MAP3K7	TLK1			FER	SRC	HCK	
				FGR	STK11		
				GSK3A	STK3		
				GSK3B	TBK1		
				IRAK4	TEC		
				JAK1			

In vivo PK study

Next, pharmacokinetic studies were performed to establish whether sufficient plasma concentrations could be obtained to inhibit the FLT3 in mice. To this end, compounds **77** and **79** were administered as a single dose in 5% DMSO, 95% SBE-B-CD (30% w/v) via tail vein injection (i.v.) or via oral gavage (p.o.) (Figure 5A). Unfortunately, the compounds displayed low oral bioavailability (F_{po} < 6 %), which can be explained by the high *in vivo* clearance (CL > 120 ml/min/kg). The volume of distribution was low (V_{ss} = 1.2-2.0 L/kg) as expected for neutral compounds. This resulted in short half live ($t_{1/2}$ < 30 min) and low plasma concentrations.

A



B

	77		79	
	i.v.	**p.o.**	**i.v.**	**p.o.**
Actual dose [mg/kg]	0.52	1.0	0.32	0.63
CL [mL/min/kg]	122 ± 7	n.a.	160 ± 6	n.a.
V_{ss} [L/Kg]	1.4 ± 0.1	n.a.	2.0 ± 0.2	n.a.
$t_{½}$ [h]	0.25 ± 0.08	0.43 ± 0	0.21 ± 0.02	0.65 ± 0.30
C_{max} [µmol/L]	0.92 ± 0.02	0.011 ± 0.001	0.38 ± 0.02	0.01 ± 0.01
T_{max} [h]	0.03	0.25	0.03	0.25
F [%]	n.a.	3.2 ± 0.1	n.a.	5.5 ± 0.8

Figure 5: Pharmacokinetic studies of **77** and **79** carried out in mice using oral and intravenous dosing. (N = 2; mouse *in vivo* pharmacokinetic studies were carried out in collaboration with AstraZeneca). (A) Plasma concentrations over time after single dosing. (B) Summary of pharmacokinetic parameters.

Conclusion

In this chapter the hit-to-lead optimization of two confirmed hits (Chapter 4) as FLT3 inhibitors for AML treatment is described. **77** and **79** were identified as highly potent and cellular active FLT3 inhibitors with low molecular weight and high lipophilic efficiency. The compounds also potently inhibited the proliferation of cells that expressed the FLT3 mutants (F691L and D835H/Y), which were previously found to confer resistance to the clinically tested drugs, such as quizartinib. Selectivity profiling of **77** and **79** using chemical proteomics in MV4-11 and U937 cells revealed that the compounds possess broad-spectrum kinase activity, comparable to the clinically approved drug midostaurin.[7,8] Pharmacokinetic profiling indicated that the chemical and metabolic stability needs to be improved before these compounds can be tested in *in vivo* models of AML.

Experimental

Biochemical Evaluation of FLT3 inhibitors

In a 384-wells plate (PerkinElmer 384 Flat White), 5 µL kinase/peptide mix (0.06 ng/µL FLT3 (Life Technologies; PV3182; Lot: 1614759F), 200 nM peptide (PerkinElmer; Lance® Ultra ULightTM TK-peptide; TRFO127-M; Lot: 2178856)) in assay buffer (50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 0.01% Tween-20, 2 mM DTT) was dispensed. Separately inhibitor solutions (10 µM – 0.1 pM) were prepared in assay buffer containing 400 µM ATP and 1% DMSO. 5 µL of these solutions were dispensed and the plate was incubated in the dark at room temperature. After 90 minutes the reaction was quenched by the addition of 10 µL of 20 mM EDTA containing 4 nM antibody (PerkinElmer; Lance® Eu-W1024-anti-phosphotyrosine(PT66); AD0068; Lot: 2342358). After mixing, samples were incubated for 60 minutes in the dark. The FRET fluorescence was measured on a Tecan Infinite M1000 Pro plate reader (excitation 320 nm, emission donor 615 nm, emission acceptor 665 nm). Data was processed using Microsoft Excel 2016, pIC_{50} values were fitted using GraphPad Prism 7.0. Final assay concentrations during reaction: 200 µM ATP, 0.03 ng/µL FLT3, 100 nM Lance TK-peptide, 0.5% DMSO. Compounds were tested in n=2 and N=2. Compounds 14, 16, 17 and 28 – 35 were tested in n=3.

In situ testing of kinase inhibitors

To evaluate inhibitor effect on cell proliferation MV4-11, U937 and Ba/F3 cell lines were grown in RPMI, supplemented with 10% fetal bovine serum in an incubator at 37°C under 5% CO_2 atmosphere. Ba/F cells (wild-type) were grown in the presence of IL-3 (10 ng/mL, PeproTech). For viability assays, 10,000 cells were seeded per well in a 96-wells plate and inhibitors were added at the indicated concentration. After three days, cell viability was measured using the Cell Titer Blue (alamarBlue) viability assay (Promega) and fluorescence was measured using the Clariostar (BMG Labtech). Relative survival was normalized to the untreated control and corrected for background signal. Data was processed using Microsoft Excel 2016, pIC_{50} values were fitted using GraphPad Prism 7.0. Experiments were performed in n=2 – 3.

In vivo pharmacokinetic studies

Mouse in vivo pharmacokinetic studies were carried out in collaboration with AstraZeneca. Compounds were prepared in a solution PO: 5% DMSO, 95% SBE-B-CD (30% w/v) in water and IV: 5% DMSO, 95% SBE-B-CD (30% w/v) in water. Male CD-1 mice (20-40 g) were administered in a single dose with test compound solution either by intravenous tail vein injection or oral gavage, in a cassette dosing fashion. Plasma levels were measured at the indicated time points using LC-MS/MS. Measured mass signal was adjusted using an internal standard and quantified using an external calibration curve from 0.5 nM to 1 µM.

Crystallography

All reflection intensities were measured at 110(2) K using a SuperNova diffractometer (equipped with Atlas detector) with Mo Kα radiation (λ = 0.71073 Å) under the program CrysAlisPro (Version CrysAlisPro 1.171.39.29c, Rigaku OD, 2017). The same program was used to refine the cell dimensions and for data reduction. The structure was solved with the program SHELXS-2014/7[30] and was refined on F2 with SHELXL-2014/7 (Sheldrick, 2015). Numerical absorption correction based on gaussian integration over a multifaceted crystal model was applied using CrysAlisPro. The temperature of the data collection was controlled

using the system Cryojet (manufactured by Oxford Instruments). The H atoms were placed at calculated positions (unless otherwise specified) using the instructions AFIX 13, AFIX 23, AFIX 43 or AFIX 137 with isotropic displacement parameters having values 1.2 or 1.5 Ueq of the attached C or N atoms.

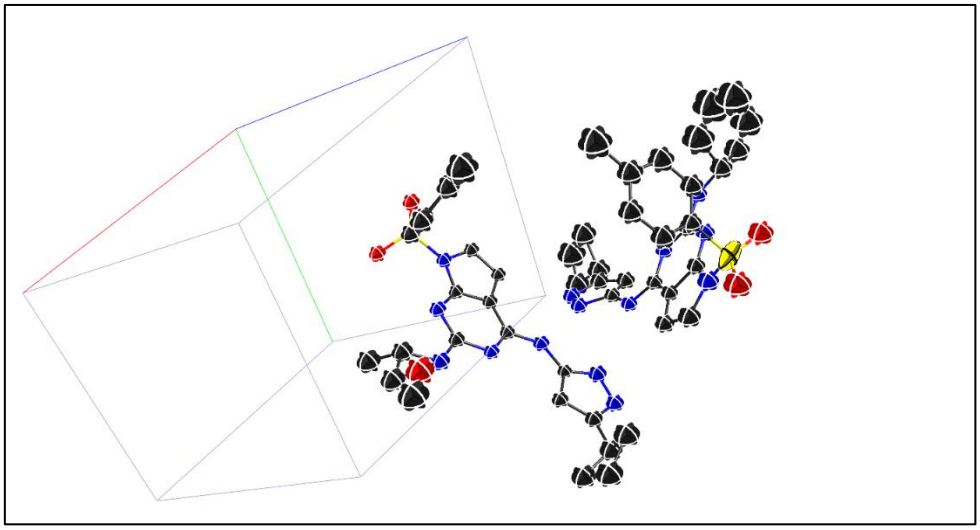
The structure is significantly disordered as the two crystallographically independent molecules A and B are disordered over two orientations. The occupancy factors of the major components of the disorder (i.e., A and B are 0.796(8) and 0.543(10)). The disorder is likely more complicated as there are some unresolved electron density peaks ranging from 0.63-1.42 e–Å–3 near the fragments (N10X→C26X, X = A and B for the major components of the disorder, X = C and D for the minor components of the disorder). This suggests those fragments are disordered over at least three orientations. As the data-to-parameter ratio is low, no attempts were made to model a three-component disorder.

The absolute configuration has been established by anomalous-dispersion effects in diffraction measurements on the crystal, and the Flack and Hooft parameters refine to 0.02(2) and 0.011(18), respectively. The chiral centers C22A/C22B have the S configuration. Used computer programs: *CrysAlis PRO* 1.171.39.29c, *SHELXS2014*/7, *SHELXL2014*/7, *SHELXTL* v6.10.

SI Table 1: Experimental details for compound **105**.

	xs1582a
Crystal data	
Chemical formula	$C_{24}H_{29}N_7O_3S$
M_r	495.60
Crystal system, space group	Triclinic, *P*1
Temperature (K)	110
a, *b*, *c* (Å)	10.0594 (3), 11.8988 (3), 12.4777 (3)
α, β, γ(°)	116.556 (2), 99.359 (2), 95.790 (2)
V (Å^3)	1292.42 (6)
Z	2
Radiation type	Mo *K*α
μ (mm^{-1})	0.16
Crystal size (mm)	0.35 × 0.18 × 0.14
Data collection	
Diffractometer	SuperNova, Dual, Cu at zero, Atlas
Absorption correction	Gaussian *CrysAlis PRO* 1.171.39.29c (Rigaku Oxford Diffraction, 2017) Numerical absorption correction based on gaussian integration over a multifaceted crystal model Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling
T_{min}, T_{max}	0.546, 1.000
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	34089, 10410, 9470
R_{int}	0.030
$(\sin \Theta/\lambda)_{max}$ (Å^{-1})	0.622

Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.074, 0.214, 1.03
No. of reflections	10410
No. of parameters	1017
No. of restraints	2528
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	1.42, -0.35
Absolute structure	Flack x determined using 4089 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Absolute structure parameter	0.02 (2)



SI Figure 1: Crystal-structure of **105**.

Synthetic Procedures

Solvents were purchased from Biosolve, Sigma Aldrich or Fluka and, if necessary dried over 3 Å or 4Å molecular sieves. Reagents purchased from chemical suppliers were used without further purification, unless stated otherwise. Oxygen or H_2O sensitive reactions were performed under argon or nitrogen atmosphere and/or under exclusion of H_2O. Microwave reactions were performed in a Biotage initiator+ microwave. Reactions were followed by thin layer chromatography analysis and was performed using TLC silica gel 60 F_{245} on aluminium sheets, supplied by Merck. Compounds were visualized by UV absorption (254 nm) or spray reagent (permanganate (5 g/L $KMnO_4$, 25 g/L K_2CO_3)). TLCMS was measured thin layer chromatography-mass spectrometer (Advion, EppressionL CMS; Advion, Plate Express). ^{1}H and ^{13}C-NMR spectra were performed on one of the following Bruker spectrometers: DPX 300 NMR spectrometer (300 MHz), equipped with 5mm-BBO-z-gradient-probe; AV-400 NMR spectrometer (400 MHz), equipped with 5mm-BBO-z-gradient-probe; AV-500 NMR spectrometer (500 MHz), equipped with BBFO-z-gradient-probe; AV-600 NMR spectrometer (600 MHz), equipped with 5mm-Cryo-z-gradient probe; AV-850 NMR spectrometer (850 MHz),. NMR spectra were measured in deuterated methanol, chloroform or DMSO and were referenced to the residual protonated solvent signals as internal standards (chloroform-*d* = 7.260 (^{1}H), 77.160 (^{13}C); methanol-d_4 = 3.310 (^{1}H), 49.000 (^{13}C); DMSO-d_6 = 2.500 (^{1}H), 39.520

(^{13}C)). Signals multiplicities are written as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), p (pentet) or m (multiplet). Coupling constants (*J*) are given in Hz. Preparative HPLC (Waters, 515 HPLC pump M; Waters, 515 HPLC pump L; Waters, 2767 sample manager; Waters SFO System Fluidics Organizer; Waters Acquity Ultra Performance LC, SQ Detector; Waters Binary Gradient Module) was performed on a Phenomenex Gemini column (5 µM C18, 150 x 4.6 mm) or a Waters XBridgeTM column (5 µM C18, 150 x 19 mm). Diode detection was done between 210 and 600 nm. Gradient: ACN in (H_2O + 0.2% TFA). HRMS (Thermo, Finnigan LTQ Orbitrap; Thermo, Finnigan LTQ Pump; Thermo, Finnigan Surveyor MS Pump PLUS Thermo, Finnigan Surveyor Autosampler; NESLAB, Merlin M25). Data acquired through direct injection of 1 mM of the sample in ACN/H_2O/*t*-BuOH (1:1:1), with mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas low 10, capillary temperature 275°C) with resolution R = 60,000 at m/z = 400 (mass range = 150-2000) and dioctylphtalate (m/z = 391.28428) as lock mass. All tested compounds were checked for purity by LCMS liquid chromatography-mass spectrometer, a Thermo (Thermo Finnigan LCQ Advantage Max; Thermo Finnigan Surveyor LC-pump Plus; Thermo Finnigan Surveyor Autosampler Plus; Thermo Finnigan Surveyor PDA Plus Detector; Phenomenex Gemini column (5 µm C18, 50 x 4.6 mm)) system and were determined to be >95% pure by integrating UV intensity recorded unless stated otherwise.

General procedure A: Nucleophilic aromatic substitution

A flask was charged with chloropyrimidine derivative (1 eq) dissolved in EtOH. Dropwise addition of aminopyrazole (1.1 - 1.2 eq) dissolved in EtOH brings the concentration of chloropyrimidine in EtOH to 0.4 M. After addition of Et_3N (1.1 – 1.4 eq) the reaction was stirred until completion as was indicated by TLC or LCMS analysis (typically 2-48 h). The reaction mixture was diluted with MeOH, concentrated onto celite and purified via silica-gel flash-column-chromatography.

General procedure B: Nucleophilic aromatic substitution

A flask was charged with chloropyrimidine derivative (eq indicated) and amine (eq indicated) dissolved in the indicated solvent (0.15 M). After addition of DiPEA or Et_3N (eq indicated), the flask was sealed and heated to the indicated temperature until completion (typically 1-4 d) was indicated by TLC or LCMS analysis. The reaction mixture concentrated and purified via silica-gel flash-column-chromatography or when the product precipitated by filtration.

General procedure C: Nucleophilic aromatic substitution

A flask was charged with chloropyrimidine derivative (1 eq) and amine (1.1 eq) dissolved in *n*-butanol (0.15 M). After addition of DiPEA (2.5 eq), the flask was sealed and heated to 120°C until completion was indicated by TLC or LCMS analysis (typically 2-4 d). The reaction mixture concentrated and purified via preparative HPLC.

General procedure D: Nucleophilic aromatic substitution

A flask was charged with chloropyrimidine derivative (1 eq) and amine (1.2 eq) dissolved in *n*-butanol (0.15 M). After addition of DiPEA (2.5 eq), the flask was sealed and heated to 120°C until completion was indicated by TLC or LCMS analysis (typically 2-4 d). The reaction mixture concentrated and purified via preparative HPLC.

General procedure E: Nucleophilic aromatic substitution

A flask was charged with chloropyrimidine derivative (1 eq) and amine (1.1 eq) dissolved in *n*-butanol (0.15 M). After addition of DiPEA (1.5 eq), the flask was sealed and heated to 120°C

until completion was indicated by TLC or LCMS analysis (typically 2-4 d). The reaction mixture concentrated and purified via preparative HPLC.

General procedure F: Nucleophilic aromatic substitution

A flask was charged with chloropyrimidine derivative (1 eq) and amine (1.2 eq) dissolved in *n*-butanol (0.15 M). After addition of DiPEA (1.5 eq), the flask was sealed and heated to 120°C until completion was indicated by TLC or LCMS analysis (typically 2-4 d). The reaction mixture concentrated and purified via preparative HPLC.

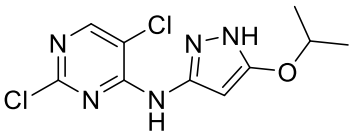
General procedure G: Nucleophilic aromatic substitution

A flask was charged with chloropyrimidine derivative (eq indicated) and amine (eq indicated) dissolved in *n*-butanol. After addition of DiPEA (eq indicated), the flask was sealed and heated to 120°C until completion was indicated by TLC or LCMS analysis. The reaction mixture concentrated and purified via preparative HPLC.

General procedure H: Nucleophilic aromatic substitution

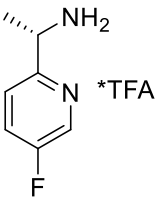
A flask was charged with chloropyrimidine derivative (eq indicated) and amine (eq indicated) dissolved in *n*-butanol. After addition of DiPEA (eq indicated), the flask was sealed and heated in the microwave to the indicated time and temperature. Completion was indicated by TLC or LCMS analysis. The reaction was mixture concentrated and purified via preparative HPLC.

2,5-Dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (3)



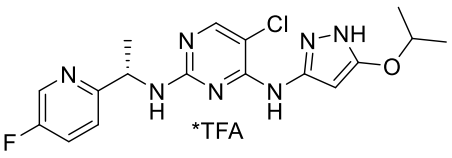
The title compound was synthesized from 2,4,5-trichloropyrimidine (**1a**) and 5-isopropoxy-1*H*-pyrazol-3-amine (**2a**) following General procedure A on a 0.30 mmol scale at RT and purified via flash-column-chromatography (dry-loading, SiO_2, 0% → 100% EtOAc in pentane) to yield the product (40 mg, 47%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.25 (s, 1H), 5.81 (bs, 1H), 4.60 (bs, 1H), 1.35 (d, *J* = 6.2 Hz, 6H). ^{13}C NMR (126 MHz, methanol-d_4) δ 158.92, 157.73, 156.14, 114.85, 83.10, 81.72, 76.28, 73.16, 22.36. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.48 min; *m/z* : 288 $[M+H]^+$.

(*S*)-1-(5-Fluoropyridin-2-yl)ethan-1-amine (4a)



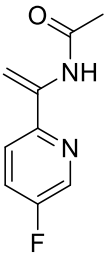
A round-bottom-flask was charged with *tert*-butyl (*S*)-(1-(5-fluoropyridin-2-yl)ethyl)carbamate (**9**) (1.17 g, 4.87 mmol, 1 eq) dissolved in $CHCl_3$ (48 mL). After cooling to 0°C and addition of TFA (12 mL) the mixture was warmed up to RT and stirred for 1 h. The solution was concentrated under reduced pressure and co-evaporated with MeOH (3x50 mL) to yield the product (1.29 g, quant.). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.52 (d, *J* = 2.9 Hz, 1H), 7.67 (td, *J* = 8.6, 2.9 Hz, 1H), 7.53 (dd, *J* = 8.7, 4.3 Hz, 1H), 4.96 (s, 3H), 4.61 (q, *J* = 6.9 Hz, 1H), 1.61 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (151 MHz, methanol-d_4) δ 160.82 (d, *J* = 255.1 Hz), 154.47 (d, *J* = 3.8 Hz), 138.54 (d, *J* = 24.9 Hz), 125.47 (d, *J* = 19.1 Hz), 124.04 (d, *J* = 4.9 Hz), 51.59, 20.43.

(*S*)-5-Chloro-*N*^{2}-(1-(5-fluoropyridin-2-yl)ethyl)-*N*^{4}-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidine-2,4-diamine (5)



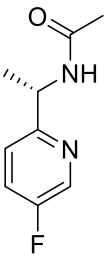
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1H-pyrazol-3-yl)pyrimidin-4-amine (**3**) and (*S*)-1-(5-fluoropyridin-2-yl)ethan-1-amine (**4a**) following General procedure D on a 0.135 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (3 mg, 4%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.42 (d, *J* = 2.9 Hz, 1H), 8.04 (s, 1H), 7.58 (td, *J* = 8.6, 2.9 Hz, 1H), 7.46 (s, 1H), 5.75 (s, 1H), 5.14 (s, 1H), 4.68 – 4.57 (m, 1H), 1.59 (d, *J* = 7.0 Hz, 3H), 1.37 (d, *J* = 6.1 Hz, 6H). ^{13}C NMR (151 MHz, methanol-d_4) δ 161.18, 158.89 (d, *J* = 254.0 Hz), 156.58, 144.95, 141.07, 136.41 (d, *J* = 24.5 Hz), 124.07 (d, *J* = 18.7 Hz), 121.69, 103.65, 73.44, 52.41, 51.73, 20.95, 19.96. HRMS calculated for $C_{17}H_{20}ClFN_7O$ 392.13964 $[M+H]^+$, found 392.1404. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.13 min; *m/z* : 392 $[M+H]^+$.

***N*-(1-(5-Fluoropyridin-2-yl)vinyl)acetamide (7)**



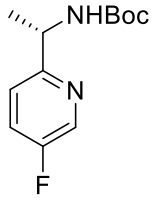
A round-bottom-flask was charged with 2-cyano-5-fluoropyrimidine (**6**) (3.00 g, 23.82 mmol, 1 eq) dissolved in dry THF (120 mL) under nitrogen atmosphere and cooled to 0°C. After dropwise addition of MeMgBr in diethyl ether (3 M, 9.5 mL, 28.62 mmol, 1.2 eq) the reaction mixture was stirred at 0°C for 50 min and after addition of Ac_2O (3.0 mL, 28.62 mmol, 1.2 eq) allowed to warm to RT. The reaction was quenched by addition of saturated $NaHCO_3$ (150 mL) and extracted with DCM (3x100 mL). The combined organic layers where dried (Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue was purified via flash-column-chromatography (SiO_2, 15% → 45% EtOAc in pentane) to yield the product (1.89 g, 44%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.06 (s, 1H), 8.36 (d, *J* = 2.7 Hz, 1H), 7.90 – 7.69 (m, 1H), 7.50 – 7.42 (m, 1H), 6.47 (s, 1H), 5.46 (s, 1H), 2.22 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 169.34, 159.37 (d, *J* = 257.1 Hz), 148.46 (d, *J* = 3.7 Hz), 136.81, 135.71 (d, *J* = 24.6 Hz), 124.34 (d, *J* = 19.1 Hz), 120.35 (d, *J* = 4.6 Hz), 99.11, 25.19.

(*S*)-*N*-(1-(5-Fluoropyridin-2-yl)ethyl)acetamide (8)



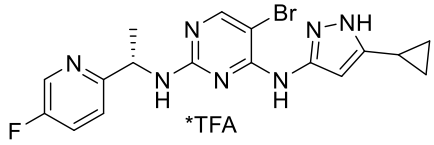
A round-bottom-flask was charged with *N*-(1-(5-fluoropyridin-2-yl)vinyl)acetamide (**7**) (1.68 g, 9.1 mmol, 1 eq) dissolved in dry Methanol (20 mL) under inert atmosphere. After addition of (+)-1,2-bis((2*S*,5*S*)-2,5-diethylphospholano) benzene(cyclooctadiene)rhodium trifluoromethanesulfonat (135 mg, 0.18 mmol, 0.02 eq) the mixture was transferred into a high-pressure reaction vessel and stirred under a 10 bar H_2 atmosphere ON. The resulting solution was concentrated under reduced pressure and purified via flash-column-chromatography (SiO_2, 0% → 1% MeOH in EtOAc) to yield the product (1.63 g, 96%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.39 (d, *J* = 2.8 Hz, 1H), 7.46 – 7.33 (m, 1H), 7.32 – 7.23 (m, 1H), 6.85 (s, 1H), 5.16 (p, *J* = 7.0 Hz, 1H), 2.03 (s, 3H), 1.45 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 169.42, 158.66 (d, *J* = 254.9 Hz), 157.15 (d, *J* = 3.8 Hz), 137.20 (d, *J* = 23.9 Hz), 123.76 (d, *J* = 18.5 Hz), 122.50 (d, *J* = 4.2 Hz), 49.30 (d, *J* = 1.0 Hz), 23.51, 22.65.

tert-Butyl (_S_)-(1-(5-fluoropyridin-2-yl)ethyl)carbamate (9)



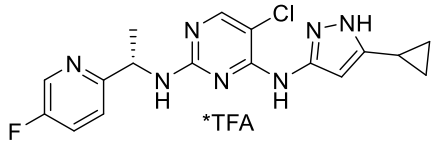
A round-bottom-flask was charged with (*S*)-*N*-(1-(5-fluoropyridin-2-yl)ethyl)acetamide (**8**) (1.24 g, 5.49 mmol, 1 eq) and DMAP (135 mg, 1.10 mmol, 0.2 eq). After addition of Boc_2O (4.20 g, 19.22 mmol, 3.5 eq) in THF (10 mL) and heating to 50°C for 3 d, the reaction was cooled to RT and LiOH (826 mg, 19.69 mmol, 3.59 eq) and H_2O (15 mL) were added. The mixture was stirred ON at RT, diluted with diethyl ether (100 mL), washed with brine (1x100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified via flash-column-chromatography (SiO_2, 0% → 20% EtOAc in pentane) to yield the product (1.40 g, quant.). ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.39 (d, *J* = 3.0 Hz, 1H), 7.39 – 7.33 (m, 1H), 7.28 – 7.23 (m, 1H), 5.54 (s, 1H), 4.85 (s, 1H), 1.63 – 1.25 (m, 12H). chiral-LC (Chiralcel OD, isocratic, 0.5 *i*PrOH in heptane): 76% (*S*). LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.76 min; *m/z* : 241 $[M+H]^+$.

(_S_)-5-Chloro-_N_^{4}-(5-cyclopropyl-1_H_-pyrazol-3-yl)-_N_^{2}-(1-(5-fluoropyridin-2-yl)ethyl) pyrimidine-2,4-diamine (10)



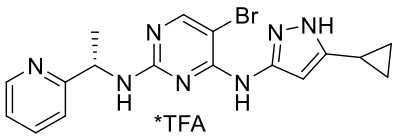
The title compound was synthesized from 5-bromo-2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**80**) and (*S*)-1-(5-fluoropyridin-2-yl)ethan-1-amine (**4a**) following General procedure C on a 0.13 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (29 mg, 42%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.43 (d, *J* = 2.9 Hz, 1H), 8.13 (s, 1H), 7.57 (td, *J* = 8.6, 2.9 Hz, 1H), 7.38 (bs, 1H), 6.06 (bs, 1H), 5.13 (bs, 1H), 2.00 – 1.93 (m, 1H), 1.59 (d, *J* = 7.0 Hz, 3H), 1.09 – 1.03 (m, 2H), 0.79 (bs, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 160.27 (d, *J* = 253.9 Hz), 159.12, 158.55, 154.30, 149.44, 145.99, 145.87, 138.06 (d, *J* = 24.5 Hz), 125.29 (d, *J* = 18.8 Hz), 123.08 (d, *J* = 4.7 Hz), 96.48, 92.48, 53.69, 21.49, 8.48, 7.84. HRMS calculated for $C_{17}H_{18}BrFN_7$ 418.07856 $[M+H]^+$, found 418.0795. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.45 min; *m/z* : 418 $[M+H]^+$.

(_S_)-5-Chloro-_N_^{4}-(5-cyclopropyl-1_H_-pyrazol-3-yl)-_N_^{2}-(1-(5-fluoropyridin-2-yl)ethyl)pyrimidine-2,4-diamine (11)



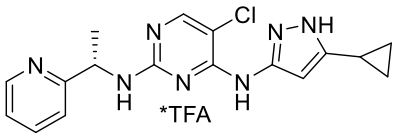
The title compound was synthesized from 2,5-dichloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and (*S*)-1-(5-fluoropyridin-2-yl)ethan-1-amine (**4a**) following General procedure C on a 0.13 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (28 mg, 44%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.43 (d, *J* = 2.8 Hz, 1H), 8.05 (s, 1H), 7.61 – 7.54 (m, 1H), 7.40 (bs, 1H), 6.07 (s, 1H), 5.14 (s, 1H), 2.00 – 1.93 (m, 1H), 1.60 (d, *J* = 7.0 Hz, 3H), 1.11 – 1.03 (m, 2H), 0.79 (s, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 160.27 (d, *J* = 253.7 Hz), 158.61, 158.57, 154.06, 149.41, 145.83, 142.93, 138.04 (d, *J* = 24.6 Hz), 125.31 (d, *J* = 18.8 Hz), 123.09 (d, *J* = 4.6 Hz), 105.55, 96.47, 53.74, 21.50, 8.48, 7.84. HRMS calculated for $C_{17}H_{18}ClFN_7$ 374.12908 $[M+H]^+$, found 374.1304. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.35 min; *m/z* : 374 $[M+H]^+$.

(*S*)-5-Bromo-N^4-(5-cyclopropyl-1*H*-pyrazol-3-yl)-N^2-(1-(pyridin-2-yl)ethyl) pyrimidine -2,4-diamine (12)



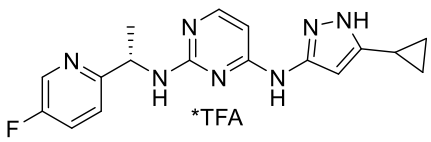
The title compound was synthesized from 5-bromo-2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**80**) and (*S*)-1-(pyridin-2-yl)ethan-1-amine (**4b**) following General procedure E on a 0.20 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (58 mg, 56%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.63 (d, *J* = 5.5 Hz, 1H), 8.27 (s, 1H), 8.15 (s, 1H), 7.78 (s, 1H), 7.71 (s, 1H), 5.96 (s, 1H), 5.23 (q, *J* = 7.1 Hz, 1H), 2.03 – 1.94 (m, 1H), 1.71 (d, *J* = 7.1 Hz, 3H), 1.13 – 1.05 (m, 2H), 0.79 (s, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 159.67, 158.91, 155.24, 149.38, 147.70, 146.17, 145.46, 144.39, 125.88, 124.19, 96.52, 92.99, 52.18, 20.06, 8.58, 7.82. HRMS calculated for $C_{17}H_{19}BrN_7$ 400.08798 $[M+H]^+$, found 400.0892. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.76 min; *m/z* : 400 $[M+H]^+$.

(*S*)-5-Chloro-N^4-(5-cyclopropyl-1*H*-pyrazol-3-yl)-N^2-(1-(pyridin-2-yl)ethyl) pyrimidine-2,4-diamine (13)



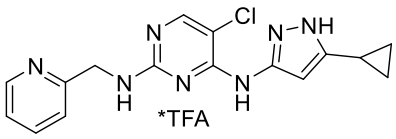
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1H-pyrazol-3-yl)pyrimidin-4-amine (**81**) and (*S*)-1-(pyridin-2-yl)ethan-1-amine (**4b**) following General procedure F on a 0.25 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (60 mg, 51%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.62 (d, *J* = 5.4 Hz, 1H), 8.24 (s, 1H), 8.05 (s, 1H), 7.77 (s, 1H), 7.68 (s, 1H), 5.98 (s, 1H), 5.23 (q, *J* = 7.1 Hz, 1H), 2.01 – 1.94 (m, 1H), 1.70 (d, *J* = 7.1 Hz, 3H), 1.11 – 1.04 (m, 2H), 0.79 (s, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 159.98, 158.20, 155.30, 149.39, 146.16, 145.75, 145.29, 144.01, 125.76, 124.06, 105.90, 96.39, 52.30, 20.14, 8.57, 7.83. HRMS calculated for $C_{17}H_{19}ClN_7$ 356.13850 $[M+H]^+$, found 356.1394. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.68 min; *m/z* : 356 $[M+H]^+$.

(*S*)-N^4-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-N^2-(1-(5-fluoropyridin-2-yl)ethyl) pyrimidine-2,4-diamine (14)



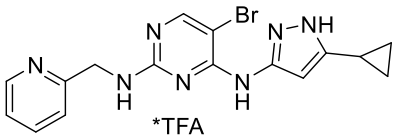
The title compound was synthesized from 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**82**) and (*S*)-1-(5-Fluoropyridin-2-yl)ethan-1-amine (**4b**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (46 mg, 34%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.44 (d, *J* = 2.8 Hz, 1H), 7.73 (d, *J* = 7.3 Hz, 1H), 7.57 (td, *J* = 8.6, 2.9 Hz, 1H), 7.49 – 7.44 (m, 1H), 6.32 (s, 1H), 6.11 (s, 1H), 5.35 – 5.19 (m, 1H), 1.96 – 1.89 (m, 1H), 1.62 (d, *J* = 7.0 Hz, 3H), 1.04 – 1.00 (m, 2H), 0.77 – 0.74 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.40, 158.25 (d, *J* = 251.6 Hz), 157.90, 153.24, 146.22, 145.90, 142.20, 136.85 (d, *J* = 22.9 Hz), 124.20 (d, *J* = 18.4 Hz), 121.46, 98.12, 93.42, 51.94, 21.46, 7.93, 6.83. HRMS calculated for $C_{17}H_{19}FN_7$ 340.16805 $[M+H]^+$, found 340.1688. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.22 min; *m/z* : 340 $[M+H]^+$.

5-Chloro-N^4-(5-cyclopropyl-1*H*-pyrazol-3-yl)-N^2-(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (15)



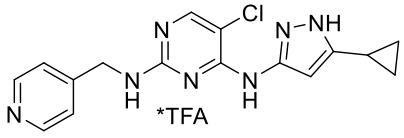
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1H-pyrazol-3-yl)pyrimidin-4-amine (**81**) and pyridin-2-ylmethanamine (**4c**) following General procedure E on a 0.185 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 10% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (45 mg, 53%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.61 (d, *J* = 5.2 Hz, 1H), 8.19 (t, *J* = 7.8 Hz, 1H), 8.04 (s, 1H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.65 (t, *J* = 6.5 Hz, 1H), 5.95 (s, 1H), 4.78 (s, 2H), 1.93 – 1.87 (m, 1H), 1.04 – 0.99 (m, 2H), 0.69 (s, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 157.69, 156.44, 149.46, 147.56, 146.51, 146.23, 143.42, 125.52, 125.34, 105.72, 95.56, 45.75, 8.47, 7.77. HRMS calculated for $C_{16}H_{17}ClN_7$ 342.12285 $[M+H]^+$, found 342.1242. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.42 min; *m/z* : 342 $[M+H]^+$.

5-Bromo-N^4-(5-cyclopropyl-1*H*-pyrazol-3-yl)-N^2-(pyridin-2-ylmethyl)pyrimidine -2,4-diamine (16)



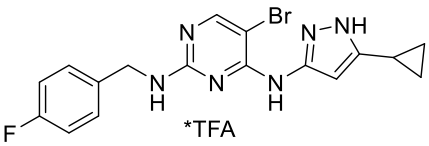
The title compound was synthesized from 5-bromo-2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**80**) and pyridin-2-ylmethanamine (**4c**) following General procedure E on a 0.20 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 10% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (71 mg, 71%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.65 (d, *J* = 5.2 Hz, 1H), 8.28 (t, *J* = 7.9 Hz, 1H), 8.16 (s, 1H), 7.79 – 7.75 (m, 1H), 7.73 (t, *J* = 6.6 Hz, 1H), 5.95 (s, 1H), 4.81 (s, 2H), 1.95 – 1.88 (m, 1H), 1.05 – 1.00 (m, 2H), 0.70 (s, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 158.63, 156.77, 155.66, 149.09, 146.48, 145.55, 144.42, 125.89, 95.96, 93.13, 45.29, 8.52, 7.74. HRMS calculated for $C_{16}H_{17}BrN_7$ 386.07233 $[M+H]^+$, found 342.12340. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.51 min; *m/z* : 386 $[M+H]^+$.

5-Chloro-N^4-(5-cyclopropyl-1*H*-pyrazol-3-yl)-N^2-(pyridin-4-ylmethyl)pyrimidine-2,4-diamine (17)



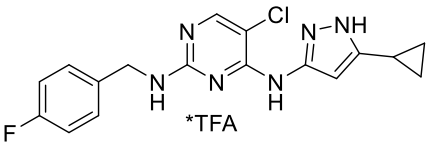
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1H-pyrazol-3-yl)pyrimidin-4-amine (**81**) and pyridin-4-ylmethanamine (**4d**) following General procedure F on a 0.25 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 10% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (49 mg, 43%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.78 – 8.75 (m, 2H), 8.14 (s, 1H), 7.94 (s, 2H), 5.84 (s, 1H), 4.85 (s, 2H), 1.89 (s, 1H), 1.06 – 1.01 (m, 2H), 0.62 (s, 2H). NO C NMR. HRMS calculated for $C_{16}H_{17}ClN_7$ 342.12285 $[M+H]^+$, found 342.1242. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.12 min; *m/z* : 342 $[M+H]^+$.

5-Bromo-*N*^{4}-(5-cyclopropyl-1*H*-pyrazol-3-yl)-*N*^{2}-(4-fluorobenzyl)pyrimidine-2,4-diamine (18)



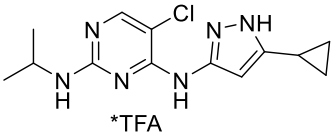
The title compound was synthesized from 5-bromo-2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**80**) and (4-fluorophenyl)methanamine (**4e**) following General procedure E on a 0.20 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (66 mg, 64%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.12 (s, 1H), 7.29 (s, 2H), 7.05 (t, *J* = 8.6 Hz, 2H), 6.09 (s, 1H), 4.55 (s, 2H), 1.94 – 1.85 (m, 1H), 1.02 – 0.93 (m, 2H), 0.60 (s, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 163.62 (d, *J* = 244.6 Hz), 159.58, 154.78, 149.23, 146.29, 145.36, 134.73, 130.33 (d, *J* = 8.1 Hz), 116.35 (d, *J* = 21.8 Hz), 96.83, 92.39, 45.37, 8.37, 7.69. HRMS calculated for $C_{17}H_{17}BrFN_6$ 403.06766 $[M+H]^+$, found 403.0686. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.28 min; *m/z* : 403 $[M+H]^+$.

5-Chloro-*N*^{4}-(5-cyclopropyl-1*H*-pyrazol-3-yl)-*N*^{2}-(4-fluorobenzyl)pyrimidine-2,4-diamine (19)



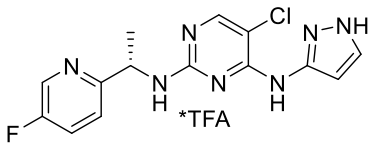
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1H-pyrazol-3-yl)pyrimidin-4-amine (**81**) and (4-fluorophenyl)methanamine (**4e**) following General procedure F on a 0.25 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (71 mg, 60%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.05 (s, 1H), 7.30 (s, 2H), 7.05 (t, *J* = 8.6 Hz, 2H), 6.10 (s, 1H), 4.56 (s, 2H), 1.92 – 1.86 (m, 1H), 1.00 – 0.94 (m, 2H), 0.61 (s, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 163.62 (d, *J* = 244.4 Hz), 159.01, 154.53, 149.19, 146.16, 142.38, 134.75, 130.32 (d, *J* = 8.3 Hz), 116.35 (d, *J* = 21.7 Hz), 105.55, 96.84, 45.41, 8.37, 7.68. HRMS calculated for $C_{17}H_{17}ClFN_6$ 359.11818 $[M+H]^+$, found 359.1194. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.21 min; *m/z* : 359 $[M+H]^+$.

5-Chloro-*N*^{4}-(5-cyclopropyl-1*H*-pyrazol-3-yl)-*N*^{2}-isopropylpyrimidine-2,4-diamine (20)



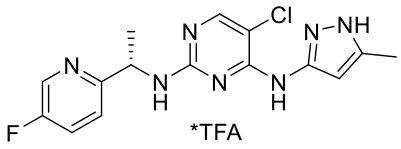
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1H-pyrazol-3-yl)pyrimidin-4-amine (**81**) and propan-2-amine (**4f**) following General procedure F on a 0.25 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (21 mg, 21%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.00 (s, 1H), 6.35 (s, 1H), 4.09 (bs, 1H), 1.98 – 1.91 (m, 1H), 1.29 (d, *J* = 6.6 Hz, 6H), 1.06 – 1.00 (m, 2H), 0.74 (s, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 158.54, 153.51, 149.20, 146.35, 142.03, 105.11, 96.07, 45.52, 22.20, 8.40, 7.71. HRMS calculated for $C_{13}H_{18}ClN_6$ 293.12760 $[M+H]^+$, found 293.1280. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.15 min; *m/z* : 293 $[M+H]^+$.

(*S*)-5-Chloro-*N*^{2}-(1-(5-fluoropyridin-2-yl)ethyl)-*N*^{4}-(1*H*-pyrazol-3-yl)pyrimidine-2,4-diamine (21)



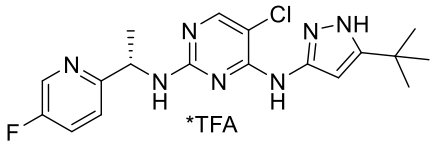
The title compound was synthesized from 2,5-dichloro-*N*-(1*H*-pyrazol-3-yl)pyrimidin-4-amine (**83**) and (*S*)-1-(5-fluoropyridin-2-yl)ethan-1-amine (**4a**) following General procedure D on a 0.25 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (79 mg, 71%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.42 (d, *J* = 2.9 Hz, 1H), 8.08 (s, 1H), 7.68 (d, *J* = 2.4 Hz, 1H), 7.59 – 7.53 (m, 1H), 7.38 (bs, 1H), 6.41 (bs, 1H), 5.20 – 5.09 (m, 1H), 1.58 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, methanol-d_4) δ 160.33 (d, *J* = 253.7 Hz), 159.01, 158.23, 153.62, 145.95, 142.15, 137.99 (d, *J* = 24.6 Hz), 131.19, 125.39 (d, *J* = 18.8 Hz), 123.42, 105.69, 100.62, 53.64, 21.50. HRMS calculated for $C_{14}H_{14}ClFN_7$ 334.09778 $[M+H]^+$, found 334.0994. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.09 min; *m/z* : 334 $[M+H]^+$.

(*S*)-5-Chloro-*N*^{2}-(1-(5-fluoropyridin-2-yl)ethyl)-*N*^{4}-(5-methyl-1*H*-pyrazol-3-yl)pyrimidine-2,4-diamine (22)



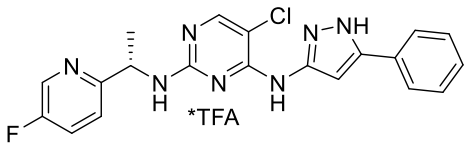
The title compound was synthesized from 2,5-dichloro-*N*-(5-methyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**84**) and (*S*)-1-(5-fluoropyridin-2-yl)ethan-1-amine (**4a**) following General procedure D on a 0.25 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (52 mg, 45%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.43 (d, *J* = 2.9 Hz, 1H), 8.06 (s, 1H), 7.58 (td, *J* = 8.6, 2.9 Hz, 1H), 7.42 (bs, 1H), 6.09 (bs, 1H), 5.13 (d, *J* = 7.2 Hz, 1H), 2.34 (s, 3H), 1.60 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, methanol-d_4) δ 160.30 (d, *J* = 253.7 Hz), 158.65, 158.58, 153.94, 146.00, 142.69, 142.08, 137.91 (d, *J* = 24.6 Hz), 125.39 (d, *J* = 18.8 Hz), 123.20 (d, *J* = 4.8 Hz), 105.61, 99.49, 53.78, 21.52, 11.05. HRMS calculated for $C_{15}H_{16}ClFN_7$ 348.11343 $[M+H]^+$, found 348.1147. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.52 min; *m/z* : 348 $[M+H]^+$.

(*S*)-*N*^{4}-(5-(*tert*-Butyl)-1*H*-pyrazol-3-yl)-5-chloro-*N*^{2}-(1-(5-fluoropyridin-2-yl)ethyl)pyrimidine-2,4-diamine (23)



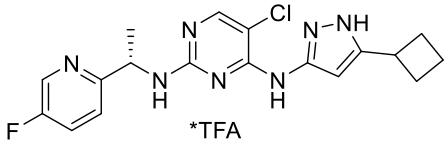
The title compound was synthesized from *N*-(5-(*tert*-butyl)-1*H*-pyrazol-3-yl)-2,5-dichloropyrimidin-4-amine (**85**) and (*S*)-1-(5-fluoropyridin-2-yl)ethan-1-amine (**4a**) following General procedure D on a 0.25 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (89 mg, 71%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.41 (d, *J* = 2.9 Hz, 1H), 8.07 (s, 1H), 7.55 (td, *J* = 8.6, 2.9 Hz, 1H), 7.37 (s, 1H), 6.38 (s, 1H), 5.22 (s, 1H), 1.60 (d, *J* = 7.0 Hz, 3H), 1.37 (s, 9H). ^{13}C NMR (151 MHz, methanol-d_4) δ 160.28 (d, *J* = 253.8 Hz), 158.63, 158.20, 155.97, 153.98, 145.78, 142.69, 138.09 (d, *J* = 24.4 Hz), 125.21 (d, *J* = 18.7 Hz), 123.18 (d, *J* = 4.6 Hz), 105.53, 96.69, 53.50, 32.30, 30.45, 21.41. HRMS calculated for $C_{18}H_{22}ClFN_7$ 390.16038 $[M+H]^+$, found 390.1614. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.37 min; *m/z* : 390 $[M+H]^+$.

(*S*)-5-Chloro-*N*^{2}-(1-(5-fluoropyridin-2-yl)ethyl)-*N*^{4}-(5-phenyl-1*H*-pyrazol-3-yl)pyrimidine-2,4-diamine (24)



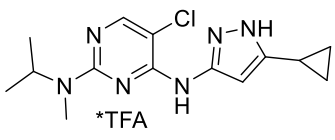
The title compound was synthesized from 2,5-dichloro-*N*-(5-phenyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**86**) and (*S*)-1-(5-fluoropyridin-2-yl)ethan-1-amine (**4a**) following General procedure D on a 0.25 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (57 mg, 44%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.24 (s, 1H), 8.10 (s, 1H), 7.75 (d, *J* = 7.7 Hz, 2H), 7.52 (t, *J* = 7.6 Hz, 2H), 7.43 (t, *J* = 7.3 Hz, 2H), 7.35 (bs, 1H), 6.70 (s, 1H), 5.19 (bs, 1H), 1.60 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, methanol-d_4) δ 160.13 (d, *J* = 253.8 Hz), 158.77, 158.74, 153.80, 146.60, 145.70, 142.53, 137.92 (d, *J* = 24.4 Hz), 130.75, 130.19, 129.88, 126.61, 125.26 (d, *J* = 18.8 Hz), 122.82, 105.57, 97.83, 54.02, 21.62. HRMS calculated for $C_{20}H_{18}ClFN_7$ 410.12908 $[M+H]^+$, found 410.1299. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.47 min; *m/z* : 410 $[M+H]^+$.

(*S*)-5-Chloro-*N*^{4}-(5-cyclobutyl-1*H*-pyrazol-3-yl)-*N*^{2}-(1-(5-fluoropyridin-2-yl)ethyl)pyrimidine-2,4-diamine (25)



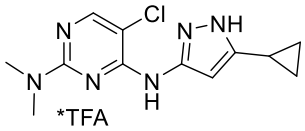
The title compound was synthesized from 2,5-dichloro-*N*-(5-cyclobutyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**87**) and (*S*)-1-(5-fluoropyridin-2-yl)ethan-1-amine (**4a**) following General procedure D on a 0.25 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (90 mg, 72%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.42 (d, *J* = 2.9 Hz, 1H), 8.06 (s, 1H), 7.55 (td, *J* = 8.5, 2.9 Hz, 1H), 7.39 (s, 1H), 6.29 (s, 1H), 5.18 (s, 1H), 3.60 (p, *J* = 8.6 Hz, 1H), 2.46 – 2.39 (m, 2H), 2.30 – 2.19 (m, 2H), 2.16 – 2.07 (m, 1H), 2.00 – 1.93 (m, 1H), 1.60 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, methanol-d_4) δ 160.27 (d, *J* = 253.9 Hz), 158.60, 158.54, 154.10, 150.85, 146.00, 142.91, 138.02 (d, *J* = 24.5 Hz), 125.25 (d, *J* = 18.8 Hz), 123.12 (d, *J* = 4.7 Hz), 105.54, 97.41, 53.66, 33.06, 30.25, 21.49, 19.50. HRMS calculated for $C_{18}H_{20}ClFN_7$ 388.14473 $[M+H]^+$, found 388.1453. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.22 min; *m/z* : 388 $[M+H]^+$.

5-Chloro-*N*^{4}-(5-cyclopropyl-1H-pyrazol-3-yl)-*N*^{2}-isopropyl-*N*^{2}-methylpyrimidine-2,4-diamine (26)



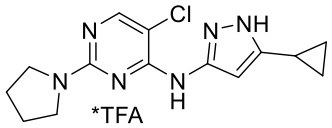
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1H-pyrazol-3-yl)pyrimidin-4-amine (**81**) and *N*-methylpropan-2-amine (**4g**) following General procedure F on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (96 mg, 76%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.01 (s, 1H), 6.25 (s, 1H), 4.78 (s, 1H), 3.02 (s, 3H), 1.99 – 1.92 (m, 1H), 1.26 (d, *J* = 6.8 Hz, 6H), 1.06 – 1.01 (m, 2H), 0.75 – 0.69 (m, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 157.76, 153.56, 149.22, 146.47, 142.49, 105.49, 96.02, 49.87, 29.10, 19.45, 8.45, 7.67. HRMS calculated for $C_{14}H_{20}ClN_6$ 307.14325 $[M+H]^+$, found 307.1431. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.18 min; *m/z* : 307 $[M+H]^+$.

5-Chloro-*N*^{4}-(5-cyclopropyl-1*H*-pyrazol-3-yl)-*N*^{2},*N*^{2}-dimethylpyrimidine-2,4-diamine (27)



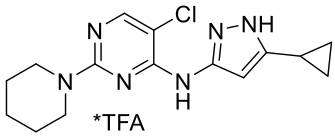
A vial was charged with NaH (60% in mineral oil, 32 mg, 0.80 mmol, 2.7 eq) dissolved in *i*PrOH (1 mL). After drop-wise addition of 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) (80 mg, 0.30 mmol, 1 eq) dissolved in *i*PrOH (0.7 mL) and DMF (1 mL), the vial was sealed and the mixture stirred at 120°C ON, concentrated under reduced pressure and purified by preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (79 mg, 67%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.01 (s, 1H), 6.32 (s, 1H), 3.22 (s, 6H), 1.98 – 1.90 (m, 1H), 1.05 – 0.96 (m, 2H), 0.76 – 0.69 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 155.01, 154.85, 147.00, 145.73, 144.53, 102.59, 94.28, 37.24, 7.44, 6.66. HRMS calculated for $C_{12}H_{16}ClN_6$ 279.11195 $[M+H]^+$, found 279.11198. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.31 min; *m/z* : 279 $[M+H]^+$.

5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(pyrrolidin-1-yl)pyrimidin-4-amine (28)



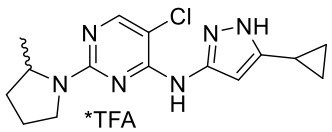
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and pyrrolidine (**4h**) following General procedure F on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (23 mg, 18%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.02 (s, 1H), 6.40 (s, 1H), 3.67 (s, 2H), 3.51 (s, 2H), 2.13 (s, 2H), 2.07 (s, 2H), 2.01 – 1.90 (m, 1H), 1.05 – 1.00 (m, 2H), 0.76 – 0.71 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 157.35, 151.78, 149.03, 146.58, 141.89, 105.39, 96.09, 49.85, 47.83, 26.67, 25.74, 8.34, 7.73. HRMS calculated for $C_{14}H_{18}ClN_6$ 305.12760 $[M+H]^+$, found 305.1267. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.03 min; *m/z* : 305 $[M+H]^+$.

5-5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(piperidin-1-yl)pyrimidin-4-amine (29)



The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and piperidine (**4i**) following General procedure F on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (95 mg, 73%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.01 (s, 1H), 6.21 (s, 1H), 3.75 – 3.69 (m, 4H), 2.02 – 1.88 (m, 1H), 1.79 – 1.72 (m, 2H), 1.72 – 1.66 (m, 4H), 1.05 – 1.00 (m, 2H), 0.75 – 0.69 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 158.01, 153.22, 149.25, 146.37, 142.92, 105.23, 96.20, 47.44, 26.39, 24.89, 8.41, 7.68. HRMS calculated for $C_{15}H_{20}ClN_6$ 319.14325 $[M+H]^+$, found 319.1441. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.56 min; *m/z* : 319 $[M+H]^+$.

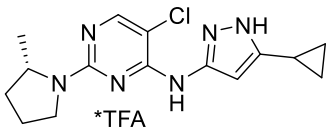
5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(2-methylpyrrolidin-1-yl)pyrimidin-4-amine (30)



The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and 2-methylpyrrolidine (**4j**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (32 mg, 25%). ^{1}H NMR (500 MHz, methanol-d_4) δ

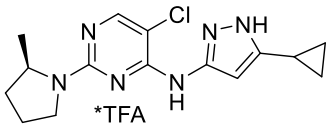
7.98 (s, 1H), 6.35 (s, 1H), 4.30 (s, 1H), 3.70 – 3.62 (m, 1H), 3.54 – 3.46 (m, 1H), 2.26 – 2.15 (m, 2H), 2.15 – 2.05 (m, 1H), 1.98 – 1.90 (m, 1H), 1.85 – 1.79 (m, 1H), 1.27 (d, *J* = 6.4 Hz, 3H), 1.06 – 0.94 (m, 2H), 0.77 – 0.68 (m, 2H). ^{13}C NMR (126 MHz, methanol-*d*$_4$) δ 157.60, 152.17, 149.34, 146.59, 142.85, 105.43, 95.94, 56.92, 33.30, 24.06, 19.08, 8.27, 8.18, 7.68. HRMS calculated for $C_{15}H_{20}ClN_6$ 319.14325 $[M+H]^+$, found 319.14322. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.41 min; *m/z* : 319 $[M+H]^+$.

(*S*)-5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(2-methylpyrrolidin-1-yl)pyrimidin-4-amine (31)



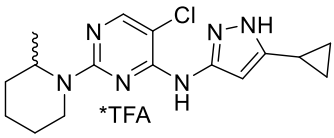
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and (*S*)-2-methylpyrrolidine (**4k**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (68 mg, 52%). ^{1}H NMR (500 MHz, methanol-*d*$_4$) δ 7.99 (s, 1H), 6.34 (s, 1H), 4.31 (s, 1H), 3.72 – 3.60 (m, 1H), 3.60 – 3.49 (m, 1H), 2.26 – 2.16 (m, 2H), 2.16 – 2.06 (m, 1H), 1.98 – 1.91 (m, 1H), 1.86 – 1.81 (m, 1H), 1.28 (d, *J* = 6.4 Hz, 3H), 1.06 – 0.99 (m, 2H), 0.75 – 0.68 (m, 2H). ^{13}C NMR (126 MHz, methanol-*d*$_4$) δ 157.65, 151.80, 149.31, 146.53, 142.27, 105.52, 96.02, 56.98, 33.27, 24.06, 19.04, 8.28, 8.19, 7.66. HRMS calculated for $C_{15}H_{20}ClN_6$ 319.14325 $[M+H]^+$, found 319.14337. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.55 min; *m/z* : 319 $[M+H]^+$.

(*R*)-5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(2-methylpyrrolidin-1-yl)pyrimidin-4-amine (32)



The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and (*R*)-2-methylpyrrolidine (**4l**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (102 mg, 79%. ^{1}H NMR (500 MHz, methanol-*d*$_4$) δ 7.98 (s, 1H), 6.34 (s, 1H), 4.31 (s, 1H), 3.71 – 3.63 (m, 1H), 3.56 – 3.47 (m, 1H), 2.26 – 2.15 (m, 2H), 2.14 – 2.08 (m, 1H), 1.99 – 1.91 (m, 1H), 1.86 – 1.80 (m, 1H), 1.28 (d, *J* = 6.5 Hz, 3H), 1.05 – 0.99 (m, 2H), 0.77 – 0.69 (m, 2H). ^{13}C NMR (126 MHz, methanol-*d*$_4$) δ 157.63, 151.85, 149.32, 146.53, 142.36, 105.50, 96.00, 56.98, 33.27, 24.06, 19.05, 8.28, 8.19, 7.67. HRMS calculated for $C_{15}H_{20}ClN_6$ 319.14325 $[M+H]^+$, found 319.14330. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.51 min; *m/z* : 319 $[M+H]^+$.

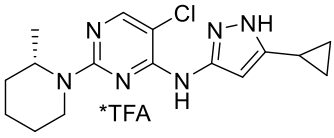
5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(2-methylpiperidin-1-yl)pyrimidin-4-amine (33)



The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and 2-methylpiperidine (**4m**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 25% → 28% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (47 mg, 35%). ^{1}H NMR (500 MHz, methanol-*d*$_4$) δ 8.01 (s, 1H), 6.21 (s, 1H), 4.65 (s, 1H), 4.30 – 4.05 (m, 1H), 3.22 (td, *J* = 13.4, 3.1 Hz, 1H), 1.98 – 1.90 (m, 1H), 1.85 – 1.74 (m, 3H), 1.73 – 1.62 (m, 2H), 1.62 – 1.48 (m, 1H), 1.30 (d, *J* = 6.9 Hz, 3H), 1.11 – 0.98 (m, 2H), 0.78 – 0.63 (m, 2H). ^{13}C NMR (126 MHz, methanol-*d*$_4$) δ 158.02,

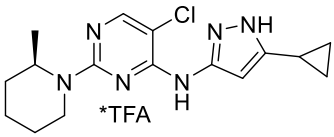
153.14, 149.25, 146.42, 142.85, 105.25, 96.04, 49.91, 41.32, 30.80, 26.05, 19.07, 15.61, 8.48, 7.68. HRMS calculated for $C_{16}H_{22}ClN_6$ 333.15890 $[M+H]^+$, found 333.15891. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.97 min; *m/z* : 333 $[M+H]^+$.

(*S*)-5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(2-methylpiperidin-1-yl)pyrimidin-4-amine (34)



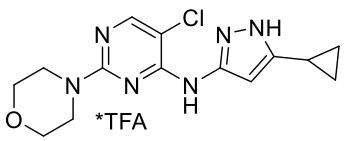
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and (*S*)-2-methylpiperidine (**4n**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 25% → 28% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (92 mg, 69%). ^{1}H NMR (500 MHz, methanol-d_4) δ 7.93 (d, *J* = 1.6 Hz, 1H), 6.12 (d, *J* = 2.9 Hz, 1H), 4.55 (s, 1H), 4.15 – 3.98 (m, 1H), 3.20 – 3.06 (m, 1H), 1.92 – 1.80 (m, 1H), 1.80 – 1.66 (m, 3H), 1.66 – 1.55 (m, 2H), 1.55 – 1.39 (m, 1H), 1.30 – 1.14 (m, 3H), 1.00 – 0.88 (m, 2H), 0.72 – 0.52 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 158.10, 152.86, 149.31, 146.36, 142.37, 105.37, 96.14, 50.05, 41.40, 30.79, 26.00, 19.03, 15.65, 8.44, 7.65. HRMS calculated for $C_{16}H_{22}ClN_6$ 333.15890 $[M+H]^+$, found 333.15909. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.96 min; *m/z* : 333 $[M+H]^+$.

(*R*)-5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(2-methylpiperidin-1-yl)pyrimidin-4-amine (35)



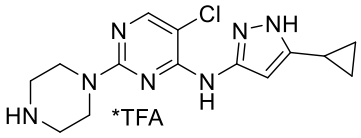
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and (*R*)-2-methylpiperidine (**4o**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 25% → 28% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (75 mg, 56%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.02 (s, 1H), 6.21 (s, 1H), 4.64 (s, 1H), 4.21 – 4.09 (m, 1H), 3.28 – 3.18 (m, 1H), 2.00 – 1.91 (m, 1H), 1.88 – 1.75 (m, 3H), 1.75 – 1.64 (m, 2H), 1.65 – 1.46 (m, 1H), 1.31 (d, *J* = 6.9 Hz, 3H), 1.14 – 0.93 (m, 2H), 0.81 – 0.61 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 158.07, 152.96, 149.30, 146.38, 142.53, 105.34, 96.11, 50.02, 41.38, 30.79, 26.01, 19.04, 15.65, 8.43, 7.66. HRMS calculated for $C_{16}H_{22}ClN_6$ 333.15890 $[M+H]^+$, found 333.15878. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.98 min; *m/z* : 333 $[M+H]^+$.

5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-morpholinopyrimidin-4-amine (36)



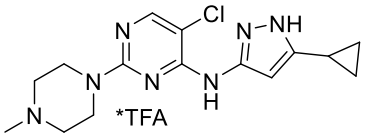
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and morpholine (**4p**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (106 mg, 81%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.09 (s, 1H), 6.21 (s, 1H), 3.83 – 3.76 (m, 4H), 3.73 – 3.68 (m, 4H), 1.96 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.06 – 1.00 (m, 2H), 0.77 – 0.71 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 157.85, 155.02, 149.52, 146.17, 145.04, 105.71, 96.28, 67.00, 46.19, 8.51, 7.75. HRMS calculated for $C_{14}H_{18}ClN_6O$ 321.12251 $[M+H]^+$, found 321.12246. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.48 min; *m/z* : 321 $[M+H]^+$.

5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(piperazin-1-yl)pyrimidin-4-amine (37)



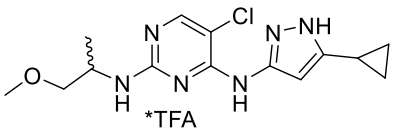
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and piperazine (**4q**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 10% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (106 mg, 65%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.11 (s, 1H), 6.21 (s, 1H), 4.07 – 3.95 (m, 4H), 3.32 – 3.30 (m, 4H), 1.98 (tt, *J* = 8.5, 5.0 Hz, 1H), 1.12 – 0.99 (m, 2H), 0.85 – 0.72 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 159.30, 157.09, 153.50, 150.24, 146.54, 105.71, 95.47, 44.17, 42.46, 8.61, 7.92. HRMS calculated for $C_{14}H_{19}ClN_7$ 320.13850 $[M+H]^+$, found 320.13869. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.37 min; *m/z* : 320 $[M+H]^+$.

5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(4-methylpiperazin-1-yl)pyrimidin-4-amine (38)



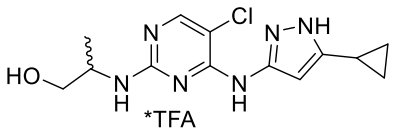
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and 1-methylpiperazine (**4r**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 10% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (136 mg, 81%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.04 (s, 1H), 6.18 (s, 1H), 4.03 (bs, 4H), 3.33 (bs, 4H), 2.93 (s, 3H), 1.94 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.09 – 0.95 (m, 2H), 0.82 – 0.67 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 159.65, 157.03, 154.31, 150.08, 146.71, 105.78, 95.38, 54.09, 43.63, 42.71, 8.58, 7.93. HRMS calculated for $C_{15}H_{21}ClN_7$ 334.15415 $[M+H]^+$, found 334.15438. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.50 min; *m/z* : 334 $[M+H]^+$.

5-Chloro-N^4-(5-cyclopropyl-1*H*-pyrazol-3-yl)-N^2-(1-methoxypropan-2-yl)pyrimidine-2,4-diamine (39)



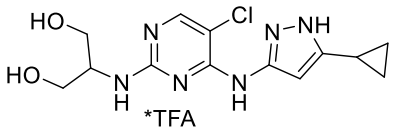
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and 1-methoxypropan-2-amine (**4s**) following General procedure F on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (75 mg, 57%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.08 (s, 1H), 6.23 (s, 1H), 4.07 (q, *J* = 6.1 Hz, 1H), 3.45 – 3.41 (m, 1H), 3.38 – 3.34 (m, 1H), 3.29 (s, 3H), 1.97 – 1.86 (m, 1H), 1.19 (d, *J* = 6.7 Hz, 3H), 0.99 – 0.91 (m, 2H), 0.74 – 0.68 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.81, 153.99, 147.27, 144.46, 143.93, 102.66, 94.23, 74.48, 57.97, 46.88, 16.49, 7.12, 6.58. HRMS calculated for $C_{14}H_{20}ClN_6O$ 323.13816 $[M+H]^+$, found 323.1391. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.81 min; *m/z* : 323 $[M+H]^+$.

2-((5-Chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)propan-1-ol (40)



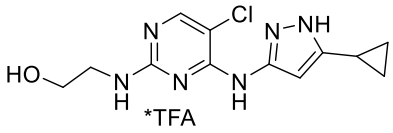
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and 2-aminopropan-1-ol (**4t**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (112 mg, 88%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.02 (s, 1H), 6.34 (s, 1H), 4.11 (s, 1H), 3.66 (dd, *J* = 11.1, 4.6 Hz, 1H), 3.57 (dd, *J* = 11.0, 6.3 Hz, 1H), 2.01 – 1.89 (m, 1H), 1.26 (d, *J* = 6.7 Hz, 3H), 1.09 – 0.94 (m, 2H), 0.92 – 0.67 (m, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 158.52, 154.06, 149.29, 146.19, 142.10, 105.24, 96.10, 65.57, 51.31, 16.79, 8.43, 7.73. HRMS calculated for $C_{13}H_{18}ClN_6O$ 309.12251 $[M+H]^+$, found 309.12245. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.18 min; *m/z* : 309 $[M+H]^+$.

2-((5-Chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)propane-1,3-diol (41)



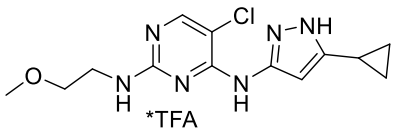
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and 2-aminopropane-1,3-diol (**4u**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 10% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (91 mg, 69%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.04 (s, 1H), 6.35 (s, 1H), 4.13 (s, 1H), 3.78 – 3.74 (m, 2H), 3.73 – 3.68 (m, 2H), 1.94 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.04 – 0.98 (m, 2H), 0.82 – 0.77 (m, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 158.40, 154.61, 149.44, 146.12, 142.16, 105.40, 96.13, 61.65, 57.06, 8.48, 7.77. HRMS calculated for $C_{13}H_{18}ClN_6O_2$ 325.11743 $[M+H]^+$, found 325.11740. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.18 min; *m/z* : 325 $[M+H]^+$.

2-((5-Chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)ethan-1-ol (42)



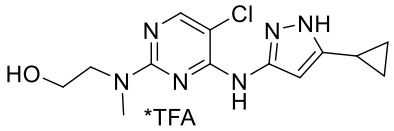
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and 2-aminopropane-1,3-diol (**4v**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (111 mg, 91%). ^{1}H NMR (500 MHz, DMSO-d_6, 1% TFA, 75°C) δ 8.14 (s, 1H), 6.30 (s, 1H), 3.59 (t, *J* = 5.7 Hz, 2H), 3.41 (t, *J* = 5.7 Hz, 2H), 1.92 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.00 – 0.90 (m, 2H), 0.76 – 0.67 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 155.93, 153.57, 147.16, 144.09, 142.49, 102.94, 94.60, 59.06, 43.74, 7.47, 6.70. HRMS calculated for $C_{12}H_{16}ClN_6O$ 295.10686 $[M+H]^+$, found 295.10714. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.71 min; *m/z* : 295 $[M+H]^+$.

5-Chloro-N^4-(5-cyclopropyl-1*H*-pyrazol-3-yl)-N^2-(2-methoxyethyl)pyrimidine-2,4-diamine (43)



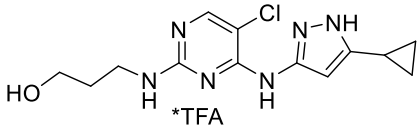
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and 2-methoxyethan-1-amine (**4w**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (59 mg, 47%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.02 (s, 1H), 6.34 (s, 1H), 3.57 (s, 4H), 3.37 (s, 3H), 2.00 – 1.89 (m, 1H), 1.10 – 0.97 (m, 2H), 0.83 – 0.67 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 155.94, 153.80, 147.16, 144.07, 143.25, 102.91, 94.51, 69.69, 57.75, 40.73, 7.41, 6.68. HRMS calculated for $C_{13}H_{18}ClN_6O$ 309.12251 $[M+H]^+$, found 309.12237. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.30 min; *m/z* : 309 $[M+H]^+$.

2-((5-Chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)(methyl)amino) ethan-1-ol (44)



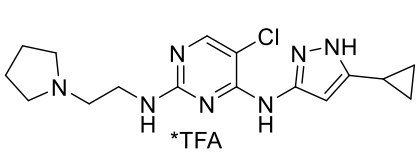
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and 2-(methylamino)ethan-1-ol (**4x**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (112 mg, 88%). ^{1}H NMR (500 MHz, DMSO-d_6, 1% TFA, 75°C) δ 8.09 (s, 1H), 6.25 (s, 1H), 3.65 (s, 4H), 3.16 (s, 3H), 1.92 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.01 – 0.92 (m, 2H), 0.75 – 0.67 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 155.03, 154.31, 147.19, 145.06, 144.44, 102.74, 94.11, 58.21, 52.05, 36.47, 7.51, 6.64. HRMS calculated for $C_{13}H_{18}ClN_6O$ 309.12251 $[M+H]^+$, found 309.12259. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.90 min; *m/z* : 309 $[M+H]^+$.

3-((5-Chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)propan-1-ol (45)



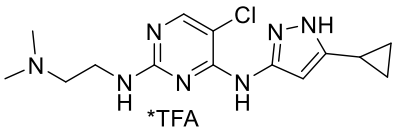
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and 3-aminopropan-1-ol (**4y**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (107 mg, 84%). ^{1}H NMR (500 MHz, DMSO-d_6, 1% TFA, 75°C) δ 8.14 (s, 1H), 6.31 (s, 1H), 3.51 (t, *J* = 6.2 Hz, 2H), 3.40 (t, *J* = 6.9 Hz, 2H), 1.93 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.73 (p, *J* = 6.4 Hz, 2H), 0.99 – 0.91 (m, 2H), 0.78 – 0.66 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 156.00, 153.24, 147.00, 144.13, 142.23, 102.84, 94.71, 58.20, 38.61, 31.32, 7.41, 6.67. HRMS calculated for $C_{13}H_{18}ClN_6O$ 309.12251 $[M+H]^+$, found 309.12280. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.94 min; *m/z* : 309 $[M+H]^+$.

5-Chloro-N^4-(5-cyclopropyl-1*H*-pyrazol-3-yl)-N^2-(2-(pyrrolidin-1-yl)ethyl)pyrimidine-2,4-diamine (46)



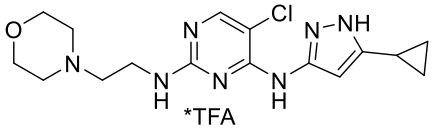
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and 2-(pyrrolidin-1-yl)ethan-1-amine (**4z**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 10% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (113 mg, 65%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.11 (s, 1H), 6.16 (s, 1H), 3.73 (t, *J* = 5.7 Hz, 2H), 3.60 (bs, 2H), 3.39 (t, *J* = 5.7 Hz, 2H), 3.01 (bs, 2H), 2.09 (bs, 2H), 2.02 – 1.93 (m, 3H), 1.08 – 1.03 (m, 2H), 0.81 – 0.75 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 156.10, 147.42, 144.12, 103.17, 94.91, 53.51, 53.07, 37.34, 22.32, 7.40, 6.79. HRMS calculated for $C_{16}H_{23}ClN_7$ 348.16980 $[M+H]^+$, found 348.16994. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.26 min; *m/z* : 348 $[M+H]^+$.

5-Chloro-N^4-(5-cyclopropyl-1*H*-pyrazol-3-yl)-N^2-(2-(dimethylamino)ethyl)pyrimidine-2,4-diamine (47)



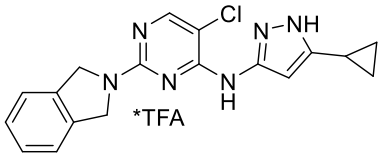
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and N^1,N^1-dimethylethane-1,2-diamine (**4aa**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 10% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (70 mg, 42%). ^{1}H NMR (500 MHz, DMSO-d_6, 1% TFA, 75°C) δ 8.14 (s, 1H), 6.19 (s, 1H), 3.63 (t, *J* = 6.0 Hz, 2H), 3.27 (t, *J* = 6.0 Hz, 2H), 2.80 (s, 6H), 1.93 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.01 – 0.90 (m, 2H), 0.79 – 0.68 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.97, 147.40, 144.16, 103.18, 94.81, 55.76, 42.53, 36.23, 7.38, 6.79. HRMS calculated for $C_{14}H_{21}ClN_7$ 322.15415 $[M+H]^+$, found 322.15397. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.11 min; *m/z* : 322 $[M+H]^+$.

5-Chloro-N^4-(5-cyclopropyl-1*H*-pyrazol-3-yl)-N^2-(2-morpholinoethyl)pyrimidine-2,4-diamine (48)



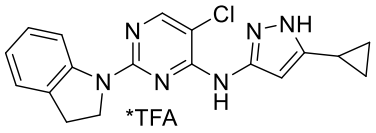
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and 2-morpholinoethan-1-amine (**4ab**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 10% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (134 mg, 75%). ^{1}H NMR (500 MHz, DMSO-d_6, 1% TFA, 75°C) δ 8.15 (s, 1H), 6.19 (s, 1H), 3.81 (s, 4H), 3.66 (t, *J* = 6.0 Hz, 2H), 3.31 (t, *J* = 6.0 Hz, 2H), 3.26 (bs, 4H), 1.99 – 1.88 (m, 1H), 1.03 – 0.91 (m, 2H), 0.78 – 0.68 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 156.06, 147.44, 144.13, 103.22, 94.88, 62.98, 55.22, 51.30, 35.46, 7.41, 6.80. HRMS calculated for $C_{16}H_{23}ClN_7O$ 364.16471 $[M+H]^+$, found 364.16477. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.12 min; *m/z* : 364 $[M+H]^+$.

5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(isoindolin-2-yl)pyrimidin-4-amine (49)



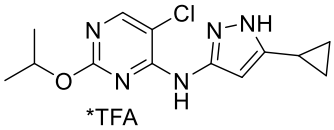
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and isoindoline (**4ac**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 30% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (108 mg, 77%). ^{1}H NMR (500 MHz, DMSO-d_6, 1% TFA, 75°C) δ 8.16 (s, 1H), 7.41 – 7.36 (m, 2H), 7.35 – 7.30 (m, 2H), 6.43 (s, 1H), 4.85 (s, 4H), 1.99 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.07 – 0.94 (m, 2H), 0.84 – 0.68 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 154.90, 154.09, 147.87, 147.39, 144.57, 135.93, 127.26, 122.49, 103.07, 94.06, 52.81, 7.59, 6.84. HRMS calculated for $C_{18}H_{18}ClN_6$ 353.12760 $[M+H]^+$, found 353.12745. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 8.49 min; *m/z* : 353 $[M+H]^+$.

5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(indolin-1-yl)pyrimidin-4-amine (50)



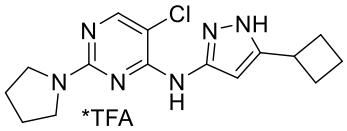
The title compound was synthesized from 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) and indoline (**4ad**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 30% → 40% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (66 mg, 47%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.14 (s, 1H), 7.97 (d, *J* = 8.1 Hz, 1H), 7.21 (d, *J* = 7.3 Hz, 1H), 7.07 (t, *J* = 7.7 Hz, 1H), 7.01 – 6.93 (m, 1H), 6.20 (s, 1H), 4.21 – 4.06 (m, 2H), 3.22 (t, *J* = 8.5 Hz, 2H), 2.00 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.09 – 1.01 (m, 2H), 0.82 – 0.75 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 165.02, 164.90, 161.75, 157.02, 154.14, 152.11, 141.43, 135.87, 133.76, 130.87, 124.65, 113.31, 104.62, 58.01, 35.96, 16.94, 16.29. HRMS calculated for $C_{18}H_{18}ClN_6$ 353.12760 $[M+H]^+$, found 353.12734. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 9.27 min; *m/z* : 353 $[M+H]^+$.

5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-isopropoxypyrimidin-4-amine (51)



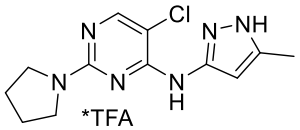
A vial was charged with NaH (60% in mineral oil, 35 mg, 0.88 mmol, 3.0 eq) dissolved in *i*PrOH (**4ae**) (1 mL) and cooled to 0°C. After drop-wise addition of 2,5-dichloro-*N*-(5-isopropoxy-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**81**) (80 mg, 0.30 mmol, 1 eq) dissolved in *i*PrOH (2 mL), the vial was sealed and the mixture stirred at 110°C for 3.5 h, concentrated under reduced pressure and purified by preparative HPLC (Gemini C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (38 mg, 31%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.24 (s, 1H), 6.29 (s, 1H), 5.24 (m, 1H), 1.96 (tt, *J* = 8.4, 5.0 Hz, 1H), 1.40 (d, *J* = 6.2 Hz, 6H), 1.08 – 1.01 (m, 2H), 0.79 – 0.72 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.99, 156.61, 152.46, 147.19, 144.52, 106.58, 94.38, 70.66, 21.32, 7.39, 6.70. HRMS calculated for $C_{13}H_{17}ClN_5O$ 294.11161 $[M+H]^+$, found 294.11171. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.51 min; *m/z* : 294 $[M+H]^+$.

5-Chloro-*N*-(5-cyclobutyl-1*H*-pyrazol-3-yl)-2-(pyrrolidin-1-yl)pyrimidin-4-amine (52)



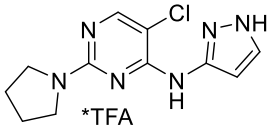
The title compound was synthesized from 2,5-dichloro-*N*-(5-cyclobutyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**87**) and pyrrolidine (**4h**) following General procedure D on a 0.176 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (66 mg, 87%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.02 (s, 1H), 6.60 (s, 1H), 3.69 (bs, 2H), 3.59 (p, *J* = 8.6 Hz, 1H), 3.51 (bs, 2H), 2.47 – 2.35 (m, 2H), 2.25 – 2.16 (m, 2H), 2.16 – 2.01 (m, 5H), 1.99 – 1.87 (m, 1H). ^{13}C NMR (126 MHz, methanol-d_4) δ 157.27, 152.00, 150.46, 146.69, 142.26, 105.29, 96.93, 49.85, 47.81, 33.03, 30.29, 26.68, 25.73, 19.50. HRMS calculated for $C_{15}H_{20}ClN_6$ 319.14325 $[M+H]^+$, found 319.14330. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.60 min; *m/z* : 319 $[M+H]^+$.

5-Chloro-*N*-(5-methyl-1*H*-pyrazol-3-yl)-2-(pyrrolidin-1-yl)pyrimidin-4-amine (53)



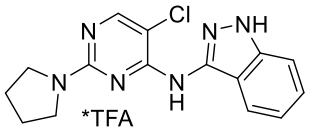
The title compound was synthesized from 2,5-dichloro-*N*-(5-methyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**84**) and pyrrolidine (**4h**) following General procedure D on a 0.11 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (39 mg, 90%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.03 (s, 1H), 6.52 (s, 1H), 3.70 (s, 2H), 3.51 (s, 2H), 2.33 (s, 3H), 2.14 (s, 2H), 2.07 (s, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 157.35, 151.54, 146.79, 141.67, 141.46, 105.43, 99.08, 49.98, 47.85, 26.68, 25.72, 10.97. HRMS calculated for $C_{12}H_{16}ClN_6$ 279.11195 $[M+H]^+$, found 279.11170. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.90 min; *m/z* : 279 $[M+H]^+$.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-(pyrrolidin-1-yl)-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (54)**



The title compound was synthesized from 2,5-dichloro-*N*-(1*H*-pyrazol-3-yl)pyrimidin-4-amine (**83**) and pyrrolidine (**4h**) following General procedure D on a 0.190 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15% → 20% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (52 mg, 72%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.03 (s, 1H), 7.67 (d, *J* = 2.4 Hz, 1H), 6.78 (d, *J* = 2.4 Hz, 1H), 3.69 (s, 2H), 3.51 (s, 2H), 2.13 (s, 2H), 2.05 (s, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 157.43, 151.68, 146.70, 141.77, 130.79, 105.38, 99.84, 49.97, 47.83, 26.68, 25.68. HRMS calculated for $C_{11}H_{14}ClN_6$ 265.09630 $[M+H]^+$, found 265.09640. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.78 min; *m/z* : 265 $[M+H]^+$.

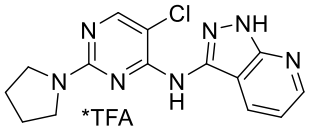
***N*-(5-Chloro-2-(pyrrolidin-1-yl)pyrimidin-4-yl)-1*H*-indazol-3-amine (55)**



The title compound was synthesized from *N*-(2,5-dichloropyrimidin-4-yl)-1*H*-indazol-3-amine (**88**) and pyrrolidine (**4h**) following General procedure D on a 0.323 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20 → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (103 mg, 74%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.09 (s, 1H), 7.64 (d, *J* = 8.2 Hz, 1H), 7.54 (d, *J* = 8.5 Hz, 1H), 7.48 – 7.37 (m, 1H), 7.23 – 7.10 (m, 1H), 3.44 (s, 2H), 3.15 (s, 2H), 2.03 (s, 2H), 1.80 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 157.47, 150.88, 142.99, 141.01, 138.25,

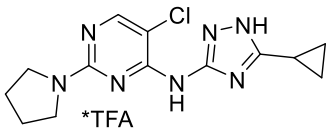
126.14, 120.96, 119.56, 117.19, 110.37, 103.02, 46.82, 24.25. HRMS calculated for $C_{15}H_{16}ClN_6$ 315.11195 $[M+H]^+$, found 315.11195. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.91 min; *m/z* : 315 $[M+H]^+$.

_N_-(5-Chloro-2-(pyrrolidin-1-yl)pyrimidin-4-yl)-1_H_-pyrazolo[3,4-_b_]pyridin-3-amine (56)



The title compound was synthesized from *N*-(2,5-dichloropyrimidin-4-yl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (**89**) and pyrrolidine (**4h**) following General procedure D on a 0.308 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (82 mg, 62%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.58 (dd, *J* = 4.5, 1.5 Hz, 1H), 8.23 (dd, *J* = 8.1, 1.5 Hz, 1H), 8.14 (s, 1H), 7.26 (dd, *J* = 8.1, 4.5 Hz, 1H), 3.47 (s, 2H), 3.19 (s, 2H), 2.07 (s, 2H), 1.84 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 157.31, 151.75, 151.36, 149.05, 143.99, 137.53, 130.88, 115.97, 109.31, 102.90, 46.84, 24.28. HRMS calculated for $C_{14}H_{15}ClN_7$ 316.10720 $[M+H]^+$, found 316.10694. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.91 min; *m/z* : 316 $[M+H]^+$.

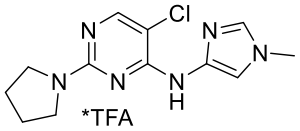
5-Chloro-_N_-(5-cyclopropyl-1_H_-1,2,4-triazol-3-yl)-2-(pyrrolidin-1-yl)pyrimidin-4-amine (57)



Step 1: 2,5-dichloro-*N*-(5-cyclopropyl-1*H*-1,2,4-triazol-3-yl) pyrimidin-4-amine was synthesized from 2,4-dichloroquinazoline (**1a**) (1 eq) and 5-cyclopropyl-1*H*-1,2,4-triazol-3-amine (**2c**) (1 eq) following General procedure A with DiPEA (3.4 eq) in THF on a 1.11 mmol scale at RT. The precipitating product was collected by filtration (43 mg, 58%). LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.05 min; *m/z* : 271 $[M+H]^+$.

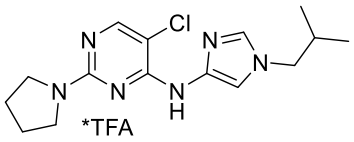
Step 2: The title compound was synthesized from the product of step 1 (1 eq), pyrrolidine (**4h**) (2.3 eq) and DiPEA (3.6 eq) in *n*-butanol (0.08 M), at 120°C for 24 h, on a 0.159 mmol scale following General procedure G and was purified by preparative HPLC (Gemini C_{18}, 28% → 31% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (5 mg, 7%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.38 (s, 1H), 3.56 (bs, 4H), 2.06 – 2.01 (m, 4H), 1.91 – 1.85 (m, 1H), 1.29 (bs, 2H), 0.95 – 0.92 (m, 2H). HRMS calculated for $C_{13}H_{17}ClN_7$ 306.12285 $[M+H]^+$, found 306.12302. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 8.49 min; *m/z* : 306 $[M+H]^+$.

5-Chloro-_N_-(1-methyl-1_H_-imidazol-4-yl)-2-(pyrrolidin-1-yl)pyrimidin-4-amine (58)



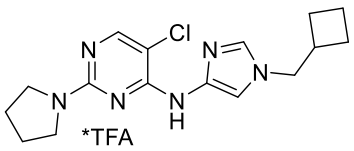
The title compound was synthesized from 2,5-dichloro-*N*-(1-methyl-1*H*-imidazol-4-yl)pyrimidin-4-amine (**90**) and pyrrolidine (**4h**) following General procedure D on a 0.246 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 5 → 15% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (101 mg, quant.). ^{1}H NMR (500 MHz, DMSO-d_6, 1% TFA, 75°C) δ 8.82 (s, 1H), 8.35 (s, 1H), 7.63 (s, 1H), 3.88 (s, 3H), 3.58 (s, 2H), 3.48 (s, 2H), 1.98 (s, 2H), 1.95 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 155.44, 151.97, 145.87, 132.87, 128.84, 112.59, 102.95, 48.16, 47.19, 35.99, 25.31, 24.63. HRMS calculated for $C_{12}H_{16}ClN_6$ 279.11195 $[M+H]^+$, found 279.11202. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.71 min; *m/z* : 279 $[M+H]^+$.

5-Chloro-*N*-(1-methyl-1*H*-imidazol-4-yl)-2-(pyrrolidin-1-yl)pyrimidin-4-amine (59)



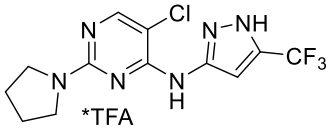
The title compound was synthesized from 2,5-dichloro-*N*-(1-isobutyl-1*H*-imidazol-4-yl)pyrimidin-4-amine (**91**) and pyrrolidine (**4h**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15 → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (90 mg, 69%). ^{1}H NMR (600 MHz, DMSO-d_6, 1% TFA, 75°C) δ 8.86 (d, *J* = 1.3 Hz, 1H), 8.33 (s, 1H), 7.68 (d, *J* = 1.5 Hz, 1H), 4.05 (d, *J* = 7.2 Hz, 2H), 3.51 (s, 2H), 3.48 (s, 2H), 2.14 – 2.08 (m, 1H), 1.97 (s, 2H), 1.93 (s, 2H), 0.88 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6, 1% TFA, 75°C) δ 155.65, 152.24, 146.29, 132.60, 129.10, 112.34, 102.89, 55.60, 47.98, 47.06, 28.95, 25.30, 24.58, 19.12. HRMS calculated for $C_{15}H_{22}ClN_6$ 321.15890 $[M+H]^+$, found 321.15912. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.06 min; *m/z* : 321 $[M+H]^+$.

5-Chloro-*N*-(1-(cyclobutylmethyl)-1*H*-imidazol-4-yl)-2-(pyrrolidin-1-yl)pyrimidin-4-amine (60)



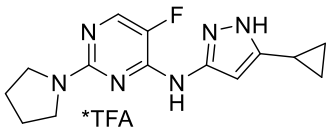
The title compound was synthesized from 2,5-dichloro-*N*-(1-(cyclobutylmethyl)-1*H*-imidazol-4-yl)pyrimidin-4-amine (**92**) and pyrrolidine (**4h**) following General procedure D on a 0.222 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 15 → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (24 mg, 24%). ^{1}H NMR (500 MHz, DMSO-d_6, 1% TFA, 75°C) δ 8.86 (s, 1H), 8.33 (s, 1H), 7.66 (s, 1H), 4.25 (d, *J* = 7.5 Hz, 2H), 3.54 (s, 2H), 3.48 (s, 2H), 2.85 – 2.69 (m, 1H), 2.05 – 1.70 (m, 10H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 155.51, 152.23, 146.25, 132.18, 129.20, 111.69, 102.92, 53.37, 48.07, 47.11, 35.07, 25.32, 24.83, 24.60, 17.59. HRMS calculated for $C_{16}H_{22}ClN_6$ 333.1589 $[M+H]^+$, found 333.15905. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.34 min; *m/z* : 333 $[M+H]^+$.

2-(Pyrrolidin-1-yl)-*N*-(5-(trifluoromethyl)-1*H*-pyrazol-3-yl)quinazolin-4-amine (61)



The title compound was synthesized from 2,5-dichloro-*N*-(5-(trifluoromethyl)-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**93**) (1 eq), pyrrolidine (**4h**) (2.8 eq) and DiPEA (2.6 eq) in *n*-butanol (0.06 M), at 120°C for 70 h, on a 0.116 mmol scale following General procedure G and was purified by preparative HPLC (Gemini C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (36 mg, 69%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.10 (s, 1H), 6.80 (s, 1H), 3.59 (s, 4H), 2.08 (s, 4H). ^{13}C NMR (151 MHz, methanol-d_4) δ 157.67, 153.60, 146.22, 122.35 (q, *J* = 266.2 Hz), 104.83, 97.29, 48.02, 26.23 (bs). HRMS calculated for $C_{12}H_{13}ClF_3N_6$ 333.08368 $[M+H]^+$, found 333.08380. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.50 min; *m/z* : 333 $[M+H]^+$.

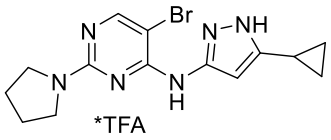
***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-5-fluoro-2-(pyrrolidin-1-yl)pyrimidin-4-amine (62)**



The title compound was synthesized from 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-5-fluoropyrimidin-4-amine (**94**) and pyrrolidine (**4h**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound

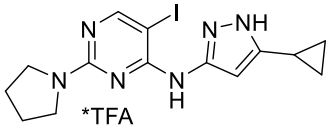
as a TFA-salt after lyophilisation (80 mg, 66%). ^{1}H NMR (600 MHz, methanol-d_4) δ 7.93 (d, *J* = 5.4 Hz, 1H), 6.44 (s, 1H), 3.68 (bs, 2H), 3.52 (bs, 2H), 2.10 (bs, 4H), 1.95 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.06 – 0.99 (m, 2H), 0.78 – 0.66 (m, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 153.04 (d, *J* = 12.8 Hz), 150.95, 148.82, 146.58, 139.92 (d, *J* = 248.8 Hz), 127.60, 127.59 (d, *J* = 31.8 Hz), 49.92, 47.81, 26.70, 25.86, 8.36, 7.71. HRMS calculated for $C_{14}H_{18}FN_6$ 289.15715 $[M+H]^+$, found 289.15690. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.72 min; *m/z* : 289 $[M+H]^+$.

5-Bromo-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-(pyrrolidin-1-yl)pyrimidin-4-amine (63)



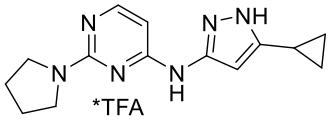
The title compound was synthesized from 5-bromo-2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**80**) and pyrrolidine (**4h**) following General procedure F on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (95 mg, 68%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.09 (s, 1H), 6.39 (s, 1H), 3.66 (s, 2H), 3.50 (s, 2H), 2.13 (s, 2H), 2.06 (s, 2H), 1.98 – 1.92 (m, 1H), 1.06 – 1.00 (m, 2H), 0.76 – 0.71 (m, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 157.71, 151.92, 149.00, 146.72, 144.80, 95.92, 92.35, 49.83, 47.84, 26.67, 25.70, 8.43, 7.76. HRMS calculated for $C_{14}H_{18}BrN_6$ 349.07708 $[M+H]^+$, found 349.0783. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.04 min; *m/z* : 349 $[M+H]^+$.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-5-iodo-2-(pyrrolidin-1-yl)pyrimidin-4-amine (64)**



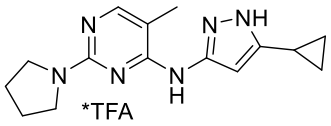
The title compound was synthesized from 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-5-iodopyrimidin-4-amine (**95**) and pyrrolidine (**4h**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 23% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (28 mg, 18%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.12 (s, 1H), 6.41 (s, 1H), 3.68 (bs, 2H), 3.50 (bs, 2H), 2.12 (bs, 2H), 2.07 (bs, 2H), 1.95 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.06 – 0.99 (m, 2H), 0.76 – 0.71 (m, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 159.32, 152.39, 150.68, 149.07, 147.13, 95.62, 62.18, 49.69, 47.77, 26.65, 25.70, 8.41, 7.76. HRMS calculated for $C_{14}H_{18}IN_6$ 397.06321 $[M+H]^+$, found 397.06254. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.25 min; *m/z* : 397 $[M+H]^+$.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-(pyrrolidin-1-yl)pyrimidin-4-amine (65)**



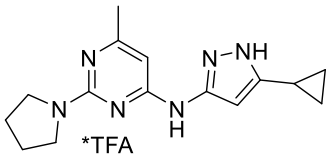
The title compound was synthesized from 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**82**) and pyrrolidine (**4h**) following General procedure F on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (85 mg, 74%). ^{1}H NMR (600 MHz, methanol-d_4) δ 7.69 (d, *J* = 7.2 Hz, 1H), 6.47 (s, 1H), 6.29 (d, *J* = 7.2 Hz, 1H), 3.81 – 3.72 (m, 2H), 3.58 – 3.48 (m, 2H), 2.19 – 2.12 (m, 2H), 2.10 – 2.03 (m, 2H), 1.96 – 1.90 (m, 1H), 1.04 – 0.99 (m, 2H), 0.75 – 0.71 (m, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 160.94, 152.68, 147.55, 144.13, 142.24, 99.14, 95.28, 49.50, 47.50, 26.63, 25.64, 8.35, 7.71. HRMS calculated for $C_{14}H_{19}N_6$ 271.16657 $[M+H]^+$, found 271.1673. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.87 min; *m/z* : 271 $[M+H]^+$.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-5-methyl-2-(pyrrolidin-1-yl)pyrimidin-4-amine (66)**



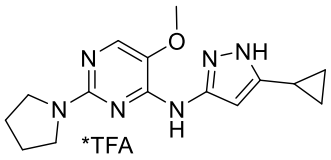
The title compound was synthesized from 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-5-methylpyrimidin-4-amine (**96**) and pyrrolidine (**4h**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (99 mg, 83%). ^{1}H NMR (600 MHz, methanol-d_4) δ 7.60 (d, *J* = 1.1 Hz, 1H), 6.42 (s, 1H), 3.68 (bs, 2H), 3.48 (bs, 2H), 2.14 (s, 3H), 2.14 (bs, 2H), 2.06 (bs, 2H), 1.95 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.04 – 0.99 (m, 2H), 0.76 – 0.71 (m, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 161.30, 152.08, 148.98, 147.12, 147.06, 140.21, 107.57, 96.28, 49.43 (bs), 47.38, 26.66, 25.70, 8.39, 7.79. HRMS calculated for $C_{15}H_{21}N_6$ 285.18222 $[M+H]^+$, found 285.18205. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.00 min; *m/z* : 285 $[M+H]^+$.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-6-methyl-2-(pyrrolidin-1-yl)pyrimidin-4-amine (67)**



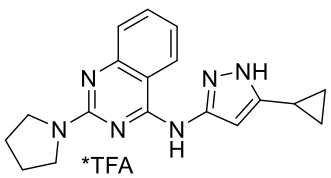
The title compound was synthesized from 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-6-methylpyrimidin-4-amine (**97**) and pyrrolidine (**4h**) following General procedure D on a 0.48 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (52 mg, 27%). ^{1}H NMR (500 MHz, DMSO-d_6, 1% TFA, 75°C) δ 10.67 (bs, 1H), 6.28 (bs, 1H), 3.66 – 3.50 (m, 4H), 2.32 (s, 3H), 2.06 – 1.98 (m, 4H), 1.96 – 1.86 (m, 1H), 1.01 – 0.89 (m, 2H), 0.73 – 0.65 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 151.44, 146.17, 145.89, 96.11, 93.65, 47.22, 24.40, 18.36, 7.35, 6.47. HRMS calculated for $C_{15}H_{21}N_6$ 285.18222 $[M+H]^+$, found 285.18211. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.09 min; *m/z* : 285 $[M+H]^+$.

***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-5-methoxy-2-(pyrrolidin-1-yl)pyrimidin-4-amine (68)**



The title compound was synthesized from 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-5-methoxypyrimidin-4-amine (**98**) and pyrrolidine (**4h**) following General procedure D on a 0.30 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (95 mg, 76%). ^{1}H NMR (600 MHz, methanol-d_4) δ 7.34 (s, 1H), 6.45 (s, 1H), 3.91 (s, 3H), 3.58 (s, 4H), 2.10 (s, 4H), 1.94 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.05 – 0.98 (m, 2H), 0.77 – 0.69 (m, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 154.75, 149.97, 148.90, 146.77, 134.91, 119.67, 95.56, 57.60, 47.61, 26.26, 8.37, 7.76. HRMS calculated for $C_{15}H_{21}N_6O$ 301.17714 $[M+H]^+$, found 301.17711. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.10 min; *m/z* : 301 $[M+H]^+$.

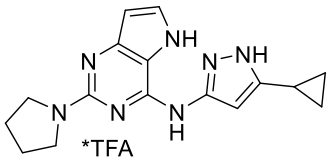
***N*-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-2-(pyrrolidin-1-yl)quinazolin-4-amine (69)**



The title compound was synthesized from 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)quinazolin-4-amine (**99**) (1 eq), pyrrolidine (**4h**) (3.5 eq) and DiPEA (4.8 eq) in *n*-butanol (0.15 M) at 120°C for 25 h on a 0.30 mmol scale following General procedure G and was purified by preparative HPLC (Gemini C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (78 mg, 60%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.32 (d, *J* = 7.5 Hz, 1H), 7.89 – 7.81 (m, 1H), 7.66 (d, *J* = 7.9 Hz, 1H), 7.54 – 7.45 (m, 1H), 6.52

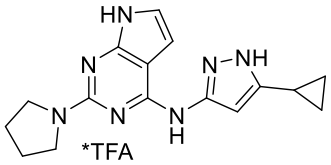
(s, 1H), 3.83 (t, *J* = 6.6 Hz, 2H), 3.68 (t, *J* = 6.6 Hz, 2H), 2.26 – 2.15 (m, 2H), 2.15 – 2.06 (m, 2H), 1.98 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.08 – 0.98 (m, 2H), 0.81 – 0.71 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 156.54, 150.02, 146.18, 145.46, 139.52, 134.88, 124.27, 116.96, 109.43, 95.12, 47.68, 24.39, 7.45, 6.55. HRMS calculated for $C_{18}H_{21}N_6$ 321.18222 $[M+H]^+$, found 321.18246. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.88 min; *m/z* : 321 $[M+H]^+$.

_N_-(5-Cyclopropyl-1_H_-pyrazol-3-yl)-2-(pyrrolidin-1-yl)-5_H_-pyrrolo[3,2-d]pyrimidin-4-amine (70)



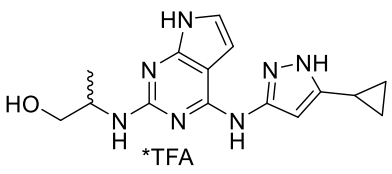
The title compound was synthesized from 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (**100**) and pyrrolidine (**4h**) following General procedure D on a 0.372 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 22% → 27% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (56 mg, 36%). ^{1}H NMR (600 MHz, methanol-d_4) δ 7.46 (d, *J* = 2.9 Hz, 1H), 6.48 (bs, 1H), 6.32 (d, *J* = 2.9 Hz, 1H), 3.63 (s, 4H), 2.11 (s, 4H), 1.95 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.09 – 0.98 (m, 2H), 0.80 – 0.69 (m, 2H). ^{13}C NMR (151 MHz, methanol-d_4) δ 150.95, 149.49, 149.01, 147.85, 137.64, 130.56, 109.49, 97.08, 94.89, 48.86, 26.28, 8.36, 7.70. HRMS calculated for $C_{16}H_{20}N_7$ 310.17747 $[M+H]^+$, found 310.17727. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.69 min; *m/z* : 310 $[M+H]^+$.

_N_-(5-Cyclopropyl-1_H_-pyrazol-3-yl)-2-(pyrrolidin-1-yl)-7_H_-pyrrolo[2,3-_d_]pyrimidin-4-amine (71)



The title compound was synthesized from 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**101**) and pyrrolidine (**4h**) following General procedure D on a 0.275 mmol scale and was purified by preparative HPLC (Gemini C_{18}, 25% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (42 mg, 36%). ^{1}H NMR (500 MHz, methanol-d_4) δ 7.03 (d, *J* = 3.7 Hz, 1H), 6.65 (d, *J* = 3.5 Hz, 1H), 5.90 (s, 1H), 3.74 – 3.65 (m, 4H), 2.20 – 2.09 (m, 4H), 1.97 (tt, *J* = 8.5, 5.0 Hz, 1H), 1.11 – 1.01 (m, 2H), 0.84 – 0.74 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6, 1% TFA, 75°C) δ 148.51, 148.18, 147.83, 147.39, 147.20, 121.23, 100.59, 94.77, 92.22, 46.71, 24.51, 7.59, 6.38. HRMS calculated for $C_{16}H_{20}N_7$ 310.17747 $[M+H]^+$, found 310.17742. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.78 min; *m/z* : 310 $[M+H]^+$.

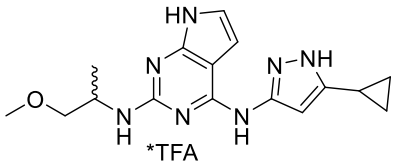
2-((4-((5-Cyclopropyl-1_H_-pyrazol-3-yl)amino)-7_H_-pyrrolo[2,3-_d_]pyrimidin-2-yl)amino)propan-1-ol (72)



The title compound was synthesized from 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**101**) (1 eq), 2-aminopropan-1-ol (**4t**) (2.7 eq) and DiPEA (1.85 eq), in *n*-butanol (0.12 M), at 200°C for 12 h, on a 0.31 mmol scale following General procedure H and was purified by preparative HPLC (Gemini C_{18}, 23% → 26% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (8 mg, 6%). ^{1}H NMR (500 MHz, methanol-d_4) δ 7.03 (d, *J* = 3.5 Hz, 1H), 6.68 (s, 1H), 5.88 (s, 1H), 4.23 – 4.13 (m, 1H), 3.71 – 3.62 (m, *J* = 24.8, 11.1, 5.4 Hz, 2H), 2.04 – 1.97 (m, 1H), 1.32 (d, *J* = 6.7

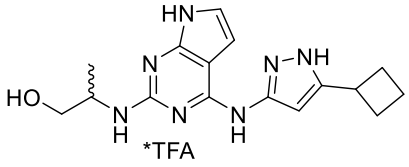
Hz, 3H), 1.11 – 1.05 (m, 2H), 0.82 – 0.77 (m, 2H). HRMS calculated for $C_{15}H_{20}N_7O$ 314.17238 $[M+H]^+$, found 314.1732. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.33 min; *m/z* : 314 $[M+H]^+$.

N^4-(5-Cyclopropyl-1*H*-pyrazol-3-yl)-N^2-(1-methoxypropan-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (73)



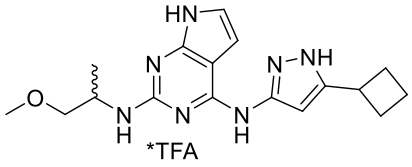
The title compound was synthesized from 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**101**) (1 eq), 1-methoxypropan-2-amine (**4s**) (2.6 eq) and DiPEA (1.85 eq), in *n*-butanol (0.12 M), at 200°C for 12 h, on a 0.31 mmol scale following General procedure H and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (9 mg, 7%). ^{1}H NMR (500 MHz, methanol-d_4) δ 7.05 – 7.00 (m, 1H), 6.68 (s, 1H), 5.89 (s, 1H), 4.35 – 4.25 (m, 1H), 3.56 – 3.45 (m, 2H), 3.41 (s, 3H), 2.04 – 1.96 (m, *J* = 8.5, 5.1 Hz, 1H), 1.31 (d, *J* = 5.6 Hz, 3H), 1.11 – 1.04 (m, 2H), 0.82 – 0.76 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 149.09, 148.54, 147.55, 121.82, 120.96, 100.05, 94.80, 90.83, 75.37, 75.07, 57.88, 46.89, 15.99, 7.18, 6.08. HRMS calculated for $C_{16}H_{22}N_7O$ 328.18803 $[M+H]^+$, found 328.1883. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.46 min; *m/z* : 328 $[M+H]^+$; purity 83%.

2-((4-((5-Cyclobutyl-1*H*-pyrazol-3-yl)amino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)amino) propan-1-ol (74)



The title compound was synthesized from 2-chloro-*N*-(5-cyclobutyl-1*H*-pyrazol-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**102**) (1 eq), 2-aminopropan-1-ol (**4t**) (2.8 eq) and DiPEA (1.85 eq), in *n*-butanol (0.12 M), at 200°C for 12 h, on a 0.30 mmol scale following General procedure H and was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (11 mg, 8%). ^{1}H NMR (850 MHz, methanol-d_4) δ 7.03 (d, *J* = 3.3 Hz, 1H), 6.70 (s, 1H), 6.11 (s, 1H), 4.25 – 4.16 (m, 1H), 3.72 – 3.68 (m, 1H), 3.68 – 3.60 (m, 2H), 2.50 – 2.38 (m, 2H), 2.31 – 2.21 (m, 2H), 2.17 – 2.08 (m, 1H), 2.02 – 1.93 (m, 1H), 1.30 (d, 3H). ^{13}C NMR (214 MHz, methanol-d_4) δ 153.01, 151.42, 150.71, 149.18, 148.74, 123.41, 101.60, 96.31, 93.58, 65.97, 50.53, 32.81, 30.37, 19.63, 17.25. HRMS calculated for $C_{16}H_{22}N_7O$ 328.18803 $[M+H]^+$, found 328.1883. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.73 min; *m/z* : 328 $[M+H]^+$.

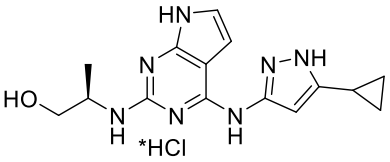
N^4-(5-Cyclobutyl-1*H*-pyrazol-3-yl)-N^2-(1-methoxypropan-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (75)



The title compound was synthesized from 2-chloro-*N*-(5-cyclobutyl-1*H*-pyrazol-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**102**) (1 eq), 1-methoxypropan-2-amine (**4s**) (2.6 eq) and DiPEA (1.85 eq), in *n*-butanol (0.12 M), at 200°C for 12 h, on a 0.31 mmol scale following General procedure H and was purified by preparative HPLC (Gemini C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (8 mg, 6%). ^{1}H NMR (850 MHz, methanol-d_4) δ 7.04 (d, *J* = 3.2 Hz, 1H), 6.70 (s, 1H), 6.11 (s, 1H),

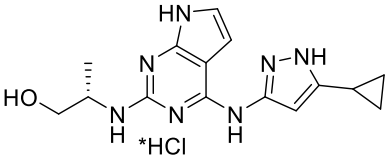
4.34 – 4.27 (m, 1H), 3.68 – 3.62 (m, *J* = 8.7 Hz, 1H), 3.55 – 3.52 (m, *J* = 9.7, 5.9 Hz, 1H), 3.51 – 3.48 (m, 1H), 3.40 (s, 3H), 2.46 – 2.41 (m, 2H), 2.30 – 2.22 (m, 2H), 2.15 – 2.09 (m, 1H), 2.01 – 1.95 (m, 1H), 1.32 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (214 MHz, methanol-d_4) δ 149.85, 149.04, 147.57, 121.86, 117.52, 100.04, 94.76, 92.08, 75.05, 57.88, 46.87, 31.27, 28.83, 18.08, 15.99. HRMS calculated for $C_{17}H_{24}N_7O$ 342.20368 $[M+H]^+$, found 342.2045. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.20 min; *m/z* : 342 $[M+H]^+$; purity 85%.

(*R*)-2-((4-((5-Cyclopropyl-1*H*-pyrazol-3-yl)amino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)amino)propan-1-ol (76)



A vial was charged with (*R*)-2-((4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)amino)propan-1-ol (**103**) (78 mg, 0.167 mmol, 1 eq) dissolved in MeOH (0.11 mL) and 1,4-dioxane (0.58 mL). After addition of aqueous NaOH (50%, 9.33 mL, 0.117 mmol, 20 eq) the reaction was stirred at 55°for 2 h and quenched with sat. aqueous NH_4Cl (3 mL) and extracted with chloroform (5x6 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) and concentrated under reduced pressure. The resulting product re-dissolved in chloroform and sat. aqueous $NaHCO_3$ and after phase separation the aqueous layer was extracted with 10% MeOH in chloroform (5x8 mL). The combined organic layers were dried (Na_2SO_4), filtered and after addition of excess HCl in 1,4-dioxane concentrated under reduced pressure to yield the compound as a HCl-salt after lyophilisation (13 mg, 22%). ^{1}H NMR (500 MHz, methanol-d_4) δ 7.04 (d, *J* = 3.6 Hz, 1H), 6.78 (d, *J* = 3.6 Hz, 1H), 6.14 (s, 1H), 4.20 – 4.11 (m, 1H), 3.82 – 3.77 (m, 1H), 3.69 – 3.63 (m, 1H), 2.08 (tt, *J* = 8.4, 5.0 Hz, 1H), 1.34 (d, *J* = 6.6 Hz, 3H), 1.27 – 1.16 (m, 2H), 0.99 – 0.86 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 153.53, 153.44, 153.40, 152.73, 145.01, 122.73, 102.01, 98.78, 92.81, 67.21, 51.52, 17.00, 9.70, 7.77. HRMS calculated for $C_{15}H_{20}N_7O$ 314.17238 $[M+H]^+$, found 314.1727. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.30 min; *m/z* : 314 $[M+H]^+$.

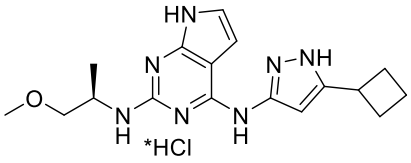
(*S*)-2-((4-((5-Cyclopropyl-1*H*-pyrazol-3-yl)amino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)amino)propan-1-ol (77)



A round bottom flask was charged with (*S*)-2-((4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)amino)propan-1-ol (**104**) (2.3 g, 5.83 mmol, 1 eq) dissolved in MeOH (16 mL) and 1,4-dioxane (20 mL). After cooling to 0°C and addition of aqueous NaOH (50%, 9.33 mL, 117 mmol, 20 eq) the reaction was allowed to warm to RT and stirred for another 90 min. The mixture was acidified with HCl (20 mL 6M) and concentrated under reduced pressure, re-dissolved in MeOH, filtered and concentrated under reduced pressure. The residue was purified by preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) and concentrated under reduced pressure. The resulting product was exchanged to a HCl salt by re-dissolving in HCl in H_2O/ACN (1/1, pH ~2) and concentrating under reduced pressure (3x) to yield the compound as a HCl-salt after lyophilisation (0.93 g, 46%). ^{1}H NMR (500 MHz, methanol-d_4) δ 7.04 (d, *J* = 3.6 Hz, 1H), 6.75 (d, *J* = 3.6 Hz, 1H), 6.08 (s, 1H), 4.20 – 4.11 (m, 1H), 3.78 (dd, *J* = 11.2, 4.2 Hz, 1H), 3.66 (dd, *J* = 11.1, 7.1 Hz, 1H), 2.06 (tt, *J* = 8.5, 5.0 Hz, 1H), 1.33 (d, *J* = 6.7 Hz, 3H), 1.22 – 1.15 (m, 2H), 0.94 – 0.88 (m, 2H). ^{13}C

NMR (126 MHz, methanol-d_4) δ 152.79, 152.75, 152.59, 152.32, 145.80, 122.85, 101.89, 98.28, 92.64, 66.95, 51.31, 17.02, 9.52, 7.72. HRMS calculated for $C_{15}H_{20}N_7O$ 314.17238 $[M+H]^+$, found 314.1723. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.51 min; *m/z* : 314 $[M+H]^+$.

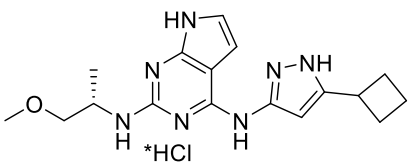
(*R*)-*N*^{4}-(5-Cyclobutyl-1*H*-pyrazol-3-yl)-*N*^{2}-(1-methoxypropan-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (78)



Step 1: A vial was charged with 2-chloro-*N*-(5-cyclobutyl-1*H*-pyrazol-3-yl)-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**110**) (0.28 g, 0.63 mmol, 1 eq), (*R*)-1-methoxypropan-2-amine hydrochloride (**4ah**) (119 mg, 0.945 mmol, 1.5 eq) dissolved in *n*-butanol (1.6 mL). After addition of DiPEA (0.33 mL, 1.89 mmol, 3.0 eq) the vial was sealed and the mixture heated in the microwave to 160°C for 13h. The reaction mixture was concentrated under reduced pressure and purified via flash-column-chromatography (SiO_2, dry-loading, 0% → 10% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product, which was used directly in step 2.

Step 2: A round bottom flask was charged with product from step 1, dissolved in MeOH (1.3 mL) and 1,4-dioxane (1.7 mL). After addition of aqueous NaOH (50%, 0.80 mL, 9.8 mmol, 20 eq) the reaction was stirred for 60 min at 55°C and quenched with sat. aqueous NH_4Cl (3 mL) and extracted with chloroform (5x6 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by preparative HPLC (Gemini C_{18}, 28% → 31% ACN in H_2O 0.2% TFA, 10 min gradient) and concentrated under reduced pressure. The resulting product re-dissolved in chloroform and sat. aqueous $NaHCO_3$ and after phase separation the aqueous layer was extracted with 10% MeOH in chloroform (5x8 mL). The combined organic layers were dried over Na_2SO_4, filtered and after addition of excess HCl in 1,4-dioxane concentrated under reduced pressure to yield the compound as a HCl-salt after lyophilisation (145 mg, 61%). ^{1}H NMR (850 MHz, methanol-d_4) δ 7.05 (d, *J* = 3.6 Hz, 1H), 6.78 (d, *J* = 3.6 Hz, 1H), 6.37 (s, 1H), 4.27 – 4.21 (m, 1H), 3.73 – 3.68 (m, 1H), 3.61 – 3.56 (m, 1H), 3.53 – 3.48 (m, 1H), 3.41 (s, 3H), 2.51 – 2.43 (m, 2H), 2.33 – 2.26 (m, 2H), 2.20 – 2.10 (m, 1H), 2.02 – 1.96 (m, 1H), 1.35 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (214 MHz, methanol-d_4) δ 153.52, 152.50, 152.08, 146.14, 145.83, 122.90, 101.91, 98.23, 94.35, 77.30, 59.44, 32.42, 29.80, 19.53, 17.29. HRMS calculated for $C_{17}H_{24}N_7O$ 342.20368 $[M+H]^+$, found 342.2043. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 8.23 min; *m/z* : 342 $[M+H]^+$.

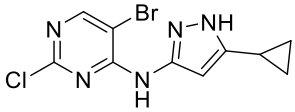
(*S*)-*N*^{4}-(5-Cyclobutyl-1*H*-pyrazol-3-yl)-*N*^{2}-(1-methoxypropan-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (79)



A round bottom flask was charged with (*S*)-*N*^{4}-(5-cyclobutyl-1*H*-pyrazol-3-yl)-*N*^{2}-(1-methoxypropan-2-yl)-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (**105**) (3.0 g, 6.05 mmol, 1 eq) dissolved in MeOH (17 mL) and 1,4-dioxane (21 mL). After cooling to 0°C and addition of aqueous NaOH (50%, 9.7 mL, 121 mmol, 20 eq) the reaction was allowed to warm to RT and stirred for another 3 h. The mixture was acidified with HCl (20 mL, 6M) and concentrated under reduced pressure, re-dissolved in MeOH, filtered and concentrated under reduced pressure. The residue was purified by preparative HPLC (Gemini C_{18}, 25% → 35% ACN in H_2O 0.2% TFA, 10 min gradient) and concentrated under reduced pressure. The resulting product

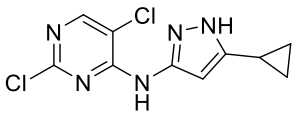
was exchanged to a HCl salt by re-dissolving in HCl in H_2O/ACN (1/1, pH ~2) and concentrating under reduced pressure (3x) to yield the compound as a HCl-salt after lyophilisation (1.07 g, 47%). ^{1}H NMR (500 MHz, methanol-d_4) δ 7.04 (d, *J* = 3.6 Hz, 1H), 6.77 (d, *J* = 3.6 Hz, 1H), 6.34 (s, 1H), 4.29 – 4.21 (m, 1H), 3.72 – 3.64 (m, 1H), 3.58 (dd, *J* = 9.6, 4.5 Hz, 1H), 3.51 (dd, *J* = 9.6, 6.8 Hz, 1H), 3.40 (s, 3H), 2.50 – 2.42 (m, 2H), 2.34 – 2.24 (m, 2H), 2.19 – 2.09 (m, 1H), 2.04 – 1.95 (m, 1H), 1.34 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (126 MHz, methanol-d_4) δ 151.97, 150.68, 150.55, 145.63, 145.00, 121.62, 100.58, 96.66, 93.10, 75.89, 58.11, 31.17, 31.15, 28.54, 18.21, 16.00. HRMS calculated for $C_{17}H_{24}N_7O$ 342.20368 $[M+H]^+$, found 342.2040. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 8.13 min; *m/z* : 342 $[M+H]^+$.

5-Bromo-2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (80)



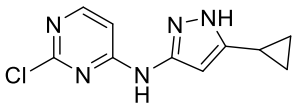
The title compound was synthesized from 5-bromo-2,4-dichloropyrimidine (**1b**) and 5-cyclopropyl-1*H*-pyrazol-3-amine (**2b**) following General procedure A on a 2.19 mmol scale at RT and purified via flash-column-chromatography (dry-loading, SiO_2, 50% → 100% EtOAc in pentane) to yield the product (523 mg, 75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.36 (s, 1H), 9.26 (s, 1H), 8.41 (s, 1H), 6.16 (s, 1H), 2.02 – 1.75 (m, 1H), 1.03 – 0.82 (m, 2H), 0.77 – 0.63 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.19, 157.95, 157.79, 146.04, 145.65, 102.76, 95.68, 7.87, 6.90.

2,5-Dichloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (81)



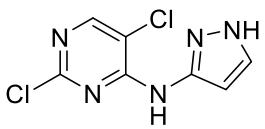
A round-bottom-flask was charged with 2,4,5-trichloropyrimidine (**1a**) (5.00 g, 27.26 mmol, 1 eq) dissolved in EtOH (35 mL). Et_3N (4.18 mL, 29.99 mmol, 1.1 eq) and 5-cyclopropyl-1*H*-pyrazol-3-amine (**2b**) (3.69 g, 29.99 mmol, 1.1 eq) dissolved in EtOH (35 mL) were added dropwise and the reaction mixture was stirred ON at RT until a colourless precipitate was formed. The formed precipitate was filtered off, washed with ice-cold EtOH and dried under reduced pressure to yield the product (7.40 g, quant.). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.37 (s, 1H), 9.70 (s, 1H), 8.32 (s, 1H), 6.19 (s, 1H), 2.03 – 1.80 (m, 1H), 1.04 – 0.87 (m, 2H), 0.81 – 0.60 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.14, 156.97, 155.25, 145.99, 145.64, 113.26, 95.69, 7.92, 6.94. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.66 min; *m/z* : 270 $[M+H]^+$.

2-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (82)



The title compound was synthesized from 2,4-dichloropyrimidine (**1c**) and 5-cyclopropyl-1*H*-pyrazol-3-amine (**2b**) following General procedure A on a 3.36 mmol scale at 80°C and purified via flash-column-chromatography (dry-loading, SiO_2, 50% → 100% EtOAc in pentane) to yield the product (290 mg, 37%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.07 (d, *J* = 6.0 Hz, 1H), 7.00 (bs, 1H), 6.08 (bs, 1H), 1.99 – 1.83 (m, 1H), 1.07 – 0.95 (m, 2H), 0.83 – 0.70 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 163.00, 161.23, 157.74, 148.69, 105.59, 94.43, 8.25, 7.62.

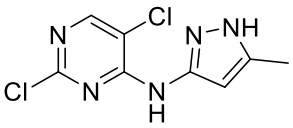
2,5-Dichloro-*N*-(1*H*-pyrazol-3-yl)pyrimidin-4-amine (83)



The title compound was synthesized from 2,4,5-trichloropyrimidine (**1a**) and 1*H*-pyrazol-3-amine (**2d**) following General procedure A on a 0.60 mmol scale at RT and purified via flash-column-chromatography (dry-loading, SiO_2, 0% → 100% EtOAc in pentane) to yield the product (123 mg, 89%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.23 (s, 1H), 7.62 (d, *J* = 2.4 Hz, 1H), 6.71 (s,

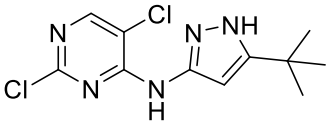
1H). ^{13}C NMR (151 MHz, methanol-d_4) δ 159.11, 158.20, 155.77, 147.53, 130.42, 114.79, 99.42. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.79 min; *m/z* : 230 $[M+H]^+$.

2,5-Dichloro-*N*-(5-methyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (84)



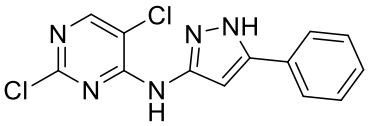
The title compound was synthesized from 2,4,5-trichloropyrimidine (**1a**) and 5-methyl-1*H*-pyrazol-3-amine (**2e**) following General procedure A on a 0.60 mmol scale at RT and purified via flash-column-chromatography (dry-loading, SiO_2, 40% → 100% EtOAc in pentane) to yield the product (128 mg, 88%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.21 (s, 1H), 6.47 (s, 1H), 2.32 (s, 3H). ^{13}C NMR (151 MHz, methanol-d_4) δ 159.11, 158.09, 155.66, 147.72, 141.29, 114.73, 98.78, 10.92. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.84 min; *m/z* : 244 $[M+H]^+$.

***N*-(5-(*tert*-Butyl)-1*H*-pyrazol-3-yl)-2,5-dichloropyrimidin-4-amine (85)**



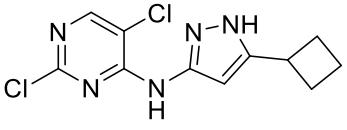
The title compound was synthesized from 2,4,5-trichloropyrimidine (**1a**) and 5-(*tert*-butyl)-1*H*-pyrazol-3-amine (**2f**) following General procedure A on a 0.60 mmol scale at RT and purified via flash-column-chromatography (dry-loading, SiO_2, 20% → 100% EtOAc in pentane) to yield the product (159 mg, 92%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.22 (s, 1H), 6.50 (s, 1H), 1.36 (s, 9H). ^{13}C NMR (151 MHz, methanol-d_4) δ 159.12, 158.06, 155.65, 155.26, 147.17, 114.73, 95.71, 32.16, 30.44. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.60 min; *m/z* : 286 $[M+H]^+$.

2,5-Dichloro-*N*-(5-phenyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (86)



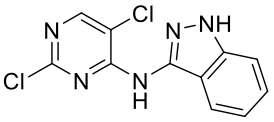
The title compound was synthesized from 2,4,5-trichloropyrimidine (**1a**) and 5-phenyl-1*H*-pyrazol-3-amine (**2g**) following General procedure A on a 0.60 mmol scale at RT and purified via flash-column-chromatography (dry-loading, SiO_2, 0% → 100% EtOAc in pentane) to yield the product (169 mg, 92%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.16 (s, 1H), 9.87 (s, 1H), 8.37 (s, 1H), 7.74 (d, *J* = 7.1 Hz, 2H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 1H), 6.89 (d, *J* = 2.2 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 157.13, 157.09, 155.37, 146.53, 142.36, 129.11, 128.67, 128.35, 125.02, 113.29, 96.83. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.02 min; *m/z* : 306 $[M+H]^+$.

2,5-Dichloro-*N*-(5-cyclobutyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (87)



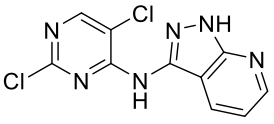
The title compound was synthesized from 2,4,5-trichloropyrimidine (**1a**) and 5-cyclobutyl-1*H*-pyrazol-3-amine (**2h**) following General procedure A on a 0.60 mmol scale at RT and purified via flash-column-chromatography (dry-loading, SiO_2, 0% → 100% EtOAc in pentane) to yield the product (156 mg, 91%). ^{1}H NMR (600 MHz, methanol-d_4) δ 8.22 (s, 1H), 6.52 (s, 1H), 3.58 (p, *J* = 8.7 Hz, 1H), 2.50 – 2.34 (m, 2H), 2.30 – 2.18 (m, 2H), 2.14 – 2.00 (m, 1H), 2.01 – 1.86 (m, 1H). ^{13}C NMR (151 MHz, methanol-d_4) δ 159.13, 158.09, 155.68, 150.15, 147.50, 114.75, 96.59, 33.07, 30.24, 19.47. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.38 min; *m/z* : 284 $[M+H]^+$.

_N_-(2,5-Dichloropyrimidin-4-yl)-1_H_-indazol-3-amine (88)



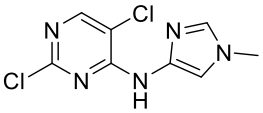
The title compound was synthesized from 2,4,5-trichloropyrimidine (**1a**) (1 eq) and 1*H*-indazol-3-amine (**2i**) (1.3 eq) following General procedure B with DiPEA (1.8 eq) in THF on a 1.0 mmol scale at RT. The crude product was purified via flash-column-chromatography (dry-loading, SiO_2, 20% → 60% EtOAc in pentane) to yield the product (174 mg, 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.01 (s, 1H), 10.08 (bs, 1H), 8.41 (s, 1H), 7.59 – 7.49 (m, 2H), 7.45 – 7.34 (m, 1H), 7.16 – 7.06 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.76, 157.30, 155.71, 141.31, 138.76, 126.68, 120.67, 120.44, 117.87, 113.41, 110.78. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.79 min; *m/z* : 280 $[M+H]^+$.

_N_-(2,5-Dichloropyrimidin-4-yl)-1_H_-pyrazolo[3,4-_b_]pyridin-3-amine (89)



The title compound was synthesized from 2,4,5-trichloropyrimidine (1 eq) (**1a**) and 1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (**2j**) (1.1 eq) following General procedure B with Et_3N (1.3 eq) in EtOH (2 mL) and THF (1.5 mL) on a 1.0 mmol scale at 60°C. The precipitating product was collected by filtration (184 mg, 65%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.55 (s, 1H), 10.22 (bs, 1H), 8.54 (dd, *J* = 4.5, 1.6 Hz, 1H), 8.41 (s, 1H), 8.10 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.19 (dd, *J* = 8.1, 4.5 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.28, 157.19, 155.92, 152.03, 149.52, 138.00, 131.00, 116.78, 113.47, 109.79. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.82 min; *m/z* : 281 $[M+H]^+$.

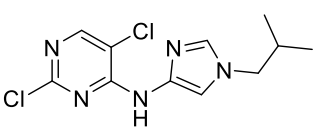
2,5-Dichloro-_N_-(1-methyl-1_H_-imidazol-4-yl)pyrimidin-4-amine (90)



Step 1: A round-bottom-flask was charged with 1-methyl-4-nitro-1*H*-imidazole (**106**) (0.25 g, 1.95 mmol, 1.15 eq) and Pd/C (10%, 207 mg) suspended in EtOH (10 mL). The mixture was degassed and H_2 gas was bubbled through while sonicating for 20 min. The reaction was stirred for another 16 h under H_2 atmosphere until full conversion was detected by TLC. The mixture was filtered and because the resulting product is unstable, the resulting filtrated was used directly in step 2.

Step 2: After Et_3N (0.353 mL, 2.53 mmol, 1.5 eq) was added to the filtrate from step 1, a solution of 2,4,5-trichloropyrimidine (**1a**) (0.32 g, 1.7 mmol, 1 eq) in EtOH (5 mL) was added dropwise and the mixture was stirred for 16 h. The mixture was concentrated under reduced pressure onto celite and purified via flash-column-chromatography (dry-loading, SiO_2, 50% → 100% EtOAc in pentane) to yield the product (117 mg, 25%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.21 (s, 1H), 7.48 (d, *J* = 1.1 Hz, 1H), 7.41 (d, *J* = 1.6 Hz, 1H), 3.76 (s, 3H). ^{13}C NMR (126 MHz, methanol-d_4) δ 159.16, 157.10, 155.16, 137.49, 135.78, 114.75, 111.38, 34.17.

2,5-Dichloro-_N_-(1-isobutyl-1_H_-imidazol-4-yl)pyrimidin-4-amine (91)



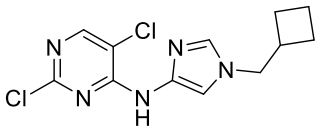
Step 1: A round-bottom-flask was charged with 4-nitro-1*H*-imidazole (**107**) (0.57 g, 5 mmol, 1 eq) and K_2CO_3 (1.04 g, 7.5 mmol, 1.5 eq) suspended in DMF (6.25 mL). After addition of 1-bromo-2-methylpropane (0.82 g, 6 mmol, 1.2 eq) the reaction mixture was stirred at 50°C overnight. The resulting mixture was filtered and the filtrate concentrated and the crude product used without further purification.

Step 2: A round-bottom-flask was charged with crude product from step 1 (0.25 g, 1.48 mmol, 1.1 eq) and Pd/C (10%) suspended in MeOH (10 mL). The mixture was degassed and H_2 gas

was bubbled through while sonicating for 20 min. The reaction was stirred for another 16 h under H_2 atmosphere until full conversion was detected by TLC. The mixture was filtered and because the resulting product is unstable, the resulting filtrated was used directly in step 2.

Step 3: Et_3N (0.30 mL, 2.15 mmol, 1.6 eq) and 2,4,5-trichloropyrimidine (**1a**) (0.25 g, 1.34 mmol, 1 eq) was added and the mixture was stirred for 16 h. The mixture was concentrated under reduced pressure onto celite and purified via flash-column-chromatography (dry-loading, SiO_2, 20% → 50% EtOAc in pentane) to yield the product (108 mg, 28%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.77 (bs, 1H), 8.30 (s, 1H), 7.56 (s, 1H), 7.33 (s, 1H), 3.81 (d, *J* = 7.0 Hz, 2H), 2.09 – 1.94 (m, 1H), 0.85 (d, *J* = 6.5 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.03, 155.56, 154.54, 136.18, 134.32, 113.21, 109.15, 53.71, 29.41, 19.49. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.4 min; *m/z* : 286 $[M+H]^+$.

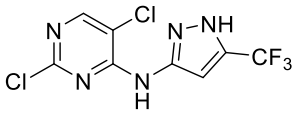
2,5-Dichloro-*N*-(1-(cyclobutylmethyl)-1*H*-imidazol-4-yl)pyrimidin-4-amine (92)



Step 1: A round-bottom-flask was charged with 1-(cyclobutylmethyl)-4-nitro-1*H*-imidazole (**108**) (0.045 g, 0.248 mmol, 1 eq) and Pd/C (10%) suspended in MeOH (2 mL). The mixture was degassed and H_2 gas was bubbled through while sonicating for 20 min. The reaction was stirred for another 16 h under H_2 atmosphere until full conversion was detected by TLC. The mixture was filtered over celite and the resulting oil used directly in step 2.

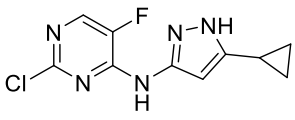
Step 2: The product from step 1 was dissolved in EtOH (7 mL) and 2,4,5-trichloropyrimideine (**1a**) (48 mg, 0.262 mmol, 1.1 eq) was added. After dropwise addition of Et_3N (50 µL, 0.360 mmol, 1.5 eq) the reaction was stirred to 35 h at RT. The mixture was concentrated under reduced pressure onto celite and purified via flash-column-chromatography (dry-loading, SiO_2, 40% → 80% EtOAc in pentane) to yield the product (66 mg, 85%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.21 (s, 1H), 7.50 (s, 1H), 7.41 (s, 1H), 4.04 (d, *J* = 7.4 Hz, 2H), 2.86 – 2.71 (m, 1H), 2.15 – 2.05 (m, 2H), 2.03 – 1.89 (m, 2H), 1.89 – 1.79 (m, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 159.15, 157.06, 155.14, 137.36, 134.93, 114.75, 110.22, 53.65, 37.58, 26.76, 18.86.

2,5-Dichloro-*N*-(5-(trifluoromethyl)-1*H*-pyrazol-3-yl)pyrimidin-4-amine (93)



The title compound was synthesized from 2,4,5-trichloropyrimidine (**1a**) (1 eq) and 5-(trifluoromethyl)-1*H*-pyrazol-3-amine (**2k**) (1.1 eq) following General procedure B with Et_3N (1.4 eq) in EtOH on a 1.0 mmol scale at 60°C. The crude product was purified via flash-column-chromatography (dry-loading, SiO_2, 20% → 60% EtOAc in pentane) to yield the product (35 mg, 19%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.34 (s, 1H), 6.69 (s, 1H). LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.62 min; *m/z* : 298 $[M+H]^+$.

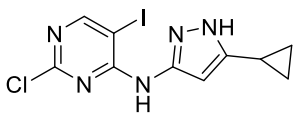
2-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-5-fluoropyrimidin-4-amine (94)



The title compound was synthesized from 2,4-dichlo-5-fluororopyrimidine (**1d**) and 5-cyclopropyl-1*H*-pyrazol-3-amine (**2b**) following General procedure A on a 1.0 mmol scale at RT and purified via flash-column-chromatography (dry-loading, SiO_2, 30% → 80% EtOAc in pentane) to yield the product (220 mg, 87%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.27

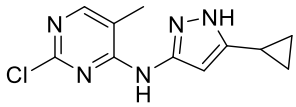
(s, 1H), 10.34 (s, 1H), 8.22 (d, *J* = 3.4 Hz, 1H), 6.24 (s, 1H), 1.96 – 1.86 (m, 1H), 0.97 – 0.90 (m, 2H), 0.72 – 0.65 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 153.23 (d, *J* = 3.1 Hz), 150.87 (d, *J* = 12.1 Hz), 145.87, 144.94 (d, *J* = 259.2 Hz), 141.38 (d, *J* = 20.6 Hz), 94.66, 48.74, 7.87, 7.02. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.00 min; *m/z* : 254 $[M+H]^+$.

2-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-5-iodopyrimidin-4-amine (95)



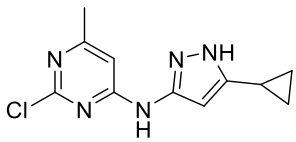
The title compound was synthesized from 2,4-dichlo-5-iodoropyrimidine (**1e**) and 5-cyclopropyl-1*H*-pyrazol-3-amine (**2b**) following General procedure A on a 1.0 mmol scale at 60°C and purified via flash-column-chromatography (dry-loading, SiO_2, 60% → 80% EtOAc in pentane) to yield the product (200 mg, 55%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.35 (s, 1H), 8.62 (s, 1H), 8.51 (s, 1H), 6.16 (s, 1H), 1.96 – 1.87 (m, 1H), 0.98 – 0.90 (m, 2H), 0.72 – 0.66 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.20, 159.88, 159.09, 146.19, 145.99, 95.20, 77.55, 7.90, 6.97. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.18 min; *m/z* : 362 $[M+H]^+$.

2-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-5-methylpyrimidin-4-amine (96)



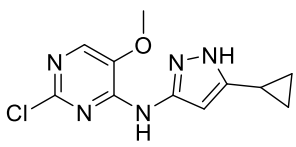
The title compound was synthesized from 2,4-dichloro-5-methylpyrimidine (**1f**) and 5-cyclopropyl-1*H*-pyrazol-3-amine (**2b**) following General procedure A on a 1.0 mmol scale at 60°C and purified via flash-column-chromatography (dry-loading, SiO_2, 70% → 80% EtOAc in pentane) to yield the product (122 mg, 49%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 9.27 (s, 1H), 8.03 – 7.91 (m, 1H), 6.27 (s, 1H), 2.12 (s, 3H), 1.92 (tt, *J* = 8.5, 5.1 Hz, 1H), 0.98 – 0.89 (m, 2H), 0.72 – 0.65 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.23, 157.43, 156.30, 147.18, 146.05, 114.36, 95.28, 13.81, 8.15, 7.39. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.70 min; *m/z* : 250 $[M+H]^+$.

2-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-6-methylpyrimidin-4-amine (97)



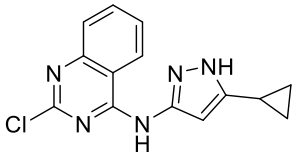
The title compound was synthesized from 2,4-dichloro-6-methylpyrimidine (**1g**) and 5-cyclopropyl-1*H*-pyrazol-3-amine (**2b**) following General procedure A on a 1.0 mmol scale at 60°C and purified via flash-column-chromatography (dry-loading, SiO_2, 40% → 60% EtOAc in pentane) to yield the product (119 mg, 48%). ^{1}H NMR (500 MHz, methanol-d_4) δ 6.84 (s, 1H), 6.02 (s, 1H), 2.31 (s, 3H), 1.90 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.01 – 0.95 (m, 2H), 0.76 – 0.71 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 160.08, 159.07, 153.08, 152.97, 120.67, 95.09, 26.12, 9.21, 8.26. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.55 min; *m/z* : 250 $[M+H]^+$.

2-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-5-methoxypyrimidin-4-amine (98)



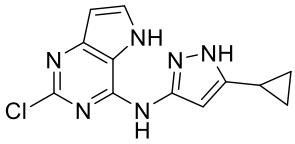
The title compound was synthesized from 2,4-dichloro-5-methoxypyrimidine (**1h**) and 5-cyclopropyl-1*H*-pyrazol-3-amine (**2b**) following General procedure A on a 1.0 mmol scale at 60°C and purified via flash-column-chromatography (dry-loading, SiO_2, 50% → 80% EtOAc in pentane) to yield the product (209 mg, 79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 9.20 (s, 1H), 7.87 (s, 1H), 6.25 (s, 1H), 3.89 (s, 3H), 2.00 – 1.84 (m, 1H), 0.99 – 0.86 (m, 2H), 0.78 – 0.60 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.31, 149.87, 146.24, 145.77, 139.62, 135.28, 94.52, 56.63, 7.83, 6.98. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.58 min; *m/z* : 266 $[M+H]^+$.

2-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)quinazolin-4-amine (99)



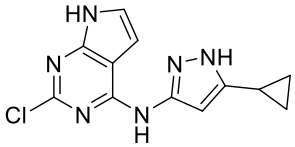
The title compound was synthesized from 2,4-dichloroquinazoline (**1i**) (1 eq) and 5-cyclopropyl-1*H*-pyrazol-3-amine (**2b**) (1.3 eq) following General procedure B with DiPEA (1.1 eq) in EtOH on a 1.0 mmol scale at RT. The precipitating product was collected by filtration (166 mg, 58%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.26 (s, 1H), 10.60 (s, 1H), 8.58 (d, *J* = 8.2 Hz, 1H), 7.87 – 7.80 (m, 1H), 7.72 – 7.64 (m, 1H), 7.60 – 7.52 (m, 1H), 6.45 (s, 1H), 1.95 (tt, *J* = 8.5, 5.1 Hz, 1H), 0.99 – 0.93 (m, 2H), 0.74 – 0.70 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.70, 156.35, 150.74, 146.76, 145.65, 133.88, 126.69, 126.49, 123.49, 113.43, 95.33, 7.56, 6.85. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.51 min; *m/z* : 286 $[M+H]^+$.

2-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (100)



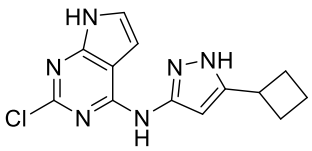
The title compound was synthesized from 2,4-dichloro-5*H*-pyrrolo[3,2-*d*]pyrimidine (**1j**) and 5-cyclopropyl-1*H*-pyrazol-3-amine (**2b**) following General procedure A on a 1.0 mmol scale at 120°C and purified via flash-column-chromatography (dry-loading, SiO_2, 2% → 7% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (123 mg, 45%). ^{1}H NMR (500 MHz, methanol-d_4) δ 7.55 (d, *J* = 3.0 Hz, 1H), 6.42 (d, *J* = 3.0 Hz, 1H), 5.26 (s, 1H), 1.93 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.04 – 0.97 (m, 2H), 0.79 – 0.74 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 152.23, 151.26, 148.88, 148.52, 131.08, 113.79, 102.11, 93.95, 89.11, 8.02, 7.70. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.58 min; *m/z* : 275 $[M+H]^+$.

2-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (101)



A round-bottom-flask was charged with 2,4-dichloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1k**) (1.91 g, 10.13 mmol, 1 eq), 5-cyclopropyl-1*H*-pyrazol-3-amine (**2b**) (2.05 g, 16.65 mmol, 1.64 eq) dissolved in *n*-butanol (30 mL). After addition of DiPEA (2.6 mL, 14.91 mmol, 1.47 eq) the mixture was stirred for 5 d at 120°C. The resulting mixture was concentrated under reduced pressure onto celite and purified via flash-column-chromatography (dry-loading, SiO_2, 0% → 10% MeOH in EtOAc) to yield the product (1.10 mg, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 (s, 1H), 11.81 (s, 1H), 10.18 (s, 1H), 7.25 – 7.05 (m, 1H), 6.75 (s, 1H), 6.40 (s, 1H), 1.99 – 1.83 (m, 1H), 1.00 – 0.84 (m, 2H), 0.76 – 0.63 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 153.80, 151.97, 151.44, 122.20, 101.65, 99.80, 94.37, 7.72, 7.03. LCMS (ESI, C_{18}, linear gradient, 0% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.42 min; *m/z* : 275 $[M+H]^+$.

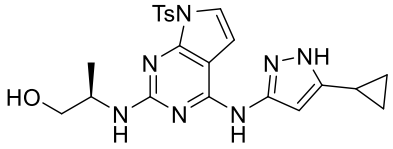
2-Chloro-*N*-(5-cyclobutyl-1*H*-pyrazol-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (102)



A vial was charged with 2,4-dichloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1k**) (0.404 g, 2.15 mmol, 1 eq), 5-cyclobutyl-1*H*-pyrazol-3-amine (**2h**) (0.487 g, 3.55 mmol, 1.65 eq) and DiPEA (0.5 mL, 2.87 mmol, 1.34 eq) dissolved in *n*-butanol. The reaction mixture was heated for 4 d at 120°C, concentrated onto celite under reduced pressure and purified via flash-column-chromatography (dry-loading, SiO_2, 40% → 100% EtOAc in pentane) to yield the product (0.35 mg, 56%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.19 (s, 1H), 11.81 (s, 1H), 10.21 (s, 1H), 7.15 (s, 1H), 6.85 (s, 1H), 6.56 (s, 1H), 3.63 – 3.42 (m, 1H), 2.37 – 2.25 (m, 2H), 2.23 – 2.07 (m, 2H), 2.06 – 1.92 (m, 1H), 1.91 – 1.76 (m, 1H). ^{13}C NMR (101 MHz, DMSO-

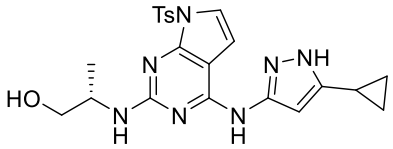
d_6) δ 152.40, 151.83, 147.95, 147.50, 122.55, 102.08, 100.24, 31.62, 29.47, 18.58. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.32 min; *m/z* : 289 $[M+H]^+$.

(*R*)-2-((4-((5-Cyclopropyl-1*H*-pyrazol-3-yl)amino)-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)amino)propan-1-ol (103)



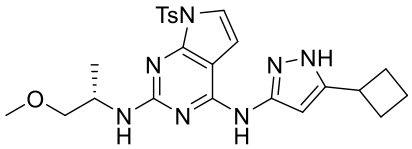
A vial was charged with 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**109**) (0.22 g, 0.47 mmol, 1 eq), (*R*)-2-aminopropan-1-ol (**4af**) (67 mg, 0.892 mmol, 1.9 eq) dissolved in *n*-butanol (1.3 mL). After addition of DiPEA (0.18 mL, 1.02 mmol, 2.2 eq) the vial was sealed and the mixture heated in the microwave to 150°C for 6 h. The reaction mixture was concentrated under reduced pressure and purified via flash-column-chromatography (SiO_2, dry-loading, 50% → 100% EtOAc in pentane) to yield the product (85 mg, 36%). ^{1}H NMR (500 MHz, methanol-d_4) δ 7.95 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 7.9 Hz, 2H), 7.16 (d, *J* = 4.0 Hz, 1H), 6.58 (d, *J* = 4.0 Hz, 1H), 5.91 (bs, 1H), 4.16 – 4.08 (m, 1H), 3.67 – 3.62 (m, 1H), 3.62 – 3.57 (m, 1H), 2.31 (s, 3H), 1.85 (tt, *J* = 8.5, 5.0 Hz, 1H), 1.23 (d, *J* = 6.7 Hz, 3H), 0.93 – 0.84 (m, 2H), 0.73 – 0.62 (m, 2H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 160.89, 154.89, 154.44, 152.25, 146.84, 145.80, 136.56, 130.70, 129.14, 119.95, 104.30, 99.22, 91.85, 67.38, 49.98, 21.55, 17.74, 8.85, 8.20. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.90 min; *m/z* : 468 $[M+H]^+$.

(*S*)-2-((4-((5-Cyclopropyl-1*H*-pyrazol-3-yl)amino)-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)amino)propan-1-ol (104)



A vial was charged with 2-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**109**) (2.57 g, 6.0 mmol, 1 eq), (*S*)-2-aminopropan-1-ol (**4ag**) (0.676 g, 9.0 mmol, 1.5 eq) dissolved in *n*-butanol (15 mL). After addition of DiPEA (2.09 mL, 12 mmol, 2 eq) the vial was sealed and the mixture heated in the microwave to 160°C for 10 h. The reaction mixture was concentrated under reduced pressure and purified via flash-column-chromatography (SiO_2, dry-loading, 0% → 8% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (2.81 g, 50%). ^{1}H NMR (500 MHz, methanol-d_4) δ 7.94 (d, *J* = 8.1 Hz, 2H), 7.26 (d, *J* = 8.2 Hz, 2H), 7.15 (d, *J* = 4.0 Hz, 1H), 6.58 (bs, 1H), 5.60 (bs, 1H), 4.17 – 4.08 (m, 1H), 3.68 – 3.63 (m, 1H), 3.61 – 3.56 (m, 1H), 2.28 (s, 3H), 1.84 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.23 (d, *J* = 6.7 Hz, 3H), 0.91 – 0.82 (m, 2H), 0.71 – 0.62 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 161.01, 154.86, 154.43, 148.89, 146.79, 136.52, 130.67, 129.11, 119.89, 104.30, 99.23, 95.54, 88.66, 67.40, 49.95, 21.55, 17.75, 8.91, 8.20. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.11 min; *m/z* : 468 $[M+H]^+$.

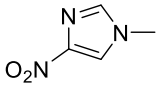
(*S*)-N^4-(5-Cyclobutyl-1*H*-pyrazol-3-yl)-N^2-(1-methoxypropan-2-yl)-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (105)



A vial was charged with 2-chloro-*N*-(5-cyclobutyl-1*H*-pyrazol-3-yl)-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**110**) (2.66 g, 6.0 mmol, 1 eq), (*S*)-1-methoxypropan-2-amine (**4ai**) (0.80 g, 9.0 mmol, 1.5 eq) dissolved in *n*-butanol (15 mL). After addition of DiPEA (2.09 mL, 12 mmol, 2 eq) the vial was sealed and the mixture heated in the microwave to 160°C for 13 h. The reaction

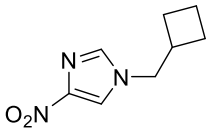
mixture was concentrated under reduced pressure and purified via flash-column-chromatography (SiO_2, dry-loading, 0% → 8% (10% of sat. aqueous NH_3 in MeOH) in DCM) to yield the product (2.36 g, 79%). ^{1}H NMR (500 MHz, methanol-d_4) δ 7.94 (d, *J* = 8.0 Hz, 2H), 7.28 (d, *J* = 8.1 Hz, 2H), 7.17 (bs, 1H), 6.60 (bs, 1H), 5.79 (bs, 1H), 4.26 – 4.18 (m, 1H), 3.56 – 3.43 (m, 2H), 3.34 (s, 3H), 3.32 (bs, 1H), 2.31 (s, 3H), 2.30 – 2.23 (m, 2H), 2.20 – 2.09 (m, 2H), 2.03 – 1.93 (m, 1H), 1.82 (bs, *J* = 9.8 Hz, 1H), 1.21 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, methanol-d_4) δ 159.57, 156.27, 154.50, 153.41, 148.50, 145.45, 135.43, 129.40, 127.88, 118.51, 103.04, 97.89, 95.47, 88.30, 75.98, 58.02, 46.41, 28.94, 20.29, 18.16, 16.85. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 6.97 min; *m/z* : 496 $[M+H]^+$.

1-Methyl-4-nitro-1*H*-imidazole (106)



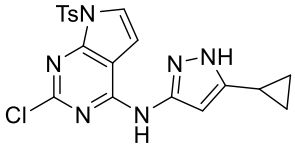
A round-bottom-flask was charged with 4-nitro-1*H*-imidazole (**107**) (0.57 g, 5 mmol, 1 eq) and K_2CO_3 (1.04 g, 7.5 mmol, 1.5 eq) suspended in ACN (6.25 mL). After addition of methyl iodine (0.85 g, 6 mmol, 1.2 eq) the reaction mixture was stirred at 65°C overnight. The resulting mixture was filtered and the filtrate concentrated after witch the crude product was recrystallized from 20 mL *i*PrOH to yield the product (0.31 g, 49%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.36 (d, *J* = 1.4 Hz, 1H), 7.81 (d, *J* = 1.2 Hz, 1H), 3.75 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 138.04, 122.55, 34.22.

1-(Cyclobutylmethyl)-4-nitro-1*H*-imidazole (108)



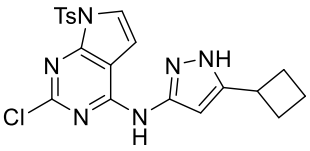
A vial was charged with cyclobutylmethyl 4-methylbenzenesulfonate (**111**) (0.288 g, 1.2 mmol, 1.13 eq) and K_2CO_3 (0.237 g, 1.7 mmol, 1.6 eq) dissolved in ACN (1.25 mL). After addition of 4-nitro-1*H*-imidazole (**107**) (0.120 g, 1.06 mmol, 1 eq) the reaction mixture was stirred for 70 h at 60°C. The reaction mixture was filtered, concentrated onto celite and purified via flash-column-chromatography (dry-loading, SiO_2, 50% EtOAc in pentane) to yield the product (61 mg g, 64%, 2 regio-isomers 4:1, major isomer reported). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.13 (d, *J* = 1.5 Hz, 1H), 7.74 (d, *J* = 1.3 Hz, 1H), 4.13 (d, *J* = 7.6 Hz, 2H), 2.86 – 2.74 (m, 1H), 2.11 – 2.03 (m, 2H), 1.98 – 1.86 (m, 2H), 1.86 – 1.76 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 148.55, 138.13, 121.46, 54.13, 37.10, 26.42, 18.73.

2-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (109)



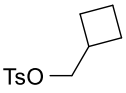
A vial was charged with 2,4-dichloro-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**112**) (2.00 g, 5.84 mmol, 1 eq), 5-cyclopropyl-1*H*-pyrazol-3-amine (**2b**) (0.90 g, 7.31 mmol, 1.25 eq) and Et_3N (1.22 mL, 8.77 mmol, 1.5 eq) dissolved in ACN (15 mL), sealed and heated in the microwave to 100°C for 2.5 h. The reaction mixture was concentrated under reduced pressure and purified via flash-column-chromatography (SiO_2, 30% → 75% EtOAc in pentane) to yield the product (1.36 g, 54%). ^{1}H NMR (500 MHz, methanol-d_4) δ 7.99 (d, *J* = 8.4 Hz, 2H), 7.49 (d, *J* = 4.0 Hz, 1H), 7.33 (d, *J* = 7.9 Hz, 2H), 6.66 (bs, 1H), 6.28 (bs, 1H), 2.34 (s, 3H), 1.89 (tt, *J* = 8.5, 5.1 Hz, 1H), 0.98 – 0.92 (m, 2H), 0.74 – 0.67 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 156.03, 155.94, 152.49, 148.60, 147.53, 135.98, 130.89, 129.33, 124.22, 105.43, 104.12, 95.86, 21.60, 8.20, 7.90. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.00 min; *m/z* : 429 $[M+H]^+$.

2-Chloro-*N*-(5-cyclobutyl-1*H*-pyrazol-3-yl)-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (110)



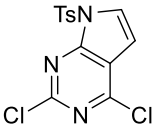
A vial was charged with 2,4-dichloro-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**112**) (2.00 g, 5.84 mmol, 1 eq), 5-cyclobutyl-1*H*-pyrazol-3-amine (**2h**) (1.00 g, 7.31 mmol, 1.25 eq) and Et_3N (1.22 mL, 8.77 mmol, 1.5 eq) dissolved in ACN (15 mL), sealed and heated in the microwave to 100°C for 4 h. The reaction mixture was concentrated under reduced pressure and purified via flash-column-chromatography (SiO_2, 30% → 80% EtOAc in pentane) to yield the product (1.52 g, 59%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.00 (d, *J* = 8.5 Hz, 2H), 7.50 (d, *J* = 4.0 Hz, 1H), 7.35 (d, *J* = 7.9 Hz, 2H), 6.65 (bs, 1H), 6.46 (bs, 1H), 3.58 – 3.49 (m, 1H), 2.36 (s, 3H), 2.38 – 2.31 (m, 2H), 2.24 – 2.15 (m, 2H), 2.09 – 1.98 (m, 1H), 1.94 – 1.86 (m, 1H). ^{13}C NMR (126 MHz, methanol-d_4) δ 156.11, 156.05, 152.53, 150.84, 147.89, 147.56, 136.03, 130.91, 129.35, 124.22, 105.45, 104.16, 96.65, 33.26, 30.23, 21.61, 19.45. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 7.51 min; *m/z* : 443 $[M+H]^+$.

Cyclobutylmethyl 4-methylbenzenesulfonate (111)



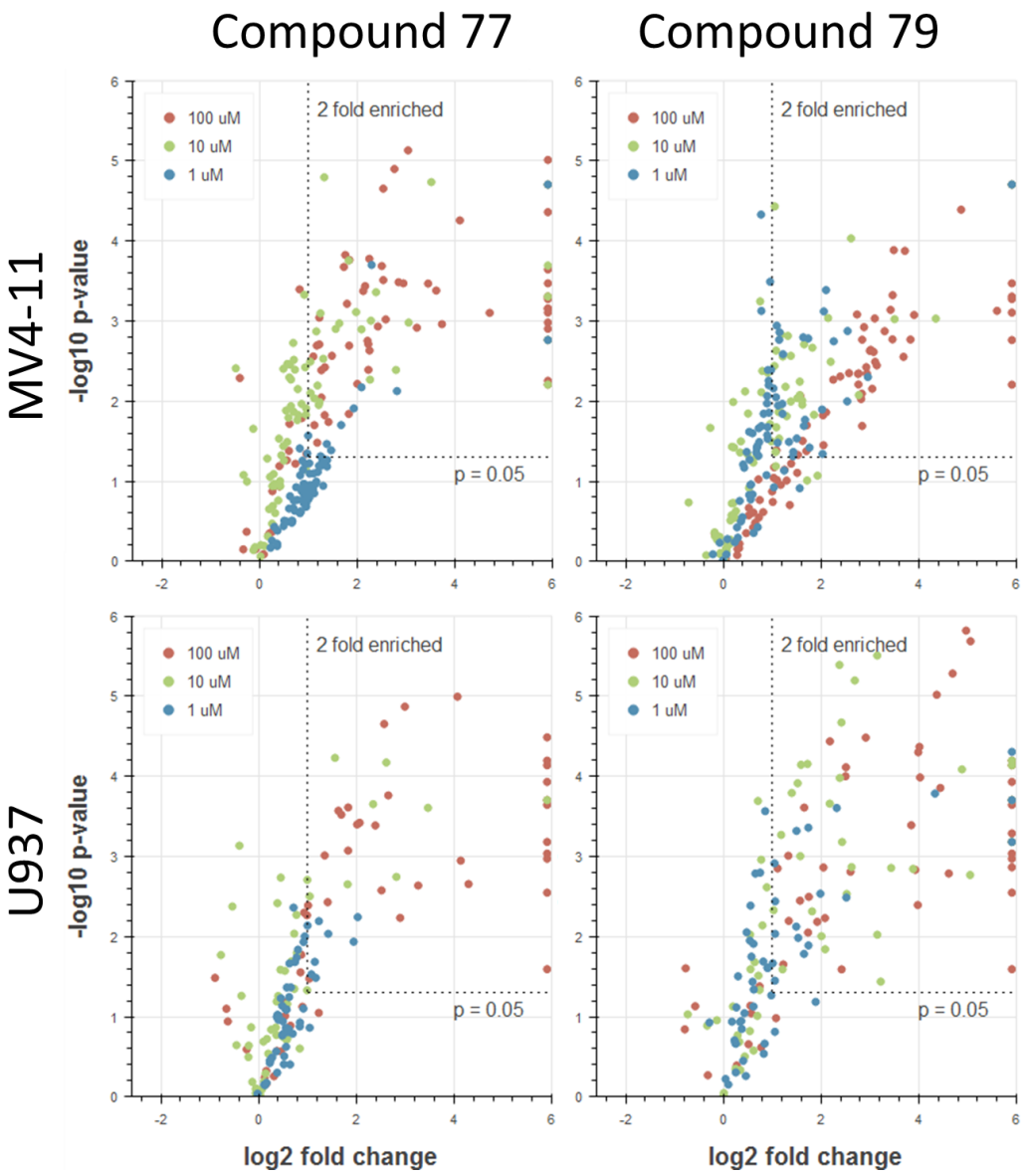
A round-bottom-flask was charged with cyclobutylmethanol (1.92 g, 22.33 mmol, 1.1 eq) dissolved in 20 mL chloroform. After dropwise addition of pyridine (3.53 g, 44.66 mmol, 2.2 mL), a solution of 4-methylbenzenesulfonyl chloride (3.87 g, 20.30 mmol, 1 eq) in 13 mL chloroform was added dropwise and the resulting mixture stirred overnight at RT. The reaction was diluted with 100 mL Et_2O, washed with aqueous HCl (0.1M, 4x40 mL) and with brine (1x100 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The resulting residue was purified via flash-column-chromatography (SiO_2, 2% → 10% EtOAc in pentane) to yield the product (1.96 g, 40%). ^{1}H NMR (300 MHz, chloroform-*d*) δ 7.79 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.7 Hz, 2H), 3.98 (d, *J* = 6.6 Hz, 2H), 2.69 – 2.54 (m, 1H), 2.45 (s, 3H), 2.08 – 1.95 (m, 2H), 1.95 – 1.78 (m, 2H), 1.77 – 1.64 (m, 2H). ^{13}C NMR (75 MHz, chloroform-*d*) δ 144.75, 133.30, 129.91, 127.98, 74.21, 33.96, 24.37, 21.75, 18.25.

2,4-Dichloro-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (112)



A round bottom flask was charged with 2,4-dichloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1k**) (6.21 g, 33.0 mmol, 1 eq), 4-methylbenzenesulfonyl chloride (6.29 g, 33.0 mmol, 1 eq) and tetrabutylammonium hydrogen sulfate (0.56 g, 1.69 mmol, 0.05 eq) suspended in DCM (124 mL). Aqueous NaOH (50%, 6.21 mL) was added and after the mixture was stirred at RT for 90 min, H_2O (120 mL) was added. The phases were separated and the organic aqueous layer was extracted with DCM (4x100 mL). the combined organic layers were washed with brine (1x80 mL), dried (Na_2SO_4), filtered over a SiO_2 plug and concentrated under reduced pressure to yield the product (11.12 g, 98%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.11 (d, *J* = 8.4 Hz, 2H), 7.76 (d, *J* = 4.0 Hz, 1H), 7.37 (d, *J* = 7.8 Hz, 2H), 6.69 (d, *J* = 4.0 Hz, 1H), 2.43 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 153.87, 153.69, 151.66, 146.85, 133.87, 130.17, 128.82, 127.77, 118.56, 102.91, 21.90.

Supplementary Information



SI Figure 2: Competition of **77** and **79** with XO44 in living cells. Volcano plot of the label-free quantification signal from IsoQuant for target kinases, pretreated with three different inhibitor concentrations in two cell lines. To enable plotting of all targets, infinite fold change (XO44 treated divided by competitor treated) was set to 60. A kinase was named a target if there was at least 50% reduction in quantification signal from probe treated samples vs inhibitor pretreated sample.

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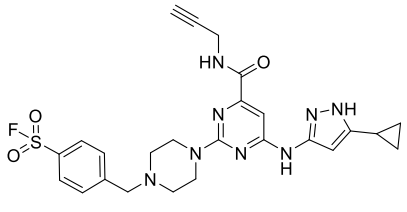
Chapter 6

Summary and future prospects*

Chapter 1 introduces the scope and relevance of the research presented in this thesis. Starting at the question of what cancer is, how it evolves and how driver mutations can be used as a starting point for drug development. AML as well as FLT3 are introduced and current treatment and challenges thereof are outlined. Mapping cellular kinase inhibition profiles of clinical compounds is important to assess their off-target activity, but suitable methodology for this purpose is not available. **Chapter 2** describes the development of a label-free chemical proteomics based kinase selectivity assay. To this end the probe XO44 (Structure shown in Figure 1)[1] was used to investigate kinase inhibitor target landscape in live cells. This assay has been applied to evaluate five clinical FLT3 inhibitors in two cell lines. Many known targets of these inhibitors could be confirmed in a cellular environment, but also new, previously not reported targets could be identified, such as SRC, SLK and STK10 for gilteritinib. While this assay allows the evaluation of cellular selectivity for the first time, there are several opportunities for improvement. High concentrations of reversible kinase inhibitors are generally required to outcompete the covalent probe. When using cell lysate, competitive chemical proteomics experiments with non-covalent inhibitors and a covalent probe can be tightly controlled by varying concentrations and incubation times.[2] This is not as easily done in live cells, where inhibitors and probes have to cross the cell membrane, before they can interact with protein targets.

* The data presented in this chapter was gathered in collaboration with Ruud H. Wijdeven, Laura de Paus, Hugo van Kessel, Eelke B. Lenselink, Gerard J. P. van Westen, Constant A. A. van Boeckel, Herman S. Overkleeft, Jacques Neefjes and Mario van der Stelt.



XO44

Figure 1: XO44, a chemical probe used for the proteomics based cellular selectivity assay used in chapter 2.

The sensitivity of the proteomics experiments can be improved by optimizing the peptide analysis and sample preparation methods. For example, chemical proteomics can be combined with whole genome sequencing. In the currently used bottom-up proteomics workflow, peptides are identified using MS/MS techniques and are further used to identify and quantify the proteins of the original sample. Since canonical proteome sequences are used small, non-functional differences in sequences could lead to substantial errors in identification and quantification of peptides, especially in cancer cells. With the continued decrease in costs in DNA sequencing, it becomes a viable option to combine whole genome sequencing with the chemical proteomics experiments, thereby ensuring that the identification and quantification algorithms work with the exact protein sequences present in the biological sample. Furthermore, potential post-translational modifications (PTMs) of the peptides should also be taken into account. This might be accomplished by simple treatment of a protein digest with broad-spectrum glycosidases, phosphatases and other PTM removing techniques.

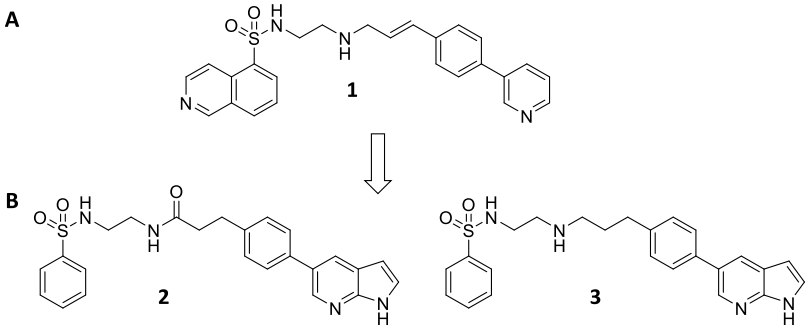


Figure 2: (A) The starting point of the structure-activity relationship study. (B) Two of the most active compounds discovered in the structure-activity relationship study of H-89 analogs as FLT3 inhibitors, described in chapter 3.

Chapter 3 describes the structure-activity relationship (SAR) of a H-89 derived chemical series as inhibitors for FLT3. During this effort several analogs were synthesized and tested against FLT3. Compound **1** was the starting point for this study (Figure 2A). Two of the most potent compounds (**2** and **3**) are shown in Figure 2B. The binding activity of these inhibitors could be substantially improved to picomolar inhibitors. Furthermore, the SAR indicated a surprising switch in binding mode, compared to the originally observed binding pose of H-89 in PKA. For example, the isoquinoline moiety, which interacts with amino acids in the hinge of PKA, was

dispensable for FLT3. Additional cellular assays with compounds **2** and **3** are required to assess their potential as leads for AML treatment. **Chapter 4** describes the search for FLT3 inhibitors with novel chemotypes that are active against drug-resistant FLT3 mutants. To this end a high throughput screen with a library of 231,152 compounds was performed. This led to the identification of 21 compounds, which were extensively profiled in various cellular assays to determine their effect of various FLT3 mutants. Two molecules, (Figure 3) were designated as suitable starting points for a hit optimization program.

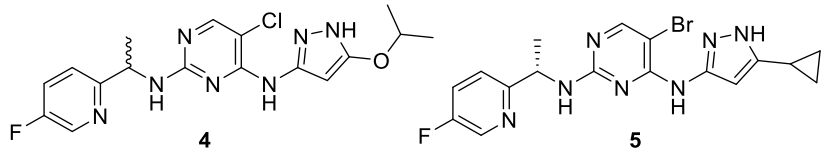


Figure 3: Structures of **4** (SPCE000476_01) and **5** (NP_004099_001), novel FLT3 inhibitors identified by HTS (Chapter 4).

Chapter 5 describes the optimization of compounds **4** and **5**. A number of compounds has been produced and biologically evaluated during this study. This efforts resulted in several cellular active, sub-nanomolar compounds with good molecular weight and lipophilicity. The structures of these compounds are shown in Figure 4 and they were further evaluated for their target profile in live cells using the chemical proteomics approach introduced in chapter 2. Subsequently these compounds were investigated in mice to evaluate their pharmacokinetic properties. Unfortunately, these studies showed that the compounds possessed poor oral bioavailability, which was due to a high intrinsic clearance. This is one of the main issues that need to be addressed for further development of these FLT3 inhibitors. This might be accomplished with either chemical of metabolic studies to identify the resulting metabolites and degradation hotspots. With this information structural changes can be introduced in the molecule to address to improve the chemical and metabolic stability.

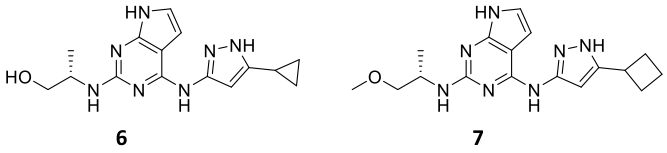


Figure 4: Structure of optimized FLT3 inhibitors (Chapter 5).

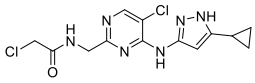
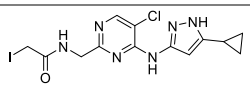
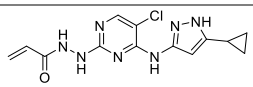
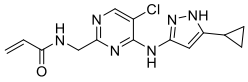
The future impact of small molecule FLT3 inhibitors on AML treatment will be revealed as more inhibitors with divers target profiles advance through clinical trials. The continued emergence of mutated AML cells in patients treated with FLT3 inhibitors also suggests that no single agent may be able to treat them all.[3,4] The optimal treatment will be chosen on the basis of the aberrant signaling pathways present in a patient. Further research is required to define the optimal therapy for each patient.

Another interesting approach is the development of covalent, irreversible inhibitors for FLT3, which may offer the possibility to have an alternative treatment strategy. Several covalent kinase inhibitors, targeting different kinases have been reported.[5] For example, ibrutinib and

acalabrutinib have been developed as covalent inhibitors for Bruton's tyrosine kinase (BTK) and are currently being used for the treatment of chronic lymphoma leukemias.[6–8]

To this end, covalent inhibitors for FLT3-ITD were designed. Structure-based studies suggested that cysteines (C694 and C828), located in the ATP-binding pocket of FLT3, could be targeted by electrophilic warheads introduced on the scaffold of compound **6**.[9] Subsequently, several inhibitors (compound **8** - **11**; Table 1) were synthesized and tested *in vitro*, as well as in the panel of cell lines. The results from this study are summarized in Table 1. Unfortunately, the compounds displayed submicromolar activity against recombinant enzyme and low activity against the cell lines. A potential reason for the low activity might either be a steric clash with the linker of the warhead or, alternatively, the replacement of the amine by an amide is not tolerated. In addition, the substitution of the amino-pyrimidine with the methyl-amino or hydrazine derivative might cause unfavorable electronic changes. Of note, different linkers or targeting C695, located outside the binding pocket, may provide alternative strategies to irreversibly inhibit FLT3.[10]

Table 1: Potential covalent FLT3 inhibitors.

		pIC_{50} ± SEM							
					Ba/F3				
Entry	**Structure**	***in vitro* FLT3**	**MV4-11**	**U937**	**wt**	**FLT3 ITD**	**FLT3 ITD F691L**	**FLT3 ITD D835H**	**FLT3 ITD D835Y**
8		6.39 ± 0.11	5.8 ± 0.2	< 5	< 5	5.4 ± 0.2	5.2 amb.	5.6 ± 0.2	5.6 ± 0.3
9		6.16 ± 0.13	6.5 ± 0.1	5.7 ± 0.2	5.5 ± 0.3	6.1 ± 0.1	6.0 amb.	6.0 ± 0.1	6.0 ± 0.1
10		6.05 ± 0.07	5.5 ± 0.1	ND	< 5	5.3 ± 0.1	< 5	5.2 ± 0.3	5.2 ± 0.1
11		6.58 ± 0.08	5.5 ± 0.1	ND	< 5	5.4 ± 0.2	5.0 ± 0.1	5.4 ± 0.3	5.6 ± 0.1

A novel strategy to indirectly disrupt FLT3 signaling is to inhibit ubiquitin carboxyl-terminal hydrolase 10 (USP10), the enzyme responsible for de-ubiquitination of FLT3. This will result in increased cellular degradation of FLT3 without the issue of the development of resistance conferring mutations in FLT3.[11]

The treatment of acute myeloid leukemia (AML) with mutated fms-like tyrosine kinase 3 (FLT3), especially internal tandem duplications in the juxtamembrane-domain (FLT3-ITD) remains a clinical challenge. The recent approval of midostaurin for FLT3-ITD in combination with cytarabine and daunorubicine benefits patients harboring FLT3 mutations.[12,13] The increase in 4-year overall survival rate from 44% (placebo treatment) to 51% (midostaurin

treatment) is, however, relatively modest. Furthermore, after remission patients are still advised to receive an allogenic bone marrow transplantation as a consolidation treatment.[14–16] AML is a genetically diverse disease with numerous mutations considered relevant for cancer progression (e. g. NPM1, CEBPA, RUNX1).[17–19] This exemplifies that FLT3 is not the only relevant drug target in AML as is also witnessed by the transient or incomplete clinical responses after FLT3-inhibitor monotherapy.[20] Thus, there remains an unmet medical need to discover new, additional therapies for FLT3-ITD AML patients.

In conclusion, the discovery of new FLT3 inhibitors is required to make AML a curable disease, but only in conjunction with other therapies. Determination of the cellular target interaction profile of kinase inhibitors will help to understand their impact on the complex signaling networks within cells. Chemical proteomics-based technologies, as developed in Chapter 2, can contribute to a better understanding of the molecular and cellular mode of action of drug candidates. Finally, novel and highly active molecules to inhibit FLT3 and its relevant mutations, such as described in this thesis, hold promise for future drug development efforts, especially when combined with other chemotherapies.

Experimental

Biochemical Evaluation of FLT3 inhibitors

In a 384-well plate (PerkinElmer 384 Flat White), 5 µL kinase/peptide mix (0.06 ng/µL FLT3 (Life Technologies; PV3182; Lot: 1614759F), 200 nM peptide (PerkinElmer; Lance® Ultra ULightTM TK-peptide; TRFO127-M; Lot: 2178856)) in assay buffer (50 mM HEPES (pH 7.5), 1 mM EGTA, 10 mM $MgCl_2$, 0.01% Tween-20, 2 mM DTT) was dispensed. Separately, inhibitor solutions (10 µM – 0.1 pM) were prepared in assay buffer containing 400 µM ATP and 1% DMSO. 5 µL of these solutions were dispensed and the plate was incubated for 90 minutes in the dark at room temperature. After 90 minutes the reaction was quenched by the addition of 10 µL of 20 mM EDTA containing 4 nM antibody (PerkinElmer; Lance® Eu-W1024-anti-phosphotyrosine(PT66); AD0068; Lot: 2342358). After mixing, samples were incubated for 60 minutes in the dark. The FRET fluorescence was measured on a Tecan Infinite M1000 Pro plate reader (excitation 320 nm, emission donor 615 nm, emission acceptor 665 nm). Data was processed using Microsoft Excel 2016, pIC_{50} values were fitted using GraphPad Prism 7.0. Final assay concentrations during reaction: 200 µM ATP, 0.03 ng/µL FLT3, 100 nM Lance TK-peptide, 0.5% DMSO. Compounds were tested in n=2 and N=2.

In situ testing of kinase inhibitors

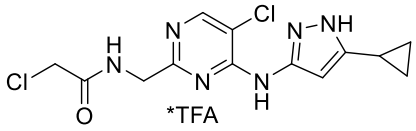
To evaluate inhibitor effect on cell proliferation MV4-11, U937 and Ba/F cell lines were grown in RPMI 10% fetal-bovine-serum. Ba/F cells (wild-type) are grown in the presence of IL-3 (10ng/ml, peprotech). All cells were cultured at 37°C under 5% CO_2. For viability assays, 10000 cells were seeded per well in a 96-wells plate and inhibitors were added at the indicated concentration. Three days later, cell viability was measured using the Cell Titer Blue viability assay (Promega), fluorescence was measured using the Clariostar (BMG Labtech). Relative survival was normalized to the untreated control and corrected for background signal.

Synthetic Procedures

Solvents were purchased from Biosolve, Sigma Aldrich or Fluka and, if necessary dried over 3Å or 4Å molecular sieves. Reagents purchased from chemical suppliers were used without further purification, unless stated otherwise. Oxygen or H_2O sensitive reactions were performed under argon or nitrogen atmosphere and/or under exclusion of H_2O. Microwave reactions were performed in a Biotage initiator+ microwave. Reactions were followed by thin layer chromatography analysis and was performed using TLC silica gel 60 F_{245} on aluminium sheets, supplied by Merck. Compounds were visualized by UV absorption (254 nm) or spray reagent (permanganate (5 g/L $KMnO_4$, 25 g/L K_2CO_3)). TLCMS was measured thin layer chromatography-mass spectrometer (Advion, EppressionL CMS; Advion, Plate Express). ^1H and ^{13}C-NMR spectra were performed on one of the following Bruker spectrometers: DPX 300 NMR spectrometer (300 MHz), equipped with 5mm-BBO-z-gradient-probe; AV-400 NMR spectrometer (400 MHz), equipped with 5mm-BBO-z-gradient-probe; AV-500 NMR spectrometer (500 MHz), equipped with BBFO-z-gradient-probe; AV-600 NMR spectrometer (600 MHz), equipped with 5mm-Cryo-z-gradient probe. NMR spectra were measured in deuterated methanol, chloroform or DMSO and were referenced to the residual protonated solvent signals as internal standards (chloroform-*d* = 7.260 (^1H), 77.160 (^{13}C); methanol-d_4 = 3.310 (^1H), 49.000 (^{13}C); DMSO-d_6 = 2.500 (^1H), 39.520 (^{13}C)). Signals multiplicities are written as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), p (pentet) or m (multiplet). Coupling constants (*J*) are given in Hz. Preparative HPLC (Waters, 515 HPLC pump M; Waters,

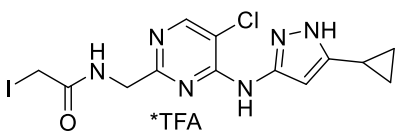
515 HPLC pump L; Waters, 2767 sample manager; Waters SFO System Fluidics Organizer; Waters Acquity Ultra Performance LC, SQ Detector; Waters Binary Gradient Module) was performed on a Phenomenex Gemini column (5 µM C18, 150 x 4.6 mm) or a Waters XBridgeTM column (5 µM C18, 150 x 19 mm). Diode detection was done between 210 and 600 nm. Gradient: ACN in (H_2O + 0.2% TFA). HRMS (Thermo, Finnigan LTQ Orbitrap; Thermo, Finnigan LTQ Pump; Thermo, Finnigan Surveyor MS Pump PLUS Thermo, Finnigan Surveyor Autosampler; NESLAB, Merlin M25). Data acquired through direct injection of 1 mM of the sample in ACN/H_2O/*t*-BuOH (1:1:1), with mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas low 10, capillary temperature 275°C) with resolution R = 60.000 at m/z = 400 (mass range = 150-2000) and dioctylphtalate (m/z = 391.28428) as lock mass. Gradient: ACN in (H_2O + 0.1% TFA). All tested compounds were checked for purity by LCMS liquid chromatography-mass spectrometer, a Thermo (Thermo Finnigan LCQ Advantage Max; Thermo Finnigan Surveyor LC-pump Plus; Thermo Finnigan Surveyor Autosampler Plus; Thermo Finnigan Surveyor PDA Plus Detector; Phenomenex Gemini column (5 µm C18, 50 x 4.6 mm)) system and were determined to be >95% pure by integrating UV intensity recorded.

2-Chloro-*N*-((5-chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)methyl)acetamide (8)



A flask was charged with HOBt (115 mg, 0.85 mmol, 1.13 eq), EDC (173 mg, 0.9 mmol, 1.2 eq) and 2-(aminomethyl)-5-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**14**) (198 mg, 0.75 mmol, 1 eq) suspended in THF (13.5 mL). The mixture was cooled down to 0°C and after addition of chloroacetic acid (82 mg, 0.87 mmol, 1.16 eq) the mixture was allowed to warm up and was stirred overnight at RT and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, dry-loading, 10% (10% of sat. aqueous NH_3 in MeOH) in EtOAc) and preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (209 mg, 61%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.49 (s, 1H), 8.74 (t, *J* = 5.8 Hz, 1H), 8.42 (s, 1H), 6.36 (s, 1H), 4.35 (d, *J* = 5.9 Hz, 2H), 4.17 (s, 2H), 1.95 – 1.84 (m, 1H), 0.97 – 0.86 (m, 2H), 0.77 – 0.67 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.55, 164.20, 155.40, 153.08, 147.15, 145.65, 112.56, 95.05, 44.75, 42.97, 8.18, 7.61. HRMS calculated for $C_{13}H_{15}Cl_2N_6O$ 341.06789 $[M+H]^+$, found 341.0690. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.16 min; *m/z* : 341 $[M+H]^+$.

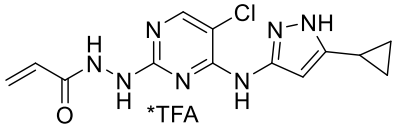
***N*-((5-Chloro-4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)methyl)-2-iodoacetamide (9)**



A flask was charged with HOBt (112 mg, 0.83 mmol, 1.11 eq), EDC (175 mg, 0.91 mmol, 1.22 eq) and 2-(aminomethyl)-5-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**14**) (198 mg, 0.75 mmol, 1 eq) suspended in THF (10 mL). The mixture was cooled down to 0°C and after addition of iodo acetic acid (156 mg, 0.84 mmol, 1.12 eq) the mixture was allowed to warm up and was stirred overnight at RT and concentrated under reduced pressure. The residue was purified via flash-column-chromatography (SiO_2, dry-loading, 10% (10% of sat. aqueous NH_3 in MeOH) in EtOAc) and preparative HPLC (Gemini C_{18}, 20% → 30% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (153 mg, 37%). ^{1}H NMR (500

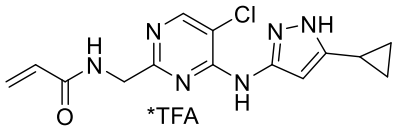
MHz, methanol-d_4) δ 8.32 (s, 1H), 6.38 (s, 1H), 4.45 (s, 2H), 3.83 (s, 2H), 2.01 – 1.90 (m, 1H), 1.05 – 0.97 (m, 2H), 0.85 – 0.72 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 169.96, 163.88, 155.49, 152.87, 148.41, 145.42, 112.77, 93.93, 44.85, 6.87, 6.77, -3.61. HRMS calculated for $C_{13}H_{15}ClIN_6O$ 433.00351 $[M+H]^+$, found 433.0047. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 4.31 min; *m/z* : 433 $[M+H]^+$.

_N_'-(5-Chloro-4-((5-cyclopropyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)acrylohydrazide (10)



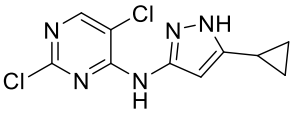
A flask was charged with 5-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-hydrazineylpyrimidin-4-amine (**8**) (80 mg, 0.30 mmol, 1 eq) dissolved in DCM (5 mL) and DMF (2 mL). After the mixture was cooled to 0°C, EDC (51 mg, 0.36 mmol, 1.2 eq) and acrylic acid (30 µL, 0.45 mmol, 1.5 eq) were added and the mixture was allowed to warm up and stirred for 2 h at RT. The reaction was diluted with brine (10 mL) and extracted with EtOAc (3x10 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure to yield the di-substituted product. The residue was re-dissolved in 10% MeOH in H_2O and stirred overnight at RT. The reaction was diluted with DCM (20 mL), $NaHCO_3$ (20 mL) and brine (10 mL) and extracted with DCM (3x20 mL). The combined organic layers were washed with brine (2x20 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by preparative HPLC (Gemini C_{18}, 15% → 25% ACN in H_2O 0.2% TFA, 10 min gradient) to yield the compound as a TFA-salt after lyophilisation (1 mg, 1%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.03 (s, 1H), 6.44 – 6.29 (m, 3H), 5.84 (dd, *J* = 6.7, 5.3 Hz, 1H), 1.95 – 1.85 (m, 1H), 1.04 – 0.93 (m, 2H), 0.82 – 0.69 (m, 2H). HRMS calculated for $C_{13}H_{15}ClN_7O$ 320.10211 $[M+H]^+$, found 320.10218. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.62 min; *m/z* : 320 $[M+H]^+$.

_N_-((5-Chloro-4-((5-cyclopropyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)methyl) acrylamide (11)



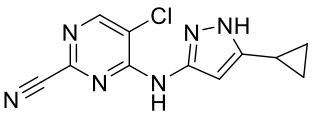
A flask was charged with HOBt (50 mg, 0.372 mmol, 1.5 eq), EDC (65 mg, 0.421 mmol, 1.7 eq), acrylic acid (18 µL, 0.262 mmol, 1 eq) and 2-(aminomethyl)-5-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**7**) (67 mg, 0.252 mmol, 1 eq) suspended in DCM (6 mL). After addition of Et_3N (70 µL, 0.502 mmol, 2 eq) the mixture was stirred overnight, quenched with aqueous HCl (to pH ~6) and extracted with DCM (3x10 mL). the combined organic layers were washed with sat. aqueous $NaHCO_3$ (1x20 mL), brine (2x20 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by preparative HPLC (C_{18}, ACN in 50 mM $NaHCO_3$) to yield the compound after lyophilisation (15 mg, 19%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.62 (s, 1H), 8.93 – 8.84 (m, 1H), 8.64 (s, 1H), 6.43 – 6.30 (m, 2H), 6.23 – 6.12 (m, 1H), 5.74 – 5.62 (m, 1H), 4.48 (d, *J* = 5.9 Hz, 2H), 1.97 – 1.85 (m, 1H), 1.04 – 0.92 (m, 2H), 0.85 – 0.71 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6, 1% TFA) δ 165.45, 163.00, 155.43, 148.65, 148.15, 144.43, 131.25, 126.26, 112.35, 94.66, 42.98, 8.30, 7.11. HRMS calculated for $C_{14}H_{16}ClN_6O_3$ 319.10686 $[M+H]^+$, found 319.10699. LCMS (ESI, C_{18}, linear gradient, 0% → 50% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.81 min; *m/z* : 319 $[M+H]^+$.

2,5-Dichloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (12)



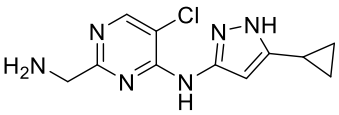
A round-bottom-flask was charged with 2,4,5-trichloropyrimidine (5.00 g, 27.26 mmol, 1 eq) dissolved in EtOH (35 mL). Et_3N (4.18 mL, 29.99 mmol, 1.1 eq) and 5-cyclopropyl-1*H*-pyrazol-3-amine (3.69 g, 29.99 mmol, 1.1 eq) dissolved in EtOH (35 mL) were added dropwise and the reaction mixture was stirred ON at RT until a colorless precipitate was formed. The formed precipitate was filtered off, washed with ice-cold EtOH and dried under reduced pressure to yield the product (7.40 g, quant.). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.37 (s, 1H), 9.70 (s, 1H), 8.32 (s, 1H), 6.19 (s, 1H), 2.03 – 1.80 (m, 1H), 1.04 – 0.87 (m, 2H), 0.81 – 0.60 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.14, 156.97, 155.25, 145.99, 145.64, 113.26, 95.69, 7.92, 6.94. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.66 min; *m/z* : 270 $[M+H]^+$.

5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-hydrazineylpyrimidin-4-amine (13)



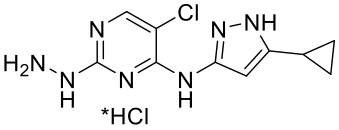
A round-bottom-flask was charged with 2,5-dichloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**12**) (0.40 g, 1.47 mmol, 1 eq), DABCO (32 mg, 0.286 mmol, 0.2 eq) and NaCN (0.105 g, 2.14 mmol, 1.5 eq) dissolved in H_2O (3 mL) and DMSO (6 mL). The mixture was stirred for 5 h at 80°C before being diluted with EtOAc (75 mL) and H_2O (50 mL). The layers were separated and the aqueous layer was extracted with DCM (2x50 mL). The combined organic layers were washed with sat. aqueous $NaHCO_3$ (1x50 mL) and H_2O (1x50 mL), dried (Na_2SO_4), filtered, concentrated under reduced pressure and the ressidue purified via flash-column-chromatography (dry-loading, SiO_2, 40% → 70% EtOAc in pentane) to yield the product (322 mg, 84%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.42 (s, 1H), 6.32 (s, 1H), 1.95 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.04 – 0.97 (m, 2H), 0.79 – 0.74 (m, 2H). ^{13}C NMR (126 MHz, methanol-d_4) δ 157.43, 155.00, 142.92, 118.89, 116.64, 95.99, 8.19, 7.74. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 5.49 min; *m/z* : 261 $[M+H]^+$.

2-(Aminomethyl)-5-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (14)



A flask was charged with 5-chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-hydrazineylpyrimidin-4-amine (**13**) (0.32 g, 1.23 mmol, 1 eq) dissolved in MeOH (12 mL) and AcOH (1.3 mL). After addition of Pd/C (10%) the mixture was degassed and H_2 gas was bubbled through while sonicating for 20 min. The reaction was stirred for another 16 h under H_2 atmosphere. The resulting mixture was filtered over celite, concentrated under reduced pressure and purified via flash-column-chromatography (SiO_2, dry-loading, 10% → 40% (10% of sat. aqueous NH_3 in MeOH) in EtOAc) to yield the product (0.24 g, 71%). ^{1}H NMR (400 MHz, methanol-d_4) δ 8.29 (s, 1H), 6.33 (s, 1H), 3.84 (s, 2H), 1.99 – 1.88 (m, 1H), 1.08 – 0.93 (m, 2H), 0.83 – 0.70 (m, 2H). ^{13}C NMR (101 MHz, methanol-d_4) δ 168.56, 156.92, 154.32, 113.75, 95.26, 47.65, 8.24, 8.08. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 1.01 min; *m/z* : 265 $[M+H]^+$.

5-Chloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)-2-hydrazineylpyrimidin-4-amine (15)



A round-bottom-flask was charged with 2,5-dichloro-*N*-(5-cyclopropyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (**12**) (0.270 g, 1.0 mmol, 1 eq) dissolved in EtOH (5.3 mL). After addition of hydrazine monohydrate (0.152 mL, 3 mmol, 3 eq) the reaction was stirred at RT for 23 h. The formed product precipitated was

collected by filtration as a HCl salt (0.235 g, 78%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.28 (s, 1H), 8.53 (s, 1H), 8.33 (s, 1H), 8.08 (s, 1H), 7.98 (s, 1H), 4.18 (s, 2H), 1.85 (m, 1H), 0.89 (m, 2H), 0.70 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, 1% TFA) δ 158.29, 155.43, 151.46, 147.45, 144.69, 106.06, 94.48, 8.01, 7.38. LCMS (ESI, C_{18}, linear gradient, 10% → 90% ACN in H_2O, 0.1% TFA, 10.5 min): t_R = 0.72 min; *m/z* : 266 $[M+H]^+$.

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List of publications

The Development of a Modular Synthesis of Teraryl-Based α-Helix Mimetics as Potential Inhibitors of Protein–Protein Interactions

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Janssen, A. P. A.; van der Vliet, D.; Bakker, A. T.; Jiang, M.; Grimm, S. H.; Campiani, G.; Butini, S.; van der Stelt, M. *ACS Chem. Biol.* **2018**, *13*, 2406–2413.

Drug Discovery Maps, a Machine Learning Model That Visualizes and Predicts Kinome–Inhibitor Interaction Landscapes

Janssen, A. P. A.; Grimm, S. H.; Wijdeven, R. H. M.; Lenselink, E. B.; Neefjes, J.; van Boeckel, C. A. A.; van Westen, G. J. P.; van der Stelt, M. *J. Chem. Inf. Model.* **2018**, DOI: 10.1021/acs.jcim.8b00640.

Activity-Based Protein Profiling Identifies α-Ketoamides as Inhibitors for Phospholipase A2 Group XVI

Zhou, J.; Mock, E. D.; Martella, A.; Kantae, V.; Di, X.; Burggraaff, L.; Baggelaar, M. P.; Al-Ayed, K.; Bakker, A.; Florea, B. I.; Grimm, S. H.; den Dulk, H.; Li, C. T.; Mulder, L.; Overkleeft, H. S.; Hankemeier, T.; van Westen, G. J. P.; van der Stelt, M. *ACS Chem. Biol.* **2019**, DOI: 10.1021/acschembio.8b00969.

Comprehensive structure-activity-relationship of azaindoles as highly potent FLT3 inhibitors

Grimm, S. H.; Gagestein, B.; Keijzer, J. F.; Liu, N.; Wijdeven, R. H.; Lenselink, E. B.; Tuin, A. W.; van den Nieuwendijk, A. M. C. H.; van Westen, G. J. P.; van Boeckel, C. A. A.; Overkleeft, H. S.; Neefjes, J.; van der Stelt, M. *Bioorg. Med. Chem.* **2019**, *manuscript accepted.*

Curriculum Vitae

Sebastian Hans Grimm was born on June 22nd 1987 in Graz, Austria. He graduated high school in 2005 at the *Bundesgymnasium und Bundesrealgymnasium Gleisdorf* in Austria. From 2005 to 2006 he performed his *Zivildienst* as an emergency medical technician at the Austrian Red Cross. In 2006 he started his Bachelor of Chemistry at the University of Graz during which he spent a semester at the *Universidad de Burgos* in Spain as part of the Erasmus student exchange programme. His undergraduate studies were concluded with an internship at the Graz University of Technology, researching photosensitive resins.

In 2011 he started his Masters in Chemistry at the University of Graz. His studies were completed with an internship at the Graz University of Technology in the research group of prof. dr. Rolf Breinbauer. His Master thesis titled *Synthesis of 5-substitutedpyridine-3-boronic acids as building-blocks for teraryl-based α-Helix mimetics* was completed in 2014.

In the same year he started his PhD research at Leiden University in the Bio-organic synthesis group under the supervision of prof. dr. H. S. Overkleeft. After a year he continued in the newly founded group Molecular Physiology, under the supervision of prof. dr. Mario van der Stelt. His research was further part of the Cancer Drug Discovery Initiative, a collaboration set up in between the Leiden Institute of Chemistry, the Pivot Park Screening Center in Oss and the Netherlands Cancer Institute and was later joined by the Leiden University Medical Centre. He presented part of his research as poster presentations at CHAINS in Veldhoven (2016 and 2017) and as an oral presentation at the SLAS conference Translating Research Ideas into Future Therapeutics in Leuven, Belgium (2016).