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Article

Changing for the Better: Discovery of the Highly Potent and Selective CDK9 Inhibitor VIP152 Suitable for Once Weekly Intravenous Dosing for the Treatment of Cancer

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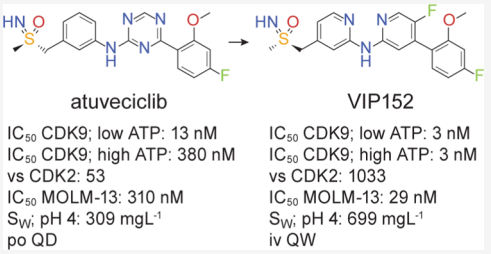
ABSTRACT: Selective inhibition of exclusively transcription-regulating positive transcription elongation factor b/CDK9 is a promising new approach in cancer therapy. Starting from atuveciclib, the first selective CDK9 inhibitor to enter clinical development, lead optimization efforts aimed at identifying intravenously (iv) applicable CDK9 inhibitors with an improved therapeutic index led to the discovery of the highly potent and selective clinical candidate VIP152. The evaluation of various scaffold hops was instrumental in the identification of VIP152, which is characterized by the underexplored benzyl sulfoximine group. VIP152 exhibited the best preclinical overall profile in vitro and in vivo, including high efficacy and good tolerability in xenograft models in mice and rats upon once weekly iv administration. VIP152 has entered clinical trials for the treatment of cancer with promising longterm, durable monotherapy activity in double-hit diffuse large B-cell lymphoma patients.



INTRODUCTION

The cyclin-dependent kinase (CDK) family consists of closely related serine/threonine kinases with specialized functions related to the regulation of the cell cycle (CDKs 1, 2, 4, and 6) or transcription (CDKs 7, 8, 9, 12, 13, and 19).[1−3] Due to their critical role in cell division and gene transcription, CDKs have been considered strong prospective targets for a new generation of anticancer drugs.[4,5] However, while multi-CDK inhibitors such as alvocidib,[6] dinaciclib,[7] or roniciclib[8] revealed promising results in xenograft models, they have displayed limited treatment responses and significant adverse events in clinical trials, with the exception of certain hematological malignancies, which have been attributed to their CDK9 inhibitory activity.[9,10] CDK9 associates with cyclins T1, T2a, and T2b to form the heterodimer positive transcription elongation factor b (PTEFb), which activates transcription via phosphorylation of RNA polymerase II.[11] Overexpression and/or hyperactivity of CDK9 is associated with a variety of human pathological conditions such as cancer, virally induced infectious diseases, and cardiovascular diseases.[12,13] PTEFb-/CDK9-mediated transcription of short-lived antiapoptotic survival proteins and oncogenes such as MCL-1 and MYC plays a critical role in a variety of cancers, especially in several hematological malignancies.[14−16] Selective, transient inhibition of CDK9 leads to a rapid depletion of short-lived messenger RNA (mRNA) transcripts of these important survival proteins and oncogenes, providing a promising rationale for cancer therapy. However, the discovery of selective CDK9 inhibitors is challenging due to the high degree of homology among the kinases of the CDK family. So far, four selective CDK9 inhibitors, atuveciclib (BAY 1143572),[17] AZD4573,[18] KB-0742,[19] and VIP152 (previously BAY 1251152),[20] are reported to have entered clinical trials (Figure 1). We previously disclosed the preclinical profile of atuveciclib, the first selective, orally available CDK9 inhibitor to enter clinical development.[17] In vitro, atuveciclib shows low nanomolar CDK9 inhibitory activity, high selectivity against other CDKs, and antiproliferative activity against various tumor cell lines with submicromolar IC_{50} values. Moreover, atuveciclib demonstrated single-agent in vivo efficacy at tolerated doses in various xenograft tumor models upon oral administration. To fully explore future treatment options using selective CDK9 inhibitors, we initiated a follow-up program to identify novel selective CDK9 inhibitors suitable for intravenous (iv) administration in patients. We now outline the discovery of the highly potent and selective CDK9 inhibitor VIP152, which has entered phase 1 clinical trials in patients.

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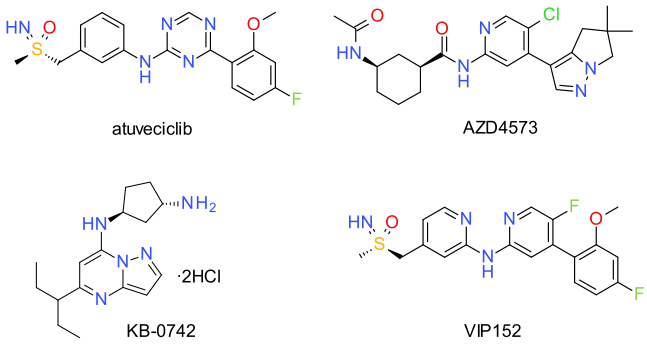


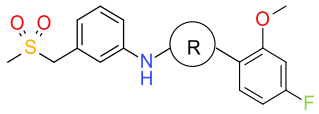
Figure 1. Structures of the clinical CDK9 inhibitors atuveciclib, AZD4573, KB-0742, and VIP152.

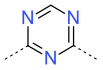
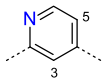
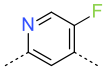
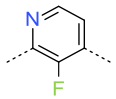
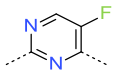
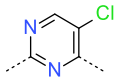
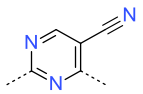
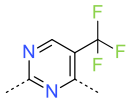
RESULTS AND DISCUSSION

Atuveciclib as a Starting Point for the Identification of the New Lead Structure BAY-332. Atuveciclib demonstrated potent and highly selective CDK9 inhibitor in our biochemical in vitro assay under low adenosine 5′-triphosphate (ATP) conditions (ATP concentration: 10 μM; IC_{50} CDK9/CycT1: 13 nM; ratio of IC_{50} values CDK2/CDK9: 100, see Table 3; and selectivity within the CDK family: >145).[17] However, under high ATP conditions (ATP concentration: 2 mM), which reflect the approximate cellular ATP concentration in humans, a significant drop in CDK9 inhibitory activity, by a factor of ca. 30, was recorded (IC_{50} CDK9/CycT1: 380 nM). Atuveciclib revealed promising antiproliferative activity against a variety of human cancer cell lines, for example, against A2780 (IC_{50}: 380 nM) and MOLM-13 (IC_{50}: 310 nM) cells, and high aqueous solubility (S_w, pH 6.5: 479 mg/L). In in vivo pharmacokinetic (PK) studies in rats, atuveciclib showed low blood clearance (CL_b: 1.1 L/h/kg), a moderate to high volume of distribution (V_{ss}: 1.0 L/kg), and a short half-life ($t_{1/2}$: 0.6 h) (see Table 3). Moreover, atuveciclib demonstrated efficacy in human xenograft tumor models [e.g., models of acute myeloid leukemia (AML) in mice and rats]. The compound is active upon once daily dosing, as well as upon certain intermittent dosing schedules (3 on/2 off). Due to the promising overall profile in vitro and in vivo, atuveciclib entered clinical trials in patients with advanced cancer and leukemia (NCT01938638 and NCT02345382). In a phase 1 study to determine the safety, tolerability, PKs, maximum tolerated dose (MTD), and preliminary antitumor activity in patients with advanced cancer, neutropenia became the most prominent adverse event upon treatment with atuveciclib.[21]

Since inhibition of MCL-1 induces apoptosis in circulating neutrophils and other white blood cell types,[22] permanent on-target inhibition of MCL-1 in circulating white blood cells by daily oral administration of atuveciclib is thought to be the main reason for the observed neutropenia in these patients. To fully explore future treatment options using selective CDK9 inhibitors, we initiated a follow-up program aimed at maximizing the therapeutic index. A highly potent and selective CDK9 inhibitor with a short half-life was envisaged to enable defined periods of acute but sufficient CDK9 inhibition for the desired antitumor effect without reducing the therapeutic window by prolonged transcription inhibition.[23,24] Moreover, switching from oral to iv administration was expected to reduce the variation in plasma levels and exposure often associated with oral administration of small-molecule kinase inhibitors[25] and thus contribute to the desired increase of the therapeutic index.

Table 1. Lead Optimization Efforts at the Hinge Binding Core and the Corresponding Key In Vitro Properties



Compd	R	CDK9/CycT1 low ATP IC_{50} [nM]	Selectivity vs CDK2, ratio of low ATP IC_{50} values	CDK9/CycT1 high ATP IC_{50} [nM]
1		18	61	1070
2		9	30	254
3		4	32	99
4		144	25	>10000
5		3	77	7
6		14	30	227
7		16	38	960
8		359	4	>20000

In preclinical studies, atuveciclib was rated not feasible for iv application in patients. In animal models, the compound revealed only limited efficacy after once weekly iv application. Moreover, the aqueous solubility of atuveciclib is not sufficient to enable the formulation of the corresponding predicted therapeutic human dose of the compound for iv administration in patients. Thus, we set out to identify a new generation of potent and highly selective CDK9 inhibitors suitable for intermittent iv treatment of patients with cancer.

According to docking experiments, binding of atuveciclib to the hinge region of CDK9 is mediated by the triazine core and the aniline NH via two hydrogen bonds (Figure 2). The methoxyphenyl substituent at the triazine points toward the ribose pocket, whereas the benzylic sulfoximine moiety is directed toward the exit of the ATP binding pocket.

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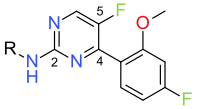
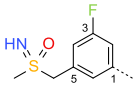
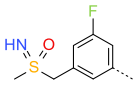
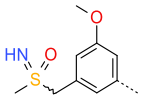
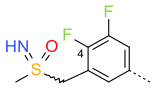
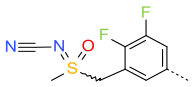
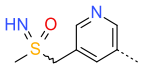
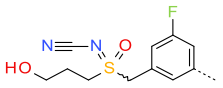
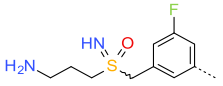
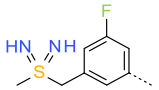
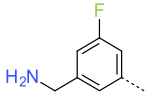
Table 2. Selected Examples of Lead Optimization Efforts at the 2-Position of the 5-Fluoropyrimidine Scaffold and the Corresponding Key In Vitro Properties



Compd	R	CDK9/CycT1 high ATP IC_{50} [nM]	Selectivity vs CDK2, ratio of high ATP IC_{50} values	A2780 IC_{50} [nM][a]	S_w, pH 6.5 [mg/L][b]	CL_h, ratHep [L/h/kg]
BAY-332		37	107	57	24[c]	1.9
9		35	85	64	24[c]	1.7
10		9	424	59	5[c]	2.3
11		23	108	144	n.d.[d]	2.5
12		3	326	37	0.2[e]	3.3
13		11	316	46	103[c]	1.1
14		3	833	262	0.5[c]	3.5
15		34	114	226	294[c]	1.1
16		104	101	184	155[c] 91[e]	2.1
17		84	14	692	17[c]	3.0

[a]Cells were treated with test compounds for 96 h. [b]The solid state of the test compounds was not characterized. [c]Determined by a high-throughput screening method using 1 mM dimethyl sulfoxide (DMSO) stock solutions.[32] [d]n.d.: not determined. [e]Determined by equilibrium shake flask solubility assay from powder.

Sulfoximines, long neglected in drug discovery, have attracted significant interest in medicinal chemistry recently.[26,27] Sulfoximines are aza analogues of sulfones, but their properties differ significantly: the introduction of the nitrogen atom creates asymmetry and offers an additional point for substitution. The nitrogen atom is basic enough to

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Table 3. Properties of the CDK9 Inhibitors Atuveciclib, BAY-332, 25, and VIP152

	atuveciclib	BAY-332	**25**	VIP152
CDK9/CycT1; IC_{50} [nM] low ATP	13	5	4	3
selectivity vs CDK2, ratio of low ATP IC_{50} values	100	54	154	150
CDK9/CycT1; IC_{50} [nM] high ATP	380	37	11	3
selectivity vs CDK2, ratio of high ATP IC_{50} values	53	107	785	1033
A2780; IC_{50} [nM][a]	380	57	126	45
MOLM-13; IC_{50} [nM][a]	310	85	80	29
S_w, pH 6.5 [mg/L][b]	479	24	2	25
S_w, pH 4 [mg/L][b]	309	30	43	699
P_{app} A–B [nm/s]	35	168	26	166
efflux ratio	6.1	0.61	9.3	1.1
CL_b ratHep [L/h/kg]	0.17	1.9	n.d.[c]	0.91
CL_b ratLM [L/h/kg]	0.15	1.6	n.d.[c]	0.82
CL_b rat in vivo, iv [L/h/kg]	1.1	1.3	n.d.[c]	1.1
V_{ss} rat in vivo, iv [L/kg]	1.0	1.6	n.d.[c]	0.74
$t_{1/2}$ rat in vivo, iv [h]	0.6	1.0	n.d.[c]	1.0
blood/plasma ratio (rat)	1.1	0.88	n.d.[c]	0.92
CYP inhibition [μM]	>20	2C8: 9.2, 2C19: 15, all others: >20	n.d.[c]	>20
CYP1A2 induction	no (up to 370 μg/L)	no (up to 41 μg/L)	n.d.[c]	no (up to 14 μg/L)

[a]Cells were treated with test compounds for 96 h. [b]Determined by equilibrium shake flask solubility assay from powder. The solid state of the test compounds was not characterized. [c]n.d.: not determined.

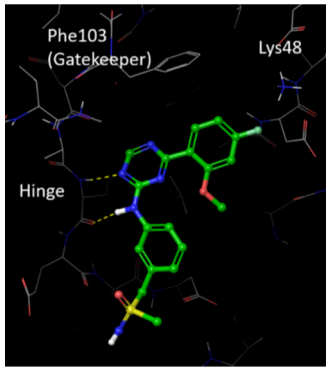

Figure 2. Docking mode of atuveciclib in complex with CDK9. The compound was docked into a published X-ray complex of CDK9/CycT1 (PDB ID: 3MY1). The aminotriazine moiety can form two hydrogen bonds to the hinge region of the kinase. For the substituted phenyl ring attached to the triazine, a π-stacking interaction with Phe103 and a weak hydrogen bond from the *para*-fluoro substituent to Lys48 may be postulated.

allow metal ion coordination or salt formation. The heteroatoms bound to the sulfur are hydrogen bond acceptors, with NH sulfoximines having dual hydrogen bond donor/acceptor functionality. Moreover, sulfoximines have often exhibited high hydrolytic and metabolic stability, as well as favorable physicochemical properties.[27−29] In the lead optimization approach that led to the identification of atuveciclib, we evaluated a broad variety of functional groups at the molecular position directed to the exit of the binding pocket, but the sulfoximine group offered the best overall profile.[17] Thus, we decided to keep the sulfoximine moiety at this position initially and rather started to investigate the structural modifications of the hinge binding core to improve the CDK9 inhibitory potency in vitro while maintaining high kinase selectivity. Since relative to sulfoximines, the synthetically less demanding sulfone analogues in general revealed comparable in vitro activity against CDK9 in the triazine inhibitor series; this evaluation was undertaken on the sulfone series. The sulfone analogue of atuveciclib, triazine **1**, was used as a point of reference (Table 1). Switching from the triazine core to pyridine **2** improved the CDK9 inhibitory activity in vitro under low and high ATP conditions (IC_{50} values: 9 and 254 nM) but also reduced the selectivity against CDK2 (ratio of low ATP IC_{50} values: 30). The selectivity against cell cycle kinase CDK2 was used as a first indicator for the overall selectivity within the family of CDKs. Introduction of a fluorine substituent at the 5-position of the pyridine core (**3**) further improved the activity against CDK9 (IC_{50} values: 4 and 99 nM), while fluorine at the 3-position (**4**) reduced the in vitro activity significantly (IC_{50} values: 144 and >10,000 nM). However, switching from triazine **1** to 5-fluoropyrimidine **5** as the central core substantially improved the CDK9 inhibitory activity under both low and high ATP conditions (IC_{50} values: 3 and 7 nM) while slightly improving the selectivity against CDK2 (ratio of low ATP IC_{50} values: 77). No further improvement of in vitro potency and selectivity against CDK2 was observed upon the introduction of a chloro (**6**), cyano (**7**), or trifluoromethyl (**8**) substituent at the 5-position.

Based on the results from our optimization efforts at the hinge binding core in the sulfone series, the initial lead optimization efforts in the analogous sulfoximine series led to the identification of the new lead compound BAY-332 which is characterized by a 5-fluoropyrimidine scaffold and an additional fluorine atom at the 3-position of the benzylic sulfoximine substituent (see Table 2). In comparison to atuveciclib, BAY-332[30] demonstrated significantly improved in vitro activity against CDK9 in the biochemical assays, especially under high ATP conditions [IC_{50} values: 380 nM (atuveciclib) vs 37 nM (BAY-332), see Tables 2 and 3]. The new lead compound also revealed improved selectivity against CDK2 based on the ratio of IC_{50} values from the corresponding biochemical in vitro assays under high ATP conditions [ratio: 53 (atuveciclib) vs 107 (BAY-332)]. BAY-332 also exhibited improved antiproliferative potency in vitro, for example, against MOLM-13 cells [IC_{50} values: 310 nM (atuveciclib) vs 85 nM (BAY-332)] and A2780 cells [IC_{50} values: 380 nM (atuveciclib) vs 57 nM (BAY-332)]. In vitro

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PK studies with BAY-332 demonstrated moderate metabolic stability in rat hepatocytes (ratHep), resulting in a moderate predicted blood clearance (CL_b) of 1.9 L/h/kg. In contrast to atuveciclib, we recorded a high permeability coefficient (P_{app} A−B) of 168 nm/s (atuveciclib: 35 nm/s) and a low efflux ratio of 0.61 (atuveciclib: 6.1) raising no concerns about a possible efflux transporter recognition of BAY-332. However, aqueous solubility at pH 6.5 of BAY-332 was significantly reduced relative to atuveciclib [S_w: 479 mg/L (atuveciclib) vs 24 mg/L (BAY-332)], presumably mainly driven by the switch from the polar triazine to the 5-fluoropyrimidine core. PK studies with BAY-332 in rats in vivo revealed a moderate blood clearance (CL_b: 1.3 L/h/kg), a high volume of distribution (V_{ss}: 1.6 L/kg), and an intermediate half-life ($t_{1/2}$: 1.0 h). In comparison to BAY-332, its enantiomer (**9**) revealed very similar in vitro properties, well within the limits of measurement accuracy.

In the MOLM-13 human AML model in mice, BAY-332 administered iv either once daily (QD, 15 mg/kg), once every 3 days (Q3D, 35 mg/kg), or once every 5 days (Q5D, 65 mg/kg) demonstrated potent antitumor efficacy with T/C ratios of 0.26 (p = 0.006), 0.26 (p = 0.007), and 0.34 (p = 0.029), respectively (see Figure 3 and Table 4). Additionally, there was good tolerability upon treatment with BAY-332, with a body weight change of less than 10%, but two fatal toxicities upon Q3D and Q5D treatment in these unoptimized dosing schedules. Nevertheless, due to the insufficient solubility properties of BAY-332, formulation of the predicted human dose for once weekly iv treatment of patients was rated not feasible.

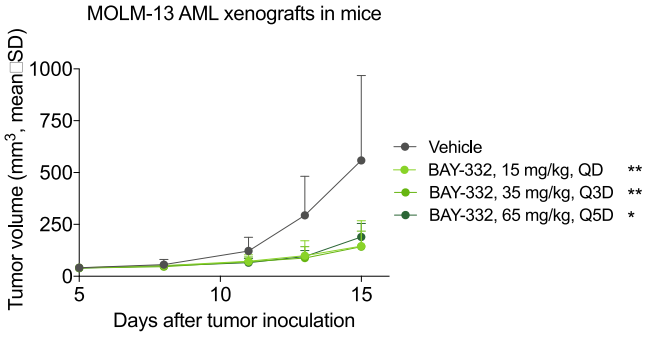

Figure 3. Antitumor efficacy of BAY-332 in the MOLM-13 human AML model in mice. Tumor growth in mice treated iv with either vehicle or BAY-332 once daily (QD), once every 3 days (Q3D), or once every 5 days (Q5D). Treatments were started 5 days after tumor cell inoculation. Statistical significances were calculated using the mean tumor volumes at the time point when the vehicle group was sacrificed. Asterisks indicate statistical significance relative to the vehicle control (*p < 0.05 and **p < 0.01).

Lead Optimization in the 5-Fluoropyrimidine Series. To enable formulation of a new generation of CDK9 inhibitors for intermittent iv administration in patients, we set out to further optimize the biochemical and cellular in vitro potency to reduce the required therapeutic dose. Complementarily, we also aimed to improve the solubility properties. In the 5-fluoropyrimidine series, we focused on structural variation of the substituent at the 2-position, which is directed to the exit of the ATP binding pocket. The introduction of polar substituents at this position to increase aqueous solubility was envisaged to be more feasible than for the aromatic substituent at the 4-position pointed toward the ribose pocket. Moreover, in the lead optimization approach to atuveciclib, the structural variation of the ribose pocket substituent had revealed a steep structure−activity relationship with respect to selectivity against CDK2 and in vitro potency.[17] Table 2 presents selected examples of our extensive lead optimization efforts at the 2-position of the pyrimidine scaffold.[31] Various structural alternatives for the fluorine at the phenyl 3-position were feasible with respect to in vitro potency. Exchange of the fluorine atom for a methoxy substituent (**10**), for instance, increased the CDK9 inhibitory activity and selectivity against CDK2, though reduced solubility. Introduction of a second fluorine atom at the 4-position (**11**) did not impact in vitro potency significantly but increased metabolic instability in rat hepatocytes. Use of an *N*-cyano sulfoximine group in combination with the additional fluorine at the 4-position of the phenyl substituent (**12**) improved the biochemical and cellular activity significantly but further reduced the metabolic stability. Moreover, the CDK9 inhibitor **12** revealed very low aqueous solubility. Introduction of an additional nitrogen atom into the aromatic substituent at the 2-position was promising with respect to the CDK9 inhibitory activity, selectivity against CDK2, and aqueous solubility, but pyridine **13** revealed low permeability (P_{app} A−B: 23 nm/s) and a high efflux ratio (>10) in the bidirectional Caco-2 permeability assay, raising concerns about transporter recognition. Introduction of a polar hydroxy group into the alkyl substituent of the *N*-cyano

Table 4. Antitumor Efficacy of BAY-332 and VIP152 in the MOLM-13 Human AML Model in Nude Mice and Athymic Rats

animal model	treatment	dose (mg/kg)	schedule	T/C_{volume} [a]	fatal toxicity	maximum body weight loss [b] (%)
MOLM-13 AML xenografts in mice	vehicle	0	QD	1.00	0/12	2.8
	BAY-332	15	QD	0.26**	0/12	4.5
	BAY-332	35	Q3D	0.26**	2/12	5.8
	BAY-332	65	Q5D	0.34*	2/12	4.8
MOLM-13 AML xenografts in rats	vehicle	0	Q3D	1.00	0/12	0.0
	VIP152	3	Q3D	0.11***	0/12	0.0
	VIP152	3.75	Q5D	0.08***	0/12	0.0
	VIP152	4.5	QW	0.15***	0/12	0.0
MOLM-13 AML xenografts in mice	vehicle	0	QD	1.00	0/12	0.1
	VIP152	12.5	QW	0.20**	0/12	4.9

[a]Treatment-to-control (T/C) ratios and statistical significances were calculated using the mean tumor volumes at the time point when the vehicle group was sacrificed. Asterisks indicate the statistical significance relative to the vehicle control (*p < 0.05, **p < 0.01, and ***p < 0.001). [b]The maximum mean body weight loss expressed as a percentage of the starting weight of the animal. Weight loss greater than 20% is considered toxic.

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sulfoximine group (**14**) led to significant improvement of CDK9 inhibitory activity and selectivity against CDK2 over BAY-332. On the other hand, hydroxy derivative **14** demonstrated only moderate antiproliferative activity against A2780 cells in vitro, low aqueous solubility, and low metabolic stability. Introduction of an NH_2 group into the alkyl chain in combination with an NH sulfoximine group (**15**) improved solubility significantly (S_w, pH 6.5: 294 mg/L); however, amine **15** revealed no permeability (P_{app} A−B: 0 nm/s) through the Caco-2 cell monolayer. The aza analogue of sulfoximine BAY-332, sulfondiimine **16**, was prepared with the hope for similar potency and selectivity but further improved aqueous solubility. Moreover, no chiral separation of enantiomers is required in the case of achiral sulfondiimine **16**. In comparison to BAY-332, matched analogue **16** revealed reduced in vitro activity against CDK9 (IC_{50}: 104 nM), as well as reduced antiproliferative activity against A2780 cells (IC_{50}: 184 nM), yet similar selectivity against CDK2 (ratio of high ATP IC_{50} values: 101) and significantly improved aqueous solubility at pH 6.5 (S_w: 155 mg/L). In the Caco-2 assay, sulfondiimine **16** demonstrated a reduced permeability coefficient [P_{app} A−B: 168 nm/s (BAY-332) vs 50 nm/s (**16**)] and an increased efflux ratio [0.61 (BAY-332) vs 5.6 (**16**)]. Moreover, sulfoximine BAY-332 and matched sulfondiimine **16** displayed comparable metabolic stability in rat hepatocytes in vitro. In a subsequent rat PK study in vivo, an increased volume of distribution (V_{ss}: 2.2 L/kg) and significantly increased blood clearance (CL_b: 3.3 L/h/kg) as well as a short half-life ($t_{1/2}$: 0.6 h) of analogue **16** relative to BAY-332 were recorded. Evaluation of non-sulfur-based functional groups at the exit of the ATP binding pocket did not lead to the identification of a compound with a suitable overall profile, similar to the findings in the lead optimization approach that led to atuveciclib.[17] Benzylamine **17**, for instance, demonstrated reduced in vitro activity in the biochemical and cellular assays compared to BAY-332, as well as low metabolic stability and no improvement of aqueous solubility.

Additional Scaffold Hops Leading to the Clinical Candidate. Since our lead optimization efforts to identify a compound with a superior preclinical overall profile to lead compound BAY-332 proved fruitless in the 5-fluoropyrimidine series, we turned our attention again to the evaluation of additional scaffold modifications. However, this time we also incorporated the neighboring aromatic substituent at the NH position in our design. The idea was to evaluate a shift of the nitrogen of the 5-fluoropyrimidine scaffold, which is not involved in hydrogen bonding to the hinge region of the ATP binding pocket, into the adjacent aromatic ring to yield *N*-(pyridin-2-yl)pyridin-2-amine **31** (Figure 4 and Scheme 2). In the triazine series, the same strategy leads to *N*-(pyridin-2-yl)pyrimidin-4-amine **24** (Scheme 1).

After a successful synthesis (see Scheme 1), the enantiomers of *N*-(pyridin-2-yl)pyrimidin-4-amine **24** exhibited very similar in vitro properties, much the same as the results previously recorded with the corresponding enantiomers in the triazine and 5-fluoropyrimidine series. The slightly more potent enantiomer **25** revealed significantly improved CDK9 inhibitory activity (IC_{50}: 11 nM) and selectivity against CDK2 under high ATP conditions (ratio of IC_{50} values: 785) in comparison to lead structure BAY-332 (IC_{50}: 37 nM and ratio of IC_{50} values: 107; Table 3). However, no increased antiproliferative activity against A2780 cells [IC_{50} values: 126 nM (**25**) vs 57 nM (BAY-332)] and MOLM-13 cells [IC_{50} values: 80 nM (**25**) vs 85 nM (BAY-332)] was recorded. Moreover, 4,6-disubstituted pyrimidine **25** displayed reduced aqueous solubility at pH 6.5 [S_w: 2 mg/L (**25**) vs 24 mg/L (BAY-332)], as well as low permeability (P_{app} A−B: 26 nm/s) and a high efflux ratio (9.3), which raised concern with respect to a potential transporter recognition of **25**. In contrast, in the corresponding *N*-(pyridin-2-yl)pyridin-2-amine series, single enantiomer VIP152 revealed a very promising preclinical overall profile.

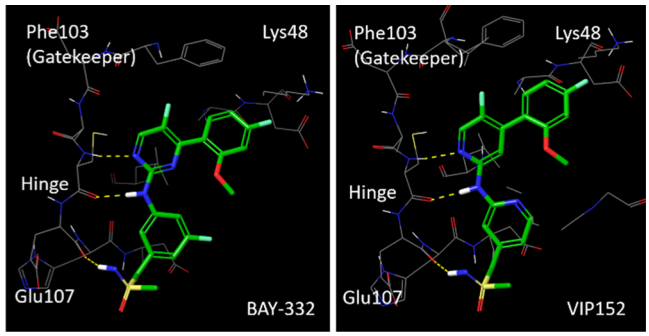

Figure 4. Docking modes of the 5-fluoropyrimidine BAY-332 and the *N*-(pyridin-2-yl)pyridin-2-amine VIP152 in complex with CDK9. The compounds were docked into a published X-ray complex of CDK9/CycT1 (PDB ID: 3MY1). BAY-332: the aminopyrimidine moiety can form two hydrogen bonds to the hinge region of the kinase. An additional hydrogen bond is formed by the sulfoximine NH to the backbone carbonyl group of Glu107. For the substituted phenyl ring attached to the pyrimidine, a π-stacking interaction with Phe103 and a weak hydrogen bond from the *para*-fluoro substituent to Lys48 may be postulated. VIP152: the central aminopyridine moiety can form two hydrogen bonds to the hinge region of the kinase. An additional hydrogen bond is formed by the sulfoximine NH to the backbone carbonyl group of Glu107. For the substituted phenyl ring attached to the central pyridine, a π-stacking interaction with Phe103 and a weak hydrogen bond from the *para*-fluoro substituent to Lys48 may be postulated.

Preclinical Properties of VIP152. *N*-(Pyridin-2-yl)-pyridin-2-amine VIP152 (see Scheme 2 and Table 3) revealed very potent CDK9 inhibitory activity (IC_{50}: 3 nM) and high selectivity against the structurally related kinases CDK2 (ratio of IC_{50} values: 1033) and CDK7 (ratio of IC_{50} values: >5000) in the Bayer kinase panel under high ATP conditions. The high kinase selectivity of VIP152 in vitro, even within the CDK family, was confirmed in the external kinase panels with a selectivity factor of at least 50 against all tested CDKs (see the Supporting Information). Outside the CDK family, substantial inhibitory activity with a selectivity factor of less than 50 was only recorded against GSK3α and IRAK1 (DiscoverX K_D values: CDK9, 1.3 nM; GSK3α, 7.4 nM; and IRAK1, 61 nM) (see the Supporting Information). In a recent comprehensive survey of CDK inhibitor selectivity in live cells with energy-transfer probes, VIP152 demonstrated the strongest target affinity and selectivity among the tested CDK9 inhibitors.[33] VIP152 exhibited very potent antiproliferative activity in vitro, especially against A2780 cells (IC_{50}: 45 nM) and MOLM-13 cells (IC_{50}: 29 nM). In comparison to VIP152, its enantiomer **32** revealed very similar in vitro properties, well within the limits of measurement accuracy. However, with multiple batches of enantiomer **32**, there was a trend toward a slightly reduced activity against CDK9 in the biochemical assay under high ATP conditions (IC_{50}: 6 nM) and antiproliferative

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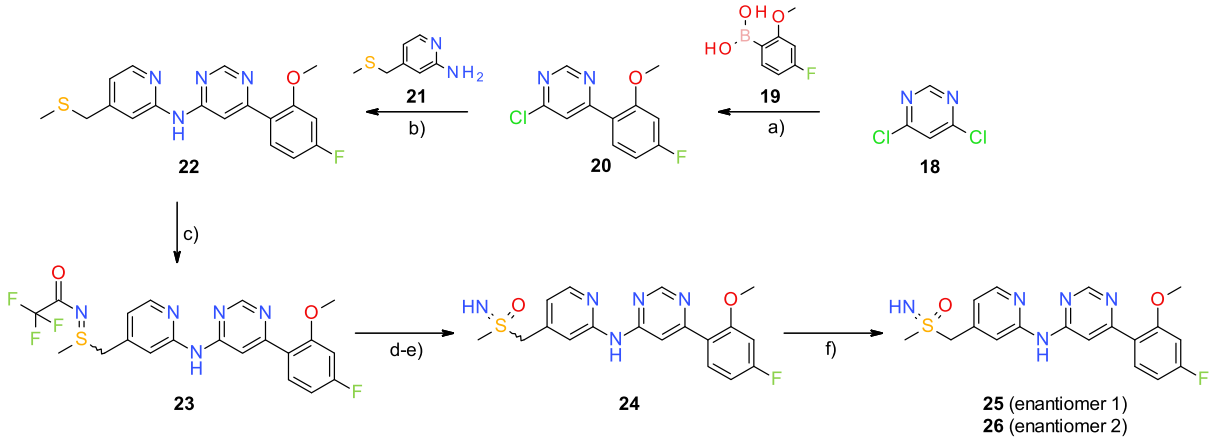
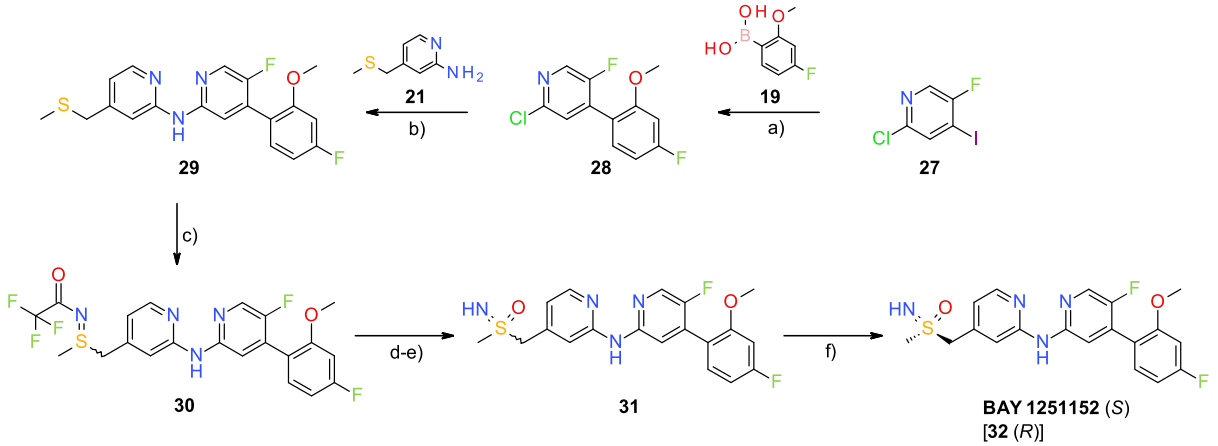
Scheme 1. Synthesis of *N*-(Pyridin-2-yl)pyrimidin-4-amines[a]



[a]Reagents and conditions: (a) $Pd(dppf)Cl_2$, K_2CO_3 (aq), 1,2-dimethoxyethane (DME), 90 °C, 150 min, and 79%; (b) Xantphos, $Pd_2(dba)_3$, Cs_2CO_3, 1,4-dioxane, 100 °C, 22 h, and 60%; (c) $CF_3C(O)NH_2$, NaO*t*-Bu, 1,3-dibromo-5,5-dimethylhydantoin, 1,4-dioxane, tetrahydrofuran (THF), 10 °C, and 63%; (d) $KMnO_4$, acetone, 50 °C, and crude; (e) K_2CO_3, MeOH, room temperature (rt), and 15% (over two steps); and (f) chiral preparative HPLC.

Scheme 2. Synthesis of VIP152[a]



[a]Reagents and conditions: (a) $Pd(PPh_3)_4$, K_2CO_3 (aq), DME, 100 °C, 4 h, and 95%; (b) Xantphos, $Pd_2(dba)_3$, Cs_2CO_3, 1,4-dioxane, 100 °C, 5 h, and 94%; (c) $CF_3C(O)NH_2$, NaO*t*-Bu, 1,3-dibromo-5,5-dimethylhydantoin, 1,4-dioxane, THF, 10 °C, and crude; (d) $KMnO_4$, acetone, 50 °C, and crude; (e) K_2CO_3, MeOH, rt, and 10% (over three steps); and (f) chiral preparative HPLC.

activity against A2780 cells (IC_{50}: 52 nM) and MOLM-13 cells (IC_{50}: 42 nM). VIP152 demonstrated low aqueous solubility at pH 6.5 (S_w: 25 mg/L), but in contrast to atuveciclib and BAY-332, *N*-(pyridin-2-yl)pyridin-2-amine VIP152 exhibited a significant increase in solubility at pH 4 (S_w: 699 mg/L), which is still within the physiologically acceptable range for iv application in humans.[34] Nevertheless, VIP152 exhibited high Caco-2 permeability (P_{app} A−B: 166 nm/s) and no efflux (efflux ratio: 1.1) in the Caco-2 assay.

In an in vivo PK study in rats (Table 3), VIP152 showed low blood clearance (CL_b: 1.1 L/h/kg), a moderate volume of distribution (V_{ss}: 0.74 L/kg), and a short to moderate half-life ($t_{1/2}$: 1.0 h). Furthermore, VIP152 exhibited a blood/plasma ratio of about 1 and did not show significant inhibition of cytochrome P450 activity, with IC_{50} values >20 μM. Induction of CYP1A2 in vitro was not observed.

The MOLM-13 xenograft model in rats was used to determine the optimum application frequency regarding the antitumor activity of VIP152. VIP152 administered iv at its respective MTD Q3D (3 mg/kg), Q5D (3.75 mg/kg), or QW (4.5 mg/kg) demonstrated similar potent antitumor efficacies with T/C ratios of 0.11, 0.08, and 0.15, respectively, and excellent tolerability (all $p < 0.001$; Figure 5A and Table 4). Antitumor activity upon once weekly iv dosing was then confirmed in a second species using the MOLM-13 xenograft model in mice. Once weekly (QW) administration of VIP152 at 12.5 mg/kg resulted in a T/C ratio of 0.20 ($p = 0.001$; Figure 5B and Table 4) and was well tolerated, as indicated by no significant body weight reduction (body weight change: −5%) and no fatal toxicities throughout the study.

The MV4-11 xenograft model in rats was used to compare the activity of daily oral administration of atuveciclib (12 mg/kg × 15 days) compared with once weekly iv dosing of VIP152 (4.5 mg/kg, Q7D × 3). Treatment with VIP152 leads to complete remission in all animals with no measurable tumor burden for an additional 30 days without treatment compared with regrowth in all of the atuveciclib-treated animals.

Since the once weekly application maybe the best regimen to allow for recovery of the treatment-induced acute reduction of circulating neutrophil levels, this application schedule was

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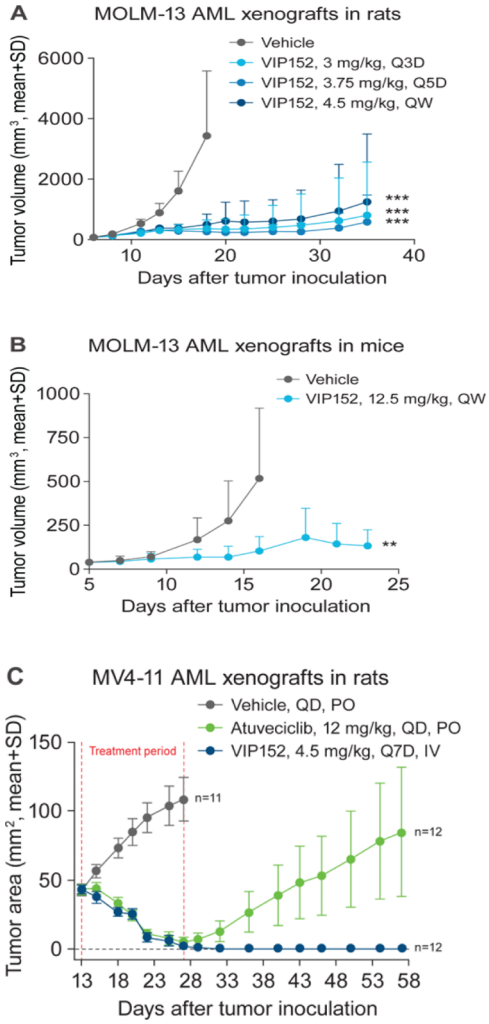

Figure 5. Antitumor efficacy of atuveciclib and VIP152 in human AML models in rats and mice. (A) Tumor growth in rats treated iv with either vehicle or VIP152 once every 3 days (Q3D), once every 5 days (Q5D), or once weekly (QW). Treatments were started 6 days after tumor cell inoculation. (B) Tumor growth in mice treated iv with either vehicle or VIP152 once weekly (QW). Treatments were started 5 days after tumor cell inoculation. (C) Tumor growth in rats treated with atuveciclib p.o. daily (15 days) dosing or VIP152 iv once weekly (3 doses). Statistical significances were calculated using the mean tumor volumes at the time point when the vehicle group was sacrificed. Asterisks indicate statistical significance relative to the vehicle control (**$p < 0.01$ and ***$p < 0.001$).

defined as the target regimen for the clinical development of VIP152.

Due to the very promising overall profile in vitro and in vivo, sulfoximine VIP152 was selected as the development candidate and entered phase 1 clinical trials in patients (NCT02635672 and NCT02745743).

Chemistry. The synthesis of racemic *N*-(pyridin-2-yl)-pyrimidin-4-amine **24** started with the reaction of 4,6-dichloropyrimidine (**18**) and boronic acid **19** under Suzuki conditions to afford building block **20**. A palladium-catalyzed cross-coupling reaction of chloropyrimidine **20** and commercial pyridin-2-amine **21** yielded 4,6-disubstituted pyrimidine **22**. The thioether was first converted into the racemic sulfilimine **23** using the conditions of Gries and Krüger.[35] Oxidation and cleavage of the trifluoroacetyl group afforded the desired sulfoximine **24**, which was separated into the enantiomers by chiral high-performance liquid chromatography (HPLC). The absolute configuration of the single stereoisomers **25** and **26** at the sulfur was not determined.

The research synthesis of *N*-(pyridin-2-yl)pyridin-2-amine **31** was accomplished with a similar synthetic strategy. Suzuki coupling of 2-chloro-5-fluoro-4-iodopyridine (**27**) and boronic acid **19** afforded the desired building block **28** in very high yield. A subsequent palladium-catalyzed cross-coupling reaction of chloropyridine **28** and commercial pyridin-2-amine **21** resulted in 2,2′-dipyridylamine **29** in very high yield, too. Finally, thioether **29** was converted into the racemic sulfoximine **31** relying again on the conditions of Gries and Krüger.[35] Chiral HPLC separation of racemate **31** yielded VIP152 and its enantiomer **32**. The stereochemistry at the sulfur of the sulfoximine group of VIP152 was determined by X-ray crystallography (Figure 6).

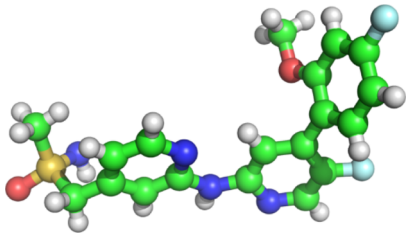

Figure 6. Small-molecule X-ray structure of VIP152 (accession code CCDC 2005811).

CONCLUSIONS

Transient modulation of CDK9 by highly selective inhibitors leads to rapid depletion of short-lived mRNA transcripts of important survival proteins and oncogenes such as MCL-1 and MYC,[36] providing a promising rationale for cancer therapy, especially for hematological malignancies. Atuveciclib (BAY 1143572), the first selective, orally available CDK9 inhibitor that entered clinical development, revealed promising results in xenograft models. In a phase 1 clinical trial in patients with advanced cancer, atuveciclib displayed a limited therapeutic index with oral administration with neutropenia being the most prominent adverse event putatively mediated, at least in part, by the on-target inhibition of MCL-1 in circulating white blood cells leading to apoptosis induction in neutrophils. With the aim of improving the therapeutic index of anticancer therapy with CDK9 inhibitors, a follow-up program was initiated to identify a new generation of highly potent and selective CDK9 inhibitors enabling once weekly iv treatment of patients by a more pronounce "oncogenic shock"-like[24] disruption of oncogene transcription, followed by a time without target inhibition needed for the recovery of circulating neutrophils. Our lead optimization efforts started with atuveciclib focused on potency, selectivity, and aqueous solubility which led to the identification of a new generation of CDK9 inhibitors. Sulfoximine containing VIP152 clearly exhibited the most promising preclinical overall profile with respect to potency, selectivity, physicochemical properties, and in vivo PK, as well as in vivo potency. VIP152 is efficacious in human xenograft tumor models of AML in mice and rats upon once weekly iv administration with good tolerability. The evaluation of various scaffold hops and the employment of the underexplored benzyl sulfoximine group were crucial for overcoming hurdles in this

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project, which highlights the ongoing important role of medicinal chemistry in small-molecule drug discovery.

The discovery of VIP152, in optimizing atuveciclib, demonstrates promising translation of our efforts in early clinical studies.[37] The improved selectivity on CDK9 starting from removal of the CDK2 activity, ATP independence, and a short-lived, iv strategy resulted in a promising 38% disease control rate in the first 31 patients in the dose escalation phase of VIP152's first-in-human study. Several patients experienced extended benefit beyond 15 cycles including two patients with double-hit diffuse large B-cell lymphoma (manuscript in prep).[37,38] This early clinical experience demonstrates that VIP152 is well tolerated with a manageable safety profile for extended periods and opens the door to test the oncogenic shock hypothesis with a once weekly dosing schedule in patients with cancer.

EXPERIMENTAL SECTION

General Methods and Materials. Commercially available reagents and anhydrous solvents were used as supplied, without further purification. All air- and moisture-sensitive reactions were carried out in oven-dried (at 120 °C) glassware under an inert atmosphere of argon. A Biotage Initiator Classic microwave reactor was used for reactions conducted in a microwave oven. Reactions were monitored by thin-layer chromatography (TLC) and ultra-performance liquid chromatography (UPLC) analysis with a Waters Acquity UPLC MS Single Quad system; column: Acquity UPLC BEH C18 1.7 μm, 50 × 2.1 mm; basic conditions: eluent A: H_2O + 0.2 vol % aq NH_3 (32%), eluent B: MeCN; gradient: 0−1.6 min 1−99% B, 1.6−2.0 min 99% B; flow: 0.8 mL/min; acidic conditions: eluent A: H_2O + 0.1 vol % formic acid (99%), eluent B: MeCN; gradient: 0−1.6 min 1−99% B, 1.6−2.0 min 99% B; flow: 0.8 mL/min; temperature: 60 °C; and DAD scan: 210−400 nm. Analytical TLC was carried out on aluminum-backed plates coated with a Merck Kieselgel 60 F254, with visualization under UV light at 254 nm. Flash chromatography was carried out using a Biotage Isolera One system with a 200−400 nm variable detector. Preparative HPLC was carried out with a Waters AutoPurification MS Single Quad system; column: Waters XBridge C18 5 μm, 100 × 30 mm; basic conditions: eluent A: H_2O + 0.2 vol % aq NH_3 (32%), eluent B: MeCN; gradient: 0−0.5 min 5% B, flow: 25 mL/min; 0.51−5.50 min 10−100% B, flow: 70 mL/min; 5.51−6.5 min 100% B, flow: 70 mL/min; acidic conditions: eluent A: H_2O + 0.1 vol % formic acid (99%), eluent B: MeCN; gradient: 0−0.5 min 5% B, flow: 25 mL/min; 0.51−5.50 min 10−100% B, flow: 70 mL/min; 5.51−6.5 min 100% B, flow: 70 mL/min; temperature: 25 °C; and DAD scan: 210−400 nm. NMR spectra were recorded at rt (22 ± 1 °C), unless otherwise noted, on Bruker Avance III HD spectrometers. ^{1}H NMR spectra were obtained at 300, 400, 500, or 600 MHz and referenced to the residual solvent signal (7.26 ppm for $CDCl_3$ and 2.50 ppm for DMSO-d_6). ^{13}C NMR spectra were obtained at 100 or 150 MHz and also referenced to the residual solvent signal (39.52 ppm for DMSO-d_6). ^{1}H NMR data are reported as follows: chemical shift (δ) in ppm, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, br = broad, and m = multiplet), coupling constant(s), and integration. High-resolution mass spectra were recorded on a Xevo G2-XS Tof (Waters) instrument. Low-resolution mass spectra (electrospray ionization, ESI) were obtained via HPLC−MS (ESI) using a Waters Acquity UPLC system equipped with an SQ 3100 Mass Detector; column: Acquity UPLC BEH C18 1.7 μm, 50 × 2.1 mm; eluent A: H_2O + 0.05% formic acid (99%), eluent B: MeCN + 0.05% formic acid (99%); gradient: 0−0.5 min 5% B, 0.5−2.5 min 5−100% B, 2.5−4.5 min 100% B; total run time: 5 min; and flow: 0.5 mL/min. Optical rotations were recorded on a JASCO P-2000 polarimeter. IR spectra were obtained on a Bruker Vertex 70 FTIR spectrometer (2 cm^{-1} resolution, 32 scans, and 400−4000 cm^{-1} range) using single-bounce diamond attenuated total reflection (ATR) or KBr techniques. The purity of all target compounds was at least 95%, as determined by LC−MS (for a detailed description of the LC−MS methods, see the Supporting Information). Compound names were generated using ICS software.

Synthetic Procedures. *Compound* ***1****. 1-[(Methylsulfanyl)-methyl]-3-nitrobenzene.* Sodium methanethiolate (13.5 g, 192 mmol) was added in two portions to a stirred solution of 1-(chloromethyl)-3-nitrobenzene (30.0 g, 175 mmol) in EtOH (360 mL) at −15 °C. The cold bath was removed, and the mixture was stirred at rt for 3 h. Then, it was diluted with brine and extracted with EtOAc (2×). The combined organic phases were washed with H_2O, dried (Na_2SO_4), filtered, and concentrated to afford the desired product (32.2 g) that was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (m, 1H), 8.11 (m, 1H), 7.66 (m, 1H), 7.50 (m, 1H), 3.75 (s, 2H), 2.01 (s, 3H).

1-[(Methylsulfonyl)methyl]-3-nitrobenzene. *m*-Chloroperoxybenzoic acid (77%; 26.9 g, 120 mmol) was added to a stirred solution of 1-[(methylsulfanyl)methyl]-3-nitrobenzene (10.0 g) in dichloromethane (DCM, 1305 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and then at rt for 2.5 h. Then, it was diluted with H_2O (300 mL) and $NaHCO_3$ (11.0 g) was added. The mixture was extracted with DCM (2×). The combined organic phases were filtered using a Whatman filter and concentrated. The residue was purified by flash chromatography (DCM/EtOH 95:5) and finally recrystallized from EtOAc to afford the desired product (6.2 g, 28.9 mmol, and 53% yield over two steps). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.28 (m, 1H), 8.22 (m, 1H), 7.83 (m, 1H), 7.69 (m, 1H), 4.68 (s, 2H), 2.93 (s, 3H).

3-[(Methylsulfonyl)methyl]aniline. $TiCl_3$ solution (ca. 15% in ca. 10% HCl, 162 mL) was added to a stirred solution of 1-[(methylsulfonyl)methyl]-3-nitrobenzene (5.1 g, 23.8 mmol) in THF (250 mL) at rt, and the mixture was stirred for 16 h. The pH was increased to 10 by the addition of 1 N NaOH; then, the reaction mixture was extracted with EtOAc (2×). The combined organic phases were washed with brine, filtered using a Whatman filter, and concentrated to afford the desired product (4.5 g) that was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.97 (m, 1H), 6.51 (m, 3H), 5.13 (br, 2H), 4.23 (s, 2H), 2.83 (s, 3H).

4-Chloro-N-{3-[(methylsulfonyl)methyl]phenyl}-1,3,5-triazin-2-amine. DIPEA (3.7 mL, 21.3 mmol) was added to a stirred solution of 2,4-dichloro-1,3,5-triazine (1.60 g, 10.7 mmol) in THF/*i*-PrOH (1:1, 20 mL) at −40 °C. Then, a suspension of 3-[(methylsulfonyl)-methyl]aniline (1.97 g, 10.7 mmol) in THF/*i*-PrOH (1:1, 10 mL) was added at this temperature. Under stirring, the temperature of the reaction mixture was slowly raised over 3 h to 0 °C. The mixture was concentrated in vacuo to afford the crude product (5.2 g) that was used without further purification.

*4-(4-Fluoro-2-methoxyphenyl)-N-{3-[(methylsulfonyl)methyl]-phenyl}-1,3,5-triazin-2-amine (****1****).* A mixture of crude 4-chloro-*N*-{3-[(methylsulfonyl)methyl]phenyl}-1,3,5-triazin-2-amine (1000 mg), (4-fluoro-2-methoxyphenyl)boronic acid (569 mg, 3.35 mmol; Aldrich), and tetrakis(triphenylphosphine)palladium(0) [$Pd(PPh_3)_4$] (580 mg, 0.50 mmol) in DME (10.3 mL) and 2 M aq K_2CO_3 (3.4 mL) was degassed using argon. The mixture was stirred under argon for 90 min at 100 °C. After cooling, the mixture was diluted with EtOAc and washed with brine. The organic phase was filtered using a Whatman filter and concentrated. The residue was purified by flash chromatography (DCM to DCM/EtOH 95:5) to afford **1** (402 mg, 1.03 mmol) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.82 (s, 1H), 7.96 (br s, 1H), 7.69−7.87 (m, 2H), 7.38−7.46 (m, 2H), 7.16 (d, J = 7.53 Hz, 1H), 6.75−6.81 (m, 2H), 4.26 (s, 2H), 3.94 (s, 3H), 2.79 (s, 3H). ESI-HRMS m/z: $[M + H]^+$ calcd for $C_{18}H_{18}FN_4O_3S$, 389.1084; found, 389.1080.

Compound ***2****. 2-Chloro-4-(4-fluoro-2-methoxyphenyl)pyridine.* A mixture of 4-bromo-2-chloropyridine (1 g, 5.20 mmol; Aldrich), (4-fluoro-2-methoxyphenyl)boronic acid (971 mg, 5.72 mmol), and $Pd(PPh_3)_4$ (600 mg, 0.52 mmol) in toluene (26 mL) and a solution of K_2CO_3 (861 mg, 6.23 mmol) in H_2O (5.2 mL) was degassed using argon. The mixture was stirred under argon for 3 h at 90 °C. After cooling, the mixture was diluted with EtOAc and washed with brine. The organic phase was filtered using a Whatman filter and

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concentrated. The residue was purified by column chromatography (hexane/EtOAc 4:1) to afford the desired product (210 mg, 0.88 mmol, 17% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.40−8.44 (m, 1H), 7.60−7.63 (m, 1H), 7.54 (dd, J = 1.52, 5.07 Hz, 1H), 7.50 (dd, J = 6.84, 8.62 Hz, 1H), 7.11 (dd, J = 2.53, 11.41 Hz, 1H), 6.92 (dt, J = 2.53, 8.36 Hz, 1H), 3.83 (s, 3H). UPLC (ESI+) m/z: $[M + H]^+$ 238.

*4-(4-Fluoro-2-methoxyphenyl)-N-{3-[(methylsulfonyl)methyl]-phenyl}pyridin-2-amine (**2**).* A mixture of 3-[(methylsulfonyl)-methyl]aniline (123 mg, 0.66 mmol), 2-chloro-4-(4-fluoro-2-methoxyphenyl)pyridine (105 mg, 0.44 mmol), tris-(dibenzylideneacetone)dipalladium(0) [$Pd_2(dba)_3$] (48 mg, 0.05 mmol), 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) (102 mg, 0.16 mmol), and sodium *tert*-butoxide (85 mg, 0.88 mmol) in DMF (4.1 mL) and THF (8.3 mL) was stirred under argon for 4.5 h at 110 °C. After cooling, the mixture was diluted with EtOAc and washed with brine. The organic phase was filtered using a Whatman filter and concentrated. The residue was purified by column chromatography (hexane/EtOAc) to afford **2** (55 mg, 0.14 mmol, 32% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.16 (s, 1H), 8.15 (d, J = 5.32 Hz, 1H), 7.78 (br d, J = 7.86 Hz, 1H), 7.63 (s, 1H), 7.39 (dd, J = 6.84, 8.36 Hz, 1H), 7.28 (t, J = 7.86 Hz, 1H), 7.07 (dd, J = 2.28, 11.41 Hz, 1H), 6.97 (s, 1H), 6.86−6.95 (m, 2H), 6.85 (dd, J = 1.27, 5.32 Hz, 1H), 4.44 (s, 2H), 3.83 (s, 3H), 2.93 (s, 3H). ^{13}C NMR (100.67 MHz, DMSO-d_6): δ 163.18 (d, $^1J_{CF}$ = 244.99 Hz, $1C_q$), 157.54 (d, $^3J_{CF}$ = 10.60 Hz, $1C_q$), 155.83 (s, $1C_q$), 147.01 (s, 1CH), 145.95 (s, $1C_q$), 141.94 (s, $1C_q$), 131.17 (d, $^3J_{CF}$ = 10.17 Hz, 1CH), 129.32 (s, $1C_q$), 128.78 (s, 1CH), 123.93 (d, $^4J_{CF}$ = 3.39 Hz, $1C_q$), 122.99 (s, 1CH), 120.05 (s, 1CH), 117.95 (s, 1CH), 115.35 (s, 1CH), 110.91 (s, 1CH), 107.25 (d, $^2J_{CF}$ = 21.19 Hz, 1CH), 100.28 (d, $^2J_{CF}$ = 25.86 Hz, 1CH), 59.72 (s, $1CH_2$), 56.17 (s, $1CH_3$), 39.50 (s, $1CH_3$). IR (diamond ATR, cm^{-1}): 3597 (br s), 3364 (w), 3290 (w), 3009 (w), 2922 (w), 1597 (s), 1529 (m), 1479 (m), 1443 (m), 1379 (m), 1279 (s), 1192 (m), 1113 (s), 1024 (s), 951 (s), 876 (m), 833 (m), 798 (m). ESI-HRMS m/z: $[M + H]^+$ calcd for $C_{20}H_{20}FN_2O_3S$, 387.1179; found, 387.1175.

*Compound **3**. 2,5-Difluoro-4-(4-fluoro-2-methoxyphenyl)-pyridine.* A mixture of 2,5-difluoro-4-iodopyridine (500 mg, 2.07 mmol; abcr), (4-fluoro-2-methoxyphenyl)boronic acid (388 mg, 2.28 mmol), and $Pd(PPh_3)_4$ (240 mg, 0.21 mmol) in DME (6.2 mL) and 2 M aq K_2CO_3 (3.1 mL) was degassed using argon. The mixture was stirred under argon for 16 h at 100 °C. After cooling, the mixture was diluted with EtOAc and washed with brine. The organic phase was filtered using a Whatman filter and concentrated. The residue was purified by column chromatography (hexane to hexane/EtOAc 3:2) to afford the desired product (420 mg, 1.76 mmol, 85% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07 (t, J = 1.32 Hz, 1H), 7.33 (br d, J = 1.51 Hz, 1H), 6.95 (dd, J = 2.64, 4.52 Hz, 1H), 6.71−6.83 (m, 2H), 3.83 (s, 3H). UPLC (ESI+) m/z: $[M + H]^+$ 240.

*5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-N-{3-[(methylsulfonyl)-methyl]phenyl}pyridin-2-amine (**3**).* To a solution of 2,5-difluoro-4-(4-fluoro-2-methoxyphenyl)pyridine (150 mg, 0.63 mmol) and 3-[(methylsulfonyl)methyl]aniline (140 mg, 0.75 mmol) in DMF (5 mL), NaH (60% suspension in mineral oil; 37 mg, 0.94 mmol) was added. The mixture was stirred for 2 h at 70 °C. After cooling, the mixture was diluted with EtOAc and washed with brine. The organic phase was filtered using a Whatman filter and concentrated. The residue was purified by preparative HPLC (basic conditions) to afford **3** (41 mg, 0.10 mmol, 16% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11 (d, J = 1.51 Hz, 1H), 7.39−7.48 (m, 2H), 7.30−7.37 (m, 1H), 7.01 (d, J = 7.16 Hz, 1H), 6.82 (d, J = 4.90 Hz, 1H), 6.70−6.79 (m, 2H), 6.51 (s, 1H), 4.22 (s, 2H), 3.83 (s, 3H), 2.78 (s, 3H). ^{13}C NMR (100.67 MHz, DMSO-d_6): δ 163.64 (d, $^1J_{CF}$ = 245.41 Hz, $1C_q$), 157.92 (d, $^3J_{CF}$ = 10.60 Hz, $1C_q$), 152.41 (d, $^4J_{CF}$ = 1.69 Hz, $1C_q$), 151.36 (d, $^1J_{CF}$ = 244.14 Hz, $1C_q$), 147.01 (s, 1CH), 145.95 (s, $1C_q$), 141.82 (s, $1C_q$), 135.00 (d, $^2J_{CF}$ = 15.68 Hz, $1C_q$), 133.60 (d, $^2J_{CF}$ = 26.28 Hz, 1CH), 131.64 (d, $^3J_{CF}$ = 10.17 Hz, 1CH), 129.40 (s, $1C_q$), 128.84 (s, 1CH), 123.10 (s, 1CH), 119.95 (s, 1CH), 118.52 (d, $^4J_{CF}$ = 3.39 Hz, $1C_q$), 117.59 (s, 1CH), 115.35 (s, 1CH), 112.63 (s, 1CH), 107.12 (d, $^2J_{CF}$ = 21.62 Hz, 1CH), 100.16 (d, $^2J_{CF}$ = 26.28 Hz, 1CH), 59.67 (s, $1CH_2$), 56.25 (s, $1CH_3$), 39.51 (s, $1CH_3$). IR (diamond ATR, cm^{-1}): 3370 (w), 3330 (w), 3105 (w), 2924 (w), 1624 (m), 1601 (s), 1483 (s), 1447 (m), 1379 (m), 1298 (s), 1213 (m), 1194 (m), 1153 (m), 1026 (m), 953 (m), 878 (w), 837 (w), 798 (m). UPLC (ESI+) m/z: $[M + H]^+$ 405. ESI-HRMS m/z: $[M + H]^+$ calcd for $C_{20}H_{19}F_2N_2O_3S$, 405.1085; found, 405.1082.

*Compound **4**. 2-Chloro-3-fluoro-4-(4-fluoro-2-methoxyphenyl)-pyridine.* A mixture of 2-chloro-3-fluoro-4-iodopyridine [1 g, 3.88 mmol; Tokyo Chemical Industry (TCI)], (4-fluoro-2-methoxyphenyl)boronic acid (660 mg, 3.88 mmol), and $Pd(PPh_3)_4$ (449 mg, 0.38 mmol) in DME (10 mL) and 2 M aq K_2CO_3 (5.8 mL) was degassed using argon. The mixture was stirred under argon for 3.5 h at 100 °C. After cooling, the mixture was diluted with EtOAc and washed with brine. The organic phase was filtered using a Whatman filter and concentrated. The residue was purified by column chromatography (hexane to hexane/EtOAc 1:1) to afford the desired product (569 mg, 2.23 mmol, 57% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.27 (d, J = 5.05 Hz, 1H), 7.45 (t, J = 5.05 Hz, 1H), 7.37 (dd, J = 6.95, 8.46 Hz, 1H), 7.09 (dd, J = 2.53, 11.37 Hz, 1H), 6.90 (dt, J = 2.53, 8.46 Hz, 1H), 3.77 (s, 3H).

*3-Fluoro-4-(4-fluoro-2-methoxyphenyl)-N-{3-[(methylsulfonyl)-methyl]phenyl}pyridin-2-amine (**4**).* A mixture of 3-[(methylsulfonyl)methyl]aniline (135 mg, 0.73 mmol), 2-chloro-3-fluoro-4-(4-fluoro-2-methoxyphenyl)pyridine (125 mg, 0.49 mmol), $Pd_2(dba)_3$ (54 mg, 0.06 mmol), BINAP (113 mg, 0.18 mmol), and sodium *tert*-butoxide (94 mg, 0.98 mmol) in DMF (4.5 mL) and THF (9 mL) was stirred under argon for 2 h at 100 °C. After cooling, the mixture was diluted with EtOAc and washed with brine. The organic phase was filtered using a Whatman filter and concentrated. The residue was purified by preparative HPLC (basic conditions) to afford **4** (78 mg, 0.19 mmol, 39% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (d, J = 5.05 Hz, 1H), 7.82 (s, 1H), 7.72 (dd, J = 1.64, 7.96 Hz, 1H), 7.38 (t, J = 7.96 Hz, 1H), 7.22−7.28 (m, 1H), 7.06 (d, J = 7.58 Hz, 1H), 6.72−6.80 (m, 4H), 4.27 (s, 2H), 3.83 (s, 3H), 2.80 (s, 3H). ^{13}C NMR (100.67 MHz, DMSO-d_6): δ 163.58 (d, $^1J_{CF}$ = 244.99 Hz, $1C_q$), 157.87 (d, $^3J_{CF}$ = 10.60 Hz, $1C_q$), 145.09 (d, $^2J_{CF}$ = 11.02 Hz, $1C_q$), 143.94 (d, $^1J_{CF}$ = 256.43 Hz, $1C_q$), 141.71 (d, $^3J_{CF}$ = 7.20 Hz, 1CH), 140.92 (s, $1C_q$), 131.65 (d, $^3J_{CF}$ = 10.17 Hz, 1CH), 131.30 (d, $^3J_{CF}$ = 11.45 Hz, $1C_q$), 129.19 (s, $1C_q$), 128.55 (s, 1CH), 124.03 (s, 1CH), 121.62 (s, 1CH), 119.28 (s, 1CH), 118.50 (d, $^4J_{CF}$ = 3.39 Hz, $1C_q$), 116.77 (s, 1CH), 107.08 (d, $^2J_{CF}$ = 21.19 Hz, 1CH), 100.10 (d, $^2J_{CF}$ = 25.86 Hz, 1CH), 59.77 (s, $1CH_2$), 56.21 (s, $1CH_3$), 39.47 (s, $1CH_3$). IR (diamond ATR, cm^{-1}): 3379 (m), 3026 (w), 3013 (w), 2920 (w), 1632 (s), 1609 (s), 1599 (s), 1535 (s), 1491 (s), 1437 (s), 1317 (m), 1283 (s), 1236 (m), 1188 (s), 1109 (s), 1030 (s), 984 (m), 951 (s), 893 (m), 835 (s), 762 (m). ESI-HRMS m/z: $[M + H]^+$ calcd for $C_{20}H_{19}F_2N_2O_3S$, 405.1085; found, 405.1085.

*Compound **5**. 2-Chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)-pyrimidine.* A mixture of 2,4-dichloro-5-fluoropyrimidine (200 mg, 1.20 mmol; Aldrich), (4-fluoro-2-methoxyphenyl)boronic acid (224 mg, 1.31 mmol), and $Pd(PPh_3)_4$ (138 mg, 0.12 mmol) in DME (3.6 mL) and 2 M aq K_2CO_3 (1.8 mL) was degassed using argon. The mixture was stirred under argon for 16 h at 90 °C. After cooling, the mixture was diluted with EtOAc and washed with brine. The organic phase was filtered using a Whatman filter and concentrated. The residue was purified by column chromatography (hexane/EtOAc 1:1) to afford the desired product (106 mg, 0.41 mmol, 35% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.47 (m, 1H), 7.51 (m, 1H), 6.82 (m, 1H), 6.73 (m, 1H), 3.85 (s, 3H).

*5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-N-{3-[(methylsulfonyl)-methyl]phenyl}pyrimidin-2-amine (**5**).* A mixture of 3-[(methylsulfonyl)methyl]aniline (108 mg, 0.58 mmol), 2-chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidine (150 mg, 0.58 mmol), $Pd_2(dba)_3$ (96 mg, 0.11 mmol), 4,5-bis-(diphenylphosphino)-9,9-dimethylxanthene (Xantphos) (88 mg, 0.15 mmol), and Cs_2CO_3 (800 mg, 2.46 mmol) in 1,4-dioxane (1.0 mL) was degassed using argon. The mixture was stirred under argon for 150 min at 100 °C. After cooling, the mixture was filtered and the filter was rinsed with DCM and EtOAc. The filtrate was concentrated in vacuo and the residue was purified by preparative HPLC (acidic conditions) to afford **5** (49 mg, 0.12 mmol, 21% yield). ^{1}H NMR

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(400 MHz, DMSO-d_6, 27 °C): δ 9.83 (m, 1H), 8.51 (m, 1H), 7.69 (m, 2H), 7.50 (m, 1H), 7.25 (m, 1H), 7.08 (m, 1H), 6.91 (m, 2H), 4.37 (s, 2H), 3.80 (s, 3H), 2.86 (s, 3H). ^{13}C NMR (100.65 MHz, DMSO-d_6): δ 164.34 (d, $^1J_{CF}$ = 247.11 Hz, 1C$_q$), 158.55 (d, $^3J_{CF}$ = 10.60 Hz, 1C$_q$), 156.53 (d, $^4J_{CF}$ = 2.54 Hz, 1C$_q$), 151.52 (d, $^3J_{CF}$ = 14.41 Hz, 1C$_q$), 150.23 (d, $^1J_{CF}$ = 250.50 Hz, 1C$_q$), 145.72 (d, $^2J_{CF}$ = 24.58 Hz, 1CH), 140.67 (s, 1C$_q$), 132.06 (d, $^3J_{CF}$ = 10.60 Hz, 1CH), 129.31 (s, 1C$_q$), 128.67 (s, 1CH), 124.15 (s, 1CH), 120.91 (s, 1CH), 119.05 (m, 1C$_q$), 118.49 (s, 1CH), 107.30 (d, $^2J_{CF}$ = 21.62 Hz, 1CH), 100.16 (d, $^2J_{CF}$ = 25.86 Hz, 1CH), 59.76 (s, 1CH$_2$), 56.34 (s, 1CH$_3$), 40.46 (s, 1CH$_3$). ESI-HRMS m/z: [M + H]$^+$ calcd for $C_{19}H_{18}F_2N_3O_3S$, 406.1037; found, 406.1041.

Compound ***6****. 2,5-Dichloro-4-(4-fluoro-2-methoxyphenyl)-pyrimidine.* A mixture of 2,4,5-trichloropyrimidine (367 mg, 2 mmol; Combi-Blocks, Inc.), (4-fluoro-2-methoxyphenyl)boronic acid (374 mg, 2.2 mmol), and [1,1′-bis(diphenylphosphino)ferrocene]-dichloropalladium(II) [Pd(dppf)Cl$_2$] (1:1 complex with DCM; 163 mg, 0.2 mmol; Aldrich) in DME (6.1 mL) and 2 M aq K_2CO_3 (3 mL) was degassed using argon. The mixture was stirred under argon for 1 h at 90 °C. After cooling, the mixture was diluted with EtOAc and washed with brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography (hexane/EtOAc 1:1) to afford the desired product (280 mg, 1.03 mmol, 52% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60 (s, 1H), 7.34 (dd, J = 6.57, 8.34 Hz, 1H), 6.80 (dt, J = 2.40, 8.27 Hz, 1H), 6.73 (dd, J = 2.27, 10.86 Hz, 1H), 3.82 (s, 3H). UPLC (ESI+) m/z: [M + H]$^+$ 273.

*5-Chloro-4-(4-fluoro-2-methoxyphenyl)-N-{3-[(methylsulfonyl)-methyl]phenyl}pyrimidin-2-amine (****6****).* To a mixture of 2,5-dichloro-4-(4-fluoro-2-methoxyphenyl)pyrimidine (136 mg, 0.498 mmol) and 3-[(methylsulfonyl)methyl]aniline (92 mg, 0.49 mmol) in *n*-BuOH, 4 M HCl in 1,4-dioxane (0.125 mL, 0.498 mmol) was added. The mixture was stirred for 65 h at 120 °C and an additional 72 h at 140 °C. Then, the mixture was evaporated to dryness and the residue was taken up in EtOAc and washed with aq $NaHCO_3$ solution, followed by brine. The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (hexane/EtOAc 1:1) to afford **6** (70 mg, 0.16 mmol, 33% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (s, 1H), 7.79 (d, J = 1.76 Hz, 1H), 7.52−7.58 (m, 1H), 7.29−7.38 (m, 2H), 7.24 (s, 1H), 7.07 (d, J = 7.53 Hz, 1H), 6.79 (dt, J = 2.38, 8.22 Hz, 1H), 6.74 (dd, J = 2.38, 10.67 Hz, 1H), 4.23 (s, 2H), 3.84 (s, 3H), 2.74 (s, 3H). ^{13}C NMR (100.67 MHz, DMSO-d_6): δ 163.90 (d, $^1J_{CF}$ = 245.84 Hz, 1C$_q$), 162.03 (s, 1C$_q$), 158.18 (s, 1C$_q$), 157.94 (d, $^3J_{CF}$ = 10.60 Hz, 1C$_q$), 156.85 (s, 1C$_q$), 140.15 (s, 1C$_q$), 131.22 (d, $^3J_{CF}$ = 10.60 Hz, 1CH), 129.38 (s, 1C$_q$), 128.72 (s, 1CH), 124.63 (s, 1CH), 121.86 (d, $^4J_{CF}$ = 2.97 Hz, 1C$_q$), 121.49 (s, 1CH), 119.78 (s, 1C$_q$), 119.14 (s, 1CH), 106.99 (d, $^2J_{CF}$ = 22.04 Hz, 1CH), 100.04 (d, $^2J_{CF}$ = 26.28 Hz, 1CH), 59.69 (s, 1CH$_2$), 56.17 (s, 1CH$_3$), 39.44 (s, 1CH$_3$). IR (diamond ATR, cm^{-1}): 3337 (s), 3074 (w), 3020 (w), 2924 (w), 1663 (w), 1607 (m), 1593 (m), 1545 (s), 1508 (m), 1445 (s), 1394 (s), 1312 (w), 1281 (s), 1198 (w), 1151 (m), 1024 (w), 953 (s), 880 (m), 849 (m), 822 (m), 789 (m). UPLC (ESI+) m/z: [M + H]$^+$ 422. ESI-HRMS m/z: [M + H]$^+$ calcd for $C_{19}H_{18}ClFN_3O_3S$, 422.0742; found, 422.0741.

Compound ***7****. N-tert-Butyl-2,4-dichloropyrimidine-5-carboxamide.* To 2,4-dichloropyrimidine-5-carbonyl chloride (5 g, 24 mmol; Combi-Blocks, Inc.) in THF (30 mL), a solution of *tert*-butylamine (1.82 g, 24.9 mmol) and triethylamine (3.45 mL) in THF (30 mL) was added slowly at −5 °C. The mixture was stirred for 4.5 h at this temperature. After removal of the precipitated salts by filtration, the filtrate was evaporated to dryness. The resulting material was used without further purification.

N-tert-Butyl-2-chloro-4-(4-fluoro-2-methoxyphenyl)pyrimidine-5-carboxamide. A mixture of *N-tert*-butyl-2,4-dichloropyrimidine-5-carboxamide (5.73 g, 23.1 mmol), (4-fluoro-2-methoxyphenyl)-boronic acid (4.32 g, 25.4 mmol), and [Pd(dppf)Cl$_2$] (1:1 complex with DCM; 1.88 g, 2.3 mmol) in DME (70 mL) and 2 M aq K_2CO_3 (34.6 mL) was degassed using argon. The mixture was stirred under argon for 16 h at 90 °C. After cooling, the mixture was diluted with EtOAc and washed with brine. The organic phase was filtered using a Whatman filter and concentrated. The residue was purified by column chromatography (DCM to DCM/EtOH 95:5 and hexane to hexane/EtOAc 1:1) to afford the desired product (618 mg) as an equimolar mixture with (*Z*)-3-amino-*N-tert*-butyl-3-(4-fluoro-2-methoxyphenyl)-2-formylprop-2-enamide.

2-Chloro-4-(4-fluoro-2-methoxyphenyl)pyrimidine-5-carbonitrile. A mixture of *N-tert*-butyl-2-chloro-4-(4-fluoro-2-methoxyphenyl)pyrimidine-5-carboxamide (564 mg) and thionyl chloride (10.3 mL) was stirred for 18 h at 80 °C. After cooling, the volatiles were removed under reduced pressure. The residue was purified by column chromatography (hexane to hexane/EtOAc 3:1) to afford the desired product (206 mg, 0.78 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.85 (s, 1H), 7.60 (dd, J = 6.50, 8.57 Hz, 1H), 6.85 (br d, J = 2.07 Hz, 1H), 6.79 (dd, J = 2.17, 10.46 Hz, 1H), 3.93 (s, 3H). UPLC (ESI+) m/z: [M + H]$^+$ 264.

*4-(4-Fluoro-2-methoxyphenyl)-2-({3-[(methylsulfonyl)methyl]-phenyl}amino)pyrimidine-5-carbonitrile (****7****).* A mixture of 2-chloro-4-(4-fluoro-2-methoxyphenyl)pyrimidine-5-carbonitrile (80 mg, 0.303 mmol), 3-[(methylsulfonyl)methyl]aniline (56 mg, 0.303 mmol), (XPhos) palladium(II) phenethylamine chloride (XPhos Pd G1, CAS 1028206-56-5) (18.8 mg, 0.023 mmol), 2-dicyclohexylphosphino-2′,4′,6′-triisopropyl-1,1′-biphenyl (XPhos) (10.8 mg, 0.023 mmol), and potassium phosphate (322 mg, 1.52 mmol) in toluene (3.2 mL) and *N*-methyl-2-pyrrolidone (NMP, 0.8 mL) was degassed using argon. The mixture was stirred under argon for 3 h at 130 °C. After cooling, the mixture was diluted with EtOAc and washed with brine. The organic phase was filtered using a Whatman filter and concentrated. The residue was purified by preparative HPLC (acidic conditions) to afford **7** (7.6 mg, 0.02 mmol, 7% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.65 (s, 1H), 7.81 (s, 1H), 7.63 (br d, J = 3.39 Hz, 2H), 7.50 (dd, J = 6.88, 8.19 Hz, 1H), 7.40 (t, J = 7.91 Hz, 1H), 7.15 (d, J = 7.72 Hz, 1H), 6.74−6.87 (m, 2H), 4.25 (s, 2H), 3.92 (s, 3H), 2.78 (s, 3H). IR (diamond ATR, cm^{-1}): 3325 (w), 3313 (w), 3296 (w), 3086 (w), 3020 (w), 2976 (w), 2931 (w), 2224 (m), 1606 (m), 1568 (s), 1441 (s), 1282 (m), 1155 (w), 1117 (w), 1030 (w), 955 (w), 800 (w). UPLC (ESI+) m/z: [M + H]$^+$ 413.

Compound ***8****. 4-Chloro-N-{3-[(methylsulfonyl)methyl]phenyl}-5-(trifluoromethyl)pyrimidin-2-amine.* To a solution of 2,4-dichloro-5-(trifluoromethyl)pyrimidine (527 mg, 2.43 mmol; Combi-Blocks, Inc.) in *t*-BuOH (8.2 mL) and DCE (8.2 mL), 1 M zinc chloride in diethyl ether (2.43 mL, 2.43 mmol) was added. The resulting mixture was stirred for 1 h at rt. Then, 3-[(methylsulfonyl)methyl]aniline (300 mg, 1.62 mmol) was added, followed by a solution of triethylamine (0.339 mL, 2.43 mmol) in *t*-BuOH (0.55 mL) and DCE (0.55 mL). After 5 h, the mixture was diluted with DCM and washed with aq citric acid solution−brine, aq $NaHCO_3$ solution, and brine. The organic phase was dried over Na_2SO_4 and concentrated to afford the desired product (662 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.59 (s, 1H), 7.76 (s, 1H), 7.59 (br dd, J = 7.54, 8.85 Hz, 2H), 7.42 (t, J = 7.91 Hz, 1H), 7.19 (d, J = 7.54 Hz, 1H), 4.28 (s, 2H), 2.84 (s, 3H). UPLC (ESI+) m/z: [M + H]$^+$ 366.

*4-(4-Fluoro-2-methoxyphenyl)-N-{3-[(methylsulfonyl)methyl]-phenyl}-5-(trifluoromethyl)pyrimidin-2-amine (****8****).* A mixture of 4-chloro-*N*-{3-[(methylsulfonyl)methyl]phenyl}-5-(trifluoromethyl)-pyrimidin-2-amine (100 mg, 0.27 mmol), (4-fluoro-2-methoxyphenyl)boronic acid (51 mg, 0.3 mmol), and bis-(triphenylphosphine)palladium(II) chloride (8 mg, 0.01 mmol) in DME (3.6 mL) and 2 M aq K_2CO_3 (1.13 mL) was degassed using argon. The mixture was stirred under argon for 96 h at 105 °C. After cooling, the mixture was evaporated to dryness, and the residue was purified by preparative HPLC (acidic conditions) to afford **8** (14 mg, 0.03 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.72 (s, 1H), 7.83 (s, 1H), 7.57 (dd, J = 1.26, 8.08 Hz, 1H), 7.47 (s, 1H), 7.39 (t, J = 7.83 Hz, 1H), 7.21 (dd, J = 6.32, 8.34 Hz, 1H), 7.13 (d, J = 7.58 Hz, 1H), 6.76 (dt, J = 2.27, 8.21 Hz, 1H), 6.71 (dd, J = 2.27, 10.61 Hz, 1H), 4.24 (s, 2H), 3.77 (s, 3H), 2.74 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 163.8, 163.6, 160.7, 157.8, 156.8, 139.3, 130.5, 129.5, 128.8, 125.7, 124.1, 122.4, 122.4, 120.1, 113.8, 106.5, 99.7, 59.6, 56.0, 39.4. ESI-HRMS m/z: [M + H]$^+$ calcd for $C_{20}H_{18}F_4N_3O_3S$, 456.1005; found, 456.1002.

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*Compound **9** and BAY-332. 1-Fluoro-3-[(methylsulfanyl)-methyl]-5-nitrobenzene.* Sodium methanethiolate (5.18 g, 73.8 mmol, 1.4 equiv) was added portionwise at 0 °C to a solution of 1-(chloromethyl)-3-fluoro-5-nitrobenzene (10.0 g, 52.8 mmol) in EtOH (110 mL). After stirring for a further 20 min at that temperature, the mixture was warmed to rt and stirred for a further 18 h. The suspension was then poured into dilute aq NaCl solution, and the mixture was extracted with EtOAc. The organic extract was filtered through a Whatman filter, and the filtrate was concentrated under reduced pressure. The residue was taken up in DCM and again concentrated under reduced pressure. The oily residue was taken up in EtOAc and washed with dilute aq NaCl solution, filtered through a Whatman filter, and concentrated under reduced pressure to afford the title compound (9.86 g, 49.0 mmol, 95% purity, 93% yield) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.00 (s, 1H), 7.80−7.85 (m, 1H), 7.42 (dt, J = 8.59, 1.89 Hz, 1H), 3.74 (s, 2H, SCH_2), 2.03 (s, 3H, SCH_3). ^{13}C NMR (151 MHz, DMSO-d_6): δ 163.8, 163.6, 160.7, 157.8, 156.8, 139.3, 130.5, 129.5, 128.8, 125.7, 124.1, 122.4, 122.4, 120.1, 113.8, 106.5, 99.7, 59.6, 56.0, 39.4.

(rac)-1-Fluoro-3-[(methylsulfinyl)methyl]-5-nitrobenzene. To a solution of 1-fluoro-3-[(methylsulfanyl)methyl]-5-nitrobenzene (10.3 g, 51.3 mmol) in MeCN (120 mL) was added $FeCl_3$ at rt (241 mg, 1.49 mmol, 3 mol %), and the mixture was stirred for 10 min at that temperature. Then, periodic acid (12.5 g, 54.8 mmol, 1.07 equiv) was added in one portion, and vigorous stirring was continued for 90 min during which the temperature of the mixture was maintained below 30 °C. The resulting suspension was poured into a solution of sodium thiosulfate pentahydrate (71.2 g) in ice−water (652 mL), and the mixture was stirred for 50 min. Then, NaCl was added until saturation, and the mixture was extracted THF (2×). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was stripped off several times with toluene and DCM and was then purified by flash chromatography (silica gel, EtOAc/hexane gradient) to afford the title compound (8.00 g, 36.8 mmol, 92% purity, 72% yield). ^{1}H NMR (300 MHz, DMSO-d_6, 27 °C): δ 8.04−8.09 (m, 2H), 7.61−7.65 (m, 1H), 4.32 (d, J = 12.81 Hz, 1H, sulfoxide−CH_2, H_A, AB system), 4.08 (d, J = 12.81 Hz, 1H, sulfoxide−CH_2, H_B, AB system), 2.46 (s, 3H, SCH_3). UPLC (ESI+) m/z: $[M + H]^+$ 218.

(rac)-2,2,2-Trifluoro-N-[(3-fluoro-5-nitrobenzyl)(methyl)oxido-λ^6-sulfanylidene]acetamide. To a suspension of (*rac*)-1-fluoro-3-[(methylsulfinyl)methyl]-5-nitrobenzene (8.00 g, 36.8 mmol), 2,2,2-trifluoroacetamide (8.33 g, 73.7 mmol, 2.0 equiv), magnesium oxide (5.94 g, 147 mmol, 4.0 equiv), and dirhodium tetraacetate (0.76 g, 3.6 mmol, 10 mol %) in DCM (1.0 L) was added iodosobenzene diacetate (17.8 g, 55.2 mmol, 1.5 equiv) at rt. The mixture was stirred at rt for 3 days, after which the mixture was filtered and the filtrate was concentrated under reduced pressure. The resulting dark oil was purified by column chromatography (silica gel, EtOAc/hexane gradient) to afford the title compound as a light yellow oil, which crystallized upon standing (10.8 g, 33.0 mmol, 90% yield). ^{1}H NMR (300 MHz, $CDCl_3$, 27 °C): δ 8.04−8.16 (m, 2H), 7.55 (dt, J = 7.91, 1.88 Hz, 1H), 4.92 (d, J = 13.94 Hz, 1H, sulfoximine−CH_2, H_A, AB system), 4.75 (d, J = 13.94 Hz, 1H, sulfoximine−CH_2, H_B, AB system), 3.32 (s, 3H, sulfoximine−CH_3). UPLC (ESI+) m/z: $[M + H]^+$ 329.

(rac)-1-Fluoro-3-[(S-methylsulfonimidoyl)methyl]-5-nitrobenzene. K_2CO_3 (22.7 g, 164 mmol, 5.0 equiv) was added at rt to a solution of (*rac*)-2,2,2-trifluoro-*N*-[(3-fluoro-5-nitrobenzyl)(methyl)-oxido-λ^6-sulfanylidene]acetamide (10.8 g, 33.0 mmol) in MeOH (700 mL). After stirring for 1 h at that temperature, the mixture was concentrated at 30 °C to 100 mL. After the addition of brine, the mixture was extracted EtOAc. The organic extract was filtered through a Whatman filter, and the filtrate was concentrated under reduced pressure to afford the crude product (9.36 g), which was directly used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6, 27 °C): δ 8.18 (s, 1H), 8.07 (dt, J = 8.84, 2.27 Hz, 1H), 7.76 (d, J = 9.12 Hz, 1H), 4.60 (d, J = 13.14 Hz, 1H, sulfoximine−CH_2, H_A, AB system), 4.50 (d, J = 13.14 Hz, 1H, sulfoximine−CH_2, H_B, AB system), 3.87 (s, 1H, NH), 2.79 (s, 3H, sulfoximine−CH_3). UPLC (ESI+) m/z: $[M + H]^+$ 233.

(rac)-Ethyl [(3-Fluoro-5-nitrobenzyl)(methyl)oxido-λ^6-sulfanylidene]carbamate. (*rac*)-1-Fluoro-3-[(*S*-methylsulfonimidoyl)methyl]-5-nitrobenzene (9.36 g) from the previous step was dissolved in pyridine (380 mL) and cooled to 0 °C. Ethyl chloroformate (5.01 mL, 52.4 mmol, ca. 1.3 equiv) was added dropwise at 0 °C to this mixture, after which the mixture was warmed to rt. After stirring for 16 h at that temperature, the mixture was concentrated under reduced pressure. The residue was taken up in EtOAc and washed with brine and then filtered through a Whatman filter. The filtrate was concentrated under reduced pressure and the crude product was purified by flash chromatography (silica gel, EtOAc/hexane) to afford the title compound (7.09 g, 23.3 mmol, 71% yield over two steps). ^{1}H NMR (300 MHz, $CDCl_3$, 24 °C): δ 8.11 (s, 1H), 8.01 (br d, J = 7.91 Hz, 1H), 7.57 (br d, J = 7.72 Hz, 1H), 4.90 (d, J = 13.94 Hz, 1H, sulfoximine−CH_2, H_A, AB system), 4.76 (d, J = 13.94 Hz, 1H, sulfoximine−CH_2, H_B, AB system), 4.08−4.22 (q, J = 7.16 Hz, 2H, OCH_2CH_3), 3.12 (s, 3H, sulfoximine−CH_3), 1.22−1.35 (t, J = 7.16 Hz, 3H, OCH_2CH_3). UPLC (ESI+) m/z: $[M + H]^+$ 305.

(rac)-Ethyl [(3-Amino-5-fluorobenzyl)(methyl)oxido-λ^6-sulfanylidene]carbamate. $TiCl_3$ solution (15% in 10% HCl; 158 mL, 186 mmol, 8.0 equiv) was added dropwise at 0 °C to a solution of (*rac*)-ethyl [(3-fluoro-5-nitrobenzyl)(methyl)oxido-λ^6-sulfanylidene]carbamate (7.09 g, 23.3 mmol) in THF (300 mL). Then, the mixture was warmed to rt and stirred for 16 h at that temperature. Then, ice was added to the mixture, which was stirred vigorously, after which K_2CO_3 was added until the evolution of gas stopped. After that, the mixture was basified with 2 N NaOH. The resulting dark mixture was saturated with NaCl and extracted with EtOAc (3 × 500 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to afford the title compound (6.37 g, quant) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 27 °C): δ 6.38−6.55 (m, 3H), 4.52−4.64 (m, 2H, sulfoximine−CH_2), 4.08−4.22 (q, J = 7.16 Hz, 2H, OCH_2CH_3), 3.91 (br s, 2H, NH_2), 3.00 (s, 3H, sulfoximine−CH_3), 1.31 (t, J = 7.16 Hz, 3H, OCH_2CH_3). UPLC (ESI+) m/z: $[M + H]^+$ 275.

(rac)-Ethyl [(3-Fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}benzyl)(methyl)oxido-λ^6-sulfanylidene]carbamate. A mixture of (*rac*)-ethyl [(3-amino-5-fluorobenzyl)(methyl)oxido-λ^6-sulfanylidene]carbamate (436 mg, 1.59 mmol, 1.0 equiv), 2-chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidine (530 mg, 2.07 mmol, 1.3 equiv), XPhos Pd G1 (98.6 mg, 0.12 mmol, 7.5 mol %), XPhos (56.8 mg, 0.12 mmol, 7.5 mol %), and finely ground potassium phosphate (1.69 g, 7.95 mmol, 5.0 equiv) in toluene (8.5 mL) and NMP (1.3 mL) was degassed and repressured with argon at rt. The mixture was heated in a sealed vial to 130 °C in a microwave reactor for 3 h. After cooling to rt, brine was added, and the mixture was extracted with EtOAc (2 × 100 mL). The combined organic extracts were filtered through a Whatman filter and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (first chromatography: DCM to DCM/EtOH 93:7 gradient; second chromatography: DCM to DCM/EtOH 95:5 gradient; third chromatography: hexane to hexane/EtOAc 1:1 gradient) to afford the title compound (271 mg, 0.55 mmol, 35% yield) as a beige solid. ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.32−8.35 (m, 1H), 7.69 (br dd, J = 11.24, 1.89 Hz, 1H), 7.47 (t, J = 7.11 Hz, 1H), 7.38 (s, 1H), 7.28−7.34 (m, 1H), 6.72−6.86 (m, 3H), 4.68 (s, 2H, sulfoximine−CH_2), 4.17 (q, J = 7.07 Hz, 2H, OCH_2CH_3), 3.87 (s, 3H, OCH_3), 3.00 (s, 3H, sulfoximine−CH_3), 1.31 (t, J = 7.07 Hz, 3H, OCH_2CH_3). UPLC (ESI+) m/z: $[M + H]^+$ 495.

*5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-N-{3-fluoro-5-[(S-methylsulfonimidoyl)methyl]phenyl}pyrimidin-2-amine [Enantiomer 1 (**9**) and Enantiomer 2 (BAY-332)].* To a solution of (*rac*)-ethyl [(3-fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)-pyrimidin-2-yl]amino}benzyl)(methyl)oxido-λ^6-sulfanylidene]-carbamate (269 mg, 0.54 mmol) in EtOH (5.0 mL) was added at rt a solution of 1.5 M ethanolic sodium ethoxide (1.81 mL, 2.72 mmol,

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5.0 equiv) freshly prepared by reacting sodium (350 mg) with EtOH (10 mL). The reaction mixture was heated to 60 °C for 4.5 h, after which the mixture was cooled to rt and diluted with brine. The mixture was extracted with EtOAc, and the combined organic extracts were filtered through a Whatman filter. The filtrate was concentrated under reduced pressure and the crude product was purified by chiral preparative HPLC [Dionex P 580 pump, Gilson 215 liquid handler, Knauer K-2501 UV detector; column: CHIRALPAK IC 5 μm, 250 × 20 mm; eluent: hexane/EtOH 80:20 (v/v); flow: 50 mL/min; rt; solution: 243 mg in 4 mL DCM/MeOH; injection: 8 × 0.5 mL; and detection: UV 280 nm].

Enantiomer 1 (**9**): t_R = 9.15 min. Yield: 85 mg, 0.20 mmol (37%). UPLC (ESI+) m/z: $[M + H]^+$ 423. $[\alpha]_D$ +16.6 ± 0.21 (c 1.00, DMSO).

Enantiomer 2 (BAY-322): t_R = 10.36 min. Yield: 91 mg, 0.22 mmol (40%). ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.35 (d, J = 1.76 Hz, 1H, aniline−NH), 7.76 (d, J = 11.47 Hz, 1H), 7.51 (t, J = 7.26 Hz, 1H), 7.30−7.37 (m, 2H), 6.77−6.87 (m, 3H), 4.35 (d, J = 13.05 Hz, 1H, sulfoximine−CH_2, H_A, AB system), 4.23 (d, J = 13.05 Hz, 1H, sulfoximine−CH_2, H_B, AB system), 3.90 (s, 3H, OCH_3), 2.97 (s, 3H, sulfoximine−CH_3), 2.73 (br s, 1H, sulfoximine−NH). ^{13}C NMR (100.56 MHz, DMSO-d_6): δ 164.25 (d, $^1J_{CF}$ = 246.90 Hz, $1C_q$), 162.13 (d, $^1J_{CF}$ = 238.47 Hz, $1C_q$), 158.73 (d, $^3J_{CF}$ = 10.73 Hz, $1C_q$), 156.30 (d, $^4J_{CF}$ = 2.68 Hz, $1C_q$), 151.70 (d, $^2J_{CF}$ = 16.10 Hz, $1C_q$), 150.53 (d, $^1J_{CF}$ = 251.50 Hz, $1C_q$), 145.69 (d, $^2J_{CF}$ = 24.92 Hz, 1CH), 142.29 (d, $^3J_{CF}$ = 12.27 Hz, $1C_q$), 133.00 (d, $^3J_{CF}$ = 9.97 Hz, $1C_q$), 132.15 (d, $^3J_{CF}$ = 10.35 Hz, 1CH), 118.98−119.10 (m, $1C_q$), 116.73−116.93 (m, 1CH), 110.24 (d, $^2J_{CF}$ = 22.62 Hz, 1CH), 107.45 (d, $^2J_{CF}$ = 21.85 Hz, 1CH), 104.73 (d, $^2J_{CF}$ = 26.45 Hz, 1CH), 100.34 (d, $^2J_{CF}$ = 26.07 Hz, 1CH), 62.39 (s, $1CH_2$), 56.46 (s, $1CH_3$), 41.27 (s, $1CH_3$). IR (KBr, cm^{-1}): 3428 (br s), 3286 (br s), 3102 (w), 2969 (w), 2923 (w), 1612 (s), 1544 (m), 1434 (s), 1316 (m), 1285 (m), 1213 (m), 1154 (m), 1030 (m), 956 (m), 837 (m), 786 (m). UPLC (ESI+) m/z: $[M + H]^+$ 423. ESI-HRMS m/z: $[M + H]^+$ calcd for $C_{19}H_{18}F_3N_4O_2S$, 423.1103; found, 423.1099. $[\alpha]_D$ −18.8 ± 0.16 (c 1.00, DMSO).

*Compound **10**. 1-Methoxy-3-[(Z)-{3-methoxy-5-[(methylsulfanyl)methyl]phenyl}-NNO-azoxy]-5-[(methylsulfanyl)-methyl]benzene.* Sodium methanethiolate (2.58 g, 36.8 mmol) was added under stirring in three portions to a solution of 1-(chloromethyl)-3-methoxy-5-nitrobenzene (5.30 g, 26.3 mmol; FCH Group) in EtOH (60 mL) at 0 °C. The cold bath was removed, and the mixture was stirred at rt overnight. Further sodium methanethiolate (0.92 g, 13.1 mmol) was added, and the mixture was stirred overnight. Further sodium methanethiolate (1.66 g, 23.6 mmol) was added, and the mixture was stirred overnight. The mixture was diluted with saturated aq NaCl solution and extracted with EtOAc (2×). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (hexane to hexane/EtOAc 7:3) to afford the product (2.9 g, 7.66 mmol, 29% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 7.85 (m, 1H), 7.73 (m, 2H), 7.68 (m, 1H), 7.08 (m, 1H), 6.96 (m, 1H), 3.91 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.73 (s, 2H, SCH_2), 3.70 (s, 2H, SCH_2), 2.04 (s, 3H, SCH_3), 2.03 (s, 3H, SCH_3). UPLC (ESI+) m/z: $[M + H]^+$ 379.

3-Methoxy-5-[(methylsulfanyl)methyl]aniline. To a solution of 1-methoxy-3-[(Z)-{3-methoxy-5-[(methylsulfanyl)methyl]phenyl}-*NNO*-azoxy]-5-[(methylsulfanyl)methyl]benzene (2.80 g, 7.40 mmol) in 1,4-dioxane (60 mL) was added iron (5.98 g, 107 mmol, 14 equiv) at rt. After heating to reflux, concd HCl (26 mL) was added dropwise over 3 h to the mixture. Then, the mixture was cooled to rt and stirring was continued for 14 h. After that, the reaction mixture was poured onto ice and the resulting mixture was basified to pH 9 by the addition of 2 N aq NaOH. The mixture was extracted with EtOAc and the organic extract was filtered through a Whatman filter. After concentration of the filtrate under reduced pressure, the crude product was purified by column chromatography (silica gel, hexane/EtOAc gradient) to afford the title compound (1.54 g, quant) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 6.28 (m, 2H), 6.12 (m, 1H), 3.76 (s, 3H, OCH_3), 3.66 (br, 2H, NH_2), 3.55 (s, 2H, SCH_2), 2.02 (s, 3H, SCH_3). UPLC (ESI+) m/z: $[M + H]^+$ 184.

5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-N-{3-methoxy-5-[(methylsulfanyl)methyl]phenyl}pyrimidin-2-amine. A mixture of 3-methoxy-5-[(methylsulfanyl)methyl]aniline (436 mg, 1.59 mmol, 1.0 equiv), 2-chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidine (840 mg, 3.24 mmol, 1.0 equiv), XPhos Pd G1 (203 mg, 0.25 mmol, 7.5 mol %), XPhos (117 mg, 0.25 mmol, 7.5 mol %), and finely ground potassium phosphate (3.48 g, 16.4 mmol, 5.0 equiv) in toluene (19 mL) and NMP (4 mL) was degassed and repressured with argon at rt. The mixture was heated in a sealed vial to 130 °C in a microwave reactor for 3 h. After cooling to rt, brine was added, and the mixture was extracted with EtOAc. The combined organic extracts were filtered through a Whatman filter and the filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography (silica gel, hexane to hexane/EtOAc 7:3 gradient), recrystallized from hexane/EtOAc, and again purified by flash chromatography (silica gel, hexane to hexane/EtOAc 8:2 gradient). The product-containing fractions were concentrated and the precipitate was collected by filtration to afford the title compound (655 mg, 1.62 mmol, 50% yield) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.30 (m, 1H), 7.51 (m, 1H), 7.36 (m, 1H), 7.15 (br, 1H), 7.03 (m, 1H), 6.80 (m, 1H), 6.74 (m, 1H), 6.55 (m, 1H), 3.86 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.63 (s, 2H, SCH_2), 2.02 (s, 3H, SCH_3). UPLC (ESI+) m/z: $[M + H]^+$ 404.

(rac)-[(3-{[5-Fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}-5-methoxybenzyl)(methyl)-λ^4-sulfanylidene]cyanamide. To a mixture of 5-fluoro-4-(4-fluoro-2-methoxyphenyl)-*N*-{3-methoxy-5-[(methylsulfanyl)methyl]phenyl}pyrimidin-2-amine (638 mg, 1.58 mmol) and cyanamide (133 mg, 3.16 mmol, 2.0 equiv) in DCM (20 mL) was added at 0 °C iodosobenzene diacetate (560 mg, 1.74 mmol, 1.1 equiv). After stirring for 3 h at that temperature, the mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (silica gel, DCM to DCM/EtOH 9:1 gradient) to afford the title compound (410 mg, 0.92 mmol, 58% yield) as a brownish solid. ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.32 (m, 1H), 7.47 (m, 1H), 7.33 (m, 1H), 7.25 (m, 2H), 6.82 (m, 1H), 6.75 (m, 1H), 6.52 (m, 1H), 4.37 (d, J = 12.6 Hz, 1H), 4.13 (d, J = 12.6 Hz, 1H), 3.86 (s, 3H), 3.81 (s, 3H), 2.71 (s, 3H). UPLC (ESI+) m/z: $[M + H]^+$ 444.

(rac)-[(3-{[5-Fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}-5-methoxybenzyl)(methyl)oxido-λ^6-sulfanylidene]-cyanamide. To a mixture of (*rac*)-[(3-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}-5-methoxybenzyl)(methyl)-λ^4-sulfanylidene]cyanamide (394 mg, 0.89 mmol) in acetone (10 mL) was added at rt $KMnO_4$ (281 mg, 1.78 mmol, 2.0 equiv), and the mixture was heated to 50 °C for 1 h. After cooling to rt, the mixture was concentrated under reduced pressure and purified by flash chromatography (silica gel, DCM to DCM/EtOH 95:5 gradient) to afford the title compound (307 mg, 0.67 mmol, 75% yield) as a light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6, 27 °C): δ 9.92 (m, 1H), 8.53 (m, 1H), 7.53 (m, 2H), 7.34 (m, 1H), 7.09 (m, 1H), 6.92 (m, 1H), 6.62 (m, 1H), 4.87 (m, 2H), 3.80 (s, 3H), 3.70 (s, 3H), 3.31 (s, 3H). UPLC (ESI+) m/z: $[M + H]^+$ 460.

*(rac)-5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-N-{3-methoxy-5-[(S-methylsulfonimidoyl)methyl]phenyl}pyrimidin-2-amine (**10**).* To a solution of (*rac*)-[(3-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)-pyrimidin-2-yl]amino}-5-methoxybenzyl)(methyl)oxido-λ^6-sulfanylidene]cyanamide (302 mg, 0.66 mmol) in DCM (30 mL) was added at 0 °C trifluoroacetic anhydride (TFAA, 0.28 mL, 1.97 mmol, 3.0 equiv). The mixture was warmed to rt and stirred for 2 h at that temperature. Then, the mixture was concentrated under reduced pressure (at 40 °C), the residue was taken up in MeOH (5 mL), and K_2CO_3 (454 mg, 3.29 mmol, 5.0 equiv) was added at rt. After stirring for 2 h at rt and 14 h at 4 °C, the mixture was diluted with EtOAc and THF and washed with brine. The organic phase was filtered through a Whatman filter, the filtrate was concentrated under reduced pressure, and the crude product was purified by preparative HPLC (basic conditions) to afford **10** (27 mg, 0.06 mmol, 9% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.30 (d, J = 2.02 Hz, 1H), 7.49 (t, J = 7.18 Hz, 1H), 7.43−7.46 (m, 1H), 7.25−7.29 (m, 2H), 7.19 (s, 1H), 6.72−6.83 (m, 2H), 6.59−6.63 (m, 1H), 4.32 (d, J = 13.14 Hz, 1H,

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sulfoximine−CH_2, H_A, AB system), 4.20 (d, J = 12.88 Hz, 1H, sulfoximine−CH_2, H_B, AB system), 3.86 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 2.93 (s, 3H, SCH_3). ^{13}C NMR (100.67 MHz, DMSO-d_6): δ 164.36 (d, $^1J_{CF}$ = 246.68 Hz, $1C_q$), 159.34 (s, $1C_q$), 158.54 (d, $^3J_{CF}$ = 10.60 Hz, $1C_q$), 156.49 (d, $^4J_{CF}$ = 2.54 Hz, $1C_q$), 151.35 (d, $^2J_{CF}$ = 16.95 Hz, $1C_q$), 150.19 (d, $^1J_{CF}$ = 251.35 Hz, $1C_q$), 145.83 (d, $^2J_{CF}$ = 24.58 Hz, 1CH), 141.52 (s, $1C_q$), 132.03 (d, $^3J_{CF}$ = 11.02 Hz, 1CH), 131.79 (s, $1C_q$), 119.1 (m, $1C_q$), 113.56 (s, 1CH), 109.59 (s, 1CH), 107.30 (d, $^2J_{CF}$ = 22.04 Hz, 1CH), 104.10 (s, 1CH), 100.17 (d, $^2J_{CF}$ = 26.28 Hz, 1CH), 62.82 (s, $1CH_2$), 56.36 (s, $1CH_3$), 55.00 (s, $1CH_3$), 41.00 (s, $1CH_3$). IR (diamond ATR, cm^{-1}): 3304 (w), 3256 (w), 3103 (w), 2964 (w), 2943 (w), 1601 (m), 1545 (m), 1454 (m), 1429 (s), 1310 (m), 1150 (m), 1026 (s), 957 (m), 851 (m), 829 (m). ESI-HRMS m/z: $[M + H]^+$ calcd for $C_{20}H_{21}F_2N_4O_3S$, 435.1303; found, 435.1300.

*Compounds **11** and **12**. (2,3-Difluoro-5-nitrophenyl)methanol.* To a solution of 2,3-difluoro-5-nitrobenzoic acid (9.00 g, 44.3 mmol) in THF (85 mL) was added dropwise at 0 °C 1 M borane in THF (177 mL, 177 mmol, 4.0 equiv). After the addition, the mixture was warmed to rt and stirred for 14 h at that temperature. Then, the mixture was cooled to 0 °C and MeOH was added carefully until evolution of gas ceased. The mixture was then diluted with EtOAc, washed with 1 N aq NaOH and brine, and then dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by flash chromatography (silica gel, hexane/EtOAc gradient) to afford the title compound as a yellowish oil which crystallized upon standing (8.20 g, 43.4 mmol, 98% yield). ^1H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.26 (m, 1H), 8.03 (m, 1H), 4.89 (br, 2H), 2.13 (br, 1H). UPLC (ESI−) m/z: $[M - H]^-$ 188.

(5-Amino-2,3-difluorophenyl)methanol. $TiCl_3$ solution (ca. 15% in ca. 10% HCl; 144 mL) was added to a stirred solution of (2,3-difluoro-5-nitrophenyl)methanol (4.00 g, 21.2 mmol) in THF (250 mL) at 0 °C, after which the mixture was warmed to rt and stirred for 16 h at that temperature. Then, the mixture was neutralized by the addition of 1 N NaOH and the resulting dark mass was extracted with EtOAc. The combined organic phases were washed with brine, filtered using a Whatman filter, and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica gel, hexane/EtOAc gradient) to afford the title compound (3.33 g, 20.9 mmol, 99% yield) as a brown solid. ^1H NMR (400 MHz, $CDCl_3$, 27 °C): δ 6.47 (m, 1H), 6.42 (m, 1H), 4.69 (br, 2H). UPLC (ESI+) m/z: $[M + H]^+$ 160.

3-(Chloromethyl)-4,5-difluoroaniline. To a solution of (5-amino-2,3-difluorophenyl)methanol (4.13 g, 26.0 mmol) in DCM (78 mL) and NMP (11 mL) was added at rt thionyl chloride (4.73 mL, 64.9 mmol, 2.5 equiv). After stirring for 14 h at that temperature, the mixture was poured onto a mixture of ice/$NaHCO_3$/NaCl and stirred for 2 h. After cessation of the reaction, the mixture was extracted with EtOAc, and the organic extracts were filtered through a Whatman filter and concentrated under reduced pressure to afford the title compound as a crude product which was directly used in the next step.

3,4-Difluoro-5-[(methylsulfanyl)methyl]aniline. Sodium methanethiolate (3.61 g, 51.6 mmol) was added portionwise to a solution of 3-(chloromethyl)-4,5-difluoroaniline from the previous step in EtOH (75 mL) at 0 °C. The mixture was warmed to rt and stirred for 14 h at that temperature. After that, the mixture was diluted with brine and extracted with EtOAc. The organic extracts were filtered through a Whatman filter and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, hexane/EtOAc gradient) to afford the title compound (1.27 g, 6.71 mmol, 26% yield over two steps) as a brownish oil. ^1H NMR (400 MHz, $CDCl_3$, 27 °C): δ 6.36 (m, 2H), 3.62 (br, 4H, SCH_2, NH_2), 2.08 (s, 3H, SCH_3). UPLC (ESI+) m/z: $[M + H]^+$ 190.

N-{3,4-Difluoro-5-[(methylsulfanyl)methyl]phenyl}-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-amine. A mixture of 3,4-difluoro-5-[(methylsulfanyl)methyl]aniline (600 mg, 3.17 mmol, 1.0 equiv), 2-chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidine (1.06 g, 4.12 mmol, 1.3 equiv), XPhos Pd G1 (197 mg, 0.24 mmol, 7.5 mol %), XPhos (113 mg, 0.24 mmol, 7.5 mol %), and finely ground potassium phosphate (3.37 g, 15.9 mmol, 5.0 equiv) in toluene (21 mL) and NMP (4 mL) was degassed and repressured with argon at rt. The mixture was heated in a sealed vial to 130 °C in a microwave reactor for 3 h. After cooling to rt, brine was added, and the mixture was extracted with EtOAc. The combined organic extracts were filtered through a Whatman filter and the filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography (silica gel, hexane to hexane/EtOAc gradient) to afford the title compound (724 mg, 1.77 mmol, 56% yield) as a yellow solid. ^1H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.30 (d, J = 2.02 Hz, 1H), 7.71 (ddd, J = 12.63, 6.82, 2.78 Hz, 1H), 7.48 (dd, J = 8.46, 6.69 Hz, 1H), 7.07−7.22 (m, 2H), 6.78−6.84 (m, 1H), 6.76 (d, J = 10.85 Hz, 1H), 3.87 (s, 3H, OCH_3), 3.70 (s, 2H, SCH_2), 2.06 (s, 3H, SCH_3).

(rac)-[(2,3-Difluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)-pyrimidin-2-yl]amino}benzyl)(methyl)-λ^4-sulfanylidene]cyanamide. To a mixture of *N*-{3,4-difluoro-5-[(methylsulfanyl)methyl]phenyl}-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-amine (715 mg, 1.75 mmol) and cyanamide (147 mg, 3.49 mmol, 2.0 equiv) in DCM (10 mL) was added at 0 °C iodosobenzene diacetate (619 mg, 1.92 mmol, 1.1 equiv). After stirring for 4 h at that temperature, the mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (silica gel, DCM to DCM/EtOH 8:2 gradient) to afford the title compound (655 mg, 1.46 mmol, 83% yield) as a pinkish solid. ^1H NMR (300 MHz, $CDCl_3$): δ 8.33 (d, J = 1.88 Hz, 1H), 7.89 (ddd, J = 12.53, 7.06, 2.64 Hz, 1H), 7.75 (s, 1H), 7.47 (dd, J = 8.29, 6.78 Hz, 1H), 7.28−7.37 (m, 1H), 6.73−6.87 (m, 2H), 4.33 (m, 2H, SCH_2), 3.87 (s, 3H, OCH_3), 2.82 (s, 3H, SCH_3). UPLC (ESI+) m/z: $[M + H]^+$ 450.

*(rac)-[(2,3-Difluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)-pyrimidin-2-yl]amino}benzyl)(methyl)oxido-λ^6-sulfanylidene]-cyanamide (**12**).* To a mixture of (*rac*)-[(2,3-difluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}benzyl)(methyl)-λ^4-sulfanylidene]cyanamide (645 mg, 1.44 mmol) in acetone was added at rt $KMnO_4$ (454 mg, 2.87 mmol, 2.0 equiv). The mixture was warmed to 50 °C for 1 h, after which it was cooled to rt and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, hexane/EtOAc gradient) to afford **12** (372 mg, 0.80 mmol, 56% yield) as a colorless solid. ^1H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.35 (d, J = 2.02 Hz, 1H), 7.94 (ddd, J = 12.44, 7.14, 2.65 Hz, 1H), 7.43−7.52 (m, 2H), 7.33 (s, 1H), 6.83 (t, J = 8.11 Hz, 1H), 6.76 (d, J = 10.68 Hz, 1H), 4.65−4.73 (m, 2H, SCH_2), 3.87 (s, 3H, OCH_3), 3.12 (s, 3H, SCH_3). ^{13}C NMR (100.67 MHz, DMSO-d_6): δ 164.44 (d, $^1J_{CF}$ = 247.11 Hz, $1C_q$), 158.59 (d, $^3J_{CF}$ = 11.02 Hz, $1C_q$), 155.96 (d, $^4J_{CF}$ = 2.54 Hz, $1C_q$), 151.68 (d, $^3J_{CF}$ = 16.11 Hz, $1C_q$), 150.48 (d, $^1J_{CF}$ = 251.77 Hz, $1C_q$), 149.15 (dd, $^1J_{CF}$ = 242.44 Hz, $^2J_{CF}$ = 13.56 Hz, $1C_q$), 145.91 (d, $^2J_{CF}$ = 25.07 Hz, 1CH), 143.43 (dd, $^1J_{CF}$ = 244.99 Hz, $^2J_{CF}$ = 13.56 Hz, $1C_q$), 137.32 (dd, $^3J_{CF}$ = 10.17 Hz, $^4J_{CF}$ = 2.97 Hz, $1C_q$), 132.07 (d, $^3J_{CF}$ = 11.02 Hz, 1CH), 118.77 (m, $1C_q$), 117.56 (m, 1CH), 115.74 (m, $1C_q$), 111.99 (s, $1C_q$), 108.30 (d, $^2J_{CF}$ = 22.04 Hz, 1CH), 107.38 (d, $^2J_{CF}$ = 22.04 Hz, 1CH), 100.25 (d, $^2J_{CF}$ = 26.28 Hz, 1CH), 56.35 (s, $1CH_3$), 53.60 (s, $1CH_2$), ∼39.5 (s, overlapped by solvent signals). IR (diamond ATR, cm^{-1}): 3292 (w), 3096 (w), 3001 (w), 2924 (w), 2193 (s), 1612 (m), 1593 (m), 1508 (s), 1423 (s), 1242 (s), 1192 (s), 1024 (s), 982 (m), 957 (m), 895 (m), 829 (m), 781 (m). UPLC (ESI+) m/z: $[M + H]^+$ 465. ESI-HRMS m/z: $[M + H]^+$ calcd for $C_{20}H_{16}F_4N_5O_2S$, 466.0962; found, 466.0958.

*(rac)-N-{3,4-Difluoro-5-[(S-methylsulfonimidoyl)methyl]phenyl}-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-amine (**11**).* To a solution of (*rac*)-[(2,3-difluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}benzyl)(methyl)oxido-λ^6-sulfanylidene]cyanamide (**12**; 344 mg, 0.74 mmol) in DCM (30 mL) was added at 0 °C TFAA (0.78 mL, 5.54 mmol, 7.5 equiv). The mixture was warmed to rt and stirred for 2 h at that temperature. Then, the mixture was concentrated under reduced pressure (at 40 °C), the residue was taken up in MeOH (5 mL), and K_2CO_3 (511 mg, 3.70 mmol, 5.0 equiv) was added at rt. After stirring for 2 h at rt, the mixture was diluted with EtOAc and THF and washed with brine. The organic phase was filtered through a Whatman filter, the filtrate

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was concentrated under reduced pressure, and the crude product was purified by flash chromatography (silica gel, DCM to DCM/EtOH 8:2 gradient) to afford **11** (300 mg, 0.68 mmol, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.30 (m, 1H), 7.86 (m, 1H), 7.47 (m, 1H), 7.26 (m, 2H), 6.82 (m, 1H), 6.76 (m, 1H), 4.42 (d, J = 13.6 Hz, 1H, sulfoximine−CH_2, H_A, AB system), 4.35 (d, J = 13.6 Hz, 1H, sulfoximine−CH_2, H_B, AB system), 3.87 (s, 3H, OCH_3), 2.96 (s, 3H, SCH_3), 2.76 (s, 1H, sulfoximine−NH). ^{13}C NMR (100.67 MHz, DMSO-d_6): δ 164.40 (d, $^1J_{CF}$ = 247.11 Hz, $1C_q$), 158.57 (d, $^3J_{CF}$ = 11.02 Hz, $1C_q$), 156.09 (d, $^4J_{CF}$ = 2.97 Hz, $1C_q$), 151.61 (d, $^3J_{CF}$ = 15.26 Hz, $1C_q$), 150.4 (d, $^1J_{CF}$ = 251.34 Hz, $1C_q$), 149.06 (dd, $^1J_{CF}$ = 241.60 Hz, $^2J_{CF}$ = 13.56 Hz, $1C_q$), 145.92 (d, $^2J_{CF}$ = 25.01 Hz, 1CH), 143.35 (dd, $^1J_{CF}$ = 242.87 Hz, $^2J_{CF}$ = 13.14 Hz, $1C_q$), 136.86 (dd, $^3J_{CF}$ = 10.17 Hz, $^4J_{CF}$ = 2.97 Hz, $1C_q$), 132.05 (d, $^3J_{CF}$ = 10.17 Hz, 1CH), 120.02 (d, $^2J_{CF}$ = 13.56 Hz, $1C_q$), 118.13 (m, $1C_q$), 117.22 (m, 1CH), 107.34 (d, $^2J_{CF}$ = 12.04 Hz, 1CH), 107.15 (d, $^2J_{CF}$ = 22.04 Hz, 1CH), 100.23 (d, $^2J_{CF}$ = 26.28 Hz, 1CH), 56.35 (s, $1CH_3$), 56.06 (d, $^3J_{CF}$ = 1.27 Hz, $1CH_2$), 41.54 (s, $1CH_3$). IR (diamond ATR, cm^{-1}): 3300 (br s), 3099 (w), 2982 (w), 2926 (w), 1634 (w), 1610 (m), 1597 (m), 1506 (s), 1431 (s), 1327 (m), 1207 (s), 1151 (m), 1026 (m), 959 (m), 870 (m), 858 (m), 829 (m), 783 (m). ESI-HRMS m/z: $[M + H]^+$ calcd for $C_{19}H_{17}F_4N_4O_2S$, 441.1009; found, 441.1007.

*Compound **13**. (5-{[5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-pyrimidin-2-yl]amino}pyridin-3-yl)methanol.* A mixture of 2-chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidine (2.13 g, 7.89 mmol), (5-aminopyridin-3-yl)methanol (1.29 g, 9.87 mmol; ABCR), XPhos Pd G1 (653 mg, 0.79 mmol), XPhos (376 mg, 0.79 mmol), and potassium phosphate (8.38 g, 39.47 mmol) in toluene (210 mL) and NMP (21 mL) was degassed using argon. The mixture was stirred under argon for 3 h at 130 °C. After cooling, the reaction mixture was partitioned between H_2O and EtOAc. The organic layer was washed with saturated NaCl, dried over Na_2SO_4, filtered, and concentrated to afford an oil. Purification by column chromatography (silica gel, hexane to hexane/EtOAc 1:1, then DCM to DCM/EtOAc to EtOAc/MeOH 8:2) afforded the desired product (3.12 g, 9 mmol, 91%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.96 (s, 1H), 8.79 (d, J = 2.53 Hz, 1H), 8.60 (d, J = 2.02 Hz, 1H), 8.08−8.14 (m, 2H), 7.55 (dd, J = 6.82, 8.59 Hz, 1H), 7.13 (dd, J = 2.40, 11.49 Hz, 1H), 6.96 (dt, J = 2.27, 8.46 Hz, 1H), 5.27 (t, J = 5.56 Hz, 1H), 4.50 (d, J = 5.56 Hz, 2H), 3.84 (s, 3H). UPLC (ESI+) m/z: $[M + H]^+$ 345.

N-[5-(Chloromethyl)pyridin-3-yl]-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-amine. (5-{[5-Fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}pyridin-3-yl)methanol (3.1 g, 9 mmol) was dissolved in DCM (66 mL) and NMP (6.6 mL). The mixture was cooled to 0 °C and thionyl chloride (1.64 mL, 22.5 mmol) was slowly added. The cold bath was removed, and the mixture was stirred at rt for 2 h. Then, the mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered, and concentrated to afford an oil (3.85 g) that was used without further purification. UPLC (ESI+) m/z: $[M + H]^+$ 363.

5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-N-{5-[(methylsulfanyl)-methyl]pyridin-3-yl}pyrimidin-2-amine. *N*-[5-(Chloromethyl)-pyridin-3-yl]-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-amine (693 mg, 1.91 mmol) was dissolved in EtOH (100 mL) and sodium methanethiolate (268 mg, 3.82 mmol) was added. The mixture was stirred at rt for 3 h. Then, it was diluted with brine and extracted with EtOAc (2×). The combined organic phases were washed with H_2O, dried (Na_2SO_4), filtered, and concentrated to afford the desired product (740 mg, 1.98 mmol) that was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.98 (s, 1H), 8.76 (d, J = 2.53 Hz, 1H), 8.60 (d, J = 2.02 Hz, 1H), 8.16 (t, J = 2.15 Hz, 1H), 8.07 (d, J = 2.02 Hz, 1H), 7.55 (dd, J = 6.82, 8.59 Hz, 1H), 7.13 (dd, J = 2.40, 11.49 Hz, 1H), 6.96 (dt, J = 2.27, 8.46 Hz, 1H), 3.84 (s, 3H), 3.68 (s, 2H), 1.96 (s, 3H). UPLC (ESI+) m/z: $[M + H]^+$ 375.

(rac)-{[(5-{[5-Fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}pyridin-3-yl)methyl](methyl)-λ^4-sulfanylidene}-cyanamide. To a cooled solution (0 °C) of 5-fluoro-4-(4-fluoro-2-methoxyphenyl)-*N*-{5-[(methylsulfanyl)methyl]pyridin-3-yl}-pyrimidin-2-amine (730 mg, 1.89 mmol) and cyanamide (159 mg, 3.78 mmol) in DCM (22 mL), iodosobenzene diacetate (670 mg, 2.08 mmol) was added. The mixture was stirred at rt for 2.5 h, then diluted with DCM, and washed with brine and sodium thiosulfate solution (10%). The organic layer was dried over Na_2SO_4, filtered, and concentrated to afford a foam. The crude product was purified by column chromatography (EtOAc to EtOAc/MeOH 4:1) to afford the desired product (672 mg, 1.62 mmol, 85%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.15 (s, 1H), 8.91 (d, J = 2.27 Hz, 1H), 8.60 (d, J = 2.02 Hz, 1H), 8.24 (t, J = 2.15 Hz, 1H), 8.16 (d, J = 2.02 Hz, 1H), 7.58 (dd, J = 6.82, 8.34 Hz, 1H), 7.13 (dd, J = 2.27, 11.37 Hz, 1H), 6.97 (dt, J = 2.53, 8.46 Hz, 1H), 4.26−4.56 (m, 2H), 3.84 (s, 3H), 2.86 (s, 3H). UPLC (ESI+) m/z: $[M + H]^+$ 415.

(rac)-{[(5-{[5-Fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}pyridin-3-yl)methyl](methyl)oxido-λ^6-sulfanylidene}-cyanamide. To a mixture of (*rac*)-{[(5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}pyridin-3-yl)methyl](methyl)-λ^4-sulfanylidene}cyanamide (650 mg, 1.56 mmol) in acetone (15.6 mL) was added at rt $KMnO_4$ (496 mg, 3.13 mmol), and the mixture was heated to 50 °C for 1 h. After cooling to rt, the mixture was concentrated under reduced pressure and purified by chromatography (silica gel, DCM to DCM/MeOH 4:1 gradient) to afford the title compound (385 mg, 0.89 mmol, 57% yield) as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.17 (s, 1H), 8.94 (d, J = 2.53 Hz, 1H), 8.61 (d, J = 2.02 Hz, 1H), 8.31 (t, J = 2.15 Hz, 1H), 8.20 (d, J = 2.02 Hz, 1H), 7.56 (dd, J = 6.82, 8.59 Hz, 1H), 7.13 (dd, J = 2.40, 11.49 Hz, 1H), 6.96 (dt, J = 2.40, 8.40 Hz, 1H), 5.01−5.10 (m, 2H), 3.84 (s, 3H), 3.41 (s, 3H). UPLC (ESI+) m/z: $[M + H]^+$ 431.

*(rac)-5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-N-{5-[(S-methylsulfonimidoyl)methyl]pyridin-3-yl}pyrimidin-2-amine (**13**).* To a cooled (0 °C) solution of (*rac*)-{[(5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}pyridin-3-yl)methyl](methyl)-oxido-λ^6-sulfanylidene}cyanamide (112 mg, 0.26 mmol) in DCM (11.3 mL), TFAA (0.11 mL, 0.78 mmol) was added. Then, the mixture was stirred at rt for 2 h. The solvent was evaporated and the residue was dissolved in MeOH (1.8 mL) and K_2CO_3 (180 mg, 1.3 mmol) was added. After 30 min, the mixture was diluted with THF and partitioned between H_2O and EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by chromatography (EtOAc/MeOH 3% to EtOAc/MeOH 40%) to afford **13** (63 mg, 0.16 mmol, 59% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.20 (s, 1H), 8.95 (br s, 1H), 8.62 (d, J = 1.52 Hz, 1H), 8.21−8.32 (m, 2H), 7.56 (t, J = 7.73 Hz, 1H), 7.14 (dd, J = 2.03, 11.41 Hz, 1H), 6.96 (dt, J = 2.28, 8.36 Hz, 1H), 4.68−4.81 (m, 2H), 3.84 (s, 3H), 3.12 (s, 3H). ^{13}C NMR (100.67 MHz, DMSO-d_6): δ 164.41 (d, $^1J_{CF}$ = 247.11 Hz, $1C_q$), 158.56 (d, $^3J_{CF}$ = 10.60 Hz, $1C_q$), 156.31 (d, $^4J_{CF}$ = 2.54 Hz, $1C_q$), 151.64 (d, $^2J_{CF}$ = 15.26 Hz, $1C_q$), 150.50 (d, $^1J_{CF}$ = 251.35 Hz, $1C_q$), 145.97 (d, $^2J_{CF}$ = 25.01 Hz, 1CH), 144.22 (s, 1CH), 140.13 (s, 1CH), 136.85 (s, $1C_q$), 132.12 (d, $^3J_{CF}$ = 10.60 Hz, 1CH), 127.07 (s, 1CH), 126.39 (s, $1C_q$), 118.92 (m, $1C_q$), 107.36 (d, $^2J_{CF}$ = 21.62 Hz, 1CH), 100.18 (d, $^2J_{CF}$ = 25.86 Hz, 1CH), 59.58 (s, $1CH_2$), 56.38 (s, $1CH_3$), 41.12 (s, $1CH_3$). IR (diamond ATR, cm^{-1}): 3302 (w), 3250 (w), 3125 (w), 2972 (w), 2924 (w), 1682 (m), 1599 (m), 1583 (m), 1543 (s), 1427 (s), 1302 (m), 1281 (s), 1198 (s), 1153 (s), 1030 (s), 953 (m), 881 (m), 837 (s), 785 (s). ESI-HRMS m/z: $[M + H]^+$ calcd for $C_{18}H_{18}F_2N_5O_2S$, 406.1194; found, 406.1145.

*Compound **14**. 3-[(3-Fluoro-5-nitrobenzyl)sulfanyl]propan-1-ol.* Cs_2CO_3 (3.1 g, 9.5 mmol) was added to a solution of 1-(chloromethyl)-3-fluoro-5-nitrobenzene (1.5 g, 7.91 mmol) and 3-mercaptopropanol (0.81 mL, 11.1 mmol) in DMF (15 mL). The mixture was stirred at rt for 90 min. Then, it was diluted with H_2O and extracted with EtOAc (2×). The combined organic phases were washed with LiCl solution (5%), saturated $NaHCO_3$ solution, and brine and dried (Na_2SO_4). The filtrate was concentrated to afford the desired product (2.32 g) that was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02 (s, 1H), 7.82 (td, J = 2.24, 8.15 Hz, 1H), 7.43 (td, J = 2.05, 8.34 Hz, 1H), 3.79 (s, 2H), 3.74 (t, J = 6.03 Hz, 2H), 2.82 (t, J = 7.06 Hz, 1H), 2.57 (t, J = 7.06 Hz, 2H), 1.77−1.90 (m, 2H). UPLC (ESI−) m/z: $[M - H]^-$ 244.

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3-[(3-Amino-5-fluorobenzyl)sulfanyl]propan-1-ol. $TiCl_3$ solution (ca. 15% in ca. 10% HCl; 19.4 mL) was added to a stirred solution of 3-[(3-fluoro-5-nitrobenzyl)sulfanyl]propan-1-ol (1.2 g, 4.89 mmol) in THF (50 mL) at rt, and the mixture was stirred for 116 h. The pH was increased to 10 by the addition of 1 N NaOH, and then the mixture was extracted with EtOAc (2×). The combined organic phases were washed with brine, filtered using a Whatman filter, and concentrated. The residue was purified by column chromatography (DCM to DCM/EtOH 95:5) to afford the desired product (685 mg, 3.18 mmol, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.40−6.45 (m, 2H), 6.27 (td, J = 2.02, 10.36 Hz, 1H), 3.73 (t, J = 6.06 Hz, 2H), 3.60 (s, 2H), 2.56 (t, J = 7.07 Hz, 2H), 1.82 (quin, J = 6.51 Hz, 2H). UPLC (ESI+) m/z: $[M + H]^+$ 216.

3-[(3-Fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)-pyrimidin-2-yl]amino}benzyl)sulfanyl]propan-1-ol. A mixture of 2-chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidine (989 mg, 3.85 mmol), 3-[(3-amino-5-fluorobenzyl)sulfanyl]propan-1-ol (638 mg, 2.96 mmol), XPhos Pd G1 (184 mg, 0.22 mmol), XPhos (106 mg, 0.22 mmol), and finely ground potassium phosphate (3.14 g, 14.8 mmol) in toluene (19 mL) and NMP (3.9 mL) was degassed and repressured with argon at rt. The mixture was heated in a sealed vial to 130 °C in a microwave reactor for 3 h. After cooling to rt, brine was added, and the mixture was extracted with EtOAc. The combined organic extracts were filtered through a Whatman filter and the filtrate was concentrated under reduced pressure. The crude product was purified by preparative HPLC (acidic conditions) to afford the title compound (846 mg, 1.94 mmol, 66% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (d, J = 2.02 Hz, 1H), 7.59 (td, J = 2.21, 11.24 Hz, 1H), 7.49 (dd, J = 6.57, 8.34 Hz, 1H), 7.29 (br s, 1H), 7.16 (s, 1H), 6.82 (dt, J = 2.40, 8.27 Hz, 1H), 6.76 (dd, J = 2.40, 10.74 Hz, 1H), 6.70 (td, J = 1.77, 9.09 Hz, 1H), 3.87 (s, 3H), 3.71 (t, J = 6.06 Hz, 2H), 3.68 (s, 2H), 2.55 (t, J = 7.07 Hz, 2H), 1.77−1.85 (m, 2H). UPLC (ESI−) m/z: $[M - H]^-$ 434.

(rac)-[(3-Fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)-pyrimidin-2-yl]amino}benzyl)(3-hydroxypropyl)-λ^4-sulfanylidene]-cyanamide. To a cooled solution (0 °C) of 3-[(3-fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}benzyl)sulfanyl]-propan-1-ol (774 mg, 1.77 mmol) and cyanamide (150 mg, 3.55 mmol) in DCM (15.4 mL), iodosobenzene diacetate (630 mg, 1.95 mmol) was added. The mixture was stirred at rt for 1 h, then diluted with DCM, and washed with brine and sodium thiosulfate solution (10%). The organic layer was dried over Na_2SO_4, filtered, and concentrated to afford a foam. The crude product was purified by column chromatography (DCM to DCM/EtOH 88:12) to afford the desired product (623 mg, 1.33 mmol, 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30−8.36 (m, 1H), 7.68−7.75 (m, 1H), 7.43−7.54 (m, 2H), 7.32 (s, 1H), 6.80−6.86 (m, 1H), 6.76 (dd, J = 2.02, 10.86 Hz, 1H), 6.64−6.73 (m, 1H), 4.11−4.36 (m, 2H), 3.87 (s, 3H), 3.74−3.84 (m, 2H), 3.02−3.31 (m, 2H), 2.04−2.13 (m, 2H). UPLC (ESI+) m/z: $[M + H]^+$ 476.

*(rac)-[(3-Fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)-pyrimidin-2-yl]amino}benzyl)(3-hydroxypropyl)oxido-λ^6-sulfanylidene]cyanamide (**14**).* (*rac*)-[(3-Fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}benzyl)(3-hydroxypropyl)-λ^4-sulfanylidene]cyanamide (534 mg, 1.12 mmol) was dissolved in acetone (11 mL). $KMnO_4$ (355 mg, 2.25 mmol) was added, and the mixture was stirred at 50 °C for 2 h. Then, additional $KMnO_4$ (89 mg, 0.56 mmol) was added and stirring was continued for 2 h. The mixture was cooled to rt and filtered. The filter cake was rinsed with acetone and EtOH. The combined organic fractions were concentrated. Purification of the crude material by column chromatography (DCM/EtOH 95:5 to 9:1) afforded 14 (11 mg, 2% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (d, J = 1.77 Hz, 1H), 7.67−7.73 (m, 1H), 7.45−7.55 (m, 3H), 6.74−6.87 (m, 3H), 4.57 (s, 2H), 3.87 (s, 3H), 3.73 (t, J = 5.56 Hz, 2H), 3.27−3.37 (m, 2H), 2.04−2.16 (m, 2H). IR (diamond ATR, cm^{-1}): 3271 (w), 3103 (w), 2920 (w), 2192 (s), 1609 (s), 1587 (s), 1506 (m), 1427 (s), 1331 (w), 1281 (m), 1211 (m), 1151 (m), 1049 (m), 1022 (s), 953 (m), 835 (m), 785 (m). UPLC (ESI+) m/z: $[M + H]^+$ 492. ESI-HRMS m/z: $[M + H]^+$ calcd for $C_{22}H_{21}F_3N_5O_3S$, 492.1318; found, 492.1320.

*Compound **15**. 3-[(3-Fluoro-5-nitrobenzyl)sulfanyl]propan-1-amine.* Cs_2CO_3 (8.93 g, 27.4 mmol) was added to a solution of 1-(chloromethyl)-3-fluoro-5-nitrobenzene (2 g, 10.5 mmol) and 3-aminopropane-1-thiol hydrochloride (1.87 g, 14.7 mmol) in DMF (30 mL). The mixture was stirred at rt for 5 h. Then, it was diluted with H_2O and extracted with EtOAc (2×). The combined organic phases were washed with LiCl solution (5%), saturated $NaHCO_3$ solution, and brine and dried (Na_2SO_4). The filtrate was concentrated to afford the desired product (1.93 g, 7.9 mmol, 74% yield) that was used without further purification.

tert-Butyl {3-[(3-Fluoro-5-nitrobenzyl)sulfanyl]propyl}-carbamate. To a solution of 3-[(3-fluoro-5-nitrobenzyl)sulfanyl]-propan-1-amine (1.93 g, 7.9 mmol) in *t*-BuOH, di-*tert*-butyl dicarbonate (2.29 g, 10.5 mmol) was added. The reaction mixture was stirred at rt for 3 h, then diluted with EtOAc, and concentrated. Purification of the remaining material by column chromatography (hexanes to hexanes/EtOH 2:1) afforded the desired product (1.84 g, 5.35 mmol, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99−8.02 (m, 1H), 7.82 (td, J = 2.24, 8.15 Hz, 1H), 7.43 (td, J = 1.89, 8.59 Hz, 1H), 4.56 (br s, 1H), 3.77 (s, 2H), 3.20 (br t, J = 6.44 Hz, 2H), 2.47 (t, J = 7.20 Hz, 2H), 1.76 (quin, J = 6.95 Hz, 2H), 1.43 (s, 9H).

tert-Butyl {3-[(3-Amino-5-fluorobenzyl)sulfanyl]propyl}-carbamate. A solution of *tert*-butyl {3-[(3-fluoro-5-nitrobenzyl)-sulfanyl]propyl}carbamate (1.33 g, 3.82 mmol) in EtOH (40.5 mL) was degassed with argon. 10% palladium on charcoal (3.86 mmol) was added, and the mixture was stirred at rt under an atmosphere of hydrogen for 18 h. The suspension was filtered using a Whatman filter and concentrated. The resulting residue was purified by column chromatography (hexanes to hexanes/EtOAc 1:1) to afford the desired product (216 mg, 0.69 mmol, 18% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.39−6.49 (m, 2H), 6.29 (br d, J = 10.55 Hz, 1H), 4.60 (br s, 1H), 3.89 (br s, 2H), 3.60 (s, 2H), 3.20 (q, J = 6.34 Hz, 2H), 2.47 (t, J = 7.25 Hz, 2H), 1.75 (quin, J = 6.92 Hz, 2H), 1.46 (s, 9H).

tert-Butyl {3-[(3-Fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}benzyl)sulfanyl]propyl}-carbamate. A mixture of 2-chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidine (806 mg, 3.14 mmol), *tert*-butyl {3-[(3-amino-5-fluorobenzyl)sulfanyl]propyl}carbamate (800 mg, 2.42 mmol), XPhos Pd G1 (150 mg, 0.18 mmol), XPhos (86 mg, 0.18 mmol), and finely ground potassium phosphate (2.56 g, 12.1 mmol) in toluene (15.6 mL) and NMP (3.1 mL) was degassed and repressured with argon at rt. The mixture was heated in a sealed vial to 130 °C in a microwave reactor for 2.5 h. After cooling to rt, brine was added, and the mixture was extracted with EtOAc. The combined organic extracts were filtered through a Whatman filter and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (hexanes to hexanes/EtOAc 2:1) to afford the desired product (1.21 g, 2.26 mmol, 93% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (d, J = 1.77 Hz, 1H), 7.65 (br d, J = 11.37 Hz, 1H), 7.52 (dd, J = 6.69, 8.46 Hz, 1H), 7.38 (br s, 1H), 7.17 (br s, 1H), 6.81−6.87 (m, 1H), 6.78 (dd, J = 2.27, 10.86 Hz, 1H), 6.71 (br d, J = 8.84 Hz, 1H), 4.60 (br s, 1H), 3.89 (s, 3H), 3.68 (s, 2H), 3.14−3.23 (m, 2H), 2.46 (t, J = 7.20 Hz, 2H), 1.75 (quin, J = 6.95 Hz, 2H), 1.45 (s, 9H).

(rac)-tert-Butyl {3-[S-(3-Fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}benzyl)-N-(trifluoroacetyl)-sulfinimidoyl]propyl}carbamate. Under an atmosphere of argon, a solution of 2,2,2-trifluoroacetamide (316 mg, 2.79 mmol) in THF (1 mL) was added dropwise to a solution of sodium *tert*-butoxide (179 mg, 1.86 mmol) in THF (1.5 mL), so that the temperature of the mixture remained below 10 °C. Subsequently, a freshly prepared solution of 1,3-dibromo-5,5-dimethylhydantoin (346 mg, 1.21 mmol) in THF (1.5 mL) was added dropwise to the stirred mixture, so that the temperature of the mixture remained below 10 °C. Then, the mixture was stirred for 10 min at 10 °C. Finally, a solution of *tert*-butyl {3-[(3-fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)-pyrimidin-2-yl]amino}benzyl)sulfanyl]propyl}carbamate (997 mg, 1.86 mmol) in THF (1.5 mL) was added dropwise to the stirred mixture, so that the temperature of the mixture remained below 10

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°C. The mixture was stirred for 1 h at 10 °C. The mixture was diluted with toluene (4.0 mL) under cooling, and a solution of sodium sulfite (235 mg, 1.86 mmol) in H_2O (7.0 mL) was added so that the temperature of the mixture remained below 15 °C. Aq NaCl solution was added, and the mixture was extracted with EtOAc (3×). The combined organic layers were filtered using a Whatman filter and concentrated. The residue was purified by column chromatography (DCM to DCM/EtOH 94:6) to afford the desired product (670 mg, 1.04 mmol, 55% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.36 (br s, 1H), 7.76 (br d, J = 11.49 Hz, 1H), 7.46−7.59 (m, 2H), 6.74−6.91 (m, 2H), 6.67 (br d, J = 7.54 Hz, 1H), 4.83 (br s, 1H), 4.45 (br d, J = 12.81 Hz, 1H), 4.22 (br d, J = 13.00 Hz, 1H), 3.89 (s, 3H), 3.14−3.30 (m, 2H), 2.89−3.03 (m, 2H), 1.91−2.04 (m, 2H), 1.44 (br s, 9H).

(rac)-tert-Butyl {3-[S-(3-Fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}benzyl)sulfonimidoyl]-propyl}carbamate. To a solution of (*rac*)-*tert*-butyl {3-[*S*-(3-fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}-benzyl)-*N*-(trifluoroacetyl)sulfinimidoyl]propyl}carbamate (561 mg, 0.869 mmol) in MeOH (17 mL), aq KOH solution (25%) was added until pH 10.5 was reached. Oxone (451 mg, 0.739 mmol) was added, and the mixture was stirred at a pH of 10−11 for 2 h. Then, the mixture was filtered, neutralized with HCl (pH 6.6), diluted with brine, and extracted with EtOAc. The organic layer was washed with aq sodium thiosulfate solution, filtered using a Whatman filter, and concentrated to afford the desired compound (433 mg, 0.77 mmol, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 (d, J = 1.77 Hz, 1H), 7.81 (td, J = 2.02, 11.37 Hz, 1H), 7.48−7.54 (m, 2H), 6.81−6.87 (m, 1H), 6.76−6.81 (m, 2H), 4.85 (br s, 1H), 4.15−4.34 (m, 2H), 3.89 (s, 3H), 3.27 (q, J = 6.40 Hz, 2H), 3.00−3.13 (m, 2H), 2.10 (td, J = 7.04, 14.46 Hz, 2H), 1.45 (s, 9H).

*(rac)-N-(3-{[S-(3-Aminopropyl)sulfonimidoyl]methyl}-5-fluorophenyl)-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-amine (**15**).* To a solution of (*rac*)-*tert*-butyl {3-[*S*-(3-fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}benzyl)-sulfonimidoyl]propyl}carbamate (27 mg, 0.048 mmol) in DCM (2 mL), trifluoroacetic acid (TFA, 36.7 μL, 0.447 mmol) was added. After 2 h, additional TFA (36.7 μL, 0.447 mmol) was added, and the mixture was stirred for 24 h. Then, the mixture was evaporated to dryness. The residue was purified by preparative HPLC (acidic conditions) to afford **15** (14.3 mg, 0.03 mmol, 64% yield). ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.17 (br s, 1H), 8.61 (s, 1H), 8.45 (br s, 1H), 7.81 (d, J = 12.1 Hz, 1H), 7.56 (t, J = 7.53 Hz, 1H), 7.46 (br s, 1H), 7.14 (d, J = 10.9 Hz, 1H), 6.97 (t, J = 7.53 Hz, 1H), 6.83 (d, J = 8.66 Hz, 1H), 4.26−4.36 (m, 2H), 3.85 (s, 3H), 3.03 (t, J = 7.15 Hz, 2H), 2.82 (br s, 2H), 1.96 (br s, 2H). ESI-HRMS m/z: $[M + H]^+$ calcd for $C_{21}H_{23}F_3N_5O_2S$, 466.1525; found, 466.1518.

*Compound **16**. 5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-N-{3-fluoro-5-[(methylsulfanyl)methyl]phenyl}pyrimidin-2-amine.* A mixture of 2-chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidine (750 mg, 2.92 mmol), 3-fluoro-5-[(methylsulfanyl)methyl]aniline (385 mg, 2.25 mmol), XPhos Pd G1 (139 mg, 0.17 mmol), XPhos (80 mg, 0.17 mmol), and finely ground potassium phosphate (2.39 g, 11.2 mmol) in toluene (15 mL) and NMP (3 mL) was degassed and repressured with argon at rt. The mixture was heated in a sealed vial to 130 °C in a microwave reactor for 3 h. After cooling to rt, brine was added, and the mixture was extracted with EtOAc. The combined organic extracts were filtered through a Whatman filter and the filtrate was concentrated under reduced pressure. The crude product was combined with the crude product from another batch of the same size and purified by column chromatography (hexanes to hexanes/EtOAc 2:1) to afford the desired product (1.62 g, 4.14 mmol, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (d, J = 2.02 Hz, 1H), 7.62 (dt, J = 11.37, 2.15 Hz, 1H), 7.49 (dd, J = 8.46, 6.69 Hz, 1H), 7.22−7.29 (m, 2H), 7.12 (s, 1H), 6.67−6.84 (m, 3H), 3.87 (s, 3H), 3.63 (s, 2H), 2.01 (s, 3H).

(rac)-[(3-Fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)-pyrimidin-2-yl]amino}benzyl)(methyl)-λ^4-sulfanylidene]-ammonium 2,4,6-Trimethylbenzenesulfonate. To a solution of ethyl (1*E*)-*N*-[(mesitylsulfonyl)oxy]ethanimidoate (1.82 g, 6.37 mmol, 1.0 equiv) in 1,4-dioxane (6.5 mL) was added dropwise at 0 °C perchloric acid (70%, 6.5 mL), and the mixture was stirred for 10 min at that temperature. Cold H_2O (30 mL) was then added to this mixture, and the resulting emulsion was extracted with DCM (3×). The combined organic extracts were washed with brine and dried over Na_2SO_4. The resulting solution of *O*-(mesitylsulfonyl)hydroxylamine was then added dropwise at 0 °C to a solution of 5-fluoro-4-(4-fluoro-2-methoxyphenyl)-*N*-{3-fluoro-5-[(methylsulfanyl)methyl]phenyl}-pyrimidin-2-amine (2.50 g, 6.39 mmol) in DCM (6.5 mL). The mixture was allowed to warm to rt overnight and then concentrated under reduced pressure to 5 mL. The precipitate was filtered off, and the residue was rinsed with diethyl ether. The combined filtrate was concentrated under reduced pressure to afford the title compound (3.04 g, 5.00 mmol, 78% yield) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6, 27 °C): δ 10.24 (s, 1H), 8.62 (d, J = 2.02 Hz, 1H), 7.85 (dt, J = 12.19, 2.12 Hz, 1H), 7.54 (t, J = 7.32 Hz, 1H), 7.48 (s, 1H), 7.14 (dd, J = 11.37, 2.27 Hz, 1H), 6.97 (td, J = 8.34, 2.27 Hz, 1H), 6.84 (d, J = 8.96 Hz, 1H), 6.73 (s, 2H), 5.96 (s, 2H, S=NH_2^+), 4.58 (d, J = 12.88 Hz, 1H, SCH_AH$_B$, AB system), 4.38 (d, J = 12.88 Hz, 1H, SCH$_A$$H_B$, AB system), 3.83 (s, 3H, OCH_3), 3.03 (s, 3H, SCH_3), 2.53 (s, 6H, mesitylsulfonate-2-CH_3, -6-CH_3), 2.16 (s, 3H, mesitylsulfonate-4-CH_3). UPLC (ESI+) m/z: $[M + H]^+$ 407 (free amine).

*5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-N-{3-fluoro-5-[(S-methylsulfonodiimidoyl)methyl]phenyl}pyrimidin-2-amine (**16**).* To a solution of (*rac*)-[(3-fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}benzyl)(methyl)-λ^4-sulfanylidene]ammonium 2,4,6-trimethylbenzenesulfonate (300 mg, 0.49 mmol) in anhyd DMF (1.3 mL) was added at 0 °C Na_2CO_3 (62.9 mg, 0.59 mmol, 1.2 equiv) and NCS (79.3 mg, 0.59 mmol, 1.2 equiv). After stirring for 15 min at 0 °C, hexamethyldisilazane (0.31 mL, 1.48 mmol, 3.0 equiv) was added, and the mixture was warmed to rt. After stirring for a further 18 h, brine was added to the solution, and the mixture was extracted with EtOAc and DCM. The combined organic extracts were filtered through a Whatman filter and concentrated under reduced pressure. The crude product was purified by preparative HPLC [Agilent Prep 1200 system (2 × Prep Pump, DLA, MWD, Prep FC); column: Waters XBridge C18 5 μm, 100 × 30 mm; eluent A: H_2O + 0.2% NH_3, eluent B: MeCN; gradient: 0−10 min 25−55% B, 10−12 min 100% B; flow: 60 mL/min; rt; solution: 243 mg in 3.2 mL DMSO; injection: 2 × 1.6 mL; detection: UV 225 nm; t_R = 5.72−6.42 min] to afford **16** (40.0 mg, 0.09 mmol, 19% yield) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6, 27 °C): δ 10.04 (s, 1H, aniline−NH), 8.59 (d, J = 2.02 Hz, 1H), 7.73 (dt, J = 12.00, 2.21 Hz, 1H), 7.54 (dd, J = 8.34, 6.82 Hz, 1H), 7.46 (s, 1H), 7.12 (dd, J = 11.37, 2.27 Hz, 1H), 6.95 (td, J = 8.46, 2.53 Hz, 1H), 6.83 (br d, J = 9.60 Hz, 1H), 4.24 (s, 2H, SCH_2), 3.83 (s, 3H, OCH_3), 2.80 (s, 3H, SCH_3). ^{13}C NMR (100.65 MHz, DMSO-d_6) δ: 164.39 (d, $^1J_{CF}$ = 247.11 Hz, 1C$_q$), 161.83 (d, $^1J_{CF}$ = 239.48 Hz, 1C$_q$), 158.57 (d, $^3J_{CF}$ = 11.02 Hz, 1C$_q$), 156.19 (d, $^4J_{CF}$ = 2.97 Hz, 1C$_q$), 151.17 (d, $^3J_{CF}$ = 14.41 Hz, 1C$_q$), 150.36 (d, $^1J_{CF}$ = 251.77 Hz, 1C$_q$), 145.88 (d, $^2J_{CF}$ = 25.00 Hz, 1CH), 141.88 (d, $^2J_{CF}$ = 12.29 Hz, 1C$_q$), 133.15 (d, $^3J_{CF}$ = 10.17 Hz, 1C$_q$), 132.04 (d, $^3J_{CF}$ = 11.02 Hz, 1CH), 118.88 (m, 1C$_q$), 116.97 (d, $^4J_{CF}$ = 1.70 Hz, 1CH), 110.41 (d, $^2J_{CF}$ = 22.04 Hz, 1CH), 107.33 (d, $^2J_{CF}$ = 22.04 Hz, 1CH), 104.47 (d, $^2J_{CF}$ = 26.70 Hz, 1CH), 100.22 (d, $^2J_{CF}$ = 25.85 Hz, 1CH), 64.32 (s, 1CH_2), 56.35 (s, 1CH_3), 44.99 (s, 1CH_3). IR (diamond ATR, cm^{-1}): 3207 (w), 3190 (w), 3108 (w), 3034 (w), 2971 (w), 2728 (w), 1716 (w), 1599 (m), 1543 (s), 1434 (s), 1317 (m), 1284 (s), 1194 (s), 1155 (s), 1031 (s), 957 (m), 837 (m), 785 (s). UPLC (ESI+) m/z: $[M + H]^+$ 422. ESI-HRMS m/z: $[M + H]^+$ calcd for $C_{19}H_{19}F_3N_5OS$, 422.1263; found, 422.1263.

*Compound **17**. 5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-pyrimidin-2-amine.* A mixture of 2-chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidine (2.00 g, 7.79 mg) in 7 N methanolic ammonia (20 mL) was heated to 130 °C in a microwave reactor for 2 h. After cooling to rt, the mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (silica gel, hexane to hexane/EtOAc 50:50 gradient) to afford the title compound (1.11 g, 4.68 mmol, 60% yield) as a beige solid. ^{1}H NMR (300 MHz, $CDCl_3$, 27 °C): δ 8.19 (d, J = 1.70 Hz, 1H), 7.43 (dd, J =

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8.48, 6.78 Hz, 1H), 6.72−6.84 (m, 2H), 5.02 (br s, 2H, NH_2), 3.86 (s, 3H, OCH_3). UPLC (ESI+) m/z: $[M + H]^+$ 238.

(3-Fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}phenyl)methanol. To a mixture of 5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-amine (130 mg, 0.55 mmol, 1.0 equiv), (3-bromo-5-fluorophenyl)methanol (112 mg, 0.55 mmol, 1.0 equiv), and 1,4-dioxane (1.3 mL) in a sealable tube was added at rt Cs_2CO_3 (214 mg, 0.66 mmol, 1.2 equiv), and the tube was purged several times with argon. Then, $Pd_2(dba)_3$ (25.1 mg, 0.027 mmol, 5 mol %) and Xantphos (31.7 mg, 0.055 mmol, 10 mol %) were added, and the tube was purged with argon again. After that, the tube was sealed and heated to 100 °C for 20 h and then cooled to rt upon which further $Pd_2(dba)_3$ (25.1 mg, 0.027 mmol, 5 mol %) and Xantphos (31.7 mg, 0.055 mmol, 10 mol %) were added. After heating to 100 °C for a further 22 h, the mixture was cooled to rt and diluted with EtOAc, washed with brine, and filtered through a Whatman filter. The filtrate was concentrated under reduced pressure and purified by flash chromatography (silica gel, hexane to hexane/EtOAc 50:50) and preparative HPLC [Waters AutoPurification system (Pump 254, Sample Manager 2767, CFO, DAD 2996, ELSD 2424, SQD 3100); column: Waters XBridge C18 5 μm, 100 × 30 mm; eluent A: H_2O + 0.2% NH_3, eluent B: MeCN; gradient: 0−8 min 10−100% B; flow: 50 mL/min; rt; solution: 149 mg in 3.0 mL DMSO; injection: 3 × 1 mL; detection: DAD scan 210−400 nm; t_R = 5.84−6.00 min] to afford the title compound (26.0 mg, 0.07 mmol, 13% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.35 (d, J = 1.88 Hz, 1H), 7.63−7.72 (m, 1H), 7.52 (dd, J = 8.48, 6.78 Hz, 1H), 7.26 (br s, 1H), 7.20 (s, 1H), 6.74−6.88 (m, 3H), 4.71 (d, J = 6.03 Hz, 2H, CH_2OH), 3.90 (s, 3H, OCH_3). UPLC (ESI+) m/z: $[M + H]^+$ 362.

N-[3-(Aminomethyl)-5-fluorophenyl]-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-amine (17). To a solution of (3-fluoro-5-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl]amino}-phenyl)methanol (20 mg, 0.055 mmol) in DMF (0.6 mL) was added dropwise at 0 °C thionyl chloride (10 μL, 0.138 mmol, 2.5 equiv). The mixture was then allowed to warm to rt over 2 h, after which it was diluted with EtOAc (100 mL) and slowly basified in an ice bath by the addition of aq $NaHCO_3$/NaCl solution until pH 8. The mixture was then extracted with EtOAc/THF, the combined extracts were filtered through a Whatman filter, and the filtrate was concentrated under reduced pressure. The crude benzyl chloride was dissolved in 7 N methanolic ammonia (7.3 mL) and heated in a sealed tube to 80 °C for 14 h. After cooling to rt, the mixture was concentrated under reduced pressure, and the crude product was purified by preparative HPLC (basic conditions) to afford **17** (9.8 mg, 0.027 mmol, 50% yield) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$, 27 °C): δ 8.32 (d, J = 1.59 Hz, 1H), 7.61 (br d, J = 11.13 Hz, 1H), 7.49 (t, J = 7.49 Hz, 1H), 7.19−7.24 (m, 1H), 7.12 (s, 1H), 6.81 (t, J = 8.05 Hz, 1H), 6.76 (d, J = 10.97 Hz, 1H), 6.69 (br d, J = 9.22 Hz, 1H), 3.87 (s, 3H, OCH_3), 3.85 (s, 2H, CH_2NH_2). ESI-HRMS m/z: $[M + H]^+$ calcd for $C_{18}H_{16}F_3N_4O$, 361.1277; found, 361.1276.

*Compound **24**, Enantiomers **25** and **26** (Scheme 1). 4-Chloro-6-(4-fluoro-2-methoxyphenyl)pyrimidine (**20**).* Under an atmosphere of argon, a mixture of 4,6-dichloropyrimidine (**18**; 5.00 g, 33.56 mmol), (4-fluoro-2-methoxyphenyl)boronic acid (**19**; 6.27 g, 36.92 mmol), and $[Pd(dppf)Cl_2]$ (1:1 complex with DCM; 2.74 g, 3.36 mmol) in 2 M aq K_2CO_3 (50 mL) and DME (101 mL) was stirred for 150 min at 90 °C. After cooling, the mixture was diluted with EtOAc and washed with dilute aq NaCl solution. The organic layer was filtered using a Whatman filter and concentrated. The residue was purified by chromatography (hexane/EtOAc 5 to 40%) to afford **20** (6.35 g, 26.61 mmol, 79% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 9.00 (m, 1H), 8.13 (m, 1H), 8.02 (m, 1H), 6.82 (m, 1H), 6.75 (m, 1H), 3.94 (s, 3H). UPLC (ESI+) m/z: $[M + H]^+$ 239.

*6-(4-Fluoro-2-methoxyphenyl)-N-{4-[(methylsulfanyl)methyl]-pyridin-2-yl}pyrimidin-4-amine (**22**).* A mixture of 4-chloro-6-(4-fluoro-2-methoxyphenyl)pyrimidine (**20**; 475 mg, 1.99 mmol), 4-[(methylsulfanyl)methyl]pyridin-2-amine (**21**; 614 mg, 3.98 mmol; UkrOrgSynthesis Ltd.), Xantphos (51 mg, 0.09 mmol), and Cs_2CO_3 (972 mg, 2.99 mmol) in 1,4-dioxane (7.1 mL) was degassed using argon. $Pd_2(dba)_3$ (27 mg, 0.03 mmol) was added under argon, and the mixture was stirred in a closed pressure tube for 8 h at 100 °C. After cooling, additional Xantphos (17 mg, 0.03 mmol) and $Pd_2(dba)_3$ (9 mg, 0.01 mmol) were added under argon, and the mixture was stirred in a closed pressure tube for 14 h at 100 °C. After cooling, the mixture was diluted with DCM and filtered. The solids were washed with DCM, and the combined filtrates were concentrated. The residue was purified by chromatography (silica gel, DCM/EtOH 95:5) to afford **22** (424 mg, 1.19 mmol, 60% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.86 (m, 1H), 8.26 (m, 1H), 8.04 (m, 1H), 7.94 (m, 1H), 7.82 (s, 1H), 7.76 (br, 1H), 6.96 (m, 1H), 6.77 (m, 2H), 3.92 (s, 3H), 3.67 (s, 2H), 2.06 (s, 3H). UPLC (ESI+) m/z: $[M + H]^+$ 357.

*(rac)-2,2,2-Trifluoro-N-{[(2-{[6-(4-fluoro-2-methoxyphenyl)-pyrimidin-4-yl]amino}pyridin-4-yl)methyl](methyl)-λ^4-sulfanylidene}acetamide (**23**).* Under argon, a solution of 2,2,2-trifluoroacetamide (200 mg, 1.77 mmol) in THF (1.0 mL) was added dropwise to a solution of sodium *tert*-butoxide (113 mg, 1.18 mmol) in THF (0.8 mL), so that the temperature of the mixture remained below 10 °C. Subsequently, a freshly prepared solution of 1,3-dibromo-5,5-dimethylhydantoin (253 mg, 0.88 mmol) in THF (1.5 mL) was added dropwise to the stirred mixture, so that the temperature of the mixture remained below 10 °C. Then, the mixture was stirred for 10 min at 10 °C. Finally, a solution of 6-(4-fluoro-2-methoxyphenyl)-*N*-{4-[(methylsulfanyl)methyl]pyridin-2-yl}-pyrimidin-4-amine (**22**; 420 mg, 1.18 mmol) in 1,4-dioxane (10.0 mL) was added dropwise to the stirred mixture, so that the temperature of the mixture remained below 10 °C. The mixture was stirred for 75 min at 10 °C. The mixture was diluted with toluene (2.0 mL) under cooling, and a solution of sodium sulfite (149 mg, 1.18 mmol) in H_2O (2.0 mL) was added so that the temperature of the mixture remained below 15 °C. Aq NaCl solution was added, and the mixture was extracted with EtOAc (3×). The combined organic layers were filtered using a Whatman filter and concentrated. The residue was purified by column chromatography (DCM/EtOH 94:6) to afford **23** (348 mg, 0.74 mmol, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.90 (s, 1H, pyrimidine-2-H), 8.38 (d, J = 5.02 Hz, 1H), 8.26 (s, 1H, NH), 8.11 (dd, J = 8.78, 7.03 Hz, 1H), 7.72 (s, 1H), 7.64 (s, 1H), 6.95 (dd, J = 5.02, 1.25 Hz, 1H), 6.84 (t, J = 8.10 Hz, 1H), 6.78 (d, J = 10.84 Hz, 1H), 4.52 (d, J = 13.05 Hz, 1H, sulfilimine−CH_2, H_A, AB system), 4.31 (d, J = 13.05 Hz, 1H, sulfilimine−CH_2, H_B, AB system), 3.95 (s, 3H, OCH_3), 2.74 (s, 3H, sulfilimine−CH_3). UPLC (ESI+) m/z: $[M + H]^+$ 468.

*(rac)-6-(4-Fluoro-2-methoxyphenyl)-N-{4-[(S-methylsulfonimidoyl)methyl]pyridin-2-yl}pyrimidin-4-amine (**24**).* $KMnO_4$ (230 mg, 1.46 mmol) was added to a stirred solution of (*rac*)-2,2,2-trifluoro-*N*-{[(2-{[6-(4-fluoro-2-methoxyphenyl)-pyrimidin-4-yl]amino}pyridin-4-yl)methyl](methyl)-λ^4-sulfanylidene}acetamide (**23**; 340 mg, 0.73 mmol) in acetone (3.4 mL) at rt. The mixture was stirred at 50 °C for 5 h, before additional $KMnO_4$ (115 mg, 0.73 mmol) was added and stirring was continued at 50 °C. After 90 min, additional $KMnO_4$ (115 mg, 0.73 mmol) was added and stirring was continued at 50 °C. After 90 min, a solution of $KMnO_4$ (230 mg, 1.46 mmol) in acetone (10 mL) was added and stirring was continued at 50 °C. After 7 h, a solution of $KMnO_4$ (115 mg, 0.73 mmol) in acetone (10 mL) was added and stirring was continued at 50 °C for 4 h. After cooling, the mixture was filtered, and the residue was washed with acetone. The combined filtrates were concentrated. MeOH (100 mL) was added, and the mixture was filtered. The filtrate was concentrated to afford the crude trifluoroacetamide (180 mg), which was used in the next step.

K_2CO_3 (29 mg, 0.21 mmol) was added to a solution of crude 2,2,2-trifluoro-*N*-{[(2-{[6-(4-fluoro-2-methoxyphenyl)pyrimidin-4-yl]-amino}pyridin-4-yl)methyl](methyl)oxido-λ^6-sulfanylidene}-acetamide (50 mg) in MeOH (9 mL) at rt. The mixture was stirred for 50 min before H_2O (100 mL) was added. Then, the mixture was extracted with DCM/*i*-PrOH (4:1) and EtOAc. The combined organic layers were filtered using a Whatman filter and concentrated. The residue was purified by preparative HPLC (basic conditions) to afford **24** (11 mg, 0.03 mmol, 15% yield over two steps).

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*6-(4-Fluoro-2-methoxyphenyl)-N-{4-[(S-methylsulfonimidoyl)-methyl]pyridin-2-yl}pyrimidin-4-amine [Enantiomer 1 (**25**) and Enantiomer 2 (**26**)].* (*rac*)-6-(4-Fluoro-2-methoxyphenyl)-*N*-{4-[(*S*-methylsulfonimidoyl)methyl]pyridin-2-yl}pyrimidin-4-amine (**24**; 33 mg) was separated into the enantiomers by chiral preparative HPLC [Sepiatec Prep SFC 100 system; column: CHIRALPAK IA 5 μm, 250 × 20 mm; eluent: CO_2/MeOH (+0.4% diethylamine) 6:4; flow: 80 mL/min; pressure (outlet): 150 bar; temperature: 40 °C; solution: 33 mg in 2 mL DMF; detection: UV 254 nm].

Enantiomer 1 (**25**): t_R = 2.90−3.50 min. Yield: 12 mg, 0.03 mmol.

Enantiomer 2 (**26**): t_R = 3.65−4.50 min. Yield: 13 mg, 0.03 mmol. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.27 (s, 1H, aniline−NH), 8.71 (d, *J* = 1.13 Hz, 1H, pyrimidine-2-H), 8.30−8.32 (m, 1H), 8.28 (d, *J* = 4.89 Hz, 1H), 8.00 (dd, *J* = 8.76, 7.25 Hz, 1H), 7.83 (s, 1H), 7.01−7.10 (m, 2H), 6.88 (td, *J* = 8.43, 2.54 Hz, 1H), 4.39 (s, 2H, sulfoximine−CH_2), 3.89 (s, 3H, OCH_3), 2.86 (s, 3H, sulfoximine−CH_3). UPLC-MS (basic): t_R = 1.14 min; MS (ESI+) *m*/*z*: $[M + H]^+$ 388.2. ESI-HRMS *m*/*z*: $[M + H]^+$ calcd for $C_{18}H_{19}FN_5O_2S$, 388.1244; found, 388.1241.

*Compound **31**, Enantiomers **32** and VIP152 (Scheme 2). 2-Chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyridine (**28**).* A mixture of 2-chloro-5-fluoro-4-iodopyridine (**27**; 1.00 g, 3.88 mmol, APAC Pharmaceutical, LLC), (4-fluoro-2-methoxyphenyl)boronic acid (**19**; 660 mg, 3.88 mmol), and $Pd(PPh_3)_4$ (449 mg, 0.38 mmol) in DME (10.0 mL) and 2 M aq K_2CO_3 (5.8 mL) was degassed using argon. The mixture was stirred under an argon atmosphere for 4 h at 100 °C. After cooling, the mixture was diluted with EtOAc and THF and washed with brine. The organic phase was filtered using a Whatman filter and concentrated. The residue was purified by column chromatography (hexane to hexane/EtOAc 50%) to afford **28** (947 mg, 3.70 mmol, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.27 (m, 1H), 7.33 (m, 1H), 7.24 (m, 1H), 6.75 (m, 2H), 3.83 (s, 3H). UPLC (ESI+) *m*/*z*: $[M + H]^+$ 256.

*5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-N-{4-[(methylsulfanyl)-methyl]pyridin-2-yl}pyridin-2-amine (**29**).* A mixture of 2-chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyridine (**28**; 400 mg, 1.57 mmol), 4-[(methylsulfanyl)methyl]pyridin-2-amine (**21**; 483 mg, 3.13 mmol), Xantphos (40 mg, 0.07 mmol), and Cs_2CO_3 (765 mg, 2.35 mmol) in 1,4-dioxane (6.0 mL) was degassed using argon. $Pd_2(dba)_3$ (21 mg, 0.02 mmol) was added under argon, and the mixture was stirred in a closed pressure tube for 5 h at 100 °C. After cooling, the mixture was diluted with aq NaCl solution and extracted with DCM (3×). The combined organic phases were filtered using a Whatman filter and concentrated. The residue was purified by chromatography (hexane to hexane/EtOAc 30%) to afford **29** (556 mg, 1.48 mmol, 94% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12−8.19 (m, 2H), 7.61 (d, *J* = 5.09 Hz, 1H), 7.36−7.46 (m, 2H), 7.24−7.31 (m, 1H), 6.71−6.84 (m, 3H), 3.82 (s, 3H, OCH_3), 3.61 (s, 2H, SCH_2), 2.03 (s, 3H, SCH_3). UPLC (ESI+) *m*/*z*: $[M + H]^+$ 374.

*(rac)-2,2,2-Trifluoro-N-{[(2-{[5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyridin-2-yl]amino}pyridin-4-yl)methyl](methyl)-λ^4-sulfanylidene}acetamide (**30**).* Under argon, a solution of 2,2,2-trifluoroacetamide (195 mg, 1.73 mmol) in 1,4-dioxane (0.5 mL) was added dropwise to a solution of sodium *tert*-butoxide (111 mg, 1.15 mmol) in 1,4-dioxane (0.6 mL), so that the temperature of the mixture remained below 10 °C. Subsequently, a freshly prepared solution of 1,3-dibromo-5,5-dimethylhydantoin (247 mg, 0.86 mmol) in 1,4-dioxane (0.6 mL)/THF (1.0 mL) was added dropwise to the stirred mixture, so that the temperature of the mixture remained below 10 °C. Then, the mixture was stirred for 10 min at 10 °C. Finally, a solution of 5-fluoro-4-(4-fluoro-2-methoxyphenyl)-*N*-{4-[(methylsulfanyl)methyl]pyridin-2-yl}pyridin-2-amine (**29**; 430 mg, 1.15 mmol) in 1,4-dioxane (1.0 mL) was added dropwise to the stirred mixture, so that the temperature of the mixture remained below 10 °C. The mixture was stirred for 60 min at 10 °C. The mixture was diluted with toluene (2.0 mL) under cooling and a solution of sodium sulfite (145 mg, 1.15 mmol) in H_2O (2.0 mL) was added so that the temperature of the mixture remained below 15 °C. Aq NaCl solution was added, and the mixture was extracted with EtOAc (3×). The combined organic phases were filtered using a Whatman filter and concentrated to afford crude **30**, which was used without further purification. UPLC (ESI+) *m*/*z*: $[M + H]^+$ 485.

*(rac)-5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-N-{4-[(S-methylsulfonimidoyl)methyl]pyridin-2-yl}pyridin-2-amine (**31**).* Acetone (6.0 mL) and $KMnO_4$ (814 mg, 5.15 mmol) were added to the residue of the previous step (crude **30**), and the mixture was stirred at 50 °C for 90 min. Additional $KMnO_4$ (223 mg, 1.42 mmol) was added and stirring was continued at 50 °C for 4 h. Finally, additional $KMnO_4$ (305 mg, 1.93 mmol) was added and stirring was continued at 50 °C for 150 min. After cooling, the mixture was filtered, the residue was washed with acetone, and the combined filtrates were concentrated. The residue was dissolved in MeOH (60 mL), K_2CO_3 (182 mg, 1.32 mmol) was added, and the reaction mixture was stirred for 20 min at rt. The mixture was diluted with aq NaCl solution and extracted with DCM (3×). The combined organic phases were filtered using a Whatman filter and concentrated. The residue was purified by preparative HPLC (basic conditions) to afford **31** (50 mg, 0.12 mmol, 10% yield over three steps). ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ 8.22 (d, *J* = 5.31 Hz, 1H), 8.13 (d, *J* = 1.52 Hz, 1H), 7.80 (s, 1H, aniline−NH), 7.46 (d, *J* = 5.05 Hz, 1H), 7.25−7.30 (m, 2H), 6.92 (d, *J* = 5.05 Hz, 1H), 6.71−6.82 (m, 2H), 4.36 (d, *J* = 12.63 Hz, 1H, sulfoximine−CH_2, H_A, AB system), 4.25 (d, *J* = 12.88 Hz, 1H, sulfoximine−CH_2, H_B, AB system), 3.83 (s, 3H, OCH_3), 3.00 (s, 3H, sulfoximine−CH_3). ^{13}C NMR (100.67 MHz, DMSO-d_6): δ 163.69 (d, $^1J_{CF}$ = 245.41 Hz, $1C_q$), 157.92 (d, $^3J_{CF}$ = 10.17 Hz, $1C_q$), 154.4 (s, $1C_q$), 151.88 (d, $^1J_{CF}$ = 254.84 Hz, $1C_q$), 150.82 (d, $^4J_{CF}$ = 2.12 Hz, $1C_q$), 147.31 (s, 1CH), 140.63 (s, $1C_q$), 134.74 (d, $^2J_{CF}$ = 15.26 Hz, $1C_q$), 134.20 (d, $^2J_{CF}$ = 26.70 Hz, 1CH), 131.59 Hz (d, $^3J_{CF}$ = 10.17 Hz, 1CH), 118.82 (d, $^4J_{CF}$ = 2.97 Hz, $1C_q$), 118.04 (s, 1CH), 113.42 (d, $^2J_{CF}$ = 43.23 Hz, 1CH), 113.18 (s, 1CH), 107.15 (d, $^3J_{CF}$ = 22.04 Hz, 1CH), 100.16 (d, $^2J_{CF}$ = 25.86 Hz, 1CH), 67.18 (s, $1CH_2$), 56.25 (s, $1CH_3$), 41.45 (s, $1CH_3$). IR (diamond ATR, cm^{-1}): 3371 (w), 3337 (m), 3289 (br s), 3020 (m), 3005 (m), 2918 (m), 2905 (m), 1622 (m), 1607 (s), 1535 (m), 1481 (m), 1416 (m), 1398 (s), 1283 (m), 1248 (m), 1196 (s), 1030 (s), 953 (s), 829 (s). UPLC (ESI+) *m*/*z*: $[M + H]^+$ 405.

*(R)-5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-N-{4-[(S-methylsulfonimidoyl)methyl]pyridin-2-yl}pyridin-2-amine (**32**) and (S)-5-Fluoro-4-(4-fluoro-2-methoxyphenyl)-N-{4-[(S-methylsulfonimidoyl)methyl]pyridin-2-yl}pyridin-2-amine (VIP152).* Separation of the two enantiomers of (*rac*)-**31** (3.468 g) via chiral preparative HPLC yielded **32** and VIP152 [Sepiatec Prep SFC 100 system; column: CHIRALPAK IC 5 μm, 250 × 30 mm; eluent: CO_2/*i*-PrOH (+0.4% diethylamine) 7:3; flow: 100 mL/min; pressure (outlet): 150 bar; temperature: 40 °C; solution: 3.468 g in 55 mL DCM/MeOH 2:1; detection: UV 254 nm].

(*R*)-Enantiomer (**32**): t_R = 7.0−8.1 min. Yield: 1.31 g, 3.24 mmol. $[\alpha]_D$ −10.5 ± 0.21 (*c* 1.00, DMSO).

(*S*)-Enantiomer (VIP152): t_R = 8.5−10.5 min. Yield: 1.32 mg, 3.26 mmol. ESI-HRMS *m*/*z*: $[M + H]^+$ calcd for $C_{19}H_{19}F_2N_4O_2S$, 405.1198; found, 405.1196. $[\alpha]_D$ +11.4 ± 0.13 (*c* 1.00, DMSO).

Pharmacology. *Kinase Assays.* The inhibitory activities of the compounds against CDK2/CycE and CDK9/CycT1 were measured in TR-FRET-based kinase activity inhibition assays using the biotinylated peptide biotin-Ttds-YISPLKSPYKISEG (C-terminus in amide form, JPT Peptide Technologies) as the substrate. Recombinant fusion proteins of GST and human CDK2 and of GST and human CycE, expressed in insect cells (Sf9) and purified by glutathione Sepharose affinity chromatography, were purchased from ProQinase GmbH (product no. 0050-0055-1). Recombinant full-length His-tagged human CDK9 and CycT1, expressed in insect cells and purified by Ni−NTA affinity chromatography, were purchased from Life Technologies (cat. no. PV4131).

For the assays, 50 nL of a 100-fold concentrated solution of the test compound in DMSO was pipetted into a black, low-volume, 1536-well microtiter plate (Greiner Bio-One), 2 μL of enzyme solution in aqueous assay buffer [50 mM Tris HCl pH 8.0, 10 mM $MgCl_2$, 1.0 mM dithiothreitol, 0.1 mM sodium orthovanadate, 0.01% (v/v) Nonidet P-40 (Sigma)] was added, and the mixture was incubated for 15 min at 22 °C to allow prebinding of the test compound to the

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enzyme before the start of the kinase reaction. Then, the kinase reaction was started by the addition of 3 μL of a solution of ATP [final concn: 10 μM (=low) or 2 mM (=high)] and substrate (final concn: 0.75 μM) in assay buffer, and the resulting mixture was incubated for a reaction time of 25 min at 22 °C. The concentrations of the enzymes were adjusted depending on the activity of the enzyme, lot, and ATP concentration and were chosen appropriate to have the assays in the linear range. Typical concentrations were in the range of 10−100 ng/mL for CDK2 and 0.5−1 μg/mL for CDK9. The reaction was stopped by the addition of 5 μL of a solution of TR-FRET detection reagents [0.2 μM streptavidin-XL665 (Cisbio Bioassays), 1 nM anti-RB (pSer807/pSer811)-antibody (BD Pharmingen, # 558389), and 1.2 nM LANCE Eu-W1024 labeled anti-mouse IgG antibody (PerkinElmer, product no. AD0077)] in an aqueous ethylenediaminetetraacetic acid (EDTA) solution [100 mM EDTA, 0.2% (w/v) bovine serum albumin in 100 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES), pH 7.5].

The resulting mixture was incubated 1 h at 22 °C to allow the formation of complex between the phosphorylated biotinylated peptide and the detection reagents. Subsequently, the amount of phosphorylated substrate was evaluated by measurement of the resonance energy transfer from the europium chelate to the streptavidin-XL. Therefore, the fluorescence emissions at 620 and 665 nm after excitation at 350 nm were measured with a PHERAstar FS reader (BMG Labtech). The ratio of the emissions at 665 and 620 nm was taken as the measure for the amount of phosphorylated substrate.

The compounds were tested on the same microtiter plate at 11 different concentrations in the range of 20 μM to 0.07 nM (dilution series prepared separately, before the assay, on the level of the 100-fold concentrated solutions in DMSO by serial dilutions) in duplicate values for each concentration. IC_{50} values were calculated using Genedata Screener software.

Merck Millipore CDK Assays. Assays were performed according to the Merck Millipore KinaseProfiler standard protocols with an ATP concentration of 10 μM.

Proliferation Assays. A2780 human ovarian tumor cells (# 93112519) were obtained from the European Collection of Authenticated Cell Cultures (ECACC) of Public Health England (Salisbury, UK) and MOLM-13 human AML cells (ACC 554) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig). Authentication of cell lines was conducted at the DSMZ via polymerase chain reaction (PCR)-based DNA profiling of polymorphic short tandem repeats. Cells were propagated under the suggested growth conditions in a humidified 37 °C incubator. Proliferation assays were conducted in 96-well plates at densities of 2500 (A2780) and 5000 (MOLM-13) cells per well in the growth medium containing 10% fetal calf serum (FCS). Cells were treated in quadruplicate with serial dilutions of test compounds for 96 h. Relative cell numbers were quantified by crystal violet staining (A2780)[39] or CellTiter-Glo luminescent cell viability assay (Promega) (MOLM-13). IC_{50} values (inhibitory concentration at 50% of maximal effect) were determined by means of a four-parameter fit on measurement data, which were normalized to vehicle (DMSO)-treated cells (=100%) and measurement readings taken immediately before compound exposure (=0%).

PK Studies. *Caco-2 Permeability Assay.* Caco-2 cells were seeded at a density of 2.5×10^5 cells/well on 24-well insert plates, 0.4 μm pore size, 0.3 cm^2 (CoStar), and grown for 13−15 days in DMEM supplemented with 10% FCS, 1% GlutaMAX (100×, Gibco), 100 U/mL penicillin, 100 μg/mL streptomycin (Gibco), and 1% nonessential amino acids (100×). Cells were maintained at 37 °C in a humidified 5% CO_2 atmosphere. The medium was changed every 2−3 days.

The bidirectional transport assay for the evaluation of Caco-2 permeability was carried out in 24-well insert plates using a robotic system (Tecan). Before the assay was run, the culture medium was replaced by the transport medium (FCS-free HEPES carbonate transport buffer pH 7.2). For the assessment of monolayer integrity, the transepithelial electrical resistance (TEER) was measured. Only monolayers with a TEER of at least 400 Ω·cm^2 were used. Test compounds were predissolved in DMSO and added either to the apical or basolateral compartment at a final concentration of 2 μM. Evaluation was done in triplicate. Before and after incubation for 2 h at 37 °C, the samples were taken from both compartments and analyzed, after precipitation with MeOH, by LC−MS/MS. The apparent permeability coefficient (P_{app}) was calculated both for the apical to basolateral (A → B) and the basolateral to apical (B → A) direction using the following equation: $P_{app} = (V_r/P_0)(1/S)(P_2/t)$, where V_r is the volume of medium in the receiver chamber, P_0 is the measured peak area of the test compound in the donor chamber at $t = 0$, S is the surface area of the monolayer, P_2 is the measured peak area of the test compound in the acceptor chamber after incubation for 2 h, and t is the incubation time. The efflux ratio basolateral (B) to apical (A) was calculated by dividing P_{app} B−A by P_{app} A−B.

In Vitro Metabolic Stability in Liver Microsomes. The in vitro metabolic stability of test compounds was determined by incubation at 1 μM in a suspension of liver microsomes in 100 mM phosphate buffer pH 7.4 ($NaH_2PO_4 \cdot H_2O$ + $Na_2HPO_4 \cdot 2H_2O$) and at a protein concentration of 0.5 mg/mL at 37 °C. The microsomes were activated by adding a cofactor mix containing 8 mM glucose-6-phosphate, 4 mM $MgCl_2$, 0.5 mM nicotinamide adenine dinucleotide phosphate, and 1 IU/mL glucose-6-phosphate dehydrogenase in phosphate buffer pH 7.4. The metabolic assay was started shortly afterward by adding the test compound to the incubation at a final volume of 1 mL. The organic solvent in the incubations was limited to ≤0.01% DMSO and ≤1% MeCN. During incubation, the microsomal suspensions were continuously shaken at 580 rpm and aliquots were taken at 2, 8, 16, 30, 45, and 60 min, to which an equal volume of cold MeOH was immediately added. The samples were frozen at −20 °C overnight and after thawing subsequently centrifuged for 15 min at 3000 rpm. The supernatant was analyzed with an Agilent 1200 HPLC system with LC−MS/MS detection. The half-life of a test compound was determined from the concentration−time plot. From the half-life, the intrinsic clearances and the hepatic in vivo blood clearance (CL) and maximal oral bioavailability (F_{max}) were calculated using the "well-stirred" liver model[35] together with the additional parameter liver blood flow, specific liver weight, and microsomal protein content. The following parameter values were used for rats: liver blood flow: 4.2 L/h/kg; specific liver weight: 32 g/kg body weight; and microsomal protein content: 40 mg/g.

In Vitro Metabolic Stability in Hepatocytes. Hepatocytes from Han/Wistar rats were isolated via a two-step perfusion method. After perfusion, the liver was carefully removed from the rat: the liver capsule was opened and the hepatocytes were gently shaken out into a Petri dish with ice-cold Williams' medium E (WME). The resulting cell suspension was filtered through sterile gauze into 50 mL Falcon tubes and centrifuged at 50*g* for 3 min at rt. The cell pellet was resuspended in WME (30 mL) and centrifuged twice through a Percoll gradient at 100*g*. The hepatocytes were washed again with WME and resuspended in the medium containing 5% FCS. Cell viability was determined by trypan blue exclusion. For the metabolic stability assay, liver cells were distributed in WME containing 5% FCS to glass vials at a density of 1.0×10^6 vital cells/mL. The test compound was added to a final concentration of 1 μM. During incubation at 37 °C, the hepatocyte suspensions were continuously shaken at 580 rpm, and aliquots were taken at 2, 8, 16, 30, 45, and 90 min, to which an equal volume of cold MeCN was immediately added. The samples were frozen at −20 °C overnight and after thawing subsequently centrifuged for 15 min at 3000 rpm. The supernatant was analyzed with an Agilent 1200 HPLC system with LC−MS/MS detection. The half-life of a test compound was determined from the concentration−time plot. From the half-life, the intrinsic clearances and the hepatic in vivo blood clearance (CL) and maximal oral bioavailability (F_{max}) were calculated using the "well-stirred" liver model[40] together with the additional parameters liver blood flow, specific liver weight, and amount of liver cells in vivo and in vitro. The same parameters for liver blood flow and specific liver weight as given above were used; liver cells in vivo: 1.1×10^8 cells/g liver, liver cells in vitro: 1.0×10^6/mL.

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Plasma Protein Binding. HT equilibrium dialysis was used to determine the plasma protein binding, as outlined by Banker et al.[41] In brief, a semipermeable membrane separated the plasma from the buffer compartment. The test compound was added to the plasma side at a concentration of 3 μM and incubated for 7 h at 37 °C and 5% CO_2 with 99% humidity and moderate shaking. Then, 10 μL of the plasma side was transferred to a deep-well plate containing 90 μL of buffer and 90 μL of the buffer side was added to 10 μL of blank plasma. All samples were precipitated with 400 μL ice-cold MeOH and frozen overnight at −20 °C. After thawing and mixing, the samples were centrifuged for 10 min at 3000 rpm. The supernatant was transferred to a 96-well plate, and LC−MS/MS measurements were undertaken. From the quotient of buffer and plasma concentration, the unbound fraction (f_u) was calculated.

CYP Inhibition Assay. The inhibitory potency of test compounds toward cytochrome P450-dependent metabolic pathways was determined in human liver microsomes (pool) by applying individual CYP isoform selective standard probes (CYP1A2, phenacetin; CYP2A6, coumarin; CYP2B6, bupropion; CYP2C8, amodiaquine; CYP2C9, diclofenac; CYP2C19, (*S*)-mephenytoin; CYP2D6, dextromethorphan; CYP2E1, chlorzoxazone; and CYP3A4, midazolam, testosterone). Reference inhibitors were included as positive controls. Incubation conditions (protein and substrate concentration and incubation time) were optimized with regard to linearity of metabolite formation. Assays were processed in 96-well plates at 37 °C using a Freedom EVO Workstation (Tecan, Crailsheim, Germany). The reactions were stopped by the addition of MeCN (100 μL) containing the respective stable labeled internal standard. Precipitated proteins were removed by centrifugation of the well plate (1800 rcf, 10 min, 10 °C). Metabolite formation was quantified by LC−MS/MS analysis (QTRAP 6500 system, Sciex, Canada), followed by inhibition evaluation and IC_{50} calculation.

CYP Induction Assay. To evaluate the CYP induction potential in vitro, cultured human hepatocytes from three separate livers were treated once daily for three consecutive days with vehicle control, one of eight concentrations of test compound, or known human CYP inducers (e.g., omeprazole, phenobarbital, and rifampin). After treatment, the cells were incubated in situ with the appropriate marker substrates for the analysis of CYP3A4, CYP2B6, and CYP1A2 activity by LC−MS/MS. Following the in situ incubation, the same hepatocytes from the same treatment groups were harvested for RNA isolation and analyzed by qRT-PCR to assess the effect of test compound on CYP1A2, CYP2B6, and CYP3A4 mRNA expression levels.

In Vivo PK Studies. All animal experiments were conducted in accordance with the German Animal Welfare Law and were approved by local authorities. For in vivo PK experiments, test compounds were administered to male Wistar rats intravenously at doses of 0.3−0.5 mg/kg and intragastrically at doses of 0.6−1 mg/kg formulated as solutions using solubilizers such as poly(ethylene glycol) 400 (PEG 400) in well-tolerated amounts. For PK after iv administration, the test compounds were given as iv bolus and blood samples were taken at 2 min, 8 min, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h, and 24 h after dosing. For PK after intragastral (ig) administration, the test compounds were given ig to fasted rats and blood samples were taken at 5 min, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h, and 24 h after dosing. Blood was collected into lithium heparin tubes (Monovette, Sarstedt) and centrifuged for 15 min at 3000 rpm. An aliquot of 100 μL from the supernatant (plasma) was taken and precipitated by the addition of cold MeCN (400 μL). The samples were frozen at −20 °C overnight and subsequently thawed and centrifuged at 3000 rpm, 4 °C for 20 min. Aliquots of the supernatants were analyzed using an Agilent 1200 HPLC system with LC−MS/MS detection. PK parameters were based on the plasma concentration and time data and calculated (e.g., using the linear-log trapezoidal rule for AUC estimation) using an Excel-based program. The PK parameters derived from the concentration−time profiles after iv administration are as follows: CL_{plasma}, total plasma clearance of test compound (in L/h/kg); CL_{blood}, total blood clearance of test compound, and $CL_{plasma} \times C_p/C_b$ (in L/h/kg) with C_p/C_b being the ratio of concentrations in plasma and blood. The PK parameters calculated from the concentration−time profiles after ig administration are as follows: C_{max}, maximal plasma concentration (in mg/L); $C_{max,norm}$, C_{max} divided by the administered dose (in kg/L); and T_{max}, time point at which C_{max} was observed (in h). The parameters calculated from both iv and ig concentration−time profiles are as follows: AUC_{norm}, area under the concentration−time curve from t = 0 to infinity (extrapolated) divided by the administered dose (in kg·h/L); $AUC_{(0-t\ last)norm}$, area under the concentration−time curve from t = 0 to the last time point for which plasma concentrations could be measured divided by the administered dose (in kg·h/L); $t_{1/2}$, apparent half-life (in h); and F, oral bioavailability, AUC_{norm} after ig administration divided by AUC_{norm} after iv administration (in %).

Physicochemical Assays. *Aqueous Solubility of Compound−DMSO Solutions.* Aqueous solubility at pH 6.5 was determined by an orientating HTS method.[32] Test compounds were applied as 1 mM DMSO solutions. After addition of buffer pH 6.5, the solutions were shaken for 24 h at rt. The undissolved material was removed by filtration. The compound dissolved in the filtrate was quantified by HPLC−MS/MS.

Equilibrium Shake Flask Solubility Assay. The thermodynamic solubility of compounds in water was determined by an equilibrium shake flask method.[42] A saturated solution of the test compound was prepared, and the solution was mixed for 24 h to ensure that equilibrium was reached. The solution was centrifuged to remove the insoluble fraction, and the concentration of the compound in solution was determined using a standard calibration curve. To prepare the test sample, solid compound (2 mg) was weighed in a 4 mL glass vial. Phosphate buffer pH 6.5 (1 mL) was added, and the suspension was stirred for 24 h at rt. Then, the solution was centrifuged. To prepare the sample for the standard calibration, solid sample (2 mg) was dissolved in MeCN (30 mL). After sonication, the solution was diluted with H_2O to 50 mL. Sample and standard were quantified by HPLC with UV detection. For each test sample, two injection volumes (5 and 50 μL) in triplicate were made. Three injection volumes (5, 10, and 20 μL) were made for the standard. HPLC conditions: column: XTerra MS C18 2.5 μm, 4.6 × 30 mm; injection volume: sample: 3 × 5 μL and 3 × 50 μL, standard: 5, 10, and 20 μL; flow: 1.5 mL/min; acidic gradient: eluent A: H_2O + 0.01% TFA, eluent B: MeCN + 0.01% TFA; 0 min 5% B, 0−3 min 65% B linear gradient, 3−5 min 65% B isocratic, 5−6 min 5% B isocratic; and UV detection: wavelength near the absorption maximum (between 200 and 400 nm). The areas of sample and standard injections, as well as the calculation of the solubility values (in mg/L), were determined using Waters Empower 2 FR software.

log D Measurement. log *D* values at pH 7.5 were recorded using an indirect method for determining hydrophobicity constants by reversed-phase HPLC.[43] A homologous series of *n*-alkan-2-ones (C_3−C_{16}, 0.02 M in MeCN) was used for calibration. Test compounds were applied as 0.67 mM DMSO stock solutions in MeCN/H_2O (1:1). The lipophilicity of compounds was then assessed by comparison to the calibration curve.

In Vivo Pharmacology Studies. *MOLM-13 Human AML Models in Mice and Rats.* All animal experiments were conducted in accordance with the German Animal Welfare Law and were approved by local authorities. MOLM-13 human AML cells were obtained from the DSMZ. Cell lines were authenticated at the DSMZ via short-tandem repeat DNA fingerprinting before use in experiments to rule out potential cross-contamination among cell lines. All cell lines were tested as being free from mycoplasma contamination using MycoAlert (Lonza) at least every four passages and before a cell line substock was generated.

For the AML mouse model, 2 × 10^6 MOLM-13 human AML cells were suspended in 100% Matrigel basement membrane matrix (BD Biosciences) and inoculated subcutaneously to female NMRI *nu/nu* mice (18−21 g, 5−6 weeks, Taconic M&B). For the AML models in rats, 2 × 10^6 MOLM-13 or MV4-11 human AML cells were suspended in 100% Matrigel and inoculated subcutaneously to female athymic nude rats (120−160 g, 5−6 weeks, Harlan). Animals were stratified into treatment and control groups (n = 12/group) based on

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primary tumor size. For the MOLM-13 models, treatments were started 5−6 days after tumor cell inoculation when the average tumor sizes were 39−41 and 73 mm^3 for mice and rats, respectively. For the MV4-11 models, treatments were started on day 13 when the tumor area was approximately 40 mm^2.

BAY-332 was administered once daily (QD, 15 mg/kg), once every 3 days (Q3D, 35 mg/kg), or once every 5 days (Q5D, 65 mg/kg). VIP152 was administered once weekly (QW, 12.5 mg/kg) for the MOLM-13 model in mice and Q3D (3 mg/kg), Q5D (3.75 mg/kg), or QW (4.5 mg/kg) for the MOLM-13 model in rats. PEG 400/EtOH/H_2O (30:10:60) or PEG 400/H_2O (80:20) was used as the vehicle control. Unless otherwise indicated, all treatments were administered intravenously and were continued until the end of the experiment. Along with body weights, tumors were measured at least twice weekly by caliper, and the volume was calculated using the formula (length × $width^2$)/2. Treatment-to-control (T/C) ratios were calculated by dividing the mean tumor volume of the treatment group by the mean tumor volume of the vehicle group at the time point when the vehicle group was sacrificed.

All statistical analyses were performed using the statistical programming language R.[44] Validity of the model assumptions was checked for each fitted statistical model.

Analyses for the MOLM-13 model in mice were performed by fitting a linear mixed effects model on the log-transformed outcome. The model included both a fixed and random intercepts and slopes for each group and subject. The data for the MOLM-13 model in rats were analyzed by group comparisons at the start of the study and on the last day of the vehicle group. Comparisons were performed based on a linear model estimated using weighted least squares that included a separate variance term for each group. Group comparisons were corrected for false positive rate using Dunnett's method. In all cases, $p < 0.05$ was considered as being statistically significant.

Docking. Docking calculations were performed using Glide[45] in the standard precision mode and default settings. The X-ray complex of CDK9/CycT1 with a benzimidazole inhibitor (PDB ID: 3MY1) was used.[46] Prior to the docking procedure, all water molecules and ligands in the protein−ligand complex were removed. Ligands were pre-processed using LigPrep.[47] Docking poses were selected based on Glide scoring.

X-ray Structure Determination of VIP152. Single crystals of VIP152 were obtained by slow evaporation of an acetone/H_2O solution of the compound at rt. A single crystal with dimensions 0.14 × 0.08 × 0.02 mm^3 was mounted on a CryoLoop using inert oil. Data collection was carried out using a Bruker diffractometer equipped with an APEX II CCD area detector, an IμS microsource with Cu Kα radiation, mirrors as a monochromator, and a Kryoflex low-temperature device (T = 110 K). The program APEX II v.2011.8-0 was used for data collection and reduction.[48] Absorption correction and scaling were performed using SADABS.[49] The crystal structure solution was achieved using direct methods as implemented in SHELXTL version 6.14[50] and visualized using the XP program.[50] Missing atoms were subsequently located from difference Fourier synthesis and added to the atom list. Least-squares refinement on F^2 using all measured intensities was carried out using the program SHELXTL version 6.14.[50] All non-hydrogen atoms were refined including anisotropic displacement parameters. Final data collection and structure refinement parameters: λ = 1.54178 Å, T = 110 K, space group = $P2(1)$, a = 16.6442(11) Å, b = 8.5659(6) Å, c = 26.1351(16) Å, β = 99.053(5)°, Z = 8, reflections collected = 59,133, independent reflections = 12,958 (R_{int} = 0.0468), completeness = 97.8%, data-to-parameter ratio = 12.7, R_1 ($I > 2\sigma$) = 0.0542, wR_2 (all data) = 0.1490, GOF = 1.032, flack parameter = 0.053(9), and largest difference peak and hole = 0.897/−0.278 e $Å^3$. CCDC 2005811 (VIP152) contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/getstructures.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c01000.

Molecular formula strings (CSV)

Kinase selectivity panel data for VIP152 and LC−MS chromatograms for determination of purity (PDF)

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Notes

The authors declare the following competing financial interest(s): U.L., D.K., N.B., A.S., P.L., G.S., K.D., R.B., H.B., I.T., U.B., M.S., S.I., D.M., and F.v.N. are or have been employees and stockholders of Bayer AG.R.I. and S.H. are employees and stockholders of Vincerx Pharma, Inc.

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■ ABBREVIATIONS

ATR, ataxia telangiectasia and Rad3-related protein; CL, clearance; CL_b, blood clearance; ECACC, European Collection of Authenticated Cell Cultures; FCS, fetal calf serum; MCL-1, induced myeloid leukemia cell differentiation protein; P_{app}, apparent permeability coefficient; RNA Pol II, RNA polymerase II; PTEFb, positive transcription elongation factor b; Q3D, every 3 days; Q5D, every 5 days; Q7D, every 7 days; ratHep, rate hepatocytes; S_w, aqueous solubility; TCI, Tokyo Chemical Industry; T/C, treatment-to-control; UPLC, ultra-performance liquid chromatography; V_{ss}, steady-state volume of distribution; WME, Williams' media E

■ REFERENCES

(1) Lim, S.; Kaldis, P. Cdks, cyclins and CKIs: roles beyond cell cycle regulation. *Development* **2013**, *140*, 3079−3093.
(2) Malumbres, M. Cyclin-dependent kinases. *Genome Biol.* **2014**, *15*, 122.
(3) Galbraith, M. D.; Bender, H.; Espinosa, J. M. Therapeutic targeting of transcriptional cyclin-dependent kinases. *Transcription* **2019**, *10*, 118−136.
(4) Sánchez-Martínez, C.; Lallena, M. J.; Sanfeliciano, S. G.; de Dios, A. Cyclin dependent kinase (CDK) inhibitors as anticancer drugs: recent advances (2015−2019). *Bioorg. Med. Chem. Lett.* **2019**, *29*, 126637.
(5) Asghar, U.; Witkiewicz, A. K.; Turner, N. C.; Knudsen, E. S. The history and future of targeting cyclin-dependent kinases in cancer therapy. *Nat. Rev. Drug Discovery* **2015**, *14*, 130−146.
(6) Zeidner, J. F.; Karp, J. E. Clinical activity of alvocidib (flavopiridol) in acute myeloid leukemia. *Leuk. Res.* **2015**, *39*, 1312−1318.
(7) Paruch, K.; Dwyer, M. P.; Alvarez, C.; Brown, C.; Chan, T.-Y.; Doll, R. J.; Keertikar, K.; Knutson, C.; McKittrick, B.; Rivera, J.; Rossman, R.; Tucker, G.; Fischmann, T.; Hruza, A.; Madison, V.; Nomeir, A. A.; Wang, Y.; Kirschmeier, P.; Lees, E.; Parry, D.; Sgambellone, N.; Seghezzi, W.; Schultz, L.; Shanahan, F.; Wiswell, D.; Xu, X.; Zhou, Q.; James, R. A.; Paradkar, V. M.; Park, H.; Rokosz, L. R.; Stauffer, T. M.; Guzi, T. J. Discovery of dinaciclib (SCH 727965): a potent and selective inhibitor of cyclin-dependent kinases. *ACS Med. Chem. Lett.* **2010**, *1*, 204−208.
(8) Lücking, U.; Jautelat, R.; Krüger, M.; Brumby, T.; Lienau, P.; Schäfer, M.; Briem, H.; Schulze, J.; Hillisch, A.; Reichel, A.; Wengner, A. M.; Siemeister, G. The lab oddity prevails: discovery of pan-CDK inhibitor (*R*)-*S*-cyclopropyl-*S*-(4-{[4-{[(1*R*,2*R*)-2-hydroxy-1-methylpropyl]oxy}-5-(trifluoromethyl)pyrimidin-2-yl]amino}-phenyl)sulfoximide (BAY 1000394) for the treatment of cancer. *ChemMedChem* **2013**, *8*, 1067−1085.
(9) Boffo, S.; Damato, A.; Alfano, L.; Giordano, A. CDK9 inhibitors in acute myeloid leukemia. *J. Exp. Clin. Cancer Res.* **2018**, *37*, 36.
(10) Fujinaga, K. P-TEFb as a promising therapeutic target. *Molecules* **2020**, *25*, 838.
(11) Paparidis, N. F. d. S.; Durvale, M. C.; Canduri, F. The emerging picture of CDK9/P-TEFb: more than 20 years of advances since PITALRE. *Mol. BioSyst.* **2017**, *13*, 246−276.
(12) Krystof, V.; Baumli, S.; Fürst, R. Perspective of cyclin-dependent kinase 9 (CDK9) as a drug target. *Curr. Pharm. Des.* **2012**, *18*, 2883−2890.
(13) Anshabo, A. T.; Milne, R.; Wang, S.; Albrecht, H. CDK9: A comprehensive review of its biology, and its role as a potential target for anti-cancer agents. *Front. Oncol.* **2021**, *11*, 678559.
(14) Galbraith, M. D.; Bender, H.; Espinosa, J. M. Therapeutic targeting of transcriptional cyclin-dependent kinases. *Transcription* **2019**, *10*, 118−136.
(15) Eyvazi, S.; Hejazi, M. S.; Kahroba, H.; Abasi, M.; Zamiri, R. E.; Tarhriz, V. CDK9 as an appealing target for therapeutic interventions. *Curr. Drug Targets* **2019**, *20*, 453−464.
(16) Espinosa, J. M. Transcriptional CDKs in the spotlight. *Transcription* **2019**, *10*, 45−46.
(17) Lücking, U.; Scholz, A.; Lienau, P.; Siemeister, G.; Kosemund, D.; Bohlmann, R.; Briem, H.; Terebesi, I.; Meyer, K.; Prelle, K.; Denner, K.; Bömer, U.; Schäfer, M.; Eis, K.; Valencia, R.; Ince, S.; von Nussbaum, F.; Mumberg, D.; Ziegelbauer, K.; Klebl, B.; Choidas, A.; Nussbaumer, P.; Baumann, M.; Schultz-Fademrecht, C.; Rühter, G.; Eickhoff, J.; Brands, M. Identification of atuveciclib (BAY 1143572), the first highly selective, clinical PTEFb/CDK9 inhibitor for the treatment of cancer. *ChemMedChem* **2017**, *12*, 1776−1793.
(18) Cidado, J.; Boiko, S.; Proia, T.; Ferguson, D.; Criscione, S. W.; San Martin, M.; Pop-Damkov, P.; Su, N.; Roamio Franklin, V. N.; Chilamakuri, C. S. R.; D'Santos, C. S.; Shao, W.; Saeh, J. C.; Koch, R.; Weinstock, D. M.; Zinda, M.; Fawell, S. E.; Drew, L. AZD4573 is a highly selective CDK9 inhibitor that suppresses MCL-1 and induces apoptosis in hematologic cancer cells. *Clin. Cancer Res.* **2020**, *26*, 922−934.
(19) Richters, A.; Doyle, S. K.; Freeman, D. B.; Lee, C.; Leifer, B. S.; Jagannathan, S.; Kabinger, F.; Koren, J. V.; Struntz, N. B.; Urgiles, J.; Stagg, R. A.; Curtin, B. H.; Chatterjee, D.; Mathea, S.; Mikochik, P. J.; Hopkins, T. D.; Gao, H.; Branch, J. R.; Xin, H.; Westover, L.; Bignan, G. C.; Rupnow, B. A.; Karlin, K. L.; Olson, C. M.; Westbrook, T. F.; Vacca, J.; Wilfong, C. M.; Trotter, B. W.; Saffran, D. C.; Bischofberger, N.; Knapp, S.; Russo, J. W.; Hickson, I.; Bischoff, J. R.; Gottardis, M. M.; Balk, S. P.; Lin, C. Y.; Pop, M. S.; Koehler, A. N. Modulating androgen receptor-driven transcription in prostate cancer with selective CDK9 inhibitors. *Cell Chem. Biol.* **2021**, *28*, 134−147.
(20) Luecking, U. T.; Scholz, A.; Kosemund, D.; Bohlmann, R.; Briem, H.; Lienau, P.; Siemeister, G.; Terebesi, I.; Meyer, K.; Prelle, K.; Valencia, R.; Ince, S.; Nussbaum, F. v.; Mumberg, D.; Ziegelbauer, K.; Brands, M. Identification of potent and highly selective PTEFb inhibitor BAY 1251152 for the treatment of cancer: from p.o. to i.v. application via scaffold hops. *Cancer Res.* **2017**, *77*, 984.
(21) Bayer AG. An open-label Phase I dose-escalation study to characterize the safety, tolerability, pharmacokinetics, and maximum tolerated dose of BAY 1143572 given in a once-daily or an intermittent dosing schedule in subjects with advanced malignancies; Clinical Trial Results Synopsis, Study no. 16519 (NCT01938638), October 9, 2017. https://clinicaltrials.bayer.com/study/?id=16519 (accessed September 4, 2020).
(22) Murphy, M. P.; Caraher, E. Mcl-1 is vital for neutrophil survival. *Immunol. Res.* **2015**, *62*, 225−233.
(23) Sonawane, Y. A.; Taylor, M. A.; Napoleon, J. V.; Rana, S.; Contreras, J. I.; Natarajan, A. Cyclin dependent kinase 9 inhibitors for cancer therapy. *J. Med. Chem.* **2016**, *59*, 8667−8684.
(24) Jain, M.; Arvanitis, C.; Chu, K.; Dewey, W.; Leonhardt, E.; Trinh, M.; Sundberg, C. D.; Bishop, J. M.; Felsher, D. W. Sustained loss of a neoplastic phenotype by brief inactivation of MYC. *Science* **2002**, *297*, 102−104.
(25) Herbrink, M.; Nuijen, B.; Schellens, J. H. M.; Beijnen, J. H. Variability in bioavailability of small molecular tyrosine kinase inhibitors. *Cancer Treat. Rev.* **2015**, *41*, 412−422.
(26) Lücking, U. Sulfoximines: a neglected opportunity in medicinal chemistry. *Angew. Chem., Int. Ed.* **2013**, *52*, 9399−9408.
(27) Lücking, U. Neglected sulfur(VI) pharmacophores in drug discovery: exploration of novel chemical space by the interplay of drug

https://doi.org/10.1021/acs.jmedchem.1c01000
J. Med. Chem. XXXX, XXX, XXX−XXX

design and method development. *Org. Chem. Front.* **2019**, *6*, 1319−1324.
(28) Frings, M.; Bolm, C.; Blum, A.; Gnamm, C. Sulfoximines from a medicinal chemist's perspective: physicochemical and in vitro parameters relevant for drug discovery. *Eur. J. Med. Chem.* **2017**, *126*, 225−245.
(29) Sirvent, J. A.; Lücking, U. Novel pieces for the emerging picture of sulfoximines in drug discovery: synthesis and evaluation of sulfoximine analogues of marketed drugs and advanced clinical candidates. *ChemMedChem* **2017**, *12*, 487−501.
(30) BAY-332 is a single stereoisomer but the absolute configuration at the sulfoximine sulfur was not determined.
(31) For additional examples, see: (a) Lücking, U.; Kosemund, D.; Scholz, A.; Lienau, P.; Siemeister, G.; Bömer, U.; Bohlmann, R. Disubstituted 5-fluoro pyrimidine derivatives containing a sulfoximine group. WO 2013037894 A1, March 21, 2013. (b) Lücking, U.; Kosemund, D.; Scholz, A.; Lienau, P.; Siemeister, G.; Bömer, U.; Bohlmann, R. Disubstituted 5-fluoro-pyrimidines. WO 2013037896 A1, March 21, 2013.
(32) For the assay description, see:Onofrey, T.; Kazan, G. *Performance and Correlation of a 96-Well High Throughput Screening Method to Determine Aqueous Drug Solubility*; Millipore Corporation: Billerica, MA, USA, 2003; Application Note Lit. No. AN1731EN00.
(33) Wells, C. I.; Vasta, J. D.; Corona, C. R.; Wilkinson, J.; Zimprich, C. A.; Ingold, M. R.; Pickett, J. E.; Drewry, D. H.; Pugh, K. M.; Schwinn, M. K.; Hwang, B.; Zegzouti, H.; Huber, K. V. M.; Cong, M.; Meisenheimer, P. L.; Willson, T. M.; Robers, M. B. Quantifying CDK inhibitor selectivity in live cells. *Nat. Commun.* **2020**, *11*, 2743.
(34) Roethlisberger, D.; Mahler, H.-C.; Altenburger, U.; Pappenberger, A. If euhydric and isotonic do not work, what are acceptable pH and osmolality for parenteral drug dosage forms? *J. Pharm. Sci.* **2017**, *106*, 446−456.
(35) Gries, J.; Krüger, J. A practical approach to N-(trifluoroacetyl)-sulfilimines. *Synlett* **2014**, *25*, 1831−1834.
(36) Scholz, A.; Oellerich, T.; Hussain, A.; Lindner, S.; Luecking, U.; Walter, A. O.; Ellinghaus, P.; Valencia, R.; von Nussbaum, F.; Mumberg, D.; Brands, M.; Ince, S.; Serve, H.; Ziegelbauer, K. BAY 1143572, a first-in-class, highly selective, potent and orally available inhibitor of PTEFb/CDK9 currently in Phase I, shows convincing anti-tumor activity in preclinical models of acute myeloid leukemia (AML). *Cancer Res.* **2016**, *76*, 3022.
(37) Diamond, J. R.; Moreno, V.; Lim, E. A.; Cordoba, R.; Cai, C.; Ince, S. J.; Lettieri, J. T.; Merz, C.; Valencia, R.; Boni, V. Phase I dose escalation study of the first-in-class selective PTEFb inhibitor BAY 1251152 in patients with advanced cancer: Novel target validation and early evidence of clinical activity. *J. Clin. Oncol.* **2018**, *36*, 2507.
(38) Moreno, V.; Cordoba, R.; Morillo, D.; Diamond, J. R.; Hamdy, A. H.; Izumi, R.; Merz, C.; Boix, O.; Genvresse, I.; Nowakowski, G. S. Safety and efficacy of VIP152, a CDK9 inhibitor, in patients with double-hit lymphoma (DHL). *J. Clin. Oncol.* **2021**, *39*, 7538.
(39) Kueng, W.; Silber, E.; Eppenberger, U. Quantification of cells cultured on 96-well plates. *Anal. Biochem.* **1989**, *182*, 16−19.
(40) Pang, K. S.; Rowland, M. Hepatic clearance of drugs. I. Theoretical considerations of a "well-stirred" model and a "parallel tube" model. Influence of hepatic blood flow, plasma and blood cell binding, and the hepatocellular enzymatic activity on hepatic drug clearance. *J. Pharmacokinet. Biopharm.* **1977**, *5*, 625−653.
(41) Banker, M. J.; Clark, T. H.; Williams, J. A. Development and validation of a 96-well equilibrium dialysis apparatus for measuring plasma protein binding. *J. Pharm. Sci.* **2003**, *92*, 967−974.
(42) See: Kerns, E. H.; Di, L. *Drug-like Properties: Concepts, Structure Design and Methods*; Elsevier Inc./Academic Press: Burlington, MA, USA, 2008; pp 276−286.
(43) Minick, D. J.; Frenz, J. H.; Patrick, M. A.; Brent, D. A. A comprehensive method for determining hydrophobicity constants by reversed-phase high-performance liquid chromatography. *J. Med. Chem.* **1988**, *31*, 1923−1933.
(44) R: A language and environment for statistical computing, R version 3.6.3, The R Project for Statistical Computing; The R Foundation. 2020, available from: https://www.r-project.org/ (accessed July 13, 2020).
(45) *Glide*, Version 5.7; Schrödinger, LLC: New York, NY, USA, 2011.
(46) Baumli, S.; Endicott, J. A.; Johnson, L. N. Halogen bonds form the basis for selective P-TEFb inhibition by DRB. *Chem. Biol.* **2010**, *17*, 931−936.
(47) *LigPrep*, Version 2.5; Schrödinger, LLC: New York, NY, USA, 2011.
(48) *APEX II*, Version 2011.8-0; Bruker AXS Inc.: Madison, WI, USA, 2011.
(49) Krause, L.; Herbst-Irmer, R.; Sheldrick, G. M.; Stalke, D. Comparison of silver and molybdenum microfocus X-ray sources for single-crystal structure determination. *J. Appl. Crystallogr.* **2015**, *48*, 3−10.
(50) *SHELXTL*, Version 6.14; Bruker AXS Inc.: Madison, WI, USA, 2003.

https://doi.org/10.1021/acs.jmedchem.1c01000
J. Med. Chem. XXXX, XXX, XXX−XXX