Development of Novel ACK1/TNK2 Inhibitors Using a Fragment-Based Approach

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Supporting Information

ABSTRACT: The tyrosine kinase ACK1, a critical signal transducer regulating survival of hormone-refractory cancers, is an important therapeutic target, for which there are no selective inhibitors in clinical trials to date. This work reports the discovery of novel and potent inhibitors for ACK1 tyrosine kinase (also known as TNK2) using an innovative fragment-based approach. Focused libraries were designed and synthesized by selecting fragments from reported ACK inhibitors to create hybrid structures in a mix and match process. The hybrid library was screened by enzyme-linked immunosorbent



assay-based kinase inhibition and ³³P HotSpot assays. Systematic structure–activity relationship studies led to the identification of compound (*R*)-**9b**, which shows potent in vitro (IC₅₀ = 56 nM, n = 3, ³³P HotSpot assay) and in vivo (IC₅₀ < 2 μ M, human cancer cell lines) ACK1 inhibition. Both (*R*)-**9b** and (*S*)-**9b** were stable in human plasma and displayed a long half-life ($t_{1/2} > 6$ h).

■ INTRODUCTION

The ACK1 tyrosine kinase is aberrantly activated, amplified, or mutated in many types of human cancers, including prostate, breast, pancreatic, ovarian, and lung cancers.^{1,2} Aberrantly activated ACK1 drives cell growth via a number of molecular mechanisms, which we have recently reviewed.³ Several recent discoveries underscore its tumor-promoting functions. For example, ACK1 phosphorylates the androgen receptor, at Tyr267 in its transactivation domain, in an androgen-independent manner to promote castration-resistant prostate cancer (CRPC) growth.^{4,5} ACK1 has been shown to promote prostate tumorogenesis by phosphorylating the WW domain-containing oxidoreductase (Wwox) tumor suppressor⁶ on Tyr287, leading to its polyubiquitination and subsequent degradation in prostate cancer models.⁷ We have also shown that ACK1 phosphorylates and activates the key signaling kinase AKT at Tyr176, which plays important roles in human physiology and disease.⁹⁻¹¹ When AKT is phosphorylated on Tyr176 by ACK1, it functionally participates in the progression of breast cancer by suppressing proapoptotic pathways.8 Mechanistically, phosphorylation on AKT Tyr176 by ACK1 activates PI3K-independent AKT activation and facilitates the survival of breast cancer cells by suppressing proapoptotic pathways.8 Conversely, knockdown of ACK1 expression by siRNA suppressed AKT Tyr176 phosphorylation and activation in breast, prostate, and pancreatic cancer cells and increased expression of proapoptotic genes such as

Bim and Fas.^{8,12} Recently, ACK1 has also been shown to phosphorylate histone demethylase KDM3A (JHDM2A) at Tyr1114 in breast cancer cells, conferring tamoxifen resistance.¹³

The importance of ACK1 activation in tumor initiation in vivo first became evident when we generated transgenic mice expressing ACK1 specifically in the prostate. These ACK1 transgenic mice developed PIN, indicating that its activation is crucial in early stages of tumor initiation.⁸ Collective evidence in preclinical models therefore validates ACK1 as an important target for anticancer drugs and has driven the development of ACK1 inhibitors.¹

Selected examples of ACK1 inhibitors are shown in Figure 1. A series of 4-amino-5,6-biarylfuro[2,3-*d*]pyrimidines (structures 1a-1c, Figure 1) were found to inhibit ACK1 and the related member of the src kinase family Lck (lymphocyte-specific kinase).^{14,15} For example, compound 1a potently inhibits both ACK1 and Lck and was useful in the development of further compounds for the treatment of T cell-mediated autoimmune and inflammatory disease as a consequence of Lck inhibition. We used compound 1b (AIM-100) as a chemical probe for ACK1 inhibition, since it was reported to inhibit Lck to a lesser extent (ACK1:Lck = 5:1) than 1a (Lck:ACK1 = 1.8:1).

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Figure 1. Structures and activities of representative ACK1 inhibitors.

We have shown that AIM-100 inhibits ACK1-dependent AKT Tyr176¹² in pancreatic cancer cells and AR Tyr267¹⁶ phosphorylation. AIM-100 also inhibits castration and radioresistant prostate xenograft tumor growth via inhibition of AR Tyr267 phosphorylation.⁵ A study of further members of the 4-amino-5,6-biarylfuro[2,3-*d*]pyrimidine series showed that the dithiolane

1c was an exceptionally potent ACK1 inhibitor ($K_i = 0.3$ nM). This compound inhibits the growth of an ACK1-dependent cell line with an IC₅₀ of 5 nM. However, its poor pharmacokinetic properties (attributed to oxidation of both the dithiolane ring and NMe₂) precluded further development. A series of pyrazolopyrimidines of type 2 have also been developed by Amgen as ACK1 inhibitors.¹⁷ For example, compound 2a potently inhibits ACK1 in vitro (IC₅₀ = 2 nM) and in intact cells, as measured by inhibition of ACK1 autophosphorylation (IC₅₀ = 20 nM). Gray and co-workers have identified the ACK1 inhibitor 3 by highthroughput kinase profiling of a focused library of pyrimidodiazepines.¹⁸ This compound abolishes EGF-induced ACK1 autophosphorylation (Tyr284) in HEK293 cells at concentrations of 2 μ M. It also inhibits A549 lung cancer cell growth at 10 μ M. A series of imidazopyrazine-based ACK1 inhibitors have been developed by Jin and co-workers at OSI/Astellas.¹⁹ For example, compound 4 is a potent and orally bioavailable ACK1 inhibitor in mouse models and possesses good experimental ADMET (absorption, distribution, metabolism, elimination, and toxicity) properties. Compound 4 inhibits ACK1-mediated phosphorylation of poly(GT) in an AlphaScreen assay with an IC_{50} of 110 nM. Moreover, it potently inhibits ACK1 in a cellular context. In NCI-H1703 human non-small-cell lung cancer cells, its $\mathrm{IC}_{\frac{50}{50}}$ for ACK1 inhibition is 35 nM as measured by an ELISA assay.

Several promiscuous kinase inhibitors have been shown to inhibit ACK1. For example, the Src/Abl kinase inhibitor bosutinib²⁰ inhibits ACK1 with an IC₅₀ of 2.7 nM.²¹ Bosutinib was found to inhibit cell migration and invasion but not cell viability in a panel of non-small-cell lung cancer (NSCLC) cell lines.²² These effects were not seen when ACK1 was knocked-down specifically in K-Ras mutant cell lines. Dasatinib, another BCR/Abl and Src family tyrosine kinase inhibitor, inhibits ACK1 with a K_D of 6 nM.²³ Dasatinib was shown to inhibit both ACK1 autophosphorylation and AR phosphorylation of Tyr-267 in heregulin-stimulated human prostate cancer LNCaP cells with IC₅₀ values of <5 nM.²⁴ Additionally, dasatinib significantly reduced the growth of LNCaP cells expressing constitutively activated ACK1 in a mouse xenograft model.²⁴ Chemical and phosphoproteomic approaches revealed ACK1 to be a target of dasatinib in human lung cancer cells.²⁵



Figure 2. (A–C) Binding modes of known ACK1 inhibitors to ACK1: (A) compound 2b taken from its crystal structure with ACK1 (PDB 3EQP), (B) compound 1b modeled with ACK1, based on similar compounds bound to Lck (PDB 2OF2), and (C) compound 5 (TAE684) modeled with ACK1, based on its crystal structure with ALK (PDB 2XB7). (D) Bisanilinopyrimidine scaffold 6 for design of novel ACK1 inhibitors.

Scheme 1. Design and Synthesis of Bisanilinopyrimidine Derivatives 9a-t with a (\pm) -Tetrahydrofurfurylamine A-Ring^a



"Method a: (±)-tetrahydrofurfurylamine, Et₃N, MeOH, 0 °C to rt, 2 h. Method b: cat. HCl (4 M) in dioxane, 2-methoxyethanol or ethanol or dioxane, sealed tube, 80–180 °C, 20–30 min, aq NaHCO₃ workup.





^aSee the Supporting Information. Method a: DIPEA, THF, 120 °C, 1 h, 77–90%. Method b: *m*CPBA, EtOAc, 0 °C to rt, 2 h, 70–96%. Method c: cat. HCl (4 M) in dioxane, microwave heating 180 °C, 30 min, 68–97%. Method d: POCl₃, 0 °C, PCl₅, 16 h, 99%. Method e: THF, 15 h, 75%.

In this paper, we describe the development of novel ACK1 inhibitors by analysis of known ACK1 inhibitors, including 1b (AIM-100, Figure 2B), the pyrazolopyrimidine derivative $2b^{17}$ (Figure 2A), and the ALK inhibitor 5 (TAE684²⁶ (Figure 2C) (which strongly cross-inhibits ACK1 from published inhibitor profiling data sets; $K_d = 2 \text{ nM}^{27}$ and $K_i = 1 \text{ nM}^{28}$). The binding modes of the three inhibitors are shown in Figure 2, as derived from the X-ray structure of 2b with ACK1¹⁷ (PDB 3EQR), 1b (AIM-100) modeled using the X-ray structure of an analogue with ACK1²⁹ (PDB 4EWH), and 5 modeled using its X-ray structure with ALK³⁰ (PDB 2XB7). These inhibitors bind the ACK1 hinge residue Ala208 via the pyrimidyl group, positioning groups in the hydrophobic pocket beyond the gatekeeper and in the ribose binding region.²⁶ The bisanilinopyrimidine scaffold has long been recognized as a classical kinase inhibitor motif.^{2,13,31} Recently, we reported our efforts in identifying novel and exceptionally potent Aurora A inhibitors using the bisanilinopyrimidine scaffold.^{32,33} In the development of novel ACK1 inhibitors, our design process incorporated an aminopyrimidine structure as the hinge binding group (Figure 2D) and fragments of **1b**, **2b**, and **5** as R^1 , R^2 , and R^3 (Figure 2D) groups to create hybrid structures in a mix and match process (Figure 2).

CHEMISTRY

Synthesis of (\pm)-9a-t as ACK1 Inhibitors. First, the synthesis of compounds (\pm)-9a-t with R¹, R², and R³ groups was achieved from a set of 2,4-dichloropyrimidine building blocks, 7 (Scheme 1), by sequential reactions with two amine fragments. The starting materials 7a-e reacted with (\pm)-tetra-hydrofurfurylamine using method a (Scheme 1) to obtain the intermediates (\pm)-8a-e, which were subsequently reacted with the requisite anilines R³NH₂ (Scheme 1) using method b to obtain the final compounds (\pm)-9a-t with A- and B-rings (Scheme 1). The synthesis of building block 7c was achieved

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from readily available dihydroxypyrimidine-5-carboxylic acid via dichloropyrimidine-5-carbonyl chloride³¹ as shown in Scheme 2 in good yield.

Synthesis of the Sulfones 12a-j and Sulfides 12k-r as ACK1 Inhibitors. The synthesis of sulfone-substituted derivatives 12a-j was undertaken from 7c as shown in Scheme 2. Our attempts to synthesize sulfone derivatives 11 by direct reaction of 7c with o-(sulfonylalkyl)aniline using microwave heating conditions (100-170 °C, 30 min, ethanol, THF, or dioxane) or conventional alkylation conditions (sodium hydride in DMF or THF at room temperature followed by heating) were unsuccessful. Therefore, we first generated the sulfide intermediates 10 by reacting building block 7c with appropriate aniline sulfides (method a, Scheme 2), which were then oxidized to sulfone intermediates 11 using mCPBA (method b, Scheme 2). The intermediates 10 (Scheme 2) were used to prepare the final sulfide analogues 12k-r with the B-ring (see the Supporting Information). The B-ring in these analogues was introduced using microwave heating in HCl (4 M in dioxane) (method c, Scheme 2) to successfully obtain the final compounds in good yields with >95% purity as determined by HPLC.

Synthesis of Enantiomers of 9b. The synthesis of (R)-9b and (S)-9b stereoisomers was achieved as shown in Scheme 3

Scheme 3. Synthesis of (R)-9b and (S)-9b as ACK1 Inhibitors



from commercially available (*R*)- and (*S*)-tetrahydrofurfurylamines. The optical rotations ($[\alpha]_D$) of commercially available (*S*)-(+)-tetrahydrofurfurylamine and (*R*)-(-)-tetrahydrofurfurylamine were measured as +11.85 and -11.33, respectively, and are in agreement with the reported optical rotation of the (*S*)- and (*R*)-stereoisomers, +12 and -12, respectively.³⁴ We were able to resolve the enantiomers of (±)-9b using chiral HPLC (see the Experimental Section) and to determine the enantiomeric purity of (*R*)-9b and (*S*)-9b using chiral HPLC as >98%.

RESULTS AND DISCUSSION

Analogue Design and in Vitro Structure–Activity Relationship (SAR). The ability of the pyrimidines 9 and 12 to inhibit ACK1 kinase activity was first examined using a novel ELISA (enzyme-linked immunosorbent assay) developed in our laboratory.⁸ This assay measured the ability of ACK1 to specifically phosphorylate a tyrosine residue in a peptide derived from AKT (of sequence ATGRYYAMKIL)⁸ followed by detection of phosphorylation by an antibody-based approach.⁸ Loss of ACK1 kinase activity was directly proportional to decreased phosphorylation of AKT peptide. Subsequently, IC₅₀ values for those compounds that inhibited ACK1 activity by >80% at a concentration of 10 μ M in the ELISA were determined in a ³³P HotSpot assay (Reaction Biology). In the present work, we performed SAR studies using the ELISA (see the Experimental Section) and dose response data obtained from the ³³P HotSpot assay. The (\pm) -tetrahydrofurfurylamine fragment (Figure 2B) from 1b was incorporated into the design and synthesis of compounds 9a-t (i.e., R^2 group, Scheme 1) as a racemic mixture with >95% purity as determined by HPLC. The R¹ substituents at the 5-position of the pyrimidine and R³ aniline fragments (Scheme 1 and Figure 2D) in compounds 9a-t were chosen from **2b** (Figure 2A) and **5** (Figure 2C). The R³ aniline building blocks shown in Scheme 1 were chosen to aid inhibitor binding interactions as well as to modulate the druglike properties such as solubility, hydrophobicity (cLogP < 5), cell permeability, and polar surface area (<140 Å). We incorporated anilines possessing polar hydrophilic groups (or water-solubilizing groups such as morpholine, piperazine, and piperidine) as R^3 .

Our initial SAR from compounds 9a-t (Scheme 1) indicated that Cl, Br, and 2-chloro-6-fluorocarboxamide groups in the 5-position of the pyrimidine ring (i.e., R^1) are important for ACK1 inhibitory activities, while methyl and F groups are unfavorable. This observation is consistent with the members of the set 9a-t. For example, compounds 13 (76%, entry 3, Table 1) and 14 (66%, entry 4, Table 1) with Br and 2-chloro-6-fluorocarboxamide, respectively, as R¹ and *m*-fluoro-*p*-hydroxyaniline as R³ showed improved ACK1 inhibition compared to the corresponding F and Me analogues 9t (41% at 10 μ M) and 9u (49%) (entries 1 and 2, Table 1). Similarly, compounds 15 (65%), 16 (83%), and 9r (85%), which contain Cl, Br, and 2-chloro-6-fluorocarboxamide fragments in the 5-position (entries 5–7, Table 1), respectively, and p-hydroxyaniline as R^3 showed 2–10-fold improvement in ACK1 inhibition compared to analogues with F (41%) and methyl (8%) as R^1 (not shown in Table 1; see the Supporting Information for synthesis). The related compounds 18 and 9q (entries 9 and 10, Table 1) with hydrophobic methoxyaniline as R³ showed moderate ACK1 inhibition of 67% and 68%, respectively, indicating hydrophilic anilines as R³ are beneficial to maintain the in vitro ACK1 inhibitory activities.

The synthesis of sulfones 12a-j (Scheme 2) was also undertaken in parallel on the basis of the initial SAR information available from compounds 9a-t (Scheme 1). We used the building block 7c as the starting point, in which a 2-chloro-6fluorocarboxamide group occupies the 5-position of the pyrimidine ring (similar to the R^1 group in compounds 9a-t) and later introduced sulfonyl-substituted anilines as R² (A-ring, Scheme 2), similar to the isopropylsulfonamide-containing A-ring of 5 (Figure 2C). Hydrophilic groups such as *p*-hydroxyaniline were introduced as the B-ring (Scheme 2). However, all the analogues from the set of sulfones 12a-j showed weak ACK1 inhibition (<60% at 10 μ M in the ELISA), and the sulfides 12k-r generated using intermediate 10 (Scheme 2) showed very little ACK1 inhibitory activity in the ELISA (see the Supporting Information). Therefore, subsequent synthetic chemistry was predominantly focused on building blocks that contain Cl, Br, or 2-chloro-6-fluorocarboxamide as R^1 (8a, 8b, Table 1. SAR and in Vitro Inhibitory Activities of Derivatives 9a-t and Related Compounds^b



			·••·		IC (³³ D
Entry	Compound ID	R ¹	\mathbf{R}^{3}		$IC_{50}(P)$
	_			10 μM ^{<i>a</i>} (ELISA)	Hotspot assay)
1	9t	F	^y st ↓ F − − − − − − − − − − − − − − − − − −	41 ± 6.3	ND
2	9u	CH ₃	^{yst} ← F oH	49 ± 2.8	ND
3	13	Br	^{у‡} СТ ОН	76 ± 14	299 nM
4	14	E H CI	^{3⁴} − − F oH	66 ± 1.5	ND
5	15	Cl	AND OH	65 ± 10.6	ND
6	16	Br	у. ОН	83 ± 2.8	164 nM
7	9r	HN -CI	а ^{ра} ОН	85 ± 0.2	54.4 nM
8	17	Br	у. С он	75.5 ± 20	99 nM
9	18	Br	Je Co	67 ± 0.2	ND
10	9q	HN CI	Je Co	68 ± 10	ND
11	9c	Cl	A A A A A A A A A A A A A A A A A A A	89 ± 1.4	67.5 nM

Table 1. continued

.	C ID	D1	D ³	% Inhibition @	IC ₅₀ (³³ P
Entry	Compound ID	R^	R	10 μM ^{<i>a</i>} (ELISA)	Hotspot assay)
12	9h	Br	A A A A A A A A A A A A A A A A A A A	89 ± 1.4	106 nM
13	9n	H H O	A A A A A A A A A A A A A A A A A A A	82 ± 7.7	47.7 nM
14	9a	Cl	N NH	82 ± 12.7	81.3 nM
15	9i	Br	AND NH	85.5 ± 0.70	94.7 nM
16	9m	HN -CI	N NH	89.5 ± 2.1	33.2 nM
17	9b	Cl	× v v v v v v v v v v v v v v v v v v v	90.5 ± 0.70	54.4 nM
18	19	Br	×××××××××××××××××××××××××××××××××××××	93 ± 3	48.0 nM
19	20	HN -CI	× ⁴ N N N	97 ± 3	48.3 nM
20	21	Н	y and the second	16 ± 4.2	ND
21	9f	CI	NH NH	79.5 ± 0.70	231 nM
22	9k	Br	A NH	84.5 ± 0.70	169 nM

Table 1. continued

		1	2	% Inhibition @	IC ₅₀ (³³ P
Entry	Compound ID	R	R	10 μM ^{<i>α</i>} (ELISA)	Hotspot assay)
23	90	HN CI	ANH NH	97	53.3 nM
24	9e	CI	N OH	93 ± 5.1	85 nM
25	9j	Br	N OH	85 ± 2.8	ND
26	9s	HN CI	N OH	98	34.2 nM
27	22			89 ± 5.6	28.4 nM

"Inhibition (%) values are given as the average of three or more determinations. ${}^{b}ND$ = not determined. IC₅₀ values were determined for compounds that showed >80% inhibition in the ELISA assay

and 8c, Scheme 1) while keeping the (\pm) -tetrahydrofurfurylamine group as \mathbb{R}^2 .

Next, we further modified the R³ group of 9 using the anilines shown in Scheme 1 while keeping R^1 as Cl, Br, or a 2-chloro-6-fluorocarboxamide group. Compounds 9c, 9h, and 9n, which contain *p*-morpholinoaniline fragments (entries 11–13, Table 1) as R³ and Cl, Br, and 2-chloro-6-fluorocarboxamide groups as R¹, showed improved ACK1 inhibitory activities with IC₅₀ values of 67.5, 106, and 48 nM, respectively, compared to earlier analogues, and this observation further suggests aniline fragments with hydrophilic/water-solubilizing moieties are well tolerated in the R³ binding region. Compounds 9a, 9i, and 9m with p-piperazinylaniline as R³ also showed excellent ACK1 inhibitory activities (entries 14–16, Table 1) with IC₅₀ values of 81, 94, and 33 nM, respectively, similar to morpholine analogues, further suggesting anilines with hydrophilic groups as R³ have a positive effect on the ACK1 inhibition. As expected, compounds 9b, 19, and 20 with (methylpiperazinyl)aniline as R^3 (entries 17–19, Table 1) showed high ACK1 inhibitory activities with IC₅₀ values of 54, 48, and 48 nM, respectively. Compound 9s (entry 26, Table 1) with piperidinecarboxylic acid showed a significantly improved ACK1 inhibitory activity with an IC₅₀ value of 34 nM, compared to its related Cl and Br analogues 9e and 9j, respectively (entries 24 and 25, Table 1). Interestingly, substitution of the *N*-methyl group of compound **20** (entry 19, Table 1) with a succinyl amide group as in compound **22** (entry 27, Table 1) led to >1.5-fold improvement in ACK1 inhibitory activity with an IC₅₀ of 28 nM. We noticed that compounds **9e**, **9s**, and **22** (entries 24, 26, and 27, Table 1) with a carboxylic acid group as the H-bond donor/acceptor overall improved ACK1 inhibitory activities, indicating its potential as a handle, for example, in amide synthesis, for further SAR exploration in the ACK1 binding region.

Overall, we noticed 2-chloro-6-fluorocarboxamide as the R¹ group significantly contributed to the ACK1 inhibitory activities in compounds **9n**, **9m**, **20**, **9o**, and **22** (entries 13, 16, 19, 23, and 27, Table 1). **9o** (entry 23, Table 1) with 2-chloro-6-fluorocarboxamide as R¹ and *p*-(4-piperidinyl)aniline as R³ showed markedly improved ACK1 inhibition with an IC₅₀ of 53.3 nM, which is 3–4-fold more potent compared to the related **9f** with an IC₅₀ of 231 nM and **9k** with an IC₅₀ of 169 nM (entries 21 and 22, Table 1), which have Cl and Br, respectively. Similarly, compound **9s** (entry 26, Table 1) with piperidine-carboxylic acid as R³ and 2-chloro-6-fluorocarboxamide as R¹ showed significantly improved ACK1 inhibition with an IC₅₀ of 34 nM in the enzymatic assay compared to its related Cl and Br

Table 2. In Vivo Inhibitory Activities of Derivatives of 9 from Scheme 1^a

compd ID	inhibition (%) of ACK1 Tyr phosphorylation at 5 μ M, 16 h	compd ID	inhibition (%) of ACK1 Tyr phosphorylation at 5 μ M, 16 h
DMSO	0	methyl ester of (\pm) -9e	(-12.0)
(±)-9b	65.3	(±)-9e	1.2
(±)-9r	34.3	(R)- 9e	83.3
(±)-9m	36.7	(S)-9c	39.3
22 (Table1)	74.5	(R)- 9b	93.5
(±)-9i	58.3	(S)- 9b	78.7
(R)- 9c	(-22.1)	23 (Table1)	74.5
(±)-9n	(-32.6)	(±)-9l	8.56
(±)-9l	(-49.3)	TAE684 (Figure 2)	86.8
AIM-100 (Figure 1)	70.0		

^{*a*}Inhibition (%) of ACK1 Tyr284 phosphorylation assessed by immunoblotting in LAPC4 cells. Negative values represent increased Tyr phosphorylation (or enhanced kinase activity).



Figure 3. Effect of ACK1 inhibitors in prostate cancer cells: (a) inhibition of ACK1 autophosphorylation in LAPC4 cells treated with 5 μ M (*R*)-**9b** and (*S*)-**9b**; inhibition of (b) LNCaP, (c) LAPC4, and (d) VCaP cell growth by AIM-100, (*R*)-**9b**, and (*S*)-**9b**.

analogues **9e** (IC₅₀ of 85 nM) and **9j** (85% inhibition at 10 μ M), respectively (entries 24 and 25, Table 1). The improved inhibitory activities of **9n**, **9m**, **20**, **9o**, and **9s** (entries 13, 16, 19, 23, and 26, Table 1) can be attributed to the 2-chloro-6-fluorocarboxamide group in the 5-position of the pyrimidine undergoing a key H-bond contact in the gatekeeper region (see Figure 4A). Altogether, our SAR demonstrates that Cl and Br groups are tolerated in the 5-position of the pyrimidine core, and substitution of the 5-position with hydrogen as in compound **21** (entry 20, Table 1) considerably lowered the in vitro ACK1 affinity (16% at 10 μ M, ELISA).

Although compounds 9n, 9m, 20, 9o, 9s, and 22 with a 2-chloro-6-fluorocarboxamide moiety showed higher potency for ACK1 in the enzymatic assay, these compounds did not display the expected cellular potency most probably due to lack of cell permeability. Selected compounds from the set (\pm) -9a-t were chosen to evaluate the effects of ACK1 inhibition in LAPC4 human prostate cancer cells with inhibition of cell growth as the readout. The piperazine-substituted chloropyrimidine (\pm) -9b (entry 17, Table 1) showed promising ACK1 inhibitory activities in cellular assays. On the basis of the findings from cell culture data, we synthesized enantiomers of 9b to understand the ACK1 inhibitory activities of individual stereoisomers. The difference in activities of the two enantiomers is small. The (R)-9b isomer (Scheme 3) has marginally better ACK1 inhibitory activity with an IC₅₀ of 56 nM, while (S)-9b has an IC₅₀ of 82 nM in the ³³P HotSpot assay. The syntheses of (R)-9b and (S)-9b are shown in Scheme 3, and other examples of enantiomerically pure compounds (Table 2) were also synthesized using the protocols in Scheme 3. Both (R)-9b and (S)-9b were able to inhibit ACK1 autophosphorylation in prostate cancer cells (Figure 3a) and inhibit cell growth (Figure 3b–d).

Cell Culture Data of Selected Pyrimidines 9. ACK1 is a tyrosine kinase that autophosphorylates at Tyr284, which is indicative of its kinase activation. To determine the inhibitory potential of the representative pyrimidines **9**, the prostate cancer derived cell line LAPC4 was either treated with EGF ligand alone or added to cells pretreated with the compounds, and equal amounts of cell lysates were subjected to immunoblotting. EGF ligand causes rapid activation of EGF receptor, which in turn leads to activation of the ACK1 kinase (Figure 3a, lane 2, top panel). In contrast, treatment of LAPC4 cells with (*R*)-**9b** and (*S*)-**9b** resulted in almost complete loss of ACK1 activation. The control ACK1 inhibitors **5** and **1b** also exhibited



Figure 4. (A) Schematic representation of compound (R)-**9b** (blue) docked to ACK1 (PDB 4EWH), showing the ACK1 amino acid residues in the hinge (black), hydrophobic pocket (green), and ATP-ribose binding pocket (red). (B) Stick model of (R)-**9b** (cyan) docked to ACK1 (PDB 4EWH), showing important ACK1 amino acid residues. (C) Surface model of (R)-**9b** (cyan) docked to the ATP binding site of ACK1. The surface is colored according to atom type (carbon, green; hydrogen, gray; oxygen, red; nitrogen, blue). The images in panels B and C were rendered using Pymol.



Figure 5. (a) SAR overview. (b) Compounds with an extended carboxylic acid tether as ACK1 inhibitors (inhibition (%) at 10 μ M in ELISA is shown).

significant reduction in ACK1 activation. Actin was used as a control, which indicates equal loading of cell lysates (Figure 3a, lower panel).

In addition to (*R*)-9b and (*S*)-9b, we have also assessed other examples of 9 (Scheme 1) for their potential to suppress ACK1 kinase activity using immunoblotting, as described above. The inhibition (%) of ACK1 kinase activity is shown in Table 2.

Effects of (R)-9b and (S)-9b in Human Cancer Cells. Three distinct human prostate cancer derived cell lines, LNCaP, LAPC4, and VCaP, were either DMSO treated or treated with 1, 2.5, 5, 7.5, and 10 µM concentrations of inhibitors AIM-100, (R)-9b, and (S)-9b for 72 h, and the number of viable cells was counted by trypan blue exclusion assay. Both (R)-9b and (S)-9b were significantly better (IC₅₀ = 1.8 μ M) than AIM-100 (IC₅₀ = 7 μ M) in their ability to inhibit cell growth in LNCaP cells (Figure 3b). However, in LAPC4 cells, all three inhibitors were comparable in their ability to suppress cell proliferation (Figure 3c). In contrast, highly metastatic CRPC-forming VCaP cells were observed to be highly sensitive to (R)-9b (IC₅₀ = 2 μ M), while AIM-100 and (S)-9b exhibited an IC₅₀ of 4 μ M (Figure 3d). Taken together, it appears that (R)-9b is a superior ACK1 inhibitor in vivo and most significantly exhibits an ability to suppress proliferation of androgen-independent or CRPC VCaP cells.

Molecular Modeling of (±)-9b. The [(tetrahydrofurfuryl)amino]pyrimidine (R)-9b was docked to the catalytic domain of ACK1 (using GOLD^{35,36} and the ACK1 crystal structure from PDB 4EWH) (Figure 4) to determine a representative binding mode and to study the structural features responsible for its binding to ACK1. In this pose, hydrogen bonding is predicted between the pyrimidine N1 atom and the B-ring NH to the ACK1 Ala208 residue (Figure 4A), typical of an aminopyrimidine type 1 kinase inhibitor.³⁷ The chlorine atom is pointing toward, but not actually occupying, the hydrophobic pocket (highlighted in green in Figure 4A). This pocket is presumably occupied by the phenyl group in those derivatives that have an amide group at the pyrimidine 5-position (e.g., 9m or 9s). The tetrahydrofuryl group in (R)-9b orients itself closer to the ribose binding pocket of ACK1 and under the P-loop.

SAR Overview and Further Analogue Design. For the compounds synthesized as ACK1 inhibitors, the R^2 group was kept constant, while the 5-positions of the pyrimidine (R^1) and aniline (R^3) groups were changed. The SAR changes are



Figure 6. Plasma stabilities of (R)-9b, (S)-9b, procainamide, and procaine using HPLC: area of the peak (*y*-axis) plotted against the time (*x*-axis). Procainamide is reported as stable and procaine unstable in human plasma.^{41,42}

highlighted in Figure 5a. Since the cell culture data indicated that (R)-9b and (S)-9b are potent in human cancer cells, we included Cl as R¹ in subsequent analogue synthesis. Therefore, on the basis of the cell culture data, SARs obtained for pyrimidines 9, and inhibitory activities of compounds that contain a carboxylic acid tether such as in 9e, 9j, 9s, and 22 (entries 24-27, Table 1), the compounds shown in Figure 5b with Cl in the 5-position of the pyrimidine as R^1 were synthesized to explore further SARs. Compounds 23, 24, 26, and 28 (Figure 5b and Supporting Information) with one- or two-carbon-tethered carboxylic acids showed moderate ACK1 inhibition. The bisamine group in compound 26 (Figure 5b) was introduced with the hope of facilitating cell permeability and solubility. Compound 29 (Figure 5b) with a piperazine amide-tethered carboxylic acid showed significant ACK1 inhibition similar to that of compounds 22 (entry 27, Table 1). The in vitro activities of compounds shown in Figure 5b demonstrate the importance of the piperazine moiety to retain potent ACK1 inhibitory activities. On the basis of the SARs obtained in this study, we will continue our medicinal/synthetic chemistry efforts to further improve this class of compounds as novel ACK1 inhibitors, and broader SAR explorations will be discussed in future publications.

In Vitro PK Properties and Profiling of (R)-9b. Our cell culture data showed both (R)-9b and (S)-9b can penetrate human cancer cell lines and inhibit cell growth in an ACK1-dependent manner (Figure 3). The *in vitro* human plasma

Table 3.	Kinase	Profiling	of (1	R)-9b	(Reaction	Biology,	HotSpot	t Assay
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kinase	inhibition (%) of 9b at 1 μ M ^{<i>a</i>}	std dev	kinase	inhibition (%) of 9b at 1 μ M ^{<i>a</i>}	std dev
ABL1	82.8	0.5	IR	46.6	1.8
ACK1	99.8	0.3	JAK2	98.6	0.4
AKT1	4.7	2.4	KDR/VEGFR2	67.3	0.0
ALK	86.0	0.1	LCK	87.7	0.0
AXL	75.9	0.0	mTOR/FRAP1	-5.6	1.8
BTK	45.1	0.2	p70S6K/RPS6KB1	24.1	1.3
c-MET	23.1	0.5	PDGFRa	42.7	0.4
CHK1	84.8	0.3	PIM1	3.6	0.2
COT1/MAP3K8	-7.7	0.1	RAF1	-0.8	3.0
CSK	26.3	0.1	ROS/ROS1	84.2	0.2
EGFR	-2.8	3.8	c-Src	71.3	b
EPHA2	31.9	0.1	SGK1	26.0	0.1
ERBB2/HER2	-1.1	1.6	TBK1	71.8	0.5
ERK1	1.9	0.2	TIE2/TEK	32.0	0.3
FGFR1	86.4	0.1	TRKA	63.2	0.0
FRK/PTK5	41.3	0.0	TYK2	98.9	0.2
GSK3a	5.1	0.7	WEE1	12.4	0.8
IKKa/CHUK	0.4	0.8			
^a ATP was used at 10	<i>u</i> M. ^{<i>b</i>} Estimated from dose response	<u>م</u>			

stabilities of compounds (R)-9b and (S)-9b were evaluated using HPLC analysis in a time-course experiment for up to 24 h (see Figure 6 and the Experimental Section). Our data showed both (*R*)-**9b** and (*S*)-**9b** were stable in human plasma (Figure 6) for up to 24 h and displayed a long half-life ($t_{1/2} > 6$ h). Procaine and procainamide were used as standards to generate the human plasma stability data of (R)-9b and (S)-9b using HPLC analysis (Figure 6).³⁸ The solubilities of (R)-9b and (S)-9b were determined as 1 and 0.9 mg/mL, respectively, in PBS with 10% DMSO (clear solution). The solubility of the mesylate salt of (*R*)-9b in PBS with 10% DMSO was determined as >5 mg/mL, further suggesting its suitability for in vivo animal experiments. The most potent compound in the cell experiments, the [(tetrahydrofurfuryl)amino]pyrimidine (R)-9b, was subjected to limited profiling against 34 kinases using the Reaction Biology ³³P HotSpot kinase profiling service (Table 3) to determine its selectivity for ACK1. At 1 μ M (R)-9b ACK1 was the kinase most potently inhibited (99.8%). Of the other kinases inhibited by >80%, the most notable were the JAK family kinases JAK2 (98.6%) and Tyk2 (98.9%). Only six other kinases were inhibited by >80%; these were ABL1 (82.8%), ALK (86.0%), CHK1 (84.8%), FGFR1 (86.4%), LCK (87.7%), and ROS/ROS1 (84.2%). Dose responses were obtained for these kinases inhibited by >80% at 1 μ M: JAK2 (IC₅₀ = 6 nM), Tyk2 (IC₅₀ = 5 nM), FGFR1 (IC₅₀ = 160 nM), ABL1 (IC₅₀ = 206 nM), CHK1 (IC₅₀ = 154 nM), ALK (IC₅₀ = 143 nM), LCK (IC₅₀ = 136 nM), and ROS/ROS1 (IC₅₀ = 124 nM). Interestingly we have seen that other diaminopyrimidines also give rise to potent JAK inhibition.^{32,33,39} To assess JAK2 activation in prostate cancer epithelial cells, LNCaP and LAPC4 were treated with heregulin or EGF ligands, respectively, followed by immunoblotting with phospho-ACK1 (Tyr284), phospho-JAK2 (Tyr1007), and phospho-STAT5 (Tyr694) antibodies. While significant ACK1 activation was observed in EGF-treated LAPC4 and LNCaP prostate cancer epithelial cells, neither JAK2 nor STAT5 activation was observed (unpublished data), suggesting that ACK1 and JAK2 kinase activations are distinct events that occur in different cellular contexts. Since several ALK inhibitors^{26,40} also inhibit ACK1 and (R)-9b was partly designed from an ALK inhibitor, its IC₅₀ for ALK inhibition was measured using the

HotSpot assay and found to be 143 nM, 3-fold less than the IC₅₀ for ACK1. The superposition of the pose of (*R*)-**9b** in ACK1 with crystal structures of both ALK and JAK2 shows a reasonable fit in each case (see the Supporting Information, Figures S1 and S2). It is also known that Src kinase is a regulator of the androgen receptor.⁴⁰ The IC₅₀ for (*R*)-**9b** against cSrc was 438 nM, 10-fold less than the IC₅₀ for ACK1. Overall, the selectivity of (*R*)-**9b** for ACK1 and JAK2 appears to be good.

CONCLUSION

A combination of a fragment-based approach of creating smallmolecule libraries using a bisanilinopyrimidine scaffold and a novel ELISA-based assay of screening the compound libraries for their inhibitory potential led to the identification of a novel class of ACK1 inhibitors. The SARs showed tetrahydrofurfurylamine as R^2 and a piperazine moiety as R^3 are critical for ACK1 inhibitory activities with (R)-9b and (S)-9b discovered as potent compounds using ELISA and ³³P HotSpot assays. Compounds (R)-9b and (S)-9b were also shown to be potent ACK1 inhibitors in vivo, suppressing the growth of the prostate cancer derived cell lines. Furthermore, (R)-9b and (S)-9b displayed selectivity toward ACK1 when compared with other related tyrosine kinases such as Src and ALK, but potently inhibited the JAK family kinases JAK2 and Tyk2. The binding mode of compound 9b described here provides further opportunities to improve this class of compounds as ACK1 inhibitors. Overall, the availability of potent and selective ACK1 inhibitors has opened the doors for preclinical animal studies that could lead to clinical trials for hormonally regulated cancers such as prostate and breast cancers.

EXPERIMENTAL SECTION

Assessment of ACK1 Inhibition via ELISA. In brief, biotinylated AKT peptide (5 μ M) was immobilized onto a streptavidin-coated 96well plate, followed by blocking with 3% BSA. ACK1 enzyme purified from SF9 cells (40 ng/reaction) was resuspended in a reaction buffer (10 mM HEPES, 20 mM MgCl₂, 75 mM NaCl, and 1 mM DTT) and added to the plate in the presence of the compounds (10 μ M). After incubation for 1 h at 37 °C, the plates were washed, blocked, and incubated with HRP-tagged anti-pTyr antibodies (1:500) for 1 h. Alternately, anti-pY176-AKT phosphoantibodies (1:100) were used, followed by secondary anti-rabbit antibodies. The plates were developed using SigmaFast OPD substrate solution (Sigma, St. Louis, MO) and read with a spectrophotometer.

General Chemistry Information. All reagents were purchased from commercial suppliers and used without further purification. ¹H NMR spectra were recorded on an Agilent-Varian Mercury 400 MHz spectrometer with $CDCl_3$, CD_3OD_4 or $DMSO-d_6$ as the solvent. ¹³C NMR spectra were recorded at 100 MHz. All coupling constants are measured in hertz, and the chemical shifts ($\delta_{\rm H}$ and $\delta_{\rm C}$) are quoted in parts per million relative to TMS (δ 0), which was used as the internal standard. High-resolution mass spectroscopy was carried out on an Agilent 6210 LC-MS (ESI-TOF) system. Microwave reactions were performed in CEM model 908005 and Biotage Initiator 8 machines. HPLC analysis was performed using a JASCO HPLC system equipped with a PU-2089 Plus quaternary gradient pump and a UV-2075 Plus UV-vis detector, using an Alltech Kromasil C-18 column (150 × 4.6 mm, 5 μ m) and an Agilent Eclipse XDB-C18 column (150 \times 4.6 mm, 5 μ m). The purities of the final compounds that underwent biological assessment were >95% as measured by HPLC. Melting points were recorded on an Optimelt automated melting point system (Stanford Research Systems). Thin-layer chromatography was performed using silica gel 60 F254 plates (Fisher), with observation under UV when necessary. Anhydrous solvents (acetonitrile, dimethylformamide, ethanol, 2-propanol, methanol, and tetrahydrofuran) were used as purchased from Aldrich. Burdick and Jackson HPLC-grade solvents (methanol, acetonitrile, and water) were purchased from VWR for HPLC and high-resolution mass analysis. HPLC-grade TFA was purchased from Fisher.

2,4-Dichloropyrimidine-5-carbonyl Chloride (Scheme 2). To POCl₃ (45 mL, 0.071 mol) in a round-bottom flask was added 2,4-dihydroxypyrimidine-5-carboxylic acid (10.00 g, 0.064 mol) portionwise at 0 °C, followed by slow addition of PCl₅ (46.60 g, 0.229 mol). The reaction mixture was warmed to rt and heated to reflux for 16 h. The mixture was concentrated to dryness and slurried with DCM (30 mL), and the solid precipitated was filtered and washed with DCM (2 × 20 mL). The filtrate was evaporated under reduced pressure to afford the title compound (13.90 g, 99%) as a yellow oil. ¹H NMR (400 MHz, CDCl₃): δ 9.25 (s, 1H).³¹

2,4-Dichloro-N-(2-chloro-6-fluorophenyl)pyrimidine-5-carboxamide (7c). To a solution of 2,4-dichloropyrimidine-5-carbonyl chloride (6.36 g, 0.030 mol) (obtained in the previous step) in THF (40 mL) under inert conditions was added 2-chloro-6-fluoroaniline (4.38 g, 0.03 mol) portionwise, and the reaction mixture was stirred at rt for 15 h. The resulting precipitate was filtered and washed with DCM (15 mL), and the solid obtained was sonicated in HCl (1 M, 100 mL), filtered, washed with water (20 mL) and DCM (20 mL) sequentially, and dried under high vacuum to obtain the title compound 7c (5.53 g) as a white solid. The first filtrate (i.e., THF and DCM mixture) was concentrated, slurried with DCM (15 mL), and filtered to get a second crop of solid. This solid was again sonicated with HCl (1 M, 30 mL), filtered, washed with water (20 mL) and DCM (15 mL) sequentially, and dried under high vacuum to obtain another crop of pure product 7c (1.70 g) also as a white solid. The combined yield of 7c was 7.23 g (75%). Mp: 207–211 °C. 1 H NMR (400 MHz, DMSO-d₆): δ 10.71 (s, 1H), 9.03 (s, 1H), 7.48-7.36 (m, 3H). ¹⁹F NMR (376 MHz, DMSO-d₆): -115.25 to -115.28 (m). LC-MS (ESI+): m/z 319.96 (M + H)⁺. HRMS (ESI+): m/zcalcd for C111H6Cl3FN3O (M + H)+ 319.9555, found 319.9562.

2,5-Dichloro-*N*-[(tetrahydrofuran-2-yl)methyl]pyrimidin-4amine [(\pm)-8a]. Tetrahydrofurfurylamine (1.251 g, 12.36 mmol) in MeOH (11 mL) was added dropwise to a solution of 2,4,5-trichloropyrimidine (2.27 g, 12.36 mmol) and triethylamine (1.251 g, 12.36 mmol) in MeOH (11 mL) at 0 °C and stirred for 1.5 h under argon. The reaction mixture was warmed to room temperature, and the solvent was removed under reduced pressure. The residue obtained was dissolved in DCM. The organic layer was washed with water, dried, and concentrated to afford 8a as a yellow solid (2.86 g, 93%). Mp: 81–84 °C. HPLC: 92.0% [t_R = 11.17 min, 30% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, methanol- d_4) δ 8.01 (s, 1H), 4.16–4.09 (m, 1H), 3.91–3.85 (m, 1H), 3.78–3.72 (m, 1H), 3.60–3.48 (m, 2H), 2.06–1.83 (m, 3H), 1.70–1.58 (m, 1H). LC–MS (ESI+): m/z 248.03536 (M + H)⁺. HRMS (ESI+): m/z calcd for C₉H₁₁Cl₂N₃O⁺ (M + H)⁺ 248.0352, found 248.0359.

5-Bromo-2-chloro-*N*-[(tetrahydrofuran-2-yl)methyl]pyrimidin-4-amine [(\pm)-8b]. This compound was synthesized using the same procedure described for 8d except using 5-bromo-2,4dichloropyrimidine (0.500 g, 2.195 mmol) in MeOH (2 mL) and tetrahydrofurfurylamine (0.222 g, 2.195 mmol) in MeOH (2 mL) and triethylamine (0.305 mL, 2.195 mmol) to afford 8b as a white solid (0.620 g, 97%). Mp: 85–86 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 8.23 (s, 1H), 7.62 (br s, 1H), 4.03–3.98 (m, 1H), 3.77–3.71 (m, 1H), 3.62–3.57 (m, 1H), 3.45–3.38 (m, 1H), 3.36–3.30 (m, 1H), 1.90– 1.75 (m, 3H), 1.60–1.52 (m, 1H). HPLC–MS (ESI+): *m*/*z* 292 and 294.1 for Br isotopes (M + H)⁺.

2-Chloro-N-(2-chloro-6-fluorophenyl)-4-[[(tetrahydrofuran-2-yl)methyl]amino]pyrimidine-5-carboxamide [(±)-8c]. To a solution of tetrahydrofurfurylamine (0.121 g, 1.2 mmol) in MeOH (2 mL) was added triethylamine (0.167 mL, 1.2 mmol) at 0 °C under an inert atmosphere. After the resulting solution was stirred at 0 °C for 10 min, 7c (0.320 g, 1.0 mmol) in DMF (0.5 mL) was added dropwise. The mixture was warmed to rt and stirred for 30 min. The solvent was removed, and the crude mixture was purified by SiO₂ chromatography (0-30% gradient elution, EtOAc/hexane) to afford 8c as a pure white solid (0.283 g, 74%). Mp: 142 °C dec. ¹H NMR (400 MHz, CDCl₃): δ 8.88 (br t, 1H), 8.55 (s, 1H), 7.58 (br s, 1H), 7.30-7.23 (m, 2H), 7.15-7.12 (m, 1H), 4.12-4.06 (m, 1H), 3.91-3.85 (m, 1H), 3.80-3.72 (m, 2H), 3.54-3.48 (m, 1H), 2.07-1.99 (m, 1H), 1.94-1.86 (m, 2H), 1.64-1.56 (m, 1H). LC-MS (ESI+): m/z 385.07 (M + H)⁺. HRMS (ESI+): m/z calcd for C₁₆H₁₆Cl₂FN₄O₂ $(M + H)^+$ 385.0629, found 385.0623.

2-Chloro-5-fluoro-N-[(tetrahydrofuran-2-yl)methyl]pyrimidin-4-amine [(±)-8d]. To a solution of tetrahydrofurfurylamine (0.607 g, 6 mmol) in MeOH (10 mL) was added triethylamine (836 μ L) under argon at 0 °C. The mixture was stirred at 0 °C for 10 min, and then 2,4-dichloro-5-fluoropyrimidine (0.835 g, 5 mmol) in MeOH (4 mL) was added dropwise at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 h. The solvent was removed, and the resulting precipitate was dissolved in EtOAc (30 mL) and washed with water (2 \times 20 mL). The organic phase was dried (Na₂SO₄), filtered, and concentrated. The crude material obtained was purified by SiO₂ chromatography (silica gel, 20 g, EtOAc/hexane, 0-25%) to afford 8d as a white solid (0.936 g, 81%). Mp: 77-78 °C. ¹H NMR (400 MHz, CDCl₃): δ 7.86 (d, J = 2.8 Hz, 1 H), 5.63 (br s, 1H), 4.08 (ddd, J = 14.8, 7.2, 3.2 Hz, 1 H), 3.92-3.76 (m, 3H), 3.37 (ddd, J = 13.6, 8.0, 4.4 Hz, 1 H), 2.10-2.02 (m, 1 H), 1.97-1.90 (m, 2H), 1.64-1.56 (m, 1H). HPLC-MS (ESI+): m/z 232.1 (M + H)⁺.

2-Chloro-5-methyl-*N*-[(tetrahydrofuran-2-yl)methyl]pyrimidin-4-amine [(\pm)-8e]. This compound was synthesized according to the procedure described for 8d except using 2,4-dichloro-5methylpyrimidine (2.680 g, 16.440 mmol) in MeOH (20 mL) and tetrahydrofurfurylamine (1.997 g, 19.730 mmol) in MeOH (5 mL) and triethylamine (2.750 mL) to afford 8e as a white solid (1.997 g, 53%). Mp: 86–89 °C. ¹H NMR (400 MHz, CDCl₃): δ 7.78 (d, *J* = 0.8 Hz,1H), 5.24 (br s, 1H), 4.08–4.04 (m, 1H), 3.90–3.81 (m, 2H), 3.80–3.74 (m, 1H), 3.33 (ddd, *J* = 13.6, 8.0, 4.4 Hz, 1H), 2.08–2.01 (m, 1H), 1.99 (app d, *J* = 0.8 Hz, 3H), 1.95–1.88 (m, 2H), 1.63–1.54 (m, 1H). HPLC–MS (ESI+): *m*/*z* 228.1 (M + H)⁺.

(*R*)-2,5-Dichloro-*N*-[(tetrahydrofuran-2-yl)methyl]pyrimidin-4-amine [(*R*)-8f]. (*R*)-(–)-Tetrahydrofurfurylamine (0.992 g, 9.81 mmol) in MeOH (10 mL) was added to a solution of 2,4,5trichloropyrimidine (1.00 g, 5.45 mmol) in MeOH (10 mL) and triethylamine (9.81 mmol) at 0 °C for 1 h under argon. The solution was then warmed to room temperature and stirred for 2 h. The solvent was removed under reduced pressure, and the solid obtained was suspended in chloroform and washed with NaHCO₃(aq). The organic layer was separated, dried (Na₂SO₄), concentrated, and further dried under vacuum to afford (*R*)-8f) as a white solid (1.354 g, 87%). Mp: 61.5–61.8 °C. HPLC: 97.3% [t_R = 3.36 min, 70% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, CDCl₃) δ 8.01 (*s*, 1H), 5.91 (*s*, 1H), 4.12–4.06 (m, 1H), 3.91–3.88 (m, 1H), 3.85–3.77 (m, 2H), 3.44–3.37 (m, 1H), 2.09–2.01 (m, 1H), 1.97–1.90 (m, 2H), 1.60–1.57 (m, 1H). LC–MS (ESI+): m/z 247.02792 (M + H)⁺. HRMS (ESI+): m/z calcd for C₉H₁₁C₁₂N₃O⁺ (M + H)⁺ 248.0352, found 248.0368.

(S)-2,5-Dichloro-N-[(tetrahydrofuran-2-yl)methyl]pyrimidin-**4-amine** [(S)-8f]. To a solution of (S)-(-)-tetrahydrofurfurylamine (0.992 g, 9.81 mmol) in MeOH (10 mL) was added triethylamine (1.3 mL, 9.81 mmol) at 0 °C. The solution was stirred for 10 min and added to a solution of 2,4,5-trichloropyrimidine (1.00 g, 5.45 mmol) in MeoH (4 mL) dropwise also at 0 °C. The solution was then warmed to room temperature and stirred for 2 h. The solvent was removed under reduced pressure, and the solid was dissolved in chloroform, washed with satd NaHCO3(aq). The organic layer was separated, dried (Na2SO4), filtered, and concentrated to afford the chloropyrimidine (S)-8f as a white solid (1.054 g, 68%). Mp: 59.2-60.0 °C. HPLC: 95.4% [t_R = 3.36 min, 70% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, CDCl₃) δ 8.01 (s, 1H), 5.91 (br s, 1H), 4.09 (ddd, J = 7.2, 4.2, 3.2 Hz, 1H), 3.94-3.88 (m, 1H), 3.85-3.77 (m, 2H), 3.44-3.37 (m, 1H), 2.09-2.01 (m, 1H), 1.97-1.89 (m, 2H), 1.62–1.54 (m, 1H). LC–MS (ESI+): m/z 247.02792 (M + H)⁺. HRMS (ESI+): m/z calcd for $C_9H_{11}C_{12}N_3O^+$ (M + H)⁺ 248.0352, found 248.0353.

5-Chloro-N²-[4-(piperazin-1-yl)phenyl]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(±)-9a]. The chloropyrimidine 8a (0.100 g, 0.403 mmol) and 1-Boc-4-(4'-aminophenyl)piperazine (0.112 g, 0.403 mmol) were mixed in EtOH (1 mL) in a 5 mL microwave vial and heated to 150 °C for 20 min in a microwave reactor, after which a drop of concentrated HCl was added before the solution was returned to the microwave for a further 20 min at 150 °C. The precipitate formed was filtered and washed with saturated NaHCO₃. The white solid was dried under reduced pressure to obtain **9a** (0.074 g, 0.190 mmol, 47%). HPLC: 97.8% [*t*_R = 6.99 min, gradient MeOH/water, 5-95% (with 0.1% formic acid), 20 min]. ¹H NMR (400 MHz, DMSO- d_6) δ 8.93 (s, 1H), 7.86 (s, 1H), 7.50 (d, J = 9.0 Hz, 2H), 7.00 (t, J = 5.7 Hz, 1H), 6.79 (d, J = 9.1 Hz, 2H), 4.10-4.03 (m, 1H), 3.75 (dd, J = 13.6, 7.5 Hz, 1H), 3.60 (dd, J = 14.5, 7.4 Hz, 1H), 3.41 (t, J = 6.0 Hz, 3H), 2.91 (t, J = 4.0 Hz, 4H), 2.79 (t, J = 4.8 Hz, 4H), 1.92–1.77 (m, 3H), 1.62–1.56 (m, 1H). HPLC– MS (ESI): m/z 195.2 $[(M + 2H)/2]^{2+}$. LC-MS (ESI+): m/z 389.19 $(M + H)^+$. HRMS (ESI+): m/z calcd for $C_{19}H_{26}ClN_6O^+$ $(M + H)^+$ 389.1851, found 389.1860.

5-Chloro-N²-[4-(4-methylpiperazin-1-yl)phenyl]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(±)-9b]. The chloropyrimidine 8a (0.200 g, 0.806 mmol) and 4-(4-methylpiperazin-1-yl)aniline (0.170 g, 0.889 mmol) were mixed in a microwave vial with HCl (200 μ L of 4 M solution in dioxane). 2-Methoxyethanol (6.50 mL) was added, the vial was sealed, and the solution was heated to 110 °C for 48 h. The resulting solution was concentrated under reduced pressure, and the product was partitioned between CHCl₃ and saturated aqueous NaHCO₃. The organic phase was then dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The resulting product was purified (silica gel, 5:95 methanol/dichloromethane) to afford (\pm) -9b as a pure white solid (0.108 g, 33%). Mp: 133 °C dec. HPLC: 95.3% [$t_{\rm R}$ = 7.10 min, gradient MeOH/water, 5– 95% (with 0.1% formic acid), 20 min]. ¹H NMR (400 MHz, CD₃OD) δ 7.77 (s, 1H), 7.46 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 4.20-4.14 (m, 1H), 3.88-3.83 (m, 1H), 3.78-3.70 (m, 1H), 3.58 (dd, J = 13.6, 4.8 Hz, 1H), 3.48 (dd, J = 13.6, 4.8 Hz, 1H), 3.14 (t, J = 4.8 Hz, 4H), 2.62 (t, J = 5.2 Hz, 4H), 2.38 (s, 3H), 2.04-1.80 (m, 3H), 1.70–1.59 (m, 1H). HPLC–MS (ESI): m/z 202.3 [(M + 2H)/ 2]²⁺. LC-MS (ESI+): m/z 403.20 (M + H)⁺. HRMS (ESI+): m/zcalcd for C₂₀H₂₈ClN₆O⁺ (M + H)⁺ 403.2007, found 403.2008.

Development of Analytical Chiral HPLC Conditions for Separation of (±)-9b. Analytical HPLC was performed using a JASCO HPLC system equipped with a PU-2089 Plus quaternary gradient pump and a UV-2075 Plus UV–vis detector using a Chiralcel OJ column (250 × 4.6 mm). The racemic mixture (±)-9b was separated with 15% IPA and 85% hexane, 1.0 mL/min, and chromatography yielded a faster eluting peak, $t_{\rm R} = 57.7$ min (*R*-isomer), and a slower eluting peak, $t_{\rm R} = 83.5$ min (*S*-isomer).

(R)-5-Chloro-N²-[4-(4-methylpiperazin-1-yl)phenyl]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(R)-9b]. The chloropyrimidine (R)-8f (0.200 g, 0.806 mmol) and 4-(4-methylpiperazin-1-yl)aniline (0.170 g, 0.889 mmol) were mixed in a microwave tube with HCl (200 μ L of 4 M solution in dioxane). 2-Methoxyethanol (6.50 mL) was added to the mixture, and the solution was warmed to 110 °C for 48 h. The reaction mixture was concentrated, and the product was partitioned between CHCl₃ and saturated aqueous NaHCO3. The organic phase was then dried over Na₂SO₄, filtered, and concentrated. The product was purified using a silica gel column with 5:95 methanol/dichloromethane to afford the pure compound (R)-9b as a white solid (0.220 g, 68%). Mp: 135 °C dec. $[\alpha]_{\rm D}$ –12 (c 0.25 in MeOH). HPLC: 96% [$t_{\rm R}$ = 6.11 min, 30% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, CD₃OD) δ 7.82 (s, 1H), 7.50 (d, J = 8.8 Hz, 2H), 6.97 (d, J = 8.8 Hz, 2H), 4.22-4.16 (m, 1H), 3.94-3.88 (m, 1H), 3.81-3.76 (m, 1H), 3.63 (dd, *J* = 13.6, 4.8 Hz, 1H), 3.53 (dd, 13.6, 4.8 Hz, 1H), 3.18 (t, *J* = 4.8 Hz, 4H), 2.67 (t, J = 5.2 Hz, 4H), 2.34 (s, 3H), 2.03-1.86 (m, 3H), 1.70-1.63 (m, 1H). ¹³C NMR (100 MHz, methanol- d_4): δ 158.38, 158.08, 152.19, 146.49, 133.46, 120.97, 116.74, 103.51, 77.46, 67.61, 54.59, 49.48, 44.66, 44.18, 28.43, 25.15. LC-MS (ESI+): m/z 403.2007 $(M + H)^{+}$. HRMS (ESI+): m/z calcd for $C_{20}H_{27}ClN_6O^{+}$ $(M + H)^{+}$ 403.2008, found 403.2004. Chiral HPLC: 96% [$t_{\rm R}$ = 43.18 min, 15% IPA and 85% hexane, 1.0 mL/min].

(S)-5-Chloro-N²-[4-(4-methylpiperazin-1-yl)phenyl]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(S)-9b]. The chloropyrimidine (S)-8f (0.372 g, 1.5 mmol) and 4-(4-methylpiperazin-1-yl)aniline (0.316 g, 1.65 mmol) were mixed in a microwave vial with HCl (0.412 mL of a 4 M solution in dioxane). The 2-methoxyethanol (8.0 mL) was added to the mixture, the vial was sealed, and the solution was heated to 110 °C for 48 h. The resulting solution was concentrated under reduced pressure, and the product was partitioned between CHCl₃ and saturated aqueous NaHCO₃. The organic phase was separated, then dried over Na2SO4, filtered, and concentrated under reduced pressure. The product obtained was purified using a silica gel column with 5:95 methanol/dichloromethane to afford the pure compound (S)-9b as a white solid (475 mg, 79%). Mp: 145 °C dec. $[\alpha]_{\rm D}$ +9.6 (c 0.25 in MeOH). HPLC: 98% [$t_{\rm R}$ = 6.85 min, 30% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, CD₃OD) δ 7.77 (s, 1H), 7.45 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 8.8 Hz, 2H), 4.17-4.14 (m, 1H), 3.89-3.84 (m, 1H), 3.77-3.71 (m, 1H), 3.63 (dd, J = 13.6, 4.8 Hz, 1H), 3.50 (dd, 13.6, 4.8 Hz, 1H), 3.14 (t, J = 4.8 Hz, 4H), 2.62 (t, J = 4.8 Hz, 4H), 2.34 (s, 3H), 2.02–1.88 (m, 3H), 1.70-1.63 (m, 1H). LC-MS (ESI+): m/z 403.20076 (M + H)+. HRMS (ESI+): m/z calcd for $C_{20}H_{27}ClN_6O^+$ (M + H)⁺ 403.19349, found 403.20079. Chiral HPLC: 100% [$t_{\rm R}$ = 32.4 min, 15% IPA and 85% hexane, 1.0 mL/min].

5-Chloro-N²-(4-morpholinophenyl)-N⁴-[(tetrahydrofuran-2yl)methyl]pyrimidine-2,4-diamine Hydrochloride [(±)-9c]. The chloropyrimidine 8a (0.100 g, 0.403 mmol) and 4-morpholinoaniline (0.072 g, 0.403 mmol) were mixed in EtOH (3 mL) in a 5 mL microwave vial and heated to 150 °C for 20 min in a microwave reactor. The solid obtained upon cooling was filtered, washed with ethyl acetate, and dried under vacuum to afford 9c as an off-white solid (0.072 g, 0.185 mmol, 46%). Mp: 221.4 °C dec. HPLC: 99.0% [t_R = 9.90 min, gradient MeOH/water, 5-95% (with 0.1% formic acid), 20 min]. ¹H NMR (400 MHz, CD₃Cl3) δ 10.32 (s, 1H), 7.67 (s, 1H), 7.45 (dd, J = 8.8 Hz, 2H), 6.90 (d, J = 8.4 Hz, 2H), 6.62 (app t, J = 5.6 Hz, 1H), 4.13-4.06 (m, 1H), 3.92-3.86 (m, 5H), 3.82-3.77 (m, 1H), 3.46-3.40 (m, 1H), 3.15(t, J = 4.8 Hz, 4H), 2.08–2.00 (m, 1H), 1.981.91 (m, 2H), 1.61–1.52 (m, 1H). LC–MS (ESI+): m/z 390.17 (M-Cl)⁺. HRMS (ESI+): m/z calcd for $C_{19}H_{25}ClN_5O_2^+$ (M - Cl)⁺ 390.1691, found 390.1694

5-Chloro- N^2 -[**4-(piperidin-1-ylmethyl)phenyl**]- N^4 -[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(±)-9d]. The chloropyrimidine 8a (0.100 g, 0.403 mmol) and 4-(piperidinylmethyl)-aniline (0.107 g, 0.564 mmol) were mixed in 2-methoxyethanol (1.5 mL), and HCl (0.05 mL of a 12 M aq solution) was added. The reaction mixture was heated in an oil bath at 120 °C with stirring for 5.5 h and then allowed to cool to room temperature. The solvent was

evaporated, and saturated NaHCO₃ (10 mL) was added, followed by EtOAc (20 mL). The organic layer was extracted with water, dried (MgSO₄), and evaporated. The resulting solid was slurried in diethyl ether and filtered to afford the required product **9d** as a white powder (0.051 g, 31%). Mp: 120.3–121.7 °C. HPLC: 98.0% [$t_{\rm R}$ = 8.09 min, gradient MeOH/water, 5–95% (with 0.1% formic acid), 20 min]. ¹H NMR (400 MHz, CD₃OD) δ 7.82 (s, 1H), 7.57 (d, *J* = 8.6 Hz, 2H), 7.22 (d, *J* = 8.6 Hz, 2H), 4.22–4.13 (m, 1H), 3.89 (dd, *J* = 13.3, 6.6 Hz, 1H), 3.75 (dd, *J* = 14.3, 7.4 Hz, 1H), 3.58 (dd, *J* = 13.6, 4.8 Hz, 1H), 3.52 (dd, *J* = 14.0, 6.8 Hz, 1H), 1.64–1.55 (m, 4H), 1.46 (br s, 2H). LC–MS (ESI+): m/z 317.12 (M – piperidine)⁺. HPLC–MS: m/z 201.7 [(M + 2H)/2]²⁺. HRMS (ESI+): m/z calcd for C₂₁H₂₉ClN₅O⁺ (M + H)⁺ 402.2055, found 402.2063.

5-Chloro-N²-{4-[4-(carboxyethyl)piperidinyl]phenyl}-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine Hydrochloride [Intermediate for (\pm) -9e]. A suspension of 8a (0.100 g, 0.403 mmol) and ethyl 1-(4-aminophenyl)piperidine-4-carboxylate (0.200 g, 0.806 mmol) in EtOH (4 mL) was heated in a microwave reactor at 150 °C for 20 min. The resulting mixture was concentrated, and the residue obtained was recrystallized with EtOAc/hexane (5 mL, 1:2). The mixture was filtered and the solid dried under high vacuum to afford the title compound 9e as a gray solid (0.273 g, 68%). Mp: 113 °C dec. HPLC: 99.0% [$t_{\rm R}$ = 5.65 min, 45% CH₃OH in 0.1% TFA/ water, 20 min]. ¹H NMR (400 MHz, CD₃OD): δ 7.88 (s, 1H), 7.49 (br s, 2H), 7.26 (br s, 2H), 4.19-4.14 (m, 3H), 3.88-3.82 (m, 1H), 3.77-3.68 (m, 3H), 3.58 (d, I = 5.6 Hz, 2H), 3.21-3.12 (m, 2H), 2.61(app t, 1H), 2.10 (app d, 2H), 2.03-1.85 (m, 5H), 1.69-1.60 (m, 1H), 1.27 (t, J = 6.8 Hz, 3H). LC-MS (ESI+): m/z 460.2 (M + H)⁺. HRMS (ESI+): m/z calcd for C₂₃H₃₁ClN₅O₃ (M + H)⁺ 460.2110, found 460.2112

5-Chloro-N²-[4-(4-carboxypiperidinyl)phenyl]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(±)-9e]. To a suspension of the compound prepared in the above step (0.272 g, 0.548 mmol) in THF (2.192 mL) was added NaOH (2 M, 1.096 mL). The reaction mixture was stirred at room temperature for 18 h. The THF was removed, water was added (2 mL), and extraction with DCM (2 mL) was performed. The aqueous solution was acidified with HCl (1 M) to pH 4-5. The resulting precipitate was filtered and washed with water (5 mL \times 4), then quickly washed with MeOH (2 mL), and dried under high vacuum to afford the first crop of the required compound (0.112 g). The solid precipitated in the filtrate was filtered, washed with water $(5 \text{ mL} \times 2)$, and dried under high vacuum to afford the second crop of the required compound (0.096 g). The solids were combined to afford 9e as a pure dark gray solid (0.208 g, 81%). Mp: 93 °C dec. HPLC: 99.0% [$t_{\rm R}$ = 8.59 min, 30% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, CD₃OD): δ 7.81 (s, 1H), 7.46 (d, J = 8.4 Hz, 2H), 7.09 (d, J = 8.8 Hz, 2H), 4.20–4.13 (m, 1H), 3.89-3.84 (m, 1H), 3.77-3.72 (m, 1H), 3.62-3.48 (m, 4H), 2.79 (dt, J = 11.2, 2.4 Hz, 2H), 2.45 (tt, J = 11.2, 4.0 Hz, 1H), 2.06-1.80 (m, 7H), 1.70–1.62 (m, 1H). LC–MS (ESI+): m/z 432.17; $(M + H)^{+}$. HRMS (ESI+): m/z calcd for $C_{21}H_{27}ClN_5O_3$ $(M + H)^{+}$ 432.1796, found 432.1796.

4-(Piperidin-4-yl)aniline [Intermediate for (±)-9f]. To a solution of 4-(4-nitrophenyl)piperidine (400 mg, 1.942 mmol) in MeOH (30 mL) was added Pd on carbon (144 mg) under argon. The argon was removed, and H₂ gas was introduced into the system. After 3.5 h at room temperature, the palladium catalyst was filtered, and the solvent was removed. The residue was purified by trituration with DCM to afford 4-(piperidin-4-yl)aniline as a white solid (340 mg, 99%). Mp: 162.7 °C. HPLC: 95.0% [$t_{\rm R}$ = 6.74 min, 30% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, methanol- d_4) δ 6.99 (d, J = 8.4 Hz, 2H), 6.69 (d, J = 8.4 Hz, 2H), 3.46–3.22 (m, 2H), 2.95 (td, J = 12.8, 3.0 Hz, 2H), 2.70–2.62 (m, 1H), 1.96–1.84 (m, 2H), 1.82–1.70 (m, 2H). LC–MS (ESI+): m/z 177.13808 (M + H)⁺. HRMS (ESI+): m/z calcd for C₁₁H₁₆N₂⁺ (M + H)⁺ 177.1386, found 177.1382.

5-Chloro- N^2 -[4-(piperidin-4-yl)phenyl]- N^4 -[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(\pm)-9f]. The chloropyrimidine 8a (80 mg, 0.32 mmol) and 4-(piperidin-4-yl)aniline (28.4 mg, 0.32 mmol) (synthesized above) were mixed in a microwave vial with EtOH (1.5 mL). Then HCl (1 mL of a 0.1 M aq solution) was added to the tube, the tube was sealed, and the solution was heated to 150 °C for 40 min. The solvent was removed under reduced pressure and the residue washed with DCM and filtered to afford **9f** as a peach solid (90 mg, 72.0%). Mp: 160 °C dec. HPLC: 85.6% [$t_{\rm R}$ = 7.51 min, 30% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, methanol- d_4) δ 7.94 (s, 1H), 7.49 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 4.16 (p, J = 6.2 Hz, 1H), 3.86–3.68 (m, 2H), 3.65–3.46 (m, 4H), 3.22–3.09 (m, 2H), 3.00–2.90 (m, 1H), 2.14–1.82 (m, 8H), 1.69–1.58 (m, 1H). ¹³C NMR (101 MHz, methanol- d_4) δ 159.20, 151.43, 141.84, 139.72, 135.04, 127.16, 122.99, 105.19, 76.74, 67.63, 45.23, 44.15, 39.15, 29.68, 28.54, 25.01. LC–MS (ESI+): m/z 388.18965 (M + H)⁺. HRMS (ESI+): m/z calcd for C₂₀H₂₆ClN₅O⁺ (M + H)⁺ 388.1899, found 388.1900.

5-Bromo-N²-(4-hydroxyphenyl)-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine Hydrochloride $[(\pm)-9q]$. A suspension of (\pm) -8b (0.073 g, 0.250 mmol) and 4-amino-2-chlorophenol (0.036 g, 0.250 mmol) in EtOH (1 mL) was heated in a microwave reactor at 150 °C for 20 min. The mixture was concentrated to dryness, EtOAc (3 mL) was then added, the resulting mixture was sonicated for 5 min and filtered, and the precipitate was washed again with EtOAc (2 mL) and dried under high vacuum to afford the title compound as a gray solid (0.071 g, 65%). Mp: 193 °C dec. HPLC: 94.0% [$t_{\rm R}$ = 6.50 min, 45% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, CD₃OD): δ 7.96 (s, 1H), 7.51 (s, 1H), 7.15 (dd, J = 8.8, 2.4 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 4.18–4.11 (m, 1H), 3.86–3.80 (m, 1H), 3.76–3.71 (m, 1H), 3.56 (app d, J = 6.0 Hz, 2H), 2.05-1.97 (m, 1H), 1.93-1.86 (m, 2H), 1.65-1.56 (m, 1H). LC-MS (ESI+): m/z 399.02 and 401.02 for Br isotopes (M + H)⁺. HRMS (ESI+): m/z calcd for C₁₅H₁₇BrClN₄O₂ (M + H)⁺ 399.0218, found 399.0213.

5-Bromo-*N*²-(4-morpholinophenyl)-*N*⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(±)-9h]. A mixture of 8b (0.100 g, 0.342 mmol) and 4-morpholinoaniline (0.067 g, 0.376 mmol) in EtOH (1 mL) was heated in a microwave reactor at 150 °C for 20 min. The precipitate was filtered, washed with ethyl acetate, and dried under high vacuum to afford the title compound 9h as a white solid (0.115 mg, 65%). Mp: 198 °C dec. HPLC: 94.7% [t_R = 5.84 min, 45% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, CD₃OD): δ 7.98 (s, 1H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.27 (d, *J* = 8.4 Hz, 2H), 4.19–4.13 (m, 1H), 3.92 (app t, *J* = 5.2 Hz, 4H), 3.86–3.80 (m, 1H), 3.76–3.71 (m, 1H), 3.61–3.56 (m, 2H), 3.35 (app t, *J* = 4.4 Hz, 4H), 2.04–1.87 (m, 3H), 1.67–1.59 (m, 1H). LC–MS (ESI+): *m*/*z* 434.13 (M + H)⁺. HRMS (ESI+): *m*/*z* calcd for C₁₉H₂₅BrN₅O₂ (M + H)⁺ 434.1186, found 434.1182.

Boc Derivative of 5-Bromo-N²-[4-(piperazin-1-yl)phenyl]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine Hydrochloride [Intermediate for (±)-9i]. A mixture of 8b (0.176 g, 0.6 mmol) and tert-butyl 4-(4-aminophenyl)piperazine-1-carboxylate (0.167 g, 0.6 mmol) in 2-propanol (3 mL) was heated at 80 °C in a sealed tube for 40 h (the reaction was monitored by HPLC-MS). The precipitate formed upon cooling the mixture was filtered, washed with 2-propanol (2 mL \times 2), and dried under high vacuum to afford the title compound 9i as a light green solid (0.300 g, 88%). Mp: 124 °C dec. HPLC: 98.5% [t_R = 7.51 min, 60% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, DMSO- d_6): δ 9.00 (s, 1H disappeared on D_2O shake), 7.94 (s, 1H), 7.53(d, J = 8.8 Hz, 2H), 6.84 (d, J =8.8 Hz, 2H), 6.76 (br t, J = 5.6 Hz, 1H, disappeared on D₂O shake), 4.10-4.03 (m, 1H), 3.78-3.73 (m, 1H), 3.63-3.58 (m, 1H), 3.43-3.40 (br m, 6H), 2.96 (t, J = 4.8 Hz, 4H), 1.90–1.77 (m, 3H), 1.62-1.56 (m, 1H), 1.40 (s, 9H). LC-MS (ESI+): m/z 533.19 and 535.19 for Br isotopes $(M + H)^+$. HRMS (ESI+): m/z calcd for $C_{24}H_{34}BrN_6O_3 (M + H)^+$ 533.1870, found 533.1862.

5-Bromo-N²-[4-(piperazin-1-yl)phenyl]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine Trifluoroacetate Salt [(\pm)-9i]. To a solution of the Boc intermediate (0.291 g, 0.511 mmol) (synthesized in the above step) in DCM (2 mL) at 0 °C was added TFA (2 mL). The reaction mixture was warmed to rt and stirred for 1 h. The solvent was removed under reduced pressure. The resulting residue was slurried in DCM/EtOAc/hexane (1:1:2) and sonicated. The precipitate obtained was filtered, washed with EtOAc/hexane (1:1, 3 mL × 2), and dried under high vacuum to afford the title compound **9i** as a gray solid (0.340 g, 98%). Mp: 200 °C dec. HPLC: 100% [t_R = 4.28 min, 35% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, DMSO- d_6): δ 9.50 (br s, 1H, disappeared on D₂O shake), 8.76 (s, 1H, disappeared on D₂O shake), 8.04 (s, 1H), 7.49 (d, J = 8.80 Hz, 2H), 6.92 (d, J = 8.85 Hz, 2H), 4.08–4.03 (m, 1H), 3.74–3.70 (m, 1H), 3.62–3.57 (m, 1H), 3.41 (t, J = 6.4 Hz, 2H), 3.24–3.22 (m, 8H), 1.91–1.74 (m, 3H), 1.61–1.54 (m, 1H). ¹⁹F NMR (376 MHz, DMSO- d_6): δ –74.19 (s). LC–MS (ESI+): m/z calcd for C₁₉H₂₆BrN₆O (M + H)⁺ 433.1346, found 433.1335.

5-Bromo-N²-{4-[(4-carboxyethyl)piperidinyl]phenyl}-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [Intermediate for (\pm) -9j]. A mixture of 8b (0.200 g, 0.683 mmol) and ethyl 1-(4-aminophenyl)piperidine-4-carboxylate (0.186 g, 0.752 mmol) in EtOH (2 mL) was heated in a microwave reactor at 150 °C for 20 min. The precipitate obtained was filtered, washed with ethyl acetate, and dried under high vacuum to afford the title compound as a brown solid (0.070 mg, 22%). Mp: 137 °C dec. HPLC: 99.7% [$t_{\rm R}$ = 6.55 min, 45% CH₃OH in water (0.1% TFA), 20 min]. ¹H NMR (400 MHz, DMSO) δ 8.96 (s, 1H), 7.94 (s, 1H), 7.50 (d, J = 8.8 Hz, 2H), 6.80 (d, J = 8.8 Hz, 2H), 6.74 (t, J = 6.4 Hz, 1H), 4.05 (q, J = 6.8 Hz, 2H), 3.75 (q, J = 7.6 Hz, 1H), 3.60 (q, J = 7.2 Hz, 1H), 3.49–3.88 (m, 5H), 2.64 (t, J = 11.2 Hz, 2H), 2.45-2.33 (m, 1H), 1.89-1.79 (m, 4H), 1.73-1.60(m, 2H), 1.17 (t, J = 7.2, 3H). LC-MS (ESI+): m/z 503.2 (M + H)⁺. HRMS (ESI+): m/z calcd for $C_{23}H_{30}BrN_5O_3$ (M + H)⁺ 504.1605, found 504.1597

5-Bromo-N²-[4-(4-carboxypiperidinyl)phenyl]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(±)-9j]. The ester intermediate (0.025 g, 0.050 mmol) (synthesized above) in THF (0.248 mL) and 2 M NaOH (0.200 mL) were stirred at room temperature for 18 h. The resulting solution was concentrated, and the product obtained was dissolved in water (2 mL). The aqueous solution was acidified with 1 M HCl to pH 4-5, and the solid mixture obtained was filtered to afford 9j as a pure light brown solid (0.019 g, 81%). Mp: 129–133 °C. HPLC: 99.7% [t_R = 6.55 min, 45% CH₃OH in water (0.1% TFA), 20 min]. ¹H NMR (400 MHz, DMSO) δ 12.19 (s, 1H), 8.95 (s, 1H), 7.93 (s, 1H), 7.49 (d, J = 9.2 Hz, 2H), 6.81 (d, J = 8.8 Hz, 2H), 6.74 (t, J = 5.6 Hz, 1H), 4.09-4.03 (m, 1H), 3.78-3.73 (m, 1H), 3.63-3.57 (m, 2H), 3.45 (dt, J = 12.4, 3.2 Hz, 1H), 3.42-3.39 (m, 1H), 2.61 (t, J = 9.2 Hz, 2H), 2.35–2.30 (m, 1H), 1.89–1.73 (m, 5H), 1.67–1.53 (m, 2H). LC–MS (ESI+): m/z 476.12 (M + H)⁺. HRMS (ESI+): m/z calcd for C₂₁H₂₆BrN₅O₃ (M + H) 476.1292, found 476.1285

5-Bromo-N²-[4-(piperidin-4-yl)phenyl]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(±)-9k]. A mixture of 8b (0.047 g, 0.161 mmol) and 4-(piperidin-4-yl)aniline (0.028 g, 0.161 mmol) (synthesis described above) in EtOH (1 mL) and HCl (1 mL, 0.1 M aq solution) was heated in a microwave reactor at 150 °C for 40 min. The solvent was removed, and the residue was slurried with DCM, filtered, and dried under high vacuum to afford the title compound 9k as a white solid (0.014 mg, 19%). Mp: 162 °C dec. HPLC: 98.8% [t_R = 2.50 min, 45% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, CD₃OD): δ 8.01 (s, 1H), 7.47 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 4.18–4.12 (m, 1H), 3.83–3.76 (m, 1H), 3.75–3.69 (m, 1H), 3.59–3.49 (m, 4H), 3.15 (t, J = 12.8 Hz, 2H), 2.96 (t, J = 7.2 Hz, 1H), 2.10–1.87 (m, 7H), 1.66–1.58 (m, 1H). LC-MS (ESI+): m/z 432.13; (M + H)⁺. HRMS (ESI+): m/z calcd for C₂₀H₂₇N₅O (M + H)⁺ 432.1394, found 432.1392.

5-[[2-Chloro-6-fluorophenyl]carbamoyl]- N^2 -[4-[(4carboxyethyl)piperidinyl]phenyl}- N^4 -[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [Intermediate for (±)-9]]. A mixture of 8c (0.200 g, 0.519 mmol) and ethyl 1-(4-aminophenyl)piperidine-4-carboxylate (0.141 g, 0.571 mmol) in EtOH (2 mL) was heated in a microwave reactor at 150 °C for 20 min. The resulting precipitate was filtered, washed with ethyl acetate, and dried under high vacuum to afford the title compound as a gray solid (0.215 mg, 71%). Mp: 137–141 °C. HPLC: 94.1% [t_R = 12.01 min, gradient 5–95% CH₃OH in water (0.1% formic acid), 20 min]. ¹H NMR (400 MHz, DMSO) δ 7.86 (s, 1H), 7.44 (d, *J* = 9.2 Hz, 2H), 6.95 (d, *J* = 9.2 Hz, 2H), 4.14 (q, *J* = 7.2 Hz, 3H), 3.88–3.86 (m, 1H), 3.77–3.72 (m, 1H), 3.62–3.44 (m, 4H), 2.73 (dt, *J* = 11.6, 2.4 Hz, 2H), 2.54–2.41 (m, 1H), 2.03–1.78 (m, 7H), 1.69–1.62 (m, 1H), 1.26 (t, *J* = 7.2 Hz, 3H). LC–MS (ESI+): *m*/*z* 597.21 (M + H)⁺. HRMS (ESI+): *m*/*z* calcd for C₃₀H₃₇ClFN₆O₄ (M + H)⁺ 597.2388, found 597.2378.

5-[(2-Chloro-6-fluorophenyl)carbamoyl]-N²-[4-(4carboxypiperidinyl)phenyl]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine $[(\pm)-91]$. The ester intermediate (0.100 g, 0.167 mmol) synthesized above in THF (1 mL) and 2 M NaOH (0.200 mL) were stirred at room temperature for 18 h. The resulting solution was concentrated, and the product obtained was dissolved in water (2 mL). The aqueous solution was acidified with HCl (1 M aq solution) to pH 4-5, and the solid mixture obtained was filtered to afford 9j as a white solid (0.75 mg, 78%). Mp: 145.1 °C. HPLC: 99.3% $[t_{\rm R} = 4.20 \text{ min}, 55\% \text{ CH}_3\text{OH in } 0.1\% \text{ TFA/water}, 20 \text{ min}]$. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.23 (s, 1H), 9.84 (s, 1H), 9.49 (br s, 1H), 8.85 (br s, 1H), 8.73 (s, 1H), 7.57 (d, J = 7.6 Hz, 2H), 7.43-7.30 (m, 3H), 6.87 (d, J = 7.6 Hz, 2H), 4.01–3.98 (m, 1H), 3.76–3.71 (m, 1H), 3.62-3.50 (m, 4H), 3.43-3.38 (m, 1H), 2.66 (app t, 2H), 2.34 (app t, 1H), 1.94–1.77 (m, 5H), 1.67–1.48 (m, 3H). LC–MS (ESI+): m/z 569.2 (M + H)⁺. HRMS (ESI+): m/z calcd for C₂₈H₃₁ClFN₆O₄ (M + H)⁺ 569.2074, found 569.2063.

Boc Derivative of 5-[(2-Chloro-6-fluorophenyl)carbamoyl]- N^2 -[4-(piperazin-1-yl]phenyl]- N^4 -[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [Intermediate for (±)-9m]. A mixture of 8c (0.308 g, 0.8 mmol) and tert-butyl 4-(4-aminophenyl)piperazine-1-carboxylate (0.222 g, 0.8 mmol) in 2-propanol (4 mL) was heated at 80 °C in a sealed tube for 5 h. The resulting precipitate was filtered upon cooling, washed with 2-propanol ($2 \text{ mL} \times 3$), and then dried under vacuum to afford the title compound as a light green solid (0.440 g, 83%). Mp: 184–186 °C. HPLC: 99.8% [$t_{\rm R}$ = 5.23 min, 70% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, DMSO- d_6): δ 10.26 (br s, 1H, disappeared on D₂O shake), 10.12 (br s, 1H, disappeared on D₂O shake), 9.31 (br s, 1H, disappeared on D_2O shake), 8.71 (s, 1H), 7.51–7.33 (m, 4H), 7.02 (app d, J = 6.0 Hz, 2H), 4.05-3.98 (m, 1H), 3.75-3.70 (m, 1H, overlapping with water peak), 3.63-3.57 (m, 1H, overlapping with water peak), 3.47-3.42 (m, 6H), 3.10 (br s, 4H), 1.93-1.88 (m, 1H), 1.83-1.76 (m, 2H), 1.56–1.48 (m, 1H), 1.40 (s, 9H). ¹⁹F NMR (376 MHz, DMSO- d_6): δ -116.16 to -116.20 (m). LC-MS (ESI+): m/z 626.25 (M + H)⁺. HRMS (ESI+): m/z calcd for $C_{31}H_{38}CIFN_7O_4$ (M + H)⁺ 626.2652, found 626.2651.

5-[(2-Chloro-6-fluorophenyl)carbamoyl]-N²-[(4-piperazin-1yl]phenyl]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine Trifluoroacetate Salt $[(\pm)-9m]$. To a solution of the Boc intermediate synthesized above (0.408 g, 0.616 mmol) in DCM (4 mL) at 0 °C was added TFA (4 mL). The reaction mixture was warmed to rt and stirred for 1 h. The solvent was removed under reduced pressure. The resulting residue was slurried in EtOAc/hexane (1:2) and sonicated. The precipitate was filtered, washed with EtOAc/ hexane (1:1) (3 mL \times 2), and dried under high vacuum to afford the title compound as a gray solid (0.400 g, 102%). Mp: 205 °C dec. HPLC: 99.5% [t_R = 4.44 min, 50% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, DMSO- d_6): δ 9.99 (s, 1H, disappeared on D₂O shake), 9.80 (br s, 1H, disappeared on D₂O shake), 9.04 (br s, 1H, disappeared on D_2O shake) 8.73 (s, 1H), 7.60 (d, I = 8.8 Hz, 2H), 7.44–7.31 (m, 3H), 6.96 (d, J = 8.8 Hz, 2H), 4.04–3.98 (m, 1H), 3.74-3.72 (m, 1H), 3.63-3.58 (m, 2H), 3.44-3.39 (m, 1H), 3.27-3.23 (m, 8H), 1.94-1.88 (m, 1H), 1.83-1.77 (m, 2H), 1.57-1.49 (m, 1H). ¹⁹F NMR (376 MHz, DMSO- d_6): δ –74.24 (s, 3H), –116.17 to -116.21 (m, 1H). LC-MS (ESI+): m/z 526.22 (M + H)⁺. HRMS (ESI+): m/z calcd for C₂₆H₃₀ClFN₇O₂ (M + H)⁺ 526.2128, found 526.2123.

5-[(2-Chloro-6-fluorophenyl)carbamoyl]- N^2 -[(4-morpholinophenyl]- N^4 -[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(±)-9n]. The chloropyrimidine 8c (100 mg, 0.26 mmol) and 4-morpholinoaniline (0.069 g, 0.38 mmol) were mixed in a microwave tube with HCl (110 μ L of a 4 M solution in dioxane). 2-Methoxyethanol

(4.00 mL) was added to the mixture and the resulting mixture heated at 110 °C for 16 h. The resulting solution was concentrated, and the product was partitioned between CHCl₃ and saturated aqueous NaHCO₃. The organic phase was then dried over Na₂SO₄, filtered, and concentrated. The resulting solid was slurried twice with dichloromethane and hexane, filtered, and dried under reduced pressure to yield **9n** as a light green solid (0.080 g). Mp: 231 °C dec. ¹H NMR (400 MHz, CD₃OD): 8.50 (s, 1H), 7.38–7.37 (m, 4H), 7.24–7.19 (m, 1H), 7.08 (d, *J* = 8.8 Hz, 2H), 4.16–4.10 (m, 1H), 3.85 (t, *J* = 4.8 Hz, 4H), 3.76–3.59 (m, 3H), 3.20 (t, *J* = 4.8 Hz, 5H), 2.01–1.91 (m, 3H), 1.66–1.58 (m, 1H). HPLC–MS (ESI+): *m/z* 527.2 (100%). HRMS (ESI+): *m/z* calcd for C₂₇H₃₁ClFN₆O₃ (M + H) 527.1968, found 527.1962.

5-[(2-Chloro-6-fluorophenyl)carbamoyl]-N²-[(4-(piperidin-4yl)phenyl]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(±)-90]. A mixture of chloropyrimidine 8c (0.062 g, 0.161 mmol) and 4-piperidinylaniline (0.028 g, 0.161 mmol) in EtOH (1 mL) and HCl (1 mL of a 0.1 M aq solution) was heated in a microwave reactor at 150 °C for 40 min. The solvent was removed, and the resulting residue was slurried with DCM, filtered, and dried under high vacuum to afford the title compound 90 as a solid (0.032 mg, 36%). Mp: 186 °C dec. HPLC: 89.4% $[t_{\rm R} = 10.37 \text{ min}, 45\%]$ CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, CD₃OD): δ 8.60 (d, J = 4.0 Hz, 1H), 7.55 (d, J = 7.6 Hz, 2H), 7.40–7.38 (m, 3H),), 7.25-7.19 (m, 1H), 4.19-4.10 (m, 1H), 3.93-3.80 (m, 1H), 3.75-3.60 (m, 3H), 3.53-3.50 (d, J = 12.0 Hz, 2H), 3.16 (t, J = 12.0 Hz, 10.4 Hz, 2H), 2.97 (t, J = 12.0 Hz, 1H), 2.11–1.89 (m, 6H), 1.67–1.60 (m, 1H). LC-MS (ESI+): m/z 525.2; (M + H)⁺. HRMS (ESI+): m/zcalcd for $C_{27}H_{31}ClFN_6O_2$ (M + H)⁺ 525.2176, found 525.2175.

5-[(2-Chloro-6-fluorophenyl)carbamoyl]- N^2 -[(3-fluoro-4-hydroxyphenyl)amino]- N^4 -[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(\pm)-9p]. This compound was synthesized according to the procedure described for 90 using 8c (0.050 g, 0.130 mmol), 4-amino-2-fluorophenol (0.017 g, 0.130 mmol), and EtOH (1 mL). A mixture of EtOAc/hexane was used for recrystallization to afford the title compound as a brown solid (0.051 g, 76%). Mp: 183 °C dec. HPLC: 99.4% [t_R = 6.53 min, 55% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, CD₃OD): δ 8.54 (s, 1H), 7.43–7.38 (m, 3H), 7.24–7.19 (m, 1H), 7.10 (d, J = 8.4 Hz, 1H), 6.96 (t, J = 8.4 Hz, 1H), 4.15–4.09 (m, 1H), 3.90–3.85 (m, 1H), 3.77–3.59 (m, 3H), 2.05–1.99 (m, 1H), 1.96–1.88 (m, 1H), 1.67–1.58 (m, 1H). ¹⁹F NMR (376 MHz, CD₃OD): δ –118.25 (d). LC–MS (ESI+): m/z 476.12 (M + H)⁺. HRMS (ESI+): m/z calcd for C₂₂H₂₁F₂N₅O₃ (M + H)⁺ 476.1296, found 476.1290.

5-[(2-Chloro-6-fluorophenyl)carbamoyl]-N²-[(4methoxyphenyl)amino]- N^4 -[(tetrahydrofuran-2-yl)methyl]-pyrimidine-2,4-diamine [(\pm)-9q]. A mixture of 8c (0.050 g, 0.130 mmol), 4-methoxyaniline (0.016 g, 0.132 mmol), and HCl (0.033 mL of a 4 M solution in dioxane, 0.132 mmol) in dioxane (1 mL) was heated in a microwave reactor at 180 °C for 30 min. The mixture was evaporated to dryness, EtOAc was added (5 mL), and the resulting mixture was sonicated. The resulting solid was filtered, washed with satd NaHCO₃ (5 mL \times 2) and water (5 mL \times 2), and dried under vacuum to afford the title compound 9q as a pure white solid (0.051 g, 84%). Mp: 201 °C dec. HPLC: 99.5% $[t_R = 4.37 \text{ min}, 65\% \text{ CH}_3\text{OH in}]$ 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, DMSO- d_6): δ 10.22 (s, 1H), 10.06 (br s, 1H), 9.27 (br s, 1H), 8.72 (s, 1H), 7.54 (app d, J = 7.6 Hz, 2H), 7.45-7.32 (m, 3H), 6.93 (d, J = 8.8 Hz, 2H), 4.04-3.98 (m, 1H), 3.75-3.70 (m, 4H), 3.61-3.55 (m, 3H), 1.95-1.87 (m, 1H), 1.82-1.76 (m, 2H), 1.56-1.48 (m, 1H). ¹⁹F NMR (376 MHz, DMSO-d₆): δ -116.16 to -116.19 (m). LC-MS (ESI+): m/z 472.14 $(M + H)^+$. HRMS (ESI+): m/z calcd for $C_{23}H_{24}ClFN_5O_3$ $(M + H)^+$ 472.1546, found 472.1548.

5-[(2-Chloro-6-fluorophenyl)carbamoyl]- N^2 -[(4-hydroxyphenyl)amino]- N^4 -[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(±)-9r]. This compound was synthesized using the procedure described for 9q using pyrimidine 8c (0.050 g, 0.130 mmol) and 4-aminophenol (0.017 g, 0.156 mmol) to obtain the title compound 9r as a beige solid (0.046 g, 78%). Mp: 263 °C dec. HPLC: 99.4% [t_R = 6.77 min, 55% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, DMSO- d_6): δ 9.82 (s, 1H), 9.41 (br s, 1H), 9.09 (s, 1H), 8.83 (br s, 1H), 8.72 (s, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.43–7.30 (m, 3H), 6.67 (d, J = 8.8 Hz, 2H), 4.02–3.98 (m, 1H), 3.76–3.71 (m, 1H), 3.62–3.57 (m, 2H), 3.42–3.37 (m, 1H), 1.93–1.87 (m, 1H), 1.84–1.75 (m, 2H), 1.56–1.47 (m, 1H). ¹⁹F NMR (376 MHz, DMSO- d_6): δ –116.15 to –116.19 (m). LC–MS (ESI+): m/z 458.13 (M + H)⁺. HRMS (ESI+): m/z calcd for C₂₂H₂₂ClFN₅O₃ (M + H)⁺ 458.1390, found 458.1390. **5-Fluoro-N²-[(4-hydroxy-3-fluorophenyl)amino]-N⁴-[(tetra-**

hydrofuran-2-yl)methyl]pyrimidine-2,4-diamine Hydrochloride [(+)-9s]. A mixture of pyrimidine 8d (0.116 g, 0.5 mmol) and 4-amino-2-fluorophenol (0.064 g, 0.5 mmol) in EtOH (2 mL) was heated in a microwave reactor at 150 °C for 20 min. The solvent was removed, and the resulting residue was slurried and sonicated with EtOAc (3 mL). The mixture was filtered, and the precipitate was washed with EtOAc (2 mL) and then dried under high vacuum to afford the title compound as a dark brown solid (0.152 g, 84%). Mp: 201 °C dec. HPLC: 98.5% [$t_{\rm R}$ = 7.72 min, 35% CH₃OH in 0.1% TFA/ water, 20 min]. ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.23 (s, 1H), 9.87 (br s, 1H), 10.18 (s, 1H), 9.19 (br s, 1H), 8.08 (d, J = 5.2 Hz, 1H), 7.43 (dd, J = 13.2, 2.0 Hz, 1H), 7.01 (dd, J = 8.8, 1.6 Hz, 1 H), 6.94 (t, I = 9.6 Hz, 1H), 4.07–4.01 (m, 1H), 3.75–3.69 (m, 1H), 3.63–3.58 (m, 1H), 1.93-1.85 (m, 1H), 1.83-1.75 (m, 2H), 1.59-1.51 (m, 1H). ¹⁹F NMR (376 MHz, DMSO- d_6): δ –134.70 (s), –163.23 (s). LC– MS (ESI+): m/z 323.14 (M + H)⁺. HRMS (ESI+): m/z calcd for $C_{15}H_{17}F_2N_4O_2$ (M + H)⁺ 323.1314, found 323.1314.

5-Methyl-N²-[(4-hydroxy-3-fluorophenyl)amino]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine Hydrochloride $[(\pm)-9t]$. A suspension of pyrimidine 8e (0.114 g, 0.5 mmol) and 4-amino-2-fluorophenol (0.064 g, 0.5 mmol) in EtOH (1 mL) was heated in a microwave reactor at 150 °C for 20 min. The mixture was concentrated, and the residue obtained was recrystallized with MeOH/ EtOAc, filtered, and washed with EtOAc (2 mL) to afford the title compound 9t as a dark brown solid. The filtrate was concentrated and recrystallized again with MeOH/EtOAc to afford a second crop of solid. The solids were combined to afford the title compound 9t as a dark brown solid (0.163 g, 92%). Mp: 199 °C dec. HPLC: 98.8% [$t_{\rm R}$ = 12.26 min, 35% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, CD₃OD): δ 7.45 (s, 1H), 7.29 (dd, J = 12.0, 1.6 Hz, 1H), 7.03-6.93 (m, 2H), 4.20-4.13 (m, 1H), 3.88-3.83 (m, 1H), 3.78-3.72 (m, 1H), 3.64-3.51 (m, 2H), 2.04 (s, 3H), 1.96-1.87 (m, 2H), 1.66-1.57 (m, 1H). ¹⁹F NMR (376 MHz, CD₃OD): δ -136.94 (s). LC-MS (ESI+): m/z 319.16 (M + H)⁺. HRMS (ESI+): m/z calcd for $C_{16}H_{20}FN_4O_2$ (M + H)⁺ 319.1565, found 319.1566.

5-Bromo-N²-[(4-hydroxy-3-fluorophenyl)amino]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine $[(\pm)-13]$. A mixture of pyrimidine 8b (0.400 g, 1.4 mmol) and 4-amino-2fluorophenol (0.174 g, 1.4 mmol) in EtOH (3 mL) in a microwave vial was heated to 150 $^\circ C$ for 20 min in a microwave reactor. The solvent was evaporated, and the residue obtained was washed with ethyl acetate, slurried with dichloromethane and hexane, and filtered to afford 13 as a pure beige solid (0.580 g, 98%). Mp: 205-207 °C. HPLC: 94% [$t_{\rm R}$ = 6.33 min, 40% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, DMSO- d_6): δ 10.20 (s, 1H), 9.83 (br s, 1H), 8.34 (br s, 1H), 8.16 (s, 1H), 7.44 (dd, J = 12.8, 2.0 Hz, 1H), 7.02 (dd, J = 8.4, 1.2 Hz, 1H), 6.91 (t, J = 8.4 Hz, 1H), 4.07-4.01 (m, 1H), 3.72-3.67 (m, 1H), 3.60-3.55 (m, 1H), 3.42-3.37 (m, 2H), 1.88-1.71 (m, 3H), 1.56-1.48 (m, 1H). HPLC-MS (ESI+): m/z 383 and 385 for Br isotopes (M + H)⁺. HRMS (ESI+): m/z calcd for $C_{15}H_{17}BrFN_4O_2$ (M + H)⁺ 383.0513, found 383.0513.

5-[(2-Chloro-6-fluorophenyl)carbamoyl]-*N*²-(3-fluoro-4-hydroxyphenyl)-*N*⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine Hydrochloride [(±)-14]. This compound was synthesized using the procedure described for 9t except using 8c (0.050 g, 0.130 mmol), 4-amino-2-fluorophenol (0.017 g, 0.130 mmol), and EtOH (1 mL). EtOAc/hexane was used for recrystallization to afford the title compound as a brown solid (0.051 g, 76%). Mp: 183 °C dec. HPLC: 99.4% [t_R = 6.53 min, 55% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, CD₃OD): δ 8.54 (s, 1H), 7.43–7.38 (m, 3H), 7.24–7.19 (m, 1H), 7.10 (d, *J* = 8.4 Hz, 1H), 6.96 (t, *J* = 8.4 Hz, 1H), 4.15–4.09 (m, 1H), 3.90–3.85 (m, 1H), 3.77–3.59 (m, 3H), 2.05–1.99 (m, 1H), 1.96–1.88 (m, 2H), 1.67–1.58 (m, 1H). $^{19}{\rm F}$ NMR (376 MHz, CD₃OD): δ –118.25 (d). LC–MS (ESI+): m/z 476.12 (M + H)⁺. HRMS (ESI+): m/z calcd for C $_{22}{\rm H}_{21}{\rm F}_2{\rm N}_5{\rm O}_3$ (M + H)⁺ 476.1296, found 476.1290.

5-Chloro-*N*²-(4-hydroxyphenyl)-*N*⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine Hydrochloride [(\pm)-15]. A mixture of 8a (0.050 g, 0.20 mmol) and 4-aminophenol (0.024 g, 0.22 mmol) in EtOH (0.500 mL) in a microwave vial was heated to 150 °C for 20 min in a microwave reactor. The precipitate formed upon cooling the mixture was filtered, washed with ethyl acetate, slurried with dichloromethane and hexane, and filtered to afford 15 as a brown solid (0.027 g, 42%). Mp: 214.0 °C dec. HPLC: 100% [t_R = 8.90 min, gradient 5–95% CH₃OH in water (0.1% formic acid), 20 min]. ¹H NMR (400 MHz, DMSO- d_6) δ 9.85 (s, 1H), 9.43 (s, 1H), 8.45 (s, 1H), 8.03 (s, 1H) 7.27 (d, J = 8.8 Hz, 2H), 6.74 (d, J = 8.8 Hz, 2H), 4.08–4.01 (m, 1H), 3.73–3.68 (m, 1H), 3.62–3.57 (m, 2H), 1.90–1.74 (m, 3H), 1.58–1.50 (m, 1H). LC–MS (ESI+): m/z320.10400 (M + H)⁺. HRMS (ESI+): m/z calcd for C₁₅H₁₇ClN₄O₂ (M + H) 321.11128, found 321.11161.

5-Bromo-*N*²-(**4-hydroxyphenyl**)-*N*⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,**4-diamine** Hydrochloride [(±)-16]. A mixture of 8b (0.500 g, 1.7 mmol) and 4-aminophenol (0.186 g, 1.7 mmol) in EtOH (5 mL) in a microwave vial was heated to 150 °C for 20 min in a microwave reactor. The solvent was evaporated, and the residue obtained was filtered, washed with ethyl acetate, slurried with dichloromethane and hexane, and filtered to afford **16** as a gray solid (0.70 g, 99%). Mp: 188 °C dec. HPLC: 95.7% [*t*_R = 5.83 min, 40% CH₃OH in water (0.1% TFA), 20 min]. ¹H NMR (400 MHz, CD3OD): δ 7.90 (s, 1H), 7.22 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 4.16–4.13 (m, 1H), 3.84–3.82 (m, 1H), 3.76–3.71 (m, 1H), 3.56 (d, *J* = 5.6 Hz, 2H), 2.01–1.89 (m, 3H), 1.63–1.58 (m, 1H). LC–MS (ESI+): *m/z* 367 (M + H)⁺. HRMS (ESI+): *m/z* calcd for C₁₅H₁₇BrN₄O₂ (M + H) 365.0607, found 365.0586.

5-Bromo- M^2 -(**4-hydroxy-3-methylphenyl**)- M^4 -[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,**4**-diamine Hydrochloride [(±)-17]. A mixture of 8b (0.400 g, 1.4 mmol) and 4-aminophenol (0.168 g, 1.4 mmol) in EtOH (3 mL) in a microwave vial was heated to 150 °C for 20 min in a microwave reactor. The solvent was evaporated, and the residue obtained was washed with ethyl acetate, slurried with dichloromethane and hexane, and filtered to afford 17 as a pure gray solid (0.60 g, 99%). Mp: 206–209 °C. HPLC: 96% [t_R = 5.64 min, 45% CH₃OH in 0.1% TFA/water, 20 min] ¹H NMR (400 MHz, CD₃OD): δ 7.86 (s, 1H), 7.11 (s, 1H), 7.03(d, *J* = 8.0 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 1H), 4.18–4.13 (m, 1H), 3.86–3.82 (m, 1H), 3.76–3.71 (m, 1H), 3.57 (d, *J* = 5.6 Hz, 2H), 2.20 (s, 3H), 2.01–1.90 (m, 3H), 1.65–1.57 (m, 1H). LC–MS (ESI+): *m/z* 367 (M + H)⁺. HRMS (ESI+): *m/z* calcd for C₁₆H₂₀BrN₄O₂ (M + H) 379.0764, found 379.0760.

5-Bromo-*N*²-(4-methoxyphenyl)-*N*⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine Hydrochloride [(±)-18]. A mixture of 8b (0.100 g, 0.34 mmol) and 4-methoxyaniline (0.040 g, 0.34 mmol) in EtOH (2 mL) in a microwave vial was heated to 150 °C for 20 min in a microwave reactor. The solvent was evaporated, and the residue obtained was washed with ethyl acetate, slurried with dichloromethane and hexane, and filtered to afford 18 as a brown solid (0.134 g, 93%). Mp: 192 °C dec. HPLC: 95.6% [t_R = 9.89 min, 45% CH₃OH in water (0.1% TFA), 20 min]. ¹H NMR (400 MHz, DMSO d_6): δ 7.91 (s, 1H), 7.34 (d, *J* = 8.8 Hz, 2H), 6.99 (d, *J* = 8.8 Hz, 2H), 4.16–4.13 (m, 1H), 3.87–3.79 (m, 4H), 3.82 (s, 3H), 3.56 (d, *J* = 5.6 Hz, 2H), 2.00–1.88 (m, 3H), 1.65–1.59 (m, 1H). LC–MS (ESI+): *m*/*z* af9 and 381 for Br isotopes (M + H)⁺. HRMS (ESI+): *m*/*z* calcd for C₁₆H₁₉BrN₄O₂ (M + H) 379.0764, found 379.0766.

5-Bromo- N^2 -[4-(4-methylpiperazin-1-yl)phenyl]- N^4 -[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(±)-19]. The piperazine 9i (0.055 g, 0.10 mmol), iodomethane (0.014 g, 0.10 mmol), and potassium carbonate (0.55 g, 0.40 mmol) were mixed in MeOH (2 mL) and stirred at room temperature for 8 h. The mixture was then concentrated under reduce pressure, and the solid was purified by flash chromatography (5 g of silica gel, 0–5% MeOH) to afford 19 as a yellow solid (0.012 g, 27%). Mp: 123 °C dec. ¹H NMR (400 MHz, DMSO- d_6) δ 8.97 (s, 1H), 7.94 (s, 1H), 7.54 (d, J = 8.8 Hz, 2H), 6.83 (d, J = 8.8 Hz, 2H), 6.75 (t, J = 5.6 Hz, 1H), 4.08–4.03 (m, 1H), 3.78–3.73 (m, 1H), 3.63–3.58 (m, 1H), 3.43–3.40 (m, 2H), 3.05 (br s, 4H), 2.54 (br s, 4H, partially overlapped with DMSO signal), 2.29 (s, 3H), 1.91–1.56 (m, 3H), 1.63–1.56 (m, 1H). LC–MS (ESI+): m/z 446.14297 (M + H)⁺. HRMS (ESI+): m/z calcd for C₁₅H₁₇ClN₄O₂ (M + H) 447.1502, found 447.1493.

5-[(2-Chloro-6-fluorophenyl)carbamoyl]-N²-[4-(4-methylpiperazin-1-yl)phenyl]-N⁴-[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(±)-20]. The chloropyrimidine 8c (0.150 g, 0.390 mmol) and 4-(4-methylpiperazin-1-yl)aniline (0.082 g, 0.430 mmol) were mixed in a microwave vial with HCl (110 μ L of 4 M solution in dioxane). 2-Methoxyethanol (4.00 mL) was added to the mixture, and the resulting solution was heated at 110 $^\circ C$ for 16 h. The resulting solution was concentrated under reduced pressure, and the product was partitioned between CHCl3 and saturated aqueous NaHCO₃. The organic phase was then dried (Na₂SO₄), filtered, and concentrated under reduced pressure. The resulting solid was slurried twice with dichloromethane and hexane, filtered, and dried under reduced pressure to yield 20 as a dark gray solid (0.097 g, 47%). Mp: 210.4 °C dec. HPLC: 90.8% [$t_{\rm R}$ = 10.2 min, gradient 5–95% CH₃OH in water (0.1% formic acid), 20 min]. ¹H NMR (400 MHz, DMSO) δ 9.83 (s, 1H), 9.46 (s, 1H), 8.85 (s, 1H), 8.73 (s, 1H), 7.598 (d, J = 8.8 Hz, 2H), 7.43-7.30 (m, 3H), 6.86 (d, J = 8.8 Hz, 2H), 4.01-3.90 (m, 1H), 3.77-3.72 (m, 1H), 3.61-3.59 (m, 2H), 3.44-3.39 (m, 2H), 3.04 (app t, J = 4.0 Hz, 4H), 2.42 (app t, J = 4.4 Hz, 4H), 2.20 (s, 3H), 1.96-1.78 (m, 3H), 1.57-1.49 (m, 1H). LC-MS (ESI+): m/z 539.22118 (M + H)⁺. HRMS (ESI+): m/z calcd for $C_{27}H_{31}ClN_7O_2$ (M + H) 540.2284, found 540.2283.

 N^{2} -[4-(4-Methylpiperazin-1-yl)phenyl]- N^{4} -[(tetrahydrofuran-2-yl)methyl]pyrimidine-2,4-diamine [(+)-21]. 2-Chloro-N-((tetrahydrofuran-2-yl)methyl)pyrimidin-4-amine (0.150 g, 0.0.70 mmol) and 4-(4-methylpiperazin-1-yl)aniline (0.148 g, 0.0.77 mmol) were mixed in a microwave vial with HCl (140 μ L of a 4 M solution in dioxane). The solvent 2-methoxyethanol (4.00 mL) was added to the mixture, and the resulting solution was heated at 110 °C for 16 h. The resulting solution was concentrated, and the product was partitioned between CHCl₃ and saturated aqueous NaHCO₃. The organic phase was then dried (Na₂SO₄), filtered, and concentrated under reduced pressure. The resulting solid was slurried twice with dichloromethane and hexane, filtered, and dried under reduced pressure to yield 21 as a dark brown solid (0.108 g, 58%). Mp: 212 °C dec. HPLC: 99.9% [$t_{\rm R}$ = 9.36 min, 55% CH₃OH in water (0.1% TFA), 20 min]. ¹H NMR (400 MHz, DMSO) δ 8.64 (s, 1H), 7.71 (br s, 1H), 7.56 (d, J = 9.2Hz, 2H), 7.16 (br s, 1H), 6.79 (d, J = 9.2, 2H), 5.88 (d, J = 5.6 Hz, 1H), 3.99-3.93 (m, 1H), 3.77-3.74 (m, 1H), 3.64-3.58 (m, 2H), 3.00 (t, J = 5.2 Hz, 4H), 2.42 (t, J = 4.8 Hz, 4H), 2.19 (s, 3H), 1.92-1.77 (m, 3H), 1.57-1.50 (m, 1H). LC-MS (ESI+): m/z 369.23246 $(M + H)^{+}$. HRMS (ESI+): m/z calcd for $C_{20}H_{28}N_6O$ (M + H) 369.2397, found 369.2407.

4-(4-{4-[[5-((2-Chloro-6-fluorophenyl)carbamoyl)-4-(((tetrahydrofuran-2-yl)methyl)amino)pyrimidin-2-yl]amino]phenyl}piperazin-1-yl)-4-oxobutanoic Acid [(±)-22]. To a suspension of 9m (0.096 g, 0.15 mmol) in chloroform (2 mL) under an inert atmosphere was added DIPEA (0.194 g, 1.5 mmol). The mixture became a clear solution and was cooled to 0 °C. Succinic anhydride (0.015 g, 0.15 mmol) was added slowly, and the reaction mixture was warmed to rt and stirred for 1 h. The solvent was removed under reduced pressure. The resulting residue was sonicated in water (5 mL), filtered, washed with water (3 mL), and dried under vacuum to afford the title compound 22 as a light green solid (0.084 g, 89%). Mp: 189 °C dec. HPLC: 99.0% [$t_{\rm R}$ = 3.71 min, 60% CH₃OH in 0.1% TFA/water, 20 min]. ¹H NMR (400 MHz, DMSO- d_6): δ 12.03 (s, 1H, disappeared on D₂O shake), 9.84 (s, 1H, disappeared on D₂O shake), 9.51 (s, 1H, disappeared on D₂O shake), 8.85 (s, 1H, disappeared on D₂O shake), 8.73 (s, 1H), 7.61 (d, J = 8.8 Hz, 2H), 7.43-7.30 (m, 3H), 6.90 (d, J = 8.8 Hz, 2H), 4.03-3.97 (m, 1H), 3.75-3.71 (m, 1H), 3.62-3.57 (m, 6H), 3.44–3.39 (m, 1H), 3.07 (br t, 2H), 3.00 (br t, 2H), 2.56 (t, J = 6.4 Hz, 2H), 2.42 (t, J = 5.6 Hz, 2H), 1.96–1.89 (m, 1H), 1.85–1.78 (m, 2H), 1.57–1.49 (m, 1H). ¹⁹F NMR (376 MHz, DMSO- d_6): δ

-116.16 to -116.19 (m). LC-MS (ESI+): m/z 626.22; (M + H)⁺. HRMS (ESI+): m/z calcd for $C_{30}H_{34}ClFN_7O_5$ (M + H)⁺ 626.2289, found 626.2289.

Plasma Stabilities of (R)-9b and (S)-9b. The in vitro human plasma stabilities of compounds (R)-9b and (S)-9b were evaluated using HPLC analysis in a time-course experiment for up to 24 h.

A compound [(*R*)-9a, (*S*)-9a, procaine, and procainamide, 5 μ L of 10 mM stock solutions in DMSO] was added to human plasma (195 μ L), and a clear solution was obtained. This mixture was incubated at 37 °C for 0 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, and 24 h. After the incubation time MeOH (400 μ L) was added to the sample vial. A white precipitate was observed, and the mixture was centrifuged for 15 min. An aliquot (400 μ L) of the supernatant was removed from the mixture, and 60 μ L was injected for analysis by HPLC. The percentage of the compound remaining vs time showed the stability of each compound in human plasma. Procaine and procainamide were run as unstable and stable standards, respectively.

ASSOCIATED CONTENT

Supporting Information

Synthetic protocols for preparation of sulfides 10a-d, sulfones 11a-d, sulfides and sulfones 12a-r, and compounds 23-42 and Figures S1 and S2 showing (R)-9b superposed in JAK2 and ALK. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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ABBREVIATIONS USED

ACK1, activated CDC42-associated kinase 1; AR, androgen receptor; CRPC, castration-resistant prostate cancer; DIPEA, *N*,*N*-diisopropylethylamine

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